

# Cytoplasmic Tails and Rossmann folds: The Ins and Outs of Cell Adhesion

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Tissue factor (TF) is structurally related to type 2 cytokine receptors, but lacks cytoplasmic motifs that typically mediate adaptor recruitment. In addition to initiating the coagulation pathway, TF is critical for tumor biology and inflammation through protease activated receptor (PAR) 2 signaling, which is modulated by phosphorylation of the TF cytoplasmic domain (TFCD). In this talk I report the structure-function relationship of the unphosphorylated and phosphorylated TFCD. Phosphorylation of Ser253 stabilizes a structure centered about hydrophobic interactions with Trp254 aromatic group, while phosphorylation of Ser258 destabilizes and unfolds a turn at the C-terminus. Phosphorylation acts as an affinity switch such that upon phosphorylation TFCD binds the prolyl isomerase Pin1, which potentially regulates the rate of de-phosphorylation of TF. Our data provide insights into how association of this key adaptor for intracellular signaling pathways is regulated by the dynamic phosphorylation and *cis / trans* proline isomerization of TFCD.

The second half of my talk concerns Murine Pactolus, which is a single chain integrin  $\beta$  subunit-like transmembrane protein identified as an apoptosis marker for neutrophils. The extracellular Pactolus amino acid sequence is similar to  $\beta$ -integrin subunits with a 60% sequence homology to  $\beta 2$  and  $\beta 7$ . In the integrin family, the Rossmann fold "I-domain" is a key binding region found in all  $\beta$ , and many of the  $\alpha$  subunits. Our NMR structure of the Pactolus I-domain indicates how two key insertions / deletions structurally distinguish this protein from the integrin I-domains. We show experimentally that Pactolus does not bind divalent metal ions, and therefore ligand binding can not be modulated through a metal ion-dependent adhesion site (MIDAS), and that the Pactolus I-domain in solution is a homodimer. This, coupled together with structural changes in the I-domain, illustrates how evolution has changed the key aspects of the integrin  $\beta$  subunit to derive a protein with novel function on an existing protein scaffold.