Phosphotyrosine Phosphatases: Regulators of Cell Signaling

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- **Tyrosine phosphorylation signal pathways**
- **Lymphocytes as a model system**
- **Positive and negative regulation of signaling by PTP**
- **PTP families: cytoplasmic and receptor type**
- **Mechanism of PTP catalysis confers sensitivity to oxidative stress**
- **Examples of oxidative stress affecting signaling by PTP**
  - **Exogenous sources of oxidative stress**
  - **Signaling induced oxidative stress**

Tyrosine Phosphorylation Signal Pathways

- **Tyrosine phosphorylation is a central regulatory mechanism for cells to transmit signals from cell surface receptors to the nucleus.**
- **Components are conserved from nematodes and Drosophila to humans**
- **Examples**
  - **Growth factor receptors: EGF, PDGF**
  - **Hormones: insulin, erythropoietin**
  - **Cytokines: IL-2, IL-4, IL-7, IL-9**
  - **G protein coupled receptors activation of Src family tyrosine kinases**
- **T cell and B cell antigen receptors, Fc receptors**

Reference:
Schessinger, J. (2000) Cell signaling by receptor tyrosine kinases. *Cell* 103, 211-225

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Lymphocytes As Model Systems

- **T and B lymphocyte responses are primarily regulated by tyrosine phosphorylation**
- **The cells are highly sensitive to oxidative stress**
- **Lymphocytes are exposed to oxidative stress at sites of inflammation, and also in the course of medical therapy**
- **Lymphocyte signal pathways, including the role of PTP, are among the best characterized**

Reference:

Schieven, G.L. (1997) Activation of lymphocyte signal pathways by oxidative stress: role of tyrosine kinases and phosphatases. Oxidative Stress, Cancer, AIDS, and Neurodegerative Diseases, L. Montagnier, R. Olivier, and C. Pasquier, eds, Marcel Dekker, Inc. New York. 35 - 44.

Negative Regulation by PTP

- **PTP hold tyrosine kinase signaling in check.**
- **Only 0.01 – 0.1% of total protein phosphorylation is on tyrosine.**
- **The level of PTP activity in cells is 10 – 100 times the tyrosine kinase activity.**
- **If PTP are inhibited, the basal level of tyrosine kinase activity will activate signal pathways in the absence of receptor stimulation**
- **Illustrated through the use of the PTP inhibitor BMOV**

Reference:

Mustelin, T. et al. (1998) Lymphocyte Activation: the coming of the protein tyrosine phosphatases. *Frontiers in Bioscience* 3: 1060-1096

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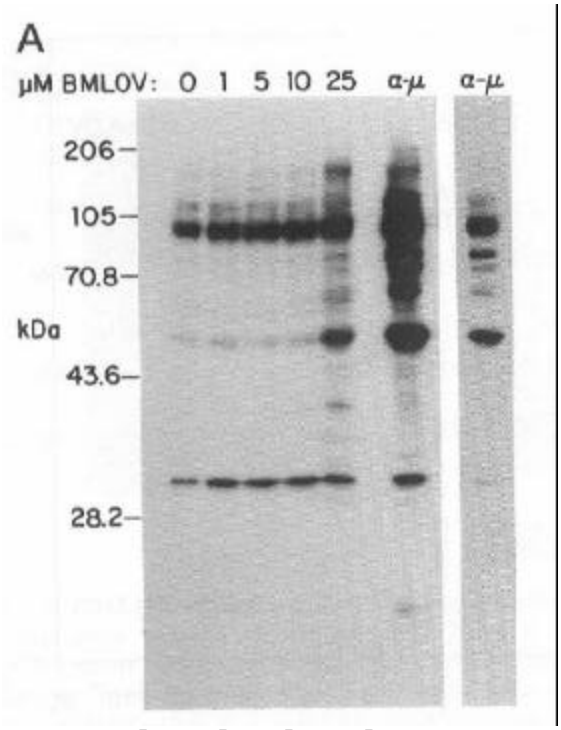
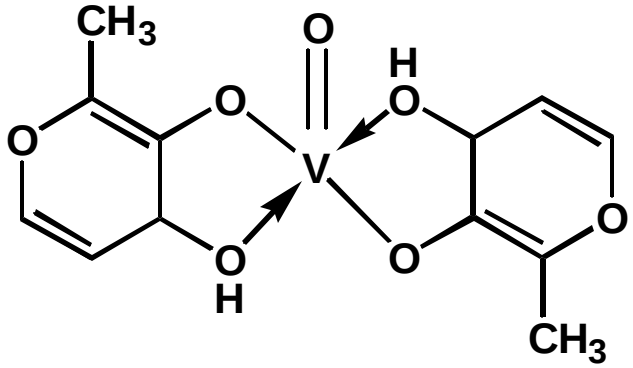
Activation of B Cell Receptor Signaling by the PTP Inhibitor BMOV



- **Ramos B cells were incubated 16 h with BMOV**
- **Cellular tyrosine phosphorylation is compared to antigen receptor stimulation (**a -m**)**



BMOV

Reference:
Schieven, G. et al., (1995) Lineage specific induction of B cell apoptosis and altered signal transduction by the phosphotyrosine phosphatase inhibitor bis(maltolato)oxovanadium(IV). *J. Biol. Chem.*, 270: 20824 – 20831

