that it permits a rapid response to changing intra- or extracellular conditions. Moreover, different sets of specific *and* common activities are required to activate a wide range of different promoter sequences. A similar situation exists for DNA repair: the cell can quickly repair the diverse DNA lesions that may occur at any time, although each type of damage requires a set of specific *and* common factors for its repair (Kowalczykowski, 2000).

Clearly, these studies push the limits of current technology in quantitative fluorescence imaging in vivo and advance our insight into nuclear function. It is expected that further in-depth investigation of the various DNA-transacting processes and their interaction will extend our understanding of nuclear function even more. This will require not only in vivo FRAP, but also protein-protein and protein-DNA interaction studies using fluorescence resonance energy transfer and fluorescence cross-correlation spectroscopy. The use of kinetic (computer) modeling to analyze and model in vivo data and to design new experimental approaches will be instrumental to properly interpret these data. In future research, the full potential of kinetic (computer) modeling may allow us to obtain an integrated view of the regulation of and interaction between processes acting on DNA (see Figure, panel B).

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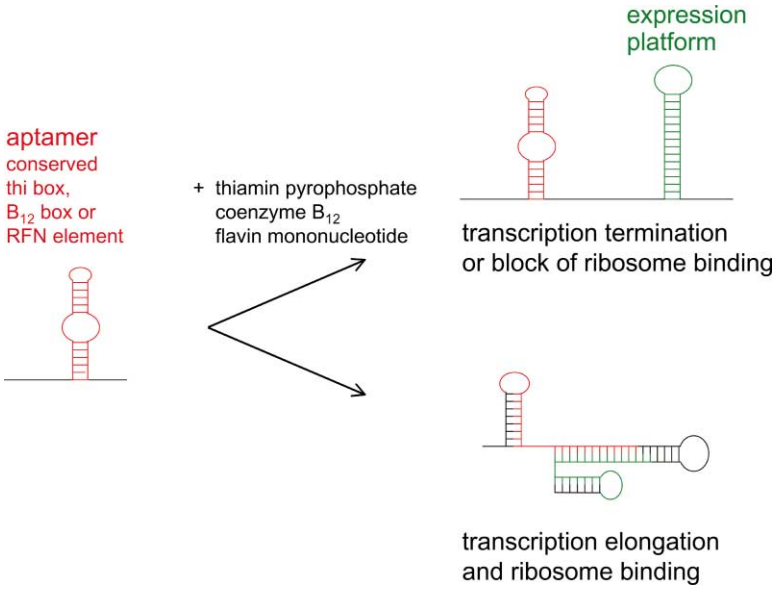
Take Your Vitamins with a Pinch of RNA

RNA "aptamers" capable of binding and discriminating among structurally related small molecules can be concocted in the laboratory. Two groups have now discovered that conserved domains in the 5′ ends of some mRNAs bind specific metabolites and respond by changing their shape in biologically useful ways, demonstrating that aptamers also are present in the natural world.

The discovery of natural aptamers began with missing regulatory proteins. These hypothetical proteins were believed to regulate the production of enzymes that catalyze the biosynthesis of several vitamins. The production of the biosynthetic enzymes is repressed by the metabolic products of the respective pathways, and repression occurs at steps that follow transcription initiation. The corresponding genes and operons are preceded by untranslated promoter-proximal regulatory sequences, which are transcribed to produce the 5′ segment of the message. The regulatory sequences for each of the biosynthetic pathways are widely conserved in prokaryotes and, more importantly, the patterns of conservation suggest that the RNAs containing the sequences can fold into conserved secondary structures. To perceptive observers, these observations suggested that the RNAs might bind the cognate metabolites without the intermediary of a protein (Gelfand et al., 1999; Nou and Kadner, 2000; Miranda-Ríos et al., 2001).

Now, two groups have confirmed this speculation. The first, led by Ronald Breaker, examined the control of the *Escherichia coli thiM* and *thiC* genes involved in thiamin synthesis (Winkler et al., 2002b), the *E. coli btuB* gene encoding a cobalamin transporter (Nahvi et al., 2002), and the *Bacillus subtilis ribGBAHT* genes involved in flavin mononucleotide and flavin adenine dinucleotide synthesis and transport (Winkler et al., 2002a). This group found that the regulatory metabolites, thiamin pyrophosphate, coenzyme B_{12} (adenosylcobalamin), and flavin mononucleotide, respectively, altered the spontaneous cleavage patterns of RNA molecules containing the cognate regulatory sequences. Since the pattern of spontaneous cleavage depends on the secondary and tertiary structure of the RNA, the small metabolites must be changing the RNA structure. The concentration dependence of cleavage alteration revealed a saturable binding site with an apparent dissociation constant in the submicromolar range. The effect is specific, since closely related compounds, such as vitamin B_{12} (cyanocobalamin), oxythiamin, and riboflavin, did not alter cleavage and certain base substitution mutations prevented metabolite binding.

