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Nucleolar dominance and maternal control of 45S rDNA expression

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Using a system of interspecies hybrids, trihybrids, and recombinants with varying proportions of genomes from three distinct *Xenopus* species, we provide evidence for de novo epigenetic silencing of paternal 45S ribosomal ribonucleic acid (rRNA) genes and their species-dependent expression dominance that escapes transcriptional inactivation after homologous recombination. The same pattern of imprinting is maintained in the offspring from mothers being genetic males (ZZ) sex-reversed to females, indicating that maternal control of ribosomal deoxyribonucleic acid (rDNA) expression is not sex-chromosome linked. Nucleolar dominance (nucleolus underdevelopment) in *Xenopus* hybrids appears to be associated with a major non-Mendelian reduction in the number of 45S rDNA gene copies rather than a specific pattern of their expression. The loss of rRNA gene copies in F_1 hybrids was non-random with respect to the parental species, with the transcriptionally dominant variant preferentially removed from hybrid zygotes. This dramatic disruption in the structure and function of 45S rDNA impacts transcriptome patterns of small nucleolar RNAs and messenger RNAs, with genes from the ribosome and oxidative stress pathways being among the most affected. Unorthodoxies of rDNA inheritance and expression may be interpreted as hallmarks of genetic conflicts between parental genomes, as well as defensive epigenetic mechanisms employed to restore genome integrity.

1. Introduction

Ribosomal DNA (rDNA) encoding rRNA, with its roots descending from the RNA world, is the most ancient part of all known genomes on the Earth, which provides unique insights into the origins of genome architecture, as well as metabolic features of life at the root of the evolutionary tree [1]. To date, however, rRNA-determining sequences and the associated chromatin remain 'dark matter' of eukaryotic genomes due to their exceptionally excessive and repetitive nature, hindering efficient characterization and experimentation. The repetitive nature of rDNA was discovered more than three decades ago when it was first isolated from *Xenopus* and found to contain 400–500 repeat units organized in tandem [2–4]. Each 45S unit consists of 18S, 5.8S, and 28S rRNA genes and spacers oriented mostly in a head-to-tail configuration. Cytogenetic and *in situ* hybridization data showed that rDNA arrays map either to a single (e.g. *Xenopus*) or multiple (e.g. mammals) chromosomal pairs and form nucleolar organizer regions (NORs) [3,5,6]. The nucleolus is a prominent, highly autonomous non-membrane nuclear compartment that supports protein synthesis machinery by actively transcribing genes for rRNAs, processing rRNAs, assembling ribosomal subunits, as well as modifying and transporting ribonucleoproteins [7–12]. Although rDNA segregation is completely dependent on the chromosome transmission mechanism, nucleolar autonomy can be perceived as almost a symbiotic mini-life form with its own minigenome, origin of replication [13,14], replication fork block [15,16], and an agglomerate of highly specialized proteins, including 45S rRNA-specific Pol I transcription machinery [17]. As one example, the nucleolus in fission and budding yeast avoids

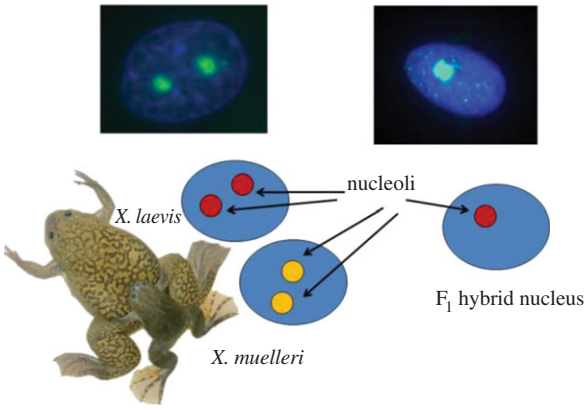

Figure 1. A pictorial representation of nucleolar dominance in *Xenopus*.

disassembly during mitotic and meiotic division and recruits new protein complexes for the heterochromatization and segregation of rDNA, which results in delayed separation in anaphase relative to other genomic regions [18–22].

