# A bouquet of chromosomes

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## Summary

**During meiotic prophase, telomeres attach to the inner nuclear envelope and cluster to form the so-called meiotic bouquet. Although this has been observed in almost all organisms studied, its precise function remains elusive. The coincidence of telomere clustering and initiation of chromosome synapsis has led to the hypothesis that the bouquet facilitates homologous chromosome pairing and synapsis. However, recent mutant analysis suggests that the bouquet is not absolutely required for either homologous pairing or synapsis but that it makes both processes much faster and more efficient. The initiation of bouquet formation is independent of the initiation of recombination. However, the progression through recombination and synapsis may be required for exit from the bouquet stage. Little is known about the mechanism of telomere clustering but recent studies show that it is an active process.**

Movies available online

Key words: Meiosis, Telomere clustering, Bouquet, Chromosome pairing, Synapsis

## Introduction

Meiosis is a specialized cell division required to halve the genome content in preparation for gamete development and ultimately fertilization. An important feature of meiosis is that homologous chromosomes find each other, pair, recombine and synapse along their whole length. This process not only leads to genetic diversity but is also important in a mechanistic sense because it leads to formation of chiasmata. These are structures that hold homologous chromosomes together until the first anaphase and thus ensures each daughter cell at meiosis I receives only one homolog from each pair. Before homologous chromosome pairing and synapsis begin, chromosome ends attach to the inner nuclear envelope (NE) and cluster to form a structure that, very loosely, resembles a bouquet of flowers. The 'bouquet' stage has been observed in all organisms studied regardless of whether they have big (maize) or small (fission yeast) genomes (Dernburg et al., 1995; Scherthan, 2001), except *Caenorhabditis elegans* and *Drosophila*, which both employ non-canonical methods of homology searching (McKee, 2004). For organisms with a bouquet, the fact that bouquet initiation just precedes the onset of homologous pairing and synapsis has led to the suggestion that bouquet formation directly facilitates homologous chromosome pairing, synapsis and homologous recombination by bringing the ends of chromosomes into close proximity and coalignment. Alternatively, it may play some other role.

We make a distinction between chromosome pairing and chromosome synapsis. By pairing, we mean whatever processes are used to bring chromosomes into co-alignment and to search base pairs for homology. Synapsis is a separate process and involves installation of the central element of the synatonemal complex (SC), which glues the two homologs together along their lengths. We know pairing and synapsis are separate events because homology is not required for synapsis (Pawlowski et al., 2004).

The mechanism of telomere clustering and the molecules needed to bring it about have remained obscure partly because it has not been possible to observe telomere clustering in living cells and few mutants exist that would aid in the identification of proteins associated with the bouquet. However, progress has been made through analysis of bouquet formation in mutant meiocytes in several model organisms, including maize, mouse, and budding and fission yeast. Below, we discuss results from studies that suggest that bouquet formation is an active process, as well as review evidence indicating that the function of the meiotic bouquet is actually to make meiotic prophase much faster and more efficient.

## The bouquet in context

The various stages of meiotic prophase historically were defined by the cytology of the chromosomes (reviewed by John, 1990); Fig. 1 shows some of these stages as they appear in maize meiocytes. Movies of the 3D data used to make these projections are available as supplemental material (http://jcs.biologists.org/supplemental/), and these show the positions of the telomeres much more clearly. In leptotene (Fig. 1A), decondensed clouds of chromatin are organized into long thin fibers by the assembly of a protein fibrous core, the axial element, onto the chromosomes. During zygotene (Fig. 1C-E), homologous chromosomes begin to associate tightly along their length or synapse when the central element of the SC is installed. It is at the beginning of zygotene, or just before, that the chromosomes form the bouquet. In maize, bouquet formation is initiated during a conspicuous change in chromatin morphology (see below) at the leptotene-zygotene transition (Fig. 1B) (Bass et al., 1997). The bouquet persists through zygotene in some organisms and through early pachytene (Fig. 1F) in maize and certain other organisms (e.g. Pfeifer et al., 2003). By mid-pachytene (Fig. 1G,H), SC formation is complete, meiotic recombination between homologs is resolved and telomeres are no longer clustered.

