Physiological regulation by protein dephosphorylation

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Phosphorylation of tyrosine residues in proteins is a major mechanism for regulation of protein structure and function, and directly affects cellular function. Tyrosine phosphorylation is a reversible process, and is controlled by the opposing activities of two major and entirely distinct families of enzymes - protein tyrosine kinases and protein tyrosine phosphatases (PTPs).

Our long-term goal is to clarify the roles of PTPs in regulating specific physiological processes at the levels of individual molecules, cells, and whole animals.

Our group studies two related PTPs - PTP Epsilon (PTPε) and PTP Alpha (PTPα). The four protein forms of PTPε are all produced from the single PTPε gene through steps regulated at the levels of transcription, translation, and post-translational proteolytic processing. All forms of PTPε share the same catalytic domains, but have unique amino termini that determine their individual subcellular locations and physiological roles.

We have shown that the receptor-type form of PTPε (RPTPε) is an assists Neu-mediated **mammary tumorigenesis** in mice. RPTPε is specifically expressed in this type of mammary tumors, and expression of RPTPε in transgenic mice causes massive mammary hyperplasia and associated tumorigenesis. In agreement, mammary tumors induced by Neu in mice genetically lacking PTPε grow slower in culture and produce smaller tumors when implanted in nude mice than do PTPε-expressing tumors. At the molecular level, the Neu kinase phosphorylates RPTPε, which then specifically dephosphorylates Src and activates it. Src is a well-known collaborator of Neu in mammary cell transformation. In the absence of RPTPε this process is less efficient, Src is less activated, and the resulting tumor cells appear less transformed.

PTPε also dephosphorylates the adaptor protein Shc, thereby sending an anti-mitogenic signal and illustrating that PTPε plays roles that are context-specific (as do many other phosphatases and kinases). In Neu-transformed mammary tumor cells, this signal would counter the pro-mitogenic effect of PTPε via activation of Src, and would create a signaling "traffic jam". We have shown that in this particular case, the dominant kinase Neu physically binds Shc and phosphorylates it, thereby preventing PTPε from gaining access to Shc. Neu therefore introduces order into its downstream signaling events and ensures that PTPε plays a coherent role that is free from internal contradiction.

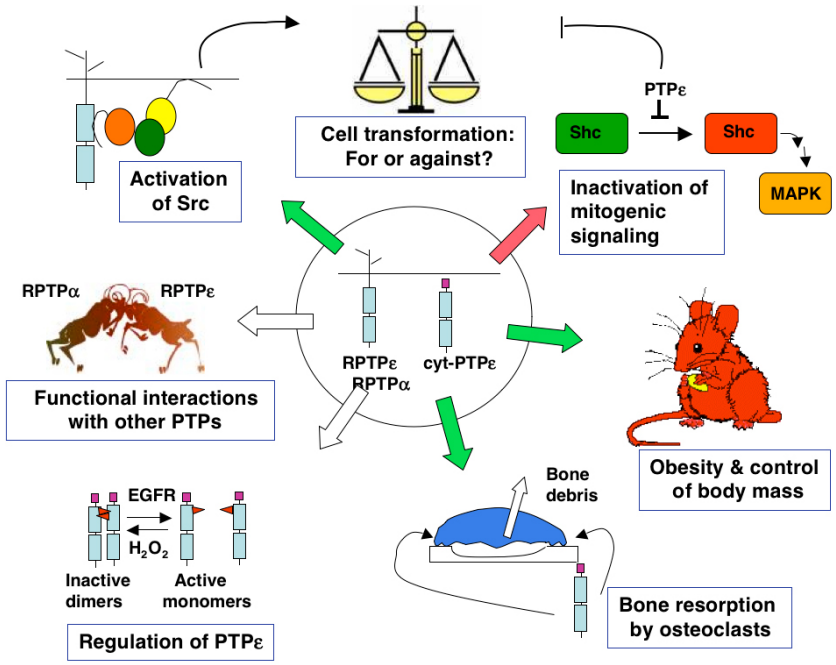

Fig. 1 *Overview of physiological systems and topics studied in our laboratory. Colored arrows indicate processes that PTP epsilon activates (green) or inhibits (red).*

A second, distinct isoform of PTPε, cyt-PTPε, is predominantly cytosolic. We have shown that cyt-PTPε is critical for **maintaining proper bone mass** by regulating the adhesion and activity of osteoclasts, the specialized cells that degrade bone *in vivo*. Cyt-PTPε is required for proper organization and function of podosomes, the adhesion structures of osteoclasts. At the molecular level cyt-PTPε participates in activating the Src kinase downstream of integrin receptor molecules. Accordingly, female mice lacking PTPε exhibit increased bone mass.

Previous studies in our lab showed that cyt-PTPε also affects **Schwann cells**, which **myelinate axons in the peripheral nervous system**. Using substrate-trapping technology we established that the voltage gated potassium channels Kv1.5 and Kv2.1 are substrates of PTPε, and that dephosphorylation of Kv2.1 predominantly at its Y124 by PTPε counters phosphorylation and upregulation of Kv2.1 by the Src and Fyn tyrosine kinases. These findings correlate with severe transient hypomyelination observed in sciatic nerves of newborn mice lacking PTPε.

