Reprinted from *J. Mol. Biol.* (1979) **132**, 69–84

Attachment of Ribosome Crystals to Intracellular Membranes

P. N. T. Unwin

J. Mol. Biol. (1979) **132**, 69–84

Attachment of Ribosome Crystals to Intracellular Membranes

P. N. T. UNWIN

Medical Research Council Laboratory of Molecular Biology
Hills Road, Cambridge, CB2 2QH, England

(Received 16 January 1979)

The attachment to membranes of ribosome crystals formed by cooling lizard oocytes and chick embryos has been investigated by electron microscopy of whole cells and by biochemical and structural experiments, using the cross-linking reagent glutaraldehyde.

It was found that the crystalline ribosomes in both animals form only on the rough endoplasmic reticulum and nuclear envelope, that they bind to these membranes through one unique site on the large ribosomal subunit, that the bond between the large subunit and the site on the membrane is sensitive to the concentration of K^+, but not of Mg^{2+}, and that this bond is selectively stabilized by mild treatment with glutaraldehyde. These results closely match those obtained from ribosomes in secretory cells, suggesting that there may be no difference between the two sets of ribosomes in their direct interaction with membranes.

The glutaraldehyde reaction was used to obtain crystals and components from which the small subunits had been preferentially released. A comparison between small subunit depleted and normal crystals led to an estimate for the positions of the subunits over the membrane surface. The side-by-side subunit assignments, "S" and "L", suggested previously (Unwin & Taddei, 1977; Unwin, 1977), were confirmed. It was deduced further that the crystalline ribosomes have the long axis of their small subunit approximately parallel to the membrane surface, and appear raised up from this surface because of interaction between their large subunits.

1. Introduction

Most experiments carried out on membrane-bound ribosomes have made use of components derived from endoplasmic reticulum in secretory cells. Ribosomes in these cells interact directly with the membranes through a specific site on their large subunit, and indirectly through the nascent protein (Sabatini *et al.*, 1966; Adelman *et al.*, 1973). The direct linkage has been characterized in several ways. For instance, it is sensitive to the concentration of potassium ions, but not magnesium ions (Adelman *et al.*, 1973); it can be stabilized by extremely mild treatment with glutaraldehyde (Kreibich *et al.*, 1978); it is normally specific for membranes of the rough endoplasmic reticulum and nuclear envelope (Palade, 1975).

Another system in which there is a significant association of ribosomes with membranes is the ovarian follicle of the lizard, *Lacerta sicula*. In this system, during winter, most of the membrane-associated ribosomes are assembled as crystalline sheets or "ribosomal bodies" (Taddei, 1972*a*). These crystals, like those in hypothermic chick embryos (Byers, 1967,1971; Morimoto *et al.*, 1972*a*,*b*), seem to be composed of inactive, but potentially functional, 80 S ribosomes (Taddei *et al.*, 1973).

0022-2836/79/210069-16 $02.00/0

In both animals, the same square, $P4$, crystal lattice and tetramer bonding of ribosomes is involved, with all ribosomes presenting the same surface away from the plane of this lattice (Unwin & Taddei, 1977). However, the lizard crystals grow as double layers, always bordered by membranes (e.g. Fig. 1) and in this respect differ from the chick embryo crystals, which grow as single layers or multi-layer stacks, and associate with membranes much more rarely.

The crystalline ribosomes of the lizard, unlike functional ribosomes in secretory cells, appear in thin sections to be raised up from the membrane surface, rather than in contact. Because of this more distant association with the membrane surface it could be argued that their interaction with membranes is not relevant to that of ribosomes in secretory cells. However, a three-dimensional analysis by electron microscopy (Unwin, 1977) showed that the distant association arises because attachment to the membrane takes place through a narrow linkage protruding from the large subunit, which touches the surface near the tetramer 4-fold axis. The three-dimensional analysis therefore suggested that the distant association of the crystalline ribosomes could be just an effect brought about by the crystal bonding, rather than an indication of interaction with the membrane by a different means.

Here I present evidence that the crystalline ribosomes do have the same direct interaction with membranes as ribosomes in secretory cells, and I determine the position of the centre of mass of the small ribosomal subunit over the membrane surface. The side-by-side subunit configuration, and assignments S and L, suggested previously (Unwin & Taddei, 1977; Unwin, 1977) are confirmed.

2. Materials and Methods

(a) *General*

Chick embryos were derived from eggs incubated for 4 days at 37°C. Lizard ovarian follicles were derived from *Lacerta sicula*, collected near Naples. Only previtellogenic material was used.

Winter lizards are defined as those containing mature crystal–membrane complexes, resulting from prolonged spells at low temperature, and summer lizards as those completely devoid of crystals. For convenience, this seasonal effect was sometimes produced artificially (Taddei *et al.*, 1973).

All experiments, unless otherwise stated, were carried out at 4°C.

(b) *Solutions*

Standard buffer was 100 mM-KCl, 5 mM-$MgCl_2$, 0·25 M-sucrose, 20 mM-triethanolamine·HCl (pH 7·6); high salt buffer was 500 mM-KCl, 5 mM-$MgCl_2$, 50 mM-Tris·HCl (pH 7·6); low salt buffer was 50 mM-KCl, 10 mM-$MgCl_2$, 20 mM-triethanolamine·HCl (pH 7·6).

(c) *Isolation of crystal–membrane complexes*

About 300 winter oocytes were required for a typical set of experiments. Their contents were released individually, using forceps and a dissecting microscope, into small (~5 μl) droplets of standard buffer, and pooled. Samples from each lizard were checked separately in the optical microscope; some contained complexes in poor yield; these were rejected or used for controls (see below). After the collection of every 100 or so good oocytes, the cytoplasm/buffer mixture was centrifuged first for a very short time (30 s at ~300 *g*) to remove isolated whole cells and tissue fragments, then again for a longer time (10 min at 700 *g*) to pellet the complexes. A clean preparation of the complexes was then obtained by repeated washing (3 times) of this pellet by resuspension in standard buffer and recentrifugation as described above. Optical and electron microscopy showed the only significant contaminants

to be mitochondria and small whole cells derived from the follicular epithelium (Fig. 6(a)); it was established, using preparations almost devoid of the complexes, that these contaminants release negligible quantities of ribosomes or subunits under the conditions described in Results. Complexes thus isolated were extremely stable and could be stored for several days at 0°C without any detectable degradation.

