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A CH domain-containing N terminus in NuMA?

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Abstract

Nuclear mitotic apparatus protein (NuMA) is an essential vertebrate component in organizing microtubule ends at spindle poles. The NuMA-dynactin/dynein motor multiprotein complex not only explains the transport of NuMA along spindle fibers but also is linked to the process of microtubule focusing. The interaction sites of NuMA to dynein/dynactin have not been mapped. In the yet functionally uncharacterized N terminus of NuMA, we predict a calponin-homology (CH) domain, a motif with binding activity for actin-like molecules. We substantiate the primary sequence analysis-based prediction with secondary structure and fold recognition analysis, and we propose the N-terminal CH domain of NuMA as a likely interaction site for actin-related protein 1 (Arp1) protein of the dynactin/dynein complex.

Keywords: NuMA protein; mitotic spindle; nuclear matrix; calponin-homology domain; Arp1 binding; dynactin; dynein

Nuclear mitotic apparatus protein (NuMA) serves at least two cell cycle-dependent functions: (1) NuMA is an essential component for the formation and maintenance of mitotic spindle poles, as well as in microtubule focusing, and thus executes an integral mitotic function (Gaglio et al. 1996; Merdes et al. 1996). The NuMA protein is transported to the spindle poles by the minus end-directed motor dynein and the activator dynactin. (2) During interphase, NuMA is thought to be a nonessential nucleoskeletal element (Merdes and Cleveland 1998).

The architecture of the protein is similar to that of many others in microtubule-organizing structures: It consists of a globular head and tail regions separated by a coiled-coil segment known as the central rod domain (residues 213–1699 in human NuMA [accession No. S23647] predicted with the method of Lupas [1997]). The coiled-coil domain mediates dimer formation as a first step of oligomerization (Harborth et al. 1999). The globular tail region contains the nuclear targeting sequence (Gueth-Hallonet et al. 1996) and

binding sites for tubulin (Haren and Merdes 2002), protein 4.1 (Mattagajasingh et al. 1999), LGN (Du et al. 2001), and Cdc2-phosphorylation sites (Saredi et al. 1997). Other binding sites of NuMA, those for the dynein/dynactin complex (Merdes et al. 1996) and especially the actin-like actin-related protein 1 (Arp1; Clark and Meyer 1999), have not been sequentially mapped.

A calponin-homology fold in the NuMA N-terminal domain

Because the role of the N-terminal head domain is less well determined, we analyzed NuMA sequences in detail. The comparison of N-terminal sequences of NuMA orthologs with substitution models of known sequence domains indicates their similarity to the calponin-homology (CH) domain. If the first 120 amino acid residues are used as a query, the position-specific scoring matrix (PSSM) CH domain model (CDD-search; Marchler-Bauer et al. 2002) is hit in its full length by region 10–104 of human NuMA, with probability of false-positive prediction $E = 0.030$, and by the homologous region in *Sus scrofa* (BF08469), with $E = 0.039$. The CH domain model is also the top hit but with a higher E value for the N terminus of orthologs in fish, *Danio rerio* (BI984078); two frogs, *Xenopus laevis*

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Fig. 1. Alignment of calponin-homolog (CH) domain proteins with nuclear mitotic apparatus protein (NuMA) orthologs. Based on a structural alignment of CH domain sequences from T-fimbrin laoa (Goldsmith et al. 1997), dystrophin Idxx (Norwood et al. 2000), utrophin Iqag (Keep et al. 1999), and calponin α 1h67 (Bramham et al. 2002), known CH domain proteins from seven subfamilies (Gimona et al. 2002) and NuMA sequences have been aligned. NuMA protein sequences are conceptual translations from expressed sequence tags (except for human and *Xenopus laevis*); their numbering corresponds to the nucleotide sequence. The α -helices are labeled A through G in the standard manner. Residue property conservation indicated by PROMOTIF (Hutchinson and Thornton 1996). The position of helices in NuMA has been predicted applying JPRED2 (Cuff et al. 1998). Triangular markers indicate specific cases of residue conservation: The hydrophobic contacts between the parallel helices C and G dominate the structural scaffolds of the domain and form the hydrophobic core. Helix C is determined in its length by a fully conserved N-terminal capping D (position 41 in the alignment) and often by a P or G in its C-terminal (position 54). Helix A is packed against the central helices C and G via hydrophobic interactions (positions 8, 11, 12, 48, 125, and 128), with the highly conserved W11 intercalating between helices C and G. Other conserved hydrophobic residues (alignment positions 39, 45, 86, 90, 112, and 129) connect structural elements to the hydrophobic core.

