



Published in final edited form as:

*Biomol Concepts*. 2013 June ; 4(3): 277–286. doi:10.1515/bmc-2012-0043.

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

Justin M. O'Sullivan<sup>1,\*</sup>, Dave A. Pai<sup>2,†</sup>, Andrew G. Cridge<sup>3</sup>, David R. Engelke<sup>2</sup>, and Austen R. D. Ganley<sup>4</sup>

<sup>1</sup>The Liggins Institute, The University of Auckland, Auckland, New Zealand <sup>2</sup>Department of Biological Chemistry, The University of Michigan, Ann Arbor, MI 48109-0600, USA <sup>3</sup>Laboratory for Evolution and Development, Biochemistry Department, University of Otago, Dunedin 9054, New Zealand <sup>4</sup>Institute of Natural Sciences, Massey University, Auckland, New Zealand

### 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 fulfil the cellular demand for rRNA, the ribosomal RNA genes (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.

### Introduction

Nucleoli are the largest non-chromosomal structures present within the eukaryotic nucleus. In yeast, the single nucleolus occupies approximately ¼ 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 organised 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.

\*Corresponding author. justin.osullivan@auckland.ac.nz.

†these authors contributed equally to this work;

In eukaryotes, the 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 *S. cerevisiae* consists of around 180 copies (8), while 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 intra-cellular 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 region (IGS) (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, 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 centre, a dense fibrillar component outside of this, and a granular component surrounding this (18, 19), although see (20)). While 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 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 noncoding 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 organisation through the sequestration and release of proteins that then, directly or indirectly, affect the organisation 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 organisation 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 GCC (37) and HiC (38), have been developed to experimentally determine global genome structure. While 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, utilising 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 localisation of rDNA has been shown to influence the organisation 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 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 shows 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). While 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, pers. communication). While 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 homogenous; 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 thirty small interspersed nuclear element (SINE) retrotransposons that derive from RNAP III-transcribed genes are found scattered throughout the human rDNA intergenic spacer (57). This opens up the question as to the effect of this transcriptional heterogeneity on the spatial organisation 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 RNAPs I and 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 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 RNA polymerase III decoded genes

The spatial organisation of the 5S rDNA genes is one of the clearest examples of the nucleolus affecting nuclear organisation. While 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 localisation (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 organisation 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 *A/tu* 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 while 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 organised as dispersed repeats. However, it is clear that the bacterial nucleoid is structured (100–108), and recent evidence suggests that the rRNA genes in *E. 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 optimise 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).

## **Conclusions**

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 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 co-ordinates 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 rDNA's sensory role. This model is conceptually similar to the rDNA theory of aging proposed by Kobayashi (2008) (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.

## Acknowledgments

JMOS and AGC are supported by the Marsden Fund. JMOS was also funded by Gravidia: National Centre for Growth and Development. DRE and DAP were funded by National Institutes of Health grant GM082875. DAP was also funded by a National Institutes of Health University of Michigan Genetics Pre-doctoral Training Grant (T32 GM07544) and a Rackham Merit Fellowship. ARDG is supported by the Marsden Fund.

## References

1. Leger-Silvestre I, Trumtel S, Noaillac-Depeyre J, Gas N. Functional compartmentalization of the nucleus in the budding yeast *Saccharomyces cerevisiae*. *Chromosoma*. May.1999 108:103. [PubMed: 10382072]
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 U S A*. Nov 23.2004 101:16495. [PubMed: 15545610]
3. Berger AB, et al. High-resolution statistical mapping reveals gene territories in live yeast. *Nat Methods*. Dec.2008 5:1031. [PubMed: 18978785]
4. Shaw P, Brown J. Nucleoli: composition, function, and dynamics. *Plant physiology*. Jan.2012 158:44. [PubMed: 22082506]
5. Hernandez-Verdun D, Roussel P, Thiry M, Sirri V, Lafontaine DL. The nucleolus: structure/function relationship in RNA metabolism. *Wiley interdisciplinary reviews RNA*. Nov-Dec;2010 1:415. [PubMed: 21956940]
6. McClintock B. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. *Zeitschrift für Zellforschung und Mikroskopische Anatomie*. 1934; 21:294.
7. Prokopowich CD, Gregory TR, Crease TJ. The correlation between rDNA copy number and genome size in eukaryotes. *Genome*. 2003; 46:48. [PubMed: 12669795]
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*. Dec 15.1998 12:3821. [PubMed: 9869636]
9. Henderson AS, Warburton D, Atwood KC. Location of ribosomal DNA in the human chromosome complement. *Proc Natl Acad Sci U S A*. Nov.1972 69:3394. [PubMed: 4508329]
10. Schmickel RD. Quantitation of human ribosomal DNA: hybridization of human DNA with ribosomal RNA for quantitation and fractionation. *Pediatr Res*. Jan.1973 7:5. [PubMed: 4687000]



