Journal of Supramolecular Structure 10:365–375 (1979)

# Ribosome Crystallization in Nuclei of Chick Embryos

**Marcello Barbieri**

*Max-Planck-Institut für Molekulare Genetik, Abt. Wittmann, D-1000 Berlin-Dahlem, Germany*

Ribosome crystallization within nuclei has been studied in chick embryos with procedures which increase its frequency by various orders of magnitude as compared to previous findings. The extrusion of ribosome microcrystals from nuclei is reported for the first time, and a model for the transfer of ribosomes from nucleus to cytoplasm is proposed.

Key words: ribosomes, crystallization, hypothermia, chick embryos, degeneration, nuclei, nucleolar extrusions

Ribosome crystallization within nuclei has been reported to occur with an extremely low frequency in respect to the frequency of formation of ribosome microcrystals in the cytoplasm. Byers [1] reported that in chick embryos submitted to a standard hypothermic treatment ribosome crystals do not normally occur within the nuclei; he found only one nucleus containing crystals and even in this case the finding had to be treated with some reservation because the nuclear membrane was not intact. Similarly, Birks and Weldon [2], in a study on ribosome crystallization that occurs at 38°C in degenerating neuroblasts, reported that "on a single occasion a large crystal was found within an apparently intact nucleus."

In different systems derived from chick embryos these findings seem to be slightly more frequent and intranuclear ribosome microcrystals have been reported, with an apparently intact nuclear membrane, in cell cultures [3] and in chorioallantoic cells infected by a virus [4]; but even in these cases the phenomenon appears to be a rare event. Byers [1] has commented on this situation by saying that "either the nucleus contains no particle which is strictly equivalent to the cytoplasmic ribosome, or conditions for crystallization of ribosomes are unfavourable within the nucleus."

Another possibility [5] is that the small number of intranuclear microcrystals reflects the fact that crystallizable ribosomes remain in the nucleus for very short periods of time

Received February 21, 1979; accepted February 21, 1979.

0091-7419/79/1003-0365$02.30 

during the transfer from nucleus to cytoplasm that accompanies the maturation of the ribosome precursors.

Whatever the true explanation, the study of intranuclear ribosome crystallization appears to be potentially able to provide information either on the environmental conditions that favor or oppose the crystallization of ribosomes, or on possible structural differences between nuclear and cytoplasmic ribosomes, or on the modalities with which ribosomes are exported from nucleus to cytoplasm, and the present study describes a first group of results that have been obtained along this line of research.

## METHODS

The preparation of the samples for electron microscopy and the statistical analysis of the ribosome microcrystals were performed according to the procedures described in a previous paper [6].

## RESULTS

Three groups of experiments were performed; each will be described in the following sections.

