F5 SPHINGOSINE-1-PHOSPHATE AND SPHINGOSYLPHOSPHOHYDROLASE MODULATION OF Ca²⁺ SIGNALING IN AIRWAY EPITHELIAL CELLS.

S. Cragg and M. Rugolo. Dipart. di Biologia E.S., Via Imerio 42, Università di Bologna, Bologna, Italy.

Sphingosine-1-phosphate (S-1-P) and sphingosinephosphohydrolase (SPC) are sphingosine metabolites involved in cell growth regulation and signal transduction. Both compounds trigger intracellular Ca²⁺ mobilization from internal stores, which may be an important event in the control of cellular proliferation. They interact with unknown receptor-mediated pathways, whose mechanisms still need to be fully elucidated. We have investigated the Ca²⁺ mobilization effect of S-1-P and SPC in immortalized cell lines derived from respiratory epithelium, by the use of fura-2 fluorescence. Both compounds induced a rapid and transient increase in intracellular free [Ca²⁺]. The maximal effect was observed at micromolar concentrations of SPC, but not S-1-P, caused a significant stimulation of total inositol phosphates accumulation, suggesting that Ca²⁺ mobilization is mediated by PLC activation. Indeed, preincubation with pertussis toxin or with the phorbol ester PMA almost completely abolished the Ca²⁺ response induced by SPC, indicating the involvement of a G protein type Gαq and a feed-back control by protein kinase C, respectively. Conversely, S-1-P failed to stimulate inositol phosphates accumulation, although it was even more efficient than SPC. Interestingly, [Ca²⁺]i elevation induced by S-1-P was also significantly inhibited (70%) by both PMA and pertussis toxin treatment, suggesting the involvement of a G protein-mediated receptor mechanism. Studies are in progress to identify the intracellular molecule generated by activation of S-1-P-sensitive membrane receptor, responsible for Ca²⁺ release from internal stores via a PLC-independent pathway. Supported by P. F. Ingegneria Genetica, CNR, Rome.

F6 THE ROLE OF PROTEIN KINASE CASCADES AND PROTO-ONCOGENE EXPRESSION AND ACTIVATION IN THE INHIBITION BY cAMP OF PDGF AND PMA-INDUCED HUMAN FIBROBLAST GROWTH.

M. Burton, M. Van Steenbrugge and M. Rase. University of Namur, Laboratory of Cellular Biochemistry and Biology, 61 rue de Bruxelles, 5000 Namur, Belgium.

Fibroblast proliferation is a key event in several normal processes like wound and tissue repair and the control of abnormal cell proliferation leading to fibrosis is a primary problem in numerous diseases. In this study, we investigated the role of protein kinase cascades leading to fibrosis as a primary problem in numerous diseases. In this study, cAMP level-raising agents were shown to inhibit human lung fibroblast proliferation induced by PDGF (Platelet-derived Growth Factor) and PMA (phorbol 12-myristate 13-acetate). We wanted to know at which level of the PDGF and PMA-signalling pathways cAMP interferes. The effects of PDGF are mediated via the activation of protein kinase cascades leading to the activation of transcription factors like wound and tissue repair and the control of abnormal cell proliferation. Fibroblast proliferation is a key event in several normal processes like wound and tissue repair and the control of abnormal cell proliferation leading to fibrosis is a primary problem in numerous diseases.

F7 METALLOTHIONEIN ISOFORM mRNA COMPARTMENTALISATION: ROLE OF 3'UNTRANSLATED REGION AND CYTOSKELETON.

Patrick Mahon1, John Beattiel, L. Anne Glover1 and John Hesketh1. Division of Biochemical Sciences, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, Scotland, U.K. and 2 Department of Molecular and Cell Biology, Marischal College, Aberdeen AB9 1AS.

The metal-binding protein metallothionein has two major isoforms (Mt-1, Mt-2) which are induced in response to metals and oxidative stress. The localisation of Mt-1 and Mt-2 mRNAs in rat hepatoma (H4) cells was investigated using two approaches, namely Northern hybridisation of total RNA extracted from free, cytoskeletal-bound and membrane-bound polysomes isolated by a sequential detergent/salt extraction procedure and in situ hybridisation. The cytoskeletal-bound polysomes were enriched in metallothionein-I (Mt-1) and c-myc mRNAs but showed a significantly lower enrichment in Mt-2 mRNA. In situ hybridisation using a biotin-labelled oligonucleotide probe revealed a predominantly perinuclear localisation for the Mt-1 mRNA. The targeting of other mRNAs has been shown to involve the 3' untranslated regions of those messages. The ability of the Mt-1 3'UTR to target a mRNA was studied in cells transfected with gene constructs in which β-globin reporter sequences were linked either to the native globin region (3'UTR) or the 3'UTR of Mt-1 mRNA. The wild-type globin mRNA was found in free polysomes and showed no localisation by in situ hybridisation. In contrast, chimaeric globin-MT transcripts were associated with cytoskeletal-bound polysomes and found in the perinuclear cytoplasm. We conclude that the Mt-1 3'UTR contains a localisation signal which can target transcripts to the cytoskeleton and the perinuclear cytoplasm.

F8 REGULATION OF INOSITOL SPECIFIC PHOSPHOLIPASE C γ1 EXPRESSION DURING CELL GROWTH INHIBITION IN HUMAN T LYMPHOCYTES.


A large number of cells respond to exogenous stimuli by activating a phospholipase C (PLC) which hydrolyzes PIP2 to two second messengers, inositol trisphosphate and diacylglycerol (1). PLCs have been identified and classified into the groups β, γ, and δ and different isoforms. The PLC γ1 has been found to be regulated during animal development indicating that its expression can be under control of cell proliferation and differentiation (2-3). Here we report the modulation of PLC γ1 expression in human T lymphocytes during growth inhibition induced by interferon beta. Increased levels of PLC γ1 became evident after 90 min. of interferon treatment and were still detectable after 24 hr. The expression of the enzyme then declined progressively reaching control levels after 48 hr of interferon administration. The catalytic activity of the enzyme, checked in terms of nmol. of radiolabeled PIP2 hydrolyzed was found to parallel the modulation of its expression. These findings strongly suggest a functional role of PLC γ1 in the biological events regulating the cell growth machinery in human T lymphocytes.

REFERENCES