The nucleolus

Yun Wah Lam*, Laura Trinkle-Mulcahy*,‡ and Angus I. Lamond

Wellcome Trust Biocentre, MSI/WTB Complex, University of Dundee, Dundee, DD1 5EH, UK

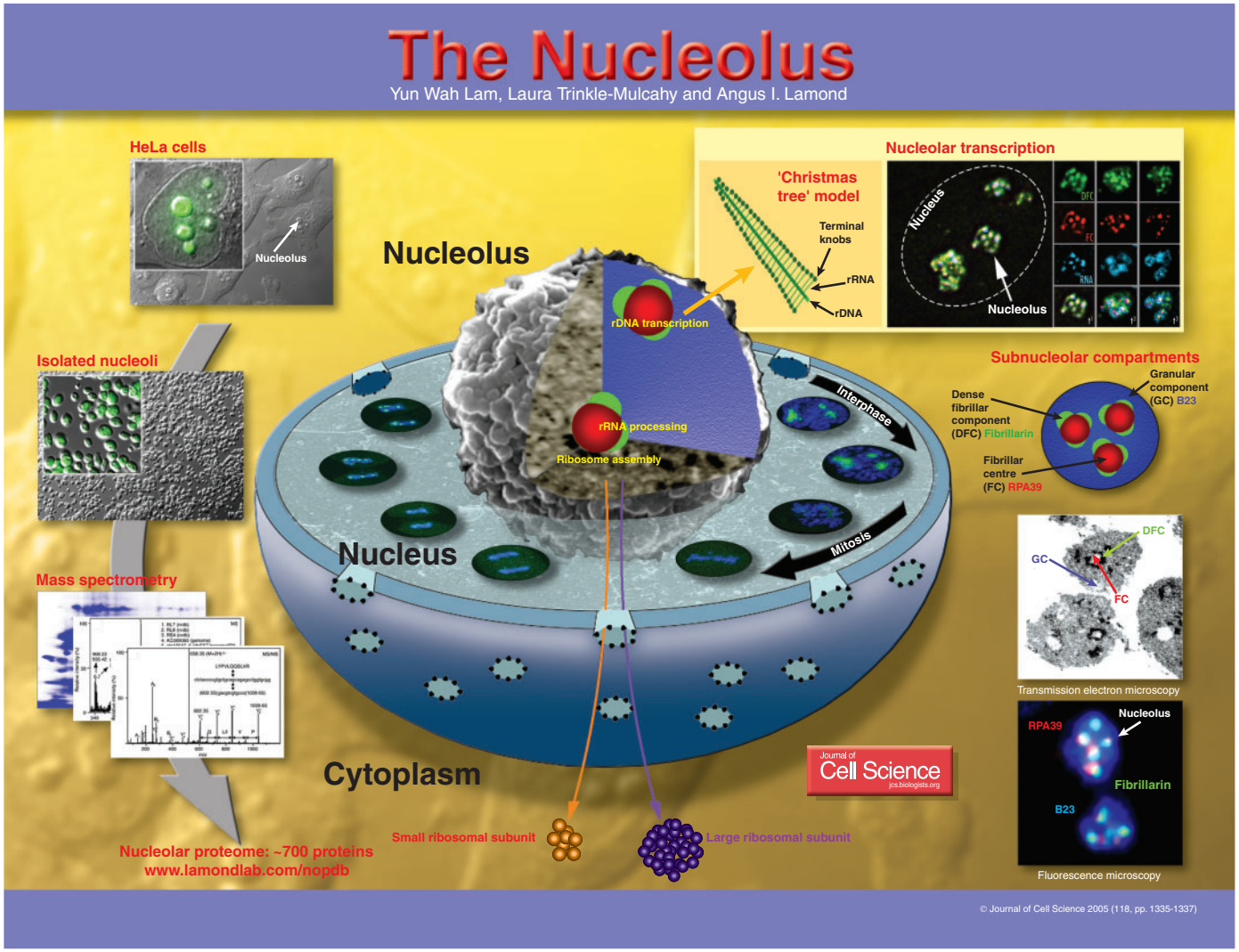
*These authors contributed equally to this work
‡Author for correspondence (e-mail: l.trinklemulcahy@dundee.ac.uk)

Journal of Cell Science 118, 1335-1337
Published by The Company of Biologists 2005
doi:10.1242/jcs.01736

The nucleolus is the most prominent structure in a cell nucleus. It is the site of ribosomal RNA (rRNA) transcription, pre-rRNA processing and ribosome subunit assembly (reviewed by Olson et al., 2002). The nucleolus is a dynamic structure that assembles around the clusters of rRNA gene repeats during late telophase, persists throughout interphase and then disassembles as cells enter mitosis. Owing to the difference in density between the nucleolus and the surrounding nucleoplasm, it is readily visible in either live or fixed cells viewed by phase contrast or differential interference contrast (DIC) optics (upper-left panel). Thanks to the advent of fluorescent protein (FP) technology, nucleoli can also be detected by fluorescence microscopy in cell lines expressing FP-tagged nucleolar proteins. An example is shown in the inset of the upper-left panel, in which PP1γ, a protein phosphatase that accumulates in the nucleolus, is tagged with YFP and stably expressed in HeLa cells (Trinkle-Mulcahy et al., 2003).

