Review

Justin M. O'Sullivan[a,*], Dave A. Pai[a], Andrew G. Cridge, David R. Engelke and Austen R.D. Ganley

The nucleolus: a raft adrift in the nuclear sea or the keystone in nuclear structure?

Abstract: The nucleolus is a prominent nuclear structure that is the site of ribosomal RNA (rRNA) transcription, and hence ribosome biogenesis. Cellular demand for ribosomes, and hence rRNA, is tightly linked to cell growth and the rRNA makes up the majority of all the RNA within a cell. To fulfill the cellular demand for rRNA, the ribosomal RNA (rDNA) genes are amplified to high copy number and transcribed at very high rates. As such, understanding the rDNA has profound consequences for our comprehension of genome and transcriptional organization in cells. In this review, we address the question of whether the nucleolus is a raft adrift the sea of nuclear DNA, or actively contributes to genome organization. We present evidence supporting the idea that the nucleolus, and the rDNA contained therein, play more roles in the biology of the cell than simply ribosome biogenesis. We propose that the nucleolus and the rDNA are central factors in the spatial organization of the genome, and that rapid alterations in nucleolar structure in response to changing conditions manifest themselves in altered genomic structures that have functional consequences. Finally, we discuss some predictions that result from the nucleolus having a central role in nuclear organization.

Keywords: genome architecture; nucleolus; rDNA.

[a]These authors contributed equally to this work.
***Corresponding author: Justin M. O'Sullivan,** The Liggins Institute, The University of Auckland, Auckland, New Zealand, e-mail: justin.osullivan@auckland.ac.nz
Dave A. Pai and David R. Engelke: Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109-0600, USA
Andrew G. Cridge: Laboratory for Evolution and Development, Biochemistry Department, University of Otago, Dunedin 9054, New Zealand
Austen R.D. Ganley: Institute of Natural and Mathematical Sciences, Massey University, Auckland, New Zealand

Introduction

Nucleoli are the largest non-chromosomal structures present within the eukaryotic nucleus. In yeast, the single nucleolus occupies approximately a quarter of the total nuclear volume in a position that is distal to the spindle pole body and in close contact with the nuclear envelope (1–3). In metazoans there can be multiple nucleoli, formed around distinct chromosomal loci, that differ from yeast in details of morphology but retain the dense staining caused by the prodigious production of ribosomes [e.g., reviewed in (4, 5)]. Nucleoli are organized around the core ribosomal RNA (rRNA) gene regions, referred to as nucleolus organizer regions (NORs) (6). NORs can, in some instances, form secondary constrictions on metaphase chromosomes during mitosis.

In eukaryotes, NORs usually consist of rRNA genes that are organized into tandem repeat arrays, collectively known as the rDNA (Figure 1). rDNA gene copy number can vary from a few copies up to tens of thousands of copies, depending on the species [see (7) for a comprehensive table]. For example, the well-characterized single rDNA array in *Saccharomyces cerevisiae* consists of around 180 copies (8), whereas in humans there are five rDNA arrays (9) that together comprise 300–400 copies per diploid genome (10). There are very few known exceptions to the tandem repeat rule: the intracellular human pathogen *Pneumocystis carinii* (11) and *Tetrahymena* (12) both appear to have just a single rDNA locus, although the latter amplifies this copy in the macronucleus (12). Nevertheless, the vast majority of eukaryotes characterized to date have the canonical rDNA organization, in which the polycistronic rRNA coding region, consisting of 18S, 5.8S, and 28S rRNA species (precise nomenclature varies somewhat between species), is interspersed with an intergenic spacer (IGS) region (13). The rDNA genes are the most highly transcribed in the genome, with rRNA accounting for approximately 80% of total RNA in a cell (14, 15). Despite this, the rDNA is a mosaic of transcribed,

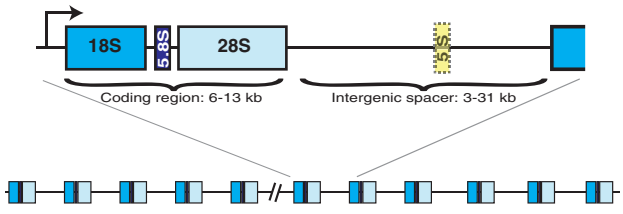

Figure 1 Structure of the eukaryotic rDNA repeat.
The structure of a typical eukaryotic rDNA repeat unit is shown in the upper part of the figure (not to scale), with the regions encoding the three major rRNA species (18S, 5.8S, and 28S) illustrated as blue boxes. The inclusion of the 5S rRNA gene (hatched box) within the rDNA repeat unit is variable and depends on the organism being investigated. The direction of RNA pol-I transcription is indicated, as is the known variation in size of the coding region and the IGS among eukaryotes. Individual rDNA repeats are usually arranged into arrays of tandem as illustrated.

typically highly, copies and completely silent copies (16). The organization of active and silent repeats within the linear rDNA array has yet to be determined. Similarly, the role of the silent copies has not been completely resolved, although they are required for efficient DNA repair in budding yeast (17).

The nucleolus is a domain of the nucleus, rather than a body delineated by a membrane or the like. Nevertheless, it has a specific structure that, in mammalian nuclei, consists of an inner fibrillar center, a dense fibrillar component outside of this, and a granular component surrounding this [(18, 19), although see (20)]. Although this is the case in mammalian nuclei, lower eukaryotes, in particular several yeast species, only have two distinctly visible components: a fibrillar component and granules. Furthermore, the fibrillar component in many yeast species is more a collection of strands, rather than a dense body (5). In either case, it has been shown that at least the non-transcribed parts of the rDNA are concentrated in the fibrillar component (FC) (21).

The nucleolus is very protein dense [e.g., reviewed in (4)] and in humans contains at least 700 different proteins (22), while being relatively DNA sparse. The nucleolus emerges from the complex mixture of proteins that associate with the rDNA, such as upstream binding factor (UBF) (23). Creation of the spatial domain of the nucleolus may result from high concentrations of binding sites in a small volume effectively causing retention of these proteins (24) by preventing movement out of the zone, as shown for ribosome movement (25). However, rapid shuttling of proteins between the nucleolus and nucleus has been observed (18, 19), suggesting that the nucleolus is a dynamic structure.

