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Review

Decoding Tat: the biology of HIV Tat posttranslational modifications

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Abstract

The Tat protein is a viral transactivator that activates HIV transcription through complex interactions with RNA and host cell factors. Tat undergoes multiple posttranslational modifications that regulate the dynamics and complexity of these interactions. The biology of these modifications and their role in Tat function are reviewed.

Keywords: HIV; Tat; Posttranslational modification

1. Introduction

As it has become apparent that the complexity of an organism is not determined by the mere quantity of its genetic information, a new focus on the role of posttranscriptional mechanisms to vary protein functions has emerged. Posttranslational modifications increase the genetic repertoire from 20 proteinogenic amino acids to more than 140 amino acids and amino acid derivatives identified in different organisms [1]. Frequent posttranslational modifications in mammalian proteins include phosphorylation, methylation, acetylation, ADP-ribosylation, glycosylation, ubiquitination, SUMOylation, and lipid additions. Each modification occurs at a distinct amino acid within a polypeptide chain and is mediated by a specific enzyme. In many cases, the catalytic activity of one modifying enzyme is counterbalanced by the action of another that reverses the reaction, establishing the highly regulated and dynamic nature of most posttranslational modifications.

Amino acid modifications range from small-scale to macromolecular additions that alter the chemical, electrical, or structural properties of the target's side chain. The functional consequences are often dramatic and can affect protein structure, stability, or localization as well as the interaction with other proteins, membranes, or nucleic acids. In recent years, emphasis shifted from an individual to a combinatorial view, and crosstalk between individual modifications has been demonstrated in many proteins, including histones. The amino-terminal extensions of core histones undergo a variety of posttranslational modifications and have served as the template for the so called "histone code" [2]. One aspect of this code is that individual tail modifications provide specific binding signals for chromatin-associated factors, which determine the accessibility and structure of chromatin during gene transcription, DNA replication, recombination, and repair. Moreover, individual modifications of one histone tail facilitate or interfere with the occurrence or function of other modifications located at the same or neighboring tails.

The principle of a dynamic protein code, which serves as a signaling platform to integrate different protein functions, can be applied to Tat. Tat is a multifunctional transactivator encoded by HIV-1 and HIV-2. The full-length open reading frame of HIV-1 Tat is composed of the two exons of the viral *tat* gene and encodes a protein of approximately 101 amino acids (130 amino acids for HIV-2). The LAI/Bru strain of HIV-1 encodes an 86–amino acid full-length Tat protein due to a premature stop codon within the second *tat* exon. In the late stage of the infection cycle, a C-terminally truncated Tat, encoded only by the first *tat* exon, is generated when unspliced viral RNAs are exported to the cytoplasm by the viral Rev

Abbreviations: CDK9, cyclin-dependent kinase 9; GCN5, general control of amino acid synthesis 5; NAD+, nicotinamide adenine dinucleotide; nuc-1, nucleosome 1 located immediately downstream of the start of transcription at the HIV-1 promoter; PCAF, p300/CBP-associated factor; PRMT, protein methyltransferase.

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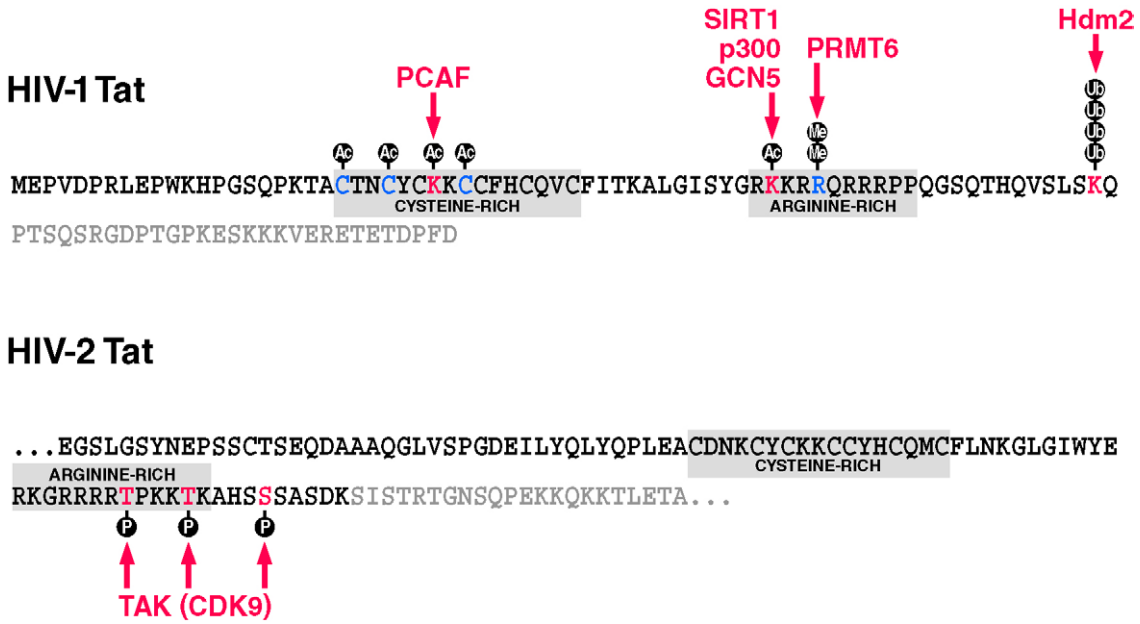

