CFTR Lifecycle Map

Subjects: Biochemistry & Molecular Biology | Cell Biology | Medicine, Research & Experimental
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Cystic Fibrosis (CF) is one of the most common genetic diseases prevalent among the Caucasian population and is caused by mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. To date, several hundred disease-causing mutations are known, resulting in a vast range of geno- and phenotypes, which makes the development of therapeutics especially challenging. To support the development of novel therapeutics, systems biological disease maps can be used. Disease maps represent existing knowledge on disease mechanisms in a computationally readable and comprehensive manner so they can then be used by clinicians and experimental scientists as well as computational scientists for different purposes, such as structuring high-throughput data, identifying disease biomarkers, developing better diagnostics and also identifying potential drug targets and drug repositioning. The CFTR Lifecycle Map in particular details the biogenesis of CFTR in cells to support ongoing drug discovery endeavours in CF research.

cystic fibrosis | CFTR | CFTR maturation | systems medicine model | trafficking | CFTR modulators

1. Introduction

Cystic fibrosis (CF) is an inherited disorder prevalent among the white European population, where, with an incidence of approximately 1 in 3000 newborns, it is one of the most common monogenic autosomal recessive diseases [1]. CF is caused by mutations of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [2], which encodes a membrane protein that serves as a chloride and bicarbonate channel in exocrine epithelia of various organs, and thereby regulates the viscosity of the mucus lining [3]. Defective CFTR, therefore, has severe implications throughout the body [2][3].

To date, more than 2100 mutations of the *CFTR* gene are known, several hundred of which are known to be disease-causing [4][5][6]. They can cause defects anywhere in CFTR's complicated and sensitive lifecycle and the wide range of mutations, resulting in different defects in the CFTR protein, makes causative treatments for CF difficult to find. As a result, there is still no mutation-specific therapeutic for some patients, especially those with rare *CFTR* mutations. The latest research efforts, therefore, focused on developing combination therapies to target multiple defects at once [7][8]. For this purpose, high-throughput (HT) screens have been performed [9][10][11][12][13][14][15][16][17][18][19][20][21]. These result in a plethora of data and various candidate compounds, often with an unclear mode of action. In order to provide an overview of already tested compounds, we previously established the

publicly available database CandActCFTR (https://candactcftr.ams.med.uni-goettingen.de/), where substances from 111 publications are listed and categorized according to their influence on CFTR function.

In order to support the elucidation of the mechanism of action for promising candidate substances and to be able to predict possible synergistic effects of substance combinations, it used a systems biology approach to create a model of the CFTR maturation pathway in cells.

This entry present a systems biological model of the CFTR lifecycle in a standardized, explorable and tractable format. The model is composed of two datasets, a core map manually curated from small-scale experiments in human cells, and a coarse map including interactors identified in high-throughput (HT) efforts [22][23][24][25]. Overall, the manually curated core map includes 170 different molecular entities and 156 reactions from 221 experimental publications. The high-throughput data layer encompasses 1384 unique interactors from four publications by Wang et al., 2006, Pankow et al., 2015, Santos et al., 2019 and Matos et al., 2019 [22][23][24][25]. The CFTR Lifecycle map provides a tool to structure and exploit existing knowledge and data, as well as develop a hypothesis regarding synergistic drug targets and novel therapeutics.

2. CFTR Map

The CFTR map encompasses information from small-scale experiments as well as high-throughput efforts, leading to differences in the degree of detail and confidence. It was therefore split into different data layers. The first data layer, the core map, was manually curated and only includes high-confidence interactors, confirmed by at least two independent small-scale experiments or acknowledged by two reviews from different research groups. As a result, the number of molecular interactions in the core map is limited but each is described with a high level of detail. The second data layer in the coarse map represents the high-throughput interactome of wt-CFTR (the interactome of F508del was excluded) as published by Wang et al., 2006, Pankow et al., 2015, Santos et al., 2019 and Matos et al., 2019 [22][23][24][25] in a structured cell layout. Here, the large-scale experimental method does not allow for conclusions regarding the nature of the interactions. Therefore, a small level of detail, but a high number of interactors, is included in the map.

2.1. Representation of the CFTR Lifecycle in the CFTR Core Map

The CFTR core map represents the molecular mechanisms affecting wt-CFTR during its lifecycle. It is the product of an exhaustive literature curation process and the manual integration of different data sources. As the whole model is represented and written in the standardized SBGN format [26], it is human understandable as well as computationally tractable. At the moment, it encompasses 262 different molecular entities and 156 reactions in 6 main cellular compartments. The biomolecules are categorized into 149 proteins, 58 complexes, 28 simple molecules, 13 ions, 6 genes, 5 RNAs, and 3 pools of degraded protein, amino acids, or nucleotides.

The CFTR core map (**Figure 1**) has a roughly cell-shaped layout, with CFTR making its way from the nucleus at the bottom all the way up to its site of action at the plasma membrane. It covers the molecular interactions CFTR

undergoes on its way from being transcribed in the nucleus to being a functional ion channel at the apical plasma membrane, including its activity and regulation there, as well as endocytosis, recycling and degradation of the mature protein. The map can be subdivided into five submaps to enable the user to either look at its whole or individual processes, depending on their focus or interest. The five submaps are guided by the subcellular location and process they focus on.

