Review

A Glimpse of Membrane Transport through Structures—Advances in the Structural Biology of the GLUT Glucose Transporters

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Abstract

The cellular uptake of glucose is an essential physiological process, and movement of glucose across biological membranes requires specialized transporters. The major facilitator superfamily glucose transporters GLUTs, encoded by the *SLC2A* genes, have been a paradigm for functional, mechanistic, and structural understanding of solute transport in the past century. This review starts with a glimpse into the structural biology of membrane proteins and particularly membrane transport proteins, enumerating the landmark structures in the past 25 years. The recent breakthrough in the structural elucidation of GLUTs is then elaborated following a brief overview of the research history of these archetypal transporters, their functional specificity, and physiological and pathophysiological significances. Structures of GLUT1, GLUT3, and GLUT5 in distinct transport and/or ligand-binding states reveal detailed mechanisms of the alternating access transport cycle and substrate recognition, and thus illuminate a path by which structure-based drug design may be applied to help discover novel therapeutics against several debilitating human diseases associated with GLUT malfunction and/or misregulation.

A brief overview of the structural biology of membrane proteins

It is estimated that approximately 20%–30% of the coding genes in the human genome encode membrane proteins (MPs), which play an essential role in numerous physiological processes in all kingdoms of life [1]. MPs also constitute major drug targets. In fact, more than 50% of the Food and Drug Administration-approved drugs act on MPs [2]. Therefore, information on the structure and mechanism of MPs is instrumental to both basic research and potential therapeutic applications. However, the fundamental importance of MPs is in sharp contrast with the small number of available structures due to the technical difficulties associated with protein production and detergent selection for the extraction and purification of MPs.

Whereas the three dimensional architectures of any protein, namely the crystal structures of myoglobin and hemoglobin, were elucidated in late 1950s [3–5], the first crystal structure of an MP was obtained in 1985, nearly 30 years after the determination of the first high-resolution protein structure [6]. Drs. Johann Deisenhofer, Robert Huber, and Hartmut Michel were awarded the Nobel Prize in Chemistry in 1988 for obtaining the structure of the photosynthetic reaction center. Since then, however, progress on structural biology of MPs has been slow compared to that of soluble proteins. As of July 12, 2017, over 130,000 structural coordinates have been deposited in the Protein Data Bank (PDB), of which merely ~1.7% are MPs. Notably, only 707 out of the 2250 MP structures are from unique proteins [7].

Prior to 2013, the predominant approach for structural determination of MPs was X-ray crystallography, although atomic structures of a few MPs, exemplified by bacteriorhodopsin and aquaporins, were obtained using electron diffraction [8–10]. The bottleneck for X-ray crystallography of MPs mainly exists in the generation of optimal samples that can yield diffracting crystals. Therefore, it is not surprising that there are two general patterns for structural biology of MPs in the past three decades: (1) structures

of prokaryotic proteins were solved ahead of their eukaryotic orthologues, and (2) the MPs whose structures were determined in early years were mostly from abundant endogenous sources, such as the complexes in electron transport chain and photosynthesis, the calcium ATPase of skeletal muscle sarcoplasmic reticulum, and bacteriorhodopsin. Following the structural determination of the K^+ channel KcsA [11], recombinantly overexpressed MPs have become the major targets for structural analysis.

In recent years, the technological breakthrough of electron cryomicroscopy (cryo-EM) has revolutionized structural biology. Structures of many biomolecules or macromolecular machineries previously insurmountable by X-ray crystallography were determined by cryo-EM to near-atomic resolutions [12]. The time of MP structures has now come [7].

Membrane transport proteins

On the basis of their primary functions, MPs may be classified into enzymes, receptors, transport proteins, and scaffold proteins, although the function of an MP may be multifaceted in many cases. Approximately 10% of the human genome encodes for transport-related functions [13]. These gene products are named membrane transport proteins, including channels and transporters.

The lipid bilayer sets a hydrophobic barrier that insulates the cellular or organellar contents from the environment. Although a small portion of low-molecular-weight molecules is able to permeate the lipid bilayer via simple diffusion and massive group translocation can be achieved through vesicular transport, most biologically important organic and inorganic molecules, such as ions, sugars, amino acids, peptides, and even lipids, can only traverse the biological membranes through active permeation mediated by specialized proteins. Membrane transport proteins thereby play a vital role in a broad spectrum of basic cellular functions, such as uptake of nutrients and release of metabolites, generation and transduction of signals, and regulation of cell mobility. A large number of diseases are correlated with malfunction of membrane transport proteins, including cardiac and neurological disorders, pain, depression, metabolic disorders, neurodegenerative diseases, and so on [14,15].

In addition to the generally referred nomenclature of transporters and channels, a few terms like pore (or porin), pump, and carrier are frequently used in the literature (Fig. 1a). Previous reviews have presented detailed explanations for the nomenclatures [16–18]. The boundary for the division of transporters and channels sometimes appears murky. The defining distinction is that an open channel is permeable to both sides of the membrane, whereas a transporter keeps at least one gate closed at any given time (Fig. 1a). Therefore, a transporter possesses at least two gates that insulate the substrate-binding site(s) from either side of the membrane, never allowing simultaneous substrate access from both sides of the membrane. This molecular principle warrants transporters the ability to couple movement of different substrates in the same (symporter) or opposite (antiporter) directions. Therefore, a transporter, but not a channel, can catalyze the uphill translocation of the substrate against its electrochemical potential by harnessing the energy released from other processes.

On the basis of energy utilization, transporters may be divided into three classes: the primary active transporters that directly utilize light or the energy released from chemical reactions such as ATP hydrolysis and electron transport to catalyze active transport, the secondary active transporters that exploit electrochemical potential of one chemical to shuttle other substrate(s) against its concentration gradient, and the facilitators or uniporters that catalyze the diffusion of the substrate down its concentration gradient (Fig. 1a).

Channels are usually named and classified on the basis of either substrate specificity or gating mechanism. On the basis of the nature of the substrates, there are water channels, cation channels, anion channels, $K^+/Na^+/Ca^{2+}/H^+/Cl^-$ channels, non-selective channels, and so on. Except for a few porins, the opening and closing of a channel are controlled by various signals, namely the gating mechanism. There are ligand-gated, voltage-gated, mechanosensing, temperature sensing channels, and so on. A number of channels are named as receptors, such as the glutamate receptors, the ryanodine receptors, and so on, because the channel gating is responsive to the namesake and related ligands.

A brief chronicle of the structural biology of membrane transport proteins

Advances in the structural biology of membrane transport proteins have reshaped our understanding of folding, evolution, classification, and functional mechanism of these miniature membrane machineries. Below I will enumerate several milestones in the structural biology of membrane transport proteins (Fig. 1b). I apologize to those whose outstanding contributions are not discussed due to word limit.

The first glance at a membrane transport protein was made in 1992, when crystal structures of the bacterial outer-membrane porins, a specific type of β-barrel channels, were determined [19,20]. The electron transport chain complexes I, III, and IV are in essence H^+ pumps, or the primary active transporters that extract the energy released from electron transfer to drive the uphill translocation of