Review

Moving fast and breaking things: Incidence and repair of DNA damage within ribosomal DNA repeats

Yana P. Blokhina[a], Abigail Buchwalter[a,b,c,*]

[a] Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA, United States
[b] Department of Physiology, University of California, San Francisco, San Francisco, CA, United States
[c] Chan Zuckerberg Biohub, San Francisco, CA, United States

ABSTRACT

The genes that code for ribosomal RNA are present in hundreds of tandemly arrayed copies in the human genome. Ribosomal DNA repeats transcribe vast amounts of ribosomal RNA in order to meet the cell's relentless demand for ribosome production. Intrinsic features of ribosomal DNA repeats render them uniquely vulnerable to DNA damage. Sensing and repairing damage to ribosomal DNA involves dramatic spatial reorganization of the nucleolus, the phase-separated nuclear subdomain where ribosomes are made. We highlight recent advances in detecting the incidence of DNA damage and defining the mechanisms of DNA repair on these essential genes.

1. Introduction

The ribosomal DNA (rDNA) repeats encode the structural RNAs of the ribosome, and are present in several hundred copies in large clusters on several chromosomes. Ribosomal RNA (rRNA) production accounts for over half of all cellular transcription [1] and generates an essential catalytic component of the ribosome. Maintaining the integrity and function of rDNA repeats thus has major effects on cellular fitness. In spite of the central and essential function of rDNA repeats, we know relatively little about the mechanisms that maintain the integrity of rDNA genes due to the fact that these repetitive loci reside within the small proportion of the human genome that remains un-mapped [2]. In recent years, new computational and experimental approaches have begun to shed light on the mechanisms that maintain and repair rDNA.

The variety of mechanisms used by eukaryotic cells to sense and repair ssDNA and dsDNA lesions have been extensively reviewed elsewhere [3,4] and have also been reviewed in the context of rDNA repair [5–7]. This review will summarize recent advances in understanding how DNA damage is generated, sensed, and repaired at the rDNA repeats, with a particular focus on the RNA polymerase I (Pol I)-transcribed 47S rDNA repeats in mammalian cells. We will highlight unique features of rDNA that make it especially vulnerable to damage, novel findings on rDNA-specific damage signaling mechanisms, and the context of DNA repair pathway choice at rDNA.

2. The genomic structure of mammalian rDNA repeats

In mammals, rDNA repeats are present in two types of tandem arrays, termed the 5S and 47S (or 45S) arrays. The 5S rDNA repeats are located in one large tandem repeat array on chromosome 1 in humans and on chromosome 8 in mice. The 47S arrays are located on the short arms of five acrocentric chromosomes in humans (chr. 13, 14, 15, 21, 22) and subcentromerically in mice (chr. 12, 15, 16, 18, 19) (reviewed in [8]. The 5S and 47S rDNA repeats are each present in hundreds of copies in mammalian genomes, with an estimated median of ~300 for humans, although the total variation in copy numbers between individuals spans two orders of magnitude [9]. This inter-individual variation is generated by frequent meiotic recombination events in the germline [10]. The 5S rDNA gene encodes a single ~120 base pair rRNA, while each human 47S rDNA repeat is ~43 kb in length and is composed of a ~13 kb transcribed region and a ~30 kb non-transcribed intergenic spacer (IGS). The intergenic spacer (IGS) carries regulatory elements and simple repeats [11]. The transcribed region of each rDNA repeat encodes a single 47S transcript that is spliced into the 18S, 5.8S, and 28S ribosomal RNAs (Fig. 1). The 5S rDNA repeats are transcribed by RNA Pol III, while the 47S repeats are transcribed by RNA Pol I. The nucleolus, a membraneless organelle within the nucleus which serves as the center for ribosome biogenesis and acts as an intracellular signaling hub, forms around Pol I-engaged 47S repeats.

---

* Corresponding author at: Cardiovascular Research Institute, University of California, San Francisco, San Francisco, CA, United States.
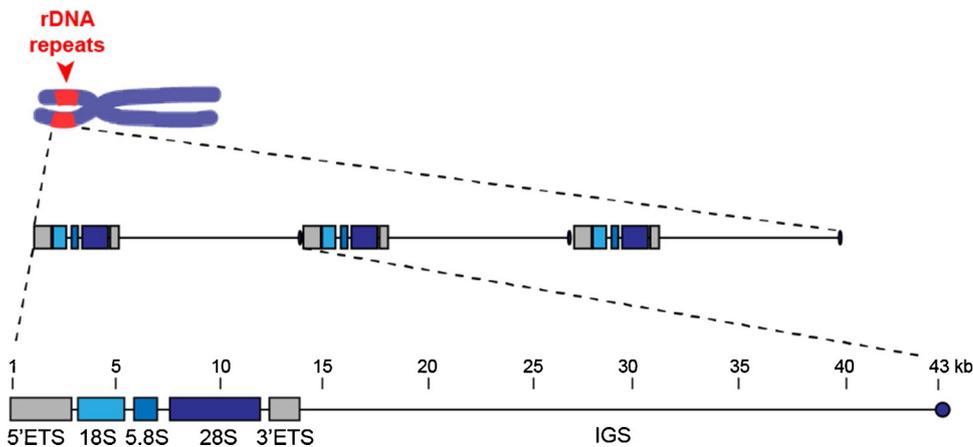E-mail address: abigail.buchwalter@ucsf.edu (A. Buchwalter).

Fig. 1. The 47S rDNA arrays are present on the short arms of the 5 acrocentric human chromosomes: 13, 14, 15, 21, and 22. These arrays can stretch for kilobases to megabases and are composed of tandem repeats of the 47S rDNA gene. Each gene codes for a single ~13 kb pre-rRNA that includes 5′ and 3′ external transcribed sequences (ETSs) and internal transcribed spacers (ITS1 and 2) that are cleaved off during rRNA processing. The pre-rRNA is further processed into the mature 18S, 5.8S, and 28S rRNAs. A ~30 kb long, non-transcribed intergenic spacer follows each transcription unit.

3. Vulnerability of rDNA repeats to DNA damage

An individual cell is estimated to endure tens of thousands of DNA lesions per day [12]. These lesions include single base changes such as deamination, depurination, and depyrimidination; crosslinks; single-stranded breaks; and double-stranded breaks (DSBs). DSBs are the least frequent but most noxious type of DNA damage [13]. DSBs are not dispersed uniformly across the genome. Rather, as recently developed direct sequencing techniques have begun to uncover, double-stranded breaks (DSBs) are highly enriched in nucleosome-depleted and active transcription start sites, as well as in sites where non-B-DNA structures such as G quadruplexes are predicted to form [14,15]. As summarized below, these features are abundant within rDNA repeats, suggesting that rDNA repeats are especially vulnerable to DNA damage.

3.1. Vulnerability #1: high transcription rate

Highly transcribed regions are particularly vulnerable to DNA damage [16]. Under homeostatic conditions, roughly half of a cell's rDNA repeats are stably silenced by packaging within heterochromatin outside the nucleolus, while active rDNA repeats engage with Pol I within the nucleolus (reviewed in [17]). This subset of rDNA repeats are extremely transcriptionally active. In fact, electron micrographs of active rDNA indicate that each repeat is extraordinarily densely covered by Pol I, with nascent chains of rRNA extending off the rDNA like bristles of a brush [18].

