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Original Article

Expression of tmp21 in normal adult human tissues

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Abstract: TMP21, known as p23 protein, is one important member of the p24 protein families. The degradation of TMP21 is mediated by the ubiquitin-proteasome pathway, as with the other presenilin-associated γ-secretase complex members. NFAT plays a very important role in regulation of human TMP21 gene expression. Compared with the function of TMP21, the studies about the distribution of this protein in human tissues are limited. We collected 19 normal adult human tissues from a healthy adult man died in a traffic accident and did examination of all the tissues collected for ICH, western blot and RT-PCR. It was shown that the expression of TMP21 is at high levels in heart, liver, lung, kidney and adrenal gland; moderate levels in brain, pancreas, prostate gland, testicle, small intestine, colon, stomach, gall bladder, thyroid gland and trachea; low levels in skeletal muscle, skin and lymphonodus. TMP21 is widely existed in normal adult human tissues. The current study provided for the first time a comprehensive expression of TMP21 in normal adult human tissues. It will benefit on helping in the design and interpretation of future studies focused on expounding the function of TMP21.

Keywords: TMP21, human tissue, immunohistochemistry, western-blotting, RT-PCR

Introduction

The secretory pathway is very important in most eukaryotic cells, which plays pivotal role in the synthesis, transport and secretion of a huge number of bioactive molecules [1]. Lots of diseases are caused by the malfunction of the secretory pathway in inter-cellular communication [2]. The p24 proteins are type I trans-membrane proteins with multiple conserved domains including a cleaved signal sequence at the amino terminus as well as a large N-terminal domain that is retained in the lumen of the cytoplasmic vesicle [3]. The p24 proteins take a vital important participate in the secretory pathway and they are major membrane components of the COPI (coat protein I, COP I) and COPII (coat protein II, COP II) coated vesicles which could mediate intracellular trafficking between the endoplasmic reticulum (ER) and Golgi compartments [4]. Widely distributing in eukaryotic cells at steady state or differentially localize to specific sets of membranes, the p24 proteins family are always transported between the diverse sub-compartments of endomembrane systems, such as ER, Golgi compartments, the COPI and COPII coated vesicles, intermediate compartment and so on [4, 5].

Be subdivided into four subfamilies: p24α, p24β, p24γ and p24δ, the p24 family conserves the domains from yeast to mammals in each family member [2, 6, 7]. However, the structure of each p24 family member has multiple conserved domains: a large N-terminal signal peptide, luminal domain, transmembrane domain and a short cytoplasmic tail [2, 3, 8, 10]. Needed to point out is that near the transmembrane domain, the luminal domain of each p24 protein contains heptad repeats of hydrophobic residues indicative of a coiled-coil protein interaction domain. And the coiled-coil protein was reported that could mediate the heteromeric

complexes between multiple p24 proteins [3, 4]. Actually, Gregory Emery et al. [3] found that proteins of each p24 proteins subfamily are mis-targeted to the ER when expressed alone except p25, which indicated that the interaction of each diverse subfamily help their destination in the cells.

It was found [9] that the p24α3- and p24δ2-trangenic frogs have different melanotrope cell phenotypes caused by the POMC transport and processing was diversely affected, and the researchers concluded that p24α3 and p24δ2 have non-redundant roles in maintaining the functional and structural integrity of the secretory pathway. Furthermore, it was concluded [8-11] that there are some specific sets of "machinery cargo" supplied by the members of p24 family to provide the proper microenvironments for efficient and correct secretory protein transport and processing.

TMP21, known as p23 protein, is one important member of the p24 protein families. It has been reported that TMP21 could maintain the integrity of the secretory pathway in mammals [5]. TMP21 is a type I protein with a receptor-like luminal domain and a short cytoplasmic tail. Further study [12] showed that this luminal domain is primarily responsible for the appearance of p23 in the plasma membrane and this cytoplasmic tail carries atypical endoplasmic reticulum (ER) retention KKXX motif that binds to COPI. TMP21 binds the presenilin complexes and acts as a modulator resulting in the selective suppression of γ-secretase cleavage [13], consequently, affects the progress of Alzheimer's disease (AD) which is a progressive neurodegenerative disorder and the most common cause of dementia worldwide [14, 15]. The pathological feature of AD pathogenesis is deposition of amyloid β protein (Aβ) [16]. Interestingly, Aβ production increases sharply after TMP21 expression was knockout by siRNA.

