TIMING OF NUCLEOLAR ACTIVITY IN MERISTEMS

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SUMMARY

The nucleoli in 4 regions of the root meristem of Zea mays have been examined and the time spent in each of the phases of the nucleolar cycle has been calculated from estimates of the rates of mitosis and the fraction of cycling cells in each region. The nucleolar cycles have been fitted to the mitotic cycles of cycling cells in the same regions.

The timing of activity of the r-RNA genes differs in relation to the chromosomal cycles of condensation and movement in the different regions of the meristem. In the stelar regions disorganization of the nucleolus starts in the middle of prophase and reorganization starts towards the end of telophase and is completed in the following interphase. In the quiescent centre of the meristem the phases of both cycles are lengthened except for telophase and reorganization of the nucleolus does not start until after the end of telophase. The acceleration of telophase in the quiescent centre is discussed in relation to inhibition of protein synthesis. In the adjacent cap initials, which have the shortest mitotic cycle, the nucleolus starts to disorganize before the onset of prophase and reorganization is completed halfway through telophase. This fits with the fact that DNA synthesis is advanced into the telophase of the previous cycle in these cells.

INTRODUCTION

An organized meristem consists of cells growing and dividing at different rates (Clowes, 1961). It also displays differences in nucleolar activity seen by comparing the size and structure of nucleoli in different regions of the meristem (Clowes, 1956; Hyde, 1967; Barlow, 1969, 1970a, b). We have now related the timing of activity of the nucleolar organizer genes in coding for ribosomal RNA with the mitotic cycles of cells with differing rates of division. We have done this by observing the times spent by nucleoli in the different phases of their cycles of activity and by fitting these times to the chromosomal cycles. Our approach is a development of that used by Giménez-Martin, Fernández-Gómez, González-Fernández & de la Torre (1971).

We have exploited the root meristems of Zea mays for this purpose since no other proliferating organ, plant or animal, has had its cell cycles so completely characterized during normal growth and under experimental conditions. We have used the silver-reducing property of nucleoli (Tandler, 1959; Fernández-Gómez, Stockert, Lopez-Saez & Giménez-Martin, 1969) to follow the timing of the formation and disorganization of the nucleoli in 4 regions of the meristem with different cell cycles (Fig. 2). These regions are as follows. (1) The initials (ci) responsible for renewing the cap cells. At 23 °C they produce some 10,000 cells a day, enough to produce a complete

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new cap every day, and have an average cell cycle of 15 h. (2) The quiescent centre
\( (qc) \) of the meristem whose 600 cells have an average cycle duration of 230 h. (3) A disk
of stelar cells \( (sti) \) just above the quiescent centre. And (4) another \( (st2) \) at 200 \( \mu \)m
from the boundary between the cap initials and the quiescent centre. The two stelar
regions have average cell cycles lasting 24 and 17 h respectively. Each of the 4 regions
has a characteristic growth fraction (fraction of cells which are cycling) which varies
from 0-4 in the quiescent centre to 1-0 in the stele at 200 \( \mu \)m (Clowes, 1971). The rates
of mitosis in cycling cells are therefore usually higher than the averages for all cells
quoted above.

The work shows that the nucleolar cycle varies considerably in the different meri-
stematic regions both in its absolute time parameters and also in relation to the mitotic
cycle.

MATERIALS AND METHODS

We have used the primary roots of \textit{Zea mays} (c.v. Golden Bantam) grown in damp sphagnum
at 23 °C in the dark for all the quantitative data.

Nucleoli in fixed roots reduce silver salts to silver as in photography and the process also
gives clearly stained cells. Our method is a slightly modified version of that used by Fernandez-
Gómez \textit{et al}. (1969):

1. Fix root tips in 1:1 mixture of 10% formaldehyde and 1% hydroquinone for 2 h.
2. Wash in distilled water 10 min.
3. Place in 2% silver nitrate solution at 65 °C overnight.
4. Wash in distilled water, 10 min.
5. Reduce in the mixture used in step 1 for 2 h.
6. Wash 10 min.
7. Place in sodium thiosulphate solution (the photographic fixer, 'Ilfofix') for 1 h.
8. Wash in distilled water 10 min.

