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### Authors

Saunders, Cosmo A

Luxton, GW Gant

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# LINCing Defective Nuclear-Cytoskeletal Coupling and DYT1 Dystonia

COSMO A. SAUNDERS and G. W. GANT LUXTON

Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455, USA

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**Abstract**—Mechanical forces generated by nuclear-cytoskeletal coupling through the linker of nucleoskeleton and cytoskeleton (LINC) complex, an evolutionarily conserved molecular bridge in the nuclear envelope (NE), are critical for the execution of wholesale nuclear positioning events in migrating and dividing cells, chromosome dynamics during meiosis, and mechanotransduction. LINC complexes consist of outer *Klarsicht*, *ANC-1*, and *Syne* homology (KASH) and inner *Sad1*, *UNC-84* (SUN) nuclear membrane proteins. KASH proteins interact with the cytoskeleton in the cytoplasm and SUN proteins in the perinuclear space of the NE. In the nucleoplasm, SUN proteins interact with A-type nuclear lamins and chromatin-binding proteins. Recent structural insights into the KASH-SUN interaction have generated several questions regarding how LINC complex assembly and function might be regulated within the perinuclear space. Here we discuss potential LINC regulatory mechanisms and focus on the potential role of the ATPases associated with various cellular activities (AAA+) protein, torsinA, as a LINC complex regulator within the NE. We also examine how defects in LINC complex regulation by torsinA may contribute to the pathogenesis of the human neurological movement disorder, DYT1 dystonia.

**Keywords**—LINC complex, TorsinA, Nuclear envelope, AAA+ protein, LAPI, LULL1, Cytoskeleton, Mechanotransduction.

## INTRODUCTION

The linker of nucleoskeleton and cytoskeleton (LINC) complex is an evolutionarily conserved molecular bridge in the nuclear envelope (NE) that is responsible for physically coupling the nucleus to the cytoskeleton in most eukaryotic cells.<sup>45</sup> LINC complexes are composed of two type II membrane proteins: the outer nuclear membrane (ONM) *Klarsicht*,

*ANC-1*, and *Syne* homology (KASH) proteins and the inner nuclear membrane (INM) *Sad1* and *UNC-84* (SUN) proteins.<sup>12</sup> All KASH proteins contain a divergent cytoplasmic domain followed by a C-terminal trans-membrane domain and a conserved short (~10–32 residue) luminal KASH domain, which is necessary and sufficient for the targeting of their unconserved cytoplasmic domains to the ONM.<sup>41</sup> Within the cytoplasm, KASH proteins bind various cytoskeletal elements as well as signaling molecules.<sup>11,41</sup> In addition, several KASH proteins also project into the nucleoplasm from the INM, where they interact with other INM proteins, A-type lamins, and chromatin-binding proteins.<sup>15,53</sup> Within the perinuclear space, KASH proteins directly interact with the conserved SUN domain of SUN proteins through their luminal KASH domain.<sup>11</sup> The divergent SUN protein C-terminus extends into the nucleoplasm, where it interacts with A-type lamins, chromatin-binding proteins, and other proteins.<sup>45</sup> It is through the KASH-SUN interaction that the LINC complex is able to transmit mechanical forces from the cytoplasm across the double membrane of the NE and into the nucleoplasm.

Over the past decade, the LINC complex has attracted much attention resulting in the identification of a growing list of KASH and SUN proteins, LINC complex accessory proteins, as well as LINC complex-dependent cellular functions including DNA damage repair, meiotic pairing of homologous chromosomes, mechanotransduction, and nuclear positioning.<sup>41,45,68</sup> In light of this wide range of cellular and developmental functions that rely upon LINC complex-dependent nuclear-cytoskeletal coupling, it should come as no surprise that mutations in the genes encoding KASH and SUN proteins are associated with an ever-expanding list of human diseases including ataxia, cancer, deafness, and muscular dystrophy.

While LINC complexes are fundamental force transducers across the NE, very little has been revealed about the regulatory mechanisms that control LINC

