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Proteomic Analysis of Interchromatin Granule Clusters

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A variety of proteins involved in gene expression have been localized within mammalian cell nuclei in a speckled distribution that predominantly corresponds to interchromatin granule clusters (IGCs). We have applied a mass spectrometry strategy to identify the protein composition of this nuclear organelle purified from mouse liver nuclei. Using this approach, we have identified 146 proteins, many of which had already been shown to be localized to IGCs, or their functions are common to other already identified IGC proteins. In addition, we identified 32 proteins for which only sequence information is available and thus these represent novel IGC protein candidates. We find that 54% of the identified IGC proteins have known functions in pre-mRNA splicing. In combination with proteins involved in other steps of pre-mRNA processing, 81% of the identified IGC proteins are associated with RNA metabolism. In addition, proteins involved in transcription, as well as several other cellular functions, have been identified in the IGC fraction. However, the predominance of pre-mRNA processing factors supports the proposed role of IGCs as assembly, modification, and/or storage sites for proteins involved in pre-mRNA processing.

INTRODUCTION

Interphase mammalian nuclei are compartmentalized into a large number of structures or organelles that are likely to contribute to the fidelity and efficiency of the many functions that occur within this compartment, including transcription, pre-mRNA processing, DNA replication, DNA repair/recombination, assembly of ribosomal subunits, and nucleocytoplasmic protein/ribonucleoprotein (RNP) trafficking (for a review, see Spector, 1993; Lamond and Earnshaw, 1998; Misteli, 2000). Although some nuclear functions can be reproduced in in vitro systems (i.e., transcription and pre-mRNA splicing), these systems may be less efficient than their in vivo counterparts (Corden and Patturajan, 1997). Therefore, in vivo spatial and temporal coordination may have a significant influence on gene expression and other nuclear processes. Among those nuclear organelles thus far identified in normal and cancer cells (for a review, see Spector, 2001) are interchromatin granule clusters (IGCs), perichromatin fibrils, nucleoli, paraspeckles, perinucleolar compartment, Cajal bodies, gemini of Cajal bodies, and promyelocytic leukemia nuclear bodies. Several of these organelles have been shown to have a relationship to various disease states, including cancer and spinal muscular atrophy (Spector *et al.*, 1992; Matera, 1999; Huang, 2000). Recently, several nuclear structures, including the nuclear pore complex (Rout *et al.*, 2000; Cronshaw *et al.*, 2002), nuclear envelope (Schirmer *et al.*, 2003), and nucleoli (Andersen *et al.*, 2002; Scherl *et al.*, 2002) have been isolated, and their protein composition was characterized by mass spectrometry analysis. In addition, in vitro-assembled spliceosomes, the U1 small nuclear ribonucleoprotein particle (snRNP), and the U4/U6.U5 tri-snRNP have been analyzed using this approach (Neubauer *et al.*, 1997, 1998; Gottschalk *et al.*, 1999; Rappsilber *et al.*, 2002; Zhou *et al.*, 2002). Analysis of the yeast nuclear pore complex (NPC) identified 174 proteins in total of which 40 were found to be associated with the NPC in the form of nucleoporins (29 proteins) or transport factors (11 proteins) (Rout *et al.*, 2000). In the case of the NPC from rat liver nuclei, 94 proteins in total were identified, 29 of which were classified as nucleoporins and 18 were classified as NPC-associated proteins (Cronshaw *et al.*, 2002). By using a subtractive proteomics approach to analyze a mouse nuclear envelope fraction, 13 known nuclear envelope integral proteins were identified as well as 67 uncharacterized open reading frames with predicted membrane spanning regions (Schirmer *et al.*, 2003). Proteomic analysis of human nucleoli has identified 271 (Andersen *et al.*, 2002) to ~350 (Scherl *et al.*, 2002) proteins, 30% of which are encoded by novel human genes (Andersen *et al.*, 2002). Analysis of in vitro assembled spliceosomes has identified 145 (Zhou *et al.*, 2002) or 311 proteins (Rappsilber *et al.*, 2002).

One of the most intensely studied nuclear substructures, the IGCs, are thought to play a role in efficiently coupling transcription and pre-mRNA splicing in nuclei (for a review, see Lamond and Spector, 2003). IGCs measure ~1.0–1.5 μm along their widest length and are composed of clusters of 20- to 25-nm granules that often seem to be connected by short fibers (for a review, see Fakan and Puvion, 1980). The IGCs were initially shown to contain a subset of pre-mRNA splicing factors by immunofluorescence and immunoelectron microscopy (for a review, see Spector, 1993). More recent studies have shown that the IGCs are enriched in a number of pre-mRNA splicing factors and the large subunit of RNA

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Table 1. Identified IGC proteins

Protein Description	Accession Code	Chromosomal Locus	RNA Binding Motif	RS	Speckle Localization	Reference	Other Domains and Motif(s)[a]	Low Complexity Region
Pre-mRNA splicing								
30 kDa splicing factor	AAC64086	10q23					TUDOR, coiled coil	Yes
45 kDa splicing factor	AAC64085	10p15.1	RRM				Coiled coil, G_patch	Yes
cdc5-related protein (KIAA0432)	BAA24862	6p21			Yes (IF)	Burns CG., 1999	SANT domains, coiled coil	Yes
DEAD/H box polypeptide 15	O43143	4p15.3					HELICc, HA2	Yes
Formin binding protein (PRP40 homolog)	AAD39463	2q24.1					Signal peptide, WW and FF domain repeats	Yes
Heterogeneous ribonucleoprotein A0	AAA65094	5q31	2 RRMs					Yes
hnRNP A2/B1	P22626		2 RRMs					Yes
hnRNP A3	P51991	10q11.21	2 RRMs					Yes
hnRNP C	A26885		RRM				Coiled coil	Yes
hnRNP C like	A44192		2 RRMs					Yes
hnRNP C1/C2	AAD03717	14q11.2	RRM				Coiled coil	Yes
hnRNP D	BAA09522	4q21.1–q21.2	2 RRMs					Yes
hnRNP E1	CAA55016	2p13–p12	3 KHs					
hnRNP E2	CAA55015		3 KHs					
hnRNP F/H	P52597	10q11.21–q11.22	3 RRMs		Yes (IF)	Matunis *et al.*, 1994		Yes
hnRNP H'	P55795	Xq22	3 RRMs					Yes
hnRNP I	P26599		4 RRMs				Signal peptide	Yes
hnRNP K	Q07244	9q21.32–q21.33	3 KHs					Yes
hnRNP K like/sub2.3	CAA82631		2 KHs					
hnRNP L	P14866	19q13.2	3 RRMs					Yes
hnRNP M	P52272	7q11	3 RRMs					Yes
hnRNP U (SAF A)	Q00839	1q44					SAP, SPRY, coiled coil	Yes
hnRNP A/B related protein	Q99020	5q35.3	2 RRMs					Yes
hnRNPA1	P09651		2 RRMs					Yes
hnRNP G	P38159	Xq26	RRM					Yes
Homolog of *C. elegans* smu-1	NP_060695	9p12					LisH, CTLH, 7 WD 40 repeats	
KH type splicing regulatory factor	AAB53222	19p13.3	4 KHs					Yes
nhp2/rs6 family protein	P55770							Yes
Nuclear matrix protein 55	AAC51852	Xq13.1	2 RRMs				Coiled coil	Yes
Nuclear RNA-binding protein 54-kD	Q15233	Xq13.1	2 RRMs				Coiled coil,	Yes
Plenty-of-prolines-101	AAC17422	1p36.11			Yes (FP)	Mintz *et al.*, 1999	PWI	Yes
PTB associated splicing factor	P23246	1p34.3	2 RRMs				Coiled coil	Yes
RNPS1	AAC39791	16p13.3	RRM	Yes	Yes (IF)	Mayeda *et al.*, 1999		Yes
SAP 114/SF3a	Q15459	22q12.2					2 SWAP, UBQ	Yes
SAP 130/SF3b (KIAA0017)	NP_036558	16q21–22			Yes (FP)	Mintz *et al.*, 1999		Yes
SAP 14/SF3b (pre-mRNA branch site protein p14)	AAK94041	2pter-p25.1	RRM					Yes
SAP 145/SF3b 150	Q13435	11q13.1					SAP, coiled coil	Yes
SAP 155/SF3b	AAC97189	2q33			Yes (FP)	Schmidt-Zachmann *et al.*, 1998	Coiled coil	Yes
SAP 49/SF3b	Q15427	1q12-q21	2 RRMs					Yes
SAP 61/SF3a	A55749						Coiled coil, 1 Znf_C2H2	
SAP 62/SF3a66	Q62203	19p13.3-p13.2					1 ZnF_U1, 1 ZnF_C2H2	Yes
SF3b14b/PHD-finger 5a	NP_116147	22q13.2						
Siah binding protein 1	AAB41656	8q24.2-qte1	3 RRMs					Yes
SnRNP Sm B/B'	P27048						Sm	Yes
SnRNP Sm D1	P13641	18q11.2					Sm	Yes
SnRNP Sm D2	P43330	19q13.2					Sm	
SnRNP Sm E	P08578	1q32					Sm	Yes
SnRNP Sm F	NP_003086	12q23.1					Sm	Yes
SnRNP Sm G	Q15357	2p13.3					Sm	
SnRNP Sm D3	P43331	22q11.23					Sm	Yes
SnRNP U1A	S42114		2 RRMs					Yes
Splicing factor 9G8	A57198	2p22-21	RRM	Yes	Yes (IF)	Caceres *et al.*, 1998	1 ZnF_C2HC	Yes
Splicing factor HCC1	AAA16347	Xp11.3	3 RRMs	Yes	Yes (IF)	Imai *et al.*, 1993		Yes
Splicing factor hPRP17	AAC39730	6q22.1					7 WD 40 repeats	Yes
Splicing factor SC35	Q01130	17q25.3	RRM	Yes	Yes (IF)	Fu *et al.*, 1992		Yes
Splicing factor SF1	AAC29484		KH					
Splicing factor SF2/ASF	S26404	17q21.3-q22	2 RRMs	Yes	Yes (IF)	Caceres *et al.*, 1997		Yes
Splicing factor SF3b10	NP_112577	6q24.1						
Splicing factor SRp20	P23152	6p21	RRM	Yes	Yes (IF)	Caceres *et al.*, 1997		Yes
Splicing factor SRp30	Q13242	15q24-25	2 RRMs	Yes	Yes (IF)	Zahler *et al.*, 1992		Yes
Splicing factor SRp40	Q13243	14q23-24	2 RRMs	Yes	Yes (IF)	Zahler *et al.*, 1992		Yes
Splicing factor SRp55	AAA93072	6 20q12-q13.1	2 RRMs	Yes	Yes (IF)	Zahler *et al.*, 1992		Yes
Splicing factor SRp75	Q08170	1p35.2	2 RRMs	Yes				Yes
Splicing factor YT521-B (KIAA1966)	NP_588611	4q13.3					Coiled coil	Yes
TLS-associated serine-arginine protein	NP_006616	1p36.11	RRM	Yes				Yes

