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Soluble Polypeptides from Root Meristems Growing at Different Temperatures

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Soluble polypeptides and their biosynthesis rate have been studied in root meristems of *Allium cepa* L. bulbs by electrophoresis in SDS polyacrylamide gels. The second mm of the root was chosen in all cases. The polypeptide composition of root meristems growing with a steady state kinetics at 15° C and 25° C appears to be roughly constant. The quantitative differences observed may be due to the different proportion of proliferative cells in the root segments studied, being higher at 15° C than at 25° C. One important fact is the existence of a polypeptide, approximately 80,000 daltons in molecular weight, which appears as an indicator of active proliferation in onion root cells.

The population of proliferating cells which constitutes the root apical meristem is characterized by considerable heterogeneity with respect to several parameters of cell growth and reproduction (9). The serial section technique allows us to select those meristematic segments in which the homogeneity of the cell population is particularly good (1). For instance, in *Allium cepa* L. the second mm of the root has approximately 90 % of proliferating cortical cells (6).

The aim of the present work is to analyse the process of protein synthesis in onion root meristems growing with a steady state kinetics at 15° C and 25° C (3). In all cases the 2nd mm of the root was selected.

The results are compared with those found in dormant meristems and in mature root regions.

Materials and Methods

Root meristems from *Allium cepa* L. bulbs grown at 15° C and 25° C were used. In all cases the second mm of the root was selected by cutting with a blade on a paper rule and discarding the rest of the root.

Labeling of roots. Bulbs with roots approximately 3 cm long were kept in a 15 μCi/ml solution of ^{3}H-leucine (58 Ci/mmol; Amersham, U.K.) at 15° C for 3 h or at 25° C for 2 h.

Extraction of proteins. This was carried out as previously described (6) but

a Potter homogenizer was used instead of a mortar and the step using $MgCl_2$ was omitted. Protein was assayed according to the procedure of LOWRY *et al.* (5).

Electrophoresis. Separation of proteins was done by electrophoresis in SDS polyacrylamide gels, according to WEBER and OSBORN's method (8), but with 10 % instead of 8 % gels in order to obtain better separations.

Gels were stained with Coomasie blue G-250, scanned in a Isco spectrophotometer at 580 nm and cut into 0.8 mm slices using a gel slicer from Mickle instruments in order to estimate the radioactivity as previously described (6).

Results

Figure 1 shows the electrophoretic profiles of all soluble polypeptides from meristems at 15° C and 25° C. Clear differences, principally quantitative, can be seen between both profiles.

The group of small polypeptides (10,000-40,000 daltons), particularly bands 2a and 2b, decreases notably at 25° C. Within the group of medium-sized polypeptides (40,000-70,000 daltons), the region of band 5b shows differences at both temperatures. With respect to the high molecular weight polypeptides, bands 8a, 8b and 9a are the most characteristic ones of this group. We can consider band 8b to be the most abundant one in the meristem at 25° C, although bands 8a and 9a appear clearly in both profiles but to a lesser extent at 25° C.

Figure 2 shows the electrophoretic profiles of soluble polypeptides which incorporated ^{3}H-leucine during the pulse at 15° C and 25° C. At both temperatures the radioactivity incorporation is lesser in the group of small polypeptides than in the other groups. At 15° C the small polypeptides show an apparent low rate of synthesis in spite of their being accumulated within the cell as shown by the total profile (fig. 1). For instance, band 2b is negligible in the radioactive profile and band 2a is very low.

Within the group of medium-sized polypeptides, the region of band 5b shows a greater radioactivity incorporation at 25° C than at 15° C. With respect to the group of high molecular weight polypeptides, band 8a shows a similar radioactivity incorporation at 15° C and 25° C, while it is much more abundant in the total profile at 15° C. This would suggest that, as in the case of small polypeptides,

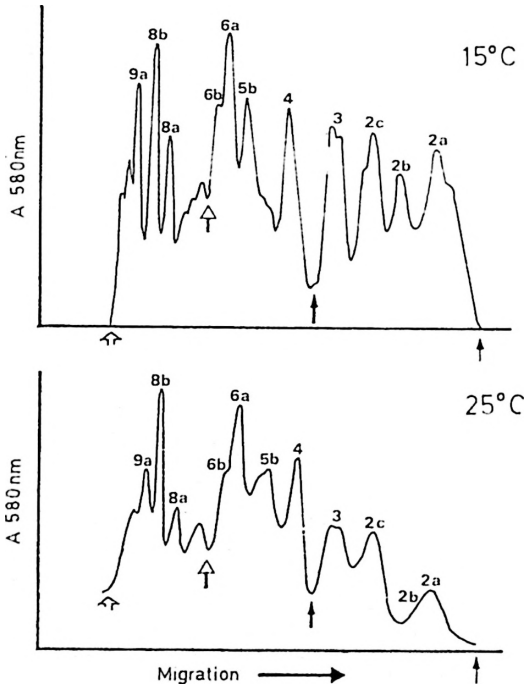

Fig. 1. *Electrophoretic patterns of soluble polypeptides from meristems at 15° and 25° C.* Electrophoresis was carried out in 8 % polyacrylamide gels with 0.1 % SDS. Gels were stained with Coomassie blue and scanned in an Isco Spectrophotometer. Arrows correspond to molecular weight:

⟶ (10,000); ➡ (40,000); ⟶|> (70,000).