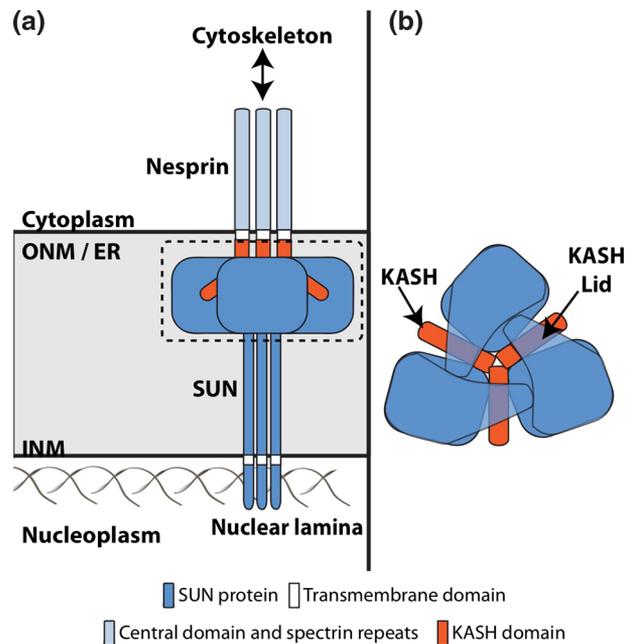
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Address correspondence to G. W. Gant Luxton, Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455, USA. Electronic mail: gwgl@umn.edu

complex assembly and function. These regulatory mechanisms must exist given the fact that the KASH domains of nesprins 1, 2, and 3 can promiscuously interact with the SUN domains of SUN1 and SUN2 within the perinuclear space with similar affinities.<sup>54,67</sup> Furthermore, LINC complexes have to switch between different cellular functions and often reorganize into higher-ordered arrays within the NE.<sup>27,39</sup> Here, we review recent structural insights into LINC complex regulation, discuss the potential role of the ATPases associated with various cellular activities (AAA+) protein torsinA as a candidate LINC complex regulator, and explore the possible relationship between defective LINC complex regulation by torsinA and the neurological movement disorder DYT1 dystonia.

### STRUCTURAL INSIGHTS INTO LINC COMPLEX FUNCTION AND REGULATION

Recently, crystal structures of the human SUN2 bound to the C-terminal 29 residues (KASH2) from synaptic NE 2 (SYNE-2)/NE spectrin repeat protein 2 (nesprin-2) were independently solved and published by two groups.<sup>63,76</sup> SUN2 is trimeric with the globular SUN domains sitting on top of a triple helical coiled-coil stalk (Fig. 1a). KASH2 interacts with the SUN2 trimer within hydrophobic grooves formed at the interface of adjacent SUN domains (Fig. 1). SUN2 further interacts with KASH2 through a “KASH-lid”, which conceals part of the KASH domain (Fig. 1b). While SUN2 trimers exist in the absence of the KASH2, the KASH-lid assumes a random, non-ordered conformation, suggesting that it may function to stabilize the KASH-SUN interaction. In addition, two interesting structural features were identified in the SUN2-KASH2 complex: a loop that surrounds and coordinates a bound cation and a conserved intermolecular disulfide bond between KASH2 and the SUN domain. While the KASH2-SUN2 interaction was shown to require the cation loop, the disulfide bond appeared to be dispensable for binding.<sup>63</sup> However, molecular dynamics modeling recently demonstrated that this intermolecular disulfide bond was crucial for the stability of the KASH-SUN interaction as well as force transmission through the LINC complex.<sup>29</sup> The crystal structure of SUN2 in a complex with the KASH domain from SYNE-1/nesprin-1 was overall quite similar to the SUN2-KASH2 complex potentially explaining the conservation of the KASH domain.<sup>63</sup> Together the multiple non-covalent and single covalent interactions between KASH and SUN domains may explain how LINC complexes resist



**FIGURE 1. LINC complexes are heterohexamers of KASH and SUN proteins. (a) Assembled LINC complexes physically couple the nucleus to the cytoskeleton via the assembly of KASH-SUN heterohexamers within the perinuclear space.<sup>63,76</sup> (b) Top-down view of the KASH-SUN heterohexamer in (a) based on recently published crystal structures of the SUN-KASH interaction.<sup>63,76</sup>**

and perhaps transmit cytoskeletal-derived mechanical forces.

Highlighting the importance of SUN domain interfaces for KASH peptide binding, SUN2 trimerization through the triple helical coiled-coil is essential for KASH domain binding, as fusion of individual SUN domains of SUN2 to an unrelated trimeric coiled-coil can restore this interaction.<sup>63</sup> SUN protein trimerization may further allow efficient force transmission across the NE and the ability to endure the high loads necessary for movement of the nucleus or chromosomes within the nucleoplasm. Intriguingly, assembly of SUN protein trimers may also promote the formation of higher-order LINC complex arrays. Moving forward, it will be important to determine the existence of SUN protein trimers or LINC complexes with a heterohexameric  $SUN_3:KASH_3$  stoichiometry within the NE of living cells. This knowledge will provide a useful intellectual framework for the future investigation of the kinetics and regulation of LINC complex assembly. While there is little question that these findings have significantly advanced our understanding of LINC complex structure and function, it is clear that they are just a preview of the increased mechanistic insights that will come in the future.