(d) *Sucrose density gradients*

The quantity of subunits, or single ribosomes, released from the crystal–membrane complexes after high salt treatment was analysed essentially according to the procedure of Falvey & Staehelin (1970). Linear sucrose gradients were prepared with sucrose concentration ranging from 0·4 M to 1M in 0·3 M-KCl, 3 mM-$MgCl_2$, 20 mM-Tris·HCl buffer (pH 7·6). Centrifugation was carried out in a Spinco SW50.1 rotor at 50,000 revs/min for 2 h. The optical density profile at 260 nm was measured discontinuously from 200-μl fractions, diluted twice, on a Perkin–Elmer model 551 spectrophotometer.

(e) *Electron microscopy and image processing*

Specimens for sectioning were fixed in 2% glutaraldehyde in 20 mM-triethanolamine buffer (and KCl and $MgCl_2$, concn according to the experiments) for 1 h, post-fixed with 1% osmium tetroxide, dehydrated in alcohol and embedded in Araldite. They were cut with a diamond knife on a Porter–Blum MT-1 ultramicrotome, and stained, after cutting, with uranyl acetate and lead citrate.

Crystals for negative staining were isolated according to the procedure of Unwin & Taddei (1977) and applied to carbon-coated grids either as described there, or (to achieve optimum release of small subunits together with minimum disordering) as follows. Grids, pretreated by soaking in 0·1% poly-L-lysine (80,000 M_r; Sigma Chemical Co.) for 5 min, were touched against small drops of the crystal solution, washed with low salt buffer and floated on drops of this buffer containing 0·05% glutaraldehyde for 5 min. They were then washed again, but in low salt buffer containing Tris in place of triethanolamine, and transferred to a drop of high salt buffer with the KCl concn at 300 mM instead of 500 mM. Following incubation in this buffer for 5 min, and a further wash in low salt buffer, the grids were stained with 2% uranyl acetate, containing 2 mg cytochrome *c*/ml, in the usual way.

Micrographs were recorded at 100 kV on a Philips EM 301 electron microscope having an objective aperture of semi-angle 5×10^{-3} radians. Minimal electron doses were used with the negatively stained specimens.

Optical diffraction, densitometry and computer processing of the micrographs were carried out as described by Unwin & Taddei (1977). As described there, the optical density measurements were taken from patches of area less than half of the 512×512 array size used in the Fourier transform calculations, in order to obtain accurate peak amplitudes and phases.

3. Results

(a) *Observations made on whole cells*

Ovarian follicles from summer lizards (see Materials and Methods), which had been incubated for various times at 4°C, were examined by electron microscopy. After 20 hours at 4°C numerous small crystals were found on membranes throughout the cytoplasm. Those membranes involved were particularly evident in the follicle cells surrounding the oocyte, where they had an appearance typical of rough endoplasmic reticulum (Fig. 1(a)). With more prolonged times at 4°C (~1 week) there was further crystallization on the same membranes, leading to the formation of small aggregates of compact, closely aligned, sheets (Fig. 1(b)). Crystal formation did not take place on any membranes other than those (rough endoplasmic reticulum, nuclear envelope) to which ribosomes were already attached.

Much larger, mature, crystal–membrane complexes ("ribosomal bodies"; Fig. 2)

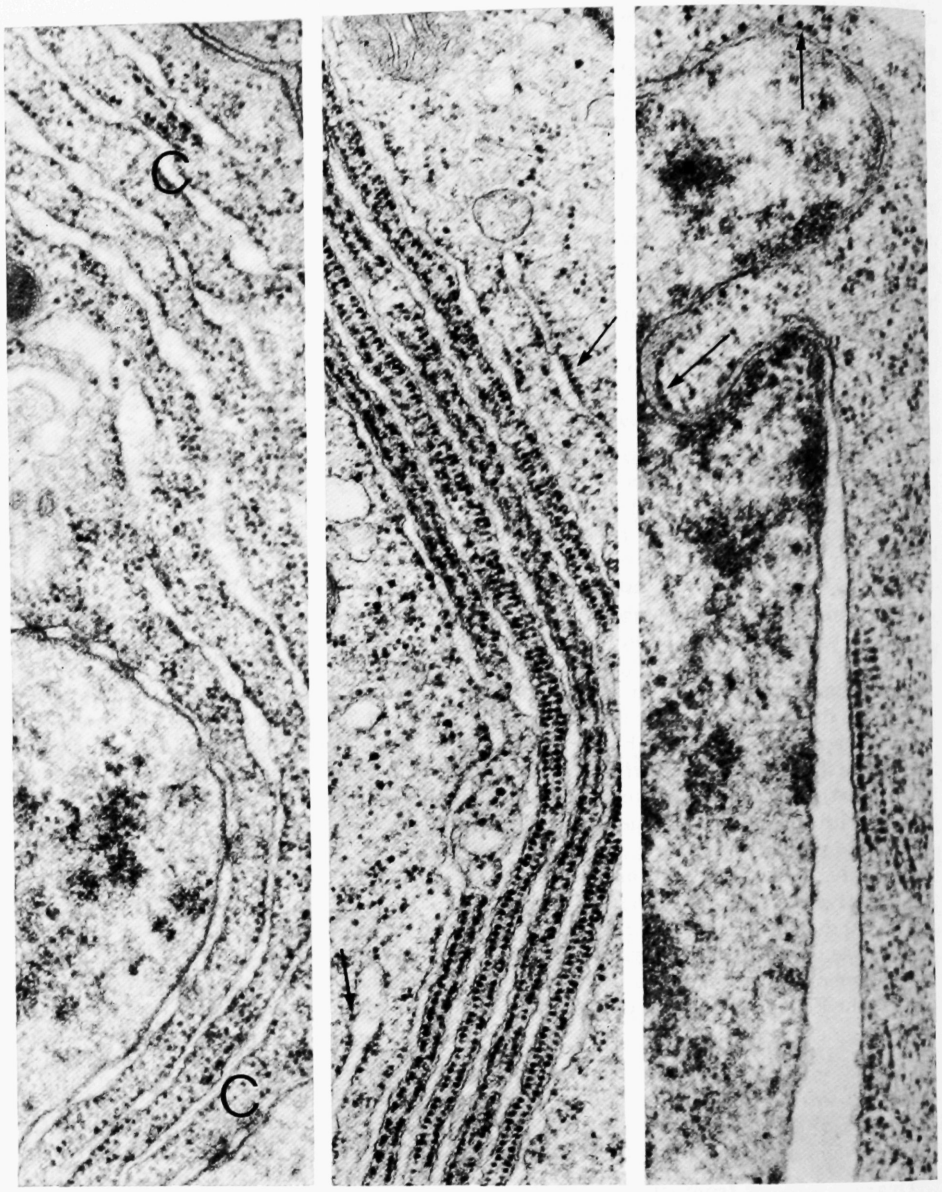

FIG. 1. Crystallization on membranes of the rough endoplasmic reticulum and nuclear envelope, following incubation at low temperature (see text): (a) and (b) lizard follicle cells; (c) chick embryo cell.

