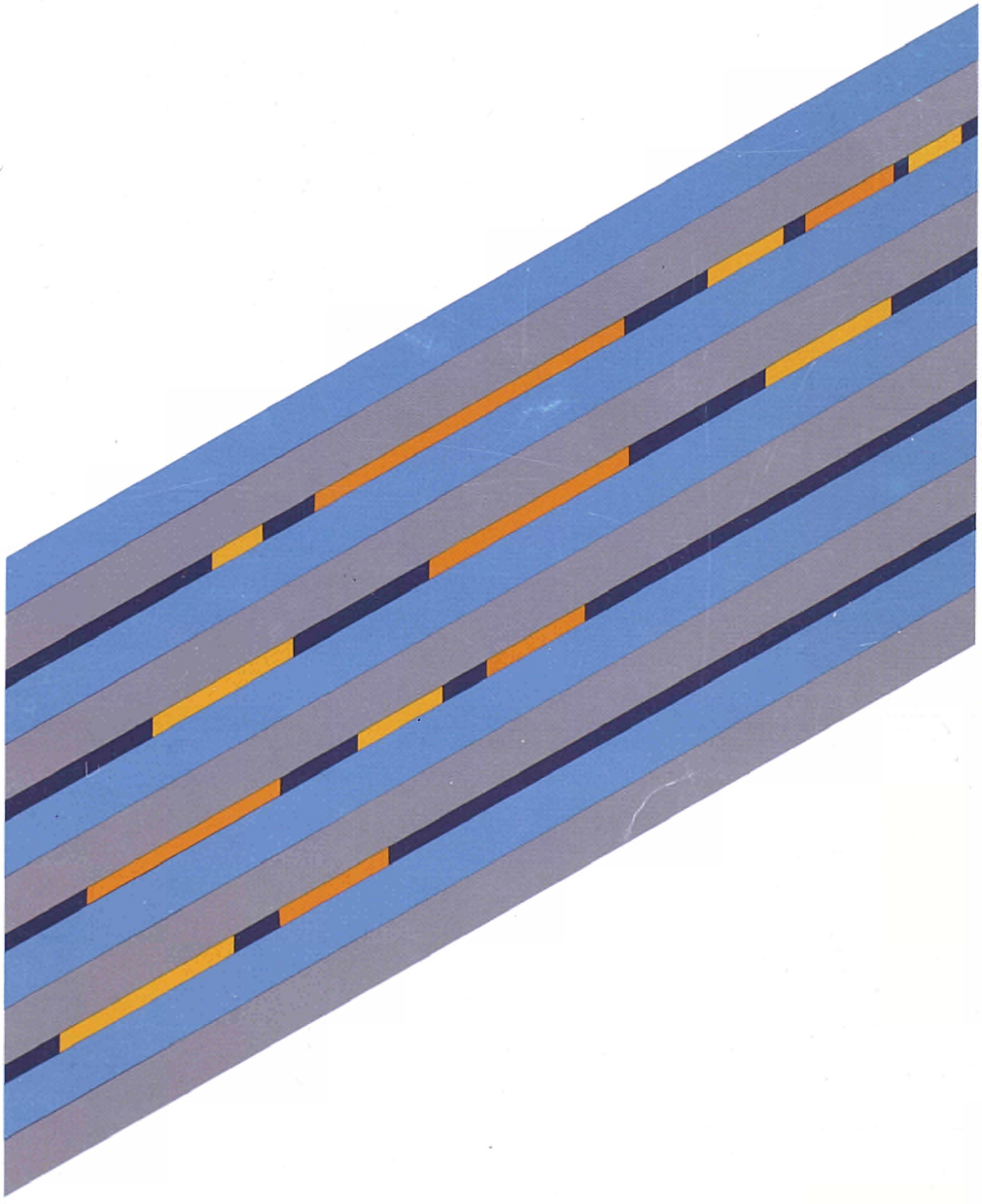
BAP

# Biotechnology action programme

## PROGRESS REPORT 1988

**Volume 1: An overview**



Commission of the European Communities
EUR 11650 EN/1

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# BAP

# 1985-89

## PROGRESS REPORT 1988

### Volume 1: An overview

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# INTRODUCTION

# I N T R O D U C T I O N

The multiannual research and training programme of the European Economic Community in the field of Biotechnology (BAP : Biotechnology Action Programme) was adopted by Council on 12 March 1985 for the period 1985 to 1989. The programme, inherently precompetitive, is oriented towards medium and long term objectives essential for the strategic strength of European industry and European agriculture. It deals with two essential tasks, namely :

- the establishment of a supportive infrastructure for biotechnology research and development in Europe ;
- the elimination, through research and through training, of bottlenecks which prevent the exploitation by industry and agriculture of the materials and methods originating from modern biology.

The programme extends over five years, with a total budget allocation of 55 Mio ECU.
The adoption by Council on 29 June 1988 of a programme revision further increased it resources by 20 Mio ECU and reinforced, without widening the range of activities in progress, some of its present weakest aspects, in particular the incomplete participation of Portugal and Spain who joined the Community after the expiration date of the calls for proposals.
The present progress report was prepared after the programme had been running for two and a half years. It outlines the preliminary results obtained by mid-1988 from transnational cost-shared research contracts between the Commission and associated public or private institutions from different Member States.

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## IMPLEMENTATION

From a total of 1,357 applications submitted in response to the calls for proposals made in 1985, 262 2-4 year shared-cost research contracts, falling in 93 transnational joint projects, were negotiated by the Commission services. The participating laboratories were selected on the basis of the interest and relevance of their proposals to the objectives of the programme, their scientific competence, the extent of industrial interest and involvement, and the provisions made for transnational cooperation. In fact all the 93 joint projects are transnational, involving from 2 to 7 partners from different Member States. Sixteen industrial firms are present in these associations, while 169 other firms expressed specific interest in one or more projects, and are now taking part in the industrial contact-groups with direct access to all scientific events taking place during the programme.
The sector on "toxicology testing in vitro" received a different treatment. A special call for proposals was launched in 1986, resulting in 158 applications out of which 16 were selected. In contrast to all other parts of BAP, this sector had a delayed start and is given here the first occasion to report on preliminary achievements.

The participating laboratories have been sorted out into 9 sectors of priority research, which correspond to a functional rearrangement of the 20 specific research lines initially composing the scope of the programme (Table 1).

One of the main tasks of the Commission services, after conclusion of the contracts, was to organize the participating laboratories into networks of small nuclei of Community expertise. These transnational groupings provide for rapid circulation and exchange of information, data and materials, and encourage mobility amongst research staff. Ideally, once

the competitive advantage resulting from the division of tasks and the gradual integration of work becomes established, these new transnational entities, composed of one or more research associations, evolve into real "European Laboratories Without Walls" (ELWWs), each entrusted with a specific research objective of the BAP programme. These ELWWs can undergo a very active transitory life, as demonstrated by increased scientific productivity (see below), and express a remarkable capacity to attract industrial interest.
Strikingly enough, ELWWs have no legal existence. They are entities larger than cooperation contracts, but smaller than entire Community research programmes. Their existence is the direct consequence of the programme and their vitality steadily increases during its implementation. By themselves they constitute a significant achievement of BAP.

The number of ELWWs, or of transnational groupings which tend to organise work on the same principle, approximates thirty, and increases in all sectors of the programme. The pace at which wide-ranging cooperations are established varies from case to case, and is logically higher when contractual partners have known each other through their participation in the former Community programme (BEP : 1982-1986), or when the scientific topics considered happen to be of a more basic nature. Such differences explain why some ELWWs reached their steady state at an early stage, while others still undertake gradual integration process.

The ELWWs listed below may be considered as particularly well established :

- genetic engineering of lactic acid bacteria,
- cellular neuroimmunology,
- mammalian minichromosomes and linear vectors,
- molecular biology of phytopathogenic Erwiniae,
- crop improvement through cell biotechnology,
- extremophile microorganisms,

- automation of DNA sequencing,
- computer-aided peptide and protein engineering software development,
- folding, assembly, stability and genetic modification of Penicillin acylase and its precursor,
- protein engineering of the Elongation factor Tu,
- biocatalysis by novel metal clusters,
- pollen biotechnology,
- molecular biology of cereal seed protein genes,
- plant hormone receptors,
- late symbiotic genes : improvement of Rhizobium strains,

etc...

In some cases the progress in research and the economic relevance of the results obtained point towards competitive developments. When this occurs, industrial pressure removes the laboratories out of the ELWW structure and inserts them, as a direct follow up of the Community programme, into an independent, often confidential, relationship of industrial cooperation.
This multiform transnational cooperation constitutes the basic justification for the programme. It would not have been possible without the sustained willingness to cooperate and the mutual esteem for the scientific competence of the laboratories involved. The role of Commission services there is modest, but essential. They provide a structure comparable to a marriage bureau which operates through three major mecanisms: meetings, visits to laboratories and publications.
From July 87 to June 1988, four sectoral meetings of contracting laboratories were organized, as well as forty smaller working seminars of ELWWs.

Seven scientific officers from the Biotechnology Division made 98 visits to contracting laboratories, to discuss progress, problems or opportunities with project leaders.

Detailed information on the activities and the results obtained by BAP contractors has been assembled and made available through a series of reports and review articles in scientific journals (cf. lists in the next section of this volume).

## PRELIMINARY ACHIEVEMENTS

The programme has now reached its half-way mark.
A panel of independent experts was invited by the Commission, at the beginning of 1988 to carry out an evaluation of its implementation mechanisms and of its early achievements. The conclusions of their analysis, both critical and encouraging, have appeared as report no. EUR 11 833 by the Office of Official Publications of the European Communities (Luxembourg). Commenting on both BEP and BAP, the evaluation panel stated that "a major achievement of the research programmes, and one that is important for the future, has been to break down national frontiers between laboratories. We commend the Commission's initiative in linking groups into European Laboratories Without Walls, a useful concept that could be applied in other domains". It also noted that programmes objectives had been sometimes over-ambitious for the resources available; and the lack of a critical mass of researchers in certain sectors was mentioned. Ambitious is the correct term, indeed, to charaterize the whole range of activities supported by the programme and reported in the following volumes. And scientific contractors themselves are not less ambitious when they embark on these many transnational ventures which are described below.

Fortunately, this Community endeavour is credited with an abundance of scientific achievements, many of them appearing already in a preliminary form at this intermediate stage of programme advancement.

As examples of some of the results, among many others, which the reader will find across the three volumes of reports, the following recent achievements can be quickly mentioned :

- Development of a kit to distinguish between vaccinated and infected pigs (pseudo rabies). The kit is now in exploitation phase.
- Isolation, for the first time, of thermotolerant methylotrophic Bacillus species with promising biotechnological potential.
- The finding that glycolysis in yeast is unregulated and that some of the enzymes are rate-limiting.
- Cloning for the very first time of a regulatory gene from cultivated higher plants by research of 2 groups supported continuously through BEP and BAP : the gene is "opaque-2" which controls zein synthesis in maize.
- In the framework of a collaboration between 7 BAP Contracts, cloning (expected to be completed by the end of 1988) of the first receptor protein gene controlling auxin perception.
- Preliminary encouraging results on the ability of engineered red blood cells to produce and release antineoplastic drugs from encapsulated pro-drugs.
- for the first time at pilot plant phase, successful achievement of co-factor regeneration.

Through a more comprehensive and statistical approach, the usual indicators of transnational activity have been studied during the reporting period, resulting in a graphical assessment of programme output which is presented in a condensed form below.

Credit must be given to several contractors for having so rapidly integrated their work that 47 joint publications have already appeared. Table 4 lists the joint publications for all sectors of the programme.

The exploitation of research results for the benefit of European agriculture and industry is one of the essential objectives of the BAP programme which can be achieved by giving all interested firms in the Community access to these results as they arise ; such access was systematically provided by the Commission services at all the sectoral contractors' meetings (see next section). In parallel to this effort, contractors were encouraged to protect, particularly through patenting, research results likely to lead to exploitation. A list of patents deposited to date in connection with the research under contract is provided in table 5. A review of development prospects will however not be possible before the end of the programme.

An obvious criterion of the success of the programme, however difficult to monitor, is the intensity of transational activities. Based on motivated statements requested from the contractors in their individual reports, an attempt has been made at quantifying the various types of cooperation agreements and exchanges taking place within the programme. Their frequency is reported in Figure 2.

Joint meetings rank very high, as a direct result of the coordinating activity of Commission services through sectoral meetings or working seminars of ELWWs. The exchange of materials is highly rated by the scientists as one of the easiest ways to initiate cooperation.

Joint experiments and staff exchange appeared to be more difficult to organize; nevertheless about 50 % of the participating laboratories were able to implement such activities which represent the ultimate phase of coordination.

## MANAGING PRINCIPLES AND FUTURE TRENDS

Since February 1985, on the eve of the adoption of BAP, the Commission services have been helped in managing the programme by the advice of the CGC-Biotechnology. This was in consequence of the decision by Council of 29 June 1984 to establish CGCs (management and coordination advisory committees) providing guidance to the Commission services on all tasks related to the preparation, implementation and evaluation of Community R & D programmes.

The services of the Commission wish to acknowledge with gratitude the help and advice which they have received from the CGC "Biotechnology" at the time of the launching phase of the programme and during its early implementation.

The CGC-Biotechnology, the composition of which is presented in table 6, has a large field of responsibility since it also covers the whole range of training activities in the field of biotechnology, COST actions related to primary aquatic biomass and to plant in vitro culture, concertation activities for the harmonization of biotechnology-related policies, and new initiatives under the Biotechnology sub-line of the Second Framework Programme.

The committee now provides the Commission with advice in connection with the preparation of the BRIDGE programme proposal. BRIDGE, which stands for Biotechnology Research for Innovation, Development and Growth in Europe, will replace BAP after 1989.

Considering the evolution of biotechnology R & D over the last ten years, and the increasing possibility of scientific breakthroughs reaching the market place, it is now essential to extend industrial participation beyond the implementation

phase and to associate European industry at all stages of the conception phases. An industrial advice, required throughout the entire preparatory phase of any new action, is being essentially provided through the expert assessments of IRDAC, the Industrial Research and Development Advisory Committee. This committee was created by a decision of the Commission of 29 February 1984. Having acknowledged the pervasiveness of biotechnology across many industrial sectors, and the specificity of products and production methods based on the exploitation of living matter, IRDAC has generated a working party on biotechnology, the composition of which is given is table 7. Members of the working party were selected on the basis of their industrial experince, but represent neither their employer nor their country of origin. They are consulted, as has been the CGC-Biotechnology, on the needs and opportunities for industrial R & D in this particular field. They issued in December 1987 a report on future R & D programmes in the field of biotechnology.

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# T A B L E S

and

# F I G U R E S

Table 1 : Organization of the programme into research sectors

| SCOPE OF THE PROGRAMME | MAJOR RESEARCH SECTORS |
|---|---|
| **I. CONTEXTUAL MEASURES** | |
| **1. Bio-informatics**<br>1.1. Data capture technologies<br>1.2. Updating and design of data banks related to biotic materials<br>1.3. Modelling techniques & algorithms<br>1.4. Advanced computer software, including expert systems | **1. BIO-INFORMATICS** |
| **2. Collections of biotic materials**<br>2.1. Upgrading of existing collections of importance as supporting resources for biotechnology R&D, and creation of new collections, required and made possible by the advances of science.<br>2.2. Development and improvement of technical methods of storage and resuscitation as well as improved methods of identification, description and classification. | **2. CULTURE COLLECTIONS** |
| **II. BASIC BIOTECHNOLOGY** | |
| **1. Enzyme engineering** | |
| 1.1. Development of bioreactors (multi-enzymatic, multiphasic, co-factor requiring or utilizing viscous media) | **3. BIOREACTORS** |
| 1.2. Stability of enzymes during industrial exploitation<br>1.3 Protein design including new concepts in enzyme catalysis, structural and functional predictions, chemical and genetic modifications, construction of artificial enzymes. | **4. PROTEIN DESIGN/ MACROMOLECULAR MODELLING** |
| **2. Genetic engineering** | |
| 2.1 Microorganisms : gene characterization and gene transfer for potential applications by industries. | **5. BIOTECHNOLOGY OF INDUSTRIAL MICROORGANISMS** |
| 2.2. Plants : Analyses of the structure and regulation of plant genomes.<br>2.3. Plants : transfer and cloning of genetic material in plant cells.<br>2.4. Microorganisms and plants : associations (in particular symbiotic relations) between crop plants and microorganisms. | **6. BIOTECHNOLOGY OF PLANTS AND ASSOCIATED MICROORGANISMS** |

Table 1 : Organization of the programme into research sectors (ctd)

| SCOPE OF THE PROGRAMME | MAJOR RESEARCH SECTORS |
|---|---|
| **II. BASIC BIOTECHNOLOGY (ctd)** | |
| 2. **Genetic engineering (ctd)** | |
| 2.5. Plants : early detection of genetic or pathological modifications in cultivated plants. | **6. BIOTECHNOLOGY OF PLANTS & ASSOC. MICROORGANISMS (ctd)** |
| 2.6. Animals (livestock, including fish) : cloning of substances important for animal husbandry, cloning vectors for animal cells, cloning of genetic material in animal cells. | **7. BIOTECHNOLOGY OF ANIMALS** |
| 3. **Technology of cells and tissues cultured in vitro** | |
| 3.1. Physiological and genetic factors governing yield and stability during continuous cultivation of microbial species important to industry. | **5. BIOTECHNOLOGY OF INDUSTRIAL MICROORGANISMS (ctd)** |
| 3.2. Control of the differentiation of plant cells and of their regeneration in entire plants. | **6. BIOTECHNOLOGY OF PLANTS & ASSOC. MICROORGANISMS (ctd)** |
| 3.3. Novel methodologies of animal cells cultures. | **7. BIOTECHNOLOGY OF ANIMALS (ctd)** |
| 4. **Assessment of risks**<br>Development of new methods for detecting contamination and for the assessment of possible risks associated with applications in industry and agriculture of biomolecular engineering. In particular, development of methods for the assessment of risks resulting from the release of genetically engineered organisms. | **8. RISK ASSESSMENT** |
| 5. **Testing methods**<br>The development of *in vitro* screening methods for the evaluation of the toxicological effects and the biological activity of molecules. | **9. IN VITRO TESTING METHODS** |

**Table 2 : Feature articles issued during the reporting period in each research sector of the BAP programme.**

| Research sector | Number of labs (1) | Number of articles (2) | Number of articles with trans-national authorship(*) | (2)/(1) in 1988 | (2)/(1) in 1987 |
|---|---|---|---|---|---|
| Bio-informatics | 42 | 47 | 3 | 1.12 | 0.61 |
| Culture collections | 13 | 3 | 2 | 0.23 | 0.15 |
| Bioreactors | 19 | 44 | 2 | 2.32 | 1.27 |
| Protein engineering | 26 | 76 | 7 | 2.92 | 1.08 |
| Biotechn. of indust. microbes | 35 | 77 | 8 | 2.20 | 1.00 |
| Biotechn. of plants & assoc. microorganisms | 64 | 171 | 19 | 2.67 | 1.27 |
| Biotechn. of animals | 43 | 85 | 6 | 1.98 | 0.69 |
| Risks assessment | 7 | 9 | 0 | 1.29 | 0.00 |
| In vitro tests | 16 | 23 | 0 | 1.44 | - |

(*) only those implying the participation of other BAP contractors.

**Table 3 : Feature articles from BAP contractors tabulated against project sizes (situation applying to the reporting period)**

| Project size (Number of participating laboratories) | Number of labs in the category (1) | Number of articles (2) | (2)/(1) in 1988 | (2)/(1) in 1987 |
|---|---|---|---|---|
| 1 | 3 | 5 | 1.67 | 0 |
| 2 | 98 | 216 | 2.20 | 0.85 |
| 3 | 66 | 125 | 1.89 | 0.90 |
| 4 | 36 | 59 | 1.64 | 0.74 |
| 5 | 50 | 103 | 2.06 | 1.14 |
| 6 | 6 | 23 | 3.83 | 1.83 |
| 7 | 7 | 4 | 0.57 | - |

**Table 4 : List of feature articles in scientific journals to which BAP contractors from different countries jointly contributed, partly or entirely as a result of their cooperation under the Community programme** (as per 1.7.1988)

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* *

## BIO-INFORMATICS

BAP-0293 (with sub-Contractors) :

**Superresolution in Confocal Scanning Microscopy.**
M. Bertero, P. Brianzi, E.R. Pike, Inverse Problems, 3, 195-212, 1987

BAP-0293 (with sub-Contractors) :

**An Analytic Inversion Formula for Confocal Scanning Microscopy.**
M. Bertero, C. De Mol, E.R. Pike. JOSA A-4, 1748-1750, 1987

BAP-0293 (with sub-Contractors) :

**Inverse Problems in Confocal Scanning Microscopy.**
M. Bertero, P. Boccacci, P. Brianzi, E.R. Pike, in Inverse Problems : An Interdisciplinary Study (Ed. P.C. Sabatier), Advances in Electronics and Electron Physics, Supplement 19, 225-239, (Academic Press, 1987)

## CULTURE COLLECTIONS

BAP-0002 & BAP-0007 :

**European Resource Centres for Plasmid Bearing Bacterial Strains.**
C. Rohde and V.M. Hugues. REGEM I. (Release of Genetically Engineered Microorganisms Conference), 1988

BAP-0004, BAP-0005 & BAP-0134 :

**Structuring strain data for storage and retrieval of information on fungi and yeasts in MINE, the Microbial Information Network Europe.**
W. Gams, G.L. Hennebert, J.A. Stalpers, D. Janssens, N.A.A. Schipper, J. Smith, D. Yarrow and D.L. Hawksworth. J. Gen. Microbiol., 1988

## BIO-REACTORS

BAP-0060 & BAP-0065 :

**Synthesis and application of water-soluble macromolecular redox coenzyme derivatives.**
A.F. Buckman and G. Carrea. Adv. Biochem. Eng. Biotechnol., in press, 1988

BAP-0056 & BAP-0055 :

**Improved metabolic properties of hexokinase overloaded human erythrocytes.**
M. Magnani, L. Rossi, M. Bianchi, G. Fornaini, V. Benatti, L. Guida, E. Zocchi and A. De Flora. Biochim. Biophys. Acta, in press, 1988

## PROTEIN ENGINEERING

BAP-052 & BAP-0246 :

**Mechanism of ferritin iron uptake : activity of the H chain and deletion mapping of the ferro-oxidase site.**
S. Levi, A. Luzzago, G. Cesareni, A. Cozzi, F. Franceschinelli, A. Albertini and P. Arosio. J. Biol. Chem., in press, 1988

BAP-0066 & BAP-0058 :

**Properties of a Genetically Engineered G-Domain of Elongation Factor Tu.**
A. Parmeggiani, G.W.M. Swart, K.K. Mortensen, M. Jensen, B.F.C. Clark, L. Dente and R. Cortese. Proc. Natl. Acad. Sci. USA 84, 3141-3145, 1988

BAP-0066 & BAP-0058 :

**Site-Directed Mutagenesis of Elongation Factor Tu.**
A. Parmeggiani, E. Jacquet, M. Jensen, P.H. Anborgh, R.H. Cool, J. Jonak and G.W.M. Swart. In : Metabolism and Enzymology of Nucleic Acids Including Gene Manipulations (J. Zelinka & J. Balan, eds) p. 175-187, Vol. 6, Slovak Academy of Sciences, Bratislava, 1987

BAP-0066 & BAP-0058 :

**The Polypeptide Chain Elongation Factor Tu : Charaterization of Mutants and Protein Engineering.**
A. Parmeggiani, P.H. Anborgh, R.H. Cool, E. Jacquet, M. Jensen, F. Gümüsel, G. Parlato and G.W.M. Swart. In : Genetics of Translation : New Approaches (M. Bolotin-Fukuhara, & M. Picard, eds) p. 259-269, NATO ASI series, 1988

BAP-0062 & BAP-0071 :

**Phospholipase A2 inhibithors : acylamino-glycero-phosphocholines derivatives.**
G.H. de Haas, M.G. van Hoort, R. Dijkman and R. Verger. Biochem. J., in press, 1988

BAP-0148 & BAP-0149 :

**Secondary structure prediction : combination of three different methods.**
V. Biou, J.F. Gibrat, J.M. Levin, B. Robson and J. Garnier. Protein Engineering, 2 (3), in press, 1988

BAP-0259 & BAP-0269 :

**Hydrogen production and deuterium-proton exchange reactions catalyzed by Desulfovibrio Ni(II) substituted rubredoxins.**
P. Saint-Martin, P.A. Lespinat, G. Fauque, Y. Berlier, J. LeGall, J.J.F. Moura, M. Teixeira, A.V. Xavier and I. Moura. Proc. Natl. Acad. Sci., in press 1988

## BIOTECHNOLOGY OF INDUSTRIAL MICROORGANISMS

BAP-0061 & BAP-0026 :

**Transformation of the yeast Kluyveromyces lactis by new vectors derived from the 1.6 um circular plasmid pKD1.**
M. Bianchi, C. Falcone, X.J. Chen, M. Wesolowski-Louvel, L. Frontali and H. Fukuhara. Cur. Gent., 1987

BAP-0061 & BAP-0026 :

**A gene cloning system for Kluyveromyces lactis and isolation of a chromosomal gene required for killer toxin production.**
X.J. Chen, M. Wesolowski-Louvel, C. Tanguy-Rougeau, H. Fukuhara, M.M. Bianchi, L. Fabiani, M. Saliona, C. Falcone and L. Frontali. J. Basic. Microbiol., in press, 1988

BAP-0061 & BAP-0026 :

**New vectors for yeast transformation : replication in different yeast genera and stability in non selective media.**
M.M. Bianchi, X.J. Chen, M. Wesolowski-Louvel, H. Fukuhara, C. Falcone, L. Fabiani, M. Saliola and L. Frontali. In : Physiological and Genetic Modulation of Product Formation. Dechema Monographs. Vol. 105-VCH. Verlag Gesellshaft, 1987

BAP-0044 & BAP-0046 :

**Conjugal gene transfer in Clostridium acetobutylicum.**
A. Davies, J.D. Pennock, D.R. Williams, D.F. Richars, N.P. Minton and M. Young. In : Genetics and Biotechnology of Bacilli. Academic Press. Orlando, USA, in press, 1988

BAP-0044 & BAP-0046 :

**Contruction of plasmid vector systems from Clostridium acetobutylicum.**
J.K. Brehm, J.D. Oultral, D.E. Thompson, T.J. Swinfield, H. Peck, M. Young and N.P. Minton. In : Genetics and Biotechnology of Bacilli. Academic Press. Orlando, USA, in press, 1988

BAP-0008 & BAP-0012 :

**Cloning and characterization of the tetracycline resistance determinant of and several promoters from within the conjugative transposon Tn919.**
C.J. Hill, G. Venema, C. Daly and G.F. Fitzgerard. Appl. Environ. Microbiol., 1988

BAP-0152 & BAP-0153 :

**Ethanol production by Zymomonas mobilis entrapped in alumina pellets.**
A.A. Koutinas, M. Kanellaki, A. Lykourghiotis, M.A. Typas and C. Drainas. Appl. Microbiol. Biotechnol., 1988

BAP-0039, BAP-0064 & BAP-0248 :

**Transformation of Aspergillus oryzae using A. niger pyrG gene.**
I.E. Mattern, S.E. Unkles, J.R. Kinghorn, P. Pouwels and C.A.M.J.J. van den Hondel. Mol. Gen. Genet., 1988

## BIOTECHNOLOGY OF PLANTS AND ASSOCIATED MICROORGANISMS

BAP-0082 & BAP-0101 :

**Recent advances in plant electroporation.**
H. Jones, M.J. Tempelaar and M.G.K. Jones, Oxford Surveys of Molecular and Cell Biology (Ed. B.J. Miflin), OUP, 4, 347-357, 1987

BAP-0082 & BAP-0101 :

**Modulation and direction of the electrofusion response in plant protoplasts.**
M.J. Tempelaar, A. Duyst, S.J. de Vlas, G. Krol, C. Symmonds, M.G.K. Jones, Plant Science, 48, 99-105, 1987

BAP-0082 & BAP-0101 :

**Electrofusion of nitrate reductase deficient Nicotiana plumbaginifolia mutants : studies on optimization and complementation.**
S.C. de Vries, E. Jacobsen, M.G.K. Jones, A.E.H.M. Loonen, M.J. Tempelaar, J. Wybrandi and W.J. Feenstra, Plant Science, 51, 105-112, 1987

BAP-0082 & BAP-0101 :

**Somatic hybridization of amino-acid analogue resistant cell lines of potato (Solanum tuberosum L.) by electrofusion.**
S.C. de Vries, E. Jacobsen, M.G.K. Jones, A.E.H.M. Loonen, M.J. Tempelaar, J. Wybrandi and W.J. Feenstra, Theor. Appl. Genet., 73, 451-458, 1987

BAP-0084 & BAP-0111 :

**Direct gene transfer in protoplasts of Nicotiana plumbaginifolia.**
F. Sala, M.L. Marchesi, S. Castiglione, J. Paszkowsky, M. Saul, I. Potrykus and I. Negrutiu, in : Biotechnology in Agriculture and Forestry (Ed. Y.P.S. Bajaj), Springer-Verlag, New York (in press)

BAP-0092 & BAP-0093 :

**Carrot somatic embryogenesis depends on the phytohormone-controlled expression of correctly glycosylated extracellular proteins.**
S.C. de Vries, H. Booj, R. Janssens, R. Vogels, L. Saris, F. LoSchiavo, M. Terzi and A. van Kammen, Genes and Development, 2, 462-476, 1988

BAP-0213- & BAP-0214 :

**Molecular cloning of the o2-m5 allele of Zea mays using transposon marking.**
M. Motto, M. Maddaloni, G. Ponziani, M. Brembilla, R. Marotta, N. Di Fonzo, C. Soave, R. Thompson and F. Salamini, Mol. Gen. Gent., 212, 488-494, 1988

BAP-0213 & BAP-0214 :

**The b-32 protein from maize endosperm, an albumin regulated by the 02 locus : nucleic acid (cDNA) and amino acid sequences.**
N. Di Fonzo, H. Hartings, M. Brembilla, M. Motto, C. Soave, E. Navarro, J. Palau, W. Rohde, F. Salamini, Mol. Gen. Genet., 212, 481-487, 1988

BAP-0213 & BAP-0214 :

**Maize seed storage proteins : genetic structure and regulation.**
N. Di Fonzo, H. Hartings, M. Maddaloni, L. Manzocchi, G. Ponziani, J. Palau, F. Salamini, C. Soave, A. Spada, R. Thompson and M. Motto, Genet. Agr. (in press).

BAP-0213 & BAP-0214 :

**Molecular studies of the high-lysine genes Opaque-2 and Opaque-6 in maize.**
N. Di Fonzo, H. Hartings, M. Maddaloni, L. Manzocchi, G. Ponziani, J. Palau, F. Salamini, C. Soave, R. Thompson and M. Motto, UCLA Symposium : The molecular basis of plant development. Steamboat Springs, Co., 1988

BAP-0190 & BAP-0212 :

**Characterization and virulance properties of Erwinia chrysanthemi lipopolysaccharide-defective, phiEC2-resistant mutants.**
E. Schoonejans, D. Expert and A. Toussaint, J. Bacteriol. 169, 4011-4017, 1987

BAP-0190 & BAP-0212 :

**Chromosonal mapping of the pel andi cel genes in Erwinia chrysanthemi strain B 374.**
M. Piecq, F. van Gijsegem, E. Schoonjans and A. Toussaint, Molec. Microbiol. 2, 297-302, 1988

BAP-0190 & BAP-0212 :

**Molecular cloning and mutagenesis in Escherichia coli of pectinase genes from Erwinia chrysanthemi.**
A. Kotoujansky, A. Diolez, M. Rouve, F. Van Gijsegem, S. Reverchon et al., Proc. 6th Int. Conf. Plant Pathogen. Bact., College Park, Maryland, pp. 139-151, Nijhoff/Junk, Dordrecht, 1987

BAP-0080 & BAP-0081 :

**Organization and partial sequence of a DNA region of the Rhizobium leguminosarum symbiotic plasmid pRL6JI containing the genes fix ABC, nifA, nifB and a novel open reading frame.**
P. Grönger, S.S. Massian, H. Reiländer, M. O'Connell, U.B. Priefer and A. Pühler, Nucleic Acids Research, 15, 31-49, 1987

BAP-0096 & BAP-0173 :

**Regulation of nodule-specific plant genes.**
E.O. Jensen, J. Stougaard, J.E. Jorgensen, N. Sandal, F.J. de Bruijn, J. Schell and K.A. Marcker, Proc. 7th Int. Cong. Nitrogen Fixation Cologne, Gustav Fisher, Stuttgart, 605-609, 1988

BAP-0096 & BAP-0173 :

**Interaction of a nodule-specific trans-acting factor with distinct DNA elements in the soybeam leghaemoglobin lbc $_3$ 5'-upstream region.**
E. O. Jensen, K.A. Marcker, J. Schell and F.J. de Bruijn, The EMBO Journal 7/5, 1265-1271, 1988

BAP-0095 & BAP-0096 :

**Plastid transformation : a progress report.**
M.J. Cornelissen, M. De Block, M. van Montagu, J. Leemans, P.H. Schreier and J. Schell, In : Gene Research-Plant DNA Infections Agents (Ed. T. Hohn and J. Schell), pp. 311-320, 1987

BAP-0202 & BAP-0204 :

**Three-dimensional image and mitochondrial distribution in sperm cells of Euphorbia dulcis.**
M. Murgia and H.J. Wilms, In : Plant sperm cells as tools for biotechnology (Ed. H.J. Wilms and C.J. Keijzer) Pudoc, Wageningen, pp. 75-79, 1988

BAP-0202 & BAP-0204 :

**Confocal scanning laser microscopy of Galanthus generative cells.**
H.J. Wilms, M. Murgia and E.A. van Spronsen, In : Plant sperm cells tools for biotechnology (Ed. H.J. Wilms and C.J. Keijzer) Pudoc, Wageningen, pp. 35-39, 1988

## BIOTECHNOLOGY OF ANIMALS

BAP-0156 & BAP-0114 :

**Analysis of the Structural Polypeptides of a Porcine Group C Rotavirus.**
M. Bremont, J. Cohen and M.A. McCrae. Journal of Virology 62, 21183-2185, 1988

BAP-0115 & BAP-0116 :

**Cis - and Trans-acting elements responsible for the cell specific expression of the human al-antitrypsin gene.**
V. De Simone, G. Ciliberto, E. Hardon, G. Palla, L. Lundberg and R. Cortese. EMBO J. 6, 2759-2766, 1987

BAP-0115 & BAP-0116 :

**Recombinant Interleukin 6 Regulates the Transcriptional Activation of a Set of Human Acute Phase Genes.**
G. Morrone, G. Ciliberto, S. Oliviero, R. Arcone, L. Dente, J. Content and R. Cortese. J. Biol. Chem. in press

BAP-0115 & BAP-0116 :

**The human al-antitrypsin gene is transcribed from two different promoters in macrophages and hepatocytes.**
E. Perlino, R. Cortese and G. Ciliberto. EMBO J. 6, 2767-2771, 1987

BAP-0123 & BAP-0157 :

**Immunity conferred to mice by anti-LPS monoclonal antibodies in murine Brucellosis.**
J.N. Limet, A.M. Plommet, G. Dubray and M. Plommet. Ann. Immunol. Inst. Pasteur, 138, 417-424, 1987

BAP-0131 & BAP-0146 :

**Genetic analysis of the role of toxins and protein A in the pathogenosis of Staphylococcus aureus infections. Properties of an unexpressed alpha-toxin locus from a clinical isolate.** T.J. Foster, M. O'Reilly, A.H. Patel, P. Nowlan, J. Bramley, in Bacterial Protein Toxins (Ed. F. Fehrenbach), Gustav Fischer, Stuttgart, New York, 1988

**Table 5 : List of patents deposited in connection with the BAP programme before the 1st July 1988 (within brackets is the name of the project leader involved, who is not necessarily the first author of the patent).**

