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TRANSLOCATOR PROTEIN (18 kDa): AN UPDATE ON ITS FUNCTION IN STEROIDOGENESIS

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Abstract

Translocator protein (18 kDa), TSPO, is a ubiquitous mitochondrial protein. Studies of its responses to drug and endogenous ligands have shown TSPO to be involved either directly or indirectly in numerous biological functions, including mitochondrial cholesterol transport and steroid hormone biosynthesis, porphyrin transport and heme synthesis, apoptosis, cell proliferation, and anion transport. Localized to the outer mitochondrial membrane of steroidogenic cells, TSPO has been shown to associate with cytosolic and mitochondrial proteins as part of a large multiprotein complex involved in mitochondrial cholesterol transport, the rate-limiting step in steroidogenesis. There is general agreement as to the structure and pharmacology of TSPO. Stimulation of TSPO has been shown to have therapeutic use as anxiolytics by inducing allopregnanolone production in the brain, and also potentially for reestablishing androgen levels in hypogonadal aging animals. Until recently, there has been general agreement as to the role of TSPO in steroidogenesis. However, recent studies involving genetic depletion of TSPO in mice have created controversy about the role of this protein in steroid and heme synthesis. We review herein the data on TSPO's structure and function, and recent results using various genetic animal models. Taken together, these studies suggest that TSPO is a unique mitochondrial pharmacological target for diseases that involve increased mitochondrial activity, including steroidogenesis. Although there is no known mammalian species that lacks TSPO, it is likely that due to the importance of this ancient protein in evolution and mitochondrial function, redundant mechanisms may exist to replace it under circumstances when it is removed.

Introduction

Translocator protein (18 kDa), TSPO, a ubiquitous mitochondrial protein, is a five transmembrane domain protein found as a monomer, dimer and polymer (1–3). TSPO

contains separate drug and cholesterol binding domains (4, 5). Recent structural studies by NMR and crystallography provided details regarding the atomic structure of TSPO (6, 7). The data presented confirmed the predicted structure and binding domains of TSPO, validated the role of the cholesterol binding domain and showed that the reported human single nucleotide polymorphism A147T, previously linked to anxiety disorders, affects cholesterol and specific drug ligand binding (3). These studies also suggested that the functional TSPO is a dimer [8]. There are numerous reviews on TSPO, its ligands, function as well as its use as a diagnostic marker of various disease states (2, 3, 8–13). Our goal herein is to summarize and provide an update of its function in steroid hormone biosynthesis.

Because of the high abundance of TSPO in the outer mitochondrial membrane (OMM) of steroidogenic cells, the role of TSPO in steroid biosynthesis has been widely and intensively studied using cell lines and animal models. It has been shown to be associated with cytosolic and mitochondrial proteins, part of a large multiprotein complex involved in mitochondrial cholesterol transport, the rate-limiting step in steroidogenesis (4, 14–17). In light of studies conducted over many years, there has been general agreement as to the structure and pharmacology of TSPO, the impact of TSPO drug ligands on in vitro and in vivo steroid production, and the therapeutic value of TSPO as anxiolytics in the brain and potentially in conditions of hypogonadism. However, recent reports of the effects of genetic depletion of TSPO have been controversial regarding the role of this protein in steroid and heme synthesis (18–21). We review herein the recent data on TSPO. Published cell and animal data suggest that lipid homeostasis, including esterified cholesterol involved in steroidogenesis, is dramatically affected by *Tspo* deletion/mutation. Changes in lipids and steroid production in response to pituitary peptide hormones vary between the fast responding adrenal cells, slow-responding testicular cells, and hormone-independent brain cells. We believe that the new data discussed herein will reinforce the goals of exploring the biological roles of TSPO, and the potential therapeutic importance of targeting this mitochondrial protein pharmacologically.

