Intramural papers of the month

By Tara Ann Cartwright, Deacqunita Diggs, Geoffrey Feld, Vijay More, and Qing Xu

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

While studying indium phosphide 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 phosphide 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 phosphide 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. **(VM)**

Citation: Gwinn WM, Qu W, Bousquet RW, Price H, Shines CJ, Taylor GJ, Waalkes MP, Morgan DL. *(http://www.ncbi.nlm.nih.gov/pubmed/25527823)*
2014. Macrophage solubilization and cytotoxicity of indium-containing particles as in vitro correlates to pulmonary toxicity in vivo. Toxicol Sci; doi:10.1093/toxsci/kfu273 [Online 19 March 2015].

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. **(GF)**

Citation: Andres SN, Appel CD, Westmoreland JW, Williams JS, NguyenY, Robertson PD, Resnick MA, Williams RS. *(http://www.ncbi.nlm.nih.gov/pubmed/25580577)*
2015. Tetrameric Ctp1 coordinates DNA binding and DNA bridging in DNA double-strand-break repair. Nat Struct Mol Biol 22(2):158-166. [Story]

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. **(QX)**

Citation: Su D, Wang X, Campbell MR, Song L, Safi A, Crawford GE, Bell DA.
(http://www.ncbi.nlm.nih.gov/pubmed/25569532)
2015. Interactions of chromatin context, binding site sequence content, and sequence evolution in stress-induced p53 occupancy and transactivation. PLoS Genet 11(1):e1004885.

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. **(TAC)**

Citation: Wang X, Chrysovergis K, Kosak J, Eling TE.
(http://www.ncbi.nlm.nih.gov/pubmed/24124102)
2014. Lower NLRP3 inflammasome activity in NAG-1 transgenic mice is linked to a resistance to obesity and increased insulin sensitivity. Obesity (Silver Spring) 22(5):1256-1263.

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. **(DD)**

Citation: Chen LY, Brown PR, Willis WB, Eddy EM.
(http://www.ncbi.nlm.nih.gov/pubmed/25181385)
2014. Peritubular myoid cells participate in male mouse spermatogonial stem cell maintenance. Endocrinology 155(12):4964-4974.

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The Environmental Factor is produced monthly by the National Institute of Environmental Health Sciences (NIEHS) *(http://www.niehs.nih.gov/)*, Office of Communications and Public Liaison. We welcome your comments and suggestions. *(bruskec@niehs.nih.gov)*

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