

Chapter 2

The Dynamic Proteome of the Nucleolus

François-Michel Boisvert, Yasmeen Ahmad, and Angus I. Lamond

2.1 Introduction

The primary function of the nucleolus is as the site of ribosome subunit biogenesis in eukaryotic cells. Nucleoli reassemble at the end of mitosis around the tandemly repeated clusters of rDNA genes forming a subnuclear compartment that locally concentrates the dedicated transcription and processing machineries that are responsible for generating ribosome subunits. The process of assembling a ribosome subunit requires the initial transcription of the ribosomal DNA (rDNA) genes by a specialized RNA polymerase – RNA pol I. These rDNA genes are arranged in arrays of head-to-tail tandem repeats, termed nucleolar organizer regions (NORs). In humans, approximately 400 copies of 43-kb repeat units are distributed along all acrocentric chromosomes (chromosomes 13, 14, 15, 21 and 22) to form NORs. In many cell types, only a subset of rDNA genes are transcriptionally active, even though inactive rDNAs are still assembled into nucleoli. The initial 47S ribosomal RNA (rRNA) precursor transcript transcribed by RNA pol I is subsequently cleaved to form the mature 28S, 18S and 5.8S rRNAs, post-transcriptionally modified through interaction with small nucleolar ribonucleoproteins (snoRNPs) and additional protein processing factors. Finally, the processed and modified rRNAs are assembled with the many ribosomal proteins, prior to interaction with the export machinery and transport to the cytoplasm.

The isolation and characterization of organelles by subcellular fractionation is a well-established technique in cell biology. Many organelles have been isolated and analysed in the past century (see, e.g. Spector et al. (1997) for reviews and protocols). These studies have provided invaluable information on the functions and properties of individual organelles. With recent advances in mass spectrometry based proteomic

A.I. Lamond (✉)

Wellcome Trust Centre for Gene Regulation and Expression,
College of Life Sciences, University of Dundee, Dundee DD15EH, UK
e-mail: angus@lifesci.dundee.ac.uk

technology, it has been possible to determine the major protein composition of various cytoplasmic organelles, for example the mitochondria (Pflieger et al. 2002), the Golgi apparatus (Bell et al. 2001; Taylor et al. 2000) and the chloroplast thylakoid membrane (Gomez et al. 2002). The isolation of subnuclear structures, in contrast with these cytoplasmic organelles, is made more difficult because they are not surrounded by membrane. Despite this limitation, isolation of several nuclear compartments, such as the nuclear envelope (Dreger et al. 2001), nuclear pore complexes (Cronshaw et al. 2002), interchromatin granule clusters (Mintz et al. 1999) and Cajal bodies (Lam et al. 2002), has been reported. The most well-studied nuclear organelle, the nucleolus, whose high density and structural stability allow effective purification using a straightforward procedure is an ideal structure for proteomic characterization. The ability to isolate nucleoli in large scale provided an excellent starting material for identifying, purifying and studying proteins in this nuclear compartment (Andersen et al. 2005; Andersen et al. 2002; Scherl et al. 2002).

Until recently, our knowledge of the protein content of nucleoli was quite limited (Fig. 2.1). However, the ability to purify nucleoli in large scale (Fig. 2.1a), combined with the major advances in the identification and analysis of proteins using mass spectrometry, has provided a wealth of information regarding the nucleolar proteome. Knowledge of nucleolar protein content has grown during the past 10 years from less than 100 proteins to well over 6,000 nucleolar proteins (Fig. 2.1b). Proteomic analyses have characterized the nucleolar proteome in both human and plant cells, identifying more than 200 plant and over 6,000 human proteins that stably co-purify with isolated nucleoli (Ahmad et al. 2009b; Andersen et al. 2002, 2005; Boisvert et al. 2010; Lam et al. 2010; Pendle et al. 2005; Scherl et al. 2002). A comparison of human and budding yeast data showed that ~90% of the nucleolus-related yeast proteins that have a clear human homologue are detected in the human nucleolar proteome (Andersen et al. 2005). This demonstrates that the nucleolar proteome is highly conserved through evolution.

