# A HEAT-SENSITIVE CELLULAR FUNCTION

# LOCATED IN THE NUCLEOLUS

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## ABSTRACT

Striking nucleolar lesions occur in cultured cells after exposure to supranormal temperatures. These lesions appear at 42°C and consist of a loss of the granular ribonucleoprotein (RNP) component and intranucleolar chromatin, and a disappearance of the nucleolar reticulum. The material remaining in the morphologically homogeneous nucleolus is a large amount of closely packed fibrillar RNP. The lesions remain identical as temperature increases to 45°C. These alterations are reversible when the cells are returned to 37°C and are associated with the reappearance of an exaggerated amount of intranucleolar chromatin and granular RNP. High-resolution radioautography indicates that after thermic shock nucleolar RNA synthesis is inhibited whereas extranucleolar sites are preserved: it also suggests that the granular RNP is reconverted to fibrillar RNP probably by simple unraveling. The results prove the existence of heat-sensitive cellular functions in the nucleolus which deal with the DNA-dependent RNA synthesis. The precise site of action is assumed to involve hydrogen bonds, resulting in configurational changes in nucleolar RNP and affecting the stability of the DNA molecule. The subsequent events in nucleolar RNA synthesis are discussed in light of the morphologic and biochemical effects of actinomycin D on the nucleolus.

## INTRODUCTION

Supranormal and infranormal temperatures affect bacterial and viral development (2-4, 10, 12). In a first attempt at a synthesis based on extensive work on poliovirus, Lwoff (13) established a critical thermosensitive event involving hydrogen bonds in the viral cycle and advanced the working hypothesis that this event is the polymerization of a monomer polymerase protein: a viral structural gene carries the information for the synthesis of this monomer, which can be synthesized at supraoptimal temperatures but cannot be polymerized into an active polymerase. Later, Gharpure (6) demonstrated that a heat-dependent cellular function also affects the developmental cycle of viruses: cells exposed before infection to supranormal temperature (15 min at 45°C) do not allow the growth of DNA viruses but RNA viruses are synthesized normally. The same author assumes that this heat-sensitive step is concerned with the DNA-dependent RNA synthesis necessary for the transcription of DNA virus-specific proteins.

It seemed logical at this point to study the effect of temperature on cells alone in order to determine the nature and the site of this cellular function. By means of ultrastructural studies, including cytochemistry and high resolution radioautography, it was found that supranormal temperature affects selectively the nucleolus and the intranucleolar chromatin and that the lesions are reversible.

## MATERIALS AND METHODS

### Cultures

The cell line (BHK) used for these experiments is derived from the kidneys of Syrian hamsters (15), and normally grows at 37°C. The cells are cultured in

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prescription bottles with a modified Eagle's solution containing, respectively, four and eight times the usual concentrations of amino acids and vitamins and supplemented with 10% calf serum and 10% tryptose phosphate. 48 hr after subculture, the cells had formed a monolayer and were transferred to a maintenance medium containing 8% calf serum.

The bottles were then totally immersed in a water bath previously warmed at the required temperature. The bottles were kept 4 or 5 cm below the water surface for a determined period of time. Within 3-4 min the temperature inside the prescription bottle had equilibrated with that of the water bath, as measured by a thermometer placed in the culture medium of the prescription bottle. The bath was maintained within 0.05°C of the desired temperature. After the thermic shock, the cells were either fixed immediately for electron microscopy or transferred to a new maintenance medium and allowed to grow at 37°C for 12-24 hr so that their ability to recover from the effect of heat shock could be studied. Control experiments were simultaneously carried out in which cells were allowed to grow normally. The temperatures employed were 38°, 39°, 40°, 41°, 42°, 43°, 44°, and 45°C, for 15, 30, 45, and 60 min and up to 120 min when no changes were noticed. The same experiments were repeated on freshly prepared cells of rat embryos in order to check whether the results differ from one cell line to another.

## Electron Microscopy

The cells were fixed *in situ* by addition of the fixative into the bottle. The following fixations have been used:

(a) 2% osmium tetroxide, phosphate-buffered at pH 7.3, for 1 hr according to Millonig (18),

(b) 2.5% glutaraldehyde, phosphate-buffered at pH 7.5, for 15 min, and

(c) 2.5% glutaraldehyde, phosphate-buffered at pH 7.3, for 15 min, followed by 2% buffered osmium tetroxide for 1 hr.

After fixation the cells were scraped off the glass with a rubber policeman and centrifuged in the fixative at 9000 *g* for 10 min.

Aldehyde-fixed cells were embedded in glycolmethacrylate (GMA) and polymerized at 4°C under an ultraviolet lamp (11). All osmium tetroxide-fixed cells were embedded in Epon. Thin sections were cut on a Porter-Blum ultramicrotome with diamond knives, stained with 2% uranyl acetate for 20 min followed by lead citrate for 10 min, and examined with a Siemens Elmiskop I at 80 kv with an objective aperture of 50 μ.

## Enzymatic Digestions

The technique used for enzymatic digestion has been given elsewhere (11). Sections of aldehyde-fixed, GMA-embedded cells were floated with a plastic ring on the following enzymatic solutions prior to staining:

(a) 0.5% pepsin in 0.1 N HCl, pH 1.5, for 1 hr at 37°C,

(b) 0.1% ribonuclease in distilled water, pH 6.8, for 1 hr at 37°C, and

(c) 0.5% pepsin for 1 hr followed by 0.1% ribonuclease for 1 hr at 37°C.

Control sections were floated either on water or on a solution of HCl at same pH and temperature. The sections were then stained with 5% buffered uranyl acetate for 1 hr followed by lead citrate for 1, 5, or 10 min.

## High-Resolution Radioautography

Monolayers of exponentially growing BHK cells were exposed to a temperature of 43°C for 1 hr, this treatment being selected in order to make sure that all cells would be affected. After the treatment, the cells were transferred to a new medium and incubated at 37°C with tritiated uridine (uridine-$^3$H) for a pulse of 5 or 30 min at a concentration of 100 μc/ml. The uridine-$^3$H utilized (The Radiochemical Centre, Amersham, England, specific activity 20 c/mmole) is labeled in the five position. Such a label would be lost, should conversion to thymidine through deoxyuridine occur. Control experiments were simultaneously carried out with the same pulses on normally growing cells at 37°C.

The cells were then washed three times with a phosphate-buffered solution containing 1.2 mg/ml of unlabeled uridine and fixed immediately.

In another type of experiment the nucleoli were labeled immediately before heat treatment. The cells were incubated with uridine-$^3$H for a pulse of 30 min, washed three times with cold uridine as above, and reincubated at both 37° and 43°C, respectively, for a chase of 1 hr in a maintenance medium containing 120 μg/ml of unlabeled uridine.

The cells were either fixed with glutaraldehyde and osmium tetroxide and embedded in Epon or fixed with glutaraldehyde alone and embedded in GMA as mentioned above. Ultrathin sections were transferred with a plastic ring to a glass slide previously covered with a collodion film. The slides were dipped into a fine grain emulsion (Scientia NUC 307, Gevaert, Anvers, Belgium, crystal size 700 Å) diluted 1:4, dried according to the technique described by Granboulan (9), and stored at 4°C in the dark for 7 wk. Development was carried out in a freshly prepared bath of D 19 (Kodak, Eastman Kodak Co., Rochester, N. Y.) for 5 min at 18°C. After fixation in buffered hyposulfite, the slides were rinsed, dried, and the collodion membrane was floated off so that 200-mesh grids could be slid under the sections.

