The Hsc70 co-chaperone CHIP targets immature CFTR for proteasomal degradation

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Protein quality control is a posttranslational process, and is essential for functional cell activity. Accumulation of misfolded polypeptides will cause many different kinds of diseases. There are two way to control the newly synthsized proteins' quality. One is refolding, the other is degradation. Here, this paper talks something about ubiquitination degradation.

Abbreviation

|	
CF	cystic-fibrosis, a most common genetic disease
CFTR	cystic-fibrosis transmembrane-conductance regulator
CHIP	C-terminal Hsp70 interacting protein (through a set of tetratricorepeat motifs)
U-box	ubiquitin ligase domain
TPR	tetratricorepeat / tetratricopeptide repeats
ALLN	N-acetyl-leucyl-leucyl-norleucinal
Hsc70	a cytosol Heat Shock Chaperone (?)

First, I want to list some abbreviations I will use during this presentation.

CFTR is a plasma membrane ion channel that exposes the majority of its mass in the cytosol. We can see its distribution from the microscopy picture.

U-box, is a 75 amino acid domain first identified in UFD2, which is a yeast E4 enzyme. Recent study showed that U-box containing proteins functions as an E3 enzyme, and takes part in the ubiquitination and degradation.

I googled, but not sure what is Hsc70, or are there any different between Hsp70 and Hsc70. I thought there are the same.
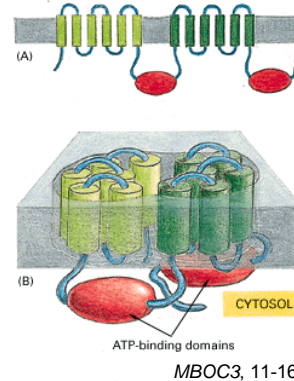
Basic knowledge
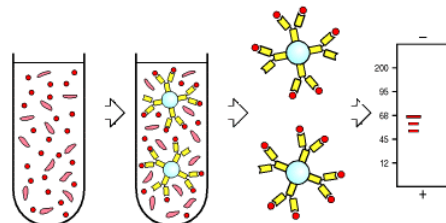
co-IP = IP + IB
re-IP = redo IP

Co-chaperone may be loosely defined as proteins that participate in the function of other chaperones.

ABC transporter family

ATP-binding domains

CYTOSOL

MBOC3, 11-16

Co-immunoprecipitation is an extension of the immunoprecipitation technique which is used to determine whether a given protein interacts physically with another given protein. Cell extracts containing the presumed interaction complex are first immunoprecipitated with antibody against one of the proteins. The material identified by this means is then tested for the presence of the other protein by immunoblotting with a specific antibody.

A typical ABC transporter consists of four domains: two highly hydrophobic domains, each with six putative membrane-spanning segments that somehow form the translocation pathway, and two ATP-binding catalytic domains (or cassettes). In some cases the two halves of the transporter are formed by a single polypeptide (*as shown*), whereas in other cases they are formed by two separate polypeptides.

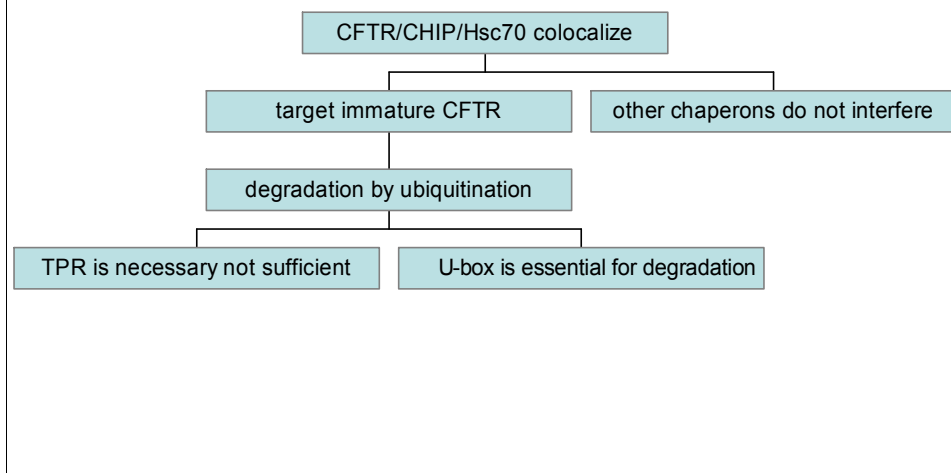Co-chaperone, I thought it is a chaperone of another chaperone.

