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Rate of DNA Synthesis during Meiotic Prophase in Lily Microsporocytes in the Presence of Deoxyadenosine and Nalidixic Acid

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The rate and period of DNA synthesis during meiotic prophase were examined using lily microsporocytes. Meiocytes at the early leptotene stage were cultured for discrete periods in the presence of inhibitors of DNA synthesis, deoxyadenosine and nalidixic acid. Deoxyadenosine, which arrests meiotic development at the early zygotene stage, markedly suppressed DNA synthesis to 35% of control at 2 mM. Nalidixic acid simply reduced the rate of DNA synthesis, resulting in prolongation of the synthetic period. The relevance of DNA synthesis to meiotic development is discussed.

Key words: Deoxyadenosine — DNA synthesis — *Lilium longiflorum* — Meiosis — Microsporocytes — Nalidixic acid.

The meiotic prophase consists of complex processes, involving synapsis and crossing over between homologous chromosomes. Synapsis occurs in the zygotene stage and crossing over in the pachytene stage of the meiotic prophase. Simultaneously, a small amount of chromosomal DNA is synthesized during the zygotene and pachytene stages (Hotta et al. 1966, Hotta and Stern 1971). The former, referred to as zygotene DNA, is synthesized typically in a semiconservative manner and represents the delayed replication of a part of the chromosomal DNA, whereas the latter has the characteristics of the repair DNA closely associated with crossing over (Howell and Stern 1971, Hotta and Stern 1976, 1984).

Zygotene DNA synthesis is believed to have significant roles in meiosis, especially in the pairing of homologous chromosomes and further meiotic development (Ito et al. 1967, Roth and Ito 1967). Electron microscopic studies have revealed that zygotene DNA synthesis takes place close to or within the SC (Kurata and Ito 1978), a unique structure associated with chromatin during synapsis. The inhibition of zygotene DNA synthesis induces the arrest or delay of meiotic development at early prophase and also interferes with the formation of SCs (Roth and Ito 1967). Thus, there is little doubt that zygotene DNA is a factor in the pairing process, but the question remains of what role zygotene DNA plays in the meiotic prophase. To answer this question, we analyzed the cytological behavior of chromosomes in lily microsporocytes cultured with various inhibitors of DNA synthesis. Thus far, the cytological effects of three types of inhibitors have been described: AdR, HU and NA (Sakaguchi et al. 1980, Takegami and Ito 1982, Takegami 1983).

Even when DNA synthesis is inhibited markedly, simple suppression of the synthetic rate or prolongation of the synthetic period may not disturb the pairing mchanisms associated with DNA

Abbreviations: AdR, deoxyadenosine; HU, hydroxyurea; NA, nalidixic acid; SC, synaptonemal complex; TdR, thymidine.

synthesis. Once the synaptic event between homologous chromosomes is initiated, further processes of SC formation might be independent of the rate and completion of DNA synthesis. To investigate this possibility, the rate of DNA synthesis in the absence or presence of inhibitors of DNA synthesis at various concentrations was examined using explanted lily microsporocytes.

Materials and Methods

Plant materials and culture methods—Microsporocytes were obtained from anthers of lily, *Lilium longiflorum* var. Georgia. Estimation of the meiotic stage of the cells was based on the correlation between bud length and the meiotic stage (Erickson 1948). Microsporocytes at the early leptotene stage were obtained from buds 12 mm long. A total of 24 cohesive strings of microsporocytes could be extruded from a bud. Most of the strings from individual buds were cultured separately in the presence of AdR or NA, inhibitors of DNA synthesis. The remaing strings were grown as control in culture medium without inhibitors. The procedure used to culture meiotic cells has been described previously (Takegami et al. 1981). To label the DNA synthesized during meiotic prophase, meiotic cells were cultured in medium containing 10 μCi/ml of (methyl-^{3}H)thymidine (spec. act. 40–60 Ci/mmol).

