REVIEW ARTICLE

Sister chromatid cohesion during meiosis: Differential mechanisms for arms and centromere coherence

Amit Bardhan

Department of Zoology, Banaras Hindu University, Varanasi 221 005, India

Sister chromatid cohesion is a fundamental aspect of chromosome behaviour which ensures faithful disjunction of chromosomes. The differential release of centromeric- and arm-cohesion during meiosis suggests that the molecular basis of cohesion in the two domains might be different. A number of meiotic mutations differentially affect cohesion in the two domains. Recent identification of two proteins, ORD and mei-S332, shows that they are involved in maintaining cohesion in two spatially different locations. While mei-S332 acts to structurally hold the sister centromeres together until second meiotic anaphase, ORD is likely to time the release of cohesion along the arms during first meiotic division.

HAD there been no physical connection between the replicated chromatids, the so-called sister chromatids, recognition of the sisters and their subsequent segregation during divisional cycle would have been an extremely complex process. Perhaps to sidestep such a complexity, the cell has evolved an apparently simple mechanism by which the sister chromatids are kept in association right from their replication in S phase. The term sister chromatid cohesion refers to this physical bonding between the replicated sisters. Fidelity of chromosome segregation in mitosis relies on the maintenance of cohesion between the sister chromatids until anaphase transition. The tension generated as a result of the interaction of sister kinetochores with spindle poles and the opposing force imparted by cohesion (between the sisters) leads to the stable bipolar arrangement of chromosomes on metaphase spindle[1]; cohesion is relieved at the onset of anaphase to enable the two sisters to move to opposite poles. In contrast to mitosis where both the centromere and arm cohesion are dissolved simultaneously, the specialized cell division of meiosis requires a *differential release of cohesion* (Figure 1). Meiosis involves two rounds of nuclear divisions following a single premeiotic S phase and results in halving the chromosome number. During the first (reductional) meiotic division homologous chromosomes segregate from each other, while during the second (equational) division of meiosis, sister chromatids disjoin. Cohesion at the centromere is maintained throughout the first meiotic division so that the two sisters are constrained to move to the same pole during anaphase-I, and only at the onset of anaphase-II the centromeric cohesion is released allowing the sisters' migration towards opposite poles. On the other hand, cohesion along homolog arms helps stabilize and maintain chiasma position[2,3], thereby enabling the bivalents to achieve a stable bipolar orientation on the metaphase-I spindle to secure reductional disjunction during anaphase-I. The arm cohesion is dissolved at metaphase-I/anaphase-I transition presumably for the release of chiasmata. Thus, sister chromatid cohesion has a two-fold significance in meiosis as compared to mitosis, to secure reductional disjunction of homologs during first meiosis and also equational segregation of the sisters during the second meiotic division, and a defect in either of the two might lead to aberrant segregation resulting in nondisjunction.

A variety of mechanisms have been proposed to explain cohesion between sister chromatids. The hypothesis that the late replicating centromeric DNA might serve to hold the sisters together proved to be untenable with the finding in budding yeast, *Saccharomyces cerevisiae* that centromeric DNA is fully replicated well ahead of the time of chromosome segregation[4]. The idea that catenation produced during DNA replication could play a role in physically holding the chromatids together[5] has received experimental support with the finding that DNA topoisomerase II (the enzyme which reversibly untangle interlocked DNA duplexes) activity is required for anaphase transition[6,7]. Also, topoisomerase II has been shown to be required for segregation of recombined chromosomes during first division of meiosis[8]. In addition to catenation that interlock two DNA helices (comprising the two sister chromatids), some protein(s) may act to reinforce cohesion as suggested by recent evidences, and they may function either as structural proteins gluing the sisters together, or as regulatory proteins in timing the release of cohesion.

