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Telomere dynamics unique to meiotic prophase: formation and significance of the bouquet

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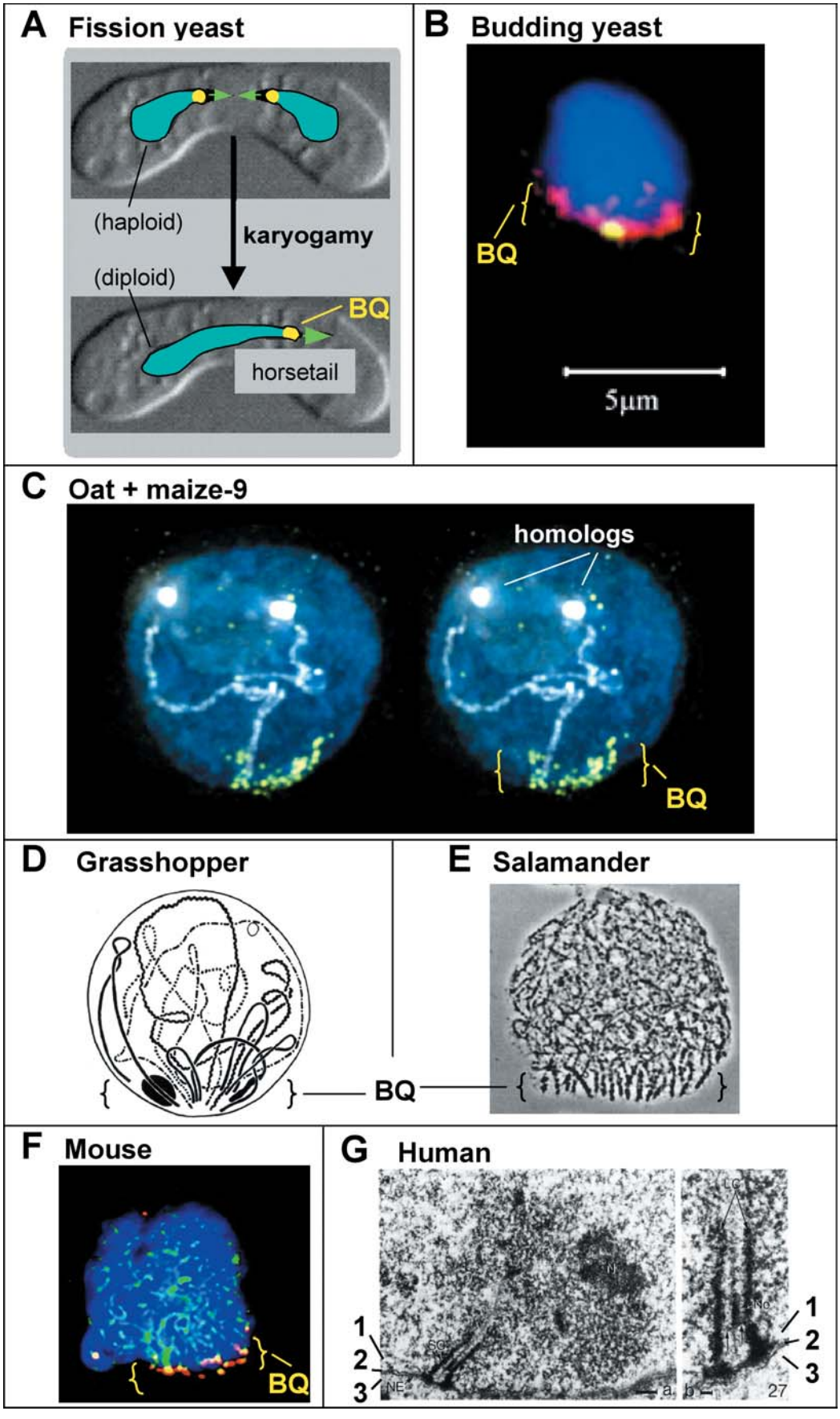
Abstract. Telomeres carry out conserved and possibly ancient functions in meiosis. During the specialized prophase of meiosis I, meiotic prophase, telomeres cluster on the nuclear envelope and move the diploid genetic material around within the nucleus so that homologous chromosomes can align two by two and efficiently recombine with precision. This recombination is in turn required for proper segregation of the homologs into viable haploid daughter cells. The meiosis-specific telomere clustering on the nuclear envelope defines the bouquet stage, so named for its resemblance to the stems from a bouquet of cut flowers. Here, a comparative analysis of the literature on meiotic telomeres from a variety of different species illustrates that the bouquet is nearly universal among life cycles with sexual reproduction. The bouquet has been well documented for over 100 years, but our understanding of how it forms and how it functions has only recently begun to increase. Early and recent observations document the timing and provide clues about the functional significance of these striking telomere movements.

