BioCreative VI PM Task: Mining protein interactions and mutations for precision medicine

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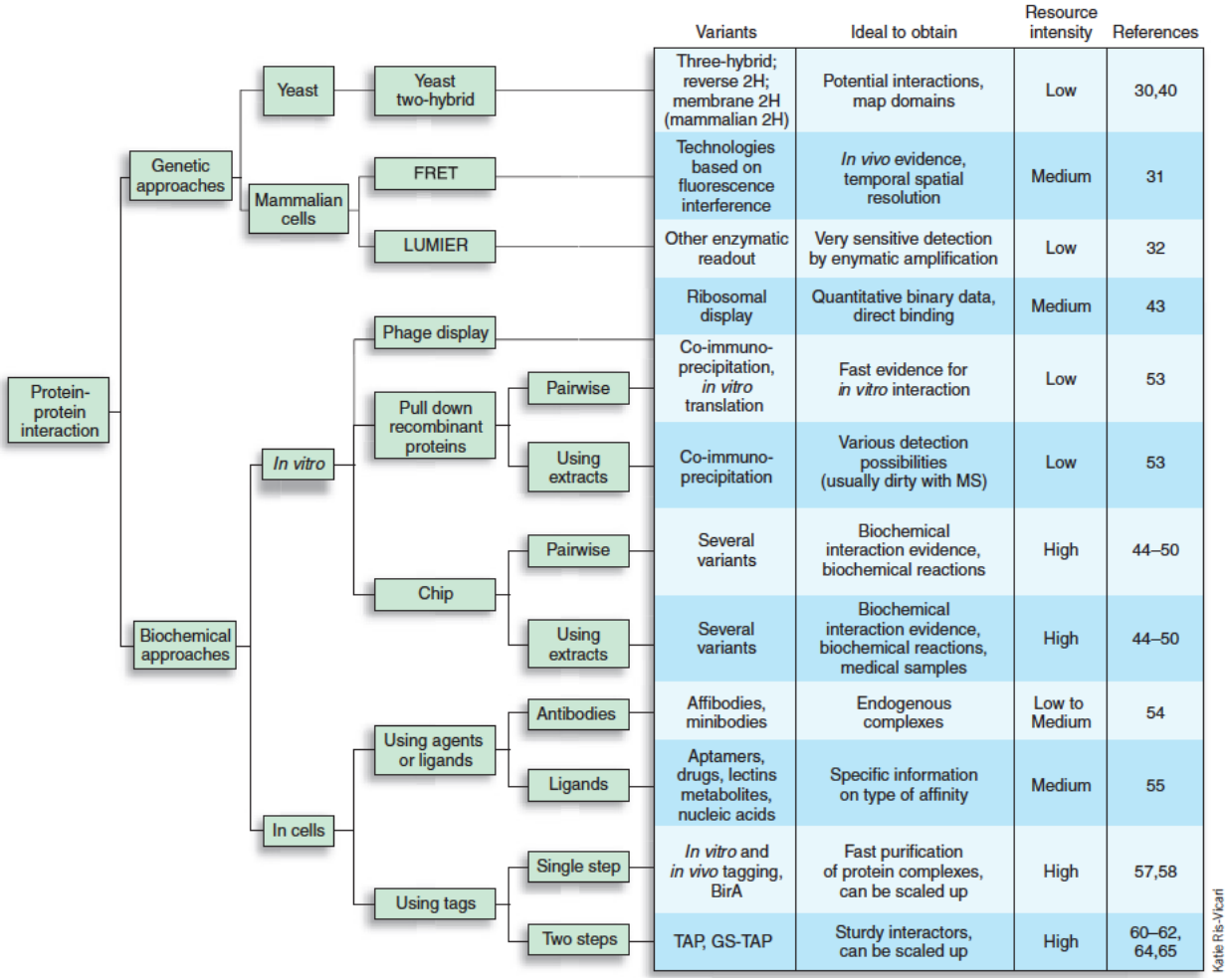
March 2017

Experimental Approaches for the Identification of Protein-Protein Interactions (PPI)

Protein interactions can be studied in both in vivo and in vitro systems. While in vitro models are more suitable for the identification of direct interactions, in vivo experiments allow to study the interactions in a biological context.
Below is a summary of the most common experimental approaches employed in the detection of PPIs.



