Published in final edited form as:

Curr Opin Cell Biol. 2008 June ; 20(3): 310–315. doi:10.1016/j.ceb.2008.04.005.

CRACKING THE COREGULATOR CODES

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Abstract

The study of the genetic code has collectively revealed that the biochemical basis of heredity is uniform for nearly all known forms of life. Genetic approaches have generated a much better appreciation and understanding of many aspects of biological processes - and in some cases provided strategies for the treatment of human diseases. Still, the enormous and undoubtedly impressive amount of information gathered on gene sequences, their myriad expression patterns and translation into proteins is insufficient to answer seemingly simpler questions such as to what sets us humans apart from much more undemanding species while sharing almost the same sets of genes. Regulation of the proteome by posttranslational modifications is beginning to be understood as a major contributing factor to the structural and functional diversity in biology and for defining cellular mechanisms in particular. Covalent, posttranslational modifications provide an astonishingly rich and specific basis for an ultra-fast regulation of cellular processes, many of which converge to transcription units to control gene expression. With this essay we intend to share with the reader the rapid growth of our knowledge of the many conjunctions that exist between posttranslational modifications and key cellular processes that have emerged by studying the nuclear receptors and their transcriptional coregulators.

INTRODUCTION

Transcriptional coregulators comprise a heterogeneous and functionally distinct group of molecules that associate with transcription factors by direct binding or in the context of multi-protein complexes and integrate cellular signals by tethering varied enzymatic activities to the transcription unit for the purpose of regulating target gene expression. The entirety of this transcriptional regulation is coactivation or corepression. Coregulator functions include the transient modification of chromatin for transcription factor binding to DNA, the mediation and regulation of proximal and distal components of the transcription 'apparatus' including transcription initiation, elongation and splicing, but also the transduction and refinement of cellular signals to the locus of transcription as well as deceptively 'transcription-unrelated' cellular processes such as protein degradation or energy homeostasis in the mitochondria. See the following references for reviews on actions of NR coregulators in integrating signal-dependent programs of transcriptional responses at the molecular level [1,10,17,26]. Because of their broad functional activities, coregulators are the preferred class of target molecules to coordinately elucidate processes of cell regulation.

The work on nuclear receptor (NR) as recipients of coregulator signaling and mediators of transcription control has unveiled the regulatory potential of posttranslational modification (PTM) of the biological processes involved, suggesting that examining their diversity is critical for understanding mechanisms of cell regulation. To characterize the coregulator complexome – a cross-talking network of transcription factors, coregulators and their regulatory enzymatic molecules – a large-scale proteomics study that involves immunoprecipitation and mass spectrometry has been initiated (www.NURSA.org). The

later provides a fundamental framework for detecting and mapping covalent modifications in proteins and quantifying their changes. Because PTMs are key to the physiologic functions of the proteins involved, it is imperative that we understand the 'coding' that these modifications impart to their complex components for their diverse activities. We therefore set out to 'crack' this coregulator code, which is to convert the PTM signals back into information that is understandable in the context of known cellular processes, and by doing so, to possibly unveil a system of principles or rules imposed by the modifications. The task is comprehensive and requires a methodical elucidation of the nature and the location of the modifications on key coregulator molecules, a determination of the composition of the complexes in which they reside, and confirmation of the diverse functions that PTMs impart on cell signaling and control of transcription. In addition to thorough proteomics approaches, this effort requires genome-wide functional analyses and sound bioinformatics strategies.

DISCUSSION

To date, the work on nuclear receptor coregulators has raised a series of important questions as to how a single primary protein can act in many diverse ways in the mammalian cell. For example, how does a coactivator become 'activated' to form distinct multimeric coactivator complexes for cellular functions? How does a coactivator differentially bind to NRs (or other transcription factors) for transcriptional activation at select promoters? How does a coactivator direct distinct subreactions of transcription (*e.g.*, chromatin remodeling, transcriptional initiation, RNA chain elongation, RNA splicing, termination, etc.)? How can one coactivator participate in regulating molecular substeps of transcription, and then function in totally different cellular compartments to control mRNA translation [2], mitochondrial biology [3], or membrane initiated cell motility [4]? How does the same coregulator function as a 'coactivator' in certain instances, and as a 'corepressor' in other instances [5]? Perhaps even more perplexing, how can a specific coactivator function as an oncogene in certain cell contexts and a tumor suppressor in others [6,7]?

