Lipin is required for efficient breakdown of the nuclear envelope in *Caenorhabditis elegans*

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Summary

The nuclear envelope (NE) is a double lipid bilayer that separates nucleus and cytoplasm. In metazoa, NE breakdown (NEBD) occurs during prophase and NE reformation around segregated chromatids occurs at anaphase-telophase. We identified *Caenorhabditis elegans* Lipin homologue (called Lpin-1) as an essential factor with roles in endoplasmic reticulum (ER) organization and NEBD. RNAi-mediated downregulation of Lpin-1 had no effect on timely entry into mitosis or on the early steps of NEBD, but Lpin-1 was required for disassembly of the nuclear lamina during late NEBD. This Lpin-1 requirement appears to be separable from the effect of Lpin-1 on the peripheral ER.

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Key words: Nuclear-envelope breakdown, Lipin, Lamin, Diacylglycerol

Introduction

The endoplasmic reticulum (ER) is differentiated into at least three major functional sub-domains; smooth ER, rough ER and nuclear envelope (NE). The NE is composed of two lipid bilayers, the inner (INM) and outer (ONM) nuclear membrane, nuclear pore complexes (NPCs) and the nuclear lamina, a meshwork of intermediate Lamin filaments underlying the INM (Gorjanacz et al., 2007a; Hetzer et al., 2005). During open mitosis in metazoa the NE breaks down to allow spindle microtubules to segregate replicated chromatids. At anaphase-telophase, new NEs form out of the ER to re-establish the nuclear compartment. Little is known about the molecular mechanisms underlying these two essential mitotic events.

Studies in *Drosophila melanogaster* embryos and starfish oocytes revealed that NEBD proceeds in two sequential steps (Kiseleva et al., 2001; Lenart et al., 2003; Terasaki et al., 2001). First, NPCs partially disassemble. Holes generated by the loss of NPCs then expand and result in large fenestrations of the NE. Concomitantly, the nuclear lamina disassembles and integral NE proteins, together with the NE membranes, are absorbed into the peripheral ER (Mattaj, 2004).

A set of kinases including Cdk-1, PKC or Aurora A are activated in prophase and are believed to contribute to NE breakdown (NEBD) by phosphorylating proteins of the INM, NPCs and nuclear lamina, thus abolishing the protein-protein interactions that support NE integrity (Burke and Ellenberg, 2002; Hachet et al., 2007; Portier et al., 2007).

In contrast to metazoa, yeast, filamentous fungi and some protists undergo closed mitosis. Expansion of the NE membranes is necessary for closed mitosis. Smp2, the yeast homologue of mammalian Lipin, is a key regulator of this NE growth (Santos-Rosa et al., 2005). Loss of Smp2 induces proliferation of NPC-containing NE membranes and nuclear expansion.

Lipins are conserved phosphatidic acid (PA) phosphatases that catalyze the dephosphorylation of PA to yield diacylglycerol (DAG), an important intermediate in lipid metabolism and a key element in lipid-mediated signaling (Carman and Han, 2006; Carrasco and Merida, 2007). Mutations in genes encoding Lipin proteins have been associated with several human diseases, such as insulin resistance, lipodystrophy, type-2 diabetes or Majeed syndrome (Reue and Brindley, 2008); however, until now the cellular consequences of the inactivation of *lipin* expression were not known.

Although the mechanisms of open and closed mitosis are different, NE expansion is a common feature. We therefore examined whether *C. elegans* Lpin-1 acts in metazoan NE dynamics. Although Lpin-1 had no detectable effect on NE assembly and expansion, it was crucially required for NEBD and Lamin de-polymerization during mitosis.

Results and Discussion

Inactivation of *C. elegans* Lpin-1 promotes bi-nucleation

C. elegans encodes a single *lpin-1* gene (H37A05.1). All lipin protein family members share a highly conserved N-terminal domain of unknown function and a C-terminal HAD-like domain containing the catalytic motif. The N-terminus of *C. elegans* Lpin-1 (residues 1-106) shows 50% identity to Smp2, whereas the C-terminus (residues 504-738) is 45% identical. The level of identity and similarity to the three human *lipin* genes is even higher, suggesting that Lpin-1 exerts an evolutionarily conserved role.

RNAi downregulation of *C. elegans lpin-1* for 48 hours or longer resulted in 100% (n=589) embryonic lethality on N2 worms. Early embryos often had lagging chromosomes and their chromosomes were always distributed into two or more nuclei per cell (data not shown). The severe effects made it impossible to find young *lpin-1* embryos to compare with control embryos. Importantly, it was impossible to exclude secondary effects in such embryos. Reducing the time of the RNAi treatment to 20 hours also resulted in high (63±24%; n=770) embryonic lethality, but left a reasonable number of analyzable young embryos. All reported *lpin-1* RNAi experiments were therefore performed for 20 hours. In these conditions 80% (n=30) of two- and four-cell-stage *lpin-1(RNAi)* embryos contained

bi-nucleated cells, in contrast to 2% (n=90) in control embryos (Fig. 1A).

