A SYK/SHC1 pathway regulates the amount of CFTR in the plasma membrane

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Extracellular signals and cellular response

- Signal
  - Plasma membrane
  - Receptor

- Signal transduction

- Cytoplasm
- Gene expression
  - Nucleus

Rapid response
- Cell shape changes
- Membrane traffic
  - Ion transport...

Prolonged response

Hypertension
Cystic Fibrosis
Cystic fibrosis

- The most frequent genetic disease in the Caucasian population
- Affect more than 70,000 individuals worldwide
- Production of abnormally thick mucus
  - Recurrent respiratory infections
  - Pancreatic insufficiency

Cystic fibrosis transmembrane conductance regulator (CFTR)

Over 2000 mutations causing cystic fibrosis

Multisystem disorder
Regulation of CFTR through a SYK pathway

Tyrosine kinase with pleiotropic effects in the human body
- Crucial role in adaptive immune receptor signaling
- Novel role in ion transport regulation

Target residue is CFTR Tyr512
Spleen tyrosine kinase (SYK)
Substrate motif
Y-D/E-D/E-X

Mendes et al. Mol Cell Biol. 2011
SYK activity modulates the cell surface expression of CFTR in human airway epithelial cells.
Identify phospho-tyrosine-binding proteins involved in the regulation of chloride transport protein and the underlying molecular mechanism.

Tyrosine phosphorylation creates binding sites for proteins containing phospho-tyrosine binding domains (SH2 or PTB domains).

Isolate and identify adaptor proteins in the human proteome, which are able to recognize SYK-phosphorylated chloride transporting protein.
Proteomic peptide-protein interaction screen

Biotinylated synthetic peptides

- IFGVS-\(p\)Y-DEYRY
- IFGVS-\(Y\)-DEYRY
- IFGVS-\(F\)-DEYRY

CFTR

SYK substrate motif Y-D/E-D/E-X

CFBE WT-CFTR cells

Mass spectrometry

Schulze and Mann 2014
EGFR-Grb2 interaction
Bioinformatic analysis of MS data identifies a network of CFTR-associated proteins potentially involved in the regulation of chloride transport.

![Diagram showing network of CFTR-associated proteins](image)

- **CFTR**: This network node represents the CFTR protein.
- **CFTR-pY**: Tyrosine phosphorylated form of CFTR.
- **CFTR-MUT**: Mutated form of CFTR.
- **CFTR-WT**: Wild-type form of CFTR.

Key statistics:
- Total nodes: 603
- CFTR-pY nodes: 61
- CFTR-MUT nodes: 26
- CFTR-WT nodes: 16
- Other nodes: 400

Authors:
- Francisco Pinto
- Paulo Matos
SHC1 binds specifically to the CFTR phosphopeptide

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Accession number</th>
<th>Protein name</th>
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<tr>
<td>CFTR-pY</td>
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<td>B box and SPRY domain-containing protein</td>
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<td>Q9NR12</td>
<td>PDZ and LIM domain protein 7</td>
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### CFBE wt-CFTR cells

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<thead>
<tr>
<th>Protein</th>
<th>Cell lysate</th>
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<tr>
<td>SHC1</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td>NCK</td>
<td>90</td>
<td>37</td>
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<tr>
<td>GRB2</td>
<td>25</td>
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</table>

SHC1 binds specifically to the CFTR phosphopeptide.
SHC1 binds to phospho-tyrosine in CFTR through its PTB domain

**SHC1**
- Important role in activation of the RAS/MAPK pathway
- Unknown role in membrane protein traffic
- p46SHC1, p52SHC1 and p66SHC1

**Diagram: SHC1 structure**

**Figure: CFTR interaction with SHC1**

**Legend:**
- SHC1
- PTB
- SH2

**Phosphorylated Tyr512 binds specifically to the SHC1 PTB domain**
SHC1 can associate with full-length CFTR

CFBE cell line stable transfected with DOX inducible wt-CFTR

SHC1 forms a complex with CFTR that depends on the activity of kinase SYK.
Does SHC1 modulate CFTR cell surface expression? 

SHC1 modulates the amount of CFTR at the cell surface
SYK and SHC1 operate as a pathway and affect the cellular chloride transport activity.

**Figure:**
- **Left panel:** Western blot analysis of CFBE wt-CFTR cells stably expressing the HS-YFP sensor.
  - CFTR, PCNA, SHC1, FLAG, PCNA bands.
- **Right panel:** Graph showing the effect of SYK Y352D and SHC Y352D on CFTR activity.
  - Surface CFTR (fold over control) and dF/dt (nM/s) measured.
  - siLuc, siSHC, siSYK treatments compared.
  - Statistical significance marked with * and **.

**Legend:**
- sILUC: siLuc + GFP-EV
- sILUC Y352D: siLuc + SYK-Y352D
- sISHC: siSHC + GFP-EV
- sISHC Y352D: siSHC + SYK-Y352D

**Graph Details:**
- x-axis: Time (s)
- y-axis: F/F₀ (%)
- Bars: siLuc, inh172, siSHC, siSYK
- Annotations: 1.0, 0.2, 2.3, 2.9
Conclusions

- Tyrosine phosphorylation by SYK downregulates the cell surface abundance of the chloride channel protein CFTR;
- SHC1 was identified as a phosphotyrosine-binding regulator of CFTR;
- SHC1 forms a complex with CFTR at the plasma membrane upon activation of SYK and modulates the amount of cell surface CFTR.

Novel role for SHC1 in membrane traffic;
Potential biomedical implications for the identification of new therapeutic targets in cystic fibrosis.
Acknowledgments

Peter Jordan
Luka Clarke

Paulo Matos
Francisco Pinto

“Oncobiology and Signaling Pathways Lab”
Patrícia Barros
Vânia Gonçalves
Andreia Henriques
Joana Pereira
Márcia Faria
Ana Matos