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Nuclear envelope localization of human UNC84A does not require nuclear lamins

Sameez Hasan, Stephan Güttinger, Petra Mühlhäusser, Fabian Anderegg, Simone Bürgler, Ulrike Kutay*

Institute of Biochemistry, ETH Zurich, Schafmattstrasse 18, CH-8093 Zürich, Switzerland

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Abstract The SUN proteins are a conserved family of proteins in eukaryotes. Human UNC84A (Sun1) is a homolog of *Caenorhabditis elegans* UNC-84, a protein involved in nuclear anchorage and migration. We have analyzed targeting of UNC84A to the nuclear envelope (NE) and show that the N-terminal 300 amino acids are crucial for efficient NE localization of UNC84A whereas the conserved C-terminal SUN domain is not required. Furthermore, we demonstrate by combining RNA interference with immunofluorescence and fluorescence recovery after photobleaching analysis that localization and anchoring of UNC84A is not dependent on the lamin proteins, in contrast to what had been observed for *C. elegans* UNC-84.

Keywords: SUN domain; Nuclear envelope; Lamin; Inner nuclear membrane; UNC-84; SPAG4

1. Introduction

An important feature of many eukaryotic cells is the capability to divide asymmetrically – a prerequisite for the generation of differentiated tissues. Nuclear migration and anchoring are important for asymmetric cell division. Both processes together help to position the nucleus at its appropriate location in the cell. This is established through complex linkage mechanisms connecting the nuclear envelope (NE) to the actin and microtubule cytoskeleton (reviewed in [1]).

Nuclear migration has been studied extensively in *C. elegans*. UNC-84 (uncoordinated) is an inner nuclear membrane (INM) protein shown to be involved in nuclear migration [2,3]. It consists of 1111 amino acids and contains one transmembrane domain located approximately in the middle of the protein. According to current models, *C. elegans* UNC-84 links the nucleus to the cytoskeleton by an interaction with UNC-83, a protein suggested to reside in the outer nuclear membrane (ONM) [4]. This interaction is proposed to be mediated by UNC-84's conserved C-terminal SUN domain (for *S*ad1p, *UN*C-84 homology) [4]. In the nucleus, UNC-84 is connected directly or indirectly to the nuclear lamina, since UNC-84 localization has been shown to be lamin-dependent in worms [5].

Four human proteins sharing UNC-84's conserved SUN domain can be identified by database searches, namely UNC84A, UNC84B, sperm associated antigen (SPAG4) and MGC33329. The homology of the four proteins is most prominent in their C-terminal SUN domain. It is, however, unclear at present what the functions of these proteins (including their SUN domains) are. A very recent study shows that UNC84A (Sun1) is localized at the NE and is important for the anchorage of Nesprin-2, a NE-associated spectrin-repeat protein [6]. Human UNC84B (Sun2) has been described as a type II transmembrane protein residing at the INM [7]. The third human SUN domain protein, SPAG4 has originally been identified as a sperm specific protein but is also expressed in a wide range of neoplastic tissues [8,9]. MGC33329 has not yet been characterized.

The mechanism of targeting membrane proteins to the INM is an area of active research (e.g. [10]). A model explaining the targeting of proteins to the INM is the ''Diffusion–Retention'' model. It suggests that proteins destined for the INM can freely diffuse from the endoplasmic reticulum (ER) via the nuclear pore membrane into the INM [11,12]. Whether or not these proteins are retained in the INM depends on their ability to engage into stable interactions with the nuclear lamina, chromatin, or both. Interestingly, no INM-specific signal sequences seem to be required for INM localization. Experimental evidence for the ''Diffusion–Retention'' model is derived from fluorescence recovery after photobleaching (FRAP) experiments. These demonstrated a high mobility of INM proteins as long as they reside in the ER, but a significantly decreased mobility as soon as they reach the INM, where they are retained [13,14].

