Old and new faces of the nucleolus

Workshop on the Nucleolus and Disease

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Although the primary function of this prominent nuclear organelle is in ribosome biogenesis, a growing body of evidence indicates that it also participates in other aspects of RNA processing, as well as in the regulation of mitosis, cell growth and death, stress responses and the cell cycle (Fig 1). Indeed, the nucleolus has emerged as a highly complex and multifunctional regulatory compartment, the roles of which in diverse biological processes we are only just beginning to understand.

The workshop began with a keynote lecture from M. Olson (Jackson, MS, USA), who gave an excellent historical account of the milestones in the field, starting with the discovery of the nucleolus more than 200 years ago. He reminded us that the acquisition of knowledge about the structure and function of the nucleolus is tightly linked with developments in microscopy, and that technological advances in the fields of fluorescent microscopy, fluorescent protein tags and proteomics are now allowing the elucidation of the complexities of this organelle.

The dynamic nucleolar proteome

Isolated nucleoli continue to transcribe ribosomal RNA (rRNA), indicating that the nucleolus is a stable structure; however, its protein content is continually in a state of flux. A. Lamond (Dundee, UK) focused on the use of second-generation proteomics to annotate this protein flux. His group have designed a ‘spatial proteomics’ protocol to characterize the protein content of the nucleolus, nucleoplasm and cytoplasm at a single given time, which involves a combination of stable isotope-labelling of amino acids in culture with cell fractionation and quantitative mass spectrometry. The Lamond group has used this method to quantitate the localization of approximately 3,000 cellular proteins and their transport in response to the induction of the nucleolar tumour-suppressor protein, ARF. Technological advances have also allowed the Lamond group to expand coverage of the human nucleolar proteome to include 80% of known ribosomal proteins.

M. Laiho (Helsinki, Finland, and Baltimore, MD, USA) is interested in changes in the nucleolar proteome in response to ultraviolet (UV)-C radiation. Her group has found that the nucleolus undergoes gross morphological changes in response to UV-C, and she presented quantitative proteomic data indicating that these changes are associated with the selective reorganization of the nucleolar proteome. The relocalization of nucleolar proteins in response to UV-C might facilitate the induction of nuclear/cytoplasmic stress-response pathways.
Nucleolar structure and assembly

The human nucleolus is sub-compartmentalized into the fibrillar centre (FC), the dense fibrillar component (DFC) and the granular component (GC), and this structure is maintained by the molecular processes of active ribosome biogenesis. During mitosis, the nucleolus disassembles and the components of the rRNA transcription complex migrate with ribosomal genes, while the processing machinery that binds to upstream binding factors (UBFs) and mimic the specialized chromatin structure of NORs—to investigate the recruitment of processing factors to NORs in the absence of transcription. They found that tUTPs—but not other components of the SSU processing complex—and the pre-rRNA processing factors Treacle, TCOF1 and NOPP140 are recruited to pseudo-NORs in a UBF-dependent manner (Prieto & McStay, 2007). These factors might provide a link between pre-rRNA processing and transcription.

Ribosome biogenesis

One of the first steps in ribosome biogenesis is the transcription and processing of pre-rRNA from rDNA, which is organized into tandem repeats known as nucleolar organizer regions (NORs). The components of the small subunit (SSU) processing complex known as tUTPs are required for the efficient transcription of pre-rRNA in humans, suggesting that there is a coupling of rRNA transcription and processing. B. McStay (Galway, Ireland) and colleagues used pseudo-NORs—which are transcriptionally silent artificial DNA arrays that bind to upstream binding factors (UBFs) and mimic the specialized chromatin structure of NORs—to investigate the recruitment of processing factors to NORs in the absence of transcription. They found that tUTPs—but not other components of the SSU processing complex—and the pre-rRNA processing factors Treacle, TCOF1 and NOPP140 are recruited to pseudo-NORs in a UBF-dependent manner (Prieto & McStay, 2007). These factors might provide a link between pre-rRNA processing and transcription.