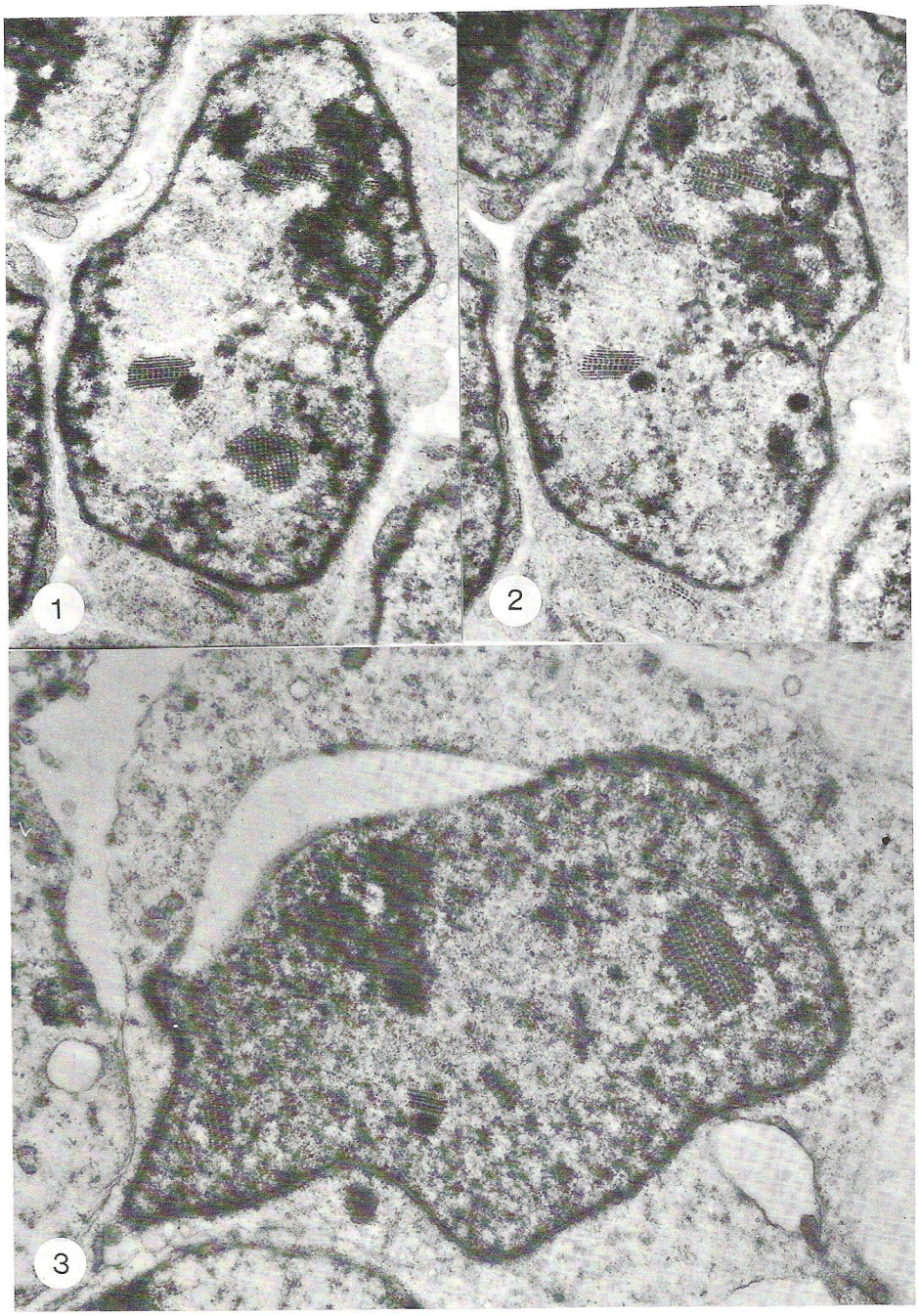
### Prolonged Hypothermic Treatments at 4°C

The standard hypothermic treatments that have been used to induce the formation of ribosome microcrystals in chick embryos consist of the incubation of eggs, or cell cultures, at temperatures of 4°C or 10°C for periods of time ranging between 3 and 24 hours [1–4]. In these conditions intranuclear ribosome crystallization is a rare event and it appeared natural, as a first step, to study what happens to it by prolonging the hypothermic treatment.

Four-day-old embryonated eggs were hypothermized at 4°C for 1–4 days, and by scanning of approximately equal tissue areas per sample (50 holes of 300-mesh grids) the number of nuclei containing crystals within apparently intact nuclear membranes amounted to 0, 4, 12, and 11, respectively, after 1, 2, 3, and 4 days of cooling. Figures 1 and 2, for example, show intranuclear crystals produced after three days of cooling and represent two different sections of the same nucleus, which show that the nuclear membrane is intact at different levels. Fairly often, however, after prolonged periods of cooling the nuclear membranes are damaged; but even in these cases the crystals appear to have a nuclear origin because they are usually far away from the damaged region and the surrounding nucleoplasm does not seem to be contaminated by cytoplasmic intrusions (Fig. 3).

In addition to the results described above the experiment with prolonged cooling provided unexpected findings that are interpreted as extrusions of nuclear material and of intranuclear ribosome microcrystals from the nucleus. The phenomena appear with a frequency which is too low to allow a quantitative evaluation, but on morphologic grounds they could be adequately documented because over 20 cases were recorded in the course of the study. They were found in sections of 4-day-old chick embryos hypothermized at 4°C for 3–4 days and appeared as special cases among the various kinds of structural alterations that are produced by degeneration processes after the death of the embryos.

These are the cases where segregated compartments associated with or delimited by fibrils, and sometimes by single-layered membranes, are formed within the nucleus. They




Figs. 1–3. Ribosome crystallization within nuclei with intact (Figs. 1 and 2) and damaged (Fig. 3) nuclear membranes in chick embryos hypothermized at 4°C for 72 hours. Figures 1 and 2 represent different sections of the same nucleus showing that the nuclear membrane is intact at various levels. × 16,000.

tend to expand until the nuclear membrane is reached and pulled apart so as to allow the extrusion into the cytoplasm of the sequestrated material. This sequence of events is illustrated in Figures 4 to 6. Figures 4 and 5 show different cases where the sequestration of nuclear material, inclusive of ribosome microcrystals, appears to be entirely confined within the nucleus. Figure 6 shows instead a case where the nuclear membrane is pulled apart and appears to indicate that the flow of material is directed from the nucleus toward the cytoplasm and not vice versa.