TSPO in structure and function in steroid hormone biosynthesis

Translocator protein (18 kDa), TSPO, is an evolutionarily well-conserved OMM protein with five transmembrane domains. TSPO was previously known as the peripheral-type benzodiazepine receptor because it was identified as a binding site for the benzodiazepine diazepam. The protein has been shown to be present in particularly high levels in the steroid-producing cells of the adrenal and testis, to localize at the OMM (22), and to be regulated by hormones (23, 24). Numerous studies conducted over the last 30 years of TSPO biochemistry and pharmacology demonstrated that TSPO specifically binds such chemicals as benzodiazepines, isoquinoline carboxamides and indole acetamides, as well as endogenous ligands including porphyrins, the polypeptide diazepam binding inhibitor (DBI), and cholesterol [reviewed in (2, 4, 8, 10, 25, 26)]. Structural studies of TSPO revealed a five helix bundle model of the protein and its further association into a homodimer and suggested that drug ligand binding can promote cholesterol movement by TSPO (3, 7, 27). These results are consistent with biochemical results.

Diazepam and other benzodiazepines were shown to modulate circulating steroid levels (1, 28). DBI (des-(Gly-Ile)-endozepine) was shown to stimulate cholesterol transport into mitochondria, thus relating steroidogenesis to benzodiazepines (29, 30). This led to pharmacological studies addressing the effects of TSPO-binding compounds on corticosteroid production by adrenal cortical cells, testosterone formation by testicular Leydig cells, and neurosteroid synthesis by glial cells (1, 10, 30–32). Exposure of steroidogenic cells and of mitochondria of steroidogenic cells to TSPO ligands, at concentrations close to their binding affinities, were found to stimulate steroid synthesis (1, 32, 33); and compounds that blocked TSPO action blocked hormone-induced steroid formation in cells and in vitro and in vivo (34–37). TSPO was shown to be a high-affinity cholesterol binding protein, with cholesterol affinity localized to the C-terminal end of transmembrane helix 5 (TM5) at a conserved cholesterol recognition amino acid consensus (CRAC) domain ((5, 38). Mutations of particular amino acids of the CRAC domain were shown to eliminate TSPO's ability to bind cholesterol (39). Though the CRAC motif, defined by the linear array (L/V)-X1-5-(Y)-X1-5-(K/R), is probably the most widely used sequence-based tool to predict the presence of a cholesterol-binding domain in a protein, an alternative cholesterol consensus motif was identified in some cholesterol binding proteins, named the "CARC" domain, which is a reverse version of the CRAC algorithm: (K/R)-X1-5-(Y/F)-X1-5-(L/V) (40). Both motifs are characterized by a triad of basic (Lys/Arg), aromatic (Tyr/Phe) and aliphatic (Leu/Val) amino acid residues within the same membrane-spanning domain. Interestingly, TSPO contains both CRAC and CARC cholesterol binding domains on the 5th transmembrane alpha-helix (Fig. 1). Although specific amino acid substitution functional studies on the CARC motif of the nicotine acetylcholine receptor indicated slowed kinetics of cholesterol binding, it is not known whether CARC is functional in TSPO (41).

Studies of the effects of TSPO drug ligands in brain suggested pharmacological means by which neurosteroid formation might be regulated both *in vitro* and *in vivo* (10, 26, 42), and by which neuropsychiatric and neurodegenerative disorders as well as neurotrauma, therefore, might be treated (24, 43). Indeed, TSPO, an OMM protein, is widely distributed in brain, dominantly in microglia cells (Fig. 2) which play a major role in neuroinflammation. Induction of neurosteroid formation in the brain by TSPO drug ligands has been used to alleviate neuropsychiatric disease symptoms (10). Etifoxine (6-chloro-N-ethyl-4-methyl-4-phenyl-4H-benzo[d][1,3]oxazin-2-amine), a TSPO/GABA_A drug ligand with anxiolytic and anticonvulsant properties, is approved and marketed in France for the treatment of anxiety disorders, and studies are underway to treat peripheral nerve injuries and axonal neuropathies by regulating neurosteroid levels (44–46). Moreover, a series of studies have supported a role for TSPO in inflammatory processes in peripheral tissues and the nervous system as a response to injury and disease; this role could be either passive, TSPO being the result of increased mitochondrial membrane biogenesis occurring during microglia proliferation, or active possibly through the regulation of reactive oxygen species (ROS) and local steroid production (47, 48). Interestingly, the process of steroid formation, mediated by cytochrome P450s monooxygenases, is one of the drivers of ROS production in steroidogenic cells (49, 50).

