Beyond the mammalian nucleolus—a comparative perspective on nucleolar dynamics in stress and through the life cycle

Ricardo Carvalho[1], Elizaveta Gubanova[1] and Gautam Dey[1*]

[1]Cell Biology and Biophysics, European Molecular Biology Laboratory, Meyerhofstrasse 1, 69117 Heidelberg, Germany

[*]Correspondence to: gautam.dey@embl.de

Abstract

Classically the site of ribosomal RNA transcription, processing and early ribosome assembly, the nucleolus is increasingly understood as a dynamic multiphase condensate reshaped by cell state. Most reviews of this plasticity are mammal-centred. Here we set mammals alongside fungi, plants and animal germlines, following the nucleolus through the life cycle under environmental change and through the programmed transitions of mitosis and meiosis. Under stress it acts in protein quality control, sequestration and transcriptional regulation. In mitosis, fungi partition a persistent nucleolus, unlike the dissolve-and-rebuild strategy of animal open mitosis. In meiosis, nucleolar fate diverges between the sexes: lost during spermatogenesis in mammals and amphibians, cycling through prenucleolar bodies in arthropods, and persisting through oogenesis to predict meiotic competence. Across all three states the same tasks recur: reducing rRNA output, managing rDNA as a specialised chromosomal domain, coupling nucleolar material to division machinery, and controlling the granular component, met differently across lineages. We argue that nucleolar identity is defined by functional logic rather than fixed morphology, and outline open questions for extending this comparative approach across the breadth of eukaryotic diversity.

Introduction

The nucleolus is the largest biomolecular compartment in the nucleus and is classically defined as the site of ribosomal RNA transcription, processing, and early ribosome subunit assembly. It forms around ribosomal DNA (rDNA) repeats and is built from a changing mixture of rDNA, nascent and processing pre-rRNAs, ribosomal proteins, assembly factors, and many other RNA-binding and regulatory proteins. In mammalian cells, this organisation is often described in terms of fibrillar centres (FCs), the dense fibrillar component (DFC), and the granular component (GC), which broadly correspond to different steps of rRNA synthesis and ribosome biogenesis. Although fungal nucleoli are often less sharply defined by ultrastructure, the same basic functional logic applies: nucleolar organisation is tightly linked to rDNA activity, RNA polymerase I function, pre-rRNA processing, and the ordered maturation of pre-ribosomal particles [1–4]. In this sense, the nucleolus is not simply a place where ribosome biogenesis happens. It is a compartment whose structure is produced and maintained by the reactions taking place within it.

This canonical biosynthetic role remains central, but it does not fully capture the place of the nucleolus in nuclear organisation. Work over the last decades has shown that the nucleolus also contributes to broader aspects of genome architecture, spatial chromatin organisation, and the regulated sequestration or release of selected factors, placing it within a wider network of nuclear control [4–7].

The nucleolus is now widely treated as a multiphase condensate whose internal organisation reflects the flow of rRNA and ribonucleoprotein intermediates through distinct but coupled functional states, helping explain how composition, material properties, and function remain linked across space and time [4,8,9]. These ideas are especially useful because they frame the nucleolus not as a fixed object with stable internal parts, but as a responsive compartment whose architecture changes together with its output.

The dynamic nature of the nucleolus becomes especially clear across the life cycle of the cell. In interphase, under favourable growth conditions, it supports sustained rRNA synthesis and ribosome production. Such steady conditions are rarely sustained for long outside the laboratory: natural environments fluctuate continuously, unlike the controlled stability of an incubator, so some degree of perturbation is the norm rather than the exception for most cells. However, under stress, the nucleolus is an environmentally responsive compartment that can rapidly remodel its organisation in terms of rDNA conformation, transcriptional output, RNA processing, and protein handling pathways [6,10–13]. During mitosis, these changes become especially striking. In many mammalian cells undergoing open mitosis, the nucleolus disassembles as mitosis proceeds and is rebuilt after chromosome segregation and mitotic exit [1,3,14,15]. In many fungi, by contrast, division occurs without complete nuclear breakdown, and the nucleolus remains visible and must be remodelled and physically partitioned rather

than fully dissolved [16–19]. Meiosis reveals additional forms of nucleolar plasticity, again linking nucleolar behaviour to chromosome organisation, nuclear architecture, and developmental state. Taken together, these observations argue that nucleolar dynamics should be treated as a central part of cell-cycle control and cell-state transitions.

Most general discussions of nucleolar dynamics have, understandably, focused on mammalian systems, where the sequence of mitotic disassembly and reassembly, together with the links between nucleolar stress and signalling pathways such as p53 control, have been studied in greatest detail and reviewed extensively elsewhere [1,3,4,14,15]. Setting these systems alongside others is informative precisely because the mammalian solution is not the only one available. Fungi are particularly useful here, because in this clade, nucleolar dynamics are often exposed not as a problem of disassembly and reassembly but as a problem of active inheritance. In budding yeast, for example, the nucleolus persists through closed mitosis and must be divided while remaining organised around a long repetitive rDNA array that segregates late and is subject to constraints imposed by ongoing transcription, DNA topology, chromosome compaction, and contacts with the nuclear envelope. More broadly, fungal systems show that the same basic task, the faithful transmission of nucleolar material and rDNA across division, can be solved through different cellular geometries and regulatory circuits. Plants and animal germlines contribute further solutions, particularly in meiosis, where nucleolar fate can differ between the two sexes of a single species.

Our goal in this review is not to survey the full diversity of nucleolar organisation across all eukaryotic lineages, which is far too broad a subject and has been treated in detail elsewhere [20,21]. Instead, we follow the nucleolus through the life cycle in a deliberately comparative way, asking how one compartment is remodelled by demands of two different kinds: environmental change, which is unpredictable and acute, and the programmed transitions of mitosis and meiosis. Three questions run through the review: how environmental change remodels nucleolar function and material state; how rDNA organisation shapes nucleolar behaviour; and how nucleolar components are reorganised, partitioned, dispersed, or rebuilt as cells pass through different division programmes. Read this way, the non-mammalian literature is more than a counterpart to mammalian-centred reviews. It provides a mechanistic basis for asking how a conserved nuclear compartment preserves core biosynthetic functions while remaining flexible enough to respond to a changing intracellular and extracellular environment.

The nucleolar stress response

One of the most consistently reported triggers of nucleolar reorganisation in interphase cells is stress (Fig. 1), although the relationship has so far been characterised mostly in metazoan systems. Understanding it more broadly will require integrating observations from across the eukaryotic tree in order to identify general patterns in the nucleolar stress response.

Given the differences in nucleolar organisation between lineages, the physiological changes seen in the nucleolus during stress also appear distinct at first glance. Under acute heat stress, the *S. cerevisiae* nucleolus shrinks and fragments into scattered granules [22], a contraction also seen in *S. pombe* and in some plants [23,24]. By contrast, mammalian nucleoli can expand upon heat stress, later forming amyloid-like structures [25–27]. In addition, nucleolar compartments in several species become less well defined and are disrupted [26,28,29].

However, despite these differences, nucleolar dynamics appear to converge at the functional level, even though they are achieved through different molecular mechanisms: the nucleolus stores misfolded proteins pending their rescue, holds other proteins in order to restrict their activity, hosts regulatory factors that slow ribosome biogenesis, and releases its own proteins to modulate the stress response.

Rescue

Under stress, the nucleolus acts as a site for protein quality control. Thermosensitive proteins from the nucleoplasm and the nucleolus enter the liquid-like granular component and bind resident proteins, which stabilise and immobilise the misfolded species while themselves becoming less mobile, allowing Hsp70-dependent refolding. Prolonged stress, however, leads to irreversible amyloid-like aggregation [27]. Another study suggests that this amyloid state is instead physiological and reversible, actively induced by low-complexity non-coding RNA transcribed from intergenic spacers in NORs [26,30].

In *S. cerevisiae* during heat stress, some nuclear and cytoplasmic misfolded proteins accumulate in the intranuclear quality control compartment (INQ). Transport from the cytoplasm occurs via the Hsp70 co-chaperone Sis1 and the aggregase Btn2 [31,32]. Interestingly, these proteins do not appear to share any common feature other than solubility [33]. They are later disaggregated with the help of Hsp104 [32]. Although the INQ is not in direct contact with the nucleolus, it is consistently found in the nucleolar region [34], resembling other Sis1-dependent condensates at the nucleolar periphery that consist of orphaned ribosomal proteins [35]. Besides preserving proteins, these foci sequester Sis1, which

leads to the release of Hsf1 and activation of the heat shock response [36,37].

Similarly, *S. pombe* forms a ring of reversibly aggregated nuclear and nucleolar proteins around its nucleolus, which requires Hsp70-Hsp104 disaggregase for resolution [24,38,39]. Moreover, in various stresses its nucleolus also hosts poly(A) RNA [40–42].

Restriction

Sequestration of proteins in the nucleolus is also used to restrict their activity. In mammalian cells, stress-specific lncRNAs transcribed from intergenic spacers sequester proteins carrying a nucleolar detention sequence [43]. Among them are MDM2 and VHL, which lose their ubiquitin-ligase activity while detained during acidosis, helping to modulate the stress response [44].

Likewise, the *S. pombe* rings contain numerous mRNA transport factors and cell cycle regulators such as Clp1, Cdc5, Ark1, Cdc13 and Plo1 [24]. While Clp1 and Cdc13 are normally found in the nucleolus, the others do not usually reside there [46]. This suggests that the nucleolus modulates cell cycle progression and the stress response by actively trapping specific regulators from the rest of the cell.

Regulation

Since the nucleolus is primarily responsible for ribosome biogenesis, stress conditions inevitably lead to its downregulation, which also often requires the sequestration of various proteins. The VHL protein mentioned above also restricts rRNA synthesis under hypoxia [45], while the CHD4/NuRD complex does so under hypotonic and heat stress [47,48]. CHD4/NuRD has histone-modifying and ATP-dependent chromatin remodelling activities, and it is recruited to the nucleolus via another long non-coding RNA, PAPAS (promoter and pre-rRNA antisense), tethered to DNA [47,48]. Similarly, in *Drosophila* the piRNA-pathway protein Piwi is sequestered from the nucleoplasm to the nucleolus during heat stress, presumably by as-yet-unidentified transcripts, where it may regulate the transposable elements located there [49].