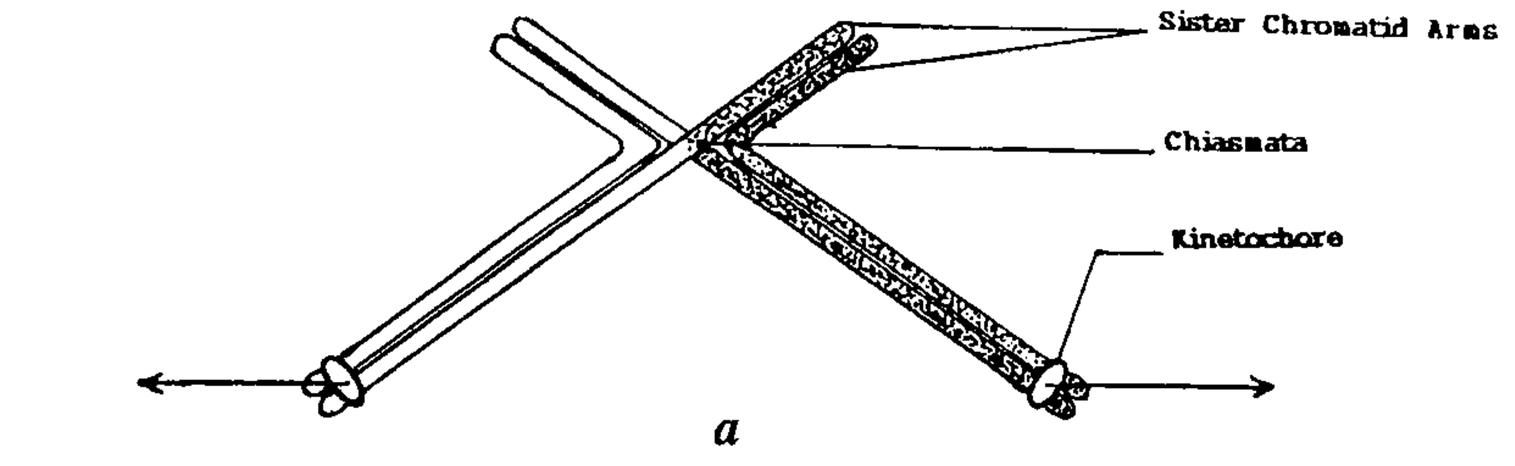

Figure 1. Chromosome behaviour during meiosis. *a*, A pair of homologous chromosomes (bivalent) connected by chiasmata, at metaphase-I. Sister chromatid cohesion is maintained both at the centromeres and along the arms; *b*, At anaphase-I, arm cohesion is dissolved (indicated by the gap between the chromatid arms) allowing the release of chiasmata, and the two homologs move to opposite poles; *c*, At metaphase-II, sister kinetochore differentiation occurs and they capture spindle microtubules from opposite poles; *d*, At anaphase-II, cohesion between the sister centromeres is released, allowing the sisters' migration towards opposite poles. In *c* and *d*, only one of the two homologous chromosomes is shown. In all the figures, arrows indicate the direction of 'pull' exerted on the kinetochore by spindle microtubules.

They include the gene products identified by a number of mutants defective in sister chromatid cohesion, and proteins identified by antibodies (see ref. 9 for a review). While catenation could provide the physical basis of cohesion that is common to both mitotic and meiotic chromosomes, the function of these proteins might be specific to either of the two divisions.

The differential release of centromeric and arm cohesion during meiosis suggests that different proteins might be involved in maintaining cohesion along the chromosome arms and at the centromere, and their mode of action might be mechanistically different. Alternatively, the same protein(s) might be shared in both the domains and only their removal/destruction is regulated differentially. That the molecular basis of cohesion at the centromere and along the arms could be different is also evident from the phenotypes of *Drosophila pim* and *thr* mutants. The *pim* and *thr* gene products are specially required for the release of cohesion between sister centromeres, but not between the arms, during mitosis[10,11].

This review addresses the probability that the mechanism of maintenance of cohesion might be different at

the centromeres and along the chromatid arms, at least during meiosis. To do this, we will survey some of the potential candidate cohesion genes which act in meiosis and try to examine whether their action is restricted to either of the two chromosomal domains (i.e. the centromere and the chromatid arms). We then discuss the identified cohesion proteins and the possible mechanisms of their action. Though the gene products which affect cohesion during mitosis are significant in their own right, they will not be discussed here for the sake of brevity.

