Intramural papers of the month

By Aleksandra Adomas, Heather Franco, Mallikarjuna Metukuri, Bailey Schug, Deepa Singh, and Sheila Yong

Assessing cockroach allergen exposure via its structure

NIEHS scientists and their collaborators have obtained the crystal structure of the cockroach allergen Bla g 1. It is the first structure to be solved for this group of insect proteins. Determining the structure of Bla g 1 allows for the standardization of assays that measure it in absolute units.

The research team used recombinant protein rather than native Bla g 1, but comparisons using ELISA dose-response curves and antibody binding properties found that the Bla g 1 made by bacteria and the natural allergen were similar. Team members then solved the structure of Bla g 1 using X-ray crystallography, which revealed a novel fold with the capacity to bind various lipids.

Further analysis using mass spectrometry and nuclear magnetic resonance indicated that Bla g 1 could accommodate a variety of hydrophobic ligands that could be important for a variety of physiological purposes, including sensitizing humans. This finding correlated well with a digestive function in the cockroach, where Bla g 1 is associated with nutrient uptake.

Overall, the authors believe that this work would allow a better assessment of Bla g 1 exposure, which is important in evaluating new intervention strategies. Additionally, the structure may be useful in designing new immunotherapies. (DS)

Citation: Mueller GA, Pedersen LC, Lih FB, Glesner J, Moon AF, Chapman MD, Tomer KB, London RE, Pomes A. *(http://www.ncbi.nlm.nih.gov/pubmed/23915714)*
2013. The novel structure of the cockroach allergen Bla g 1 has implications for allergenicity and exposure assessment. J Allergy Clin Immunol; doi:10.1016/j.jaci.2013.06.014 [Online 31 July 2013].

Study finds pregnancy length varies by up to 5 weeks

A recent study published by researchers in the NIEHS Epidemiology Branch found that the length of a normal human pregnancy can vary naturally by as much as five weeks. The researchers gathered information from the North Carolina Early Pregnancy Study, which took place 1982-1985, and followed the pregnancies of 125 women from conception through birth.

Using urinary hormone measures, the researchers were able to identify the precise point at which a woman ovulates and an embryo implants in the womb. They found that the median time for ovulation to live birth was 268 days, or 38 weeks and 2 days, but most surprising was the 37-day range of gestational length among healthy pregnancies.

The researchers took information from daily urine samples and analyzed the samples for three hormones connected with the onset of pregnancy: estrone-3-glucoronide, pregnanediol-3-glucoronide, and human chorionic gonadotropin (hCG). Implantation was determined as the first day of a sustained rise in hCG levels.

Women are normally given a due date that is calculated as 280 days after the onset of their last menstrual period, but these findings indicate that a range of dates may better communicate to women when they are likely to deliver. Also, biologic variability may be greater than previously thought. (BS)

Citation: Jukic AM, Baird DD, Weinberg CR, McConnaughey DR, Wilcox AJ. *(http://www.ncbi.nlm.nih.gov/pubmed/23922246)*
2013. Length of human pregnancy and contributors to its natural variation. Hum Reprod 28(10):2848-2855.

Role of arginine in polyemerase beta-catalyzed nucleotidyl transfer reactions

NIEHS scientists showed that arginine in position 254 (Arg254) has a crucial role for stabilizing the active site of mammalian polymerase beta and enabling proton transfer during gap-filling DNA synthesis. Proton transfer from the primer O3' group to a nearby carboxylate group of aspartate in position 256 (Asp256) in the enzyme active site is a key step of the nucleotidyl transfer reaction catalyzed by polymerase beta. Knowing how the catalytic action of polymerases works is important for understanding factors controlling efficiency of correct nucleotide insertion and fidelity in DNA repair and replication.

The researchers mutated the Asp256 residue to glutamate and observed that the mutant was more than 1000 times less active than the wild-type enzyme and that the crystal structures were subtly different in the active site. Computational analyses determined that the O3' proton in the mutated polymerase still transferred to the nearby carboxylate in position 256, but it happened late in the reaction path. Since this late transfer was due to repositioning of Arg254 side chain, Arg254 was not able to stabilize the proton transfer from O3.' Also, the glutamate-containing enzyme did not undergo charge reorganization associated with the proton transfer, which is mediated by the catalytic magnesium ion in the active site. (AA)

Citation: Batra VK, Perera L, Lin P, Shock DD, Beard WA, Pedersen LC, Pedersen LG, Wilson SH.
(http://www.ncbi.nlm.nih.gov/pubmed/23647366)
2013. Amino acid substitution in the active site of DNA polymerase beta explains the energy barrier of the nucleotidyl transfer reaction. J Am Chem Soc 135(21):8078-8088.