Fig. 1. Amino acid sequence and modifications of HIV-1 and HIV-2 Tat proteins. Modified residues, which have been precisely mapped, are shown in red, modifications with approximate positions are shown in blue. Cysteines in the cysteine-rich region are nonenyzmatically acetylated in the presence of acetyl coenzyme A. Amino acids encoded by the second *tat* exon are marked in gray.

protein. This Tat form (72 amino acids for HIV-1, 99 for HIV-2) is sufficient to transactivate the HIV promoter (Fig. 1).

Tat is a unique viral transactivator that binds an RNA stem-loop structure called TAR that spontaneously forms at the 5′ extremities of all viral transcripts. Tat functions as an adaptor protein for cellular cofactors, many of which exhibit intrinsic enzymatic activities. Tat binds the cyclinT1 component of the positive transcription elongation factor b and recruits the kinase activity of cyclin-dependent kinase 9 (CDK9) to elongating HIV transcripts [3]. CyclinT1 binding involves a highly conserved cysteine-rich region, which is part of the transactivating domain of Tat. An equally conserved arginine-rich motif is essential for direct contact of Tat with TAR RNA (Fig. 1). Tat and cyclinT1 bind TAR RNA cooperatively and induce phosphorylation of the C-terminal domain of RNA polymerase II by CDK9.

Recent studies demonstrated the control of CDK9 activity by extracellular stressors [3]. CDK9 kinase activity is naturally suppressed by interaction with 7SK RNA, an abundant and evolutionarily conserved small nuclear RNA, and its interaction partner, the hexamethylene bisacetamide-induced protein 1 (named MAQ1, for ménage a quatre). Ultraviolet irradiation and treatment with actinomycin D induced rapid release of 7SK RNA from CDK9 and activated the CDK9 kinase activity.

Other Tat cofactors include a number of transcriptional coactivators with intrinsic histone acetyltransferase activity, including p300/CBP, p300/CBP-associated factor (PCAF), TAFII250 and Tat-interacting protein 60 [3]. Since Tat also induces remodeling of a single nucleosome (nuc-1) positioned at the HIV promoter, it was proposed that Tat stimulates transcriptional elongation of HIV both by increasing the intrinsic ability of the RNA polymerase II complex to elongate efficiently and by recruiting histone-modifying enzymes to remodel the elongation block caused by nuc-1.

2. Phosphorylation of HIV-2 Tat by the Tat-associated kinase

The discovery that HIV-1 and HIV-2 Tat associate with a cellular kinase, now identified as CDK9, triggered experiments to determine if Tat itself is the substrate of this kinase activity. Recombinant HIV-2, but not HIV-1, Tat was phosphorylated by immunoprecipitated Tat-associated kinase activity [4]. HIV-2 Tat was also phosphorylated in vivo, while no specific phosphoprotein was immunoprecipitated from cells expressing HIV-1 Tat [5]. The phosphorylation sites were mapped to threonine 85, threonine 89, and serine 94 within the first exon of HIV-2 Tat, but the combined mutation of these residues did not alter Tat function in cotransfection experiments [6]. However, threonine 85 within the arginine-rich motif is highly conserved among HIV-2 isolates, and the influence of its phosphorylation remains to be determined in infection (Fig. 1).

The possibility that Tat is phosphorylated by a cellular kinase is intriguing because it links Tat modifications to extracellular signals. Since the enzymatic activity of CDK9 is activated by ultraviolet irradiation and treatment with actinomycin D, phosphorylation of HIV-2 Tat should increase in response to these stimuli. The possibility exists that Tat phosphorylation regulates the interaction of Tat with TAR RNA because threonine 85 is located in the arginine-rich motif of HIV-2 Tat, and phosphorylation of CDK9 regulates RNA-binding to 7SK RNA [7].