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Rescue of F508del CFTR: Commentary on "F508del CFTR with two altered RXR motifs escapes from ER quality control but its channel activity is thermally sensitive"

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The cystic fibrosis transmembrane conductance regulator (CFTR) belongs to the ATP-binding cassette superfamily and mediates the transport of chloride and bicarbonate through epithelial cell membranes. Mutations in the human *CFTR* gene cause cystic fibrosis (CF) [1], and more than 90% of CF patients are either homozygous or compound heterozygous for the 3-bp in-frame deletion F508del [2]. F508del *CFTR* is the most common, potentially fatal mutation in Caucasian populations. Although data is conflicting to what extent F508del CFTR can be processed and remains functional in CF patients' epithelia, in vitro studies consistently demonstrated that F508del CFTR is a temperature-sensitive mutant, defective in protein maturation [3]. F508del CFTR is unable to adopt a protease-resistant mature conformation that enables exit from the endoplasmic reticulum (ER) and processing in the Golgi compartment. The ER quality control [4] leads to ER retention, retrotranslocation and ultimately to cytoplasmic degradation of mutant CFTR by the ubiquitin-proteasome pathway [5,6]. Wild-type and F508del-CFTR undergo ER-associated degradation (ERAD) from two distinct checkpoints, the mutant being disposed of independently of N-glycosidic residues and calnexin, probably by the Hsc70/Hsp70 machinery, and wild type CFTR undergoing glycan-mediated ERAD [7]. If F508del CFTR is processed at the permissive temperature and delivered to the plasma membrane, the mutant exhibits reduced channel open probability and sensitivity to stimulation with cAMP agonists [8], and rapidly disappears from, and does not return to the cell surface due to decreased conformational stability [9,10]. Mutant CFTR is the target for the treatment of the basic defect in CF and researchers are currently screening for correctors of defective F508del CFTR folding/cellular processing ("correctors") and channel gating ("potentiators") [11].

John R. Riordan co-discovered the *CFTR* gene and its major CF mutation in 1989 [12] and has published numerous seminal papers on CFTR structure and biochemistry. His group has now solved another mystery of CFTR that is relevant for any future pharmacological therapy of CF. Based on a previous study [13], Hegedus et al. report in this issue of the *Journal* that the inactivation of two of the four arginine-framed tripeptide signals in CFTR, R29-Q30-R31 at the N-terminus and R553-A554-R555 in the cytosolic nucleotide-binding fold NBF1 is necessary and sufficient to promote trafficking of F508del CFTR towards the plasma membrane. The rescued mutant, however, remains thermolabile indicating that the deletion of F508 which is also located in NBF1 causes both a defect in protein folding and stability at 37 °C.

Arginine-based ER-localization signals are sorting motifs that are involved in the biosynthetic transport of multimeric membrane proteins [14]. These motifs are located in the cytoplasmic regions of polytopic membrane proteins that are subunits of membrane protein complexes; the presence of the signal maintains improperly assembled subunits in the ER until it is masked as a result of heteromultimeric assembly [14]. CFTR is the first case demonstrating that the Arg-based ER sorting motif couples the control of folding with the forward transport of membrane proteins. As long as the two RXR signals in the N-terminal tail and in the dodecapetide motif are exposed during folding, CFTR is retained in the ER. Simultaneous substitution of one arginine by a lysine in each of the two RXR tripeptides of F508del CFTR was necessary and sufficient to escape the ER quality control and to reach the plasma membrane. This finding is surprising in the light of a recent report [15] that the diacidic D565-A566-D567 motif in NBF1 is essential for the exit of CFTR from the ER. According to the new data by Hegedus et al., the diacidic signal motif is less relevant to the fate of F508del CFTR than the two RXR motifs in the N-terminal tail and the dodecapeptide.

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Hegedus' finding also provides a clue why second-site mutations in the RXR motif in the dodecapetide of NBF1 such as R553Q, R553M and R555K partially corrected the F508del CFTR mutant phenotype in model systems [16] and in CF patients [17]. Some 20 years ago the commentator made the clinical diagnosis of CF in a 7-year old patient in whom CF had been suspected before but had been excluded because of normal chloride concentrations in sweat tests. *CFTR* mutation analysis uncovered the mutation R553Q on one F508del CF chromosome of this patient. In light of the newly gained knowledge, it can be suspected that the inactivation of one RXR ER retention motif partially rescued F508del CFTR from the ER quality control and retained substantial residual function of CFTR in the patient's sweat glands. This case illustrates how basic CFTR research may unintentionally resolve puzzling CF disease phenotypes.

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