The nucleolus is not just a site of ribosome biogenesis: it functions in a myriad of other nuclear processes, including cell cycle control [reviewed in (4)]. Several proteins are known to localize to the nucleolus in a cell cycle-specific manner, including several that are associated with human disease (26). Furthermore, nucleolar localization of viral proteins involved in viral replication, including HIV, appears to be necessary for replication (19). Additionally, nucleolar structure changes in response to both environmental conditions and the cell cycle (18, 26). Such structural alterations, as well as alterations in the numbers of rDNA repeats, would relieve or exacerbate the retention of proteins sequestered in the nucleolus as a result of changes in the spatial clustering of binding sites. Strikingly, several non-coding RNA transcripts from the rDNA IGS appear to bind and sequester proteins in the nucleolus, and are regulated by stress (27). Given its dynamic nature, and the central role it plays in responding to cellular and environmental challenges, we hypothesize that the nucleolus has a direct role in coordinating nuclear structural organization.

The nucleolus as an organizer of genome structure

The nucleolus can contribute to nuclear organization through the sequestration and release of proteins that then, directly or indirectly, affect the organization of the nucleus. However, for the remainder of this review we are going to consider the issues surrounding the possibility that the nucleolus plays a direct role in the regulation of genome structure and how this might be achieved. In this context, we refer to genome structure as the spatial organization of the genome within the nucleus, thus this form of organization focuses on the DNA, although obviously all the attendant proteins and other factors are also part of this.

There is growing evidence that the genome takes on a specific structural arrangement within the nucleus. In human cells, different chromosomes are found to occupy chromosome 'territories', which have different positions in different cell types (28, 29). Genes are also observed to inhabit specific locations in the nucleus (3). Gene loops, that bring linearly distant enhancers in close spatial proximity to promoters, are also thought to be important for regulation of gene expression [e.g., (30–33)]. Recently developed techniques derived from proximity-based ligation (34–36), such as genome conformation capture (GCC) (37) and Hi-C (38), have been developed to experimentally determine global genome structure. Although extremely powerful, these techniques suffer from limitations when it comes to aligning sequences from repetitive elements.

Essentially because repetitive elements cannot be positioned to a unique position, they provide potentially confusing information in proximity-based ligation assays and are typically ignored (38–40). However, the rDNA is a special case and useful information can be obtained by collapsing the rDNA sequences to a single locus (37, 41, 42).

Computational-based approaches, utilizing proximity-ligation data and biophysical characteristics, have been taken to model global genome structure [e.g., (38, 40, 43, 44)]. Interestingly, few restraints are required to impart a crude order on *in silico* polymer-based reconstructions of the budding yeast nucleus (43, 44). However, one restraint that is required is the positioning of the nucleolus opposite to the spindle pole body (43), suggesting the nucleolus is a significant landmark for spatial organization of the genome.

Nucleolar localization of rDNA has been shown to influence the organization of other genomic loci in the malaria parasite, *Plasmodium falciparum* (45). Despite this, a structured nucleolus is not essential for nuclear function in yeast, as the rDNA genes can be deleted from their chromosomal locus and replaced with plasmid-encoded copies (46). These extra-chromosomally encoded rDNA genes form multiple, tiny dispersed nucleoli (47), and the growth of these strains is compromised. However, it remains unknown whether the growth defects stem from disruption of nuclear organization, or from attenuated rRNA transcription/processing (46). Nucleolar structure is also disrupted when yeast are forced to transcribe the chromosomal rDNA repeats with RNA polymerase (RNAP) II, rather than RNAP I (48). The entire yeast rDNA array can be shifted to another location within the genome, but in this case only minor phenotypic changes are observed, despite the nucleolus changing its position in the nucleus (49). This is consistent with a limited amount of published data that show that specific rDNA:non-rDNA interactions are sequence specific and independent of the chromosomal position of the non-rDNA locus (42). Thus, more work is required to deduce the effects of changes in nucleolar position on genome structure and function.

If the nucleolus directly regulates nuclear structure then it stands to reason that interactions between the rDNA repeats and other non-nucleolar loci are central to this. This is borne out experimentally in budding yeast where a majority of DNA-DNA interactions involve the rDNA (37). Although it can be argued that this interpretation is simplistic and does not take into account the copy number of the rDNA, any interactions between rDNA and non-rDNA loci are candidates for interactions by which the nucleolus shapes genome organization. These interactions should involve rDNA loci that are directly accessible from the nucleoplasm and are not protected by being internalized within the nucleolar structure.

The division of rDNA units into highly transcribed copies and completely silenced copies may reflect a functional distinction between units buried in the nucleolar interior and those located at the nuclear-nucleolar interface, respectively (T. Kobayashi, personal communication). Although it is almost certain that a main driver for nucleolar organization is the centralization of massive biosynthesis of ribosomes, we speculate that the tandem repeat organization of eukaryotic rDNA genes also enables the conservation of contacts at the nuclear:nucleolar boundary while still maintaining dedicated transcription units within the nucleolus. Such a system would allow the flexible assignment of rDNA repeats to the different functional categories: transcription, repair, replication, and structural associations, the latter having hitherto largely gone unrecognized. Therefore, the maintenance in eukaryotes of rDNA repeats with identical sequences [notably the non-coding regions (50)], at a much greater copy number than is needed for transcription alone, may ultimately stem from the ability of this system to seamlessly replace one repeat with another, ensuring that critical functions are maintained.

Transcription and nucleolus directed organization

The rDNA is not transcriptionally homogeneous; instead, all three classes of RNA polymerase are present in the nucleolus, in at least some organisms. Aside from RNAP I transcription, RNAP II transcription appears to be widespread in eukaryote rDNA (27, 51–55). Furthermore, RNAP III-transcribed 5S rDNA genes are located within the rDNA repeat in several species, including yeast [Figure 1; (56)]. Moreover, around 30 small ĩnterspersed nuclear ēlement (SINE) retrotransposons that derive from RNAP III-transcribed genes are found scattered throughout the human rDNA IGS (57). This opens up the question as to the effect of this transcriptional heterogeneity on the spatial organization of the nucleolus/nucleus.

Transcription-induced clustering represents a simple mechanism for spatial genome organization (58–60). Thus, polymerase class-dependent association of active or primed promoters in the rDNA may contribute to the coordination of nuclear-nucleolar structure. In support of this idea, structures consistent with RNAP I transcription factories involving rDNA repeats have been observed in metazoan cells (60). Furthermore, RNAP III forms foci within the nucleoplasm, and not the nucleoli, of human

cells, although it is possible that this is the result of SINE transcription (61). Transcription by all three eukaryotic RNA polymerases on overlapping regions of the rDNA repeat complicates this picture.

