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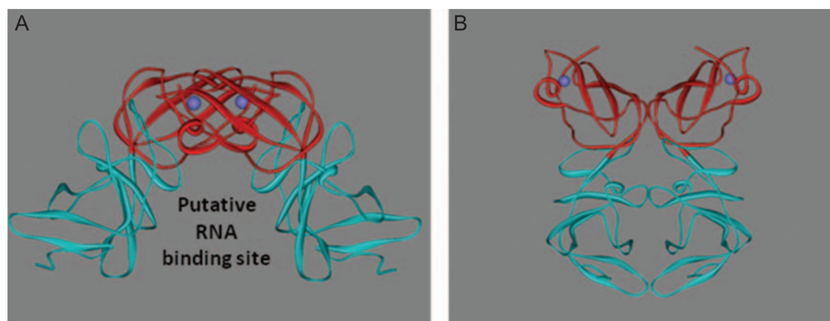

Figure 1.3 Rice dimer (A) and Love dimer (B). The first 100 amino acids (putative inhibitors binding site) are colored red. The Zn^{2+} ions are shown as purple balls.

conserved, their modes of dimerization were different and involved non-overlapping contact surfaces. A positively charged groove created by the dimeric interface of the X-ray structure reported by Rice's group, which had the appropriate dimensions to support the hypothesis that it could be an RNA-binding site, is fully exposed in Love's X-ray structure. The reason for the differing modes of dimerization and how well either one may reflect a biochemically relevant structure of the NS5A protein, especially since about half of the protein is missing from the structural analyses, is not apparent at this stage. Some have postulated that the two dimeric modes may represent snapshots of an oligomeric state, the functional significance of which has yet to be revealed. In general agreement with the X-ray structural findings, a glutathione-*S*-transferase (GST)-tagged NS5A Domain I was able to pull down a His-tagged NS5A protein, presumably through a dimeric interaction, whereas this was not possible with GST alone. This interaction does not appear to be mediated by the presence of nucleic acids and yet, interestingly, there is a similarity between the minimal NS5A fragment required to effect this pull-down and the minimal fragment required to maintain the RNA-binding affinity of the full-length NS5A protein. It is also noteworthy that the minimal peptide fragment required to effect the dimerization in the pull-down study was longer than the peptide constructs used in the X-ray studies (amino acids 1–240 *versus* 25–215 for the Rice dimer and 33–202 for the Love dimer), and although the reason for this disparity is not apparent, it could be a result of the distinct physical states that the two studies are dealing with and of differences in experimental parameters, such as protein concentration.[11]

In another study, a glutaraldehyde cross-linking experiment demonstrated that either Domain I or the full-length version of NS5A (but not Domain II–III) dimerize in solution, that the dimer is in equilibrium with the monomer and that the presence of uracil-rich RNA, which is known to bind to NS5A, shifts the equilibrium in favor of the dimer.[5] Interestingly, in the same study, NS5A–RNA cross-linking followed by the mapping of the amino acids involved in the cross-linking on to either of the two X-ray structures indicated