

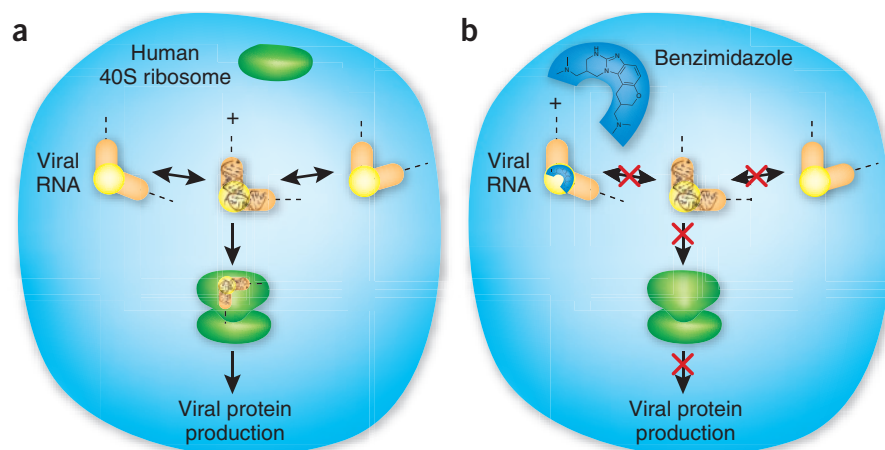
## Locking out viral replication

Darren W Begley & Gabriele Varani

**Few antimicrobial drugs function by directly targeting RNA. A small molecule that binds the hepatitis C viral genome by 'locking' in a particular RNA conformation to inhibit viral protein production suggests a new paradigm for drug design.**

Over 3% of the world population is infected with the hepatitis C virus (HCV)<sup>1</sup>. There is no vaccine for this, and the best treatment available (interferon-ribavirin combination) is effective in fewer than half of patients. Several drug candidates are currently in clinical trials that inhibit the virally encoded HCV protease, a classical target for intervention. However, this member of the *Flaviviridae* virus family is prone to frequent mutation, making the development of prophylactic and curative treatment options with sustained efficacy particularly challenging. Interestingly, some of the most highly conserved regions of the HCV genome are found in untranslated regions. The HCV virus encodes an internal ribosomal entry site (IRES) at the 5' end of its genomic RNA that is responsible for initiation of protein synthesis. A small molecule recently discovered at Ibis Therapeutics binds a critical structure within the HCV IRES and inhibits its function<sup>2</sup>, and a study in this issue reveals its mechanism of inhibition. The benzimidazole derivative locks the RNA conformation and prevents the IRES from assuming its functional structure during initiation of protein synthesis. Viral replication studies by Parsons *et al.* suggest that this molecule may represent a new class of antivirals that function by directly binding to viral RNA<sup>3</sup>.

IRESs are highly structured RNA elements present in the genome of many retroviruses (and a few cellular genes) that control the initiation of protein synthesis. The HCV IRES directly interacts with the host 40S ribosomal subunit and helps recruit eukaryotic translational initiation factors<sup>4</sup>. This mechanism bypasses the canonical protein synthesis pathway, which



**Figure 1** Subdomain IIa of the hepatitis C virus (HCV) IRES (orange-yellow) forms an L-shaped structure that adopts multiple conformations due to intrinsic flexibility within the internal loop 'elbow'. (a) The 40S human ribosome (green) recognizes the HCV IRES, with subdomain IIa inducing a conformational change required for initiation of viral protein synthesis. (b) Binding of a benzimidazole derivative (cyan) to the 'elbow' structure captures a single conformation of the IIa subdomain, which prevents mutual conformational adaptation with the assembling ribosome. Locking in this structure leads to decreased viral protein production, both *in vitro* and in infected cells. Crystal structure of subdomain IIa (black) adapted from ref. 7 (PDB ID: 2nok).

depends on the recognition of the 5' and 3' ends of mature cellular mRNAs. Although the HCV IRES adopts conserved secondary and tertiary structures, like many other RNAs it retains conformational flexibility to recognize and adapt to various cellular targets (Fig. 1). By monitoring the distance between fluorescent tags on a viral RNA construct, Parsons *et al.* show that the benzimidazole derivative discovered at Ibis captures a specific structure within the HCV IRES domain, locking it into a conformation that is unable to interact productively with the host translation machinery (Fig. 1).

Subdomain IIa of the HCV IRES adopts an L shape and induces significant conformational changes in the 40S ribosomal subunit when it binds<sup>5–7</sup>. The benzimidazole derivative analyzed by Parsons *et al.* binds to the 'elbow' of subdomain IIa, and in doing so it fixes the interhelical

angle at a conformation similar to that induced by a single A→U point mutation<sup>3</sup>. Both the benzimidazole derivative and the mutation reduce the production of viral proteins by approximately 80%, likely by preventing formation of the complete 80S eukaryotic ribosome. Hence this small molecule does not function by competitively inhibiting the interaction between the IRES and its target. Instead, it affects IRES-driven viral protein synthesis by altering RNA conformational properties at a step where mutual adaptation between the IRES and its ribosomal target is required for activation.

