Much remains a mystery. For example, we would dearly like information on the identity of the environmental cues that affect the RetS and Gac two-component regulatory systems. Is it the presence or absence of the unknown signals that leads to chronic infections in the CF lung? Do *retS* mutations accumulate during the course of a chronic infection? Are both of the response regulator domains phosphorylated in the presence of signal? Is the sensor histidine kinase domain responsible for the phosphorylation of both response regulator domains? Although many questions remain RetS is a strong candidate for the Holy Grail that regulates the fine balance between acute and chronic infection.

Timothy L. Yahr and E. Peter Greenberg
Department of Microbiology and
The W.M. Keck Microbial Communities and Cell Signaling Program
University of Iowa Carver College of Medicine
Iowa City, Iowa 52242

Selected Reading

Dacheux, D., Attree, I., and Toussaint, B. (2001). Infect. Immun. *69*, 538–542.

Goodman, A.L., Kulasekara, A., Rietsch, A., Boyd, D., Smith, R.S., and Lory, S. (2004). Dev. Cell *7*, 745–754.

Heurlier, K., Williams, F., Heeb, S., Dormond, C., Pessi, G., Singer, D., Camara, M., Williams, P., and Haas, D. (2004). J. Bacteriol. *186*, 2936–2945.

Parkins, M.D., Ceri, H., and Storey, D.G. (2001). Mol. Microbiol. *40*, 1215–1226.

Rodrigue, A., Quentin, Y., Lazdunski, A., Mejean, V., and Foglino, M. (2000). Trends Microbiol. *8*, 498–504.

Singh, P.K., Schaefer, A.L., Parsek, M.R., Moninger, T.O., Welsh, M.J., and Greenberg, E.P. (2000). Nature *407*, 762–764.

Stock, A.M., Robinson, V.L., and Goudreau, P.N. (2000). Annu. Rev. Biochem. *69*, 183–215.

Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warrener, P., Hickey, M.J., Brinkman, F.S., Hufnagle, W.O., Kowalik, D.J., Lagrou, M., et al. (2000). Nature *406*, 959–964.

mRNA Turnover Meets RNA Interference

By using two very different approaches, recent work by Gazzani et al. (2004) and Souret et al. (2004) reveal a fundamental link between mRNA degradation and RNA silencing pathways in *Arabidopsis*.

In plants and animals, two classes of small RNAs, which are similar in structure but differ in their biogenesis, can negatively regulate target gene expression. MicroRNAs (miRNAs) arise via endonucleolytic processing by the enzyme Dicer from highly structured precursor RNAs that are transcribed from endogenous non-protein-coding genes by RNA polymerase II. On the other hand, small interfering RNAs (siRNAs) are generated by processing (also by Dicer) of long double-stranded RNA (dsRNA) precursors that arise as a result of viral replication, activity of cellular RNA-dependent RNA polymerases (RdRPs), or transcription of inverted repeats in the genome.

siRNA or miRNA-containing RNA-induced silencing complex (RISC) can negatively regulate gene expression via transcriptional repression (heterochromatin assembly), translational repression, and also via mRNA cleavage. While how the RISC complex can silence transcription and translation remains largely mysterious, its modus operandi in mRNA cleavage is being rapidly unraveled. It is now clear that the key endonuclease component of mammalian RISC is its Ago2 subunit (Liu et al., 2004), and that the 5′ fragment and 3′ fragments that are generated by the endonucleolytic cleavage by RISC in *Drosophila* embryo lysates bear 3′ hydroxyl and 5′ phosphate, respectively (Schwarz et al., 2004). However, the subsequent fate of these mRNA cleavage products remains unclear. It is nonetheless obvious that they must be eliminated, since translation of truncated messages is potentially deleterious because they may encode toxic polypeptide fragments. This view is supported by the existence of elaborate mRNA surveillance and decay pathways that have evolved to remove mRNAs that contain premature stop codons and thus can also result in truncated, toxic products if translated (reviewed in Maquat, 2004).