Like all other intranuclear structures, the nucleolus is not membrane enclosed, but the combination of its unique density and robust structure makes it one of the most convenient subcellular structures to purify. Thus, when mammalian nuclei are physically disrupted (e.g. by sonication) in a solution of low salt concentration, nucleoli remain intact even under conditions that disintegrate most other subnuclear structures. Nucleoli can therefore be isolated in essentially pure form by centrifuging sonicated nuclei through a density cushion (middle-left panel). The isolated nucleoli are intact, similar in size and morphology to the nucleoli in live cells and even retain transcriptional activity to some extent (Cheutin et al., 2002).

Thanks to the ability to isolate large amounts of purified nucleoli, recent studies have analysed the nucleolar protein composition in great detail using high-throughput mass-spectrometry (MS)-based proteomic techniques



(See poster insert)

Journal of Cell Science

(lower-left panel). For example, initial proteomic studies of HeLa nucleoli reported the identification of over 400 proteins (Andersen et al., 2002; Scherl et al., 2002). Ongoing improvements in protein separation methods prior to MS, and in the sensitivity of MS, continue to expand the number of nucleolar proteins identified. An online database describing close to 700 human proteins detected in purified nucleoli is now available (www.lamondlab.com/Nopdb) (Andersen et al., 2005). A proteome of the *Arabidopsis thaliana* nucleolus has also been identified recently (http://bioinf.scri.sari.ac.uk/cgi-bin/atnopdb/home) (Pendle et al., 2005). One striking discovery from these proteomic studies is that up to 30% of the nucleolar proteins are encoded by previously uncharacterised genes (Andersen et al., 2002; Andersen et al., 2005). This suggests that despite the extensive previous research on the nucleolus extending over almost two centuries, there is still much to be learned about its structure and function. Moreover, with the new availability of human and plant nucleolar proteomes, bioinformatic studies have already started to reveal new insights concerning common motifs found in nucleolar proteins (Leung et al., 2003) and the evolution of this nuclear organelle (Staub et al., 2004).

Examination of the proteome provides a glimpse into the functional complexity of the nucleolus. Its central role in ribosome subunit biogenesis is confirmed by the presence of many proteins (more than one third of the nucleolar proteome) involved in different steps in rRNA transcription, rRNA processing and modification, as well as the large and small ribosome subunit proteins themselves (Andersen et al., 2005). However, there are also many proteins that have no obvious relationship with these 'classical' nucleolar processes. For example, many proteins related to cell cycle regulation (about 3.5% of the identified proteome), DNA damage repair (about 1%) and pre-mRNA processing (about 5%) are detected in isolated nucleoli. This is consistent with the idea that the nucleolus performs additional roles beyond generating ribosomal subunits (reviewed by Olson et al., 2002; Pederson, 1998). More recent examples of cellular activities linked to the nucleolus include RNA editing (Sansam et al., 2003), DNA damage repair (van den Boom et al., 2004), telomere metabolism (Kieffer-Kwon et al., 2004; Zhang et al., 2004), tRNA processing (Paushkin et al., 2004) and regulation of protein stability (Mekhail et al., 2004; Rodway et al., 2004).

The internal structure of the nucleolus has been studied in detail by both transmission and scanning electron microscopy. For example, field-emission scanning electron microscopy (FESEM) analysis of isolated HeLa nucleoli provides a high-resolution (~1 nm) view of the 3D contour of the nucleolar surface and the interface between the nucleolus and the nucleoplasm (main central image). The internal structure of the nucleolus is revealed by transmission electron microscopy (TEM) of thin sections cut through nucleoli. Based on the morphology revealed by such TEM images, three subcompartments have been identified within the interior of the nucleolus. These include fibrillar centres (FCs), which are surrounded by dense fibrillar components (DFCs), and the FC-DFC complexes are embedded in the granular component (GC). Immuno-EM experiments show that many nucleolar proteins accumulate in one or two of these subcompartments, suggesting they each have distinct protein compositions and functions (Schwarzacher and Mosgoeller, 2000). For example, the RNA polymerase I subunit RPA39 is predominantly localised in the FC, whereas fibrillarin, a protein that is involved in ribose 2′-O-methylation of rRNA, accumulates in the DFC, and nucleolar phosphoprotein B23 is found in the GC. Recently, cell lines have been established that simultaneously express these three proteins tagged with different fluorescent proteins. This allows the assembly and disassembly of each subnucleolar compartment to be analysed in live cells during mitosis by high-resolution light microscopy (Leung et al., 2004). Recent data have also revealed that some factors in nucleoli are localised in subnucleolar regions that do not precisely correlate with any of the three well-known subcompartments (Politz et al., 2002), which suggests that the nucleolus is more complex than a sum of FCs, DFCs and the GC.