The extrusion of nuclear material, and even of whole nucleoli from the nuclei, has been observed before and interpreted in various ways [8], but this is the first time that the phenomenon is reported to occur at 4°C and that ribosome microcrystals have been described among the extruded material.

### Degeneration Treatments at 20°C

These were the water treatments and the shock treatments at 20°C that were described in a previous paper [6], except that in this case the study was specifically concentrated on intranuclear crystallization. The above treatments have made it possible to examine a large number of intranuclear ribosome crystals for the first time, and a statistical analysis was carried out with the nearly quantitative procedure described in Methods.

The first result of this study is that all intranuclear crystals consist of three-dimensional P422 stacks of P4 layers, whereas the great majority of cytoplasmic crystals are two-dimensional P4 layers. This indicates that at least a slight difference exists between nuclear and cytoplasmic crystallization that may reflect different rates in the kinetics of crystallization but not in the symmetry groups of the crystals.

A precise evaluation of the increase in intranuclear ribosome crystallization is difficult, but an approximate computation was done by considering that with the above treatments an average of one nucleus containing microcrystals was found for each hole of 300-mesh grids, whereas with standard cooling at 4°C for equivalent times the average was one such nucleus every 6–8 grids, which corresponded to the scanning of the sections of several hundred holes. The treatments described therefore increase the frequency of intranuclear ribosome crystals by an order of magnitude that ranges between $10^2$ and $10^3$. A qualitative evaluation of this effect is provided by occasional findings, like that represented in Figure 7, which shows various adjacent nuclei containing microcrystals. Similar cases have never been observed with conventional methods.

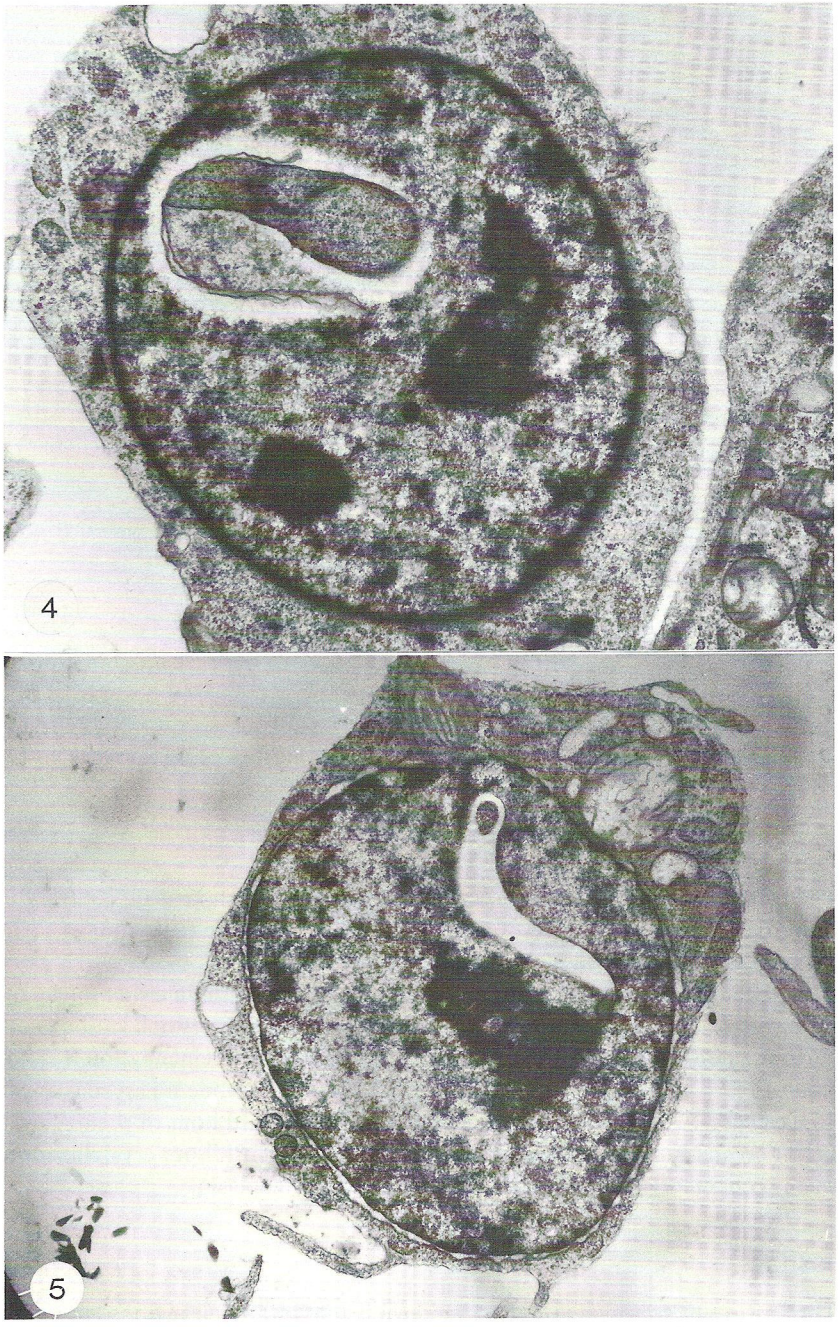
### Degeneration Treatments at 38°C Followed by Cooling at 4°C

