The Relation of Chromosome Compaction to DNA Synthesis During Meiosis in *Lilium*

Kiyoharu Oono* and Yasuo Hotta

*Department of Biology, University of California, San Diego, La Jolla, California 92093, USA*

**ABSTRACT.** Three intervals of DNA synthesis in *Lilium* microsporocytes—premeiotic S-phase, delayed zygotene replication and pachytene repair synthesis—were analyzed with respect to chromosome compaction. Chromatin was prepared from isolated nuclei and fractionated into a condensed and a diffuse component. Glutaraldehyde was used in a parallel procedure to crosslink chromatin that might be compacted but unstable in the fractionation procedure. Nuclei in premeiotic S-phase had a much larger fraction of condensed chromatin rescued by glutaraldehyde than those in any other stage. Virtually no DNA replication occurred in this fraction until the last interval of S-phase. Zygote replication occurred mainly in the diffuse chromatin fraction and the distribution remained so after completion of replication. Pachytene repair synthesis occurred mainly in condensed chromatin. Generally, sequences with high C\textsubscript{ot} value fractionated preferentially with diffuse chromatin but the opposite was the case for pachytene nuclei. Digestion with staphylococcal nuclease revealed no significant differences in nucleosome organization of condensed and diffuse chromatin.

The approximate constancy in molecular rate of DNA replication, or fork migration, and the tissue-specific differences in duration of S-phase have led to the suggestion that chromosome compaction might regulate duration by occluding initiation sites. Compacted chromatin would thus function as a passive regulatory agent inasmuch as DNA replication would not occur until a replication fork migrated in from an adjacent region. A more active role might be played by compacted chromatin if decompaction were separately regulated and if it were also the immediate factor for initiating replication (2). However, convincing evidence has yet to be obtained which would directly tie DNA replication to the physical state of chromatin despite the general evidence for late replication in heterochromatic regions (7).

The accessibility of origins for DNA replication and its relation to chromosome compaction is of particular interest for meiosis because of the evidence from several organisms that premeiotic S-phase is considerably longer than the S-phase of other cell types (5). Moreover, nuclei from meiocytes of a number of species display many condensed regions of chromatin during premeiotic interphase, including S-phase (24). It is tempting to attribute the duration of premeiotic S-phase to the prevalence of heterochromatic regions, and we decided to study the problem with *Lilium* micro-

*Present address: National Institute of Agricultural Sciences, Division of Genetics, Hiratsuka, Kanagawa 254, Japan.*

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sperocytes because of their suitability for examining such a relationship. Since microsporocytes are characterized by three distinctive types of DNA synthesis (22), each of these occurring under different conditions of chromosome compaction, our study was addressed to separating condensed and diffuse chromatin at different meiotic stages and to measure the distribution of DNA synthesis among the two fractions. Specifically, we sought to determine whether the transient compaction of chromatin during premeiotic S-phase was related to its attenuation, and whether the much later syntheses during zygotene and pachytene showed any preferential localization among the chromatin fractions.

Much attention has been paid to the relationship between DNA transcription and chromosome compaction (17). Yet, despite the numerous studies of active and inactive chromatin, the major procedural difficulty confronted in this study was finding an unequivocal method for fractionating chromatin into condensed and diffuse components. In this respect, success has only been partial, but enough circumstantial evidence has been obtained to indicate that there is indeed a relationship between chromosome compaction and DNA synthesis. The relationship is such that the attenuation of S-phase in meiocytes could be attributed to a poverty of DNA synthesis in regions of compacted chromatin. However, the results also indicate that the relationship may involve more than a regulation of overall rates of synthesis. It would appear that just as DNA transcription requires an appropriate physical state of chromatin, so does the replication of a particular set of DNA sequences that presumably has a specific function in meiosis.

MATERIALS AND METHODS

The following cultivars of hybrid Lilium were used in this study: Bright Star, Cinnabar, Nutmeg and Black Beauty. Since no significant differences in their behavior were found, the cultivars used in each of the individual experiments will not be indicated. The purchase and handling of lilies have been described earlier (11, 14). Callus cultures were started from young anthers, at least two cell cycles prior to meiosis, and maintained on an agar substrate. For radioactive labelling, however, callus material was transferred to the liquid medium of Murashige and Skoog (18). Callus cultures, and also young anthers directly, were used for analyzing chromatin patterns in somatic cells. Data obtained from young anthers and from callus tissue were essentially the same.