Table 1. Continued

Protein Description	Accession Code	Chromosomal Locus	RNA Binding Motif	RS	Speckle Localization	Reference	Other Domains and Motif(s)[a]	Low Complexity Region
Tra-2 beta homolog	AAC28242	3q28	RRM	Yes	Yes (IF)	Beil *et al*., 1997		Yes
U1 small ribonucleoprotein 1	AAF19255	14q24	RRM	Yes			PWI, coiled coil, RD/E dipeptide repeats	Yes
U1 snRNP 70	P08621	19q13.3	RRM	Yes			Coiled coil, RD/E dipeptide repeats	Yes
U1 snRNP C	P09234	6p21.31					1 ZnF_U1	Yes
U2 snRNP-A'	P09661						LRRcap	Yes
U2AF35	Q01081	15q12-13	RRM	Yes			2 ZnF_C3H1	Yes
U2AF65	P26368	19q13.4	3 RRMs	Yes				Yes
U4/U6-associated RNA splicing factor (PRP3)	AAC09069	1q21.1					PWI	Yes
U5 snRNP 200kD protein (KIAA0788)	O75643	2q11.2					2DEXDc, 2HELICc, SEC63	Yes
U5 snRNP 220kD protein	NP_006436	17p13.3					JAB_MPN	Yes
U5 snRNP 40 kDa protein (38 kDa splicing factor)	AAC69625	1p35.1					7 WD 40 repeats	Yes
U5 snRNP 116 kDa protein (KIAA0031)	AAC53299	17q21			Yes (IF)	Fabrizio *et al*., 1997	1 ZnF_NFX	Yes
U5 snRNP-associated 102 kDa protein	AAF66128	20q13.33					Coiled coil, 13 HAT repeats	Yes
RNA-associated proteins								
ATP dependent RNA helicase A	Q08211	1q25	2 DSRMs				DEXDc, HELICc, HA2	Yes
DAM1 (breast carcinoma amplified sequence 2)	BAA34863	1p13.3-21					Coiled coil	Yes
DEAD/H box polypeptide 3	O00571	Xp11.3-p11.23		Yes			HELICc	Yes
DEAD/H box RNA helicase p68	Q61656	17q21					HELICc	Yes
DEAD/H box RNA helicase p72	Q92841						HELICc	Yes
Double-stranded RNA binding nuclear protein, DRBP76	CAC01405	19p13.2	2 DSRMs				DZF	Yes
E1B-55 kDa associated protein	CAA07548	19q13.31					SAP, SPRY	Yes
Elav-like 1	P70372		3 RRMs					
Interleukin enhancer binding factor 3	AAC71052	19p13.2	2 DSRMs				DZF	Yes
Matrin 3	P43244		2 RRMs				1 ZnF_U1, 1 ZnF_C2H2	Yes
Nuclear cap binding protein 20 kDa (CBP20)	P52298	3q29	RRM					Yes
Nuclear cap binding protein 80 kd	Q09161	9q34.1					MIF4G, coiled coil	Yes
Nuclear protein NP220	BAA11748	2p13.2-p13.1	2 RRMs	Yes	Yes (IF)	Inagaki *et al*., 1996	2 ZnF_C2H2, 2 ZnF_U1, scattered 9-meric repeats	Yes
Nuclear RNA helicase BAT1	Q13838	6p21.3					DEXDc, HELICc	
Pleiotropic regulator 1	AAD24799	7q22					7 WD 40 repeats	
Poly(A) binding protein II	AAC39596	14q11.2-q13	RRM		Yes (IF)	Bregman *et al*., 1995	Coiled coil	Yes
Ribonucleoprotein L	BAA24237	19q13.2	RRM					
RNA binding motif protein 14	NP_063922	11q13.1	2 RRMs					Yes
RNA binding motif protein 5	AAH02957	3p21.3	2 RRMs				1 ZnF_RBZ	Yes
RNA binding motif protein EWS	Q01844		RRM				1 ZnF_RBZ	Yes
RNA binding protein FUS/TLS	P35637	16p11.2	RRM				1 ZnF_RBZ	Yes
RNA binding protein HuR	AAB41913	19p13.2	3 RRMs					
RNA binding protein Raly/Merc	A47318	20q11.21-q11.23	RRM					Yes
RNA helicase (KIAA0801)	NP_055644	5q31.2		Yes	Yes (FP)	This study	DEXDc, HELICc, coiled coil	Yes
Rnpc2	AAH04000		3 RRMs					Yes
Son protein (KIAA1019)	P18583	21q22.11	DSRM	Yes	Yes (FP)	This study	11 mer repeats, 16 tandem decameric repeats, 12 tandem heptameric repeats, 15 heptameric repeats, 3 tandem 11 mer repeats, 13 heptameric repeats, G_patch, coiled coil	

Table 1. Continued

Protein Description	Accession Code	Chromosomal Locus	RNA Binding Motif	RS	Speckle Localization	Reference	Other Domains and Motif(s)[a]	Low Complexity Region
SR140: U2-associated SR140 protein (KIAA0332)	BAA20790	3q23	RRM	Yes	Yes (IF)	Will *et al.*, 2002	SWAP, coiled coil, RPR, 5 octamer repeats	Yes
SYT interacting protein (RNA binding motif protein 14)	NP_006319	11q13.1	2 RRMs			Brett *et al.*, 1997		Yes
Zinc finger RNA binding protein, ZFR (KIAA1086)	AAC25762	5p13.3					3 ZnF_U1, 3 ZnF_C2H2, DZF	Yes
Cleavage and polyadenylation								
CPSF 100 kDa subunit	AAB66830	14q31.1					Coiled coil	Yes
CPSF 160 kDa subunit	Q10569							Yes
CPSF 30 kDa subunit	AAC53567						5 ZnF_C3H1	Yes
CPSF 73 kDa subunit	AAB70268	2p25.2						
CSTF 64 kDa	P33240	Xq22.1	RRM					Yes
Pre-mRNA cleavage factor Im	NP_008938	12q13.2	RRM	Yes			RD/E dipeptide repeats	Yes
RNA polymerase II subunits								
RNA polymerase II 16 kDa subunit	O15514	2q21					RPOL4c	
RNA polymerase II 19 kDa subunit	P52433	11q13.1	S1 (Ribosomal protein S1-like RNA binding domain)					
RNA polymerase II 23 kDa subunit	P19388	19p13.3						Yes
RNA polymerase II 140 kDa subunit	P30876	4q12						Yes
RNA polymerase II Largest subunit	P24928	17p12-13			Yes (IF)	Bregman *et al.*, 1995	RPOLA_N, coiled coil, C-terminal 7 residue repeats	Yes
Transcription								
POZ domain protein FBI-1	NP_056982	19p13.3			Yes (IF)	Pendergrast *et al.*, 2002	BTB, 4 ZnF_C2H2	Yes
POZ/zinc finger transcription factor, ODA-8	NP_062752	3q13.2					BTB, 5 ZnF_C2H2	Yes
Skip	Q13573	14q24.3					Coiled coil	Yes
Tho2	AAM28436	Xq25-q26.3					Coiled coil	Yes
RNApolymerase II holoenzyme component SRB7	Q13503	12p12.1						
mRNA export, NMD								
Aly	AAD09608	17q25.3	RRM		Yes (IF)	Zhou, *et al.*, 2000		Yes
Mago-nashi homolog	NP_002361	1p34-p33			Yes (IF)	Kataoka *et al.*, 2001		
Rae1/mRNP41	P78406	20q13.31					4 WD 40 repeats	Yes
RNA binding motif protein 8 (Y14)	AAD21089	14q22-23	RRM	Yes	Yes (IF)	Kataoka *et al.*, 2000		Yes
Apoptosis								
Acinus/SAP152 (KIAA0670)	NP_055792	14q11.2	RRM	Yes	Yes (FP)	This study	SAPdomain, coiled coil, RD/E dipeptide repeats	Yes
Bcl-2-associated transcription factor, Btf (KIAA0164)	AAH34300	6q22-23		Yes	Yes (FP)	This study		Yes
Others								
actin	P02571	17q25			Yes (IF)	Spector, unpublished data		
APOBEC-1 stimulating protein	CAB94754	10q21.1	3 RRMs					Yes
CAF1/p48	Q09028	1p34.3			Yes (FP)	Saitoh, N. unpublished data	6 WD 40 repeats	
Cell division cycle 2-like 1, Clk	NP_277025	1p36			Yes (FP)	Sacco-Bubulya *et al.*, 2002	Coiled coil	Yes
eIF4A III (KIAA0111)	P38919	17q25.3			Yes (FP)	Sacco-Bubulya, P. unpublished data	DEXDc, HELICc	Yes
Galectin	O08573				Yes (IF)		GLECT	
Glutathione transferase	S-P08011				Yes (IF)	Bennett *et al.*, 1986	MAPEG	
Hsp 70/Hsc 70	NP_005338	9q33-q34.1			Yes (IF)	Maheswaran *et al.*, 1998	Signal peptide	
Nuclear matrix protein NMP200	CAB51857	11q12.2					U box, 7 WD 40 repeats	Yes
Pinin	NP_002678	14q13.3			Yes (IF)	Brandner *et al.*, 1997	Coiled coil	Yes
Protein phosphatase 1, regulatory subunit 10/ FB19 protein	JE0291	6p21.3	RRM				TFS2N, 1 ZnF_C3H1	Yes
Rod1	BAA75465	5q22	4 RRMs					Yes
SAF B	AAC29479	19p13.2-13.3	RRM		Yes (FP)	Nayler *et al.*, 1998	Coiled coil	Yes
SCAF10	JC5314			Yes	Yes (IF)	Mortillaro *et al.*, 1998	Pro_isomerase	Yes
SCAF6/DAN16	AAN77183	19p13.1		Yes			SWAP, RPR, Trp containing repeat region, G_patch,	Yes
SRm300 (KIAA0324)	AAF21439	16p13.3		Yes				Yes
Wilms' tumour 1-associating protein, WTAP (KIAA0105)	NP_004897	6q25-q27			Yes (IF)	Little *et al.*, 2000	Coiled coil	Yes

IF, Immunofluorescence; FP, fluorescent protein.