Redundancy in DNA repair mechanisms protects cells from UV-induced DNA damage

Using G2-arrested budding yeast, NIEHS scientists have identified redundancy in the mechanisms that repair UV-induced DNA damage. Given the conservation of repair pathways and links with various human pathologies including cancer, the finding may lead to new therapies for treating these diseases.

The researchers found that mutations in either translesion DNA synthesis (TLS) or homologous recombination (HR) reduced the repair efficiency of UV lesions, as surmised from formation and disappearance of repair intermediates. However, inactivation of both pathways led to accumulation of long-lived intermediates and loss of survival. Based on a physical assay they previously developed to detect recombination between sister chromatids, UV-induced recombination was not due to formation of DNA double-strand breaks. They concluded that TLS and HR are functionally redundant in protecting G2 cells from UV-induced DNA damage.

The authors proposed that repair of UV damage normally begins with formation of a small single-stranded DNA gap produced by nucleotide-excision repair. The TLS pathway can fill the gap, but in its absence, the HR pathway is initiated by the gaps and completes the repair. Identification of this pathway provides new insights into understanding genome instability and other environmental health risks associated with lesions such as those due to UV exposure. (HF)

Citation: Ma W, Westmoreland JW, Resnick MA.
(http://www.ncbi.nlm.nih.gov/pubmed/23858457)
2013. Homologous recombination rescues ssDNA gaps generated by nucleotide excision repair and reduced translesion DNA synthesis in yeast G2 cells. Proc Natl Acad Sci U. S. A. 110(31):E2895-E2904.

Crystal structures reveal how flame retardants may disrupt estrogen metabolism

In a recent study, NIEHS and National Cancer Institute researchers revealed how brominated flame retardants (BFRs), chemicals widely used in consumer products to reduce the spread of fire, may disrupt estrogen metabolism. The research impacts public health since these compounds are released into the environment from existing and discarded products, and can be detected in various living organisms.

The authors used tetrabromobisphenol A (TBBPA), the most widely used BFR, and BFR metabolite 3-OH-BDE-47, to demonstrate how BFRs bind human estrogen sulfotransferase (SULT1E1). SULT1E1 normally binds to estrogen to regulate its concentration and excretion from the body. Interestingly, TBBPA and 3-OH-BDE-47 share a similar phenolic ring structure with estrogen that allows them to bind to SULT1E1.

The scientists unveiled three-dimensional crystal structures of SULT1E1 as a complex with estrogen, TBBPA, and 3-OH-BDE-47, respectively. They showed that both TBBPA and 3-OH-BDE-47 can bind to the same substrate-binding site in SULT1E1 as estrogen. Moreover, TBBPA and 3-OH-BDE-47 are structurally more flexible, and the bromine atoms on their phenolic ring enhance their binding affinity to SULT1E1. These findings show that SULT1E1 can accommodate structurally diverse halogenated compounds, suggesting that SULT1E1 can be inhibited by a wide range of BFRs at low doses. (SY)

Citation: Gosavi RA, Knudsen GA, Birnbaum LS, Pedersen LC.
(http://www.ncbi.nlm.nih.gov/pubmed/23959441)
2013. Mimicking of estradiol binding by flame retardants and their metabolites: a crystallographic analysis. Environ Health

Perspect 121(10):1194-1199.

Probiotics use during pregnancy many reduce diseases in children

Based on data from the Norwegian Mother and Child Cohort Study (MoBa), a research team led by NIEHS scientists have determined that, for the general population, probiotics consumed during pregnancy and infancy may help prevent atopic eczema and rhinoconjunctivitis in early childhood.

The researchers used data from more than 40,000 mother and children pairs participating in the MoBa study, which recruited Norwegian women between 1999 and 2008. The authors assessed the consumption of Tine SA Biola and Cultura., two types of probiotic-enriched milk and yogurt, in pregnancy and infancy, using a pregnancy food frequency questionnaire and a postnatal questionnaire. These products contain lactobacilli and bifidobacteria species that are safe, and were the only probiotic foods widely available on the market during the time of the study.

