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ELYS/MEL-28 Chromatin Association Coordinates Nuclear Pore Complex Assembly and Replication Licensing

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Summary

Xenopus egg extract supports all the major cell-cycle transitions in vitro. We have used a proteomics approach to identify proteins whose abundance on chromatin changes during the course of an in vitro cell cycle. One of the proteins we identified was ELYS/MEL-28, which has recently been described as the earliest-acting factor known to be required for nuclear pore complex (NPC) assembly [1–4]. ELYS interacts with the Nup107-160 complex and is required for its association with chromatin. ELYS contains an AT-hook domain, which we show binds to chromatin with a high affinity. This domain can compete with full-length ELYS for chromatin association, thereby blocking NPC assembly. This provides evidence that ELYS interacts directly with chromatin and that this interaction is essential for NPC assembly and compartmentalization of chromosomal DNA within the cell. Furthermore, we detected a physical association on chromatin between ELYS and the Mcm2-7 replication-licensing proteins. ELYS chromatin loading, NPC assembly, and nuclear growth were delayed when Mcm2-7 was prevented from loading onto chromatin. Because nuclear assembly is required to shut down licensing prior to entry into S phase, our results suggest a mechanism by which these two early cell-cycle events are coordinated with one another.

Keywords

DNA; CELLCYCLE

Results and Discussion

Identification of ELYS/MEL-28 as a Chromatin-Associated Protein

We have undertaken a proteomic analysis of chromatin passing through interphase of the cell cycle in *Xenopus* egg extract. The abundance of proteins associated with chromatin at different times was assessed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Full details of this analysis will be published elsewhere. The behavior of known proteins was as expected: Mcm2-7 peaked in G1, DNA polymerase δ peaked during S

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phase, and nuclear pore complex (NPC) proteins, such as Nup153, increased as nuclei assembled and grew throughout interphase (Figure 1A).

One protein identified in this screen was ELYS/MEL-28, whose abundance on chromatin resembled that of Nup153 (Figure 1A). We were particularly interested in ELYS because we had also identified it in a screen for factors associating with the replication licensing proteins Mcm2-7 on chromatin (see below). ELYS was originally identified in a screen for genes involved in mouse hematopoiesis [5] but was subsequently found to have an essential function early in development [6]. In *Caenorhabditis elegans*, an ELYS homolog, MEL-28, was identified in a screen for genes that affect nuclear morphology [1, 2]. A fragment of *Xenopus* ELYS was also identified in a screen for proteins that rescue mitotic catastrophe [7].

We prepared an antibody against a synthetic peptide corresponding to an internal sequence of *Xenopus* ELYS/MEL-28. The antibody recognized a protein in egg extract at the predicted molecular weight for the full-length protein that could be resolved as a discrete doublet (Figure S1A in the Supplemental Data available online). We used this antibody to examine the association of ELYS with chromatin at different stages of the cell cycle. Consistent with the results obtained by proteomic analysis, ELYS chromatin association was first detected after maximal Mcm2 binding and increased through interphase (Figure 1B). ELYS was not detectably bound to mitotic chromatin.

ELYS Is Required for Nuclear Pore Complex Assembly

We immunoprecipitated ELYS from egg extract and identified coprecipitating proteins by LC-MS/MS (Figure S2). This showed eight previously characterized members of the Nup107-160 nucleoporin complex [8–10] and another nucleoporin, Nup50, coprecipitating with ELYS. We therefore examined whether ELYS is required for the assembly of the NPC by using the antibody to immunodeplete ELYS from egg extract (Figure S1B). Figure 2A shows that not only was there a strong reduction in Nup133 chromatin binding when ELYS was depleted, but there was also a strong reduction in the binding of the mAb414 nucleoporins, Nup62, Nup153, Nup214, and Nup358 [11–13]. This suggests that ELYS is required for the association of the Nup107-160 complex with chromatin and for NPC formation.

Although membranes were assembled round the nuclei in ELYS-depleted extract, the nuclei were smaller than those in control extract (Figure 2C). In control extract, ELYS colocalized at the nuclear rim with the mAb414 antibody, but in ELYS-depleted extract, this mAb414 staining was absent (Figure 2D). These results suggest that ELYS is localized to the nuclear periphery and is required for NPC formation but not for nuclear envelope assembly. Consistent with this, ELYS depletion abolished DNA replication (Figure 2B), which is known to be dependant upon nuclear assembly and protein import [14]. Because Nup107-160 chromatin association is, to date, the earliest characterized step in NPC formation [9, 10], these results suggest that ELYS is involved very early in NPC assembly.