Importantly, the stimulatory effects of TSPO drug ligands on adrenal, brain and gonadal steroids were relatively minor in normal (control) *in vivo* settings. A possible explanation is that gonadal and adrenal cells are regulated by pituitary hormones, and brain cells by local factors, and these are the same hormones and local factors that control TSPO activity. In contrast, with reduced central or local control, it appears that the ligand activation of TSPO in effect replaces central or local control and has notable effects on cholesterol import into mitochondria. For example, recent *in vitro* and *in vivo* studies of aging Brown Norway rats, in which there is primary hypogonadism, demonstrated that the pharmacologic activation of TSPO with drug ligands induced testosterone formation (51). Testosterone formation also was induced in the case of secondary hypogonadism in young rats. In the latter studies, the GnRH antagonist Cetrorelix was used to induce chemical castration. Treatment of Cetrorelix-treated animals for four days with the TSPO specific drug ligand FGIN-1-27 resulted in the recovery of testicular testosterone production (52). These results were repeated using the TSPO ligand XBD173 (4), suggesting that these compounds can induce testosterone production by hypofunctional Leydig cells in an LH-independent manner.

Several proteins have been identified that are associated with TSPO, forming functional complexes (53, 54). Together, these proteins have been proposed to function as an OMM signal transduction complex, or “transduceosome,” identified by protein crosslinking in steroidogenic cells. The transduceosome is linked to a mitochondrial “metabolon” (16, 55), a term that refers to multiprotein complexes that perform a series of reactions on a particular substrate. The metabolon links the OMM transduceosome to the inner mitochondrial membrane (IMM) CYP11A1 and therefore acts as an intermediate between cholesterol import into the mitochondria and its conversion to pregnenolone. The mitochondrial metabolon overlaps with the transduceosome at the OMM, with TSPO and voltage-dependent ion channel (VDAC1) considered members of both complexes.

Genetic deletion of *Tspo* in cell models

Despite studies conducted over the course of many years that have produced strong evidence for a highly significant role of TSPO in steroid hormone biosynthesis, recent studies based largely on genetic animal models have called this conclusion into question.

Knockdown of TSPO in tumor cell models has been achieved by homologous recombination (56), antisense oligodeoxynucleotides (57), and antisense knockdown (58). In each case, steroid biosynthesis in steroidogenic cells was reduced. Admittedly, however, it is technically challenging to be certain as to whether the effects seen on steroidogenesis in such studies were specifically a consequence of TSPO knockdown or reduced cell viability (58).

A recent study reported no effect of TSPO knockdown in MA-10 cells (20). In this study, no change in progesterone production was seen 48 hours after knockdown of TSPO expression by transfection with siRNAs. This was in striking contrast to previous reports showing significant reduction of steroid production in the same cell line after TSPO knockdown using antisense oligodeoxynucleotides (59) or antisense knockdown (58). As yet, the explanation for the difference in results is uncertain. In subsequent studies, Selvaraj and

