

7-2. 294-191

Net 340.-

Hormones and Cell Regulation (n° 14)

Hormones et Régulation Cellulaire (n° 14)

0275 81969

12/90/1859

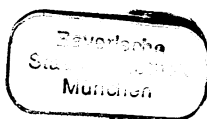
Colloques INSERM

ISSN 0768-3154

Other *Colloques* published as co-editions by John Libbey Eurotext and INSERM

- 133** Cardiovascular and Respiratory Physiology in the Fetus and Neonate. *Physiologie Cardiovasculaire et Respiratoire du Fœtus et du Nouveau-né.*
Scientific Committee : P. Karlberg,
A. Minkowski, W. Oh and L. Stern;
Managing Editor : M. Monset-Couchard.
ISBN : John Libbey Eurotext 0 86196 125 0
INSERM 2 85598 340 1
- 134** Porphyrins and Porphyrrias. *Porphyries et Porphyries.*
Edited by Y. Nordmann.
ISBN : John Libbey Eurotext 0 86196 087 4
INSERM 2 85598 281 2
- 137** Neo-Adjuvant Chemotherapy. *Chimiothérapie Néo-Adjuvante.*
Edited by C. Jacquillat, M. Weil and D. Khayat.
ISBN : John Libbey Eurotext 0 86196 125 0
INSERM 2 85598 340 1
- 139** Hormones and Cell Regulation (10th European Symposium). *Hormones et Régulation Cellulaire (10^e Symposium Européen).*
Edited by J. Nunez, J.E. Dumont and R. J.B. King.
ISBN : John Libbey Eurotext 0 86196 125 0X
INSERM 2 85598 340 1
- 147** Modern Trends in Aging Research. *Nouvelles Perspectives de la Recherche sur le Vieillessement.*
Edited by Y. Courtois, B. Fauchoux, B. Forette,
D.L. Knook and J.A. Tréton.
ISBN : John Libbey Eurotext 0 86196 126 0X
INSERM 2 85598 340 1
- 149** Binding Proteins of Steroid Hormones. *Protéines de liaison des Hormones Stéroïdes.*
Edited by M.G. Forest and M. Pugeat.
ISBN : John Libbey Eurotext 0 86196 125 0
INSERM 2 85598 340 1X
- 151** Control and Management of Parturition. *La Maîtrise de la Parturition.*
Edited by C. Sureau, P. Blot, D. Cabrol, F. Cavallé
and G. Germain.
ISBN : John Libbey Eurotext 0 86196 125 0
INSERM 2 85598 340 1

Suite page 123



Hormones and Cell Regulation

Hormones et Régulation Cellulaire

Proceedings of the 14th INSERM European Symposium on Hormones and Cell Regulation, held at Mont Sainte-Odile (France), September 25-28, 1989

Sponsored by the Institut National de la Santé et de la Recherche Médicale (INSERM)

Edited by

J. Nunez

J.E. Dumont

LES EDITIONS
INSERM 

 John Libbey
EUROTEXT
LONDON · PARIS

British Library Cataloguing in Publication Data

INSERM European Symposium on Hormones and
Cell Regulation (*14th, 1989, Sainte-Odile, France*)

Hormones and cell regulation

1. Organisms. Cells. Metabolism. Regulation.

Role of Hormones

I. Title. II. Nunez, J. (Jacques), 1927

III Dumont, J.E. (Jacques E.), 1931

IV. Séries

574.87'61

ISBN 0-86196-229-X

First published in 1989 by

John Libbey Eurotext Ltd

6 rue Blanche, 92120 Montrouge, France. (1) 47 35 85 52
ISBN 0 86196 229-X

John Libbey & Company Ltd

13 Smiths Yard, Summerley Street, London SW18 4HR,
England.
(1) 947 27 77

**Institut National de la Santé et de la Recherche
Médicale**

101 rue de Tolbiac, 75654 Paris Cedex 13, France.
(1) 45 84 14 41
ISBN 2 85598 400 9

ISSN 0768-3154

© 1989 Colloques INSERM/John Libbey Eurotext Ltd,
All rights reserved
Unauthorized publication contravenes applicable laws

Proceedings of the annual European Symposia on **Hormones and Cell Regulation** held every autumn since 1976 have been published by Elsevier Biomedical Press in a series co-edited by INSERM (*) from Volume 1 to Volume 9.

The 10th Symposium held in 1985 and the next ones are now published as co-editions by John Libbey Eurotext and INSERM in the series "Colloques INSERM" (Vol. 139, Vol. 153, Vol. 165, Vol. 176, Vol. 198).