In budding yeast, rDNA silencing may follow from the sequestration of TORC1, which regulates rDNA accessibility, into cytosolic stress granules [51]. TORC1 inhibition causes condensation of the rDNA array (*RDN1*, chromosome XII) in metaphase-arrested cells, which has been proposed to slow rRNA synthesis [50].

Release

The nucleolus, as rRNA production slows, also gains a pool of orphaned ribosomal proteins, which it releases to regulate the cell cycle. For example, in mammalian cells undergoing nucleolar disruption, such as after UV damage, some ribosomal proteins exit the nucleolus and cause cell cycle arrest and apoptosis via p53 stabilisation [52–56]. Although no studies have addressed heat stress directly, there is evidence for both nucleolar disruption and p53 accumulation during it [57,58], which suggests that, as in budding yeast, orphaned ribosomal proteins may contribute to activation of the stress response, albeit by a different mechanism.

Taken together, these examples show that, beyond ribosome biogenesis, the nucleolus is deeply integrated into stress response pathways during interphase (Fig. 1). Nucleolar stress in mammalian cells is closely studied, given its links to diseases ranging from cancer [59] to neurodegeneration [60]. The specific molecular routes, however, appear not to be broadly conserved, and although comparatively little is known about nucleolar dynamics in other eukaryotes, particularly beyond model organisms, current evidence suggests that similar functional outcomes can arise through different molecular mechanisms rather than through a single conserved pathway.

This leaves open the question of whether there are shared underlying principles defining nucleolar behaviour during stress, and whether the nucleolus actively contributes to cellular stress response, or whether it is passively affected by global stress-induced changes.

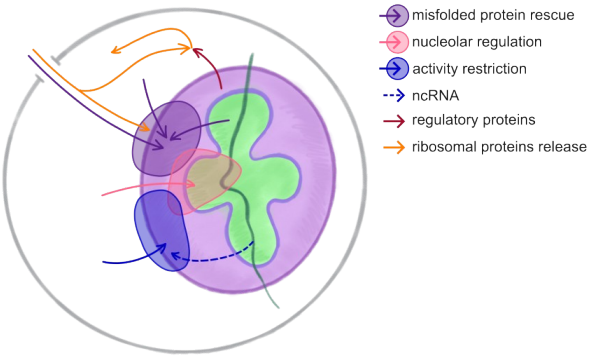

Fig. 1. Generalised scheme of reported stress-related protein rearrangements in discussed species. Dark green – rDNA, lime – FC, lavender – DFC, purple – GC, grey – nuclear envelope.

Nucleolar dynamics in mitosis

Across eukaryotes, mitosis forces a basic choice in nucleolar behaviour. In many animal cells undergoing open mitosis, the nucleolus vanishes as mitosis progresses and is rebuilt after chromosome segregation [1,3,4,14,15]. In contrast, many fungi carry out mitosis with an intact nucleus, and the nucleolus remains visible and must be physically partitioned rather than dismantled [16–19]. These two regimes shift the main problem to be solved. If the nucleolus disassembles, the challenge is controlled dissolution and faithful post-mitotic reassembly at the rDNA loci. If it persists, the challenge is to remodel and split a nucleolar domain built around rDNA while chromosomes segregate.

Work in human cells shows that nucleolar disassembly is an ordered event. Live-cell analyses indicate that FC-associated Pol I components dissociate first, whereas DFC and GC markers redistribute later, closely aligned with loss of nuclear compartmentalisation and nuclear envelope breakdown [4,14,61]. Many of the ribosome biogenesis and nucleolar scaffolding proteins relocate to chromosome-associated and perichromosomal material during mitosis and later pass through prenucleolar bodies (PNBs) before rejoining reforming nucleoli [1,3,4,14,15,62,63]. Nucleolar reassembly is likewise staged: rDNA transcription restarts at NORs in telophase, while processing factors are initially organised in PNBs and are then recruited to reform FC/DFC/GC organisation in early G1 [1,4,14,61,62].

Because mammalian mitotic nucleolar dynamics are discussed elsewhere [1,3,4,14,15], here we will focus on fungi, where genetics and imaging experiments have mapped how rDNA, nucleolar components, the nuclear envelope, and the spindle are coordinated to partition a persistent nucleolus during mitosis [17,19,64–66].

Nucleolar division in *S. cerevisiae*

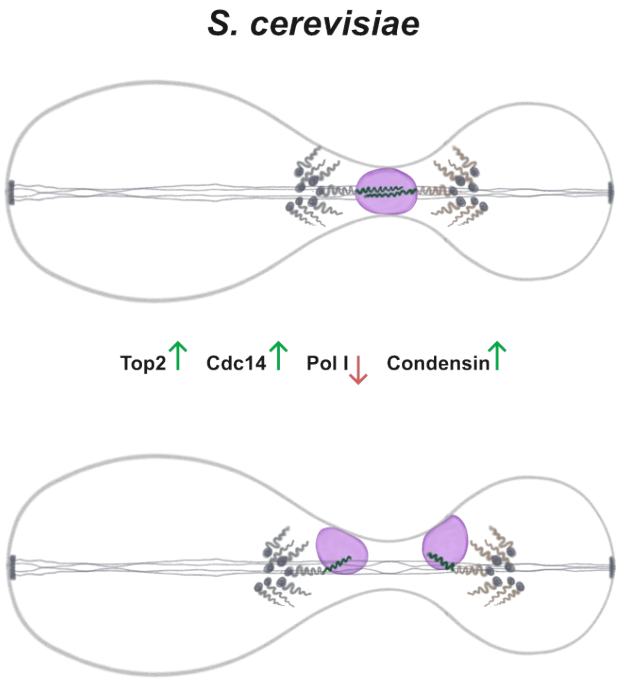

Fig. 2. Mitotic partitioning of the rDNA–nucleolar domain in *Saccharomyces cerevisiae*. During anaphase, the rDNA array and associated nucleolar material are among the last nuclear domains to segregate. Their separation requires coordinated changes in rDNA topology, transcription and chromosome organisation. Top2-dependent resolution of DNA linkages, Cdc14 release, reduced RNA polymerase I (Pol I) transcription and condensin-dependent compaction promote the separation of the rDNA array and partitioning of the associated nucleolar material between the mother and daughter nuclei. Green and red arrows indicate increased and decreased activity or association, respectively. Purple – nucleolus, dark green – rDNA, grey and brown – chromosomes, NE, spindle, kinetochores.

In *S. cerevisiae*, the nucleolus remains present throughout closed mitosis and must be divided rather than disassembled (Fig. 2) [16–18]. This is non-trivial because the nucleolus is built around a very long rDNA repeat array on chromosome XII, which behaves as a distinct chromosomal domain [16,17,65]. In addition, the nucleolus has liquid–liquid phase-separated properties, and division of one droplet into two is not thermodynamically spontaneous but a regulated process that requires active inputs.

Imaging studies show that a defining feature of budding yeast mitosis is that segregation of the rDNA and associated nucleolar markers lags behind much of the genome [16,17,65,67]. In metaphase, rDNA can appear as an extended structure, often positioned apart from bulk chromatin [17,18]. Tracking of multiple loci shows that markers within the rDNA array segregate late, whereas flanking regions can segregate on time [17,68]. Morphologically, the rDNA transitions from an extended loop-like structure to a compact mass and then to a stretched bar aligned with the spindle axis before splitting into two separated bodies [17,68,69]. Nucleolar proteins show similarly delayed dynamics relative to non-rDNA chromosomal loci [16,18].

Mechanistically, two classes of constraints are at play during segregation itself. First, the rDNA is long and prone to persistent DNA intertwines after replication, so efficient separation requires Top2 activity in anaphase [64,65,67,68]. Second, high transcription and co-transcriptional ribosome assembly at rDNA oppose shortening and condensin occupancy [68–70]. When transcription remains high, condensin binding is reduced and rDNA fails to shorten efficiently [69–72]. Together, these constraints make rDNA a “last-to-segregate” region whose completion can define the end of mitosis (Fig. 2) [16,17,68,69].

A central event that helps relieve these constraints is the regulated release and activation of Cdc14, which is sequestered in the nucleolus by Net1 as part of the RENT complex [64,65,73–75]. At anaphase onset, FEAR provides an early trigger for Cdc14 release, while MEN later ensures completion if early steps are delayed [17,64,66,76,77]. Functionally, Cdc14 activation gates entry into an anaphase remodelling programme at rDNA: condensin becomes enriched at the nucleolar/rDNA region, and rDNA condensation and segregation proceeds [64–66,78].

At the same time, high Pol I transcription is not compatible with efficient rDNA shortening and separation, and this constraint must be relieved [68,70,71,79]. Experiments that reduce rRNA synthesis improve rDNA segregation in condensin- or Cdc14-compromised backgrounds

[68,70,79]. In addition, Cdc14 inhibits Pol I transcription during anaphase, linking mitotic phosphoregulation to reduced transcriptional output from rDNA [80]. A stepwise model is therefore supported by the literature: anaphase entry triggers Cdc14 activation (via Net1 release and FEAR/MEN timing), Cdc14 promotes condensin enrichment at rDNA, condensin drives rDNA condensation/shortening, and Top2 resolves remaining DNA linkages so sisters can separate [64–66,71,78]. In parallel, transcription downshift contributes both to reducing rDNA-associated constraints and to improving condensin binding [68–72].