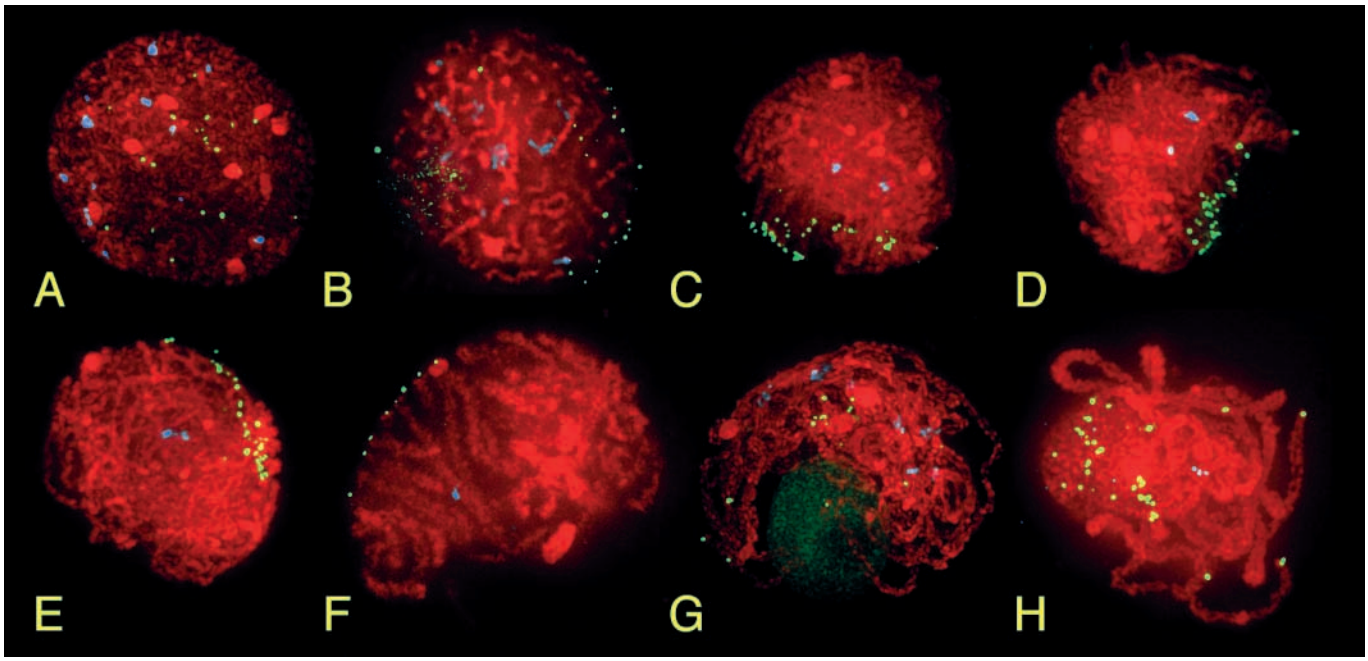


**Fig. 1.** Chromatin rearrangement and bouquet formation in meiotic prophase of maize. Images of each nucleus were acquired using a DeltaVision Microscopy system (Applied Precision) followed by computational deconvolution (Golubovskaya et al., 2002). This results in a 3D data stack. Shown here are 2D projections from whole or partial 3D data stacks. We encourage you to view the movies of rotating 3D images of these same nuclei in the supplemental material (http://jcs.biologists.org/supplemental/). Chromatin (stained with DAPI) is shown in red; telomeres (FITC) are shown in green; centromeres (Cy3 or Cy5) are shown in blue in A, B and G; and the single 5SrRNA (Cy3 or Cy5) locus is shown in blue in C, D, E, F and H. (A) Leptotene. Telomeres and centromeres are distributed throughout the nuclear volume. Chromosomes can be seen as thin threads almost like beads on a string, and heterochromatin knobs appear spherical. (B) Late leptotene. Telomeres are attached to the inner surface of the nuclear envelope (NE). Centromeres are distributed throughout the nuclear volume. Chromosomes are still completely unsynapsed but appear more condensed. (C) Early zygotene. Telomeres are gathered in a narrow patch on the NE, but are not yet completely clustered. Chromatin is more condensed, and some regions are beginning to synapse. This can be seen more easily in the supplemental material as thicker and apparently paired regions of the chromosomes. Some knobs are round, and some are elongated. The two 5SrRNA foci, seen here as blue dots, one on each homolog, are obviously not paired. (D,E) Classic zygotene. Telomeres are clustered in the bouquet. These cells are very typical of the maize bouquet. Chromatin structure has not changed from earlier zygotene, except that more chromosome regions are paired. This is readily apparent in the supplemental material. The 5SrRNA foci are still not paired, but we imagine that they are in the process of pairing in E (compare distances between two foci in C, D and E). (F) Pachytene. All chromosomes are completely paired and synapsed. This can be seen as a doubled chromatin width (compare E and F), and there is only one 5SrRNA focus. All telomeres are still on the NE, but they are beginning to disperse from the bouquet. (G,H) Late pachytene. All chromosomes are still completely paired and synapsed, but telomeres are being released from the NE. Centromeres (G) are dispersed throughout the nucleus, and the 5SrRNA foci is paired (H). The chromatin appears thinner in G owing to a different fixation.