While our work was in progress, experiments were published describing a role for ELYS/MEL-28, in association with the Nup107-160 complex, in NPC assembly in the *C. elegans* and human systems [1–3]. Further characterization of ELYS by Franz et al. [4] showed that it is recruited onto chromatin in *Xenopus* egg extracts. Because ELYS was shown to be required for Nup107-160 complex chromatin association, Franz hypothesized that ELYS chromatin association might serve as a seeding point for NPC formation on the chromatin surface.

ELYS Chromatin Association Directs Nuclear Pore Complex Assembly

A number of features suggest that ELYS might interact directly with chromatin and direct other nuclear pore components to these sites. First, ELYS binds early to chromatin (Figure 1) and is required for other nucleoporins to associate with chromatin (Figure 2). Second, we also found ELYS associating on chromatin with Mcm2-7, suggesting a close association with chromatin (see below). Finally, ELYS contains a conserved AT-hook DNA-binding domain (residues 2329–2337); AT hooks in other proteins have been shown to mediate their direct interaction with DNA or chromatin [15, 16].

To further characterize ELYS chromatin association, we investigated whether it could associate with chromatin in egg extract lacking membranes. Whereas both ELYS and Nup133 were recovered on chromatin isolated from membrane-free extract, the association of the mAb414 antigens was severely compromised (Figure 3A). Therefore, in contrast to the mAb414 nucleoporins, both ELYS and the Nup107-160 complex can associate with chromatin independently of nuclear-membrane formation. This suggests that ELYS chromatin association mediates Nup107-160 complex chromatin association and directs NPC formation.

To investigate the potential role of the AT-hook domain in mediating ELYS chromatin association, we produced a recombinant protein (rATH) corresponding to the C-terminal 208 aa of ELYS containing the AT-hook domain (Figure S3). rATH could bind efficiently, and with a high affinity, to sperm chromatin in the absence of other factors (Figure 3B), consistent with the idea that the AT-hook domain allows ELYS to interact directly with chromatin. When the recombinant rATH protein was titrated into egg extract, rATH also bound to chromatin despite the presence of competing chromatin proteins (Figure 3C). The addition of rATH to egg extract inhibited not only ELYS chromatin association but also that of Nup133 and the mAb414 antigens, but it left Mcm2 chromatin association unaffected. Thus, the addition of a recombinant protein corresponding to the AT-hook-containing region of ELYS to egg extract competes with ELYS for chromatin binding, thereby restricting the chromatin loading of ELYS-dependant nucleoporins.

The addition of rATH to egg extract inhibited DNA replication at concentrations similar to those that inhibited nucleoporin assembly (Figure 3D). Nuclei assembled in rATH-treated extract were surrounded by membrane but were considerably smaller than those formed in the untreated extract (Figure 3E) and showed no discernable nuclear rim staining with mAb414 (Figure 3F). They therefore closely resembled nuclei assembled in ELYS-depleted extract (Figure 2C).

These experiments provide evidence that ELYS can associate directly with chromatin without the need for other NPC components and that this interaction is at least in part mediated by its AT-hook domain. Taken together with our previous results and the recent results of other groups [1–4], this suggests that the chromatin association of ELYS is the initiating event that directs nuclear pore assembly at that site and, in this way, explains the means by which chromosomes are compartmentalized within the cell.

ELYS Interacts with the Replication Licensing System

Mcm2–7 are loaded onto chromatin in late mitosis and early G1 to license origins for use in the subsequent S phase [17]. The loading of Mcm2-7 onto *Xenopus* sperm requires four other proteins: nucleoplasmin, ORC, Cdc6, and Cdt1 [18]. In addition to its role in licensing DNA replication, Mcm2-7 loading is also required for the association of cohesin with chromatin [19, 20]. Thus, it is likely that Mcm2-7 coordinates the assembly of other chromatin-associated proteins that play important roles in the cell-division cycle. As noted above, we also identified ELYS in a screen for chromatin proteins associating with Mcm2-7.

Chromatin was recovered from extract in mid S phase, digested with nuclease, and immunoprecipitated with either Mcm3 or control antibody. Precipitated proteins were identified by LC-MS/MS. Figure 4A shows that ELYS and the replication fork protein Sld5 were well recovered in the anti-Mcm3 immunoprecipitation. In contrast, Orc2, a component of ORC, was not well recovered upon precipitation, suggesting that this technique enriches proteins associated with Mcm2-7. Precipitation with Mcm2 antibody gave similar results (unpublished data).

We were unable to detect an interaction between ELYS and the licensing proteins ORC, Cdc6, Cdt1, or Mcm2-7 in solution, either by reciprocal immunoprecipitation or by analysis of an anti-ELYS immunoprecipitate by LC-MS/MS (unpublished data). This suggests that the ELYS Mcm2-7 interaction occurs only on chromatin. When rATH was added to the extract, it was not efficiently recovered on Mcm3 immunoprecipitated chromatin (Figure 4B), suggesting that the AT-hook domain of ELYS does not mediate its interaction with Mcm3.