colleagues suggested that the stimulation of progesterone formation in MA-10 cells by incubating the cells with the TSPO ligand PK 11195 may be unrelated to the ability of the ligand to bind TSPO (20). This conclusion was reached by their studies reporting that the ligand was able to stimulate progesterone production in *Tspo* KO MA-10 cell lines generated via CRISPR/Cas9 technology (20, 60). Unfortunately, no PK 11195 binding data was shown in the study, making it unclear how the conclusion could be reached that the TSPO deletion was complete and therefore that the effect of PK 11195 was not through TSPO. Moreover, TSPO drug ligands, when used at high micromolar concentrations, have been shown to have non-specific effects on various cellular functions (4). At nanomolar and low micromolar concentrations, however, the actions and effects of drug ligands such as PK 11195 are specific to TSPO, as shown by stereospecific chemical crosslinking, detailed pharmacological binding studies, and screening for other known drug targets (61–64). These factors were not considered in the Selvaraj paper. Moreover, we recently generated *Tspo* KO MA-10 cell lines via CRISPR/Cas9 technology and observed that these cells make little to no steroids and that PK 11195 stimulation did not affect steroid biosynthesis in the TSPO-deficient cells (65).

One of the main arguments used by Selvaraj and colleagues that TSPO plays no role in steroidogenesis is that the adrenal tumor H295R cells do not contain TSPO but produce steroids (66). Indeed, the authors cite this observation as the “gold standard” to debunk the role of TSPO in steroid biosynthesis. However, previous studies reported that H295R cells do indeed express TSPO and that its levels were regulated by cAMP (67). We confirmed this finding by immunoblot analysis (unpublished), and previous studies demonstrated high levels of expression of TSPO in normal human adrenal specimens (68).

Genetic deletion of *Tspo* in animal models

Deletion of the *Tspo* gene in embryonic stem cells used to generate mouse knockouts resulted in significant embryonic lethality (69). Examination of the *Tspo* locus revealed that two genes, tubulin tyrosine ligase-like family member 12 (*Tll12*) and mitochondrial malonyl-CoA:ACP acyltransferase (*Mcat*), have partial overlap at this locus (4). It also should be noted that the *Tspo* gene locus encodes 48 predicted miRNAs, and the TSPO coding DNA sequence is the target of 23 miRNAs (4). Thus, a number of biological systems are networked. It is possible, therefore, that the deletion of *Tspo* or changes in the networked genes may be responsible for the observed embryonic lethality.

Tspo deletion by P-element insertion, whole body dsRNA knockdown, and inhibition by PK 11195 in *Drosophila* resulted in flies resistant to apoptosis induced by H₂O₂ exposure, prolonged fly lifespan, and protected the flies from neurodegeneration (70). These phenotypes all are related to altered mitochondrial function that includes reduced mitochondrial respiration and increased mitochondrial oxidative stress (71). However, the latter does not increase the ROS-generated macromolecular damage (70), indicating that the effect of TSPO loss is reversible and condition-dependent. Given that the TSPO is an OMM conserved protein throughout evolution, it seems likely that it would play important roles in rodents and human. Indeed, TSPO has been suggested to be involved in controlling lifespan (72).

TSPO also has been proposed to play a role in the mitochondrial permeability transition pore (mPTP), which is an inner membrane event associated with specific interactions with TSPO on the OMM during the permeability transition (73, 74). TSPO drug ligands were shown to modulate mPTP (75, 76). The same author(s) made the *AlbCre* (2.34 kb mouse albumin enhancer/promoter)-mediated conditional KO (cKO) of the gene and then concluded that TSPO plays no role in the regulation or structure of the mPTP (73). The data presented show no changes between wild-type (WT) vs. cKO, including basic mitochondria properties such as morphology, the rate of respiration, mitochondrial membrane potential, and Ca^{2+} retention capacity. This was a surprising finding considering that numerous papers have reported that TSPO drug ligands and anti-TSPO antibodies affect mPTP function and that TSPO is the main component of mPTP (75–79). The question raised is whether there is a compensatory mechanism capable of replacing TSPO function in regulating the permeability transition pore.