Interestingly, the closely-related PTPα performs similar roles in Schwann cells vis-à-vis Kv channels, and affects myelination in the peripheral nervous system even more than cyt-PTPε. The molecular details of this activity, however, differ somewhat from those

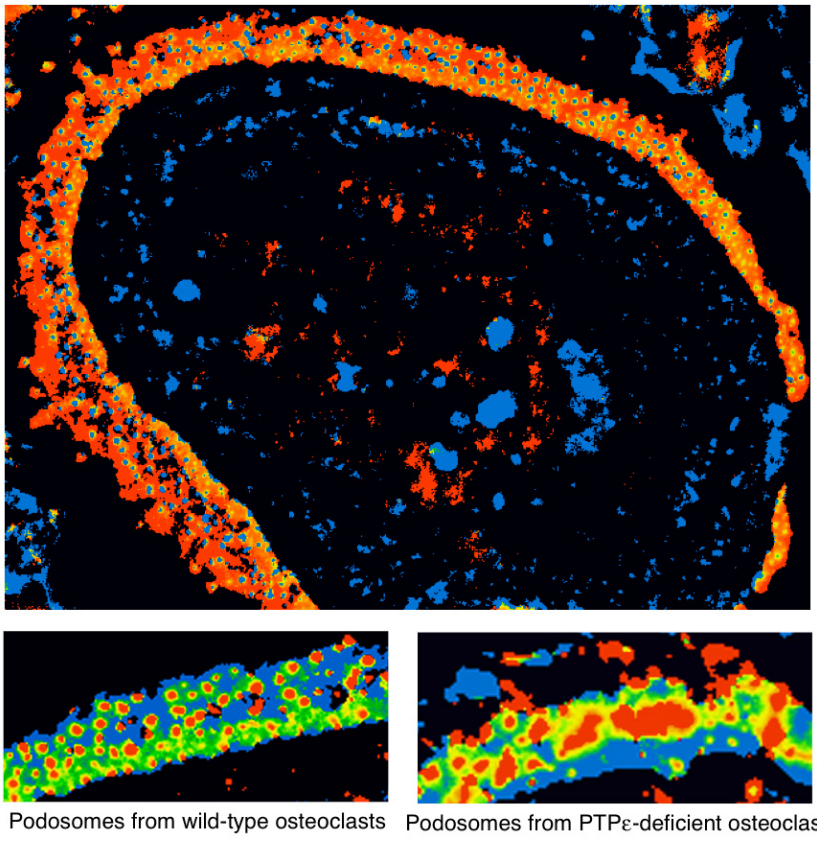

Fig. 2 *Podosomes – adhesion structures of bone-resorbing osteoclasts – are disrupted in cells from mice lacking PTP epsilon. Top: mature, bone resorbing osteoclast, in which podosomes are arranged as an array at the cell periphery. Podosomes contain an actin core (Blue) surrounded by proteins such as vinculin (Red). Bottom: magnified image of podosomes from osteoclasts of wild-type (left) and PTP epsilon-deficient (right) mice. Note evenly sized- and spaced podosomes in WT sample vs. total disarray in PTPε-deficient sample. Red-actin, blue-vinculin. (in collaboration with Chen Luxenburg/Benny Geiger).*

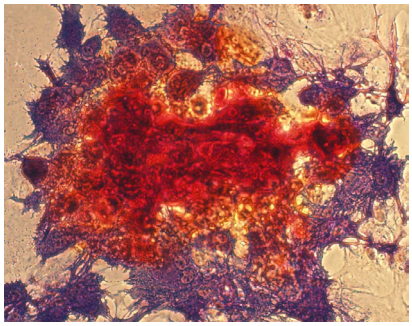

Fig. 3 *Osteoblasts – the cells that produce bone matrix – as viewed in culture. Along with osteoclasts, osteoblasts determine overall bone mass and turnover. Bone marrow cells were grown for thee weeks in the presence of dexamethasone and ascorbic acid. Cells were stained for alkaline phosphatase (purple, a marker of osteoblasts in this system) and Alizarin Red (red, bone matrix secreted by osteoblasts).*

of cytosolic cyt-PTPε and are caused by PTPα being an integral receptor-type molecule. This study illustrates that the physiological roles of these closely-related PTPs *in vivo* overlap in some aspects, but are distinct in others.

Regulation of PTP activity is not properly understood at present. We have characterized novel mechanisms for regulation of this PTP – **inhibitory dimerization** and **inhibitory interactions with microtubules** *in vivo*. Dimerization and interaction with tubulin are regulated by physiological processes in cells, such as increased oxidative stress or activation of the epidermal growth factor (EGF). PTPε is also regulated by tyrosine phosphorylation at its C-terminal – phosphorylation drives PTPε to activate Src and related kinases, thereby providing PTPε with the ability to influence indirectly multiple cellular processes via Src. PTPε is phosphorylated by integrins, as well as by Neu and the related EGF receptor.

In all, these and other studies indicate that PTPε and PTPα participate in diverse physiological processes. Our future studies are aimed at understanding at the molecular level how PTPs regulate processes such as bone homoestasis, obesity, and malignant transformation, while addressing broader issues of regulation of PTP activity and uniquness vs redundancy between related PTPs.

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