The experiment was carried out with 4-day-old embryonated eggs that were immersed in water at 38°C and kept there for periods of time ranging between 1 and 12 hours. In order to determine the amount of ribosomes that remained available for crystallization after any prefixed period of preincubation in water at 38°C, the eggs were transferred to the cold room and hypothermized at 4°C for 48 hours, after which the embryos were extracted and prepared for electron microscopy examination as described in Methods.

The results show that the formation of ribosome microcrystals in the cytoplasm decreases in all tissues in a rather discontinuous but monotonic way: After 1 hour of preincubation in water at 38°C the pattern is more or less the same as that which characterizes hypothermized but nonpreincubated embryos (Fig. 8), but after 3 hours of preincubation it is profoundly changed and many cells contain scattered ribosome aggregates, many of which appear to consist of 4 or 8 ribosomes (Figs. 9 and 10).

Perhaps one of the best morphologic indications of this general decrease is the finding that after three hours of preincubation crystals started disappearing even in mitotic cells

(Fig. 9), which had never failed to produce ribosome crystals in healthy embryos after a standard cooling [1] and which consistently had crystals after one hour of preincubation (Fig. 8).




Figs. 4 and 5. Intranuclear sequestration of material that contains ribosome microcrystals with associated fibrils or membranes. Figure 4 × 14,080; Figure 5 × 10,550

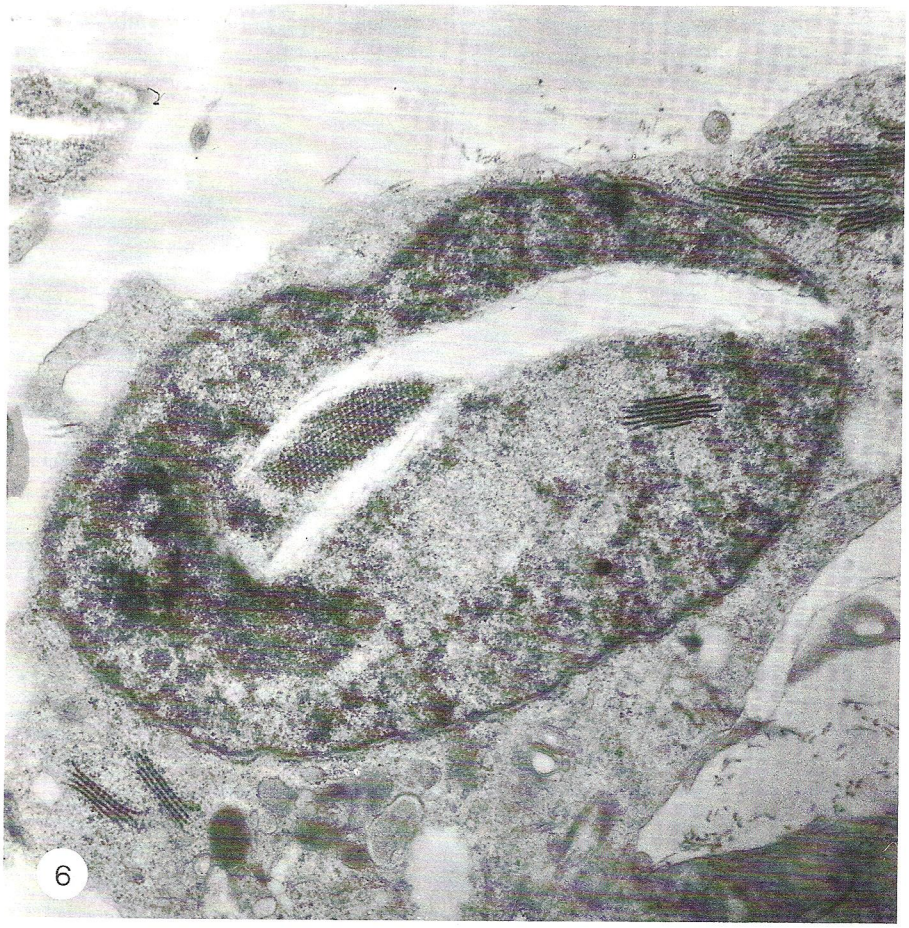



Fig. 6. Extrusion of ribosome microcrystals from the nucleus at the stage of the rupturing of the nuclear membrane. × 17,000

The formation of ribosome microcrystals inside the nuclei, instead, contrary to what happens in the cytoplasm, increases steadily from the third to the fifth hour of preincubation, and only after this period is the tendency reversed.

