10 Folding of the Spike Protein and its association with the Membrane Protein during Mouse Hepatitis Virus Assembly.

Dirk-Jan Opstelten, Pieter de Groote, Marian Horzinek and Peter Rottier. Institute of Virology, Veterinary Faculty, University of Utrecht, Yalelaan 1, 3582 TD Utrecht, The Netherlands. The spike (S) protein of the mouse hepatitis virus (MHV) is a type I glycoprotein. The protein is involved in at least two types of interactions: (i) S forms oligomers and (ii) it associates with the MHV membrane (M) protein in the process of virus assembly. The oligomerization of S takes place with a relatively long half-time of 40-60 min and its association with M is even slower. We studied the possibility that the folding of S is the rate-limiting step in these processes. By analyzing S under non-reducing conditions we found that the protein forms disulfide bonds co- and post-translationally. The diulfide bond formation coincided with conformational changes as observed by differences in mobility in non-reducing gels. We were able to manipulate the disulfide bond formation in vivo by adding the reducing agent dithiothreitol (DTT) to the culture medium. This resulted in the reduction of already oxidized S and the inhibition of disulfide bond formation in newly synthesized proteins. The reduction of S affected its conformation as observed by a change in mobility in non-reducing gels and a change in antigenic epitopes. The reduced S was not competent to associate with M and did not leave the endoplasmic reticulum (ER). In contrast, M appeared to be insensitive to in vivo reduction and was transported efficiently out of the ER indicating that cellular processes, such as glycosylation and transport, were unaffected by DTT. Subsequently, S became oxidized and (re)folding was observed. Interestingly, during reoxidation the major fraction of S transiently aggregated into large disulfide complexes from which folded S molecules dissociated.

11 **Expression of Rh1 Opsin from *Calliphora erythrocephala* using a recombinant baculovirus as a vector**

Philipp Sander, Uwe Wolfrum and Reinhard Paulsen
Institut für Zoologie I, Universität Karlsruhe, Kornblumenstr. 13, W-7500 Karlsruhe, FRG

We have employed the baculovirus vector expression system (1) to generate large amounts of non-fusion *Calliphora* Rh1 opsin for further biochemical and structural studies. Westernblot analysis reveals that the recombinantly expressed opsin displays the same electrophoretic mobility as does its native counterpart. Expression levels reach a maximum of 10 μg per 10^6 cells 48 hours after infection as estimated by laser densitometry of SDS-PAGE gels. In contrast to native rhodopsin the recombinantly expressed opsin cannot be extracted from total cell membranes with the detergent digitonin, indicating that the expression product is in a misfolded state. Immunohistological experiments (immunofluorescence labeling, immunogold labeling) show, that the expressed protein is not located in the plasma membrane but rather is retained in cytoplasmic membranes, probably the endoplasmatic reticulum. We discuss that posttranslational events like action of a cyclophilin (2), glycosylation (3) and attachment of the chromophore are essential for proper folding and processing of *Calliphora* Rh1 opsin.

Supported by the DFG (Pa 274/3-3).

(1) Summers HD and Smith GE (1987), Tex Agr Exp Stat Bull No. 1555
(2) Stamnes MA *et al.* (1991), Cell 65: 219-227
(3) Huber A *et al.* (1991), JBC 265: 17906-17906

12 Plasmodia of **Physarum polycephalum** contain large complexes of poly-ß-L-malate and nuclear proteins

Bernhard Angerer, Eggehard Holler, Institut für Biophysik und physikalische Biochemie, Fakultät III, Universität Regensburg, D-8400 Regensburg/Federal Republic of Germany
Giant plasmodia of Physarum polycephalum contain as many as 10^8 nuclei, which divide synchronously in an endomitotic fashion. Poly-ß-L-malate (PMA) is specific for the plasmodial stage of the life cycle. It occurs in pico gram quantities in these nuclei. It has high affinities for DNA polymerase-α-primase and histones under cell-free conditions (1). We present here evidences that these among other complexes of PMA exist in vivo and can be demonstrated in extracts from plasmodial nuclei. Complexes of 200-700 kD with histones and of 600-900 kD with DNA polymerase-α-primase (half-lives 30-60 min) are demonstrated by gel permeation FPLC. [^{14}C]PMA added to nuclei before rupture did not mix with the complex-bound PMA demonstrating that these complexes exist in vivo. Complexation inhibits reversibly activities of both DNA polymerase α and primase. By prolonged standing or increase in ionic strength, the complexes are dissociated into PMA (200 kD, proteins as standards) and proteins (less than 240 kD). Results of cabodiimid-crosslinking with nuclei confirms that PMA is bound to proteins. The size of the complexes varies during cell-cycle being highest in S-phase. We propose that PMA functions as a matrix for stockpiling of certain nuclear proteins to maintain the plasmodial nature of the organism.
Reference: (1) Fischer, H., Erdmann, S., Holler, E.: An unusual polyanion from Physarum polycephalum that inhibits homologous DNA polymerase α in vitro. Biochemistry 28, 5219-5226 (1989).