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PERSPECTIVES

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News from Cairo: 18th International Conference on Arginine and Pyrimidines (ICAP 2002; 13–16 October 2002)

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The Arginine/Pyrimidine Conferences are biennial meetings that started as Arginine Workshops 36 years ago, organized by a group of microbiologists led by Werner Maas (New York University). Over the years they have become increasingly biochemical, but the microbiological flavor still prevails. In the year 2000 I was the organizer of the 17th meeting, held in Valencia, Spain, where it was decided that the next meeting would be organized by Ahmed Abdelal, then at Georgia State University at Atlanta, and now at Northeastern University, Boston. The meeting was held at the Agricultural Biotechnology Institute of Giza, in Cairo University, on October 13–16, 2002 under the auspices of the Egyptian Ministry of Agriculture. Ahmed was helped in the organization by his former associates at Georgia State, Chung Dar-Lu and Shebab Hashim and, locally, by Magdy Madkour (Agricultural Research Center, Cairo). One special invitee to the meeting was Professor John L. Ingraham (University of California at Davis), a senior and highly respected figure of world microbiology (Fig. 1).

The meeting consisted of half-hour oral presentations, with ample time for discussions. The first two presentations dealt with exotic organisms. Guy Hervé and Zoran Minic (Université Pierre et Marie Curie, Paris, France) reported studies on pyrimidine metabolism in *Riftia pachyptila*, a deep-sea hydrothermal-vent giant tubeworm hosting a bacterial endosymbiont that extracts metabolic energy from the oxidation of sulfide. They demonstrated the absence in the worm, and the presence in the endosymbiont, of the three initial enzymes of de novo pyrimidine biosynthesis, and the converse for the enzymes of the pyrimidine salvage pathway. Pyrimidine and arginine metabolism genes and enzymes of the extreme psychrophilic bacteria (maximal growth rates at 0–4°C) *Moritella profunda* and *Moritella abyssi* were reported by Ying Xu and Nicholas Glansdorff (Vrije Universiteit and Institute JM Wiame, Brussels, Belgium) to resemble those of enteric bacteria. These enzymes generally exhibit cold-adaptation, and a gene was identified that encodes a bifunctional protein having both argininosuccinase and acetylglutamate synthetase activities, which are the initial and final enzyme steps of the route of arginine biosynthesis. From the study of purified enzymes they concluded that cold-adaptation may become constrained by natural limits to the optimization of catalytic efficiency.

Following a more biotechnological trend, pyrimidine metabolism in the cheese starter culture microorganism *Lactococcus lactis* was the subject of three presentations from the Danish group of the Biocentrum-DTU in Lyngby (Jan Martinussen, Casper Jorgensen, Karin Hammer and Mogens Kilstrup). They have characterized the regulation of the expression of *pyrG* (the gene encoding CTP synthase) in *L. lactis* and have also described the existence of two nucleoside uptake systems and their impact on nucleotide pools. In addition, they reflected on the lack in *L. lactis* of the gene for nucleotide diphosphate kinase, an enzyme believed to be responsible for the synthesis of nucleotide triphosphates in all organisms. They concluded that, in *L. lactis*, this crucial enzyme is functionally replaced by pyruvate kinase, which provides a link between glycolysis and nucleotide metabolism.

Mammalian pyrimidine metabolism was the subject of the presentation by Frederic and Severine Sigoillot, David Evans and Hedeel Guy, from Wayne State University (Detroit, Mich.). They examined in cultured mammary normal and tumor cell-lines the phosphorylations by MAP and cAMP-dependent kinases that they have recently described in the pyrimidine-specific carbamoyl phosphate synthetase of the multifunctional protein CAD (a trifunctional single-polypeptide protein with carbamoyl phosphate synthetase, aspartate transcarbamylase and dihydroorotase activities). They

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Fig. 1 John L. Ingraham, Professor Emeritus, University of California at Davis, special invitee to the ICAP 2002 meeting. (Photograph published in Annu. Rev. Microbiol., vol 55, 2001)

concluded that these phosphorylations play an important role in the regulation of pyrimidine biosynthesis, thus explaining some of the differences between the pathway in normal and tumor cells.