- Patients suffering from CF have mutations in both of their genes encoding CFTR.
- There are hundreds of different mutations that can result in CF with varying degrees of severity.
- The most common mutation, ΔF508, results in a single amino acid deletion. CFTR-ΔF508 is thought to fold much more slowly than the wildtype CFTR protein.

Cystic fibrosis is the most common disease in U.S. And it relates to the mutation of CFTR.

One common mutation, delta F508 is a single amino acid deletion. And the author use this mutation as a compare in this paper, and I thought we can rule out the possible mechanism of how this mutation causes disease.

As in other literature, they showed that CFTR-delta F508 folds much slower than wild type, combine with the conclusion from this paper, I thought that the aberrant folding of CFTR-delta F508 leads to the aberrant degradation, which may interfere with CFTR's function.

Research strategy

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                    ┌─────────────────────────────┐
                    │   CFTR/CHIP/Hsc70 colocalize │
                    └─────────────────────────────┘
                    ┌──────────────┴──────────────────────┐
          ┌──────────────────────┐         ┌────────────────────────────┐
          │  target immature CFTR │         │ other chaperons do not interfere │
          └──────────────────────┘         └────────────────────────────┘
          ┌──────────────────────────┐
          │ degradation by ubiquitination │
          └──────────────────────────┘
        ┌──────────┴───────────────────┐
┌──────────────────────────┐  ┌────────────────────────────┐
│ TPR is necessary not sufficient │  │ U-box is essential for degradation │
└──────────────────────────┘  └────────────────────────────┘
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As there are so many co-IP in this paper, I would like to introduce the main structure of this paper first.

First, use immunofluorescent microscopy, they found CFTR/CHIP/Hsc70 are co-localized.

Second, they found that this degradation is not interfered by other chaperons, and have a high specificity to target immaturely glycosylated CFTR, which is called B form.

Third, the authors found that the degradation of immature CFTR is through ubqiuitinating, probably in proteasome.

Finally, they mentioned that the U box of CHIP is essential for the degradation, while TPR motif is necessary but not sufficient.

CHIP co-localizes with CFTR and Hsc70 at the ER

Green: GFP-CFTR; Blue: nuclear; Red: Myc-CHIP/the 20S proteasome/Hsp70; Yellow: co-localization.
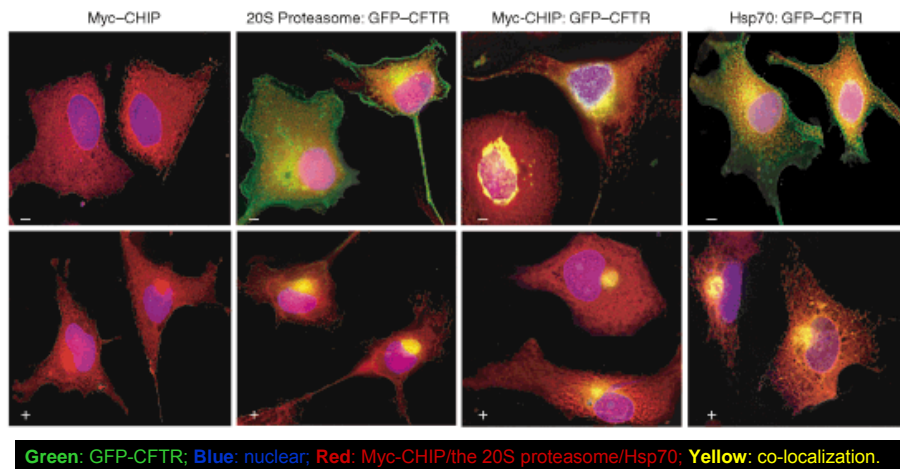
+, means treated with ALLN, an inhibitor of the 20s proteasome. If the green and red co-localize, it will show yellow.

Elevated CHIP levels, through the expression of exogenous Myc–CHIP, prevent the cell-surface localization of CFTR and cause green fluorescent protein (GFP)–CFTR14 to accumulate in a perinuclear location.

When cells are treated with the proteasome inhibitor ALLN, degradation of misfolded CFTR is inhibited and it accumulates in intracellular inclusions termed aggresomes. In ALLN-treated cells, the localization of CHIP is altered, and it can be colocalized in the aggresome with proteasomes, Hsc70 and CFTR.

Thus, CHIP has the potential to interact with CFTR in the ER or in aggresomes. It is important to note, however, that CFTR degradation initiates in the ER and that CFTR is not normally detected in aggresomes. Thus, CHIP probably functions at the ER to influence GFP–CFTR localization.

