Contributions of restricted length reporting original and significant findings of immediate interest

RIBOSOME CRYSTALLIZATION INDUCED IN CHICK EMBRYO TISSUES BY HYPOTHERMIA

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During studies of hypothermic effects upon cytoplasmic microtubules (1) in chick embryos, a highly ordered aggregate of ribosomes was discovered in the developing fiber cells of the 10-day embryonic lens (Fig. 1). More extensive electron microscope studies revealed greater numbers of these aggregates in the annular and epithelial cells of the lens. In all cases the morphology of this crystal was that of a "sheet," one ribosome thick. Because the concentration of such sheets seemed to be proportional to the native density of ribosomes in the cell studied, I decided to chill and examine the cells of early (1 to 2 day) chick embryos, where ribosomal density is severalfold higher. Here, this hypothermic crystallization was extensive (Fig. 2); all cell types—epidermal and neural ectoderm, mesoderm, and endoderm—possessed crystalline sheets.

The procedures were as follows. Fertile eggs of White Rock domestic fowl were incubated to desired stages at 38°C and then transferred to various chambers maintained at 20°, 15°, 10°, or 5°C. After such hypothermic treatment for periods from ½ to 8 hr long, some of the embryos were fixed in phosphate-buffered glutaraldehyde (3% at pH 7.2) for electron microscope studies. The fixed tissues were rinsed in buffer, postfixed in osmium tetroxide, dehydrated, and embedded in Epon. Thin sections were stained with solutions of uranyl acetate and lead citrate for viewing in a Philips EM 200. Other cooled embryos were returned to the 38°C incubator for later examination, either for viability or for fine structural changes.

Crystalline sheets of ribosomes appeared in all tissues of both the 10-day embryonic lens and the 1 to 2 day embryo only after 3 or more hours of hypothermic treatment at 5°C or 8 or more hours at 10°C. No sheets were found in embryos treated with the higher temperatures (15° or 20°C), nor after 5° or 10°C treatments for periods shorter than those indicated above.[1] Whereas these ribosomal sheets in lens cells were about 0.25 μ in maximum dimensions, many in ribosome-rich tissues, like the 24 hr embryonic epidermal ectoderm (Fig. 3), exceeded 1.5 μ in their greatest dimension. On the other hand, all tissues possessed many aggregates of just four ribosomes (see Figs. 1 and 3) in a square array: an aggregate which might be considered a fundamental unit of the larger sheets.

The control experiments demonstrated reversibility of these hypothermic effects. First, no such aggregates were found in embryos fixed from eggs which were cooled to 5°C and then rewarmed to 38°C. Moreover, hatchability of embryos cooled to 5°C for 24 hr after 24 hr of incubation was 80%, just 5% below the normal for these eggs. Ten-day embryos did not fare so well under this experimental regime (about 60% hatchability), but

[1] The temperature decrease within these eggs was very slow. Insertion of a thermometer into eggs cooling in the 5°C chamber demonstrated that the internal temperature was still above 10°C after 2 hr.