One transcription-linked vulnerability that rDNA repeats face is the production of R-loops. As RNA synthesis proceeds, nascent RNA can hybridize with the DNA that encodes it in *cis*, preventing reannealing of dsDNA and creating an RNA:DNA hybrid structure known as an R-loop [19]. R-loops expose a region of displaced ssDNA to mutagenic processes such as deamination and depurination [19]. If not resolved, R-loops can stall the progression of RNA and DNA polymerases, eventually leading to double-stranded DNA breaks [20]. Unique sequence features render rDNA repeats especially vulnerable to transcription-linked R-loop formation. In particular, the relatively high GC content of the rDNA repeats (~56 % [21],) compared to the genome-wide average (~41 % [22],) increases the likelihood that stable RNA:DNA hybrids will form and decreases the likelihood that they will be resolved before they are encountered by another DNA-templating enzyme [19]. The movement of RNA polymerases through DNA promotes partial uncoiling of the DNA strand; this topological stress promotes the formation of R-loops. The topoisomerase I enzyme plays an important role in resolving R loops by generating ssDNA nicks to relieve strain, including at rDNA [23–25]. A recent study that mapped R-loops genome-wide revealed that topoisomerase I inhibition causes the rapid accumulation of R-loops along several kilobases of the rDNA locus [25]. This observation suggests that R-loops and R-loop-related genomic instability

may be abundant on rDNA repeats.

3.2. Vulnerability #2: replication stress

Genomic regions with high GC content, such as the rDNA, are challenging for the DNA replication machinery to navigate [26]. In addition, the presence of simple repeats and microsatellites can cause replication fork "slippage" as nascent DNA anneals to another nearby region of homology [26]. The large intergenic spacer region of each rDNA repeat contains several types of simple repeats [11] that challenge the replication machinery. Collisions between replication and transcription machineries present an additional problem [27]. As rDNA repeats are extremely densely covered with actively transcribing Pol I they may be especially vulnerable to transcription-replication collisions. If any of these types of replication stress persist for a significant period of time, endonucleases will generate either ssDNA or dsDNA breaks in an attempt to resolve stalled or collapsed replication forks, inducing DNA damage [28]. Rapidly proliferating cells, such as hematopoietic progenitor cells, are particularly vulnerable to the accumulation of DNA damage arising from each round of DNA replication. Indeed, unresolved damage arising from replication stress appears to accumulate on rDNA repeats within hematopoietic stem cells during mammalian aging [29].

3.3. Vulnerability #3: non-B-DNA structures in ribosomal DNA repeats

Regions of DNA with high GC content are prone to form a non-B-DNA structure known as a G-quadruplex. G-quadruplexes form from single-stranded stretches of guanine nucleotides that interact in *cis* to form stacking interactions rather than participating in Watson-Crick base pairing [30]. As Pol I moves through the template strand of an rDNA repeat, the non-template strand forms G-quadruplexes [31]. G-quadruplexes may actually accelerate transcription by decreasing the likelihood that the template and non-template strands re-anneal after a Pol I molecule passes through. However, G-quadruplexes also present an obstacle to the subsequent movement of the DNA replication machinery [32]. Intriguingly, the abundant nucleolar protein nucleolin binds to G-quadruplexes within rDNA with high affinity, likely stabilizing these structures and promoting Pol I activity [31]. It remains unclear how nucleolin is displaced and G4 quadruplexes are resolved in advance of DNA replication, but the stabilization of quadruplexes in rDNA by nucleolin binding may make these sites more vulnerable to quadruplex-replication collisions and subsequent DSBs. Ligands that stabilize G-quadruplexes induce replication stress and disrupt rDNA transcription, and rapidly proliferating cancer cells with DNA repair pathway mutations are selectively vulnerable to these drugs [33]. The high propensity of rDNA repeats to damage makes it especially important to understand how damage is repaired in the context of this

highly transcribed, repetitive region.

4. Sensing rDNA double-stranded breaks

We next focus on the unique features of the rDNA damage response, which range from initial events of rDNA DSB signaling to the dramatic reorganization of nucleolar structure in response to damage. Active rDNA repeats have an unusual, decondensed chromatin structure, with very few stable nucleosomes in the transcribed region [34]. Active rDNA is instead bound densely by the transcription factor UBF, Pol I, and TCOF1. The phase-separated state of nucleoli as well as a dearth of nucleosomes in the transcribed region of active rDNA repeats may be some of the features that require modification of the DSB response in this region.

4.1. Initiation of rDNA double-stranded break signaling

When a DSB is generated, Ku70/80 and MRE11-RAD50-NBS1 (MRN) complexes are rapidly recruited to the exposed dsDNA ends [35]. These complexes initiate signaling cascades that will eventually direct the mechanism used for DNA repair: Ku70/80 promotes non-homologous end joining (NHEJ), while MRN promotes homologous recombination (HR). These signals are orchestrated by two DNA damage response kinases: DNA-dependent protein kinase catalytic subunit (DNA-PKcs), which is recruited and activated by Ku70/80, and ataxia-telangiectasia mutated (ATM), which is recruited and activated by MRN [36]. Both DNA-PKcs and ATM can relay the DNA damage signal by phosphorylating H2AX at Ser 139 (referred to as γH2AX) [37]. The γH2AX motif is recognized by several factors, most prominently MDC1, which interacts with the NBS1 subunit of the MRN complex and further amplifies ATM signaling [38].

While both Ku70/80 and MRN have been detected within nucleoli after rDNA damage [39,40], γH2AX phosphorylation is less prominent within the transcribed portion of rDNA repeats, likely because of the unusually low nucleosome occupancy there [41]. In fact, γH2AX appears to be dispensable for the recruitment of the MRN complex subunit NBS1 to damaged rDNA [40]. MDC1 may also play a lesser role in rDNA damage signaling, although the evidence is mixed. While MDC1-/- cells have an impaired ability to inhibit Pol I transcription in response to irradiation-induced DNA damage [42], RNAi-mediated depletion of MDC1 has little effect on Pol I's sensitivity to DNA damage [43]. Recent evidence indicates that MDC1 is not detectable within nucleoli, and depletion of MDC1 does not abrogate NBS1 focus formation on enzymatically damaged rDNA [40]. MDC1 is eventually recruited to damaged rDNA that has become exposed to the nucleoplasm in nucleolar cap structures (described in more detail below) [40]. The discrepancies between these studies may arise from the MDC1 disruption method used, the DNA damage method used (see Section 8), the time frames analyzed, and/or the cell types under study.

4.2. The unique role of the Treacle/TCOF1 protein in rDNA damage signaling

In recent years, it has become clear that the abundant nucleolar phosphoprotein Treacle/TCOF1 is a key mediator of several unique features of rDNA damage signaling. TCOF1 is associated with rDNA throughout the cell cycle [44,45] and is phosphorylated by ATM in response to rDNA damage [40]. TCOF1 can directly bind and recruit the MRN complex component NBS1 to rDNA [40,46,43,47]. Intriguingly, this interaction is mediated by an MDC1-like motif within TCOF1 and by binding to the same region of NBS1 that associates with MDC1, which opens the possibility that TCOF1 or MDC1 binding to NBS1 are mutually exclusive [43,46]. This stands in contrast to the DSB response elsewhere on the genome, which requires MDC1 to bridge the γH2AX signal and recruit additional MRN (Fig. 2) [38].

TCOF1 further amplifies the rDNA damage signal by recruiting

TOPBP1, a cofactor for the kinase ATR (ATM and Rad3-related kinase) [48], co-opting ATR activity for rDNA damage signaling. Typically, ATR is activated when ssDNA is exposed during replication stress and/or during the process of repairing a DSB. While ATR is a "first responder" to ssDNA damage, it is also a secondary effector that is activated when DSBs are processed by homologous recombination (HR), a repair process that involves resection and exposure of a tract of ssDNA. ATR phosphorylates a wide range of targets that partially overlap with ATM [37]. This TOPBP1-dependent mode of activating ATR in the nucleoli appears to be quite unique, as the TOPBP1:TCOF1 interaction occurs also in the absence of damage and does not depend on the presence of exposed ssDNA [48]. In fact, overexpression of TOPBP1 is sufficient to induce downstream responses to rDNA damage, including nucleolar cap formation (see below) and transcriptional arrest even in the absence of DNA damage [49].

