



## Under the magnifying glass: The ups and downs of *rDNA* copy number

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### ABSTRACT

The ribosomal DNA (*rDNA*) in *Drosophila* is found as two additive clusters of individual 35 *S* cistrons. The multiplicity of *rDNA* is essential to assure proper translational demands, but the nature of the tandem arrays expose them to copy number variation within and between populations. Here, we discuss means by which a cell responds to insufficient *rDNA* copy number, including a historical view of *rDNA* magnification whose mechanism was inferred some 35 years ago. Recent work has revealed that multiple conditions may also result in *rDNA* loss, in response to which *rDNA* magnification may have evolved. We discuss potential models for the mechanism of magnification, and evaluate possible consequences of *rDNA* copy number variation.

In this review, we will refer to the entire cluster of arrayed 35 *S* (also called the 45 *S*, depending on the organism) rRNA transcription units as the *bobbed* gene, and an individual copy of the repeated transcription unit and linked control sequences as a cistron. Likewise, *rDNA* array is synonymous with the *bobbed* gene locus and *rDNA* with a single cistron; *rDNA* copy number, then, means the number of cistrons in an organism or a cell.

We will not discuss the control of copy number of the ribosomal RNA (rRNA) cistrons within the rRNA gene cluster locus (i.e., the *rDNA*) in *Saccharomyces cerevisiae*. This is perhaps an odd decision, given what all is known about *rDNA* copy number regulation in budding yeast. However, much of the biology of yeast *rDNA* seems driven by features unique to that organism. Yeast has a genetic system necessary to increase copy number within a “short” cluster (that is, a locus containing few copies of the rRNA cistron) [42], and the rare form of closed-mitosis during daughter bud formation makes extrachromosomal *rDNA* accumulation a problem that is particularly pronounced in budding yeast [5]. Truly excellent reviews have been published on the yeast *rDNA* [43,70], and those should suffice for readers curious about *rDNA* copy number regulation in that organism.

What follows here is mostly from *Drosophila*, in which research on *rDNA* copy number is the oldest and, outside of yeast, best researched. Our sincerest apologies to colleagues whose work on the *rDNA* of other organisms, or even *Drosophila*, was excluded here.

### 1. The *bobbed* locus of *Drosophila melanogaster*

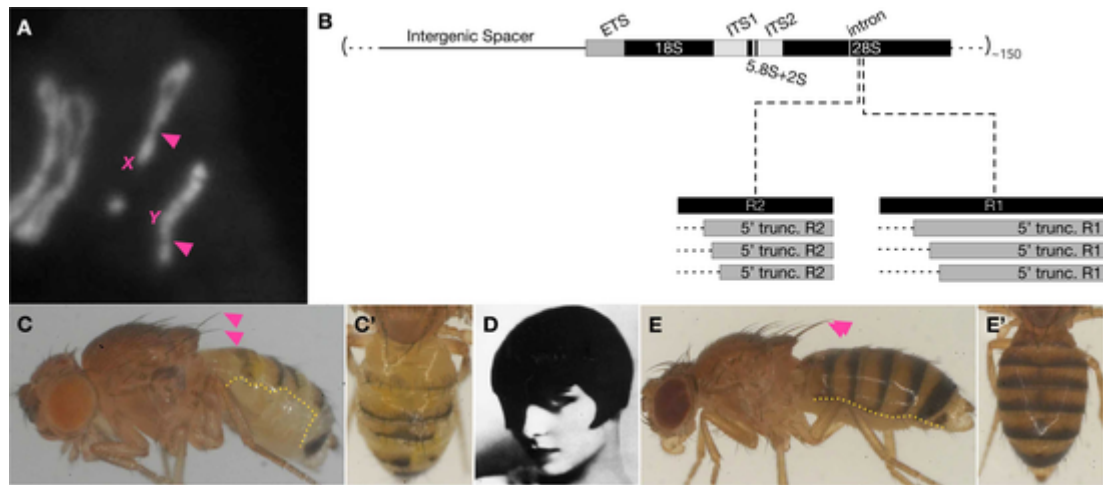
Alfred Sturtevant discovered the seminal X-linked “*bobbed*” (*bb*) mutation in 1915, and subsequently identified multiple *bobbed* alleles with variable expressivity ranging from wild-type to lethal [99]. Curt Stern later found that males or females bearing a normal Y chromosome were suppressed for an X-linked *bobbed* phenotype, and correctly inferred that the Y also contains a *bobbed* gene that could completely compensate for mutations of the X-linked *bb* gene, even if lethal [12,102]. Further work found Y-linked *bobbed* alleles, and showed that the X-linked *bobbed* and Y-linked *bobbed* alleles were additive/redundant in their functions [68,101]. Before identifying *bobbed* as the *rDNA* array, its unusual genetics – its redundancy, its correlation between copy number, expressivity, and penetrance, and its proclivity to revert – probably confused understanding of the nature of the gene that was coalescing at the time.

The *bobbed* loci map to the X pericentric heterochromatin and to the Y chromosome short arm, both near the cytological “secondary constrictions” of the Nucleolus Organizing Regions (NORs) ([14], see also [72]) (Fig. 1A). Later it was Ferruccio Ritossa that showed that the *bobbed* loci, the NORs, and the *rDNA* arrays were one-and-the-same by showing that the two largest ribosomal RNAs (the 28 *S* and 18 *S*) hybridized to DNA from flies harboring deficiencies of/within the NORs of both X and Y chromosomes [88,89]. Whereas we refer to the X-linked *bobbed/rDNA* array as X-*rDNA* (and that of the Y as Y-*rDNA*), mutations of those arrays that confer a *bobbed* phenotype are X-*bb* and Y-*bb*. The complementarity further demonstrated how *rDNA*

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**Fig. 1.** The Ribosomal DNA (*rDNA*) and bobbed phenotype of *Drosophila melanogaster*. (A) Partial karyotype of *Drosophila* showing the X and Y chromosomes and the linked X-*rDNA* and Y-*rDNA* loci. Pink “X” and “Y” identify chromosomes and locations of respective centromeres (“primary constrictions”). Pink arrowheads show locations of “secondary constrictions,” the *rDNA* loci. (B) Schematic of a *rDNA* repeat unit. The primary rRNA transcript (35 S) is processed into three mature rRNAs, the 18 S, 5.8 S + 2 S, and 28 S, by removal of the External Transcribed Spacer (ETS), two Internal Transcribed Spacers (ITS1 and ITS2), an intron in the 28 S, and a small cleavage product demarcating the 5.8 S and 2 S. *Drosophila rDNA* cistrons can harbor retrotransposable element insertions (R1 and R2, or 5’ truncations of full-length R1 and R2 elements) at stereotyped sites in the 28 S. (C) Bobbed phenotype showing two common phenotypes: wispy, trimmed/ truncated, askew bristles (pink arrowheads), and incomplete dorsal abdominal cuticle deposition leading to herniations and exposed epidermis (lateral extent of dorsal cuticular deposition is indicated by yellow dotted line); compare both to (E). (C’) shows a dorsal view of the abdomen where uneven and askew bristles at the segmental boundaries are evident. (D) American silent film star Mary Louise Brooks (1906–1985) sporting her namesake “lulu-bob” haircut, whose evocation by the close-trimmed bristle phenotype gave the *bobbed* locus its name. (E) Wild-type *Drosophila* showing normal scutellar bristle morphology (pink arrowheads) and extent of dorsolateral cuticular deposition (yellow dotted line). (E’) shows a dorsal view of the abdomen; compare both to (C). (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)

comprised 0.3% of the genome and consisted of hundreds of structurally-repetitive rRNA gene copies, and finally provided a clear explanation of the allelic series, additivity, and redundancy of the X-*rDNA* and Y-*rDNA* array loci.

We now know from molecular cloning that the *rDNA* array loci consist of hundreds of copies of the 35 S primary transcription unit which comprises the 18 S, 5.8 S, and 28 S subunits, preceded by an “External Transcribed Spacer” (ETS) and separated by intervening “Internal Transcribed Spacers” (ITSs) [70] (Fig. 1B). Individual transcription units are separated by Intergenic Spacer sequences (IGS; historically, these were called Non-Transcribed Spacers, NTSS, until they were found to indeed be transcribed [64]). In *Drosophila* as well as other insects, the 5.8 S is cleaved into “5.8 S” and “2 S” components. These function analogously to the single 5.8 S of other organisms, as the *Drosophila* 5.8 S and 2 S pair in much the same way that the 5.8 S self-anneals in other organisms [35,76]; it is not known if the 2 S or its cleavage product provides some unique functional role or whether the cleavage is essentially inconsequential.

The allelic series of *bobbed* and additive rescue demonstrated that the *rDNA* “polygene” has dose-requirements, with high copy numbers of the cistron necessary for full viability and cuticular/bristle protein production, fewer copies resulting in etched cuticles and truncated or insubstantial bristles (the “bobbed” gene was named for its resemblance to the then-vogue haircut) (Fig. 1C-E), and fewer still was lethal [88]. *bobbed* mutations also expressed delayed development in comparison to *wild-type* siblings. It is clear from decades of work that a certain copy number of *rDNA* is necessary for viability, though which tissues are most-sensitive remains unknown [10,65,124]. In *bobbed* alleles the cuticle, in particular the posterior dorsal abdominal tergites, show the phenotype most clearly. With the important caveat that not all *rDNA* copies are equal, it appears from ours and others’ work that about 90 *rDNA* copies are necessary to confer viability, and about 120 are needed to avoid a bobbed phenotype altogether [75, 106,110]. One difficulty in this determination stems from common retrotransposon insertions that inactivate expression of individual *rDNA* (see below). An additional (but understudied) source of varia-

tion comes from the expression control exerted by different IGS sequences [64], as individual *rDNA* expressed at a higher rate are expected to be more important in determining a phenotype than those that are expressed a little or not at all. The common view of the *rDNA* array is that epigenetic control is enforced to express some *rDNA*, and package others as unexpressed heterochromatin. This has not been subject to careful scrutiny in *Drosophila*, and in fact it remains possible that *rDNA* are expressed under direct control of the RNA Polymerase I enhancer sequences in the IGS [31] and polymorphisms within the transcription units, much like more-conventional genes.

We will return to the consideration of phenotypes that are affected by *rDNA* copy number. The case is unequivocal for *bobbed*, of course, and more-broadly for protein translation, but for other phenotypes this is an active area of research by many laboratories. It is extremely difficult work because of the pleiotropy expected for any locus that affects a biological process as fundamental as translation: experiments that probe “necessity” and “sufficiency” are largely beyond reach. It is also extremely difficult work because manipulating and determining *rDNA* copy number are laborious processes.

## 2. Ascertaining *rDNA* copy number

Understanding regulatory mechanisms controlling the maintenance of *rDNA* copy number rely on accurate quantification. The more sensitive, the better, since in general small changes could be meaningful, and it remains likely that different cells or tissues possess different *rDNA* copy numbers [13,75]. One reliable method for quantification is hybridization with *rDNA* probes. In the case of fluorescence (e.g., [119]), great care must be taken to assure linearity and prevent bleaching artifacts, although cell-by-cell measurements are feasible with this approach. In the case of radiolabeled probes, either liquid-phase or competitive membrane-phase hybridization [88] and slot- or dot-blot Southern detection, oftentimes whole animals are required [54], obscuring variation within organisms. The technical demands and use of radioisotopes have made these approaches less common.

