Caenorhabditis elegans Nucleoporins Nup93 and Nup205 Determine the Limit of Nuclear Pore Complex Size Exclusion In Vivo^{□□}

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Nuclear pore complexes (NPCs) span the nuclear envelope and mediate communication between the nucleus and the cytoplasm. To obtain insight into the structure and function of NPCs of multicellular organisms, we have initiated an extensive analysis of *Caenorhabditis elegans* nucleoporins. Of 20 assigned *C. elegans* nucleoporin genes, 17 were found to be essential for embryonic development either alone or in combination. In several cases, depletion of nucleoporins by RNAi caused severe defects in nuclear appearance. More specifically, the *C. elegans* homologs of vertebrate Nup93 and Nup205 were each found to be required for normal NPC distribution in the nuclear envelope in vivo. Depletion of Nup93 or Nup205 caused a failure in nuclear exclusion of nonnuclear macromolecules of ~70 kDa without preventing active nuclear protein import or the assembly of the nuclear envelope. The defects in NPC exclusion were accompanied by abnormal chromatin condensation and early embryonic arrest. Thus, the contribution to NPC structure of Nup93 and Nup205 is essential for establishment of normal NPC function and for cell viability.

INTRODUCTION

In all eukaryotes, the two membranes that form the nuclear envelope (NE) define the two largest distinct, but connected, cellular compartments, the nucleus and cytoplasm. This compartmentalization is essential to regulate access to chromatin and provides scope for the control of gene expression. The physical separation of the genome and RNA transcription from protein synthesis generates a requirement for high-capacity macromolecular transport between the nucleus and the cytoplasm. This is fulfilled by nuclear pore complexes (NPCs), which are large proteinaceous structures spanning the nuclear envelope and through which receptor-mediated transport of macromolecules and passive exchange of ions and metabolites occur (Mattaj and Englmeier, 1998; Görlich and Kutay, 1999; Conti and Izaurralde, 2001).

NPCs have a modular architecture with substructures that include an eightfold symmetric spoke-ring complex anchored in the membrane, cytoplasmic and nuclear annular rings, and asymmetric cytoplasmic and nuclear filamentous structures (Stoffler *et al.*, 1999; Bagley *et al.*, 2000). The yeast NPC is smaller and may be simpler than the NPC of higher eukaryotes. Most NPC components and associated proteins were initially identified in *Saccharomyces cerevisiae* by using genetic and biochemical approaches. An exhaustive pro-

teomic analysis of the yeast NPC identified 29 nucleoporins (Rout *et al.*, 2000). More recently, analysis of the components of a highly purified nucleoporin fraction from rat liver nuclei, by using mass spectrometry, revealed essentially the same number of vertebrate nucleoporins (Cronshaw *et al.*, 2002). Two thirds of the ~30 nucleoporins are recognizably conserved between the two species, suggesting they might carry out similar functions. In addition, mammals and yeast each express specific nucleoporins with no evident orthologs.

The localization of most nucleoporins within the NPC has been determined in yeast whereas fewer nucleoporins have been localized within the vertebrate NPC (Rout *et al.*, 2000; Walther *et al.*, 2001, 2002; and references therein). Biochemical and genetic evidence indicates that several nucleoporins are organized in discrete interconnected subcomplexes. Several of these NPC subcomplexes are conserved from yeast to vertebrates (Stoffler *et al.*, 1999; Vasu and Forbes, 2001). Despite the sequence similarities of several nucleoporins and conservation of NPC subcomplexes, the function of these conserved sets of nucleoporins is still poorly understood.

Functional analysis of individual nucleoporins and NPC subcomplexes has mainly been done in either *S. cerevisiae* or in *Xenopus* cell-free extracts that support the reconstitution of functional nuclei (Doye and Hurt, 1997; Grandi *et al.*, 1997; Vasu and Forbes, 2001; Walther *et al.*, 2001). Among the conserved NPC subcomplexes, the yeast Nup84p complex (Siniosoglou *et al.*, 1996, 2000) and the equivalent Nup107-160 complex in vertebrates (Fontoura *et al.*, 1999; Belgareh *et al.*, 2001; Vasu and Forbes, 2001) play an essential role in NPC assembly (Boehmer *et al.*, 2003; Harel *et al.*, 2003; Walther *et al.*, 2003).

A second group of yeast nucleoporins includes Nup188p that interacts genetically with Nic96p, Nup170p, Nup192p, and POM152p (Nehrbass *et al.*, 1996; Kosova *et al.*, 1999).

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Abbreviations used: DIC, differential interference contrast; GFP, green fluorescent protein; ORF, open reading frame; NE, nuclear envelope; NPC, nuclear pore complex; npp, nuclear pore complex protein; RNAi, RNA interference.

Together, these are the most abundant proteins of the yeast NPC. Temperature-sensitive mutants in yeast Nic96p or Nup192p show a reduced number of NPCs assembled per nucleus (Gomez-Ospina *et al.*, 2000), suggesting that yeast Nic96p and Nup192p may either play a role in NPC assembly and/or in forming major structures of the NPC. In addition, cells lacking Nup188p or Nup170p show higher rates of passive diffusion of green fluorescent protein (GFP) multimers (Shulga *et al.*, 2000), consistent with these proteins playing a role in establishing the resting diameter of the channel within the NPC through which macromolecular transport occurs. The three vertebrate proteins related to yeast Nic96p, Nup188p, and Nup192p, namely, Nup93, Nup188, and Nup205, also exist in a complex (Grandi *et al.*, 1997; Miller *et al.*, 2000). Immunodepletion of the vertebrate complex from *Xenopus* extracts, followed by *in vitro* nuclear assembly, results in nuclei that contain a reduced quantity of specific nucleoporins, suggesting the formation of incomplete NPCs (Grandi *et al.*, 1997).

Interestingly, recent results show that some nucleoporins and NPC subcomplexes have important functions distinct from their role in nucleocytoplasmic transport, influencing both nuclear organization and chromatin segregation (Kerscher *et al.*, 2001; Feuerbach *et al.*, 2002). Moreover, a subset of nucleoporins has been shown to redistribute in part to kinetochores during mitosis in mammalian cells (Belgareh *et al.*, 2001). The function, if any, of this specific mitotic localization is not yet known.

Analysis of NPC morphology and structure by thin-section transmission electron microscopy in early embryos of the nematode *Caenorhabditis elegans* revealed that NPCs are larger in *C. elegans* than in yeast, with dimensions closer to those of higher eukaryotes (Cohen *et al.*, 2002). Moreover, *C. elegans* is more closely related to humans than is yeast and its nucleoporins consequently show higher sequence conservation to those of human. Information obtained in the nematode will thus be likely to reflect metazoan nucleoporin function in general. A rapid and well-defined embryogenesis combined with very efficient inhibition of gene expression by double-stranded RNA-mediated interference (RNAi) makes *C. elegans* an attractive model system, in addition to yeast, to study nucleoporin function *in vivo* during interphase and mitosis.

