Meiotic Aspects of Chromosome Organization

(meiosis, pachytene DNA)

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SUMMARY

Homologous chromosome pairing regulates the expression of a set of metabolic activities that is unique to the zygotene-pachytene interval. These activities probably relate to crossing-over and they involve specific proteins and specific chromosome regions. The recombination-related proteins include an endonuclease, a DNA-unwinding protein, and a reassociation protein. These proteins act primarily on specific DNA sequences, their activity being manifest in the formation of nicks and gaps accompanied by repair synthesis. The site-specificity of nick-repair activity is determined by site-specific changes in chromatin organization. These changes do not occur in the absence of homologous pairing. The DNA undergoing nick-repair is housed in a set of families of moderately repeated sequences ("P-DNA"). They range in length from 800-4000 bp; they show very small sequence divergence and share homology with corresponding repeats across a broad phylogenetic spectrum. They are unevenly distributed in the genome. About 60% of the Lilium genome has no P-DNA sequences within at least 350 kb from one another; the remainder of the genome has P-DNA sequences spaced 30-350kb apart. Nick-repair activity is mainly confined to the end regions of P-DNA ("PsnDNA") which measure 125-400 bp in length. PsnDNA is housed in chromatin of distinctive composition. The chromatin has little, if any, histones; instead, the DNA is associated with a snRNA ("PsnRNA") and a non-histone protein. The PsnRNA is complementary to the PsnDNA and the protein binds specifically to PsnRNA. A model is briefly discussed to account for the regulation of pachytene DNA metabolism.

INTRODUCTION

Crossing-over and reductional disjunction have long been recognized as the principal functions of meiosis. Although the regularity of crossing-over during meiosis invites the view that meiocytes are abundantly equipped with recombinational mechanisms, it is more fruitful in studies of higher eukaryotes to emphasize the mechanisms regulating recombination rather than to dwell on the recombination mechanisms themselves. In terms of DNA content, a high frequency of recombination is not a general feature of meiosis; genetic evidence on 'positive interference' points in the opposite direction. Per DNA nucleotide, recombination frequencies are much lower in large than in small genomes. In *Drosophila* a meiocyte has about 1.7 crossovers per 100 kilobase pairs ('kbp') whereas in *Lilium* the corresponding value is 0.03 (STERN & HOTTA 1978). The lily genome is about 300 times bigger than the *Drosophila* genome but has 1/50th the concentration of crossovers. Meiotic regulation of recombination has two important features: it assures regularity so that there is at least one crossover per bivalent, and it is selective in distribution so that some chromosomal regions, such as heterochromatin, are excluded from a significant level of crossovers. These characteristics of regularity and selectivity need to be addressed in approaching meiosis from a molecular or biochemical standpoint, the approach being followed in our studies.

Meiocytes, being the gateway to gametogenesis, do not necessarily confine their activities to the purely meiotic requirements of chromosomes. In animal phyla, the products of meiosis are directly transformed into gametes and during prophase there is considerable overlap in activities related to meiosis proper and those related to gametogenesis. In plants and fungi a haploid generation of varied duration interrupts the transformation of the meiotic products into gametes. Among microbial eukaryotes meiosis may be tied to sporulation and among plants, processes essential to gametophyte development may originate in the meiocyte. Thus, from a molecular standpoint, events observed in meiocytes are not necessarily specific to meiosis even if they are specific to the meiocyte. In the discussion that follows we presume that the events analyzed are directly related to meiosis. We recognize that other possibilities cannot be rigorously excluded, but they will not be discussed.

Physiological, biochemical and radioautographic studies have contributed some useful pointers to the mechanisms underlying chromosome behavior during meiotic prophase. In *Lilium* and *Trillium* an irreversible commitment to meiosis occurs between late S-phase and leptotene (ITO & TAKEGAME 1982, WALTERS 1980, NINNEMANN & EPEL 1973). In yeast, reversal can occur even after recombination (SIMCHEN et al. 1972). In certain insects perturbation of meiocytes by temperature shock at stages as advanced as early pachytene can cause an elimination of crossing-over (PEACOCK 1968). Genetic analyses of diverse organisms have shown that events during meiotic

prophase are critical to crossing-over and to normal disjunction. There is also growing evidence that DNA synthesis, though small in amount, occurs during meiotic prophase and that a major fraction of such synthesis occurs in the vicinity of the synaptonemal complex (KURATA & ITO 1978, MOSES et al. 1981). In male meiocytes of lily and mouse, the occurrence of prophase DNA synthesis has been demonstrated by biochemical analysis. In the case of lily, it has also been demonstrated that during zygotene there is a delayed semiconservative synthesis of specific sequences. During pachytene, DNA synthesis is entirely of the repair type (HOTTA & STERN 1971). What emerges from all these studies is that DNA metabolism is a major activity of cells in meiotic prophase. Such metabolism is not random and its occurrence thus implies that specific chromosome regions play particular roles in meiosis. How these regions are organized and how their activities are regulated are the targets of this presentation.

