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Membrane composition influences the topology bias of bacterial integral membrane proteins

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ABSTRACT

Small multidrug resistance (SMR) protein family members confer bacterial resistance to toxic antiseptics and are believed to function as dual topology oligomers. If dual topology is essential for SMR activity, then the topology bias should change as bacterial membrane lipid compositions alter to maintain a "neutral" topology bias. To test this hypothesis, a bioinformatic analysis of bacterial SMR protein sequences was performed to determine a membrane protein topology based on charged amino acid residues within loops, and termini regions according to the positive inside rule. Three bacterial lipid membrane parameters were examined, providing the proportion of polar lipid head group charges at the membrane surface (PLH), the relative hydrophobic fatty acid length (FAL), and the proportion of fatty acid unsaturation (FAU). Our analysis indicates that individual SMR pairs, and to a lesser extent SMR singleton topology biases, are significantly correlated to increasing PLH, FAL and FAU differences validating the hypothesis. Correlations between the topology biases of SMR proteins identified in Gram+ compared to Gram− species and each lipid parameter demonstrated a linear inverse relationship.

1. Introduction

Bacterial membrane compositions vary significantly when comparing the lipids of Gram positive (Gram+) to Gram negative (Gram−) bacteria [1]. Due to this variation, the composition of membrane phospholipids, including acyl chain length, the degree of unsaturation and charged polar head groups are often used to aid bacterial classification. Phospholipid diversity is essential to understanding factors that influence the function, insertion, and topology of integral membrane proteins. Features such as the degree of phospholipid head group charge (as reviewed by [2]), fatty acid chain length and fatty acid unsaturation (as reviewed by [3]) have all been shown to alter the function and folding of membrane proteins, emphasizing the importance of membrane composition with integral membrane protein activity. In particular, the balance of anionic phospholipids within the membrane, namely phosphatidic acid (net −1 charge), phosphatidyl glycerol (net −1), and cardiolipin (net −2 charge), appears to play an important role in the insertion and transmembrane segment arrangements of integral membrane proteins such as leader peptidase (Lep) [4], phenylalanine permease (PheP) [5], gamma-aminobutyric acid permease (GabP) [6], lactose permease Y transporter (LacY) [7] and potassium channel protein KcsA [8]. These studies indicate that increasing anionic phospholipid content in the membrane can alter the folded state of the protein and impact overall function.

In general, integral membrane protein topology and transmembrane segment (TMS) insertion can be determined from the primary sequence and secondary structure content following 'the positive inside rule' (as reviewed by [9]). This rule states that the amount of positively charged residues (lysine; K and arginine; R) will determine the orientation of protein TMS insertion in the membrane [10,11] (Fig. 1). Loops and/or termini with the greatest abundance of positive residues are expected to reside inside the cell, facing the cytoplasm and away from the proton enriched periplasm [12]. Hence, the topology of any membrane protein can be calculated using the equations provided in Fig. 1. This calculation has enabled examination of entire bacterial membrane proteomes and determined that the vast majority of integral membrane proteins adopt a preference or bias for one orientation within the membrane [13,14]. However, a small proportion of membrane proteins can adopt a dual topology orientation, where they show a neutral topology bias that permits protein membrane insertion in either direction (Fig. 1B).

An example of a dual topology integral membrane protein, belongs to a family of multidrug transporters known as small multidrug resistance (SMR) proteins. Members of this family confer host resistance to antiseptics and antimicrobials that possess a permanently charged cation(s) referred to as a quaternary cation compound (QCC) though proton motive force (as reviewed by [15]). The SMR protein family is one of 14 other phylogenetically distinct secondary active transporter protein families classified within drug and metabolite

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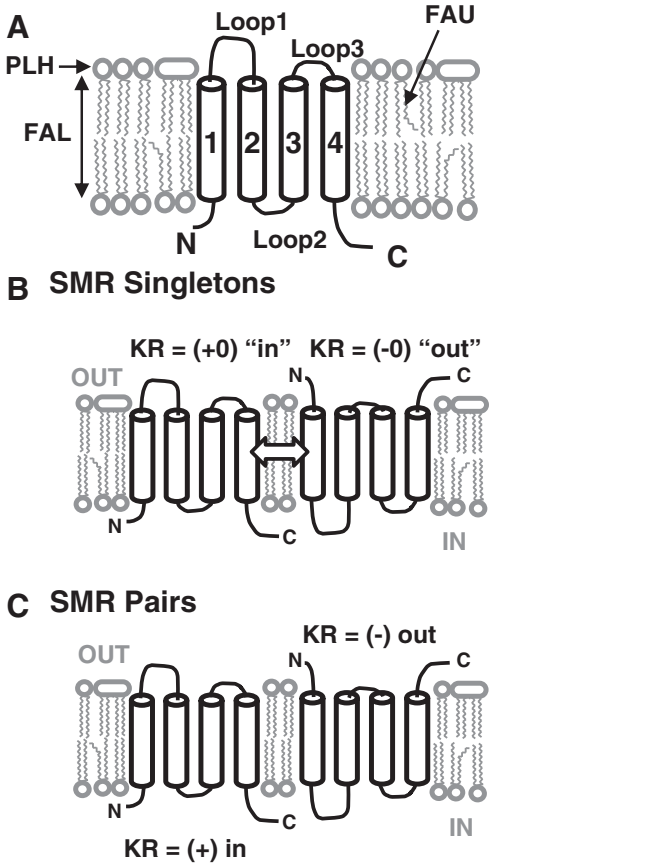