## TORSINA AS A CANDIDATE LINC COMPLEX REGULATORY FACTOR

An often-discussed potential regulator of the LINC complex from within the perinuclear space is the evolutionarily conserved ATP-binding protein, torsinA.<sup>18,50,63</sup> Torsin proteins have been identified in all metazoans examined to date, but none have yet been found in unicellular eukaryotes or prokaryotic genomes.<sup>3,64</sup> Recently, torsin proteins have attracted much attention as they have been implicated in multiple fascinating and important NE-localized functions ranging from the maintenance of proper ER/NE morphology,<sup>22,49,60</sup> the regulation of nuclear pore complex (NPC)-mediated nuclear import,<sup>75</sup> as well as the execution of a nuclear export pathway through which large ribonucleoprotein granules and herpesvirus capsids exit the nucleus *via* budding through the NE.<sup>30,42</sup> The significance of torsinA function is illustrated by the fact that mice lacking torsinA die shortly after birth.<sup>22</sup> TorsinA belongs to the AAA+ protein superfamily, members of which typically function as ring-shaped hexameric molecular chaperones that utilize energy derived from ATP-hydrolysis to remodel protein complexes.<sup>25,56</sup> Since torsinA has been shown to interact with the KASH domains from several nesprins, it has been proposed that torsinA acts as a molecular chaperone to control LINC complex assembly and/or disassembly.<sup>50</sup> Importantly, torsinA and the three additional mammalian torsin proteins (torsinB, torsin2, and torsin3) are the only AAA+ proteins known to reside within the lumen of the endoplasmic reticulum (ER) and the contiguous perinuclear space of the NE.<sup>3</sup> Accumulating evidence suggest that torsinA exhibits chaperone-like behaviors both *in vitro* and *in vivo*.<sup>7,51</sup> Notwithstanding these insights, the identity of the substrate(s) potentially remodeled by torsinA, and thus its precise cellular function(s), remain unidentified.

## TORSINA IS AN ATYPICAL AAA+ PROTEIN

AAA+ proteins are defined by the presence of a 200–250 amino acid ATP-binding AAA domain, which contains Walker-A and -B motifs, as well as other motifs that differentiate them from classic P-loop NTPases.<sup>25</sup> Walker-A motifs bind ATP while Walker-B motifs hydrolyze ATP.<sup>25</sup> Mutation of the glutamate residue in the Walker-B motif prevents ATP-hydrolysis while leaving ATP-binding unaffected. Since ATP is required for substrate binding by most AAA+ proteins this mutation generates a useful reagent known as a ‘substrate trap’.<sup>77</sup> AAA+ proteins possess additional motifs including sensor-1, sensor-2, and arginine fin-

gers, all of which participate in ATP-hydrolysis. Furthermore, the sensor-2 motif is also necessary for ATP-binding while the arginine fingers contribute to AAA+ protein oligomerization.<sup>25</sup>

Sequence-based analysis places torsinA in the classic AAA+ protein family and reveals a close relation to the C-terminal AAA+ domain of ClpB.<sup>28</sup> Upon sequence comparison, torsinA differs structurally from other AAA+ proteins in five major ways. First, unlike most AAA+ proteins, which are typically soluble, the first 20 amino acids of torsinA encode a signal sequence that directs the translocation of torsinA into the lumen of the ER and the contiguous perinuclear space of the NE.<sup>10,34</sup> Second, torsinA possess a hydrophobic N-terminal domain (NTD) that follows the signal sequence allowing torsinA to monotonically associate with membranes and be retained within the secretory pathway by exclusion from ER exit sites.<sup>73</sup> Third, torsinA harbors critical differences in two conserved AAA+ motifs important for ATPase activity including the ATP-binding Walker-A motif and the sensor-2 motif, which promotes ATP-binding and -hydrolysis. Whereas the Walker-A motif is usually defined by the sequence motif GxxGxGK(T/S), torsinA contains an asparagine instead of threonine or serine.<sup>47</sup> Substituting the Walker-A threonine with asparagine in the bacterial AAA+ chaperone ClpB, which serves to reactivate aggregated proteins, results in a partial inhibition of ATPase activity.<sup>47</sup> The sensor II motif of torsinA atypically lacks the conserved arginine that interacts with the  $\gamma$ -phosphate of ATP in other AAA+ proteins.<sup>82</sup> This motif is referred to as the redox-sensing sensor (RSS) because it contains a unique cysteine-containing redox-sensing sensor-2 motif that is critical for both nucleotide- and partner-binding.<sup>82</sup> Fourth, no arginine fingers are found in torsinA.<sup>82</sup> Fifth, unlike other AAA+ proteins, torsinA lacks an identifiable substrate-binding domain or other functional domains outside of its ATPase domain, making it unclear how torsinA selectively interacts with and remodels potential substrates.<sup>3</sup>