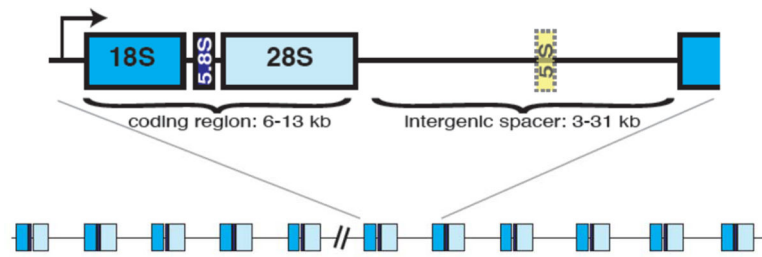
11. Tang X, Bartlett MS, Smith JW, Lu JJ, Lee CH. Determination of copy number of rRNA genes in *Pneumocystis carinii* f. sp. *hominis*. *Journal of Clinical Microbiology*. 1998; 36:2491. [PubMed: 9705380]
12. Yao MC, Blackburn E, Gall JG. Amplification of the rRNA Genes in Tetrahymena. *Cold Spring Harbor Symposia on Quantitative Biology*. 1979; 43:1293. [PubMed: 290442]
13. Long EO, Dawid IB. Repeated genes in eukaryotes. *Annual review of biochemistry*. 1980; 49:727.
14. Grummt I. Regulation of mammalian ribosomal gene transcription by RNA polymerase I. *Progress in Nucleic Acid Research and Molecular Biology*. 1999; 62:109. [PubMed: 9932453]
15. Warner JR. The economics of ribosome biosynthesis in yeast. *Trends in Biochemical Sciences*. 1999; 24:437. [PubMed: 10542411]
16. McStay B, Grummt I. The epigenetics of rRNA genes: from molecular to chromosome biology. *Annu Rev Cell Dev Biol*. 2008; 24:131. [PubMed: 18616426]
17. Ide S, Miyazaki T, Maki H, Kobayashi T. Abundance of ribosomal RNA gene copies maintains genome integrity. *Science*. Feb 5.2010 327:693. [PubMed: 20133573]
18. Raska I, Shaw PJ, Cmarko D. New insights into nucleolar architecture and activity. *International review of cytology*. 2006; 255:177. [PubMed: 17178467]
19. Sirri V, Urcuqui-Inchima S, Roussel P, Hernandez-Verdun D. Nucleolus: the fascinating nuclear body. *Histochemistry and cell biology*. Jan.2008 129:13. [PubMed: 18046571]
20. Thiry M, Lafontaine DLJ. Birth of a nucleolus: the evolution of nucleolar components. *Trends in Cell Biology*. 2005; 15:194. [PubMed: 15817375]
21. Goessens G. Nucleolar structure. *International review of cytology*. 1984; 87:107. [PubMed: 6201455]
22. Andersen JS, et al. Nucleolar proteome dynamics. *Nature*. Jan 6.2005 433:77. [PubMed: 15635413]
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*. Jan 1.2005 19:50. [PubMed: 15598984]
24. Fritsch CC, Langowski J. Anomalous diffusion in the interphase cell nucleus: the effect of spatial correlations of chromatin. *J Chem Phys*. Jul 14.2010 133:025101. [PubMed: 20632774]
25. Politz JC, Tuft RA, Pederson T. Diffusion-based transport of nascent ribosomes in the nucleus. *Mol Biol Cell*. Dec.2003 14:4805. [PubMed: 12960421]
26. Boisvert FM, van Koningsbruggen S, Navascues J, Lamond AI. The multifunctional nucleolus. *Nat Rev Mol Cell Biol*. Jul.2007 8:574. [PubMed: 17519961]
27. Jacob MD, Audas TE, Mullineux ST, Lee S. Where no RNA polymerase has gone before: Novel functional transcripts derived from the ribosomal intergenic spacer. *Nucleus*. 2012; 3:1.
28. Parada L, McQueen P, Misteli T. Tissue-specific spatial organization of genomes. *Genome Biology*. 2004; 5:R44. [PubMed: 15239829]
29. Bolzer A, et al. Three-Dimensional Maps of All Chromosomes in Human Male Fibroblast Nuclei and Prometaphase Rosettes. *PLoS Biology*. May 1.2005 3:e157. [PubMed: 15839726]
30. O'Sullivan JM, et al. Gene loops juxtapose promoters and terminators in yeast. *Nat Genet*. Sep. 2004 36:1014. [PubMed: 15314641]
31. Tan-Wong SM, Wijayatilake HD, Proudfoot NJ. Gene loops function to maintain transcriptional memory through interaction with the nuclear pore complex. *Genes Dev*. Nov 15.2009 23:2610. [PubMed: 19933151]
32. Laine JP, Singh BN, Krishnamurthy S, Hampsey M. A physiological role for gene loops in yeast. *Genes Dev*. Nov 15.2009 23:2604. [PubMed: 19933150]
33. Ansari A, Hampsey M. A role for the CPF 3'-end processing machinery in RNAP II-dependent gene looping. *Genes Dev*. Dec 15.2005 19:2969. [PubMed: 16319194]
34. Dekker J, Rippe K, Dekker M, Kleckner N. Capturing chromosome conformation. *Science*. Feb 15.2002 295:1306. [PubMed: 11847345]
35. Cullen KE, Kladde MP, Seyfred MA. Interaction between Transcription Regulatory Regions of Prolactin Chromatin. *Science*. Jul 9.1993 261:203. [PubMed: 8327891]