The association between ELYS and the Mcm2-7 complex suggests a functional interaction between replication licensing and NPC assembly. To investigate this, we treated egg extract with geminin, an inhibitor of Cdt1 that prevents the loading of Mcm2-7 onto chromatin [21–23]. As expected, treatment of egg extract with geminin abolished Mcm2 and SMC1 chromatin association (Figure 4C). Most interestingly, geminin significantly delayed, although did not abolish, the chromatin association of ELYS, Nup133, and the mAb414 nucleoporins. Nuclei prepared in a geminin-treated egg extract also grew at lower rate than did those prepared in a control extract (Figure 4D). Thus, in the absence of Mcm2-7 chromatin association, the affinity of ELYS for chromatin is reduced, which in turn slows NPC assembly and nuclear growth.

This unexpected link between NPC assembly and replication licensing could play an important role in ensuring that events occur in the correct sequence during the early cleavage divisions. It is essential that the replication licensing system is inactivated before entry into S phase, so that Mcm2-7 are not reloaded onto DNA that has already been replicated, which would allow the DNA to rereplicate. Geminin plays a central role in the downregulation of replication licensing in metazoans [17]. In early cleavage embryos, geminin protein levels remain approximately constant throughout the cell cycle, but its ability to bind and inhibit Cdt1 fluctuates [24–27]. On exit from metaphase, geminin activity is inhibited, which allows Mcm2-7 to be loaded onto DNA [26]. Once nuclei are assembled, geminin is imported into nuclei, leading to its reactivation [24, 27, 28]. Because the presence of Mcm2-7 on chromatin promotes ELYS and nuclear pore association, it also promotes the activation of geminin (Figure 4E). This creates a feedback loop that promotes geminin activation once origin licensing has taken place, thereby ensuring rapid but error-free progression through the early embryonic cell-division cycle.

Supplemental Data

Refer to Web version on PubMed Central for supplementary material.

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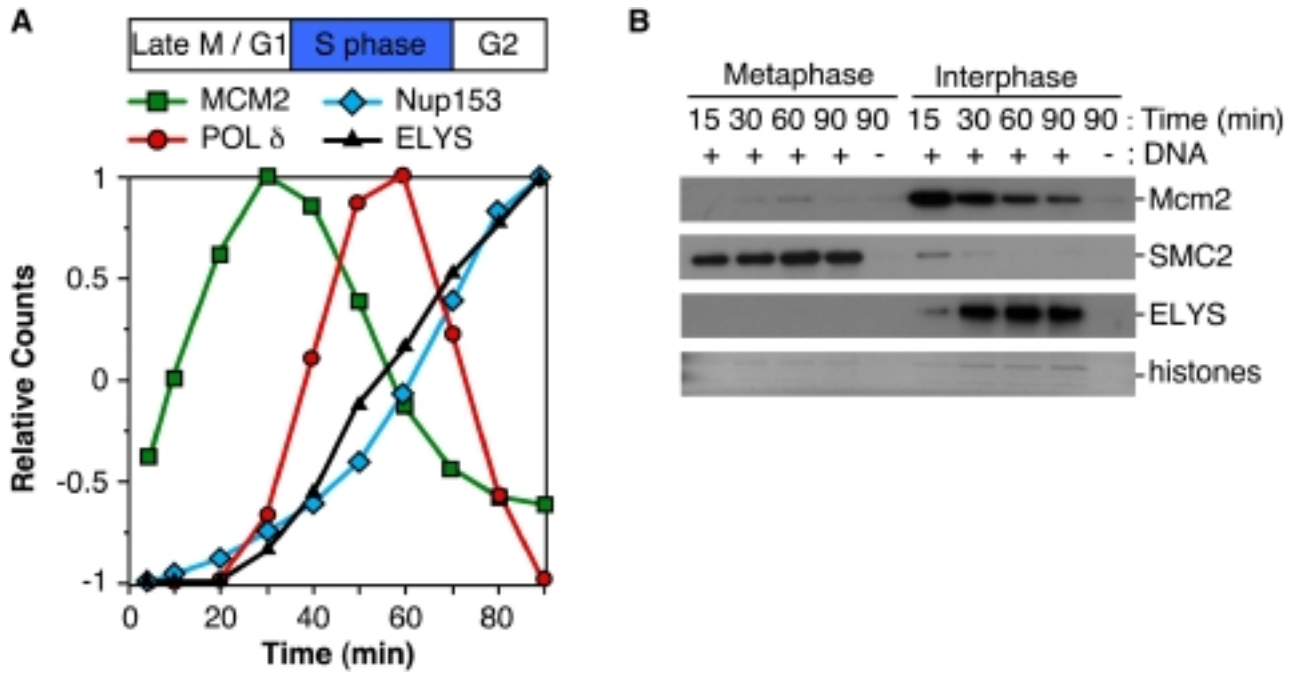
Acknowledgments