Recently, it has been found that TMP21 is a PKCδ-interacting protein. The silencing of TMP21 leads to enhanced translocation of PKCδ to the plasma membrane [17]. PKCδ is the mediator of PMA-induced apoptosis in LNCaP cells [18], Wang HB et al. [17] speculated that the potentiating effect of p23 depletion on PMA-induced apoptosis should be mediated by PKCδ and they confirmed that potentiating effect of p23 depletion on PMA-induced apoptosis is mediated through PKCδ.

Compared with the function of TMP21, the studies about the distribution of this protein in human tissues are limited. In order to describe the distribution of TMP21 in human tissues, we collected the normal human tissues in one healthy person who died in a traffic accident. ICH, western blot and RT-PCR were performed as a relative quantitative analysis.

Materials and methods

Tissue specimens

Human tissues were obtained from an adult man who died in a traffic accident and who was healthy before his death. After communication with family of the deceased, they donated the deceased body into science research. The autopsy specimens were derived from post-mortem examination carried out within 1h of death. The tissues of different organs were kept in an ice case immediately, and then parts of them were fixed with 4% paraformaldehyde and the rest of them put into an -80°C low temperature refrigerator, prepared for IHC, Western blot, and RT-PCR. We analyzed a total of 19 normal human tissues from the same body as follow: liver, brain, lung, spleen, pancreas, heart, kidney, prostate, skeletal muscle, testicle, small intestine, colon, stomach, gall bladder, thyroid gland, adrenal gland, trachea, lymphonodus and skin. Human tissue collecting was approved by Chongqing Medical University Ethic Committee and the process was abided by the rules of human tissue using of ethic committee.

Immunohistochemistry

To qualitative detect the distribution of TMP 21 protein in normal human tissues by IHC, we used rabbit-anti-human TMP21 polyclonal antibody (supplied by Prof. Weihong Song, University of British Columbia), rabbit two-step IHC detection reagent (Invitrogen Corp., Carlsbad, USA). The process was according to the introduction of PV-6001 two-step IHC detection reagent as follow: (1) Dewaxing, hydration; (2) Antigen repair: Tissue slices were immersed into a box with citrate buffer solution, baked in moderate heat of micro-wave oven for 6 to 8 minutes, then washed by PBS solution for 3 times (10

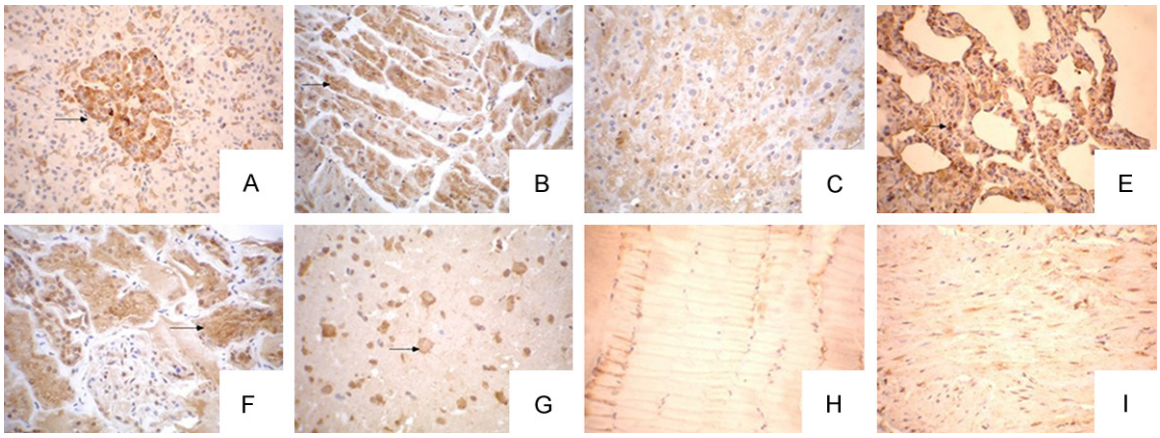

Figure 1. TMP21-immunoreactivity in human. The expression of TMP21 is at high level in pancreas (A), heart (B), liver (C), lung (E), kidney (F) and cerebral cortex (G). It is at low level expression in prostate (I) and it seems like no expression in skeletal muscle (H) (×400). (The arrows show the expression in each picture of diverse tissue).

