

Proteomic Analysis Reveals a Role for the GTPase RPAP4/GPN1 and the Cochaperone RPAP3 in Biogenesis of All Three Nuclear RNA Polymerases

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Abstract Biogenesis of nuclear RNA polymerases (RNAP) is a poorly understood, yet central molecular process in eukaryotes. Recent analysis of interaction partners of RNAP II, the enzyme that synthesizes protein-coding mRNAs, in the soluble fraction of cell extracts identified a series of factors that play central roles in RNAP II biogenesis. The GPN loop GTPase RPAP4/GPN1 was shown to be required for nuclear import of RNAP II, and the HSP90 co-factor RPAP3 is essential for cytoplasmic assembly of this multisubunit enzyme. Examination of the list of interactors for RNAP II as well as RPAP4/GPN1 and RPAP3 reveals the presence of many specific subunits of RNAP I and III, which synthesize most of the cell's non-coding transcripts. This finding suggests that biogenesis of all three nuclear RNAPs may be coupled. Silencing of RPAP4/GPN1 and RPAP3 further indicates that both factors are essential for normal nuclear localization of the three polymerases. We present a model in which biogenesis of RNAP I, II and III is integrated through the action of assembly and nuclear import factors.

Keywords RNA polymerase • RNA polymerase II-associated proteins (RPAP) • Biogenesis • Nuclear import • Assembly

Introduction

The nucleus of eukaryotic cells contains three types of RNA polymerases (RNAP). RNAP I is located in the nucleolus where it synthesises the 45S ribosomal RNA (rRNA) precursor that makes up the core of the ribosome. RNAP II and RNAP III

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can both be found in the non-nucleolar nuclear space. RNAP II synthesizes mRNAs as well as a number of small nuclear RNAs (snRNA) and microRNAs (miRNA). RNAP III directs the synthesis of rRNA 5S, tRNAs and other small, non-coding RNAs. Each RNAP is assisted by a general transcription machinery which serves for promoter recognition, RNA chain elongation and response to transcriptional regulators. Work performed over the last three decades has revealed numerous mechanisms by which transcription is regulated in eukaryotes, many of the regulatory factors being involved in helping RNAPs to cope with the nucleosomal structure of chromatin [1–4; and references therein].

Much less is known about regulation of RNAP molecules prior to and following transcription. Biogenesis and recycling of these key enzymes have only been addressed recently. Two discoveries dealing with RNAP II, which has been the most intensively studied nuclear RNAP, have had a particularly important impact on our understanding of RNAP biogenesis. First, RNAP II molecules involved in transcription on chromatin are recycled at each cell cycle [5, 6]. Indeed, the bulk of RNAP II is released from chromatin during mitosis, more precisely at metaphase, and is re-imported to the nucleus after mitosis. Because the nuclear envelope reforms at late anaphase [7], this result implies that recycled RNAP II molecules have to re-enter the nucleus through nuclear import mechanisms. Recent results from our laboratory indicate that the same scenario is true for RNAP I and RNAP III (Forget et al., in preparation). Second, a group of factors that interact with RNAP II in the soluble cell fraction has been identified and at least some of them were shown to act as regulators of RNAP II biogenesis [8, 9]. The RNAP II-associated protein 4 (RPAP4; also termed GPN1) is member of a novel GTPase family characterized by the presence of a Glu-Pro-Asn (GPN) loop motif [10]. RPAP4/GPN1 shuttles between the cytoplasm and the nucleus in a CRM1-dependent manner [11, 12]. Silencing of RPAP4/GPN1 results in abnormal accumulation of RNAP II in the cell's cytoplasm, suggesting a role in nuclear import of this polymerase [11–13]. Substitutions in the RPAP4/GPN1 GPN loop or GTP binding motif provoke cytoplasmic retention of RNAP II, an indication that the GTPase activity is required for RNAP II nuclear import [12]. Treatment of the cells with benomyl, a compound that interferes with microtubule assembly/integrity, also interferes with RNAP II nuclear localization [12]. Notably, treatment of yeast strains having substitutions in RPAP4/GPN1 that produce slow growth phenotypes with sub-lethal concentrations of benomyl completely abolished growth. These results indicate that microtubule assembly is somehow involved in RNAP II nuclear import. The RNAP II-Associated Protein 3 (RPAP3) is a HSP90 co-factor that is part of a multisubunit complex consisting of RPAP3 itself, a TPR domain-containing factor that mediates direct interaction with HSP90, as well as other components that may have a chaperone function of their own, including prefoldin-like proteins and the RUVBL1 and RUVBL2 AAA+ATPases [14–16]. The canonical prefoldin complex is well known for its role in the chaperoning and polymerization of actin and tubulin [17, 18], while RUVBL1 and RUVBL2 are required for the assembly of other multimeric protein complexes, like snoRNPs [19]. Boulon et al. [20] have shown that HSP90 and RPAP3 are involved in assembly of RNAP II in the cell's cytoplasm prior to import