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References

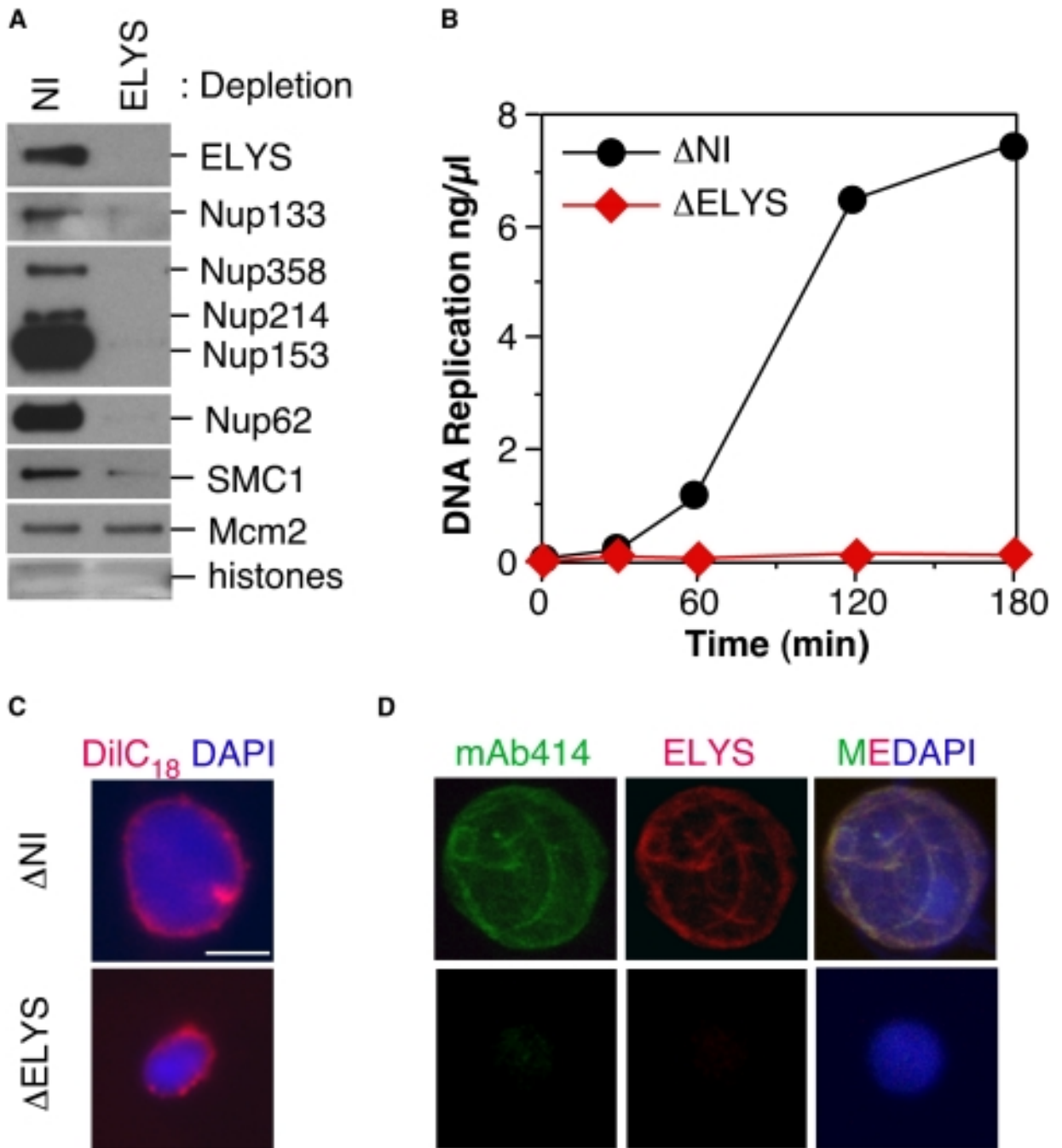
1. Fernandez A.G. Piano F. MEL-28 is downstream of the Ran cycle and is required for nuclear-envelope function and chromatin maintenance. *Curr. Biol.* 2006;16:1757–1763. [PubMed: 16950115]
2. Galy V. Askjaer P. Franz C. Lopez-Iglesias C. Mattaj I.W. MEL-28, a novel nuclear-envelope and kinetochore protein essential for zygotic nuclear-envelope assembly in *C. elegans*. *Curr. Biol.* 2006;16:1748–1756. [PubMed: 16950114]
3. Rasala B.A. Orjalo A.V. Shen Z. Briggs S. Forbes D.J. ELYS is a dual nucleoporin/kinetochore protein required for nuclear pore assembly and proper cell division. *Proc. Natl. Acad. Sci. USA* 2006;103:17801–17806. [PubMed: 17098863]
4. Franz C. Walczak R. Yavuz S. Santarella R. Gentzel M. Askjaer P. Galy V. Hetzer M. Mattaj I.W. Antonin W. MEL-28/ELYS is required for the recruitment of nucleoporins to chromatin and postmitotic nuclear pore complex assembly. *EMBO Rep.* 2007;8:165–172. [PubMed: 17235358]
5. Kimura N. Takizawa M. Okita K. Natori O. Igarashi K. Ueno M. Nakashima K. Nobuhisa I. Taga T. Identification of a novel transcription factor, ELYS, expressed predominantly in mouse foetal haematopoietic tissues. *Genes Cells* 2002;7:435–446. [PubMed: 11952839]
6. Okita K. Kiyonari H. Nobuhisa I. Kimura N. Aizawa S. Taga T. Targeted disruption of the mouse ELYS gene results in embryonic death at peri-implantation development. *Genes Cells* 2004;9:1083–1091. [PubMed: 15507119]
7. Su J.Y. Maller J.L. Identification of a *Xenopus* cDNA that prevents mitotic catastrophe in the fission yeast *Schizosaccharomyces pombe*. *Gene* 1994;145:155–156. [PubMed: 8045419]
8. Vasu S. Shah S. Orjalo A. Park M. Fischer W.H. Forbes D.J. Novel vertebrate nucleoporins Nup133 and Nup160 play a role in mRNA export. *J. Cell Biol.* 2001;155:339–354. [PubMed: 11684705]
