

[Biochem. J., 267, 91 (1990)]

**Mitogenic Signalling Pathways of Tumour Necrosis Factor Involves the Rapid Tyrosine Phosphorylation of 41000-Mr and 43000-Mr Cytosol Proteins.**

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Tumour necrosis factor (TNF) is a potent mitogen for some fibroblast cell lines. Here we have examined the TNF-mediated changes in protein phosphorylation in Swiss 3T3 and human FS-4 fibroblasts, and compared them with changes observed after the treatment of cells with other mitogens, such as PDGF and bombesin. TNF stimulated the rapid phosphorylation of two 41000-Mr and 43000-Mr cytosol proteins on tyrosine, threonine and/or serine, as did PDGF, epidermal growth factor; the increased levels of this mitogen-induced protein-tyrosine phosphorylation correlated well with the extent of mitogen-induced DNA synthesis

[Biochemistry, 29, 10555 (1990)]

**Characterization of Interleukin 2 Stimulated 65-Kilodalton Phosphoprotein in Human T Cells.**

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We have characterized the cellular proteins which are rapidly phosphorylated by interleukin 2 (IL2) in a human IL 2 dependent cell line. When treated with IL 2, the phosphorylation of five proteins, 65, 50, 37, 24, and 21 kDa, was found in IL 2 dependent cell lines by two-dimensional gel electrophoretic analysis. After cell conversion from an IL 2 dependent state to an IL 2 independent state, one of the five phosphoproteins, the 65-kDa protein, became constitutively phosphorylated even without addition of IL 2. Also, in other IL 2 independent cell lines, such as KUT-2 and HUT-102, constitutive phosphorylation of the 65-kDa protein occurred without IL-2 stimulation.

[Biochemistry, 29, 8319 (1990)]

**65-Kilodalton Protein Phosphorylated by Interleukin 2 Stimulation Bears Two Putative Actin-Binding Sites And Two Calcium-Binding Sites.**

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We have previously characterized a 65-kDa protein (p65) as an interleukin 2 stimulated phosphoprotein in human T cells and showed that three endopeptide sequences of p65 are present in the sequence of l-plastin. In this paper, we present the complete primary structure of p65 based on cDNA isolated from a human T lymphocyte (KUT-2) cDNA library. Analysis of p65 sequences and the amino acid composition of cleaved p65 N-terminal peptide indicated that the deduced p65 amino acid sequence exactly coincides with that of l-plastin over the C-terminal 580 residues and has a 57-residue extension at the N-terminus to l-plastin.