



REVIEW ARTICLE

Bioinformatic analysis of the nucleolus

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The nucleolus is a plurifunctional, nuclear organelle, which is responsible for ribosome biogenesis and many other functions in eukaryotes, including RNA processing, viral replication and tumour suppression. Our knowledge of the human nucleolar proteome has been expanded dramatically by the two recent MS studies on isolated nucleoli from HeLa cells [Andersen, Lyon, Fox, Leung, Lam, Steen, Mann and Lamond (2002) *Curr. Biol.* **12**, 1–11; Scherl, Coute, Deon, Calle, Kindbeiter, Sanchez, Greco, Hochstrasser and Diaz (2002) *Mol. Biol. Cell* **13**, 4100–4109]. Nearly 400 proteins were identified within the nucleolar proteome so far in humans. Approx. 12 % of the identified proteins were previously shown to be nucleolar in human cells and, as expected, nearly all of the known housekeeping proteins required for ribo-

some biogenesis were identified in these analyses. Surprisingly, approx. 30 % represented either novel or uncharacterized proteins. This review focuses on how to apply the derived knowledge of this newly recognized nucleolar proteome, such as their amino acid/peptide composition and their homologies across species, to explore the function and dynamics of the nucleolus, and suggests ways to identify, *in silico*, possible functions of the novel/uncharacterized proteins and potential interaction networks within the human nucleolus, or between the nucleolus and other nuclear organelles, by drawing resources from the public domain.

Key words: amino acid composition, bioinformatics, nucleolus, profiling, proteomics, sequence homology.

BACKGROUND

A eukaryotic cell, by definition, segregates its DNA content within an internal compartment known as the nucleus. As early as in the 1800s, it was already obvious from light-microscopy studies that the interior of the nucleus was not uniform, notably with the discovery of the nucleolus. As noted by Gabriel Gustav Valentin, who first described the presence of the nucleolus in somatic cells [1,2], “*In every cell, without exception, there exists a somewhat darker-appearing and compact nucleus of a round or nearly round shape. Mostly it is located in the centre of a specific cell, composed of finely granular material and containing in its interior an exactly spheroidal body which in this way forms a kind of secondary nucleus within the first one.*”

Because the nucleolus is dense and highly refractile, it was the first subnuclear organelle intensely studied by light microscopists (Figure 1), and both its variability in size and number and the dynamic aspects of this organelle during mitosis were noted within 50 years of its discovery [3]. It was, however, not until the 1960s that the role of the nucleolus was established as the ‘ribosome factory’, where the rDNAs are kept and transcribed and the rRNA transcripts are processed and assembled with ribosomal proteins to form ribosomes. Although all rDNA repeats are housed within nucleoli, only half of the rDNA repeat copies are utilized even at the maximal transcriptional output [4]. Yet, surprisingly, this expends about 50–80 % of the total transcriptional activity in transcribing the rDNA and making other small nucleolar RNAs required for rRNA processing in an actively cycling eukaryotic cell [5]. However, an exclusive role of the nucleolus as a ribosome factory may not explain the recent discovery of viral, cell-cycle regulatory and tumour-related proteins within this structure [6–8], where the nucleolus acts as a molecular ‘safe’ or ‘sink’ to retain proteins and thus prevent them from interacting with their potential downstream partners until a specific cell-cycle stage, or

metabolic state. This view is consistent with analyses showing that many human nucleolar proteins, identified independently in two recent organelle-directed proteomic studies, are not obviously related to ribosomal biogenesis [9,10].

Owing to their inherent high density, human nucleoli can be isolated by sucrose-gradient centrifugation following the disruption of nuclei by sonication. The recent advances in protein-separation strategies and MS allow high-throughput, reproducible identification of the human nucleolar proteome [9,10]. In these studies, the proteins in purified samples of human nucleoli were separated by either one-dimensional (1D) or two-dimensional (2D) SDS/PAGE, followed by in-gel trypsinization, and the peptides were analysed by tandem mass spectrometry (MS/MS) to identify not only the peptide masses, but also their amino acid sequences [11,12]. Alternatively, the isolated nucleoli were solubilized, then trypsinized in solution and the resulting peptides were separated by liquid chromatography (LC) before analysing by MS/MS. The identified peptides from both 1D/2D gel-MS/MS and LC-MS/MS were then used to interrogate the NCBI (National Center for Biotechnology Information) database and, in some cases, the human-genome database to identify the corresponding proteins [13].

Nearly 300 nucleolar proteins were identified using MALDI-TOF (matrix-assisted laser-desorption ionization–time-of-flight) and nanoelectrospray MS on proteins separated by a combination of 1D and 2D gel analyses [9]. Recently, the coverage of nucleolar proteins was extended to over 400 members by performing LC-MS/MS ([10]; J. S. Andersen, Y. W. Lam, A. K. Leung, A. I. Lamond and M. Mann, unpublished work). More than 90 % of the proteins were identified multiple times by three MS studies of the human nucleolar proteome, suggesting that the current data are reproducible and rigorous ([9,10]; J. S. Andersen, Y. W. Lam, A. K. Leung, A. I. Lamond and M. Mann, unpublished work). Approx. 12 % of the identified proteins were known

Abbreviations used: 1D, one-dimensional; 2D, two-dimensional; LC, liquid chromatography; MS/MS, tandem mass spectrometry; PP1, protein phosphatase-1; PSP1, paraspeckle protein 1; RNP, ribonucleoprotein particle; RRM, RNA-recognition motif; snRNP, small nuclear RNP.

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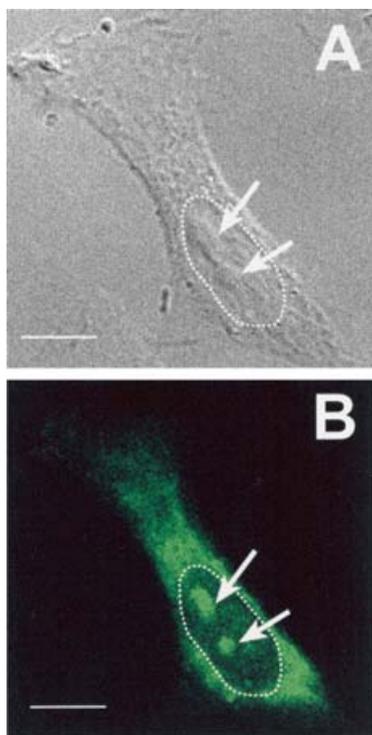


Figure 1 The nucleolus

(A) Because nucleoli are denser and more refractile than the nucleoplasm, they appear as darker bodies under the light microscope. (B) A fluorescent micrograph depicting the same cells expressing a GFP-ribosomal protein which localizes both in nucleoli and endoplasmic reticulum in the cytoplasm. Arrows indicate nucleoli and dotted lines indicate the boundaries of nuclei. Scale bar, 5 μ m.

previously to be nucleolar in human cells (see the Expansion of the known human nucleolar proteome section). Surprisingly, approx. 30% represented either novel or uncharacterized proteins (see the Bioinformatic analyses section). As expected, nearly all of the known housekeeping proteins required for ribosomal biogenesis were identified in these analyses. So, the immediate question is what are the remaining 88% of proteins doing in the nucleolus? The present review focuses on our current understanding of the nucleolar proteome and how to apply bioinformatic analyses of this proteome database to ask questions and, more importantly, design experiments that explore the function and dynamics of the nucleolus [6,15–19].

EXPANSION OF THE KNOWN HUMAN NUCLEOLAR PROTEOME

Before 2002, 121 human proteins had been reported to localize in the nucleolus in the published literature (Table 1). In all these cases, the proteins were first identified biochemically and then their subcellular localizations were shown subsequently to be nucleolar by antibody staining and/or tagging with fluorescent proteins. This is in stark contrast with the recent proteomic approach, in which nucleolar protein components were identified en masse from highly purified samples of nucleoli. Examining Table 1, we note that more than 90% of the human proteins that were previously reported to be involved in ribosomal biogenesis were identified multiple times, both from the 1D/2D gel and the LC approaches (Tables 1a and 1d). In general, the LC-MS/MS approach appears more sensitive than the 1D/2D gel approach in detecting peptides and thus gave rise to a higher coverage of nucleolar proteins (indicated by ticks in the LCMS/MBC columns rather than the CB02 column). Approx. 70% of the proteins

identified by 1D/2D gel-MS/MS analyses were also detected by the LC-MS/MS studies.

Even so, only less than 10% of proteins that were previously reported to show facultative nucleolar localization, i.e. to be localized in nucleoli during only part of the cell cycle, or under certain metabolic conditions, were identified in any of the proteomic analyses (Tables 1b and 1c). This may be because the nucleoli analysed were isolated from unsynchronized HeLa cells and hence such facultative nucleolar proteins may only constitute a minor fraction of the proteins isolated. Although LC-MS/MS has already increased the sensitivity of detection, it may still not be sensitive enough to detect very low abundance factors. Future analyses will therefore focus on nucleoli isolated at specific cell-cycle stages and under particular metabolic conditions (see the Dynamic proteome and Proteome profiling sections). This should help to increase the total coverage of the human nucleolar proteome.