[a] Database for motif and domain searches: SMART (*http://smart.embl-heidelberg.de*), (Schultz *et al.*, 1998; Letunic *et al.*, 2002). Only those proteins containing SR dipeptides were manually searched for RD/E dipeptide repeats and other repetitive amino acid sequences.

polymerase II (Bregman *et al.*, 1995; Mortillaro *et al.*, 1996), however, transcription and pre-mRNA splicing do not generally seem to take place within these nuclear regions (Cmarko *et al.*, 1999; Misteli and Spector, 1999). Instead, splicing factor assembly, modification and/or storage are thought to occur within these nuclear compartments (for a review, see Misteli and Spector, 1998; Lamond and Spector, 2003). IGCs are dynamic nuclear structures from which splicing factors have been shown to be recruited to sites of active transcription in living cells (Misteli *et al.*, 1997; Janicki *et al.*, 2004). Studies using fluorescence recovery after photobleaching have shown that there is a continuous flux of proteins between the IGCs and the nucleoplasm (Kruhlak *et al.*, 2000; Phair and Misteli, 2000). However, it is unclear whether the IGC proteins move as monomers, small complexes, or as a large complex such as individual 20- to 25-nm granules to sites of transcription. In addition, the specific composition of individual interchromatin granules remains to be determined.

We have previously established a protocol to biochemically isolate IGCs from mouse liver nuclei (Mintz *et al.*, 1999) and in our initial characterization of this fraction by mass spectrometry, we identified 33 protein constituents of IGCs. Here, we have extended these studies to saturation and have identified 146 IGC proteins as well as 32 novel protein candidates. We have characterized the 146 proteins based upon their motifs and localization. Our analysis has identified 31 RS domain-containing proteins as well as proteins involved in other aspects of mRNA metabolism. Interestingly, we have found a significant overlap (63%) between our analysis and the recently reported analyses of the protein composition of spliceosomes (Neubauer *et al.*, 1998; Rappsilber *et al.*, 2002; Zhou *et al.*, 2002). Our findings support a proposed role of IGCs in the assembly, modification, and/or storage of proteins involved in pre-mRNA processing.

MATERIALS AND METHODS

IGC Purification and Mass Spectrometry Analysis

Approximately 3 mg of IGCs was purified from 120 5- to 6-wk-old female Swiss Webster mice (27–30 g) according to a procedure described previously (Mintz *et al.*, 1999). The purified IGC fraction was directly dissolved in 2 M urea-phosphate-buffered saline-0.1 mM EDTA, allowing us to recover IGC proteins with high efficiency, rather than our previous approach, whereby we resuspended proteins in TM5 (0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$). In addition, in the present study we started with 6 times the number of mice relative to our previous report, which yielded ~10 times more IGC proteins based on measurement of protein concentrations by mass spectrometry analysis. One-third of the dissolved IGC proteins were biotinylated at Cys residues with the chemical cross-linker Biotin-HPDP followed by trypsin digestion, whereas the remaining two-thirds of the IGC proteins were directly digested with trypsin. Cys-containing peptides were selected through avidin chromatography to reduce the complexity of the peptide mixture, thus increasing the chances of detecting low abundant peptides with Cys residues that are normally masked by abundant peptides (Spahr *et al.*, 2000). The selected Cys-containing peptides, as well as a mixture of trypsin-digested peptides without Cys selection, were analyzed by liquid chromatography and tandem mass spectrometry (MS/MS). Fragment ion spectra were batch searched against nonredundant protein sequences in databases. Resulting peptide matches were manually evaluated and confirmed. Motif analysis of each identified protein was performed using SMART (http://smart.embl-heidelberg.de/) (Schultz *et al.*, 1998; Letunic *et al.*, 2002). Database for Tables 1–4 is available at http://spectorlab.cshl.edu.

Transient Transfection of Cells and Immunofluorescence Microscopy

Four cDNA clones that correspond to newly identified IGC proteins (KIAA0164, 0670, 0801, and 1019) were kindly provided by Dr. Nagase (Kazusa DNA Research Institute, Chiba, Japan). The clones were fused in frame, to enhanced yellow fluorescent protein at their N termini by using the pEYFP-C expression vector (BD Biosciences Clontech, Palo Alto, CA). A431 cells were transfected with the resultant constructs using FuGENE6 transfection reagent (Roche Diagnostics, Indianapolis, IN) according to the manufacturer's instructions and incubated for 16 h. Cells were processed for immunofluorescence as described previously (Spector *et al.*, 1998). Antibody to SC35 (Fu and Maniatis, 1990) was used at 1:1000 dilution to label IGCs, followed by Texas Red-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Images were acquired on an Axioplan 2i fluorescence microscope (Carl Zeiss, Thornwood, NY)with a plan-APO 100×/1.4 numerical aperture objective lens using Openlab Software (Improvision, Lexington, MA) and an Orca charge-coupled device camera (Hammamatsu, Middlesex, NJ).

RESULTS

The IGC Proteome

We have previously reported on the development of a biochemical strategy to purify and characterize IGCs from mouse liver nuclei. Using this approach combined with mass spectrometry analysis, we identified 33 known proteins (Mintz *et al.*, 1999) and expressed sequence tags (ESTs) encoding at most 16 proteins after searching a nonredundant protein database or dbEST (National Center for Biotechnology Information and DNA Data Bank of Japan/European Molecular Biology Laboratory/GenBank) with the uninterpreted MS/MS spectra. We have now extended this study by scaling up our purification and optimizing the sample preparation (see MATERIALS AND METHODS) to identify a larger complement of IGC proteins. The IGC fraction was digested with trypsin and subjected to liquid chromatography electrospray ionization MS/MS followed by uninterpreted fragment ion searching of nonredundant and expressed sequence tag databases (dbEST) in a data-dependent manner. Our analysis will identify proteins that are enriched in IGCs and therefore localize in a speckled pattern by immunofluorescence microscopy (i.e., snRNPs and serine-arginine proteins), as well as other proteins that may be equally distributed throughout the nucleoplasm, including the IGCs and diffuse nuclear pools (i.e., hnRNP A and C). We performed five rounds of the analysis and reached saturation as we repeatedly obtained the same set of peptide sequences. As a result, 2214 peptide sequences were obtained, which correspond to 360 proteins. We categorized the proteins based upon their known function, motifs, and/or localization: identified IGC proteins (41%), potential IGC proteins (19%), novel IGC protein candidates (9%), and unexpected IGC proteins (31%) (Tables 1–4).

Identified IGC Proteins

The group of identified IGC proteins (Table 1, 146 proteins) contains the most frequently detected proteins and is composed of previously identified IGC proteins, as well as proteins whose functions are similar to well-characterized IGC proteins. Because many of the proteins that have been localized to IGCs contain RNA binding motifs and RS domains that are stretches of dipeptide repeats of arginine (R) and serine (S) (Birney *et al.*, 1993), we systematically surveyed all of the detected proteins with regard to these motifs. Nineteen percent of the identified IGC proteins contain an RS domain, and 50% contain one to four RNA binding motifs (Table 1). The presence of an RS domain and/or basic region has been reported to act as a speckle localization signal for some pre-mRNA splicing factors as well as a protein interaction domain (for a review, see Fu, 1995; Graveley, 2000). In addition, each of the identified proteins was characterized with regard to the presence of other motifs and its localization to nuclear speckles. Twenty-seven percent of the identified IGC proteins have previously been reported to localize

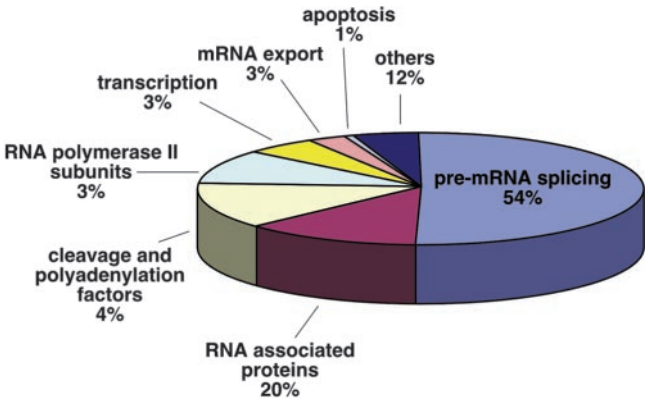

Figure 1. Profile of the Identified IGC proteins. One hundred forty-six identified IGC proteins are categorized based upon their proposed functions; 81% of the proteins are involved in activities related to RNA metabolism.

in nuclear speckles. We did not detect any sequence motifs common to all identified IGC proteins. Two frequently detected motifs in this group are the DEAD box helicase motif (Linder *et al.*, 1989; Luking *et al.*, 1998) and an RNA binding motif (Birney *et al.*, 1993). The absence of a specific localization signal, aside from the RS domain contained within a subset of proteins, may reflect a more transient interaction of many proteins with nuclear speckles or may indicate that these proteins are targeted to and/or associate with nuclear speckles through other RS-domain–containing interaction partners.

A profile of this protein group (Figure 1) indicates that 54% of the identified IGC proteins have a role in pre-mRNA splicing, 20% of the proteins are classified as RNA-associated proteins, and 7% have roles in other aspects of pre-mRNA processing, such as 3′ RNA processing, mRNA export, and nonsense-mediated decay (see DISCUSSION). Together, 81% of the IGC proteins likely participate in pre-mRNA/mRNA metabolism.

IGCs have been proposed to be important for the coupling of RNA polymerase II transcription and pre-mRNA splicing, because numerous proteins are recruited from nuclear speckles to sites of transcription (for a review, see Lamond and Spector, 2003). Six percent of the identified IGC proteins are involved in transcription (Table 1). Several subunits of RNA polymerase II, including the largest subunit, which has previously been localized to nuclear speckles (Bregman *et al.*, 1995; Mortillaro *et al.*, 1996), and several transcription factors have been identified in this fraction. Most general transcription factors were diffusely distributed throughout the nucleoplasm and were not identified in the IGC fraction. However, the proportion of transcription factors may be underrepresented, because we have categorized many proteins as potential IGC proteins (Table 2) due to the lack of information on their specific subnuclear localization. As expected, we did not detect RNA polymerases I or III in the IGC fraction.

Interestingly, several proteins were identified that have previously been characterized as having structural roles in cells. These proteins include actin (Nakayasu and Ueda, 1984), matrin 3 (Belgrader *et al.*, 1991; Nakayasu and Berezney, 1991), lamin A/C (Jagatheesan *et al.*, 1999), and pinin (Ouyang and Sugrue, 1996; Brandner *et al.*, 1997; Ouyang *et al.*, 1997). Although all of these proteins have been localized to IGCs, they do not form an underlying protein scaffold for attachment of IGCs (Sacco-Bubulya and Spector, 2002). Instead, they may be integral components of individual interchromatin granules and their role(s) is yet to be determined.

In addition, our analysis identified several proteins that were recently shown by others to have roles in pre-mRNA splicing or to be localized to nuclear speckles. These include acinus (Boucher *et al.*, 2001; Schwerk *et al.*, 2003), eIF4Aiii (Li *et al.*, 1999; Holzmann *et al.*, 2000), RNA binding motif protein 8 (Y14) (Kataoka *et al.*, 2001), and the RNA export protein Aly (Zhou *et al.*, 2000). Surprisingly, our analysis did not reveal some proteins that have previously been reported to localize to nuclear speckles, for example, casein kinase II and protein phosphatase 1 (Trinkle-Mulcahy *et al.*, 2001). Protein phosphatase 1 has only one trypsin cleavage site, so it would likely be underrepresented in our peptide identification by mass spectrometry. Other proteins that were not identified associate with IGCs with low affinity and therefore may dissociate during the purification procedure. Alternatively, association of proteins such as kinases and phosphatases may be more sensitive to changes in phosphorylation state during IGCs purification.