4.3. Kinase signaling in response to rDNA damage

A shared consequence of ATM and ATR activation is the local inhibition of transcription, which allows repair processes to proceed without interference from RNA polymerases, both in the context of RNA Pol II [50,51], and RNA Pol I [52,40–42,47]. Whether DNA-PKcs can inhibit Pol II's transcriptional activity in response to DNA damage is less clear [50,51], and DNA-PKcs is dispensable for inhibition of Pol I activity in response to rDNA damage [52,41,42,47].

ATM and ATR kinases activate cell cycle checkpoint regulators CHK2 and CHK1, respectively, leading to cell cycle arrest to allow the cell to repair damage [53,54]. Whether rDNA damage induces a robust cell cycle arrest is currently muddied by conflicting data. Breaks at rDNA induced by I-PpoI (see Section 8) lead to ATM-dependent CHK2 activation and ATR-dependent CHK1 activation within 2 h [48]. Furthermore, CHK1 and CHK2 are required for effective repression of rRNA transcription [48]. However, breaks induced by CRISPR-Cas9 at rDNA caused only transient CHK1 activation and no CHK2 activation, and did not affect cell cycle progression [40]. It is unlikely that the method of damage induction underlies the contradiction between these two studies, as an earlier study demonstrated that either I-PpoI or Cas9 induced breaks at rDNA result in a reduction in mitotic entry, indicating checkpoint activation [55]. Intriguingly, this earlier study reported that rDNA-focused damage induced a more complete cell cycle arrest than that achieved by genome-wide irradiation, suggesting that cells may be acutely sensitive to rDNA damage. Whether rDNA damage consistently induces a cell cycle arrest response, or what factors influence the length of cell cycle arrest, remain open questions.

5. Damaged rDNA reorganizes into nucleolar caps

The nucleolus is formed by liquid-liquid phase separation around actively transcribing rDNA repeats, and contains three distinct phase-separated compartments: the fibrillar center (FC), the dense fibrillar component (DFC), and the granular component (GC) [56]. The FC is the innermost compartment and is enriched with UBF, Pol I, and rDNA; the DFC is interspersed with the FC and enriched with nascent rRNA and RNA processing factors; and the GC encircles the FC and DFC and is enriched with ribosome assembly factors (Fig. 3).

Disruptions to rDNA can cause the formation of "nucleolar caps": a dramatic inversion of the nucleolus so that both the protein and DNA components of the FC and DFC condense and pop outside of the GC (Fig. 3). Nucleolar cap formation is a rapid (< 1 h) response to pharmacological disruption of Pol I transcription by actinomycin D treatment [57–59], and a slower (2–6 hours) response to rDNA damage [40,47,48,60]. Damage induced anywhere in the rDNA repeat, including the IGS, but not in genomic DNA flanking the rDNA arrays, induces nucleolar cap formation [47]. Nucleolar cap formation in response to any stimulus coincides with and depends on transcriptional inhibition. When rDNA damage is induced, ATM and ATR are activated
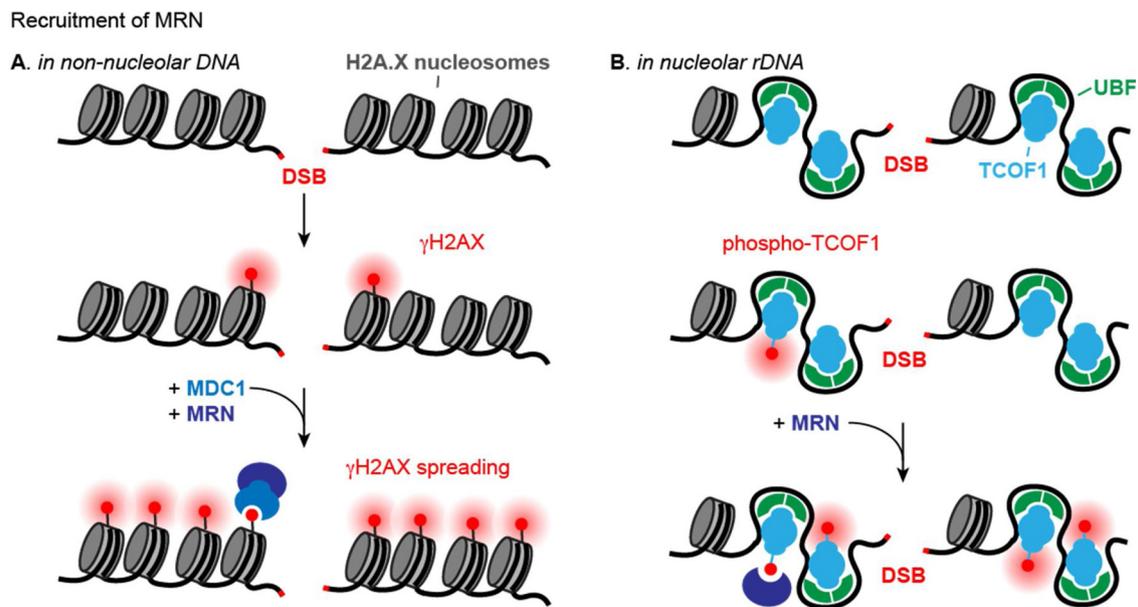
Fig. 2. Comparison of MRN's recruitment mechanism to damaged DNA outside (A) versus inside (B) the nucleolus. (A) when a double-stranded break (DSB) occurs in non-nucleolar DNA, the MRN complex binds directly to DNA near the break (not shown) and recruits the kinase ATM, which phosphorylates the histone variant H2A.X in nearby nucleosomes. γH2AX then recruits additional MRN along with MDC1. Increased MRN and ATM activity promotes spreading of the γH2AX signal outward from the break site. (B) When a DSB occurs in actively transcribing rDNA within the nucleolus, the MRN complex binds directly to DNA near the break (not shown) and recruits ATM. H2A.X-containing nucleosomes are sparse within rDNA repeats, while the rDNA-associated protein TCOF1 is abundant. ATM phosphorylates TCOF1, which directly recruits additional MRN, promoting increased local ATM activity and expanded TCOF1 phosphorylation.

and mediate transcriptional inhibition [48]. This explains why direct disruption of transcription (e.g. with actinomycin D) rapidly induces cap formation, while the response to DSBs has a latency period. Disruption of either ATM or ATR, but not DNA-PKcs, preserves Pol I activity and blocks nucleolar cap formation [52,47,55,40,41,48]. While transcription inhibition is clearly required for nucleolar cap formation in response to damage, recent evidence suggests that active movement of rDNA to the nucleolar boundary may also be involved [41]. Intriguingly, the linker of nucleoskeleton and cytoskeleton (LINC) complex and the actin network, which are involved in active movement of DSBs during HR of other genomic loci [61], are also necessary for cap formation, but not transcriptional inhibition, in the context of rDNA damage [41].

