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Research Progress on the Structural Basis and Transport Mechanism of MFS Superfamily Transporters

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Abstract: *The transmembrane transport of substances in organisms is one of the key processes for cell life activities. Through the mediation of various transporters, cells can regulate the transmembrane transport of substances, maintain intracellular and extracellular homeostasis, and ensure the normal function of cells. Among many transporter families, MFS (Major Facilitator Superfamily) superfamily is undoubtedly the largest, most widely distributed and most important protein family. The MFS superfamily is a family of active transporters widely present in prokaryotes and eukaryotes, and its members are widely involved in the transport of various small molecules (carbohydrates, amino acids, nucleotides, organic acids, drugs, etc.) Inside and outside the cell. MFS transporters provide the transport driving force by coupling the reverse transport of ions (such as H + or Na +) to realize the transmembrane transport of transported substances. The typical members of MFS superfamily include glucose transporter (GLUT), lactate transporter (MCT), amino acid transporter (APC), nucleotide transporter (NUP) and so on, which play an important role in biomedical research. In this paper, the classification of membrane transporters, the introduction of MFS superfamily proteins, the history of structural biology of MFS superfamily proteins, the structural characteristics of MFS superfamily transporters, and the transport mechanism of MFS superfamily proteins were reviewed. It is expected to provide a reference for further understanding of the functional regulation mechanism of MFS superfamily transporters.*

Keywords: *MFS superfamily transporters; structural basis; Transport mechanism*

1. Introduction

Membrane transporters are important participants in physiological and chemical processes in vivo, which regulate the transmembrane transport of various substances through energy dissipation or electrochemical gradient, and play a key role in maintaining cellular homeostasis, material metabolism, signal transduction and so on. With the deepening of biological research, the understanding of membrane transporters has been improved, and a large number of different types of membrane transporter families have been found. Among them, MFS (Major Facilitator Superfamily, mainly facilitated transport superfamily), as a kind of membrane transport protein superfamily with wide distribution and diverse functions, has been one of the research hotspots in the field of membrane transport. In this paper, the classification of membrane transporters and the structure and function of MFS superfamily proteins are systematically reviewed, in order to provide theoretical support for further exploration of the molecular mechanism of membrane transport.

1.1 Classification of Membrane Transport Proteins

Hu Chaoyang (2021) pointed out that membrane transporters widely exist in prokaryotic and eukaryotic cell membranes, and can be divided into several superfamilies according to their functional and structural characteristics. For example, ATP-binding cassette (ABC) transporter superfamily: This family includes a variety of ATP-driven active transporters, which are widely involved in the transmembrane transport of various ions, metabolites, lipids and so on. Classical representatives are P-glycoprotein, CFTR, etc.; active transporter (P-type, V-type, F-type) superfamily: these transporters use ATP hydrolysis energy to drive ion gradients, such as Na +/K + -ATPase, H + -ATP synthetase, etc.; Secondary transporter superfamily: It includes solute carrier superfamily (MFS), ionization channel superfamily, hemichannel superfamily, etc., and realizes transport by using ion electrochemical gradient

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and other ways without direct drive of ATP.

1.2 Structure and function of MFS superfamily proteins

The MFS superfamily is one of the largest known membrane transport protein families, which is widely distributed in organisms and has a large number of members in both prokaryotes and eukaryotes. MFS proteins play a key role in maintaining cell homeostasis, nutrient uptake, metabolic waste excretion, drug toxicity elimination and other processes by using ion electrochemical gradient to achieve the transmembrane transport of various small molecules through passive diffusion or carrier-assisted. Rasmussen S G (2011) pointed out that the basic structure of MFS protein is usually composed of 12-14 hydrophobic transmembrane helices (TMH), and the N and C termini are located at the cytoplasmic side of the transmembrane helices, which are usually connected by hydrophilic connecting loops. Topological analysis showed that MFS protein could be divided into two symmetrical half-transmembrane domains, forming a hydrophobic gap in the middle, which might be the channel of substrate transport. MFS protein also contains some conserved domains, such as MFS family signature sequence (EIVRFLNR), GT-rich region (Gly-Thr rich region), etc. As shown in Figure 1.These structural elements play an important role in the process of substrate recognition and transport.

