Heterochromatic Segment of *Rumex acetosa* Autosome

Masahide Kurita and Yûzô Kuroki

Biological Institute, Faculty of Science, Ehime University,
Matsuyama, Japan

Received July 27, 1973

According to Graham and Barr (1952), Schwarzacher (1963) and Mittwoch *et al.* (1965), a Barr body or a sex chromatin body was found to vary in number from cell to cell. Zuk (1969) reported that the heterochromatic Y-chromosomes of *Rumex thyrsiflorus* undergo DNA replication without a loss of their heterochromatic coiled state. There were, however, made few references to the state of chromocenter, which was originated from the heterochromatic segment (H-segment) of autosome, under the DNA replication. By the present authors (1971), there were published two studies of the chromocenters in somatic nucleus of *Rumex acetosa*. The chromocenters were variable in number from cell to cell in a single clone, the number of chromocenter being not always coincident with that of H-segment. The Y-chromosome seemed to have a tendency to gather chromatin into a chromocenter-like mass.

It is profitable to elucidate a detailed behavior of H-segment in *Rumex* autosome through an interphase and a prophase. Then, using a clone suitable for study, the present authors have done research on the above behavior.

Materials and method

The materials are three female clones of *Rumex acetosa* having a few H-segments. They are as follows: 1) clone No. 441 (karyotype, $2X+10A^{st}+a^m+a^{sm}$) with one H-segment which is situated on a distal end of one arm of the a^m -chromosome (Fig. 1), 2) clone No. 434 ($2X+A^{sm}+9A^{st}+2a^{sm}$) with one H-segment on the distal end of short arm of the A^{sm} -chromosome (Fig. 7), and 3) clone No. 439 ($2X+2A^{sm}+8A^{st}+a^m+a^{sm}$) with three H-segments, among which two are situated on the short arms of two A^{sm} -chromosomes and the remaining one on one arm of the a^m -chromosome (Fig. 13). Cytological preparations were made from a root tip following the same aceto-orcein squash method as employed in our earlier study (Kuroki and Kurita 1969). A count of chromocenter was carried out on nuclei which were selected for reasons of having no such chromatin mass as was doubtful whether it was a chromocenter or not. These nuclei must be interphase ones, because of their appearance within a cell group which presents many dividing nuclei.

Observation

Clone No. 441: At interphase nucleus, chromocenter varies in number from zero to two (Table 1) and is darkly stained. The nucleus with one chromocenter

(Fig. 3) is often observed. Fairly many nuclei have no chromocenter (Fig. 2), they being lower in frequency than the one chromocenter-bearing nuclei. Two chromocenters are very rarely found in a single nucleus.

At early prophase where each of chromosomes is not yet identified, H-segment (Fig. 5 h) originated from chromocenter is more darkly stained, much thicker, and bolder in outline than the other euchromatic segment of chromosome arm. In some cases, however, the former segment shows the same intensity of staining as the latter segment. The H-segment at early prophase is usually elongated in contrast with the spherical chromocenter at interphase, and seems to become slightly light in staining. When the H-segment shows two chromatids after splitting longitudinally, both the chromatids are connected with each other by a hyaline substance which is not found in the other split segment of chromosome arm. As shown in Table 1, the number of H-segment at early prophase varies from zero (Fig. 4) to two as is counted the chromocenter at interphase.

Table 1. Number of nucleus whose chromocenter or H-segment was strongly confirmed

Clone No.	Stage	Chromocenter or H-segment				
		0	1	2	3	4
441	Interphase	58	101	2	0	0
	Early prophase	10	43	2	0	0
	Mid-prophase	0	100	0	0	0
434	Interphase	10	121	7	0	0
	Early prophase	11	49	1	0	0
	Mid-prophase	0	100	0	0	0
439	Interphase	2*	18	66	101	14
	Early prophase	0	10	26	34	0
	Mid-prophase	0	0	0	100	0

* refer to text.