Changes to the rDNA chromatin environment have also been linked to nucleolar segregation in response to rDNA damage. The human silencing hub (HUSH) complex, a recently identified complex that participates in transcriptional silencing by promoting H3K9 trimethylation [62], is required for effective rDNA silencing and nucleolar cap formation in response to DNA damage [41]. Specifically, H3K9me3

deposition induced by HUSH activity appears to mediate the shutdown of rRNA transcription [41]. Phosphorylation of histone H2B on Serine 14 (H2BS14) also plays an important role in the nucleolar response to rDNA damage. Elsewhere in the genome, H2BS14 has been observed coincident with γH2AX on damaged DNA [63]; this mark is also deposited by the MST1 kinase during apoptosis [64]. Intriguingly, the MST1-related kinase MST2, which is constitutively localized to nucleoli and is activated by ATM, phosphorylates H2B in response to rDNA damage [60]. This appears to be a rapid and transient response, peaking within an hour of DNA damage. The transient accumulation of this modification is required for rDNA transcriptional inhibition and nucleolar segregation [60]. Disruption of MST2 prevents resolution of rDNA DSBs, underscoring the functional importance of this signal [60].

While the function of nucleolar caps has not been conclusively proven, several possibilities have been proposed. One possibility is that these structures serve to separate rDNA arrays on different chromosomes from each other in order to prevent aberrant recombination. Another possibility is that condensation of rDNA into nucleolar caps exposes damaged rDNA to the nucleoplasm, enabling DSB signaling and
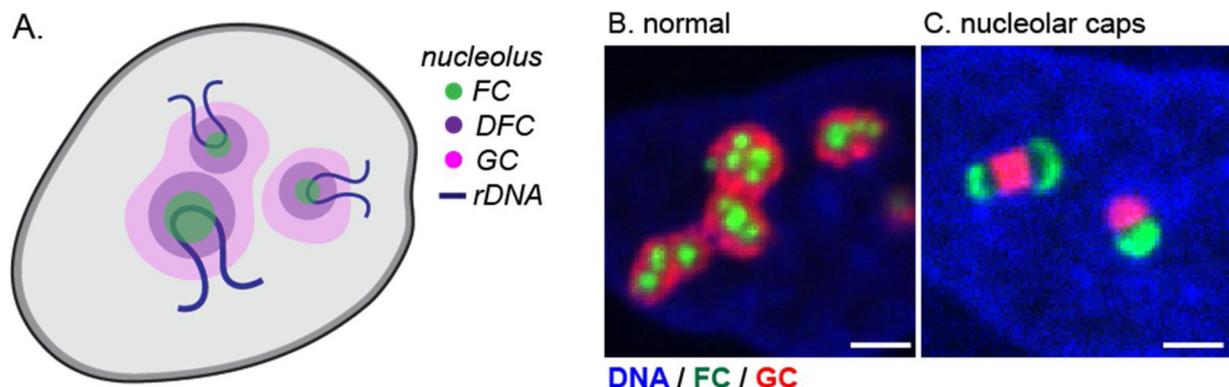

Fig. 3. (A) Tripartite organization of the nucleolus around rDNA repeats. FC, fibrillar center; DFC, dense fibrillar component; GC, granular component. (B-C) Examples of nucleolar organization visualized by fluorescence microscopy in normal (B) and transcriptionally inhibited (C) cells. The FC is marked by UBF, while the GC is marked by nucleophosmin.

repair factor recruitment [47]. Consistent with this idea, several repair signals and factors (including γH2AX and HR factors) are not detectable at rDNA breaks until nucleolar caps form [52,47,55,40,41]. The observation that γH2AX is only prominent at nucleolar caps, and not within the nucleolar interior, might suggest that this DSB signal is produced only after nucleolar caps have formed. However, this could also reflect the low density of the H2AX histone variant within rDNA [41]. Separately, it may be inaccurate to conflate lack of visible repair factor accumulation within nucleoli with lack of function within nucleoli. In fact, nucleolar proteomics studies suggest that several DNA damage sensing and repairing proteins can access nucleoli (reviewed in [6]). Overall, it seems that some "first responders" to rDNA damage do engage with damaged rDNA within nucleoli and before nucleolar cap formation, indicating that rDNA repair and changes in nucleolar morphology are interdependent.

6. Repair of double-stranded breaks in rDNA

There are two major mechanisms for repairing DSBs: non-homologous end joining (NHEJ) and homologous recombination (HR) [65]. NHEJ directly ligates broken ends together, often resulting in small deletions or insertions. HR is less error-prone as it uses homologous sequence, usually on a sister chromatid, as a template. However, HR can introduce mutations as a consequence of recombination resulting in dramatic genomic rearrangements when an inappropriate template is used for repair [66], such as repetitive sequences present in *cis* on the same chromosome or on a non-homologous chromosome. DSBs can be repaired by several alternative pathways, such as single-strand annealing (SSA) and microhomology-mediated end joining (MMEJ) under particular conditions [38]. These pathways are more mutagenic and error-prone than NHEJ and HR and could negatively impact rDNA integrity. However, rDNA repair by SSA and MMEJ has been much less extensively explored than by NHEJ and HR in mammalian cells.

The choice between NHEJ and HR is influenced by multiple factors, including the cell cycle, the type of damage, the chromatin environment of the break, and the transcriptional activity of the damaged locus (recently reviewed in [38]). NHEJ is active throughout the cell cycle, while HR is typically not used for repair outside S and G2 phases when a sister chromatid is available [65]. Intriguingly, HR is the favored repair mechanism specifically for active genes in G2 [67]. Current data indicate a complex interplay between NHEJ and HR in rDNA repair.

6.1. Evidence for NHEJ at rDNA

Evidence indicates that NHEJ plays an integral role in DSB repair at rDNA, although it seems to primarily act within the nucleolar interior and not at nucleolar caps. NHEJ is initiated by binding of the Ku70/80 complex independently of MRN. Ku70/80 is broadly distributed throughout the nucleoplasm, with only a small amount of the complex engaged with DSBs at any given time. When free Ku70/80 is removed by pre-extraction, a subpopulation of Ku70/80 becomes visible within nucleoli shortly after rDNA damage, indicating that this NHEJ-promoting complex can access nucleoli [39]. However, Ku70/80 is not found concentrated at nucleolar caps [47]. During NHEJ, Ku70/80 recruits downstream repair factors such as DNA-PK, DNA ligase IV, and XRCC4 [38,68]. Surprisingly, while DNA-PK is not detectable in nucleoli or at nucleolar caps after rDNA damage [47], inhibiting the NHEJ repair pathway by depleting DNA-PK or XRCC4 leads to an increase in rDNA DSBs, transcriptional inhibition, and nucleolar cap formation [41,52]. This clearly indicates a functional dependence of rDNA repair on the NHEJ pathway. Furthermore, DNA-PK-null cells, but not ATM-null cells, are acutely sensitive to rDNA damage and undergo growth arrest and cell death. It is likely that these phenotypes arise from persistent breaks and transcriptional inhibition when NHEJ is compromised [52].

While this evidence indicates that rDNA relies on NHEJ for effective

DSB repair, NHEJ factors are only occasionally detectable within nucleoli. It is possible that this is because of how quickly NHEJ proceeds. NHEJ takes approximately 30 min to complete, while HR takes 7 h or longer [69]. NHEJ may act rapidly to repair rDNA breaks within the nucleoli while persistent breaks may be moved to nucleolar caps to be repaired by HR. Consistent with this idea, a number of HR-specific factors are recruited to nucleolar caps, while NHEJ factors are absent from these structures [52,47,55]. One exception to this is the NHEJ-promoting factor 53BP1, which has been reported at nucleolar caps [52]. However, since 53BP1 can also prevent over-resection in HR by antagonizing BRCA1 [38], this observation could indicate that 53BP1 participates in HR at nucleolar caps.