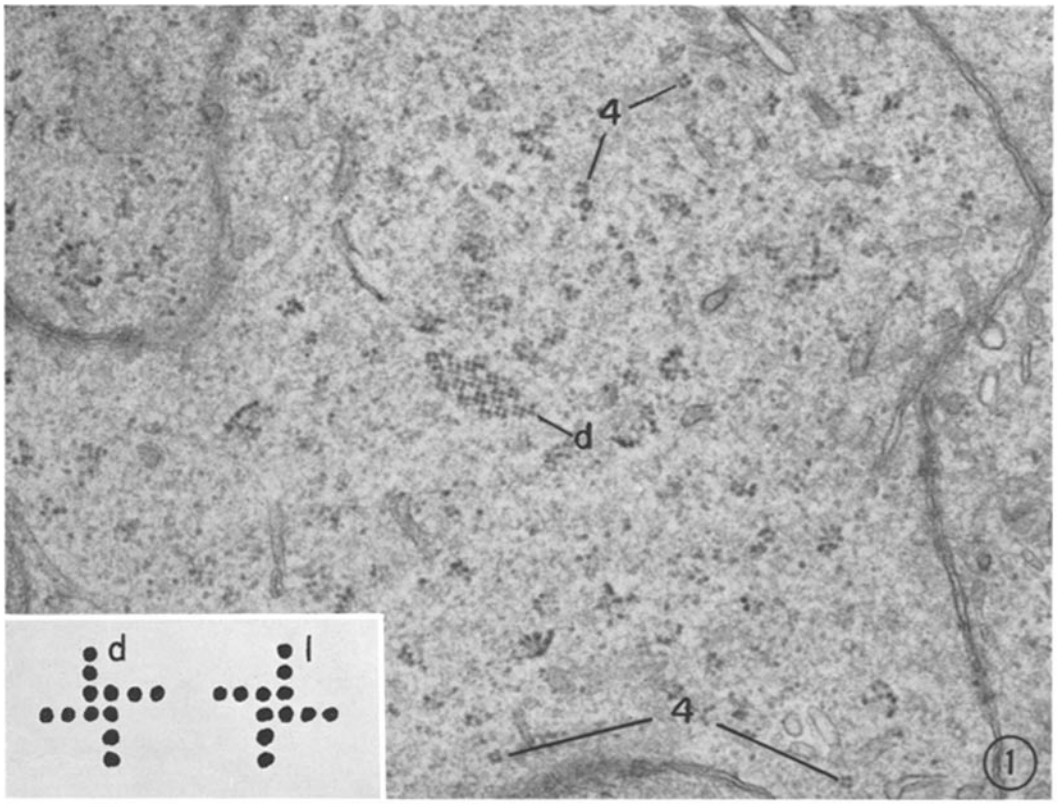

FIGURE 1 A portion of a fiber cell from the 10-day embryonic chick lens after 5°C hypothermic treatment, displaying a crystalline sheet of the dextro (*d*) pattern and several four-ribosome aggregates (*4*). *Inset* Diagrams of the dextro (*d*) and levo (*l*) patterns displayed by the hypothermic sheet (see text). Fig. 1, × 45,000; inset, × 125,000.

some of the fatalities were caused by microbial infection.

The basic pattern of ribosomal crystallization, as viewed from one side of the surface lattice, is diagrammed in Fig. 4. The perpendicularity of packing justifies a consideration of the bonding characteristics of four sides. These sides in the diagram are coded, in clockwise order, as (*1*) clear, (*2*) bold lines, (*3*) fine lines and (*4*) dots. Every side except the clear one, which faces the "void," has a neighbor at a distance of 240 A, center-to-center spacing. It is to be noted that the four ribosomes in the unit square are united uniformly by sides with dots facing sides with fine lines, i.e. by *tetramer bonds* between sides which are 90° counterclockwise from the void and sides which are 180° from the void. The unit square is always joined to an adjacent square by sides with bold lines facing their own kind, i.e., by *dimer bonds* between mutually apposed sides which are 90° clockwise from the void. Therefore, the bonding pattern of any ribosome may be superimposed upon that of any other by two operations: (*a*) successive 240 A translations, and (*b*) successive rotations in multiples of 90° about an axis normal to the sheet. This congruence of bonding patterns implies a similar pattern of asymmetry on the part of the ribosomes themselves. Such a sheet, or net, is described crystallographically as a member of plane group *p4*. The unit cell, indicated in Fig. 4, is a square of side length 537 A with vertices at the centers of the four-ribosome squares. The center of a ribosome is 170 A from a vertex at an angle of 18.4° from the side of the cell.

Viewing the pattern in Fig. 4 from beneath would obviously yield a mirror image, in which the four coded sides listed above would be presented in a counterclockwise, rather than a clock-

wise, order. To facilitate description, I shall henceforth refer to the pattern in Fig. 4 as "dextro" and to its mirror image as "levo." These two patterns are depicted in the inset of Fig. 1. A portion of each pattern has been "graphically extracted" from the complete net to reveal the arrangement of four-ribosome rows with which the sides of a unit square are continuous. The mirror images–dextro (*d*) and levo (*l*)–might be visualized as pinwheels pointing clockwise and counterclockwise, respectively. Turning attention to the micrographs, one finds the dextro lattice in the lens fiber cell in Fig. 1, and both forms in the epidermal ectoderm in Figs. 2 and 3. Just as the originally dextro pattern in the diagram (Fig. 4) becomes levo when viewed from beneath, so should the form displayed by the actual ribosomal sheets depend simply upon the side from which they are viewed in the electron microscope. Therefore, the occurrence of both images, dextro and levo, does not imply the presence of two classes of sheets. On the other hand, neither does it exclude the possibility of such dimorphism.

