motifs. Alternatively, because FAN1 preferentially binds monoubiquitinated FANCD2 (ref. 13), the monoubiquitinated FANCI subunit of the FANCD2–FANCI complex may be critical for chromatin binding. Hence, the identification of other ubiquitin-binding partners for FANCD2 and FANCI will be important. Second, how the FAN1 and SNM1A nucleases are released from monoubiquitinated FANCD2 and monoubiquitinated PCNA is unknown, but the release may be accomplished by the USP1–UAF1 deubiquitinating complex[28,29]. Third, it remains unclear how FANCD2–FANCI regulates TLS machineries and whether nuclease events come before or after the TLS events[9]. Finally, it will be important to determine whether FAN1 is itself an FA gene (that is, a gene with biallelic germline mutations in FA patients) or it is somatically mutated or silenced in human cancers with ICL hypersensitivity.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Kick-sTARting HIV-1 transcription elongation by 7SK snRNP deporTATion

Matjaz Barboric & Tina Lenasi

The HIV-1 Tat protein promotes viral transcription elongation by recruiting P-TEFb to RNA element TAR on the viral mRNA. Recent work from D'Orso and Frankel uncovers unexpected aspects of this process.

Transcription of the human immunodeficiency virus 1 (HIV-1) genome is controlled primarily at the level of RNA polymerase II (Pol II) elongation. Recent work by D'Orso and Frankel[1] provides a new perspective on how the transactivation response element (TAR) on the nascent viral transcript and the HIV-1 transactivator of transcription (Tat) cooperate to derepress their cellular cofactor on paused Pol II and promote the transition from promoter-proximal pausing into transcription elongation.

To propagate proficiently in its host, HIV-1 has evolved multiple diplomatic ties with its challenging environment. A testament to this dynamic relationship is a complex HIV-1 genome that, besides the structural genes (*gag*, *pol* and *env*), contains four accessory (*vif*, *vpr*, *vpu* and *nef*) and two regulatory (*tat* and *rev*) genes. In effect, the nonstructural proteins interact with diverse machineries of the host cell, enabling the virus to progress through its life cycle. One such exemplary interaction is between the HIV-1 Tat protein, its cognate TAR-hairpin structure present at the 5′ ends of viral transcripts and the host positive transcription elongation factor b (P-TEFb) kinase, composed of the catalytic subunit Cdk9 and the regulatory subunit Cyclin T1. A classic study by Wei *et al.*[2] revealed that the formation of this tripartite protein:RNA complex is key for viral transcription and has led to the the widely accepted model whereby Tat recruits P-TEFb to a promoter-proximal paused Pol II through TAR to relieve a blockade to efficient Pol II elongation[3]. However, other studies have found P-TEFb or Tat in HIV-1 transcription complexes before the synthesis of TAR[4–6], and it has been unclear why the HIV-1 promoter remained refractory to transcription elongation. Now D'Orso and Frankel[1] report the unexpected findings that Tat assembles with the inhibitory 7SK small nuclear ribonucleoprotein (snRNP) harboring the catalytically inactive form of P-TEFb and that this hybrid complex occupies the HIV-1 promoter with the transcription preinitiation complexes (PICs). Once Pol II clears the promoter, however, TAR captures Tat and P-TEFb from 7SK snRNP, resulting in the ejection of the inhibitory 7SK snRNP components and productive transcription elongation. These observations redefine our view of Tat transactivation and provide a new mechanism for how a noncoding RNA can regulate a switch between Pol II pausing and elongation.

HIV-1 Tat has been the vanguard of Pol II transcription elongation control for more than two decades[3]. Nevertheless, its mechanism of action has ceased to be recognized as a viral peculiarity only recently, when global analyses showed that most genes contain disproportionally high levels of Pol II at the promoter-proximal regions and that thousands of these genes experience transcription initiation but not elongation[7]. The latter phenomena constitute the hallmarks of every textbook model describing HIV-1 Tat transactivation[8]. In the absence of Tat, PIC containing general transcription factors (GTFs) that include transcription factor IIA (TFIIA), TFIIB, TFIIE, TFIIH, TFIIF, Mediator and nonphosphorylated Pol II assembles on the HIV-1 promoter due to the core promoter recognition complex TFIID, which comprises TATA-binding protein (TBP) and 10–14 evolutionary conserved TBP-associated factors. Upon PIC assembly, the Cdk7 of TFIIH phosphorylates the C-terminal domain (CTD) of Pol II's largest subunit, Rpb1, on Ser5 and Ser7, coinciding with transcription initiation