In (a) the crystals (C) have only just begun to develop, whereas in (b) the result of a more prolonged spell at low temperature, they have more nearly matured. (b) and (c) show depletion of membrane-bound ribosomes (arrows) in regions neighbouring the crystals. In (c) each crystalline layer on the membrane is "capped" by a curved cytoplasmic layer. Since members of such pairs of layers always have their right-handed appearing surfaces apposed (Byers, 1967), the layer against the membrane must be right-handed when viewed from the cytoplasmic side. The splitting of the nuclear envelope in (c) is typical with the fixation conditions used and may be due to the outer membrane (which serial sections show to be populated by numerous small crystals) becoming mechanically rather different from the inner one. Magnification, (a) and (b) 40,000×; (c) 50,000×.

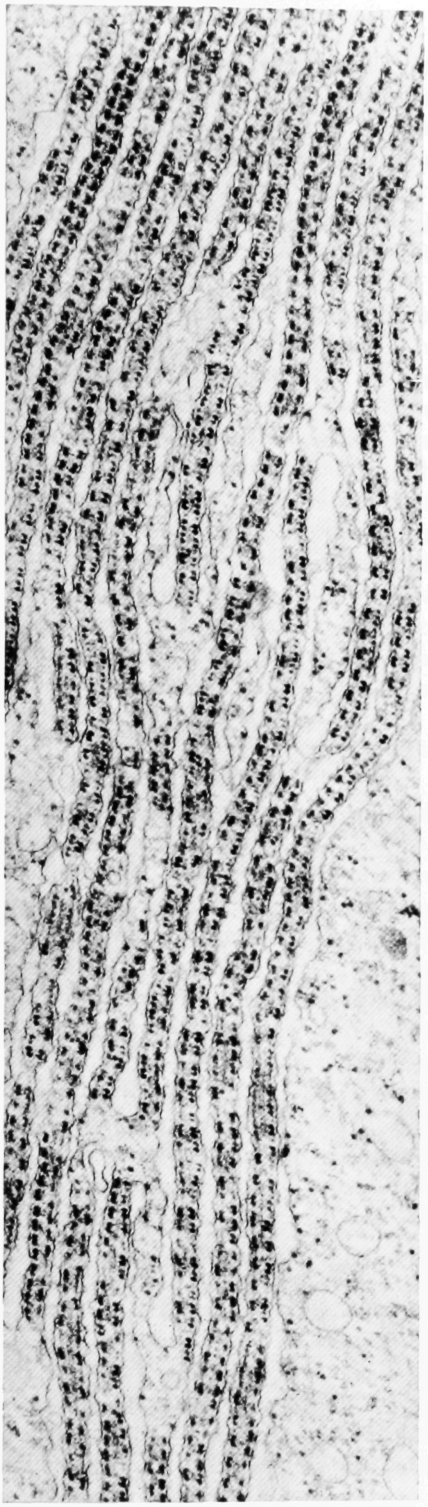

FIG. 2. A portion of a mature crystal–membrane complex, as found in the lizard oocyte during winter. There are large patches of smooth membrane in regions neighbouring the crystals. Magnification, 35,000×.

are found in the subcortical region of the oocyte during winter (Taddei, 1972*a*), and also in the follicle cells themselves if the lizards are maintained in a cool laboratory environment throughout the summer (Unwin, unpublished results). Presumably, these originate from smaller aggregates, as in Figure 1(a) and (b). Mature complexes are distinguished by an almost complete lack of non-crystalline membrane-bound ribosomes (less than 1% of the total number) and an abundance of "smooth" membrane. Consistently, about 30% of the total membrane surface in these complexes is smooth.

The short cold treatment, when applied to four-day old chick embryos, led to extensive crystallization of ribosomes in the cytoplasm and some on membranes. Careful observation (see below) indicated that when membranes were involved, they were also part of the rough endoplasmic reticulum or nuclear envelope (Fig. 1(c)).

In both animals, as evident in the Figures, the crystallization was accompanied by depletion, in neighbouring regions, of other bound ribosomes and by straightening of the implicated membranes. The straightening phenomenon was particularly striking with the normally highly irregular membranes of the chick embryo, and was used in this case to differentiate crystals which were properly attached from those which were closely juxtaposed but non-interacting.

By identifying features which distinguish the two crystal faces (Byers, 1967; see the legend to Fig. 1(c)) and performing tilting experiments in the electron microscope (results not shown), it was found that the chicken crystals, observed with the membrane behind, always formed a right-handed *P*4 lattice, as in Figure 3 (not a left-handed lattice, as they would do if they were the other way up). The same relationship applies in the lizard (Unwin & Taddei, 1977). Therefore, in either case, the crystals and the ribosomes composing them are the same way up with respect to the membranes on which they lie.

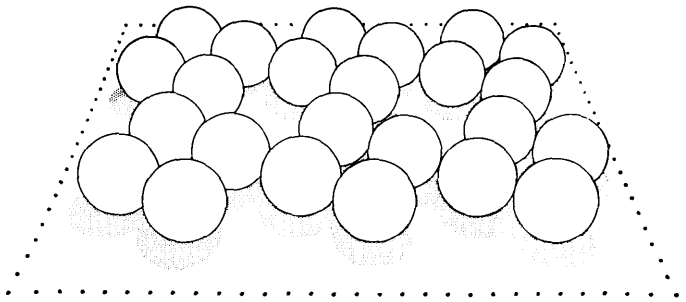

FIG. 3. Relation between the *P*4 crystal lattice and the membrane surface. The lattice viewed with the membrane behind always has this right-handed configuration, indicating that there is only one form of crystal–membrane interface.

(b) *Sensitivity of attachment to K^+ and Mg^{2+} concentration*

Ovarian follicles were extracted from summer lizards, punctured with a fine tungsten needle, and soaked for 30 hours at 4°C in a buffer containing 0·25 M-sucrose, 2 mM-$MgCl_2$, 20 mM-triethanolamine·HCl (pH 7·6) and various concentrations of KCl. Test experiments carried out concurrently, with dyes, confirmed that the membranes did not reseal during that 30-hour period.

