SHORT PAPER

Achiasmate meiosis in the *Fritillaria japonica* group II. Formation of synaptonemal complexes in microsporocytes

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ABSTRACT

An achiasmatic meiosis is observed in microsporocytes of *Fritillaria japonica* group, in which paired chromosomes devoid of chiasmata are continuously maintained through meiotic prophase. Experiments were aimed at examining whether the chromosome pairing is associated with normal synaptonemal-complex (SC) structure. Examination in the electron microscope of pachytene cells showed that the SC was present in all cross-sections through the chromosomes, and the SC seemed to be normal in appearance and dimension.

Two distinctive events in typical meiosis are synapsis and crossing-over between the homologous chromosomes. These events are generally accepted to occur during the respective zygotene and pachytene stages, and their occurrences assure the chiasmate condition which is required to maintain the bivalents through prophase for regular segregation of the homologous chromosomes. However, several types of achiasmate meioses found in various kinds of animals adequately showed that the bivalents are not always sustained mechanically by the chiasmata alone (cf. Table 4 in John and Lewis 1965). On one type of these achiasmate meioses, the paired chromosomes devoid of chiasmata are continuously maintained by some "side-by-side" or lateral association until metaphase I. In higher plants the achiasmate meiosis has been found to be confined to microsporocytes of the *Fritillaria japonica* group (Liliaceae), in which the paired chromosomes are sustained by the lateral attraction alone (Noda 1975).

The maintenance of associated chromosomes raises a number of interesting questions, but before the association can be meaningfully interpreted, it is necessary to establish whether the occurrence of the persistent association of homologues is due to pairing associated with normal synaptonemal complex structure (SC) or not. Since the SC is the only well ordered structurally

unique element thus far found between synapsed chromosomes, we examined its presence in zygonema-pachynema microsporocytes of this species.

The bulbous of *Fritillaria koidzumiana* Ohwi which is included into the *F. japonica* group were collected from two natural populations at Shitanomyo, Yatsuo, Toyama Prefecture, and in Mt. Iwo-zen, Kanazawa, Ishikawa Prefecture. The somatic chromosomes were observed in root-tip cells which were prepared by use of aceto-orcein squash method. All the plants from two populations examined were found to have $2n=24$ chromosomes with or without the small telocentric B chromosome, B_2 . The karyotype was well consistent to that of the plants observed in various local populations of this species (Noda, 1968). Meiotic observations in the microsporocytes under a light microscope were made according to the schedules described in Noda (1975). The achiasmate meiosis was confirmed in the plants from both populations. For electron microscopy, the living anthers in the plants from Shitanomyo were exclusively used after determining of the meiotic stages. The anthers were cut into 2 mm cubes using a clean razor blade in a drop of 3% glutaraldehyde in cacodylate buffer at pH 7, and quickly transferred to a large volume of the same solution at 4°C for 12 h. They were then post-fixed in 2% osmium tetroxide in cacodylate buffer for 3 h, dehydrated and embedded for ultrastructural studies in spurr resin. Thin sections were stained with uranyl acetate and lead citrate, and viewed with a Hitachi HU-11D-S electron microscope. At least 10 nuclei were examined for each anther.

When viewed under a light microscope, the pachytene cells showed full, or almost full, synapsis of homologous chromosomes along the entire chromosome length (cf. Fig. 1 in Noda 1975). At the later stages, the bivalents are charac-

Fig. 1. Meiosis in microsporocytes with 12_{II} in *F. Koidzumiana* from Mt. Iwo-Zen. 1a. Late post-pachytene, showing twelve diffused bivalents. 1b. Metaphase I. All the twelve bivalents devoid of chiasma are maintained by parallel association. 1a. $\times 500$. 1b. $\times 560$.

Fig. 2. Electron micrographs of pachynema cell of *F. koidzumiana*. 2a. Numerous segments of SC embedded in the masses of chromatin. 2b. A typical SC structure with two lateral elements and medial complex. 2a. $\times 9300$. 2b. $\times 60000$.

terized by the absence of opening-out between paired segments, giving them the appearance of being composed of a single element (Fig. 1a). At metaphase I no chiasma is usually found in the bivalents which revealed parallel apposition of the homologous chromosomes (Fig. 1b). Further detailed descriptions of the bivalent structure and fate of the structure can be seen in a previous paper (Noda 1975).