Here, we show that most known vertebrate nucleoporins have a potential homolog in *C. elegans*. The majority of these nucleoporins are essential for viability and nuclear abnormalities are observed upon their depletion *in vivo*. We analyzed in detail the effect of the depletion of the homologs of the vertebrate nucleoporins Nup205 and Nup93. These two proteins are essential in *C. elegans* and in both cases RNAi-treated embryos show a clear defect in the nuclear exclusion of nonnuclear macromolecules of 70 kDa and in NPC distribution. This suggests that these nucleoporins are functionally connected and involved in NPC structure, thereby controlling passive nuclear envelope permeability in a manner similar to that demonstrated for yeast Nup188p and Nup170p (Shulga *et al.*, 2000). Surprisingly, the lack of proper nuclear exclusion in early development does not completely block the first cell divisions. It does, however, strongly induce peripheral chromatin condensation.

MATERIALS AND METHODS

Worm Strains

The *C. elegans* Bristol strain N2 was used for analysis of RNAi effects on brood size and viability. DP38 *unc-119(ed3)* (Maduro and Pilgrim, 1995) and AZ212

GFP::hisH2B (Praitis *et al.*, 2001) were provided by the *Caenorhabditis* Genetic Center (University of Minnesota, Minneapolis, MN). WH204 [*GFP::tbb-2*] and XA3501 [*GFP::hisH2B/GFP::tbb-2*] have been described previously (Strome *et al.*, 2001; Askjaer *et al.*, 2002).

Generation of Transgenic Worms

Using sequence information from ACeDB (<http://www.wormbase.org>), we polymerase chain reaction (PCR) amplified the genomic DNA sequence of the *C. elegans* lamin gene (*lmn-1*, DY3.2) and the *C. elegans* MAN1 gene (*lem-2*, W01G7.5). The *lmn-1* and *lem-2* PCR products were used to replace *hisH2B* downstream of *GFP* in pJH4.52 (Strome *et al.*, 2001) to generate plasmids pPAG4 and pPAG10, respectively. pPAG4 was further modified by replacing *GFP* with a sequence encoding yellow fluorescent protein (YFP) from plasmid pPD133.86 (Miller *et al.*, 1999). Plasmid pUP9 is derived from pJH4.52 by insertion of *unc-119* from plasmid pDP#MM051 (Maduro and Pilgrim, 1995) and by replacing *GFP* with cyan fluorescent protein (CFP) from plasmid pPD133.82 (Miller *et al.*, 1999). Transgenic worms were generated using the gold particle bombardment method (Praitis *et al.*, 2001) with modifications (Askjaer *et al.*, 2002). Strain XA3507 expressing *GFP::MAN1* fusion protein from an integrated transgene was obtained by cobombardment of DP38 worms with plasmids pDP#MM051 (Maduro and Pilgrim, 1995) and pPAG10. XA3502 was generated by cobombardment with plasmids pPAG6 and pUP9 and expresses YFP::LMN-1 from an integrated transgene. XA3502 harbors in addition a silenced CFP::hisH2B transgene.

Identification of *C. elegans* Nucleoporins

Mammalian nucleoporin protein sequences (Cronshaw *et al.*, 2002) were used as queries in BLASTP searches at Wormbase (www.wormbase.org) mainly against WormPep database releases 85 and 86. Predicted and confirmed gene products obtained were validated by several means: The gene products obtained were examined for protein motifs such as FG dipeptide repeats and Ran binding domains, and predicted molecular weights were compared with the query proteins. Furthermore, when used as queries in WU-Blast2 searches against the *sptrmb1* and *swissprot* databases at the European Bioinformatics Institute (www.ebi.ac.uk) and/or in BLASTP searches against the nonredundant mammalian database at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov), all npp genes identified in this work identified as best match the starting query nucleoporin. Finally, nine of the predicted *C. elegans* nucleoporins have been confirmed to localize to the nuclear periphery as expected (see Supplementary Figure 1).

RNAi

RNA-mediated interference was performed by feeding the worms with bacteria that express double-stranded RNA (Fraser *et al.*, 2000). Plasmids for RNAi were generated by either PCR or reverse transcription-PCR amplification of target sequences and cloning into pPD129.36 L4440 (Timmons and Fire, 1998). A list of primer sequences is available at <http://www.embl-heidelberg.de/ExternallInfo/mattaj/elegans/primers.html>.

RNAi constructs were designed to target all predicted isoforms of the individual npp genes. RNAi plates were prepared as described previously (Askjaer *et al.*, 2002). For combinatorial RNAi, the double-stranded RNA-expressing bacterial cultures were grown separately and mixed equally immediately before seeding the plates.

To analyze the effect of RNAi on brood size and viability, L2/L3 larvae were incubated on RNAi plates at 16°C for 70 h. Young adults from these plates were then transferred to individual RNAi plates and incubated at 20°C for 20 h. The adult hermaphrodites were removed from the plates. After further incubation of the plates the number of oocytes, embryos and larvae on the plates was determined and postembryonic phenotypes were scored. To obtain RNAi embryos for imaging, L3 and L4 larvae from the GFP strains were incubated on the RNAi plates at 20°C for 32 h.

Live Embryo Imaging

Embryos were mounted in M9 buffer on 2% agarose pads and covered with a coverslip. Recording of epifluorescence and transmitted light was with a Leica (Wetzlar, Germany) confocal microscope TCS SP2 with a HCX PL APO 63×/1.4 objective. Images were captured using integrated Leica software and processed with ImageJ. The laser intensity was adjusted so that no effect on development was seen. Images were collected at 20-s intervals for a total of 20–40 min.