After 10 hours of preincubation at 38°C no crystal is found in either nuclei or cytoplasms, but the decline of intranuclear in respect to cytoplasmic crystallization is definitely delayed, to the point that from the fifth to the eighth hour of preincubation it is not uncommon to find extensive crystallization within the nuclei while no crystals appear in the cytoplasm (Fig. 11). By contrast, from the first to the third hour of preincubation no intranuclear crystal is found, whereas from the third to the fifth hour crystallization within the nuclei is consistently less abundant than that in the cytoplasm.

## DISCUSSION

The first result to which attention is drawn is that intranuclear ribosome crystallization always takes place in cells that are undergoing some kind of degeneration process.

Given this circumstance, the possibility that the microcrystals represent cytoplasmic contamination was considered first, but the detailed examination of hundreds of cases that was performed during the study has allowed us to consider this as very unlikely.

Another possibility is that degeneration processes alter the nuclear environment and produce a shift toward cytoplasmic-like conditions, which are more favorable to crystallization. This hypothesis, however, implies that ribosome crystallization can occur only in a restricted set of environmental conditions and this does not seem to be the case.

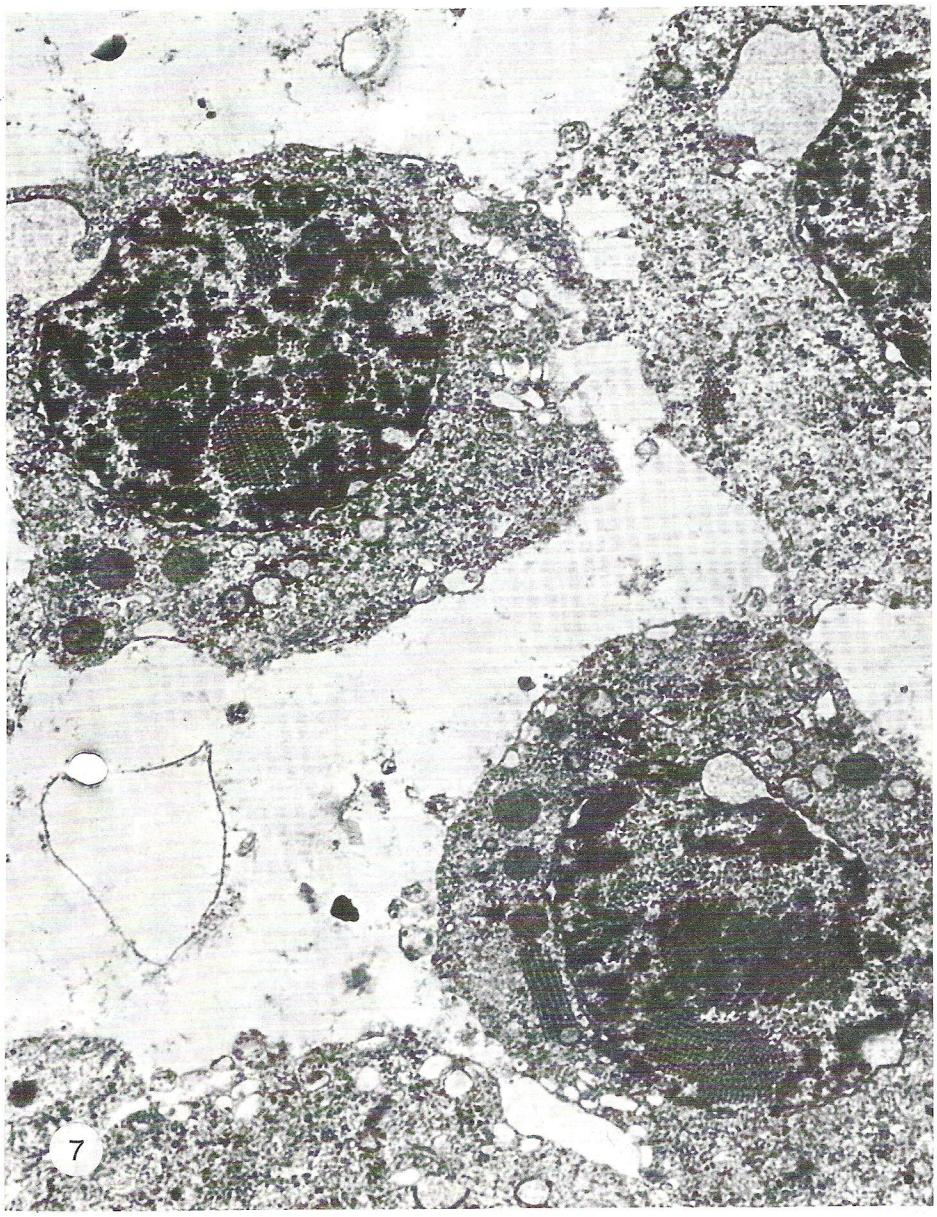
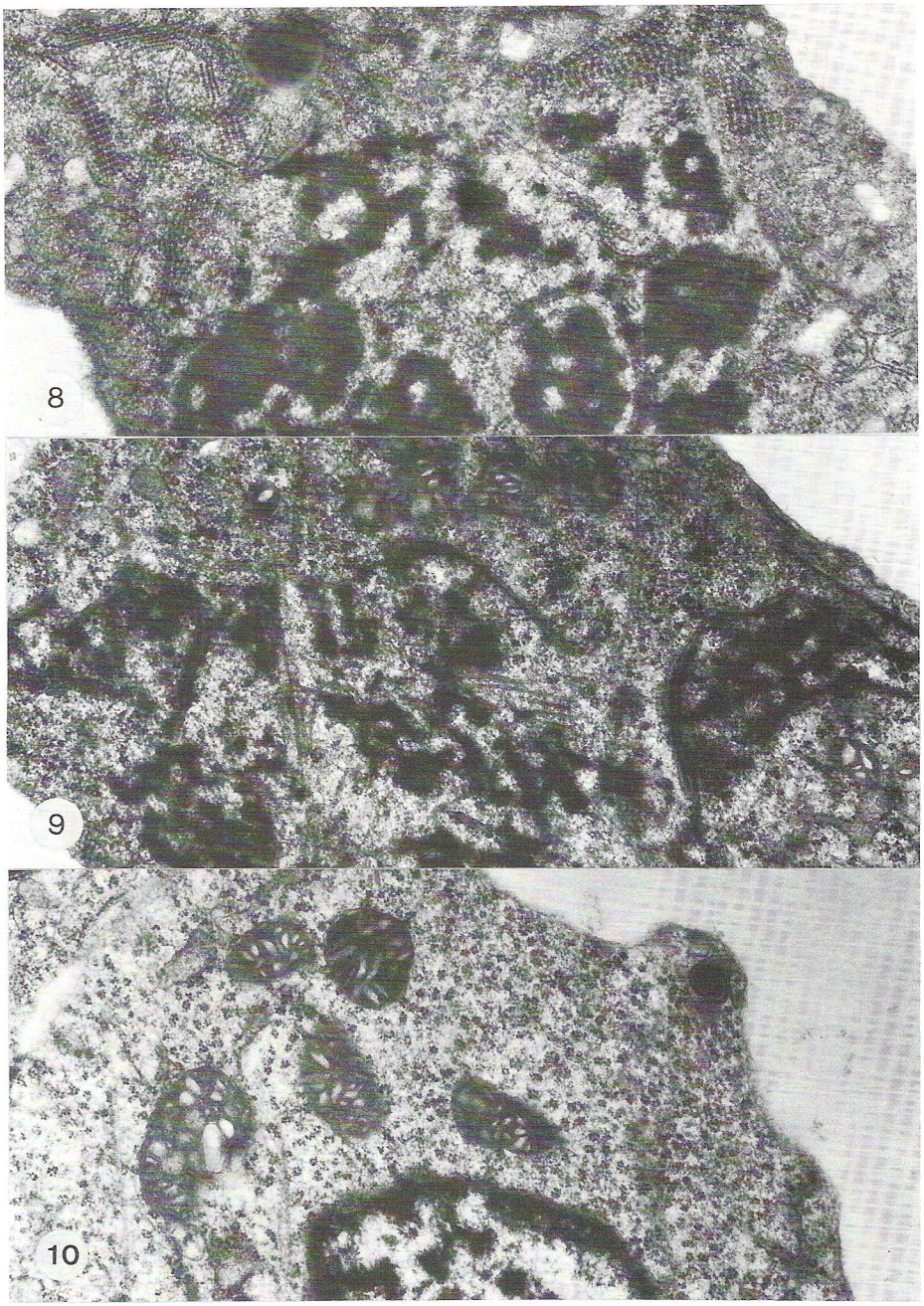