Potential and Unexpected IGC Proteins

We found 70 proteins whose nuclear localizations, for the most part, have not been characterized, although these proteins have been studied at the biochemical and/or molecular levels (Table 2). We categorized this group of proteins as potential IGC proteins. Many of these potential IGC proteins have roles in transcription, such as DNA *cis*-element binding factors (i.e., transcription factor NF-AT45, nuclear factor I-X, and C/EBPs), components of a chromatin remodeling complex (BAF53A and BAF57), and transcription mediators (transcriptional coactivator CRSP77, thyroid hormone receptor-associated proteins, and transcriptional intermediary factors). Seven percent of the potential IGC proteins are possible molecular chaperones because they contain either a cyclophilin type peptidyl-prolyl *cis-trans*-isomerase motif or AAA ATPase family motif. Four percent are DNA repair proteins, and the remaining proteins have varied functions or they have not been studied at the molecular level. Although the subnuclear distribution of each protein remains to be determined, the identification of these proteins in the IGC fraction suggests that IGCs may be major sites for coupling transcription and pre-mRNA processing, thus promoting efficient gene expression. Furthermore, some of the molecular chaperone proteins included in this category may be responsible for the formation/maintenance of the structure of IGCs.

To determine whether these proteins are bona fide IGC constituents, we made cDNA fusion constructs to tag them with yellow fluorescent protein and expressed them in A431 cells. Four representative cDNAs shown in Figure 2 all encoded for proteins that localize to IGCs (KIAA0670, KIAA0801, and KIAA1019) or their periphery (KIAA0164), further confirming the specificity of our preparation. Because we now have evidence that they are bona fide IGC proteins, we have included these four proteins in Table 1.

In our previous study, we showed that the IGC fraction was highly purified and free of detectable contaminants, such as other nuclear structures. When examined using transmission electron microscopy, the final fraction was significantly homogeneous, containing granules measuring 20–25 nm in diameter that were immunolabeled with anti-SC35 antibody, a marker protein for IGCs (Mintz *et*

Table 2. Potential IGC proteins

Protein Description	Accession Code	RNA Binding Motif	RS	Other Domains and Motif(s)[a]	Low Complexity Region
A-kinase anchor protein 8K	Q63014			1 ZnF_C2H2	Yes
Aladin (Adracalin).	P58742			4 WD 40 repeats	Yes
Aquarius (KIAA0560)	AAB50008				Yes
Ash2	AAC13564			SPRY	Yes
Ataxin-1	P54254			AXH	Yes
BAF53A	AAC94992			Actin	
BAF57	AAC04509			HMG, coiled coil	Yes
BMAL1(HLH/PAS protein)	O00327				Yes
C/EBPa	P53566			BRLZ	Yes
C/EBPb	P28033			BRLZ	Yes
Calsyntenin 1 (KIAA0911)	NP_075538			Signal peptide, cadherin repeats, transmembrane, coiled coil	Yes
CyP-60 (cyclophilin-like protein)	S64705			Ubox, pro_isomerase	Yes
Dna J protein homolog 2	P31689			Dna J, DnaJ CXXCXGXG, DnaJ C	Yes
dpy-30-like protein	NP_115963			Dpy-30	
Early lymphoid activation protein	I56219				
eIF4AI	P04765			DEXDc, HELICc	
FB19 protein	JE0291			TFS2N, 1 ZnF_C3H1	Yes
G10 protein	AAC14190				
GC-rich sequence DNA-binding factor candidate	AAD34617				Yes
General transcription factor IIIC, polypeptide 2	NP_001512			4 WD 40 repeats	Yes
Hepatocyte nuclear factor 1 alpha	P15257			HOX	Yes
Hepatocyte nuclear factor 4 alpha	P41235			1 ZnF_C4, HOLI	Yes
Homeobox protein zhx-1	JC4863			2 ZnF_C2H2, 5 HOX	Yes
Interleukin enhancer binding factor 2	NP_080650			DZF	Yes
IRA1	AAG44738			LisH, 8 WD 40 repeats	Yes
LIM-domain protein LMP-1	AAD13197			PDZ, 3 LIM	Yes
Lupus La protein	P32067	RRM		LA	Yes
Mader/NAB	S31927				Yes
MAX-like bHLHZIP protein, transcription factor-like 4	NP_037515			HLH	
mRNA associated protein MRNP 41 (RAE1 homolog)	P78406			4 WD 40 repeats	Yes
mRNA export factor TAP	Q99JX7	RRM		LRR, LRRcap, NTF2, TAPC	Yes
Ngfi-A binding protein 1	NP_032693			NCD1, NCD2, Nab1	Yes
Nuclear Factor I-X	P09414			DWA	Yes
Nuclear protein ZAP3	Q9R0I7			Coiled coil	Yes
Nuclear receptor coactivator 5	NP_066018			HGTP anticodon, coiled coil	Yes
Nuclear VCP-like protein NVLp.1	AAB70460			AAA	Yes
NuMA	A42184			Coiled coil	Yes
p150TSP (KIAA0155)	BAA09925			9 TPR, coiled coil	Yes
PCAF-associated factor 400, PAF400	AAD04629			FAT, PI3Kc, FATc	Yes
Peptidylprolyl isomerase (cyclophilin)-like 1	NP_057143				
Polymyositis/Scleroderma autoantigen 1, PM/SCL-75	Q9JH17			RNase_PH, RNase_PH_C	Yes
Predicted osteoblast protein	BAA13251			Signal peptide	
Prox1	Q92786				
RAD50	AAC52894			Rad50_zn_hook, coiled coil	Yes
RuvB like DNA helicase	NP_035434			AAA	
SEC13-related protein	NP_109598			6 WD 40 repeats	
Symplekin, Huntingtin interacting protein I	XP_017129				Yes
SYT interacting protein SIP	AAC64058	2 RRMs			Yes
TAFII30 protein	CAB59510			Signal peptide	Yes
TAR DNA binding protein	NP_663531	2 RRMs			Yes
TAR DNA-binding protein-43	I38977	2 RRMs			Yes
Thyroid hormone receptor-associated protein 100 kDa- (KIAA0130)	NP_035999				Yes
Thyroid hormone receptor-associated protein 150 kDa	AAD22034		Yes		Yes
Transcription elongation factor B (SIII) polypeptide 2, elongin B	NP_112391			UBQ	
Transcription factor NF-AT 45	A54857			DZF	
Transcription factor-like protein 4	JC5333			HLH	Yes
Transcription intermediary factor 1-beta, TIF1-beta	Q62318			Signal peptide, 2 RING, 2 BBOX, BBC, PHD, BROMO	Yes
Transcriptional co-activator CRSP77	XP_048386				
Transcriptional intermediary factor 2	CAA66263			HLH, PAS, PAC	Yes
Transducin (beta) like 1 protein	CAA73319				Yes
Trf-proximal protein	NP_064432				
Tuftelin-interacting protein 33	NP_061253			G_patch	Yes
Tumor protein D52	P55327			TPD52	
WD repeat domain 5 protein	NP_060058			7 WD40 repeats	Yes
WD repeat protein BIG-3	AAL27006			7 WD40 repeats	Yes
XPA-binding protein 2, XAB2 (KIAA1177)	BAB15807			11 HAT	Yes
XPE UV-damaged DNA binding protein	CAA05770				Yes
ZAN75	BAA31522			2 ZnF_C2H2	Yes
Zinc finger DNA binding protein 99 ZBP-99	AAD21084			4 ZnF_C2H2	Yes
Zinc finger protein	CAB70967			4 ZnF_C2H2	Yes

[a] Database for motif and domain searches: SMART (*http://smart.embl-heidelberg.de*), (Schultz *et al.*, 1998; Letunic *et al.*, 2002). Only those proteins containing SR dipeptides were manually searched for RD/E dipeptide repeats and for other repetitive amino acid sequences.

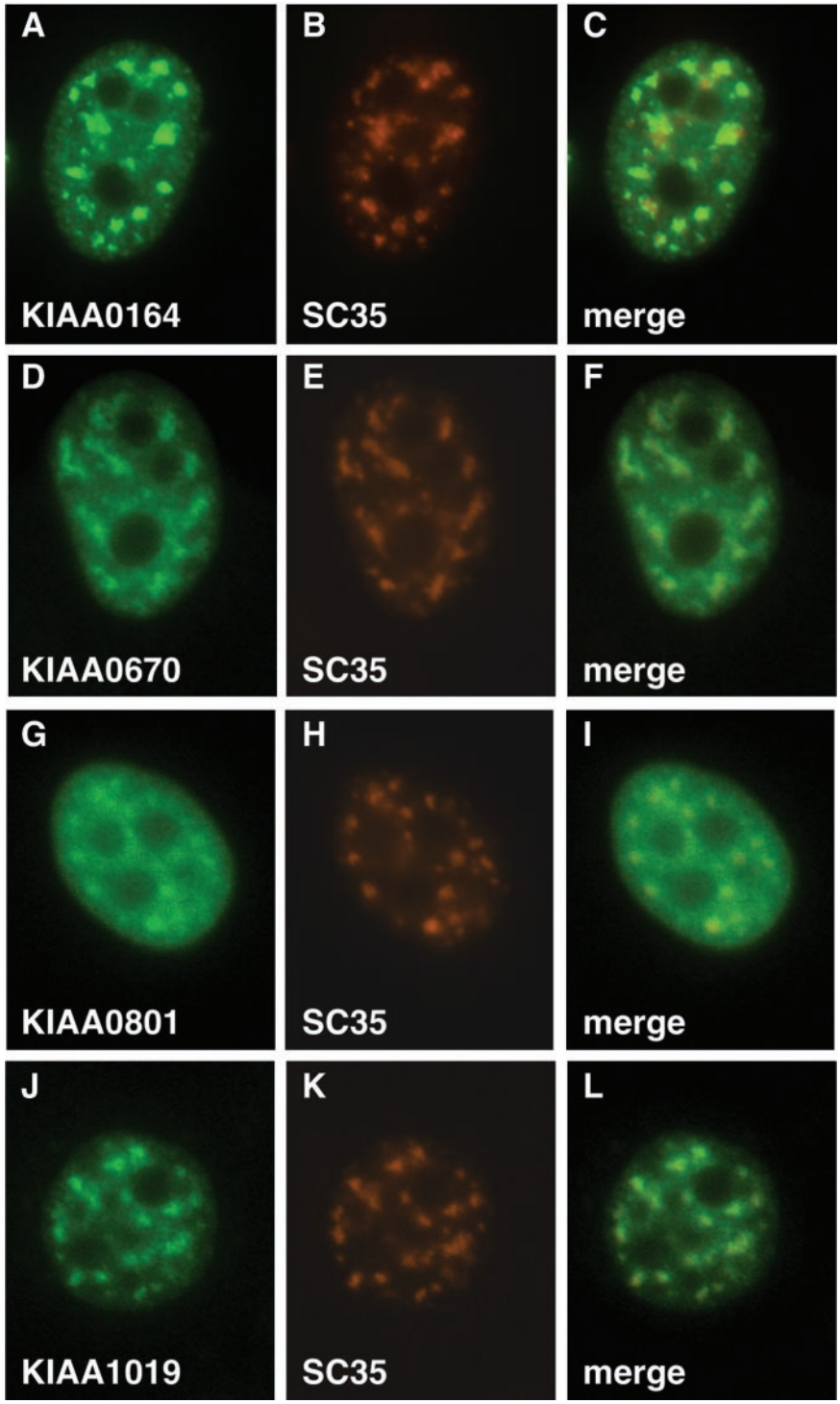

Figure 2. In vivo localization of several novel IGC proteins. The cDNAs for several novel IGC protein candidates (KIAA0164 = Btf, KIAA0670 = acinus, KIAA0801 = RNA helicase, KIAA1019 = son protein) were fused to yellow fluorescent protein and expressed in A431 cells. The cells were fixed and labeled with an antibody to the pre-mRNA splicing factor SC35 (Fu and Maniatis, 1990), which localizes in IGCs.