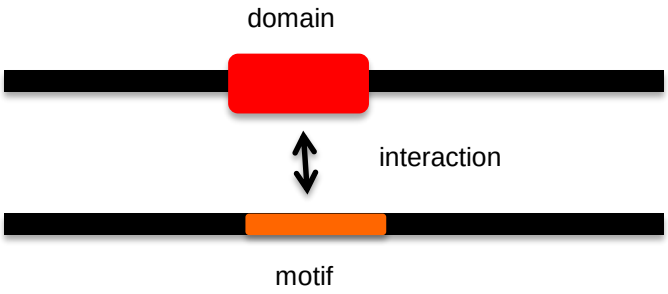
Variants	Ideal to obtain	Resource intensity	References
Three-hybrid; reverse 2H; membrane 2H (mammalian 2H)	Potential interactions, map domains	Low	30,40
Technologies based on fluorescence interference	*In vivo* evidence, temporal spatial resolution	Medium	31
Other enzymatic readout	Very sensitive detection by enymatic amplification	Low	32
Ribosomal display	Quantitative binary data, direct binding	Medium	43
Co-immuno-precipitation, *in vitro* translation	Fast evidence for *in vitro* interaction	Low	53
Co-immuno-precipitation	Various detection possibilities (usually dirty with MS)	Low	53
Several variants	Biochemical interaction evidence, biochemical reactions	High	44–50
Several variants	Biochemical interaction evidence, biochemical reactions, medical samples	High	44–50
Affibodies, minibodies	Endogenous complexes	Low to Medium	54
Aptamers, drugs, lectins metabolites, nucleic acids	Specific information on type of affinity	Medium	55
In vitro and *in vivo* tagging, BirA	Fast purification of protein complexes, can be scaled up	High	57,58
TAP, GS-TAP	Sturdy interactors, can be scaled up	High	60–62, 64,65

Kocher & Superti-Furga, Nature Methods (2007)

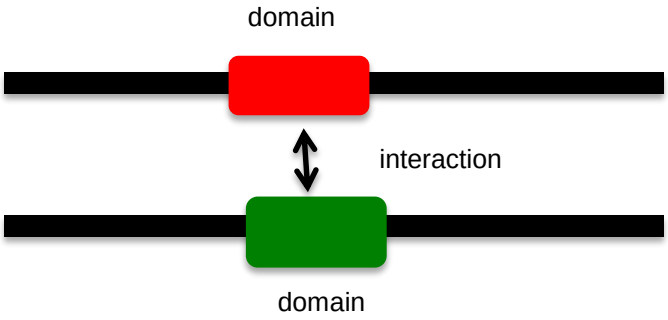
Regulation of Protein-Protein Interactions (PPI) – 1

PPI occurs mainly between protein regions carrying domains or linear motifs and can be regulated by post translational modifications.