Bibliographic and bioinformatic analyses of the proteomic data have allowed the classification of nucleolar proteins into functional groups and suggested potential functions for ~150 previously uncharacterized human proteins (Ahmad et al. 2009b; Coute et al. 2006; Hinsby et al. 2006; Leung et al. 2003). A classification of the molecular functions of the nucleolar proteins shows that only approximately 30% have a function obviously related to the production of ribosome subunits (Boisvert et al. 2007). However, the diverse identities and functions of many of the other nucleolar proteins are consistent with additional processes occurring within the nucleolus. This includes many pre-mRNA processing factors and proteins that are involved in cell-cycle control as well as DNA replication and repair (reviewed in Boisvert et al. 2007). An additional dimension has been added to the analysis of the nucleolar proteome by studies characterizing the dynamic changes in the proteome of the nucleolus under different metabolic conditions, such as inhibition of transcription following treatment of cells with actinomycin D (Andersen et al. 2005), in response to DNA damage (Boisvert et al. 2010; Boisvert and Lamond 2010) or following viral infection (Lam et al. 2010). The ability to analyse quantitatively and with high throughput the parallel increases and decreases in levels of many protein components has highlighted just how dynamic the nucleolar proteome can be.

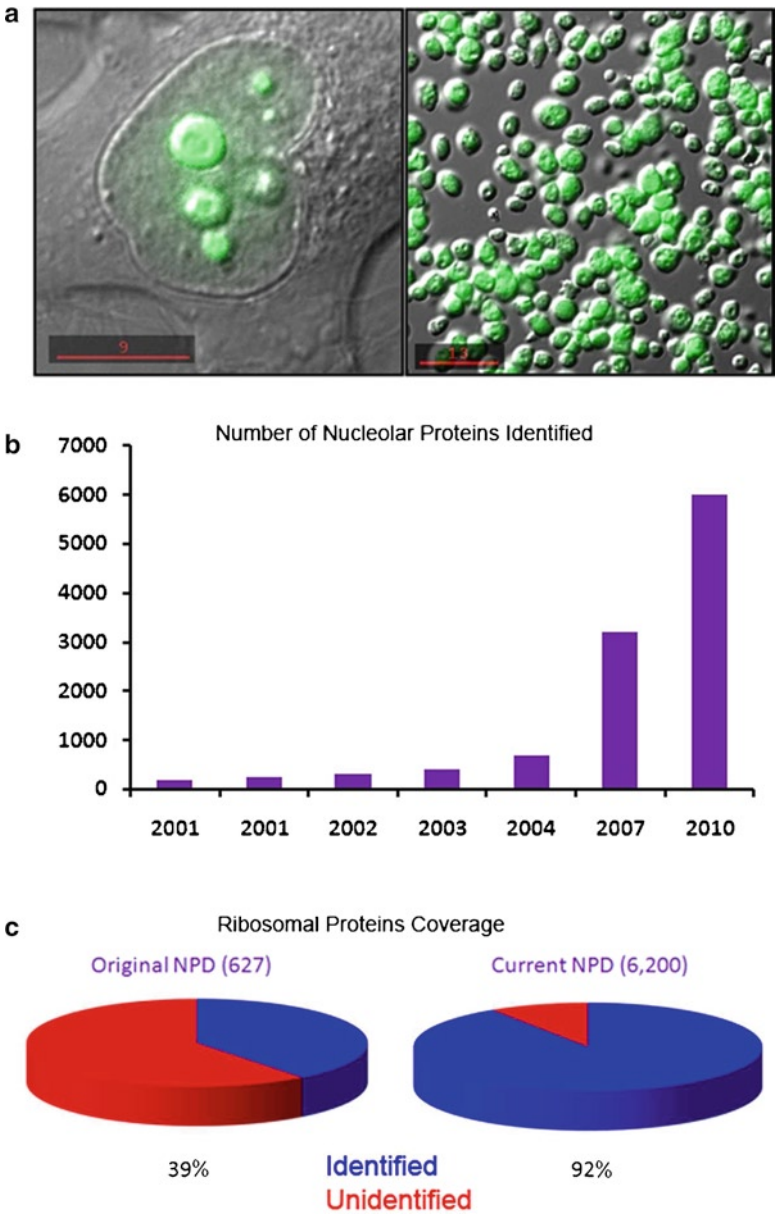


Fig. 2.1 Nucleolar proteome. (a) Overlay of a GFP-tagged nucleolar protein as shown over the DIC image in the whole cell (*left*) or following isolation of nucleoli showing intact structure and the absence of any visible contaminants from other cellular compartments. (b) Number of proteins that have been identified by mass spectrometry on purified nucleoli over the years following the improvement of methods and instruments. (c) Comparison of the number of ribosomal proteins that have been identified from the original published Nucleolar Protein Database to the present database

2.2 Isolation of Nucleoli

The starting point for the proteomic study of a cellular organelle or complex is the ability to isolate it intact, in high purity and in ideally large quantities. The relatively high density and structural stability of the nucleolus, as compared with other cellular structures, facilitates its efficient isolation even though it is not enclosed by a lipid membrane. Since the initial purification of nucleoli from human tumour cells and rodent liver cells in the early 1960s (Busch et al. 1963; Maggio 1966), several studies have been reported on the characterization of isolated nucleoli. Nucleoli have been purified from a large variety of mammalian tissues, including liver, thyroid (Voets et al. 1979) and brain (Banks and Johnson 1973) and from cells of non-mammalian species such as *Xenopus* (Saiga and Higashinakagawa 1979) and *Tetrahymena* (Matsuura and Higashinakagawa 1992).

