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Commentary

Eubacterial signal transduction by ligands of the mammalian peripheral benzodiazepine receptor complex

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Benzodiazepine receptors have been widely studied because they represent the sites of action for many commonly used anxiolytic, anticonvulsant, muscle-relaxant, and hypnotic drugs (1–4). In the central nervous system, the plasma membrane benzodiazepine receptor is the primary target for these drugs (1). However, the mitochondria of all tissues tested contain a second, so-called peripheral benzodiazepine receptor that often binds both benzodiazepines and isoquinolines with high affinity (2–5). Despite the pharmacological importance of the peripheral benzodiazepine receptors, relatively little is known about the molecular events that result from ligand binding. The significant amino acid sequence similarity between a peripheral benzodiazepine receptor subunit that binds isoquinolines (pk18, an 18-kDa subunit of the mitochondrial benzodiazepine receptor) and proteins from purple photosynthetic bacteria (6–9) was previously used to suggest that prokaryotes might have related activities. In a potentially landmark set of experiments, this issue of the *Proceedings* shows that pk18 can function in a eubacterial signal transduction pathway. Specifically, Yeliseev *et al.* (10) demonstrate that rat pk18 complements a mutant which lacks a member of the pk18 family (the tryptophan-rich sensory protein, TspO, from the photosynthetic bacterium *Rhodobacter sphaeroides*), that pk18 binds a characteristic isoquinoline ligand in this foreign host, and that pk18 allows isoquinoline-dependent gene expression. These observations suggest the exciting possibility that fundamental aspects of the receptor and the downstream signal transduction pathway are conserved in eubacteria and higher eukaryotic mitochondria.

Similarities in *trans*-Membrane Signaling by pk18 and TspO

The location of pk18 in the mitochondrial outer membrane allows it to transmit signals from the cytoplasm to the mitochondrial inner membrane or matrix (2–4). By analogy, the existence of TspO in the outer membrane of Gram-negative bacteria like *R. sphaeroides* (9) positions it to pass external signals to the periplasm, inner membrane or cytoplasm. The ability of pk18 to function in *R. sphaeroides* suggests fundamental similarities in the process of *trans*-membrane information transfer (10).

In considering how pk18 could participate in *R. sphaeroides* signal transduction, it is significant to note that current views of the peripheral benzodiazepine receptor depict it as a multisubunit complex that crosses the outer and inner mitochondrial membranes (2, 5). A ternary complex of pk18, the voltage-dependent anion channel, and the mitochondrial adenine nucleotide carrier is believed to be required for a functional response to the numerous ligands that control different downstream pathways (2–5, 11–13). Isoquinoline binds to pk18 at a high affinity, whereas benzodiazepines react with subunits believed to comprise part of the mitochondrial voltage-dependent anion channel (5). By analogy, *R. sphaeroides* outer membranes also contain a 47-kDa protein that binds the benzodiazepine, flunitrazepam (8). Thus, it is possible that TspO and pk18 each interact with the 47-kDa benzodiazepine-binding protein to form a complex with properties reminiscent of the peripheral benzodiazepine receptor (5). Formation of such a complex could allow ligand-induced conformational changes to alter activity of downstream components in the presumed TspO signal transduction apparatus.

Both pk18 and TspO Are Positioned to Transmit Signals Across a Membrane

It is also remarkable that pk18 is found in the *R. sphaeroides* outer membrane (10), similar to its location in mitochondria (2–5). Movement of pk18 to the mitochondrial outer membrane without a N-terminal targeting sequence is not surprising since it is nuclear-encoded (4). Export of pk18 to the bacterial outer membrane (10) indicates that it must have retained targeting information from a presumed eubacterial ancestor like TspO.

The ability of pk18 to restore TspO-dependent gene expression in *R. sphaeroides* also suggests that this mammalian protein allows a presumed receptor to respond to a signal normally recognized by its prokaryotic counterpart. Current models suggest that the five short α-helical regions of pk18 are preferentially associated with the external leaflet of the mitochondrial outer membrane (14). If pk18 and TspO were similarly located on the external side of the bacterial outer membrane, receptor complex function could respond to environmental signals like oxygen (8). In this regard, function of outer membrane signal transduction receptors could dictate a structure and topology for TspO and pk18 that is different from well-studied bacterial porins (15).

Isoquinoline Binding by pk18 or TspO

Despite the potential of mitochondrially targeted drugs to interfere with peripheral benzodiazepine complex function, relatively little is known about what domains of pk18 form the high-affinity isoquinoline binding site, its stoichiometry in the ternary complex, how it interacts with other subunits, or how ligand binding alters function (2–4). Specific isoquinoline binding to pk18 and its ability to stimulate TspO-dependent target genes in a TspO mutant should provide a genetic system to identify domains that ligands or other subunits of the presumed signal transduction apparatus.

pk18 Family Members Are Not Ubiquitous

Database searches indicate that pk18 homologues exist in vertebrates (see ref. 10 for a recent sequence alignment) and invertebrates (*Caenorhabditis elegans* GenBank accession number Z81048). In contrast, *Saccharomyces cerevisiae* lacks

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proteins related to pk18 (16), and mitochondria isolated from this yeast do not exhibit high-affinity isoquinoline binding characteristic of mitochondria containing peripheral benzodiazepine receptors (17). Thus, it will be interesting to see if similar signal transduction pathways exist in other eukaryotic microbes. The apparent lack of related proteins in the genome of *Escherichia coli* and other prokaryotes (unpublished observations from scanning databases with TspO or pk18) also raises the interesting possibility that eubacteria and Archaea either never contained TspO-like signal transduction pathways or lost them during evolution.

If not all prokaryotes contain pk18 family members, is there any similarity among those organisms in which they exist? The answer to this question may lie in the observation that bacterial members of the pk18 family are only currently known to exist in purple photosynthetic bacteria (6, 8, 9) and the cyanobacterium *Synechocystis* sp. (17). Descendants of purple bacteria are thought to be the evolutionary ancestors of the mitochondrion (18), so Yeliseev *et al.* (10) propose that TspO and its cognate signal transduction chain is the progenitor of mammalian peripheral benzodiazepine receptors. If this were true, it would be interesting to see if other eubacterial members of the TspO/pk18 family were retained in related prokaryotes and if they function in similar *trans*-membrane signal transduction pathways. In addition, by asking if analogous receptor complexes respond to oxygen (like *R. sphaeroides* TspO; ref. 9) or different ligands, one could determine how their function might have adapted to the metabolic capabilities and environmental niches of their respective hosts.

Are TspO/pk18 Just the Tip of a New Signal Transduction Iceberg?

The work of Yeliseev *et al.* (10) is likely to provide a broad spectrum of the scientific community with an important new way to think about signal transduction pathways and the function of a pharmacologically important group of drug receptors. The systems available to probe bacterial gene expression, signal transduction pathways, and the organization of membrane proteins will be of enormous benefit to deciphering different aspects of TspO/pk18 function in *R. sphaeroides*. Such information is essential to understand the normal physiological function of TspO in this facultative phototroph and understand why this type of signal transduction pathway was conserved during the evolution of higher eukaryotic mitochondria. As additional information from eubacterial, Archaeal and eukaryotic genome sequences is compiled, it seems likely that prokaryotes will provide other new and equally exciting opportunities to dissect conserved functions and signal transduction pathways.

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