Ribosomal gene transcription is regulated by modulation of the transcriptional apparatus and by epigenetic silencing. H. Bierhoff (Heidelberg, Germany) demonstrated that the Pol I co-factor TIF-1A is phosphorylated by CK2 in response to external signals, and that this disrupts the interaction of TIF-1A with Pol I, promoting the elongation of pre-rRNA transcripts and cell proliferation (Bierhoff et al., 2008). In Arabidopsis, silencing at rDNA repeats involves small interfering RNA (siRNA)-directed DNA methylation. C. Pikaard (Washington, DC, USA) showed that the 24-nucleotide (nt) siRNAs required for this process are generated in Cajal body-like nucleolar dots that he termed nucleolar siRNA-processing centres (Pontes et al., 2006). Pikaard also presented data demonstrating that in interspecies genetic hybrids, in which only one set of rRNA genes is active (a phenomenon known as nucleolar dominance), these 24-nt siRNAs bind to intergenic spacer regions of the non-active rRNA genes and are required for epigenetic silencing. He suggested that the siRNAs afford sequence specificity to de novo DNA methylation at these regions. In mammalian cells, the epigenetic silencing of rDNA involves the nucleolar remodelling complex (NoRC). Analogous to the situation in Arabidopsis, NoRC-mediated silencing requires 100–350 nt RNA molecules, which originate from an intergenic spacer region upstream of the pre-rRNA promoter (pRNA) and form a conserved stem–loop structure (Mayer et al., 2006). I. Grummt (Heidelberg, Germany) showed that the secondary structure of pRNA is crucial for binding to TIP5, which is the large subunit of NoRC, targeting NoRC to nucleoli and facilitating rRNA gene silencing. Grummt also demonstrated that TIP5 acetylation is required for its silencing functions, although the interaction between pRNA and TIP5 is impaired by this post-translational modification. She presented a model to reconcile these data that involved acetylation-mediated displacement of pRNA on binding of TIP5 to chromatin (Fig 2).
Regulation of cell cycle/apoptosis by nucleolar sequestration
- Cdc14—mitotic exit
- Tumour suppressor proteins p53/MDM2/ARF
- RelA component NF-κB—apoptosis
- SUMO-specific protease SENP-5—cell division
- Redistribution of telomeric components—ageing
- c-Myc oncogene—cell growth
- PP1γ—cell cycle progression

Assembly of RNP production (plants)
- Signal recognition particle
- RNPase enzymes (RNP) in tRNA processing
- Telomerase RNP

Processing of RNA
- Some tRNAs
- Polycistronic snoRNAs
- Maturation of snRNAs

mRNA surveillance/ non-sense-mediated decay (plants)
- Nucleolar transport of HIV mRNA/Rev/Tat
- Animal and plant virus proteins

Virus replication
- Nucleolar transport of HIV mRNA/Rev/Tat
- tRNA, small-nucleolar ribonucleoprotein
- qβ isoform of protein phosphatase 1
- rRNA, ribosomal DNA
- RelA, a subunit of NF-κB
- PP1γ, γ-isoform of protein phosphatase 1
- HIV, human immunodeficiency virus

Sensor of cell stress
- JNK2-Tif-1A mediated inhibition
- rRNA transcription
- Stabilization of p53

Nucleolar transport of HIV mRNA/Rev/Tat
- Animal and plant virus proteins

Active surveillance mechanisms are in place to monitor the synthesis of RNA, including that of tRNA. D. Tollervey (Edinburgh, UK) demonstrated that in Saccharomyces cerevisiae, non-coding RNAs from rDNA intergenic spacer regions (such as IGS1-R) are targets for exonome-mediated degradation. Trf4, which is a component of the TRAMP4 complex, the exosome protein Mtr3, the nuclear-specific exosome component Rrp6, and the RNA-binding proteins Nrd1 and Nab3 are required for this degradation. Tollervey also demonstrated a role for the Sβ exonuclease Rat1 in efficient transcription termination of tRNA genes. These results reveal potentially important links between the transcriptional and post-transcriptional steps of RNA synthesis.

Eukaryotic 18S ribosomal RNA processing is mediated by the SSU processome, which comprises U3 small-nucleolar ribonucleoprotein (snORNP), IUTP, bUTP and MPP10 subcomplexes, as well as many additional factors. Interestingly, mutations in SSU processome proteins are linked to several diseases, including male infertility and childhood cirrhosis; however, little is known about how the SSU processome is assembled. S. Baserga (New Haven, CT, USA) focused on the architecture of one of the bUTP subcomplexes of the SSU processome using S. cerevisiae as a model system. She presented a comprehensive map of interactions between six proteins within the subcomplex, and defined a crucial role of the interaction between UTP6 and UTP21 for its function. She also showed that the amino-terminal domain of UTP6 interacts with UTP18, whereas the UTP6 HAT domain interacts with UTP21. Mutational analysis of the UTP6 HAT domain indicated that the latter interaction is essential for pre-rRNA processing and cell growth (Champion et al, 2008).