With KCl concentrations in the range 25 to 250 mM, this treatment produced small, two-layer, crystals in regions throughout the oocyte and follicle cells. At or below 100 mM-KCl, these were, without exception, formed on membranes, as *in vivo*. Above this concentration, however, crystals also appeared which were only partly associated with membrane, or were altogether free (Fig. 4). The higher the K^+ concentration, the smaller the fraction of crystals which were associated with membrane.

The same effects as above were observed with varying KCl concentrations using buffers containing 5 mM and 10 mM-$MgCl_2$. No dependence of membrane association on Mg^{2+} concentrations between 2 mM and 10 mM could be detected. Association of the crystalline ribosomes with membranes therefore is sensitive only to K^+ concentration. This qualitative finding is in agreement with the results obtained from *in vitro* studies using membrane-bound ribosomes from rat liver (Adelman *et al.*, 1973).

The finding that membrane-free crystals can be created in the lizard supports the arguments put forward earlier (Unwin & Taddei, 1977) that the membranes are not directly responsible for bringing about crystallization in this animal, and that crystallization in the lizard and the chick embryo are closely related processes. Differences in relative amounts of rough endoplasmic reticulum and differences in the effects of cooling on the mobilities of membrane proteins are two factors which might contribute to the fact that all crystals in the lizard, but only a small proportion in the chick embryo, are normally associated with membranes.

(c) *Preferential release by high salt of the small subunit after mild treatment with glutaraldehyde*

Three 300-μl portions of mature crystal–membrane complexes (each ~1 O.D. unit at 260 nm; see Materials and Methods) were used in a typical set of experiments. Glutaraldehyde was added to two of these, generally to make 0·005%, and allowed to react at 20°C for various lengths of time. The reactions were quenched by dilution to 1 ml with standard buffer containing 50 mM of Tris·HCl instead of triethanolamine·HCl, and pellets of the crystal–membrane complexes were recovered by a low speed spin (1000 *g* for 10 min). These pellets were resuspended and incubated at 20°C in 300 μl of high salt buffer for 30 minutes, and then given a further low speed spin. The supernatants were layered on 0·4 M to 1 M linear sucrose gradients, centrifuged, and analysed spectrophotometrically as described in Materials and Methods. The pellets were examined by electron microscopy.

The high salt release was found to give significant quantities of subunits, but only negligible amounts of any larger aggregate. In the absence of the cross-linking reaction, the optical density profiles indicated release of approximately equal numbers of both subunits (Fig. 5(a)). Puromycin was not required for the release, confirming that the lizard crystals, as with the chicken crystals (Morimoto *et al.*, 1972*a*), do not contain nascent protein. After short cross-linking times, release only of the large subunit was impaired (Fig. 5(b)). After a rather longer cross-linking time, release of both subunits became impaired, but with the large subunit being much more severely affected than the small one (Fig. 5(c)). The same selectivity for the large subunit—membrane bond and a quantitatively very similar overall sensitivity of the cross-linking reaction have been recently demonstrated for membrane-bound ribosomes from rat liver (Kreibich *et al.*, 1978).

Examination of the high salt leached pellets showed most of the large subunits

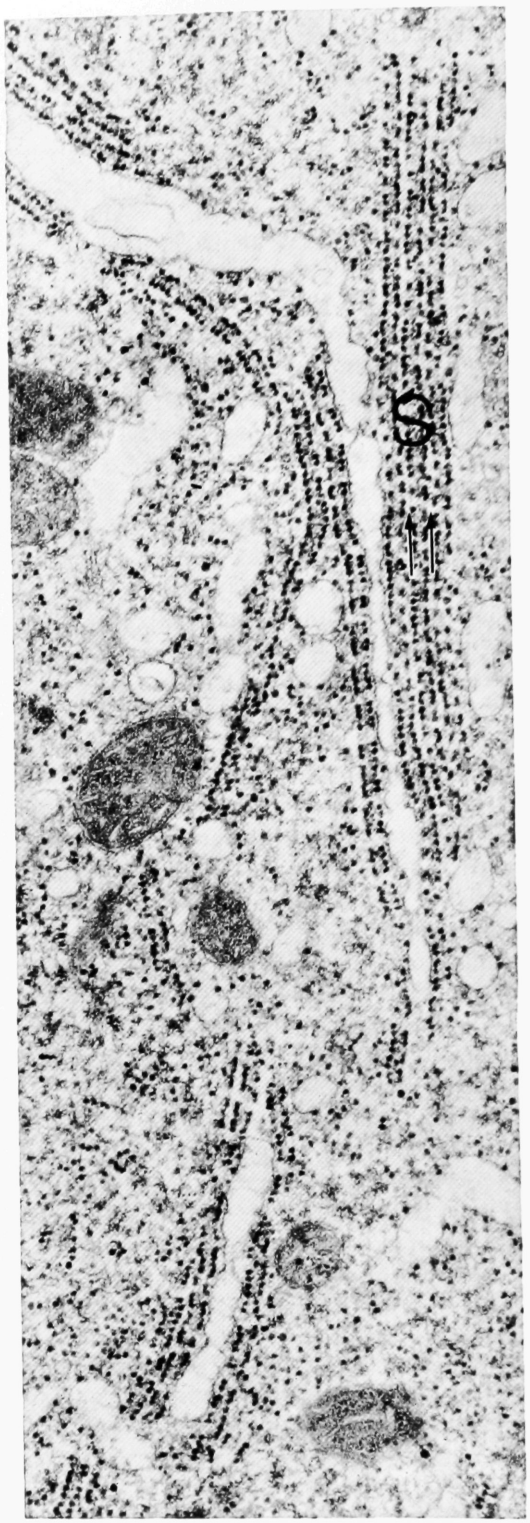

FIG. 4. Crystallization in a punctured oocyte soaked for 30 h at 4°C in buffer containing 150 mM-KCl. With elevated K^+ concentrations a large fraction of the crystalline sheets are found only partly in association with, or entirely free from, membranes. This effect is independent of Mg^{2+} concentration.