36. Mukherjee S, Brieba LG, Sousa R. Discontinuous movement and conformational change during pausing and termination by T7 RNA polymerase. *Embo J*. Dec 15.2003 22:6483. [PubMed: 14657021]
37. Rodley CD, Bertels F, Jones B, O'Sullivan JM. Global identification of yeast chromosome interactions using Genome conformation capture. *Fungal Genet Biol*. Nov.2009 46:879. [PubMed: 19628047]
38. Lieberman-Aiden E, et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science*. Oct 9.2009 326:289. [PubMed: 19815776]
39. Sexton T, et al. Three-dimensional folding and functional organization principles of the Drosophila genome. *Cell*. Feb 3.2012 148:458. [PubMed: 22265598]
40. Duan Z, et al. A three-dimensional model of the yeast genome. *Nature*. May 2.2010 465:363. [PubMed: 20436457]
41. Rodley CD, et al. 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. [PubMed: 22292080]
42. Rodley CD, Pai DA, Mills TA, Engelke DR, O'Sullivan JM. tRNA gene identity affects nuclear positioning. *PLoS ONE*. 2011; 6:e29267. [PubMed: 22206006]
43. Gehlen LR, et al. Chromosome positioning and the clustering of functionally related loci in yeast is driven by chromosomal interactions. *Nucleus*. Jul 1.2012 3
44. Tjong H, Gong K, Chen L, Alber F. Physical tethering and volume exclusion determine higher-order genome organization in budding yeast. *Genome Res*. Jul.2012 22:1295. [PubMed: 22619363]
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 U S A*. Aug 24.2010 107:15117. [PubMed: 20696894]
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*. *Nucleic acids research*. 2000; 28:3524. [PubMed: 10982872]
47. Oakes M, et al. Mutational analysis of the structure and localization of the nucleolus in the yeast *Saccharomyces cerevisiae*. *Journal of Cell Biology*. 1998; 143:23. [PubMed: 9763418]
48. Oakes M, Siddiqi 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. *Molecular and Cellular Biology*. 1999; 19:8559. [PubMed: 10567580]
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*. *Molecular and Cellular Biology*. 2006; 26:6223. [PubMed: 16880531]
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 research*. 2007; 17:184. [PubMed: 17200233]
51. Kobayashi T, Ganley AR. Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. *Science*. Sep 2.2005 309:1581. [PubMed: 16141077]
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 and Development*. 2002; 16:2755. [PubMed: 12414727]
53. Li C, Mueller JE, Bryk M. Sir2 represses endogenous polymerase II transcription units in the ribosomal DNA nontranscribed spacer. *Molecular biology of the cell*. 2006; 17:3848. [PubMed: 16807355]
54. Kermekchiev M, Ivanova L, Ribin, a protein encoded by a message complementary to rRNA, modulates ribosomal transcription and cell proliferation. *Molecular and Cellular Biology*. 2001; 21:8255. [PubMed: 11713263]
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. *Molecular cell*. Aug 28.2009 35:414. [PubMed: 19716787]