GENERAL COMMENTS ON ACTIVITIES DURING MEIOTIC PROPHASE

The behavior of DNA-related enzyme activities during the interval spanning premeiotic S-phase through pachytene provides certain pointers on the nature of chromosome activity at meiotic prophase. The profiles of these enzyme activities are illustrated in Figure 1. It is at once apparent that the enzymes can be classified into two groups on the basis of their activity profiles. One group is active during premeiotic S-phase, its members displaying greater or lesser fluctuations in activity during zygotene and pachytene. The second group is essentially inactive during the prelepotene interval, rises markedly in activity during zygotene and returns to a negligible activity level at the end of pachytene. It is significant that activities of the second group can be viewed as essential to recombination, whereas those of the first group can be assigned to DNA synthesis even though they are also involved in recombinational activities. To the extent that the proteins of the second group have been characterized, they all appear to be specific to meiotic cells, similar activities in somatic cells being associated with different proteins. A brief description of the characteristics of these meiotic-specific proteins will serve as a point of departure for the discussion that follows. References are given in the legend to Figure 1.

The meiotic endonuclease is programmed to introduce nicks into chromosomal DNA beginning at late zygotene-early pachytene. The question raised in a recent review of mitotic recombination as to whether the higher frequency in meiotic cells is attributable to an accumulation of unrepaired nicks is answered unambiguously (KUNZ & HAYNES 1981). Nicks are not accumulated; they are deliberately introduced at about the time that the process of pairing is completed. The action of the DNA-unwinding protein ("U-protein") is dependent on the presence of nicks. The U-protein unwinds duplex DNA at nicked sites for distances up to about 400 bp if a 3' OH terminus is available, the action requiring ATP. Since meiotic

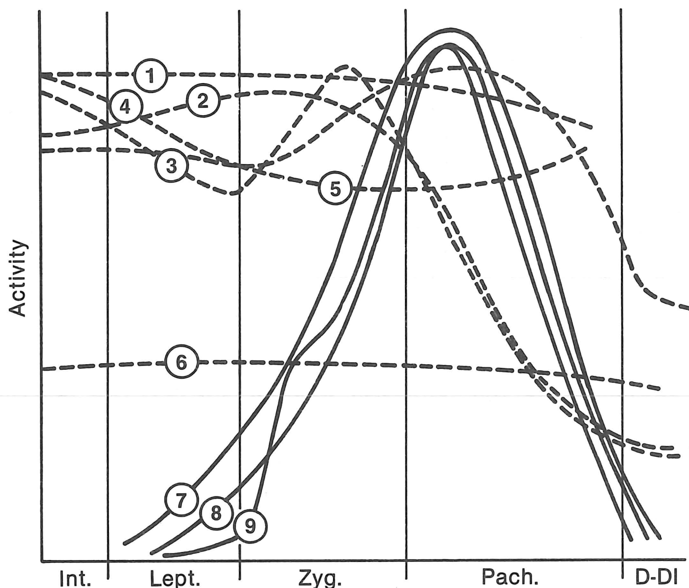

Figure 1. Activity profiles of DNA-related enzymes during the preprophase and prophase intervals of meiosis in *Lilium*. 1: DNA polymerase B (SAKAGUCHI et al. 1980); 2: Polynucleotide kinase (HOWELL & STERN 1971); 3: Polynucleotide ligase (HOWELL & STERN 1971); 4: Topoisomerase II (HOTTA unpublished); 5: Acid phosphatase (HOWELL & STERN 1971); 6: Topoisomerase I (HOTTA unpublished); 7: R-protein (HOTTA & STERN 1979); 8: U-protein (HOTTA & STERN 1978b); 9: Meiotic endonuclease (HOWELL & STERN 1971). The profiles are adapted from the references shown. The stages included in this comparison are premeiotic interphase (primarily S-phase), leptotene, zygotene, pachytene, and diplotene-diakinesis (D-D).

endonuclease forms 3'-P termini, neither unwinding nor repair synthesis can occur unless the phosphates are removed. We do not know whether the phosphatase action is subject to special meiotic regulation; as shown in Figure 1, phosphatase activity is fairly uniform over the entire interval studied. The combination of endonuclease, U-protein, and phosphatase should make available single-strand DNA at the nicked sites and thus make possible duplex formation between homologous chromatids. It is certain, however, that exonuclease activity either precedes or follows unwinding; many, if not all the discontinuities introduced into DNA at pachytene cannot be repaired in

isolated nuclei by ligase alone as would be the case if only nicks were present. The requirement for DNA polymerase to effect repair may be taken as evidence for the presence of gaps at the nicked sites (STERN & HOTTA 1978). Reassociation of single-strand DNA is effectively catalyzed by the R-protein, which has several distinctive characteristics. Unlike the two other proteins, it is part of the lipoprotein complex of the nucleus. Its capacity to reassociate DNA is regulated by phosphorylation, and its activity falls markedly in the absence of homologous pairing. Although these different proteins have not been assembled into an in vitro recombination system such as has been achieved by Radding and colleagues (RADDING 1981), their absence during the premeiotic S-phase and their transient presence during zygotene-pachytene point to their having a specific role in DNA metabolism during meiotic prophase. Whether that role is partly or entirely related to recombination cannot yet be determined, but it is clear that the achievement of chromosome synapsis is associated with an active period of distinctive DNA metabolism.

GENERAL FEATURES OF PACHYTENE DNA METABOLISM

The sites of pachytene DNA nicking are unevenly distributed in the genome and, consequently, so is pachytene repair synthesis. This distribution can be characterized in two ways. With respect to the genome as a whole, it is clear that very large segments of DNA are regularly excluded from the nicking process. Nicking does not occur in mouse satellite sequences which constitute about 10% of the genome (HOTTA & STERN 1978a) nor does it occur in the satellite sequences of the Arabian oryx which account for 40% of the genome (STERN & HOTTA 1980). In lilies, approximately 50-60% of the DNA is excluded from nicking at pachytene (HOTTA & STERN 1974), an exclusion which is associated with an absence of the specific group of sequences in which nicking normally occurs (unpublished). These sequences, which we refer to as pachytene or 'P-DNA' sequences, are the sites that determine the distribution of pachytene nick-repair activity (HOTTA & STERN 1975). However, as will be detailed later, P-DNA regions are themselves differentiated into sub-regions, nick-repair activity being localized in relatively small but specific sub-regions. The coordinated appearance of the three recombination-related enzymes is thus matched by a substantial degree of organization of the DNA sites at which their activities are presumably directed. The nature of that organization must be one of the major keys to an understanding of the meiotic process.

