THE QUANTITATIVE RELATIONSHIP OF
THE FIBRILLAR CENTRES AND OTHER
NUCLEOLAR COMPONENTS TO CHANGES
IN GROWTH CONDITIONS, SERUM
DEPRIVATION AND LOW DOSES OF
ACTINOMYCIN D IN CULTURED DIPLOID
HUMAN FIBROBLASTS (STRAIN MRC-5)

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SUMMARY

The proportions of the 4 components of nucleoli: namely, granular component, fibrillar
component, vacuolar space and the fibrillar centre material, were calculated by electron
microscopical stereological procedures for human diploid fibroblasts (strain MRC-5) under 6
different culture conditions. Using nucleolar volumes obtained by light microscopy of un-
sectioned cells, estimates of the volume of each constituent per nucleolus were obtained.

From the size and number of fibrillar centres encountered it was possible to estimate approxi-
mately their numbers per nucleolus. This ranged from an average of $42 \pm 7$ to $234 \pm 25$,
depending on the level of cell activity, the number rising with increasing cell activity. Their
volumes were inversely proportional to their number per nucleolus, indicating a possible
fusion with cell inactivation. The number of fibrillar centres exceeds the number of nucleolus
organizers in man (which is ten) and is nearer the number of ribosomal genes, which has been
quoted at between 100 and 400 for diploid cells.

The volumes of granular and fibrillar components also reflect changes in cell activity. A
different response follows drug-induced inactivity when compared with the less artificial
inactivation resulting from confluence or serum starvation. There was less fibrillar component
in the actinomycin D-inactivated nucleoli. It is suggested that the nature of the fibrillar com-
ponent may not be the same in cells in different states and that the simple interpretation that
this is the transcriptional component may need to be revised.

The change to fewer larger fibrillar centres upon nucleolar inactivation may be a conse-
quence of 3 simultaneous processes. First, that the organizers increase in size by the condensation
of previously active organizer chromatin, which is withdrawing from its transcriptional con-
figuration. Secondly, this process may be accompanied by the fusion of the resultant larger
nucleolar organizer regions. And finally, the increase in sizes of fibrillar centres may be further
affected by an accretion of some non-chromatin material, possibly matrix or skeletal protein
material, onto the organizers.

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INTRODUCTION

It is now generally concluded that the 2 main components of nucleoli, namely the granular component and the fibrillar component, relate to 2 stages in the formation of ribosomes. The most extensive component of nucleoli, the granular component, is composed of ribosome precursor particles in their later maturation stages, while the generally less abundant fibrillar component represents the earlier stages in the formation of the constituent ribonucleoprotein, notably RNA transcription (Jordan, Timmis & Trewavas, 1980).

A further component of nucleoli has been more difficult to interpret and remains the subject of some conjecture. This part of the nucleolus is most commonly called 'the fibrillar centre', a term coined by Recher and others in 1969 but one that has led to its confusion with the fibrillar component. The different nomenclatures adopted by workers in the field has hindered the development of unifying concepts and interpretations. To alleviate this problem we will make clear our understanding of the terms we employ.

The zones referred to as the granular component and the fibrillar component have already been partly explained. We use these terms in the manner defined by the Nucleolus Nomenclature Committee that met at Montevideo in 1965 chaired by H. Swift (Swift, 1966). Further to this we avoid using the terms granular 'zone', 'part' or 'region' and also the terms 'pars granulosa' and 'pars amorpha'. The Committee used the words 'particulate component' rather than 'granular component' but 'granular component' is now more or less universally employed and not open to misunderstanding.

