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Series

A cross-eyed geneticist's view VI. Segregation distortion in *Drosophila melanogaster*: Recent progress in solving 'an esoteric puzzle'

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Segregation distortion refers to an unusual genetic phenomenon in diploid organisms by which the two alleles at a locus in a parent are not recovered in the classical 1:1 Mendelian ratio in its offspring. The *Drosophila melanogaster* neogene *Sd* was created by a duplication breakpoint on the left arm of chromosome 2 (2L), and encodes a truncated RanGAP protein with normal GTPase activity but which mis-localizes to the nucleus and disrupts Ran gradients. Male flies carrying *Sd* exhibit segregation distortion for the *Rsp* locus on the right arm of chromosome 2 (2R). Specifically, spermatids inheriting chromosome 2 with the Sd^{+} $Rsp^{s(s)}$ genotype, in heterozygous males of genotype *Sd* Rsp^{i} / Sd^{+} $Rsp^{s(s)}$, fail to develop properly so that the majority of progeny (approaching 100%) carry just the *Sd* Rsp^{i} chromosome. One recent paper reported novel RNAi-expressing transgenes with *Sd*-mimicking properties; and another reported localization of an X-linked suppressor which restores Mendelian transmission. This article highlights how *Drosophila* genetics resources made this possible, and the significance of these findings to nucleus-cytoplasm transactions of interest to the wider cell biology community.

Keywords. Nucleus-cytoplasm transactions; Ran gradient; transmission ratio distortion

1. Introduction

The Ran (Ras-related nuclear regulatory) protein, which plays an important role in transport into and out of the cell nucleus, can exist in two nucleotide-bound forms, Ran-GTP and Ran-GDP. Inside the nucleus, the chromatin-bound protein RCC1 converts Ran-GDP to Ran-GTP. Out in the cytoplasm, Ran GTPase activating protein (RanGAP) and RanBP (Ran binding protein), form a complex with Ran-GTP to activate Ran's intrinsic GTPase activity, and thus converts Ran-GTP to Ran-GDP. This creates high Ran-GTP and low Ran-GDP concentrations inside the nucleus, and vice versa in the cytoplasm. Other proteins that differentially bind to Ran-GTP versus Ran-GDP, thus, exhibit differential activities in the nucleus and cytoplasmic, and are harnessed for directional transport. In 1999, Barry Ganetzky and colleagues discovered that *Drosophila melanogaster* flies bearing a particular ∼12 kb tandem duplication in the left arm of chromosome 2 (2L) uniquely produced a truncated RanGAP homologue, called Sd-RanGAP, from the novel *Sd* gene created by truncation of the 3′ end of one RanGAP gene copy by a duplication breakpoint, while the other copy was intact and encoded full-length RanGAP. The Sd-RanGAP protein has normal GTPase activity but is mis-localized to the nucleus, and presumably, it disrupts the Ran gradients by decreasing intra-nuclear RanGTP concentration. Male flies carrying *Sd* exhibit segregation distortion (SD) for the *Rsp* (*Responder*) locus in the heterochromatin of the proximal right arm of chromosome 2 (2R). SD refers to an unusual genetic phenomenon in diploid organisms by which the two alleles at a locus in a parent are not recovered in the classical 1:1 Mendelian ratio in its offspring. Specifically, in heterozygous males of genotype *Sd* Rsp^{i} / Sd^{+}

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$Rsp^{s(s)}$, spermatids inheriting chromosome 2 with the Sd^{+} $Rsp^{s(s)}$ genotype fail to develop properly so that the majority of progeny (approaching 100%) carry the *Sd* Rsp^{i} chromosome. Ordinarily, Mendelian segregation is expected to segregate Rsp^{i} and $Rsp^{s(s)}$ 1:1 in the progeny. *Rsp* is composed of repeats of an AT-rich 120 base pair satellite sequence whose copy number correlates with sensitivity to SD. The Rsp^{ss} (supersensitive) alleles contain ~2500 copies, sensitive alleles (Rsp^{s}) contain ~700, and Rsp^{i} (insensitive) alleles contain < 20. We do not understand why $Rsp^{s(s)}$spermatids are more sensitive than Rsp^{i} to the Ran gradient's putative disruption. As predicted by theory, *Sd* chromosomes have accumulated upward modifiers of distortion such as *E(SD)* (*Enhancer of SD*), *M(SD)* (*Modifier of SD*), and *St(SD)* (*Stabilizer of SD*), whereas the X and third chromosomes have accumulated dominant suppressors (*Su(SD)*). The modifiers are not yet molecularly characterized.

