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TFII-I and USF (RBF-2) regulate Ras/MAPK-responsive HIV-1 transcription in T cells

Ivan Sadowski *, David A. Mitchell

Department of Biochemistry and Molecular Biology, University of British Columbia, 2146 Health Sciences Mall, Vancouver, BC, Canada V6T 1Z3

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Abstract

The HIV-1 long terminal repeat (LTR) is stringently controlled by T cell activation signals, and binds a variety of transcription factors whose activities are regulated downstream of the T cell receptor. One of the most highly conserved *cis*-elements on the LTR, designated RBEIII, binds the factor RBF-2 which is comprised of a USF-1/USF-2 heterodimer and a co-factor TFII-I. RBF-2 is necessary for transcription from the LTR in response to RAS-MAPK activation through T cell receptor engagement, but is also required for repression of viral expression in unstimulated cells. Considering the defined activities of USF and TFII-I, RBF-2 may be responsible for regulating promoter context by controlling chromatin organisation, thereby coordinating opportunity for transcriptional activation by additional factors bound to the enhancer region.

Keywords: Transcription; Ras; USF1; USF2; TFII-I; HIV-1; Chromatin; T cells

1. Introduction

Our current understanding of eukaryotic transcription has benefited from studies with the model eukaryote *Saccharomyces cerevisiae* as well as mammalian DNA viruses, including Adenovirus, Herpes, SV40 and Polyoma. These classes of virus have evolved to efficiently utilise the cellular transcription machinery, sometimes at the expense of host gene expression, and consequently analysis of their *cis*-elements and *trans*-acting factors has contributed to an understanding of the basic mechanisms for transcriptional activation. More recently, heightened interest in the role of chromatin and histone modification has produced a realisation that regulation of genes controlling eukaryotic cell growth and development requires a combination of mechanisms that manipulate chromatin organisation as well as template utilisation in response to environmental cues. Unlike DNA viruses, lentivirus replication is completely subject to cellular mechanisms controlling chromatin organisation because they integrate into the host chromosome as part of their life cycle. For the human immunodeficiency virus (HIV) this relationship contributes to pathology of acquired immunodeficiency syndrome (AIDS) because integrated provirus produces latently infected resting T helper cells that are impenetrable by current therapies, and which also act as an inducible reservoir for viral production in response to T cell activation [1,2]. The mechanisms causing repression of proviral transcription in resting T cells have not been defined, and are likely to involve repressive chromatin [2]. HIV-1 has evolved to coordinate its replication with signals controlling T cell activation [3,4], and this implies that reactivation of the virus in response to T cell signalling must also include mechanisms that relieve the repressive effect of chromatin. Consequently, apart from its obvious importance for disease, HIV-1 offers a model for understanding regulation of chromatin structure by

* Corresponding author. Tel.: +1 604 822 4524; fax: +1 604 822 9311.
E-mail address: sadowski@interchange.ubc.ca (I. Sadowski).

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cell signalling pathways. One factor, known as RBF-2, appears to contribute to both repression of chromosomally integrated HIV-1 in unstimulated T cells and reactivation of transcription in response to T cell signalling. RBF-2 binds to the most highly conserved *cis*-element on LTRs from infected individuals (RBEIII); this same *cis*-element is necessary for stimulation of transcription in response to RAS-MAPK signalling in activated T cells. RBF-2 has recently been identified as a USF1/USF2 heterodimer whose binding to the highly conserved RBEIII element requires a cofactor represented by TFII-I. In this article, we review current knowledge regarding these proteins and build the argument that this combination of factors may function to coordinate chromatin organisation of the integrated LTR in response to T cell signalling mechanisms.