The structures that received the name of fibrillar centres from Recher would have formed one of the classes of structure grouped together under the title of nucleolar vacuole by the Montevideo Committee and now deserve a separate identification. These structures are always noticeably more weakly staining than condensed chromatin or either the granular or fibrillar components, and contain 10 nm fibrils (Jordan & Chapman, 1971). The size of the structures in MRC-5 fibroblasts ranges from 0.21 to 0.44 μm in diameter. They are most commonly seen adjacent to or embedded within the fibrillar component and frequently show attachments to chromatin. They contain DNA as demonstrated by autoradiography, cytochemistry and in situ hybridization studies. They also contain protein, including a protein with silver affinity. Transcription does not occur within them but is initiated at their peripheries (Goessens & Lepoint, 1974; Mirre & Stahl, 1981; Lafontaine & Lord, 1973).

Over 20 different terms have been used to describe this part of the nucleolus (Goessens & Lepoint, 1979), but at a meeting to discuss nomenclature in 1977 (Jordan & Loening, 1977) it was felt that sufficient evidence had emerged to use the term nucleolus organizing region (that part of the chromosome that contains the ribosomal genes and from which nucleoli are known to arise).

Since then, however, publications have appeared using the older less committal terms, indicating that some uncertainty still remains concerning the correct identification.
Fibroblast nucleoli

Some reasons for this uncertainty can be summarized as follows. First, although DNA has been demonstrated within these 'fibrillar centres' it is in rather small quantities. Precise numerical values have not been obtained, but the low levels of DNA label in autoradiographs (Lafontaine & Lord, 1973) and the difficulties of demonstrating DNA in nucleoli using the Feulgen technique (McLeish, 1964) contribute to this view.

Secondly, no transcription has ever been demonstrated in the fibrillar centres (Goessens, 1976; Mirre & Stahl, 1978), yet they are known to contain diffuse chromatin.

Thirdly, the nucleolus, like the nucleus, is now known to contain a scaffold or skeletal matrix material (Todorov & Hadjiolov, 1979). If such a material was associated with the nucleolus organizer it could give rise to some of the difficulties in identifying the DNA and also make a simple equating of such compound structures with nucleolus organizers misleading.

Fourthly, sometimes nucleoli show no structures corresponding to these fibrillar centres or their supposed mitotic counterpart, the secondary constriction of metaphase chromosomes. The structure therefore seems not to be an essential feature of nucleoli (Goessens & Lepoint, 1979).

Fifthly, bodies that have the same structure as fibrillar centres occur outside the nucleolus and even outside the nucleus after treatment with inhibitors of transcription (Moreno Diaz’de la Espina & Risueno, 1977).

In view of the need to clarify such areas of uncertainty, we have undertaken a stereological analysis to find the proportional relationships and amounts of the various nucleolar components in nucleoli of cells in different phases of growth in cell culture and under the influence of low doses of the transcription inhibitor actinomycin D. We have used the diploid human fibroblasts, MRC-5 cells, which have a stable karyotype, so that we can examine the relationship between the number of fibrillar centres and the number of nucleolus organizers.

MATERIALS AND METHODS

Cell cultures

MRC-5 human diploid fibroblast cells were supplied by Dr J. P. Jacobs of the National Institute for Medical Research, London.

The cells were grown in Eagle's basal medium (Flow Laboratories) supplemented with 10% foetal calf serum, gentamycin (50 μg/ml) and amphotericin B (2.5 μg/ml), and utilized between passages 22 and 27.

Cultures were fixed and embedded at 1 and 3 days after subculture.

Cells maintained in medium depleted of serum were fixed after 8 days; the medium was changed, however, every 3 days.

Cells receiving drug treatment were subcultured and after 1 day were treated with 1 of 2 doses of actinomycin D; namely, 0.08 μg/ml or 1.0 μg/ml for 1 h.

Trypsin-treated cells were suspended for 1 min in a solution of 0.25% trypsin. The trypsin was then inactivated by adding fresh medium containing serum, and the cells were fixed.
Light microscopy

For light microscopy cells were grown on glass slides that were cut longitudinally and inserted into glass culture vessels before sterilization. The slides were removed and the cells were fixed in 1-6% glutaraldehyde for 30 min, washed in phosphate-buffered saline and mounted in 90% glycerol.