- Sampling of material (M.S. Beck)
- Monofilament sampling (M.S. Beck)
- Resilient strip (M.S. Beck)
- Editeur de séquences biologiques (the 1D editor) (J.F. Sallantin)
- PDOS:the emulation of DOS for the communication board (J.F. Sallantin)
- PC DRA : the 3D editor (J.F. Sallatin)
- Tool Box Crim (graphic tool box) (J.F. Sallantin)
- GOR III, Homologue, Bit pattern, Combine : Programmes (J. Garnier)
- Process for the production of $N^6$ substituted NAD, NADP or FAD (A.F. Bückmann)
- Procédé de dosage des lipases (R. Verger)
- Lipases et extraits lipasiques, leur procédé de préparation et leur application, notamment en thérapeutique (R. Verger)
- Viral gene and enzyme = two patents (M. Gasson)
- Vecteurs de clonage et d'expression comportant le génome du virus de l'érythroblastose aviaire ou le génome du virus helper RAV-1 (V.M. Nigon)
- Inhibiteur de la synthèse protéique, procédé d'isolement, utilisation et composition pharmaceutique en contenant (F. Stirpe)
- Immunotoxines, procédé de préparation et compositions pharmaceutiques en contenant (F. Stirpe)

Table 6 : Members of the Advisory Committee for the Management and Coordination of the Biotechnology Action Programme.

| | |
|---|---|
| **BELGIQUE - BELGIE** | **IRELAND** |
| M. Bienfet | E.P. Cunningham (+) |
| J. de Brabandère (**) | B. Finucane (**)(+) |
| A.M. Prieels (+) | B. McSweeney (+) |
| (G. Thiers) | J. O'Grady (****) |
| | J. Ryan (****) |
| **BUNDESREPUBLIK DEUTSCHLAND** | **ITALIA** |
| N. Binder (+) | A. Albertini |
| H. Klein (*) | M. Moretti |
| R. Wandel | (M. Lener) |
| (E. Warmuth) | (G. Magni) |
| **DANMARK** | **LUXEMBOURG** |
| P.O. Larsen (+) | F. Arendt |
| K.A. Marcker (****) | A. Betz |
| I. Petersen | |
| **ELLINIKI DIMOKRATIA** | **NEDERLAND** |
| C.E. Sekeris | H.J. Grande (+) |
| A.L. Stavropoulos (+) | B.A. Heide (***) |
| A.S. Tsaftaris (**) | M.C.F. van den Bosch |
| | R.R. van der Meer (<) |
| | (E. Veltkamp) |
| **ESPANA** | **PORTUGAL** |
| A. Albert (**) | F.J.A. Carvalho Guerra (**) |
| R. Revilla Pedreira (**) | A. Xavier (**) |
| **FRANCE** | **UNITED KINGDOM** |
| P. Douzou (+) | R.H. Aram (+) |
| M. Lelong | D.A. Jonas (+) |
| P. Printz (*) | D.G. Lindsay (***) |
| (G. Pelsy) (+) | A.F. Lott (*) |
| | (F.P. Woodford) (+) |
| | (H. Pickles) (****) |
| **COMMISSION** : | F. Van Hoeck |
| | D. de Nettancourt |

(*) from 1985
(**) from 1986
(***) from 1987
(****) from 1988

(<) Chairman
( ) Substitutes
(+) resigned

Table 7 : Members in 1988 of the Biotechnology Working Party of IRDAC.

| | |
|---|---|
| K. Baker | P. Mangold |
| R. Brown | B. McSweeney |
| Dr. Castell | G. Mignoni |
| G. Delheye | M. Miller |
| Dr. Dornauer | M. Nominé |
| D. Gunary | K. Powell |
| A. Herrero | J.P. Raynaud |
| M. Hilmer Nielsen (*) | V. Rubio |
| Dr. Hirsinger | E. Shejbal |
| B. Jarry | A. Stavropoulos |
| R. Jeambourquin | M.A. van Damme |
| N. Kossen | R.R. van der Meer |
| B. Le Buanec | J.E. Veldhuijzen van Zanten |
| M. Le Hodey | D. von Wettstein |
| W. Leuchtenberger | |

(*) Chairman of the working party

**Table 8 : Commission staff for the implementation of BAP**

DIRECTORATE-GENERAL XII
SCIENCE, RESEARCH AND DEVELOPMENT
Director-General : **P. Fasella**

COST-SHARED ACTIONS
(Directorates C, E, F and FUSION)
Deputy-Director-General : **H. Tent**

DIRECTORATE F
BIOLOGY
Director : **F. van Hoeck**

DIVISION F-2
BIOTECHNOLOGY
Head : **D. de Nettancourt**

| **B A P research actions : 1985 - 1989** | |
|---|---|
| Bio-informatics | P. Reiniger (1), B. Nieuwenhuis (2) |
| Culture collections | P. Reiniger (1), A. Aguilar (2) |
| Bioreactors | A. Goffeau (3), B. Nieuwenhuis (4), I. Economidis (5) |
| Protein engineering | B. Nieuwenhuis, A. Goffeau (3) |
| Industrial microorganisms | A. Aguilar, A. Goffeau (3) |
| Plants & assoc. microorganisms | E. Magnien |
| Animals | P. Larvor (6), H. Bazin, A. Klepsch (7) |
| In vitro testing methods | P. Larvor (6), A. Klepsch (7) |
| Risk assessment | U. Bertazzoni, I. Economidis (5) |
| **BAP training actions : 1985 - 1989** | |
| All sectors | A. Goffeau (3), D. de Nettancourt (8) |

| **Future actions** |
|---|
| B R I D G E : 1990 - 1994 |

(1) until 31.03.87
(2) from 01.04.87
(3) until 31.12.86
(4) until 15.09.87
(5) from 16.09.87
(6) until 31.07.87
(7) from 01.08.87
(8) from 01.01.87

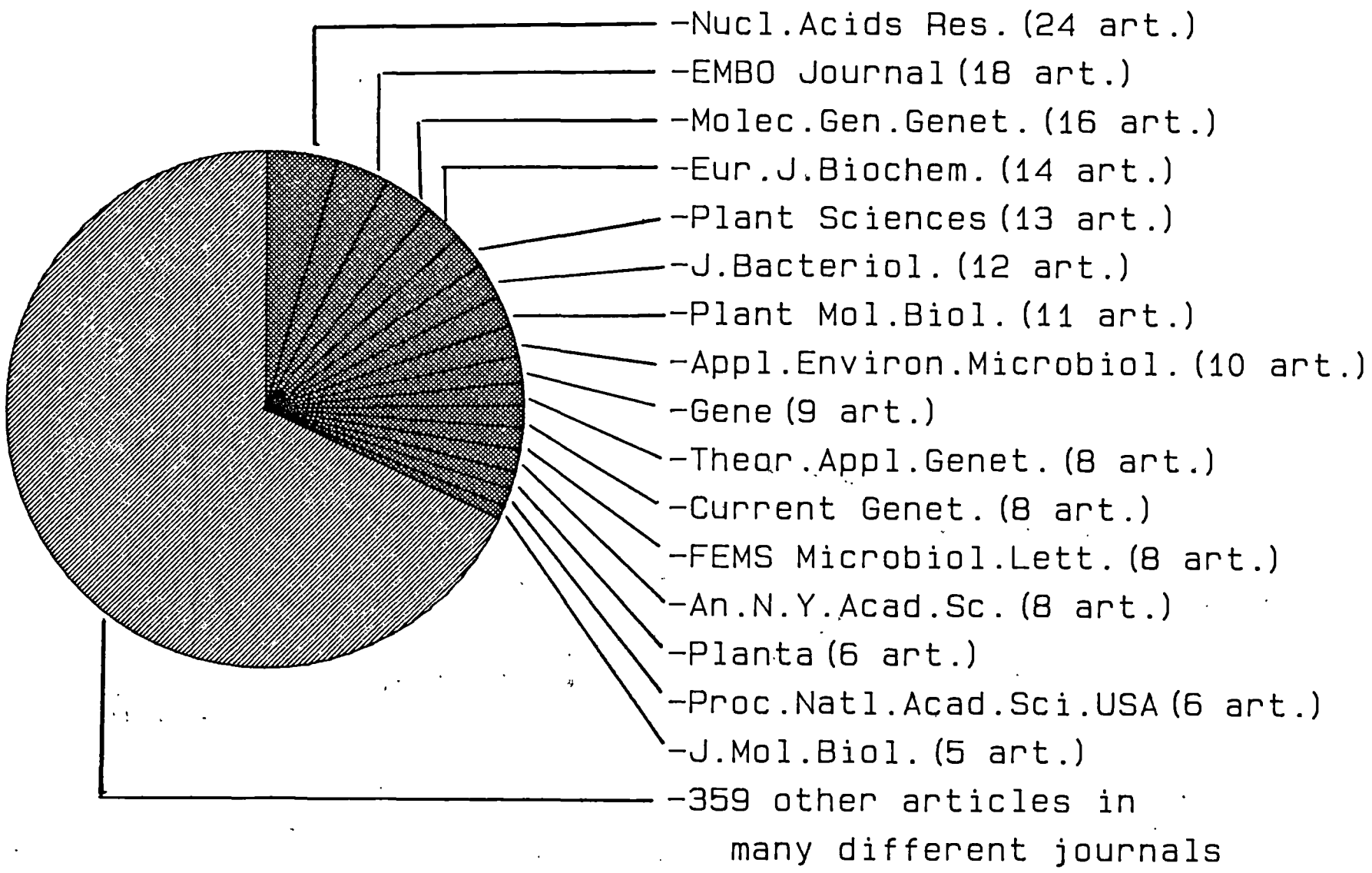
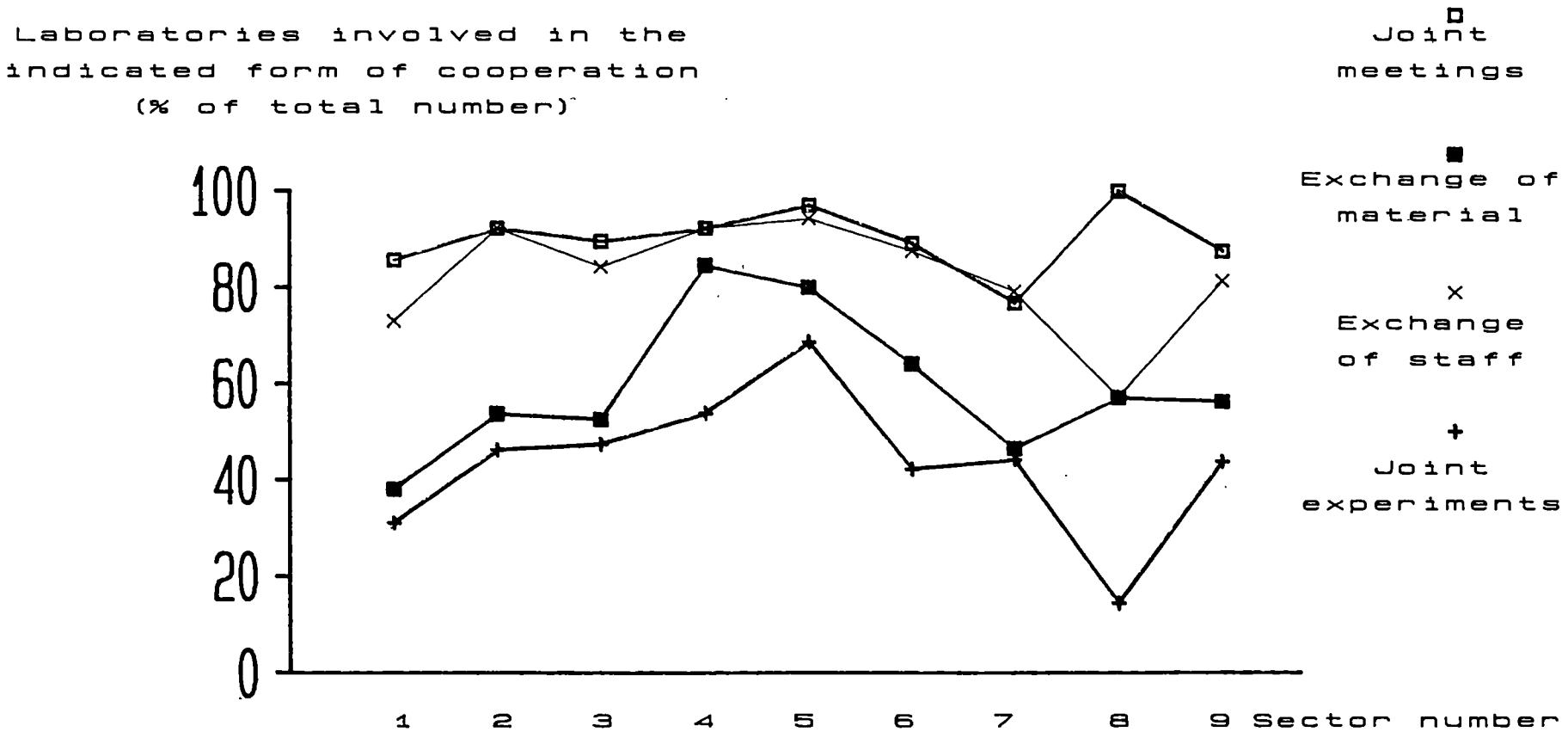



Fig.1: Frequency distribution of feature articles in major scientific journals during the reporting period

Fig.2: Frequency distribution, per research sector, of laboratories committed to one or another form of cooperation.

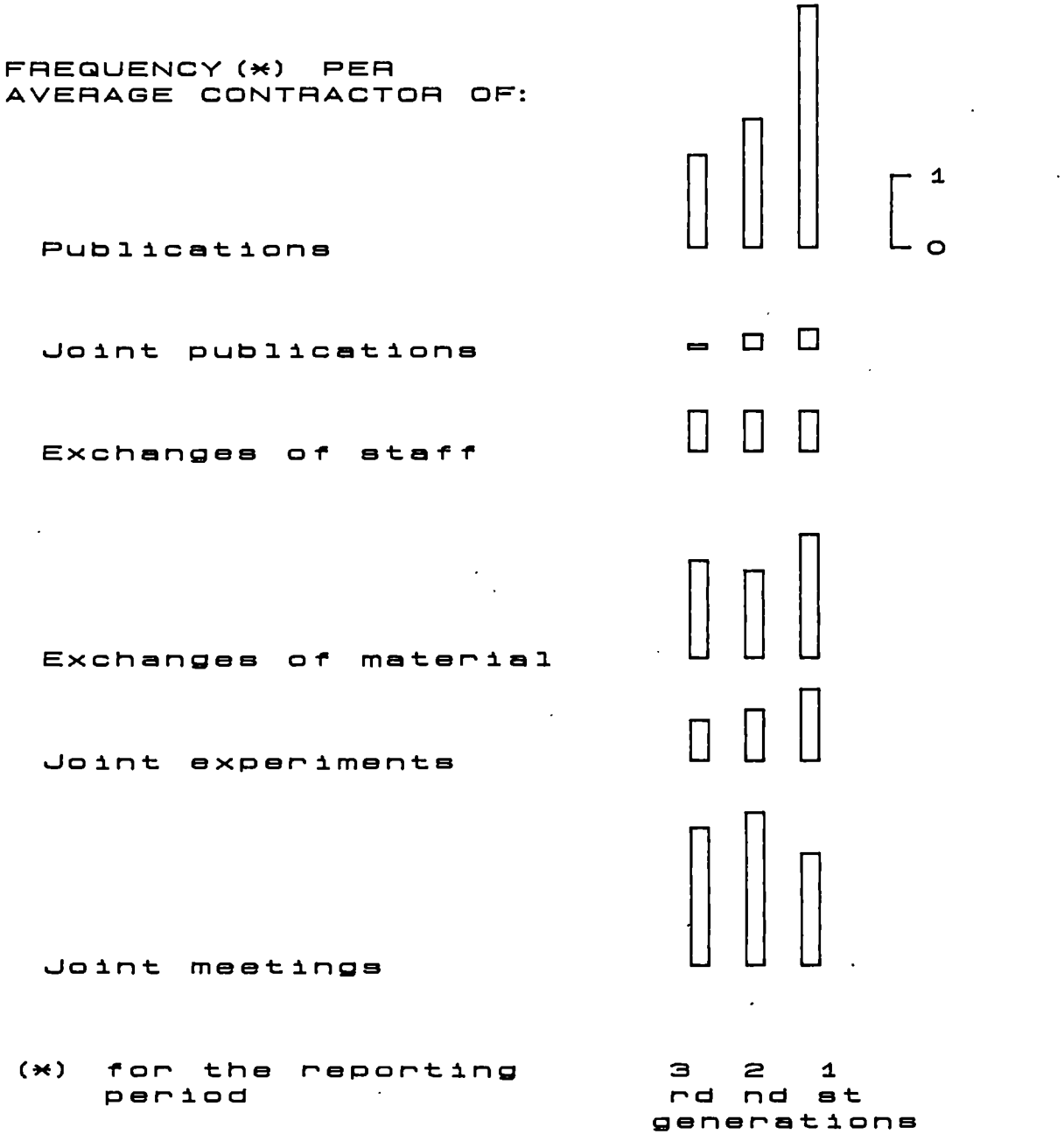



1-Bio-informatics
2-Culture collections
3-Bioreactors
4-Protein engineering
5-Biotech.of indust.microorg.
6-Biotech.of plants and associated microorg.
7-Biotechnology of animals
8-Risk assessment
9-In vitro testing methods

Fig.3: Scientific productivity and cooperation profiles within groups of contractors of 3 successive generations

1st generation contractors joined the Community programmes with BEP (1982-1986). They logically yield more output presently in BAP and base cooperation more on practical exchanges.

2nd/3rd generation contractors arrived with two successive rounds of BAP proposals and tend to prefer meetings before integrating further research work.



# C O O R D I N A T I N G  A C T I V I T I E S

Scientific meetings, visits to contracting laboratories, publication of reports and surveys have been the three main actions on which the coordinating activity by the services of the Commission has been based during the initial implementation of the Biotechnology Action Programme.

Meetings were organized on the site of participating laboratories on different occasions, to allow the constitution or the reinforcement of study groups working on a list of priority topics.

Visits to laboratories were carried out throughout the reporting period, with the aim to facilitate, whenever requested, the administrative steps of contract implementation and to contribute to the establishment of a network of individual scientists willing to increase their involvement in cooperative research.

Publications in scientific journals constitute the normal and most efficient way for the dissemination of results obtained in the framework of the programme (see references at the end of each individual report presented in Volume 2) ; in addition, the services of the Commission initiate, whenever useful, surveys of activities and of specific results which characterize one or several research areas in the Community. They also circulate reports or analyses prepared on their request by selected contractants to cover activities carried out within particular groups.

## Scientific meetings

Four sectoral meetings were organized to bring together all contractants, connected with a particular research sector of BAP. In addition, 40 meetings were convened upon specific requests : in the latter cases, the objectives of the meetings were to strengthen transnational cooperation and integrate further the work of ELWWs (European Laboratories Without Walls), or to provoke cross-fertilization between remote contracting groups which considered that the confrontation of their scientific ideas and of their particular skills would be a mutual benefit. These other meetings were convened in smaller configurations, adapted to the detailed discussion of very specific needs : they are more of the spontaneous type.

Finally, the Commission services also organized several exploratory meetings of experts for the assessment of new promising areas of R&D. Sixteen such meetings took place during the reporting period. They included the participation of BAP contractors and of scientists outside the programme as well as of experts nominated by the CGC Biotechnology and by the Commission, and of representatives from industrial organizations or learned societies. The conclusions of these brainstorming activities have been reported to the Commission and can be made available upon request. The corresponding reports are listed with all publications appearing in the last section of this chapter on coordinating activities.

## 1. SECTORAL MEETINGS

### SALAMANCA, 25-28 October 1987

55 participants from 10 countries and the Commission.

**Subject :**

Genetic engineering for animal husbandry.

**Local organizer :**

E. Vinuela, Centro de Biologia Molecular (CSIC-UAM), Madrid (Spain).

**Dimension and structure :**

34 individual presentations including 3 invited lectures, one working group session.

**Objectives :**

First meeting whereby contractants from 34 laboratories working under the sector "Genetic engineering for animal husbandry" could convene and disseminate their preliminary results. During the meeting, there was a working session to discuss future programmes (BRIDGE).

### BAYREUTH, 26-28 October 1987

52 participants from 12 countries and the Commission.

**Subject :**

Risk assessment for the release of genetically manipulated microorganisms.

**Local organizer :**

Prof. W. Klingmüller, University of Bayreuth (Germany)

**Dimension and structure :**

22 lectures of invited speakers and BAP contractors.

**Objectives :**

First meeting of BAP contractors working in the sector of risk assessment. The meeting was open to participants of many other European laboratories (including non-EC members) active in the same field. Various research approaches were discussed and different views were expressed about the important problem of safety evaluation in biotechnology.

## DOURDAN, 25-27 February 1988

31 participants from 11 countries and the Commission.
11 industrial representatives from different firms.

**Subject :**

Biotechnology of Gram-positive industrial microorganisms.

**Local organizer :**

S.D. Ehrlich, Institut Jacques Monod, Paris (France).

**Dimension and structure :**

23 oral presentations, one round-table discussion on future trends and industrial involvement within the framework of BRIDGE, and a presentation by DG XII Commission staff.

**Objectives :**

To encourage groups towards a stronger collaboration in the framework of ELWW (European Laboratories Without Walls) and to allow opinions and suggestions to be exchanged with industrial partners.
A report on the round-table is inserted with reports by organizers of meetings in the following section.

## BAD IRSEE, 30 March - 1 April 1988

68 participants from 13 countries, including 8 industrial firms, and the Commission.

**Subject :**

*In vitro* evaluation of the toxicity and pharmacological activity of molecules.

**Local organizer :**

T. Krieg, Ludwig-Maximillians-Universität München (Germany)

**Dimension and structure :**

3 invited lectures, 16 oral communications, 17 posters on display, one round-table and a working group session.

**Objectives :**

First occasion for 16 contracting laboratories in the sector "*In vitro* evaluation of the toxicity and pharmacological activity of molecules" to meet and to discuss research strategies and collaboration at the beginning of their contract period. At the round-table presentation, the state of the art on reconstruction of skin was reviewed. The working group session gave the opportunity to discuss future programmes (BRIDGE).

## 2. SPONTANEOUS MEETINGS

Due to the usually informal approach to the organization of spontaneous meetings, some have also taken place with little information passed to the Commission. The description below does not therefore include all events having occurred in the framwork of BAP, but only a list of well documented examples.

Many of the well documented spontaneous meetings took place in European Laboratories Without Walls (ELWWs). The participants in ELWWs usually convened in turn at the site of each associated laboratory to review the progress of research and to plan future experiments jointly. Depending on the number of partners and the type of cooperation established between them, spontaneous meetings of ELWWs were trimestrial, semestrial or annual.

In contrast with other spontaneous meetings organized upon specific requests justified by their conveners, the periodic seminars of ELWWs led, in particular, to written up-to-date conclusions on on-going activities. Summaries of these conclusions appear in the next section of the volume with reports by organizers of meetings.

Spontaneous meetings are normally supported with travel funds raised from the BAP budget. But in the case of ELWWs, an extra-support is only provided once for the initial integration of work to be promoted. On other occasions, meetings of ELWWs are financed from the contract money perceived by each participating laboratory.

## BRUSSELS, 14-15 January 1987

7 participants from 2 countries.

**Subject :**

Spontaneous discussion on cloning in Pseudomonas.

**Local organizer :**

J. Davison, ICP, Brussels (Belgium)

**Dimension and structure :**

Discussion of mutual results and of the work of a visiting scientist from Groningen. Institute seminar by M. Kok.

**Objectives :**

Development of mutual interests and ideas. Exchange of materials and techniques.

## PARIS, 7-9 February 1987

14 participants from 3 countries.

**Subject :**

Analysis of replication and stability functions of plasmids in B. subtilis.

**Local organizer :**

S.D. Ehrlich, Université Paris-VII (France)

**Dimension and structure :**

Presentations by the two collaborating groups and common discussions including the invited speakers.

**Objectives :**

Discussing the joint research programme.

**BAARN, 19 May 1987**
**BRUSSELS, 11 June 1987**
**KEW, 21 September 1987**
**BRAUNSCHWEIG, 25 January 1988**
**KEW, 30 April 1988**

**Common subject :**

Implementation of MINE.

**Local organizers :**

The meetings were locally organised respectively by M.A.A. Schipper ; J. de Brabandere ; D.L. Hawksworth ; D. Claus and D.L. Hawksworth.

**Dimension and structure :**

All the MINE general meetings, except the one on 30 April 1988, were attended by 3-5 scientists from each node of MINE. Representatives from the Working Party on Culture Collections attended the meetings in Brussels, Kew (September) and Braunschweig. Representatives from culture collections from Canada, Sweden and Israel participated in some of the meetings.

**Objectives :**

Development and implementation of the MINE project. In addition to the general MINE meetings, a number of specialised meetings took place :

**Computer expert meetings**

BAARN, 21 May 1987 (NL)

KEW, 3 August 1987 (UK)

**Format meetings :**

5 (2 for bacteria and 3 for fungi/yeast).

**MINE-MiCIS-CEC meeting** : 22 September 1987, London.

**Computer expert meeting** : (MINE, MiCIS, MSDN, CEC) December 10, 1987, Brussels (Belgium).

**ORSAY, 7 - 12 July 1987**

17 participants from 4 countries.

Subject :

EC-CECAM Workshop on Force-fields for computer simulations on macromolecules.

Local organizer :

S. Wodak, ULB, Brussels (Belgium)

Dimension and structure :

Oral presentations, round-table discussions, benchmark calculations.

Objectives :

A collaborative effort between European and US scientists for improving force-fields, to standardise simulation procedures and to discuss recent developments.

**VAALS, 15 - 17 July 1987**

10 participants from 4 countries.

Subject :

Computer modelling and control of bioreactors and fermentors.

Local organizer :

Dr. J. Thio, DSM/Stamicarbon (The Netherlands)

Dimension and structure :

Opening lecture on the activities of DSM in biotechnology. Presentations by the four project leaders and discussions. Demonstration of GIDS (Graphic Interactive Dynamic Simulation) a computer based simulation system.

Objectives :

Review of the progress made by each group, discussion on the necessary relationships with industry, establishment of future plans.

**TUTZING, 22 July 1987**

18 participants from 2 countries.

Subject :

Folding, assembly, stability and genetic modification of penicillin acylase and its precursors.

Local organizer :

G. Schumacher, Boehringer Mannheim, Tutzing (D)

Dimension and structure :

Working group session.

Objectives :

Discussion on research progress, organization of the collaborative programme of the four laboratories, planning of future research.

**GHENT, 24 August 1987**

4 participants from 2 countries.

Subject :

Transformation of fungi.

Local organizer :

W. Fiers, L.M.B. Ghent (Belgium)

Dimension and structure :

Presentation of results obtained so far by each laboratory and a general discussion.

Objectives :

Coordination of the research efforts, and development of protocols for efficient transformation in A. niger and A. oryzae. Delineation of priorities in both laboratories.

## ROMA, 15-16 September 1987

15 participants from 4 countries.

Subject :

Hairy root and plant cell differentiation.

Local organizer :

P. Costantino, University of Roma "La Sapienza" (Italy)

Dimension and structure :

**First day** : presentations by each participating group of the latest achievements in the field ;
**Second day** : discussions on the prospects of hairy root research and definition of collaborations.

Objectives :

Setting up of a new ELWW, partly anchored in the BAP programme and including contributors from other non-contracting institutions.

## NORWICH, 18 September 1987

15 participants from 5 countries.

Subject :

Mitochondrial molecular genetics in relation to crop improvement.

Local organizer :

D.R. Davies, John Innes Institute, Norwich (United Kingdom)

Dimension and structure :

Three sessions of oral presentations by the participating groups on structure/function of the mitochondrial genome, mitochondrial transformation and organelle transfer.

Objectives :

Enlargement of the ELWW, initially with 5 partners, to 2 new laboratories with expertise in the development of transformation vectors. Activation of transnational exchanges.

## GHENT, 25-29 September 1987

4 participants from 2 countries.

Subject :

Regulatory elements for heterologous gene expression.

Local organizer :

W. Fiers, L.M.B. Ghent (Belgium)

Dimension and structure :

Round-table discussions.

Objectives :

Coordination of research in both laboratories. Discuss the participation of Dr. Unkles and Dr. Campbell in Ghent to sequence the Nia-regulatory (promoter) region.

## DUBLIN, 15-16 October 1987

11 participants from 4 countries.

Subject :

Symbiotic genes in Rhizobium : construction of improved strains.

Local organizer :

M. O'Connell, NIHE, Dublin (Ireland)

Dimension and structure :

Small-scale working seminar of those scientists working on the project. Presentations from the 4 participating groups followed by an extensive round-table discussion.

Objectives :

Second periodic meeting of the ELWW. New cooperation agreements established, and industrial contacts explored.

## ORSAY, 19-23 October 1987

25 participants from 6 countries.

Subject :

EC-CECAM Workshop on Electrostatics in recognition processes between biological macromolecules.

Local organizer :

E. Westhof, IMBC-CNRS, Strasbourg (France).

Dimension and structure :

Oral presentations, round-table discussions, informal exchange.

Objectives :

A collaborative effort of European and US scientists to describe new methods for the calculation of the electrostatic field around proteins and to discuss new approaches in this area.

## SALAMANCA, 1-5 November 1987

104 participants from 13 countries and the services of the Commission.

Subject :

African Swine Fever and pig immunology.

Local organizer :

E. Vinuela, Centro de Biologia Molecular (CSIC-UAM), Madrid (Spain)

Dimension and structure :

8 sessions with 38 communications, 70 posters and 1 panel discussion.

Objectives :

Meeting on pig immunology with emphasis on African Swine Fever reviewing the current research efforts and discussing future research activities.

## COPENHAGEN, 5 November 1987

7 participants from 3 countries.

Subject :

Embryogenesis in plant tissue culture.

Local organizer :

P. Olesen, De Danske Sukkerfabrikker, Copenhagen (Denmark)

Dimension and structure :

5 oral presentations and discussion.

Objectives :

Bringing together 4 research contracts that were initially negotiated in isolation and could be cross-fertilizing.

## HAREN, 6-7 November 1987

19 scientists from 6 countries.

Subject :

Crop improvement through cell biotechnology.

Local organizer :

M.J. Tempelaar, University of Groningen (The Netherlands)

Dimension and structure :

11 oral presentations, with repeated discussion times.

Objectives :

Enlargement of the ELWW (originally with 6 participating laboratories) to 2 new groups contributing work on cereals more particularly.

## VILLEURBANNE, 12-13 November 1987

2 participants from the Belgian laboratory and all French research staff.

Subject :

Molecular biology of plant pathogenic and plant beneficial bacteria.

Local organizer :

J.M. Robert-Baudouy, INSA, Villeurbanne (France).

Dimension and structure :

Working group sessions, plus Institute seminars by F. Brunel and J. Davison. Development of mutual interests and ideas of possible future collaboration. Exchanges of materials and techniques, discussions.

Objectives :

Discussion on the molecular mechanisms of plant pathogenesis and on the antagonism between phytopathogens and plant beneficial bacteria.

## DUBLIN, 14-17 November 1987

10 participants from 2 countries.

Subject :

Replication functions of B. subtilis plasmids.

Local organizer :

K. Devine and D. McConnell, Trinity College, Dublin (Ireland)

Dimension and structure :

Presentations by the two collaborating groups and common discussions.

Objectives :

Discussion on research progress, planning of future research.

**MANCHESTER, 28 November - 2 December 1987**

12 participants from 2 countries.

Subject :

Automation in DNA sequencing.

Local organizer :

J.W Martin, UMIST, Manchester (United Kingdom)

Dimension and structure :

Working group session.

Objectives :

Discussions on research progress, coordination of activities of the four centres involved.

**LONDON, 13 December 1987**

2 participants from 2 countries.

Subject :

Genetic regulation of yeast glycolysis

Local organizer :

B.S. Hartley, Imperial College, London (United Kingdom)

Dimension and structure :

Working sessions.

Objectives :

Review of progress and strategy for joint project.

## MONTPELLIER, 28-29 January 1988

14 participants from 7 countries.

Subject :

Expression of cereal seed protein genes.

Local organizer :

P. Joudrier, INRA, Montpellier (France)

Dimension and structure :

12 oral presentations to cover planned activities of contributing laboratories, 10 reports on achievements in 4 major cereals, followed by a general discussion to assess needs and opportunities for closer work integration in Europe.

Objectives :

The launching of a ELWW on the molecular biology of seed proteins.

## LYON, 1-2 February 1988

17 participants from 3 countries.

Subject :

Pollen biotechnology.

Local organizer :

C. Dumas, CNRS/University of Lyon (France)

Dimension and structure :

Review of data produced in 1987 (10 presentations), planning of scientific exchanges six months ahead.

Objectives :

To activate further the life of this ELWW, newly established in BAP.

## RHENEN, 28 February - 1 March 1988

16 participants from 4 countries and the services of the Commission.

Subject :

Genetic engineering of foot-and-mouth disease virus antigen.

Dimension and structure :

21 short communications with discussion.

Objectives :

Discussion of different research strategies and organisation of collaboration between the different groups.

## COPENHAGEN, 24-25 March 1988

22 participants from 8 countries, including 2 industrial firms.

Subject :

Fundamental aspects of plant cell regeneration.

Local organizers :

S. de Vries, The Agricultural University, Wageningen (The Netherlands)
P. Olesen, De Danske Sukkerfabrikker, Copenhagen (Denmark)

Dimension and structure :

8 scientific reports, a round-table discussion, a concluding session with the view of industry and future-oriented talks.

Objectives :

To set up a new ELWW on plant cell regeneration, where industry expects more coordinated work. To identify gaps in basic knowledge which should be addressed in priority.

## LONDON, 24-25 March 1988

8 participants from 2 countries.

Subject :

Electrophoresis of proteins : data capture, analysis and construction of databanks.

Local organizer :

L.R. Hill, NCTC, London (United Kingdom)

Dimension and structure :

Working group session.

Objectives :

Discussion on research progress, organization of the collaborative programme of the four laboratories, planning of future research.

## ATHENS, 25-26 March 1988

9 participants from 3 countries.

Subject :

Bioconversion of hydrophilic and hydrophobic compounds by enzyme systems.

Local organizer :

Dr. F. Kolisis, The National Hellenic Research Foundation, Athens (Greece)

Dimension and structure :

Progress reports and discussions by the three collaborating groups.

Objectives :

Organization of collaborative work and common assessments on opportunities for an enlargement of this area of activity.

## BAD HONNEF, 11-14 April 1988

33 participants from 4 countries, including 2 industrial firms.

**Subject :**

Plant hormone receptors.

**Local organizer :**

D. Klämbt, University of Bonn (D)

**Dimension and structure :**

Oral presentations from all 5 groups in the same ELWW and from a guest scientist. Discussion with all contributors and the industrial representatives.

**Objectives :**

To review progress at cloning genes for plant hormone receptors. To rationalize cooperation during the coming year. This was the first meeting of a newly created ELWW.

## COVENTRY, 14-16 April 1988

27 participants from 3 countries, including 2 industrial representatives.

**Subject :**

Molecular biology of phytopathogenic Erwiniae.

**Local organizer :**

G.P.C Salmond, University of Warwick (United Kingdom).

**Dimension and structure :**

Nineteen oral presentations falling under the areas of plant pathogenesis, protein targetting and industrial production. Detailed discussions on further collaborations.

**Objectives :**

Semestrial meeting for the 5 participating groups in this ELWW. Exchange of materials, joint assessment of data. Definition of a common strategy to organize the involvement of interested companies.

## BRUSSELS, 19 - 20 May 1988

18 participants from 6 countries and the services of the Commission.

**Subject :**

Mammalian minichromosomes and linear vectors.

**Local organizer :**

J. Rommelaere, Université Libre de Bruxelles (Belgium)

**Dimension and structure :**

1 invited lecture, oral presentations by all participating groups, workshop and a site visit at Solvay.

**Objectives :**

To share expertise and material from different model systems and to discuss future cooperation.

## SIENA, 27 - 28 May 1988

22 participants from 6 countries, including 4 industrial firms.