BIOINFORMATIC ANALYSES

The large amount of data acquired from these proteomic studies requires a systematic way to analyse and integrate it with the information already deposited in the databases that are publicly available. To facilitate these analyses, several databases were downloaded to our Unix server and were interrogated with software such as stand-alone BLAST [20,21] and in-house customized Perl scripts. The resulting information was presented in a searchable database to provide a one-stop, user-friendly gateway to other relevant databases for non-specialists where each protein was given a summary of up-to-date, annotated information (Figure 2; <http://www.dundee.ac.uk/lifesciences/lamonddatabase/>). The most abundant motifs within the nucleolar proteome were the RNA-binding RRM (RNA-recognition motif) domain, DEAD/H (Asp-Glu-Ala-Asp/His) box helicase domain and the protein-protein interaction WD (Trp-Asp) domain (Table 2). These motifs support the role of the nucleolus being an organelle of multiple interlinked complexes acting in both ribosomal maturation and other RNA-processing pathways [22].

An overview of the separate classes of proteins identified in the purified nucleoli is presented in Figure 3. Surprisingly, the subsequent addition of more than 100 newly identified nucleolar proteins from the LC-MS/MS data resulted in minimal change to the distribution of proteins between categories, as previously assigned based on the analysis of 271 proteins [9]. DNA- and RNA-binding proteins that are involved in DNA repair, transcription, the unwinding of nucleic acid and RNA modification, including splicing, constitute roughly a quarter of the nucleolar proteome. Approx. 16% of the human nucleolar proteome is accounted for by translation-related factors, i.e. ribonucleoprotein particles/RNAs related to translation, such as SRP (signal recognition particle), tRNA and 5S RNA, and nearly all the ribosomal proteins [9,10,17,23,24]. However, there is no evidence that this is related to the controversial idea of mRNA translation in the nucleus [25,26]. Yet, it should be noted that, during the 1960s, data were published reporting that amino acids could be incorporated into peptides within isolated nucleoli [27–29]. The high protein complexity of the nucleolus implies that either the biogenesis of ribosomes is a surprisingly complex process and/or that the nucleolus carries out additional functions, consistent with the theory of a plurifunctional nucleolus [30,31].

Surprisingly, approx. 30% of the nucleolar proteins are either uncharacterized or else are the products of novel open reading frames. This partly reflects the currently limited status of the human genome annotation and also presents a challenge for us to unravel the functional complexity of the nucleolus [9]. However,

if we pool information from different sources, including gene-expression activity and information on homologues in other species, this approach should help speed up our understanding of the human nucleolar proteome, as discussed in the following sections.

PATHWAY PREDICTION

The ribosomal biogenesis pathway was so far studied mainly in yeast, while its characterization in other eukaryotes, especially humans, lags far behind [32,33]. The accumulated knowledge

Table 1 Known nucleolar proteome

In order to compare our nucleolar proteome with the list of previously published nucleolar proteins, it was first necessary to define this comprehensive list. Known nucleolar proteins were identified and extracted from PubMed (1978–2002) using the MeSH (medical subject heading) key words 'amino acid sequences' and word 'nucleol*' in the abstract field. In total, 123 nucleolar proteins were thus identified; however, two of these proteins were not annotated with amino acid sequences and hence were not compared with our MS results. Part (a) shows nucleolar proteins identified in more than one MS analysis. Proteins known to be localized in nucleoli during only part of the cell cycle and/or under certain metabolic conditions are shown in parts (b) and (c) respectively. Other known proteins localized in nucleoli are shown in part (d). The column 'Accession' shows the corresponding NCBI GI (GenInfo Identifier) number of the protein. A tick (✓) in the column 'CB02', 'LCMS' or 'MBC' signifies the identification of the protein by the 1D/2D gel–MS/MS approach [9], by our LC–MS/MS approach (J. S. Andersen, Y. W. Lam, A. K. Leung, A. I. Lamond and M. Mann, unpublished work) or by the MS/MS approach described by Scherl et al. [10]. The literature regarding the nucleolar localization of each protein is documented under the column 'Literature'. Note that some proteins fall into multiple categories.

(a) Known nucleolar proteins found in more than one MS analysis

Protein	Name	Accession	CB02	LCMS	MBC	Literature
BLM	Bloom syndrome	4557365	✓	✓		[64], Table 1(b)
BRIX	BRIX	19311012	✓		✓	[88]
CGI-94	CGI-94 protein	7705809	✓	✓		[89]
DDX21	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 21	13787209	✓	✓	✓	[90]
DDX5	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68 kDa)	4758138	✓	✓	✓	[91], Table 1(b)
DKC1	Dyskeratosis congenita 1, dyskerin	4503337	✓	✓	✓	[92]
FBL	Fibrillarin	12056465	✓	✓	✓	[93]
H2BFE	H2B histone family, member E	4504263	✓	✓	✓	[94]
hPOP1/KIAA0061	KIAA0061 protein	23097292	✓	✓		[95]
HSPA1B	Heat shock 70 kDa protein 1B	5123454	✓		✓	[96], Table 1(c)
HUMAUANTIG	Nucleolar GTPase	3334276	✓	✓	✓	[97]
Ku70/G22P1	Thyroid autoantigen 70 kDa (Ku antigen)	4503841	✓	✓	✓	[98]
MKI67	Antigen identified by monoclonal antibody Ki-67	19923217	✓	✓		[99]
MKI67IP	MKI67 (FHA domain) interacting nucleolar phosphoprotein	21314753	✓	✓	✓	[100]
MPHOSPH10	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)	2230873	✓	✓	✓	[101]
NCL	Nucleolin	4885511	✓	✓	✓	[102]
NHP2L1	NHP2 non-histone chromosome protein 2-like 1 (<i>S. cerevisiae</i>)	4826860	✓	✓	✓	[103]
NOH61	Putative nucleolar RNA helicase	9506931	✓	✓	✓	[104]
NOL1	Nucleolar protein 1 (120 kDa)	5453792	✓	✓	✓	[105]
NOL5A	Nucleolar protein 5A (56 kDa with KKE/D repeat)	5453794	✓	✓	✓	[106]
NOLC1	Nucleolar and coiled-body phosphoprotein 1	4758860	✓	✓		[107]
NOP5/NOP58	Nucleolar protein NOP5/NOP58	7706254	✓	✓	✓	[108]
Nop52/D21S2056E	DNA segment on chromosome 21 (unique) 2056 expressed sequence	4503247	✓	✓	✓	[109]
NPM1	Nucleophosmin (nucleolar phosphoprotein B23, numatrin)	10835063	✓	✓	✓	[110]
Nrap	Nucleolar RNA-associated protein	18644728	✓	✓	✓	[111]
PMSCL1	Polymyositis/scleroderma autoantigen 1 (75 kDa)	4826922	✓	✓		[112]
PMSCL2	Polymyositis/scleroderma autoantigen 2 (100 kDa)	4505917	✓	✓	✓	[113]
PPP1CC	Protein phosphatase 1, catalytic subunit, γ isoform	4506007	✓	✓		[114]
PTBP1	Polypyrimidine tract-binding protein 1	4506243	✓	✓	✓	[115]
RPL22	Ribosomal protein L22	4506613	✓	✓	✓	[116]
RPL5	Ribosomal protein L5	14591909	✓	✓		[117]
RPL6	Ribosomal protein L6	16753227	✓	✓	✓	[118]
RPL9	Ribosomal protein L9	15431303	✓	✓	✓	[119]
RPS6	Ribosomal protein S6	17158044	✓	✓		[120]
SURF6	Surfeit 6	19557702	✓	✓		[121]
TCOF1	Treacher Collins–Franceschetti syndrome 1	4507411	✓	✓		[122]
TOP2B	Topoisomerase (DNA) II β (180 kDa)	19913408	✓	✓		[123]
U3-55K	U3 snoRNP (small nucleolar RNP)-associated 55 kDa protein	4759276	✓	✓	✓	[124]
UBTF	Upstream binding transcription factor, RNA polymerase I	7657671	✓	✓		[125]

(b) Known proteins localized in nucleoli during part of the cell cycle

Protein	Name	Accession	CB02	LCMS	MBC	Literature
S phase						
BLM	Bloom syndrome	4557365	✓	✓		[64]
PCNA	Proliferating cell nuclear antigen	4505641				[93]
G ₂ /M phase						
CENPC1	Centromere protein C1	4502779				[126]
STK18	Serine/threonine kinase 18	21361433				[127]
Telophase						
DDX5	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (RNA helicase, 68 kDa)	4758138	✓	✓	✓	[91]

Table continues on following page.

Table 1 (contd.)

