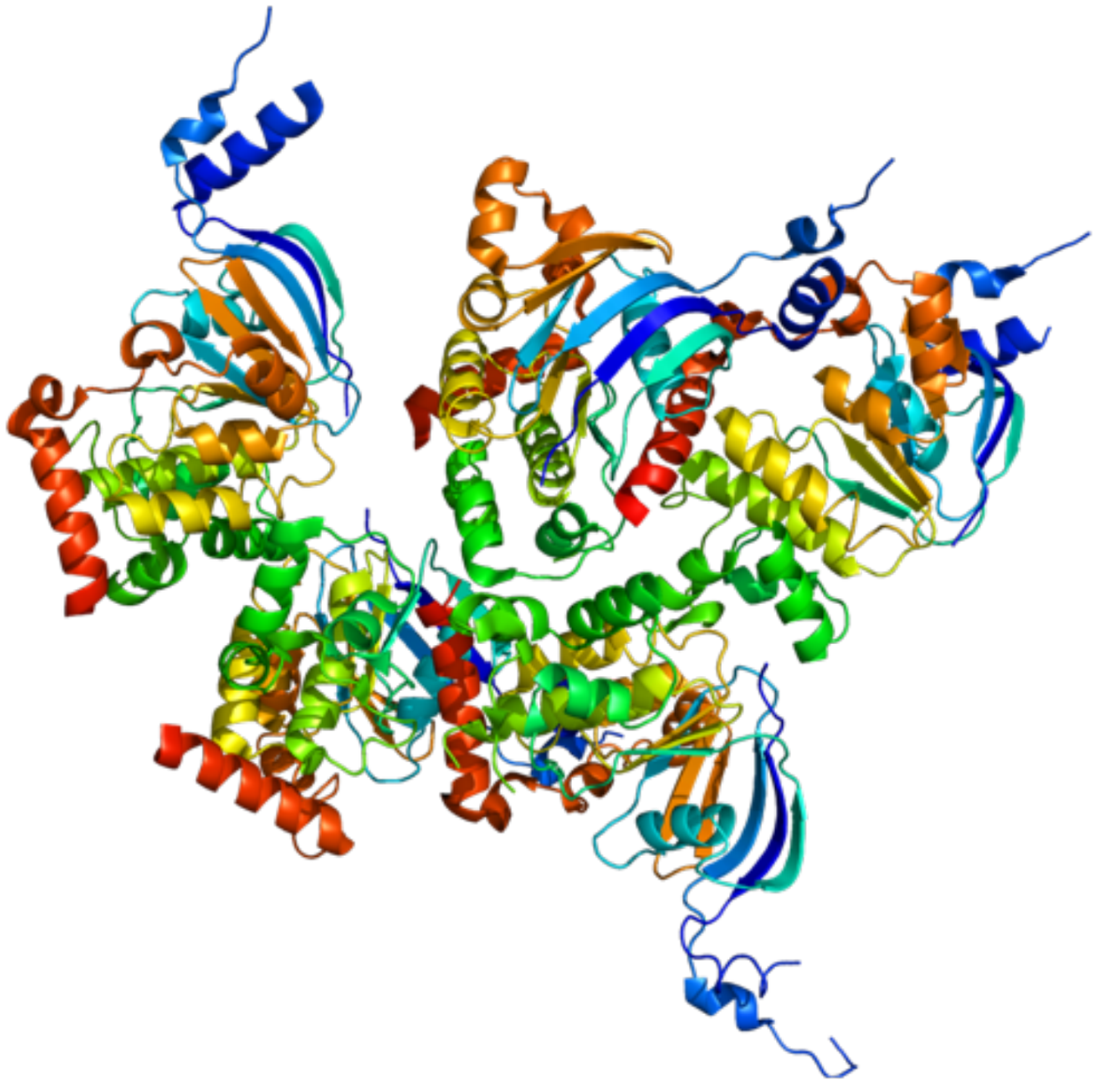
CFTR



(Cystic Fibrosis Transmembrane Conductance Regulator)

What is this thing?

- CFTR is a transmembrane channel protein. It exists in all jawed vertebrates, and modulates the passage of chloride [3] and thiocyanate ions [1] through phospholipid bilayers.

- Mutations in CFTR are responsible for the etiology of cystic fibrosis. There are ~2000 documented mutations, but most belong to a general subset of CFTR dysfunctions.

- Cystic Fibrosis (CF) is an autosomal recessive disease characterized by an overproduction of mucus in all of an afflicted individual's mucosa, causing male sterility, pancreatic/cardiovascular/digestive dysfunction, pulmonary infection and failure.

- Interesting as both an etiological factor and a model for understanding autosomal disorders.

Its History

- Much like other homozygous diseases, there exists a set of evolutionary explanations for CFTR mutations.