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.* May.1995 12:481. [PubMed: 7739390]
57. Gonzalez IL, Sylvester JE. Complete sequence of the 43-kb human ribosomal DNA repeat: analysis of the intergenic spacer. *Genomics.* 1995; 27:320. [PubMed: 7557999]
58. Bartlett J, et al. Specialized transcription factories. *Biochem Soc Symp.* 2006:67. [PubMed: 16626288]
59. Cook PR. Predicting three-dimensional genome structure from transcriptional activity. *Nat Genet.* Nov.2002 32:347. [PubMed: 12410231]
60. Cook PR. The organization of replication and transcription. *Science.* Jun 11.1999 284:1790. [PubMed: 10364545]
61. Pombo A, et al. Regional specialization in human nuclei: visualization of discrete sites of transcription by RNA polymerase III. *EMBO J.* Apr 15.1999 18:2241. [PubMed: 10205177]
62. Cioci F, et al. Silencing in yeast rDNA chromatin: reciprocal relationship in gene expression between RNA polymerase I and II. *Molecular cell.* 2003; 12:135. [PubMed: 12887899]
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. [PubMed: 20038608]
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. *Molecular and Cellular Biology.* 2003; 23:1558. [PubMed: 12588976]
65. Daniels LM, Delany ME. Molecular and cytogenetic organization of the 5S ribosomal DNA array in chicken (*Gallus gallus*). *Chromosome research: an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology.* 2003; 11:305.
66. Wimber DE, Steffensen DM. Localization of 5S RNA genes on *Drosophila* chromosomes by RNA-DNA hybridization. *Science.* Nov 6.1970 170:639. [PubMed: 5470721]
67. Cloix C, et al. Analysis of 5S rDNA arrays in *Arabidopsis thaliana*: physical mapping and chromosome-specific polymorphisms. *Genome Res.* May.2000 10:679. [PubMed: 10810091]
68. Sorensen PD, Frederiksen S. Characterization of human 5S rRNA genes. *Nucleic Acids Res.* Aug 11.1991 19:4147. [PubMed: 1870970]
69. Metzberg RL, Stevens JN, Selker EU, Morzycka-Wroblewska E. Identification and chromosomal distribution of 5S rRNA genes in *Neurospora crassa*. *Proc Natl Acad Sci U S A.* Apr.1985 82:2067. [PubMed: 3157192]
70. Mao J, et al. The 5S RNA genes of *Schizosaccharomyces pombe*. *Nucleic Acids Res.* Jan 22.1982 10:487. [PubMed: 6278416]
71. Fedoriw AM, Starmer J, Yee D, Magnuson T. Nucleolar association and transcriptional inhibition through 5S rDNA in mammals. *PLoS Genet.* Jan.2012 8:e1002468. [PubMed: 22275877]
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.* Jun.1995 129:1181. [PubMed: 7539809]
73. Haeusler RA, Engelke DR. Spatial organization of transcription by RNA polymerase III. *Nucl Acids Res.* Oct 18.2006 34:4826. [PubMed: 16971453]
74. Stutz F, Gouilloud E, Clarkson SG. Oocyte and somatic tyrosine tRNA genes in *Xenopus laevis*. *Genes Dev.* Aug.1989 3:1190. [PubMed: 2792760]
75. Kuhn RM, Clarke L, Carbon J. Clustered tRNA genes in *Schizosaccharomyces pombe* centromeric DNA sequence repeats. *Proc Natl Acad Sci U S A.* Feb 15.1991 88:1306. [PubMed: 1996332]
76. Scott KC, Merrett SL, Willard HF. A heterochromatin barrier partitions the fission yeast centromere into discrete chromatin domains. *Curr Biol.* Jan 24.2006 16:119. [PubMed: 16431364]
77. Dunham MJ, et al. Characteristic genome rearrangements in experimental evolution of *Saccharomyces cerevisiae*. *PNAS.* 2002; 99:16144. [PubMed: 12446845]
78. McFarlane RJ, Whitehall SK. *tRNA* genes in eukaryotic genome organization and reorganization. *Cell Cycle.* 2009; 8:3102. [PubMed: 19738425]

