Poster Presentation

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PRL phosphatases and their interactions with CNNM magnesium transporters

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The phosphatases of regenerating liver (PRLs) are highly over-expressed in metastatic cancers yet their mechanism of action is poorly understood. The PRL family belongs to the PTP (protein tyrosine phosphatase) superfamily and is comprised of three closely related proteins: PRL1, PRL2, and PRL3. They all contain a C-terminal prenylation site and a single catalytic domain of roughly 170 amino acids. Like other members of the family of protein tyrosine phosphatases, their phosphatase activity occurs through a two-step catalytic cycle involving the transient phosphorylation of a catalytic cysteine residue. In PRL phosphatases, this intermediate is extremely long-lived leading to the accumulation of a cysteine-phosphorylated form of the enzyme both in vitro and in cells [1,2]. While a number of different cellular substrates have been proposed, there is no consensus about their physiological substrate due, in part, to their slow rate of overall catalysis. The catalytic cysteine of PRLs readily forms a disulfide with the adjacent cysteine residue, which further decreases their effectiveness in dephosphorylating physiological substrates [1]. Recently, CNNM proteins, a family of membrane proteins involved in magnesium homeostasis, were identified as PRL-binding partners [3]. Disruption of the PRL-CNNM interaction promotes tumor formation and invasiveness in animal and cellular models, strongly suggesting that the physiological function of PRLs is to regulate CNNM magnesium transport.

Here, we determined five crystal structures of PRL3 or PRL2 bound to the CBS-pair domain of CNNM3. In the structures, the CBS-pair domain is present as a dimer in the head-to-head arrangement that is typical for other CBS-pair domains. The CNNM3 CBS-pair domain contains a long loop that extends away from the dimerization interface and contacts the PRL catalytic site. The side chain of aspartic acid 426 sits in the pocket formed by the phosphatase P-loop and WPFDD motif and likely mimics the negatively charged phosphate of a bound substrate. Mutagenesis showed that Asp426 of CNNM3 is required for high affinity binding, suggesting that the CNNM CBS-pair domain might act as a pseudo-substrate. Addition of the CBS-pair domain inhibited phosphatase activity and CNNM3 binding was blocked by phosphorylation of the PRL active site cysteine. In addition to the many polar contacts, Pro427 and Tyr429 provide a hydrophobic surface that contacts Leu105 of PRL2 (Leu108 of PRL3). The structures reveal why disulfide formation dramatically decreases binding affinity and confirm that all three PRLs bind to CNNMs. We used isothermal titration calorimetry (ITC) experiments and extensive mutagenesis to probe the importance of PRL residues for CNNM binding. Comparison of binding activity and in vitro phosphatase activity shows that they are strongly correlated with the notable exception of the PRL3 R138E mutant which showed weak CNNM3 binding but normal phosphatase activity. These results support the hypothesis that PRLs function as pseudophosphatases in regulating the action of CNNM proteins in cancer.

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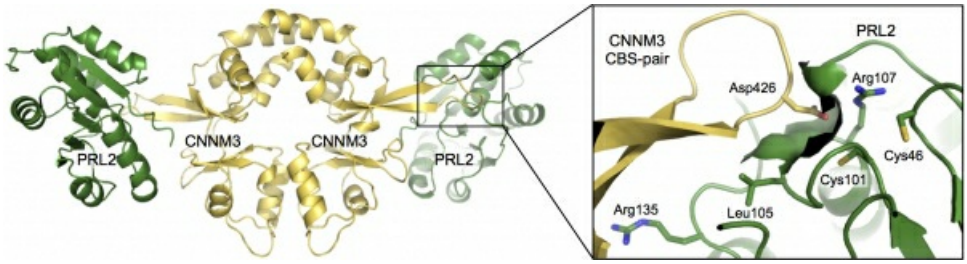

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