Unexpected chromosome behavior in wheat: general rule or an exception?

Lukaszewski AJ
University of California, Riverside, USA

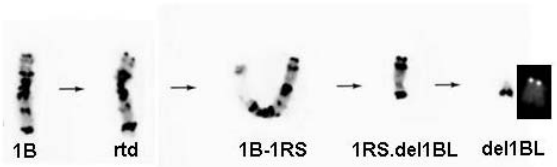
At the present level of technology, microscopic observations provide a rather coarse resolution and seem to be hopelessly outdated. Yet, sometimes they offer some interesting insights into various aspects of chromosome behaviour. This article will briefly discuss three recent observations of unexpected chromosome behavior in wheat.

THE CENTROMERE

The concept of the centromere appears to be getting fuzzier as technology advances. Disregarding holocentric chromosomes, it used to be the primary constriction. If the centromere is understood as a region of a chromosome responsible for the motoric function, the old definition of "kinetochore" will suffice. However, if it is understood as a segment of a chromosome responsible for its proper behavior in cell divisions, and this includes both the movement itself as well as timing of this movement, the centromere may turn out to be a rather large part of a chromosome with ill defined borders.

Francki (2001) isolated a rye-specific centromeric probe that proves to be of considerable value in the study of the centromere, especially when used on wheat chromosomes with rye centromeres. Such centromeric introgressions were developed by repeated centric fission-fusion of wheat and rye univalents (Zhang at al, 2001). In *in situ* probing on these chromosomes, the Francki probe hybridizes only to the part of chromosomes underlying the kinetochore; no interaction with the spindle apparatus of the flanking non-labelled parts of the chromosome has ever been observed even though the origin of the introgressions suggests that some minor flanking kinetochore regions of wheat origin must have been retained. Observations of misdivision of univalents with thus labelled kinetochore regions imply that the kinetic function of the centromere, located strictly in the primary constriction, is likely separated from sister chromatid cohesion. The latter function appears allocated to chromosome segments flanking the kinetochore region outside of the primary constriction, and may spread out considerable distances from the kinetochore. The kinetochore region itself does not appear to provide cohesion of sister chromatids or, at least, it does not provide sufficient cohesion for a normal behavior of a chromosome. Chromosomes with kinetochore regions reduced by breakage show in unmodified metaphases (the spindle apparatus present) two clearly separated signals of the Francki probe (see figure below, the last chromosome on the left). During centric misdivision, the spindle apparatus often tears the kinetochore region out of univalents but such free kinetochore regions have never been recovered among progeny. All viable midget chromosomes have the kinetochore region flanked by the adjacent regions unlabelled by the Francki probe, and the longer the flanking regions the higher the transmission rate of a midget to progeny. At present it would be risky to define any physical length of the sister chromatid cohesion regions, but in chromosomes with replacement centromeres studied in detail, they may well stretch out to ca. 10-20% of a chromosome arm length. This is based on the positions of breaks in univalents, when the pulling forces of the spindle fibers attached to the kinetochore act against the sister cohesion.

The hybridization pattern of the Francki probe, as well as of some other centromere-specific probes, suggests that the chromosome region responsible for forming the kinetochore is composed of specific DNA sequences. Cohesion of sister chromatids, on the other hand, does not appear to be an inherent function of a specific chromosome region. Instead, it appears to be imposed by the kinetochore region on the adjacent chromosome segments, and must be a facultative function. This is based on observations of mitotic and meiotic behavior of deletion wheat chromosomes and inversions covering almost complete chromosome arms. The deletion wheat chromosomes [the term "deletion" here is used in the sense of Bridges (1917) as a loss of an intercalary segment] were produced (Lukaszewski, 1997) by a combination of centric fission of univalents across the kinetochore region, breakage of another chromosome in some intercalary position produced in anaphase I or II by the pairing pattern of a reverse tandem duplication (rtd), and fusion of the two breakage products (see illustration below). Reverse tandem duplications generate chromatid bridges in meiotic anaphase I or II, depending on which segment is involved in a crossover. If intercalary breakage caused by the rtd and centromere fission of a univalent occur in the same division, a dicentric chromosome is produced, and such chromosomes break in mitotic anaphase with frequencies dependent on the distance between the two centromeres. Eventually, monocentric chromosomes can be recovered that have portions of the original kinetochore region from the univalent, and attached to it an intercalary or terminal segment from the rtd chromosome arm. Such monocentric chromosomes can then be broken across the kinetochore region to generate deletion telocentrics. Deletion chromosomes 1BS and 1BL were recovered