al., 1999). In addition, immunoblot analysis showed that a subset of known IGC proteins are highly enriched in the IGC fraction, whereas minimal contamination of protein components of other nuclear structures, such as the nuclear envelope, promyelocytic leukemia bodies, or Cajal bodies were detected in the IGC fraction (Mintz *et al.*, 1999). Nonetheless, by mass spectrometry we did detect numerous proteins, which have previously been characterized as components of other cellular structures, and therefore we have classified them as unexpected proteins (Table 3). Because these proteins are relatively abundant and mass spectrometry is a highly sensitive technique, it is likely that they are protein contaminants in our preparation.

Novel IGC Protein Candidates

In addition, and most interestingly, we found 32 proteins for which no available biological information is available, except for sequence information (Table 4). Each of these pro-

teins was analyzed for known motifs. Four proteins have various similarities to other proteins involved in RNA metabolism. These examples include a protein with a RNA helicase C-terminal domain (KIAA0052), a protein slightly similar to cleavage and polyadenylation stimulation factor (KIAA0663), a putative splicing factor (RIKEN cDNA 2410002M20), and a protein with similarity to SAF-B (similar to KIAA0138 gene product), which is known to be in IGCs. Thus, these proteins are highly likely to be IGC components. Two other proteins contain an SAP motif, one also with a poly-A binding domain (RIKEN cDNA 2610511G16) and the other with SPRY and Ffh domains (similar to hypothetical protein). The SAP motif is named after *S*AF-A/B, *a*cinus and *P*IAS (Aravind and Koonin, 2000). SAF-B and acinus are localized in the IGCs (Table 1 and Figure 2), and PIAS has been shown to be associated with RNA helicase II/ATP-dependent RNA helicase (Valdez *et al.*, 1997). The SAP motif is defined as a sequence homologous to the N-terminal DNA binding region of SAF-A and has been found in several other nuclear proteins (Aravind and Koonin, 2000). Proteins with a SAP domain often contain an additional motif that is involved in the assembly of RNA-processing complexes (Aravind and Koonin, 2000). Therefore, it has been proposed that such proteins are associated both with chromatin and RNA. Additionally, they may function to deliver the RNA processing machinery to the site of transcription (Aravind and Koonin, 2000), which overlaps with a proposed function of IGCs.

RS Domain-containing Proteins

In the IGC proteomic analysis, we detected 31 proteins with RS dipeptide motifs, including two novel IGC candidates (Tables 1, 2, and 4). Of these, 17 proteins have actually been shown to localize to IGCs by either immunofluorescence analysis or expression of the fluorescently tagged proteins in cells (Table 1). By comparing these proteins, based upon the organization of their other motifs relative to the RS domains, we sorted them into three major groups (Figure 3). The first group (Figure 3A) represents proteins with an RS motif and one to three RNA recognition motifs (RRMs). This group can be further divided into three subgroups. Proteins in the first subgroup, from SRp20 to SRp75, are small proteins with N-terminal RRMs and a C-terminal RS motif. Among this group are members of the SR family of pre-mRNA splicing factors (SRp20, SF2/ASF, SC35, 9G8, SRp30, SRp40, SRp55, and SRp75). Proteins in the second sub-group, from the tra-2 beta homologue to splicing factor HCC1, are also small splicing factors, but they have an N-terminal RS motif and a C-terminal RRM(s). Proteins in the third subgroup are related to the first two subgroups because they have N-terminal RRM and C-terminal or middle region RS motifs; however, they are larger proteins and their RS motifs are continuous to RD or RE dipeptides, which could provide them with additional functional properties (see DISCUSSION).

Proteins in the second group (Figure 3B) are medium-to-large proteins, ranging from 663 to 2297 amino acids. All (except for acinus) do not have a recognizable RRM motif, and they are characterized by the presence of compositionally biased regions. Among them, Btf and a protein called "similar to TRAP150" have significant sequence similarities to TRAP150 (60 and 33% sequence identity, respectively). TRAP150 has been shown to be a transcriptional mediator component (Johnson *et al.*, 2002). Proteins categorized in this second group contain additional domains, such as a cyclophilin type peptidyl-prolyl *cis-trans*-isomerase (proisomerase) domain, a SAP domain, and a DEAD box helicase motif, thus they may have additional interactions and/or functions. Indeed, SRm 300 is a splicing coactivator (Blencowe *et al.*, 2000), and acinus is involved in chromatin condensation in the late stage of apoptosis (Sahara *et al.*, 1999) as well as in pre-mRNA processing (Schwerk *et al.*, 2003). Btf also was reported to be involved in apoptosis (Kasof *et al.*, 1999).

The third group (Figure 3C) also represents proteins of medium-to-large (917–2427 amino acid length) size with interesting repetitive sequences. Especially notable is son protein, which contains six types of repetitive sequences that cover approximately one-third of its sequence. The functions of these proteins are not well characterized; however, NP220 was reported to be a DNA and nuclear matrix binding protein (Inagaki *et al.*, 1996), and SR140 is associated with U2 snRNP (Will *et al.*, 2002).

DISCUSSION

We have performed an in-depth analysis of the protein composition of IGCs derived from mouse liver nuclei. As expected, we detected numerous proteins involved in pre-mRNA processing. In addition, we detected transcription factors, RNA polymerase II subunits, and proteins with unexpected roles in apoptosis and DNA repair. We also identified numerous novel IGC protein candidates.

IGCs and Spliceosomes

Extensive evidence has suggested that the nucleus is compartmentalized with respect to gene expression (for a review, see Spector, 2003). IGCs are enriched in pre-mRNA splicing factors, yet these nuclear regions are not sites of splicing or transcription. Rather, they are sites of splicing factor assembly/modification and/or storage (for a review, see Lamond and Spector, 2003) from which factors are recruited to nearby sites of active transcription. The C-terminal domain of the large subunit of RNA polymerase II and phosphorylation of the RS domain of SR splicing factors play a major role in supplying these factors to the site of active transcription (Misteli *et al.*, 1998; Misteli and Spector, 1999). However, it has not been determined whether different splicing factors are targeted to a site of transcription individually, or as subcomplexes as needed for different stages of pre-mRNA processing. The latter is a possibility, because individual interchromatin granules are of a consistent size with ribosomes and are therefore large enough to contain such subcomplexes of proteins. When we made a comparison of protein components of the spliceosome (Zhou *et al.*, 2002) versus IGC components, we found significant (63%), but not total overlap, between these two structures, although each complex was initially purified from an entirely distinct nuclear fraction.

Because there is considerable overlap of IGC components (modification/assembly and/or storage sites) with spliceosome components (functional sites), there is a possibility that interchromatin granules move from the IGCs to the site of active transcription, rather than each protein moving individually. It has been shown that fluorescently tagged splicing factors are highly mobile in living cells, but they move slowly enough to suggest that the proteins move in a complex, rather than as a monomer (Kruhlak *et al.*, 2000). By time-lapse microscope analysis, it was shown that "spheres" seem to bud off of the surface of nuclear speckles when cells