The role of TSPO in mediating mitochondrial cholesterol import for steroid hormone production has been questioned after a report on testis-specific *Tspo* KO using *Amhr2-Cre* (18). The authors reported that genetic knockout of TSPO in the Leydig cells had no effect on testosterone production, and concluded that “TSPO function is not essential for steroid hormone biosynthesis.” As we were unsuccessful in obtaining the generated mouse line from the authors, we generated the same *Amhr2-Cre* line of mice ourselves to produce testis-specific *Tspo* KO. Though we tried several times, were unable to generate the *Tspo* cKO mouse that had been described (18) due in part to early embryonic lethality/adverse effects. However, all the mice with the cKO-genotypes had the same TSPO expression as the WT controls (80). Additionally, we obtained an only small percentage of mice with such a cKO-genotype, which seems to be the survival from the “Cre-loxP” breeding system. After we published the results, we realized that the two genes, *Tspo* and *Amhr2-Cre*, have strong genetic linkage with 18.1 cM genetic distance, which makes the system difficult to be used for the testis tissue-specific cKO. Moreover, the *Amhr2* gene is expressed at early pre-implantation stages in the mouse. These observations suggest that the *Tspo* gene could be deleted at early preimplantational stages. We are continuing this work so as to identify the specific role of TSPO in preimplantation in relation to the identified genetic linkage. It is curious that Selvaraj and colleagues failed to have or to report the same problem using *Amhr2-Cre*.

To further examine the role of TSPO in steroidogeneses in a mouse model, we obtained a different Cre line, the *Nr5a1(Sf-1)-Cre* mouse, to generate the *Tspo* cKO mouse (80). The *Nr5a1* gene was shown to be specific to the testis, adrenal, and brain, the steroidogenic tissues in which we were interested. We were successful in obtaining mice with adrenal gland-specific *Tspo* cKO in which TSPO was almost completely removed in the cortex area. In the testes of these mice, TSPO was only partially depleted, as seen by immunofluorescence staining, and there was no TSPO depletion in the brain. These patterns of TSPO depletion were found to correlate well with increased presence of cholesterol-enriched lipid droplets in steroid synthesizing cells and with reduced steroid production.

A human glial fibrillary acidic protein (*hGFAP*)-Cre-mediated *TSPO* KO mouse was generated to produce an astrocyte- and neural precursor- specific TSPO cKO (81). Although

TSPO^{-/-} mice showed reduced astrogliosis and clinical symptoms in experimental autoimmune encephalomyelitis, accompanied by significant decreases in mRNA levels of cytokines TNF α and CXCL10, the GFAP was also found to be expressed in kidneys, testis, bone, cartilage, pancreas and liver (82–85). No data on brain steroid levels were presented, and no effects of TSPO drug ligands on the mutant animals were shown.

Mouse *Tspo* exon2/3 was deleted globally for the first time via C57BL/6 Cre deleter mice (the mouse used was not specified) to make a *Tspo* global knockout (gKO) mouse (86). The authors verified the TSPO depletion by immunostaining as well as by the use of TSPO radioligands, including [³H]PK 11195. The *Tspo*^{-/-} mice showed no significant changes in body length, body weight, blood pregnenolone concentration, blood protoporphyrin IX, or expression of *Star* or *Cyp11a1* mRNAs. However, there was a significant reduction in mitochondrial metabolism (oxygen consumption rate, OCR; and extracellular acidification rate, ECAR) in the *Tspo*^{-/-} mice, consistent with an impaired mitochondrial function that also had been observed in *Drosophila* (70). In this study, likely compensatory mechanisms were proposed though not specifically defined (87).

Following the *Tspo* cKO using *Amhr2*-Cre, Selvaraj and colleagues reported a second *Tspo* gKO using a global “Cre deleter” - *Ddx4*-cre. The data presented showed that *Tspo* gKO was identical to the WT phenotype (88). In this report, the authors described no difference in the plasma level of steroid hormones, steroidogenic gene expression in the adrenal glands, the ultrastructural morphology of adrenal cortex and mitochondria, and lipid deposition in the adrenal cortex. A few genes related to immunity were reported to be differentially expressed by *Tspo*^{fl/fl} and *Tspo*^{-/-}. Interestingly, *Tspo* was not among the genes listed as having differential expression. The same animals were shown to have no difference in heme biosynthesis, but the authors reported low OCR, low ATP production, and low proton leaking in fibroblasts, as previously reported in microglia (21, 86). Unlike the data presented by Banati and colleagues (86), the authors did not observe that mice with TSPO depletion possessed a low metabolic rate. The authors proposed the likely presence of an unknown compensatory mechanism replacing TSPO in steroidogenesis.