9. Harel A. Orjalo A.V. Vincent T. Lachish-Zalait A. Vasu S. Shah S. Zimmerman E. Elbaum M. Forbes D.J. Removal of a single pore subcomplex results in vertebrate nuclei devoid of nuclear pores. *Mol. Cell* 2003;11:853–864. [PubMed: 12718872]
10. Walther T.C. Alves A. Pickersgill H. Loiodice I. Hetzer M. Galy V. Hulsmann B.B. Kocher T. Wilm M. Allen T. The conserved Nup107–160 complex is critical for nuclear pore complex assembly. *Cell* 2003;113:195–206. [PubMed: 12705868]
11. Davis L.I. Blobel G. Nuclear pore complex contains a family of glycoproteins that includes p62: Glycosylation through a previously unidentified cellular pathway. *Proc. Natl. Acad. Sci. USA* 1987;84:7552–7556. [PubMed: 3313397]
12. Meier E. Miller B.R. Forbes D.J. Nuclear pore complex assembly studied with a biochemical assay for annulate lamellae formation. *J. Cell Biol.* 1995;129:1459–1472. [PubMed: 7790348]
13. Walther T.C. Pickersgill H.S. Cordes V.C. Goldberg M.W. Allen T.D. Mattaj I.W. Fornerod M. The cytoplasmic filaments of the nuclear pore complex are dispensable for selective nuclear protein import. *J. Cell Biol.* 2002;158:63–77. [PubMed: 12105182]
14. Blow J.J. Control of chromosomal DNA replication in the early *Xenopus* embryo. *EMBO J.* 2001;20:3293–3297. [PubMed: 11432816]
15. Reeves R. Nissen M.S. The A.T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure. *J. Biol. Chem.* 1990;265:8573–8582. [PubMed: 1692833]
16. Metcalf C.E. Wassarman D.A. DNA binding properties of TAF1 isoforms with two AT-hooks. *J. Biol. Chem.* 2006;281:30015–30023. [PubMed: 16893881]
17. Blow J.J. Dutta A. Preventing re-replication of chromosomal DNA. *Nat. Rev. Mol. Cell Biol.* 2005;6:476–486. [PubMed: 15928711]