Resolution of this ambiguity may depend upon whether or not the other two perpendicular faces of the ribosome, which face the surfaces of the sheet, can be defined. Referral to Fig. 2 reveals a pertinent feature in this regard. Several of the sheets are cut in transverse section, revealing curved profiles. In this case, the concave surfaces are bounded by a relatively electron-opaque cytoplasmic matrix, whereas the convex surfaces meet a lighter matrix. In other micrographs, where curvature is not seen in profiles of sheets, the dense and light matrices are still evident. This differentiation between surfaces now permits inquiry into the possibility of dimorphism. One may ask, for example, which pattern, dextro or levo, is dis-

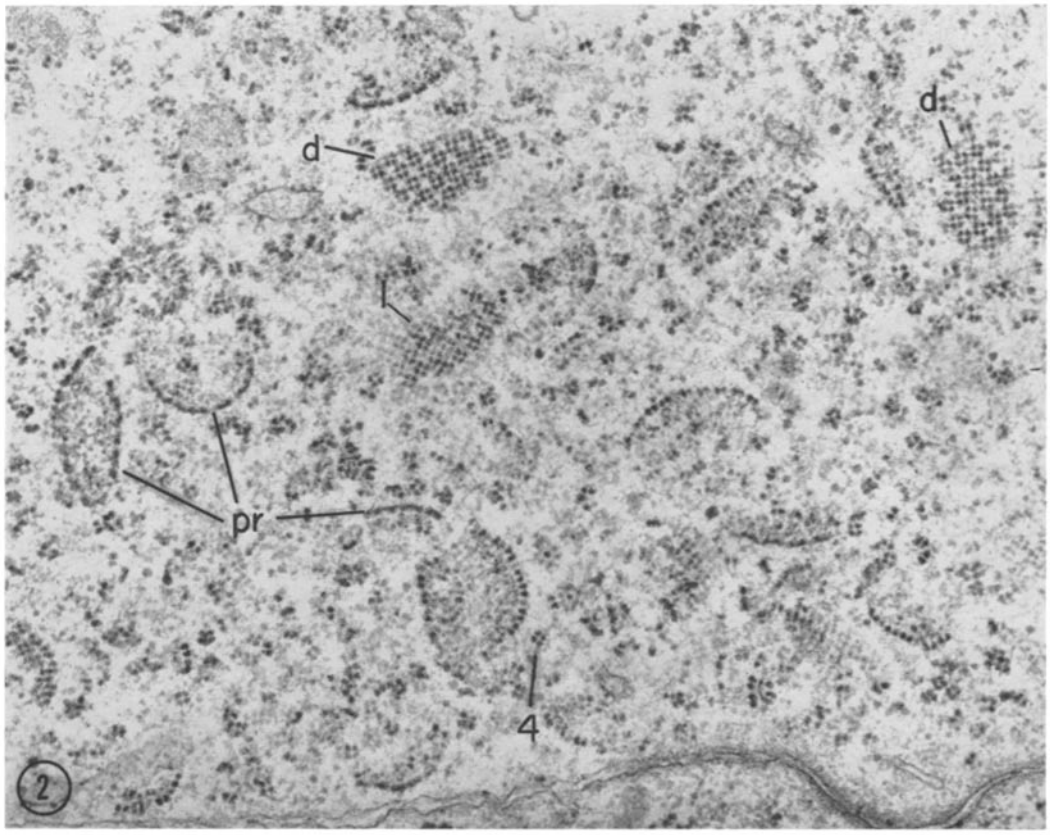

FIGURE 2 A portion of an epidermal ectoderm cell of the 24 hr chick embryo after hypothermia. Both dextro (*d*) and levo (*l*) patterns of ribosomal sheets are present. Curved profiles (*pr*) of such sheets are abundant. One isolated set of four ribosomes in a square array is indicated (*4*). × 45,000.

