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Review

Biosynthesis of cystic fibrosis transmembrane conductance regulator☆

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ABSTRACT

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride (Cl^-) channel. Mutations of its gene lead to the disease of cystis fibrosis (CF) among which the most common is the deletion of phenylalanine at position 508 (Phe508del). CFTR is a multi-domain glycoprotein whose biosynthesis, maturation and functioning as an anion channel involve multi-level post-translational modifications of CFTR molecules and complex folding processes to reach its native, tertiary conformation. Only 20–40% of the nascent chains achieve folded conformation, while the remaining molecules are targeted for degradation by endoplasmic reticulum, lysosomes, or autophagy. A large number of mutations causing CF impair processing of CFTR. Growing knowledge of CFTR biosynthesis has enabled understanding the cellular basis of CF and has brought to light various potential targets for novel, promising therapies.

This article is part of a Directed Issue entitled: Cystic fibrosis: From o-mics to cell biology, physiology, and therapeutic advances.

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1. Introduction

The cystic fibrosis transmembrane conductance regulator (CFTR or ABCC7) is a cAMP-regulated chloride (Cl^-) channel that belongs to the ATP-binding cassette (ABC) transporter superfamily (Gadsby et al., 2006). The mutations of *CFTR* gene lead to the disease of Cystic Fibrosis (O'Sullivan and Freedman, 2009), among which the most common is the deletion of phenylalanine at position 508 (Phe508del) in the population of Caucasians (Riordan et al., 1989). The CFTR protein is expressed principally in the apical membrane of epithelial cells in the airways, intestine, pancreas, testis, and exocrine glands, where it plays a critical role in transepithelial fluid and electrolyte transport. CFTR forms a transmembrane, anion-selective pore, which transports mainly Cl^- (Sheppard and Welsh, 1999), but is also permeant to other anions such as I^-, Br^-, F^-, and bioactive substrates such as glutathione or ATP (Akabas, 2000; Linsdell and Hanrahan, 1998). CFTR channel also functions as a regulator of other ion transporters in epithelial cells (Bakouh et al., 2013; Gabriel et al., 1993; Gentzsch et al., 2010; Stutts et al., 1995) and potentially as a regulator of epithelial innate immune function and inflammation (Cohen and Prince, 2012; Pezzulo et al., 2012).

A large spectrum of various regulatory and signaling events are involved in CFTR biogenesis and trafficking. Quality control mechanisms modulate CFTR folding, stability and targeting to the plasma membrane. Additionally, CFTR regulates a significant number of intracellular signaling cascades that influence the physiological state of a whole tissue and organ. This explains multiple post-translational modifications of CFTR.

2. Short overview of transcriptional and post-transcriptional regulation of CFTR expression

CFTR expression is regulated developmentally or by pathologic conditions and depends on the cell type and tissue. The predominant sites of CFTR expression are the epithelial surfaces throughout the body which correspond with the sites of cystic fibrosis disease: the submucosal glands and airway surface epithelium, the pancreatic epithelium, the epithelium of the crypts throughout the gastrointestinal tract, the epithelium of sweat glands, the epithelium of the developing genital ducts and vas deferens, the cervical and uterine epithelium, the epithelium of the salivary glands, and the epithelial lining of the intrahepatic bile ducts and gall bladder. The airway surface epithelium is a site of developmental regulation of CFTR expression. In these cells CFTR is highly expressed during embryonic and fetal development, but significantly decreases at birth. CFTR is also regulated by various chemical or pathological factors, for instance the female sex hormones estrogen and progesterone regulate CFTR expression (Sweezey et al., 1998).