with missing long proximal segments of the normal arms (up to 80%), and with fragments of the kinetochore region from rye chromosome 1R. In the context discussed here, a fragment of a rye kinetochore region was translocated to a distal point on a wheat chromosome arm, well beyond the estimated 10-20% distance from the kinetochore that provides sister chromatid cohesion in mitosis in a standard location of the kinetochore. Such deletion chromosomes are stable and there are no indications that the sister cohesion function is impaired. Translocation of the kinetochore region to the middle of an arm brings the cohesion of sister chromatids with it. Similarly, in a chromosome 1R with an inverted long arm in such a way that what used to be the distal region is now immediately adjacent to the kinetochore region and what was the centromeric region now is adjacent to the telomere, sister chromatids adhere close to the kinetochore region and not the telomere. The same thing happens in wheat reverse tandem duplications covering most of the arms. In other words, removal of a chromosome segment from the vicinity of the kinetochore region eliminates cohesion of sister chromatids. Hence the conclusion that the sister chromatid cohesion is not an intrinsic function of specific regions of a chromosome; it radiates out of the kinetochore region wherever it happens to be located. Therefore, arguably, it is the kinetochore region, the region responsible for the interaction with the spindle apparatus, which determines and dictates the centromere functions in a chromosome. Consequently, the original definition of the centromere (= kinetochore) suffices.

DISTRIBUTION OF CROSSING OVER

An interesting aspect of chromosome behavior in wheat, and probably in many other species, is the distribution of crossing over. It is now well established that in the Triticeae it concentrates in the distal regions of chromosomes. This is thought to be a direct consequence of the terminal initiation of pairing and synapsis that give preference to terminal chiasmata, and strong positive chiasma interference that limits the number of crossovers in the proximal regions. It can also be thought of as a centromeric effect; specifically, interference from sister chromatid cohesion in the vicinity of the kinetochore, or the effect of the concentration of repetitive DNA sequences that have to be prevented from crossing over lest non-homologues pair and recombine. Attempts at manipulation of the cross-over pattern in wheat seemingly confirmed these conclusions (Jones at al., 2002; Qi et al., 2002). In these two experiments, genetic mapping in deficiency chromosomes has shown dramatic increases in crossover rates in the distal region that, when present in normal chromosomes, recombined infrequently. This led to a speculation that any part of a wheat chromosome could be saturated with crossovers, if it could only be placed in the distal region of an arm (Lukaszewski, 2003). However, in an inverted arm of rye chromosome 1R in wheat, chiasmata are not formed by the telomere even though synapsis still appears to be initiated distally and progress toward the centromere. Chiasmata are formed in the immediate vicinity of the kinetochore, in the same exact region as before the inversion. Moreover, the distribution of chiasmata within the recombining region of the arm appears to have been retained in the inversion so their highest frequency is now next to the kinetochore region and drops off quickly toward the middle of the arm.

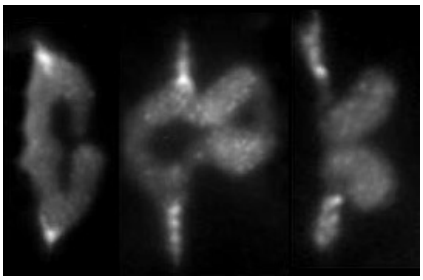
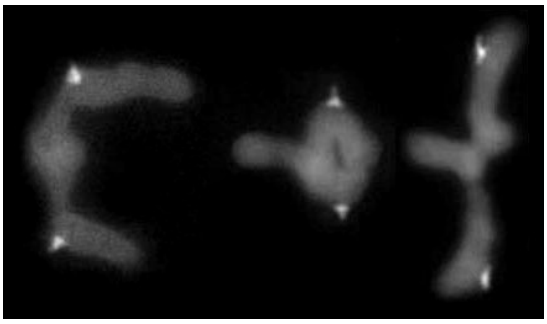


Figure1 : A ring biva-lent of a normal 1R; center: a bivalent of $1R_{inv}$, right: a bivalent of $1RL_{inv}$.