**Subject :**

Pollen biotechnology.

**Local organizer :**

M. Cresti, University of Siena (I)

**Dimension and structure :**

6 oral presentations to cover the work of 3 laboratories in the same ELWW and of 2 guest scientists. A poster session and a final discussion have been foreseen as well.

**Objectives :**

Planning of future work, with the possible participation of Spanish and Portuguese laboratories and in the light of industrial needs. This was the fourth meeting of the ELWW.

## ERICE, 29 May - 7 June 1988

3 Greek, 2 Portuguese and 1 Spanish scientists sponsored by BAP.

**Subject :**

Crystallography of molecular biology.

**Local organizer :**

W. Hol, University of Groningen (The Netherlands).

**Dimension and structure :**

Lectures, poster sessions, round-table discussions.

**Objectives :**

A course for young scientists from all over the world (total number of participants being 210 from 36 countries) to acquire knowledge in this area. Financial contribution from the Commission was furthermore for travel expenses of some of the speakers.

## HEIDELBERG, 30 May 1988

4 participants from 2 countries.

**Subject :**

G-binding proteins in neuro-endocrine cells

**Local organizer :**

P. Gierschik, University of Heidelberg (D)

**Dimension and structure :**

Working group session.

**Objective :**

G-binding proteins recently became of interest in processing neurotransmitter signals. The antibodies available in Heidelberg are of great interest for studies carried out in Paris. Exchange of material and collaboration between the groups were discussed.

**NANCY, 30-31 May 1988**

24 participants from 2 countries.

**Subject :**

Biochemical-physical, knowledge, control, optimization of animal cell cultures in bioreactors.

**Local organizer :**

J.M. Engasser, Institut National Polytechnique de Lorraine (France).

**Dimension and structure :**

10 scientific presentations with discussions.

**Objectives :**

To review and discuss recent results of the different groups and to coordinate future activities.

**CAMOGLI, 2 - 4 June 1988**

28 participants from 7 countries.

**Subject :**

Crop improvement through cell biotechnology.

**Local organizer :**

F. Sala, University of Pavia (Italy)

**Dimension and structure :**

17 oral presentations to review the contributions of all eight associated laboratories, including one presentation from a Spanish guest. General discussion on the present state of cooperation, and on prospects created by the future BRIDGE programme proposal.

**Objectives :**

Periodic (semestrial) assessment of progress within this ELWW, which held here its 4th meeting from the time BAP started.

## GRENOBLE, 6 - 7 June 1988

10 participants from 3 countries.

**Subject :**

Advanced monitoring and computer control of biotechnological processes.

**Local organizer :**

Prof. A. Cheruy, Ecole Nationale Supérieure d'Ingénieurs Electriciens de Grenoble (France).

**Dimension and structure :**

Progress reports and discussions by the four collaborating groups.

**Objectives :**

Organization of collaborative work and coordinative planning for the Bioreactors-Biotransformations meeting in Athens on 18-21 October 1988.

## PARIS, 17-18 June 1988

22 participants from 7 countries, including representatives from 3 industrial firms, and the services of the Commission.

**Subject :**

Cellular neuro-immunology.

**Local organizer :**

C. Kordon, INSERM, Paris (France)

**Dimension and structure :**

5 workshop sessions.

**Objectives :**

Information about material and know-how that can be exchanged and used by laboratories following different research strategies. Discussions on future collaborations between research groups, and relations with industry.

**GRONINGEN, 30 June 1988**

4 participants from 2 countries.

**Subject :**

Progress on gene cloning in Pseudomonas.

**Local Organizer :**

B. Witholt, Groningen Biotechnology Centre (The Netherlands).

**Dimension and structure :**

Working discussions.

**Objectives :**

Discussions on past progress, future ventures, joint publications, possible future ELWW meeting. Views on homologies and computer modelling of ferredoxins.

## Visits to contracting laboratories and affiliated firms

Personal contacts of CEC staff with project leaders and their co-workers can favour the construction of a European scientific network based primarily on the quality of human relationships. The direct connections established in this way also prove beneficial to the management planning of the entire programme.

The visits facilitate monitoring of the administrative steps necessary for the correct implementation of the contracts, as well as discussions of the latest results of the research activities. They also provide a unique opportunity for contractants to influence later developments of Community R&D programmes by their personal feelings and suggestions. They constitute a major information feedback for the overall coordination activity.

By June 1988, 98 contracting laboratories had been visited by CEC staff. Each of these visits gave rise to the preparation of a standard itemized report, used by Commission services for their evaluations. These reports were not circulated outside the Commission ; they contain confidential information which can only be exploited in the framework of the Division of Biotechnology. Indeed, the visits are exclusively meant to serve two specific purposes :

- to facilitate the participation of contractants in the on-going programme,
- to sustain new developments in biotechnology R&D which serve the interest of the Community.

The list of items covered by each report from a site-visit is detailed below :

- Personal contacts, expertise covered ;
- Insertion of the laboratory in the local academic environment;
- Facilities and special equipment available in the laboratory ;
- Precise stage of the research programme reached at the time of the visit ;
- Existence of an important obstacle to research progress (if applicable) ;

- Important and recent breakthroughs claimed by the laboratory (if applicable) ;
- List of publications issued during the preceding year ;
- Pending questions related to the administrative and financial management of the contract ;
- Tendency to diverge from the conditions foreseen by the contract (if applicable) ;
- Suggestions by the contractant (prospective studies, possible meetings, evolution of the content and the implementation methods of the Community programme) ;
- Industrial interest and type of relationships established with industrial firms.

An essential outcome of the programme of visits has been an increase in mutual knowledge throughout the Community : contractants become more closely acquainted with activities and operational mechanisms at Community level ; Commission staff has more direct insight into European science and cooperation prospects ; contractants may be invited to explore other contacts in Europe for any reason that would emerge from the detailed discussions between scientists and the programme manager.

More recently, Commission services strongly responded to their commitment to sustain a proper information flow from and to industrial firms with an interest in the programme. Apart from regular invitations to affiliated firms to attend contractors meetings, Commission staff made themselves as much as possible available to informal meetings between industrial representatives and the services of the Commission. On these occasions, discussions are held on factors which determine the support of the firms to the Community projects, the prospects for exploitation of the results of the programme are envisaged, and more prospective thinking is developed with regard to the future trends of European cooperation in biotechnology R&D. Meetings of this kind have already taken place with 30 different companies and more will be planned.

## Publications and reports

Next to 535 scientific articles and 16 patents published by contractants themselves (see volumes 2-3-4 of this progress report), the services of the Commission produced informative materials describing BAP structure and activities, initiated reviews covering entire sectors of the Biotechnology Action Programme, entrusted experts with the task of reporting on specific scientific events.

The list of these reports and publications issued before July 1988 is provided below.

**MOLECULAR BIOLOGY AND CROP IMPROVEMENT : A CASE STUDY OF WHEAT, OILSEED RAPE AND FABA BEANS.** R.B. Austin.
With R.B. Flavell, I.E. Henson, H.J.B. Lowe, Cambridge University Press for the CEC, 114 p., 1986

**BIOMOLECULAR ENGINEERING IN THE EUROPEAN COMMUNITY.**
Achievements of the Research Programme (1982-1986).
Final Report. Ed. E. Magnien.
Martinus Nijhoff published for the Commission of the European Communities. 1172 p., 1986

**ALTERNATIVE USES FOR AGRICULTURAL SURPLUSES.** Eds. W.F. Ragman and P. Larvor.
Elsevier Applied Science. CEC, 134 p., 1986

**BIOTECHNOLOGIES COMMUNAUTAIRES.** Un programme chasse l'autre.
D. de Nettancourt.
Biofutur, 45: 3-4, 1986

**SECTORAL CONTRACTANT MEETING.**
Risks assessment in biotechnology. U. Bertazzoni, Brussels, 18 March 1986.
DG XII. CEC, 1986

**TRAINING ACTIVITIES IMPLEMENTED IN THE FRAMEWORK OF THE BIOMOLECULAR ENGINEERING PROGRAMME. FACTS AND STATISTICS.**
DG XII/F/2, CEC, 1986

**EC-BIOTECHNOLOGY : A EUROPEAN CHALLENGE.**
R. van der Meer.
Trends in Biotechnology. 4 : 277-279, 1986

**DOSSIER 1986.**
Biotechnologie: la carta europea. L'apporto della CEE. Direzione Generale Informazione, Communicazione, Cultura, CEC, 15 p., 1986

**THE EUROPEAN COMMUNITY OF RESEARCH AND TECHNOLOGY. BEP-BAP. BIOTECHNOLOGY.**
DG XII. CEC, 16 p., 1986

**IMPULSE TO EUROPEAN COOPERATIVE RESEARCH IN THE FIELD OF AGRICULTURAL MICROORGANISMS : INTERACTIVE PROGRAMME FOR INTERACTIVE MICROBES.**
E. Magnien.
Symbiosis, 2 : 265-274, 1986
Idem : Chimica oggi, Gennaio-Febbraio 1987, 69-72

**LIST OF RESEARCH CONTRACTS IN THE BIOTECHNOLOGY ACTION PROGRAMME (1985-1989).**
DG XII/F/2. XII/62/87-EN, 89 p., 1987

**BOOK OF ABSTRACTS.**
Culture collections and genetic engineering of microorganisms. Sectoral meeting of contractors. Ioannina, 23-25 April 1987.
DG XII. CEC, 133 p., 1987

**BOOK OF ABSTRACTS.**
Genetic and cellular engineering of plants and microorganisms important for agriculture.
Sectoral meeting of contractors. Louvain-la-Neuve, 23-26 March 1987.
DG XII. CEC, 228 p., 1987

**BOOK OF ABSTRACTS.**
Enzyme engineering : protein design and applications in biocatalysis. Sectoral meeting of contractors. Capri,
2-6 May 1987
DG XII. CEC, 135 p., 1987

**BOOK OF ABSTRACTS.**
Novel methodology for animal cell cultures.
Sectoral meeting of contractors. Seillac, 25-27 May 1987.
DG XII. CEC, 86 p., 1987

**BOOK OF ABSTRACTS.**
Genetic engineering for animal husbandry.
Sectoral meeting of contractors. Salamanca,
26-27 October 1987.
DG XII, CEC, 1987 (in preparation)

**PLANT BIOTECHNOLOGY AND COMMUNITY DEVELOPMENT.**
E. Magnien
Proceedings of the NATO A.S.I. on Plant Cell Biotechnology
M.S. Pais Ed., Albufeira 30 March - 10 April 1987 (in press)

**L'EUROPE DES BIOTECHNOLOGUES EXISTE : JE L'AI RENCONTREE.**
E. Magnien.
Biofutur 59, p. 24-26, juillet 1987

**IN VITRO CULTURE OF STRAWBERRY PLANTS.**
Proceedings of a workshop organized in the framework of action COST 87 in Brussels, 18-19 December 1984.
EUR 10871, 100 p., 1987

**COMMUNITY RESEARCH IN BIOTECHNOLOGY : TOWARDS A STRONGER INVOLVEMENT OF INDUSTRIES.**
D. de Nettancourt.
DG XII, CEC, 4 p., 1986

**RESEARCH AND TRAINING ACTIVITIES OF THE EUROPEAN COMMUNITIES IN BIOTECHNOLOGY.**
D. de Nettancourt.
Proceedings of the meeting of the Belgian Chemical Society : "Genetic engineering in Belgium, research and perspective of industrial applications", Brussels, 20 February 1987 (in press)

**THE EUROPEANIZATION OF BIOTECHNOLOGY R & D AND THE MEDITERRANEAN DIMENSION : COMMON INTERESTS, COMMON DESTINY.**
E. Magnien
Proceedings of the International Conference on Biotechnology and Agriculture in the Mediterrean basin.
G. Tzotzos and A. Saint-Rémy Eds.
Athens 26-28 June 1986 (in press, 1987)

**ELWWs : 'LABORATORI APERTI' PER L'AGRICOLTURA E L'INDUSTRIA NEL CAMPO DELLE BIOTECNICHE.**
E. Magnien e D. de Nettancourt
Biotec 4/87, p. 50-53, 1987

**ACTIVITIES OF THE EUROPEAN COMMUNITIES IN THE AREA OF BIO-INFORMACTICS.**
B. Nieuwenhuis, Proceedings of the Royal Irish Academy (section B), in press

**REPORT ON BAP SECTORAL MEETING IN CAPRI.**
A.R. Rees, Protein engineering 1 (4), 271-274, 1987

BOOK OF ABSTRACTS.
Extremophiles.
Horizontal meeting of BAP contractors. London, 2 December 1987.
DG XII. CEC, 18 p., 1987

A BRIDGE PROJECT IN STREPTOMYCES MOLECULAR BIOLOGY ?
Report of an expert meeting to the Commission.
DG XII, CEC, 4 p., 1987

THE USE OF RFLPs FOR BASIC RESEARCH AND PLANT BREEDING.
Report of an expert meeting to the Commission.
DG XII, CEC, 7 p., 1987

MANIPULATION OF CORE PROCESSES IN PLANT BREEDING.
Report of an expert meeting to the Commission.
DG XII, CEC, 11 p., 1987

EUROPEAN LABORATORIES WITHOUT WALLS : FOCUSED PRECOMPETITIVE RESEARCH.
R. van der Meer, E. Magnien and D. de Nettancourt.
Trends in Biotechnology, 5 : 318-321, 1987

EUROPEAN LABORATORY WITHOUT WALLS IN THE FIELD OF CROP IMPROVEMENT THROUGH CELL BIOTECHNOLOGY.
E. Magnien
DG XII. CEC, 8 p., 1987

IRDAC OPINION ON FUTURE R&D PROGRAMMES IN THE FIELD OF BIOTECHNOLOGY.
DG XII. CEC, 16 p., 1987

THE APPROACH OF THE BIOTECHNOLOGY INDUSTRY TO BRIDGE.
European Biotechnology Co-ordination Group.
c/o CEFIC, Brussels, 5 p., 1987

BIO-INFORMATICS IN EUROPE.
CEFIC position paper, Brussels, 14 p., 1987

**SAFETY EVALUATION THROUGH RISK ASSESSMENT IN BIOTECHNOLOGY.**
European Biotechnology Co-ordination Group.
c/o CEFIC, Brussels, 9 p., 1987

**RISK ASSESSMENT.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 6 p., 1988

**BIOREACTORS AND BIOTRANSFORMATION.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 9 p., 1988

**PROTEIN ENGINEERING.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 4 p., 1988

**CULTURE COLLECTIONS, DATABANKS, MEGASEQUENCING AND BIOINFORMATICS.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 4 p., 1988

**PHYSIOLOGY AND MOLECULAR GENETICS OF INDUSTRIAL MICROORGANISMS.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 6 p., 1988

**BIOTECHNOLOGY OF CROP SPECIES AND ASSOCIATED ORGANISMS.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 5 p., 1988.

**BIOPROCESSING OF AGRICULTURAL PRODUCTS FOR INDUSTRIAL EXPLOITATION/DOWNSTREAM PROCESSING.**
Brainstorming group report to the CGC Biotechnology.
DG XII, CEC, 3 p., 1988

**IN VITRO TESTING METHODS.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 5 p., 1988

**ANIMAL PRODUCTION AND HEALTH CARE.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 4 p., 1988

**RESEARCH AVENUES FOR BRIDGE.**
Brainstorming group report to the CGC Biotechnology.
DG XII. CEC, 3 p., 1988

**BOOK OF ABSTRACTS.**
Biotechnology of Gram-positive industrial microorganisms.
Sectoral meeting of contractors. Dourdan, 26-27 February 1988.
DG XII, CEC, 43 p., 1988

**BOOK OF ABSTRACTS.**
Microbiology of traditionally fermented foods in the Mediterranean countries.
ELWW meeting.
DG XII. CEC, 28 p., 1988

**BOOK OF ABSTRACTS.**
Plant molecular pathology.
ELWW meeting.
DG XII. CEC, 45p., 1988

**CONTROL OF DIFFERENTIATION AND MORPHOGENESIS IN PLANTS.**
Report of an expert meeting to the Commission.
DG XII. CEC, 16 p., 1988

**LA STRADA DELLA RICERCA PER UNA VALUTAZIONE RAZIONALE DEL RISCHIO.**
U. Bertazzoni.
Biotec, 1/88, 18-19, 1988

**LE PROTEINE DEL SEME DEI CEREALI;**
M. Motto
Biotec, 2/88, 25-27, 1988

**LA RESISTENZA AI PATOGENI.**
M. Buiatti.
Biotec, 4/88, 36-38, 1988

**IL MIGLIORAMENTO DEI RACCOLTI.**
M. Racchi.
Biotec, 4/88, 39-40, 1988

**LABORATOIRES EUROPEENS SANS MURS : UNE RECHERCHE PRECOMPETITIVE CIBLEE.**
R. van der Meer, E. Magnien and D. de Nettancourt.
Biofutur, 70, 53-56, 1988

**EUROPESE LABORATORIA ZONDER MUREN.**
B. Nieuwenhuis.
Biotechnologie in Nederland, 1988/1, 13-16, 1988

**PLANT BIOTECHNOLOGY RHYMES WITH EUROPE.**
E. Magnien.
Plants Today, 1988 (in press)

**VERS DES PROTEINES SUR MESURE.**
A. Sari.
Science & Technologie, N0 4 - avril 1988, 40-50, 1988

**OPINION AND RECOMMENDATIONS OF GIBiP ON THE PRIORITIES, GENERAL LINES AND TRENDS IN THE BRIDGE-PROGRAMME OF THE COMMISSION OF THE EC.**
Green Industry Biotechnology Platform.
c/o HOM business & venture development, Den Haag, 3 p., 1988

**THE FUTURE FOR ANIMAL CELL BIOTECHNOLOGY.**
R. Spier.
Report of the Symposium at the opening of the Wolfson Cytotechnology Laboratory, 19-20 May 1988.
DG XII. CEC, 5 p., 1988

**BIOTECHNOLOGY ACTION PROGRAMME : AUTOPSY OF A CALL FOR PROPOSALS.**
D. de Nettancourt and M. Mongini.
DG XII. CEC, 23 p., 1988

**EVALUATION OF THE BIOMOLECULAR ENGINEERING PROGRAMME, BEP (1981-85), AND OF THE BIOTECHNOLOGY ACTION PROGRAMME, BAP (1985-89).**
DG XII. CEC, 15 p. and annexes, 1988

R E P O R T S

b y

organizers of meetings

# FORCE-FIELDS FOR COMPUTER SIMULATIONS ON MACROMOLECULES

Short report on the CECAM-EC workshop held in July 1987
and
proposal for a follow-up workshop in 1988
April 14, 1988
Shoshana Wodak

With the increasing use of molecular modelling in the fields of drug design and protein engineering, the issue of developing reliable models for simulating properties of biological molecules and, in particular macromolecules, becomes a crucial one.

The basic methodologies for these simulations - computation of the conformational energy, energy minimization, Molecular Dynamics, Monte-Carlo sampling - are essentially well established and more or less standard. But the particular energy functions used, the parameters and simulation conditions may vary widely from one laboratory to the next.

An earlier CECAM meeting (October, 1986) discussed the urgent need for a systematic effort aimed at setting standards for simulation conditions and for deriving improved force-fields, and recommended to hold a workshop on this subject that would set the pace for a coordinated effort among European and American laboratories active in the field.

This workshop was sponsored jointly by CECAM and the EC and held on July 7-12 1987. 17 scientists (see enclosed list) from the major laboratories engaged in computer simulations of biological molecules attended. Discussions were focused on force fields and computer simulations of protein-solvent systems.

Ongoing efforts to improve and test force fields in different laboratories (Harvard-Karplus; Univ. of Pitsburg-Brooks; NIH-Brooks; Biosym-Dinur; UCSF-Kollman; Groningen-Van Gunsteren; Cornell-Brady; Univ. of Lille-Vergoten; Univ. of Michigan-Krimm) were presented and discussed. Noteworthy were the detailed presentations on the collaborative efforts already well underway on optimizing force fields with respect to vibrational spectra of N-methylacetamide, alanine dipeptide, sugars, simple alkanes and aromatic molecules, in the gas phase as well as crystalline state.

Progress and remaining bottlenecks in simulations of liquid water and protein-water systems were also extensively discussed. The problem of handling long-range forces electrostatic interactions in these systems received particular attention, the use of flexible versus rigid water models was debated, and the question of how to adjust force fields and simulation conditions so as to fit satisfactorily not only equilibrium properties, but also dynamic parameters was examined.

Recent methods for computing differences in solvation free energies and free energies changes in closely related conformational states by Molecular Dynamics simulations were analyzed. Free energy values computed by these procedures can be readily compared to experimental quantities and possibly used to calibrate force fields and energy parameters down stream of other well established calibration procedures.

The use of experimental data from inelastic neutron scattering of proteins and peptides in solutions to test force-fields and parameters was also brought up, as indeed, theoretical calculations of the picosecond dynamic behaviour of BPTI were not able to reproduce measured neutron spectra without fine adjustments of the force-fields : use of explicite positions for non polar hydrogens and better truncation methods for electrostatic interactions.

The use of ab-initio quantum mechanical calculations in deriving better force-fields has also been discussed, albeit less extensively. They were retained as very useful for computing and calibrating rotational barriers and electronic partial charges, with a note of caution for the latter of fitting potentials rather than charge distributions. Simulations in which a combined quantum mechanical (semi-empirical) and molecular mechanical potential is used to describe chemical reactions in condensed phase were described, and it has been suggested that the method, which stipulates a quantum mechanical treatment for special portions of the system (atoms involved in a chemical reaction), may remove some of the needs to derive classical force-fields and energy parameters that mimick the special behaviour of these portions.

Last but not least, benchmark calculations - Molecular Dynamics and energy minimization - were performed on the alanine crystal (zwitter ion form) using five parameter sets : "old CHARMM" (1984- 1985), "new CHARMM" (1987-1988), OPLS/AMBER (1986-1987); "old AMBER" (1983-1984) and GROMOS (1987). Simulation results were distributed among all workshop participants and are being presently analyzed by the different groups.

In conclusion, consensus has been reached among workshop participants to consider this event as a starting point for a larger collaborative effort for improving force-fields, standardizing simulation procedures and critically overviewing recent developments.

Standard formats for energy parameter library and, in general, the constitution of a data base of parameters were discussed. More important still, an overall strategy for improving force-fields and energy parameters was proposed:

1) Perform ab-initio quantum mechanical calculations on small molecules in the gaz phase to fit rotational barrier, partial electronic charges and bond distance and angles

2) Fit potential energy function (most certainly include Urey Bradly cross terms) and force constants to gaz phase experimental vibrational spectra for a sufficiently large spectrum of small molecules so that the different functional groups in proteins are well represented

3) With the intra-molecular force-field obtained from 1-2, non-bonded force-field should be adjusted by fitting parameters in the condensed ordered phase (structural parameters and vibrational spectra of crystals)

4) Compare the results of steps 1-3, iterate if necessary, to obtain the best compromise between intra molecular and non-bonded parameters

5) Test the performance of the obtained parameters in simulations of solvated systems for which experimental data are available

6) Emphasis should be given to testing transferability of derived force fields, and to devising standard protocol for achieving transferability for cases in which it does not apply directly.

The fact that points 1-3 are already an active area of endeavour in a number of laboratories and that improved force fields which fit well spectral data start to be available was considered a very positive point from which further efforts should benefit.

In line with these efforts, a proposal by the Harvard group (J. Smith and M. Fields) to estabilish a data bank of model systems well suited for calibrating computational procedures against experimental data was retained as particularly valuable. These model systems should be composed of small molecules, with functional groups relevant to proteins on which experimental data, ranging all the way from crystal structure, to solvation energies, to vibrational spectra, etc., are available.

In view of these conclusions, of the impact they may have on future activities in the field, and in view of the enthusiasm and interest expressed by all participants to pursue activities in this area, we propose to hold a follow-up workshop on force-fields in the summer of 1988, immediately after the planned workshop on Free Energy Calculations by Perturbation Methods, as a number of participants in the latter event are also implicated in the force-field effort.

We hope very much that the EC will recognize the importance of keeping up the momentum of this very important area of interest by subsidizing the travel expenses of scientists participating in the follow-up workshop.

**CECAM Workshop**
**on**
**ELECTROSTATICS IN RECOGNITION PROCESSES**
**BETWEEN**
**BIOLOGICAL MACROMOLECULES**
**ORSAY, October 19-23, 1987**
**With the support of the**
**Centre National de la Recherche Scientifique**
**and the**
**Biotechnology Action Programm of the European Communities**

Organized by
Eric WESTHOF
IBMC-CNRS
15, rue R. Descartes
F - 67084 Strasbourg

This workshop follows a preceding CECAM workshop held in Orsay in June 1986 on "Nucleic acids and Solvent Structure". That workshop was itself a follow-up of a workshop held during July 1983 and organized by Wilma OLSON and Herman BERENDSEN. The organizers of 1983 workshop brought together scientists with expertise either in experimental and theoretical work on nucleic acid conformations and dynamics. During the 1983 workshop, the electrostatic problem related to the polyelectrolyte nature of nucleic acids was much discussed. During the 1986 workshop , the emphasis was more on the analysis of the solvation of nucleic acids both from an experimental and a theoretical point of view. The appropriate way to compare results from simulation studies and from experimental work, especially crystallography was a main point of discussion.

Due to the advances made in the developments of method and software for the calculation of the electrostatic field around proteins, the 1987 workshop was devoted to the description of the new methods, their advantages, and drawbacks as well as their applictions mainly to proteins. Two methods appeared strongly similar, those of Malcolm DRUMMOND and of Randy ZAUHAR where the interaction between the solute and the polarizable dipoles of the solvent are calculated through the induced surface charge distribution on surface elements of the protein. The differences in applicability with the finite difference method of Jim WARWICKER and Barry HONIG were made clear. Several other approaches to the elctrostatic problem were also addressed and discussed.

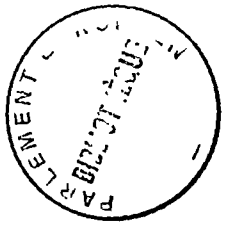
As in the previous workshops, the set-up of the workshop and its organization left time for informal discussions and exchanges between participants.

# WORKING SEMINARS

# O F

# EUROPEAN LABORATORIES WITHOUT WALLS

# (E.L.W.W.)



# BIO-INFORMATICS

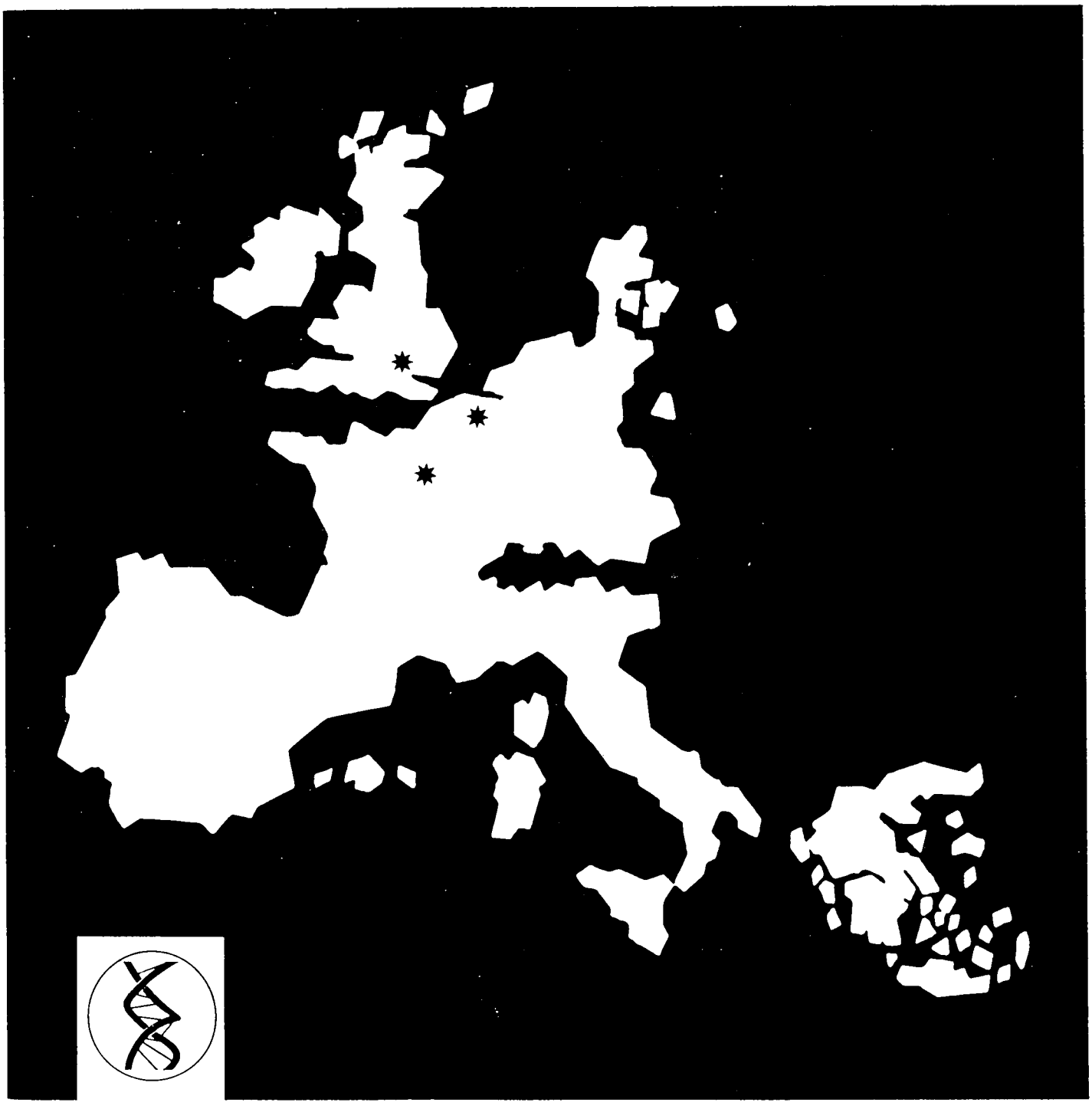
EUROPEAN LABORATORY WITHOUT WALLS

BAP-0138-B
BAP-0133-UK
BAP-0142-UK
BAP-0145-F

Queen Mary College,
London

## In the field of

ELECTROPHORESIS OF PROTEINS



BAP Contractors Meeting

24-25 March, 1988, at NCTC Colindale, London NW9 5HT.

Electrophoresis of proteins: data capture, analysis and construction of databanks

Day 1 Attendence:

K. Kersters, B. Pot, (LMG, Gent) BAP 0138-B
L.R. Hill, M. Costas, L. Sloss, S. On (NCTC, London) BAP 0133-UK
M.J. Dunn (RPMS, London) BAP 0142-UK
K. Smith (QMC, London), participant
(R.G. Whalen, Paris, BAP 0145-F unable to attend due to prior commitments)

Progress in the participating laboratories was reviewed. At NCTC, a 2-D LKB laser scanner interfaced with a COMPAQ micro-computer, programmes translated to TURBO-PASCAL. Standard protocol for protein extraction and electrophoresis applied to strains of: Campylobacter laridis/UPTC, Campylobacter jejuni subsp. doylei, Campylobacter pylori, Listeria denitrificans, Mycoplasma mycoides.

L. Sloss has evaluated a radio-labelling system AMBIS, with, however, poor results. Strategy of databanks was discussed: pro tem advisable to store both actual patterns of centrotypes of EP-types, as well as hypothetical or average patterns of EP types. At NCTC, Bio Image's VISAGE 110 image analyser system was recently installed and, especially for 2-D work, will greatly enhance data capture and analysis.

At LMG, an interpolation program up-and-running, differing in detail from NCTC software but achieving the same end. Interpolation involves 400 data points and positions of selected reference peaks are statistical averages of 100 + runs of LMG 1125 reference strain. Database software developed on mainframe. Groups studied include further Xanthomonas spp., Bacillus spp., lactic acid bacteria, pseudomonads and rhizobia.

At RPMS, PD Quest installed, software locally operational at database level; data acquisition being done in USA and returned to RPMS. Databases being constructed of composite 2-D gels, and system has capacity to compare up to to 30 2-D gels. The subjectivity of choosing reference spots was discussed, and will arise also in the NCTC VISAGE system and agreed direct comparison of the two systems should be made.

At QMC (unit renamed Centre for Parallel Computing), a holistic approach is being developed, a statistical classification pattern recognition system. Attention was drawn to the Advanced Informatics in Medicine (AIM) document of the CEC. (Meeting adjourned for a demonstration of VISAGE.)

Day 2: LMG and NCTC Groups only

LMG and NCTC actual gels were exchanged to separately analyse on respective systems. Detailed comparison made of how the NCTC and LMG software packages operate, and further discussion took place of the shared standard protocol for preparation of gels. Prospected joint work: to explore in more detail differences noted in 1-D separations between pigmented and non-pigmented variants of Serratia rubidea, to extend to 2-D work and, if practicable, to restriction endonuclease digests of DNA.

EUROPEAN LABORATORY WITHOUT WALLS

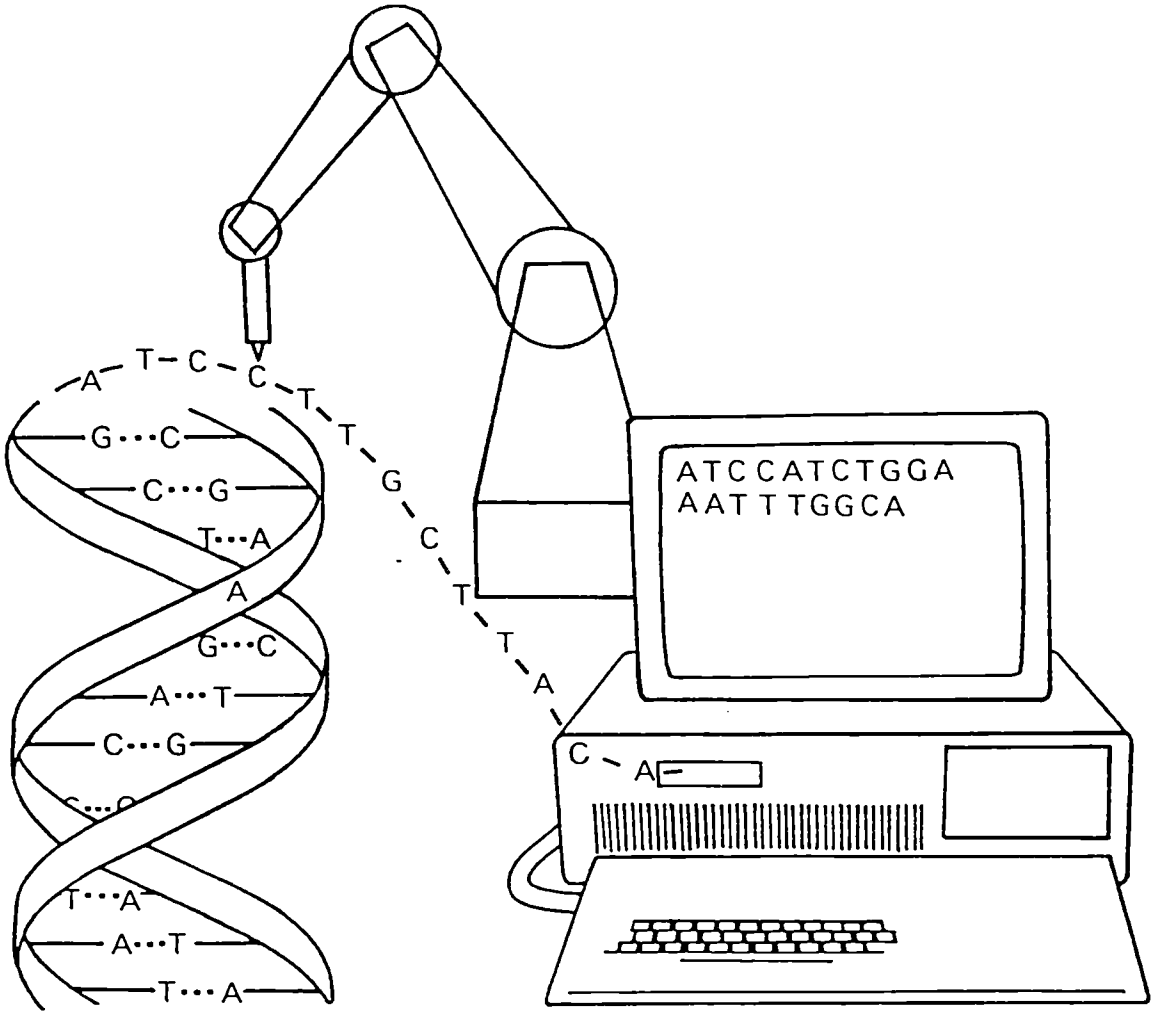
BAP-0029-UK
BAP-0035-UK
BAP-0036-D
BAP-0135-D

# In the field of

AUTOMATION OF DNA SEQUENCING

# BIOTECHNOLOGY ACTION PROGRAMME

## Automation of DNA Sequencing




**UK**

UMIST

RAL

**FRG**

University of Constance

TCCIDP

## "AUTOMATION OF DNA SEQUENCING" PROJECT
## CO-ORDINATION MEETING NO 3

The third meeting of the contractors from the four centres engaged in the "Automation of DNA Sequencing" project took place in Manchester Biotechnology Centre from 28 November to 2 December 1987. There were 12 participants, ten from the centres involved and two guests (one, Dr. M. Browne from DIAS, UMIST and the other Dr. S. Beck from MRC, Cambridge who previously worked with Prof. Pohl at Konstanz).