# Elevation of CHIP reduces CFTR-C, but not accumulates CFTR-B

Mutant causes aberrant folding and defective trafficking.
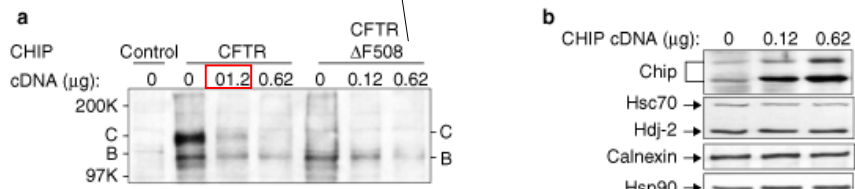
C form: maturely glycosylated, plasma-membrane-localized CFTR;
B form: immaturely glycosylated, ER-localized CFTR.

Elevation of CHIP activity does not cause detectable changes in co-chaperones of Hsc70 seems to alter the fate of immature CFTR (B form).
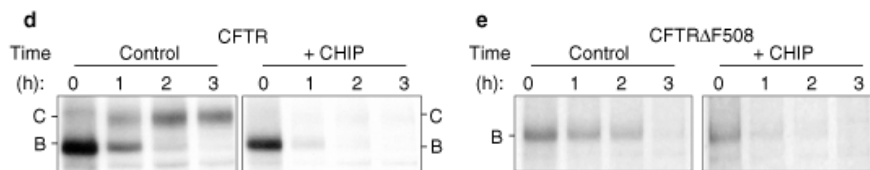
Degradation CFTR may involve the proteasome
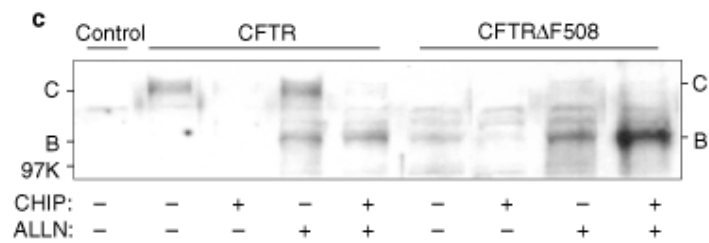

Proteasome inhibitor ALLN blocks CFTR maturation and degradation.

CHIP does not involve in the proteolysis, but may help target CFTR for degradation.

CFTR promotes the degradation of newly synthesized CFTR
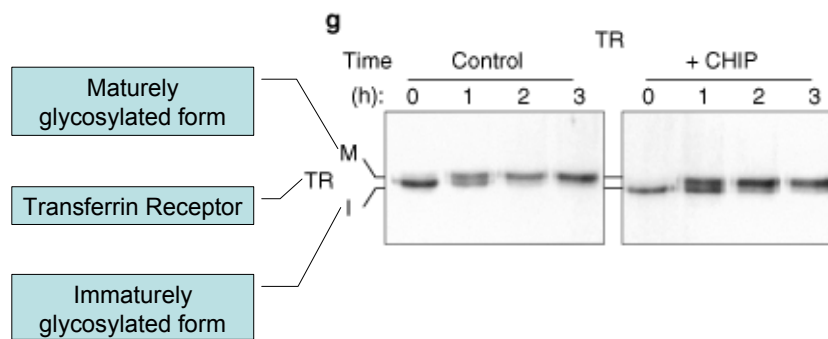

CHIP blocks the maturation of CFTR and causes almost all the CFTR-B to be degraded within the first 1 hr.
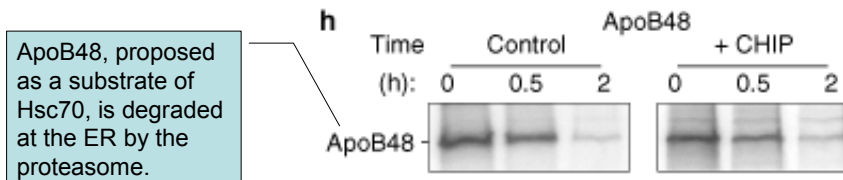
TPR & U-box are necessary not sufficient for CHIP's function
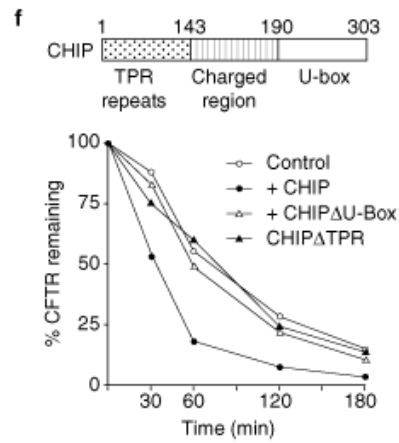
# CFTR's degradation by CHIP is not interfered with ER insertion