Extraction of DNA—DNA was extracted from the meiocytes by the method of Hotta et al. (1965) with some modifications. Cultured cells were washed several times with 95% ethanol at −20°C. The cells were then suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 0.5 M NaCl, 10 mM EDTA and 2% sodium dodecyl sulfate, and then homogenized in a Potter-Elvehjem teflon-glass homogenizer. The homogenate was heated at 60°C for 10 min. Pronase E (Kaken Chemical Inc. Japan) was added to the homogenate to a final concentration of 250 μg/ml, and the mixture was incubated at 37°C for 5–6 h. After incubation, the solution was deproteinized with chloroform : isoamyl alcohol (24 : 1). Subsequent ethanol precipitation, RNase treatment and deproteinization were performed according to the procedure of Marmur (1961). The purified DNA was dissolved in 0.15 M NaCl–0.015 M sodium citrate to a concentration of 100–200 μg/ml. An aliquot of the labelled DNA (50–100 μg) was precipitated with 5% cold TCA, collected on a GF/C filter (Whatman) and its radioactivity was determined using a liquid scintillation counter.

Expression of synthetic rate—The specific activities of labelled DNA prepared from cells cultured for six days usually ranged from 200 to 220 cpm/μg. Since the time course of DNA synthesis was reproducible, the synthetic rate in the presence or absence of the inhibitors was expressed as a percentage relative to the radioactivity of DNA at six days of culture without inhibitors.

Results

Period of DNA synthesis during meiotic prophase—When microsporocytes in early leptonema were cultured without inhibitors, most of the living meiotic cells went through the first metaphase within eight days of culture with few abnormalities (Fig. 1). Cytological observations showed that the zygotene stage lasts for the first two days and the following three-day period corresponds to the pachytene stage. When microsporocytes in early leptonema were incubated with ^{3}H-TdR, the radioactivity incorporation showed that DNA synthesis during meiotic prophase stopped three days after explantation (Fig. 1). These results are consistent with the cytological observation that meiocytes explanted at the early leptonema still appeared to be in the middle pachytene stage after three days of culture. It has been estimated that the relative amount of zygotene DNA is about 0.3% of the total genomic DNA and that of pachytene DNA is one-third of the zygotene DNA (Hotta and Stern 1976). Since the syntheses of zygotene and pachytene DNA occur successively in spite of their different functions in meiotic processes, the exact distinction between their synthetic periods was not possible, but zygotene DNA synthesis was assumed to have occured

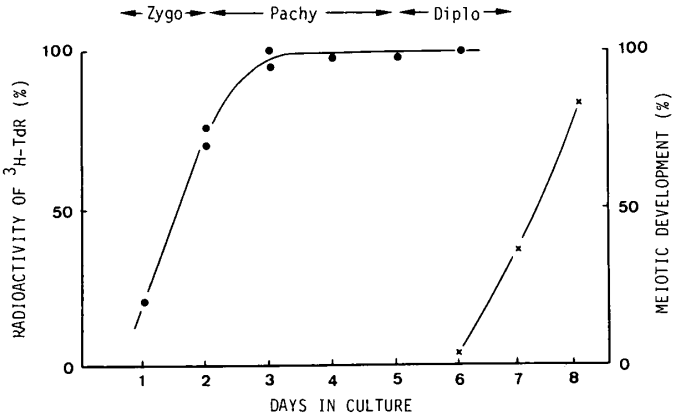

Fig. 1

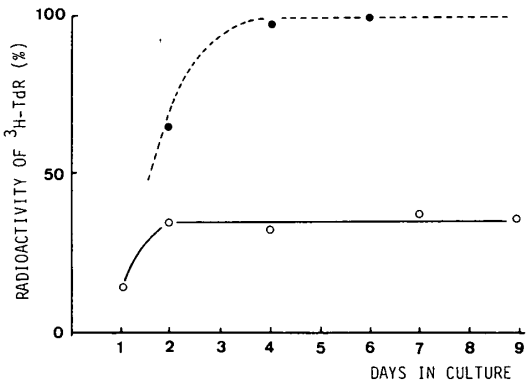

Fig. 2

Fig. 1 Rate of DNA synthesis during meiotic prophase in explanted lily microsporocytes (*Lilium longiflorum*). Meiocytes at early leptonema were cultured for indicated periods in medium containing ^{3}H-thymidine. The radioactivity in DNA (●) is expressed as a percentage of that at six days after explantation. Meiotic development (×) is expressed as the cumlative percentage of cells which passed through the first meiotic division relative to the total cells in the culture.

