Mechanisms of thalidomide teratogenicity

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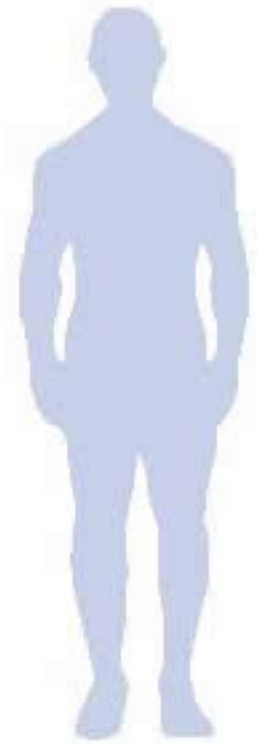
History of Thal

- Developed and commercialized as a sedative by Grünenthal in the late 1950s

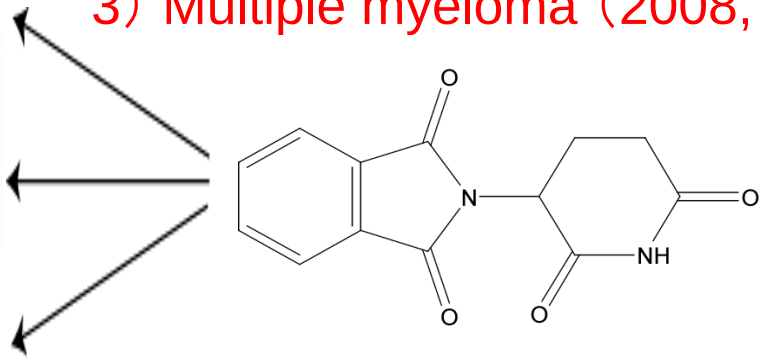
  - Thal was withdrawn from the global market in 1961, after its negative effects on newborns became clear

    - In a half century, various useful actions of Thal were identified

Development and sales by Celgene in the US



1）Hansen's disease（1998, approved in the US）
2）Multiple myeloma（2006, approved in the US）
3）Multiple myeloma（2008, approved in Japan）

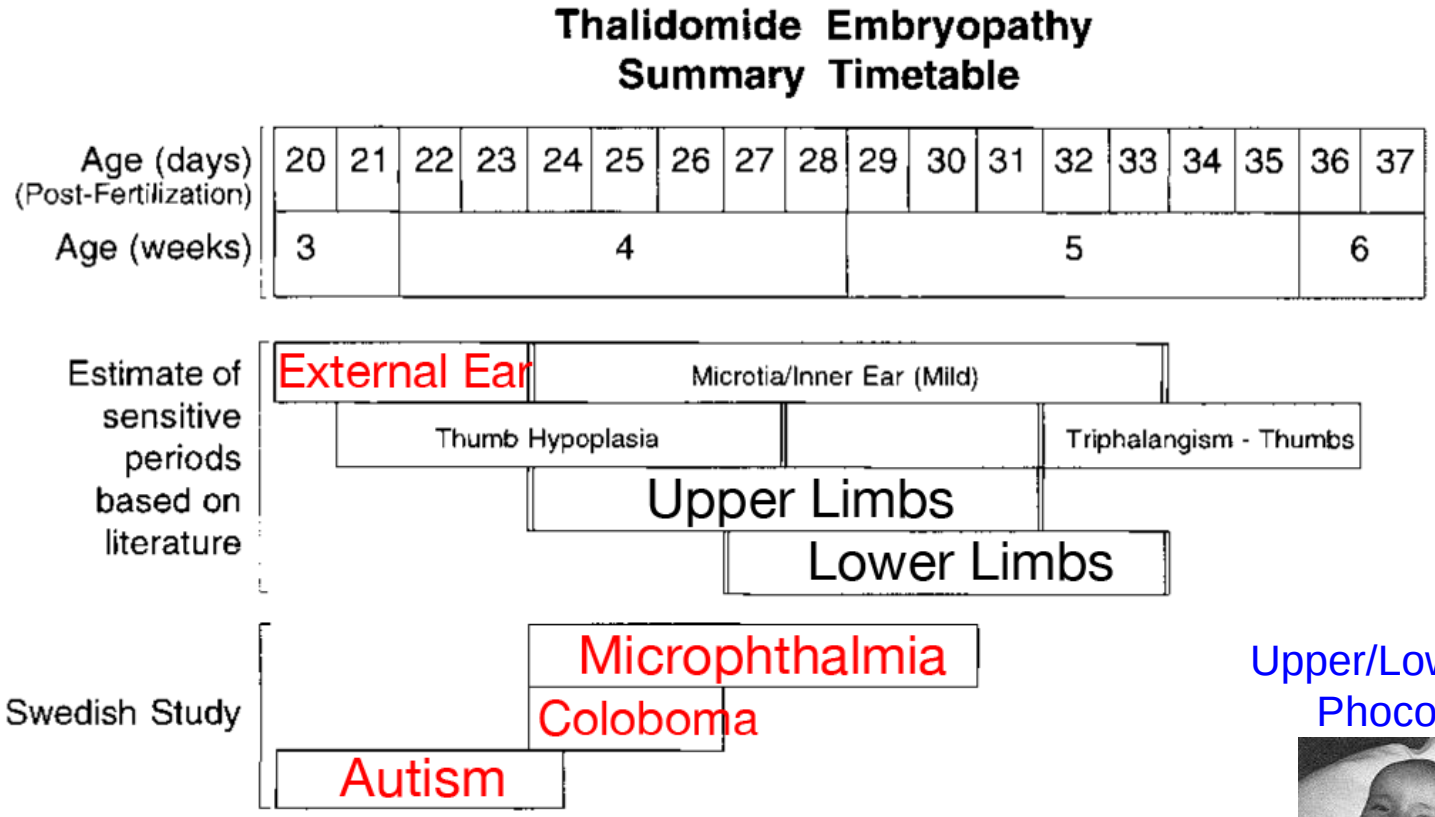


Thalidomide (Thal)
MW: 258

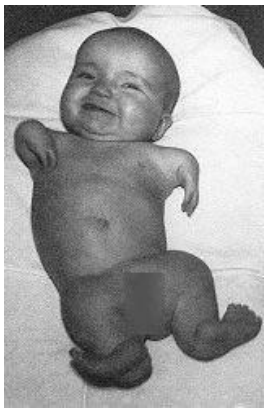
Main effects：Sedative-hypnotic properties, anti-cancer effects, immunomodulatory effects, etc.
Side effects: Embryopathy (teratogenicity of limbs and otic vesicles)

Thal is now prescribed under strict control.

Teratogenicity by Thal exposure in humans

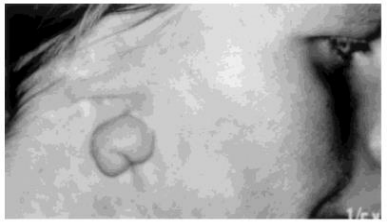


Upper/Lower limbs
Phocomelia



Autism

Absence of external ear



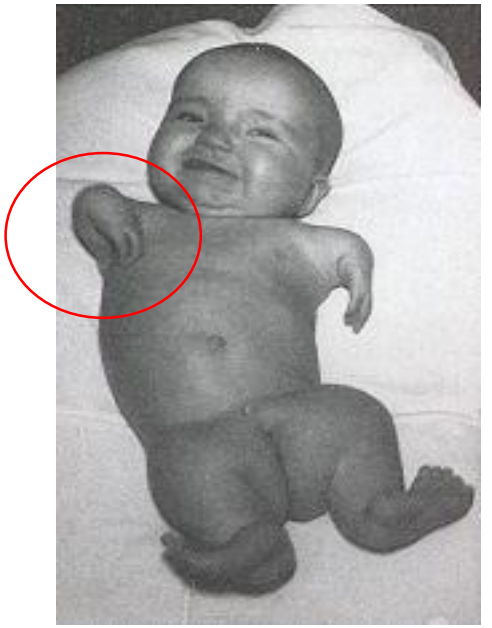
Microphthalmia

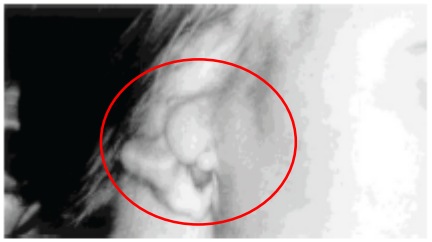
Miller and Strömland ***TERATOLOGY*** (1999)

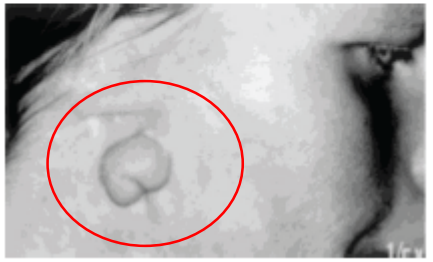
Thal teratogenicity

	Anomalies or site of anomaly	No. (%) affected	
	Thumbs	70 (81%)	
*	Upper limb (excluding thumb)	59 (69%)	
	Lower limb	21 (24%)	
*	Ears/hearing	33 (38%)	
	Facial nerve palsy	17 (20%)	
	Kidney	12 (14%)	by history or medical record
	Cardiovascular	7 (8%)	
	Chest/lung	4 (5%)	
	Genitalia	3 (3%)	
	Anal atresia	4 (5%)	
	Choanal atresia	2 (2%)	
	Dental anomalies	4 (5%)	
	Mental retardation (moderate to severe)	5 (6%)	
	Autism	4 (5%)	

Teratology (1999)







Thal causes developmental anomalies in limbs and ears with high frequency.

The pathogenic mechanism is not yet known, although various hypotheses have been proposed.

Multiple hypotheses on mechanisms of Thal action

TABLE 1. ACTIVE HYPOTHESES TO EXPLAIN THE MECHANISM OF THALIDOMIDE (1966–2003)

Hypothesis	*Authors*
Acylation of macromolecules	Jonsson (1972)
Ascorbic acid synthesis	Vaisman *et al.* (1983)
DNA intercalation	Jonsson (1972)
Disruption of angiogenesis	Stephens and Fillmore (2000) Jurand (1966) D'Amato *et al.* (1994) Sauer *et al.* (2000)
Down-regulation of adhesion receptors	Neubert *et al.* (1996)
Alteration of cytokine synthesis (tumor necrosis factorα)	Sampaio *et al.* (1991)
Folic acid antagonism	Kemper (1962)
Inhibition of DNA synthesis	Bakay and Nyhan (1968)
DNA oxidation	Parman *et al.* (1999)
Interference of glutamate metabolism	Faigle *et al.* (1962)
Mesonephros-stimulated chondrogenesis	Lash and Saxen (1971) Lash and Saxen (1972) Stephens and McNulty (1981) Stephens and Pugmire (1986)
Oxidative stress	Hansen *et al.* (1999) Hansen *et al.* (2002) Hansen *et al.* (2002) Parman *et al.* (1999) Sauer *et al.* (2000)

Inhibition of angiogenesis

Oxystress

Hansen and Harris (2004)

1) Target proteins are unidentified.
2) Why does teratogenicity occur at particular sites in the body?

Contents

1. Background of thalidomide (Thal)
2. Identification of cereblon (CRBN) as a target of Thal teratogenicity using our affinity bead technology
3. Involvement of CRBN in the anti-cancer effects of Thal and its analogs
4. Mechanisms of the therapeutic effects of Thal and its analogs
5. Current research on the mechanisms of Thal teratogenicity

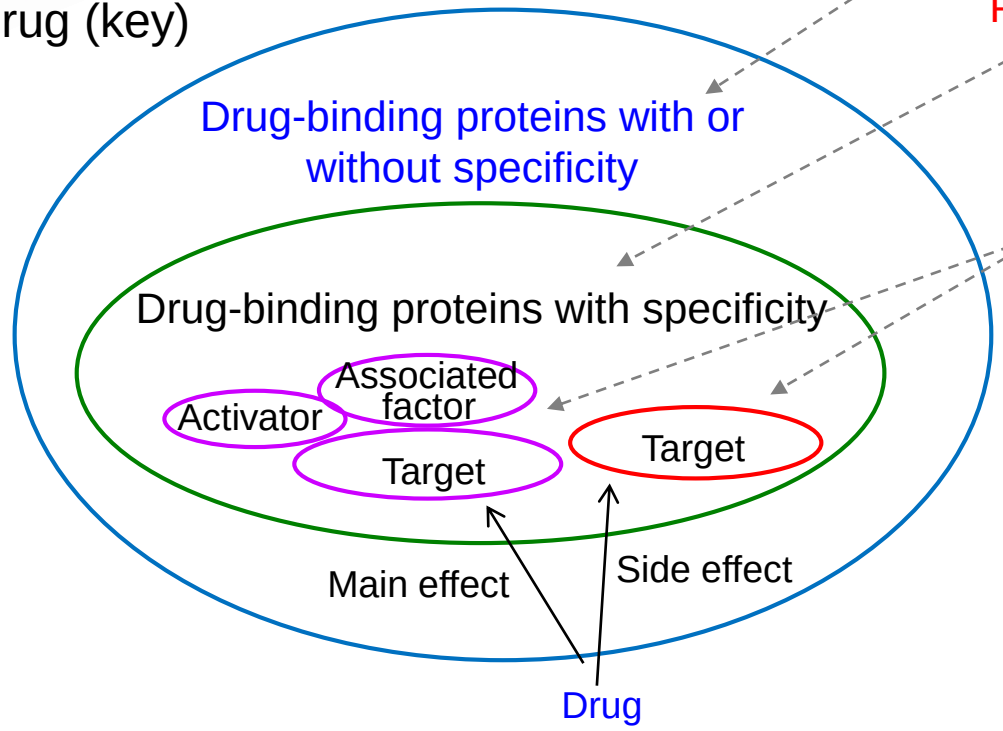
Strategy of isolation of drug targets based on drug-target interactions

A target binds directly and specifically to the drug and is involved in a particular drug action.

Target (keyhole)

Drug (key)

Effective drugs bind strongly and specifically to a target.



Protein library (>10^5 proteins)

⇩

Drug-binding proteins with or without specificity (～100 proteins)

⇩

Proteins binding directly and specifically (<10 proteins)

⇩

Protein with strongest affinity (1 protein)

⇩

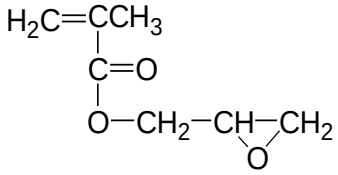
← **Our affinity bead technology can perform up to here**

A true target needs to be confirmed by multidisciplinary life sciences

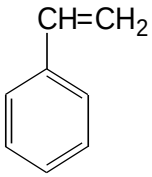
⇩

Target

Development of two types of nanosized beads as novel matrices for affinity chromatography

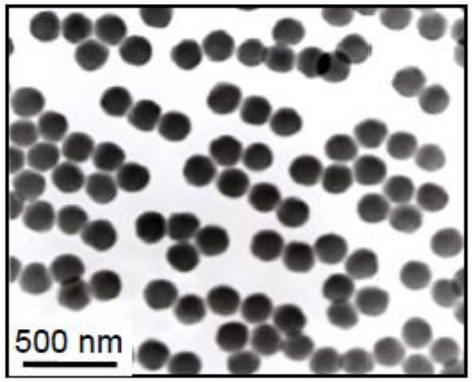


Glycidyl methacrylate (GMA)

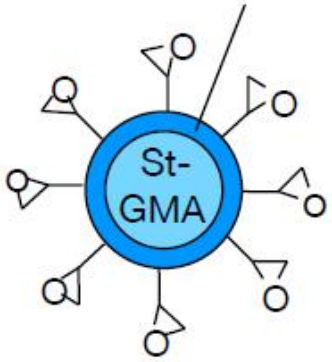


Styrene (St)

Latex (SG) beads

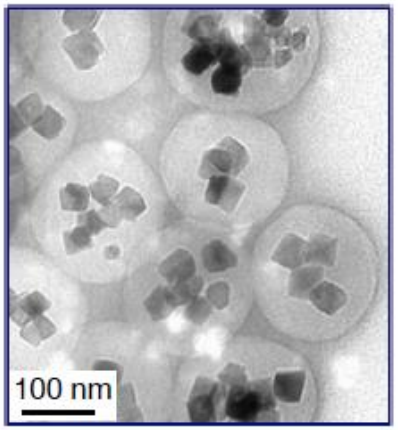


Poly-GMA

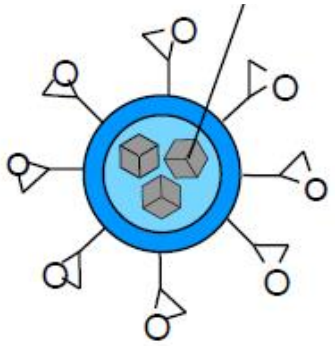


Kawaguchi *et al.*, ***NAR*** (1989)
Shimizu *et al*., ***Nat Biotechnol*** (2001)

Magnetic (FG) beads



Magnetite nanocrystals



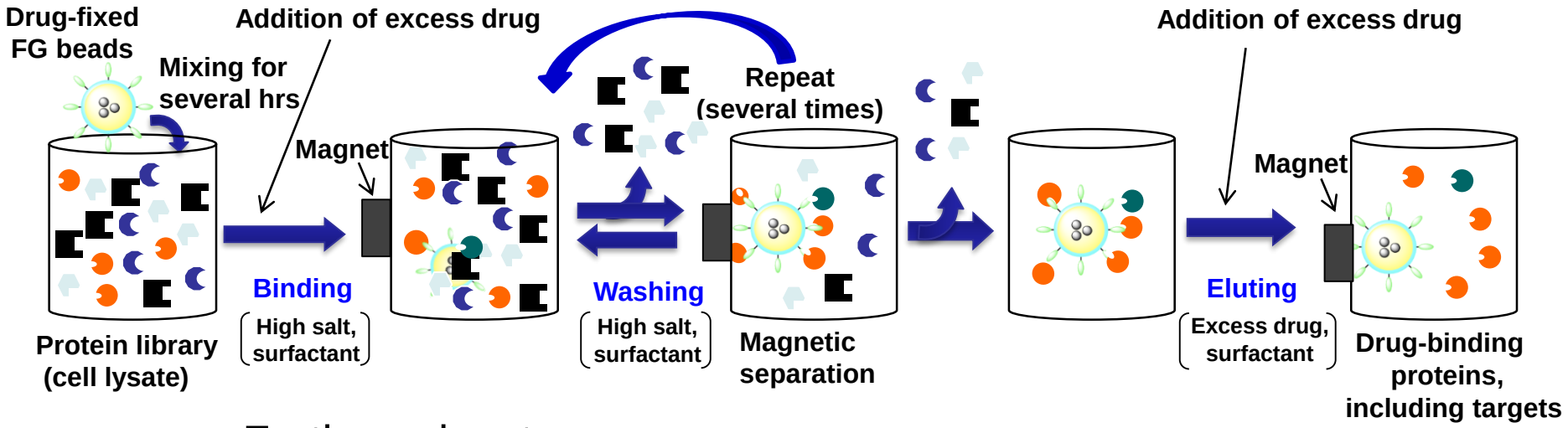
Nishio *et al.*, ***Colloids Surf B Biointerface*** (2008)