6.2. Evidence for HR at rDNA

In contrast to rDNA repair by NHEJ which seems to occur within nucleoli, repair by HR appears to be most prominent at nucleolar caps. HR is initiated when the MRN complex binds and catalyzes resection of broken DNA ends. MRN works in conjunction with the enzymes CtBP-interacting protein (CtIP), exonuclease 1 (EXO1), endonuclease DNA2, and Bloom syndrome helicase (BLM) to excise nucleotides and expose a stretch of resected ssDNA. This ssDNA is immediately bound by the RPA complex, which can be phosphorylated to regulate the extent of resection [70]. RPA is subsequently displaced by RAD51 to form a filament for homologous sequence invasion facilitated by BRCA2 [38]. Once the homologous template and the resected ssDNA anneal, the homologous sequence is used as a template for *de novo* DNA synthesis to extend and replace the damaged sequence; this is achieved by several distinct mechanisms in mammalian cells [38].

A wide array of HR factors concentrate at nucleolar caps, but are not detectable within nucleoli [40,47,52]. However, recent evidence suggests that some steps of HR take place within nucleoli and before nucleolar caps form. For instance, phosphorylated TCOF1 recruits NBS1 and MRE-11 to foci within the nucleolar interior that are presumptive rDNA breaks [40,43,46]; these MRN components then move into nucleolar caps along with rDNA [40,41]. RPA foci, which indicate presence of resected ssDNA, have been reported both in the nucleolar interior and at caps [41,48], or only at the caps [40,47]. As RPA foci are larger and brighter at nucleolar caps, the discrepancy between these studies may be due to differences in sensitivity of fluorescence detection.

Whether HR factors actively contribute to the formation of nucleolar caps has begun to be determined. These recent studies suggest that early HR signaling factors (MRN and ATM) both precede and influence nucleolar cap formation. For instance, depletion of either NBS1 or MRE-11 inhibits nucleolar cap formation [40,41]. However, the role of other HR factors in nucleolar cap formation presents a more complex picture. Depletion of CtIP, a resection cofactor, does not affect nucleolar cap formation, but does disrupt the recruitment of other HR factors to these structures [40,48]. Intriguingly, CtIP depletion appears to abrogate movement of NBS1 foci to caps [40], suggesting a feedback mechanism from CtIP to the MRN complex. Given that NBS1 is required for the formation of nucleolar caps, it is not entirely clear how CtIP depletion can inhibit the movement of NBS1 to caps without preventing their formation. On the other hand, it has also been reported that depleting the resection factors BLM/DNA2 and RPA2 prevents nucleolar cap formation [41]. It has been suggested that resection could promote nucleolar cap formation by generating a signal that activates ATR: RPA-bound ssDNA. However, the fact that ATR can be activated as a consequence of a direct TOPBP1:TCOF1 interaction indicates that ATR activation can occur independently of resection within nucleoli [48].

Because HR usually relies on a sister chromatid to serve as a homologous donor for repair, this process is typically restricted to the S and G2 phases of the cell cycle. An earlier analysis of HR factor recruitment to nucleolar caps concluded that HR is active on damaged rDNA throughout the cell cycle, and proposed the hypothesis that other

rDNA repeats could serve as homology donors in the absence of a recently replicated sister chromatid [47]. In contrast, recent studies have asserted that RPA is only observed at nucleolar caps during S/G2, which would indicate that as in nuclear DNA, HR-mediated repair of rDNA occurs predominantly in G2 [41,48].

Overall, the functional importance of HR for rDNA repair remains murky. While HR factors clearly influence nucleolar morphology in response to damage, inhibition of the HR pathway does not prolong DSBs or inhibit cell cycle progression. Rather, inhibition of HR has actually been suggested to facilitate the resolution of rDNA DSBs by NHEJ [52,55].

7. Repair of single stranded DNA lesions on rDNA

ssDNA lesions at rDNA have not been extensively studied, though they are likely to be abundant given the frequency of R-loops within active rDNA repeats. The nucleotide excision repair (NER) pathway excises and repairs single-stranded lesions throughout the genome. The types of lesions recognized by NER include UV-induced crosslinks, bulky modifications to single bases, and R-loops. NER involves excision of damaged ssDNA and *de novo* DNA synthesis using the undamaged ssDNA as a template. Specific arms of the NER pathway identify and repair lesions on transcribed or non-transcribed strands of DNA [71]. The global genomic repair (GGR) pathway relies on the XPF/ERCC1 nuclease to excise lesions on either strand of DNA [71]. The transcription-coupled repair (TCR) pathway specifically repairs lesions on the transcribed strand of DNA and relies on the enzymes Cockayne Syndrome A and B (CSA/CSB). TCR was first described as a specific pathway that is induced when Pol II is stalled by a lesion on the template strand [71]. Interestingly, unlike Pol II-transcribed regions of the genome, rDNA never undergoes TCR. Instead, rDNA relies entirely on GGR for repair of ssDNA lesions induced by UV radiation or chemical modification [72,73]. However, it remains unclear whether NER resolves R-loops within rDNA. Elsewhere in the genome, the NER nucleases XPF/ERCC1 and XPG have been implicated in processing R-loops into DSBs which are then recognized by DSB repair pathways [74,75]. Notably, conversion of R-loops to DSBs increases the likelihood of ineffective or error-prone repair, leading to genome instability. Whether rDNA R-loops are similarly processed into DSBs has not yet been determined.

8. Methods of DNA damage induction used for studying rDNA damage and repair

Irradiation has been widely used to generate DNA damage and to dissect damage response and repair pathways. However, the random nature of the damage inflicted limits the utility of this approach for studying damage sensing and response at specific loci, such as at rDNA repeats. Several researchers have used site-specific endonuclease enzymes to inflict damage at rDNA repeats. The endonucleases I-PpoI and AsiSI have been used most extensively for this purpose. I-PpoI recognizes the 15-base-pair sequence CTCTCTTAA ▼GGTAGC (where ▼represents the cut site). This sequence is found within the 28S rRNA-coding portion of each rDNA repeat, as well as at up to 15 additional sites elsewhere in the human genome [50]. When expressed within human cells, I-PpoI accesses ∼10 % of its target sites within rDNA [76]; with a median number of 300 rDNA repeats per human cell, I-PpoI expression can be expected to induce ∼30 rDNA DSBs. The AsiSI endonuclease recognizes an 8-base-pair sequence GCGAT ▼CGC, a sequence that is found at ∼1200 locations across the human genome, including within the 5′ external transcribed spacer (5′ETS) of each 47S rDNA repeat [77]. AsiSI has been demonstrated to effectively cleave 174 non-rDNA sites [77] and is able to cleave rDNA [41], but it is unknown what proportion of rDNA repeats it typically cleaves. Interestingly, AsiSI cannot cleave CpG-methylated DNA. Since a significant proportion of rDNA repeats are silenced by DNA methylation, AsiSI

cleavage likely is restricted only to active, demethylated repeats.

CRISPR/Cas9 targeted nuclease activity paired with rDNA-specific guide RNAs have also been used to generate breaks within rDNA repeats [47,55,40]. This approach allows precise control of the location of damage induction, allowing comparisons of the response to rDNA damage within transcribed, non-transcribed, and flanking sequences [47]. Cas9 more effectively cleaves actively transcribed loci and can be impeded when nucleosomes are positioned over the target sequence [78]; it is thus likely that active rDNA repeats are preferentially cleaved by Cas9, although this has not been demonstrated. The frequency of off-target Cas9 binding and cleavage events varies with the guide RNA used, but in many cases results in cleavage and repair events at 5–10 off-target sites [79].