The nucleolar isolation procedure is robust, and therefore the general strategy has been essentially unchanged over almost 40 years. Isolated cell nuclei are subjected to sonication, adjusting the power so that the nucleoli remain intact while the rest of the nuclei are fragmented as judged by microscopy. Then the nucleoli are isolated by centrifugation through a density gradient, on the basis of their high density compared with other nuclear components. Modifications of the basic procedure cater to the isolation of nucleoli from different cell types and organisms. For example, the procedure for isolating nucleoli from adherent HeLa cells was not suitable for suspension cultured HeLa S3 cells (our unpublished results). A critical factor is the salt concentration, especially, magnesium ion concentration used in the buffer during sonication, because the structural intactness of nucleoli decreases if salt concentration is too low (Vandelaer et al. 1996). However, if magnesium concentration is too high, nuclei cannot be efficiently disrupted by sonication (Lam et al. 2002) and hence the purity of the isolated nucleoli is compromised. In our experience, nuclei from adherent HeLa cells can be effectively sonicated in 0.35 M sucrose containing 0.5 mM $MgCl_2$, which lies between the large range of magnesium concentrations reported in other studies involving nucleolar isolation (Cheutin et al. 2002; Scherl et al. 2002).

It is essential to assess the purity and intactness of the isolated nucleoli before MS analysis. The quality of isolated HeLa nucleoli can be assessed using several criteria. First, the fraction isolated contains round or ovoid particles of uniform size, more than 95% of which can be labelled by the RNA dye Pyronin Y and by anti-nucleolar antibodies. These particles are morphologically similar to nucleoli detected in intact HeLa cells, as judged by both light and electron microscopy (Fig. 2.2). The ultrastructure of the isolated nucleoli shows that the internal nucleolar substructures (FC, DFC and GC) remain intact (Fig. 2.2). The purity of the isolated nucleoli can be further confirmed by western blotting, which should show that proteins known to be largely excluded from the nucleolus are virtually undetectable in the isolated nucleoli, while known nucleolar proteins (e.g. nucleolin and fibrillarin) are highly enriched. This was confirmed in an initial MS analysis, conducted to estimate the purity of the isolated nucleoli (Andersen et al. 2005). Of the 80 proteins found in

