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Chromosome behaviour during meiosis*

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Abstract

Pairing of homologous chromosomes and genetic recombination are key features of meiosis. DNA repair events at the pachytene interval of mammalian germ cells is very distinct and are often linked to the genetic recombination processes. The DNA polymorphism observed could be generated by the meiotic recombination-associated events. During the last few years our laboratory has been studying some of the aspects of DNA repair processes that occur in rat pachytene spermatocytes and also the behaviour of lamina structure during spermatogenesis. Some of our salient findings have been discussed here.

Key words: Pachytene spermatocytes, DNA repair sites, lamina structure.

1. Introduction

Most of the eukaryotic cells have two life cycles. The first one is the mitotic cell cycle wherein a diploid cell undergoes cell division to give rise to two more identical daughter cells. In the second meiotic cell cycle, a diploid cell undergoes reductive cell division to give rise to four haploid cells containing half the number of chromosomes. This meiotic division occurs during gametogenesis in higher eukaryotic cells. Before the onset of meiotic division, the chromosomes of the pregametic cell are replicated to yield two pairs of sister chromatids after which they enter a long meiotic prophase interval. This prophase is divided into five distinct stages which are termed as (a) leptotene, (b) zygotene, (c) pachytene, (d) diplotene, and (e) diakinesis[1]. The chromosomes condense along their axial elements at the leptotene interval. During the zygotene stage, the parental homologous chromosomes pair with the help of a structure known as the 'synaptonemal complex' (SC)[2]. These SCs have a tripartite structure, comprising a central element bounded by two lateral elements. The pachytene interval is the longest stage of the meiotic prophase, during which time crossing over and genetic recombination takes place between the paired homologous chromosomes. With the separation of the parental chromosome pairs in the

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subsequent stages, the first meiotic division results in the reduction of chromosomes by half, which is followed by another division, analogous to mitosis to produce haploid gametes.

Genetic recombination between the paired homologous chromosomes during the meiotic prophase, in addition to providing genetically diverse haploid gametes, which contribute to the species diversity, may also serve a role of correcting DNA sequence alterations that arise during proliferation and development of germinal cells. In addition, recombination is vital for normal chromosomal disjunction because mutants lacking recombination exhibit chromosome malsegregation.

Historically, there have been three general approaches for the study of meiosis, (1) morphological and cytological investigations, (2) genetic dissection of the recombinational events, and (3) characterization of the biochemical processes of meiosis. Early studies concentrated on the morphological and cytological aspects of meiosis. These studies clearly defined the behaviour of chromosomes during prophase leading to the discovery of the synaptonemal complexes which play a central role in bringing chromosomes together[2]. It is now very clear that these structures are an integral part of meiosis in all the systems studied. The second phase of development was concerned with genetic studies wherein several mutants particularly in lower eukaryotic systems like yeast, *Neurospora crassa* and Ascobolus were analyzed by looking at the meiotic products. Several genes have also been identified which are essential for the meiotic process. However, more recently, studies on the biochemical aspects of meiosis, namely, (1) DNA metabolic events, (2) characterization of the enzymatic machinery involved in genetic recombination, and (3) characterization of the proteins involved in chromosome pairing have been initiated. Our laboratory, over the last few years has been investigating some of the aspects of the biochemistry of meiosis in mammals using rat spermatogenesis as the model system. Here, some of our recent findings have been summarized and discussed in the context of more recent developments in literature.

2. DNA metabolism in meiosis

One of the features distinguishing meiosis from other types of cellular development is the associated DNA metabolic events. While other types of development necessarily involve DNA in an informational context and may lead to changes in utilization of information, meiosis, in addition, results in reassortment of the informational content and orderly segregation of a total complement of chromosomes into gametes.

Much of the work on DNA metabolic events in higher eukaryotes has come from the laboratory of Herbert Stern[3], using lilium gametogenesis and mouse spermatogenesis as the experimental model systems. A distinctive and functionally significant feature of pre-meiotic DNA replication is its incompleteness. About 0·1–0·2% of the genome is replicated at the zygotene interval and is termed as 'Zyg-DNA'. These DNA sequences have a low copy number but may include a fraction of repeated sequences. One distinctive feature of Zyg-DNA replication is that the ends

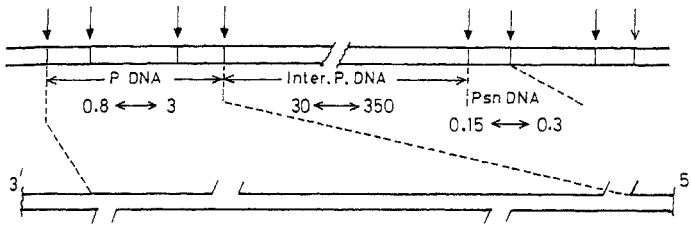

FIG. 1. A diagrammatic model representing the organisation of pachytene repair DNA proposed by Herbert Stern.

of Zyg-DNA segments remain unreplicated and completion of their replication does not occur until after the pachytene stage. About 1 to 2% of Zyg-DNA is transcribed into poly A(+) RNA, the translation products of which are believed to be the components of synaptonemal complexes.