Characteristics of these beads

- Resistant to organic solvent
- Immobilize large numbers (> 10^7) of chemicals
- Highly dispersive in binding reactions
- Reduce nonspecific protein binding

One-step affinity isolation using FG beads by the batch method

Competitive inhibition assay | **Competitive drug elution assay**



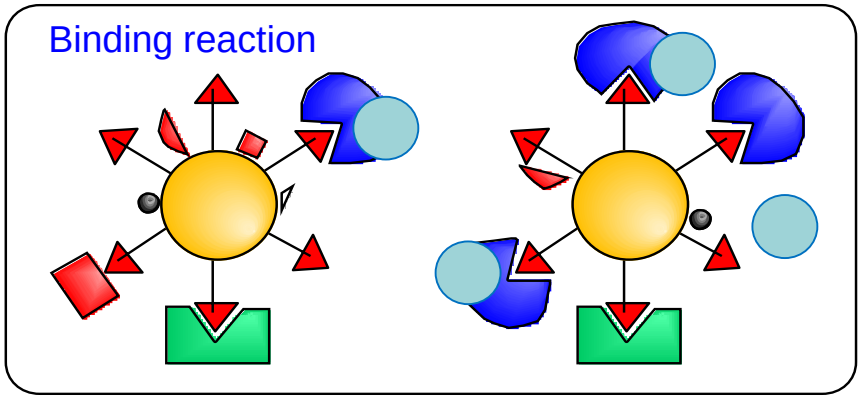
Further advantages

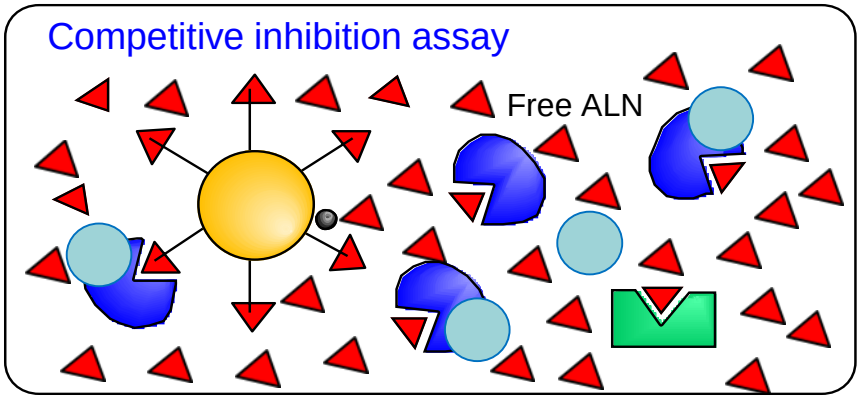
- Binding specificity
- Binding mode and binding affinity
- High concentration efficiency (> 1,000 times)
- Targets for other ligands besides chemicals

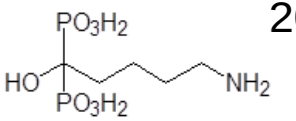
➡ A target, involved in drug actions, can be identified from several drug-binding proteins.

Sandhu and Handa ***IOP eBOOKs*** Online ISBN 978-0-7503-1584-5 (2018)

Superiority of our affinity bead technology

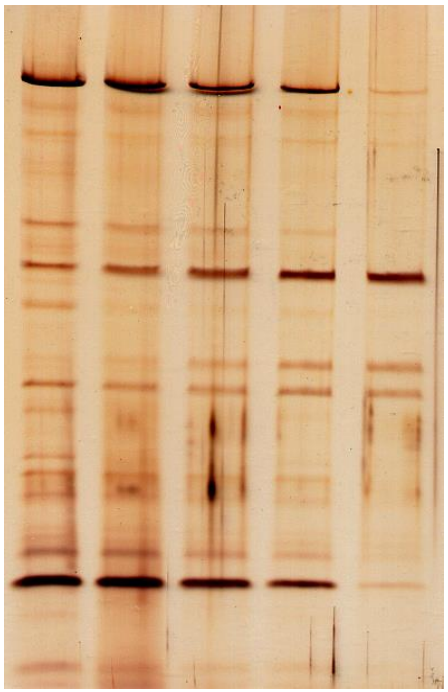






Alendronate (ALN) (osteoporosis drug)

Proteins binding to ALN-fixed beads were analyzed by SDS-PAGE and silver staining



Time-dependent change of binding proteins

Nonspecific binding

Specific binding

Specific binding

Nonspecific binding

Nonspecific binding

← Dynamin 2 (binding protein)

← Sorting nexin 9 (associated protein)

Clarification of binding specificity

List of various ligands for which we have identified targets using our bead technology

Pharmaceuticals

Methotrexate: Uga *et al.*, ***Mol Pharmacol*** (2006)
Thalidomide: Ito *et al.*, ***Science*** (2010), Chamberlain *et al.*, ***Nat Struct Mol Biol*** (2014)
Matyskiela *et al.*, ***Nature*** (2016), Nguyen *et al.*, ***Mol Cell*** (2016)
Alendronate: Masaike *et al.*, ***Mol Pharmacol*** (2010)
E3330: Shimizu *et al.*, ***Nat Biotechnol*** (2000)
FK506: Shimizu *et al.*, ***Nat Biotechnol*** (2000)
Vesnarinone: Hotta *et al.*, ***Mol Pharmacol*** (2013)
Salicilic acid: Gupta *et al.*, ***Mol Pharmacol*** (2013)
Trifluorothiazoline compound: Perez-Perarnau *et al.*, ***Angew Chem Int Ed*** (2014)

Biomolecules

(Metabolites)
Vitamin K2: Karasawa *et al.*, ***Mol Pharmacol*** (2013)
Amino acids: Kume *et al.*, ***Genes Cells*** (2010)
Heme: Azuma *et al.*, ***PLoS One*** (2008), Kabe *et al.*, ***Nat Commun*** (2016)
Protoporphyrin IX: Kabe *et al.*, ***J Biol Chem*** (2006)
Capsaicin: Kuramori *et al.*, ***Biochem Biophys Res Commun*** (2009)

(dsDNA)
ATF/CREB site: Wada *et al.*, ***Methods Enzymol*** (1995), Wada *et al* ***J Virol*** (1991)
Ad4BP/SF-1 site: Morohashi *et al.*, ***J Biol Chem*** (1992)
E4TF1/GABP site: Watanabe *et al.*, ***EMBO J*** (1990)
Oct1/4 site: Kang *et al.*, ***Genes Dev*** (2009)

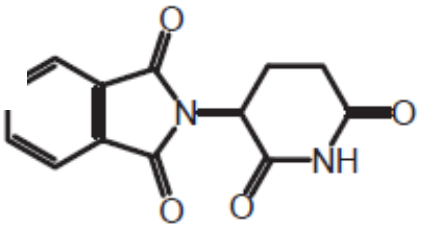
(Protein)
TFIIA: Usuda *et al.*, ***EMBO J*** (1991), Ma *et al.*, ***Genes Dev*** (1993)
EspB (toxin of enteropathogenic *E. coli*): Iizumi *et al.*, ***Cell Host & Microbe*** (2007)

(Peptide)
FKBP12: Ohtsu *et al.*, ***Anal Biochem*** (2005)
Nocistatin: Okuda-Ashitaka *et al.*, ***J Biol Chem*** (2012)

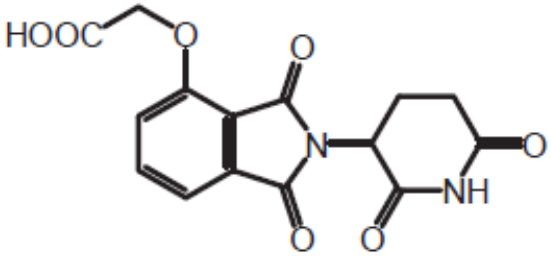
Toxic chemicals

Mono-(2-ethylhexyl) phthalate: Kuramori *et al.*, ***Toxicological Sciences*** (2009)
Atrazine: Hase *et al.*, ***Biochem Biophys Res Commun*** (2008)
Bisphenol A: Ito *et al.*, ***PLoS One*** (2012)

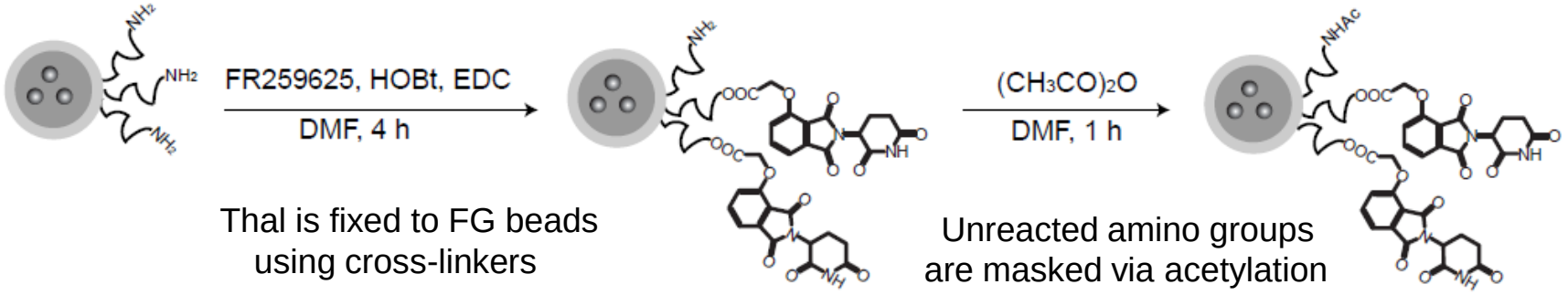
Fabrication of Thal-fixed beads



Thalidomide (Thal)



FR259625
(provided by a pharmaceutical company)



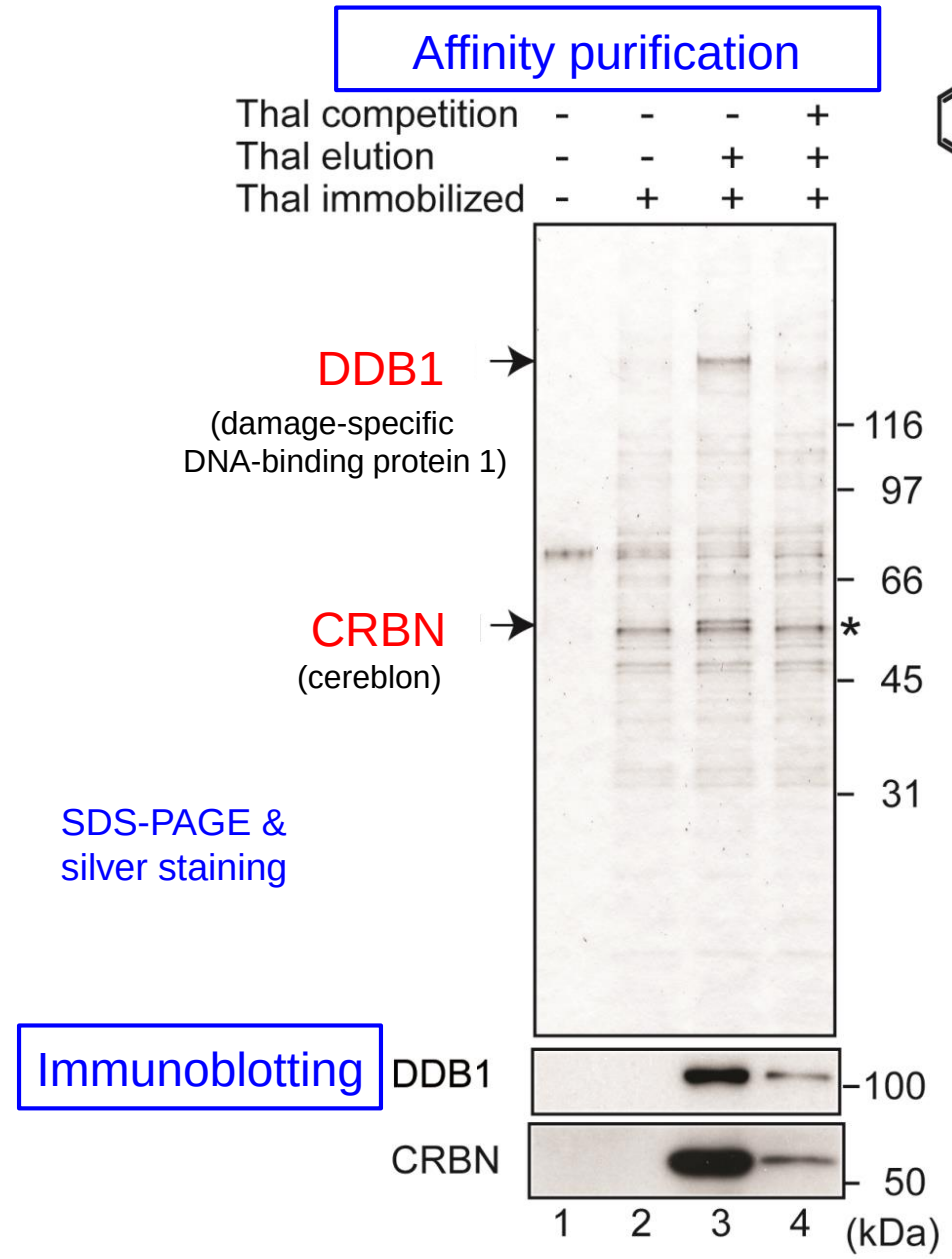
Thal is fixed to FG beads
using cross-linkers

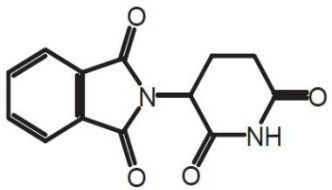
Unreacted amino groups
are masked via acetylation

