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Review

Pseudo-NORs: A novel model for studying nucleoli

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ABSTRACT

Nucleolar organiser regions (NORs) are comprised of tandem arrays of ribosomal gene (rDNA) repeats that are transcribed by RNA polymerase I (Pol I), ultimately resulting in formation of a nucleolus. Upstream binding factor (UBF), a DNA binding protein and component of the Pol I transcription machinery, binds extensively across the rDNA repeat in vivo. Pseudo-NORs are tandem arrays of a heterologous DNA sequence with high affinity for UBF introduced into human chromosomes. In this review we describe how analysis of pseudo-NORs has provided important insights into nucleolar formation. Pseudo-NORs mimic endogenous NORs in a number of important respects. On metaphase chromosomes both appear as secondary constrictions comprised of undercondensed chromatin. The transcriptional silence of pseudo-NORs provides a platform for studying the transcription independent recruitment of factors required for nucleolar formation by this specialised chromatin structure. During interphase, pseudo-NORs appear as distinct and novel sub-nuclear bodies. Analysis of these bodies and comparison to their endogenous counterpart has provided insights into nucleolar formation and structure.

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1. Introduction

The eukaryotic nucleus is highly compartmentalised. Partially decondensed chromosomes occupy discrete chromosome territories [1–3]. Regulatory proteins are often present in discrete nuclear bodies [4]. This compartmentalisation has fuelled a large body of research into how genes interact with nuclear bodies or their constituent proteins resulting in the formation of transcription factories. The nucleolus is both the most prominent body and the largest transcription factory and represents a paradigm for studying organisation of gene expression within the nucleus (for recent reviews see [5–7]). The primary function of nucleoli is ribosome biogenesis. They form around ribosomal gene (rDNA) repeats and their formation is strictly dependent on transcription by RNA polymerase I (Pol I). Uniquely, the genetic loci containing rDNA repeats, termed Nucleolar Organiser Regions (NORs), can be visualised throughout the cell cycle. During mitosis when transcription is inactivated and the nucleolus is broken down, NORs are undercondensed and visible as a secondary constriction on metaphase chromosomes [8]. Pseudo-NORs are novel artificially constructed arrays that behave in many respects like true NORs but are transcriptionally silent [9,10]. Here we will review how analysis of pseudo-NORs has provided important insights into both secondary constriction and nucleolar formation.

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2. Organisation of human rDNA

Human ribosomal gene (rDNA) repeats comprise ~43 kb [11,12]. Sequences encoding pre-rRNA (13 kb) are separated by long intergenic spacers (IGSs) of approximately 30 kb. Regulatory elements, including gene promoters and transcription terminators, are located in the IGS. The 47S pre-rRNA is processed by a series of endo and exonucleolytic cleavages to generate one molecule each of 18S, 5.8S and 28S rRNA. In situ hybridisation experiments have revealed that clusters of rDNA repeats (NORs), are located on the short arms of the five human acrocentric chromosomes, chromosomes 13, 14, 15, 21 and 22 [13]. Pulse-field gel electrophoresis of genomic DNA digested with enzymes that do not cut human rDNA, such as EcoRV and Sse83871, revealed a major rDNA band of 3 Mb as well as several minor bands of 1 and 2 Mb [14]. This implies that most human NORs are comprised of approximately 70 copies of rDNA repeats and demonstrates that NORs contain only rDNA. rDNA repeats are oriented in a telomere to centromere direction [15,16]. More recently this view of rDNA organisation within NORs has proven to be too simplistic. Single DNA molecule analysis by molecular combing has revealed that NORs comprise a mosaic of canonical and non-canonical rDNA repeats [17]. As much as one third of rDNA repeats are non-canonical, apparently forming palindromic structures. In human chromosomes rDNA repeats appear to be the only genes present on acrocentric short arms, thus isolating them from genes transcribed by Pol II. Isolation of rDNA is further reinforced by the heterochromatised satellite repeats that comprise much of the DNA sequence on either side of NORs [18–21].