Potential sister-chromatid cohesion mutations

Non-disjunction in meiosis-I might occur in either of two ways: absence or reduction of reciprocal genetic exchange (for a review, see ref. 12), or failure in maintenance of chiasmata. Although the mechanism of chiasma maintenance is still a matter of dispute (reviewed by Carpenter[13]), cohesion between sister chromatids could be one of the major contributing factors[3]. The reduced genetic exchange, or early loss of chiasma (or both), could lead to formation of univalents in diakinesis/metaphase-I and subsequent random movement of the univalents on first meiotic spindle. On the other hand, mis-segregation in meiosis-II might result from precocious release of cohesion between the sister centromeres followed by their independent movement, and an earlier incidence of failure of genetic exchange does not seem to affect anaphase-II segregation. However, it is known that presence of univalents in metaphase-I spindle could delay anaphase transition and the univalents could segregate equationally; the resulting sisters again mis-segregate during second meiosis. Therefore, it seems difficult to specify whether a nondisjunction event results primarily from a failure of cohesion, or is a manifestation of reduced genetic exchange. The cases where recombined chromosomes mis-segregate might be informative, and could be the result of precocious dissociation of sisters, since here the resulting (from recombination) chiasmata fails to bind and (thus) to regularize disjunction. However, an early dissociation (between sisters) event could affect recombination by affecting homolog association, thus making it probable that mis-segregation of non-recombinant chromosomes could result primarily from lack of cohesion instead of lack of genetic recombination *per se*. Identification of genes which regulate meiotic cohesion has been done by isolating mutants that mis-segregate during meiotic division(s). Though a direct visual observation of chromosome behaviour in mutants could undoubtedly demonstrate the involvement of a specific gene in cohesion, identification of genes has been hindered by poor chromosome morphology in systems which offer a powerful genetic tool, e.g. the yeast and *Drosophila*, although in the latter case the male meiosis has made cytological approach amenable. Therefore, to understand the involvement of specific genes in cohesion, we will survey some meiotic mutations (Table 1) which result in mis-segregation during meiotic division(s), and examine the likelihood that the primary defect is at the level of sister chromatid cohesion. Special emphasis will be put on situations where recombined chromosomes mis-segregate, and, where recombination frequency is not significantly reduced by the mutation.

A number of mutations show a rather pleiotropic effect, affecting frequency of genetic recombination and synaptonemal complex (SC) structure/function, besides affecting chromosome disjunction during meiosis. This group includes the *red1*, *rec8*, *spo76* and *ord*[14–21]. All of them produce highly aneuploid meiotic products. Following the segregation behaviour of a number of centromere-linked heterozygous markers, it has been found that the mutations primarily affect reductional (meiosis-I) disjunction. Majority of the disomes were heterozygous for the centromere-linked markers, suggesting that the mutations affect meiosis-I segregation (non-disjunction in meiosis-II would result in disomes with sister centromeres, rather than with homologous centromeres). Though the mutations reduce frequency of meiotic recombination and thus raise the possibility that non-disjunction results from lack of genetic exchange, genetic analyses have shown that even recombined chromosomes undergo mis-segregation in the mutants[14–16,19,21]. The latter observations suggest that mis-segregation results from failure in maintaining chiasmata which, in turn, might be the consequence of precocious release of cohesion between the sister chromatids early in meiosis-I. Fluorescent *in situ* hybridization analysis of meiotic prophase chromosomes in the *rec8* mutant of fission yeast has shown that sister chromatids get frequently separated during first meiotic prophase[16]. Cytological observations in *spo76* and *ord* mutants also show precocious separation of sister chromatids in early prophase-I of meiosis[18,20,21]. It has also been shown that meiotic synapsis is aberrant in the mutants with defective SC formation[15,18], except in *ord* where synaptic progression has not been analysed. Even in *ord* it is highly probable that meiotic synapsis gets affected, since the mutant reduces recombination frequency. In fission yeast where classical tripartite SCs are not formed, and instead a structure called linear elements (which are thought to be equivalent structure to the lateral element of SC of other organisms) are formed along the length of meiotic prophase chromosomes, formation of the latter also gets affected in the *rec8* mutant[16]. One reasonable possibility could be that component(s) of SC confer sister chromatid cohesion (as suggested by Maguire[22–24]), and deformed (or absence of) SC formation reduces the probability of meiotic recombination. Alternatively, it may be possible that sister