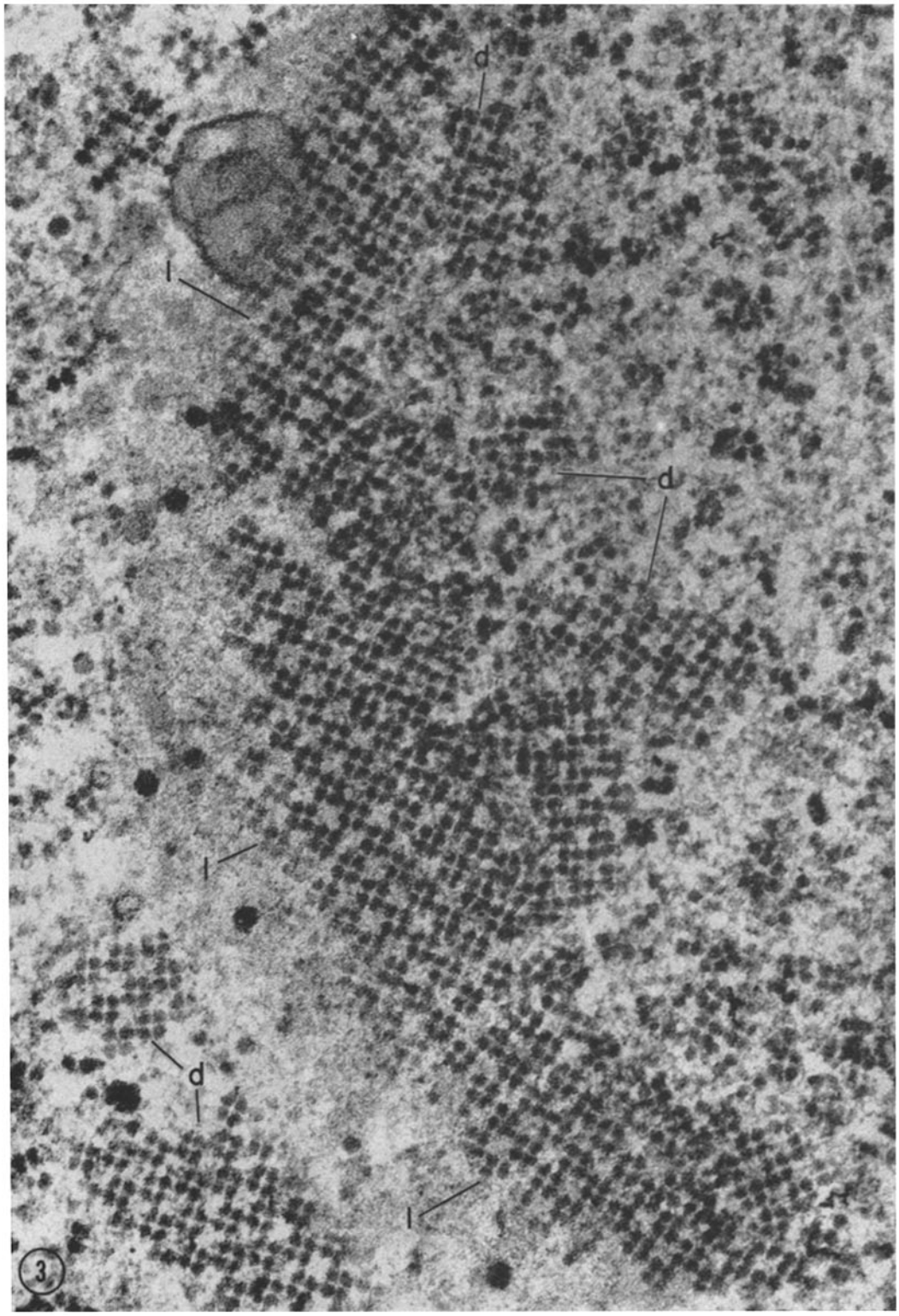

FIGURE 3 A higher magnification view of ribosomal crystals in the epidermal ectoderm of the 24 hr chick embryo. Both dextro (*d*) and levo (*l*) patterns are indicated. × 100,000.

