NMR AND STRUCTURAL CHARACTERIZATION OF NUDC, A CO-CHAPERONE INVOLVED IN CLIENT-TRANSFER BETWEEN HEAT SHOCK PROTEINS

F. DELHOMMEL [1], M. BIEBL [2], M. SATTLER [1], J. BUCHNER [2]

[1]Bavarian NMR Center, Technical University of Munich / Institute of Structural Biology, Helmholtz Zentrum München - München (Germany), [2]Center for Integrated Protein Science, Technical University of Munich - München (Germany)

One of the primordial requirement for cell survival is to ensure the correct folding of all proteins under changing environment conditions. Hence, all organisms possess intricate systems that safeguard proteostasis and cell homeostasis. Among others, this function is carried out by molecular chaperones that assist protein clients in acquiring their native, active form. Hsp70 and Hsp90 organize large molecular machines that are central to the chaperoning activity. These two heat shock proteins work in concert: First, clients are recognized by Hsp40 which recruits them to Hsp70. The chaperones then initiate a cycle of unfolding-refolding that drives clients toward their natively folded state. Specific meta-stable clients interact with the Hsp70-Hsp40 complex and are subsequently transferred to Hsp90 by the co-chaperone HOP. However, recent data suggest that there may be additional factors than can mediate the transfer and functionally replace HOP.

Our present work focuses on NudC, a 40 kDa protein a putative co-chaperone of Hsp90 composed of a N-terminal helical dimerization region and a so-called CS domain. Biochemical approaches were combined with backbone amide NMR spectroscopy to characterize the interaction of NudC with Hsp90, model the complex and validate it in the full length, methyl-labeled proteins. A similar strategy coupling 15N-labeled truncated constructs and methyl-labeled full-length proteins was further used to determine that NudC interacts also with multiple Hsp40 co-chaperones and with the client GR, but not with Hsp70. In addition to extensive solution experiments, our crystal structure of the NudC-Hsp40 complex revealed that a structured motif of NudC directly competes with Hsp70 for Hsp40 binding. Finally, methyl-labeling was used to confirm that the newly identified binding sites of NudC simultaneously interacts with the Hsp40, its bound client GR, and with Hsp90.

Consistent with our structural data, the presence of NudC drastically increases client activation by accelerating the transfer from the Hsp40-Hsp70 system to Hsp90, even in absence of HOP. This was further confirmed in cell assays in which NudC was shown to be essential for survival as well as for GR activation.

Thus, our structural, biochemical and in cell data demonstrate a new, unexpected chaperone transfer mechanism that is independent of either Hsp70 or HOP, in which NudC recruits Hsp40-bound clients and directly connects to Hsp90 for the final step of their maturation.