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RNA: Jack of All Trades and Master of All

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Noncoding RNAs have regulatory capabilities that evolution harnesses to fulfill diverse functions. Lee et al. show that a noncoding RNA from Epstein-Barr virus recruits a host transcription factor to silence virus gene expression and propose that it does this through base-pairing with nascent viral transcripts.

As noted years ago by François Jacob, a broad set of processes that regulate gene expression appear to be the product of evolutionary tinkering (Jacob, 1977). For decades these mechanisms were thought to be exclusively protein-driven, but, as would be predicted by unfettered tinkering, many are now known to involve regulatory RNAs. These RNAs employ simple yet highly flexible modes of interaction with proteins and other nucleic acids to regulate every aspect of gene expression and function. In this issue of *Cell*, Lee et al. (2015) from the Steitz laboratory add a new trick in the repertoire of regulatory RNAs. The authors examine the function of an Epstein-Barr virus (EBV) noncoding RNA, *EBER2*, and, using capture hybridization analysis of RNA targets (CHART) (Lee et al., 2015 and references therein), find that *EBER2* localizes to the tandem terminal repeats (TRs) in the EBV genome, in the vicinity of where the PAX5 host transcription factor binds (Arvey et al., 2012). The authors go on to show that *EBER2* interacts with PAX5, albeit indirectly. Based on structure predictions, phylogenetic conservation in other related gamma herpesviruses, and experimental data, they also propose that *EBER2* forms an 18 bp hybrid with intronic TR sequences in viral *LMP2* nascent transcripts. This RNA-RNA interaction brings the *EBER2* associated PAX5 to the vicinity of its DNA binding site to enhance repression of LMP genes likely through chromatin remodeling (Figure 5 in Lee et al. 2015).

This provides a possible answer to the long open question regarding the function of the abundant *EBER*s. In that regard, several interesting questions are raised by the manuscript, does *EBER1* also interact with PAX5? Indeed, careful inspection of Figure 2B in Lee et al. suggests that this may be the case. Could this explain the small effect of *EBER2* knockdown on PAX5 binding to the TR? As the authors themselves ponder—what about EBV strains deleted for *EBER2* (or both *EBER*s)? It is interesting to wonder whether the phenotypes observed with these strains (and there is controversy here) could be partially rescued by directly enhancing the PAX5 TR DNA interaction. These experiments would address the importance of *EBER*-mediated PAX5 recruitment for EBV replication and latency. As interesting as these questions are, the model of Lee et al. raises even more fascinating possibilities with general impact on RNA biology.

The model proposed in Figure 5 of Lee et al. represents a remarkable example of the versatile ability of RNAs to build complexes required for constitutive and regulated gene function. It also raises interesting questions. Can *EBER2* base pair with TR sequences in DNA, which would be accessible only when the region is transcribed? This scenario is not mutually exclusive with base-pairing to nascent RNAs, and one could imagine how the *EBER2* ribonucleoprotein would be handed from nascent RNA to DNA to bring PAX5 very close to its DNA binding site. Given the high density of nascent transcripts in many genomic regions, it is possible to imagine nascent RNAs as nets of binding sites that localize transactivators near their eventual site of action. The ideas provoked by this manuscript add one more chapter to the rapidly evolving RNA story.

It is now clear that RNAs participate in almost every facet of the biology of cells and viruses, and based on their function, RNAs have been categorized as protein-coding mRNAs or noncoding, which lack discernable open reading frames. Although this division is arbitrary and in many cases based on the absence of evidence, it has been widely used and serves as practical way to organize our rapidly changing understanding of RNA biology (Mercer et al., 2009). Excellent comprehensive reviews on noncoding RNAs (ncRNAs) and their many functions have been published (Mercer et al., 2009; Guttman and Rinn, 2012; Cech and Steitz, 2014).

Indeed, ncRNAs have many properties of adaptable regulators (Figure 1A): (1) RNAs, like DNAs, can "read" sequences by base-pairing and this ancient mode of nucleic acid-nucleic acid recognition provides very high specificity with minimal investment of genetic material. In contrast, proteins that "read" nucleic acid sequence generally do so by building complex binding domains (such as Puf proteins) (Wang et al., 2002). Additionally, RNAs have a proclivity to form structures that enhance base pairing and their 2′ OH provides opportunities for hydrogen bonding. (2) RNAs interact with proteins using sequence, chemical modification of bases and sugars, and their secondary or tertiary structure. (3) RNAs, like proteins, are modular and can use domains or different surfaces within one domain to interact with other molecules (Guttman and Rinn, 2012). Furthermore, discrete interaction domains can be connected to form flexible modular scaffolds (Figure 1A). The modular nature of RNAs and the versatility of each module for diverse interactions dramatically expand the repertoire of regulatory RNAs and explain their exquisite specificity.