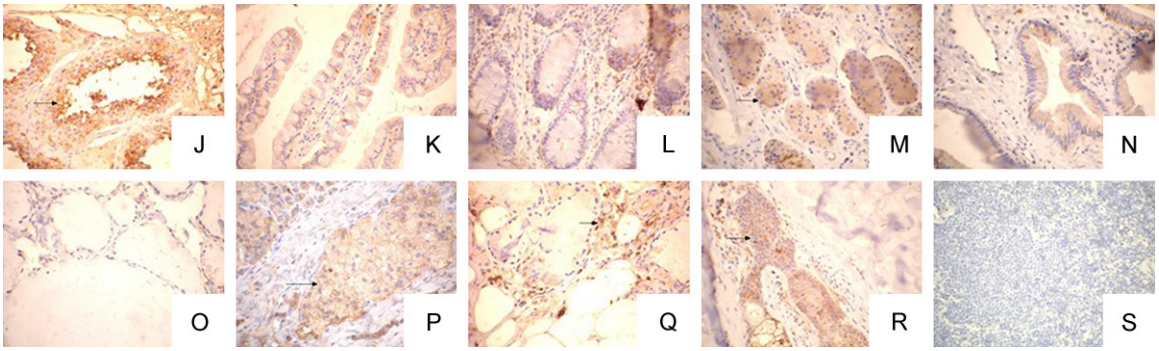

Figure 2. TMP21-immunoreactivity in human. The expression of TMP21 is at low level in testis (J), small intestine (K), colon (L), stomach (M), gallbladder (N), thyroid (O), adrenal (P), trachea (Q) and skin (R). It seems like no expression in lymph node (S) (×400). (The arrows show the expression in each picture of diverse tissue).

minutes per time) after 30 minutes natural cooling; (3) Hatch in 3% H_2O_2 for 5 to 10 minutes (eliminate the affection of endogenous hyperoxide), then washed by PBS solution for 3 times (10 minutes per time); (4) Dropwise added the primary antibody (diluted by PBS solution for 1:500), then washed by PBS solution for 3 times (10 minutes per time) after staying overnight in 4°C refrigerator; (5) Dropwise added the relevant second antibody for 30 minutes in room temperature or 37°C, then washed by PBS solution for 3 times (10 minutes per time); (6) DAB staining, and observation under microscope, then end staining in times, swashing by distilled water or running water; (7) Re-staining, dehydration, transparent; (8) Finally blocked the tissue slice with suitable reagent. The staining results were measured qualitatively on positive or negative. The positive result showed that puce plaques or grains are stained in the tissue cells. The control negative result could not find puce staining in tissue cells using PBS instead of first-antibody.

Western blot

1.) Preparation: Human tissue was operated upon the ice, and tissue homogenate was mixed by a glass homogenizer with sufficient splitting. Then, the sample was transferred into an 1.5 ml centrifuge tube for centrifuging with 10 minutes by 12000-14000× g under 4°C. The supernate was available and quantified by BCA methods and kept under -20°C. Denature and reduce protein complexes before loading onto gel. 2.) Protein SDS-PAGE gel electrophoresis: (1) 5% spacer gel and 8% separation gel were poured onto a slab. (2) Protein sample preparation: Enough Glycine running buffer