Electron microscopy

The MRC-5 fibroblasts were fixed in 1-6% glutaraldehyde in Sorensen’s phosphate buffer (pH 7-3) at 4 °C for 30 min. After fixation the cells were washed in phosphate buffer and then scraped from the culture vessel with a rubber policeman and harvested by centrifugation; the pellets were resuspended in 1% osmium tetroxide and post-fixed for 1 h.

The cells were dehydrated in an alcohol series and embedded in Epon resin.

Ultra-thin sections of silver interference colour were cut with a Dupont diamond knife on an LKB III ultramicrotome. The sections were stained with uranyl acetate for 30 min and then with Reynolds’s lead citrate for 4 min. The sections were viewed with an AEI EM 6B electron microscope.

Stereology

The stereological analysis was carried out using the method of point counting. For this procedure a test lattice containing points at 0-5-cm intervals was constructed.

To perform the analysis maps of nucleoli from electron micrographs were traced onto transparent sheets; the test lattice was then superimposed and the number of test points falling on each nucleolar component recorded.

The majority of electron micrographs were recorded at a primary magnification of 10000. A total of 745 micrographs were analysed.

Sampling of tissue

To ensure that the sampled tissue represented a random distribution of cells, the fibroblasts were divided between a number of resin blocks and sections were cut from each. Electron micrographs were recorded from all the cells within the section containing nucleoli and nucleolar profiles at all levels were selected, not just equatorial profiles.

To obtain estimates of the numbers of fibrillar centres in nucleoli (Table 1) we took the total area of fibrillar centres for each cell type and divided it by the number of fibrillar centres encountered. Making the assumption that they were spherical we calculated a volume enabling us to find their average number in the volume of fibrillar-centre material derived from the stereological analysis. In this calculation we corrected the size of the fibrillar centres to take account of the fact that the size of the sphere calculated from a series of random sections has only π/4 of the true diameter. Such a correction is not required for the volume of the nucleoli measured unsectioned by light microscopy.

RESULTS

To obtain an estimate of the total nucleolar volumes, nuclei having only a single nucleolus, and therefore from the latter part of the cell cycle, were measured by light microscopy (Anastassova-Kristeva, 1977). The nucleolar volumes thereby computed are shown in Table 1.

The amount and distribution of the 4 nucleolar components in cells grown under different conditions were studied using stereological methods on electron micrographs and the results are presented in Table 1.
Table 1. Data for cells grown under different conditions

<table>
<thead>
<tr>
<th>Cell type*</th>
<th>Nucleolar† volume</th>
<th>Granular‡ component</th>
<th>Fibrillar‡ component</th>
<th>Fibrillar‡ centre</th>
<th>Vacuolar‡ space</th>
<th>Area of nucleoli analysed (μm² × 10⁴)</th>
<th>Average§ volume of fibrillar centre (μm² × 10⁻²)</th>
<th>Approx. no.§ of fibrillar centres per nucleolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log phase</td>
<td>76 (130)</td>
<td>74 (56)</td>
<td>17 (13)</td>
<td>1.6 ± 0.17 (1.2)</td>
<td>7.5 (5.7)</td>
<td>4.0</td>
<td>0.51</td>
<td>234 ± 23</td>
</tr>
<tr>
<td>Confluent</td>
<td>79 (167)</td>
<td>80 (63)</td>
<td>16 (13)</td>
<td>2.8 ± 0.26 (2.2)</td>
<td>1.7 (1.4)</td>
<td>3.8</td>
<td>1.46</td>
<td>150 ± 14</td>
</tr>
<tr>
<td>Serum-starved</td>
<td>50 (118)</td>
<td>53 (27)</td>
<td>32 (16)</td>
<td>3.5 ± 0.32 (1.8)</td>
<td>1.1 (0.6)</td>
<td>2.6</td>
<td>1.15</td>
<td>156 ± 14</td>
</tr>
<tr>
<td>0.08 μg/ml AMD</td>
<td>40 (152)</td>
<td>89 (36)</td>
<td>5.7 (2.3)</td>
<td>3.7 ± 0.44 (1.5)</td>
<td>1.6 (0.3)</td>
<td>2.9</td>
<td>3.28</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>1 μg/ml AMD</td>
<td>36 (138)</td>
<td>86 (31)</td>
<td>5.2 (1.9)</td>
<td>5.2 ± 0.45 (1.9)</td>
<td>3.3 (1.2)</td>
<td>4.3</td>
<td>4.56</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>Trypsin-treated</td>
<td>— — 72</td>
<td>— — 22</td>
<td>— — 3.2</td>
<td>— — 2.8</td>
<td>— — 2.0</td>
<td>— —</td>
<td>— —</td>
<td>— —</td>
</tr>
</tbody>
</table>