The nucleolus is a structure in which the interactions and translocation of a large number of proteins and RNAs are conducted and coordinated (reviewed by Fromont-Racine et al., 2003). During ribosome subunit biogenesis, pre-rRNA transcripts are transcribed by RNA polymerase I from the repeated clusters of rDNA genes. This process is vividly demonstrated when eukaryotic cells are swollen and spread in an alkaline, hypotonic solution in the presence of detergent (e.g. Chooi and Leiby, 1981; Mougey et al., 1993; Osheim et al., 2004). Under these conditions (Miller spreads), the rDNA repeats are fully extended and, along this central DNA axis, nascent growing rRNA transcripts can be seen emerging from each rDNA unit. As transcription proceeds from the initiation point, the rRNA transcripts become increasingly longer, resulting in a 'Christmas tree' model (reviewed by Trendelenburg et al., 1996). It is not clear how this complex structure is assembled in the nucleolus. The initiation of transcription probably occurs either within the FCs or at the FC-DFC boundary. The resulting pre-rRNA transcripts then emerge into the DFC region, where they are cleaved and modified by the small nucleolar RNPs (snoRNPs) and other processing enzymes. The rRNAs also begin the pathway of assembly with ribosomal proteins in the DFC and continue this as they pass through the GC and are exported to the cytoplasm. The sequential movement of rRNA through the FC, DFC and GC subcompartments can be demonstrated when cells are labelled with a short pulse of halogenated nucleotide, which reveals a wave of nascent rRNA spreading from the FC-DFC complexes to the GC regions (this is shown in three stages in the panel labelled 'Nucleolar transcription').

Despite major advances in recent years, many important questions remain to be answered about the nucleolus. For example, it is still unclear what components provide the structural integrity of the nucleolus. It is also not known how nucleolar assembly and disassembly is regulated during mitosis, and much remains to be learned concerning the full range of biological processes that either occur within, or else

involve, the nucleolus. Similarly, a clearer picture is needed at the molecular level between subnucleolar structure and specific functions. Although a detailed description of the protein content of the nucleolus is now emerging, we still do not know what functions many of these proteins perform, and less is known about the full spectrum of RNAs and DNA sequences that associate with nucleoli. The nucleolus is therefore likely to remain a source of interesting new discoveries and undoubtedly some more surprises for the foreseeable future.

We thank Anthony K. L. Leung and Carol E. Lyon for providing some of the images, and other members of the Lamond lab for help and discussions.

References

Andersen, J. S., Lyon, C. E., Fox, A. H., Leung, A. K., Lam, Y. W., Steen, H., Mann, M. and Lamond, A. I. (2002). Directed proteomic analysis of the human nucleolus. *Curr. Biol.* **12**, 1-11.

Andersen, J. S., Lam, Y. W., Leung, A. K. L., Ong, S. E., Lyon, C. E., Lamond, A. I. and Mann, M. (2005). Nucleolar proteome dynamics. *Nature* **433**, 77-78.

Cheutin, T., O'Donohue, M. F., Beorchia, A., Vandelaer, M., Kaplan, H., Defever, B., Ploton, D. and Thiry, M. (2002). Three-dimensional organization of active rRNA genes within the nucleolus. *J. Cell Sci.* **115**, 3297-3307.

Chooi, W. Y. and Leiby, K. R. (1981). An electron microscopic method for localization of ribosomal proteins during transcription of ribosomal DNA: a method for studying protein assembly. *Proc. Natl. Acad. Sci. USA* **78**, 4823-4827.

Fromont-Racine, M., Senger, B., Saveanu, C. and Fasiolo, F. (2003). Ribosome assembly in eukaryotes. *Gene* **313**, 17-42.

Kieffer-Kwon, P., Martianov, I. and Davidson, I. (2004). Cell-specific nucleolar localization of TBP-related factor 2. *Mol. Biol. Cell* **15**, 4356-4368.

Leung, A. K., Andersen, J. S., Mann, M. and Lamond, A. I. (2003). Bioinformatic analysis of the nucleolus. *Biochem. J.* **376**, 553-569.

Leung, A. K., Gerlich, D., Miller, G., Lyon, C., Lam, Y. W., Lleres, D., Daigle, N., Zomerdijk, J., Ellenberg, J. and Lamond, A. I. (2004). Quantitative kinetic analysis of nucleolar breakdown and reassembly during mitosis in live human cells. *J. Cell Biol.* **166**, 787-800.

