How Much REST Is Enough?

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Recent papers published in *Nature* by Guardavaccaro et al. and Westbrook et al. describe a nexus of two masters of negative regulation of protein levels. Both of these studies establish that the transcriptional repressor REST/NRSF is regulated by the highly versatile ubiquitin protein ligase (E3) SCF$^{\beta\text{-TrCP}}$, adding a new dimension to the relationship between the ubiquitin-proteasome system and epigenetic regulation of transcription. These studies elucidate a critical means of regulation for REST, with implications for neuronal stem cell differentiation and the dual roles of this protein as a tumor suppressor and oncogene. These findings and their significance are discussed herein.

REST (repressor element 1 silencing factor), or NRSF (neuron-restrictive silencing factor), is a transcriptional repressor regulating a myriad of genes. It binds to a 21–23 base pair repressor element (RE1) of which there are ~1900 copies in the human genome. REST plays critical roles in preventing differentiation and maintaining the self-renewal capability of neuronal stem cells. In accordance with its function in silencing of both neuronal and nonneuronal genes, REST is essential for embryonic development and for a number of cellular responses in neurons and other cell types.

How REST serves as a master repressor of genes is complex and fascinating. This 1097 amino acid protein includes a DNA binding domain, followed by lysine- and proline-rich domains, sandwiched between repressor domains (RD1 and RD2) located at its N and C termini (Figure 1A). These RDs recruit distinct arrays of proteins that either repress or epigenetically silence gene expression. REST activity can be modulated by a RE1-like dsRNA that turns REST from a repressor to an activator. REST interacts with and is inactivated by *huntington*, with Huntington's disease mutations abrogating this interaction. Recently, proteasomal degradation has been implicated in downregulating REST during neural stem cell differentiation (Ballas and Mandel, 2005; Coulson, 2005; Majumder, 2006; Ooi and Wood, 2007).

REST can function as either a tumor suppressor or an oncogene depending on the cellular context. Diminished REST expression is associated with colon cancer and transformation of human mammary epithelial cells (HMEC) (Westbrook et al., 2005). High levels of forms truncated in the DNA binding domain due to alternative splicing, which are similar to a normally occurring alternative splice product, are implicated in small-cell lung cancer and neuroblastoma (Coulson et al., 2000; Palm et al., 1999). Likewise, a frameshift mutant truncated just beyond the DNA binding domain (REST-FS) (Figure 1A) is found in colon cancer and can transform epithelial cells (Westbrook et al., 2005). All of these truncated forms can presumably function to some extent as "dominant negatives." An oncogenic role for REST has been established in medulloblastoma, an aggressive childhood malignancy of neural progenitors, where high REST levels coupled with Myc overexpression drive cells toward proliferation and tumorigenesis rather than differentiation (Majumder, 2006; Ooi and Wood, 2007; Coulson, 2005).

In recent papers by Guardavaccaro et al. (2008) and Westbrook et al. (2008), using unbiased coimmunoprecipitation and siRNA screens, respectively, REST was identified as a substrate for a member of the skp1-cullin-F box (SCF) family of ubiquitin ligases (E3s). Substrate recognition by SCF E3s is determined by the F box protein that binds the core ligase complex. There are ~70 human F box proteins, of which only a handful have defined substrates. Perhaps the best known of the SCF E3s is SCF$^{\beta\text{-TrCP}}$, which recognizes conserved phospho-degrons in ~20 substrates, including components of the Rel family of transcription factors, β-catenin, and other critical regulatory molecules, many of which are involved in cell-cycle regulation (Cardozo and Pagano, 2004). REST now joins this prominent list of β-TrCP substrates that are degraded by the proteasome. Interestingly, the two groups identified, and established as important, two adjacent yet distinct β-TrCP binding sites in REST (Figure 1A). Two similar sites have previously been identified in Cdc25A (Busino et al., 2003; Ray et al., 2005). Notably, the more C-terminal site in REST terminates only 30 amino acids from RD2. While each group established "their" site as critical for REST degradation, neither group explored both sites.

Beyond identifying distinct degrons, the two groups took different approaches to evaluating the relationship of β-TrCP with REST and its cellular consequences. Westbrook et al. demonstrated, using HMEC, that overexpression of β-TrCP, which is known to be oncogenic in some tissues, results in decreased REST that is accompanied by anchorage-independent growth, indicating cellular transformation. Transformation was largely prevented by re-expression of REST and essentially abrogated by a stable degron-mutant REST. They similarly established a causal role between increased β-TrCP, decreased REST, and neural differentiation in a well-characterized in vitro model, demonstrating that suppression of neural differentiation markers by β-TrCP knockdown was completely prevented by concomitant knockdown of REST. Strikingly, as embryonic stem cells were stimulated to undergo neuronal differentiation, β-TrCP expression increased 13-fold,

correlating with decreased REST stability. Thus, their findings provide in vitro genetic data implicating β-TrCP-mediated ubiquitylation and proteasomal degradation of REST in abrogating the tumor suppressor role of REST and in facilitating neuronal differentiation (Figure 1B).