- CFTR discovered and characterized 1989 by Messrs. Riordan, Rommens, Kerem, et al. by means of cDNA expression measurement in epithelial cells of CF patients. They discover the most common mutation, ΔF508. [3]

- Better methodologies and increased interest spur the characterization of hundreds of other mutant CFTR.

- The natural history: CFTR appears in aquatic vertebrates (exact time uncertain) for osmoregulation, and take a secondary role in organ development of epithelial systems. The gene propagated to the other chordates. The phylogeny illuminates function. [1]

ΔF508

- ΔF508 is a CFTR mutation characterized by a deletion of a single phenylalanine residue at position 508.

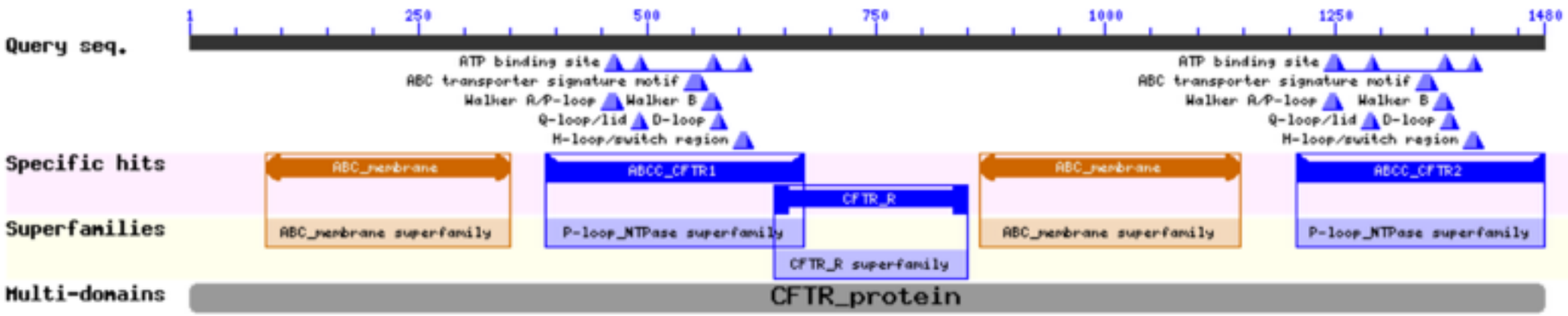
- Heterozygosity may be evolutionarily advantageous—cholera toxin, diarrhea, asthma hypotheses.
    - …but homozygosity causes CF and is fatal.
    - And the data suggest that negative selection may also explain the prevalence of the mutation. [2]

- Single residue deletion in transmembrane region, mutant-type CTFR cannot be exported from endoplasmic reticulum to epithelial cell membranes.

The Interesting Parts

- CFTR https://www.youtube.com/watch?v=_j99-xgOIaw
- Structure: Two transmembrane domains, two nucleotide binding domains (ATP), a regulatory site.
- Mutation types:
    - Splice error: Most protein produced is nonfunctional and cannot embed into ER for trafficking to apical membrane.
    - Premature stop codon: Truncated, nonfunctional protein.
    - Trafficking defect: ΔF508 and others. Protein misfolds, never leaves ER.
    - Gating defect & narrow chloride channel: Cl- cannot pass through the protein as in wild-type
    - Decreased stability: Proteins don't stay in the cell membrane as long as wild-type, and are degraded.
- Net effect is osmotic dysregulation: hyperabsorption of some ions in some tissues, underabsorption of some ions in others (depending upon how CFTR functions in the cell)

The Families & Their Structures



- ABC Transporter Superfamily & membrane superfamily, also its own personal superfamily
- cAMP-dependent Chloride Channel (NB: the regulatory site)
- ATP binding are well-conserved, ABC transporters have a signature motif
- http://www.ncbi.nlm.nih.gov/protein/P13569.3

Why Are The Interesting Parts Interesting?

- Development of novel therapies.
    - Potentiators
        - A class of pharmaceuticals modifies gate defects CFTR such that the protein no longer requires ATP to mediate flux. Improves pancreatic function in human models. [6]
- CRISPR-Cas9
    - Homozygous hereditary illnesses are sensitive to gene therapies.
    - CRISPR-Cas9 + HIV-derived factors + small hairpin RNA can knockdown CFTR mutant expression in airway cells. [5]

Question Time

- Say we use a CRISPR-Cas9 knockdown therapy in individuals with CF, and use a CRISPR-Cas9 therapy inducing expression of wild-type CFTR. Would lung function return to normal?
    - Animal models (sorry)
- Would organismal knockdown of the trait adversely effect downstream systemic functionality?
- Can CF CRISPR-Cas9 gene therapeutic model be abstracted to the other autosomal diseases? (Huntington's, Tay-Sachs, etc.)

References.

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