(c) Known proteins localized in nucleoli under certain metabolic conditions

Protein	Name	Accession	CB02	LCMS	MBC	Literature
Apoptosis						
DEDD	Death-effector-domain-containing	14670394				[128]
VIL2	Villin 2 (ezrin)	31283				[129]
Growth-related						
ANG	Angiogenin, ribonuclease, RNase A family, 5	4557313				[130]
AREG	Amphiregulin (schwannoma-derived growth factor)	4502199				[131]
FGF2	Fibroblast growth factor 2 (basic)	15451898				[132]
FGF3	Fibroblast growth factor 3 [murine mammary tumour virus integration site (v-int-2) oncogene homologue]	4885233				[133]
PTHLH	Parathyroid-hormone-like hormone	4506269				[134]
Heat shock						
HSP105B	Heat shock 105 kDa	2495344				[135]
HSPA1B	Heat shock 70 kDa protein 1B	5123454	✓		✓	[96]
HSPB1	Heat shock 27 kDa protein 1	4504517				[136]
PPID	Peptidylprolyl isomerase D (cyclophilin D)	4826932				[137]
Interferon induction						
PRKR	Protein kinase, interferon-inducible double-stranded RNA dependent	4506103				[138]
SP110a	SP110 nuclear body protein isoform a	17986254				[139]
SP110b	SP110 nuclear body protein isoform b	17986256				[139]
SP110c	SP110 nuclear body protein isoform c	17986252				[139]
UV induction						
ING1	Inhibitor of growth family, member 1	19923771				[140]
RAD17	RAD17 homologue (<i>Schizosaccharomyces pombe</i>)	4506383				[103]
Others						
ARL4	ARF-like protein 4	5031603				[141]
CALR	Calreticulin	4757900				[142]
CBFA2T3	Core-binding factor, runt domain, α subunit 2; translocated to, 3	20127532				[143]
DNAJB1	DnaJ (Hsp40) homologue, subfamily B, member 1	1706473				[144]
GAS41	Glioma-amplified sequence-41	5729838				[145]
MDM2	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)	4505137				[146]
OXR1	Oxidation resistance 1	8922241				[147]
p14ARF	p14 ARF	17738294				[148]
PNMA1	Paraneoplastic antigen MA1	14719430				[149]
SE20-4	Cutaneous T-cell lymphoma-associated tumour antigen se20-4; differentially expressed nucleolar TGF- β 1 target protein (DENTT)	14861044				[150]
TP53	Tumour protein p53 (Li-Fraumeni syndrome)	8400738				[151]
YY1	YY1 transcription factor	4507955				[152]
ZFP37	Zinc-finger protein 37 homologue (mouse)	4507963				[153]
ZNF259	Zinc-finger protein 259	4508021				[154]

(d) Other known proteins localized in nucleoli

Protein	Name	Accession	CB02	LCMS	MBC	Literature
Ribosomal proteins						
MRPL3	Mitochondrial ribosomal protein L3	6005862				[155]
RPS7	Ribosomal protein S7	4506741		✓		[156]
RNA-processing factors						
POP4	POP4 (processing of precursor, <i>S. cerevisiae</i>) homologue	5729986		✓		[95]
RNASE3	Ribonuclease, RNase A family, 3 (eosinophil cationic protein)	4506551				[157]
RPP14	Ribonuclease P (14 kDa)	5902066				[158]
RPP38	Ribonuclease P (38 kDa)	5454026				[158]
SKIV2L	Superkiller viralicidal activity 2-like (<i>S. cerevisiae</i>)	20631987				[159]
Rpp21/FLJ22638	Hypothetical protein FLJ22638	15080755				[160]
FTSJ2	FtsJ homologue 2 (<i>Escherichia coli</i>)	7019377				[161]
GEMIN4	Gem (nuclear organelle)-associated protein 4	7657122				[162]
Translation-related factors						
SRP19	Signal-recognition particle 19 kDa	4507213				[163]
SRP68	Signal-recognition particle 68 kDa	7657617				[163]
SRP72	Signal-recognition particle 72 kDa	5902124				[163]
DEAD box proteins						
DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homologue, <i>S. cerevisiae</i>)	13787200				[164]
DDX12	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homologue, <i>S. cerevisiae</i>)	1517818				[164]
Nucleotide or nucleic-acid-binding proteins						
hCAP-C/SMC4L1	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	21361252		✓		[165]
hCAP-H/KIAA0074	KIAA0074 protein	559715				[165]

Table continues on following page.

Table 1 (contd.)

Protein	Name	Accession	CB02	LCMS	MBC	Literature
SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	5453591				[165]
ERCC5	Excision repair cross-complementing rodent repair deficiency, complementation group 5 (xeroderma pigmentosum, complementation group G (Cockayne syndrome))	4503601				[166]
FMR1	Fragile X mental retardation 1	4503765				[167]
FXR1	Fragile X mental retardation, autosomal homologue 1	4826736				[168]
FXR2	Fragile X mental retardation, autosomal homologue 2	4758410				[168]
WRN	Werner syndrome	5739524				[169]
ANP32B	Acidic (leucine-rich) nuclear phosphoprotein 32 family, member B	5454088				[170]
JAZ	Double-stranded RNA-binding zinc-finger protein JAZ	6912440				[171]
KHDRBS3	KH-domain-containing, RNA-binding, signal-transduction-associated 3	5730073		✓		[172]
SLC29A2	Solute-carrier family 29 (nucleoside transporters), member 2	10835019				[173]
STAU	Staufen, RNA-binding protein (<i>Drosophila</i>)	4759176				[116]
TARBP2	TAR (HIV) RNA-binding protein 2	19743840				[174]
TERT	Telomerase reverse transcriptase	4507439				[175]
TOP3A	Topoisomerase (DNA) III α	10835218				[176]
ZNF274	Zinc-finger protein 274	7706507				[177]
RB1	Retinoblastoma 1 (including osteosarcoma)	4506435				[178]
Others						
CDC2	Cell-division cycle 2, G ₁ to S and G ₂ to M	4502709				[179]
PPM1D	Protein phosphatase 1D magnesium-dependent, δ isoform	4505997				[180]
PPP1R10	Protein phosphatase 1, regulatory subunit 10	4506009				[181]
ASE-1	CD3- ϵ -associated protein; antisense to ERCC-1	6912246		✓		[182]
ASNA1	arsA arsenite transporter, ATP-binding, homologue 1 (bacterial)	4757796		✓		[38]
COIL	Coilin	4758024	✓			[183]
HIC p40	Human I-mfa-domain-containing protein (HIC p40)	14739599				[184]
HSA6591	Nucleolar cysteine-rich protein	7657196				[185]
MCRS1	Microspherule protein 1	5453694				[186]
NOL3	Nucleolar protein 3 (apoptosis repressor with CARD domain)	4505419				[187]
NOL4	Nucleolar protein 4	4505421				[188]
SC65	Nucleolar autoantigen (55 kDa) similar to rat synaptonemal complex protein	3183090				[189]
SENP3	Sentrin/SUMO-specific protease 3	11245811		✓		[190]
SSB	Sjogren syndrome antigen B (autoantigen La)	10835067				[191]
SSRP1	Structure-specific recognition protein 1	4507241		✓		[192]

during the last decade of genetic defects in the yeast ribosome-synthesis pathway, as well as the recent success in the identification of pre-ribosomes using MS, has expanded our understanding of the ribosome-synthesis pathway dramatically (<http://www.proteome.com>; <http://www.pre-ribosome.de>). In particular, the large-scale systematic purification of protein complexes using tandemly arranged, high-affinity tags has led to tremendous progress in dissecting how the pre-60 S particles are formed. This advance is particularly impressive given that the half-lives of these transient structures are only approx. 1 min *in vivo* [34–37]. To define better the pathway of 60 S subunit synthesis, a series of particles was pulled out using tagged versions of several previously identified components from the yeast pre-60 S particles, revealing a series of ‘snapshots’ of the pre-60 S ribosomes as they move from the nucleolus to the cytoplasm.

Drawing on published data, as well as identifying homologues from the yeast ribosome-biogenesis pathway, Figure 4 shows all the possible 90 human homologues in the nucleolar proteome that could potentially be involved in ribosomal biogenesis, e.g. POP4, FIB and NOP52, organized here in Figure 4(A) according to how these proteins assist along the pathway. In this analysis, nearly 40 novel/uncharacterized proteins, constituting a third of this category in the nucleolar proteome, could potentially have functions related to ribosomal biogenesis based on the function of their yeast homologues. For other human proteins with limited characterization, e.g. many DEAD/H box helicases, we can now pinpoint more precisely their possible functions and

ask whether they perform a similar function in the human system as their yeast counterparts. This illustrates the potential for using the information from the proteomic studies to elucidate human ribosomal biogenesis further.

To survive, a cell requires energy, and nucleotides such as ATP and GTP are commonly utilized as the major energy currency for nuclear export, transcription and other enzymic activities, e.g. unwinding DNA/RNA. So far one putative ATP transporter has been identified within the human nuclear proteome, namely ASNA1, a human homologue of a bacterial arsenite ATP-binding transporter *arsA* ([38]; J. S. Andersen, Y. W. Lam, A. K. Leung, A. I. Lamond and M. Mann, unpublished work). In addition, putative GTPases (NNP47, NNP51 and NNP57/NGB) and AAA-type ATPases (NNP86 and MDN1) can be identified and we propose that they may be involved in human ribosome biogenesis, especially in the export of pre-ribosomal particles. However, there are proteins, such as nucleophosmin (NPM/B23), that have been shown previously to be involved in ribosomal biogenesis, which are human- or higher metazoan-specific and thus were unavoidably excluded in this approach.

