Short communication

Distribution of mRNA encoding Tat-binding protein-1 (TBP-1), a component of 26S proteasome, in the rat brain

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Abstract

Cellular localization of Tat-binding protein-1 (TBP-1) mRNA is studied in the rat central nervous system (CNS) by in situ hybridization histochemistry. TBP-1 is one of the molecules which interact with HIV Tat and influence HIV amplification. Also, TBP-1 is recognized as a component of a 19S regulatory subunit of the 26S proteasome which degrades ubiquitinated proteins and is essential for the degradation of substrates which are not modified by the 20S proteasomal core.
HIV Tat-binding protein-1, TBP-1, has been identified as a cellular factor that cooperates with the human immunodeficiency virus-1 (HIV-1) protein Tat, which is crucial for HIV amplification [11]. TBP-1 is also demonstrated as a component of the 26S proteasome [1,3], which is an essential multiprotein complex that degrades ubiquitinated proteins in an ATP-dependent fashion. It is responsible for the bulk of protein turnover as well as for the degradation of regulatory short half-life proteins such as transcription factors, key-regulatory metabolic enzymes, cyclins, and for the production of antigenic peptides presented by the class I major histocompatibility complex [5,7,15]. The 26S proteasome is composed of at least two functionally interdependent parts: the 20S, a core catalytic subunit of the protease that can act as a peptidase in isolation, and 19S regulatory subunit (also called as the PA700) that is required for degradation of proteins [2,8,9,16].

Several lines of evidence suggest that 20S proteasome, also called as MCP (multicatalytic proteinase), plays an important role in the nervous system [4,10]. The 20S proteasome-like immunoreactivity is observed heterogeneously in the rat CNS, primarily observed in the nuclei and axonal processes (including some myelinated axons) as well as in synaptic boutons [10]. In addition, the specific proteasome inhibitor lactacystin promotes neurite outgrowth of N2A neuroblastoma cell line in vitro, indicating that the 20S proteasome is implicated in the promotion or maintenance of the differentiated neuronal phenotype [4].

However, the role of 19S regulatory subunit, a counterpart of 26S proteasome, in the CNS is still open at present; even the distribution of 19S regulatory subunit in the CNS has not been studied. Since TBP-1 is considered to be a component of 19S regulatory subunit, it is essential to examine the cellular distribution of TBP-1 to elucidate the distribution of 19S regulatory subunit. It is likely that 19S subunit and 20S proteasome work interdependently if 19S subunit shows a similar distribution to 20S proteasome. Conversely, if the localization of 19S subunit is different from that of 20S proteasome, it is possible that 19S subunit (or a component of 19S subunit) could work differentially from 20S proteasome in vivo. In addition, it has been reported that TBP-1 (a component of 19S subunit) could work as a transcriptional factor in vitro [11,13].

Therefore it is significant to examine the cellular localization of TBP-1 to elucidate its role in vivo. The in vivo expression pattern of TBP-1 has not been studied, regardless of whether it is or is not expressed in vivo. Thus, we obtained a rat TBP-1 partial cDNA fragment, and then investigated the distribution of TBP-1 mRNA in the rat CNS by in situ hybridization histochemistry (ISHH).

The rat TBP-1 partial cDNA obtained by PCR is highly homologous to the human TBP-1 cDNA. The nucleotide sequence of acquired DNA is approximately 88% homologous to that of the human TBP-1 cDNA (Fig. 1) corresponding to the C-terminal region of TBP-1 and its deduced amino acid sequence is approximately 99% homologous to the human sequence. Therefore the acquired DNA is considered to be a partial cDNA of the rat TBP-1. We used this DNA fragment as a template to synthesize probes for ISHH. Although the probe contains the ATPase domain that is conserved among ATPase family genes, our ISHH study for the mouse brain with the probe designed to avoid the conserved ATPase domain (based on our newly cloned mouse TBP-1 full-length cDNA structure) shows the same results (data not shown).

