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Short communication

Transcriptional regulation of the mouse microtubule-associated protein tau

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Abstract

The microtubule-associated protein (MAP) tau is found primarily in neurons and errors in its regulation are associated with Alzheimer's disease and other neurodegenerative disorders. Tau expression is transcriptionally regulated and tissue-specific. In this study, starting with a ~7500-bp fragment from the mouse tau gene, which includes tau exon −1, we define regions preferentially conferring tissue-specific expression. Furthermore, gel shift assays indicate that transcriptional regulators SP-1 and AP-2 are important for basal expression but not necessary for neuron-specific expression of the tau transcript.

Keywords: Mouse tau; Promoter specificity; Transcriptional regulation; Factors AP-2, SP-1

Tau is a microtubule-associated protein (MAP) enriched in axons of mature and growing neurons [1,2]. Tau is also found in the distal ends of growing neurons [3,4], in oligodendrocytes [5] and in some non-neuronal tissues [6,7]. Aberrant forms of tau protein are the major components of neurofibrillary tangles (NFTs), a hallmark of several neurodegenerative diseases [8].

Tau is encoded by a single copy gene [9,10]. It produces three transcripts of 2, 6 and 9 kilobases (kb) which are differentially expressed in the nervous system, depending upon stage of neuronal maturation and neuron type [11–16]. Mutations in the tau gene cause inherited frontotemporal dementia with parkinsonism associated with chromosome 17 (FTDP-17) [8]. Two conserved tau gene haplotypes, H1 and H2, have been identified by single nucleotide polymorphisms in the tau promoter and entire gene sequences [17–19]. The H1 haplotype promotes higher tau gene expression than the H2 [19] and is associated with progressive supranuclear palsy, corticobasal degeneration and Parkinson's disease [17–24].

Tau promoters have been characterized for the human [25] and rat gene [26]. In both studies, one promoter was identified directly upstream of untranslated exon −1 by sequence inspections, primer extensions and reporter assays. This promoter is GC-rich, lacks a TATA box (leading to heterogeneous transcription starts) and contains SP-1 and AP-2 binding sites. However, the human promoter does not appear to confer neuronal specificity [19,25], whereas the rat does [26]. Also, tau (almost certainly in its nuclear guise) is widely expressed in tissues [25,27]. To help resolve these points, we employed the methods used by the previous studies to investigate a region of the mouse tau promoter which is more extensive than the regions analyzed for either the human or the rat. Our results show that the approximate 3′ half of this region can confer fourfold higher expression in neuroblastoma than HeLa cells and that it contains binding sites for SP-1 and AP-2.

We analyzed 7.5 kb upstream of the mouse MAP tau exon −1 using the PIPS algorithm [28]. Sequence comparisons of this region between mouse and rat showed that they are more than 75% homologous except for three areas (−5

Abbreviations: bp, base pairs; CAT, chloramphenicol acetyltransferase; kb(p), kilobase (pairs); MAP, microtubule-associated protein; MTP, mouse tau promoter; NFTs, neurofibrillary tangles; nt, nucleotides

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to −6 kb; −3.5 to −4.5 kb; and −1 to −2 kb) where homology is between 50% and 70% (Fig. 1A). Additionally, a region of ~500 bp in mouse (between −3 and −4 kb) is absent in rat and a region of ~ 300 bp in mouse (between −1 and −2 kb) differs from that in rat.

Sequence comparison of the same region between mouse and human shows that they share three regions where homology is higher than 50%, in agreement with the dot matrix shown by Poorkaj et al. [29]. The first is 1 kb upstream of exon −1, the second is in the −2 to −4 kb region and the third is in the −5.5 to −6.5 kb region (Fig. 1B). Fig 1C shows the region of uninterrupted homology centered around exon −1. The significant sequence conservation between mouse and human includes exon −1 itself and continues till 1.4 kb downstream of exon −1. Interestingly, however, the GT microsatellite located ~400 nucleotides (nt) downstream of the human exon −1 [25] is not present in the mouse.

The mouse tau promoter region, like its human and rat counterparts, lacks a TATA box but is GC-rich throughout exon −1 and upstream of it. By sequence inspection, we identified 10 AP-2 and 20 SP-1 potential binding sites in the 7.5-kb region (Table 1). Of these, three AP-2 and twelve SP-1 sites are in the −5 to −7 kb region upstream of exon −1, and four AP-2 and six SP-1 binding sites are in the 500-bp region proximal to exon −1.