* Log phase cells are cells taken after 1 day in culture with complete medium. Confluent cells have been 3 days in culture. Serum-starved cells have been allowed to grow for 3 days with complete medium but then kept for 7 days in serum-free medium. 0.08 μg/ml AMD: 1 day in culture followed by 0.08 μg/ml actinomycin D for 2 h. 1 μg/ml AMD: 1 day in culture followed by 1 μg/ml actinomycin D for 2 h. Trypsin-treated: 3 day cells, 0.25% trypsin for 1 min.

† Nucleolar volumes were calculated from phase-contrast micrographs of cells fixed in situ. Only nucleoli that were single in a nucleus were measured. n is the number of nucleoli measured in each case.

‡ The % values for nucleolar components were obtained by stereological procedures as described in Materials and methods. The volumes of components were obtained using these values and the nucleolar sizes were obtained from light microscopy.

§ The no. of fibrillar centres is calculated from the total number and area encountered in the stereological investigation of sections. Their average cross-sectional area is corrected to give the medium cross-section of a sphere and then a volume. Their number per nucleolus is calculated for the large single nucleoli measured by light microscopy, which represent nucleoli at their maximal development at the end of the cell cycle. The error indicated for these values is a re-expression of the S.E. calculated for the proportion of fibrillar centre in the earlier column.

The standard errors have only been included for the fibrillar centre column because of its low percentage value.
Fibroblast nucleoli

Nucleoli of cells after 1 day in culture

The nucleoli of cells that had been in culture for only 1 day had variable shapes and a loose reticulate structure, showing 7.5% space (Table 1); such nucleoli are commonly called nucleolonemal (Figs. 1, 2), the nucleolonema simply being the thread-like structures seen in nucleoli irrespective of which components comprise them (Swift, 1966). The nucleoli were composed largely of granular component with fibrillar centres and their associated fibrillar component evenly distributed throughout. The fibrillar centres were small, measuring around 0.21 μm in diameter, and accounted for only (1.6 ± 0.17)% of the nucleolus. An approximate figure of 234 ± 25 for their number was obtained. Nucleoli were also found with a more compact nature, showing that the cultures could not be considered entirely uniform, the illustrations showing typical examples.

Nucleoli of cells after 3 days in culture

After 3 days in culture, the time when the cells were usually passaged, the nucleoli were noticeably different from the 1 day condition (Figs. 3, 4). The granular component was still the most abundant, but the whole nucleolus was more compact with a marked reduction of vacuolar space (17%). The granular component was a larger proportion of the nucleolus at 80%, and more in real terms totalling 63 μm³, than in the cells 1 day after sub-culture. The fibrillar centres were larger and accounted for more of the nucleolus and totalled 2.2 μm³ (2.8 ± 0.26%), their number being estimated as 150 ± 14 per nucleolus (Table 1).

The nucleoli of cells at confluence deprived of serum

The nucleoli of cells grown in the medium lacking serum were smaller, nucleolonemal and about 2 thirds of the size of those after 1 day or 3 days in the complete medium (Figs. 5, 6). There was more fibrillar component, but it was less distinct from the granular component. The fibrillar centres accounted for a higher proportion of the nucleolus (3.5 ± 0.32%) and numbered 156 ± 14.