In the decade-long search for small-molecule drugs that target RNA, the focus has often been on disrupting the interactions between RNA and proteins. As with protein–protein complexes, abrogating such interactions has proven very difficult. Proteins have large surface areas and

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typically slow 'off' rates, making the target site on either protein or RNA inaccessible to small-molecule inhibitors. The task is even more challenging when targeting functional RNAs because of their intrinsic molecular flexibility. The present work illustrates a much more attractive strategy that exploits this very property of RNA. Nature has already provided us with examples of this principle in the aminoglycosides—antibiotics that function by stabilizing a conformation of bacterial ribosomal RNA prone to misincorporation. By capturing one structural state, a small molecule can affect RNA conformational equilibria without having to compete with proteins for binding energy or steric access.

The essential roles of viral RNA regulatory elements from HCV, HIV, influenza and other viruses make them attractive drug targets, but this feature remains completely unexploited.

These RNA sequences encode not just a defined functional structure but also the ability to direct conformational changes in response to ligand binding to form alternative structures that are equally essential. This property of RNA is found in structures as diverse as the HCV IRES, the HIV *trans*-activation response element, and bacterial riboswitches<sup>8–11</sup>. This combination of genetic rigidity (high sequence conservation) and structural flexibility (the need to change structure for function) could turn out to be the Achilles' heel of RNA viruses. Small-molecule inhibitors, such as the benzimidazole inhibitor described in this study, suggest a new strategy for the development of candidate RNA-binding antivirals. For this to happen, it will be necessary to substantially improve their potency and specificity while creating pharmacological characteristics conducive to successful preclinical

and clinical development. If this can be done, perhaps anti-infective drug discovery will have found a new gold mine.

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## The physical chemistry of membrane curvature

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**Membrane curvature sensing by amphipathic helices is an emergent property of the ensemble of molecules and membrane sites. New data suggest that individual molecules do not experience stronger binding to curved membranes.**

Controlling the spatial arrangement of their constituent molecules is a defining characteristic of living organisms. Among the most fundamental of life's organizational motifs is the lipid bilayer membrane, and regulation of membrane curvature is central to innumerable biochemical processes<sup>1,2</sup>. In this issue, Hatzakis *et al.*<sup>3</sup> reveal new insights into the chemical mechanisms of membrane curvature recognition. The authors make highly refined observations of membrane binding as a function of membrane curvature, which avoid the intrinsic ambiguities of ensemble average measurements through the use of recently developed single-vesicle assays. The results run counter to traditional hypotheses and reveal a richer underlying chemistry of membrane curvature recognition.

Amphipathic  $\alpha$ -helices (AHs) are a class of protein structural motifs commonly associated with membrane curvature recognition. AHs fold upon contact with membranes and insert their hydrophobic face into the lipid bilayer<sup>4,5</sup>.

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This process is facilitated by curvature-induced defects in lipid packing, resulting in an overall higher affinity of AHs for positively curved membranes<sup>5,6</sup>. Working with the well-characterized AH-forming peptide sequence from endophilin A1 (eAH), the authors obtain data illustrating that there is more to the story of membrane curvature sensing than just molecular affinity.

By performing what the authors refer to as a single liposome curvature (SLiC) assay, they were able to obtain binding curves and thus measure the free energy ( $\Delta G$ ) for eAH binding to vesicles of known curvature. These measurements were made on a vesicle-by-vesicle basis whereby binding and vesicle size are both independently measured by fluorescence. This technical advance sidesteps the fundamental problem that even the best vesicle preparations yield highly polydisperse distributions of vesicle sizes. The results reveal a surprisingly modest gain in  $\Delta G$  binding with increased curvature of only  $\sim 1k_B T$ , which is comparable to thermal fluctuations and could not possibly amount to much curvature recognition. The amount of binding at saturation, however, proved to be highly dependent on curvature.

To fully understand this discovery, we must first remind ourselves of some basic statistical thermodynamics. When we say 'affinity', we are

typically referring to  $\Delta G$ , the Gibbs free energy. This includes an enthalpic component,  $\Delta H$ , that refers to actual energetic differences between the two states under consideration (for example, bound and free for the case of membrane curvature recognition). Free energy also includes an entropic component,  $-T\Delta S$ , where  $T$  is the temperature and  $\Delta S$  refers to the entropy difference between the two states. Here is the rub: what is  $\Delta S$  between the bound and unbound states? Conventionally, this is proportional to the log ratio of the number of microscopic configurations ( $W$ ) that the protein and membrane may adopt in the bound state over the number they have in the free state ( $\Delta S = k_B \ln(W_{\text{bound}}/W_{\text{free}})$ ), where  $k_B$  is the Boltzmann constant. Each state is an ensemble of microscopic configurations, and the fact that there may be more configurations in one state than another has direct impact on free energy and on the observed equilibrium of a chemical reaction.

All data agree that more eAHs bind to positively curved membranes, so what does the observation of no difference in  $\Delta G$  mean? When membranes bend, defects in lipid packing form in response to mechanical stresses in the bilayer itself. These defects provide binding sites for eAHs. The data suggest that defect structure, as measured by  $\Delta G$  of eAH binding, is relatively similar for membranes curved to