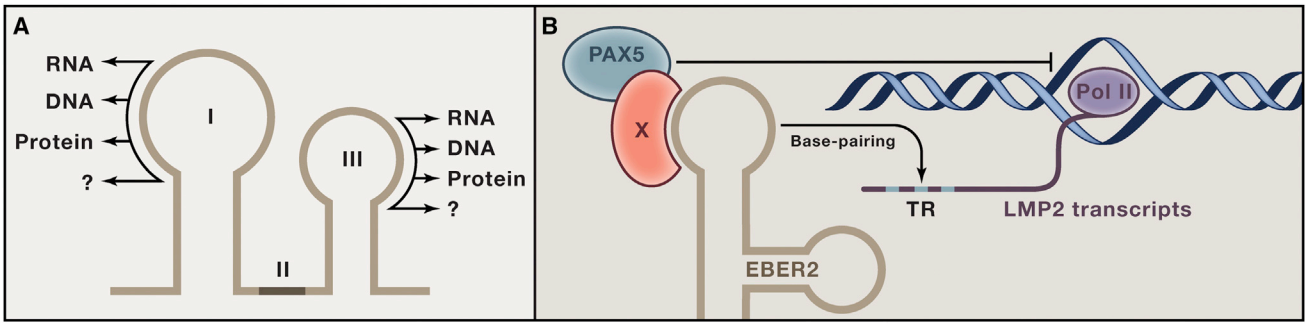

Figure 1. RNA Modularity and Interaction Versatility

(A) The schematic presents an RNA with two interaction modules (I and III) connected via a linker (II), which could be a hybrid linker in cases where I and III are in different molecules (e.g., CRISPRs). Each interaction module can interact with a diverse set of types of ligands. It is very likely that RNAs, like proteins, will be found to interact with every other type of macromolecule and small molecule present in cells (represented by the '"?) (as already predicted by riboswitches and by the ability to select for binding to very different ligands in vitro).

(B) The example discovered in Lee et al. (2015) is presented in which an RNA molecule (*EBER2)* bridges between a protein (PAX5) and a second RNA (*LMP2)*.

A modulary RNA code, whereby discrete interaction domains can be combined into flexible modular scaffolds (I-III in Figure 1A) (Guttman and Rinn, 2012; Mercer et al., 2009), makes RNA a highly malleable substrate for evolutionary tinkering. This has been particularly apparent where rapid evolution is required as in host-pathogen interactions, such as the EBER2-PAX5 interaction. In fact, ncRNAs, encoded by both host and pathogen, play important roles in the control of the innate and acquired immune systems by altering every step of gene expression (Cech and Steitz, 2014). An excellent example of the modular evolution of RNA domains involved in host-pathogen interactions is provided by flaviviruses, such as dengue viruses, which cleave >90% of the genomes in infected cells to form a ncRNA derived from the 3′ UTR. Elements in the 3′ half of the ncRNA are conserved to serve in regulating translation of these viruses but elements in the 5′ half, also known as the variable region, evolve rapidly to counter different components of host innate immunity (Bidet and Garcia-Blanco, 2014).

The resourcefulness of partner recognition by RNAs is exemplified by the EBER2: nascent TR:(X):PAX5 ribonucleoprotein (Figure 1B). EBER2 assembles with unknown factors (X) and hijacks PAX5. Additionally, EBER2 base-pairs with nascent RNAs to bring PAX5 in the vicinity of its DNA binding site—a new twist for *trans*-acting RNA. Small nuclear RNAs (snRNAs) are known to base-pair with nascent transcripts (pre-mRNAs) to mediate RNA splicing, as suggested by the Steitz group 35 years ago (Lerner et al., 1980), and HIV-1 Tat protein binds nascent TAR RNAs to recruit the cellular transcription factor P-TEFb to the lentiviral LTR (Wei et al., 1998). The modules described by Lee et al. are not new but the combination is—tinkering with any available part to build a new machine. We argue that the versatility of RNA makes it an exceptionally adept at sampling many forms and interactions that can assemble into a diverse array of machines, some of which will be selected. Whether or not RNA-based machines that recognize nascent transcripts are widely used is unclear. What is a foregone conclusion, however, is that there are many RNA-based surprises ahead.

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REFERENCES

Arvey, A., Tempera, I., Tsai, K., Chen, H.-S., Tikhmyanova, N., Klichinsky, M., Leslie, C., and Lieberman, P.M. (2012). Cell Host Microbe *12*, 233–245.

Bidet, K., and Garcia-Blanco, M.A. (2014). Biochem. J. *462*, 215–230.

Cech, T.R., and Steitz, J.A. (2014). Cell *157*, 77–94.

Guttman, M., and Rinn, J.L. (2012). Nature *482*, 339–346.

Jacob, F. (1977). Science *196*, 1161–1166.

Lee, N., Moss, W.N., Yario, T.A., and Steitz, J.A. (2015). Cell *160*, this issue, 607–618.

Lerner, M.R., Boyle, J.A., Mount, S.M., Wolin, S.L., and Steitz, J.A. (1980). Nature *283*, 220–224.

Mercer, T.R., Dinger, M.E., and Mattick, J.S. (2009). Nat. Rev. Genet. *10*, 155–159.

Wang, X., McLachlan, J., Zamore, P.D., and Hall, T.M.T. (2002). Cell *110*, 501–512.

Wei, P., Garber, M.E., Fang, S.M., Fischer, W.H., and Jones, K.A. (1998). Cell *92*, 451–462.