Key words. Bouquet; cytology; nuclear envelope; synapsis; meiosis.

The bouquet is a telomere cluster on the nuclear envelope at meiotic prophase

Nuclei in early meiotic prophase exhibit a highly polarized and clustered arrangement of chromosome ends called the bouquet, recognized long ago as unique to meiosis [1, for early reviews, see 2–4]. Beautiful and numerous examples of the bouquet arrangement are found in early microscopic studies, in which telomere organization was documented and noted but not always the purpose of the study [5, and references therein for a timeline of bouquet research]. Many of the micrographs and drawings captured remarkably clear images of the bouquet arrangement showing telomeres grouped on the nuclear envelope (NE) with, in some cases, the centrioles in the cytoplasm nearby [6–12]. Although the bouquet has been described now for over a century, recent advances in cell and molecular biology of meiosis have provided a renewed interest in meiotic telomeres, as evidenced by several recent reviews that focus on the bouquet [5, 13–16]. The biological significance of the bouquet is generally accepted to be its role in promoting homologous chromosome interactions, but exactly how the all the movements and clustering are accomplished is still being resolved. The causal relationships between overlapping meiotic processes such as those involved in homolog pairing, synapsis and crossing over can be difficult to discern experimentally [17–19]. The complexity of meiotic prophase is reflected in its subdivision into five stages on the basis of chromosome fiber morphology. In order, they are the leptotene, zygotene, pachytene, diplotene and diakinesis stages. The telomere clustering starts late in the leptotene stage, always overlaps with the zygotene stage and usually persists into the pachytene stage. These three stages are classically delineated by whether homologous chromosome synapsis is yet to start, in progress or completed, respectively. Several examples of direct and indirect detection of the bouquet are shown in figure 1A–F and include examples from fungi, plants, insects and mammals.


A Fission yeast
(haploid)
karyogamy
(diploid)
BQ
horsetail
B Budding yeast
BQ
5μm
C Oat + maize-9
homologs
BQ
D Grasshopper
BQ
E Salamander
F Mouse
BQ
G Human
1
2
3
NE
a
b
27
LC
No
1
2
3

Ultrastructural observations of the regions of telomere-NE attachment

The synaptonemal complex (SC) is unique to meiotic prophase and permits telomere identification because it consists of a continuous axial structure that joins synapsed homologous chromosomes from end to end in pachytene-stage chromosomes [20–22]. Analysis of meiotic prophase nuclei by electron microscopy (EM) revealed that the bouquet involves direct connections (fig. 1G) between the ends of the meiotic chromosome cores and the inner membrane of the NE (reviewed in [15]). Attachments at the early bouquet and zygotene stage provide a mechanism for large-scale chromosome end alignment (for examples see fig. 1C–E), an arrangement conducive to the processes of homology search and initiation of synapsis [23]. These telomere-NE attachments are a hallmark of early meiotic prophase nuclei [21, 24–29]. The persistence of the NE associations well into pachytene (fig. 1 D) suggest that they play another role subsequent to the initial gathering of ends to initiate synapsis [30]. The precise role of the telomeric repeat sequences and the role of the telomeric complex in forming and stabilizing these NE attachments is not entirely clear, but some telomere-associated proteins have been localized to the bouquet or implicated in its formation and may be involved [31–38].

The timing of the bouquet

The EM-based observations of the bouquet stage were based on detection of telomeres as the ends of continuous chromosome fibers [39, 40]. The ending point of the bouquet stage, when the telomeres disperse, was therefore clearly established early on to be in the pachytene stage in most species. In contrast, the timing of the initial steps of bouquet formation have only recently been determined with the aid of fluorescence microscopy methods capable of detecting telomere locations at any stage of the cell cycle. These studies showed that the telomere cluster forms de novo in most species, during meiotic prophase just before the leptotene-zygotene transition [14, 41–44]. The kinetics of the telomeres in the first half of meiotic prophase indicate that telomeres may have one or more specific roles during this time of dramatic and global changes in chromatin structure, nuclear architecture and regulated DNA metabolism. Comparative analysis of the general features of bouquets from different species reveals a nearly universal pattern for the occurrence of the bouquet – from just before to just after the zygotene stage, when synapsis is underway [30, 33, 34, 43, 45–51].