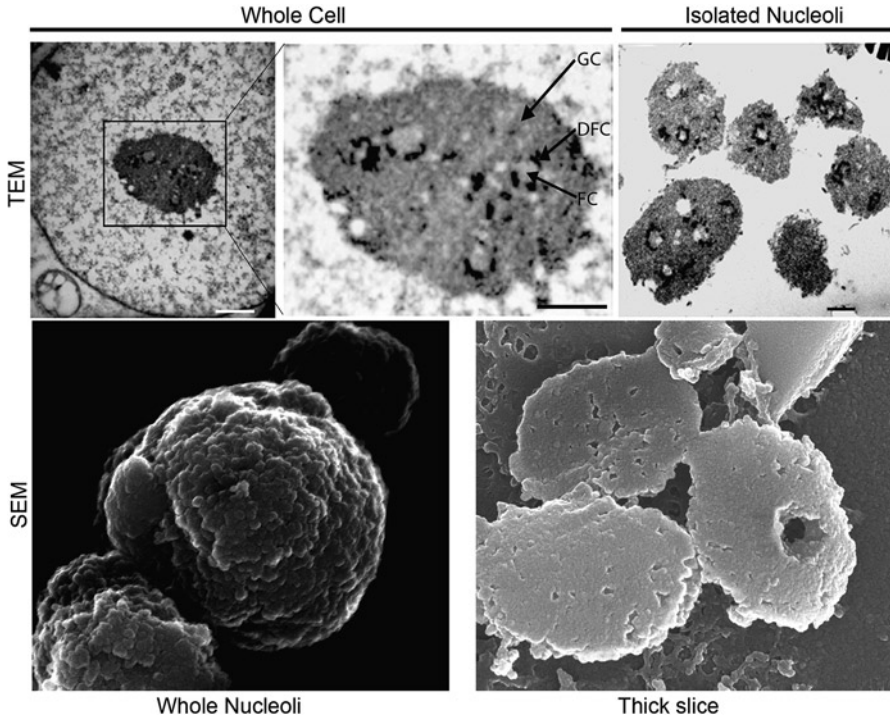


Fig. 2.2 Electron microscopy images of isolated nucleoli. Purified nucleoli are morphologically similar to nucleoli in intact cells. Transmission electron microscopy (TEM – *upper panels*) and scanning electron microscopy (SEM – *lower panels*) of isolated nucleoli were used to image nucleoli within intact HeLa cells (*left*) and nucleoli purified from HeLa cells (*right*). The central panels are enlargements of the indicated nucleoli in the intact cells. The isolated nucleoli are morphologically intact, retaining a clearly defined granular compartment (GC), dense fibrillar centre (DFC) and fibrillar centre (FC). Scale bars are 1 μ m

the initial screen, many were known nucleolar proteins, while obvious protein contaminants were absent. The detection of fibrillarin and nucleolin in the non-nucleolar fraction by Western blotting likely reflects the physiological presence of these proteins in a diffuse pool in the nucleoplasm (Chen and Huang 2001; Phair and Misteli 2000). It is, however, also possible that there may be some “leakage” of nucleolar proteins during the isolation procedure.

One indication of the intactness of the isolated HeLa nucleoli is that these nucleoli can incorporate BrUTP *in vitro* (Andersen et al. 2005). The incorporated BrUTP is located in distinct foci inside the nucleoli, similar to the published nascent RNA pattern in nucleoli *in vivo* (Masson et al. 1996). The *in vitro* incorporation of BrUTP in isolated nucleoli was inhibited by actinomycin D, but not by α -amanitin, which selectively inhibits RNA polymerase II (Andersen et al. 2005). This confirms that

nucleolar BrUTP incorporation was due to the action of RNA polymerase I, and that the isolated nucleoli, in agreement with previous reports, were transcriptionally active, at least for elongation (Cheutin et al. 2002). The properties of isolated nucleoli may vary greatly according to cell types, as Vandelaer et al. (Vandelaer et al. 1996) reported that nucleoli isolated from ELT cells using this method showed damage, especially in fibrillar centres, and were inefficient in transcription. In our experience, this nucleolar isolation (Vandelaer et al. 1996) method gave an extremely low yield and therefore was unsuitable for HeLa cells. Altogether, the combination of morphological, biochemical and functional studies demonstrate it is possible to isolate nucleoli that are both structurally and functionally intact.