(CAA68905) and *Silviana tropicalis* (AL628703); and *Mus musculus* (BB866521).

Further hints came from analyses of NuMA fold predictions. The consensus fold recognition method authored by Fischer (available at www.cs.bgu.ac.il/bioinbgu/; Fischer 2000) was used for assignment of the NuMA N terminus to the most closely related known three-dimensional fold (score of 12 being reported as the significance threshold). The CH domain fold represented by the structure of chicken calponin- α 1h67a (Bramham et al. 2002) was obtained as a significant first rank, by far the best hit in all six species studied with the consensus scores 28.4 (*S. tropicalis*), 27.4 (*X. laevis*), 20.7 (pig), 19.7 (mouse), 16.3 (human), and 11.2 (*Drosophila melanogaster*). This CH domain structure is the first (and only) structural investigation of a CH domain from a protein containing only a single CH domain.

The relatedness of N-terminal NuMA segments to the CH domain family is also supported by conservation of the hydrophobic pattern and of specific residues (markers in the structure-based alignment; see Fig. 1), as well as by positional coincidences of predicted secondary structures with those observed in CH domain crystal structures (see Figs. 1, 2). The architecture of the CH domain is all α -helical and dominated by four helices (A, C, E, and G), which are 11 to 18 residues in length and are connected by long loops. Three are roughly parallel, and the fourth is almost perpendicular to the others. Three short and less regular helices (B, D, and

F) can be found as minor structural elements (Bramham et al. 2002). NuMA proteins show common conservation of hydrophobic core residues that are likely key factors in stabilizing the CH domain's three-dimensional structure (see Figs. 1, 2).

Function of the NuMA N terminus

CH domain proteins show a high degree of functional variability. From sequence conservations and differences among eight classes of CH domain-containing proteins, Gimona et al. (2002) concluded that helices A and B and their interconnecting loop tend to contain the class-specific information for biological function, whereas the more C-terminal helices are suggested to contribute to the fixation of fold. Tandem CH domains in cytoskeletal and cytoskeleton-membrane linkage proteins are involved in binding of actin-like molecules. The hydrophobic sequences in helices A, F, and G of the first CH domain and in helix A of the CH domain 2 have been implied in forming the actin-binding site (Moores et al. 2000). The binding mode is less well understood for single CH domain proteins. The single CH domain protein IQGAP apparently binds actin as a dimer (Epp and Chant 1997). Additionally, single CH domain proteins are known to be involved in signaling processes in which they can show adaptor functions. This is the case for CaP family members that have an autonomous actin-binding site C-terminally to the CH domain (Gimona et al. 2002). The single CH domain of calponin was shown to be dispensable for actin interaction, but it is an important locator for the protein on actin filament and augments the actin-binding affinity of the other binding sites (Gimona and Mital 1998).

Experimental data on NuMA and the theoretical considerations reported here indicate a role for the N terminus in NuMA. Differential interactions with NuMA have been observed for wild-type and mutant Arp1 that could still recruit other dynactin/dynein complex subunits (Clark and Meyer 1999). Considering that Arp1 is an actin-related protein that has been shown to bind the tandem CH domain containing β III-spectrin (Holleran et al. 2001), an interaction between Arp1 and NuMA in the latter's CH domain region appears plausible. There are two possible scenarios: (1) Tandem CH domain arrangement is provided after dimerization of NuMA; and (2) possibly, a single CH domain may suffice for actin-like molecule binding (Hanein et al. 1998). Further, the secondary structure of residues 110–210 in human NuMA and of the homologous region in orthologs is predicted α -helical with long polar loops, and helical packing reminiscent of CH domains is not excluded. The complete binding site might be formed in the same manner as in tandem CH domain proteins involving helices from the segment 110–210 (Hanein et al. 1998; Gimona et al. 2002). Directed experiments can test these hypotheses.

Fig. 2. The hydrophobic core in the structure of 1H67 (Bramham et al. 2002). The cartoon of secondary structures is colored from the N terminus to the C terminus with a red-to-blue gradient. The structure is shown top-view down onto the parallel helices C and G. Residues with alignment positions 8, 11, 12, 35, 39, 45, 48, 86, 90, 112, 125, and 128 (Fig. 1), which are among the conserved hydrophobic residues in the domain core, are displayed in ball-and-stick mode (in violet). The α -helices and the strictly conserved Trp11 are labeled.

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