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Structural conservation of the autoinhibitory domain in SUN proteins

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ABSTRACT

LINC complexes span across the nuclear envelope and are assembled by SUN and KASH proteins. SUN1 and SUN2 are the two most abundant SUN proteins in mammals. In SUN2, the predicted coiled-coil domain preceding the SUN domain forms a three-helix bundle that constitutes an autoinhibitory domain (AID) to lock down the SUN domain. Here, we found that SUN1 also contains an AID preceding the SUN domain and solved the structure of the AID-SUN tandem of SUN1. SUN1 AID also adopts a three-helix bundle conformation that interacts with the SUN domain and keeps it in an autoinhibited state. Disruptions of the interaction interface in the AID-SUN tandem restored the SUN domain activity for binding to the KASH peptide. Structural comparison further demonstrated that the autoinhibited conformations of the AID-SUN tandems from SUN1 and SUN2 are similar and the intramolecular interdomain packing in SUN1 is slightly more compact than that in SUN2 due to minor variations of the residues in the interaction interface. Thus, AID is a conserved functional domain in SUN proteins and this work provides the structural evidence to support the conversation of the AID-mediated autoinhibition of SUN proteins.

1. Introduction

The nuclear envelope (NE) is composed of the inner and outer nuclear membrane (INM and ONM) and forms a selective physical barrier for separating the cytoplasm from the nucleus [1]. The integrity and other biophysical properties of the NE play pivotal roles in controlling cellular homeostasis and signaling and in an organization of intracellular architecture [2,3]. A number of proteins are specifically localized in the NE and often function together to assemble various protein complexes to organize the NE and regulate its integrity [4,5]. Among them, LINC (Linker of Nucleoskeleton and Cytoskeleton) complexes span across the NE and are composed of ONM-resident KASH (Klarsicht, ANC-1, and Syne Homology) and INM-resident SUN (Sad1, UNC-84) proteins that bridge the perinuclear space [6–8].

In mammals, LINC complexes are assembled by at least four KASH proteins (Syne/Nesprin-1 to −4) and two widely-expressed SUN proteins (SUN1 and SUN2) [5,9]. KASH proteins are tail-anchored ONM proteins. At their C-termini, KASH proteins expose a conserved luminal peptide (~30 residues) into the perinuclear space that directly interact with SUN proteins [10–12]. In contrast to KASH proteins, SUN proteins are INM proteins and the C-terminal SUN domain binds to KASH proteins within the NE lumen [13–15]. Subcellular localization results also indicate that SUN1 and SUN2 have the similar topologies with the N-terminal domains in the nucleoplasm and the SUN domains in the NE lumen [14]. Consistent with their essential roles for the LINC complex assembly, defects in SUN and/or KASH proteins contribute to various human diseases such as muscular dystrophy, ataxia, progeria, lissencephaly and cancer [16–19].

Formation of LINC (SUN-KASH) complexes in the NE provides the mechanical coupling of the structural components between the nucleus and cytoskeleton [20]. The information about the interactions between SUN-KASH complexes in detail is critical for understanding their precise roles. Recently determined structure of SUN2 SUN domain reveals that SUN domain together with a

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preceding short helix forms a homotrimer and this is sufficient for KASH binding [21,22]. The structure of the SUN2-KASH2 complex further reveals that the flexible KASH-lid in the apo-SUN domain becomes well folded β-hairpin, which pairs with the KASH domain and anchors it into the protomer interfaces of the SUN domain [23].

Based on the primary sequence analysis, SUN proteins contain at least one coiled-coil domain preceding the SUN domain that would presumably aid in multimerization [24,25]. Some studies have proposed that the coiled-coil domains in SUN1 and SUN2 could mediate their homodimerization [4,26]. Moreover, there is another unexplored possibility that SUN proteins might form heterodimers or tetramers mediated by these coiled-coil domains [25,27]. However, recent studies showed that, in SUN2, the two predicted coiled-coil domains (CC1 and CC2) play distinct roles in the SUN-KASH complex formation, i.e., CC2 is a monomer and inhibits the SUN domain activity, while CC1 forms a trimer and functions as an activation segment to release CC2-mediated inhibition [28]. The structure of the CC2-SUN monomer of SUN2 reveals that CC2 is broken into three short helices that form a three-helix bundle to lock the SUN domain in an inactive conformation. In contrast, CC1 forms a coiled-coil trimer to promote the trimerization of SUN domain for KASH binding [28]. Thus, the coiled-coil domains of SUN2 are the intrinsic dynamic regulators and the predicted CC2 acts as an autoinhibitory domain (AID) to inhibit the SUN domain activity. However, it remains to be determined whether SUN1 also contains this AID for mediating its autoinhibition.