Termination of Signaling

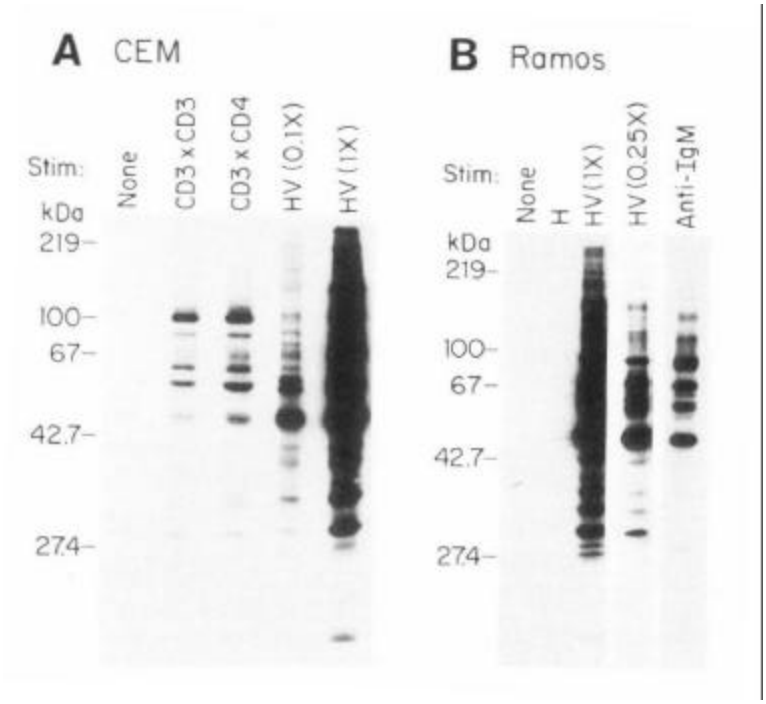
- **PTP act selectively to terminate tyrosine kinase signals**
- **PTP display substrate specificity: peptide Km's vary 100-1000 fold**
- **PTP use adapter domains to bind to signal complexes**
  - **SH2 domains (SHP1 and SHP2)**
  - **Pro-rich sequences to bind SH3 domains (PTP-PEP and PEST)**
  - **ERM and PDZ domains to bind cytoskeleton (PTP H1, MEG1, PTP36)**
- **Catalytic-like domains confer binding specificity (RPTP such as CD45)**

References:

Li, L. and Dixon, J.E. (2000) Form, function, and regulation of protein tyrosine phosphatases and their involvement in human diseases. *Seminars in Immunology* 12: 75-84.

Mustelin, T. et al. (1998) Lymphocyte Activation: the coming of the protein tyrosine phosphatases. *Frontiers in Bioscience* 3: 1060-1096

Pervanadate Induces Strong Signaling by Activating PTK While Inhibiting PTP



- **CEM T cells or Ramos B cells were treated with hydrogen peroxide plus vandate, which forms pervanadate.**
- **Cellular tyrosine phosphorylation is strongly induced by pervanadate compared to receptor stimulation.**

Reference:

Schieven et al., (1993) Reactive oxygen intermediates activate NF-κB in a tyrosine kinase dependent mechanism and in combination with vanadate activate the $p56^{lck}$ and $p59^{fyn}$ tyrosine kinases in human lymphocytes. *Blood*, 82, pp. 1212-1220

PTP Positively Regulate Signaling by Activating Src Family Kinases

- **Src family kinases (Src, Lck, Fyn, Yes, Hck, Fgr, and Blk) are involved in many signal pathways**
  - **Antigen receptor, FcR, RPTK, GPCR**
- **Src kinases are activated by autophosphorylation in the active site (Y394 in Lck)**
- **Src kinases are inhibited by C-terminal tyrosine phosphorylation (Y505 in Lck), which binds the protein's SH2 domain to hold the enzyme in an inactive conformation.**
- **PTP dephosphorylate the C-terminal phosphotyrosine to activate the Src kinases.**

Reference:

Schlessinger, J. (2000) New roles for Src kinases in control of cell survival and angiogenesis. *Cell*, 100: 293-296

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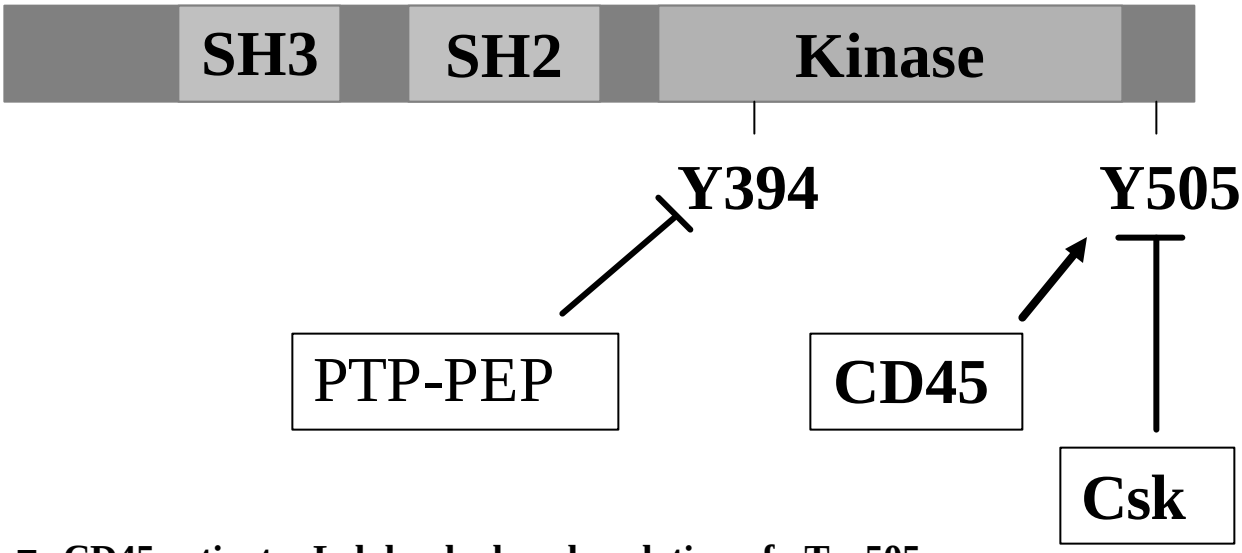
Regulation of Lck