The sheets have a tendency to form stacks (S), even in the absence of membranes. The sheets

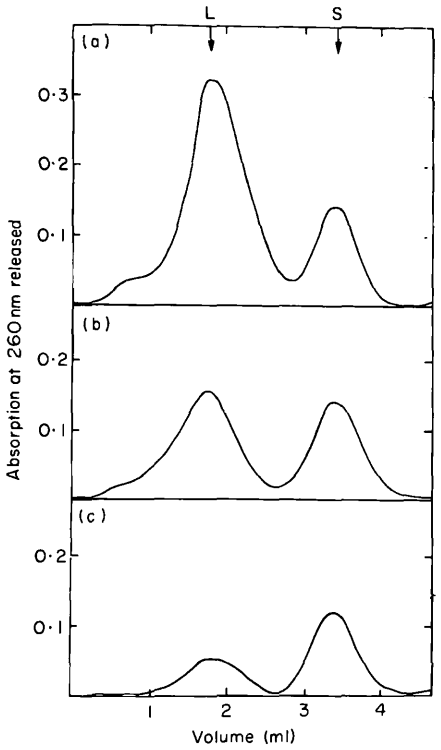

FIG. 5. Sucrose density gradient profiles, showing the effect of high salt on fully developed crystal–membrane complexes isolated from the lizard: (a) before; (b) after 6 min; and (c) after 24 min incubation with 0·005% glutaraldehyde at 20°C.

The cross-linking reaction impairs release of the large subunit (L) and allows the small subunit (S) to be released preferentially.

remaining on the membranes after the short cross-linking treatment to be separated and randomly arranged (Fig. 6(b)), and most of them remaining after the longer treatment to be in the tetramer configuration, with the tetramers randomly arranged (Fig. 6(c)). Therefore the large subunit—membrane bond and the tetramer bonds are both stabilized more readily by the cross-linking reaction than any bond involving the small subunits. The large subunits must also be responsible for the tetramer bonds, a deduction previously made by Byers (1971) using tetramers derived from chick embryos.

The raised up configuration of the crystalline ribosomes on the membrane surface is retained when the lizard crystals are broken down into the large subunit tetramers (Fig. 6(c)), but is lost when they are broken down into the isolated particles (Fig. 6(b)). This is a reversible phenomenon, as was demonstrated by dialysing the preparation of large subunits, cross-linked to the membrane surface in high salt buffer as in Figure 6(b), against low salt buffer. The low salt treatment re-establishes the raised up configuration, and the tetramer bonding as well (Fig. 6(d)). Thus there is a close relationship between tetramer bond formation and proximity of the ribosome to the membrane surface.

composing these stacks do not appear to approach one another as closely as they do the membrane, presumably because the zones between them (arrows) involve two rows of protrusions rather than one (see Unwin, 1977). Magnification, 35,000×.

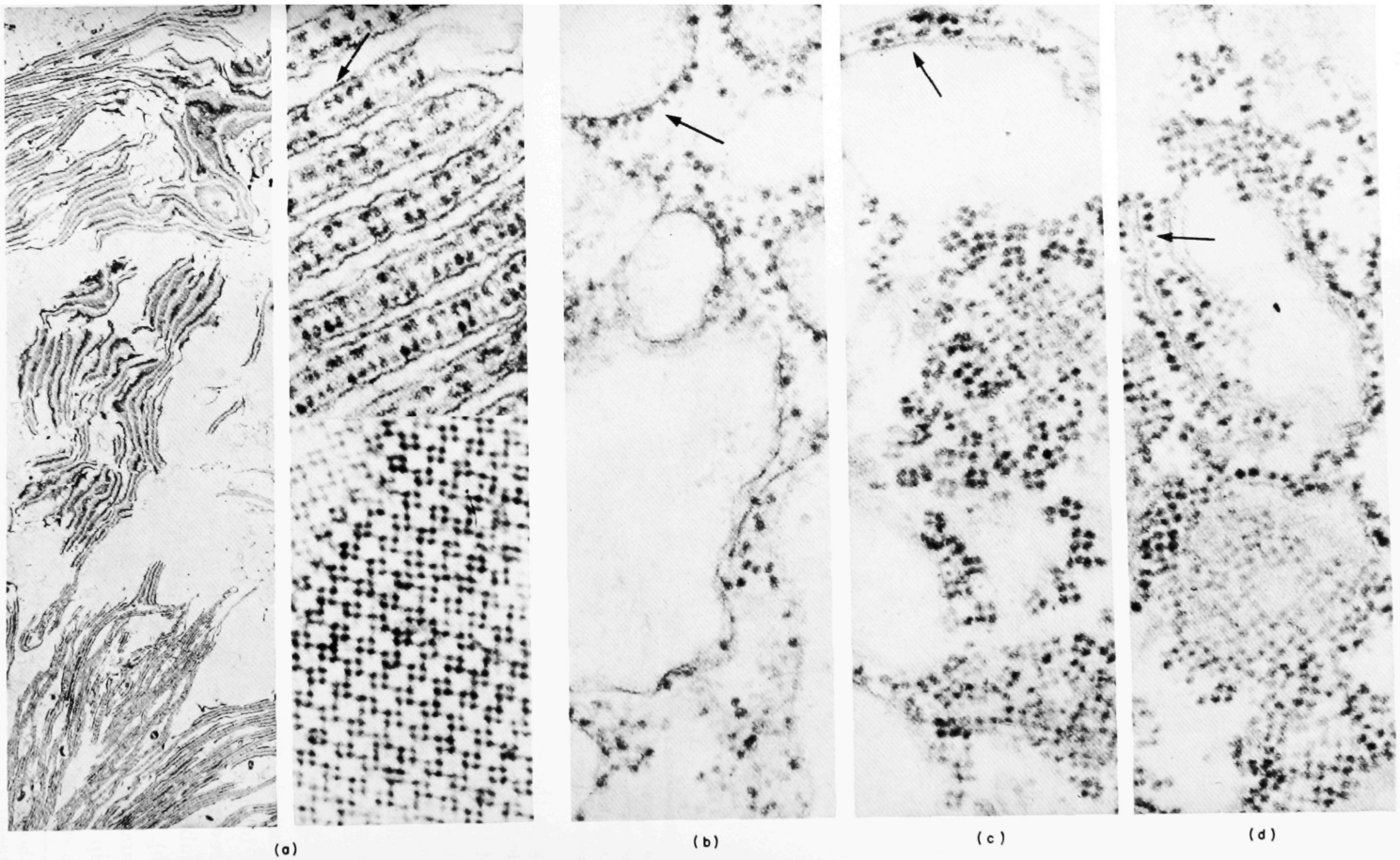

FIG. 6. Sections from pellets of isolated crystal–membrane complexes.

(a) Before the cross-linking, high salt, treatments in Fig. 5; (b) after the short treatment; (c) after the longer treatment; (d) after the short treatment followed by dialysis against low salt buffer.