DISTRIBUTION OF P-DNA REGIONS

In those regions of the lily genome that undergo pachytene nicking, the distances between nicks range from about 30 to 350 kilobases ('kb'). The numbers are derived from measurements made on DNA gently extracted under alkaline conditions from pachytene cells. We are uncertain as to whether the intervals between the P-DNA sites are interrupted by a different set of discontinuities and, if so, the distance

between nicks may be as high as 750 kb. It is important to note that the sites of nicking on complementary DNA strands of the DNA duplex are coordinately spaced. In an alkaline glycerol gradient of total DNA from pachytene cells, the majority of P-DNA sequences is found in the 63S region which houses DNA strands having a modal length of 160 kb (unpublished; see Fig. 2). The presence of a substantial fraction of single-strand DNA having a modal length of 160 kb is a distinguishing characteristic of alkaline extracts from pachytene cells (HOTTA & STERN 1974). The residual DNA, which accounts for more than half the genome does not house P-DNA sequences and, on extraction, is present as longer DNA strands most of which accumulate at the bottom of the gradient. Only a small fraction of the single-strand DNA extracted from pre-zygotene cells bands in the 63S region of an alkaline glycerol gradient. P-DNA sequences can be excised from bulk DNA by treating isolated pachytene nuclei with DNase II; nuclei from stages other than pachytene or late zygotene do not yield the sequences when similarly treated (HOTTA & STERN 1981). Duplex DNA extracted from pachytene nuclei so treated displays a bimodal profile when sedimented in a neutral glycerol gradient. One of the two peaks, which accounts for about 40% of the DNA, consists of duplexes that have a modal length of about 160 kbp. These duplexes are formed by the cutting action of DNase II in the P-DNA regions. Their correspondence in length to the single strands resulting from in vivo nicking indicates that the nicks on complementary strands must be positioned within distances no greater than the length of the P-DNA regions. These distances must nevertheless be great enough to provide sufficient hydrogen bonding because duplex DNA extracted from untreated pachytene nuclei does not display the bimodal profile. The relationship of P-DNA sequences to the sedimentation profiles of DNA extracted from pachytene nuclei is illustrated in Figure 2.

ORGANIZATION OF P-DNA REGIONS

In our studies of the reassociation properties of P-DNA it was found that the modal length of the reassociated fragments after S1 nuclease digestion was about 2000 bp (BOUCHARD & STERN 1980). That length is considerably greater than the earlier estimate of 200 bp which was inferred from buoyant density measurements of BrdU-labelled P-DNA (HOTTA & STERN 1975). The evidence now available indicates that pachytene DNA synthesis is localized within short segments of the P-DNA regions (unpublished). We will refer to these sub-regions of P-DNA as 'PsnDNA'. We have been able to isolate what we presume to be intact P-DNA regions by very brief digestions of isolated pachytene nuclei with DNase II. The fragments thus isolated measure 800-4000 bp in length, the labelled PsnDNA ends being rapidly removed by treating with exonuclease. The PsnDNA sequences at the ends of the P-DNA fragments can be recovered without degradation by longer DNase II digestion of the nuclei (HOTTA & STERN 1981). The fragments thus obtained range from 125-400 bp in length. Our tentative model of their organization within the P-DNA region is shown in Figure 3B. The diagram also shows the positions of the gaps generated by

