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Synthetic RNA targets for HIV-1 *tat* and *rev* proteins.

François Hamy, Ulysse Asseline, Shigenori Iwai, Derek A. Mann, Clare Pritchard, Michael J. Gait, and Jonathan Karn, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK.

In the search for novel drugs for the treatment of AIDS, much attention is now being given to specific HIV targets other than the viral enzymes such as reverse transcriptase and proteinase. One of these targets is the gene regulatory protein *tat* which is essential to viral replication and binds to a specific HIV RNA sequence. The *trans*-activator protein *tat* binds with high affinity *in vitro* to the *trans*-activation response region RNA (TAR). Specific recognition of TAR involves the two base pairs G_{26}:C_{39} and A_{27}:A_{38} flanking the U-rich bulge as well as U_{23} in the bulge itself. When mutations are introduced at any of these bases, the loss of tat binding is energetically equivalent to the loss of at least one hydrogen bond. In order to determine more precisely which functional groups may be involved in hydrogen bonding and whether such bonding may occur in the major or minor groove, we have constructed a synthetic RNA model into which modified nucleoside residues can be readily incorporated by chemical synthesis. *Tat* binds to this RNA model with high affinity. Replacement of individual ribonucleosides by 2'-deoxyribonucleosides at any of positions 21 to 28 had no significant effect on *tat* binding suggesting that 2'-hydroxyl groups are not involved in hydrogen bonding interactions at these sites. There was no loss of binding affinity when G_{21}, G_{26} or G28 were replaced by I, dI or by N^7-deazadG. However, replacement of G_{26} by N^7-deazadG resulted in a dramatic loss of tat binding. Similarly when A_{22} or A_{27} was replaced by N^6-methyldA or by N^7-deazadA, only N^7-deazadA_{27} gave rise to significant loss of *tat* binding. The results suggest that *tat* binding requires hydrogen bonding to the N^7 positions of G_{26} and A_{27} in the major groove. The effect of methylphosphonate substitution of phosphodiesters in the U-rich bulge was also determined. The results showed that methylphosphonate substitution between A_{22} and U_{23} caused substantial loss of *tat* binding ability whereas no effect was seen for methylphosphonate substitution of any of the other 3 phosphodiesters in the U-rich bulge. An electrostatic contact between *tat* and TAR involving $A_{22}pU_{23}$ is therefore likely. This is in agreement with chemical protection data of TAR by *tat* and related peptide. As a prelude to any possible use of such duplex RNAs as potential anti-HIV agents, we have found that substitution of all 4 of these phosphates simultaneously by phosphorothioates (which would be expected to stabilize the bulge to nuclease attack) had no effect on tat binding ability.