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ScienceDirect
Gene 388 (2007) 102–109
GENE
SECTION
FUNCTIONAL GENOMICS
www.elsevier.com/locate/gene

Transcriptional and subcellular regulation of the TRIP-Br family

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Received 20 July 2006; received in revised form 22 September 2006; accepted 12 October 2006
Available online 24 October 2006
Received by A.J. van Wijnen

Abstract

TRIP-Brs are a recently discovered set of proteins whose functions remain poorly characterized. Here we report the identification of TRIP-Br3 as a member of the TRIP-Br family along with evidence showing that TRIP-Brs interact with bromodomain-containing transcriptional cofactors PCAF, STAF65γ, and KAP1. PCAF, a histone acetyltransferase; STAF65γ, a protein associated with histone acetylation activity; and KAP1, a corepressor, influence the transcriptional activity of TRIP-Brs differentially. Finally, while all three TRIP-Brs are localized to the nucleus, TRIP-Br2 and TRIP-Br3 are also present in the cytoplasm through interaction with CRM1. Our results suggest that different TRIP-Brs function by interacting with a wide variety of bromodomain-containing transcriptional regulators in different subcellular locales.

Keywords: Bromodomain; PCAF; STAF65γ; KAP1

1. Introduction

TRIP-Br proteins are a recently discovered set of proteins with the potential to function in both transcriptional control and cell cycle regulation. Both TRIP-Br1 and TRIP-Br2 activate transcription when recruited to a target promoter (Hsu et al., 2001). Moreover, increased amounts of TRIP-Br1 and TRIP-Br2 activate the transcriptional activities of E2F/DP1, suggesting that they may function as coactivators.

In addition to transcriptional regulation, several lines of evidence suggest that TRIP-Brs also play a role in the regulation of the cell cycle. TRIP-Br1, by interacting with CDK4, affects the kinase activity of CDK4 (Sugimoto et al., 1999; Li et al., 2005). At low serum levels, TRIP-Br1 promotes cell growth by antagonizing the function of the cdk inhibitor p16INK4a. Moreover, TRIP-Brs activate the transcriptional activity of E2F/DP1 by interacting with DP1. Interestingly, the expression level of TRIP-Br1 is cell cycle-dependent, and TRIP-Brs increase the expression level of p21 (Watanabe-Fukunaga et al., 2005). However, the molecular and cellular bases of these cell cycle regulatory activities remain poorly understood.

Both TRIP-Br1 and TRIP-Br2 contain a conserved PHD-Bromo binding domain, an interaction module for bromodomain-containing proteins. A number of bromodomains that have been characterized in detail recognize acetylated-lysines on the tails of core histones, which mark for transcriptionally active regions on the chromatin (Zeng and Zhou, 2002; Yang, 2004; de la Cruz et al., 2005). It is logical that TRIP-Brs would function as transcriptional activators, since their PHD-Bromo binding domains bind bromodomains of transcriptional coactivators, which in turn bind to acetylated histones associated with a loosened chromatin structure. However, TRIP-Br1 also interacts with KAP1, a bromodomain-containing corepressor (Friedman et al., 1996), suggesting that not all bromodomains are associated with transcriptional activation only.

In this report, we analyzed a new member of the TRIP-Br family, TRIP-Br3, as well as TRIP-Br1 and TRIB-Br2, for their transcriptional activity, protein–protein interaction with

Abbreviations: TRIP-Br, transcriptional regulator interacting with the PHD-bromodomain; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; GST, glutathione *S*-transferase; GFP, green fluorescent protein.

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doi:10.1016/j.gene.2006.10.008

bromodomain-containing regulators, and their subcellular localization. Our report is the first functional comparison of TRIP-Br proteins at both transcriptional and cellular levels, and suggests a molecular mechanism for their functions via subcellular localization.

2. Materials and methods

2.1. Plasmids

Human cDNAs for TRIP-Br1 and TRIP-Br3 were cloned from human HEK293 cells by RT-PCR. Human TRIP-Br2 cDNA (KIAA0127) was obtained from Kazusa DNA Research Institute. TRIP-Br cDNAs were then subcloned into pcDNA3.1-HA, pM, pGSTag, and pEGFP (Clontech) to express HA-TRIP-Brs, Gal4-TRIP-Brs, GST-TRIP-Brs, and GFP-TRIP-Brs. The expression vector for Flag-PCAF has previously been described (Yao and Yang, 2003), to contain amino acids 352–832 of PCAF. STAF65γ (KIAA0764) obtained from Kazusa DNA Research Institute cDNA was fused to a Flag tag expression vector (Yao and Yang, 2003) to make Flag-STAF65γ. HA-KAP1 was constructed by inserting the coding region of KAP1 into pcDNA3.1-HA. All plasmid constructs were confirmed by sequencing. TKLuc and G5TKLuc have previously been described (Yang et al., 2002). TKLuc contains a thymidine kinase promoter from the herpes simplex virus upstream of the luciferase reporter. G5TKLuc is essentially the same as TKLuc with an additional five tandem Gal4 DNA binding sites upstream of the 5′ Sp1 site on the TK promoter.

