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Structural Features and Chaperone Activity of the NudC Protein Family

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The NudC family consists of four conserved proteins with representatives in all eukaryotes. The archetypal *nudC* gene from *Aspergillus nidulans* is a member of the *nud* gene family that is involved in the maintenance of nuclear migration. This family also includes *nudF*, whose human orthologue, Lis1, codes for a protein essential for brain cortex development. Three paralogues of NudC are known in vertebrates: NudC, NudC-like (NudCL), and NudC-like 2 (NudCL2). The fourth distantly related member of the family, CML66, contains a NudC-like domain. The three principal NudC proteins have no catalytic activity but appear to play as yet poorly defined roles in proliferating and dividing cells. We present crystallographic and NMR studies of the human NudC protein and discuss the results in the context of structures recently deposited by structural genomics centers (i.e., NudCL and mouse NudCL2). All proteins share the same core CS domain characteristic of proteins acting either as cochaperones of Hsp90 or as independent small heat shock proteins. However, while NudC and NudCL dimerize *via* an N-terminally located coiled coil, the smaller NudCL2 lacks this motif and instead dimerizes as a result of unique domain swapping. We show that NudC and NudCL, but not NudCL2, inhibit the aggregation of several target proteins, consistent with an Hsp90-independent heat shock protein function. Importantly, and in contrast to several previous reports, none of the three proteins is able to form binary complexes with Lis1. The availability of structural information will be of help in further studies on the cellular functions of the NudC family.

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Abbreviations used: NudCL, NudC-like; NudCL2, NudC-like 2; PDB, Protein Data Bank; HSQC, heteronuclear single-quantum coherence; SeMet, selenomethionine; CS, CHORD-Sgt1; NOE, nuclear Overhauser enhancement; MDH, malate dehydrogenase; ITC, isothermal titration calorimetry; GST, glutathione *S*-transferase; MBP, maltose binding protein; TEV, tobacco etch virus.

Introduction

The *nud* (*nu*clear *d*istribution) gene family was originally identified in the filamentous fungus *Aspergillus nidulans* (or *Emericella nidulans* in its sexual form) as a set of genes associated with dynein-dependent nuclear migration.[1–6] In a healthy organism, the nuclei migrate towards the growing tip of hyphae during vegetative growth,[5] whereas in mutants with impaired *nud* genes, the nuclei are clustered together following karyokinesis and are unable to undergo translocation.[2] The *nud* family became a focus of intense interest when it was discovered that one of its members, *nudF*, encoded a close homologue (42% amino acid sequence identity) of the mammalian Lis1 protein, which is mutated in a debilitating developmental genetic syndrome known as Miller–Dieker lissencephaly.[3,7–9] In afflicted individuals, the brain cortex is smooth (without typical groves or sulci) because the layer structure is partly or wholly disrupted due to the inability of young neurons to migrate from the ventricular zone to their target destinations. Thus, there has been considerable speculation that the nuclear migration pathway observed in *Aspergillus* has been evolutionarily conserved and acquired a new function in fetal brain development.

As expected, some of the *nud* genes code for the components of cytoplasmic motor dynein and dynactin, which are directly responsible for the translocation of the nucleus along microtubules.[10] Three *nud* genes do not belong to this group: *nudF*, *nudE*, and *nudC*. As already stated, the 45-kDa NudF is a homologue of the mammalian Lis1 protein, which is now known to be a dynein regulator and a component of the brain isoform of the platelet-activating factor acetylhydrolase II.[11,12] The NudE protein is represented in mammalian genomes by two paralogues, NudE and NudEL, currently renamed Nde1 and Ndel1.[13–15] Each protein, just over 300 residues in length, contains a conserved 160-residue-long parallel coiled-coil domain at the N-terminus, which binds the homodimeric Lis1.[16] The details of how this complex interacts with and regulates dynein are still being debated on, but recent data suggest that Lis1 alone, or with Nde1/Ndel1, induces a persistent force state in dynein.[17]

Of all the *nud* gene products, NudC has been the most elusive with respect to function. Deletion of the *nudC* gene in *Aspergillus* produces a severe phenotype with a much thicker cell wall compared to wild type.[18] Recently published data suggest that NudC and NudF form a complex in the fungus that is essential for the proper functioning of spindle pole bodies.[19] Orthologues of NudC have been identified in higher eukaryotes, including *Caenorhabditis elegans*,[20] *Drosophila melanogaster*,[21] amphibians (newt),[22] and mammals. In most—if not all—Metazoa, three paralogues are found: hNudC,[23,24] hNudC-like (NudCL, also annotated as NudC domain containing protein 3),[25] and hNudC-like 2 (NudCL2, or NudC domain containing protein 2).[26] The lengths of polypeptide chains differ among the three, but they all contain a single globular domain with significant amino acid sequence conservation.[27] A similar domain is also found in the fourth member of this family, NudCD1 (NudC domain containing protein 1).[28,29] This enigmatic protein (also known as CML66) is a tumor antigen that has been implicated in stimulating tumor cell proliferation, invasion, and metastasis, but its function is unknown.[28]

The physiological functions of the mammalian NudC paralogues are far from understood. The vast majority of the reported research focused on NudC. It is expressed in all tissues, both in the fetus and in the adult organism,[24] but at a particularly high level in the cell lines and proliferating cells of normal tissues,[30] indicating a possible role in cell division. Indeed, downregulation of human NudC mRNA results in impairment of both cell proliferation and mitotic spindle formation.[31] There is also evidence that NudC is involved in cytokinesis in a phosphorylation-dependent fashion.[32–34] As might be expected, a protein involved in mitotic cell division is also found to play a role in cancer. For example, there is an inverse correlation between NudC expression and nodal metastasis in esophageal cancer,[35] and an adenovirus expressing NudC was able to inhibit the growth of prostate tumors by blocking cell division.[36] In apparent contrast, NudC was identified as one of the overexpressed genes in cutaneous T-cell lymphoma[37] and was found to be expressed in neuroectodermal tumors, but not in nonneoplastic brain tissues.[38] Moreover, high expression levels were associated with cells infiltrating white matter or undergoing division.[38]

Unfortunately, the details of the specific molecular mechanisms in which NudC is involved are very elusive. Several reports implicate NudC in direct interactions with Lis1;[39–42] others suggest interactions with kinesin-1[43] and polo-like kinase.[32,44] A recent study lists 131 (sic) proteins identified by mass spectrometry as potential binding partners of NudC.[40] Furthermore, there is evidence that NudC may function as a chaperone, effectively stabilizing its target proteins or enhancing folding. This potentially includes both the Hsp90-dependent pathway[40] and direct chaperone activity.[40,44] Finally, there is a set of studies exploring the hypothesis that NudC is a secreted protein that functions by binding to the extracellular domain of the thrombopoietin receptor.[45–50]

The remaining two paralogues, NudCL and NudCL2, were discovered only recently and are just beginning to attract attention. So far, NudCL has been implicated in enhancing the stability of the dynein intermediate chain,[25] while NudCL2 has