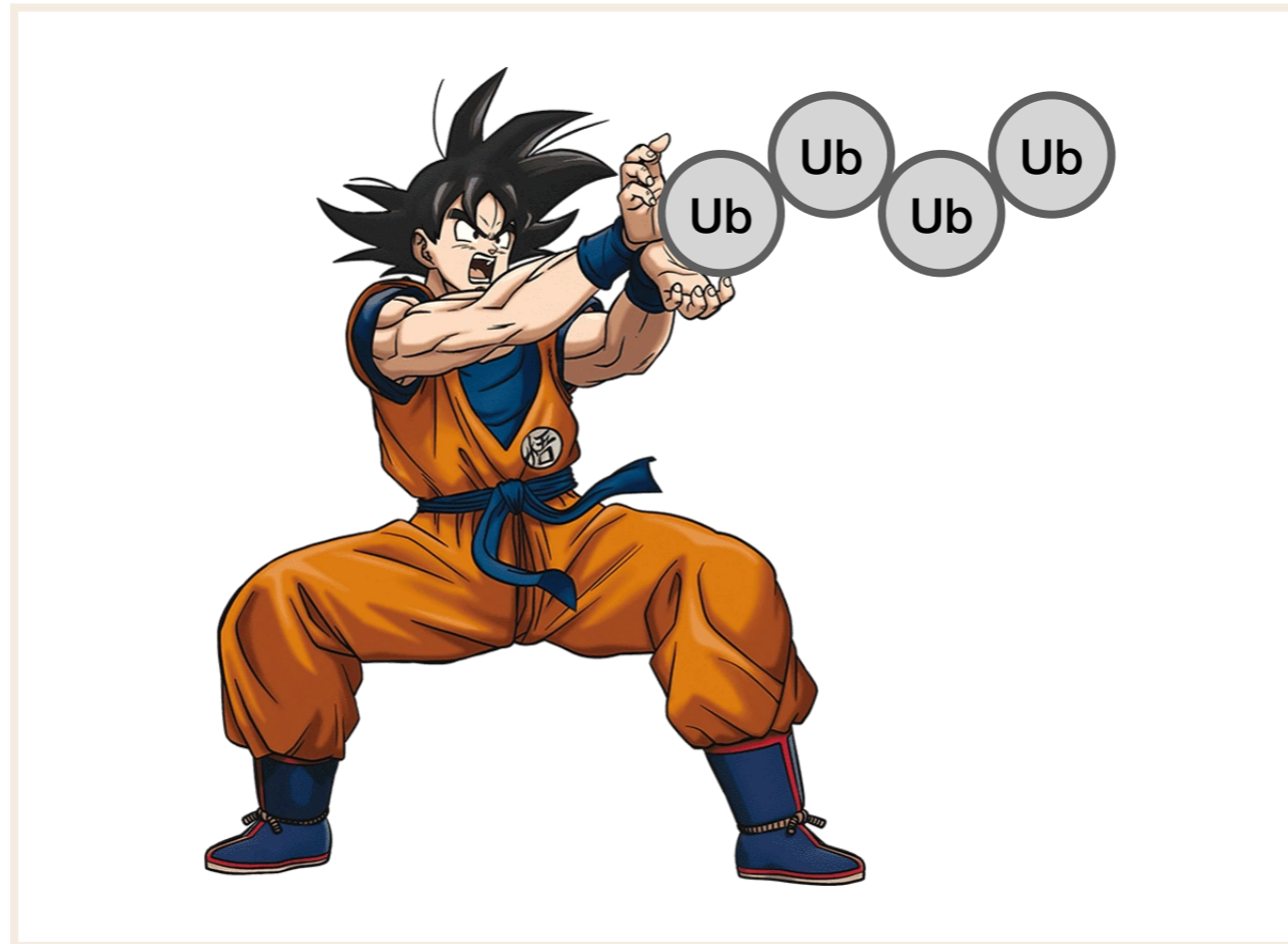


The Ubiquitin Code



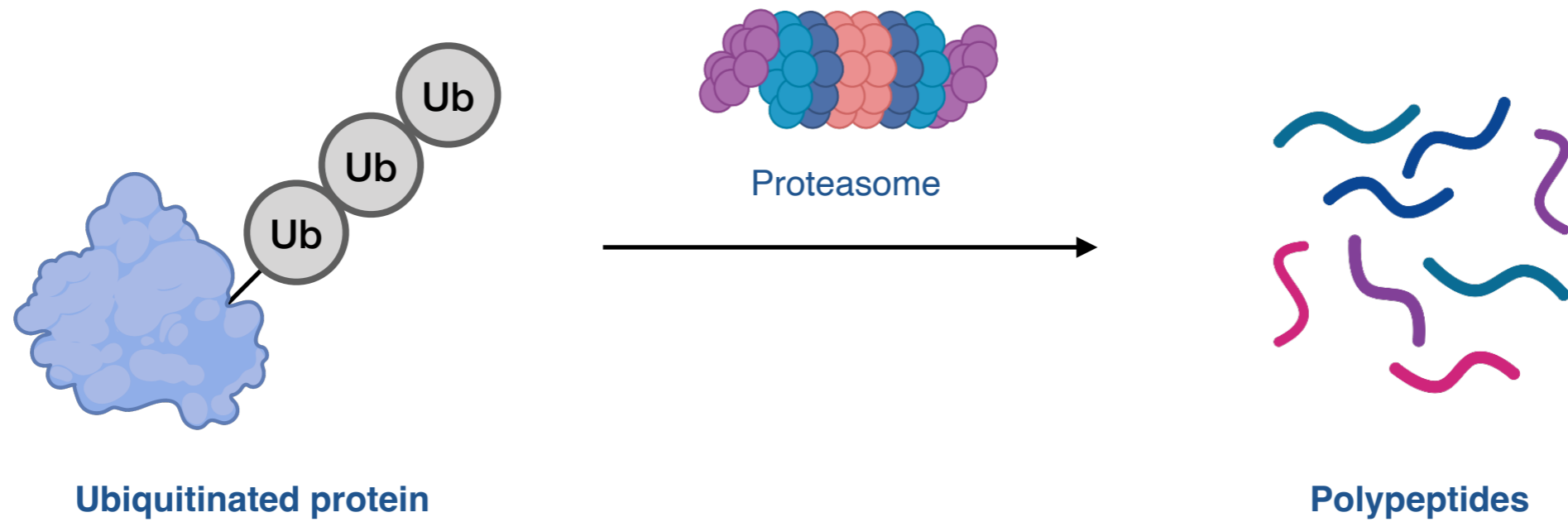
Benito F. Buksh

MacMillan Research Group

Group Meeting

January 24th, 2023

The Ubiquitin Code

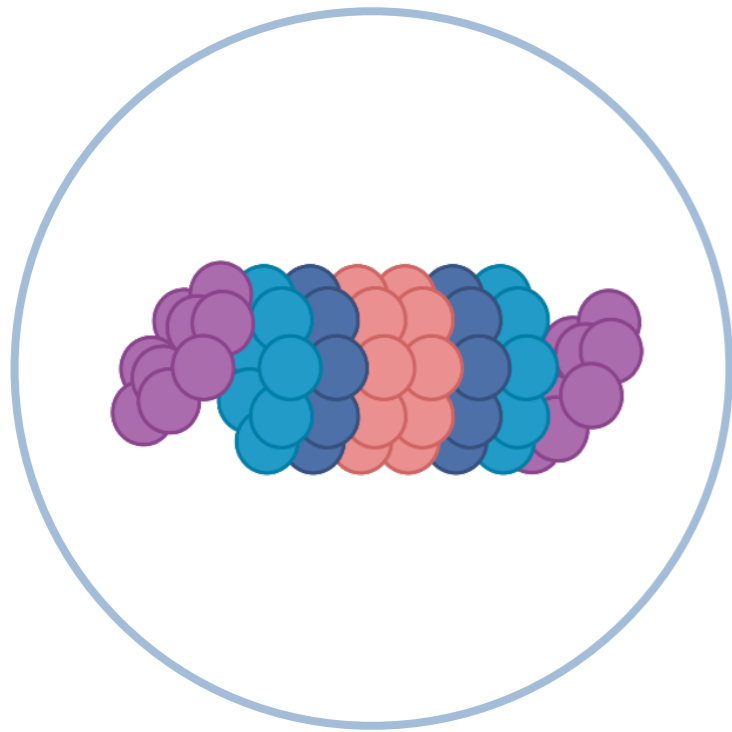


Ubiquitinated protein

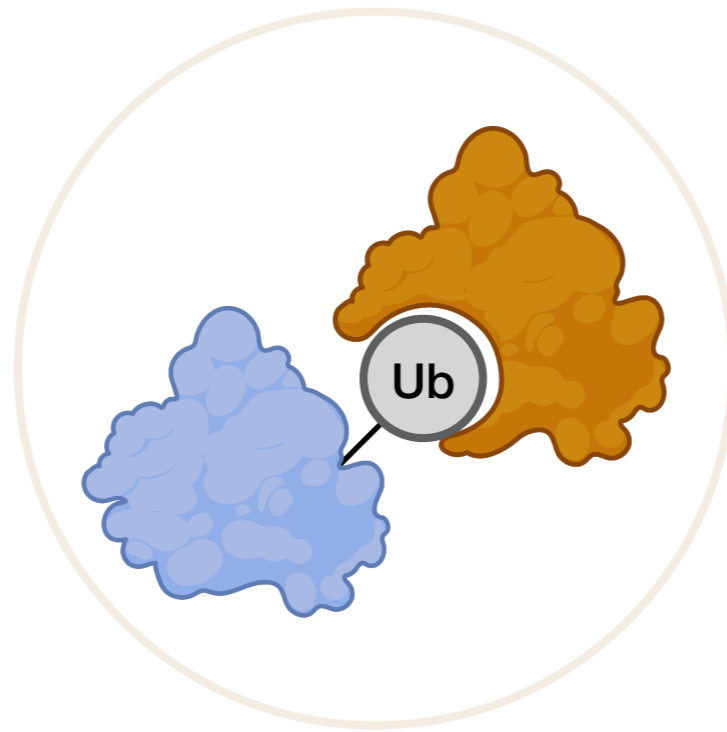
Polypeptides

Ubiquitinated proteins are brought to the proteasome and undergo proteolysis

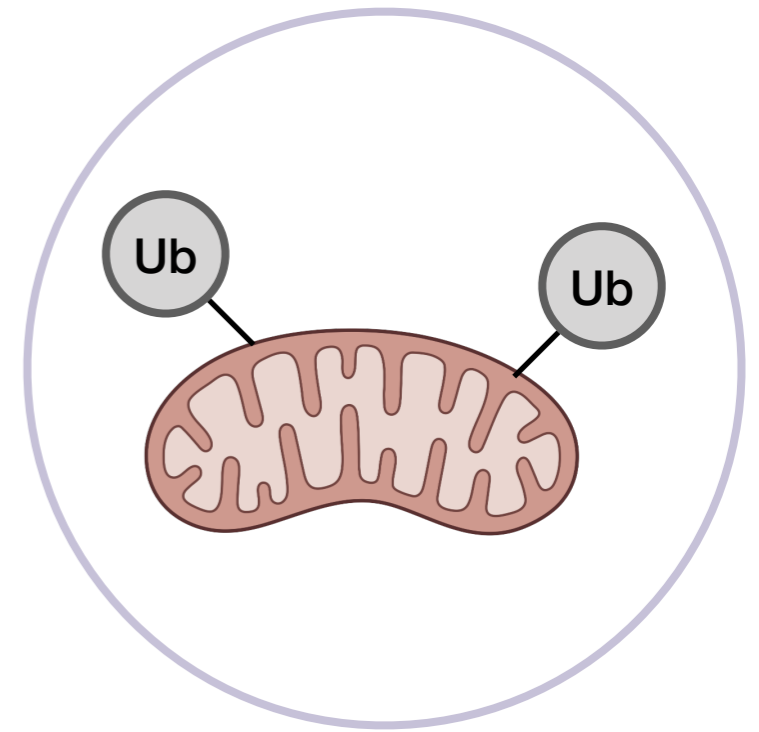
The Ubiquitin Code



**Ubiquitin-Mediated
Proteolysis**



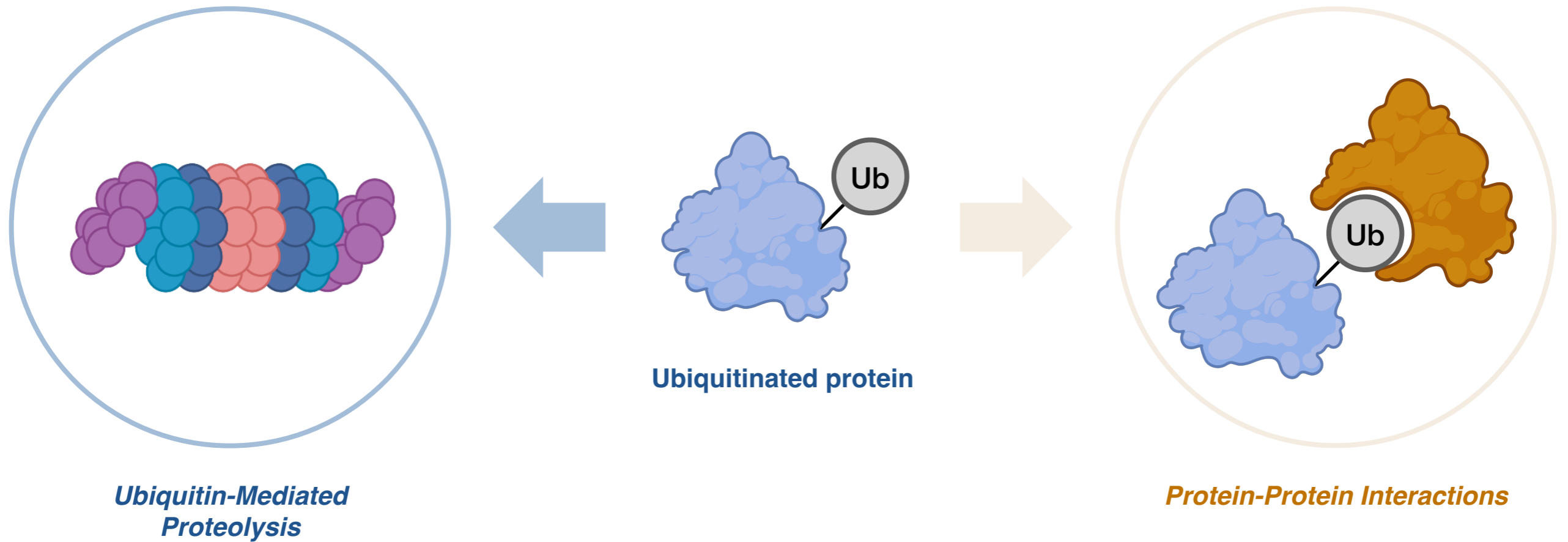
Protein-Protein Interactions



Mitophagy
(mitochondrial degradation)

In addition to degradation, protein ubiquitination can trigger additional signals

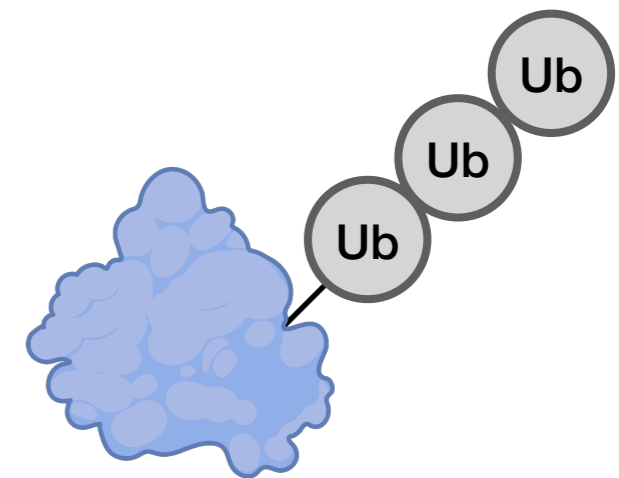
The Ubiquitin Code



Question: When does ubiquitin enable protein-degradation, a protein-interaction, or another process?

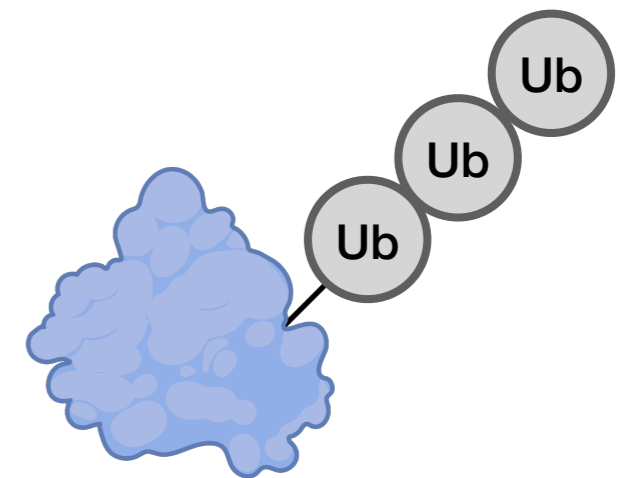
The Ubiquitin Code

- The History of Ubiquitin and How Substrates are Ubiquitinated
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 - DNA damage response
 - Protein-localization
- Therapeutic Outlook



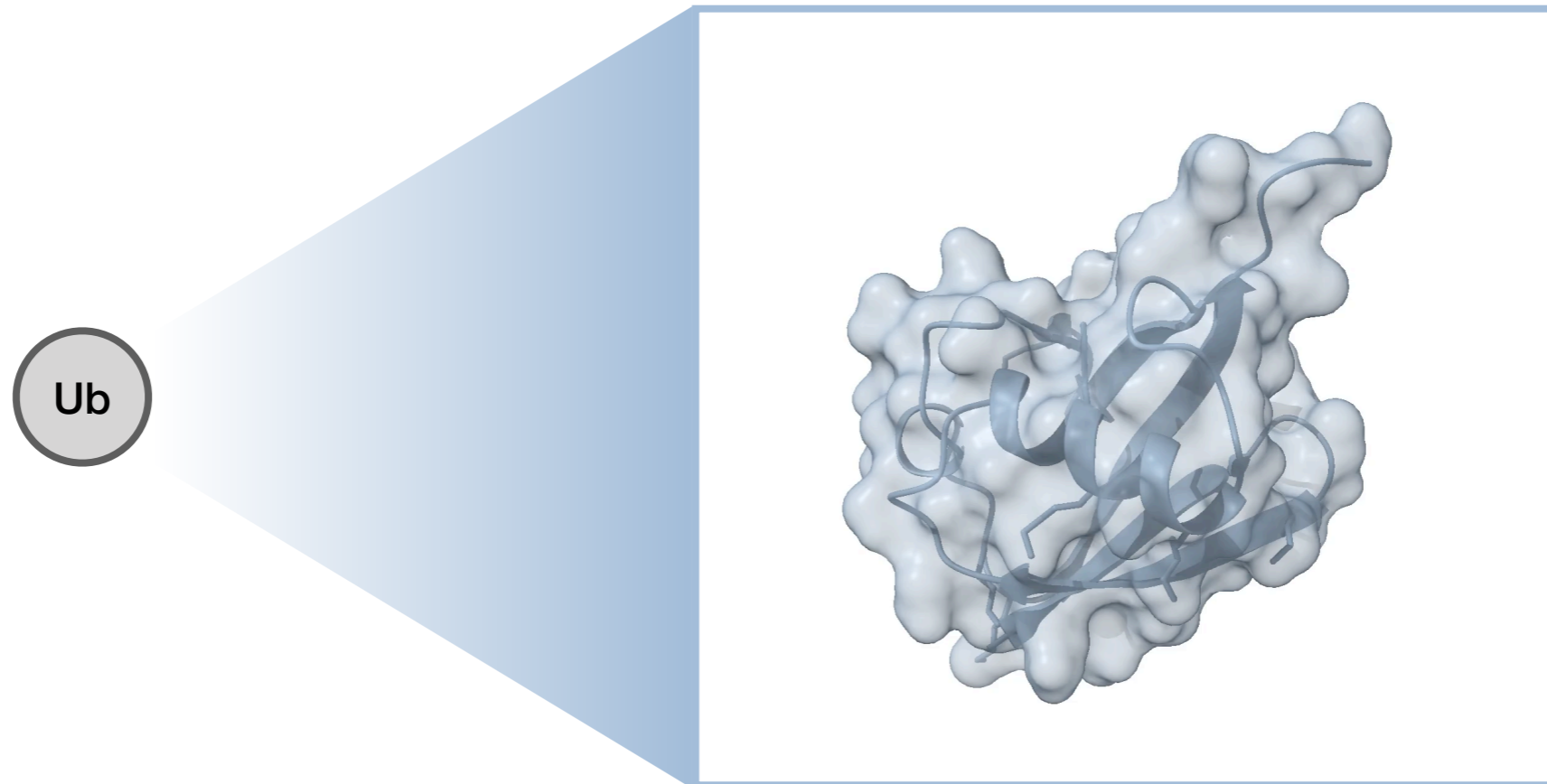
The Ubiquitin Code

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What is Ubiquitin?

Ubiquitin



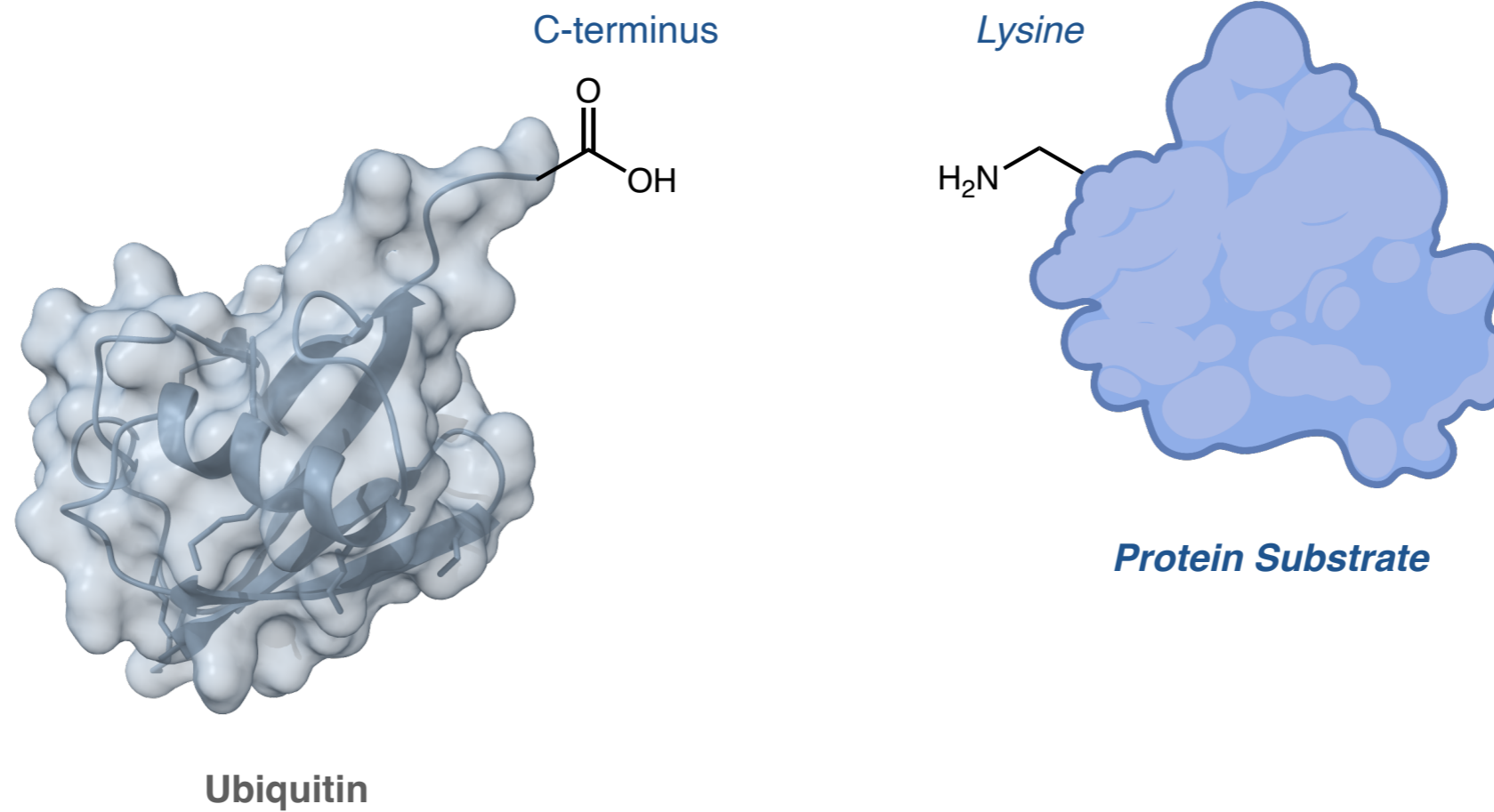
■ 76 amino acids

■ 8.6 kDa

■ Expressed in all Eukaryotic cells

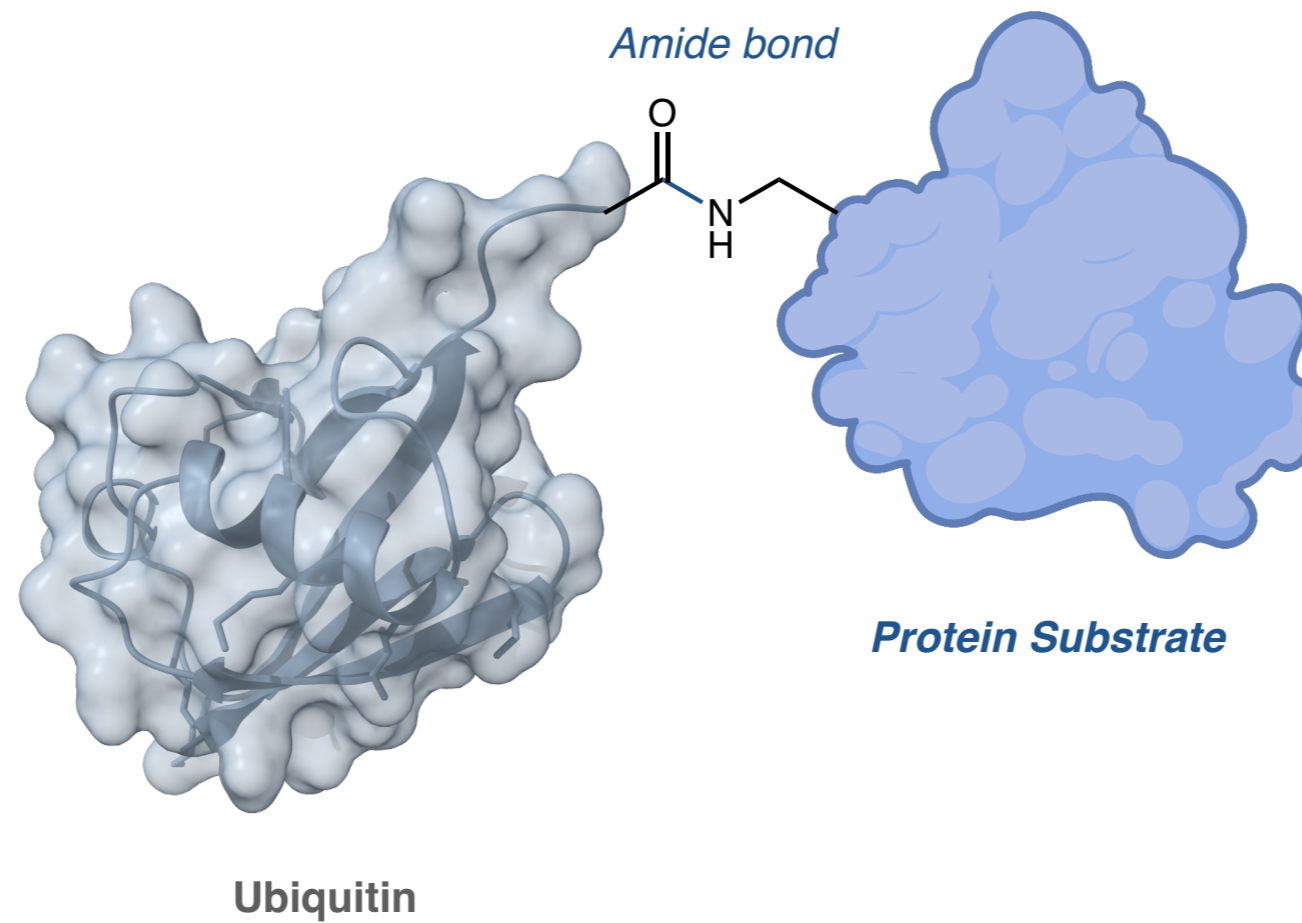
RCSB PDB: 1UBQ

What is Ubiquitin?



