COMMENTARY

Unravelling meiotic chromosomes: topoisomerase II and other proteins

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Introduction

With the advent of commercial electron microscopes, it was discovered by M.J. Moses (1956) and D.W. Fawcett (1956) that chromosomes at prophase of meiosis have well-defined axial elements that synapse homologously to form the synaptonemal complex, SC. This arrangement would appear to be eminently suited to the analysis of chromosome structure and function because each bivalent is distinct and the chromatin is still in a relatively decondensed interphase state. Indeed, much was learned from observations of meiocytes in a large number of sexually reproducing species (von Wettstein *et al.* 1984; John, 1990). However, it proved to be difficult to isolate and purify these chromosomes for molecular analysis (Walmsley and Moses, 1984; Ierardi *et al.* 1983), not least because of the attachment of the SC ends to the nuclear envelope. Consequently, initial progress in studying chromosome structure was made with the analysis of somatic metaphase chromosomes rather than meiotic prophase chromosomes.

Relevant to this commentary is the discovery that a major component of the residual scaffold of histone-depleted metaphase chromosomes was topoisomerase II, topo II (Earnshaw *et al.* 1985; Earnshaw and Heck, 1985; Gasser *et al.* 1986; Boy de la Tour and Laemmli, 1988). The enzyme provides a structural anchorage for the chromatin loops to the axial scaffold of the metaphase chromosome (Cockerill and Garrard, 1986; Mirkovitch *et al.* 1988; Izaurralde *et al.* 1988; Earnshaw, 1988). topo II is required to condense metaphase chromosomes (Uemura *et al.* 1987) and to untangle the replicated DNA strands so that the sister chromatids can segregate at anaphase of mitosis (Holm *et al.* 1989). It seems reasonable to expect that topo II is also a component of the meiotic chromosome.

Topoisomerase II at meiosis

In addition to chromatin condensation and chromatid segregation, meiotic chromosomes have two characteristics that may demand topo II activity. Not long after Moses and Fawcett had discovered the axial elements, G.F. Meyer (1964) correlated SCs with chiasmata (crossing-over, genetic recombination) by showing that a number of Diptera with SCs had chiasmata and those without SCs did not. Biochemically, Y. Hotta *et al.* (1979) confirmed the dependence of recombination on homologous chromosome synapsis by showing that in an achiasmatic *Lilium* hybrid that lacked SCs, pachytene DNA nicking and repair was suppressed in the presence of normal nuclease levels, but in colchicine-treated meiocytes, where the doubling of chromosomes permits normal synapsis and chiasma formation, the nicking and repair processes were restored. Reciprocal recombination connects a maternally derived chromosome with the paternal homologue and thereby introduces topological constraints on the eventual segregation of chromosomes at the first meiotic division. Indeed, diploid yeast with a genetic *top2/top2* topo II deficiency fail to complete chromosome segregation at the meiosis I division but in the presence of *rad50/rad50*, which blocks meiotic recombination, chromosome segregation at meiosis I is restored (Rose *et al.* 1990).

Another need for topo II in the segregation of chromosomes at meiosis I is generated by the interlocking of bivalents during synapsis. Early observations on metaphase I of meiosis did not give evidence of interlocks and it was assumed that by some process of homologous prealignment, interlocking was avoided. However, three-dimensional reconstructions of serially sectioned zygotene spermatocytes as well as whole-mount surface spreads gave abundant evidence of interlocked axial elements during the early stages of synapsis (Rasmussen, 1986). Apparently, by some mechanism or another, all the interlocks are resolved by the time the cells reach metaphase I. When S. W. Rasmussen (1977) described these interlocks at the meeting, 'A Discussion of the Meiotic Process' in 1975, H. Stern pointed out that the *Escherichia coli* type II topoisomerase, topo II, by the induction of reversible, protein-linked, double-strand DNA breaks could, if present in meiocytes, catalyse the passage of one chromosome across another. Evidence in favour of this prediction is now accumulating.

Studies of the biochemistry and molecular biology of meiotic chromosomes are limited by the lack of sufficient quantities of synchronous meiocytes. An effective but uncommon source is in the pollen mother cells of Easter lilies, in which Stern and coworkers were able to assay the activity of nuclear enzymes at successive stages of meiosis (Stern and Hotta, 1983). topo II proved to be present continuously throughout meiotic prophase with a peak at pachytene and a decrease at diplotene. The profile is consistent with a requirement for topo II activity in the resolution of interlocks at early prophase, in the condensation of chromatin during prophase, and in the resolution of recombined chromatids (Rose *et al.* 1990).