Table 1. Features of the mutations

Mutation	Level of mis-segregation	Recombination frequency	Type of mis-segregation	Meiotic synapsis	Somatic mitosis	Others
Saccharomyces cerevisiae						
red1	Meiosis-I	Reduced	Both recombined and non-recombined chromosomes	No SC assembly	Unaffected	
med1	Predominantly meiosis-I, also meiosis-II	Reduced	Recombined and non-recombined	Normal	Unaffected	
dis1	Meiosis-I	Unaffected	Recombined	Not detected	Increased non-disjunction	
Schizosaccharomyces pombe						
rec8	Meiosis-I; also meiosis-II?	Reduced	Recombined and non-recombined	Aberrant linear element formation	Unaffected ?	
Sordaria macrospora						
spo76	Meiosis-I	Reduced in heterozygotes	Recombined; also non-recombined?	Aberrant	Unaffected ?	Defective in UV damage repair; no meiosis-II
Zea mays						
dy	Meiosis-I; also meiosis-II?	Unaffected	Recombined	Precocious desynapsis	Unaffected ?	
Drosophila melanogaster						
ord	Predominantly meiosis-I	Reduced in females	Recombined and non-recombined	Not detected	Unaffected	Gonial mitosis slightly affected
mei-S332	Predominantly meiosis-II	Unaffected	Recombined	Not detected	Unaffected	Gonial mitosis slightly affected

chromatid cohesiveness is needed for proper homolog association and SC formation, and a defect in the former could lead to aberrant SC formation with reduced genetic recombination. However, the pleiotropic nature of the mutations suggests that they might as well be some regulatory components of meiosis, parallely affecting different meiotic events independent of each other. Indeed, isolation of one allele of *ord* (*ord*[6]) that affects recombination to the same extent as do the other alleles but affects disjunction less frequently[21] suggests that the gene can differentially influence the two processes, strengthening the direct involvement of the gene in maintaining cohesion.

The yeast *med1* mutation is also somewhat similar to the above discussed ones in the sense that it results in high level of meiosis-I, and to some extent meiosis-II, non-disjunction, and also reduces frequency of recombination[25]. But, in contrast to the mutations already described, SC formation is apparently normal in the *med*[1] mutant[25]. Increased spore viability of *med1 spo13* double mutant suggests that *med1* function is required for reductional division of meiosis[25] (the *spo13* mutant undergoes a single meiotic division of predominantly equational nature; thus, meiotic lethal mutants which are defective in recombination produce viable meiotic products in a *spo13* background since *spo13* skips first meitoic division[26]). Although reduced recombination elevates meiosis-I non-disjunction in the mutant, a good number of the disomes result from precocious separation of recombined chromatids followed by their random segregation through both meiotic divisions[25]. Though it has been suggested that the defects in cohesion as well as recombination are secondary consequences of perturbed chromatin structure caused by the mutation[25], it cannot be ruled out that MED1 is a regulatory protein independently affecting cohesion and recombination. It may even be possible that MED1 function is required specifically for arm cohesion and a defective cohesion along the arms reduces probability of meiotic recombination by affecting homolog association in early meiosis-I. The *med1* mutant phenotype appears to be very similar to that of *ord* in *Drosophila*. Cloning and sequencing of *med1* will show whether the gene is homologous to the *Drosophila ord* gene.

Another good candidate for meiotic cohesion is the maize *dy* gene. In the homozygous *dy* mutants, sporadic precocious desynapsis of bivalents follows apparently normal pachytene synapsis. By diakinesis, bivalents often separate to univalents which lag for some time on the spindle and then segregate equationally during anaphase-I[27,28]. Frequency of meiotic crossing-over appears to be unaffected in the mutant (a distal heterozygous marker, a heterochromatic 'knob', was found to be separated equationally in most of the cases, confirming the occurrence of an exchange event between the knob and the centromere[28]). The inferred crossover frequencies in

the mutant were equal and consistent with the expected wild type crossover frequency. The results show that the mutation has no apparent effect on meiotic recombination but it affects maintenance of chiasmata. Ultrastructural observations of synaptic progression showed that the central region of the SC disintegrates early, causing precocious desynapsis in the mutant. It has been suggested that one of the late functions of SC is to provide for sister chromatid cohesion, and that the lack of sister cohesiveness results in early loss of chiasmata[22–24, 27–29].