Nucleoli following treatment of 1 day cultures with actinomycin D

After treatment with low doses of actinomycin D (0.08 μg/ml) for 1 h the nucleoli showed the characteristic segregation (the separation and redistribution of nucleolar components). These nucleoli should be compared with those of the 1 day actively growing cells seen in Figs. 1 and 2, for they represent the appearance of such nucleoli 1 h after treatment with actinomycin D. The degree of segregation was not as pronounced as that observed following the higher dose; the fibrillar component tended to be localized within the body of the granular component (Figs. 7, 8).

Figs. 1, 2. Nucleoli of MRC-5 fibroblasts 1 day after sub-culture showing loose nucleolonemal structure with scattered fibrillar centres (fc) surrounded by the denser material of the fibrillar component (f). The nucleoli are composed predominantly of granular component (g). Fig. 1, × 33 000. Fig. 2, × 36 000.
Fibroblast nucleoli

With the higher dose of actinomycin D (1.0 μg/ml for 1 h) the segregation was more marked, fibrillar centres were extruded onto the surface and the nucleolonemal structure was lost (Figs. 9, 10). There was also a rapid loss of both granular and fibrillar components, which must largely account for the reduction in nucleolar size (Table 1). There was, however, an increase in the total amount of fibrillar centre material, which was 1.5 and 1.9 μm³ after 0.08 and 1.0 μg of actinomycin D, respectively. However, after the higher doses of actinomycin D, some disaggregation of the components and dispersal into the nucleoplasm occurred. There was a marked drop in the calculated number of fibrillar centres to 46 ± 5 and 42 ± 4 in the 0.08 and 1.0 μg/ml actinomycin D treatments, respectively (Table 1).

Nucleoli of cells following treatment with trypsin

The short treatment with trypsin only lasting 1 min had interesting consequences for nuclear structure. The nuclear envelope, which was normally regular showing more or less elliptical outlines, became very convoluted (Fig. 11). It was not possible to measure the nucleolar volume by the method used for non-trypsinized cells because the change in cell morphology obscured the nucleolus. But from the stereological analysis it was possible to conclude that the granular component comprised a lower and the fibrillar component a higher proportion of the nucleolus, when compared with the nucleoli of the 3 day cultures of the cells at the corresponding stage to the trypsin-treated ones.

The proportion of the nucleolus accounted for by the fibrillar centres shows a rise when compared with that for the 3 day culture (Table 1).

Fibrillar centre frequency

The numbers of fibrillar centres in nucleoli of cells under different conditions are reflected in their frequency in sectioned nucleoli (Table 2). Few sections (12.5 %) showed more than 3, and most commonly only 1, under all conditions except for the cells 1 day after sub-culturing, when 2 per section was more frequent. There were also many more, 22 % showing more than 3 per section in these cells, when compared with the confluent and serum-starved cells.

DISCUSSION

The changes in nucleolar structure we have found here may be interpreted in relation to the RNA metabolism of the cells (Emerson, 1971; Johnson, Abelson, Green & Penman, 1974).

The structural changes seen emphasize the dynamic nature of the organelle, as even a short treatment with trypsin caused changes. The rapid changes following low

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Figs. 3, 4. Nucleoli from confluent MRC-5 fibroblast cells 3 days after sub-culture. The nucleoli are more tightly condensed and not displaying such a clear nucleolonemal texture. Granular component (g) predominates and fibrillar centres (fc) with surrounding fibrillar component (f) are scattered throughout. Fig. 3, x 27000. Fig. 4, x 35000.
Table 2. Frequency of fibrillar centres in sectioned nucleoli