2.3 Proteomic Analysis of Nucleolar Proteins

While nucleoli have been studied for over two centuries, it is only recently that an extensive list of proteins present within nucleoli at different times has emerged, thanks to the advance in the techniques of mass spectrometry based proteomics (reviewed by Coute et al. 2006) as well as better purification procedures. Adaptation of nucleolar purification procedures by sedimentation over sucrose cushion of sonicated nuclei led to the isolation of relatively intact and pure nucleoli, which were then used in high throughput proteomic-based experiments (Andersen et al. 2002). From the ~300 proteins identified in the first experiments (Andersen et al. 2002; Scherl et al. 2002), improvements in mass spectrometers have now identified over 50,000 peptides from more than 6,000 human proteins that co-purify with isolated nucleoli, providing significantly enhanced coverage of the nucleolar proteome (Ahmad et al. 2009b) (<http://www.lamondlab.com/NOPdb3.0/>). Interestingly, however, despite such an increase in the number of identified nucleolar proteins, when the proteins are categorized in terms of their functions, the distribution of functional categories is not altered significantly (Boisvert et al. 2007). This suggests that while the nucleolar proteome identified may still not be complete, it nonetheless fairly reflects the distribution of protein categories in the nucleolus, which is unlikely to change dramatically, even if more nucleolar proteins are discovered in the future. The functional categories indicated by proteomic studies are therefore likely to be a realistic reflection of nucleolar functions.

Interpretation of protein inventories derived using proteomics to identify proteins in purified organelles is complicated by the fact that many proteins are not exclusive to one compartment but instead partitioned between separate subcellular locations (Gauthier and Lazure 2008; Hall et al. 2009). Recent developments in quantitative proteomics allow the subcellular spatial distribution of proteins to be mapped and thus have led to a better definition of a nucleolar protein (Boisvert et al. 2010; Boisvert and Lamond 2010). The measurements using the spatial proteomics method allow classification of proteins according to whether they are enriched in the nucleolus compared to other compartments, or whether they are less abundant in that organelle. It is also important to recognize that these values are not fixed and can

change over time. This highlights the importance of not only identifying the presence of a protein in any specific cellular organelle or structure, but also measuring its relative abundance in different locations and assessing how this subcellular localization can change between different compartments under different cell growth and physiological conditions.

Global proteomic analyses of the different proteins have identified components that have been associated with functions unrelated to ribosome subunit biogenesis. Several proteomic analyses have also been undertaken to characterize the nucleolar proteome in non-mammalian species such as trypanosomes (Degrasse et al. 2008), *Arabidopsis* (Brown et al. 2005) and budding yeast (Huh et al. 2003). A comparison of human and budding yeast nucleolar data shows that over 90% of yeast proteins with clear human homologues can be detected in the human nucleolar proteome. This demonstrates that the core nucleolar proteome is largely conserved through evolution. Bibliographic and bioinformatic analyses of the proteomic data have allowed the classification of nucleolar proteins into functional groups and suggested potential functions for several previously uncharacterized human proteins. This shows that approximately 30% have a function related to the production of ribosome subunits (Boisvert et al. 2007). However, the diverse identities and functions of many of the other nucleolar proteins are consistent with additional processes occurring within the nucleolus. This includes many pre-mRNA processing factors and proteins that are involved in cell-cycle control as well as DNA replication and repair. The most striking feature of the functional distribution of the nucleolar proteome is the high proportion of novel and previously uncharacterized factors, a surprising fact for an organelle intensively investigated for over two centuries. Of the known proteins, the most common functional motifs found in approximately 20% of these proteins are nucleic acid and nucleotide binding domains. The DEAD-box helicase motifs in particular, characteristic of the superfamily of RNA dependent ATPases, were also represented highly in the nucleolar proteome, consistent with the control of RNA interactions being an important feature of nucleolar function. Consistent with its major role in transcription and processing of rRNAs and their subsequent assembly into ribosomal subunits, the nucleolar proteome includes many ribosomal proteins, processing factors and components required for transcription of the rRNA gene clusters, as well as human homologues of genes known to be involved in these processes in other organisms.