The DNA metabolic events at the pachytene interval are very characteristic in nature and are believed to centre around the various recombination-related activities. Experiments carried out in several organisms have demonstrated an increase in DNA repair synthesis at the pachytene interval. The DNA repair activity is the result of programmed introduction of single-stranded nicks. In both mouse and lilium pachytene meiocytes, sites undergoing repair have been termed as 'pachytene DNA' or 'P-DNA'. These sequences were shown to belong to the class of middle repetitive DNA. The structural organization of P-DNA is distinctive and falls into two unequal size classes. The smaller size class ranges from 150 to 300 bases in length and is termed as 'PSn-DNA', while the larger size class ranges from 800 to 3000 bases in length referred to as P-DNA. A diagrammatic representation of these findings of Stern and coworkers[3] is presented in Fig. 1. They have also hypothesized that these repair sites might serve as initiators of recombination process. However, no direct evidence in its support is available. Despite preliminary characterization of the repair activity, very little is known about the events leading to the repair synthesis and its actual significance in the context of the various events occuring at the pachytene interval. We therefore initiated studies on the characterization of these repair sites in rat pachytene spermatocytes with an emphasis on DNA sequence characterization. Initially, we had shown that the chromatin of pachytene spermatocytes is extensively poly (ADP-ribosyl)ated. Poly(ADP-ribosyl)ation of nuclear proteins is greatly stimulated by the introduction of nicks in the DNA. By using a novel approach we showed that the DNA-repair domains could be isolated by making use of an affinity column made up of anti-poly(ADP-robose)IgG[4]. Such affinity-purified chromatin domains had single-stranded DNA gaps within the duplex DNA. However, since these domains also contained transcriptionally active chromatin domains, we had to develop an alternative approach for the study of DNA repair sites. Recently, we have made use of two independent techniques to specifically isolate DNA repair sites from rat

pachytene spermatocytes. It is known from the extensive work carried out on DNA replication intermediates that if there are two nicks on the same strand in the DNA duplex which are separated by less than 25 kb, upon mild alkali (30 mM NaOH) treatment and subsequent neutralization, the DNA strands between the nicks are released into the supernatant as ssDNA fragments[5]. Secondly, bromodeoxyuridine (BrdUrd) can be specifically incorporated into the DNA repair sites and BrdUrd containing ssDNA fragments can be isolated using anti-BrdUrd antibodies[6]. By combining these two techniques, we have now shown that approximately 0·7% of the genomic DNA undergoing repair activity in pachytene spermatocytes can be isolated as ssDNA. An analysis of the sizes of these ssDNA fragments revealed that they were of two size classes, namely, 4-7S and 8-12S. The possibility of the repair DNA being contaminated with replication intermediates in these DNA preparations was eliminated by two experimental findings: (1) the size distributions of ssDNA fragments released from bone marrow cells were much more heterogeneous in nature (7–20 S), and (2) there was very little cross-hybridisation (only 2%) between replication intermediates and the DNA repair sites in a slot blot hybridisation study.

We have also further analysed the DNA sequences in the rat genome that harbour the DNA repair sites by carrying out Southern blot hybridisation of DNA digested with various restriction enzymes with radioactive probes made against BrdUrd containing ssDNA fragments. Such an analysis revealed that in addition to some background hybridisation signals, strong hybridisation signals were observed with *Eco*RI (1·3 and 2·4 kb), *Hind*III (5 kb) and *Bam*HI (9 kb) repetitive DNA families. The *Eco*RI 1·3 kb repeat family DNA was subsequently subcloned in M13mp19 among which one repair-positive and one repair-negative clones were selected for sequence analysis. The nucleotide sequence of the repair-positive clone revealed several interesting features like the presence of: (1) $(CAGA)_6$ repeat, (2) $(CA)_{22}$ repeat, and (3) four sequences highly homologous to hypervariable minisatellite sequences (HVMS). One of these sequences contained within it a sequence 5′ GGCAGG 3′ which has been shown recently to be responsible for the instability of minisatellites in mouse[7].