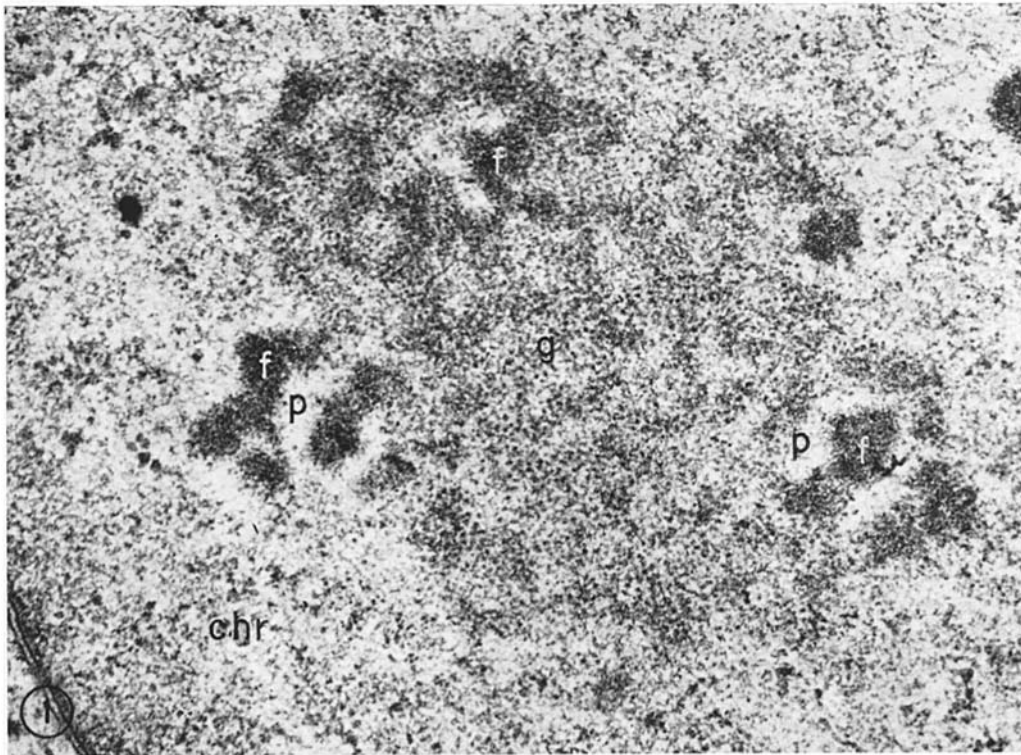After removal of gelatin by means of acetic acid

FIGURE 1 Nucleolus of a normal hamster fibroblast at 37°C. The dense granular RNP (*g*) is embedded in a loose network of fibrillar RNP (*f*). Within the meshes, small cavities are filled with a light amorphous material (*p*). In this section of OsO₄-fixed, Epon-embedded specimen, the nuclear chromatin (*chr*) is diffusely distributed. × 36,000.

diluted 1:40, the sections were stained with 5% veronal-acetate-buffered uranyl acetate at 37°C (pH 5) for 2 hr followed by lead citrate for 10 min.

RESULTS

*Ultrastructure at 37°C of Normal BHK Cells*

Normal hamster kidney fibroblasts in exponential growth at 37°C are large elongated cells. The cytoplasm displays numerous fibrillar elements, scattered phagocytic vacuoles, and little glycogen. Mitochondria have rudimentary cristae and an occasional DNA fiber. Rough endoplasmic reticulum membranes are rare but free ribosomes are fairly abundant with little formation of polysomes. The nucleus is large, and the chromatin widely distributed with condensation on the nuclear membrane or around the nucleolus. Perichromatin

granules are rare but the 200–250 A interchromatin granules are sparsely distributed in a finely fibrillar nucleoplasmic matrix. The nucleolus is prominent and the four usual nucleolar components can be identified (Fig. 1):

(*a*) 150–200 A dense granules randomly dispersed in the nucleolus,

(*b*) a loose fibrillar reticulum (nucleolonema) composed of 50–80 A fibrils (16, 26),

(*c*) an amorphous matrix of low electron opacity, and

(*d*) the nucleolus-associated chromatin with intranucleolar ramifications (7).

The granules and fibrils are completely digested by ribonuclease: they will be referred to as granular and fibrillar ribonucleoproteins (RNP). The amorphous matrix is attacked by pepsin alone. The nucleolus-associated and intranucleolar chromatin can be seen in GMA-embedded material following a short action of ribonuclease
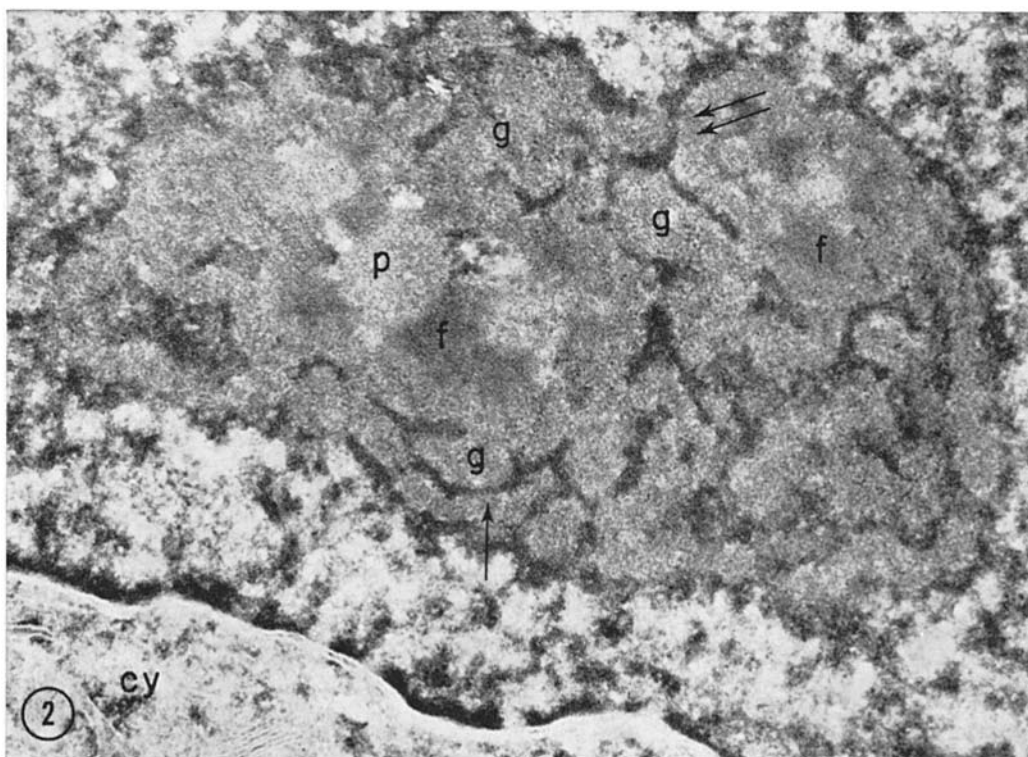
which is used to remove the ribonucleoproteins and also to enhance the contrast of desoxyribonucleoproteins; as suggested by Yotsuyanagi (28), ribonuclease, a basic protein, exerts its specific digestive activity toward RNA and also binds to DNA and increases its stainability with uranyl acetate (Fig. 2).

*Effect of Supranormal Temperature*

At temperatures of 38°, 39°, and 40°C, no noticeable lesion occurs in hamster fibroblasts even after an incubation of 120 min. The cells remain attached to the glass wall, and continue to grow normally when transferred to a new medium and cultured at 37°C for 24–48 hr. The first ultrastructural changes appear at 41°C after 1 hr of incuba-

tion: they involve some but not all nucleoli. The normal fibrillar network becomes blurred in outline and a fading out of its reticular aspect occurs. Simultaneously the granular RNP particles decrease in number and become fuzzy and cloudy spots. Some forms of granular RNP seem to unravel while proportionally the fibrillar ones increase. However, most of the cells possess a nearly normal nucleolus, and no definite conclusions can be made on the basis of the few lesions observed.