In some interspecies hybrids, rRNA genes from only one parental species are transcribed and the number of nucleoli formed per nucleus tends to be halved relative to progenitors, an enigmatic phenomenon known as nucleolar dominance [23]. Unlike X chromosome silencing, which is random with respect to the origin of parental chromosome (except marsupials [24]), nucleolar dominance is progenitor dependent, with rRNA genes from one parental species consistently dominant over the other [25–27]. For example, a hierarchy of dominance has been observed in reciprocal crosses between allotetraploid *Brassica* species, in which *B. nigra* dominates over *B. rapa* and *B. oleracea*, and *B. rapa* dominates only over *B. oleracea* [23]. Similar species-dependent nucleolar dominance has been believed to occur in a palaeo-allotetraploid amphibian system, *Xenopus* frogs, with parental *X. laevis*, *X. muelleri*, and *X. borealis* having two nucleoli per cell, but their F_1 hybrids developing only one nucleolus under *X. laevis* dominance (figure 1) [25–27]. Nucleolar dominance was mimicked in *Xenopus* using *X. laevis* and *X. borealis* minigenes with intergenic rRNA spacers co-injected into oocytes, resulting in suppression of transcription from *X. borealis* minigenes [28]. This has led to the hypothesis of enhancer imbalance postulating that either intergenic enhancer repeats alone [28] or enhancer repeats and spacer promoters confer *X. laevis* dominance [29,30]. However, we have recently re-examined the patterns of 45S rRNA transcription in F_1 *Xenopus* hybrids and found it to be under maternal rather than species control [31]. Here, we dissect nucleolar dominance with respect to rRNA expression, rDNA inheritance, as well as global responses at the transcriptome level in a variety of hybrid recombinants among three *Xenopus* species. Together, these observations shed light on the genetic basis of nucleolar dominance and its genome-wide impacts.

2. Material and methods

See electronic supplementary material, S1.

3. Results

(a) Number of nucleoli and 45S rDNA expression patterns

Unlike parental species that have two nucleoli per nucleus forming from two homologous NORs, F_1 hybrid, backcross, and trihybrid frogs tend to develop only one nucleolus per nucleus, regardless of tissue type or developmental stage, and in spite of the presence of both homologous NORs (table 1 and figure 2; electronic supplementary material, figure S1). Based on next generation sequencing (Ion Torrent, Life Technologies) of transcriptomes, as well as allele-specific pyrosequencing and droplet digital polymerase chain reaction (PCR) assays, we demonstrate that F_1 hybrids are characterized by the predominantly (91–97%) maternal expression of 45S rDNA in all stages and tissues (table 1; see also [31]). Similarly, all backcrosses with grandmaternal *X. laevis*, including those from sex-reversed mothers F_1(L × M) being genetic males (having ZZ sex chromosomes), resulted in expression patterns consistent with maternal dominance (table 1). However, backcross offspring with grandmaternal *X. borealis* (i.e. crosses F_1(B × L)♀ × L♂ and F_1(B × L)♀ × B♂) exhibited *X. laevis* (grandpaternal) dominance in spite of their mothers having *X. borealis* expression (table 1). Trihybrids from the cross (L × M)♀ × B♂ had *X. laevis* (maternal) expression.

(b) 45S rDNA copy numbers and parental genomic proportions

F_1 hybrids had between 46–55% and 43–70% fewer 45S rRNA genes relative to the mid-parent value in reciprocal crosses that combined *X. laevis* and *X. muelleri*, and *X. laevis* and *X. borealis* genomes, respectively (table 1). A similar pattern was observed in backcross hybrids that tended to lose 20–27% and 38–49% of rRNA genes relative to the mid-parent value in crosses combining *X. laevis* and *X. muelleri*, and *X. laevis* and *X. borealis* genomes, respectively. Assuming Mendelian ratio expectations, *X. laevis* rRNA genes were 20–42% underrepresented in F_1 hybrid zygotes (table 1).

(c) Expression profiling of messenger RNAs and small nucleolar RNAs in *Xenopus laevis*, *Xenopus muelleri*, and their hybrid F_1

