The Reaction to C-Banding of C-Banded Constituents during Mitotic Cycle of *Crepis capillaris*

JUNKO NOGUCHI* AND TOSHIHIDE OHNO**

*Department of Botany, Faculty of Science, Kyoto University, Kyoto 606 ; **Furiura, Chigusa-ku, Nagoya 485

The reaction to C-banding was investigated throughout the mitotic cycle of *Crepis capillaris* (2n = 6): (1) 18-22 C-bodies or C-bands were found during mid telophase and interphase to prophase and metaphase, and also 12-14 at late anaphase to early telophase in the mitotic cycle. Fewer C-bands in late anaphase to early telophase were due to the absence of minute bands; (2) large and medium sized C-bands were strongly stained by Giemsa, while small and minute bands stained palely. It is suggested that in *Crepis capillaris* the difference of color in C-banded segments following Giemsa staining is referable to the amount of constitutive heterochromatin rather than to the difference in the condensation and decondensation; (3) the size of C-bodies changed during telophase to interphase and prophase. It is inferred that the extent of C-bodies is regulated by both the length of DNA sequences of constitutive heterochromatin and the amount of proteins combined with C-banded DNA. It was shown that the reaction to C-banding is neither due to the differential condensation of chromatin nor to a higher concentration of DNA in the C-banded regions, in the C-banding mechanism as has been suggested so far at least.

Key words: Cell cycle — *Crepis capillaris* — Reaction to C-banding.

Many previous reports have shown that the number and the extent of C-bands in metaphase chromosomes are substantially in accordance with the counts and sizes of C-bodies in interphase nuclei following C-banding treatment: *Anemone* and *Hepatica* (Marks and Schweitzer, 1974), *Tulipa* (Filion, 1974), Rye (Weismark, 1975), *Scilla* (Greilhuber and Speta, 1976, 1978), and *Anacyclus* (Schweitzer and Ehrendorfer, 1976). La Cour (1978) also showed in *Fritillaria* that the stainability of C-bands, as well as their number and size, was also the same between metaphase and interphase. These results suggest that C-banded bodies are stable during the mitotic cycle in number, as well as in size and stainability, i.e., a C-band constantly appears during a cell cycle.

On the other hand, a few reports have provided evidence that the number and morphology of C-bodies in a nucleus change during the mitotic cell cycle, e.g., in *Crepis vesicaria* ssp. *taraxacifolia* (Komatsu and Tanaka, 1978) and in *Liriodendron tulipifera* (Morawets, 1981), and also that the volume of heterochromatin in an interphase nucleus of *Lathyrus tingitanus* is lower than in the metaphase (Verma, 1978). Conclusions drawn so far, however, have been based on indirect proof between nuclei.
stained by Giemsa C-banding and nuclei stained by orcein except for the result of Greilhuber (1979). Furthermore, most of the previous observations have not been made in detail throughout a cell cycle.

The purpose of this paper is to clarify the reaction to C-banding throughout a cell cycle: (1) Do specific chromosome segments in a complement react positively to C-banding throughout the cell cycle? (2) Does the reaction of C-banding qualitatively change during a cell cycle? (3) Does the size of C-banded segments change during a cell cycle? These three points can also be ascertained by the following observations: (1) Is the number of C-banded bodies in a complement constant during a cell cycle? (2) Is the stainability of a C-banded body the same during a cell cycle? (3) To what extent do C-banded bodies change during a cell cycle? The material used in this study is *Crepis capillaris*, which has several large or medium-sized C-bands in the somatic complement that will permit the measurement of changes in the total size of C-banding in a cell cycle.

**Material and Methods**

Achines of *Crepis capillaris* were germinated on moist filter papers in petri dishes in the dark at 20°C. The root tips of seedlings about 5 mm in length on the fourth day were pretreated with 0.002 M 8-hydroxyquinoline for 1.5 hr at 18°C and fixed in 45% acetic acid for 10 to 15 min at 4 to 8°C. The root tips were directly fixed without pretreatment in order to allow the study of anaphase and telophase nuclei. The procedure for the C-banding treatment after an aceto-orcein squash was carried out according to the method of Noguchi and Tanaka (1981). Thus both the C-banding profile and one stained by aceto-orcein of the same cell were obtained.

The size of cells, nuclei, heteropycnotic bodies and C-bodies

The size of a cell and a nucleus was measured as follows: a tracing paper was superimposed on a photograph of an orcein stained cell enlarged 2500 times, the outline of the cell and the nucleus were drawn, and then the areas were measured with an automatic area meter. The number of cells and nuclei drawn was 10-20 per each phase. In order to measure the size of heteropycnotic bodies (or chromocenters) and C-bands in a nucleus, only nuclei showing the maximum banding response were selected, and were enlarged up to 4000 times. Fused C-bodies were separately counted by comparing an orcein specimen with one stained by Giemsa. The size of heteropycnotic bodies and C-bodies in telophase nuclei to interphase and prophase ones were measured in the slides without pretreatment. The measurement of heteropycnotic bodies and C-bodies was done in telophase to interphase and prophase including synthetic phase. The outlines of all heteropycnotic bodies stained by orcein and C-bodies by Giemsa in a nucleus were drawn by the same procedure mentioned above. Furthermore, the sketches were photographed, enlarged 1000 times, and the cut-out areas were measured with an area meter. The number of nuclei measured was 5-10 per each phase by orcein and Giemsa, respectively.