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THE EFFECT OF THERMAL SHOCK ON THE DIVISION CYCLE OF MERISTEMATIC CELLS

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ABSTRACT

The dynamic adaptation of a meristem to a thermal change from 15 to 35°C has been studied. A gradual drop occurs in the mitotic index during the first 6 hr after the change, while the percentage of prophases drops less steeply. At the same time the phenomenon of nucleolar segregation is observed.

By making use of a cell population morphologically differentiated as binucleate (by 'labelling' with caffeine), it was possible (a) to prove that prophase is specifically prolonged and (b) to locate a highly thermosensitive segment of interphase, situated in the middle of the S period, and apparently coinciding with the sub-period S_2, described in the same material as characterized by a decrease in the rate of DNA synthesis.

The lengthening of prophase and nucleolar segregation resemble the effects produced by drugs which inhibit RNA synthesis.

Intermittent thermal shocks are suggested as a possible means for synchronizing the division cycle of plant cells.

INTRODUCTION

Temperature is an important factor in determining the duration of the mitotic cycle. Brown (1951), Evans & Savage (1959), Van't Hof & Sparrow (1963), Van't Hof & Ying (1964) and López-Sáez, Giménez-Martín & González-Fernández (1966) have shown by different methods that in plant material the speed of the division cycle increases with temperature up to 25 or 30°C.

On the other hand, various writers have drawn attention to a specific temperature influence on certain stages of the process, which modify their relative velocities within the whole cycle (Agrell, 1958; Evans & Savage, 1959).

The sensitivity of certain parts of the division cycle to temperature variations has led to the application of thermal shocks at sub-lethal levels and, intermittently, to bring about an experimental synchronization in the development of a cell population in *Tetrahymena* and

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in cultures of animal cells (Scherbaum & Zeuthen, 1954; Newton & Wildy, 1959; Newton, 1964).

The maintaining of a constant speed at a given temperature that remains unaltered throughout the cycle, taken in conjunction with the variations that the cycle undergoes when thermal shocks are applied at different periods of the cycle, suggests that certain portions of the cycle differ from others in thermosensitivity. The object of this work was to study this possibility.

MATERIALS AND METHODS

The general method of culturing and preparing the *Allium cepa* L. meristems and the caffeine labelling procedure were described in detail in the preceding paper (Giménez-Martín, González-Fernández & Torre, 1971).

For morphological study of the nucleolus, the meristems were fixed in hydroquinone-formol and stained by the silver impregnation method of Fernández-Gómez *et al.* (1969). About 1500 interphase cells were scored for each 1 hr interval.

Thermal change. This was effected by the instantaneous transfer of bulbs which had sprouted and grown in an incubator at 15°C to another incubator at 35°C in which other environmental conditions were kept identical.

Thermal shock. A thermal shock was considered to have been applied when, after being transferred to the 35°C incubator for 3 hr, the bulbs were returned to the 15°C incubator. The effect of shocks applied at different points in interphase was judged by measuring any variation induced in its duration.

RESULTS AND DISCUSSION

Temperature and the cell division cycle

The mitotic index (MI) and the percentage of meristematic cells in each of the mitotic phases observed in the meristem of roots that have sprouted and are growing with steady-state kinetics, at different constant temperatures, are given in Table 1, from which it is apparent that the durations of the different periods of the division cycle maintained the same relative proportions, whatever the constant temperature may be.

Adaptation of the cell division cycle to thermal changes

The roots that have sprouted and grown either at 15°C or at 35°C maintain a constant percentage duration for each phase throughout their cycle. However, when these parameters

TABLE 1. Relative durations of the phases of mitosis at different temperatures

	5°C	10°C	15°C	20°C	25°C	30°C	35°C
Prophases (%)	6·3	6·5	6·6	6·1	6·0	6·1	5·9
Metaphases (%)	2·0	1·8	1·7	1·8	1·7	1·9	1·8
Anaphases (%)	1·3	1·3	1·3	1·5	1·2	1·3	1·2
Telophases (%)	3·9	3·7	4·1	3·9	3·9	4·0	3·9
Mitotic index	13·5	13·3	13·7	13·3	12·8	13·3	12·8

are studied at successive hours after a thermal change from 15 to 35°C, a dynamic response is found on the part of the meristem to accommodate itself to the new temperature. Fig. 1 shows the gradual falling off that takes place in mitotic index from the moment when the thermal change occurs, and this continues at successive hours, to a minimum of cells in mitosis at the sixth hour, after which the curve begins to rise again, with values for the MI and for the percentage of cells in each mitotic phase reaching values above the normal at the twelfth hour.