Another group used the protamine-Cre deleter to produce a *Tspo* gKO mouse line (89). A normal Mendelian ratio from heterozygote (HE) $\times$ HE pairs was observed, as was normal morphology in the *Tspo* gKO mice. Interestingly, the RNA-seq data from lung tissues of *Tspo* gKO mice were found to have a transcriptomic profile identical to the WT mice. It is difficult to understand how TSPO depletion with low metabolic rate would fail to be reflected in the gene expression profile of lungs (86). Nevertheless, this study reported, using immunoblot analyses, that TSPO was expressed at an extremely low level in brain, which is in contrast to what was reported using immunofluorescence staining of TSPO, radioligand binding studies and positron electron tomography imaging (86, 90–92). It is unfortunate that the authors did not try to use complementary approaches to validate their findings.

Conclusions and perspectives

As noted earlier, it is clear from biochemical and pharmacological analyses that TSPO is a mitochondrial protein that can be targeted to increase steroid production in various states of

reduced endogenous steroid synthesis and/or for hormone-independent steroid production, including neuropsychiatric disorders and hypogonadism. With the exception of one report (20) that we were unable to replicate, studies of steroidogenic cells of various origins and cell lines demonstrate clearly that TSPO is a cholesterol-binding protein that plays an important role in steroidogenesis. The discrepant finding in the literature makes it apparent that serious attention should be paid in future studies to such methodology and materials as anti-TSPO antibodies, incubation conditions, the presence of other factors that could affect TSPO levels, and action of its ligands. Complementary methodology (e.g. immunoblots coupled to ligand binding studies, chemical crosslinking, displacement studies), as well as detailed pharmacology using drugs of various affinities (e.g. stereospecific, modified, devoid of affinity for TSPO) should be used to confirm and validate data obtained with any single method. The recent discovery of different occupancy times for various TSPO drug ligands for TSPO (93, 94) should be seriously considered in future in vitro and in vivo studies of TSPO drug ligands.

The only cell-specific deletion of TSPO in steroidogenic cells achieved, using *Nr5a1(Sf-1)-Cre*, clearly demonstrated a role for TSPO in steroidogenic cell lipid homeostasis in adrenal and testis, and a significant effect on ACTH-induced steroid formation. Although there is a tendency to equate the steroidogenic ability of the adrenal, gonadal and brain tissues, they are distinct, with the adrenal being a highly active and rapid responder (stress-response occurring within minutes), the testis a slower responder (hours to days), and the brain a constitutive responder (hormone-independent). These differences may also reflect differences between faster and slower lipid homeostasis and steroid formation. It is possible that TSPO functions somewhat differently in these three tissues.

Global deletion of *Tspo* appears to have a limited effect on animal phenotype, though as yet there have been no studies performed under various challenges (food, stress, drugs). It seems likely that there are compensatory mechanisms when TSPO is deficient. Maintaining separate breeding colonies for *Tspo*^{-/-} and *Tspo*^{fl/fl} mice (88) may not be appropriate. As *Tspo*^{-/-} mice represent the research subject under investigation, perhaps HE paired with WT mice should be used to maintain colonies, and HE × HE pairs used to produce the homozygous (HO) mice for experiments. This would avoid adaptive compensatory mechanisms. Control animals should not be from HE × HE pairs, but from the “born to be” WT mice. It should be kept in mind that deletion of TSPO might trigger the expression of a replacement protein, or a cholesterol-binding protein already present might replace the function of TSPO. Still to be determined is why removal of the entire *Tspo* gene versus disruption of the gene resulted in two distinct phenotypes (lethal vs WT-like).