The *CFTR* gene is localized on the seventh chromosome (7q31.2) and is composed of 27 exons spreading over 190 kb which encodes a 6.5 kb mRNA transcript (Riordan et al., 1989). *CFTR* expression is complex and involves multiple, tissue-specific transcription start sites, alternative first exons and alternatively spliced transcripts. Moreover, various DNA elements important for regulating, inhibiting or inducing *CFTR* gene expression were found *in vivo* or *in vitro*. The hCFTR intron 1 is responsible for *CFTR* expression in the intestinal crypt epithelium (Rowntree et al., 2001). A nuclear factor (NF) κB element located at position −1103 bp responds to interleukin-1β signaling *via* binding of NF-κB and increases *CFTR* expression in Calu-3 cells (Brouillard et al., 2001). Human histone acetyl transferase GCN5 and transcription factor ATF-1 can potentiate CFTR transcription through an inverted CCAAT (Y-box) element at +2 bp. The CCAAT displacement protein/cut homologue (CDP/cut) acts as a repressor of *CFTR* transcription through the Y-box element by competing for the sites of binding of transactivators hGCN5 and ATF-1, and leads to histone deacetylation (Li et al., 1999). A functional cAMP response element (CRE) located at position −48 in the *CFTR* promoter and adjacent Y box at position −60 are important for induction of *CFTR* expression. CRE element, when bound by CRE-binding protein (CREB), confers cAMP-dependent activation of basal *CFTR* transcription and thereby mediates increased *CFTR* expression in response to cAMP. Mutations of the CRE or the Y box decreased the activity of the promoter in transient transfections of T84 or JEG-3 cells (Matthews and McKnight, 1996). Furthermore, various promoter mutations, belonging to the class V CF mutations, significantly reduce transcription of CFTR, leading to production of only small amounts of functional channels (Rogan et al., 2011).

Various *CFTR* mutations provoking CF also affect the post-transcriptional processing of CFTR mRNA. The nonsense and frameshift mutations, belonging to the Class I, lead to creation of premature termination codons (PTCs) such as W1282X, G542X, Y122X, and result either in the synthesis of truncated and unstable protein or in the decrease of the half-lives of mutant mRNAs. The nonsense-mediated mRNA decay (NMD) pathway eliminates the abnormal mRNA containing premature stop codons. However, several studies demonstrated that aminoglycoside antibiotics can read through PTCs in the *CFTR* gene, and lead to production of functional full-length CFTR proteins (Bedwell et al., 1997; Howard et al., 1996; Sermet-Gaudelus et al., 2007; Wilschanski et al., 2003). Most of the class V mutations are splicing mutations, which affect the normal splicing of the pre-mRNA and thus reduce the levels of correctly spliced mRNA, by partial exon skipping or inclusion of intronic sequences (Rogan et al., 2011). These splicing mutations (*e.g.* 3849 + 10 kb C → T, 3272-26 A → G, IVS8-5T, D565G and G576A) lead to variable levels of correctly spliced transcripts among different patients and among different organs of the same patient.

3. Post-translational CFTR processing

3.1. Complexity of CFTR structure and folding

CFTR is a multi-domain glycoprotein of 1480 amino acids with a molecular weight of ∼170 kDa. It has a modular structure consisting of two membrane-spanning domains (MSD1 and MSD2, each comprising six transmembrane segments), two nucleotide-binding domains (NBD1 and NBD2) and a central regulatory (R) domain, which is a unique feature of CFTR among other ABC transporters. Twelve helical segments of MSD1 and MSD2, linked by intermediate extracellular loops and four cytoplasmic loops (CLs 1–4), assemble together to form the transmembrane channel, while the NBDs have the capacity to bind and hydrolyze ATP. The R domain contains serine residues that are phosphorylated by the cAMP-dependent PKA (protein kinase A) or PKC (protein kinase C) and regulates the channel gating together with the NBDs (Hunt et al., 2013; Riordan et al., 1989). The interactions between the NBDs and CLs of MSDs are critical for the proper assembly and Cl^- channel function of CFTR (Anderson et al., 1991; Riordan et al., 1989; Riordan, 2008). NBD1 forms an interface with the coupling helix of cytoplasmic loop 4 (CL4) and loop 1 (CL1) in MSD2 and MSD1, respectively, whereas the NBD2 associates with CL2 and CL3 of MSD1 and MSD2, respectively (Gadsby et al., 2006; He et al., 2008).

The first step of CFTR entry in the secretory pathway is the translocation of newly synthesized polypeptide into the endoplasmic reticulum (ER) membrane (Fig. 1). Translation occurs at a rate of 2.7 residues per second. Consequently, synthesis of the whole CFTR takes ∼9 min, which applies to both wild-type (WT) and Phe508del-CFTR (Ward and Kopito, 1994). CFTR folding that starts early during translation is a complex and hierarchical process, which takes place in multiple cellular compartments along the secretory pathway (Fig. 1) and involves several folding

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