Regulation of Nuclear Receptor Mediated Transcription

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Short Abstract — **Single-cell based studies of nuclear receptor mediated transcription demonstrate a range of responses not previously appreciated by population-based studies. We aim to characterize cell-to-cell variation demonstrating a range of responses not previously appreciated by population-based studies. We will measure the variability of nuclear translocation using an established HeLa cell line containing GFP tagged androgen receptor. We will measure spatiotemporal promoter occupancy of key coregulators using a HeLa cell variant having stable integration of a multi-copy prolactin promoter responsive to estrogen receptor. Finally, we will develop a mathematical model describing how the variability relates to the probabilistic interactions to explore underlying mechanisms for gene regulation control.**

I. Objectives and Significance of Research

Single-cell based gene regulation studies can demonstrate the range of responses to different environmental and physiological stimuli defining the relevant differences between cells not previously appreciated by population-based studies. Nuclear receptors (NRs) are the largest superfamily of transcription factors and are responsible for mediating numerous physiological responses through the regulation of gene expression. Estrogen receptor alpha (ER) and androgen receptor (AR) are prototypical examples of Type 1 NRs that mediate the actions of estradiol and testosterone. Recently, chromatin immunoprecipitation (ChIP) analyses have revealed that gene regulation at the promoter level involves sequential assembly/disassembly of macromolecular complexes. Further fluorescent recovery after photobleaching (FRAP) studies suggest the process is dynamic. Interestingly, the output of transcription, mRNA, has also been shown to vary over time and from cell to cell. We expect that examining cells at the single cell level will highlight the source(s) of this variation amongst cells in a population and provide insight into causes of the deregulation of transcription often associated with development of certain diseases.

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II. Aims

Utilizing high throughput and deconvolution image acquisition and analysis my project focuses on two single cell models of NR mediated gene regulation.

A. Variation in Androgen Receptor Translocation

First, I will examine the variability of nuclear translocation of AR in response to ligand treatment in an established HeLa cell line that contains GFP tagged AR. This will be done by comparing the coefficient of variation (standard deviation/mean) of wild type cells to cells with known mutations.

B. Promoter Occupancy of Estrogen Receptor

Next, I will use high throughput microscopy (HTM) to determine the spatiotemporal dynamics of promoter occupancy of ER coregulators (CoRs) using multi-copy integrated prolactin promoter array containing HeLa line expressing GFP-ER. Characterization of NR/CoR promoter array occupancy, array size, and mRNA synthesis will be performed after treatment with agonist/antagonists and will be followed by additional studies using RNAi.

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C. Mathematical model describing mRNA production

Finally, to further our understanding of how the observed variability relates to the probabilistic interaction of proteins, I will develop a mathematical model that complements the ER experimental data and extracting additional key information, such as identifying a sensitive step of activation or indicating additional mechanisms that can be expanded to explore gene regulation control.

References

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