Biochimica et Biophysica Acta 1829 (2013) 1184–1190

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagrm

Transcriptional coactivator CIITA, a functional homolog of TAF1, has kinase activity

Katherine C. Soe, Ballachanda N. Devaiah, Dinah S. Singer *

Experimental Immunology Branch, National Cancer Institute, NIH, Bethesda, MD 20892, USA

ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form 3 September 2013
Accepted 4 September 2013
Available online 13 September 2013

Keywords:
CIITA
MHC class II
MHC class I
TAF7
Transcription
Kinase

ABSTRACT

The Major Histocompatibility Complex (MHC) class II transactivator (CIITA) mediates activated immune responses and its deficiency results in the Type II Bare Lymphocyte Syndrome. CIITA is a transcriptional co-activator that regulates γ-interferon-activated transcription of MHC class I and class II genes. It is also a functional homolog of TAF1, a component of the general transcription factor complex TFIID. TAF1 and CIITA both possess intrinsic acetyltransferase (AT) activity that is required for transcription initiation. In response to induction by γ-interferon, CIITA and it's AT activity bypass the requirement for TAF1 AT activity. TAF1 also has kinase activity that is essential for its function. However, no similar activity has been identified for CIITA thus far.
Here we report that CIITA, like TAF1, is a serine–threonine kinase. Its substrate specificity parallels, but does not duplicate, that of TAF1 in phosphorylating the TFIID component TAF7, the RAP74 subunit of the general transcription factor TFIIF and histone H2B. Like TAF1, CIITA autophosphorylates, affecting its interaction with TAF7. Additionally, CIITA phosphorylates histone H2B at Ser36, a target of TAF1 that is required for transcription during cell cycle progression and stress response. However, unlike TAF1, CIITA also phosphorylates all the other histones. The identification of this novel kinase activity of CIITA further clarifies its role as a functional homolog of TAF1 which may operate during stress and γ-IFN activated MHC gene transcription.

Published by Elsevier B.V.

1. Introduction

The Major Histocompatibility Complex (MHC) class I and II molecules are cell surface glycoproteins that bind and present intracellular-derived peptide antigens. Aberrant expression of MHC genes is associated with autoimmune diseases and tumorigenesis [1,2]. The loss of expression of MHC class II in Type II Bare Lymphocyte Syndrome (BLS) has been linked to the loss of expression of the MHC transactivator CIITA, a 135 kD protein characterized as the master regulator of MHC gene expression. Exogenous CIITA has been shown to restore class II expression in class II-depleted cells, such as those of Type II BLS patients [3,4]. Like MHC class II, CIITA is constitutively expressed only in antigen presenting cells, where it regulates expression of both class I and class II genes. However, in other cell types, CIITA expression can be induced by γ-IFN [5].

Interferon induction of CIITA leads to both *de novo* activation of MHC class II transcription and increased transcription of the constitutively expressed MHC class I gene. CIITA functions both as a coactivator and transcription factor. As a coactivator, CIITA nucleates an enhanceosome complex which binds to upstream enhancer elements in both MHC class I and class II genes [6]. As a transcription factor, CIITA interacts with components of the general transcription factor complex, TFIID [7]. The assembly of the transcription pre-initiation complex is nucleated by the association of TFIID with core promoters, followed by the recruitment of the remaining general transcription factors, RNA polymerase II and elongation factors. The TFIID complex consists of the TATA binding protein (TBP) and several transcription-associated factors (TAFs). Among the TAFs, only the largest, TAF1, has enzymatic activity. It has both acetyltransferase (AT) and kinase activities [8,9]. The AT activity is required for initiation of basal MHC class I transcription [10]. Constitutive MHC class I transcription, in the absence of interferon, is dependent on this canonical TFIID. In the presence of interferon, CIITA is induced and interacts with TBP and a number of TAFs including TAF7, TAF6 and TAF9, suggesting that it forms a TFIID-like complex [7]. Like TAF1, CIITA has AT activity, which during interferon activated transcription functionally replaces TAF1 AT activity, bypassing the requirement for TAF1 [11]. Thus, both TAF1 and CIITA are involved in MHC transcription: TAF1 during constitutive expression of MHC class I and CIITA during interferon-activated expression of both MHC class I and class II.

