The innate immune system can distinguish between RNAs of viral and cellular origin, but the basis for this discrimination is not known. A new paper by Calderon and Conn demonstrates that conformational plasticity determines the ability of one RNA sequence to bind to and activate the pattern recognition receptor OAS1/RNase L. In identifying a novel mode through which the immune response is naturally controlled, this finding opens new avenues toward developing approaches for the management of a wide range of viral infections.

“Form follows function” (1) is a principle typically associated with modernist architecture and industrial design. It is also an apt maxim for biomolecules. At a basic level, shape determines affinity, whereas plasticity enables regulation. This scalable principle applies to all four molecular food groups: proteins, nucleic acids, carbohydrates, and lipids. Among the nucleic acids, the double-stranded nature of DNA places strong constraints on conformational complexity. In contrast, RNA’s ability to form novel base-pairing interactions confers a large degree of structural diversity (2). Such diversity lends itself to the ability to assume multiple metastable structures, i.e. structural plasticity. RNA structural plasticity underlies a diversity of molecular outcomes, including post-transcriptional modifications (3), riboswitches (4), translational recoding (5), and IRES elements (6). The notion that form follows function is central to our existence.

Viral infections often result in production of double-stranded RNAs (dsRNAs), which are recognized as pathogen-associated molecular patterns by cytosolic pattern recognition receptors. These include a subset of Toll-like receptors, as well as PKR, RIG-I, and the OAS enzyme families (7). OAS enzymes become activated upon contact with dsRNAs, causing conformational changes that result in the synthesis of a 2′–5′-linked oligoadenylate (2′–5′A). This activates RNase L, which initiates innate immunity by cleaving all cellular RNAs, thus inhibiting translation and mobilizing signaling cascades (8). On the surface, this process is quite straightforward. However, cells normally harbor many nonviral dsRNAs, e.g. tRNAs, lncRNAs, rRNAs, etc. How do cells distinguish between these “self” and “non-self” RNAs so as not to constitutively initiate the antiviral response? Understanding how this discrimination is achieved requires a better understanding of the general mechanisms by which these proteins recognize RNA.

The ubiquitously expressed human noncoding RNA 886 (nc886) was initially identified as an inhibitor of spurious activation of the dsRNA response by both PKR and OAS1. Subsequent work by the Conn laboratory showed that nc886 can adopt two stable conformational states, and that activation and inhibition of PKR is conformation-dependent (9). In the current paper (10), Calderon and Conn (a) show that only one of these conformers is able to activate OAS1, (b) map the activation region to the apical stem-loop region (Fig. 1), and (c) present a detailed biophysical characterization of the molecule. The general finding that RNA conformation controls innate immunity is quite novel and will have an impact on efforts toward controlling the acute pathologies associated with infection by many RNA viruses.

The authors take a methodical and logical approach in addressing the importance of nc886 conformation in OAS/RNase L activation. Exploiting the stability of the two RNA conformers enabled them to purify each to homogeneity by native PAGE. These were then used in an in vitro time-course assay that monitored OAS1/RNase L activation as measured by production of PPi, the byproduct of 2′–5′A synthesis. This revealed that only one conformer (conformer 1) strongly activated OAS/RNase L. A series of careful biochemical approaches were then used to determine kinetic (Ksyn, Vmax) parameters for RNA–OAS1 binding and OAS1 catalytic activity for each of the two conformers. These revealed that they have different affinities for OAS1, which in turn impact its activation and catalytic activity. The authors next extended from these in vitro experiments to a more definitive demonstration of biological activity, by transfecting cells with the purified nc886 conformers or with carefully chosen positive and negative control RNAs. Using rRNA cleavage by RNase L to monitor OAS activation (cells express three closely related OAS proteins), they recapitulated the in vitro results confirming that the active form was conformer 1.

Having established the active RNA species, the investigation then turned to the structural dissection of the molecule. nc886 is roughly L-shaped, comprising terminal stem and apical stem-loop domains (Fig. 1). A careful mutagenesis approach coupled with detailed in vitro biochemical assays revealed that the OAS1 activation requires the apical stem-loop of conformer 1, but not its terminal stem. Finally, the cell-based assay was employed to demonstrate that this apical stem-loop feature of
nc886 is required to activate the cellular OAS/RNase L pathway in vivo.

So what does this mean and where is the research heading? Although the reader is left to wonder about the structural differences that are clearly revealed by ITC, UV melting profiles, and SHAPE analyses, this merely serves to whet our appetite in anticipation of the high resolution structures to come. Additionally, although both conformers are highly stable, we do not know which conformer is most predominant in vivo. It is also clear that the energy barrier separating them is high. Thus, it is reasonable to posit that switching between the two conformers may be effected by a trans-acting “factor” and that it should be regulated. Viewed in the larger context, although the list of noncoding RNAs keeps growing, we have barely scratched the surface in our understanding of most of these at the structural level, let alone how their plasticity may be exploited to ensure regulation at the cellular and organismal levels. The importance of this paper is that it will serve as a template for future investigations of these questions.

On an editorial note, this manuscript was a joy to read. The background and significance are well contextualized, the experiments are well-designed and controlled, and they are presented in a logical, easy-to-follow order. Technically, the work is first class, and the writing is clear. Please consider using this as a journal club or classroom article as an example of what a well-written paper should look like.

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