To address this fundamental gap in our understanding of torsinA function, several groups have focused on determining the molecular mechanism of torsinA ATPase activation. However, the fact that torsinA is found within the oxidizing environment of the ER lumen, is glycosylated, and contains 6 conserved cysteine residues has posed a considerable challenge for biochemical analysis.<sup>83</sup> Demonstrative of this difficulty are the inconsistent results obtained by different laboratories regarding the ATPase activity of purified torsinA.<sup>33,35,59</sup> The critical evaluation of these conflicting results is hindered by the use of different expression systems (bacterial vs. insect), dissimilar torsinA constructs, and different assay conditions.

Recently, pioneering work from the Schlieker laboratory provided significant insight into the mechanism of torsinA ATPase activation. They identified the major torsinA-interacting proteins lamina-associated polypeptide 1 (LAP1) and luminal domain like LAP1 (LULL1) as stimulatory co-factors for torsinA.<sup>80</sup>

### MECHANISM OF TORSINA ATPASE ACTIVATION

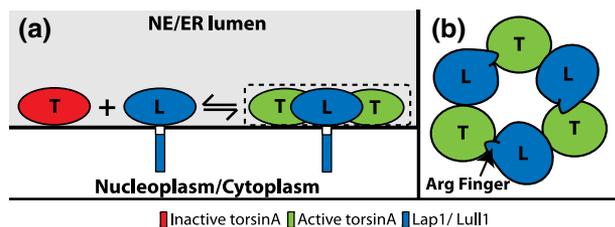
LAP1, a type II membrane protein, was the first torsinA-interacting protein identified in the NE<sup>21</sup> (Fig. 2). LAP1 binds tightly to an ATP-hydrolysis defective 'substrate trap' mutant of torsinA (E171Q),<sup>21,48</sup> which accumulates within the NE in cells.<sup>20,49</sup> The N-terminus of LAP1 localizes to the nucleoplasm where it binds nuclear lamins, while its C-terminus resides within the perinuclear space where it interacts with torsinA.<sup>21</sup> Like torsinA, mice lacking LAP1 also exhibit perinatal lethality.<sup>32</sup> BLAST analysis revealed the existence of a novel protein, LULL1, with 60% identity to the luminal domain of LAP1.<sup>21</sup> LULL1 is also a type II membrane protein, the C-terminus of which resides within the ER/NE lumen where it interacts with torsinA in an ATP-dependent manner<sup>21,48</sup> (Fig. 2). However, the N-terminus of LULL1 directs its localization to the peripheral ER and/or ONM, excluding it from the INM.<sup>48</sup> While LULL1 knockout mice have yet to be reported, there is evidence that functional segregation exists between LAP1 and LULL1. For example, LULL1 is required for the efficient growth of herpes simplex virus 1 and works together with torsinB to maintain proper ER morphology through undefined mechanisms.<sup>60,70</sup>

Using torsinA purified from baculovirus, Zhao *et al.* demonstrate that this AAA+ protein has negligible ATPase activity.<sup>80</sup> Surprisingly though, when the luminal domains of either LAP1 or LULL1 purified from bacteria were added to isolated torsinA, they found a significant stimulation of torsinA ATPase

activity (Fig. 2a). This ATPase activator function was also shown to control the activities of torsinB, torsin2, and torsin3, albeit to different extents. Substrates of AAA+ proteins often stimulate their ATPase activity<sup>25</sup>; however the lack of torsinA-mediated unfolding of LAP1 or LULL1 argues that these proteins most likely function as cofactors.<sup>80</sup> Two recent publications from the Schlieker and Schwartz laboratories further support this idea through experimental and computational evidence showing that the luminal domains of LAP1 and LULL1 adopt a fold similar to an AAA+ domain.<sup>6,64</sup> While neither protein possesses identifiable nucleotide-binding motifs, both contain a strictly conserved arginine finger that is dispensable for torsinA-binding but is required for the stimulation of torsinA ATPase activity<sup>6,64</sup> (Fig. 2b).