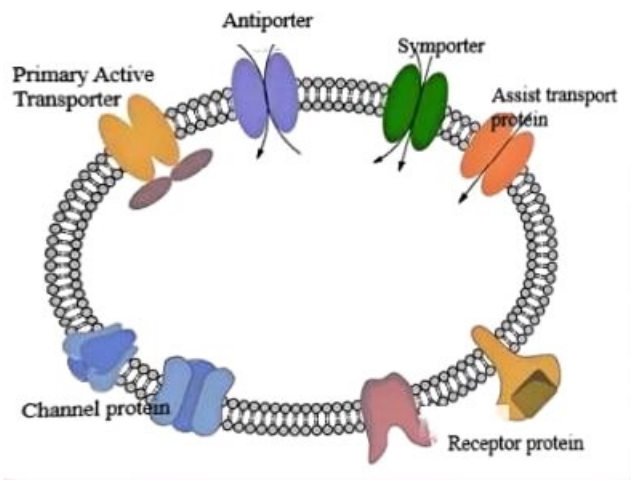

Figure 1 Schematic diagram of the structure of MFS superfamily protein.

The MFS superfamily can be further divided into several subfamilies according to the differences in substrate types and transport modes, such as Sugar Porter (SP) and GLUT family, which are widely involved in the uptake and secretion of monosaccharides. Oligosaccharide: H + Symporter (OHS), such as the lactose transporter LacY, mediates the proton cotransport of oligosaccharides. Drug: H + Antiporter (DHA): For example, yeast cell membrane drug resistance protein Pdr5 is involved in the excretion of various toxic substances from cells. Amino Acid/Amine Transporter (AAT): Such as the neurotransmitter glutamate transporter, which regulates the balance of amino acid levels inside and outside the cell. Abramson J (2023) pointed out that different subfamilies of MFS proteins have differences in substrate recognition, transport mechanism and cell function, but they all follow similar structural and topological characteristics. The MFS superfamily has a large number of members, and the structure and function of a large number of representative proteins have been studied in depth, such as lactose transporter LacY, glucose transporter GlcP in Escherichia coli, glucose transporter GLUT family in human, neurotransmitter transporter EAAT and so on. These studies will help us to fully understand the important role of MFS superfamily in life activities.

2. History of Structural Biology of MFS Superfamily Proteins

In this paper, we will review the history of structural biology of MFS superfamily proteins and look forward to the future research directions.

2.1 Discovery and early research of MFS superfamily proteins

MFS superfamily proteins were first proposed by Major et al. In the early 1990s. Through

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comparative analysis of the E. coli genome, they found that many transmembrane transporters have similar topology and sequence conservation, so they were classified as MFS superfamily. The main characteristics of MFS superfamily proteins include: 1) they are usually composed of 12-14 transmembrane helices; 2) they have highly conserved domains, such as two domains mainly at the N-terminus and C-terminus; 3) It is responsible for transporting various small molecules, such as sugars, amino acids, nucleotides, etc. In the 1990s, with the rapid development of bioinformatics, scientists have found and identified a large number of coding genetic sequence of MFS superfamily proteins in different organisms. These studies lay a foundation for the structural biology of MFS superfamily proteins.

2.2 Structural analysis of MFS superfamily proteins

Due to the transmembrane properties of MFS superfamily proteins, their structural elucidation has been a major challenge in the field of structural biology. In the 1990s, scientists mainly relied on bioinformatics methods, combined with hydrophobicity analysis, secondary structure prediction and other methods, to construct the preliminary structure model of MFS superfamily proteins. For example, in 1994, Major et al. Proposed a topological model consisting of 12 transmembrane helices based on the MFS transmembrane protein encoded by the MLR gene of Escherichia coli. This model lays a foundation for the subsequent structural study of MFS protein. It was not until the early 21st century that scientists resolved the crystal structure of MFS superfamily proteins through advanced protein structure determination technology. In 2003, Abramson et al. First reported the crystal structure of Escherichia coli lactate transporter (LacY), which is the first high-resolution crystal structure of the MFS superfamily. The structure shows that LacY is composed of 12 transmembrane helices and has two domains, which roughly conforms to the topological model established before. Subsequently, more crystal structures of MFS superfamily proteins have been reported, such as the glycerol-3-phosphate transporter GlpT of Escherichia coli and the sulfate transporter YjdL of Escherichia coli. These studies provide direct experimental evidence for understanding the structural characteristics of MFS superfamily proteins.