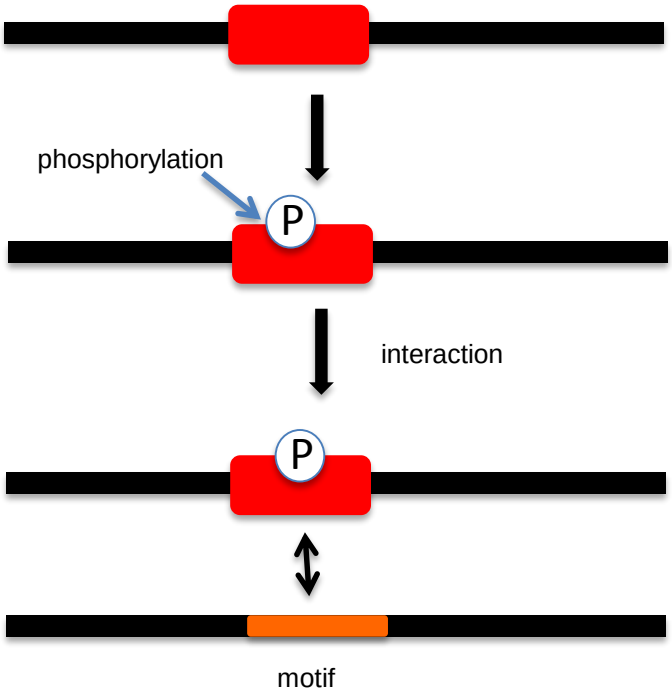
A) Domain-linear motif interaction



B) Domain-domain interaction



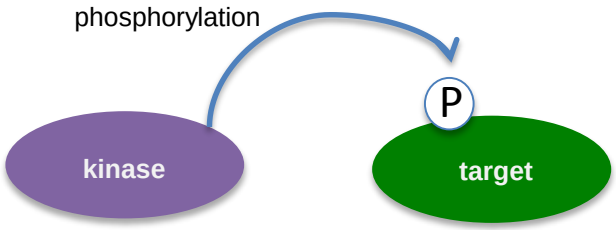
C) Interaction depending on phoshorylation



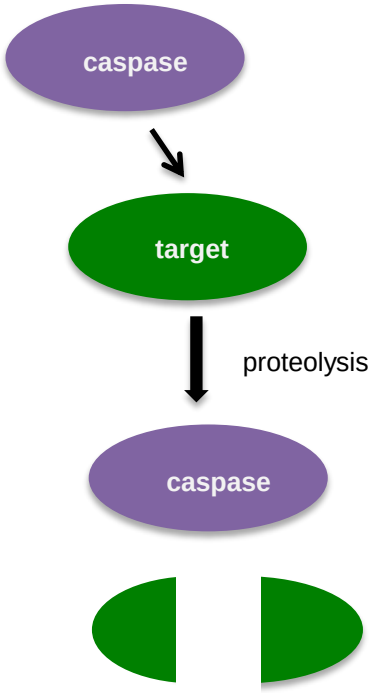
Regulation of Protein-Protein Interactions (PPI) – 2