A critical point is reached at 42°C with the appearance of striking nucleolar lesions. The changes involve most of the nucleoli rather suddenly after only 15 min of treatment and are, of course, more pronounced after 1 hr of treatment. The nucleolus becomes round and sometimes has
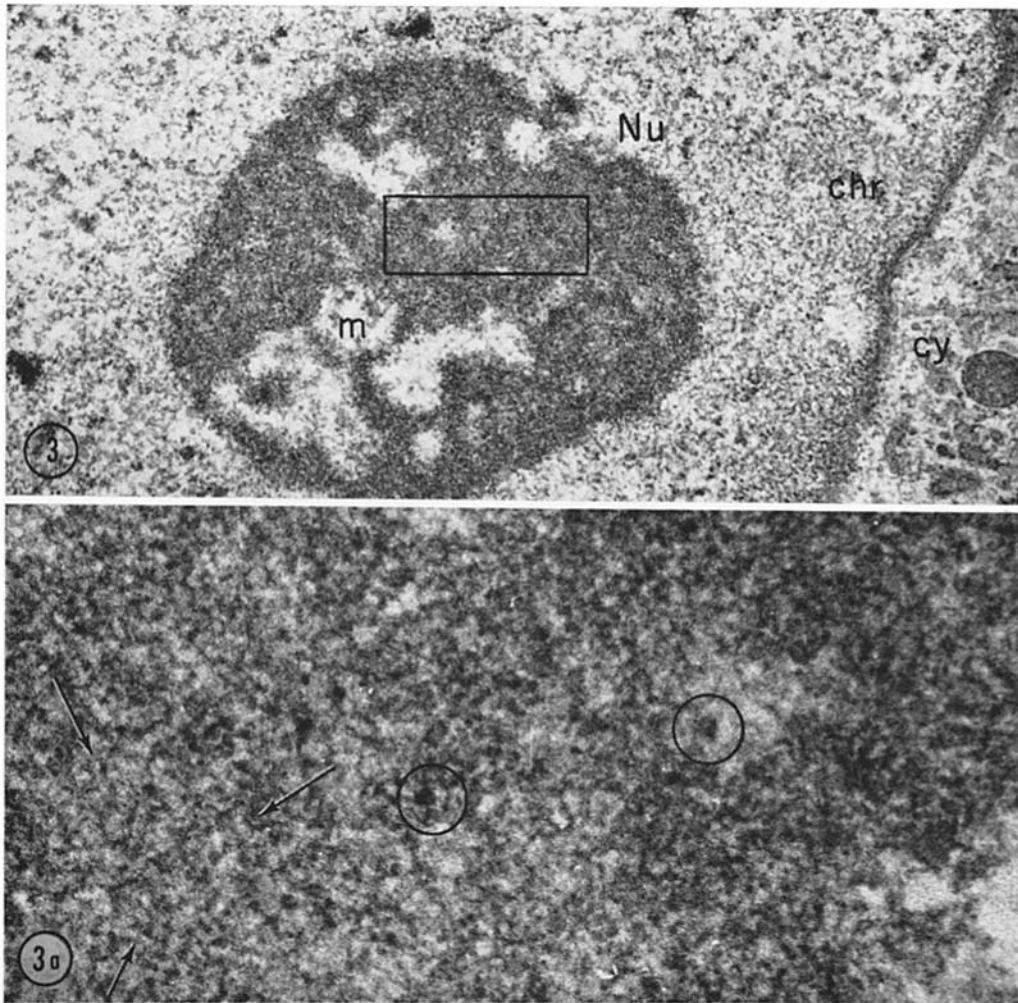
FIGURES 3 and 3 *a*   Effect of a thermic shock of 42°C for 1 hr on hamster fibroblasts. Simple OsO₄ fixation. Epon.

FIGURE 3   The nucleolus (*Nu*) has almost lost its reticular aspect and consists of closely packed fibrils with wide open meshes (*m*). The granular component has also disappeared, and the bulk of the nuclear chromatin (*chr*) retains its finely fibrillar appearance. Cytoplasm, *cy*. × 30,000.

FIGURE 3 *a*   is an enlargement of the enclosed area in Fig. 3. Arrows delimit a region where many fibrils are cut longitudinally. The length of the fibrils reaches 200–300 A while their diameter averages 60–100 A. Some granules are still evident (circles) but rather poorly delimited. They become surrounded by tiny threads as their unraveling proceeds. × 166,000.

wide open meshes (Fig. 3). The fibrillar reticulum has totally disappeared from the nucleolus and the granular form of ribonucleoproteins has been completely lost. The nucleolus exclusively consists of closely packed fibrils. The size of these fibrils grossly correlates with that of the fibrils of the normal nucleolus, as they average 60–100 A in diameter, but their number has considerably increased since no appreciable reduction of the nucleolar size has occurred (Fig. 3 *a*). Otherwise in the nucleus, few changes can be seen apart from early and nonspecific chromatin clumping. Occa-
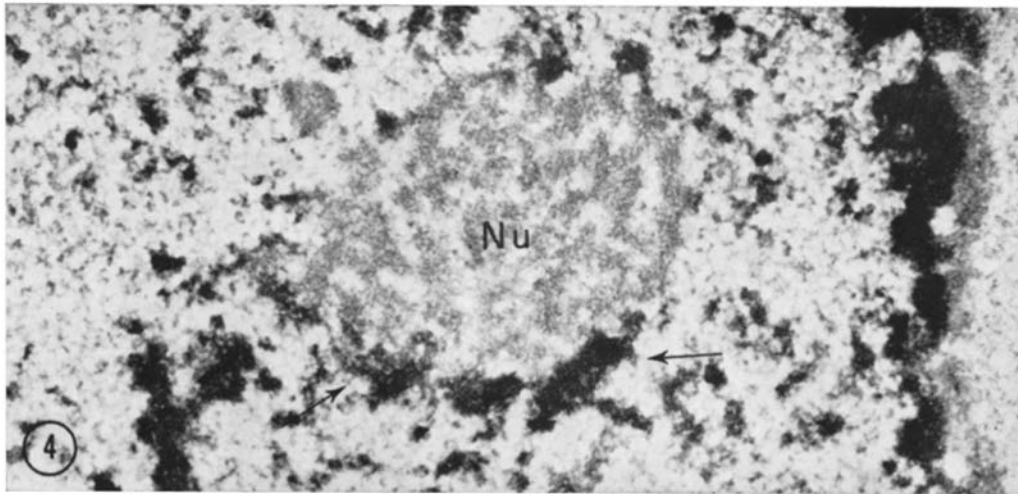
FIGURE 4  Thermic shock of 43°C for 1 hr. The RNA fibers of the treated nucleolus (Nu) are totally digested by treatment with a solution of 0.1% RNase for 1 hr. This reveals that whereas the perinucleolar chromatin (arrows) is preserved, the intranucleolar bands of chromatin have completely disappeared. Glutaraldehyde-fixed, GMA-embedded cell. × 35,000.
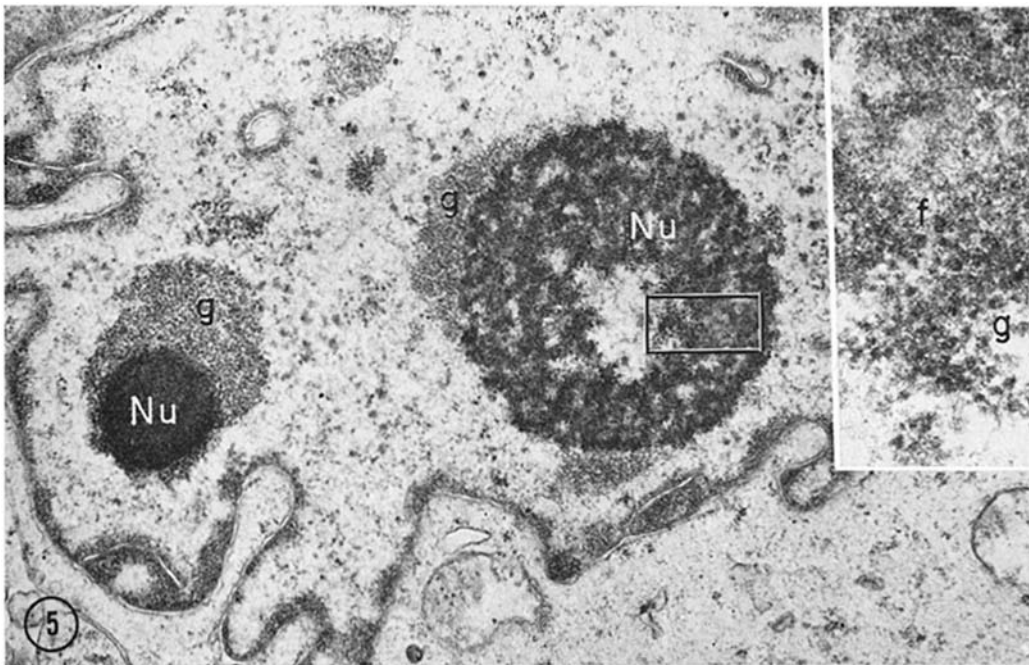