to the nucleus. Indeed, silencing of RPAP3, a mainly cytoplasmic protein, also causes abnormal accumulation of RNAP II in the cytoplasm. Because silencing of RPAP4/GPN1 and RPAP3 has similar effects on RNAP II localization, we propose that RNAP II assembly and nuclear import are tightly coupled. RNAP II molecules that are either recycled at mitosis or newly assembled in the cytoplasm through the action of HSP90 and RPAP3 are imported to the nucleus through the action of RPAP4/GPN1 in a process that requires microtubule assembly/integrity.

Biogenesis of RNAP I and III has not been characterized in much detail [21, 22]. For example, we do not know whether the same set of RNAP II-specific factors are involved or else, whether distinct machineries are at play [23]. What is established, however, is that all three nuclear RNAPs share some subunits [24, 25], suggesting that their biogenesis could somehow be interconnected. In this chapter, we report on results of affinity purification coupled with mass spectrometry (AP-MS) showing that RPAP4/GPN1 and RPAP3 are part of complexes containing subunits of all three nuclear RNAPs, including both shared and specific RNAP subunits. Moreover, silencing experiments reveal that both RPAP4/GPN1 and RPAP3 are necessary for normal nuclear import of all three nuclear RNAPs. These results strengthen the conclusion that biogenesis of RNAP I, II and III is tightly coupled, requiring some common factors including RPAP3 and RPAP4/GPN1.

Materials and Methods

Protein Affinity Purification Coupled with Mass Spectrometry

Generation of cell lines expressing tandem affinity peptide (TAP) or FLAG tagged RNAP or RPAP subunits and tandem affinity purification were performed as previously described [9, 12, 14, 26, 27]. After TCA precipitation, the eluates were digested with trypsin, and the resulting tryptic peptides were purified and identified by tandem mass spectrometry (LC-MS/MS) using a microcapillary reversed-phase high pressure liquid chromatography coupled LTQ-Orbitrap (ThermoElectron) quadrupole ion trap mass spectrometer with a nanospray interface, as we recently described [28]. Protein database searching was performed with Mascot 2.2 (Matrix Science) against the human NCBI nr protein database. Mascot scores and spectral counts were used to select specific interactors for Fig. 1.

Antibodies

The antibodies used in this study were obtained from various sources: POLR1A monoclonal antibody (Santa Cruz); anti-FLAG monoclonal antibody (Sigma); anti-RPAP4 antibody (CIM Antibody Core, Arizona State University, Tempe, Arizona);

Interactors	Tagged proteins					
	POLR1A	POLR2A	POLR3A	RPAP4	RPAP3	
POLR2A	135/23	8149/912		6030/324	254/8	RNAP II subunits
POLR2B	159/6	5202/498		4863/335	1566/72	
POLR2C		1023/70		1043/60		
POLR2D		972/95		69/1		
POLR2G		651/36		651/29		
POLR2I		506/30		380/18		
POLR2J		269/22		133/7		
POLR2E	281/12	599/29	539/22	486/19	362/18	Shared subunits RNAP I, RNAP II and RNAP III
POLR2F		134/9	171/8	66/5		
POLR2H	383/11	743/122	522/20	538/13	118/4	
POLR2K		55/4	82/7	60/3		
POLR2L		56/4	127/7	95/4		
POLR3A	288/14	143/4	3727/177	2738/127	1918/88	RNAP III subunits
POLR3B			2213/105	2115/623	169/8	
POLR3C			1213/45			
POLR3D			469/21	466/21		
POLR3E			1703/82	1598/70	92/4	
POLR3F			895/40			
POLR3G			476/22			
POLR3H			396/18	204/7		
POLR3K			259/13	107/3		
CRCP			494/23			
POLR1C	550/21	339/15	819/41	505/17	112/5	Shared subunits RNAP I and RNAP III
POLR1D			384/17	80/4		
POLR1A	7552/711	1362/26		92/3	226/7	RNAP I subunits
POLR1B	1083/57	443/11		229/5	39/1	
POLR1E	255/11	268/10				
TWISTNB	78/4	54/2				
CD3EAP	112/5	366/19				
ZNRD1	110/2					
RPAP4/GPN1		427/33	70/2	1612/331	312/17	RPAP4/GPN1
RPAP3	1242/54	1303/60	794/25	1848/68	2882/175	RPAP3/R2TP/PFDL complex
PDRG1	51/3	227/11	142/4	144/12	186/10	
PFDN2	199/6	245/8	185/7	250/7	209/6	
PFDN6		162/8	225/12	174/15	184/9	
PIH1D1	489/14	563/15	196/07	634/25	281/9	
RUVBL1	1155/61	1414/72	583/18	1158/53	1755/122	
RUVBL2	1281/60	1686/86	546/17	1554/68	1943/16	
URI1	606/22	622/24	510/20	1095/45	1199/65	
UXT	52/2	267/5	86/4	74/10	204/6	
WDR92	773/30	303/9	721/27	727/26	1038/52	
HSP90AA1	1160/45	749/35	190/8	698/27	938/40	
HSP90AB1	1204/51	987/45	232/9	1284/52	1246/52	
HSPA8	1710/88	2868/260	1018/42	2107/99	1748/100	