- **CD45 activates Lck by dephosphorylation of pTyr505**
- **Lck is further activated by autophosphorylation of Tyr394**
- **Csk inactivates Lck by phosphorylation of Tyr505**
- **PTP-PEP, in a complex with Csk, inactivates Lck by dephosphorylation of pTyr394**

References:

Alexander, D.R. (2000) The CD45 tyrosine phosphatase: a positive and negative regulator of immune cell function. *Seminars in Immunology,* 12: 349-359

Dustin, M.L. and Chan, A.C. (2000) Signaling takes shape in the immune system. *Cell,* 103: 283-294

Gjorloff-Wingren et al., (1999) Characterization of TCR-induced repetor-proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. *Eur. J. Immunol.* 29: 3845-3854

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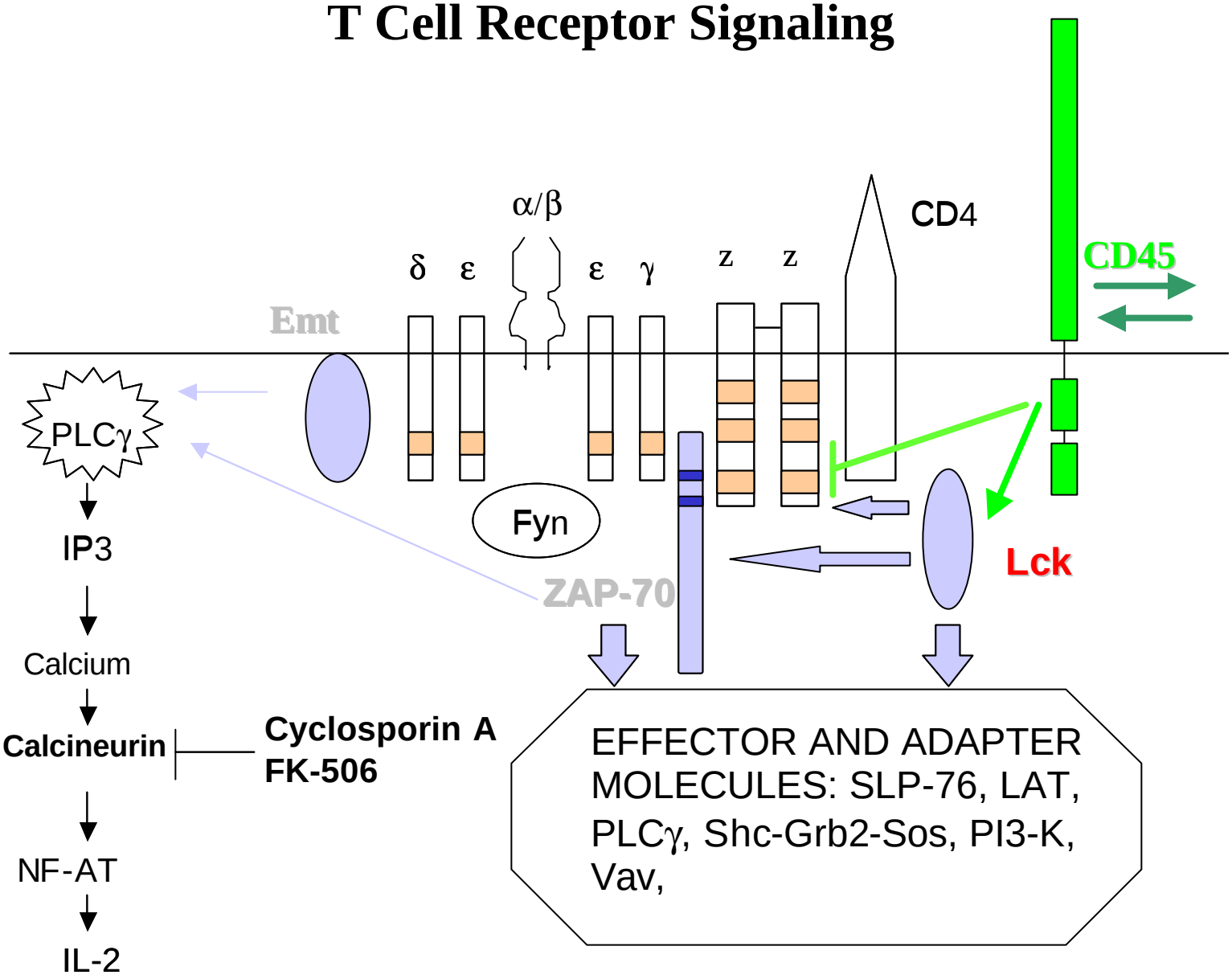
T Cell Receptor Signaling