The molecular answer to the above series of questions is now becoming clear from recent work in the NR/coregulator and related fields. Logical interpretations of limited available data from a variety of sources suggest that the key to understanding the diversity of coregulator functions is an unexpectedly extensive posttranslational 'coding' of the coregulator proteins [8,9]. Such covalent modifications include, but are not limited to, phosphorylation, methylation, ubiquitylation, sumoylation and acetylation. In the case of coregulators, it has been observed that a combination of such modifications can lead to functionally distinct activities for the same primary sequence protein. The 'differentially coded' coactivator is now predisposed to form different multimeric coactivator complexes, and thereby, directed to interact with distinct genes and even to perform different compartmental functions in a cell [2,10]. Because such PTMs are the key to the physiologic functions of these proteins, it is imperative that we understand the 'coding' that these modifications impart to the coregulators for their diverse functions, and associate the kinases and enzymes that impart the PTM coding signals to the proteins in different cell types, and learn about the relationships of these coregulator modifications in the context of human pathologies.

Although known to exist for many decades, posttranslational modifications of proteins only recently have been subject to intensive study in terms of their regulatory potential. The power of phosphorylation was realized decades ago, beginning with studies of enzymes that demonstrated increased enzymatic activity when phosphorylated in response to small molecules [11]. The era of phosphokinase discovery further popularized the role of phosphorylation and that reaction dominated our thinking about regulatory protein

modifications for a considerable period of time [12]. It is only recently that we have realized the critical role that multiple interdependent posttranslational modifications can play in the activation and deactivation of regulatory proteins [13]. Rough estimates suggest that ~25,000 human genes could produce ~120,000 primary sequence proteins after RNA splicing takes place. Of this total, at least 15% are likely to be transcriptional regulatory factors.

However, to focus on coregulators for this commentary, we need only consider a total of ~300 proteins (www.NURSA.org). The fact that many coregulators undergo extensive posttranslational modifications is no longer in question. So, if we accept that all coregulatory proteins contain PTMs, and that an average coregulator (~80,000 daltons in size) has ~8 different PTMs, an extremely conservative estimate, then the 'potential' for coregulator complex diversity in cells could be well in excess of one billion molecules per cellular gene [14]. It is highly unlikely that this potential is ever fully realized, and currently, there is no way of determining the exact amount of this potential ever used in a cell. However, since each single modification (PTM) can result in a protein with an altered function, it is an inescapable conclusion that multiple combinations of modifications can bestow a large array of distinct functions to any one regulatory protein. In reality, then, we potentially have an enormous number of different proteins within our body that can be used for the myriad of distinct regulatory functions required of mammals, and especially in humans.

PTMs can arise from transient interactions of coregulator complexes with enzymes in signaling pathways, or by intra-complex modifications by co-coregulators, most of which are themselves enzymes. The exact functional implications of PTMs of most coregulators are unclear at present. A few molecules, however, have been studied in greater detail. The SRC-3 (NCOA3; ACTR/AIB1/RAC3/pCIP/TRAM-1) coactivator is an example of one such molecule. Using proteomic approaches, over 40 separate modifications of the SRC-3 coregulator have been determined to date. These include phosphorylations, methylations, acetylations, ubiquitylations, sumoylations; in total, this plethora of PTMs creates enormous combinatorial potential for distinct functions in just this one coregulator.

To dissect the PTM coding within coactivators, it is necessary to methodically elucidate the nature and the location of the modifications on one molecule, to determine their function by mutational analyses, to determine the signaling enzymes/pathways responsible for their modifications, and to determine the interrelationships of modifications at one site on modifications occurring at more distant sites. This has not been done completely for any molecule yet but work is in progress. For example, phosphorylation of SRC-3 leads to formation of 'active' high molecular weight complexes with other coactivators, and the specific sites of phosphorylation can determine the co-coactivator partners that comprise the larger coactivator functional complexes [15]. Since coactivator complexes are dynamic, separate modifications then direct SRC-3 to dissociate from its partners in the coactivator complex. Examples of modifications inducing dissociation of SRC-3/ACTR coactivator complexes include acetylations and methylations [9,16]. In this way, rapid temporal enzymatic modifications promote a dynamic association and dissociation of active coactivator complexes. This equilibrium can be shifted by both ligands and signaling pathway modulations.

