Cytological Studies on *Luzula* Chromosome VI. Migration of the Nucleolar RNA to Metaphasic Chromosomes and Spindle

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The nucleolus, as a spherical body, lacks continuity during the life cycle of the cell; it usually disappears from view at late-prophase and is reformed at telophase. Many earlier investigators¹⁾ asserted that in prophase the nucleolar material moves on chromosomes²⁾, and in telophase it reappears from chromosomal matrix or pre-nucleolar material which is derived from the peri-chromosomal plasm appearing as a superficial coating on the early telophase chromosome³⁾.

The present investigation, using autoradiographic techniques, was undertaken in an attempt to throw light on the problem of the behaviour of the nucleolar material during mitosis, particular attention being paid to its fate after the nucleolar disappearance during late-prophase.

Material and Methods

Root meristematic cells of *Luzula purpurea* were used. *Luzula purpurea* is known to have marked cytological characteristics such as low chromosome number $n=3$, large chromosome, large nucleolus and lack of constriction⁴⁾. The coiling cycle of mitotic chromosomes can clearly be traced during the course of mitosis. The process of disappearance of the nucleolus at late-prophase can also be traced.

Tritiated cytidine (cytidine- H^3) was used as a precursor for RNA labeling. Seedlings grown in the culture medium (Hoagland's mineral nutrient solution) were treated with cytidine- H^3 ($2.2 \mu\text{C}/\text{ml}$) mixed with unlabeled thymidine ($5\sim 10 \mu\text{M}$) for 10 min.⁵⁾ After this pulse labeling the roots were collected either immediately or after 5~60 min. cultivation in the culture medium. Root tips were smeared and gently squashed on slides. The smeared roots were fixed with acetic-alcohol (1:3) solution. The slide was covered with Kodak AR-10 autoradiographic stripping film after removal of the cover slip by the dry-ice method⁶⁾. The film was exposed for 1 week to 3 months. All preparations were stained with pyronin-methylgreen after the photographic processing. The method for RNA extraction was reported in a previous paper.⁷⁾

Results and Discussion

Incorporation of cytidine- H^3 into chromosomes completely ceased at mid-prophase⁷⁾. In nucleolus, however, there is a slight increase of incorporation of

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cytidine- H^3 at early-prophase as compared with that observed at interphase (Fig. 1-a). Incorporation of cytidine- H^3 slightly declined at mid-prophase, and abruptly declined at late-prophase, i. e., just before the nucleolus disappeared. No incorporation of cytidine- H^3 was observed in metaphasic and/or anaphasic nuclei which were fixed immediately after the treatment of cytidine- H^3 for 10 min.

Fig. 1. Autoradiographs of interphase and mitotic nuclei.

- a. Interphase (top and bottom) and early-prophase (middle) nuclei fixed immediately after the treatment with cytidine- H^3 for 10 min.
- b. Late-prophase cell fixed immediately after the treatment with cytidine- H^3 for 10 min. The radioactive RNA is evenly distributed over the nucleus and cytoplasm.
- c. Metaphase cell treated with cytidine- H^3 for 10 min. and further cultivated in radioactivity free medium for 30 min. The radioactive RNA appears on all chromosomes.
- d. Autoradiograph shows the same metaphase after subsequent treatment with 5 per cent perchloric acid at 4° for 24 hrs.

Labeled metaphase nuclei appeared in cells which allowed further culture in radioactivity free medium at least for 30 min. after the treatment with cytidine- H^3 for 10 min. Fig. 1-c shows the heavily labeled chromosomes which were observed in metaphase nucleus. Fig. 2-a shows the labeled chromosomes and spindles seen in metaphase nuclei which were cultured in radioactivity free medium for 34 min. after the treatment with cytidine- H^3 . In these nuclei, the completed spindles were heavily labeled.

Fig. 2. Autoradiographs of mitotic nuclei.

- a. Autoradiograph of metaphase nuclei (side view), showing silver grains on chromosomes and spindle bodies. Cells were treated with cytidine- H^3 for 10 min. and further cultured in radioactivity free medium for 34 min.
- b. Autoradiograph of anaphase nucleus, showing silver grains on disjoined chromosomes and half-spindle. Cell was treated with cytidine- H^3 for 10 min. and further cultured in radioactivity free medium for 40 min.

Labeled anaphase nucleus was observed in cells which were cultured in radioactivity free medium beyond at least 30~40 min. after the treatment with cytidine- H^3 for 10 min. Fig. 2-b shows anaphase cells which are labeled in both disjoined chromosomes and half-spindle.