The AT activities of CIITA and TAF1 are regulated by TAF7, which binds to both and inhibits their acetyltransferase activity [12,13]. Thus, TAF7 inhibits transcription initiation until the transcription pre-initiation complex is fully assembled. Autophosphorylation of TAF1 results in the release TAF7 from the TFIID complex, revealing the TAF1 AT activity and allowing transcription to initiate [14]. Although TAF7 also binds and inhibits CIITA AT activity, the mechanism involved in its release from CIITA has not been identified thus far. We therefore

* Corresponding author at: Experimental Immunology Branch, NCI, NIH, Bethesda, MD 20892, USA. Tel.: +1 301 496 9097; fax: +1 301 480 0977.
E-mail address: Dinah.singer@nih.gov (D.S. Singer).

1874-9399/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.bbagrm.2013.09.001

investigated whether CIITA, like TAF1, has kinase activity that regulates its interaction with TAF7.

Here we report that CIITA has intrinsic kinase activity, allowing it to both auto- and trans-phosphorylate. Our data suggests that CIITA is an atypical serine/threonine kinase which can utilize both ATP and GTP as phosphate donors. CIITA phosphorylates TAF7, TFIIF and histone H2B, all of which are known TAF1 substrates. Although sharing the same substrates, CIITA and TAF1 phosphorylate different sites on TAF7. Importantly, like TAF1, CIITA's ability to autophosphorylate regulates its interaction with TAF7. We propose that the kinase activity associated with CIITA serves a function similar to that of TAF1, in which it regulates TAF7 binding, release and thus MHC transcription initiation.

2. Materials and methods

2.1. Cell lines and culture—Drosophila

Sf9 cells were maintained in TNM-FH insect medium (Pharmingen) at 27 °C. HeLa and CHO cells were grown as described previously [13].

2.2. Plasmid constructs

Full-length FLAG-WT CIITA in the baculovirus transfer vector pVL1393 was as described previously [11]. CIITA mutant AAE and truncation mutant 1–428 in the baculovirus transfer vector pVL1393 were generated from full-length Flag-WT CIITA with the following primers: 1–428, 5′ TGATCACGGATCAGCCTGAGATGA 3′ and 5′ ATAGCTCTTGCC CTGACCAGCTTT 3′; AAE, 5′ AGG**CC**G**G**AGAGCTATTGGGCTGG 3′ and 5′ GACCAGCTTTGGCCAGCACAGC 3′, and the *PfuUltra* high-fidelity DNA polymerase (IDT). The full-length WT CIITA in the mammalian pCDNA vector used here has been described previously [11]. The TAF7 WT and mutants in the pET and pGEX bacterial expression vectors have also been described previously [14] The CIITA 700–1130 mutant was a kind gift from Dr. Jenny Ting, University of North Carolina, Chapel Hill, NC.

2.3. Protein purification

Flag-tagged full-length and mutant CIITA proteins were expressed and purified in Sf9 cells as described previously [13] with minor changes. Briefly, Sf9 cells were harvested 32–40 h after infection with recombinant baculovirus for optimal protein expression. Uninfected Sf9 cells, harvested and subjected to the same purification steps as the infected Sf9 cells, were used to generate control extract. HeLa cells were transfected with the CIITA pcDNA constructs through Lipofectamine (Invitrogen) mediated transfection and harvested after 48 h. The recombinant proteins were immunoprecipitated with anti-FLAG M2-agarose beads (Sigma) and eluted using 100 μg/ml FLAG peptide. The Flag peptide was eliminated on a microcon column (Millipore) and proteins recovered in HKEG buffer (20 mM Hepes, pH 7.9, 100 mM KCl, 0.2 mM EDTA, 20% vol/vol glycerol).

Flag- and GST-tagged TAF7 were purified from a bacterial expression system as described previously [14]. Highly purified (>99%) human histone substrates were obtained from New England Biolabs as recombinant proteins expressed in *Escherichia coli*; their purity was confirmed by the company by mass spectrometry analysis (ESI-TOF MS) and peptide sequencing. Similarly, TBP, TFIIB, TFIIE and TFIIF recombinant protein/complexes expressed and purified from *E. coli*, with purity confirmed at >95%, were purchased from ProteinOne.