This division programme operates within a defined architectural context at the nuclear periphery. In *S. cerevisiae*, the rDNA is tethered to the inner nuclear membrane through the CLIP–cohibin system, linking the rDNA/nucleolar domain to INM proteins including Nur1 and Heh1/Src1 [81–84]. Importantly, this linkage is regulatable: TORC1 inactivation reveals CLIP–cohibin-dependent separation of rDNA from nucleolar proteins [82,83], and rDNA breaks trigger phosphorylation/SUMOylation events that promote Cdc48/Ufd1-dependent disassembly of CLIP–cohibin [84]. Nur1 is also a cell-cycle-regulated phosphorylation target of Cdk1 and is opposed by Cdc14, providing a direct route by which mitotic control can tune rDNA–NE coupling [84,85]. Together, these studies establish that the rDNA/nucleolar domain is both positioned and mechanically constrained by NE contacts, and that those contacts can be actively remodelled.

After segregation, the two daughter nuclei re-establish nucleolar architecture and resume ribosome biogenesis, visible as restoration of a nucleolar domain around the rDNA in each daughter nucleus [16–18].

Nucleolar inheritance in other fungi

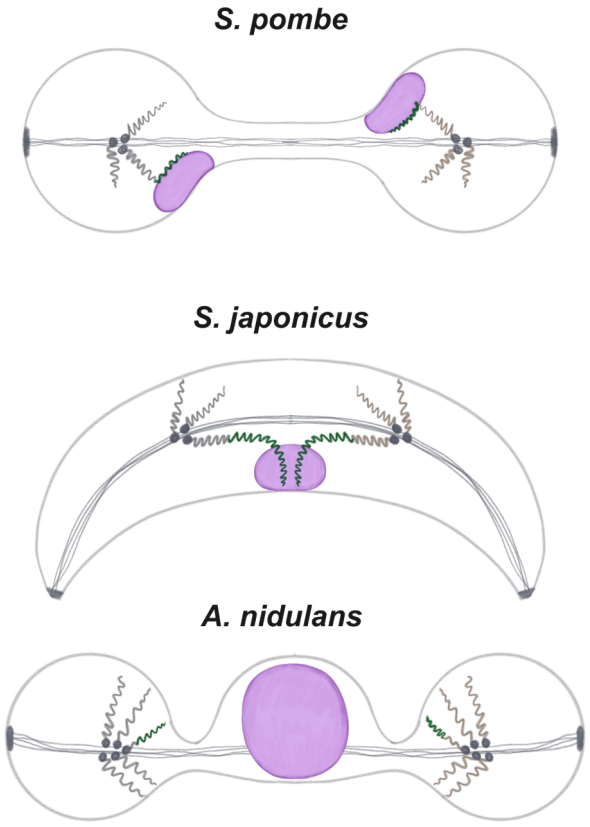

Fig. 3. Distinct modes of nucleolar inheritance during fungal mitosis. In *Schizosaccharomyces pombe*, closed mitosis preserves nuclear compartmentalisation, and the rDNA-associated nucleolar domain is divided between the two daughter nuclei. In *Schizosaccharomyces japonicus*, rDNA undergoes late-anaphase compaction and separation during a division programme that includes nuclear-envelope rupture; nucleolar material is transiently dispersed and subsequently rebuilt. In *Aspergillus nidulans*, partially open mitosis permits chromosome segregation while nucleolar material persists transiently as a central remnant between the separating chromatin masses before daughter nucleoli are re-established. Purple – nucleolus, dark green – rDNA, grey and brown – chromosomes, NE, spindle, kinetochores. Schematics are not to scale.

In other fungi, the same core problems must be solved: partitioning rDNA, handling the nucleolar protein mass, and coordinating these events with the division machinery (Fig. 3). However in these systems the geometry and control logic differ.

In *S. pombe*, mitosis is also closed, yet the regulatory wiring around rDNA is different. rDNA resides within H3K9-methylated chromatin sustained by heterochromatin pathways, providing an alternative way to stabilise the repeat array [86–88]. Consistent with this divergence, the budding-yeast FEAR→Cdc14 logic is not conserved in the same way: FEAR orthologues are not required for release of the Cdc14-family phosphatase Clp1/Flp1 from the nucleolus at mitotic entry, and *clp1Δ* cells segregate the nucleolus without any delay [89].

Distinct mitotic networks still control nucleolus-associated regulators in fission yeast; for example, SIN signalling regulates localisation of Clp1/Flp1 [89]. In parallel, the mechanics of nuclear division in *S. pombe* are tightly linked to membrane growth and lipid control, with nuclear envelope expansion required for proper chromosome/nuclear division [90–92]. This provides a clear example of "same task, different implementation," combining heterochromatin-based rDNA control with NE remodelling and distinct phosphoregulation.

In *S. japonicus*, the geometry differs more strongly, as nuclear-envelope rupture and rebuilding are part of the division programme [90,93,94]. Yam et al. (2013) show that rDNA remains embedded within the nucleolar mass through most of anaphase, then undergoes late-anaphase axial compaction and exits the nucleolar mass shortly before rapid dispersal of nucleolar signal [94]. Nucleoli then reform in the newly formed nuclei as the NE re-establishes its integrity [90,93]. Strikingly, the INM protein and orthologue of *S. cerevisiae*'s Heh1, Man1 contributes to symmetric nucleolar inheritance; when Man1 is perturbed, nucleolar material is frequently inherited unequally by the two daughter nuclei [90,94]. In this system, nucleolar inheritance is therefore tightly coupled to NE remodelling and a late rDNA remodelling step.

A. nidulans provides another example where nucleolar material is remodelled into intermediate states during division. Live imaging shows that nucleolar material becomes spatially uncoupled from separating chromatin masses and can persist transiently between them before nucleolar organisation is re-established in daughter nuclei [19,95]. This occurs in a "partially open" mitosis created by partial NPC disassembly: core pore structures remain at the NE, while peripheral nucleoporins disperse and regulated nuclear transport is transiently suppressed [96–98]. In addition, the INM protein MtgA influences nucleolar morphology and FC/GC arrangement, linking NE composition to nucleolar shape during division [99]. This division mode therefore resembles *S. japonicus*, in which nucleolar material is transiently reorganised and then rebuilt, rather than the *S. cerevisiae*/*S. pombe* pattern where nucleolar partitioning is largely achieved by splitting the rDNA-associated nucleolar domain inside an intact nucleus.

In *C. albicans*, imaging studies highlight persistent nucleolus–NE proximity and a tight spatial relationship to the spindle in both yeast and filamentous growth forms [100–103]. While mechanistic wiring is less well defined in these studies, the geometry supports the idea that physical coupling of the nucleolus to division-driving structures can be especially strong in this system.

Shared mitotic requirements across eukaryotes

Despite the clear difference between "dissolve and rebuild" (many animals) and "partition a persistent nucleolus" (many fungi), the same core requirements recur across systems. The main difference is the route used to satisfy each requirement.

1) Reduce rRNA gene output and remodel nucleolar activity at the right time

In budding yeast, high Pol I transcription and ongoing co-transcriptional assembly at rDNA oppose rDNA shortening and segregation. Experimental reduction of rRNA synthesis improves segregation in backgrounds compromised for key mitotic regulators [68,70,79]. In addition, in *S. cerevisiae*, anaphase regulation includes direct inhibition of Pol I transcription by Cdc14 [80]. In mammals, rDNA transcription is shut down as cells enter mitosis, an event linked to CDK1-dependent regulation of Pol I initiation factors [3,4,14,15]. Together, data from fungi and animals supports the idea that mitosis requires a coordinated downshift in rDNA transcriptional output, even though the regulatory logic might differ.

2) Make rDNA physically separable in the context of chromosome segregation

In *S. cerevisiae*, the rDNA array is a late-segregating domain whose separation requires condensin-driven compaction and Top2-dependent resolution of DNA linkages [64–66,71,78]. In fission yeast, condensin recruitment to rDNA is wired differently but remains essential for proper rDNA behaviour [104,105]. In mammals, rDNA remains anchored at NORs on mitotic chromosomes while nucleolar components redistribute, and nucleolar reassembly is organised around NORs when transcription restarts [1,4,14,61,62]. These observations support a shared requirement to control the rDNA locus as a specialised chromosomal region through mitosis, whether by compaction and segregation within an intact nucleus (fungi) or by maintaining NOR identity during nucleolar dissolution and reassembly (animals).

3) Couple nucleolar material to division-driving structures and nuclear architecture

In fungi with persistent nucleoli, coupling to the spindle, chromosomes, and the nuclear envelope is central because nucleolar material must be physically partitioned during nuclear division. In *S. cerevisiae*, segregation of the rDNA/nucleolar domain occurs late and in defined morphologies aligned to spindle elongation [17,68,69]. In *S. japonicus*, nucleolar inheritance is embedded in a rupture-and-rebuild division programme, and rDNA shows late-anaphase remodelling followed by rapid dispersal of nucleolar signal [90,93,94]. In *A. nidulans*, nucleolar material can persist transiently between separating chromatin masses during a partially open mitosis with partial NPC disassembly, and INM

composition can influence FC/GC arrangement [19,96–99]. In mammalian open mitosis, nucleolar components redistribute to mitotic chromosome-associated material and later pass through PNBs, placing the chromosome surface and post-mitotic nuclear import at the centre of nucleolar reassembly [1,4,14,15,61–63].

4) Control the GC as a physical and functional entity during partitioning and reassembly

Across fungi, GC behaviour differs: in some systems nucleolar material largely tracks the rDNA-associated territory, whereas in others it disperses or persists as a remnant and is then rebuilt [16,19,69,94,95]. Mammalian mitosis provides a complementary view in which GC components show defined kinetics of disassembly and a delayed return during reassembly, with GC formation later than FC/DFC in the reconstruction sequence [1,4,14,61,62]. Biophysical work in mammalian cells further shows that nucleolar disassembly is preceded by measurable changes in GC material state, including increased mobility and reduced effective interaction strength, and a drop in surface tension and bending rigidity before rapid dissolution at nuclear envelope breakdown [106]. More broadly, nucleolar material properties are linked to rRNA flux and the progression of ribosome maturation, defining distinct biophysical states [4,107,108]. In fungi, Rio1 in *S. cerevisiae* and Ani1/Fkbp39 in *S. pombe* provide examples in which regulators of rRNA processing and pre-ribosome handling influence nucleolar state in ways consistent with a coupling between ribosome maturation and GC behaviour [72,109].