Active NORs remain undercondensed during mitosis and have a distinct chromatin structure appearing as a secondary constriction on metaphase chromosomes. rDNA in active NORs is approximately ten-fold less condensed than the adjacent satellite DNA [22]. This undercondensation results in reduced dye binding when chromosomes are stained, giving rise to an apparent gap in the chromosome (Fig. 1). Often, an axis of condensed chromatin is found within the secondary constriction. This condensed chromatin within the constriction has been proposed to contain either IGS sequences [23] inactive rDNA repeats [24] or non rDNA AT-rich DNA [25]. In addition to their decondensed state, a long recognised feature of active NORs is that they can be selectively stained with silver nitrate [8]. The strongest evidence that secondary constrictions correlate with the transcriptional competence of rDNA is that components of the Pol I transcription machinery including UBF (upstream binding factor) remain associated with NORs on mitotic chromosomes [26–28]. A common feature of nucleolar proteins, including UBF (Fig. 2A), is that they contain runs of acidic residues thus providing an explanation for silver staining of active NORs. On inactive NORs, rDNA appears to be packaged in a form that is indistinguishable from the surrounding heterochromatin. During interphase silent NORs can be visualised as condensed foci of rDNA that lack associated Pol I transcription machinery.

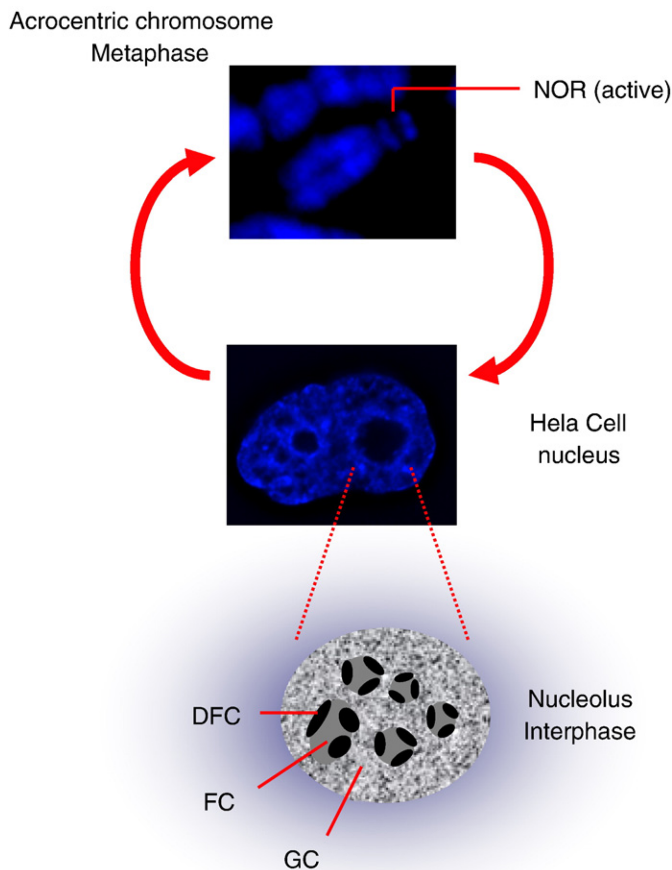

Fig. 1. Nucleolar cycle and structure. The upper panel shows a DAPI stained human acrocentric chromosome with an active NOR appearing as a secondary constriction (gap in staining). The middle panel shows a DAPI stained HeLa cell nucleus. The chromatin density in nucleoli is lower than the surrounding nucleoplasm consequently they appear unstained by DAPI. A cartoon version of a typical nucleolus as observed by electron microscopy is shown below. FC, DFC and GC refer to fibrillar centre, dense fibrillar and granular components of the nucleolus, respectively.

3. RNA polymerase I transcription machinery

Transcription of rDNA by Pol I requires the formation of a pre-initiation complex (PIC) on the promoter, that includes UBF and the promoter selectivity factor, SL1 in human cells or TIF-IB in the mouse (see [29–31] for recent reviews). PICs formed in vitro are stable and support multiple rounds of transcription initiation. In contrast work in yeast demonstrates that PICs are recycled after each round of initiation [32]. The stability of PICs on chromatinised templates in vivo is currently unknown and remains an open question. UBF directly affects Pol I transcription at a number of levels, functioning as a transcription activator [33,34] and as a regulator of transcription elongation [35]. Promoter specificity is conferred by SL1/TIF-1B, a complex that contains TBP (TATA-box binding protein) and at least three Pol I-specific TAF_Is (TBP-Associated Factors (TAF_Is)), TAF_I110/95, TAF_I68 and TAF_I48 [36–38]. Recently, two more TAF_Is have been identified, TAF_I41 and TAF_{II}12, that are required for efficient Pol I transcription initiation [39,40]. TAF_Is interact with UBF and recruit Pol I to rDNA by binding to TIF-1A/Rrn3, a basal regulatory factor that is associated with the initiation-competent subpopulation of Pol I (Pol Iβ) [41–43]. In addition, UBF can also directly interact with PAF53 and PAF49 subunits of Pol I [44,45].