FG beads

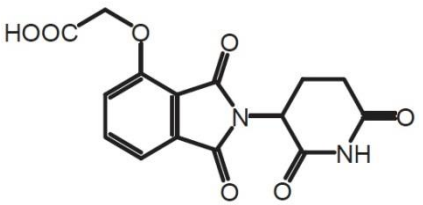
Thal-fixed FG beads

Identification of CRBN and DDB1 as Thal-binding proteins

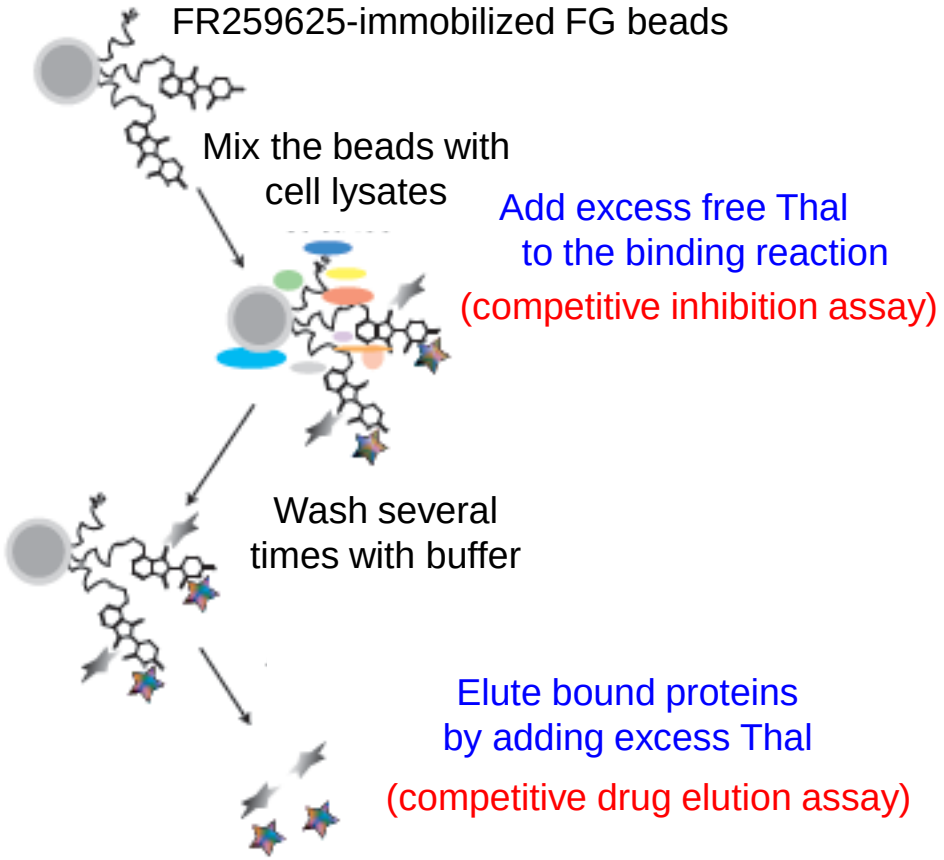




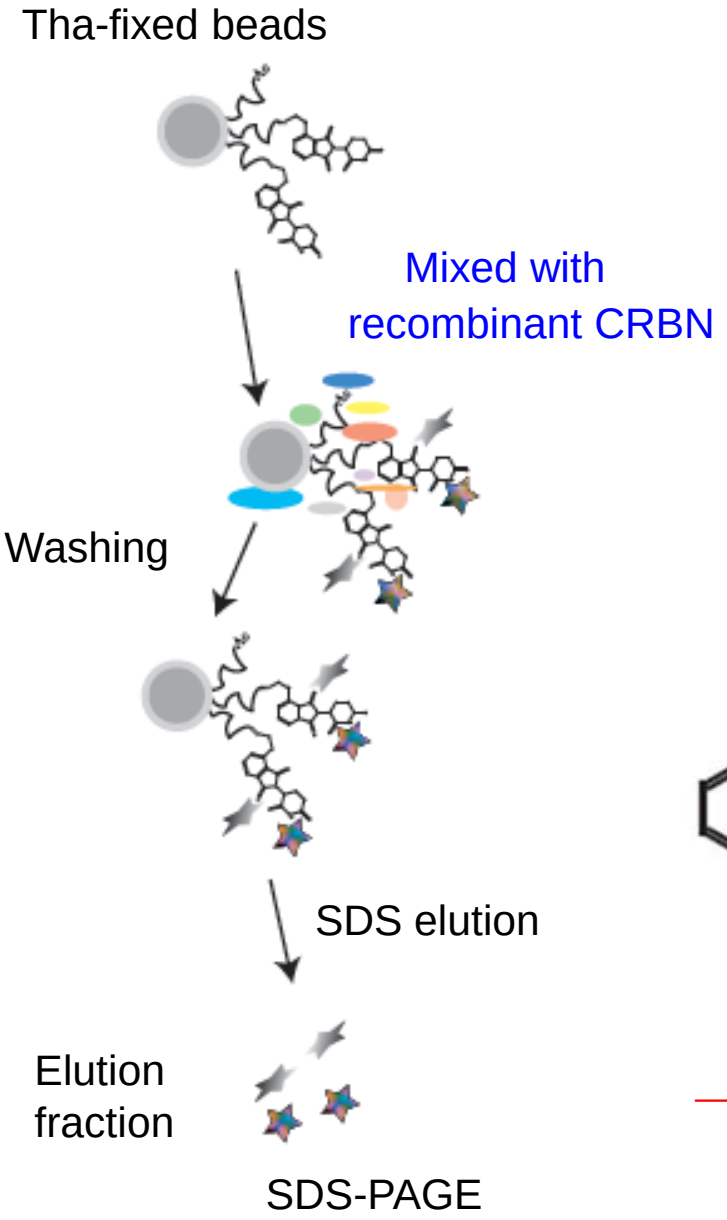
Thal

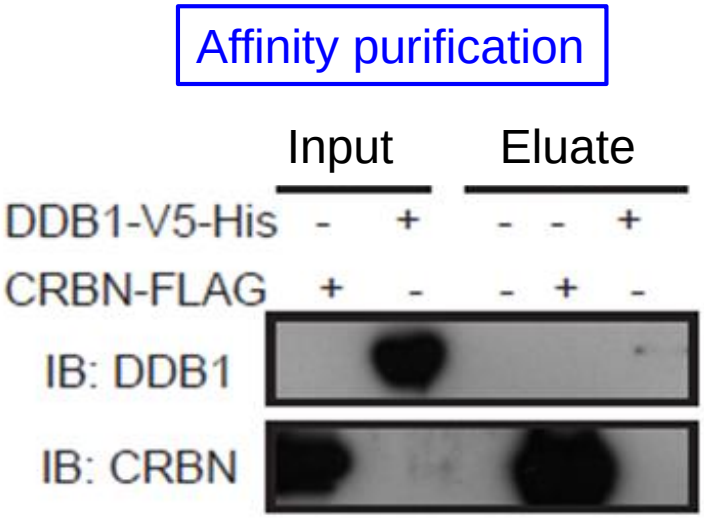


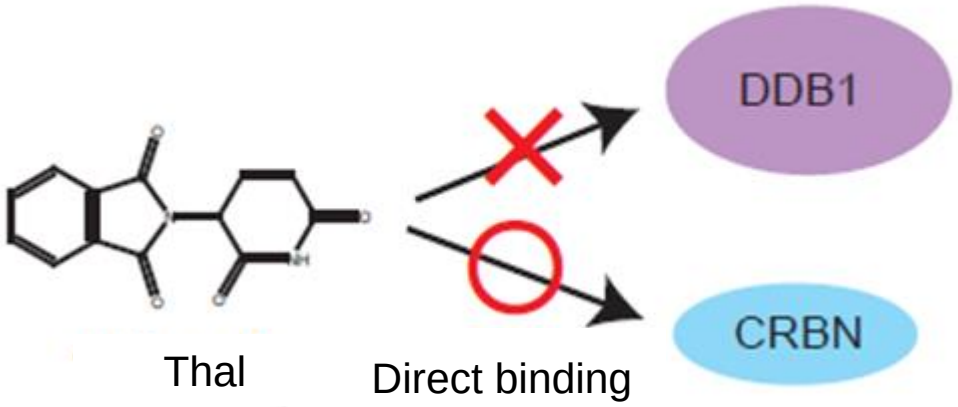
FR259625
(carboxylated derivative)



Identification of a protein binding directly to Thal



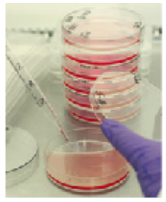




→ Thal binds directly to CRBN,
but not to DDB1.

Interaction of DDB1 with CRBN

293T cells expressing FLAG-HA-CRBN



Anti-FLAG Ab-fixed beads

Cell lysis with 0.5%NP-40

Cell lysates

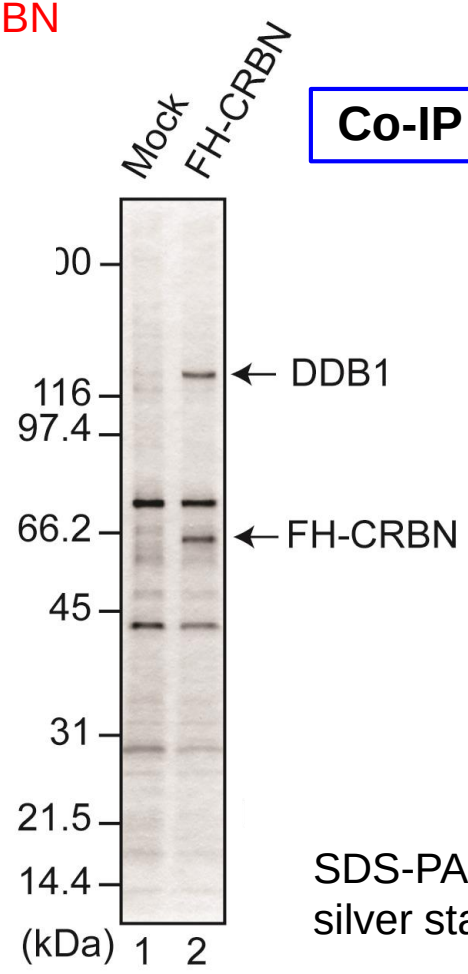
FLAG-HA

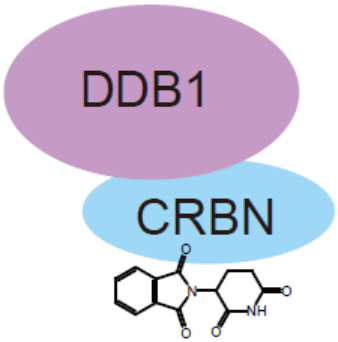
Washing

FLAG-HA

Elution with FLAG peptides

FLAG-HA



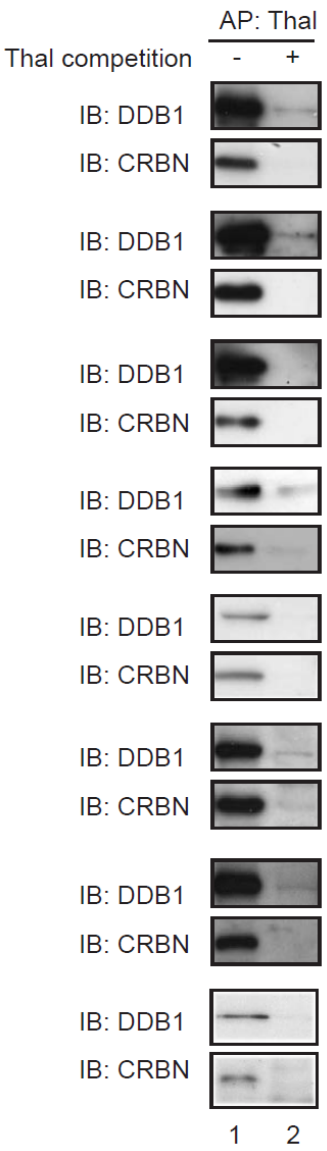


SDS-PAGE & silver staining

→ DDB1 forms a complex with CRBN.

Ubiquitous expression of CRBN in various cells

(analysis using Thal-fixed beads)



Cell lines

Jurkat (human **T-cell**)

THP-1 (human **monocyte**)

U266 (human **B-cell**)

HUVEC (human **endocapillary** cell)

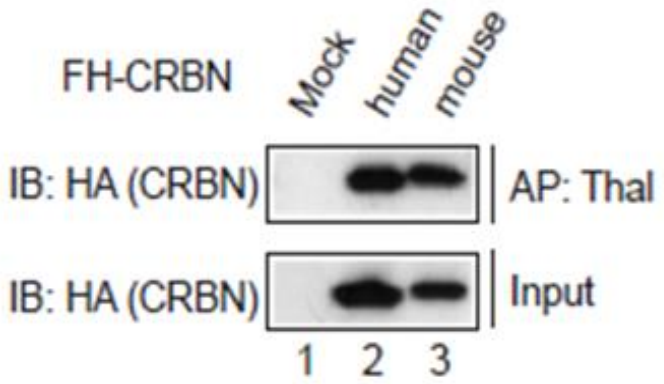
LP101 (human **stromal** cell)

293T (human **kidney** cell)

SH-SY5Y (human **neuronal** cell)

N1E-115 (mouse **neuronal** cell)

Affinity purification
using Thal-fiexed beads

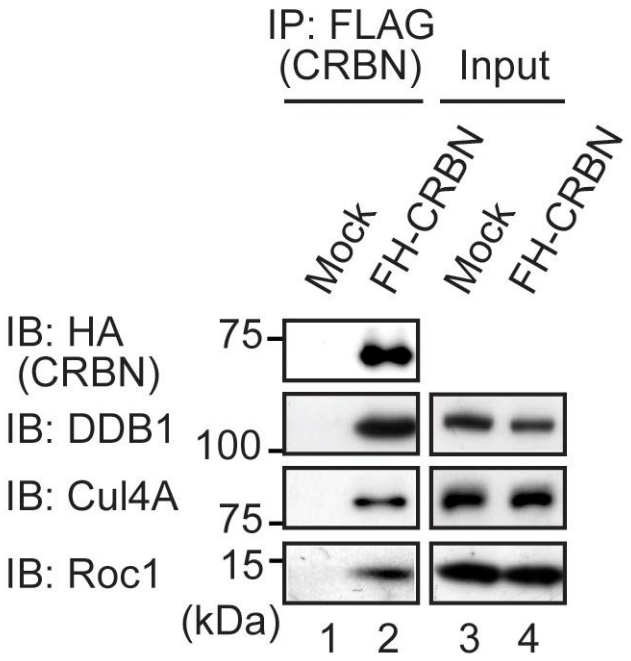


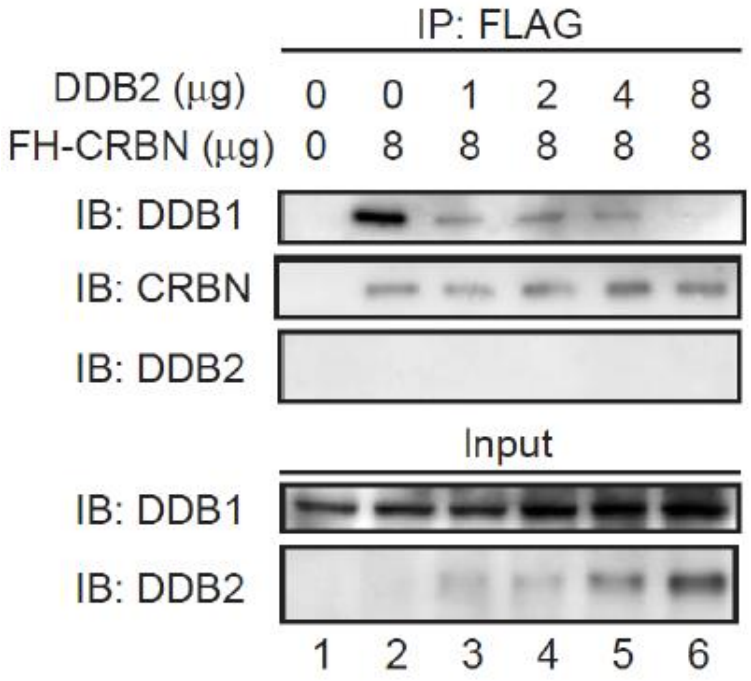
→ CRBN and DDB1 were expressed ubiquitously in all the cells tested.

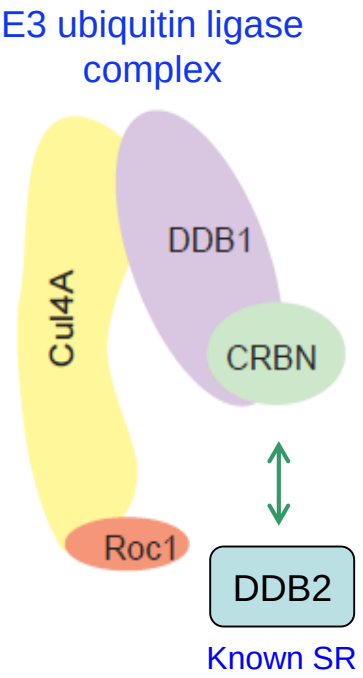
→ Although rodents do not show teratogenicity by Thal, mouse CRBN binds to Thal.

CRBN forms an E3 ubiquitin ligase complex with DDB1, Cul4A, and Roc1, and works as a substrate receptor

Co-immunoprecipitation (Co-IP)







Identification of proteins co-immunoprecipitating with CRBN

DDB2, known as a substrate receptor (SR) competes with CRBN for binding to DDB1

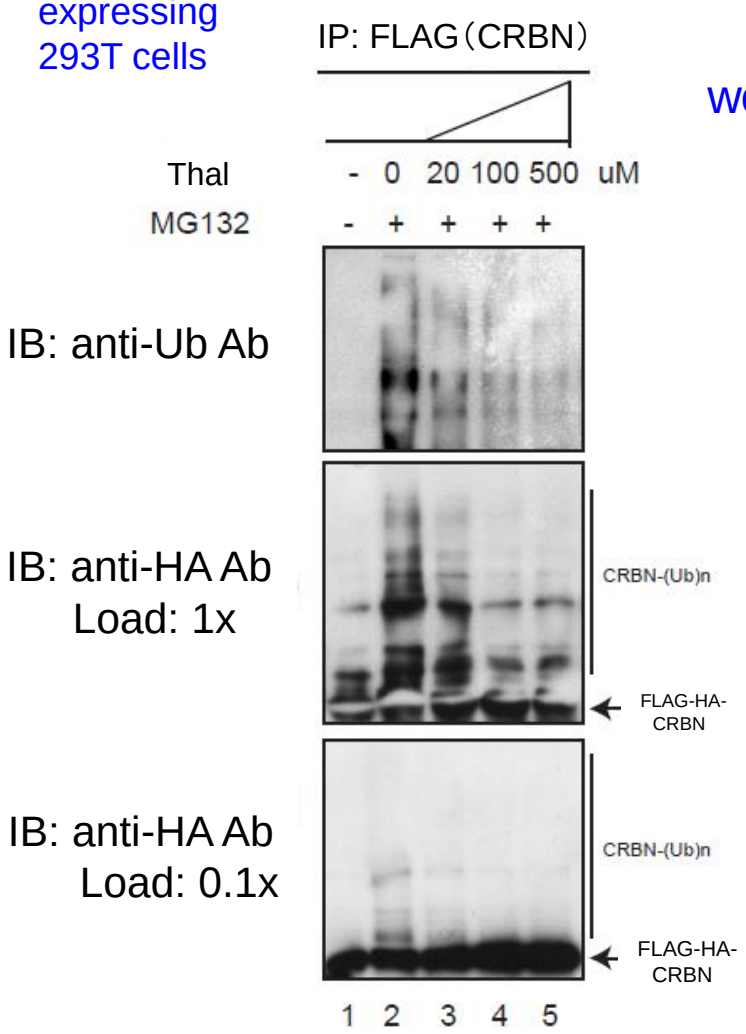
→ CRBN forms an E3 ubiquitin ligase complex with DDB1, Cul4A, and Roc1.

→ CRBN is suggested to works as a SR.

Thal inhibitis the auto-ubiquitination of CRBN

Co-IP and IB

FLAG-HA-CRBN-expressing 293T cells



As known SRs, such as DDB2, CSA, CDT2, etc. are reported to be auto-ubiquitinated, we tested whether CRBN is also auto-ubiqutinated.

→ CRBN is auto-ubiquitinated in a Thal-sensitive manner, which was also confirmed by *in vitro* experiments.

↓

Thal targets the CRBN E3 ubiquitin ligase, via binding to CRBN.

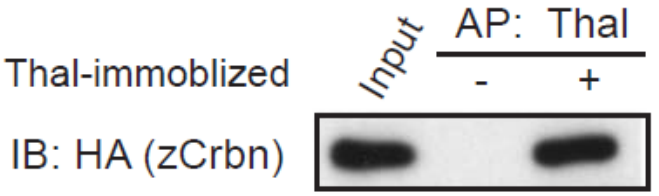
Possible mechanisms

① Thal inhibits the binding of CRBN to SR.

② Thal alters the substrate specificity of CRBN.

Analyses of Thal action and CRBN function using zebrafish as an experimental animal

Affinity purification

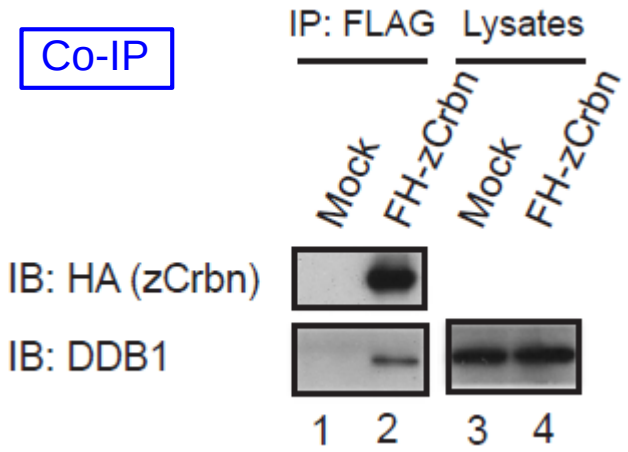


→ zCrbn binds to Thal.

Advantages of zebrafish

1) Transparency, easy observation
2) Fast ontogeny of 2.5 days
3) Easy genetic engineering (KD/OE)
4) Processability of multiple individuals
5) Widely used in pharmacology and toxicology studies
6) Whole genome sequence available

Co-IP

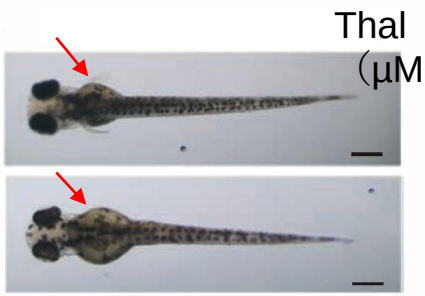


→ zCRBN binds to hDDB1.