Also enzymatic activities and aggregates are considered PPI

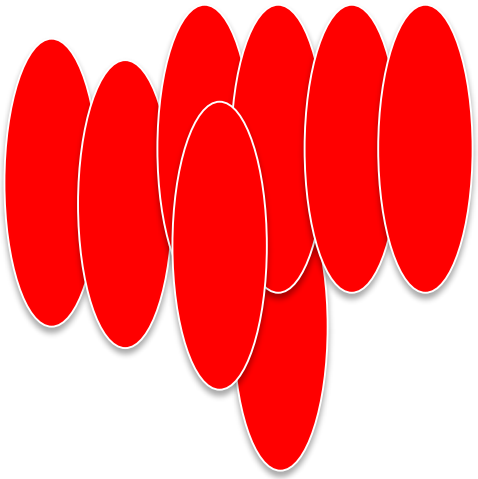
A) Phosphorylation



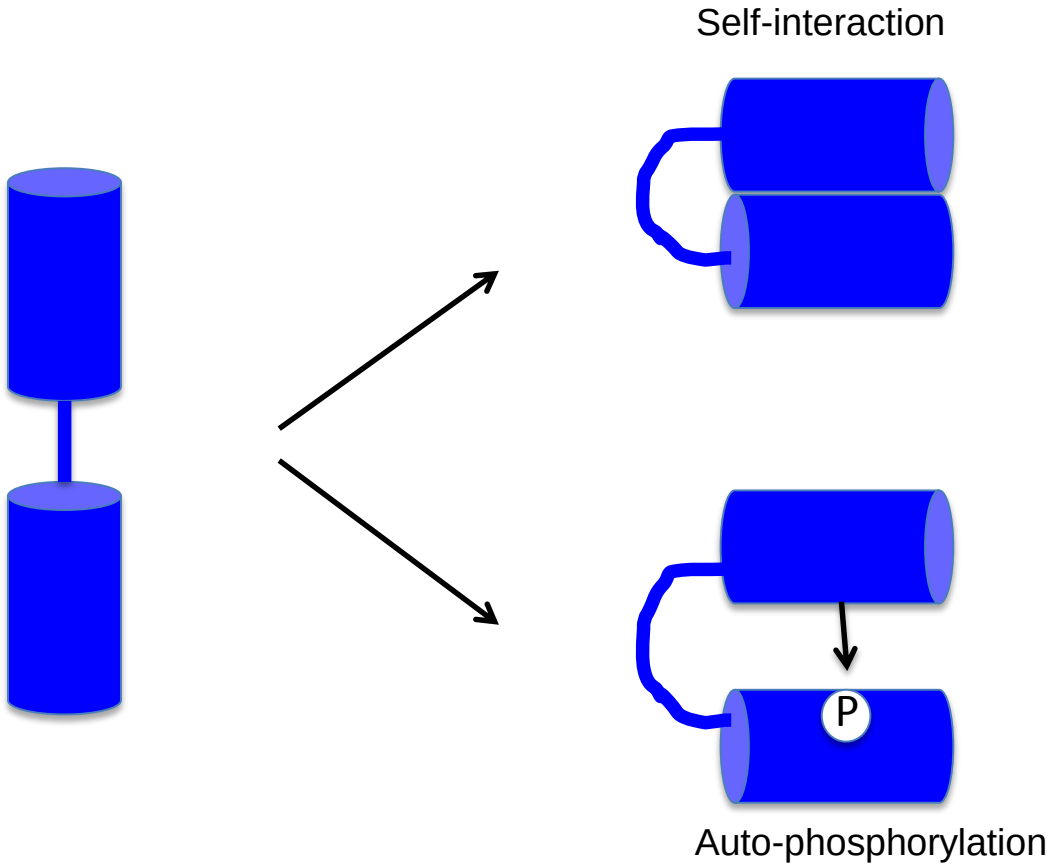
B) Proteolysis



C) Aggregates

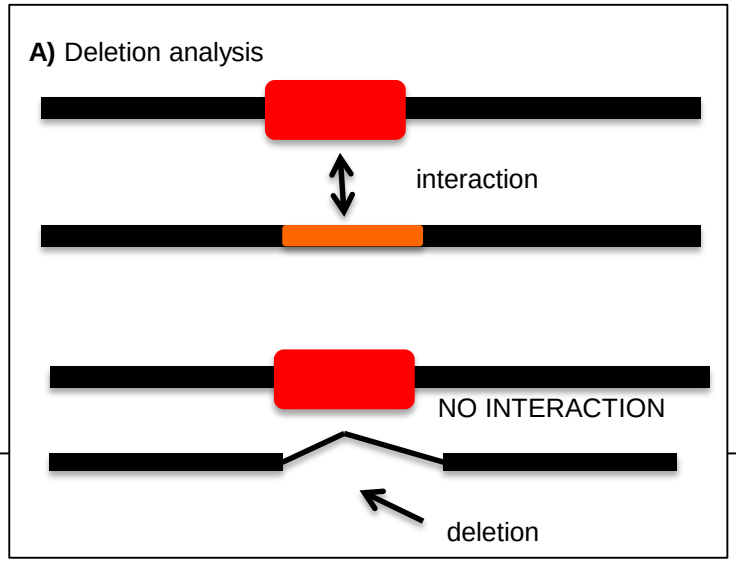