(*) except Vol. 7 published by Elsevier

Contents

Sommaire

- VII Préface
- IX Foreword
- X *Avant-propos*

- XII List and address of speakers
Liste et adresse des orateurs

SURFACE RECEPTORS AND CYCLASES *RÉCEPTEURS DE SURFACE ET CYCLASES*

- 3 **M. Chinkers**
Guanylate cyclase as a cell surface receptor
La guanylate cyclase comme récepteur de surface cellulaire
- 7 **P.J.M. Van Haastert**
Transmembrane signal transduction in *Dictyostelium mutans*
Transduction transmembranaire du signal chez Dictyostelium mutans
- 15 **Y. Salomon**
Ca²⁺ requirement of MSH-receptor function : an unusual example among G-protein associated peptide hormone receptors
Exigence en Ca²⁺ de la fonction récepteur de la MSH : un exemple inhabituel parmi les récepteurs d'hormones peptidiques associés à la protéine G
- 21 **F. Fahrenholz, H. Luzius and D.A. Jans**
Irreversible V2 vasopressin receptor activation : effect on down-regulation of the c-AMP mediated cellular response
Activation irréversible du récepteur V2 de la vasopressine : effet sur le rétrocontrôle négatif de la réponse cellulaire médiée par l'AMPc
- 27 **J.M. Henley and E.A. Barnard**
AMPA-sensitive excitatory aminoacid receptors in the chick brain
Récepteurs d'acides aminés excitateurs sensibles à l'AMPA dans le cerveau de poulet
- 33 **F. Libert, M. Parmentier, A. Lefort, C. Dinsart, J. Van Sande, C. Maenhaut, M.J. Simons, J.E. Dumont and G. Vassart**
Homology cloning of cDNAs amplified by the polymerase chain reaction. Identification of four new members of the G-protein coupled receptor family
Clonage par homologie d'ADNc amplifiés par la réaction en chaîne de la polymérase. Identification de quatre nouveaux membres de la famille des récepteurs couplés à la protéine G

REGULATION OF GENE EXPRESSION
RÉGULATION DE L'EXPRESSION GÉNÉRIQUE

- 39 **M. Schatt, S. Wieland and S. Rusconi**
Promoter and enhancer activation properties of the glucocorticoid receptor
Propriétés d'activation du promoteur et de l'amplificateur du récepteur des glucocorticoïdes
- 45 **M. Boshart, F. Weih, A. Schmidt, D. Nitsch, F. Stewart and G. Schütz**
Transcriptional regulation of the tyrosine aminotransferase gene : structure of a regulatory switch
Régulation transcriptionnelle du gène de la tyrosine aminotransférase : structure d'un commutateur régulateur
- 49 **E.R. Simpson, S. Graham-Lorence, C.J. Corbin, J.C. Merrill, M.S. Mahendroo and C.R. Mendelson**
Regulation of expression of the gene encoding aromatase cytochrome P-450
Régulation de l'expression du gène codant pour l'aromatase cytochrome P-450
- 57 **P. Flores, A. Gutman, J.L. Imler, A. Lloyd, J. Schneikert, C. Wasyluk and B. Wasyluk**
AP1 and PEA3 are nuclear targets for transcription activation by non-nuclear oncogenes
AP1 et PEA3 sont des cibles nucléaires de l'activation de la transcription par des oncogènes non nucléaires

GROWTH FACTORS
FACTEURS DE CROISSANCE

- 65 **T.J. Velu, P. Martin, W.C. Vass, K. Helin, A. Ritzhaupt, L. Beguinot, J.T. Schiller and D.R. Lowy**
The epidermal growth factor receptor and its role in cell transformation
Le récepteur du facteur de croissance épidermique et son rôle dans la transformation cellulaire
- 71 **M. Mallat, B. Chamak, C. Theyry and D. Leroy**
Microglial cell functions during brain development
Fonctions des cellules microgliales pendant le développement du cerveau

CONTROL MECHANISMS IN OTHER SYSTEMS
MÉCANISMES DE CONTRÔLE DANS D'AUTRES SYSTÈMES

- 79 **A. Diu, M. Fevrier, P. Mollier and J. Thèze**
Control of B cell activation in humans
Contrôle de l'activation des cellules B chez l'homme

- 85 **R. Bernards, J.M. O'Brien, D.M. Marcus, D.M. Albert, T. Jacks and R.A. Weinberg**
Towards an animal models for retinoblastoma
Vers un modèle animal du rétinoblastome
- 91 **H.R. Schöler, R. Balling, A.K. Hatzopoulos, N. Suzuki and P. Gruss**
Transcriptional activity of the octamer motif in embryonic stem cells and preimplantation embryos
Activité transcriptionnelle du motif octamérique dans les cellules souches embryonnaires et les embryons de préimplantation