Fig. 1 Summary of affinity purification coupled with mass spectrometry data using POLR1A, POLR2A, POLR3A, RPAP4/GPN1 and RPAP3. Total mascot score/spectral counts are provided for each interaction. Interactors are grouped in various classes as shown on the *right*

anti-RPAP3 antibody (Abnova); horseradish peroxidase-conjugated secondary antibody (GE Healthcare); anti- β -tubulin monoclonal antibody (Sigma) and Alexa Fluor 488 (Invitrogen).

Transfection and siRNA Silencing

Transfection experiments for generating stable HeLa cell lines expressing FLAG-tagged versions of POLR2A and POLR3A used lipofectamine, as described by the supplier (Invitrogen) [12]. RPAP4/GPN1 (ON-TARGETplus SMART pool), RPAP3 (ON-TARGETplus SMART pool), and control (siCONTROL Non-targeting pool) siRNAs (Dharmacon) were doubly transfected into HeLa cells using oligofectamine (Invitrogen) at a siRNA final concentration of 100 nM [12]. The efficiency of silencing was monitored for each experiment using western blotting.

Immunofluorescence and Imaging

Immunofluorescence and imaging using HeLa cells were performed as previously described [12]. Immunofluorescence studies used an anti-FLAG antibody to localize exogenously expressed FLAG-POLR2A and FLAG-POR3A, whereas a monoclonal antibody raised against POLR1A was used to monitor localization of endogenous POLR1A. Indeed, we have been unable to generate a cell line expressing a FLAG tagged version of POLR1A that localizes normally to the nucleolus.

Results

RPAP4/GPN1 and RPAP3 Interact with Subunits of RNA Polymerase I, II and III

Affinity purification of RPAP4/GPN1 and RPAP3, followed by identification of binding partners by mass spectrometry, revealed that both factors interact with all three nuclear RNAPs, namely RNAP I, II and III subunits (Fig. 1). In addition to RNAP shared subunits (POLR1C, POLR1D, POLR2E, POLR2F, POLR2H, POLR2K and POLR2L) that copurified with RPAP4/GPN1 and/or RPAP3, specific RNAP I (POLR1A and POLR1B), RNAP II (POLR2A, POLR2B, POLR2C, POLR2D, POLR2G, POLR2I and POLR2J) and RNAP III (POLR3A, POLR3B, POLR3D, POLR3E, POLR3H and POLR3K) subunits were identified as well in these purifications. Interaction of the RPAPs with subunits of all three RNAPs suggests that they either interact independently with all three enzymes or with a

megacomplex containing the three polymerases. Interestingly, affinity purification of the largest RNAP subunits, POLR1A, POLR2A and POLR3A, resulted in purification of other large RNAP subunits (e.g. presence of POLR2A, POLR2B and POLR3A in the POLR1A purification, and of POLR1A and POLR3A in the POLR2A purification). These copurifications argue in favour of the existence of a megacomplex containing many subunits of all three RNAPs during nuclear RNAP biogenesis. However, the data in Fig. 1 also show that not all RNAP subunits copurify with all tagged RNAP subunits (for example, POLR3A was the only RNAP III subunit found to copurify with POLR2A in our experiments). Whether this finding reflects the formation of a megacomplex containing only a selection of RNAP subunits during the assembly process or reflects a lack of sensitivity of our AP-MS technology is not known at this time. Of note, POLR1A, POLR2A and POLR3A interact with the assembly chaperone HSP90 (HSP90AA1 and HSP90AB1). RPAP4/GPN1 also interacts reciprocally with the RPAP3-R2TP-PFDL complex. Together, these results indicate that RNAP I and III interact with the RPAPs involved in biogenesis of RNAP II.

