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Extracellular phosphorylation and the control of kinase action: vitronectin, PKA and KSMP/meprin β

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Our research is aimed at the elucidation of regulatory processes involved in the concerted action of cells and tissues. We focus on protein kinases, on their role in the modulation of cell adhesion via vitronectin (Vn) and on proteases that may regulate kinase action. Since de-regulation of such processes causes many of the major human diseases (heart failure, stroke, cancer, abnormal growth, etc.), our research will hopefully improve diagnosis and the design of new drugs.

Phosphorylation of Vitronectin by Casein Kinase II

The cell adhesion protein Vn is the major target in human blood for extra-cellular phosphorylation. Our recent work shows (i) that the CKII phosphorylation of Vn has a Km of 0.5-2μM (lower than the Vn concentration in blood, 3-6μM); (ii) that it is targeted to Thr50 and Thr57 which are vicinal to the Arg-Gly-Asp (RGD) site of Vn (Fig. 1); (iii) that the phosphorylation of Thr57 facilitates the phosphorylation of Thr50; (iv) that the CKII phosphorylation of wt-Vn enhances the adhesion of bovine aorta endothelial cells; (v) that the double mutant Thr50,57Glu (in which the neutral Thr residues are replaced by the negatively charged Glu residues considered analogs of Thr-P) has an enhanced capacity to promote cell adhesion and to accelerate cell spreading; and (vi) that in endothelial cells, the Thr50,57Glu mutant exhibits an enhanced adhesion which is due to an increased affinity towards the αvβ3 integrin.

Vitronectin Binds to Fibrin and can thus Anchor to Blood Clots

Vn constitutes together with fibrin and fibronectin the provisional matrix. Work in our laboratory shows that Vn specifically interacts with fibrin, and that this interaction involves the amino-terminal edge (Asp1-Thr44) of this adhesion protein as well as its carboxy terminal domain (Lys348-Arg379) (Fig. 1). The binding of Vn to fibrin enhances the adhesion of endothelial cells and of platelets onto the fibrin, but prevents the Vn phosphorylation by Protein Kinase A (PKA). Our results suggest that Vn may interact with fibrin at wound healing areas, and that this interaction may enhance cell adhesion, an essential step in blood vessel repair.

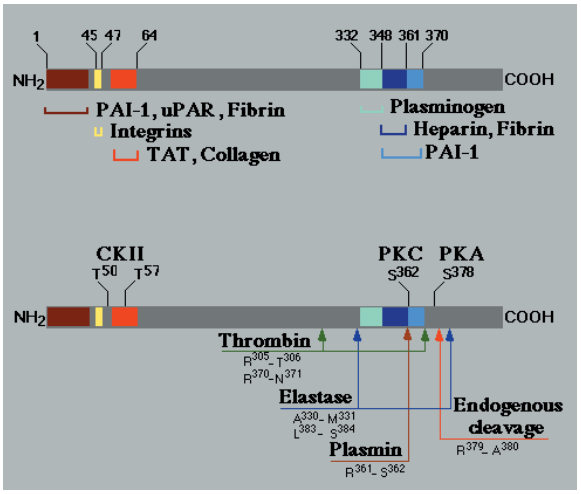

Fig. 1. Upper panel: The linear structure and the binding domains of Vn. Lower panel: The proteolytic cleavage and the phosphorylation sites of Vn as determined in our laboratory.

The C-Terminal Tail of KSMP/Meprin β is Involved in the Intracellular Trafficking of this Membranal Proteinase

The Kinase Splitting Membranal Proteinase (KSMP) discovered and characterized in our laboratory, is identical to the β subunit of meprin-a metalloendoproteinase located in brush border membranes. Using truncated mutants of rat meprin β expressed in Cos-7 and HEK 293 cells, we showed here that the cytoplasmic tail of KSMP/meprin β is indispensable for its exit from the ER. A meprin β mutant lacking the last 25 amino acids is shown to be transport-incompetent, although it does not contain any of the known ER-retention signals. Systematic analysis of the rate of the ER-to-Golgi transport using a series of mutants with Ala or Pro substitutions in the tail, suggests that while no specific amino acid residue by itself is imperative for normal intracellular trafficking of meprin β, the insertion of a bend at a distinct position

in the tail (specifically by a Y685P mutation) suffices to retain this protein in the ER. We propose that the very length of the cytoplasmic tail, as well as its secondary structure are essential for the ER-to-Golgi transport of meprin β, possibly by allowing an interaction with a cargo receptor.

The Negative Charge of Glu 127 in Protein Kinase A and its Biorecognition

A set of mutants of PKA in which Glu-127 was replaced by Gln, Asp, Asn, and Arg was prepared. The Km and Vmax values of these mutants show that the negative charge of Glu-127 (not merely its hydrogen bonding capacity) is indispensable for the kinase activity, since Glu127/Gln is inactive, in spite of the fact that it can form hydrogen bonds, and is very similar in bulkiness and conformation to wt-PKA. Glu-127 is involved in the biorecognition of PKA, interacting ionically with the positively charged guanido group of Arg P-3 (Fig. 2). In support of this conclusion, we showed that a regression of the Glu-127 carboxylate by 1.54 Å (as in Glu127/Asp) results in an active kinase with a similar thermal stability and susceptibility to conformation-dependent proteolysis, a similar Vmax, an identical Km for ATP, but a >20-fold higher Km for Kemptide. The two inactive mutants of PKA, Glu127/Gln and Glu127/Asn, are potentially useful for studying protein-protein interactions of PKA, e.g. for monitoring enzymatically the displacement of active PKA from its complexes.

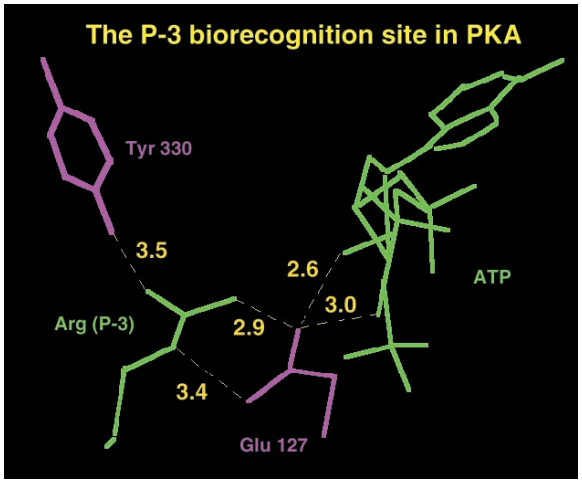

Fig. 2. *Distances in the 3-D structure of the catalytic subunit of PKA between the carboxylate group of Glu 127 (pink) and the guanido nitrogens of Arg (P-3), as well as the 2' and 3' hydoxyls of the ribose ring of ATP.*

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