Movements of meiotic telomeres

For decades, indirect and circumstantial evidence suggested that the bouquet-stage telomeres interact with microtubules or the microtubule organizing centers of cells. Genetic and cytological data reveal that clustering and telomere-led nuclear movements in budding yeast and fission yeast are associated with microtubules [16, 46, 48, 52]. Interestingly, recent colchicine treatment studies shed light on another telomere rearrangement mechanism that is not compromised by microtubule disruption [49, 50]. In rye plants with large subtelomeric regions of heterochromatin, 0.1 mM colchicine or podophyllotoxin

Figure 1. Various views of the bouquet. The bouquet, where telomeres are clustered and attached to the nuclear envelope, has been detected by various cytological techniques. In these examples the region of the bouquet (BQ) is enclosed by braces. (*A*) Bouquets from fission yeast, before and after karyogamy. The clustering of telomeres occurs just prior to karyogamy and is present throughout the horsetail stage [42]. Diagrams of the relative positions of the nuclei (blue) and the telomeres (yellow dots) are drawn over a phase-contrast micrograph of a cell. The direction (green arrows) of telomere cluster movement within the cell is indicated. (*B*) A bouquet from budding yeast, detected by fluorescence in situ hybridization (FISH). The nucleus (blue) is from a mildly spread tetraploid SK1 strain (reproduced with permission from figure 8C of [46]). The telomere cluster is detected after hybridization with XY′ probe (red). Immunocytochemical detection of tubulin with anti-Tub4 shows the location of the spindle pole body (green, or yellow where overlapping with the red FISH signals; reproduced with permission from [46]). (*C*) A bouquet at the zygotene-stage nucleus from a maize-chromosome-addition line of oat, detected by three-dimensional telomere FISH [44]. A stereo projection of a bouquet-stage nucleus (blue) shows the telomere FISH signals (green dots) and partial synapsis of the maize chromosome 9 stained by whole chromosome FISH paint (white fibers). Synapsis has begun near the bouquet side of the nucleus. (*D*) A bouquet at the early pachytene stage from *Chlorthippus parallelus*, detected and drawn from light microscopy (from [59]). This nucleus shows that the bouquet persists in the pachytene stage, well after the initiation of synapsis. (*E*) A bouquet at the early zygotene stage from a plethodontid salamander, detected by phase-contrast microscopy (reproduced with permission from figure 3C of [60]). Note the roughly parallel alignment of the terminal segments of ends of the chromosome. (*F*) A bouquet at the late leptotene/early zygotene stage from rat, detected by immunolocalization with an antibody raised against the human telomeric protein hRap1 (reproduced with permission from figure 8B of [37]). The image shows the DNA (blue) and immunodetection of the SC protein SCP3 (green) or hRap1 epitopes (red) from a mildly spread nucleus. (*G*) An ultrastructural view of the region of telomere attachment to the nuclear envelope of a synapsed bivalent at early pachytene from human, detected by thick-section uranyl-acetate staining. The bivalent is of the X and Y chromosomes, but the morphological features of the end-on attachments are typical for telomeres at the bouquet stage (reproduced with permission from figure 27 of [61]). The axial cores of the chromosomes have formed a synaptonemal complex, and a slight thickening of densely staining material is evident where the lateral elements (which resemble the rails of a train track) of the synaptonemal complex contact the nuclear envelope. The locations of the nucleus (1), nuclear envelope (2), and cytoplasm (3) are indicated. The attachment is shown at two different magnifications.