Table 3. Unexpected proteins

Protein Description	Accession Code	Protein Description	Accession Code	Protein Description	Accession Code
14-3-3 protein	P31946	Endo/exonuclease Mre 11	AAB04955	Nuclear receptor co-repressor N-CoR	S60254
40s ribosomal protein s4, X isoform	P12750	Enhancer of rudimentary homolog	Q14259	Nucleolar phosphoprotein p130	I38073
40s ribosomal protein S10	P46783	Exosome complex exonuclease RRP45/PMSCL1	Q06265	Nucleolar protein family A, member 1	NP_080854
40s ribosomal protein S14	P13471	Fibrillarin	P22087	Nucleolar protein NAP57/CBF5	O60832
40s ribosomal protein S16	P17008	Fibrinogen, alpha polypeptide	XP_130931	Nucleolar protein NOP10	NP_061118
40s ribosomal protein s2 (s4) (llrep3 protein)	P15880	Glucocorticoid receptor	P06537	Nucleolar protein NOP5/NOP58	AAD27610
40s ribosomal protein S28	P25112	Glucokinase regulatory protein	Q07071	Nucleolar protein NOP56	O00567
40s ribosomal protein s3a. 12/1998	P49241	Histone deacetylase (HD1)	Q13547	Nucleoporin Nup75	NP_079120
40s ribosomal protein s5. 7/1999	P46782	Histone H1	P15864	Nucleoporin Nup84	AAB52419
40s ribosomal protein s6 (phosphoprotein np33)	P10660	Histone H2a	P02262	Nuclear Pore Complex Protein NUP155	O75694
40s ribosomal protein S7	P06584	Histone H2b	P02278	O-linked GlcNAc transferase	AAB63466
40s ribosomal protein s8	P09058	Histone H3	P06351	PCAF associated factor 400	AF110377
40s ribosomal protein S9	P29314	Histone H4	P02304	PML	AAA97601
60s acidic ribosomal protein p0	P05388	Host cell factor C1 HCF	P51610	Protein disulfide isomerase A3 precursor, ER-60	P30101
60s acidic ribosomal protein p1	P47955	HP1	P45973	RAD50 homolog	NP_033038
60s ribosomal protein L12	P30050	Immunoglobulin Heavy Chain Binding Protein	P11021	Ran GAP1	P46061
60s ribosomal protein L13	P41123	Importin alpha	P52294	Ran GTPase	NP_033417
60s ribosomal protein L14	P50914	Importin beta	Q14974	RanBP2 (Nup 358)	P49792
60s ribosomal protein L15	P39030	Integral membrane glycoprotein gp210	P11654	Recombination signal binding protein	AAA16254
60s ribosomal protein L19	P14118	Lamin A	P02545	RelA-associated inhibitor	XP_030918
60s ribosomal protein L23	P23131	Lamin B1	P14733	REST corepressor (KIAA0071)	NP_055971
60s ribosomal protein L24	P38663	Lamin B2	P21619	Ribosomal protein S30	AAD1774
60s ribosomal protein L27a.	P46776	Lamin B3	P48680	S164/presenilin	AAC97961
60s ribosomal protein L31	P12947	Lamin C	P02545	SAP18 (sin3 associated polypeptide p18)	AAD41090
60s ribosomal protein L35	P42766	Lamina-associated polypeptide 2 LAP2	P42166	Sin3	AAB01610
60s ribosomal protein L4	P36578	Metalloproteinase inhibitor 1 precursor	P01033	SWI/SNF BAF155	AAC50693
60s ribosomal protein L7a	P11518	Methyl-CpG binding domain-containing protein MBD3	AAC68877	SWI/SNF related, BAF170, Rsc8	NP_003066
60s ribosomal protein L8	P25120	Mi2 chromodomain helicase-dna-binding protein 4	Q14839	SWI/SNF related, member 5	BAA25173
Acetyl-CoA carboxylase	Q13085	Microfibrillar-associated protein 1	P55081	TPR protein	S33124
aryl sulfotransferase	P52840	Mitotic phosphoprotein 44	AAL86380	Transcription repressor p66 (KIAA1150)	AAL39081
Clathrin heavy chain 1 (CLH-17)	Q00610	MTA1-like protein (KIAA1266)	BAAC36562	Tryptophan 2,3-dioxygenase	P48776
Coilin p80	P38432	myb-binding protein p160	AAC39954	Tubulin b	P07437
CRM1	BAA23415	Myosin light chanin alkali, non-muscle isoform	P16475	Ubiquinol cytochrome C reductase complex protein 2	Q9DB77
Cytochrome c oxidase polypeptide VIB	P56391	Nuclear pore complex protein Nup84	AAB52419	Ubiquitin-conjugating enzyme E2L 3	NP_003338
Cytochrome p450	Q64458	Nuclear pore complex protein Nup153	P49791	Ubiquitin-like protein SMT3A	P55854
DNA polymerase e	Q07864	Nuclear pore complex protein nup155	O75694	UDP-glucuronosyl transferase	P09875
DNA ligase I	P37913	Nuclear pore complex protein Nup50	AAC53278	Vimentin	P08670
DNA repair protein XRCC4	NP_071801				

are actively transcribing (Eils *et al.*, 2000). It remains to be determined whether these spheres correspond to an individual granule or clusters of IGC granules.

Apoptosis and Other Functions

In addition to proteins functioning in pre-mRNA splicing and transcription, we detected proteins that are involved in other nuclear functions. For example, acinus (KIAA0670) has been reported to be involved in a late step of an apoptotic pathway (Sahara *et al.*, 1999). An in vitro system using permeabilized cells and apoptotic cell lysates revealed that acinus is activated by caspase 3 cleavage, and it induces apoptotic chromatin condensation in the absence of DNA fragmentation (Sahara *et al.*, 1999). It was also shown that

Table 4. Novel IGC protein candidates

Protein Description	Accession Code	RNA Binding Motif	RS	Other Domains and Motif(s)[a]	Low Complexity Regions	Notes
DNA segment, Chr 6, Wayne State University 176	NP_613053			Transmembrane		
Epidermal Langerhans cell protein LCP1	NP_075923			HMG box	Yes	
GC-rich sequence DNA-binding factor candidate	NP_037461			Coiled coil	Yes	
Hypothetical protein FLJ10637	NP_060634			Coiled coil		
Hypothetical protein FLJ11305	BAA91611				Yes	Similar (89% identity) to unnamed protein product (AK001302)
Hypothetical protein MGC28864	AAH17152			Coiled coil, HGTP anticodon domain	Yes	
KIAA0052 protein	AAH28604.2			DEXDc, HELICc, coiled coil	Yes	Homologous to "putative helicase", "RNA helicase Mtr4"
KIAA0460 protein	T00074				Yes	
KIAA0663 protein	T00368			3 ZnF_C3H1	Yes	Slightly similar to "lacunin", large multidomain extracellular matrix Zinc finger protein, CPSF (clipper/cleavage and polyadenylation stimulation factor)
KIAA1160 protein	BAA86474			Coiled coil		
mKIAA1125 protein	BAC41468			PHD, BROMO, PWWP, 1 ZnF_NFX, coiled-coil	Yes	
Putative 40-2-3 protein	AAH28253				Yes	
RIKEN cDNA 1110015K06	AAH10333				Yes[a]	
RIKEN cDNA 1700016A15	XP_127067			1 ZnF_NFX[a]	Yes	Similar (97% identity) to nuclear protein UKp68
Wdr18 protein	AAH32968.1			4 WD40 repeats	Yes	Similar (74% identity) to hypothetical protein R32184_1
RIKEN cDNA 2410002M20	NP_766285			PRP38 family	Yes	Weakly similar to splicing factor, arginin/serine-rich 2
RIKEN cDNA 2410008G02 (KIAA0095)	AAH23140			NIC	Yes	Related to NIC96
RIKEN cDNA 2500003M10	NP_075704				Yes	
RIKEN cDNA 2610015J01	NP_081625	RRM			Yes	
RIKEN cDNA 2610034N24	NP_081532			HEAT PBS	Yes	
RIKEN cDNA 2610511G16	NP_080477		Yes	SAP, coiled coil	Yes	
RIKEN cDNA 2610528A15 (KIAA0052)	NP_082427			DEXDc, HELICc, coiled coil	Yes	
RIKEN cDNA 2810013E07	NP_835213			TPR	Yes	Similar (91% identity) to hypothetical protein FLJ20530
RIKEN cDNA 5730555F13/modulator of estrogen induced transcription	NP_079966			Coiled coil	Yes	
RIKEN cDNA 9330151F09 gene	NP_666265				Yes	Similar (60% identity) to thyroid hormone receptor-associated protein, 150 kDa subunit
Similar to a C.elegans protein encoded in cosmid K12D12(Z49069) (KIAA0225 protein)	BAA13214				Yes	
Similar to alcohol dehydrogenase PAN1B-like protein	XP_223159				Yes	Short-chain alcohol dehydrogenase
Similar to CG11943 gene product	AAH45524				Yes	
Similar to hypothetical protein	XP_290525			SAP, SPRY	Yes	Similar (92% identity) to nuclear calmodulin-binding protein
Similar to KIAA0138 gene product	XP_128733	RRM		SAP, coiled coil	Yes	Similar (75% identity) to scaffold attachment factor B
Similar to thyroid hormone receptor-associated protein, 150 kDa	XP_233523		Yes		Yes	
Unnamed protein product	BAA96656			LisH, CTLH, 6 WD40 repeats		Homologous to "brain-enriched WD-repeat protein"

[a] Database for motif and domain searches: SMART (*http://smart.embl-heidelberg.de*) (Schultz *et al.*, 1998; Letunic *et al.*, 2002). Only those proteins containing SR dipeptides were manually searched for RD/E dipeptide repeats and other repetitive amino acid sequences.

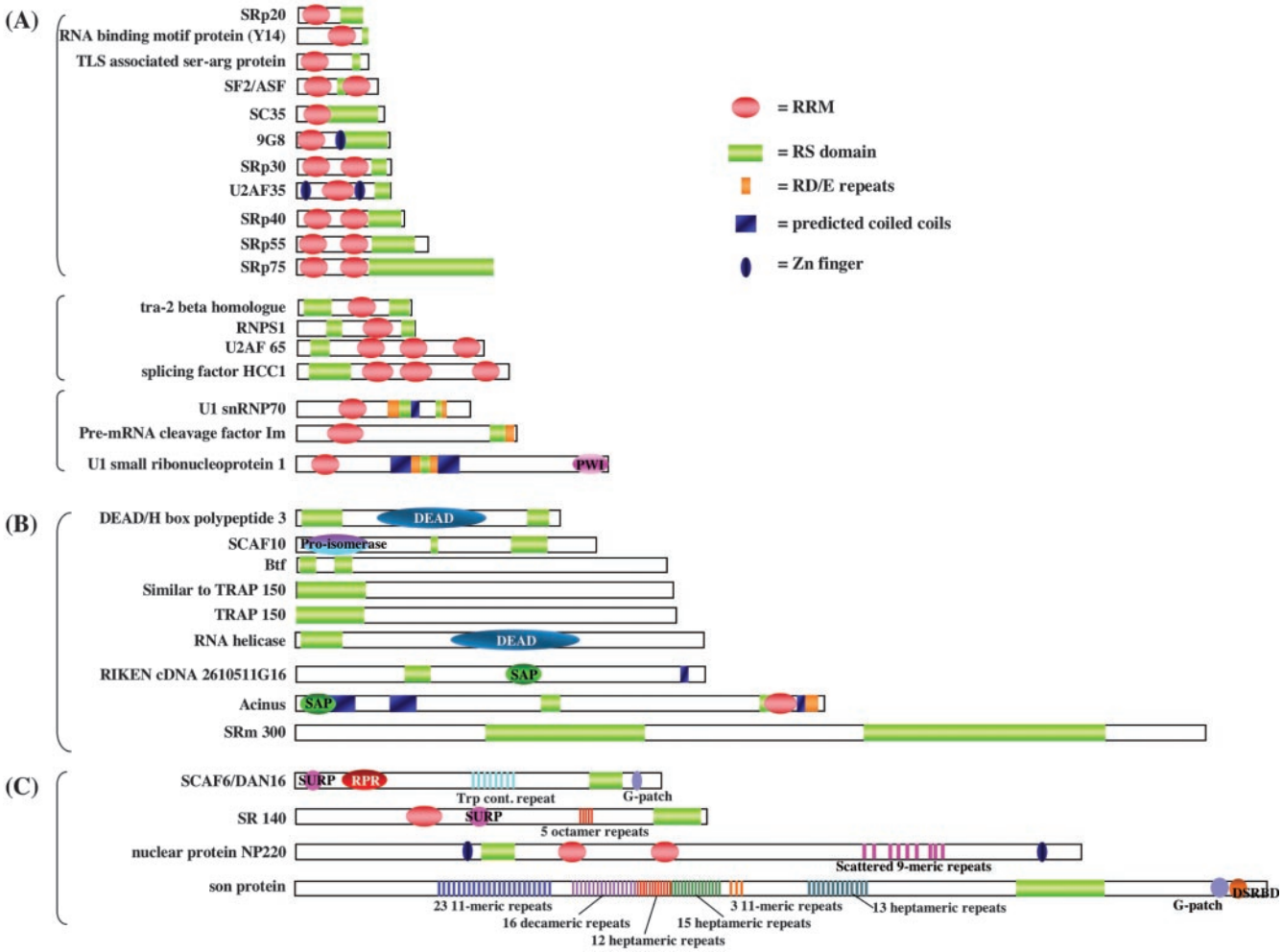

Figure 3. RS domain-containing proteins detected in the IGCs. Thirty-one proteins with RS motifs were detected in the IGC fraction and were categorized into three subgroups. Proteins in the first group (A) are of relatively low molecular mass, contain one or more RRMs, and many are founding members of the SR protein family. Proteins in the second group (B) are of larger molecular mass, and most do not contain an RRM but do contain additional motifs. Proteins in the third group (C) are also of higher molecular mass and contain repetitive sequences.

acinus is important for apoptotic chromatin condensation in vivo by using antisense RNA (Sahara *et al.*, 1999). Recently, a complex called ASAP, containing RNPS1 (splicing factor), acinus and SAP18 (Sin3-associated protein; a component of a histone deacetylase complex), was isolated and the complex was shown to promote both pre-mRNA splicing and apoptosis, suggesting a possible link among apoptosis, splicing, and chromatin modification (Schwerk *et al.*, 2003). Interestingly, acinus contains an RS domain (Boucher *et al.*, 2001) that accounts for its localization to IGCs (Figure 2).

