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SUMO as a nuclear hormone receptor effector

New insights into combinatorial transcriptional regulation

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Animal development is driven by robust, cell-specific gene expression programs. Understanding mechanistically how a single transcription factor (TF) can govern distinct programs with exquisite precision is a major challenge. We view TFs as signal integrators, taking information from co-regulator interactions, post-translational modifications, other transcription factors, chromatin state, DNA sequence and in some cases, specific noncovalent ligands, to determine the collection of genes regulated by a TF at any given time. Here, we describe a reductionist approach to combinatorial transcriptional regulation, focusing on a single C. elegans TF, the nuclear hormone receptor NHR-25, and a single post-translational modification, SUMO. We suggest that the ratio of sumoylated to unsumoylated NHR-25 could specify a switch-like cell-fate decision during vulval development. Direct examination of this “SUMO ratio” in vivo is challenging and we discuss possible solutions going forward. We also consider how sumoylation of multiple substrates might be coordinated during vulval development. Finally, we note that iteration of this approach could leverage our sumoylation findings to define the roles of other effectors of NHR-25 in the developing vulva and in other tissues.

Introduction: Combinatorial Regulation of Gene Expression

Animal development demands intricate coordination of gene regulation across many cell types, tissues and organs. Genes must be expressed at the correct time and place during development. Aberrations in this regulation can cause developmental abnormalities or animal death. Transcription factors (TFs) play a major role in coordinating metazoan gene regulation, creating gene expression networks that govern development, physiology, homeostasis and other complex biological processes. TFs must integrate information from diverse sources, such as co-regulatory proteins and other TFs, post-translational modifications, chromatin modifications, the DNA sequence to which they bind, and in some cases, noncovalent ligands. The integrated output of a given TF is the precise and reproducible activation or repression of tens to thousands of genes, with the expression of each gene specified by its unique combinatorial input code.

Given the vast permutations possible from these myriad inputs, unraveling combinatorial regulation of gene expression is a daunting challenge. In a recent PLoS Genetics article,1 we described a reductionist approach to this question. We focused on a single, evolutionary conserved, broadly expressed TF, the C. elegans nuclear hormone receptor NHR-25, examining the consequences of a single post-translational modification, sumoylation, on development of a single organ, the vulva. Our results enhance our understanding of mechanisms by which post-translational modifications modulate TF activities at the cellular and organ levels during development in C. elegans and other metazoans. In turn, this approach will provide insights on the
**Sumoylation of NR5 Family Nuclear Hormone Receptors**

Humans and *C. elegans* share similar small ubiquitin-like modifier (SUMO) proteins, which serve as post-translational modifications directly conjugated onto substrate proteins, similar to ubiquitin. Sumoylation affects a broad range of cellular processes including DNA repair, transcription, chromatin dynamics, and metabolism. SUMO is conjugated onto lysines in substrate proteins using E1 and E2 enzymes, similar to ubiquitination. Unlike ubiquitination, E3 ligases and ubiquitin, SUMO is not essential for SUMO conjugation, and sumoylation is not a typical signal for proteasomal degradation. Like all post-translational modifications, SUMO is a dynamic modification. Indeed, the SUMO protease that renders SUMO competent for conjugation can also release SUMO from substrate proteins.

We explored the interplay between the single *C. elegans* SUMO gene, *smo-1*, and the conserved nuclear hormone receptor, *nhr-25*, during vulval development. We identified that SMO-1 interacts with NHR-25 through an unbiased yeast two-hybrid screen for novel regulators of NHR-25. In vitro biochemical analyses revealed that NHR-25 is sumoylated, and we demonstrated that sumoylation of NHR-25 is important for ensuring correct cell fate determination or maintenance during vulval development. Our data show that sumoylation restricts NHR-25 activity in vivo: a reporter gene that contains NHR-25 binding elements is hyper-activated in *smo-1* deficient animals. This observation was further supported in heterologous cell culture assays: mutations in NHR-25 at SUMO acceptor sites on the human orthologs SF-1 and LRH-1 impaired NHR-25 binding to and activation at canonical SF-1 response elements, 1 whereas sumoylation of SF-1 protein displayed a dominant gain-of-function in mice, causing endocrine abnormalities and cell fate changes, presumably through binding genomic sites deviating from the consensus. NHR-25 can bind to an NR5 family consensus site upstream of the *lin-3* gene, and our studies demonstrated that sumoylation impaired NHR-25 binding to and activity at canonical SF-1 response elements, whereas sumoylation of SF-1 has no effect on binding to this site in mammalian cells.

**How Does Sumoylation Regulate Cell Fate in Vulval Precursor Cells?**

The *C. elegans* vulva is a paradigm of organogenesis. From six vulval precursor cells, the mature 22 cell organ is formed through a coordinated series of cell division, fate choice, migration, and fusion to produce the final 22-cell organ. The P3.p, P4.p, and P8.p cells divide once and their daughter cells execute a 3° fate, indicated by the blue bars. The remaining VPCs divide an additional two times with P6.p executing a 1° fate (marked by the red bar) and P5.p and P7.p executing 2° fates, marked by the yellow bars. A *smo-1*-dependent gradient of NHR-25 forms during the course of vulval development. Using an integrated, low-copy NHR-25::GFP translational fusion we observed decreasing levels of NHR-25 in 2° and 3° cells, relative to 1° cells. This gradient is abolished following inactivation of *smo-1*, and the 3° cells execute a 1° and/or 2° fate (marked by the orange bar). The “SUMO ratio” model. The NHR-25 gradient combined with constant, limiting sumoylation results in high levels of unsumoylated NHR-25 relative to sumoylated NHR-25 in 1° cells. As the NHR-25 levels drop in 2° and 3° cells, an increasing fraction of the total NHR-25 protein is sumoylated. At a particular set point, there is a sufficient amount of sumoylated NHR-25 relative to unsumoylated NHR-25 to allow adoption or maintenance of the 3° fate. Modified from Ward et al., 2013.