Since torsins lack arginine fingers,<sup>82</sup> the authors proposed and provided evidence for a model whereby the luminal domains of LAP1 or LULL1 activate torsinA through an active site complementation mechanism.<sup>6,64</sup> In this model, these stimulatory cofactors interact with torsinA through a defined torsinA-cofactor interface that juxtaposes their arginine finger near the nucleotide bound in torsinA resulting in the assembly of an alternating heterohexameric (torsinA-LAP1/LULL1)<sub>3</sub> holoenzyme (Fig. 1). These important findings have perhaps generated more questions than they have answered. For example, the kinetic properties of torsinA ATPase activation by LAP1 and LULL1 are more similar to those observed for small GTPases where GAPs (GTPase-activating proteins) induce GTP hydrolysis above a negligible background.<sup>61</sup> This raises the possibility that torsinA functions mechanistically as a GTPase-like molecular switch rather than an AAA+ protein. In addition, the functional assembly state of torsinA remains undefined.

While the proposed active site complementation model requires the existence of an alternating heterohexameric (torsinA-LAP1/LULL1)<sub>3</sub> holoenzyme (Fig. 2b), this may represent only one piece of a much more complicated puzzle as demonstrated by the conflicting published results from other groups studying torsinA oligomerization. For example, *in vitro*, isolated torsinA has been reported to be both monomeric and multimeric<sup>34,59</sup> while *in cellulo* experiments demonstrate that torsinA can self-associate into dimers and higher order oligomers consistent with the formation of a homo-hexamer.<sup>19,24,31,69,74</sup> These seemingly disparate results are not entirely mutually exclusive, as the oligomerization of HSP/Clp AAA+ protein family members are known to form homo- and/or heterohexameric assemblies.<sup>72</sup> Understanding torsinA oligomerization is further complicated by the fact that torsinB associates with and appears to be functionally



**FIGURE 2. Stimulation of torsinA ATPase activity by LAP1 or LULL1.** (a) The ATPase activity of torsinA is stimulated by LAP1 or LULL1 through the formation of heterohexamers on the INM and ER membranes, respectively.<sup>6,64</sup> (b) Top-down view of the alternating torsinA-LAP1/LULL1 heterohexameric assembly in (a).<sup>6,64</sup>

redundant with torsinA in cells.<sup>26,32</sup> To resolve these issues, it is important that a structure of torsinA alone and in complex with LAPI or LULL1 be solved. Furthermore, structures of these proteins and complexes in the presence of transition state analogs would be helpful. It will also be interesting to investigate the potential role for the NTD during torsinA ATPase activation by LAPI and LULL1, since a small fraction of torsinA has been shown to exist off the membrane in a soluble state.<sup>73</sup>

### TARGETING OF TORSINA TO THE INM

Previously, a transient interaction between LULL1 and torsinA was shown to relocalize torsinA from the peripheral ER to the NE.<sup>74</sup> More recently, the Hanson laboratory demonstrated that the LULL1-torsinA interaction targets torsinA to the INM and destabilizes torsinA oligomers within the peripheral ER.<sup>23</sup> This finding is consistent with the fact that torsinA exists as a stable, slowly diffusing oligomer in the ER lumen that is unable to freely exchange between ER subdomains.<sup>74</sup> The targeting of torsinA to the INM by LULL1 was blocked by mutations in either protein that interfered with the stimulation of ATPase activity by active site complementation.<sup>23</sup> Remarkably, the forced dimerization of the N-terminus of LULL1 within the cytoplasm triggered this process, indicative of the existence of a signaling pathway within the cytoplasm that may control torsinA localization within the ER. These results suggest that LULL1 oligomerization leads to the transient disassembly of torsinA oligomers within the peripheral ER so that torsinA can reach LAPI within the INM. Thus, while LULL1 and LAPI both interact with and stimulate torsinA ATPase activity *in vitro*, the cellular consequence(s) of these interactions may be quite different. In the future, it will be interesting to further investigate the physical process responsible for the movement of torsinA from the peripheral ER to the INM following interaction with LULL1. The elucidation of the signaling pathway(s) that impinges upon the sub-organellar localization of torsinA will illuminate novel communication mechanisms that exist between the cytoplasm and ER.