Silencing of RPAP4/GPN1 and RPAP3 Results in Abnormal Accumulation of RNA Polymerase I, II and III Subunits in the Cytoplasm

To address a putative function of RPAP4/GPN1 and RPAP3 in biogenesis of RNAP I and III, we used siRNA-directed silencing of both factors and monitored the intracellular localization of POLR1A and POLR3A, the largest subunit of RNAP I and III, respectively. As mentioned in the Introduction section, silencing of either RPAP4/GPN1 or RPAP3 results in cytoplasmic accumulation of RNAP II subunits. Figure 2 shows that independent silencing of RPAP4/GPN1 and RPAP3 has a similar effect on POLR2A, POLR3A and POLR1A, all three largest polymerase subunits showing an accumulation in the cytoplasm, as determined by immunofluorescence. Control siRNA did not alter RNAP localization. A western blot showing the efficiency of siRNA silencing is also included. These results indicate that similar mechanisms are at play to regulate biogenesis of nuclear RNAPs, and that they involve at least some of the same regulatory factors, including RPAP4/GPN1 and RPAP3.

Discussion

Our results indicate that biogenesis of all three eukaryotic nuclear RNAPs, RNAP I, RNAP II and RNAP III, uses a common set of factors. Indeed, silencing of the GTPase RPAP4/GPN1 and the HSP90 cochaperone RPAP3 are essential to

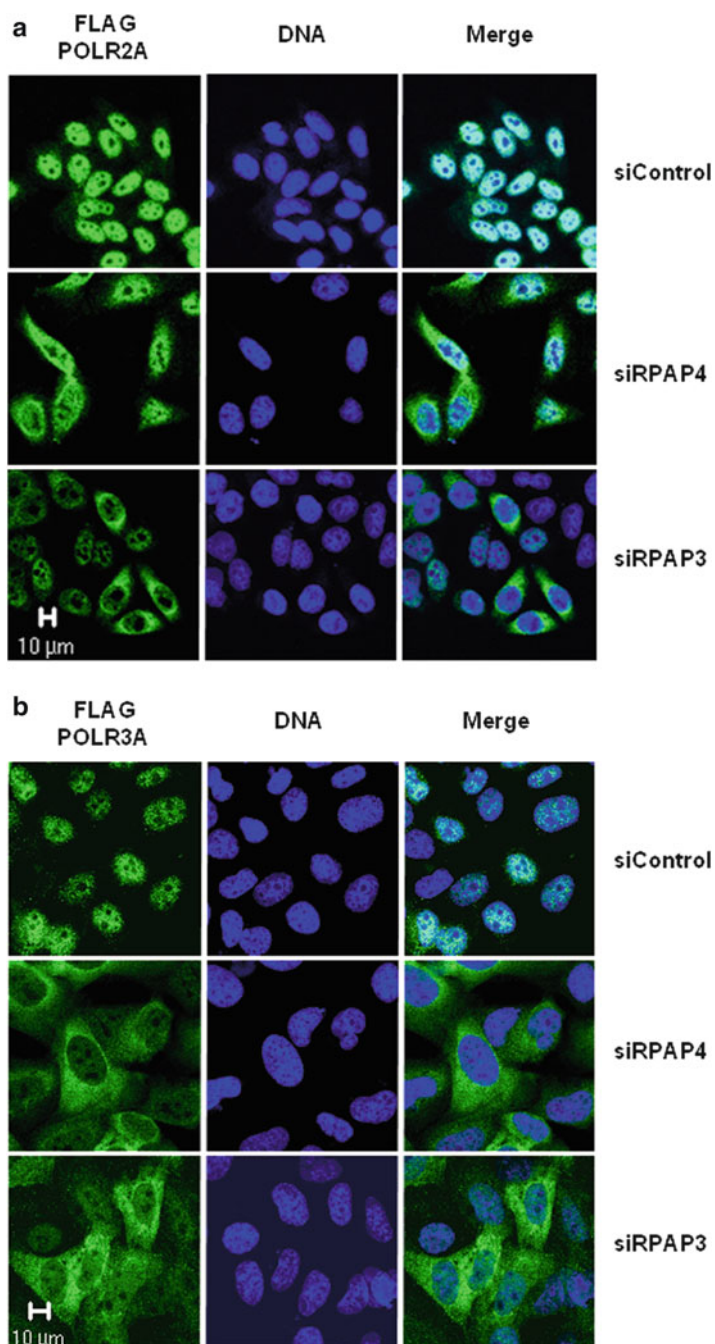


Fig. 2 Immunofluorescence experiments showing the intracellular localization of POLR2A (a), POLR3A (b) and POLR1A (c) following RPAP4/GPN1 and RPAP3 silencing. In each case, a control experiment is shown for comparison. DNA staining with TO-PRO-3 iodide served to visualize nuclei. Silencing efficiencies have been monitored by western blotting (d)