We used Illumina (HiSeq) RNA-sequencing of *X. laevis*, *X. muelleri*, and their hybrid F_1 to assess transcriptome-wide changes associated with observed rDNA and rRNA unorthodoxies (electronic supplementary material, figure S2). The largest fraction (18–31%) of the 116 and 279 differentially expressed genes (false discovery rate (FDR $p < 0.05$) in, respectively, *X. laevis* × *X. muelleri*, and *X. muelleri* × *X. laevis* hybrids relative to parental species belonged to Gene Ontology (GO) categories that were directly related to ribosome structure, rRNA binding, and the polysome (electronic supplementary material, tables S1–S3). Ribosome-related categories were the only significantly over-represented GO terms at the FDR $p < 0.05$ level. Ribosomal protein genes (e.g. *rplp1*, *rplp2*, *rplp3*, *rplp11*, *rps3a-a*, *rps15*, and *rpl28-a*) were among the most overexpressed (fivefold to 107-fold) genes in hybrids, presumably due to overcompensation for rDNA depletion and rDNA-related incompatibilities. However, *ATP5A1*, a nuclear gene encoding a subunit of mitochondrial ATP-synthase, was the most significantly anomalously expressed gene (FDR $p < 2.3 \times 10^{-21}$) in *X. laevis* × *X. muelleri* hybrids (electronic supplementary material, table S1). Similar to ribonucleoprotein complex genes, *ATP5A1* and *COX7A2*, genes from the oxidative phosphorylation pathway, were both highly upregulated in hybrid genomes. A number of other

Table 1. The relationship between the number of nucleoli, 45S rRNA gene copies, parental genome, and expression proportions (SR, sex-reversed).

mother	father	nucleoli/cell	gene copy no. ± s.e.	L rDNA % ± s.e.	M rDNA ± s.e.	B rDNA % ± s.e.	L rRNA % ± s.e.	M rRNA % ± s.e.	B rRNA % ± s.e.
X. laevis (L)	*X. laevis* (L)	2	761 ± 7	100	0	0	100	0	0
X. muelleri (M)	*X. muelleri* (M)	2	486 ± 8	0	100	0	0	100	0
X. borealis (B)	*X. borealis* (B)	2	686 ± 13	0	0	100	0	0	100
X. laevis (L)	*X. muelleri* (M)	1	335 ± 4	20 ± 0.04	80 ± 0.04	0	96 ± 0.01	4 ± 0.01	0
X. muelleri (M)	*X. laevis* (L)	1	334 ± 36	23 ± 0.10	77 ± 0.10	0	4 ± 0.00	96 ± 0.00	0
X. laevis (L)	*X. borealis* (B)	1	418 ± 5	10 ± 0.01	0	90 ± 0.01	97 ± 0.19	0	3 ± 0.68
X. borealis (B)	*X. laevis* (L)	1	218 ± 8	10 ± 0.02	0	90 ± 0.02	9 ± 0.01	0	91 ± 0.01
F_1(L × M)	*X. laevis* (L)	1	427 ± 0	46 ± 2.40	54 ± 2.40	0	98 ± 0.600	2 ± 0.600	0
F_1(L × M)	*X. muelleri* (M)	1	313 ± 5	25 ± 1.07	75 ± 1.07	0	83 ± 0.564	17 ± 0.564	0
SR F_1(L × M)	*X. muelleri* (M)	1	400 ± 21	19 ± 1.80	81 ± 1.80	0	77 ± 2.470	23 ± 2.470	0
F_1(L × B)	*X. laevis* (L)	1	306 ± 36	59 ± 2.40	0	41 ± 2.400	100 ± 0.010	0	0 ± 0.010
F_1(L × B)	*X. borealis* (B)	1	356 ± 16	18 ± 1.40	0	82 ± 1.400	97 ± 0.541	0	3 ± 0.541
F_1(B × L)	*X. laevis* (L)	1	240 ± 23	84 ± 2.60	0	16 ± 2.600	98 ± 0.100	0	2 ± 0.100
F_1(B × L)	*X. borealis* (B)	1	260 ± 18	13 ± 4.40	0	87 ± 4.400	56 ± 0.560	0	44 ± 0.560
F_1(L × M)	*X. borealis* (B)	1	355 ± 14	3 ± 0.70	5 ± 0.700	92 ± 0.300	91 ± 1.800	3 ± 1.000	6 ± 1.000

