ANChors away: an actin based mechanism of nuclear positioning

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Summary

Mechanisms for nuclear migration and nuclear anchorage function together to control nuclear positioning. Both tubulin and actin networks play important roles in nuclear positioning. The actin cytoskeleton has been shown to position nuclei in a variety of systems from yeast to plants and animals. It can either act as a stable skeleton to anchor nuclei or supply the active force to move nuclei. Two *C. elegans* genes and their homologues play important roles in these processes. Syne/ANC-1 anchors nuclei by directly tethering the nuclear envelope to the actin cytoskeleton, and UNC-84/SUN functions at the nuclear envelope to recruit Syne/ANC-1.

Key words: Nuclear migration, Nuclear anchorage, UNC-84, SUN domain, ANC-1, Muscular dystrophy, Nuclear envelope

Introduction

When envisioning a typical eukaryotic cell, one usually imagines the nucleus occupying a central location from where it communicates with the rest of the cell. However, the nucleus and other organelles are in fact quite dynamic. They often migrate through the cytoplasm and then occupy specific locales, often far from the center of the cell. For example, in a newly fertilized zygote, pronuclei must first migrate towards one another, and then they migrate to a species-specific location before undergoing the first mitosis. Nuclear positioning is also essential to a variety of polarized cells, such as intestinal brush-border cells and many secreting endocrine cells. In other cases, nuclear migration events reposition nuclei to distant regions of the cell. Examples include growing plant pollen tube cells (Hepler et al., 2001) and developing *C. elegans* hypodermal cells (Sulston et al., 1983). Disruption of nuclear migration in the cell bodies of the developing cerebral cortex lead to the human neurodevelopmental disease lissencephaly (Lambert de Rouvroit and Goffinet, 2001). Even in single celled organisms, such as budding and fission yeast, tight controls exist to position the nucleus correctly prior to cell division (Morris, 2000; Tran et al., 2001).

A wide variety of organisms have syncytia, which are formed either when multiple nuclear divisions occur without cell divisions or as a result of cell fusion. Normally, syncytial nuclei are located in specific regions or evenly spaced through the cytoplasm. For instance, a vertebrate myotube has hundreds of nuclei; most are evenly spaced apart; however, a group of 4-8 cluster at the neuromuscular junction (Couteaux, 1973). A second striking example is in the *Drosophila* blastoderm, where over 6000 nuclei, derived from 14 rounds of nuclear division without cytokinesis, are carefully positioned at the periphery of a single cell. A disruption in the precise spacing of these nuclei at the cortex has catastrophic results, including tripolar spindles and disruptions of patterning (Foe and Alberts, 1983).

Two related processes are required to control the specific positioning of nuclei. First, nuclei must migrate through the cytoplasm to the appropriate locales within a cell, and then they must be anchored at that position so they do not drift. These processes are controlled by a combination of forces from microtubule and actin-based networks. In most studied cases, microtubules and associated dynein and kinesin motors play a central role in the positioning of nuclei. For example, the female pro-nucleus migrates as a result of dynein on the nuclear envelope pulling the nucleus towards the male centrosome. Since the role of microtubules in nuclear migration and positioning has been recently reviewed (e.g. Bloom, 2001; Suelmann and Fischer, 2000; Morris, 2000; Reinsch and Gonczy, 1998), here we concentrate on the important contributions of actin-based networks for positioning of nuclei. Specifically we focus on a newly identified family of proteins, Syne/ANC-1.