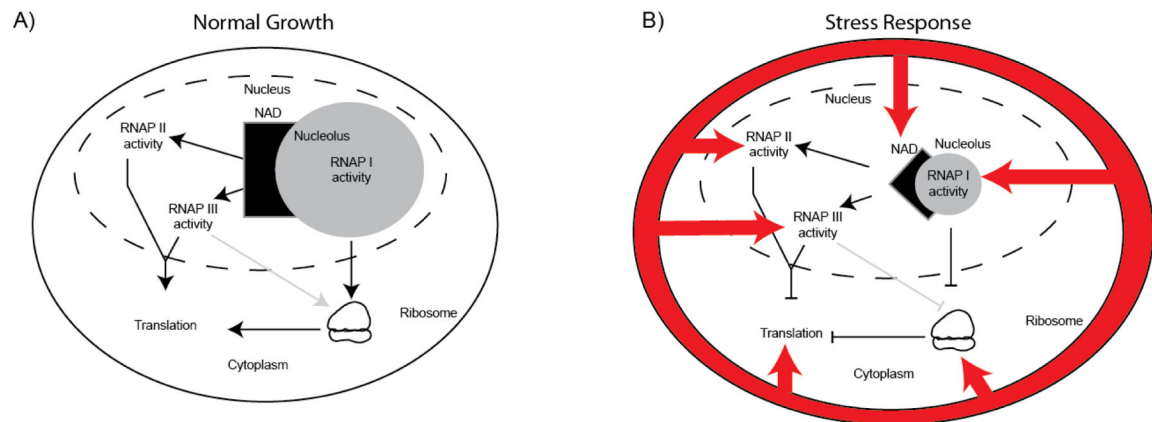
79. Thompson M, Haeusler RA, Good PD, Engelke DR. Nucleolar clustering of dispersed tRNA genes. *Science*. Nov 21.2003 302:1399. [PubMed: 14631041]
80. Wang L, et al. Silencing near tRNA genes requires nucleolar localization. *J Biol Chem*. Mar 11.2005 280:8637. [PubMed: 15654076]
81. Haeusler RA, Pratt-Hyatt 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*. Aug 15.2008 22:2204. [PubMed: 18708579]
82. Iwasaki O, Tanaka A, Tanizawa H, Grewal SI, Noma K. Centromeric localization of dispersed Pol III genes in fission yeast. *Mol Biol Cell*. Jan 15.2010 21:254. [PubMed: 19910488]
83. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res*. Mar 1.1997 25:955. [PubMed: 9023104]
84. Kramerov DA, Vassetzky NS. Short retroposons in eukaryotic genomes. *International Journal of Cytology*. 2005; 247:165.
85. Deininger PL, Batzer MA. Mammalian retroelements. *Genome Res*. Oct.2002 12:1455. [PubMed: 12368238]
86. Belancio VP, Hedges DJ, Deininger P. Mammalian non-LTR retrotransposons: for better or worse, in sickness and in health. *Genome Res*. Mar.2008 18:343. [PubMed: 18256243]
87. Kaplan FS, et al. The topographic organization of repetitive DNA in the human nucleolus. *Genomics*. Jan.1993 15:123. [PubMed: 8432523]
88. Pai DA, Engelke DR. Spatial organization of genes as a component of regulated expression. *Chromosoma*. Feb.2010 119:13. [PubMed: 19727792]
89. Moqtaderi Z, et al. Genomic binding profiles of functionally distinct RNA polymerase III transcription complexes in human cells. *Nat Struct Mol Biol*. May.2010 17:635. [PubMed: 20418883]
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*. *Journal of Biological Chemistry*. 1998; 273:5023.
91. Hernandez-Verdun D. Assembly and disassembly of the nucleolus during the cell cycle. *Nucleus*. May-Jun;2011 2:189. [PubMed: 21818412]
92. Drosopoulou E, et al. Sex chromosomes and associated rDNA form a heterochromatic network in the polytene nuclei of *Bactrocera oleae* (Diptera: Tephritidae). *Genetica*. Jul 24.2012
93. Peng JC, Karpen GH. H3K9 methylation and RNA interference regulate nucleolar organization and repeated DNA stability. *Nature Cell Biology*. 2007; 9:25. [PubMed: 17159999]
94. Paredes S, Maggert KA. Ribosomal DNA contributes to global chromatin regulation. *PNAS*. 2009; 106:17829. [PubMed: 19822756]
95. Zhang LF, 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. [PubMed: 17512404]
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. [PubMed: 18120749]
97. Nemeth A, et al. Initial genomics of the human nucleolus. *PLoS Genet*. Mar.2010 6:e1000889. [PubMed: 20361057]
98. O'Sullivan JM, Sontam DM, Grierson R, Jones B. Repeated elements coordinate the spatial organization of the yeast genome. *Yeast*. Feb.2009 26:125. [PubMed: 19235779]
99. van Koningsbruggen S, et al. High-resolution whole-genome sequencing reveals that specific chromatin domains from most human chromosomes associate with nucleoli. *Mol Biol Cell*. Nov 1.2010 21:3735. [PubMed: 20826608]
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 U S A*. Mar 16.2010 107:4991. [PubMed: 20194778]
101. Postow L, Hardy CD, Arsuaga J, Cozzarelli NR. Topological domain structure of the *Escherichia coli* chromosome. *Genes Dev*. Jul 15.2004 18:1766. [PubMed: 15256503]