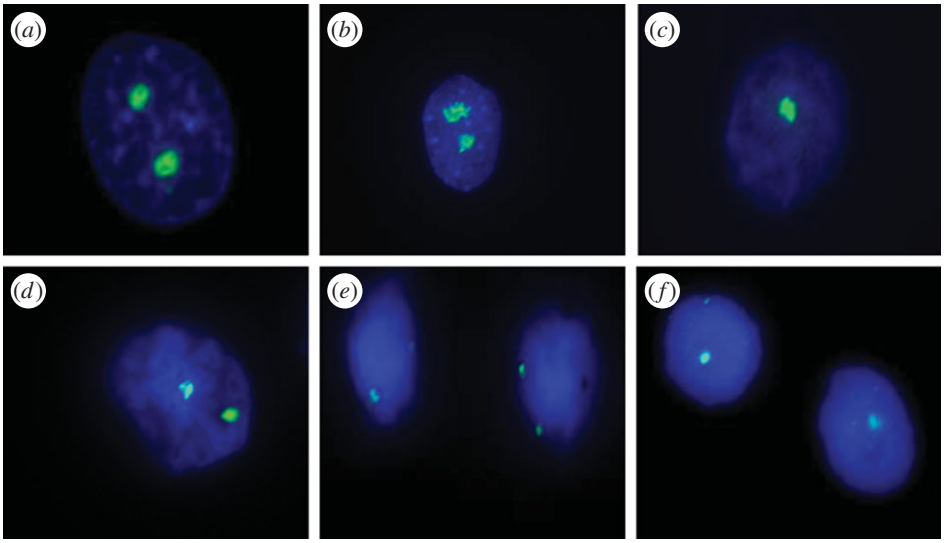

Figure 2. Fluorescent imaging of nucleoli and NORs in *Xenopus* kidney cells. Anti-nucleolin staining: (*a*) *Xenopus laevis*, (*b*) *X. borealis*, and (*c*) F_1(*X. laevis* × *X. borealis*). 18S rDNA FISH assays: (*d*) *X. laevis*, (*e*) *X. borealis*, and (*f*) F_1(*X. laevis* × *X. borealis*).

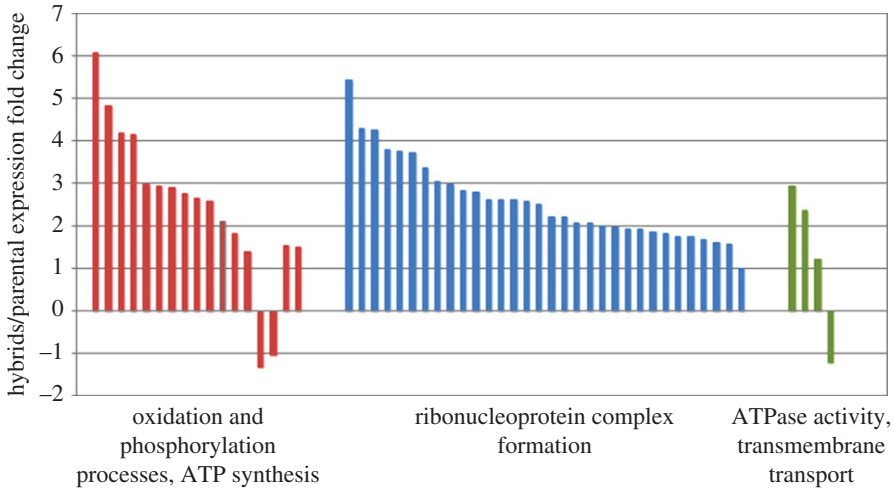

Figure 3. Relative changes in expression in functional groups over-represented among genes with significantly differential expression (FDR $p < 0.05$). Each bar represents a single gene.

genes associated with oxidative stress were also upregulated in hybrids, including three ATPases, *ALDH1L1*, *AASS*, and LOX (figure 3; electronic supplementary material, table S1). Overall, very few genes had maternally biased transcript frequencies (*adam10*, *cwf19l2*, *myrf*, and *pacsin3*; however, one gene, *ppp1r14c*, was paternally biased).

Based on SOLiD (ABI) sequencing of small RNAs, we found that two small nucleolar RNAs (snoRNAs), SNORD22 and SNORD96, were consistently differentially expressed in F_1 hybrids relative to their parental *X. laevis* and *X. muelleri*. Three additional snoRNAs (SNORD49, snoR38, and SNORA73) were downregulated; with only one upregulated in F_1 hybrids (SNORD29) relative to parental *X. laevis* (electronic supplementary material, table S4). At least two of the snoRNAs are derived from introns of the U22 host gene whose primary role seems to be transcriptional production of snoRNA [32]. No snoRNAs were subject to parental imprinting with the possible exception of snosnR60_Z15, expression of which was consistent with paternal (or *X. muelleri*) imprinting.