After the bouquet is dispersed, the cell continues through diplotene, when the SC disassembles and chiasmata, which hold the homologs together until anaphase 1, become visible. In diakinesis, the chromosomes undergo a final stage of chromosome condensation just before NE breakdown and the initiation of metaphase.

In maize, at the leptotene-zygotene transition, there is an abrupt shift in telomere and centromere behavior. Before zygotene, centromeres are confined to one nuclear hemisphere; afterwards, they have no obvious nuclear organization. Telomeres behave the opposite way: prior to zygotene there is no observable polarity; afterwards the telomeres are both polarized and clustered on the NE (Carlton and Cande, 2002). In this context, polarized means that centromeres and/or telomeres localize to one hemisphere of the nucleus. At the leptotene-zygotene transition, this shift is ‘caught in the act’, such that most telomeres lie in one nuclear hemisphere and most centromeres lie in the other (because this is difficult to see in flat projections, the reader is encouraged to see the supplemental movies, http://jcs.biologists.org/supplemental/). Similar dramatic changes in polarity have been described in other organisms. For example, in fission yeast, centromeres are normally clustered near the spindle pole body (SPB) in haploid cells, and telomeres are loosely grouped at the other end of the nucleus; as cells mate and nuclei fuse and meiosis begins, the telomeres cluster at the SPB and the centromeres are released (Chikashige et al., 1997). The polarization switch in maize coincides with the transient elongation of large blocks of heterochromatin called knobs and of centromeric heterochromatin, which is consistent with the notion that the large-scale changes in nuclear organization might be mediated by changes at a lower-order level of chromatin organization (Carlton and Cande, 2002).

The meiotic bouquet is readily visualized at the light microscope level in many organisms, in which one can follow the path of the condensed threadlike zygotene chromosomes

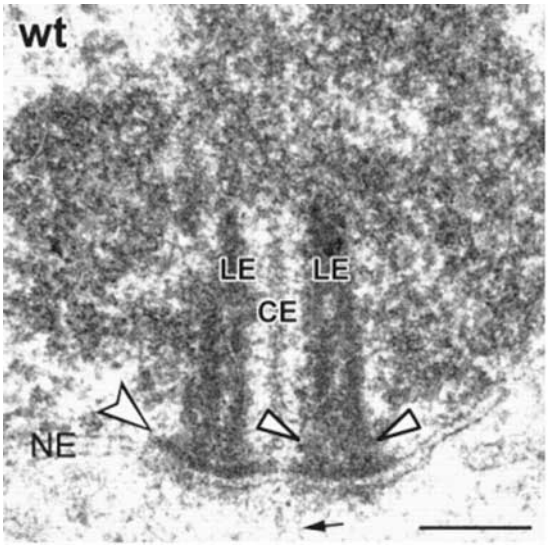


**Fig. 2.** Transmission electron microscopy (TEM) of telomere attachments in the wild-type mouse spermatocyte I nucleus. This TEM shows electron-dense lateral elements (LE) and the central element (CE) of the synatonemal complex (SC) terminate with a conical thickening (arrowheads) at the inner nuclear envelope (NE). An electron-dense plate connects the wide end of the LE thickening with the inner NE (indented arrow). Bar, 0.2 μm. Reproduced from Molecular Biology of the Cell (Liebe et al., 2004), with permission from The American Society for Cell Biology.