Carrier-free 32P-orthophosphate and 3H-thymidine (SA = 30 Ci/m mole) were purchased from New England Nuclear Corp. (Boston, Mass.).

Isolation of nuclei. Microsporocyte nuclei were isolated as previously described (12). Callus tissues were washed with 0.01M Tris or phosphate buffer (pH, 7.0-7.5), frozen in liquid nitrogen and lyophilized. The dried callus material was pulverized in a blender and then suspended in the glycerin: sucrose medium used in nuclear isolation. The suspension was layered over 10 ml of 1.8 M sucrose which was layered over 10 ml of 2.6 M sucrose. The sample was centrifuged in a Sorvall H-4 rotor at 8,000 rpm for 20 minutes. Nuclei were recovered at the interface between the 1.6 and 2.8 M layers.

Since the use of hypertonic sucrose solutions prior to chromatin fractionation might have effects on chromosome condensation, an alternative but less satisfactory procedure was tested in which hypertonic solutions were omitted. Microsporocytes or callus powder were suspended with the aid of a homogenizer in a solution containing 0.5 M sucrose, 1 mM each of CaCl2, MgCl2 and MnCl2, 0.2 % Triton X-100 and 0.01 M Tris-HCl buffer (pH, 7.5). In the case of microsporocytes, the homogenization was essential to achieve cell breakage. Nuclei were
pelleted by centrifuging the suspension at $1,500 \times g$ for seven minutes. After two resuspensions of the pellet and washing with the sucrose medium, the fraction obtained was highly enriched with nuclei but was appreciably contaminated with cytoplasm, starch grains and cell walls. Since further purification would have required the use of hypertonic media, the nuclear fraction was analyzed as such. It will be seen that the pattern of chromatin compaction was unaffected by the tonicity of the medium used in preparing nuclei.

**Crosslinking of chromatin.** About 1 mg DNA equivalents of nuclei were suspended in two or more ml of 0.2% glutaraldehyde : 0.01 M phosphate buffer (pH, 7.4) and kept at 0°C for 20–30 minutes. The reaction was stopped by adding approximately one-tenth the suspension volume of 10% glycine at 0°C, and centrifuging the mixture for 10 minutes at $1,000 \times g$. The pellet was washed thoroughly with 0.01 M phosphate buffer. This particular schedule of treatment was adopted after testing several crosslinking schedules in order to obtain a fractionation that most closely reflected the *in vivo* pattern of compaction at the different meiotic stages. Technical problems confronted in the search are discussed under Results. Efforts to use formaldehyde in place of glutaraldehyde were unsuccessful.

**Fractionation of chromatin.** The nuclear pellet obtained by either of the methods described was suspended in 0.01 M Tris-HCl buffer (pH 8.0) and washed twice in buffer using $1,000 \times g$ spin to recover the nuclei. About 1 mg DNA equivalent of nuclei was suspended in 10–20 ml of Tris buffer and homogenized with a conventional tissue grinder. The homogenate was layered over 15 ml each of 1.3 M and 1.6 M sucrose in a tube fitted for the SW 27 Spinco rotor and centrifuged for four hours at 25,000 rpm. Although, as expected from the procedure (10), a significant amount of zygotene-labelled DNA is released from the nuclei into the medium, it is recovered in the pellet. The pellet was dispersed in a 15 ml Corex tube with about 2.5 ml of Tris buffer, and sonicated for three minutes in a Sonifier Cell Disrupter (Model W 140 D, Plainview, Long Island, New York) using a small probe and a power output of 25%. The suspension was chilled with an ice bath throughout the sonication. After the sonication, the sample was dialyzed overnight against Tris buffer and centrifuged at $10,000 \times g$ to remove debris. A 1.5–0.5 mg DNA equivalent of chromatin in 1 ml of medium was loaded on a 7.6 to 76.0% linear glycerol gradient in 0.01 M Tris buffer (pH 8.0) and centrifuged in an SW 27 Spinco rotor at 22,500 rpm for 15 hours at 4°C. Fractions were obtained by dripping from the bottom of the tube.