Together, these results indicate that nucleolar dominance is not only associated with a distinct rDNA expression pattern, but also with rDNA hereditary instability, having profound impacts on the rest of transcriptome. As discussed below, *Xenopus* provide a unique model system to investigate the relationship between these phenomena in animals.

4. Discussion

(a) Number of nucleoli and 45S rDNA expression patterns

In agreement with earlier studies [25–27], we show that *X. laevis*, *X. muelleri*, and *X. borealis* typically develop two nucleoli per nucleus, whereas F_1 hybrids among the species tend to suppress one of the nucleoli, a pattern interpreted as nucleolar dominance (figures 1 and 2; and electronic supplementary material, figure S1). However, the early reports have incorrectly linked this phenomenon to species-specific genome dominance, claiming that only *X. laevis* 45S rRNA gene copies are transcribed, regardless of the cross direction. We have established that 45S rRNA (but not 5S rRNA) in F_1 hybrids is under genomic imprinting, and maternal copies are predominantly expressed in all stages and tissues [31].

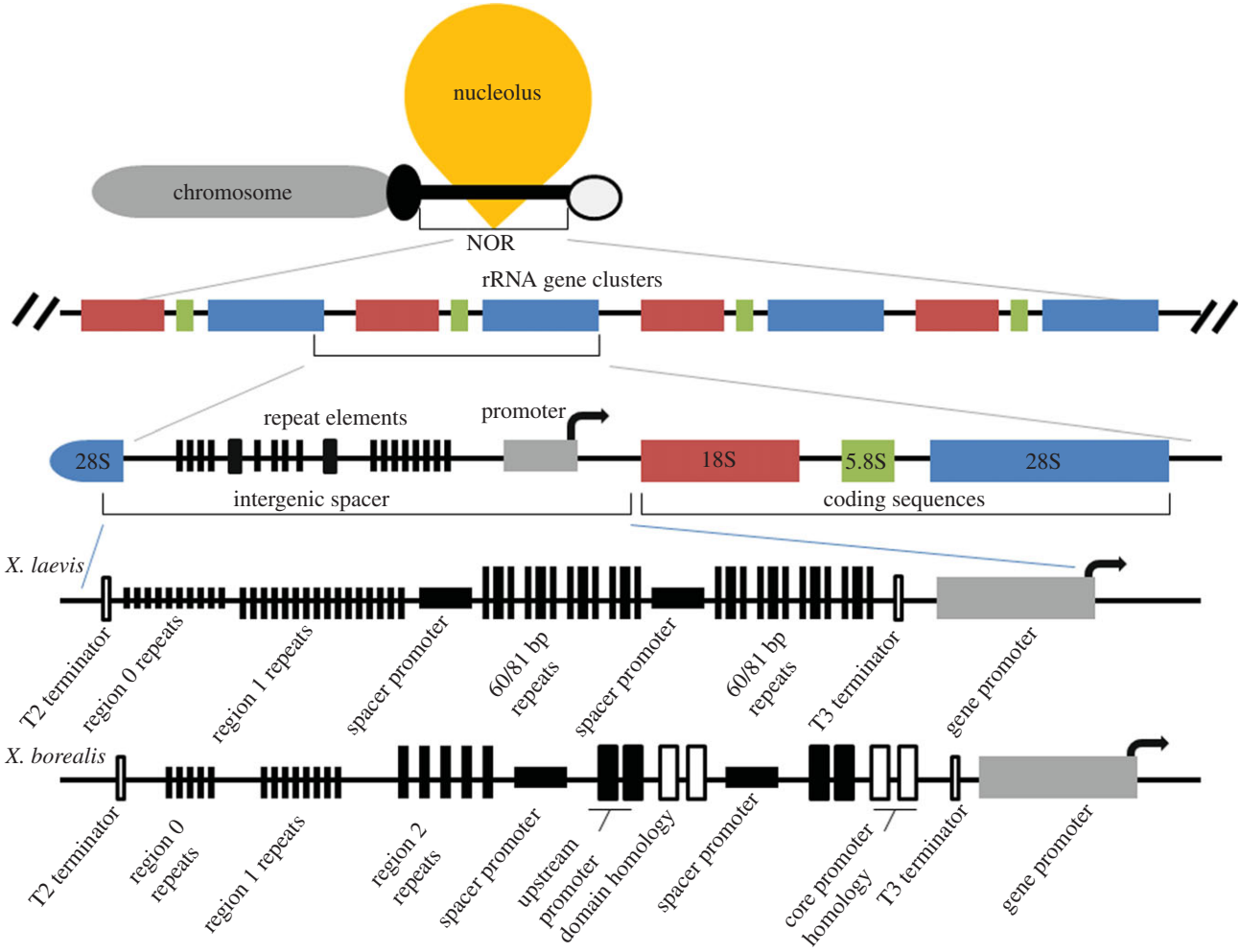

Figure 4. NOR of *Xenopus*. An annotated close-up of the *X. laevis* and *X. borealis* enhancer regions are shown on the lowest portion (after Caudy & Pikaard [30]).

