RIBOSWITCHES

(Meta-)genome mining for new ribo-regulators

RNA regulatory elements are potential antibiotic targets and synthetic biology building blocks

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Riboswitches are small structural elements within messenger RNA (mRNA) that can change their conformation in response to specific environmental exposures. These changes can alter mRNA transcription or translation. Such ribo-regulators are emerging as a substantial contributor to bacterial gene control. Yet such RNA-based regulation remains challenging to study, in part because of a lack of effective high-throughput technologies for their unbiased identification. On page 187 of this issue, Dar *et al.* describe a novel method for genome-wide experimental identification of genes that are regulated by conditional transcription termination, which likely is a result of RNA structural switching (*1*). This work further enhances our understanding of bacterial gene regulation and expands the universe of RNA-based regulatory devices that can be deployed in synthetic biology applications.

In 2002, discoveries involving small RNAs were honored by *Science* as the Breakthrough of the Year: "RNA, long upstaged by its more glamorous sibling, DNA, is turning out to have star qualities of its own..." (*2*). Among the plethora of regulatory RNAs discovered in the past decade, riboswitches (and attenuators) represent a particularly intriguing group of cis-acting regulators. They reside in the untranslated region of the mRNA they control and can fold into mutually exclusive structures. In this way, one structural configuration allows gene expression, whereas the other structural configuration does not. The trigger for this conformational change is often a direct binding of a ligand.

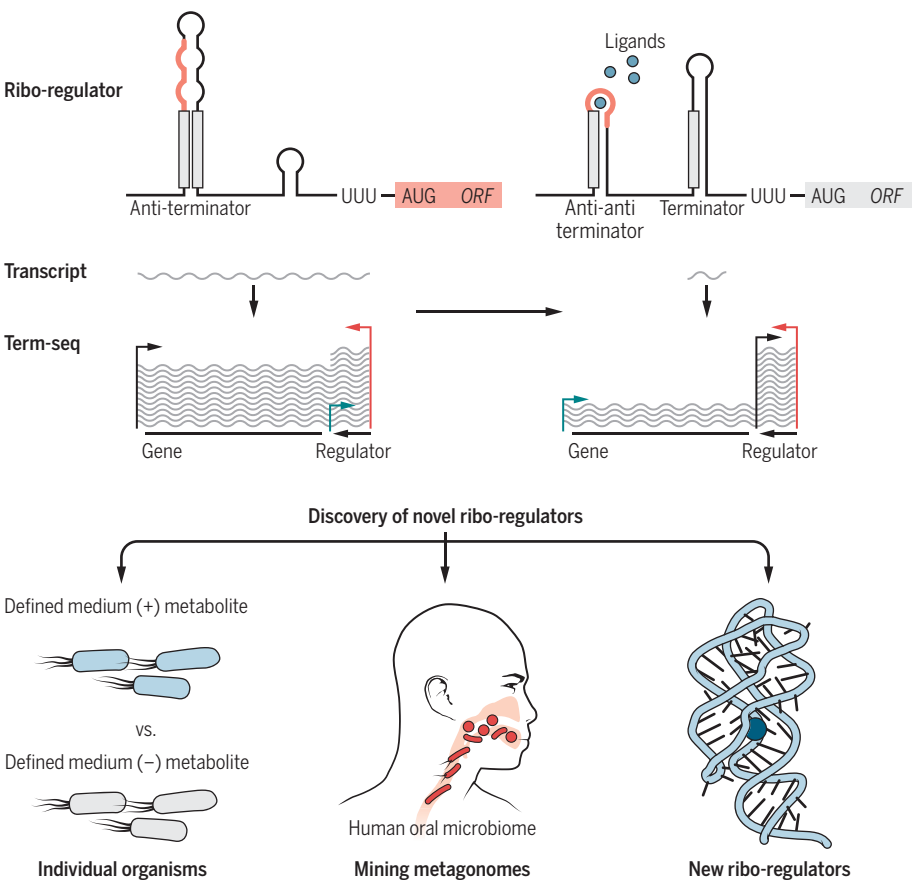
The majority of the riboswitches discovered until the work of Dar *et al.* were found as conserved elements in the untranslated regions of gene transcripts. About 25 different classes of riboswitches have been discovered using this approach and have been experimentally verified in bacteria, archaea, algae, fungi, and plants (*3*).

Riboswitches control a multitude of biological pathways, including bacterial vitamin and amino acid biosynthesis. Their mode of gene regulation primarily involves the control of transcription termination or the initiation of translation. However, riboswitches also exist that control splicing, mRNA degradation, or ribozyme activation. Riboswitches selectively respond to metabolites but can also be triggered by temperature or metal ion binding (*4*).

It is generally believed that the currently known riboswitches represent only the tip of the iceberg; however, the identification of new riboswitches by conservation-based approaches is not likely to continue to be a driver for discovery. For instance, non-conserved ribo-regulators, which are only present in one species or a specific clade of bacteria, are not likely to be discovered using comparative bioinformatics.

Dar *et al.* present a method that allows the discovery of RNA regulation irrespective of its evolutionary conservation. The methodology combines RNA sequencing with a library preparation method, term-seq, that identifies the 3′ end of the transcript. By applying this method to an organism grown in the presence and absence of a particular ligand, genes that are regulated at the RNA level leading to conditional premature termination of transcription can be identified (see the figure). The power of the approach is demonstrated by the identification of more than 90% (49 of 53) of known riboswitches as well as the discovery of 18 new potential regulatory elements in *Bacillus subtilis*.