4. The nucleolus

The nucleolus is a dynamic structure. It is disassembled when cells enter mitosis and transcription shuts down and reassembles around individual NORs as cells exit mitosis and transcription of rDNA resumes. During prophase cyclin dependent kinase1-cyclin B (CDK1-cyclin B) levels rise and transcription is inhibited by phosphorylation of components of the Pol I machinery [46,47]. Accumulation of partially processed pre-rRNA during this period suggests that inhibition of processing precedes total repression of Pol I transcription possibly by an independent mechanism [48]. Many processing components are redistributed from the nucleolus to the cytoplasm and others associate with the surface of condensing chromosomes [49]. During metaphase UBF and SL1 are retained on NORs [26–28]. In contrast at least some Pol I subunits (RPA194, 39, 20 and 16) transiently dissociate from NORs and reassociate during anaphase [50,51]. In late anaphase-early telophase when CDK1-cyclin B levels drop, transcription resumes. Pre-rRNA processing components (both chromosome associated and cytoplasmic) accumulate in prenucleolar bodies (PNBs) on the surface of decondensing chromosomes. Processing components are sequentially released from PNB to the now transcriptionally active NORs (see [6,7] for reviews).

The mature interphase nucleolus can be divided into three subcompartments the fibrillar centre (FC), the dense fibrillar component (DFC) and the granular component (GC) [5] (Fig. 1). Pre-rRNA processing intermediates move through these subcompartments. rDNA and pools of factors required for transcription and early steps in pre-rRNA processing are found in the FC. Transcription is proposed to take place either at FC/DFC interface or entirely within the DFC. Early steps in pre-rRNA processing occur in the DFC with the later steps in processing and ribosome assembly taking place in the GC.

Advances in live cell imaging and proteomics have revealed further dynamic aspects of the nucleolus. Most nucleolar proteins studied are continually exchanging between the nucleoplasm and the nucleolus [52–54]. Furthermore, the protein composition and the morphology of subnucleolar compartments can change in response to alterations in the levels of transcription [55]. This dynamic nature of the nucleolus argues for its structure arising solely as a consequence of the functional interaction of its constituents. In this view, factors are enriched in nucleoli as a consequence of functional interactions with other nucleolar components. This model for assembly of large sub-cellular structures is often referred to as self-organisation [56]. In this respect the Pol I transcription machinery

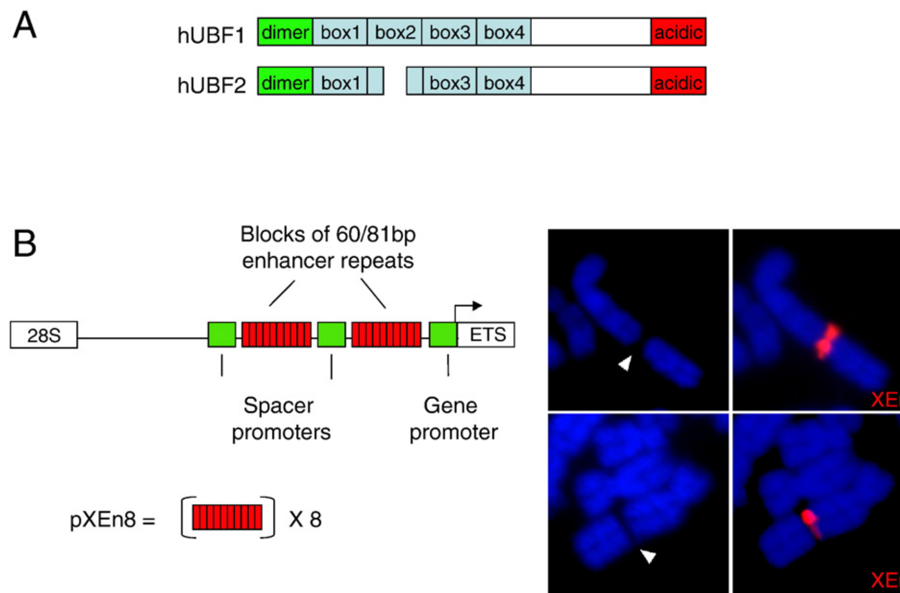

Fig. 2. Generation of pseudo-NORs by integration of UBF binding sequence arrays. (A) Cartoon depicting UBF domain structure. Dimer refers to dimerisation domain, box to HMG box DNA binding motif and acidic to the C-terminal domain enriched in acidic residues. (B) DNA sequence elements present in the intergenic spacer from the *Xenopus laevis* ribosomal gene repeat are illustrated in cartoon form. The plasmid pXEn8 contains 8 blocks of enhancer elements (eighty 60/81 bp repeats, red boxes). This plasmid insert was transfected into human HT1080 cells, together with a blasticidin resistance marker. DAPI stained pseudo-NOR containing chromosomes from one of the derived cell lines are shown on the right. Pseudo-NORs were detected by FISH with an XEn DNA probe (red). Novel secondary constrictions associated with pseudo-NORs are indicated by arrowheads.