FIGURE 5  Recovery at 37°C for 12 hr, after a thermic shock of 43°C for 1 hr. The two nucleoli (Nu) seen here are mostly fibrillar as a result of the treatment. But the granular RNP particles (g) have reappeared and form a cap over the nucleolus. × 20,000. Inset shows the junction between the dense fibrillar RNP (f) and the particulate granular component (g). × 80,000.
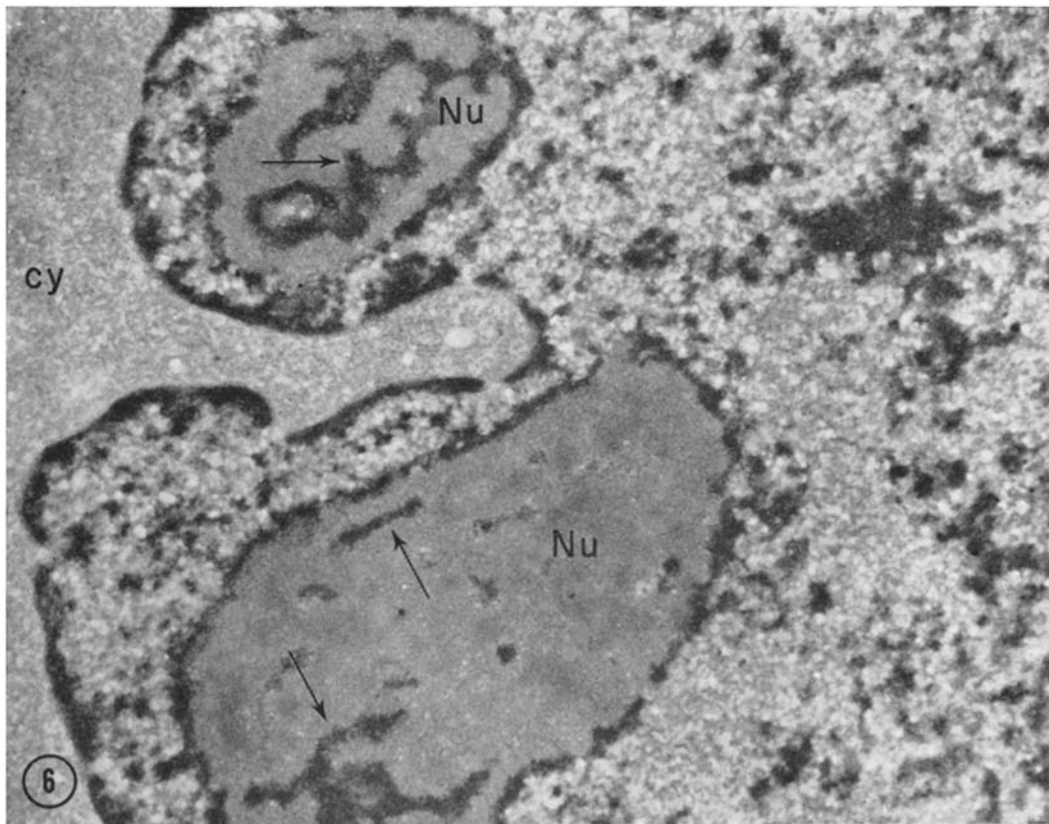
FIGURE 6  Same treatment as in FIG. 5. The section was floated on a solution of 0.5% pepsin for 1 hr, and of 0.1% RNase for 1 hr. Most nucleolar (*Nu*) components have been extracted and the reappearance of intranucleolar chromatin (arrows) has begun. Cytoplasmic (*cy*) proteins and ribosomes have been digested. Glutaraldehyde-fixed, GMA-embedded cell. × 24,000.

sionally, the interchromatin granules are grouped in clusters.

The cytoplasmic structures are perfectly preserved; the only lesions seen are dilatation of the cisternae of the endoplasmic reticulum and moderate swelling of the mitochondria. The ribosomes retain their usual configuration.

The lesions remain identical at 43°, 44°, and 45°C, for most of the times of treatment employed. But after treatment at 45°C for 1 hr, many cells become detached from the glass and are presumed to undergo necrosis.

A study of the ability of heated cells to recover from these nucleolar lesions proved that recovery is possible. Even after a thermic shock for 1 hr at 42°, 43°, and 44°C, the cells, when returned to 37°C for 24 hr, not only grow normally but again

build up in an exaggerated manner their lost nucleolar structure. The nucleolus then becomes larger than normal and exhibits a wide variety of shapes. The enlargement of the nucleolus is chiefly due to the reappearance of the granular RNP in a remarkably larger amount than is found normally in relation to the fibrillar component. This process of reappearance of granular RNP can be seen 12 hr (Fig. 5) after the thermic shock but is most striking after 24 hr (Fig. 7). At this time, evidence of active protein synthesis is seen in the cytoplasm where large numbers of free ribosomes and polysomal formation can be found (Fig. 8).

The effect of supranormal temperature on normal diploid rat embryonic cells in exponential growth is entirely similar, the lesions appearing also at 42°C.

## Enzymic Extraction

Enzymic digestions carried out on glutaraldehyde-fixed, GMA-embedded material demonstrate that the fibrillar component left in the nucleolus after thermic shock is completely digested by pepsin and RNase and therefore consists of ribonucleoproteins. Noteworthy is the fact that absolutely no intranucleolar chromatin can be seen at this stage whereas the nucleolus-associated chromatin is intact (Fig. 4). However, during the period of recovery the intranucleolar chromatin reappears after 12 hr (Fig. 6), at the time of reappearance of the granular RNP. Subsequently, it develops into a dense and intricate network after 24 hr, at the time when granular RNP particles are overwhelmingly numerous as compared with the fibrillar ones. This intranucleolar chromatin network clearly originates from the nucleolus-associated chromatin (Fig. 9).