Recently sequencing technology has evolved to a point where investigators can sequence an *rDNA* array almost in its entirety [34,41,71,98]. This allows direct counting of copy number, but relies on known genomic context and a relatively short *rDNA* array, which is not common and certainly not of broad experimental applicability. Other approaches come from shotgun or random sequencing, and assessing copy number by multiplicity of coverage ([21,67], Sharma and colleagues, unpublished, available at BioRxiv [97]). The general applicability of this approach remains unclear, however, as the apparent copy number can span more than 2-fold across an idealized contig of the 35 *S rDNA*. Such approaches have not yet been validated by comparison with some second approach, which may lead to artifacts stemming from data acquisition or processing [28]. For example, in quantifying *rDNA* copy number, CHEF gel estimation remains the gold standard for validation [67]. Alas, CHEF analysis is accurate to unequivocally determine *rDNA* copy number only if the flanks of the *rDNA* array are known and the length of each *rDNA* cistron is invariant; neither of these conditions is true outside of yeast, and so other PCR-based techniques have become standard.

Real-time (“quantitative”) PCR [75,113] and Digital Drop PCR [123] are two that are easily employed, though they do require specialized equipment. In the case of the former, linearity and correspondence with a *bobbed* allelic series have given some degree of confidence in the *rDNA* copy numbers obtained from PCR methods [75].

### 3. Reversion of the bobbed phenotype

Various researchers have commented on instability of the bobbed phenotype, and generally there are at least five mechanisms by which *bobbed* can be unstable. First, *Y* chromosomes are subject to hyperploidy through spontaneous (primary) nondisjunction of either sex chromosome in males or females [7,44]. Importantly, nondisjunction may be more likely in males with chromosomes bearing *bobbed* alleles [62] and the lack of genetic markers on the *Y* makes its presence essentially invisible. Since the bobbed phenotype is dependent on the sum of *rDNA* copy number, even *Y-bb* chromosomes can complement a *bobbed* genotype in *X-bb/X-bb/Y-bb* females and *X-bb/Y-bb/Y-bb* males (compared to, respectively, *X-bb/X-bb* and *X-bb/Y-bb*). Such supernumerary *Y*s are used intentionally to maintain *bobbed* strains in the laboratory.

Second, in genotypes in which crossovers between two *X*s or an *X* and *Y* can occur, the *X-rDNA* locus can become the recombination point of an unequal homologue exchange. In female *Drosophila*, which are recombination competent, the products are an *X-bb* reverted to wild-type, and a more-extreme *X-bb* allele; the latter can be lost due to cell lethality, erasing any evidence of the reciprocity. In males, which do not undergo meiotic recombination, a mitotic exchange event can create paired *X-Y* and *Y-X* translocation chromosomes [18]. Either product can be the recipient of the lion’s share of the *X-rDNA* and *Y-rDNA*, and thus complement any *bobbed* alleles in the genotype. The smaller *X-Y* chromosome is essentially unmarked and can be considered like a *rDNA*-containing “B” chromosome that would be invisible without cytological examination or careful analysis of secondary nondisjunctional progeny [7,57].

Third, a purely-somatic form of phenotype reversion can occur in clones or individuals which bear mutations that genetically interact with the *rDNA* [61], for example those that alter *rDNA* expression [153]. In these cases, although *rDNA* copy number may remain unchanged, the expression of one or more cistrons can be increased. Debate still exists whether these are cases of derepression of previously-silent cistrons, are increased output of the already-expressed cistrons (as in yeast [95]), or a mix of both possibilities. The appearance of recessive *suppressors-of-bobbed* in some laboratory strains may be common, and a general reason why the mechanism of true reversion had been debated for so long ([61,127]).

In a fourth (and fifth) type of instability, Ritossa described the bobbed phenotype reverting to wild-type in offspring of males with a moderate *X-bb* allele and a *Y-bb* allele that was either severe (*Y-bb*-) or entirely devoid of *rDNA* (*Y-bb0*). Ritossa monitored this “*rDNA* magnification” and found that nearly all of the offspring were reduced for *bobbed* expressivity, and the expressivity further lessened from generation to generation. It appeared that the *bobbed* locus was slowly healing itself over multiple generations, restoring the mutant allele to full function. Strikingly, within these slowly-magnifying populations were some offspring that fully-restored *bobbed* to *wild-type* in a single generation. Thus, magnification seemed to have two different but simultaneous manifestations.

Ritossa used sensitive competitive DNA/RNA hybridization to search for clues to the mechanisms of *rDNA* magnification [88,90]. As expected, he found that reversion of the bobbed phenotype correlated with an increased *rDNA* copy number ([59]). However, in the “slow” magnification, he noted that the increase of *rDNA* copies was not inherited at the *bobbed* locus, but rather was recreated every generation *de novo*. Further, in this “slow” process the reversion usually back-reverted to a bobbed phenotype – in fact to the original bobbed phenotype – if taken out of magnifying conditions. Ritossa reasoned that the basis for reversion was the presence of extrachromosomal *rDNA*, either circular or linear mini-arrays, that were expressed but lost during gametogenesis [86,87]. Kenneth Tartof [105,106] studied the genetic requirements for this “slow” magnification, and showed that *rDNA* copy number could increase even at a normal *X-rDNA* array, and he named this form of magnification “disproportionate replication.” Disproportionate replication did not require a bobbed phenotype, but did require magnifying conditions, that is one *rDNA* array on the *X*, *bobbed* or not, and a homologous *Y*-linked *rDNA* array with a severe bobbed allele (i.e., *X/0* males, *X/X-bb* females, or *X/Y-bb* males).

The “fast” type of magnification behaved differently. Here, revertants were sporadic (on the order of 15% of offspring showed reversion), complete (the reversion was to *wild-type*, not just to a milder bobbed phenotype), and permanent and heritable. Increases in *rDNA* copy number also attended this type of magnification, and restored *rDNA* were indeed found incorporated into the natural *X-bb* locus. This magnification required a *bobbed* allele on the *X* chromosome and magnifying conditions including a *Y-bb*- or *Y-bb0* allele [6,19], and possibly some still-unknown locus on the tip of the *Y* chromosome far from the *Y-rDNA* locus [45]. It was therefore evident that the “slow” and “fast” forms of magnification were different, although they coincided in magnifying males, suggesting that they may be different manifestations of the same mechanism, or at least two types of magnification induced by the same conditions.

### 4. Observations of magnification and likely mechanism

To deduce the timing of magnification, Tartof and R. Scott Hawley [29,30] created a pair-matched set of chromosomes, a *Y-bb*- chromosome and a revertant, *Y-bb+*. This pair allowed them to discriminate between magnification and disproportionate replication by comparing offspring from *X-bb/Y-bb-* and *X-bb/Y-bb+* fathers. The former genotype produced clusters of identical magnification-bearing sperm chromosomes, indicating that magnification occurred in a stem cell or spermatogonium early in spermatogenesis. In contrast, the latter genotype produced only singular events, indicating that disproportionate replication occurred late in spermatogenesis (perhaps as late as meiosis). Both stages required some canonical DNA Damage Response genes, as mutations of the DNA repair gene *mus108* inhibited both forms of reversion, but other mutations could distinguish between disproportionate replication and true magnification [29]. They homed in on the latter process.

True magnification occurred in X/0 males, which ruled out recombination between homologous *rDNA* arrays. Tartof put forth the hypothesis that magnification relied on unequal sister chromatid exchange [107,108]. He predicted that such mitotic recombination events would produce sister chromatids with complementary losses and gains of *rDNA*, and promptly showed that magnifying *X-bb* chromosomes produced more severe *X-bb* chromosomes under magnifying conditions. He could not establish that these genetically-reciprocal events were both derived from the same event, and they did not appear in equal number. Nonetheless, it was a key piece of evidence that magnification and *rDNA* loss were linked.

Strong support for unequal sister chromatid exchange came from analysis of a magnifying *ring-X-bb* chromosome (a circular chromosome without telomeres and that bore a *bobbed* mutation, in a male genotype with a *Y-bb* chromosome to induce magnification). If magnification required unequal sister chromatid exchange, then exchange at the *rDNA* would produce unstable dicentric ring chromosomes as the two sisters formed a continuous figure-∞. This is contrasted to the alternative, if magnification did not involve sister-exchange, magnification should be able to revert individual sister *ring-X* chromatids without conjoining them. Tartof reported no magnification in 1500 offspring, which he interpreted as the obligate formation of dicentric double-rings, lethal in subsequent mitoses, and support for unequal sister chromatid exchange [107,108]. Sharyn Endow analyzed magnification products and directly observed the unstable dicentric ring chromosomes, cytologically proving unequal sister chromatid exchange in magnifying conditions and not in non-magnifying conditions [18].

The co-existence of “fast” and “slow” magnification fed debate over the nature and mechanism of magnification, injecting doubt about the role of unequal exchange, and it wasn’t until 1986 that the issue was satisfactorily resolved. Marcus and Hawley carefully analyzed the conditions and properties of “slow” and “fast,” and showed that “slow” magnification was not magnification at all, but rather a suppression of the *bobbed* phenotype by other mutations in the strain [61]; the aforementioned third and fourth types of reversion were the same. Finally, magnification fell neatly under a simple explanation. Unequal sister chromatid exchange appears to be the main, perhaps only, mechanism for the formation of the final *rDNA* magnification chromosomes ([19,127]). However the unequal sister exchange in the premeiotic divisions of the male germline requires a double-strand break to initiate exchange.

## 5. Origin of the magnification-inducing DNA break

The *I-CreI* meganuclease fortuitously recognizes the *Drosophila* *rDNA* sequence, and a heat-shock inducible transgenic *I-CreI* is sufficient to create chromosome exchanges between the *X-rDNA* and *Y-rDNA* loci [56]. Additionally, expression could be used to damage the *rDNA* and recover an allelic series of *bobbed* [74]. Upon a second exposure to *I-CreI*, stable and heritable *rDNA* revertants could be recovered from *bobbed* mutants. We cannot assert that the mechanism is the same as true magnification, but we can conclude that a *rDNA* break in premeiotic divisions is sufficient to cause *rDNA* expansion.

Similar results have been obtained in mutations of the Bloom Helicase (*blm/mus309*), which is required to replicate “hard-to-replicate” DNAs [91]. Defects in *blm* function cause replication fork collapse and double-strand breaks during S phase. We have recently found that *blm* mutants produce *bobbed* offspring, enhance existing *bobbed* alleles, produce offspring with X-Y exchanges at the *rDNA*, and increase the frequency of *bobbed* reversion (manuscript in preparation). This strongly suggests that any *rDNA* damage is sufficient to alter *rDNA* copy number through homologue, sister, or intrachromatid repair.

If we accept that any break is sufficient, then the mystery becomes what about the “magnifying condition” makes the break. Whatever the

answer, it must explain why unequal sister chromatid exchange happens only to *X-bb* alleles when the *Y* is even-more severely *bobbed*. What about those two conditions conspire to elicit DNA damage in the *X-rDNA*? It may have to do with the mode of chromosome-specific *rDNA* regulation in male *Drosophila*.