To analyze bi-nucleation in *lpin-1(RNAi)* embryos we monitored chromosomes during the first zygotic division using GFP-fused hisH2B (Fig. 1B). In control zygotes the oocyte and sperm pronuclei met, attached and migrated into the center of the embryo, where they underwent the first zygotic division. During this division the maternal and paternal chromosomes mixed. However, in *lpin-1(RNAi)* embryos the maternal and paternal chromosomes remained separate on the metaphase plate and during cell division (Fig. 1B, arrowhead). This led to formation of two nuclei per daughter cell.

To demonstrate that this phenotype was caused by specific downregulation of *lpin-1*, we generated four anti-Lpin-1 antibodies. Anti-Lpin-1 (4FRP) antibody was generated against the highly conserved N-terminal domain (residues 25-161), whereas the other three anti-Lpin-1 (4FMI, 4FLZ and 4FFA) antibodies were raised against a less-conserved central region of Lpin-1 (residues 162-415). All four anti-Lpin-1 antibodies detected a protein at the expected size in N2 embryonic extract (supplementary material Fig. S1A) that was reduced in *lpin-1(RNAi)* embryos to ~25% of the wild-type amount (Fig. 1C,D). To determine the subcellular localization of Lpin-1, *control(RNAi)* and *lpin-1(RNAi)* embryos expressing GFP-fused signal peptidase SP-12 (GFP–SP-12) were immunostained with all four anti-Lpin-1 antibodies. Anti-Lpin-1 (4FRP and 4FFA) antibodies showed that the soluble Lpin-1 was localized weakly throughout the cytoplasm and nucleoplasm and was enriched in the ER, where it colocalized with GFP–SP-12 (Fig. 1E; supplementary material Fig. S1B,E). The anti-Lpin-1 (4FMI and 4FLZ) antibodies showed similar staining but, in addition, showed an enrichment of signal in the NE compared with the ER (Fig. 1F; supplementary material Fig. S1D,C). Two of our antibodies thus exhibited equal staining in ER and NE and two exhibited NE enrichment. The reductions of the all these Lpin-1 signals in *lpin-1(RNAi)* embryos demonstrate the specificity of the subcellular localizations (Fig. 1E,F; supplementary material Fig. S1B-E). We presume that Lpin-1 has different epitopes whose accessibility is different in the NE versus ER. Because the less-accessible epitope could either be in the NE or the ER, we cannot conclude whether Lpin-1 is more concentrated in the NE or the ER, but can be confident that the protein is present in both membrane compartments.

Lpin-1 affects dynamics of the peripheral ER and NE during mitosis

Localization of *C. elegans* Lpin-1 to the ER and NE membranes is consistent with the mammalian Lipin paralogs (Cascales et al., 1984; Grimsey et al., 2008; Harris et al., 2007), and inactivation of yeast ortholog Smp2 affected both ER and NE morphology (Santos-Rosa et al., 2005). We therefore monitored the dynamics of the peripheral ER network using GFP–SP-12. In controls, the ER network