<table>
<thead>
<tr>
<th>Cell type*</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>Log phase</td>
<td>12</td>
<td>23</td>
<td>30</td>
<td>13</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
<td>113</td>
</tr>
<tr>
<td>Confluent</td>
<td>23</td>
<td>34</td>
<td>23</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td></td>
<td>140</td>
</tr>
<tr>
<td>Serum-starved</td>
<td>25</td>
<td>31</td>
<td>23</td>
<td>12</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
<td></td>
<td>119</td>
</tr>
<tr>
<td>0.08 µg/ml AMD</td>
<td>30</td>
<td>40</td>
<td>18</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>2</td>
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<td>6</td>
<td>2</td>
<td></td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Trypsin-treated</td>
<td>18</td>
<td>32</td>
<td>25</td>
<td>12</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>113</td>
<td></td>
</tr>
<tr>
<td>Total all types</td>
<td>206</td>
<td>335</td>
<td>22</td>
<td>112</td>
<td>45</td>
<td>35</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>26</td>
<td>754</td>
</tr>
</tbody>
</table>

* As detailed for Table 1.

Doses of actinomycin D demonstrate this further and enable us to conclude that the structure of the nucleolus is a consequence of concurrent transcriptional activity.

In all the cells with lower activity there is a higher proportion of fibrillar centres in the nucleoli, which indicates that it is a structural or essential component in contradistinction to a product.

The numbers and sizes of these fibrillar centres enable us to find out if there is any relationship with nucleolar organizers. Human nuclei are known to have 5 pairs of organizers (Henderson, Warburton & Atwood, 1972). Cells in culture during the active growth phase show the highest number (234 ± 25 per nucleolus) and the smallest average size of 0.51 x 10^-2 µm^3. The number of fibrillar centres is much higher than the number of organizers and is not consistent with a simple relationship. The nature of the calculations depends upon accurate measurements and homogeneity of size and distribution, and therefore can only be considered as giving a general guide to the numbers. The numbers are similar to those found in mouse oocytes (Mirre & Stahl, 1981).

In both the confluent and serum-starved cells, assumed to have become arrested in G1 (Emerson, 1971), the number of fibrillar centres is lower but their size greater. We were particularly interested to use naturally inactivated nucleoli in our analysis and employed the serum starvation method for this comparison. We conclude that the confluent cells deprived of the essential growth factor found in the serum (Lambert & Pirt, 1979) are in a more advanced stage of inactivation. Both of these types have about 150 fibrillar centres, suggesting some fusing of fibrillar centres, which is also supported by their size. It must certainly be considered that each organizer may pass through many fibrillar centres.

Figs. 5, 6. Nucleoli of MRC-5 fibroblasts grown for 8 days in the absence of serum. The nucleoli are smaller, display the nucleolonemal structure and have a higher proportion of fibrillar component (f). The junction between granular (g) and fibrillar components is not so distinct. The nucleolus has large spaces but the fibrillar centres (fc) are very clear and account for a higher proportion of the nucleolar structure. Fig. 5, × 51,000. Fig. 6, × 50,000.
For legend see p. 387.
For legend see p. 387.
For legend see opposite page.
Fibroblast nucleoli

We found no evidence that larger nucleoli have a different proportion of fibrillar centre material, so even if a simple one-to-one relationship held at earlier stages it was lost with nucleolar growth in the same way as in the oocytes (Mirre & Stahl, 1981).

The high numbers found suggest a relationship with the number of genes, which in man is reported to be between 100 and 400 in diploid cells (Long & Dawid, 1980).

The proportion of fibrillar centre in a nucleolus is a sensitive indicator of cell activity, and confirms earlier studies of this relationship in plant and animal cells (Recher, Briggs & Parry, 1971; Fakan, 1971; Jordan & Chapman, 1971, 1973; Goessens, 1978). Active organizers are obscured by the products of transcription and the associated processing (Miller & Beatty, 1969; Franke et al. 1979), so fibrillar centres may only represent nucleolus organizers in a non-active form.

Other explanations for the behaviour of fibrillar centres may be considered. In particular, the possibility that fibrillar centres represent at least in part some structural or skeletal elements of the nucleolus.