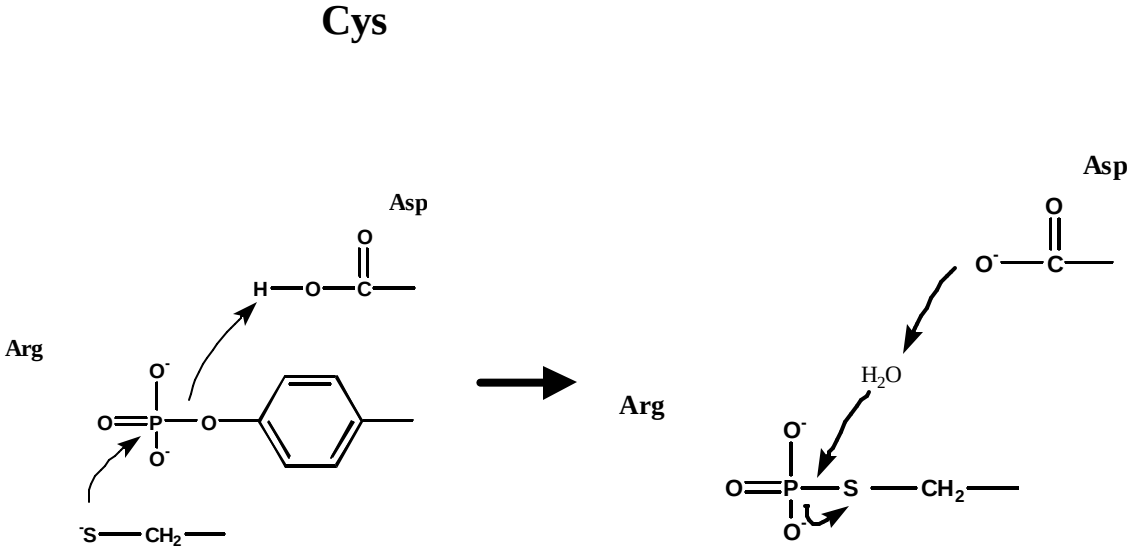
References:

Dustin, M.L. and Chan, A.C. (2000) Signaling takes shape in the immune system. *Cell*, 103: 283-294

Kane, L. et al. (2000) Signal transduction by the TCR for antigen. *Curr. Opinion in Immunol.* 12: 242-249

PTP Mechanism of Action

- **PTP are sensitive to oxidation of the highly reactive cysteine (pKa=4.7) that is essential for catalytic activity**



- **The cysteine must be reduced to act as the phosphoacceptor.**
- **The Asp residue acts as a general acid catalyst**
- **The conserved Arg residue binds the phosphate**

Reference:

Li, L. and Dixon, J.E. (2000) Form, function, and regulation of protein tyrosine phosphatases and their involvement in human diseases. *Seminars in Immunology* 12: 75-84.

RPTP: CD45

Alternate Splicing | **Trans-membrane** | **D1 catalytic** | **D2 Domain**

- **CD45 expression is limited to hematopoietic cells**
- **D1 is the catalytic domain**
- **D2 PTP-like domain confers substrate specificity**
- **Eight alternatively spliced forms are known, with developmentally regulated expression**
- **CD45 is required for lymphocyte development**
- **Human patient deficient in CD45 has SCID phenotype**
- **CD45 is responsible for activation of Src-family kinases**

References:

Alexander, D.R. (2000) The CD45 tyrosine phosphatase: a positive and negative regulator of immune cell function. *Seminars in Immunology,* 12: 349-359

Johnson, K. et al., (2000) A supramolecular basis for CD45 tyrosine phosphatase regulation in sustained T cell activation. *Proc. Nat. Acad. Sci. USA* 97: 10138-10143

RPTPa

- **RPTPa crystal structure suggests dimerization causes the active site of each component to be blocked by an N-terminal wedge of the other component**
- **This finding has led to a model where RPTP dimerization leads to inactivation.**
- **RPTPa regulates Src kinases in fibroblasts**
- **Knockout mice have impaired integrin mediated adhesion, cell spreading and FAK phosphorylation**

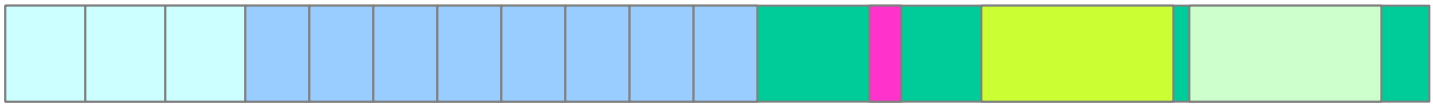
References:

Bilwes et al. (1996) Structural basis for inhibition of receptor protein-tyrosine phosphatase-α by dimerization. *Nature,* 382: 555-559.

Harder K. et al. (1998) Protein-tyrosine phosphatase alpha regulates Src family kinases and alters cell-substratum adhesion. *J. Biol. Chem.* 273: 31890-31900.