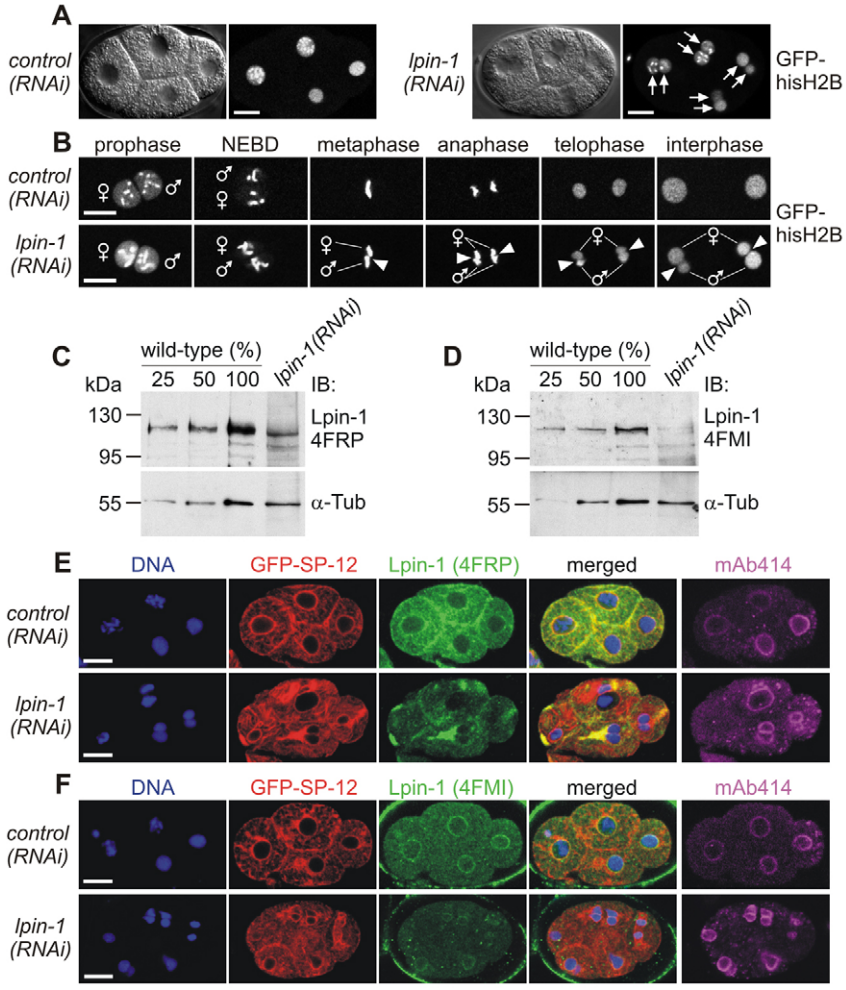

Fig. 1. Subcellular localization of Lpin-1 and its effect on parental-chromosome mixing. (A) *control(RNAi)* (left) and *lpin-1(RNAi)* (right) embryos at the four-cell stage expressing GFP-hisH2B, observed by differential interference contrast (DIC) and fluorescent microscopy. In this and all figures, embryos are oriented with anterior to the left. Arrows point to twinned nuclei. (B) Confocal still images of the first zygotic division of *control(RNAi)* (top) and *lpin-1(RNAi)* (bottom) embryos expressing GFP-hisH2B. Maternal and paternal chromosomes stay separated during mitosis in *lpin-1(RNAi)* embryos (arrowheads). (C,D) Western blotting was used to determine the *lpin-1(RNAi)* efficiency and to demonstrate the specificity of (C) anti-Lpin-1 (4FRP) and (D) anti-Lpin-1 (4FMI) antibodies. α-tubulin was a loading control. (E,F) GFP–SP-12 (red)-expressing *control(RNAi)* and *lpin-1(RNAi)* embryos were immunostained with (E) anti-Lpin-1 (4FRP) (green) or (F) anti-Lpin-1 (4FMI) antibodies and with mAb414 and Hoechst to visualize NPCs (magenta) and chromatin (blue), respectively. Scale bars: 10 μm.

undergoes dramatic cell-cycle-regulated morphological transitions (Poteryaev et al., 2005). Interphase ER mostly consists of short tubules that are interconnected by three-way junctions and small patches. However, during mitosis, starting slightly before NEBD, the peripheral ER forms a reticular network with long, radially orientated thick tubules and sheets (Fig. 2A,B). Downregulation of Lpin-1 dramatically altered ER morphology. Although thick tubules still formed during mitosis, ER membranes accumulated in mitotic clusters and patches, which were considerably larger than in control embryos (Fig. 2A,B; supplementary material Movie 1). During interphase, the difference in ER organization was even more pronounced. We observed fewer short interconnected ER tubules; instead, there were large membrane sheets, patches and mitotic ER clusters, which failed to disperse and persisted into the following division (Fig. 2A,B). Thus, we conclude that Lpin-1 is required for the organization of the peripheral ER.

Perturbation of the cyclic transitions of the peripheral ER interferes with NE disassembly (Audhya et al., 2007) and reformation (Anderson and Hetzer, 2008), demonstrating that ER morphology and NE organization are tightly linked. To determine whether inhibition of Lpin-1 might also alter NE dynamics, we used embryos coexpressing both a GFP-fused INM protein (GFP–LEM-2) and RFP-fused hisH2B. In control zygotes, the oocyte and sperm pronuclear envelopes attached and, during pro-metaphase, the NE membranes dissociated from chromosomes (Fig. 2C). This allowed mixing of maternal and paternal chromosomes. Subsequently a single NE assembled around them. In *lpin-1(RNAi)* embryos the removal of pronuclear-envelope membranes from the vicinity of the chromosomes was inhibited (Fig. 2C, arrow). This physical barrier prevented the mixture of oocyte- and sperm-derived chromosomes, resulting in the assembly of two new NEs and bi-nucleate daughter cells. NE-membrane clearance around the chromosomes was perturbed in subsequent divisions in *lpin-1(RNAi)* embryos, demonstrating that this phenotype is not specific to the first zygotic division. We conclude that Lpin-1 is required for proper NE disassembly but is dispensable for NE assembly.

