easily studied, since *dPatj* mutant flies are viable and show no detectable apical-basal polarity defects (Pielage et al., 2003). Loss of *dPatj* activity does not randomize the R3/R4 decision, but, interestingly, symmetrical R3/R3 ommatidia are seen in *dPatj* mutant flies. R3/R3 ommatidia are associated with high Fz activity. Furthermore, a 2-fold reduction of *dPatj* activity enhances the gain-of-function phenotypes induced by Fz overexpression. Thus, dPatj appears to antagonize Fz PCP signaling. Whether this effect of dPatj on Fz activity is mediated via its direct interaction with Fz, however, is not entirely clear since a Fz-GFP C-terminal fusion protein lacking the C-terminal PBM involved in dPatj binding localizes apically and rescues a complete loss of *fz* activity (Strutt, 2001).

What could be the functional significance of the aPKC- and dPatj-mediated inhibition of apical Fz? It is important to note that Fz localizes at the apical cortex of eye epithelial cells long before they differentiate (i.e., anterior to the eye morphogenetic furrow) and acquire their second polarity axis. Although it is not entirely clear when Fz signals to establish PCP (Strutt and Strutt, 2002), Fz appears to signal to establish PCP only during a brief period of time preceding the R3/R4 decision. Thus, one hypothesis is that aPKC-mediated phosphorylation of Fz defines this temporal window of Fz signaling by inhibiting Fz prior to and after this period. Consistent with this hypothesis, the level of dPatj accumulation is specifically downregulated in the R3/R4 precursor cells when PCP signaling is thought to occur. Moreover, this downregulation of dPatj does not depend on Fz signaling, as it is still observed in PCP mutant flies. Additionally, the level of Bazooka (Baz; the *Drosophila* Par3 homologue) is upregulated in the R3/R4 precursor cells, and this upregulation also does not depend on PCP signaling. Loss of *baz* activity in clones results in symmetrical R4/R4 ommatidia (associated with low Fz signaling), and a 2-fold reduction of *baz* activity suppresses Fz overexpression phenotypes. These data, therefore, suggest that Baz positively regulates Fz signaling. Baz does not appear to act by regulating the levels of dPatj. Whether Baz acts by antagonizing aPKC activity or by yet another mechanism remains to be determined. Together, these observations suggest a model whereby the downregulation of dPatj and upregulation of Baz release Fz from aPKC-mediated inhibition and thus define when Fz signaling is active and PCP is established (Figure 1). One prediction of this model is that PCP, as reflected by the asymmetric distribution of Fz at the apical cortex of R3/R4 cell pairs, may be established earlier in developing *dPatj* mutant eyes.

The notions that PCP signaling is inhibited by components of apical polarity complexes and that this inhibition is important to define when PCP is established are novel. Moreover, inhibition of Fz PCP signaling by apical-basal polarity complexes may reflect a more general property of cell polarity regulation, which is that cells may more easily interpret a single polarity cue at one time. Accordingly, one first response of polarized cells to a novel polarity information such as PCP may be to downregulate preexisting polarity cues. Future studies will no doubt test whether PCP formation in the eye actually requires a transient downregulation of apical-basal polarity in R3/R4 cells.

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No Rest for REST: REST/NRSF Regulation of Neurogenesis

Epigenetic strategies control the orderly acquisition and maintenance of neuronal traits. A complex network of transcriptional repressors and corepressors mediates gene specificity for these strategies. In this issue of *Cell,* a study by Ballas and coworkers (Ballas et al., 2005) provides insight into the early lineage commitment events during neurogenesis. This study demonstrates that regulation of the REST/NRSF transcriptional repressor plays a fundamental role in the progression of pluripotent cells to lineage-restricted neural progenitors.

The molecular basis for diversity in the function of the various cell types in multicellular organisms is cell-type-specific gene expression. Neurons differ from any other cells in the organism by containing a specific set of proteins that are critical for execution of the specialized functions in the nervous system and are encoded by genes that must be expressed in a neuron-specific manner. Neuronal differentiation and active regulation of the differentiated state are controlled by the balance between negative and positive regulators, which are critical for ensuring continuous control of neuron-specific gene transcription in every neuron throughout adulthood.