Individual enzymes of pyrimidine metabolism were the subject of several presentations. Eukaryotic dihydroorotate dehydrogenase, an integral inner mitochondrial membrane flavoprotein that catalyzes the fourth step of pyrimidine biosynthesis, was studied by Alexandra Ullrich, Monika Löeffler, Kerstin Dieckert (Philipps University, Marburg, Germany), Wolfgang Knecht, Joh Rawls and Jure Piskur (BioCentrum-DTU, Lyngby, Denmark). They reported the first cloning, overexpression and characterization of a plant dihydroorotate dehydrogenase, that of *Arabidopsis thaliana*. They also reported that, in the *Drosophila melanogaster* enzyme, the N-terminal targeting sequence is important for biological function in vivo, but not for catalysis. Orotidine 5′-monophosphate decarboxylase, which catalyzes the last step of UMP synthesis, was studied by Jens-Christian Navarro Poulsen, Pernille Harris, Kaj Frank Jensen and Sine Larsen (University of Copenhagen and Technical University of Denmark, Lyngby). By using site-directed mutagenesis and X-ray crystallography of mutants they provided insight on how this enzyme is able to accelerate the reaction by a factor of 10^{17}. The mechanism of allosteric activation by GTP of CTP synthetase (the enzyme that makes CTP from UTP using glutamine and one ATP molecule) was studied by Martin Willemoës and Bent Sigurskjold (University of Copenhagen). They concluded that GTP promotes the coordination of UTP phosphorylation with glutamine hydrolysis. Frank Raushel, Tamiko Neal (College Station, Texas), Hazel Holden and James Thoden (University of Wisconsin) described the crystallographic 3-D structure of *Escherichia coli* dihydroorotase, the third enzyme of the pyrimidine biosynthesis pathway and a member of the amidohydrolase family; the enzyme also has a binuclear metal center. They also clarified the mechanism of substrate/product binding and proposed a mechanism of catalysis. Raymond Cunin, Sigrid Van Boxtael, Shakil Khan and Dominique Maes (Vrije Universiteit, Brussels, Belgium) reported the structure, at 1.8 Å resolution, of aspartate transcarbamylase (which catalyzes the second pyrimidine biosynthesis step) of the extreme hyperthermophilic archaeon *Pyrococcus furiosus*. They compared this structure with that of *E. coli* aspartate transcarbamylase, and used this comparison as a basis to reflect on the structural determinants of catalysis, cooperativity and thermostability in this enzyme. Octavian Barzu, Anne Marie Gilles, Helene Munier-Lehman, *Gilles Labesse and Liliane Assairi (Institut Pasteur and *Université de Montpellier, France) presented a structural model of *E. coli* UMP kinase, based on the structure of carbamate kinase determined in my laboratory, that can explain the results of site-directed mutagenesis and immunochemical studies. Partial characterization of the UTP allosteric effector site by tryptophan and nucleotide analog fluorescence and by site-directed mutagenesis was also reported.

The enzymes of arginine metabolism were the subject of several talks. There were three talks on the arginine biosynthetic enzyme acetylglutamate kinase, which catalyzes the second step of the arginine biosynthetic pathway and is, in many cases, the controlling point, being feedback inhibited by arginine. Richard L. Weiss, Suhn-Kee Chae, Jessica Chung, Catherine McKinstry, Carey Fei Li and Gloria E. Turner (University of California at Los Angeles) reported the use of mutants of the *arg-6* locus of *Neurospora crassa* as well as the two-hybrid system to map, in the C-terminal domain of acetylglutamate kinase, the sites of interaction with acetylglutamate synthetase. These two enzymes form a metabolon in both *N. crassa* and yeast. Katia Pawels, Agnes Abadjieva, Pierre Hilven, *Anna Stankiewicz and Marjolaine Crabeel (Vrije Universitiet, Brussels; and *Warsaw University, Poland)—the group that described the acetylglutamate kinase/synthetase metabolon in yeast—reported that, by knocking out arginine inhibition of yeast acetylglutamate kinase, the synthetase also becomes refractory to feedback inhibition. Conversely, they reported that deletion of the synthetase gene results in decreased arginine sensitivity of the kinase, stressing that the metabolon promotes coordination of the catalytic activities and of the feedback regulation of these two enzymes. Leonor Fernández-Murga, Santiago Ramón Maiques and myself (Instituto de Biomedicina de Valencia) reported that the acetylglutamate kinases that are inhibited by arginine have a N-terminal extension of 17–36 residues in comparison with the sequence of those acetylglutamate kinases that are not inhibited. By using the enzyme from *Pseudomonas aeruginosa* we showed that this extension is essential for feedback inhibition by arginine and is involved in arginine binding. By determining the crystal structure of *P. aeruginosa* acetylglutamate kinase we found that this enzyme forms a trimer of dimers in the shape of a doughnut, the three

dimers being connected by interlacing of the N-terminal extensions. In contrast, the structure of *E. coli* acetylglutamate kinase, which is not inhibited by arginine, is purely dimeric [Structure (2002) 10:329–342].

Human pathology of urea synthesis was the subject of a presentation by Igor Yefimenko, Vicente Rubio and Javier Cervera (Instituto de Investigaciones Citológicas and Instituto de Biomedicina, Valencia). In *E. coli* carbamoyl phosphate synthetase, they tested the effects of clinical mutations that have been identified in patients with carbamoyl phosphate synthetase I deficiency (a rare urea cycle defect), and rationalized the observations on the basis of the known structure of the *E. coli* enzyme.