AMINO ACID COMPOSITION ANALYSES

Taking advantage of the recent nucleolar proteome expansion based on these organelle-directed MS studies, we analysed whether or not certain amino acids are enriched, as compared with either total-nuclear or total-cellular protein sequences (Figure 5).

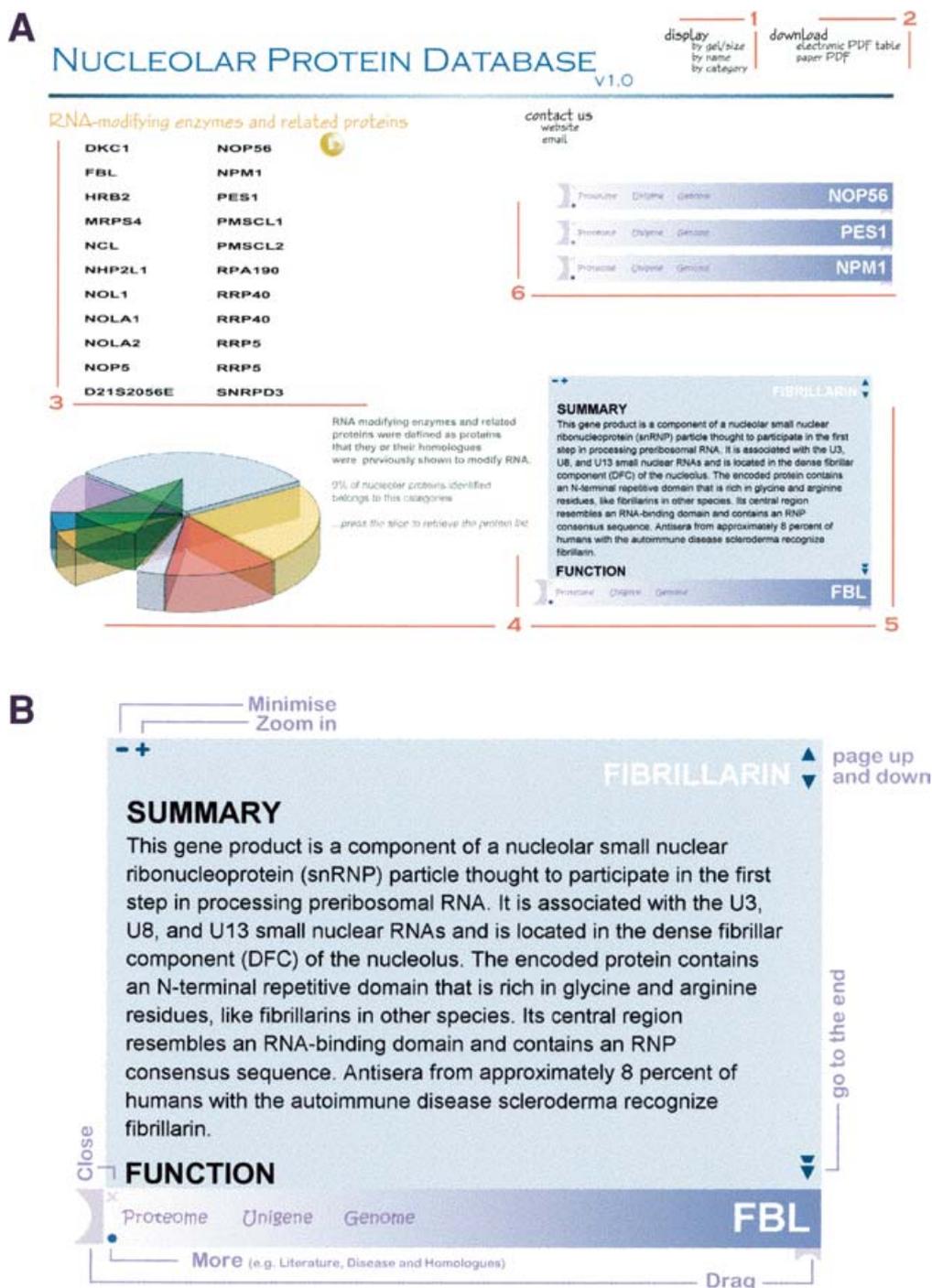


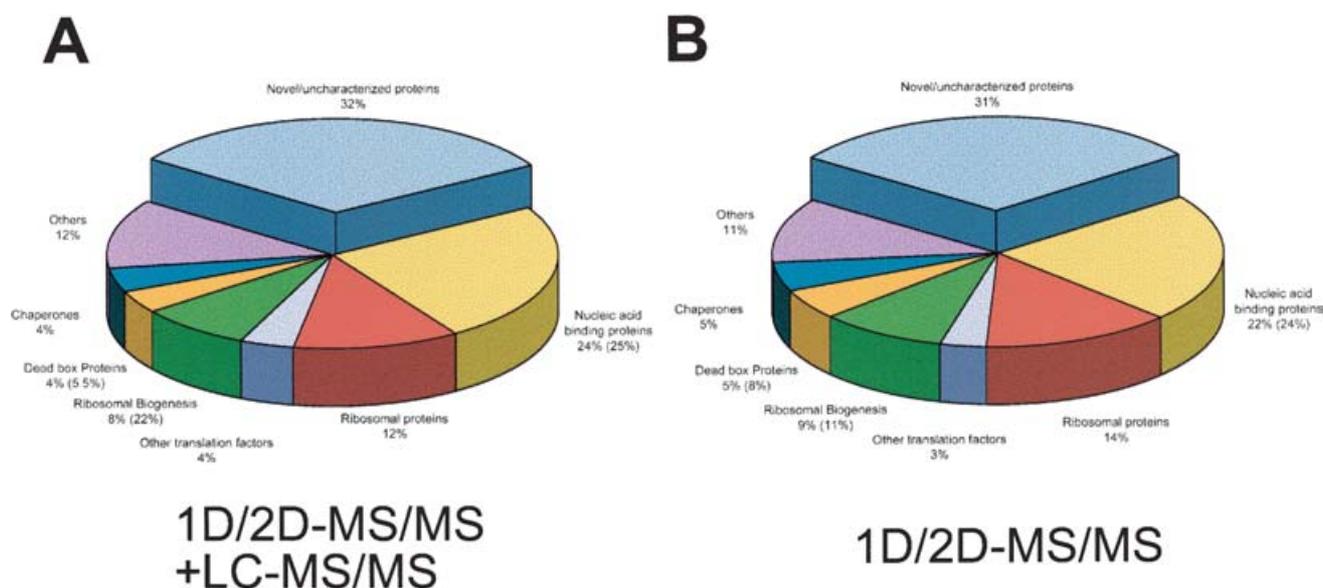
Figure 2 Nucleolar Protein Database

(A) A snapshot of the Nucleolar Protein Database (<http://www.dundee.ac.uk/lifesciences/lamonddatabase/>) showing the proteome by category, e.g. RNA-modifying enzymes and related proteins. (1) The database provides three options to display, i.e. by gel/size, by name or by category; (2) the complete dataset can be downloaded electronically; (3) the current set of proteins under a particular category (4); (5) the information of a particular protein is provided in details in a 'console window' on the lower, right-hand corner by clicking the protein name, where different protein console windows can be minimized as demonstrated in (6). (B) A protein 'console window' showing the information of a particular protein, e.g. fibrillarlin. The construction of the Nucleolar Protein Database is summarized as follows: the peptide sequences obtained from MS were assigned to a particular protein sequence either by BLAST or, more commonly, by Mascot (<http://www.matrixscience.com>). All the sequences were then retrieved by Batch Entrez from the NCBI database. The protein sequences were then assigned to their corresponding gene by BLAST analysis against the UniGene database (<http://www.ncbi.nlm.nih.gov/UniGene/>). The UniGene entry provides a starting point leading to various other relevant databases, such as LocusLink (<http://www.ncbi.nlm.nih.gov/LocusLink>) and OMIM (Online Mendelian Inheritance in Man; <http://www.ncbi.nlm.nih.gov/OMIM/>), to provide the cognate genomic and disease-linked information of that protein respectively. Other information is provided, including the data regarding corresponding homologues and the published literature and linking to other proteome databases.

Table 2 The most abundant motifs in the human nucleolar proteome

The frequency of the appearance of the 'motif' is indicated as a/b , where a indicates the number of proteins containing that particular motif and b indicates the total count of the motif within the human nucleolar proteome. 'Proteins' shows the corresponding human nucleolar proteins containing such a motif.