The root tips were then dehydrated, embedded in paraffin and sectioned at 5 \( \mu \)m. The median
sections of 40 roots were scored.

For the chromosomal cycle similarly grown roots fixed in 1:3 acetic acid: ethanol, and stained
in Feulgen were scored in median sections. Nucleolar vacuoles were studied in unstained
sections after fixation in Helly's fluid.

RESULTS

\textbf{Nucleolar morphology}

After silver impregnation the fully organized nucleoli of interphase cells are brown
in a yellow nucleus in pale yellow cytoplasm. Most of the cells (91-2\% in \( ci \), 97-3\% in
\( qc \), 89-5\% in \( st1 \) and 88-3\% in \( st2 \)) contain fully organized nucleoli, but these vary in
volume in the ratio 2:1:8:8 in \( ci \), \( qc \), \( st1 \) and \( st2 \) respectively after the preparative
procedure used here (Fig. 2). In this diploid cultivar there are 2 nucleoli which fuse in
some cells. For the measurements of volume, cells with unfused nucleoli were used.

In the quiescent centre nucleoli have the minimal volume and silver-reducing
capacity. They are fairly homogeneous, but some have a tiny central vacuole. Some
nucleoli in this region have a segment protruding from the main sphere (Fig. 3). This is
the object sometimes called a karyosome by electron microscopists (Sankaranarayanan
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Table 1. Percentages of cycling cells in phases of the nucleolar and chromosomal cycles

<table>
<thead>
<tr>
<th></th>
<th>Cap initials</th>
<th>Quiescent centre</th>
<th>Stele 1</th>
<th>Stele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophase</td>
<td>1.9</td>
<td>3.6</td>
<td>6.4</td>
<td>7.1</td>
</tr>
<tr>
<td>Metaphase and anaphase</td>
<td>2.6</td>
<td>1.1</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Telophase</td>
<td>6.4</td>
<td>0.5</td>
<td>3.9</td>
<td>2.7</td>
</tr>
<tr>
<td>No. of all cells scored</td>
<td>1102</td>
<td>508</td>
<td>3505</td>
<td>3572</td>
</tr>
<tr>
<td>Disorganized</td>
<td>3.8</td>
<td>2.9</td>
<td>4.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Dispersed</td>
<td>2.6</td>
<td>2.2</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Reorganizing</td>
<td>3.3</td>
<td>1.6</td>
<td>4.2</td>
<td>3.4</td>
</tr>
<tr>
<td>No. of all cells scored</td>
<td>2077</td>
<td>1030</td>
<td>6756</td>
<td>5808</td>
</tr>
<tr>
<td>Growth fraction</td>
<td>0.9</td>
<td>0.4</td>
<td>0.8</td>
<td>1.0</td>
</tr>
</tbody>
</table>

& Hyde, 1965). Although only 40% of the cells are cycling, there are no other differences in nucleolar morphology within the region.

Nucleoli of the cap initials are only slightly bigger and they also have poor affinity for silver although this region has the highest rate of mitosis in the meristem. However, they have more vacuoles than the nucleoli of the quiescent centre (Fig. 4) and show some internal differentiation in the localization of affinity for silver.

Nucleoli of the stele outside the quiescent centre are much bigger, contain more vacuoles and show greater differences in silver affinity within each nucleolus.

The disorganization of the nucleolus, which occurs around prophase, is characterized by reduction in silver affinity and the acquisition of an irregularly lobed periphery which is less argyrophilic than the centre. The condensing chromosomes become darker at this stage. When the nuclear membrane breaks down, the poorly stained remnants of the nucleolus appear to disperse quickly throughout the protoplasm and remain like this through metaphase and anaphase. The chromosomes on the metaphase plate are argyrophilic throughout their lengths.