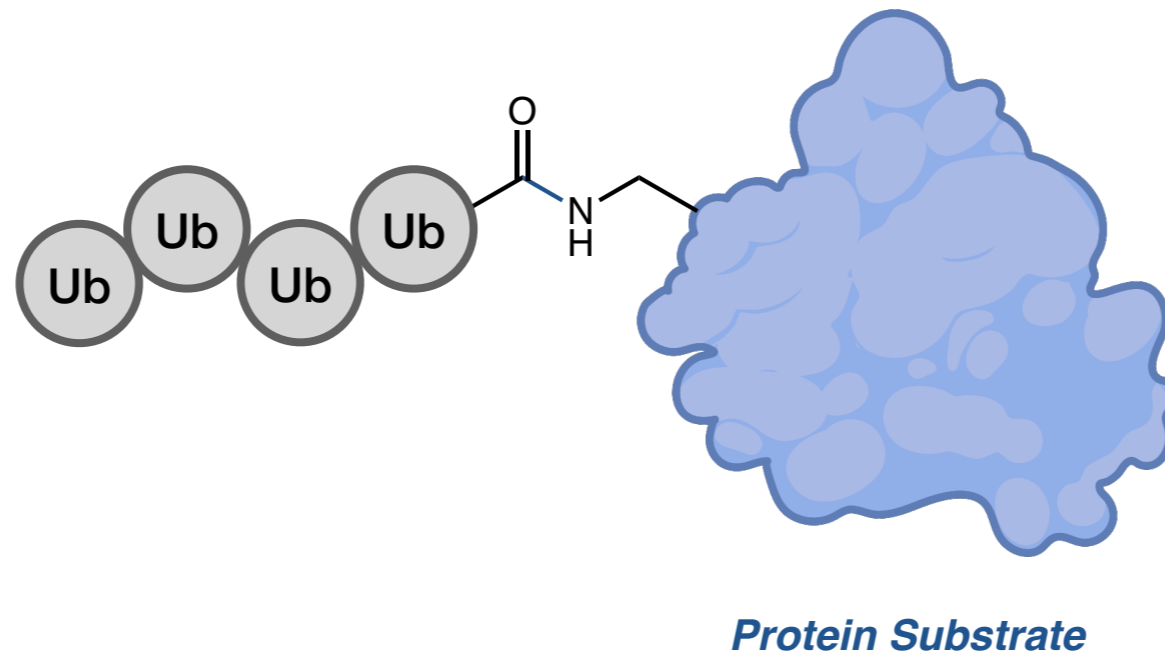
Attachment of ubiquitin occurs at the C-terminus of Ubiquitin and a nucleophilic residue of the substrate

What is Ubiquitin?



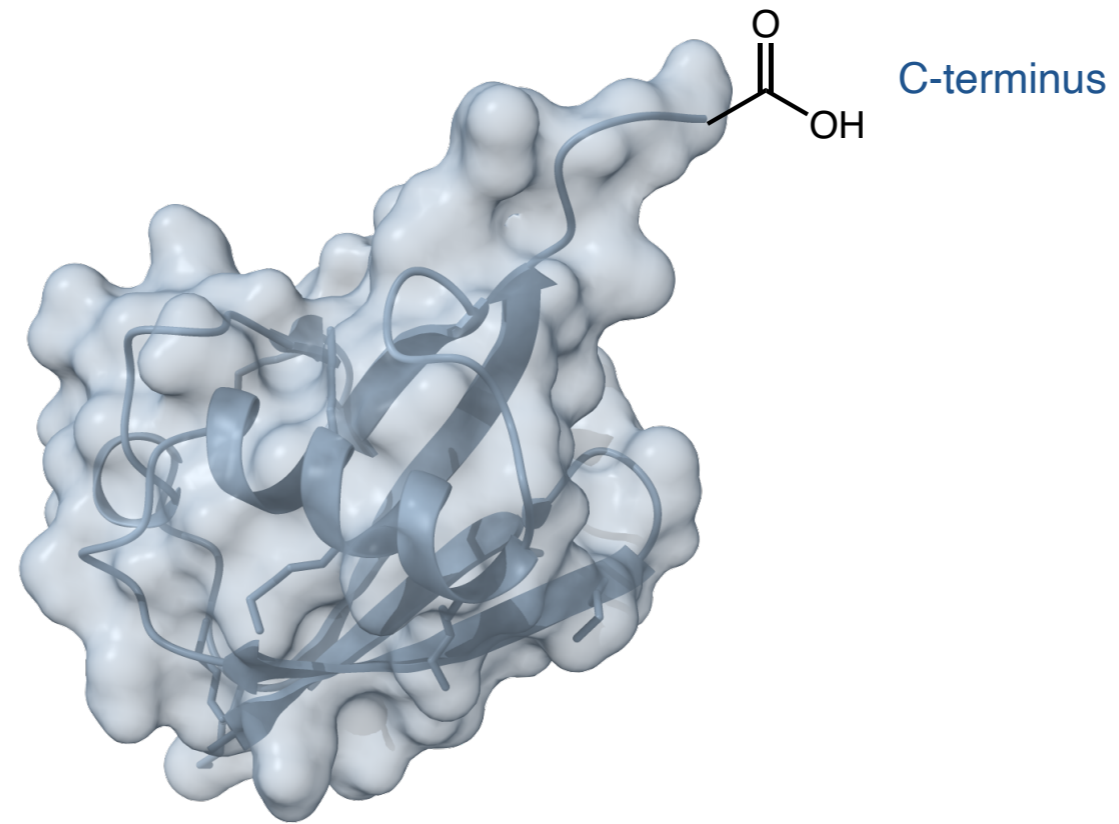
Attachment of ubiquitin occurs at the C-terminus of Ubiquitin and a nucleophilic residue of the substrate

Ubiquitin Chains



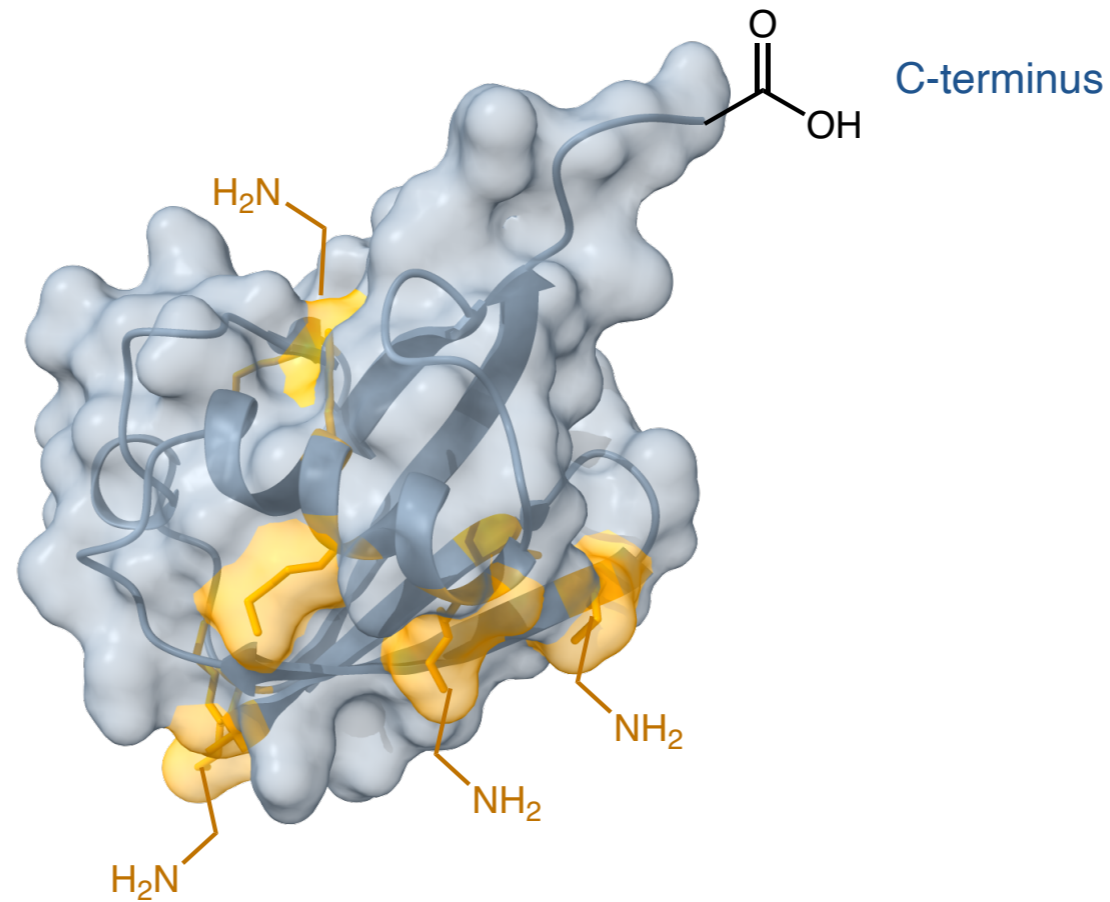
Ubiquitin can be conjugated to ubiquitin to form chains

Ubiquitin Chains



Ubiquitin Chains

Additional ubiquitin proteins can be conjugated to lysine on ubiquitin



Ubiquitin peptide sequence

MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**AKIQD**K**EGIPPD
QQRLIFAG**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRRLRGG

Ubiquitin Chains

Additional ubiquitin proteins can be conjugated to lysine on ubiquitin

Points of Attachment

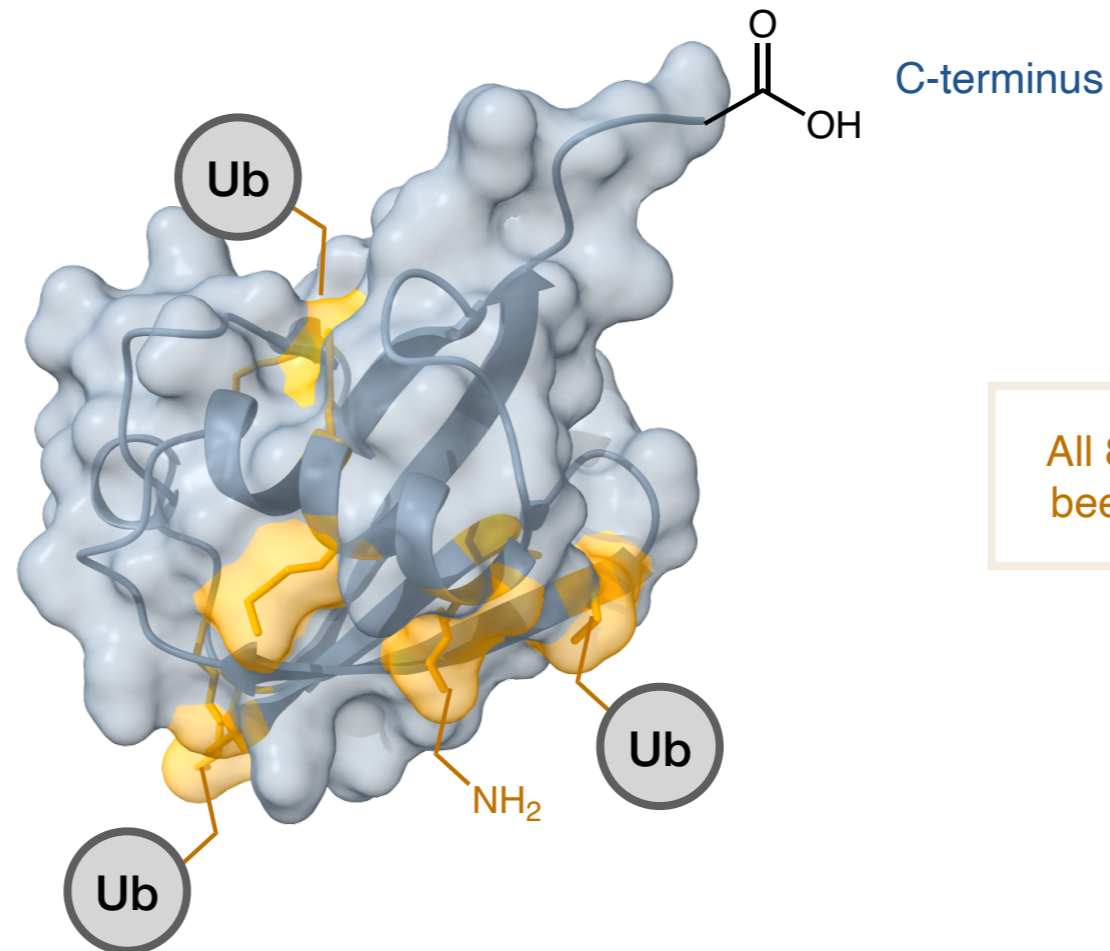
M1 (N-term)

K6

K11 K33

K27 K48

K29 K63



All 8 Ub linkages have been detected in cells

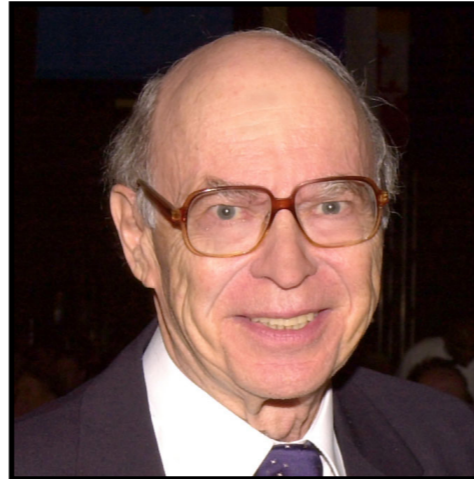
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MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**AKIQD**K**EGIPPD
QQRLIFAG**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRRLRGG

The History of Ubiquitin



2004 Nobel Prize in Chemistry



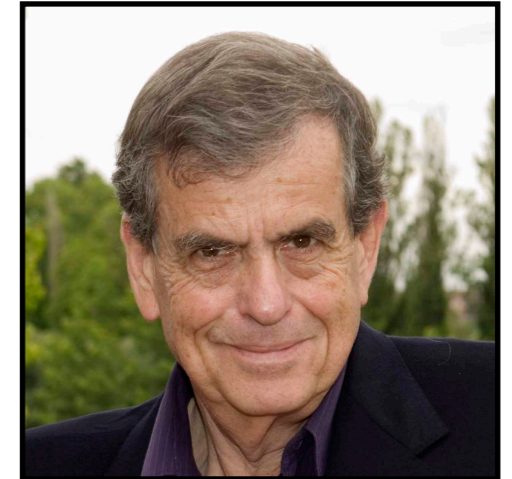
Irwin Rose

Fox Chase Cancer Center



Avram Hershko

Technion - Israel Institute
of Technology



Aaron Ciechanover

Technion - Israel Institute
of Technology

“For the discovery of Ubiquitin-mediated protein degradation”

The History of Ubiquitin

ATP-dependent conjugation of reticulocyte proteins with the polypeptide required for protein degradation

(protein/breakdown/energy requirement/covalent linkage of polypeptides)

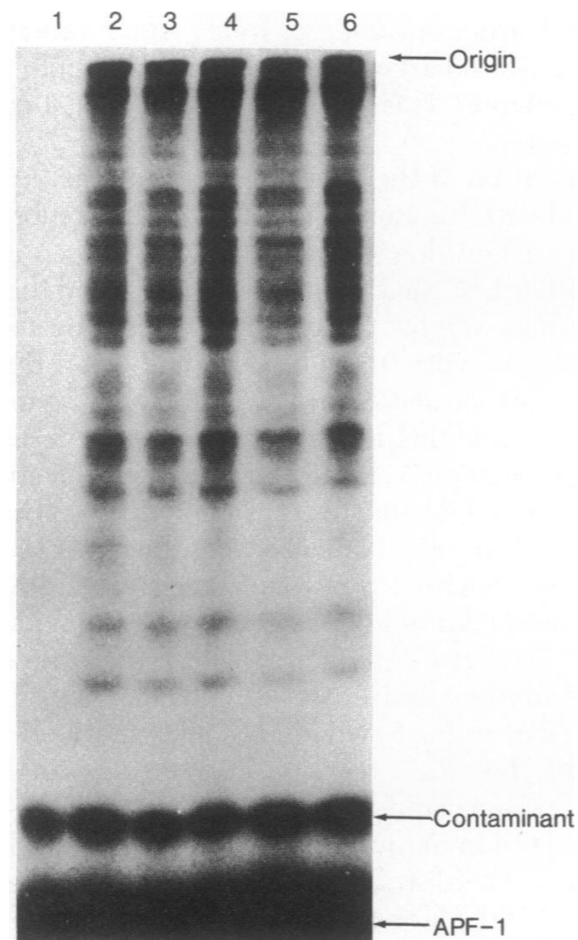
AARON CIECHANOVER*, HANNAH HELLER*, SARAH ELIAS*, ARTHUR L. HAAS†, AND AVRAM HERSHKO*§

*Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel; and †The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

Communicated by Irwin Rose, December 10, 1979

The History of Ubiquitin

ATP + radiolabeled-Ub + lysate fraction - SDS-PAGE



- 1) No ATP
- 2) Complete reaction
- 3-6) SDS + BME + Heat (different concentrations / durations of heating)

■ **Observation:** Multiple bands by SDS-Page

■ **Observation:** Linkage is stable under denaturing conditions

Conclusion: Suggests covalent bond between ubiquitin and multiple different proteins

The History of Ubiquitin

Proposed role of ATP in protein breakdown: Conjugation of proteins with multiple chains of the polypeptide of ATP-dependent proteolysis

(protein/turnover/energy dependence/isopeptide linkage)

AVRAM HERSHKO*, AARON CIECHANOVER*, HANNAH HELLER*, ARTHUR L. HAAS, AND IRWIN A. ROSE

The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

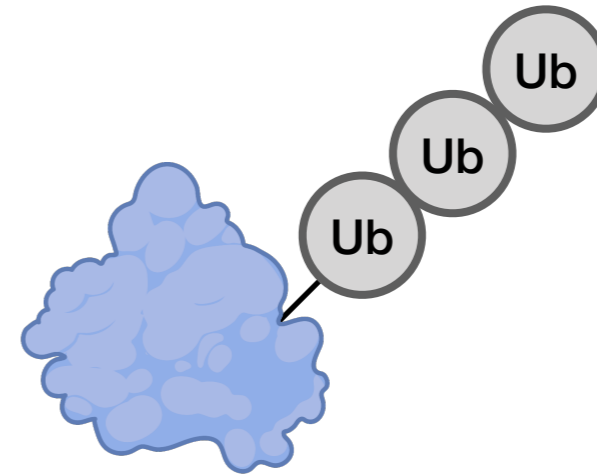
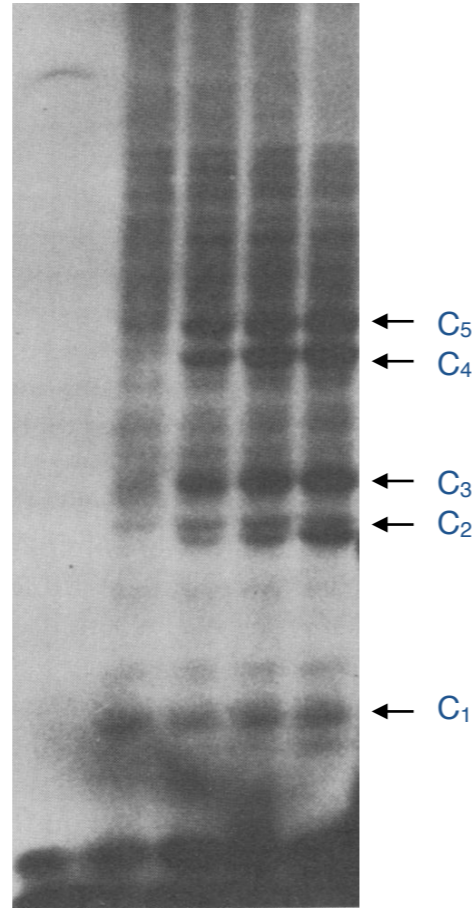
Contributed by Irwin Rose, December 10, 1979

“A sequence of reactions in which the linkage of [ubiquitin] to the substrate is followed by the proteolytic breakdown of the substrate is proposed.”

The History of Ubiquitin

ATP + radiolabeled-Ub + lysate fraction - SDS-PAGE

ATP	-	+	+	+	+
With unlabeled lysozyme	-	-	+	+	+



Conclusion:
Ubiquitin forms multiple conjugates
on protein substrates

- The linkage between ubiquitin and the protein substrate is stable to hydroxylamine and alkali (amide bond!)
- Removal of ATP after formation of ubiquitin conjugates caused the regeneration of ubiquitin (deubiquitinases!)

The History of Ubiquitin

The Discovery of E1

Activation of the heat-stable polypeptide of the ATP-dependent proteolytic system

(ubiquitin/adenylate/thiolester/high-energy bond)

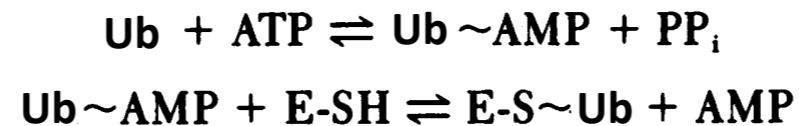
AARON CIECHANOVER, HANNAH HELLER, RACHEL KATZ-ETZION, AND AVRAM HERSHKO*

Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

Communicated by Irwin Rose, October 17, 1980

“We now describe an enzyme that carries out the activation by ATP of the polypeptide with pyrophosphate displacement.”

In the presence of ATP, “E1” catalyzes the following reaction:



The History of Ubiquitin

The Discovery of E1

Activation of the heat-stable polypeptide of the ATP-dependent proteolytic system

(ubiquitin/adenylate/thiolester/high-energy bond)

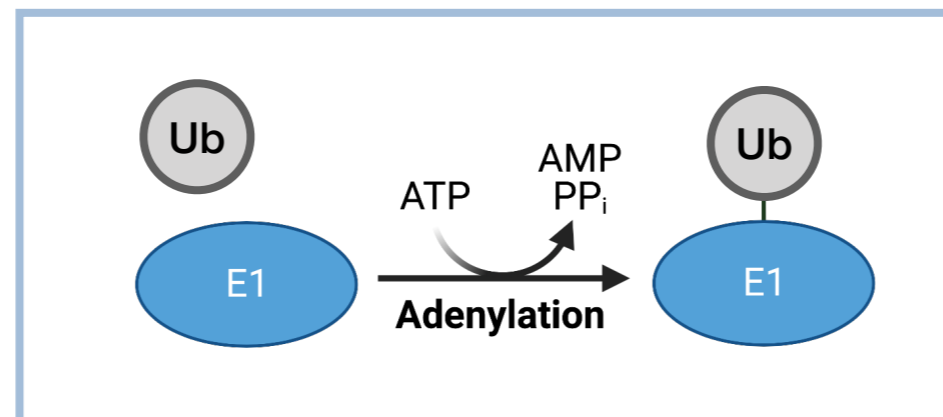
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In the presence of ATP, “E1” catalyzes the following reaction:



The History of Ubiquitin

The Discovery of E2 and E3

Components of Ubiquitin-Protein Ligase System

RESOLUTION, AFFINITY PURIFICATION, AND ROLE IN PROTEIN BREAKDOWN*

(Received for publication, December 27, 1982)

Avram Hershko[‡], Hannah Heller, Sarah Elias, and Aaron Ciechanover

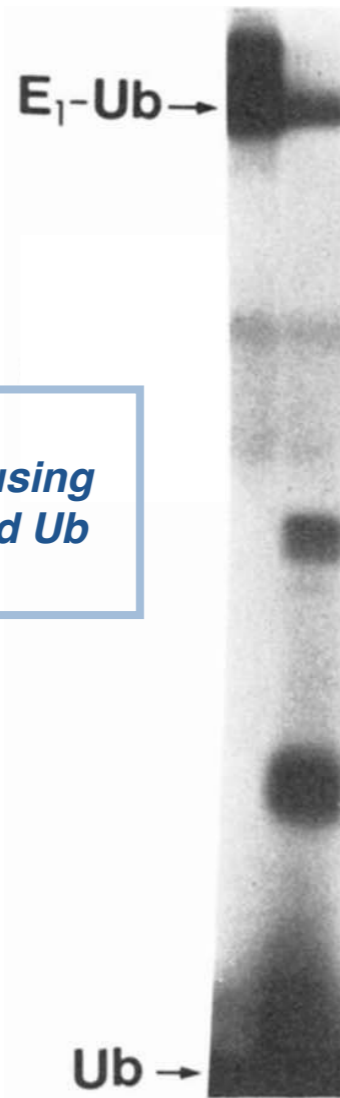
From the Unit of Biochemistry, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

By affinity chromatography of a crude reticulocyte extract on ubiquitin-Sepharose, three enzymes required for the conjugation of ubiquitin with proteins have been isolated. One is the ubiquitin-activating enzyme (E_1), which is covalently linked to the affinity column in the presence of ATP and can be specifically eluted with AMP and pyrophosphate (Ciechanover, A., Elias, S., Heller, H., and Hershko, A. (1982) *J. Biol. Chem.* 257, 2537–2542). A second enzyme, designated E_2 , is bound to the ubiquitin column when E_1 and ATP are present, and is eluted with a thiol compound at high concentration. The third enzyme, designated E_3 , is adsorbed to the affinity column by noncovalent interactions and can be eluted with high salt or increased pH. The presence of all three enzymes is absolutely required for the conjugation of ^{125}I -ubiquitin with proteins. All three affinity-purified enzymes are also required for the breakdown of ^{125}I -albumin to acid-soluble material in the presence of ubiquitin, ATP, and the unadsorbed fraction of the affinity column.