**Impact of NHR-25 and its sumoylation in other cells, other organs and other points in development, as well as the effects of other signaling inputs on these activities.** In this commentary, we (i) compare the mechanisms of sumoylation of NHR-25 to its mammalian homologs (SF-1 and LRH-1), and (ii) consider challenges and possible solutions for testing our model that sumoylation of NHR-25 produces a switch-like behavior that regulates developmental processes.
The Challenge of SUMO Ratios

The biggest question raised by our work is what proportion of NHR-25 (or any other protein for that matter) is sumoylated at any given time? This question is difficult enough to address using cell culture methods in which homogenous populations of cells can be assayed. The question becomes significantly more challenging when different cell types and time points in development are considered. Moreover, as sumoylation is reversible, sumoylation ratios may be highly dynamic. Although it may be illuminating to examine E1, E2 and SUMO protease expression across the vulval precursor cells, protein levels may not correlate with activity. Development of methodologies to measure enzymatic outputs of sumoylation and desumoylation enzymes in vivo, are sorely needed. Current methods used to monitor sumoylation in vivo typically give static snapshots. Mass spectrometry-based methodologies can report on global sumoylation and on changes in response to stimuli, however determination of sumoylation ratios would require internal peptide standard spanning the modification site. Alternatively, sumoylation ratios could be deduced from quantitative immunoblots, assuming the sumoylated species is sufficiently represented. Given the emerging power of phage-display generated Fab antibodies, it may be possible to generate an antibody highly specific to the sumoylated NHR-25 isoform(s). Thus, the ratio of unsumoylated to sumoylated NHR-25, rather than the absolute amount of sumoylated NHR-25, could generate a robust, switch-like developmental decision (Fig. 1C). The switch point could be set either at steady-state, or through a kinetic determinant such as a molecular timer.

When the unsumoylated form is in excess a particular cell fate might be adopted (1° or 2°), and when the sumoylated form is in excess an alternate cell fate (3°) is adopted. Notably, such a scheme could sensititize the system to levels of other sumoylated proteins. Overexpression of another highly sumoylated substrate, such as RanGAP or LIN-11, might squelch NHR-25 sumoylation, and phenocopy the 3° cell phenotype caused by loss of smo-1 or overexpression of NHR-25.

Regulation of Sumoylation during Vulval Development

We could not detect NHR-25 sumoylation by western blot or immunoprecipitation-western blots from worm lysates, and NHR-25 was not identified in a proteomic screen of C. elegans sumoylated proteins. Yet, our in vivo NHR-25 activity reporter indicates smo-1 depletion causes activation of NHR-25 in major tissues in which NHR-25 is expressed. A “memory” of NHR-25 sumoylation while the protein is actually unsumoylated is an attractive model; this idea was invoked to explain the “SUMO enigma”: the paradoxical discrepancy between the functional importance of sumoylation of a given substrate and the difficulty in biochemically detecting its sumoylation. As sumoylation is dynamic, SUMO can be removed from a substrate but perhaps imprint a “memory” on the protein or complex. Thus the function of the protein/complex can be regulated by sumoylation without depleting the limiting amounts of SUMO. Potential mechanisms in which a sumoylation “memory” could be imparted include altered subcellular localization, SUMO-dependent post-translational modifications, recruitment of co-regulators, or altered complex composition. Sumoylation “memory” would provide an interesting mechanism to extend the functional output of this single modification and allow the regulatory impact of sumoylation to persist.

Many of the best-characterized C. elegans sumoylation substrates are involved in vulval development. It is clear that sumoylation must be correctly controlled temporally and spatially, as loss of LIN-11 sumoylation or overexpression of an allele...
that mimics constitutive sumoylation both caused distinct vulval defects. It is unclear, however, how sumoylation of multiple substrates during organogenesis might be separately modulated from a limiting pool of SUMO and perhaps a handful of E3s. Substrate localization or scaffolding may bring specific substrates into proximity of sumoylation machinery, thus organizing sumoylation pathways similarly to those for protein kinases. Sumoylation of multiple components of a complex is another route of regulation. Three of the ten dosage compensation complex proteins are sumoylated, which is thought to enhance interactions between factors that target this complex to the X chromosome and condensin subunits. Interestingly, sumoylation of these condensin subunits is specific to the dosage compensation complex; sumoylation did not occur in the context of chromosome condensin complexes involved in chromosome segregation. The notion of coordinated complex sumoylation in response to developmental stimuli, in turn producing enhanced complex formation and function, is a framework concept from which to view global sumoylation during vulval development.

Perspectives

Determination that sumoylation regulates NHR-25 opens new avenues for future investigation in which sumoylation (or the memory of it) can serve as a “lever” for exploration of the roles of other posttranslational modifications and co-regulators, which together, serve as determinants of NHR-25 activity in vulval precursor cells and in other tissues. Building on this reductionist strategy will inform our understanding of how these signals are integrated by NHR-25 during development and will provide new insight into combinatorial regulation of transcription.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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