The membrane component of the ribosomal bodies from the lizard *Lacerta sicula:* a freeze-etching study

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Summary. We have studied, using the freeze-etching technique, the cytomembranes of the flat cystenae, regularly interposed between the cristalline sheets of ribosomes, inside the ribosomal bodies of the hibernating lizard *Lacerta sicula.* The results show the presence, on the protoplasmic face (PF), of numerous IMPs (intramembrane particles) which can be separated into two populations. The flatter ones appear to be arranged in a regular pattern, which, with the support of the optical diffraction analysis, can be correlated with the disposition of the ribosomes in the cristalline sheets.
Key words. Ovarian follicles; *Lacerta sicula;* ribosomal bodies; cytomembranes; freeze-etching.

During the hibernation of the lizard *Lacerta sicula,* inside the ovarian follicle ribosomes which are not engaged in protein synthesis aggregate into crystalline sheets regularly flanked by cisternae, so as to form structural complexes named 'ribosomal bodies'[1,2]. An interesting aspect of these structures is represented by the interaction between the ribosomes of the crystalline layer and the underlying membrane of the cisternae. Although a narrow (50 Å) interspace is evident in cross section (fig. 1), Unwin[3] has shown that the ribosomes are directly attached to the membranes through a salt-sensitive linkage as in rough endoplasmic reticulum (RER). Furthermore, on the basis of three-dimensional analysis, the binding occurs by a protrusion from the large subunit of the ribosomes[4]. According to Unwin[3,4], the distant association of the crystalline ribosomes is likely to be just an effect brought about by the crystal bonding, rather than an indication of interaction with the membrane different from that found in the RER.

Since it is well known that in RER intrinsic membrane proteins act as ribosome binding sites[5], we undertook a study of the ribosomal bodies by the freeze-etching technique; with this method it is possible to visualize the intrinsic membrane proteins as intramembrane particles (IMPs), our aim being to identify those IMPs that could correspond to intrinsic proteins responsible for the binding of the ribosomes. In fact in this system, unlike the RER, the known disposition of the ribosomes allows us to correlate the feature of the hydrophobic interior of the membrane with the crystalline arrangement on its surface.

Materials and methods. Ovarian follicles of *Lacerta sicula* were dissected from lizards collected near Naples during the winter. Specimens were fixed in phosphate-buffered formaldehyde-glutaraldehyde pH 7.4, postfixed with phosphate buffered 2% osmium[6], dehydrated with ethanol and embedded in Epon. Ultrathin sections, obtained with a Porter-Bloom MT2-B ultramicrotome and stained with uranyl acetate and lead citrate, were observed under a Philips 301 electron microscope.

For the freeze-etching electron microscopy, the ovarian follicles were fixed in phosphate-buffered formaldehyde-glutaraldehyde pH 7.4, glycerinated (25% glycerol in phosphate buffer) and transferred onto gold carriers. The specimens were frozen in melting Freon 22 and stored in liquid nitrogen. The freeze-fracture was performed in a Balzers appara-

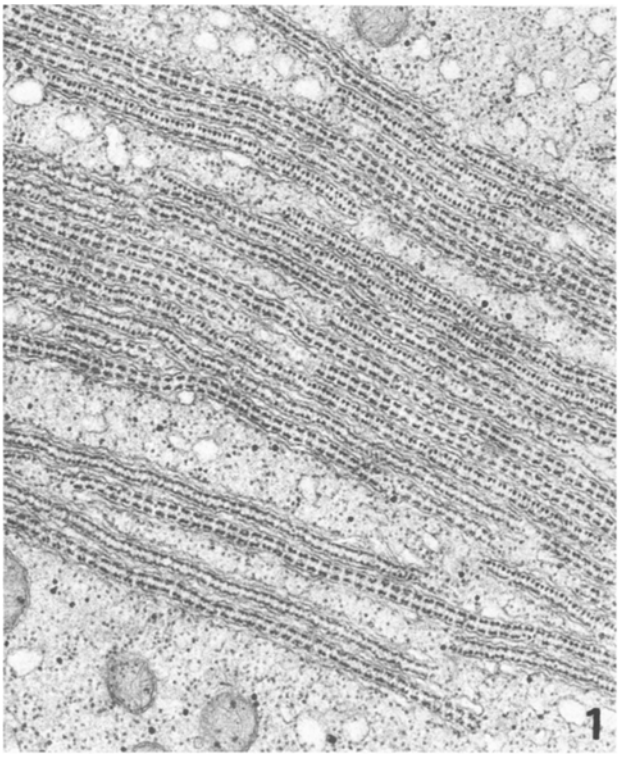

Figure 1. A cross-section of a ribosomal body in an oocyte of a hibernating lizard. The flat and parallel cisternae and the interposed ribosome crystalline sheets are evident. × 24,000.

