



Signal Transduction and Reversible Phosphorylation

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BIOGRAPHY

Tomas Mustelin earned his M.D. and Ph.D. degrees from University of Helsinki in 1987. He trained as a postdoctoral fellow at The Scripps Research Institute in La Jolla, 1988-1990. Dr. Mustelin returned to Finland for two years in clinical practice and research as a Junior Scientist with the Finnish Academy of Sciences at University of Helsinki. He was appointed Docent at University of Helsinki in 1992, an appointment he maintains to this day. From 1992-1998, Dr. Mustelin worked at La Jolla Institute of Allergy and Immunology in San Diego, as Assistant, then Associate Member. He was affiliated briefly with the Sidney Kimmel Cancer Center in San Diego prior to his recruitment to The Burnham Institute in September 1999.

Dr. Mustelin investigates a family of genes called protein tyrosine phosphatase (PTPases), many of which act as tumor suppressors in numerous types of human cancer. It is anticipated that damage or loss of many additional family members will be found to underlay human disease, particularly cancers of white blood cells (e.g. leukemias and lymphomas). Dr. Mustelin has generated the tools to study some 35 different PTPases, representing nearly half of the genes in this family in the human genome. Dr. Mustelin's work aims at understanding the exact function of each of these PTPases in the cell's machinery for growth, survival, and death, in the white blood cell system. The results of Dr. Mustelin's research will help him and others to design rational approaches for the combat of cancer.

This laboratory investigates the molecular mechanisms of signal transduction from the T cell antigen receptor that lead to gene activation and initiation of cell growth.

Tyrosine phosphorylation

Phosphorylation of key proteins and enzymes on specific tyrosine residues is a fundamental mechanism whereby cells control their growth, proliferation and differentiation. This control is lost in cancer. Extracellular growth stimuli, such as growth factors or antigens, affect cells by inducing a rapid but transient tyrosine phosphorylation of a number of regulatory proteins. We have shown that inhibition of this phosphorylation prevents antigen receptor-initiated lymphoid cell activation

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and proliferation altogether, indicating that enhanced tyrosine phosphorylation is a prerequisite for initiating the cascade of biochemical events leading to activation of the lymphocyte's effector functions and clonal proliferation. A deeper understanding of these events will form a basis for a rational design of pharmacological or other means to manipulate T cell activation and proliferation as part of treatment of various diseases or conditions where the lymphoid system plays a part, such as T cell leukemias, dysfunction of the immune system in cancer patients, other immunodeficiencies, autoimmunity etc. Our progress may also be helpful in a broader sense since the same or similar molecules and pathways also operate in other cells.

Protein tyrosine phosphatases (PTPases)

Since phosphorylation of cellular proteins on tyrosine residues plays a very fundamental role both in determining the responsiveness of T cells to receptor triggering, as well as the initiation and maintenance of cell proliferation, the role of PTPases in T cell physiology is obviously crucial. It is important to note, however, that PTPases may function both as positive and negative regulators of T cell functions, and a more detailed understanding of the T cell-expressed PTPases and their physiological substrates and roles will add to our knowledge on the overall function of these proteins.

Following earlier work on CD45 and its function in T cell activation, we recently entered this field more broadly by systematically determining which of all the known PTPases are expressed in T lymphocytes. Subsequently, the cDNAs for the PTPases that were found to be present were isolated in the lab or obtained from other scientists. Using these cDNAs (currently CD45, SHP1, SHP2, TCPTP, HePTP, PEP,