3. Structural Characteristics of MFS Superfamily TransportersP

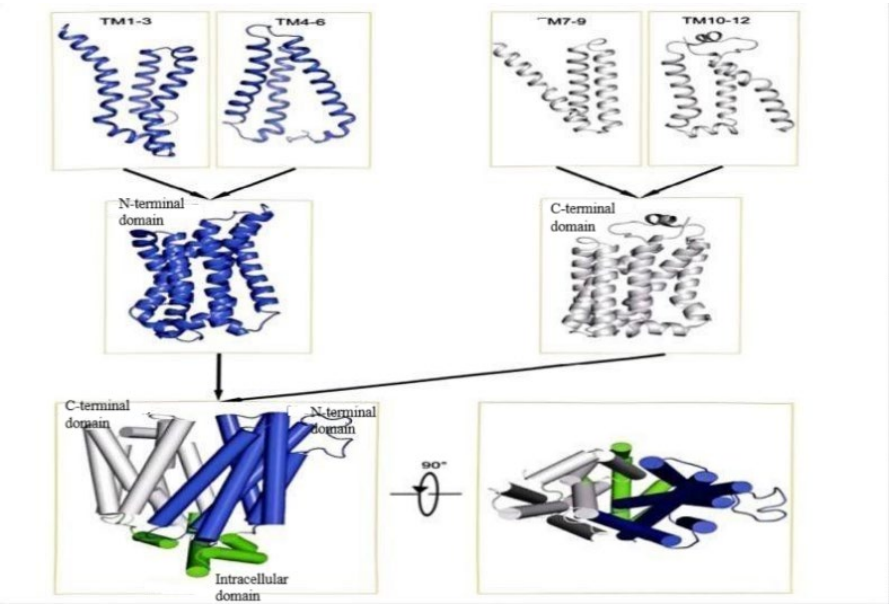

Figure 2.Structural characteristics

At present, crystal structures of several MFS transporters have been analyzed, which provides an important basis for further understanding of their structure-function relationships. As shown in Figure 2.We will start from the structural characteristics of several typical MFS transporters to explore the relationship between their structure and function.

Quistgaard E M (2023) pointed out that lactose/H + cotransporter LacY is one of the most classical MFS transporters in Escherichia coli, which is widely used in the study of the structure and function of MFS transporters. LacY has the typical structural characteristics of MFS: there are 12 transmembrane helical domains, and the N and C termini are symmetrically arranged to form a large central channel. The

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crystal structure shows that the 12 transmembrane helical domains of LacY can be divided into two topologically symmetrical half-domains, forming a deep substrate-binding cavity in the middle. Substrate lactose and H + binding sites are mainly located in this central binding cavity, and key amino acids such as Glu126 and His322 are involved in substrate recognition and binding.

Zheng H (2023) pointed out that GlpT is another classical MFS transporter in E. coli, which is responsible for catalyzing the coordinated transmembrane transport of glycerol-3-phosphate (G3P) and inorganic phosphate (Pi). Similar to LacY, GlpT also has 12 transmembrane helical domains, and the N and C termini are symmetrically arranged. However, the central binding cavity of GlpT is more open, and the substrate binding sites are concentrated on the transmembrane helices TM1, TM4, and TM11. Key substrate-binding amino acids include Arg45, Arg269, Tyr340, etc., which bind to substrates through the formation of hydrogen bonds or electrostatic interactions.