Motif	Frequency	Proteins
RNA-recognition motif (RRM)	40/51	NNP18, NNP59, NNP6, NNP64, NNP84, NNP88, NNP90, GRSF1, HNRPA0, HNRPA1, HNRPA2B1, HNRPA3, HNRPA4, HNRPA5, HNRPA6, HNRPA7, HNRPA8, HNRPA9, HNRPA10, HNRPA11, HNRPA12, HNRPA13, HNRPA14, HNRPA15, HNRPA16, HNRPA17, HNRPA18, HNRPA19, HNRPA20, HNRPA21, HNRPA22, HNRPA23, HNRPA24, HNRPA25, HNRPA26, HNRPA27, HNRPA28, HNRPA29, HNRPA30, HNRPA31, HNRPA32, HNRPA33, HNRPA34, HNRPA35, HNRPA36, HNRPA37, HNRPA38, HNRPA39, HNRPA40, HNRPA41, HNRPA42, HNRPA43, HNRPA44, HNRPA45, HNRPA46, HNRPA47, HNRPA48, HNRPA49, HNRPA50, HNRPA51, HNRPA52, HNRPA53, HNRPA54, HNRPA55, HNRPA56, HNRPA57, HNRPA58, HNRPA59, HNRPA60, HNRPA61, HNRPA62, HNRPA63, HNRPA64, HNRPA65, HNRPA66, HNRPA67, HNRPA68, HNRPA69, HNRPA70, HNRPA71, HNRPA72, HNRPA73, HNRPA74, HNRPA75, HNRPA76, HNRPA77, HNRPA78, HNRPA79, HNRPA80, HNRPA81, HNRPA82, HNRPA83, HNRPA84, HNRPA85, HNRPA86, HNRPA87, HNRPA88, HNRPA89, HNRPA90, HNRPA91, HNRPA92, HNRPA93, HNRPA94, HNRPA95, HNRPA96, HNRPA97, HNRPA98, HNRPA99, HNRPA100
DEAD/DEAH box helicase	23/25	BLM, CHD4, DDX10, DDX15, DDX17/P72, DDX18, DDX21, DDX24, DDX31, DDX33, DDX5, DDX9, DDXL, GU2, NOH61, NNP33, NNP40, NNP41, NNP48, NNP75, NNP121, EIF4A1, IF4N
Helicase conserved C-terminal domain	20/22	BLM, CHD4, DDX10, DDX17/P72, DDX18, DDX21, DDX24, DDX31, DDX33, DDX5, DDX9, DDXL, GU2, NOH61, NNP33, NNP41, NNP48, NNP75, EIF4A1, IF4N
WD domain	12/13	NNP116, NNP24/BING4, NNP30, NNP36, NNP42, NNP43/SAZD, NNP62, NNP63, NNP34, CSTF1, NMP200, U3-55K
Intermediate filament proteins	9/9	RAD50, SMC4L1, DSP, KRT17, KRT18, KRT8, LMNA, LMNB1, VIM
Myosin tail	9/10	CSPG6, RAD50, SMC1L1, SMC4L1, DSP, KRT17, KRT18, KRT8, LMNB1, NNP19
Elongation factor Tu GTP-binding domain	6/6	NNP69, EEF1A1, EEF1A2, EEF2, EIF2S3, TUFM

**Figure 3** Distribution of conserved motifs and putative functional categories of the identified proteins in the nucleolar proteome

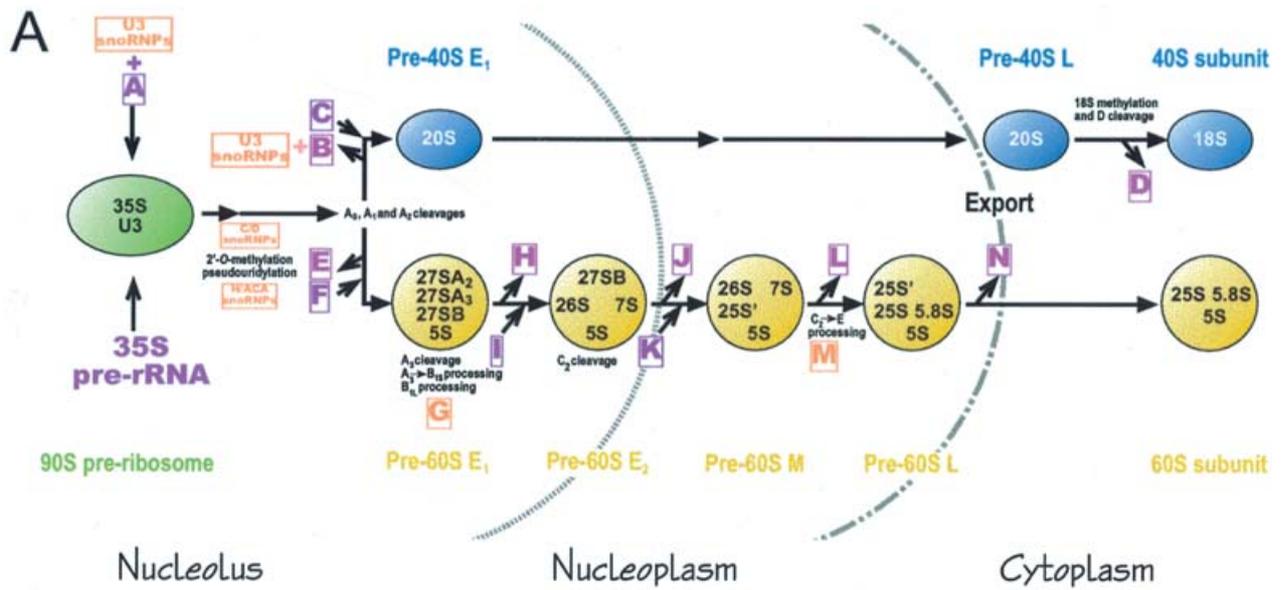
(A) Current analysis and (B) 1D/2D gel analysis data [9]. The percentages in brackets indicate the maximum number of proteins that could possibly be classified in the category in question. For example, 22% of the proteins may be related to ribosomal biogenesis from the current analysis (A), due to their yeast orthologues being present in the ribosomal synthesis pathway.

Apparently, charged amino acids, such as glutamate, aspartate, lysine and arginine, are more favoured with respect to nuclear-specific, or total-cellular, protein sequences, whereas neutral amino acids, such as proline and cysteine, are highly disfavoured. Surprisingly, the polar amino acid serine, which is an important target for phosphorylation, was also not common within the nucleolar protein sequences. These apparent amino acid biases may reflect nucleolar targeting motifs, although these remain poorly characterized [39]. The initial search for such targeting motifs using software, such as MEME (Multiple Expectation Maximization for Motif Elicitation; <http://meme.sdsc.edu/meme/website/intro.html>; [40]), was only sensitive enough to retrieve motifs, such as RRM, or WD domains, as mentioned in the Bioinformatic analyses section.

Although it was not possible to define nucleolar-specific motifs shared by all nucleolar proteins, it was possible to determine whether or not some short peptide motifs show any specific

enrichment in the human nucleolar proteome. For example, the protein phosphatase-1 (PP1)-binding motif, (Lys/Arg)-Val-Xaa-Phe, constitutes 0.032% of all tetrapeptide sequences present in the nucleolar proteome. This is approx. 1.4-fold more common than in the total nuclear proteome, suggesting that a protein containing one or more PP1-binding site is more common in the nucleolus than in other parts of the nucleus. In contrast, the SUMO (small ubiquitin-related modifier)-conjugation site, (Ile/Val/Leu/Met/Phe)-Lys-Xaa-(Glu/Asp), constitutes approx. 0.2% of tetrapeptides in nucleolar protein sequences, a value similar to its frequency in the total nuclear proteome.

Unlike arginine, glycine does not show a particular preference within the nucleolar proteome (Figure 5); however, the tripeptides GRG and RGG are enriched in the nucleolar proteome at least 2- and 3-fold respectively, as compared with either total cellular, or nuclear-specific, proteins (Figure 6A) and roughly a third of the human nucleolar proteome contains the RGG motif at least once



B

Gene Name	Yeast GN	In	Out	Sc	Sp	Ce	Dm	Remarks
NNP59	BMS1	A	B					GTPase
DDX37	DHR1	A	B					DEAD/H box Helicase
NNP53	DIP2	A	B					
NNP7	EMG1	A	B					
HRB2	KRR1	A	B					
NNP57	NOP14	A	B					
NNP52	PWP2	A	B					
2NAC	RCL1	A	B					
NNP41	ROK1	A	B					DEAD/H box Helicase
RRP5	RRP5	A	B					
NNP90	RRP7	A	B					
U3-50K	RRP1	A	B					
NNP13	UTP11	A	B					
NNP43/SAZD	UTP13	A	B					
NNP120	UTP15	A	B					
NNP117	UTP19	A	B					
NNP49	UTP21	A	B					
NNP27	UTP22	A	B					
NNP114	UTP4	A	B					
NNP105	UTP6	A	B					
NNP24/BING4	UTP7	A	B					
NNP52	YGR145W	A	B					
NNP46	YKJ060W	A	B					
NNP96	KRE33	A, B, E						
NNP71	UTP10	A, B, E						
BYSL	ENP1	A, D						
NNP108	YOR145C	C, D						