The simplest explanation for the overlapping polymerase activities within rDNA repeats is that the different RNA polymerase activities are temporally and spatially separated. This is supported by evidence that suggests a reciprocal relationship between RNAP I and II transcription in the rDNA (62, 63). Thus, the presence of rDNA repeats on the nucleus/nucleolus interface may free them up to be transcribed by RNAP II and/or III. However, in a yeast strain where rDNA repeat number is reduced to the extent that most copies are likely to be transcribed by RNAP I (64), RNAP II transcription is also high (51). This suggests that transcription by these two polymerases is not mutually exclusive.

In the case of RNAP I and RNAP III, it is clear that the transcription units can co-exist. Not only are the 5S rRNA genes and 35S rRNA genes (transcribed by RNAP III and I, respectively) interspersed in the linear repeats but there is also substantial evidence in the literature that 5S rRNA genes are associated with nucleoli even when located at distant sites in the linear genome (see below). Thus, the dynamics of rDNA repeat transcription is an important area for future research.

The nucleolus and RNAP III decoded genes

The spatial organization of the 5S rDNA genes is one of the clearest examples of the nucleolus affecting nuclear organization. Whereas in *S. cerevisiae* the 5S rDNA are located with the large rDNA repeats, in most eukaryotes they are not, and instead are present either as one or more clusters of repeats (e.g., *Drosophila melanogaster*, chicken, *Arabidopsis thaliana*, and human), in other repeat clusters (e.g., crustaceans and dinoflagellates), or entirely linearly dispersed (e.g., *Neurospora crassa* and *Schizosaccharomyces pombe*) (56, 65–70). However, these differences in the linear organization of the 5S genes between species belie commonalities in their spatial localization. For example, in mice ectopic 5S rDNA gene sequences have been shown to promote nucleolar localization (71). Similarly, in humans, one of the transcribed, linear clusters of 5S genes on chromosome I was shown to localize to a perinucleolar compartment (72). Moreover, the linearly dispersed 5S genes in many other eukaryotes have been shown to co-localize with nucleoli in three dimensions (73), suggesting that there are benefits to co-localizing the 5S genes with the other ribosomal genes. This is strong evidence for the nucleolus playing a direct role in the spatial organization of the nucleus.

The co-localization of RNAP III decoded loci with nucleoli is not restricted to the 5S rDNA – tRNA genes also show interesting patterns of spatial organization. Eukaryotic tRNA genes are generally dispersed throughout the linear genomes, although in rare cases there are isolated linear clusters of tRNA genes. *Xenopus laevis* oocytes have developmentally regulated tRNA genes that are found in clusters (74), and multiple clusters of tRNA genes in *S. pombe* are located within the centromeric heterochromatin (73, 75, 76). They are also frequent sites of genomic rearrangements (77, 78). In *S. cerevisiae*, both microscopy and crosslinking proximity analysis show that tRNA genes cluster together and co-localize with the nucleolus (37, 42, 79–81). In addition, a smaller cluster of tRNA genes has also been identified at the centromere of *S. cerevisiae* (40, 43), consistent with the observation that the tRNA genes in *S. pombe* are primarily clustered at the centromere at a position offset from the nucleolus (82). As previously noted for 5S rDNA sequences, yeast tRNA coding regions confer interaction specificity with the nucleolus (42), indicating that position alone is insufficient to explain this phenomenon.

Little is known about the three-dimensional organization of tRNA genes in most eukaryotes, however, and whether they co-localize with nucleoli. This is important to determine, as metazoan nuclei can be 100 times larger than yeast but have only 2–3 times as many tRNA genes (83). Thus, there is a significantly greater structural problem to overcome, and the relative effect of tRNA gene clustering on overall genome organization will be much less. In this context, if RNAP III transcription units are key components for spatial organization, a significantly more frequent DNA element would be needed in complex eukaryotes. In this context, it is interesting to consider that SINEs, retrotransposons derived from RNAP III transcripts (usually tRNA and 7SL RNA), are found in great quantities in large eukaryotic genomes (84–86). There is evidence that SINEs can form clusters in mammalian nuclei (87, 88) and substantial evidence that at least some SINEs bind RNAP III complex components *in vivo* (89). It will be interesting to test whether some subset of these SINE clusters co-localize with nucleoli, especially in light of the finding that *Alu* SINEs are processed in the nucleolus (90).

By definition, rDNA:non-rDNA interactions must involve interplay between different loci, but it need not be direct and may involve RNA, proteins or other factors (e.g., epigenetic modifications) that facilitate either directed or self-assembled interactions. Irrespective of how the associations are stabilized, they must be flexible enough to

allow reassignment of the rDNA repeat to another function without interfering with the primary function of the nucleolus – ribosome production. A simple model to explain the origin of these interactions is that the act of transcription or transcriptional regulation is responsible for interaction formation and/or maintenance. This is consistent with polymerase class-dependent association of different regions of the rDNA, and more generally with the idea of transcription factories. However, in the yeast strain where all the rDNA repeats are transcriptionally active due to enforced reductions in copy number, little or no phenotype is observed (17). It is possible that interactions with the rDNA may function to position non-nucleolar loci during nuclear division (91), when the rDNA are transcriptionally or replicatively inactive and accessible to other factors. In this case, transcription would not be the sole driver of interactions that involve the rDNA repeats.

The nucleolus and heterochromatin

The nucleolus appears to influence the chromatin structure of the DNA that surrounds it. In metazoans, the nucleolus is commonly observed to be surrounded by shell of late-replicating heterochromatin. Similarly, tephritids (fruit flies) and other dipterans (true flies) also exhibit preferential associations of the rDNA with heterochromatin-rich chromosomes (92). In *Drosophila*, there appears to be a direct relationship between the nucleolus and non-rDNA heterochromatin (93, 94). Furthermore, nucleolar association seems to be an important factor to maintain the heterochromatic state of the inactive human X chromosome (95), with the Barr body originally being known as the 'nucleolar satellite' (96). Analysis of nucleolus-associated chromatin domains (NADs) in two human cell lines (i.e., HeLa and HT1080) identified satellite repeats as being the major components of the NADs (97). Repetitive elements have also been implicated as forming part of the NAD in yeast (98).