Taken together, these comparisons suggest that mitotic nucleolar dynamics can be described by a shared list of tasks: (i) reduce rDNA transcriptional output, (ii) manage rDNA as a specialised chromosomal domain, (iii) coordinate nucleolar material with the division machinery and nuclear architecture, and (iv) tune the GC so that nucleolar material can either be partitioned directly or redistributed and rebuilt. Fungi emphasise the “partition” solution, whereas many animals emphasise a “dissolve and rebuild” solution, but both satisfy the same underlying requirements using different cellular geometry and control logic [1,4,15,17,61–63,94,106].

Meiotic dynamics

Sexual reproduction involves two opposite processes – halving cell ploidy via meiosis to form gametes and restoring it via gamete fusion. Meiosis consists of two rounds of divisions with the same stages as mitosis, and results in formation of four haploid cells from one diploid.

Chromosome pairing during prophase I

The longest and most elaborate stage is prophase I, which is subdivided into several substages (Fig. 4). At leptotene, chromosomes condense, double-strand breaks for crossover are introduced, and the synaptonemal complex begins to form as homologous chromosomes approach each other. The transition to zygotene is often marked by bouquet formation, in which telomeres cluster at the nuclear envelope. Synapsis is completed at zygotene, and at pachytene all double-strand breaks are repaired, producing crossovers. Finally, at diplotene the synaptonemal complex disassembles and homologous chromosomes remain linked only through chiasmata.

When the bouquet forms, nucleoli follow the NOR-bearing chromosomes to the nuclear envelope and fuse, physically connecting them [110–112]. This connection remains between homologous pairs even after the bouquet disassembles [113,114].

The nucleolus also promotes pairing when the bouquet is absent: in *Arabidopsis*, initial telomere pairing occurs through the nucleolus [115], and telomere release at leptotene is an active process requiring ASK1, a component of the SCF ubiquitin-ligase complex [116], together with SUN1 and SUN2, two components of the LINC (linker of nucleoskeleton and cytoskeleton) complex [117]. NOR-bearing chromosomes retain their ability to pair even when nucleus-wide pairing stabilisation is disrupted [118]. In rice, telomeres also associate with the nucleolus during pairing [119].

Another non-trivial task is to pair the X and Y chromosomes. In *Drosophila* males, this pairing is mediated by shared rDNA, through either sequence repetition or transcriptional activity [120,121].

In *S. pombe*, which lacks a synaptonemal complex, robust chromosome pairing is achieved with the help of phase-separated lncRNA–protein complexes; although these are structures distinct from the nucleolus, the nucleolus has also been proposed to contribute to pairing [122–125].

After prophase I

Further nucleolar fate varies not only across species but also between the sexes within a single species. During spermatogenesis, nucleolar size and rRNA synthesis peak around mid-pachytene before declining from late pachytene onward, and the nucleolus fragments; it has been proposed, on the basis of correlated timing and morphology, that some of this material contributes to the chromatoid body that forms in early spermatids [126,127] – a link also described in the frog *Dendropsophus minutus* [128] and reviewed across mammals [129]. In some arthropods, by contrast, nucleolar material persists by cycling through prenucleolar bodies (PNBs): it

disperses from metaphase I to telophase I, when PNBs coat the chromatin surface [130]; these coalesce into transient nucleoli at interkinesis, disperse again at prophase II, and reappear at telophase II to seed the spermatid nucleolus [131,132].

In oogenesis, by contrast, nucleoli become active at mid-to-late pachytene and remain so even during the prolonged diplotene arrest, which can last years [133], despite being unnecessary for oocyte maturation. Only the maternal nucleolus is inherited, and it is important for chromatin organisation in early embryonic development in both the female and the male pronucleus [134,135]. This activity reaches an extreme in amphibians: *Xenopus* oocytes amplify rDNA extrachromosomally, generating roughly 1,000–1,500 free nucleoli per germinal vesicle to meet the ribosome demand of oogenesis [136–138]. As oocytes complete growth, the active nucleolus converts into a transcriptionally silent nucleolus-like body surrounded by a heterochromatin rim – the karyosphere, or “surrounded nucleolus” (SN) configuration – and this transition correlates with meiotic competence: SN oocytes resume meiosis and reach metaphase II at substantially higher rates than oocytes retaining the immature “non-surrounded nucleolus” (NSN) configuration [139–141].

In *S. cerevisiae*, which shows distinct nucleolar behaviour during mitosis, the meiotic nucleolus remains intact until anaphase I, disassembles and reassembles at telophase I, and repeats this cycle during meiosis II; nucleolar proteins are retained in the ascus and excluded from the new spores, which may reset cellular ageing [18,142,143].

Set against this fungal pattern, the divergence in the animal germline described above stands out. In *S. cerevisiae* a single nucleolus disassembles and reassembles at each meiotic division regardless of which gamete lineage it occupies, whereas in animals nucleolar fate splits sharply by sex: lost during spermatogenesis, but retained and in many species amplified during oogenesis. This restates, for meiosis, the same fungi-versus-animals contrast drawn for mitosis: fungal systems repeatedly solve nucleolar continuity through active, division-coupled remodelling of a persisting structure, while the animal germline instead allows nucleolar fate itself to diverge between sexes. Whether other fungi show germline-specific asymmetries in meiotic nucleolar behaviour, or whether the symmetric pattern seen in budding yeast is the more general rule, remains an open question.

Regulation of meiosis timing

As in mitosis, the nucleolus also controls the timing of meiotic progression by sequestering key regulators.

In *S. cerevisiae*, Csm1 and Lrs4, components of the monopolin complex involved in the proper orientation of sister kinetochores during meiosis I, usually reside in the nucleolus until the polo-like kinase Cdc5 promotes their exit after prophase I [144,145]. The same kinase, together with Spo12 and Slk19, drives nucleolar division and releases Cdc14 from the nucleolus, but, unlike in mitosis, only briefly during anaphase I, in order to dephosphorylate Cdk1 substrates and promote spindle disassembly [146,147]. For a more comprehensive review of Cdk regulation in meiosis, see [148].

Similarly, in *Drosophila* the nucleolus hosts the centromere-specific histone variant CENP-A, and its assembly factor CAL1 resides both at centromeres and in the nucleolus, where it forms a complex with the nucleoplasmin homologue Modulo [149–151]. For proper centromere assembly, the timely release of CENP-A and CAL1 in prophase I has to be controlled by a further assembly factor, CENP-C, and, in male meiosis, by nucleolar transcriptional activity [151].

As in mitotic division, during meiosis the nucleolus facilitates the coordination of successive steps, and it is also involved in the spatial organisation of homologous chromosomes during the early stages of meiosis. It is worth noting that not only nucleolar protein composition and transcriptional activity, but also the biophysical properties of the nucleolus as a phase-separated organelle, appear to matter for chromosome pairing. This contribution is clearest where canonical mechanisms are absent or insufficient, as in *S. pombe*, which lacks a synaptonemal complex, and in X–Y pairing in *Drosophila*.

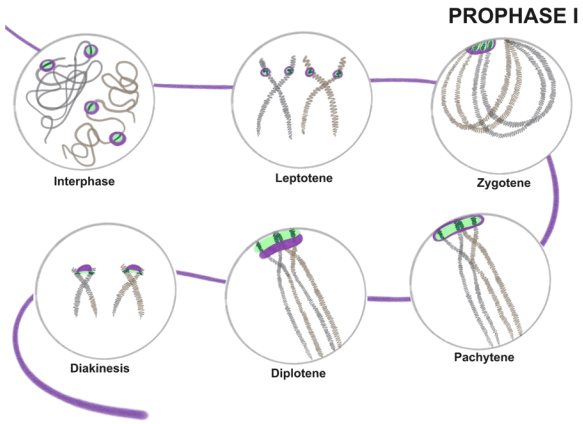

Fig. 4. Nucleolar dynamics during prophase I of meiosis in human spermatocytes. Meiotic stages adapted from [152]. Dark green – rDNA, lime – FC, dark purple – DFC and GC, grey and brown – chromosomes, bearing nucleolar regions, NE.

Conclusion

Following nucleolar dynamics through the life cycle has helped shift the view of the nucleolus from a stable ribosome-producing compartment to a responsive

nuclear domain whose organisation changes with transcriptional activity, chromosome state, nuclear architecture, stress, and developmental programme. Two classes of demand emerge from the comparison. Environmental change acts on the nucleolus opportunistically, at whatever point in the cycle it arrives, whereas mitosis and meiosis act on it on schedule. In both cases, nucleolar dynamics are not simply a consequence of what the cell is doing but part of how the cell reorganises nuclear function across changing states. They also show that nucleolar remodelling is an active process: during division, cells must coordinate transcriptional downregulation, rDNA segregation, chromosome mechanics, and nuclear-envelope interactions in order to partition or rebuild the nucleolus. A similar case for activity can be made in interphase: the regulated sequestration of specific proteins, the signalling roles of ribosomal subunits released from the nucleolus, and the active condensation of rDNA chromatin under stress all point toward the nucleolus as a participant in, rather than a passive casualty of, the stress response – though this remains a conclusion drawn mostly from case-by-case observations rather than from a systematic cross-species test of whether the response is actively driven or passively imposed.