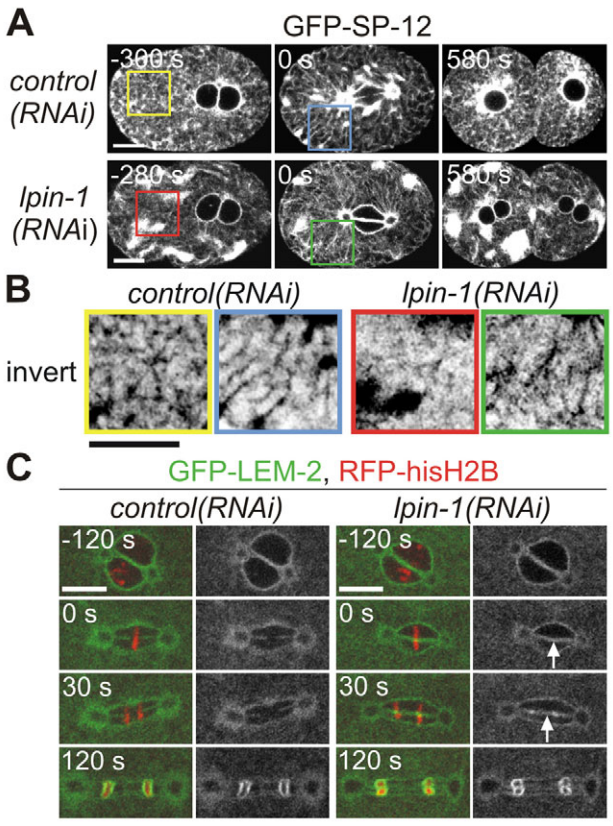

Fig. 2. Lpin-1 depletion perturbs ER morphology and NEBD. (A) Images from recordings of *control(RNAi)* (top) and *lpin-1(RNAi)* (bottom) embryos expressing GFP–SP-12. Time in all figures is indicated in seconds relative to anaphase onset. (B) Color-inverted and higher magnification (3×) views of the two different ER morphological states in *control(RNAi)* (left) and *lpin-1(RNAi)* (right) embryos. (C) Images from recordings of *control(RNAi)* (left) and *lpin-1(RNAi)* (right) embryos coexpressing GFP–LEM-2 and RFP-hisH2B. Arrows point to persistent stacked NE. Scale bars: 10 µm.

Lpin-1 promotes NEBD via lamina de-polymerization

NE stability during interphase requires the nuclear lamina, which is disassembled late in *C. elegans* NEBD (Gorjanacz et al., 2007a). We examined whether the lamina is properly disassembled in Lpin-1-downregulated embryos. We performed control and *lpin-1* RNAi experiments on embryos expressing GFP-fused BAF-1 protein, a conserved soluble component of the nuclear lamina. In control embryos GFP-BAF-1 was localized to the INM (Fig. 3A), where it has a role in stabilizing the NE by simultaneously binding to chromatin and to a set of integral INM proteins, until NEBD. During NEBD, BAF-1 is phosphorylated and released from the NE and chromatin (Fig. 3A) (Gorjanacz et al., 2007b). GFP–BAF-1 dynamics was not altered by depletion of Lpin-1, suggesting that disassembly of the network of Lamin-interacting proteins is properly initiated in *lpin-1(RNAi)* embryos (Fig. 3A).

The major components of the nuclear lamina are the Lamin (called LMN-1) filaments (Gruenbaum et al., 2005). We performed control and *lpin-1* RNAi experiments on embryos coexpressing YFP-fused LMN-1 and RFP-hisH2B. In control embryos, YFP–LMN-1 filaments were completely de-polymerized just before metaphase (Fig. 3B, left). In Lpin-1-downregulated embryos, disassembly of LMN-1 filaments was always delayed. In the majority of embryos (8/10 recordings) we found that the LMN-1 polymers were either never disassembled, or only disassembled during anaphase or telophase (Fig. 3B, right). In 2/10 cases we observed only a slight defect, with LMN-1 filaments disassembling during anaphase onset (Fig. 3B, middle). In these latter cases we did not observe bi-nucleate daughter cells. LMN-1 immunostaining revealed that endogenous LMN-1 filaments also remained polymerized throughout mitosis upon Lpin-1 downregulation (Fig. 3C). The relative amount of LMN-1 was not changed after Lpin-1 depletion as analyzed by western blotting (see below) or immunostaining (Fig. 3C). We conclude that Lpin-1 regulates the timely disassembly of LMN-1 filaments.

Integral INM proteins become mobile during NEBD and are absorbed by the ER network, where they remain until NE re-formation (Ellenberg et al., 1997). Since LMN-1 can bind to several integral INM proteins (e.g. Emerin, LEM-2), it is conceivable that persisting LMN-1 filaments in *lpin-1(RNAi)* embryos might prevent their release into the ER during mitosis. To analyze this we used GFP-Emerin. In *control(RNAi)* embryos, GFP-Emerin was dispersed into the ER during mitosis (Fig. 3D). When Lpin-1 was downregulated the GFP-Emerin signal significantly increased in NE membranes during mitosis, but not during interphase (Fig. 3D). This was even more obvious when the endogenous Emerin protein was visualized with specific antibody (Fig. 3E; supplementary material Fig. S2). These results suggest that failure to de-polymerize LMN-1 filaments during mitosis results in NE stabilization and prevention of NEBD.