Overlap between some metazoan NADs and reported lamina-associated domains suggests that specific genomic regions could alternate between associating with the nucleolus and the nuclear periphery, either in different cells or at different times (97, 99). The regulation of this recruitment would necessarily affect the organization of the remainder of the genome too. However, large-scale relocations are not a necessity if relative long-range positioning can be maintained through alterations to the compaction levels of intervening regions, rather than simply by physical relocation of the DNA. In effect, some contacts can be broken whereas others are maintained. However, direct recruitment of non-rDNA loci to the nucleolar boundary remains to be demonstrated. Therefore, regulation of these interactions in response to specific signals or pathways is still a hypothesis that requires testing.

Do bacteria have nucleoli and do they also function to organize the nucleoid?

It has traditionally been thought that bacteria lack the equivalent of a nucleolus as their repetitive ribosomal DNA genes are organized as dispersed repeats. However, it is clear that the bacterial nucleoid is structured (100–108), and recent evidence suggests that the rRNA genes in *Escherichia coli* may be transcribed in specific foci in the cell, opening up the idea that bacteria contain a nucleolus-like structure (109, 110) to facilitate recycling of RNA polymerase and coordination of ribosome assembly (111).

The different copies of bacterial ribosomal RNA genes, including the spacer regions, have high levels of sequence similarity. This finding was unexpected given the apparent dispersal of these genes in the genome. It has been proposed that sequence similarity is maintained through a process of gene conversion (112). Therefore, putative bacterial nucleoli may serve not only to optimize rRNA transcription and hence growth (111) but also to juxtapose ribosomal DNA genes to facilitate gene conversion between the disparate copies.

Whether the bacterial equivalent of a nucleolus actually exists is an important area for future study as it will shed light on critical aspects of bacterial growth rate regulation (111).

Conclusion

Accepting that the nucleolus is not simply a raft adrift the nuclear landscape, what advantage is there in the nucleolus controlling nuclear structure? We contend that the answer lies in the central position that ribosomes have within cellular metabolism (Figure 2). Stresses of all kinds affect ribosome activity [e.g., reviewed in (113)], the production of ribosomes, and consequently the nucleolus itself. Responses to stress [e.g., reviewed in (114)] may sometimes involve gross alterations to nucleolar structure [e.g., (115)]. These alterations have been related to the release and stabilization of

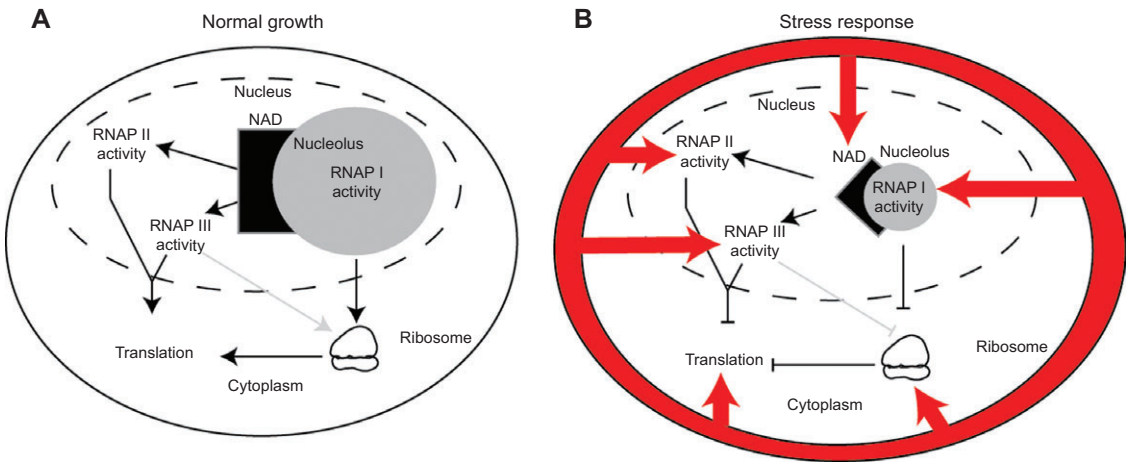

Figure 2 Nucleolar structure and NADs act as an intermediary between the genomic structural network that coordinates transcription and the cytoplasmic translational network.
(A) In a permissive environment, the structure of the nucleolus is dictated largely by the RNAP I transcription levels. In turn, this sets the organization of the rDNA repeats and interactions with the genomic loci, in particular the NADs, which affect RNAP II and RNAP III transcription patterns [i.e., genes that are transcribed and also whether this transcription is efficient (occurring in factories) or less efficient (dispersed)] and levels (depicted by black arrows). Cytoplasmic translation also feeds back to the nucleus and all facets of RNAP activity (for simplicity these linkages have been omitted from this cartoon). The net effect of this is that nucleolar structure acts as a link to help coordinate nuclear processes, whereas the nucleolar product (the ribosome) is the central facet in the cytoplasmic network. (B) In a non-permissive (i.e., stress) environment (depicted by the red zone), environmental signals (red arrows) target nuclear (i.e., RNAP I, RNAP II, RNAP III transcription) and cytoplasmic processes. By targeting RNAP I transcription, alterations are affected in nucleolar structure (depicted by smaller nucleolus) including changes in the NADs (depicted by alteration to shape). The net effect of this is to reinforce the signaling to the RNAP II and RNAP III transcription and subsequently effect a change in cytoplasmic translation.

proteins from the nucleolus [e.g., (115) and reviewed in (114)]; therefore, it is likely that alterations to the NADs associated with the nucleolar boundary also occur during stress response, but direct evidence for this is lacking. Our hypothesis predicts that such alterations occur and cause stress-related alterations to the associated genes, and these events are part of how the stress response is relayed to appropriate transcriptional networks outside the nucleolus (Figure 2). Thus, nucleolar structure acts as an intermediary between the genomic structural network that coordinates transcription, and the cytoplasmic translational network (Figure 2). The fact that regions of the nucleolus are acted on by the three different polymerases supports the sensory role of the rDNA. This model is conceptually similar to the rDNA theory of aging proposed by Kobayashi (116). In this theory, the repetitive nature of the rDNA makes it uniquely prone to instability, and this instability acts as an early warning system for general genomic instability, triggering the aging pathway. Therefore, we propose that nucleolar structure is the keystone that synchronizes expression and cellular responses by linking the distinct genomic and cytosolic protein networks within cells.