Fig. 7. Intranuclear ribosome crystallization in adjacent nuclei of chick embryos submitted to water treatment at ∿20°C for 60 hours. × 14,100




Figs. 8–10. Decrease of ribosome crystallization as a function of the preincubation time in the degeneration treatments at 38°C followed by cooling at 4°C for 48 hours. After 1 hour of preincubation ribosome crystallization is induced by cooling as with untreated embryos (Fig. 8), while after 3 hours a substantial percentage of cells in mitosis (Fig. 9) as well as in interphase (Fig.10) show only scattered ribosome aggregates. × 17,400

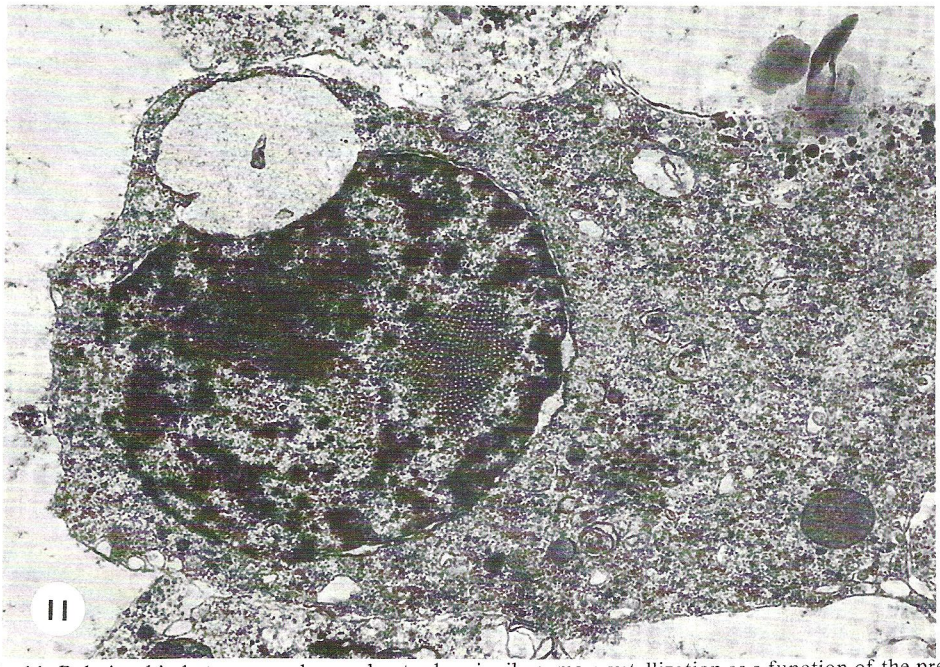


Fig. 11. Relationship between nuclear and cytoplasmic ribosome crystallization as a function of the preincubation time at 38°C in water. After 6 hours ribosome crystallization is more pronounced within the nucleus than in the cytoplasm. × 15,150

It has already been noticed [5] that ribosome crystallization takes place in a variety of environments, and a recent report has shown that it occurs even in homogenates and cell extracts after a profound disruption of the cytoplasmic environment, with the addition of salts and in the presence of nucleases [7].

The interpretation that is favored here is that degeneration processes are required in order to interrupt or damage the physiologic transport of ribonucleoproteins from nucleus to cytoplasm and keep inside the nucleus ribosome precursors that slowly mature, at low temperatures, into crystallizable ribosomes. This interpretation, which was first proposed in a previous paper [5], naturally explains a variety of experimental results. It accounts, first of all, for the fact that with the standard hypothermic treatments described by Byers [1] ribosome crystallization takes place exclusively in the cytoplasm. These treatments do not damage the cellular mechanisms irreversibly because after reincubation at 38°C the embryos continue to grow normally, and according to the model ribosome precursors are matured into crystallizable ribosomes while their transport from nucleus to cytoplasm is also taking place.

Second, the model accounts for the fact that by prolonging the hypothermic treatments from one to four days intranuclear crystallization is eventually observed but still remains a rare event. This is because the majority of the potentially crystallizable ribosomes have already been exported from the nucleus during the first 24 hours of cooling. Furthermore, the nuclear precursors that still remain in the nuclei after the first 24 hours of cooling continue their maturation very slowly at 4°C, and a rapid increase in crystallization is not expected.

