1573 THE TAK1-TAO1 COMPLEX MEDIATES STRESS-ACTIVATED SIGNALING.

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Environmental stress stimuli such as osmotic shock and UV irradiation trigger activation of cell signaling to adapt the cells to or resist the stress. Mitogen-activated protein kinases (MAPs) JNK and p38 are major mediators of stress signaling, which are activated through the MAP kinase cascade, a protein kinase cascade composed of MAP3K, MAP2K, and MAPK. A number of MAP3Ks that can activate JNK/p38 have been identified. However, which MAP3K is involved in each stress and how MAP3K is regulated are largely unidentified yet. Here we found that TAK1 MAP3K was activated by osmotic stress. Furthermore, we isolated a kinase TAO1 (thousand and one amino acid protein kinase 1) as a TAK1 binding protein from the yeast two-hybrid screen with TAK1 bait. TAO1 has been reported to have a MAP3K-like activity. TAO1 was also activated by osmotic stress, suggesting that the TAK1-TAO1 complex participated in osmotic stress signaling. It was demonstrated that TAK1 could activate JNK/p38 as well as NF-kB pathway. However, osmotic stress solely activates JNK/p38 but not NF-kB pathway. We found that TAO1 did not activate NF-kB, but rather inhibited TAK1-induced NF-kB activation. These results suggest that TAK1, when activated by osmotic stresses, forms a complex with TAO1 resulting in activation of JNK/p38 but not NF-κB pathway.

1574 USE OF AFFINITY CHROMATOGRAPHY TO IDENTIFY NOVEL PROTEINS THAT MEDIATE STRESS-INDUCED GENE ACTIVATION.

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Exposure of cells to mitogenic or stress stimuli, including a broad range of toxicants, results in the induction of immediate-early (IE) protein kinase cascades and gene activation. The resulting changes in gene expression induce alterations in cell growth, division, differentiation, and metabolism. The mechanisms by which IE cascade activation promotes transcription are yet to be clearly defined, but involve the rapid modification of the histone components of chromatin. In particular, the N-terminal tail of histone H3 is phosphorylated at serine 10 (S10p) and acetylated at lysines 9 and 14 (K9ac and K14ac). However, the way in which these post-translational modifications promote the remodelling of chromatin required for gene activation is unclear. Using affinity chromatography, we have identified human proteins that recognise histone H3 tails containing stress- and mitogen-associated modifications. Synthetic peptides corresponding to unmodified (H3um), diacetylated (H3K9acK14ac), and phospho-diacetylated (H3K9acK14acS10p) histone H3 tails were immobilised onto agarose beads and incubated with HeLa cell nuclear extract. Six proteins bound in a modification-specific manner and were identified by peptide mass spectrometry. RbAp46/48, nucleophosmin, and NAP1, bound preferentially to H3K9acK14ac or H3um. Three proteins with molecular masses of 27.7, 28.3 and 29.1 kDa bound only to the H3K9acK14acS10p peptide, suggesting that they may play a role in mediating the stress and mitogen-induced transcriptional response. Peptide binding competition assays using surface plasmon resonance confirmed that these proteins bind to H3K9acK14acS10p with high affinity and revealed that the S10p modification was the major determinant of binding. Identification of these proteins will shed light on mechanisms by which toxicants induce changes in chromatin structure that facilitate gene activation and the pleotropic stress response.

1575 INHIBITION OF NUCLEAR FACTOR KAPPA B BY PHENOLIC ANTIOXIDANTS: INTERPLAY BETWEEN ANTIOXIDANT SIGNALING AND INFLAMMATORY CYTOKINE EXPRESSION.

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Phenolic antioxidants inhibit the induction of inflammatory cytokines by inflammatory stimuli. Here, we analyzed the mechanism by which the antioxidants inhibit LPS-induced expression of TNFα in macrophages. Hydroquinone (HQ) and t-butyl hydroquinone (tBHQ), prototypes of phenolic antioxidants, block LPS-induced transcription of TNFα and a NF-κB-mediated reporter gene expression, suggesting NF-κB as a target in the inhibition. Analyses of the NF-κB activation pathway revealed that the antioxidants do not inhibit LPS-induced activation of the IκB kinase activity, degradation of IκBα, or translocation of activated NF-κB into the nucleus, but block the formation of NF-κB/DNA binding complexes. *In vitro* experiments showed that the antioxidants do not directly interfere with DNA binding of NF-κB. Structure-activity analyses suggest that inhibition of NF-κB function involves the redox cycling property of the antioxidants. These findings implicate a redox-sensitive factor important for the binding of NF-κB to its DNA recognition sequence as a target molecule in the inhibition of NF-κB function and inflammatory cytokine expression by phenolic antioxidants.