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References

1. Leger-Silvestre I, Trumtel S, Noaillac-Depeyre J, Gas N. Functional compartmentalization of the nucleus in the budding yeast Saccharomyces cerevisiae. Chromosoma 1999; 108: 103–13.

2. Bystricky K, Heun P, Gehlen L, Langowski J, Gasser SM. Long-range compaction and flexibility of interphase chromatin in budding yeast analyzed by high-resolution imaging techniques. Proc Natl Acad Sci USA 2004; 101: 16495–500.

3. Berger AB, Cabal GG, Fabre E, Duong T, Buc H, Nehrbass U, Olivo-Marin JC, Gadal O, Zimmer C. High-resolution statistical mapping reveals gene territories in live yeast. Nat Methods 2008; 5: 1031–7.
4. Shaw P, Brown J. Nucleoli: composition, function, and dynamics. Plant Physiol 2012; 158: 44–51.
5. Hernandez-Verdun D, Roussel P, Thiry M, Sirri V, Lafontaine DL. The nucleolus: structure/function relationship in RNA metabolism. Wiley Interdiscipl Rev RNA 2010; 1: 415–31.
6. McClintock B. The relation of a particular chromosomal element to the development of the nucleoli in Zea mays. Zeitschr Zellforsch Mikrosk Anatom 1934; 21: 294–326.
7. Prokopowich CD, Gregory TR, Crease TJ. The correlation between rDNA copy number and genome size in eukaryotes. Genome 2003; 46: 48–50.
8. Kobayashi T, Heck DJ, Nomura M, Horiuchi T. Expansion and contraction of ribosomal DNA repeats in Saccharomyces cerevisiae: requirement of replication fork blocking (Fob1) protein and the role of RNA polymerase I. Genes Dev 1998; 12: 3821–30.
9. Henderson AS, Warburton D, Atwood KC. Location of ribosomal DNA in the human chromosome complement. Proc Natl Acad Sci USA 1972; 69: 3394–8.
10. Schmickel RD. Quantitation of human ribosomal DNA: hybridization of human DNA with ribosomal RNA for quantitation and fractionation. Pediatr Res 1973; 7: 5–12.
11. Tang X, Bartlett MS, Smith JW, Lu J-J, Lee C-H. Determination of copy number of rRNA genes in Pneumocystis carinii f. sp. hominis. J Clin Microbiol 1998; 36: 2491–4.
12. Yao M-C, Blackburn E, Gall JG. Amplification of the rRNA genes in tetrahymena. Cold Spring Harb Symp Quantit Biol 1979; 43: 1293–6.
13. Long EO, Dawid IB. Repeated genes in eukaryotes. Annu Rev Biochem 1980; 49: 727–64.
14. Grummt I. Regulation of mammalian ribosomal gene transcription by RNA polymerase I. Progr Nucl Acid Res Mol Biol 1999; 62: 109–54.
15. Warner JR. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 1999; 24: 437–40.
16. McStay B, Grummt I. The epigenetics of rRNA genes: from molecular to chromosome biology. Annu Rev Cell Dev Biol 2008; 24: 131–57.
17. Ide S, Miyazaki T, Maki H, Kobayashi T. Abundance of ribosomal RNA gene copies maintains genome integrity. Science 2010; 327: 693–6.
18. Raska I, Shaw PJ, Cmarko D. New insights into nucleolar architecture and activity. Int Rev Cytol 2006; 255: 177–235.
19. Sirri V, Urcuqui-Inchima S, Roussel P, Hernandez-Verdun D. Nucleolus: the fascinating nuclear body. Histochem Cell Biol 2008; 129: 13–31.
20. Thiry M, Lafontaine DLJ. Birth of a nucleolus: the evolution of nucleolar components. Trends Cell Biol 2005; 15, 194–9.
21. Goessens G. Nucleolar structure. Int Rev Cytol 1984; 87: 107–58.
22. Andersen JS, Lam YW, Leung AK, Ong SE, Lyon CE, Lamond AI, Mann M. Nucleolar proteome dynamics. Nature 2005; 433: 77–83.
23. Mais C, Wright JE, Prieto JL, Raggett SL, McStay B. UBF-binding site arrays form pseudo-NORs and sequester the RNA polymerase I transcription machinery. Genes Dev 2005; 19: 50–64.
24. Fritsch CC, Langowski J. Anomalous diffusion in the interphase cell nucleus: the effect of spatial correlations of chromatin. J Chem Phys 2010; 133: 025101.
25. Politz JC, Tuft RA, Pederson T. Diffusion-based transport of nascent ribosomes in the nucleus. Mol Biol Cell 2003; 14: 4805–12.
26. Boisvert FM, van Koningsbruggen S, Navascues J, Lamond AI. The multifunctional nucleolus. Nat Rev Mol Cell Biol 2007; 8: 574–85.
27. Jacob MD, Audas TE, Mullineux S-T, Lee S. Where no RNA polymerase has gone before: novel functional transcripts derived from the ribosomal intergenic spacer. Nucleus 2012; 3: 315–9.
28. Parada L, McQueen P, Misteli T. Tissue-specific spatial organization of genomes. Genome Biol 2004; 5: R44.
29. Bolzer A, Kreth G, Solovei I, Koehler D, Saracoglu K, Fauth C, Müller S, Eils R, Cremer C, Speicher MR, Cremer T. Three-dimensional maps of all chromosomes in human male fibroblast nuclei and prometaphase rosettes. PLoS Biol 2005; 3: e157.
30. O'Sullivan JM, Tan-Wong SM, Morillon A, Lee B, Coles J, Mellor J, Proudfoot NJ. Gene loops juxtapose promoters and terminators in yeast. Nat Genet 2004; 36: 1014–8.
31. Tan-Wong SM, Wijayatilake HD, Proudfoot NJ. Gene loops function to maintain transcriptional memory through interaction with the nuclear pore complex. Genes Dev 2009; 23: 2610–24.
32. Laine JP, Singh BN, Krishnamurthy S, Hampsey M. A physiological role for gene loops in yeast. Genes Dev 2009; 23: 2604–9.
33. Ansari A, Hampsey M. A role for the CPF 3′-end processing machinery in RNAP II-dependent gene looping. Genes Dev 2005; 19: 2969–78.
34. Dekker J, Rippe K, Dekker M, Kleckner N. Capturing chromosome conformation. Science 2002; 295: 1306–11.
35. Cullen KE, Kladde MP, Seyfred MA. Interaction between transcription regulatory regions of prolactin chromatin. Science 1993; 261: 203–6.
36. Mukherjee S, Brieba LG, Sousa R. Discontinuous movement and conformational change during pausing and termination by T7 RNA polymerase. EMBO J 2003; 22: 6483–93.
37. Rodley CD, Bertels F, Jones B, O'Sullivan JM. Global identification of yeast chromosome interactions using Genome conformation capture. Fungal Genet Biol 2009; 46: 879–86.
38. Lieberman-Aiden E, van Berkum NL, Williams L, Imakaev M, Ragoczy T, Telling A, Amit I, Lajoie BR, Sabo PJ, Dorschner MO, Sandstrom R, Bernstein B, Bender MA, Groudine M, Gnirke A, Stamatoyannopoulos J, Mirny LA, Lander ES, Dekker J. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. Science 2009; 326: 289–93.
39. Sexton T, Yaffe E, Kenigsberg E, Bantignies F, Leblanc B, Hoichman M, Parrinello H, Tanay A, Cavalli G. Three-dimensional folding and functional organization principles of the Drosophila genome. Cell 2012; 148: 458–72.
40. Duan Z, Andronescu M, Schutz K, McIlwain S, Kim YJ, Lee C, Shendure J, Fields S, Blau CA, Noble WS. A three-dimensional model of the yeast genome. Nature 2010; 465: 363–7.
41. Rodley CD, Grand RS, Gehlen LR, Greyling G, Jones MB, O'Sullivan JM. Mitochondrial-nuclear DNA interactions contribute to the regulation of nuclear transcript levels as part of the inter-organelle communication system. PLoS ONE 2012; 7: e30943.