Here, we analyzed the primary sequence of SUN1 and found that the region preceding the SUN domain is predicted to be not a coiled-coil domain but a presumable AID with a high sequence similarity to that of SUN2. The structure of the AID-SUN tandem of SUN1 confirmed this prediction and revealed that SUN1 AID also forms a three-helix bundle that interacts with the SUN domain and locks it in an inactive state. Mutations of the essential residues in the interdomain packing interface of the AID-SUN tandem restored the SUN domain activity for binding to the KASH peptide. Compared with the structure of the CC2/AID-SUN tandem of SUN2, the structure of the AID-SUN tandem of SUN1 is slightly more compact due to minor variations of the residues in the interdomain interaction interface. Thus, AID is a structurally conserved domain in both SUN1 and SUN2 for controlling their potential autoinhibitions.

2. Materials and methods

2.1. Protein expression and purification

DNA sequence of the AID-SUN tandem (residues 668–913) was amplified by PCR from the full length mouse SUN1 and then inserted into a modified pET32a vector (with an N-terminal GB1-His_6 tag). Mutations of the AID-SUN tandem were created using the standard PCR-based mutagenesis method and confirmed by DNA sequencing. Recombinant proteins were expressed in *Escherichia coli* BL21-Codon Plus host cells at 16 °C. The GB1-His_6-tagged proteins were purified using Ni^{2+}-NTA affinity chromatography (GE Healthcare) followed by size exclusion chromatography (Superdex 200, GE Healthcare). After cleavage of the tag, the proteins were further purified by another run of size-exclusion chromatography.

2.2. Crystallization, data collection, and structure determination

Crystals of AID-SUN tandem were obtained in 0.1M MES pH 6.3, 0.6M $MgSO_4$ using the sitting-drop vapor diffusion method at 16 °C. Before being flash-frozen in liquid nitrogen, crystals were cryo-protected with the mother liquor supplemented with 15% ethylene glycol. Diffraction data were collected at the beamline BL17U of the Shanghai Synchrotron Radiation Facility (SSRF) with a wavelength of 0.979 Å at 100 K [29]. The initial phase was determined by molecular replacement using the structure models of the CC2/AID-SUN tandem of SUN2 (PDB code: 5ED8) as the searching models with PHASER [30]. An incomplete structure model was further manually built with COOT [31] and refined with PHENIX [32]. The statistics for data collection and structural refinement were summarized in Table 1.

2.3. Yeast-two-hybrid experiment

SUN1 and KASH2 were cloned into vector pGAD-T7 and PGBK-T7, respectively. The GAL4 AD-SUN1 (WT, △α1, △α1/α2 and the

Table 1
Data collection and refinement statistics.

Crystal	AID-SUN tandem of SUN1
Data Collection	
Space group	$P3_121$
Unit cell dimensions(Å)	a = 73.6 b = 73.6 c = 97.4
Resolution range (Å)	50–2.10 (2.18–2.10)
Unique reflections	18348 (1779)
Redundancy	10.1 (10.3)
Data completeness (%)	99.9 (100)
R_{merge} (%)[a]	9.9 (70.7)
I/σ(I)	24.9 (4.1)
Wilson B-value ($Å^2$)	35.5
Structure Refinement	
Resolution range (Å)	34.44–2.20 (2.34–2.20)
R_{work}/R_{free} (%)	18.1 (27.9)/22.2 (24.4)
No. of reflections R_{work}/R_{free}	17021/1010
Atoms (non-H protein/solvent)	1908/180
RMS bond length (Å)	0.010
RMS bond angle (°)	1.10
Mean B-value ($Å^2$)	27.7
Ramachandran plot (%) (favored/additional/disallowed)[b]	97.1/2.9/0
Maximum likelihood coordinate error	0.19

Data for the outermost shell are given in parentheses.
[a] Rmerge = 100 ΣhΣi|Ih, I −⟨Ih⟩|/ΣhΣiIh, i, where the outer sum (h) is over the unique reflections and the inner sum (i) is over the set of independent observations of each unique reflection.
[b] As defined by the validation suite MolProbity [33].