

## Intramural papers of the month

By Kelly Lenox, Kristin Lichti-Kaiser, Zack McCaw, and Mallikarjuna Metukuri

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### Scientists closer to understanding stem cell self-renewal and differentiation

A research team, led by NIEHS scientists, determined that the mRNA export complex THO, present in embryonic stem cells (ESCs), preferentially interacts with and regulates pluripotency gene expression. Pluripotency is the ability of a cell to develop into any type of cell, and self-renewal is the ability of a cell to proliferate while maintaining its developmental potential. ESCs that lack Thoc5 or Thoc2, two components of THO, lose this self-renewal capacity and differentiate into various cell types. This research is vitally important to basic and clinical research because scientists use ESCs to derive cells for disease modeling, drug discovery, and the development of cell-based therapies.

The authors cultured ESCs and transfected cells with either Thoc2 or Thoc5 small interfering RNAs (siRNAs) in 96-well plates. Other experimental techniques included immunofluorescence, microarray, and RNA immunoprecipitation and sequencing.

The researchers found that THO, via Thoc5, is needed for ESC self-renewal. Furthermore, they discovered that a decrease of THO inhibited somatic cell reprogramming and blastocyst development, making THO a required complex in the establishment of pluripotency. The finding that ESC self-renewal and differentiation occurs at the post-transcriptional level brings scientists closer to understanding the mechanisms involved in stem cell fate specification. **(ZM)**

*Citation:* Wang L, Miao YL, Zheng X, Lackford B, Zhou B, Han L, Yao C, Ward JM, Burkholder A, Lipchina I, Fargo DC, Hochedlinger K, Shi Y, Williams CJ, Hu G.

(<http://www.ncbi.nlm.nih.gov/pubmed/24315442>)

2013. The THO complex regulates pluripotency gene mRNA export and controls embryonic stem cell self-renewal and somatic cell reprogramming. *Cell Stem Cell* 13(6):676-690. [[Story](#)]

### Estrogen receptor alpha involved in DES-induced gene expression in male mice

In a recent issue of *Environmental Health Perspectives*, NIEHS researchers are the first to report a novel estrogen receptor alpha (ERalpha) dependent correlation between DNA methylation patterns and levels of the *Svs4* and *Ltf* genes, after neonatal diethylstilbestrol (DES) exposure in male mice. This work provides more detail on the negative effects of endocrine-disrupting chemicals on mammals.

Researchers used a neonatal DES exposure mouse model to examine changes in the methylation patterns of androgen-dependent gene *Svs4* and estrogen-dependent gene *Ltf* in the seminal vesicles of male mice. They found that DNA methylation of the *Svs4* gene promoter changed from methylated to unmethylated during development, and that DES exposure prevented this change. In the *Ltf* gene promoter, DES exposure altered the methylation status from methylated to unmethylated at two specific CpGs. Alterations in methylation status correlated with decreased levels of *Svs4* and increased levels of *Ltf* gene expression in an ERalpha-dependent manner. In addition, DES exposure increased expression of epigenetic modifiers. **(KLK)**

*Citation:* Li Y, Hamilton KJ, Lai AY, Burns KA, Li L, Wade PA, Korach KS.

(<http://www.ncbi.nlm.nih.gov/pubmed/24316720>)

2013. Diethylstilbestrol (DES)-stimulated hormonal toxicity is mediated by ERalpha alteration of target gene methylation patterns and epigenetic modifiers (DNMT3A, MBD2, and HDAC2) in the mouse seminal vesicle. *Environ Health Perspect*; doi:10.1289/ehp.1307351 [Online 6 December 2013].

### Identification of a novel mechanism that suppresses glucocorticoid signaling

A recent study conducted by researchers at NIEHS identified hairy and enhancer of split-1 (HES1) as a novel regulator of glucocorticoids, the primary stress hormones essential for life. HES1 is a key regulator of development and organogenesis, and the authors suggest abnormal expression of HES1 may contribute to forms of glucocorticoid resistance seen in some patients.

The authors demonstrated that glucocorticoids silence HES1 gene and protein expression in multiple cell types and tissues.

Overexpression of HES1 in human cells led to reduced glucocorticoid-mediated changes in gene expression and vice versa. In addition, a mutated form of HES1 could not impair glucocorticoid signaling. To assess the effects *in vivo*, the authors generated HES1 liver knockout mice, which displayed abnormal glucocorticoid-dependent signaling profiles that resulted in impaired glucose tolerance. This metabolic phenotype was corrected by the removal of endogenous glucocorticoids by adrenalectomy, whereas injection of exogenous glucocorticoids restored it.

The findings suggest that the dismissal of HES1 cooperates with the glucocorticoid receptor to regulate a large component of the transcriptional targets of glucocorticoids through a transcriptional derepression mechanism. The authors propose that HES1 is a master regulator of glucocorticoid signaling profile and silencing of HES1 is required for proper glucocorticoid signaling.

**(MM)**

*Citation:* [Revollo JR, Oakley RH, Lu NZ, Kadmiel M, Gandhavadi M, Cidlowski JA.](#)

<http://www.ncbi.nlm.nih.gov/pubmed/24300895>

2013. HES1 is a master regulator of glucocorticoid receptor-dependent gene expression. *Sci Signal* 6(304):ra103.

## Factors critical during early stages of heart development clarified

NIEHS researchers studying heart development in mammalian embryos reported several important findings that reveal specialized and novel cardiac-enriched Switch/Sucrose NonFermentable (SWI/SNF) chromatin-remodeling complexes. Because these complexes are required for heart formation and critical for cardiac gene expression regulation at the early stages of heart development, the results will be valuable in improving cardiac reprogramming strategies and elucidating the mechanisms that contribute to congenital heart disease.

Studying mouse embryos at distinct developmental stages, researchers demonstrated that the SWI/SNF complex subunits exhibit differential patterns of expression in early development, and that specific BRG1-associated factors were elevated in the early heart compared with head and trunk. Subsequent studies revealed that the BAF250a subunit was required for fully functional cardiomyocyte differentiation *in vitro*. Results suggest that the subunit BAF250a plays a regulatory role in early heart formation, via the direct repression of cardiac-specific gene expression. Molecular analyses suggest that BAF250a physically interacts with repressor proteins, including CHD4 and HDAC1, to alter the chromatin architecture and epigenetic signature of cardiac related genes.

Defining a previously unknown SWI/SNF complex composition and spatiotemporal expression pattern, these findings provide novel insight into the role of the complex in cardiac lineage decision and subsequent heart formation during early development.

**(KL)**

*Citation:* [Singh AP, Archer TK.](#)

<http://www.ncbi.nlm.nih.gov/pubmed/24335282>

2013. Analysis of the SWI/SNF chromatin-remodeling complex during early heart development and BAF250a repression cardiac gene transcription during P19 cell differentiation. *Nucleic Acids Res*; doi:10.1093/nar/gkt1232 [Online 13 December 2013].

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