Immunofluorescence

Worms from RNAi plates were dissected directly on poly-L-lysine-coated glass slides. The eggshell was opened by freeze cracking and the embryos were fixed in methanol for 20–30 min at –20°C. After rehydration in phosphate-buffered saline (PBS) with 0.1% Tween 20 and blocking with 3% milk the embryos were incubated with a 1:500 dilution of monoclonal antibody (mAb) 414 against nucleoporins (Jackson Immunoresearch Laboratories, West Grove, PA). After incubation for 120 min at room temperature, embryos were

Table 1. Identification of *C. elegans* nucleoporins

<i>C. elegans</i> gene		Mammalian nucleoporin ^a	P _N ^b	Embryonic phenotypes		Other phenotypes ^c	Phenotypes previously reported ^f
				Emb (%) ^c	Nmo ^d		
<i>npp-1</i>	K07F5.13b	Nup54	1.2e ⁻³⁹	99 ± 2 (n = 672)	+	Lva, Lvl, Stp	Emb ^{1,2,3} ; Stp ¹
<i>npp-2</i>	T01G9.4	Nup85	9.6e ⁻²⁵	37 ± 28 (n = 4285)	+	Lva, Stp	Emb ^{3,4,5} ; Gro ⁴ ; Nmo ⁶ ; wt ¹
<i>npp-3</i>	K12D12.2	Nup205	1.2e ⁻³⁸	100 ± 0 (n = 378)	+	Lva, Ste	Emb ² ; Rup ² ; Clr ² ; Unc ²
<i>npp-4</i>	Y54E5A.4	Nup45/Nup58	1.4e ⁻¹⁷	87 ± 19 (n = 651)	+	Gro, Stp	wt ²
<i>npp-5</i>	F07A11.3	Nup107	6.7e ⁻¹¹	0 ± 0 (n = 2224)	–	(Gro)	wt ^{2,3}
<i>npp-6</i>	F56A3.3a	Nup160	3.1e ⁻⁰⁵	98 ± 4 (n = 776)	++	Lva, Lvl	Emb ^{4,5} ; Nmo ⁶
<i>npp-7</i>	T19B4.2	Nup153	3.0e ⁻²³	96 ± 9 (n = 1404)	++	Lva	Emb ⁴ ; Nmo ⁶ ; Ste ⁴
<i>npp-8</i>	Y41D4B.19b	Nup155	2.3e ⁻⁷¹	99 ± 2 (n = 392)	++	Lva, Lvl	Emb ² ; Pvl ²
<i>npp-9</i>	F59A2.1	Nup358	2.7e ⁻⁶⁵	100 ± 0 (n = 854)	++	ND	Emb ^{2,3,7} ; Nmo ⁷ ; Pvl ²
<i>npp-10</i>	ZK328.5b	Nup98/96	1.1e ⁻⁹⁹	99 ± 4 (n = 324)	++	Lva, Lvl, Ste	Emb ^{2,5,7} ; Nmo ⁷ ; Ste ²
<i>npp-11</i>	F53F10.5	Nup62	7.1e ⁻²⁹	87 ± 17 (n = 808)	+	Lva, Lvl	Emb ⁴ ; Nmo ⁶
<i>npp-12</i>	T23H2.1	gp210	6.2e ⁻¹³⁵	0 ± 0 (n = 714)	ND	wt	wt ^{3,4} ; Emb ^{1,5,8} ; Lva ⁵
<i>npp-13</i>	Y37E3.15a	Nup93	5.6e ⁻⁶³	95 ± 4 (n = 786)	+	ND	Emb ⁵
<i>npp-14</i>	C03D6.4	Nup214	3.4e ⁻⁴⁶	1 ± 1 (n = 608)	ND	wt	wt ⁴
<i>npp-15</i>	C29E4.4	Nup133	9.0e ⁻¹⁴	1 ± 1 (n = 1536)	–	(Gro)	wt ^{2,3,7}
<i>npp-16</i>	Y56A3A.17b	Nup50	2.1e ⁻¹²	0 ± 0 (n = 894)	ND	wt	wt ^{2,5,7}
<i>npp-17</i>	F10G8.3	RAE1	2.1e ⁻⁹⁷	7 ± 11 (n = 304)	ND	Ste, Stp	Pvl ⁴ ; Stp ⁴ ; Hya ⁴
<i>npp-18</i>	Y43F4B.4	Seh1	1.0e ⁻⁷²	0 ± 0 (n = 272)	ND	wt	wt ^{2,3,5,7}
<i>npp-19</i>	R06F6.5a	Nup35	1.3e ⁻¹¹	96 ± 4 (n = 1232)	++	Stp	Emb ⁵ ; wt ²
<i>npp-20</i>	Y77E11A.13a	Sec13R	4.6e ⁻⁷⁰	100 ± 0 (n = 588)	++	Ste, Lva, Lvl	Emb ² ; Lvl ² ; Gro ² ; Stp ²

ND, not determined.

^a Cronshaw *et al.* (2002) and references therein; Harel *et al.* (2003). No clear *C. elegans* homologs were found for the mammalian nucleoporins Aladin, NLP1/hCG1, Nup37, Nup43, Nup88, Nup188, POM121, or TPR. See text for details.

^b Probability values obtained in BLAST searches with mammalian protein sequences against the WormPep database releases 85 and 86 at <http://www.wormbase.org>.

^c Emb, embryonic lethal; average percentage of embryos that do not hatch ± SD. The number of embryos counted is given in brackets. Lethality of control embryos was 0% (n > 1000).

^d Nmo, (pro-)nuclear morphology alteration in early embryo; ++, absence of nuclei; +, spherical, but smaller nuclei.

^e Clr, clear; Gro, slow growth; Hya, hyper active; Lva, larval arrest; Lvl, larval lethal; Pvl, protruding vulva; Rup, ruptured; Ste, sterile; Stp, sterile progeny; Unc, uncoordinated; wt, wild type. Brackets indicate weak penetrance. Only phenotypes present in >10% of analyzed individuals are reported.

^f For Emb only lethality >20% in previous studies is reported. ¹ Colaiacovo *et al.* (2002); ² Kamath *et al.* (2003); ³ Piano *et al.* (2002); ⁴ Fraser *et al.* (2000); ⁵ Maeda *et al.* (2001); ⁶ Zipperlen *et al.* (2001); ⁷ Gönczy *et al.* (2000); ⁸ Cohen *et al.* (2003).

washed two times for 30 min in PBS with 0.1% Tween 20. Alexa Fluor 633 secondary antibody (Molecular Probes, Eugene, PA) was used at 1:2000 dilution in PBS for 45–120 min. The embryos were washed as before and mounted. Confocal images were obtained on a Leica TCS SP2 microscope as described above.

Injection of Fluorescently Labeled Dextran

Worms from RNAi plates were injected in one syncytial gonad with mixes of fluorescently labeled dextrans. Dextran (10 kDa) coupled to Alexa-488 (Molecular Probes) was mixed with either 70-kDa dextran coupled to rhodamine (Sigma-Aldrich, St. Louis, MO) or 160-kDa dextran coupled to tetramethylrhodamine B isothiocyanate (Sigma-Aldrich) to a final concentration of 2 mg/ml in injection buffer (20 mM KPO₄, pH 7.5, 3 mM K citrate, 2% polyethylene glycol-6000). Injected worms were incubated for 5 h at 25°C before dissection to allow incorporation of the dextrans into the newly formed embryos. Dextran distribution was determined by confocal microscopy.