**Purification of DNA.** Standard procedures were used for DNA purification (9). In the case of chromatin that had been digested with staphylococcal nuclease, the digest was made 1 M in sodium chloride by addition of a 5 M solution and treated with pronase as in the regular procedure. The mixture was then deproteinized with chloroform-amyloalcohol, and the aqueous layer dialyzed against distilled water and lyophilized.

**Agarose gel analysis.** DNA equivalents of 0.5 mg of isolated nuclei or chromatin were suspended in 2 ml of 0.01 M Tris buffer (pH 7.5), 5 mM each of CaCl$_2$ and MgCl$_2$, and treated with staphylococcal nuclease (15). The purified DNA fragments were dissolved in water at a concentration of 300 μg/ml. A 50 μl DNA solution was loaded on a 1.4% agarose gel. Details of enzyme concentration and times of digestion are given in the appropriate figure.

**RESULTS**

**Effectiveness of chromatin fractionation.** The method of chromatin fractionation used was essentially the procedure described by Berkowitz and Doty (1). Modifications were introduced to achieve a partitioning of chromatin into condensed and diffuse fractions that reflected as faithfully as possible the cytological cycle of chromatin compaction during meiosis. The solid line in Fig. 1 traces the proportion of condensed to total chromatin through meiotic development as measured by our *in vitro* procedure.
The first point in the curve was obtained from young anthers which include both somatic and microsporocyte precursor cells; the same value was obtained using cultured callus tissues. Chromatin from the microsporocytes themselves showed a progressive increase in proportion of condensed material (as defined by sedimentation behavior) with meiotic development. A peak value in the ratio of condensed/diffuse chromatin may be seen to coincide with Divisions I and II, after which there is a sharp decline. Although it would have been extremely difficult to obtain a quantitative comparison between the fractionation data and the cytological observations, it is evident that the solid curve in Fig. 1 corresponds roughly to the actual cycle of chromosome compaction and decompaction. A significant defect in the curve is the lack of any evidence for appreciable chromosome compaction during premeiotic interphase as would be expected from the prevalence of heterochromatic regions in the premeiotic nuclei (24). Fortunately, a procedure for rescuing the heterochromatin was found (dashed curve, Fig. 1) and its details are described after first evaluating some features of the basic procedure.

Although the method proved useful for a comparative study of chromosome compaction, it was found to be virtually useless for absolute measurements of compacted chromatin. The in vitro partitioning of chromatin was found to be extremely sensitive to time of sonication. With no sonication, all the chromatin sedimented as though it were condensed; with a 5-minute sonication, all the chromatin behaved as though it

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**Fig. 1.** The proportion of condensed chromatin in nuclei from cells at successive stages of microsporogenesis. The data were obtained from sedimentation analysis, an example of which is given in Fig. 2. Other details are given in Table 1 and under Materials and Methods. +G indicates pretreatment of nuclei with glutaraldehyde.
were diffuse. The 45-second period of sonication used by Berkowitz and Doty (1), displayed only small differences between the various stages of meiosis. The 3-minute sonication period which was adopted for this study provided maximum differentiation between meiotic stages. A sedimentation profile, typical of profiles used to construct the curve in Fig. 1, is shown in Fig. 2. Direction of sedimentation is from right to left.

Fractions were pooled into three groups, A, B and C, as shown in the top horizontal line. Curve with solid circles represents chromatin from untreated nuclei of premeiotic cells. The shape of this curve is similar to the one obtained from nuclei in early meiotic prophase except for a small shift of absorbancy from the C to the B region. The curves from glutaraldehyde-treated nuclei (+G) of premeiotic (I) and meiotic prophase (P) cells indicate the much more prominent formation of a third peak in the premeiotic chromatin. Postmeiotic and somatic chromatin profiles are virtually unaffected by the glutaraldehyde treatment.

Since tonicity of the isolation medium affects nuclear volume, and superficially at least, chromosome compaction, we tested the effects of tonicity on the partitioning of chromatin by fractionating nuclei from each of the meiotic stages that had been prepared by two different methods. The results were unambiguous. Nuclei isolated by the hypertonic glycerin : sucrose procedure yielded sedimentation profiles that were indistinguishable from those obtained using nuclei isolated in isotonic sucrose media. In all cases, the critical factor was duration and intensity of sonication.