An additional dimension to the analysis of the nucleolar proteome involves characterizing the dynamic changes in the proteome of the nucleolus under different metabolic conditions, such as inhibition of transcription following treatment of cells with actinomycin D (Andersen et al. 2005), following viral infection (Cawood et al. 2007; Hirano et al. 2009; Hiscox 2007; Hiscox et al. 2010; Lam et al. 2010), following DNA damage (Boisvert et al. 2010; Boisvert and Lamond 2010) or through studies of protein turnover (Lam et al. 2007). The ability to analyse quantitatively and with high throughput the parallel increases and decreases in levels of many protein components has highlighted just how dynamic the nucleolar proteome can be. It will be interesting in the future to compare in detail how the nucleolar proteome varies between transformed human cell lines and primary cells.

The tumour suppressor p53 plays an important role involving the nucleolus in regulating aspects of stress responses and control of cell cycle progression. Under normal conditions, p53 is a short-lived protein that is present in cells at a barely detectable level. Exposure of cells to various form of exogenous stress, such as DNA damage, heat shock, hypoxia, etc., triggers the stabilization of p53, which is then responsible for an ensuing cascade of events, resulting in either cell cycle arrest, or in apoptosis. Accumulation of p53 induces the p21-mediated inhibition of cyclin D/cdk4 and cyclinE/cdk2, resulting in cell cycle arrest in G1. The stability of the p53 protein in mammals is primarily regulated in non-transformed cells by the interplay of two proteins, hdm2 and p14Arf in humans (the equivalent mouse proteins are mdm2 and p19Arf) (Prives 1998). Hdm2 functions as a specific E3 ubiquitin ligase for p53, resulting in a low level of p53 due to proteasome-mediated degradation of ubiquitin-conjugated p53 in the cytoplasm. A variety of stimuli, including stress pathways and oncogenic signals, increase expression of Arf, which then associates with hdm2 to inhibit the ubiquitination, nuclear export and subsequent degradation of p53. It has been proposed that Arf physically sequesters hdm2 in nucleoli, thereby relieving nucleoplasmic p53 from hdm2-mediated degradation (Weserska-Gadek and Horky 2003). Arf is predominantly a nucleolar protein and might also regulate ribosome biogenesis by retarding the processing of early 47S/45S and 32S rRNA precursors, perhaps through interaction with B23 (Bertwistle et al. 2004). Exposure of cells to various forms of stress, such as DNA damage, heat shock and aberrant ribosome subunit biogenesis results in an increase in p53 level and hence cell cycle arrest. Thus, the nucleolus acts as a sensor for cellular stress signals through p53 stabilization.

In p53 wild-type cells, p53 appears to cause a shut-down of nucleolar activity, which results in a specific segregation of nucleolar proteins within the nucleolus (Boisvert and Lamond 2010). However, this seems to be dependent on p53, because the effect is reduced in p53 knock-out cells (Boisvert and Lamond 2010). One consequence is that ribosomal proteins no longer accumulate in the nucleolus following DNA damage. This suggests a possible early role for p53 in shutting down the rDNA transcription machinery, as well as stopping the nucleolar recruitment, and/or retention of ribosomal proteins in the nucleolus, indicating that cells rapidly stop ribosome subunit production following DNA damage. Several recent reports showed that p53 becomes activated after silencing of ribosomal proteins such as RPL23 (Zhang et al. 2010), RPL11 (Lohrum et al. 2003), RPS6 (Volarevic et al. 2000) and TIF1A (Yuan et al. 2005). Other evidence emerging from a number of mouse models supports the existence of this ribosomal dependent p53 checkpoint *in vivo* (Fumagalli et al. 2009). During normal cellular growth, ribosomal proteins are assembled into ribosome subunits, but several ribosomal proteins including RPL11, RPL5, RPL23, RPS7 and RPS9 have now been shown to be released from the nucleolus following stress and to bind HDM2, resulting in stabilization of p53 (Fumagalli et al. 2009; Lindstrom and Nister 2010; Ohashi et al. 2010; Zhang et al. 2006). However, proteomic analysis suggests that p53 is actually necessary for the initial release of ribosomal proteins from the nucleolus following stress, and that this release probably results in an amplification of the p53 response by preventing HDM2-mediated degradation of p53 (Boisvert and Lamond 2010).