Examples of actin-based nuclear-positioning events

Nuclei must be carefully positioned throughout oogenesis and embryogenesis of *Drosophila*. During the cytoplasmic dumping stage of oogenesis, when the 15 nurse cells rapidly squeeze their cytoplasm into the oocyte through narrow ring canals, nuclei must remain anchored away from the ring canals. Normally, an array of striated actin bundles extends from the plasma membrane to nurse cell nuclei. These bundles shorten as dumping progresses and the nurse cells shrink (Guild et al., 1997). Mutations in the actin-monomer-binding protein profilin, or in the actin-filament-bundling proteins villin and fascin, disrupt these filaments, generating free-floating nuclei (Robinson and Cooley, 1997). These mutations block oocyte development because, as cytoplasmic dumping from the nurse cells begins, nuclei become physically stuck in ring canals. A second example is the early embryonic nuclear migrations towards the periphery of the

syncytial blastoderm. The actin gel-like network around migrating nuclei depolymerizes. This has been hypothesized to contribute to the force required for migration as nuclei passively 'surf' the depolymerizing front (von Dassow and Schubiger, 1994).

Actin networks can also function actively to reposition nuclei. Chytilova et al. (Chytilova et al., 2000) recently described a dramatic example. Actin depolymerizing drugs completely abolished rapid, long-distance intracellular nuclear migration in *Arabidopsis* root hairs, whereas drugs that disrupted microtubules had no effect (Chytilova et al., 2000). Because of the speed and distance of the nuclear migrations in these cells, the actin network must be functioning actively to move nuclei, in contrast to the above examples of passive mechanisms. Budding yeast provides another example: both actin filaments and microtubules are required for proper localization of the nucleus and spindle at the bud neck to ensure normal cell division (Palmer et al., 1992; Bloom, 2001).

C. elegans genes required for nuclear positioning

C. elegans is an excellent model system for the studies of the mechanisms of nuclear positioning. The invariant cell lineages are easily observed, and nuclear migrations and positions have been thoroughly documented (Sulston et al., 1983). In addition, four large syncytial hypodermal cells, containing over 100 nuclei, cover most of the adult body. Before fusing, dramatic nuclear migrations take place in many of these cells (Sulston et al., 1983).

As in other systems, both actin filaments and microtubules probably work together to postion organelles in *C. elegans*. There is a large body of evidence that microtubules play important roles in nuclear positioning in the early embryo (reviewed by Reinsch and Gonczy, 1998). In addition, a disruption in the actin cytoskelton by a weak loss-of-function mutation in the *C. elegans* homologue of cofilin, *unc-60(r398)* (Ono et al., 1999), leads to a defect in positioning of mitochondria (Starr and Han, 2002). Although it has not been directly tested with pharmaceutical agents in *C. elegans*, actin is also probably required for positioning of nuclei in a variety of cell types.

Two genes, *anc-1* and *unc-84*, are required in *C. elegans* for proper nuclear anchorage in syncytial cells. Normally, nuclei are evenly spaced throughout the hypodermal syncytia. They move slightly as the underlying muscles of the worm move but return to their original position when the worm relaxes. Mutations in *anc-1* or *unc-84* lead to an Anc (nuclear anchorage defective) phenotype, in which the nuclei float freely within the cytoplasm and multiple nuclei group together (Hedgecock and Thomson, 1982; Malone et al., 1999). Mitochondria also fail to localize properly in *anc-1*, but not *unc-84*, mutant cells (Hedgecock and Thomson, 1982; Starr and Han, 2002). Both *unc-84* and a third gene, *unc-83*, are required for many nuclear migration events in *C. elegans*. Null *unc-83* or *unc-84* mutations lead to an Unc (uncoordinated) phenotype because failed nuclear migrations lead to the death of P-cells, which normally give rise to ventral neurons (Horvitz and Sulston, 1980). *unc-83* encodes a novel protein; its transcripts are temporally and spatially controlled. UNC-83 first appears during development at the nuclear envelope of migrating nuclei (Starr et al., 2001).