2. The HIV-1 long terminal repeat and T cell signalling

The HIV-1 long terminal repeat is probably the most intensely investigated nucleotide sequence; over 50 different DNA binding proteins have been observed to interact with one or more variants of the LTR *in vitro* or *in vivo* [5–7]. During the course of infection, a fraction of activated T cells revert to a resting G_0 state where propagation of chromosomally integrated virus is repressed to produce a latently infected population [8]. The mechanisms that cause silencing of the integrated provirus are not well understood, but considering that its promoter is densely populated with binding sites for transcription factors that are constitutive in most cell types [7], they must include its organisation into repressive chromatin [9,10]. Consistent with this model, the LTR is known to have phased nucleosomes positioned immediately downstream of the transcriptional start site and at approximately 140 nucleotides upstream (Nuc 0 and Nuc 1, Fig. 1) [1]. Transcription of the integrated virus can be induced by engagement of the T cell receptor (TCR) during antigen presentation or stimulation with TNFα [8], a process that is accompanied by disruption of nucleosome phasing [3].

Engagement of the T cell receptor (TCR) through antigen presentation causes activation of several associated protein tyrosine kinases ($p56^{lck}$ and Zap70, see Fig. 2) that signal production of the second messengers inositol triphosphate (IP_3) and diacylglycerol (DAG) [11,12]. IP_3 causes release of intracellular calcium and activation of calineurin, while DAG activates PKCθ,

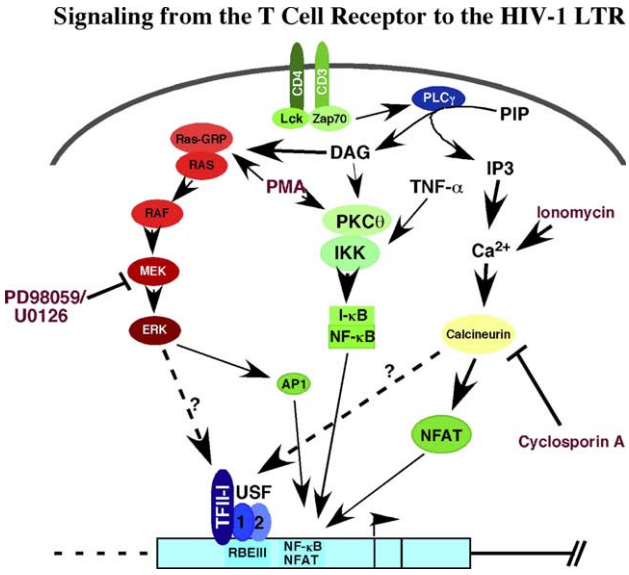

Fig. 2. Signalling pathways stimulated by the T cell receptor affecting HIV-1 transcription. Engagement of the T cell receptor (CD3/CD4) causes activation of associated $p56^{lck}$ and Zap70 protein tyrosine kinases and production of the second messengers diacylglycerol (DAG) and inositol triphosphate (IP_3) through hydrolysis of phosphatidylinositol phosphate (PIP) by phospholipase Cγ (PLCγ). DAG activates the RAS–RAF–MEK–ERK MAP kinase cascade and the protein kinase Cθ (PKCθ) pathways. The transcription factor AP1 is phosphorylated and activated by ERK, while NF-κB is activated upon destruction of the inhibitor I-κB through a phosphorylation mediated by the I-κB kinase (IKK). IP_3 causes calcium release and activation of calcineurin, which dephosphorylates NFAT to allow its accumulation in the nucleus. The inhibitors PD98059 and U0126 inhibit MEK of the MAP kinase pathway, while cyclosporin A inhibits calcineurin. Ionomycin causes activation of calcineurin by causing release of intracellular calcium. Phorbol esters such as PMA cause activation of PKC and Ras-GRP.

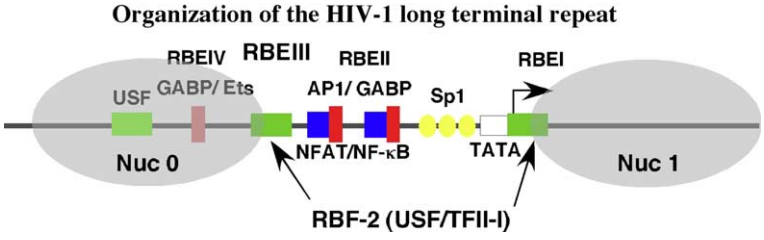

Fig. 1. Organisation of the HIV-1 long terminal repeat (LTR). The locations of transcription factor-binding sites on the LTR are indicated as follows: open box, TBP/TFIID; yellow, Sp1; blue, NFAT/NF-κB; red, AP1/GABP/Ets; dark green, RBF-2; light green, USF. Elements required for full responsiveness to v-Ras are indicated; RBEI, RBEII, RBEIII, and RBEIV. The locations of nucleosomes positioned in unstimulated T cells are shown (Nuc 0 and Nuc 1). The site of transcriptional initiation is indicated with an arrow.