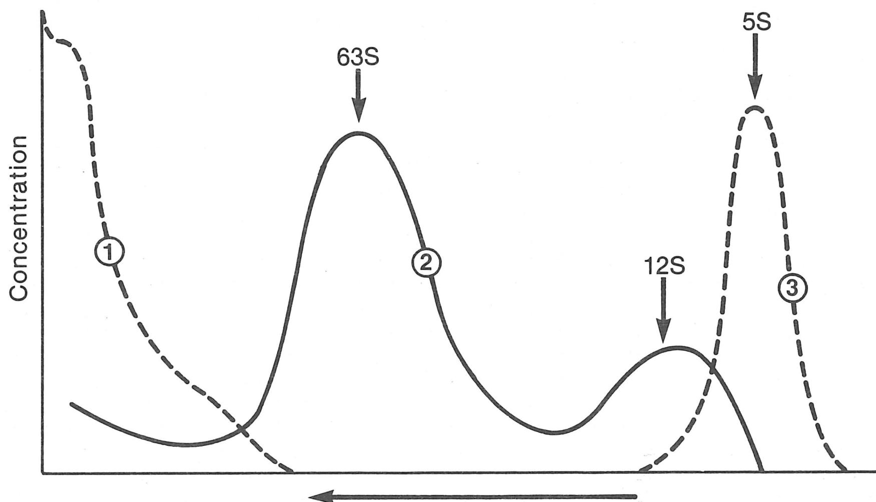

Figure 2. Hybridization of 'PsnDNA' to variously treated DNA extracts from meiotic cells. The simplified sedimentation profiles are based on studies discussed in the text; they track only the levels of hybridization to successive fractions in the gradient. The PsnDNA probe represents the families of repeated sequences undergoing nick-repair activity during pachytene. Its isolation is described by Hotta and Stern (1981). 1: Profile of hybridization to either single-strand or duplex DNA extracted from prezygotene or postpachytene cells. There is little DNA in the 60S region of the gradient and the probe accurately reflects the distribution of total DNA. 2: Single-strand DNA extracts from pachytene cells show almost all the hybridizable sequences in the 63S region of the gradient although 60% of the total DNA is at the bottom of the centrifuge tube. The 12S region contains a small fraction of the PsnDNA sequences and a minor fraction of the total DNA. 3: Duplex DNA extracted from pachytene nuclei after treatment with DNase II. About 40% of the DNA is present in the 55S region of the gradient and most of the remainder is at the bottom of the tube. The PsnDNA sequences are released by the DNase II treatment in duplexes ranging from 125-400 bp.

the actions of the endo- and exonucleases. These are inferred from in vitro labelling experiments in which the pachytene gaps were resealed by treating isolated pachytene nuclei with

alkaline phosphatase, DNA polymerase and ligase. The DNA so labelled was indistinguishable from in vivo labelled P-DNA. The gaps must lie entirely, or nearly so, within the PsnDNA regions.

Figure 3 contains a tentative model of P-DNA organization with respect to the genome as a whole. Three, or possibly four, units of organization comprise the model. The smallest unit is that containing the PsnDNA which is the principal site of nick-repair activity at pachytene. The PsnDNA units flank the larger P-DNA units which were first characterized by their distinctive reassociation behavior. The P-DNA units are interspersed within a defined region of the genome at intervals ranging from about 30-350 kbp. We have not yet characterized the DNA spanning the P-DNA units, but initial evidence indicates one interruption in the DNA between the P-DNA units which is not due to pachytene nicking. P-DNA nicks have 3'P termini so that interruptions cannot be repaired unless the phosphates are first removed. Treatment of isolated pachytene nuclei with DNA polymerase and ligase but without phosphatase results in an upward shift in 50% or more of what were considered to be P-DNA flanked segments. Inasmuch as most, if not all, P-DNA gaps cannot be sealed unless nuclei are first treated with alkaline phosphatase, we infer that the increase in S value is due to the sealing of non-P-DNA gaps located between P-DNA regions. We are unable to estimate the proportion of such non-P-DNA interruptions because we do not know the efficiency of the polymerase-ligase treatment and the increase in S value is insufficient to provide for a clear separation between precursors and products in the treatment. An attractive speculation is that Zyg-DNA sequences lie between the P-DNA regions, a possibility that needs to be explored.

P-DNA comprises a relatively large number of families of moderately repeated sequences. For *Lilium* we estimate about 600 such families (BOUCHARD & STERN 1980). The Psn-DNA components of the P-DNA sequences have been at least partially purified and have been used as probes in a number of the analyses referred to above. The occurrence of moderately repeated sequences in plants or animals is in itself unexceptional, but quite apart from their programmed activity during meiotic prophase there are two other characteristics which make these P-DNA sequences distinctive. First, sequence divergence within the families of P-DNA repeats is very low whereas commonly, the divergence among moderately repeated families is of the order of 10%. The contrast is illustrated in the melting profiles of reassociated P-DNA and of middle repeat DNA (Figure 4). Divergence among P-DNA families is similar to the divergence within *E. coli* genomes. The relatively high degree of sequence conservation very probably reflects a stringency in sequence requirements for effective meiotic function, and thus an importance of that function to the meiotic process.

Sequence conservation of P-DNA has been found not only within the *Lilium* genome, but also across a broad phylogenetic

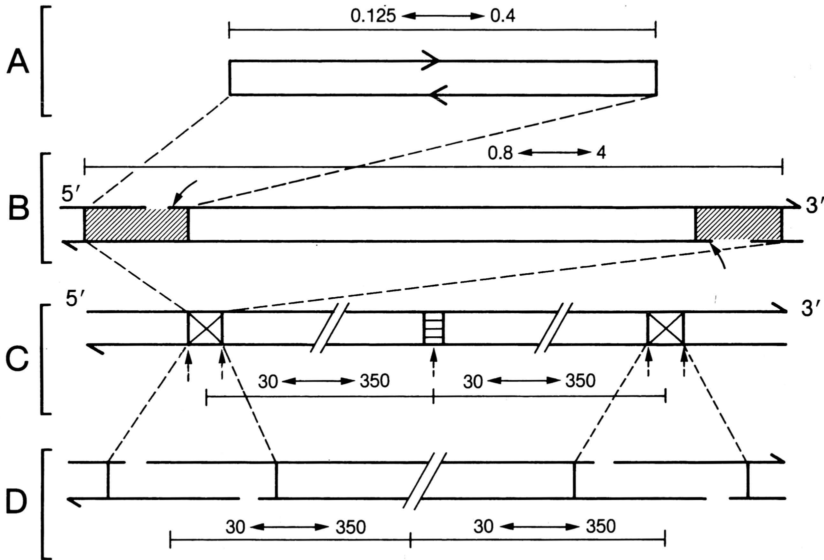

Figure 3. A diagrammatic representation of the organization of P-DNA regions in the *Lilium* genome. The evidence in support of the diagram is discussed in the text. The numbers shown are in kb. A: The PsnDNA segment of P-DNA. It is associated with a specific RNA (PsnRNA) and a specific nonhistone protein (Psn-protein). B: The P-DNA sequence is flanked by the PsnDNA segments (hatched regions). Nicking, exonuclease action, and repair synthesis during pachytene occur within this region. C: Location of P-DNA sequences within the genome. Distances shown are based on the lengths of single-strand DNA present in the 63S region of alkaline glycerol gradients of pachytene extracts. The center region (horizontal hatching) is a region that houses single strand interruptions; its nature is unknown. The dashed arrows are the positions of chromatin sites that are highly sensitive to DNase II. D: A speculative model of the sites of pachytene nicking within the P-DNA region. There are at least two nicks in each PsnDNA region.

