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Preformed Protein-binding Motifs in 7SK snRNA: Structural and Thermodynamic Comparisons with Retroviral TAR

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The 7SK small nuclear RNA is a highly conserved non-coding RNA that regulates transcriptional elongation. 7SK utilizes the HEXIM proteins to sequester the transcription factor P-TEFb by a mechanism similar to that used by retroviral TAR RNA to engage Tat and P-TEFb. Tat has also recently been shown to bind 7SK directly and recruit P-TEFb to TAR. We report here the solution structures of the free and arginine-bound forms of stem loop 4 of 7SK (7SK-SL4). Comparison of the 7SK-SL4 and TAR structures demonstrates the presence of a common arginine sandwich motif. However, arginine binding to 7SK-SL4 is mechanistically distinct and occurs via docking into a pre-organized pocket resulting in a 1000-fold increased affinity. Furthermore, whereas formation of the binding pocket in TAR requires a critical base-triple, hydrogen-bond formation between the equivalent bases in 7SK-SL4 is not essential and the pocket is stabilized solely by a pseudo base-triple platform. In addition, this theme of preformed protein binding motifs also extends into the pentaloop. The configuration of the loop suggests that 7SK-SL4 is poised to make ternary contacts with P-TEFb and HEXIM or Tat. These key differences between 7SK-SL4 and TAR present an opportunity to understand RNA structural adaptation and have implications for understanding differential interactions with Tat.

Introduction

RNA polymerase II is highly regulated at all phases of transcription. The elongation phase, in particular, has recently received renewed attention resulting in the identification and functional characterization of numerous regulatory factors.[1,2] One of the key negative regulators of this process is the non-coding 7SK small nuclear RNA (snRNA) that inhibits the cyclin-dependent kinase P-TEFb (positive transcription elongation factor b).[3–8] 7SK sequesters P-TEFb by forming a ternary complex with the cyclin T1 subunit of P-TEFb and the HEXIM proteins.[9] The significance of this process is further highlighted by P-TEFb interactions with retroviral TAR (transactivation response region) RNA and Tat protein, which have evolved strategies to mimic the 7SK RNA and HEXIM protein, respectively.[10,11] In contrast to 7SK, however, TAR promotes retroviral transcription by antagonistic positive transactivation of P-TEFb and subsequent stabilization of the elongating polymerase II complex. Recent work has elucidated the interactions of Tat with various cellular host factors, one of which is a direct interaction between 7SK and Tat.[12–13] More

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Abbreviations used: HMQC, heteronuclear multiple-quantum coherence; HSQC, heteronuclear single-quantum coherence; NOE, nuclear Overhauser effect; NOESY, NOE spectroscopy; RDC, residual dipolar coupling; snRNP, small nuclear ribonucleoprotein complex; TAR, transactivation response region.