Figure 2. A survey picture of a freeze-etched ribosomal body showing the parallel membranes of the cisternae. The IMPs are more numerous on the P face (P) than on the E face (E). × 23,000.

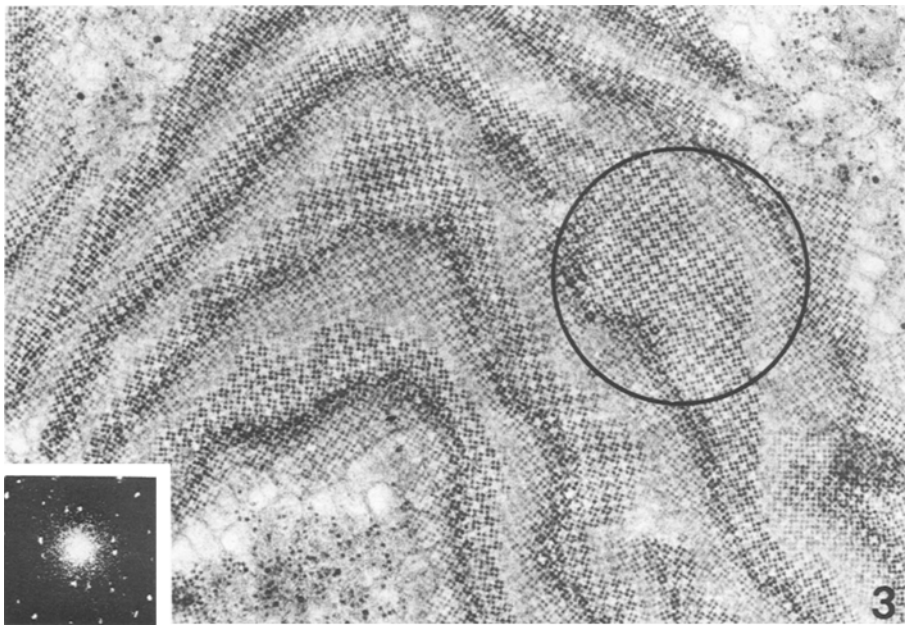

Figure 3. An oblique section showing the typical P4 crystalline arrangement of the ribosomes, × 28,000. In the insert the optical diffraction pattern of the encircled area.

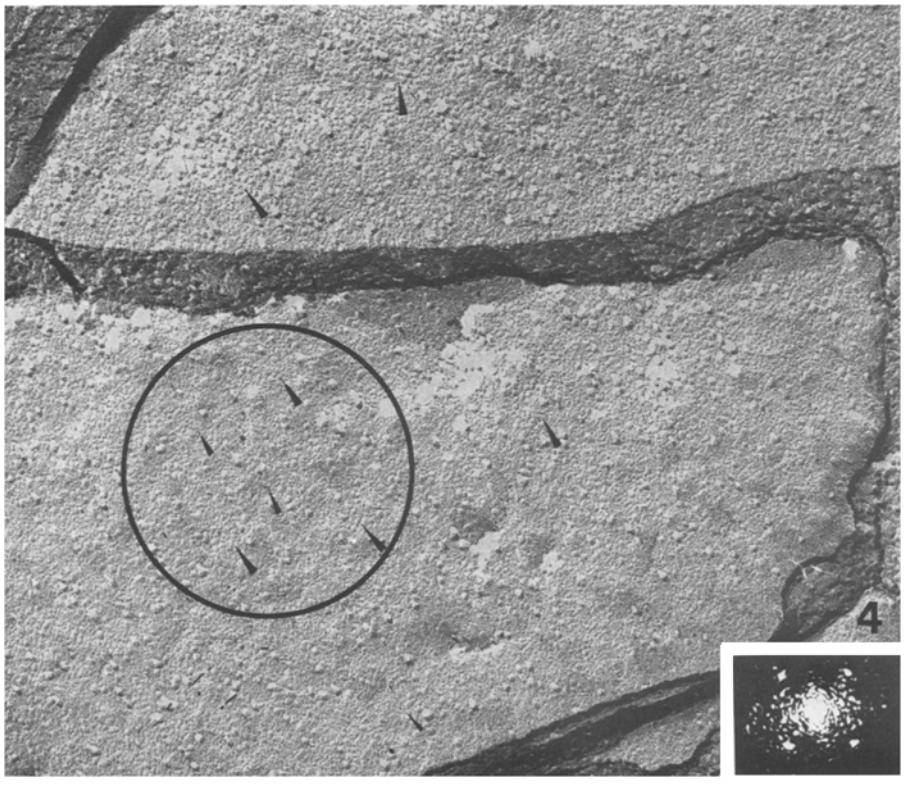

Figure 4. P faces of cisternal membranes at higher magnification. Some IMPs appear prominent and randomly dispersed; the others are flat and more densely packed, forming crossed rows (arrows). × 75,000. In the insert the optical diffraction pattern of the encircled area.

tus at −105 °C and 2×10^{-6} Torr. Replicas, obtained by shadowing the fracture surface with platinum at an angle of 45° followed with carbon at 90°, were examined with a Philips 301 electron microscope.

