clude that knockdown of *hda-1* levels with RNA interference (RNAi) in *skn-1* mutants leads to upregulation of *end-1* transcripts and a decrease in its variability. Indeed, the coefficient of variation for *end-1* levels does decrease with RNAi knockdown of *hda-1* in *skn-1* mutants as compared to the variation in *skn-1* mutants alone. However, this is still markedly higher than the variation observed in the wild-type. It appears that the double-mutant phenotype is complex. Not only is *end-1* derepressed, but the variation is also partially limited. Is the partial decrease in variation simply a byproduct of derepression of this gene? Or do multiple mechanisms feed into the control of variation? Or both? These questions are complex, but this paper lays the groundwork for addressing them.

The work of Raj and colleagues begins to address the mechanisms that cause incomplete penetrance. Their highly quantitative single molecule approach is new to developmental biology, which typically makes use of reporter transgenes, antibody staining, and in situ hybridization to assess gene expression. With this methodology, Raj et al. clearly show that variation in expression occurs in specific mutant conditions and that the architecture of a developmental network is able to compensate for noisy expression. However, one of the most challenging problems in the field of gene expression (in wild-type or mutant conditions) is to identify the source of transcriptional stochasticity. Although a role for chromatin state is proposed in this paper, this is hardly surprising given that its regulation is so fundamental to gene expression in general. The next challenge will be to show how these alterations affect variability in gene expression at individual loci.

Robustness compensates for variation caused by the stochastic low level expression of key regulators. However, mechanisms that ensure robustness also provide a buffer in the wild-type, which allows for the evolution of new regulatory interactions (for a review, see Masel and Siegal, 2009). It will be exciting to determine not only how variation occurs due to the break down of wild-type biological programs but also how novel cryptic modes of regulation are revealed when robustness mechanisms are impaired.

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MicroRNAs: From Decay to Decoy

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MicroRNAs interact with Argonaute proteins to guide posttranscriptional gene silencing. Eiring et al. (2010) now show that *miR-328* has a second function, acting as a decoy by binding to hnRNP E2 and lifting its translational repression of an mRNA involved in myeloid cell differentiation.

Although microRNAs (miRNAs) were discovered a decade ago, a detailed understanding of these tiny gene regulators is still in its infancy. Genes encoding miRNAs are transcribed as primary miRNA transcripts (pri-miRNAs) by RNA polymerase II. Pri-miRNAs are processed by Drosha to produce stem-loop-structured miRNA precursors (pre-miRNAs). Pre-miRNAs are exported to the cytoplasm, where Dicer generates ∼21 nucleotide double-stranded RNA intermediates. Such double-stranded RNAs are processed further, and one strand, the mature miRNA, interacts with Argonaute (Ago) proteins to form miRNA-protein complexes (miRNPs) (Bartel, 2009; Carthew and Sontheimer, 2009). miRNAs are able to silence gene expression posttranscriptionally by binding to partially complementary target sites in the 3′ untranslated region (UTR) of target messenger RNAs (mRNAs), leading to repression of translation or destabilization of the mRNA by deadenylation. Almost perfectly complementary target sites in the mRNA can be cleaved by the miRNA through an RNA interference-like mechanism (Filipowicz et al., 2008). In contrast to these

inhibitory effects on gene expression, miRNAs can also stimulate the expression of target genes (Ørom et al., 2008; Vasudevan et al., 2007). In this issue, Eiring et al. (2010) report a new function for the human miRNA, *miR-328*. They show that *miR-328* can act as a decoy by binding to a regulatory RNA binding protein and preventing it from blocking translation of mRNAs. Thus, *miR-328* has a dual role in the regulation of gene expression.

The RNA binding protein hnRNP E2, also called poly(rC) binding protein, interacts with a conserved C-rich binding site in the 5′ UTR of the *C/EBPα* mRNA and inhibits its translation (Figure 1A). *C/EBPα* mRNA encodes a transcription factor that is important for the differentiation of myeloid progenitor cells into white blood cells called granulocytes in the bone marrow. The activity of hnRNP E2 is induced by the kinase activity of BCR/ABL, a fusion protein derived from the reciprocal translocation of chromosomes 9 and 22. This chromosomal abnormality (called the Philadelphia chromosome) and the BCR-ABL oncoprotein it encodes are present in about 95% of all patients with chronic myeloid leukemia (CML). BCR/ABL prevents granulocytic differentiation, and thus allows myeloid progenitor cells to proliferate. The authors analyzed miRNA expression profiles in myeloid progenitor cells from CML patients in blast crisis that strongly express the BCR/ABL oncoprotein and found reduced expression of *miR-328*. They realized that the sequence of *miR-328* is very similar to the hnRNP E2 binding sequence found in the *C/EBPα* mRNA and hypothesized that the mature *miR-328* might bind to hnRNP E2. Using several experimental approaches, they demonstrate that *miR-328* interacts with hnRNP E2 under physiological conditions and that surprisingly this interaction is independent of Ago proteins and other proteins associated with the gene silencing machinery.

