Virology 378 (2008) 371–376

Contents lists available at ScienceDirect

Virology

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

TOJ3, a v-*jun* target with intrinsic oncogenic potential, is directly regulated by Jun via a novel AP-1 binding motif

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ARTICLE INFO

Article history:
Received 22 April 2008
Returned to author for revision
20 May 2008
Accepted 2 June 2008
Available online 7 July 2008

Keywords:
Avian sarcoma virus 17
AP-1
Cell transformation
Transcriptional activation
MSP58, PTEN

ABSTRACT

The *TOJ3* gene was originally identified on the basis of its specific activation in avian fibroblasts transformed by the v-*jun* oncogene of avian sarcoma virus 17 (ASV17). Overexpression of *TOJ3* induces cellular transformation of embryonic avian fibroblasts, revealing an intrinsic oncogenic potential. Transforming activity has also been demonstrated for *MSP58*, the human homolog of *TOJ3*, and oncogenic cell transformation by *MSP58* is specifically inhibited by the tumor suppressor PTEN. To investigate the mechanism of aberrant *TOJ3* gene activation in *jun*-transformed fibroblasts, the entire quail *TOJ3* gene including 13 exons and the 5′ regulatory region was isolated. Functional analyses of the promoter by transcriptional transactivation assays revealed that the specific induction of *TOJ3* is mediated by a cluster of three noncanonical AP-1 binding motifs (5′-CAGCTCA-3′ or 5′-CACCTCA-3′) which share the 3′ half-site with the consensus motif (5′-TGA$^{C}/_{G}$TCA-3′). Electrophoretic mobility shift assays and chromatin immunoprecipitation analyses showed that Jun binds to these motifs with an affinity similar to that observed for binding to an AP-1 consensus site. Noncanonical binding sites are also present in the chicken and human *TOJ3*/*MSP58* promoter regions. These results confirm and extend the previous observation that *TOJ3* represents an immediate effector gene of Jun and may point to an essential role of *TOJ3*/*MSP58* in carcinogenesis involving aberrant AP-1 expression.

Introduction

The dimeric AP-1 (activator protein-1) transcription factor complex containing c-Jun, c-Fos, or related proteins regulates the expression of genes relevant for cell proliferation and differentiation (Rauscher et al., 1988; Vogt, 2001, 2002; Eferl and Wagner, 2003). AP-1 binds with high affinity to the consensus sequence 5′-TGA$^{C}/_{G}$TCA-3′, or variants thereof, in the promoters of specific target genes (van Dam and Castellazzi, 2001; Eferl and Wagner, 2003). Numerous genes have been identified that are differentially expressed in cells transformed by the v-*jun* oncogene (Maki et al., 1987) of avian sarcoma virus 17 (ASV17), including genes which are directly regulated by AP-1 (van Dam and Castellazzi, 2001; Vogt, 2001; Hartl et al., 2003; Black et al., 2004; Iacovoni et al., 2004). However, only a few AP-1 targets have been identified that display intrinsic oncogenic potential, including *TOJ3*, originally discovered on the basis of its immediate and specific activation in avian fibroblasts transformed by the ASV17 v-*jun* oncogene (Bader et al., 2001). The 530-amino acid protein product (TOJ3) of the *TOJ3* gene is the avian homolog of microspherule protein 1 (MSP58/MCRS1), containing a bipartite nuclear localization motif and a phosphopeptide binding module (forkhead-associated domain, FHA) (Bader et al., 2001). Human MSP58 is localized in the nucleus and presumably acts in concert with distinct protein binding partners as a cell cycle-dependent transcriptional cofactor important for cell proliferation (Lin and Shih, 2002; Shimono et al., 2005; Du et al., 2006). Furthermore, MSP58 interacts with transformation associated proteins (Bruni and Roizman, 1998; Ren et al., 1998) or tumor suppressors (Okumura et al., 2005), including PTEN that frequently shows a loss of function in various human cancer types (Simpson and Parsons, 2001). Direct interaction with PTEN abrogates the oncogenic function of MSP58 (Okumura et al., 2005), suggesting that the *TOJ3*/*MSP58* genes may play an important role in human cancer.