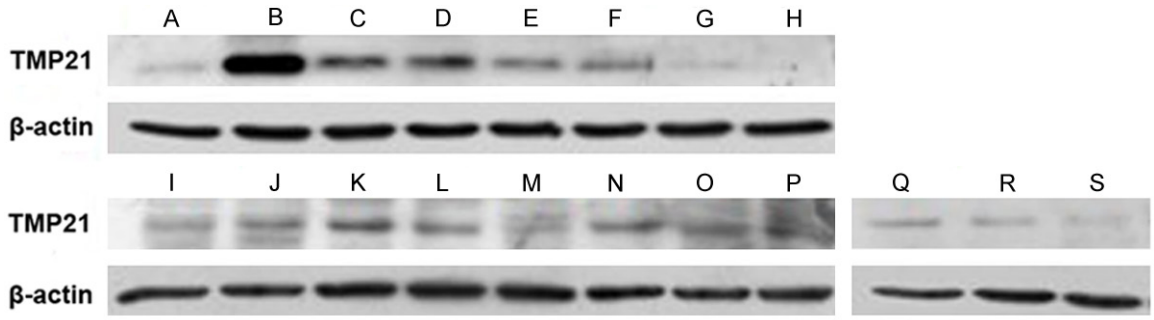

Figure 3. Western blot of TMP21. It shows TMP21 protein was highly expressed in human heart (B), liver (C), spleen (D), lung (E), kidney (F), small intestine (K) and adrenal (P) tissues, and moderately expressed in pancreas (A), cerebral cortex (G), prostate (I), testis (J), colon (L), stomach (M), gallbladder (N), thyroid (O) and trachea (Q), but was barely detected in skeletal muscle (H), skin (R) and lymph node (S).

were filled into the electrophoresis tank which were added with 2× buffer by 1:1 ratio, 100°C for 5 minutes, then fast cooled and centrifuged for 30 s by 10 000× g. (3) Protein electrophoresis was carried out to separate proteins according to their molecular weight (size), and stop when bromophenol blue arrived on the line between spacer gel and separation gel. 3.) Transfer of separated proteins onto PVDF membrane: "Sandwich" folder was made by filter paper and PVDF membrane, which preprocessed by the blotting buffer (Glycine 144.0 g + Tris 33.3 g + ddH2O2). 4.) Blocking the membrane with skimmed milk for 1-2 h under room temperature. 5.) The primary antibody (1:500) incubated with TMP21 protein. 6.) Relevant secondary antibody was incubated. 7.) Finally, the PVDF membrane developing was tested and scanned under ECL reaction liquid, and the protein strip was analyzed and stored.

RT-PCR

1.) RNA Preparation (the process was in strict accordance with the introduction of TaKaRa RNA extraction kit): (1) Grinding and homogenate of sample; (2) Extraction of total RNA; (3) Cleaning of RNA sediment; (4) RNA solution. 2.) Reverse transcription Reverse transcription condition: 37°C for 15 minutes, 85°C for 5 seconds. Production of reverse transcription was kept under -80°C refrigerator. 3.) PCR The primer sequence of TMP21 shows as follow: (1) Forward primer: 5'-cgggatccgccaccatgtctggtttgtctggcccac-3'; (2) Reverse primer: 5'-ggaattcctcaatcaatttcttggccttg-3'. β-actin served as an input control, the primer sequence shows as follow: (1) Forward primer: 5'-cgaggatccggacttcgagcaagagatgg-3'; (2) Reverse primer: 5'-cagtctagagaagcatttgcggtggacg-3'. PCR system: 2.5 μl 10× buffer; 2.0 μl dNTP; 0.3 μl Tap; 1.5 μl MgCl; 1.0 μl per primer; 2.0 μl cDNA; 14.7 μl H2O2. PCR condition: 95°C 5 min; then 94°C 30 sec, 55°C 30 sec and 72°C 45 sec for 30 Cycles; 72°C 5 min. Product of PCR was analyzed by agarose gel electrophoresis (AGE).

Results

Immunohistochemistry

In our study, ICH shows that TMP21 distributes widely in normal human tissues. The TMP21 protein is expressed at high levels in pancreas islet, heart, liver, lung, kidney, brain, and low levels in prostate gland, testicle, small intestine, colon, stomach, gall bladder, thyroid gland, adrenal gland, skin and trachea (**Figures 1**, **2**). It seems like no expression in skeletal muscle and lymphonodus. In pancreas, TMP21 expression concentrates in pancreas islet (**Figure 1A**) and low expression in exocrine gland. Renal tubule cells are the main residence of TMP21 protein in the kidney, and neuron cells in the cerebral cortex (**Figure 1E** and **1F**). Unfortunately, we lost the spleen sample in the process of ICH examination as our results were lack of the spleen.