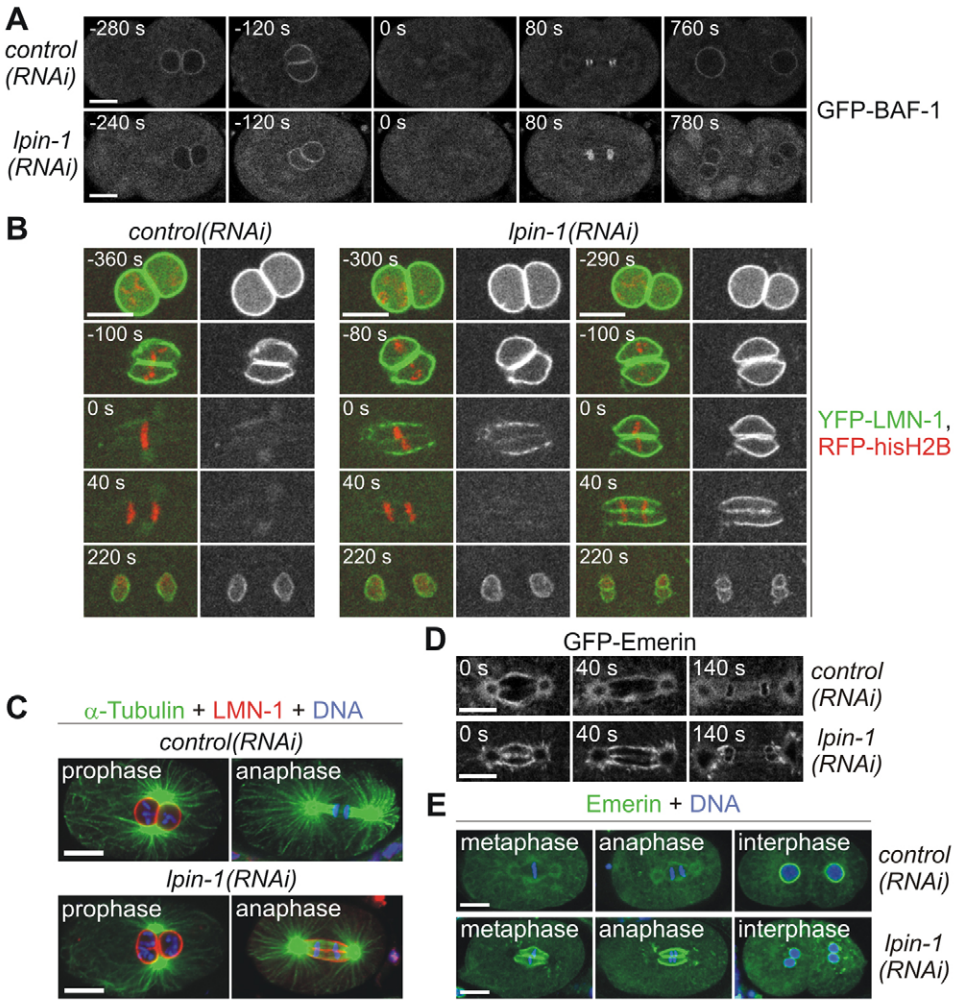

Fig. 3. Lpin-1 depletion affects lamina disassembly. (A,B) Images from recordings of *control(RNAi)* and *lpin-1(RNAi)* embryos expressing (A) GFP–BAF-1 or (B) YFP–LMN-1 and RFP-hisH2B. (C) Fixed *control(RNAi)* (top) and *lpin-1(RNAi)* (bottom) embryos were immunostained to visualize α-tubulin (green), LMN-1 (red) and DNA (blue). (D) Images from recordings of *control(RNAi)* and *lpin-1(RNAi)* embryos expressing GFP-Emerin. (E) Fixed *control(RNAi)* (top) and *lpin-1(RNAi)* (bottom) embryos were immunostained to visualize DNA (blue) and Emerin (green). Scale bars: 10 μm.

We next investigated the mechanism of Lpin-1 action. Recently, centrosomes have been implicated in promoting timely NEBD (Hachet et al., 2007; Portier et al., 2007). Microtubule-based processes that assist in removal of the NE membrane from chromosomes during NEBD have also been described (Beaudouin et al., 2002; Muhlhausser and Kutay, 2007). We examined whether Lpin-1 might affect these processes. Visualizing microtubules in fixed *control(RNAi)* and *lpin-1(RNAi)* embryos revealed that Lpin-1 did not substantially alter either the integrity of centrosomes or their attachment to the NE (Fig. 3C). *lpin-1(RNAi)* embryos also assembled normal astral and spindle microtubules (Fig. 3C).

NEBD is thought to be initiated by NPC disassembly (Lenart et al., 2003; Terasaki et al., 2001). This was examined by analyzing embryos expressing YFP-fused Nup107. In control embryos, NPCs disassembled during prophase and, in *lpin-1(RNAi)* embryos, YFP-Nup107 showed similar dynamics (Fig. 4A, –160 seconds). Furthermore, GFP-Nup155 and mAb414 antigens were disassembled normally (our unpublished results) (Fig. 4B).

gp210, a transmembrane nucleoporin, plays a role in lamina disassembly and NEBD (Audhya et al., 2007; Galy et al., 2008). Downregulation of Lpin-1 had no effect on gp210 localization to the NE; however, during mitosis gp210 was substantially enriched in the NE and, similarly to Emerin, persisted in these membranes (Fig. 4B, anaphase). Similarly, downregulation of gp210 had no effect on Lpin-1 localization and no synthetic lethality was observed between *gp210* and *lpin-1* (data not shown). Cumulatively, the above set of experiments demonstrates that Lpin-1 acts in lamina disassembly and NEBD independently of centrosomes, NPCs and gp210.