As soon as the chromosomes reach the spindle poles dark particles begin to condense all along every one of them. These particles are the so-called pre-nucleolar bodies that later fuse to form the 2 nucleoli of early interphase. The pronucleolar substance appears to collect around the chromosomes as layers rather than in droplets (Rattenbury & Serra, 1952).

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We have scored the number of cells in each region of the meristem with (1) organized nucleoli, (2) with nucleoli in the process of disorganization, (3) with dispersed nucleolar material, and (4) with nucleoli in the process of reorganization. We also scored the percentage of cells in each phase of mitosis in roots grown in similar conditions, but stained in Feulgen. We have used the growth fractions calculated by Clowes (1971) from pulse-label data to adjust the figures for both the phases of mitosis and the
Table 2. Durations of mitotic cycles, mitosis and time spent by nucleolus not in fully organized condition in hours at 23 °C

<table>
<thead>
<tr>
<th>Cap initials</th>
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<th>Stele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-doubling time</td>
<td>9.1</td>
<td>20.3</td>
<td>20.8</td>
</tr>
<tr>
<td>Cycle duration in cycling cells</td>
<td>8.2</td>
<td>8.1</td>
<td>16.6</td>
</tr>
<tr>
<td>Duration of mitosis</td>
<td>0.9</td>
<td>4.2</td>
<td>2.1</td>
</tr>
<tr>
<td>Time spent without fully organized nucleoli</td>
<td>0.8</td>
<td>5.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

nucleolar phases associated with them in order to relate these to cycling cells in each region of the meristem (Table 1).

We shall assume steady-state kinetics for the purpose of timing the phases and so the percentages of cells in each phase are directly proportional to the fraction of each cell cycle occupied by the phase. The cell cycles have been measured as averages or cell-doubling times by using colchicine to accumulate metaphases in roots grown in similar conditions. These values have been transformed to durations of the cell cycles of cycling cells by multiplying by the growth fraction (Table 2).

In fitting the nucleolar cycles to the chromosome cycles we have used the fact that all prophase nuclei contain nucleoli and that no metaphase nuclei do so. The prophase-metaphase boundary is therefore taken to occur at the same time as the disorganization-dispersion boundary. Special care was taken to check that the disorganized phase did not end before the breakdown of the nuclear membrane at the end of prophase especially in the quiescent centre. The relation of the 2 cycles is given in Fig. 1.

DISCUSSION

Silver impregnation of the nucleoli shows up at the resolution of the light microscope many of the features observed under the electron microscope. Our observations confirm the work of others concerning the size of nucleoli, different numbers of nucleolar vacuoles and different proportions of fibrillar and granular regions in cells of different metabolism (Clowes, 1956; Chouinard, 1966; Hyde, 1967; Barlow, 1970b). The object described by Sankaranarayanan & Hyde (1965) as the karyosome appears most commonly but not exclusively in the quiescent centre. Hyde (1967) noted it also in the quiescent centre of roots of Plantago and it is probably the same as the 'L Zone' seen also in dormant cells of Helianthus by Jordan & Chapman (1971). It seems to be derived from a special part of the nucleolar organizer chromatin and lies at the surface of the nucleolus in inactive cells and is said to move to the interior and to disperse into small parts when there is a demand for r-RNA.

The most interesting fact that emerges from fitting the nucleolar phases to the chromosomal phases is that specialization within the meristem leads to changes in the
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Fig. 1. Chromosomal and nucleolar cycles in 4 regions of the root meristem of *Zea mays* grown at 23 °C. The radius of each circle is proportional to the average duration of the mitotic cycle of cycling cells except for the quiescent centre which is drawn at half the scale of the other regions. The angle subtended by each arc at the centres of the circles is proportional to the length of each phase which is given in hours.

relation of the timing of activity of the genes coding for r-RNA to the condensation pattern and movements of the chromosomes.