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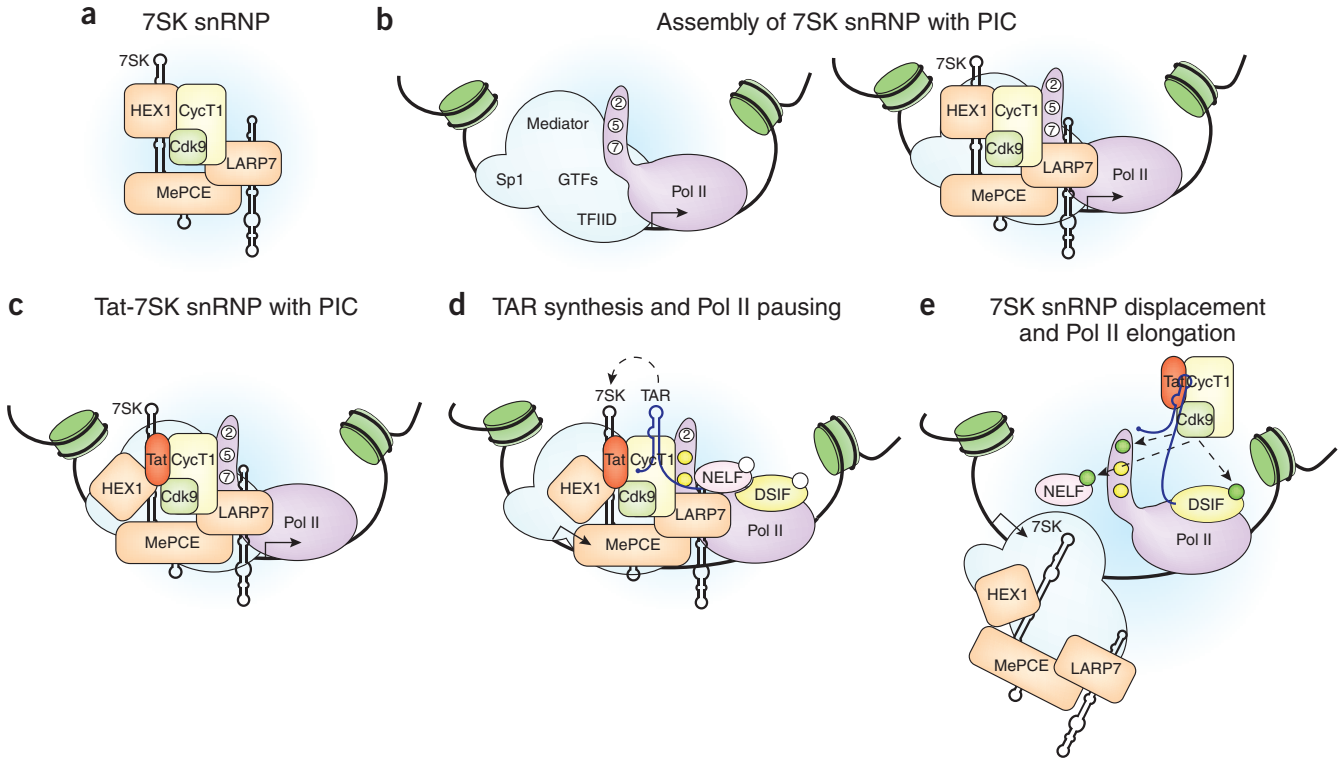

Figure 1 Revised model of HIV-1 Tat transactivation. (**a**) Schematic of 7SK snRNP. For simplicity, monomeric HEXIM1 is depicted. 7SK (black) is presented as a structure containing multiple hairpins. P-TEFb is presented as a heterodimer between CycT1 and Cdk9, because Tat does not form a tripartite Tat:P-TEFb:TAR complex with other regulatory subunits of P-TEFb. (**b**) In the absence of Tat, 7SK snRNP interacts with PIC on the HIV-1 core promoter. Although Sp1 and TATA elements are critical for this assembly, the mechanism of 7SK snRNP recruitment remains unknown. The kinase activity of P-TEFb is repressed within 7SK snRNP, and the Ser2, Ser5 and Ser7 residues of the multiple Pol II CTD tandem heptapeptides with the consensus sequence YSPTSPS are in a nonphosphorylated form (white circles). (**c**) As Tat is synthesized upon the first rounds of inefficient HIV-1 transcription, it assembles into the PIC-bound 7SK snRNP on the HIV-1 promoter. Alternatively, Tat might assemble with 7SK snRNP outside of nuclear speckles, where 7SK snRNP formation takes place[36], and joins the PIC as part of 7SK snRNP. Within 7SK snRNP, Tat may replace HEXIM1 from the 7SK 5′-hairpin structure and P-TEFb via its ARM and activation domain, respectively, and thus remove it from its preferred position. (**d**) Upon Cdk7-mediated phosphorylation of the Pol II CTD on Ser5 and Ser7 (yellow circles), Pol II starts transcription and clears the promoter, but DSIF and NELF cooperatively arrest transcription soon afterward. Coinciding with the Pol II promoter-proximal pausing, the nascent viral transcript containing TAR is synthesized (blue hairpin). (**e**) TAR may competitively dislodge 7SK from the Tat:P-TEFb complex to form a transcriptionally active Tat:P-TEFb:TAR complex. As a result, P-TEFb phosphorylates the Pol II CTD on Ser2, DSIF and NELF (green circles), resulting in the transition from Pol II pausing to elongation. This pivotal event coincides with the displacement of 7SK snRNP from DNA due to an unknown mechanism. Whereas LARP7 and MePCE remain with 7SK in core 7SK snRNP, HEXIM1 may reassociate with 7SK snRNP or could be replaced by hnRNP proteins[37,38] (not shown).