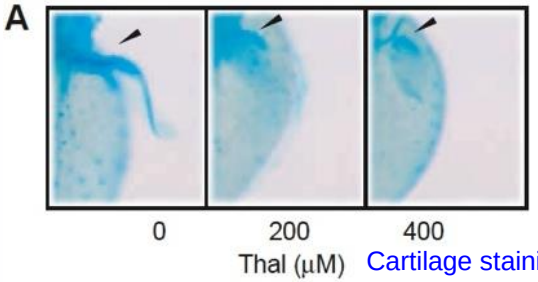
Zebrafish CRBN (zCRBN) shares 70% homology with human CRBN.

CRBN E3 ubiquitin ligase complex is involved in early development and is a target of Thal teratogenicity

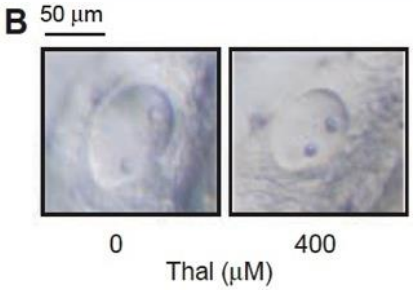
Thal treatment



Pectoral fin (75 hpf)

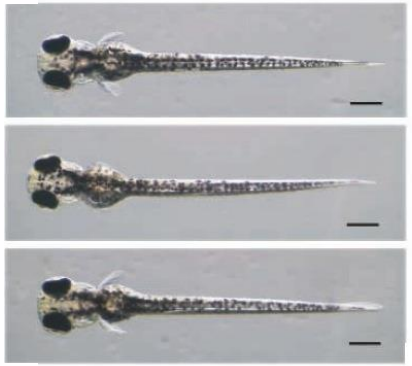


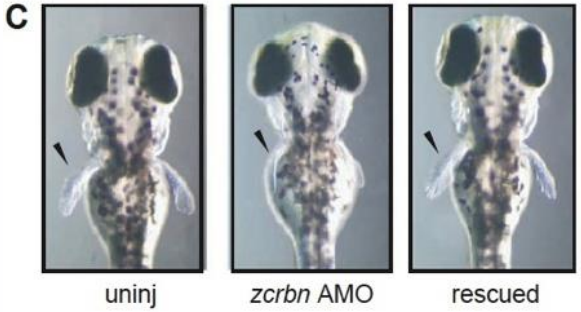
Otic vesicle (30 hpf)



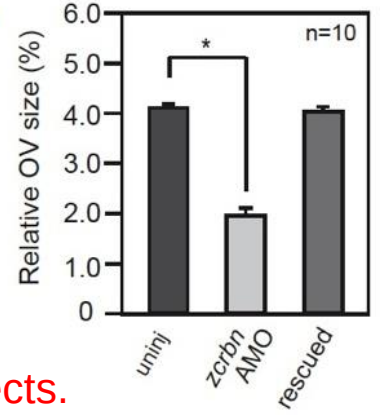
→ Thal causes similar developmental defects in zebrafish as in humans.

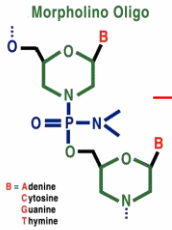
CRBN KD





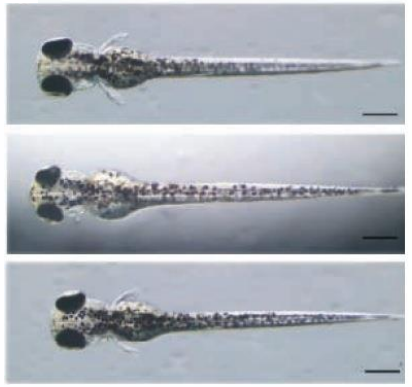
Quantification of otic vesicle size

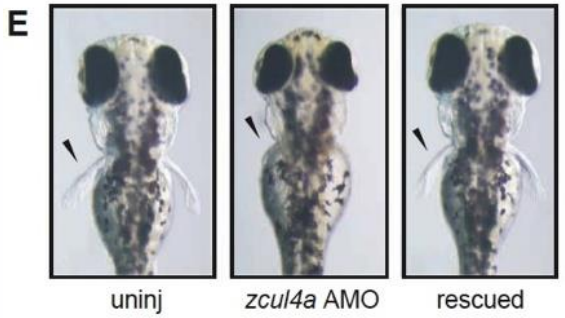


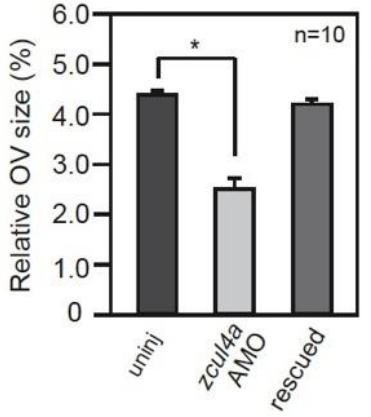


→ Knockdown of CRBN or Cul4A causes similar developmental defects.

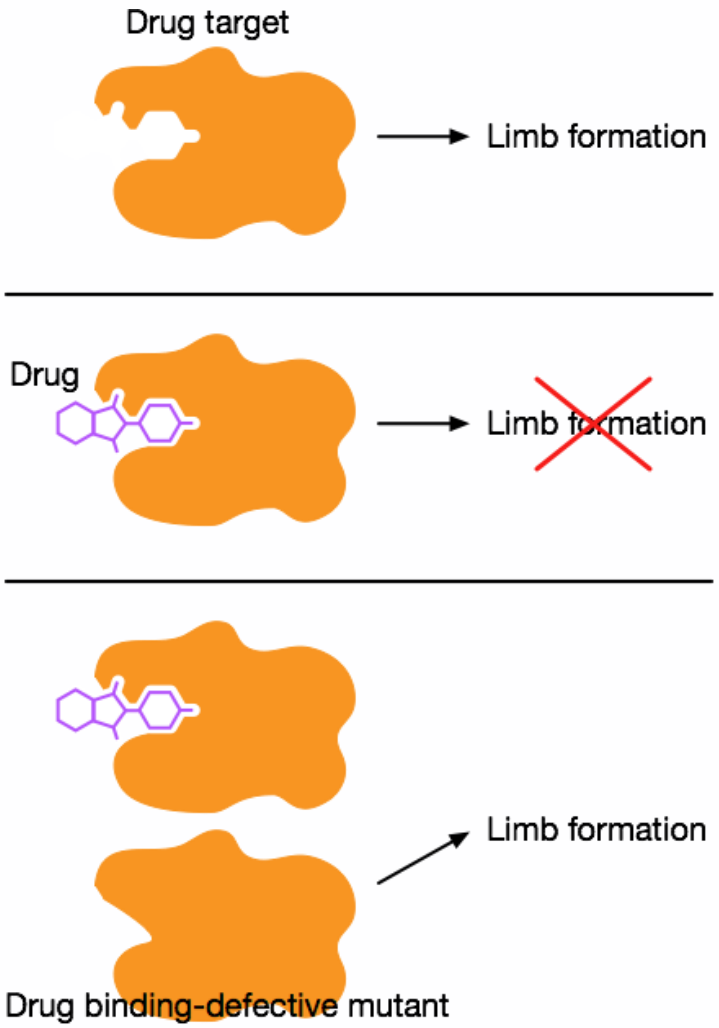
Cul4a KD







Proof of CRBN as a true target of Thal teratogenicity



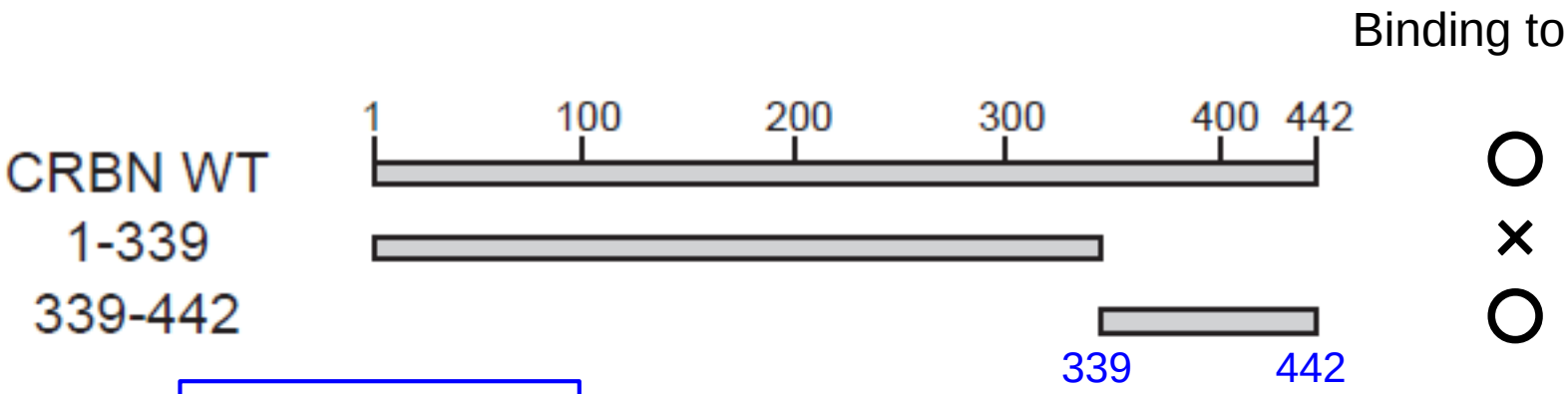
- So far, the association between the silencing of CRBN and Thal teratogenicity has been clarified.

- How can we confirm that CRBN is a true target of Thal teratogenicity?

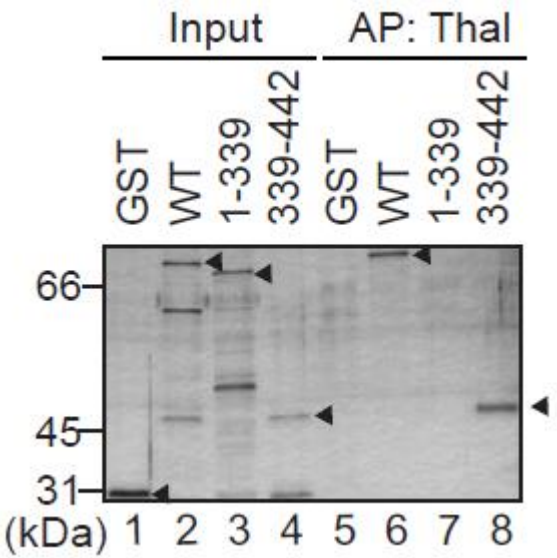
- If Thal teratogenicity is suppressed by a CRBN mutant that does not bind to Thal, but is otherwise fully functional, we can conclude that CRBN is a bona fide target of Thal teratogenicity.

- Accordingly, we designed CRBN mutants that do not bind to Thal.

Identification of the Thal-binding region of CRBN



Affinity purification

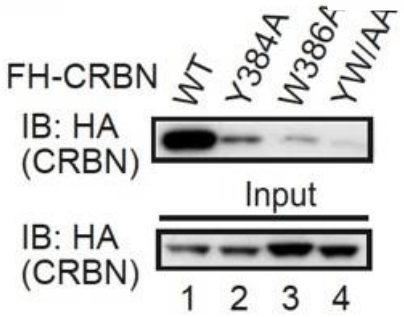


→ Thal-binding region of CRBN is in the C-terminal region.

Recombinant GST-CRBN and its mutants were tested for their interaction with Thal using affinity bead technology.

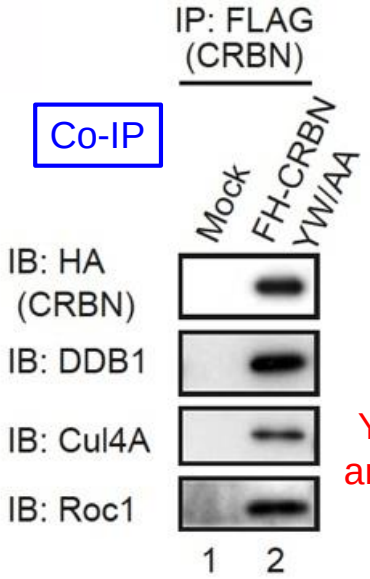
Identification of a CRBN mutant, CRBN$^{YW/AA}$, that does not bind to Thal but is otherwise fully functional

AP



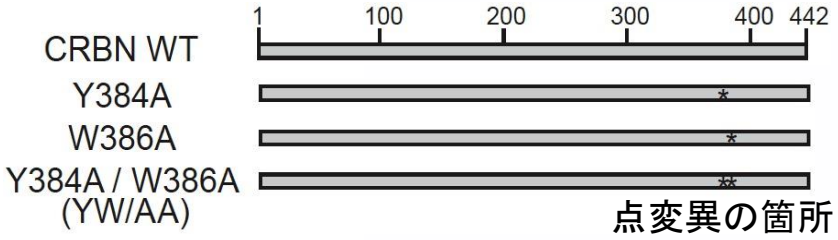
YW/AA does not bind to Thal.

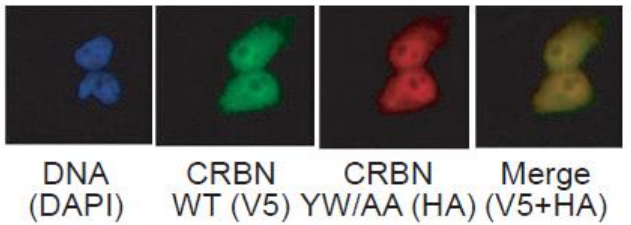
Co-IP



YW/AA forms an E3 complex.

Making point mutations in the C-terminal region

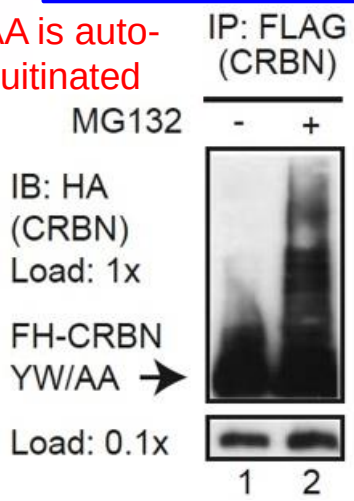


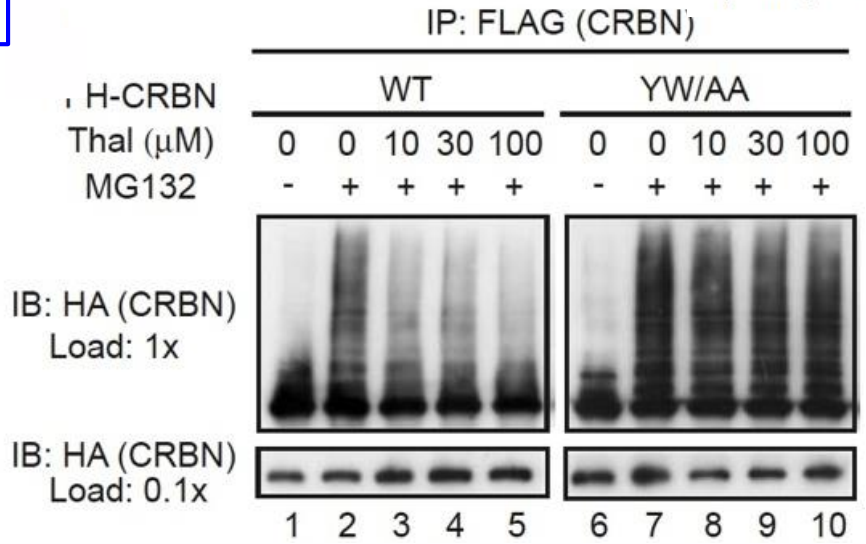


Colocalization

Auto-ubiquitination

YW/AA is auto-ubiquitinated





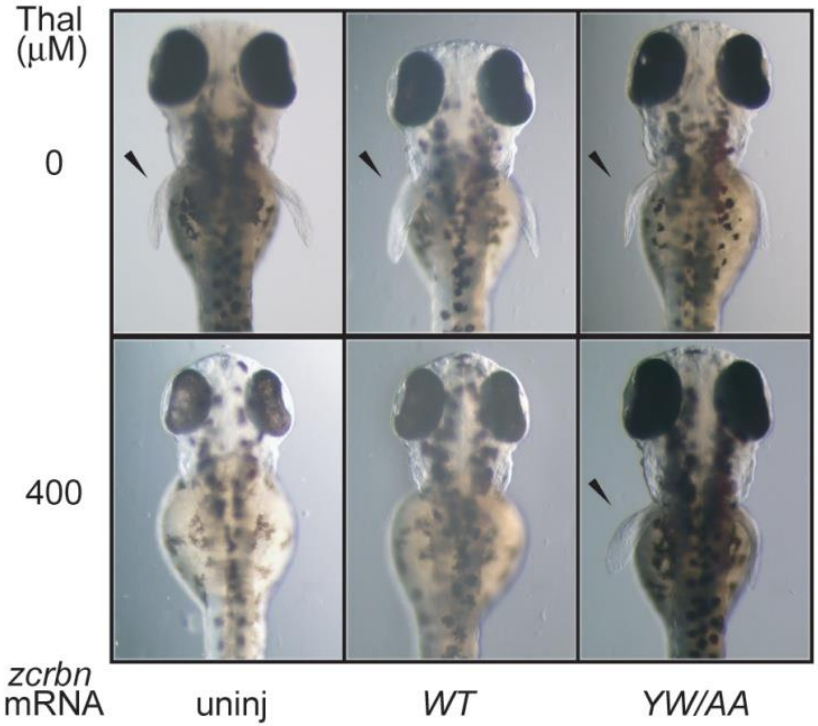
IB

Auto-ubiqutination of YW/AA is resistant to Thal.

Expression of CRBN$^{YW/AA}$ inhibits Thal teratogenicity

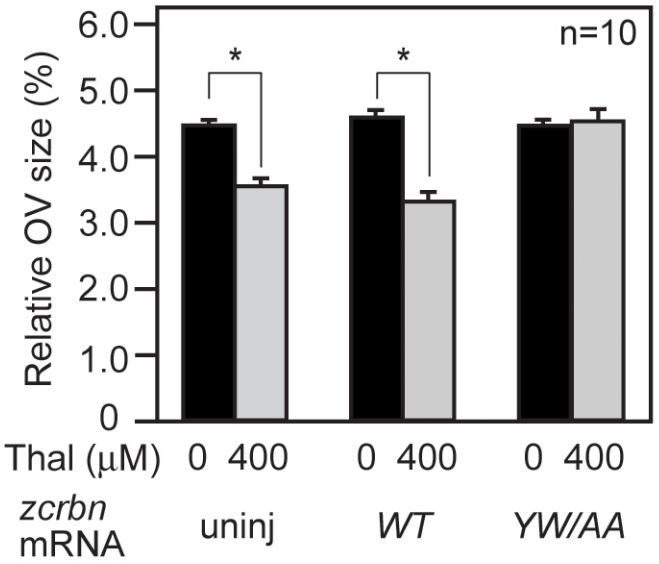
Zebrafish fertilized eggs were injected with zCRBN^{WT} or CRBN$^{YW/AA}$ mRNA and then treated with Thal.