The yeast *dis1* and *Drosophila mei-S332* mutations apparently affect cohesion specifically at the centromere region, but the time of their expressions differs. While *dis1* predominantly affects meiosis-I segregation[30], *mei-S332* impairs meiosis-II disjunction[20,31,32]. Both the mutants have no apparent effect on recombination[30–32], suggesting that they do not interfere with homolog association, and non-disjunction involves almost all the chromosomes of the complement. Genetic analysis in *dis1* suggests that non-disjunction results from precocious separation of sister chromatids at meiosis-I followed by their equational, rather than random, segregation[30]. Cytological observation of meiotic segregation in *mei-S332* mutant males reveals that sister chromatids frequently dissociate in mid- to late-anaphase-I, resulting in non-disjunction or lagging chromosomes in anaphase-II[20,31,32]. The results suggest that although both the mutations affect specifically the centromeric cohesion (disrupted cohesion along the arms would reduce the probability of exchange event), the mechanism of their action most likely differs. *Dis1* might act to regulate functional differentiation of sister kinetochores, and an early differentiation in the mutant leads to premature separation of sister chromatids (if *dis1* were to maintain cohesion after sister kinetochore differentiation, the mutant would cause only meiosis-II non-disjunction). On the other hand, *mei-S332* is likely to act to physically hold the sister kinetochores, after their functional differentiation at prometaphase-I and early anaphase-I. *Dis1* differs from *mei-S332* in another respect, in that, it induces high level of mitotic non-disjunction as well[30], suggesting that *dis1* function might be shared between the two types of nuclear divisions.

It seems probable that a number of mutations could affect cohesion along the chromatid arms during meiosis, viz. *red1*, *spo76*, *med1*, *rec8*, *dy* and *ord*. All of them (except *dy*) reduce frequency of meiotic recombination, and also affect maintenance of chiasmata. This is expected from an aberrant association between the arms of sister chromatids during meiosis-I. A few of them might also act to maintain centromeric cohesion. However, it seems untimely to draw any conclusion about their involvement in centromeric cohesion, except to mention safely that if they are to maintain cohesion at the centromeres, the mechanism might be different compared to what operates for arm cohesion (discussed later).

Molecular mechanism of cohesion during meiosis

Recent identification of two proteins from *Drosophila*, mei-S332 and ORD, which are shown to be involved in maintaining sister chromatid cohesion in meiosis has provided a critical insight into the molecular basis of cohesion[33,34].

Kerrebrock *et al.*[33] have cloned the *Drosophila mei-S332* gene and have shown that the gene encodes a novel 44 kDa protein. Genetic and cytological analyses suggest a role for the protein in promoting cohesion specifically at the centromere region, after differentiation of sister kinetochores. This has been corroborated by the localization of the mei-S332 protein (fused to jelly fish Green Fluorescent Protein) on meiotic chromosomes. The localization pattern of the protein (on meiotic chromosomes) is consistent with the idea that the protein functions as a structural component to hold the sister centromeres together. Mei-S332.GFP localizes to the centromeric region of the meiotic chromosomes, starting from prophase-I onwards until metaphase-II (ref. 33). Strikingly, at the onset of anaphase-II the protein suddenly disappears from the centromeres and is no longer detectable[33], which is consistent with the requirement for the release of cohesion if the protein is to act to physically hold the two sisters together.