On the other hand, the repair-negative clone contained a $(CA)_6$ repeat and only one sequence having a high homology to the HVMS sequences was reported. This sequence, however, did not contain a GGCAGG sequence within it. Therefore, the most striking difference between the repair-positive and the repair-negative clones was the presence of an HVMS sequence containing GGCAGG motif responsible for germ line instability of minisatellites only in the repair-positive clone. Interestingly, all the sequence motifs present in the repair-positive clone have characteristic functional properties. The $(CAGA)_6$ and $(CA)_n$ repeats belong to the microsatellite DNA family and can detect DNA polymorphism in human individuals. The $(CAGA)_6$ repeat has been shown to be present within the recombination hot spot of the MHC locus[8]. The alternating purine/pyrimidine stretches like the $(CA)_{22}$ repeat are known to attain Z-DNA conformation in negatively supercoiled plasmids[9]. Furthermore, they have been shown to stimulate recombination in the His-3-locus of yeast and in somatic

cells[10,11]. HVMS sequences are highly polymorphic and have been extensively used in DNA fingerprinting. A consensus HVMS sequence has also been shown to stimulate both reciprocal recombination and gene conversion events[12]. Because of the homology of HVMS sequence with the bacterial CHI sequence it has been postulated that these sequences may be eukaryotic counterparts of CHI, defining recombination hot spots in the eukaryotic genome. Recently, Jeffreys and coworkers reported the exchange of flanking markers in the region of an HVMS sequence in the human genome[13]. However, more direct evidence in support of its recombinator role is necessary. The significance of the presence of $(CA)_n$ and $(CAGA)_n$ sequences in the repair-positive clone is yet to be investigated. The presence of the germ line instability sequence within an HVMS sequence in the repair-positive clone possibly suggests that this locus is very important and the repair activity at this locus might be related to the events leading to generation of polymorphism.

Of the four HVMS-like sequences present in the repair-positive clone, we have demonstrated that one (18 mer) can indeed detect polymorphism in human individuals. DNA fingerprinting was carried out in the DNA samples consisting of a mother, father and twins and was probed with the 18 mer sequence. The twins shared identical fingerprints with bands corresponding to fragments either from the mother or the father. Four DNA samples from random individuals showed fingerprints, each distinct from the other. While the progress in DNA metabolic events during meiosis in higher animals and plants has been slow, there has been a rapid progress in our understanding of meiotic events in the yeast, *S. cerevesiae*. This has been possible particularly due to the ease with which sporulation can be induced in a synchronized manner. Several mutants have been described which are defective in the recombination process in *S. cerevesiae*. One class of genes is required for both mitotic and meiotic events like Rad 50–57 series of genes. Genes of the second class are required specifically for recombination and chromosome segregation which do not have any functional role in mitotic cells. The majority of such genes are expressed only during meiosis. One of the major findings in the recombination process in *S. cerevesiae* is the occurrence of double-strand breaks in the genome which has been shown to be an intermediate step in the recombination process[14]. Similar double-strand breaks have been shown to occur during V(D)J recombination of the immunoglobulin genes[15]. However, no double-strand breaks have yet been demonstrated during meiosis in animal and plant systems. It remains to be seen as to which of the models of recombination, the recombination events in spermatogenesis will fit into.

As mentioned earlier, another important aspect of chromosome behaviour during meiosis is the pairing of homologous chromosomes at the pachytene interval with the help of synaptonemal complexes. For several years, scientists have been interested in identifying the structural proteins of this important subnuclear structure. This was greatly hampered by the lack of suitable methods to isolate the SCs and characterize their protein components. However, in 1985, Heyting and coworkers[16] developed a method to isolate these structures and subsequently have identified two polypeptides of 30 and 33 kDa, which are specifically localized to the lateral elements of SCs[17] In addition, topoisomerase II has also been shown to be localized to SCs implicating

a role in chromosome disjunction[18]. By using genetic studies, Hollingsworth and Beyers[19] identified a HOP1 gene in *S. cerevisiae* whose protein product was shown to be essential for chromosome pairing. The HOP1 gene codes for a 70 kDa protein having a Cys_2/His_2 finger motif. A mutation in this motif disrupted the pairing process.