C

Gene Name	Yeast GN	In	Out	Sc	Sp	Ce	Dm	Remarks
NNP56	KRE33	A	B, E					
NNP71	UTP10	A	B, E					
NNP66	RRP12	A	E					
NOH61	DBP9	F	H					DEAD/H box Helicase
NNP59	NOP4	F	H					
NNP125	RPF2	F	H					
NNP37	SSF1	F	H					
NNP81	YKL082C	F	H					
NNP3	BRX1	F	J					
NNP48	DRS1	F	J					DEAD/H box Helicase
NNP17	EBP2	F	J					
NNP76	MAX16	F	J					
NNP23	NSA1	F	J					
NNP22	RPF1	F	J					
ITG848P	TIF6	F	J					
NNP30	YTM1	F	J					
NNP54/BOP1	ERB1	F	L					
DDX18	HAS1	F	L					DEAD/H box Helicase
NNP96	NP7	F	L					
NOL1	NOP2	F	L					SAM-dependent methyltransferase
PES1	NOP7	F	L					Ribosomal subunit export
NNP51	NOG1	F	N					GTPase
NOP52	RRP1	F	N					
NNP18	NOP15	I	J					
NNP125/RRP50	MRT4	I	L					
NNP12	NSA2	I	L					
NNP86	RX1	I	L					Ribosomal subunit export
FTSJ3	SPB1	I	L					SAM-dependent methyltransferase
NNP107	RLP24	I	N					
NNP76	DBP10	K	L					DEAD/H box Helicase
MDN1	MDN1	K	L					
NNP56	NOC2	K	L					Ribosomal subunit export
NNP110	NOC3	K	L					Ribosomal subunit export
NNP47	NUG1	K	L					GTPase, Ribosomal subunit export
NNP50	SOA1	K	L					
NNP116	YCR072C	K	L					
NNP38	YPL146C	K	L					
NNP57/NGB	NOG2	K	N					GTPase, Ribosomal subunit export

D

Gene Name	Yeast GN	Complex	Sc	Sp	Ce	Dm	Remarks
XRN2	RAT1	G					
NNP61	POP1	G, RNase MRP					
POP4	POP4	G, RNase MRP					
NNP95	DIS3	M, Exosome					
NNP67	MTR4	M, Exosome					DEAD/H box Helicase
NNP10	RRP4	M, Exosome					
RRP40	RRP40	M, Exosome					
PMSCL1	RRP45	M, Exosome					
RRP46	RRP46	M, Exosome					
PMSCL2	RRP6	M, Exosome					
NNP8	SK36	M, Exosome					
DKC1	CBF5	HAC/AsuRNP					
NOLA1	GAR1	HAC/AsuRNP					
NOLA2	NHP2	HAC/AsuRNP					
NOLA3	NOP10	HAC/AsuRNP					
MRP84	IMP3	U3 snoRNP					
NNP14	IMP4	U3 snoRNP					
NNP96	LCP5	U3 snoRNP					
MPHOPSH10	MPP10	U3 snoRNP					
NNP34	SOF1	U3 snoRNP					

Gene Name	Yeast GN	Complex	Sc	Sp	Ce	Dm	Remarks
FBL	NOP1	U3 snoRNP, C/D snoRNP					SAM-dependent methyltransferase
NOP5/NOP58	NOP58	U3 snoRNP, C/D snoRNP					
NOP56	SIK1	U3 snoRNP, C/D snoRNP					
NHP2L1	SNU13	U2 snoRNP, C/D snoRNP					

Figure 4 For legend see facing page.

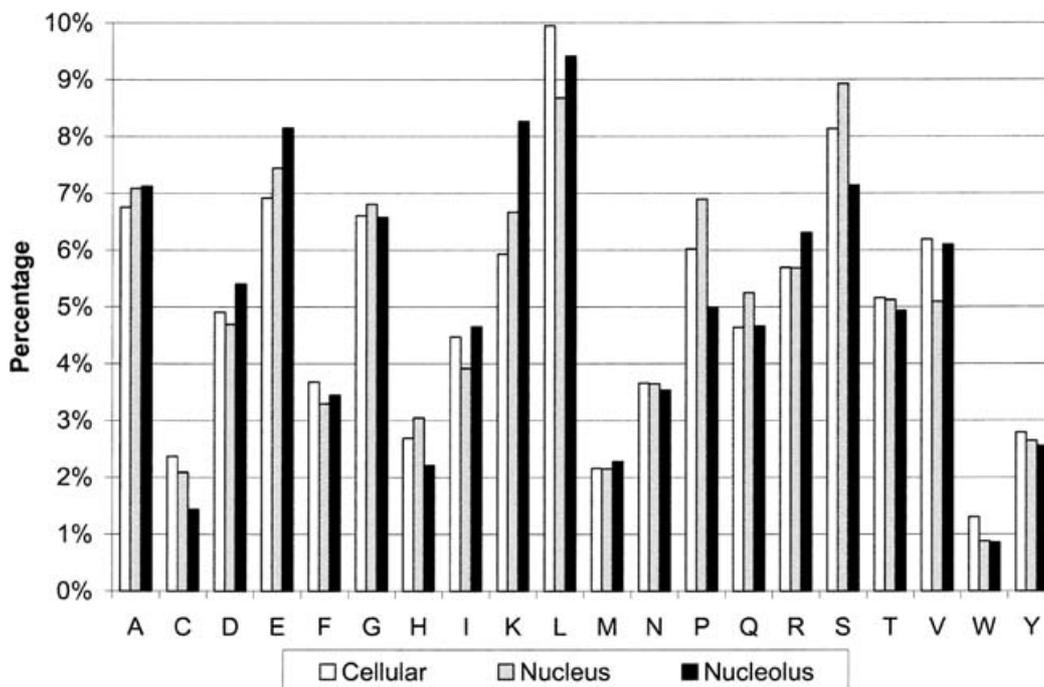


Figure 5 Abundance of individual amino acids in the human nucleolar proteome

For comparison, we generated sets of protein sequences extracted from either the human-specific total cellular proteomes or the human nuclear-specific proteomes and each set contains roughly the same number of sequences and amino acids (approx. 30 000) as the identified nucleolar proteome.

(Figure 6B). Previous studies have suggested that SMN (survival of motor neuron 1), a component of the nucleolar accessory bodies, recently renamed as Cajal bodies after the name of their discoverer [41,42], utilizes this RG-rich domain to interact with a range of proteins involved in pre-rRNA processing, ribosome production, pre-mRNA splicing, transcription and recruitment to Cajal bodies. The close association of the Cajal body with the nucleolus has been well established since its discovery. It is noted that Cajal body components containing RG-rich domains, such as Sm proteins, have been shown to traffic through the nucleolus [43]. It remains to be established whether such trafficking behaviour is due to their involvement with various RG-containing proteins within the nucleolus.

More surprisingly, the tetrapeptides FGGR and RGGF have over 10- and 20-fold enrichment, respectively, within the nucleolus, compared with either total-cellular or nuclear-specific proteins (Figure 6 and results not shown), and these two particular peptide sequences fall into the consensus motif, i.e. (G/F)-GGRGG(G/F), for asymmetric dimethylation of arginine residues

[44]. This is in line with the recent result from a budding yeast study showing that several nucleolar factors are modified by arginine methyltransferase *in vivo* (Figure 6B; [45]). Although methylation does not change the overall charge on an arginine residue, addition of methyl groups increases steric hindrance and removes potential hydrogen bonds from the amino hydrogens and hence may affect potential protein-protein interactions [46]. The enrichment of such motifs possibly reflects unknown functional properties of the human nucleolus that are distinct from other subnuclear structures or, alternatively, may reflect a common mechanism for specific nucleolar localization. Consistent with the latter idea, a direct role for the asymmetric dimethylation of arginine residues within RG-rich domains was shown to be essential for nucleolar localization in the higher-molecular-mass form of basic fibroblast growth factor [47]. On the other hand, the change in interaction between proteins mediated by arginine methylation can macroscopically affect nuclear body formation, as recently documented in the case for p80 coilin, the marker protein of Cajal bodies [48,49].