42. Rodley CD, Pai DA, Mills TA, Engelke DR, O'Sullivan JM. tRNA gene identity affects nuclear positioning. PLoS ONE 2011; 6: e29267.
43. Gehlen LR, Gruenert G, Jones MB, Rodley CD, Langowski J, O'Sullivan JM. Chromosome positioning and the clustering of functionally related loci in yeast is driven by chromosomal interactions. Nucleus 2012; 3: 370–83.
44. Tjong H, Gong K, Chen L, Alber F. Physical tethering and volume exclusion determine higher-order genome organization in budding yeast. Genome Res 2012; 22: 1295–305.
45. Mancio-Silva L, Zhang Q, Scheidig-Benatar C, Scherf A. Clustering of dispersed ribosomal DNA and its role in gene regulation and chromosome-end associations in malaria parasites. Proc Natl Acad Sci USA 2010; 107: 15117–22.
46. Wai HH, Vu L, Oakes ML, Nomura M. Complete deletion of yeast chromosomal rDNA repeats and integration of a new rDNA repeat: use of rDNA deletion strains for functional analysis of rDNA promoter elements in vivo. Nucl Acids Res 2000; 28: 3524–34.
47. Oakes M, Aris JP, Brockenbrough JS, Wai H, Vu L, Nomura M. Mutational analysis of the structure and localization of the nucleolus in the yeast Saccharomyces cerevisiae. J Cell Biol 1998; 143: 23–34.
48. Oakes M, Siddiql I, Vu L, Aris J, Nomura M. Transcription factor UAF, expansion and contraction of ribosomal DNA (rDNA) repeats, and RNA polymerase switch in transcription of yeast rDNA. Mol Cell Biol 1999; 19: 8559–69.
49. Oakes ML, Johzuka K, Vu L, Eliason K, Nomura M. Expression of rRNA genes and nucleolus formation at ectopic chromosomal sites in the yeast Saccharomyces cerevisiae. Mol Cell Biol 2006; 26: 6223–38.
50. Ganley ARD, Kobayashi T. Highly efficient concerted evolution in the ribosomal DNA repeats: total rDNA repeat variation revealed by whole-genome shotgun sequence data. Genome Res 2007; 17: 184–91.
51. Kobayashi T, Ganley AR. Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. Science 2005; 309: 1581–4.
52. Coehlo PSR, Bryan AC, Kumar A, Shadel GS, Snyder M. A novel mitochondrial protein, Tar1p, is encoded on the antisense strand of the nuclear 25S rDNA. Genes Dev 2002; 16: 2755–60.
53. Li C, Mueller JE, Bryk M. Sir2 represses endogenous polymerase II transcription units in the ribosomal DNA nontranscribed spacer. Mol Biol Cell 2006; 17: 3848–59.
54. Kermekchiev M, Ivanova L. Ribin, a protein encoded by a message complementary to rRNA, modulates ribosomal transcription and cell proliferation. Mol Cell Biol 2001; 21: 8255–63.
55. Gagnon-Kugler T, Langlois F, Stefanovsky V, Lessard F, Moss T. Loss of human ribosomal gene CpG methylation enhances cryptic RNA polymerase II transcription and disrupts ribosomal RNA processing. Mol Cell 2009; 35: 414–25.
56. Drouin G, de Sa MM. The concerted evolution of 5S ribosomal genes linked to the repeat units of other multigene families. Mol Biol Evol 1995; 12: 481–93.
57. Gonzalez IL, Sylvester JE. Complete sequence of the 43-kb human ribosomal DNA repeat: analysis of the intergenic spacer. Genomics 1995; 27: 320–8.
58. Bartlett J, Blagojevic J, Carter D, Eskiw C, Fromaget M, Job C, Shamsher M, Trindade IF, Xu M, Cook PR. Specialized transcription factories. Biochem Soc Symp 2006; 73: 67–75.
59. Cook PR. Predicting three-dimensional genome structure from transcriptional activity. Nat Genet 2002; 32: 347–52.
60. Cook PR. The organization of replication and transcription. Science 1999; 284: 1790–5.
61. Pombo A, Jackson DA, Hollinshead M, Wang Z, Roeder RG, Cook PR. Regional specialization in human nuclei: visualization of discrete sites of transcription by RNA polymerase III. EMBO J 1999; 18: 2241–53.62.
62. Cioci F, Vu L, Eliason K, Oakes M, Siddiqi IN, Nomura M. Silencing in yeast rDNA chromatin: reciprocal relationship in gene expression between RNA polymerase I and II. Mol Cell 2003; 12: 135–45.
63. Cesarini E, Mariotti FR, Cioci F, Camilloni G. RNA polymerase I transcription silences noncoding RNAs at the ribosomal DNA locus in Saccharomyces cerevisiae. Eukaryotic Cell 2010; 9: 325–35.
64. French SL, Osheim YN, Cioci F, Nomura M, Beyer AL. In exponentially growing Saccharomyces cerevisiae cells, rRNA synthesis is determined by the summed RNA polymerase I loading rate rather than the number of active genes. Mol Cell Biol 2003; 23: 1558–68.
65. Daniels LM, Delany ME. Molecular and cytogenetic organization of the 5S ribosomal DNA array in chicken (Gallus gallus). Chromosome Res 2003; 11: 305–17.
66. Wimber DE, Steffensen DM. Localization of 5S RNA genes on Drosophila chromosomes by RNA-DNA hybridization. Science 1970; 170: 639–41.
67. Cloix C, Tutois S, Mathieu O, Cuvillier C, Espagnol MC, Picard G, Tourmente S. Analysis of 5S rDNA arrays in Arabidopsis thaliana: physical mapping and chromosome-specific polymorphisms. Genome Res 2000; 10: 679–90.
68. Sorensen PD, Frederiksen S. Characterization of human 5S rRNA genes. Nucleic Acids Res 1991; 19: 4147–51.
69. Metzenberg RL, Stevens JN, Selker EU, Morzycka-Wroblewska E. Identification and chromosomal distribution of 5S rRNA genes in Neurospora crassa. Proc Natl Acad Sci USA 1985; 82: 2067–71.
70. Mao J, Appel B, Schaack J, Sharp S, Yamada H, Söll D. The 5S RNA genes of Schizosaccharomyces pombe. Nucleic Acids Res 1982; 10: 487–500.
71. Fedoriw AM, Starmer J, Yee D, Magnuson T. Nucleolar association and transcriptional inhibition through 5S rDNA in mammals. PLoS Genet 2012; 8: e1002468.
72. Matera AG, Frey MR, Margelot K, Wolin SL. A perinucleolar compartment contains several RNA polymerase III transcripts as well as the polypyrimidine tract-binding protein, hnRNP I. J Cell Biol 1995; 129: 1181–93.
73. Haeusler RA, Engelke DR. Spatial organization of transcription by RNA polymerase III. Nucl Acids Res 2006; 34: 4826–36.
74. Stutz F, Gouilloud E, Clarkson SG. Oocyte and somatic tyrosine tRNA genes in Xenopus laevis. Genes Dev 1989; 3: 1190–8.
75. Kuhn RM, Clarke L, Carbon J. Clustered tRNA genes in Schizosaccharomyces pombe centromeric DNA sequence repeats. Proc Natl Acad Sci USA 1991; 88: 1306–10.
76. Scott KC, Merrett SL, Willard HF. A heterochromatin barrier partitions the fission yeast centromere into discrete chromatin domains. Curr Biol 2006; 16: 119–29.
77. Dunham MJ, Badrane H, Ferea T, Adams J, Brown PO, Rosenzweig F, Botstein D. Characteristic genome rearrangements in experimental evolution of Saccharomyces cerevisiae. Proc Natl Acad Sci USA 2002; 99: 16144–9.