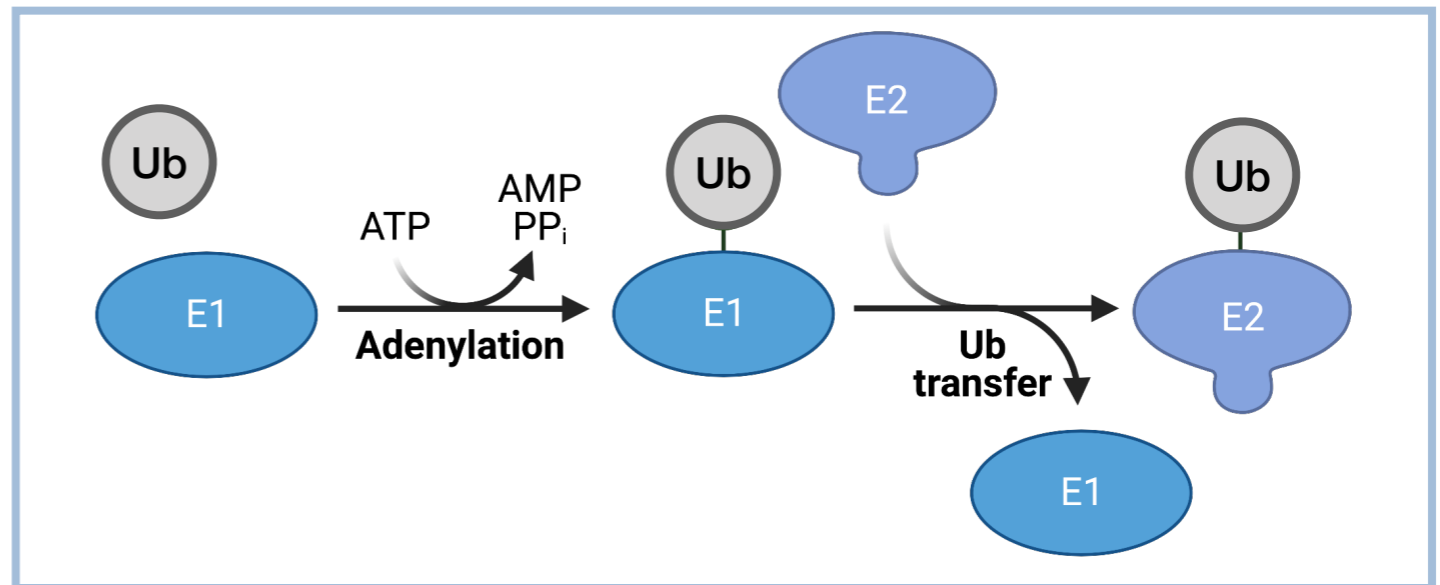
The History of Ubiquitin

The Discovery of E2 and E3

E1	+	+
E2	-	+
E3	-	-
Substrate	+	+



SDS-PAGE using
Radiolabeled Ub

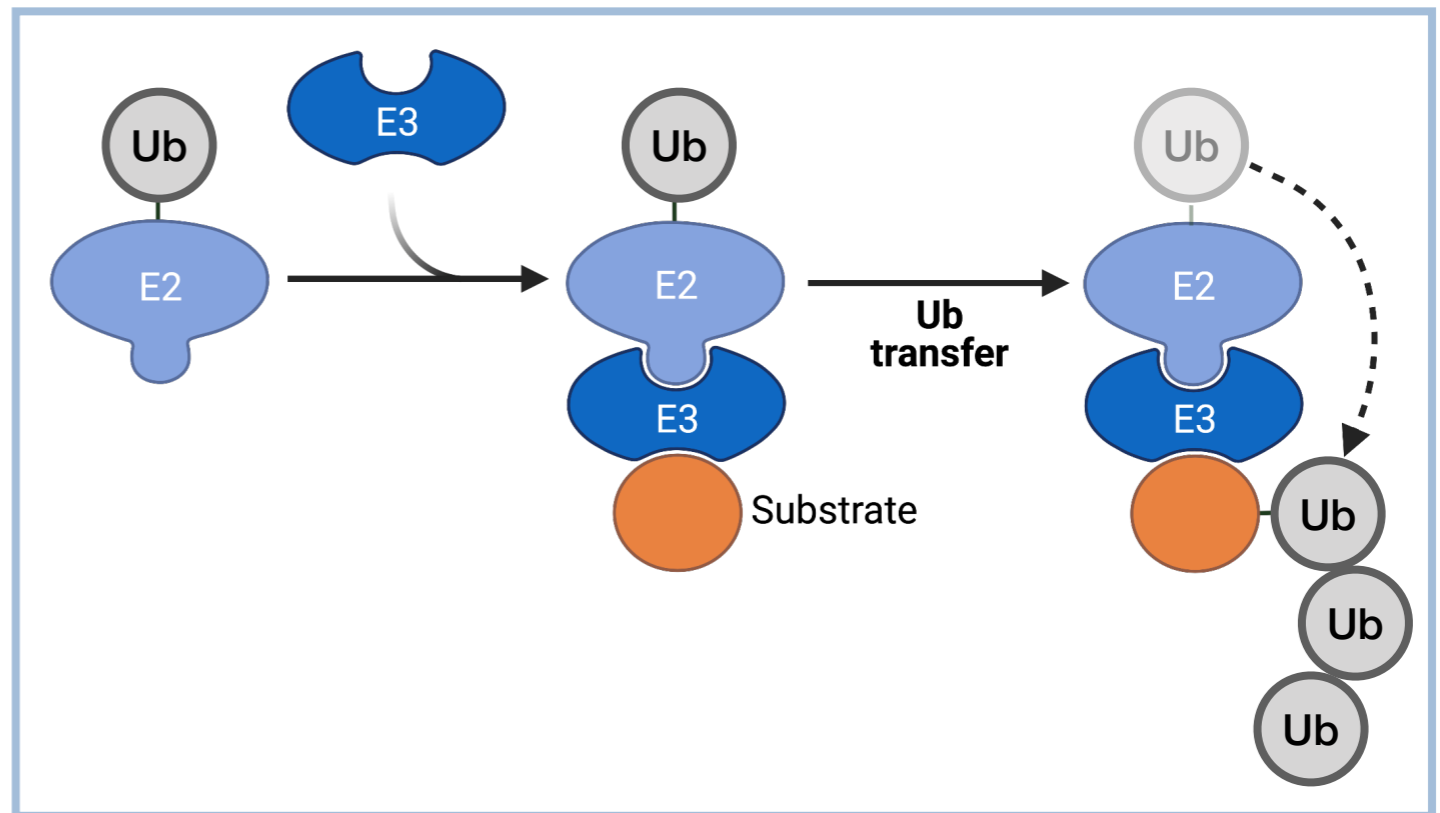
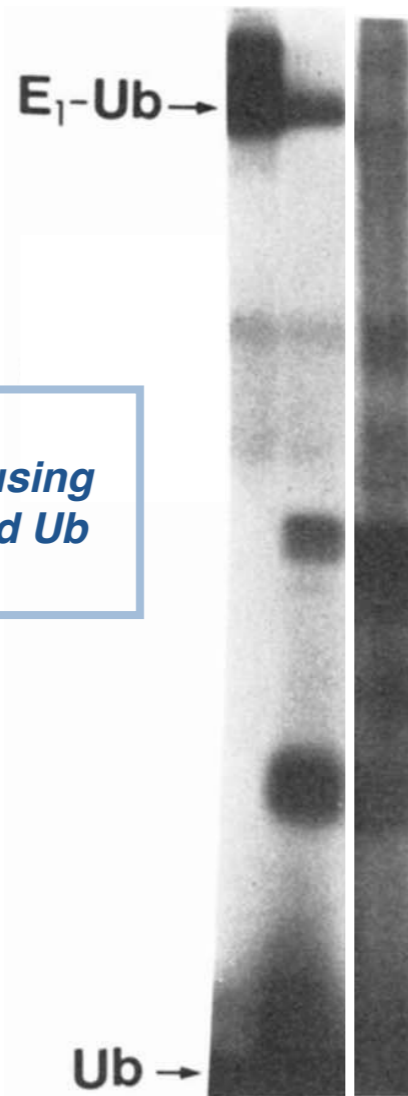


Transfer of radiolabeled Ub to E2 observed with E2 addition

The History of Ubiquitin

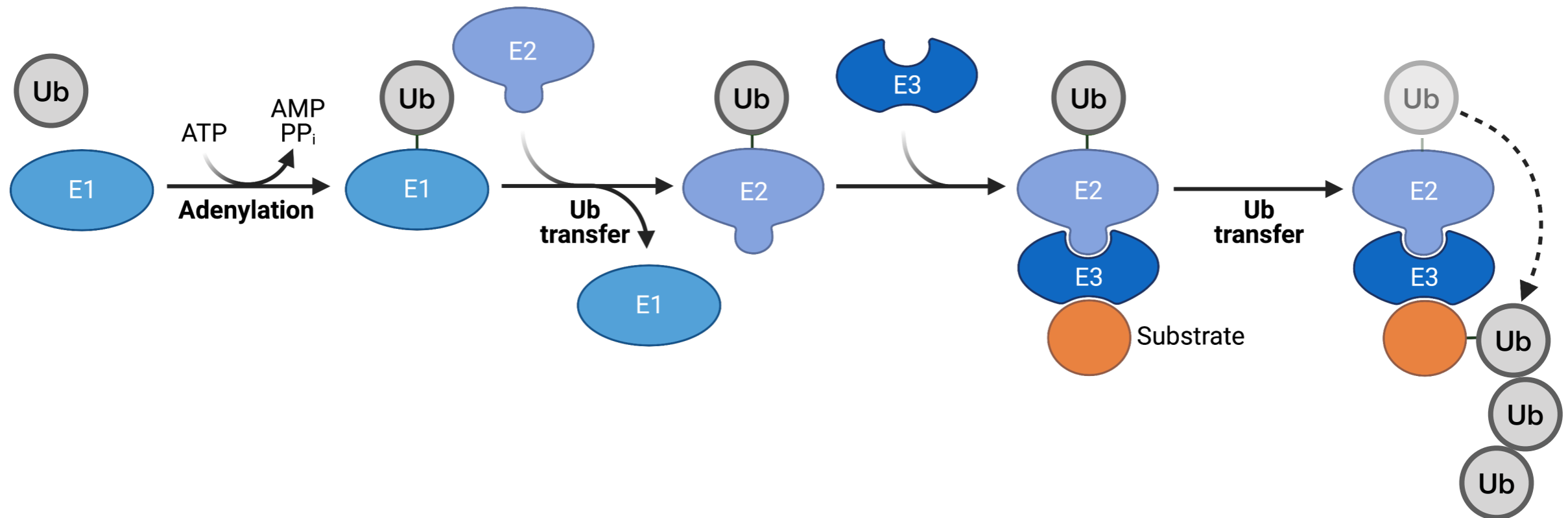
The Discovery of E2 and E3

E1	+	+	+
E2	-	+	+
E3	-	-	+
Substrate	+	+	+



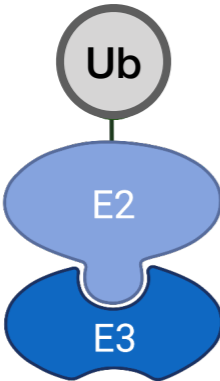
Numerous Ub-bands observed with E3 addition

Full Mechanism of Substrate Ubiquitination



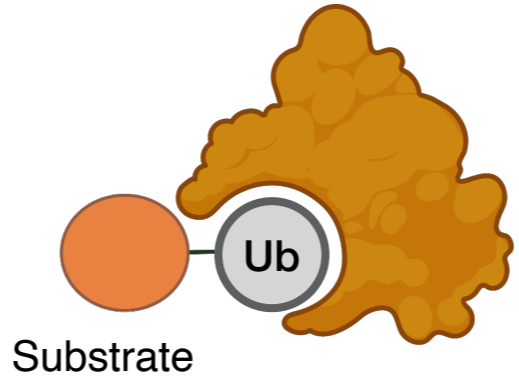
Deubiquitinases (DUBs)

Writers



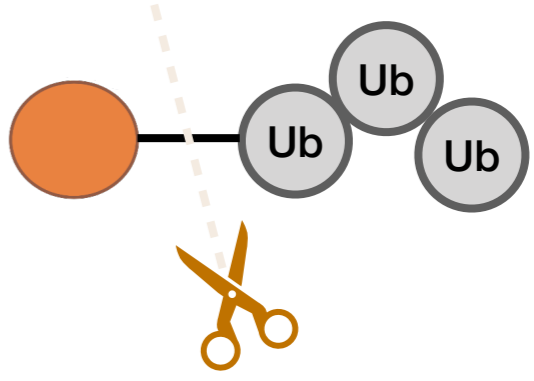
E2 enzyme / E3 ligase complex

Readers



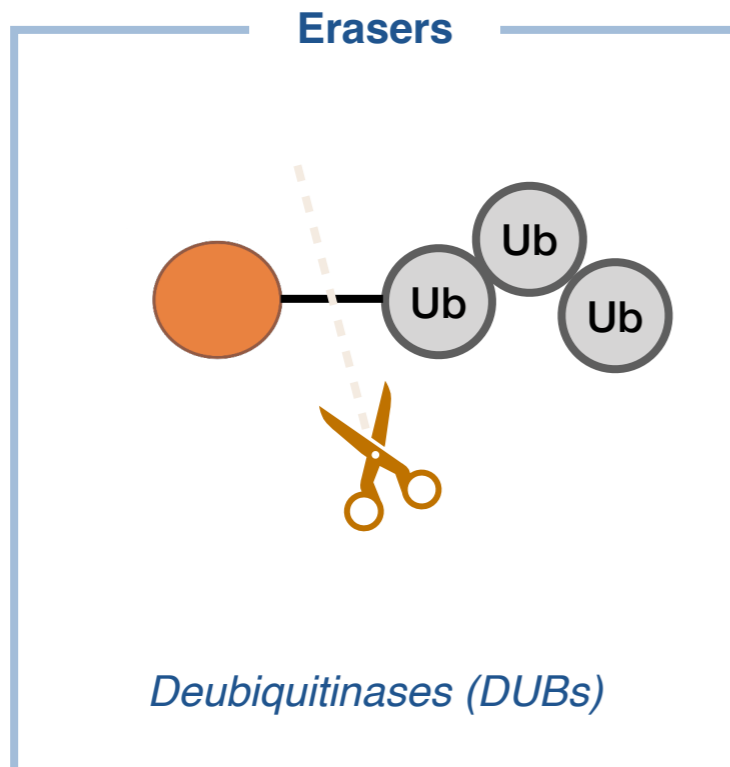
Proteins with Ub-binding domains

Erasers



Deubiquitinases (DUBs)

Deubiquitinases (DUBs)



Class I: Cysteine proteases

Ub-specific protease (USP)

Ub C-terminal protease (UCH)

Machado-Josephin protease (MJD)

Ovarian tumor protease (OTU)

Class II: Metalloproteases

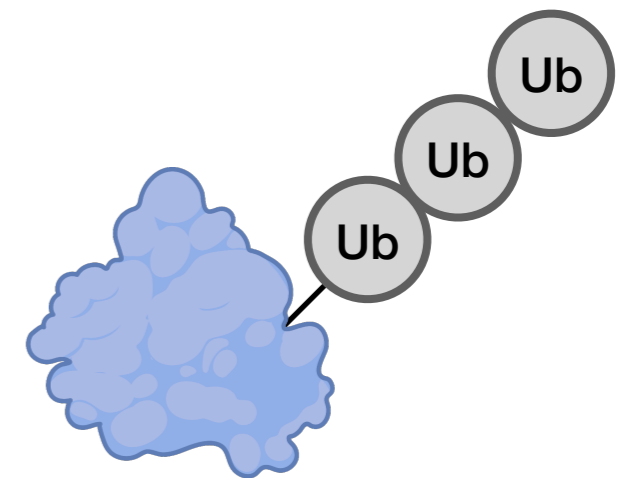
Jab1/Mov34/Mpr1 protease (JAMM)

Approximately 100 DUB genes in humans

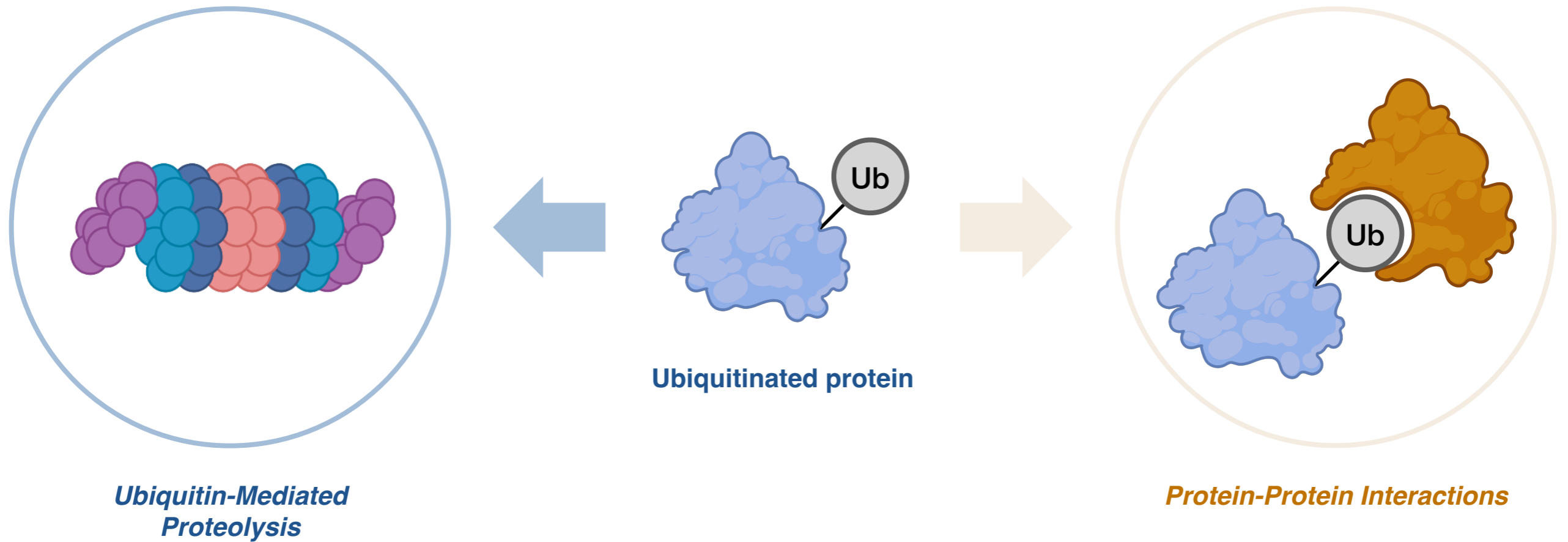
Deubiquitinases can hydrolyze ubiquitin conjugates from proteins

The Ubiquitin Code

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- Therapeutic outlook



The Ubiquitin Code



Question: When does ubiquitin enable protein-degradation, a protein-interaction, or another process?

The Ubiquitin Code: Linkage Type

Additional ubiquitin proteins can be conjugated to lysine on ubiquitin

Points of Attachment

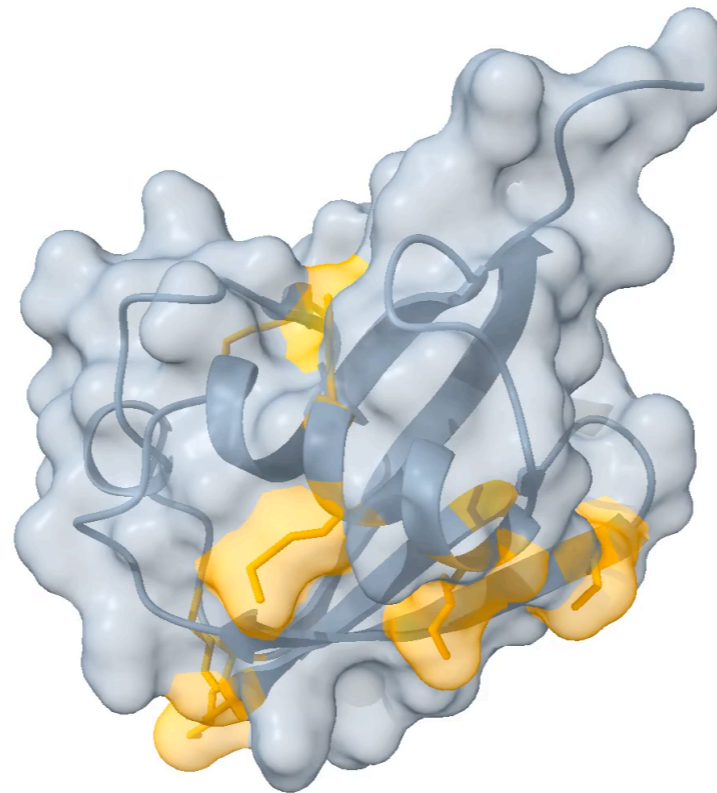
M1 (N-term)

K6

K11 K33

K27 K48

K29 K63



All 8 Ub linkages have been detected in cells

Ubiquitin peptide sequence

MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**AKIQD**K**EGIPPD
QQRLIFAG**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRRLRGG

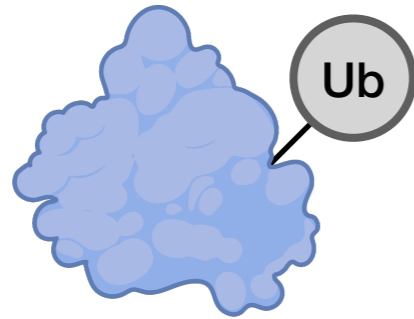
The Ubiquitin Code: Linkage Type

Linkage	Associated Function
K48 K11	Degradation to the 26S Proteasome
M1 K63	Can induce protein-interactions; DNA repair and transcription factor activation
K6	Mitophagy
K27	Innate Immunity from viral and bacterial infection
K29	Signaling and neurodegenerative disorders
K33	Non-degradative

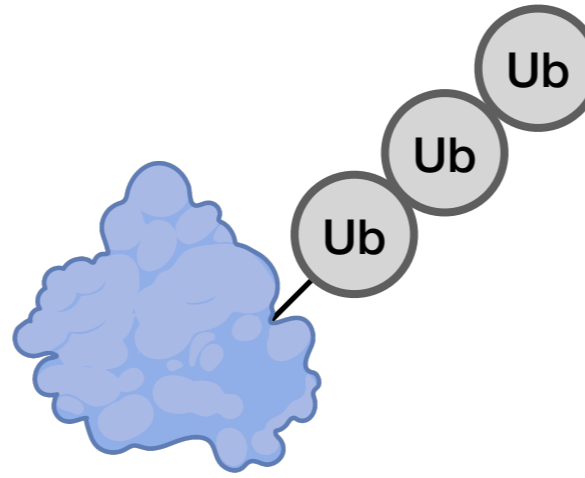
Less Characterized

Type of ubiquitin attachment can determine function

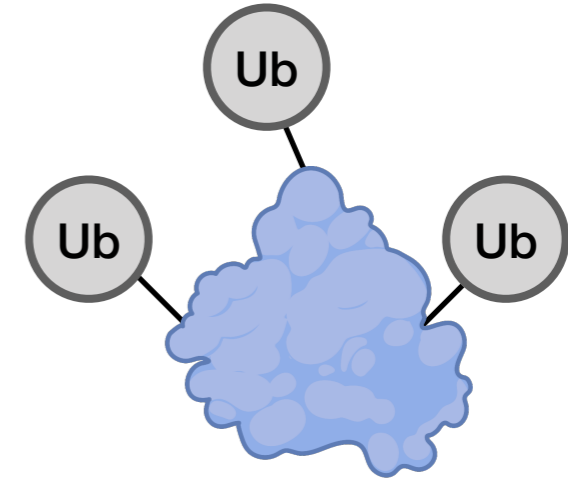
Types of Ubiquitin Chains



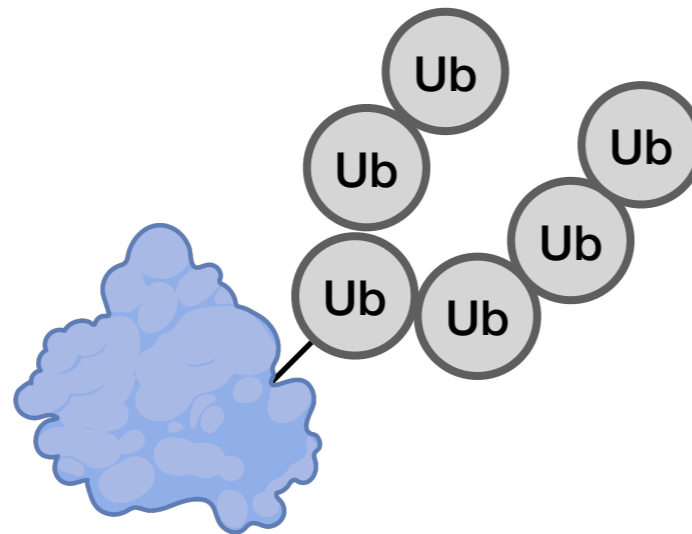
Monoubiquitination



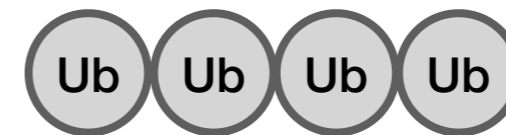
Linear ubiquitin chain



Multimonoubiquitination



Branched ubiquitin chain

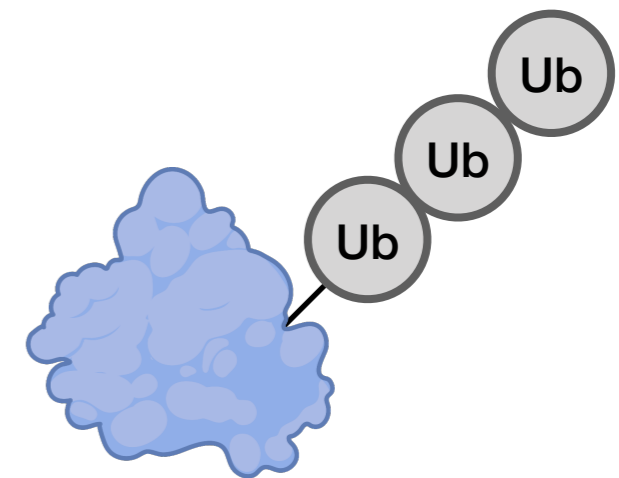


Unanchored ubiquitin chain

Number of ubiquitins on a protein can also determine function

The Ubiquitin Code

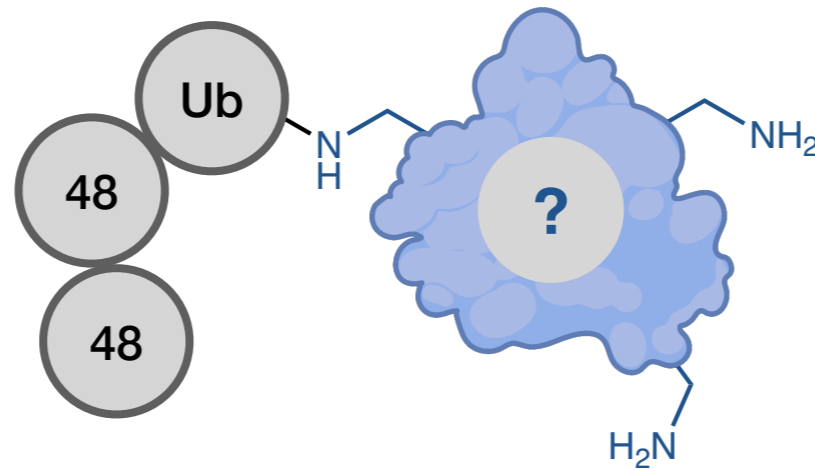
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Methods to Study Covalent Modifications by Ubiquitin


How can we identify

1) ubiquitinated substrates, 2) the site of ubiquitination, and 3) the type of Ub-chain?