RESULTS

Identification of *C. elegans* Nucleoporins

To study nucleoporins of *C. elegans*, we first sought to identify as many *C. elegans* nucleoporins as possible by database mining. To date, 28 mammalian genes encoding in total 30 nucleoporins have been described (Cronshaw *et al.*, 2002; and references therein). Protein sequences of these nucleoporins were used to search the WormPep database (www.wormbase.org; WormPep releases 85 and 86), which contains

confirmed and predicted *C. elegans* open reading frames (ORFs). To validate the genes obtained, we confirmed that when used to search mammalian data sets, the individual *C. elegans* ORFs reciprocally identified the mammalian nucleoporin originally used. We used as criteria the predicted molecular weights and characteristic motifs such as FG dipeptide repeats. By this approach, we could match the majority (20/28) of mammalian nucleoporin genes to specific *C. elegans* ORFs (Table 1). Furthermore, we and others have localized nine of the predicted nucleoporins to the nuclear envelope of *C. elegans* embryos by using specific antibodies or GFP fusion proteins (Supplementary Figure 1). For eight mammalian nucleoporins, we could not unambiguously identify *C. elegans* homologs (Table 1; see DISCUSSION). In accordance with standard *C. elegans* nomenclature, we have proposed the gene designation *npp* for nuclear pore complex protein, because the preferred gene name *nup* already refers to genes involved in nuclear positioning in the worm. Similarly, rather than assigning gene numbers to the *C. elegans* nucleoporins based on molecular weight as in yeast and vertebrates, we conform to standardized consecutive numbering used in naming genes in this organism. For simplicity and clarity, we will use the name of the homologous vertebrate nucleoporins with a Ce prefix in the rest of the text.

Depletion of Nucleoporins Affects Nuclear Morphology and Causes Embryonic Lethality

Having identified most of the *C. elegans* nucleoporins, we investigated their importance for early development by RNAi (Timmons and Fire, 1998). We followed the fate of embryos from nematodes that were fed on bacteria expressing double-stranded RNA corresponding to the individual npp genes (Fraser *et al.*, 2000). Of the 20 npp genes tested, we found that 12 were absolutely required for embryonic viability (CeNup35, -45/58, -54, -62, -93, -98/96, -153, -155, -160, -205, -358, and CeSec13R; depletion leading to 87% or greater embryonic lethality), whereas depletion of CeNup85 caused ~37% lethality (Table 1). Depletion of CeRAE1 did not interfere with embryonic viability but 100% of the progeny of the treated worms were sterile. Only six npp genes did not show clear phenotypic defects (CeNup50, -107, -133, -214, Cegp210, and CeSeh1). It should be noted that all 20 npp genes have also been targeted as part of large-scale RNAi studies performed by other laboratories (Gönczy *et al.*, 2000; Maeda *et al.*, 2001; Colaiacovo *et al.*, 2002; Piano *et al.*, 2002; Kamath *et al.*, 2003). Comparing our data to recently published studies generally showed good agreement in embryonic lethality phenotypes despite differences in the sequences used for RNAi and methods of delivery, thereby supporting the observations (Table 1). However, we repeatedly found that depletion of CeNup45/58 (vertebrate Nup45 and Nup58 are encoded by a single gene) resulted in ~87% embryonic lethality, whereas a previous study (Kamath *et al.*, 2003) reported no embryonic phenotype for CeNup45/58 (Table 1). We suggest that the explanation for this is that we obtained a higher depletion efficiency for CeNup45/58, perhaps because of the choice of target sequence for RNAi.

Recent work from our laboratory and those of others has reported that depletion of nucleoporin Nup107 or Nup133 from mammalian tissue culture cells causes codepletion of several other nucleoporins (Boehmer *et al.*, 2003; Harel *et al.*, 2003; Walther *et al.*, 2003). If this was also the case in *C. elegans*, it would obviously interfere with our estimates of essential npp genes. To address this possibility, we analyzed by immunoblotting the expression of CeNup153, CeNup358, CeNup98, and CeNup96 in control and nonviable RNAi embryos. Out of six nucleoporin genes targeted (CeNup62, CeNup98/96, CeNup54, CeNup45/58, CeNup155, and CeNup153), none led to cross-depletion of other nucleoporin gene products (Supplementary Figure 2). We therefore conclude that our observations can most likely be attributed to depletion of single nucleoporins.

The 13 npp genes that were found to be required for embryonic development (Table 1) were next investigated by DIC time-lapse microscopy. Strikingly, in all 13 cases defects were already observed in one- and two-cell embryos. Depletion of nucleoporins CeNup54, -45/58, -62, -85, -93, or -205 led to production of embryos with spherical pronuclei and nuclei that were 12–34% smaller than nuclei of control embryos (Figure 1, B–G, and Table 1). RNAi against CeNup35, -98/96, -153, -155, -160, or -358 caused an even more severe phenotype where neither pronuclei nor nuclei were recognizable by DIC microscopy (Figure 1, H–M and Table 1). In embryos depleted of CeSec13R, nuclear morphology was very irregular (Figure 1N and Table 1). However, in contrast to the other genes analyzed here, RNAi against CeSec13R caused additional severe defects in embryonic appearance and blocked cytokinesis. Presumably these effects are due to pleiotropic functions of Sec13, which is localized both to the NPC and to the COPII coat involved in endoplasmic retic-

ulum transport (Siniosoglou *et al.*, 1996; Antonny and Schekman, 2001). For six of the 13 essential npp genes, similar DIC phenotypes have been reported previously (Table 1), whereas the remaining seven npp genes have so far not been analyzed in this way. These observations suggest that several nucleoporins play crucial roles in establishing a functional nuclear envelope.

Combinatorial RNAi Demonstrates Interactions between Nucleoporins of the Conserved Nup107-160 Complex

For seven npp genes, we and others, using different techniques or RNAi constructs, detected no defects in embryonic development (CeNup50, -107, -133, -214, Cegp210, CeSeh1, and CeRAE1; Table 1). This reduces the possibility that RNAi was simply inefficient in these cases. Indeed, in the case of CeNup107, we confirmed that the protein was depleted by using specific antibodies (Supplementary Figure 1). From studies with yeast, it is known that certain individual nucleoporins are dispensable for growth, but can become critical if another nucleoporin is mutated or absent (Doye and Hurt, 1997). To address whether this was also the case in *C. elegans*, we depleted combinations of nucleoporins.