presents a number of puzzles. The Pol I machinery is present in vast excess within nucleoli. For example, only 5–10% of nucleolar Pol I is actively engaged in transcriptional elongation [54]. Human cells can also contain a vast excess of UBF over the number of active rDNA promoters [57]. If we accept the above model for nucleolar targeting we need to explain how components of the Pol I machinery not incorporated into PICs or engaged in transcriptional elongation are localised to FCs within nucleoli.

5. UBF binds across the rDNA repeat

UBF is a member of HMG (High Mobility Group) proteins, containing multiple HMG box DNA binding motifs, at least four of which are involved in DNA binding [58–61] (Fig. 2A). In addition to HMG boxes UBF comprises two other distinct domains. It contains a dimerisation domain at its N-terminus and 79 out of the C-terminal 90 residues are negatively charged with 57 being acidic residues (glutamic and aspartic) and 22 serines that are likely to be phosphorylated. The acidic nature of its C-terminus makes UBF highly argyophilic and likely to be one of the targets for the silver staining associated with active NORs. A characteristic feature of the HMG box DNA binding motif is its ability to bend DNA. A dimer of UBF can organise naked, i.e. nucleosome free, promoter DNA into a 360° loop, establishing a structure that resembles the core nucleosome both in mass and DNA content [62,63]. This structure has been termed the 'enhancesome' [64]. In vitro DNA binding assays have failed to identify a consensus other than a preference for binding to GC-rich sequences [65]. This apparent lack of sequence specificity contrasts greatly with its highly specific targeting to rDNA repeats throughout the cell cycle.

UBF exists in two splice variants, UBF1 and UBF2 [66] (Fig. 2A). Despite representing approximately 50% of the UBF present in all mammalian cells, UBF2 cannot function in transcriptional activation as 37 residues are missing from the second HMG box. This observation together with UBF's abundance and binding characteristics suggested that UBF performs roles that do not involve binding to promoter DNA. An indication that this is the case comes from the recognition that UBF plays a role in transcriptional enhancer function [67,68]. Many vertebrate rDNA intergenic spacers have repetitive DNA elements that enhance transcription of the adjacent of the gene promoter [69]. In vitro studies demonstrated that UBF binding to these repetitive elements is required for their function in transcriptional enhancement [68].

Chromatin Immunoprecipitation (ChIP) is a commonly used assay to study the distribution of transcription factors in vivo. In this assay cells are typically treated with 1% formaldehyde for 10 min. This treatment generates protein–DNA and protein–protein crosslinks such that the distribution of factors bound both directly and indirectly to chromatin can be determined. Following formaldehyde treatment a soluble chromatin fraction is prepared by sonication in the presence of detergents. Nucleoli are remarkably dense in protein and RNA and resistant to sonication even in the absence of formaldehyde cross-linking [70]. The concern that standard ChIP protocols would not quantitatively release rDNA chromatin, or would release an unrepresentative fraction prompted the development of a protocol specific for nucleolar chromatin [71]. In this altered protocol cells are treated with a lower percentage of formaldehyde (0.1–0.2%), then nucleoli are isolated by sonication and sedimentation through sucrose. Dispersal of nucleolar chromatin by addition of detergent is monitored microscopically. Finally a soluble nucleolar chromatin fraction is generated by further sonication. A soluble chromatin fraction cannot be prepared with nucleoli isolated from cells treated with 1% formaldehyde (B. McStay, unpublished observation). Application of nucleolar ChIP provided the first demonstration that UBF binds throughout the intergenic spacer and the pre-rRNA coding region in vivo [71]. This suggested that UBF plays an important role in organising rDNA chromatin on active NORs. At the time it was speculated that extensive binding of UBF across the rDNA repeat could be directly responsible for the formation of secondary constrictions at active NORs.