Similarly, wheat chromosomes with reverse tandem duplications covering almost entire arms pair (however infrequently) in the middle of these very long arms, that is, in the same regions that pair (form chiasmata) in normal arms. These two cases demonstrate that in a normal chromosome arm only the distal region is capable of crossing over and the proximal part is not. This proximal part does not crossover even when placed in the vicinity of the telomere where it undergoes early synapsis. Moreover, the pattern of chiasmata, hence crossing over, in the region capable of it, is not a consequence of the pattern of synapsis: its most distal regions, those normally close to the telomere, form chiasmata with a high frequency not because they synapse first, but because they have some inherent capability for high crossing over. When placed by the inversion next to the kinetochore region, they still form chiasmata with the highest frequency. It appears that while the actual crossover rate in the region capable of crossing over can be enhanced, by elimination of regions with a higher rate still, regions that do not crossover by nature will not do so even when placed in the position seemingly most favourable for recombination.

So far there is only one inversion chromosome arm for cytological studies, and it is a rye chromosome in wheat. Whether this is representative also of wheat, or other species, remains to be seen. There are good indications that it is. There are two reverse tandem duplications in wheat, on 2BS and 4AL, which involve essentially entire arms: one breakpoint is in the vicinity of the telomere

and the other by the kinetochore region so that the region immediately adjacent to the kinetochore in a normal arm, in an rtd forms the terminal segment of the arm. These chromosome arms are capable of fold-back pairing and chiasmata are always in the vicinity of the original telomere, that is, in the middle of the rtd arm. In rtd homozygotes, chiasmata are never formed by the telomeres. If they are formed at all, they are located in the same region that recombines in a normal arm. In rtd heterozygotes (Figure below), the overall pairing of the rtd arm is rare (bivalent on the left) but when it takes place (bivalents on the right), chiasmata are formed only between the telomeric end of the normal arm and the mid point of the rtd arm; the end of the rtd arm has never been observed to pair with proximal regions of the normal arm.



The question that needs to be answered is whether the proximal halves of the chromosome arms discussed here are inherently incapable of crossing over, or are prevented from it by some genetic mechanism. Published data seem to favour the latter explanation: in a wide hybrid of rye, Jones (1967) identified a family with random distribution of chiasmata, some of which were formed very close to the centromeres. In a *ph1*-induced recombination, Lukaszewski et al (2004) recovered wheat-rye recombinants with breakpoints very close to the centromeres. So, both in wheat and in rye, under some genetic conditions, proximal recombination is permitted. Therefore, there must exist a genetic system that under normal conditions prevents the proximal regions from crossing over. It would be an interesting challenge to identify this mechanism and work out its nature. Perhaps *Aegilops speltoides* would the a good place to start, as it may well be the extreme case of distal chiasma distribution (Luo et al., 2005)

The observations on chiasma distribution in the inverted arms revive the concept of pairing centers or zygomeres (Sybenga, 1966), specialized structures or regions responsible for homologue recognition and pairing initiation, and may explain some observations on the so-called recombination hot-spots and their characteristics. Regardless, if the proximal halves of all Triticeae chromosome arms are prevented from crossing over by some genetic mechanism(s), some planned research may have to devise strategies independent of crossing over.

INITIATION OF CHROMOSOME PAIRING

While the centromere's role in homologue recognition is invoked in wheat with some fanfare, it seems more plausible that it is the telomeric regions that initiate pairing and synapsis and the centromeres follow (Corredor et al., 2007). The configuration responsible for the initiation of pairing is the leptotene (or telomere) bouquet: a congregation of all telomeres on the nuclear envelope in early meiotic prophase. It is the presence of the telomeric sequence that compels a specific chromosome region to enter the bouquet (Carlton and Cande, 2007). There is little doubt that this pattern of pairing/synapsis initiation is predominant in wheat. The above-mentioned inverted 1RL clearly shows it: inverted telocentrics, which obviously have telomeric repeats in the centromeres (kinetochore regions), must enter the bouquet with both ends. In heterozygotes, this helps them find the corresponding segment of a normal arm hence increases their MI pairing success. However, with low but consistent frequency, the terminal region of a normal homologue will find, synapse and crossover with the centromeric region of the inverted arm in a two-armed chromosome. Since the telomeres and the centromeres are physically separated on the opposing poles of an early meiotic nucleus, some mechanism other than the telomere bouquet must be present that permits scans of the entire nuclear volume. Low frequency of the telomere-to-centromere pairing suggests that this is not the main mechanism for homologue recognition and/or alignment.

Whether these cases are peculiar to wheat or follow a general pattern for all Triticeae or even a wider group of organisms is far from clear at this point. However, it appears that some terms commonly used in cytogenetics need new definitions.

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