**Fig. 3.** The telomere bouquet in *Schizosaccharomyces pombe*: the horsetail stage. Heterochromatic centromeres and telomeres are visualized with swi6-GFP (bright dots), which also lightly stains the entire nucleus. Here, the nucleus is elongated, as it is whipped about the cell in a swishing horsetail-like movement. To see this movement, please view our supplemental movies (http://jcs.biologists.org/supplemental/). Telomeres are clustered at one point in the nucleus, on the left, which is adjacent to the spindle pole body (SPB) attached to cytoplasmic microtubules (not visible here). Two centromeric dots can be seen in the interior of the nucleus. The third centromere is out of the plane of focus. Image courtesy of Ye Jin, UC Berkeley.

within the nucleus (Scherthan, 2001). In some organisms, including maize, visualization of the bouquet is difficult in squashes, where chromosome orientation is perturbed. In other organisms such as yeast (Trelles-Sticken et al., 1999) and *Arabidopsis* (Armstrong et al., 2001), the bouquet can be seen in chromosome squashes. However, fluorescent in situ hybridization (FISH) using telomere and centromere probes, together with 3D reconstructions of nuclei imaged by deconvolution or confocal laser scanning fluorescence microscopy, have rendered the analysis of the bouquet much easier. In addition, transmission electron microscopy (TEM) has shown that, in metazoans and plants, attachment plaques form on the inside of the NE just before the bouquet stage and the axial elements that form the lateral elements of the SCs appear to insert into the plaques (Fig. 2). These structures move closer together as the bouquet forms. The proteins involved in forming this attachment are not known. Although mouse spermatocytes lacking the SC protein SCP3 lack axial elements and conical thickenings, telomeres are still attached to the inner NE through an electron-dense plate that contains telomere DNA repeats and several cohesins (Liebe et al., 2004).

## The meiotic bouquet and the Rabl arrangement

The Rabl and bouquet arrangements of chromosomes in the nucleus were first described at the turn of the 19th century (reviewed in Scherthan, 2001). These two classes of polarized arrangement of centromeres and telomeres in the nucleus are easy to distinguish. The Rabl arrangement of telomeres and centromeres on opposite sides of the somatic interphase nucleus is a product of anaphase chromosome movement. The centromeres are pulled towards the spindle pole during anaphase and reach the side of the nucleus adjacent to the centrosome, and the telomeres are at the other side of the reformed nucleus. This relationship is maintained in some cases after NE reformation and chromosome decondensation. By contrast, in the bouquet organization, a tight cluster of telomeres is attached to the inner NE, and the centromeres are distributed throughout the nucleus. Other components of the nucleus are also rearranged at this stage – for example, the nuclear pores. The telomere cluster lies opposite the cluster of nuclear pores in rye (Cowan et al., 2001) and nuclear pores are excluded from the telomere-enriched domains in mouse meiocytes (Scherthan et al., 2000b). In meiotic cells in which centrosome position can be determined, the telomeres are adjacent to the centrosome and the centromeres are distributed elsewhere in the nucleus, usually in the other nuclear hemisphere. An extreme example is fission yeast. During the bouquet stage, which is extended compared with that in other organisms, the telomeres are attached in a tight cluster to the inner NE immediately adjacent to the SPB; by contrast, during mitotic interphase, centromeres are associated with the SPB and telomeres lie dispersed elsewhere in the nucleus (Davis and Smith, 2001) (Fig. 3, and supplemental movies, http://jcs.biologists.org/supplemental/). Thus, in most if not all organisms, the bouquet is not a modification of the Rabl chromosome arrangement, but rather a de novo arrangement that may be associated with other gross nuclear architectural changes (Bass et al., 1997). The clustering of telomeres on the NE might be part of an extensive global remodeling of nuclear architecture that occurs during zygotene, including the clustering of nuclear pores away from the site of telomere clustering.