In 2012, Frauke Greil and Kami Ahmad reported that the *X-rDNA* locus is silenced by a still-unknown process in *Drosophila* males, while the *Y-rDNA* array is active ([25], also [119]). This observation is reminiscent of nucleolar dominance seen in interspecific hybrids of plants and animals [63,78]. It also poses a conundrum: if only the *Y-rDNA* array is expressed, then the *bobbed* phenotype should reflect the *Y-bb* allele alone; the *X-rDNA* status (*bobbed* or not) should be recessive rather than additive in males. *Y-bb* alleles that are lethal when the sole source of rRNA should be dominant-lethal. X/0 males should die from lack of rRNA. And the magnifying conditions used by Ritossa, Tartof, and others should be impossible! So, it must be that a *Y-bb* allele that is incapable of sustaining sufficient *rDNA* expression triggers the derepression of the *X-rDNA* array in males. It is tempting to further propose that when the *X-rDNA* array is active in males, magnification occurs. In fact, perhaps *X-rDNA* expression is sufficient to make the necessary DNA breaks. How the *Y-rDNA* and *X-rDNA* arrays are regulated, specifically how a repressed *X-rDNA* array is derepressed in magnifying conditions, is an outstanding and persisting mystery of *rDNA* biology. Happily, Greil and Ahmad reported that *X-rDNA* loci are active in some strains, opening the possibility of mapping this “derepression” phenotype. Experiments to deduce whether monoallelic expression is a property of the *X-rDNA*, the *Y-rDNA*, or of some unlinked mutation may well provide the insight needed to understand why magnifying conditions lead to magnification.

A tantalizing clue may come from other mutations that cause damage at the *rDNA* – heterozygous mutations in *Su(var)3–9* and *Su(var)205* (which encode a histone H3 Lysine-9 methyltransferase and the H3K9-methyl binding protein HP1a, respectively) deregulate the *rDNA* and cause permanent *rDNA* loss ([1,25,75,77]). It is as hard to imagine a loss of *rDNA* without DNA breaks as it is to imagine magnification without DNA breaks. The loss of *rDNA* copy number by these mutations may indicate an *rDNA* instability that leads to primary loss, but also gains/magnification; to our knowledge, it has not been determined if these *Su(var)* mutations exhibit increased magnification rates, or could be sufficient to create magnifying conditions, as does mutation of *blm*.

One might assume that in a normal male, whose *X-rDNA* is silent and whose *Y-rDNA* is active, the *Y-rDNA* is subject to constant magnifying conditions. But we know this is not so. If it were no *Y-bb* alleles could be maintained as stable strains, yet multiple strains are, including our own. Specifically, a *Y-bb* allelic series has been maintained in stock for over a decade with no sign of changes in the *bobbed* expressivity or the *rDNA* copy number [75]. Therefore something must discriminate *X-rDNA* from *Y-rDNA*, even though the ribosomal RNAs that come from them appear to be identical.

## 6. Heterogeneities

Within the *rDNA* arrays are multiple dimensions of heterogeneity. As alluded to before, the IGSs are variant in their length, reflecting differences in copy number and sequence of individual sub-repeats. These appear to be regulatory [31], suggesting that a shared selective pressures may act upon individual cistrons to assure sufficient rRNA output of an active array.

In arthropods, an additional dimension of variation exists as multiple classes of retrotransposable elements are found within the transcription unit; in *Drosophila* the two – R1 and R2 (historically called Type-I and Type-II inserts, respectively) – both are found within the 28 S subunit at specific sites [22,23,37] (Fig. 1B); R1s are slightly enriched in the X-linked *rDNA* array, and the R2 slightly enriched

within the Y-linked *rDNA* array [104,121]. Common for retrotransposable elements, both classes are subject to abortive reverse-transcription during integration. Thus, based on retrotransposable elements alone, the *rDNA* cistrons can have a dramatic amount of variation: uninserted *rDNA* cistrons, R1-inserted, R2-inserted, double-inserted, and 5' truncations of those latter three classes [3]. As Oscar Miller first noted, inserted *rDNA* cistrons are poorly expressed ([38], also see [51,125]). What keeps inserted cistrons at this low level of expression is not fully elucidated, but most-likely includes a combination of sequence-specific [26,121] and regional factors such as proximity or “epigenetic” memory [16,50,53]. Silencing R1- and R2-inserted cistrons is desirable because if expressed, they are expected to alter the strict stoichiometry that naturally arises from the co-expression of the 18 S, 5.8 S, and 28 S portions of the 35 S primary rRNA transcript [50]. As before, the connection between expression and sequence remains to be thoroughly understood.

Retrotransposition is stereotyped, with the R1s and R2s inserting reliably at base pairs 6851 and 6777, respectively, of the primary rRNA transcript. The POL subunit’s strong sequence-specificity is better understood for R2, where it generates a single-strand break and primes the reverse-transcriptase-mediated DNA polymerization. During the final step of maturation and ligation, R2 POL creates the second break to create an end-protected double-strand break that finally incorporates the R2 element [17,37,122]. This event could potentially go awry and create the necessary double-stranded break that repairs from a sister chromatid in S- or G2-phase of the cell cycle.

Others have noted that R1 and R2 expression are weakly correlated with magnifying conditions, although causality has not been determined [48,50,51,58,112]. On the one hand, it is possible that magnification sufficiently remodels the *rDNA* chromatin and derepresses R1- and/or R2-containing cistrons. Recent investigations into the expression of R1 and R2 have shown that both are induced by stress [32, 85]. However, the authors argue that this may be a consequence of increased generalized *rDNA* transcription in pre-magnifying *bobbed* males since the level of transcription of the insertions is still well-below the level of uninserted cistron transcription. de Cicco and Glover [15] also reported an increase in R1- and R2- inserted *rDNA* during the first generation of *rDNA* magnified males, but the increase was the same as the level of magnification of uninserted *rDNA*, implying R1 and R2 activity were not an essential part of magnification.

An investigation of the *rDNA* units that were lost during *rDNA* magnification showed that only uninserted cistrons were lost, while cistrons containing R1 and R2 were retained, arguing for a non-random distribution of uninserted and R1-/R2-containing carrying *rDNA* [92]. A different analysis showed that uninserted *rDNA* increased more so than did R1-containing *rDNA*, and R2-containing *rDNA* barely changed at all [110]. A similar conclusion was reached by a follow-up study [111]. Overall, these conflicting results likely indicate that R1 and R2 elements are not randomly distributed in the *rDNA* arrays ([4,125]) and suggest that R1 and R2 mobilization per se is not necessary for magnification.

However, it is possible that retrotransposable element expression is critical for magnification. Recent yet-unreviewed work from Yukiko Yamashita’s laboratory suggests this. In that work, R2 expression was sufficient to increase *rDNA* copy number in a *bobbed* allele, and silencing RNAs directed at reducing R2 expression decreased magnification (Nelson and colleagues, unpublished, available at BioRxiv [69]). If this is confirmed, it would be an exciting demonstration of an adaptive use of the R2 retrotransposon to create and maintain *rDNA* variation. The “purpose” of *rDNA* magnification may really be to assist “selfish DNA” elements increase their numbers, and its role in offsetting potential losses of *rDNA* a secondary benefit.

We consider the formation of the magnification-initiating break to be the “regulated” step, but it need not be so. Magnification and loss require the sister chromatid exchange to be unequal, which it normally is

not. It is therefore a possibility that breaks are common in the *rDNA*, but they are faithfully repaired from allelic *rDNA* cistrons except in magnifying conditions. Some plausible support come from mutations in *CTCF* which recruits cohesin as an effector of silencing [9,83], and mutants of *Su(var)3–9* and *Su(var)205* which reduce the function of the naturally cohesive heterochromatin. The same may be considered for any condition that alleviates repression of the *X-rDNA* array. Magnification may be blocked in normal conditions by the cohesin- and heterochromatin-induced pairing of the inactive *X-rDNA*. Loss of *Y-rDNA* and subsequent derepression of the *X-rDNA* may diminish pairing and allow out-of-register unequal sister chromatid exchange. The difference in magnification between active *X-bb* and *Y-bb* may indeed stem from nucleolar dominance, but because of pairing rather than DNA double strand break formation on only one array.

### 6.1. *rDNA* magnification in humans

As we are aware, there are no descriptions of *rDNA* magnification in other organisms, including mammals. This itself is an important point because one must always express caution when applying discoveries made in model systems to ecology, human disease, or otherwise. If *rDNA* magnification does occur in humans, it would impact how we consider the *rDNA* as a locus of induced variation, or as a locus important for epistasis, expressivity, penetrance, or heritability of other phenotypes. Can we apply what is known about *Drosophila* (or *S. cerevisiae*) *rDNA* magnification to humans?

We do not conclude that magnification is unique to *Drosophila* (and *S. cerevisiae*), and in fact it seems clear that the lack of magnification could be explained entirely by a difficulty in detecting it. In humans, the 35 S/45 S are on the short arms of the acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22), and the evidence suggests that they are all active [100,115], and entire arrays can be lost through chromosome fusions [81] or terminal deficiencies [80,115]. This indicates that humans are capable of making much more rRNA than needed to avoid any obvious phenotype, and without an easy-to-assess phenotype (like death in yeast, or *bobbed* in *Drosophila*), it would be extremely difficult to detect magnification.

But we do think it’s fair to say that there is no reason to think that magnification occurs in humans. There is no sign of programmed DNA damage at the *rDNA* loci, either from particular gene activities or from disruptions to natural nucleolar dominance [63,66,78,109]. There are no obligatory *rDNA*-linked transposable elements that could damage *rDNA*. Without DNA damage, magnification cannot proceed, at least by any mechanism that we imagine or that data support. As in *Drosophila*, mutations in *blm* lead to hypervariability in the *rDNA* [40], though it is not clear if these are driven by intra- or inter-array exchanges. *CTCF* is a known regulator of the *rDNA* in humans [36,114], suggesting also that a loss of cohesion may contribute to unequal sister exchange. But these mutant conditions are exceptional, and cannot be taken as evidence for a naturally-evolved magnification system [116]. While it is true that many studies have described variation in human *rDNA* copy number, these are adequately explained by progressive or stochastic losses in the soma. There is precious little longitudinal work to show progressive losses (or gains) of *rDNA* across a lifespan of an individual, or even within cloned cell cultures. In disease conditions, such as cancer, there is also plenty of evidence for changes in *rDNA* copy number, but these losses (and they are primarily losses) are part of a disease state, and so again it would be unlikely that a mechanism to magnify would evolve from the conditions occurring in a somatic disease of age.

If *rDNA* magnification does exist in humans, how would we know? First, to claim discovery, one should require magnification to be regulated, involving gene activities that are largely-dedicated to *rDNA* copy number changes: in yeast this is the *fob1* gene, in *Drosophila* the mechanism is not known but as we argue probably involves *rDNA*

derepression, nucleolar dominance, and potentially retrotransposable element expression. Second, magnification should be induced by particular conditions (such as a detectable decrease in rRNA levels). Alternatively, it could be that *rDNA* magnification is constitutive to offset a constitutive *rDNA* copy number decrease, but a balance must have evolved to well-match these rates.