The UNC-84/SUN proteins at the nuclear envelope

unc-84 encodes a novel protein that localizes to the nuclear envelope in all *C. elegans* somatic cells after the 24 cell stage (Lee et al., 2002; Malone et al., 1999). The C-terminal SUN (for Sad1p, UNC-84 homology) domain and the predicted transmembrane region of UNC-84 are conserved in *S. pombe* Sad1p, *Drosophila* predicted protein CG18584 and two human proteins, SUN-1 and SUN-2 [see Fig. 1 (Hagan and Yanmagida, 1995; Malone et al., 1999); D.A.S., unpublished]. Tagged versions of both human SUN-1 and SUN-2 localize in tissue culture cells to what appears to be the inner nuclear envelope (Dreger et al., 2001; Hoffenberg et al., 2000), although this hypothesis has not

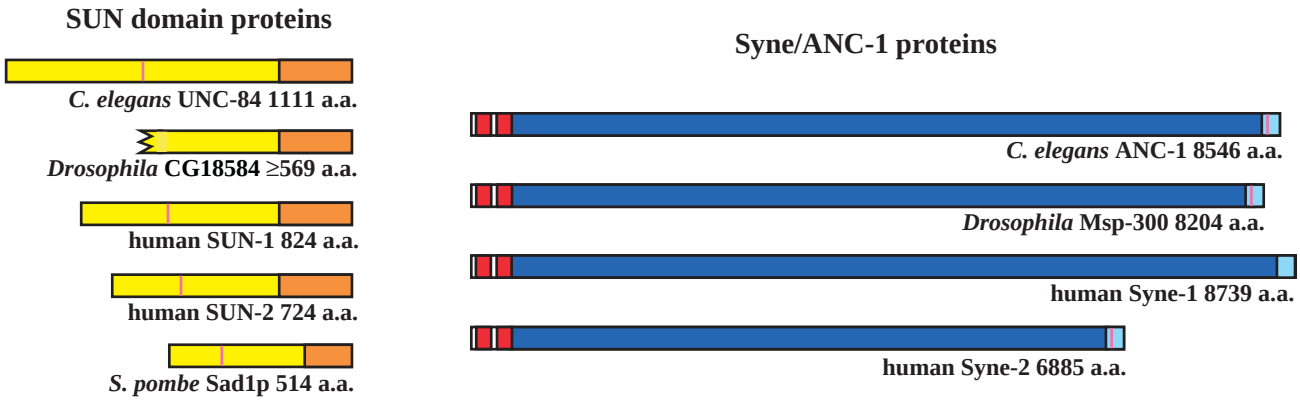

Fig. 1. UNC-84 and ANC-1 are conserved. On the left, the family of SUN domain-containing proteins is depicted. The SUN domains (tan) are between 34 and 47% identical to the UNC-84 SUN domain. All of these proteins, except the *Drosophila* member (which might not be full length) have a predicted trans-membrane domain (pink). On the right, the Syne/ANC-1 proteins are depicted. The last 60 residues are the KASH domain (light blue) with a predicted trans-membrane domain (pink). These domains are between 50 and 60% identical to one another. The N-termini of these proteins contain two calponin-like domains of about 100 residues each (red). The large central region (blue) of ANC-1 consists of mostly novel repetitive stretches, while central regions of the others consist of mostly spectrin repeats. These domains are both predicted to form extended rod-like structures.

