

## **Intramural papers of the month**

By Raj Gosavi, Mallikarjuna Metukuri, Jacqueline Powell, Staton Wade, and Sheila Yong

- [NTP review framework addresses environmental health questions](#)
- [Polymerase beta complements aprataxin function by catalyzing a deadenylation reaction](#)
- [RORgamma regulates hepatic glucose metabolism and insulin sensitivity](#)
- [INO80 regulates embryonic stem cell fate and blastocyst development](#)
- [Novel structural insights into HIV reverse transcriptase](#)

### **NTP review framework addresses environmental health questions**

Scientists in the NTP Office of Health Assessment and Translation (OHAT) recently published a flexible 7-step process to streamline the development of hazard identification conclusions. The principles of this systemic review process are intended to help environmental health scientists integrate evidence from a variety of sources. While systemic review methodologies are well-established in the area of clinical medicine, particularly for human clinical trials, environmental health questions involve unique challenges, due to the breadth of the relevant data, including results of human, animal, and mechanistic studies.

In 2011, OHAT began consulting with a variety of sources, including technical experts, the NTP Board of Scientific Counselors, and the public, to develop an efficient and standardized systematic review approach for literature-based environmental health science assessments. The resulting 7-step framework not only increases transparency and objectivity in the process of collecting and synthesizing scientific evidence for reaching conclusions, but also provides methods to increase data management efficiency.

The 7 steps provide guidance on problem formulation and protocol development, searching and selecting studies for inclusion, extracting data from studies, assessing the quality of individual studies, rating confidence in the body of evidence, translating confidence ratings into evidence of health effects, and integrating evidence to develop hazard identification conclusions. **(JP)**

*Citation:* [Rooney AA, Boyles AL, Wolfe MS, Bucher JR, Thayer KA.](#)

(<http://www.ncbi.nlm.nih.gov/pubmed/24755067>)

2014. Systemic review and evidence integration for literature-based environmental health science assessments. *Environ Health Perspect*; doi:10.1289/ehp.1307972 [Online 22 April 2014].

### **Polymerase beta complements aprataxin function by catalyzing a deadenylation reaction**

A study published by NIEHS researchers found that human DNA polymerase beta (pol beta) removed the 5'-adenosine monophosphate group (5'-AMP) on the abasic site (5'-dRP) of base excision repair (BER) substrates. For aborted ligation products, pol beta, together with flap endonuclease 1 (FEN1), is reported to have the potential to complement the function of aprataxin (APTX) in cells that are deficient in APTX. Considering that lack of functional APTX is associated with neurodegenerative disorder ataxia oculomotor apraxia type 1 (AOA1), these findings highlight important cellular pathways that can take over during APTX deficiency.

The authors confirmed catalysis of pol beta in removing 5'-AMP-dRP from the DNA substrates, using *in vitro* assays, along with a new crystal structure of pol beta in complex with the substrates. Further verification of pol beta function was obtained by *in vivo* studies using *S. cerevisiae*. Studies with yeast strains containing deletion of Hnt3 or Rad27, yeast homologs of APTX and FEN1, respectively, affirmed complementary function of pol beta and FEN1 to that of APTX. Furthermore, live cells deficient in Hnt3 and Rad27 showed hypersensitivity to the genotoxic agent methyl methanesulfonate, which was rescued when pol beta was present.

The *in vivo* and *in vitro* studies both suggest alternative enzymatic processes in cells to fix aborted ligation products, which, if left unrepaired, may result in DNA double-strand breaks. **(RG)**

*Citation:* [Caglayan M, Batra VK, Sassa A, Prasad R, Wilson SH.](#)

(<http://www.ncbi.nlm.nih.gov/pubmed/24777061>)

2014. Role of polymerase beta in complementing aprataxin deficiency during abasic-site base excision repair. *Nat Struct Mol Biol* 21(5):497-499.

### **RORgamma regulates hepatic glucose metabolism and insulin sensitivity**

A recent study conducted by NIEHS researchers determined that retinoic acid-related orphan receptor gamma (RORgamma) regulates diurnal hepatic gluconeogenesis and insulin sensitivity. RORgamma is a nuclear receptor that functions as a ligand-dependent transcription factor by binding to ROR-responsive elements in target genes. The circadian clock plays a critical role in the regulation of many physiological processes, including metabolism and energy homeostasis. Disruption of circadian rhythm increases the risk for several diseases. In the liver, RORgamma exhibits a robust circadian pattern of expression that is under the direct control of the hepatic circadian clock.

In the present study, using ubiquitous and liver-specific RORgamma-deficient mice, the authors demonstrated that mice deficient in RORgamma exhibit improved insulin sensitivity and glucose tolerance. This is associated with reduced hepatic gluconeogenesis, particularly in the daytime, and is due to a reduced peak expression of several glucose metabolic genes. By using genome-wide cistronic profiling, gene expression, and promoter analysis, the authors further demonstrated that RORgamma directly regulates glucose metabolic genes downstream of the hepatic clock machinery.