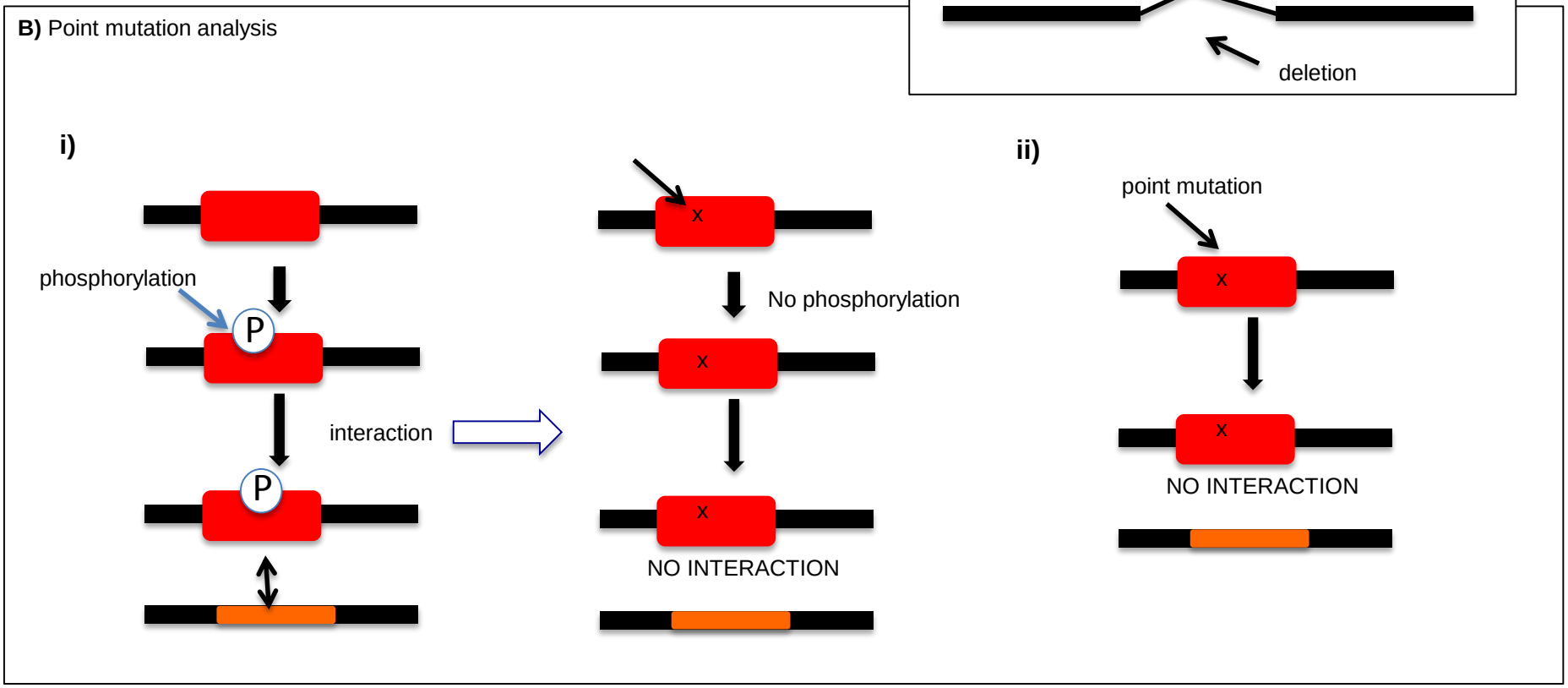
Self Interactions



How are regions crucial for PPI identified?

1- “Direct” methods like x-ray crystallography
2- Mutational analysis:
    A) Deletions
    B) Point mutations





How are these concepts addressed in the text?