YW/AA: Y384A/W386A

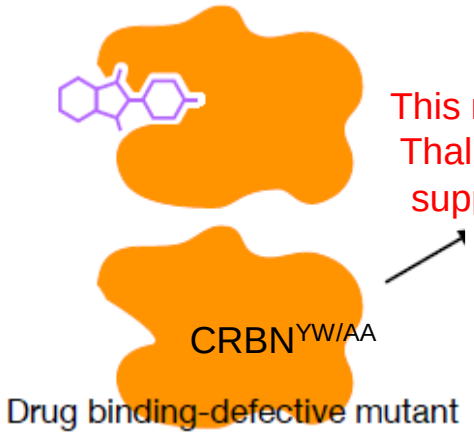


Pectoral fin formation (72 hpf)

*p<0.001



Otic vesicle size (30 hpf)



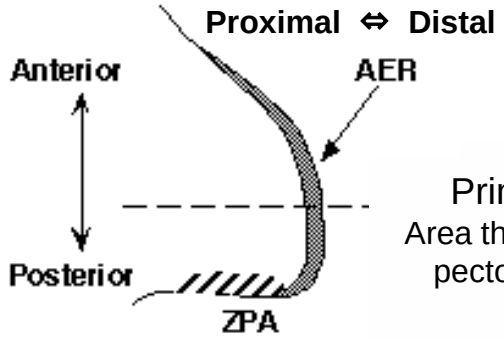
This mutant that does not bind to Thal, but is fully functional, can suppress Thal teratogenicity.

→ CRBN is a bona fide target of Thal teratogenicity.

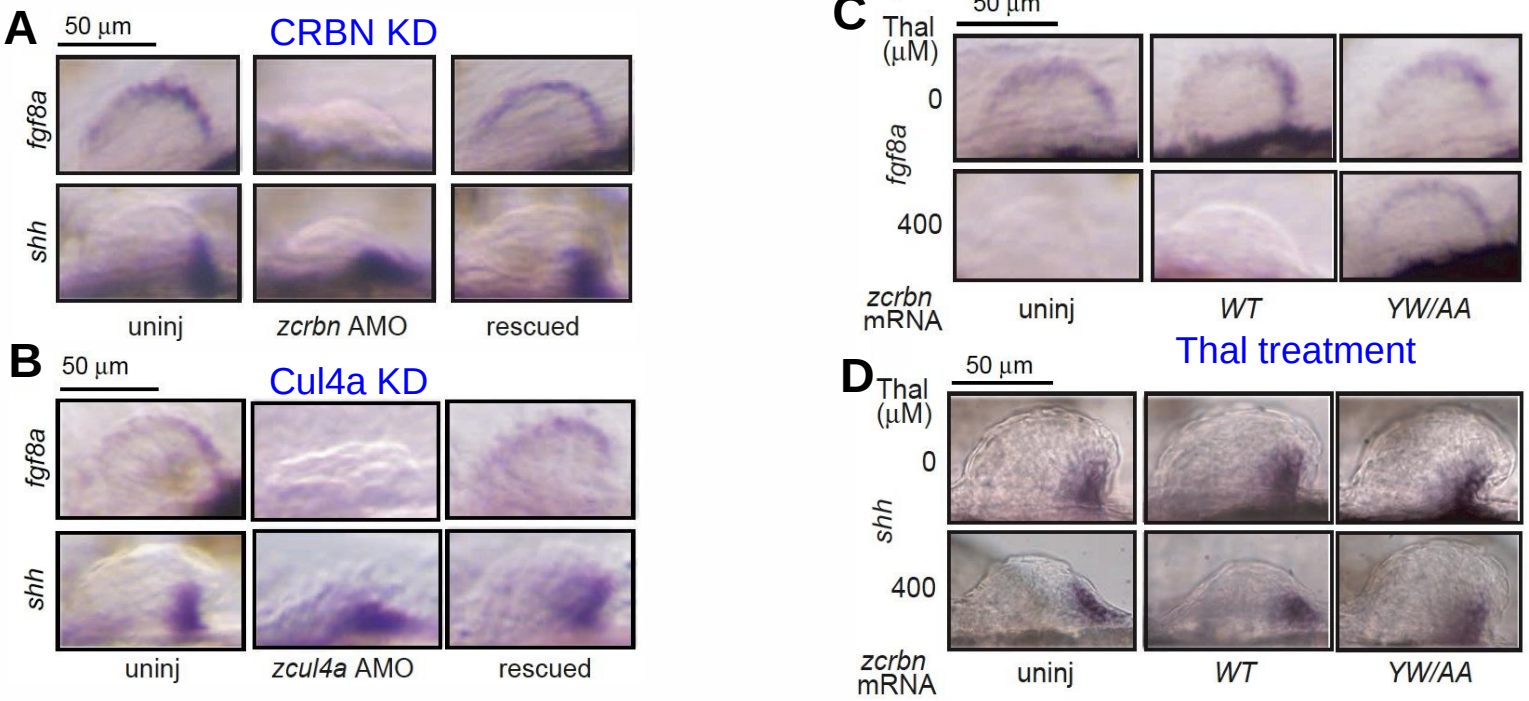
Downstream factors of CRBN

Fgf8（fibroblast growth factor 8）
- Essential for the pectoral fin formation along the PD axis and expressed in the apical ectodermal ridge (AER).

Shh（sonic hedgehog）
- Essential for the formation of pectoral fins along the AP axis and expressed in the zone of polarizing activity (ZPA).



Primordium
Area that will form the pectoral fin

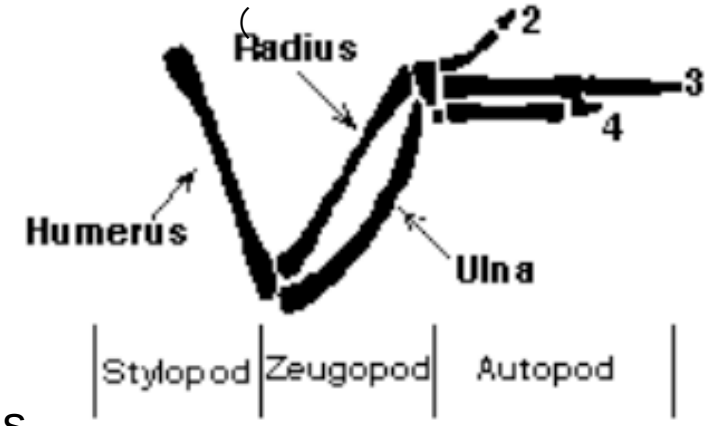


Whole mount *in situ* hybridization of fin buds (48 hpf)

→ ・Fgf8 is a downstream factor of CRBN and Thal
・$CRBN^{YW/AA}$ reverses Thal-induced suppression of Fgf8 expression.

Verification using chicken embryos

Chicken upper limb

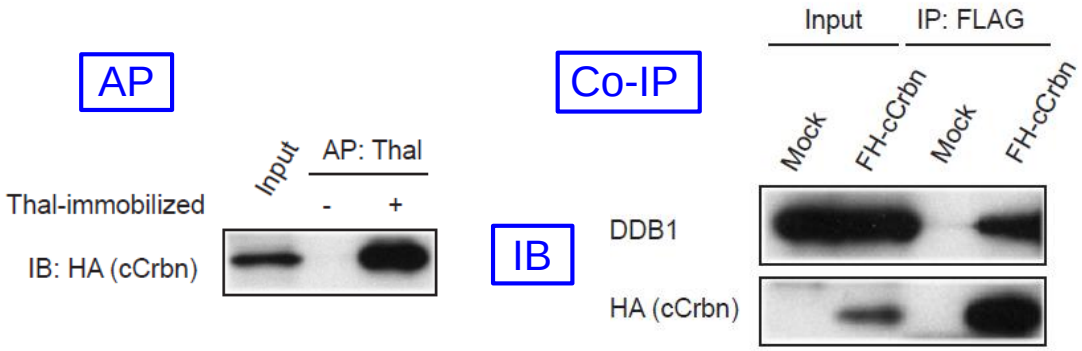


Why further validation is required using chickens

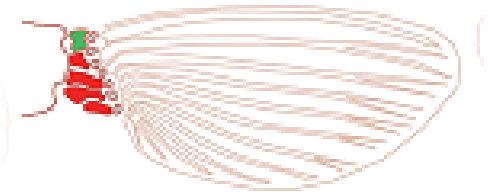
1) Established animal model for Thal teratogenicity
2) Anatomically closer to humans than zebrafish

Problems of zebrafish:

1）Body structure is quite different from that of humans
2）Short research history and few reports on Thal teratogenicity



Zebrafish pectoral fin



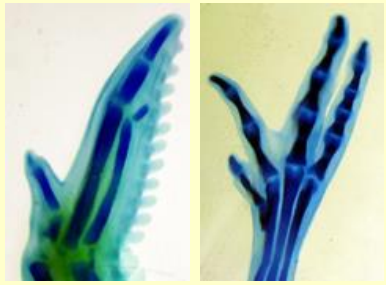
→ cCrbn binds to Thal and interacts with human DDB1.

Investigation of the role of CRBN in Thal teratogenicity in chicks

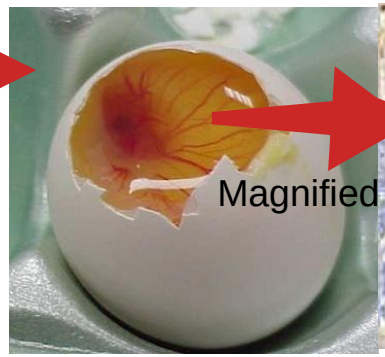
Administration of Thal and introduction of foreign genes by electroporation into the anterior limb bud of the embryo

Chicken

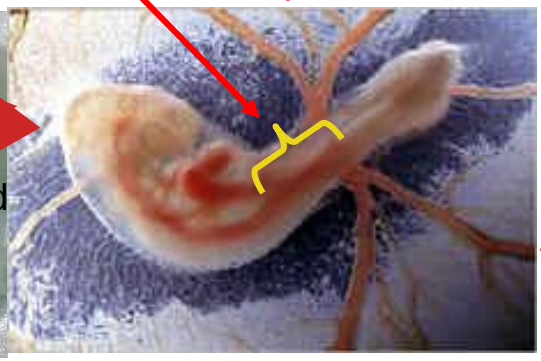
Upper limb Lower limb



3 days after fertilization



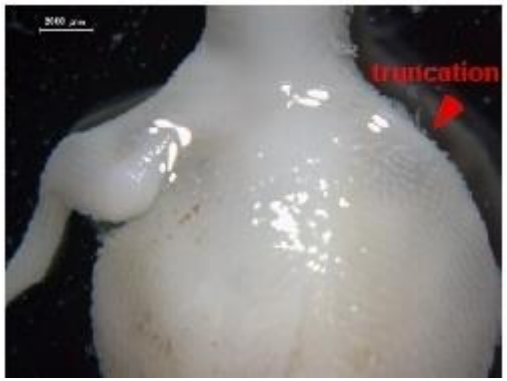
Magnified

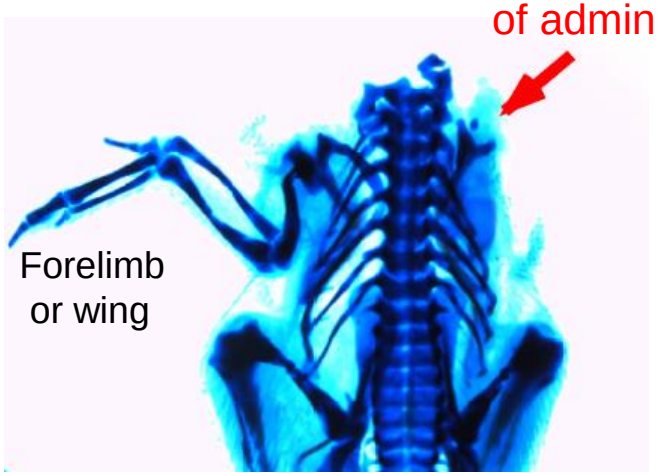


This region (anterior limb bud) develops into the forelimb

→ Thal clearly causes developmental defects specifically at the site of administration.

10 days after fertilization

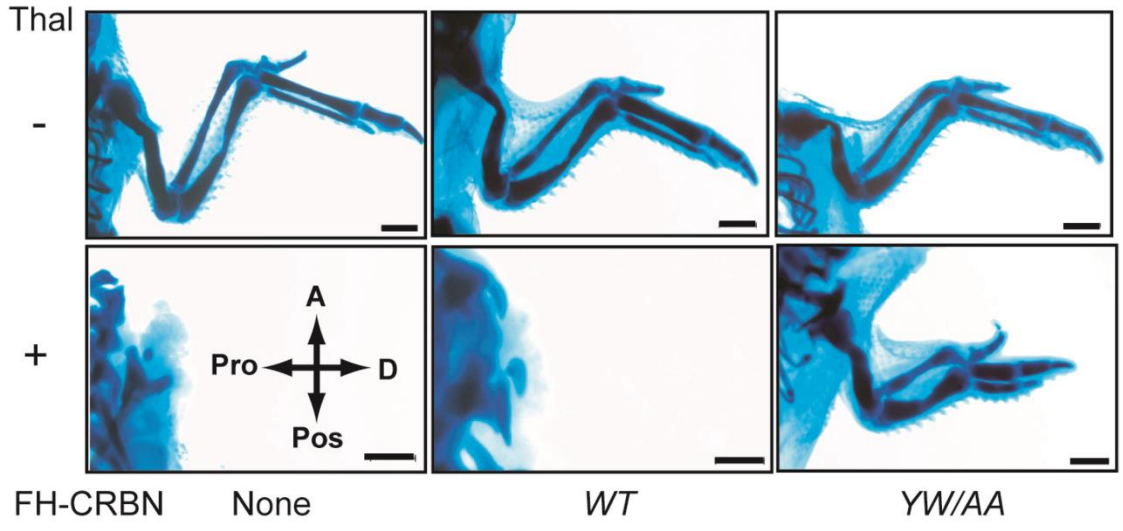




Forelimb or wing

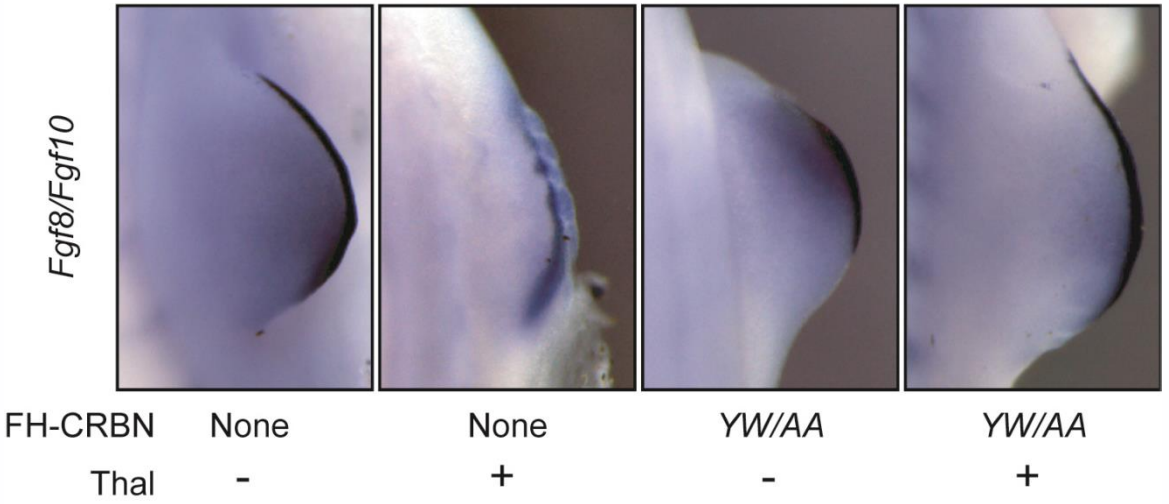
Bone staining

CRBN$^{YW/AA}$ *suppresses Thal teratogenicity in chicks*



Stage 36

Whole mount *in situ* hybridization of limb buds



Stage 20

→ ▪ CRBN$^{YW/AA}$ suppresses Thal teratogenicity and rescues Thal-induced repression of Fgf8 expression.

▪ Our findings in zebrafish were validated in chickens.

Thal-induced developmental defects of the pectoral fins precede angiogenesis

Thal-induced angiogenesis inhibition is responsible for the developmental defects of pectoral fins.