Vertebrate nucleoporins Nup107 and Nup133 are known to be part of a subcomplex that is required for NPC formation, presumably functioning as a scaffold for assembly of the pore (Boehmer *et al.*, 2003; Harel *et al.*, 2003; Walther *et al.*, 2003). Interestingly, it has so far not been possible to dissect the role of each of the seven components of the Nup107-160 complex in vertebrates, mainly due to codepletion as mentioned above. When we targeted CeNup107 in combination with CeNup133, we did not observe an increase in embryonic lethality, indicating that these nucleoporins are dispensable for the activity of the Nup107 complex at least in *C. elegans* (Figures 1O and 2). Another component of the Nup107 complex, CeNup85 showed an intermediate degree of embryonic lethality when depleted alone (Table 1). Importantly, when either CeNup107 or CeNup133, or both, were targeted in combination with CeNup85 embryonic lethality increased to ~100% (Figure 2). We suggest that this dramatic increase in lethality reflects a collapse of the Nup107-160 complex, which renders it inactive. Depletion of CeNup85 in combination with the nucleoporins CeNup214 or CeRAE1 also caused a significant and strong increase in embryonic lethality (Figure 2). This clearly demonstrates important roles for all five nucleoporins and suggests that CeNup85 might have a crucial function in the Nup107-160 complex by interacting with other components of the NPC. Combinatorial RNAi against CeNup85 and either Cegp210, CeNup50, CeSeh1, or the inner nuclear membrane protein CeMAN1/LEM-2 did not result in a significant increase in embryonic lethality (Figure 2; our unpublished data). At least in the case of MAN1 RNAi, independent experiments have confirmed that MAN1 is efficiently depleted (our unpublished data). This demonstrates specificity in the interaction between CeNup85 and the aforementioned nucleoporins, CeNup107, -214, -133, and CeRAE1.

Depletion of CeNup205 or CeNup93 Does Not Prevent Nuclear Import or Dramatically Affect Nuclear Growth

Of the 12 npp genes absolutely required for embryonic viability, seven show dramatic defects in nuclear morphology at the two-cell stage. The observation that depletion of CeNup205 or CeNup93 resulted in ~100% embryonic lethality (Table 1) but only caused a minor defect in nuclear size at the two-cell stage (Figure 1) led us to

Figure 1. Depletion of nucleoporins causes nuclear morphology alterations in early embryos. Still images from time-lapse DIC microscopy are shown of a wild-type two-cell embryo (A), or of two-cell embryos depleted of CeNup54/NPP-1 (B), CeNup85/NPP-2 (C), CeNup205/NPP-3 (D), CeNup45/58/NPP-4 (E), CeNup62/NPP-11 (F), CeNup93/NPP-13 (G), CeNup160/NPP-6 (H), CeNup153/NPP-7 (I), CeNup155/NPP-8 (J), CeNup358/NPP-9 (K), CeNup98/96/NPP-10N/C (L), CeNup35/NPP-19 (M), CeSec13/NPP-20 (N), or triple depleted of CeNup85/NPP-2, CeNup107/NPP-5, and CeNup133/NPP-15 (O). Embryos are oriented with anterior on the left and similar time points relative to cytokinesis are displayed for all embryos. Bar, 10 μ m.

further investigate the function of these two nucleoporins. The vertebrate Nup205 and Nup93 can be coprecipitated from cell extracts (Grandi *et al.*, 1997). In vitro nuclear reconstitution in *Xenopus* eggs extract depleted for the Nup93-complex leads to smaller nuclei with no detectable defect in nuclear import of a reporter substrate (Grandi *et al.*, 1997). However, due to the physical interaction between the components of this complex and their codeple-

tion from extracts, it has not been possible so far to address the function of each individual protein in vitro, neither have they been investigated in living animal cells. We decided to address the function in vivo of each nucleoporin by RNAi depletion in *C. elegans*.

We analyzed whether depletion of either of the two nucleoporins has an effect on nuclear import in vivo. Active transport through NPCs into the newly assembled nuclei

Figure 2. Combinatorial RNAi reveals essential combinations of nucleoporins in the Nup107/Nup160 complex. The effect of RNAi on embryonic lethality was measured as described in MATERIALS AND METHODS. Embryonic lethality was measured upon RNAi against CeMAN1/LEM-2, CeNup107/NPP-5, CeNup133/NPP-15, CeNup107+CeNup133, CeNup214/NPP-14, or CeRAE1/NPP-17 alone (black bars, values from Table 1) or in combination with depletion of CeNup85/NPP-2 (hatched bars). CeNup85/NPP-2 alone resulted in 37% lethality, whereas targeting CeNup85/NPP-2 together with CeNup107/NPP-5, CeNup133/NPP-15, CeNup214/NPP-14, or CeRAE1/NPP-17 caused a significant increase in lethality. Triple RNAi against CeNup85/NPP-2, CeNup107/NPP-5, and CeNup133/NPP-15 similarly enhanced the lethality compared with CeNup85/NPP-2(RNAi) alone or CeNup107/NPP-5(RNAi) together with CeNup133/NPP-15(RNAi). Error bars indicate standard deviations.

after mitosis is required for nuclear growth and rapid import of lamin (Loewinger and McKeon, 1988). We monitored these processes *in vivo* by using a strain expressing YFP-lamin (YFP-LMN-1). The specificity of RNAi against CeNup205 and CeNup93 was verified by reverse transcription-PCR (our unpublished data). Control or RNAi-treated embryos were recorded using time-lapse confocal microscopy. To allow direct comparison of the different recordings, we performed all recordings the same day and at constant temperature. For each RNAi experiment, three embryos from independent worms were analyzed. Similar to control RNAi embryos, YFP-lamin occurred in nuclei during telophase of embryos depleted for CeNup205 and CeNup93. The distribution and intensity of YFP-lamin in interphase was also unaffected by CeNup205 or CeNup93 depletion (Figure 3A). These results show that YFP-lamin is imported into the nuclei of CeNup205(RNAi) and CeNup93(RNAi) embryos, suggesting that nuclear import is not strongly affected. Nevertheless, the final sizes of P1 nuclei before entry into the next mitosis were slightly reduced in CeNup205(RNAi) and CeNup93(RNAi) embryos compared with the control RNAi embryos (Figure 3B). We also noticed a significant delay in cell division. CeNup93- and CeNup205-depleted embryos showed a delayed second division, measured either from P0 metaphase/anaphase transition to AB metaphase/anaphase transition or from there to P1 metaphase/anaphase transition (Figure 3C). Together, these results suggest that despite the essential role of these two proteins in *C. elegans* embryonic development, CeNup205 and CeNup93 do not play major roles in nuclear protein import.