and the Ras–Raf–MEK–ERK pathway through RasGRP [13,14] (see Fig. 2). Like other cytokine and growth factor-stimulated genes, induction of the LTR by these signalling mechanisms involves a complex interplay of a combination of DNA binding factors. The enhancer region of the LTR (−40 to −100) has binding sites for a variety of signal-responsive factors including NFAT, AP1, NF-κB, GABP/Ets, in addition to sites for SP1 which is generally considered to be constitutive in most cell types [7,15] (see Fig. 1).

3. Ras and MAP kinase signalling to transcriptional activator proteins

MAP kinase signalling pathways are universally conserved in eukaryotes for transmission of extracellular signals to nuclear gene regulatory factors. The small GTPase Ras plays a pivotal role in activation of the downstream Raf-MEKK-MEK-MAP/ERK protein kinase cascade in response to membrane-associated receptor protein tyrosine kinases [16,17] (Fig. 2). A variety of sequence-specific DNA binding transcriptional activators are known substrates for MAP kinases, including members of the Elk/Ets, Fos/Jun (AP-1) and CREB/ATF families [17–20]. Most genes responsive to MAP kinase signalling are regulated by a combination of promoter elements and DNA binding factors. Furthermore, many transcription factors that are responsive to cytokine and growth factor-stimulated signalling function within complexes bound cooperatively to adjacent *cis*-elements. Most of these proteins are considered to be transcriptional activators in the classical sense in that they are thought to recruit general transcription factor complexes to the promoter in response to cellular signals, thereby inducing signal responsive gene expression. For example, the Ets family members Elk-1, SAP-1 and SAP-2 bind the serum response element (SRE) on the *c-fos* promoter in cooperation with the serum response factor (SRF) [19]. Various Ets family transcription factors are responsive to the ERK, JNK/SAPK and p38 MAP kinases downstream of growth factor- and stress-stimulated pathways [19], while SRF is responsive to signalling from RhoA small GTP binding protein-induced actin assembly [21]. Likewise, AP-1, comprised of fos/jun heterodimers binds cooperatively to enhancer elements with the nuclear factor of activated T cells (NFAT) on promoters of a variety of genes responsive to T cell receptor engagement, including *IL-2*, *GM-CSF*, *TNF-α* and the HIV-1 long terminal repeat (Fig. 1) [22,23]. Fos and jun proteins are phosphorylated by ERK and JNK MAP kinases, whereas the NFATs are regulated by calcium mobilisation through calcineurin [4,11,22] (Fig. 2). As with all *bona fide* transcriptional activator proteins, this group of factors typically have specific modular domains or regions that are capable of activating transcription when fused to a heterologous DNA binding domain, generally independent of any promoter context other than their cognate binding site. Furthermore, because their modular activation domains are regulated by signal transduction, often through direct phosphorylation, a number of LexA or GAL4 DNA binding domain-signal responsive activation domain fusion proteins are sometimes used as reporters for monitoring cell signalling [24–26]. Although specific general transcription factor machinery targets have not been identified for many of these proteins, they are presumed to recruit components of the RNA Polymerase II holoenzyme or mediator complex [27–29], often in addition to histone acetyl transferase or SWI/SNF chromatin reorganising machines [30,31].