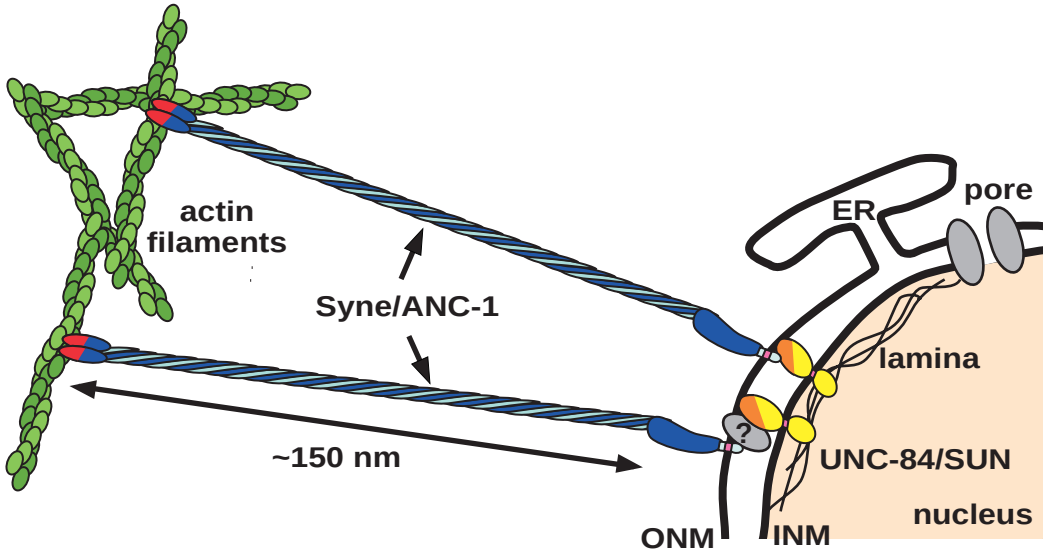

Fig. 2. A model for nuclear anchorage and migration is shown. The KASH domain (light blue) is targeted to the outer nuclear membrane (ONM) through an interaction with the SUN domain (tan) of UNC-84 (yellow), which may occur through an intermediating protein or complex (gray circle). This predicts that Syne/ANC-1, UNC-84, the nuclear lamina, and other unknown proteins create a bridge across both nuclear membranes. Such a novel mechanism would require that the outer nuclear membrane be independently specified from the endoplasmic reticulum (ER) or nuclear pores (gray). The calponin domains of Syne (red) attach to actin microfilaments (green) to effectively anchor nuclei in the cytoplasm.

been tested by either digitonin extraction methods or by immunoelectron microscopy, and the locations of endogenous SUN-1 or SUN-2 have not been examined. Therefore, the topology of UNC-84/SUN in the nuclear envelope remains to be determined. One model suggests that as an integral component of the inner nuclear membrane, the SUN domain of UNC-84 extends into the periplasmic space where it interacts with components of the outer nuclear membrane (Fig. 2). Then the SUN domain could effectively retain other proteins at the outer nuclear envelope. This mechanism could be similar to the way lamin traps components at the inner nuclear membrane (for a review, see Holaska et al., 2002).

The switch between nuclear anchorage and nuclear migration must be a tightly coordinated event. Before a nucleus can migrate through the cytoplasm of the cell, the nuclear anchor must be released. Mutations in *unc-84* disrupt both nuclear anchorage and migration, suggesting that UNC-84 is intimately involved with this switch. Interestingly, the SUN domain of UNC-84 is required to target both ANC-1 (through an interaction that is not known to be direct or indirect) and UNC-83 (through a direct interaction) to the nuclear envelope (Starr and Han, 2002; Starr et al., 2001). It is not known whether ANC-1 and UNC-83 can interact with UNC-84 simultaneously, although both antigens are detected at the nuclear envelope of adult hypodermal cells. A simple model would be that, when UNC-83 binds to UNC-84, it displaces ANC-1, perhaps through an unknown mediating protein or complex, which frees the nucleus and allows migration to proceed. In reality, the mechanism is probably more complex, involving unknown signaling molecules, since overexpression of UNC-83 or the KASH domain of ANC-1 does not displace ANC-1 or UNC-83, respectively (Starr et al., 2001) (D.A.S., unpublished).

The Syne/ANC-1 family of proteins

Recent work has determined that ANC-1 belongs to a family of proteins conserved in humans, *Drosophila* and *C. elegans* (Fig. 1) (Starr and Han, 2002). These huge proteins (>8000 residues) contain two N-terminal calponin-homology (CH) domains, which bind to actin (Korenbaum and Rivero, 2002), and a conserved C-terminal domain that targets the protein to the outer nuclear envelope (Apel et al., 2000; Mislow et al., 2002a; Rosenberg-Hasson et al., 1996; Starr and Han, 2002; Zhang et al., 2001; Zhen et al., 2002). The large central region, which is predicted to be highly helical, consists of mostly spectrin repeats in the mammalian and *Drosophila* proteins, whereas in *C. elegans*, it consists of unique repetitive regions.

