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Series

A cross-eyed geneticist's view

III. Mouse chromosomes take a drive

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1. Introduction: Meiosis and meiotic drive

Mendel's Law of Segregation asserts that the paternally- and maternally-derived alleles of a gene are equally likely to pass into a gamete. That is, if an individual has the alleles *A* and *a* at a genetic locus, then a gamete is as likely to inherit allele *A*, as it is allele *a*. But what if more *a*-bearing than *A*-bearing gametes are produced? Then, either allele '*A*' is linked to a mutation conferring poorer gamete survival, or allele '*a*' is linked to a locus showing meiotic 'drive'. Two recent papers (Akera *et al*. 2017; Wu *et al*. 2018) reported mechanisms underlying drive of mouse chromosomes bearing 'strong centromeres', that results in their ending up more often in the egg rather than the polar body, and thus were inherited by more than 50% of the pups. Centromere strength depended on the number of repeats of a DNA sequence called the minor satellite. Greater the number, greater was the strength (Iwata-Otsubo *et al*. 2017). The difference between strong and weak centromeres acted as a *cis*-asymmetry. Both groups also found *trans*-asymmetry between the meiotic spindle's cortical and central halves. The strong centromere preferentially segregated to the central pole and hence into the egg, and thence into the next generation, while the weak centromere segregated to the cortical pole and was extruded into the polar body which does not contribute to the next generation. The *cis*- and *trans*-asymmetries (strong versus weak centromeres, and central versus cortical spindle poles) connived to break Mendel's Law, and produced the kind of thriller that holds geneticists in its thrall.

The diploid cell that undergoes meiosis in females is called the primary oocyte, and in males, the primary spermatocyte. At the onset of meiosis the chromosomes are replicated and the newly made sister chromatids are held together by cohesin proteins. A multi-protein complex called the kinetochore assembles on their centromeric DNA sequences. In the first meiotic division (MI) the sister chromatid pairs derived from the paternally- and maternally-derived chromosomes segregate to opposite spindle poles, with the sister chromatids retaining their centromeric cohesins and kinetochore attachment. Following MI, the primary oocyte produces a large cell called the secondary oocyte and a small cell called the polar body I (PBI), while the primary spermatocyte produces two equal sized secondary spermatocytes. In the second meiotic division (MII) the sister chromatids lose their centromeric cohesins and segregate to opposite spindle poles. Following MII the secondary oocyte produces a large cell called the ovum and a small cell called the polar body II (PBII), and if the PBI also undergoes MII, two more PBII are produced. MII of the secondary spermatocytes produces four equal-sized spermatids, which then differentiate into the spermatozoa. Fertilization of the haploid ovum by a haploid spermatozoon establishes a novel diploid zygote cell. The zygote, and its daughter cells, undergo about 20–40 rounds of mitotic cell divisions, and thereby generate the trillions of cells of the multi-cellular adult animal or plant. For almost all practical purposes (e.g. DNA profiling) the DNA and chromosomes of the mitotically-produced cells are identical to that of the zygote, although minor differences are known to accumulate between the genome sequences of monozygotic twins (Weber-Lehmann *et al*. 2014).

2. The devil's in the dTALE

Oocytes from F1 hybrid female mice from the C57Bl/6 x SJL cross show a >75 : <25 segregation bias with which the SJL-derived chromosomes 17 and 4 are retained in the egg while the C57Bl/6-derived homologues go into the polar body. SJL centromeres have more minor satellite repeats than the C57Bl/6 centromeres and this *cis*-asymmetry drives the bias. Wu *et al*. (2018) found a greater density of

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microtubules in the cortical than central half of the meiotic spindle, that appeared to serve as the *trans*-asymmetry. Additionally, they showed that more MTOCs (microtubule organizing centres), as measured by the fluorescence intensity from tagged MTOC protein Cep192-GFP, come together to form the cortical than the central spindle pole, which presumably resulted in the microtubule asymmetry in the spindle's cortical versus central half. Thus the drive appeared to result from unequal sized kinetochores and an asymmetry in microtubule density across the spindle.

The major satellite repeat in each chromosome was labeled using a fluorescently-tagged dTALE-mClover protein (designer transcription activator-like-effector tagged with mClover) that was translated from cRNA generated *in vitro* and microinjected into the oocytes. Detection of the dTALE-mClover by fluorescent in-situ hybridization (FISH) and time-lapse imaging enabled them to track chromosome fate in the 9 hours from the time of nuclear envelope breakdown (NEBD) till the onset of anaphase I. The C57Bl/6-derived chromosome 17 homologue, containing the smaller number of minor satellite repeats, also fortuitously contained the larger number of major satellite repeats, whereas the SJL-derived chromosome 17 homologue hardly had any major satellite repeats. Thus, staining of the C57Bl/6-derived chromosome 17 by the dTALE was intense, while there was practically no signal from the SJL-homologue. To a lesser extent they could also distinguish between the C57Bl/6- and SJL-derived chromosome 4 homologues, although the other bivalents showed a lower level of major satellite asymmetry. The C57Bl/6-derived chromosome 17 was also found to give a more intense signal for the outer kinetochore protein Spc24.

The initial attachment of the chromosome 17 bivalent to the meiotic spindle was found to be unbiased. However, if the initial attachment placed the more intense FISH signal toward the egg-ward pole (the Maj. Sat.-Centre orientation), then ~60% of the time by anaphase the bivalent rotated relative to the spindle axis to assume a cortex-ward pole orientation (a Maj. Sat.-Cortex orientation). There was no rotation if the initial attachment placed the homologue with the more intense FISH signal toward the cortical spindle pole, and no further rotations were observed once the bivalent was correctly orientated to show drive. This re-orientation required Aurora kinase activity because it was blocked by a dominant-negative Aurora C construct, or by use of an Aurora kinase inhibitor. Significantly, the re-orientation preceded migration of the spindle from the oocyte's centre to the cortex.