In one of our earlier studies, we set out to identify a protein specific to SCs in rat pachytene spermatocytes. By comparing the proteins of nuclear matrices isolated from pachytene and liver nuclei, we could identify a 110 kDa polypeptide that was specific to pachytene nuclear matrix[20]. However, subsequently this protein was shown to be a dimer of a 60 kDa polypeptide and identified as germ cell-specific lamin[21]. This was rather surprising since earlier workers had shown, by using antibodies to somatic lamins, that there is no laminar structure in meiotic and post-meiotic germ cells. We have shown that this germ cell-specific lamin is related but not identical to lamin B. It is present in the laminar structure of pre-meiotic spermatogonial cells. As the germ cells enter the meiotic prophase concomitant with the dissolution of the laminar structure, there is a redistribution of the $lamin_g$ within the pachytene nucleus. Following meiotic division, $lamin_g$ is the sole component of the laminar structure in round spermatids. Another interesting feature of $lamin_g$ is its conservation in the germ cells of all the species in the eukaryotic kingdom[20]. More recently, we have obtained monoclonal antibodies against $lamin_g$. Using these monoclonal antibodies, we have now been able to show it to be localised to the telomeric ends of the chromosomes possibly interacting with the inner nuclear membrane of the pachytene nucleus. Figure 2 represents the model we have proposed for the behaviour of laminar structure during spermatogenesis.

The pairing of homologous chromosomes through synaptonemal complexes is one of the landmarks of meiosis and recombination. The important question that is often raised is the mechanism of chromosome pairing during prophase. Although many of the earlier cytological and genetic evidences suggested that recombination ensues, following pairing, recent evidences generated by Nancy Kleckner's group using *S. cerevesiae* as the experimental system suggest that the recombination process involving homology search may be the mechanism by which pairing is initiated[21]. These experiments have clearly shown that double-strand breaks and recombination events precede the appearance of synaptonemal complexes. More recently, the results obtained from chromosome pairing technique have also favoured such a hypothesis[22]. However, it remains to be seen whether similar mechanisms operate in animal and plant system as well.

3. Conclusions and perspectives

We have seen over the last five years, tremendous advancement in our understanding of molecular aspects of mitotic cell division. This has been particularly possible with the availability of recombinant DNA technology and specific monoclonal antibodies. The same techniques are now being employed to unravel the mechanisms involved in meiotic cell division. This area of research which was purely confined to cytological

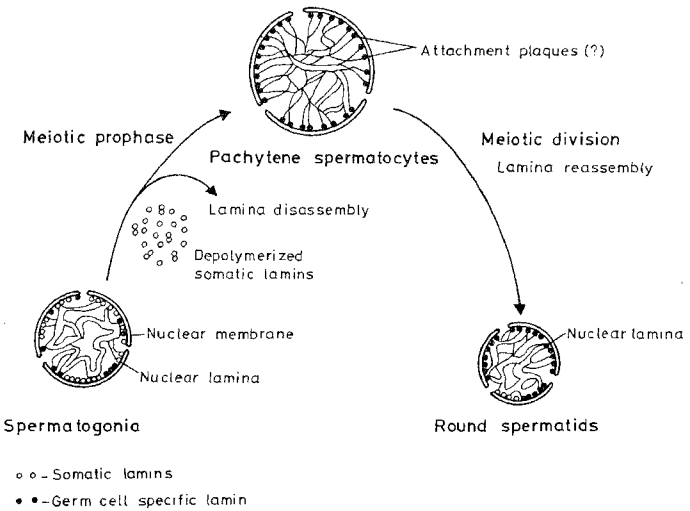

FIG. 2. A model describing the behaviour of lamina structure during meiotic division of mammalian germ cells (Sudhakar and Rao[21]).

techniques is now amenable for molecular approaches. The underlying phenomenon during meiosis, namely, chromosome pairing and genetic recombination will be understood in greater detail in the years to come. The sporulation event in the yeast *Saccharomyces cerevesiae* is being exploited to the fullest extent employing both molecular and genetic approaches. However, on the other hand, the meiotic division in higher eukaryotes, particularly mammals, is much more complex. There are many repetetive DNA elements present in higher eukaryotic cells that are absent in yeast. Furthermore, there is much more shuffling of the genome during meiosis involving minisatellite and microsatellite sequences generating polymorphism in the genome. There have been speculations that these events may involve the same machinery as of classical recombinational events. Some believe that these minisatellite sequences may be the site and cause of meiotic recombination while some others believe that the polymorphism generated at these loci are the effects of the meiotic events. Some of our recent studies in the rat spermatogenesis model system reviewed briefly in this article have given a DNA molecular probe to study many of these aspects of meiotic DNA transactions.

Another important aspect of chromosome behaviour during meiosis is the pairing

of homologous chromosomes and behaviour of the laminar structure. Some proteins are now being identified as structural components of synaptonemal complexes. The molecular cloning of their genes and their structure–functional relationships will give valuable insights into the molecular events of chromosome pairing. We have identified in our laboratory a unique germ cell-specific lamin which is antigenically conserved in the germ cells across eukaryotic kingdom. Similar cloning and structure–function studies on this protein will tell us its role in the organization of meiotic chromosomes.

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