At mid-prophase, one H-segment per nucleus is found and it is situated on the a^m -chromosome (Fig. 6). The H-segment is always bold in outline, and it is darkly stained in some cases and is as light as the proximal segment of the opposite arm in the other cases.

Clone No. 434: The chromocenter at interphase varies in number from zero (Fig. 8) to two, being darkly stained (Fig. 9). The H-segment at early prophase also varies from zero to two. All the mid-prophases observed show each one H-segment which is situated on the A^{sm} -chromosome (Fig. 12). The observation on this clone is closely similar in result to that on clone No. 441. The interphase nuclei without chromocenter in clone No. 434, however, seem to be lower in frequency than those in clone No. 441.

Clone No. 439: As shown in Table 1, the chromocenter at interphase varies in number from zero to four. The nucleus with three chromocenters (Fig. 17) are often observed.

The H-segment at early prophase varies in number from one to three (Figs.

18–20). Some of these H-segments are found to be darkly stained and the others to be lightly stained. For instance, in some cells with three H-segments, three are all dark, and in the other cells, two are dark and the remaining one is light, and in the still other cells, one is dark and the remaining two are light. There is, however, no case where the three H-segments are all light. As observed in clone No. 441, the H-segment is always thicker, and bolder in outline than the other segment of chromosome arm.

Figs. 1–6. Clone No. 441. 1, metaphase. 2, interphase without chromocenter. 3, ditto with one chromocenter. 4, early prophase showing no H-segment. 5, ditto showing one H-segment. 6, mid-prophase showing one H-segment. arrow: position of H-segment. c: chromocenter. h: H-segment. $\times$ ca. 1600.

Figs. 7–12. Clone No. 434. 7, metaphase. 8, interphase without chromocenter. 9, ditto with one chromocenter. 10, early prophase showing no H-segment. 11, ditto showing one H-segment. 12, mid-prophase showing one H-segment. $\times$ ca. 1600.

At mid-prophase, there are recognized three H-segments among which two are situated on the two A^{sm} -chromosomes and the remaining one on the a^m -chromosome (Fig. 21). The three H-segments are always bold in outline. Each of them, however, is darkly stained in some cases and is lightly stained in the other cases.

Between the two H-segments on the homologous A^{sm} -chromosomes, the difference in staining is found (Fig. 21) or not.

Discussion

As already mentioned, clone No. 441 has only one H-segment on the a^{sm} -chromosome (Fig. 1) and No. 434 also has only one on the A^{sm} -chromosome (Fig. 7). In both the clones, however, there were sometimes found interphase nuclei without chromocenter (Figs. 2 and 8), together with those showing one chromocenter (Table 1). This denotes that the chromocenter originated from H-segment of auto-

Figs. 13–21. Clone No. 439. 13, metaphase. 14, interphase without chromocenter. 15–17, ditto with one, two, three chromocenters respectively. 18–20, early prophase showing one, two, three H-segments respectively. 21, mid-prophase showing three H-segments, two (middle, right) of them being darkly stained. $\times$ ca. 1600.

some is wholly despiralized during the DNA replication in some cases. Thus, the report of Back (1969) must be supported. It is, however, not denied that the DNA replication with a heterochromatic coiled state is undergone in the other cases as noted by Žuk (1969). As known from Table 1, the chromocenter of clone No. 434 seems to be stabler in condensed state than that of clone No. 441. The clone No. 439 has three H-segments (Fig. 13). In this clone, only two interphase nuclei were found to have no chromocenter (Fig. 14), besides many nuclei with one to four chromocenters. The two nuclei were recognized through the observation on many cytological preparations some of which were not used for counting of nuclei with

chromocenter. Therefore, in this clone the interphase nuclei without chromocenter are sure to be very rare or rather to be almost zero in number, compared with the other nuclei. This denotes, on one hand, that all the three chromocenters were very rarely overlapped at a short period of despiralized state under the DNA replication, and on the other hand that at least some one of three chromocenters almost always undergoes the DNA replication with the heterochromatic coiled state. There are fairly many cases where, out of three chromocenters, one or two despiralize wholly.

