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The changing landscape in translocator protein (TSPO) function

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Translocator protein (TSPO), previously known as the peripheral benzodiazepine receptor (PBR), is an outer mitochondrial membrane protein. TSPO has been shown to cooperate with steroidogenic acute regulatory protein (StAR) and function in the transport of cholesterol into mitochondria. TSPO has also been considered as a structural component of the mitochondrial permeability transition pore (MPTP). However, recent advances have changed these views of TSPO's functions and have prompted a re-evaluation of established concepts. This review summarizes the history of TSPO, key elements of the debate, and functional experiments that have changed our understanding. Moving forward, we examine how this fundamental change impacts our understanding of TSPO and affects the future of TSPO as a therapeutic and diagnostic target.

TSPO: a protein with a long history

The function of TSPO, previously known as the PBR (see Glossary), has been a topic of active research for the past 25 years. It was initially described in 1977 as a peripheral receptor for benzodiazepines distinct from the central nervous system benzodiazepine receptor [the gamma-aminobutyric acid type A ($GABA_A$) receptor] and since then its pharmacological characterization has been extensive [1,2]. The specificity of chemicals like PK11195 (an isoquinoline carboxamide derivative) and Ro5-4864 (4′-chlorodiazepam) that could bind to TSPO with high affinity but not to the $GABA_A$ receptor was exploited to study potential cellular actions mediated by TSPO such as in steroidogenesis and apoptosis [3]. The protein sequence of TSPO is relatively conserved from bacteria to humans (Figure 1), suggesting a possible evolutionarily conserved fundamental role for TSPO in cellular and organismal physiology [4].

Early studies highlighted predominant TSPO localization to the mitochondrial outer membrane. Although TSPO was widely detected in multiple organs, its expression appeared particularly high in steroid hormone-producing cells of the adrenal glands, testes, and ovaries. Two key studies linked TSPO function to steroid hormone biosynthesis. First, TSPO binding by PK11195 and Ro5-4864 could induce steroid hormone production in both adrenocortical and Leydig tumor cell lines [5,6]. Second, TSPO knock down or disruption by homologous recombination in rat Leydig tumor cells could decrease steroid hormone production [7,8]. These studies were deemed highly significant because they demonstrated that TSPO's effects were independent of StAR, a key player in mitochondrial cholesterol transport required for steroidogenesis (Box 1). As a result, TSPO was thought to function as the channel that receives cholesterol from StAR and mediates its transport to the mitochondrial inner membrane. This steroidogenic function for TSPO was the most studied among all of its implicated properties. Given this putative link, up- or downregulation of TSPO expression in different tissues has been considered a direct indication of steroid hormone production in numerous studies [9–11]. Results that describe TSPO and steroidogenesis have been highlighted in recent literature reviews on this topic [12,13].

Copurification of TSPO with key molecular components previously implicated to form the MPTP resulted in its inclusion as a regulator of this process [14]. The mitochondrial permeability transition (MPT) refers to a sudden

Glossary

Benzodiazepines: a class of drugs that are clinically used as muscle relaxants, anticonvulsants, and sedative–hypnotics. The pharmacology of benzodiazepines is primarily mediated by binding to the 'central' benzodiazepine receptor (the $GABA_A$ receptor) located in the central nervous system and potentiating inhibitory neurotransmission.
Mitochondrial cholesterol transport: mitochondria are cellular organelles that comprise a double membrane creating two distinct internal compartments. For steroid hormone production, cholesterol must be transported through the outer mitochondrial membrane across the aqueous intermembrane space to the matrix side of the inner mitochondrial membrane before it can be converted to pregnenolone. This is an essential and rate-limiting step in steroid hormone biosynthesis.
Mitochondrial permeability transition (MPT): an increase in the permeability of the inner mitochondrial membrane to any molecule <1.5 kDa due to opening of a nonspecific pore. The MPT has been associated with the cell death seen in numerous pathological conditions.
Mitochondrial permeability transition pore (MPTP): the structural components that form the pore in the inner mitochondrial membrane during the MPT. Recent evidence suggests that this is formed by dimers of F_0F_1 ATP synthase.
Peripheral benzodiazepine receptor (PBR): benzodiazepines were observed to also occupy 'peripheral' sites distinct from the central $GABA_A$ receptor on the outer mitochondrial membrane. The PBR was the protein characterized based on this distinct pharmacology that was subsequently renamed as TSPO.
Steroidogenesis: the biosynthesis of steroid hormones. In this process, steroid hormone-producing cells convert cholesterol into pregnenolone in an enzymatic step catalyzed by CYP11A1/P450scc that occurs in the mitochondrial matrix. Pregnenolone is then converted into various classes of steroid hormones by subsequent enzymatic steps within the endoplasmic reticulum and the mitochondria.