Guardavaccaro et al. focused on a better understanding of the oncogenic role of REST. In assessing multiple nonneuronal cell lines, they found REST protein levels decreased during the G2 phase of the cell cycle, which was dependent on an intact degron, β-TrCP, and proteasome activity. Screening important cell-cycle regulators, *MAD2* expression was found to be negatively regulated by REST, with the *MAD2* RE1 implicated in this downregulation. MAD2 is integral to the mitotic checkpoint complex/spindle-assembly checkpoint, which plays a crucial role in maintaining the anaphase-promoting complex/cyclosome (APC/C^{Cdc20}) in an inactive form until all chromosomes are correctly aligned on mitotic spindles. Thereafter, this highly complex E3 executes its role in initiating sister chromatid separation and progression to anaphase (Musacchio and Salmon, 2007). They demonstrated that overexpression of either degron-mutant REST or oncogenic REST-FS (Figure 1A) phenocopies MAD2 haploinsufficiency. This is manifested by accelerated onset of anaphase and by a host of chromosomal abnormalities indicative of premature APC/C^{Cdc20} activation. This was not observed with wild-type REST, which was efficiently degraded during G2 even when overexpressed. These findings of genomic instability are consistent with a role for nondegradable REST in tumorigenesis (Figure 1B).

Collectively, these two studies elegantly establish a direct connection between REST and $SCF^{β\text{-}TrCP}$. Westbrook et al. substantially advance our understanding of how REST could be regulated during neural development and provide evidence for β-TrCP-mediated transformation in nonneuronal cells via REST degradation. On the other hand, Guardavaccaro et al. identify at least one mechanism by which stable forms of REST may contribute to oncogenesis by genetic instability. These findings together at face value lead toward different conclusions regarding the role of nondegradable REST in cancer. However, their results are certainly not mutually exclusive given the different assays, cells, and mutations utilized, not to mention the range of potential REST targets and varied expression levels of interacting proteins that contribute to gene repression/silencing. It would therefore be premature to conclude a single unifying hypothesis regarding the role of β-TrCP-mediated degradation REST in tumorigenesis. Ultimately, in vivo analyses will be required to understand the relative importance of these observations. Similarly, while the finding of altered *MAD2* expression and accompanying chromosomal abnormalities are striking, the importance of *MAD2* as a REST target in tumors arising from neural stem cells also awaits assessment.

An important issue raised by these studies relates to the nature of the signaling pathways that activate REST's two β-TrCP degrons and to what extent they are regulated in the context of neural differentiation and potentially dysregulated in malignancy. Further, given the proximity of these two degrons to RD2 (Figure 1A), one cannot disregard the potential for functionally important interactions between the two degrons, RD2, and their associated proteins.

In addition to potential implications in regulating neural stem cell differentiation, the findings of Westbrook et al. in particular suggest the potential for targeting β-TrCP to activate REST tumor suppressor function in nonneuronal tumors. Such an approach, however, must be considered with caution, as β-TrCP substrates are highly varied in function and therefore may result in unpredicted effects on signal transduction and the cell cycle in both tumors and normal tissues. Additionally, even when considering approaches to increasing REST activity directly to enhance its tumor suppressor function, the question of what converts it from a tumor suppressor to an oncogene and how this is affected by cellular context requires further study. Considering the findings of genomic instability associated with REST overexpression, it would appear that even in nonneuronal cells too much REST is not necessarily a good thing.

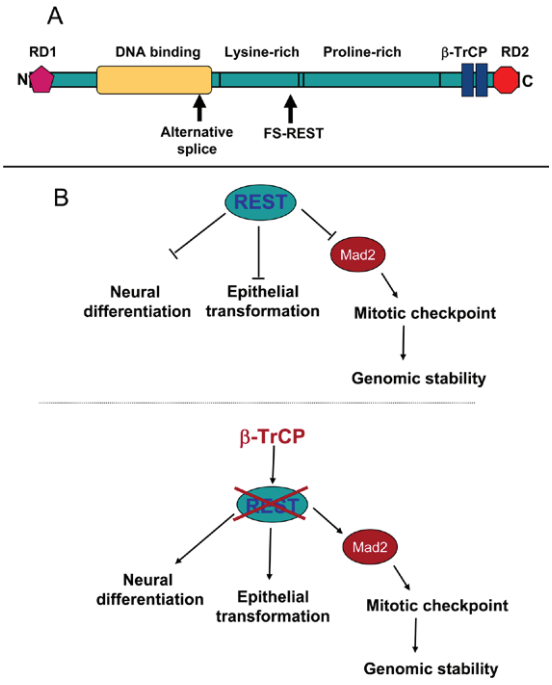

Figure 1. Domains and Proposed Functions of REST

(A) Schematic representation of REST/NRSF. REST has N and C terminal repressor domains (RD1 and RD2) that serve as scaffolds for distinct gene repressor/silencing complexes. The DNA binding domain is followed by lysine- and proline-rich domains and two β-TrCP binding sites. Alternative splicing leads to truncated forms of REST that terminate in the region indicated by the arrow. These include a naturally occurring neuron-specific form and forms associated with small-cell lung cancer and neuroblastoma. The position of an oncogenic truncation found in colon cancer resulting from a frameshift (REST-FS) is also indicated by an arrow.

(B) Proposed functions of REST. (Upper panel) In neuronal stem/progenitor cells, REST suppresses expression of neuron-specific genes maintaining cells in an undifferentiated state. Likewise, in normal epithelial cells, REST protein level is maintained, and it functions as a tumor suppressor. REST also suppresses expression of MAD2, a component of the mitotic checkpoint complex/spindle-assembly checkpoint, until degradation of REST by $SCF^{β\text{-}TrCP}$ during the G2 phase of the cell cycle. (Lower panel) During neuronal differentiation, REST is degraded in a $SCF^{β\text{-}TrCP}$-dependent manner, allowing for expression of genes necessary for differentiation. When β-TrCP is overexpressed, as occurs in some epithelial cancers, REST levels are dysregulated, contributing to transformation.

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