LAR, RPTPs, RPTPd

LAR



Ig-like domains **Type III fibronectin-like repeats** **TM** **D1** **D2**

- **Members of this family play roles in adhesion and CNS function**
- **LAR localizes to focal contact sites, and associates with Trio, a protein with GEF domains for Rac and Rho**
- **LAR knockouts have defective mammary gland development, knockouts have defective mammary gland development, defects in regulation of glucose by insulin and decreased cholinergic neuron size**

- **RPTPs knockouts have neuronal defects lethal within two weeks**
- **RPTPd knockouts have learning defects**

References:

Ren, J. et al. (1998) Transgenic mice deficient in the LAR protein-tyrosine phosphatase exhibit profound defects in glucose homeostasis. *Diabetes* 47: 493-497

Schaapveld R. et al. (1997) Impaired mammary gland development and function in mice lacking LAR receptor-like tyrosine phosphatase activity. *Dev. Biol.* 188: 134-146

Uetani N et al. (2000) Impaired learning with enhanced hippocampal long-term potentiation in PTPdelta-deficient mice. *EMBO J.* 19: 2775-2785

Elchebly M. et al. (1999) Neuroendocrine dysplasia in mice lacking protein tyrosine phosphatase sigma. Nat. Genet. 1999 21:330-333.

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RPTPe

- **PTPe substrate trapping mutant associates with the voltage-gated potassium channel Kv2.1**
- **PTPe reduces Src- or Fyn-stimulated Kv2.1 currents and tyrosine phosphorylation**
- **PTPe knockouts exhibit hypomyelination of sciatic nerve axons**
- **PTPe knockouts exhibit hyperphosphorylation of Kv1.5 and Kv2.1 Kv channel alpha-subunits in sciatic nerve tissue**

Reference:

Peretz A. et al. (2000) Hypomyelination and increased activity of voltage-gated K(+) channels in mice lacking protein tyrosine phosphatase epsilon. *EMBO J.* 19: 4036-4045.

Cytoplasmic PTP: PTP1B

PTPase domain

- **PTP1B was the first PTP to be purified (1988), and the first to have a crystal structure determined (1994)**
- **PTP1B dephosphorylates the insulin receptor, negatively regulating insulin receptor signaling.**
- **PTP1B knockouts display increased sensitivity to insulin**
- **PTP1B knockouts have reduced food intake and reduced weight gain when fed a high fat diet**
- **PTP1B is an attractive target for the treatment of Type 2 diabetes and obesity**

References:

Jia Z,et al. (1995) Structural basis for phosphotyrosine peptide recognition by protein tyrosine phosphatase 1B. *Science*. 268:): 1754-1758.

Elchebly M, et al. (1999) Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. *Science*. 283:1544-1548.

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TCPTP

PTPase domain **ER retention signal**

- **TCPTP was originally identified in T cells but is widely expressed**
- **Closely related to PTP1B, 85% sequence similarity in PTPase domain**
- **Alternatively spliced forms have either an ER retention signal or a nuclear localization signal in the C-terminus (48 and 44 kDa)**
- **Knockout mice die at 3 – 5 weeks, with hematopoietic cell defects**
- **TCPTP may dephosphorylate a variety of receptor tyrosine kinases**

Reference:

Ibarra-Sanchez et al. (2000) The T cell protein tyrosine phosphatase. *Seminars in Immunology*, 12: 379-386.

HePTP

PTPase domain

- **HePTPis expressed only in hematopoietic cells**
- **Three other family members (STEP, PCPTP1, and Typ) are expressed in a variety of tissues but not leukocytes**
- **HePTP binds Erk2 and p38 kinase via its N-terminus**
- **Erk2 and p38 phosphorylate HePTP**
- **HePTP dephosphorylates the critical tyrosine in the Erk2 and p38 activation loops**
- **Overexpression of HePTP impairs TCR-induced Erk2 and p38 activation**
- **A variety of dual specificity MAP kinase phosphatases (MKP) also dephosphorylate Erk, Jnk, and p38 kinases.**

Reference:

Saxena M. et al. (1999) Inhibition of T cell signaling by mitogen-activated protein kinase-targeted hematopoietic tyrosine phosphatase (HePTP). *J. Biol. Chem.* 274: 11693-11700

SH2 phosphatases: SHP-1

- **SHP-1 contains two SH2 domains which bind phosphotyrosine in ITIM sequences in FcgRIIB, CD22, and CD72 in B cells, and pTyr signal complexes in other hematopoietic cells.**
- **SHP-1 associates with CD5 in T cells and dephosphorylates TCR components**
- **SH2 binding activates SHP-1**
- **Knockout mice have the *motheaten* phenotype due to severe inflammation and autoimnunity arising from excessive antigen receptor and cytokine signaling.**

Reference:

Zhang et al., (2000) Roles of the SHP-1 tyrosine phosphatase in the negative regulation of cell signaling. *Seminars in Immunology,* 12: 361-378.