The *Drosophila ord* gene has been cloned and shown to encode a novel 55 kDa protein[34]. *Ord* transcribes only in adult ovary and testis, and in no other tissues or developmental stages, suggesting its meiosis-specific function. The low abundance of *ord* message suggests that the protein is likely to maintain cohesion in a regulatory manner, rather than as a structural component[34]. The effect of *ord* mutation on meiotic recombination is consistent with a regulatory role of the protein. Some of the *ord* alleles exhibit a unique phenomenon of negative complementation where one allele poisons the activity of another. When the mild allele ord^4, which retains a high amount of residual activity even in *trans* to a deficiency for the locus, is placed *trans* to some other *recessive* alleles (ord^1, ord^2, ord^6), the level of malsegregation increases significantly[34]. However, other recessive alleles (ord^3, ord^5) do not interfere with residual function of ord^4. Sequence analyses have shown that in ord^1, ord^2 and ord^6 (which impair ord^4 function), the mutations residue in the C-terminal halves of the proteins, while the ones that do not interfere with ord^4 function lack the C-terminal halves from missense mutations. This suggests that the C-terminal half of the protein is crucial for its function. Bickel *et al.*[34] have suggested that functioning of ORD requires protein–protein interaction through C-terminal halves. They have proposed that the ORD protein is having two functional domains, one for protein binding and another for exert-

ing cohesion function, and that cohesion requires binding but binding does not ensure cohesion (see Figure 5 in ref. 34). Since ORD dimerization could not be detected, it is most likely that the protein interacts with some other protein molecule, which is yet to be identified[34]. The alleles which poison the activity of *ord*[4] might have altered 'cohesion domain' but intact 'protein binding domain' and form non-functional complex with the interacting partner, thus outcompeting *ord*[4] (which might have slightly altered 'protein binding domain' but intact 'cohesion domain') to form functional complex with the protein partner[34].

Another protein, Cor1, isolated from hamster has been suggested to maintain cohesion on the basis of observation that it localizes between sister chromatids of meiotic chromosomes (but not mitotic chromosomes)[35]. Cor1 is a component of the lateral element of SCs (ref. 35), and its localization pattern on meiotic chromosomes is consistent with a role of the protein in maintaining cohesion until second meiotic anaphase. Cor1 has been shown to localize axial to metaphase-I chromosomes with a substantial amount in association with pairs of sister centromeres. Interestingly, the centromeric Cor1 persists up to metaphase-II and dissociates at the onset of anaphase-II, which suggests that the protein might have a role in ensuring co-segregation of sister chromatids at anaphase-I (ref. 35). However, the evidence is rather correlative and it seems difficult to analyse directly the involvement of Cor1 in sister chromatid cohesion. However, the large body of circumstantial evidences for a role of some SC components in imparting cohesion is consistent with the role attributed to Cor1.

Concluding remark

A handful of potential candidate genes have been identified from diverse organisms, but till date only two candidate proteins could be identified which are unambiguously involved in maintaining sister chromatid cohesion during meiosis. With the identification of mei-S332 and its localization on meiotic chromosomes, it is evident that this particular protein is required specifically to maintain centromeric cohesion during meiosis, and the gene product has no apparent contribution in maintaining arm cohesion. On the other hand, the *ord* mutant phenotype manifests at a time when arm cohesion is very crucial (for maintaining chiasmata), and it is most likely that the ORD protein acts to maintain cohesion at least along the chromatid arms. Though mei-S332 localizes to meiotic chromosomes from prophase-I onwards, the mutant phenotype expresses very late, at a time when the sister kinetochores are functionally duplicated. It is probable that during first meiotic division, maintaining cohesion at the centromeres is not very crucial (as the sister kinetochores are not differentiated, see Goldstein[36]), and ORD can very well maintain the structural integrity of bivalents, even in absence of mei-S332, to secure proper reductional disjunction. Only when the sister kinetochores differentiate, proper functioning of mei-S332 becomes crucial to physically hold the two sisters together until anaphase-II transition. There is some evidence that ORD could promote centromeric cohesion as well[21]. However, the mechanisms of its functions at the centromere and along the chromatid arms might be different, and requires that during metaphase-I/anaphase-I transition function of ORD be preferentially removed from the chromatid arms while at the same time be retained at the centromere. It may be possible that different forms of ORD exist and their function might be mechanistically different[34]. ORD along chromatid arms might act through regulating the release of topoisomerase II inhibition, while ORD at the centromeric region might regulate functional duplication of sister kinetochores (the release of arm cohesion requires topoisomerase II, but the activity of the enzyme has been shown to be dispensable for the release of centromeric cohesion; see ref. 37).