The variation of nucleolar morphology accompanying reduced cellular activity may indicate changes in an underlying structural matrix. The nucleolonemal structure of the active nucleolus (Figs. 1, 2) is in marked contrast with the compact structure of the nucleolus in the confluent cells. Interpretations of nucleolonemal structure will be more meaningful after a further characterization of nucleolar skeletal elements or matrix proteins (Todorov & Hadjiolov, 1979).

The nucleolus of the confluent cells maintains the same size and the same proportions of the 2 most extensive nucleolar components (fibrillar and granular) as the actively growing cells. This is at first sight surprising, but explanations are possible: first, that there has been a coordinate reduction of all nucleolar processes including transport of pre-ribosomes so that the size of all components is unchanged. Alternatively, the same level of nucleolar activity has been maintained but is accompanied by an increased turnover or wastage of its products, in coordination with the reduced levels of activity in other cellular processes. A lengthening of the time required for

Figs. 7, 8. Nucleoli of MRC-5 fibroblasts in cultures 1 day after sub-culture in complete medium. Actinomycin D (0.08 µg/ml) had been added 1 h before fixation. There has been a collapse or aggregation of the components of the nucleolus. No trace of nucleolonemal organization is discernable and the fibrillar component is no longer evenly distributed around the fibrillar centres. Fig. 7, x 39000. Fig. 8, x 50000.

Figs. 9, 10. Nucleoli of MRC-5 fibroblast cultures 1 day after sub-culture in complete medium. Actinomycin D (1.0 µg/ml) had been added to the culture 1 h before fixation. Fig. 9, x 48000. Fig. 10, x 26000.

Fig. 9 shows clear segregation into the 3 basic components, the fibrillar centre (f), the fibrillar component (f) and the granular component (g).

Fig. 10 shows that there is some fragmentation at this dose and that the fibrillar centres with associated fibrillar component move to the surface of the granular component.

Fig. 11. Nucleus of trypsinized MRC-5 fibroblast showing the convoluted nucleus. x 13000.

Fig. 12. Nucleolus of trypsinized MRC-5 fibroblast. The fibrillar centres (f) account for a higher proportion of the nucleolus than in the cells which are fixed before the treatment with 0.25% trypsin for 1 min. x 57000.
transcription (Emerson, 1971) and an increase in the instability of ribonucleoprotein (Abelson, Johnson, Penman & Green, 1974) have been reported for fibroblasts at this stage, showing that both explanations may be relevant.

The residual structures in the serum-starved cells can be thought of as resulting from a change towards an 'essential' nucleolus, resulting from a depletion of those constituents, such as RNA (Unuma, Senda & Muramatsu, 1972), that can be considered as stages in the formation of ribosome particles themselves, i.e. the products of manufacture, leaving only the manufacturing machinery. Hence the fibrillar components of nucleoli that are inactive (Jordan & Chapman, 1971; Berger & Schweiger, 1975) may lack transcription complexes and be quite different from the fibrillar components visualized as 'Christmas trees' in spread preparations from active nucleoli.

Actinomycin D is thought to have its primary effect upon transcription through the detaching of nascent transcripts from the genes (Scheer, Trendelenburg & Franke, 1975). It is difficult to envisage how such an organizer-associated activity could so rapidly affect the skeletal or nucleolonemal aspect of the 'post-transcriptional' part of the nucleolus, i.e. the maturing granular component. An explanation of segregation induced by actinomycin that involves a skeletal component may be supported by the findings of Lindell (Lindell, 1976; Lindell, O'Malley & Puglisi, 1978) that the primary effect of the drug is on a rapidly turning over messenger RNA, not directly on ribosomal RNA synthesis. A nucleolonemal morphology independent of organizer chromatin is seen in Spirogyra (Godward & Jordan, 1965).

It is well known that the fibrillar centres contain protein (Recher et al. 1971; Hernandez-Verdun, Hubert, Bourgeois & Bouteille, 1980), and only a little DNA (Goessens, 1976). Their increasing size with nucleolar inactivation may also, therefore, be due in part to a redistribution of a skeletal component.

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REFERENCES


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