Figure 4 Ribosome-biogenesis pathway in yeast

The 90 S pre-ribosomal complex is proposed to contain the 35 S rRNA and the U3 snoRNP (small nucleolar RNP). The early pre-rRNA cleavages at sites A_0 to A_2 lead to the separation of the pre-40 S and pre-60 S particles. In both pathways, a series of predicted intermediates are drawn, which are designated early (E), middle (M) and late (L), according to their positions on the proposed pathway [32]. The processing steps envisaged to be associated with each of these complexes are indicated in (B)–(D), as is the likely time of export from the nucleolus to the cytoplasm [33]. Nucleolar proteins found in this study that are putative ribosomal biogenesis factors based on homology with their yeast counterparts are represented in the following panels: (B) 40 S ribosomal subunit maturation pathway; (C) 60 S ribosomal subunit maturation pathway and (D) other processing complexes. Note that it is very probable that other pre-ribosomal complexes exist in addition to those shown in (A), and it is not clear in what order the components are gained and lost between the complexes. The colour indicated on the left of each entry corresponds to the classification of the nucleolar proteome as denoted in Figure 3. The pre-60 S pathway includes only proteins for which corroborating data exist, supporting a direct role in ribosome synthesis. 'Yeast GN' shows the closest matching yeast gene to the corresponding proteins. 'In' and 'Out' in (B) and (C) indicate the corresponding input and output complex during the maturation pathway as illustrated in (A). The shaded panels represent the results of BLASTp searches for each protein against the species-specific proteome database from EBI/SGD databases: *S. cerevisiae* (Sc), *Schizosaccharomyces pombe* (Sp), *Drosophila melanogaster* (Dm) and *Caenorhabditis elegans* (Ce). The results are shaded such that a black panel represents the expectancy value (e-value) of 0.0 and thus indicates a nearly perfect match; 80% grey for $e\text{-value} < 1e^{-100}$; 50% for $1e^{-100} < e\text{-value} < 1e^{-75}$; 40% for $1e^{-75} < e\text{-value} < 1e^{-50}$; 25% for $1e^{-50} < e\text{-value} < 1e^{-25}$ and a white panel for $e\text{-value} > 1e^{-25}$. It should be noted that the e-value between known yeast homologues of human protein can be as high as $1e^{-10}$.

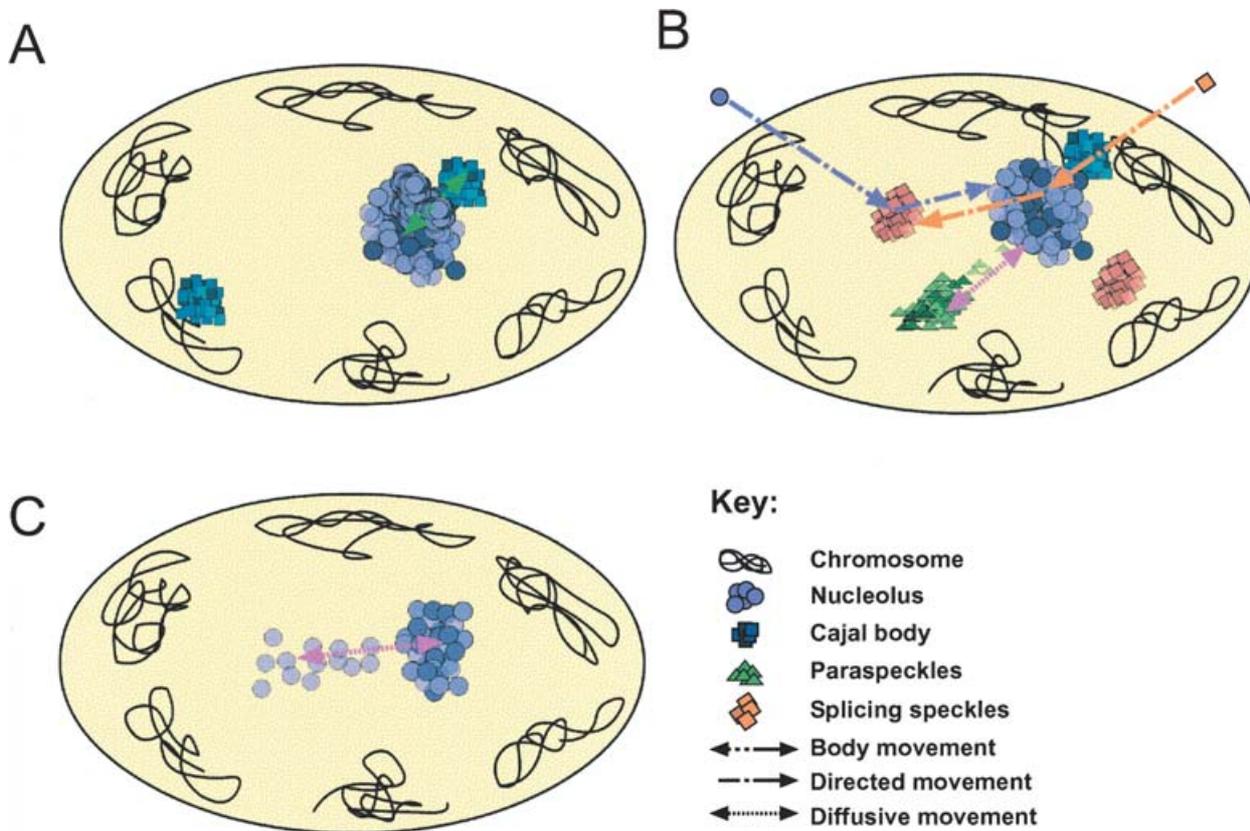


Figure 7 Dynamic nuclear proteome (animated versions to illustrate the dynamic behaviour can be found at <http://www.BiochemJ.org/bj/376/bj3760553add.htm>)

(A) The Cajal body (blue squares) has a close association with the nucleolus on the periphery and are even sometimes located within the nucleolus. (B) Splicing factor Sm (orange square in the top-right corner) travels via the nucleolus/Cajal body before accumulating in the splicing speckles, whereas nucleolar protein NHPX (purple circle in the top-left corner) transiently interacts with the splicing speckles before entry into the nucleolus. Marker protein of paraspeckles PSP1 (green triangle) continually exchanges between the nucleolus and the paraspeckles. (C) All the nucleolar proteins analysed so far continually exchange with the nucleoplasm, according to recent fluorescence recovery after photobleaching (FRAP) data.

DYNAMIC PROTEOME

An important aspect of subnuclear structures is their dynamic and regulated association with one another (Figure 7; [43,50,51]). This is especially evident for the nucleolus. Even at the light-microscopy level, a close association between nucleoli and Cajal bodies had been observed in the early twentieth century (Figure 7A; [41,42]). They are often associated with the nucleolar periphery, or even located inside the nucleolus [52–55]. Tracking of Cajal body movement in both plant and human cells showed that Cajal bodies can enter into nucleoli [56–58]. Recent studies have shown that mechanisms are operating to organize the temporal distribution of proteins and RNPs (ribonucleoprotein particles) to distinct subnuclear bodies, including nucleoli, Cajal bodies and splicing speckles, a nuclear domain where the splicing factors are accumulated [59–61]. For example, the Sm proteins of spliceosome subunits (snRNPs; small nuclear RNPs) localize to Cajal bodies and nucleoli before accumulating in splicing speckles (Figure 7B; orange squares [59,60]), whereas nucleolar protein NHPX travels transiently to the splicing speckles before accumulating in nucleoli (Figure 7B; purple circles [61]). This directed nucleolar entry may also be subject to cell-cycle regulation. For example, both the p68 DEAD box RNA helicase and the Bloom syndrome candidate gene product, BLM,

accumulate within the nucleolus specifically at late telophase and S phase respectively [62–64].

So far only a few examples of directed intranuclear pathways have been uncovered, and the underlying mechanisms are still not known. In future, when more nuclear organelle-specific proteomes are available, comparative analyses may help to identify other proteins that undergo similar intricate traffic within the nucleus. It should be noted that both Sm proteins and NHPX have previously been identified in the organelle-specific proteomic studies on the nucleolus and splicing speckles/spliceosome ([9,10,65–69]; J. S. Andersen, Y. W. Lam, A. K. Leung, A. I. Lamond and M. Mann, unpublished work). The link between splicing factors and the nucleolus was reinforced further by two recent reports showing transient accumulation of more splicing factors within the nucleolus [70,71].