We may take the stelar cells as typical of meristematic cells, for these show a relationship between the cycles which is similar to that reported for whole meristems of *Allium, Vicia* and other plant material by La Fontaine & Lord (1969), Stockert, Fernández-Gómez, Giménez-Martín & Lopez-Saez (1970) and Giménez-Martín *et al.* (1971). Here the nucleolus starts to disorganize some way through prophase and starts to reorganize towards the end of telophase and completes reorganization in interphase. This fits with our observations that some prophase nuclei have fully organized nucleoli.
In the cap initials, however, the nucleolus starts to disorganize before the onset of prophase. The process takes less time here than in the other regions, but prophase takes considerably less time. Both the dispersed phase of the nucleolus and metaphase-anaphase are shortened and they end at the same time. Reorganization is also shortened, but telophase is not greatly shortened. The timing implies that the nucleolus must be fully organized for the last half of the telophase in this region. This is confirmed by the discovery of fully organized nucleoli in some telophases in the cap initials.

This fact is particularly interesting because the cap initials, and no other cells in Zea roots, have the DNA-synthetic period brought forward into the telophase of the previous cycle thus eliminating the G1 phase (Clowes, 1967). Such a precocious S period is also known to occur in a few other kinds of cells with short mitotic cycles notably in early sea-urchin embryos (Hinegardner, Rao & Feldman, 1964). The early completion of nucleolar reorganization might be expected in such cells also, for the timing of the activity of the different genes must be the motor that drives the cell cycle itself.

In the quiescent centre all of the phases of both cycles are lengthened except for telophase, but the unorganized part of the nucleolar cycle extends further into G1 than in any other region and telophase ends before reorganization starts. In this region the nuclei spend the shortest fraction of the cell cycle with condensed chromosomes and unorganized nucleoli. Telophase not only occupies a smaller part of the cell cycle, but it is also shorter than in any other region. It is interesting in this context that Fernández-Gómez, de la Torre & Giménez-Martín (1972) report that using inhibitors to lower the rate of protein synthesis to 10–16% of normal also accelerates telophase and the reorganization of nucleoli. We know that the rate of protein synthesis is conspicuously lower in the quiescent centre than in the rest of the root apex (Clowes, 1958). This may be linked with the small size of the nucleoli here and it could be that, during the formation of nucleoli, the nucleolar organizer genes have a double function of synthesizing r-RNA and of reorganizing the nucleolar material itself. If the demand of the cytoplasm for r-RNA is minimal here, as in the experimental inhibition of protein synthesis, the reorganization of the nucleolus may be faster than the slowing of the nucleolar and mitotic cycles would lead us to expect. Experimental inhibition of RNA synthesis prevents the reorganization of nucleoli and so the effect observed in the quiescent centre is more likely to be linked with its low protein synthesis although we know that the cells of the quiescent centre also have less cytoplasmic and nucleolar RNA and a lower rate of RNA synthesis than other cells (Clowes, 1956; Barlow, 1970a).

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REFERENCES


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Figs. 2–4. Root meristem of *Zea mays* under Nomarski interference optics. *cb*, cap boundary; *ci*, cap initials; *qc*, quiescent centre; *st 1*, stele just above quiescent centre.

Fig. 2. Part of the meristem showing 3 of the regions discussed in the text. The quiescent centre is almost a hemispherical group of cells on the root axis above the cap boundary. The cap initials form a disk 3–4 cells deep below the cap boundary. Stele 1 is a disk of cells lying between the arbitrarily chosen parallel lines. × 1000.

Fig. 3. A cell in the quiescent centre with a nucleolar 'karyosome'. × 2500.

Fig. 4. Cap initials with several nucleolar vacuoles. Nucleoli of the quiescent centre (above the cap boundary) have few or none. × 2500.
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