Two related proteins exist in mammals and have been called Syne-1 and -2 (Apel et al., 2000), Nesprin-1 and -2 (Zhang et al., 2001), Myne-1 and -2 (Mislow et al., 2002a), or NUANCE (Zhen et al., 2002). Since the same two genes appear to encode all of these proteins, we use the original names, Syne-1 and -2 (for synaptic nuclear envelope). Single homologues have been identified in *Drosophila*, Msp-300 (Rosenberg-Hasson et al., 1996; Starr and Han, 2002), and *C. elegans*, ANC-1 (Starr and Han, 2002). A second protein in *Drosophila*, Klarsicht, contains the conserved C-terminal domain but has no additional features in common with the other proteins. Klarsicht localizes to the nuclear envelope, where it has been proposed to organize dynein and/or kinesin motors to control nuclear and lipid vesicle migration along microtubules (Mosley-Bishop et al., 1999; Welte et al., 1998). We call the conserved C-terminal 60 residues the KASH domain (for **K**larsicht/**A**NC-1/**S**yne-1 **h**omology) and refer to this family of proteins as the Syne/ANC-1 proteins.

Antibodies against Syne-1, Syne-2 and ANC-1 localize to the nuclear envelope (Apel et al., 2000; Mislow et al., 2002a; Starr and Han, 2002; Zhang et al., 2001; Zhen et al., 2002). In

mammals, they specifically localize to the nuclear envelope of muscle cell nuclei throughout development. Interestingly, Syne-1 is enriched at the nuclear envelope of myonuclei clustered at the neuromuscular junction, which suggests a role in positioning of nuclei towards the synapse (Apel et al., 2000). The KASH domain probably localizes Syne/ANC-1 proteins to the nuclear envelope. The C-terminal 64 and 59 residues of Syne-1 and Syne-2, respectively, are sufficient to localize tags to the nuclear envelope of mammalian tissue culture cells (Zhang et al., 2001; Zhen et al., 2002). The KASH domains of ANC-1 and Klarsicht are also been sufficient for nuclear envelope localization. Furthermore, the predicted transmembrane region of the KASH domain is required for localization to the nuclear envelope (Zhang et al., 2001).

Whether Syne/ANC-1 proteins localize to the outer or inner nuclear membrane is still a matter of debate. Most of the data suggest that Syne/ANC-1 proteins localize to the outer nuclear envelope and the cytoplasm. Digitonin extraction experiments that allow antibodies to detect epitopes on the outside of the nuclear envelope, but block epitopes inside the nuclear envelope, indicate that Syne-2 is a component of the outer nuclear envelope (Zhen et al., 2002). This evidence implicates the KASH domain in making Syne-2 the first protein to localize to the outer nuclear envelope but not the connected endoplasmic reticulim, although it does not address whether Syne-2 is also on the inside of the nuclear envelpe. Furthermore, the vast majority of ANC-1 antibody staining in *C. elegans* and MSP-300 antibody staining in *Drosophila* is cytoplasmic and excluded from the nucleoplasm (Volk, 1992; Starr and Han, 2002). Other data suggest that Syne-1 functions as part of the nuclear matrix scaffold as an inner nuclear membrane component. Syne-1 interacts directly with lamin A and nesprin, both components of the inner nuclear matrix, in in vitro protein blot overlay experiments (Mislow et al., 2002b). Therefore, Syne/ANC-1 proteins could function both inside and outside the nucleus. Further investigations, including immunoelectron microscopy localization of endogenous protein, are required to demonstrate the exact locations of Syne/ANC-1 proteins with respect to the nuclear envelope.