The lack of correspondence between in vitro fractionation data and the heterochromatic property of premeiotic nuclei was approached by testing methods for cross-linking chromatin. Under the conditions used, formaldehyde was found to be ineffective because it did not enhance the proportion of compacted chromatin at any of the stages shown in Fig. 1. Glutaraldehyde treatment, on the other hand, provided a suitable procedure. Like sonication, the time of treatment was found to be critical since duration of exposure to glutaraldehyde strongly affected sedimentation profile. The 20-minute exposure selected for use increased the condensed fraction slightly at some stages and none at others. However, as shown by the dotted line in Fig. 1, an
emphatic rescue of condensed chromatin occurred at premeiotic interphase. Moreover, the sedimentation profile (Fig. 2) indicated both a qualitative and quantitative response of the chromatin to glutaraldehyde. The pronounced new peak at the heavy end of the gradient (curve I, Fig. 2) was only weakly present in chromatin of cells in meiotic prophase and virtually absent from somatic tissues. Most of the increase in the condensed fraction of meiotic chromatin occurred as a broadening of the intermediate peak. A summary of the quantitative changes which occurred in the fractionated chromatin as a result of glutaraldehyde pretreatment is given in Table 1. The much higher response of nuclei prepared from cells in premeiotic interphase is patent and contrasts sharply with the response of chromatin from somatic interphase cells.

**DNA synthesis in condensed and diffuse chromatin.** Distribution of DNA synthesis between the two chromatin fractions was determined by exposing S-phase, zygotene and pachytene cells to $^3$H-thymidine for intervals ranging from 12 to 24 hours. In the case of S-phase, some 6-hour exposures were also used. Shorter labelling periods of 1–2 hours, though highly desirable, were impractical because of the low specific activities attained. However, since each of the intervals of DNA synthesis studied extends for at least 48 hours (20, 22), the data obtained provides information not only about the net distribution of label for each individual labelling period, but also about

![Distribution of labelled DNA between condensed and diffuse chromatin from somatic, premeiotic, zygotene and pachytene cells. Chromatin was prepared and centrifuged in a glycerol gradient as outlined in Fig. 2. Labelling procedures are described in the text. Each of the fractions collected by dripping from the bottom of the tubes was made 0.2 N in NaOH and heated at 70°C for 20 minutes. The acid precipitate fraction was recovered by making the solution 10% in trichloracetic acid (TCA) and the precipitate washed twice with ethanol. It was suspended in 0.02 M Tris-HCl (pH 7.5), 5 mM MgCl$_2$. A portion was heated in 10% TCA for 20 minutes and the insoluble radioactivity measured. A second portion was precipitated with 5% TCA and assayed for radioactivity. The difference between the two counts was considered to be $^3$H-DNA. The values so obtained corresponded with those obtained by measuring the radioactivity released on treating the preparation with 100 μg/ml of pancreatic DNase. The dotted lines extending beyond 48 hours indicate the probable course of isotope distribution prior to the extensive chromatin condensation in relation to Divisions I and II. This is discussed in the text.](image-url)
any trend in the accumulation of label among the chromatin fractions over the total period of labelling.

Regardless of meiotic stage, the diffuse fraction of chromatin was always preferentially labelled in cells exposed to isotope for up to 12 hours. As shown in Fig. 3, condensed chromatin contained from 0.3 to 0.6 of the radioactivity it would have had if the distribution were uniform. The cumulative distribution of label, however, was found to be different for each of the meiotic intervals analyzed. The trends are evident in Fig. 3. In the case of S-phase replication, the overall trend is simple. The proportion of label in condensed chromatin increases until its distribution becomes uniform. The endpoint is the same for somatic and meiotic cells. The endpoints for the zygotene and pachytene intervals are different from one another and from the S-phase. Zygotene label remains preferentially in diffuse chromatin; pachytene label accumulates preferentially in condensed chromatin. Thus, although the bulk of DNA label, represented by S-phase synthesis, shows the expected trend toward uniform distribution, the small but specialized categories of meiotic DNA synthesis appear to have distinctive relationships to chromosome condensation.

