The Journal of Physiology

PERSPECTIVES

# Beyond cystic fibrosis transmembrane conductance regulator (CFTR) single channel kinetics: implications for therapeutic intervention

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Cystic Fibrosis (CF) is the most common, life threatening, recessive monogenetic disease in the Caucasian population. It is now over 25 years since the first report of the molecular identity cystic fibrosis transmembrane conductance regulator (CFTR). It is firmly established that CFTR mutation and variants, of which there are over 1900 (Spielberg & Clancy, 2016) and the most common of which is F508del, underlie the disease. Furthermore, recent studies have shown that non-CF diseases may cause dysfunctional activity of wild-type CFTR resulting in acquired CF-like disease state, notably in chronic obstructive pulmonary disease (COPD) (Solomon *et al.* 2016), asthma and acute pancreatitis (Saint-Criq & Gray, 2016).

CFTR is a member of the ATP-binding cassette (ABC) transporter family, and is a large protein with complex biophysical characteristics that forms an ion channel that is permeable to $Cl^-$ and $HCO_3^-$. CFTR is involved in both fluid absorption and secretion in a number of epithelia including airway, gastrointestinal and reproductive, sweat and salivary glands. The channel structure contains several domains; two membrane spanning domains, two nucleotide binding domains (NBD), and a regulatory domain. The gating of CFTR is ATP dependent, and channel activity is regulated by phosphorylation and intracellular pH (Chen *et al.* 2009). Considerable research has led to our understanding of wild-type and mutant channel activity. Distinct CFTR mutations are present throughout the channel protein and have been divided into six classes dependent on their molecular consequences (Ikpa *et al.* 2014). The mutations alter the synthesis, processing, function, or half-life of CFTR.

In this issue of *The Journal of Physiology*, Chen *et al.* (2017) present an elegant and thorough study which enhances our knowledge of the intraburst kinetic behaviour of CFTR, and how this is influenced by alteration of intracellular pH. While single channel gating kinetics have been studied extensively, we still do not have a complete understanding of how wild-type or mutant CFTR functions. Chen *et al* demonstrate how subtle changes in intracellular pH can impact ATP-dependent intraburst open and closed times, and reveal a second mechanism that further controls intraburst gating. This is important because understanding how specific regions/amino acid residues impact channel open time will provide a molecular roadmap and will enable focused drug discovery efforts. Thus, knowledge of regions important for regulation, interactions of NBD domains and pore gating extends beyond the biophysicist's natural curiosity to understand channel behaviour. This insight provides a broader impact for therapeutic intervention in both CF and acquired CF-like disorders.

Targeting CFTR for small molecule modulators has received significant attention in recent years. Traditional high throughput screening of chemical libraries with structure–function optimization has identified compounds designed to be potentiators (compounds that increase channel open probability), or correctors (proteins that improve trafficking to the apical surface of epithelial cells) (Zhang *et al.* 2012). Efforts have focused on both functional and biochemical cell-based assays with little attention to the biophysical interplay of CFTR domains. To date, one potentiator (VX-770, ivacaftor, Kalydeco) and one corrector (VX-809, lumacaftor; when in combination with ivacaftor called Orkambi) have been approved by the FDA for CF patients with specific CFTR mutations. Others are in various stages of drug development, providing an arena of intense focus for active compound discovery.

A natural progression in drug discovery is to apply an *in silico* approach wherein knowledge of the 3-D structure of CFTR can be combined with small molecule chemical structure to identify compounds targeted against specific portions of the channel protein. The complexity of the structure of CFTR has added difficulty for investigators who are attempting to elucidate the three-dimensional structure of the protein (Moran, 2014; Callebaut *et al.* 2016). Our understanding of how small molecule interactions can impact CFTR activity has the potential for enhancement based on the work of Chen *et al.* (2017). The authors concentrated on intraburst gating using the intrinsic pH sensitivity as a probe together with NBD specific mutations to interrogate ATP binding/hydrolysis-dependent and -independent modulation. Notably, identification of residues which impact channel gating, particularly by influencing the intraburst open-time provide further clues that direct us towards, or away from, particular regions of the protein. In this manner, we can make educated predictions regarding the potential effect of drugs on channel gating, and increase throughput for the discovery of bioactive molecules capable of enhancing CFTR function or trafficking.

When CF was first reported in 1938, predicted survival was only 6 months. Today, due to a combination of early diagnosis, improvement in the patient's nutritional status, and control of airway infection life expectancy has increased to ~40 years. With new therapies emerging, this is likely to increase even further. Additionally, as a deeper understanding of acquired CF-like disease emerges with an understanding of the underlying biophysical modification, and the clinical assessment of CFTR potentiators proceed (Solomon *et al.* 2016), it is highly likely that CFTR modulators will have a broader target patient population.

Going forward, greater knowledge of CFTR regulation and gating will be paramount in making informed decisions regarding which portion of the channel protein to target for small-molecule intervention and critical evaluation of the potential functional implications. Chen, Xu and Sheppard (Chen *et al.* 2017) have significantly contributed to this end.

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