Methods to Study Covalent Modifications by Ubiquitin

Trypsin

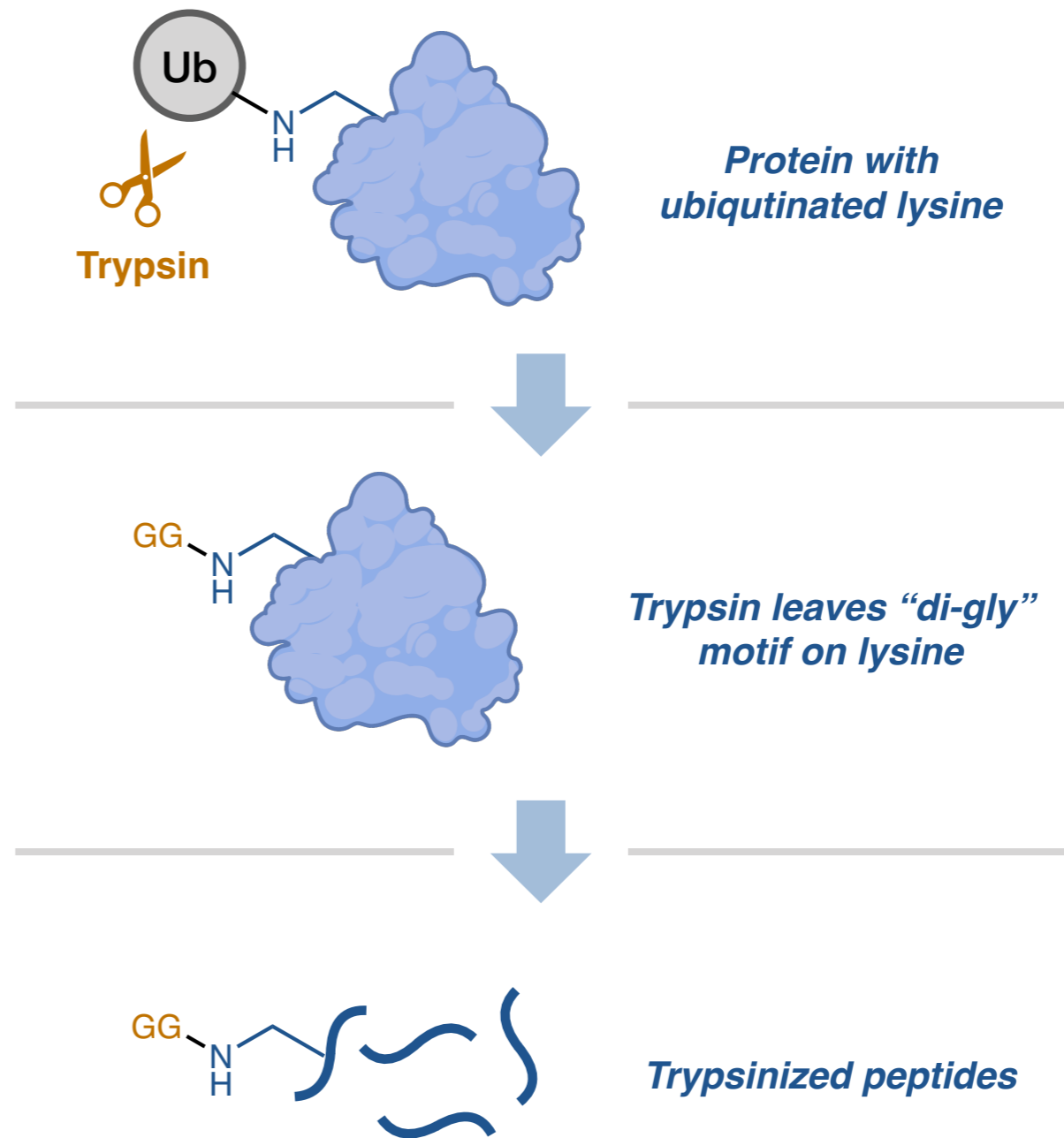


cleaves peptides after lysine (K) and arginine (R)

Ubiquitin Peptide Sequence

MQIFVKTLTGKTITLEVEPSDTIENV
KAKIQDKEGIPPDQQRLIFAGKQLE
DGRTLSDYNIQKESTLHLVLRRL**RGG**

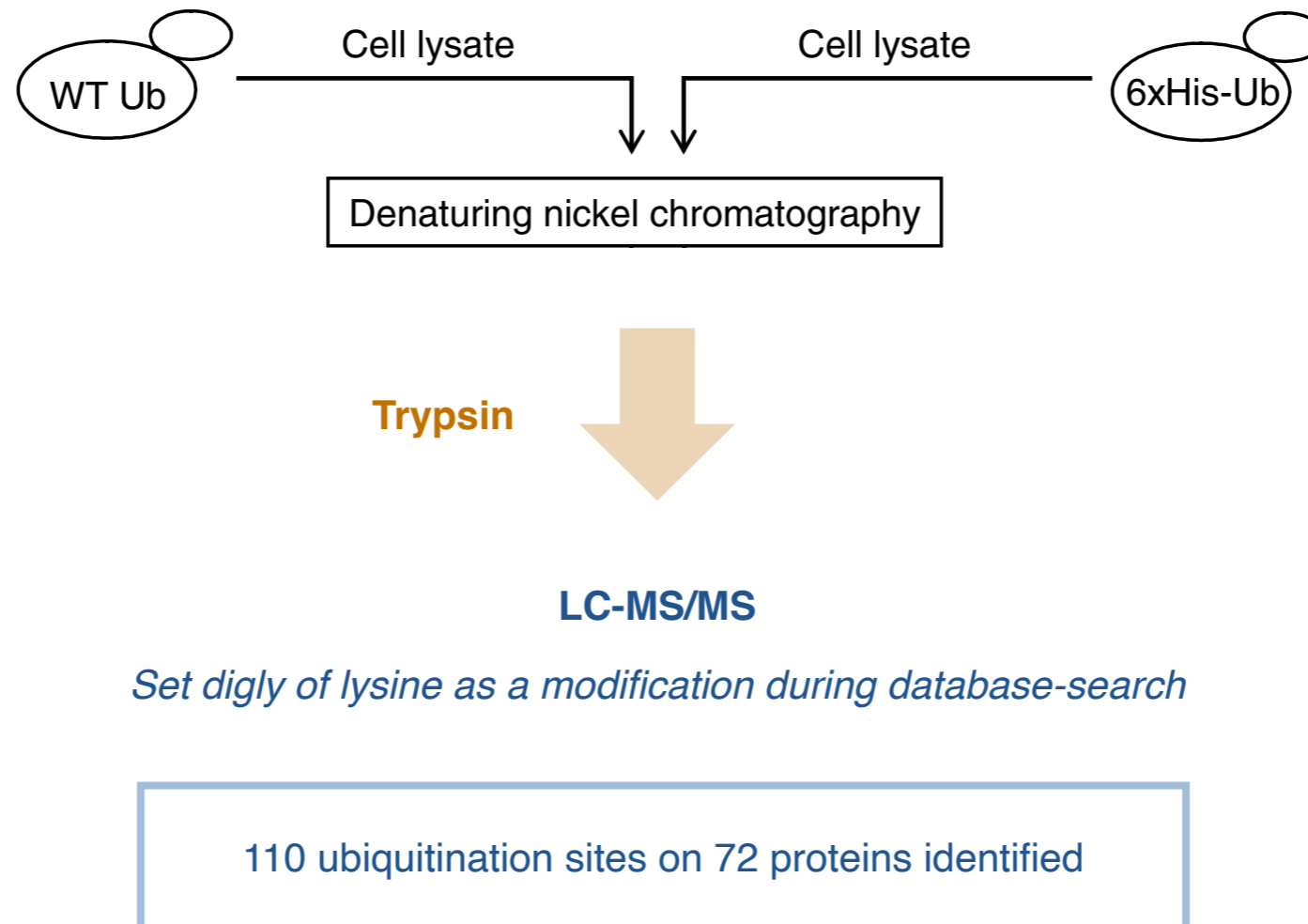
GG = 114.1 Da mass shift



Di-gly modification on lysine is identified via MS/MS

Methods to Study Covalent Modifications by Ubiquitin

Proteomics-based identification of ubiquitination sites by the Gygi Lab



Set digly of lysine as a modification during database-search

Limitation: *Requires expression of His-tag at N-terminus of ubiquitin*

Methods to Study Covalent Modifications by Ubiquitin

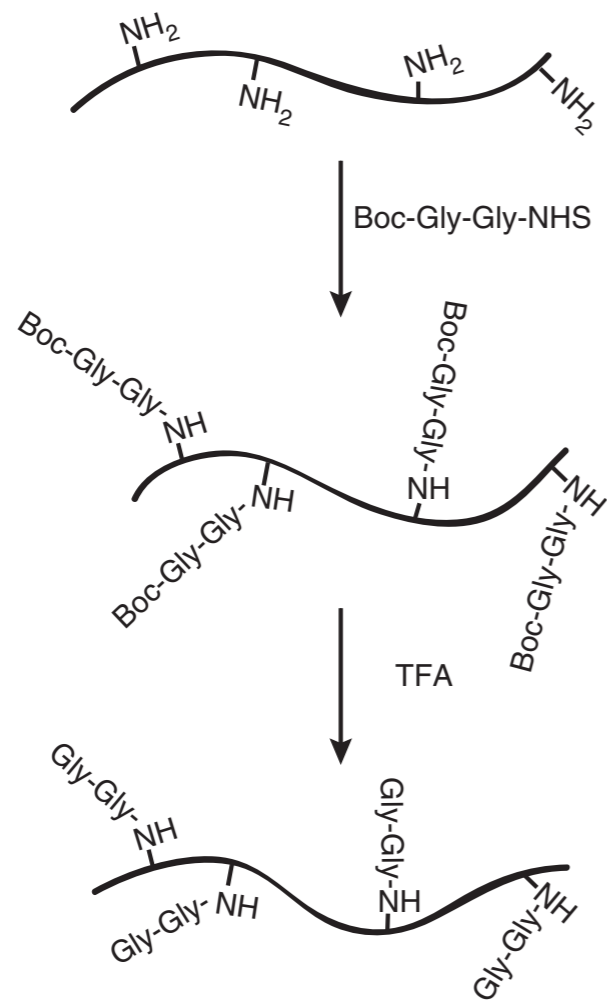
Ubiquitin-Remnant Profiling

Problem: How to identify ubiquitinated peptides without expressing N-terminally tagged ubiquitin?

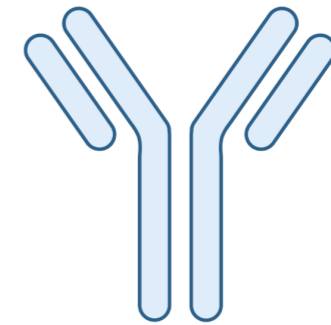
Methods to Study Covalent Modifications by Ubiquitin

Ubiquitin-Remnant Profiling

Preparation of digly-K antigen



Monoclonal antibody
generation



anti-digly antibody

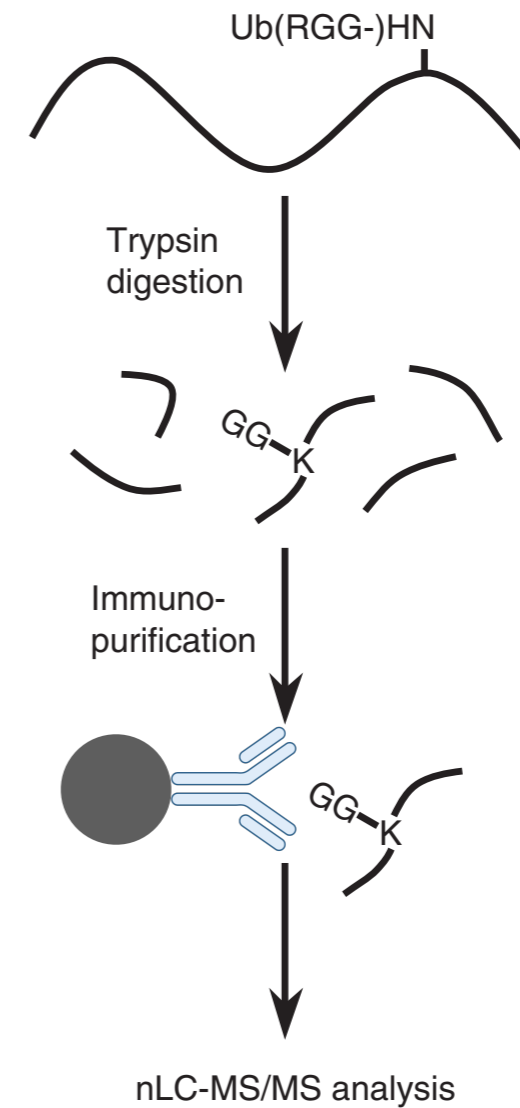
recognizes digly-conjugated lysine

Methods to Study Covalent Modifications by Ubiquitin

Ubiquitin-Remnant Profiling

Advantage

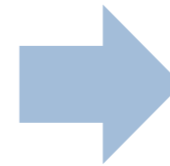
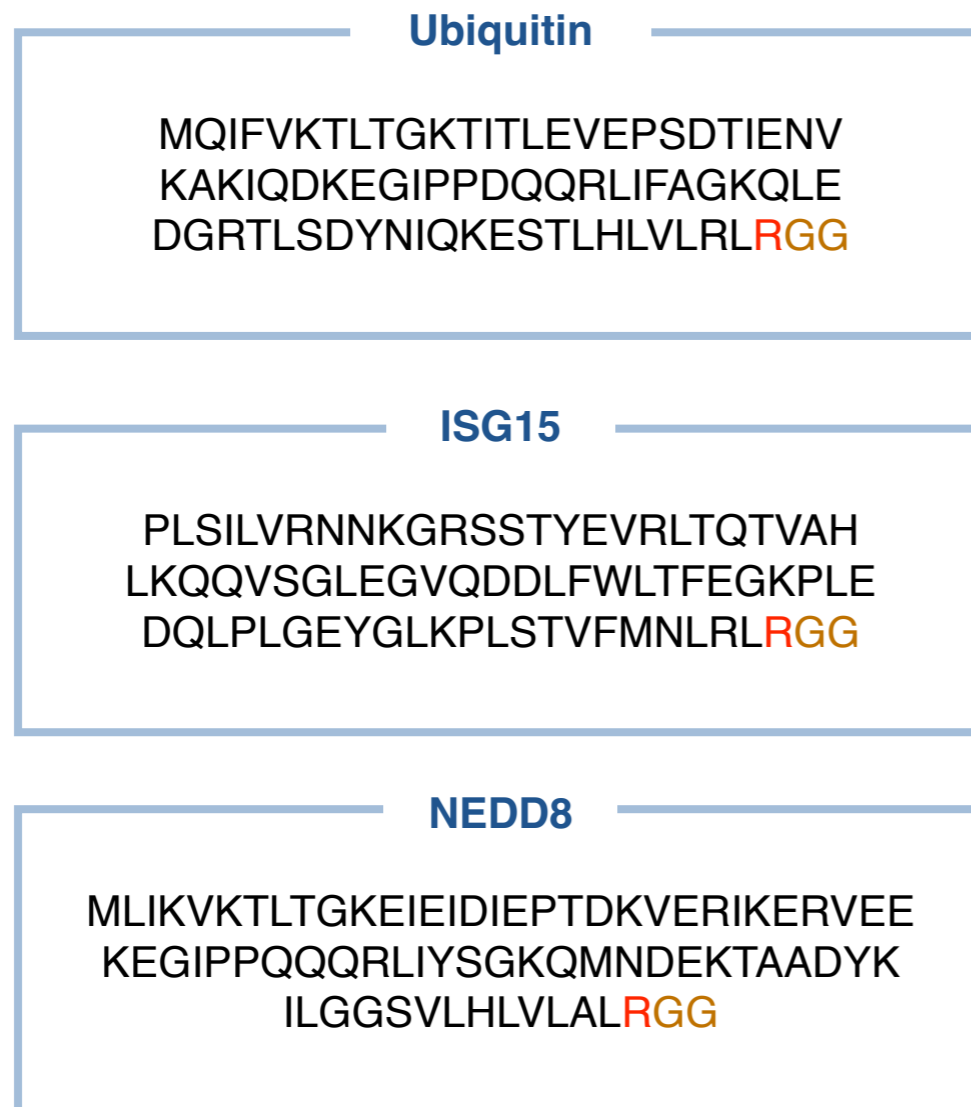
Enables use of lysate as input
(no expression of epitope-tagged ubiquitin)



Identified 374 diglycine-modified lysines on 236 ubiquitinated proteins

Methods to Study Covalent Modifications by Ubiquitin

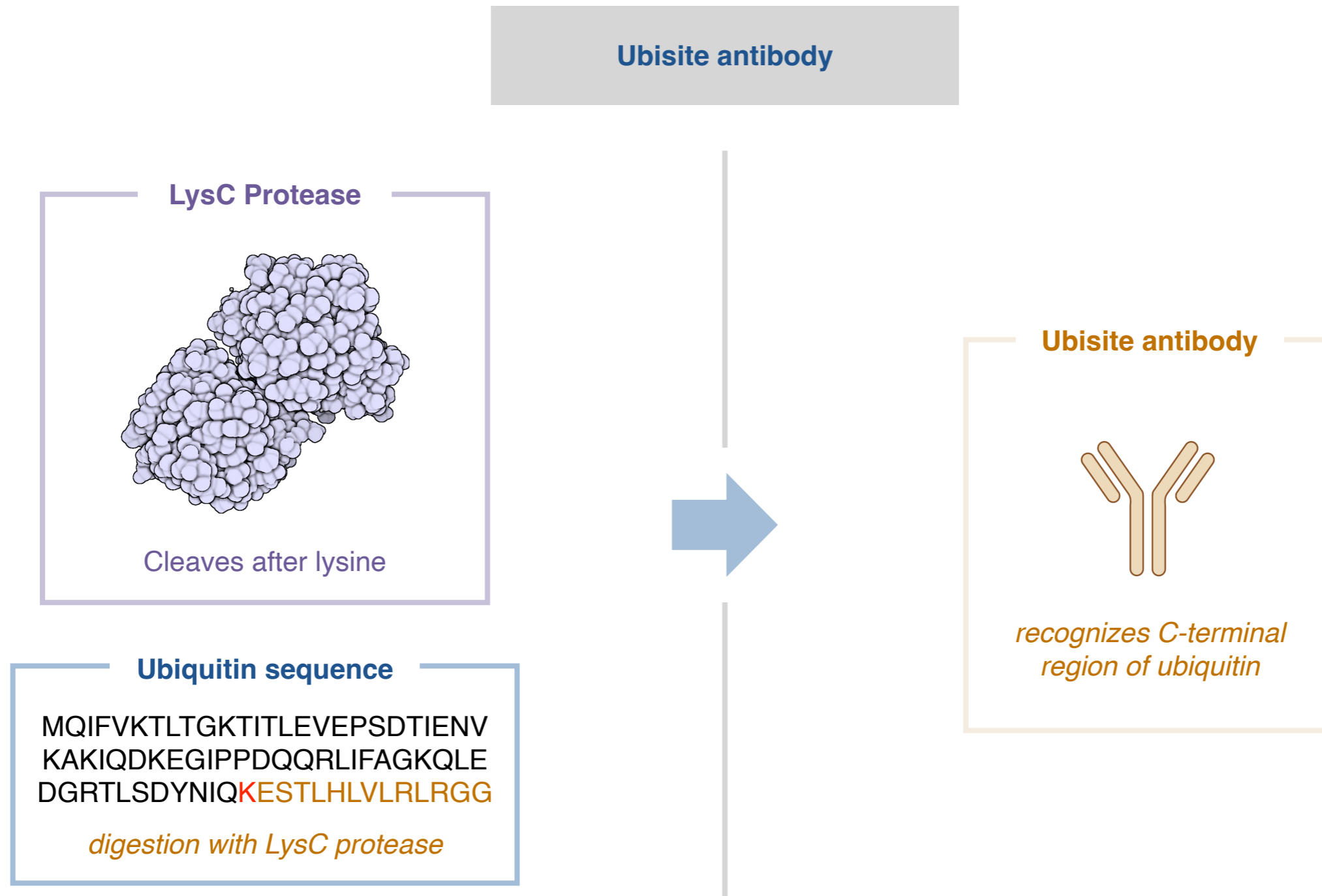
Ubiquitin-Remnant Profiling



Diglycine motif is also generated by lysine modified with ISG15 and NEDD8

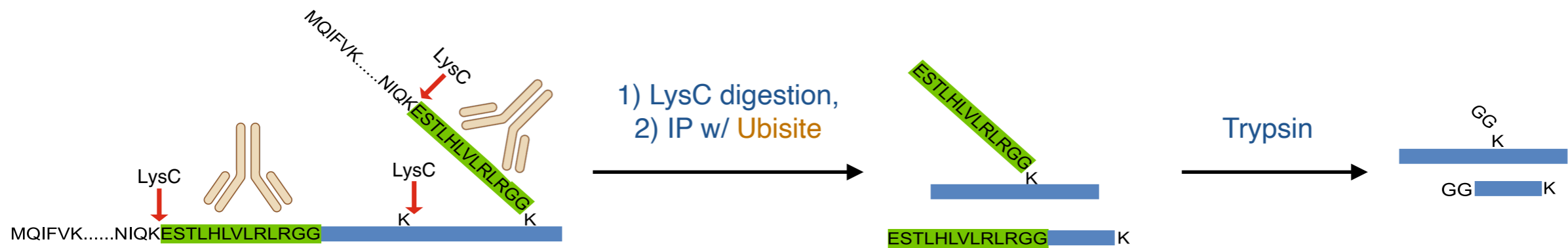
Limitation: Ubiquitin-like proteins (ISG15 and NEDD8) generate the same remnant

Methods to Study Covalent Modifications by Ubiquitin



Methods to Study Covalent Modifications by Ubiquitin

Ubisite antibody

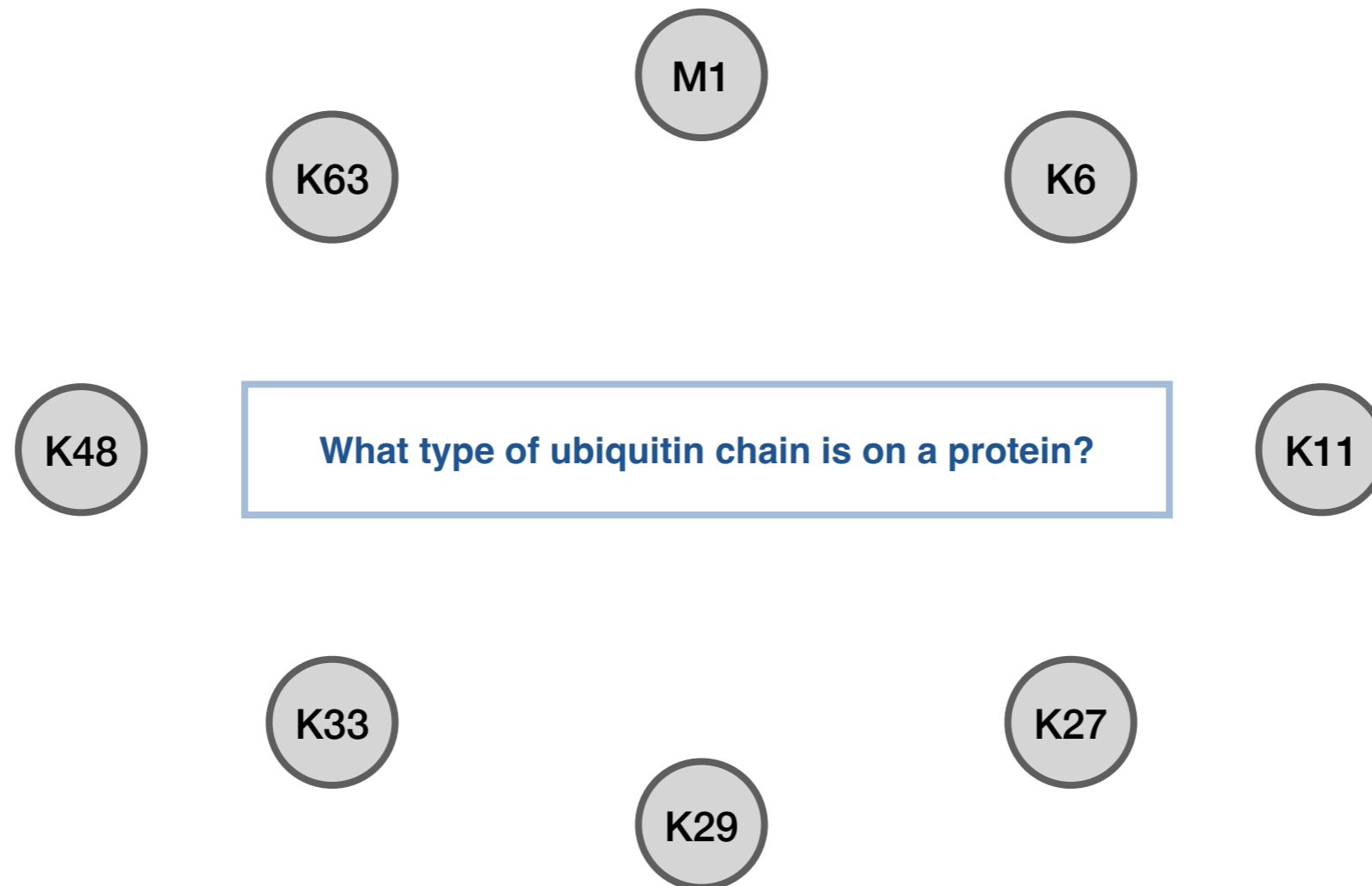


Advantage: Enrichment of ubiquitin-specific “di-gly” peptides

Advantage: Can be used for detection of N-terminal ubiquitination (No N-terminal tag)

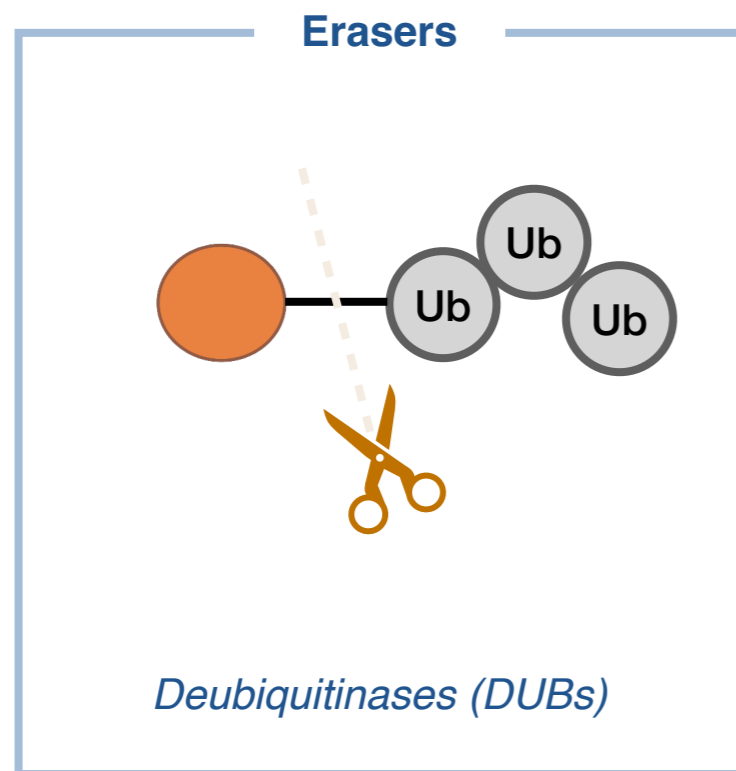
Results: 63,000 unique ubiquitination sites on 9200 proteins in two human cell lines

