Viral RNA gymnastics

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Retroviruses have a stretch of RNA that dimerizes during viral particle formation. A new study suggests that RNA flexibility in the monomeric form may facilitate dimerization or other RNA-dependent viral functions.

Retroviruses encode their genomes as small (7–12 kb) single-stranded RNA molecules that are packaged as dimers into virions, which are the extracellular form of the virus. It is believed that a primary role of RNA dimerization is to facilitate recombination for the repair of defective genomes and to increase genetic variation[1,2]. To maintain an economical genome, retroviruses often use overlapping open reading frames or locate multiple RNA regulatory elements within a small region of the RNA. Indeed, the region responsible for dimerization in many retroviruses is packed with functional elements that regulate processes such as reverse transcription, genome packaging, translation, and RNA splicing and export that occur at very different times in the viral life cycle[1]. How does a retrovirus manage to coordinate these diverse functions in one small piece of RNA? In this issue, Badorrek and Weeks address the question by characterizing the secondary structure of the RNA dimerization region of the Moloney murine sarcoma virus (MuSV) using a new chemical probing method that monitors the conformation of each nucleotide[3]. The study suggests that conformational rearrangements needed to generate multiple overlapping regulatory elements may be mediated by the inherent flexibility of part of the RNA, which perhaps acts as an 'RNA control center'.

The phenomenon of RNA dimerization is highly conserved among retroviridae, but the mechanisms of dimerization are diverse, probably reflecting differences in the regulatory sequences contained in each region. Many of the mechanistic insights have come from *in vitro* studies beginning in the early 1990s with dimerization elements from human immunodeficiency virus-1 (HIV-1), respiratory syncytial virus (RSV) and murine viruses[1,2,4]; these are supported by *in vivo* analyses of viral mutants, particularly those addressing the coupling between dimerization and RNA packaging or reverse transcription[4]. In general, the dimerization domains are located in the 5′ untranslated region of the unspliced genomic mRNA and contain several complementary sequences that allow base pairing between two parallel RNA strands. In most cases, it is believed that dimer formation initiates at a site where RNA hairpins containing palindromic sequences in their loops can form 'kissing-loop' interactions, in which two loops become base paired (**Fig. 1**). The kissing-loop complex is then converted into a more stable extended

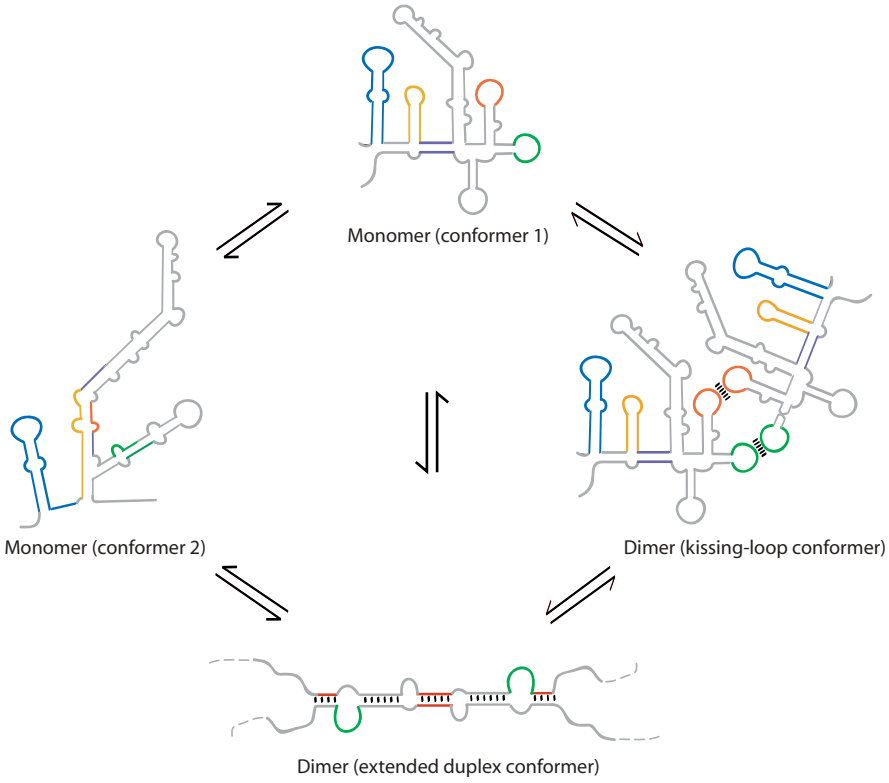

Figure 1 A general view of structural rearrangements of retroviral dimerization elements. For simplicity, only two conformers each of the monomeric and dimeric states are shown, with possible conformational transitions indicated by arrows. Many more conformational states are likely, each supporting a different set of viral functions depending on the conformation of individual RNA control elements.