The role of Lpin-1 in NEBD is separable from its effect on the peripheral ER

Because Lpin-1 depletion alters both organization of peripheral ER and NE disassembly, it was conceivable that the effect on the NE was a consequence of the improperly structured peripheral ER. Recently, it was shown that both the GTPase RAB-5 and the ER proteins YOP-1 and RET-1 in combination indirectly contribute to NEBD through their function in structuring the peripheral ER (Anderson and Hetzer, 2008; Audhya et al., 2007). It was shown that depletion of RAB-5 and YOP-1 plus RET-1 inhibits the formation of thick tubules, sheets and mitotic ER clusters (Audhya et al., 2007), and thus their effect on the shaping of peripheral ER is different to that of Lpin-1 depletion. Furthermore, expression of the dominant negative RAB-5^{Q78L} mutant perturbed the peripheral ER similarly to *lpin-1* but without affecting NEBD (Audhya et al., 2007), whereas depletion of gp210 was shown to prevent NEBD without affecting the peripheral ER (Audhya et al., 2007; Galy et al., 2008). We therefore speculated

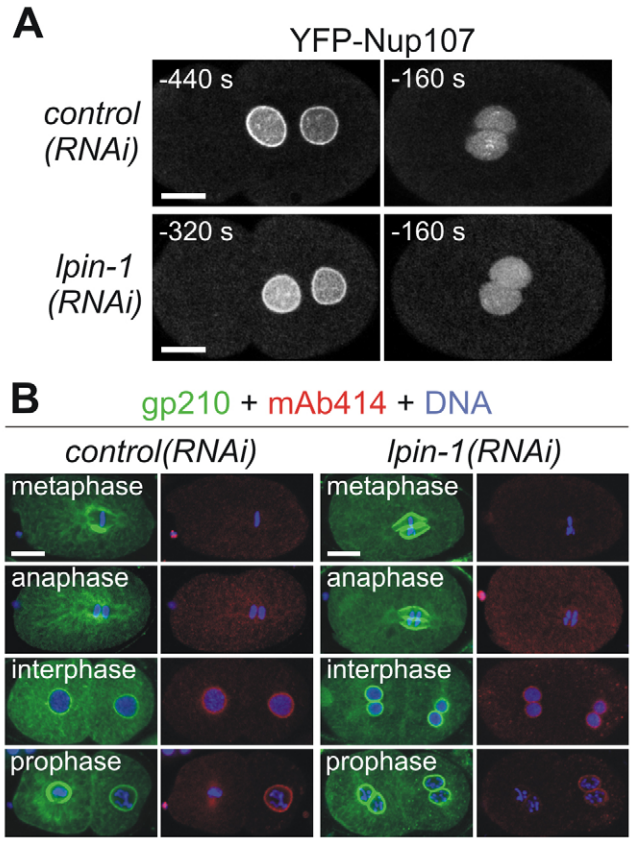

Fig. 4. Lpin-1 acts independently of NPCs and gp210. (A) Images from recordings of *control(RNAi)* (top) and *lpin-1(RNAi)* (bottom) embryos expressing YFP-Nup107 show the loss of NPCs during prophase in both. (B) *Control(RNAi)* (left) and *lpin-1(RNAi)* (right) embryos from the first zygotic division to the second prophase were immunostained with gp210 antiserum, mAb414 and Hoechst 33258 to visualize gp210 (green), NPCs (red) and chromatin (blue), respectively. Note that, in two-cell-stage embryos (bottom), the anterior AB cell enters earlier into mitosis then the posterior P1 cell. Scale bars: 10 µm.

that Lpin-1 might exert a role in NEBD that is independent of the peripheral ER. To investigate whether the effect of Lpin-1 on NEBD is direct or indirect we examined whether co-depletion of LMN-1 with Lpin-1 might rescue the NEBD defect. Worms expressing GFP–LEM-2 were treated with control RNAi bacteria, or with mixtures of bacteria expressing *lpin-1* and control, *lpin-1* and *lem-2* or *lpin-1* and *lmn-1* RNAi bacteria. Embryos were collected, immunostained with antibodies specific for Emerin and nucleoporins, and the percentage of nuclear twinning in two- and four-cell-stage embryos was measured (Fig. 5A). We found 2% (2/90) nuclear twinning in *control(RNAi)* embryos, 79% (41/52) in *lpin-1(RNAi)*, 81% (56/69) in *lpin-1(RNAi)* + *control(RNAi)*, 80% (60/75) in *lpin-1(RNAi)* + *lem-2(RNAi)* and 30% (13/43) in *lpin-1(RNAi)* + *lmn-1(RNAi)* embryos. The efficiency of the above RNAi experiments was determined by western blotting (Fig. 5B). These results clearly demonstrate that co-depletion of Lpin-1 and LMN-1 significantly rescued the effect of Lpin-1 depletion on NEBD.