6. UBF and chromatin

The fact that UBF binding is observed across the entire rDNA repeat, raises the question as to whether UBF binding and the presence of nucleosomes are compatible. ChIP experiments do not provide sufficient resolution to distinguish whether UBF binds to nucleosomal DNA or forms enhancesomes interspersed with

nucleosomes. However there is evidence that UBF can associate with nucleosomal DNA in vitro [72]. Furthermore, UBF can displace histone H1 from histone octamers in vitro, thereby promoting decompaction of chromatin [72]. The interaction of the linker histone H1 with nucleosomes is known to stabilize compact higher order chromatin structures and impede the access of regulatory factors (for review, see [73]). These results suggest that UBF binds to rDNA on nucleosomes, possibly in a manner similar to that proposed for the HMG box protein HMGB [74]. In support of UBF promoting decompaction of chromatin, RNAi-mediated depletion of UBF has been found to increase the level of histone H1 on rDNA (E Sanij and R Hannan, personal communication). Further evidence indicating that UBF binds to nucleosomal DNA comes from the analysis of pseudo-NORs (see below).

7. Pseudo-NORs mimic true NORs

A commonly used strategy to study the consequences of interaction between specific genes and their regulatory factors at the microscopic level is to construct artificial arrays that contain many copies of the regulatory sequences of the gene in question. In the case of NORs this approach is particularly appropriate due to their large size (~3 Mb) and repeated sequence content. The construction of large UBF binding DNA sequence arrays on non-NOR bearing human chromosomes has provided compelling support for a UBF role in secondary constriction formation [9]. The UBF binding sequences used were 60/81 bp repeats from the IGS of *Xenopus* rDNA (Fig. 2B). UBF is sufficiently conserved between *Xenopus* and humans that it can bind efficiently to these so called XEn repeats [33,68]. The largest of these arrays was ~2 Mb in length, approximating the size of endogenous NORs. These ectopic UBF binding site arrays, termed pseudo-NORs due to the absence of promoter sequences, are associated with UBF throughout the cell cycle and adopt the key morphological features of active NORs during metaphase, i.e. they are undercondensed, appearing as achromatic regions on DAPI stained chromosomes (Fig. 2B). Notably, pseudo-NORs are positive in silver staining despite their transcriptional silence [9]. It can be inferred from this that the appearance of secondary constrictions at NORs on metaphase chromosomes results from binding of argyophilic (strongly silver staining) proteins that prevent full condensation. Furthermore, this process is not dependent on transcription of rDNA in the previous cell cycle. In support of a direct role for UBF, its depletion by siRNA leads to both loss of secondary constrictions and silver staining at pseudo-NORs ([10] and unpublished observations). Thus, besides its role in Pol I transcription, UBF plays an important role in promoting under-condensation of active NORs [10] and maintaining an active chromatin structure through cell divisions.

Further analysis of pseudo-NORs provides additional evidence for UBF binding to nucleosomal DNA. XEn DNA sequences appear as a classical nucleosomal ladder when nuclei from pseudo-NOR containing cells are digested with micrococcal nuclease [75]. Moreover, pseudo-NORs can be readily visualised when cells are stained with antibodies against acetylated histone H4, consistent with pseudo-NORs exhibiting a euchromatic structure (Wright and McStay, unpublished observation).

8. UBF binding promotes recruitment of the Pol I machinery to NORs

As stated above, components of the Pol I transcription machinery are present in excess yet predominantly colocalise with rDNA in nucleoli. Given that SL1 and Pol I make direct protein–protein contacts with UBF in vitro it is possible that this excess of factors is recruited to nucleoli by interaction with UBF bound not only at the promoters but at other sites across the rDNA repeat. Support for this view has come from the finding that Pol I machinery could be found associated with rDNA chromatin across the IGS as revealed by ChIP [9]. In these experiments it appeared that larger amounts of Pol I were associated with IGS chromatin than with the gene promoter and transcribed sequences, respectively.

Results from live cell imaging experiments with GFP-tagged Pol I fully support the above conclusion. Inverse fluorescent recovery after photobleaching (iFRAP) quantifies the loss of fluorescence of the region of interest after complete bleaching outside the region. This provides a direct measure of the residency time of the proteins in the region of interest. iFRAP demonstrated that the majority of each Pol I subunit examined associated with ribosomal genes is not engaged in transcription and rapidly dissociates from rDNA [54]. Only 10% of nucleolar Pol I is engaged in transcription elongation as evidenced by its slower dissociation kinetics. The rapidly dissociating fraction of Pol I observed by iFRAP is most likely the same Pol I fraction observed by ChIP associated with the human intergenic spacer chromatin. A high local Pol I concentration presumably aids efficient Pol I recruitment at the gene promoter required to support the high rates of transcription initiation observed on rDNA.

