Nucleolar dominance, a locus-level regulation of ribosomal DNA expression, in *D. melanogaster* females

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Abstract

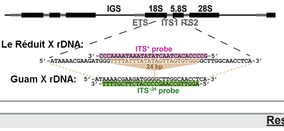
Ribosomal DNA (rDNA) codes for the catalytic RNA components of ribosomes and is organized in tandem repeats of in eukaryotic genomes. In *Drosophila*, rDNA loci are on the X and Y chromosomes where each locus contains ~200-250 copies. A large-scale regulation of rDNA expression called nucleolar dominance, where rDNA locus is entirely silenced or activated, operates to regulate the dosage of rRNA. In male *D. melanogaster*, Y rDNA is preferentially transcribed while the entire X rDNA locus is silenced. In females, both rDNA loci are transcribed in larval brains. Previous studies were unable to characterize female nucleolar dominance in other tissues and developmental stages due to technical limitations. Here we identify sequence variation in an X rDNA locus and utilize these sequence differences with fluorescent in situ hybridization to characterize nucleolar dominance in females. We expand on previous studies and show that nucleolar dominance does not occur in X/X females in multiple tissues and throughout development. Using various chromosome complements and compound chromosomes, we found that nucleolar dominance is not limited to Y chromosome or male cells. This study begins to unravel factors dictate the rDNA expression pattern in both female and male and will help us understand how nucleolar dominance occurs.

Introduction

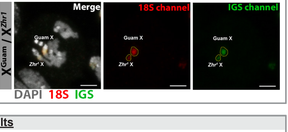
rDNA codes for essential RNA catalytic components of ribosomes, and its transcription is important for ribosome functions [1]. Thus, the regulation of rDNA transcription is critical to meet cellular metabolic demand. Nucleolar dominance was originally found to occur in interspecies hybrids [2-5], and has been shown to occur within a species [6-8]. In male *D. melanogaster*, previous studies found that Y rDNA dominates over X rDNA expression [6, 8]. Utilizing SNPs between X and Y rDNA loci and RNA in situ hybridization, we found that Y rDNA dominance is established developmentally [9]. In female *D. melanogaster*, both X rDNA loci are expressed in larval neuroblasts [3, 9]. However, due to the high sequence homology, no SNPs were found between X rDNA loci, limiting the understanding of nucleolar dominance in females. This study expands on the knowledge of female nucleolar dominance to various tissues and developmental stages using RNA in situ and explored potential factors impacting nucleolar dominance.

Methods

RNA *in situ* Hybridization
- RNA in situ protocol used from Natalie et al. (2020) [9],modified from Levesque et al. (2013) [10].

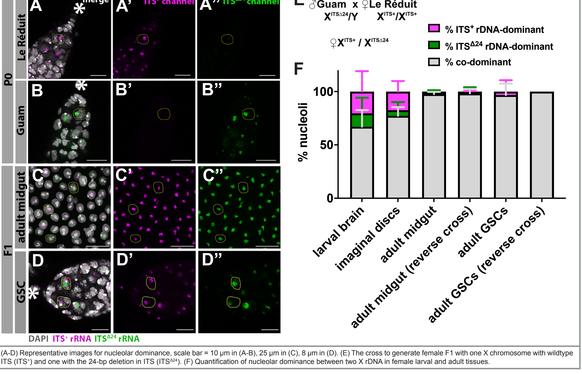


DNA Fluorescence *in situ* hybridization (DNA FISH)
- relative rDNA size was quantified using a modified protocol from Lu et al. (2018) [11].