The current status of the Automatic Plaque Selection and Culture Inoculation Robot (APSCIR) was demonstrated by Mr. P. Courtney. The TCCIDP (Konstanz) designed and built VME based imaging computer, which runs under an OS/9 operating system, had arrived some time previously in MBC. The imaging software, originally formulated by Mr. P. Courtney in Pascal on a Magiscan II image processor, had been transposed to "C", and loaded in the TCCIDP computer. Aspects of the XYZ robot plaque selection device were also operational. The Automatic Reagent Manipulating System (ARMS) which instruments primer/template annealing and the Sanger Sequencing reactions was demonstrated by Dr. P. Jasiobedzki, who also presented a report on commercially available vision systems for colony selection.

Dr. J.E. Bateman read a paper describing progress to date at Rutherford Appleton Laboratory on the design and constructuion of the EEC funded multi-wire proportional counter for imaging radiolabelled sequencing gels. Mr; C. Heller presented an account of work in progress on the Direct Blotting Electrophoresis device currently under development in Prof. Pohl's laboratory at the University of Konstanz. Prof. Massen's group discussed current activity in the TCCIDP on real-time symbol extraction with fast greylevel vision preprocessors. An imaging system developed by Dr. M. Browne was demonstrated and Dr. S. Beck read a paper on his work in Cambridge on Direct Blotting Electrophoresis.

Discussions were held to coordinate activity between the four centres and a June 1988 date for the next meeting (at Konstanz) was agreed.

Dr. W.J. Martin
Manager,
Manchester Biotechnology Centre

# CULTURE COLLECTIONS

EUROPEAN LABORATORY WITHOUT WALLS

BAP-0143-GR
BAP-0144-F

## In the field of

LACTIC ACID CULTURES

COLLECTION

# CREATION D'UNE COLLECTION DE SOUCHES DE BACTERIES LACTIQUES

# MODELISATION ET CONTROLE AUTOMATIQUE

# DE CULTURES MIXTES THERMOPHILES

Compte rendu de la réunion de coordination scientifique

La réunion s'est tenue à Grignon le 3 septembre 1987.

Participants : C. BEAL, M. DESMAZEAUD, A. ZOURARI
G. KALATZOPOULOS, E. SPINNLER

Suite à un problème de conservation de souches, l'équipe du Professeur KALATZOPOULOS n'a pu poursuivre les essais de mesure d'acidification des souches de sa collection. Néanmoins, cette équipe a travaillé à la mise en place des protocoles d'estimation des qualités technologiques de ces souches :

- mesure de vitesse d'acidification
- mesure d'activité peptidasique
- mesure d'activité estérasique

- Les profils électrophorétiques des differentes souches seront comparés.

- Un travail sur l'influence sur traitement thermique des laits sur la croissance et une étude des plasmides de ces souches complétera le travail.

L'équipe de G.CORRIEU, a pour sa part, travaillé à l'amélioration des techniques de mesure d'activité acidifiante afin de pouvoir comparer entre eux des ferments de diverses origines.

Ce travail a consisté à effectuer des cinétiques d'acidification avec des concentrations variables d'un même ferment. Toutes les 1/2 heures et tous les 1/4 d'heure pendant la phase exponentielle, un tube est utilisé pour faire les mesures suivantes :

- nombre de germes vivants / ml
- ATP intracellulaire
- pH

Parmi ces mesures, seules les deux dernières ont une reproductibilité stisfaisante. De plus le pH peut être facilement mesuré en ligne. Cette mesure sera donc priviligiée par la suite.

Le travail concernant le suivi en ligne des populations de streptocoques s'est aussi poursuivi en travaillant non plus sur les concentrations en $CO_2$ dissous, mais sur les volumes de $CO_2$ libérés à partir d'urée. Ces mesures impliquent l'ajout de 15 g d'urée par litre de lait et permettent de faire une estimation des populations de streptocoques au delà de $5\text{-}10^8$ bact/ml en cultures pures et en cultures mixtes.

Le travail se poursuit aussi sur la quantification des effets coopératifs entre Streptococccus thermophiles et Lactobacillus bulgaricus sur un milieu à base de lactosérum.

L'équipe de Monsieur DESMAZEAUD (avec A. ZOURARI) a terminé le travail commencé sur les souches isolées de yagourts grecs. Parmi les 360 souches étudiées (180 Streptococcus thermophilus et 180 Lactobacillus bulgaricus) dans une même espèce, on n'observe aucune variablité en ce qui concerne la consommation au moins une dizaine de sucres différents. Par contre, d'autres critères plus technologiques semblent différencier certaines d'entre elles (variants à vitesse de croissance lente par exemple :

Un travail va être entrepris sur ces souches concernant :

- leurs courbes d'acidification
- les électrophorégrammes de leurs protéines
- la viscosité engendrée
- la postacidification

Un séjour de Madame Ellena KAMBARAKI (équipe de G. KALATZOPOULOS) est prévu du 4 au 17/10/87 au Laboratoire de Microbiologie Laitière (Jouy-en Josas) pour mettre en place les conditions d'isolement et de conservation des bactéries lactiques thermophiles isolées de produits traditionnels grecs.

Un voyage d'Eric SPINNLER à Athènes est prévu début décembre pour mettre en place le bioréacteur acheté par l'ESAA. Des lyophilisats de cultures pures et mixtes produits à Grignon seront testés en fabricatioon de yagourt à Athènes.

Eric SPINNLER

EUROPEAN LABORATORY WITHOUT WALLS

BAP-0143-GR
BAP-0144-F

## In the field of

MICROBIOLOGY OF TRADITIONALLY FERMENTED FOODS IN THE MEDITERRANEAN COUNTRIES

## MINUTES OF THE MEETING ON MICROBIOLOGY OF TRADITIONALLY FERMENTED FOODS IN THE MEDITERRANEAN COUNTRIES.

The days 28th and 29th of April,1.988, the E.L.W.W. meeting on Microbiology of traditionally fermented foods in the Mediterranean countries was held in Madrid at the Instituto del Frío (Spanish Council for Scientific Research).

Financial support for the meeting was provided by the Commission of the European Communities, Directorate General for Science,Research and Development, and in part by the Spanish Council for Scientific Research.

The list of participants was as follows:

Dr.Alfredo Aguilar(Belgium), in the Chair; M.Barbosa(Portugal);V.Bottazzi(Italy); M.Desmazeaud(France); G.Kalatzopoulos(Greece); J.C.Le Jaouen(France); B.Moreno (Spain); M.Núñez(Spain); C.Peláez(Spain); H.E.Spinnler (France)

Also attended as invited researchers:

Dr.M.Ramos(Spain); M.Juárez(Spain);M.Medina(Spain).

### Welcome and Introduction

Dr. Aguilar welcomed the participants and began the meeting describing the present and future Biotechnology Programmes of the European Communities. He explained the basic orientation of the BAP Programme which started in 1.986 and has been recently revised for an enlargement in 1.988-90 with the attachment of Spain and Portugal.

The joint Spanish and Portuguese proposals to the different subjects have been 82 and 22 respectively, including 2 Spanish proposals to the subject "culture collection", highly related to the topic of this meeting. Some other subjects

to which proposals have been presented too, are: Bio-informatics, including Information Technology and Protein Engineering Risk Evaluation;Plants;Microorganisms;Animal Cells;Pharmacology and Biorreactors.Moreover,Dr.Aguilar reported valuable data referring to the financial support for the BAP revision,standing out the budget assigned to Spain and Portugal, as well as the Training Contracts.

Finally, Dr. Aguilar referred to the actions, conditions, budget and topics, in relation to the BRIDGE Programme foreseen for 1.990-94

Short Oral Contributions

Dra. Barbosa presented a report about the research carried out in Portugal on sheep's and goat's milk cheeses standing out the work on physicochemical and microbiological composition, technological procedure and ripening conditions of the cheeses.

Dr. Bottazzi described the objectives and the different steps which might be pointed out in a common project within the EEC, dealing with the traditional fermented products from the Mediterranean countries. He referred as well to the inadequacy of the available data on this subject and the necessity of studies describing the products, chemical composition, characterization of the lactic microflora and the creation of a culture collection. Furthermore, the different tasks of the different research teams working together in a possible transnational cooperation were also described.

Dr.Desmazeaud summarized the work carried out in his Institute as an actual BAP contractor, in relation to the characterization of thermophilic streptococci and lactobacilli isolated from yoghourt produced in Greece. He pointed out the possibility of a future BAP Programme in cooperation with the other Mediterranean countries, explaining to what extent he could contribute to the creation of a microorganisms collection through a biochemical and genetic characterization of the isolated strains.

In the same way, Dr.Kalatzopoulos presented the proposal of a potential future common Programme on the subject already mentioned, suggesting the possibility of making it extensive to other fermented products, such as wine, bread, fermented sausages etc. In these products do exist a specific microflora which might be known in order to improve the quality and at the same time, extent the storage life of the products.

Dr. Le Jaouen referred to a better standardization of the traditional sheep's and goat's products from France, achieved by an improvement of the technological parameters of production, either in the farm or in the small industries. At the same time, a better characterization of their mioroflora and understanding of legislative aspects would be necessary.

Dr. Moreno reported the research carried out in the Food Hygiene and Food Technology Department of the Veterinarian School in León(Spain). Concerning the dairy products, he mentioned the work on the microbiological quality of Spanish -- sheep's and goat's milk cheeses, as well as the effect of growth of lactic acid bacteria on the development of Staphylococcus aureus and its enterotoxin production.

Dr. Núñez considered that lot of work has still to be done in order to improve the bacteriological quality of the raw milk cheese varieties by means of inoculation of selected lactic - acid bacteria (Str. lactis and Lb. plantarum) as biological inhibitors of undesirable microorganisms.

Dra. Peláez described the situation of the Spanish cheese-making production made from sheep's and goat's milk; the main current problems involved in it and the multiple aspects which should be approached. She pointed out the validity of the characterization of the products, selection of microorganisms, antagonistic properties of lactic acid bacteria against pathogens and freezing of curds as a technology used to standardize the products troughout the year.

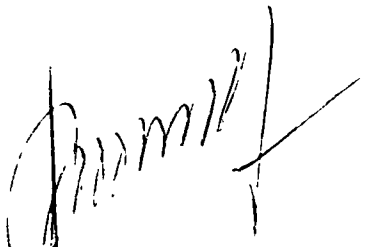
Finally, Dr. Spinnler reported the results obtained on the development of a method to measure the kinetics of acidification by thermophilic lactic acid bacteria (Str. thermophilus CNRZ 404 and Lb. bulgaricus CNRZ 398, using different inoculum levels.

Besides the oral contributions, all the participants took part in a round table and it was agreed that the main objective of a possible future common project within the"European Laboratory Without Walls" Programme, would be to preserve our traditional Mediterranean products with"appellations d'origines controleés". The participants focused the attention especially in those products which presented a potential economical interest in the Mediterranean area and susceptible to be improved by means of the Biotechnology.

Once the meeting was over, a visit to the Instituto del Frío and the Instituto de Fermentaciones Industriales ( both from the Spanish Council for Scientific Research) took place.

The day after the meeting, all the participants visited a Manchego cheese-making factory, nearby Madrid (Toledo).

Dra.Carmen Peláez



Prof. G.Kalatzopoulos

EUROPEAN LABORATORY WITHOUT WALLS

BAP-0002-UK
BAP-0007-D

## In the field of

EUROPEAN PLASMID DATABASE

EUROPEAN PLASMID DATABASE - Expert meeting

Braunschweig, 19/20 May 1988

Participants: A. Aguilar (CEC, Brussels), V. Hughes (NCTC, London), C. Jones (NCTC, London), E. Kampert (Phabagen, Utrecht), C. Leslie (NCIMB, Aberdeen), D. Osborne (NCTC, London), K. Painting (NCYC, Norwich), F. Pfeiffer (MIPS at MPI, Martinsried/Munich), A. Pindar (MiCIS, London), M. Vicente (CIB, Madrid). D. Claus, E. Breyel, M. Kracht, A. Regensburger, C. Rohde, E. Senghas (members of DSM, Braunschweig)

Opening:

C. Rohde, the chairperson of the meeting, welcomes the participants and thanks the Services of the Commission of the European Communities for financial assistance. This first meeting to discuss questions on the establishment and organization of a European plasmid database takes place with the aim to discuss general and detailed technical aspects and has the definite aim to create a standardized common database format.

Introduction by A. Aguilar:

A. Aguilar presents biotechnological projects supported by the CEC (BEP and BAP as the pioneer and the current programme) and the BRIDGE project which is planned for the near future and which could support the establishment of the European plasmid database. The preconditions for participating in the BRIDGE project are well defined, very important aspects for the European plasmid database are transnationality, precompetitiveness, and the database has to be user-orientated and beneficial for all scientists. A possible culture collections' use by itself would not be supported, but of course the data information would have to come from the participating collections.

Brief presentations, given by the participants:

DSM:
C. Rohde gives a short overview on the tasks and projects of the DSM's plasmid group, which was extended at the beginning of 1988. So far a plasmid database at DSM does not exist, but it is just in the process to be established, together with the preparation of the new DSM catalogue edition (being published probably by autumn 1988). A format for the European plasmid database was worked out at DSM. This format was created to be a basis for the discussions during the meeting.
D. Claus describes the new aspects of DSM's service and research activities in connection with the DSM expansion.

NCTC:
V. Hughes presents the organization of NCTC's plasmid database established 2 years ago. 2 major files are the heart of the system: a plasmid file and an accession file. The necessity of having a house-keeping file for a culture collection is pointed out, but similar aspects have to be taken into consideration for the European database. V. Hughes lays stress on the importance of literature data and on the aspect of flexibility of the European database.
MiCIS:
A. Pindar describes the history of MiCIS, the UK national system for microbial strain data, designed 3 years ago. A. Pindar mentions that MiCIS is not necessarily an initiative for the participants from the UK exclusively, but it will be open for European projects like the plasmid database, sharing costs and enhancing collaboration.
MIPS:
F. Pfeiffer gives a short report on the MIPS activities. At the Martinsried Institute for Protein Sequence Data the protein data bank was established 2 years ago, financed by the BMFT (the German Ministry for Research and Technology) and by the CEC, as a 5 years project, being part of the PIR-International (International Association of protein sequence data banks).
F. Pfeiffer describes the collaboration of MIPS (Europe) with JIPID (Asia) and NBRF-PIR (America), having one common protein sequence data set and using one standardized format. F. Pfeiffer also describes VecBase, a cloning vector sequence data base containing only vectors which are sequenced completely.
NCYC:
K. Painting briefly presents the relational database system at NCYC, being connected to a VAX system. The necessity of a special order of the fields for the European plasmid database is pointed out.
NCIMB:
C. Leslie describes the plasmid database situation at NCIMB and its integration into the MiCIS system. For the future NCIMB would like to increase the capacity of the database system in respect to genetical data.
CIB:
M. Vicente demonstrates the capacity of the user-orientated database at CEDIG, the Spanish database on genetic engineering. An updated catalogue was elaborated. The database files include fields for plasmids and cosmids, phages, recombinant vectors, genotheques, bacteria for genetics, cells and laboratory data. So far CEDIG is a system open for recherches by Spanish scientists. The CEDIG database includes the indexes of the Elsevier catalogue.
PHABAGEN:
E. Kampert describes the situation at the Phabagen collection with respect to a plasmid database, which is not yet established, though a detailled proposal for its format does exist.

Minutes of the general discussions about the establishment of a European plasmid and phage database

C. Rohde mentions questions, which may be difficult to be answered at this early stage but which should be discussed:
- DSM proposes a centralized database, situated at any location where experts are working. Are there arguments for a node structure instead?
- Should the database offer its service to anybody?
- Who enters the data? Who updates the data and how often should they be updated?
- Should plasmids and phages be integrated into one single database?

During the presentations given by the participants it became obvious that successfully running databases do exist at the different institutions. In some way they have to be "combined", and, in order to have a single plasmid database in Europe, certainly a collaboration between MINE (Microbial Information Network Europe), MiCIS (Microbial Culture Information Service) and MSDN (Microbial Strain Data Network) will be necessary. This is agreed by all participants.
M. Kracht mentions that for interconnecting the databases a special field for linkages might be created and - at a later stage - a database host has to be discussed. The advantages of a centralized database are mentioned: it would be faster, cheaper and less complicated.
A. Pindar stresses the importance of an identical format and suggests a centralized structure, because a network would be too complicated.
F. Pfeiffer describes how recherches at VecBase can be done: on-line recherches are not possible, recherches are sent to PIR, Washington, by electronic mail. Depending on the inquiries copies of the database are distributed.
A. Aguilar proposes that the databases of bacteria and of plasmids have to be harmonized. Data should be entered by culture collections. The importance of the establishment of the proposed European information centre - besides the "node" system for the MINE bacteria/fungi database - at one of the culture collections is mentioned.
It is generally agreed that the question of charges (for database users) is not relevant at this stage.
C. Jones outlines the necessity of updating the database regularly and as often as possible. It is of course a question of money. Each collection should send updates regularly.
It is agreed that plasmids and phages should be integrated into one database, as most of the fields representing genetic data refer to plasmids and phages.
A. Aguilar mentions that the CEC has great interest in having this European database, and financial support will be possible, although in what form is open at the moment.
F. Pfeiffer points out that database updates should be done regularly, having a "combined movement", e.g. a 3 months cycle. He proposes - for reasons of flexibility and data exchange - to have an identifier or subidentifier for combining different systems.

V. Hughes offers the NCTC's plasmid database for "export" to the European database, to make the first steps of the establishment easier, or to function as a pilot format.

General discussion about the format

It is generally agreed that the database should be organized as a menu file structure, being the easiest possibility for the user. All the fields referring to collection data should be one block of fields. A splitting point within the format is the field "element type", giving the user the possibility to enter either the database for plasmids or phages or transposons.
E. Kampert mentions the importance of the transposons, which make some specific fields necessary.
M. Vicente, E. Kampert and A. Regensburger outline the different vector types to be mentioned, a well-devised structure of fields for this kind of application is necessary.
V. Hughes mentions the absolute need to have a common and a correct nomenclature for phenotypic characteristics etc. at a very early stage, otherwise problems cannot be avoided.
V. Hughes mentions the publications of Novick et al. (1976), Bacteriol. Reviews 40, 168-189 and Demerec et al. (1966), Genetics 54, 61-76.
M. Vicente proposes to ask a phage expert for advice, as there exist aspects specific for phage genetics.
E. Senghas proposes to create a specific field for the transposon type, referring to the classification of transposons. Other Tn specific data are integrated in the other fields.

The format structure proposed by DSM is discussed in detail. The revised format (enclosed) contains all the ideas which originated during the expert meeting. It was decided that a minimal data set should not be created for the European plasmid database.

The participants of the meeting recommend
- to continue as fast as possible with the establishment of the European plasmid database
- to contact scientists being experts on plasmids/phages and to invite their critical comments on the format proposal before the database is going to be established
- DSM and NCTC will start to combine their databases (a possible pilot format)
- a publication of the format in its final structure is planned, a genetic journal should be chosen (GENETICS or NUCLEIC ACIDS RESEARCH or a similar one)
- a small article should then be published in a European journal, describing this database project

Braunschweig, June 14, 1988

EUROPEAN DATABASE : PROPOSAL FOR THE PLASMID/PHAGE/TRANSPOSON - FORMAT (worked out during the database congress at DSM, May 1988)

New modifications (proposed by DSM):
- organization of fields under special headings (A - F),
- abbreviated names for each field (in relation to the MINE format)

In DSM's opinion there is probably no need at this early stage to define the detailed organization of each field, e.g. its different possibilities for answering with yes/no or with +/-. But this has to be done as soon as the general structure of the database has been agreed. It should be presumed that a common standardized format has to be used by the individual collections to establish their plasmid database, and thereafter, based on these standardized individual databases, the European integrated database can be established. Some problems have to be discussed then: who will write an integration program; which fields have to be integrated and checked for redundant information, which fields can simply be added, etc.

Numbers : field numbers,
i: field should be indexed
ph: field for phages only
pl: field for plasmids only
tn: field for transposons only

A. Database administration
B. Name
C. Element administration
D. Host and history
E. Biological properties
F. Applications

A. Database administration

| | | | |
|---|---|---|---|
| 1 | ACCN | (i) | : code number (accession number, record number) |
| 2 | EDI | | : date of input into database |
| 3 | EDU | | : date of update |
| 4 | ID | | : responsible scientist |
| 5 | ET | (i) | : element type (Ph = phage, Pl = plasmid, Tn = transposon) |

B. Name

| | | | |
|---|---|---|---|
| 6 | NAME | (i) | : element name |
| 7 | PLREF | (pl) | : plasmid reference center |

(+ registered, - not registered)
8 OTHNAM (i) : other names (synonyms)

C. Element administration

9 NUM (i) : element number in collection(s)
subfield: distributed in
10 OCC (i) : other culture collection numbers
11 EDA : date of accession
12 CHK : collection check
subfield: last update
13 PRE : preservation method
14 SUPPLY : form of supply
15 ISOM (ph): isolation method (propagation method)
subfield: recommended host
16 PREC : precautions (hazard group)
17 REG : restrictions (regulations concerning supply)
18 LIT : literature (first publication and a new one)
19 REM : remarks and comments

D. Host and history

20 CLASS (i) : element classification
(natural or constructed element)
21 ORIHST (i) : original host
22 RNGHST : host range
subfield: host specificity
23 CONSTR : constructed by
24 CONSTRFR : constructed from (sources)
25 DEP : depositor
26 HIS : history

E. Biological properties

27 PHAMORPH(i)(ph): phage morphology
subfield: particle size
28 LYSO (i)(ph): lysogenic (+/-)
29 PLAFO (i)(ph): plaque forming phage
30 PLAMORPH(i)(ph): plaque morphology
31 SOLVSEN (i)(ph): solvent sensitivity
32 IMM (i)(ph): immunity
33 NT (i)(ph): nucleic acid type (dsDNA, ssDNA, RNA, unknown)
34 INSERT : insert (cloned fragment)
35 MWT : molecular weight (in kb and Mdal)
subfield: method of determination
(EM contourlength, agarose gel electrophoresis, sequence analysis, restriction fragment lengths)
36 INC (i)(pl): incompatibility group
37 REPL (i)(pl): replicon
38 COPYNR (i)(pl): copy number (high, low)
39 AMPL (i)(pl): amplifiable

subfield: method

40 RESTR (i) : unique restriction sites
either:
subfield: for cloning
or:
mark restriction enzyme(s) with a sign.
In brackets: enzyme(s) for inactivated resistance gene

41 MAP : restriction map (reference)

42 SEQU : sequence (reference number, code link for entering sequence database)

43 ILLUSTR : illustration (graphical)
linear, not circular form!?

44 TRANS (i)(pl): transfer
subfields: conjugative (+/-)
mobilizable/non-mobilizable
unknown

45 PATH : pathogenicity (like toxin production, $T_i$ plasmid)

F. Applications

46 PHATYPE (i)(ph): phage type
subfields: transducing phage
(generalized/specialized)
pilus specific

47 TNTYPE (i)(tn): transposon type
subfields: IS element
composite Tn
other Tn

48 VECTYPE (i) : vector type
subfields: cloning vector
shuttle vector
expression vector
promotor probe vector
suicide vector
replacement vector
cosmid
phasmid
Charon vector
other vector

49 MARKER (i) : markers
subfields: phenotype
production of (pathway)
degradation of (pathway)
resistance to (mechanism)
genetic markers
F primes

50 PATENT : patent (free patent, patent number)

EUROPEAN LABORATORY WITHOUT WALLS

BAP-0072-I
BAP-1137-D
BAP-0006-NL
BAP-0001-UK

## In the field of

EUROPEAN COLLECTION FOR
BIOMEDICAL RESEARCH

Report to Biotechnology Action Program section 2.1: Culture Collections.
Subject: meeting on 24.02.87 in Genova (Italy) at National Institute for Cancer Research (Prof. Ferrara).

Participants: Prof.G.B. Ferrara and coworkers;
Prof. Dr.H. Grosse-Wilde (Essen, GFR);
Dr.A.Doyle (Salisbury, G.B.);
Prof.dr.M.F.Niermeijer (Rotterdam, The Netherlands)

Greneral Aim of the meeting
This meeting was organised at the request of Dr. H.Reiniger to discuss various points in the coordination of the culture collection program of the EEC, including diagnostic strategies for AIDS in samples submitted to and distributed by culture collections, and on the organisation of a data network.

1. Format and organisation of participating cell culture collections
   a. The institute for Immunogenetics, University Hospital, Essen (Prof. Dr.H.Grosse-Wilde) has a collection of:
      . HLA-homozygous lymphoblastoid lines, for reference, typing, biochemical and genetic research on the HLA-locus; these cells are also present in the institute in Genova.
      . lymphoblastoid cell lines of patients with HLA-associated diseases, concentrating on families with at least two-affected patients with diabetes mellitus, coeliac disease, congenital adrenal hyperplasia and its variant forms, multiple sclerosis, ankylosing spondylitis, Alzheimer disease, SLE, haemochromatosis, etc.

   b. The Laboratory of Immunogenetics, Natl. Institute for Cancer Research, Genova (Italy) (Prof. G.B.Ferrara):
      . HLA homozygous typing lymphoblastoid lines (see above); for protection against loss these strains are preserved

in two independent facilities.

. Lymphoblastoid cell lines from families with HLA-related diseases (see earlier).

. Lymphoblastoid cell lines from various genetic cancer syndromes (including familial breast , lung and colonic carcinoma, a.o. polyposis coli).

c. European Collection of Animal Cell cultures (at the Public Health Laboratory Services establishment, Salisbury, Great Britain) (Dr.A.Doyle).
. animal cells from a large number of spiecies and tissues;
. human tumour fibroblasts and cells from a large variety of tumours.
. HLA-typing lymphoblastoid lines, in cooperation with the UK transplantation program.
. lymphoblastoid cell lines from various human genetic diseases, in cooperation with the European Human Genetic Mutant Cell Bank (Rotterdam and Salisbury).

d. Dept. Clinical Genetics, Erasmus University, Rotterdam (the Netherlands) (prof.Dr.H.Galjaard, Prof.Dr.M.F. Niermeijer).
. Fibroblasts of patients, carriers and other informative relatives of genetic Mendelian and chromosomal disorders.

The EEC biotechnology programs support would allow establishing both fibroblast-cultures (in Rotterdam) and long-term lymphocyte cultures (at Salisbury) simultaneously, from the same individuals. The availability of such well defind cell strains is of paramount importance with the introduction of DNA-markers identifying disease-genes (either directly or through linkage) and will enable future generations of relatives of studied persons to use these new predictive methodologies in genetic counseling and family planning.

The joint efforts of the Salisbury and Rotterdam group would be directed towards the storage of:
. rare diseases and their variants
. index cases of recessive, X-linked and dominant diseases, for reference to future studies using DNA-marker studies (by linkage) in the future;
. well studied families, wherein certain genetic diseases with severe health implications and of an important frequency are segregating:
f.i.: X-linked diseases: fragile X-syndrome (a few well studied families only);
Duchenne muscular dystrophy, haemofilia, etc.
Autosomal dominant diseases (some larger and well analysed families):
Huntington's disease, Alzheimer's disease,

neurofibromatosis, tuberous sclerosis, familial cancer syndromes (polyposis coli, von Hippel Lindau's disease, etc.);
- cases of new mutations of some of the previous diseases;
- chromosomal translocations of selected chromosomes (related to disorders mentioned above);
- patients with two "rare" diseases (by allowing gene localisation or detection of deletions).

2. Coordination of activities as European Collection for Biomedical Research
The participants at the meeting concluded that their collaboration could result in the institution of an European Collection for Biomedical Research. This would be a facility offering exchange of biomedical materials and information in the areas of research on genetics, cancer and ageing. Exchange and availability of the various cell strains would be the first goal, facilitated by easy excess to catalogues of several centers by computer. At the next meeting of the Biomedical Action Program, it would be decided if it is desirable to extend the ECBR to include :
   - collection of gene probes;
   - collection of tumor tissues;
   - collection of monoclonal antibodies and hybridomas (possibly to be coordinated with the facility in Nice (France).

   This approach, of course, will need the approval and financial support fom the EEC Biotechnology program, or other action program within the EEC (as on cancer).

3. Overlap in the present activities
There is no significant overlap between the participating centers. In contacts with the UK transplantation program it will be decided if supplementary activities for storage of HLA homozygous cells is needed in the UK, in the presence of storage elsewhere.
In the collection of cell strains the centres will critically review cases/diseases submitted for banking, as to relevance to future family studies, level of data andstudies already done in the respective families, availability of cells from relatives, frequency of disease, developments in research, etc.

4. Diagnostic tests for AIDS in samples submitted to and distributed by the cell banks
With an incidence of 0,1 to 0,01 AIDS-positive blood donors in CCC ÅC various Western countries, it is of paramount importance to :
   a. protect workers in laboratories against unexpected exposure;
   b. to be able to guarantee that no AIDS-infected materials may

be distributed through cell banks.

Since B-lymphocytes (in longterm lymphoblastoid lines) may harbour the AIDS virus, those centers handling blood (for establishing cultures) or handling lymphoblastoid cultures must have facilities available for testing at various stages.

i. Testing for AIDS in blood samples, submitted for culturing.
Laboratories (Essen, Salisbury, Genova) plan to introduce AIDS antibody screening in serum by indirect immunofluorescence staining. This is a convenient and sensitive test and is more practical for small numbers of samples as the ELISA test.

Positive cases will have to be confirmed by the Western blotting technique.
Incoming samples will be handled under PII conditions, which are thought to be satisfactory, if laboratory routine is strictly applied. The low expected incidence of AIDS in the incoming samples would make the routine use of PIII facilities prohibitively laborious and expensive (even if available in some places).

The donors of the blood samples will be informed, either directly or through the physicians referring the samples, that tests for infectious substances will be done, for the protection of workers working the donor's cells. If a positive test is found and confirmed, information of the donor will be by his general practitioner or by the referring physician.
An information leaflet for donors will be prepared on this subject by the various centers.

Financing of diagnosis in incoming samples has not been provided for in the EEC Biotechnology program. The cost-estimate is about 10.000 DM for each of the three centres. The EEC Biotechnology program is requested to provide this supplementary funding.

The situation may change when techniques will become available in the future for direct identification of the virus in blood specimens.

ii. Testing for AIDS in lymphoblastoid cultures handled, stored and distributed by the cell banks.
Since this is a very specific area, still in the research phase, it was decided that the centre at Salisbury will perform AIDS testing in lymphoblastoid cultures. There is, at Salisbury, availability of high level containment facilities (PIII) when required, and collaboration is possible with local expertise from the PHLS.
Testing techniques will involve (on ± 1000 cultures/year): reverse transcriptase; various techniques for direct detec-

tion of the virus, by immunological and other techniques.

The EEC Biotechnology program is asked to give additional funding for one molecular biologist/virologist and two technicians and laboratory expenses during the next four years.
During this phase the most efficient and reliable technology may become established.

When other techniques allowing easier identification of HIV-virus (f.i. by the DNA-probing) become available, the necessity for continuing this additional funding may be reconsidered, provided the techniques become sufficiently specific and easy to be conducted by all local cell banks.

5. Communication between and with cell banks

Various needs and utilisations of electronic data handling and communication were reviewed at the meeting.
The Essen centre is already connected to the MINE data net, the Genova centre will be in short. The Essen centre sees utilisation of such a net for rapid communication.

For general cell banking purposes, a computerized catalogue is essential. This is available in Essen and Salisbury, and in preparation in Genova and Rotterdam.
Access to the catalogue may be through periodical distribution of a printed catalogue (like from Salisbury), or through acces by a data network. Especially in the latter situation, strict rules for privacy protection are needed, since a registry containing for example diagnosis and date of birth may easily lead to patient identification.

The acceptance by the general public of any form of genetic registry will be dependent upon guarantees for maintaining privacy and confidentiality.
Even if a catalogue will not be immediately perceived as a registry by most scientists, it is prudent to develop regulations for electronic registries of genetic data on individuals.

It was proposed to discuss this point at the coming Biotechnology meeting in Tours and to see if institution of privacy protection rules and a steering committee might be desireable.

It was agreed, that depending upon the main focus of each collaborating service, the need for direct electronic communication differs. It is more important in a HLA-typing laboratory (also involved in transplantation) than in a pure cell banking facility.

Financing: additional staff for data introduction- and management will be outside the EEC Biotechnology program's support for most centres and additional funding would become needed for staff,

equipment, maintenance and subscription/user fees for data distribution networks.

Coordination in cataloguing will be discussed at the meeting in Tours:

- the format of already existing computer cataloguing systems: Salisbury, Rotterdam, Essen.
- utilisation of identical codes for genetic disease (like: the Mc Kusick numbers for Mendelian disorders).
- coordination with other networks, like the Italian Interlab system.

6. Conclusion, continuation of the program
The participants agreed that this program is an important step towards guaranteing an increasing number of the European population to profit of the new advances in genetics, and to use these findings in their personal life (by informed genetic counseling) and reproductive decisions. For continuous support to such international research activities, facilities for storage of cells, tissues, biomedical reagents (like gene probes and monoclonal antibodies) will be essential for future generations. Only through continuing international support, the European population may benefit in the next decades.

MF Niermeijer

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Rotterdam

# PROTEIN DESIGN /

# MACROMOLECULAR MODELLING

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BAP-0041-D

## In the field of

"FOLDING, ASSEMBLY, STABILITY AND GENETIC MODIFICATION OF PENICILLIN ACYLASE AND ITS PRECURSORS"

Group Meeting of the European Communities
Biotechnology Action Programme

"Folding, assembly, stability and genetic modification of penicillin acylase and its precursor"

Tutzing, 22nd of July, 1987

Participants:

1. University of Newcastle upon Tyne

Dr. C. Lindsay
Prof. R. H. Pain
Dr. A. Slade
Dr. R. Virden

2. University of York

Dr. C. Hill
Mr. S. Smerden

3. University of Munich

Prof. Böck
Fr. D. Sizmann

4. Boehringer Mannheim GmbH

Dr. K. Beaucamp
Dr. P. Buckel
Dr. Dvorak
Dr. S. Fischer
Dr. M. Jarsch
Dr. Prinz
Dr. G. Schumacher
Dr. Tischer
Dr. Popp
Dr. H. Lenz

**Report**

of the Group Meeting of the European Communities Biotechnology Action Programme on

"Folding, Assembly, Stability and Genetic Modification of Penicillin Acylase and its Precursor"

The group meeting was attended by the three EC contrators and by two guest groups from Newcastle upon Tyne (biochemical characterization of the enzyme reaction) and from York (crystallography and X ray analysis of penicillin acylase).