The other components of the Pol I machinery required for efficient transcription initiation, SL1 and TIF-1A/Rrn3, are highly abundant within nucleoli. For example HeLa cells contain at least two orders of magnitude excess of SL1 over the number of active promoters (derived from quantitative western blots, Wright and McStay unpublished observation). This level of abundance indicates that only a fraction of SL1 is engaged in PICs at any given time. ChIP experiments reveal that non PIC engaged SL1 and TIF-1A/Rrn3 associate with IGS chromatin [9]. The presence of high local concentrations of SL1 makes little sense if PICs are highly stable, supporting many rounds of transcription and possibly staying intact through the cell cycle. If on the other hand PICs recycle after each initiation event, high local concentrations of SL1 would aid rapid formation of new PICs.

A number of lines of evidence support a model in which recruitment of the Pol I machinery to NORs is independent of promoters and transcription but dependent on UBF. Expression of a chimeric lac repressor-UBF fusion protein can result in sequestration of a fraction of endogenous Pol I at large arrays of lac operator sequence that are 30 and 45 fold larger that NORs and pseudo-NORs, respectively [76]. Pseudo-NORs are more biologically relevant in size, chromatin context and the fact that they recruit endogenous factors. In every case examined the Pol I machinery is highly enriched at pseudo-NORs [9]. Importantly, siRNA experiments reveal that this recruitment is strictly UBF dependent [10]. The ability of pseudo-NORs to sequester the Pol I machinery is independent of the chromosomal integration site of the XEn array or its position within the interphase nucleus. This supports the conclusion that nucleolar localisation of the

Table 1
Components of ribosome biogenesis machinery tested for pseudo-NOR association

Present at pseudo-NORs	Absent at pseudo-NORs
UBF1/2	SSU components
RNA Polymerase I subunits	hU3–55K
RPA195	MPP10
RPA135	Imp3
PAF53	Imp4
PAF49 (CAST)	UTP12
RPA43	Box C/D and H/ACA snoRNP components
SL1 components	Fibrillarin
TBP	Nap57
TAF_I110	Nucleolin
TIF-IA/Rrn3	Nucleophosmin
SSU components	
tUTP4 (Cirhin)	
tUTP5	
tUTP10	
tUTP17	
Treacle	
Nopp140	

Pol I machinery is driven by its affinity for UBF loaded chromatin and provides an explanation for localisation of the Pol I machinery within the FC that is not engaged in PICs or transcriptional elongation.

9. Pseudo-NORs recruit factors required for early events in pre-rRNA maturation

Studies using electron microscopy have provided support for the contention that early processing events and transcription of pre-rRNA are intimately connected. The clearest illustration of this fact is that a structure, termed the terminal knob, that forms on the 5′ end of nascent transcripts and is observed in Miller spreads, contains the U3 small nucleolar ribonucleoprotein (snoRNP) required for the cleavages that yield 18S rRNA [77]. The terminal knob, also called the SSU (Small Sub-Unit) processome is now known to contain not just the U3 snoRNP but a large collection of other factors some previously implicated in 18S rRNA maturation as well as a collection of other open reading frames of previously unknown function termed UTPs (U Three proteins) [78–80]. Work in yeast [81] and more recently in human cells [10] has described a subset of these proteins, termed tUTPs (transcription UTPs) that represent an SSU sub-complex (UTP-A) [80] and are required for both transcription and SSU formation. Intriguingly these tUTPs, but no other components of the mature SSU processome (including U3 snoRNA), are efficiently recruited to pseudo-NORs [10] (see Table 1). This finding together with the observation that tUTPs associate with IGS chromatin in endogenous NORs [10] is consistent with their proposed role in coupling the transcription and processing machineries [81].

