Intramural papers of the month

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- NTP finds that indium-induced pulmonary toxicity depends on particle solubilization
- Ctp1 acts as a bridge over troubled DNA
- Genome-wide p53 binding is independent of chromatin state, but response depends on it
- The role of the NLRP3 inflammasome in obesity resistance and insulin sensitivity of NAG-1 mice
- Testosterone and peritubular myoid cells involved in maintenance of spermatogonial stem cell microenvironment

**NTP finds that indium-induced pulmonary toxicity depends on particle solubilization**

While studying indium phosphate and indium-tin oxide-induced pulmonary toxicity, researchers from the National Toxicology Program revealed that the lung toxicity of these compounds was dependent upon particle solubilization, not total indium content. The research found that particle solubilization and cytotoxicity data, generated in vitro with macrophages, accurately predicted pulmonary toxicity in vivo. Since indium-containing particles are widely used in the semiconductor and microelectronics industries, the work has a bearing on occupational health.

Macrophage solubilization of particles and cytotoxicity in vitro correlated positively with findings from in vivo studies in which mice were exposed to indium phosphate or indium-tin oxide via oropharyngeal aspiration. Owing to greater particle solubilization by macrophages, cytotoxicity, and hence greater release of the toxic constituent, ionic indium, indium phosphate was far more toxic than indium-tin oxide, both in vitro and in vivo. The authors propose an in vitro model that can be used for toxicity predictions for indium-containing particles and possibly other metal-containing particles, potentially leading to reduced use of animals for pulmonary toxicity testing.


**Ctp1 acts as a bridge over troubled DNA**

Using X-ray crystallography, biophysical techniques, and yeast genetics, NIEHS scientists revealed that Ctp1/CtIP/Sae2, a critical protein involved in the eukaryotic DNA damage response, binds and bridges DNA. The discovery illuminates a new function for the enzyme. Ctp1 family proteins coordinate with the Mre11/Rad50/Nbs1 nuclease complex, a central component of DNA double-strand break (DSB) repair machinery. Mutations in CtIP, the human homologue of Ctp1, are linked to Seckel and Jawad syndromes, two genetic diseases characterized by microcephaly and dwarfism.

In describing Ctp1molecular architecture, the authors identified three key regions with interconnected functionality. First, they determined the crystal structure of a conserved N-terminal tetrameric helical dimer-of-dimers domain (THDD) that enables Ctp1 to form a functional tetramer. Next, they demonstrated that both the THDD and a conserved C-terminal CxxC-RHR motif (RHR) possess DNA binding properties with preference for forked DNA, and that Ctp1 can bind two separate DNA molecules simultaneously. Finally, a flexible, intrinsically disordered region containing multiple DSB-responding protein-binding motifs separates the THDD and RHR. Thus, Ctp1 is a flexible multivalent DNA binding protein capable of recruiting repair partners to and bridging the broken stands of a DSB.

The ability of Ctp1 to bind DNA is paramount to its repair function in vitro and in vivo to protect cells from endogenous and environmental DNA damage. For example, yeast strains lacking Ctp1 DNA binding capacity show DNA repair deficiencies, and are sensitive to genotoxic insult. Future studies into the DNA binding properties of Ctp1/CtIP may provide a molecular basis for CtIP-linked diseases, opening the door for future therapeutics.


**Genome-wide p53 binding is independent of chromatin state, but response depends on it**

Researchers from NIEHS and Duke University have characterized binding of p53 response elements (p53REs) and the
corresponding chromatin landscape across the whole genome, in a cell model exposed to a chemotherapeutic drug. The findings provide new knowledge for developing approaches to prevent or treat cancer.

Tumor suppressor p53 is well known for its role in carcinogenesis. When activated by DNA damage, p53 binds to its DNA response elements and regulates transcription of genes involved in DNA repair and cell death.

In this study, the researchers analyzed stress-induced changes of p53 binding, chromatin state, and gene expression, after treating human lymphoblastoid cells with the DNA-damaging agent doxorubicin, and then mapped p53 binding and the chromatin activation mark, H3K4me3, by ChIP-seq. They discovered that p53-responsive genes showing the largest changes in expression had low levels of H3K4me3 and were repressed at baseline. Binding sites with greater similarity to p53RE consensus sequence correlated with increased p53 occupancy. However, the chromatin landscape strongly influenced the relationship between occupancy and gene induction.

Surprisingly, p53 strongly bound to thousands of DNA elements located in repressed chromatin that have recently evolved from human retroviral transposons. Characterizing the chromatin-mediated p53 stress response and the deregulation of transposons may prove to be clinically relevant for understanding outcomes in cytotoxic therapy for cancer.

The role of the NLRP3 inflammasome in obesity resistance and insulin sensitivity of NAG-1 mice

Researchers from NIEHS have conducted the first study to characterize the association of the NLRP3 inflammasome with diet-induced obesity and improved insulin sensitivity in NSAID activated gene-1 (NAG-1) transgenic mice. The NLRP3 inflammasome is a multiprotein complex that activates caspase-1, leading to the secretion of the proinflammatory cytokines Interleukin (IL)-1beta and IL-18. Both IL-1beta and IL-18 have been associated with obesity, insulin resistance, and type 2 diabetes.

The study demonstrated that, compared with their wild-type littermates, NAG-1 Tg mice, whether fed with a low fat or high fat diet, had lower NLRP3 inflammasome activity and lower expression of NLRP3 proteins, caspase-1, and apoptosis-associated speck-like protein. Expression of IL-1beta, IL-18, and TNFalpha in white adipose tissue (WAT) was also reduced. Furthermore, NAG-1 Tg mice exhibited significantly lower levels of leptin, more insulin sensitivity, and reduced mRNA levels of macrophage infiltration markers F4/80, CD11b, and CD11c in WAT.

Taken together, this study suggests that NAG-1 may be an important regulator in the development of obesity. The mechanism by which NAG-1 plays a protective role in obesity appears, in part, to be mediated by changes in NLRP3 inflammasome activity.

Testosterone and peritubular myoid cells involved in maintenance of spermatogonial stem cell microenvironment

NIEHS scientists suggest that testosterone-dependent regulation of glial cell line-derived neurotrophic factor (GDNF) in peritubular myoid (PM) cells influences spermatogonial stem cell (SSC) maintenance in vitro. GDNF is a protein that is involved in the maintenance, proliferation, and self-renewal of SSCs, and is produced by PM cells in vitro. Since conditions in the microenvironment determine whether SSCs undergo self-renewal or differentiation, this work may provide a better understanding of how male mice sustain sperm production throughout their reproductive years.

PM cells and Sertoli cells form the cellular boundary of the SSC microenvironment, but scientists did not know what role PM cells played in SSC maintenance. The researchers employed an adult mouse PM cell primary culture system and germ cell transplantation to find out. They determined that testosterone induced GDNF expression in PM cells. They also cocultured PM cells, with and without testosterone, and thymocyte antigen 1-positive spermatogonia. When the cells were transplanted to the testes of germ cell-depleted mice, the SSCs cocultured with testosterone-treated PM cells rendered significantly more transplant-derived colonies.

This research confirms other studies that assert GDNF is necessary for the ability of SSCs to undergo proliferation and self-renewal in vitro, but it also supports the hypothesis that PM cells exert a heavy influence on SSC maintenance.

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