## High-Resolution Radioautography

Incorporation of uridine-$^3$H into hamster fibroblasts at 37°C occurs mostly in the fibrillar nucleolar RNP during a pulse of 5 min (Fig. 10), but radioactivity is found in both the granular and fibrillar RNP during a pulse of 30 min (Fig. 12). Incorporation is also evident to a lesser extent in the nucleus whereas the cytoplasm is almost unlabeled. Following a thermic shock of 43°C for 1 hr the uptake of uridine-$^3$H by the altered nucleolus is almost absent after a 5 min pulse while the incorporation over the nuclear dispersed chromatin is much less affected (Fig. 11). An approximate grain count reveals a 90% reduction in the nucleolar incorporation of the precursor whereas the nuclear uptake is cut by only 20%. The same order of effect was found for a 30 min pulse after

a thermic shock but more labeling is evident because of the length of the pulse (Fig. 13).

When the nucleoli were given a 30 min pulse of uridine-$^3$H just prior to heat treatment, to label both the fibrillar and granular RNP, subsequent chases for 1 hr with cold uridine at both 37° and 43°C resulted in a heavy nucleolar labeling in both experiments without any appreciable difference in grain count. Moderate labeling is found in the nucleus at this stage and early labeling of the cytoplasm is evident both in the control experiment at 37°C and after the thermic shock (Figs. 14 and 15).

## DISCUSSION

The morphologic lesions appearing in the nucleolus after thermic shock clearly point to the nucleolus as one of the sites of a thermosensitive cellular function. Experiments were carried out on two different cell lines, and in both of them the following structural lesions were found in the nucleolus in the absence of any other cytological alteration: (a) fading out of the nucleolar reticulum, and (b) total disappearance of the granular RNP particles.

The remaining material consists of a large amount of closely packed, fibrillar RNP. Enzymatic digestions demonstrate that intranucleolar chromatin also disappears whereas the perinucleolar associated chromatin is preserved. At this point, especially in view of the disappearance of the intranucleolar chromatin, one can already suspect that profound disturbance of RNA synthesis has occurred after the thermic shock since all cellular RNA synthesis requires a DNA template. This suspicion is confirmed by high-resolution radioautography; little incorporation of tritiated uridine

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FIGURES 7, 8, and 9   Recovery at 37°C for 24 hr, after a thermic shock of 43°C for 1 hr.

FIGURE 7   The bulk of nucleolar mass (Nu) is remarkably enlarged, mostly because of the large increase in granular RNP (g). The electron-opaque fibrillar network (crossed arrows) has been reorganized. OsO₄. Epon. × 12,000.

FIGURE 8   In the cytoplasm, a large amount of free ribosomes is evident. In addition, polysomal spirals (arrows) can easily be seen. × 81,000.

FIGURE 9   This section was floated on a solution of 0.5% pepsin for 1 hr and of 0.1% ribonuclease for 1 hr. Intranucleolar associated chromatin (arrows) originating from the perinucleolar associated chromatin has increased remarkably and forms an intricate network permeating the whole nucleolus. Glutaraldehyde. GMA. × 28,000.
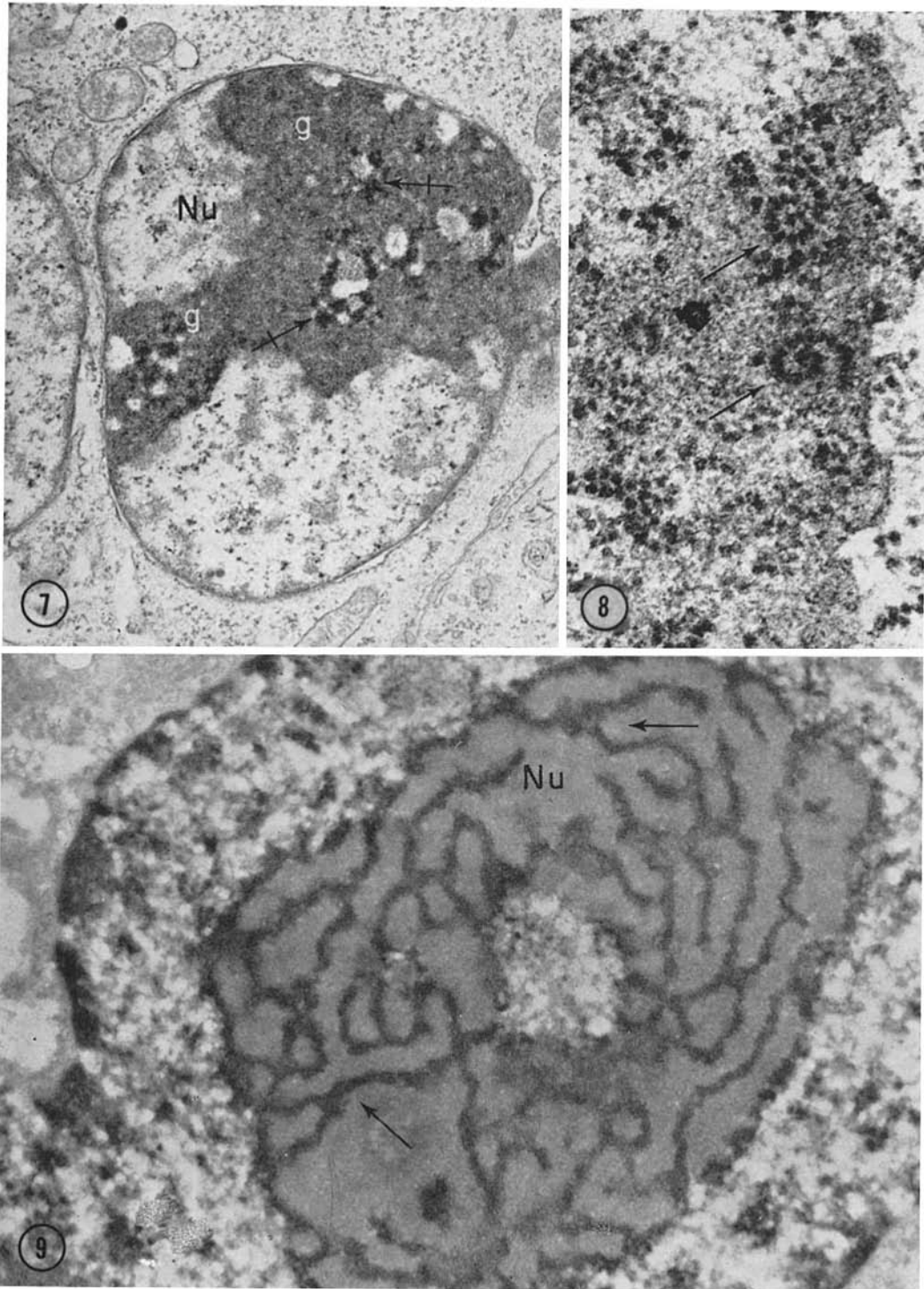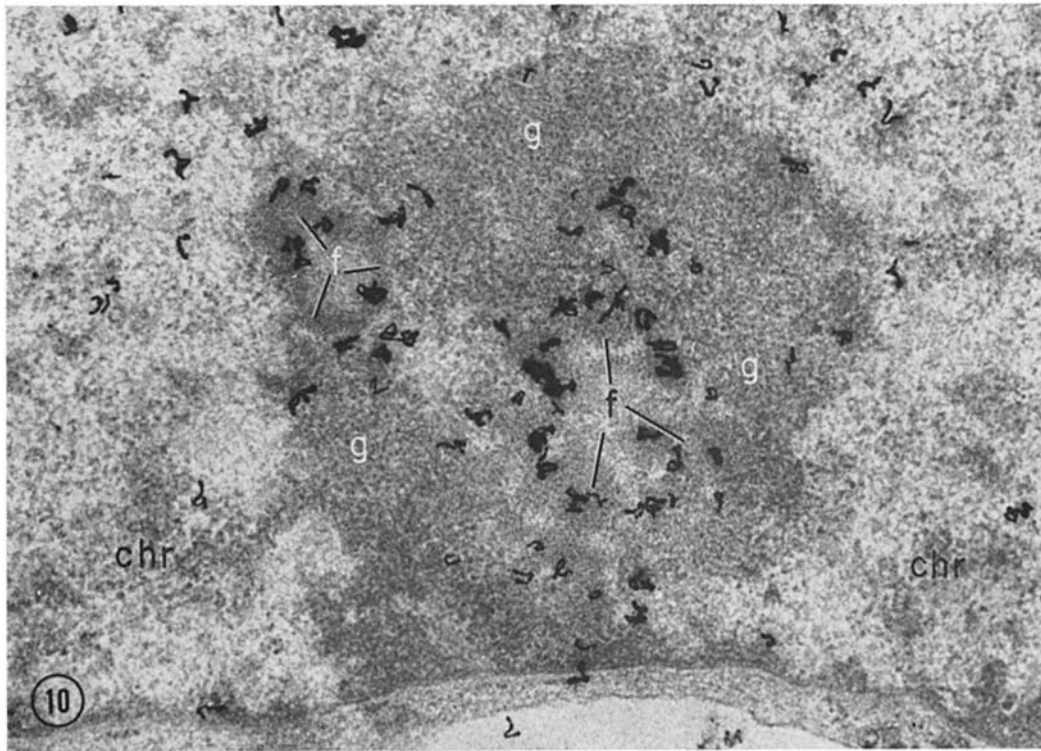
FIGURE 10  Pulse of uridine-$^3$H for 5 min; untreated cell growing normally at 37°C. The incorporation is located mostly over the fibrillar zone of the nucleolus whereas most of the granular zone ($g$) is unlabeled. Extranucleolar incorporation is also evident on the dispersed chromatin ($chr$). Double fixation. Epon. Fibrillar RNP, $f$. × 36,000.