In *C. elegans*, localization of ANC-1 to the nuclear envelope requires UNC-84 (Starr and Han, 2002). This fact, taken in context of the above model for UNC-84 function at the nuclear envelope, suggests that Syne/ANC-1, UNC-84 and perhaps other proteins effectively bridge both membranes of the nuclear envelope (Fig. 2). In support of such a bridge hypothesis, Syne-1 co-immunoprecipitates with the major nuclear matrix component lamin A/C (Mislow et al., 2002a).

Overexpression of the C-terminal domain of ANC-1 causes an Anc phenotype as severe as that of null alleles of *anc-1* (Starr and Han, 2002). In mammalian tissue culture cells, the overexpressed C-terminus of Syne-2 is able to displace endogenous Syne-2 from docking sites at the nuclear envelope (Zhen et al., 2002). Therefore, disrupting the localization of endogenous Syne proteins to the nuclear envelope probably produces the dominant negative phenotype. Since no direct physical interaction between UNC-84 and any Syne proteins has been detected, UNC-84 may function through other proteins to recruit or maintain Syne proteins at the nuclear envelope. It will be informative to investigate whether overexpression of the KASH domains of Syne-1 or Msp-300 in mice or flies causes a nuclear anchorage defect, and if so, to analyze the consequences.

The second major feature of the Syne/ANC-1 proteins is the conserved N-terminal CH domains. CH domains are found in a large family of proteins, including dystrophin and α-actinin; when found in pairs, as they are in the Syne/ANC-1 proteins, they usually bind to actin (Gimona et al., 2002). The CH domains of Syne-2 and ANC-1 have been shown to bind to actin in vitro and to co-localize with actin in vivo (Starr and Han, 2002; Zhen et al., 2002). In addition, Msp-300 co-sediments with actin out of an embryonic extract and co-localizes with actin (Volk, 1992). Finally, overexpression of the N-terminus of ANC-1 causes a weakly penetrant, dominant negative Anc phenotype (Starr and Han, 2002). Thus, when overexpressed, the N-terminus of ANC-1 can block the function of endogenous ANC-1. These data and the high degree of conservation of the CH domains suggest that binding to actin is critical for the function of the Syne proteins.

The bulk of ANC-1 consists of vast stretches of repetitive, mostly helical domains, with short stretches of predicted coiled-coil domains throughout the protein (Starr and Han, 2002). ANC-1 might thus fold to form an elongated myosin-tail-like structure. Syne-1, Syne-2 and Msp-300 all have large central domains containing multiple spectrin-like repeats (Apel et al., 2000; Mislow et al., 2002a; Volk, 1992; Zhang et al., 2001; Zhen et al., 2002). Spectrin repeats are ~106 residues long and fold to form highly coiled, 5 nm long, triple helical bundles (Yan et al., 1993). Syne-2 has 22 spectrin repeats interspersed with other predicted coiled regions. Therefore, the central extended coiled domain of Syne-2 could extend well over 150 nm, and Syne-1 and Msp-300 could be 25% longer. Presumably, the spectrin repeats of the Syne and Msp-300 proteins function analogously to the long coiled domains of ANC-1. Some of the Syne-1 spectrin repeats directly interact with each other in two-hybrid and blot-overlay experiments, which suggests that at least Syne-1 forms antiparallel dimers (Mislow et al., 2002b). In an experiment designed to test the requirement of the large central region of Syne-2, Zhen et al. (Zhen et al., 2002) fused the N-terminal CH domain to the C-terminal KASH domain and transfected the chimera into mammalian tissue culture cells. This resulted in an ectopic recruitment of actin to the cytoplasmic side of the nuclear envelope (Zhen et al., 2002), suggesting that the role of the huge central domain is to separate the actin-binding domain from the nuclear envelope domain. Therefore, we hypothesize that the evolutionary conservation of size in Syne/ANC-1 is due to a selective advantage.