To characterize the mouse tau promoter, we first constructed a series of 5′ deletions from −7 to −1 kb in the CAT reporter gene (Fig. 2A). The promoter activity of MTP7000 was fourfold higher in HeLa than in SY5Y cells. Successive deletions of 1 kb from the 5′ end of the promoter region decreased activity from 12-fold (MTP7000) to threefold (MTP1000) in HeLa cells and increased activity from threefold (MTP7000) to 10-fold (MTP1000) in SY5Y cells. The shifts in activity are discontinuous and differ between the two cell types: The major activity shift in HeLa cells occurs between MTP6000 and MTP5000, whereas the major shift in SY5Y cells occurs between MTP3000 and MPT1500. This implies that the region between −6000 to −5000 may harbor elements which promote constitutive expression, whereas the region between −3000 and −1500 may contain elements which suppress neuronal expression.

The −1 kb upstream of exon −1 must be a key region to tau expression because it is highly conserved in human, mouse and rat. So the next generation of deletions probed that region roughly in 200-bp intervals (Fig. 2B). The promoter activity of MTP1000 and MTP800 is ~4-fold higher in SY5Y than in HeLa cells. MTP200 shows the highest absolute (20-fold) and relative (~7-fold) activity in SY5Y cells. Finally, MTP500 and MTP100 show equivalent amounts of promoter activity in both cell types. This analysis defines two putative successive elements: (1) a core promoter in the −110 to +58 region, which is equally active in both cell types, and (2) an element proximally upstream of the core promoter which extends to −186 and confers significant neuronal specificity.

To further define regions involved in tau transcriptional regulation, we also constructed internal deletions of the 7.5-kb region (Fig. 2C). The internal deletion, which removes the region from −186 to +58 (MTP0.5 ΔS-X), loses promoter activity in both SY5Y cells and HeLa cells, confirming the conclusion that the region from −110 to +58 defines a core promoter. In contrast, the next 3′ deletion (MTP0.2 ΔS-X, which still lacks the core element) is active and shows threefold more activity in SY5Y than HeLa cells, confirming the conclusion that this region contains neuronal-specific elements and suggesting that it can also substitute for the core promoter directly upstream of it.

MTP7.6 ΔA-S, in contrast to its internally undeleted progenitor MTP7000, shows similar levels of activity in both cell types (~10-fold), suggesting that elements which suppress neuronal activity have been removed by the internal deletion. This is in agreement with the large increase in promoter activity shown by MPT2000 and MTP1500 in SY5Y cells. MTP2.0 ΔS-S and MTP0.5 ΔS-S show absolute and relative activity levels similar to their respective internally undeleted predecessors MTP2000 and MTP500.

To start identifying the factors that regulate tau transcriptional activity, we did gel shift assays using HeLa and SY5Y extracts and the proximal region of the MAPT promoter (Fig. 3A). The first 168 nt immediately upstream of and overlapping with exon −1 (construct MTP100) is shifted by both extracts and shows two shifted bands. The lower one is stronger with HeLa, the higher one stronger with SY5Y (the bands are indicated as L and H in Fig. 3, and the extracts as HL and SY, respectively). The L shifted band is of same size for both HeLa and SY5Y whereas the H shifted band is slightly smaller in size with HeLa than with SY5Y.

The next 76 nt upstream (construct MTP0.2 ΔS-X) does not significantly shift with either extract, though the intensity of the unbound probe decreases. This construct shows significant activity in the CAT assays (Fig. 2C), so it may bind factors which activate transcription but binds them weakly and/or transiently. The next 261 nt upstream (construct MTP0.5 ΔS-X) shows a single shifted band, with SY5Y stronger than HeLa. As with the H band of MTP100, the shifted band seen with MTP0.5 ΔS-X is also slightly smaller in size with HeLa than with SY5Y. This construct shows practically no activity with the CAT reporter (Fig. 2C), so it may bind factors which inhibit expression of the tau promoter.

The region proximal to exon −1 harbors many SP-1 and AP-2 binding sites (Table 1). To determine if these two factors form the complexes we observed with our probes, we added oligonucleotides that reproduce their binding sites to our binding reactions (Fig. 3B, C; probe MTP0.2 ΔS-X is not shown, since it does not show a strong shift with either cell extract).

With MTP100 (Fig. 3B), the L band remains in the presence of SP-1 and AP-2 competitors in both HeLa and