(a) Shows predominantly edge-on and face-on views of the complexes, as isolated, both at low (3500×) and high (80,000×) magnification. The proximity of the ribosomes to the membrane surface can be seen in the edge-on views (arrows). Some reformation of the inter-tetramer bonds is evident in (d). Magnification, (b), (c) and (d) 80,000×.

(d) *Location of small subunit*

Isolated lizard crystals were attached to polylysine-coated carbon grids, subjected to a similar cross-linking, high salt, treatment as described above, negatively stained and examined by electron microscopy (see Materials and Methods). The high salt treatment partially disrupted the crystals; however, some order was retained mainly in the layer resting on the grid (Fig. 7). The best preserved patches from many of these crystals were selected by optical diffraction on the basis of symmetry and resolution, and then analysed further by calculation of their Fourier transforms.

Detail from the Fourier transforms of six glutaraldehyde, high salt, treated crystals is given in Table 1. The diffraction peaks extend only to the index 1,3, but to this resolution all are of the same phase and have similar amplitudes. Since the relative orientation of the two layers was different in each example, the orientation of the second layer could not have had a significant effect on the peaks of the layer being analysed.

Comparison of the data from the glutaraldehyde, high salt, treated crystals with the low resolution data obtained previously (Unwin & Taddei, 1977; Unwin, 1977) from normal crystals (Table 1) showed equivalent peaks to have no changes in phase,

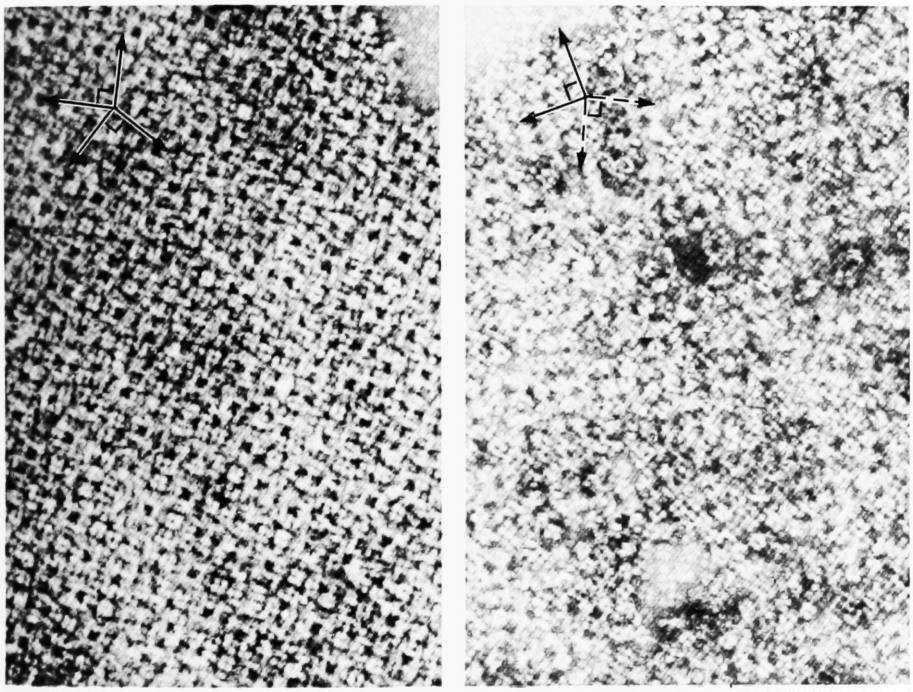

FIG. 7. Ribosome crystals isolated from the lizard and negatively stained with uranyl acetate. (a) Normal crystal; (b) small subunit depleted crystal obtained by treating the normal crystal with glutaraldehyde, then high salt, on the microscope grid (see text). The [1,0] and [0,1] directions for the two layers in each sheet are indicated. In (b) the high salt treatment has badly disordered the layer (arrows with broken lines) furthermost from the carbon film; the juxtaposed layer is still more or less intact. Magnification, 43,000×.

TABLE 1

Low resolution structure factor phases and amplitudes for normal and glutaraldehyde, high salt, treated crystals

			Normal crystals			GHS crystals				
h	k	Phase	$\|F\|_p$†	$\|F\|_{3D}$‡	$\|F\|_1$§	$\|F\|_2$	$\|F\|_3$	$\|F\|_4$	$\|F\|_5$	$\|F\|_6$
0	1	+	18·7	16·4	11·5	9·5	8·6	11·6	10·6	8·9
1	1	−	6·1	6·0	0·8	—	—	—	0·6	—
0	2	−	5·5	6·5	4·1	4·5	3·9	3·5	3·6	4·9
1	2	−	12·5	14·2	8·9	8·4	8·9	9·3	9·4	9·0
2	1	+	2·7	2·5	—	—	—	—	—	—
2	2	+	1·7	2·4	—	—	—	—	—	—
0	3	+	1·0	1·1	—	—	—	—	—	—
1	3	−	—	0·9	2·2	2·1	2·3	2·5	3·2	1·8
3	1	−	1·6	1·2	—	—	—	—	—	—

† Amplitudes determined by Unwin & Taddei (1977); average of up to 8 independent measurements.

‡ Amplitudes determined for a different set of crystals by Unwin (1977) after drawing continuous curves through 3-dimensional data.

§ Average amplitudes from 2 independent measurements.

GHS, glutaraldehyde, high salt treated.

but significant changes in amplitude. In particular the 1,3 peak, which is weak or absent with the normal crystals, becomes relatively strong, and the 1,1 peak, which is strong with the normal crystals, is greatly weakened or lost altogether.

All sets of data were recorded under the same conditions and scaled according to the areas from which they were obtained. The summed diffracted intensities for each set therefore give an idea of the amount of biological matter present. The ratio of the summed intensities normal/glutaraldehyde, high salt, treated crystals is, on average, about 2:1, indicating that there is about $\sqrt{2}$ times more contrast from the normal crystals. Since a single ribosome has approximately 1·5 times the mass of a large subunit, this is about the figure one would expect for complete release. However, neither this analysis nor a method using a negative stain is likely to provide an accurate figure. It is therefore only possible to deduce that a substantial fraction of the small subunits are removed when the cross-linking, high salt, treatment is applied to crystals on the grid.

Fourier syntheses of the data from the glutaraldehyde, high salt, treated crystals give projections which are similar to those from the normal crystals (Fig. 8(a) and (b)), but in which the maxima corresponding to the centres of the ribosomal particles are displaced by some 15 to 25 Å. The actual positions for these centres are plotted in Figure 9. It is seen that the centres for the glutaraldehyde, high salt, treated crystals are grouped together away from those for the normal crystals on a line pointing roughly towards the tetramer 4-fold axis.