4. TFII-I, a general transduction and assembly factor

Recent observations implicate an additional factor, designated TFII-I, as a critical regulator of protein tyrosine kinase and stress-stimulated signal-responsive transcription. However, most evidence suggests that TFII-I operates through mechanisms that are distinct from typical transcriptional activator proteins, and unlike those factors mentioned above, requires specific promoter contexts. TFII-I was initially purified as an initiator (Inr) element-binding protein [32], but was subsequently observed bound cooperatively to upstream elements with a variety of sequence specific DNA binding transcriptional activator proteins, including several of the factors mentioned above (see Table 1, [33]). TFII-I protein has several notable features, including its six directly reiterated I-repeats (R1–R6, Fig. 3(a)) resembling helix-loop-helix (HLH) motifs. One of the repeats (R2) has a preceding basic region, which in combination with a putative leucine zipper motif in the N-terminus, is necessary for binding initiator elements [34,35] (see note, Table 1). TFII-I is also capable of directly binding to upstream promoter sequences, including the serum response element (SRE) and *c-sis*/PDGF induction element (SIE) of the *c-fos* promoter (Table 1), these interactions have been attributed to similarities within these elements to the Inr consensus sequence. Intriguingly, TFII-I also binds to the upstream E-box in the Adenovirus major late (AdML) promoter, the endoplasmic reticulum stress response element (ERSRE) of the *Grp78* promoter, and more recently was shown to bind the upstream RBEIII element of the HIV-1 LTR ([23], Fig. 1), none of which bear obvious similarity to an Inr element, or to each other (Table 1). Consequently, one possibility is that TFII-I may have multiple DNA binding domains, perhaps represented by the additional I-repeats, for recognition of different DNA sequence elements [35]. Three alternative TFII-I isoforms are produced using combinations of two additional short

Table 1
Interaction of TFII-I with factors bound to upstream *cis*-elements

Factor	Promoter	Element	Location[a]	Sequence[b]	Interaction[d]	Stimulates[e]	Ref.
USF1	AdML	E Box	−61	C**CACGTG**A	IP, CB, S	Y	[34,73]
	HIV-1 LTR	RBEIII	−120	**ACTGCTGA**[c]	IP, CB, S	Y	[46]
Myc	AdML	E box	−61	C**CACGTG**A	IP, CB	Y	[40]
SRF	*c-fos*	SRE	−310	ATGT**CCATATTAGG**	IP, CB, S	Y[f]	[41,43]
Phox1	*c-fos*	SRE	−310	**ATGTCCATATTAGG**	IP, CB, S	Y[f]	[41,74]
STAT1, 3	*c-fos*	SIE	−340	**TTCCCG TCAATCC**	IP, S	ND	[43]
ATF6	*GRP78*	ESRE	−95	CCATCGGAGGCCT**CCACG**	IP, S	ND	[44,45]

Not shown: TFII-I binds cooperatively with NF-κB, USF-1, and Myc at initiator elements (Inr, YAYTCYYY).
[a] Location of the element relative to transcriptional start site.
[b] Sequence of element bound by factor (bold) and by TFII-I (underlined).
[c] Recognition site not defined for TFII-I at RBEIII.
[d] *Interaction demonstrated by*: IP, co-immunoprecipitation; CB, cooperative binding to DNA; S, synergistic activation of transcription (*in vitro* or *in vivo*).
[e] TFII-I stimulates binding of the factor to DNA *in vitro*: Y, yes; ND, no data.
[f] TFII-I stimulates formation of a Phox1/SRF complex on the SRE.