The existence of topo II in meiotic chromosomes was confirmed by immunolocalization with antibodies to topo II (Moens and Earnshaw, 1989). The presence of the

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antigen in the chromatin and axial elements at the early stages of chromosome synapsis is compatible with its role in the resolution of interlocked chromosomes. At later stages, the antigen concentrates more on the SC of the bivalents, consistent with the resolution at the SC of recombinant chromosomes and condensation of the chromatin adjacent to the SC. A similar distribution of topo II over the chromatin and SCs has been observed in *Bombyx* (moth) with anti-*Drosophila* topo II antibody (S. W. Rasmussen, personal communication) and in yeast with anti-yeast topo II antibody (F. Klein, personal communication). Thus the presence and localization of topo II in meiotic chromosomes is consistent with its proposed functions and it is constant across diverse species.

Other non-histone proteins of meiotic chromosomes

Whereas the $170\times10^3 M_r$ topoisomerase II is a prominent non-histone protein in SDS–PAGE gels of isolated somatic metaphase chromosomes and scaffolds (Earnshaw *et al.* 1985; Gasser *et al.* 1986), it is not a dominant band in gels from purified SCs (Heyting *et al.* 1985, 1989). Furthermore, monoclonal and polyclonal antibodies raised against purified SCs detect a number of proteins other than topo II in immunoblots. The most prominent and most frequently generated antibody is against a pair of related peptides of $30\times10^3 M_r$ and $33\times10^3 M_r$. These peptides appear to be structural components of the lateral elements (Moens *et al.* 1987), they are SC-specific, and they are conserved in a number of vertebrates tested. Monoclonal antibodies (mAbs) against a $125\times10^3 M_r$ SC protein recognize the central element pairing region of the SC, and mAbs against a $190\times10^3 M_r$ protein recognize lateral and central element components (Heyting *et al.* 1989). To an extent, the apparent failure to elicit anti-topo II antibodies may be an artifact. During screening of antibodies raised against purified SCs, those that react with chromatin as well as SCs are regularly found but are usually not pursued due to their lack of SC specificity. Anti-topo II displays such a staining pattern and would therefore be ignored.

The limitations of a search for and analysis of chromosomal proteins with antibodies raised against purified meiotic chromosomes are the inability to generate antibodies against low abundance proteins and the lack of information on the function of the proteins that are found. In the yeast, *Saccharomyces cerevisiae,* genetic manipulation offers the possibility of correlating functional and structural aspects. The cytological expression of meiotic mutants and the distribution of the gene products can be monitored in whole-mount spreads of meiotic chromosomes (Dresser and Giroux, 1988). During meiosis, the *RAD50* gene is required for recombination and the effect at the chromosomal level of the *rad50/rad50* genotype is the failure of axial elements to synapse during prophase (Alani *et al.* 1990). The *RAD50* gene product, however, does not appear to be a structural component of the meiotic chromosome. On the other hand, the *HOP1* gene product which is required for synapsis and consequently for recombination between non-sister chromatids, is a structural component of the SC. The zinc-finger motif of the HOP1 protein suggests a function in the binding of DNA to the SC (Hollingsworth *et al.* 1990).

Metaphase chromosome scaffolds contain, in addition to topo II, several unidentified proteins as well as the proteins of the centromere. Centromere proteins are recognized in whole-mount metaphase chromosomes and in immunoblots of scaffold proteins by anti-kinetochore antibodies from the sera of some patients with the CREST autoimmune disease (Earnshaw *et al.* 1984). Although meiotic prophase chromosomes are not undergoing division, centromere proteins are closely associated with the SC at the centromeric region of the SC, as identified by CREST serum (Moens *et al.* 1987).

Chromatin–SC interactions

The interaction between non-histone proteins of the meiotic chromosome and the chromatin or the DNA has not been defined. From visual evidence, it is clear that the chromatin is attached in loops to the SC (Weith and Traut, 1980; Rattner *et al.* 1981) but the type of scaffold attachment regions of the DNA loops, SARs, described in mitotic metaphase chromosomes has not yet been identified for meiotic chromosomes. Chromatin-to-SC attachment may be more flexible at meiosis because of the dynamic functions in homologous chromosome pairing and sequence matching for recombination. Of a variety of DNA probes tested so far, only two have been found that associate preferentially with the SC rather than the chromatin. Telomere DNA is localized at the ends of the SCs and a centromere-specific mouse (*Mus musculus*) satellite DNA is concentrated in the centromeric region of the SC. These associations probably depend on telomere and centromere proteins for binding to the SC and they suggest a general protein–protein interaction for the SC–chromatin interaction (Moens and Pearlman, 1989, 1990, and unpublished data).

From this survey, the conviction arises that the meiotic chromosomes are now living up to the original promise that they can give detailed insights into the structure and function of the chromosome.

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