Methods to Study Covalent Modifications by Ubiquitin



Methods to Study Covalent Modifications by Ubiquitin

Ubicrest



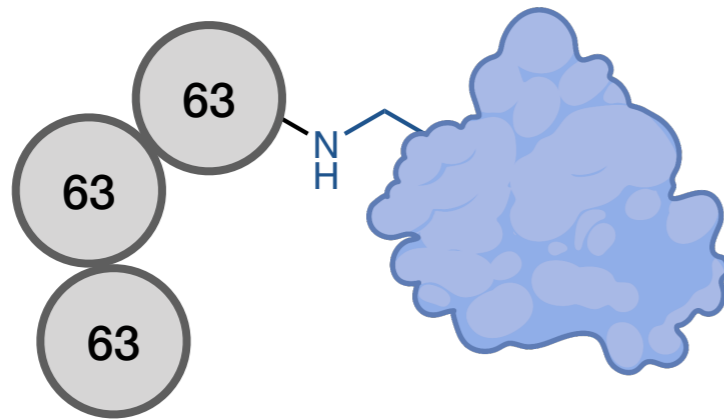
Approximately 100 DUB genes in humans

Ub linkages	DUBs (preferred Ub linkages)
Lys6	USP21 (unspecific)
Lys11	vOTU (unspecific, no Met1)
Lys27	OTUD3 (Lys6, Lys11)
Lys29	Cezanne (Lys11)
Lys33	OTUD2 (Lys11, Lys27, Lys29, Lys33)
Lys48	TRABID (Lys29, Lys33, Lys63)
Lys63	OTUB1 (Lys48)
Met1	OTUD1 (Lys63)
Substrate-/unbound	OTULIN (Met1)

Certain DUBs prefer deubiquitination of specific Ub linkages

Methods to Study Covalent Modifications by Ubiquitin

Ubicrest



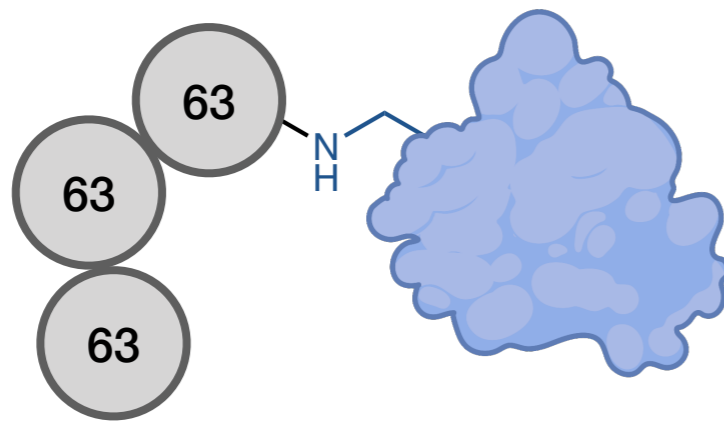
Example: K63-Ubiquitinated protein

Ub linkages	DUBs (preferred Ub linkages)
Lys6	USP21 (unspecific)
Lys11	vOTU (unspecific, no Met1)
Lys27	OTUD3 (Lys6, Lys11)
Lys29	Cezanne (Lys11)
Lys33	OTUD2 (Lys11, Lys27, Lys29, Lys33)
Lys48	TRABID (Lys29, Lys33, Lys63)
Lys63	OTUB1 (Lys48)
Met1	OTUD1 (Lys63)
Substrate-/unbound	OTULIN (Met1)

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Methods to Study Covalent Modifications by Ubiquitin

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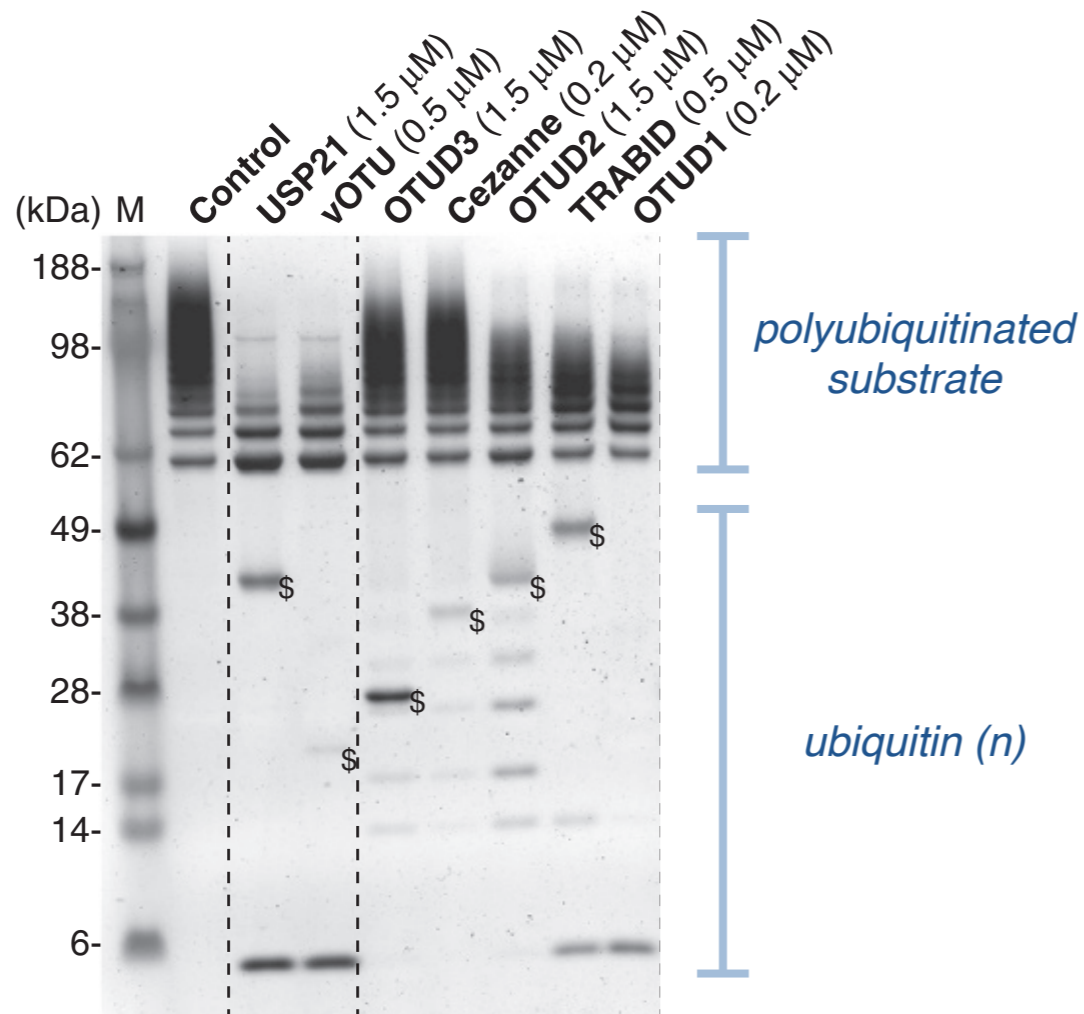
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Methods to Study Covalent Modifications by Ubiquitin

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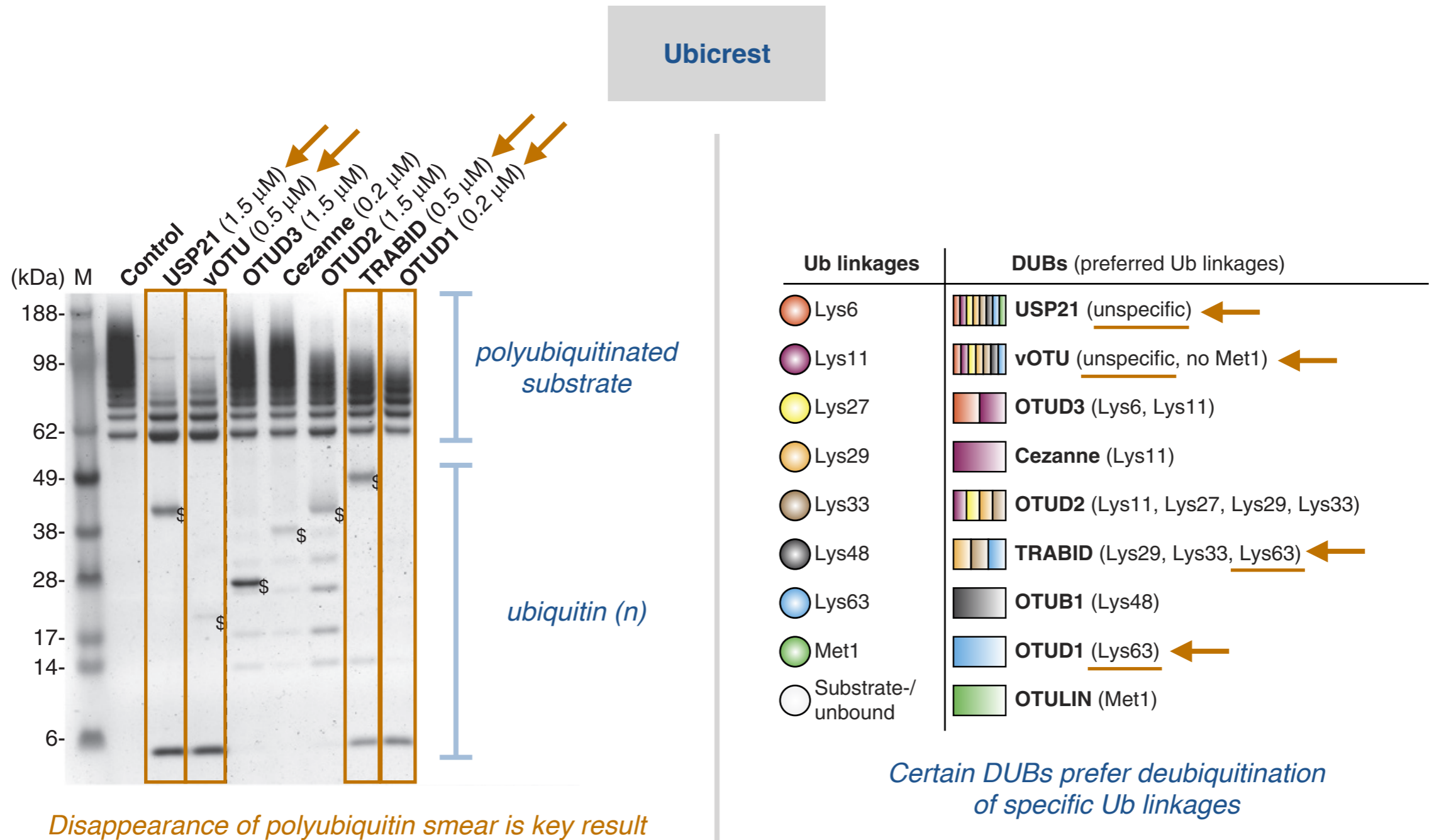


Disappearance of polyubiquitin smear is key result

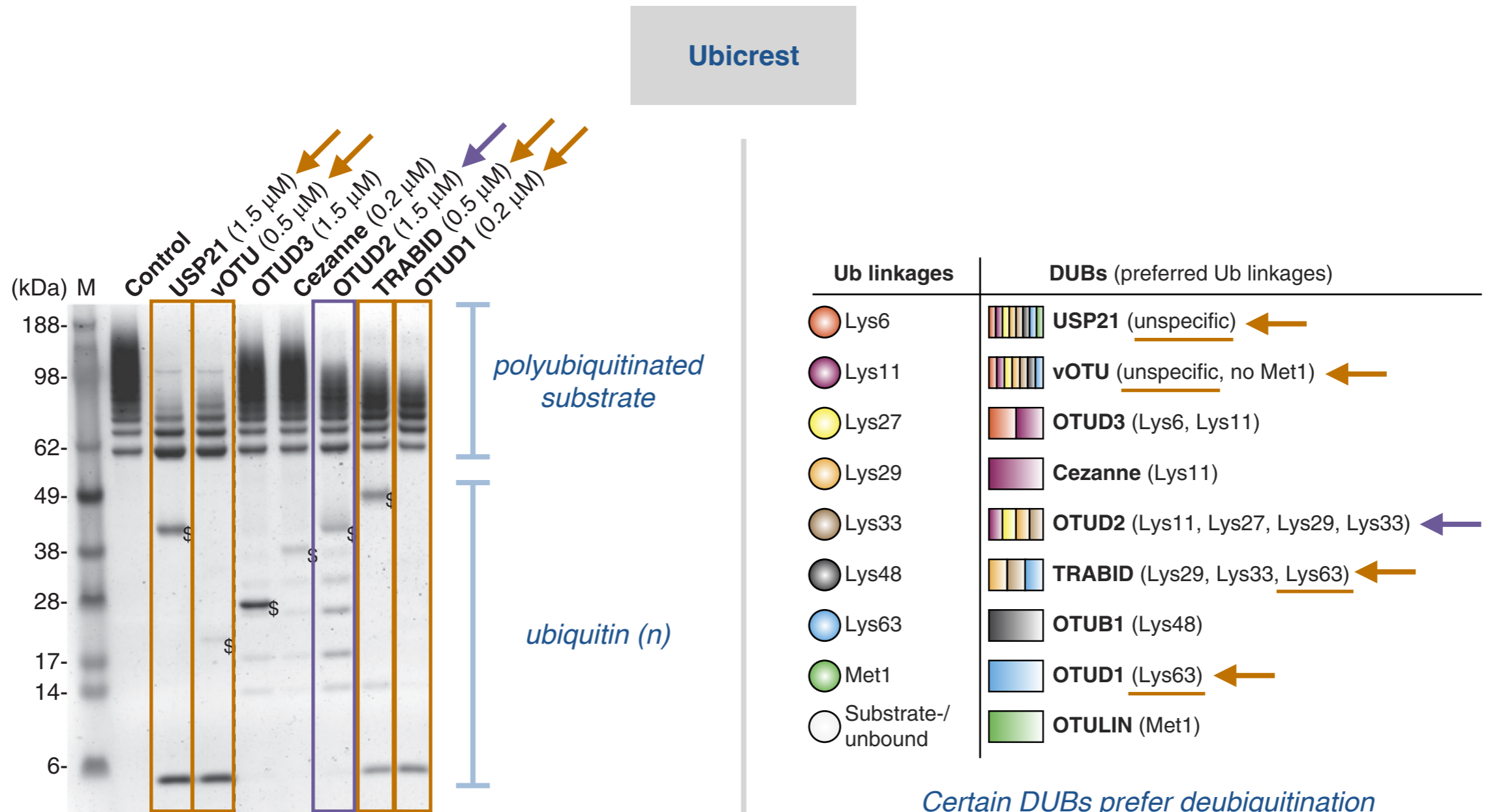
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Methods to Study Covalent Modifications by Ubiquitin



Methods to Study Covalent Modifications by Ubiquitin



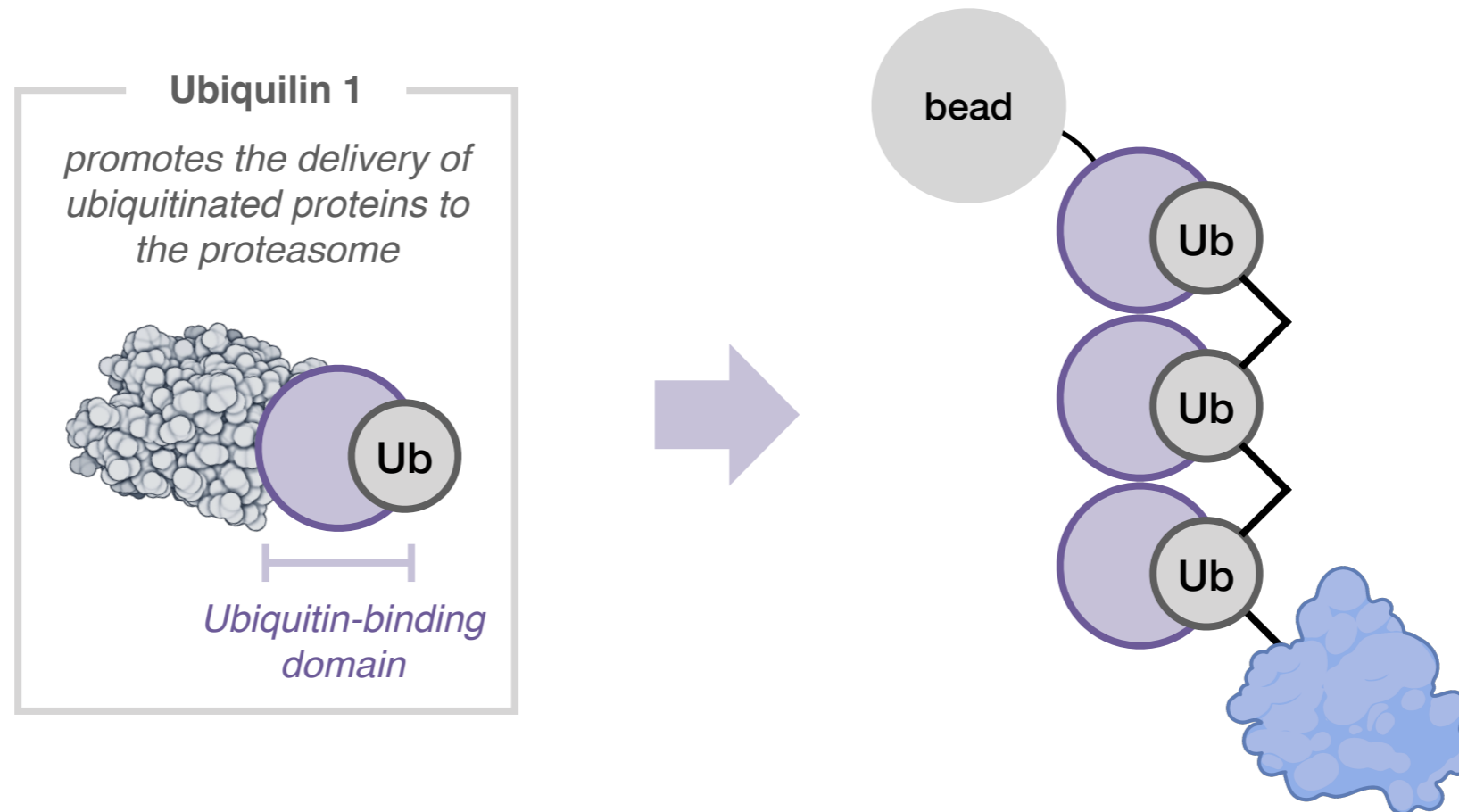
Disappearance of polyubiquitin smear is key result

Some deubiquitinases can behave nonspecifically to release longer chains

Certain DUBs prefer deubiquitination of specific Ub linkages

Methods to Study Covalent Modifications by Ubiquitin

TUBES (Tandem-Repeated Ubiquitin-Binding Entities)

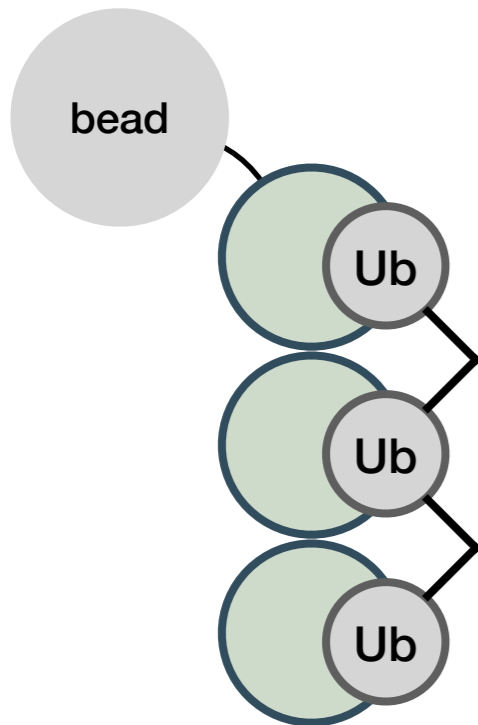


Advantages: *Protects ubiquitin sites from proteasomal degradation and deubiquitinase cleavage*

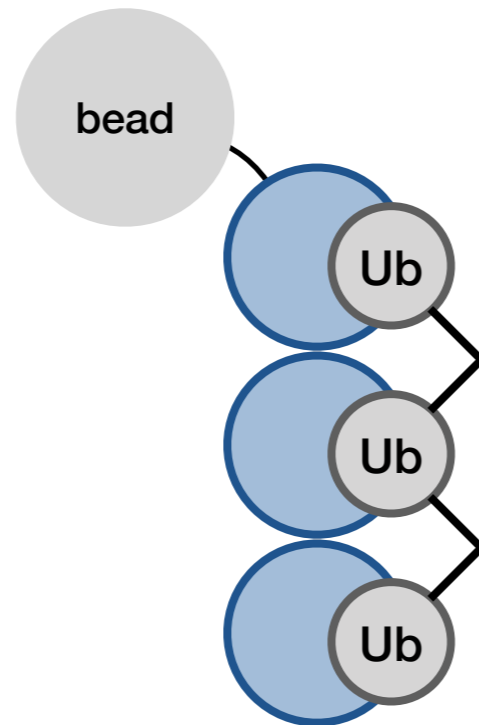
Methods to Study Covalent Modifications by Ubiquitin

TUBES (Tandem-Repeated Ubiquitin-Binding Entities)

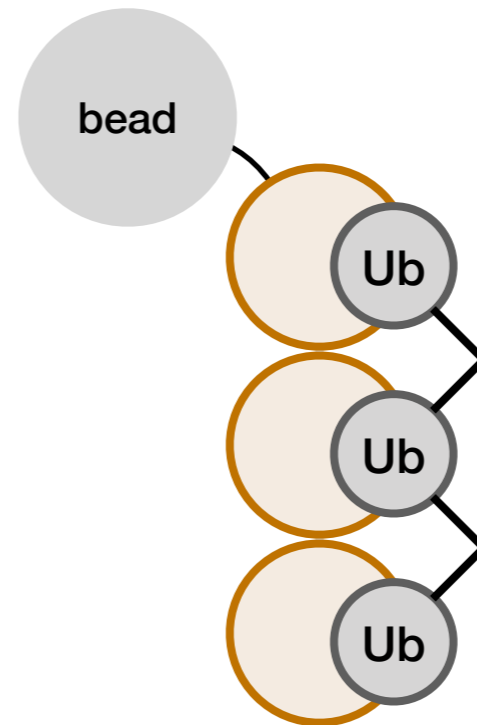
M1-selective



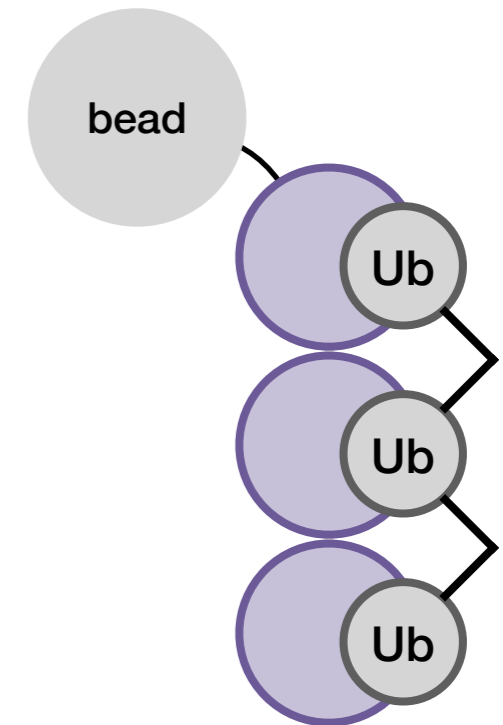
K48-selective



K63-selective



Pan-selective

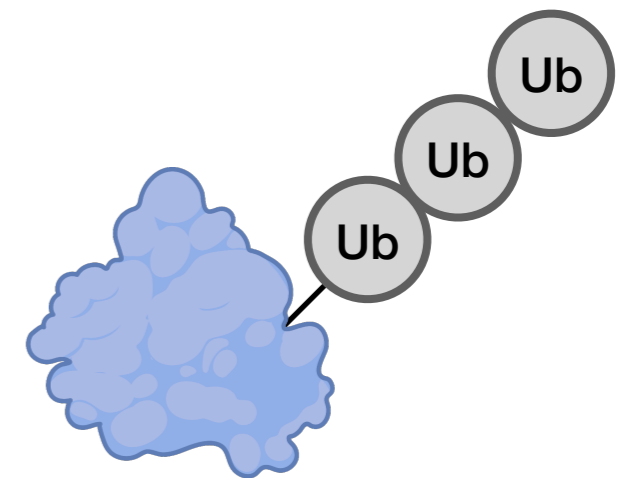


Can be selective for specific ubiquitin conjugates

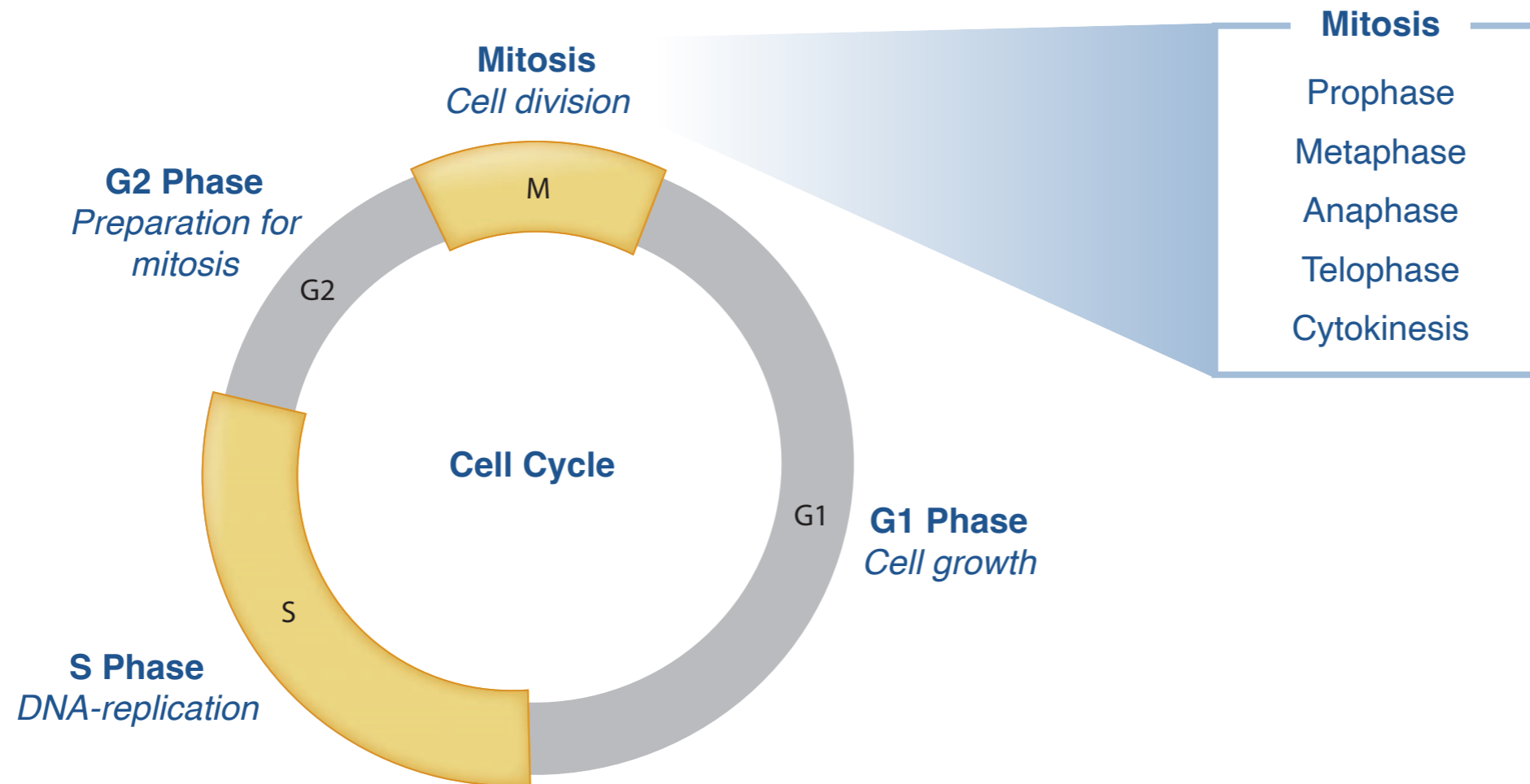
Commercially available

The Ubiquitin Code

- The History of Ubiquitin and How Substrates are Ubiquitinated
- The Ubiquitin Code
- Methods to Study Covalent Modifications by Ubiquitin
- How the Cell uses Ubiquitin for Regulation
 - Cell cycle / The APC/C
 - Transcription
 - DNA damage response
 - Protein-localization
- Therapeutic outlook