The optical diffraction analysis of the micrographs was carried out as described by DeRosier and Klug[7].

Results and discussion. Figure 1 represents a cross section of a ribosomal body showing the crystalline ribosomal sheets

flanked by membranes of flat and parallel cisternae; the narrow interspace between the ribosomes and the membranes is evident[1]. The oblique section (fig. 3) shows the crystalline arrangement of the ribosomes in tetramers forming a P4 lattice, for which the optical diffraction pattern (fig. 3, insert) provides good evidence. At low magnification, the replicas obtained by the freeze-etching technique give some more information on the morphology of the ribosomal body cisternae, which appear to be flat, continuous and parallel. Figure 2 is a survey picture of a ribosomal body in which it is possible to observe that the IMPs are more numerous on the protoplasmic face (PF) of the membranes while on the endoplasmic face (EF) the particles are scanty and dispersed, as described in the RER. At higher magnification, in samples in which the fracture plane reveals a more extensive internal area of the membrane (fig. 4), two particle populations are recognizable on the PF: a more prominent and randomly dispersed one, and another formed by numerous flat IMPs arranged in a regular pattern. This pattern can often be visualized as parallel rows of particles with an interspace of about 600 Å. Occasionally, as in figure 4, a not well resolved 'square arrangement' of the IMPs can be seen, formed by intersecting perpendicular rows, which can be analysed by optical diffraction (fig. 4, insert).

The freeze-etching data, supported by the optical diffraction analysis, show a regular arrangement of the IMPs inside the membranes of the ribosomal bodies that can be correlated with the arrangement of the ribosomes in the crystalline sheets. In the three-dimensional model proposed by Unwin[4], each ribosome in a tetramer interacts with the membrane through a protrusion of the large subunit, and the four protrusions of a tetramer come together and touch the membrane at a site near the 4-fold axis.

Hence, the expected distance between two neighboring IMPs corresponding to ribosome binding sites should have the same value as the unit cell dimension (595Å) of the ribosome P4 crystal lattice[2]). As the interspace between two parallel rows of IMPs is about 600 Å, we can hypothesize that the ill-defined particles regularly arranged on the PF correspond to intrinsic proteins which bind to the ribosomes. The low resolution of these particles could depend on the absence of nascent polypeptides, since the ribosomes are functionally inactive[8].

It is to be noted that in the ovarian follicles of hibernating lizards, as well as in hypothermically treated lizard embryos, the ribosome crystals are always associated with membranes[9], unlike hypothermically treated chick embryos, where ribosome crystals are free in the cytoplasmic matrix[10,11]. This latter finding, together with the data in this report, suggests a direct role of the membranes in the process of ribosome crystallization in lizard tissues.

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Fusion polypeptides in gene cloning: Potential problems due to conformational alterations at the junction

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Summary. Many eukaryotic genes are cloned in bacterial hosts as fusion polypeptides. Prediction of the secondary structures for some common prokaryotic fusion polypeptides shows that many junction sites correspond to important secondary structures. It is suggested that such structures could affect (hinder, etc.) the conformation or drive the folding of the neighboring eukaryotic counterparts. Thus the prokaryotic junction should be better performed in random coil regions, or short fusion prokaryotic polypeptides should be used.
Key words. Molecular cloning; fusion polypeptides; protein conformation; protein folding.

The fusion of eukaryotic to bacterial genes, thus producing a fused polypeptide, is commonly used to obtain cloned eukaryotic gene products in bacteria. Fused proteins are prepared for a better expression or secretion, and also for acquiring special antigenicity to facilitate screening procedures of the cloned products. Usually, the gene of interest is fused to the N-terminal portion of a bacterial or phage gene, and the hybrid gene is then transcribed from a strong bacterial promoter. These chimeric fusion proteins sometimes produce at levels of 10% or more of the total cellular protein, but there are also many examples of instability or lack of expression of the cloned protein, although its DNA sequence is in the correct reading frame. In other examples the cloning process yields polypeptide aggregates (termed inclusion bodies) other than native states. In fact, each protein poses a different problem[1].

An explanation for a number of the effects reported may be related to the protein folding pathway when this is driven or altered by the prokaryotic fused polypeptide. The present view of the protein folding process[2] assumes that the conformational information is dispersed through the polypeptide sequence, some positions being much more important than others in determining conformation. The folding process implies the formation of local reversible structures or nucleation centers: short stretches of secondary structures like α-helices of β-bends. Further stabilization comes from the establishment of interaction between neighboring structures. It is commonly thought that folding starts independently and more or less simultaneously in many regions of the polypeptide chain (autonomous folding domains). Thus, the prokaryotic fused polypeptide could affect the folding pathway by destabilizing a kinetically important intermediate in the