At early prophase in three clones studied, the H-segment is not always stained more darkly than the other euchromatic segment of chromosome arm. But the former is always thicker and bolder in outline than the latter. The H-segment similar in staining to the other segment is highly probable to have been originated from the chromocenter-forming segment which lost a heterochromatic coiled state under the DNA replication.

At mid-prophase in three clones, the H-segments are always bolder in outline than the other euchromatic segments, especially the proximal segment connecting with the H-segment and the distal segment of arm without H-segment. The H-segment is not always darkly stained (Fig. 21) as found at early prophase. The lightly stained H-segment may have been originated from the chromocenter-forming segment which lost the condensed state under the DNA replication as mentioned already. In clone No. 439 with three H-segments, each of them is darkly stained in some cases and is lightly stained in the other cases. The staining is sometimes different even between the two H-segments situated on the two homologous Asm-chromosomes (Fig. 21). This denotes that an intensity of staining is not constant for a single H-segment, and the dark or the light staining of H-segment may be due to whether it took a figure of chromocenter under the DNA replication or not.

As mentioned above, the variation was found in number of chromocenter and then in staining of H-segment. It is probably due to a delicate condition in nucleus whether a chromocenter loses its condensed state during the DNA replication or not. The chromocenter figure may be not constant for a single H-segment.

As mentioned already, in the three clones some interphase nuclei have no chromocenter. Some of early prophase nuclei show a lightly stained H-segment which is thick and bold in outline, and all the mid-prophase nuclei present each the fixed number of H-segments which are bold in outline. Then, it is considered that the H-segment taking no chromocenter figure under the DNA replication is more rapidly spirialized than any other euchromatic segment through early- and mid-prophase.

In each clone, there were found a few nuclei in which the chromocenter or the H-segment number is more than the H-segment number specific for a clone by one. It is considered that any proximal segment of euchromatic arm appears rarely to be chromocenter from an unknown cause.

Summary

A study was made on H-segments of autosomes in female clones of *Rumex*

acetosa. Chromocenters at interphase as well as H-segments at early prophase are variable in number, in contrast with the H-segments at mid-prophase where their number is constant to a clone. It was considered that the chromocenter undergoes the DNA replication with a heterochromatic coiled state in some cases and without the coiled state in the other cases. At early- and mid-prophase, each of the H-segments is darkly stained or is lightly stained as an euchromatic segment is. The dark H-segment must have been originated from the chromocenter kept through an interphase, and the light H-segment from a chromocenter-forming segment which lost the coiled state under the DNA replication. The H-segment taking no chromocenter figure under the replication seems to be more rapidly coiled than an euchromatic segment through early- and mid-prophase.

Literature cited

- Back, F. 1969. X chromosome activity in prophase. Suppl. Heredity **24** (Chrom. Today 2): 116-120.
- Graham, M. A. and Barr, M. L. 1952. A sex difference in the morphology of metabolic nuclei in somatic cells of the cat. Anat. Rec. **112**: 709-718.
- Kurita, M. and Kuroki, Y. 1971. Chromocenter in *Rumex acetosa* nucleus. Bot. Mag. Tokyo **84**: 18-23.
- Kuroki, Y. and Kurita, M. 1969. Karyotypes of *Rumex acetosa* L. Mem. Ehime Univ., Sci., Ser. B **4**: 41-49.
- and — 1971. Chromocenter number in *Rumex acetosa* L. Japan. J. Genetics **46**: 295-300.
- Mittwoch, U., Lele, K. P. and Webster, W. S. 1965. Relationship of Barr bodies, nuclear size and deoxyribonucleic acid value in cultivated human cells. Nature **205**: 477-479.
- Schwarzacher, H. G. 1963. Sex chromatin in living human cells *in vitro*. Cytogen. **2**: 117-128.
- Žuk, J. 1969. Analysis of Y chromosome heterochromatin in *Rumex thyrsiflorus*. Chromosoma (Berl.) **27**: 338-353.
-