Lyp1, Lyp2, PTP-MEG2, PTP-MEG1, PTPH1, PTP36, VHR, MKP-1, PTEN, PRL-1, OV-1 and LMPTP-A, -B, and -C), we are in the process of analyzing their potential role in the early events of TCR-mediated signal transduction. So far, results suggest that as many as eleven of these enzymes participate at various steps in signal transmission. We will investigate these in detail and determine their exact sites of action, their regulation and their importance. The planned work includes the generation of mice deficient in the expression of specific PTPases ('knock-out') as well as basic molecular biology and biochemical characterization of the enzymes. Our group will also continue to study the observation that 1) several PTPases (including CD45, SHP1, SHP2 and LMPTP) are regulated by tyrosine phosphorylation and therefore must interact with PTKs, 2) the finding that HePTP associates physically with, and regulates, the MAP kinases Erk and p38, and 3) that phosphorylation of SHP2 induces its association with three proteins that are important in T cell activation, namely PI3K, Grb2 and Vav. This suggests that PTPase targeting can be actively regulated during signal transmission. We are also interested in studying PTPases involved in integrin signaling, adhesion, cell migration, malignant transformation, and other physiological processes regulated by reversible tyrosine phosphorylation.

Protein tyrosine kinases (PTKs)

We have a long-standing interest in the PTKs that are instrumental to T cell activation. Several of these kinases are encoded by protooncogenes and have been shown to cause malignant transformation in laboratory models as well as in patients suffering from T cell lymphomas and leukemias. We have published papers documenting the role and regulation of the Src family kinases Lck and Fyn, the

Syk-family kinases Syk and Zap-70, and the Csk kinase. Current work focuses on the physical and functional interactions between these PTKs, their regulation and their substrates. Experiments will also address the redundancy versus specificity of family members and their differential expression in subsets of lymphocytes.

Phosphatidylinositol 3-kinase and the PTEN tumor suppressor

Phosphatidylinositol 3-kinase (PI3K), a signal transducing molecule regulated by PTKs and PTPases, has been another center of attention in our laboratory for several years. This lipid kinase is also known to participate in signals that govern cell growth and differentiation in many other cell types. We have found that PI3K plays an active positive role in T cell activation by augmenting the TCR-induced activation of the MAP kinase Erk2. Studies on the mechanism of this involvement have revealed that TCR-induced tyrosine phosphorylation of the 36-38-kDa LAT protein is responsible for the recruitment and activation of PI3K. Current work addresses the role of the PTEN phosphatase, which antagonizes PI3K by dephosphorylating its products. This protein is a well-established tumor suppressor, which is deficient in a large portion of human cancers. PTEN expression induces rapid apoptosis in T cells. Other areas under investigation are: 1) the regulation of PI3K by phosphorylation of one of its SH2 domains, 2) the role of PI3K products in the recruitment of other signaling molecules (e.g. PLC γ 1, Vav and Itk) to the plasma membrane, 3) the relationship between PI3K, Ras and Rac, 4) the association of PI3K with SHP2, and 5) the hypothesis that the PI3K-stimulated MEKK2 kinase is a component in MAP kinase activation in T cells.

Subcellular location of PTPases in T cells (confocal microscopy)

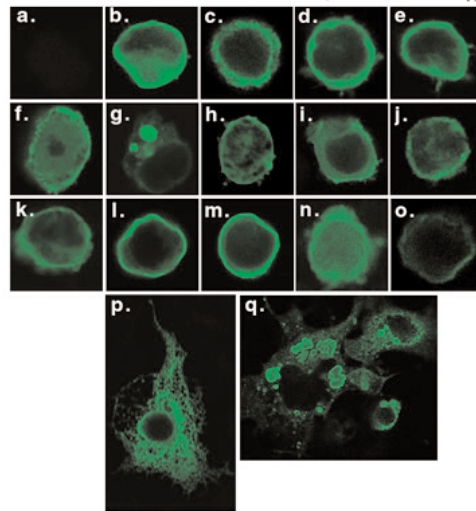


Figure Legend

Confocal microscopy of Jurkat cells transfected with empty pEF/HA vector (panel a), or expression plasmids encoding the indicated PTPases and stained with a FITC-anti-HA mAb (panels a - o). Panels p and q represent COS cells expressing TCPTP or PTP-MEG2 and stained with the FITC-anti-HA mAb.

Publications

Mustelin, T., Coggeshall, K.M., Isakov, N. and Altman, A. Tyrosine phosphorylation is required for T cell antigen receptor-mediated activation of phospholipase C. *Science* 247:1584-1587, 1990.

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