Title: The role of sulfur-transferring proteins associated with the centrosome

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Sulfur is an essential element to all living organisms. In the last years a lot of progress has been made towards the understanding of sulfur transfer in the cells and the major enzymes involved in these pathways including cysteine desulfurases and rhodanese homology domain proteins. These enzymes are characterized by a persulfide group (R-S-SH) on a conserved cysteine residue that serves as a sulfur donor for the biosynthesis of sulfur-containing cofactors like iron-sulfur clusters, thiamin, biotin, lipoic acid and molybdopterin in addition to the thio modification of tRNA. In eukaryotes, the L-cysteine desulfurase NFS1 plays a versatile role and is crucial for mitochondrial iron-sulfur cluster biosynthesis, for thio-modification of mitochondrial and cytosolic tRNAs and for molybdenum cofator biosynthesis in the cytosol. The majority of NFS1 is localized in mitochondria for its main function in iron-sulfur cluster biosynthesis. While the nuclear localization is not completely clear so far, the cytosolic localization has only recently been described for its role outside of FeS cluster biosynthesis. In addition to its localization in mitochondria, the nucleus and the cytosol, NFS1 was recently also mapped on the centrosome. The centrosome is the main microtubule-organizing center in animal cells, fungi and lower eukaryotes. As such it is of primary importance for organelle positioning and the fidelity of chromosome segregation during mitosis. In addition, the centrosome regulates cytokinesis and cell cycle progression through G1. Although proteomic approaches have recently revealed an impressive number of centrosomal components (~100 different proteins), the functional role of the majority of these proteins is still unknown. NFS1 is not the only sulfurtransferase found at the centrosome. The rhodanese-like protein Cep41, which is involved in ciliary diseases and required for tubulin glutamylation at the primary cilium was also localized at the centrosome. This suggests that sulfurtransfer plays a role in centrosomal functions.
In this project we want to investigate the proposed role of sulfurtransferases in centrosomal function. In human cells, we will investigate the cell cycle-dependent localization of NFS1 and Cep41 by light microscopy. We will suppress expression of these proteins by RNAi and analyze the consequences on cell division and cell cycle progression. We will also create point mutations in the catalytic center of these proteins in order to assess the consequences of the lack of sulfurtransferase activity while proteins are still present at the centrosome. Our analyses will be complemented by a comparative approach where we will investigate orthologues of centrosomal sulfurtransferases in Dictyostelium amoebae, whose centrosome is functionally similar to that of higher cells but complety different in its ultrastructure.

Required background:
Basic methods in molecular biology, protein biochemistry and cell biology, such as cloning, PCR, transfection, cell culture, light microscopy.

Paper:
Marelja, Z., Stöcklein, W., Nimtz, M., and Leimkühler, S. (2008). J Biol Chem *283*, 25178-25185.
Chowdhury, M.M., Dosche, C., Löhmannsroben, H.G., and Leimkühler, S. (2012). J Biol Chem *287*, 17297-17307.
Kuhnert, O., Baumann, O., Meyer, I., and Gräf, R. (2012). Cell Mol Life Sci *69*, 3651-3664.
Kuhnert, O., Baumann, O., Meyer, I., and Gräf, R. (2012). Cell Mol Life Sci *69*, 1875-1888.
Lee, J.E., et al. (2012). Nature genetics *44*, 193-199.

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