spectrum of plants (FRIEDMAN et al. 1982). In a comparative study of moderately repeated sequences it has been found that DNA from the cereal grains as well as from *Vicia faba* could drive the reassociation of P-DNA sequences from lily but had insignificant effect on the reassociation of even the high

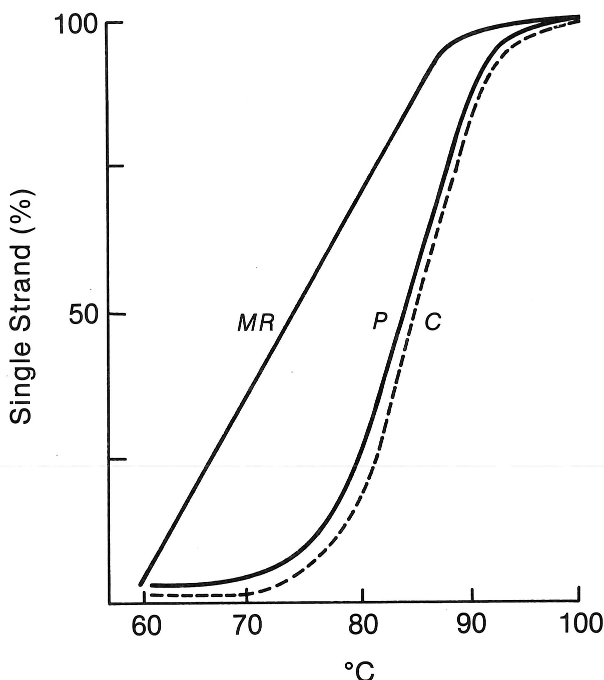

Figure 4. Melting profiles of reassociated DNA from *Lilium*. MR: moderately repeated sequences; P: P-DNA sequences; C: native DNA. The contrast in fidelity of reassociation between MR and P groups of sequences is apparent from the shapes of the remelt curves. The profiles are adapted from the results of Bouchard and Stern (1980).

thermal stability fraction of the other moderate lily repeats. The study also indicated that although considerable variation occurred between different species with respect to the number of repeats per family, the number of families was approximately constant. Such conservation of individual family sequences reinforces the conclusion that the P-DNA sequences have a fundamental function in meiosis.

PROPERTIES OF P-DNA CHROMATIN

In progressing from leptotene to pachytene chromosomes become fitted with lateral elements which are then organized into synaptonemal complexes. Whether any structural relationship exists between components of the synaptonemal complex and P-DNA regions is unknown, but there is sufficient evidence to demonstrate that synapsis is accompanied by distinctive changes in the chromatin housing the PsnDNA segments of the

P-DNA sequences (HOTTA & STERN 1981). These changes are important from a functional standpoint inasmuch as they regulate the accessibility of the PsnDNA regions to the endonuclease and, probably, other proteins. The site-selective nicking which occurs at pachytene is effected, not by sequence specificities of the meiotic endonuclease, but by those factors that regulate the site-specific changes in chromatin organization. 'Psn-chromatin' contains little, if any, histone (unpublished). Instead, PsnDNA is associated with a nonhistone protein from which it can be dissociated in presence of 0.4 M NaCl (HOTTA & STERN 1981). It is also associated with what will be referred to as PsnRNA. The RNA shares homology with the DNA although no RNA-DNA hybrid has been found in the Psn regions. The length of the homologous region in the RNA is at least 125 nucleotides, the length found after purifying Psn-chromatin fragments by treating DNase II-released chromatin with RNases. Recent studies (unpublished) have shown that the native PsnRNA molecule is significantly bigger, 370 or perhaps even close to 600 nucleotides. The 125-nucleotide residue is due to the protective action of the 'Psn-protein'. Preliminary evidence indicates that Psn-protein binds specifically to PsnRNA, thus protecting it from nuclease action.

PsnRNA is synthesized during zygotene-pachytene. It is not detectable in somatic tissues nor is any significant concentration demonstrable in the meiocytes at prezygotene or postpachytene stages. We do not yet have a specific reagent to test for the presence of Psn-protein at stages other than zygotene-pachytene, but since the protein can be made radioactive by pulsing cells with [^{35}S]methionine at zygotene it seems probable that Psn-protein, like Psn RNA, is synthesized during meiotic prophase. Synthesis of the two components at zygotene-pachytene is almost certainly addressed to the role of rendering PsnDNA accessible to endonuclease action. Using isolated nuclei it has been shown that PsnRNA is a necessary and effective factor in altering Psn-chromatin so as to provide access to the endonuclease (HOTTA & STERN 1981). Just how this change occurs is still under study. It requires ATP and a cytoplasmic factor that has not yet been identified. The factor is also a prophase-specific component. There is clearly a coordination in the activities of several factors to make sites available for pachytene nicking. Two of the factors, PsnRNA and Psn-protein, the only ones thus far identified, are themselves components of the altered chromatin. It is highly probable that PsnRNA is also the factor responsible for the specificity of site alterations.

