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TRANSCRIPTION AND THE NAVIGATION OF NUCLEAR SPACE

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Recent advances in light microscopic approaches to single cell analyses have allowed new insights into the processes underlying nuclear metabolism, including nuclear receptor-based transcription and related issues involving protein folding and turnover. While standard biochemical approaches have for decades suggested various degrees of molecular dynamics (key molecular complexes can be stable or unstable), only in the last few years has it become technically feasible to actually test these ideas directly in living cells. Over the last few years, we have used fluorescently-tagged estrogen receptor-α (ER) and several coregulators to examine the hypothesis that regulation of both molecular dynamics and compartmentalization are necessary and codependent aspects of transcriptional activation.

Time lapse and fluorescence recovery after photobleaching (FRAP) studies of bulk nuclear ER and the SRC-1 coactivator have revealed that spatial, solubility and molecular dynamic changes are differentially dependent upon ligand and, surprisingly, proteasome activity (Mol Endo 14:518, 2000; Nat Cell Biol 3:15, 2001). Whereas biochemical transcription assays usually measure effects of receptor activation hours after stimulation (indeed, generally overnight), time lapse studies show ligand can cause significant subnuclear reorganization within minutes. Utilizing an integrated lac operator array and functional lac repressor fusions to ER in a novel protein-protein recruiting assay in living cells, we found that receptor-coactivator complexes remain highly dynamic in live cells even in the presence of agonist (Mol Cell Biol 21:4404; 2001). These findings suggest that transcription complexes are more dynamic than previously appreciated.

Recently we have extended our studies to include evaluation of an additional coactivator, SRC-3, and the androgen receptor (AR), both of which further underscore the complexity of intracellular dynamics. Although highly homologous to SRC-1 and similarly nuclear in localization, SRC-3 FRAP and real time biochemical results show distinct differences as SRC-3 is much more mobile and soluble in the nucleus, being completely susceptible to low salt extractions. FRAP approaches also indicate SRC-3 can rapidly shuttle between the cytoplasm and nucleus, with increased nuclear retention observed in the presence of ER plus estradiol, or after stimulation by epidermal growth factor. Similar to ER, androgen receptor (AR) studies have revealed an agonist-specific effect upon its subnuclear mobility, organization and solubility; however, AR dynamics are particularly dependent upon expression levels. At ~endogenous levels, time lapse, FRAP and real time extraction studies reveals that while agonist (R1881) and antagonist (Casodex, hydroxyflutamide, estradiol) can cause cytoplasmic to nuclear translocation of AR, only agonist leads to a marked reduction in AR mobility and solubility. Importantly, increasing relative AR expression levels (2.5-50 fold; still below extreme levels routinely found in transient transfections) led to a parallel drop in agonist-induced mobility, and increased insolubility. At higher expression levels, ligand-dependent differences in

mobility and solubility were greatly reduced. These results suggest that agonists and antagonists can differentially influence AR intranuclear dynamics and solubility, and that non-physiological expression levels can artificially reduce and obscure these measurements.

The overall goal of these and similar studies being undertaken in several laboratories is to increasingly improve upon the ability to monitor multiple mechanistic events at the single, live cell level. Integration of well-studied promoters driving fluorescent transcriptional reporters now allow observation and measurement of transcription factor dynamics, protein-protein interactions, and chromatin modeling at the site of action, with direct monitoring of transcription readout. Taken together, combined approaches that link spatial, mobility and solubility issues to nuclear receptor/coactivator function should continue to provide important and novel insight into the complexity of nuclear metabolism.

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