Ubiquitin-Mediated Regulation: Control of the Cell-Cycle



Cell-cycle checkpoints are critical for inhibiting cell growth

Deregulation of this process can lead to cancer

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

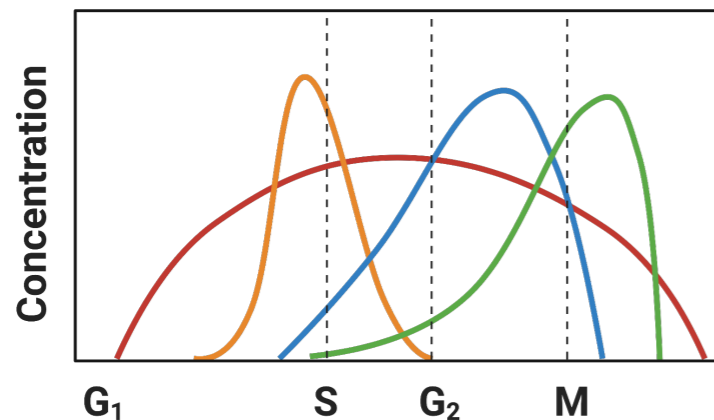
Cyclin-Dependent Kinase (CDK)

Kinase that controls cell cycle progression through phosphorylation

Stay constant throughout the cell cycle

Expressed at specific times during the cell cycle

Cyclins



Positive regulators of CDKs

Form Cyclin-CDK complexes to enable substrate phosphorylation

CDK Inhibitors (CKIs)

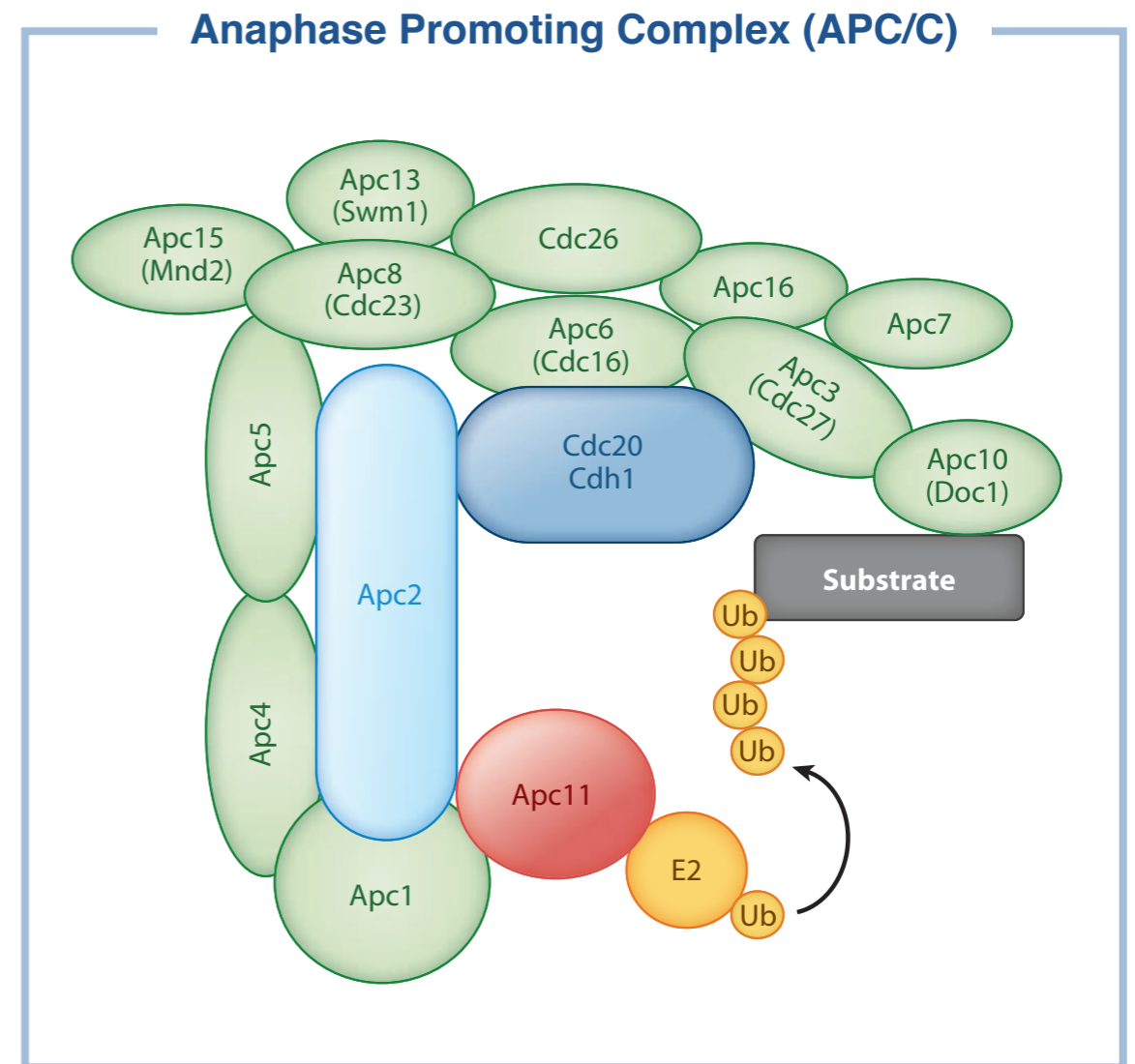
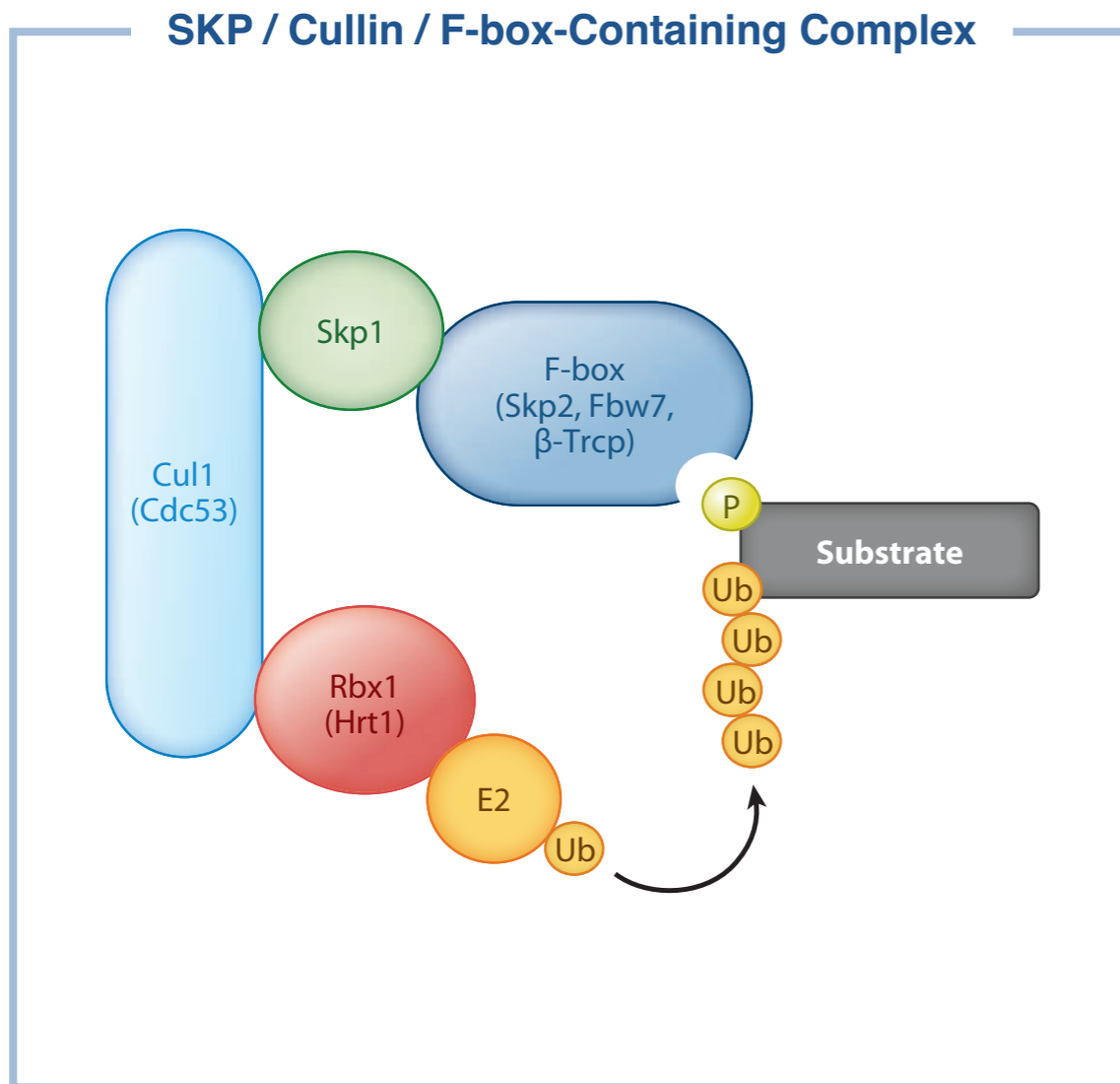
Negative regulators of CDKs

Interact with Cyclin-CDK complexes to block kinase activity

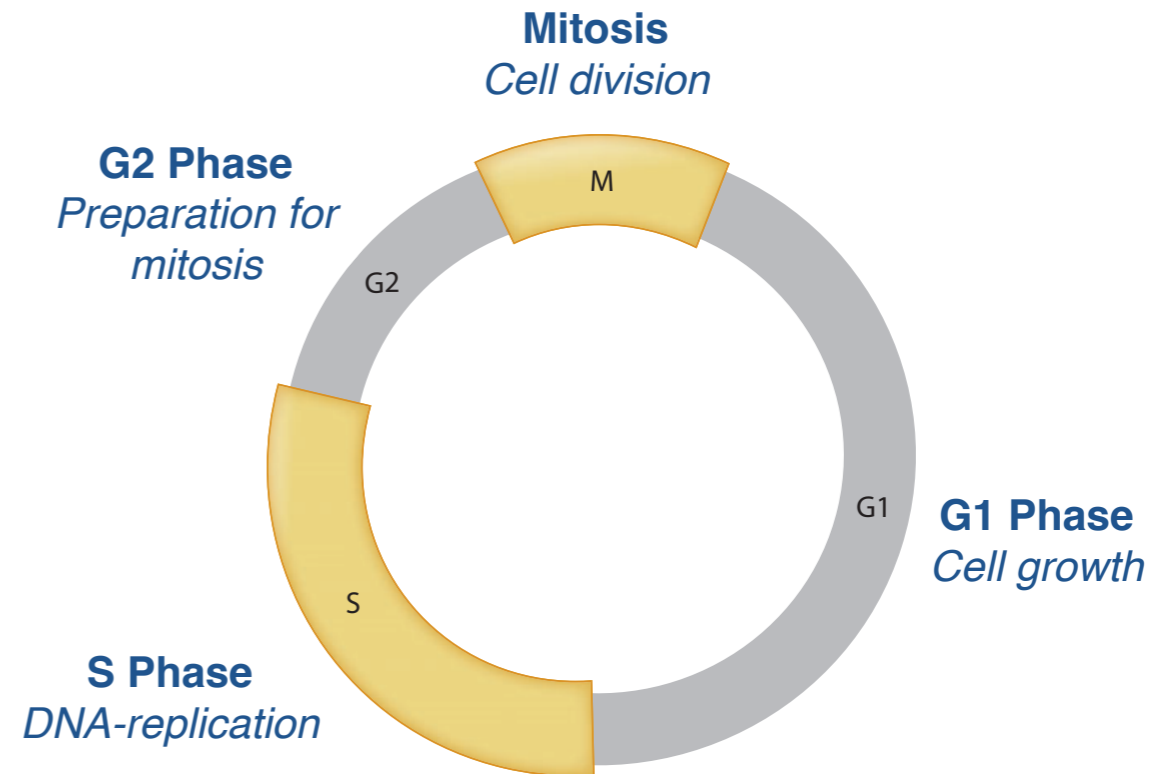
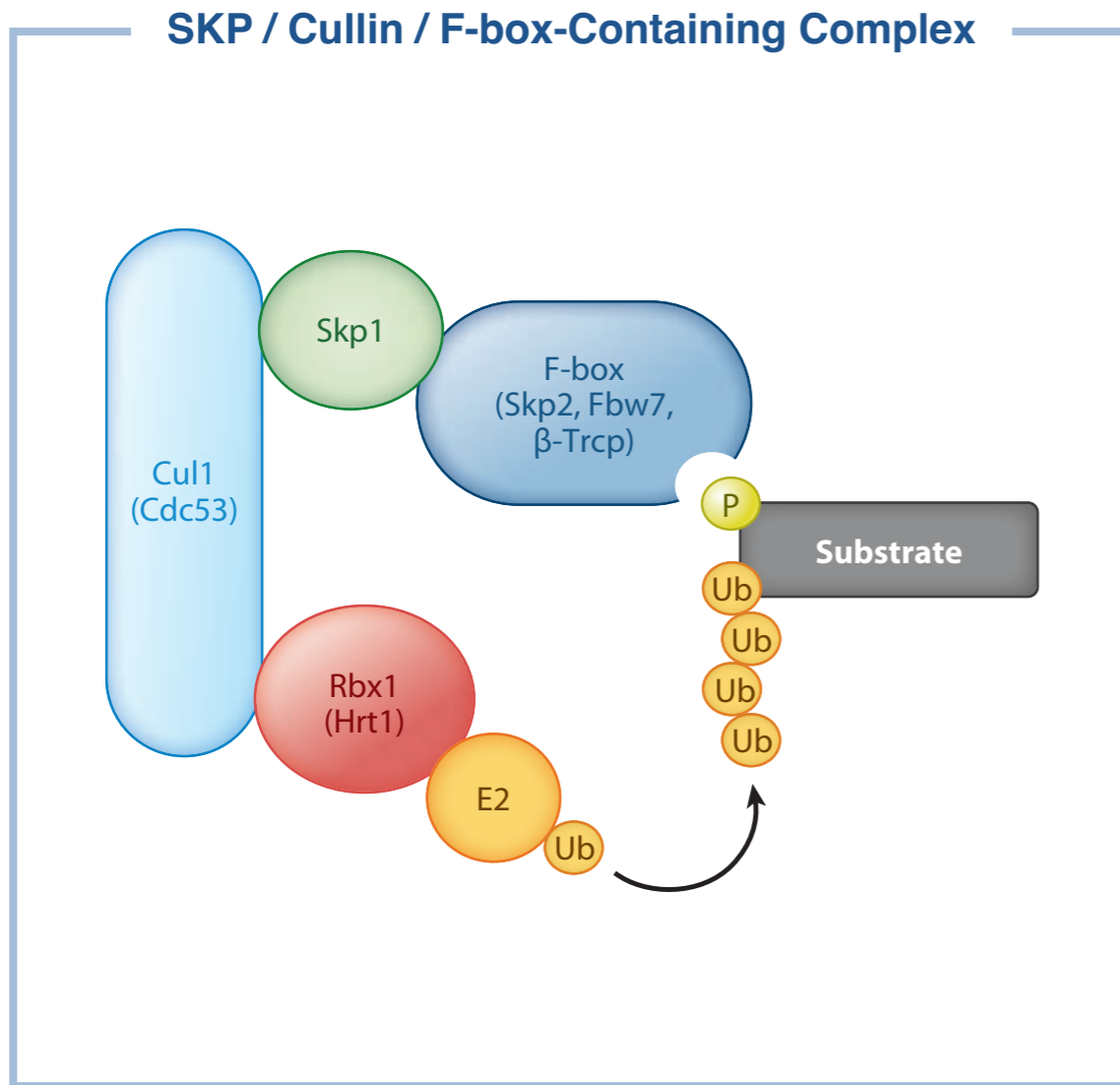
Orchestrated synthesis and degradation of cyclins and CKIs control cell-cycle progression

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

The primary E3 ligase complexes that control degradation of cyclins and CKIs

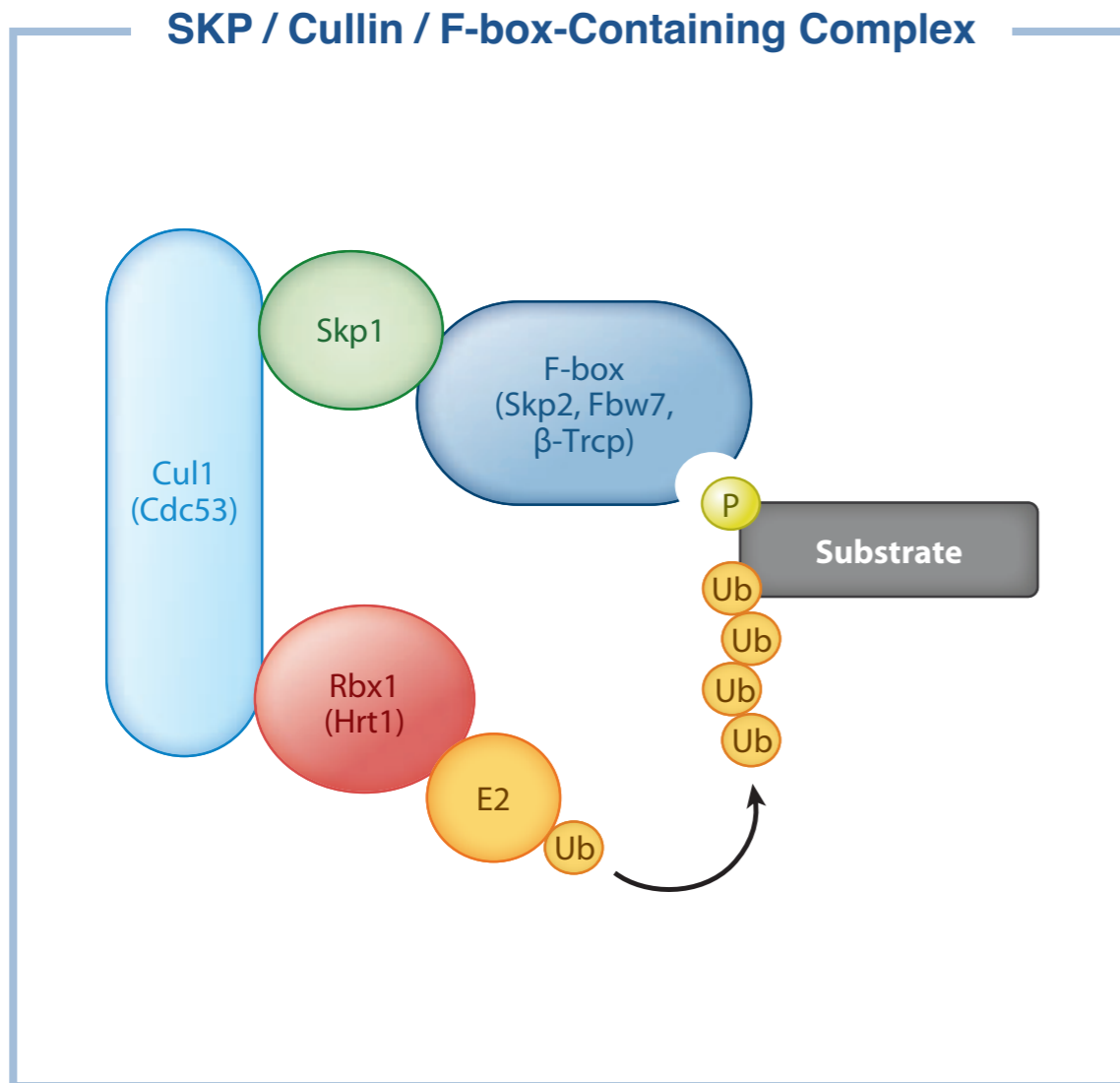


Ubiquitin-Mediated Regulation: Control of the Cell-Cycle



Promotes progression through S and G2 phase

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle



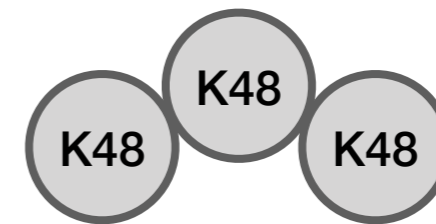
CUL1: cullin-like scaffolding protein

RBX1: Recruits E2

SKP1: Recruits F-box proteins

F-box Degron: Phosphodegrons

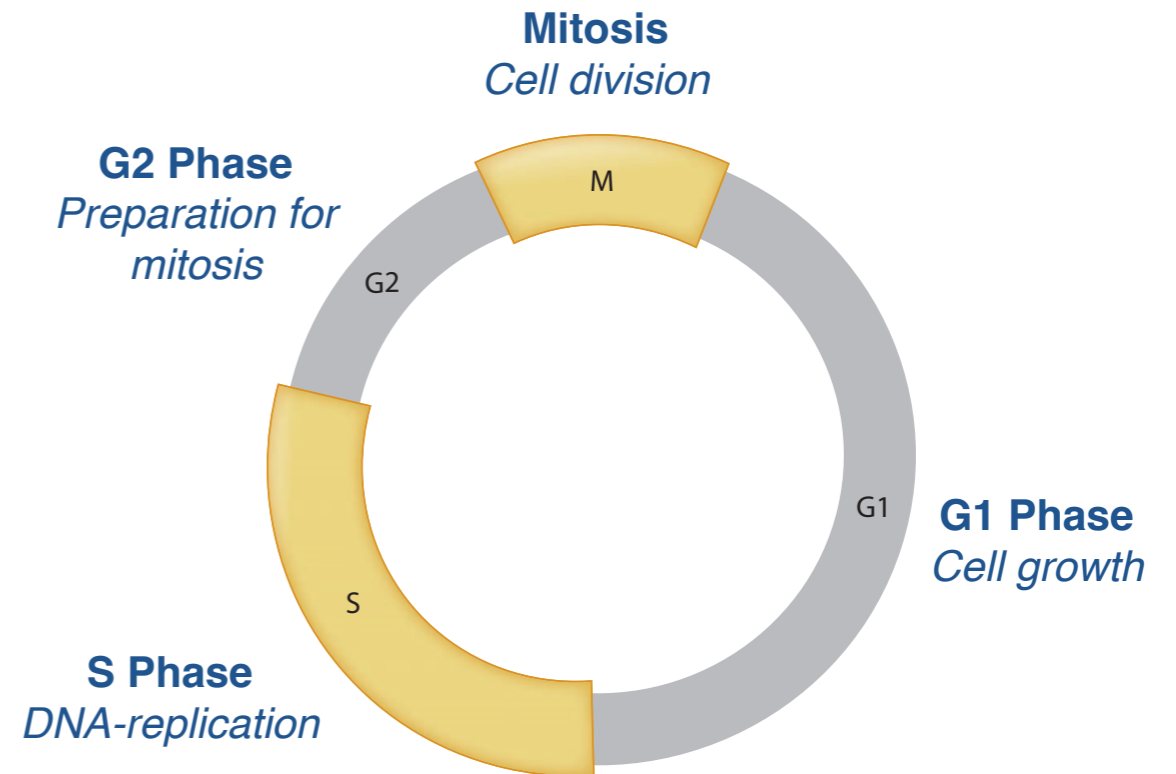
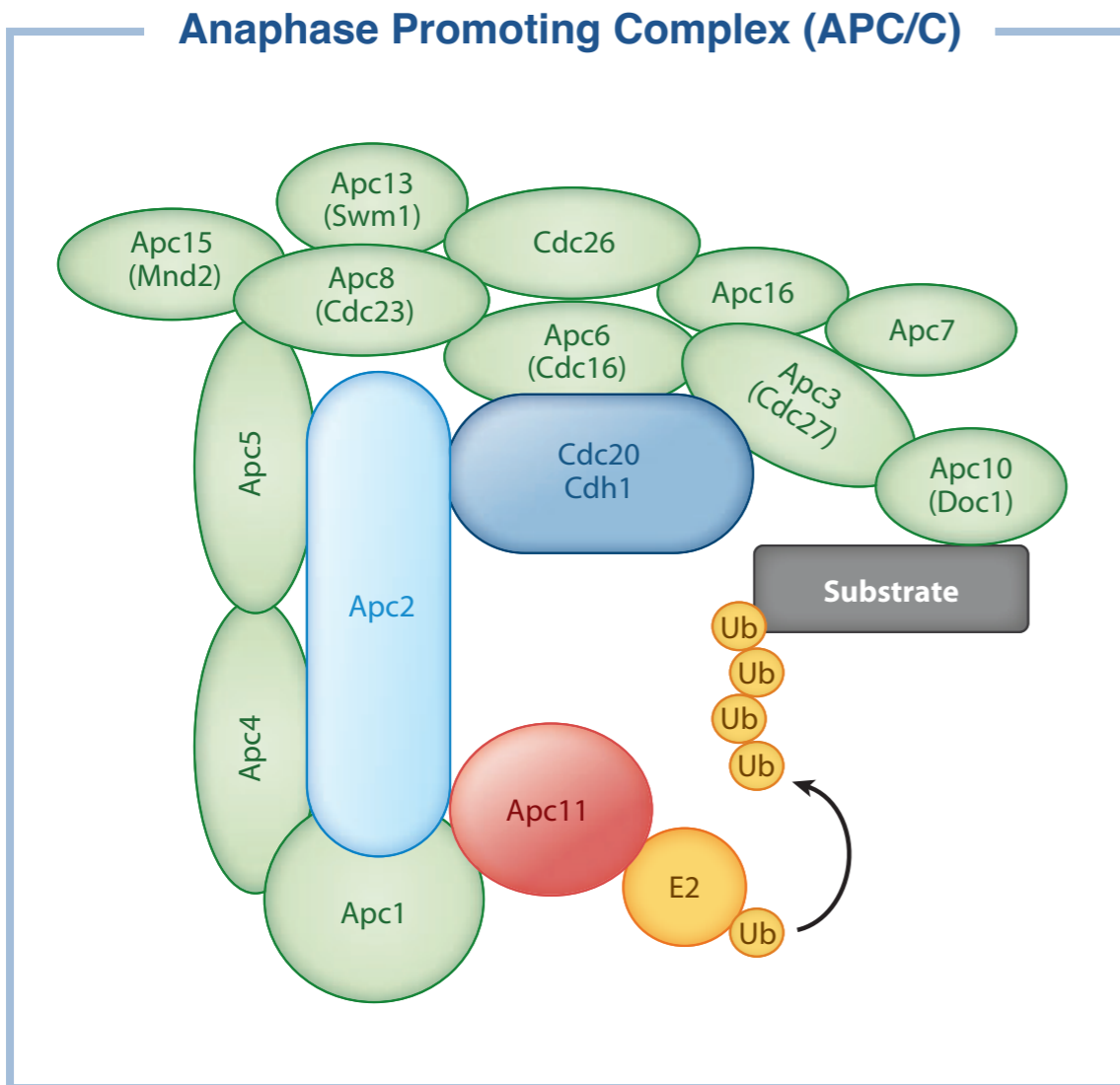
Specific phosphorylated sequences on substrates