We used a strain expressing GFP-histone-H2B to examine the terminal stage of development of CeNup205 and CeNup93-depleted embryos. The embryos stopped dividing before the ~100-cell stage and, unlike control embryos, their nuclei were of irregular size (Figure 3D). The CeNup205(RNAi) and CeNup93(RNAi) embryos also showed abnormal chromatin condensation in the nuclear periphery (Figure 3D), which could have indicated that the cells were undergoing apoptosis. We therefore investigated potential defects during cell division by monitoring GFP-histone H2B and GFP- β -tubulin distribu-

tion during early development. Embryos were followed by time-lapse confocal microscopy and synchronized using P0 metaphase/anaphase transition as a reference (Figure 3E and Movie 1). Before congression to the metaphase plate, the GFP-histone H2B-stained chromosomes do not show any particular localization in the nuclear space in wild-type embryos. In contrast, CeNup205 or CeNup93 depletion led to a strong and reproducible abnormal peripheral chromatin condensation (Figure 3E and Movie 1). Thus, the aberrant chromatin morphology observed in arrested embryos (Figure 3D) is most likely not related to apoptosis but rather a general feature in CeNup205(RNAi) and CeNup93(RNAi) embryos at all stages.

CeNup205 and CeNup93 Are Required for Normal NPC Distribution in the Nuclear Envelope (NE)

We then investigated the effect of depletion of CeNup205 or CeNup93 on NPC assembly and distribution by immunofluorescence by using mAb mAb414 (Davis and Blobel, 1987). In control embryos, a continuous rim of mAb414 signal surrounded each nucleus (Figure 4A, left column). In contrast, when we depleted either CeNup205 (Figure 4A, middle column) or CeNup93 (Figure 4A, right column) or both (Supplementary Figure 3A) by RNAi, mAb414 staining seemed discontinuous and distinct mAb414 foci were observed around the chromatin. This phenotype was even more pronounced in older embryos (our unpublished data). There was no preferential condensation of chromatin at mAb414-stained foci (Supplementary Figure 3A, first and second rows). Importantly, in the depleted embryos, the overall intensity of mAb414 staining was similar to control embryos (Figure 4A), suggesting that the punctate staining reflects NPC clustering rather than a reduced number of NPCs. NPC clustering phenotypes are frequently observed on mutation or removal of yeast nucleoporins (Doye and Hurt, 1997).

Depletion of CeNup205 or CeNup93 Cause Nuclear Exclusion Defect

To analyze NE integrity, we examined the distribution of MAN1, an inner nuclear envelope protein (Lin *et al.*, 2000). GFP-MAN1 was localized, as expected, to the NE of inter-

Figure 3. Depletion of CeNup205 or CeNup93 delays cell division and induces abnormal peripheral chromatin condensation. (A) Embryos expressing YFP-lamin from control worms or worms depleted of CeNup205/NPP-3 or CeNup93/NPP-13 were observed by time-lapse confocal microscopy. Still images of two-cell embryos are shown with indication of time (minutes:seconds) relative to anaphase onset. Anterior of the embryos is on the left. In all cases, lamin accumulated at the nuclear periphery and in the nucleoplasm. (B) Maximum diameter of P1 nuclei was measured. Shown is the average of control, CeNup205(RNAi) or CeNup93(RNAi) embryos ($n = 3$ for each). (C) Timing of cell division was measured from P0 anaphase onset to AB anaphase onset (dark gray) and from AB anaphase onset to P1 anaphase onset (light gray). Shown is the average of control, CeNup205(RNAi) or CeNup93(RNAi) embryos ($n = 3$ for each). (D) Embryos expressing GFP-histone H2B from control worms or from worms depleted of CeNup205/NPP-3 or CeNup93/NPP-13 were observed by confocal microscopy. Shown are projections from z-scan acquisitions (z step = $0.5 \mu\text{m}$) of representative CeNup205(RNAi) or CeNup93(RNAi) embryos collected 16 h after oviposition or a wild type embryo with a comparable cell number. Note the heterogeneous distribution and the abnormal peripheral condensation of chromatin in CeNup205(RNAi) and CeNup93(RNAi) embryos. (E) Embryos expressing GFP- β -tubulin and GFP-histone H2B from either control worms or worms depleted of CeNup205/NPP-3 or CeNup93/NPP-13 were observed by time-lapse confocal microscopy. Still images are shown with indication of time (minutes:seconds) relative to anaphase onset. Note the abnormal peripheral condensation of chromatin in P1 of 2-cell CeNup205(RNAi) and CeNup93(RNAi) embryos (indicated by arrows). Bars, $10 \mu\text{m}$.

phase cells (Figure 4B). GFP-MAN1 also localized around the chromatin in embryos depleted of CeNup205 or CeNup93 (Figure 4B) or both CeNup205 and CeNup93 (Supplementary Figure 3B), suggesting the presence of normal

NEs. GFP-emerin also localized normally in CeNup205 and CeNup93-depleted embryos (our unpublished data).

NE and NPC integrity are essential for nuclear exclusion of macromolecules above roughly 45 kDa. We tested nuclear

Figure 4. Depletion of CeNup205 or CeNup93 induces abnormal NPC distribution in the NE. (A) Embryos from control worms or worms depleted of CeNup205/NPP-3 or CeNup93/NPP-13 were fixed and analyzed with the anti-nucleoporin mAb414. Projections from z-scan acquisitions by using confocal microscopy (z step = 0.5 μ m) at low (first row; bar, 10 μ m) or higher (second row; bar, 1 μ m) magnification are shown. NPCs are clustered in the NE of CeNup205/NPP-3- or CeNup93/NPP-13-depleted embryos. (B) Embryos expressing GFP-MAN1 from control worms or worms depleted of CeNup205/NPP-3 or CeNup93/NPP-13 were fixed and observed by confocal microscopy. Single confocal sections are shown. Note that the GFP-MAN1 distribution was uniform in the NEs of all embryos.

exclusion in CeNup205(RNAi) and CeNup93(RNAi) embryos expressing GFP- β -tubulin. In control embryos, soluble GFP- β -tubulin was cytoplasmic and excluded from the pronuclei and the nuclei of daughter cells from telophase to prometaphase (Figure 5A, left column, and Movie 2). In contrast, in CeNup205 or CeNup93-depleted embryos, nuclear exclusion of GFP- β -tubulin was not observed after the first mitosis (Figure 5A, middle and right columns, and Movie 2). The lack of exclusion was already apparent before the first mitosis of CeNup205(RNAi) embryos, whereas CeNup93(RNAi) embryos showed normal pronuclear exclusion of GFP- β -tubulin. The lack of nuclear exclusion of GFP- β -tubulin was also clearly visible in older CeNup205(RNAi) or CeNup93(RNAi) embryos (Figure 5B). Young or old embryos codepleted of both CeNup205 and CeNup93 do not show nuclear exclusion of GFP- β -tubulin (Supplementary Figure 3C). To test whether the lack of exclusion visualized with GFP- β -tubulin was a general defect in nuclear exclusion, we examined the behavior of inert molecules coupled