9. Remaining questions

Both the likelihood that a locus incurs a DSB and the subsequent choice of repair mechanism depend on the transcriptional state and chromatin environment of the locus. rDNA can exist in highly active, inactive, or stably repressed states [17], and it is currently not known whether active rDNA repeats undergo a different repair mechanism from inactive or repressed ones, since most damage-inducing methods do not make a distinction between these states. One exception may be the AsiSI endonuclease, which targets unmethylated sites in rDNA and may be biased toward active repeats. While active rDNA resides exclusively within nucleoli, super-resolution microscopy has revealed that inactive repeats can be found both within and outside of nucleoli [80]. It is very likely that damage that occurs on rDNA in these different transcriptional states and subcellular locales arises in response to distinct insults and uses distinct repair mechanisms.

What we know about the incidence of damage and mechanisms of repair at rDNA comes primarily from actively replicating cells. Much less is known about how slowly dividing or postmitotic cells respond to rDNA damage. To understand how quiescent and senescent cells handle DNA damage, it will be important to analyze the mechanisms of rDNA repair in postmitotic cells. Key differences are a lower transcription rate potentially leading to different stressors causing the majority of damage at rDNA, and the lack of an S/G2 phase, suggesting that contribution of HR to rDNA repair may be less important in those cell types. DNA damage is a known contributor to the progression of aging [81], and rDNA is a hub of incurred damage over lifespan [29]. Knowledge of how different types of tissues and cell types repair damage at rDNA can facilitate development of targeted interventions to improve relevant DNA repair pathways for therapeutic benefit.

References

[1] Tom Moss, Victor Y. Stefanovsky, At the center of eukaryotic life, Cell 109 (5) (2002) 545–548.

[2] Brian. McStay, Nucleolar organizer regions: genomic 'Dark matter' requiring illumination, Genes Dev. 30 (14) (2016) 1598–1610.

[3] Rachel Abbotts, David M. Wilson 3rd, Coordination of DNA single strand break repair, Free Radic. Biol. Med. 107 (June) (2017) 228–244.

[4] Emil Mladenov, Simon Magin, Aashish Soni, George Iliakis, DNA double-strand-Break repair in higher eukaryotes and its role in genomic instability and cancer: cell cycle and proliferation-dependent regulation, Semin. Cancer Biol. 37-38 (June) (2016) 51–64.

[5] Daniël O. Warmerdam, Rob M.F. Wolthuis, Keeping ribosomal DNA intact: a repeating challenge, Chromosome Res. 27 (1-2) (2019) 57–72.

[6] L.M. Ogawa, S.J. Baserga, Crosstalk between the nucleolus and the DNA damage response, Mol. Biosyst. 13 (3) (2017) 443–455.

[7] Mikael S. Lindström, Deana Jurada, Sladana Bursac, Ines Orsolic, Jiri Bartek, Sinisa Volarevic, Nucleolus as an emerging hub in maintenance of genome stability and Cancer pathogenesis, Oncogene 37 (18) (2018) 2351–2366.

[8] Tamara A. Potapova, Jennifer L. Gerton, Ribosomal DNA and the nucleolus in the context of genome organization, Chromosome Res. 27 (1-2) (2019) 109–127.

[9] Matthew M. Parks, Chad M. Kurylo, Randall A. Dass, Linda Bojmar, David Lyden, C. Theresa Vincent, Scott C. Blanchard, Variant ribosomal RNA alleles are conserved and exhibit tissue-specific expression, Sci. Adv. 4 (2) (2018) eaao0665.

[10] Dawn M. Stults, Michael W. Killen, Heather H. Pierce, Andrew J. Pierce, Genomic architecture and inheritance of human ribosomal RNA gene clusters, Genome Res. 18 (1) (2008) 13–18.

[11] Saumya Agrawal, Austen R.D. Ganley, The conservation landscape of the human ribosomal RNA gene repeats, PLoS One 13 (12) (2018) e0207531.

[12] T. Lindahl, D.E. Barnes, Repair of endogenous DNA damage, Cold Spring Harb. Symp. Quant. Biol. 65 (2000) 127–133.

[13] Anthony Tubbs, André Nussenzweig, Endogenous DNA damage as a source of genomic instability in Cancer, Cell 168 (4) (2017) 644–656.

[14] Stefanie V. Lensing, Giovanni Marsico, Robert Hänsel-Hertsch, Enid Y. Lam, David Tannahill, Shankar Balasubramanian, DSBCapture: in situ capture and direct sequencing of dsDNA breaks, Nat. Methods 13 (10) (2016) 855–857.

[15] Nicola Crosetto, Abhishek Mitra, Maria Joao Silva, Magda Bienko, Norbert Dojer, Wang Qi, Elif Karaca, et al., Nucleotide-resolution DNA double-strand break mapping by next-generation sequencing, Nat. Methods 10 (4) (2013) 361–365.

[16] Aline Marnef, Sarah Cohen, Gaëlle Legube, Transcription-coupled DNA double-strand break repair: active genes need special care, J. Mol. Biol. 429 (9) (2017) 1277–1288.

[17] Brian McStay, Ingrid Grummt, The epigenetics of rRNA genes: from molecular to chromosome biology, Annu. Rev. Cell Dev. Biol. 24 (2008) 131–157.

[18] O.L. Miller Jr, B.R. Beatty, Visualization of nucleolar genes, Science 164 (3882) (1969) 955–957.

[19] JoséM. Santos-Pereira, Andrés Aguilera, R loops: new modulators of genome dynamics and function, Nat. Rev. Genet. 16 (10) (2015) 583–597.

[20] Wenjian Gan, Zhishuang Guan, Jie Liu, Ting Gui, Keng Shen, James L. Manley, Li. Xialu, R-loop-Mediated genomic instability is caused by impairment of replication fork progression, Genes Dev. 25 (19) (2011) 2041–2056.

[21] Juan S. Escobar, Sylvain Glémin, Nicolas Galtier, GC-Biased Gene Conversion Impacts Ribosomal DNA Evolution in Vertebrates, Angiosperms, and Other Eukaryotes, Mol. Biol. Evol. 28 (9) (2011) 2561–2575.

[22] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, K. Devon, et al., Initial sequencing and analysis of the human genome, Nature 409 (6822) (2001) 860–921.

[23] Aziz El Hage, Sarah L. French, Ann L. Beyer, David Tollervey, Loss of topoisomerase I leads to R-Loop-Mediated transcriptional blocks during ribosomal RNA synthesis, Genes Dev. 24 (14) (2010) 1546–1558.

[24] Sandie Tuduri, Laure Crabbé, Chiara Conti, H.élène Tourrière, Heidi Holtgreve-Grez, Anna Jauch, V.éronique Pantesco, et al., Topoisomerase I suppresses genomic instability by preventing interference between replication and transcription, Nat. Cell Biol. 11 (11) (2009) 1315–1324.

[25] Stefano G. Manzo, Stella R. Hartono, Lionel A. Sanz, Jessica Marinello, Andrea Cossarizza Sara De Biasi, Giovanni Capranico, Frederic Chedin, DNA topoisomerase I differentially modulates R-Loops across the human genome, Genome Biol. 19 (1) (2018) 100.

[26] Hervé Técher, Stéphane Koundrioukoff, Alain Nicolas, Michelle Debatisse, The impact of replication stress on replication dynamics and DNA damage in vertebrate cells, Nat. Rev. Genet. 18 (9) (2017) 535–550.

[27] Stephan Hamperl, Karlene A. Cimprich, Conflict resolution in the genome: how transcription and replication make it work, Cell 167 (6) (2016) 1455–1467.