Results and discussion

To explore the mechanism of aberrant transcriptional *TOJ3* activation, we have isolated the entire quail *TOJ3* gene. Using a 5′-fragment of *TOJ3* cDNA as a probe, a genomic quail library was screened leading to the isolation of two overlapping DNA fragments of 1141 and 3926 bp, respectively. The assembled 4538-bp nucleotide sequence encompasses the promoter region and 13 exons of the *TOJ3*

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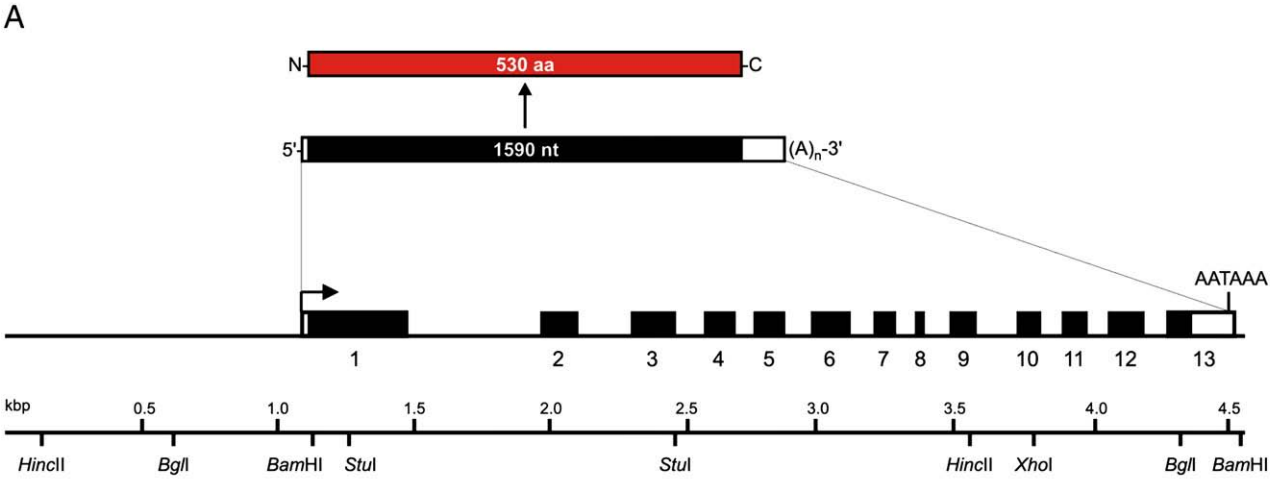
doi:10.1016/j.virol.2008.06.001

gene (Fig. 1A). The promoter region contains 1089-bp of 5′-untranscribed region, the previously mapped transcription start site (Bader et al., 2001), and a potential TATA box at nucleotide positions −28 to −23. No consensus AP-1 binding site (5′-TGA$^{C}/_{G}$TCA-3′) was found, but 19 potential nonconsensus AP-1 binding motifs with the sequence 5′-CAGCTCA-3′ (18×) or 5′-CACCTCA-3′ (1×). In both motifs, the 3′-half (CTCA) of the consensus site is retained (Fig. 1B). Three of the 19 motifs are located in close proximity to the transcription start site (−100 to −94, −81 to −75, −62 to −56), referred to as the distal (D), central (C), or proximal (P) site. Comparison of the quail *TOJ3* promoter with the corresponding region of the chicken *TOJ3* gene (from contig NW_001471752 of the chicken genome sequence) showed a high degree of conservation from nucleotide positions −69 to +8 including the proximal 5′-CACCTCA-3′ motif (Fig. 1C). Furthermore, six 5′-CAGCTCA-3′ motifs are present in the chicken *TOJ3* promoter. *MSP58*, the human *TOJ3* homolog, is located on chromosome 12 and contained within contig NT_029419.11 of the human genome sequence. Notably, the overall *MSP58* gene topology is very similar to that of *TOJ3*, encompassing 15 exons and displaying a cluster of 15 variant AP-1 binding sites in the 5′ upstream region (not shown), some of which have been described as functional sites in other AP-1 targets.

For functional promoter analysis, DNA fragments encompassing the quail or chicken *TOJ3* promoter were inserted into chloramphenicol acetyltransferase (CAT) reporter plasmids yielding the constructs pCAT-qTOJ3 and pCAT-cTOJ3. Transient transfection of the pCAT-qTOJ3 or pCAT-cTOJ3 plasmids into ASV17-transformed quail embryo fibroblasts (QEF) revealed that both avian *TOJ3* promoters are strongly activated, even stronger than the promoter of the direct AP-1 target