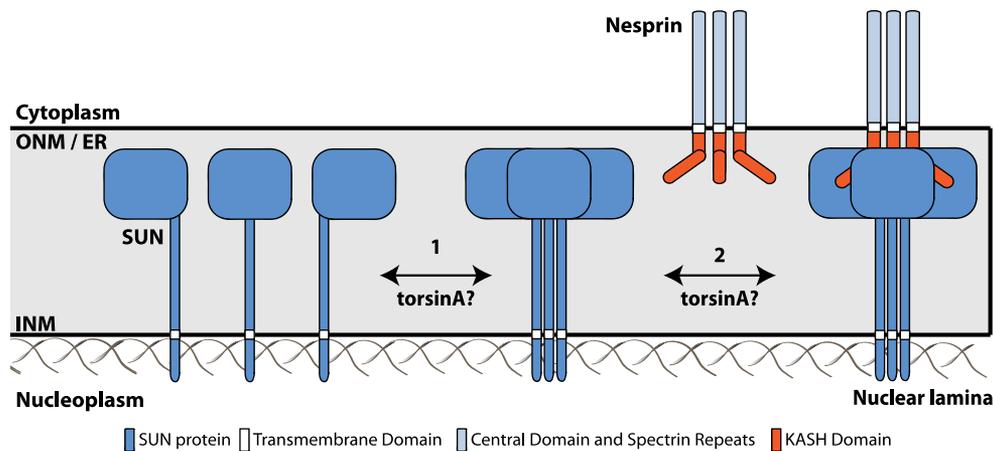
### EVIDENCE FOR TORSINA-MEDIATED LINC COMPLEX REGULATION

TorsinA was initially postulated to be a candidate regulator of the LINC complex because expression of a GFP-tagged torsinA construct encoding the ATP-hydrolysis-defective E171Q mutation in CHO cells resulted in the formation of NPC devoid stretches of

NE with an abnormally narrow perinuclear space.<sup>49</sup> This close apposition of nuclear membranes was the opposite of what was observed in HeLa cells simultaneously depleted of SUN1 and SUN.<sup>75</sup> Therefore, since AAA+ proteins frequently remodel protein interactions, it was suggested that torsinA might modulate the interactions between nesprins and SUN proteins.<sup>18</sup> Consistent with this hypothesis are the following five results: (1) torsinA directly interacts with the KASH domains of several nesprins<sup>50</sup>; (2) torsinA is required for the localization of nesprin-3 $\alpha$  to the NE<sup>50</sup>; (3) LULL1-mediated concentration of torsinA within the NE displaces nesprin-2G, nesprin-3, and SUN2<sup>74</sup>; (4) SUN1 is involved in the proper localization of torsinA to the NE<sup>8</sup>; and (5) the *Caenorhabditis elegans* torsin protein OOC-5 is required for the NE localization of the KASH protein ZYG-12 in germ cells.<sup>75</sup>

Although it is becoming increasingly clear that torsinA is involved in some aspect of LINC complex assembly and/or function, the mechanism responsible for this potential regulation is undefined. Based on the structural studies of the KASH-SUN interaction described above, we hypothesize that LINC complex assembly can be regulated by torsinA or other potential regulatory factors by influencing the ability of SUN proteins to homotrimerize and/or heterohexamerize with KASH proteins within the perinuclear space (Fig. 3). An attractive potential mechanism for torsinA-mediated LINC complex regulation is provided by the fact that the C-terminus of torsinA is involved in binding the KASH domain.<sup>50</sup> Within this region of torsinA resides the previously discussed RSS motif that is important for nucleotide and partner binding.<sup>82</sup> When taken together with the existence of the KASH-SUN disulfide bond,<sup>63</sup> this raises the prospect that torsinA may use the RSS motif to break apart the SUN-KASH interaction in a fashion similar to a protein disulfide isomerase (PDI).<sup>2</sup> However, the feasibility of this potential mechanism is complicated by the fact that KASH peptides bind to the domain interfaces of trimeric SUN proteins.<sup>63,76</sup> Effectively, this shields the KASH domain, making it unclear how homo- or hetero-hexameric torsinA might access the KASH domain to regulate LINC complexes following their assembly. This gives rise to the alternative mechanism whereby torsinA may interact with and remodel the KASH domain of nesprins, preparing them for engagement with SUN proteins.

Another perplexing mechanistic issue regarding how torsinA could regulate the LINC complex is that torsinA is targeted to the INM in cells.<sup>23</sup> Since the width of the perinuclear space is ~ 30-50 nm,<sup>9</sup> it is unclear how torsinA would access the KASH domains of nesprins on the ONM (Fig. 3). Models of the LINC complex suggest that the KASH domain extends no



**FIGURE 3. Working model of torsinA-mediated LINC complex regulation.** (a) The two hypothesized steps of LINC complex assembly that may be regulated by torsinA within the perinuclear space are (1) homotrimerization of SUN proteins, and (2) heterohexameric assembly of KASH-SUN proteins. SUN proteins form homotrimers in the absence of KASH proteins.<sup>63,76,81</sup> While torsinA may positively and/or negatively regulate one or both of these steps, the precise regulatory mechanism remains unclear.