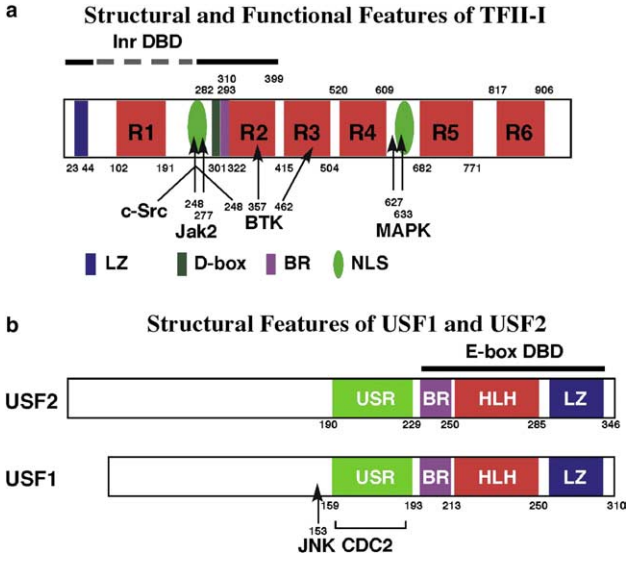

Fig. 3. RBF-2 is comprised of USF1, USF2 and TFII-I. (a) Schematic representation of TFII-I structural and functional features. The location of six HLH-like repeats are indicated (R1–R6). Abbreviations are: LZ, leucine zipper region; D-box, aspartic acid-rich region; BR, basic region; NLS, nuclear localisation signal. Features within the N-terminus required for specific binding to initiator elements are indicated (Inr DBD). Amino acid residue numbers are indicated, and the location of phosphorylation sites identified in B cells and fibroblasts. (b) Schematic representation of USF1 and USF2 functional and structural features. *Abbreviations*. USR, USF-specific region; LZ, leucine zipper region; BR, basic region; HLH, helix–loop–helix region. The location of phosphorylation sites identified on USF1 in HeLa cells are indicated. A CDC2-dependent phosphorylation was localised within the USR [75].

exons a and b, between the leucine zipper region and the first I-repeat (TFII-Iα, β, and γ) [36]. Furthermore, two additional TFII-I protein family members have been identified, designated GTF2IRDI/BEN/MusTRD1 and GTF2IRD2, which are similarly organised with N-terminal leucine zipper motifs and multiple I-repeats [37,38]. Although the function of the alternative TFII-I isoforms and additional family members have not been characterised, GTF2IRDI/BEN is known to regulate immunoglobulin gene expression through a downstream control element [39]. The putative leucine zipper motif within the N-terminal 90 amino acids mediates homodimer and heterodimer formation between TFII-I and its three alternatively spliced isoforms [36], and the possibility also exists for heterodimer formation between the three TFII-I family members [38].

TFII-I interacts with an expanding list of sequence-specific transcription factors (Table 1), and binds cooperatively with USF and Myc at initiator elements and the upstream E-box of the AdML promoter [34,40], Phox1 and SRF at the SRE of the *c-fos* promoter [41], and NF-κB at the HIV-1 initiator elements [42]. Interactions between TFII-I and USF1, Myc, Phox1, SRF, STAT 1 and 3, and ATF6 have also been demonstrated by co-immunoprecipitation [33]. Additionally, TFII-I causes synergistic activation of transcription with ATF6 and the STATs from the ERSRE and SIE elements, on the *Grp78* and *c-fos* promoters respectively [43–45]. Recombinant TFII-I protein has a unique ability to stimulate the binding of a variety of transcription factors to DNA *in vitro*, without itself becoming part of a detectable ternary complex. In this respect, it can increase the affinity of USF1 and USF1/USF2 heterodimers for the HIV-1 RBEIII element [46] (Fig. 1), and also of USF1 to initiator elements [34]. Similarly, TFII-I promotes the formation of ternary complexes between SRF and Phox1 on the *c-fos* SRE [41], and also stimulates binding of Myc to initiator and E box elements [40] (see Table 1). The mechanism for this quasi-catalytic assembly function has not been determined, but is likely to be achieved through its specific direct interactions with these factors and with sequences overlapping their DNA binding sites.