Mutational analyses have shown that different centromeric sequences differentially affect the segregation behaviour of yeast mitotic and meiotic chromosomes. While some sequences are required for sister chromatid cohesion and faithful segregation of chromosomes during meiosis, others are important for fidelity of mitotic disjuction[38–44]. Recently, Murphy and Karpen[45] have carried out molecular and functional characterization of *Drosophila* centromere on a minichromosome, Dp1187. They have shown that besides the sequences which are necessary for minimal centromeric function, an additional ~200 kb of heterochromatic pericentromeric sequences on either side of the minimal centromere is required for faithful disjunction of chromosomes. Moreover, partial deletion of the ~200 kb repetitive sequence differentially affects segregation of chromosomes in male and female meiosis, which suggests that there are meiosis-specific functions residing in the pericentromeric heterochromatin. They have proposed a model in which the ~200 kb heterochromatic sequences are involved in maintaining sister chromatid cohesion by binding with cohesion proteins (Figure 7 *a* in ref. 45). The model is consistent with the demonstration that heterochromatin is involved in meiotic chromosome segregation[46]. Sekelsky and Hawley[47] have suggested that the mei-S332 protein might bind to the ~200 kb pericentromeric heterochromatic sequences to exert its function. However, such a binding is yet to be demonstrated. This can be done by examining the binding of mei-S332 *in vitro* to the -AATAT- satellite which comprises the ~200 kb pericentromeric sequences. Alternatively, localization of the protein on derivatives of Dp1187 might clarify this point. Lastly, it needs to be determined whether the removal of the function of mei-S332 and

ORD requires degradation of the proteins. The abundance of PEST sequences in both the proteins has been implicated as an indication that the proteins might undergo degradation at the onset of anaphase transition, through an ubiquitin independent pathway[33,34].

The recent identification of two proteins involved in maintaining cohesion between sister chromatids promises a better understanding of some basic aspects of meiosis research, particularly, the factor(s) determining chromosome segregation and the mechanism of chiasma binding. The molecular analysis of centromere function in *Drosophila*, and the identification of ORD and mei-S332 proteins has already laid the foundation for the identification of additional protein(s) which might be involved in maintaining sister chromatid coherence.