B

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          PstI
-1089  ...CTGCAGTTGGGTTTCTTCGCCGGCTCGTTACGTGCCCCCAGCCGTCCCTCAATACCACGCAGTGAAGATCCCAAGGGAGGAACGGCAGCA -1000
ATACCGCGAGGAGAAGATGGGAACCATCCAGAGGAAGGAGTGGGCTGTCAACTGGAGCGAGCCCAGCGTCGGGCACGTCACCCCCAGCTCAGCATAACCC  -900
CATGGACACTTATTGTGCTGCTGGACTGAAGGGGGATGATGTATGTGGGACCCCCCCCCTGCTTCTGGCTGTGCCCTATAAGGACCCTCTGGTGACACGT  -800
GGGGATGGCTCTGCCCCAAAGTGGTGCCTTGTCCCAGAGGATCGGCTCAGCTCCATTGAACAGGGCATGGCCACAAGGGGATGGTGTTTCTCCAAAGTGA  -700
CGGCTTGTCCTCAATGGGTACAGAGACAGCTCGGCCCCTATAGGGACAGTCCCATCCTACGAGCATGGCTTGAACCCAAAGTGATGGCTCCTAAAGGCTC  -600
AGCTCAGCCCCATGGCACAGGGCTTGTCTCCAAGGCACAGCCACAGAGAGATGGCTTGTCCTATAGGGACAGATTGTCCCCCAAAGGACAGCTGAGCCCT  -500
ACAGGGACAGCTCAACCCCATAGGGCCCTATGGGCACGGCTCTGCCTATAGGCTGCCATGTCCCTACAGTGCTGCCATATCCCACAGGGGTTCCTCGTCC  -400
CTAAAGTGCTGCAATGGCTCAGCCCTATAGGGACAGCTCAACCCCATAGGGACAGCTCAGCCCTATAGGAACAGCTCAGCACTATAGGGACAGCTCAGCC  -300
CTATAGGGACAGCTCAGCCCCATGGGGACAGCTCAGCCCTATAGGGACAGCTCAGCCCCATAGGGACAGCTCAGCCCTATAGGAACAGCTCAGCCCTATA  -200
GGGACAGCTCAGCCCTATAGGGACAGCTCAGCCCTATAGGGACAGCTCAGCCCTAAAGGGACAGCTCAGCCCTATAGGAATAGCTCAGCCCCATAGGGAC  -100
AGCTCAGCCCTATAGGAACAGCTCAGCCCTATAGGGACACCTCACCCCCCCACGCAAGTGCCGCTCTTCCCAATAAAAGGAGAATCCCTCCACGGTTCCA   +1
                        M  T  V  T  A  L  L  G  I ...
GCCTCGTTCATTGCATCACACTCATGACGGTCACGGCCCTATTGGGGATCC...                                               +52
                    BspHI                    BamHI
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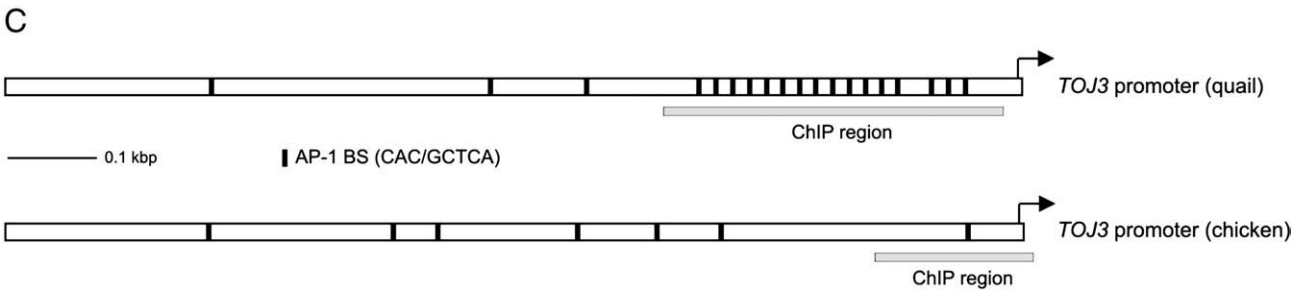

Fig. 1. Structure of the quail *TOJ3* gene. (A) The schematic diagram indicates the 13 exons with the coding region shown in black, the transcriptional initiation site (arrow), a polyadenylation signal, restriction enzyme cleavage sites, and the structures of mRNA and protein product. (B) Nucleotide sequence of a 1141-bp *TOJ3* promoter/exon1 fragment, containing 19 nonconsensus AP-1 binding motifs (5′-CA$^{C}/_{G}$CTCA-3′), a TATA box (5′-AATAAA-3′), the previously mapped transcriptional start site at position +1 (Bader et al., 2001), and the translational start site. (C) Diagram of the chicken and quail *TOJ3* promoters showing the positions of potential AP-1 binding sites (BS) and of the transcriptional start sites (arrows). BLAST analysis of quail *TOJ3* promoter sequences was used to identify the corresponding region in the chicken genome. The regions amplified for chromatin immunoprecipitation (ChIP) analyses are indicated. The nucleotide and deduced amino acid sequences reported here have been deposited in the GenBank database (accession no. EU116502).