Mitochondrial function is a critical component of cellular function. Thus, the presence of redundancy in mechanisms that mediate crucial mitochondrial functions would be expected. This is true of the OMM VDAC for example. Mice in which *Vdac* has been knocked out nonetheless are viable and display no apparent changes in phenotype (95, 96). Consistent with this, mitochondria from *Vdac1*^{-/-} and *Vdac3*-null mice and from cell lines in which *Vdac1*, 2 and 3 were knocked out had no changes in mitochondrial permeability transition pore activity (96, 97); this although VDAC has a key role in this activity. These results suggest redundancy, with other proteins taking over VDAC function when VDAC is deleted.

This also is true of the IMM adenine nucleotide translocator 1 (ANT1), a critical component of mitochondrial oxidative phosphorylation (98). Thus, *Ant1*^{-/-} mice were viable and fertile, with growth characteristics comparable to those of WT mice (98). More recently, redundancy in the mitochondrial carrier system transporting small molecules between mitochondria and the cytoplasm was shown (99) and loss of one could be replaced by another gene or genetic network depending on the methodology used (100, 101). These findings suggest functional redundancies for key mitochondrial proteins including VDAC1, ANT1, and TSPO, assuring that their functions are carried out.

The generation of animal models with genetic deletions of domains critical for TSPO function, such as the CRAC domain, and the use of other animals, e.g. rats, to generate *Tspo* KO mice would be of great use in addressing some among the many mechanistic issues involved in steroid formation as well as other critical processes. However, the ultimate model is the human. No human has been identified who lacks *TSPO*. However, the presence of rs6971 polymorphism (A147T) on the *TSPO* gene results in an altered binding affinity of TSPO for specific ligands (102) and influences pregnenolone production in lymphomonocytes of healthy individuals (103). Rs6971 also has been proposed to affect the hypothalamic-pituitary-adrenal axis, predisposing carriers to psychiatric disorders (9, 103–105). Moreover, the presence of rs6971 was shown to affect the response of patients to anxiolytic TSPO drug ligands (106). These observations raise critical questions of pathophysiological significance. For example, do carriers of rs6971 have differences in circulating steroid levels or do they respond in a different way to hormone stimuli or stress?

TSPO is a 3.5-billion-year-old protein (17). Defining its structure, function and pharmacology will allow us to better understand the evolution of a protein, an organelle, and processes (such as steroidogenesis) that are critical for life. We hope that this review will provide the reader a critical evaluation of the state of the field, and help advance the science of TSPO.

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Figure 1.

TSPO structure, cholesterol binding and topology. A. The two cholesterol-binding domains of TSPO, CRAC and CARC, are illustrated in the three-dimensional NMR structure. The ability of the TSPO CRAC, but not CARC, motif to bind cholesterol has been experimentally demonstrated. The theoretical prediction indicates an evolutionary origin, and likely cholesterol “flip-flop”; both domains can bind cholesterol from the outer phospholipid bilayers of mitochondrial membrane. PK 11195, a diagnostic ligand of TSPO, is indicated. B. Transmembrane topology of TSPO in the OMM, with an evolutionary view of the topologic transition from bacterial to mammalian TSPO (107). A bacterial like-topology is presumably present in mammalian TSPOs.

Figure 2.

TSPO, an OMM protein widely distributed in brain. A. A confocal image of DsRed-tagged mouse TSPO. The presence of TSPO on the OMM is shown by confocal live-cell imaging. MA-10 cells were used to express the DsRed-TSPO protein in red; mitochondria were marked as mito-roGFP in green; and DNA was stained by Hoechst 33342 in blue. B. Immunofluorescence staining of brain TSPO using rabbit anti-TSPO monoclonal antibody. The presence of TSPO in brain hippocampus is likely dominantly in glia and microglia. Rabbit anti-TSPO monoclonal Ab stains TSPO in red; co-staining with DAPI for DNA.