It may be, therefore, assumed that the labeled RNA in both chromosomes and spindle from metaphase to anaphase shifted from the labeled nucleolar RNA into which cytidine- H^3 was incorporated before cessation of RNA synthesis in the nucleolus before mid-prophase. This assumption is supported by the observation on prometaphase nucleus, especially nuclei just after the nucleolar breakdown. Labeled RNA, immediately after the nucleolar breakdown, dispersed over the nucleus and into the cytoplasm. It was, however, noticed that some labeled RNA locally associated with the chromosomes (Fig. 1-b).

All labeled RNA was easily removed by the treatment with perchloric acid (Fig. 1-c, -d).

Cytological trace of the nucleolar RNA during prophase in mitosis has been made

by many authors in various materials. Kaufmann, McDonald and Gay⁸⁾ considered from their studies using RNase that accumulation and subsequent dispersion of chromosomal RNA take place, correlated with the appearance and disappearance of the nucleolus during the course of mitosis. Jacobson and Webb²⁾ found that ribonucleoprotein began to accumulate on chromosomes at late-prophase. These reports suggest that nucleolar RNA or ribonucleoprotein migrates to metaphase chromosomes during prophase.

Meanwhile, recent advance in autoradiographic studies enriched our knowleges on the fate of nucleolar RNA during the course of mitosis. Woods and Taylor⁹⁾ elucidated from the results using cytidine-H³ as a marker for RNA labeling that, when the nucleoli disappeared in prophase, labeled nucleolar RNA apparently became associated with the prophase chromosomes, but they did not believe that the nucleolar RNA remained associated with chromosomes for long. Woodard, Rasch and Swift¹⁰⁾ have obtained such results that there was a sharp increase in the amount of chromosomal and nucleolar RNA and protein in very early interphase. This seems to be difficult to reconcile with the preceding results. La Cour¹¹⁾ has confirmed, from the study using adenosine-H³ as a precursor for RNA, that the labeled precursor was incorporated in the nucleoli during prophase and that aggregation of labeled material appeared on the chromosomes in the vicinity of nucleolus when it disappeared in later stages of prophase. In the present study, the migration of the nucleolar RNA to metaphasic chromosomes and spindle was confirmed. Although this should again be checked using other materials and better geometry conditions, at present every experiments seem to indicate that the chromosomal RNA in metaphase and the spindle RNA¹²⁾ as well as the spindle protein¹³⁾ are synthesized before cell division or in very early stages of cell division.

Summary

Using cytidine-H³ as a marker for RNA synthesis and behaviour, the behaviour of the nucleolar RNA after the nucleolar dissolution was traced. Mitotic cells in root tips treated with cytidine-H³ for short period (10 min.) were observed immediately after the treatment or after 5~60 min. cultivation.

No incorporation of cytidine-H³ was observed in metaphasic and/or anaphasic chromosomes and in the spindle body. Labeled anaphase chromosomes and spindle were observed in cells which were cultured in radioactivity free medium for 30~40 min. after the treatment with cytidine-H³ for 10 min.

It has become clear that the nucleolar RNA moves on metaphasic chromosomes during the late-prophase or pro-metaphase and associates with metaphasic chromosomes. It is also clear that the nucleolar RNA seems to be distributed in the spindle body, and in the cytoplasm after the nucleolar dissolution.

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摘 要

草 薙 昭 雄 : *Luzula* 染色体の細胞学的研究 VI. 仁-RNA の中期染色体や紡錘体への移行

Cytidine-H³ で仁-RNA をラベルし、オートラジオグラフ法を用いて核分裂過程、特に仁消失の前後における仁-RNA の行動を追跡した。

Cytidine-H³ の染色体へのとりこみは前期のはじめで完全にとまるが、仁へのとりこみは仁消失の直前、すなわち前期のおわりごろまで持続した。

Cytidine-H³ で 10 分間処理し直ちに固定した材料の染色体や紡錘体はまったくラベルされなかったが、処理後数十分間 H³ を含まない培養液中で培養した材料の染色体や紡錘体は強くラベルされていた。この事実は仁の消失以前にラベルされた仁-RNA が仁の解体後染色体や紡錘体に移行したものと考えられる。また仁解体直後の細胞の観察結果から、仁-RNA の一部は細胞質中にも分散することが明らかにされた。(東京都立アイソトープ総合研究所)