Phosphorylation of TFII-I appears to regulate its function, and accordingly TFII-I is phosphorylated

by ERK1 and c-Src or JAK2 in serum-stimulated fibroblasts [47,48] and by Bruton's tyrosine kinase (BTK) following B-cell receptor engagement in B-cell lines [49] (Fig. 3(a)). More recently TFII-I was found to be phosphorylated in response to thapsigargin, which induces the endoplasmic reticulum unfolded protein response [45], and in Jurkat T cells stimulated with PMA [46]. Phosphorylation of TFII-I Y248 (Fig. 3(a)) is necessary for activation of the *c-fos* promoter in response to serum stimulation in fibroblasts [47,50] and BCR engagement in B-cells [49], as well as activation of the *Grp78* promoter by the endoplasmic reticulum stress response element through stimulation of c-Src [45]. In these cases, phosphorylation of Y248 correlates with TFII-I nuclear translocation. ERK1 interacts with TFII-I at a MAP kinase interaction domain (D box, Fig. 2), and apparently phosphorylates serines S627 and S633 within MAP kinase consensus sequences [48] (Fig. 3(a)). Phosphorylation of these residues is necessary for activation of the *c-fos* promoter in serum-stimulated fibroblasts [48]. These observations demonstrate an essential role for TFII-I in activation of signal responsive transcription. This is a particularly surprising notion given that mechanisms for regulation of the *c-fos* promoter by the transcriptional activator proteins Ets/SRF at the SRE and the STATs at the SIE are generally accepted. How does TFII-I intercede within these established functions to regulate signal responsive transcription? Recent identification of TFII-I as a component of RBF-2, a factor that is necessary for Ras-responsive transcription of the HIV-1 long terminal repeat (LTR) provides some additional clues towards an answer to this question.

5. RBF-2, a context dependent factor for HIV transcription

Four regions of the HIV-1 LTR contribute to stimulation of its transcription by activated protein tyrosine kinases and v-Ras in co-transfection experiments [51], designated the Ras-response factor binding elements (RBEI, RBEII, RBEIII and RBEIV, Fig. 1). RBEII and RBEIV were found to bind a factor with identical sequence specificity and mobility in electrophoretic mobility shift assays, designated RBF-1 (Ras-response element binding factor 1). Similarly RBEI, overlapping the initiator element, and RBEIII at −120 also bound a factor with identical sequence specificity and mobility, but which was distinct from that bound by RBEII and RBEIV; this second factor was termed RBF-2. RBF-1 was subsequently shown to be identical to the Ets-family member GABPα associated with its β1 subunit [52], a finding that is consistent with the defined role of Ets family members in mediating responsiveness to Ras-MAPK signalling (see above).

RBF-2 was more of an enigma, as the upstream RBEIII element (ACTGCTGA) did not have obvious similarity to *cis*-elements for previously described factors. Furthermore, mutations in RBEIII on their own were found to cause enhanced activation from transiently transfected LTR templates, but prevented induction of the LTR by co-transfected v-Ras expression plasmids in combination with mutations in the other RBE sites [51]. Mutation of the RBEIII element on integrated LTR reporter genes causes elevated basal transcription in unstimulated Jurkat T cells, but also prevents induction of the LTR by stimulation with PMA or TCR cross-linking [46]. In contrast, the RBEIII mutation does not prevent induction by TNFα [46], which causes activation of the LTR through NF-κB [53] (see Fig. 2). RBEIII is one of the most highly conserved *cis*-elements on the HIV-1 LTR in infected individuals, along with the NFAT/NF-κB sites, and the core promoter elements (Fig. 1). This stringent conservation is underscored by the observation that LTRs with mutations in RBEIII invariably are accompanied by a duplication that precisely reiterates a functional binding site for RBF-2. Such duplications are observed in approximately 40% of infected individuals [52,54–58]. Even more striking is the fact that although duplications of a variety of lengths are observed, typically 20–40 nucleotides, the position of a functional RBF-2 binding site is always retained at precisely −120 nucleotides upstream of the site of transcriptional initiation [58] (Fig. 1). Spatial positioning of RBEIII is also conserved in LTRs from all HIV-1 subtypes (A–G, [59]). Additionally, although there are significant differences between the organisation of HIV-1 and HIV-2 LTRs, all of the HIV-2 sequences reported to date have a conserved binding site for a factor that was termed peri-κB, at the identical position as RBEIII in HIV-1 LTRs [60]. Several experiments indicate that peri-κB may be identical to RBF-2 [51,61]. These observations, demonstrating precise spatial conservation of its upstream *cis*-element, indicate that RBF-2 function must be dependent upon a specific promoter context, and are consistent with the fact that mutations in RBEIII can have both a negative or positive effect on transcription, depending on the status of other *cis*-elements on the LTR. We argue below that this context-dependent mechanism may relate to chromatin organisation of the integrated LTR.