PTMs are not designed only for kinetic association/dissociation of coactivator proteins from their functioning complexes, but also are involved in activation/inactivation, assembly of specific partners in the functional complex, targeting specific transcription factors for regulation, and eventually for degradation of the coregulator proteins [10]. Coregulators are not primarily regulated at the level of DNA transcription, and that is logical since they appear to be the 'master genes' for regulating all other 'producer genes' [17]. If they were

regulated solely by transcription, then they would require coregulators for their own genes, and then those coregulators would need other coregulators, and a never-ending circle would result. Consequently, coregulator levels usually are controlled primarily at the level of their degradation. PTMs are the effectors for this regulation and can either dictate more rapid degradation or prevent it, depending on the precise signaling enzymes involved [17]. PTMs are again at the heart of posttranslational regulation of coregulator degradation.

An activated coregulator complex must bind and regulate its appropriate target transcription factors. As one example of the combinatorial possibilities, inactive SRC-3 coactivator contains two key amino acids (aa.723; 786) that are sumoylated; they must be de-sumoylated for activation so that the molecule can bind with high affinity to the appropriate receptors. This event is followed by mono-ubiquitylations at these two sites which fully activates the molecule to bind to select NRs. These mono-ubiquitylations, however, are dependent upon two prior phosphorylations at separate downstream sites in the coactivator (aa. 505; 509), and by a specific kinase (GSK3). The mono-ubiquitylated sites are processively polyubiquitylated during subsequent rounds of transcription. When the ubiquitin chain reaches 4–5 ubiquitins, it is then subject to binding by the 19S cap of the proteasome and the protein undergoes degradation via the 26S proteasome [18] (Fig. 1). This example illustrates the combinatorial interplay of separate protein PTMs, each of which plays its own role, but their roles are interdependent. In terms of transcription, the results of the processive PTM ubiquitylations at amino acids 723/786 reveal the existence of a transcriptional 'time clock' which begins with activation of the coactivator molecule and ends with its proteasomal destruction [18]. We also found that peptidyl prolyl isomerase 1 (PIN1) catalyzes a conformational change of phosphorylated SRC-3 to promote its degradation [19]. On the other hand, phosphorylation of C-terminal residues of SRC-3 by atypical protein kinase C (aPKC), which is frequently overexpressed in cancers, stabilizes the coregulator in a selective ER-dependent manner by inhibiting its interaction with the 20S proteasome core alpha C8 subunit, thereby promoting SRC-3 function and powerfully enhancing ER-dependent target gene transcription and estradiol-dependent cell growth [20]. These examples show a coordinate enzymatic organization of proteolytic pathways by different PTMs, using different cellular signals and enzymes (GSK3, PIN1, aPKC in these examples), strongly suggesting a fundamental importance for controlling cellular levels of key molecules such as SRC-3.

Clearly, SRC-3 is not the only coregulator of particular interest that has been shown to be subjected to PTMs for obtaining multivariate functions. PELP1, separately cloned and termed MNAR (Modulator of nongenomic activity of estrogen receptor), is a good example of a multitasking transcriptional coregulator that participates in both enhancement and repression of gene function and is regulated by PTMs [21]. Phosphorylation of PELP1/MNAR for example, triggers estrogen activation of important signaling pathways such as the Src/MAPK and PI3K/Akt tracts to regulate cell proliferation and apoptosis [22,23]. NR-induced activation of specific intracellular kinases that control vital cellular functions has increasingly become a focus of NR/coregulator investigation. Such "nongenomic," or extranuclear, actions of NRs differ from the classical, transcription-dependent hormone action and involve the production of second-messenger molecules and the rapid activation of signal transduction pathways (e.g., ERK/MAPK, p38/MAPK, PI3K/AKT, PLC/PKC). These signaling cascades may occur in series or in parallel with NR-mediated transcription, and may cross-talk with other pathways, but converge at the level of modification of transcriptionally relevant molecules such as NRs and their coregulators. This synergistic coupling between extranuclear and genomic actions of hormones is accompanied by highly specific PTMs that serve as triggers but also as mediators and promoters of the signaling cascades. This scenario fundamentally revises the paradigms of classical cell signaling. In fact, covalent modifications are the only common trait the coregulators share in addition to