farther into the NE lumen than the SUN domain of SUN2, which represents only a fraction of the distance between the outer and inner nuclear membranes.<sup>63</sup> Perhaps torsinA is involved in the proper ONM targeting of nesprins from the INM where several nesprin isoforms reside, projecting their N-terminus into the nucleoplasm to interact with the INM protein emerin and nuclear lamins.<sup>46</sup> Interestingly, LAP1 was recently demonstrated to interact with emerin within the nucleoplasm, and this interaction was shown to be essential for skeletal muscle maintenance in mice.<sup>62</sup> Emerin has often been considered a LINC complex component due to its ability to interact with both nesprins and SUN proteins<sup>44</sup>; thus, torsinA may regulate LINC complex assembly through modulating nesprin-emerin and/or SUN-emerin interactions at the INM. While interactions between LAP1 and LINC complex components have yet to be reported, they remain a formal possibility. It is also possible, although quite speculative, that the interaction between torsinA and LAP1 at the INM dislodges torsinA from the membrane allowing it to diffuse across the perinuclear space to interact with KASH domains on the ONM. Future experimental efforts should be focused on the biophysical, biochemical, and cellular mechanisms of LINC complex regulation by torsinA. It will also be interesting to investigate the role of redox potential and PDIs during LINC complex remodeling.

### LINC COMPLEX DYSFUNCTION AND DYT1 DYSTONIA

TorsinA was first identified by the Breakefield laboratory in 1997 as the protein encoded by the *DYT1/*

*Tor1a* gene, mutations of which cause DYT1 dystonia.<sup>57</sup> Dystonia is the third most frequent human neurological movement disorder behind essential tremor and Parkinson's disease.<sup>16</sup> It is characterized by repetitive muscle contractions that result in involuntary twisting of the extremities and abnormal posturing.<sup>1,17</sup> People afflicted with dystonia often experience serious disruptions in their ability to perform routine tasks including walking and sitting. Despite its prevalence, the pathogenesis of dystonia remains poorly understood. DYT1 dystonia is the most common and severe form of inherited dystonias, with autosomal-dominant inheritance and a penetrance of 30–40%.<sup>5</sup> The symptoms of DYT1 dystonia first appear at a mean age of 12.5 years.<sup>55</sup> Our understanding of DYT1 dystonia pathogenesis is complicated by the fact that torsinA is ubiquitously expressed, yet only the central nervous system appears to be affected in patients suffering from this disease.<sup>4</sup> Intriguingly, several other tissue-specific movement disorders (*i.e.* muscular dystrophies and cerebellar ataxias) are caused by mutations in widely expressed proteins that reside within the NE.<sup>78</sup> Furthermore, the lack of gross morphological defects in the brains of DYT1 patients does not provide obvious clues regarding disease mechanisms;<sup>1</sup> though recently a role for sensorimotor circuit neurodegeneration has been identified.<sup>37</sup> It is important to note that mutations in the *TOR1AIP1* gene, which encodes LAP1, are also associated with severe dystonia, further highlighting the significance of the torsinA-LAP1 interaction.<sup>14</sup>

DYT1 dystonia results from the deletion of a single glutamic acid codon ( $\Delta E302/303$  or  $\Delta E$ ) in *DYT1/Tor1a*, the gene that encodes torsinA.<sup>56</sup> While the precise mechanism through which the  $\Delta E$  mutation

causes DYT1 dystonia is unclear, increasing evidence suggests that it is a loss-of-function mutation. For example, torsinA knockout mice are phenocopied by mice homozygous for the  $\Delta E$  mutation,<sup>22</sup> which impairs the ability of torsinA to interact with either LAP1 or LULL1, thereby disrupting its ATPase activity.<sup>48,80</sup> This is surprising since the  $\Delta E$  mutation causes torsinA to accumulate within the NE where LAP1 resides.<sup>20,49</sup> Additionally, the efficiency of torsinA-mediated SUN2 displacement following LULL1-dependent NE targeting was significantly reduced by the presence of the disease mutation.<sup>73</sup> However, a gain-of-function mechanism has also been proposed since the  $\Delta E$  mutation promotes an association between torsinA and SUN1, which is different from the interaction of ATP-bound torsinA with LAP1.<sup>8</sup> In addition, the interaction of nesprin-3 with torsinA bearing the  $\Delta E$  mutation was shown to be stronger than for wild type torsinA.<sup>50</sup> Taken together, these results suggest that LINC complex dysfunction may contribute to DYT1 dystonia pathogenesis.