Interestingly, parental genome imprinting was believed to have evolved in response to parental conflicts involving embryonic growth; and therefore are absent from amphibians, animals devoid of intimate parent–offspring interactions during the embryo development [33]. We thus ask if this dramatic epigenetic phenomenon, likely affecting more than 50% of all transcriptional output, is a transient feature limited to F_1 hybrids or a more general, although overlooked, trait of *Xenopus* chromatin. To address the question, we backcrossed F_1 hybrid females (F_1 hybrid males are sterile [34,35]) to all three parental species. Similar to F_1 hybrids, backcross hybrids also develop only one nucleolus (electronic supplementary material, figure S1).

We were surprised to observe that in offspring from a backcross of F_1(*X. borealis* ♀ × *X. laevis* ♂) ♀ × *X. laevis* the expression pattern completely shifted to *X. laevis* dominance (table 1), despite the fact that their F_1 mother had *X. borealis* expression. Even in a backcross between both parents having *X. borealis* expression, i.e. F_1(*X. borealis* ♀ × *X. laevis* ♂) ♀ × *X. borealis* ♂, offspring showed a slight bias towards *X. laevis* expression (56%) that originated from grandpaternal genomic contribution. Unlike paternal copies, grandpaternal *X. laevis* copies appear to escape silencing because they segregate through maternal chromosomes. Since all other backcrosses also predominantly expressed *X. laevis* copies regardless of the paternal species, we conclude that (i) although paternal genes are silenced, (ii) once maternal NOR contains genes from *X. laevis*, these genes transcriptionally dominate over genes from the other species, even if *X. laevis* is in the minority (table 1), and (iii) nucleolar dominance, or the number of expressed nucleoli, is uncoupled from gene expression patterns and genomic imprinting.

The expression patterns are consistent with de novo silencing of 45S rDNA from the sperm pronucleus within the maternal cytoplasm, which may be an ancient epigenetic feature of amphibian chromatin. The *Xenopus* sperm nucleus is rapidly reprogrammed by the egg cytoplasm following fertilization to form the paternal pronucleus, and both egg and sperm pronuclei start very intense DNA replication within 20 min of fertilization [36,37]. The process takes place in an environment extremely enriched for maternal rRNAs, associated small RNAs, and proteins, as amphibian oocytes undergo rDNA amplification leading to 500–2 500 extrachromosomal nucleoli containing at least 2 million rRNA gene copies [38]. This transcriptional repression of paternal genes does not prevent meiotic cross-overs between homologous clusters in the F_1 germline, consistent with the fact that recombination has been long known to play a critical role in the stabilization of 45S rDNA repeats [39]. Once recombined into maternal chromosomes, *X. laevis* 45S rDNA alleles transcriptionally outcompete *X. muelleri* and *X. borealis* alleles in backcross hybrid genomes. Although 18S, 5.8S, and 28S rDNA sequences are well conserved among taxa, intergenic spacer regions (IGSs) are highly variable among *Xenopus* species [40]. Unlike *X. laevis*, which has about 10 60 or 81 bp repeats between the gene promoter and the nearest IGS promoter, *X. borealis* and *X. muelleri* have only two of these elements in the analogous location (figure 4) [41,42].

These repeats contain 42 bp enhancer elements in all three species, but because *X. laevis* has more than five times more of them, its alleles transcriptionally dominate in experiments with minigene constructs [28–30] or when combined in backcross (but not F_1) hybrids, as demonstrated in this study.