A comparative approach is especially valuable here, because comparison across systems helps separate core requirements from lineage-specific implementations. For example, the need to reduce rDNA transcriptional output, to manage the rDNA locus as a special chromosomal region, and to coordinate nucleolar material with nuclear architecture appears broadly shared, even though different organisms meet these requirements by different means. A related molecular logic recurs across all three contexts discussed in this review: regulated sequestration and release of factors from the nucleolus – seen as reversible protein sequestration during stress, as Cdc14 release from Net1/RENT to drive rDNA segregation in mitosis, and as the nucleolar residence and prophase-I exit of the monopolin components Csm1 and Lrs4 in meiosis – repeatedly couples nucleolar state to cell-cycle and stress signalling, even where the specific factors involved differ. At the same time, the comparative view prevents mammalian organisation from being treated as the default state. Terms such as FC, DFC, and GC remain useful, especially for mammalian ultrastructure and for framing conserved functional logic, but fungal and other non-mammalian systems remind us that nucleolar organisation can be achieved with different morphologies and different spatial constraints. A compartment can remain recognisably nucleolar while changing its shape, its degree of internal partitioning, its relation to the nuclear envelope, or its mode of inheritance. This argues that nucleolar identity should be defined less by a fixed appearance than by a linked set of functions, molecular interactions, and dynamic behaviours.

More broadly, the work discussed here shows the value of combining genetics, cell biology, imaging, biochemistry, and biophysics. Together, these approaches make it possible to connect changes in nucleolar shape and material state with rDNA regulation, RNA processing, chromosome mechanics, protein handling, and nuclear-envelope coupling. This kind of integration will be essential for moving from descriptive accounts of nucleolar remodelling to a more mechanistic understanding.

The studies discussed here also make clear that environmental and developmental context must be treated as central. The nucleolus does not behave the same way in a rapidly growing mitotic cell, a heat-shocked interphase cell or a meiotic nucleus. This point has practical implications for future work. A major risk in the field is to infer general principles from cells studied in only one condition, growth regime, or developmental stage. Many open questions may look different when examined under nutrient limitation, proteotoxic stress, altered ploidy, meiosis, quiescence, or species-specific division programmes. The same is true across evolutionary distance. What appears as a special case in one model may instead reflect a broader principle that only becomes visible when the nucleolus is studied across different physiological and developmental contexts.

What lies ahead? One obvious goal is a more mechanistic account of how nucleolar state changes are triggered and executed. We still lack a full causal map linking cell-cycle kinases, rDNA chromatin regulators, Pol I machinery, RNA processing factors, condensin and Top2 function, nuclear-envelope coupling, and condensate material properties. In mitosis, for example, it remains important to understand how transcriptional shutdown, chromosome compaction, changes in GC behaviour, and remodelling of rDNA–nuclear-envelope contacts are coordinated in time. In stress, the field still needs a clearer picture of how nucleolar remodelling interfaces with proteostasis pathways and how general the budding yeast logic is across fungi and other eukaryotes. In meiosis, especially outside *S. cerevisiae*, the basic descriptive groundwork is still incomplete. A second goal is broader evolutionary coverage. Budding yeast has provided a rich mechanistic framework, but it cannot stand in for all fungal or all non-animal nucleolar biology. Fission yeasts, filamentous fungi, and other microbial eukaryotes are likely to reveal additional solutions to the problem of maintaining or rebuilding nucleolar function through changing nuclear states. A broader comparative base should make it easier to distinguish universal tasks from species-specific details.

In sum, following the nucleolus through the life cycle – across stress, mitosis and meiosis – shows that nucleolar organisation is inseparable from cell state. Different organisms use different structural solutions, but often to solve the same underlying problems. For that reason, nucleolar dynamics provide a useful framework for understanding how nuclear compartments remain functional while the cell around them changes.

Funding and acknowledgements

RC, EG, and GD acknowledge the support of the European Molecular Biology Laboratory. GD is supported by a FEBS Excellence Award and the EMBO Young Investigator Programme.

Declaration on the use of Generative AI

A Large Language Model (LLM; Anthropic 2026; Claude Opus 5.0. https://claude.ai) was used to check grammar, spelling, the accuracy of reference callouts, and document formatting, in the main text of this manuscript at the final stage before submission for peer review. No LLM was involved in drafting, editing of content or figure generation.

References

1 Hernandez-Verdun D (2011) Assembly and disassembly of the nucleolus during the cell cycle. *Nucleus* **2**, 189–194.

2 Woolford JL & Baserga SJ (2013) Ribosome biogenesis in the yeast Saccharomyces cerevisiae. *Genetics* **195**, 643–681.

3 Farley KI, Surovtseva Y, Merkel J & Baserga SJ (2015) Determinants of mammalian nucleolar architecture. *Chromosoma* **124**, 323–331.

4 Lafontaine DLJ, Riback JA, Bascetin R & Brangwynne CP (2021) The nucleolus as a multiphase liquid condensate. *Nat Rev Mol Cell Biol* **22**, 165–182.

5 Boisvert F-M, van Koningsbruggen S, Navascués J & Lamond AI (2007) The multifunctional nucleolus. *Nat Rev Mol Cell Biol* **8**, 574–585.

6 Boulon S, Westman BJ, Hutten S, Boisvert F-M & Lamond AI (2010) The nucleolus under stress. *Mol Cell* **40**, 216–227.

7 O'Sullivan JM, Pai DA, Cridge AG, Engelke DR & Ganley ARD (2013) The nucleolus: a raft adrift in the nuclear sea or the keystone in nuclear structure? *BioMolecular Concepts* **4**, 277–286.

8 Feric M, Vaidya N, Harmon TS, Mitrea DM, Zhu L, Richardson TM, Kriwacki RW, Pappu RV & Brangwynne CP (2016) Coexisting Liquid Phases Underlie Nucleolar Subcompartments. *Cell* **165**, 1686–1697.

9 Correll CC, Bartek J & Dundr M (2019) The Nucleolus: A Multiphase Condensate Balancing Ribosome Synthesis and Translational Capacity in Health, Aging and Ribosomopathies. *Cells* **8**, 869.

10 Mayer C & Grummt I (2005) Cellular Stress and Nucleolar Function. *Cell Cycle* **4**, 1036–1038.

11 Matos-Perdomo E & Machín F (2019) Nucleolar and Ribosomal DNA Structure under Stress: Yeast Lessons for Aging and Cancer. *Cells* **8**, 779.

12 Pirogov SA, Gvozdev VA & Klenov MS (2019) Long Noncoding RNAs and Stress Response in the Nucleolus. *Cells* **8**, 668.

13 Gavrilova AA, Neklesova MV, Zagryadskaya YA, Kuznetsova IM, Turoverov KK & Fonin AV (2024) Stress-Induced Evolution of the Nucleolus: The Role of Ribosomal Intergenic Spacer (rIGS) Transcripts. *Biomolecules* **14**, 1333.

14 Zharskaya OO & Zatsepina OV (2007) The dynamics and mechanisms of nucleolar reorganization during mitosis. *Cell Tiss Biol* **1**, 277–292.

15 Archambault V, Li J, Emond-Fraser V & Larouche M (2022) Dephosphorylation in nuclear reassembly after mitosis. *Front Cell Dev Biol* **10**, 1012768.

16 Granot D & Snyder M (1991) Segregation of the nucleolus during mitosis in budding and fission yeast. *Cell Motil Cytoskeleton* **20**, 47–54.

17 Torres-Rosell J, Machín F, Jarmuz A & Aragón L (2004) Nucleolar Segregation Lags Behind the Rest of the Genome and Requires Cdc14p Activation by the FEAR Network. *Cell Cycle* **3**, 494–500.

18 Fuchs J & Loidl J (2004) Behaviour of nucleolus organizing regions (NORs) and nucleoli during mitotic and meiotic divisions in budding yeast. *Chromosome Res* **12**, 427–438.

19 Ukil L, De Souza CP, Liu H-L & Osmani SA (2009) Nucleolar Separation from Chromosomes during **Aspergillus nidulans** Mitosis Can Occur Without Spindle Forces. *MBoC* **20**, 2132–2145.

20 Heath IB (1980) Variant Mitoses in Lower Eukaryotes: Indicators of the Evolution of Mitosis? In *International Review of Cytology* pp. 1–80. Elsevier.

21 Raikov IB (1982) *The Protozoan Nucleus: Morphology and Evolution* (Cell Biology Monographs, Volume 9). Springer-Verlag, Vienna and New York.

22 Webster DL & Watson K (1993) Ultrastructural changes in yeast following heat shock and recovery. *Yeast* **9**, 1165–1175.

23 Fransolet S, Deltour R, Bronchart R & Van de Walle C (1979) Changes in ultrastructure and transcription induced by elevated temperature in Zea mays embryonic root cells. *Planta* **146**, 7–18.

24 Gallardo P, Real-Calderón P, Flor-Parra I, Salas-Pino S & Daga RR (2020) Acute Heat Stress Leads to Reversible Aggregation of Nuclear Proteins into Nucleolar Rings in Fission Yeast. *Cell Reports* **33**, 108377.

25 Welch WJ & Suhan JP (1985) Morphological study of the mammalian stress response: characterization of changes in cytoplasmic organelles, cytoskeleton, and nucleoli, and appearance of intranuclear actin filaments in rat fibroblasts after heat-shock treatment. *J Cell Biol* **101**, 1198–1211.

26 Wang M, Tao X, Jacob MD, Bennett CA, Ho JJD, Gonzalgo ML, Audas TE & Lee S (2018) Stress-Induced Low Complexity RNA Activates Physiological Amyloidogenesis. *Cell Rep* **24**, 1713-1721.e4.

27 Frottin F, Schueder F, Tiwary S, Gupta R, Körner R, Schlichthaerle T, Cox J, Jungmann R, Hartl FU & Hipp MS (2019) The nucleolus functions as a phase-separated protein quality control compartment. *Science* **365**, 342–347.

28 Hayashi K & Matsunaga S (2019) Heat and chilling stress induce nucleolus morphological changes. *J Plant Res* **132**, 395–403.

29 Picart-Picolo A, Picart C, Picault N & Pontvianne F (2020) Nucleolus-associated chromatin domains are maintained under heat stress, despite nucleolar reorganization in Arabidopsis thaliana. *J Plant Res* **133**, 463–470.

30 Wang M, Bokros M, Theodoridis PR & Lee S (2019) Nucleolar Sequestration: Remodeling Nucleoli Into Amyloid Bodies. *Front Genet* **10**.

