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GUEST COMMENTARY

Regulatory Small RNAs: the Key to Coordinating Global Regulatory Circuits

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Until recently, only a dozen small RNAs that do not function as tRNAs or rRNAs were known for *Escherichia coli*. In the last 3 years, systematic screens based on computation, microarrays, and cloning have led to the discovery of more than 50 novel small-RNA (sRNA)-encoding genes in this bacterium (8, 9, 23, 27, 29, 31, 34). Computational characterization of the sRNA genes revealed few shared genomic and sequence features of possible functional significance (13), and the physiological roles of the majority of the sRNAs are still unclear. For those sRNAs that have been characterized, expression and functional studies have indicated that each of the sRNA genes belongs to a specific regulon. In addition, many of them affect multiple targets and/or proteins, which in turn act as regulators of other genes, suggesting an involvement of sRNAs in global control (8, 12, 26, 27, 29, 31). Intriguingly, these studies revealed that an sRNA that is part of one regulon and is abundant under a specific environmental condition acts in turn to modulate the expression of genes that are part of other physiologically associated regulons. Most often, these globally regulating sRNAs act in *trans* by base pairing with their target mRNAs to increase or decrease their expression by affecting translation or stability. These observations demonstrate that sRNAs add complexity to the interplay between factors involved in global control in response to environmental changes. Therefore, some important tasks for future study are to functionally characterize this fairly novel and exciting class of RNAs, to discover their target genes, and to elucidate their physiological roles.

The studies by Opdyke et al. (22) and Chen et al. (10) in this issue focus on the biological functions of two newly discovered sRNA genes. Jason Opdyke and colleagues present the characterization of *gadY*, a small-RNA-encoding gene whose expression is *rpoS* dependent. The *gadY* gene is located between two regulators of the acid response, *gadX* and *gadW*, and overlaps the 3′ end of *gadX*. The overexpression of GadY RNA results in the accumulation of *gadX* mRNA. Increased levels of the transcriptional activator GadX in turn lead to an increase in the levels of two glutamate decarboxylase enzymes, GadA and GadB, which are responsible for deacidifying the cytoplasm. Thus, the stationary-phase sRNA GadY is a regulator of genes affecting resistance to acid conditions.

Chen et al. present the characterization of another small RNA, denoted MicC. Under conditions of low temperature and in minimal medium, *micC* expression rises. This increase in the level of MicC results in the repression of OmpC, a major outer membrane protein. It is interesting that the expression of two abundant porins, OmpC and OmpF, is controlled by two sRNAs, MicC and the previously characterized sRNA MicF (6, 11). The expression pattern of MicC is a mirror image of MicF expression: MicF levels increase at a high temperature, high osmolarity, or exposure to toxic agents, while MicC is induced at low temperatures and in minimal medium (10, 11). Thus, the repression of OmpC and OmpF by MicC and MicF leads to changes in the porin ratio that affect membrane properties in response to temperature or osmolarity.

Other small RNAs have been shown to affect multiple targets or regulator genes. One example is RyhB, also known as SraI, which is induced in response to iron depletion. This RNA, whose expression is controlled by Fur, down-regulates the mRNA levels of three enzymes involved in the tricarboxylic acid cycle, aconitase A (encoded by *acnA*), fumarase A (*fumA*), and succinate dehydrogenase (*sdhCDAB*); two iron storage proteins, bacterioferritin (*bfr*) and ferritin (*ftnA*); and the enzyme superoxide dismutase (*sodB*) that is part of the response to oxidative stress (20). Another example is provided by the newly discovered small RNAs IstR-1 and IstR-2, which are encoded opposite and upstream of the *tisAB* operon (previously called *ysdAB*), which encodes an SOS-induced toxic peptide (J. Vogel, L. Argaman, E. G. H. Wagner, and S. Altuvia, unpublished results). Although IstR-2 is induced in response to DNA damage while IstR-1 is present throughout growth, it is IstR-1 that inhibits the synthesis of the SOS-induced toxic peptide by base pairing with the *tisAB* mRNA (Vogel et al., unpublished results).

The previously identified small RNAs OxyS, DsrA, and RprA have also been shown to be induced under specific environmental conditions and to act on regulator genes. The induction of OxyS in response to oxidative stress results in the regulation of >40 *E. coli* genes and in protection against DNA damage (3). Two known target genes, *rpoS* and *fhlA*, encode transcriptional regulators of stationary phase and formate metabolism (3, 4, 32). DsrA and RprA stimulate the expression of RpoS in response to low temperatures and cell surface stress

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(14–18, 25). In addition to regulating *rpoS*, DsrA inhibits translation of the transcription modulator H-NS (14, 17).

It is interesting that all currently characterized globally regulating sRNAs bind the Sm-like Hfq protein and act by base pairing to target mRNAs (21, 24, 26, 28, 33, 34). These antisense or base-pairing sRNAs act in *trans* to increase or decrease the expression of their target mRNAs by affecting translation or stability. OxyS base pairing across the ribosome-binding region of *fhlA* inhibits translation of this mRNA (4, 7). Likewise, the binding of MicF and MicC to the *ompF* and *ompC* mRNAs, respectively, blocks the translation of these proteins (1, 5, 10). DsrA and RprA stimulate the translation of *rpoS* by base pairing with part of an inhibitory structure that normally occludes the ribosome-binding site to prevent its formation (15–18). IstR-1 inhibits translation of the toxic peptide by binding to a short region in the *tisAB* mRNA, leading to its cleavage (Vogel et al., unpublished results). The base pairing of RyhB with its targets facilitates degradation of the mRNAs (19, 20), and GadY represents the first example of a bacterial sRNA that binds its target mRNA to stabilize it (22).

All of the base-pairing sRNAs perform their functions by forming relatively short and usually discontinuous hybrids with their target mRNAs, as determined by the regions of complementarity. These short hybrids are fast to form (2, 7, 30), and more than one target mRNA can be regulated by the same sRNA via different regions (2, 14, 15). The characteristics of rapid response and the regulation of multiple target genes make this exciting class of genes suitable for responses to sudden changes in the environment. It is conceivable, therefore, that many more global regulatory circuits in bacteria will be found to be controlled by small-RNA-encoding genes.

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