the substrate and coenzyme (pyridoxal-5-phosphate) binding region and the histidine moiety susceptable to covalent modification by the enzyme activated irreversible inhibitor α-difluoromethylornithine (DFMO); 157-170: "Asp(Glu)-Glu-Leu-Met-Lys-Val-Ala-Lys(Arg)-Ala(Thr)-His-Pro-Lys-Ala-Lys" (possible variations in parenthesis).

Antibodies raised against various epitopes of this particular peptide may help to answer the question why *Tetrahymena* ODC has a similar K_i-value for DFMO as *Trypanosoma* ODC but is almost insensitive to irreversible inhibition[1].

Antibodies against epitopes of the other peptides may help to decide whether degradation or modification processes are involved in the rapid inactivation of ODC or whether activation of preformed inactive ODC is the mechanism of the rapid stimulation of ODC - which is unlikely after preliminary cyclohexiimide inhibition assays[3].

1) Eichler, W. (1989) *J. Protozool.* **36,** 577-582.
2) Eichler, W. (1989) *Biol. Chem. Hoppe-Seyler* **370,** 1113-1126.
3) Eichler, W. (1990) *J. Protozool.* **37,** 273-277.
4) Hickok, N.J. et al. (1987) *DNA* **6,** 179-187.
5) van Kranen, H.J. et al. (1987) *Gene* **60,** 145-155.
6) Coffino, P. & Chen, E.L. (1988) *Nucleic Acids Res.* **16,** 2731-2732
7) Fonzi, W.A. & Sypherd, P.S. (1987) *J. Biol. Chem.* **262,** 10127-10133.
8) Phillips, M.A. et al. (1987) *J. Biol. Chem.* **262,** 8721-8727.
9) Eichler, W. (1987) *Biol. Chem. Hoppe-Seyler* **368,** 1036.

Wolfgang Eichler, Institut für Biochemie I der Universität, Im Neuenheimer Feld 328, D-6900 Heidelberg.

W. Eichler and B. Schertel

Regulation of Polyamine Biosynthesis by L-Arginine: A Common Regulative Principle in Eucaryotes

Previous studies have shown that the activity of ornithine decarboxylase (ODC, EC 4.1.1.17), the regulatory key enzyme of polyamine biosynthesis, is regulated at three levels in the protozoan *Tetrahymena thermophila*[1, 2, 3]: 1. the induction concomitant with growth stimulation; 2. the inactivation depending on polyamine excess; 3. the limitation of stimulation depending on the available substrate concentration (arginine/ornithine) independently of polyamine abundance.

The stimulation of growth starts a time program for ODC stimulation/inactivation which is modulated by substrate and product concentrations: L-arginine - and to a minor extent L-ornithine - concentrations in the medium influence ODC specific activity in a reciprocal relation; the lower arginine concentration, the higher is the ODC stimulation. Putrescine concentrations do not determine the degree of ODC stimulation but the duration of the stimulated state.

After the elucidation of the regulatory relationships, assays were performed to study whether - besides the known regulatory effects of growth and polyamines - regulatory effects of arginine can be observed in mammalian organisms. Actually, in juvenile rats which were fed an arginine free diet, hepatic ODC specific activity exceeded that of control animals, on the other hand, after arginine refeeding of those animals, ODC activity was decreased even below the control levels in either case without corresponding alterations of the hepatic polyamine content. These results are strong evidence that in mammalian organisms ODC activity is also regulated at the substrate level suggesting that this mechanism is a common regulative principle in eucaryotes.

What still remains to be studied is whether the regulation by polyamines of arginine degradation to ornithine and the regulation of ornithine concentration by ornithine-δ-transaminase which have been reported for *Tetrahymena thermophila*[4, 1] is also relevant in mammalian cells as supposed in a previous report[5].

1) Eichler, W. (1989) *Biol. Chem. Hoppe-Seyler* **370,** 1113-1126.
2) Eichler, W. (1990) *Biol. Chem. Hoppe-Seyler* **371,** 273-282.
3) Eichler, W. (1990) *J. Protozool.* **37,** 273-277.
4) Eichler, W. (1989) *Biol. Chem. Hoppe-Seyler* **370,** 1127-1131.
5) Matsuzaki, S. et al. (1981) *Acta Endocrinol.* **98,** 57-61.

Wolfgang Eichler and Bodo Schertel, Institut für Biochemie I der Universität, Im Neuenheimer Feld 328, D-6900 Heidelberg.

U. Evers[1], F. Triana[2], K. Nierhaus[2] and A. Yonath[1,3]

Crystallographic Studies on Complexes Mimicking Protein Biosynthesis

Models of bacterial ribosomes were reconstructed at low resolution from two-dimensional sheets. These revealed features which were not detected previously in prokaryotic ribosomes, including an empty space, comprising of 15-20% of the volume of the ribosome and located at the interface between the two ribosomal subunits, a tunnel of about 100Å in length and up to 25Å in diameter, which leads from the intersubunit space to a location compatible with the exit site of the nascent protein chain, and a groove in an RNA-rich region within the 30S subunit, in a position suitable for binding mRNA. Model-building experiments showed that tRNA molecule can bridge between the groove and the tunnel, so that the anticodon end of the tRNA is close to the mRNA and the CCA-terminus is positioned so that the peptidyl group may extend into the tunnel. Space considerations show that the intersubunit space is large enough to accommodate three separate sites for tRNA molecules, with room to spare for other factors involved in protein biosynthesis.