The second group, led by Evgeny Nudler, examined the control of the *B. subtilis* riboflavin operon as well as the *B. subtilis* thiamin operon (Mironov et al., 2002). Mironov et al. found that the addition of flavin mononucleotide or thiamin pyrophosphate to in vitro transcription reactions with purified components led to increased termination of the corresponding transcripts, and point mutations of the conserved bases in the leader sequences abolished this effect. These authors also



Model for the Effects of Thiamin Pyrophosphate, Coenzyme B_{12}, and Flavin Mononucleotide Binding on the Structures of the 5′ Leaders of mRNAs Encoding Proteins Required for Vitamin Synthesis and Transport

The 5′ untranslated regions of select mRNAs are proposed to have two domains: an aptamer domain, encompassing the highly conserved "thi box," "B_{12} box," or "RFN element" (red), and an expression platform, encompassing the ribosome binding site and/or transcription termination and antitermination sequences (green). The conserved boxes of the aptamer domains bind thiamin pyrophosphate, coenzyme B_{12}, or flavin mononucleotide, presumably as soon as this part of the leader is synthesized. In the presence of the metabolite, a structure is formed that terminates transcription or blocks ribosome binding. In the absence of the coeffector, an alternative structure is formed that allows transcription elongation and ribosome binding.

showed that flavin mononucleotide, but not riboflavin, interfered with hybridization of a complementary oligonucleotide to the regulatory RNA. Additional evidence that flavin mononucleotide binds to RNA came from the observation that the intrinsic fluorescence of the flavin was quenched upon synthesis of the RNA.

The RNA structural changes following metabolite binding to the native aptamers have biological consequences, and the RNAs therefore have been named "riboswitches" (Figure 1). The consequences differ depending on the metabolic pathways. Binding of coenzyme B_{12} inhibits translation by favoring sequestration of the ribosome binding site of the downstream *btuB* gene in a base-paired structure, while binding of flavin mononucleotide favors formation of an intrinsic transcription terminator that reduces transcription of the downstream *rib* operon. Binding of the same metabolite to different riboswitches can also have different consequences: thiamin pyrophosphate binding leads to sequestration of the ribosome binding site of the *E. coli thiM* gene but enhances transcription termination prior to the *B. subtilis* thiamin operon. Although the signal readout can differ, segments of the riboswitches and their predicted structures are, as noted earlier, highly conserved among RNAs that respond to and presumably bind the same metabolite.

Do riboswitches work as well as regulatory proteins? As mentioned above, the native aptamers can be incorporated into more than one regulatory scheme. The binding studies carried out by both the Breaker and Nudler groups indicate that the specificity of the natural aptamers is exquisite. A clear advantage of riboswitches over proteins is that they are cheaper: regulation through direct binding of a signal to the leader of a message should require less energy than synthesis of an intermediary protein. Thus a riboswitch may be particularly desirable for regulating the synthesis of metabolites that are only needed in very limited quantities or whose synthesis does not require a high expenditure of energy. This leads to the related question of whether riboswitches arose recently due to their selective advantage in the regulation of some systems, or whether riboswitches are "molecular fossils," whose invention predates that of regulatory proteins and which have been retained in the few cases where their properties were advantageous.

Finally, if riboswitches are so good at their job, why aren't they more widespread? So far, the vitamin binding aptamers have been found only in prokaryotes. However, sequence gazing suggests that they are also present in archaea and eukaryotes (Winkler et al., 2002b). Perhaps more riboswitches will be found now that we know how to look for them. The extent of conservation of 5′ and 3′ untranslated sequences apparent from the comparison of the genomic sequences of related organisms suggests that these sequences are targets for far more regulation than was previously appreciated (Gottesman et al., 2001). This conclusion is echoed by the recent findings that the structures of mRNA leaders are modulated by temperature and *trans*-acting, noncoding RNAs (Gottesman et al., 2001; Johansson et al., 2002). It will be exciting to see what other aptamers and mechanisms of riboswitching remain to be discovered.

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