Eukaryotic mRNAs are degraded through one of the three principal pathways (reviewed in Parker and Song, 2004): (1) poly(A) shortening followed by decapping and 5′ to 3′ decay by XRN1-like enzymes, (2) poly(A) shortening followed by 3′ to 5′ decay mediated by exosome complex, and (3) endonucleolytic cleavage followed by (presumably) exonucleolytic reactions mediated by the same components also involved in pathways (1) and (2). In *Arabidopsis*, XRN1-like enzymes are represented by multiple isoforms, only one of which, AtXRN4, is cytoplasmic (Kastenmayer and Green, 2000). In order to address its cellular function, Souret et al. (2004) have isolated null mutations in *AtXRN4* gene and characterized the changes in the transcriptome-wide mRNA decay profiles in these plants by using cDNA microarrays.

Interestingly, in drastic contrast to the inactivation of *S. cerevisiae XRN1* that leads to very slow growth, loss of *AtXRN4* does not cause any obvious phenotype at the whole plant level. In agreement with the lack of an overt phenotype, decay rates of only a small subset of cellular transcripts (a total of 14) are altered in the *xrn4* mutants. Moreover, several transcripts among those that are upregulated in the *xrn4* background show accumulation of the truncated fragments. Upon closer examination, these fragments turned out to correspond to the stabilized 3′ segments of the respective transcripts. Souret et al. (2004) then surmised that these fragments could represent the mRNA remnants derived from the siRNA- or miRNA-directed cleavage, a conjecture that was subsequently borne out in several (although not all) cases. A simple and elegant model that naturally follows

from these findings is that one of the important functions of AtXRN4 is to exonucleolytically degrade the 3′ fragments that are produced as a result of the endonucleolytic cleavage of the mRNA by RISC.

Several important questions arise that warrant further inquiry. First, it remains to be established why the 3′ fragments of some transcripts were NOT stabilized in the *xrn4* mutant plants. Perhaps an alternative exonuclease (5′–3′ or 3′–5′) is involved in these cases. If so, it would be important to determine the underlying basis of the division of labor between it and AtXRN4. Second, for some of the 3′ fragments that were stabilized, including the most extensively characterized AtFBL6 transcript, neither miRNA nor siRNA could be found in existing databases (e.g., cgrb.orst.edu/smallRNA/db) that could direct RISC to the presumed cleavage site. It is therefore possible that the presumed endonuclease is not RISC in this case. Third, the identity of the enzymatic activity responsible for the degradation of the corresponding 5′ fragments remains unknown, although the *Arabidopsis* exosome complex (Chekanova et al., 2000) is a likely candidate. A recent finding (Shen and Goodman, 2004) further suggests that the 5′ fragments may be marked for decay via oligouridylation of their 3′ ends. Finally, it is notable that only the cleavage products of the endogenous mRNAs, resulting from the activity of siRNA/RISC complex, but not the full length decapped mRNA accumulated in the *xrn4* plants. Could it be that the major pathway of mRNA decay in plants proceeds without decapping as an early step (e.g., via pathway (2) described above)?

In contrast to an intriguing lack of accumulation of the decapped full-length transcripts derived from the endogenous genes, decapped full-length transcripts from transgene constructs do accumulate in *xrn4* mutant plants in the study by Sablowski group (Gazzani et al., 2004), who homed in on the AtXRN4 using completely different strategy. They have engineered an ectopic, glucocorticoid hormone-regulated conditional overexpression of the *Arabidopsis* transcription factor STM, via creating transgenic plants expressing STM-glucocorticoid receptor (GR) fusion under the control of a strong, constitutively active 35S promoter. Treatment of the STM-GR plants with synthetic glucocorticoid dexamethazone causes activation of the meristem developmental program and consequent inhibition of cotyledon and leaf development. A genetic suppressor screen aimed at finding mutations capable of reversing this phenotype then led to an isolation of—you guessed it—loss-of-function alleles of *XRN4*. Subsequently, the knockout (null) allele was shown to have a similar phenotype.

Curiously, *STM-GR* mRNA levels are lower, rather than higher, in the *xrn4* mutant background compared with the wild-type plants. While consistent with the observed phenotype, this may seem surprising given the proposed role of AtXRN4 in mRNA turnover. Explanation of this seemingly paradoxical finding lies in the fact that *xrn4* mutation leads to the degradation of *STM-GR* mRNA, which proceeds via the RNA silencing pathway and is dependent on the functional RNA silencing machinery, and specifically on RdRP. Indeed, degradation of the STM-GR transgene-encoded mRNA in the *xrn4* background correlates with the RdRP-dependent appearance of siRNAs corresponding to the *STM-GR*. Therefore, the model that is proposed by the authors is that AtXRN4 antagonizes RNAi, most likely by degrading the template for RdRP. The identity of this template that causes RNA silencing unless degraded by AtXRN4 is not yet unambiguously established, but full-length, decapped *STM-GR* mRNA and/or RISC-cleaved *STM-GR* mRNA fragments are likely candidates.