Since RORgamma enhances gluconeogenesis and decreases insulin sensitivity and glucose tolerance, the authors propose that suppressing RORgamma activity by antagonists might be beneficial in controlling glucose homeostasis and the management of metabolic diseases, such as type 2 diabetes. **(MM)**

*Citation:* [Takeda Y, Kang HS, Freudenberg J, DeGraff LM, Jothi R, Jetten AM.](#)

(<http://www.ncbi.nlm.nih.gov/pubmed/24831725>)

2014. Retinoic acid-related orphan receptor gamma (RORgamma): a novel participant in the diurnal regulation of hepatic gluconeogenesis and insulin

## INO8o regulates embryonic stem cell fate and blastocyst development

NIEHS researchers and their collaborators discovered that the INO8o complex maintains the pluripotency of embryonic stem cells (ESCs), allowing them to remain undifferentiated. Their findings provide insights into how ESCs selectively activate pluripotency genes and repress differentiation genes.

Using a combination of molecular biology, biochemistry, and systems biology techniques, the scientists showed that INO8o occupies promoters of genes involved in ESC self-renewal and pluripotency. INO8o does so with help from transcription factor OCT4 and histone methyltransferase complex component WDR5 - two other key pluripotency proteins. INO8o binding to the promoters maintains an open chromatin structure that allows transcription to occur. Subsequently, the Mediator protein and RNA polymerase Pol II are recruited, leading to increased transcription and expression of pluripotency genes.

Interestingly, when the researchers reprogrammed differentiated cells to produce induced pluripotent stem cells (iPSCs), they found that INO8o expression rapidly increased, followed by increased expression of pluripotency genes. Hence, they proposed that INO8o activates the pluripotency network in iPSCs. The researchers also observed an increase in INO8o expression during early embryonic development, reaching its peak at the blastocyst stage. INO8o expression in the blastocyst is required to establish pluripotency in the inner cell mass, which ultimately forms the embryo. **(SY)**

*Citation:* Wang L, Du Y, Ward JM, Shimbo T, Lackford B, Zheng X, Miao YL, Zhou B, Han L, Fargo DC, Jothi R, Williams CJ, Wade PA, Hu G. (<http://www.ncbi.nlm.nih.gov/pubmed/24792115>)

2014. INO8o facilitates pluripotency gene activation in embryonic stem cell self-renewal, reprogramming, and blastocyst development. Cell Stem Cell 14(5):575-591.

## Novel structural insights into HIV reverse transcriptase

NIEHS researchers have provided the first detailed characterization of the structural changes that occur during the formation of HIV reverse transcriptase, the enzyme that makes DNA copies of the viral RNA genome. This process, which allows the subsequent integration of viral DNA into the host genome, is critical to the HIV life cycle. Structural insights provided by this work identify potential targets for the development of novel therapeutics for HIV.

HIV reverse transcriptase is constructed from two p66 peptides, which form an initial p66/p66' homodimer. Then, a complex series of conformational transformations result in partial unfolding of one subunit. This action makes its cleavage site available to HIV protease, producing the mature p66/p51 heterodimer. The researchers used NMR and X-ray data to construct a model of the p66 monomer, and to identify many of the complex conformational changes that both precede and follow formation of the p66/p66' homodimer.

The research team determined that the p66/p66' homodimer exists as a conformational heterodimer, in which the two chains, although having identical sequences, adopt different conformations. The conformational changes that occur in the p66' subunit ultimately result in the destabilization of one of the p66' domains - the ribonuclease H - so that it unfolds and is selectively destroyed by viral HIV protease. **(SW)**

*Citation:* Zheng X, Pedersen LC, Gabel SA, Mueller GA, Cuneo MJ, Derosé EF, Krahn JM, London RE. (<http://www.ncbi.nlm.nih.gov/pubmed/24574528>)

2014. Selective unfolding of one ribonuclease H domain of HIV reverse transcriptase is linked to homodimer formation. Nucleic Acids Res 42(8):5361-5377.

(Raj Gosavi, Ph.D., is a research fellow in the NIEHS Structure and Function Research Group. Mallikarjuna Metukuri, Ph.D., is a research fellow in the NIEHS Metabolism, Genes, and Environment Group. Former NIEHS postdoctoral fellow Jacqueline Powell, Ph.D., is a writer and analyst with Education and Training Systems International. Staton Wade, Ph.D., is an Intramural Research Training Award fellow in the NIEHS Chromatin and Gene Expression Group. Sheila Yong, Ph.D., is a visiting fellow in the NIEHS Inositol Signaling Group.)

---

The Environmental Factor is produced monthly by the [National Institute of Environmental Health Sciences \(NIEHS\)](http://www.niehs.nih.gov/)

(<http://www.niehs.nih.gov/>)

, Office of Communications and Public Liaison. The content is not copyrighted, and it can be reprinted without permission. If you use parts of Environmental Factor in your publication, we ask that you provide us with a copy for our records. We welcome your [comments and suggestions](#). ([bruskec@niehs.nih.gov](mailto:bruskec@niehs.nih.gov))

This page URL: NIEHS website: <http://www.niehs.nih.gov/>  
Email the Web Manager at [webmanager@niehs.nih.gov](mailto:webmanager@niehs.nih.gov)