CHIP does not influence the degradation of apolipoprotein B48

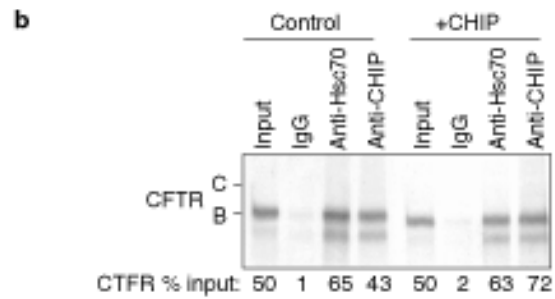ApoB48, proposed as a substrate of Hsc70, is degraded at the ER by the proteasome.

CHIP & Hsc70 exhibit specificity in the selection substrates that they target for degradation.

Hsc70/CHIP recognizes CFTR conformation-specifically
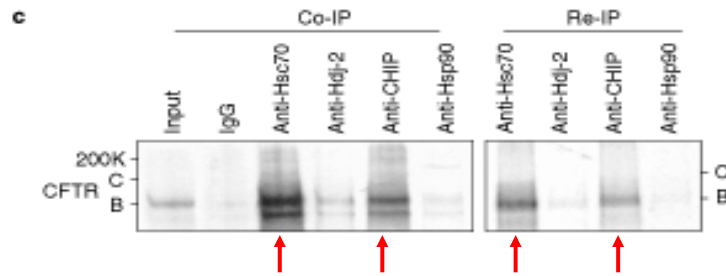

C form can not be IP with Hsc70/CHIP
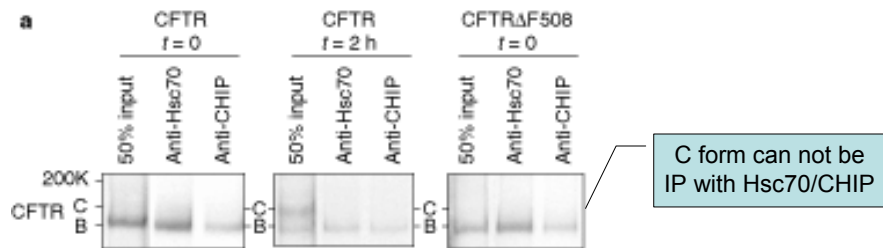
CHIP does not interfere the binding of Hsc70 to CFTR

Overexpression of CHIP has little influence on the total quantity of CFTR-B that could be co-IP with Hsc70.
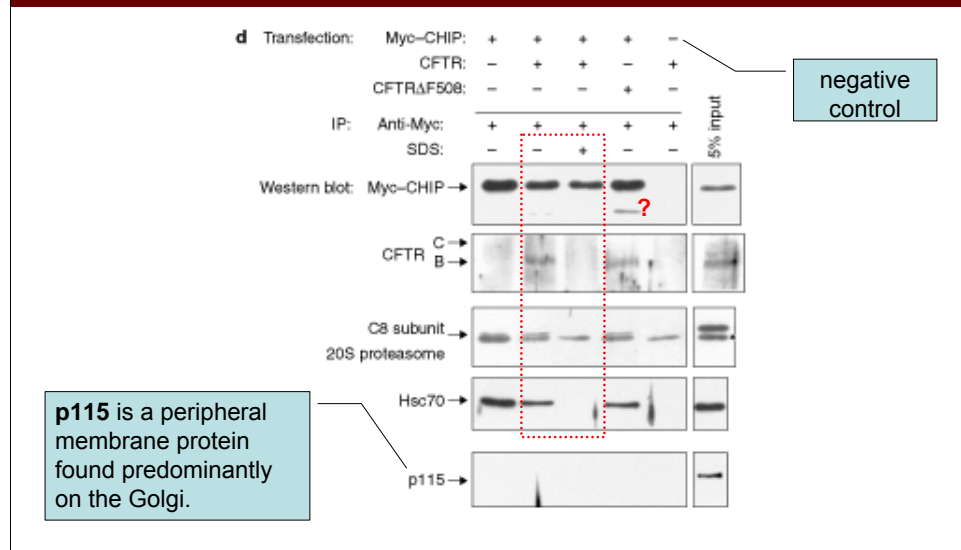
CHIP & Hsc70 function independently to CFTR