transducing regulatory signals for the modulation of transcription. This also is true for the most unusual NR coregulator, the RNA, SRA [24], which is modified posttranscriptionally by the pseudouridine synthases Pus1p and Pus3p to trigger a coactivator-corepressor switch in the RNA [25], affording another means to regulate transcription.

A roundworm has <1000 cells but has a similar total gene count to humans. We have little genomic differences compared to mouse and other species. We are 99% similar to mouse - but look at us!! How did we gain the proteome diversity that allows such differential evolutionary advancement compared to all other species? It is clear that the human species differs not just because of the number of different genes, but by how they are used. Humans have gained the capacity to multitask in terms of cell regulation. Multitasking leads to the grouping of gene expressions in a way so as to control major metabolic functions - and this requires proteome diversity. The coregulators are an example of such diversity; each PTM makes them different, and each mutation provides a distinction in function, and each distinction in function allows rapid regulation of multiple gene sets which have been evolutionarily grouped together to effect inter-compartmental regulation of physiologic processes. We believe that mammals and humans may have made a leap in evolution, in part, by accumulating combinations of modifiable amino acids and signaling pathway specializations within the coregulators. This approach permitted the evolution of gene groupings by leaps and bounds - and without much change in primary DNA and primary protein sequences. Moreover, if PTMs are playing such a role, then it follows that we humans also have the potential for unlimited future diversity without generation of new genes, but simply by adding more PTMs to our existing proteins to create new functions.

Since the coregulators can be considered to be 'master genes' that have evolved to coordinate multiple diverse cellular reactions to implement major physiologic processes [17], they also contain the potential to efficiently promote cellular pathologies by coordinately misdirecting multiple independent functions [26]. Evidence indicates that PTMs of coactivators can influence pathologic inflammation, aberrant adipogenesis, cell replication abnormalities and oncogenesis, etc. In fact, of the ~300 coregulators identified to date, >165 of them already have been associated with some disease state in humans [26] and (www.NURSA.org). Since most of these associations have been published only in the past 5 years, we suspect it represents only the tip of the iceberg of what remains to be learned for these master genes in terms of human diseases. Finally, since coregulator (and NR) PTMs are a result of prior enzymatic reactions, and thus, are subject to inhibition/activation with drugs, it is only a matter of time before the pharmaceutical industry realizes the potential of this new information for therapeutic intervention.

CONCLUSION

In conclusion, the recent advances in coregulator-controlled transcription and their extensive PTM patterns have opened opportunities to explore the intricate aspects of cell system biology in great detail. New knowledge such as that gained from the study of the SRC family of coregulators in our laboratory underline the key role that PTMs play as a cellular mechanism in providing and maintaining regulatory circuits so far not described in simpler organisms. While modern biology alone can't possibly suffice in offering plausible answers to the question of what makes us human, it certainly will provide a new framework in which other questions will find answers and new ideas for therapies for diseases, and provide a new platform for us scientists to concur and diverge on new hypotheses. Is the latter not an inherited human trait above all?

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Figure 1.

(A) A cellular stimulus such as estrogen induces kinases to cause phosphorylations of the SRC-3 coactivator that induce formation of a functional coactivator complex. Fbw (E3 ligase) enters complex and (B) mono-ubiquitylates SRC-3 to 'super-activate' it; other intra-complex modifications occur during the same time frame. (C) One additional ubiquitin molecule is added to SRC-3 during each round of transcription and the chain grows in number. (D) The polyubiquitin chain eventually extends to 5 ubiquitins. (E) At that point, the 19S Cap binds the polyubiquitylated coactivator and takes it to the 26S proteasome for degradation. The time period from activation to degradation is ~30 seconds (if sequential) and represents a 'Ubiquitin-Dependent Transcriptional Time Clock' [14,18].