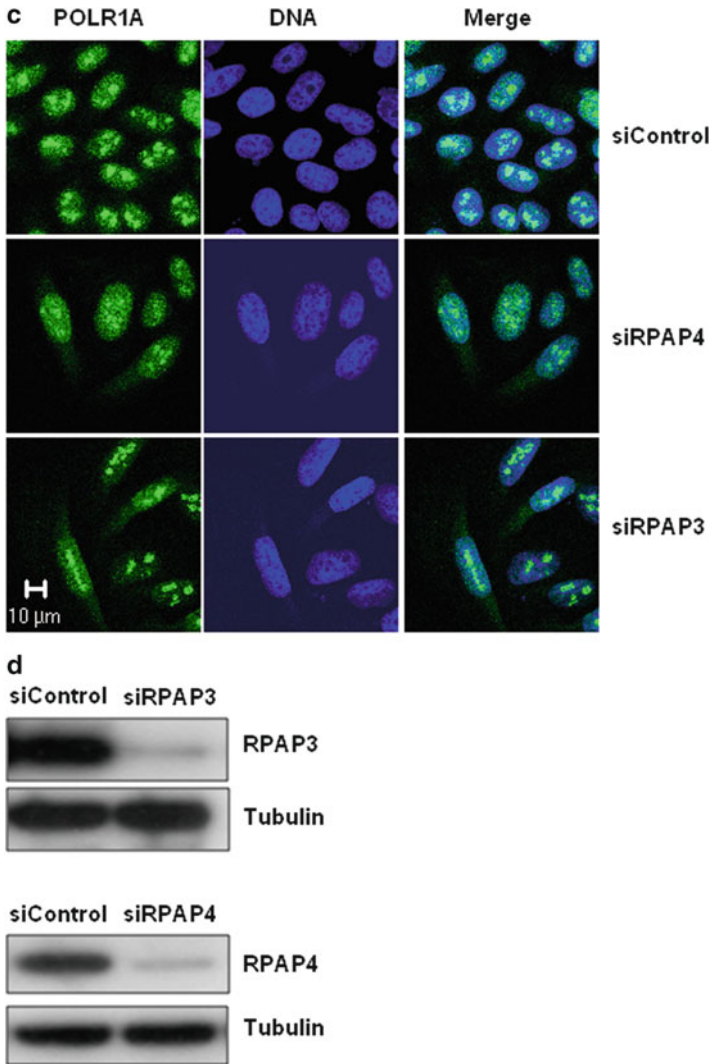


Fig. 2 (continued)

maintain normal nuclear localization of all three enzymes. Silencing of either factor resulted in the cytoplasmic accumulation of all three RNAPs. These results further suggest that nuclear RNAPs are assembled and imported to the nucleus in a tightly coordinated manner.

Contrary to RNAP II and III, RNAP I molecules have to be targeted to the nucleolus after accessing the nuclear space. It is interesting to note that RPAP4/GPN1 and RPAP3 silencing both lead to accumulation of POLR1A in the cytoplasm, although the effect not being as striking as in the case of POLR2A and POLR3A.

