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Review

Voltage-dependant anion channels: Novel insights into isoform function through genetic models☆

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ABSTRACT

Voltage-dependant Anion Channels, also known as mitochondrial porins, are pore-forming proteins located in the mitochondrial outer membrane (MOM) that, in addition to forming complexes with other proteins that localize to the MOM, also function as the main conduit for transporting metabolites between the cytoplasm and mitochondria. VDACs are encoded by a multi-member gene family, and the number of isoforms and specific functions of VDACs varies between species. Translating the well-described *in vitro* characteristics of the VDAC isoforms into *in vivo* functions has been a challenge, with the generation of animal models of VDAC deficiency providing much of the available information about isoform-specific roles in biology. Here, we review the approaches used to create these insect and mammalian animal models, and the conclusions reached by studying the consequences of loss of function mutations on the genetic, physiologic, and biochemical properties of the resulting models. This article is part of a Special Issue entitled: VDAC structure, function, and regulation of mitochondrial metabolism.

Contents

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1. Introduction

Voltage-dependent Anion Channels (VDACs) are the most abundant proteins in the mitochondrial outer membrane (MOM) [1], a key region of the organelle that plays a central role in various cellular processes, including metabolite flux, metabolic compartmentation, and apoptosis. Not surprisingly, VDACs have been implicated in all of these processes via differing mechanisms: facilitating metabolite flux by enabling transport of ATP, ADP, phosphocreatine and other small ions across the MOM [2,3], anchoring hexokinases to the MOM during glycolysis [4] and aiding release of cytochrome c from the intermembrane space, either directly regulation pore opening and closure [5,6] and/or indirectly by interacting with Bcl-2 family members [7] (reviewed in Ref. [8]). The diversity in function is matched by the evolution of different isoforms of the protein, each with distinct roles in mitochondrial biology, and comparing and contrasting the functional characteristics of these variants in different model systems will be the focus of this review. Since the first studies to isolate the relevant genes and characterize the functional roles of porins were conducted in yeast, and later studies were conducted in flies and mammals, we will analyze the models for porin deficiency in that order.

2. Yeast VDAC isoforms

VDACs have been characterized in a variety of eukaryotes, with the number of isoform variants ranging from one to perhaps five, depending on the species [1,9,10,11]. The yeast (*Saccharomyces cerevisiae*) variants of VDAC; YVDAC1 and YVDAC2, are encoded by the single copy genes POR1 and POR2, respectively, with the proteins having a 49% amino acid identity. POR1 is considered the major isoform in yeast, with the ability to form channels in phospholipid membranes [12]. The POR1 deletion strain (Δ*por1*) exhibited a delayed growth adaptation in non-fermentable glycerol at 30 °C, and a complete lack of growth at 37 °C [13]. In addition to the growth phenotypes, the deletion strain also demonstrated significantly reduced levels of mitochondrial cytochromes; cytochrome c,c_1,b and aa_3, along with COXIV, while containing normal levels of other mitochondrial proteins. The ability of the deletion strain Δ*por1* M22-2 to grow at lower temperature led to a multi-copy suppressor screen for a functional substitute for YVDAC1, resulting in the discovery of YVDAC2. The YVDAC2 isoform, however, was neither able to form channels in reconstituted *in vitro* systems nor rescue the growth phenotype, and its true function remains to be determined [12]. Yeast has been used extensively as a model system for various mitochondrial dependent processes including, amongst others, the release of cytochrome c during apoptosis. Conflicting reports have argued for [14,15,16,17] and against the necessity of YVDAC1 [18,19] for cytochrome c release during apoptosis. Other processes where a role for YVDAC1 has been proposed include as a channel for metabolites such as NADH, and as a conduit for reactive oxygen species (ROS) signaling via redox regulation and consequent regulation of mRNA and protein levels in the cell. [20,21,22,23]. YDAC1 has been hypothesized to control cellular redox states by the transport of ROS species such as the superoxide anion (O_2^-) across the MOM, and this in term is known to affect cellular mRNA and protein levels (reviewed in Ref. [21]). Over-expression or POR1 complementation in yeast of yeast, fruit fly and mammalian VDACs has also been an important tool in isolating abundant amounts of purified VDAC protein and examining the consequences of engineered point mutations with regard to structure and function [10,24,25].