Summary of the presented talks:

1. **Dr. Schumacher, Boehringer, Tutzing:**
   The DNA sequence of the penicillin acylase structural gene reveals that the two dissimilar subunits of the mature enzyme, α and ß, are derived from a common precursor polypeptide. Plasmids wee constructed coding for α , for ß, for both subunits in an operon, for an N-terminally extended α subunit and for a precursor protein lacking the signal peptide. Problems of penicillin acylase overproduction were discussed.

2. **D. Sizmann, Munich:**
   α and ß subunit as well as a stabilized form of the precursor without signal peptide were purified as inclusion bodies. A model for the processing pathway of the penicillin acylase precursor was presented derived from in vitro processing experiments. Three mutant precursor polypeptides showing impaired processing behaviour have been obtained and investigated. Constructions of further mutant precursor proteins were discussed.

3. **Dr. Virden, Newcastle upon Tyne:**
Approaches for the chemical and kinetic characterization of the active site of penicillin acylase were presented. Different inhibitors were investigated for their inhibition potential. Inhibition of enzyme activity by PMSF and re-activation of activity by 6-APA were studied and the reaction constants were determined. The catalytic pathway proceeds via an acyl-intermediate. Enzyme-substrate interactions were discussed.

4. **Dr. Slade, Newcastle upon Tyne:**
Chemical studies of the active site using the irreversible inhibitor PDCP and the reversible inhibitor PMSF were reported. Identification of the active site with the help of $^{35}$S-radiolabeled PMSF proved to be difficult due to loss of the label. A method for thiol modification of the active site was presented; the data allocate the catalytic center to the ß-subunit.

5. **Dr. Hill, York:**
A method for purification of penicillin acylase by hydrophobic interaction chromatography was discussed. Crystallization of the pure penicillin acylase has been achieved and studies of these crystals at 7 Å and 2.8 Å resolution were reported. Approaches to obtain heavy metal derivatives of the crystals were explained.

6. **Prof. Pain, Newcastle upon Tyne:**
A model for folding of proteins from a fully denatured form to the native state was presented. The implications in protein synthesis and in protein export as well as factors affecting the refolding were discussed.

7. **Dr. Linsey, Newcastle upon Tyne:**
The characteristic solution conformation of penicillin acylase and the process of unfolding was investigated by circular dichroism and fluorescence spectrometry studies. Intermediate

concentrations of the denaturing agent resulted in precipitation of penicillin acylase whereas the fully denatured form of the protein stays soluble.

In order to coordinate the scientific activities of the participating groups a rough time schedule for future experiments was set up for the period of a year.

It was the general feeling of all participants that the meeting was extremely useful for the exchange of ideas and for the coordination of efforts. There is hope that penicillin acylase will be one of the best studied models on protein design since excellent expertises from many directions flow together in this joint project. It was felt that the group should meet immediately again when the 3D structure of the enzyme is available, at least, however, after one year.

The organizers and all participants thank the EC BAP programme for the financial support of this workshop.

# BIOTECHNOLOGY

# O F

# INDUSTRIAL MICROORGANISMS

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BAP-0010-D
BAP-0011-NL
BAP-0012-NL

## In the field of

GENETIC ENGINEERING LACTIC ACID BACTERIA

# P R O G R A M M E

Workshop/Meeting of contractants of the EC Biotechnology Action Programme

'GENETIC MANIPULATION OF LACTIC ACID BACTERIA FOR IMPROVED DAIRY FERMENTATION'
at
Institute of Microbiology, Federal Dairy Research Centre, Kiel, Federal Republic of Germany

September 17 to 19, 1987

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## Thursday, September 17

18:00 (6 p.m.) - 19:30
Opening: 1. Representation of activities of the Federal Dairy Research Centre at Kiel (Teuber)
2. Presentation of Kiel-group
   a) Phage systematics (Teuber)
   b) Phages of thermophilic lactic streptococci (Krusch/Neve)
   c) Phage resistance of mesophilic streptococci (Geis/Neve)
   d) Specificity of protease (Bockelmann)

20:00 (8 p.m.)
Dinner at local pub 'Forstbaumschule'(very informal and relaxing)

## Friday, September 18

9:00 to 10:30
Presentation of Cork-group
1. Phage resistance
2. Transposons
3. Proteinase
4. Lactobacillus
   (Daly, Vaughan, Hill, Coffey)

10:30 to 11:00 Coffee break

11:00 - 12:30
Presentation of Groningen-group
1. The proteinases of lactic streptococci: a genetical approach (Kok)
2. Characterization and use of Streptococcus cremoris promoter sequences (van der Vossen)
3. Deletion formation of pAMß in Bacillus subtilis (van der Lelie)
4. Campbell-type integration of plasmid DNA in Streptococcus lactis (Leenhouts)

12:30 to 13:30
Lunch at cafeteria of the Federal Dairy Research Centre

13:30 to 15:00
Presentation of Ede-group

1. Genetic Characterization of Leuconostoc spp. from Dutch Starter Cultures (David)
2. Expression Vectors for Lactic Streptococci (Simons)
3. Organization of the S.cremoris SK11 proteinase gene (Vos, P.)
4. Replication of the S.lactis plasmid pSH71 (de Vos)
5. Characterization of related S.cremoris SK11 phages (de Vos)

15:00 to 15:30 coffee break

15:30 to 17:00
Presentation of Reading/Norwich-group (Gasson)
Programme to be anounced

17:00 to 18:30
General discussion

20:00 Buffet-dinner at Flintbek (Invitation to the home of M. Teuber)

Saturday, September 19

10:00 to 15:00 (open end)
Workshop at the Institute:
Electronmicroscopy of phage and plasmids

# LIST OF PARTICIPANTS

(as of August 28, 1987)

1. University college Cork

   Charly Daly
   Elene Vaughan
   Colin Hill
   Eden Coffey

2. NIZO, Ede

   Willem M. de Vos
   Silke David
   Pieter Vos
   Guus Simons

3. University Groningen

   Gerard Venema
   Jan Kok
   Jos van der Vossen
   Niels van der Lelie
   Kees Leenhouts
   Maarten van de Guchte
   Marco van Belkum
   Alfred Haandrikman

4. National Food Research Institute Reading/Norwich

   Michael Gasson
   further participants to be anounced

5. Bundesanstalt für Milchforschung Kiel

   Michael Teuber
   Arnold Geis
   Horst Neve
   Uli Krusch
   Sylvia Sellmer
   Rolf Braun
   Wilhelm Bockelmann
   Barbara Kiefer
   Sven Paul
   Stefan Hertwig
   Kwang-hee Lee

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BAP-0026-F
BAP-0199-D

## In the field of

BIOLOGY OF THE YEAST Kluyveromyces

PROGRAMME

May 27 Friday

Aims of the meeting; Proposition of the schedule;
Introducing each other. (H.Fukuhara) 30'

9:30-12:00 What is Kluyveromyces ? (Chair Whittaker)
M.Smith : Classification of the genus Kluyveromyces v.d.Walt 30'
Steensma : Electrophoretic karyotype of K.marxianus and K.lactis strains. 30'
F.Sor : Chromosomes of Kluyveromyces 30'
P.Whittaker: Protoplast fusion 30'

-Lunch- *

14:00-16:00 Transformation and cloning systems (Chair Frontali)
M.Bianchi : 2u-like plasmmid pKD1 and chromosomal ARS 30'
L.Frontali: ARS elements of K.lactis 15'
X.J.Chen : pKD1-derived vectors 30'
M.Wésolowski-Louvel/C.Tangy-Rougeau: KEX1 protease of K.lactis 30'

-Pause-

16:30-18:30 Regulation related topics (Chair Breunig)
K.Breunig : Regulation of the yeast LAC genes 30'
A.Reynal/C.Gerbaud/F.Iborra: Promoter of the B-glucosidase gene from K.fragilis 30'
C.Falcone : Alcohol dehydrogenase genes of K.lactis 30'
A.Dominguez : Acide phophatase of yeast 30'

-Dinner- *

May 28 Saturday

9:00-11:00 Mitochondria related topics (Chair Ferrero)
A.Ragnini : Mitochondrial DNA of K.lactis 30'
P.Whittaker: Problems with mitochondrial antibiotics/ Pathways of alternative respiration. 30'
I.Ferrero/P.Goffrini: RAG genes 30'

11:00-12:00 Coffee and general discussion
K.lactis as a genetic system, origin of our strains, known genes and mutations, nomenclature, genetic techniques, prospects for K.lactis and related species, making a list of concerned labs, future meetings, etc.
-End-

The detailed content of the seminar will not be published. A short report will be sent to the CEC.

* The lunch will be taken at the university cantine (36.50 F); the dinner will be served at the "Restaurant des Pins" in the campus near the building 400 ( 110 F).

# REPORT OF THE ELWW SEMINAR "BIOLOGY OF THE YEAST KLUYVEROMYCES"

ORSAY, 27-28 May 1988

23 participants (10 laboratories including 3 contractants) from 6 countries.

Subject:
Biology of the yeast Kluyveromyces.

Local organizer:
H.Fukuhara, Institut Curie, Orsay.

Dimension and structure:
One invited lecture, 14 oral presentations, a general discussion.

Objectives:
This is the first attempt to make a working network specialized for this area of research, although several important groups could not be included this time. The general objectives are (i) to put into contact the laboratories to allow direct exchange of information on their activities, and (ii) to prepare future gatherings of greater dimension. Specific objectives are (i) to construct a coherent genetic system for Kluyveromyces lactis, (ii) to evaluate the possibilities of cooperation between laboratories, and (iii) to have an overview of the current research in this field and, identify, if any, difficulties and deficiencies.

Conclusions

Among the species of Kluyveromyces, K.lactis is the only species for which a workable genetic system has been known. However, the other species are taxonomically well studied, and molecular approaches allow us to exploit their physiological particularities. Molecular analysis of chromosomes and mitochondrial DNA confirmed earlier taxonomic studies and added new insights into the relatedness between these species. Vector systems have become available for several of them, and being used for cloning of genes. A few studies on gene regulation are quite advanced, especially for the lactose metabolism which is one of the major interests of this group of yeast. Concerning the mitochondria and their role in the general biology of K.lactis, new aspects seem to emerge with respect to what has been known in Saccharomyces. Active participation of researchers from the applied field was most appreciated.

We were aware of other progresses in the field such as killer system, secretion and their applications, which are the leading topics in Kluyveromyces research. They should be discussed in next meetings with participation of representative laboratories. In order to set up a formal genetic system of K.lactis, it was proposed to standardize the nomenclature of mutations isolated in different laboratories. A list of available K.lactis gene clones will be prepared. It was a generally expressed wish of the participants that another European meeting of this type be organized within a year.

ELWW SEMINAR: Biology of the yeast Kluyveromyces

May 27-28 1988, Institut Curie, Section de Biologie, Building 111, University of Paris, Orsay Campus, France.

PARTICIPANTS

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BAP-0200-D

# In the field of

BIOTECHNOLOGICAL APPLICATIONS

OF Zymomonas

WORKING SEMINAR OF THE ELWW

in the field of : BIOTECHNOLOGICAL APPLICATIONS OF ZYMOMONAS

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* *

1. Local organizer : Dr. Milton A. Typas

2. Proposed dates for the seminar : 19-20th December , 1987

3. Proposed site for the seminar : Department of Biochemistry, Cell & Molecular Biology and Genetics , Athens University

4. Designation of laboratories whose participation would be requested :

4.1. From the BAP programme : Athens University , Dept.Biochemistry, Cell & Molecular Biology and Genetics

Ioannina University , Dept of Chemistry , Div.of Biochemistry and Organic Chemistry

Jülich , Kernforschungsanalage , Biotechnologie

4.2. From outside the BAP programme :

Invited speaker

4.3. From interested firms : "BIOHELLAS" , Biotechnology Company in Greece

The Athens meeting on "Biotechnological applications of Zymomonas" under the scheme of E.L.W.W. seminars, was organized in order to bring the collaborating groups together and enable them to exchange recent results, discuss in detail experimental difficulties, new ideas and approaches towards the common final goal. The overall progress was discussed and the groups came up with the following conclusions :

a) techniques for helped conjugation are of common use now by all researchers involved in the common project, with satisfactory rates and they are the best tool for genetic recombination in the bacterium for the moment.

b) the structural analysis of Z.mobilis natural plasmids is now nearly finished and physical maps of all plasmids are available

c) the promoter of the pyruvate decarboxylase gene from Zymomonas has been isolated and various recombinant vectors have been constructed

d) similarly, expression vectors have been constructed and the transfer of foreign genes (e.g.alanine dehydrogenase) show some promissing results

e) auxotrophic markers of all different strains have been produced, by various ways, and these mutants show a greater stability than antibiotic resistance markers. However, stable glutamine non-utilizing mutants have not been isolated yet .

f) transposons have been succesfully transferred in Zymomonas but they show great instability. Similarly, several indications for genetic instability and rearrangements in Zymomonas have been presented

g) gene banks have been established for the study of the recombination system in the bacterium

h) although an operational transformation system has not been established, results show that radioactively labeled DNA is uptaken by the bacterium, but it is rapidly degraded in the host cells

i) optimum protoplast formation and fusion conditions have been established in the organism

j) immobilization of Zymomonas on γ-alumina pellets shows that this system has several advantages over the traditional yeasts for continuous ethanol production.

It was generally agreed that the organism still presents several difficulties, which should be looked into, more carefully. The transformation system of the organism will be a valuable tool and research into this field should be carried on, bearing in mind that the sofar published systems do not work with Zymomonas. The failure to isolate any strain specific phages or to succeed in infecting the organism with a wide variety of phages tested shows that more work on this field should be done. Gene stability and genetic rearrangements present great difficulties for all genetic studies and thus, apart from further research to find the possible reasons for these phenomena, only genetically stable markers and auxotrophs should be used. The succesful transfer of transposons in the organism and their subsequent instability gives more ground for

transposon mutagenesis experiments which should be also expanded to strain construction. Curing of plasmid species in the various strains of Z.mobilis proved to be a very difficult task and this is approached now with hybridization experiments using probes from some of these natural plasmids. In view of all these, the collaborating groups have decided to join their efforts in a study of the recombination system of the organism. Gene banks of the genome of Z.mobilis have been made and several U.V.-sensitive mutants have been isolated. For a more efficient and effective approach in this study, a visit of the two leaders of the Greek research groups to Julich, in late June, has been organized. It was also decided that along with the above study the same gene banks should be used for complementation experiments with a large selection of auxotrophs in order to isolate the genes responsible for various types of auxotrophy.

| NAMES | LABORATORY | CITY and COUNTRY |
|---|---|---|
| M.A.Typas | Athens University , Dept.of Biochemistry, Cell & Molecular Biology , and Genetics | Athens , Greece |
| E.Alexandri | " " | " " |
| I.Galani | " " | " " |
| B.J.Mouzak | " " | " " |
| S.Kolyva | " " | " " |
| C.Drainas | Ioannina University, Dept. of Chemistry Div. of Biochemistry and Organic Chemistry | Ioannina , Greece |
| A.Afendra | " " | " " |
| G.Bartholomatos | " " | " " |
| G.Kollios | " " | " " |
| A.Perisynakis | " " | " " |
| A.Skordaki | " " | " " |
| J.Roussis | " " | " " |
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| A.Lykouriotis | " " | " " |
| H.Sahm | Jülich , Kernforschungsan lage , Biotechnologie | Jülich , FRG |
| G.Sprenger | " " | " " |
| M.Reynen | " " | " " |
| U.Uchlenbusch | " " | " " |

EUROPEAN LABORATORY WITHOUT WALLS

BAP-0141-F BAP-0271-E
BAP-0012-NL
BAP-0262-IRL
BAP-0045-D
BAP-0263-IRL
BAP-0046-UK
BAP-0044-UK

## In the field of

BIOTECHNOLOGY OF GRAM-POSITIVE INDUSTRIAL MICROORGANISMS

Thursday, February 25th, 1988

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| 15:00 | Registration |
| 20:00 | Dinner |

Friday, February 26th

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| 9:00 - 9:05 | **S.D. EHRLICH** - Opening remarks |
| 9:05 - 9:25 | **A. AGUILAR** - General introduction |

**CHAIRMAN : J.P. AUBERT**

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|---|---|
| 9:25 - 9:50 | **D. McCONNELL** : Solutions to the problem of genetic instability in engineered strains of Bacillus |
| 9:50 - 10:15 | **G. VENEMA** : Use of genetically engineered Bacillus for fermentation purposes |
| 10:15 - 10:40 | **S.D. EHRLICH** : Structurally stable plasmid vectors for DNA cloning in Bacillus subtilis |

BREAK 10:40 - 11:00

**CHAIRMAN : D. McCONNELL**

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| 11:00 - 11:25 | **J. ERRINGTON** : Bacteriophage vectors for gene cloning and genetic analysis in Bacillus subtilis |
| 11:25 - 11:50 | **P. DHAESE** : Interaction of the ϕ105 repressor with operator DNA : a mutational analysis and in vitro binding studies |
| 11:50 - 12:15 | **S. LE GRICE** : Repressible B. subtilis expression systems and their use in production of biologically active heterologous protein. |
| 12:15 - 12:40 | **H. de LENCASTRE** : The arabinose regulon |

LUNCH

Friday, February 26th, 1988

**CHAIRMAN : G. VENEMA**

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| 15:00 - 15:25 | G. **RAPOPORT** : Use of the sacB system for the secretion of proteins from B. subtilis |
| 15:25 - 15:50 | H. **HESLOT** : Induction of levansucrase by sucrose in Bacillus subtilis : involvement of an antitermination mechanism negatively controlled by the PTS |
| 15:50 - 16:15 | A. **GALIZZI** : The Bacillus subtilis outB gene is autogenously regulated |
| 16:15 - 16:40 | W. **SCHUMANN** : Isolation of heat-shock genes from Bacilli |

BREAK 16:40-17:00

**CHAIRMAN : H. HESLOT**

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|---|---|
| 17:00 - 17:25 | G. **GRANDI** : The B. subtilis neutral protease precursor is autocatalytically processed |
| 17:25 - 17:50 | B. **DIDERICHSEN** : Construction and analyses of amylase hybrids |
| 17:50 - 18:15 | A. **KLIER** : Characterization of the 130 kDa protein genes of the Bacillus thuringiensis israelensis crystals |
| 18:15 - 18:40 | J. **MAHILLON** : Class II transposon Tn4430: involvement of an integrase-like protein in the cointegrate-resolution process |

DINNER

Saturday, February 27th, 1988

CHAIRMAN : M. YOUNG

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|---|---|
| 9:00 - 9:25 | J.C. **PATTE** : Genetic transfer and molecular biology of corynebacteria |
| 9:25 - 9:50 | P. **YEH** : Isolation and characterization of the genes specifically involved in the meso-diaminopimelate/lysine biosynthetic pathway of Corynebacterium glutamicum |
| 9:50 - 10:15 | J.F. **MARTIN** : Molecular genetics of amino acid-producing corynebacteria : structural and functional analysis of the cloned tryptophan and threonine operons of Brevibacterium lactofermentum |
| 10:15 - 10:40 | K. **DUNICAN** : Genetic studies on Corynebacterium glutamicum |

BREAK 10:40 - 11:00

CHAIRMAN : J.F. MARTIN

| | |
|---|---|
| 11:00 - 11:25 | M. **YOUNG** : Efficient transfer of plasmids and transposons into Clostridium acetobutylicum |
| 11:25 - 11:50 | W.L. **STAUDENBAUER** : Development of thermophilic clostridial vector plasmids |
| 11:50 - 12:15 | N.P. **MINTON** : Vector development in Clostridium acetobutylicum |
| 12:15 - 12:40 | J.P. **AUBERT** : Organization and expression of Clostridium thermocellum cellulose degradation (cel) genes |

LUNCH

Saturday, February 27th, 1988

15:00

## ROUND TABLE - TRENDS AND PERSPECTIVES

**CHAIRMAN :**

S.D. EHRLICH

**PARTICIPANTS :**

A. AGUILAR, J.P. AUBERT, G.A. van den BERG, B. DIDERICHSEN, G. GRANDI, H. HESLOT, B. JARRY, A. KLIER, J.F. MARTIN, J.C. PATTE, A. SABATIER, G. VENEMA, M. YOUNG

# ROUND TABLE

The final session of the meeting was a round table, centered on two main aspects : (1) the quality of research in the field of Gram positive organisms in Europe, (2) the involvement of inductry in that research, within the frame work of BRIDGE.

The session was chaired by S.D. Ehrlich (INRA, France), the participants were :

A. AGUILAR (CEE, Bruxelles)
J.P. AUBERT (Institut Pasteur, France)
G.A. van den BERG (Gist-Brocades N.V., The Netherlands)
B. DIDERICHSEN (NOVO Industry, Danemark)
J. DUARTE (LNETI, Portugal)
G. GRANDI (ENI Ricerche, Italy)
H. HESLOT (INA-PG, France)
B. JARRY (Eurolysine, France)
J.F. MARTIN (Leon University, Spain)
D. McCONNELL (Trinity College, Ireland)
G. RAPOPORT (Institut Pasteur, France)
A. SABATIER (Rhone Poulenc, France)
G. VENEMA (Groningen University, The Netherlands)
M. YOUNG (University College of Wales, United Kingdom)

(1) The general feeling, expressed explicitely by several of the participants (van den Berg, Diderichsen, McConnell, Sabatier), was that the research presented at the meeting, which reflects the research in Europe, was excellent. Studies on DNA metabolism, including stability of genetic information, were given as an example where European research has a leading position world-wise (Sabatier, Venema), while in the field of protein secretion the

research was perceived to be as good as in the United states (McConnell, Venema). Support for the two domains was called for (van den Berg, Martin, Sabatier). Further suggestions included support for research in physiology of growth at high temperatures (Sabatier), development of genetic methods in less well known bacteria (Martin) and studies on sugar transport and Krebs cycle, leading towards controlling energy flow in bacteria (Jarry).

(2) Several of the participants with industrial affiliations (Diderichson, Jarry, Sabatier) stressed the importance of good basic research for the industry in Europe. The potential for involvement of industry appears, however, limited by the fact that industry can not invest major amounts of funds in "pre-competitive" research (van den Berg). Difficulties related to confidentiallity are also clearly preceived by both industrial and public sectors scientists (Diderichsen, Grandi, Heslot, Martin, Venema), particularly in the context of industrial micro-organisms, where it is not easy to drow a line between the pre-competitive and the competitive research (Jarry). Experience gained from currently run national programs of cooperation between public and industrial sectors, such as CHVP in France, which covers several microorganisms of industrial importance (Bacilli, Streptpmuces, yeast), was quoted as potentially useful for solving some of the above difficulties (Rapoport). Several participants presented their own experience with cooperative programs of that kind (Grandi, Helsot, Venema). Negotiations between the industrial and public contractants to define, on a case to case basis, the results to be kept confidential and those to be published seems to be common to many of these. A question which may be raised is whether it would be possible to consider a provision of that nature in the BRIDGE contracts involving industry.

Increasing the role of industry in conception of programs and choice of projects (Diderichsen) and creating mixed teams, composed of university and industry scientists (Sabatier) were also seen as potentially furthering cooperation between the industrial and the public research. A suggestion, interesting in a more global context, to create a European research center in microbiology, which would treat problems of industrial importance and be capable of providing graduate training, was also made (Diderichsen).

| | | |
|---|---|---|
| A. AGUILAR | Commission of the European Communities, DG XII, F-2, SDME 3/64, 200 rue de la Loi, B-1049 Bruxelles, Belgique | |
| J.P. AUBERT | Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris cedex 07, France | 23 |
| G.A. van den BERG | Gist-Brocades N.V., P.O. Box 1, 2600 Ma Delft, The Netherland | |
| R. COCKER | BIOCON Ltd, Kilnagleary, Carrigaline, Co Cork, Ireland | |
| T. DAUVRIN | Laboratoire d'Enzymologie, UCL, 1 place de la Croix du Sud, 1348 Louvin la Neuve, Belgique | |
| P. DHAESE | Lab. Genetics, State University of Ghent, B-9000 Ghent, Belgique | 5 |
| B. DIDERICHSEN | NOVO Research Institute, Novo Allé, DK-2880 Bagsvaerd, Denmark | 13 |
| J. DUARTE | Biotechnology, LNETI, Queluz-de-Baixo, P-2745 Queluz, Portugal | |
| L.K. DUNICAN | Department of Microbiology, University College, Galway, Ireland | 19 |
| S.D. EHRLICH | Institut Jacques Monod, T43, 2 place Jussieu, 75251 Paris cedex 05, France | 3 |
| J. ERRINGTON | Microbiology Unit, Dept. Biochemistry, University of Oxford, United Kingdom | 4 |
| S. GALIZZI | Dept Genetica e Microbiologia "A. Buzzati-Traverso", Università di Pavia, I-27100 Pavia, ITALIE | 10 |
| G. GRANDI | ENI Ricerche, Via San Salvo 1, San Donato Milanese, 20097 Milano, ITALIE | 12 |

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|---|---|---|
| H. HESLOT | Laboratoire de Génétique, I.N.R.A., Centre des Biotechnologies Agro-industrielle, 78850 Thiverval Grignon, France | 9 |
| B. JARRY | Laboratoire de Recherche Eurolysine, Centre Scientifique d'Orsay, Bâtiment 403, 91405 Orsay cedex, France | |
| A. KLIER | Institut Pasteur, Service de Biochimie Microbienne, 28 rue du Docteur Roux, 75725 Paris cedex 15, France | 14 |
| J. KREFT | Institut für Genetik und Mikrobiologie, der Universität Würzburg, Lehrstuhl für Mikrobiologie, Röntgenring 11, 87 Würzburh, den, R.F.A. | |
| S. LE GRICE | Central Research Units, F. Hoffmann-La Roche & Co, CH-4002 Basel, Switzerland | 6 |
| H. de LENCASTRE | Laboratorio de Genética Molecular, Instituto Gulbenkian de Ciência, Oeiras, Portugal | 7 |
| J. MAHILLON | Plant Genetic Systems N.V., J. Plateaustraat 22, B-9000 Ghent, Belgique | 15 |
| J.F. MARTIN | Departamento de Microbiologia, Facultad de Biologia, Universidad de Léon, Léon, Espagne | 18 |
| D. MCCONNELL | Dept. Genetics, Trinity College, Lincoln Place Gate, Dublin 2, Ireland | 1 |
| N.P. MINTON | Microbial Technology Laboratory, CAMR PHLS, Porton Down, Salisburry SP4 OJG Wiltshire, United Kingdom | 22 |
| J.C. PATTE | Laboratoire de Chimie Bactérienne, C.N.R.S., 31 chemin Joseph Aiguier, 13402 Marseille cedex 9, France | 16 |

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|---|---|---|
| G. RAPOPORT | Institut Pasteur, Service de Biochimie Microbienne, 28 rue du Docteur Roux, 75724 Paris cedex 15, France | 8 |
| A.M. SABATIER | Rhone Poulenc Sante, Centre de Recherche de Vitry sur Seine, 94407 Vitry sur Seine, France | |
| W. SCHUMANN | Lehrstuhl für Genetik, Universität Bayreuth, D-8580 Bayreuth, R.F.A. | 11 |
| W.L. STAUDENBAUER | Technische Universität München, Lehrstuhl für Mikrobiolgie, Arcisstrasse 21, 8000 München, R.F.A. | 21 |
| G. VENEMA | Dept. Genetics, Center of Biological Sciences,. Kerklaan 30, 9751 NN Haren (Gn), The Netherlands | 2 |
| P. YEH | Genetica, 160 quai de Polangis, 94340 Joinville-le-Pont, France | 17 |
| M. YOUNG | The University College of Wales, Dept. Botany and Microbiology, School of Biological Sciences, Aberystwyth SY23 3DA, United Kingdom (present adress: Institut Jacques Monod T43, 2 place Jussieu, 75251 Paris cedex 05, France) | 20 |

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BAP60270-UK

## In the field of

ENVIRONMENTAL CONTROL OF METABOLIC FLUXES AS A BASIS FOR BIOTECHNOLOGICAL PROCESSES

Programme for the Working Seminar of ELWWs "Environmental Control of Metabolic Fluxes as a Basis for Biotechnological Processes"

July 6, 7 and 8, 1988; Biological Centre, Haren, The Netherlands

**Wednesday evening, July 6:** Arrival at Amsterdam Airport. Departure times of direct trains from the Airport to **Assen:** 16.05 and 17.05 h. Arrival of trains at the Railway station **Assen:** 18.35 and 19.35 h. We will meet you at the station. Accommodation has been arranged at Hotel Wapen van Leiden, Oude Rijksweg 1, VRIES (Tel. 05921 - 41316)

**July 7 and 8:** Scientific sessions in the Biological Centre Haren

**Thursday, July 7:** Chairman Professor W. Harder

9.30 h: Introduction

9.45 h: Physiology and biochemistry of methanol utilization by thermotolerant methylotrophic Bacillus sp.
N. Arfman, University of Groningen

10.30 h: Coffee

11.00 h: Environmental control of metabolic fluxes in thermotolerant methylotrophic Bacillus sp.
A.G. Brooke, E.M. Watling, M.M. Attwood, University of Sheffield

11.45 h: Physiology of a thermotolerant methylotroph NCIB 12522 in chemostat systems
N. Al-Awadhi, EAWAG

12.30 h: Lunch

13.30 h: Further studies on the genetics of thermotolerant methylotrophic Bacillus sp.
G.E. de Vries, University of Groningen

14.15 h: Possible applications of metabolic control analysis in methylotrophic organisms
J.R. Small, University of Groningen

15.00 h: Tea

15.30 h: Effect of heat shock on the growth of cultures of Klebsiella pneumoniae and a thermotolerant methylotroph NCIB 12522
A. Heitzer, EAWAG

16.15 h: General Discussion

**Friday, July 8:** Chairman Professor D.W. Tempest

9.30 h: Bioenergetic aspects of thermophilic bacteria
W. de Vrij, University of Groningen

10.15 h: Further studies on the growth of *Bacillus stearothermophilus*
R. Burke, University of Sheffield

11.00 h: Coffee

11.30 h: Is potassium essential for the growth of bacteria under all conditions?
D.W. Tempest*, J. Pennock*, O.M. Neijssel#, M.J. Teixeira de Mattos# and E.T. Buurman#, *University of Sheffield; #University of Amsterdam

12.15 h: Lunch

13.30 h: What does a batch growth curve look like?
Th. Egli, EAWAG

14.15 h: General Discussion

15.00 h: Tea

15.30 h: Future Research and Funding

**Saturday, July 9:** Programme of social activities

**Sunday, July 10:** Departure

WORKING SEMINAR UNDER THE BAP PROGRAMME

"ENVIRONMENTAL CONTROL OF METABOLIC FLUXES AS A BASIS FOR BIOTECHNOLOGICAL PROCESSES"

Biological Centre, University of Groningen, Haren, July 6-9 1988.
Local Organizers: Dr. L. Dijkhuizen/ Prof W. Harder

A meeting was arranged between the two participating laboratories in the BAP programme (Prof. W. Harder, Department of Microbiology, University of Groningen, The Netherlands; Prof. D.W. Tempest, Department of Microbiology, University of Sheffield, UK). In addition, the groups of Prof. O.M. Neijssel (Department of Microbiology, University of Amsterdam, The Netherlands) and Prof. G. Hamer (EAWAG, ETH Zürich, Switzerland), were invited. Sixteen members of these groups participated in the meeting, ten of which presented a research seminar, relating to the physiology, biochemistry or genetics of the thermotolerant/ thermophilic bacilli under investigation.

Bacilli are currently used in a variety of industrial processes based on carbohydrates as feedstocks. In order to be able to fully exploit their biotechnological potential, a detailed knowledge of the mechanisms involved in controlling metabolic fluxes in these organisms, particularly those leading to useful products, is required. It is our intention to examine in a few bacterial species the regulation of carbon substrate metabolism, its bioenergetic consequences and the carbon flow into metabolites with an industrial potential. The organisms selected for these studies are the thermophile Bacillus stearothermophilus and recently isolated thermotolerant, methanol-utilizing, Bacillus species.

In the first year that the programme has been running, a detailed quantitative study of energy transduction and carbon assimilation in Bacillus stearothermophilus and methanol-utilizing Bacillus species has been undertaken. For this purpose the organisms were grown in continuous cultures under a variety of nutrient-limiting conditions. This approach also allowed an assessment of the potential of these organisms to overproduce compounds of possible industrial importance. With cultures of the methylotrophic bacilli grown under, for instance,

potassium-limiting and methanol-excess conditions, a substantial part of the carbon supplied in the feed was indeed found to accumulate in extracellular products.

Several presentations dealt with an evaluation of the general properties of the Bacillus species and the enzymology of growth on methanol, glucose or glycerol. In the methylotrophic bacilli an NAD-dependent alcohol dehydrogenase appears to be involved in the initial oxidation of methanol to formaldehyde. In Bacillus stearothermophilus, glycerol metabolism was found to proceed via the so-called phosphorylative pathway, involving a glycerol kinase. Both these enzymes were synthesized upto extremely high levels. The characteristic properties of these enzymes, and the possible application of the underlying powerful expression systems for heterologous gene expression, were discussed.

During the meeting sufficient time was set aside to discuss future joint experiments and publications. In addition, a considerable exchange took place of techniques used by the various groups.

The overall feeling was that the work programme was progressing well and that the meeting had been most successful.
It was agreed to plan the next (biannual) meeting of the participating groups in March 1989.

List of Participants of the Working Seminar under the BAP Programme "Environmental Control of Metabolic Fluxes as a Basis for Biotechnological Processes", Haren July 6 - 9, 1988.

| | |
|---|---|
| Professor W. Harder | University of Groningen, The Netherlands |
| Dr. L. Dijkhuizen | |
| Dr. G.E. de Vries | |
| Dr. J.R. Small | |
| Mr. N. Arfman | |
| Dr. W. de Vrij | |
| Professor D.W. Tempest | University of Sheffield, United Kingdom |
| Dr. M.M. Attwood | |
| Dr. A.G. Brooke | |
| Dr. R. Burke | |
| Miss L. Watling | |
| Professor O. Neijssel | University of Amsterdam, The Netherlands |
| Dr. M.J. Teixeira de Mattos | |
| Dr. T. Egli | EAWAG, ETH Zürich, Switzerland |
| Mr. N. Al-Awadhi | |
| Mr. A. Heitzer | |
| Mr. R. Schneider | |

EUROPEAN LABORATORY WITHOUT WALLS

BAP-0267-NL
BAP-0270-UK
BAP-0290-D
BAP-0291-UK
BAP-0249-I

## In the field of

EXTREMOPHILES

HORIZONTAL BAP MEETING ON EXTREMOPHILES

LONDON, December 2, 1987

In connection with a symposium entitled "Extremophiles: Exploration and Exploitation" organized by the EFB Working Party on Microbial Physiology (and sponsored by the Society of Chemical Industry in collaboration with the Society for General Microbiology) in London on December 3 and 4, 1987, a so-called horizontal meeting of BAP contractors and experts working on extremophiles was held at the premises of the Society of Chemical Industry (London) on December 2, 1987. Present at the meeting were Prof. M. De Rosa, Napoli, Italy, Dr. J.C. Duarte (Lisboa, Portugal), Dr. L. Dijkhuizen (Haren, The Netherlands), Prof. C. Grandi (Padova, Italy), Prof. W. Harder (Haren, The Netherlands, Chairman), Dr. A. de Leeuw (Delft, The Netherlands), Dr. F. Meussdörfer (Düsseldorf, W. Germany), Prof. C. Ratledge (Hull, United Kingdom), Prof. M. Rossi (Napoli, Italy), Dr. S. Struwe (Copenhagen, Denmark) and Prof. K.O. Stetter (Regensburg, W. Germany). Unfortunately Prof. Tempest (Sheffield, United Kingdom) was unable to attend, but a short presentation of his project was given by Prof. W. Harder.