This species-dependent expression from a maternal chromosome is a confounding factor in backcrosses, as only two alleles are available for the analysis, and it is difficult to determine if the recombinants from backcrosses in fact undergo de novo silencing of paternal genes. To introduce a third allele as a marker for the paternal chromosome, we produced trihybrids by crossing an *X. laevis* × *X. muelleri* F_1 female with an *X. borealis* male. Despite the fact that 45S rRNA gene clusters consisted mainly of paternal *X. borealis* alleles (more than 90%, table 1) in such trihybrids, they still predominantly expressed maternal *X. laevis* alleles (90%), similar to F_1 and backcross hybrids, and consistent with the hypothesis of paternal gene inactivation. However, we still lack direct evidence for the silencing of paternal rDNA in backcrosses or trihybrids, and alternative explanations need to be taken into consideration. For example, additional smaller clusters of 45S rRNA genes on other chromosomes may go undetected, creating a more complex rDNA landscape shifting expression from one direction to another. Indeed, *Xenopus* are pseudo-polyploids with very complex genomes [43], and even though they have lost 50–75% of all duplicated genes [44], remnants of 45S rRNA clusters may exist in addition to the main NOR.

Female *Xenopus* frogs have heterogametic sex chromosomes (ZW), whereas males are homogametic (ZZ). We asked a question whether maternal control of the imprinting pattern extends to a cross in which the mother is a genetic male (ZZ) sex-reversed to phenotypic female to resolve if the system is dependent upon sex chromosomes, which are prone to genetic conflicts [45]. To address this question, we used a sex-reversed *X. laevis* × *X. muelleri* F_1 hybrid male that was a fertile female [35] and backcrossed it to a *X. muelleri* male. Offspring from such a cross are all male, and their expression pattern of 45S rDNA was predominantly *X. laevis* (77%), consistent with maternal control of expression observed in other crosses. However, in crosses with sex-reversed mothers, maternal effects are sex-chromosome independent.

(b) 45S rDNA copy numbers and parental genomic proportions

We hypothesized that nucleolus underdevelopment (nucleolar dominance) results from disruptions in hybrid genomes and hereditary instability of rRNA genes, a hypothesis that could unambiguously be tested in frogs because NOR has been relatively well described in this system [3,4,40]. To test this hypothesis, we quantified gene copy variation and discovered that F_1 hybrids tended to lose nearly half of all 45S rRNA genes relative to the mid-parent value in reciprocal crosses that combine *X. muelleri*, and *X. laevis* and *X. laevis* and *X. borealis* genomes, respectively. This dramatic rDNA volume reduction corresponds well with the number of nucleoli being halved in F_1 and backcross hybrids relative to parental species.

Interestingly, *X. laevis* rRNA genes were preferentially excluded from all F_1 hybrid zygotes, leading to a consistent 20–42% deficit in this allele compared to Mendelian ratio expectations, although this segregation disadvantage of *X. laevis* alleles ceased after recombination (table 1). Longer IGSs give *X. laevis* a transcriptional advantage, but presumably at the cost of compromised stability during replication, recombination, or repair. Ribosomal DNA transcription and replication are not completely independent, as overexpression of the chromatin remodelling complex NoRC not only silences rDNA transcription, reduces the size and the number of nucleoli, but also impairs cell proliferation and resets replication timing from early to late, as observed in mice [46]. In yeast, rDNA recombination and replication are dependent on transcription from a non-coding bidirectional promoter (E-pro) within the rDNA spacer, which stimulates the dissociation of cohesin, a DNA-binding protein complex suppressing sister-chromatid-based changes in the rDNA copy number [39]. Histone deacetylase *Sir2* controls both E-pro transcription [39] and replication timing [47]. Uniparental loss of 45S rDNA was also reported in the allotetraploid grass *Zingeria trichopoda* [48], allotetraploid *Tragopogon* (Asteraceae) [49], and allopolyploid yeast *Pichia sorbitophila* [50]. All these observations from hybrid (allopolyploid) genomes suggest that parent-of-origin effects may be a pervasive feature of rDNA chromatin.