## 7. *rDNA* magnification offsets *rDNA* loss – sometimes

One might surmise that magnification has evolved to offset natural losses of the *rDNA*. Instability and loss in the soma has been known for some time [13], and its contribution to penetrance and expressivity should be considered. However our work and others suggest that the average *rDNA* amount between individuals varies very little, and so the detection of somatic *rDNA* loss may be limited to some cells.

Recently, Yukiko Yamashita and colleagues reported a very high level of loss of the *rDNA* through normal male germ line stem cell divisions, progressive with age [52]. Because no bobbed phenotypes were reported in the offspring of aged fathers, it seems unavoidable to propose a new form of reversion that occur every generation to offset the described germ line loss. This reversion must also occur in two phases in cells of the soma and the germ line. In the soma, reversion must be an early embryonic event to explain why offspring of old males were not bobbed or show decreases in *rDNA* copy number. Whatever the mechanism, the reversion was seen to offset *rDNA* loss in the previous germline and return the *rDNA* copy number to the same level it was in the prior generation's soma. This is reminiscent of Ritossa's "slow" magnification, as it was not inherited, and may rejuvenate his hypothesis of extrachromosomal *rDNA*, or it may also be the consequence of some other factor that was not considered in the experiments. In the germ line, the observations were different. The *rDNA* in the pole cells of the embryo stayed with less *rDNA* until after coalescing into the testis germ line stem cells in the adult, when they quickly returned to the *rDNA* level of the previous generation, and began the cycle of loss and gain over again. This observation, if repeated, would represent an unprecedented dynamism and memory of *rDNA* copy number.

Until now *rDNA* has been considered to be very stable from generation to generation. This is informally seen by the maintenance of multiple bobbed strains through the decades of work on *rDNA* magnification and molecular biology; surely if bobbed alleles were unstable in non-magnifying conditions – either by sporadic magnification to suppress the phenotype, or by progressive loss to enhance the phenotype – it should have been noted [4]. Some studies have directly tested for instability leading to *rDNA* loss and found none ([29,30,60,106–108,127]).

In our work, we detect *rDNA* loss in only three conditions: the expression of enzymes that specifically damage the *rDNA*; in genetic mutants that affect chromatin structure, rRNA expression, or DNA repair at the *rDNA* array; and when subject to profound nutritional stress conditions that alter rRNA expression ([1,2,26,39,75]). Otherwise, we find that bobbed alleles are remarkably robust.

*rDNA* stability is true even for arrays that straddle the bobbed-lethal/extreme-bobbed threshold [74,75]. When chromosome *Y-bb*<sup>484</sup> is the sole array (by placing it over an *X* chromosome devoid of all *rDNA*) it expresses as bobbed-lethal with rare extremely-bobbed escapers. *Y-bb*<sup>183</sup> expresses only moderate sub-viability when culture conditions are not optimal, and the flies bearing *Y-bb*<sup>183</sup> as the sole *rDNA* array are extremely-bobbed with gross loss of abdominal cuticle. These strains are normally kept in the presence of a normal *X-rDNA* array, but every time males are crossed to generate offspring with these *rDNA* arrays as sole *rDNA* array, their phenotypes reappear exactly as they did before, and quantifications are indistinguishable from previous determinations over decades, regardless of the age or conditions of the culture.

We have performed experiments investigating gradual loss in mutants and in conditions in which *rDNA* is destabilized by dietary manipulation [2], and do not observe loss in subsequent broods of males. It remains unresolved whether the phenomenon described by Lu and colleagues affects only a subset of chromosomes, acts only under a subset of conditions, is a consequence of other mutations in the genotype, or some other explanation.

## 8. A brief mention of the 5 *S rDNA*

Relatively little work has been done on the biology of the 5 *S* array. The 5 *S* is also a repeat array, comprising about 50 copies per homologue in *Drosophila* (the locus is not sex-linked, instead being on the left arm of chromosome 2), is transcribed by RNA Polymerase III, and might be expected to show some complex genetic interactions with alleles of the 35 *S* or ribosomal proteins. Changes in 5 *S* copy number are not known to cause a bobbed-like phenotype in *Drosophila*, however increases in *rDNA* copy number are linked to dramatic growth phenotypes in plants ([94]).

It is possible that ribosomal RNA stoichiometry may be maintained by coordinating magnification and loss between the 35 *S* and 5 *S rDNA* arrays [21] however those reports have come under question [28]. Still, this locus might yet reveal some fascinating biology.

## 9. Phenotypes

High intra- and inter-individual *rDNA* copy number variability makes it difficult to assertively assign phenotypic consequences of deficient *rDNA* arrays in most organisms, including *Drosophila*, and even yeast. *rDNA* transcription and ribosome biogenesis manifest in the formation of the nucleolus, the most transcriptionally active site in the genome. This alone may influence all cellular and metabolic processes through sheer transcriptional and translational demand [118]. Such effects may alone exert metabolic phenotypes [8,33]. Limited *rDNA* might also be expected to cause limited ribosome biogenesis, and thus defects in translation. The characteristic abnormalities of a delay in time of development and reduction in the rate of egg laying may be consequences of reduced protein synthesis [65], but specific phenotypes have yet to be ascribed to this.

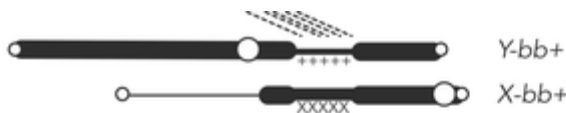
Many groups have shown that deletions within the *Y-bb rDNA* array results in the destabilization of heterochromatin and suppression of heterochromatin-induced gene silencing (Position Effect Variegation, PEV) ([49,75,126] however see [25]). This effect, discovered in induced *rDNA* copy number variants and reproduced in naturally occurring polymorphic *Y rDNA* arrays, may also lead to changes in gene expression across the genome [73] Again, though *rDNA* has been linked to subtle expression differences, whether these steady-state mRNA differences result in selectable phenotypes has yet to be demonstrated.

Research in other organisms has shown that perturbations in *rDNA* copy number may be a cause or consequence of many cancers and age associated diseases. The highly proliferative nature of malignant transformation and the cumulative stressors of cellular aging could be aggravated by *rDNA* limitation, metabolic perturbations, or otherwise. An inconsistent pattern of *rDNA* copy number changes along with hypo/hyper-methylation in promoters and coding regions of rRNA genes has been observed in cancerous and aged tissues when compared to healthy controls in mammals, plants and fish [28,96,103,113,120,123]. The lack of a clear pattern of losses or gains across all cancer types could merely reflect tissue-specific differences in *rDNA* regulation. It could also be that cancer compromises *rDNA* stability rather than copy number, allowing both gains and losses, the latter of which may be more likely ([113]).

In some conditions, *rDNA* copy number may aggravate disease states through specific genetic interactions. Deficiencies and mutations

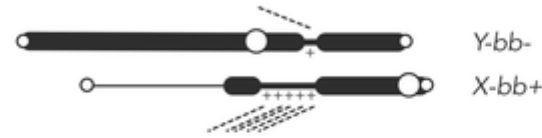
**Normal Condition:**

The male Y-rDNA is active and is the sole source of rRNA, as the X-rDNA is silenced by nucleolar dominance.



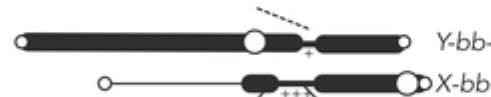
**"Magnifying" Condition:**

Inadequate rRNA output from the Y-rDNA triggers X-rDNA derepression through an unknown mechanism.



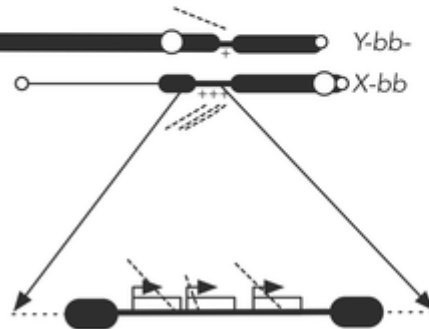
**"Magnifying" bobbed Condition:**

Here, the derepressed X-rDNA itself has limited rDNA copy number; this genotype expresses a mild bobbed phenotype that can be easily monitored for magnification.



**X-rDNA-Localized DNA Damage and Repair:**

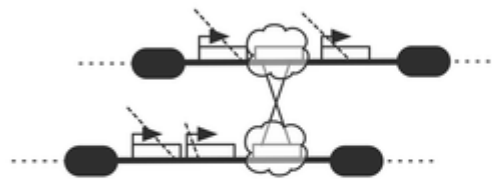
Something about this condition – hyperexpression, R1 or R2 expression, polymerase collisions, etc. – causes increased DNA damage at the rDNA locus.



DNA damage may be limited to S or G2 (left), or may occur in G1 then enter S phase (right), generating substrates for homology-dependent repair.



Repair proceeds out-of-register, perhaps encouraged by loss of cohesion, resulting in unequal sister-chromatid exchange.



One exchange product possesses fewer rDNA copies, and is underrepresented or lost owing to cell-lethality, slow cell growth rate, etc.



The other exchange product possesses more rDNA copies, and confers a growth advantage compared to cells that inherit the reciprocal exchange product or chromosomes on which unequal sister-chromatid exchange has not occurred, giving the illusion of "intentional" magnification.



Fig. 2. Synthesis of Potential rDNA Magnification Mechanisms.

in DNA repair factors or chromatin components that lead to fluctuations in rDNA copy number may make replication, repair, or heterochromatin formation more reliant on yet other gene products. It is plausible that rDNA copy number may genetically interact with these genes and contribute to the etiological basis of diseases such as Bloom syndrome, Werner syndrome, ataxia-telangiectasia, and even growth rate [40,47, 93]. Furthermore rDNA copy number changes that have been implicated in disease may act through a metabolic dysregulation that arises through rDNA copy number changes [2,8,33].

There is a recently emerging consideration that rDNA defects play a pivotal role in neuronal biology, and may underly some neurological disorders ([46]). Whether it might do so through alterations to transcription, cell cycle and development, heterochromatin and

transposon control, or metabolism is an area of active inquiry [11, 79].

10. Where are we at?

Magnification of the rDNA is well-accepted, and supported by decades of work by many laboratories, working in collaboration or independently. Unequal sister chromatid exchange appears fully explanatory as a mechanism (Fig. 2). However, how widespread is this phenomenon – are all chromosomes susceptible, whether it happens in normal genotypes – seems still open for discussion (and disagreement). The balance of information suggests that DNA breaks are involved in initiating magnification, but it is unknown whether this is a

specific and regulated process (as, e.g., it is in *Saccharomyces cerevisiae*), or whether it is a consequence of misregulation of the *rDNA* under what we now call “magnifying” conditions. Perhaps evolution would not discriminate: why would a specific system evolve under conditions that cause it to happen anyway?