Two aspects of premeiotic DNA synthesis distinguish it from the behavior of somatic cells. Within an individual flower bud, premeiotic DNA synthesis may extend over 3–4 days, during which time the cells are neither randomly phased nor synchronous with respect to the progress of DNA replication (24). Interpretation of the labelling trend is thus somewhat equivocal and should not be compared with the trend observed in the randomly phased somatic cells. Also, it may be seen in Fig. 3 that the distribution of label in meiotic chromatin remains constant between 12 and 24 hours. This characteristic was observed in all six preparations of premeiotic cells so analyzed. Since the specific activity of total DNA approximately doubled between 12 and 24 hours and was unaccompanied by any appreciable change in proportion of condensed chromatin, the constancy in distribution of label might be explained in one of two ways. Synthesis may proceed independently at unequal rates in the two chromatin fractions, or it may proceed at different rates but with a requirement for compacted regions to decondense temporarily during their replication. In the latter case, the rate of labelling in condensed chromatin would be governed by the rate of decompaction and might thus account for the extended premeiotic S-phase, a possibility considered by Callan (5).

Since considerations of the relationship between premeiotic DNA synthesis and chromosome compaction in Lilium microsporocytes should take into account the cytological data on transient heterochromatinization, we checked the radioactivity of condensed chromatin that had been rescued with glutaraldehyde. The sedimentation profiles in Fig. 4 clearly show that the chromatin thus rescued had virtually no radioactivity even after a 20-hour period of exposure. Labelling on the heavy side of the diffuse chromatin peak is assignable to the original fraction of condensed chromatin; the profile of that fraction is displayed by the solid curve in Fig. 2. It is particularly significant that after glutaraldehyde treatment, only one-third of the chromatin is recovered in the diffuse fraction (Table 1), but calculations from the data in Table 1 and Fig. 3 indicate that 80–85% of the DNA radioactivity is housed in that diffuse fraction. To the extent that recovery of condensed chromatin by the glutaraldehyde method reflects the transient heterochromatinization observed cytologically, the correlation between DNA replication and the diffuse state of chromatin is striking.
If the correlation is real, it lends strong support to the view that chromosome compaction is a key factor in the attenuation of the premeiotic S-phase. The steep rise in radioactivity of the condensed chromatin that is recovered without glutaraldehyde treatment after a 48-hour labelling period (Fig. 3) may be due to the more stable heterochromatin that is generally associated with late labelling in somatic tissues (16).

Unlike the S-phase, the timing of DNA synthesis during meiotic prophase has no obvious association with chromosome compaction. The DNA replicated during zygotene (Z-DNA) is the last component of the genome to be replicated, but it is evident from Fig. 3 that only a small fraction is present in condensed chromatin. At the end of a 48-hour labelling period only 25% of the total Z-DNA is present in the condensed fraction; glutaraldehyde has no appreciable effect on the distribution. Since Z-DNA can be identified by its distinctive buoyant density (22) it was possible to follow its distribution after termination of zygotene, but no significant change was detected until major compaction of chromatin began in association with diakinesis and later meiotic stages. By contrast, the DNA labelled during pachytene, a repair synthesis of endogenously nicked DNA (13), accumulates preferentially in the condensed chrom-
The data were obtained from sedimentation analyses such as those shown in Fig. 2. The numbers for each of the stages are based on 4-6 separate sedimentation runs. Deviations from the values shown were no greater than 5% for any individual determination. Fractions collected from the gradients were pooled into groups A, B and C as indicated in Fig. 2. “A” is referred to as the “heavy peak” and “B” as the “intermediate peak.” A and B combined have been designated as the fraction of condensed chromatin. I and II indicate methods of nuclear isolation whether hypertonic (I) or isotonic (II). “Young anthers” include both premeiotic and adjacent somatic cells. Details of glutaraldehyde treatment are given under Materials and Methods.

The proportion of condensed chromatin and the specific radioactivity of such chromatin both increase as cells progress through the pachytene stage (Figs. 1 and 3). It may be calculated that the combined effect of the two events is a localization of 60-70% of the pachytene label in the condensed chromatin fraction.

C₀t characteristics of DNA in condensed and diffuse chromatin. It has been shown in C₀t analyses of chromatin-DNA from various animal tissues that the more rapidly reassociating DNA fractions are preferentially concentrated in condensed chromatin (17). A similar distribution was observed for total Lilium DNA as measured by absorbancy at 260nm or by radioactivity incorporated during meiotic or somatic S-phase (Fig. 5). The differences in reassociation kinetics are small but real. Chromatin from meiocytes is in this respect indistinguishable from somatic chromatin, plant or animal.