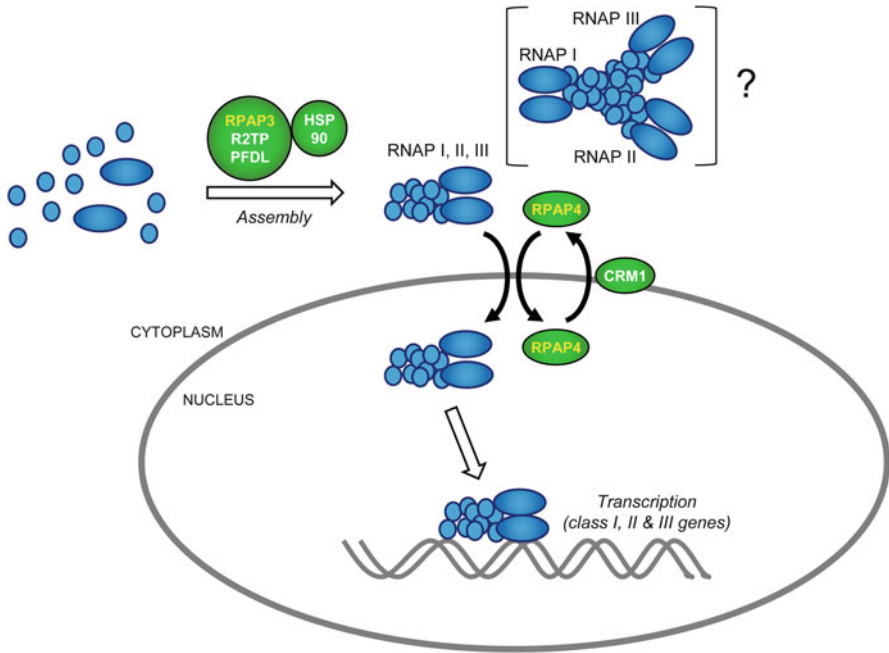


Fig. 3 Model depicting that RNAP I, RNAP II and RNAP III biogenesis is coordinated through the action of common factors. RPAP3 and RPAP4/GPN1 are essential for biogenesis of all three nuclear RNAPs as their silencing results in abnormal cytoplasmic accumulation of the largest subunit of all three polymerases. The model takes into account previous results showing that RPAP3 is a HSP90 cochaperone involved in RNAP II assembly, and RPAP4/GPN1 is a GTPase involved in RNAP II nuclear import. The existence of a putative multi-RNAP megacomplex as an intermediate in RNAP assembly is shown, but remains mainly speculative at this point

Figure 3 presents a model in which RNAP I, RNAP II and RNAP III biogenesis proceeds through a common pathway involving the same set of regulatory factors. In this model, RPAP3 is involved in assembly of all three nuclear enzymes, most likely through the action of HSP90, and RPAP4/GPN1 participates in nuclear import of the three polymerases. Whether these factors interact independently with each RNAP or else with a megacomplex composed of RNAP I, II and III subunits is not known, although our proteomic data argues in favour of the existence of such a megacomplex as an intermediate in nuclear RNAP assembly. We expect that one or more not yet identified additional factors might be required to target RNAP I to the nucleolus.

Identification of factors required for biogenesis of nuclear RNAPs has been mainly the result of targeted proteomics studies. Our own group published a number of AP-MS datasets which differ by the use of an always increasing number of affinity purified tagged components [9, 12, 14, 27]. Figure 4 shows an interaction network defined by our laboratory using classical AP-MS from soluble whole cell extracts [12]. In these experiments, chromatin is discarded prior to protein

