A thiol-disulfide switch in the regulation of cytoskeletal dynamics.

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Redox modifications of protein cysteinyl residues “protein thiol switches” specifically and reversibly switch proteins between different conformational and functional states and thereby control numerous cellular functions (1). We have previously shown that a cytosolic isoform of the vertebrate-specific oxidoreductase glutaredoxin 2 (Grx2c, refs 2,3) is essential for brain development (4). We have identified CRMP2, a mediator of semaphorin/plexin signaling, as redox-regulated target and demonstrated that this regulation is required for normal axonal outgrowth and brain development (5,6). Our preliminary results demonstrate that the CRMP2 redox switch does not only control neuronal development, but also cell migration, invasion and cytoskeletal dynamics in general. We demonstrate a specific and reversible intermolecular Cys504-Cys504 thiol-disulfide switch in homo-tetrameric CRMP2. This switch determines two conformations of the quaternary CRMP2 complex. Our work in progress provides also evidence that the semaphorin/plexin-activated flavin monooxygenases of the MICAL family could be responsible for oxidation of CRMP2 thiols, while Grx2c catalyzes the reduction. We therefore propose one of the first thiol-disulfide switches operated by a specific oxidase and a specific reductase, comparable to kinase/phosphatase pairs controlling protein functions. Within the strong network that will be provided by the Priority Program 1710, we aim at clarifying the molecular mechanism of this redox switch. More specifically, we will clarify whether MICAL proteins operate the CRMP2 thiol-disulfide switch as specific oxidases (task 1) and if this occours in response to semaphorin

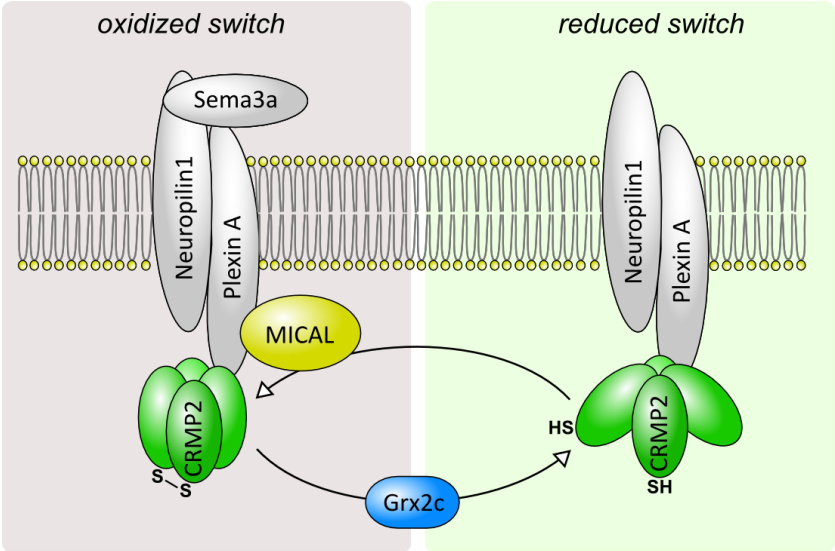

Figure 1: The proposed redox switch in CRMP2 controlled by a specific reductase and oxidase.

signaling (task 2). We shall clarify the molecular mechanism of CRMP2 oxidation (task 3). We will identify the molecular basis of the thiol disulfide switch and how this affects the interaction of CRMP2 with downstream effectors (task 4). We propose to investigate the intersection of this thiol-disulfide switch with phosphorylation signaling cascades in the regulation of cytoskeletal dynamics using quantitative proteomic approaches (task 5). And, we will investigate the role of the regulatory iron-sulfur cluster in Grx2 (7,8) in the regulation of CRMP2 activity and cytoskeletal dynamics (task 6). Our project aims at deciphering the precise biochemical mechanism of this thiol-disulfide switch, how it is specifically operated *in vivo*, and how this affects the protein's function and interaction with other proteins and signaling pathways, i.e. its physiological role.

Scientists involved in the project

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