demonstrated by the detection of ligand-induced Ca2+ mobilization, chemotaxis, and ligand-induced receptor endocytosis. Surface CXCR4 expression was downregulated by cytokines IL-4, IL-13 and GM-CSF and upregulated by IL-10 and TGF-β1. Downregulation was mediated post-translationally, in the absence of protein degradation, through an endocytotic mechanism. In contrast to SDF-1α–induced CXCR4 endocytosis, cytokine-induced endocytosis of this receptor was independant of actin filament polymerization. GM-CSF increased the expression of GRK3, β-arrestin–1, Pyk2, and FAK. Cytokine treatment also increased the total and tyrosine-specific phosphorylation of CXCR4 as well as the phosphorylation of FAK on tyrosine-397. It also induced the formation of GRK3-CXCR4 or FAK-CXCR4 complexes. Infection of macrophages by primary R5X4 and X4 isolates of HIV-1 was inhibited by IL-4, IL-13, and GM-CSF, an effect that was associated with down-regulation of surface CXCR4 expression. These data indicate that ligand-dependent and ligand-

diated by different mechanisms. ıokine receptors may be of mmation, tumor metastasis, and

675.3

Arsenic-induced c-Src Activation in Uroepithelium

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Environmental or occupational exposure to arsenic is associated with a greatly increased risk of skin, urinary bladder and respiratory tract cancers in arseniasis-endemic areas throughout the world. Arsenic shares many properties of tumor promoters by affecting specific cell signal transduction pathways responsible for cell proliferation. In the current studies, we demonstrate that arsenic activates c-Src in a human uroepithelial cell line. Using pharmacological and genetic inhibition of Src, we found that c-Src activity is a prerequisite for arsenic-induced EGFR and ERK activation in these cells. Consistent with these in vitro observations, exposure of mice to arsenic in drinking water, which has been found previously to be associated with AP-1 activation and epithelial proliferation, induces interaction of EGFR and c-Src in the urinary bladder. This response is also accompanied with an increase in ERK activation. The findings represent a potential pathway for mediating arsenic-induced phenotypic changes in the uroepithelium.

675.4

Phosphorylation of the Ras-GRF1 Exchange Factor Integrates Heterotrimeric G Protein Signaling with Ras Activation

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Combinatorial signaling through integrators of multiple signal transduction pathways is critical to biological response. The Ras-GRF1 exchange factor is a key orchestrator of signaling. The ability of Ras-GRF1 to activate Ras is increased by multiple serine/threonine phosphorylation events that occur in response to stimulation of G protein-coupled receptors (Mattingly & Macara, Nature 382:268), and is also regulated by calcium/calmodulin (Farnsworth et al. Nature 376:524). Ras-GRF1, when it is tyrosine phosphorylated by Src, can also act as an exchange factor for Rac (Kiyono et al. JBC 275:5441). Through CNBr digestion, 2-D tryptic mapping and MALDI/MS, we have now identified 4 of the residues at which muscarinic receptor stimulation increases Ras-GRF1 phosphorylation in intact cells. Phosphorylation of Serine-916, which is required for full activation of the Ras exchange factor (Mattingly, JBC 274:37379), we now show to be a regulated phosphorylation event induced by PKA in rat cortical brain slices. These results therefore provide further mechanistic understanding of the control of the Ras-GRF1 exchange factor in its role as a critical component in combinatorial signaling. (RO1 CA-81150)

675.5

WITHDRAWN

675.6

Glucocorticoids Stimulate Calcineurin in Jurkat T Cells Throu Non Genomic Pathway

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We have shown that dexamethasone (Dex) activates calcin phosphatase activity in Jurkat T cells (JTC) through a non-gen pathway involving phospholipase C (PLC), IP_3 and release of intrace calcium. We show that the activation of calcineurin by Dex is depende iCa^{+2} by pretreating JTC with BAPTA (25uM),an iCa^{+2} chelator. BA completely blocked Dex-induced activation of calcineurin: [cor 993+120; Dex: 1417+218; Dex/BAPTA: 1075+130]. Furthermore, induced activation of PLC involves tyrosine phosphorylation incubating JTC with genistein, a non-specific tyrosine kinase inhit blocked the Dex-induced activation of calcineurin:

(Min)	0	0.25	0.5	5	10
Dex:	1200+60	2149+246	1787+265	2312+239	2274+348
+Gen:	1200+60	1250+250	1640+450	1675+320	1700+200

Specifically, Dex-induced activation of calcineurin involves tyro phosphorylation of PLCγ1 and γ2 isoforms. Using JTC treated with I tyrosine-phosphorylated proteins were immunoprecipitated with monoclonal antibody PY-20, and western blots using PLCγ1 and antibodies showed that Dex increased tyrosine phosphorylation of I isoforms within 15 seconds. Finally, the pro-apoptotic protein BAI dephosphorylated by Dex. JTC were incubated with $^{32}PO_4$ and BAD immunoprecipitated. Autoradiography demonstrated that dephosphorylates BAD at 15 and 30 minutes.

ENDOPLASMIC RETICULUM STRESS RESPONSI (676.1-676.4)

676.1

Induction of organelle membrane biogenesis by Ca $^{2+}$-ATI (SERCA1a) expression.

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The mechanisms by which sarcoplasmic reticulum (SR) arises dur skeletal myogenesis are unknown. SR has been proposed to emerge fi the endoplasmic reticulum (ER) and the biosynthesis of SR/ER C ATPase, SERCA1a, is among the earliest events of skeletal myogene We hypothesize that SERCA1a expression is a stimulus for organ membrane biosynthesis. Overexpression of avian SERCA1a in transfec mouse Ltk$^-$ fibroblasts elicited compact masses of intracellular membr ("plaques") that were enriched in SERCA1a protein. Unfixed c transfected with cDNA encoding a SERCA1a/Green Fluorescent Prot fusion protein demonstrated that membrane plaques were not fixati induced artifacts. Immunofluorescent labeling indicated co-localization SERCA1a with the ER marker calreticulin, although western blots shov that the level of endogenous ER protein expression was not altered SERCA1a-transfected cells. These data suggest that SERCA1a express triggered the generation of specialized membrane structures that contiguous with the ER and contain ER proteins. Supported by University of Delaware Research Foundation and Pfizer, Inc.

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ABSTRACTS
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