18. Gillespie P.J. Li A. Blow J.J. Reconstitution of licensed replication origins on *Xenopus* sperm nuclei using purified proteins. *BMC Biochem.* 2001;2:15. [PubMed: 11737877]
19. Gillespie P.J. Hirano T. Scc2 couples replication licensing to sister chromatid cohesion in *Xenopus* egg extracts. *Curr. Biol.* 2004;14:1598–1603. [PubMed: 15341749]
20. Takahashi T.S. Yiu P. Chou M.F. Gygi S. Walter J.C. Recruitment of *Xenopus* Scc2 and cohesin to chromatin requires the pre-replication complex. *Nat. Cell Biol.* 2004;6:991–996. [PubMed: 15448702]
21. McGarry T.J. Kirschner M.W. Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell* 1998;93:1043–1053. [PubMed: 9635433]
22. Tada S. Li A. Maiorano D. Mechali M. Blow J.J. Repression of origin assembly in metaphase depends on inhibition of RLF-B/Cdt1 by geminin. *Nat. Cell Biol.* 2001;3:107–113. [PubMed: 11175741]
23. Wohlschlegel J.A. Dwyer B.T. Dhar S.K. Cvetcic C. Walter J.C. Dutta A. Inhibition of eukaryotic DNA replication by geminin binding to Cdt1. *Science* 2000;290:2309–2312. [PubMed: 11125146]
24. Hodgson B. Li A. Tada S. Blow J.J. Geminin becomes activated as an inhibitor of Cdt1/RLF-B following nuclear import. *Curr. Biol.* 2002;12:678–683. [PubMed: 11967157]
25. Maiorano D. Rul W. Mechali M. Cell cycle regulation of the licensing activity of Cdt1 in *Xenopus laevis*. *Exp. Cell Res.* 2004;295:138–149. [PubMed: 15051497]
26. Li A. Blow J.J. Non-proteolytic inactivation of geminin requires CDK-dependent ubiquitination. *Nat. Cell Biol.* 2004;6:260–267. [PubMed: 14767479]
27. Li A. Blow J.J. Cdt1 downregulation by proteolysis and geminin inhibition prevents DNA re-replication in *Xenopus*. *EMBO J.* 2005;24:395–404. [PubMed: 15616577]
28. Yoshida K. Takisawa H. Kubota Y. Intrinsic nuclear import activity of geminin is essential to prevent re-initiation of DNA replication in *Xenopus* eggs. *Genes Cells* 2005;10:63–73. [PubMed: 15670214]