N. Watkins (Newcastle, UK) found that in humans, the box C/D motif of U3 snoRNP is essential for recruitment into the SSU processome, and both the box B/C motif and the 3’ hinge are needed for binding to the MPP10 complex and subsequent localization to the GC. In further studies, Watkins found that the inhibition of RNA transcription and/or processing—using actinomycin D or tUTP depletion—resulted in the accumulation of a new 50S U3 snoRNP-processing intermediate. The 50S complex contains nucleolin but no other crucial SSU factors, and is located in the DFC where processing takes place. He concluded that tUTP is required for the recruitment of this crucial intermediate into the SSU processome.

RNA processing in the nucleolus
Further functions are being discovered for the nucleolus in a wide range of RNA-processing and RNP-assembly activities (Fig 1). J. Brown (Dundee, UK) demonstrated a role for the plant nucleolus in messenger RNA (mRNA) surveillance and nonsense-mediated decay (NMD) by showing that there are similar amounts of total mRNA in the nucleoplasmic and nucleolar compartments, but that aberrantly spliced mRNAs are enriched in the nucleolar fractions. Most of these aberrant transcripts contain premature termination codons, which are generally the target of NMD. Consistent with the hypothesis that NMD takes place in the Arabidopsis nucleolus, it was found that UPF2 and UPF3, which are core components of the SSU processome, which comprises U3 small-nucleolar ribonucleoprotein (snORNP), IUTP, bUTP and MPP10 subcomplexes.
of the functional NMD complex, localize to this compartment. Furthermore, the expression of mutated forms of UPF2 and UPF3 induces the nucleolar accumulation of aberrantly spliced mRNA. This work provides evidence for a new nucleolar function in plants, and raises interesting questions about the pathway of NMD, and about where and how mRNA surveillance occurs in this species.

M. Carmo-Fonseca (Lisbon, Portugal) argued for the presence of a new nucleoplasmic compartment that functions as a quality-control centre for mRNA. In S. cerevisiae, it has been shown that 3′-end formation of mRNA is monitored by a pathway that requires the nuclear exosome component Rrp6 (Hilleren et al., 2001). Carmo-Fonseca showed that yeast strains lacking Rrp6 or other components of the nuclear exosome accumulate polyadenylated RNA, U14 snoRNA and snoRNP proteins in a nucleoplasmic foci that are distinct from the nucleolus (Carneiro et al., 2007). It was proposed that these foci represent quality-control centres.

The nucleolus in viral infection
Many viruses target nucleolar functions as part of their infection strategy. Several talks at the meeting focused on recent advances that improve our understanding of how viruses use nucleolar proteins and functions for their own benefit.

The nucleolar localization of a protein is determined by various factors, including nucleolar-localization signals (NoLSs). J. Hiscox (Leeds, UK) studied the nucleolar transport of three viral proteins from diverse viruses: nucleocapsid protein from infectious bronchitis virus and avian coronavirus, the ORF57 protein of herpesvirus saimiri and the REV protein of HIV-1. These proteins were also used to construct chimeric proteins in which the NoLS was replaced with that of another virus (Emmott et al., 2008), which allowed Hiscox to show that NoLSs are responsible for distinct nucleolar localizations and transport rates. Nuclear import/export rates also contribute to nucleolar localization: the rapid nuclear import and slower nuclear export of the N protein explain its nucleolar localization. D. Matthews (Bristol, UK) focused on the nucleolar localization of the viral proteins that are encoded by adenoviruses. He showed that three adenoviral proteins, pMu, Protein V and pVII, are targeted to the nucleolus, and he identified their NoLSs. In addition, he showed that Protein V regulates the ARF, HDM2, p53 pathway by initiating a decrease in ARF levels and the misdistribution of HDM2. As Protein V is a component of incoming virus particles, Matthews speculated that it might have an opportunity to affect the host cell immediately after the virus has gained entry.