Since function and localization properties of UNC84A (Sun1), the closest human homolog of *C. elegans* UNC84, are unclear, we set out to investigate these. Here we report a detailed analysis of the localization properties of human UNC84A. Moreover, we demonstrate that, in contrast to *C. elegans* UNC-84, localization and anchoring of human UNC84A is not dependent on the presence of lamins.

2. Materials and methods

2.1. Molecular cloning and transient transfection

The coding regions of human UNC84A (Sun1) and SPAG4 were amplified by PCR using HeLa cell cDNA as template. The PCR fragments were cloned into the *Bgl*II–*Eco*RI and *Bam*HI–*Eco*RI sites of pEGFPN3 (Clontech), respectively. Subclones were generated by

*Corresponding author. Fax: +41 44 632 15 91.
E-mail address: ulrike.kutay@bc.biol.ethz.ch (U. Kutay).

Abbreviations: NE, Nuclear envelope; RNAi, RNA interference; FRAP, fluorescence recovery after photobleaching; INM, inner nuclear membrane; ER, endoplasmic reticulum; GFP, green fluorescent protein; LAP2β, lamina-associated polypeptide 2 beta

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PCR using the full-length clones as template. The green fluorescent protein-lamina-associated polypeptide 2 beta (GFP-LAP2β) clone was a gift of T.A. Rapoport (Boston, USA). HeLa cells were transfected using the FuGene transfection reagent (Roche). 24–48 h after transfection, cells were fixed in 1% paraformaldehyde for 10 min. To visualize GFP fusion proteins, coverslips were washed in PBS and mounted.

2.2. RNA interference, immunofluorescence, antibodies

Stable cell lines expressing UNC84A-GFP and GFP-LAP2β were transfected at 30% confluency with siRNAs specific for lamin A/C and lamin B1 using Oligofectamin (Invitrogen) [15,16]. After 72 h, indirect immunofluorescence was performed essentially as described [17]. The antibodies used were anti-lamin A/C (Novocastra), anti-lamin B1 (Zymed), anti-lamin B2 (LN43, abcam) and anti-mouse Texas-Red (Molecular Probes). The anti-β-tubulin antibody was from Sigma.

2.3. FRAP

Experiments were performed on a LSM510 confocal microscope equipped with an Ar 488 nm laser, a 500–550 nm bandpass filter and a 40×1.3 oil objective (Carl Zeiss). Regions of interest (ROI) were bleached, and subsequent fluorescence recovery was quantified over the ROI using the Zeiss LSM510 software.

3. Results

3.1. UNC84A localizes to the NE independently of its SUN domain

C. elegans UNC-84 as well as human UNC84B (Sun2) have been reported to localize to the NE [3,7]. The localization of human UNC84A was analyzed by expressing a GFP-tagged version in HeLa cells. As expected, the full-length protein (1–917) localized almost exclusively to the nuclear rim (Fig. 1). To address which parts of the coding region of UNC84A contribute to its nuclear rim localization, several deletion constructs were generated, each C-terminally fused to GFP (Fig. 1A). First, we deleted the conserved C-terminal SUN domain. This deletion mutant (1–723) localized exclusively to the nuclear rim, identical to the full-length protein (Fig. 1B).

3.2. Two different domains are required for proper localization of human UNC84A

According to the "Diffusion–Retention" model, INM proteins require interactions with the nuclear lamina or chromatin to be retained at the NE [12]. To identify putative nuclear retention domains within human UNC84A, a more extensive localization analysis of different deletion mutants was performed (Fig. 1B). Extending the C-terminal deletion to amino acid 500 (1–499) did not abolish nuclear rim localization of UNC84A, but the amount of protein found in membranes other than the NE (resembling ER and Golgi compartment) was significantly higher compared to the full-length protein. This finding suggests that the region between amino acids 500 and 723 of UNC84A weakly contributes to the localization of UNC84A to the NE.

