

Protein kinases WNK4 and SYK constitute a signalling pathway regulating cell surface expression of CFTR

The subfamily of With No Lysine [K] (WNK) protein kinases is characterised by a unique sequence variation in the catalytic site and contains four genes in the human genome. The discovery that mutations in WNK1 or WNK4 cause familial hypertension led to the identification of a role for WNK1, WNK3 and WNK4 in the regulation of a variety of ion transporters, including the chloride channel CFTR. An important aspect of ion channel biology in several diseases is the actual amount of channel expressed at the cell surface. We have found that human WNK4 is part of a signalling pathway modulating CFTR surface expression. WNK4 was isolated in a complex with the tyrosine protein kinase Syk, which phosphorylated CFTR at Y512 *in vitro*. The presence of WNK4 or its kinase-dead mutant blocked the phosphorylation of CFTR by Syk. In BHK cells expressing CFTR, the effect of Syk and WNK4 on surface expression of this ion channel was analysed by immunofluorescence and cell-surface biotinylation and its activity assessed by iodide efflux measurements. We found that expression of Syk inhibits surface expression of CFTR and ion efflux activity. In contrast, the presence of kinase-dead Syk mutant as well as co-expression of Syk with WNK4, increased surface CFTR and ion transport activity. Consensus Syk phosphorylation motifs are also present in some other chloride transporters. Taken together, our data reveal a novel signalling pathway involved in the regulation of some ion channels.

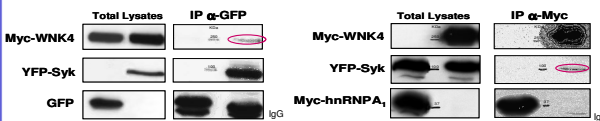
1. Antibody array approach

Nitrocellulose array with 400 antibodies to signalling molecules

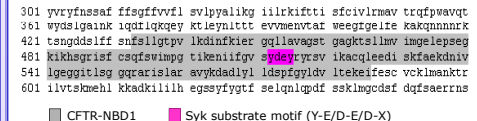


Incubated with HEK293 cell lysate expressing myc-WNK4
Incubated with anti-myc peroxidase only (no lysate)

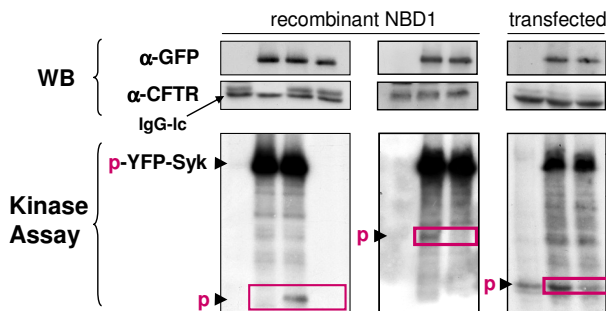
2. Myc-WNK4 and YFP-Syk co-immunoprecipitate in HEK293 cells



3. Unique Syk substrate motif present in NBD1 of CFTR



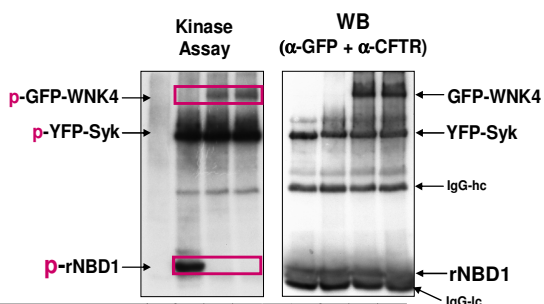
4. Syk phosphorylates Tyr512 in NBD1 of CFTR *in vitro*