6. USF1, USF2 and TFII-I are components of RBF-2

RBF-2 purified from Jurkat T cell nuclear extracts by specific affinity to concatamerised RBEIII oligonucleotide was found to contain multiple polypeptides [62], including species of approximately 50 and 120 KDa which were found to represent USF1, USF2

and TFII-I [46]. Antibodies against USF1, USF2 and TFII-I prevent binding of RBF-2 from Jurkat nuclear extracts to RBEIII oligos in EMSA. Furthermore, USF1/USF2 heterodimers, produced by co-translation *in vitro*, or by co-expression in insect cells, produced a complex in EMSA that was identical in size as RBF-2 from nuclear extracts. USF1/USF2 bound to the RBEIII element on their own with approximately 160-fold weaker affinity than to a consensus E box. However, recombinant TFII-I is capable of stimulating binding of USF1 homodimers and USF1/USF2 heterodimers, but not USF2 homodimers, to the RBEIII element *in vitro* by at least 20-fold [46]. TFII-I is also capable of weak association with the RBEIII element *in vitro* on its own, although the specific sequences recognised on this element have yet to be identified. These results demonstrate that RBF-2 is comprised of a USF1/USF2 heterodimer, the binding of which to RBEIII element is dependent upon TFII-I as a cofactor.

7. USF, ubiquitous but underappreciated

USF-1 and 2 are members of the basic HLH-leucine zipper (b-HLH-zip) family, and are similar in structure and DNA-binding specificity to Myc/Max/Myn, Mad/Mxi, and TFE3 [63,64]. These proteins are expressed constitutively in many tissues and bind predominately as heterodimers to "E-box" *cis*-elements containing the core sequence "CACGTG" [63]. USF1 and USF2 have similar C-termini within the USF-specific region (USR) and the b-HLH-zip domains, but have unique N-terminal sequences (Fig. 1). Transcriptional regulation by USF is context dependent in that activation may require expression of specific co-activators, or cooperative interactions with other transcription factors [65]. Additionally, in some promoter contexts USF has a repressive effect on transcription [66,67]. It was recently shown that chicken USF1/USF2 heterodimers bind a divergent E-box element (CACGGG) in the 5′ HS4 insulator sequence of the β-globin gene, and recruit the SET7/9 methyltransferase and the histone H3 acetyl transferase PCAF, which contributes to the barrier effect of the HS4 insulator in preventing encroachment of heterochromatin onto the β-globin locus [68]. Should USF also recruit histone modifying complexes to promoters in mammalian cells, these observations may in part explain the apparent context-dependent function of USF in gene regulation.

The finding that RBF-2 is comprised of a USF1/USF2 heterodimer was intriguing because these factors had not previously been implicated in activating transcription in response to MAP kinase signalling. However, USF1 and USF2 were shown to have anti-proliferative activities when overexpressed in cell lines transformed with v-Myc or v-Ras [65,66,69]. Additionally, expression of dominant interfering USF and TFII-I mutant proteins prevents induction of integrated wild type HIV-1 LTR reporter genes in cells stimulated by T cell receptor cross-linking [46]. Furthermore, USF1, USF2 and TFII-I all become hyperphosphorylated in Jurkat T cells stimulated with PMA [46], although the location or function of these phosphorylated residues have not been identified.