The function of Syne proteins

In Fig. 2, we propose a model in which Syne/ANC-1 anchor nuclei by tethering the nucleus to the actin cytoskeleton. According to this tethering model, Syne/ANC-1 extends away from the nucleus to where the N-terminus directly binds to the actin cytoskeleton. In this model, the C-terminus of Syne/ANC-1 is recruited to the outer nuclear envelope by UNC-84/SUN. It is not known whether Syne/ANC-1 directly interacts with UNC-84/SUN or whether other proteins mediate this interaction. Note that multiple transcripts of *Syne-1* and *Syne-2* have been identified, most without the N-terminal actin-binding domain (Apel et al., 2000; Mislow et al., 2002a; Zhang

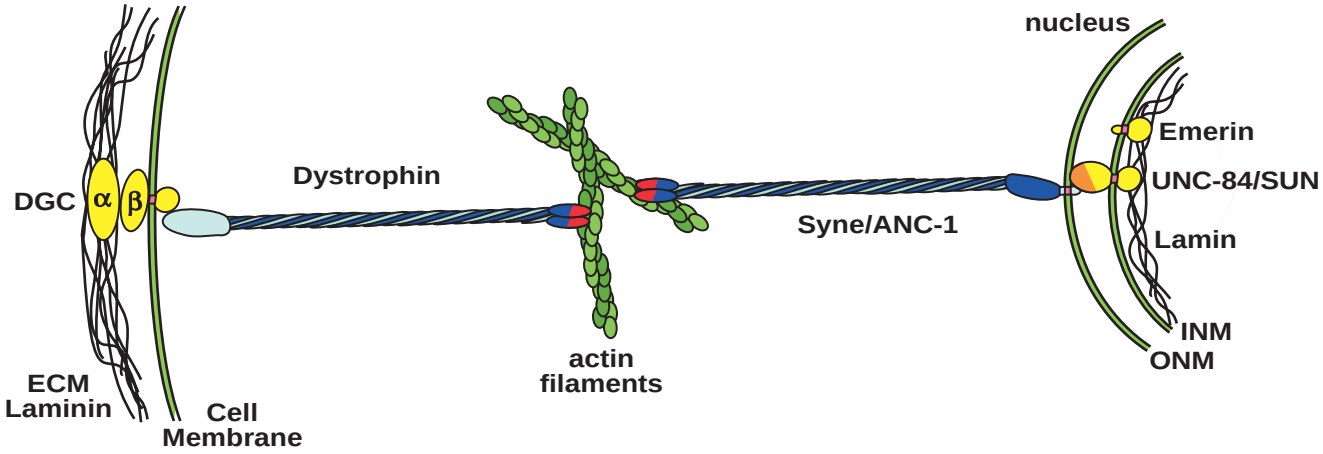

Fig. 3. Is there a potential role for Syne/ANC-1 in Muscular Dystrophy? This model points out the similarities between the mechanisms of Syne/ANC-1 and dystrophin. Both of these huge proteins bind to actin through N-terminal calponin domains, and extend spectrin repeats through the cytoplasm. Both then function as part of a bridge across membranes. Mutations in many of these components lead to muscular dystrophy (see text).

et al., 2001; Zhen et al., 2002), and there are alternative transcripts of *msp-300* that lack the sequence encoding the C-terminal KASH domain (Rosenberg-Hasson et al., 1996). Therefore, Syne/ANC-1 proteins may have additional roles that do not require the conserved actin-binding or nuclear-envelope-localizing domains.

Genetic studies in *C. elegans* and *Drosophila* have provided the best insight into the function of the Syne proteins. As mentioned above, null mutations in the *C. elegans* gene *anc-1* disrupt the uniform spacing of nuclei in multi-nucleated syncytial cells, leading to an Anc phenotype (Hedgecock and Thomson, 1982). Therefore, ANC-1 is required for positioning of syncytial nuclei. ANC-1 is also expressed in mononucleated cells, and its function is required for anchorage of mitochondria (Starr and Han, 2002). It is thus conceivable that it positions nuclei in a wide variety of cells. The *anc-1* nuclear anchorage defect does not have a drastic effect on the animal. In contrast, mutations in the *Drosophila* gene *msp-300* are lethal. Embryos die because of a defect in muscle morphogenesis (Rosenberg-Hasson et al., 1996; Volk, 1992). The exact nature of the lethal phenotype has not been completely described. For instance, it is not known whether nuclei are properly positioned in mutant muscles. In addition, owing to the maternal contribution, whether MSP-300 anchors nuclei during early embryogenesis or oogenesis has not been examined.