31 Malinovska L, Kroschwald S, Munder MC, Richter D & Alberti S (2012) Molecular chaperones and stress-inducible protein-sorting factors coordinate the spatiotemporal distribution of protein aggregates. *Mol Biol Cell* **23**, 3041–3056.

32 Miller SB, Ho C-T, Winkler J, Khokhrina M, Neuner A, Mohamed MY, Guilbride DL, Richter K, Lisby M, Schiebel E, Mogk A & Bukau B (2015) Compartment-specific aggregases direct distinct nuclear and cytoplasmic aggregate deposition. *EMBO J* **34**, 778–797.

33 Kumar A, Mathew V & Stirling PC (2022) Nuclear protein quality control in yeast: The latest INQuiries. *Journal of Biological Chemistry* **298**, 102199.

34 Sontag EM, Morales-Polanco F, Chen J-H, McDermott G, Dolan PT, Gestaut D, Le Gros MA, Larabell C & Frydman J (2023) Nuclear and cytoplasmic spatial protein quality control is coordinated by nuclear–vacuolar junctions and perinuclear ESCRT. *Nat Cell Biol* **25**, 699–713.

35 Ali A, Garde R, Schaffer OC, Bard JAM, Husain K, Kik SK, Davis KA, Luengo-Woods S, Igarashi MG, Drummond DA, Squires AH & Pincus D (2023) Adaptive preservation of orphan ribosomal proteins in chaperone-dispersed condensates. *Nature cell biology* **25**, 1691.

36 Feder ZA, Ali A, Singh A, Krakowiak J, Zheng X, Bindokas VP, Wolfgeher D, Kron SJ & Pincus D (2021) Subcellular localization of the J-protein Sis1 regulates the heat shock response. *J Cell Biol* **220**, e202005165.

37 Rubio LS, Mohajan S & Gross DS (2024) Heat Shock Factor 1 forms nuclear condensates and restructures the yeast genome before activating target genes. *eLife* **12**, RP92464.

38 Boronat S, Cabrera M & Hidalgo E (2021) Spatial sequestration of misfolded proteins as an active chaperone-mediated process during heat stress. *Curr Genet* **67**, 237–243.

39 Boronat S, Cabrera M, Vega M, Alcalá J, Salas-Pino S, Daga RR, Ayté J & Hidalgo E (2023) Formation of Transient Protein Aggregate-like Centers Is a General Strategy Postponing Degradation of Misfolded Intermediates. *Int J Mol Sci* **24**, 11202.

40 Tani T, Derby RJ, Hiraoka Y & Spector DL (1995) Nucleolar accumulation of poly (A)+ RNA in heat-shocked yeast cells: implication of nucleolar involvement in mRNA transport. *MBoC* **6**, 1515–1534.

41 Ideue T, Azad AK, Yoshida J, Matsusaka T, Yanagida M, Ohshima Y & Tani T (2004) The nucleolus is involved in mRNA export from the nucleus in fission yeast. *Journal of Cell Science* **117**, 2887–2895.

42 Yoshida J & Tani T (2005) Hsp16p Is Required for Thermotolerance in Nuclear mRNA Export in Fission Yeast Schizosaccharomyces pombe. *Cell Structure and Function* **29**, 125–138.

43 Audas TE, Jacob MD & Lee S (2012) The nucleolar detention pathway: A cellular strategy for regulating molecular networks. *Cell Cycle* **11**, 2059–2062.

44 Mekhail K, Khacho M, Carrigan A, Hache RRJ, Gunaratnam L & Lee S (2005) Regulation of ubiquitin ligase dynamics by the nucleolus. *J Cell Biol* **170**, 733–744.

45 Mekhail K, Rivero-Lopez L, Khacho M & Lee S (2006) Restriction of rRNA Synthesis by VHL Maintains Energy Equilibrium under Hypoxia. *Cell Cycle* **5**, 2401–2413.

46 Carme P, Rutherford K, Bähler J, Mata J & Wood V (2026) PomBase in 2026: expanding knowledge, modeling connections. *Genetics* **232**, iyag001.

47 Zhao Z, Sentürk N, Song C & Grummt I (2018) lncRNA PAPAS tethered to the rDNA enhancer recruits hypophosphorylated CHD4/NuRD to repress rRNA synthesis at elevated temperatures. *Genes Dev* **32**, 836–848.

48 Zhao Z, Dammert MA, Grummt I & Bierhoff H (2016) lncRNA-Induced Nucleosome Repositioning Reinforces Transcriptional Repression of rRNA Genes upon Hypotonic Stress. *Cell Reports* **14**, 1876–1882.

49 Mikhaleva EA, Leinsoo TA, Ishizu H, Gvozdev VA & Klenov MS (2019) The nucleolar transcriptome regulates Piwi shuttling between the nucleolus and the nucleoplasm. *Chromosome Res* **27**, 141–152.

50 Matos-Perdomo E & Machín F (2018) The ribosomal DNA metaphase loop of Saccharomyces cerevisiae gets condensed upon heat stress in a Cdc14-independent TORC1-dependent manner. *Cell Cycle* **17**, 200–215.

51 Takahara T & Maeda T (2012) Transient Sequestration of TORC1 into Stress Granules during Heat Stress. *Molecular Cell* **47**, 242–252.

52 Lohrum MAE, Ludwig RL, Kubbutat MHG, Hanlon M & Vousden KH (2003) Regulation of HDM2 activity by the ribosomal protein L11. *Cancer Cell* **3**, 577–587.

53 Rubbi CP & Milner J (2003) Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J* **22**, 6068–6077.

54 Jin A, Itahana K, O'Keefe K & Zhang Y (2004) Inhibition of HDM2 and Activation of p53 by Ribosomal Protein L23. *Molecular and Cellular Biology* **24**, 7669–7680.

55 Fumagalli S, Di Cara A, Neb-Gulati A, Natt F, Schwemberger S, Hall J, Babcock GF, Bernardi R, Pandolfi PP & Thomas G (2009) Absence of nucleolar disruption after impairment of 40S ribosome biogenesis reveals an rpL11-translation-dependent mechanism of p53 induction. *Nat Cell Biol* **11**, 501–508.

56 Zhang Y & Lu H (2009) Signaling to p53: ribosomal proteins find their way. *Cancer Cell* **16**, 369–377.

57 Matsumoto H, Shimura M, Omatsu T, Okaichi K, Majima H & Ohnishi T (1994) p53 proteins accumulated by heat stress associate with heat shock proteins HSP72/HSC73 in human glioblastoma cell lines. *Cancer Letters* **87**, 39–46.

58 Liu Y, Liang S & Tartakoff AM (1996) Heat shock disassembles the nucleolus and inhibits nuclear protein import and poly(A)+ RNA export. *EMBO J* **15**, 6750–6757.

59 Weeks SE, Metge BJ & Samant RS (2019) The nucleolus: a central response hub for the stressors that drive cancer progression. *Cell Mol Life Sci* **76**, 4511–4524.

60 Hua L, Yan D, Wan C & Hu B (2022) Nucleolus and nucleolar stress: from cell fate decision to disease development. *Cells* **11**, 3017.

61 Leung AKL, Gerlich D, Miller G, Lyon C, Lam YW, Lleres D, Daigle N, Zomerdijk J, Ellenberg J & Lamond AI (2004) Quantitative kinetic analysis of nucleolar breakdown and reassembly during mitosis in live human cells. *The Journal of Cell Biology* **166**, 787–800.

62 Dundr M, Misteli T & Olson MOJ (2000) The Dynamics of Postmitotic Reassembly of the Nucleolus. *J Cell Biol* **150**, 433–446.

63 Stenström L, Mahdessian D, Gnann C, Cesnik AJ, Ouyang W, Leonetti MD, Uhlén M, Cuylen-Haering S, Thul PJ & Lundberg E (2020) Mapping the nucleolar proteome reveals a spatiotemporal organization related to intrinsic protein disorder. *Molecular Systems Biology* **16**, e9469.

64 D'Amours D, Stegmeier F & Amon A (2004) Cdc14 and Condensin Control the Dissolution of Cohesin-Independent Chromosome Linkages at Repeated DNA. *Cell* **117**, 455–469.

65 Sullivan M, Higuchi T, Katis VL & Uhlmann F (2004) Cdc14 Phosphatase Induces rDNA Condensation and Resolves Cohesin-Independent Cohesion during Budding Yeast Anaphase. *Cell* **117**, 471–482.

66 Wang B-D, Yong-Gonzalez V & Strunnikov AV (2004) Cdc14p/FEAR Pathway Controls Segregation of Nucleolus in *S. cerevisiae* by Facilitating Condensin Targeting to rDNA Chromatin in Anaphase. *Cell Cycle* **3**, 958–965.

67 Titos I, Ivanova T & Mendoza M (2014) Chromosome length and perinuclear attachment constrain resolution of DNA intertwines. *Journal of Cell Biology* **206**, 719–733.

68 Machín F, Torres-Rosell J, De Piccoli G, Carballo JA, Cha RS, Jarmuz A & Aragón L (2006) Transcription of ribosomal genes can cause nondisjunction. *Journal of Cell Biology* **173**, 893–903.

69 Dauban L, Kamgoué A, Wang R, Léger-Silvestre I, Beckouët F, Cantaloube S & Gadal O (2019) Quantification of the dynamic behaviour of ribosomal DNA genes and nucleolus during yeast Saccharomyces cerevisiae cell cycle. *Journal of Structural Biology* **208**, 152–164.

70 Tomson BN, D'Amours D, Adamson BS, Aragon L & Amon A (2006) Ribosomal DNA Transcription-

Dependent Processes Interfere with Chromosome Segregation. *Mol Cell Biol* **26**, 6239–6247.

71 Wang B-D, Butylin P & Strunnikov A (2006) Condensin Function in Mitotic Nucleolar Segregation is Regulated by rDNA Transcription. *Cell Cycle* **5**, 2260–2267.