2.4. Immunoprecipitation and immunoblots

TAF7 binding to CIITA was detected by immobilizing equimolar amounts (1 μg) of unphosphorylated and auto-phosphorylated CIITA proteins on anti-Flag M2 agarose beads and incubating with 300 ng of GST-TAF7 for 2 h at 4 °C. The beads were washed twice with 50 mM Tris (pH 8.0), 150 mM NaCl, and 0.2% NP-40 and bound GST-TAF7 was immunoblotted with anti-TAF7 antibody. All immunoblot analyses were performed using the Odyssey infrared scanner and secondary antibodies from Li-Cor. The TAF7 antibody (Abcam), CIITA polyclonal antibody (Santa Cruz Biotech), M2 Flag antibody (Sigma), Histone H2B and Phos-S36 H2B antibodies (ECM Biosciences) were used in these analyses.

2.5. Kinase assays

FLAG-CIITA (1 μg) or control extract and protein substrates were incubated in kinase buffer (50 mM, Tris–HCL (pH7.5), 5 mM DTT, 5 mM $MnCl_2$, 5 mM $MgCL_2$) in the presence of 10 μCi of [γ-^{32}P]ATP or [γ-^{32}P] GTP and phosphatase inhibitors. The kinase reactions were incubated for 1 h at 30 °C, following which the proteins were resolved by SDS-PAGE and the extent of phosphorylation quantitated by a phosphorimager. Equimolar concentrations of all proteins were used in the kinase assays unless otherwise indicated. When kinase inhibitors were used, appropriate dilutions of the inhibitor were added at the start of the kinase reaction. Mock treated kinase reactions were treated with equivalent volumes of DMSO.

2.6. In-gel kinase assay

The in-gel kinase assays were done as described earlier [15] with minor modifications. Briefly, three sets of 3 μg purified CIITA and control cell extract (CE) were run on a 6% SDS-PAGE gel which was then cut into strips with one set of proteins each. One set was denatured with 6 M guanidine hydrochloride for 1.5 h and renatured for 16 h, following which the gel strip was soaked in kinase buffer supplemented with 20 Ci/ml γ^{32}P ATP for 1.5 h. The gel was washed stringently for 1 h with 1% SDS in the final wash, dried, and phosphorylated proteins detected by a phosphorimager. The autoradiograph was aligned with the other two gel strips with Flag-CIITA and CE that was silver stained or immunoblotted with anti-Flag antibody.

2.7. Histone acetyltransferase (HAT) assays

HAT assays were done as described previously [11]. Reactions were stopped with SDS sample buffer, proteins resolved on 15% SDS-gels and fixed overnight. The gels were dried and acetylation quantified by a phosphorimager.

2.8. Mass spectrometry analysis of CIITA

Purified rCIITA protein was submitted for mass spectrometry to the NCI Laboratory of Proteomics for identification of contaminating proteins. Purified rCIITA protein was digested with chymotrypsin (Roche), in 100 mM Tris, pH 7.8 with 10 mM CaCl2 for 16 h at 25 °C or with ArgC (Roche), in 100 mM Tris, pH 7.6 with 10 mM CaCl2 for 16 h at 37 °C. Digested samples were desalted by C18 ZipTip (Millipore), lyophilized and re-suspended in 16 μL of 0.1% formic acid for LC–MS analysis. 6 μL sample was loaded on an Easy nLC II nano-capillary HPLC system (Thermo Scientific) with a 10 cm integrated μRPLC-electrospray ionization (ESI) emitter columns, coupled online with a dual-pressure linear ion trap (LTQ Velos Pro) mass spectrometer (Thermo Scientific) for μRPLC-MS/MS analysis. Peptides were eluted using a linear gradient of 2% mobile phase B (acetonitrile with 0.1% formic acid) to 42% mobile phase B within 45 min at a constant flow rate of 200 nL/min. The fifteen most intense molecular ions in the MS scan were sequentially selected for collision-induced dissociation (CID) using a normalized collision energy of 35%. The mass spectra were acquired at the mass range of m/z 380–2000. The Easy Nano Spray ion source (Thermo Scientific) capillary voltage and temperature were set at 1.7 kV and 275 °C, respectively. The dynamic exclusion function on the mass spectrometer was enabled during the

Download English Version:

https://daneshyari.com/en/article/1946479

Download Persian Version:

https://daneshyari.com/article/1946479

Daneshyari.com