We next performed the same combinatorial RNAi experiments on worms coexpressing GFP–LEM-2 and RFP-hisH2B. In contrast to the nuclear twinning observed in *lpin-1(RNAi)* + *control(RNAi)* embryos (in 6/7 recordings), we found few bi-nucleate cells when Lpin-1 was co-downregulated with LMN-1 in embryos (in 1/5 recordings) (Fig. 5C). As expected, co-downregulation of LMN-1 resulted in misshapen nuclei and aggregated LEM-2 signals (Fig. 5C, arrowheads). However, it had no obvious effect on the appearance of *lpin-1*-induced mitotic ER clusters (data not shown). In addition, lagging membranes located between the two daughter nuclei were still observed (Fig. 5C, arrow). In the remaining 30% of the embryos in which *lmn-1(RNAi)* did not overcome the *lpin-*

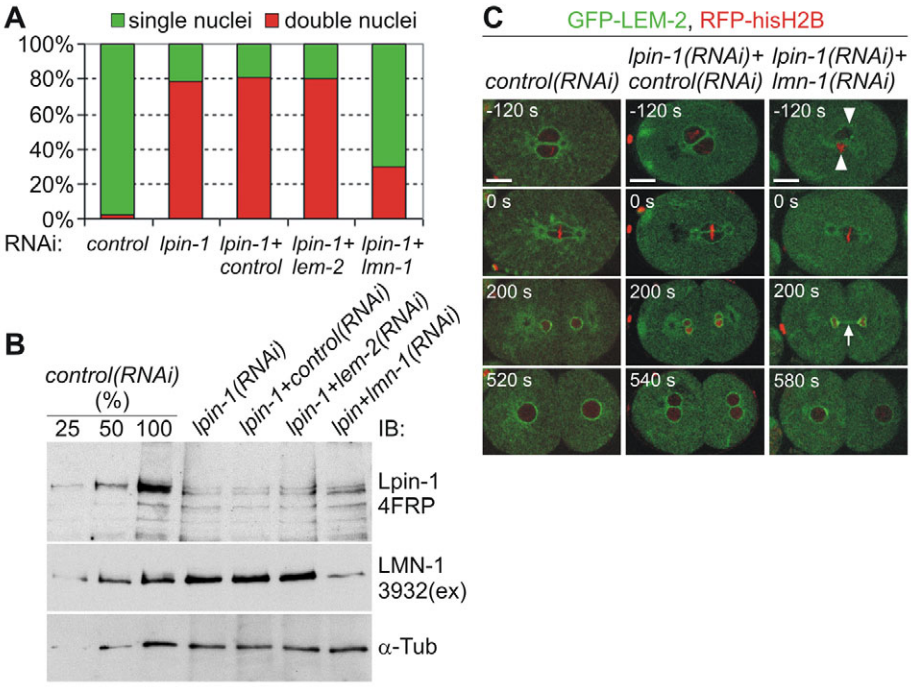

Fig. 5. Lpin-1 has a peripheral ER-independent role in NEBD. (A) The ratio of single- and bi-nucleation was determined in *control(RNAi)*, *lpin-1(RNAi)*, *lpin-1(RNAi)* + *control(RNAi)*, *lpin-1(RNAi)* + *lem-2(RNAi)* and *lpin-1(RNAi)* + *lmn-1(RNAi)* embryos. (B) Western blotting demonstrates the efficiency of Lpin-1 (top) and LMN-1 (middle) downregulation. α-tubulin (bottom) was a loading control. (C) Images from recordings of *control(RNAi)* (left), *lpin-1(RNAi)* + *control(RNAi)* (middle) and *lpin-1(RNAi)* + *lmn-1(RNAi)* (right) embryos coexpressing YFP–LMN-1 and RFP-hisH2B.Arrowheads point to misshapen pro-nuclei, arrow to lagging NE membranes. Scale bars: 10 µm.

1-induced NEBD defect, LMN-1 might not have been efficiently depleted. These results demonstrate that Lpin-1 has a specific role in NEBD through regulating the timely de-polymerization of LMN-1 filaments during mitosis and that, at least in part, this role of Lpin-1 is separable from its effect on organization of peripheral ER.

Smp2, the yeast homolog of Lipin, acts as a transcriptional repressor of key lipid biosynthesis genes during interphase, but its phosphorylation by Cdk1 in mitosis leads to transcriptional de-repression and a pulse of membrane synthesis (O'Hara et al., 2006; Santos-Rosa et al., 2005). Surprisingly, our experiments clearly demonstrate that Lpin-1 depletion had no detectable effect on NE-membrane growth and expansion during *C. elegans* mitosis.

The other molecular mechanism through which Lpin-1 might affect biogenesis of the NE membrane lies in its known enzymatic properties. Lpin-1 de-phosphorylates PA, yielding DAG and inorganic phosphate (Carman and Han, 2006; Carrasco and Merida, 2007). DAG is used for the production of the membrane phospholipids phosphatidylcholine and phosphatidylethanolamine, and for the storage lipid triacylglycerol. Consequently, mutations in mammalian *lipin* genes prevent normal development of adipose tissue and result in lipodystrophy, whereas Lipin overexpression promotes obesity (Peterfy et al., 2001; Phan and Reue, 2005). Similarly, *C. elegans* treated with *lpin-1* RNAi for 1 week were thinner (data not shown), suggesting that they have less stored fat (Golden et al., 2009). However, it is unclear how a reduction in membrane phospholipids or storage lipids could exert an effect on NEBD.