While many of the proteins identified in nucleolar proteomic studies are either known nucleolar proteins or else homologues of nucleolar proteins in other species, there are still a large number of proteins that are either previously unidentified, or else have not been shown previously to be localized in the nucleolus. To confirm whether these proteins are indeed localized in nucleoli, and not contaminants, systematic tagging of putative nucleolar proteins with fluorescent proteins and subcellular localization in cells following transient transfection have been analyzed by fluorescence microscopy. The relatively small number of FP-tagged proteins that did not localize in the nucleolus are not necessarily contaminants, however, because a protein may only be accumulated in the nucleolus during a particular phase of the cell cycle or under specific metabolic conditions. For example, microscopy analysis has showed that many proteins rapidly cycle between the nucleolus and nucleoplasm (e.g. Chen and Huang 2001). It is also possible that the fluorescent protein attached to the nucleolar protein interferes with the correct localization. The isolated nucleoli may therefore contain factors that are predominately localized in the nucleoplasm but transiently cycle through the nucleolus. Mass spectrometry is sufficiently sensitive to detect these low abundant proteins. For example, PSP1, a protein first identified in the proteome of the nucleolus, was present in a previously unknown nuclear domain “paraspeckles” and was apparently not nucleolar. However, drug treatment and fluorescence loss in photobleaching (FLIP) experiments confirmed that this protein interacted dynamically with the nucleoplasm and nucleolus in a transcriptional-dependent manner (Fox et al. 2005). To fully confirm the presence of a protein in the nucleolus, it is therefore necessary to take this into consideration and perform FLIP experiments on the transfected cells. This demonstrates that most of the identified proteins were nucleolar of steady state in inter-phase cells, and that confirms the proteomic approach is highly reliable in discovering nucleolar proteins.

2.4 Presentation and Publication of Data

The large amount of data acquired from proteomics studies requires a systematic way to analyse and integrate them with the information already deposited in publicly available databases. To facilitate this, a Nucleolar Online Proteomics Database (NOPdb) was created and published in 2006 (Leung et al. 2006). More recently, this database has been updated and revamped to version 3.0 (Ahmad et al. 2009a). The NOPdb consists of a backend database and a frontend interface to allow researchers to search for nucleolar proteomics data (Fig. 2.3).

The NOPdb archives all human nucleolar proteins identified to date by the Lamond group and their collaborators using MS analyses performed on purified preparations of human nucleoli (Andersen et al. 2005; Boisvert et al. 2010; Boisvert and Lamond 2010; Lam et al. 2007; Leung et al. 2006). The current version 3.0 of the NOPdb includes over 50,000 peptides contained in over 6,200 human proteins identified in different human cells lines. The coverage of the human nucleolar

The screenshot displays the NOPdb web interface. On the left, a 'Protein List' shows various proteins with their IDs and names. The main panel shows 'general details' for the protein 'Serine/threonine-protein phosphatase PP1-alpha catalytic subunit (PP1CA)'. The details include:

- Protein Name: Serine/threonine-protein phosphatase PP1-alpha catalytic subunit (PP1CA)
- UniProt ID: P60554
- Gene Symbol: PP1CA
- Gene Name: Serine/threonine-protein phosphatase PP1-alpha catalytic subunit
- Sequence: MSISDIALSDGSLLEVGSGPFGKIVLTENRGLKLSREFLSQPLLELAPKQZHQYVLAFTYGGPFDENYLSLQYVQSGHLEKLLA18KPEPTFLSGHCAINRISVFDECPHNAIKLTTCTNCLPAMVDEKCHGSLPFLQSGHQRHWPFTENPQGLGCLLH12PKDQVQSGENQKQSTFGVLEVWFHHLQSLCAHQVDEG
- Molecular Weight: 37488.00000
- pI: 6.017
- Number of Peptides Identified: 39
- Peptides: AHQVEDQIEFFAK, EFLVQPLLELAPK, GISTTGAEW

On the right, a 'NOPdb Search Results' window shows a table of search results:

Protein	Gene	Molecular Weight	pI
Serine/threonine-pr	PP1CA	37488.00000	6.017
Isoform Gamma-1a	PP1CC	36983.79000	6.018
Serine/threonine-pr	PP1CB	37188.83000	6.018
U3 small nucleolar	SNHG2P110	78863.79000	6.008

Below the search results, there is a 'Search History' window showing 'PP1' and a 'NOPdb Search' window with fields for 'Protein Name', 'Amino Acid Sequence', 'Size Range', 'pI Range', 'Motif', and 'Gene Ontology (GO) Annotation'.