78. McFarlane RJ, Whitehall SK. tRNA genes in eukaryotic genome organization and reorganization. Cell Cycle 2009; 8: 3102–6.
79. Thompson M, Haeusler RA, Good PD, Engelke DR. Nucleolar clustering of dispersed tRNA genes. Science 2003; 302: 1399–401.
80. Wang L, Haeusler RA, Good PD, Thompson M, Nagar S, Engelke DR. Silencing near tRNA genes requires nucleolar localization. J Biol Chem 2005; 280: 8637–9.
81. Haeusler RA, Pratt-Hyatt M, Good PD, Gipson TA, Engelke DR. Clustering of yeast tRNA genes is mediated by specific association of condensin with tRNA gene transcription complexes. Genes Dev 2008; 22: 2204–14.
82. Iwasaki O, Tanaka A, Tanizawa H, Grewal SI, Noma K. Centromeric localization of dispersed Pol III genes in fission yeast. Mol Biol Cell 2010; 21: 254–65.
83. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucl Acids Res 1997; 25: 955–64.
84. Kramerov DA, Vassetzky NS. Short retroposons in eukaryotic genomes. Int J Cytol 2005; 247: 165–221.
85. Deininger PL, Batzer MA. Mammalian retroelements. Genome Res 2002; 12: 1455–65.
86. Belancio VP, Hedges DJ, Deininger P. Mammalian non-LTR retrotransposons: for better or worse, in sickness and in health. Genome Res 2008; 18: 343–58.
87. Kaplan FS, Murray J, Sylvester JE, Gonzalez IL, O'Connor JP, Doering JL, Muenke M, Emanuel BS, Zasloff MA. The topographic organization of repetitive DNA in the human nucleolus. Genomics 1993; 15: 123–32.
88. Pai DA, Engelke DR. Spatial organization of genes as a component of regulated expression. Chromosoma 2010; 119: 13–25.
89. Moqtaderi Z, Wang J, Raha D, White RJ, Snyder M, Weng Z, Struhl K. Genomic binding profiles of functionally distinct RNA polymerase III transcription complexes in human cells. Nat Struct Mol Biol 2010; 17: 635–40.
90. Chen Y, Sinha K, Perumal K, Gu J, Reddy R. Accurate 3′ end processing and adenylation of human signal recognition particle RNA and Alu RNA in vitro. J Biol Chem 1998; 273: 35023–31.
91. Hernandez-Verdun D. Assembly and disassembly of the nucleolus during the cell cycle. Nucleus 2011; 2: 189–94.
92. Drosopoulou E, Nakou I, Síchová J, Kubícková S, Marec F, Mavragani-Tsipidou P. Sex chromosomes and associated rDNA form a heterochromatic network in the polytene nuclei of Bactrocera oleae (Diptera: Tephritidae). Genetica 2012; 140: 169–80.
93. Peng JC, Karpen GH. H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. Nat Cell Biol 2007; 9: 25–35.
94. Paredes S, Maggert KA. Ribosomal DNA contributes to global chromatin regulation. Proc Natl Acad Sci USA 2009; 106: 17829–34.
95. Zhang L-F, Huynh KD, Lee JT. Perinucleolar targeting of the inactive X during S phase: evidence for a role in the maintenance of silencing. Cell 2007; 129: 693–706.
96. Barr ML, Bertram EG. A morphological distinction between neurones of the male and female, and the behaviour of the nucleolar satellite during accelerated nucleoprotein synthesis. Nature 1949; 163: 676–77.
97. Németh A, Conesa A, Santoyo-Lopez J, Medina I, Solovei I, Cremer T, Dopazo J, Längst G. Initial genomics of the human nucleolus. PLoS Genet 2010; 6: e1000889.
98. O'Sullivan JM, Sontam DM, Grierson R, Jones B. Repeated elements coordinate the spatial organization of the yeast genome. Yeast 2009; 26: 125–38.
99. van Koningsbruggen S, Gierlinski M, Schofield P, Martin D, Barton GJ, Ariyurek Y, den Dunnen JT, Lamond AI. High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli. Mol Biol Cell 2010; 21: 3735–48.
100. Wiggins PA, Cheveralls KC, Martin JS, Lintner R, Kondev J. Strong intranucleoid interactions organize the Escherichia coli chromosome into a nucleoid filament. Proc Natl Acad Sci USA 2010; 107: 4991–5.
101. Postow L, Hardy CD, Arsuaga J, Cozzarelli NR. Topological domain structure of the Escherichia coli chromosome. Genes Dev 2004; 18: 1766–79.
102. Fiebig A, Keren K, Theriot JA. Fine-scale time-lapse analysis of the biphasic, dynamic behaviour of the two Vibrio cholerae chromosomes. Mol Microbiol 2006; 60: 1164–78.
103. Valens M, Penaud S, Rossignol M, Cornet F, Boccard F. Macrodomain organization of the Escherichia coli chromosome. EMBO J 2004; 23: 4330–41.
104. Espeli O, Mercier R, Boccard F. DNA dynamics vary according to macrodomain topography in the E. coli chromosome. Mol Microbiol 2008; 68: 1418–27.
105. Nguyen HH, de la Tour CB, Toueille M, Vannier F, Sommer S, Servant P. The essential histone-like protein HU plays a major role in Deinococcus radiodurans nucleoid compaction. Mol Microbiol 2009; 73: 240–52.
106. Maurer S, Fritz J, Muskhelishvili G. A systematic in vitro study of nucleoprotein complexes formed by bacterial nucleoid-associated proteins revealing novel types of DNA organization. J Mol Biol 2009; 387: 1261–76.
107. Skoko D, Yoo D, Bai H, Schnurr B, Yan J, McLeod SM, Marko JF, Johnson RC. Mechanism of chromosome compaction and looping by the Escherichia coli nucleoid protein Fis. J Mol Biol 2006; 364: 777–98.
108. Boccard F, Esnault E, Valens M. Spatial arrangement and macrodomain organization of bacterial chromosomes. Mol Microbiol 2005; 57: 9–16.
109. Cabrera JE, Cagliero C, Quan S, Squires CL, Jin DJ. Active transcription of rRNA operons condenses the nucleoid in Escherichia coli: examining the effect of transcription on nucleoid structure in the absence of transertion. J Bacteriol 2009; 191: 4180–5.
110. Cabrera JE, Jin DJ. Active transcription of rRNA operons is a driving force for the distribution of RNA polymerase in bacteria: effect of extrachromosomal copies of rrnB on the in vivo localization of RNA polymerase. J Bacteriol 2006; 188: 4007–14.
111. Jin DJ, Cagliero C, Zhou YN. Growth rate regulation in Escherichia coli. FEMS Microbiol Rev 2012; 36: 269–87.
112. Liao D. Gene conversion drives within genic sequences: concerted evolution of ribosomal RNA genes in bacteria and archaea. J Mol Evol 2000; 51: 305–17.