Whether Lpin-1 affects NEBD directly and independently of the peripheral ER or whether it is just a consequence of the perturbed peripheral ER is not clear. However, the remarkable differences in the organization of the peripheral ER of different genetic backgrounds, which show similar NEBD phenotypes, suggests that the dynamics of these membrane sub-domains might be separately controlled. One possible explanation could be that Lpin-1 exerts its effect on NEBD by virtue of producing DAG, a well known second messenger. Lipins, when dephosphorylated, are active and mainly localized to membranes (Grimsey et al., 2008; Harris et al., 2007; Kim et al., 2007; O'Hara et al., 2006; Santos-Rosa et al., 2005). Nuclear DAG level rises to a peak at G2-M phase transition (Banfic et al., 1993; Deacon et al., 2002; Sun et al., 1997). Concomitantly, members of classical and novel PKC families bind to DAG, leading to their activation and translocation to the membranes and nucleus (Deacon et al., 2002; Neri et al., 1998; Sun et al., 1997). These PKC isoenzymes were shown to phosphorylate LMN-1 filaments in different experimental systems, resulting in their de-polymerization (Collas, 1999; Goss et al., 1994; Muhlhausser and Kutay, 2007; Sun et al., 1997). Additionally, DAG is the lipid with the greatest negative curvature effect and might thus promote additional membrane instability during NEBD. The function of Lpin-1 reported here is probably unique to metazoa, which produce Lamin filaments to stabilize the NE; these filaments must be depolymerized during NEBD.

Materials and Methods

Nematode strains and RNAi

All worm strains were cultured using standard methods at 20°C. The following strains were used: *GFP::hisH2B* [AZ212 (Praitis et al., 2001)], *GFP::SP12* (Poteryaev et al., 2005), *GFP::emerin* [XA3504 (Askjaer et al., 2002)], *YFP::Nup107* [XA3506 (Franz et al., 2005)], *GFP::BAF-1* [XA3556 (Gorjanacz et al., 2007b)], *GFP::LEM-2; RFP::hisH2B* [OD83 (Audhya et al., 2007)] and *YFP::LMN-1; RFP::hisH2B* [OD139 (Portier et al., 2007)]. RNAi experiments and embryo lethality tests were performed by feeding L4 worms with bacteria expressing dsRNA for 20-48 hours as described (Gorjanacz et al., 2007b). Control RNAi experiments used empty pL4440 vector; other RNAi experiments used *lpin-1* ORF nucleotides 33-1161, *gp210* ORF nucleotides 2701-3601, *lem-2* ORF nucleotides 1-1503, *lmn-1* ORF nucleotides 1-765 and *emr-1* full-length ORF cloned into pL4440. In combinatorial RNAi, worms were first treated with control, *lem-2* or *lmn-1* RNAi bacteria for 29 hours followed by an additional 21-24 hours of co-RNAi treatment with 1:1 mixtures of the above bacteria with *lpin-1* RNAi bacteria.

Antibody production

Anti-Lpin-1 4FRP antibodies were raised in rabbits against residues 25-161 and anti-Lpin-1 4FMI, 4FLZ and 4FFA against residues 162-415. Antibodies against Emerin (2MBA) were raised in rabbits against residues 141-381. All antibodies were affinity purified with the soluble antigens.

Immunofluorescence

Embryos were fixed, immunostained and recorded as described (Gorjanacz et al., 2007b). Primary antibodies were diluted as follow: anti-Lpin-1 (4FRP, 4FMI, 4FLZ, 4FFA) antiserum 1:200; anti-emerin antiserum 1:300; anti-gp210 antiserum 1:200 (Galy et al., 2008); anti-LMN-1 antiserum 1:500 (Liu et al., 2000); monoclonal antibody (mAb) 414 against nucleoporins 1:500 (Babco, Cambridge, UK) and anti-α-tubulin mAb 1:400 (Sigma-Aldrich, St Louis, MO). We used Alexa Fluor 488, 546 and 633 secondary antibodies diluted 1:800 (Molecular Probes, Eugene, OR). For DNA staining, Hoechst 33258 (Sigma, Deisenhofen, Germany) was used at 1 μg/ml.

Western blot

Embryos for western blot analysis were obtained by hypochlorite treatment. As during such treatment the youngest embryos are frequently lost, 24-hour-long RNAi treatment was used for *lpin-1*. Immunoblotting was performed using standard methods. The following primary antibodies were used: anti-Lpin-1 (4FRP, 4FMI, 4FLZ, 4FFA) antiserum 1:300; anti-LMN-1 antiserum 1:500 (Liu et al., 2000) and anti-α-tubulin mAb 1:800 (Sigma-Aldrich, St Louis, MO).

Live embryo imaging

Embryos were analyzed by dual DIC and fluorescence microscopy with Leica confocal microscope AOBS SP2 as described (Gorjanacz et al., 2007b).

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