The key unanswered question is why the endogenous STM mRNA does not suffer the same fate as *STM-GR* transgene encoded mRNA in the *xrn4* mutant background, despite the presence of the siRNAs corresponding to the *STM* sequences. Gazzani et al. (2004) suggest that a possible explanation may lie in the difference in translational efficiency. It is known that translation of STM requires certain meristem-specific factors, therefore *STM-GR* mRNA that is expressed ectopically may be translated poorly and, thus, vulnerable to decapping (because decapping and translation machineries compete for mRNA access), and in the *xrn4* background, to silencing. Differences in the RNA silencing phenotypes triggered by transgene-derived and endogenous gene-derived transcripts have been observed previously, for example, transgene-derived RNAs are capable of causing systemic silencing that is dependent on the RdRP activity, while the endogenous gene-derived transcripts only lead to localized effects (Himber et al., 2004, Parizotto et al., 2003).

The importance of these contributions is severalfold. First, until now, "conventional" messenger RNA degradation and RNA silencing pathways have always been considered in separate contexts. The studies by Souret et al. (2004) and Gazzani et al. (2004) change that; moreover, their implications potentially reach across the phylogenetic boundaries, because similar functional intersections between mRNA turnover and RNAi pathways are likely to be found in Metazoa. Second, with the exception of poly(A) specific ribonuclease PARN (Reverdatto et al., 2004), mRNA turnover pathways and enzymes in plants remain poorly understood, and therefore unequivocal attribution of mRNA turnover function to AtXRN4 represents an important step forward. Finally, these studies suggest that the ability to manipulate mRNA turnover machinery may impact future applications of RNA silencing in basic and applied research, and potentially in clinical settings as well.

Dmitry Belostotsky
Department of Biological Sciences
State University of New York at Albany
Albany, New York 12222

Selected Reading

Chekanova, J.A., Shaw, R.J., Wills, M.A., and Belostotsky, D.A. (2000). J. Biol. Chem. *275*, 33158–33166.

Gazzani, S., Lawrenson, T., Woodward, C., Headon, D., and Sablowski, R. (2004). Science *306*, 1046–1048.

Himber, C., Dunoyer, P., Moissiard, G., Ritzenthaler, C., and Voinnet, O. (2003). EMBO J. *22*, 4523–4533.

Kastenmayer, J.P., and Green, P.J. (2000). Proc. Natl. Acad. Sci. USA *97*, 13985–13990.

Liu, J., Carmell, M.A., Rivas, F.V., Marsden, C.G., Thomson, J.M., Song, J.J., Hammond, S.M., Joshua-Tor, L., and Hannon, G.J. (2004). Science *305*, 1437–1441.

Maquat, L.E. (2004). Curr. Genomics *5*, 175–190.

Parker, R., and Song, H. (2004). Nat. Struct. Mol. Biol. *11*, 121–127.

Parizotto, E.A., Dunoyer, P., Rahm, N., Himber, C., and Voinnet, O. (2004). Genes Dev. *18*, 2237–2242.

Reverdatto, S.V., Dutko, J.A., Chekanova, J.A., Hamilton, D.A., and Belostotsky, D.A. (2004). RNA *10*, 1200–1214.

Schwarz, D.S., Tomari, Y., and Zamore, P.D. (2004). Curr. Biol. *14*, 787–791.

Shen, B., and Goodman, H.M. (2004). Science *306*, 997.

Souret, F.F., Kastenmayer, J.P., and Green, P.J. (2004). Mol. Cell *15*, 173–183.

Good Fences Make Good Neighbors: Barrier Elements and Genomic Regulation

In the genome, it is essential to maintain a physical barrier between active and inactive regions; however, the nature of this barrier has been elusive. In a recent issue of *Molecular Cell*, West et al. (2004) shed light on mechanisms underlying these molecular "fences."

