The nuclear envelope protein, LAP1B, is a novel Protein Phosphatase 1 substrate

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Reversible protein phosphorylation is a major mechanism controlling key intracellular events that are essential for cell health and viability. This process involves the action of both protein kinases and protein phosphatases. Among the protein phosphatases, protein phosphatase 1 (PP1) is a ubiquitous serine/threonine phosphatase that is estimated to dephosphorylate about one third of all proteins in eukaryotic cells. Therefore, PP1 is involved in many cellular events including glycogen metabolism, transcription, protein synthesis, cellular division and meiosis. The versatility of PP1 is largely determined by the binding of its catalytic subunit to different regulatory subunits, which are responsible for the targeting of PP1 to a particular subcellular compartment and also determine its substrate specificity and activity. To date, more than 200 PP1 regulators have been described and most of them interact with the PP1 catalytic subunit through a conserved PP1 binding motif termed the RVxF motif.

Recently, we described a novel PP1 binding protein, the lamina associated polypeptide 1B (LAP1B), which belongs to a family of integral proteins of the inner nuclear membrane. The function of LAP1B is poorly understood but it is known that it binds to lamins and chromosomes and is phosphorylated during interphase and mitosis. Moreover, LAP1 was found to interact with torsinA, this is a central protein in the neurological movement disorder known as DYT1 dystonia.

The interaction between LAP1B and PP1 isoforms (PP1α and PP1γ) was validated using in vitro and in vivo techniques. The LAP1B:PP1 complex was immunoprecipitated from cultured cells and rat cortex and further validated by yeast co-transformation (Figure 1A and B). PP1, which is enriched in the nucleus, binds to the N-terminal nuclear domain of LAP1B, as shown by immunocolocalization (Figure 1C). The relevant domains for this interaction were also identified. Further, the PP1 binding motif responsible for the interaction was also mapped (REVRF). Functionally this complex determines LAP1B phosphorylation state in vitro (Figure 2). These findings place PP1 at a key pivotal position, participating in the pathogenesis of DYT1 dystonia and related nuclear envelope-based diseases.

FIGURE 1
Validation of the LAP1B:PP1 interaction. A- Co-immunoprecipitation of the LAP1B:PP1 complex in rat cortex. B- Yeast co-transformation assay in SD/QDO/X-α-gal medium. Full-length LAP1B and its deletion mutants are represented. PP1 binding domains are identified; red boxes - RVxF motifs, green boxes - SILK motif, yellow boxes - transmembrane domain. Positive interactions between PP1γ isoform and LAP1B are detected by the presence of blue yeast colonies. C- Subcellular distribution of the LAP1B:PP1 complex in HeLa cells. Green - MycLAP1B; Red - endogenous PP1α, Blue - Nucleus.

FIGURE 2
LAP1B dephosphorylation by PP1 in vitro. SH-SY5Y cells were incubated with okadaic acid (OA, protein phosphatase inhibitor) and immunoprecipitated with LAP1 antibody. Immunoprecipitates were incubated with or without 100 ng of PP1γ1 protein. IP, immunoprecipitation. IB, immunobloting.