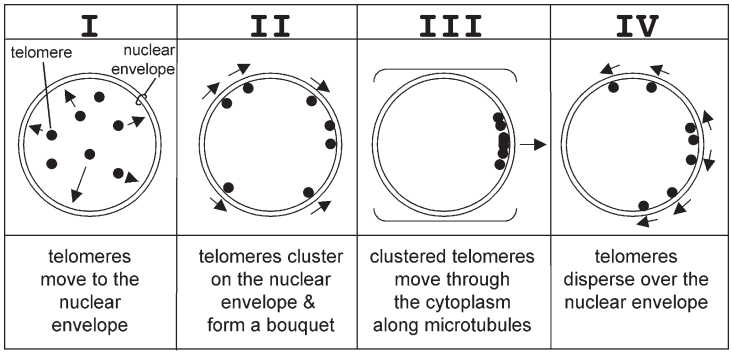


Figure 2. Four sequential types of telomere movements that can occur in meiotic prophase of multicellular eukaryotes are indicated. The four different movement classes are indicated at the top as I–IV. Diagrams indicate the positions of the telomeres relative to the nuclear envelope and the relative direction of telomere movements (arrows). Descriptions of the movements are given at the bottom. Classes I and II coincide with the late leptotene stage. Class III occurs mostly during the zygotene stage, which is coincident with the bouquet stage. This type of movement is based on microtubules in the fission yeast [16, 52]. Class IV usually occurs during pachytene. Classes II and IV can be distinguished cytologically in fixed cells by criteria that differentiate between the leptotene and pachytene stages of meiotic prophase.

treatment disrupted bouquet formation but did not result in a loss of microtubules. Furthermore, disruption of the rye microtubules by other drugs did not block bouquet formation. These findings suggest that a colchicine-sensitive target, but not intact microtubules, may be important for bouquet formation. Colchicine treatments can reduce pairing, synapsis and chiasmata formation during early meiotic prophase (reviewed in [15, 23]). The microtubule-dependent telomere movements in yeast and the colchicine-sensitive formation of the bouquet raise some interesting questions. These include whether yeast and plants have different telomere motility systems or whether telomeres move by one mechanism to form the bouquet and by another to move the bouquet. Indeed, telomere movements within meiotic prophase can be divided into four discernable classes (illustrated here as I–IV, fig. 2), leaving open the possibility that two or more different types of forces direct meiotic telomere movements.

Functional significance of the bouquet and some questions

Several lines of evidence establish that one of the most likely functions of the bouquet is to ensure the efficient initiation of synapsis of homologous chromosomes [27, 51, 53–55]. Other aspects of the bouquet can be experimentally addressed now. The telomeric complex contains a number of proteins that have various roles in DNA metabolism, including signal transduction, DNA repair, chromatin silencing and recruitment and regulation of telomerase, as summarized in the accompanying reviews (Wei and Price, and Kanoh and Ishikawa). To what extent, if any, does the bouquet produce a localized concentration of telomeric proteins, and if it does, how might it affect the homology search, synapsis or recombination? As the proteins of the telomeric complex are identified, it will be important to investigate which of them play a direct role in meiosis. Proteins such as TRF1, TRF2, Rap1, Ndj1 and Taz1 are found at meiotic telomeres, and genetic studies indicate that these proteins are important for meiotic telomere functions [6, 37, 42, 46, 56–58]. A critical question at this point is whether these proteins play a direct role in meiotic telomere functions or an indirect role, such as, for example, by their effect on the overall regulation of telomere length.

Another line of questions involves the possible role of telomeres in crossover control. The maize mutant *desynaptic* (*dy*) reduces crossover recombination but appears to have a normal bouquet [30]. The *dy* mutant exhibits precocious telomere-NE detachment at pachytene, raising, for example, the question of whether telomeres have multiple and separable functions for synapsis and recombination in plants or other organisms. As a potentially informative exception, the *Drosophila* telomeres do not have telomerase-derived telomeres or their associated set of telomeric repeat-binding proteins (see Biessmann and Mason, this issue), and *Drosophila* chromosomes do not form a bouquet at meiotic prophase. Which meiotic peculiarities of *Drosophila* meiosis, if any, are related to its lack of a common-type telomeric complex or bouquet? These and related questions on the role of the nuclear envelope are beginning to be addressed, uncovering some of the long-held secrets of the bouquet.

Clearly the field of meiosis is rapidly gaining the ability to solve some of the mysteries of the bouquet, assisted by recognition of the conserved features of meiotic prophase nuclei. Future investigations into the functional significance of the bouquet structure will be important for understanding of the broader evolutionary significance of telomeres and linear chromosomes in the evolution of meiosis and sexual reproduction in eukaryotes.

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