A persistent question that arises when considering magnification is of the consequence. Broadly, there seem to be three “reasons” for magnification. One is to avoid lethality from the loss of translational capacity. This itself is important, but perhaps no different from any form of DNA repair that offsets mutation. Magnification may be a special case as, for example, repeat sequences are more susceptible to loss than more complex sequences. A second reason would be to “tune” other phenotypes that depend on *rDNA* copy number. This is perhaps the most exciting possibility, as the wide variation in *rDNA* copy number that exists within populations may modify yet-unknown phenotypes. It could, then, explain some degree of heritability “missing” from GWAS studies that struggle to consider epistatic interactions [82,84]. Our laboratory, and many others, are exploring this possibility since the correlation between *rDNA* copy number, aging, cancer, and neurological disorders are too compelling, and too important, to leave alone. Finally, it is possible that *rDNA* magnification is neither important nor adaptive, but merely the consequence of the unusual biology of repeat DNAs. Perhaps our understanding of *rDNA* copy number regulation is hindered by our expectation that it is “unique,” and not simply following the same rules of satellite DNA and other repetitive elements. Magnification may be a phenomenon without a reason [55], one of the many spandrels that arise during the evolution of other complex biological systems [24]. Whatever the case, many exciting studies are being done to work it out.

## References

- [1] J.C. Aldrich, K.A. Maggert, Simple quantitative PCR approach to reveal naturally occurring and mutation-induced repetitive sequence variation on the *Drosophila* Y chromosome, *PLoS One* 9 (10) (2014), e109906 <https://doi.org/10.1371/journal.pone.0109906>, PMID: 25285439; PMCID: PMC4186871.
- [2] J.C. Aldrich, K.A. Maggert, Transgenerational inheritance of diet-induced genome rearrangements in *Drosophila*, *PLoS Genet.* 11 (4) (2015), e1005148 <https://doi.org/10.1371/journal.pgen.1005148>, PMID: 25885886; PMCID: PMC4401788.
- [3] K.T. Averbeck, T.H. Eickbush, Monitoring the mode and tempo of concerted evolution in the *Drosophila* melanogaster rDNA locus, *Genetics* 171 (4) (2005) 1837–1846 <https://doi.org/10.1534/genetics.105.047670>, Epub 2005 Sep 2. PMID: 16143606; PMCID: PMC1456108.
- [4] A. Bianciardi, M. Boschi, E.E. Swanson, M. Belloni, L.G. Robbins, D.N.A. Ribosomal, organization before and after magnification in *Drosophila* melanogaster, *Genetics* 191 (3) (2012) 703–723 <https://doi.org/10.1534/genetics.112.140335>, Epub 2012 Apr 13. PMID: 22505623; PMCID: PMC3389968.
- [5] B. Boettcher, Y. Barral, The cell biology of open and closed mitosis, *Nucleus* 4 (3) (2013) 160–165, <https://doi.org/10.4161/nucl.24676>.
- [6] E. Boncinelli, F. Graziani, L. Polito, C. Malva, F. Ritossa, rDNA magnification at the bobbed locus of the Y chromosome in *Drosophila* melanogaster, *Cell Differ.* 1 (2) (1972) 133–142, [https://doi.org/10.1016/0045-6039\(72\)90036-x](https://doi.org/10.1016/0045-6039(72)90036-x).
- [7] C.B. Bridges, Non-disjunction as proof of the chromosome theory of heredity, *Genetics* 1 (1) (1916) 1–52 <https://doi.org/10.1093/genetics/1.1.1>, PMID: 17245850; PMCID: PMC1193653.
- [8] F. Bughio, K.A. Maggert, The peculiar genetics of the ribosomal DNA blurs the boundaries of transgenerational epigenetic inheritance, *Chromosome Res.* 27 (1–2) (2019) 19–30 <https://doi.org/10.1007/s10577-018-9591-2>, Epub 2018 Dec 4. PMID: 30511202; PMCID: PMC6393165.
- [9] G.A. Busslinger, R.R. Stocsits, P. van der Lelij, E. Axelsson, A. Tedeschi, N. Galjart, J.M. Peters, Cohesin is positioned in mammalian genomes by transcription, CTCF and Wapl, *Nature* 544 (7651) (2017) 503–507 <https://doi.org/10.1038/nature22063>, Epub 2017 Apr 19. PMID: 28424523; PMCID: PMC6080695.
- [10] M. Buszczak, R.A. Signer, S.J. Morrison, Cellular differences in protein synthesis regulate tissue homeostasis, *Cell* 159 (2) (2014) 242–251 <https://doi.org/10.1016/j.cell.2014.09.016>, PMID: 25303523; PMCID: PMC4222182.
- [11] I.V. Chestkov, E.M. Jestkova, E.S. Ershova, V.E. Golimbet, T.V. Lezheiko, N.Y. Kolesina, L.N. Porokhovnik, N.A. Lyapunova, V.L. Izhevskaya, S.I. Kutsev, N.N. Veiko, S.V. Kostyuk, Abundance of ribosomal RNA gene copies in the genomes of schizophrenia patients, *Schizophr. Res.* 197 (2018) 305–314 <https://doi.org/10.1016/j.schres.2018.01.001>, Epub 2018 Jan 12. PMID: 29336872.
- [12] T.W. Cline, A lesson from flex: consider the Y chromosome when assessing *Drosophila* sex-specific lethals, *Development* 128 (6) (2001) 1015–1018, <https://doi.org/10.1242/dev.128.6.1015>.
- [13] S. Cohen, K. Yacobi, D. Segal, Extrachromosomal circular DNA of tandemly repeated genomic sequences in *Drosophila*, *Genome Res.* 13 (6A) (2003) 1133–1145 <https://doi.org/10.1101/gr.907603>, PMID: 12799349; PMCID: PMC403641.
- [14] K.W. Cooper, Cytogenetic analysis of major heterochromatic elements (especially Xh and Y) in *Drosophila melanogaster*, and the theory of “heterochromatin”, *Chromosoma* 10 (1959) 535–588, <https://doi.org/10.1007/BF00396588>.
- [15] D.V. de Cicco, D.M. Glover, Amplification of rDNA and type I sequences in *Drosophila* males deficient in rDNA, *Cell* 32 (4) (1983) 1217–1225, [https://doi.org/10.1016/0092-8674\(83\)90304-5](https://doi.org/10.1016/0092-8674(83)90304-5).
- [16] D.G. Eickbush, T.H. Eickbush, Transcription of endogenous and exogenous R2 elements in the rRNA gene locus of *Drosophila* melanogaster, *Mol. Cell Biol.* 23 (11) (2003) 3825–3836 <https://doi.org/10.1128/MCB.23.11.3825-3836.2003>, PMID: 12748285; PMCID: PMC155226.
- [17] T.H. Eickbush, D.G. Eickbush, Integration, regulation, and long-term stability of R2 retrotransposons, *Microbiol Spectr.* 3 (2) (2015) <https://doi.org/10.1128/microbiolspec.MDNA3-0011-2014>, PMID: 26104703; PMCID: PMC4498411.
- [18] S.A. Endow, D.J. Komma, K.C. Atwood, Ring chromosomes and rDNA magnification in *Drosophila*, *Genetics* 108 (4) (1984) 969–983 <https://doi.org/10.1093/genetics/108.4.969>, PMID: 6439601; PMCID: PMC1224277.
- [19] S.A. Endow, D.J. Komma, One-step and stepwise magnification of a bobbed lethal chromosome in *Drosophila* melanogaster, *Genetics* 114 (2) (1986) 511–523 <https://doi.org/10.1093/genetics/114.2.511>, PMID: 3095184; PMCID: PMC1202954.
- [20] J.G. Gibbons, A.T. Branco, S.A. Godinho, S. Yu, B. Lemos, Concerted copy number variation balances ribosomal DNA dosage in human and mouse genomes, *Proc. Natl. Acad. Sci. USA* 112 (8) (2015) 2485–2490 <https://doi.org/10.1073/pnas.1416878112>, Epub 2015 Jan 12. PMID: 25583482; PMCID: PMC4345604.
- [21] D.M. Glover, D.S. Hogness, A novel arrangement of the 18S and 28S sequences in a repeating unit of *Drosophila* melanogaster rDNA, *Cell* 10 (2) (1977) 167–176, [https://doi.org/10.1016/0092-8674\(77\)90212-4](https://doi.org/10.1016/0092-8674(77)90212-4).
- [22] D.M. Glover, R.L. White, D.J. Finnegan, D.S. Hogness, Characterization of six cloned DNAs from *Drosophila* melanogaster, including one that contains the genes for rRNA, *Cell* 5 (2) (1975) 149–157, [https://doi.org/10.1016/0092-8674\(75\)90023-9](https://doi.org/10.1016/0092-8674(75)90023-9).
- [23] S.J. Gould, R.C. Lewontin, The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme, *Proc. R. Soc. Lond. B Biol. Sci.* 205 (1161) (1979) 581–598, <https://doi.org/10.1098/rspb.1979.0086>.
- [24] F. Greil, K. Ahmad, Nucleolar dominance of the Y chromosome in *Drosophila* melanogaster, *Genetics* 191 (4) (2012) 1119–1128 <https://doi.org/10.1534/genetics.112.141242>, Epub 2012 May 29. PMID: 22649076; PMCID: PMC3415996.
- [25] P.A. Guerrero, K.A. Maggert, The CCCTC-binding factor (CTCF) of *Drosophila* contributes to the regulation of the ribosomal DNA and nucleolar stability, *PLoS One* 6 (1) (2011), e16401 <https://doi.org/10.1371/journal.pone.0016401>, PMID: 21283722; PMCID: PMC3024428.
- [26] A.N. Hall, T.N. Turner, C. Queitsch, Thousands of high-quality sequencing samples fail to show meaningful correlation between 5S and 45S ribosomal DNA arrays in humans, *Sci. Rep.* 11 (1) (2021) 449 <https://doi.org/10.1038/s41598-020-80049-y>, PMID: 33432083; PMCID: PMC7801704.
- [27] R.S. Hawley, C.H. Marcus, M.L. Cameron, R.L. Schwartz, A.E. Zitron, Repair-defect mutations inhibit rDNA magnification in *Drosophila* and discriminate between meiotic and premeiotic magnification, *Proc. Natl. Acad. Sci. USA* 82 (23) (1985) 8095–8099 <https://doi.org/10.1073/pnas.82.23.8095>, PMID: 3934666; PMCID: PMC391449.
- [28] R.S. Hawley, K.D. Tartof, A two-stage model for the control of rDNA magnification, *Genetics* 109 (4) (1985) 691–700 <https://doi.org/10.1093/genetics/109.4.691>, PMID: 3921426; PMCID: PMC1202502.
- [29] D.C. Hayward, D.M. Glover, Analysis of the *Drosophila* rDNA promoter by transient expression, *Nucleic Acids Res.* 16 (10) (1988) 4253–4268, <https://doi.org/10.1093/nar/16.10.4253>.
- [30] F. He, A. James, H. Rajé, H. Ghaffari, P. DiMario, Deletion of *Drosophila* Nopp140 induces subcellular ribosomopathies, *Chromosoma* 124 (2) (2015) 191–208 <https://doi.org/10.1007/s00412-014-0490-9>, Epub 2014 Nov 11.
- [31] M.L. Holland, R. Lowe, P.W. Caton, C. Gemma, G. Carbajosa, A.F. Danson, A.A. Carpenter, E. Loche, S.E. Ozanne, V.K. Rakyan, Early-life nutrition modulates the epigenetic state of specific rDNA genetic variants in mice, *Science* 353 (6298) (2016) 495–498 <https://doi.org/10.1126/science.aaf7040>, Epub 2016 Jul 7. PMID: 27386920; PMCID: PMC5734613.
- [32] Y. Hori, A. Shimamoto, T. Kobayashi, The human ribosomal DNA array is composed of highly homogenized tandem clusters, *Genome Res.* 31 (11) (2021) 1971–1982 <https://doi.org/10.1101/gr.275838.121>, Epub 2021 Aug 18. PMID: 34407983; PMCID: PMC8559705.
- [33] Y. Hori, T. Tanaka, Y. Kikuchi, In vitro cleavage of *Drosophila* 2S rRNA by M1 RNA, *Nucleic Acids Symp. Ser.* 44 (2000) 93–94, <https://doi.org/10.1093/nass/44.1.93>.
- [34] K. Huang, J. Jia, C. Wu, M. Yao, M. Li, J. Jin, C. Jiang, Y. Cai, D. Pei, G. Pan, H. Yao, Ribosomal RNA gene transcription mediated by the master genome regulator protein CCCTC-binding factor (CTCF) is negatively regulated by the condensin complex, *J. Biol. Chem.* 288 (36) (2013) 26067–26077 <https://doi.org/10.1074/jbc.M113.486175>, Epub 2013 Jul 24. PMID: 23884423; PMCID: PMC3764810.
- [35] J.L. Jakubczak, Y. Xiong, T.H. Eickbush, I. Type (R1) and type II (R2) ribosomal DNA insertions of *Drosophila* melanogaster are retrotransposable elements closely



