

Charakterisierung von NUANCE, einem Protein
der α -Aktinin Superfamilie

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Summary

NUANCE was identified as a protein with an α -actinin-like actin binding domain, 22 spectrin repeats and a transmembrane domain which is embedded in a so called *klarsicht* domain. A human 21 kb cDNA of NUANCE spreads over 373 kb on chromosome 14q22.1-q22.3 and predicts a protein of 798 kDa. At the subcellular level NUANCE is present predominantly at the nuclear membrane, in the nucleoplasm and in the vicinity of the nucleus in the cytoplasm. NUANCE binds F-actin *in vivo* and *in vitro*. The fusion protein 6xHis-ABD containing the actin binding domain shows actin bundling and actin polymerization activity. The dissociation constant $K_d = 3,8 \pm 1 \mu\text{M}$ of NUANCE is similar to the one of the closely related enaptin. Yeast two hybrid analysis shows a direct interaction with lamin A. Selective disruption of the lamin A/C network in COS7 cells using a dominant negative lamin B mutant resulted in the redistribution of NUANCE. Furthermore, the siRNA knockdown of lamins A/C in keratinocytes caused the dispersal of NUANCE from the nuclear envelope. Using biochemical techniques, we demonstrate that NUANCE binds directly to a C-terminal common region of lamins A/C implicating that NUANCE is also localised in the inner nuclear membrane. Experiments regarding the *klarsicht* domain of NUANCE show that this domain is sufficient for proper localisation and guidance to the nuclear envelope. GFP fusion proteins containing the *klarsicht* domain localise at the nuclear envelope, are able to displace endogenous NUANCE and cause a significant shrinkage of the nucleus. Using binding assays, we show that the luminal part of NUANCE and the nuclear envelope protein SUN1/UNC84 are interacting directly. Expression of dominant negative NUANCE constructs or NUANCE knockdown with RNAi in COS7 cells revealed that the presence of NUANCE at the nuclear envelope is necessary for the proper localization of emerin. Thus, NUANCE and enaptin are the first α -actinin related proteins that have the potential to link the microfilament system with the nucleus. The data imply a scaffolding function of NUANCE at the nuclear membrane and suggest a potential involvement of this protein in muscular dystrophies.