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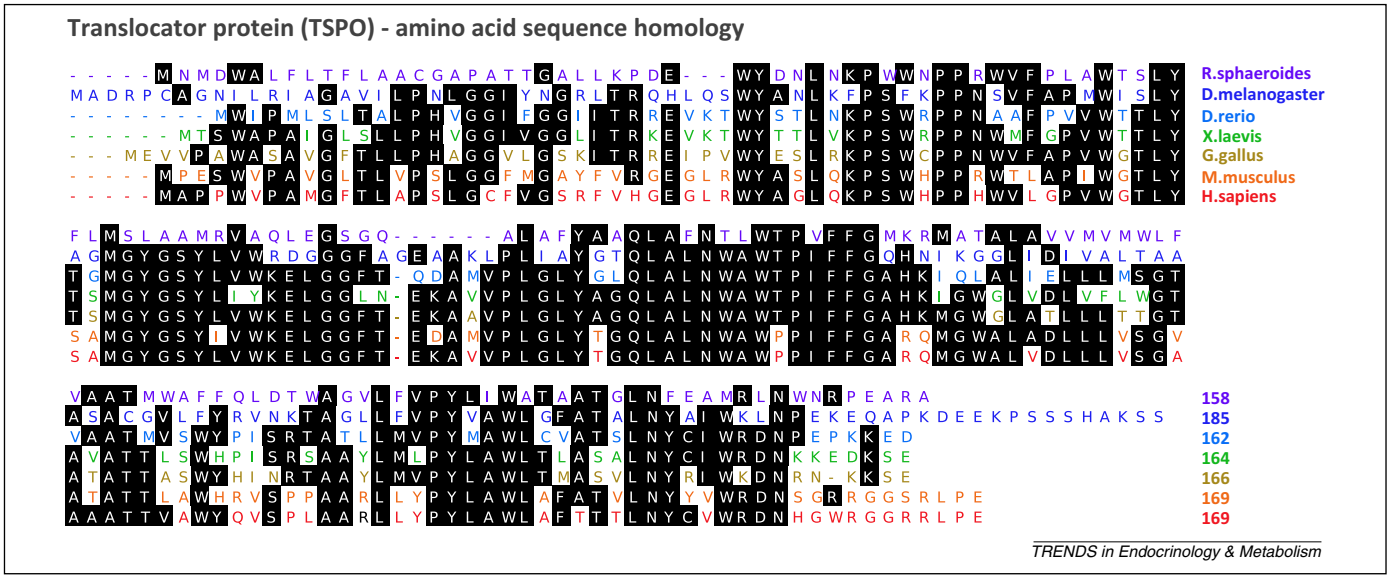

Figure 1. Translocator protein (TSPO) amino acid sequence homology. TSPO amino acid sequence comparisons showing relatively conserved consensus sequences (shaded) in various model organisms. Percentage identity with *Homo sapiens*: *Rhodobacter sphaeroides*, 33.5%; *Drosophila melanogaster*, 42.6%; *Danio rerio*, 54.3%; *Xenopus laevis*, 57.3%; *Gallus gallus*, 60.4%; and *Mus musculus*, 81.1%.

increase in permeability of the inner mitochondrial membrane, a mechanism that has been associated with the cell death seen in various human pathologies (Box 2). Early biochemical studies seeking core MPTP components identified the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane, the adenine nucleotide transporter (ANT) in the inner mitochondrial membrane, and Cyclophilin D (CyPD) in the matrix [15]. The demonstration that TSPO could be copurified with the VDAC and ANT resulted in attempts to dissect the effects of TSPO-binding chemicals as a means to regulate the MPT. The chemicals PK11195 and Ro5-4864 were found to affect MPTP opening [16,17]. The endogenous protoporphyrin IX, a heme precursor that binds with high affinity to TSPO, could also mediate MPTP opening [18]. As a result, the VDAC/ANT/TSPO model became widely accepted as the structure of the MPTP.

Subsequent genetic analysis of the putative core components of the MPTP systematically excluded a role for all genes encoding VDAC [19] and ANT [20] in the MPT. These findings set the field on a new course that led to the recent definition of the molecular nature of the MPTP as being formed by dimers of F_0F_1 ATP synthase [21]. Although a role for TSPO was not discussed in the new model, it remains to be explained how TSPO-binding chemicals could modulate cell death processes. The pharmacological evidence supporting a TSPO link to the MPTP appears well documented [22–25]. Developments in the field of the MPT were extensively described in a recent review on this topic [26].

TSPO as a biomarker in human disease pathology

TSPO expression in the central nervous system is very low under normal physiological conditions and restricted to astrocytes and microglia. However, in response to brain injury and inflammation, TSPO levels dramatically increase in these glial cells [27]. First identified for their pharmacological binding profile, use of selective and high-affinity TSPO-binding chemicals like PK11195 labeled with radioisotope tracers aided in the visualization of affected brain regions in several neurodegenerative diseases [2,28]. This prospect led to extensive efforts to develop novel synthetic chemicals that bind with high affinity and specificity to TSPO for diagnostic imaging *in vivo* [28]. Use of TSPO as a diagnostic marker has been reliable and several TSPO-binding chemicals as positron emission

Box 1. Mitochondrial cholesterol import and steroidogenesis

The first enzymatic step in the biosynthesis of steroid hormones involves the cleavage of the side chain of cholesterol to form pregnenolone. The cytochrome P450 side chain cleavage (CYP11A1) that performs this reaction resides on the matrix side of the inner mitochondrial membrane. To produce steroid hormones in response to trophic hormone stimulation, it is essential that cholesterol crosses the outer mitochondrial membrane, the aqueous intermembrane space, and the inner mitochondrial membrane. This cholesterol import mechanism also forms a regulatory step that is rate limiting for steroid hormone production. It was discovered that rapid *de novo* synthesis of StAR plays an indispensable role in the mitochondrial cholesterol import required for adrenal and gonadal steroidogenesis (reviewed in [75]). Mutations in StAR were found to be the basis of lipoid congenital adrenal hyperplasia and, similar to this human condition, StAR gene deletion in mice led to an almost complete inability to synthesize steroid hormones. Nevertheless, the gap in understanding of the precise mechanism by which StAR mediates mitochondrial cholesterol import, and the fact that StAR is not expressed in steroidogenic cells of the human placenta, led to speculation that another player that works at the level of the outer mitochondrial membrane in cooperation with StAR may be essential. TSPO filled the void in this model with overwhelming pharmacological indicators but without direct evidence. The recent demonstration that TSPO may not be involved in steroidogenesis has reopened this informational gap in the cholesterol import model.