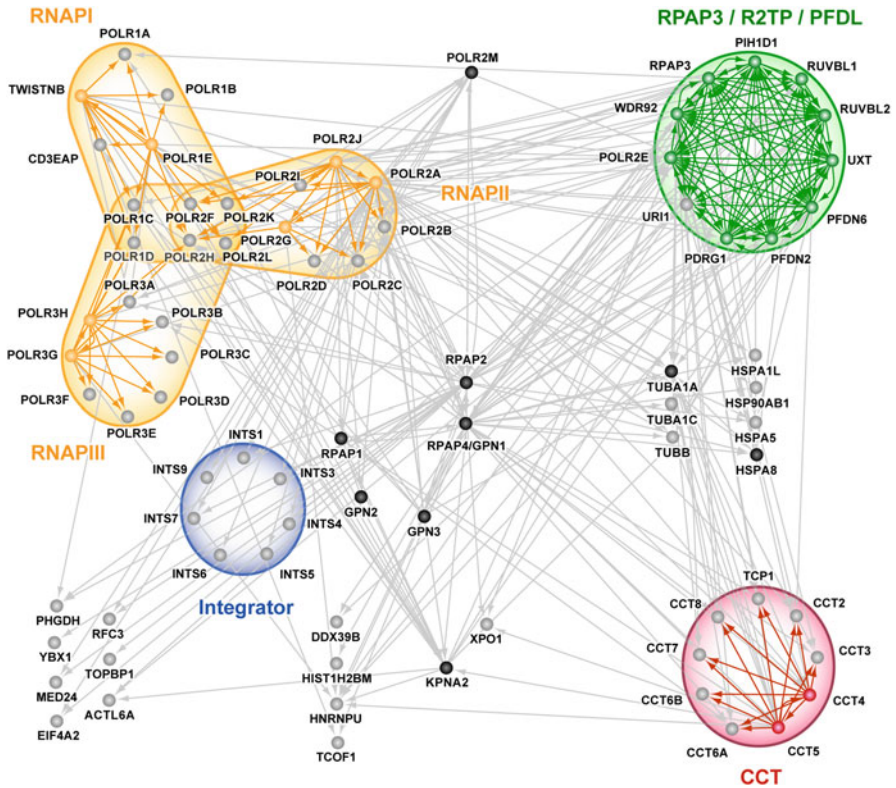


Fig. 4 Network of interactions formed by nuclear RNAP and RPAP subunits in the soluble cell fraction. Components of previously characterized multisubunit complexes are grouped. In this diagram affinity tagged proteins used in AP-MS experiments are coloured and their copurified interactors are represented by an edge

extraction. As a consequence the resulting network is largely enriched in soluble factors (i.e. factors that interact with RNAP during transcription on chromatin are mostly absent). Other methods designed specifically to characterize protein complexes on chromatin are more suited to characterize transcription relevant complexes [28]. This procedural aspect explains why the network presented in Fig. 4 mainly contains factors involved in RNAP biogenesis. The network in Fig. 4 integrates high confidence AP-MS data obtained with 28 tagged proteins (coloured nodes, as opposed to grey nodes). Only interactions that obtained high interaction reliability (IR) scores are included, as we described previously [12].

Examination of this network not only reveals interactions made by subunits of all three nuclear RNAP, with shared subunits identified through a Venn diagram-like presentation, but also interactions connecting RNAP to other proteins, including the RPAP3/R2TP/PFDL, the chaperonin/CCT and the Integrator complexes. Not surprisingly, the RPAP3/R2TP/PFDL complex is itself connected to HSP90 as these proteins were shown to act in concert in RNAP II assembly [20]. Presence of the

chaperonin/CCT complex, which has previously been shown to play a central role in microtubule assembly, may be explained by our finding that microtubule assembly/integrity is required for RNAP II nuclear import [12]. The Integrator complex has been shown to interact with RNAP II and regulate snRNA processing [29]. Other RPAPs occupy a central position in this network.

In conclusion, a large network of factors associates with RNAP in the soluble cell fraction. Some of these factors, namely RPAP4/GPN1 and RPAP3, play a role in biogenesis of all three nuclear RNAPs. Additional work is required to define putative roles of other network components in assembly or nuclear import of these important molecular machines.

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References

1. Conaway JW, Shilatifard A, Dvir A, Conaway RC. Control of elongation by RNA polymerase II. *Trends Biochem Sci.* 2000;25(8):375–80.
2. Coulombe B, Burton ZF. DNA bending and wrapping around RNA polymerase: a “revolutionary” model describing transcriptional mechanisms. *Microbiol Mol Biol Rev.* 1999;63(2):457–78.
3. Hampsey M. Molecular genetics of the RNA polymerase II general transcriptional machinery. *Microbiol Mol Biol Rev.* 1998;62(2):465–503.
4. Kornberg RD. The molecular basis of eukaryotic transcription. *Proc Natl Acad Sci USA.* 2007;104(32):12955–61.
5. Dey A, Nishiyama A, Karpova T, McNally J, Ozato K. Brd4 marks select genes on mitotic chromatin and directs postmitotic transcription. *Mol Biol Cell.* 2009;20(23):4899–909.
6. Parsons GG, Spencer CA. Mitotic repression of RNA polymerase II transcription is accompanied by release of transcription elongation complexes. *Mol Cell Biol.* 1997;17(10):5791–802.
7. Tseng LC, Chen RH. Temporal control of nuclear envelope assembly by phosphorylation of lamin B receptor. *Mol Biol Cell.* 2011;22(18):3306–17.
8. Czeko E, Seizl M, Augsberger C, Mielke T, Cramer P. Iwr1 directs RNA polymerase II nuclear import. *Mol Cell.* 2011;42(2):261–6.
9. Jeronimo C, Forget D, Bouchard A, Li Q, Chua G, Poitras C, et al. Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. *Mol Cell.* 2007;27(2):262–74.
10. Gras S, Chaumont V, Fernandez B, Carpentier P, Charrier-Savournin F, Schmitt S, et al. Structural insights into a new homodimeric self-activated GTPase family. *EMBO Rep.* 2007;8(6):569–75.
11. Carre C, Shiekhhattar R. Human GTPases associate with RNA polymerase II to mediate its nuclear import. *Mol Cell Biol.* 2011;31(19):3953–62.
12. Forget D, Lacombe AA, Cloutier P, Al Khoury R, Bouchard A, Lavalley-Adam M, et al. The protein interaction network of the human transcription machinery reveals a role for the conserved GTPase RPAP4/GPN1 and microtubule assembly in nuclear import and biogenesis of RNA polymerase II. *Mol Cell Proteomics.* 2010;9(12):2827–39.
13. Reyes-Pardo H, Barbosa-Camacho AA, Perez-Mejia AE, Lara-Chacon B, Salas-Estrada LA, Robledo-Rivera AY, et al. A nuclear export sequence in GPN-loop GTPase 1, an essential