to fluorochromes. A mixture of 10-kDa dextran coupled to a green fluorochrome and 70-kDa dextran coupled to a red fluorochrome was injected into gonads of either control or RNAi-treated hermaphrodites. After 5 h, dextrans were incorporated into newly formed embryos. As expected, in the control embryos, the 70-kDa dextran was excluded from all interphase nuclei, whereas the 10-kDa dextran freely diffused into the nuclear space. In CeNup205(RNAi) embryos, however, the 70-kDa dextran was not excluded from the nuclei (Figure 5C). The lack of exclusion of 70-kDa dextran in CeNup205-depleted embryos confirmed the defect observed in the GFP- β -tubulin strain. The lack of nuclear exclusion could be due to a defect either in the NE or in NPC structure. A defect in NE integrity would be predicted to cause unrestricted access to the nucleus whereas an alteration in NPC structure might be expected to result in a change in permeability up to a new size limit. We therefore examined the distribution of a 160-kDa dextran, injecting a mixture of 160-kDa

Figure 5. Depletion of CeNup205 or CeNup93 increases the size range of macromolecules that can diffuse freely across the NPC. (A) Young embryos expressing GFP- β -tubulin from control worms or worms depleted of CeNup205/NPP-3 or CeNup93/NPP-13 were observed by time-lapse confocal microscopy. Still images are shown with indication of time (minutes:seconds) relative to anaphase onset. Anterior of the embryos is on the left. Soluble GFP- β -tubulin was not excluded from the nuclear space in CeNup205(RNAi) or CeNup93(RNAi) embryos. Pronuclei in CeNup205(RNAi) embryos also failed to exclude GFP- β -tubulin. (B) Soluble GFP- β -tubulin was not excluded from the nuclear space in older CeNup205(RNAi) or CeNup93(RNAi) embryos. (C and D) Gonads of control worms or worms depleted of CeNup205 were injected with a mix of fluorescent dextrans of 10 kDa (left) and 70 kDa (right) (C) or a mix of fluorescent dextrans of 10 kDa (left) and 160 kDa (right) (D). Dextran distribution in embryos was analyzed by confocal microscopy. Single confocal images are shown. The 10-kDa dextran diffused freely into all nuclei, whereas the 70-kDa dextran was only excluded from nuclei of control embryos and not from nuclei of CeNup205(RNAi) embryos (C). The 160-kDa dextran is excluded from the nuclei of both control embryos and CeNup205(RNAi) embryos. Bar, 10 μ m.

red dextran and 10-kDa green dextran into control and CeNup205-depleted embryos. As expected, in the control embryos, the 160-kDa dextran was excluded from all nuclei in contrast to the 10-kDa dextran, which freely dif-

fused into nuclear space (Figure 5D). A similar distribution was seen in CeNup205(RNAi) embryos, arguing that CeNup205 depletion causes a defect in NPC structure rather than in NE integrity.

DISCUSSION

We have annotated 20 predicted *C. elegans* genes as encoding nucleoporins and begun their functional characterization. The number of nucleoporin genes is lower than found in mammals, where 28 genes have been described. It is possible that the nematode genome encodes fewer nucleoporins than higher eukaryotes. However, because yeast also contain ~30 different nucleoporins, we find this unlikely. There are, however, no additional nematode genes that exhibit nucleoporin-like sequence features. Electron microscopy of the NE in *C. elegans* embryos has recently revealed that the NPC in this organism seems to have intermediate dimensions between vertebrate and yeast NPCs (Cohen *et al.*, 2002). We therefore suggest that the lack of additional nucleoporins in the database may be due to one or more of the following reasons. 1) Although the WormPep database has a high fidelity a certain number of genes are mispredicted (Reboul *et al.*, 2001) and perhaps existing genes are not yet included in the list of predicted ORFs. 2) Some *C. elegans* nucleoporins might be so divergent from their mammalian counterparts that they will only be identified by direct functional analysis. 3) Eight of the 20 *C. elegans* nucleoporin genes are predicted to encode two to three different isoforms, which could potentially increase the total number of nematode nucleoporin proteins without requirement for additional nucleoporin genes.

We have demonstrated that depletion of 12 of 20 individual nucleoporins causes almost complete embryonic lethality (CeNup35, -45/58, -54, -62, -93, -98/96, -153, -155, -160, -205, -358, and CeSec13R). This is in agreement with yeast data showing that ~50% of nucleoporin genes are essential for viability (Fabre and Hurt, 1997). Combinatorial RNAi revealed that a further five npp genes are important for early nematode development (CeNup85, -107, -133, -214, and CeRAE1). Only three npp genes did not reveal any phenotype when targeted alone or in combination (CeNup50, CeSeh1, and Cegp210). Based on our results and those of others, we speculate that CeNup50 and CeSeh1 might be dispensable for embryonic development, whereas Cegp210 may have escaped depletion by RNAi. Reverse transcription-PCR analysis of mRNA from CeNup50(RNAi) and CeSeh1(RNAi) embryos show a specific although limited reduction in the amount of targeted mRNA, whereas we did not detect any significant decrease of Cegp210 mRNA in Cegp210(RNAi) embryos (our unpublished data). Other laboratories have reported Cegp210(RNAi) phenotypes ranging from wild-type (Fraser *et al.*, 2000; Piano *et al.*, 2002) to weak embryonic lethality (Maeda *et al.*, 2001; Colaiacovo *et al.*, 2002; Cohen *et al.*, 2003), probably reflecting differences in RNAi efficiency. Cegp210 is the only transmembrane nucleoporin recognizable from its sequence and it might therefore be expected to be important for NPC anchoring, as indeed suggested by recent work from Gruenbaum and colleagues (Cohen *et al.*, 2003).

When analyzed by DIC microscopy the RNAi phenotypes came in two categories. When CeNup54, -45/58, -62, -85, -93, or -205 were targeted by RNAi, the nuclei of early embryos were slightly smaller than wild type but seemed otherwise normal. However, because the majority [$\geq 87\%$ except for CeNup85(RNAi)] of these embryos died before hatching, these particular nucleoporins are essential for development. A more striking phenotype was observed when another set of nucleoporins was depleted. RNAi depletion of CeNup35, -98/96, -153, -155, -160, -358, or CeSec13 seemed to completely block nuclear formation after the first mitosis. This

indicates that certain nucleoporins in addition to their expected roles in nucleocytoplasmic transport are required for nuclear reassembly after mitosis. We have previously analyzed the defects in nuclear envelope formation upon depletion of CeNup358 (Askjaer *et al.*, 2002) and are currently investigating the remaining nucleoporins with this particular phenotype. It should be noted that for six nucleoporin genes DIC phenotypes similar to those presented here have been reported previously (CeNup98/96 and -358, Gönczy *et al.*, 2000; CeNup62, -85, -153, and -160, Zipperlen *et al.*, 2001). Our description of the remaining seven npp genes with identifiable early DIC phenotypes demonstrates that a large number of nucleoporins are required for postmitotic nuclear reassembly and function. In particular, our analysis provides a first demonstration of a nuclear-related function of the novel nucleoporin CeNup35 (Cronshaw *et al.*, 2002). Finally, the observation that combinatorial RNAi against CeNup85, -107, and -133 causes dramatic nuclear morphology defects suggests that these nucleoporins, which are known to physically interact in other organisms, are individually dispensable but are together essential for nuclear assembly similar to the situation with components of the analogous yeast Nup84p complex (Fabre and Hurt, 1997).