Examination in the electron microscope of pachytene cells from another anther of the same bud as was used for light microscopy showed that the SC was present in all cross-sections through the chromosomes (Fig. 2a). Structurally, the SC is embedded in most masses of chromatin, and recognized as consisting of two thick dense lateral elements separated from each other by a less dense medial region in which lies a medial complex (Fig. 2b). Arising from the lateral components, the chromatin appears to radiate into surrounding nucleoplasm. The SC appeared to be normal in appearance and dimension, compared with those for *Lilium* (Moens 1968 a, b).

Thin sections of pachytene cells had numerous pieces full of SC. The relative abundance of SC structure among pachytene nuclei from different buds showed no significant difference in mean number of SC segments per section. Although evidence in support of full pairing is absent, the abundance of SC in these cells must have been maximal or nearly so. From these observations, it is evident that chromosome pairing for microsporocytes of this fritillary is associated with normal SC structure.

In typical meiosis, the SC disassociates from the bivalents at diplonema, and subsequently the maintenance of bivalents depends upon chiasmata up to anaphase I separation. As shown in some mantid (Gassner 1969), however, the SC in achiasmatic meiosis may remain coincident with the chromosomes until the bivalents break transversely during anaphase I. Attempts to demonstrate the precise stage of the disassociation of the SC led to equivocal results, because of restriction in satisfactory abundance of bulbs used as material.

The physical presence of the SC between synapsed chromosomes is a necessary condition to implicate this structure in the process of crossing-over, but it is not a sufficient one. The inference that pairing associated with normal SC structure does not assure crossing-over has been demonstrated in a number of study. This study also shows the apparent normalcy of SC structure in paired regions as viewed by electron microscopy, but the ineffectiveness of the pairing manifested in the achiasmate is not documented. The possibility that the achiasmatic behavior in microsporocytes of this plant derives from a genic defect is unlikely because of typically chiasmatic meiosis in macrosporocytes (embryo-sac mother cells) of this species (Noda 1970, 1975). The pairing in microsporocytes might be also effective on crossing-over. Even if so, however, the prolonged presence of the SC or the persistence of biva-

lents by the lateral attraction during late prophase and metaphase I suppresses the disjunction of homologues, thus rendering the presence of chiasmata undetectable. Alternatively, it would also imply that pairing is ineffective in promoting chiasma formation. Precocious release of the SC might be a factor giving rise to achiasmate. These issues remain open, and they are ones which can be resolved experimentally.

REFERENCES

- JOHN, B. and LEVIS, K. R. (1965) The meiotic System. In *Protoplasmatologia*, 6, Fl. Springer-Verlag, Wien and New York.
- GASSNER, G. (1969) Synaptonemal complexes in the achiasmatic spermatogenesis of *Bolbe nigra* Giglio-Tos (Mantoidea). *Chromosoma* **26**, 22-34.
- NODA, S. (1968) Cytology in the genus *Fritillaria*. II. Karyotypes and B-chromosomes in *F. japonica* var. *japonica* and var. *koidzumiana*. *Bull. Osaka Gakuin Univ.* **10**, 127-141. (In Japanese with English summary).
- NODA, S. (1970) Meiotic process in EMCs of *Fritillaria amabilis*. *Bull. Osaka Gakuin Univ.* **14**, 107-116. (In Japanese with English summary).
- NODA, S. (1975) Achiasmate meiosis in the *Fritillaria japonica* group. I. Different modes of bivalent formation in the two sex mother cells. *Heredity* **34**, 373-380.
- MOENS, P. B. (1968a) The structure and function of the synaptonemal complex in *Lilium longiflorum* sporocytes. *Chromosoma* **23**, 418-451.
- MOENS, P. B. (1968b) Synaptonemal complexes of *Lilium tigrinum* (triploid) sporocytes. *Can. J. Genet. Cytol.* **10**, 799-807.