The multidrug/avidin transporter EmrD is an important member of the MFS superfamily in Escherichia coli, which can transport a variety of hydrophobic small molecules, such as a variety of antibiotics, dyes and so on. Shao Kehua (2021) pointed out that compared with the first two classical MFS proteins, the structure of EmrD has some unique features: for example, it still maintains the typical MFS topology of 12 transmembrane helices; the central binding cavity is more hydrophobic, which is suitable for transporting hydrophobic substrates; Substrate binding sites are mainly composed of hydrophobic amino acids, such as Phe24, Phe110, Leu154, etc.

Jiao Xuyao (2021) pointed out that UhpT is an MFS transporter that transports phosphomonoester nucleotides in Escherichia coli. In contrast to the aforementioned MFS proteins, UhpT also maintains the typical topology of a 12-transmembrane helix in the overall structure. However, the central binding cavity of UhpT is more open, and the substrate binding sites are mainly concentrated on TM2, TM5, and TM11 transmembrane helices. The key substrate binding amino acids include Arg45, Arg269, His355, etc. They bind to phosphate monoester substrates by forming hydrogen bonds, salt bridges, etc.

4. Transport mechanism of MFS superfamily proteins

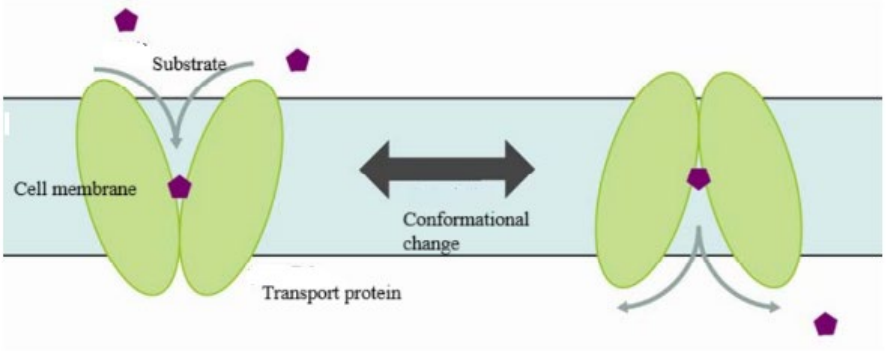

Figure 3. Transport mechanism

MFS transporters are widely involved in the transport of various small molecules inside and outside the cell, showing strong substrate specificity. Generally speaking, uniporters have high substrate specificity. As shown in Figure 3.For example, GLUT, a glucose transporter, can only transport monosaccharide glucose. Coupled transporters and antiporters usually have a broad substrate spectrum and can transport a variety of small molecules with similar structures, such as amino acid transporters, which can transport a variety of amino acids. The transport mechanisms of MFS transporters are as follows: passive diffusion, ion-coupled transport, potential-coupled transport and ATP-driven transport. Different types of MFS transporters have different transport mechanisms, but they all follow the principle of quantum mechanics to achieve directional and selective transport of substrates through conformational changes and energy coupling of substrate binding sites.

5. Research Prospect

As an important executor of intracellular and extracellular transport, MFS transporters play a key role in maintaining cell homeostasis and coordinating life activities. In this paper, the structure, substrate specificity, transport mechanism, and regulation mechanism of MFS transporters are reviewed. With the further study of the structure and function of MFS transporter, we will have a more comprehensive

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understanding of its mechanism in life activities. Future studies should further explore the molecular mechanism of MFS transporters and disease development, and provide new targets and strategies for the prevention, diagnosis and treatment of related diseases. It is believed that MFS transporter will play a more important role in life science research and clinical application through the unremitting exploration of scholars.

6. Conclusions

MFS superfamily transporters, as a widespread family of membrane transporters in the biological world, have unique structural basis and transport mechanism. In this review, the structural characteristics, transport mechanisms, physiological roles, research progress and challenges of MFS superfamily transporters are summarized. In the future, with the continuous development of new technologies and the deepening of interdisciplinary research, the study of MFS superfamily transporters will achieve more fruitful results, providing more in-depth understanding and strong support for understanding cell physiological activities, disease mechanisms and drug development.

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