Therapontos *et al.*, ***PNAS***（2009）

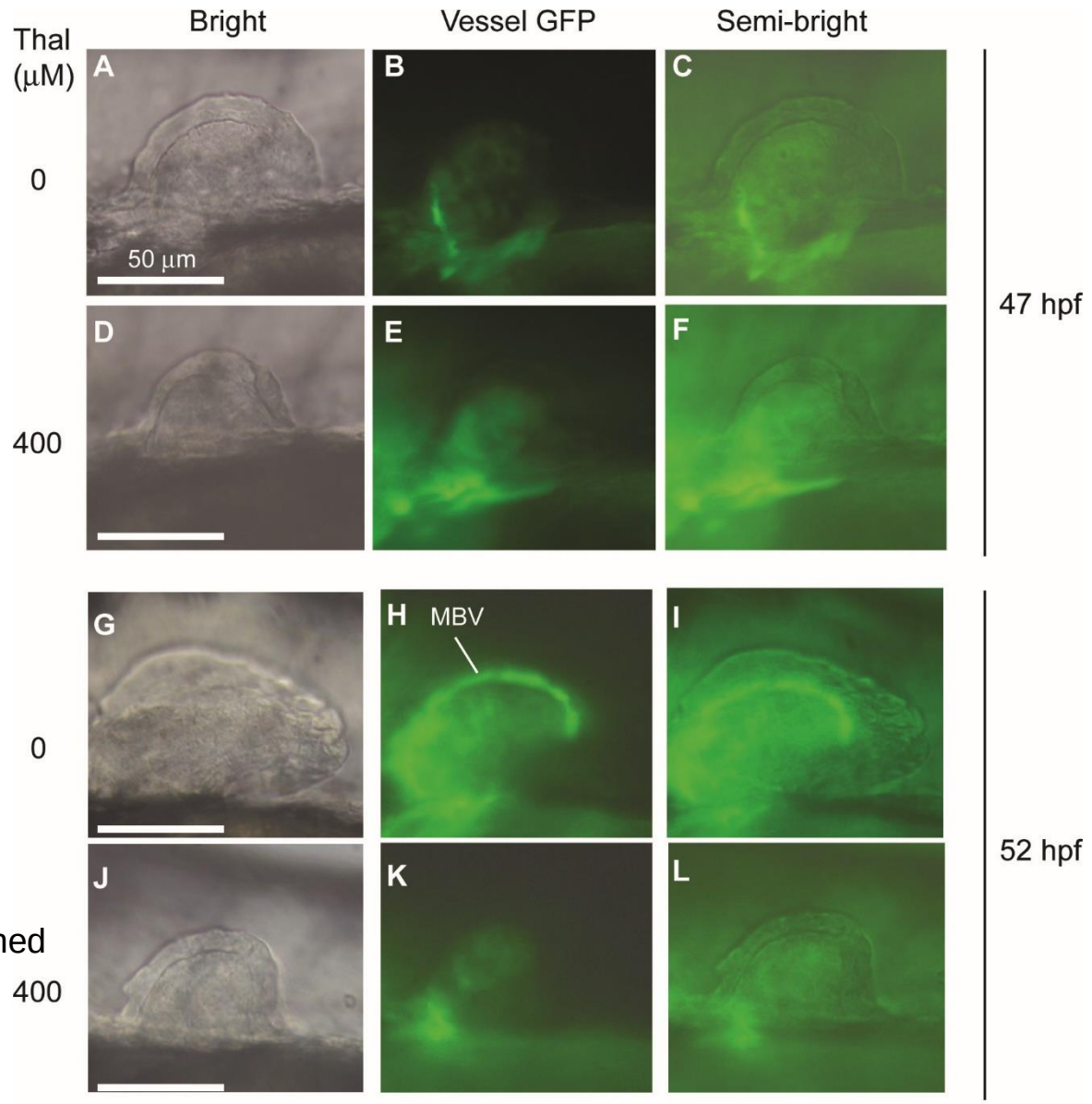
Angiogenesis inhibition is not involved in the deformity of pectoral fins.

At 52 hpf, both angiogenesis and pectoral fin development were inhibited by Thal.

At 47 hpf, marginal blood vessel (MBV) was not yet formed, but the pectoral fins clearly showed developmental defects.

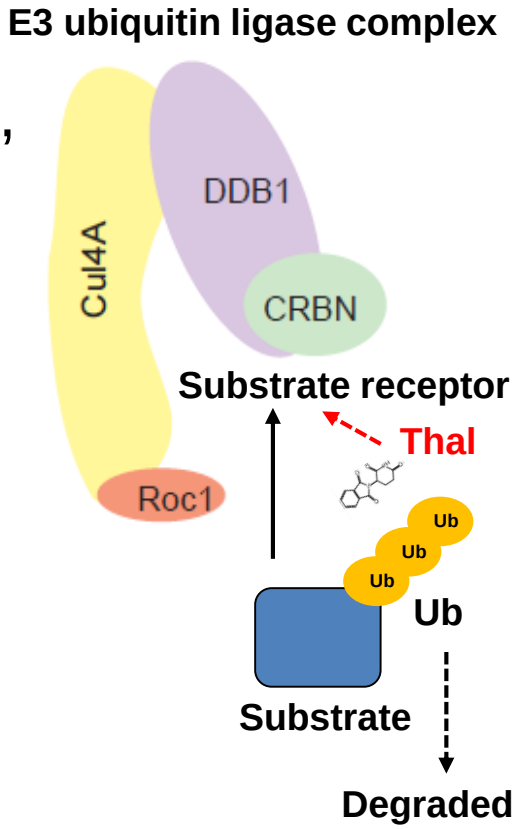
Fli1a:EGFP transgenic zebrafish, established by Professor Kawakami at NIG, Japan

GFP is selectively expressed in endothelial cells of the MBV.



Summary of our major findings regarding CRBN

1. Identification of CRBN as a Thal-binding protein using our affinity bead technology.
2. CRBN forms an E3 ubiquitin ligase complex, works as its substrate receptor and contributes to the normal development of the limbs and otic vesicles in zebrafish and chick models.
3. CRBN is a target of Thal teratogenicity, and Thal exerts its teratogenic effects by binding to CRBN and altering CRBN E3 ubiquitin ligase activity.



Ito *et al.*, ***Science*** (2010)

This work has received great responses around the world and led to our collaboration with Celgene corporation in the US.

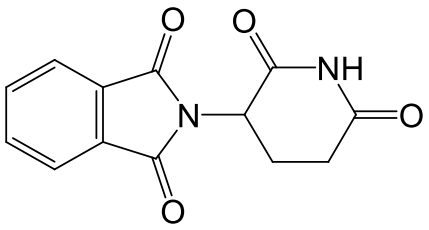
Contents

1. Background of thalidomide (Thal)
2. Identification of cereblon (CRBN) as a target of Thal teratogenicity using our affinity bead technology
3. Involvement of CRBN in the anti-cancer effects of Thal and its analogs
4. Mechanisms of the therapeutic effects of Thal and its analogs
5. Current research on the mechanisms of Thal teratogenicity

Immunomodulatory drugs（IMiDs）

Thal and its derivatives with immunomodulatory activity

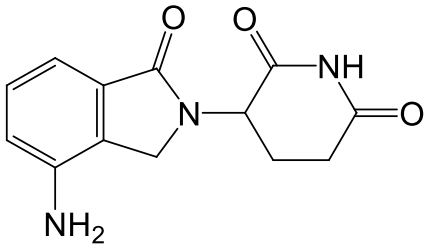
First generation



Thalidomide (Thal)

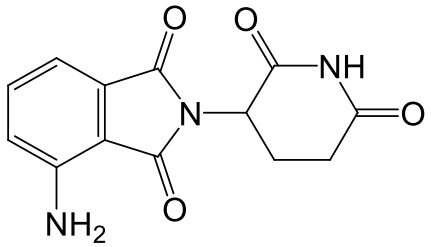
- Hansen's disease
- Multiple myeloma (MM)

Second generation



Lenalidomide (Len)

- Multiple myeloma (MM)
- Myelodysplastic syndrome (particularly, 5q− syndrome)
- Adult T-cell leukemia/lymphoma
- Chronic Lymphocytic Leukemia
- Non-Hodgkin Lymphoma



Pomalidomide (Pom)

- Multiple myeloma (MM)
- Myelofibrosis

Len and Pom are much more effective against MM than Thal.

*approved in the US and Japan
*currently undergoing clinical testing

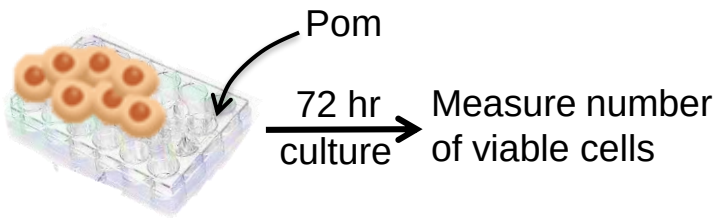
Anti-cancer effects (main effects)

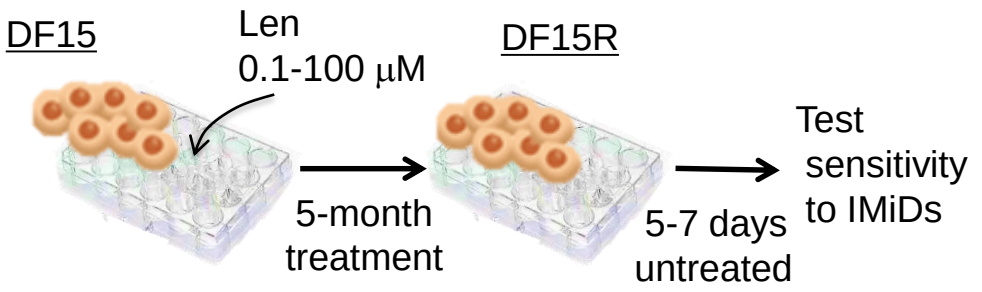
1. Inhibition of MM cell growth
2. Immunomodulatory effects (T-cell activation)

We performed a joint study on the main effects of IMiDs with Celgene in the US.

CRBN is involved in anti-cancer effects of IMiDs

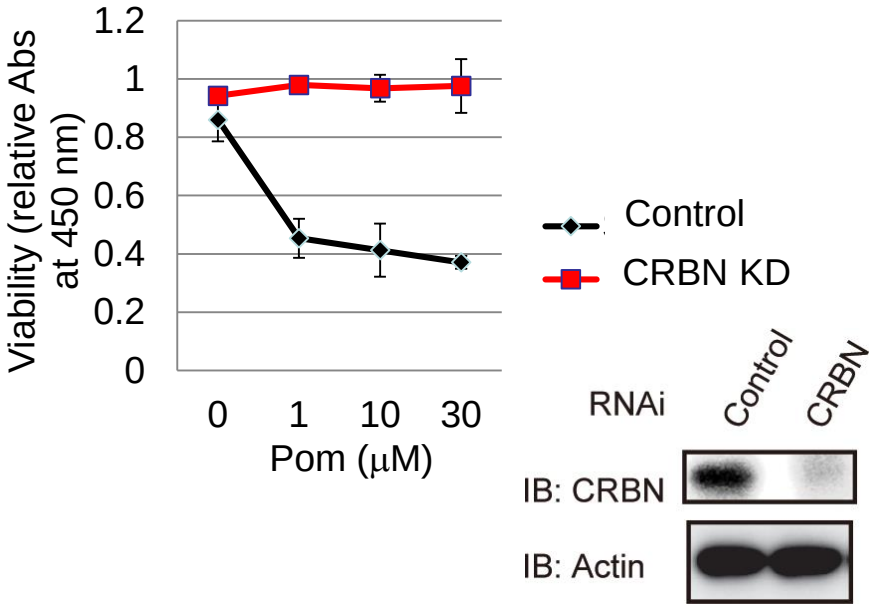
OPM2: MM cells sensitive to IMiDs

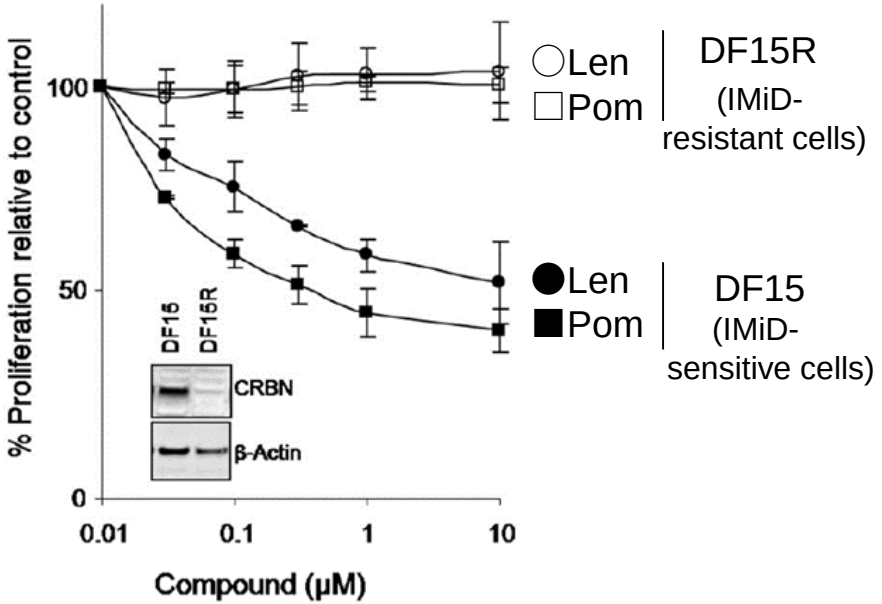




Inhibition of MM cell growth

OPM2 viability 3 days after Pom treatment



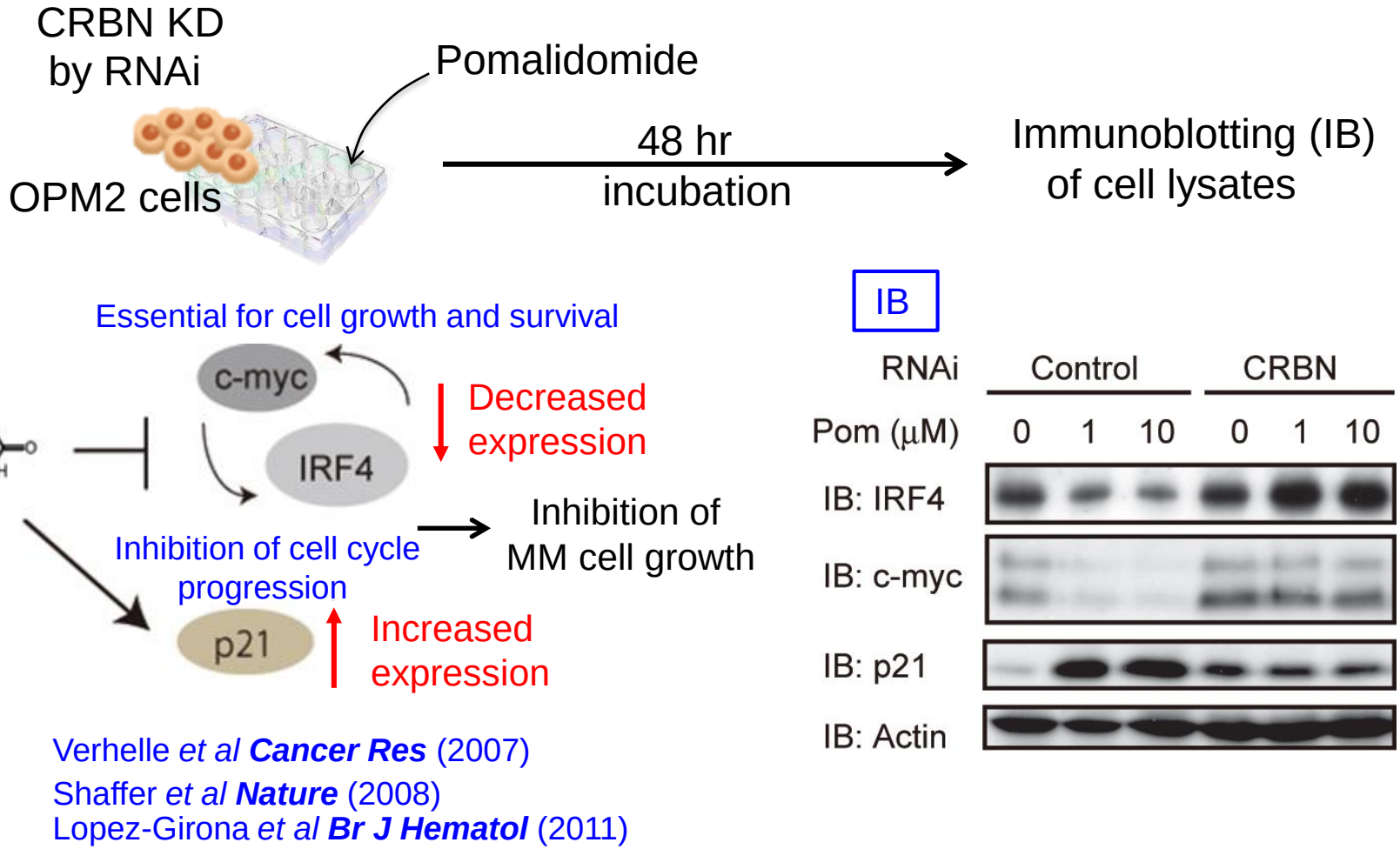


→ CRBN KD cells are resistant to Pom.

→ Expression of CRBN is decreased in Len/Pom-resistant cells

Lopez-Girona *et al.*, ***Leukemia*** (2012)

CRBN is a target of the main effects of IMiDs

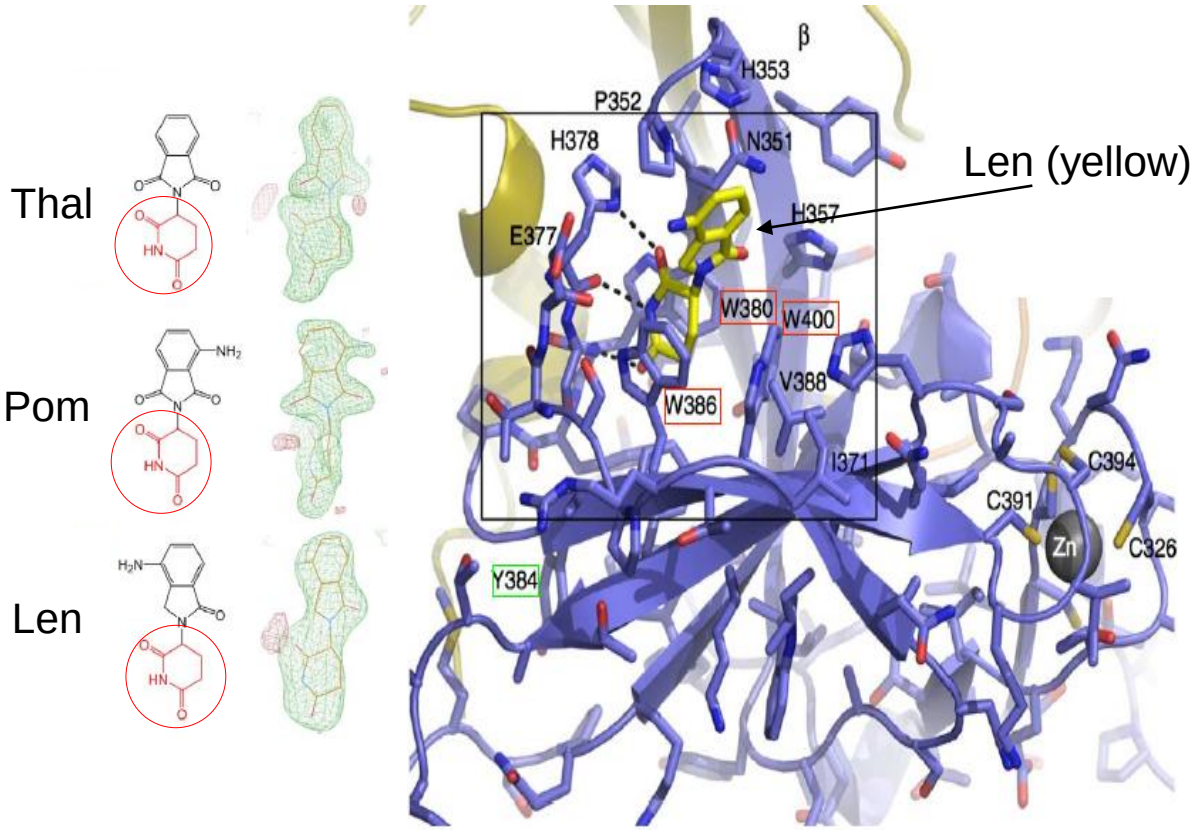


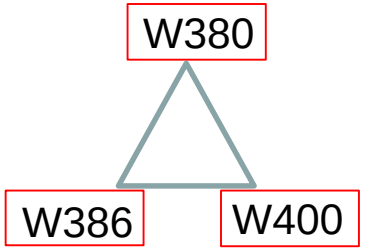
Verhelle *et al* ***Cancer Res*** (2007)
Shaffer *et al* ***Nature*** (2008)
Lopez-Girona *et al* ***Br J Hematol*** (2011)

→ IMiDs regulate the expression of IRF4 and c-myc via CRBN, which are essential for the growth and survival of MM cells.