- protein for nuclear targeting of RNA polymerase II, is necessary and sufficient for nuclear export. *Biochim Biophys Acta*. 2012;1823(10):1756–66.
14. Cloutier P, Al Khoury R, Lavalleye-Adam M, Faubert D, Jiang H, Poitras C, et al. High-resolution mapping of the protein interaction network for the human transcription machinery and affinity purification of RNA polymerase II-associated complexes. *Methods*. 2009;48(4):381–6.
 15. Gstaiger M, Luke B, Hess D, Oakeley EJ, Wirbelauer C, Blondel M, et al. Control of nutrient-sensitive transcription programs by the unconventional prefoldin URI. *Science*. 2003;302(5648):1208–12.
 16. Sardiù ME, Cai Y, Jin J, Swanson SK, Conaway RC, Conaway JW, et al. Probabilistic assembly of human protein interaction networks from label-free quantitative proteomics. *Proc Natl Acad Sci USA*. 2008;105(5):1454–9.
 17. Martin-Benito J, Gomez-Reino J, Stirling PC, Lundin VF, Gomez-Puertas P, Boskovic J, et al. Divergent substrate-binding mechanisms reveal an evolutionary specialization of eukaryotic prefoldin compared to its archaeal counterpart. *Structure*. 2007;15(1):101–10.
 18. Simons CT, Staes A, Rommelaere H, Ampe C, Lewis SA, Cowan NJ. Selective contribution of eukaryotic prefoldin subunits to actin and tubulin binding. *J Biol Chem*. 2004;279(6):4193–203.
 19. McKeegan KS, Debieux CM, Watkins NJ. Evidence that the AAA+ proteins TIP48 and TIP49 bridge interactions between 15.5K and the related NOP56 and NOP58 proteins during box C/D snoRNP biogenesis. *Mol Cell Biol*. 2009;29(18):4971–81.
 20. Boulon S, Pradet-Balade B, Verheggen C, Molle D, Boireau S, Georgieva M, et al. HSP90 and its R2TP/Prefoldin-like cochaperone are involved in the cytoplasmic assembly of RNA polymerase II. *Mol Cell*. 2010;39(6):912–24.
 21. Dundr M, Hoffmann-Rohrer U, Hu Q, Grummt I, Rothblum LI, Phair RD, et al. A kinetic framework for a mammalian RNA polymerase in vivo. *Science*. 2002;298(5598):1623–6.
 22. Hardeland U, Hurt E. Coordinated nuclear import of RNA polymerase III subunits. *Traffic*. 2006;7(4):465–73.
 23. Wild T, Cramer P. Biogenesis of multisubunit RNA polymerases. *Trends Biochem Sci*. 2012;37(3):99–105.
 24. Archambault J, Friesen JD. Genetics of eukaryotic RNA polymerases I, II, and III. *Microbiol Rev*. 1993;57(3):703–24.
 25. Young RA. RNA polymerase II. *Annu Rev Biochem*. 1991;60:689–715.
 26. Gingras AC, Gstaiger M, Raught B, Aebersold R. Analysis of protein complexes using mass spectrometry. *Nat Rev Mol Cell Biol*. 2007;8(8):645–54.
 27. Jeronimo C, Langelier MF, Zeghouf M, Cojocaru M, Bergeron D, Baali D, et al. RPAP1, a novel human RNA polymerase II-associated protein affinity purified with recombinant wild-type and mutated polymerase subunits. *Mol Cell Biol*. 2004;24(16):7043–58.
 28. Lavalleye-Adam M, Rousseau J, Domecq C, Bouchard A, Forget D, Faubert D, et al. Discovery of cell compartment specific protein-protein interactions using affinity purification combined with tandem mass spectrometry. *J Proteome Res*. 2013;12(1):272–81.
 29. Baillat D, Hakimi MA, Naar AM, Shilatfard A, Cooch N, Shiekhattar R. Integrator, a multi-protein mediator of small nuclear RNA processing, associates with the C-terminal repeat of RNA polymerase II. *Cell*. 2005;123(2):265–76.