STUDIES ON CHANGES IN THE NUCLEAR HELICES OF AMOEBA PROTEUS DURING THE CELL CYCLE

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SUMMARY

The fine structure of the nuclei of synchronously growing cell populations of Amoeba proteus was studied at 1-h intervals during the interphase. This study showed that the nuclear helices undergo increases in their number at certain stages during interphase. These changes were found to correlate with ultrastructural changes occurring in the nucleoli.

INTRODUCTION

The occurrence of helical structures in the nuclei of Amoeba proteus was first reported by Pappas (1956). Later studies (Stevens, 1967; Wise, Stevens & Prescott, 1972) showed that the helices radiate from a common central core forming star-shaped clusters in cross-sections.

On the basis of enzyme digestion experiments with ribonuclease (Stevens, 1967) and also labelling with radioactive precursors of RNA (Stevens, 1967; Wise et al. 1972) and protein (Stevens, 1967), the chemical composition of amoeba helices was thought to consist of RNA and protein. In recent years Georgiev & Samarina (1971) in their review of D-RNA, used the presence and composition of the amoeba helices to support their discovery of the RNP particles in the nuclei of tissue culture cells. These particles consisted of D-RNA (RNA with a DNA-like base composition) and protein.

The possibility of the helical structures being the morphological basis for the movement of RNA coupled with protein from the nucleus to the cytoplasm, emphasizes the importance of clarifying the role of these structures in an interphase nucleus.

The present study represents an attempt to obtain information regarding the site of synthesis or formation of these helices and the fate of these structures after leaving the nucleus. Particular attention has been paid during this investigation to changes in the helices with time and also to the relationship of the helices with other nuclear components. Attempts have been made to correlate some of those changes with certain concurrent biochemical events in the nucleus (Ord, 1973).
MATERIALS AND METHODS

Cultures

Mass cultures of *A. proteus*, strain *P*<sub>0</sub> *X*<sub>6</sub>, were maintained at 18–20 °C using the *Tetrahymena pyriformis* feeding technique of Prescott & James (1955).

The synchronization method

A synchronized population of the cells of *A. proteus* was obtained by picking up the division spheres from mass cultures. After cleavage cells of the same age were grouped together.

Cell cycle

Earlier studies on nuclear DNA synthesis throughout the cell cycle (Ord, 1968) showed that *A. proteus* strain *P*<sub>0</sub> *X*<sub>6</sub> had a 3-phase cell cycle. The total cell cycle took approximately 48–54 h: DNA synthesis, occupying the first fifth of the cell cycle, was followed by a long *G*<sub>1</sub> lasting some 38–44 h, with mitosis taking approximately 30–35 min. There was no significant *G*<sub>0</sub> period.

The strain *P*<sub>0</sub> *X*<sub>6</sub> used during this investigation was slightly different in that it had a longer *G*<sub>1</sub>, making the total cell cycle approximately 58–60 h.

Preparation for electron microscopy

Cells were either fixed by osmium tetroxide or double fixed with Karnovsky's mixture (1965) followed by osmium tetroxide.

Single fixation was carried out by fixing cells in a 1 % solution of osmium tetroxide in 0.1 M cacodylate buffer pH 6.2 for 60–90 min. After fixation cells were washed and processed for electron microscopy.

Double fixation was done by firstly fixing the cells in Karnovsky's mixture — glutaraldehyde and paraformaldehyde in 0.1 M cacodylate buffer — at pH 7.2 for 30 min. Then the cells were washed in distilled water and postfixed for 1 h in 1 % Os<sub>4</sub>O<sub>6</sub> in 0.1 M cacodylate buffer at pH 7.2. The cells were then dehydrated in a graded series of ethanols, immersed in propylene oxide and embedded in Araldite. Sections of 60–90 nm thickness were cut and stained with uranyl acetate and lead citrate before examination with a Philips EM300 electron microscope.

Microscopic examination

At least 35 sections of each nucleus were examined. This generally covered the entire nucleus. Scanning each nuclear section for single helices or helical groups was done at 25,000 times magnification. The total number of groups in the nucleus would be impossible to obtain without building up a 3-dimensional model from serial sections of each nucleus. However, after careful examination of many nuclei it was felt that, providing edge sections of a nucleus, and nuclei which were cut at very odd angles, were excluded, results obtained using either one of the following methods gave a typical picture for each age.