In addition to SSU processome formation, pseudouridylation and 2′-*O* methylation of pre-rRNA carried out by box H/ACA and box C/D

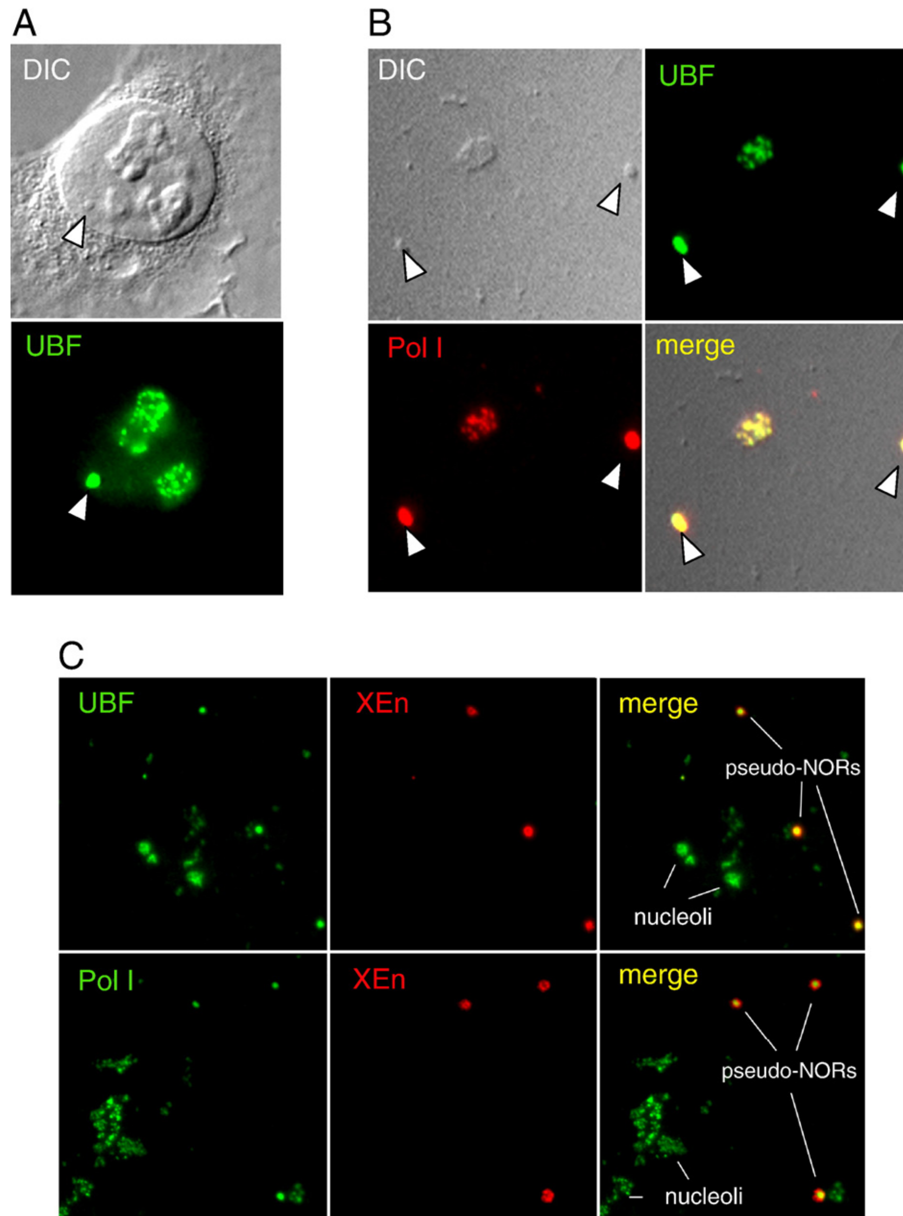

Fig. 3. Pseudo-NORs can be released intact from interphase cells. (A) DIC image and immunofluorescent staining of a pseudo-NOR containing cell line with anti-UBF antibodies. Pseudo-NORs are indicated by arrowheads. (B) Pseudo-NORs and nucleoli were released from interphase cells by repeated sonication and sedimented through sucrose. The resuspended material was fixed onto poly-lysine coated slides and probed with antibodies against UBF (green) and Pol I (red). Arrowheads indicate the presence of pseudo-NORs on DIC and fluorescent images. Note the additional staining of nucleolar material. (C) Slides containing released pseudo-NORs and nucleoli were subject to combined immuno FISH using an XEn DNA probe (red) and antibodies to UBF (upper panels) or Pol I (lower panels). Positions of pseudo-NORs and nucleoli are indicated.