72 Iacovella MG, Golfieri C, Massari LF, Busnelli S, Pagliuca C, Dal Maschio M, Infantino V, Visintin R, Mechtler K, Ferreira-Cerca S & De Wulf P (2015) Rio1 promotes rDNA stability and downregulates RNA polymerase I to ensure rDNA segregation. *Nat Commun* **6**, 6643.

73 Shou W, Sakamoto KM, Keener J, Morimoto KW, Traverso EE, Azzam R, Hoppe GJ, Feldman RMR, DeModena J, Moazed D, Charbonneau H, Nomura M & Deshaies RJ (2001) Net1 Stimulates RNA Polymerase I Transcription and Regulates Nucleolar Structure Independently of Controlling Mitotic Exit. *Molecular Cell* **8**, 45–55.

74 Huang J & Moazed D (2003) Association of the RENT complex with nontranscribed and coding regions of rDNA and a regional requirement for the replication fork block protein Fob1 in rDNA silencing. *Genes Dev* **17**, 2162–2176.

75 Tanny JC, Kirkpatrick DS, Gerber SA, Gygi SP & Moazed D (2004) Budding Yeast Silencing Complexes and Regulation of Sir2 Activity by Protein-Protein Interactions. *Molecular and Cellular Biology* **24**, 6931–6946.

76 Geil C, Schwab M & Seufert W (2008) A Nucleolus-Localized Activator of Cdc14 Phosphatase Supports rDNA Segregation in Yeast Mitosis. *Current Biology* **18**, 1001–1005.

77 Manzano-López J & Monje-Casas F (2020) The Multiple Roles of the Cdc14 Phosphatase in Cell Cycle Control. *IJMS* **21**, 709.

78 de los Santos-Velázquez AI, de Oya IG, Manzano-López J & Monje-Casas F (2017) Late rDNA Condensation Ensures Timely Cdc14 Release and Coordination of Mitotic Exit Signaling with Nucleolar Segregation. *Current Biology* **27**, 3248-3263.e5.

79 Elliott SG & McLaughlin CS (1979) Regulation of RNA synthesis in yeast III: Synthesis during the cell cycle. *Molec Gen Genet* **169**, 237–243.

80 Clemente-Blanco A, Mayán-Santos M, Schneider DA, Machín F, Jarmuz A, Tschochner H & Aragón L (2009) Cdc14 inhibits transcription by RNA polymerase I during anaphase. *Nature* **458**, 219–222.

81 Mekhail K, Seebacher J, Gygi SP & Moazed D (2008) Role for perinuclear chromosome tethering in maintenance of genome stability. *Nature* **456**, 667–670.

82 Mostofa MdG, Rahman MA, Koike N, Yeasmin AM, Islam N, Waliullah TM, Hosoyamada S, Shimobayashi M, Kobayashi T, Hall MN & Ushimaru T (2018) CLIP and cohibin separate rDNA from nucleolar proteins destined for degradation by nucleophagy. *Journal of Cell Biology* **217**, 2675–2690.

83 Mostofa MdG, Morshed S, Shibata R, Takeichi Y, Rahman MA, Hosoyamada S, Kobayashi T & Ushimaru T (2019) rDNA Condensation Promotes rDNA Separation from Nucleolar Proteins Degraded for Nucleophagy after TORC1 Inactivation. *Cell Reports* **28**, 3423-3434.e2.

84 Capella M, Mandemaker IK, Martín Caballero L, Den Brave F, Pfander B, Ladurner AG, Jentsch S & Braun S (2021) Nucleolar release of rDNA repeats for repair involves SUMO-mediated untethering by the Cdc48/p97 segregase. *Nat Commun* **12**, 4918.

85 Godfrey M, Kuilman T & Uhlmann F (2015) Nur1 Dephosphorylation Confers Positive Feedback to Mitotic Exit Phosphatase Activation in Budding Yeast. *PLOS Genetics* **11**, e1004907.

86 Allshire RC & Ekwall K (2015) Epigenetic Regulation of Chromatin States in *Schizosaccharomyces pombe*. *Cold Spring Harb Perspect Biol* **7**, a018770.

87 Yague-Sanz C (2024) Shaping the chromatin landscape at rRNA and tRNA genes, an emerging new role for RNA polymerase II transcription? *Yeast* **41**, 135–147.

88 Iglesias N, Paulo JA, Tatarakis A, Wang X, Edwards AL, Bhanu NV, Garcia BA, Haas W, Gygi SP & Moazed D (2020) Native Chromatin Proteomics Reveals a Role for Specific Nucleoporins in Heterochromatin Organization and Maintenance. *Molecular Cell* **77**, 51-66.e8.

89 Chen C-T, Peli-Gulli M-P, Simanis V & McCollum D (2006) *S. pombe* FEAR protein orthologs are not required for release of Clp1/Flp1 phosphatase from the nucleolus during mitosis. *Journal of Cell Science* **119**, 4462–4466.

90 Zhang D & Oliferenko S (2013) Remodeling the nuclear membrane during closed mitosis. *Current Opinion in Cell Biology* **25**, 142–148.

91 Takemoto A, Kawashima SA, Li J-J, Jeffery L, Yamatsugu K, Elemento O & Nurse P (2016) Nuclear envelope expansion is crucial for proper chromosomal segregation during a closed mitosis. *Journal of Cell Science* **129**, 1250–1259.

92 Khondker S, Han G-S & Carman GM (2022) Phosphorylation-mediated regulation of the Nem1-Spo7/Pah1 phosphatase cascade in yeast lipid

synthesis. *Advances in Biological Regulation* **84**, 100889.

93 Yam C, He Y, Zhang D, Chiam K-H & Oliferenko S (2011) Divergent Strategies for Controlling the Nuclear Membrane Satisfy Geometric Constraints during Nuclear Division. *Current Biology* **21**, 1314–1319.

94 Yam C, Gu Y & Oliferenko S (2013) Partitioning and Remodeling of the Schizosaccharomyces japonicus Mitotic Nucleus Require Chromosome Tethers. *Current Biology* **23**, 2303–2310.

95 Larson JR, Facemyer EM, Shen K-F, Ukil L & Osmani SA (2014) Insights into Dynamic Mitotic Chromatin Organization Through the NIMA Kinase Suppressor SonC, a Chromatin-Associated Protein Involved in the DNA Damage Response. *Genetics* **196**, 177–195.

96 De Souza CPC, Osmani AH, Hashmi SB & Osmani SA (2004) Partial Nuclear Pore Complex Disassembly during Closed Mitosis in *Aspergillus nidulans*. *Current Biology* **14**, 1973–1984.

97 Osmani AH, Davies J, Liu H-L, Nile A & Osmani SA (2006) Systematic Deletion and Mitotic Localization of the Nuclear Pore Complex Proteins of Aspergillus nidulans. *MBoC* **17**, 4946–4961.

98 De Souza CPC & Osmani SA (2007) Mitosis, Not Just Open or Closed. *Eukaryotic Cell* **6**, 1521–1527.

99 Chemudupati M, Osmani AH & Osmani SA (2016) A mitotic nuclear envelope tether for Gle1 also affects nuclear and nucleolar architecture. *MBoC* **27**, 3757–3770.

100 Chibana H, Tanaka K & Yanagida M (1996) Analysis of the cell cycle in the budding yeast *Candida albicans* by positioning of chromosomes by fluorescence *in situ* hybridization (FISH) with repetitive sequences. *Genes to Cells* **1**, 727–740.

101 Finley KR & Berman J (2005) Microtubules in *Candida albicans* Hyphae Drive Nuclear Dynamics and Connect Cell Cycle Progression to Morphogenesis. *Eukaryot Cell* **4**, 1697–1711.

102 Thomson DD, Berman J & Brand AC (2016) High frame-rate resolution of cell division during *Candida albicans* filamentation. *Fungal Genetics and Biology* **88**, 54–58.

103 Reza MH, Dutta S, Goyal R, Shah H, Dey G & Sanyal K (2024) Expansion microscopy reveals characteristic ultrastructural features of pathogenic budding yeast species. *J Cell Sci* **137**, jcs262046.

104 Nakazawa N, Nakamura T, Kokubu A, Ebe M, Nagao K & Yanagida M (2008) Dissection of the essential steps for condensin accumulation at kinetochores and rDNAs during fission yeast mitosis. *Journal of Cell Biology* **180**, 1115–1131.

105 Ishida K, Tanaka K & Kawakami K (2022) Generation and characterization of a temperature-sensitive mutant allele of the second largest subunit of RNA polymerase I in *Schizosaccharomyces pombe*. *microPublication Biology* 2022, 000586.

106 Pham AT, Mani M, Wang X & Vafabakhsh R (2024) Multiscale biophysical analysis of nucleolus disassembly during mitosis. *Proc Natl Acad Sci USA* **121**, e2312250121.

107 Riback JA, Eeftens JM, Lee DSW, Quinodoz SA, Donlic A, Orlovsky N, Wiesner L, Beckers L, Becker LA, Strom AR, Rana U, Tolbert M, Purse BW, Kleiner R, Kriwacki R & Brangwynne CP (2023) Viscoelasticity and advective flow of RNA underlies nucleolar form and function. *Molecular Cell* **83**, 3095-3107.e9.

108 Sheu-Gruttadauria J, Yan X, Stuurman N, Ogrean RO, Lin S, Floor SN & Vale RD (2023) Nucleolar dynamics are determined by the ordered assembly of the ribosome. *bioRxiv*, 2023.09.26.559432. Preprint.

109 Ugolini I, Bilokapic S, Ferrolino M, Teague J, Yan Y, Zhou X, Deshmukh A, White M, Kriwacki RW & Halic M (2022) Chromatin localization of nucleophosmin organizes ribosome biogenesis. *Molecular Cell* **82**, 4443-4457.e9.

110 Berrios S & Fernández-Donoso R (1990) Nuclear architecture of human pachytene spermatocytes: quantitative analysis of associations between nucleolar and XY bivalents. *Hum Genet* **86**, 103–116.