[28] Michelle K. Zeman, Karlene A. Cimprich, Causes and consequences of replication stress, Nat. Cell Biol. 16 (1) (2014) 2–9.

[29] Johanna Flach, Sietske T. Bakker, Mary Mohrin, Pauline C. Conroy, Eric M. Pietras, Damien Reynaud, Silvia Alvarez, et al., Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells, Nature 512 (7513) (2014) 198–202.

[30] Michelle L. Duquette, Priya Handa, Jack A. Vincent, Andrew F. Taylor, Nancy Maizels, Intracellular Transcription of G-Rich DNAs Induces Formation of G-Loops, Novel Structures Containing G4 DNA, Genes Dev. 18 (13) (2004) 1618–1629.

[31] L.A. Hanakahi, Hui Sun, Nancy Maizels, High affinity interactions of Nucleolin with G-G-Paired rDNA, J. Biol. Chem. 274 (22) (1999) 15908–15912.

[32] Katrin Paeschke, John A. Capra, Virginia A. Zakian, DNA replication through G-Quadruplex motifs is promoted by the Saccharomyces cerevisiae Pif1 DNA helicase, Cell 145 (5) (2011) 678–691.

[33] Hong Xu, Marco Di Antonio, Steven McKinney, Veena Mathew, Brandon Ho, Nigel J. O'Neil, Nancy Dos Santos, et al., CX-5461 is a DNA G-Quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours, Nat. Commun. 8 (February) (2017) 14432.

[34] Gabriel E. Zentner, Alina Saiakhova, Pavel Manaenkov, Mark D. Adams, Peter C. Scacheri, Integrative genomic analysis of human ribosomal DNA, Nucleic Acids Res. 39 (12) (2011) 4949–4960.

[35] Logan R. Myler, Ignacio F. Gallardo, Michael M. Soniat, Rajashree A. Deshpande, Xenia B. Gonzalez, Yoori Kim, Tanya T. Paull, Ilya J. Finkelstein, Single-molecule imaging reveals how Mre11-Rad50-Nbs1 initiates DNA break repair, Mol. Cell 67 (5) (2017) 891–898 e4.

[36] Andrew N. Blackford, Stephen P. Jackson, ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response, Mol. Cell 66 (6) (2017) 801–817.

[37] Shuhei Matsuoka, Bryan A. Ballif, Agata Smogorzewska, E. Robert McDonald 3rd, Kristen E. Hurov, Luo Ji, Corey E. Bakalarski, et al., ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage, Science 316 (5828) (2007) 1160–1166.

[38] Ralph Scully, Arvind Panday, Rajula Elango, Nicholas A. Willis, DNA double-strand break repair-pathway choice in somatic mammalian cells, Nat. Rev. Mol. Cell Biol. 20 (11) (2019) 698–714.

[39] S.ébastien Britton, Julia Coates, Stephen P. Jackson, A new method for high-resolution imaging of ku foci to decipher mechanisms of DNA double-strand break repair, J. Cell Biol. 202 (3) (2013) 579–595.

[40] Lea M. Korsholm, Zita Gál, Lin Lin, Oliver Quevedo, Diana A. Ahmad, Ekaterina Dulina, Yonglun Luo, Jiri Bartek, Dorthe H. Larsen, Double-strand breaks in ribosomal RNA genes activate a distinct signaling and chromatin response to facilitate nucleolar restructuring and repair, Nucleic Acids Res. 47 (15) (2019) 8019–8035.

[41] Aline Marnef, Anne-Laure Finoux, Coline Arnould, Emmanuelle Guillou, Virginie Daburon, Vincent Rocher, Thomas Mangeat, Philippe E. Mangeot, Emiliano P. Ricci, Gaëlle Legube, A cohesin/HUSH- and LINC-Dependent pathway controls ribosomal DNA double-strand break repair, Genes Dev. 33 (17-18) (2019) 1175–1190.

[42] Michael Kruhlak, Elizabeth E. Crouch, Marika Orlov, Carolina Montaño, Stanislaw A. Gorski, André Nussenzweig, Tom Misteli, Robert D. Phair, Rafael Casellas, The ATM repair pathway inhibits RNA polymerase I transcription in response to chromosome breaks, Nature 447 (7145) (2007) 730–734.

[43] Dorthe H. Larsen, Flurina Hari, Julie A. Clapperton, Myriam Gwerder, Katrin Gutsche, Matthias Altmeyer, Stephanie Jungmichel, et al., The NBS1-Treacle complex controls ribosomal RNA transcription in response to DNA damage, Nat. Cell Biol. 16 (8) (2014) 792–803.

[44] Benigno C. Valdez, Dale Henning, Rolando B. So, Jill Dixon, Michael J. Dixon, The treacher collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor, Proc. Natl. Acad. Sci. U.S.A. 101 (29) (2004) 10709–10714.

[45] Eliezer Calo, Bo Gu, Margot E. Bowen, Fardin Aryan, Antoine Zalc, Jialiang Liang, Ryan A. Flynn, et al., Tissue-selective effects of nucleolar stress and rDNA damage in developmental disorders, Nature 554 (7690) (2018) 112–117.

[46] Alberto Ciccia, Jen-Wei Huang, Lior Izhar, Mathew E. Sowa, J. Wade Harper, Stephen J. Elledge, Treacher collins syndrome TCOF1 protein cooperates with NBS1 in the DNA damage response, Proc. Natl. Acad. Sci. U.S.A. 111 (52) (2014) 18631–18636.

[47] Marjolein van Sluis, Brian McStay, A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage, Genes Dev. 29 (11) (2015) 1151–1163.

[48] Clémence Mooser, Ioanna-Eleni Symeonidou, Pia-Amata Leimbacher, Alison Ribeiro, Ann-Marie K. Shorrocks, Stephanie Jungmichel, Sara C. Larsen, et al., Treacle Controls the Nucleolar Response to rDNA Breaks via TOPBP1 Recruitment and ATR Activation, Nat. Commun. 11 (1) (2020) 123.

[49] Miiko Sokka, Kirsi Rilla, Ilkka Miinalainen, Helmut Pospiech, Juhani E. Syväoja, High levels of TopBP1 induce ATR-Dependent shut-down of rRNA transcription and nucleolar segregation, Nucleic Acids Res. 43 (10) (2015) 4975–4989.

[50] Tibor Pankotai, C.éline Bonhomme, David Chen, Evi Soutoglou, DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks, Nat. Struct. Mol. Biol. 19 (3) (2012) 276–282.

[51] Niraj M. Shanbhag, Ilona U. Rafalska-Metcalf, Carlo Balane-Bolivar, Susan M. Janicki, Roger A. Greenberg, ATM-dependent chromatin changes silence transcription in Cis to DNA double-strand breaks, Cell 141 (6) (2010) 970–981.

[52] Shane M. Harding, Jonathan A. Boiarsky, Roger A. Greenberg, ATM Dependent Silencing Links Nucleolar Chromatin Reorganization to DNA Damage Recognition, Cell Rep. 13 (2) (2015) 251–259.

[53] Poorwa Awasthi, Marco Foiani, Amit Kumar, ATM and ATR signaling at a glance, J. Cell. Sci. 128 (23) (2015) 4255–4262.

[54] Joanne Smith, Lye Mun Tho, Naihan Xu, DavidA. Gillespie, The ATM–Chk2 and ATR–Chk1 pathways in DNA damage signaling and cancer, Chapter 3, in: George F.Vande Woude, George Klein (Eds.), Advances in Cancer Research, Vol. 108 Academic Press, 2010, pp. 73–112.