### Rules of telomere behavior revealed by studying chromosome derivatives

Analysis of the behavior of chromosomal derivatives in several organisms suggests that the mechanism underlying the rearrangement of chromosomes that leads to bouquet formation acts specifically on telomeres and requires telomeric or subtelomeric sequences. When telomeres and centromeres are placed in novel positions on chromosomes after arm breakage and fussion, they still behave as centromeres and telomeres. This suggests that their inherent properties are more important than their position along the chromosome or within the nucleus. In a maize line containing chromosome derivatives generated from a rare centromere misdivision event followed by de novo telomere addition at the broken centromere, the regions adjacent to the centromere now become competent to enter the bouquet, whereas previously they were excluded from the bouquet. Since the only change that is known to have occurred is the addition of telomere repeats to the broken centromere, the new telomere must be responsible for the centromere entering the bouquet (Carlton and Cande, 2002). The behavior of ring chromosomes during meiosis in mouse and maize confirms that telomeric or subtelomeric sequences, but not a physical end, are required for the chromosome to participate in the bouquet. Circular human chromosomes lacking telomeric sequences are faithfully passed from one generation to the next in a transchromosomal mouse; however, cytological analysis shows that the circular minichromosome does not participate in the bouquet (Voet et al., 2003). By contrast, a maize ring chromosome that contains interstitial telomeric sequences but no physical ends is drawn into the bouquet and associates with other telomeres on the NE (Carlton and Cande, 2002). Sadaie et al. observed a similar phenomenon in fission yeast. Provided the ring chromosome contains telomeric or subtelomeric sequences, it associates with the SPB as part of the bouquet (Sadaie et al., 2003).

### The mechanism of bouquet formation

The behavior of telomeres during meiotic prophase in maize, humans and mice is consistent with a model in which the telomeres attach to the NE randomly at the end of leptotene and then ‘skate’ around the inner surface of the NE until they approach each other (Bass et al., 1997; Scherthan et al., 1996). Telomere association with the NE therefore is likely to be highly regulated and an active mechanism must be required for movement of telomeres on the inner NE. At pachytene, the process that holds telomeres together in the bouquet must be reversed or switched off as telomeres and nuclear pores become dispersed over the surface of the NE. A quantitative study of telomere clustering in meiocytes in rye anther culture strongly suggests that, in order to form the bouquet, the movement of telomeres must be directionally biased, thus implicating an active process. Homology between chromosome ends at the telomeric and subtelomeric region is mathematically predicted to encourage the formation of mini-clusters, which are intermediates actually seen in maize *pam1* mutant meiocytes as well as in rye (Carlton and Cande, 2002; Carlton et al., 2003; Golubovskaya et al., 2002).

What drives the polar movement of telomeres? In algae and animals, the centrosome is found directly outside the NE, adjacent to the telomere cluster (Scherthan, 2001). In fission yeast, telomeres form a tight cluster on the NE adjacent to the SPB, which is not yet inserted into the NE (Chikashige et al., 1994). These observations have led to the suggestion that cytoplasmic microtubules interacting with components in the NE drive telomere clustering, but there is no evidence that confirms this model. It is equally plausible that the centrosome is attracted to the bouquet site on the NE. Higher plants do not have obvious microtubule-organizing centers (MTOCs), and depolymerization of cytoplasmic microtubules by vinblastine and amiprophos-methyl (APM), a plant herbicide, does not inhibit telomere clustering in meiocytes in rye anther culture. This suggests that the telomere clustering is not dependent on cytoplasmic microtubules in plants (Cowan and Cande, 2002).

Interestingly, colchicine blocks bouquet formation in plants at low concentrations that do not depolymerize the cytoplasmic microtubule arrays. Although colchicine is a well-studied microtubule-depolymerizing drug, it also affects the function of other proteins, including at least one transmembrane protein (reviewed by Cowan and Cande, 2002). Addition of colchicine to rye anthers in culture at various times during prophase I (leptotene through pachytene) specifically disrupts the lateral movement of telomeres on the NE but does not affect other nuclear reorganizations, such as nuclear pore rearrangement (Cowan and Cande, 2002). When applied to plants and animals early in meiotic prophase, colchicine causes improper synapsis, decreased chiasmata formation and a greatly reduced frequency of recombination, yielding univalent chromosomes followed by infertility (Loidl, 1990; Zickler and Kleckner, 1998). In higher plants and mice, the leptotene-zygotene transition may be the most colchicine-sensitive stage: treatment with colchicine after zygotene has no effect on meiosis (Loidl, 1990). Since colchicine blocks telomere clustering in the rye system, and disrupts homologous synapsis in many other cell types, the two processes might be causally related.