Western blot

Western blot shows that TMP21 is expressed at high levels in heart, liver, spleen, lung, kidney, small intestine and adrenal gland, expressed at moderate levels in pancreas, cerebral cortex, prostate gland, testicle, colon, stomach, gall bladder, thyroid gland and trachea examined. There is no expression of TMP21 in skeletal

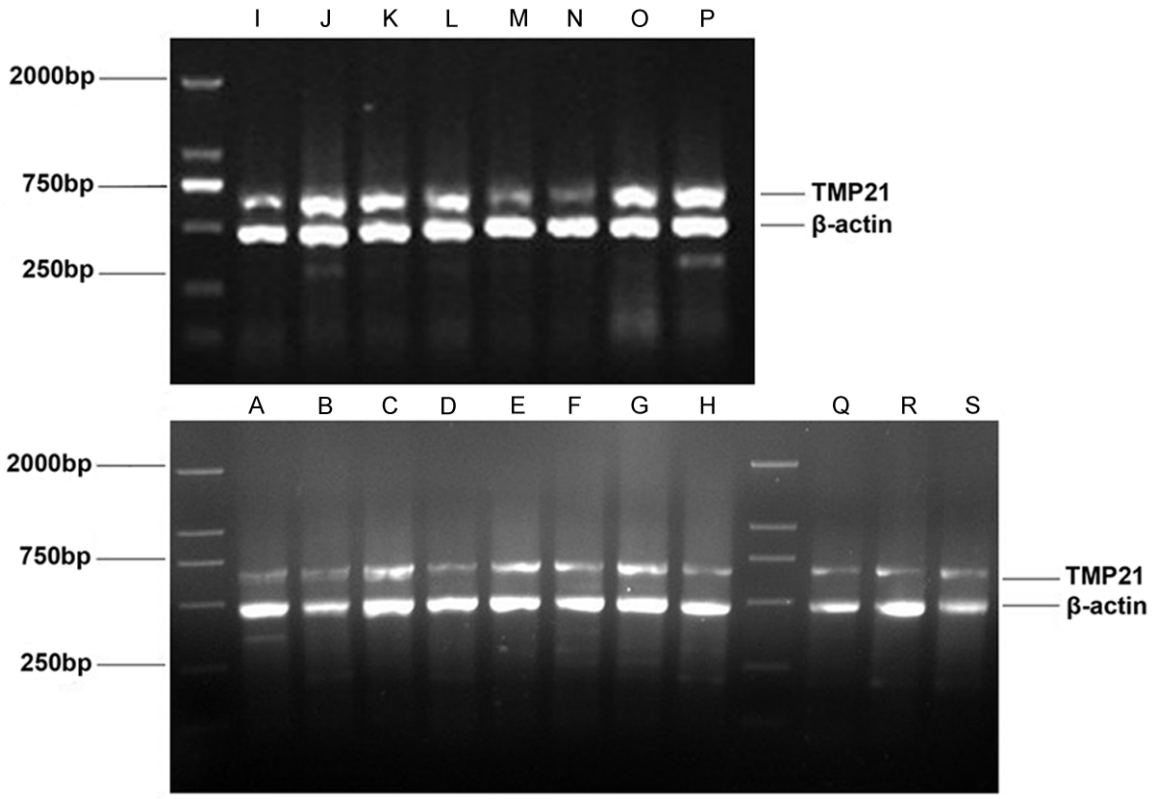

Figure 4. The expression analysis of TMP21 mRNA in human tissues. It shows that TMP21 mRNA is widely expressed in most normal human tissues. It is highly expressed in heart (B), liver (C), lung (E), skeletal muscle (H), testis (J), small intestine (K), thyroid (O), adrenal (P), and lymph node (S). It is low expressed in Pancreas (A), spleen (D), kidney (F), cerebral cortex (G), colon (L), prostate (I), stomach (M), gallbladder (N), trachea (Q). However, the expression of TMP21 mRNA is not apparently found in skin (R).

muscle, lymphonodus and skin examined (**Figure 3**).

RT-PCR

The TMP21 mRNA is also widely expressed in most normal human tissues by RT-PCR, mostly like the results of ICH. Among the previous 19 normal human tissues examined, TMP21 mRNA is relatively higher expressed in those organs: heart, livers, lung, skeletal muscle, testicle, small intestine, adrenal gland, thyroid gland and lymphonodus (**Figure 4**). No expression was found in skin examined.