Preferentially assembles Lys48-linked chains

Promotes progression through S and G2 phase

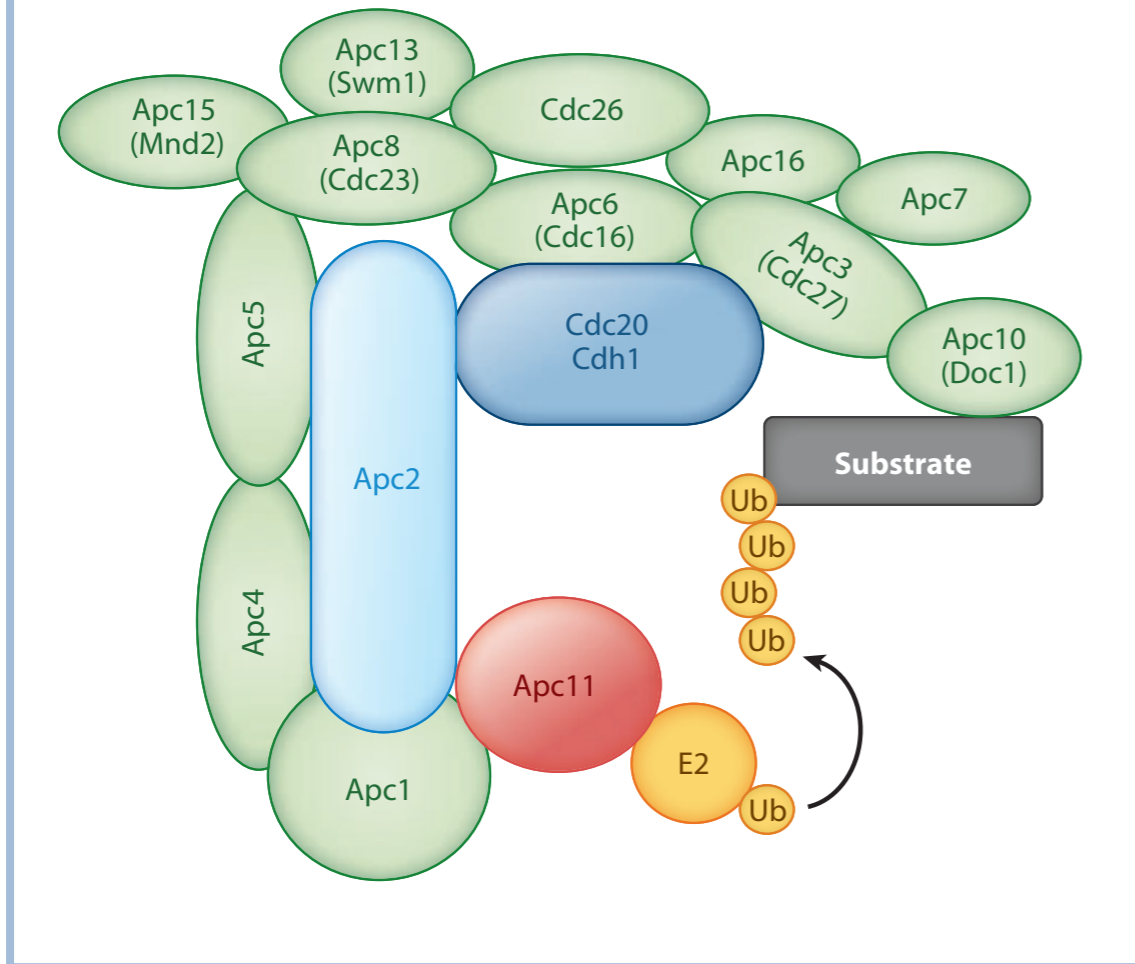
Ubiquitin-Mediated Regulation: Control of the Cell-Cycle



Promotes progression through Mitosis and G1 phase

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

Anaphase Promoting Complex (APC/C)



APC2: cullin-like scaffolding protein **APC11:** Recruits E2

Proteins in green: scaffolding proteins

APC/C adaptor

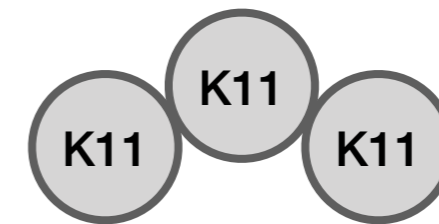
Degrans

CDC20

D-box: RXXLXXXXN

CDH1

KEN-BOX: KENXXXN



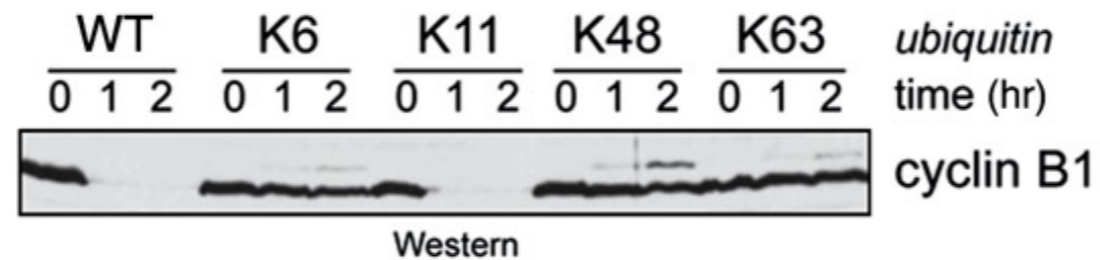
Preferentially assembles Lys11-linked chains

Promotes progression through Mitosis and G1 phase

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

The APC/C triggers degradation by assembling K11-linked ubiquitin

Experiment: Mitotic extracts supplemented with wt or single-lysine Ub and APC/C activated with addition of E2



Ubiquitin with all lysines mutated except for the one listed

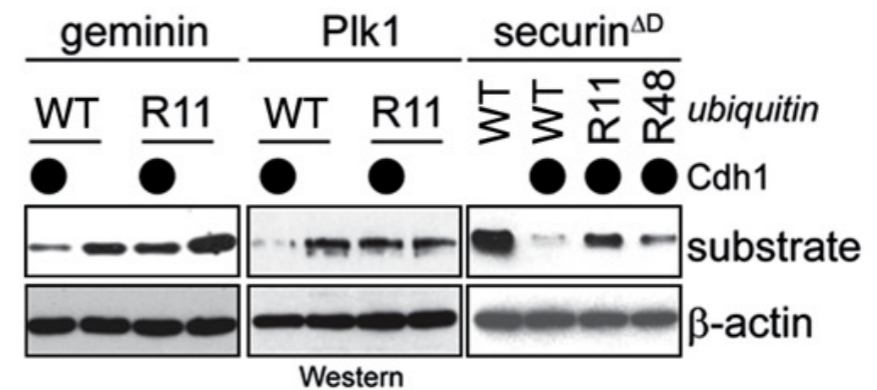
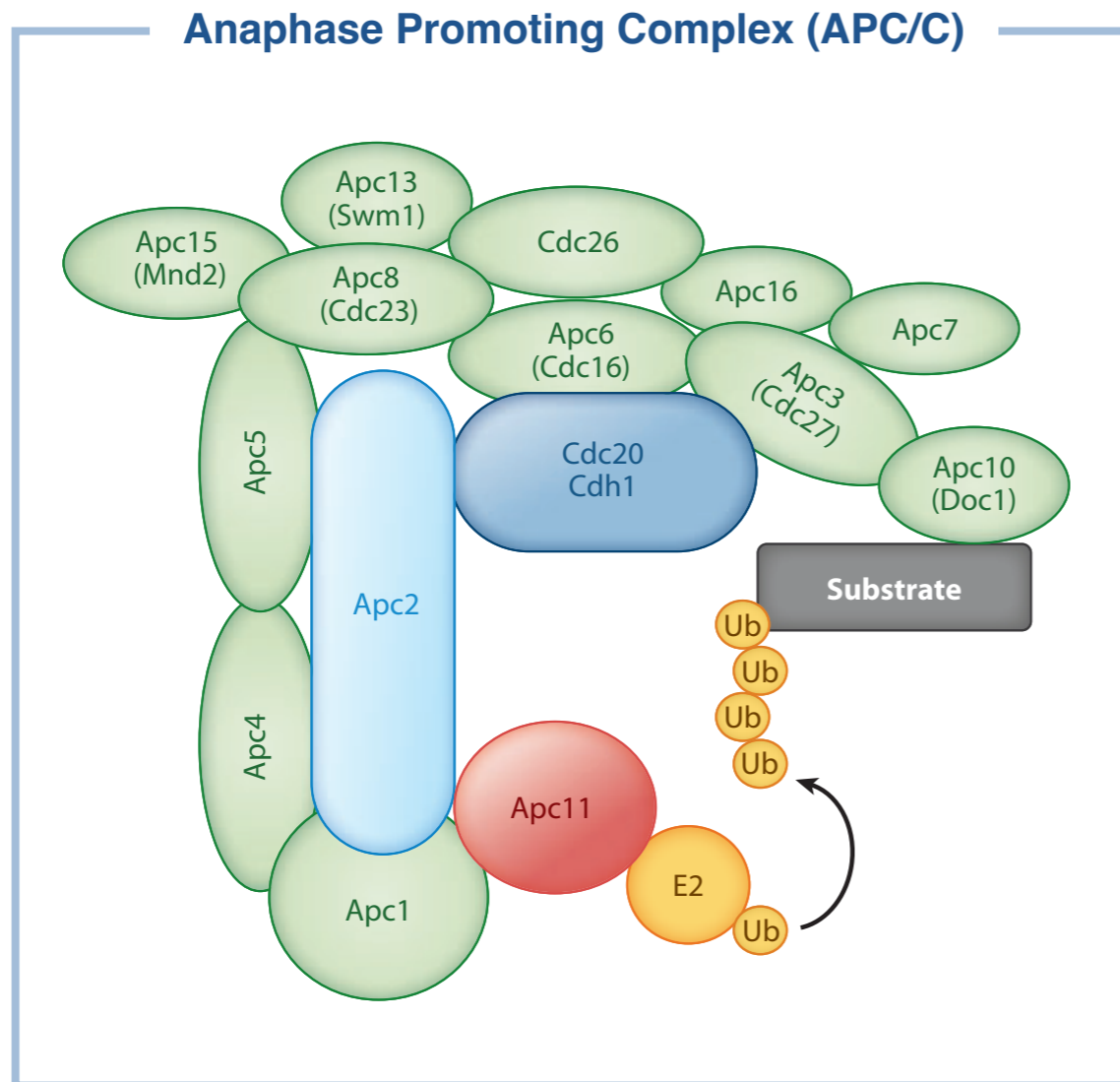
Only wild type and K11 ubiquitin triggers cyclin B1 degradation

Conclusion: APC/C assembles K11-linked chains

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

The APC/C triggers degradation by assembling K11-linked ubiquitin

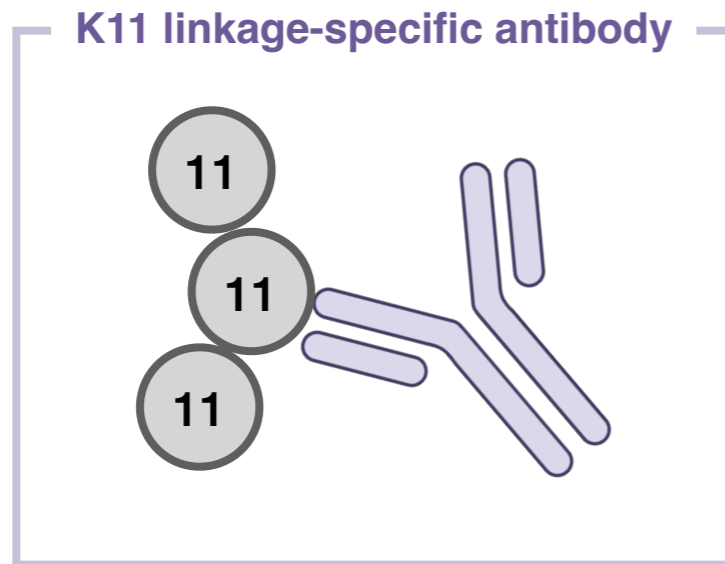
Experiment: Expression of wt or mutant ubiquitin in 293T cells ± CDH1 adaptor expression



Conclusion: APC/C assembles K11-linked chains on multiple substrates in cells

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

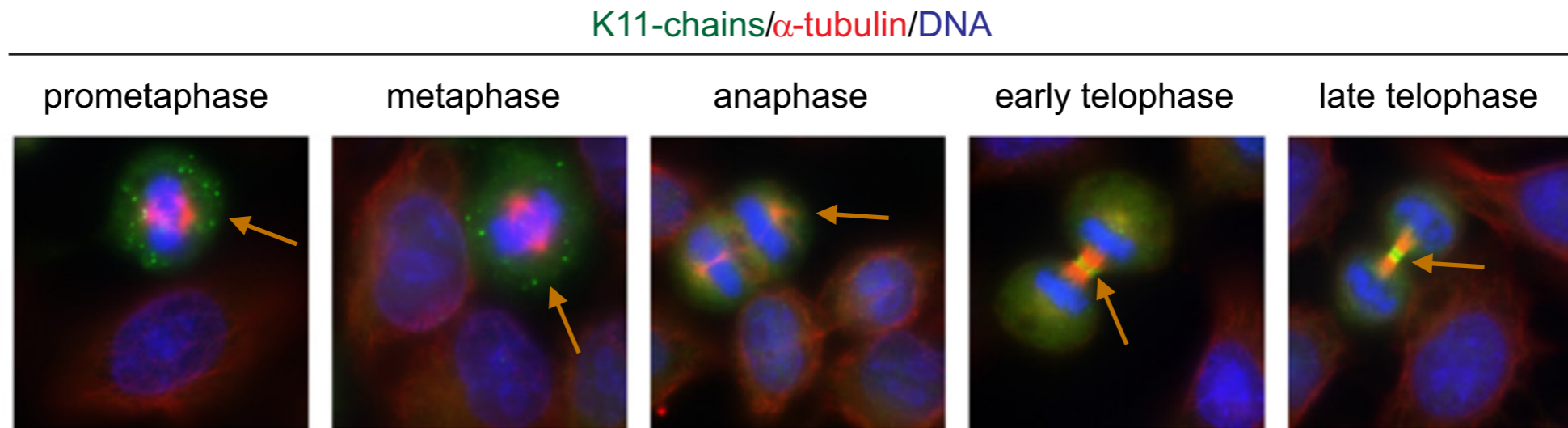
Where do K11-Ub chains accumulate during different stages of mitosis?



Genentech
A Member of the Roche Group

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

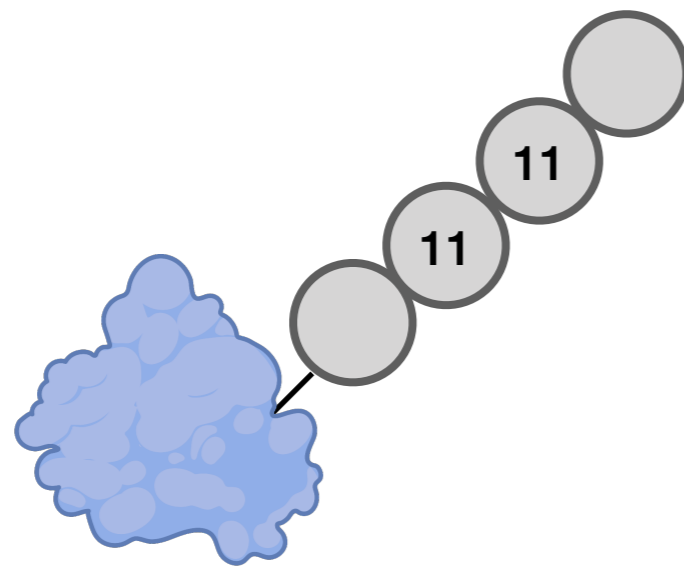
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K11 linkages are upregulated in mitosis

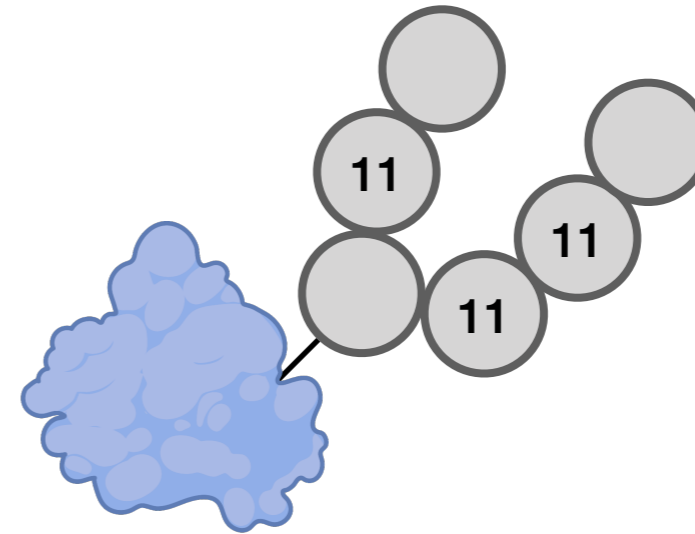
K11-linked chains accumulate at spindle midbody from late anaphase on

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle



Linear ubiquitin

OR

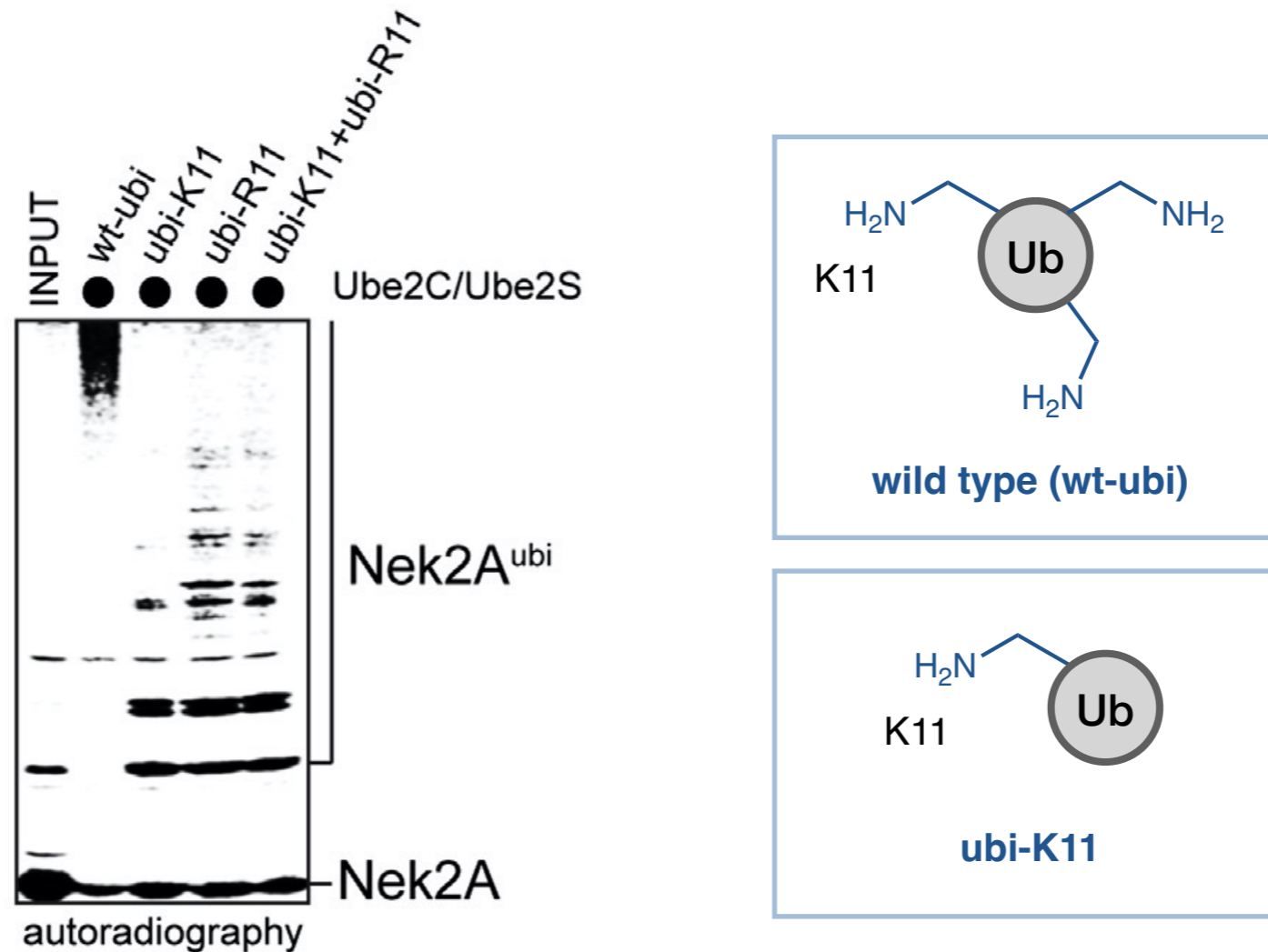


Branched ubiquitin

Are linear or branched chains formed by the APC/C E3 ligase complex?

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

Experiment: APC/C-mediated in vitro ubiquitination of Nek2A

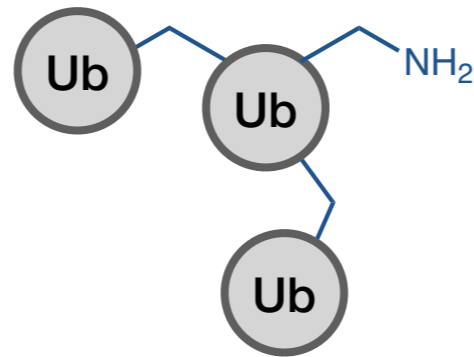


ubi-K11 is not sufficient for polyubiquitination of Nek2A

Wild-type ubiquitin required for polyubiquitination of Nek2A

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

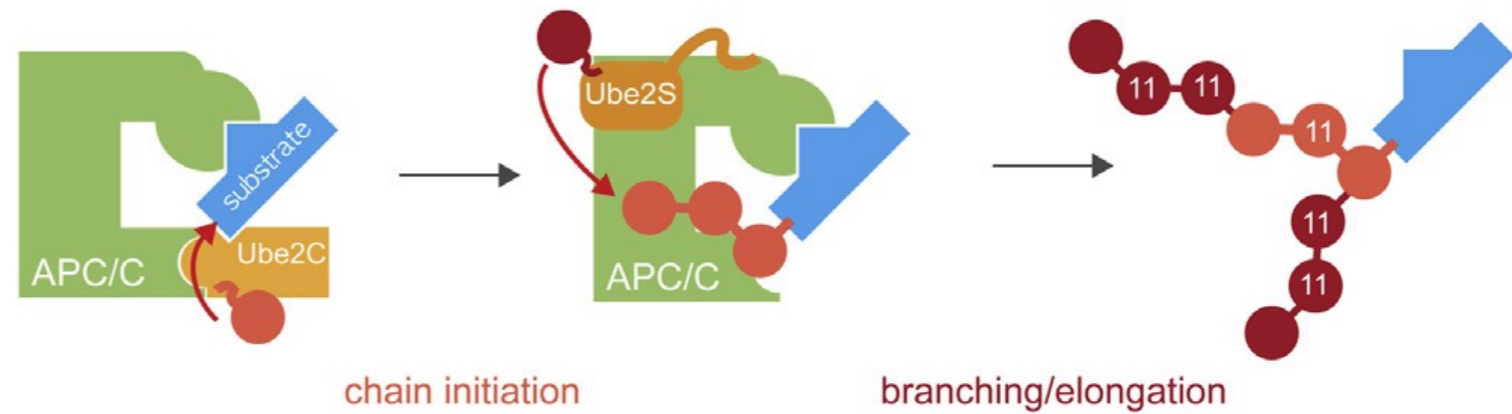
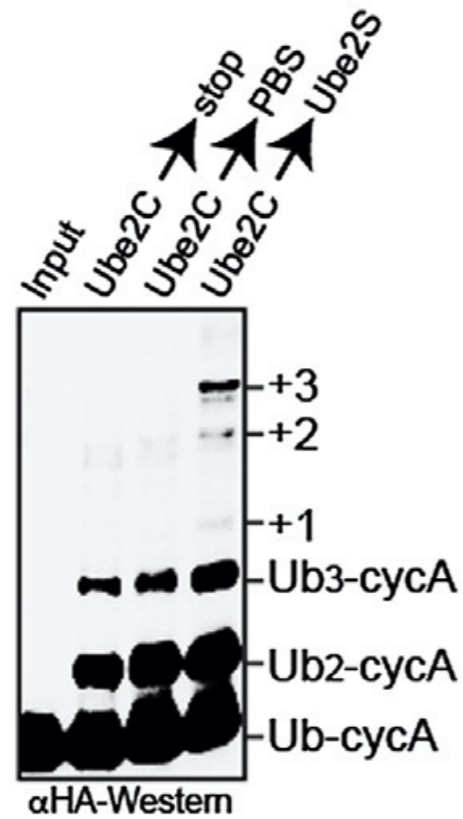
Experiment: APC/C-mediated *in vitro* ubiquitination of Nek2A