A remaining question concerns the identity of the colchicine-sensitive target. Notably, inhibition of bouquet formation in rye anthers treated with colchicine is very similar to the block in bouquet formation in *pam1* mutant maize meiocytes, which also exhibit aberrant synapsis (see below); thus, it is possible that cloning of the *pam1* gene will shed light on this target. We speculate that the bouquet-specific colchicine target is an as-yet-unidentified cytoskeletal component that is associated with the inner face of the NE. The drug studies discussed above demonstrate that the mechanism responsible for generating the chromosomal movements associated with bouquet formation are autonomous to the nucleus and do not appear to rely on the cytoplasmic cytoskeleton.

### The bouquet in fission yeast plays an important role in homologous chromosome alignment

Fission yeast is an excellent organism in which to use molecular genetics to identify proteins involved in the formation and maintenance of the bouquet. Fission yeast chromosomes do not undergo synapsis, and an alternative mechanism has evolved in this organism to keep homologous chromosomes closely aligned as they undergo recombination. A tight bouquet is formed in early meiotic prophase, in which telomeres are attached to the SPB through an as-yet-unidentified nuclear-membrane-spanning linker (Chikashige et al., 1997). During S phase and meiotic prophase, the whole

nucleus is whipped around within the cell, in what is called the horsetail stage (Chikashige et al., 1994) (see supplemental movies, http://jcs.biologists.org/supplemental/). These movements stretch the nucleus out and help to align the chromosomes. Mutations in telomere-binding proteins that disrupt telomere length regulation, such as *taz1* and *rap1* (Cooper et al., 1998; Kanoh and Ishikawa, 2001; Nimmo et al., 1998), and mutations such as *dot2* and *kms1* that affect SPB integrity, also affect telomere clustering (Niwa et al., 2000; Shimanuki et al., 1997; Jin et al., 2002). Taz1p binds to the telomeric repeat sequence and has a role in maintaining telomere length. When Taz1p is depleted, telomeres become longer than those in wild-type cells and fail to cluster completely, forming multiple telomeric foci during meiotic prophase and lowering recombination frequency (Cooper et al., 1997; Cooper et al., 1998; Nimmo et al., 1998). Interestingly, Taz1 mutants do not have normal horsetail movement. The aberrant horsetail movement and reduced genetic recombination they display is very similar to the phenotype of the dynein heavy chain mutant, *dhc1*, in which horsetail movement is abolished (Yamamoto et al., 1999). This raises the possibility that the function of the bouquet in fission yeast is to allow all the chromosomes to be grabbed at once, attached to the SPB, and vigorously moved around by the cytoplasmic microtubules at the SPB to align homologs. How the telomeres become associated with the SPB is not known.

## Bouquet dissolution is highly regulated

The formation of the bouquet is distinct from initiation of recombination since recombinant-deficient mutants have bouquet-stage nuclei (Trelles-Sticken et al., 1999), and the bouquet mutant *pam1* initiates recombination in the absence of a bouquet (Golubovskaya et al., 2002). However, bouquet dissolution in late zygotene or early pachytene may be dependent on the progression or completion of recombination and may be regulated by some of the same signaling pathways that monitor recombination. ATM is a serine-threonine kinase that phosphorylates several crucial checkpoint proteins involved in DNA repair, including repair of the double-strand breaks that initiate meiotic recombination (reviewed by Kastan and Lim, 2000; Kastan et al., 2000; Shiloh, 2003a; Shiloh, 2003b). Inactivation of ATM results in aberrant telomere clustering in mouse meiocytes. The defect is manifest as an inability of telomeres to disperse once clustered on the NE, and these meiocytes also display aberrant synapsis (Pandita et al., 1999; Scherthan et al., 2000a). This suggests that bouquet dissolution might be dependent on recombination checkpoints. One of the substrates of the ATM kinase is the histone variant H2AX. The phosphorylated version of this histone seems to be present at the sites of double-strand breaks (Pilch et al., 2003). Male H2AX-knockout mice are sterile and also display an aberrant bouquet (a delayed in dissolution phenotype?), which suggests that H2AX is a downstream effector of the ATM kinase in regulating telomere movement during meiotic prophase (Fernandez-Capetillo et al., 2003). Likewise, yeast *spo11* mutants, which lack double-strand breaks and thus cannot initiate or complete recombination, make a fine bouquet but cannot exit the bouquet stage (Trelles-Sticken et al., 1999).