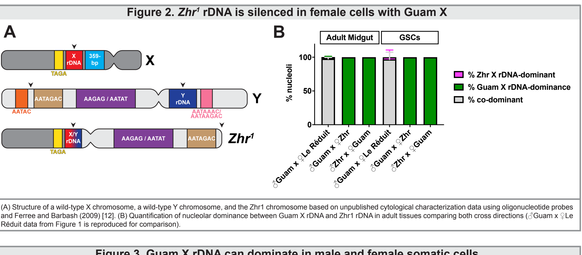
Results

Figure 1. Co-dominance occurs in X/X females throughout development



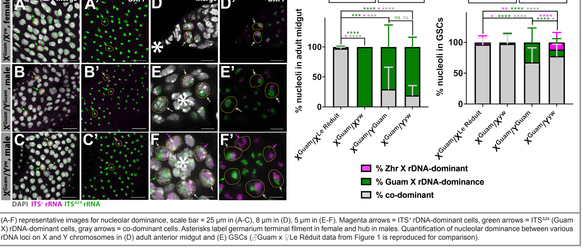
(A-D) Representative images for nucleolar dominance, scale bar = 10 μm in (A-B), 25 μm in (C), 8 μm in (D). (E) The cross to generate female F1 with one X chromosome with wildtype ITS (ITS¹) and one with the 24-bp deletion in ITS (ITS^Δ24). (F) Quantification of nucleolar dominance between two X rDNA in female larval and adult tissues.

Figure 2. *Zhr¹* rDNA is silenced in female cells with Guam X



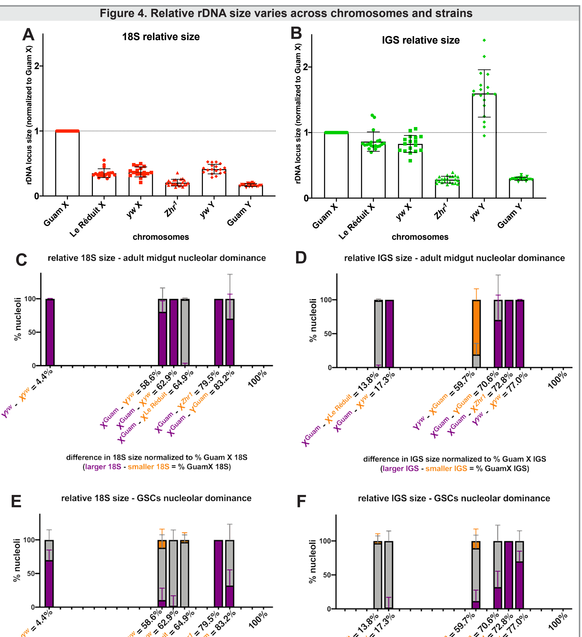
(A) Structure of a wild-type X chromosome, a wild-type Y chromosome, and the Zhr1 chromosome based on unpublished cytological characterization data using oligonucleotide probes and Ferree and Barbash (2009) [12]. (B) Quantification of nucleolar dominance between Guam X rDNA and Zhr1 rDNA in adult tissues comparing both cross directions (♂Guam x ♀Le Réduit data from Figure 1 is reproduced for comparison).

Figure 3. Guam X rDNA can dominate in male and female somatic cells



(A-F) representative images for nucleolar dominance, scale bar = 25 μm in (A-C), 8 μm in (D), 5 μm in (E-F). Magenta arrows = ITS¹ rDNA-dominant cells, green arrows = ITS^Δ24 (Guam X) rDNA-dominant cells, gray arrows = co-dominant cells. Asterisks label germarium terminal filament in female and hub in males. Quantification of nucleolar dominance between various rDNA loci on X and Y chromosomes in (D) adult anterior midgut and (E) GSCs (♂Guam x ♀Le Réduit data from Figure 1 is reproduced for comparison).

Figure 4. Relative rDNA size varies across chromosomes and strains

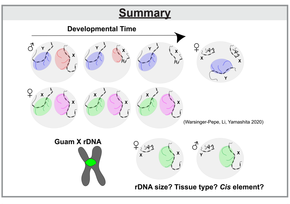


Quantification of relative rDNA size using ratio of raw integrated for (A) 18S and (B) IGS signal density separately (see Methods). (C-F) Relationship between relative rDNA size (18S or IGS) difference and nucleolar dominance (in adult midgut or GSC). yw X and yw Y rDNA nucleolar dominance data referenced from Warsinger-Pepe et al. (2020) [9]. All other nucleolar dominance data replicated from previous figures.

Discussion

We characterized nucleolar dominance in female *D. melanogaster*, expanding on our previous knowledge to other tissue types and developmental stages. Using a compound chromosome and various chromosome complements, we identified an X chromosome that is able to dominate over both X and Y rDNA loci. Our data suggests that rDNA size may play a tissue-specific role in establishing dominance. Furthermore, our data suggests that elements within the Guam X rDNA may strongly dictate its ability to dominate over other rDNA loci. What these elements are and how they influence nucleolar dominance await future investigation.

Summary



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