The majority of antibiotics that target mRNA translation have RNA binding prop-



A ribo-regulator "Big Bang." Ribo-regulators can regulate the transcription of a gene in response to ligand binding. This regulation leads to a conditional termination of transcription of the gene, such that only the first part of the transcript is completed. Accordingly, a decrease in the full gene transcript will be noted by the term-seq approach of Dar *et al.*, along with a new transcription stop site. In this way, ribo-regulators can be discovered in individual organisms as well as metagenomes, leading to the experimental discovery of ribo-regulators that can be used as new components in synthetic biology.

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erties. Accordingly, it has been suggested that RNA-based regulation of antibiotic resistance genes would be important. The authors identify a wide range of genes that are conditionally terminated by various antibiotics targeting translation. From the perspective of the bacterium, it is important to rapidly activate the expression of an antibiotic resistance gene in response to that particular antibiotic. Having such regulation occur at the RNA level enables exactly this kind of rapid response to antibiotic exposure.

The discovery of such new regulatory mechanisms controlling antibiotic resistance genes provides an improved understanding of antibiotic resistance and also suggests that these ribo-regulators might be potential drug targets. Furthermore, integration of such regulatory elements into reporter systems could create biosensors that enable characterization of drug exposures at the single-cell level. These tools are likely to aid in the study of the mode of action of antibiotics as well as the evolution of antibiotic resistance.

Because the approach developed by Dar *et al.* is dependent only on access to RNA samples, the approach can be deployed to study metagenomic RNA samples. As a proof of concept, the authors sampled the human oral microbiome and exposed samples to various antibiotics. In this way, the authors identified several genes ribo-regulated by antibiotics, highlighting the generality of the approach.

In addition to improving our understanding of bacterial gene regulation and response to antibiotic treatment, the study also opens new avenues for building synthetic biology tools. The versatility and modularity of RNA-based regulation has spurred the development of RNA regulatory devices for building genetic circuits and even creating regulatory elements de novo (*5*). These efforts have led to the construction of complex genetic circuits, yet their applicability remains limited by several factors, including the spectrum of ligands for which RNA regulators exist. Given the immense diversity of the biological world, it is very likely that we have only scratched the surface of this diversity in terms of mining biological systems for regulatory devices that can be deployed in synthetic biology. Approaches such as the one described by Dar *et al.* will allow researchers to expand the repertoire of such elements available to synthetic biologists. ■

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CANCER

How neutrophils promote metastasis

Neutrophils may be cellular targets for cancer therapy

By **Thomas Tüting**[1] *and* **Karin E. de Visser**[2]

Metastatic disease is the main cause of death in cancer patients. Despite its devastating effects, the complex processes that lead to metastasis are poorly understood. During their journey to distant organs, cancer cells encounter various types of normal cells, including immune cells. Neutrophils are the most abundant immune cells in our blood, and they protect us from infections and facilitate wound healing. Intriguingly, neutrophils frequently accumulate in cancer patients. Recent studies have addressed the causal link between neutrophils and cancer in mouse tumor models, and point to a key role of neutrophils in promoting the most deadly aspect of cancer—its dissemination to distant organs (*1–4*).

"Are tumor-associated neutrophils just innocent bystanders...or do they actually influence cancer behavior?"

Observations in cancer patients have linked elevated neutrophil counts in blood with increased risk for metastasis (*5*). Furthermore, ulceration of melanomas and subsequent neutrophilic inflammation are associated with invasiveness and a high probability for metastatic dissemination (*1*). Notably, a gene expression meta-analysis of ~18,000 human tumors across 39 cancer types linked an intratumoral neutrophil-related gene signature with poor prognosis (*6*). Are tumor-associated neutrophils just innocent bystanders that accumulate in aggressive tumors, or do they actually influence cancer behavior?

In two independent transgenic mouse models that mimic human breast cancer, primary breast tumors induce neutrophil accumulation in distant organs before the arrival of cancer cells, where they enhance the early steps of metastasis formation (*2, 4*). In one model, neutrophils localize to the lung where they produce leukotrienes that facilitate colonization by selectively propagating cancer cells with higher tumorigenic potential (*4*). In the other breast cancer mouse model, neutrophils promote lung metastasis by dampening antitumor T cell immunity (*2*). In both studies, neutrophil accumulation in the (pre)metastatic niche was initiated by signals emanating from the primary tumor.

Environmental stimuli can also trigger the prometastatic functions of neutrophils (*1, 3*). In a transgenic mouse model of melanoma, ultraviolet irradiation induced neutrophil activation in the skin, which promoted invasive and migratory behavior of melanoma cells, resulting in their expansion along blood vessel endothelial surfaces and distant metastasis formation (*1*). Additionally, bacterial lipopolysaccharide-induced acute lung inflammation initiated recruitment of neutrophils, which release proteases that can degrade thrombospondin-1, a matrix glycoprotein. Thrombospondin-1 inhibits tumorigenesis; thus, its destruction enhances metastatic outgrowth (*3*). Taken together, neutrophils can exert prometastatic functions in response to an inflammatory trigger from either the primary tumor or an environmental stimulus.

These experimental and clinical findings provide a scientific basis for therapeutically targeting the prometastatic role of neutrophils in cancer (see the figure). Importantly, such approaches must be developed with caution because neutrophils also exert antimetastatic activity in other experimental mouse models (*7, 8*). The ability to inhibit or promote tumor growth and metastatic spread of cancer cells in different experimental systems illustrates the context dependency and plasticity of the neutrophil phenotype. It is generally believed that progressively growing tumors perturb the process of granulopoiesis in the bone marrow, and switch neutrophils from tumor-protective to disease-promoting, more-immature phenotypes (*9*). The molecular and cellular mechanisms orchestrating this

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