1. The helical group frequency for a nucleus was obtained from that section of the nucleus which had the highest number of groups: bearing in mind that this does not give the total number of groups in the nucleus, since no one cut will take in all the helical groups of a nucleus. The final result for any age was then obtained by averaging the values obtained from the individual nuclei of that age.

2. The helical group frequency for a nucleus was obtained by averaging the number of helical groups from all the sections of that nucleus (omitting only edge sections). The final result for any age was then obtained as in (1) by averaging the values obtained from all individual nuclei of that age.

Histograms were made from the results obtained using both methods (1) and (2). These showed an identical peak and dip pattern. In the present paper values and illustrations are based on method (2).
Nuclear helices of A. proteus

When searching the cytoplasm for helices a slightly lower magnification was used to cover larger areas, but final identification was made at 25000. No attempt was made to estimate the numbers of helices in the whole cytoplasm of an amoeba.

RESULTS

Electron-microscopic observations on the occurrence and distribution of the helical structures

Ultrastructural studies showed that the helical structures of A. proteus appeared in the nucleus either as single helices or in groups. The groups appeared in 2 configurations, the commonly seen star-shaped grouping with 7–10 helices (Fig. 2), and the much longer structure shown in Fig. 3 with 20–30 or more helices. These 2 configurations may represent the same structure, and the difference in shape may be due to the angle at which the sections were cut. If the structure seen in Fig. 2 represents a cross-section and the clustering seen in Fig. 3 represents a longitudinal section of a group of helices, then one would expect all cuts except the very few which pass from end to end to appear as a star or distorted star.

Table 1. Measurements of nuclear and cytoplasmic helices

<table>
<thead>
<tr>
<th>Region</th>
<th>Length of helix, ( \mu m )</th>
<th>Width of helix (pitch of the coil), ( \mu m )</th>
<th>Width of filament, ( \mu m )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear helices (in groups)</td>
<td>0.265</td>
<td>0.038</td>
<td>0.019</td>
</tr>
<tr>
<td>Nuclear helices (singles in close association with the nucleoli)</td>
<td>0.270</td>
<td>0.037</td>
<td>0.02</td>
</tr>
<tr>
<td>Helices in the honeycomb layer</td>
<td>0.261</td>
<td>0.039</td>
<td>0.02</td>
</tr>
<tr>
<td>Helices in the cytoplasm</td>
<td>0.264</td>
<td>0.039</td>
<td>0.01</td>
</tr>
</tbody>
</table>

A close examination of the nucleus was made at different stages during interphase, to investigate the origin or the site of formation of the helical structures. This showed that helices, in single forms, occurred in very close association with the nucleoli (Fig. 5). Occasionally helices being assembled from small particles at the periphery of the nucleoli were detected (Fig. 4). The close association with the nucleoli was not restricted to single helices. Groups of helices were also seen to be in close proximity to the peripherally located nucleoli. Only very occasionally was a group of helices seen in the central region of the nucleus.

Throughout this ultrastructural survey of the amoeba nucleus, helical structures in single forms were seen near the nuclear envelope and some were detected within the honeycomb layer (Fig. 8B). This suggested that the helical structures moved out to the cytoplasm. Examination of the cytoplasm showed that structures morphologically resembling nuclear helices were occasionally seen in it (Fig. 8C). Close examination of these cytoplasmic helices showed that while at low-power magnification (up to
the nuclear helices and cytoplasmic helices seemed to be morphologically identical (Fig. 7A), at high power they differed. At magnification of 50,000 or more the nuclear helix appeared as a uniform filament coiled into a helix (Fig. 7B). On the other hand, a cytoplasmic helix appeared more particulate, having the shape of 2 rows of ribosomes arranged in a staggered manner with a thin filament connecting the subunits (Fig. 7C).