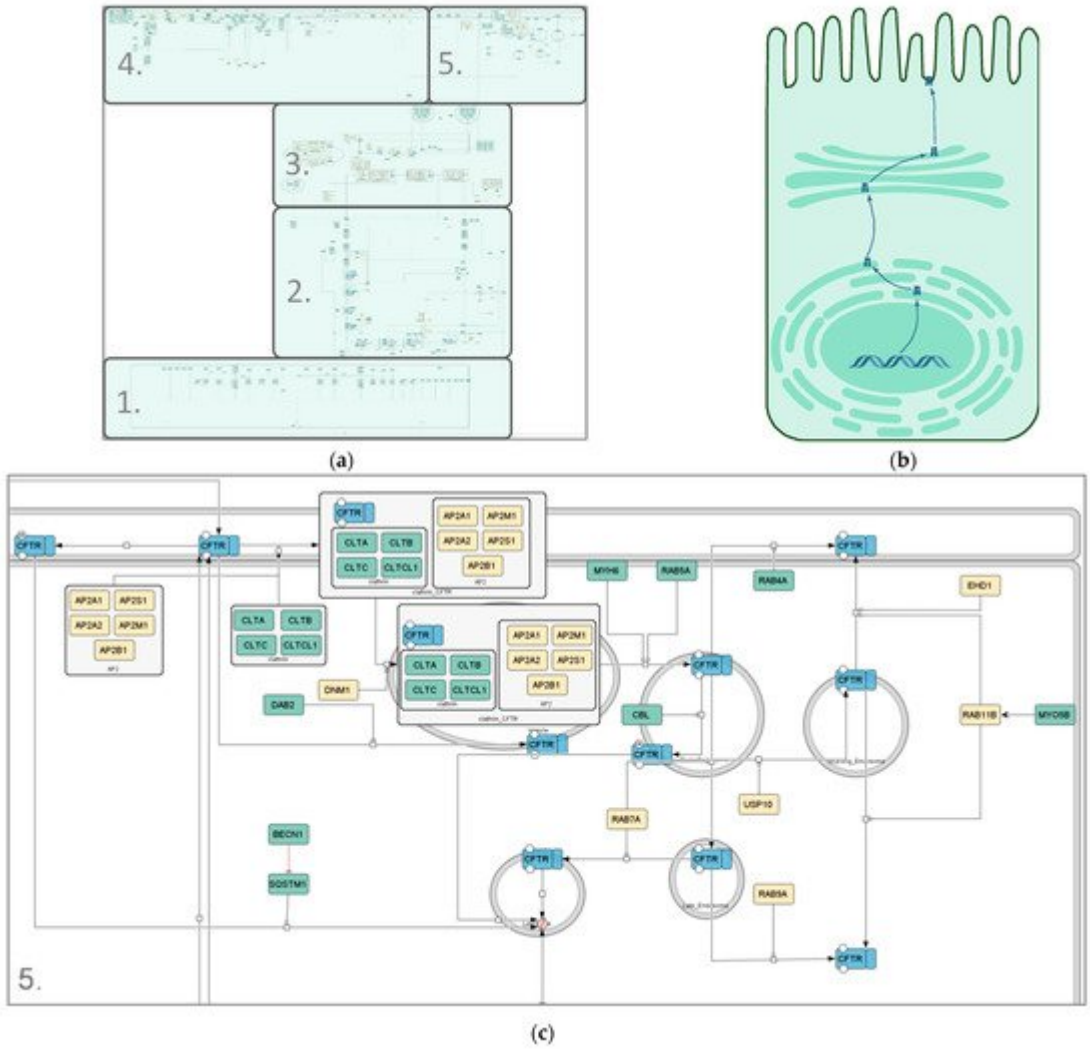

Figure 1. Different representations of the CFTR lifecycle. (**a**) Image of the SBGN-compliant manually curated CFTR lifecycle core map; (**b**) cartoon representation of the CFTR lifecycle in an apical epithelial cell; (**c**) zoomed-in section of the endocytosis pathway in the manually curated CFTR lifecycle core map (submap 5).

3. Visualization of the wt-CFTR Interactome as Coarse Model

In addition to the extensive manually curated interaction pathways a second data layer was included for interactors with lower confidence and detail. The second data layer represents information from large-scale experiments, namely the wt-CFTR core interactomes published by Wang et al., 2006, Pankow et al., 2015, Santos et al., 2019 and Matos et al., 2019 [22][23][24][25]. The coarse map is also written in the SBGN format, but is represented in the

SBGN Activity Flow notation, which lacks mechanistic information. In order to structure the information into an intuitive, cell-based layout, the interactors were grouped according to their function and subcellular localization. Again, the model was divided into several submaps, based on the functional categorization. Each submap focuses on one main step or area of function in the CFTR-lifecycle, abstracted into a state-transition reaction. Six of them correspond to those from the core map, with an extra map for mRNA processing between the “Transcription” and “Translation, Folding and ER Quality control”. Additional maps focus on interactions with the cytoskeleton, as well as immune-related and other interactors.

4. Conclusion

The CFTR Lifecycle Map is part of the CandActCFTR project, which established a publicly available database of candidate cystic fibrosis therapeutics, combining data from different sources, such as high-throughput- and small scale screens, data from relevant databases and unpublished primary data (candactcftr.ams.med.uni-goettingen.de/). The CFTR Lifecycle Map, as a second part of the project, aims to provide the means to identify promising drug targets and elucidate the mode of action for candidate substances. Furthermore, it will be used to predict possible additive effects of different substance combinations. It thereby simultaneously provides a backbone to structure available data as well as a tool to develop hypotheses regarding novel therapeutics.

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