The C/EBPα protein drives myeloid cell differentiation, and hnRNP E2 inhibits this differentiation by blocking the translation of *C/EBPα* mRNA. Consequently, the authors surmised that ectopic expression of *miR-328* should influence myeloid differentiation. Using a number of in vitro and in vivo assays, they dem-
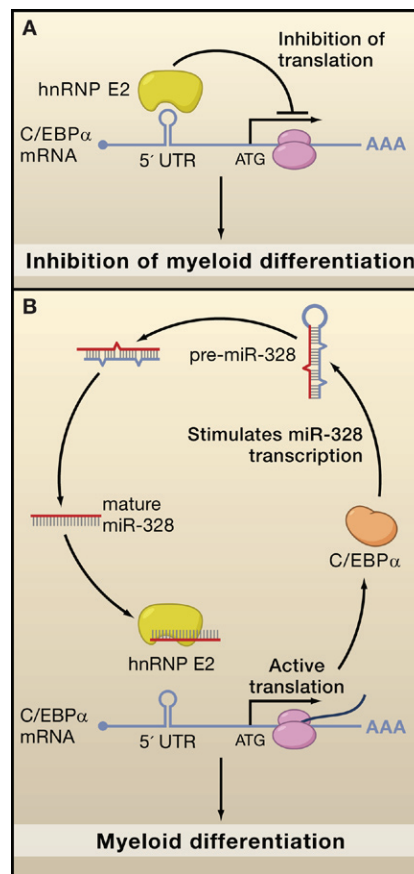

Figure 1. Regulation of hnRNP E2 by *miR-328*

(A) Myeloid progenitor cells from chronic myeloid leukemia (CML) patients express the fusion protein BCR/ABL kinase. In these cells, BCR-ABL activates the RNA binding protein hnRNP E2, which interacts with the 5′ UTR of the *C/EBPα* mRNA leading to inhibition of its translation. C/EBPα is a transcription factor that drives the differentiation of myeloid progenitor cells into granulocytes in the bone marrow. Reduced C/EBPα protein levels in leukemic blast cells blocks their differentiation into granulocytes.

(B) The sequence of mature *miR-328* is similar to the hnRNP E2 binding site in *C/EBPα* mRNA, enabling this miRNA to interact directly with hnRNP E2. The binding of *miR-328* to hnRNP E2 releases *C/EBPα* mRNA from translational repression, rescuing C/EBPα protein production and resulting in differentiation of blast cells into granulocytes. In addition, C/EBPα stimulates *miR-328* transcription, thus forming a positive feedback loop that fine tunes the regulation of myeloid differentiation.

onstrate that *miR-328* is able to lift translational inhibition of *C/EBPα* mRNA and drive CML blast cells to differentiate into granulocytes. The ability of *miR-328* to rescue the differentiation of CML blast cells is due to its interaction with hnRNP E2 leading to the sequestration of hnRNP E2 and the restoration of *C/EBPα* mRNA

translation. Furthermore, the authors identified a positive regulatory feedback loop, which highlights the complexity of miRNA regulatory networks. They show not only that *miR-328* regulates C/EBPα expression, but also that the C/EBPα protein induces *miR-328* expression by binding directly to the *miR-328* promoter (Figure 1B).

Is *miR-328* a typical miRNA, or does it just act as a decoy for hnRNP E2? Searching for targets using common miRNA target prediction algorithms, Eiring and colleagues validated the oncogene PIM1 as a direct target of *miR-328*. PIM1 is a regulator of the cell cycle and apoptosis and is therefore important for the survival of leukemic blast cells expressing BCR/ABL. Therefore, *miR-328* has a dual function: on the one hand, it guides silencing of target genes, and on the other, it acts as a decoy blocking translational inhibition mediated by hnRNP E2.

It is well established that RNA binding proteins can modulate the function of miRNAs in different systems. For example, proteins like Deadend 1 or HuR (Hu antigen R, also known as ELAV1) can compete with miRNA binding sites on mRNAs and modulate miRNA function (Bhattacharyya et al., 2006; Kedde et al., 2007). The Eiring et al. findings are intriguing because an miRNA-mediated regulatory function associated with RNA binding proteins has not been reported before. Moreover, the general view of miRNA functions entails a model in which miRNAs serve as guides for Ago protein complexes, the actual mediators of posttranscriptional gene silencing. Eiring and coworkers show that *miR-328* can act independently of Ago proteins by interacting directly with hnRNP E2.

The discovery of this new function for miRNAs raises a number of fascinating questions. How many other miRNAs are similar to the binding sequences of RNA binding proteins? What is the impact of miRNAs acting as decoys in the pathogenesis of cancer and other diseases? How are miRNAs released from Ago proteins and how is the interplay between the two pathways regulated? Future studies will be necessary to unravel the mechanistic details underlying the diverse functions of miRNAs both in healthy as well as diseased tissue.

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