Fig. 2.3 The Nucleolar Protein Database (NOPdb). The NOPdb3.0 is an online resource available at <http://www.lamondlab.com/NOPdb3.0/>. It is searchable by protein names, gene names, amino acid or nucleotide sequences, sequence motifs or by limiting the range for isoelectric points and/or molecular weights. This web-based database displays interactive entries for each nucleolar protein identified in our studies. Information for each protein includes a summary of the known features, genomic location, unigene entry and proteome. The database is continually updated to include newly identified nucleolar proteins

proteome has increased over the years, as demonstrated by coverage of ribosomal proteins increasing from ~28% in the earlier versions of the NOPdb (version 2.0) to incorporate over 80% in NOPdb3.0. It is estimated that NOPdb3.0 contains over 80% of the main human nucleolar proteins. The proteins in the database are regularly updated as more experiments are performed in the Lamond laboratory.

The NOPdb3.0 is an online resource available at <http://www.lamondlab.com/NOPdb3.0/>. It is searchable either by protein names, gene names, amino acid or nucleotide sequences, sequence motifs or by limiting the range for isoelectric points and/or molecular weights. The database is also searchable by Interpro motif numbers (database of protein families, domains and functional sites) (Bateman et al. 2004; Letunic et al. 2004; Mulder et al. 2003) and by gene ontology (GO) terms (describe gene products in terms of their associated biological processes, cellular components and molecular functions in a species-independent manner) (Ashburner et al. 2000).

The NOPdb3.0 provides a range of information on proteins, including protein name, accession number, gene symbol, gene name, sequence, molecular weight, isoelectric point (PI), peptides identified, experiments in which the protein was identified, motifs and GO annotation.

The NOPDB application facilitates mining of stored data thanks to the data being stored in a relational structure that is well documented. Thus, tools can be built to search, analyse, read and interpret the data. This mining capability is evident within the search feature of the application. Furthermore, the NOPdb3.0 uses application programming interface (API) to create dynamically generated graphs, allowing researchers to visualize the data produced from experiments and enabling cross analysis between experiments.

2.5 Perspectives

The nucleolus can be isolated intact from mammalian cells using a simple and straightforward procedure. This makes the nucleolus a model nuclear organelle for proteomic studies. The continuing advances in mass spectrometry techniques toward high sensitivity and automation enable identification of most of the proteins present in isolated organelles. The basic map of HeLa nucleolar proteins is therefore now largely charted. Future analyses of cell nucleoli will identify also some cell type-specific nucleolar proteins, through the analysis of nucleoli purified from a variety of sources, including primary cells and cell lines derived from different tissues. Some proteins may interact with nucleoli under only specific metabolic conditions and therefore have not been detected in current studies. For example, it will be important to isolate and analyse nucleoli from cells at specific cell cycle stages, during different cell differentiation states, following various forms of cell transformation and during senescence. One challenge of these experiments is the need not only to detect the identity of proteins but also to quantitate the changes in their abundance under different conditions. More quantitative methods have now allowed measurement of the relative protein enrichment in nucleoli, which should provide a standard for annotating nucleolar proteins (Boisvert et al. 2010).

In conclusion, although more work remains to be done, we believe that the human nucleolar proteome detailed so far represents a significant advance toward defining a comprehensive inventory of nucleolar proteins. These data should be of value for future studies on the range of biological roles performed by the nucleolus, including, for example, stress responses as well as ribosome subunit biogenesis, and the mechanisms involved in its assembly and function. Future studies will expand our knowledge of the nucleolar proteomics in other model organisms and will provide a more detailed quantitative picture of the levels of each protein and how this changes under a range of metabolic conditions.

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