Recent advances in light microscopy and, in particular, the use of live cell fluorophores revealed the surprising result that many components within the nucleolus are not static, but rather are dynamic (Figure 7C; [19,72–74]). The dynamic behaviour of nucleolar proteins is also manifested in the nucleolar proteome. For example, a subset of proteins that were shown to accumulate within nucleoli on transcriptional inhibition [9,75], and several proteins normally localized in Cajal bodies, including snRNP proteins and the Cajal body marker protein p80 coilin, are

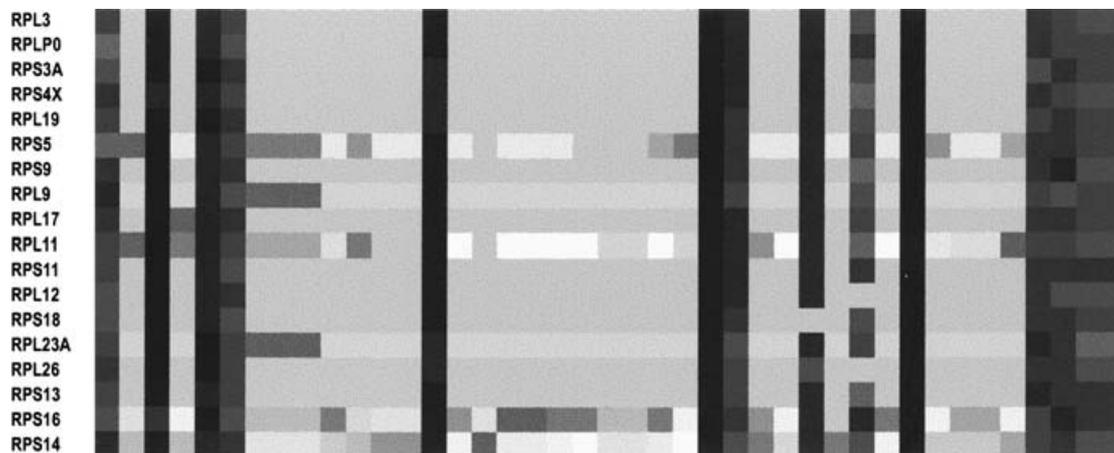


Figure 8 Nucleolar proteome profiling in terms of its homology with other model organisms

The data presented here represent a subset of 20 randomly chosen ribosomal proteins within the human nucleolar proteome. To analyse and present homologues from different completed genome sequences, we can view them as a profile, where each profile is a single row consisting of multiple elements that individually encode either the presence (100% black) or absence (0% black) of sequence homologues in a particular genome. Each profile is a representation of the homologues across different species; matching profiles identifies proteins with similar patterns of inheritance. When placing all the profiles parallel to one another, each row represents a single protein, whereas each column represents a single genome and the blackness of each element represents the degree of homology between the human gene and orthologue from that particular organism. Note that no homology in either primary sequence, or in tertiary structure, is required among the proteins with similar BLASTp profiles in this approach to reveal a possible functional relationship between two proteins.

accumulated in the nucleolus when dephosphorylation is inhibited [54]. During the identification of the human nucleolar proteome, a new subnuclear structure known as ‘paraspeckles’ was identified and three distinct paraspeckle proteins were all shown to associate with the nucleolus in a transcription-dependent manner (Figure 7B, green triangles; [75]). The marker protein, paraspeckle protein 1 (PSP1), is continuously exchanging between the nucleolus and paraspeckles, but PSP1 accumulates at the nucleolar periphery upon transcriptional inhibition. Further comparative analyses between specific organelle proteomes at different cell-cycle stages/metabolic conditions may therefore identify additional complexes that have either cell-cycle-specific or metabolically dependent interactions with the nucleolus.

PROTEOME PROFILING

Most bioinformatics analyses performed so far have analysed either individual proteins or genes and have determined whether certain motifs are present or whether the respective orthologues in other model organisms perform certain functions. In an alternative approach, proteins can be analysed either pairwise or in clusters, because proteins rarely operate alone, but usually function together in a network/complex. Proteins that form a common complex are often expressed co-ordinately in multiple organisms because many pathways or complexes are crippled by the loss of any of the individual components. For example, a set of nucleolar proteins, which are also found in the ribosome, is profiled in Figure 8, according to how they are inherited in different genomes. The similarity of the profile of each individual protein within this subset suggests a potential interdependency in different organisms, where they associate in a complex. Similar phenomena have been observed in yeast, where co-inherited proteins are usually functionally related [76]. Likewise, proteins are often expressed at either the same time, or place, as functionally related proteins. By either choosing different cell tissues [77], or by varying the growth conditions of the cells [78], enough variation in gene expression can be observed to identify co-expressing genes that may exist as complexes within the nucleolar proteome. By

exercising these profiling techniques within the human nucleolar proteome, we may get a glimpse of the possible interaction networks within the human nucleolus. The recent expansion of protein–protein interaction data in yeast, both from large-scale yeast two-hybrid assays and from high-throughput MS, provides a basis for evaluating such potential associations identified from proteome profiling within the nucleolus [79,80].

As discussed in the Dynamic proteome section, the nucleolar proteome is highly dynamic and therefore the 400 human nucleolar proteins identified could facilitate an understanding of how the nucleolus responds to changes in metabolic activity. As recently demonstrated, the inhibition of transcription, e.g. by actinomycin D treatment, causes a set of 11 proteins to accumulate within the nucleolus [75]. Because the main advantage of MS is its high mass accuracy, it is possible to add a quantitative dimension to proteomic experiments by incorporating into proteins an isotope with a molecular mass that is different from the isotope used in the control experiment [81]. The chemically identical analytes with different stable isotope compositions can then be differentiated by their mass differences and the signal intensities between the two forms used to calculate their relative abundance ratio. This quantitative approach provides another dimension to our understanding of how the composition of the nucleolus changes under different metabolic states and at specific cell-cycle stages. It may also be possible to identify whether or not certain proteins move either into or out of the nucleolus as a complex using this type of proteome-profiling technique. For example, upon actinomycin D treatment, proteins that are normally localized in the paraspeckles accumulate into the nucleolus at roughly the same rate, suggesting that these proteins may potentially exist as a complex (J. S. Andersen, Y. W. Lam, A. K. Leung, A. I. Lamond and M. Mann, unpublished work).

PERSPECTIVES

In the present review, we have surveyed the previously established and recently identified nucleolar proteins known in human cells (c.f. Table 1; [9,10]). This includes over 500 proteins, which

are encoded by approx. 0.6–3% of the human genome, assuming that the current estimate of 15 000–80 000 human genes is correct. Surprisingly, considering that the nucleolus has been studied for more than 150 years, approx. 30% of the proteins are still defined as either novel, or previously uncharacterized, according to their genome annotations. By mining the databases that are publicly available, we could obtain interesting clues regarding the functions within the nucleolus. The nucleolus is known to be the place where ribosomal subunit biogenesis occurs. However, the pathway of ribosome synthesis in humans has still not been defined in detail, although this situation may be improved in the near future, especially now with the corresponding human homologues of budding-yeast factors being mapped in the nucleolus (see the Pathway prediction section). In comparison with the known ribosomal biogenesis pathway from yeast, mainly *Saccharomyces cerevisiae*, roughly one-third of the novel or uncharacterized human nucleolar proteins were annotated to be related to ribosomal biogenesis (c.f. Figure 4). The future analyses of these putative ribosomal biogenesis factors, which comprise approx. 20% of the nucleolar proteome, should advance our understanding of how the ribosomal subunits form in human cells and how conserved this process is. The roles of these putative ribosome biogenesis factors can then be tested rigorously by biochemical methods.

As proteins rarely work alone within the cell, nucleolar proteins may work in a complex with one or more partner proteins to perform a specified function. The proteome-profiling technique, by incorporating a multitude of information deposited in public databases, provides a basis for studying biological process modularly, rather than individually (see the Proteome profiling section; [76,82,83]). The fortuitous discovery of nucleolar functions through studying a single protein requires a systematic way to analyse its interaction partners, either along a pathway or within a complex [84]. Even though the number of the human nucleolar proteins is of the order of hundreds, it is still relatively simple to use computational methods to map potential relationships of proteins with one another. The future mining of the wealth of existing and continually expanding information in databases, especially together with the forthcoming quantitative proteomic data, requires efforts to develop good database structures and user-friendly interfaces [85,86]. Good visualization tools are also important, especially to allow non-specialists to have ready access to the existing data. Moreover, standardization of protocols for archiving data is also crucial for transferring information effectively between laboratories and such procedures can be modelled on existing systems provided, for example, by NCBI (<http://ncbi.nlm.nih.gov/>) and EBI (European Bioinformatics Institute; <http://www.ebi.ac.uk/>) for genomic data and OME (Open Microscopy Environment; <http://www.openmicroscopy.org/>) for microscopic data [87].

Another approach for studying dynamic changes in the nucleolar proteome would be to carry out parallel studies using microarray techniques. Gene chips can be tailor-made to contain the 500 corresponding nucleolar cDNAs, allowing changes in the transcriptional levels of the cognate genes to be studied under different growth conditions. Correlative studies with the quantitative measurement of the relative protein abundance derived from proteomic data may provide insights into possible post-transcriptional regulation of the respective nucleolar genes. Moreover, stable cell lines that label multiple subcompartments within the nucleolus with fluorescent-protein-tagged marker proteins may provide a means to study changes in subcellular level under different growth conditions in live cells. Concurrent isolation of RNA and nucleoli from these cell lines in parallel allows quantitative comparisons of the localization pattern with the expression of genes at both transcription and translation levels.

FINAL WORDS

As described by Valentin back in 1836, the nucleolus is like 'a secondary nucleus within the nucleus'. In fact, its complexity, as revealed by its subcompartmentalization, may provide a good model to study how the nucleus is organized. The number of nucleolar proteins (> 500) is ideal for identifying modular sets of proteins that perform specific functions by large-scale profiling of the proteins under many different conditions. The opportunity to isolate functionally intact nucleoli using a well-established protocol, including from established cell lines with fluorescently labelled subcompartments, provides a reproducible *in vitro* system to test models both biochemically and microscopically. The current understanding of the human nucleolar proteome and dynamics may be nothing but a teaser trailer, yet we are all welcome to the show.

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