The meeting was opened at 2.00 p.m. After a word of welcome from the chair, each participant introduced himself, stated his particular interest in extremophiles and explained how his research projects were related and integrated into the BAP programme. Subsequently 10-15 minutes oral presentations of the various research projects were given by Professors Rossi, Grandi and De Rosa on properties of enzymes and membranes of thermophiles, by Dr. Dijkhuizen on the physiology of methylotrophic thermotolerant bacilli and by Dr. Meussdörfer on fatty acid modification by microbial enzymes. Whereas the first three projects have already been running for 3 years, which was evident from the considerable amount of information that was presented, the latter 2 projects and that of Prof. Tempest had just been started. There was, however, no shortage of ideas and it is expected that rapid progress will also be made in these programmes. Each of the presentations was followed by discussions. See for a summary of the presentations the enclosed abstracts.

From these discussions it became clear that all participants were enthusiastic about the initiative taken by the Commission to bring the present group together. Ample scope for future collaboration was apparent in several areas of the existing programme and it was decided to submit to the commission a proposal to form a club of scientists working on extremophiles within the EEC countries. The present BAP contractors could function as a nucleus for setting up this club and are prepared to initiate an attempt to organize future collaboration in the context of "European Laboratories without Walls" as part of the BAP programme.

It was decided to focus our attention on a limited number of aspects of Extremophiles which were considered as being of immediate importance. These are: a) physiology and metabolic regulation, b) membranes and proteins c) whole cell biocatalysis and d) genetics. A suggestion was made to meet again prior to the forthcoming FEMS symposium on "The Microbiology of extreme environments" to be held September 18/23, 1988 in Lisboa, Portugal. This next meeting should be planned well ahead of time and should preferably last one day.

Towards the end of the meeting Dr. S. Struwe presented various research projects on extremophiles that are currently underway in Denmark. Interaction of several projects with similar programmes in other EEC countries is certainly possible and would be welcomed by the participants of this meeting.

Haren, December 8, 1987

Prof. W. Harder
Chairman of the Meeting

EUROPEAN LABORATORY WITHOUT WALLS

BAP-267-NL
BAP-270-UK

## In the field of

PHYSIOLOGY OF THERMOPHILIC BACTERIA

EEC Meeting Sheffield November 30th - December 1st 1987

Held at University of Sheffield, Department of Microbiology, Room F35.

Programme

| | |
|---|---|
| November 30th 1987 | Chairman Professor D.W. Tempest (Sheffield) |
| 10.00 - 10.15 | Introduction |
| 10.15 - 10.45 | Miss J. Pennock (Sheffield) - The regulation of carbon metabolism in thermophilic Bacilli. |
| 10.45 - 11.15 | Dr M.J. Teixeira de Mattos (Amsterdam) - Regulation of carbon substrate flux in anaerobic chemostat culture. |
| 11.15 - 11.30 | Coffee |
| 11.30 - 12.00 | Dr A.G. Brooke (Sheffield) - A study of metabolic fluxes in thermotolerant methylotrophic Bacilli. |
| 12.00 - 12.30 | Miss E.M. Watling (Sheffield) - The physiology of thermotolerant methylotrophic Bacilli. |
| 12.45 - 14.00 | Lunch |
| 14.15 - 14.45 | Dr L. Dijkhuizen (Haren) - Applied aspects of growth on methanol. |
| 14.45 - 15.15 | Mr N. Arfman (Haren) - The purification and characterization of a novel methanol dehydrogenase. |
| 15.15 - 15.45 | Dr G. De Vries (Haren) - The genetics of thermotolerant methylotrophic Bacilli. |
| 15.45 - 14.00 | Tea |
| 16.00 - 16.30 | Mr N. Al-Awadhi (Zurich) - Growth of thermotolerant and thermophilic solvent utilizing bacteria. |
| 16.30 - 17.30 | Overall Discussion |
| December 1st 1987 | Chairman Professor W. Harder (Haren) |
| 10.15 - 11.15 | Future Research: further coordination of research topics. |
| 11.15 - 11.30 | Coffee |
| 11.30 - 12.30 | Future funding possibilities |
| 12.45 - 14.00 | Lunch |
| 14.00 - | Free to discuss closer collaboration and writing of a joint paper.<br>Free afternoon |

Report of the meeting held in Sheffield University, Department of Microbiology, November 30th and December 1st 1987.

Local organisers: Professor D.W. Tempest/Dr M.M. Attwood,
EEC contact number BAP-0270-UK (HI).

A meeting was arranged between the two participating laboratories in the above BAP programme; that is, the Department of Microbiology, University of Sheffield, U.K. and University of Groningen, The Netherlands. Pertinent work groups from ETH-Zurich, Sutherland and The Department of Microbiology, University of Amsterdam were invited. The list of participants and the agenda for the meeting are enclosed.

All the lectures were well presented and the discussions were lively and well directed. The overall feeling was that the meeting had been most successful and the work programme was progressing very satisfactorily. Further work schedules were planned and the next research meeting will be on July 7th-8th 1988 at the University of Groningen, Biological Centre, Haren, The Netherlands.

List of Participants EEC Meeting Sheffield November 30th - December 1st 1987

| | | |
|---|---|---|
| Professor W. Harder | University of Groningen | The Netherlands |
| Dr L. Dijkhuizen | University of Groningen | |
| Dr G. De Vries | University of Groningen | |
| Mr N. Arfman | University of Groningen | |
| | | |
| Professor O.M. Neijssel | University of Amsterdam | |
| Dr M.J. Teixeira de Mattos | University of Amsterdam | |
| | | |
| Professor D.W. Tempest | University of Sheffield | U.K. |
| Dr M.M. Attwood | University of Sheffield | |
| Dr A.G. Brooke | University of Sheffield | |
| Dr R. Burke | University of Sheffield | |
| Dr A. Moir | University of Sheffield | |
| Miss J. Pennock | University of Sheffield | |
| Miss L. Watling | University of Sheffield | |
| Mr P. Mallinder | University of Sheffield | |
| | | |
| Dr T. Egli | ETH Zurich | Switzerland |
| Mr N. Al-Awadhi | ETH Zurich | |

# BIOTECHNOLOGY OF PLANTS

# AND

# ASSOCIATED MICROORGANISMS

EUROPEAN LABORATORY WITHOUT WALLS

# In the field of

MITOCHONDRIAL MOLECULAR GENETICS

IN RELATION TO

CROP IMPROVEMENT

BAP-0019-B
BAP-0016-F
BAP-0017-UK
BAP-0020-NL
BAP-0022-F
BAP-0075-D
BAP-0102-UK

## Working Seminar of the ELWW in the field of: Mitochondrial Genetics in Relation to Crop Improvement

The discussions in this workshop held at the John Innes Institute, Norwich, UK on September 18, 1987 were centered on three topics; structure and function of the mitochondrial genome; mitochondrial transformation; organelle transfer. In the first of these discussion sessions Dr. C. Thomas of the John Innes described his work on the mitochondrial plasmids of sugarbeet and in particular the information acquired on their sequences and transcripts. Certain common sequences among the plasmids were observed which might indicate a role as replication origins. Two transcripts have been detected from one of the plasmids, one transcript from another and several from a third. Putative promoter sequences are highly conserved on all three plasmids. It is hoped that the determination of sequences which promote replication and transcription will be useful in the development of vectors for transforming mitochondria. Of particular interest in the report of the work of the Orsay group provided by Professor Quetier was that of an analysis of the changes induced in the mitochondrial genome of two varieties of wheat during culture of callus derived from immature embryos. The changes observed in non embryogenic callus differed consistently from those observed in embryogenic callus and the implications of these observations are indeed far reaching. From this same laboratory Dr. Lejeune described his observations on a region of chloroplast DNA found in wheat mitochondrial DNA; this has been highly conserved during the evolution of wheat, being present in the progenitors of this crop. The possible mechanisms of mitochondrial recombination are being investigated and initial experiments aimed at studying this process were described. Recent work in the laboratory of Professor Briquet at Louvain-le-Neuve has included studies of a variant polypeptide unique to cytoplasmic male sterile strains of Vicia faba. In this same species mitochondrial plasmids have been sequenced and transcripts detected from them. Origin of replication like sequences are also observed in two of these plasmids. The origin of cytoplasmic male sterility in sugarbeet has always been a paradox; evidence from the Dijon laboratory described by Dr. Berville suggested that the Owen form of cms may have arisen from a hybrid of garden beet and sugarbeet. This hypothesis is based on the observations of the chloroplast DNA of the two forms; garden beet has a similar chloroplast DNA to cms forms of sugarbeet whereas the fertile forms of sugarbeet have a different chloroplast DNA. Of particular interest was the observation from this same laboratory that in maize the information coding for susceptibility to the T-toxin of Helminthosporium maydis can be separated from the information coding for cytoplasmic male sterility.

The second section of the meeting dealt with mitochondrial transformation and clearly this work is at a very early stage. Dr. de Haas from the laboratory of the Free University in Amsterdam described a strategy for isolating replication origins from chloroplast and mitochondria. Their ability to act as autonomously replicating

sequences (ARS) in yeast is a part of the strategy for their recognition. Chloroplast sequences isolated from Nicotiana tabacum, Petunia hybrida and Euglena gracilis show considerable homology to ARS sequences. Candidate ARS sequences from mitochondria have also been identified and a putative origin of replication from P. hybrida mitochondria shows many similarities to mitochondrial replication origins from Saccharomyces cerevisiae. In addition they exhibit similar secondary structures to yeast replication origins and highly conserved sequences on sugarbeet minicircle DNAs. The problems of transforming mitochondria were also discussed by Mr. Moore from Edinburgh University. Vector constructs incorporating selectable markers and putative promoters were described with the particular problem being highlighted of the difficulty of selecting markers which will be expressed in mitochondria and not in the nucleus or chloroplast. To aid integration, homologous sequences from mitochondria were being incorporated into the construct. Dr. Vassarotti from Louvain-la-Neuve described an essentially similar approach being undertaken in their laboratory to produce suitable constructs for mitochondrial transformation; problems of copy number, transfer, delivery and of selection procedures were highlighted by all the participants in this section.

In the final section on organelle transfer Dr. Lawrence of the John Innes Institute described her work to develop a system for mitochondrial transfer in sugarbeet. Suitable procedures have been devised to allow microinjection of mitochondria and the indications are that a successful regeneration of callus from microinjected protoplasts can now be obtained. The implications of the successful application of this technology are very significant in terms of the genetic analysis of mitochondria and also in terms of crop improvement of those species in which cytoplasmic male sterility is an important component of the production of new varieties. Mrs. Jensen of the Free University, Amsterdam is attempting to transfer organelles by protoplast fusion. Suitable donor or recipient strains with appropriate markers are being constructed and methods of inactivating the nuclei prior to fusion are being developed.

The success of a meeting of this kind ultimately depends on the readiness with which participants exchange information; in these terms the workshop was outstandingly successful and at the end a joint decision was taken to circulate preprints amongst participants, to encourage exchange of students and research workers between laboratories, to identify the specialised techniques which each laboratory has and which might be of value to the others, to exchange clones wherever possible and to undertake joint experiments in all instances where benefits could accrue from this. The network of existing collaborations among the participants is already very substantial indeed and is indicative of the value of the concept of the European laboratory at large.

Professor D. R. Davies.

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BAP-0013-D
BAP-0015-F
BAP-0018-I
BAP-0076-NL
BAP-0085-F
BAP-0099-UK

# In the field of

HAIRY ROOT

a n d

PLANT CELL DIFFERENTIATION

ELWW meeting on "Hairy root and plant cell differentiation".

Università "La Sapienza", Dip. Genetica e Biologia Molecolare, Rome, Sept. 15-16/1987.

Tue, Sept. 15

9.00-11.00 Session I. State of the art of hairy root research.

Updating on:
-Ri plasmids: types, maps, functions, homologies with Ti.....
-Opines

11.00-13.00 Session I, cont.d

-T-DNA: structure, homologies with Ti, various Ri DNAs, CT-DNA, genetic loci, ORFs, ORF cloning, transcription.....
-Hairy root tissues and plants: in vitro cultures, regeneration, hairy root phenotypes of cultures and plants, transgenic plants.....

13.00-15.00 Lunch

15.00-17.00 Session I, cont.d
Role of hormones in hairy root induction.

17.00-17.20 Coffee break

17.20-19.00 Session II. Current research trends on hairy root.

Short presentations of ongoing hairy root research in the various laboratories.

Wed, Sept. 16

9.00-11.00 Session III.

Discussion on research perspectives based on hairy roots.

11.00-11.20 Coffee break

11.20-13.00 Definition of collaborative projects between participating laboratories. Establishment of the ELWW.

-ELWW meeting on "Hairy root and plant cell differentiation".

-Meeting report.

On September 15-16/1987 an ELWW working seminar on "Hairy root and plant cell differentiation" was held in Roma, Dipartimento di Genetica e Biologia Molecolare, Università "La Sapienza", with the participation of several european laboratories currently involved in hairy root research (see list of participants).
Scope of the meeting was to discuss state of the art research on hairy roots, define collaborative projects between the participating laboratories and establish an ELWW.
After a thorough updating on what achieved so far on the various aspects of this biological system and a totally outspoken description of the projects and approaches currently underway in the different laboratories (see the scientific programme of the meeting), a very fruitful discussion has taken place on the involvement of plant hormones in the hairy root syndrome. From the data presented by various groups and the above mentioned discussion it stood clear to all participants -including molecular biologists, microbiologists, biochemists, plant physiologists, cell biologists and morphologists- that the hairy root system bears enormous potentialities for the study of the molecular events underlying plant root differentiation. In particular, the role of just a very limited number of Ri plasmid T-DNA genes in conferring to plant cells the competence to respond to the plant hormone auxin was pinpointed. These genes, capable of controlling root differentiation and other plant morphological traits, have been already cloned in expression vectors and transgenic plants obtained, providing unique biological material to approach complex problems of plant developmental biology.
All participants agreed that the greatest interest of hairy root research lays on the unique tools it provides to get into the molecular physiology of root development. It has therefore been unanimously decided that the area of common interest to be covered by the ELWW should be defined in broader terms as "The genetic control of root differentiation"; within this cultural framework, approaches, projects and transnational collaborations not necessarily limited to hairy root work should be included in the envisaged ELWW. On the other hand, hairy root work not oriented towards or relatable to the understanding of basic problems of plant developmental biology should be regarded as outside the scope of this ELWW.

-Proposal for the establishment of the ELWW on "The genetic control of root development".

The participants to the meeting on "Hairy root and plant cell differentiation", held in Rome, Sept. 15-16/1987,

unanimously welcome the opportunity to establish an ELWW according to the following criteria:

-The area covered by and the denomination of the ELWW should be "The genetic control of root development".

-Two levels of participation to the ELWW are envisaged:

a) ELWW membership.

The member laboratories are not necessarily limited to those represented at the meeting in Rome (see list). Members can be accepted provided their scientific level and interests meet the scope of the ELWW as judjed by an appropriate advisory board.

Members of the ELWW agree on:

i) Meeting at least once a year to exchange results and perspectives.

ii) Exchanging papers at the latest when accepted for publication on the basis of an established mailing list extended to all ELWW members.

iii) Exchanging clones, strains and other relevant material at the latest when the work describing it is accepted for publication.

iv) Exchanging personnel for training on new techniques according to the possibilities of the recipient laboratory.

b) ELWW joint projects.

Beyond the above described obligations of the ELWW members, the recognized purpose of the ELWW is to foster transnational collaborations between member laboratories. These latter should, however, be free of collaborating individually with each other. Joint projects should therefore be established, within the framework of the ELWW, between any number of member laboratories. Collaborative projects within the ELWW should be approved by an appropriate advisory board.

ELWW meeting on 'Hairy root and plant cell differentiation'
Rome Sept. 15/16/1987.

Foreign participants:

- J. Schell, A. Spena
  Max-Planck-Institut für Züchtungsforschung, Köln, FRG
- F. Casse-Delbart, L. Jouanin, F. Vilaine
  Lab. Biologie Cellulaire, INRA, Versailles, France
- J. Tempé, A. Petit, J. Brevet
  Université de Paris-Sud, Orsay, France
- D. Chriqui
  Lab. Cytologie Végétale, Univ. P.et M. Curie, Paris 6, France
- J. Guern, C. Morel, H. Barbier
  Lab. Physiologie Végétale, CNRS, Gif-sur-Yvette, France
- J.H. Hoge
  Dept. Plant Mol. Biol., University of Leiden, The Netherlands
- G. Ooms
  Rothamsted Exp. Station, Harpenden, UK

Convener:

- P. Costantino
  Dip. Genetica e Biol.Mol., Università di Roma "La Sapienza", Italy

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# In the field of

CROP IMPROVEMENT THROUGH

CELL BIOTECHNOLOGY


BAP-013-D H. LÖRZ
BAP-014-F Y. DATTEE
BAP-082-NL M.J. TEMPELAAR
BAP-083-NL L.VON VLOTEN-DOTING
BAP-084-I F. SALA
BAP-085-F M. CABOCHE
BAP-101-DK M.G.K. JONES
BAP-111-B M. JACOBS


program BAP meeting Haren, the Netherlands, November '87:
Presentations of contractants labs in EC sponsored ' Biotechnology Action Program ' no 165 : Genetic Manipulation and Regeneration of model and crop plants in vitro.

Friday 6.11.'87
(8.45 transport hotel-Biological Centre)

MORNING: Biological Centre, green room.

9.00-9.15 MJ Tempelaar (Haren, NL)
Opening remarks

9.15-9.45 B Junker/ E Kranz (Cologne, FRG)
Strategies in cereal transformation

9.45-10.15 M Jacobs/ I Negrutiu (Brussels, B)
Gene tranfer experiments

10.15 Coffee break, free discussion

10.45-11.15 MGK Jones (Harpenden, UK)
Production and characterization of somatic hybrid plants of potato.

11.15-11.45 P Guerche/ Y Chupeau or C Bellini (Versailles, F)
Direct gene transfer in Brassica and other crops

11.45-12.15 Y Henri/ Y Dattee/ D Negri (Orsay, F)
Relationship between mitochondrial genome organisation and regeneration ability in long term wheat somatic tissue culture

12.15-12.45 M Sala

Lunch

AFTERNOON, Biological Centre, orange room

Lectures on projects in the Cell and Plant Genetics group of the Genetics Department of the Biological Faculty of the University of Groningen.

13.30-14.00 WJ Feenstra (Head of Genetics Dept and CP group): Overview

14.00-14.30 LP Pijnacker: Chromosome research in potato

14.30-15.00 E Jacobsen: Different classes of pea mutants with an altered symbiotic interaction.

15.00 tea break, subsequently opportunity for informal discussions, visit of the lab and contact with members from various research groups

18.15 Joint dinner, Informal discussions etc

Saturday 7-11-'87

MORNING, conference room at the hotel

9.00-9.30 HA Verhoeven/ HCPM van der Valk (Wageningen, NL)

Flow cytometric analysis and sorting of metaphase chromosomes and micronuclei from N. plumbaginifolia.

9.30-10.00 H Jones (Harpenden, UK)

Direct gene transfer into potato protoplasts by electroporation

10.00-10.30 G vd Steege (Haren, NL)

characteristics of protoplasts in electric fields : uptake of molecules and fusion

10.30 Concluding discussion;

coffee & meal

departure

REPORT OF BAP MEETING OF PROJECT #165, NOVEMBER 6/7 IN HAREN, NL

The second 1987 meeting of EC-sponsored BAP project #165 has been held in Haren, NL on November 6 and 7.

Now comprising 8 groups, this ELWW meeting had each lab contributing with presentations on their present projects. Progress had been made on various fronts, such as crop plants obtained from electrofusion (potato) and electroporation (rape), more insight in electrical conditions and membrane events in electric field manipulations for fusion and DNA uptake, large-scale preparation of purified plant chromosomes for transformation and analysis. In some instances, the first results from joint experiments could be presented.

In addition, one afternoon was devoted to lectures dealing with topics worked on in the host lab (chromosomes and mutants of pea and potato) and to visits and exchanges in various parts of the lab.

In the informal parts of the meeting, scientific discussions were held, joint experiments were evaluated and future collaborative work was planned.

Finally, decisions were taken on the following points:

- Location and date of the next ELWW meeting:

The next meeting will be held on June 2 and 3 in a suitable location in Italy, to be selected by local organizer Dr. F. Sala. Extra funds from the EC might be made available for this occasion (see previous reports), and Dr. Sala will look into this possibility.

- Participation of industrial representatives:

The main topic of discussion has been on how to communicate with industrial representatives. It was decided, that a special once-a-year meeting of the group leaders with representatives from industry (with present or future interests) would be the best way of interaction. This is thought to be preferable to changing the format of the present informal and small ELWW meetings. In addition, the proposed conferences could have a wider scope than the project-bound scientific meetings.

These procedures could start form the 2nd meeting in 1988 onwards and participants could then invite their sponsors to take part.

LIST OF PARTICIPATING SCIENTISTS BAP MEETING NOVEMBER 1987, HAREN, NL

B. Junker and E. Kranz,
Max-Planck-Institut für Züchtungsforschung,
Abt.Genetische Grundlagen der Pflanzenzüchtung,
Egelspfad,
D-5000 KÖLN 30,
W-Germany.

M. Jacobs, I. Negrutiu and T. Wichai,
Laboratorium voor Plantengenetica,
Vrije Universiteit Brussel,
Paardenstraat 65,
B-1640 SINT-GENESIUS-RODE,
Belgium.

M.G.K. Jones and H. Jones,
Biochemistry Dept.,
Rothamsted Experimental Station,
Harpenden, Herts,
England, AL5 2JQ.

L. van Vloten-Doting, H.A. Verhoeven and H.C.P.M. van der Valk,
ITAL,
Postbus 48,
6700 AA WAGENINGEN,
The Netherlands.

F. Sala,
University of Pavia,
Dept. of Genetics and Microbiology,
Via S. Epifanio,
1-27100 PAVIA,
Italy.

P. Guerche and C. Bellini,
INRA,
Route de Saint Cyr,
F-7800 VERSAILLES,
France.

Y. Dattee, Y. Henri and N. Negri,
Université Paris-Sud,
Lab d'Amélioration des Plantes,
Bat. 360,
F-91405 ORSAY CEDEX,
France.

M.J. Tempelaar, G. van der Steege and W.J. Feenstra,
Department of Genetics,
Centre of Biological Sciences,
University of Groningen,
Kerklaan 30,
9751 NN HAREN,
The Netherlands.

CAMOGLI 2-4 JUNE 1988
E.L.W.W. MEETING

PROGRAMME

Friday June 3
Morning

9.00 M. VALDEZ: - "Protoplast isolation from calli of the wild rice Oryza longistaminata"
STOLARTZ: - "Cell and protoplast culture of triticale"
M. VICENTE: - "Implementation of biotechnological techniques in a Mediterranean crop: the olive tree"
10.30 Break
10.45 C. TONELLI: - "Analysis of somaclonal variants in tomato"
H. LORTZ: - "In vitro culture and transformation studies in barley"
M. JONES: - "Further characterization of somatic hybrids of S.tuberosum and S. brevidens (chromosomes, nuclear and chloroplast DNA contributions)"

Afternoon

16.00 H. VERHOEVEN - "Isolation and characterization of micro protoplasts"
H. van der VALK- "Towards chromosome mediated gene transfer. Vital staining and regeneration of immobilized protoplasts"
I. NEGRUTIU: - "Transient expression: observations and speculations"
17.30 Break
17.50 R. CELLA: - "Intracellular localization of dihydrofolate reductase (DHFR-TS) in carrot cells resistant to methotrexate"
M. JACOBS: - "More facts about gamma-fusion"
H. JONES: - "Application of electroporation for stable integration and transient gene expression studies in potato protoplasts"

Saturaday, JUNE 4
Morning

9.00 M.J. TEMPELAAR:- "Progress in genome transfer into potato protoplasts"
H. VAUCHERET: - "Improvement of electroporation by the use of Lambda recombinant DNA"
W.J. FEENSTRA: - "Genetic analysis of starch biosynthesis in potato"
10.30 Break
10.45 E. NIELSEN: - "The valine-resistance of a Nicotiana plumbaginifolia cell line as a tool to study regulation of branched-chain amino acid pathway"
M. TERZI: - "Developmental mutant in somati embryogenesis"
11.45 - Discussion on the future of the C.E.C. programme in the BRIDGE perspectives

REPORT OF THE MEETING OF THE BAP GROUP :

"Genetic manipulation and regeneration in model and crop plants in vitro",

CAMOGLI (Italy), 2-4 June 1988.

Research teams :

**M.G.K. Jones**, Biochemistry Department, Rothamsted Experimental Station, U.K. ;
**M. Tempelaar**, Genetics Department, University of Groningen, The Netherlands ;
**L. van Vloten-Doting**, ITAL, Wageningen, The Netherlands ;
**M. Jacobs**, Molecular Biology Department, Free University of Brussels, Belgium ;
**F. Sala**, Genetics and Microbiology Department, University of Pavia, Italy ;
**M. Caboche**, INRA, Cell Biology Laboratory, Versailles, France;
**H. Lörz**, Max-Planck-Institute, Köln, FRG ;
**Y. Dattée**, University of Orsay, France.

Our research teams meet at six-month intervals to transfer information and technology and to plan joint experiments.

The fifth meeting has been organized by the Italian group (University of Pavia) and held at Camogli (Genova) on June 2-4.

Twenty-seven participants coming from the eight laboratories presented data on the progress of their research. Dr. Miguel Vicente was a guest scientist and took actively part in the scientific discussion, also in view of his application to join, with his "Centro de Investigaciones Biologicas" (Madrid), our BAP research group.

Progress has been made in different fronts. The Köln group presented data on efficient and reproducible methods for induction of somatic embryogenesis in Triticosecale. The Pavia group greatly extended their genetic and molecular analysis of somaclonal variants in tomato, showing their usefullness in plant breeding, demonstrated that the in vitro culture can lead to extensive nuclear DNA amplification in rice and performed a biochemical characterization of valine-resistant mutant plant cell lines. Encouraging data have been obtained at Köln on the culture of barley protoplasts and on their genetic transformation, while at Rothamsted further somatic hybrids between Solanum tuberosum and S. brevidens have been obtained and characterized and a second field experiment is in progress. Asymmetric hybrids have been obtained at Sint Genesius Rode and demonstrations has been given that gamma-irradiation of one of the two partner protoplasts can lead to the transfer of a few genetic components.

Gene transfer is being pursued at ITAL where the development of chromosome transfer by micronuclei has been advanced up to the fusion stage, while (in a collaborative research between Rothamsted and Groningen) detailed work has been carried out on electroporation with potato protoplasts. Electroporation is also being utilized at Versailles where lambda recombinant DNA has come out as a most effective gene vector. Sint Genesius Rode produced data intended to elucidate the patterns of integration and expression of foreign genes following direct gene transfer into protoplasts of two Nicotiana species.

## PARTECIPANTS

Camogli 2 4 June 1988
E.L.W.W. Meeting

| | |
|---|---|
| PAVIA | Francesco SALA |
| | Erik NIELSEN |
| | Giuseppe FORLANI |
| | Stefano CASTIGLIONE |
| | Rino CELLA |
| | Paola MIRANDA |
| | Maria Luisa MARCHESI |
| | Giuseppe GAVAZZI |
| | Chiara TONELLI |
| | Mario TERZI |
| KOLN | STOLARTZ |
| | STOLARTZ |
| | H. LORTZ |
| ORSAY | Marta VALDEZ |
| | D. NEGRI |
| MADRID | Miguel VICENTE |
| HARPENDEN | Michael G.K. JONES |
| | Hedduryn JONES |
| GRONINGEN | G. Van der STEEGE |
| | M.J. TEMPELAAR |
| | W.J. FEENSTRA |
| WAGENINGEN | Henry Van der VALK |
| | Harrie VERHOEVEN |
| | Van VLOTEN-DOTING |
| SAINT GENESIUS RODE | M. JACOBS |
| | Ioan NEGRUTIU |
| VERSAILLES | Catherine BELLINI |
| | Hervé VAUCHERET |

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BAP-079-F P. BOISTARD
BAP-080-IRL M. O'CONNELL
BAP-081-D A. PÜHLER
BAP-100-UK P.R. HIRSCH


## In the field of

# SYMBIOTIC GENES IN RHIZOBIUM : CONSTRUCTION OF IMPROVED STRAINS

Comparison of late symbiotic genes in Rhizobium species and construction of improved strains.

Pierre Boistard, CNRS-INRA, Toulouse, France
Penelope Hirsch, Rothamstead, Harpenden, U.K.
Michael O'Connell, NIHE, Dublin, Ireland
Alfred Puhler, Bielefeld, West Germany

Participants in the above contract met in Dublin on October 15th and 16th 1987. The meeting was attended by P. Boistard, D. Kahn, J. Batut, M. David (Toulouse), P. Hirsch (Rothamstead), A. Puhler, U. Priefer, (Bielefeld), M. O'Connell, M. Hynes, G. Reigh and D. Donnelly (Dublin).

The Toulouse group reported on the genetic analysis of a cluster of nitrogen fixation genes located on the symbiotic megaplasmid of Rhizobium meliloti. The role of fixL fixJ genes as positive regulators of nitrogen fixation was determined using lac fusions and the expression of fixN, a gene belonging to the cluster was found to be regulated by oxygen.

At Rothamsted experiments were carried out with R. leguminosarum biovar viceae to assess the influence of different chromosomal backgrounds and different symbiotic plasmids on the efficiency of the symbiosis. Strains with different chromosomal backgrounds but the same symbiotic plasmid varied in the onset of nitrogenase activity while strains with different plasmids in the same background varied in final yields as measured by plant dry weight. Chromosomal substitutions were made for comparison, using the plasmid R68.45 as a vector.

The Bielefeld group reported the cloning of genes involved in lipopolysaccharide production in R. leguminosarum biovar viceae and genetic mapping of the regions involved. Lipopolysaccharide mutants have a fix$^-$ phenotype and are non motile.

The Dublin group reported the isolation of a Fix$^-$ mutant of R. leguminosarum biovar viceae which is altered in exopolysaccharide production. Also, siderophore production was found in different species of Rhizobium as a strain specific phenomenon.

The following transnational collaborations were planned during the meeting. Arrangements were made to accommodate visiting scientists at Toulouse for both long and short periods. Joint experiments were planned, specifically aimed at locating genes in R. leguminosarum biovar viceae corresponding to the fix regulatory genes L and J of R. meliloti. Exchange of clones and mutants between Dublin and Bielefeld was arranged to facilitate a comparative analysis of the mutants isolated. Increased industrial participation in the contract was planned. Finally, it was agreed that the four laboratories remain open to expansion of the collaboration to include other groups, in particular any interested Spanish or Portuguese groups.

EUROPEAN LABORATORY WITHOUT WALLS

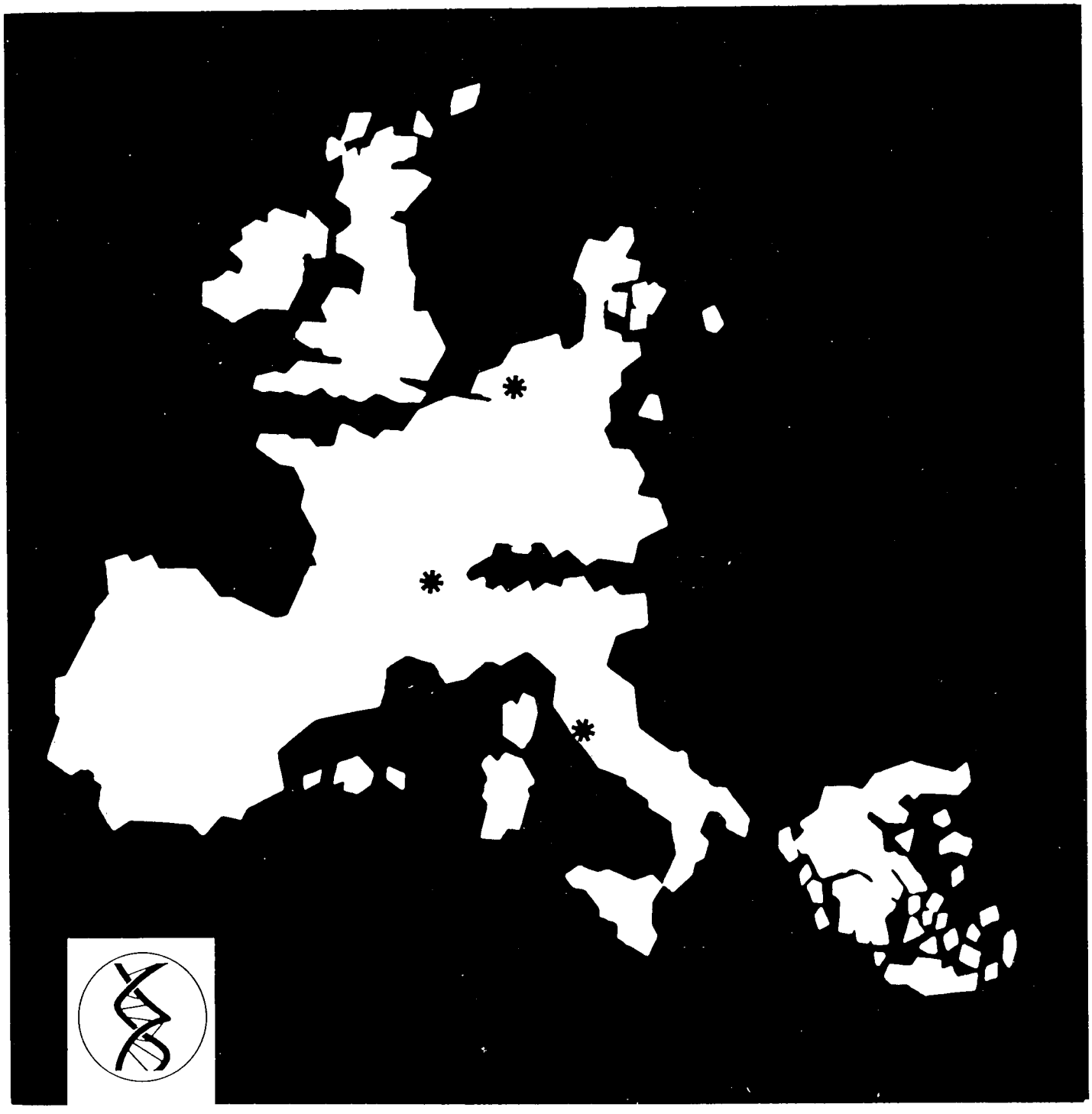
BAP-202-NL J.L. VAN WENT

BAP-203-F CH. DUMAS

BAP-204-I M. CRESTI

## In the field of

POLLEN BIOTECHNOLOGY



**POLLEN BIOTECHNOLOGY - ELWW ACTIVITY** BAP - 0202 NL
- 0203 F
- 0204 I

(CRESTI, Siena - DUMAS, Lyon - WILMS, Wageningen)

This 3rd workshop held in Lyon (1st and 2nd of februardy 1988) followed those previously organized in Lyon (19-20 may 1987) and Wageningen (august 1987).

This workshop was organized in 3 parts.

- 1- Main data obtained in 1987
- 2- Scientific exchanges planed during the 1st semester of 1988
- 3- Prospects for 1988.

## I- MAIN DATA OBTAINED DURING 1987

Different subjects were presented during the 1st day through several talks followed by informal discussion. In addition to the activity of this ELWW several additionnal talks from invited speakers belonging to Lyon's group.

- Pollen quality (Dr. C. DIGONNET-KERHOAS and P. ROECKEL, Lyon)

- Sperm cell (SC)

. Isolation
  - Corn (Dr. E. MATTHYS-ROCHON, Lyon)
  - Spinach (K. THEUNIS, Wageningen)
  - *Brassica* ( Dr. E. MATTHYS-ROCHON, S. DETECHEPARE, Lyon)

Quality
  - Corn (P. ROECKEL, V. WAGNER, Lyon)

- Male programme
  - *Brassica* ( S. DETECHEPARE, Lyon)
  - Wheat (P. VERGNE, Lyon)

- Cytoskeleton

- *Nicotiana* (A. TIEZZI. Siena)

- Invited speakers

- Molecular biology of the male programme in *Brassica* (C.M. GUILLY, and P. HEIZMANN, Lyon)

- Use of M. abs in self-incompatibility in *Brassica* (T. GAUDE, Lyon)

Publications: A book and several papers in preparation.