## Bouquet function: what does it do?

Because the bouquet is so universally conserved, it probably has a function important enough to keep. The fact that initiation of telomere clustering immediately precedes chromosome pairing and synapsis has led to the hypothesis that the bouquet facilitates both of these processes (Bass et al., 2000; Scherthan et al., 1996). Consistent with this potential role is the general observation that homologous synapsis is typically initiated near the telomeres (Pfeifer et al., 2001; Stack and Anderson, 2002). Also, synapsis requires close proximity of chromosomes, and the optimal spacing of chromosomes may be promoted by the movement of chromosome ends into the bouquet. Thus, it is reasonable to speculate that bouquet formation is involved in synapsis, as has been previously suggested (e.g. Golubovskaya et al., 2002; MacQueen et al., 2002; Scherthan et al., 2000a). Likewise, clustering of telomeres might promote homologus pairing, by aligning the ends of the chromosomes and placing homologous regions of chromosomes in vectorial alignment. It could also serve to restrict the homology search to a much smaller volume of the nucleus. Is there a relationship between pairing and synapsis? In the maize meiotic mutant *poor homologous synapsis* (*phs1*), pairing and synapsis can be separated and are not dependent on one another (Pawlowski et al., 2004). However, the bouquet could affect both pairing and synapsis even if these processes are not causally linked. Other roles have also been suggested for the bouquet, including chromosome interlock resolution and regulation of recombination (Zickler and Kleckner, 1998). These are addressed below.

Perhaps the best way to determine the function of the bouquet is to analyze the phenotype of mutants that cannot make a bouquet. There are now such bouquet initiation mutants from several organisms, but deduction of bouquet function is complicated by the fact that many meiotic mutants have mild bouquet problems in addition to other larger problems. Defining mutants whose primary lesion is the inability to create a bouquet is difficult, and thus deducing the bouquets normal function remains problematic. In maize, several mutants have a mild telomere-clustering aberration – for example, *phs1* (Pawlowski et al., 2004) and *dsy1* (Bass et al., 2003) – yet have severe defects either in pairing and recombination or in synapsis. In our opinion, there are two mutants that appear to have problems in bouquet formation as their primary defect: *pam1* in maize and *ndj/tam1* in budding yeast.

As its name implies, the *pam1* mutant of maize has *plural abnormalities of meiosis*. Golubovskaya et al. found that telomeres attach normally to the NE, and are normally polarized in the nucleus (Golubovskaya et al., 2002). They undergo some initial stages of clustering by making several small clumps of telomeres (similar to those seen in rye anthers treated with colchicine), but cannot cluster into a normal tight bouquet. This is the earliest observable defect found in the *pam1* mutants because leptotene looks completely normal under both deconvolution 3D microscopy and TEM. Mutant *pam1* meiotic nuclei also have aberrant synapsis including non-homologous synapsis, partner switches and foldbacks, and loss of interlock resolution. There is also a dramatic reduction in homologous pairing (which can be monitored by determining the location of 5SrRNA loci in pachytene nuclei). In addition, there is an extreme asynchrony of meiosis such that, in old anthers that would normally contain only pollen (the product

of two post-meiotic mitotic divisions), meiocytes are found in all stages of meiosis from zygotene to the tetrad stage. This could be due to arrest of meiocytes at various points along the meiotic cell cycle, or it could be due to a slowing down of meiotic events. The *pam1* mutant is male sterile and almost completely female sterile; however, a few female meiocytes (<1%) do complete normal meiosis. This suggests that formation of a bouquet in maize greatly facilitates pairing, synapsis and resolution of interlocks, but is not absolutely required for these processes, because some meiocytes can ultimately produce normal gametes.

What is the effect on recombination in the *pam1* mutant? Interestingly, RAD51 foci on zygotene chromosomes look completely normal, which suggests that the early stages of recombination do not require bouquet initiation and that these two processes can be separated. The converse has also been shown (formation of the bouquet does not require initiation of recombination): *spo11* mutants in yeast and *Sordaria*, which lack recombination, make fine bouquets (Storlazzi et al., 2003; Trelles-Sticken et al., 1999). This implies that initation of the bouquet and of recombination are independent events.