Measurements performed on nuclear and cytoplasmic helices to find whether they underwent changes in their size as they moved from the site of origin in the nucleus to their final distinction in the cytoplasm are given in Table 1. The measurements show that, although there was a gradation of length of helices ranging from 0.12 to 0.49 μm in both the nucleus and the cytoplasm, the average length in both regions was the same. Measurements made on the width of the helix (pitch of the coil) also showed no difference between nuclear and cytoplasmic helices. The only component which appeared to differ was the filament making up the helix. The filament of the cytoplasmic helix measured half that of the nuclear helix.

It is worth mentioning here that the cytoplasmic helices (Fig. 8C), besides having morphological resemblance to nuclear helices (Fig. 8A), have features in common with cytoplasmic polysomes. The subunits which make up the cytoplasmic helix are of a size comparable to ribosomes (Fig. 8D). These findings suggest that cytoplasmic helices may be the intermediate stage of a transformation of nuclear helices to cytoplasmic ribosomes.

Changes in nuclear helices with time

To study changes in the helical structures with time, groups of cells were fixed at 1-h intervals starting from mitosis. Because of the length of the cell cycle this investigation covered only the first 17 h starting from division, that is S-phase plus the first quarter of the G2. The remainder of the G2 was examined at 2 intervals only, at 23 h of age and at a pre-division stage, where cells were approximately 58–60 h old.

The ultrastructural survey showed that the helices first appeared in the nucleus 2 h after mitosis. They were generally in single forms in close proximity to the nucleoli. Only occasionally were clusters of helices found at this young age. When the helices were present in clusters no more than 2 clusters per nucleus were seen (Fig. 9).

Sampling groups of cells at hourly intervals and counting the number of helical groups at each period revealed that the changes in the number of helical groups with time were in the form of peaks and dips (Fig. 1). This study showed that after their first appearance in the nucleus the number of helical groups progressively increased with time, reaching a peak of 6.5 groups per nucleus in 5-h-old amoebae (Fig. 10). This was followed by a marked reduction in the number of groups so that some nuclei at 6 h had as few as one group. An increase in the number of helical groups was observed in cells fixed 8 h after division. During the following 9–14 h age there was again a marked reduction in the number of helical groups. A third increase in the number of helical groups was found in amoebae fixed at 15 h after mitosis. This increase, unlike the 2 previous ones, did not drop immediately, but was still apparent at 16 h age. By 17 h age there was again a drop in the number of helical groups. The 2 later G2 periods
Nuclear helices of A. proteus

examined, i.e. 23 h age and the pre-division period, both showed large numbers of helical groups.

It is worth mentioning here that some of the ages with peaks in the number of helical groups (8 and 15–16 h after mitosis) were preceded by changes in nucleolar fine structure. Observations made on cells fixed 7 and 14 h after division showed the appearance of dark irregular patches randomly distributed over the nucleolar surface (Fig. 6). These timings have been marked on the histogram of Fig. 1 by vertical arrows. Changes in nucleolar fine structure were not detected in unstained sections.

The study of the relationship between the nuclear helices, cytoplasmic helices and polysomes was made using cells 16 and 23 h old. These were both periods of peaks in the number of nuclear helices. This examination showed that a considerable number of helices could be found in the cytoplasm at a time when the nucleus was undergoing an increase in its number of helices.

![Histogram showing changes in the number of helical groups in the nuclei of A. proteus with increasing cell age. Arrows indicate positions where changes in nucleolar fine structure occurred.](image)

**DISCUSSION**

Our ultrastructural observations indicate that the nuclear helices of *A. proteus*, which are thought to be RNA and protein complexes (Stevens, 1967; Wise *et al.* 1972), increase in number at certain stages during interphase. These increases turn up regularly at 5, 8 and 15–16 h after mitosis and correlate with certain biochemical events occurring in the cell cycle of *A. proteus*: the helices' peaks coincide approximately with peaks in uridine incorporation reported by Ord (1973) in another strain of *A. proteus*.

A brief note by Leonhardt & Nilsson (1974) reports that examination of *A. proteus* nuclear helices at 0, 2, 4, 5, 8, 16 and 20 h showed a peak in helices at the 5-h age period only. It is difficult to compare our results with their report, since a different strain of *A. proteus* was used and no details were given of the temperature at which the cultures were maintained. Differences in temperature cause differences in the length of the cell cycle, and this may alter the timing of peaks for nuclear helices.