PMID	ABSTRACT
21751375	In the Wnt/beta-catenin pathway, p300/CBP (CREB-binding protein) is recruited by nuclear beta-catenin to regulate a wide array of T-cell factor (TCF)-dependent gene expression. Previous studies have indicated that CBP/beta-catenin complex-mediated transcription is critical for cell proliferation. Both CBP and beta-catenin are phosphoproteins. The interaction domain has been mapped to the N-terminal region of CBP (amino acids 1-111) and the C-terminal region of beta-catenin, but it is unclear whether phosphorylation on specific residues of these regions is required for the interaction. To address this unmet challenge, phosphoproteomic profile of the critical N-terminus of CBP was determined by utilizing TiO(2) affinity chromatography followed by LC-MS/MS analysis. Two unique and novel phosphorylation sites Ser77 and Ser92 were identified. Further studies aided by site-directed mutagenesis, immunoprecipitation and mammalian two-hybrid assay have concluded that the phosphorylation of a Proline-directed Ser92 residue modulates the selective binding ability of CBP with beta-catenin. The specific Mitogen-activated protein kinase inhibitor PD98059, which promotes cell cycle G1 arrest, concomitantly inhibits the interaction, and the evidences suggest that the MEK/ERK (extracellular signal-regulated kinase) cascade activation is the upstream signal required for Ser92 phosphorylation, leading to enhancement of the interaction of CBP with beta-catenin. CI - Copyright (c) 2011 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
21763312	VirB8 is a critical component of the Brucella suis type IV secretion system (T4SS). We previously showed that the transmembrane (TM) domain plays an essential role in interactions of this protein with itself and the other proteins of the T4SS. We report that a point mutation in this TM domain stabilizes homodimers of VirB8 and heterodimers with VirB10. A similar variant of Agrobacterium tumefaciens VirB8 showed the same phenotype. The B. suis VirB8 variant was unable to complement a virB8 mutant and displayed a dominant negative phenotype when expressed in wild type B. suis. We suggest that interaction of VirB8 with VirB10 could play a major role in the correct function of the B. suis VirB T4SS. CI - Copyright (c) 2011 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved
21763481	Nearly every ciliated organism possesses three B9 domain-containing proteins: MKS1, B9D1, and B9D2. Mutations in human MKS1 cause Meckel syndrome (MKS), a severe ciliopathy characterized by occipital encephalocele, liver ductal plate malformations, polydactyly, and kidney cysts. Mouse mutations in either Mks1 or B9d2 compromise ciliogenesis and result in phenotypes similar to those of MKS. Given the importance of these two B9 proteins to ciliogenesis, we examined the role of the third B9 protein, B9d1. Mice lacking B9d1 displayed polydactyly, kidney cysts, ductal plate malformations, and abnormal patterning of the neural tube, concomitant with compromised ciliogenesis, ciliary protein localization, and Hedgehog (Hh) signal transduction. These data prompted us to screen MKS patients for mutations in B9D1 and B9D2. We identified a homozygous c.301A>C (p.Ser101Arg) B9D2 mutation that segregates with MKS, affects an evolutionarily conserved residue, and is absent from controls. Unlike wild-type B9D2 mRNA, the p.Ser101Arg mutation failed to rescue zebrafish phenotypes induced by the suppression of b9d2. With coimmunoprecipitation and mass spectrometric analyses, we found that Mks1, B9d1, and B9d2 interact physically, but that the p.Ser101Arg mutation abrogates the ability of B9d2 to interact with Mks1, further suggesting that the mutation compromises B9d2 function. Our data indicate that B9d1 is required for normal Hh signaling, ciliogenesis, and ciliary protein localization and that B9d1 and B9d2 are essential components of a B9 protein complex, disruption of which causes MKS. CI - Copyright (c) 2011 The American Society of Human Genetics. Published by Elsevier Inc. All rights reserved
19236849	Previous analyses of the sirtuin family of histone deacetylases and its most prominent member SIRT1 have focused primarily on the identification of cellular targets exploring the underlying molecular mechanisms of its implicated function in the control of metabolic homeostasis, differentiation, apoptosis and cell survival. So far, little is known about the regulation of SIRT1 itself. In the study presented herein, we assigned the main region of SIRT1 in vivo phosphorylation to amino acids 643-691 of the unique carboxy-terminal domain. Furthermore, we demonstrate that SIRT1 is a substrate for protein kinase CK2 both in vitro and in vivo. Both, deletion construct analyses and serine-to-alanine mutations identified SIRT1 Ser-659 and Ser-661 as major CK2 phosphorylation sites that are phosphorylated in vivo as well