Hybrid genomes from interspecies crosses tend to be disrupted by genetic incompatibilities among genes having their own distinct evolutionary histories, brought together in a new epigenomic environment (Bateson–Dobzhansky–Muller effect) [51]. This instability helps uncover the hidden world of selfish genetic elements and understand how illusive the unity of the organism is, being continuously challenged by genomic conflicts [45]. Indeed, rDNA shows many characteristics of 'selfish' genetic elements, capable of violating Mendelian rules of fair segregation, and spreading in genomes without contributing to organismal fitness. First, rDNA seems to be highly redundant, as many of the rRNA genes remain heterochromatinized and are never transcribed. In chickens, for example, a mutant genotype with about 160 out of the typical complement of 290 45S rRNA genes (56%) still supports normal development [52]. The phenotypic effect of a new mutation in one of the multiple copies is thus expected to be negligible, and biased gene conversion may play a major role in the fate of new mutations [45]. Second, rDNA is subject to very rapid copy number evolution between species [53–55] and within species [56–58]. It can extrachromosomally circularize [59,60], attract transposable elements [58,61] as well as invade other selfish elements, such as B chromosomes [62–64] and germline-limited DNA [65]. Finally, rRNA gene clusters are recombinational hotspots in cancer [66], and abnormalities in the nucleolar morphology of cancer cells attracted the attention of tumour pathologists as early as the nineteenth century [67,68].

(c) Expression profiling of messenger RNAs and small nucleolar RNAs in *Xenopus laevis*, *Xenopus muelleri*, and their hybrid F_1

Since snoRNAs are critical for the processing of 5.8S, 18S, and 28S rRNAs [69], a question arises as to how such a large disruption of rRNA expression due to nucleolar dominance affects snoRNA profiles. Other studies have found that some snoRNAs could indeed be subject to parental imprinting, at least in mammals [70]. Although we observed significant alterations in snoRNA expression, genome imprinting does not affect this system of small RNAs. It

appears that imprinting the sperm pronucleus does not extend beyond Pol I transcription, as Pol III-transcribed 5S rDNA was not uniparentally silenced [31], nor were genes under Pol II transcriptional control, among which we found very few with maternally biased transcript frequencies. In addition to affecting the pattern of snoRNAs expression, nucleolar dominance and the de novo genome imprinting of 45S rDNA had a significant impact on mRNA levels, especially those from genes related to ribosome structure, rRNA binding, the polysome, and oxidative stress. Mitochondrial-encoded proteins (maternal) and nuclear-encoded proteins (maternal and paternal), therefore, must work in concert when forming functional complexes for efficient oxidative phosphorylation and energy production. Maternal–paternal cytonuclear incompatibilities may thus lead to oxidative shock and severe hybrid dysfunctions [71]. Also, direct interactions between ribosomes and mitochondria via ribosome-sensing receptors on the outer mitochondrial membrane are central to co-translational and post-translational protein transport [72], which may provide a novel link between mitochondrial and ribosome pathways, consistent with the observed patterns of transcriptional disruption.

Nucleolar architecture and rDNA transcription respond to cellular stresses such as UV irradiation, viral infection, and temperature shock [73]. Given the transcriptome signatures, it might be tempting to speculate that maternal rDNA imprinting could be an epigenetic defensive response to mitonuclear incompatibilities and oxidative stress. However, this explanation would be inconsistent with the observed pattern of rDNA expression in backcrosses (i.e. *X. laevis* dominance), as in one backcrossing direction maternal cytoplasm matches the paternal genome and is expected to reduce mitonuclear conflict, whereas in the reciprocal direction, maternal cytoplasm and paternal genome mismatch and are thus predicted to increase mitonuclear incompatibilities relative to F_1 hybrids.

5. Conclusion

Earlier investigations of nucleolar dominance were mainly focused on the mechanistic aspects of rDNA expression control. By extending our explorations to rDNA inheritance patterns, as well as their consequences on global changes in transcriptomes, including snoRNAs, we showed that nucleolar dominance results in a major disruption at both genomic and transcriptional levels. We also discovered maternal control over rDNA expression, which can be an ancient epigenetic mechanism or its relic to keep genomic parasites and genetic conflicts in check. Highly repeatable rDNA not only harbours multiple mobile elements [74] but itself has all the major features of selfish DNA [45], and its stability poses a constant metabolic challenge to cells.

Ethics. All animal work meets the legal requirements relating to animal welfare in the United States of America and the Commonwealth of Virginia (IACUC protocol no. 14–061 (VBI)).

Data accessibility. Sequence data are available via NCBI's SRA, accession no. SRP064976.

Authors' contributions. K.M. participated in the design of the study, conducted research and participated in the analysis of the data and drafting the manuscript. S.M. conducted research and participated in the analysis of the data. Y.B.K., J.H.O., and L.K. participated in the analysis of sequence data. G.S. was responsible for the animal husbandry. H.R.G. helped draft the manuscript. P.M. conceived of the study, coordinated the study, and helped draft the manuscript.

Competing interests. We have no competing interests.

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