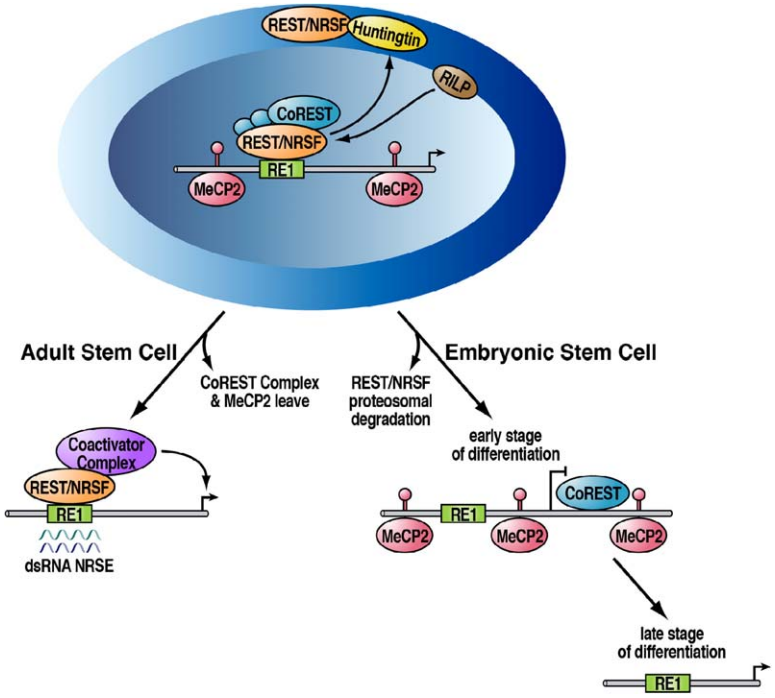

Figure 1. Multiple Strategies for Derepression of REST/NRSF-Regulated Neuronal Genes

The regulatory mechanisms that restrict the expression of these genes exclusively to the central nervous system are therefore fundamental for the development and function of the brain.

In 1995, two groups independently identified a gene encoding a zinc finger protein that was suggested to function as a master regulator of the neuronal phenotype. The transcription factor REST, an RE1-silencing *t*ranscription factor (Chong et al., 1995), also known as *n*euron-*r*estrictive *s*ilencer *f*actor NRSF (Schoenherr and Anderson, 1995) blocks transcription of its target genes by binding to a specific consensus 21 bp RE1 binding site/neuron-restrictive silencer element (RE1/NRSE) that is present in the target genes' regulatory regions. REST/NRSF functions very effectively as a transcriptional repressor at a distance and is able to repress transcription despite location or orientation of the binding site within a gene.

Many surprising discoveries in the field of REST/NRSF-dependent transcriptional regulation have been identified in recent years, and the current study of Ballas and colleagues documented in this issue of *Cell* (Ballas et al., 2005) presents an additional layer of insight into its functionality. While structurally REST/NRSF resembles many known transcriptional regulators, functionally, it still remains an enigma despite intensive studies in many labs around the world. REST/NRSF contains a DNA binding domain localized within the cluster of eight zinc fingers, as well as repressor domains at the N and C termini. Both repressor domains are transferable to heterologous DNA binding domains and function from proximal and distal positions. Interestingly enough, the two repressor domains differentially interact with several cellular cofactors, including Co-REST, N-CoR, mSin3A, and SCPs, which can be used to recruit histone deacetylases, histone methyltransferases, and LSD1-containing complexes (reviewed in Ballas et al., 2005 and Lunyak et al., 2002; Shi et al., 2004; Yeo et al., 2005) to the vicinity of the REST/NRSF-regulated promoters or to their genomic loci, thus facilitating nonneuronal lineage restriction. The ability of REST/NRSF to mediate such differential recruitment of molecular machineries indicates the possible existence of two modes of action which can be used by REST/NRSF to establish either active repression or gene silencing. Both of these modes tightly link to chromatin status and chromatin modifiers in use. Results from the knockout of REST/NRSF in mouse also strongly suggest that tissue/cell type, as well as genetic context, is important for determining the effect of REST/NRSF on gene expression (Chen et al., 1998).

