A new protein interaction network involving the DNA damage sensing protein kinase NEK1 and the putative transcriptional regulator FEZ1

Eliana M. Assmann, Marcos R. Alborghetti, Maria E. R. Camargo, and Jörg Kobarg

Laboratório Nacional de Luz Síncrotron, Centro de Biologia Molecular Estrutural, 13084-971 Campinas-SP, Brasil

NEK protein kinases are evolutionarily conserved kinases structurally related to the *Aspergillus nidulans* mitotic regulator NIMA. To date eleven members of the NEK family have been described in vertebrates. The pleiotropic deleterious effects including the formation of kidney cysts caused by NEK1 mutation in mice emphasize its involvement in the regulation of diverse cellular processes and especially in the etiology of polycystic kidney disease (PKD). In a previous study we identified NEK1 interacting proteins by the yeast two-hybrid system. These take part in the development of PKD (KIF3A, tuberin, alpha-catzulin), in the double-strand DNA break repair at the G2/M transition phase of the cell cycle (ATRX, MRE11) or in neuronal cell development (FEZ1). FEZ1 (Fasciculation and elongation protein zeta 1) is a mammalian orthologue of the *C. elegans* protein UNC-76, necessary for axon growth. FEZ1 was reported to interact with PKCzeta, DISC1, the agnoprotein of polyomavirus JC virus and E4B. We identified 16 proteins that interact with human FEZ1(221-396) in a yeast two-hybrid assay. Some of these proteins take part in transcription regulation (DRAP1, BAF60a, SAP30L, Tlk2, Bromodomain containing protein 1, Zn-Finger 251) and neuronal cell development (FEZ1, RAB3A GTPase activating protein). We were able to confirm 8 interactions by pull down assays and found that FEZ1 coiled-coil region is necessary for its dimerization, and majority of protein interactions (10/16). Our results emphasize the functional involvement of FEZ1 in neuronal development, but suggest further, that FEZ1 may also be involved in a transcriptional control, which may prove critical for neuronal cell differentiation. Furthermore, it was shown in a recent study that NEK1 expression and activity are up-regulated upon ionizing radiation induced DNA damage. The overall scenario of the newly discovered protein-protein interaction network of regulatory proteins described here, may suggest that FEZ1 among other NEK1 interacting proteins, could be substrates for phosphorylation by NEK1. Future functional and structural studies will address the way by which phosphorylation through NEK1 may regulate the activity of the interacting proteins in the cell and what are the roles of both the coiled-coiled regions and the dimerization of both NEK1 and FEZ1.

Financial support: FAPESP, CNPq, LNLS.