Though widely speculated, the relationship between DYT1 dystonia and LINC complex dysfunction remains undefined. Nevertheless, torsinA containing the  $\Delta E$  mutation disrupts some LINC complex-dependent functions such as cell migration and polarization, which may be important for neuronal function. The orientation of the centrosome to a position between the nucleus and leading edge is a hallmark of cell polarity in several directionally migrating cell types including fibroblasts and some neurons.<sup>40</sup> LINC complex-dependent nuclear-cytoskeletal coupling is critical for centrosome orientation and the efficient migration of these cells.<sup>38,40,79</sup> Interestingly, mouse embryonic fibroblasts from torsinA knockout mice and fibroblasts derived from DYT1 patients are defective in centrosome orientation.<sup>50,52</sup> Centrosome orientation in fibroblasts is achieved through the coordinated positioning of the centrosome and the nucleus. The centrosome is maintained at the cell center through a microtubule-dependent mechanism while the nucleus is moved towards the cell rear by retrograde flowing actin cables on the dorsal nuclear surface, which are harnessed by linear arrays of nesprin-2G/SUN2 LINC complexes or transmembrane actin-associated nuclear (TAN) lines.<sup>38</sup> Currently, it is unknown if torsinA controls the positioning of the centrosome or the nucleus. While the brains of torsinA knockout mice lack obvious developmental defects normally associated with impaired neuronal migration,<sup>22</sup> subtle alterations in neuronal migration in the embryonic forebrain and from explants of the medial ganglionic eminence were observed in the absence of torsinA.<sup>43</sup> Although controversial, a correlation between the position of the centrosome and axon initiation has been demonstrated

in developing hippocampal neurons.<sup>13</sup> Since many of the cellular components and mechanisms required for axon initiation are similar to those required for centrosome orientation during directional cell migration,<sup>36</sup> torsinA might also affect the establishment of axon-dendrite polarity in neurons. The decreased number of projections observed by group tractography in the cerebellothalamocortical pathway of mice heterozygous for  $\Delta E$  mutation further supports this idea.<sup>71</sup>

A more speculative connection between DYT1 dystonia and LINC complex dysfunction was recently uncovered in *Drosophila*. Groundbreaking work from the Budnik laboratory identified a new mechanism through which NE budding drives the export of large ribonucleoprotein particles (RNPs) from the nucleus.<sup>65</sup> Interestingly, in neurons from homozygous torsinA knockout or  $\Delta E$  knock-in mice the INM blebs into the perinuclear space.<sup>22</sup> This neural-specific NE morphology defect was proposed to be the result of the higher levels of torsinB expression found in non-neuronal cells vs. neurons.<sup>31,32</sup> Interestingly, when RNP budding is disrupted in *Drosophila* torsinA mutants, large RNPs accumulate in the perinuclear space and synaptic mRNAs fail to traffic efficiently to their proper sites of protein synthesis, thereby compromising synaptic development.<sup>30</sup> TorsinA appears to function in the nuclear envelope budding process at a step involving scission of the inner nuclear membrane.<sup>30</sup> The movement of RNPs from muscle nuclei to post-synaptic sites in flies was shown to require the nesprin-1 orthologue, dNesp1.<sup>58</sup> This research raises several questions relevant to the etiology of DYT1 dystonia. For example, it will be critical to determine if the involvement of torsinA in RNP export by NE budding occurs in neurons. It will also be of interest to resolve how RNPs are chosen and then selected for torsinA-dependent export. Finally, the identification of signaling pathways that regulate this torsinA-dependent process may represent novel therapeutic targets for DYT1 dystonia.

## CONCLUSIONS

Our understanding of the structure and function of KASH-SUN interactions has dramatically increased since their original description 13 years ago.<sup>66</sup> Since then the list of cellular, developmental, and disease-related roles of LINC complexes continues to grow at an exciting rate. Despite this explosion of interest, it remains unclear how LINC complexes are assembled and disassembled in a regulated manner. The identification of torsinA as a potential regulator of LINC complexes within the perinuclear space of the NE

represents significant progress toward this end. However, the exact mechanism of torsinA-mediated LINC complex regulation remains an important mystery, the solution of which promises significant insight into LINC complex biology as well as the pathogenesis of DYT1 dystonia. A comprehensive mechanistic understanding of torsinA function will lead to the development of potential treatments for dystonia and related disorders.

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### CONFLICT OF INTEREST

Cosmo A. Saunders and G.W. Gant Luxton have no conflicts on interest.

### ETHICAL STANDARDS

The authors for this article carried out neither animal nor human studies for this study.

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