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Meeting Report

Function, structure and genetics of prokaryotic and eukaryotic elongation factors

EMBO – NATO workshop organized by L. Bosch and A. Parmeggiani in Thiverval-Grignon, France, July 16–20, 1984

Barend Kraal, Reuben Leberman* and Mathias Sprinzl+

*Department of Biochemistry, State University of Leiden, Leiden, The Netherlands, * EMBO Laboratory Grenoble Outstation, Grenoble, France,* and +*Department of Biochemistry, University of Bayreuth, Bayreuth, FRG*

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During the last 30 years our insight into the mechanism of protein synthesis has steadily increased to the extent that we now are well informed about most essential components and the sequence of events in, at least, bacteria. However, a multitude of fundamental mechanistic questions have remained unanswered. Essentially, we do not understand many intricate details of the elongation cycle such as the ribosomal switch mediating translocation.

It is gratifying, however, that our experimental tools to tackle these classical problems have increased considerably due to the recent developments in genetic and biophysical techniques. This became apparent at a recent EMBO–NATO workshop devoted to factors involved in polypeptide elongation. Cloning of the genes coding for these factors from various eukaryotic organisms such as cytoplasmic EF-1α from *Artemia salina* (Möller, Leiden) and from yeast (Kaziro, Tokyo; Buhler, Gif-sur-Yvette), mitochondrial EF-Tu from yeast (Kaziro), and chloroplast EF-Tu from *Euglena gracilis* (Stutz, Neuchatel; Spremulli, Chapel Hill) provided a wealth of new information on amino acid sequences which all show remarkably conserved regions of homology with bacterial EF-Tu. This primary structure information can now be discussed more usefully in the light of the 3-D structure of the *E. coli* EF-Tu·GDP presented by Nyborg (Aarhus). The analysis of this structure to a resolution of 2.6 Å provides the necessary basis for the understanding of many of the functional reactions of EF-Tu and is outstanding in being the first high-resolution structure determination of a high-affinity guanosine nucleotide binding protein.

In relation to the 3-D model, Kraal (Leiden) described a novel characteristic of EF-Tu, namely, a second tRNA-binding site which only becomes apparent upon association of EF-Tu with the ribosome or the antibiotic kirromycin. The data demonstrate that EF-Tu has a dual interaction with the tRNAs in both ribosomal sites, thus positioning the two tRNAs both with respect to each other and to the ribosome–tRNA complex. Other aspects of EF-Tu–tRNA complex formation concerning the role of several histidine residues and the accessibility of the phosphodiester bonds were discussed by Jonák (Prague) and Ebel (Strasbourg), respectively.

In the past, the work with kirromycin-resistant EF-Tu mutants has proved to be very informative. A new type of such a mutant was described by Wittinghofer (Heidelberg). Particular mutations of *tufA* and *tufB*, the two EF-Tu encoding genes, suppress nonsense mutations. Surprisingly, suppression only occurs in cells harbouring two

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distinct mutant EF-Tu species, one of which is defective in the second tRNA-binding site (Bosch, Leiden). Ribosome mutants are also used in studies on translational fidelity. Enhanced accuracy is obtainable at the cost of enhanced GTP hydrolysis and a decreased efficiency of protein synthesis (Kurland, Uppsala). Using the non-hydrolyzable analog GTPγS, Thompson (Boulder) also showed that speed and accuracy are inversely related. Pingoud (Hannover) presented a computer simulation of the elongation cycle using kinetic data for 29 individual reactions. One of the conclusions of this approach is that, in vivo, the concentration of reagents appears to be optimal for the cycle. The model also explains the role of ppGpp in slowing down protein synthesis while preserving translational accuracy.

Site-directed mutagenesis has now become one of the major tools of studying structure-function relationships. Substitution of residue Glu-68 by Gly in the polypeptide region likely to interact with aminoacyl-tRNA was reported by Parmeggiani (Palaiseau). Genetic manipulation has also deepened our insight into the relationship between the expression of translational genes and cell growth. A general introduction on the regulation of ribosomal genes was given by Lindahl (Rochester, NY). Zengel (Rochester), discussing *tufA* and *tufB* expression, described a secondary *tufA* promoter, located in the adjacent EF-G gene. This promoter, in contrast to the major one (*Str* promoter) is not under stringent control. Autogenous regulation of *tufB* expression was found to occur through feedback inhibition of translation by EF-Tu (Bosch, Leiden). Evidence for translational repression of the synthesis of the eukaryotic factor EF-1α by protein in mRNP particles was presented by Slobin (Jackson).

The rewards of biophysical approaches also became highly apparent at this meeting. Fluorescence spectrometry has been used extensively for measuring the interaction between factors, tRNA and the ribosome under equilibrium conditions (Eccleston, London; Remy, Strasbourg; Johnson, Oklahoma; Sprinzl, Bayreuth). The application of spin-labelled guanine nucleotides as sensitive probes for conformational changes in EF-Tu on its way through the elongation cycle (Faulhammer, Bayreuth) may also be useful for the investigation of other GTP-binding proteins. High-resolution NMR (500 MHz) revealed partial melting in the middle of tRNA (yeast) upon interaction with EF-Tu (Hilbers, Nijmegen). The location of EF-Tu on the ribosome (near the base of the L7/L12 stalk) was reported by Hardesty (Austin) using fluorescence energy transfer. Robertson and Wintermeyer (Munich) showed a simultaneous displacement of peptidyl-tRNA to the P-site and exit of the deacylated tRNA upon addition of EF-G. The controversial views concerning the number and nature of the tRNA-binding sites on the ribosome (Nierhaus, Berlin; Spirin, Pushchino; Wintermeyer, Munich) led to very stimulating discussions and the promise of fruitful collaborations to resolve these problems. With regard to the elongation factor EF-G, the observation by Girshovich (Pushchino) should be mentioned about its preferential cross-linking to 23 S ribosomal RNA, as well as the communication by Parlato (Naples) on the induction of its GTPase activity in the absence of ribosomes.

Although the study of eukaryotic factors has not as yet acquired the depth and sophistication of that of their prokaryotic counterparts, several reports dealt with their isolation and characterization (and cloning, see above). A common feature of all translational factors is their affinity towards RNA, which may explain their loose compartmentalization in the cell on the exposed regions of polysomes (Spirin, Pushchino). In the case of EF-2 the binding affinity for ribosome and nucleotide is regulated by ADP-ribosylation, although unanimity on the mechanism involved has not yet been reached (Bermek, Istanbul; Ovchinnikov, Pushchino; Abraham, Bergen). Recognition of the unique site for ADP-ribosylation is dependent on post-translational modification of histidine into diphthamide. By studying defective mutants, Bodley (Minneapolis) found indications for 5 proteins involved in diphthamide synthesis. In yeast an extra elongation factor EF-3 was isolated from thermosensitive mutants (McLaughlin, Moldave, Irvine). It was reported to function in the regeneration of EF-1$\alpha\cdot$GDP to EF-1$\alpha\cdot$GTP by transphosphorylation with ATP (Chakraburthy, Milwaukee).

In conclusion this meeting showed that genetic and biophysical approaches can complement each other in understanding the mechanism and control of the complex protein synthesis machinery.