3. Fly VDAC/porin isoforms

As described earlier in this review, while VDAC has been classically viewed as the major determinant of MOM since the 1970s, studies over the past 15 years have clearly demonstrated that VDAC also interacts with both cytosolic and MOM proteins (reviewed in Ref. [26]). For example, VDAC integrates mitochondrial and cytoplasmic energy metabolism through its binding of hexokinases, effectively linking glycolysis and mitochondrial oxidative phosphorylation, in addition to being the possible mitochondrial docking site for glycerol kinase and creatine kinase [26]. The fruit fly provides a powerful genetic model system to identify and further characterize VDAC functions. *Drosophila melanogaster* contains a cluster of four genes (*porin*, *CG17137* [*Porin2*], *CG17139*, and *CG17140*) that encode proteins that are homologous to known VDACs [25]. *porin* exhibits the greatest homology to mammalian VDACs and is ubiquitously expressed in the fruit fly, while the other three fly VDACs have a more spatially restricted expression pattern, predominantly in the male reproductive tract [25]. A series of hypomorphic alleles has been generated and characterized using imprecise excision of a P element inserted in the 5′ untranslated region [27] of *porin* [28]. Flies lacking *porin* exhibit a variety of mutant phenotypes including partial developmental lethality, abnormal mitochondrial respiration, partial complex I deficiency, fertility defects, skeletal muscle abnormalities and neurological dysfunction. The fertility defects include reduced fertility and fecundity in females and infertility in males associated with sperm immotility. The muscle phenotype consists of abnormal mitochondrial morphology with unusual inclusions by electron microscopy as well as a functional deficit manifested as a defect in a climbing assay. The neurological dysfunction in porin-deficient flies is manifested by an increased sensitivity to mechanical stress ("bang sensitivity"), progressive retinal dysfunction demonstrated by an abnormal electroretinogram (ERG), and an aberrant electrophysiological response at the larval neuromuscular junction (NMJ). The neurological dysfunction at the larval NMJ is also characterized by an abnormal distribution of mitochondria within the motor neuron. Using a mitochondria-targeted GFP transgene expressed in the motor neuron, the quantity of mitochondria in the presynaptic termini and axons is significantly reduced, with concomitant accumulation of mitochondria in motor neuronal cell bodies [28]. Park et al. also recently reported phenotypic characterization of fly mutants deficient for porin which confirms the neurological and male infertility phenotypes, as well as demonstrating that the observed abnormal mitochondrial morphology can be rescued with overexpression of Drp1, a regulator of mitochondrial fission [29]. This observation suggests that porin may play an important role in the regulation of mitochondrial dynamics [29]. The phenotypes of defective energy metabolism with perturbed mitochondrial dynamics, male infertility with sperm immotility, and neurological dysfunction in porin-deficient fruit flies are reminiscent of abnormal phenotypes seen in mitochondrial diseases and suggest that porin deficiency in the fly is a valid model of mitochondrial dysfunction that is relevant both for rare primary mitochondrial diseases and for common adult neurological and metabolic diseases for which mitochondrial dysfunction has been implicated to play a pathogenic role.

4. Mammalian VDAC isoforms

4.1. Gene organization

A prerequisite to understanding the functional characterization of mammalian VDACs is a description of the organization of the three VDAC isoforms in mammals. In mammals, three VDAC isoforms; VDAC1, VDAC2 and VDAC3, have been characterized, with VDAC1 and VDAC3 shown to have 9 exons and VDAC2 having 10, the additional exon encoding part of the 5′-UTR region [30,31]. There is roughly 70% nucleotide sequence identity amongst the three VDAC isoforms and around 90% nucleotide identity between the human and mouse variants (henceforth referred to as mVDACs for mice and hVDACs for humans) [30]. All three isoforms in humans and mice have the start codon located in the second exon, span varying lengths of