There is as yet no method of isolating the helical structures (Wise *et al.* 1972).
Therefore, there is no definite method of identifying biochemically the class of RNA in these structures. However, the results of the present ultrastructural survey suggest that the RNA is of the ribosomal type, or includes the ribosomal type, for the following reasons. (1) In a few instances helices appeared to be assembled at the marginal zones of the nucleoli (Fig. 5); this zone corresponds to the area occupied by the perinucleolar chromatin (Minassian & Bell, unpublished). (2) Helical clusters were almost always in the neighbourhood of nucleoli and single helices were associated with the nucleoli. (3) The increase in the number of helices at 2 instances was preceded by changes in the nucleolar fine structure. However, since messenger RNA can also have close association with the nucleolus (Deak, Sidebottom & Harris, 1972; Deak, 1973), it is not possible to rule out the presence of other forms of RNA within the helices on the basis of ultrastructural studies only.

The fate of the nuclear helices after passing through the nuclear envelope is not certain. However, the morphological resemblance found between the nuclear helices and cytoplasmic helices, on one hand, and the resemblance found between the cytoplasmic helices and the linear chains of ribosomes, on the other, suggest that the cytoplasmic helices may represent an intermediate stage in a possible transformation of nuclear helices to single short chains of ribosomes (Fig. 8A–D).

Experiments using emetine on A. proteus (Flickinger, 1972) and N-hydroxy-2-fluorenylacetamide on rat liver cells (Popp & Shinozuka, 1974) showed that an increase in cytoplasmic helices (helical polysomes) followed treatment with such protein inhibitors. From this work both Flickinger (1972) and Popp & Shinozuka (1974) suggest that the single short chains of ribosomes are the form taken when the cell is actively synthesizing proteins.

No detailed study has yet been made on changes in the cytoplasmic helices with time, so it is not possible to say whether the cytoplasmic helices of A. proteus follow a pattern similar to that of nuclear helices, or whether they have a different pattern dependent on the rates of protein synthesis.

REFERENCES


(Received 16 July 1975)
Fig. 2. Cross-section through a helical structure in a nucleus of a 6-h-old cell. × 62,500.

Fig. 3. Longitudinal section through the helical structure in a nucleus of a 6-h-old cell. × 62,500.

Fig. 4. Electron micrograph of a nucleus suggesting that helices (h) are assembled from particles at the peripheral zones of the nucleoli (no). × 50,000.

Fig. 5. Ultrastructural section of a portion of a nucleus, illustrating close association between a helix (h) and the marginal zones of a nucleolus (no). × 59,850.

Fig. 6. Section of nucleus fixed 7 h after division, showing dark patches (d) which appear over the nucleolar surface. × 20,600.
Nuclear helices of A. proteus
Fig. 7 A–C. Electron micrographs showing the relationship between nuclear and cytoplasmic helices. 

A, at low-power magnification the nuclear helix (h) and cytoplasmic helix (x) are morphologically identical. × 20600. B, at high-power magnification (× 50000) each nuclear helix (h) appears as a uniform filament coiled into a helix. C, at the same magnification (× 50000) the cytoplasmic helix (x) appears more particulate, having the shape of 2 rows of small spheres arranged in a staggered manner with a thin filament connecting the subunits.
Fig. 8 A–D. Electron micrographs showing the movement of helices from the nucleus through the nuclear envelope into the cytoplasm. c, cytoplasm; h, nuclear helix or helices; he, honeycomb layer of nuclear envelope; n, nucleus; no, nucleolus; p, polyribosomes; x, cytoplasmic helix. A, helices in groups in the nucleus. × 50000. B, 2 helices in the nuclear envelope. Note that the upper helix is entering the cytoplasm and has partially uncoiled, while the lower helix is still in the honeycomb layer. × 50000. C, a helix in the cytoplasm. × 50000. D, polyribosomes in the cytoplasm. × 50000.
Nuclear helices of *A. proteus*
Fig. 9. Section of a 2-h-old nucleus, showing 2 groups of helices. No helical clusters were found in younger cells. $\times 41000$.

Fig. 10. Section of a 5-h-old nucleus. At this age there is a marked increase in the number of helical groups (h). $\times 41000$. 
Nuclear helices of *A. proteus*