is seen in the nucleolus after the thermic shock. This lack of incorporation is striking and indicates an almost complete inhibition of nucleolar RNA synthesis whereas extranucleolar sites are less affected. Let us examine how this blocking of RNA synthesis occurs. Two possibilities have to be considered:

(*a*) Since it is a well known fact that high temperature denatures proteins and enzymes, an inactivation of RNA polymerase as a result of a thermic shock has to be considered, especially in view of the protein monomer–multimer hypothesis of Lwoff (13). But such an inactivation should affect equally all forms of RNA polymerases, and blocking of all forms of RNA synthesis should occur. This is not the case, however, since extranucleolar sites of RNA synthesis can incorporate tritiated uridine after thermic shock. Although these data do not permit us to reject this hypothesis

definitively, a second hypothesis can be proposed which, for the time being, appears more attractive.

(*b*) The disappearance of all intranucleolar DNA after the thermic shock and the fact that the rate of DNA synthesis decreases in heated cells (24) strongly suggest that supranormal temperatures act at this level. It is conceivable that the intranucleolar DNA is most sensitive to supranormal temperature, since it is already known that the behavior of this DNA is different from that of nuclear DNA (17). Such an action would probably involve heat-sensitive hydrogen bonds responsible for the structural stability in a DNA double helix. The reversibility of the lesions would not come as a surprise since it has been already postulated that supra- and infranormal temperatures displace in one direction or the other the equilibrium of a reaction affecting macromolecules (14).

Whatever the precise site of action is, the result is evident: nucleolar RNA synthesis is blocked by
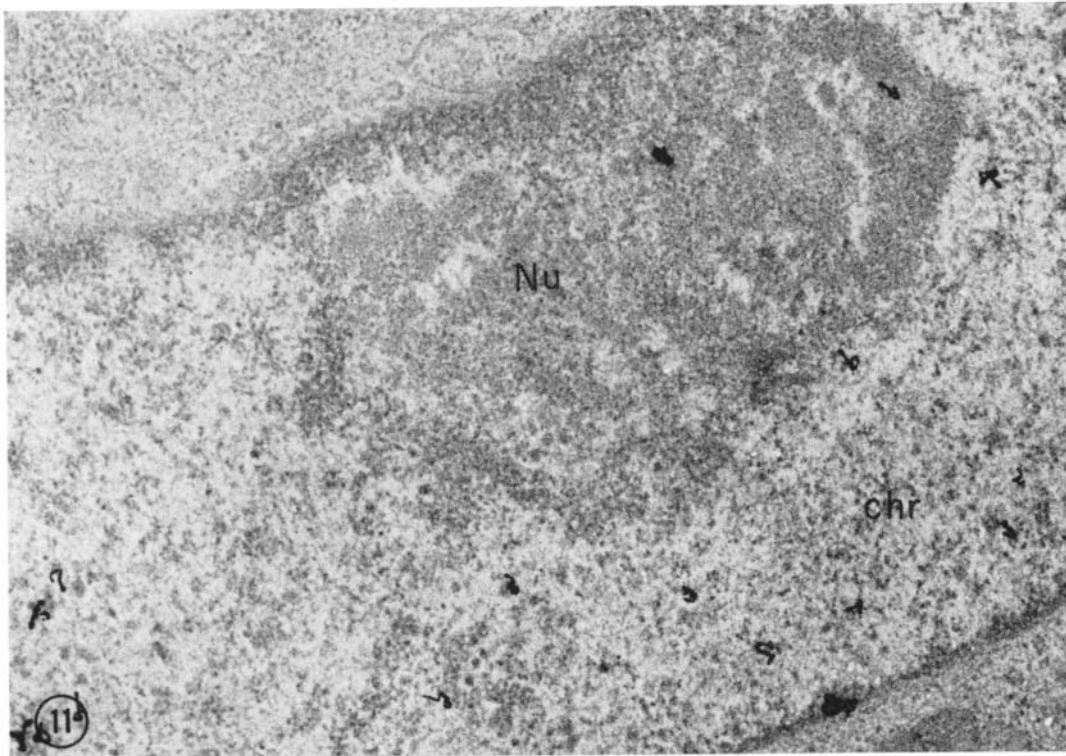
FIGURE 11   Pulse of uridine-$^3$H for 5 min after a thermic shock of 43°C for 1 hr. The nucleolar ($Nu$) incorporation is severely depressed whereas the nuclear chromatin ($chr$) has retained part of its ability to metabolize the precursor. Double fixation. Epon. × 39,000.

supranormal temperature, and this phenomenon is associated with the disappearance of the granular RNP and the augmentation and condensation of the fibrillar RNP. What has happened to the RNP granules? Again, two possibilities exist: (a) they have been released in the nucleoplasm or simply lost from the cell; or (b) they have been converted into fibrils by unraveling. The pulse-chase experiment was designed to answer this question. If the granular RNP is extruded into the nucleus during a thermic shock, one should expect to see marked increase in *nuclear labeling*, since labeled granular RNP would be found in the nucleus, and a decreased *nucleolar labeling*, since only labeled fibrillar RNP would be left in the nucleolus. On the other hand, if the thermic shock produces a simple loss of nucleolar RNA, this should also result in a loss of radioactivity in the nucleolus. The results shown in Fig. 14 and 15 permit us to reject the first possibility and to interpret the findings in terms of the second one.