Eukaryotic genomes are separated into gene-rich euchromatin and gene-poor heterochromatin. Transgenes inserted near heterochromatin undergo stochastic inactivation, indicating that heterochromatin has a tendency to spread into neighboring DNA (Elgin and Grewal, 2003). Thus, natural barriers to spreading, i.e., "insulator" elements, are critical when active genes are nearby. Insulators actually exhibit two functions: they demarcate heterochromatin/euchromatin boundaries, and they prevent inappropriate crossactivation of neighboring genes (i.e., enhancer blocking). The first mutation affecting a chromatin boundary was observed in *Drosophila* by Ed Lewis in the 1970s, but the concept of boundary function was formed in the early 1990s (Gdula et al., 1996; Vazquez et al., 1993). The crucial nature of general barrier function is indicated by its evolutionary conservation, ranging from single-celled budding yeast (Donze et al., 1999) to humans (West et al., 2002). However, despite two decades of intensive investigation and many exciting discoveries, the mechanism of insulator function remains largely unknown.

Until about 7 years ago, histone proteins making up chromatin were widely regarded as inert building blocks that package the unwieldy DNA into the relatively tiny nucleus. Indeed, transcription was simplistically viewed as individual transcriptional activators or repressors communicating with the basal transcriptional machinery via coactivators or corepressors. Recent progress in the chromatin field has fundamentally reshaped our views of gene activation. For example, histone modifications are now known to play a central role in gene activation and repression. Acetylation and methylation of certain lysine residues (Lys-4 of histone H3) are usually associated with and required for gene activation, whereas generalized deacetylation and distinct methylated residues (such as Lys-9 of H3) underlie repression (Fischle et al., 2003). Thus, combinations of different modifications may mark local chromatin for activation or repression, likely through both direct alteration of local chromatin structure and recruitment of effector proteins. For example, H3 Lys-9 methylation recruits a major heterochromatin protein called HP1 (Elgin and Grewal, 2003; Fischle et al., 2003). It turns out that histone modifications appear also to be key to the function of barriers, as first suggested in yeast (Elgin and Grewal, 2003), and now more explicitly revealed through West et al.'s work in higher eukaryotes.

In vertebrates, the β-*globin* locus is an excellent model to examine the interplay between histone modifications, chromatin structure, and insulator elements. The locus control region (LCR), located upstream of the coding genes, directs temporally programmed activation of fetal, and then adult, genes within the locus. The LCR also demarcates heterochromatin, which lies further upstream of the chicken β-*globin* locus, from euchromatin within the locus (Figure 1). Pioneering work by Gary Felsenfeld's group spanning more than a decade identified a chromatin boundary within the LCR located at a DNase I hypersensitive site (a hallmark of regulatory elements), called HS4 (West et al., 2002). Then, as described in a series of elegant papers, Felsenfeld's group showed that the HS4 region works as an insulator both to set up a chromatin boundary to prevent the encroachment of heterochromatin into the coding genes and to provide enhancer blocking activity that requires CTCF protein (West et al., 2002). In another landmark study, the Crane-Robinson lab showed that DNAaseI hypersensitivity within the chicken β-*globin* locus correlates with histone acetylation (Hebbes et al., 1994). Finally, the Felsenfeld lab previously observed acetylated H3 and methylated H3 K4 in the open euchromatin, while K9-methyl correlated with closed heterochromatin, which led to the model that histone modifications may play a crucial role in barrier function (Litt et al., 2001).

In the current study, West et al. tested this model and provide exciting evidence for the underlying mechanism. The authors previously identified a 250 bp functional insulator core within HS4 containing five distinct protein binding regions or "footprints" (Bell et al., 1999). Through deletion mutagenesis, the source of the histone modifications is traced to a particular DNA sequence (CACGGG) known as an "E box", occurring within footprint IV (FIV). Thus, deletion of FIV (and not other footprints) abolishes the histone modifications and causes concomitant loss of chromatin barrier function. They then use a combination of classical chromatography and E box DNA affinity purification to identify the binding activities as USF proteins, which are ubiquitously expressed transcriptional activators of previously unknown mechanism. The USF proteins form a complex with PCAF (H3 acetylation) and SET7/9 (H3 K4 methylation) enzymes in erythrocyte extracts (where β-*globin* is expressed), and both USF and the enzymes bind to the HS4 region in vivo. Finally, and persuasively, the authors use RNAi to reduce the levels of USF, leading to a dra-