On the other hand, Fig. 1 shows that only the percentage of prophases at the second hour remains normal, and that thereafter prophases fall less steeply than the total number of cells in mitosis, until the extreme case is reached at the sixth hour, when practically all (88·5%) of the cells in mitosis are in prophase.

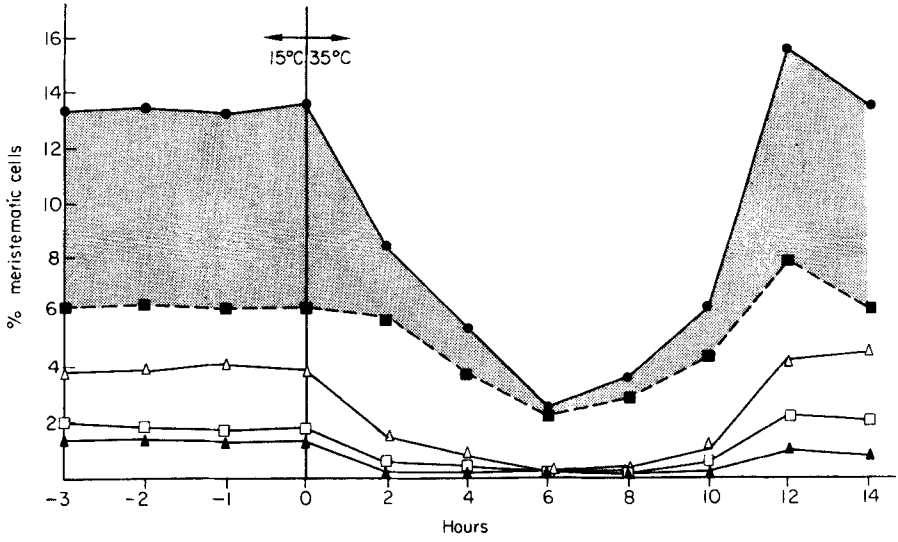

FIG. 1. Changes in mitotic index, ●; prophases, ■; metaphases, □; anaphases, ▲; and telophases, △; following a thermal change from 15 to 35°C.

We may note a number of peculiarities in the adaptation of cells going through the division cycle resulting from the change of temperature from 15 to 35°C:

(a) A marked decrease in the number of cells in mitosis, which could imply the existence of a period in the division cycle sensitive to temperature changes, this period being outside that of mitosis itself. This hypothesis is tested below by using a synchronous binucleate population.

(b) A prolongation of, or specific arrest in prophase, reflected in the lack of parallelism between the drop in mitotic index and the number of prophases following the thermal change. This is the subject of a later test.

(c) The gradual reduction in the percentage of metaphases, anaphases or telophases to values approaching 0 at the sixth hour after the change. In a few bulbs the 15–35°C thermal change inhibited the process of cytokinesis which takes place during telophase, giving rise to binucleate cells.

(d) An increase in the percentage of cells in each mitotic phase to values above normal at the twelfth hour, which also suggests that there may be a thermosensitive area in the interphase period.

Interphase thermosensitivity

In order to detect whether there is any thermosensitive period during interphase and to define its limits, we used a synchronous population produced by the action of caffeine, which was morphologically distinguished as consisting of binucleate cells (Giménez-Martín, González-Fernández & López-Sáez, 1965). In control bulbs, the fastest binucleate cells