113. Spriggs KA, Bushell M, Willis AE. Translational regulation of gene expression during conditions of cell stress. Mol Cell 2010; 40: 228–37.
114. Suzuki A, Kogo R, Kawahara K, Sasaki M, Nishio M, Maehama T, Sasaki T, Mimori K, Mori M. A new PICTure of nucleolar stress. Cancer Sci 2012; 103: 632–7.
115. Rubbi CP, Milner J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. EMBO J 2003; 22: 6068–77.
116. Kobayashi T. A new role of the rDNA and nucleolus in the nucleus – rDNA instability maintains genome integrity. Bioessays 2008; 30: 267–72.

Justin M. O'Sullivan studied Cellular and Molecular Biology at Canterbury University, obtained his PhD at Otago University, and completed post-doctoral work at the University of Kent and the University of Oxford. He was appointed a Senior Lecturer at Massey University, New Zealand. He is currently a Senior Research Fellow at the Liggins Institute at the University of Auckland.

Dave Pai studied Biophysics at Johns Hopkins University before attending the University of Michigan, obtaining a PhD in Biological Chemistry in the group of David Engelke.

Andrew Cridge graduated in New Zealand from Lincoln University (B.Sc., Hons.) and Otago University (PhD). He completed post-doctoral studies in post-transcription regulation of gene expression at the University of Manchester (UK) and Massey University (New Zealand). Additional research at Massey University focused on examining the spatial organization of gene structure in its role in gene regulation. Currently, he is a post-doctoral fellow at the Laboratory for Evolution and Development, at the University of Otago.

David Engelke trained in Biochemistry as an undergraduate at the University of Wisconsin, obtained his PhD in Molecular Biology from Washington University in St. Louis, and completed post-doctoral work at the University of California, San Diego and the California Institute of Technology. He is a Professor of Biological Chemistry at the University of Michigan, where for 30 years his group has studied the biochemistry, genetics, and cell biology of small RNA biosynthesis in eukaryotic nuclei.

Austen Ganley has a PhD in Molecular Genetics from Massey University, New Zealand. He was a Clark Postdoctoral Fellow in Molecular Evolution and Comparative Genomics at Duke University, NC, USA. He also worked in the National Institute for Basic Biology and the National Institute of Genetics in Japan with Takehiko Kobayashi before returning to Massey University (Albany) in New Zealand to establish his own group in 2008. His main interests are in understanding the biology and evolution of the ribosomal DNA repeats.