In the budding yeast bouquet mutant *ndj/tam1* (Chua and Roeder, 1997; Conrad et al., 1997; Trelles-Sticken et al., 2000), telomeres do not attach to the NE, as they do in the wild type, and thus telomeres cannot cluster opposite the SPB. This indicates that attachment to the NE is required for bouquet formation. Ndj1p localizes to telomeres and probably facilitates attachment to the NE; however, it has not been shown to be part of the attachment plaque. The phenotype of the *ndj* mutant is surprisingly similar to that in the *pam1* maize mutant; there is a severe delay of the onset and completion of synapsis, an extreme delay in all stages of meiotic prophase, non-homologous synapsis, decreased homologous pairing (monitored by FISH), and some sterility. Interestingly, chromosome condensation is not effected in either *pam1* or *ndj*, and thus condensation is probably not related to the bouquet.

Given the similarity of phenotypes of the maize *pam1* and yeast *ndj/tam1* mutants, we propose that the bouquet is required for efficient homologous pairing and synapsis. The bouquet is not absolutely required for pairing (because the *pam1* and *ndj* mutants do achieve some homologous pairing), for synapsis (again, both mutants can achieve some degree of synapsis), or for completion of meiosis (in both mutants there are always some survivors). However, the rate and efficiency of all these processes are severely reduced in these bouquet mutants. We can also conclude that the bouquet is required for timely initiation of synapsis because synapsis is severely delayed in both the *pam1* mutant and the yeast *ndj* mutant. Analysis of the phenotype of *phs1* mutants, which synapse at the normal time and place, albeit non-homologously, demonstrates that homologous pairing is not required for synapsis. So, the bouquet might affect these two processes in different manners.

Regarding the contribution of the bouquet to recombination, our view is that the initial stages of recombination – double-strand breaks, followed by DNA strand invasion facilitated by RAD51 and DMC1 – contribute more to homologous pairing than does the bouquet. However, it is likely that the bouquet, and the movements associated with it (such as in the *Schizosaccharomyces pombe* horsetail), is required to bring the chromosomes into close enough proximity for strand invasion. The bouquet could almost be evolutionarily dispensable because mutants and colchicine-treated meiocytes are not 100% sterile; some exceptional meiocytes are able to complete normal meiosis. This is in contrast to mutants that, for example, have a disrupted homology search, such as *phs* in maize (Pawlowski et al., 2004), or are unable to synapse at all, such as *zip1* in yeast (Sym et al., 1993). Meiosis in these mutants is never completed. Moreover, *C. elegans* and *Drosophila* lack a bouquet yet are able to complete meiosis (McKee, 2004), and in other organisms, such as *Sordaria* (Tesse et al., 2003) and polyploid wheat (Aragon-Alcaide et al., 1997), homologs have already found each other by the time the bouquet has formed.

One simple model of how the bouquet could act in meiosis can be proposed. At leptotene or the leptotene-zygotene transition, recombination is initiated by the generation of double-strand breaks, which are resected and then loaded with RAD51 and other proteins. Concurrently and independently, the telomeres, which have attached to the NE earlier in leptotene, cluster first in small clumps, and then into a tight bouquet. Following this, RAD51-coated single-strand DNA overhangs begin to search for homology, which we speculate begins at the chromosome ends. Synapsis follows closely, also starting from the chromosome ends, but does not require a signal that homology has been established. Exit from the bouquet stage probably does require a signal from the recombination pathway [perhaps upon completion of the noncrossover (Allers and Lichten, 2001) recombination pathway], and possibly from the synapsis process. When pairing and synapsis are complete, and recombination is well underway, the telomeres exit the bouquet stage. This is followed by further condensation, SC breakdown and chiasmata maturation, all leading to the uniquely meiotic separation of homologous chromosomes at anaphase 1.

## Concluding remarks

The inheritance, maintenance and function of interchromosomal order in the nucleus have been topics of major intellectual interest since the early 20th century (reviewed by Bickmore and Chubb, 2003). Studies have been hampered by a lack of knowledge about how chromosomes are moved around in the interphase somatic nucleus and by an inability to pinpoint when such movements occur. The bouquet is a special and very dramatic example of establishment of chromosome domain polarity within the nucleus and, unlike somatic chromosomal rearrangements, its functional importance has been established and the movements well described. Further progress in our understanding of bouquet formation and function, in particular identifying the machinery responsible for bringing telomeres into close proximity on the NE, will have implications not only for our understanding of meiosis and the homology search but also for our understanding of how chromosome domains are set up and maintained within the somatic interphase nucleus.

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