Next, we examined the effect of N-terminal deletions. UNC84A-GFP lacking the first 200 amino acids (200–917) displayed nuclear rim localization and, in addition, localized outside the NE, in an ER/Golgi-like pattern, similar to the phenotype of the C-terminal deletion mutant (1–499). Combining this N-terminal deletion with the deletion of the SUN domain (200–723) showed the same phenotype. These findings argue for a second localization domain within the N terminus of UNC84A. The importance of both domains for proper

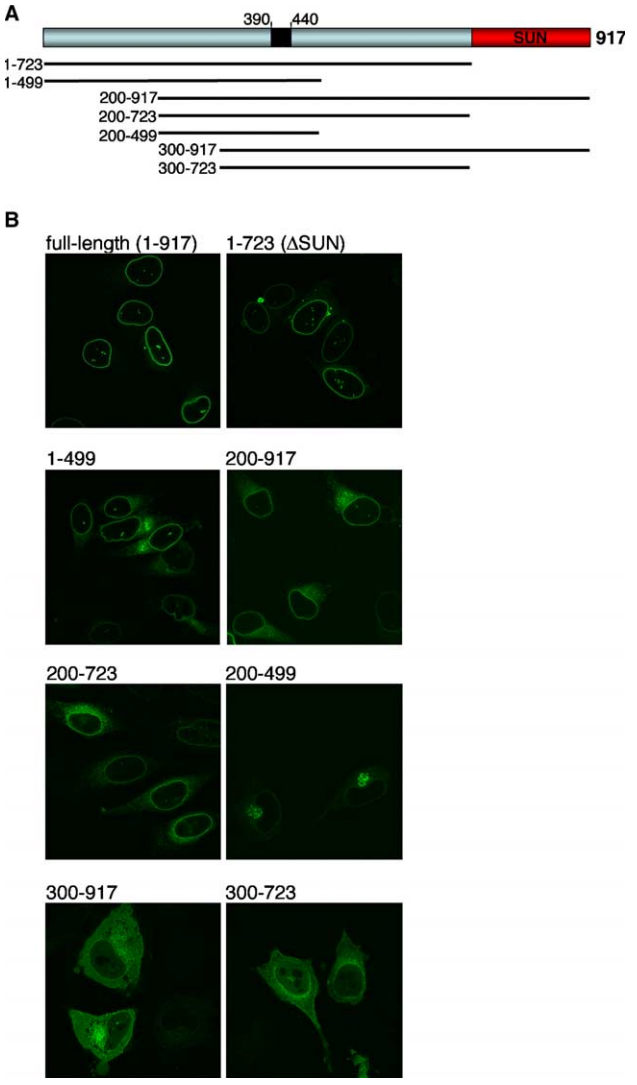

Fig. 1. Distinct regions in the N-terminal and C-terminal domains of human UNC84A contribute to NE localization. (A) Schematic representation of UNC84A depicting its conserved SUN domain (red) and hydrophobic regions comprising the transmembrane segment(s) (black). Black lines indicate the length of deletion constructs. (B) Transient transfection analysis of UNC84A-GFP deletion derivatives in HeLa cells. Pictures were obtained using confocal microscopy. Note that we analyzed the localization of these GFP fusion proteins in cells with both low and high GFP signal and found that localization was not severely altered by expression levels.

UNC84A targeting is underlined by the fact that the combined deletion of both domains (200–499) almost completely impaired NE localization. Instead, most of the protein was found in the Golgi.

The most striking effect on NE targeting was observed when the first 299 amino acids of UNC84A were deleted. This mutant (300–917) showed a very faint rim staining and most of the fusion protein was found in membranes outside the nuclear region, mostly within the ER and even at the plasma membrane. This demonstrates that the N-terminal domain of UNC84A is necessary for NE localization. As expected, additional deletion of the SUN domain (300–723) did not further contribute to mislocalization. Taken together, our data suggest that at least two distinct domains of UNC84A are required for exclusive nuclear rim localization. The N-terminal 299 amino