Correlation of Structure and RNA Synthesis in the Nucleolus-Organizing Polytene Chromosomes of *Phaseolus vulgaris*

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Abstract. The relationship between RNA synthesis and morphology of the nucleolus-organizing polytene chromosomes in the highly endopolyploid suspensor cells of *Phaseolus vulgaris* has been studied by actinomycin D treatment, temperature lowering, and H²-uridine autoradiography. Actinomycin D and low temperature induce a condensation of the giant chromosomes, particularly of the nucleolus organizers and of the intranucleolar regions of the chromosomes. RNA synthesis occurs in the extended state of the chromosomes, but it ceases in the highly condensed state caused by the treatment of the cells either with actinomycin D or with low temperature.

Introduction

Chromosomal RNA synthesis is known to be more intense in extended chromatin than in condensed chromatin. Particularly the puffs of the salivary gland chromosomes in *Diptera* and the loops of lampbrush chromosomes are well known as loci active in RNA synthesis. On the other hand, the high morphological variability of endopolyploid nuclei in plants did not hitherto allow a functional interpretation of specific structures which were observed. However, as the author already suggested (Nagl, 1969 a, b), the reversible, temperature-dependent structural changes found in the polytene chromosomes of the suspensor cells in *Phaseolus* seem to be an expression of functional differences. These giant chromosomes are in an extended state at optimal temperatures, and in a condensed state at extreme low or high temperatures. The nucleolus organizers and the intranucleolar regions of the chromosomes exhibit these structural modifications in a particularly striking manner (Nagl, 1969 b). Therefore the nucleolus-organizing polytene chromosomes in the suspensor cell nuclei of *Phaseolus vulgaris* were considered to be especially suited to studies involving the relationship between RNA synthesis and structural changes of the chromatin in a plant.
In this study two agents, temperature lowering and actinomycin D were used in combination with H\textsuperscript{3}-uridine autoradiography in order to elucidate the relationship between structure and function of plant giant chromosomes.

**Materials and Methods**

Plants of Phaseolus vulgaris, variety Hild's Marona, were maintained in phyto-chambers at 22--27°C as described earlier (Nagl, 1969a, b). Mature suspensors were taken out of the ovules. They were either directly fixed in ethanol-acetic acid (3:1) or used for further treatments.

The morphology of the giant chromosomes was influenced by lowering of the growth temperature to 8--12°C as in earlier experiments (Nagl, 1969a, b), and by culture of the isolated suspensors in the B5 medium of Gamborg, Miller and Ojima (1968) to which actinomycin D (Serva, Heidelberg) was added at a final concentration of 3, 30, and 300 µg/ml. Controls were cultured without actinomycin D.

RNA synthesis was autoradiographically tested using H\textsuperscript{3}-uridine as a precursor (The Radiochemical Centre, Amersham; Spec. Act. 6.1 Ci/mM and 26.5 Ci/mM). The suspensors were cultured in the B5 medium, to which 10 µCi/ml of the tritiated uridine were added, for 20 and 60 min respectively. Squash preparations were made, some of which were digested with RNase (Serva, Heidelberg; 6 hours at 40°C) as controls. The slides were covered with Kodak stripping film and exposed for 14 days at 4°C. After the development, they were stained with toluidine blue (0.25%, pH 4.0). 10 suspensors originating from 5 plants were used for each experiment.

**Results**

**Actinomycin D treatment**

The polytene chromosomes of *Phaseolus vulgaris* normally have a granular appearance; the nucleolus organizing regions split up into numerous fine threads (Fig. 1), similar to what has been found in the giant chromosomes of *Acricotopus* (cf. Mechelke, 1953). These threads traverse the nucleolus ending at the satellites (Nagl, 1965, 1969b).

A treatment with 3 µg/ml actinomycin D induces a condensation of the polytene chromosomes, particularly of the nucleolus organizers, within 3 hours. After 11 hours of treatment the organizers are compact (Fig. 2), and the intranucleolar threads appear to have condensed. Higher concentrations of actinomycin (30 and 300 µg/ml) generally lead to the same structural changes. However, the action seems to be more rapid and causes a more distinct condensation, especially of the intranucleolar chromatin (Fig. 3). Longer treatment up to 20 hours, using any of the concentrations mentioned, results in condensation of the entire polytene chromosome complement in most cells. The nucleolus organizers