Fig. 1. Diagrams of various SMR protein topology biases and equations used to calculate insertion biases. A) A diagram of a lipid bilayer containing a SMR protein. A single SMR protein monomer is shown in black, where labelled lines indicate N- or C-termini and loop (1–3) regions connected to each of the four transmembrane α-helices (numbered cylinders) of the protein. Phospholipids are represented in grey, where polar head groups (circles) are connected to two fatty acids tails. Other polar lipids such as cardiolipin, are represented as oval (polar head group) connected to four fatty acid tails. Each lipid composition parameter examined in this study is indicated by an arrow and shows mean polar lipid head groups (PLH), relative fatty acid length (FAL) and relative fatty acid unsaturation (FAU). Panels B and C show a membrane orientation diagram of an SMR singleton (B) and SMR pair (C) according to the positive inside rule KR bias calculation provided in D. D) Equations used to determine KR topology bias and net topology bias. Each bias estimates the orientation according to the sum of charged amino acid residues, (KR bias calculates K/R residues only; net charge calculates the net charge difference between K/R from E/D) located at each N–C-termini and in loop region.

transporter (DMT) superfamily [16,17]. Members of the SMR protein family are composed of four highly hydrophobic α-helical TMS linked together by relatively short loops. The genes encoding SMR proteins are frequently located on conserved 3′ regions of mobile genetic elements known as integrons, enhancing SMR transmission to unrelated bacterial species [18,19]. The SMR protein family is divided into three major subclasses; the small multidrug protein (SMP), the suppressor of *groEL* mutation (SUG), and paired small multidrug resistance protein (PSMR) subclasses. Both SMP and SUG subclasses confer host resistance as a single protein, known as a 'singleton' based on previous SMR topology studies [13,14]. The majority of experimental studies of SMP subclass members have focused on the archetypical protein, EmrE in *Escherichia coli*, which confers host resistance to a broad range of structurally diverse QCC. SUG subclass members, such as *E. coli* SugE, confer host resistance to a narrow subset of QCC in comparison to SMP [20] but have a broader bacterial distribution than SMP [21]. Finally, PSMR subclass members, MdtI (YdgE) and MdtJ (YdgF) in *E. coli*, confer host resistance to QCC by the expression of two distinct SMR genes [22]. PSMR proteins reside within the membrane as a paired heterooligomeric complex where each protein adopts a fixed antiparallel topology/orientation from the other [14]. Topological analyses of other SMR pairs, have mainly focused on the Gram positive *Bacillus subtilis* SMR pair EbrA and EbrB, which also demonstrates an antiparallel orientation [23–27].

The focus of this study is to determine if the host bacterial lipid composition influences integral membrane protein topology and determine what lipid parameter(s) are specifically involved. Our working hypothesis is that fixed/single orientation integral membrane proteins are expected to have topology biases that shift in value with respect to membrane composition. By extension, if a dual topology orientation is an essential feature for protein function then the neutral topology bias should also vary in response to changing membrane compositions to maintain neutrality. The SMR protein family is an ideal candidate for such an investigation since members of this family exist as either dual topology (or antiparallel topology) singletons or as single/fixed topology pairs. To test this hypothesis, SMR topology biases were determined from a bioinformatic analysis of 1320 bacterial SMR family protein sequences from diverse taxa representing major bacterial phyla. A matrix of positively (and negatively) charged amino acid residues (Lys and Arg) found within N- and C-termini and loops 1–3 of each protein was assembled (Fig. 1). In parallel, a phospholipid composition dataset was collected for bacteria known to encode an SMR sequence. This lipid dataset summarized the proportion of polar lipid head group charges (PLH), the relative fatty acid tail length (FAL), and the proportion of fatty acid unsaturation (FAU) in various bacterial plasma membranes (Fig. 2). After statistical assessment of the topology-lipid matrices by correlational analyses and hierarchical clustering, we propose that all three lipid composition parameters influence the positive inside rule [12] and thus support the overall hypothesis.

2. Materials and methods

2.1. SMR protein sequence topology bias dataset acquisition and matrix assembly

A total of 1320 bacterial SMR protein sequences (as of May 2011) were collected for this study by expanding upon a SMR protein dataset of 685 sequences assembled from a previous study [21]. All additional SMR protein sequences were identified by performing tBLASTn searches of the NCBI microbial genome sequencing database using either *E. coli* EmrE (P23895) or *E. coli* SugE (AAC46453) protein sequences. All SMR protein sequences were initially aligned using ClustalW (2.0) and then manually edited using the multiple alignment programme Jalview [28,29]. Aligned SMR proteins were separated into five groups based on SMR homology with known representatives from each subclass (SMP, SUG, and PSMR: YdgEF, EbrAB, YkkCD) using the phylogenetic Neighbour Joining analysis method available in the PHYLIP software package [30]. A summary of SMR distribution within each representative phylum included in this study is provided in Supplementary Table 1.

Estimated TMS regions in each protein within the SMR protein dataset were predicted using the TMHMM v2.0 programme [31,32] and the online SOSUI server [33]. All SMR protein sequences in the