Hybridization analyses indicate that at least 80% of the PsnDNA sequences are complementary to those of PsnRNA. The number of different PsnRNA sequences must be similar to the number of PsnDNA families (unpublished). A single species of PsnRNA is incompatible with this result, but the presence of a short sequence that is common to all the PsnRNAs is not excluded. The preliminary evidence cited earlier on the specific affinity of Psn-protein for PsnRNA makes the presence of such a sequence probable. The regulatory role of PsnRNA

families invites the speculation that inter- or intrachromosomal regulatory mechanisms in meiosis may operate by transcribing each species of small RNA molecule at a master site, multiple transcripts of individual PsnRNA species interacting with the dispersed members of the complementary families of PsnDNA sequences. The evidence available makes it probable that such a mechanism operates at pachytene, thus coordinating the opening of different chromosome sites to endonuclease action. How general this type of mechanism might be in meiotic regulation is open to speculation. The recent report on the presence of repeated sequences in meiosis-specific poly(A)+RNA transcripts is compatible with an snRNA regulatory mechanism, but no evidence has been adduced for the formation of complementary snRNA sequences nor has the relationship of the repeat regions to mRNAs been clarified (APPELS et al. 1982). It is probable that the sequences do play a significant role in meiotic metabolism inasmuch as they are abundantly present during part or all of the prophase and preleptotene intervals, but are absent, or nearly so, from mitotically dividing cells in the premeiotic anthers. This analysis of meiotic transcription and the analysis of meiotic nicking both point to the conclusion that chromosomes house certain sets of repeated DNA sequences which are specifically active in transcription and repair synthesis during meiosis. To the extent that these sequences are uniquely active during meiosis, they can be considered as meiotic-specific sequences having a distinctive and fundamental role in regulating meiotic events.

HOMOLOG SYNAPSIS AS A REGULATORY FACTOR IN PROPHASE METABOLISM.

Synapsis of homologous chromosomal regions is a precondition for crossing-over, and it is generally believed that zygotene pairing coupled with SC formation effect such synapsis. It has been shown, however, that heterologous pairing can occur without any obvious abnormality in the morphology of the synaptonemal complex (RASMUSSEN & HOLM 1980; MOSES & POORMAN 1981). Since heterologous pairing as distinct from homoeologous pairing, does not result in crossovers, it is clear that pairing per se under otherwise normal meiotic conditions does not assure their occurrence. It is reasonable to suppose that the ineffectiveness of heterologous pairing is entirely due to a lack of complementarity between the DNA strands of the paired heterologous chromosomes. However, the evidence accumulated points to a more pervasive role of homologous pairing in the metabolic events of meiotic prophase. Synapsis of homologous regions controls the expression of a complex set of events associated with programmed nick-repair activity at pachytene. The grounds for this conclusion are based upon the observed consequences of limited homologous pairing in a lily hybrid cultivar, "Black Beauty", and of reduced synapsis in otherwise normal meiocytes effected by treatment with colchicine (HOTTA et al. 1979).

Obvious effects of reduced homologous pairing on pachytene metabolism are illustrated diagrammatically in Figure 5.

The most prominent effect of pairing failure is a reduction in pachytene DNA nicking and, as expected, a corresponding reduction in pachytene repair synthesis. The extent of nicking is expressed as the fraction total single-strand DNA banding in the 60S region of a glycerol gradient. This fraction is necessarily an approximate measure of the nicks formed in vivo because of the background level of DNA in the 60S region of all preparations. Repair activity is a more accurate measure of nick-repair events at pachytene. Nevertheless, regardless of the criterion used, it is clear that meiocytes with normal chiasma frequency ("Ch") have a much higher level of nick-repair activity than those with a low chiasma frequency as in the microsporocytes of Black Beauty ("2n-BB") or in those treated with colchicine. The behavior of Black Beauty microsporocytes that have been made tetraploid ("4n-BB") by treating premeiotic anthers in situ with colchicine is of particular interest. The high proportion of 60S DNA in extracts of these cells, shown to be chiasmatic, directly demonstrates that it is synapsis and not some genic factor that is responsible for the absence of nick-repair activity in the diploid BB hybrid. The effect of reduced synapsis is also seen in the activity of the R-protein. The low chiasmatic BB hybrid has low R-protein activity which is fully restored in the tetraploid cells (HOTTA et al. 1979).

The reduced level of the R-protein in 2n-BB cells does not reflect a general reduction in the three proteins that display the transient rise in activity during meiotic prophase (Figure 1). The endonuclease and U-protein are totally unaffected by the level of chiasma frequency. R-protein, unlike the other two, is part of a nuclear lipoprotein complex and thus physically associated with the nuclear structure, but this difference in association points to no obvious explanation for the big difference among these proteins in response to synapsis. Whether the lipoprotein complex that houses the R-protein undergoes a change in response to pairing failure has not been determined. It is clear, however, that directly or indirectly synapsis has an effect on chromatin structure. In absence of homologous pairing the changes that otherwise occur in chromatin structure at PsnDNA sites do not take place. The much reduced level of PsnRNA in 2n-BB (Figure 5) is associated with that failure. It could be argued that synthesis of R-protein and of PsnRNA are reduced in the absence of homologous pairing. Evidence on this possibility is lacking and, tentatively, it seems preferable to suppose that homologous pairing protects these components against accelerated degradation. A turnover of these proteins must occur normally in meiosis, a behavior that may be inferred from their virtual disappearance following completion of meiotic prophase.