Zygotene-labelled DNA consists of single or few copy sequences (20) and, as expected, no major difference was found between condensed and diffuse chromatin with respect to the C₀t characteristics of Z-DNA. The situation was found to be different, however, in the case of pachytene-labelled DNA of which 70-80% of the sequences are moderately repeated and reannealed at a C₀t value of less than 10⁴ mole sec./liter (19). As already discussed, pachytene label is concentrated in the condensed fraction of chromatin and thus conforms to the general rule that condensed chromatin has the higher proportion of repeated sequences. Analysis of the separated condensed and diffuse fractions nevertheless revealed deviations from the rule (Table 2). The more rapidly reassociating DNA label was twice as concentrated as the more slowly reassociating one in the condensed chromatin, but four times as concentrated in the diffuse chromatin. This pattern of preferential localization of repeated sequences in diffuse chromatin is contrary to the behavior of the genome as a whole. Most probably, the distribution relates to the function of these sequences in the meiotic process. It is difficult to provide a meaningful interpretation of the distribution inasmuch as we still lack an understanding of how the moderate repeats might contribute to the

<table>
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<th>Stage</th>
<th>Chromatin in condensed fraction (%)</th>
<th>Untreated</th>
<th>Treated</th>
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<td></td>
<td></td>
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<td>Young anthers</td>
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TABLE 1. EFFECT OF GLUTARALDEHYDE TREATMENT ON CHROMATIN FRACTIONATION
Distribution of radioactivity was calculated on the basis of a 45/55 ratio of condensed/diffuse chromatin (Fig. 1). The fraction of radioactivity in condensed chromatin = 0.45 ± 1.35 = 0.61 (Fig. 3).

Radioactivity distribution among DNA C_{0t} fractions was determined from Fig. 5. Sixty-five percent of DNA labelled in condensed chromatin but 80% of DNA labelled in diffuse chromatin had C_{0t} values of less than 10^3 \text{m sec/l}.

The mechanism of crossing-over. The data, nevertheless, do permit the conclusion that the distribution of pachytene-labelled DNA within chromatin is not a consequence of factors such as C_{0t} characteristics or time of replication which appear to influence the

![Graph](image)

**Fig. 5.** C_{0t} curves of pachytene-labelled DNA isolated from diffuse and condensed chromatin. Microsporocytes in early-mid pachytene were incubated in the presence of ^3H-thymidine for 48 hours. Chromatin was prepared and resolved on a glycerol gradient as described in the legend of Fig. 2. The fractions from regions A and B were pooled for condensed chromatin, and those from region C were pooled for diffuse chromatin. The DNA was isolated from each of the fractions, dissolved in 0.12 M phosphate buffer (pH-6.8) and sonicated to an average length of 500 base pairs. Reassociation kinetics were determined by standard procedures (4). The upper three curves (solid lines) represent the reassociation behavior as determined by absorbancies at 260 nm; the lower three curves (dashed lines) were determined by radioactivities. Solid circles, unfractionated chromatin; open circles, diffuse chromatin; triangles, condensed chromatin. The upper three curves reflect the reassociation behavior of microsporocyte chromatin regardless of the stage of extraction.

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<th>Chromatin</th>
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<td>Diffuse</td>
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distribution of DNA as a whole among chromatin fractions. The physical state of chromatin would appear to have a specific role in meiotic physiology.

*Action of staphylococcal nuclease.* The apparently unorthodox properties of meiotic chromatin with respect to DNA synthesis and compaction, led us to look for some distinctive features in the response of meiotic chromatin to staphylococcal nuclease. The results were unimpressive. Several studies of somatic chromatin have indicated that the condensed form is more resistant to nuclease action (3). Our experiments lead to the same conclusion. Higher concentrations of enzyme or longer periods of incubation were required to obtain the same degree of DNA fragmentation in the condensed as in the diffuse fraction (Fig. 6). The agarose gel patterns were essentially the same for condensed and diffuse chromatin regardless of the stage of meiosis. A few distinguishing characteristics of the products of nuclease action have been reported for meiotic chromatin (21), but these account for only a very small percentage of the total. The distinguishing characteristics of meiotic chromatin would appear to be based in properties other than basic nucleosome organization.