Fig. 2 Effect of AdR on the rate of DNA synthesis during meiotic prophase in lily microsporocytes explanted at early leptonema. Microsporocytes were cultured in the presence or absence of 2 mM AdR in medium containing ^{3}H-thymidine as described in Materials and Methods. The radioactivity in DNA is expressed as a percentage of that at six days in the absence of AdR. (●), control; (○), 2 mM AdR. The broken line shows the curve of DNA synthesis drawn from Fig. 1.

during the first two days, followed by pachytene DNA synthesis for one day. This means that cells completing DNA synthesis reach metaphase-I four days later.

Effect of AdR on the synthetic rate—Microsporocytes exposed to 2 mM AdR at explantation displayed a characteristic response, a marked suppression of meiotic development. The affected cells stayed in the early zygotene stage during 7–10 days of culture, and the progression of chromosome condensation was also arrested at the early stage of zygonema (Ito et al. 1967). DNA synthesis in the presence of 2 mM AdR was suppressed to 35% of the control (Fig. 2): DNA synthesis reached a plateau at the second day of culture and thereafter no increase was observed. Since the zygotene DNA was synthesized within two days after explantation, DNA synthesized in the presence of 2 mM AdR was a part of the zygotene DNA. Thus we conclude that if part of the zygotene DNA is not replicated, the cells cannot progress through meiotic prophase and are arrested at the early zygotene stage.

Effect of NA on the synthetic rate—Microsporocytes cultured in the presence of 0.1 mM NA exhibited delayed meiotic development. Cells in early leptonema reached metaphase about three days later than the controls, but could progress through prophase without marked damage to their chromosome structure, except for the appearance of univalent chromosomes. Such univalent chromosomes are observed only when cells at late G2 or early leptonema are exposed to NA (Takegami 1983). The univalency is a result of inhibition of SC formation during the zygotene stage, but not of inhibition of chiasma formation itself.

The response of cells exposed to 0.4 mM NA was complete suppression of meiotic development. Consequently, the affected cells stayed in early prophase for ten days or longer. These results are similar to those for cells exposed to 2 mM AdR and also to several kinds of agents that inhibit DNA and RNA synthesis, as described previously (Sakaguchi et al. 1980).

The effects of NA on the rate of DNA synthesis differd from those of AdR. In the presence of 0.1 mM NA, the synthesis slowed down markedly and reached a plateau three days later than

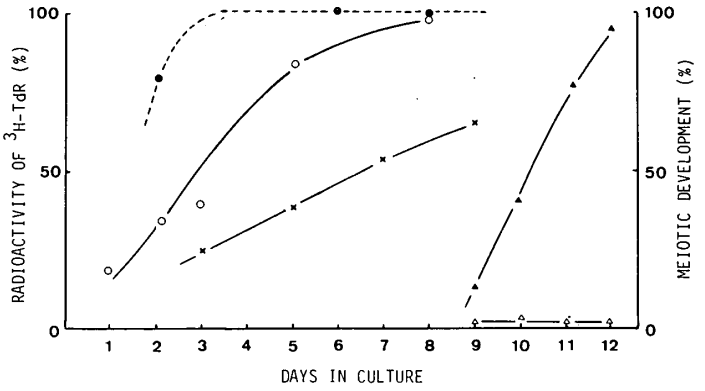

Fig. 3 Effect of NA on the rate of DNA synthesis during meiotic prophase in lily microsporocytes explanted at early leptonema. Cells were cultured for indicated periods in medium containing ^{3}H-thymidine and 0.1 mM or 0.4 mM NA. The radioactivity in DNA is expressed as a percentage of that at six days in the absence of the inhibitor. (●), control; (○), 0.1 mM NA; (×), 0.4 mM NA. The broken line shows the curve of DNA synthesis from Fig. 1. Meiotic development of the microsporocytes is expressed as the cumulative percentage of cells which have passed through the first meiotic division to the total cells in culture. (▲), 0.1 mM NA; (△), 0.4 mM NA.