**Figure 1.****ELYS/MEL-28 Is a Chromatin-Associated Protein**

(A) Chromatin was isolated from egg extract at discrete time points throughout interphase and subjected to LC-MS/MS. The relative abundance of ELYS/MEL-28, polymerase δ , Mcm2, and Nup153 are indicated. The duration of S phase in this extract is indicated above the graph.

(B) Sperm chromatin was incubated in either a metaphase or interphase egg extract. At the indicated times, chromatin was isolated and immunoblotted for Mcm2, SMC2, and ELYS. As a control, extract was incubated without sperm chromatin for 90 min. The lower portion of the gel was stained with Coomassie blue so that the level of histones could be determined as a control for chromatin recovery.

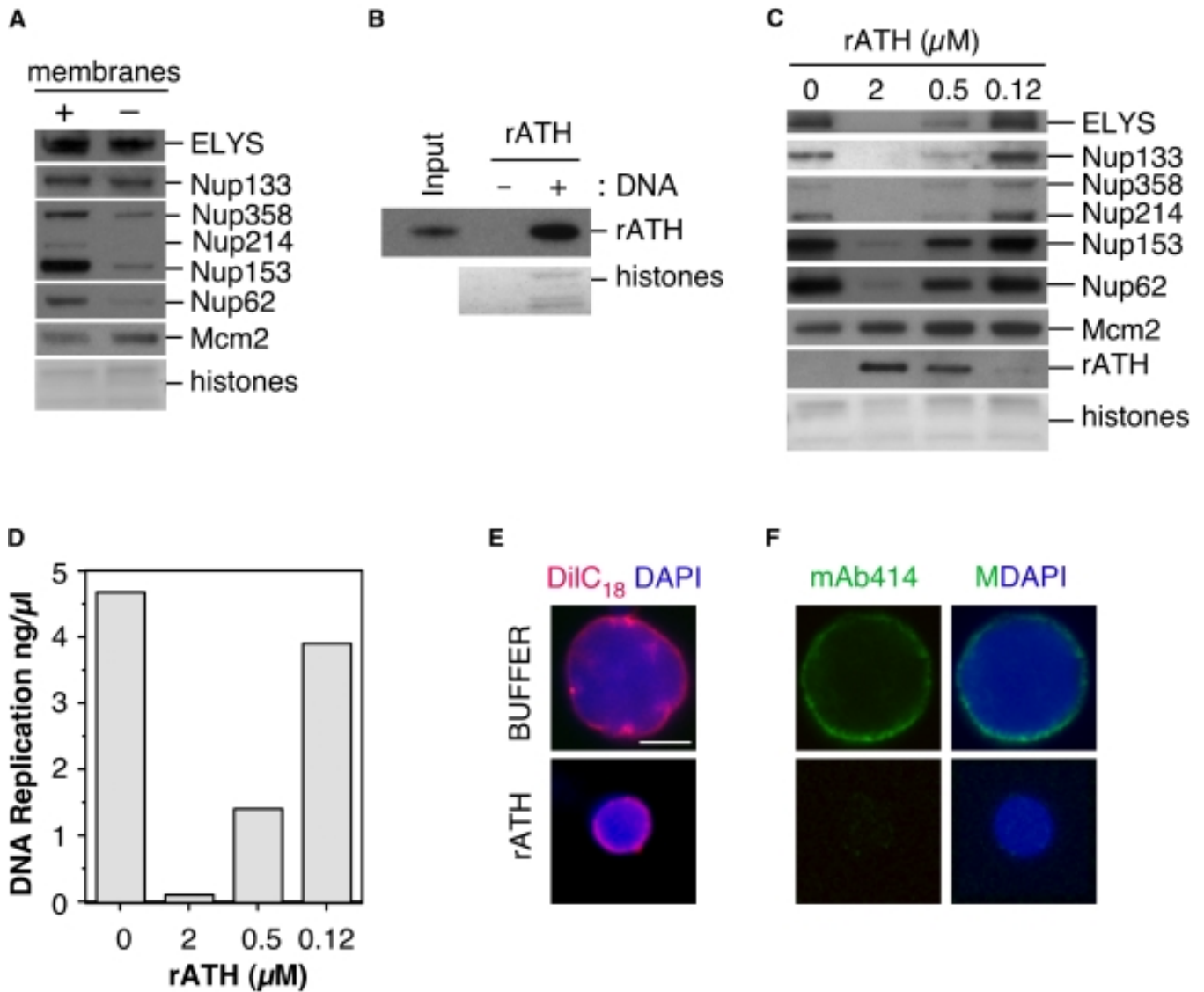
**Figure 2.****ELYS Is Required for Nuclear Pore Complex Assembly**