There is already strong evidence that fibrillar RNP is a precursor of the granular RNP particles (8, 5). Moreover, Marinozzi (16), in his description of the two morphological RNP nucleolar components, estimates that granules are wrapped-up fibrils and that intermediate forms can be seen.

We now propose that the already labeled granular RNP has been reconverted to a fibrillar form of RNA, and that the process of transformation, fibrillar RNP $\underset{43°C}{\overset{37°C}{\rightleftharpoons}}$ granular RNP, is temperature-dependent: the transformation probably involves only configurational changes in the molecules. Whether or not an enzyme is responsible for this process cannot be ascertained here.

In the period of recovery at 37°C, the intra-nucleolar chromatin reappears after 12 hr and develops into a dense network after 24 hr. This phenomenon is associated with a nucleolus of considerable size partly due to the excessive amount of granular RNP, indicating that the
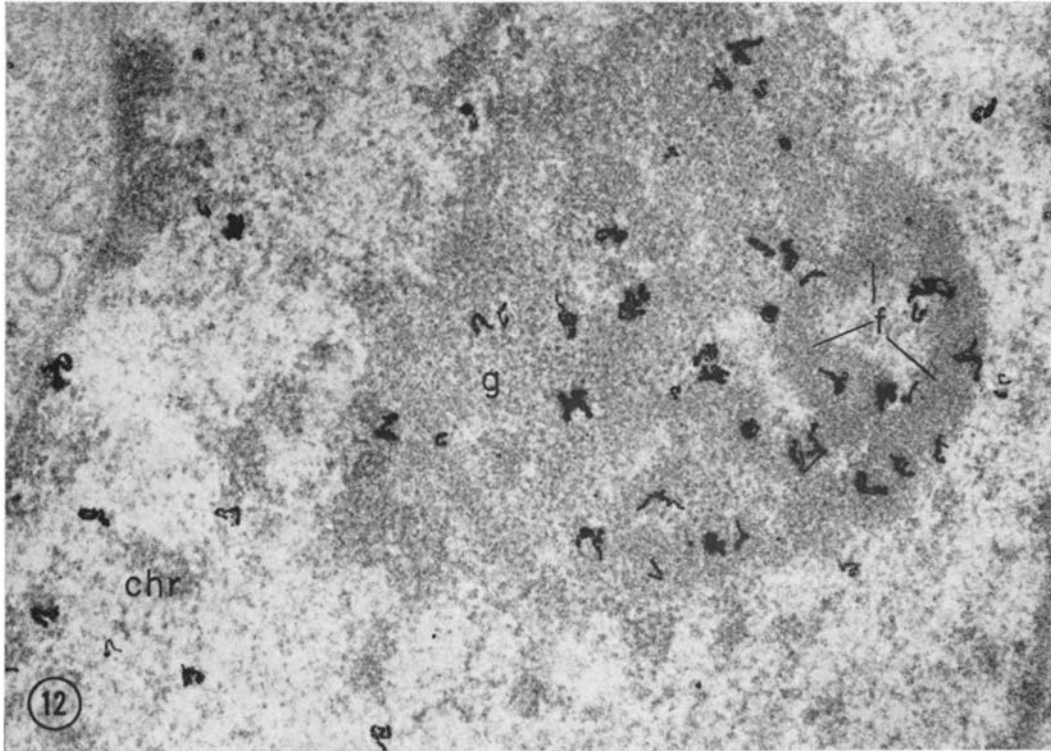
FIGURE 12    Pulse of uridine-$^3$H for 30 min at 37°C. The radioactivity is located in both the granular (g) and fibrillar (f) nucleolar RNP and in the nuclear chromatin (chr). Double fixation. Epon. × 36,000.

effect of supranormal temperature is a reversible one. An excessive amount of intranucleolar DNA appears followed by new RNA synthesis. This RNA is transformed from fibrillar to granular RNP, as is the already existing fibrillar RNP, leading therefore to a proportionally larger amount of granular RNP. Hence, the following direction of reaction in the period of recovery at 37°C is favored:

DNA →
    Fibrillar (Newly synthe-
    RNP    sized and al-
            ready existing)  →  Granular
                                    RNP
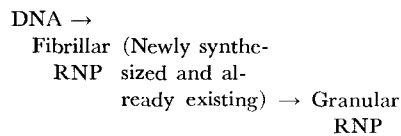
The increase in cytoplasmic free ribosomes and polysomes can be interpreted as evidence that active protein synthesis occurs in the phase of recovery from the thermic shock. Since Caspersson's work (1), many observations already have led to the generalization that active protein synthesis is correlated with an increase in size of the nucleolus.

## Hypotheses and Biochemical Considerations

Polycistronic transcription of nucleolar RNA on organizer chromosomes results in the synthesis of 45S molecules which are precursors to ribosomal RNA. This transcription is blocked by actinomycin D (21). The evidence presented here on the effect of supranormal temperature indicates that nucleolar RNA synthesis is also blocked by thermic shock. However, at this stage the morphologic results of these two types of inhibition are quite different: actinomycin D produces a segregation of granular and fibrillar RNP particles until the end stage where only fibrillar RNP is discernible (25), whereas the effect of supranormal temperature does not lead to segregation and results in an over-accumulation of fibrillar RNP with no evidence of granular RNP.
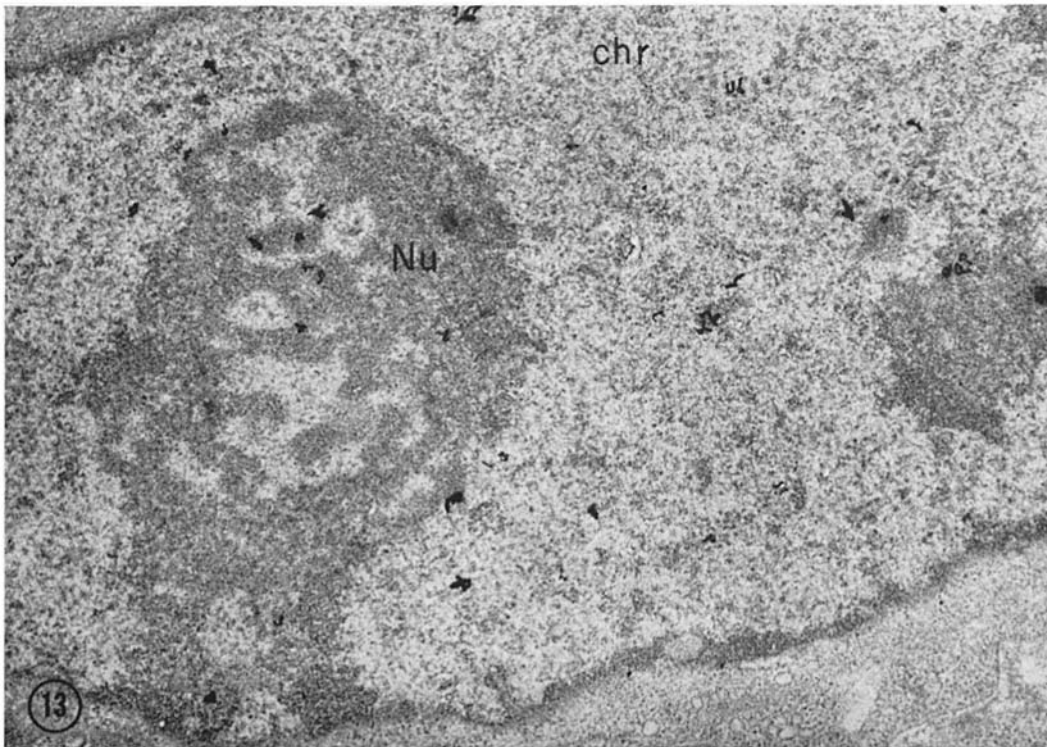
FIGURE 13   Pulse of uridine–³H for 30 min after a thermic shock of 43°C for 1 hr. The radioactivity is strongly depressed over the nucleolus (*Nu*) which shows the structural effect of the thermic shock. The incorporation into the nuclear chromatin is much less affected (*chr*). Double fixation, Epon. × 24,000.