Thirty-seven percent of pregnant mothers in the study consumed these probiotic milk and yogurt products, and 18 percent of them also gave their child probiotic milk products after 6 months of age. The researchers found that maternal probiotic milk consumption in pregnancy, as well as consumption during early childhood, was associated with a slightly reduced relative risk of atopic eczema at 6 months, and rhinoconjunctivitis symptoms between 18-36 months.

The findings indicate that probiotic intake might be beneficial for the prevention of atopic eczema and rhinoconjunctivitis in the general population of children. Previous studies focused on high risk children. The fact that only two probiotic products were available during the study period likely facilitated identification of potential health effects. (BS)

Citation: Bertelsen RJ, Brantsaeter AL, Magnus MC, Haugen M, Myhre R, Jacobsson B, Longnecker MP, Meltzer HM, London SJ.
(http://www.ncbi.nlm.nih.gov/pubmed/24034345)
2013. Probiotic milk consumption in pregnancy and infancy and subsequent childhood allergic diseases. J Allergy Clin Immunol; doi:10.1016/j.jaci.2013.07.032 [Online 10 September 2013].

Dermatomyositis shares genetic background with other autoimmune diseases

NIEHS researchers, together with American and European collaborators, conducted the first genome-wide association study of any form of myositis, and found that dermatomyositis shares common genetic features with other autoimmune disorders. Pathogenesis of dermatomyositis, which is characterized by muscle and skin inflammation, is largely unknown, and identification of disease genetic predispositions could lead to novel diagnostic and therapeutic advances.

The scientists performed a genome-wide association study and examined genetic variants, or single nucleotide polymorphisms (SNPs), to determine if any of these variants were associated with the disease. Genotyping of more than 1,000 adult and juvenile patients of European ancestry, and 4,000 healthy control individuals, confirmed a strong association of dermatomyositis with major histocompatibility complex (MHC). MHC molecules mediate recognition of body cells by lymphocytes, and are involved in autoimmune diseases.

The researchers then focused on SNPs outside of the MHC region that are specifically associated with autoimmune diseases. They were able to identify three genes - phospholipase C like 1 (PLCL1), B lymphoid tyrosine kinase (BLK), and chemokine ligand 21 (CCL21) - that could be linked with dermatomyositis. (AA)

Citation: Miller FW, Cooper RG, Vencovsky J, Rider LG, Danko K, Wedderburn LR, Lundberg IE, Pachman LM, Reed AM, Ytterberg SR, Padyukov L, Selva-O'Callaghan A, Radstake T, Isenberg DA, Chinoy H, Ollier WE, O'Hanlon TP, Peng B, Lee A, Lamb JA, Chen W, Amos CI, Gregersen PK; Myositis Genetics Consortium.
(http://www.ncbi.nlm.nih.gov/pubmed/23983088)
2013. Genome-wide association study of dermatomyositis reveals genetic overlap with other autoimmune disorders. Arthritis Rheum; doi:10.1002/art.38137 [Online 27 August 2013].

Identification of novel immune regulatory elements and epigenetic plasticity in memory lymphocytes

A collaborative team led by NIEHS investigators determined that the global reprogramming of the epigenome, or the epigenetic modifications of DNA, permits adult somatic cells to differentiate into diverse cell types. This reprogramming occurs when naive B cells enter the germinal-center (GC) reaction in secondary lymphoid organs and contributes to the cells' ability to rapidly differentiate into plasma cells when the body is rechallenged by antigens during a secondary immune response.

The authors purified four different B cell populations - naive, GC, memory, and plasma cells (PC) - from inflamed tonsils of eight individuals and performed global DNA methylation analysis using the methylated CpG island recovery assay (MIRA).

To investigate the function of DNA methylation changes associated with immune activation, the authors determined whether activation-induced differentially methylated regions were enriched for regulatory elements and also found that Alu elements display differential methylation patterns, correlated with decreased expression of the de novo methyltansferase DNMT3A in GC B cells.

The authors propose that the loss of DNA methylation during the naive to GC B cell transition permits these cells to differentiate toward memory or PC fates, and to generate the differential response to antigenic challenge. (MM)

Citation: Lai AY, Mav D, Shah R, Grimm SA, Phadke D, Hatzi K, Melnick A, Geigerman C, Sobol SE, Jaye DL, Wade PA. *(http://www.ncbi.nlm.nih.gov/pubmed/24013550)*
2013. DNA methylation profiling in human B cells reveals immune regulatory elements and epigenetic plasticity at Alu elements during B-cell activation. Genome Res; doi:10.1101/gr.155473.113 [Online 6 September 2013].

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