The key observation that points to a link between homologous chromosome pairing and the occurrence of structural changes in chromatin is the absence of nick-repair activity in chiasma-deficient pachytene meiocytes that nevertheless have normal levels of endonuclease activity. The poor accessibility of meiotic endonuclease or other nucleases to the PsnDNA

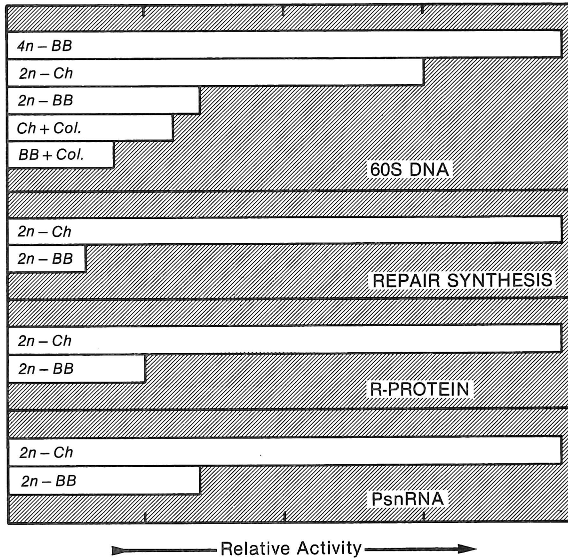

Figure 5. The effect of homologous chromosome pairing on activities at pachytene. "60S DNA" reflects the level of endogenous pachytene nicking. "Repair synthesis", "R-protein", and "PsnRNA" are all significantly higher in normal chiasmatic cells (2n-Ch) than in those of the Black Beauty cultivar (2n-BB), a hybrid deficient in homologous pairing. Reduction in nicking activity is observed not only in 2n-BB cells but also in chiasmatic cells treated with colchicine to reduce pairing (Ch + Col) or in 2n-BB cells treated with colchicine to reduce residual pairing (BB+ Col). An important feature of this diagram is the very high level of nicking which occurs in tetraploid BB cells (shown to be chiasmatic). Most of the data for the diagram obtained from Hotta et al. (1979). Data on PsnRNA are unpublished.

sites of chiasma-deficient cells is demonstrable in isolated pachytene nuclei of the BB cultivar. Limited incubation of these nuclei with meiotic endonuclease or DNase II results in very low levels of DNA nicking or cutting (HOTTA et al. 1979). Addition of relatively high concentrations of PsnRNA with appropriate supplements to the incubation medium significantly increases nuclease accessibility in PsnDNA regions. It may be concluded from these and preceding observations that homologous pairing regulates pachytene DNA metabolism by

facilitating the interaction of PsnRNA and Psn-protein with PsnDNA sequences.

CONCLUDING COMMENTS

It is generally assumed that the major meiotic event at pachytene is the consummation of recombinational processes. Whether this is the only significant event at pachytene must remain an open question. Our studies demonstrate that the zygotene-pachytene interval is characterized by a distinctive pattern of metabolism, one that can be distinguished from the metabolic activities directed at oogenesis and spermiogenesis which in animal species overlap at least part of the prophase interval. A major feature of this meiotic prophase metabolism is the introduction of discontinuities in chromosomal DNA and their repair in the presence of a complement of proteins that have the capacity to promote recombination. The discontinuities, which result from the combined effects of endo- and exonucleolytic action, are regulated not only temporally but also spatially. They occur at specific chromosomal sites, sites from which very large segments of the genome are excluded. Regular patterns of organization are apparent for the DNA intervals spanning the sites and for the DNA sequence organization within the sites themselves.

Coordination of events at the zygotene-pachytene interval is achieved not only by stage-specific synthesis of essential proteins but also by pairing of homologous chromosomes. Synapsis regulates events at specific DNA sites in which pachytene nick-repair activities are localized. The organization of P-DNA sequences within the genome affords mechanisms whereby synapsis can effect a broad control on prophase metabolism. P-DNA sequences consist of families of repeats, each family being matched with a set of complementary snRNA molecules which make possible a single type of interaction for all family members. The snRNA, in turn, appears to have a specific affinity for a "Psn-protein", both components invading the P-DNA sites during zygotene-pachytene. An inviting model of regulation is one in which master control sites are activated to transcribe PsnRNA during the zygotene-pachytene interval. The PsnRNA diffuses through the nucleus and is stably accommodated in P-DNA regions but only if homologous pairing has occurred. In the absence of homologous pairing, the RNA is not accommodated and the changes which make P-DNA accessible to endonuclease do not take place, thus preventing the occurrence of nicking and related activities.

The above speculations are relatively secure when compared with the uncertainties generated by a lack of evidence concerning the relationship between P-DNA metabolism and a principal meiotic event--recombination. If P-DNA metabolism relates to other meiotic events, we have not yet identified them. If it relates only to recombination, defining the nature of that relationship is beyond the reach of available information. The gap between P-DNA metabolism and recombination nodules (RASMUSSEN & HOLM 1980, CARPENTER 1981) is very

large. It seems most unlikely to us that the highly organized and highly regulated events in DNA metabolism at pachytene have no meiotic function. If recombination is that function we must somehow account for the fact that the number of P-DNA sites exceeds the number of crossovers by several orders of magnitude. We can only rationalize our observations by drawing an analogy with other aspects of gametogenesis. Excessive production to assure ultimate adequacy is a strong characteristic of microsporogenesis, spermatogenesis, and oogenesis. It could be argued that the apparently excessive provision of potential recombination sites at pachytene is no more than a mechanism for assuring a regularity of recombination in meiosis.

