Analysis of function in the carboxyl-terminal domain of the skeletal muscle chloride channel, ClC-1

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Human ClC-1 has a 398 amino acid cytoplasmic carboxyl-tail, which includes two cystathionine β-synthase (CBS) domains, confirmed as being very important by various function-affecting truncations, mutations and functional complementation studies. To investigate the mechanism of its significant functional role, we used deletion scanning mutagenesis, alanine scanning mutagenesis, split channel strategy and GST-pulldown methods to determine the essential protein-protein interaction positions. We found that the strong interaction between $N_{1\text{-}720}$ (containing CBS1) and $C_{721\text{-}988}$ (containing CBS2) was not affected when important amino acids, in terms of channel function and gating, were deleted, including $C_{721\text{-}988\Delta(800\text{-}806)}$, $C_{721\text{-}988\Delta(807\text{-}813)}$ and $C_{721\text{-}988\Delta(863\text{-}888)}$. However, when CBS2 was deleted from $C_{721\text{-}988}$, the interaction was abolished ($N_{1\text{-}720}$ + $C_{721\text{-}988\Delta(820\text{-}870)}$). Similarly, when CBS1 was deleted from $N_{1\text{-}720}$, the interaction disappeared ($N_{1\text{-}720\Delta(592\text{-}662)}$ +$C_{721\text{-}988}$ and $N_{1\text{-}720\Delta(607\text{-}662)}$ + $C_{721\text{-}988}$), which demonstrated that the functional channel gained by co-expressing $N_{1\text{-}720}$ and $C_{721\text{-}988}$ was realized mainly by the interaction between CBS1 and CBS2. This does not eliminate the possibility that the carboxyl tail could interact with other parts of the transmembrane region, such as loops between transmembrane domains, so we went on to study the significance of the cytoplasmic loops. Alanine scan mutants of different cytoplasmic loops were produced in $N_{1\text{-}720\Delta(607\text{-}662)}$ (a transmembrane region construct truncated after S720 and missing CBS1 which retained some interaction with $C_{598\text{-}988}$). Alanine scans of loops DE (199-206), FG1 (251-255), FG2 (257-261), HI1 (293-296), HI2 (297-300) and JK (380-385)) were co-expressed with $C_{598\text{-}988}$ (containing both CBS1 and CBS2), but no difference in interaction was observed compared to positive control, $N_{1\text{-}720\Delta(607\text{-}662)}$ + $C_{598\text{-}988}$. To conclude, interaction between CBS1 and CBS2 is strong, but, although other interacting positions are conceivable, significant binding of the carboxyl tail to the cytoplasmic loops of the transmembrane region of the protein appears to have been eliminated.