## II- SCIENTIFIC EXCHANGES

a)- Between BAP participants, 2 nd semester 1987 workshops not included.

| | |
|---|---|
| Wageningen | A. Tiezzi |
| | M. Murgia |
| Siena | |
| Lyon | A. Moscatelli (to test Mabs on SC, cytoskeleton) |
| Siena | H. Wilms |
| Wageningen | |
| Lyon | K. Theunis (to learn SC isolation) |

b)- Lyon ----> Wageningen E. Matthys-Rochon (2nd workshop)

c)- Participants to the 3rd workshop in Lyon:

Siena (M. Cresti, A. Tiezzi)
Wageningen ( K. Theunis, J. Van Went, H. Wilms)
Lyon (S. Detchepare, C. Dumas. I. Dupuis, T. Gaude, CM Guilly, P. Heizmann, N. Jnoud, C. Kerhoas-Digonnet, E. Matthys-Rochon, P. Roeckel, P. Vergne, V. Wagner).

Invitated and excused· ORSAN (Jarry), ICI (Schuch), DGXII Staff (Magnien, Nettancourt de).

## III- PROSPECTS FOR 1988

a)- To solve technical problems met in 1987 on.

- pollen quality
- SC isolation
- MGU characterization

b)- To improve SC characterization, and storage

c)- To elucidate MGU formation (EM studies. Mabs)

d)- Exchnages between scientists

e)- Meeting in Siena (June 1988)

- Organization of a common poster on "Pollen Biotechnology" (BAP participants)
- Workshop n°4 - Monday 30th may (morning session for BAP participants)
- BAP dinner (30th evening) with invited presons.

OGGETTO: Meeting BAP-Siena 27 - 28 maggio 1988.

PARTECIPANTI

Siena: Cresti M., Tiezzi A., Ciampolini F., Murgia M., Moscatelli A.

Lione: Matthys-Rochon E., Dupuis F., Said C., Roeckel P.

Wageningen: Van Went J.L., Willemse M.T.M., Wilms H.J., Theunis C.H., Keijzer C.J..

Hanno partecipato alla riunione anche i Proff. Risueno M.C. (Spagna) e Pais M.S.S. (Portogallo).

Sono state invitate anche le seguenti Ditte:

Soporcel: Ferreira G., Santos I.

I.C.I.: Schuch W., Greenland A.

Nunhem's Zaden B.V.: De Wit J.P.C.

Royal Sluis: Wilms H.

Nella riunione ufficiale alla quale hanno partecipato i titolari dei contratti e collaboratori sono stati presi in esame i risultati conseguiti negli ultimi sei mesi del 1988. Vengono inoltre riassunti da ciascun relatore le comunicazioni che verranno presentate al Simposio Sexual Reproduction in Higher Plants che si è tenuto dopo il meeting BAP.

Le comunicazioni riguardanti il progetto BAP che sono state presentate al Simposio sono le seguenti:

- MOSCATELLI A., TIEZZI A., VIGNANI R. and CRESTI M.
  Presence of kinesin in the pollen tube of Nicotiana.

- TIEZZI A., MOSCATELLI A., CIAMPOLINI F., MILANESI C., MURGIA M. and CRESTI M.
  The cytoskeletal apparatus of the generative cell in several Angiosperm species.

- THEUNIS C.H., VAN WENT J.L. and H.J. WILMS
  A technique to isolate sperm cells of mature spinach pollen.

- KEIJZER C.J.
  Artificial fertilization in Torrenia.

- MATTHIS-ROCHON E., DETCHEPARE S., WAGNER V., ROEKEL P. and DUMAS C.
  Isolation and characterization of viable sperm cells from tricellular pollen species.

- SONG Y., WAGNER V., MATTHYS-ROCHON E. and DUMAS C.
  Observations on isolated embryo sacs of corn.

Dei tre gruppi sono inoltre state presentate delle dimostrazioni poster. Un poster sul progetto BAP è stato allestito.

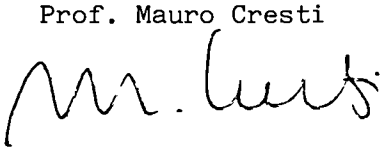
Si è discusso inoltre con i Proff. Pais M.S.S. e Risueno M.C. del loro probabile inserimento nel progretto BAP.

Al termine della discussione si è convenuto che i due nuovi Ricercatori invieranno a ciascuno dei tre laboratori (Siena, Lione, Wageningen) il programma inviato alla C.E.E. per una visione completa.

Successivamente qualora le nuove proposte vengano accettate dalla C.E.E. verrà convocata una nuova riunione per programmare la ricerca dell'ultimo anno.

Al Simposio, che ha seguito questo meeting, hanno partecipato anche le Industrie sopra citate.

Prof. Mauro Cresti



EUROPEAN LABORATORY WITHOUT WALLS

BAP-076-NL R.A. SCHILPEROORT
BAP-090-D H.G. SARX
BAP-091-DK D. VON WETTSTEIN
BAP-099-UK M. KREIS
BAP-106-UK R.B. FLAVELL
BAP-106-F P. JOUDRIER
BAP-213-D W. ROHDE
BAP-214-I M. MOTTO

## In the field of

EXPRESSION OF CEREAL SEED

PROTEIN GENES

# EXPRESSION OF CEREAL PROTEIN GENES

## WORKSHOP SEMINAR HELD AT MONTPELLIER (FRANCE)

## 28-29 JANUARY 1988

The scope of this two days workshop was to convene, for the first time, the contractants working in the field of molecular biology of cereal seed protein genes.

In addition of the contractant laboratories :

Cambridge : PBI, Prof. FLAVELL, England
Rothamsted : Harpenden Station, Dr. KREIS, England
Copenhagen : Carlsberg Res. Lab., Dr. BRANDT, Denmark
Leiden : Drs. HENSGENS, de PATER, The Netherlands
Andernach : MALZFABRIK, M. K. BRENNER, BRD
Köln : Max Planck I., Dr. THOMPSON, BRD
Bergamo : I. Sper. Cerealicoltura, Dr. MOTTO, Italy
Montpellier : INRA, Drs M.F. GAUTIER, P. JOUDRIER, France

other laboratories were invited because of their interest and works in this area of research :

Milano : CNR, Dr. VIOTTI, Italy
Barcelona : I. biologia CSIC, Dr. PUIGDOMENECH, Spain
Madrid : ETS Ing. Agronomos, Dr. GARCIA-OLMEDO, Spain
Perpignan : University, Dr. M. DELSENY, France

The aim of the workshop was to initiate and create an European Laboratory Without Wall (ELWW) in the field defined previously.

The workshop was divided in three sessions :

- The first one was a presentation of the research intentions of each participating laboratory for the next few years.

- The second one dealed with current researches undertaken in each laboratory.

- The third one was a general discussion about the ELWW.

It would be too long to relate all the objectives and programmes of each laboratory, it is why we are going to present the main themes emerging from all research teams.

Several research themes are concerned with the research activity of all laboratories such as :

+ Structure, expression and regulation of plant nuclear genome with storage protein genes as a model for these studies.

+ Protein structure and functionality.

- others are rather specific of the activities of laboratories such as :

+ Isolation and characterization of genes encoding proteins or enzymes involved in technological quality of cereal products : - malting and brewing quality (Carlsberg Research laboratory, Malz Fabrik).
- pasta and breadmaking quality (PBI, INRA-Montpellier, Rothamsted).

+ Cell structure analysis (PBI, Carlsberg, Leiden, Barcelona).

+ Embryogenesis (Leiden, Barcelona, Rothamsted).

(All these informations are gathered in table 1 and 2)

In the **second session,** current works have been presented. Most of them are concerned with the isolation and characterization of genes encoding storage proteins or enzymes in order to have a better knowledge on the expression and regulation mechanisms of these genes. (cf. list of scientific talks).

On **maize,** research are under progress concerning a better understanding of the O2 locus (MOTTO, THOMPSON) and on the characterization of organ-specific genes (embryo) during germination. VIOTTI's group is studying the expression and regulation of zein genes in yeast and also the methylation of zein genes in connexion with MOTTO's and SHEWRY's groups.

On **wheat,** the characterization of clones belonging to different classes (gliadin and glutenin) of storage proteins is under way (GAUTIER), and works are done on the expression of these clones after transfer into tobacco (FLAVELL). This strategy is used to determine what specific sequences are important for the expression of these genes.

GARCIA-OLMEDO's group is working on wheat sucrose synthase genes and at a lesser extend on barley and agropyron in order to study light influence, tissue specificity (coleoptile and roots) and growth conditions on sucrose synthase gene expression.

On **barley**, studies of regulation and expression of barley seed protein genes are the main objectives of the research conducted either by the group of KREIS or BRANDT.

Their results underlined the importance of sequence regions located upstream the coding region of a gene.

On **rice**, researches are concerned with tissue specific expressed genes (HENSGENS, De PATER).

**Third session : general discussion about the ELWW.**

After the agreement of all participants to constitute an ELWW, a discussion took place about what the group would like to do.

This ELWW will gather laboratories working on seeds (small grain cereals, maize) and wish to enlarge the group to laboratories working on legumes or oilseeds.

Research will focuse on quality problems such as baking quality, malting and brewing quality, nutritional quality.

It was also decided to write a pamphlet describing the research teams involved in this preliminary ELWW, the aim, the areas of research of overlapping interest and then to illustrate the research in progress by several demonstrative example.

Tables 1 and 2 list the different research themes, techniques used and expertises for each laboratory.

Although plant transformation techniques (with plasmid Ti) are used with success by several laboratories, there is no equivalent monocot transformation system.

However, considerable efforts to circumvent this problem are under progress.

It is an important limitation for research programmes which aims are to transfer genes to cereals crops in order to improve them.

All participants agreed to have another meeting, at the end of 1989.

Last, but not least, we acknowledge the generous help from the CEC which made the meeting possible.

SCIENTIFIC PROGRAM OF THE ELWW WORKING SEMINAR

"EXPRESSION OF CEREAL PROTEIN GENES"

**First Session : Research intentions of each participating laboratory**

A. BRANDT : CARLSBERG RESEARCH LABORATORY
COPENHAGEN, DENMARK.

K. BRENNER : WEISSHEIMER MALZ
ANDERNACH, GERMANY.

M. DELSENY : UNIVERSITE DE PERPIGNAN
PERPIGNAN, FRANCE.

R. FLAVELL : INSTITUTE OF PLANT SCIENCE
RESEARCH. CAMBRIDGE, ENGLAND.

F. GARCIA-OLMEDO : E.T.S. INGENIEROS
AGRONOMOS. MADRID, SPAIN.

L. HENSGENS : MOLBAS RESEARCH GROUP
LEIDEN, THE NETHERLANDS.

P. JOUDRIER : LABORATOIRE DE TECHNOLOGIE DES
CEREALES. MONTPELLIER, FRANCE.

M. KREIS : ROTHAMSTED EXPERIMENTAL STATION
HARPENDEN, ENGLAND.

M. MOTTO : ISTITUTO SPERIMENTALE PER LA
CEREALICOLTURA, BERGAMO, ITALY.

P. PUIGDOMENECH : CENTRO DE INVESTIGACION Y
DESARROLLO. BARCELONA, SPAIN.

R. THOMPSON : MAX PLANCK INSTITUT FÜR
ZÜCHUNGSFORSCHUNG, KöLN, BRD.

A. VIOTTI : INSTITUTE OF PLANT BIOSYNTHESIS
C.N.R. MILANO, ITALY.

**Second Session : Scientific talks :**

**1) MAIZE :**

M. MOTTO - Molecular studies of the high lysine genes opaque-2.

R. THOMPSON - The opaque-2 locus of maize.

P. PUIGDOMENECH - Characterization of organ-specific genes in maize.

A. VIOTTI - The zein gene of maize : expression and regulation in endosperm cell and in the yeast S. cerevisiae.

**2) WHEAT :**

R. FLAVELL - Control of wheat storage protein expression.

M.F. GAUTIER - Cloning of wheat storage protein genes.

F. GARCIA-OLMEDO - Mapping and differential expression of linked sucrose synthase genes in wheat, barley and agropyron.

**3) BARLEY :**

M. KREIS - Regulation and expression of barley seed protein genes.

A. BRANDT - Structure and expression of B, C and gamma-hordein genes.

**4) RICE :**

S. de PATER - Tissue specific expressed genes in rice.

**Third Session :** GENERAL DISCUSSION ABOUT THE ELWW.

TABLE 1

| techniques/expertise | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DNA Cloning | + | + | + | + | + | + | | + | + | + | + | + |
| DNA Sequencing | + | + | + | + | + | + | | + | + | + | + | + |
| Protein Sequencing | | | | | | | | | | + | | |
| Plant Transformation | + | + | + | | + | + | | + | | + | + | + |
| Brewing | | | | | + | | + | | | | | |
| Breadmaking | | | + | + | | | | | | | + | |
| Immunology | + | + | + | + | + | + | | + | + | | + | |
| Protein Purification | + | | + | + | + | + | | + | + | + | + | |
| Plant Breeding (formal genetics) | + | | + | | + | | | | | + | + | + |
| Cell structure analysis | | | + | | + | + | | | + | | | |
| Tissue culture | + | + | + | | | + | | + | + | | | + |
| Heterologous expression of proteins. | | | + | | + | + | | | | | + | |

1 = BERGAMO : ISTITUTO PER LA CERALICOLTURA, Dr MOTTO

2 = MILANO : ISTITUTO BIOSYNTHESI VEGETALI, Dr VIOTTI

3 = CAMBRIDGE : INSTITUTE OF PLANT SCIENCE RESEARCH, PROF. FLAVELL

4 = MONTPELLIER : INRA, LAB. TECHNOLOGIE DES CEREALES, Dr GAUTIER, JOUDRIER

5 = COPENHAGEN : CARLSBERG LABORATORY, Dr BRANDT

6 = LEIDEN : MOLBAS RESEARCH GROUP, Dr HENSGENS, DE PATER

7 = ANDERNACH : WEISSHEIMER MALZ, K. BRENNER

8 = PERPIGNAN : UNIVERSITE, LAB. PHYSIOLOGIE VEGETALE, Dr DELSENY

9 = BARCELONA : INSTITUTO DE BIOLOGIA, CSIC, Dr PUIGDOMENECH

10 = MADRID : ETS INGENIEROS AGRONOMOS, Dr GARCIA-OLMEDO

11 = HARPENDEN : ROTHAMSTED EXPERIMENTAL STATION, Dr KREIS

12 = KöLN : MAX PLANCK INSTITUTE, Dr THOMPSON

TABLE 2

| THEME/ TEAM | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Protein functionality | | + | + | + | + | | + | | + | + | + | |
| Enzymes seed formation | + | | | | + | + | + | + | | + | + | + |
| seed germination | | | + | | + | + | + | + | + | | | |
| Embryogenesis | | | | | | + | | | + | | + | |
| Quality related : | | | | | | | | | | | | |
| nutritional | + | | + | | + | | | | | + | + | + |
| starch structure | + | | | | + | | + | | | | | |
| protein structure | + | + | + | + | + | | + | + | + | + | + | + |
| brewing quality | | | | | + | | + | | | | | |
| breadmaking quality | | | + | + | | | | | | + | + | |

EUROPEAN LABORATORY WITHOUT WALLS

## In the field of

PLANT HORMONE RECEPTORS

BAP-0205-UK M.A. VENIS

BAP-0206-NL K.R. LIBBENGA

BAP-0207-D D. KLÄMBT

BAP-0208-UK M.A. HALL

BAP-0209-I A. BALLIO

Short report on the working seminar on "Plant Hormone Receptors" held from 11th to the 14th of April 1988 at the Physik Zentrum in Bad Honnef, FRG.

Thirty participants from the five working groups of this joint project actively discussed the reported results on auxin-, ethylene- and fusicoccin-receptors. Three guests, two from BASF, Limburger Hof, and Bayer, Monheim, and one from Jeff Schell's group at the MPI für Züchtungsforschung, Köln, joint us.

The most progressive report was on the isolation of cDNA clones of the maize membrane associated auxin binding protein from a $\lambda$gt 10 library. After a period of two years of frustrating research Thomas Hesse could report on a successful isolation of these clones using oligonucleotide probes. These were constructed from sequenced N terminal region and several tryptic peptides of the purified assumed auxin receptor. These clones will help to elucidate the structure and functions of the auxin receptor related to cell elongation.

There are many data for the existence of a soluble, cytoplasmic auxin receptor related to cell division. Direct evidences are still missing. Bert van der Zaal selected cDNA clones from 2,4D treates tobacco cell suspension cultures which do not cross hybridize with cDNA from auxin free tobacco cells. Seven of these are characterized further. The mRNA of one of these are induced very rapid within 15 min and also by the use of IAA instead of 2,4D. Although the nucleotide sequence is known, the gene could not identified yet. It is expected that this gene should be auxin-dependent regulated.

There were reports on the solubilization and further characterization of a ethylene binding protein which seems to be an integral membrane protein and of a fusicoccin binding protein, which may be only associated to the membrane. Investigations were started to search for endogenous ligands for the fusicoccin binding protein and for the N-1-naphthylphtalamic acid binding protein as well by immuno affinity chromatography.

The transnational contacts and cooperations are well developped and will be increased within the next year.

EUROPEAN LABORATORY WITHOUT WALLS

# In the field of

EMBRYOGENESIS

IN PLANT TISSUE CULTURE


BAP-0077-DK R. RAJAGOPAL

BAP-0078-DK P. OLESEN

BAP-0093-NL S.C. DE VRIES

BAP-0098-UK K. ROBERTS


# M I N I S Y M P O S I U M

EMBRYOGENESIS IN PLANT TISSUE CULTURE

A/S DE DANSKE SUKKERFABRIKKER, BIOTECHNOLOGY SECTION,

THURSDAY, NOVEMBER 5, 1987 - 13:15 O'CLOCK

PROGRAMME

| | |
|---|---|
| 13:15 - 13:45: | Louise Jannick, DDS/Biotechnology Section: "Identification and purification of embryogenic cells from cellsuspension of carrot." |
| 14:45 - 14:30: | Sacco de Vries, Agricultural University of Wageningen, Holland: "Carrot somatic embryogenesis depends on the phytohormone controlled presence of correctly glycosylated extracellular proteins." |
| 14:30 - 15:15: | Morten Jørsboe, DDS/Biotechnology Section: "Peroxidases as a marker for early event in somatic embryogenesis." |
| 15:15 - 15:30: | Break |
| 15:30 - 16:15: | Roger Pennell, John Innes Institute, UK: "The potential of an anti-AGP monoclonal antibody in the molecular analysis of plant cell plasma membranes" |
| 16:15 - 17:00 | Ole Schou, Risø Research Laboratories: "Detection of embryo specific marker in the plasma membrane by means of monoclonal antibodies." |

EUROPEAN LABORATORY WITHOUT WALLS

## In the field of

FUNDAMENTAL ASPECTS OF

PLANT CELL REGENERATION

BAP-013-D
BAP-014-F
BAP-076-NL
BAP-078-DK
BAP-083-NL
BAP-085-F
BAP-088-I
BAP-092-I
BAP-093-NL
BAP-098-UK
BAP-099-UK
BAP-101-UK
BAP-103-UK
BAP-111-B
BAP-205-UK
BAP-206-NL

BAP workshop on fundamental aspects of

Plant Regeneration

*****

De Danske Sukkerfabrikker, Copenhagen

24 and 25 March 1988

*

* *

Significance of the topic :

Plant regeneration from somatic cells is only the happy end of a long process of cell development and differentiation with many unpredictable traps. This process is far from being understood, but it can be controlled in a limited number of species, and even in a few fortuitous genotypes of otherwise recalcitrant species, by disrupting in vitro the cell communication channels and by applying massive hormonal doses exogenously. However, irrespective of the fact that some well-known regenerating systems have successfully been applied on a large scale (among the Solanaceae in particular), most research approaches failed over the last decade to achieve any significant progress in controlling regeneration at will.

This is because research in this field has been too empirical and was barely given the means to elucidate the switch mechanisms and molecular mediators which, in the intimates of plant cells, govern the activation or repression of developmental sequences. Plant regeneration is nothing but the emerged side of an iceberg built on the hidden principles of plant development.

Potential users of plant regeneration in agriculture and industry attribute a top priority to basic research designed at controlling the underlying processes. This priority is based on the fact that genetic engineering has made tremendous progress, which often cannot be applied until any manipulated cell is given a chance of reproducing itself into a fully reconstituted and fertile organism. The first bottlenecks have been, in the early 80's, the lack of transformation methods applicable to new species of interest : this has now been largely overcome (J. Schell, pers. commun.). In the mid-eighties, the bottlenecks have been shortages of useful genes to engineer plants with : this is still an important question, but no more the actual limiting factor, considering the number of cloned genes that await further uses until proper recipient systems can be designed (1987 report of calgene for example mentions over 60 genes and a dozen of promotors already cloned or licensed).

Potential users of plant genetic engineering therefore recommend that significant means be given to public research in the area of plant cell regeneration (see reports to the Commission of IRDAC, of GIBiP, of CEFIC, etc.). While stressing the urgency of the task, they are not in a position to suggest what the right approaches would be. Although it is clear that plant regeneration is just an aspect of plant developmental biology, there is not a programme with time limitations which could reasonably embark on developmental biology in a broad sense. The purpose of this meeting, therefore, was to identify sharp priorities for research on very early "signals" that trigger cell differentiation and morphogenesis.

## Current effort in the BAP programme

The first meeting day was an occasion to review work under way in contracting laboratories. The programme is appended as annex 1. Contributors were from 11 different laboratories, distributed as follows :

4 labs from the ELWW (European Laboratory Without Walls) on "Genetics and molecular biology of somatic embryogenesis" ;

3 labs from the ELWW on "Cell biotechnology for crop improvement" ;

2 labs from the ELWW on "Plant hormon receptors" ;

1 lab from the ELWW on "Hairy root and plant cell differentiation" ;

1 lab from the ELWW on "Proteins in seed development".

None of these ELWWs established under BAP adequately cover the topic of plant cell regeneration, although all of them have significant contributions towards the same goal.

- From a genetic point of view :

  Isolation and characterization of temperature sensitive developmental mutants in carrot.

- From a molecular biology point of view :

  Isolation and characterization of genes induced in tobacco by growth promoting signals (auxins, cytokinins, ...), or specifically expressed with the early steps of either somatic embryogenesis in carrot and sugar beet or seed development in barley. Further isolation of transacting factors controlling their specific expression.

- From a biochemical/physiological point of view :

  Identification of hormon receptors and elucidation of transducing sequences in maize and tobacco.

- From a cell engineering point of view :

  Cell markers of regenerative potential, cell/tissue fractionation, *in vitro* selection of "competent" cells, etc.

All these approaches are the different facettes of plant cell regeneration research. They do not constitute yet a plant cell regeneration programme. This would require more focus and further rationalization.

A plant cell regeneration programme for BRIDGE

The second meeting day (annex 1) brought the scientific debate under the BRIDGE perspective. A number of conclusions have been reached as follows :

- Experimental species : to profit from multidisciplinarity, complementary approaches should apply to the same experimental species. Twenty-eight different experimental species such as in BAP presently cannot be afforded any more. As there are no E. coli or Drosophila-like plant models whatsoever, a unique experimental species cannot be easily chosen. Rather than a total exclusion of alternative models, the advise would be to use, for each particular scientific objective, the experimental species which is best adapted. Carrot, tomato, potato, Brassica, maize, alfalfa are frequently quoted, each with a particular advantage.

- Industrial needs : These were reviewed by the responsible scientist for plant biotechnology in the seed company Zaadunie (Dr. A.J. Kool). He stressed the objective of regeneration could not be isolated from that of stability and homogeneity of products. The interference of regenerative procedures with genetic stability will have to be taken into account. He mentioned embryogenesis (somatic or zygotic) as a more reliable procedure than in vitro organogenesis, with the implication that basic research could concentrate on embryogenesis. He also advocated the choice of experimental species best adapted to each particular type of investigation. These could preferably be crop species, but not a priori crop species. The choice of crop species in principle, without looking at the needs of research, could be both a scientific and a political error.

- Genetic background : Preference should go to systems with proper and accessible genetic controls. Small genomes, mutants available or easily inducible, rapidly cycling populations, amenability to in vitro manipulation and transformation, etc.

- Functional analysis : Many genes expressed during gametogenesis or embryogenesis, or triggered by external stimuli, will be cloned by the time BRIDGE starts. Any new programme in this field should already make provisions for an increased emphasis on the products of these genes and on the higher levels of "trans-"regulation.

- Plant cell interfaces : The above requirement leads immediately to the plant plasmalemma and to the cell wall. Many of the genes mentioned, related to development and differentiation, either direct their product to the membrane/wall, are switched by transductional chains initiated from membrane/wall, or both. As brilliantly described by S. Fry, the cell wall metabolism, at the crossroads of differentiation and plant-microbe interactions, will appear to be the site of primary events that control major developmental routes : an obvious target for BRIDGE.

In conclusion, the organizers of the meeting reached the following decisions :

1. With regard to their activity in BAP, a special ELWW on "Fundamental aspects of plant cell regeneration" will be established, with cross-representations of the other ELWWs listed above. It will hold an annual meeting, the one in 1989 being hosted by K. Roberts at John Innes Institute.

2. With regard to the preparation of BRIDGE, S. de Vries will draft a scientific report to cover promising research areas as discussed here. His draft, being amended by all participants in this meeting, eventually will be transmitted by Commission services to three target groups for final improvement and criticism if necessary.

   . Individual experts

   . CGC brainstorming group in plant biotechnology

   . GIBiP industrial platform

# EEC-BAP workshop on fundamental aspects of plant regeneration

**to be held at De Danske Sukkerfabrikker, Copenhagen, Denmark**

Program for Thursday March 24

Chair: P. Olesen, Copenhagen, Denmark / S.C. de Vries, Wageningen, Netherlands

| | |
|---|---|
| 09.00-09.10 | P. Olesen, Copenhagen, Denmark<br>Welcome to participants |
| 09.10-09.40 | M.G.K. Jones, Rothamstead, UK<br>Events involved in plant regeneration in vitro and some culture systems for their study |
| 09.50-10.20 | D.J. James, East Malling, UK<br>Severe reduction in regeneration ability in regeneration-competent genotypes after transformation of apple and strawberry plants with disarmed Ti binary vectors |
| 10.30-11.00 | Coffee |
| 11.00-11.30 | M. Terzi, Naples, Italy<br>Developmental mutants for studying plant development |
| 11.40-12.10 | S.C. de Vries, Wageningen, Netherlands<br>Extracellular proteins and carrot somatic embryogenesis |
| 12.20-13.30 | Lunch (courtesy of DDS) |
| 13.30-14.00 | J.H.C. Hoge, Leiden, Netherlands<br>Plant genes regulated by auxin and cytokinin |
| 14.10-14.40 | P. Olesen / J. Mollerup Andersen, DDS, Denmark<br>Detection of regenerative potential |
| 14.50-15.20 | Thea |
| 15.20-15.50 | M. Kreis, Rothamstead, UK<br>Molecular analysis of early events in barley embryo and endosperm differentiation and development |
| 16.00-16.30 | P. Lazzeri, MPI Cologne, BRD<br>Somatic embryogenesis in barley and other cereals |

Program for Thursday March 24 (continued)

| | |
|---|---|
| 17.00-20.00 | Dinner |
| 20.00-22.00 | Round table discussion on regeneration.<br>- Dr. Pennell, John Innes Inst., UK will present some of his recent data on sugarbeet regeneration.<br>- Dr. Lazzeri, MPI, Cologne will summarize the in-house workshop on "Somatic embryogenesis in crop plants" held at the MPI on 22-24 February 1988<br>- Further topics to be discussed will be prepared by the chairmen |

## BRIDGE expert meeting on "Control of differentiation and morphogenesis in plants"

**to be held at De Danske Sukkerfabrikker, Copenhagen, Denmark**

Program for Friday March 25

Chair: S.C. de Vries, Wageningen, Netherlands

| | |
|---|---|
| 09.00-09.30 | E. Magnien, EEC, Brussels<br>The importance of this meeting for the preparatory work of the EEC-BRIDGE program |
| 09.30-10.15 | Report by the chairmen of the previous day on priority areas selected and conclusions arrived at during the round table discussion |
| 10.15-10.45 | Coffee |
| 10.45-11.45 | View of experts ( C. Lloyd and K. Roberts, J.I.I., Norwich, UK; S. Fry, Edinburgh, UK; M. Jacobs, Belgium ) contacted for this meeting on research areas to be developed in the field of plant cell differentiation and development |
| 11.45-12.15 | A.J. Kool, Zaadunie, Netherlands<br>The expectations and role of industry |
| 12.15-12.45 | S.C. de Vries, Wageningen, Netherlands<br>Tentative conclusion; future EEC-BAP meetings on fundamental aspects of regeneration; preparation of BRIDGE document in the area of "Control of development and differentiation in plants" |
| 12.45-13.30 | Lunch and farewell |

**List of participants EEC workshop 24-25 March 1988, Copenhagen, Denmark**

1 . dr. M. Buiatti, Dip. di Biol. Animale e Genetica, Via Romana 17, 50125 Firenze, Italia
2 . dr. P. Constantino, Univ. di Roma c/o Ist. Fisiologia Generale, piazzale A. Moro 5, I-00185 Roma, Italia
3 . dr. S. Fry, Dept. of Botany, Univ. Edinburgh, King's buildings, Mayfield Road, Edinburgh, EH9 3JH, U.K.
4 . dr. Ch. H. Hänisch ten Cate, I.T.A.L., P.O. Box 48, 6700 AA Wageningen, Netherlands
5 . dr. J.H.C. Hoge, Dept. of Plant Mol. Biol., Univ. Leiden, Wassenaarseweg 64, 2333 AL Leiden, Netherlands
6 . dr. J. Hollerup Andersen, Biotechnology Section DDS, 1 Langebrogade, P.O. Box 17, DK-101 Copenhagen K, Denmark
7 . dr. M. Jacobs, Inst. Mol. Biol., V.U. Brussel, Paardenstraat 65, B-1640 St. Genesius-Rode, Belgium
8 . dr. D.J. James, East Malling Res. Station, East Malling, Maidstone, Kent ME19 6BJ, U.K.
9 . dr. M.G.K. Jones, Rothamstead, Harpenden AL5 2JQ, U.K.
10. dr. M. Jullien, I.N.R.A., Route de St. Cyr, F78000 Versailles, France
11. dr. A.J. Kool, Biotechnology Section, Zaadunie B.V., Westeinde 62, P.O. Box 26, 1600 AA Enkhuizen, Netherlands
12. dr. M. Kreis, Rothamstead, Harpenden AL5 2JQ, U.K.
13. dr. P. Lazzeri, M.P.I., 30 Vogelsang, den Egelspfad, D-5000 Köln, B.R.D.
14. dr. C. Lloyd, J.I.I., Colney Lane, Norwich NR4 7UH, U.K.
15. dr. A. Maan, Dept. of Plant Mol. Biol., Botanical Laboratory, Nonnensteeg 3, 2311 VJ Leiden, Netherlands
16. dr. E. Magnien, C.E.C. Dir. Biology, Div. Biotechnology, Rue de la Loi 200, B-1049 Brussels, Belgium
17. dr. P. Olesen, Biotechnology Section DDS, 1 Langebrogade, P.O. Box 17, DK-101 Copenhagen K, Denmark
18. dr. K. Pennell, J.I.I., Colney Lane, Norwich NR4 7UH, U.K.
19. dr. P. Puidgomenech, Dept. Molecular Genetics, C.S.I.C., Jorge Girona Salgado 18-26, 08034 Barcelona, Spain
20. dr. E. Teoule, Lab. du d'amélioration des plantes, Univ. de Paris-Sud, Centre d'Orsay, F91405 Orsay, France
21. dr. M. Terzi, Dept. of Genetics, Univ. degli Studi di Napoli, Via Mezzocannone 8, 80134 Napoli, Italia
22. dr. S.C. de Vries, Dept. of Molecular Biology, Agricultural University Wageningen, De Dreijen 11, 6703 BC Wageningen, Netherlands

EUROPEAN LABORATORY WITHOUT WALLS

| | |
|---|---|
| BAP-0074-NL | P. DE WIT |
| BAP-0088-I | M. BUIATTI |
| BAP-0103-UK | S. FRY |
| BAP-0105-UK | D. INGRAM |
| BAP-0209-I | A. BALLIO |

## In the field of

MOLECULAR PLANT PATHOLOGY

PROGRAMME OF THE SEMINAR

4 May ,Morning (9 -12 )

Factors involved in fungal pathogenicity/compatibility including fungal transformation

Chairman: D.S.Ingram

Speakers: J.Johnstone: Towards a molecular biological analysis of the pethogenicity of Pyrenopeziza brassicae; R.Oliver: Transformation and molecular aspects of pathogenicity of the tomato pathogen Fulvia fulva; C.Caten: Molecular analysis of the pathogenicity of Septoria nodorum to wheat; M.S.Wolfe: Population genetics of powdery mildews; Ingram D.S.: Sexual reproduction in pathogenic fungi.

General discussion

4 May, Afternoon (14.30 -18)

Response of the host with emphasis on changes at protein and mRNA and protein level

Chairman: P.DeWit

Speakers: P.DeWit: Elicitors and basic compatibility factors in the host fungus interaction Cladosporium fulvum-tomato; J.F.Bol: Structure and function of virus inducible plant genes; J.Friend: The role of lignification as a defense mechanism; P.Ricci: Elicitors of plant defense reactions in Phytophtora diseases of tobacco and carnation;

S.Kauffmann: Isolation,characterization and biological functions of PR-proteins

General discussion

5 May ,Morning (9-12)

Plant receptors for fungal toxins and elicitors

Chairman: A.Ballio

Speakers: A.Ballio: Fusicoccin binding sites; J.Ebel: Soybean-membrane receptors for glucan elicitors from the fungus P.megasperma f.sp.glycinea; S.Green: Apoplastic enzymes as markers of early pathogenesis in the host-fungus interaction

General discussion on proposed models of plant-pathogen interactions

5 May ,Afternoon (14.30-18)

In vitro manipulation and selection for resistance:

Chairman: M.Buiatti

Speakers: M.Buiatti: In vitro markers of resistance and their use in selection programmes; B.Foroughi-Wehr: In vitro selection for resistance in potatoes and cereals; J.Grisvard: Strategy to study the interaction between Phaseolus vulgaris and Colletothricum lindemuthianum using RFLP;

General discussion on the feasibility of practical applications of present knowledge on plant-pathogen interactions

6 May ,Morning (9-12)

Round table with industries on the applications of concepts and methods discussed in other sessions. The round table will be chaired by E.Magnien and introduced by the four chairmen of earlier sessions with a summary of their contents. The discussion will then be open particularly to firm representatives.

This was a meeting with two purposes. In the first place, it was the occasion for all BAP contractors working in molecular plant pathology to meet together and develop further their transnational links. The two first days were dedicated to this workshop activity, and to reviewing current progress in the field within Europe. The last day was left for a different purpose : taking advantage of the participation of 13 industrial representatives from 6 different Member State origins, preliminary views on future BRIDGE research were exposed, and experts invited to assess possibilities for strengthening this specific field in the light of industrial expectations.

## 1. BAP workshop (4-5 May 1988) :

### 1.1. Attendance

Five BAP contractors have been the initial nucleus for the organization of the workshop. Contrary to other research areas in BAP, this one could not be sufficiently funded and suffers from the non participation of key European experts. Still, it was a priority, as the area was considered by industry one of high significance and one where biotechnology breakthrough could lead to qualitative research leaps. Next to the five contracting participants were also invited 9 other recognized European experts, as well as the industrial scientists mentioned above, to reach the critical mass which would be needed if the topic was to receive a decisive impetus in the framework of BRIDGE. In this case, therefore, the Commission is not dealing with a BAP-ELWW (European Laboratory Without Walls), but with a ELWW rooted in BAP and open to many non-contracting participants.