Four adult male Wistar rats (purchased from Kchen, Japan) weighing approximately 200 g were deeply anes-
Fig. 2. In situ localization of TBP-1 mRNA in the rat brain. Coronal sections of rat brain are labeled with ³⁵S-labeled TBP-1 antisense RNA. Hybridization signals are visualized as the accumulation of silver grains. Dark-field illumination. Lateral is to the left, medial is to the right. A: olfactory bulb. TBP-1 mRNA transcripts are localized in the anterior olfactory nucleus, mitral cell layer and medial border of external plexiform layer. B: cerebral cortex. Positive hybridization signals are observed in layers II–VI. C: hippocampus. Strong accumulation of the hybridization signals is observed in the CA1–CA3 fields of Ammon’s horn and dentate gyrus. D: cerebellum. Purkinje cells are positive for TBP-1 mRNA. E: strong hybridization signals are observed in the locus coeruleus, mesencephalic trigeminal nucleus and the motor trigeminal nucleus. AOE, anterior olfactory nucleus, external part; AOL, anterior olfactory nucleus, lateral part; EPI, external plexiform layer; Mi, mitral cell layer; I–VI, layer 1–6; CA1–3, field CA1–3 of Ammon’s horn; DG, dentate gyrus; Pur, Purkinje cell; 4V, the fourth ventricle; LC, locus coeruleus; Me5, mesencephalic trigeminal nucleus; Mo5, motor trigeminal nucleus. Scale bar: 500 µm.
thetized by intraperitoneal injection of sodium pentobarbital (Nembutal, 50 mg/kg b.wt.) and perfused transscardially with saline followed by ice-cold 4% paraformaldehyde in 0.1 M sodium phosphate buffer (PB). After perfusion, the rat brains were postfixed with the same fixative at 4°C for 1 day and immersed in 30% sucrose in 0.1 M PB at 4°C for 2–3 days. They were then frozen with powdered dry-ice and 15-μm-thick frozen sections were made on a cryostat. Sections were thaw-mounted onto glass-slides coated with TESTA (3-aminopropyltriethoxysilane; Sigma, USA). The protocol for ISHH and the criteria for identification of positive cells were based on the published method [12,17]. In brief, sections were fixed with formaldehyde, digested with proteinase K, acetylated with acetic acid anhydride and dehydrated. [α-35S]UTP-labeled single-strand RNA synthesized with the Ncol-digested template plasmid containing the rat TBP-1 fragment and SP6 RNA polymerase and that synthesized with the Nol-digested template plasmid containing the rat TBP-1 fragment and T7 RNA polymerase were used as antisense and sense probes, respectively. Signals were visualized by micro-autoradiography using emulsion (Irford, UK). Terminology is based on the atlas of Paxinos and Watson [14].

We investigated TBP-1 mRNA positive cells in the prosencephalon, mesencephalon and rhombencephalon by ISHH. Most positive cells were identified as neurons based on their morphological features. No significant signals were observed with sense probes (control probes). Results obtained were consistent throughout the animals used.

Fig. 3. Diagrams showing the localization of TBP-1 mRNA in the rat brain (A–L). Frontal sections. Large dots indicate neurons express TBP-1 mRNA very strongly, while small dots indicate neurons express TBP-1 mRNA firmly.
In the olfactory bulb, strong hybridization signals were observed in the anterior olfactory nucleus, mitral cell layer and accessory olfactory bulb. Positive cells were detected in the medial border of external plexiform layer (Fig. 2A and Fig. 3). In the neocortex, the cingulate cortex and the retrosplenial cortex, positive hybridization signals were observed in layers II–VI (Fig. 2B and Fig. 3). Strong hybridization signals were observed in the tenia tecta, layer II of both the piriform and entorhinal cortices, and the dorsal endopiriform nucleus. In the hippocampal formation, robust accumulation of hybridization signals was detected in the CA1–CA3 fields of Ammon’s horn and dentate gyrus. Scattered positive signals were seen in other regions (Fig. 2C and Fig. 3). In the amygdaloid complex, strong hybridization signals were observed in the lateral and basolateral amygdaloid nucleus. Positive cells were detected in the medial amygdaloid nucleus. Positive neurons with moderate signal intensity were detected in the globus pallidus. Scattered positive signals were seen in the caudate–putamen.