[55] Daniël O. Warmerdam, Jeroen van den Berg, RenéH. Medema, Breaks in the 45S rDNA lead to recombination-mediated loss of repeats, Cell Rep. 14 (11) (2016) 2519–2527.

[56] Carl C. Correll, Jiri Bartek, Miroslav Dundr, The Nucleolus: A Multiphase Condensate Balancing Ribosome Synthesis and Translational Capacity in Health, Aging and Ribosomopathies, Cells 8 (8) (2019), https://doi.org/10.3390/cells8080869.

[57] Yaron Shav-Tal, Janna Blechman, Xavier Darzacq, Cristina Montagna, Billy T. Dye, James G. Patton, Robert H. Singer, Dov Zipori, Dynamic sorting of nuclear components into distinct nucleolar caps during transcriptional inhibition, Mol. Biol. Cell 16 (5) (2005) 2395–2413.

[58] R.C. Reynolds, P.O.'B. Montgomery, Ben Hughes, Nucleolar 'Caps' produced by actinomycin D, Cancer Res. 24 (7) (1964) 1269–1277.

[59] I. Floutsakou, S. Agrawal, T.T. Nguyen, C. Seoighe, A.R.D. Ganley, B. McStay, The shared genomic architecture of human nucleolar organizer regions, Genome Res. (2013), https://doi.org/10.1101/gr.157941.113.

[60] Dafni Eleftheria Pefani, Maria Laura Tognoli, Deniz Pirincci Ercan, Vassilis Gorgoulis, Eric O'Neill, MST2 kinase suppresses rDNA transcription in response to DNA damage by phosphorylating nucleolar histone H2B, EMBO J. 37 (15) (2018), https://doi.org/10.15252/embj.201798760.

[61] Francisca Lottersberger, Roos Anna Karssemeijer, Nadya Dimitrova, Titia de Lange, 53BP1 and the LINC complex promote microtubule-dependent DSB mobility and DNA repair, Cell 163 (4) (2015) 880–893.

[62] Iva A. Tchasovnikarova, Richard T. Timms, Nicholas J. Matheson, Kim Wals, Robin Antrobus, Berthold Göttgens, Gordon Dougan, Mark A. Dawson, Paul J. Lehner, Gene silencing. Epigenetic silencing by the HUSH complex mediates position-effect variegation in human cells, Science 348 (6242) (2015) 1481–1485.

[63] Oscar Fernandez-Capetillo, C. David Allis, André Nussenzweig, Phosphorylation of histone H2B at DNA double-strand breaks, J. Exp. Med. 199 (12) (2004) 1671–1677.

[64] Wang L. Cheung, Kozo Ajiro, Kumiko Samejima, Malgorzata Kloc, Peter Cheung, Craig A. Mizzen, Alexander Beeser, et al., Apoptotic phosphorylation of histone H2B is mediated by mammalian sterile twenty kinase, Cell 113 (4) (2003) 507–517.

[65] Ketki Karanam, Ran Kafri, Alexander Loewer, Galit Lahav, Quantitative live cell imaging reveals a gradual shift between DNA repair mechanisms and a maximal use of HR in mid S phase, Mol. Cell 47 (2) (2012) 320–329.

[66] Josée Guirouilh-Barbat, Sarah Lambert, Pascale Bertrand, Bernard S. Lopez, Is Homologous Recombination Really an Error-Free Process? Front. Genet. 5 (June) (2014) 175.

[67] François Aymard, Beatrix Bugler, Christine K. Schmidt, Emmanuelle Guillou, Pierre Caron, S.ébastien Briois, Jason S. Iacovoni, et al., Transcriptionally active chromatin recruits homologous recombination at DNA double-strand breaks, Nat. Struct. Mol. Biol. 21 (4) (2014) 366–374.

[68] Hélène Bordelet, Karine Dubrana, Keep moving and stay in a good shape to find your homologous recombination partner, Curr. Genet. 65 (1) (2019) 29–39.

[69] Zhiyong Mao, Michael Bozzella, Andrei Seluanov, Vera Gorbunova, Comparison of nonhomologous end joining and homologous recombination in human cells, DNA Repair 7 (10) (2008) 1765–1771.

[70] Michael M. Soniat, Logan R. Myler, Hung-Che Kuo, Tanya T. Paull, Ilya J. Finkelstein, RPA phosphorylation inhibits DNA resection, Mol. Cell 75 (1) (2019) 145–153 e5.

[71] Graciela Spivak, Nucleotide excision repair in humans, DNA Repair 36 (December) (2015) 13–18.

[72] F.C. Christians, P.C. Hanawalt, Lack of transcription-coupled repair in mammalian ribosomal RNA genes, Biochemistry 32 (39) (1993) 10512–10518.

[73] Yanyan Yang, Jinchuan Hu, Christopher P. Selby, Wentao Li, Askar Yimit, Yuchao Jiang, Aziz Sancar, Single-nucleotide resolution analysis of nucleotide excision repair of ribosomal DNA in humans and mice, J. Biol. Chem. 294 (1) (2019) 210–217.

[74] Julie Sollier, Caroline Townsend Stork, María L. García-Rubio, Renee D. Paulsen, Andrés Aguilera, Karlene A. Cimprich, Transcription-coupled nucleotide excision repair factors promote R-Loop-Induced genome instability, Mol. Cell 56 (6) (2014) 777–785.

[75] Nodar Makharashvili, Sucheta Arora, Yizhi Yin, Qiong Fu, Xuemei Wen, Ji-Hoon Lee, Chung-Hsuan Kao, Justin Wc Leung, Kyle M. Miller, Tanya T. Paull, Sae2/CtIP prevents R-Loop accumulation in eukaryotic cells, eLife 7 (December) (2018), https://doi.org/10.7554/eLife.42733.

[76] R.J. Monnat Jr, A.F. Hackmann, M.A. Cantrell, Generation of highly site-specific DNA double-strand breaks in human cells by the homing endonucleases I-PpoI and I-CreI, Biochem. Biophys. Res. Commun. 255 (1) (1999) 88–93.

[77] Thomas Clouaire, Vincent Rocher, Anahita Lashgari, Coline Arnould, Marion Aguirrebengoa, Anna Biernacka, Magdalena Skrzypczak, et al., Comprehensive mapping of histone modifications at DNA double-strand breaks deciphers repair pathway chromatin signatures, Mol. Cell 72 (2) (2018) 250–62.e6.

[78] Sebald An Verkuijl, Marianne G. Rots, The influence of eukaryotic chromatin state on CRISPR-Cas9 editing efficiencies, Curr. Opin. Biotechnol. 55 (February) (2019) 68–73.

[79] Xuebing Wu, David A. Scott, Andrea J. Kriz, Anthony C. Chiu, Patrick D. Hsu, Daniel B. Dadon, Albert W. Cheng, et al., Genome-wide binding of the CRISPR endonuclease Cas9 in mammalian cells, Nat. Biotechnol. 32 (7) (2014) 670–676.

[80] Run-Wen Yao, Guang Xu, Ying Wang, Lin Shan, Peng-Fei Luan, Yang Wang, Man Wu, et al., Nascent Pre-rRNA sorting via phase separation drives the assembly of dense fibrillar components in the human nucleolus, Mol. Cell 76 (5) (2019) 767–783 e11.

[81] Xiao Tian, Denis Firsanov, Zhihui Zhang, Yang Cheng, Lingfeng Luo, Gregory Tombline, Ruiyue Tan, et al., SIRT6 is responsible for more efficient DNA double-strand break repair in long-lived species, Cell 177 (3) (2019) 622–638 e22.