- related to those of *Bombyx mori*, *J. Mol. Biol.* 212 (1) (1990) 37–52, [https://doi.org/10.1016/0022-2836\(90\)90303-4](https://doi.org/10.1016/0022-2836(90)90303-4).
- [38] M. Jamrich, O.L. Miller Jr, The rare transcripts of interrupted rRNA genes in *Drosophila melanogaster* are processed or degraded during synthesis, *EMBO J.* 3 (7) (1984) 1541–1545 PMID: 6430695; PMCID: PMC557556.
- [39] J. Ji, X. Tang, W. Hu, K.A. Maggert, Y.S. Rong, The processivity factor Pol32 mediates nuclear localization of DNA polymerase delta and prevents chromosomal fragile site formation in *Drosophila* development, *PLoS Genet.* 15 (5) (2019), e1008169 <https://doi.org/10.1371/journal.pgen.1008169>, PMID: 31100662; PMCID: PMC6542543.
- [40] M.W. Killen, D.M. Stults, N. Adachi, L. Hanakahi, A.J. Pierce, Loss of Bloom syndrome protein destabilizes human gene cluster architecture, *Hum. Mol. Genet.* 18 (18) (2009) 3417–3428 <https://doi.org/10.1093/hmg/ddp282>, Epub 2009 Jun 19.
- [41] J.H. Kim, V.N. Noskov, A.Y. Ogurtsov, et al., The genomic structure of a human chromosome 22 nuclear organizer region determined by TAR cloning, *Sci. Rep.* 11 (2021) 2997, <https://doi.org/10.1038/s41598-021-82565-x>.
- [42] T. Kobayashi, T. Horiuchi, A yeast gene product, Fob1 protein, required for both replication fork blocking and recombinational hotspot activities, *Genes Cells* 1 (5) (1996) 465–474, <https://doi.org/10.1046/j.1365-2443.1996.d01-256.x>.
- [43] T. Kobayashi, M. Sasaki, Ribosomal DNA stability is supported by many ‘buffer genes’—introduction to the Yeast rDNA Stability Database, *FEMS Yeast Res.* 17 (1) (2017) fox001, <https://doi.org/10.1093/femsyr/fox001>.
- [44] K.E. Koehler, L.C. Boulton, H.E. Collins, R.L. French, K.C. Herman, S.M. Laceyfield, L.D. Madden, C.D. Schuetz, R.S. Hawley, Spontaneous X chromosome MI and MII nondisjunction events in *Drosophila melanogaster* oocytes have different recombinational histories, *Nat. Genet.* 14 (4) (1996) 406–414, <https://doi.org/10.1038/ng1296-406>.
- [45] D.J. Komma, S.A. Endow, Incomplete Y chromosomes promote magnification in male and female *Drosophila*, *Proc. Natl. Acad. Sci. USA* 84 (8) (1987) 2382–2386 <https://doi.org/10.1073/pnas.84.8.2382>, PMID: 3104913; PMCID: PMC304655.
- [46] M. Krzyzanowska, K. Rebała, J. Steiner, M. Kaliszczak, D. Pieśniak, K. Karnecki, M. Wiergowski, R. Brisch, K. Braun, Z. Jankowski, M. Kosmowska, J. Chociej, T. Gos, Reduced ribosomal DNA transcription in the prefrontal cortex of suicide victims: consistency of new molecular RT-qPCR findings with previous morphometric data from AgNOR-stained pyramidal neurons, *Eur. Arch. Psychiatry Clin. Neurosci.* 271 (3) (2021) 567–576 <https://doi.org/10.1007/s00406-021-01232-4>, Epub 2021 Jan 26. PMID: 33501518; PMCID: PMC7981327.
- [47] E.X. Kwan, E.J. Foss, S. Tsuchiyama, G.M. Alvino, L. Kruglyak, M. Kaerberlein, M.K. Raghuraman, B.J. Brewer, B.K. Kennedy, A. Bedalov, A natural polymorphism in rDNA replication origins links origin activation with calorie restriction and lifespan, *PLoS Genet.* 9 (3) (2013), e1003329 <https://doi.org/10.1371/journal.pgen.1003329>, Epub 2013 Mar 7. PMID: 23505383; PMCID: PMC3591295.
- [48] T. Labella, L. Vicari, A. Manzi, F. Graziani, Expression of rDNA insertions during rDNA magnification in *D. melanogaster*, *Mol. Gen. Genet* 190 (3) (1983) 487–493, <https://doi.org/10.1007/BF00331081>.
- [49] K. Larson, S.J. Yan, A. Tsurumi, J. Liu, J. Zhou, K. Gaur, D. Guo, T.H. Eickbush, W.X. Li, Heterochromatin formation promotes longevity and represses ribosomal RNA synthesis, *PLoS Genet.* 8 (1) (2012), e1002473 <https://doi.org/10.1371/journal.pgen.1002473>, Epub 2012 Jan 26. PMID: 22291607; PMCID: PMC3266895.
- [50] E.O. Long, M. Collins, B.I. Kiefer, I.B. Dawid, Expression of the ribosomal DNA insertions in bobbed mutants of *Drosophila melanogaster*, *Mol. Gen. Genet.* 182 (3) (1981) 377–384, <https://doi.org/10.1007/BF00293925>.
- [51] E.O. Long, I.B. Dawid, Expression of ribosomal DNA insertions in *Drosophila melanogaster*, *Cell* 18 (4) (1979) 1185–1196, [https://doi.org/10.1016/0092-8674\(79\)90231-9](https://doi.org/10.1016/0092-8674(79)90231-9).
- [52] K.L. Lu, J.O. Nelson, G.J. Watase, N. Warsingher-Pepe, Y.M. Yamashita, Transgenerational dynamics of rDNA copy number in *Drosophila* male germline stem cells, *Elife* 7 (2018), e32421 <https://doi.org/10.7554/eLife.32421>, PMID: 29436367; PMCID: PMC5811208.
- [53] Y. Luo, E. Fefelova, M. Ninova, Y.A. Chen, A.A. Aravin, Repression of interrupted and intact rDNA by the SUMO pathway in *Drosophila melanogaster*, *Elife* 9 (2020), e52416 <https://doi.org/10.7554/eLife.52416>, PMID: 33164748; PMCID: PMC7676866.
- [54] E.M. Lyckegaard, A.G. Clark, Ribosomal DNA and Stellate gene copy number variation on the Y chromosome of *Drosophila melanogaster*, *Proc. Natl. Acad. Sci. USA* 86 (6) (1989) 1944–1948 <https://doi.org/10.1073/pnas.86.6.1944>, PMID: 2494656; PMCID: PMC286821.
- [55] M. Lynch, The frailty of adaptive hypotheses for the origins of organismal complexity, *Proc. Natl. Acad. Sci. USA* 104 (Suppl 1) (2007) 8597–8604 <https://doi.org/10.1073/pnas.0702207104>, Epub 2007 May 9. PMID: 17494740; PMCID: PMC1876435.
- [56] K.A. Maggert, K.G. Golic, Highly efficient sex chromosome interchanges produced by I-Cre1 expression in *Drosophila*, *Genetics* 171 (3) (2005) 1103–1114 <https://doi.org/10.1534/genetics.104.040071>, Epub 2005 Jul 14. PMID: 16020774; PMCID: PMC1456814.
- [57] K.A. Maggert, Reduced rDNA copy number does not affect “competitive” chromosome pairing in XYY males of *Drosophila melanogaster*, *G3 (Bethesda)*. 4 (3) (2014) 497–507 <https://doi.org/10.1534/g3.113.008730>, PMID: 24449686; PMCID: PMC3962488.
- [58] M. Makni, M. Marrakchi, N. Prud’homme, The occurrence of long ribosomal transcripts homologous to type I insertions in bobbed mutants of *Drosophila melanogaster*, *Genet Res.* 54 (2) (1989) 127–135, <https://doi.org/10.1017/s0016672300028494>.
- [59] C. Malva, F. Graziani, E. Boncinelli, L. Polito, F. Rotossa, Check of gene number during the process of rDNA magnification, *Nat. New Biol.* 239 (92) (1972) 135–137, <https://doi.org/10.1038/newbio239135a0>.