SH2 phosphatases: SHP-2

- **SHP-2 has two SH2 domains that bind pTyr sequences in a variety of receptor tyrosine kinases such as EGF and PDGF receptor**
- **SHP-2 plays a positive role in signaling by RPTK such as EGF and PDGF receptor, and by cytokine receptors such as IL-5**
- **SHP-2 is tyrosine phosphorylated at its C-terminus and binds Grb-2, leading to translocation of Sos and activation of the Ras-Raf-MAPK pathway**
- **SHP-2 may inhibit signaling by other receptors such as c-Kit**

Reference:

Mustelin, T. et al. (1998) Lymphocyte Activation: the coming of the protein tyrosine phosphatases. *Frontiers in Bioscience* 3: 1060-1096

PEST group of PTP

PEP

PTPase domain **PEST regions**

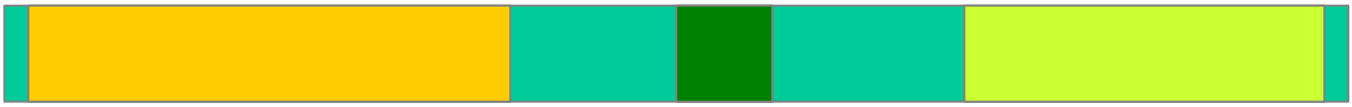
- **PEST group named for proline, glutamic acid, serine and threonine rich regions**
- **Includes PEP (hematopoietic cells), PTP-PEST, and PTP-HSCF**
- **The PEST regions allow association with other proteins' SH3 domains that bind proline rich regions**
- **PEP associates with Csk, the kinase that down regulates Src kinase activity**
- **PEP may dephosphorylate the tyrosine in the activation of loop of Src kinases, thereby inactivating them**

Reference:

Gjorloff-Wingren et al., (1999) Characterization of TCR-induced repetor-proximal signaling events negatively regulated by the protein tyrosine phosphatase PEP. *Eur. J. Immunol.* 29: 3845-3854

Erm domain PTP

MEG-1



Erm domain **PDZ** **PTPase domain**

- **Family includes 7 members, all containing an ERM domain, homologous to cytoskeletal protein band 4.1**
- **Several members also contain PDZ domains, which organize signal complexes at membranes**
- **PTP-MEG1 associates with the plasma membrane and the particulate fraction in T cells, suggesting cytoskeletal interactions**
- **The other family members examined, PTPH1 and PTP36, show similar distribution**

Reference:

Gjorloff-Wingren et al. (2000) Subcellular localization of intracellular protein tyrosine phosphatases in T cells. Eur. J. Immunol. 30: 2412-2421

Oxidation of PTP

- **The active site cysteine residue is highly reactive, and therefore much more sensitive to oxidation than most Cys residues**

- **The Cys -SH can be reversibly oxidized to sulfenic acid, Cys-SOH**

- **Potent oxidizing agents such as pervanadate may irreversibly oxidize Cys-SH to sulfinic acid (Cys-SOOH); or sulfonic acid (SO_3H)**

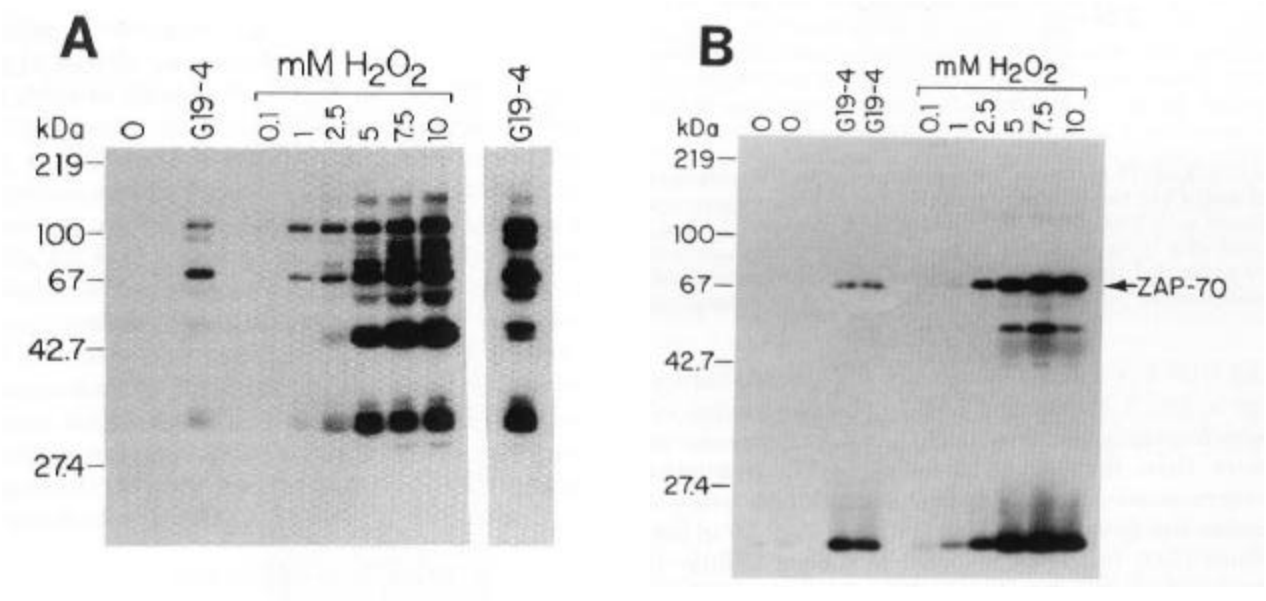
- **Cells are exposed to a variety of exogenous sources of oxidative stress that can activate signal pathways**

- **Hydrogen peroxide and UV radiation are two examples of sources of oxidative stress that impact signaling and phosphotyrosine phosphatases**

Reference:

Denu, J. and Tanner, K. (1998) Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry*, 37:5633-5642.