The difference map between the data obtained by Unwin & Taddei (1977) and the average of the glutaraldehyde, high salt, treated crystals shows a single positive peak lying over the assignment, S, given previously (Fig. 8(c)). Trial difference maps, treating each example in Table 1 separately, showed only the shape and magnitude of this peak, not its position, to be sensitive to the scaling factor used, or to the positions of the particle centres (Fig. 9). I therefore take the position given in Figure 8(c) to be a reliable estimate for the location of the centre of mass of the small subunit.

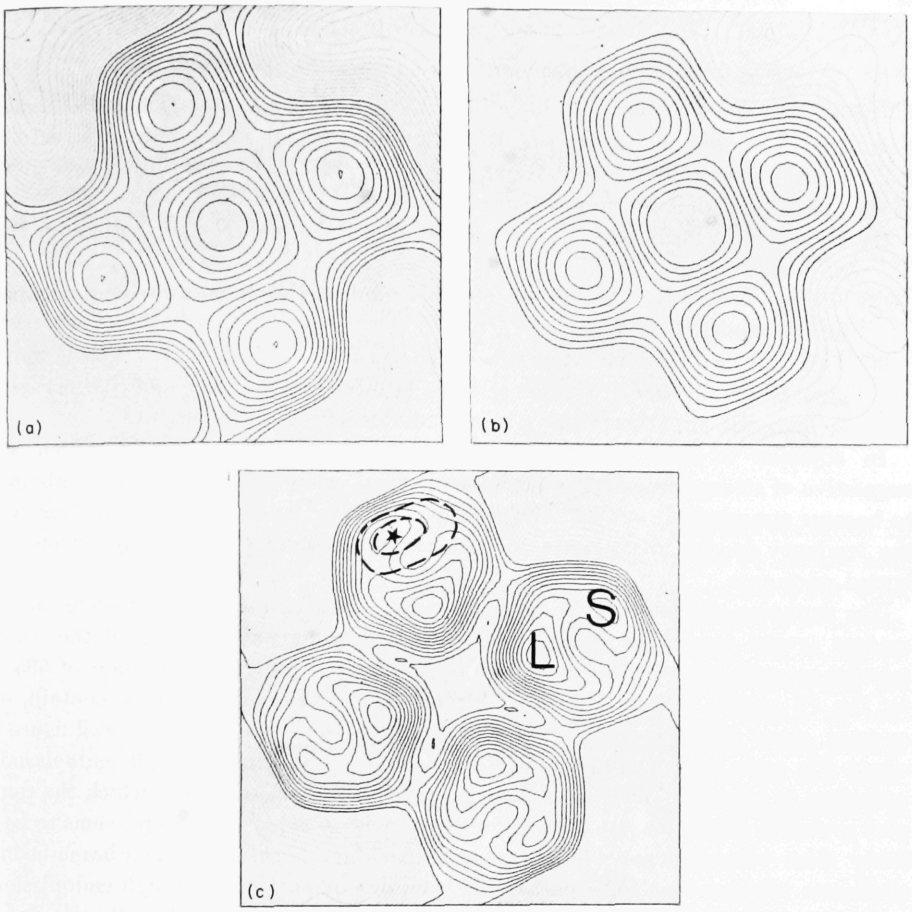

FIG. 8. Projection maps of ribosome tetramers obtained from negatively stained crystals of the lizard. (a) Using the low resolution data of Unwin & Taddei (1977); (b) using the averaged data obtained from the glutaraldehyde, high salt, treated crystals; (c) using the complete set of data of Unwin & Taddei (1977) and assigning the same positions for S and L. Also plotted over one ribosome in (c) is the peak obtained from a difference map between (a) and (b). No $F(0,0)$ terms were included in the Fourier syntheses; contours corresponding to stain-excluding regions are indicated by thicker lines.

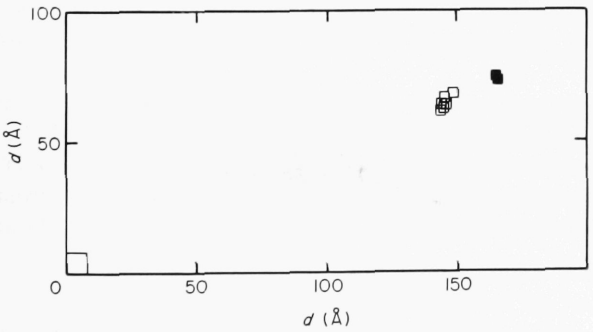

FIG. 9. Positions for the centre of one of the ribosomal particles plotted against distance, d, in the [1,0] and [0,1] directions, from the tetramer 4-fold axis. Open squares are from the glutaraldehyde, high salt, treated crystals; filled squares are from the normal crystals.

4. Discussion

(a) *Interaction of crystalline ribosomes with membranes*

The chicken and lizard crystals have the same raised up configuration and the same orientation with respect to the same type of membrane. This common structural relationship means that the interactions involved are almost certainly independent of the animal and that the results can be generalized as follows.

(1) The crystals form only on the membranes of the endoplasmic reticulum and nuclear envelope.

(2) The ribosomes composing the crystals bind to the membranes through one unique site on their large subunit.

(3) This site interacts with the site on the membrane in a way which is indistinguishable from that of ribosomes from secretory cells in terms of (i) sensitivity to high salt, (ii) susceptibility to cross-linking by glutaraldehyde.

In addition to these findings, two secondary effects were noted, which are suggestive of strong and specific interaction. One was the tendency of the membranes to become straightened out by the (presumably more rigid) crystals. The other was the tendency for the membranes to become depleted, in neighbouring regions, of bound ribosomes.

The depletion phenomenon is most pronounced, and apparently complete, in the mature crystal–membrane complex of the lizard, where about 30% of the whole membrane surface is smooth (see Results). Assuming a unit cell dimension of 595 Å for the *P*4 crystal lattice (Unwin & Taddei, 1977), the complex must contain, on average, about 800 ribosomes/square μm of membrane surface. This overall figure is about the same as one obtains for the most densely packed rough endoplasmic reticulum in summer follicle cells (Unwin, unpublished results) from which the complex probably originates (see Results; also Taddei, 1972*b*). Hence there seems to be a fairly close correspondence in the overall packing densities of membrane-bound ribosomes whether they form part of the complex or part of the rough endoplasmic reticulum. Since inactive ribosomes are known to bind to available sites on endoplasmic reticulum membranes (Adelman *et al.*, 1973) and since these sites are known to be mobile (Ojakian *et al.*, 1977), this correspondence is easily explained by a situation in which each crystalline ribosome occupies an equivalent binding site on the membrane surface to a functional one. There are other potential explanations (for instance, that the crystalline ribosomes occupy additional, more favoured sites and that the old sites become inaccessible to the numerous cytoplasmic ribosomes which are still present), however, the available evidence does not seem to argue in favour of them.