Diencephalon

In the epithalamus, no distinct positive signals were identified. In the thalamus, strong hybridization signals were observed in the anterodorsal thalamic nucleus and positive cells with moderate signal intensity were detected in the rest of the thalamic nuclei. Many positive cells were
immunoreactivity is mainly localized in the nucleus in the hypothalamus, strong hybridization signals were observed in the supraoptic nucleus. Positive cells were localized in the supraoptic hypothalamic nucleus and the paraventricular hypothalamic nucleus, whereas the positive cells were sparsely distributed in the remaining area.

Mesencephalon

Strong hybridization signals were observed in the red nucleus, the compact part of substantia nigra, the oculomotor nucleus and the trochlear nucleus.

Metencephalon and myelencephalon

In the cerebellar cortex, distinct hybridization signals were observed in the layer of the Purkinje cells (Fig. 2D and Fig. 3). Positive hybridization signals were detected in the interposed and lateral cerebellar nuclei. In the pontine nuclei, many positive cells were detected. Strong hybridization signals were observed in the locus coeruleus, mesencephalic trigeminal nucleus and motor trigeminal nucleus (Fig. 2E and Fig. 3). In addition, strong hybridization signals were also detected in the facial nucleus, nucleus ambiguus, dorsal vagus nucleus, hypoglossal nucleus and lateral reticular nucleus. Positive cells were localized in the superior and inferior vestibular nuclei, inferior olivary nucleus, cuneate nucleus and the spinal trigeminal nucleus.

Our ISHH study revealed that TBP-1 mRNA is distributed widely but heterogeneously in the CNS. The pattern of localization of TBP-1 mRNA coincides well with that of 20S proteasome-like immunoreactivity; the 20S proteasome-like immunoreactivity is mainly observed in the neocortex, the CA1–CA3 fields of Ammon’s horn and dentate gyrus, and the layer of the Purkinje cells [10]. This fact suggests that TBP-1 co-localizes with 20S proteasome in the CNS. It implies that the expression of TBP-1 and 20S proteasome is regulated by the similar manner in the CNS and that the 19S regulatory subunit and 20S proteasome cooperate, probably as the 26S proteasome in the CNS, since TBP-1 is recognized as a component of the 19S regulatory subunit. Unlike other non-lysosomal protease, 20S protease-like immunoreactivity is observed in the nucleus [6,10]. We have confirmed that TBP-1-like immunoreactivity is mainly localized in the nucleus in other tissues by immunohistochemistry (manuscript in preparation). Its well-coincident existence with 20S proteasome suggests that the major role of TBP-1 in the brain is related to 26S proteasome activity, although we could not exclude the possibility that TBP-1 works independently from 26S proteasome, e.g. as a transcriptional factor that is suggested in other species; even TBP-1 and 20S proteasome co-localize in the same nucleus.

The fact that some cells do not express a detectable amount of TBP-1 mRNA raises the possibility that TBP-1 is not essential for all the cells; some cells (including some glial cells) might utilize an alternative molecule(s) or the possibility that if TBP-1 has a long lifetime, then TBP-1 mRNA is transcribed only under a specific circumstance. More studies are required to elucidate these points.

It is demonstrated that the 26S proteasome is involved in a remarkably wide range of cellular processes by degrading the regulatory short half-life proteins that are related to the vesicle fusion, proteolysis, peroxisomal and mitochondrial biogenesis and transcription [7]. Our histochemical results implies that major role of TBP-1 in the brain is related to the 26S proteasome activity. Hence, it is difficult to specify the primary role of TBP-1 in the CNS. Rather, it is likely that its role in the CNS is diverse and its localization reflects the sum of a large number of cellular events related to the 26S proteasome activity.

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References