- [60] C. Malva, A. La Volpe, G. Gargiulo, Comparison between rDNA magnification and bb lethal mutation frequencies in *Drosophila melanogaster*, *Mol. Gen. Genet* 180 (3) (1980) 511–515, <https://doi.org/10.1007/BF00268054>.
- [61] C.H. Marcus, A.E. Zitron, D.A. Wright, R.S. Hawley, Autosomal modifiers of the bobbed phenotype are a major component of the rDNA magnification paradox in *Drosophila melanogaster*, *Genetics* 113 (2) (1986) 305–319 <https://doi.org/10.1093/genetics/113.2.305>, PMID: 3087814; PMCID: PMC1202840.
- [62] B.D. McKee, G.H. Karpen, *Drosophila* ribosomal RNA genes function as an X-Y pairing site during male meiosis, *Cell* 61 (1) (1990) 61–72, [https://doi.org/10.1016/0092-8674\(90\)90215-z](https://doi.org/10.1016/0092-8674(90)90215-z).
- [63] B. McStay, Nucleolar dominance: a model for rRNA gene silencing, *Genes Dev.* 20 (10) (2006) 1207–1214, <https://doi.org/10.1101/gad.1436906>.
- [64] J.R. Miller, D.C. Hayward, D.M. Glover, Transcription of the ‘non-transcribed’ spacer of *Drosophila melanogaster* rDNA, *Nucleic Acids Res.* 11 (1) (1983) 11–19 <https://doi.org/10.1093/nar/11.1.11>, PMID: 6306560; PMCID: PMC325687.
- [65] J. Mohan, F.M. Ritossa, Regulation of ribosomal RNA synthesis and its bearing on the bobbed phenotype in *Drosophila melanogaster*, *Dev. Biol.* 22 (3) (1970) 495–512, [https://doi.org/10.1016/0012-1606\(70\)90165-x](https://doi.org/10.1016/0012-1606(70)90165-x).
- [66] G. Mohannath, F. Pontvianne, C.S. Pikaard, Selective nucleolus organizer inactivation in Arabidopsis is a chromosome position-effect phenomenon, *Proc. Natl. Acad. Sci. USA* 113 (47) (2016) 13426–13431 <https://doi.org/10.1073/pnas.1608140113>, Epub 2016 Nov 7. PMID: 27821753; PMCID: PMC5127337.
- [67] E.A. Morton, N.Hall Ashley, Elizabeth Kwan, Calvin Mok, Konstantin Queitsch, Vivek Nandakumar, John Stamatoyannopoulos, Bonita J. Brewer, Robert Waterston, Christine Queitsch, Challenges and Approaches to Genotyping Repetitive DNA, *G3 Genes=Genomes/Genet. Volume 10 (Issue 1) (2020) 417–430*, <https://doi.org/10.1534/g3.119.400771>.
- [68] Muller, H.J. 1932. Further studies on the nature and causes of gene mutations. *Proceedings of the 6th International Congress of Genetics*, pp. 213–255.
- [69] Nelson JO, Slicko A., Yamashita, Y.M. The retrotransposon R2 maintains *Drosophila* ribosomal DNA repeats. *BioRxiv* doi: (<https://doi.org/10.1101/2021.07.12.451825>), currently not certified by peer review.
- [70] J.O. Nelson, G.J. Watase, N. Warsingher-Pepe, Y.M. Yamashita, Mechanisms of rDNA Copy Number Maintenance, *Trends Genet.* 35 (10) (2019) 734–742 <https://doi.org/10.1016/j.tig.2019.07.006>, Epub 2019 Aug 5. PMID: 31395390; PMCID: PMC6744303.
- [71] S. Nurk, S. Koren, A. Rhie, M. Rautiainen, A.V. Bizakadze, A. Mikheenko, M.R. Vollger, N. Altemose, L. Uralsky, A. Gershman, S. Aganezov, S.J. Hoyt, M. Diekhans, G.A. Logsdon, M. Alonge, S.E. Antonarakis, M. Borchers, G.G. Bouffard, S.Y. Brooks, G.V. Caldas, N.C. Chen, H. Cheng, C.S. Chin, W. Chow, L.G. de Lima, P.C. Dishuck, R. Durbin, T. Dvorkina, I.T. Fiddes, G. Formenti, R.S. Fulton, A. Functammasan, E. Garrison, P.G.S. Grady, T.A. Graves-Lindsay, I.M. Hall, N.F. Hansen, G.A. Hartley, M. Haukness, K. Howe, M.W. Hunkapiller, C. Jain, M. Jain, E.D. Jarvis, P. Kerpedjiev, M. Kirsche, M. Kolmogorov, J. Korlach, M. Kremitzki, H. Li, V.V. Maduro, T. Marschal, A.M. McCartney, J. McDaniel, D.E. Miller, J.C. Mullikin, E.W. Myers, N.D. Olson, B. Paten, P. Peluso, P.A. Pevzner, D. Porubsky, T. Potapova, E.I. Rogaeve, J.A. Rosenfeld, S.L. Salzberg, V.A. Schneider, F.J. Sedlazeck, K. Shafin, C.J. Shew, A. Shumate, Y. Sims, A.F.A. Smit, D.C. Soto, I. Sović, J.M. Storer, A. Streets, B.A. Sullivan, F. Thibaud-Nissen, J. Torrance, J. Wagner, B.P. Walenz, A. Wenger, J.M.D. Wood, C. Xiao, S.M. Yan, A.C. Young, S. Zarate, U. Surti, R.C. McCoy, M.Y. Dennis, I.A. Alexandrov, J.L. Gerton, R.J. O’Neill, W. Timp, J.M. Zook, M.C. Schatz, E.E. Eichler, K.H. Miga, A.M. Phillippy, The complete sequence of a human genome, *Science* 376 (6588) (2022) 44–53 <https://doi.org/10.1126/science.abj6987>, Epub 2022 Mar 31.
- [72] G. Palumbo, R. Caizzi, F. Ritossa, Relative orientation with respect to the centromere of ribosomal RNA genes of the X and Y chromosomes of *Drosophila melanogaster*, *Proc. Natl. Acad. Sci. USA* 70 (6) (1973) 1883–1885 <https://doi.org/10.1073/pnas.70.6.1883>, PMID: 4198277; PMCID: PMC433618.
- [73] S. Paredes, A.T. Branco, D.L. Hartl, K.A. Maggert, B. Lemos, Ribosomal DNA deletions modulate genome-wide gene expression: “rDNA-sensitive” genes and natural variation, *PLoS Genet* 7 (4) (2011) e1001376, <https://doi.org/10.1371/journal.pgen.1001376>.
- [74] S. Paredes, K.A. Maggert, Expression of I-Cre1 endonuclease generates deletions within the rDNA of *Drosophila*, *Genetics* 181 (4) (2009) 1661–1671 <https://doi.org/10.1534/genetics.108.099093>, Epub 2009 Jan 26. PMID: 19171942; PMCID: PMC2666528.
- [75] S. Paredes, K.A. Maggert, Ribosomal DNA contributes to global chromatin regulation, *Proc. Natl. Acad. Sci. USA* 106 (42) (2009) 17829–17834 <https://doi.org/10.1073/pnas.0906811106>, Epub 2009 Oct 12. PMID: 19822756; PMCID: PMC2764911.
- [76] G.N. Pavlakis, B.R. Jordan, R.M. Wurst, J.N. Vournakis, Sequence and secondary structure of *Drosophila melanogaster* 5.8S and 2S rRNAs and of the processing site between them, *Nucleic Acids Res.* 7 (8) (1979) 2213–2238, <https://doi.org/10.1093/nar/7.8.2213>.
- [77] J.C. Peng, G.H. Karpen, H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability, *Nat. Cell Biol.* 9 (1) (2007) 25–35 <https://doi.org/10.1038/ncb1514>, Epub 2006 Dec 10. PMID: 17159999; PMCID: PMC2819265.
- [78] C.S. Pikaard, The epigenetics of nucleolar dominance, *Trends Genet* 16 (11) (2000) 495–500, [https://doi.org/10.1016/s0168-9525\(00\)02113-2](https://doi.org/10.1016/s0168-9525(00)02113-2).
- [79] L. Porokhovnik, Individual copy number of ribosomal genes as a factor of mental retardation and autism risk and severity, *Cells* 8 (10) (2019) 1151 <https://doi.org/10.1016/j.cel.2019.08.011>.