Lopez-Girona *et al.*, ***Leukemia*** (2012)

X-ray crystallography of a complex of IMiDs with CRBN





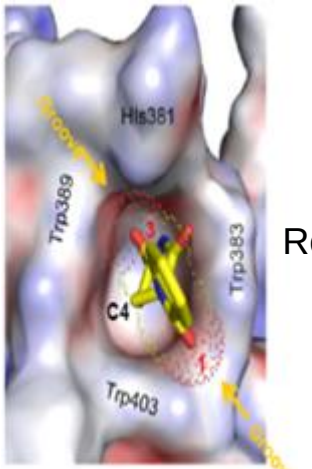
A triple tryptophan pocket (tri-trp pocket) is formed by 3 tryptophan residues, present in the C-terminal region (319–428 aa) of CRBN.

→ The glutarimide ring, common to IMiDs, is inserted into the tri-trp pocket.

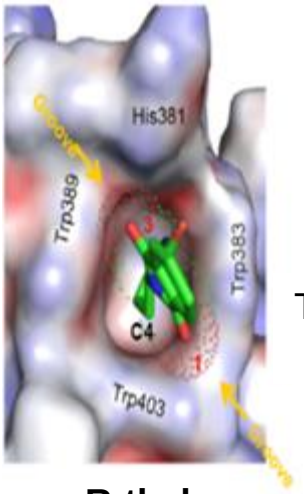
All IMiDs (Thal, Len, Pom) have optical isomers (S-IMiDs, R-IMiDs).

Chamberlain *et al.*, ***Nat Struct Mol Biol*** (2014)

3D structure of the complex of CRBN with S-Thal or R-Thal



S-thal



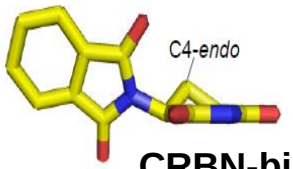
R-thal

Relaxed conformation (stable)

Twisted conformation (unstable)

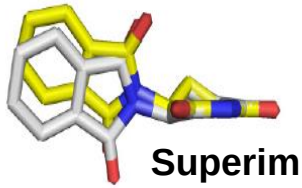
Comparison of the structure of the free form and CRBN-binding form of Thal isomers

S-Thal

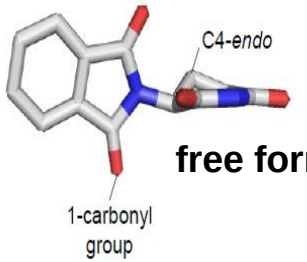


CRBN-binding form

Slightly twisted (stable)

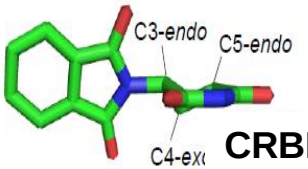


Superimposed



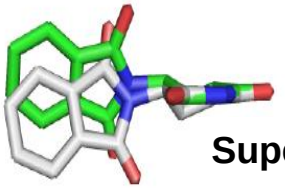
free form

R-Thal

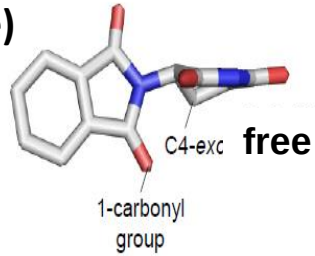


CRBN-binding form

Highly twisted (unstable)



Superimposed



free form

→ S-Thal fits more stably into the tri-trp pocket of CRBN than R-Thal.

Mori *et al.*, ***Sci Rep*** (2018)

Our conclusion regarding the optical isomers of IMiDs

Biochemical and 3D-structural analyses indicated that S-IMiDs have a much higher affinity for CRBN than R-IMiDs.

Mori *et al.*, ***Sci Rep*** (2018)
Chamberlain *et al.*, ***Nat Struct Mol Biol***（2014）

S-IMiDs are primarily involved in both the main effects and side effects.

Mori *et al.*, ***Sci Rep*** (2018)

IMiDs are easily racemized under physiological conditions (pH 7.4, 37 ºC).

Mori *et al.*, ***Sci Rep*** (2018)
Nishimura *et al.*, ***Chem Pharm Bull*** (1994)

S-IMiDs preferentially bind to CRBN, and then the remaining R-IMiDs will be readily racemized, leading to a supply of S-IMiDs.

→ Optical isomers of IMiDs are not a crucial issue for their drug actions.

Contents

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5. Current research on the mechanisms of Thal teratogenicity

Identification of novel substrates that bind to CRBN in the presence of IMiDs

Outline of UbiScan analysis

Treatment of MM cells with or without IMiDs.

↓

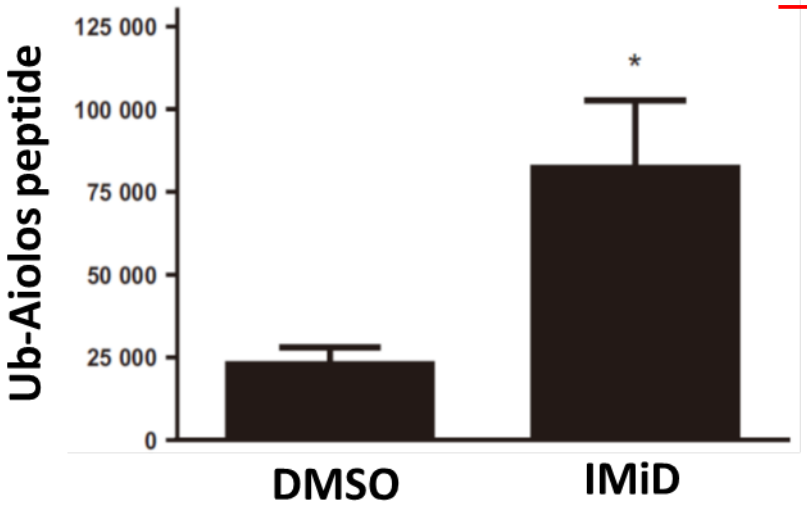
Preparation of cell lysates and collection of ubiquitinated peptides by immunoaffinity chromatography using a specific antibody

↓

Analysis by tandem mass spectrometry

↓

Screening of ubiquitinated peptides enriched in cells by upon treatment with IMiDs



→ Identification of Aiolos as a novel CRBN substrate that shows significantly increased ubiquitination when treated with IMiDs

Ikaros family (IKZF)

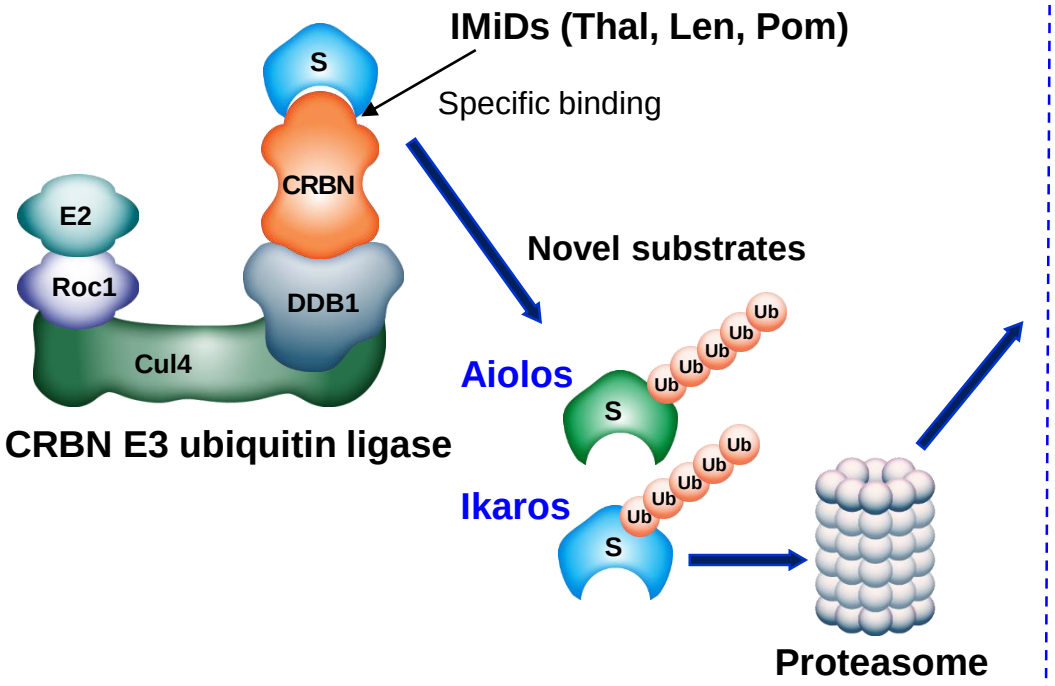
Ikaros (IKZF1)
Helios (IKZF2)
Aiolos (IKZF3)
Eos (IKZF4)
Pegasus (IKZF5)

Aiolos and Ikaros are transcription factors essential for the differentiation and maturation of blood cells.

Gandhi *et al.*, ***Br J Haematol*** (2014)

Mechanisms of actions of IMiDs

Aiolos and Ikaros are known to be repressors of IL-2 and activators of IRF-4/c-myc.



Pharmacological effects

T-cells

- ↑ **IL-2**
- ↑ **T-cell activation (IL-2, IFN-γ)**
- ↑ **Monocytes (TNFα, IL-2, IL-6)**

Immunomodulatory actions

MM cells

- ↓ **IRF-4, c-myc**
- ↓ **Cell cycle/ proliferation**
- ↑ **Apoptosis**

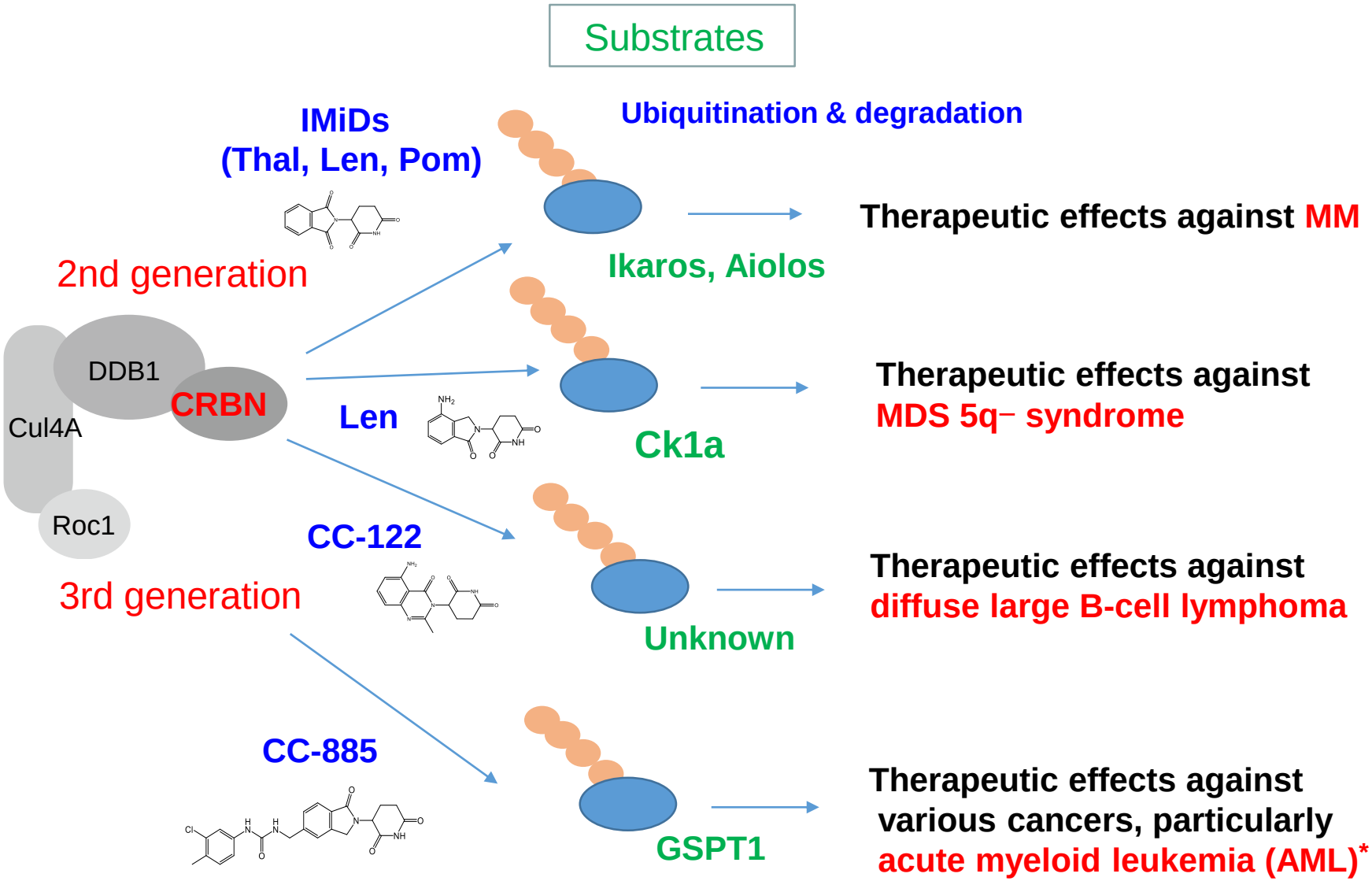
Antitumor actions

Multifaceted therapeutic effects

→ Binding of IMiDs to CRBN recruits Aiolos and Ikaros as novel substrates to the CRBN E3 ubiquitin ligase complex and induces their ubiquitination and degradation, leading to multifaceted therapeutic effects.

Krönke *et al.*, ***Science*** (2013), Lu *et al.*, ***Science*** (2013), Gandhi *et al.*, ***Br J Haematol*** (2014)

Development of CRBN modulators

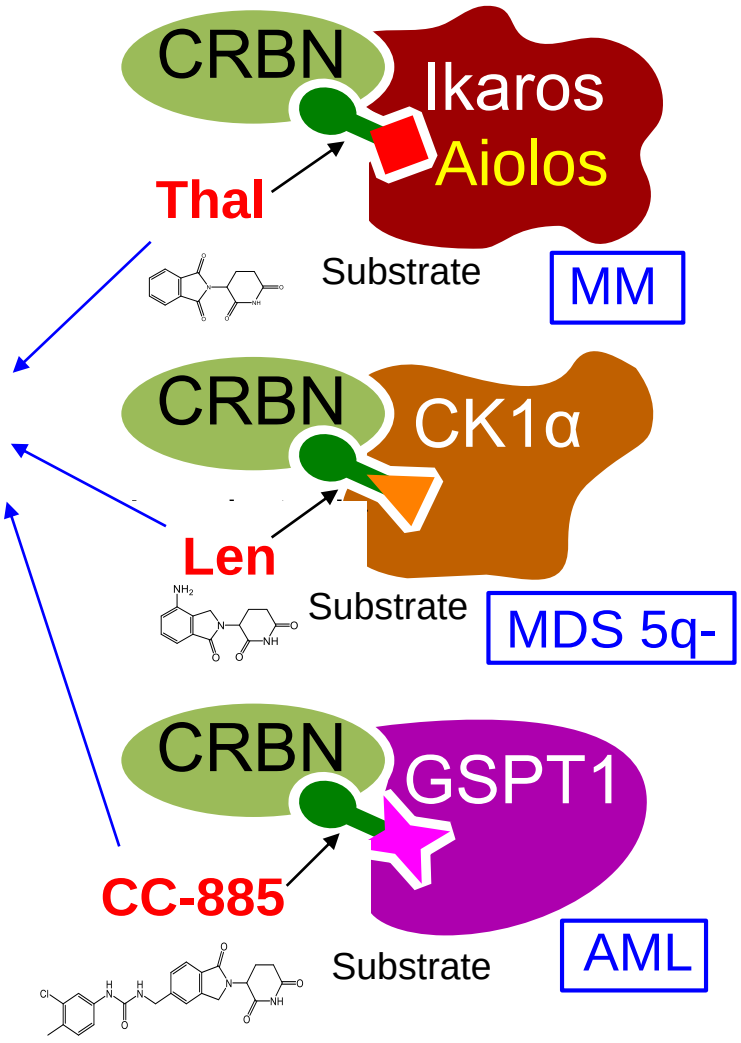


*Matyskiela *et al.*, ***Nature*** (2016)

CRBN modulators

Therapeutics

Thal and its subsequently developed analogs are collectively called “CRBN modulators”, which bind to CRBN, recruit their unique substrate, and exert therapeutic effects on disorders via degradation of the substrate.



Novel substrates recognize and bind to a complex of CRBN and a CRBN modulator, which serve as a molecular glue.