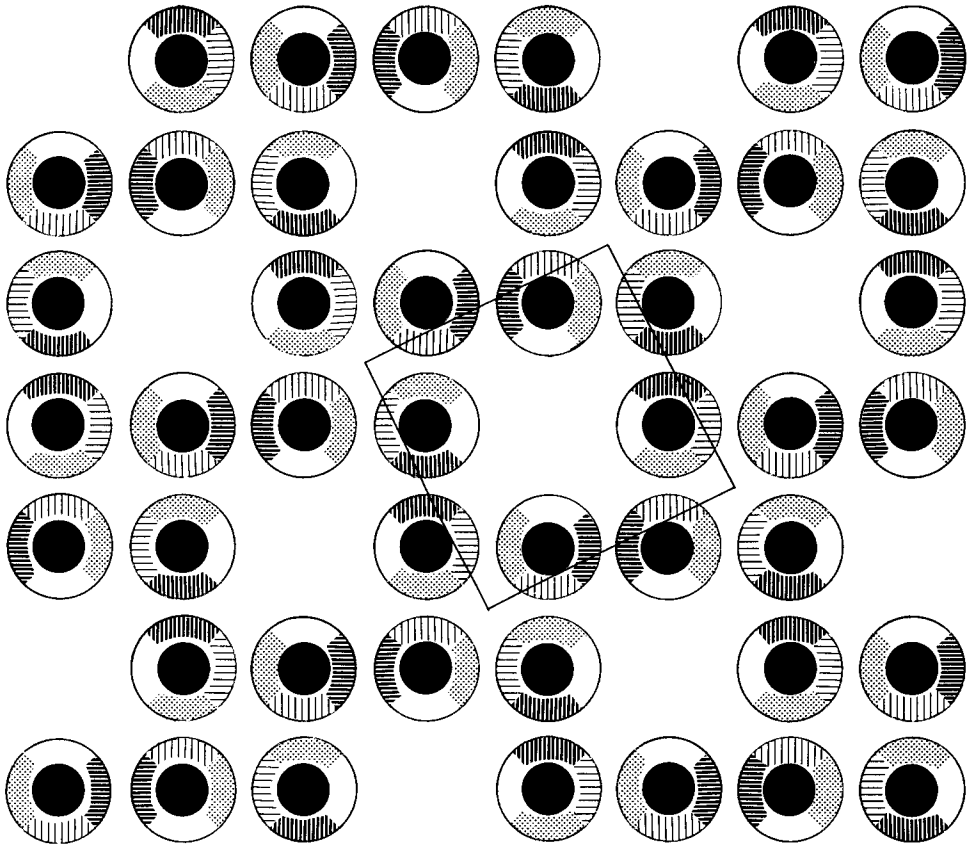

FIGURE 4 This diagram represents the bonding pattern of ribosomes within a hypothermic ribosomal sheet of the dextro pattern. The levo form would be obtained by viewing it from beneath. The unit cell is represented by the square. Coded faces indicate that every ribosome possesses the same order of bonds with its neighbors. × 640,000.

played by a sheet viewed from the side with a dense matrix. If the same pattern is discovered in every case, there is but one form of the lattice; if both are found, there are two forms. Unfortunately, an electron micrograph is a planar projection of a three-dimensional specimen. This projection does not contain the information necessary to determine the pattern of a sheet from a specified surface. Therefore, new kinds of evidence will have to be brought to bear on this problem in the future.

There are occasional deviations from the bonding pattern described above, but they generally leave the four-ribosome square intact. For instance, a square may be rotated as far as 15° from its usual orientation in the lattice. Here, as in other cases, the tetramer bonds (dots to fine lines) exhibit greater stability than the dimer bonds (bold lines to bold lines).

These cohesive four-ribosome squares (tetramers) also occur free in the cytoplasm and are probably identical to the "stable polysomes" encountered in sucrose gradient polysome profiles of chick tissue chilled before homogenization (2, 3). Such aggregates are insensitive to ribonuclease treatment sufficient to fragment messenger-bound polysomes. Electron micrographs of these four-ribosome aggregates by negative staining present an image very similar to that which I find by thin sectioning. More recent experiments (4) demonstrate that such stable "fours" are derived from inactive monosomes during hypothermic tissue preparation.

As noted earlier, the bonding pattern suggests that any individual ribosome, if asymmetric, must be rotated about an axis normal to the plane of the sheet if it is to be superimposed upon its nearest neighbors. Such is probably not the case

for adjacent ribosomes of a polysome lying against the membrane of the endoplasmic reticulum. Nevertheless, the center-to-center spacing of both aggregates is the same, 240 A, in the tissues studied here. This may simply suggest that the ribosome possesses effective radial dimensions of 120 A, regardless of which sides are apposed to one another. One may also wonder whether these bonding sites may represent thermal transitions of sites normally functional in maintaining the form of "helical polysomes."

A model for a different sort of ribosomal crystal, in the chromatoid bodies of *Entamoeba invadens* (5, 6), has recently been obtained by optical diffractometry of electron micrographs (7). Perhaps the hypothermic crystal described here will provide another system for the analysis of ribosomal morphology. Moreover, the hypothermic crystallization of ribosomes may find application in the in vitro purification and manipulation of these familiar, but still mysterious, cell components.

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