	recombinant NBD1				transfected							
WB α-GFP	+	-	-	-	+	-	-	-	+	-	-	-
WB α-CFTR	+	-	-	-	+	-	-	-	+	-	-	-
WB IgG-Ic	+	-	-	-	+	-	-	-	+	-	-	-
Kinase Assay p-YFP-Syk	+	-	-	-	+	-	-	-	+	-	-	-
Kinase Assay p-rNBD1	+	-	-	-	+	-	-	-	+	-	-	-
GFP-vector	+	-	-	-	+	-	-	-	+	-	-	-
YFP-Syk wt	-	+	+	-	-	+	+	-	-	+	+	-
YFP-Syk kd	-	-	-	+	-	-	-	+	-	-	-	+
rNBD1 wt	+	-	+	+	-	-	-	-	-	-	-	-
rSumo-NBD1 wt	-	-	-	-	+	+	-	-	-	-	-	-
rSumo-NBD1 Y512F	-	-	-	-	-	-	+	-	-	-	-	-
Myc-NBD1 wt	-	-	-	-	-	-	-	-	+	+	-	-
Myc-NBD1 Y512F	-	-	-	-	-	-	-	-	-	-	+	-

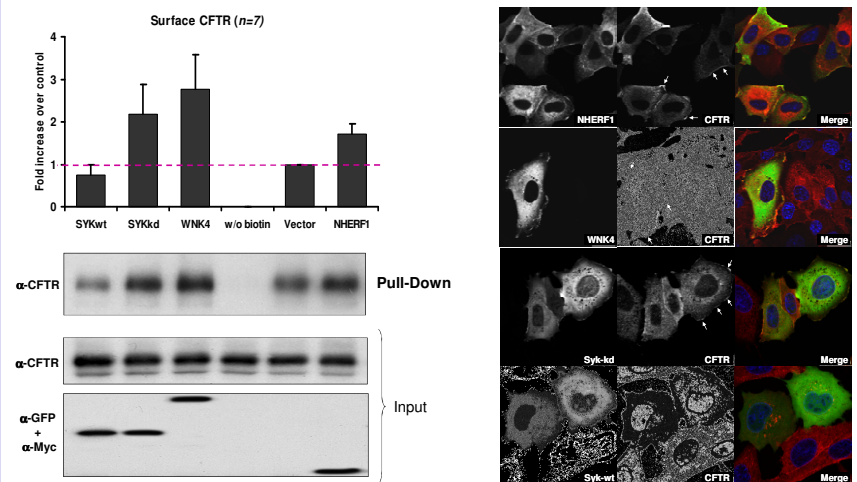
Human rNBD1 wt was kindly provided by Philip J. Thomas Laboratory, University of Texas Southwestern Medical Center at Dallas of the CFTR Folding Consortium

5. WNK4 inhibits the phosphorylation of CFTR-NBD1 by Syk

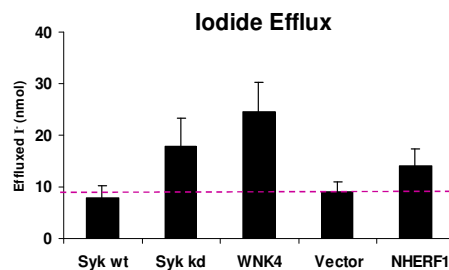


YFP-Syk kd	+	-	-	-	+	-	-	-
YFP-Syk wt	-	+	+	+	-	+	+	+
GFP-WNK4 wt	-	-	+	-	-	-	+	-
GFP-WNK4 kd	-	-	-	+	-	-	-	+
rNBD1 wt	+	+	+	+	+	+	+	+

6. Syk and WNK4 have opposing effects on the surface expression of CFTR in BHK cells...



7. ... and also on CFTR ion efflux activity



In summary:

- ✓ we identified the interaction of the protein kinase WNK4 with the Spleen tyrosine (y) kinase, Syk;
- ✓ Syk phosphorylates Tyr512 in the NBD1 domain of the WNK-regulated chloride channel CFTR *in vitro*;
- ✓ WNK4 inhibits the phosphorylation of CFTR-NBD1 by Syk;
- ✓ Syk decreases, whereas WNK4 increases, CFTR surface expression and resulting ion efflux activity in BHK cells expressing CFTR,;
- ✓ these data identify a signalling pathway involved in the regulation of ion transporters by WNK4.