1576 RESTRAINT STRESS ACTIVATES STAT3 VIA ADRENOCEPTOR STIMULATION AND IL-6 PRODUCTION IN MOUSE LIVER.

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In vivo stress causes an activation of the JAK-STAT signaling pathway as evidenced by the large magnitude increases in phosphorylated STAT3 in the livers of mice subjected to restraint. Restraint can elevate IL-6 levels in the liver and this cytokine is known to instigate signaling through the JAK-STAT pathway but the role of the stress mediators, epinephrine, norepinephrine and corticosterone in this cascade is not well understood. Using a combination of focused microwave irradiation to preserve protein phosphorylation state, and phospho-specific immunoblotting, we found that the greater than 10 fold increase in liver PSTAT3tyr705 caused by 2 hrs of restraint is blocked by both alpha and beta adrenergic antagonists. Adrenergic agonists (phenylephrine 10 mg/kg or isoproterenol 10 mg/kg s.c.) which mimic the stress response *in vivo* also activate liver STAT3. Liver responses to both restraint stress and the adrenergic agonists were reduced in IL-6 deficient animals, suggesting that IL-6 is required for signal transduction initiated by activation of adrenoceptors. Pretreatment of IL-6 deficient mice with exogenous IL-6 (1 mg/kg i.p. 60 min before restraint) restored liver STAT3 activation in response to restraint stress. Hepatocyte growth factor and epidermal growth factor were ruled out as potential mediators of restraint stress-induced activation of STAT3 using real time PCR as only message for IL-6 was elevated. Altogether, these data suggest that restraint stress and adrenergic agonist-induced activation of liver STAT3 are dependent on IL-6 synthesis mediated by activation of adrenoceptors in liver. Stress is known to modulate the liver toxicity of numerous compounds. Stress-induced changes in JAK-STAT signaling and subsequent protein transcription will help to clarify the molecular basis of stress-induced modulation of liver toxicity. This work was supported by intramural funds of CDC-NIOSH

1577 EVALUATION OF THE DERMAL BIOAVAILABILITY OF AQUEOUS XYLENE IN F344 RATS AND HUMAN VOLUNTEERS.

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Xylene is a clear, colorless liquid used as a solvent in the printing, rubber, and leather industries and is commonly found in paint thinners, paints, varnishes, and adhesives. Although humans are most likely to be exposed to xylene *via* inhalation, xylene is also found in well and surface water. Therefore, an assessment of the dermal contribution to total xylene uptake is useful for understanding human exposures. To evaluate the significance of these exposures, the dermal absorption of o-xylene was evaluated in F344 male rats and human volunteers using a combination of real-time exhaled breath analysis and physiologically based pharmacokinetic (PBPK) modeling. Animals were exposed to o-xylene at a 0.2 mg/ml aqueous concentration using a 2.5-cm diameter occluded glass patch attached to a clipper-shaved area on the back of the rat. Immediately following the initiation of exposure, individual animals were placed in a glass off-gassing chamber and exhaled breath was monitored. Human volunteers participating in the study placed both legs into a stainless steel hydrotherapy tub containing an initial concentration of approximately 0.5 mg/L o-xylene. Exhaled breath was continually analyzed from each volunteer before, during, and after exposure to track absorption and subsequent elimination of the compound in real time. In both animal and human studies, a PBPK model was used to estimate the dermal permeability coefficient (Kp) to describe each set of exhaled breath data. Rat skin was found to be approximately 12 times more permeable to aqueous o-xylene than human skin. The estimated human and rat aqueous o-xylene Kp values were 0.005 +/- 0.001 cm/hr and 0.058 +/- 0.009 cm/hr, respectively. (Supported by NIEHS grant 1-P42-ES10338-01).

1578 A NEW QSAR MODEL FOR HUMAN SKIN ABSORPTION.

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The objective of this study was to create a model for the quantitative prediction of percutaneous absorption based on physicochemical properties of chemicals. Such a model is valuable as a tool for screening and prioritization purposes for safety evaluation and risk assessment. It is very beneficial to the industry to be able to predict the skin permeability of compounds before they are made or on occasions when no experimental values exist. A five-parameter QSAR model to predict the permeability coefficient Kp, was completed using a training set of 270 chemicals and their

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Preface

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