8. The function of TFII-I for RBF-2 and regulation of the HIV-1 LTR

The prototypical LTR represented by the initial LAI/HTLV-III sequence has an upstream E box binding site for USF at −160 (Fig. 1). However, this element is only conserved in approximately 30% of viral sequences from infected individuals [46,58]. It seems peculiar then that the RBEIII element, a non-consensus binding site for USF is so stringently conserved at such a precise location [58]. Interaction of USF at RBEIII requires TFII-I [46], and therefore this implies that a combined function of USF and TFII-I, at that specific location, may play a critical role in regulation of the HIV-1 life cycle. There is no direct evidence that TFII-I is itself a transcriptional activator in the classical sense, and its function in stimulating transcription could be mediated solely through its ability to assemble activators onto their upstream *cis*-elements (Table 1), and general transcription factor complexes onto initiator elements [32]. As TFII-I is regulated by protein tyrosine kinases and ERK, such an "assembly" function might enhance the activity of sequence specific activators that are regulated by the same or parallel signalling pathways. In chromatin immunoprecipitation experiments USF1, USF2 and TFII-I were observed bound to the wild type LTR in both unstimulated Jurkat cells, and cells stimulated with PMA [46]. As indicated above, mutation of the RBEIII element on the integrated LTR causes elevation of basal expression in unstimulated cells, and also prevents interaction of USF1, USF2 and TFII-I *in vivo*. These observations demonstrate that RBF-2 (TFII-I/USF1/USF2) is necessary for induction of the LTR in response to T cell signalling, but is also required for full repression of the integrated LTR in unstimulated cells. Consistent with this view, TFII-I has been shown to interact with HDAC3, which inhibits its activation of the *c-Fos* and *Vβ* promoters [70], although HDAC3 has not been shown to be a component of RBF-2. Therefore, RBF-2 bound to RBEIII may differentially regulate transcription in unstimulated and stimulated cells; this effect could be regulated by post-translational modification, or association with other regulatory factors.

9. Model for RBF-2 and RBEIII for regulation of HIV transcription

Considering the available evidence, most of which points to a role for RBF-2 in regulation of promoter context, it seems likely that factors bound at RBEIII primarily function to regulate chromatin organisation of the integrated LTR. TFII-I may play two roles: assembly of USF at the non-consensus binding site RBEIII; and recruitment of chromatin modifying factors to influence the positioning and interaction between phased nucleosomes. In unstimulated cells, TFII-I may function in repression through recruitment of HDAC3 to cause deacetylation of histones [70], and considering the spatial conservation of RBEIII may even contribute to positioning of nucleosomes on the LTR. This effect could also be mediated by interaction of TFII-I with the PIASxβ SUMO isopeptide ligase, which is thought to have a role in chromatin organisation [71,72]. Stimulation of T cells through T cell receptor engagement causes phosphorylation of TFII-I, USF1 and USF2; these modifications may cause alterations in protein interactions, perhaps including allowing recruitment of the histone acetyl transferases by USF [68]. Acetylation of the phased nucleosomes may facilitate the formation of open chromatin, allowing interaction of additional factors with the enhancer element (NF-κB, NFAT, GABP, SP1, Fig. 1) to enable transcriptional activation by recruitment of mediator and RNA Polymerase II. This model can explain the effect of RBEIII on repression of the LTR in unstimulated cells and also the requirement of TFII-I and USF for activation of the LTR in response to T cell signalling. Regulation of cellular genes by TFII-I may also involve a combination of its assembly function in promoting binding of sequence specific DNA binding proteins (Table 1), and recruitment of chromatin modifying complexes. The list of sequence-specific DNA binding factors that interact with TFII-I is almost certain to expand, and consequently may play a significant role in global responses to cell growth and differentiation signals.

10. Concluding remarks

Characterisation of the HIV-1 LTR has revealed a wealth of information concerning regulation of gene expression by protein tyrosine kinase and Ras-stimulated signalling. This viral promoter appears to have pirated most of the significant factors that are responsive to second messengers generated by T cell receptor signalling. TFII-I plays a central role in regulating expression of the LTR, perhaps through coordinating chromatin organisation. If this does turn out to be the case, it seems likely that TFII-I would have a similar role in regulation of cellular genes. Considering that this protein appears to be directly regulated by protein tyrosine kinases in addition to MAP kinases, global identification of its target genes and full spectrum of interacting transcription factors will be important in understanding cellular responses to growth factor signalling.

Conflict of interest statement

None declared.

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