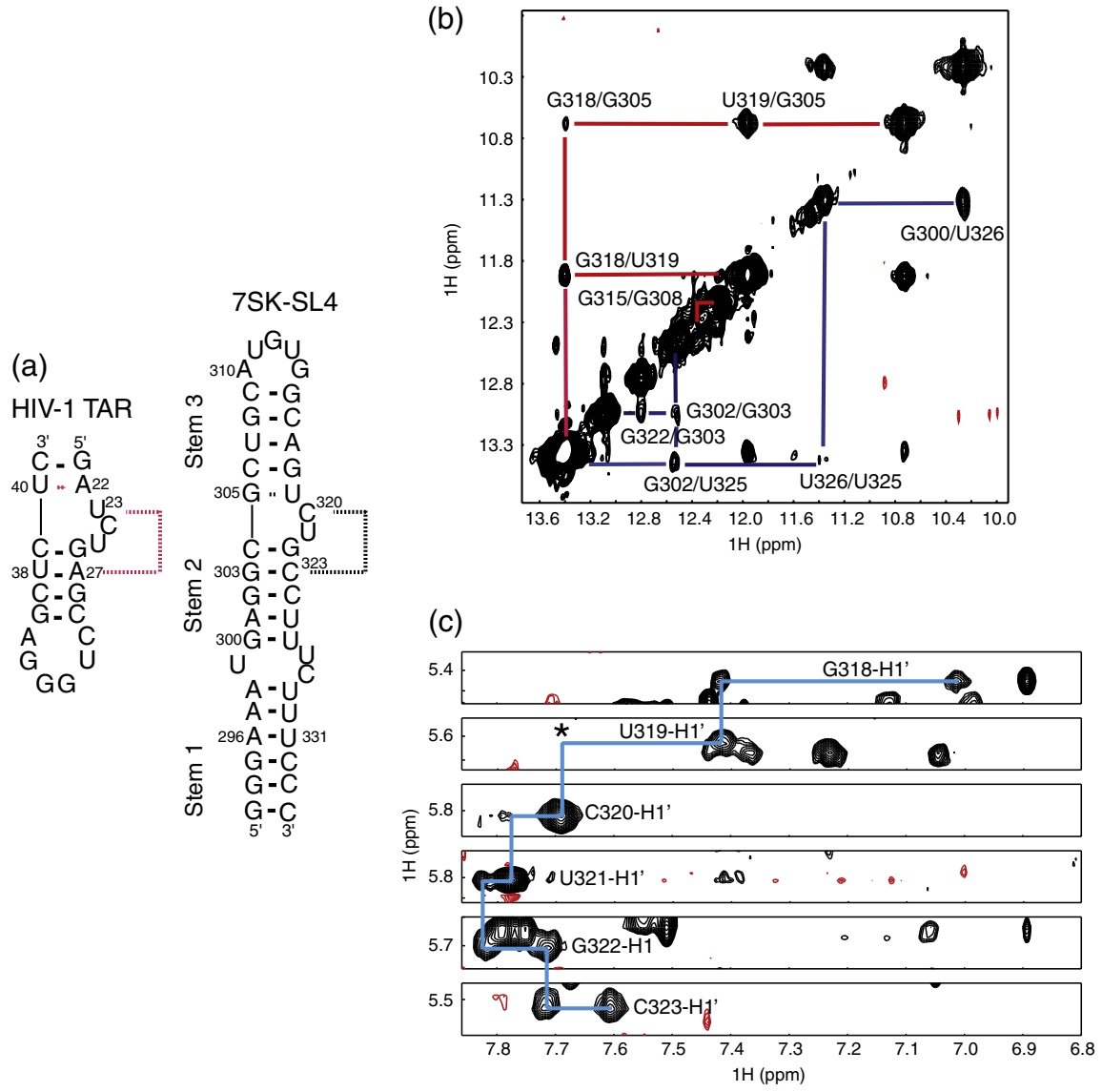

Fig. 1. Secondary structure and assignments of 7SK-SL4. (a) Secondary structure of HIV-1 TAR and 7SK-SL4. (b) Imino region of a 2D NOESY spectrum of 7SK-SL4 recorded at 278 K. The sequential assignment pathways are indicated in red (stem 2) and blue (stem3). Most importantly, the terminal U319 and G305 residues are base paired. (c) Excerpt of ^{13}C-^{1}H NOESY-HMQC sequential assignment data for 7SK-SL4 showing connectivities for the H1′ and H6/H8 protons in the G318-C323 segment. The asterisk denotes a break in the canonical connectivity pattern between bulge residues U319 and C320.

significantly, Tat has been shown to bind 7SK RNA by expelling HEXIM from the ternary complex, prompting a re-evaluation of our understanding of P-TEFb equilibrium between host and viral transcription. [13,14]

The unique mechanism by which TAR interacts with cyclin T1 and Tat has been outlined. Specifically, two distinct regions of TAR promote formation of the ternary complex: the flexible apical loop binds both cyclin T1 and Tat while the flexible bulge region recognizes Tat.[15,16] Collectively these interactions proceed via a cooperative induced-fit mechanism, the structural basis of which has yet to be determined. Tat contains an arginine-rich motif (ARM) in which a single arginine residue has been shown by biochemical and *in vivo* transactivation assays to bind to the TAR bulge region.[17,18] Several NMR studies have elucidated the major conformational transition that argininamide (a tight-binding analog of arginine) induces in the TAR structure upon complex formation.[19-23] In the free form the bulged residues and the terminal A22 and U40 residues are not involved in hydrogen bonding interactions but upon