the control (Fig. 3). In this case, synthesis of both zygotene and pachytene DNA seemed to have been completed, since the treated cells eventually incorporated the tritiated precursor at the same level as the untreated cells. One point to be emphasized is the relationship between the completion of DNA synthesis and prophase development; that is, completion is necessary for further chromosome condensation during meiotic prophase.

Stronger suppression of DNA synthesis occured in the presence of 0.4 mM NA, the amount synthesized during ten days came to only 60% of the control. This was less than the amount of zygotene DNA which should have been synthesized. The main effect of NA on DNA synthesis was simple suppression of the synthetic rate causing prolongation of the synthetic period. The strong suppression of the synthetic rate, as seen with 0.4 mM NA, was sufficient to suspend cells at early prophase for over ten days.

Discussion

The biochemical properties of two types of DNA synthesis during meiotic prophase have been reported in many papers (Hotta et al. 1966, Hotta and Stern 1971, Howell and Stern 1971, Hotta and Stern 1976, 1984). Zygotene DNA synthesis is the semiconservative replication of a part of chromosomal DNA which did not replicate during the premeiotic S-phase, while pachytene DNA synthesis is of repair type associated with genetic recombination (Hotta and Stern 1971, Howell and Stern 1971, Bouchard and Stern 1980). The former has been assumed to be closely correlated with the pairing of homologous chromosomes and further meiotic development (Ito et al. 1967, Roth and Ito 1967); its initiation is necessary for SC formation, and its completion is required for the maintenance of chromosome integrity (Takegami and Ito 1982).

The general response of microsporocytes exposed to inhibitors of DNA synthesis at early leptonema is the arrest or suppression of meiotic development (Sakaguchi et al. 1980). In the arrested cells, SC formation is also inhibited. In the present study, two different types of inhibitors, AdR and Na, were chosen because of their characteristic effects on meiotic development and the synaptic process (Roth and Ito 1967, Takegami 1983). One of the conclusions derived from our experiments is that the arrest or marked suppression of meiotic development by DNA-inhibitory agents is due to the suppression of zygotene DNA synthesis.

When microsporocytes are kept in a state where the zygotena DNA is insufficiently replicated, as was the case with 2 mM AdR or 0.4 mM NA, they cannot progress through meiotic prophase and remain in the zygotene stage. The delay of meiotic development in the cells exposed to 0.1 mM NA is attributed to the decrease in the synthetic rate of zygotene DNA. This decrease results in prolongation of the synthetic period. These results suggest that the completion of zygotena DNA synthesis is an essential factor for meiotic development in lily microsporocytes. Since the zygotene DNA is estimated to account for 0.3% of the total genomic DNA, the replication of 99.9% chromosomal DNA would still be insufficient for the progression of the meiotic cycle.

Serious suppresion of DNA synthesis in microsporocytes inhibits SC formation. However, it is interesting that 0.1 mM NA effectively induced the formation of univalent chromosomes in spite of its weak suppression of zygotene DNA synthesis. The inhibition of SC formation by NA depends on the stage of the cells exposed to the agent: cells in late G2 or early leptonema form univalent chromosomes, but cells in later stages do not (Takegami 1983). This characteristic effect of NA on the synaptic process may reflect its indirect action on chromosome structure which has already been condensed. The disturbance of organized chromosome condensation during the presynaptic process due to NA action may make the alignment of homologous sites within chromosomes impossible. More detailed studies on NA action are necessary to elucidate the synaptic process.

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