A second protein implicated in apoptosis, Btf (KIAA0164), was identified as a protein associated with the adenovirus oncoprotein E1B 19K as well as Bcl-2 family members. Btf has a transcriptional repression activity and its sustained overexpression induces apoptosis and suppresses transformation by E1A and E1B-19K or mutant p53 (Kasof *et al.*, 1999). Although we have found that acinus colocalized within IGCs, Btf is localized at the periphery of IGCs (Figure 2).

As potential IGC proteins, we detected DNA repair proteins such as XPE UV-damaged DNA binding protein and XPA-binding protein 2 (Table 2). It is also interesting that we detected several types of "chaperone" proteins such as Hsp70, Dna J protein homolog, or RuvB like DNA helicase. In the developing kidney, Hsp70 is colocalized with Wilms tumor suppressor WT-1 in a speckled nuclear distribution pattern (Maheswaran *et al.*, 1998). In the plant *Brassica napus*, it was shown that Hsp70 becomes associated with RNP structures in the interchromatin region and the nucleolus upon stress treatment to induce embryogenesis of microspores (Segui-Simarro *et al.*, 2003). Although the localization of Hsp70 in IGCs remains to be confirmed, it would be interesting to analyze the changes in protein components in IGCs throughout the stages of development, oncogenesis, or environmental changes.

Recently, it has been suggested that transcription and translation are coupled. A small amount of translation, which might be important for quality control of gene products, has been reported to take place in the nucleus before

export of mRNAs to the cytoplasm where the majority of translation occurs (Iborra *et al.*, 2001). Thus far, we have detected two isoforms of eukaryotic initiation factor 4A, eIF4Ai and iii, in our proteomics analysis of IGCs. We and others also have found that fluorescently tagged eIF4Aiii is localized to IGCs (Holzmann *et al.*, 2000). It has been shown that eIF4Ai, ii, and iii all confer RNA-dependent RNA helicase and ATP-dependent RNA helicase activities. However, they seem to function differently because eIF4Ai and ii facilitate translation, but eIF4Aiii inhibits translation in a reticulocyte lysate (Li *et al.*, 1999). Recently, eIF4Aiii has been shown to be involved in nonsense-mediated decay (NMD) (Ferraiuolo *et al.*, 2004). NMD is an RNA surveillance mechanism that serves to degrade mRNAs containing premature translation termination codons (for a review, see Maniatis and Reed, 2002; Wilkinson and Shyu, 2002; Singh and Lykke-Andersen, 2003). In our IGC fraction, we identified numerous members of the exon-exon junction complex that contains factors that are required for both mRNA export and NMD [Aly, RNPS1, RNA binding motif protein 8 (Y14), and mago-nashi homolog (MAGOH)]. This finding raises the possibility that proteins involved in these processes may be recruited from IGCs to transcription sites.

Motif Analysis

As expected, we detected many proteins with RNA binding motifs, RS motifs, and RNA helicase motifs, including ATP binding DEAD box helicases. However, thus far we have not detected a single sequence motif that is common among all IGC proteins. Therefore, aside from the RS domain, which serves to target certain proteins to IGCs, many other IGC-associated proteins may assemble into these structures by specific protein–protein and/or protein–RNA interactions rather than by a single targeting signal. Interestingly, 82% of the identified IGC proteins contain low complexity regions, such as a long stretch of a single type of amino acid, which could be involved in interactions with RNA or other proteins.

Because the RS motif seems to be unique among IGC proteins, we focused on a more in depth analysis of proteins containing an RS domain. We found that this group of proteins can be divided into several subgroups (Figure 3). In addition to the typical small RS domain-containing proteins that contain one or more RRMs, among which are members of the SR family of pre-mRNA splicing factors, there are larger RS domain-containing proteins containing additional domains and/or regions containing short repeats. It is plausible to imagine that these repeats are likely to perform a scaffolding function, as is found for certain HEAT repeat-containing proteins (Neuwald and Hirano, 2000). Also interesting are four proteins, U1 snRNP70, pre-mRNA cleavage factor Im, U1 small ribonucleoprotein 1, and acinus, that have degenerated RS domains in which the RS repeat itself contains, or is continuous with, RD/E dipeptides. RE repeats were previously found in the splicing factor YT521-B and were shown to be important for localization to the YT body, a subnuclear structure that is similar to but distinct from nuclear speckles (Nayler *et al.*, 2000). The RD/E dipeptide motif is reminiscent of a phosphorylated RS domain, because the serine residue in RS is replaced with a negatively charged aspartic acid or glutamic acid. Interestingly, YT521-B was shown to localize to transcriptionally active sites and was suggested to play a role in grouping genes into higher order structures (Nayler *et al.*, 2000). Thus, proteins with both RS and RD/E motifs may bridge sites of active transcription with IGCs.

In summary, we have characterized the proteome of IGCs purified from mouse liver nuclei. Although the protein identification supports a role of these nuclear domains in events relating to pre-mRNA processing, a significant number of new proteins have been identified, as well as interesting domains of known proteins. These will provide the impetus for future studies aimed at deciphering the organization and additional function(s) associated with this nuclear organelle.

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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.

Aravind, L., and Koonin, E.V. (2000). SAP - a putative DNA-binding motif involved in chromosomal organization. Trends Biochem. Sci. *25*, 112–114.

Belgrader, P., Dey, R., and Berezney, R. (1991). Molecular cloning of matrin 3. A 125-kilodalton protein of the nuclear matrix contains an extensive acidic domain. J. Biol. Chem. *266*, 9893–9899.

Birney, E., Kumar, S., and Krainer, A.R. (1993). Analysis of the RNA-recognition motif and RS and RGG domains: conservation in metazoan pre-mRNA splicing factors. Nucleic Acids Res. *21*, 5803–5816.

Blencowe, B.J., Bauren, G., Eldridge, A.G., Issner, R., Nickerson, J.A., Rosonina, E., and Sharp, P.A. (2000). The SRm160/300 splicing coactivator subunits. RNA *6*, 111–120.

Boucher, L., Ouzounis, C.A., Enright, A.J., and Blencowe, B.J. (2001). A genome-wide survey of RS domain proteins. RNA *7*, 1693–1701.

Brandner, J.M., Reidenbach, S., and Franke, W.W. (1997). Evidence that "pinin", reportedly a differentiation-specific desmosomal protein, is actually a widespread nuclear protein. Differentiation *62*, 119–127.

Bregman, D.B., Du, L., van der Zee, S., and Warren, S.L. (1995). Transcription-dependent redistribution of the large subunit of RNA polymerase II to discrete nuclear domains. J. Cell Biol. *129*, 287–298.

Cmarko, D., Verschure, P.J., Martin, T.E., Dahmus, M.E., Krause, S., Fu, X.D., van Driel, R., and Fakan, S. (1999). Ultrastructural analysis of transcription and splicing in the cell nucleus after bromo-UTP microinjection. Mol. Biol. Cell *10*, 211–223.

Corden, J.L., and Patturajan, M. (1997). A CTD function linking transcription to splicing. Trends Biochem. Sci. *22*, 413–416.

Cronshaw, J.M., Krutchinsky, A.N., Zhang, W., Chait, B.T., and Matunis, M.J. (2002). Proteomic analysis of the mammalian nuclear pore complex. J. Cell Biol. *158*, 915–927.

Eils, R., Gerlich, D., Tvarusko, W., Spector, D.L., and Misteli, T. (2000). Quantitative imaging of pre-mRNA splicing factors in living cells. Mol. Biol. Cell *11*, 413–418.

Fakan, S., and Puvion, E. (1980). The ultrastructural visualization of nucleolar and extranucleolar RNA synthesis and distribution. Int. Rev. Cytol. *65*, 255–299.

Ferraiuolo, M.A., Lee, C.S., Ler, L.W., Hsu, J.L., Costa-Mattioli, M., Luo, M.J., Reed, R., and Sonenberg, N. (2004). A nuclear translation-like factor eIF4AIII is recruited to the mRNA during splicing and functions in nonsense-mediated decay. Proc. Natl. Acad. Sci. USA *101*, 4118–4123.

Fu, X.D. (1995). The superfamily of arginine/serine-rich splicing factors. RNA *1*, 663–680.

Fu, X.D., and Maniatis, T. (1990). Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. Nature *343*, 437–441.

Gottschalk, A., Neubauer, G., Banroques, J., Mann, M., Luhrmann, R., and Fabrizio, P. (1999). Identification by mass spectrometry and functional anal-

ysis of novel proteins of the yeast [U4/U6.U5] tri-snRNP. EMBO J *18*, 4535–4548.

Graveley, B.R. (2000). Sorting out the complexity of SR protein functions. RNA *6*, 1197–1211.

Holzmann, K., Gerner, C., Poltl, A., Schafer, R., Obrist, P., Ensinger, C., Grimm, R., and Sauermann, G. (2000). A human common nuclear matrix protein homologous to eukaryotic translation initiation factor 4A. Biochem. Biophys. Res. Commun. *267*, 339–344.

Huang, S. (2000). Review: perinucleolar structures. J. Struct. Biol. *129*, 233–240.

Iborra, F.J., Jackson, D.A., and Cook, P.R. (2001). Coupled transcription and translation within nuclei of mammalian cells. Science *293*, 1139–1142.

Inagaki, H., Matsushima, Y., Nakamura, K., Ohshima, M., Kadowaki, T., and Kitagawa, Y. (1996). A large DNA-binding nuclear protein with RNA recognition motif and serine/arginine-rich domain. J. Biol. Chem. *271*, 12525–12531.

