An RNA-Binding Peptide from Bovine Immunodeficiency Virus Tat Protein Recognizes an Unusual RNA Structure†

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ABSTRACT: The human immunodeficiency virus (HIV) Tat protein binds specifically to an RNA hairpin, TAR, located at the 5′ end of its mRNA. Tat uses a single arginine residue within a short region of basic amino acids to recognize a bulge region in TAR. Here we show that a 17 amino acid arginine-rich peptide from the bovine immunodeficiency virus (BIV) Tat protein also binds to an RNA hairpin at the 5′ end of its mRNA (BIV TAR), but recognizes different structural features of the RNA. Mutagenesis, RNase mapping, and chemical interference experiments indicate that bulge and stem regions of BIV TAR are recognized simultaneously by the BIV peptide and that the RNA adopts an unusual structure. BIV Tat binds to its TAR site with high affinity and specificity and, unlike HIV Tat, does not appear to use cellular proteins to stabilize RNA binding in vivo. Thus, two related viral activators have evolved rather distinct ways to recognize their RNA targets.

The folding of an RNA molecule determines the precise arrangement of its functional groups and thus plays an important role in sequence-specific RNA–protein recognition. The cocrystal structures of two tRNA synthetase–tRNA complexes have shown not only that the RNAs share structural complementarity with their cognate proteins but that they change conformation upon binding, repositioning groups on the bases and backbone for specific interactions (Rould et al., 1989, 1991; Ruff et al., 1991; Cavarelli et al., 1993). Similarly, RNA-binding studies using model peptides from the human immunodeficiency virus (HIV) Tat and Rev proteins have shown that tertiary features of the RNA and conformational changes are important for specific recognition (Frankel, 1992; Tan et al., 1993).

Tat and Rev are members of a class of RNA-binding proteins, including bacterial antiterminators and ribosomal proteins, that contain an arginine-rich motif approximately 10–20 amino acids in length (Lazinski et al., 1989). Tat binds to TAR RNA (the trans-acting response element), and Rev binds to RRE RNA (the Rev response element). In each case, a short peptide containing just the arginine-rich domain (9 amino acids from Tat and 17 amino acids from Rev) binds to its RNA site with specificity similar to that of the intact protein (Weeks et al., 1990; Calnan et al., 1991a; Kjems et al., 1992). In Tat, a single arginine is responsible for specific recognition of a bulge region in TAR (Calnan et al., 1991b; Puglisi et al., 1992; Tao & Frankel, 1992), with surrounding basic amino acids needed to raise the RNA-binding affinity and enhance the arginine-binding specificity (Tao & Frankel, 1993). The arginine-rich domain of Tat appears to be unstructured, even in the context of the intact protein (Calnan et al., 1991a,b). In contrast, the arginine-rich domain of Rev binds specifically to the RRE only when in an α-helical conformation and appears to use six amino acids (four arginines, one threonine, and one asparagine) for specific recognition (Tan et al., 1993). Thus, while it may be convenient to classify Tat and Rev together as members of the arginine-rich class of RNA-binding proteins, it seems clear that the structural motifs used for recognition are quite distinct.

Because Tat and Rev recognize their RNA-binding sites in such different ways, studies of other arginine-rich domains are likely to reveal different features of RNA–protein recognition and to provide interesting comparisons. In this study, we examine the RNA-binding properties of a peptide from the bovine immunodeficiency virus (BIV) Tat protein. BIV is a recently characterized lentivirus, related to the human and simian viruses (HIV and SIV), that causes persistent lymphocytosis, lymphadenopathy, and central nervous system lesions in infected cows (Gonda, 1992). The BIV genome contains the essential retroviral structural genes, *gag*, *pol*, and *env*, flanked by the LTR on the 5′ and 3′ termini in addition to at least five accessory genes analogous to *tat*, *rev*, *vif*, *vpr*, and *vpu* of HIV (Gonda, 1992). BIV Tat is encoded by two exons and shares sequence similarity to the HIV and SIV proteins (Garvey et al., 1990). A Tat-like factor from BIV-infected cells has been shown to activate the viral LTR (Pallansch et al., 1992), and BIV Tat cDNA clones have been shown to activate both the BIV and HIV promoters (Liu et al., 1992). Here we show that, as with HIV Tat, the arginine-rich region of BIV Tat recognizes an RNA target (BIV TAR) located at the 5′ end of the viral mRNAs. However, both in vitro and in vivo results suggest that while certain recognition features are shared by the BIV and HIV Tat proteins, the TAR RNA structures formed by the two viruses are rather distinct and their dependence on host RNA-binding factors appears to be different. The results emphasize that a simple "recognition code" is unlikely to exist for RNA–protein recognition and that, as for DNA–protein recognition, structural comparisons of related and unrelated motifs (both protein and RNA) will be needed to more completely define the interactions that contribute to sequence-specific binding.

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