Third, the model accounts for the fact that when degeneration processes start simultaneously with the lowering of temperature, a dramatic increase of intranuclear ribosome crystallization does take place as happens in the water treatment and the shock treatment experiments at 20°C. In this case the exportation mechanisms are damaged or interrupted before the nuclear reservoir of potentially crystallizable ribosomes is depleted, and the model foresees, therefore, that a large number of crystallizable ribosomes are trapped inside the nucleus, as the evidence shows.

It will be noticed that the model presumes a high turnover of nuclear ribonucleoproteins but this assumption, far from being an obstacle, is in good agreement with the experimental facts if it is considered that from the third to the fith day the RNA content of chick embryos increases approximately ten times. It will also be noted that the model does not relate the amount of ribosome crystals found at any time to the amount of ribosome precursors present in the nuclei at any one time, but with the amount of ribonucleoproteins that have gone through the maturation stages during the whole period that elapses between the removal of the eggs from the incubator and the moment at which the embryos are extracted for analysis.

The model has implications for cell biology because it implies not only that the transport of ribosomes from nucleus to cytoplasm is conceptually independent from the transformation of precursors into mature ribosomes but also that one of these two processes can in practice be altered without affecting the other.

The second result to which attention is drawn is the fact that intranuclear ribosome crystallization is consistently delayed with respect to the cytoplasmic one. The best evidence, up to now, comes from the experiments in which embryonated eggs are hypothermized at 4°C after various periods of preincubation in water at 38°C. These experiments show that cytoplasmic crystallization appears first and begins to decrease after the first hour of preincubation while intranuclear crystallization begins to appear only after three hours of preincubation and its decline takes place only after the fifth hour.

This pattern is interpreted by the proposed model because on the one hand, nuclear ribonucleoproteins are at earlier stages of ribosome biogenesis and on the other hand, time is also required for the preincubation in water at 38°C to damage effectively the exportation mechanisms and trap potentially crystallizable ribosomes inside the nucleus.

It will also be appreciated that the model proposed [5] foresees the decline observed in both nuclear and cytoplasmic crystallization, because one of its assumptions is that ribosomes are crystallizable only in a transient period of their life cycle. According to the model, in fact, subphysiologic temperatures are necessary for crystallization precisely because they prolong substantially the duration of this period.

Finally, the extrusion of ribosome microcrystals from the nuclei which has been reported above deserves some comment.

Some researchers have suggested that nucleolar extrusions represent the normal way in which ribosomes are exported from nucleus to cytoplasm, but this view is now generally disregarded and said extrusions are considered by most as an agonal phenomenon [8]. The present study concurs with this last conclusion, because the extrusion of ribosome microcrystals was never observed before cell death but only after that event and in the presence of clear signs of cell degeneration. However, although massive extrusions can be disregarded as the normal way of exporting ribosomes from the nuclei, it is possible that they represent the amplification of a process that normally takes place at a macromolecular level. In fact, if massive extrusions can take place in the presence of cell degeneration and at the low

temperature of 4°C, it is possible that the nucleus reacts strongly toward an abnormal accumulation of mature ribosomes.

One can think, therefore, that at a physiologic temperature and in viable cells, the mentioned nuclear activity is exercised as soon as a small excess of ribosomes is accumulated – a mechanism which would bring about an almost continuous displacement of ribonucleoproteins across the nucleus as the result of small scale "extrusionlike" reactions induced in the nucleoplasm by various maturation stages of the ribosome precursors. According to this model, the exportation of ribosomes from nucleus to cytoplasm would be realized neither by diffusion mechanisms nor by the displacement of ribosomes along preexisting substrates, but as the result of specific interactions between ribonucleoproteins and nucleoplasm that are triggered during ribosome biogenesis.

At the present stage of the research the extrusion of ribosome microcrystals from the nuclei may seem to represent only a morphologic curiosity, but the alternative possibility which has been described above deserves further investigation.

## ACKNOWLEDGMENTS

I am deeply grateful to Professor H.G. Wittmann for continued discussions and support. I also wish to acknowledge the skillful help of Dr. A.W. Siddiqui for the electron microscopy preparations.

## REFERENCES

1. Byers B: J Mol Biol 26:155, 1967.
2. Birks RI, Weldon PR: J Anat 109:143, 1971.
3. Barbieri M, Simonelli L, Simoni P, Maraldi NM: J Submicrosc Cytol 2:33, 1970.
4. Moretti GF, Zitelli A, Baroni A: J Submicrosc Cytol 4:215, 1972.
5. Barbieri M: J Theor Biol 47:269, 1974.
6. Barbieri M: J Supramol Struct 10:359, 1979.
7. Barbieri M: J Supramol Struct 10:349, 1979.
8. Bush H, Smetana K: "The Nucleolus." New York: Academic, 1970, p 145.