ION CHANNELS
CANAUX IONIQUES

- 99 **S.J.H. Ashcroft and F.M. Ashcroft**
The role of the ATP-sensitive K-channel in stimulus-response coupling in pancreatic β -cell
Le rôle du canal potassium sensible à l'ATP dans le couplage stimulus-réponse de la cellule β du pancréas
- 105 **E.E. Strehler, R. Heim, R. Fisher, P. James, T. Vorherr, G. Vogel, M.A. Strehler-Page and E. Carafoli**
The plasma membrane Ca^{2+} pump : structural, functional and genetic aspects of isoform diversity
La pompe Ca^{2+} de la membrane plasmique : aspects structuraux, fonctionnels et génétiques de la diversité d'isoformes
- 111 **R. Serrano and R. Perona**
Growth control by proton transport : evolutionary considerations and novel approaches based on the cloned yeast proton pump
Contrôle de la croissance par le transport de protons : considérations évolutionnistes et nouvelles approches basées sur la pompe à protons clonée de la levure
- 117 **C. Frelin, P. Vigne and C. Van Renterghem**
Membrane events involved in the action of endothelin and other vasoconstricting hormones
Événements membranaires impliqués dans l'action de l'endothéline et d'autres hormones vasoconstrictives

Transcriptional regulation of the tyrosine amino-transferase gene : structure of a regulatory switch

Michael Boshart, Falk Weih, Andrea Schmidt, Doris Nitsch, Francis Stewart and Günther Schütz

Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG

INTRODUCTION

To understand the differentiation processes which yield the various cellular phenotypes, it is necessary to elucidate mechanisms by which genes are selectively expressed. Of particular importance for the establishment of a given pattern of gene activity is the interplay between controlling genes and signaling molecules such as hormones. Furthermore, it seems clear that tissue-specific patterns of gene activity depend on both positive and negative regulatory factors. The tyrosine aminotransferase (TAT) gene is an example of a gene whose expression is controlled by positive and negative factors and by hormones. TAT expression is regulated not only by glucocorticoids and by the peptide hormone glucagon acting via the cAMP pathway, but also by two genetically defined, trans-acting loci (Fig. 1).

First, a dominant negative regulator, termed tissue-specific extinguisher (*Tse-1*), has been defined by intertypic somatic cell hybrids and mapped to a small region of human chromosome 17 (Killary and Fournier, 1984; Lem et al. 1989).

Second, a positive regulator has been postulated to account for the lethal phenotype of certain albino mice carrying a homozygous deletion around the albino locus on chromosome 7. TAT as well as several other liver-specific enzymes are not expressed in the liver of these newborn albino mice (Gluecksohn-Waelsch, 1979). Since the structural gene for TAT is not deleted, and has been mapped to a chromosome other than 7, it has been suggested that the deletion at the albino locus removes a regulatory locus (Schmid et al., 1985).

Previously, we have shown that glucocorticoid responsiveness of TAT expression is conferred by a conditional enhancer 2500 bp upstream of the transcription start site (Jantzen et al., 1987). Here we show that a liver-specific enhancer even further upstream of the TAT promoter, mediates the effect of the dominant negative regulator *Tse-1* as well as responsiveness to cAMP. This enhancer has structural and functional characteristics of a signal transducer and we suggest that it works as a regulatory switch which controls the hormone triggered timed onset of expression of the TAT gene in the newborn liver.

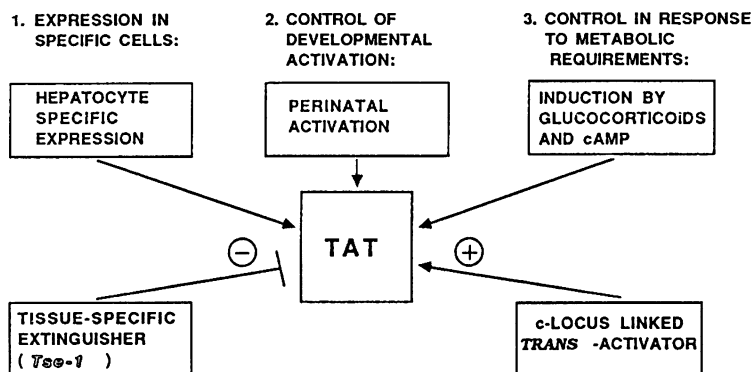


Fig. 1 Control of TAT gene expression

RESULTS AND DISCUSSION

To identify *cis*-acting sequences mediating the complex transcriptional regulation of the TAT gene, we fused the TAT promoter including different portions of its 5'-flanking region to the universal reporter gene chloramphenicol acetyltransferase (CAT) and transiently transfected these constructs into various cell lines. First, we found that a sequence 3600 bp upstream of the transcription start site strongly activated CAT expression in well differentiated rat hepatoma cells (FT02B) but not in rat fibroblasts. A series of 5'- and 3'-deletion mutants defined a sequence of 80 bp absolutely essential for transcriptional stimulation. This sequence conferred hepatoma-specific activation to the heterologous TK promoter. The same sequence also mediated response to hormonal signals transduced via the cAMP pathway. We also investigated whether this sequence was the target for dominant negative regulation by the product of the tissue-specific extinguisher (*Tse-1*) locus. To this end we transfected the same constructs used to define the liver-specific enhancer into a hepatoma microcell hybrid line (Lem et al., 1989) which contains a small segment of human fibroblast chromosome 17, carrying and expressing the *Tse-1* locus. The enhancer was inactive in this cell line. However, induction with cAMP was able to overcome extinction completely, thus revealing a functional antagonism between *Tse-1* and the signal transduction pathway.