The lethality associated with essential nucleoporin genes that are not required for nuclear formation in early embryos could be caused by defects in nucleocytoplasmic transport. Imbalance in concentrations of macromolecules across the nuclear envelope would then in turn presumably have consequences on e.g., chromatin maintenance or gene expression patterns. Our analysis of CeNup205 and CeNup93 has demonstrated that chromosome behavior inside the nucleus is influenced by these nucleoporins. In all embryonic nuclei the chromosomes displayed a strong and abnormal peripheral condensation upon depletion of CeNup205 or CeNup93. In contrast, nuclear protein import was not blocked in these embryos, as visualized by nuclear accumulation of YFP-lamin, indicating that the changes in chromatin morphology were probably not caused by a lack of protein import. These observations extend and provide important support for previous *in vitro* data. Nup93 interacts with Nup188 and Nup205 in *Xenopus* egg extracts (Grandi *et al.*, 1997; Miller *et al.*, 2000). Depletion of Nup93 from such extracts leaves nuclear protein import unaffected while causing a delay in DNA replication (Grandi *et al.*, 1997), which might be related to the perinuclear chromatin condensation defect observed here.

However, we also observed significant differences in the phenotypes caused by depletion of these nucleoporins compared with previous data. Depletion of CeNup205 or CeNup93 led to strong aggregation of NPCs in the NE, as judged by NPC staining by mAb mAb414. The total mAb414 signal was however not noticeably decreased, indicating that the NEs in affected embryos contained roughly the same number of NPCs but in a clustered distribution. In contrast, nuclei assembled *in vitro* in *Xenopus* extracts immunodepleted for Nup93 react much less with mAb414 (Grandi *et al.*, 1997). Due to the stability of NPC subcomplexes, depletion by affinity purification in general leads to codepletion of several components concomitantly, thereby obscuring the assignment of specific functions to individual nucleoporins. We therefore propose that the more dramatic effects seen on the number of NPCs upon immunodepletion of Nup93 compared with our RNAi depletion of CeNup93 may have resulted from codepletion of other factors with Nup93 from the *in vitro* assembly reactions. RNAi is in general considered as an efficient tool to deplete single proteins *in vivo*.

However, depletion of nucleoporins in mammalian tissue culture cells by RNAi has recently revealed an unexpected cross-interference with the accumulation of other nucleoporins, presumably at the posttranslational level (Boehmer *et al.*, 2003; Harel *et al.*, 2003; Walther *et al.*, 2003). To address this, we analyzed the protein concentrations of four different nucleoporins in embryos depleted of either of six essential nucleoporins. In none of these experiments did we observe cross-interference, which leads us to conclude that our observations can most likely be attributed to depletion of individual nucleoporins.

In the absence of CeNup205 or CeNup93 a dramatic failure in the nuclear exclusion of ~70-kDa fluorescent reporter molecules (GFP- β -tubulin and fluorescent dextrans) was observed. However, a larger dextran of ~160 kDa was excluded from the nuclei of both depleted and control embryos, indicating that the nuclei were enclosed by intact NEs. This was further supported by the finding that the inner NE proteins MAN1 and emerlin were present around nuclei of CeNup205(RNAi) and CeNup93(RNAi) or doubly depleted embryos. In combination, these data demonstrated that CeNup205 and CeNup93 are involved in determination of the size exclusion limit of the NPC permeability barrier, possibly by forming part of the central channel in the NPC. In *S. cerevisiae*, Nup188p has previously been implicated in control of nuclear pore resting size (Shulga *et al.*, 2000). Although we were unable to identify a *C. elegans* homolog of Nup188p, it is intriguing that Nup188p, which itself is non-essential, interacts with the two yeast homologs of CeNup205 and CeNup93, Nup192p and Nic96p, respectively.

Conditional mutations in the *S. cerevisiae* genes *NUP192* and *NIC96* cause defects in NPC assembly leading to a lower number of NPCs in the NE (Kosova *et al.*, 1999; Gomez-Ospina *et al.*, 2000). This might seem contradictory to our results. However, neither of the two mutations are null alleles (null mutants of *NUP192* and *NIC96* are nonviable), which means that mutant protein is produced in these strains. A likely possibility, based on the published data and our results, is that the mutant yeast proteins are unable to be integrated into the NE or NPCs but can still interact with at least some of their normal interaction partners, thereby sequestering nucleoporins from the NE. In contrast, in RNAi-depleted embryos the absence of CeNup205 or CeNup93 does not prevent NPC biogenesis but the NPCs formed have an increased resting pore size.

The fact that perinuclear chromatin condensation and lack of proper nuclear exclusion were both already observed in two-cell embryos suggest a connection between the two processes. Chromosome condensation needs to be precisely regulated to allow proper access of transcription and replication factors to the DNA in interphase and S phase and efficient chromosome alignment and segregation during mitosis (Jessberger, 2002). In vertebrates, the five-subunit condensin complex is responsible for chromosome condensation and condensation is at least partially controlled by the nucleocytoplasmic distribution of condensin (Schmiesing *et al.*, 2000). In the light of the observations presented here, it is possible that the increase in permeability seen in CeNup205(RNAi) and CeNup93(RNAi) embryos allows premature entry of condensin-like factors.

In summary, our study demonstrates that the *C. elegans* genome encodes orthologs of many of the nucleoporins found in vertebrates. More than 50% of these proteins are required for embryonic development. Detailed functional analysis of CeNup205 and CeNup93, two components of a conserved NPC subcomplex, demonstrates that they are

important structural components of the NPC that participate in the establishment of the NPC resting pore size. The NPC diffusion limit is controlled during the cell cycle (Feldherr and Akin, 1990) and increases as a very early step in nuclear envelope breakdown in starfish oocytes (Lenart *et al.*, 2003). Interestingly, this latter increase in pore size was correlated with partial NPC disassembly. Further work is required to analyze whether Nup205 and Nup93 have a role in this process.

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