and promoter clearance[9], whereas many of the GTFs and Mediator remain at the promoter for rapid Pol II reinitiation[10]. Critically, DRB sensitivity–inducing factor (DSIF/Spt4-Spt5) and a multisubunit negative elongation factor (NELF) arrest Pol II soon after promoter clearance, precluding transcription elongation[11]. To antagonize this block, Tat recruits P-TEFb to a paused Pol II by interacting cooperatively with TAR on the emerging HIV-1 transcript, leading to the stimulation of transcript elongation and processing, including 5′-end capping[3,12]. All these events are stimulated by the kinase activity of P-TEFb, which targets Ser2 and Ser5 of the Pol II CTD[13], the C-terminal region of the Spt5 subunit of DSIF[14,15] and the NELF-E subunit of NELF[16]. Whereas CTD phosphorylation triggers the recruitment of pre-mRNA processing components and the elongation/splicing factor Spt6 (ref. 17), the phosphorylation of DSIF converts it into a positive elongation factor and the phosphorylation of NELF releases it from elongating Pol II[3].

The study by D'Orso and Frankel[1] provides valuable insights that warrant rethinking how Tat, TAR and P-TEFb collaborate in stimulating HIV-1 transcription (**Fig. 1**). In agreement with previous findings, P-TEFb and Tat associate with PICs on the HIV-1 core promoter independently of TAR or transcription itself [4–6]. Strinkingly, P-TEFb did not show the kinase activity toward the Pol II CTD because the PICs contained LARP7 and HEXIM1, two protein components of the P-TEFb inhibitory 7SK snRNP complex[18] (**Fig. 1a–c**). Namely, only a fraction of cellular P-TEFb is catalytically active, and most of it is repressed within the 7SK snRNP, which consists of the noncoding 7SK snRNA (7SK), HEXIM1 and/or HEXIM2, LARP7 and MePCE, a 5′-end 7SK capping enzyme[18]. Therein, 7SK is a scaffold that is stabilized by LARP7 bound to its 3′ end and MePCE[19–22], which together constitute the core 7SK snRNP[22]. However, the 5′-hairpin structure of 7SK binds HEXIM1 and/or HEXIM2 directly. As a result, the P-TEFb–interacting region in HEXIM proteins gets unmasked, which enables them to bind and repress P-TEFb[18]. D'Orso and Frankel[1] not only found 7SK snRNP in purified PICs but also on the HIV-1 promoter in cells. This is an intriguing finding because only the active form of P-TEFb was thought to interact with transcription complexes. A critical solution to the remaining puzzle as to what activates P-TEFb and Pol II elongation was provided by the demonstration that the synthesis of the nascent HIV-1 transcript containing TAR triggered the removal of the inhibitory components of 7SK snRNP from Pol II during the transition into

productive elongation (**Fig. 1d,e**). Thus, in this revised model of Tat transactivation, TAR does not function merely as a recruiter of Tat and P-TEFb to paused Pol II; rather, it provides a critical protein:RNA binding step, transferring the preloaded Tat and P-TEFb to the emerging transcript and concomitantly ejecting 7SK snRNP. Finally, Sp1 or TATA elements were essential for loading PIC, 7SK snRNP and Tat on the HIV-1 core promoter, suggesting that the basal transcription machinery and Sp1 recruit 7SK snRNP and Tat for the subsequent TAR-mediated activation of Pol II elongation.