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.* Jun.2006 60:1164. [PubMed: 16689793]
103. Valens M, Penaud S, Rossignol M, Cornet F, Boccard F. Macrod domain organization of the *Escherichia coli* chromosome. *EMBO J.* Oct 27.2004 23:4330. [PubMed: 15470498]
104. Espeli O, Mercier R, Boccard F. DNA dynamics vary according to macrodomain topography in the *E. coli* chromosome. *Mol Microbiol.* Jun.2008 68:1418. [PubMed: 18410497]
105. Nguyen HH, et al. The essential histone-like protein HU plays a major role in *Deinococcus radiodurans* nucleoid compaction. *Mol Microbiol.* Jul.2009 73:240. [PubMed: 19570109]
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.* Apr 17.2009 387:1261. [PubMed: 19254726]
107. Skoko D, et al. Mechanism of chromosome compaction and looping by the *Escherichia coli* nucleoid protein Fis. *J Mol Biol.* Dec 8.2006 364:777. [PubMed: 17045294]
108. Boccard F, Esnault E, Valens M. Spatial arrangement and macrodomain organization of bacterial chromosomes. *Molecular Microbiology.* 2005; 57:9. [PubMed: 15948945]
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.* Jul.2009 191:4180. [PubMed: 19395497]
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.* Jun.2006 188:4007. [PubMed: 16707692]
111. Jin DJ, Cagliero C, Zhou YN. Growth rate regulation in *Escherichia coli*. *FEMS Microbiol Rev.* Mar.2012 36:269. [PubMed: 21569058]
112. Liao D. Gene conversion drives within genic sequences: concerted evolution of ribosomal RNA genes in Bacteria and Archaea. *Journal of Molecular Evolution.* 2000; 51:305. [PubMed: 11040282]
113. Spriggs KA, Bushell M, Willis AE. Translational regulation of gene expression during conditions of cell stress. *Mol Cell.* Oct 22.2010 40:228. [PubMed: 20965418]
114. Suzuki A, et al. A new PICTure of nucleolar stress. *Cancer science.* Apr.2012 103:632. [PubMed: 22320853]
115. Rubbi CP, Milner J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J.* Nov 17.2003 22:6068. [PubMed: 14609953]
116. Kobayashi T. A new role of the rDNA and nucleolus in the nucleus—rDNA instability maintains genome integrity. *Bioessays.* 2008; 30:267. [PubMed: 18293366]



**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 intergenic spacer amongst eukaryotes. Individual rDNA repeats are usually arranged into arrays of tandem as illustrated.



**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 RNAPII and RNAPIII 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 while 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 signalling to the RNAP II and RNAP III transcription and subsequently effect a change in cytoplasmic translation.