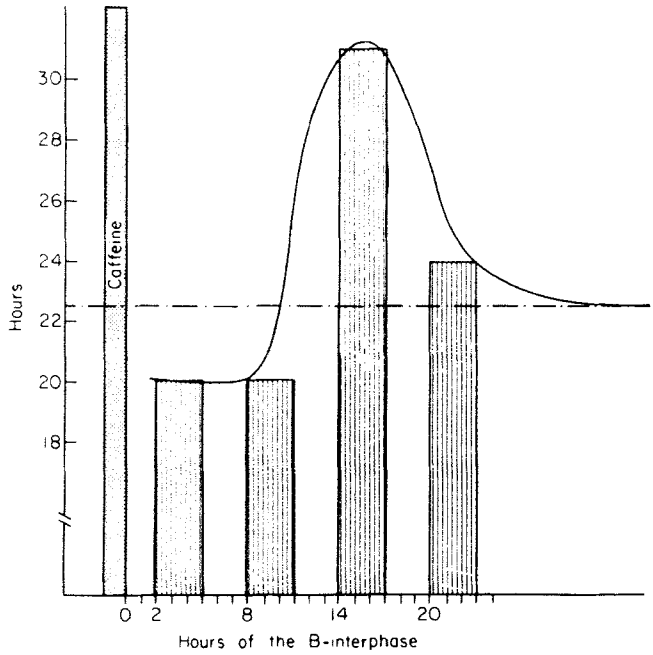

FIG. 2. Effect on the duration of interphase of 3 hr thermal shocks given at different periods of interphase in a binucleate population.

going through the cycle at 15°C constant temperature initiate their bimitosis 22 or 23 hr after the end of the 1 hr caffeine treatment.

After trying thermal shocks of different durations, we chose a 3 hr shock as being the shortest that produced a drop in the mitotic index curves similar to that seen in Fig. 1. A few bulbs with a MI of 7% or more at the end of the shock period, were not included in the study.

Binucleate cells belonging to bulbs in dynamic equilibrium at 15°C were subjected, in different meristems, to 3 hr shocks at 35°C at the second, eighth, fourteenth and twentieth hours following the end of the caffeine labelling (B interphase). As shown in Fig. 2, the duration of the B interphase, reckoned as ending with the first bimitosis to be detected, was slightly diminished by thermal shocks administered at the second and eighth hours, which seems to indicate that these two periods are hardly, if at all, sensitive to temperature variations. The