The subsequent fate of the *45S* precursors is still a controversial subject. What appears to be certain is that this 45S molecule cleaves rapidly to a 35S molecule and that the labeling of these molecules is transferred to the 28S, 18S, and 4S fractions after a period of time (22, 23). The transformation of 45S to 35S and 28S is believed to take place in the nucleolus (19, 20). This process is not blocked by actinomycin D (27). Morphologic and radio-autographic experiments indicate that early labeling of nucleoli starts on the fibrillar RNP and is later transferred from fibrillar to granular RNP (8). This process is not blocked by actinomycin D (5). At this level, the effect of the thermic shock cannot yet be compared with that of actinomycin D, but the evidence presented here suggests a completely different mode of action. Since the transformation of already synthesized fibrillar to granular RNP cannot proceed in heated cells, accumulation of a large amount of precursors can

be suspected. This should permit fractionation and sedimentation analysis in the ultracentrifuge and perhaps lead to final biophysical and biochemical identification of nucleolar RNA.

The hypothesis can already be advanced that *fibrillar* RNA consists of a *heterogenous* population of *RNA's*, some of which deal with the production of granular RNP whereas others are concerned with other activities. Since the effect of supranormal temperature on nucleolar DNA–dependent RNA synthesis and its reversibility is more physiological that that of actinomycin, the temperature system of study is believed to be of special value for the critical analysis of nucleolar function and identification of its components.
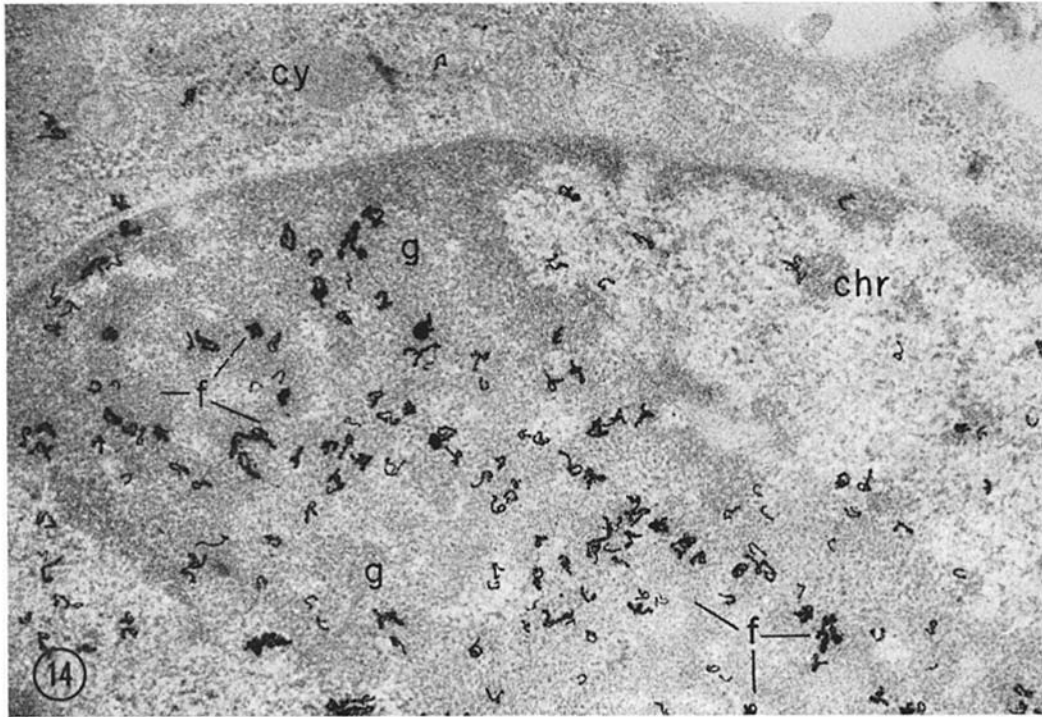
FIGURE 14 Pulse of uridine-³H for 30 min followed by a chase at 37°C for 1 hr. A strong radioactivity is evident over the nucleolus both in the granular (g) and in the fibrillar (f) zone. The nucleus is also heavily labeled whereas the cytoplasm (cy) shows early incorporation. Nuclear chromatin, chr. Double fixation. Epon. × 36,000.

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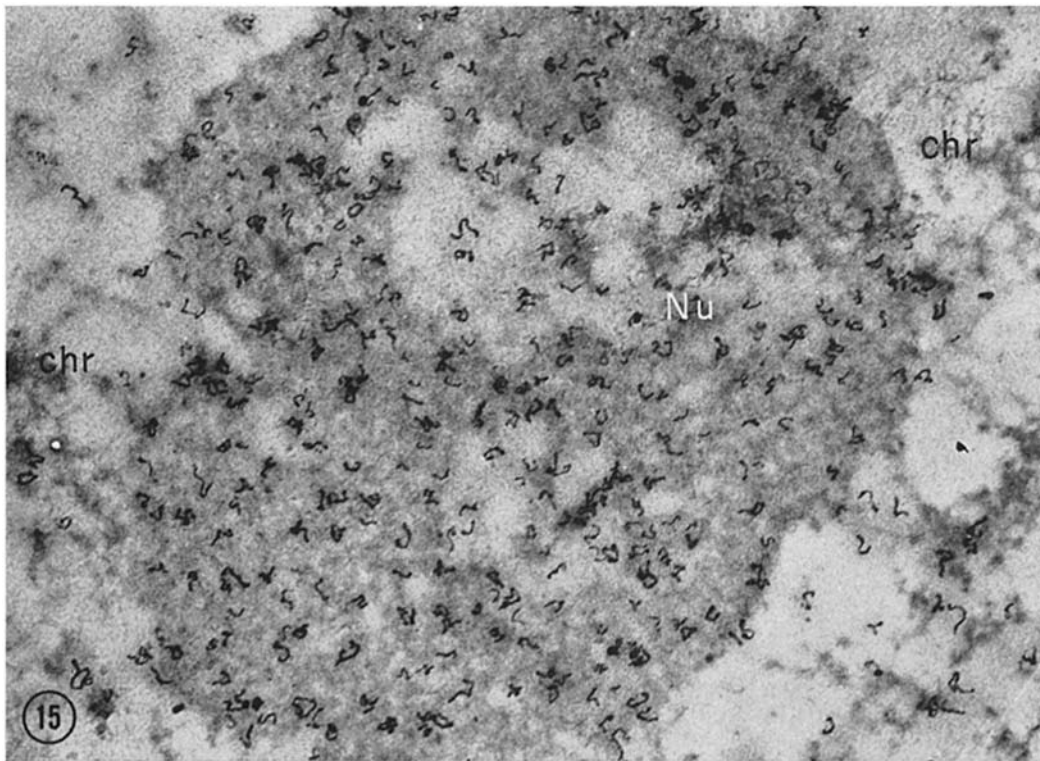
FIGURE 15 Pulse of uridine-$^3$H for 30 min followed by a chase at 43°C for 1 hr. The radioactivity over the nucleolus (Nu) is as strong as in Fig. 14, except that the granules are no longer evident as a result of the thermic shock. Glutaraldehyde fixation, GMA embedding. Nuclear chromatin, chr. × 30,000.

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