As a first step towards understanding the mechanism of extinction of enhancer function by *Tse-1*, we sought to identify the specific sequences required for liver-specific enhancer activity, for induction by cAMP and for extinction by *Tse-1*. A complete set of clustered point mutants covering the enhancer was constructed and transfected into rat hepatoma cells. Within the 80 bp minimal enhancer fragment two short DNA sequences were found, each of which was absolutely essential for enhancer function. Each of the two sequences was inactive on its own, if placed in front of the heterologous TK promoter. However, multiple copies of these sequences conferred strong transcriptional stimulation onto the TK promoter.

A multimer of the distal 26 bp sequence was responsive to cAMP and was subject to negative regulation by *Tse-1*. Again the functional antagonism between *Tse-1* and the cAMP-pathway was observed; cAMP induction completely reversed the

negative regulation by Tse-1. In vivo footprinting revealed characteristic changes in DMS-reactivity at this sequence element which correlated with (i) cAMP induction, (ii) the presence of the Tse-1 carrying chromosome fragment, and (iii) the relief from extinction by cAMP.

A multimer of the proximal 18 bp element behaved as a liver cell specific activator of transcription. In the natural context of the enhancer the distal cAMP- and Tse-1-responsive element must cooperate with the proximal liver-specific element to overcome the requirement for multimerisation. Artificial combination of the 26 bp- and 18 bp-sequences created an element with all the regulatory properties of the entire TAT enhancer.

Thus, the structure of the enhancer exemplifies the concept of the modular architecture of regulatory elements: a cAMP responsive module and a liver-specific module must cooperate to make up a functional unit with regulatory properties unique to the combination of both. The strict cell-type specificity of this combination is guaranteed by a double control and by the absence of redundancy: both modules are absolutely essential and both are inactive in fibroblasts. In the case of the cAMP-responsive element, which might be recognized by an ubiquitous factor, Tse-1 exerts negative control in fibroblasts.

As the balance between Tse-1 activity and cAMP induction is critical for activity of one component which is synergistically interacting with a second component, the TAT enhancer must have the properties of a sensitive switch responding to changes in the Tse-1/cAMP balance with dramatic changes in overall activity. Therefore, one attractive model would give Tse-1 a central role in prenatal repression of TAT. Activity of Tse-1 in the liver would decrease during liver development rendering the repressed TAT gene increasingly responsive to hormonal stimulation towards the end of gestation. The timed onset of TAT expression around birth would then be triggered by the strong release of gluconeogenic hormones as a consequence of neonatal hypoglycemia. In fact, TAT can be induced prematurely, days before birth by administration in utero of glucagon (acting via cAMP) (Greengard, 1970).

REFERENCES

- Gluecksohn-Waelsch, S. (1979): Genetic control of morphogenetic and biochemical differentiation: lethal albino deletions in the mouse. *Cell* 16, 225-237.
- Greengard, O. (1970): The developmental activation of enzymes in rat liver. In *Mechanisms of Hormone Action, Vol. I*, G. Litwack, ed. (New York: Academic Press), pp. 53-85.
- Jantzen, H.M., Strähle, U., Gloss, B., Stewart, F., Schmid, W., Boshart, M., Miksicek, R., Schütz, G. (1987): Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. *Cell* 49, 29-38.
- Killary, A.M. and Fournier, R.E.K. (1984): A genetic analysis of extinction: trans-dominant loci regulate expression of liver-specific traits in hepatoma hybrid cells. *Cell* 38, 523-534.
- Lem, J., Chin, A.C., Thayer, M.J., Leach, R.J. and Fournier, R.E.K. (1989): Coordinate regulation of two genes encoding gluconeogenic enzymes by the trans-dominant locus Tse-1. *Proc. Natl. Acad. Sci. USA* 85, 7302-7306.
- Schmid, W., Müller, G., Schütz, G. and Gluecksohn-Waelsch, S. (1985): Deletions near the albino locus on chromosome 7 of the mouse affect the level of tyrosine aminotransferase mRNA. *Proc. Natl. Acad. Sci. USA* 82, 2866-2869.