Dystrophin and the associated dystrophin-glycoprotein complex (DGC) connect the actin cytoskeleton to the extracellular matrix (Ehmsen et al., 2002); mutations in any of these components lead to Duchenne or Becker muscular dytrophies (Burke et al., 2001; Rando, 2001). Although Syne/ANC-1 is proposed to connect the actin cytoskeleton to the nuclear matrix, whereas dystrophin connects actin to the extracellular matrix, there are some striking similarities between these two mechanisms (Fig. 3). Dystrophin and Syne/ANC-1 both bind to actin through an N-terminal CH domain and extend through the cytoplasm towards the plasma or nuclear membranes, respectively. Through associated proteins, the DGC or UNC-84/SUN and other proteins, dystrophin and Syne/ANC-1 eventually connect to related underlying matrices, either the basement membrane component laminin or the nuclear lamina. Compared with dystrophin, which does not have a predicted transmembrane domain, Syne/ANC-1 could circumvent the problem of the double membrane at the nuclear envelope by using its own transmembrane domain to pass through the outer nuclear membrane. Proteins associated with the KASH domain of Syne-1 are likely eventually to bind lamin in the nuclear matrix, creating a bridge across the nuclear envelope. Anti-Syne-1 antibodies co-immunoprecipitate a component of the nuclear matrix, lamin A/C (Mislow et al., 2002a), which suggests there is a more complete connection between the Syne proteins and the nuclear matrix. We therefore propose that lamin, UNC-84/SUN, Syne/ANC-1 and perhaps other proteins bridge the nuclear envelope and connect the nuclear matrix to the actin cytoskeleton.

Mutations in lamin A/C or emerin lead to Emmery-Dreifuss muscular dystrophy (Burke et al., 2001; Burton and Davies, 2002; Hutchison et al., 2001; Nagano and Arahata, 2000). An expression array experiment that identified messages that were up- or downregulated in tissue from Duchenne muscular dystrophy patients showed that SUN-2 is downregulated an average of three to fourfold (Chen et al., 2000). Since, lamin and UNC-84 have been linked to muscular dystrophy, it would be worth investigating whether disruptions in the Syne/ANC-1 also contribute to muscular dystrophy.

Concluding remarks

In the past few years, much as been learned about the mechanisms of nuclear positioning in animals. ANC-1 plays an important role in the anchoring of nuclei in *C. elegans*, probably by directly tethering the nuclear envelope to the actin cytoskeleton. ANC-1 is recruited to the nuclear envelope by UNC-84. Potential mammalian ANC-1 homolgues have also been shown to bind to actin and the nuclear envelope in a manner similar to that in which dystrophin connects the actin cytoskeleton to the plasma membrane. There are also

homologues of UNC-84 at the nuclear envelope of mammalian cells. However, these two families of proteins are likely to represent just the tip of the iceberg, and many questions about their functions remain. The most important issue to address is the function of Syne-1 and Syne-2 in mammals and whether they contribute to any human neuromuscular diseases. Another area to explore is the switch between nuclear migration and anchorage, which is likely to have many implications in developmental biology. Other major questions include the following: What is the function of the apparent nucleoplasmic pool of Syne/ANC-1 proteins? Does UNC-84 directly recruit ANC-1 or are there intermediate components? Why are there single members of Syne/ANC-1 and UNC-84 families in *C. elegans* and *Drosophila*, but two of each in mammals? Clearly these questions will keep the field busy for many years to come.

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