Hydrogen Peroxide Activates Signal Pathways



H_2O_2 induces T cell tyrosine phosphorylation and activates the ZAP-70 tyrosine kinase, similar to TCR stimulation

Reference:

Schieven, G. et al. (1994) ZAP-70 tyrosine kinase, CD45 and T cell receptor involvement in UV and H_2O_2 induced T cell signal transduction. *J. Biol. Chem.*, **269**, 20718-20726.

Hydrogen Peroxide Inhibits PTP

- **H_2O_2 is generated at sites of inflammation by neutrophils and macrophages**

- **H_2O_2 can readily diffuse through cells and has the potential to act as a second messenger**

- **H_2O_2 inhibits PTP in vitro and in cells**

- **Serine/threonine phosphatases are not inhibited by H_2O_2**

- **H_2O_2 reversibly oxidizes the active site cysteine thiolate to sulfenic acid in PTP**

References:

Schieven, G.L. (2000) Induction of protein tyrosine phosphorylation by oxidative stress and its implications. Antioxidant and Redox Regulation of Genes, C.K. Sen, H. Sies, and P. Baeuerle eds., Academic Press, San Diego. 129-146.

Schieven, G.L. (1997) Tyrosine phosphorylation in oxidative stress. Oxidative Stress and Signal Transduction, E. Cadenas and H. J. Forman, eds., Chapman & Hall, New York. 181-189.

Denu, J. and Tanner, K. (1998) Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. *Biochemistry*, 37:5633-5642.

Lee, S.R. et al. (1998) Reversible inactivation of protein tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. J. Biol. Chem., 273: 15366-15372.

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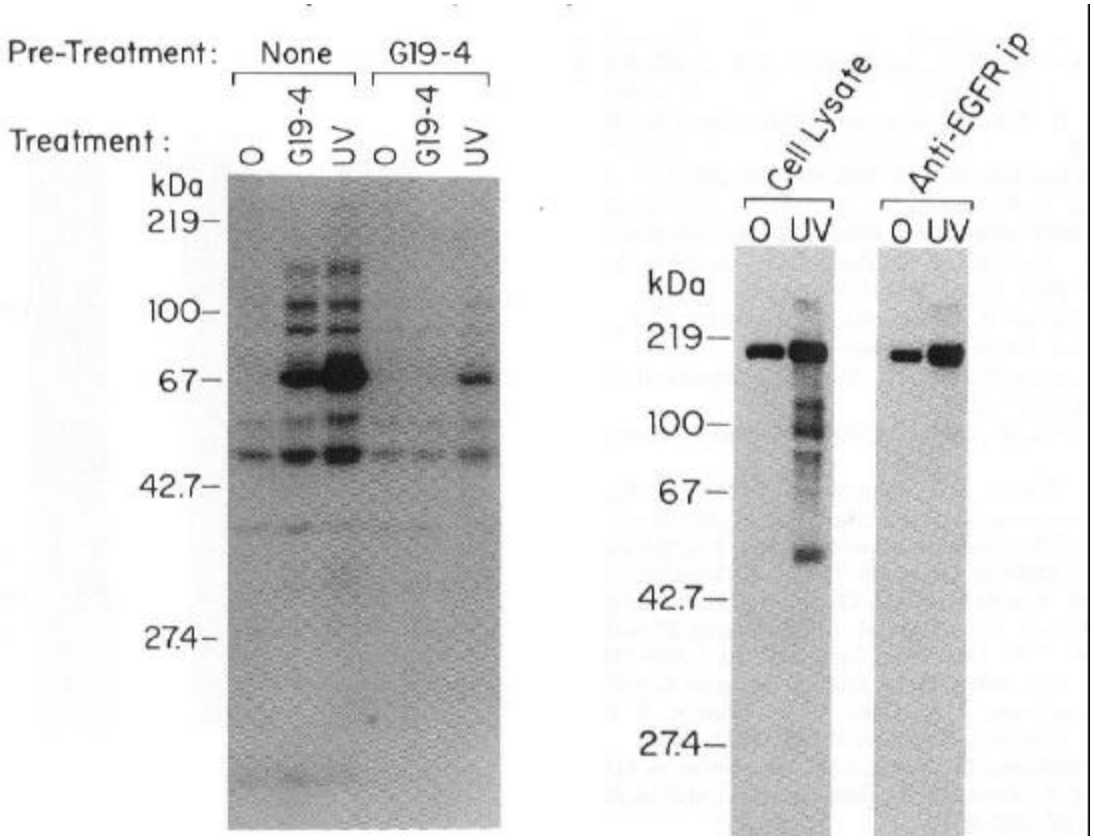
UV Induced Signals



- **UV activates T cell signaling in a pattern very similar to TCR stimulation, and also induces EGF receptor signaling**

Reference:

Schieven, G. et al. (1994) ZAP-70 tyrosine kinase, CD45 and T cell receptor involvement in UV and H_2O_2 induced T cell signal transduction. *J. Biol. Chem.*, **269**, 20718-20726.

UV Induced Signaling and PTP Inhibition

- **UV induces immediate early gene expression, known as the mammalian UV response**
- **UV activates RPTK such as EGF and PGDF receptors and antigen receptor signal pathways in T and B cells independent of ligand**
- **UV has been reported to induce clustering of receptors**
- **UV inhibits PTP in cells (SHP-1, RPTP**a**, RPTP**d**)**
- **UV inhibits dephosphorylation of PDGF receptor in cells**
- **N-acetyl cysteine protects PTP from UV inactivation in cells, suggesting that UV affects the active site thiol**

References:

Sachsenmaier, C. et al. (1994) Involvement of growth factor receptors in the mammalian UV response. *Cell,* 78: 963-972.