- [doi.org/10.3390/cells8101151](https://doi.org/10.3390/cells8101151), PMID: 31561466; PMCID: PMC6830322.
- [80] T.A. Potapova, J.R. Unruh, Z. Yu, G. Rancati, H. Li, M.R. Stampfer, J.L. Gerton, Superresolution microscopy reveals linkages between ribosomal DNA on heterologous chromosomes. *J. Cell Biol.* 218 (8) (2019) 2492–2513 <https://doi.org/10.1083/jcb.201810166>, Epub 2019 Jul 3. PMID: 31270138; PMCID: PMC6683752.
- [81] M. Poot, R. Hochstenbach, Prevalence and phenotypic impact of robertsonian translocations. *Mol. Syndromol.* 12 (1) (2021) 1–11 <https://doi.org/10.1159/000512676>, Epub 2021 Feb 17. PMID: 33776621; PMCID: PMC7983559.
- [82] M.O. Press, A.N. Hall, E.A. Morton, C. Queitsch, Substitutions are boring: some arguments about parallel mutations and high mutation rates. *Trends Genet.* 35 (4) (2019) 253–264 <https://doi.org/10.1016/j.tig.2019.01.002>, Epub 2019 Feb 20. PMID: 30797597; PMCID: PMC6435258.
- [83] E.M. Pugacheva, N. Kubo, D. Loukinov, M. Tajmou, S. Kang, A.L. Kovalchuk, A.V. Strunnikov, G.E. Zentner, B. Ren, V.V. Lobanenko, CTCF mediates chromatin looping via N-terminal domain-dependent cohesin retention. *Proc. Natl. Acad. Sci. USA* 117 (4) (2020) 2020–2031 <https://doi.org/10.1073/pnas.1911708117>, Epub 2020 Jan 14. PMID: 31937660; PMCID: PMC6995019.
- [84] Rabanal FA, Nizhynska V., Mandáková T., et al. Unstable Inheritance of 45S rRNA Genes in Arabidopsis thaliana. G3 (Bethesda). 2017;7(4):1201–1209. Published 2017 Apr 3. ([doi:10.1534/g3.117.040204](https://doi.org/10.1534/g3.117.040204)).
- [85] H.S. Raje, M.E. Lieux, P.J. DiMario, R1 retrotransposons in the nucleolar organizers of Drosophila melanogaster are transcribed by RNA polymerase I upon heat shock. *Transcription* 9 (5) (2018) 273–285 <https://doi.org/10.1080/21541264.2018.1506682>, Epub 2018 Aug 21. PMID: 30063880; PMCID: PMC6150621.
- [86] F. Ritossa, C. Malva, E. Boncinelli, F. Graziani, L. Polito, The first steps of magnification of DNA complementary to ribosomal RNA in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* 68 (7) (1971) 1580–1584 <https://doi.org/10.1073/pnas.68.7.1580>, PMID: 5283949; PMCID: PMC389245.
- [87] F. Ritossa, Crossing-over between X AND Y chromosomes during ribosomal DNA magnification in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* 70 (7) (1973) 1950–1954 <https://doi.org/10.1073/pnas.70.7.1950>, PMID: 4198661; PMCID: PMC433640.
- [88] F.M. Ritossa, K.C. Atwood, S. Spiegelman, A molecular explanation of the bobbed mutants of Drosophila as partial deficiencies of “ribosomal” DNA. *Genetics* 54 (3) (1966) 819–834 <https://doi.org/10.1093/genetics/54.3.819>, PMID: 5970623; PMCID: PMC1211204.
- [89] F.M. Ritossa, S. Spiegelman, Localization of dna complementary to ribosomal rna in the nucleolar organizer region of Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA* 53 (4) (1965) 737–745 <https://doi.org/10.1073/pnas.53.4.737>, PMID: 14324529; PMCID: PMC221060.
- [90] F.M. Ritossa, Unstable redundancy of genes for ribosomal RNA. *Proc. Natl. Acad. Sci. USA* 60 (2) (1968) 509–516 <https://doi.org/10.1073/pnas.60.2.509>, PMID: 5248808; PMCID: PMC225077.
- [91] J.M. Ruchert, M.M. Brady, S. McMahan, K.J. Lacey, L.C. Latta, J. Sekelsky, E.P. Stoffregen, Bln helicase facilitates rapid replication of repetitive DNA sequences in early Drosophila development. *Genetics* 220 (1) (2022) iyab169 <https://doi.org/10.1093/genetics/iyab169>, PMID: 34849849; PMCID: PMC8733427.
- [92] G. Salzano, C. Malva, Non-random loss of uninterrupted ribosomal DNA repeating units upon induction of a bobbed mutation. *J. Mol. Biol.* 177 (1) (1984) 189–200.
- [93] J. Schawaldner, E. Paric, N.F. Neff, Telomere and ribosomal DNA repeats are chromosomal targets of the bloom syndrome DNA helicase. *BMC Cell Biol.* 4 (2003) 15. <https://doi.org/10.1186/1471-2121-4-15>.
- [94] R.G. Schneeberger, C.A. Cullis, Specific DNA alterations associated with the environmental induction of heritable changes in flax. *Genetics* 128 (3) (1991) <https://doi.org/10.1093/genetics/128.3.619>, PMID: 1678726; PMCID: PMC1204535.
- [95] D.A. Schneider, A. Michel, M.L. Sikes, L. Vu, J.A. Dodd, S. Salgia, Y.N. Osheim, A.L. Beyer, M. Nomura, Transcription elongation by RNA polymerase I is linked to efficient rRNA processing and ribosome assembly. *Mol. Cell.* 26 (2) (2007) 217–229 <https://doi.org/10.1016/j.molcel.2007.04.007>, PMID: 17466624; PMCID: PMC1927085.
- [96] F. Shao, X. Liu, X. Zhang, Q. Wang, W. Wang, Methylation of 45S ribosomal DNA (rDNA) is associated with cancer and aging in humans. *Int J. Genom.* 2021 (2021), 8818007 <https://doi.org/10.1155/2021/8818007>, PMID: 33575316; PMCID: PMC7861956.
- [97] Sharma D., Hermann-Le Denmat S., Matzke NJ, Hannan K., Hannan RD, O’Sullivan JM, Ganley ARD. A new method for determining ribosomal DNA copy number shows differences between Saccharomyces cerevisiae populations. *BioRxiv* doi: <https://doi.org/10.1101/2021.01.21.427686>, currently not certified by peer review.
- [98] J. Sims, G. Sestini, C. Elgert, A. von Haeseler, P. Schlögelhofer, Sequencing of the Arabidopsis NOR2 reveals its distinct organization and tissue-specific rRNA ribosomal variants. *Nat. Commun.* 12 (1) (2021) 387 <https://doi.org/10.1038/s41467-020-20728-6>, PMID: 33452254; PMCID: PMC7810690.
- [99] N.P. Sivertsev-Dobzhansky, T. Dobzhansky, Deficiency and duplications for the gene bobbed in drosophila melanogaster. *Genetics* 18 (3) (1933) 173–192. <https://doi.org/10.1093/genetics/18.3.173>.
- [100] E. Smirnov, P. Trosan, J.V. Cabral, P. Studeny, S. Kerešič, K. Jirsava, D. Cmarko, Discontinuous transcription of ribosomal DNA in human cells. *PLoS One* 15 (3) (2020), e0223030 <https://doi.org/10.1371/journal.pone.0223030>, PMID: 32119673; PMCID: PMC7051091.
- [101] C. Stern, Ueber die additive Wirkung multipler Allele. *Biol. Zent.* 49 (1929) 261–290.
- [102] C. Stern, Ein genetischer und zytologischer Beweis für Vererbung im Y-Chromosom von Drosophila melanogaster. *Z. Ver. erbungslehre* 44 (1927) 187–231. <https://doi.org/10.1007/BF01740987>.
- [103] D.M. Stults, M.W. Killen, E.P. Williams, J.S. Hourigan, H.D. Vargas, S.M. Arnold, J.A. Moscow, A.J. Pierce, Human rRNA gene clusters are recombinational hotspots in cancer. *Cancer Res.* 69 (23) (2009) 9096–9104 <https://doi.org/10.1158/0008-5472.CAN-09-2680>, Epub 2009 Nov 17.
- [104] K.D. Tartof, I.G. Dawid, Similarities and differences in the structure of X and Y chromosome rRNA genes of Drosophila. *Nature* 263 (5572) (1976) 27–30. <https://doi.org/10.1038/263027a0>.
- [105] K.D. Tartof, Increasing the multiplicity of ribosomal RNA genes in Drosophila melanogaster. *Science* 171 (3968) (1971) 294–297. <https://doi.org/10.1126/science.171.3968.294>.
- [106] K.D. Tartof, Regulation of ribosomal RNA gene multiplicity in Drosophila melanogaster. *Genetics* 73 (1) (1973) 57–71 <https://doi.org/10.1093/genetics/73.1.57>, PMID: 4631601; PMCID: PMC1212882.
- [107] K.D. Tartof, Unequal mitotic sister chromatid exchange as the mechanism of ribosomal RNA gene magnification. *Proc. Natl. Acad. Sci. USA* 71 (4) (1974) 1272–1276 <https://doi.org/10.1073/pnas.71.4.1272>, PMID: 4208547; PMCID: PMC388208.
- [108] K.D. Tartof, Unequal mitotic sister chromatid exchange and disproportionate replication as mechanisms regulating ribosomal RNA gene redundancy. *Cold Spring Harb. Symp. Quant. Biol.* 38 (1974) 491–500. <https://doi.org/10.1101/sqb.1974.038.01.053>.
- [109] N.A. Tchurikov, D.V. Yudkin, M.A. Gorbacheva, A.I. Kulemzina, I.V. Grischenko, D.M. Fedoseeva, D.V. Sosin, Y.V. Kravatsky, O.V. Kretova, Hot spots of DNA double-strand breaks in human rDNA units are produced in vivo. *Sci. Rep.* 6 (2016) 25866 <https://doi.org/10.1038/srep25866>, PMID: 27160357; PMCID: PMC4861929.
- [110] R. Terracol, N. Prud’homme, Differential elimination of rDNA in bobbed mutants of Drosophila melanogaster. *Mol. Cell Biol.* 6 (4) (1986) 1023–1031 <https://doi.org/10.1128/mcb.6.4.1023-1031.1986>, PMID: 3023865; PMCID: PMC367611.
- [111] R. Terracol, Differential magnification of rDNA gene types in bobbed mutants of Drosophila melanogaster. *Mol. Gen. Genet* 208 (1–2) (1987) 168–176. <https://doi.org/10.1007/BF00330438>.
- [112] R. Terracol, Transcription of rDNA insertions in bobbed mutants of Drosophila melanogaster. *Genet. Res.* 48 (3) (1986) 167–174.
- [113] V. Valori, K. Tus, C. Laukaitis, D.T. Harris, L. LeBeau, K.A. Maggert, Human rDNA copy number is unstable in metastatic breast cancers. *Epigenetics* 15 (1–2) (2020) 85–106 <https://doi.org/10.1080/15592294.2019.1649930>, Epub 2019 Aug 12. PMID: 31352858; PMCID: PMC6961696.
- [114] S. van de Nobelen, M. Rosa-Garrido, J. Leers, H. Heath, W. Soochit, L. Joosen, I. Jonkers, J. Demmers, M. van der Reijden, V. Torrano, F. Grosveld, M.D. Delgado, R. Renkawitz, N. Galjart, F. Sleutels, CTCF regulates the local epigenetic state of ribosomal DNA repeats. *Epigenetics Chromatin.* 3 (1) (2010) 19 <https://doi.org/10.1186/1756-8935-3-19>, PMID: 21059229; PMCID: PMC2993708.
- [115] M. van Sluis, C. van Vuuren, H. Mangan, B. McStay, NORs on human acrocentric chromosome p-arms are active by default and can associate with nucleoli independently of rDNA. *Proc. Natl. Acad. Sci. USA* 117 (19) (2020) 10368–10377 <https://doi.org/10.1073/pnas.2001812117>, Epub 2020 Apr 24. PMID: 32332163; PMCID: PMC7229746.
- [116] D.O. Warmerdam, R.M.F. Wolthuis, Keeping ribosomal DNA intact: a repeating challenge. *Chromosome Res.* 27 (1–2) (2019) 57–72 <https://doi.org/10.1007/s10577-018-9594-z>, Epub 2018 Dec 17. PMID: 30556094; PMCID: PMC6394564.
- [118] J.R. Warner, The economics of ribosome biosynthesis in yeast. *Trends Biochem. Sci.* 24 (11) (1999) 437–440. [https://doi.org/10.1016/s0968-0004\(99\)01460-7](https://doi.org/10.1016/s0968-0004(99)01460-7).
- [119] N. Wirsinger-Pepe, D. Li, Y.M. Yamashita, Regulation of Nucleolar Dominance in Drosophila melanogaster. *Genetics* 214 (4) (2020) 991–1004 <https://doi.org/10.1534/genetics.119.302471>, Epub 2020 Mar 2. PMID: 32122935; PMCID: PMC7153946.
- [120] E. Watada, S. Li, Y. Hori, K. Fujiki, K. Shirahige, T. Inada, T. Kobayashi, Age-dependent ribosomal DNA variations in mice. *e00368-20. Mol. Cell Biol.* 40 (22) (2020) <https://doi.org/10.1128/MCB.00368-20>, PMID: 32900821; PMCID: PMC7588874.
- [121] P.K. Wellauer, I.B. Dawid, K.D. Tartof, X and Y chromosomal ribosomal DNA of Drosophila: comparison of spacers and insertions. *Cell* 14 (2) (1978) 269–278. [https://doi.org/10.1016/0092-8674\(78\)90113-7](https://doi.org/10.1016/0092-8674(78)90113-7).
- [122] Y.E. Xiong, T.H. Eickbush, Functional expression of a sequence-specific endonuclease encoded by the retrotransposon R2Bm. *Cell* 55 (2) (1988) 235–246. [https://doi.org/10.1016/0092-8674\(88\)90046-3](https://doi.org/10.1016/0092-8674(88)90046-3).
- [123] B. Xu, H. Li, J.M. Perry, V.P. Singh, J. Unruh, Z. Yu, M. Zakari, W. McDowell, L. Li, J.L. Gerton, Ribosomal DNA copy number loss and sequence variation in cancer. *PLoS Genet* 13 (6) (2017), e1006771 <https://doi.org/10.1371/journal.pgen.1006771>, PMID: 28640831; PMCID: PMC5480814.
- [124] Q. Zhang, N.A. Shalaby, M. Buszczak, Changes in rRNA transcription influence proliferation and cell fate within a stem cell lineage. *Science* 343 (6168) (2014) 298–301 <https://doi.org/10.1126/science.1246384>, PMID: 24436420; PMCID: PMC4084784.
- [125] J. Zhou, T.H. Eickbush, The pattern of R2 retrotransposon activity in natural populations of Drosophila simulans reflects the dynamic nature of the rDNA locus. *PLoS Genet.* 5 (2) (2009), e1000386 <https://doi.org/10.1371/journal.pgen.1000386>, Epub 2009 Feb 20. PMID: 19229317; PMCID: PMC2637433.
- [126] J. Zhou, T.B. Sackton, L. Martinsen, B. Lemos, T.H. Eickbush, D.L. Hartl, Y chromosome mediates ribosomal DNA silencing and modulates the

chromatin state in *Drosophila*, Proc. Natl. Acad. Sci. USA 109 (25) (2012) 9941–9946 <https://doi.org/10.1073/pnas.1207367109>, Epub 2012 Jun 4. PMID: 22665801; PMCID: PMC3382510.

*Drosophila*, Annu Rev Genet. 23 (1989) 87–120 PMID: 2694947., <https://doi.org/10.1146/annurev.ge.23.120189.00051>.

[127] RS Hawley, CH Marcus, Recombinational controls of rDNA redundancy in

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