2.2. Cell culture, transfection, and luciferase assay

HEK293 cells and HeLa cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and penicillin–streptomycin. 10^6 cells were seeded into 60 mm-diameter tissue culture dishes. 16 h later, 5 μg of the reporter plasmid, 0.5 μg of pRL-TK, 10 μg of the Gal4-fusion plasmid, and 10 μg of the overexpression plasmid were transfected into cells using the calcium phosphate coprecipitation method (Graham and van der Eb, 1973). 48 h after transfection, cells were harvested and luciferase assays were performed using the Dual Luciferase Assay system (Promega).

2.3. In vitro protein–protein interaction assays

GST fusion proteins were expressed in *E. coli* strain DH5α and captured onto glutathione–agarose beads (Sigma). Cells expressing HA-tagged or Flag-tagged proteins were lysed in PBS with the addition of 0.2% NP-40 for 0.5 h, and the resulting cell extracts were mixed with the beads in the presence of PBS and 0.2% NP-40 at 4 °C for 1 h. Beads were then washed extensively in a mixture of PBS and 0.2% NP-40. Bound proteins were eluted by boiling in Laemmli sample buffer, separated by SDS-PAGE, and detected by Coomassie blue staining and Western blot analysis.

2.4. Immunoprecipitation and Western blot analysis

Immunoprecipitation of Flag-tagged proteins were carried out using anti-Flag M2 affinity gel (Sigma) following the manufacturer's suggestions and as previously described (Yao and Yang, 2003). Western blot analyses were performed using standard protocols.

2.5. Fluorescence microscopy and leptomycin B treatment

HEK293 cells were seeded on chamber slides and grown for 18 h. 5 μg of expression plasmids for various GFP fusion proteins were transfected into cells using the calcium phosphate coprecipitation method (Graham and van der Eb, 1973). 24 h later, cells were washed with PBS, fixed with 4% paraformaldehyde, rinsed with PBS again, and dried. One drop of anti-fade mounting medium with 4′, 6′-diamidino-2-phenylindole (Vector) was added to the cells before coverslips were applied. To inhibit the CRM1-dependent nuclear export pathway, cells were transfected with appropriate GFP fusion plasmids. 24 h later, transfected cell were treated with 10 ng/mL of Leptomycin B (Sigma) for 2 h and their images analyzed using a fluorescence microscope (Yang et al., 2002).

3. Results

3.1. A new member of the TRIP-Br family

Using protein sequences of human TRIP-Br1 and TRIP-Br2 to search the NCBI databases, we identified a protein we termed TRIP-Br3 as the third member of the TRIP-Br family. TRIP-Br3 has been independently identified as Hepp (*he*matopoietic *p*rogenitor *p*rotein) from a screen using subtracted cDNA libraries; it has a low expression level in most tissues but elevates in hematopoietic progenitors and mature blood cells (Abdullah et al., 2001). However, the function of TRIP-Br3 and its unique qualities compared to the other two TRIP-Brs was to date unknown. To begin analyzing their functions, we aligned the protein sequences of TRIP-Brs and found four conserved regions: cyclin A-binding domains, SERTA domains, PHD-Bromo binding domains, and the C-terminal region (Fig. 1A). The cyclin A-binding domain was also found in E2F (Hsu et al., 2001). SERTA domains appear in several proteins including *SE*I-1 (later renamed TRIP-Br1), *R*BT1 (RPA-binding transactivator) (Cho et al., 2000), and *TA*RA (Marchler-Bauer and Bryant, 2004). This domain is required for the interaction between TRIP-Br1 and CDK4 (Sugimoto et al., 1999; Li et al., 2005), but the function of the SERTA domain remains unclear. The PHD-Bromo binding domain is required for protein–protein interaction between TRIP-Br1 and KAP1 (Hsu et al., 2001). The C-terminal region of TRIP-Br proteins is rich in acidic amino acids, and recruitment of this region to a target promoter activates transcription (Hsu et al., 2001). Taken together, the four conserved regions of the TRIP-Br proteins can be categorized into two possible functional groups: the cyclin A-binding domain and the SERTA domain are likely to be involved in the regulation of the mitotic cell cycle whereas the