snoRNPs, respectively are also thought to occur co-transcriptionally [82,83]. Nucleolar phosphoprotein Nopp140 associates with box H/ACA snoRNPs [84] and a related nucleolar phosphoprotein Treacle interacts with box C/D snoRNPs [85]. They are not required for the catalytic activity of their associated snoRNPs. The finding that Nopp140 and Treacle but not core constituents of H/ACA and box C/D snoRNPs (fibrillarin and NAP57 respectively) are targeted to pseudo-NORs suggests that like tUTPs, recruitment to rDNA, chromatin provides a means of coordinating transcription with pre-rRNA maturation [10]. As with the Pol I machinery, recruitment of tUTPs, Nopp140 and Treacle to pseudo-NORs is strictly dependent on UBF, occurring independent of both promoters and transcription. Treacle can associate directly with UBF and remains associated with NORs through metaphase [86]. Nopp140 has been reported to interact with Pol I [87], is not found associated with NORs during metaphase, but is recruited during telophase. The mechanism and timing of tUTP recruitment is still under investigation. A full list of the proteins tested for pseudo-NOR association is shown in Table 1.

10. What pseudo-NORs tell us about nucleolar formation and structure

To understand how nucleoli reform after cell division we need a detailed description of the order of events. Two key events are the resumption of transcription and pre-rRNA processing. It appears that reactivation of these processes occurs by independent mechanisms. Premature inactivation of CDK1-cyclin B by treatment of metaphase cells with roscovotine leads to reactivation of transcription but not pre-rRNA processing [88]. Nevertheless, it is essential that these two processes are reactivated in a highly coordinated or coupled manner, otherwise unprocessed precursor RNAs would accumulate. The notion that transcription and processing are coupled is reinforced by the identification of tUTPs and the recent finding that transcription elongation by Pol I is linked to efficient rRNA processing and ribosome assembly [89]. One way of coordinating these two processes is for NORs to sequester components of both the transcription and processing machineries prior to the onset of transcription. The retention of certain factors on mitotic NORs clearly identifies some of these, UBF, SL1 and Treacle. The transcriptional silence of pseudo-NORs and their separation from nucleoli has allowed identification of other factors recruited to NORs independent of transcription, including tUTPs and Nopp140. Further analysis of pseudo-NORs has demonstrated that recruitment of these transcription and processing components to NORs is absolutely dependent on the specialised chromatin structure specified by UBF [10].

During metaphase when transcription is repressed, pseudo-NORs appear similar in morphology to active endogenous NORs. During interphase, pseudo-NORs appear as novel nuclear bodies and their protein composition most closely resembles the FC component of mature nucleoli (Fig. 3A, Table 1). Treatment of cells with low concentrations of actinomycin D specifically inhibits Pol I transcription. This results in the separation of the nucleolar components. FC and DFC components condense and migrate to the periphery of the nucleolus forming caps associated with a central body derived from the GC. FC and DFC components remain distinct within these caps (reviewed in [6]). Pseudo-NORs bear a striking resemblance in appearance to caps in the segregated nucleoli of actinomycin D treated cells [10]. We propose that the fusion of FCs observed in actinomycin D treated cells and the appearance of pseudo-NORs as visible nuclear bodies are related phenomena, both depending on UBF. The notion that pseudo-NORs are equivalent to a protein dense component of the nucleolus (i.e. the FC) is reinforced by the finding that, like nucleoli, they can be released intact from cells by repeated sonication (Fig. 3B and C).

To summarise, the resumption of gene expression after cell division requires decondensation of specific DNA sequences followed by recruitment of the factors required for reformation of transcription factories. NORs achieve these aims by maintaining rDNA chromatin in an undercondensed state and retaining key factors throughout metaphase. The generation and analysis of pseudo-NORs has revealed the central role that UBF performs in maintenance of this primed state.

11. Future analysis of pseudo-NORs

The fact that pseudo-NORs are visible by light microscopy and can be released from cells apparently intact (Fig. 3A) suggests that their protein composition may be more complex than described in Table 1. We anticipate that proteomic analysis of pseudo-NORs identifies the fraction of the nucleolar proteome that is recruited to NORs independent of transcription and provides further insights into the biology of the nucleolus.

Pseudo-NORs provide a powerful tool for establishing how factors are recruited to rDNA chromatin in a transcription and sequence independent manner. This is not only restricted to those described in this review. An ever increasing numbers of factors, particularly those regulating chromatin structure have been implicated in the regulation of ribosomal gene transcription [90]. We anticipate that a fraction of these will recognise the specialised chromatin structure specified by UBF rather than the underlying DNA sequence. Pseudo-NORs provide a convenient way of discriminating this fraction. For example SIRT7 is an NAD-dependent histone deacetylase, enriched in nucleoli [91] and a positive regulator of Pol I transcription [92]. Endogenous SIRT7 is highly enriched in pseudo-NORs (Wright and McStay unpublished observation).

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