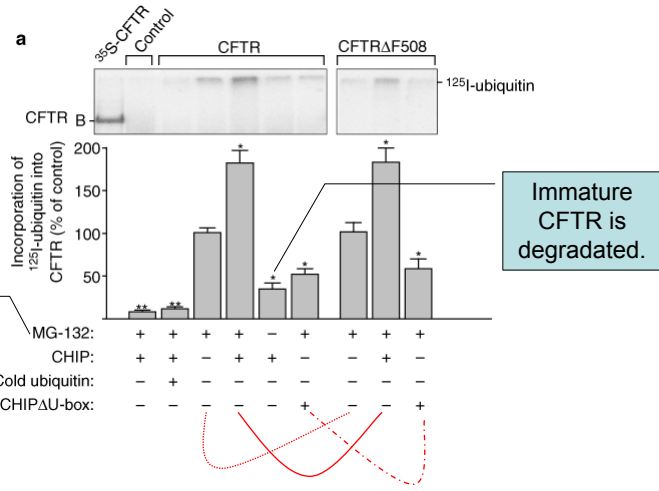The interactions between CFTR and CHIP/Hsc70 are more stable than other chaperons.

Other chaperons have weak interactions with CFTR.

CHIP forms complex with Hsc70 and subunit from the proteasome

negative control
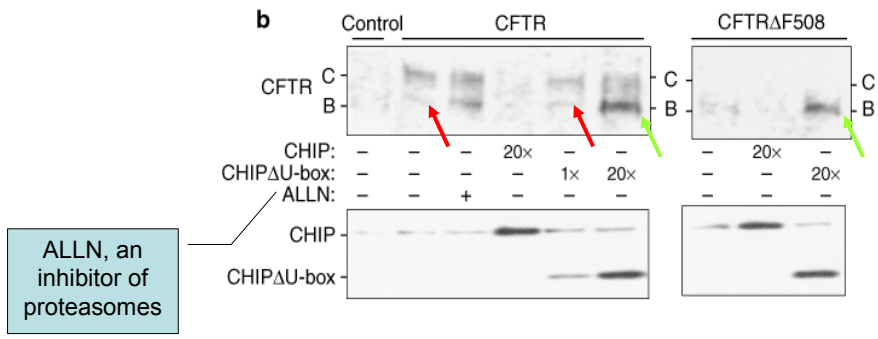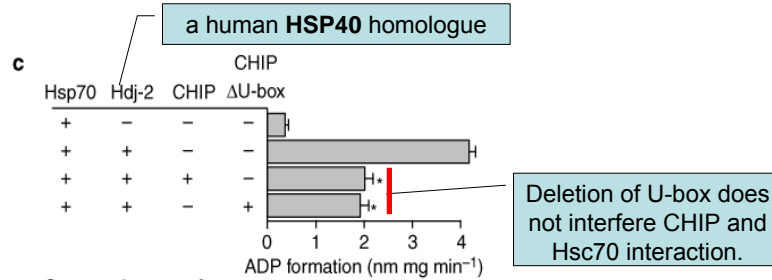
p115 is a peripheral membrane protein found predominantly on the Golgi.

SDS

The U-box is required for CHIP to promote CFTR ubiquitination

Overexpression of CHIP U-box blocks CFTR degradation

ALLN, an inhibitor of proteasomes

CHIP & CHIP ΔU-box function similarly to Hsc70

a human **HSP40** homologue

c

Hsp70	Hdj-2	CHIP	CHIP ΔU-box
+	−	−	−
+	+	−	−
+	+	+	−
+	+	−	+

ADP formation (nm mg min⁻¹)
0   1   2   3   4

Deletion of U-box does not interfere CHIP and Hsc70 interaction.

Control experiments:
1. CHIP & CHIP ΔU-box can bind Hsp70 in the same manner;
2. The binding of CHIP to Hsc70 reduced the extent to which **Hsp40** proteins simulate the ATPase of Hsp70.

The influence of CHIP on CFTR is not caused by nonspecific inhibition of the function of Hsc70.

Conclusions

- **CHIP functions with Hsc70 to target the immature CFTR for degradation by ubiquitination.**
- **The U-box appears essential for this process.**
- **Hsc70/CHIP may function specifically in ER quality control.**

? How distinguish the protein for degradation

In this paper, they found out that:

I thought that because CFTR delta-F508 has a lower folding velocity, which will accumulated unfolded protein, and may intrigue the degradation.

But there are still many questions remained. One big question is that how CHIP/Hsc70 complex distinguish between misfolded protein and canonical protein.

Q & A

Nov. 25, 2003

Thanks for your attention.