The intersubunit free space allows for the internal motion of ribosomes, providing the dynamics involved in biosynthesis of proteins. It also contributes to the poor internal order of

all crystals of 70S ribosomes. A series of complexes was designed, each of which mimics a stage in protein biosynthesis and contains ribosomes with limited motional freedom. Producing such complexes in quantities suitable for crystallographic studies is time consuming and requires significant sophistication, the level of which is determined by the nature of the mRNA. A simple complex of 70S ribosomes from Thermus thermophilus together with 1.5-1.8 equivalents of phetRNAphe and a chain of about 35 uridines, was crystallized. Considering the reproducibility of their growth, their internal order and their shape, the crystals of the complex are superior to those of isolated ribosomes, thus showing that the complex is less flexible than the isolated 70S ribosomes.

Despite the marked improvement in the crystal order, this complex suffers from imperfections. Being a homopolynucleotide, poly(U) does not contain a powerful signal for attachment and may bind at random locations along its chain, and protrude into the solution in an uncontrolled fashion. Thus, complexes with heteropolynucleotides of defined sequences have been designed and are currently being produced in high purity and large amounts. Such complexes facilitate the construction of uniform populations, containing either one of the two main conformers of the elongation cycle, namely the pre- or the post-translational ribosomes.

(1) Max-Planck Res. Unit for Struct. Mol. Biol., D-2000 Hamburg 52.
(2) Max-Planck-Inst. for Mol. Genetics, D-1000 Berlin 33.
(3) Dept. Structural Chemistry, Weizmann Inst., Rehovot, Israel.

Fetscher, B. and Adam, G.

Functional Dynamics of Intracellular Ca-Compartments of 3T3 Mouse and Rat Embryo Fibroblasts

A certain subset of growth-stimulating factors (the "competence growth factors") may be characterized by their ability to induce a fast Ca-efflux ($\leq$ 2 min) originating from an intracellular Ca-compartment[1]. For 3T3 cells, these Ca-effluxes are specifically blocked by application of the socalled Ca-channel blockers (such as nifedipin or verapamil), which concomitantly inhibit cell proliferation[2].
We have extended this work with the following results:
I) Similar results pertain to rat embryo fibroblasts REF, for which epidermal growth factor (EGF) acts as a competence factor according to the criteria given above and is inhibited by the antagonist TMB-8.
II) For 3T3-cells, different Ca-channel blockers act selectively on Ca-effluxes elicited by different growth factors:
- Ca-efflux induced by vasopressin is not inhibited by TMB-8, 50% inhibited by nifedipine or verapamil[2], and 100% inhibited by flunarizine.
- Ca-efflux induced by fetal calf serum is not inhibited by TMB-8 but 100% inhibited by flunarizine.

We conclude that different signal paths and/or functional intracellular Ca-compartments may be differentiated by different Ca-channel blockers.

1) Rozengurt,E. and Mendoza,S.A. (1985) J.Cell Sci. Suppl. 3, 229-242.
2) Hartmann, T. and Adam,G. (1988) Europ.J. Cell Biol. 46 (Suppl.22) 25.

Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz.

A.A.H. Fischer , K. Müller and C. Scholtissek

Specific Inhibition of the Synthesis of Influenza Virus Late Proteins and Stimulation of Early, M2 and NS2 Protein Synthesis by 3-Deazaadenosine

3-Deazaadenosine (3DA-Ado) specifically inhibits the synthesis of late influenza virus A proteins, while it causes an overproduction of early proteins and of the non-structural proteins NS2 and M2. This effect can be significantly enhanced by homocysteine thiolactone. Except for the M gene, synthesis of viral mRNA is not significantly affected by 3DA-Ado. We conclude that 3DA-Ado acts via its homocysteine derivate by interfering with a specific posttranscriptional modification of viral mRNA and on splicing of specifically the M mRNA.

Other tested RNA viruses without a nuclear phase multiply normally in the presence of 3DA-Ado. In contrast to amantadine, which is another specific and potent inhibitor of influenza A virus replication, even under the most stringent conditions we completely failed to obtain any 3DA-Ado-resistant influenza virus variants.

A.A.H. Fischer, K. Müller and C. Scholtissek, Institut für Virologie (FB 18), Frankfurter Strasse 107, D-6300 Giessen.

E. Fischer, M. Wagner and R. Brossmer

Specificity of a lectin from *Cepaea hortensis*

The specificity of the sialic acid-binding lectin from the snail *Cepaea hortensis*, purified