Matyskiela *et al.*, ***Nature*** (2016)

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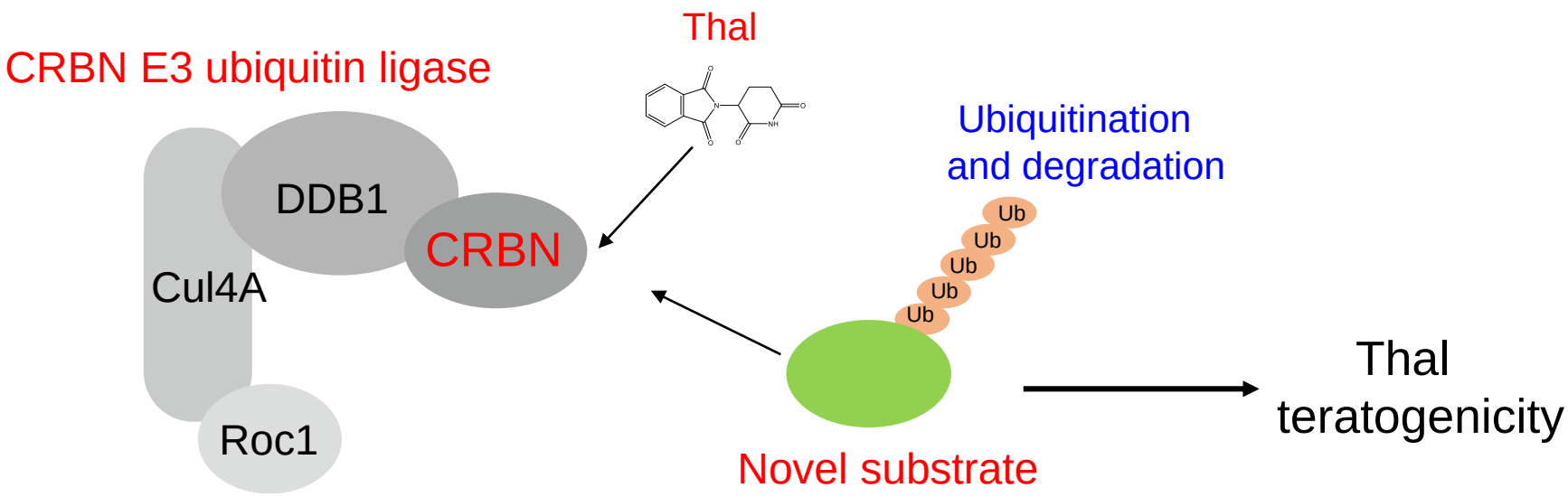
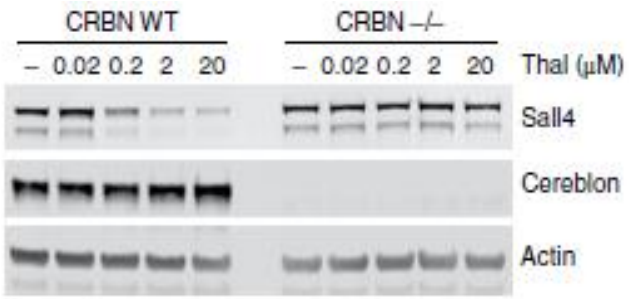
Novel substrates responsible for Thal teratogenicity



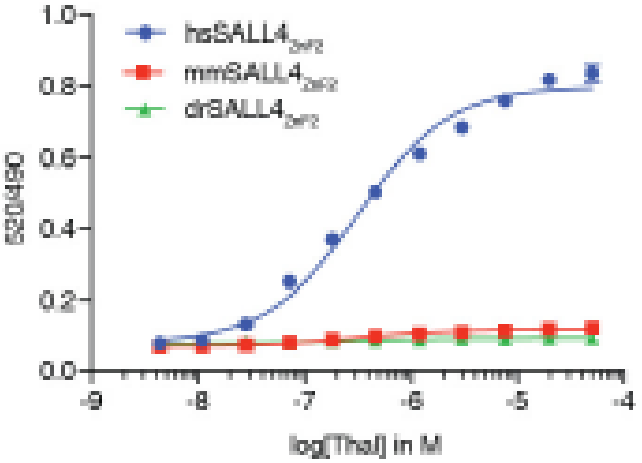
Neosubstrates associated with Thal teratogenicity

IB

Thal induces Sall4 degradation
(human iPS cells)



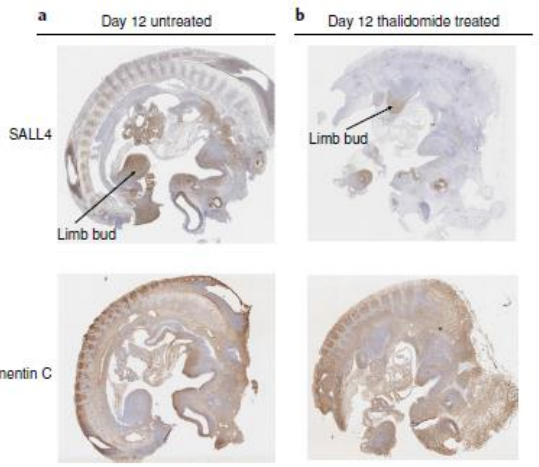
hCRBN



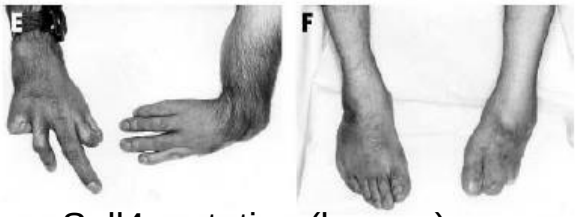
Interaction

IB

Thal-induced Sall4 degradation
in rabbit embryos

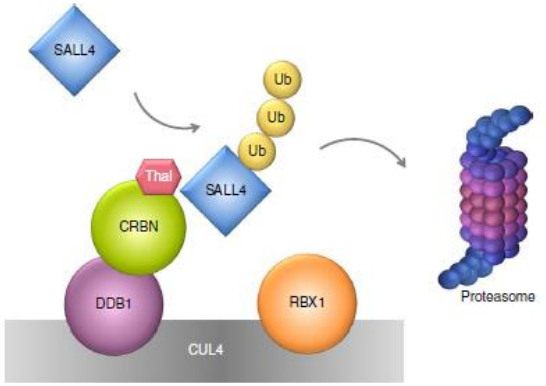


Okihiro/Duane-Radial-ray syndrome



Sall4 mutation (human)

Borozdin *et al.*, ***J Med Genet*** (2004)



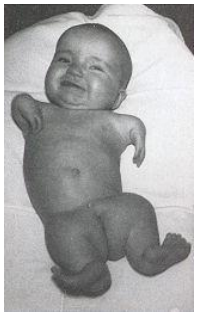
・Thal teratogenicity has been observed among primates, rabbits, chicks and zebrafish. However, Sall4 degradation does not occur in zebrafish and chicks.

・There might be additional neosubstrates responsible for Thal teratogenicity.

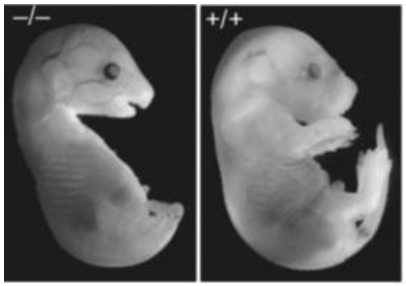
Donovan *et al.*, ***eLife*** (2018)
Matyskiela *et al.*, ***Nat Chem Biol*** (2018)

Novel CRBN substrate LD1

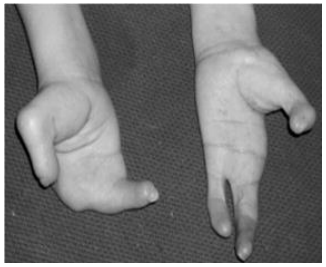
Sorry!!
The actual name of LD1 cannot be disclosed because this study is not yet published (under review).



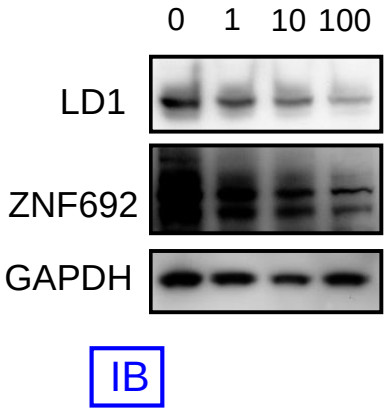
Small Limb (Phocomelia)

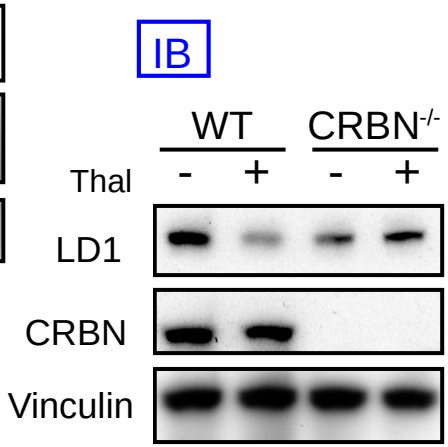


LD1 mutation (Limb deformity 1) (Mouse)

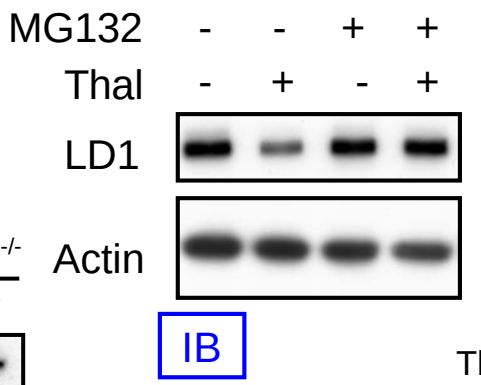


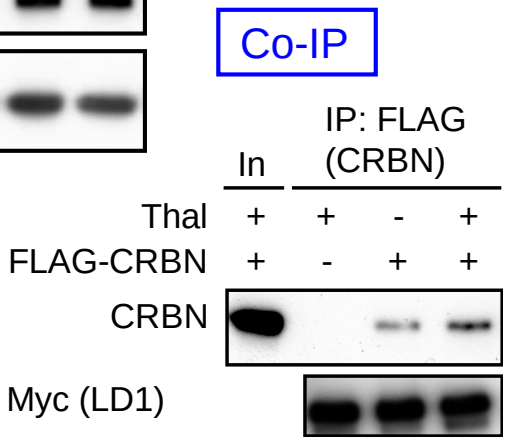
LD1 mutation (Human)

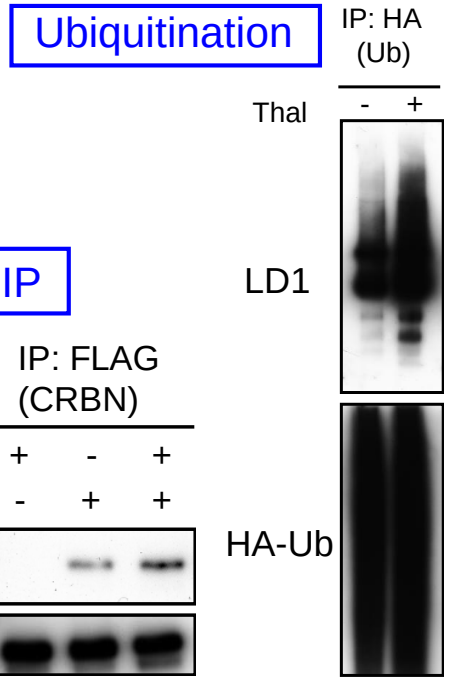




HaCat細胞





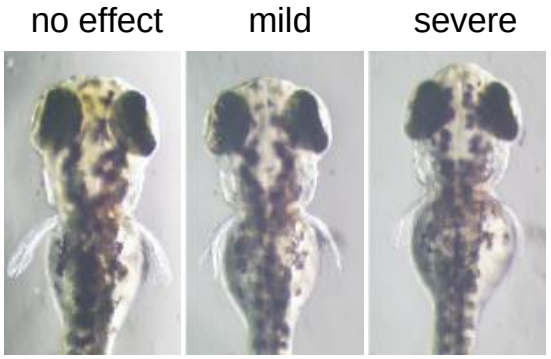


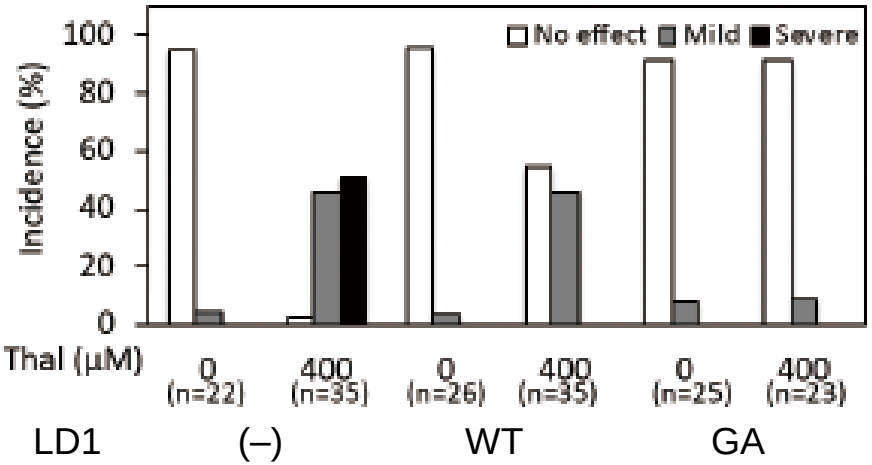
→ Thal induces CRBN-dependent ubiquitination and degradation of LD1.

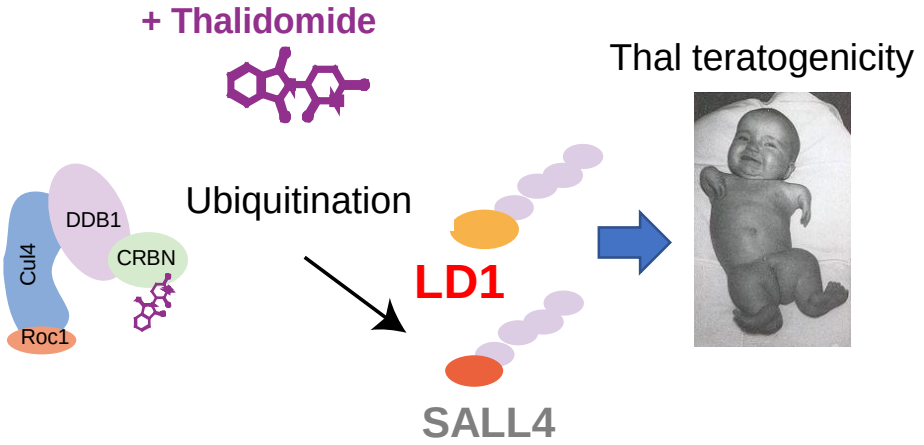
Collaboration with a group in Milan Univ., Italy

Thal causes fin teratogenicity through LD1 degradation

Inhibition rate of Thal teratogenicity







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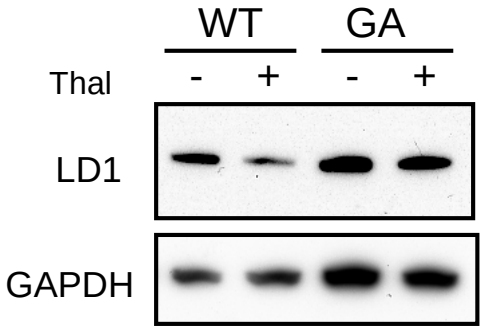
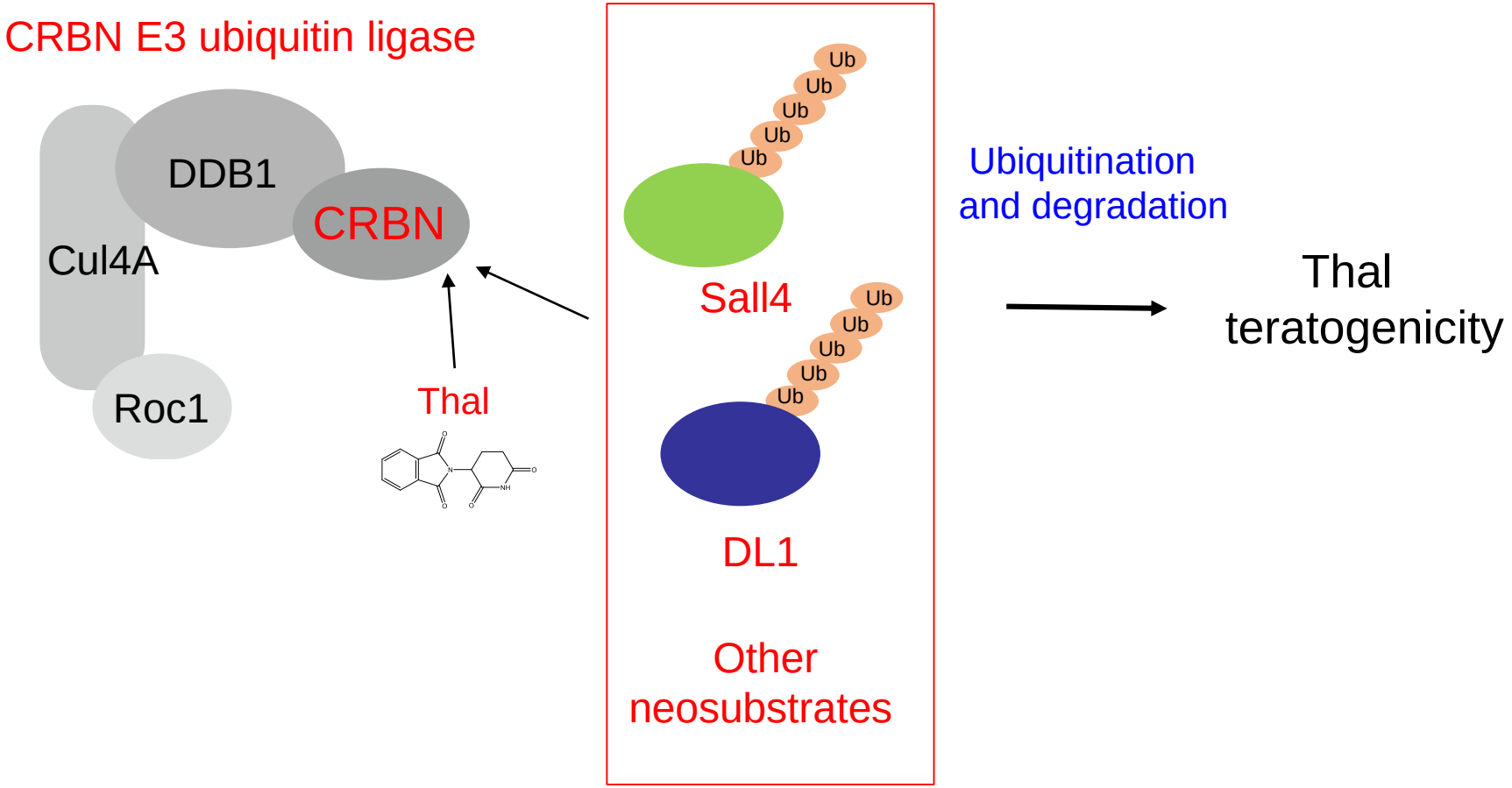


→ The LD1 mutant GA, which is resistant to Thal-induced degradation, more efficiently blocks Thal teratogenicity than WT LD1 in zebrafish.

Asatsuma-Okumura *et al.*, in revision

Neosubstrates responsible for Thal teratogenicity



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Thank you for your kind attention

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