## 1.2. Host-pathogen relations under present focus

As indicated in the following table :

| | Plant side | Microbial side | Participant | BAP Contract |
|---|---|---|---|---|
| Solanaceae | Tomato | Fusarium oxysporum | Buiatti | YES |
| | Tomato | Cladosporium fulvum | de Wit | YES |
| | Tomato | Cladosporium fulvum | Green | YES |
| | Tomato | Cladosporium fulvum | Oliver | NO |
| | Potato | Fusarium, Helminthosporium, Phytophthora | Foroughi-Wehr | NO |
| | Potato | Phytophthora infestans | Friend | NO |
| | Tobacco | Tobacco Mosaic Virus | Bol | NO |
| | Tobacco | Tobacco Mosaic Virus | Kauffmann | NO |
| | Tobacco | Phytophthora spp. | Ricci | NO |
| | Pepper | Phytophthora spp. | Ricci | NO |
| Legumes | Soybean | Phytophthora megasperma | Ebel | NO |
| | French bean | Colletothricum lindemuthianum | Grisvard | NO |
| Cereals | Barley | Erysiphe graminis (mildew) | Wolfe | NO |
| | Barley | Fusarium, Helminthosporium | Foroughi-Wehr | NO |
| Ornementals | Carnation | Fusarium oxysporum | Buiatti | YES |
| | Carnation | Phytophthora spp. | Ricci | NO |
| Trees | Peach, Almond tree | Fusicoccum amygdali | Ballio | YES |
| Others | Brassica | Pyrenopeziza brassicae | Ingram | YES |

## 1.3. Major research lines :

Five lines received attention (see also the scientific programme in annex 1).

- **Applications of Restriction Fragment Length Polymorphisms (RFLP)**
  The possibility to pinpoint, with specific DNA probes, variable sequences revealed on electrophoretic patterns of digested fragments from the total genome. A systematic study of this DNA polymorphism can be conducted parallel to segregation analyses of breeding lines tested for a specific resistance trait.

The close correlation of the expression of this trait with unique sets of variable sequences in the DNA will give access to loci in the genome which may contain genes controlling resistance. Prior to any gene identification, the DNA probes, thus associated to a defined genetic character, can find powerful applications as diagnostic tools in breeding, to sharply orient selection.

**Transformation methods for pathogenic frungi.**
A presentation by Oliver of newly developed methods to transform Cladosporium fulvum reminds us that, dealing with an interaction between host and pathogen, the definition of resistance is always relative to recognition and virulance genes on the fungal side, implying molecular tools to study these genes in the pathogen and how they function. Transformation methods in combination with the analysis of non-pathogenic mutant strains will make this study possible.

**Population genetics in relation to fungal resistance to chemicals.**
This is an essential part of crop protection strategy; it concerns an interaction which is not between host and pathogen as above, but between populations of this pathogen and defined chemicals used to control them. From a strictly scientific point of view, this is out of the scope of the meeting although not irrelevant.

**In vitro selection for resistance.**
The selection of plant cells can be either for survival under exposure to a purified fungal toxin, or for the expression of biochemical features normally associated to resistance reactions (phytoalexin production, endoglucanases, lignin,...) This is very empirical science, as potentially resistant cell lines may result so for completely unknown reasons, and may also fail to express the desired level of resistance when selected cells are regenerated into plants grown in the field. The position of industrial firms was quite clear on this type of work : some simply do not believe in it, others do think good breeding material could occasionally come out of it but, in this latter case, consider in vitro screening as their own development work which is not in the domain of public research.

- **Recognition steps and activation of plant defences.** This point is listed here as the last one because of its overwhelming importance. It occupied 2/3 of the discussion time in the meeting, appeared to be the most rewarding one from a scientific point of view and the one attracting the highest interest from industry. Dominating speakers were de Wit, Green, Kauffmann, Ebel, Bol, Ricci and Ballio. Three of them (underlined) are presently BAP contractors. The attention of these groups is focused on specific and rare signal molecules which can be traced at very early stages of both compatible and incompatible interactions. Plant genes and/or products characteristic of these 2 types of interactions are described, mainly in two classes : the phytoalexins which are secondary metabolites, and PR (pathogenesis-related) proteins which belong to 4 immunological groups and appear to be hydrolases. An immense interest is concentrated on the plant wall where the initial events may take place and which was shown to release oligosaccharides with an eliciting power. The plant cell wall/membranes have already been identified as important targets in the coming BRIDGE programme.

- **BRIDGE round table with industry (6 May 1988).**

The list of participating industrial scientists is provided as annex 2.
Three major questions were debated :

- Where to focus collaborative research ?
- Is concentration on few experimental species recommended ?
- What sort of involvement in Community research is industry expecting ?

In conclusion, and with some oversimplification may be, industry in this BAP workshop urges again the Commission to support international cooperation.

Their expectations in basic research is that the Commission prepares BRIDGE with new objectives, but relying on implementation methods mainly inherited from BAP. The situation would be totally different with a programme of product-oriented research, like ECLAIR or EUREKA, where industry would easily step in and be prepared to take leadership. The message of these 13 industrial representives in Florence was essentially that both types of programmes are equally important from their point of view, but should not be mixed up as far as implementation mechanisms are concerned.

MOLECULAR PLANT PATHOLOGY

Florence, 4-6 May, 1988

## List of industrial participants

| | | |
|---|---|---|
| B.C. Baldwin | ICI Seeds | Bracknell (UK) |
| G. Biasini | Montedison | Massa (I) |
| T.K. Bradshaw | SHELL Research Ltd. | Sittingbourne (UK) |
| A. Burggraaf | Phytotec | Louvain-la-Neuve (B) |
| M. Dubois | SANOFI-Elf | Labège (F) |
| G. Freyssinet | Rhône-Poulenc Agrochimie | Lyon (F) |
| B. Grezes-Besset | SANOFI-Elf | Labège (F) |
| L. Martinelli | Montedison | Massa (I) |
| F. Massardo | Montedison | Milano (I) |
| R. Pontzen | BAYER AG | Monheim (D) |
| R. Shields | UNILEVER | Sharnbrook (UK) |
| P. Van den Elzen | MOGEN International | Leiden (NL) |
| F. Vecchio | ORIS/SIPCAM | Milano (I) |
| J. Wall | Agricultural Genetic Company | Cambridge (UK) |

# BIOTECHNOLOGY OF ANIMALS

EUROPEAN LABORATORY WITHOUT WALLS

BAP-127-F HACHE
BAP-128-F ENGASSER
BAP-129-F NABET
BAP-130-D LEHMANN
BAP-132-D SCHÜGERL

## In the field of

BIOCHEMICAL-PHYSICAL KNOWLEDGE, CONTROL OPTIMISATION OF ANIMAL CELL CULTURES IN BIOREACTORS

**ELWW - meeting** in the field of

**Biochemical-physical knowledge, control, optimization of animal cell cultures in bioreactors**

NANCY, 30 - 31 May 1988

List of participants:

K. Schügerl, D. Wentz, G. Kretzmer, H. Graf
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Lehrstuhl - Biotechnik tierischer Zellen
Universität Bielefeld
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B. Röder, J. Vorlop
G.B.F.
Mascheroderweg 1
D-3300 Braunschweig

D. Duval, C. Demangel
Bertin et Cie
Chemical and Biochemical Engineering Division
F-78373 Plaisir Cedex

P. Nabet, J.-M. Bour, D. Carbonell, D. Marsic, J. Capiaumont, F. Derouiche, C. Legrand
Université de Nancy
INSERM
Faculté de Médecine
F-54505 Vandoeuvre-les-Nancy Cedex

J.-M. Engasser*, A. Marc, L. Helbert, V. Geaugey, A. Martial, E. Mailly, J.-L. Goergen
Institut National Polytechnique
de Lorraine
E.N.S.A.I.A.
2, Avenue de la Forêt de Haye
F-54505 Vandoeuvre-Les-Nancy

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* Local organizer

## Scientific presentations

| | |
|---|---|
| J. Vorlop | : Oxygen transfer and carrier mixing in the 100 l membrane stirrer cell culture reactor |
| H. Büntemeyer | : Amino-acid estimation in mammalian cell culture media |
| B. Röder | : Nutrient requirements of BHK 21 cells |
| C. Demangel | : Influence of ultrasonic treatment on hybridoma growth and monoclonal antibodies production |
| H. Graf | : Development of the influence of physical stress on mammalian cells |
| D. Wentz | : Determination of biological parameters by on-line methods |
| J.-M. Bour | : Use of whey as a substitute for FCS in culture media - New biochemical markers for cell proliferation and death - Design and assay with a reactor in which cells are entrapped inside a foam |
| L. Helbert | : Kinetics and modelling of BHK cell growth on microcarriers |
| V. Geaugey | : Nutrient requirements of hybridoma cells and membrane bioreactor assays (ultra and microfiltration) |

# EUROPEAN LABORATORY WITHOUT WALLS

BAP-121-B Rommelaere
BAP-122-I Riva
BAP-236-I Donini
BAP-237-UK Bostock
BAP-256-D Lipps
E Azorin
P Costa

## In the field of

MAMMALIAN MINICHROMOSOMES
AND LINEAR VECTORS

**European Laboratory Without Walls**
**in the field of Genetic Engineering for Animal Husbandry**

**Area of research:** Mammalian minichromosomes and linear vectors

**First meeting:** May 19-20, Université Libre de Bruxelles and Solvay Co.,
Brussels, Belgium

1. PARTICIPANTS

- C.J. BOSTOCK and A. SHERVINGTON, Animal Virus Research Institute, Pirbright, UK
- P. DONINI,A.M. GUERRINI and F. ASCENZIONI, Università di Roma, Italy
- S. RIVA and G. BIAMONTI, Istituto di Genetica, Biochimica ed Evoluzionistica del CNR, Pavia, Italy
- WEFES, Universität Tübingen, FRG
- F. GRUMMT, Universität Würzburg, FRG
- J. VANCONCELOS-COSTAS, Instituto Gulbenkian de Ciencia, Oeiras, Portugal
- F. AZERINE, Escuella Tecnica Superior de Ingenieras Industriales, Barcelona, Spain
- Ph. d'OULTREMONT, Solvay Co, Bruxelles, Belgium
- J. ROMMELAERE, A. BRANDENBURGER, P. CAILLET-FAUQUET, F. DUPONT and P. SEGELAERE, Université Libre de Bruxelles, Belgium

2. AIM

The group developed from a common commitment in the construction of linear vectors which could be used as artificial minichromosomes for the transfer and maintenance of foreign genes in animal cells.

Minimal requirements for such constructs consist of an origin of replication, telomeres and a centromere allowing the initiation and completion of DNA replication and the proper segregation of the vector, respectively. Given our poor present knowledge of these 3 components of mammalian chromosomes, the different teams are using homologous elements from lower eukaryotes or animal viruses, hoping that they will be functional in mammalian cells after cloning in an appropriate vector.

The participating teams therefore share a common goal and use similar biotechnologies, yet they study different model systems. The groups therefore decided to join together, in order to profit by their unity at the technical level and their complementarity at the biological material level.

3. SYNERGISTIC RESEARCH ACTIVITIES OF THE DIFFERENT GROUPS

This ELWW makes it its ambition to deliver recommendations

state of the art still requires one to assess a variety of candidate DNA sequences from different sources, as putative components of animal minichromosomes. This diversity was apparent from the progress reports but does not mean a lack of coordination of different activities. Actually, research programmes are concerted at three levels:

a) The work is distributed among participants so that unnecessary duplications are avoided, complementary lines of investigation are followed and a variety of biological systems (ranging from viruses to mammalian cells) are screened. Research in the ELWW encompasses work on the three essential elements of artificial minichromosomes mentioned above, i.e. origins of DNA replication, chromosomal telomeres and centromeres. Different model systems are used to clone and test candidate DNA sequences for these 3 components. The distribution of work on these systems is as follows:

| | Vector constituents | | | | |
|---|---|---|---|---|---|
| Teams | Telomeres | Origins of replication | Centromeres | Others | Systems from which vector constituents are derived |
| Pirbright | x | x | | | Bovine papilloma virus: Capriopoxvirus; Tetrahymena, Yeast |
| Roma | x | x | x | | Bovine papilloma virus; Tetrahymena; Plasmodium; Yeast, Mammalian cells |
| Tübingen | x | x | | | Bovine papilloma virus; Tetrahymena; Yeast |
| Pavia | | x | | | Mammalian cells |
| Brussels | x | | | | Parvoviruses |
| Oeiras | x | x | | | African swine fever virus |
| Barcelona | | | | x | Simian virus 40; Mammalian cells |

b) In addition, scientists and biological materials are exchanged between different teams in order to perform joint experiments which aim, in particular, at combining some of these DNA elements on a single vector. For example, recombinants comprising putative origins of DNA replication from mammalian cells and genomic telomeres of animal parvoviruses, respectively cloned in Pavia and Brussels, are in the process of construction and testing. Several other examples of cooperations of this sort were given in the progress reports.

c) Another area of cooperation is methodology, since technical problems to be faced are often similar, irrespective of the system studied. As an example, the Pirbright's group discussed during this meeting the conditions of filed inversion gel electrophoretic methods suitable for measuring the sizes and analyzing the structures of larger constructs.

4. THE INDUSTRIALIST'S POINT OF VIEW
P. d'Oultremont

Why industrial companies should participate to BAP or later on BRIDGE, ECLAIR ... EEC research programmes.

As far as SOLVAY is concerned these are some reasons of which we want to indicate the most important ones.

1) By definition industry is not performing basic but applied research in order to transform scientific discoveries in useful products. It is therefore essential to stay in close contact with Universities and Scientists.

2) It seems important to indicate to the scientific community how industry sees the future needs in some market segments and thus in which direction Scientists should concentrate their efforts.

As a resume SOLVAY will always be prepared to participate to such kind of action provided we can work with first class research teams and provided the programmes fit with our product lines.

EUROPEAN LABORATORY WITHOUT WALLS

BAP-272-UK MILLER
BAP-284-UK GILLHAM
BAP-285-F KORDON

# In the field of

CELLULAR NEUROIMMUNOLOGY

# PROCEEDINGS OF THE MEETING "PERSPECTIVES IN CELLULAR NEUROIMMUNOLOGY"

Held in Paris on June 17 and 18 1988

## INTRODUCTION

Thirty scientists representing eighteen academic and four industrial laboratories from eight countries of the European Economic Community met on June 17 and 18 1988 in Paris. They reviewed current trends and work in progress in their own laboratories within the field of neuroimmunoloyg.

The analysis of common properties of interactions between the nervous, the endocrine and the immune systems is developing very rapidly. It requires multi-disciplinary approaches, and is likely to yield important clinical applications in particular a better understanding of

1) immunological processes underlying aging,
2) mechanisms involved in natural or drug induced immunomodulation,
3) the relationship between coping behaviour and immune disorders, including autoimmune diseases and facilitation of tumoral growth,
4) the transfer to the brain of feedback information concerning challenges to the immune system, and
5) use by viruses or bacteria of signal molecules involved in communication within the nervous of the immune system, and which may thus mediate infection of neural or immune cells.

In addition, neuroimmunological research is also of potential biotechnological interest. It includes definition of new cellular models useful for toxicology, in particular immunotoxicology, as well as for the design of new drugs (immunomodulators; substances acting directly on postreceptor transduction levels, but also molecules which may affect access of toxins or infectious vectors to the nervous system.

Most data generated in this area over the last two years originate from North-American laboratories, and have mostly raised interest so far from US pharmaceutical companies. The participants to the meeting agreed however that, taken as a whole, the European research potential is far from negligeable; its efficiency could be significantly increased by procedures aimed at facilitating cooperation between teams developing complementary approaches, and by closer contacts between those laboratories and European companies.

## MAJOR ORIENTATIONS OF PROSPECTIVE INTEREST

Four major orientations were reviewed during the meeting:

### 1. General aspects of neuroimmune communication

Data accumulated over recent years show that discrete lesions of the nervous system, changes in the activity of determined neural networks or behavioral parameters can affect immune responses. More recently, those data were substantiated by observations indicating that neuropeptides can affect directly various functions, such as the rate of mitosis or of gene expression of T or B lymphocytes and conversely, that interleukins can have a direct impact on hypothalamic or hypophyseal structures responsible for neuroendocrine regulation.

Those discoveries are important, because they provide the first cellular and molecular correlates likely to account for interactions between the brain and the immune system. A certain number of observations however require further confirmation. In particular, actions of lymphokines on the brain or the endocrine system do not necessarily involve transfer of information from the immune system; they could be produced locally, or involve direct communication between lymphocytes and non immune tissue under particular homing conditions only. Mapping of brain circuits involved in neuroimmune regulation has never been attempted with modern neuroanatomical methods. Characterization of neuronal networks involved in either neuroendocrine mediated or direct (via the peripheral innervation of lymphoid organs) effects on the immune system should thus be encouraged. Finally, improvement of immune parameters used for studying immunological correlates of copying would yield further insight into the mechanisms involved, and provide models for studying some of its important practical of theoretical aspects, as the hemispheric dependence of immune disorders.

### 2. Expression of neuropeptide genes and receptors by lymphocytes

Among receptors expressed by lymphocytes, several correspond to peptide or transmitters released by nerve or epithelial cells within the thymus or other lymphoid organs. These signalling systems; as well as tight junctions present in the thymus, have been shown to play an important role in lymphocyte differentiation. This process could be an important level of neurogenic effects on immune cells. For instance, immunodeficient states could be due to inadequate migration of nursing cells; tumoral cells have been shown to secrete immunomodulators interfering with thymic maturation of lymphocytes; tachykinins are likely involved in the pathogeny of rheumatoid diseases; antidromic release of neuropeptides in the thymus has been reported to occur under the influence of nociceptive stimuli, and thymocytes have been reported to undergo suicide processes when exposed to excess levels of corticosteroids. In addition,

different antigens or toxins can induce expression of specific hormone genes. Differentiated lymphocytes are also sensitive to circulating peptide hormones as Beta-endorphin, ACTH, prolactin and several others. Beta-endorphin for instance influences the expression of the T3 complex by a differential action on its subunits, an action which parallels that of the hormone on lymphocyte proliferation; the process might involve changes in the recognition capacity of antigens by T cells.

Several of those aspects await further investigation. In particular, the types of Beta-endorphin receptors underlying effects of the hormone on lymphocytes are not satisfactorily characterized; some of them are not naloxone dependent, suggesting mediation by a possible non opiate receptor. Formal characterization of prolactin receptors is still pending. Those questions will have to be answered before the relevance of effects mentioned above can be assessed.

3. Membrane transduction of lymphocyte signals

Postreceptor coupling mechanisms and transduction signals have been less extensively studied in the immune system than in several other tissues, as for instance the nervous or the endocrine systems. They are likely to provide interesting endpoints for analyzing the mechanisms involved in sequential stimulation or double check of immunocompetent cells, in immunomodulation, or in the onset of refractory periods following discrete patterns of stimulation. In addition, they could provide new tools for immunotoxicology or the design of immunomodulatory drugs.

So far, a few data suggest that antibodies direct against CD3 receptors or interleukin 2 may affect phospholipase C and generate phosphoinositide and diacylglycerol signals which, in turn, end up in protein kinase C activation. Preliminary data suggest that some of those processes might depend upon GTP binding proteins, in particular since they appear affected by pertussis or by cholera toxins. In parallel, these or other signals affecting lymphocytes modulate the activity of calcium channels. Systematic studies of the nature and the possible specificity of G proteins subunits expressed by lymphocytes and on the potentialisation or the reciprocal inhibition of second messengers upon exposure to combined signals are still lacking. Complete characterization of second messengers could provide early signals to analyze the impact of natural or artificial immunomodulators, as for instance Beta-endorphin, prolactin, steroid hormones or immunosuppressors.

4. Cytokine actions on non immune tissue

Actions of cytokines on the brain raises several unanswered problems. The inventory of IL1 or neuroleukin effects on neurons or glial cells in culture, or on the brain itself, must now be approached in a more systematic manner. Questions to be asked

concern for instance: the possibility that IL1 could be produced within neural or glial cells themselves, a hypothesis which could be approached by biochemical or in situ hybridization techniques; characterization of IL1 receptors and receptor expression in the brain by appropriate molecular probes and their localization within brain structures; the possible function of sequence homologies between neuroleukin and viral or bacterial agents able to infect the nervous system. Similar questions can be addressed to other tissues which receive lymphocyte information, as neutrophils, platelets or erythrocytes.

PROPOSALS

1. As a conclusion ot the scientific discussion, the participants to the meeting identified topics ("burning questions") which could be immediately and efficiently addressed on the basis of discrete cooperative projects between members of the panel. Those are:

   - Pharmacological characterization of peptide or transmitter receptors on lymphocytes and assessment of their identity to corresponding neuronal receptors.
   - Pharmacological characterization of lymphokine receptors in the nervous system.
   - Regulation of the expression of cytokin receptors in non immune tissue or neuropeptide receptors in lymphocytes.
   - Characterization of coupling mechanisms involved in lymphocyte signalling.
   - Inventory and role of lymphocyte (and thymocyte) G proteins.
   - Mechanism of action of peripheral hormones (Beta-endorphin, prolactin, steroid hormones) on lymphocytes.
   - Regulation of the production of neuropeptides by lymphocytes.
   - Functional neuroanatomy of neuroimmunological circuits and of thymic innervation.
   - Characterization of signalling processes in homing models or during pregnancy.

2. On those topics, rapidly developing short term cooperations could be initiated by light procedures. A limited number of initial cooperative studies between members of the panel are already under way; the project coordinators listed below have committed themselves to propose further pilot studies before the end of 1988. Generation of preliminary data would greatly benefit from support of a few investigator exchanges or postdoctoral fellowships.

3. At a later stage, cooperative research within European teams could be greatly enhanced by developing three distinct subprogrammes within the general area of cellular neuroimmunology. Each of those subprogrammes or networks would associate a more restricted number of participating

laboratories and approach the field at a distinct level:

a. Selection of appropriate models; analysis of immunomodulatory effects of antigens, antibodies, cytokines, neuropeptides and hormones on thymocytes and lymphocytes; characterization of receptors and expression of the corresponding genes, coupling proteins, second messengers, protein kinases and ionic channels; discriminative approach of the coupling mechanisms involved respectively in cell proliferation and expression of specific genes.

Proposed coordinator: P. GIERSCHIK, assisted by A. ENJALBERT and G. FILLON.

Participating laboratories*:

1. (Characterization of vasopressin and oxytocin receptors, of their coupling properties and their effect on T lymphocytes).
2. (G proteins technology).
3. (Measurement of second messengers after discrete sequences of T cell stimulation).
4, 9, 11. (Models of CDE2, CD3, IL2 receptor stimulation).
5. (Gamma interferon messengers).
7. (Neuropeptide expression).
8. (Pharmacology of 5HT 1 and 2 and of prolactin receptors).
14. (Selection of cell lines).
16. (cDNA probes for receptors and oncogenes).
17. (Role of polyamines and ionic channels).

b. Characterization of biosynthesis, the distribution and the receptors of lymphokines in the nervous system, by both in vivo (quantitative autoradiography, in situ hybridization) and in vitro (primary cultures of nervous and glial cells). Analysis of their effects on neuronal growth and differentiation, on neurotransmitter secretion and release and on the hypothalamo-hypophyseal unit. Neurotransmitter regulation of cytokine secretion in the nervous system.

Proposed coordinator: C. KORDON, assisted by J. MARJAN.
Participating laboratories:

3. (Neurons and glial cells in primary culture).
8. (In situ hybridization techniques).
10. (Mutant models, glial cell lines).
15. (Perifusion of hypothalamic slices).

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laboratory number refers to the list of participants

c. Improvement of in vivo models available for the study of neuroimmune interactions. Mapping of neuronal circuits controlling hormone mediated immunomodulation or affection the extrinsic peripheral innervation of immune tissues, immunological correlates of behavioral patterns or coping and non coping situations, effect of cytokine on higher neural functions.

Proposed coordinator: R. DANTZER, assisted by K. MILLER

Participating laboratories:

1. (Human cytokine radioimmunassays, thymus immunocytochemistry).
3, 8. (Receptor autoradiography).
5. (Role of hormones and transmitters in autoimmunity).
10. (Genetic models).
12, 15, 18. (Neuroanatomical techniques in humans and rodents).
14. (Brain immune relationships after specific immune suppression by viruses, bacteria and parasites).
16. (Pharmacology of the thymus).

Industrial laboratories will be associated to the development of each topic.

Each of those subprogrammes could be coordinated separately; a general meeting of the three programmes should be organized every year or every second year to review work in progress in the whole area and adjust the objectives of the action.

## PERSPECTIVES IN CELLULAR NEUROIMMUNOLOGY

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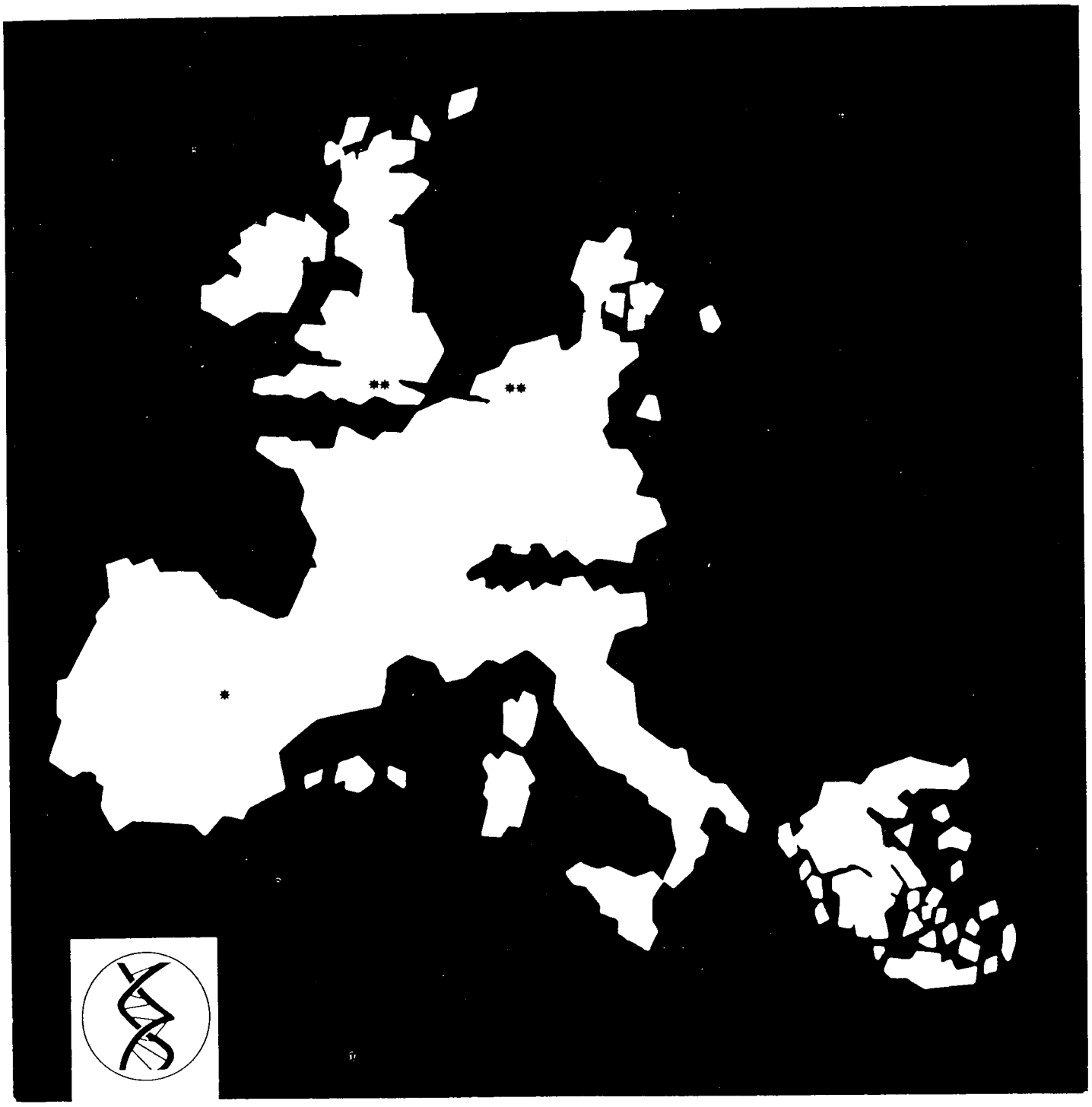
E DOMINGO

# In the field of

GENETIC ENGINEERING OF
FOOD- AND-MOUTH DISEASE VIRUS ANTIGEN



Report on working seminar of ELWW entitled
"Genetic Engineering of Foot-and-Mouth Disease Virus Antigen" held at Rhenen, NL, February 28 - March 1, 1988

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| Klepsch, A. | CEC observer. |

**DAY 1. RESEARCH REPORTS**

The field was divided into the five subject areas, listed below. This programme attracted a total of 21 contributions. Each was followed by a period of discussion. Main highlights were as follows.

1) Synthesis, processing and assembly of capsid proteins in heterologous expression systems. Virion proteins can be made in a cell-free translation system, processed, and assembled into structures resembling capsids, although the products appear to be unstable. N-terminal myristoylation of VP0 was not necessary for assembly. Much attention was paid to finding

how much of the nonstructural protein coding region of the genome must be included to ensure proper processing. Two virus-coded proteolytic activities (L and 3C) were characterised, and a third (presumably 2A) implicated. Unfortunately, these activities are extremely fastidious; even a minor alteration to the substrate, or to a protein encoded next to the putative enzyme, can inhibit processing.

The use of recombinant DNA viruses (vaccinia virus or baculovirus) to express the complete capsid protein region in animal cells was described. Synthesis of the capsid precursor, and limited proteolytic processing, was demonstrated using both vectors. However, it has so far proved impossible to make recombinants of either vector containing the L protease gene, implying that its product is toxic. Moreover, we are a long way from achieving the high level of expression that was planned with baculoviruses. Ways of solving both problems were discussed.

As an alternative to expressing whole capsids, the immunogenic region of VP1, residues 140-160, was inserted into the pre-S2 sequence attached to hepatitis B virus surface antigen, and expressed in yeast. The immunogenicity of the product has still to be determined.

2) Engineering live virus. A method for recovering live virus from cDNA clones of foot-and-mouth disease virus continues to elude us. Two groups are currently working on this important problem, following quite different approaches; both described encouraging recent progress.

3) Virion structure and sequence alignments. We can expect the complete atomic structure of foot-and-mouth disease virus shortly; 80% of the x-ray reflections, which are of exceptional quality, have already been measured. Currently, the information available is still sketchy, although we are told that the deep cleft in the virion surface, and the elevated 5-fold axis of symmetry which it surrounds, are less pronounced than in rhinovirus. This was confirmed independently by model building. On sequence alignments, much molecular epidemiological data, and a mathematical model of evolutionary changes, were described.

4) Antigenic structure. The five speakers in this section described the use of monoclonal antibodies for studying variation among natural virus isolates and mapping neutralizable epitopes. Interestingly, variation among a group of type C viruses occurred at epitopes recognised by neutralising antibodies, but not nonneutralisable epitopes, a good illustration of the driving force of antibody selection in nature. Four Groups had tried to locate antigenic sites by mapping antibody-escape mutations. All agreed that there are several, maybe five, independent sites but only one site, the 140-160 region of VP1, was identified unequivocally as an antibody-binding site. There was also evidence for a site at residues 43-48, and a third in VP2. Conflict was noted between the results of this genetic method of mapping antigenic sites, which has been well authenticated with other picornaviruses, and those obtained by peptide competition studies. It is hoped that these conflicts will be resolved when we know the structure of the virus.

5) Host immune response. There is now a wealth of detailed information on the immune response of model animal species (mice and guinea pigs) to one particular epitope, viz the 140-160 region of VP1. It is estimated that 10-40% of the neutralising antibodies in cattle sera are directed against this site. Increasing the molecular size of the peptide-containing immunogen generally increases potency but may decrease coverage. Recent work

showing that the ability to respond to the peptide depends on inclusion of an epitope recognised by the host's helper T-cells was reviewed. The most potent peptide vaccine yet developed consists of residues 140-160 of VP1 fused to the amino terminus of hepatitis B virus core antigen.

DAY 2 (am) DECISIONS

The local organiser introduced the proceedings by commending not only the excellence of the work that had been described the previous day, but also the candour with it was reported. He noted that there is a long and happy tradition of cooperation in the international fight against foot-and-mouth disease. A considerable amount of collaborative research already occurs outside formal CEC contracts. A good example, which members of the ELWW would do well to follow, is the epitope mapping work, described by G Belsham, which is to be published with an authorship drawn from five different countries. The main conclusions of the meeting were as follows:

1) The need for a vaccine. Foot-and-mouth disease is still enzootic in parts of the European Community, and poses a threat to Europe's eastern border. For the foreseeable future it will be imperative to maintain an ability to vaccinate. For this purpose, and for the eventual control of the disease worldwide, a safe and stable vaccine is needed.

2) Current status of research. Research into new vaccines had been following two strategies, one aimed at expressing the entire virus capsid, the other, individual epitopes. The first was proving difficult owing to the functional complexities of the virus genome, toxicity of one gene product, and disappointing levels of expression. Nevertheless, these projects were at a very early stage, and encouraging progress had already been made. The second approach, with many more years' work behind it, had made great progress, with at least one potentially marketable product on the horizon. Here, the main problem is that few of the data on peptide immunogenicity, or antigenic sites, relate to the animal species we wish to protect, the cow and the pig. The meeting concluded that there is an urgent need for more basic immunology in these ungulates.

3) Future cooperation between members of the ELWW. The following decisions were taken:

a) Expert advice/assistance will be sought from bovine/porcine immunologists. Action: F. Brown

b) A monthly foot-and-mouth disease virus bibliography will be made available to ELWW members by the IAH library at Pirbright. Action: A. King

c) The computer database of foot-and-mouth disease virus nucleic acid sequences, set up by Mr. N.J. Knowles, World Reference Laboratory, IAH, Pirbright, will be made available to members. Action: A. King

d) It was agreed to convene a second meeting of the ELWW, with the same number of participants, in one year's time. Action: E. Domingo

A.M.Q. KING
Local Organiser

# INDEXES FOR VOLUMES 2-3-4

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# PROJECT LEADERS

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* : volume
** : page

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# KEYWORDS

European Communities — Commission

**EUR 11650 — Biotechnology action programme – Progress report 1988 Volume 1: An overview**

Edited by: *E. Magnien*

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The biotechnology action programme (BAP) was adopted on 12 March 1985 for a period of five years and led to the conclusion, throughout the year 1986 and early in 1987, of a total of 262 research contracts. The purpose of this action is to allow the continuation of a previous biomolecular engineering programme (BEP) and its extension to new areas considered as essential for the development of biotechnology in the Community. Further supported have been the sectors on second generation bioreactors, on genetic engineering — covering the whole range of methods applying to animal husbandry, veterinary medicine, plant improvement, crop protection, beneficial soil micro-organisms — and on risk assessment. New domains, which BAP now also addresses, include contextual measures for the pooling and improvement of infrastructures for R&D (storage and treatment of biological data, collections of biotic materials), as well as specific projects for protein design, the applications of genetic engineering to industrial micro-organisms, the development and upscaling of novel methods for *in vitro* cell cultures. A special effort is also being initiated in the area of *in vitro* systems for the screening and assessment of pharmacological properties and toxicological effects of new molecules.

A. This is the second of a series of four progress reports, to cover on a yearly basis the period 1985-89 of the execution of the BAP programme. The first of this series, published last year, only presented two thirds of the activities foreseen by the programme, at a very preliminary stage of implementation. Due to a stepwise start of contracts, it is only with this second publication that all contributions of the 262 scientific contractors who participate in BAP can be reviewed in detail. One striking development, resulting from gradual integration of research in the framework of 93 transnational projects, certainly is the constitution of multipartner European Laboratories Without Walls (ELWWs), with provisions for exchanges and with division of tasks. This is how the Community hopes to be able to circumvent some of the academic and geographical barriers which obstruct this multidisciplinary field.

B. The programme will be revised by Council before the end of 1988, and may then expand further by an increase of activities in selected areas and by the entry of Portuguese and Spanish participants. In addition to this research programme, a training programme is run in parallel with a capacity of 60 grants a year covering all biotechnology related fields. Reports on the training programme are issued separately on a periodical basis.

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