1. Nicklas, R. B., *Genetics*, 1974, **78**, 205–213.
2. Darlington, C., *Recent Advances in Cytology*, Churchill, London, 1932.
3. Maguire, M. P., *Maydica*, 1993, **38**, 93–106.
4. McCarroll, R. M. and Fangman, W. I., *Cell*, 1988, **54**, 505–513.
5. Murray, A. W. and Szostak, J. W., *Annu. Rev. Cell Biol.*, 1985, **1**, 289–315.
6. Holm, C., Goto, T., Wang, J. C. and Botstein, D., *Cell*, 1985, **41**, 553–563.
7. Uemura, T. and Yanagida, M., *EMBO J.*, 1986, **5**, 1003–1010.
8. Rose, D., Thomas, W. and Holm, C., *Cell*, 1990, **41**, 553–563.
9. Miyazaki, W. Y. and Orr-Weaver, T. I., *Annu. Rev. Genet.*, 1994, **28**, 167–187.
10. D'Andrea, R. J., Stratmann, R., Lehner, C. F., John, U. P. and Saint, R., *Mol. Cell. Biol.*, 1993, **4**, 1161–1174.
11. Stratmann, R. and Lehner, C. F., *Cell.*, 1996, **84**, 25–35.
12. Hawley, R. S., in *Genetic Recombination* (eds. Kucherlapati, R. and Smith, G. R.), American Society for Microbiology, Washington DC, 1988, pp. 497–527.
13. Carpenter, A. T. C., *Cell*, 1994, **77**, 959–962.
14. Rockmill, B. and Roeder, G. S., *Proc. Natl. Acad. Sci. USA*, 1988, **85**, 6057–6061.
15. Rockmill, B. and Roeder, G. S., *Genetics*, 1990, **126**, 563–574.
16. Molnar, M., Bahler, J., Sipiczki, M. and Kohli, J., *Genetics*, 1995, **141**, 61–73.
17. De Veaux, L. C. and Smith, G. R., *Genes Dev.*, 1994, **8**, 203–210.
18. Moreau, P. J. F., Zickler, D. and Leblon, G., *Mol. Gen. Genet.*, 1985, **198**, 189–197.
19. Mason, J. M., *Genetics*, 1976, **84**, 545–572.
20. Goldstein, L. S. B., *Chromosoma*, 1980, **78**, 79–111.
21. Miyazaki, W. Y. and Orr-Weaver, T. I., *Genetics*, 1992, 1047–1061.
22. Maguire, M. P., *Exp. Cell Res.*, 1978, **112**, 297–308.
23. Maguire, M. P., *Chromosoma*, 1979, **70**, 313–321.
24. Maguire, M. P., *Chromosoma*, 1982, **84**, 675–686.
25. Rockmill, B. and Roeder, G. S., *Genetics*, 1994, **136**, 65–74
26. Klapholz, S. and Esposito, R. E., *Genetics*, 1980, **96**, 589–6
27. Maguire, M. P., *Chromosoma*, 1978, **65**, 173–183.
28. Maguire, M. P., Paredes, A. M. and Riess, R. W., *Gen* 1991, **34**, 879–887.
29. Maguire, M. P., *Biochem. Cell Biol.*, 1990, **68**, 1231–1242.
30. Rockmill, B. and Fogel, S., *Genetics*, 1988, **119**, 261–272.
31. Davis, B., *Mol. Gen. Genet.*, 1971, **113**, 251–272.
32. Kerrebrock, A. W., Miyazaki, W. Y., Birnby, D. and Weaver, T. L., *Genetics*, 1992, **130**, 827–841.
33. Kerrebrock, A. W., Moore, D. P., Wu, J. S. and Orr-We T.L., *Cell*, 1995, **83**, 247–256.
34. Bickel, S. E., Wyman, D. W., Miyazaki, W. Y., Moore, I and Orr-Weaver, T. L., *EMBO J.*, 1996, **15**, 1451–1459.
35. Dobson, M. J., Pearlman, R. F., Karaiskakis, A., Spyropo B. and Moens, P. B., *J. Cell Sci.*, 1994, **107**, 2749–2760.
36. Goldstein, L. S. B., *Cell*, 1981, **25**, 591–602.
37. Funabiki, H., Hagan, L., Uzawa, S. and Yanagida, M., *J. Biol.*, 1993, **121**, 961–976.
38. Sears, D., Hegemann, J., Shero, J. and Hieter, P., *Gen* 1995, **139**, 1159–1173.
39. Murphy, M. R., Fowlkes, D. M. and Fitzgerald-Hayes, *Chromosoma*, 1991, **101**, 189–197.
40. Jehn, B., Niedenthal, R. and Hegemann, J. H., *Mol. Cell. I* 1991, **11**, 5212–5221.
41. Hahnenberger, K., Carbon, J. and Clarke, L., *Mol. Cell. I* 1991, **11**, 2206–2215.
42. Clarke, L. and Baum, M., *Mol. Cell. Biol.*, 1990, **10**, 1863–1872
43. Gaudet, A. and Fitzgerald-Hayes, M., *Genetics*, 1989, **121**, 477–
44. Cumberledge, S. and Carbon, J., *Genetics*, 1987, **117**, 203–
45. Murphy, T. and Karpen, G., *Cell*, 1995, **82**, 599–609.
46. Dernburg, A. F., Sedat, J. W. and Hawley, R. S., *Cell*, 1996 135–146.
47. Sekelsky, J. J. and Hawley, R. S., *Cell*, 1995, **83**, 157–160.

ACKNOWLEDGEMENTS. I thank Prof. T. Sharma, Prof. S Lakhotia and Dr Rajiva Raman, for their suggestions and inter also thank T. K. Rajendra, Girdhar G. Sharma, Koushalya Sha Satheesh C. R. and M. Subbarayan for helpful comments or manuscript. I also acknowledge the Cytogenetics Laboratory providing the facilities. Financial support from UGC, New Delh the form of fellowship is gratefully acknowledged.

Received 24 December 1996; accepted 13 February 1997