Devary, Y. et al. (1992) The mammalian ultraviolet response is triggered by activation of src tyrosine kinases. *Cell* 71: 1081-1091.

Rosette C. and Karin, M. (1996) Ultraviolet light and osmotic stress: activation of the JNK cascade throughmultiple growth factor and cytokine receptors. *Science* 274:1194-1197.

Grob, S. et al. (1999) Inactivation of protien-tyrosine phsphatases as mechanism of UV-induced signal transduction. J. Biol. Chem. 274: 26378-26386.

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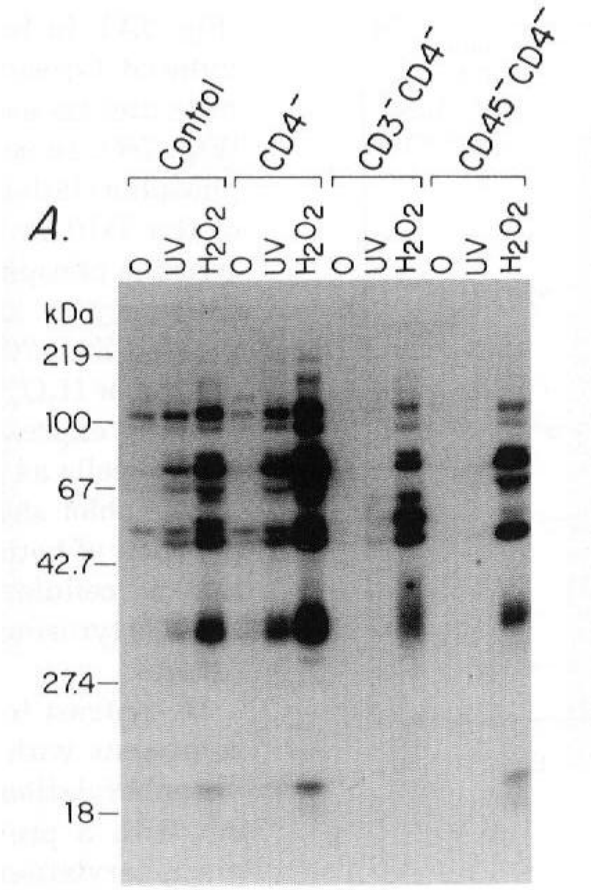
Differential effects of UV and H_2O_2



- **UV requires the presence of the T cell receptor and CD45 to induce cellular tyrosine phosphorylation**
- **In contrast, hydrogen peroxide does not require the TCR or CD45 to induce tyrosine phosphorylation**
- **These results suggest a difference in the mechanism of action between UV and hydrogen peroxide**

Reference:

Schieven, G. et al. (1994) ZAP-70 tyrosine kinase, CD45 and T cell receptor involvement in UV and H_2O_2 induced T cell signal transduction. *J. Biol. Chem.*, 269: 20718-20726.

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Endogenous Production of H_2O_2 in Signaling

- **Receptor tyrosine kinases induce H_2O_2 production**
- **PDGF receptor induced H_2O_2 is required for mitogenesis**
- **EGF receptor induces H_2O_2 production that augments tyrosine phosphorylation signaling**
- **EGF receptor acts via a pathway involving PI-3 kinase and Rac to induce H_2O_2 production.**
- **EGF receptor induced H_2O_2 production reversibly inhibits PTP1B in cells**
- **This provides a mechanism by which receptors can augment their signaling by transiently inhibiting the PTP that would reduce tyrosine phosphorylation.**

References:

Sundaresan, M. et al. (1995) Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. *Science* 270: 296-299.

Bae, Y. et al. (2000) Platelet-derived grrowth factor-induced H_2O_2 production *J. Biol. Chem.* 275: 10527-10531

Bae, Y. et al. (1997) Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. *J. Biol. Chem.* 272: 217-221

Lee, S. R. et al. (1998) Reversible inactivation of protein-tyrosine phosphatase 1B in A431 cells stimulated with epidermal growth factor. *J. Biol. Chem.* 273: 15366-15372

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Implications for H_2O_2 in Signaling

- **PTP appear to be regulated during signaling by intracellular production of H_2O_2**
- **This regulation can augment signals produced by receptor tyrosine kinases such as EGF and PDGF receptor**
- **This regulation might inhibit signaling in other cell types such as T cells, where catalase is an autocrine growth factor, due to the requirement for CD45 to activate Src kinases**
- **Exogenous H_2O_2 production at sites of inflammation appears to have the potential to augment or inhibit signal pathways outside of normal receptor control**

Conclusions

- **PTP play both positive and negative roles in signal pathways**
- **Numerous receptor and non-receptor PTP have been identified**
- **PTP display substrate specificity and are targeted to signaling complexes**
- **Cellular localization of PTP is regulated by adapter domains and dynamic interactions during signaling**
- **Oxidative stress provides a regulatory mechanism during normal signaling, but can also bypass normal receptor control of tyrosine phosphorylation signal pathways**
- **PTP are highly sensitive to oxidative stress due to their essential active site cysteine**

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