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duplex, in a process probably facilitated by the viral nucleocapsid protein and surrounding RNA sequences[1].

The three-dimensional structures of some elements of a dimerization domain, including kissing hairpins and extended duplexes, have been solved, in one recent case bound to nucleocapsid proteins[5]. Although these snapshots have revealed important RNA-RNA interactions within isolated segments of the dimer linkage sequence, they have not generally captured how conformational rearrangements between elements in the larger RNA context are coordinated[6]; indeed, even the requirement for the individual RNA elements in dimerization is complex and not completely resolved for any virus (see ref. 7, for example).

The article by Badorrek and Weeks investigates the structure of the monomer in solution. The authors first defined the boundaries of the minimal MuSV dimerization region using a competition assay that assesses the importance of each element, at least *in vitro*, in a relatively quantitative fashion. They then probed this dimerization region using chemical modification of 2′-OH groups to assess whether each nucleotide sugar is in a flexible conformation, typical of an RNA loop or bulge, or is more rigid, as in a duplex[3]. They found one particular element, important for dimerization and previously modeled as a stable hairpin, that shows unexpected flexibility and no stable secondary structure, suggesting that it may be largely unstructured or exist in multiple states.

How might this flexibility be functionally important for dimerization? The authors propose that the inherent flexibility of the element allows this portion of the dimerization region to sample appropriate conformers during different functional states, without the energetic or kinetic costs that would be associated with RNA unfolding during conformational remodeling. This flexibility could be seen facilitating either formation of kissing-loop interactions or allowing dimer formation through other, as yet un-defined, pathways (**Fig. 1**). This more dynamic view naturally couples dimerization to the regulation of other important viral functions through structurally coupled RNA elements, and starts to answer the question of how a small stretch of RNA may access the diverse conformations necessary for participating in diverse functions.

This sort of RNA flexibility is observed in many biological settings where complicated assemblies must be formed, as in the ribosome, or where dynamic rearrangements are needed, as in the spliceosome[8]. Protein interactions or changes in the folding environment that, for example, alter the electrostatic conditions can markedly alter RNA conformational behavior and are essential for the proper functioning of such ribonucleoprotein machines. This undoubtedly will be true for retroviral dimerization *in vivo* as well. Much remains to be learned about the (probably many) functional conformers of retroviral dimerization elements (**Fig. 1**), including where and when they exist within retroviral life cycles, how they differ among viruses, and how longer-range RNA interactions and viral or cellular proteins influence their conformational states. Nonetheless, this article and many previous studies clearly indicate that the conformational gymnastics of retroviral dimerization regions are central to controlling a variety of essential RNA-based viral functions.

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Probing cell death by chemical screening

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Apoptosis occurs through precise cellular pathways, whereas necrosis is generally thought of as a nonspecific cellular response to external damage. However, identification of a chemical inhibitor of necrotic events suggests that specific molecular pathways can also trigger necrosis.

Histological and molecular studies have led to the current distinction between two basic forms of cell death: 'apoptosis', characterized by maintenance of cell-membrane integrity and ATP production in the dying cell, and 'necrosis', in which there is early swelling of organelles and loss of plasma-membrane integrity, associated with cessation of ATP generation. Apoptosis is mediated by a set of specific molecular pathways. In contrast, necrosis is widely believed to occur independently of predestined molecular mechanisms[1]. In this issue, Degterev *et al.* describe a small organic molecule that specifically blocks a set of cellular necrotic processes, suggesting involvement of a distinct molecular pathway in this death form as well[2] (**Fig. 1**). A potential therapeutic application of the finding is demonstrated in mice by an effective decrease in ischemic brain injury after treatment with the inhibitor.

The study addresses the induction of necrosis by death receptors (DR) of the TNF/NGF family[3]. These receptors also induce apoptotic death, which—as in other apoptotic processes—involves activation of a cascade of cysteine proteases, the caspases. In apoptosis, the way this cascade leads to death, and the mechanism of cascade initiation through direct interaction of the receptor complex with caspase-8 and caspase-10, have been studied in detail[4,5]. In contrast, almost nothing is known about the mechanism of DR-induced necrotic death. It does not involve caspase activation; in fact, interference with DR-induced apoptosis by blocking caspase action greatly facilitates necrosis[6].

Why does blocking DR-induced apoptosis enhance necrosis? In line with the prevalent view of necrosis as a 'nonprogrammed' death that occurs upon failure of vital functions, DR-induced necrosis can be viewed as 'frustrated apoptosis', meaning disintegration of the cell when its orderly steps toward death are arrested en route. Alternatively, induction of necrosis by DR might reflect activation of a distinct 'fail-safe' pathway to death. Degterev *et al.* investigated this question by screening for a chemical inhibitor of DR-induced necrosis. The apparent specificity of the necrosis inhibitor identified—dubbed 'necrostatin-1' (Nec-1)

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