The work by D'Orso and Frankel[1] spurs several questions. First, what is the mechanism of the TAR-triggered acquisition of Tat and P-TEFb from 7SK snRNP during Pol II pausing? Given that (i) Tat directly displaces HEXIM1 from Cyclin T1 or P-TEFb and from 7SK *in vitro* and in cell lysates[8,23,24], (ii) Tat induces structural rearrangements in P-TEFb where otherwise HEXIM1 may bind[25,26] and (iii) Tat forms a stable complex with P-TEFb and 7SK snRNP[1,27], it seems likely that the RNA-binding arginine-rich motif (ARM) of Tat and its P-TEFb–interacting activation domain initially cooperate to displace HEXIM1 from its preferred position within 7SK snRNP. Once TAR emerges from the Pol II, it may capture a preformed Tat:P-TEFb complex from 7SK snRNP by competitively dislodging 7SK. Although the Tat:P-TEFb complex might be fully active before binding TAR, its repositioning onto the nascent transcript could be the final step that enables the subsequent phosphorylation events. Second, it remains unclear what causes the ejection of the inhibitory 7SK snRNP components from the HIV-1 promoter-proximal region upon the formation of the Tat:P-TEFb:TAR complex. Does productive transcription itself strip the inhibitory 7SK snRNP components from elongating Pol II, thus paralleling the divorce of GTFs and Pol II after transcription initiation? Stimulation of HIV-1 transcription by TNF-α, which operates in a Tat:TAR–independent manner through the recruitment of P-TEFb by NF-κB and Brd4 (refs. 28–30), would address this entertaining possibility. Alternatively, the physical act of transferring the Tat:P-TEFb complex to TAR might cause the release. This could be the case if P-TEFb, alone or in concert with another component(s) of 7SK snRNP, directed the recruitmentof 7SK snRNP to PIC by, for example, associating with Sp1 (ref. 31) or the Cdk8-containing Mediator complex[32]. Clearly, deciphering the assembly of 7SK snRNP with PIC is another pressing issue. By understanding it, we may discover that PICs with preloaded but repressed P-TEFb constitute cellular activator-specific transcription networks that would require promoter-proximal RNA intervention for unleashing Pol II elongation. Finally, preventing the 7SK snRNP loading into PIC should resolve a remaining unknown: is the capture of P-TEFb from the PIC-bound 7SK snRNP an essential prerequisite for Tat transactivation? In other words, could Tat, in addition to or independently of the pool of P-TEFb within the PIC enclave, transactivate HIV-1 promoter using P-TEFb from alternative cellular sources, such as 7SK snRNP outside of PIC[23,24], the Brd4:P-TEFb complex[33,34] or the recently identified HIV-1 Tat multifunctional transcription elongation complex[27,35] (**Fig. 2**)? If Tat failed to stimulate Pol II elongation from HIV-1 promoter lacking 7SK snRNP, the now-revised model of Tat transactivation would gain even further momentum.

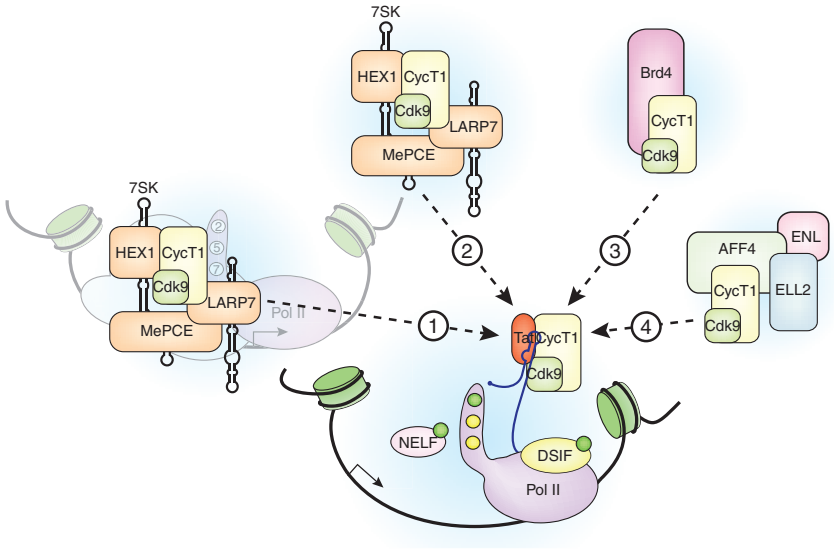

Figure 2 Alternative pools of P-TEFb for HIV-1 Tat transactivation. HIV-1 Tat may capture P-TEFb from different cellular complexes to form the tripartite Tat:P-TEFb:TAR complex for stimulating transcription elongation. The P-TEFb–containing complexes include 7SK snRNP assembled with PIC on the HIV-1 promoter (1), 7SK snRNP that may not be part of PIC (2), the Brd4:P-TEFb complex (3) and the HIV-1 Tat-containing multifunctional transcription elongation complex (4). For simplicity, the schematic of the latter complex lacks additional components, such as AF9, AFF1 or the PAF1 complex. The sequence of the different routes of P-TEFb acquisition by Tat and the relationships between them remain to be determined.

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