Discussion

In this study, we have investigated the expression of TMP21 in normal adult human tissues. TMP21 was identified as a new member of the presenilin-associated complex [13, 19] which regulates two independent intramembranous cleavage activities, γ-secretase and ε-secretase activities. TMP21 binds the presenilin complexes and acts as a modulator resulting in the selective suppression of γ-secretase cleavage [13]. Our recent study showed that TMP21 is ubiquitinated and the degradation of TMP21 is mediated by the ubiquitin-proteasome pathway, as with the other presenilin-associated γ-secretase complex members [20] and NFAT plays a very important role in regulation of human TMP21 gene expression [21]. This study shows that TMP21 is widely and diversely distributed in 19 normal human tissues by examination of ICH, western blot and RT-PCR, which show the similar results that expression of TMP21 is at high levels in heart, liver, lung, kidney and adrenal gland, moderate levels in brain, pancreas, prostate gland, testicle, small intestine, colon, stomach, gall bladder, thyroid gland and trachea, low levels in skeletal muscle, skin and lymphonodus.

Human TMP21 protein coding gene locates at 14q24.3, nearby the coding gene of presenilin [22, 23]. TMP21 protein, a member of p24 fam-

ily protein with names of p24δ1 and p23 [2], is a type I trans-membrane protein with typical p24 family protein structures [5, 24]. In previous studies, the expression of TMP21 was reported in experimental animal tissues or a few of localizations in several human tissues. Strating et al. [25] investigated the presence of transcripts of the p24 family members in mouse tissues by RT-PCR. The results showed that the expression of TMP21 is intense in the heart, liver, lung, brain, spleen, kidney, small intestine, colon and muscle. Another study [26] has been reported on neurobiology of disease examined the distribution of TMP21 in rat brain. They found that TMP21 is expressed in major areas of adult rat brain including the septum, cortex, hippocampus, striatum, amygdala, thalamus, hypothalamus, cerebellum and brainstem. At the cellular level, there is more intense staining in the neuronal cells than in glial cells and strong staining was obvious in neo-cortex, hippocampus and pyramidal neuronal cell. We found that the expression of TMP21 protein locates at neuron cells in the cerebral cortex in adult human normal brain tissue.

In 1996, with Northern blot in rat tissues, it was found that TMP21 protein exists mostly in pancreatic acinar cells [27] and is enriched in microsomal fraction. Hosaka et al. [28] found that TMP21 protein is ubiquitously expressed at high levels in endocrine cells (pancreatic β-cells) among the mouse tissues and localized on the insulin granule membrane. Those previous studies show the similar results with our study, although we performed the examination in adult normal human tissues. We found that TMP21 protein expression is at obviously high level in pancreas islet and nearly no expression in the exocrine gland of pancreas, we deduced that maybe the reason why the expression of this protein is at moderate level expression by Western blot and RT-PCR.

The distribution and location of TMP21 protein in human tissue could indicate that it maybe has relationship with some disorders in diverse organs, such as AD, diabetes [13, 17, 28, 29]. The function of TMP21 protein is not clear now, however, it was found that the content of TMP21 protein keeps high-steady-state level during embryonic development and then declines after birth and the decline in TMP21 expression during postnatal development may significantly contribute to enhance β-amyloid production in the adult brain [27]. In diabetic patients, it has been reported [29] that about 85% of them happened with deposition of pancreatic islet amyloid [30, 31]. We deduced that TMP21 maybe plays fatal important role in progress of diabetes. In our study, we found that the expression of TMP21 is at high level in kidney and it is located in the renal tube cells. It seems like no expression in other parts of kidney. Kidney is one of the most important organs in human body, and the kidney disorders are usually tough in the clinical work [32]. So we speculate some renal disorders maybe involved in the abnormal expression of TMP21.

In conclusion, the present study demonstrates the expression of TMP21 widely in adult human tissue, especially in heart, liver, lung, kidney, pancreas islet, and in brain. Some studies were reported about the relationship between the TMP21 and diabetes, AD. Maybe there are sorts of association between this protein with some disorders with heart, liver, kidney and lung. Together, we provide for the first time a comprehensive expression of TMP21 in normal adult human tissues. Our findings will benefit on helping in the design and interpretation of future studies focused on expounding the function of TMP21.

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Disclosure of conflict of interest

None.

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