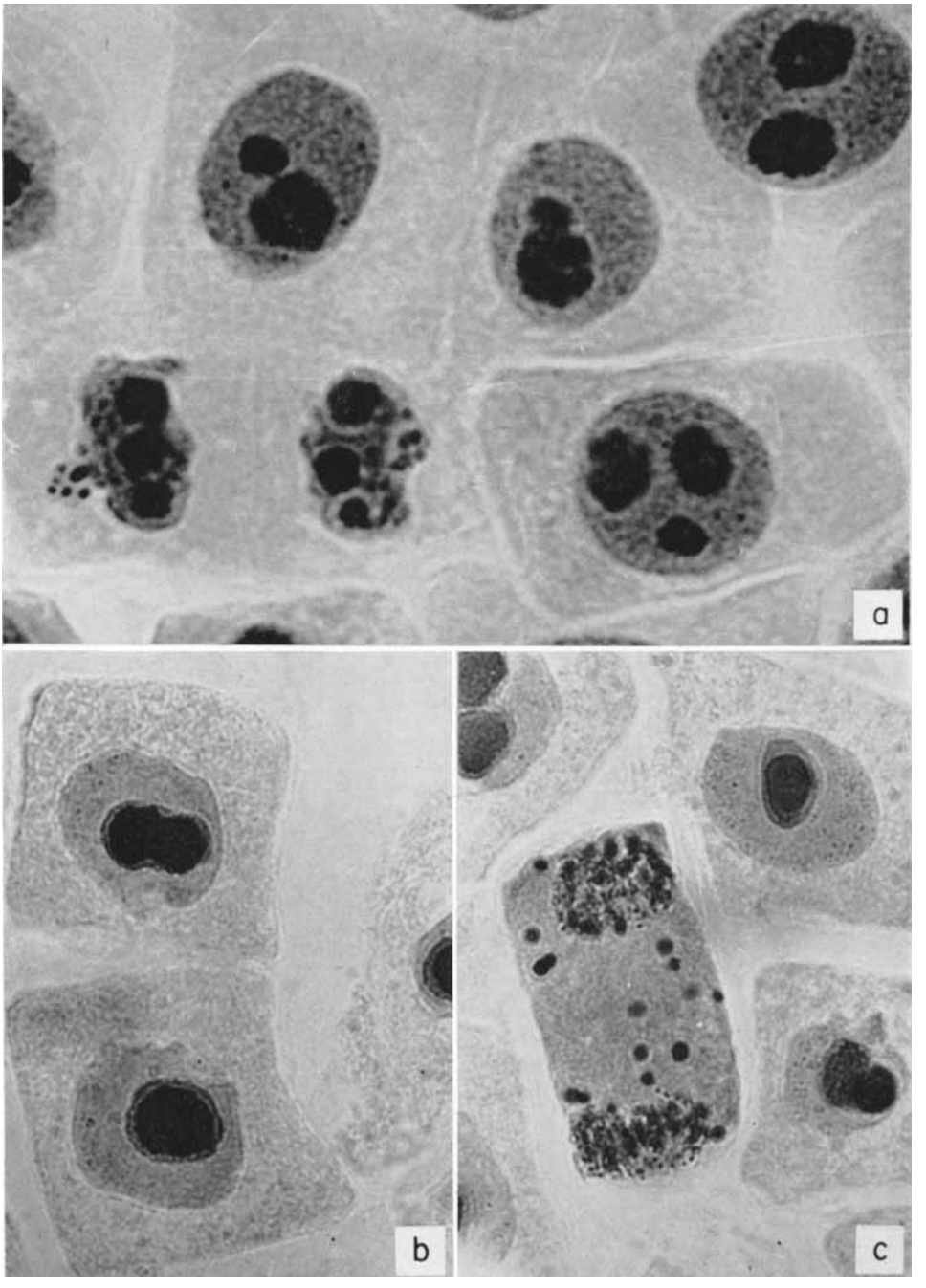

FIG. 3. Meristematic cells stained by the silver impregnation method to demonstrate nucleoli. (a) Untreated meristem; (b) thermal change from 15 to 35°C, showing nucleolar segregation; (c) abnormal telophase with argyrophilic bodies scattered about the cytoplasm.

reduction in the duration of the B interphase at 15°C may be due to the greater speed of the cycle at 35°C, the temperature maintained during the 3 hr shock.

On the other hand, 3 hr thermal shocks administered at the fourteenth hour in the B interphase produce a marked prolongation of interphase (of almost 9 hr), indicating a considerable degree of thermosensitivity characterizing this small segment of the interphase. Unevenness of DNA synthesis in the course of the S period is a well-known fact (Howard & Dewey, 1961) and this highly thermosensitive period, occurring at about the middle of the synthesis period, appears to coincide with a sub-period S_2, which has been described by some of us in the same material, and is characterized by a drop in the rate of DNA synthesis (Fernández-Gómez, González-Fernández & Giménez-Martín, 1968, 1969).

Lastly, thermal shocks beginning at the twentieth hour in the B interphase bring about a slight prolongation of the cycle, possibly reflecting a depressive effect on the cells in the G_2 period.

Prophase thermosensitivity

Another experiment was carried out in order to ascertain whether the different response of prophase cells to the thermal change from 15 to 35°C was due either: (a) to the constancy in the rate of entry and exit of cells to and from prophase, or (b) to a specific prolongation of prophase.

In control bulbs at 15°C the binucleate cells initiate their biprophase 22–23 hr after the 1 hr caffeine treatment, and the earliest bimetaphases make their appearance 3 hr later (twenty-fifth or twenty-sixth hours).

Binucleate cells at the twenty-third hour of their B-interphase (about 6% of biprophases) were subjected to the thermal change. The first bimetaphases made their appearance, in this case, at the twenty-eighth hour. Thus prophase increases markedly in duration (from 3 to 5 hr), despite the greater speed of the cycle at 35°C. The lengthening of this mitotic phase points to the existence of some thermosensitive function located in the prophase.

Effect of thermal changes on the nucleolus

We also studied the morphology of the nucleoli of cells in the process of adapting themselves to the new temperature during a thermal change from 15 to 35°C, and observed the occurrence of segregation between the fibrillar and granular elements of nucleoli (Fig. 3b). This effect is also produced by inhibitors of RNA synthesis (Stockert *et al.*, 1970), and fits in with the finding of a thermo-labile function located in the nucleolus of animal cells (Simard & Bernhard, 1967). This phenomenon was found to reach completion in interphase cells 2 hr after the change (see its dynamics in Table 2), while the effect was totally reversed after 10 hr.

TABLE 2. Nucleolar segregation at different times after a thermal change from 15 to 35°C

	Hours after thermal change					
	0	2	4	6	8	10
% interphase cells with segregated nucleoli	0	94·1	63·8	23·3	8·4	0·0

At the second hour after the thermal change we also detected abnormalities in a number of telophases (19%) in the reconstitution of the nucleolus which takes place at this stage. These abnormalities consisted in the presence of prenucleolar material scattered about the cytoplasm in this period of mitosis, reminiscent of the descriptions given by Díez *et al.* (1970) (Fig. 3c).

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