(A) Sperm chromatin was incubated in nonimmune or ELYS-depleted egg extract for 90 min. Chromatin was then isolated and immunoblotted for ELYS, Nups133, 358, 214, 153, 62, and Mcm2. The lower portion of the gel was stained with Coomassie blue so that the level of histones could be determined as a control for chromatin recovery.

(B) DNA synthesis was assayed in nonimmune and ELYS-depleted extracts.

(C) Sperm chromatin was incubated in nonimmune or ELYS-depleted egg extract treated with 10 μg/ml DilC₁₈ (red). Nuclei were fixed and spun onto coverslips and counterstained with DAPI (blue). The scale bar represents 10 μm.

(D) Nuclei assembled in nonimmune or ELYS-depleted egg extract were fixed, spun onto coverslips, and stained with mAb414 (green) and ELYS antibodies (red) and DAPI (blue).

**Figure 3.****ELYS Chromatin Association Directs Nuclear Pore Complex Assembly**

(A) Sperm chromatin was incubated in complete or membrane-free egg extract for 60 min. Chromatin was isolated and immunoblotted for ELYS, Nups133, 358, 214, 153, 62, and Mcm2. The lower portion of the gel was stained with Coomassie blue so that the level of histones could be determined as a control for chromatin recovery.

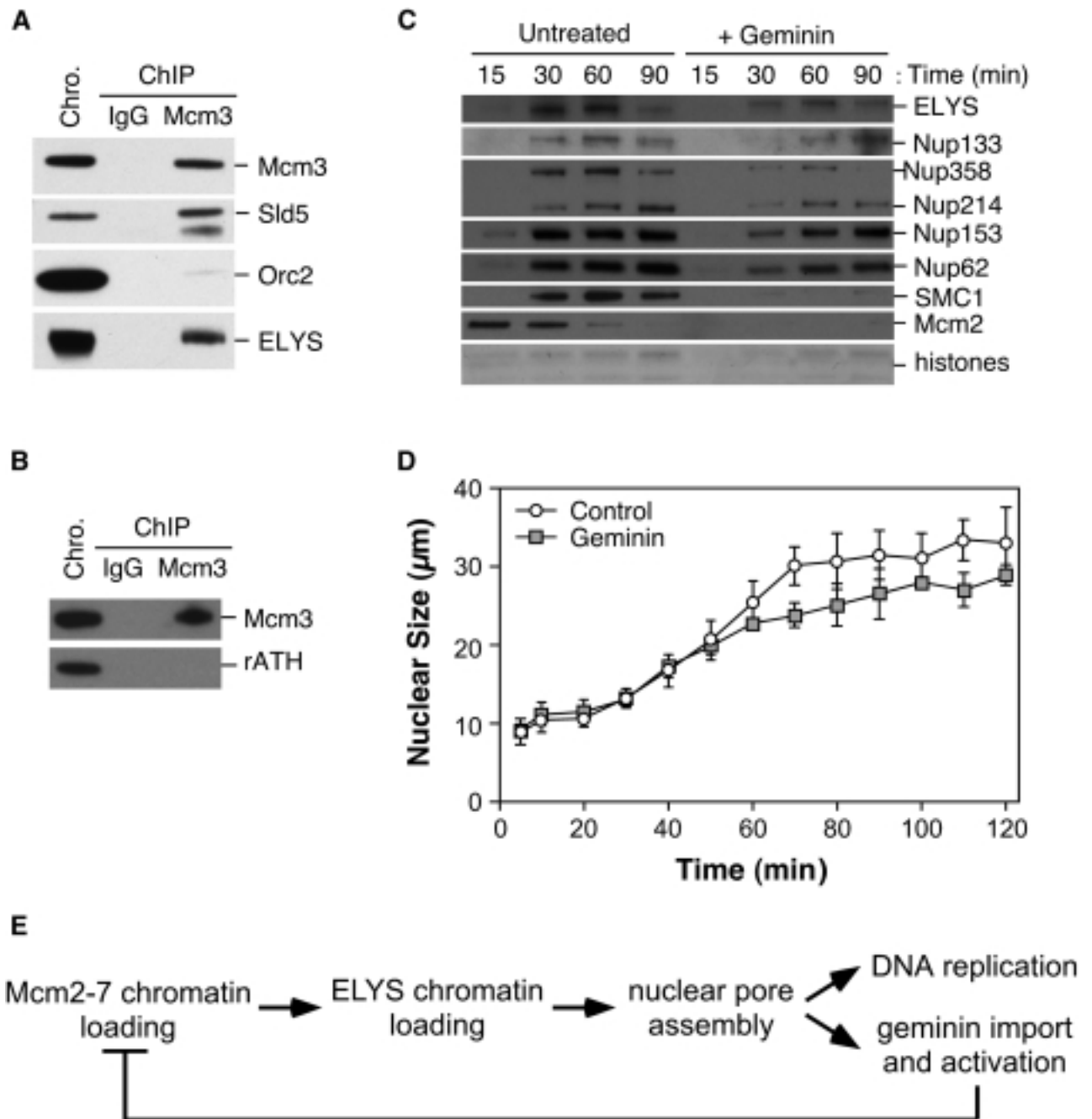
(B) rATH was incubated in the presence or absence of sperm chromatin for 30 min. Chromatin was isolated and immunoblotted for rATH. The lower portion of the gel was stained with Coomassie blue so that the level of histones could be determined as a control for chromatin recovery. Twenty-five percent of input rATH is shown as control for protein recovery.

(C) Sperm chromatin was incubated for 90 min in egg extract treated with either buffer alone or rATH at the indicated concentrations. Chromatin was isolated and immunoblotted for rATH, ELYS, Nups133, 358, 214, 153, 62, and Mcm2. The lower portion of the gel was stained with Coomassie blue so that the level of histones could be determined as a control for chromatin recovery.

(D) DNA synthesis was assayed in rATH-treated extracts.

(E) Sperm chromatin was incubated in extract treated with 10 $\mu\text{g/ml}$ DilC₁₈ (red) and either buffer or 2 μM rATH. Nuclei were fixed and spun onto coverslips and counterstained with DAPI (blue). The scale bar represents 10 μm .

(F) Nuclei assembled in egg extract treated with buffer alone or 10 μM rATH were fixed, spun onto coverslips and stained with mAb414 (green) and DAPI (blue).

**Figure 4.****ELYS Interacts with the Replication Licensing System**

(A) Sperm chromatin was incubated in egg extract for 45 min. Chromatin was then isolated by centrifugation through a sucrose cushion, digested with nuclease, and immunoprecipitated with either nonimmune IgG or Mcm3 antibodies. Precipitated proteins were immunoblotted with antibodies to Mcm3, Sld5, Orc2, or ELYS. An equal sample of chromatin is shown as control for protein recovery.

(B) Chromatin isolated from rATH-treated egg extract was digested with nuclease and immunoprecipitated with either nonimmune IgG or Mcm3 antibodies as in (A). Precipitated proteins were identified with antibodies to Mcm3 and rATH.

(C) Sperm chromatin was incubated in egg extract treated with buffer alone or 50 nM geminin^{DEL}. At the indicated times, chromatin was isolated and immunoblotted for ELYS, Nups133, 358, 214, 153, 62, SMC1, and Mcm2.

(D) Sperm chromatin was incubated in egg extract treated with buffer alone or geminin^{DEL}. At the indicated times, the size of randomly selected nuclei were measured. Values are shown \pm the standard deviation.

(E) Model for the interaction of ELYS with the replication licensing system.