Mekhail, K., Gunaratnam, L., Bonicalzi, M. E. and Lee, S. (2004). HIF activation by pH-dependent nucleolar sequestration of VHL. *Nat. Cell Biol.* **6**, 642-647.

Mougey, E. B., O'Reilly, M., Osheim, Y., Miller, O. L., Jr, Beyer, A. and Sollner-Webb, B. (1993). The terminal balls characteristic of eukaryotic rRNA transcription units in chromatin spreads are rRNA processing complexes. *Genes Dev.* **7**, 1609-1619.

Olson, M. O., Hingorani, K. and Szebeni, A. (2002). Conventional and nonconventional roles of the nucleolus. *Int. Rev. Cytol.* **219**, 199-266.

Osheim, Y., French, S. L., Keck, K. M., Champion, E. A., Spasov, K., Dragon, F., Baserga, S. J. and Beyer, A. L. (2004). Pre-18S ribosomal rna is structurally compacted into the SSU processome prior to being cleaved from nascent transcripts in Saccharomyces cerevisiae. *Mol. Cell* **16**, 943-954.

Paushkin, S. V., Patel, M., Furia, B. S., Peltz, S. W. and Trotta, C. R. (2004). Identification of a human endonuclease complex reveals a link between tRNA splicing and pre-mRNA 3′ end formation. *Cell* **117**, 311-321.

Pederson, T. (1998). The plurifunctional nucleolus. *Nucleic Acids Res.* **26**, 3871-3876.

Pendle, A. F., Clark, G. P., Boon, R., Lewandowska, D., Lam, Y. W., Andersen, J., Mann, M., Lamond, A. I., Brown, J. W. and Shaw, P. J. (2005). Proteomic analysis of the Arabidopsis nucleolus suggests novel nucleolar functions. *Mol. Biol. Cell* **16**, 260-269.

Politz, J. C., Lewandowski, L. B. and Pederson, T. (2002). Signal recognition particle RNA localization within the nucleolus differs from the classical sites of ribosome synthesis. *J. Cell Biol.* **159**, 411-418.

Rodway, H., Llanos, S., Rowe, J. and Peters, G. (2004). Stability of nucleolar versus non-nucleolar forms of human p14(ARF). *Oncogene* **23**, 6186-6192.

Sansam, C. L., Wells, K. S. and Emeson, R. B. (2003). Modulation of RNA editing by functional nucleolar sequestration of ADAR2. *Proc. Natl. Acad. Sci. USA* **100**, 14018-14023.

Scherl, A., Coute, Y., Deon, C., Calle, A., Kindbeiter, K., Sanchez, J. C., Greco, A., Hochstrasser, D. and Diaz, J. J. (2002). Functional proteomic analysis of human nucleolus. *Mol. Biol. Cell* **13**, 4100-4109.

Schwarzacher, H. G. and Mosgoeller, W. (2000). Ribosome biogenesis in man: current views on nucleolar structures and function. *Cytogenet. Cell Genet.* **91**, 243-252.

Staub, E., Fiziev, P., Rosenthal, A. and Hinzmann, B. (2004). Insights into the evolution of the nucleolus by an analysis of its protein domain repertoire. *Bioessays* **26**, 567-581.

Trendelenburg, M. F., Zatsepina, O. V., Waschek, T., Schlegel, W., Troster, H., Rudolph, D., Schmahl, G. and Spring, H. (1996). Multiparameter microscopic analysis of nucleolar structure and ribosomal gene transcription. *Histochem. Cell Biol.* **106**, 167-192.

Trinkle-Mulcahy, L., Andrews, P. D., Wickramasinghe, S., Sleeman, J., Prescott, A., Lam, Y. W., Lyon, C., Swedlow, J. R. and Lamond, A. I. (2003). Time-lapse imaging reveals dynamic relocalization of PP1gamma throughout the mammalian cell cycle. *Mol. Biol. Cell* **14**, 107-117.

van den Boom, V., Citterio, E., Hoogstraten, D., Zotter, A., Egly, J. M., van Cappellen, W. A., Hoeijmakers, J. H., Houtsmuller, A. B. and Vermeulen, W. (2004). DNA damage stabilizes interaction of CSB with the transcription elongation machinery. *J. Cell Biol.* **166**, 27-36.

Zhang, S., Hemmerich, P. and Grosse, F. (2004). Nucleolar localization of the human telomeric repeat binding factor 2 (TRF2). *J. Cell Sci.* **117**, 3935-3945.

Cell Science at a Glance on the Web
Electronic copies of the poster insert are available in the online version of this article at jcs.biologists.org. The JPEG images can be downloaded for printing or used as slides.