By labeling the major satellite repeat and the outer kinetochore, respectively, with dTALE-mClover and Spc24-mCherry they measured the distance between the two structures as a proxy for the tension between them. Sister kinetochores appeared to experience greater tension on the cortical than the central pole. Thus, the asymmetry in tension appeared to favour establishment of the Maj. Sat.-Cortex orientation.

3. Also RAN: Tracing spindle asymmetry to a cortex-based $CDC42^{GTP}$ gradient and a preceding chromatin-based RAN^{GTP} gradient

Oocytes from F1 hybrid females from the CF-1 x CHPO cross show a 62 : 38 bias in retaining CF–derived centromeres in the egg (Iwata-Otsubo *et al*., 2017). The CF-1-derived centromeres contain more minor satellite DNA, whereas CHPO-derived ones have less minor satellite DNA. Thus, the *cis*-asymmetry in this system appears to be the same as in the C57Bl/6 x SJL F1 hybrid. To distinguish between the two centromere types, Akera *et al*. (2017) used a fluorescently tagged centromere-specific histone H3 variant CENP-B, which was incorporated in the positioned nucleosomes that form in the minor satellite DNA. They found that in late MI metaphase, when the spindle had migrated close to the cortex, its cortical side was enriched in tyrosinated (Tyr) α-tubulin whereas the egg side was enriched for detyrosinated α-tubulin, and just before anaphase I the CF-1 centromeres were preferentially oriented toward the egg pole. Attachment of the CF-1 centromeres to tyrosinated microtubules on the cortical side of the spindle was more unstable and prone to detachment than that of the CHPO centromeres. Laloraya (2018) suggested that a tyrosination-sensitive property of microtubule-associated proteins might affect their kinetochore association. Alternatively, centromere-associated proteins, with different abundance on CF-1 versus CHPO centromeres, might influence the stability of the interaction with tyrosinated microtubules.

Advancing spindle migration by using cytochalasins to induce actin depolymerization also advanced the attainment of spindle asymmetry, which suggested that the tyrosination asymmetry might be set up in response to signals from the cortex. A chromatin-based guanosine triphosphate-bound RAN (RAN^{GTP}) gradient polarizes the cortex overlying the spindle, and the polarized cortex is enriched in active $CDC42^{GTP}$. Expression of the constitutively active RANQ69L or dominant-negative CDC42T17N mutations resulted in loss of the Tyr-α-tubulin asymmetry, and also abolished the biased orientation of strong centromeres to the egg pole. Thus the drive appeared to depend on the asymmetry in spindle tyrosination induced by cortical polarization.

A tour de force 'light-induced dimerization' experiment strengthened the model that cortically localized CDC42 activity leads to enrichment of Tyr-α-tubulin in the cortical half of the spindle. For this experiment, an anchor protein containing a PACT domain fused to enhanced green fluorescent protein (EGFP) and HaloTag was localized to spindle poles via the PACT domain. A small molecule dimerizer composed of a HaloTag ligand linked to a photocaged trimethoprim (TMP), which is an *E. coli* dihydrofolate reductase (eDHFR) ligand, also was localized to the spindle poles via binding of the HaloTag ligand with the HaloTag on the anchor protein. Selective uncaging of trimethoprim at one pole by a targeting a laser resulted in recruitment of

eDHFR fused to constitutively active CDC42Q61LΔCAAX and mCherry to that pole (the CAAX motif deletion abolishes plasma membrane targeting), and resulted in tyrosination of α-tubulin in the microtubules in the corresponding spindle half. Further, they showed that overexpression of tubulin tyrosine ligase (TTL), which catalysis α-tubulin tyrosination, destabilized spindle microtubules and increased their sensitivity to low temperature, whereas depleting TTL decreased Tyr-α-tubulin and stabilized them. Thus, the asymmetry in microtubule stability could underlie the differential interaction of the stronger CF-1 centromeres with the two halves of the spindle.

4. Sorting it out: A matter of time?

At this point we do not know how to explain the differences between the Akera *et al*. (2017) and Wu *et al*. (2018) findings. The 2017 study suggested that migration of the spindle to the cortex sets up a cortical polarization, which, in turn, sets up differential microtubule stability between the spindle's cortical and central halves via CDC42-dependent tubulin tyrosination, which results in more stable attachments of the stronger centromeres with microtubules in the central than cortical half. The 2017 study also examined the β-tubulin distribution and found it to be symmetrical, while Tyr α-tubulin distribution was asymmetrical, they also quantified the cortical/egg tubulin signal ratio and found it to be $\sim$ 1 for β-tubulin while it was >1 for Tyr α-tubulin, indicating that the microtubule density across the spindle was indeed uniform in this case and the trans-asymmetry in this case arose from differences in tyrosination of α-tubulin, not from changes in microtubule density per se, unlike the observation made by the 2018 study. The 2018 paper also suggested that the greater microtubule density that occurs in the cortical than central half of the spindle is established well before the spindle migrates to the cortex, presumably because more MTOCs assemble to form the future cortical pole than central pole. This, together with unequal sized kinetochores and Aurora kinase activity, favours the Maj. Sat.-Cortex orientation. Strain-specific differences in the 'evolutionary arms race' between drive and mechanisms that suppress it might account for why the two groups obtained different results. To obtain more clarity regarding the related but different findings made by Akera et al. and Wu et al., it would be very helpful if each group could test for 'their' mechanism using the strains used by the other.

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