We have called each band by the whole number of its molecular weight $\times 10^{-4}$ and a letter as a subindex beginning with the lightest band as previously described (6).

band 8a at 15° C has an apparently low rate of synthesis and is accumulated within the cell. Band 8b, which is very high at both temperatures, shows the greatest radioactivity incorporation of all the bands at 25° C.

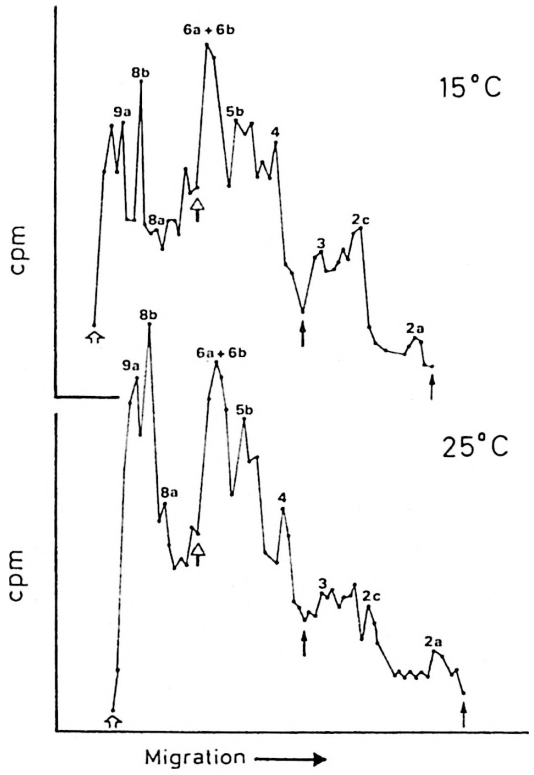

Fig. 2. *Electrophoretic profiles of labelled polypeptides from meristems at 15° C and 25° C.*
Gels scanned, were cut into 0.8 mm slices and the radioactivity estimated. Arrows, numbers and letters have the same meaning as in figure 1.

Discussion

To explain the differences found between both temperatures, it must be remembered that both kinds of meristems are in a steady kinetics state, and that in spite of the differences in cycle time, the cell frequency in cycle phases is apparently constant with temperature (3). On the other hand, the number of meristematic cells per meristem file decreases with a rise in temperature (2), so that the length of the meristem is about 2,000 μm at 15° C and 1,500 μm at 25° C (fig. 3). When the 2nd mm of the root is selected at 15° C, it constitutes a segment with 90 % of its cortical cells in active proliferation. At 25° C, a similar segment would also include about 90 % of cortical cells according to their morphology, but a certain proportion of them would be going through their last division cycle and preparing for the elongation process, while the rest would be non-proliferative (4).

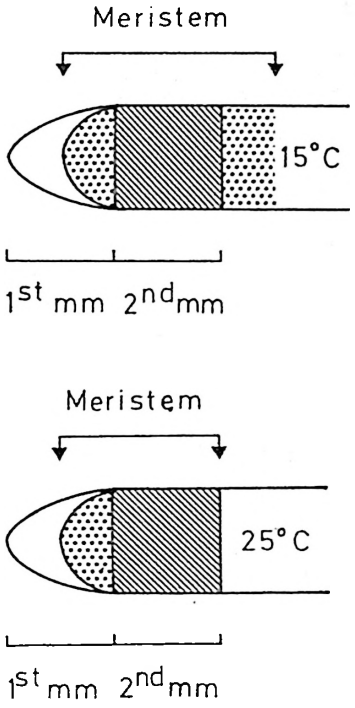

Fig. 3. *Schematic representation of the apical region of the root in* Allium cepa *L.*
It is considered as root meristem the region after the cap where mitosis can be observed.

Examination of the electrophoretic profiles, shows that the number of polypep-

tides is the same in meristems growing at 15° C and 25° C. This constancy in the number of polypeptides occurs not only in meristems in any steady state but is also well preserved in the mature region (6) and in meristems from dormant roots, where growth is negligible (7).

Nevertheless, the quantity and rate of biosynthesis of each polypeptide are characteristic for each of the previously named root segments. Band 8a, for example, in dormant meristems is negligible (7); its maximum content corresponds to the meristem at 15° C; it decreases in the meristem at 25° C and is again negligible in the mature zone, at any temperature (6). In our opinion, band 8a may be considered as an indicator of the proliferative activity of onion root cells.

We suggest that the different polypeptide composition shown by meristems at 15° C and 25° C is due to the different proportion of proliferative cells located in the selected root segment which in turn is conditioned by the growth temperature. In fact the proliferative fraction in the second millimeter is higher at the lower of temperatures studied (2).

Resumen

Por medio de electroforesis en geles de poliacrilamida con SDS se estudia la fracción soluble proteica de meristemos radicales de *Allium cepa* L., tanto respecto al contenido de polipéptidos como a su biosíntesis. En todos los casos se ha seleccionado el segundo milímetro de la raíz. La composición polipeptídica de meristemos radicales que se hallan creciendo en cinética de equilibrio a 15° C es prácticamente idéntica. Las diferencias cuantitativas observadas pueden deberse a la distinta proporción de células proliferantes en los segmentos radicales estudiados, siendo esta proporción mayor a 15° C que a 25° C. Un hecho importante es la existencia de un polipéptido de aproximadamente 80.000 daltones de peso molecular, que puede considerarse como un indicador de la actividad de proliferación en las células de la raíz de *Allium cepa*.

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