111 Stahl A, Wachtler F, Hartung M, Devictor M, Schöfer C, Mosgöller W, de Lanversin A, Fouet C & Schwarzacher HG (1991) Nucleoli, nucleolar chromosomes and ribosomal genes in the human spermatocyte. *Chromosoma* **101**, 231–244.

112 Berríos S, Fernández-Donoso R, Pincheira J, Page J, Manterola M & Cristina Cerda M (2004) Number and Nuclear Localisation of Nucleoli in Mammalian Spermatocytes. *Genetica* **121**, 219–228.

113 Tres LL (1975) Nucleolar RNA synthesis of meiotic prophase spermatocytes in the human testis. *Chromosoma* **53**, 141–151.

114 Holm PB & Rasmussen SW (1983) Human meiosis V. Substages of pachytene in human spermatogenesis. *Carlsberg Res Commun* **48**, 351–383.

115 Armstrong SJ, Franklin FC & Jones GH (2001) Nucleolus-associated telomere clustering and pairing precede meiotic chromosome synapsis in Arabidopsis thaliana. *J Cell Sci* **114**, 4207–4217.

116 Yang X, Timofejeva L, Ma H & Makaroff CA (2006) The Arabidopsis SKP1 homolog ASK1 controls meiotic chromosome remodeling and release of

chromatin from the nuclear membrane and nucleolus. *J Cell Sci* **119**, 3754–3763.

117 Cromer L, Tiscareno-Andrade M, Lefranc S, Chambon A, Hurel A, Brogniez M, Guérin J, Le Masson I, Adam G, Charif D, Andrey P & Grelon M (2024) Rapid meiotic prophase chromosome movements in Arabidopsis thaliana are linked to essential reorganization at the nuclear envelope. *Nat Commun* **15**, 5964.

118 Stronghill P, Pathan N, Ha H, Supijono E & Hasenkampf C (2010) Ahp2 (Hop2) function in Arabidopsis thaliana (Ler) is required for stabilization of close alignment and synaptonemal complex formation except for the two short arms that contain nucleolus organizer regions. *Chromosoma* **119**, 443–458.

119 You H, Tang D, Liu H, Zhou Y, Li Y, Shen Y, Gong Z, Yu H, Gu M, Jiang J, Zhang T & Cheng Z (2024) Chromosome ends initiate homologous chromosome pairing during rice meiosis. *Plant Physiol* **195**, 2617–2634.

120 McKee BD (1996) The license to pair: Identification of meiotic pairing sites inDrosophila. *Chromosoma* **105**, 135–141.

121 McKee BD, Yan R & Tsai J-H (2012) Meiosis in male Drosophila. *Spermatogenesis* **2**, 167–184.

122 Olson LW, Edén U, Egel-Mitani M & Egel R (1978) Asynaptic meiosis in fission yeast? *Hereditas* **89**, 189–199.

123 Ding D-Q, Okamasa K, Yamane M, Tsutsumi C, Haraguchi T, Yamamoto M & Hiraoka Y (2012) Meiosis-Specific Noncoding RNA Mediates Robust Pairing of Homologous Chromosomes in Meiosis. *Science* **336**, 732–736.

124 Ding D-Q, Okamasa K, Katou Y, Oya E, Nakayama J, Chikashige Y, Shirahige K, Haraguchi T & Hiraoka Y (2019) Chromosome-associated RNA–protein complexes promote pairing of homologous chromosomes during meiosis in Schizosaccharomyces pombe. *Nat Commun* **10**, 5598.

125 Ding D-Q, Haraguchi T & Hiraoka Y (2013) The role of chromosomal retention of noncoding RNA in meiosis. *Chromosome Res* **21**, 665–672.

126 Schultz MC & Leblond CP (1990) Nucleolar structure and synthetic activity during meiotic prophase and spermiogenesis in the rat. *Am J Anat* **189**, 1–10.

127 Villalobos-Arellano JR, Valencia DM, Ortiz-Hernández R, Torres-Ramírez N, Echeverría-Martínez OM, Hernández-Hernández A & Vázquez-Nin GH (2019) Nucleolus structural integrity during the first meiotic prophase in rat spermatocytes. *Exp Cell Res* **383**, 111587.

128 Peruquetti RL, Taboga SR, Santos LR de S, de Oliveira C & de Azeredo-Oliveira MTV (2011) Nucleolar cycle and chromatoid body formation: Is there a relationship between these two processes during spermatogenesis of *Dendropsophus minutus* (Amphibia, Anura)? *Micron* **42**, 87–96.

129 Peruquetti RL, Taboga SR & Azeredo-Oliveira MTV de (2012) Morphological Changes of Mammalian Nucleoli during Spermatogenesis and Their Possible Role in the Chromatoid Body Assembling. *International Scholarly Research Notices* **2012**, 829854.

130 Suja JA & Rufas JS (1987) Nucleolar meiotic cycle in orthoptera. *Cell Biology International Reports* **11**, 289–299.

131 Teruel M, Cabrero J, Perfectti F & Camacho JPM (2007) Nucleolus size variation during meiosis and NOR activity of a B chromosome in the grasshopper Eyprepocnemis plorans. *Chromosome Res* **15**, 755–765.

132 Morielle-Souza A & de Azeredo-Oliveira MTV (2008) Study of the nucleolar cycle and ribosomal RNA distribution during meiosis in triatomines (Triatominae, Heteroptera). *Micron* **39**, 1020–1026.

133 Vagner-Capodano AM, Hartung M & Stahl A (1987) Nucleolus, nucleolar chromosomes, and nucleolus-associated chromatin from early diplotene to dictyotene in the human oocyte. *Hum Genet* **75**, 140–146.

134 Ogushi S, Palmieri C, Fulka H, Saitou M, Miyano T & Fulka J (2008) The Maternal Nucleolus Is Essential for Early Embryonic Development in Mammals. *Science* **319**, 613–616.

135 Ogushi S & Saitou M (2010) The Nucleolus in the Mouse Oocyte is Required for the Early Step of Both Female and Male Pronucleus Organization. *Journal of Reproduction and Development* **56**, 495–501.

136 Brown DD & Dawid IB (1968) Specific Gene Amplification in Oocytes. *Science* **160**, 272–280.

137 Gall JG (1968) Differential synthesis of the genes for ribosomal RNA during amphibian oögenesis. *Proc Natl Acad Sci U S A* **60**, 553–560.

138 Brangwynne CP, Mitchison TJ & Hyman AA (2011) Active liquid-like behavior of nucleoli determines their size and shape in Xenopus laevis oocytes. *Proceedings of the National Academy of Sciences* **108**, 4334–4339.

139 Zuccotti M, Piccinelli A, Giorgi Rossi P, Garagna S & Redi CA (1995) Chromatin organization during mouse oocyte growth. *Mol Reprod Dev* **41**, 479–485.

140 Inoue A, Nakajima R, Nagata M & Aoki F (2008) Contribution of the oocyte nucleus and cytoplasm to the determination of meiotic and developmental competence in mice. *Hum Reprod* **23**, 1377–1384.

141 Nikolova V, Markova M, Zhivkova R, Chakarova I, Hadzhinesheva V & Delimitreva S (2024) How the Oocyte Nucleolus Is Turned into a Karyosphere: The Role of Heterochromatin and Structural Proteins. *J Dev Biol* **12**, 28.

142 Ünal E, Kinde B & Amon A (2011) Gametogenesis eliminates age-induced cellular damage and resets lifespan in yeast. *Science* **332**, 1554–1557.

143 Li P, Jin H & Yu H-G (2014) Condensin suppresses recombination and regulates double-strand break processing at the repetitive ribosomal DNA array to ensure proper chromosome segregation during meiosis in budding yeast. *MBoC* **25**, 2934–2947.

144 Clyne RK, Katis VL, Jessop L, Benjamin KR, Herskowitz I, Lichten M & Nasmyth K (2003) Polo-like kinase Cdc5 promotes chiasmata formation and cosegregation of sister centromeres at meiosis I. *Nat Cell Biol* **5**, 480–485.

145 Rabitsch KP, Petronczki M, Javerzat J-P, Genier S, Chwalla B, Schleiffer A, Tanaka TU & Nasmyth K (2003) Kinetochore Recruitment of Two Nucleolar Proteins Is Required for Homolog Segregation in Meiosis I. *Developmental Cell* **4**, 535–548.

146 Buonomo SBC, Rabitsch KP, Fuchs J, Gruber S, Sullivan M, Uhlmann F, Petronczki M, Tóth A & Nasmyth K (2003) Division of the Nucleolus and Its Release of CDC14 during Anaphase of Meiosis I Depends on Separase, SPO12, and SLK19. *Developmental Cell* **4**, 727–739.

147 Marston AL, Lee BH & Amon A (2003) The Cdc14 Phosphatase and the FEAR Network Control Meiotic Spindle Disassembly and Chromosome Segregation. *Developmental Cell* **4**, 711–726.

148 MacKenzie AM & Lacefield S (2020) CDK Regulation of Meiosis: Lessons from S. cerevisiae and S. pombe. *Genes* **11**, 723.

149 Chen C-C, Greene E, Bowers SR & Mellone BG (2012) A Role for the CAL1-Partner Modulo in Centromere Integrity and Accurate Chromosome Segregation in Drosophila. *PLOS ONE* **7**, e45094.

150 Unhavaithaya Y & Orr-Weaver TL (2013) Centromere proteins CENP-C and CAL1 functionally interact in meiosis for centromere clustering, pairing, and chromosome segregation. *Proceedings of the National Academy of Sciences* **110**, 19878–19883.

151 Kwenda L, Collins CM, Dattoli AA & Dunleavy EM (2016) Nucleolar activity and CENP-C regulate CENP-A and CAL1 availability for centromere assembly in meiosis. *Development* **143**, 1400–1412.

152 Wang L, Wu B, Ma Y, Ren Z & Li W (2022) The blooming of an old story on the bouquet†. *Biol Reprod* **107**, 289–300.