Among the arginine catabolic enzymes, studies on the arginine deiminase from *Giardia intestinalis* were the subject of a communication from Abbas Koaik, Francoise van Vliet, Victor Stalon and Catherine Tricot (Université Libre and Institute JM Wiame, Brussels). They reported cloning of the gene and its expression in *E. coli*, characterizing the enzyme and having crystallized it in native and Se-Met derivative forms, with the goal of determining its 3-D structure by X-ray diffraction studies. Gloria E. Turner and Richard L. Weiss (University of California at Los Angeles) reported on the developmental expression of two major forms of arginase in *N. crassa*, and presented evidence that arginase plays a role in conidiagenesis and again in early germination.

Arginine metabolic pathways and their regulation at the level of gene expression was the subject of most other presentations. Yoshifumi Itoh and Yuji Nakada (National Food Research Institute, Tsukuba, Japan) studied the arginine dehydrogenase and decarboxylase pathways in *P. aeruginosa*. They reported the involvement of the former in D-arginine catabolism and of the latter in both agmatine catabolism and polyamine biosynthesis. Francoise Bringel and Jean-Claude Hubert (Université Louis Pasteur, Strasbourg, France) analyzed arginine auxotrophy in mesophilic *Lactobacillus*, in which they sought for gene alterations in the different isolates. The group of Georgia State University (Ahmed Abdelal, now at Northeastern University, Boston; Chung-Dar Lu, Mohamed Hegazy, Shehab Hashim and Hassan Wally) reported studies on the regulation of arginine metabolism in *P. aeruginosa*. They showed that ArgR, the homodimeric protein that controls arginine metabolism in this organism (and which does not resemble the enterobacterial regulators of the ArgR/AhrC family), is subject to carbon catabolite repression. They also characterized the effects of arginine and of ArgR on the regulation of L-glutamate metabolism and of the *adcAB* operon that encodes arginine decarboxylase. They identified the arginine decarboxylase gene and showed that its induction depends on ArgR and on an ArgR operator preceding the *adcAB* operon. The same group reported the use of a GeneChip from Affimetrix for *P. aeruginosa* to identify 23 ArgR-inducible and 22 ArgR-repressible genes. Daniel Charlier (Vrije Universiteit, Brussels) summarized the biochemical and structural determinants of the specific binding to DNA of ArgR (enterobacterial type) regulators, and of their mechanisms of action and requirements in recognition sequences. Hervé Nicoloff, Jean-Claude Hubert, *Michiel Kleerebezem, *Willem M. de Vos and Francoise Bringel (Université Louis Pasteur, Strasbourg, and *Wageningen Centre for Food Science, The Netherlands) reported the existence of two enterobacterial-like ArgR paralogs in *Lactobacillus plantarum* and showed

Fig. 2 A solid background for the participants in the ICAP2002: the old Pyramids

that both were required in arginine regulation. ArgR of *Thermotoga neapolitana* was the subject of the presentation of Amelie Morin, Frederique Braun, Diliana Dimova and Vehary Sakanyan (Université de Nantes, France), who shed light on the DNA binding specificity and arginine requirements of this regulator, and provided insight into understanding its evolutionary relationship with ArgR from other organisms. Protein-DNA and protein-protein interactions involved in transcriptional regulation of bacterial genes was the subject of another talk of the Nantes group (Marina Snapyan, Laetitia Guevel, Michele Lecoq, Marie-Claire Arnaud and Vehary Sakanyan), in which they described the use of a fancy protein chip methodology, utilizing probes labeled with near-infrared fluorescence dyes, to show that the *Bacillus stearothermophilus* PargCo promoter-operator region binds four types of regulatory proteins: ArgR repressors, the RNA polymerase sigma and alpha subunits and cAMP binding protein. Alexandre Wohlkonig, Victor Stalon and Corinne Vander Wauven (Université Libre and Institute JM Wiame, Brussels, Belgium) reported on another transcriptional regulator, ArcR, encoded in *Bacillus licheniformis* as a part of the arginine fermentative catabolic operon *arcABDCR*, which binds at 60 bp from the transcription origin and is required for transcription of this operon. They showed that *arcR* is expressed both as part of the operon and also monocistronically, and that the ArcR protein is active only in the reduced state (the oxidized form was insoluble). In this way, *arcR* promotes transcription only under conditions of oxygen restriction. Agnieszka Dzikowska, Joanna Empel, Rafal Tomecki and Piotr Weglenski (Warsaw University) enquired whether the Zn_2C_6 binuclear cluster transcriptional regulator of *Aspergillus nidulans* activates the expression of arginine catabolism genes together with some MADS-box protein. Apparently, it does.

At intervals during the conference there was some opportunity to practice camel riding, pyramid viewing (Fig. 2) and shop or bazaar bargaining. In one of these intervals the meeting participants decided that the next Arginine/Pyrimidine meeting, due in summer/fall 2004, will take place near Marburg, Germany, with Monika Leffler as the head organizer.