LITERATURE CITED

APPELS, R., BOUCHARD, R.A. and STERN, H. 1982 cDNA clones from meiotic-specific poly(A)+ RNA in Lilium:Homology with sequences in wheat, rye, and maize. Chromosoma (Berl.) 85: 591-602.

BOUCHARD, R.A. and STERN, H. 1980 DNA synthesized at pachytene in Lilium: A non-divergent subclass of moderately repetitive sequences. Chromosoma (Berl.) 81:349-363.

CARPENTER, A.T.C. 1981 EM autoradiographic evidence that DNA synthesis occurs at recombination nodules during meiosis in Drosophila melanogaster females. Chromosoma (Berl.) 83: 59-80.

FRIEDMAN, E., BOUCHARD, R.A. and STERN, H. 1982 DNA sequences repaired at pachytene exhibit strong homology among distantly related higher plants. Chromosoma,(Berl.) in press.

HOTTA, Y. and STERN, H. 1971 Analysis of DNA synthesis during meiotic prophase in Lilium. J. Mol. Biol. 55: 337-355.

HOTTA, Y. and STERN, H. 1974 DNA scission and repair during pachytene in Lilium. Chromosoma (Berl.) 46: 279-296.

HOTTA, Y. and STERN, H. 1975 Zygotene and pachytene-labeled sequences in the meiotic organization of chromosomes. Pp. 283-300. The Eukaryote Chromosome. (Peacock, W.J. and R.D. Brock, Eds.) Australian Nat., Univ. Press.

HOTTA, Y. and STERN, H. 1978a Absence of satellite DNA synthesis during meiotic prophase in mouse and human spermatocytes. Chromosoma (Berl.) 69: 323-330.

HOTTA, Y. and STERN, H. 1978b DNA unwinding protein from meiotic cells of Lilium. Biochemistry 17: 1872-1880.

HOTTA, Y. and STERN, H. 1979 The effect of dephosphorylation on the properties of a helix-destabilizing protein from meiotic cells and its partial reversal by a protein kinase. Eur. J. Biochem. 95: 31-38.

HOTTA, Y., BENNETT, M.D., TOLEDO, L.A. and STERN, H. 1979 Regulation of R-protein and endonuclease activities in meiocytes by homologous chromosome pairing. Chromosoma (Berl.) 72: 191-201.

HOTTA, Y. and STERN, H. 1981 Small nuclear RNA molecules that regulate nuclease accessibility in specific chromatin regions of meiotic cells. Cell 27: 309-319.

HOWELL, S.H. and STERN, H. 1971 The appearance of DNA breakage and repair activities in the synchronous meiotic cycle of

Lilium. J. Mol. Biol. 55: 357-378.
ITO, M. and TAKEGAMI, M.H. 1982 Commitment of mitotic cells to meiosis during the G_2 phase of premeiosis. Plant and Cell Physiol. 23: 943-952.
KUNZ, B.A. and HAYNES, R.H. 1981 Phenomenology and genetic control of mitotic recombination in yeast. Ann. Rev. Genetics 15: 57-89.
KURATA, N. and ITO, M. 1978 Electron microscope autoradiography of ^{3}H-thymidine incorporation during the zygotene stage in microsporocytes of Lily. Cell Structure and Function 3: 349-356.
MOSES, M.J. and POORMAN, P.A. 1981 Synaptonemal complex analysis of mouse chromosomal rearrangements. II. Synaptic adjustment in a tandem duplication. Chromosoma (Berl.) 81: 519-536.
MOSES, M.J., DRESSER, M.E. and POORMAN, P.A. 1981 DNA synthesis associated with synaptonemal complexes in meiotic prophase. J. Cell Biol. 91: 70a.
NINNEMANN, H. and EPEL, B. 1973 Inhibition of cell division by blue light. Exp. Cell. Res. 79: 318-326.
PEACOCK, W.J. 1968 Chiasmata and Crossing Over. Pp. 242-252. Replication and Recombination of Genetic Material (Peacock, W.J. and R.D. Brock, Eds.) Austr. Acad. Science.
RADDING, C.M. 1981 Recombination activities of E. coli Rec A protein. Cell 25: 3-4.
RASMUSSEN, S.W. and HOLM, P.B. 1980 Mechanics of meiosis. Hereditas 93: 187-216.
SAKAGUCHI, K., HOTTA, Y. and STERN, H. 1980 Chromatin-associated DNA polymerase activity in meiotic cells of lily and mouse: its stimulation by meiotic helix-destabilizing protein. Cell Struc. Func. 5: 323-334.
SIMCHEN, G., PINON, R. and SALTS, Y. 1972 Sporulation in saccharomyces cerevisiae: premeiotic DNA synthesis, readiness, and commitment. Exp. Cell. Res. 75: 207-218.
STERN, H. and HOTTA, Y. 1978 Regulatory mechanisms in meiotic crossing-over. Ann. Rev. Plant Physiol. 29: 415-436.
STERN, H. and HOTTA, Y. 1980 The organization of DNA metabolism during the recombinational phase of meiosis with special reference to humans. Mol. Cell. Biochem. 29: 145-158.
WALTERS, M.S. 1980 Premeiosis and meiosis in Lilium "Enchantment". Chromosoma (Berl.) 80: 119-146.

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