Thyroid

THYROID HORMONE METABOLISM AND ACTION

T_3-Dependent Transcriptional Programming by TRβ in Thyroid Cells Requires SWI/SNF Chromatin Remodelers

Noelle Gillis, BS, Jennifer Tomczak, MPH, Seth Frietze, PhD, Frances E. Carr, PhD.
University of Vermont, Burlington, VT, USA.

Transcriptional regulation in response to thyroid hormone (T_3) is a dynamic and cell-type specific process that maintains cellular homeostasis and identity. A bimodal switch model, where T_3 binding alters the co-regulator profile of a constitutively DNA-bound thyroid hormone receptor (TR) to affect downstream gene expression, is widely used to describe the interaction between TRβ and chromatin. To test this model on a genome-wide scale, we used an integrated genomics approach to profile and characterize the cistrome of TRβ by CUT&RUN, map changes in chromatin accessibility by ATAC-seq, and capture the transcriptomic changes in response to T_3 by RNA-seq in the normal thyroid cell line, Nthy-ORI. Our CUT&RUN data demonstrated that T_3 binding causes significant shifts in TRβ genomic occupancy; these shifts are associated with differential chromatin accessibility. Most of the T_3-induced differentially expressed genes have a TRβ binding site associated with the proximal protomer region within one kilobase of the transcriptional start site, suggesting that these are direct TRβ regulatory target genes. Remarkably, the majority of TRβ binding sites were found in transgenic regions and distal regulatory elements. In order to identify the co-regulatory proteins that are required for execution of a T_3-dependent transcriptional program in our thyroid cells, we used a TRβ-miniTurboID fusion construct to perform a proximity ligation assay followed by mass spectrometry. We identified 1,138 nuclear proteins that interact with TRβ. Of these proteins, 75 interact preferentially in the presence of T_3 and 68 in the absence of T_3. All of the core SWI/SNF complex subunits from both of the major subtypes (BAF and PBAF) were identified as TRβ. Interestingly, we found that the PBAF-specific subunit, PBRM1, was significantly enriched in the presence of T_3. BAF complex-specific subunits, such as ARID1A, were also present in our dataset. To test whether TRβ could differentially recruit BAF and PBAF complexes to its binding sites, we performed CUT&RUN targeting BRG1, PBRM, and ARID1A to determine the degree of co-occupancy with TRβ. Based on our comprehensive genomic and proteomic analyses, we propose a new model for selective recruitment of BAF and PBAF SWI/SNF complexes to TRβ binding sites for differential functions in regulating chromatin accessibility.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

T3 Enters Axon Terminals of Mouse Cortical Neurons, Is Retrogradely Transported to the Cell Nucleus and Activates Gene Expression

Federico Salas-Lucia, PhD[1], Barbara M.L.C Bocco, PhD[1], Kristof Rada, MSc[2], Csaba Fekete, MD,PhD DsC[2], Balazs Gereben, PhD[2], Antonio C. Bianco, MD, PhD[1].
[1]Section of Endocrinology and Metabolism, University of Chicago, Chicago, IL, USA, [2]Institute of Experimental Medicine, Budapest, Hungary.

Thyroid hormone (TH) is critical for brain development and function. T3 enters neurons through membrane transporters and reaches the cell nucleus where it binds to receptors (TR) to regulate gene transcription. However, neurons also express the type 3 deiodinase (D3), which is located in the cellular and nuclear membranes and inactivates T3. Here, we investigated the fate and biological impact of T3 that enters neurons through axons. Primary cortical neurons were isolated from E16.5 embryos of the TH action indicator (THAI) mice, which were engineered with a TH-responsive transgene where three copies of a T3-responsive element drive a luciferase (Luc) reporter. Neurons were seeded on a microfluidic device consisting of two independent compartments: (i) cellular, where about 70-90,000 cell bodies were located, and (ii) axonal, where a few hundred distal axons were located. Fluidic isolation of the compartments was monitored with Alexa Fluor 594 hydrazide. In the first set of experiments (repeated 3 times), 8-10-day old cultures were incubated for 48h with medium containing 1% charcoal-stripped serum (Tx-medium). Subsequently, 10nM T3 was added to the axonal compartment, and 24h later cell bodies were harvested and Luc mRNA measured by RT-qPCR. There was a 2.4 ± 0.7-fold increase in Luc mRNA levels, but the addition of 2uM Silychristin (MCT8 inhibitor) to the axonal compartment reduced T3 induction of Luc mRNA by 32 ± 4.2%. In the second set of experiments (repeated 3 times), 4.9 ± 2.2pM ^{125}I-T3 (final concentration) was added to the cellular or axonal compartments. Medium was sampled and ^{125}I-T3 and its metabolites were separated/quantified via UPLC linked to a flow scintillation detector. After 72h of adding ^{125}I-T3 to the axonal compartment, about 0.73 % ^{125}I-T3 (0.052 ± 0.025pM) was found in the cellular compartment. In addition, 3,3'-^{125}I-T2 and ^{125}I (0.011 ± 0.003 and 0.052 ± 0.023pM, respectively) were also detected. When ^{125}I-T3 was added to the cellular compartment, about 1.6% ^{125}I-T3 (0.048 ± 0.027pM), no metabolites, was detected in the axonal compartment. Only background radioactivity was detected in the opposing compartment when ^{125}I-T3 was added in the absence of cells. We conclude that T3 can be taken up by neuronal axons, partly via MCT8, and transported retrogradely to the cell nucleus to initiate TH signaling. D3-generated T3 metabolites exit the cell body alongside with small amounts of intact T3. This pathway could explain how D2-generated T3 in tanycytes is taken up by TRH-secreting neurons to mediate negative T3 feedback. Anterograde T3 transport was also detected, the significance of which remains unknown.

Thyroid

THYROID HORMONE METABOLISM AND ACTION

The Effects of Energy Restriction on Thyroid Hormone Dynamics

Joselyne Tessa Tonelu, BS[1], Skand Shekhar, MD[2], Chinelo Okigbo, MD PhD[1], Helen Leka, BS[1], Anne Kim, MD[1], Bona Purse, MSW[1], Katie Hirsch, MA, EP-C, CISSN[3], Bin Wei, MS[4], Brian Stolze,