Jagatheesan, G., Thanumalayan, S., Muralikrishna, B., Rangaraj, N., Karande, A.A., and Parnaik, V.K. (1999). Colocalization of intranuclear lamin foci with RNA splicing factors. J. Cell Sci. *112*, 4651–4661.

Janicki, S.M., *et al*. (2004). From silencing to gene expression: real-time analysis in single cells. Cell *116*, 683–698.

Johnson, K.M., Wang, J., Smallwood, A., Arayata, C., and Carey, M. (2002). TFIID and human mediator coactivator complexes assemble cooperatively on promoter DNA. Genes Dev. *16*, 1852–1863.

Kasof, G.M., Goyal, L., and White, E. (1999). Btf, a novel death-promoting transcriptional repressor that interacts with Bcl-2-related proteins. Mol. Cell. Biol. *19*, 4390–4404.

Kataoka, N., Diem, M.D., Kim, V.N., Yong, J., and Dreyfuss, G. (2001). Magoh, a human homolog of *Drosophila* mago nashi protein, is a component of the splicing-dependent exon-exon junction complex. EMBO J. *20*, 6424–6433.

Kruhlak, M.J., Lever, M.A., Fischle, W., Verdin, E., Bazett-Jones, D.P., and Hendzel, M.J. (2000). Reduced mobility of the alternate splicing factor (ASF) through the nucleoplasm and steady state speckle compartments. J. Cell Biol. *150*, 41–51.

Lamond, A.I., and Earnshaw, W.C. (1998). Structure and function in the nucleus. Science *280*, 547–553.

Lamond, A.I., and Spector, D.L. (2003). Nuclear speckles: a model for nuclear organelles. Nat. Rev. Mol. Cell. Biol. *4*, 605–612.

Letunic, I., Goodstadt, L., Dickens, N.J., Doerks, T., Schultz, J., Mott, R., Ciccarelli, F., Copley, R.R., Ponting, C.P., and Bork, P. (2002). Recent improvements to the SMART domain-based sequence annotation resource. Nucleic Acids Res. *30*, 242–244.

Li, Q., Imataka, H., Morino, S., Rogers, G.W., Jr., Richter-Cook, N.J., Merrick, W.C., and Sonenberg, N. (1999). Eukaryotic translation initiation factor 4AIII (eIF4AIII) is functionally distinct from eIF4AI and eIF4AII. Mol. Cell. Biol. *19*, 7336–7346.

Linder, P., Lasko, P.F., Ashburner, M., Leroy, P., Nielsen, P.J., Nishi, K., Schnier, J., and Slonimski, P.P. (1989). Birth of the D-E-A-D box. Nature *337*, 121–122.

Luking, A., Stahl, U., and Schmidt, U. (1998). The protein family of RNA helicases. Crit. Rev. Biochem. Mol. Biol. *33*, 259–296.

Maheswaran, S., *et al*. (1998). Inhibition of cellular proliferation by the Wilms tumor suppressor WT1 requires association with the inducible chaperone Hsp70. Genes Dev. *12*, 1108–1120.

Maniatis, T., and Reed, R. (2002). An extensive network of coupling among gene expression machines. Nature *416*, 499–506.

Matera, A.G. (1999). RNA splicing: more clues from spinal muscular atrophy. Curr. Biol. *9*, R140–R142.

Mintz, P.J., Patterson, S.D., Neuwald, A.F., Spahr, C.S., and Spector, D.L. (1999). Purification and biochemical characterization of interchromatin granule clusters. EMBO J *18*, 4308–4320.

Misteli, T. (2000). Cell biology of transcription and pre-mRNA splicing: nuclear architecture meets nuclear function. J. Cell Sci. *113*, 1841–1849.

Misteli, T., Caceres, J.F., Clement, J.Q., Krainer, A.R., Wilkinson, M.F., and Spector, D.L. (1998). Serine phosphorylation of SR proteins is required for their recruitment to sites of transcription in vivo. J. Cell Biol. *143*, 297–307.

Misteli, T., Caceres, J.F., and Spector, D.L. (1997). The dynamics of a pre-mRNA splicing factor in living cells. Nature *387*, 523–527.

Misteli, T., and Spector, D.L. (1998). The cellular organization of gene expression. Curr. Opin. Cell Biol. *10*, 323–331.

Misteli, T., and Spector, D.L. (1999). RNA polymerase II targets pre-mRNA splicing factors to transcription sites in vivo. Mol. Cell *3*, 697–705.

Mortillaro, M.J., Blencowe, B.J., Wei, X., Nakayasu, H., Du, L., Warren, S.L., Sharp, P.A., and Berezney, R. (1996). A hyperphosphorylated form of the large subunit of RNA polymerase II is associated with splicing complexes and the nuclear matrix. Proc. Natl. Acad. Sci. USA *93*, 8253–8257.

Nakayasu, H., and Berezney, R. (1991). Nuclear matrins: identification of the major nuclear matrix proteins. Proc. Natl. Acad. Sci. USA *88*, 10312–10316.

Nakayasu, H., and Ueda, K. (1984). Small nuclear RNA-protein complex anchors on the actin filaments in bovine lymphocyte nuclear matrix. Cell Struct. Funct. *9*, 317–325.

Nayler, O., Hartmann, A.M., and Stamm, S. (2000). The ER repeat protein YT521-B localizes to a novel subnuclear compartment. J. Cell Biol. *150*, 949–962.

Neubauer, G., Gottschalk, A., Fabrizio, P., Seraphin, B., Luhrmann, R., and Mann, M. (1997). Identification of the proteins of the yeast U1 small nuclear ribonucleoprotein complex by mass spectrometry. Proc. Natl. Acad. Sci. USA *94*, 385–390.

Neubauer, G., King, A., Rappsilber, J., Calvio, C., Watson, M., Ajuh, P., Sleeman, J., Lamond, A., and Mann, M. (1998). Mass spectrometry and EST-database searching allows characterization of the multi-protein spliceosome complex. Nat. Genet. *20*, 46–50.

Neuwald, A.F., and Hirano, T. (2000). HEAT repeats associated with condensins, cohesins, and other complexes involved in chromosome-related functions. Genome Res. *10*, 1445–1452.

Ouyang, P., and Sugrue, S.P. (1996). Characterization of pinin, a novel protein associated with the desmosome-intermediate filament complex. J. Cell Biol. *135*, 1027–1042.

Ouyang, P., Zhen, Y.Y., and Sugrue, S.P. (1997). Cloning and analysis of cDNA encoding murine pinin. Gene *197*, 115–120.

Phair, R.D., and Misteli, T. (2000). High mobility of proteins in the mammalian cell nucleus. Nature *404*, 604–609.

Rappsilber, J., Ryder, U., Lamond, A.I., and Mann, M. (2002). Large-scale proteomic analysis of the human spliceosome. Genome Res. *12*, 1231–1245.

Rout, M.P., Aitchison, J.D., Suprapto, A., Hjertaas, K., Zhao, Y., and Chait, B.T. (2000). The yeast nuclear pore complex: composition, architecture, and transport mechanism. J. Cell Biol. *148*, 635–651.

Sacco-Bubulya, P., and Spector, D.L. (2002). Disassembly of interchromatin granule clusters alters the coordination of transcription and pre-mRNA splicing. J. Cell Biol. *156*, 425–436.

Sahara, S., Aoto, M., Eguchi, Y., Imamoto, N., Yoneda, Y., and Tsujimoto, Y. (1999). Acinus is a caspase-3-activated protein required for apoptotic chromatin condensation. Nature *401*, 168–173.

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.

Schirmer, E.C., Florens, L., Guan, T., Yates, J.R., 3rd, and Gerace, L. (2003). Nuclear membrane proteins with potential disease links found by subtractive proteomics. Science *301*, 1380–1382.

Schultz, J., Milpetz, F., Bork, P., and Ponting, C.P. (1998). SMART, a simple modular architecture research tool: identification of signaling domains. Proc. Natl. Acad. Sci. USA *95*, 5857–5864.

Schwerk, C., Prasad, J., Degenhardt, K., Erdjument-Bromage, H., White, E., Tempst, P., Kidd, V.J., Manley, J.L., Lahti, J.M., and Reinberg, D. (2003). ASAP, a novel protein complex involved in RNA processing and apoptosis. Mol. Cell. Biol. *23*, 2981–2990.

Segui-Simarro, J.M., Testillano, P.S., and Risueno, M.C. (2003). Hsp70 and Hsp90 change their expression and subcellular localization after microspore embryogenesis induction in *Brassica napus* L. J. Struct. Biol. *142*, 379–391.

Singh, G., and Lykke-Andersen, J. (2003). New insights into the formation of active nonsense-mediated decay complexes. Trends Biochem. Sci. *28*, 464–466.

Spahr, C.S., Susin, S.A., Bures, E.J., Robinson, J.H., Davis, M.T., McGinley, M.D., Kroemer, G., and Patterson, S.D. (2000). Simplification of complex peptide mixtures for proteomic analysis: reversible biotinylation of cysteinyl peptides. Electrophoresis *21*, 1635–1650.

Spector, D.L. (1993). Macromolecular domains within the cell nucleus. Annu. Rev. Cell Biol. *9*, 265–315.

Spector, D.L. (2001). Nuclear domains. J. Cell Sci. *114*, 2891–2893.

Spector, D.L. (2003). The dynamics of chromosome organization and gene regulation. Annu. Rev. Biochem. *72*, 573–608.

Spector, D.L., Goldman, R.D., and Leinwand, L.A. (1998). Cells: A Laboratory Manual, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Spector, D.L., Lark, G., and Huang, S. (1992). Differences in snRNP localization between transformed and nontransformed cells. Mol. Biol. Cell *3*, 555–569.

Trinkle-Mulcahy, L., Sleeman, J.E., and Lamond, A.I. (2001). Dynamic targeting of protein phosphatase 1 within the nuclei of living mammalian cells. J. Cell Sci. *114*, 4219–4228.

Valdez, B.C., Henning, D., Perlaky, L., Busch, R.K., and Busch, H. (1997). Cloning and characterization of Gu/RH-II binding protein. Biochem. Biophys. Res. Commun. *234*, 335–340.

Wilkinson, M.F., and Shyu, A.B. (2002). RNA surveillance by nuclear scanning? Nat. Cell Biol. *4*, E144–E147.

Will, C.L., Urlaub, H., Achsel, T., Gentzel, M., Wilm, M., and Luhrmann, R. (2002). Characterization of novel SF3b and 17S U2 snRNP proteins, including a human Prp5p homologue and an SF3b DEAD-box protein. EMBO J. *21*, 4978–4988.

Zhou, Z., Licklider, L.J., Gygi, S.P., and Reed, R. (2002). Comprehensive proteomic analysis of the human spliceosome. Nature *419*, 182–185.

Zhou, Z., Luo, M.J., Straesser, K., Katahira, J., Hurt, E., and Reed, R. (2000). The protein Aly links pre-messenger-RNA splicing to nuclear export in metazoans. Nature *407*, 401–405.