Larracuente and Presgraves (2012) comprehensively reviewed the SD system, and also suggested that the *Rsp* repeats might produce short repeat-associated small interfering RNA (rasiRNA), that form RNP complexes in the cytoplasm, whose import into the nucleus might be rendered inefficient by the disrupted Ran gradient, consequently, impairing post-meiotic chromatin remodeling, and because $Rsp^{s(s)}$ alleles have many more repeats, spermatids bearing them are disproportionately affected. However, as yet there is no experimental evidence for this model. Curiously, the *Sd* gene was found at only 1–5% frequency in *D. melanogaster* populations across the world. Wong and Holman (Wong and Holman 2020) have discussed the selection pressures that might contribute to maintaining it at this low level. Here I will summarize the findings from two recent papers, namely, Gingell and McLean (2020), who reported novel RNAi-expressing transgenes with *Sd*-mimicking properties; and Temin (2020), who reported localization of an X-linked suppressor, *Su(SD)*, which restores Mendelian transmission.

2. Brief note on *Drosophila* chromosomes and genetic nomenclature

'X chromosome' and 'chromosome 1' are synonymous, chromosome 4 is small and has relatively very few genes, and the Y chromosome carries male fertility factors but does not determine sex. Females have two X chromosomes whereas males have an X and a Y (XO males are sterile), and both sexes have two copies each of chromosomes 2, 3, and 4. Chromosome genotypes are written with the mutant loci listed from left to right (e.g., $Dp(1;2)eag^{x6}$*der* Rsp^{i} *cn bw*), a '/'separates the genotypes of the two homologues, and a semi-colon (;) separates the genotype of the different chromosome as in the sequence X; 2; 3; 4. The chromosomes undergo about 1024-fold amplification in salivary gland cells of third instar larvae. The amplified copies are maintained in register and form a readily visible banding pattern that demarcates chromosomal segments and is characteristic of the individual chromosomes, thus providing a cytogenetic map of the genome. Segments 1–20 correspond to the X chromosome, 21–40 to 2L, 41–60 to 2R, 61–80 to 3L, 81–100 to 3R, and 101 and 102 to chromosome 4. Landmarks on the polytene chromosome map have a one-to-one correspondence with loci on the genetic map and the DNA sequence (Kaufman 2017).

3. Transgenes that mimic *Sd*

SD-5 is a strong distorting chromosome bearing the mutations *Sd*, *E(SD)*, Rsp^{i}, *M(SD)*, and *St(SD)*, whereas $SD\text{-}5^{r7}$ is its non-distorting revertant deleted for the *Sd* allele. Gingell and McLean (2020) showed that an $SD\text{-}5^{r7}$ strain transgenically expressing RNAi against the RCC1 homologue (which is required to convert RanGDP to RanGTP in the nucleus) produces segregation distortion comparable to that of the original *SD-5* chromosome. In retrospect this may not be too surprising because both *Sd* and the transgene act to disrupt the Ran gradient. Additionally, transgenic expression in $SD\text{-}5^{r7}$ of anti-protamine RNAi also produced a comparable distortion. Protamines replace histones in sperm, and thus facilitate chromatin condensation during spermatid development. However, protamine-knockout or -knockdown genotypes did not distort in the absence of the upward modifiers borne on $SD\text{-}5^{r7}$. Thus, disrupting the Ran gradient or reducing protamine alone has either a modest or no distorting effect, but requires the upward modifiers to trigger distortion. And, as evidenced by the $SD\text{-}5^{r7}$ phenotype, the modifiers alone do not distort segregation.

4. Discovery of a new *Su(SD)*

An insertional translocation of the X chromosome polytene region 13A2; 13E4-8 into 21E1-2 on chromosome 2L was obtained in unrelated studies, to wit, as a gamma-ray-induced allele of the X chromosomal locus *ether a go-go* (*eag,* named for its ether-induced leg shaking phenotype). Segregation of the recipient

chromosome 2L with a normal X chromosome resulted in duplication of the translocated segment called *Dp(1;2)eag^{x6}*. Subsequently, a spontaneous derivative, *Dp(1;2)eag^{x6}der*, arose that removed the 13A6-B1;13C2-6 segment from the original insert.

Temin (2020) found that *eag^{x6}der* /SD-Mad males do not show segregation distortion, despite the fact that SD-Mad is a strong distorter chromosome. She used the cleverly designed 'suicidal' *Sd Rsp^{ss}* chromosome, which shows strong self-distortion when opposite an *Rsp^{s}* or *Rsp^{i}* homologue, to rule out the presence of an *Rsp^{i}* allele on the *eag^{x6}der* chromosome, since *eag^{x6}der* /*Sd Rsp^{ss}* males did not exhibit self-distortion of *Sd Rsp^{ss}*. Non-occurrence of distortion was also not because of increased dosage of polytene segment 13A-E, since deletion of the corresponding region from the X chromosome did not restore distortion. The SD suppression phenotype co-segregated with the *eag^{x6}der* insertion.