18769030	GABAergic and glycinergic function is dependent on neuronal intracellular chloride. The neuron-specific electroneutral potassium (K(+)) and chloride (Cl(-)) cotransporter (KCC2), is a key regulator of neuronal Cl(-), yet little is known about KCC2 regulation. Using yeast two-hybrid, we identified Protein Associated with Myc (PAM) as a binding partner of KCC2. The RCC1 (Regulator of Chromatin Condensation) domain of PAM binds to the carboxyl terminus of KCC2, as demonstrated through yeast two-hybrid and GST-pull-down assays. RCC1/PAM and full-length KCC2 coimmunoprecipitate following heterologous co-expression in HEK293 cells. Additionally, (86)Rb/K(+) uptake assays in this model system show that RCC1/PAM causes increased KCC2-mediated flux. After narrowing down RCC1/PAM binding to a 20 amino acid region on the KCC2 carboxyl terminus, we created a point mutant in this region to eliminate interaction between the KCC2 carboxyl terminus and RCC1/PAM. This same mutation abolishes N-ethylmaleimide activation of KCC2, suggesting that PAM plays a role in modulating KCC2 function. CI - Copyright 2008 S. Karger AG, Basel
19765080	Besides being activated by G-protein beta/gamma subunits, G-protein activated potassium channels (GIRKs) are regulated by cAMP-dependent protein kinase. Back-phosphorylation experiments have revealed that the GIRK1 subunit is phosphorylated in vivo upon protein kinase A activation in Xenopus oocytes, whereas phosphorylation was eliminated when protein kinase A was blocked. In vitro phosphorylation experiments using truncated versions of GIRK1 revealed that the structural determinant is located within the distant, unique cytosolic C-terminus of GIRK1. Serine 385, serine 401 and threonine 407 were identified to be responsible for the incorporation of radioactive (32)P into the protein. Furthermore, the functional effects of cAMP injections into oocytes on currents produced by GIRK1 homooligomers were significantly reduced when these three amino acids were mutated. The data obtained in the present study provide information about the structural determinants that are responsible for protein kinase A phosphorylation and the regulation of GIRK channels
19712061	Mutations in the gene encoding leucine-rich repeat kinase 2 (LRRK2) are the most common cause of autosomal-dominant familial and late-onset sporadic Parkinson's disease (PD). LRRK2 is a large multi-domain protein featuring a GTP-binding C-terminal of Ras of complex proteins (ROC) (ROCO) domain combination unique for the ROCO protein family, directly followed by a kinase domain. Dimerization is a well-established phenomenon among protein kinases. Here, we confirm LRRK2 self-interaction, and provide evidence for general homo- and heterodimerization potential among the ROCO kinase family (LRRK2, LRRK1, and death-associated protein kinase 1). The ROCO domain was critically, though not exclusively involved in dimerization, as a LRRK2 deletion mutant lacking the ROCO domain retained dimeric properties. GTP binding did not appear to influence ROCO(LRRK2) self-interaction. Interestingly, ROCO(LRRK2) fragments exerted an inhibitory effect on both wild-type and the elevated G2019S LRRK2 autophosphorylation activity. Insertion of PD mutations into ROCO(LRRK2) reduced self-interaction and led to a reduction of LRRK2 kinase inhibition. Collectively, these results suggest a functional link between ROCO interactions and kinase activity of wild-type and mutant LRRK2. Importantly, our finding of ROCO(LRRK2) fragment-mediated LRRK2 kinase inhibition offers a novel lead for drug design and thus might have important implications for new therapeutic avenues in PD
18583962	LIM-homeodomain (LIM-HD) transcription factors form a combinatorial 'LIM code' that contributes to the specification of cell types. In the ventral spinal cord, the binary LIM homeobox protein 3 (Lhx3)/LIM domain-binding protein 1 (Ldb1) complex specifies the formation of V2 interneurons. The additional expression of islet-1 (Isl1) in adjacent cells instead specifies the formation of motor neurons through assembly of a ternary complex in which Isl1 contacts both Lhx3 and Ldb1, displacing Lhx3 as the binding partner of Ldb1. However, little is known about how this molecular switch occurs. Here, we have identified the 30-residue Lhx3-binding domain on Isl1 (Isl1(LBD)). Although the LIM interaction domain of Ldb1 (Ldb1(LID)) and Isl1(LBD) share low levels of sequence homology, X-ray and NMR structures reveal that they bind Lhx3 in an identical manner, that is, Isl1(LBD) mimics Ldb1(LID). These data provide a structural basis for the formation of cell type-specific protein-protein interactions in which unstructured linear motifs with diverse sequences compete to bind protein partners. The resulting alternate protein complexes can target different genes to regulate key biological events