It was originally thought that the REST/NRSF-RE-1/NRSE system served as a molecular switch that helped distinguish neural from nonneural cell types. Although REST/NRSF is expressed mainly in nonneural cells, the expression of REST/NRSF in neuronal progenitor cells, neurons, and neuronal cell lines has been a matter of controversy (Nishimura et al., 1996). In particular, bioinformatics analysis predicts more then 1000 putative gene targets (Lunyak et al., 2002), which encode many proteins with fundamental importance in brain function, including neuronal receptors and synaptic vesicle proteins, adhesion molecules, and signaling and channel proteins. How then would REST/NRSF, whose usual function is to act as a transcriptional repressor complex, go on to allow the activation of neuron-specific gene expression? It is plausible that the expression of REST/NRSF in cells of neuronal origin might reflect alternative roles for REST/NRSF in these cells and suggests that neurons may express additional factors that either cancel or modify the effects of REST/NRSF.

Kuwabara and colleagues have recently made the surprising discovery that noncoding RNAs could function in activating REST/NRSF-regulated gene expression (Kuwabara et al., 2004), as shown in Figure 1. The sequence of this novel, small, noncoding RNA matched the RE1/NRSE, which is the binding site for REST/NRSF. These double-stranded RNA molecules are specifically expressed in adult multipotent neural progenitor cells early in their differentiation. At the onset of neuronal differentiation, NRSE dsRNAs are proposed to interact with the REST/NRSF complex and trigger a conformational change, which prevents its association with corepressor proteins, such as HDACs and methyl-DNA binding proteins, thus converting REST/NRSF from a repressor to an activator. Another proposed model accounts for the expression within the CNS of one of the isoforms of REST/NRSF, REST4, which functions as a dominant-negative regulator by competing with REST/NRSF for DNA binding in neurons (for references, see Zuccato et al., 2003). In addition, both REST/NRSF and REST4 interact with RILP, a LIM domain protein, for nuclear translocation (Shimojo and Hersh, 2003), suggesting that REST/NRSF action could depend on its cellular and physiological environment. Recently, the wild-type huntingtin protein was found to bind to REST/NRSF and thereby sequester REST/NRSF in the cytoplasm (Zuccato et al., 2003). It was postulated that in the pathology of Huntington's disease, the REST/NRSF-huntingtin protein interaction is lost, causing REST/NRSF to enter the nucleus and repress its target genes. Thus, several mechanisms have the potential to regulate the activity of REST/NRSF within CNS.

In this issue of *Cell*, Ballas et al. (2005) come forward with an alternative model by providing direct evidence for the role of REST/NRSF during neurogenesis, when the progenitor cells traverse through the early stages of the neuronal differentiation pathway. The results from Mandel's group indicate that REST/NRSF expression is regulated on the posttranslational level, and although REST/NRSF mRNA levels stay relatively constant, its protein level is downregulated via a proteosomal pathway when cells progress on their way to lineage-restricted neural progenitors. Intriguingly enough, even upon loss of REST/NRSF from its regulatory sites, a subset of neuronal genes still maintains repression based on REST/NRSF-mediated recruitment of repressive factors, resulting in chromatin modifications. These additional epigenetic events help to maintain the genes in a state that is permissive for subsequent activation during neurogenesis and are important for the differentiation capacity of neuronal progenitors.

In conclusion, the numerous research studies underscore the fact that the cellular context is critical for understanding and defining the function(s) of the REST/NRSF-regulated system. Not only is the distinction between neuronal and nonneuronal cell types important, but, perhaps, equally important is the distinction between stages of differentiated precursor. In this context, the work described by Ballas et al. provides ample evidence that molecular mechanisms of REST/NRSF action are extremely complex and is revealed on different conceptual levels (cellular, network, and system as a minimum distinction), thus hindering simplistic interpretations.

This study, together with recent findings in the field, raises additional questions for future investigations. What processes initiate the alterations in chromatin structure of neuronal genes during neurogenesis that result in a switch from their silent mode to the active state? Is there a molecular memory of the REST/NRSF-mediated events that influence the orchestrated regulation of neuronal genes in mature neurons to fulfill the properties unique for CNS? Clearly, there will be no rest until further detailed investigations can answer these questions and ultimately bring together the pieces of the REST/NRSF puzzle.

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