5. Eyes on the flies

The haplo-insufficient *Star* locus exhibits a 'rough-eye' phenotype in deletion heterozygotes, and maps close to the *eag^{x6}der* insertion in polytene segment 21E. Novel *Star* deletion heterozygotes were isolated by screening for flies inheriting an X-ray mutagenised *eag^{x6}der* chromosome that conferred the rough-eye phenotype. A subset of rough-eyed mutants was also deleted for the neighboring *eag^{x6}der* insert and showed a concomitant loss of the suppression phenotype, whereas rough-eyed progeny retaining the insert continued to suppress SD. These results indicated that the *eag^{x6}der* insert contains a dominant suppressor of SD (*Su(SD)*). Previously, the *SD-Roma* strain was shown to contain a moderately distorting chromosome 2, and a revertant of it, *R57*, was found to harbor a strong X-linked dominant suppressor called *Su(SD)^{R57}*. Temin mapped *Su(SD)^{R57}* to X chromosome map position 49.9 cM, between the markers *garnet eyes* (*g*) and *scalloped wings* (*sd*), which placed it approximately in polytene position 13D-E. The *eag^{x6}der* insertion contains sequence from 13A2; 13E4-8, and therefore the new *Su(SD)* and the previously known *Su(SD)^{R57}* are either in the same gene or closely linked.

Males have only a single X chromosome; hence, it is challenging to use X-ray mutagenesis to cytogenetically localize an X-linked locus with a male-limited phenotype, such as *Su(SD)^{R57}*, because deletion of neighboring genes can potentially cause inviability. In contrast, deletions are easily obtained in autosomal loci, such as *eag^{x6}der*, since they are rescued by heterozygosity. Temin devised an elegant strategy to obtain X-ray-induced *eag^{x6}der* deletions by employing the chromosome 2 recessive eye color markers *cn* (*cinnabar*) and *bw* (*brown*). Adult *cn* and *bw* single mutant flies have vividly different eye colors (respectively, bright scarlet and brown) compared to either the wild type (red eyes) or the *cn bw* double mutant (white eyes). The *Dp(1;2)eag^{x6}der Rsp^{i} cn bw* chromosome was generated by recombination, mutagenized by X-rays, and segregated into F1 males of genotype *Dp(1;2)eag^{x6}der Rsp^{i} cn bw*/*Sd Rsp^{ss} bw*. The F1 males (brown-eyed) were individually crossed to *cn bw* (white-eyed) females. Since *Su(SD)* in the *eag^{x6}der* insert suppresses distortion equal number of brown-eyed (genotype *Sd Rsp^{ss} bw / cn bw*) and white-eyed (*Dp(1;2)eag^{x6}der Rsp^{i} cn bw / cn bw*) progeny are generated, but suppression is lost if *Su(SD)* is deleted or inactivated, and the consequent self-distortion of *Sd Rsp^{ss} bw* results in a large excess of white-eyed progeny. Mere visual inspection of the cross vials for a white-eyed brood was used to identify mutants with complete or partial loss of the insert. Mutants with visible cytogenetic changes allowed localization of *Su(SD)* to polytene segment 13C7-13E4, whereas those with no obvious cytological change likely contain either a very small deletion or point mutation. These will serve as landmarks to delimit the *Su(SD)* locus when this region is sequenced.

Future studies might include examining *Dp(1;2)eag^{x6}* (from which *Dp(1;2)eag^{x6}der* was derived) for the suppressor phenotype. If *Dp(1;2)eag^{x6}* does not suppress, then the *Su(SD)* mutation likely arose with the internal deletion that produced *eag^{x6}der*, and confirm its independence from *Su(SD)^{R57}*. Transposon tagging mutagenesis of *Su(SD)* in the brown-eyed F1 males might provide an approach to fish out the gene sequence.

6. Conclusions

A heterozygous cross showing deviation from Mendelian 1:1 segregation is exceptional. Hence, any deviation when first seen is serendipitous, and the mechanism underlying it need not be related to the reasons for doing the cross and analyzing its progeny in the first place. But no geneticist would ignore such an exceptional observation (recall William Bateson advising geneticists to 'treasure your exceptions!'), although to solve it might demand diverting resources

and time from one's original research objectives, and thus risk upsetting funding agencies by embarking on tangential research. Setting out on such tangents is also risky if the 'genetics resources' are not suitably developed. Examples of genetics resources used in the work described here were polytene chromosome maps, the *SD-5*^{r7} revertant, the *Dp(1;2)eag*^{x6} translocation, the haplo-insufficient *Star* phenotype, *cn* and *bw* interaction in the double mutant, and the suicidal *Sd Rsp*^{ss} *bw* chromosome. Such resources are built up over many years by scientists pursuing diverse research objectives. Gingell and McLean (2020) described *SD* as being initially 'an esoteric puzzle'. Molecularly defining *Su(SD)* and its upward modifiers will identify new players in Ran signaling and nuclear transport, a subject that is anything but esoteric and is of wide interest.

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