The results just summarized, together with these supporting observations, lead me to conclude that there are unlikely to be any fundamental differences between crystalline ribosomes and ribosomes in secretory cells in their direct interaction with membranes. The apparent differences can be considered to arise from a redistribution of ribosomes and their binding sites, brought about by the crystallization.

(b) *Orientation of ribosomal subunits*

In previous papers (Unwin & Taddei, 1977; Unwin, 1977) it was established that the large subunit–small subunit axis of the crystalline ribosomes lies approximately parallel to the membrane surface and that the large subunit is located closest to the

centre of the tetramer. More precise assignments than this were not feasible. In this paper, by comparing crystals composed mainly of large subunits with normal crystals, I have determined the position of the centre of mass of the small subunit. This position is in agreement with the assignment suggested previously and permits one to relate, with confidence, views obtained from crystalline ribosomes with those obtained from isolated ones.

The view in Figure 8(c) is equivalent to the "right-featured frontal" view of isolated ribosomes (Nonomura *et al.*, 1971; Lake *et al.*, 1974) in which the long axis of the small subunit is perpendicular to the viewing direction and the "head" part of the small subunit is in the region where the division between subunits is most clearly seen. This means that in the crystals the long axis of the small subunit must be approximately parallel to the membrane surface and the head part must be next to the intertetramer link.

(c) *Mode of attachment to the membrane surface*

It was shown previously (Unwin, 1977) that ribosomes of a tetramer are raised up from the membrane by protrusions which come together and touch its surface near the 4-fold axis. It is demonstrated here that the raised up configuration is lost when the tetramers are broken down into single particles, and that the effect is reversible. These findings together imply a rather loose sort of linkage between the crystalline ribosomes and the membrane surface. They suggest that the tetramer bonds may act to stabilize the ribosomes in a position away from the membrane surface by constraining their protrusions to point outwards, and that the ribosomes, when freed of this constraint, simply tend to be closer. Possible configurations for the two cases, which are compatible with known structural details are drawn schematically in Figure 10.

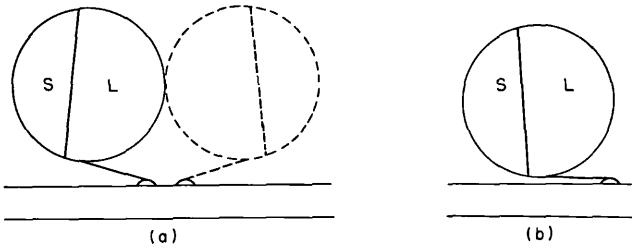

FIG. 10. Possible differences in configuration between (a) tetrameric and (b) isolated membrane-bound ribosomes. Bonds on the large subunits constrain and stabilize the tetrameric ribosomes into a raised up configuration over the membrane surface, and isolated ribosomes approach the membrane surface more closely because this constraint is lost.

Since the direct interaction with membranes of the crystalline ribosomes closely matches that of ribosomes in secretory cells, it is plausible that the latter also attach loosely through their large subunits in the way indicated in Figure 10. It is clear that only in the special circumstance where ribosomes bind specifically to one another would the protrusions be easy to detect.

5. Conclusions

Crystalline ribosomes from lizard oocytes and chick embryos have a similar direct interaction with sites on the membrane as ribosomes in secretory cells in terms of:

(1) involvement of a unique site on the large ribosomal subunit;

(2) sensitivity to K^+, but not to Mg^{2+}, concentration:

(3) susceptibility to cross-linking by glutaraldehyde;

(4) specificity for the endoplasmic reticulum and nuclear envelope.

The crystalline ribosomes lie with their subunits side-by-side over the membrane surface and with the long axis of the small subunit approximately parallel to it. They appear more distantly associated with the membrane than ribosomes in secretory cells because of interactions between their large subunits.

The mode of attachment to the membrane surface of the crystalline ribosomes, and of ribosomes in secretory cells, may be the same.

I thank Dr Carlo Taddei, Institute of Histology and Embryology, University of Naples, and colleagues at this Laboratory for helpful discussions.

REFERENCES

Adelman, M. R., Sabatini, D. D. & Blobel, G. (1973). *J. Cell Biol.* **56**, 206–229.
Byers, B. (1967). *J. Mol. Biol.* **26**, 155–167.
Byers, B. (1971). *Proc. Nat. Acad. Sci., U.S.A.* **68**, 440–444.
Falvey, A. K. & Staehelin, T. (1970). *J. Mol. Biol.* **53**, 1–19.
Kreibich, G., Freienstein, C. M., Pereyra, B. N., Ulrich, B. L. & Sabatini, D. D. (1978). *J. Cell Biol.* **77**, 488–506.
Lake, J. A., Sabatini, D. D. & Nonomura, Y. (1974). In *Ribosomes* (Nomura, M., Tissières, A. & Lengyel, P., eds), pp. 543–557, Cold Spring Harbour Laboratory Press, New York.
Morimoto, T., Blobel, G. & Sabatini, D. D. (1972*a*). *J. Cell Biol.* **52**, 338–354.
Morimoto, T., Blobel, G. & Sabatini, D. D. (1972*b*). *J. Cell Biol.* **52**, 355–366.
Nonomura, Y., Blobel, G. & Sabatini, D. D. (1971). *J. Mol. Biol.* **60**, 303–323.
Ojakian, G. K., Kreibich, G. & Sabatini, D. D. (1977). *J. Cell Biol.* **72**, 530–551.
Palade, G. E. (1975). *Science (Wash. D.C.)*, **189**, 347–358.
Sabatini, D. D., Tashiro, Y. & Palade, G. G. (1966). *J. Mol. Biol.* **19**, 503–524.
Taddei, C. (1972*a*). *Exp. Cell Res.* **70**, 285–295.
Taddei, C. (1972*b*). *Exp. Cell Res.* **72**, 562–566.
Taddei, C., Gambino, R., Metafora, S. & Monroy, A. (1973). *Exp. Cell Res.* **78**, 159–167.
Unwin, P. N. T. (1977). *Nature (London)*, **269**, 118–122.
Unwin, P. N. T. & Taddei, C. (1977). *J. Mol. Biol.* **114**, 491–506.