**DISCUSSION**

The original objective of this study was to determine whether changes in chromosome compaction could be related to one or more of the distinctive features of meiotic DNA synthesis. Although direct evidence for a functional relationship between the two processes was not obtained, the circumstantial evidence is compelling. The weak
feature of that evidence is the arbitrary dividing line that had to be drawn between diffuse and condensed chromatin. The method chosen to yield separate fractions of condensed and diffuse chromatin had uncertain endpoints. In the absence of any unambiguous physical or chemical criteria, we settled on cytological ones in judging the effectiveness of a particular procedure. The one ultimately adopted made possible an in vitro profile of meiotic chromosome condensation that was in satisfactory correspondence with the meiotic sequence of cytological changes. Thus, despite the arbitrariness in differentiating between in vitro fractions of condensed and diffuse chromatin, a real relationship has been identified between the method of fractionation and meiotic chromosome behavior.

Judged by the pattern of label distribution between condensed and diffuse chromatin, each of the three intervals of DNA synthesis in Lilium microsporocytes has a special relationship to chromosome compaction. During the early and middle phases of the premeiotic S-period, DNA replication is excluded from the condensed regions even though they account for two-thirds of the total chromatin; during zygotene, the interval of chromosome pairing, most of the late replicating Z-DNA remains in the diffuse chromatin fraction; and during pachytene, the interval of crossing-over, DNA synthesis is centered in condensed chromatin. We speculate that each of these relationships reflects an essential meiotic function.

The most impressive feature of premeiotic S-phase behavior is the exclusion of chromosome replication from a fraction of chromatin that behaves as condensed only if nuclei are pretreated with glutaraldehyde. Since over 99% of the genome is replicated during this interval (22), the exclusion cannot persist, and we estimate that it roughly extends for the initial one-half to two-thirds of S-phase. Regardless of the precise duration of the exclusion, the behavior is impressive because glutaraldehyde appears to act principally on the transient heterochromatin that is so prominent cytologically in premeiotic nuclei. If true, the evidence presented is in line with the radioautographic observations of Holm (8), who noted in Lilium that heterochromatic DNA granules were being labelled almost as late as leptotene.

A most distinctive feature of zygotene replication is its being a very late event which, unlike other late replicating fractions, is poorly represented in condensed chromatin. We speculate that the housing of Z-DNA in the diffuse fraction is tied to its activities in chromosome synapsis. The logic of this speculation is based on the general observation that actively transcribing chromatin is maintained in a diffuse form (17). Z-DNA may not be so situated for purposes of transcription. We presume that a decompacted chromatin, regardless of its precise nature, is essential to the role of DNA in synapsis. It would appear that at least in the case of Z-DNA, late replication need not be tied to chromosome compaction. The conclusion needs further testing by determining the distribution of Z-DNA sequences between the chromatin fractions during the interval spanning S-phase to zygotene.

The relationship between pachytene repair replication and chromosome compaction is the most puzzling of the three intervals of DNA synthesis. Not only is there an appreciable increase in condensed chromatin during the pachytene stage, but its ^3^H-DNA has the higher specific activity. Moreover, it is the moiety of low repeat sequences that is most strongly localized in the condensed chromatin, a localization that is precisely opposite to what occurs in the rest of the chromosome. A highly tentative explanation is that regions with a potential for crossing-over are confined to condensed
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chromatin. The latter might reflect a special relationship between synaptonemal complex and the chromatin in regions of crossing-over. It is tempting to involve Carpenter’s “recombination nodules” (6) in the process, but so small a fraction of repair replication is translated into crossovers that the actual materials of the crossover would be chemically undetectable by present methods (13).

The fact that we have found each of the three categories of meiotic DNA synthesis to be distinctively partitioned between compact and diffuse chromatin supports the belief that the relationships observed are real. To the extent that they are, they point to the importance of compaction factors in the meiotic behavior of chromosomes. And, indeed, if behavior is so governed, then specific mechanisms must exist to regulate the timing and location of the compaction process. The nature of these mechanisms is an inviting target for future studies.

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REFERENCES


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