M. Taliansky (Dundee, UK) examined the role of the nucleolar protein fibrillarin in the systemic infection of the plant virus, groundnut rosette virus (GRV). He showed that the ability of the GRV ORF3 protein to move viral RNA long distances through the phloem—the specialized plant-transport system—depends strictly on its interaction with fibrillarin. The ORF3 protein enters the nucleolus, where it forms complexes with fibrillarin that are relocalized and...
to the cytoplasm at a later stage. In the cytoplasm these complexes interact with viral RNA to form viral RNP that is able to move long distances (Canetta et al., 2008). A. Whitehouse (Leeds, UK) showed that γ-2 herpesviruses also use the nucleolus for viral mRNA transport. The ORF57 protein that is encoded by these viruses is able to shuttle between the nucleus and the cytoplasm, bind to viral mRNA, and interact with various nuclear import and export factors. Moreover, this protein is responsible for the relocation of some nuclear export factors into the nucleolus, in particular the hTREX proteins; this indicates that the ORF57 protein either assembles the export-competent viral RNP particle within the nucleolus, or travels through the nucleolus to modify these proteins or the viral mRNA. M. Lymberopoulos (Laval, Canada) also demonstrated the redistribution of several nucleolar proteins during infection with another herpesvirus, herpes simplex virus 1. However, the mechanisms of such redistribution are distinct for different nucleolar proteins.

The practical implications of virus–nucleolus interactions were discussed by J. Rossi (Duarte, CA, USA), who presented evidence that HIV is a sensitive target for inhibitory small RNAs that localize to the nucleolus. Using a library of nucleolar localizing ribozymes, Rossi selected two that targeted HIV and provided potent inhibition to the nucleolus. Using a library of nucleolar localizing ribozymes, Rossi selected two that targeted HIV and provided potent inhibition to the nucleolus. Using a library of nucleolar localizing ribozymes, Rossi selected two that targeted HIV and provided potent inhibition to the nucleolus. M. Lymberopoulos (Laval, Canada) also demonstrated the redistribution of several nucleolar proteins during infection with another herpesvirus, herpes simplex virus 1. However, the mechanisms of such redistribution are distinct for different nucleolar proteins.

The nucleolus in development, cell growth, death and cancer

In contrast to the well-defined structure of the somatic-cell nucleolus (see above), nucleoli from fully grown mammalian oocytes that are competent to mature are transcriptionally inactive and form compact fibrillar masses known as nucleolar precursor bodies. S. Ogushi (Kobe, Japan) and colleagues set out to determine whether this nucleolar material contributes to embryogenesis (Ogushi et al., 2008). In an elegant series of experiments that involved removing the nucleolus from mouse oocytes, they showed that the zygotic nucleolus is maternally inherited and is essential for further embryonic development. They also revealed that this nucleolar material is in some way special, as embryonic development progressed when the oocytes were reconstituted with oocyte nucleoli but not with nucleoli from somatic cells.

Several talks at the meeting contributed to our understanding of the nucleolus as a regulator of cell growth and death, and the role of the organelle in carcinogenesis. J. Milner (York, UK) examined the effects of RNA interference-induced silencing of the cell-survival genes SIRT1, JNK2 and p53 under basal non-stress conditions of cell growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependent deacetylase with growth. Mammalian SIRT1 is an NAD-dependen...
required for the targeting of endogenous PML proteins to this organelle. Spontaneous or oncogene-retrieval-induced senescence is associated with the formation of large PML nuclear bodies that initially contain nucleolar components. Later, poly-ubiquitin conjugates are found on the outer shell or within most of these senescence-associated PML bodies.

M. Hetman (Louisville, KY, USA) identified the nucleoli of postmitotic neurons as sensors of DNA damage. In camptothecin-treated cultured cortical neurons, a selective reduction of rRNA transcription disrupted nucleolar integrity, leading to p53-mediated apoptosis that was dependent on the de novo expression of protein-coding genes (Kalita et al., 2008). Therefore, the rDNA selectivity of DNA damage-induced transcriptional inhibition might determine its ability to induce neuronal apoptosis. Notably, extensive nucleolar disruption has also been observed in a rat model of cortical ischaemia, suggesting that nucleolar stress might contribute to the pathology of neurological diseases, including stroke.

**Concluding remarks**

The nucleolus was once thought to be merely a ribosome-producing factory. However, this second EMBO meeting on the nucleolus highlighted the diverse roles of the organelle in both health and disease. The meeting brought together participants from a wide range of backgrounds to discuss the growing repertoire of functions of the nucleolus. The meeting was lively and well organized, and allowed for much discussion. Although it was agreed that rapid progress is being made in this area, it is clear that there is still much to be discovered, which will undoubtedly be discussed at future meetings.

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