Multiple lysines required for ubiquitination of Nek2A

Conclusion: APC/C synthesizes branched conjugates

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

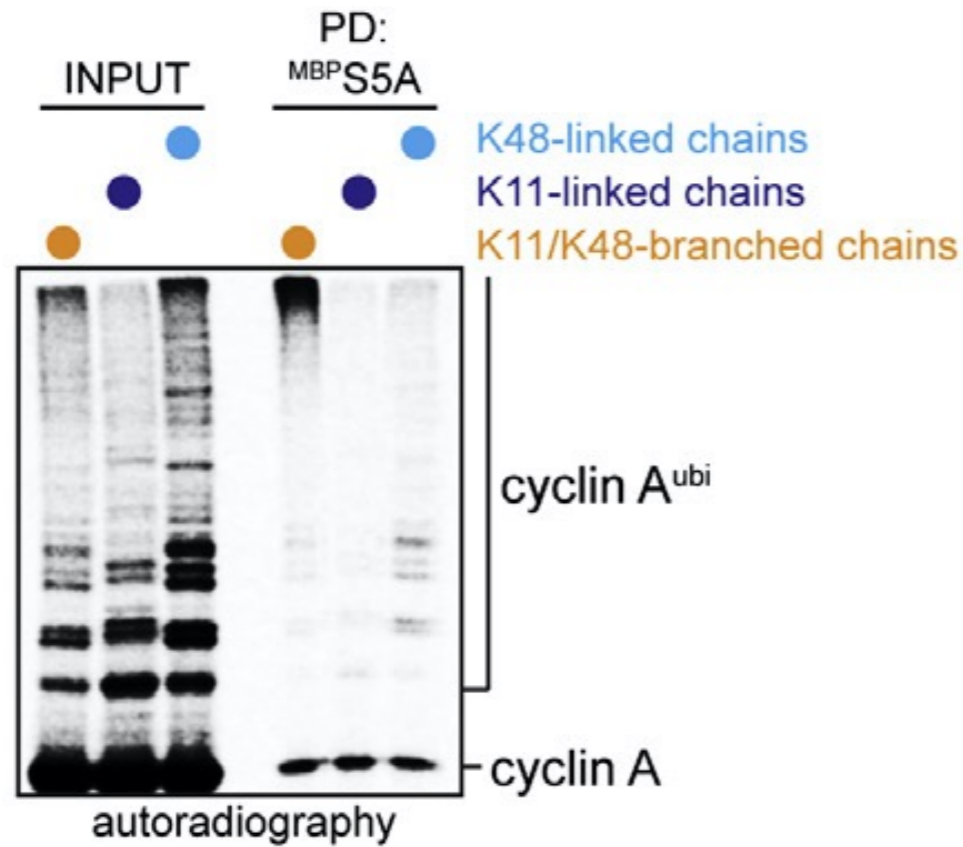


UBE2C (E2): synthesizes K11, K48, and K63 linkages

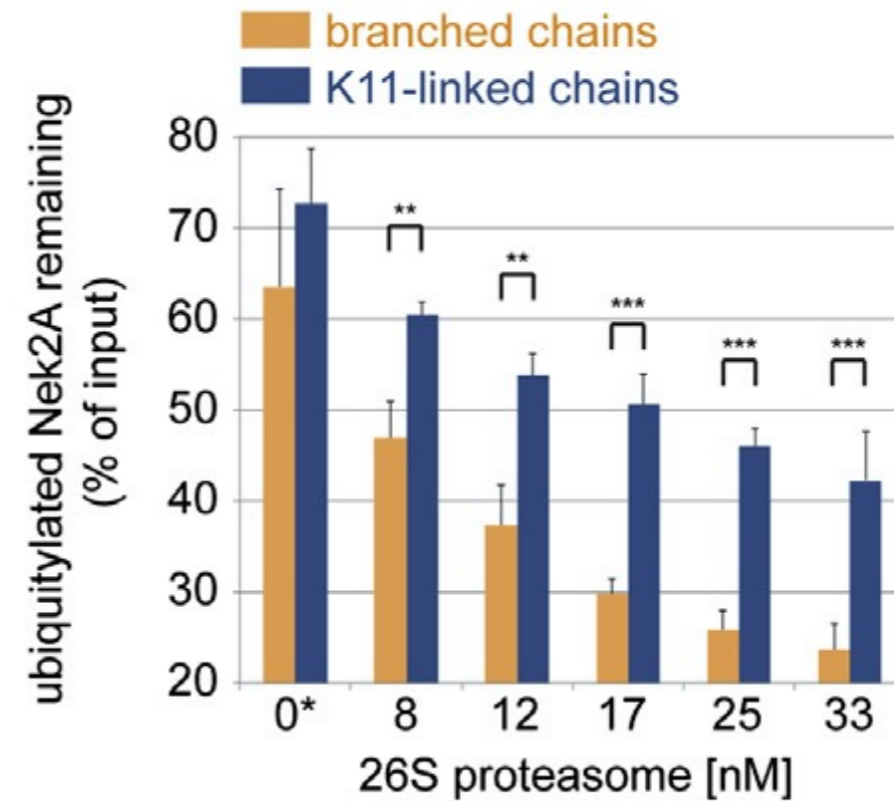
UBE2S (E2): Adds blocks of K11-linked ubiquitin to moieties with unmodified K11

The E2 ubiquitin-conjugating enzyme UBE2S adds ubiquitin to ubiquitin chains formed by the E2 UBE2C

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle



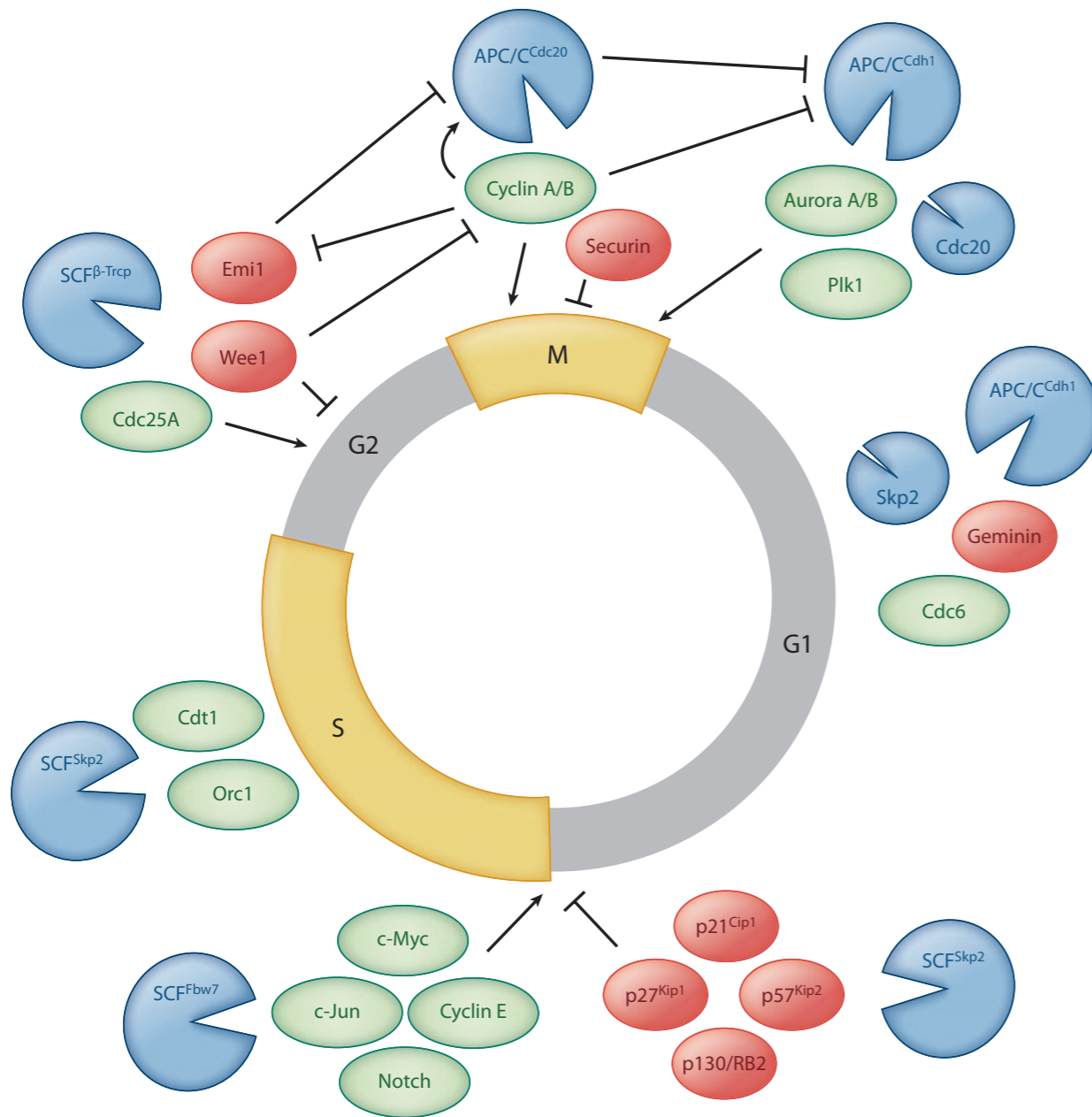
Branched conjugates enhance binding to S5A, which directs ubiquitinated proteins to the proteasome



Conjugation of Nek2A with branched chains enhances proteasomal degradation

Branched conjugates assembled by the APC/C enhance substrate recognition by the proteasome

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle



E3 ligase^{Adaptor}

Cell-cycle activators

Cell-cycle inhibitors

Key takeaways

- 1) APC/C performs ubiquitination through M and G1
- 2) SCF performs ubiquitination through S and G2

Ubiquitin-Mediated Regulation: Transcription

Histone Ubiquitination

The first protein known to be ubiquitinated: Monoubiquitination of H2A at K119

Isolation and Characterization of Protein A24, a “Histone-like” Non-Histone Chromosomal Protein*

(Received for publication, April 7, 1975)

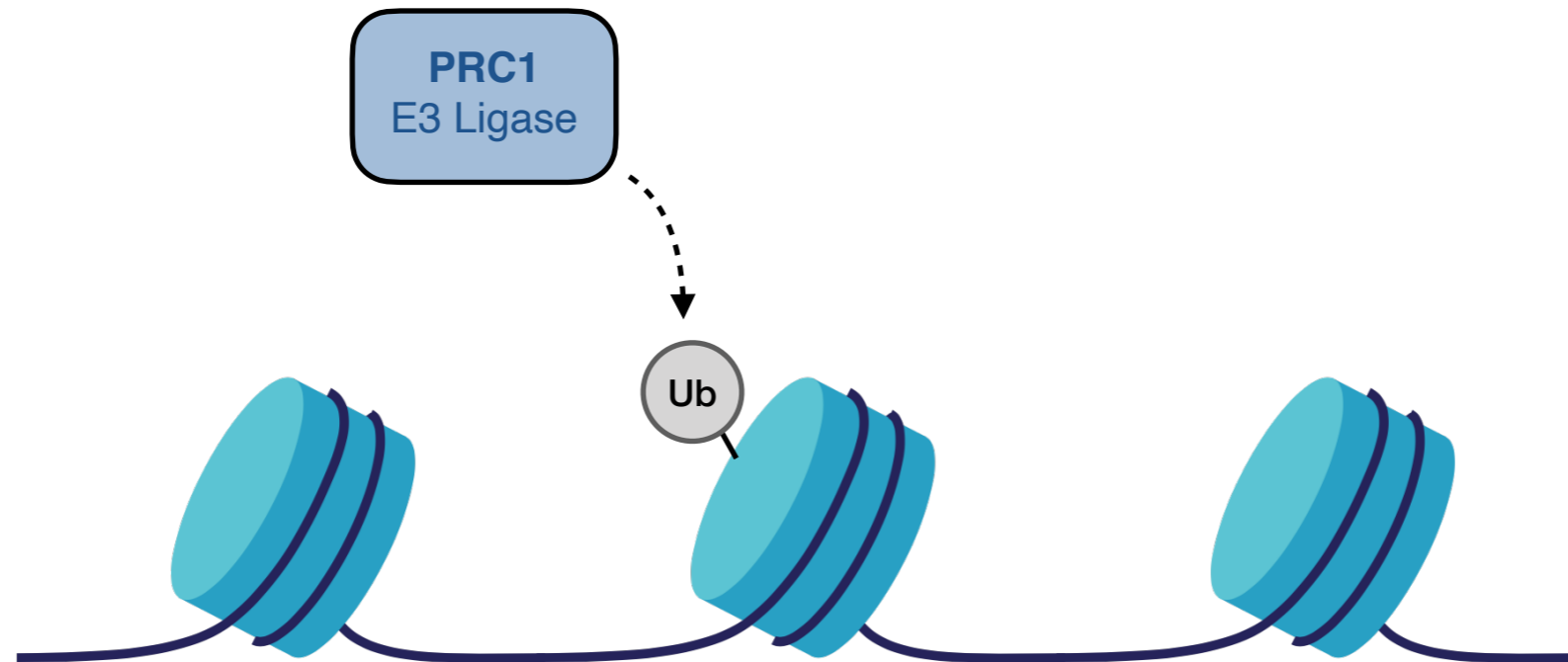
IRA L. GOLDKNOPF,[‡] CHARLES W. TAYLOR, RONALD M. BAUM, LYNN C. YEOMAN, MARK
O. J. OLSON, ARCHIE W. PRESTAYKO, AND HARRIS BUSCH

*From the Nuclear Protein Laboratory, Department of Pharmacology, Baylor College of Medicine,
Houston, Texas 77025*

“The present results show that A24 is a non-histone chromosomal protein
with solubility properties similar to those of histones”

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2A: Transcriptional Repression

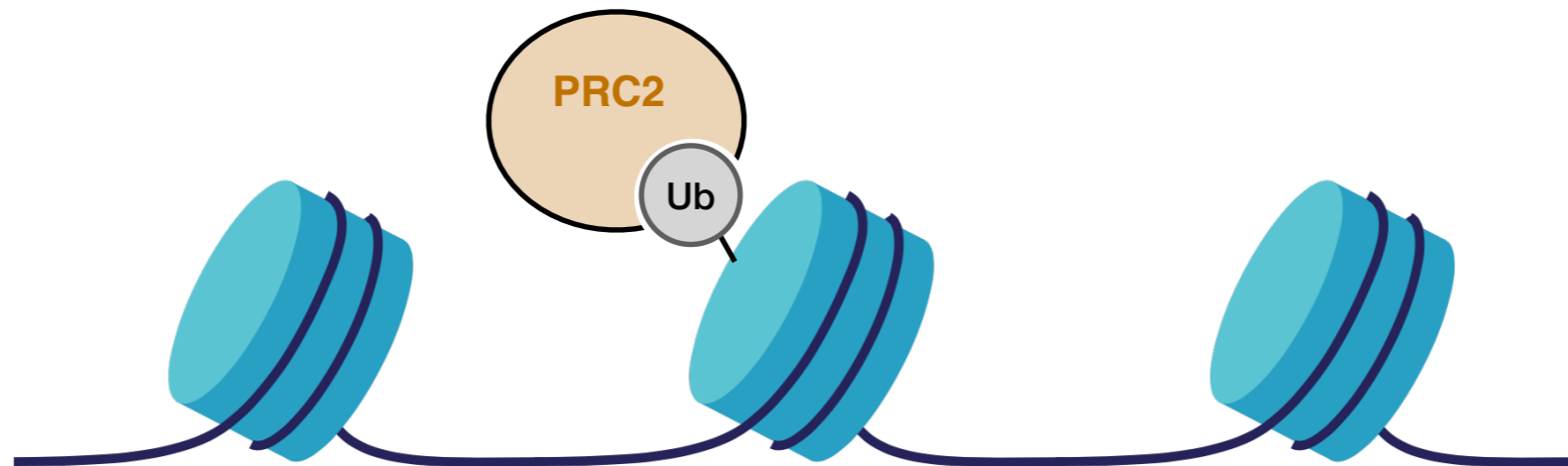


1) PRC1 ubiquitinates K27 on H2A

Monoubiquitination of H2A facilitates transcriptional repression of cell cycle inhibitors to increase proliferation

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2A: Transcriptional Repression

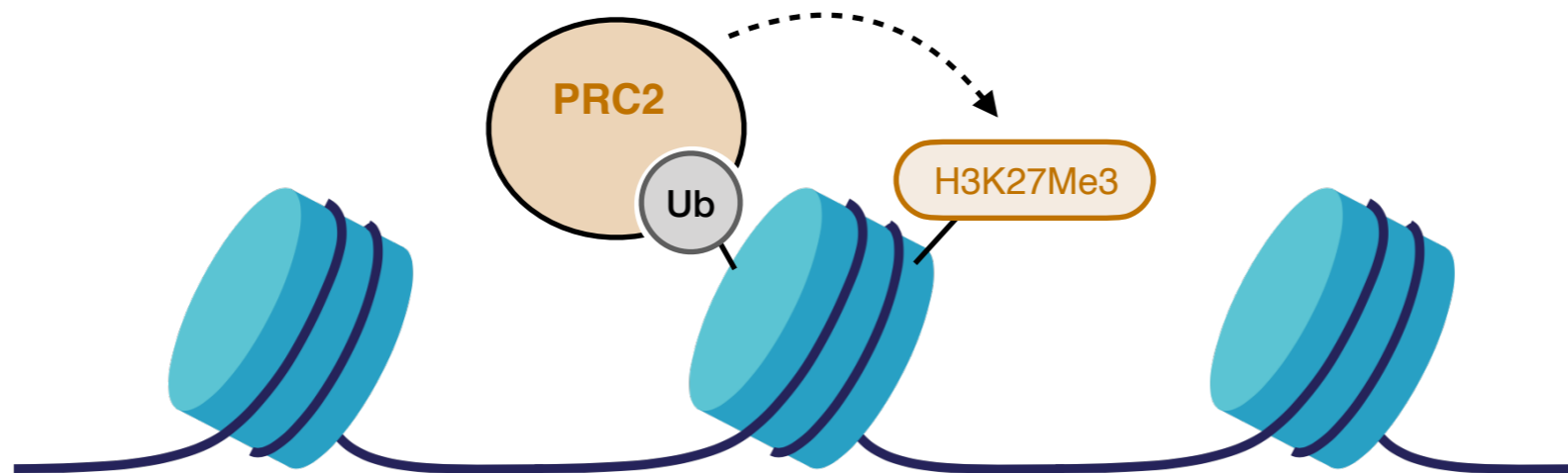


- 1) PRC1 ubiquitinates K27 on H2A
- 2) PRC2 recognizes monoubiquitinated H3 and tri-methylates K27 on H3

Monoubiquitination of H2A facilitates transcriptional repression of cell cycle inhibitors to increase proliferation

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2A: Transcriptional Repression

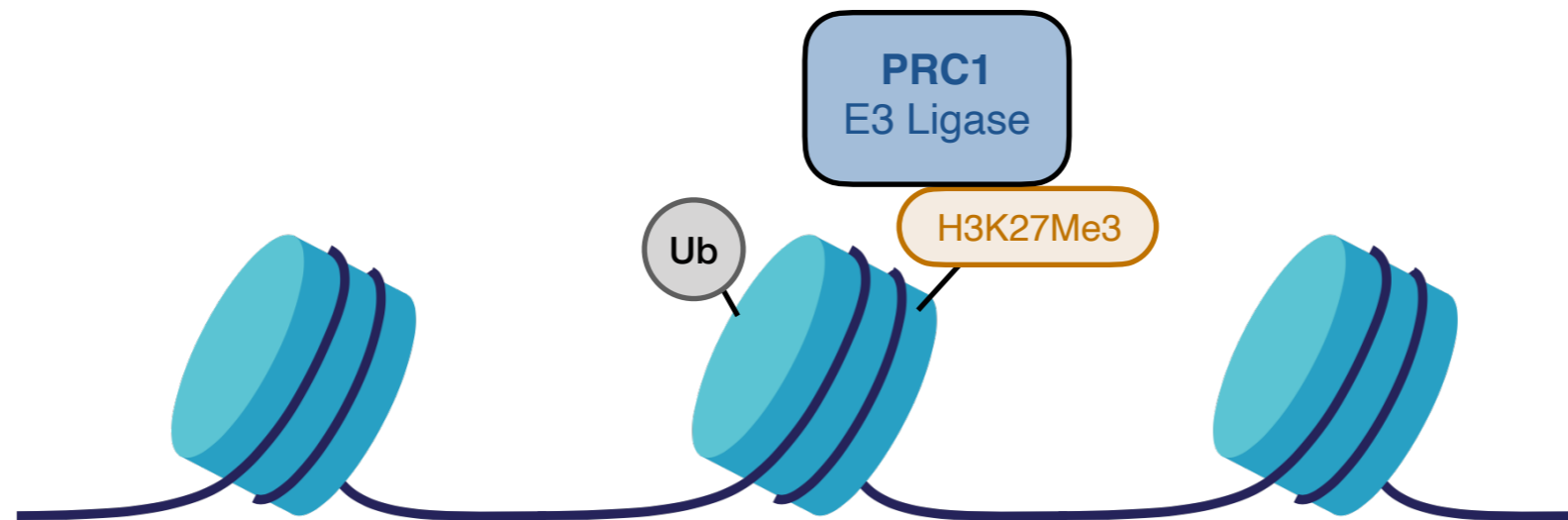


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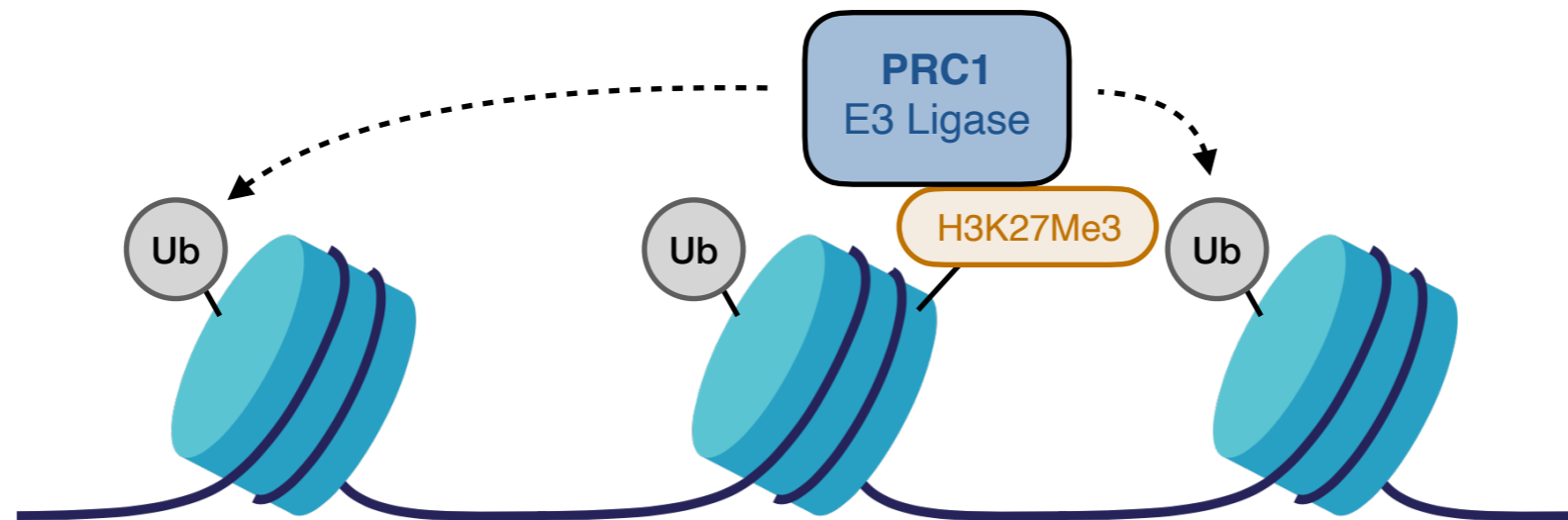


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Ubiquitin-Mediated Regulation: Transcription

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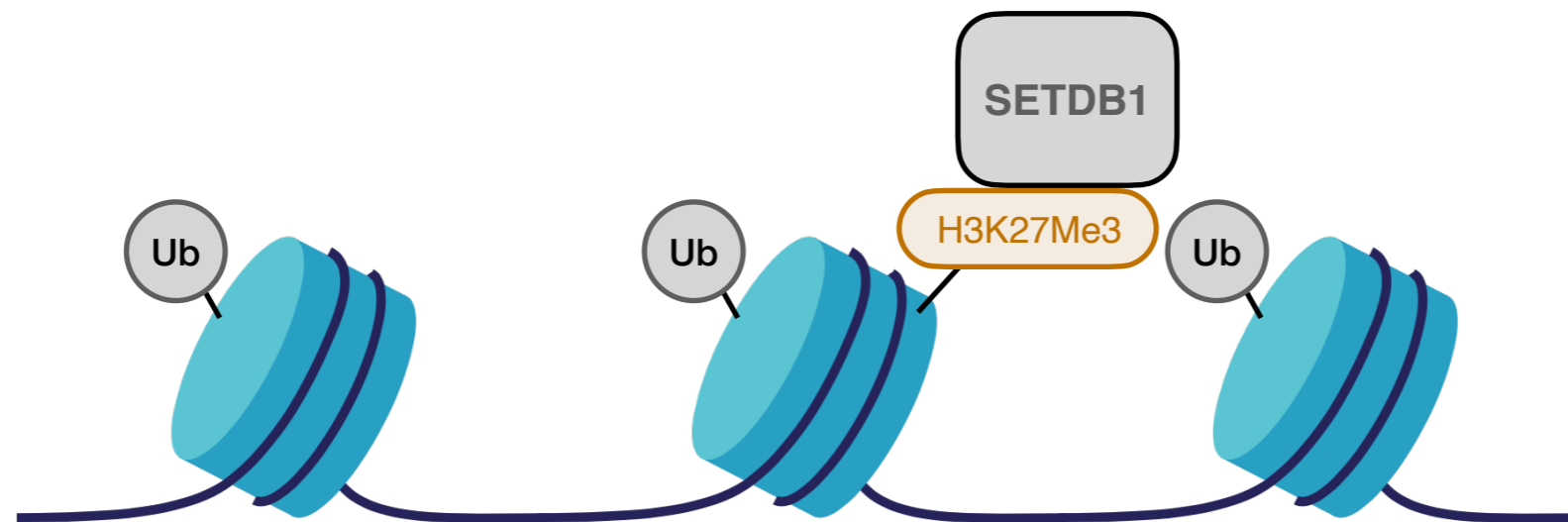


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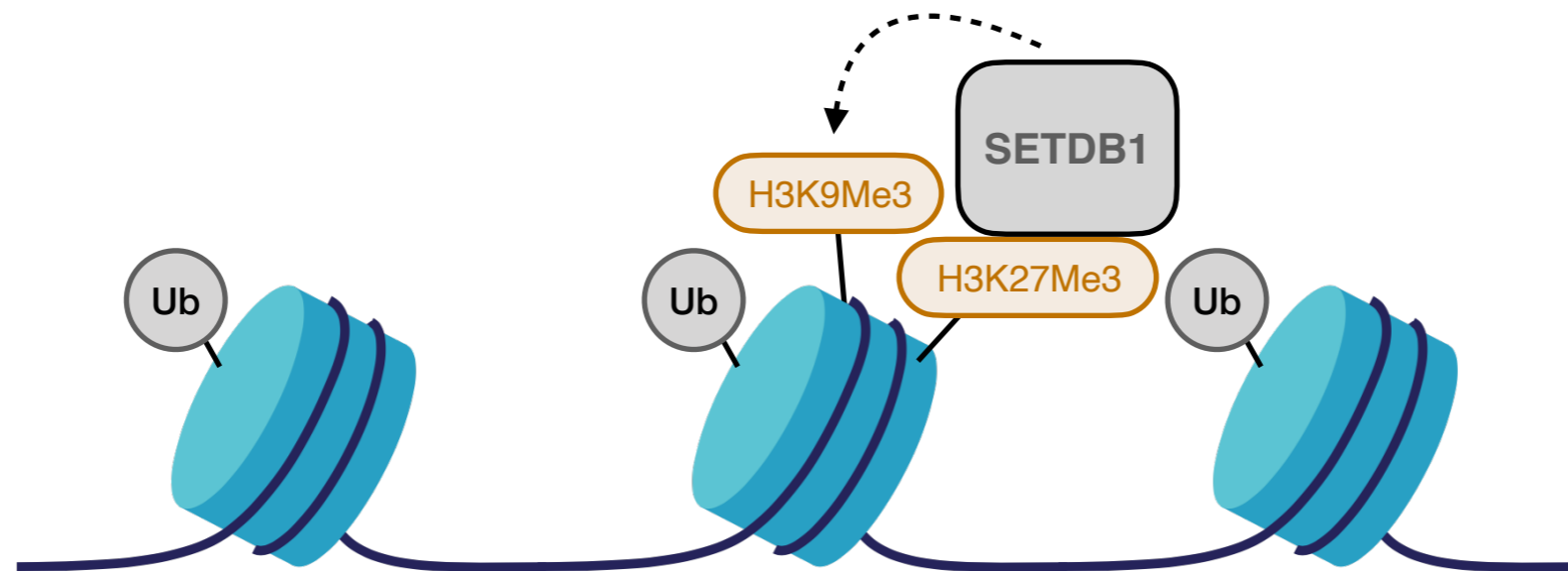


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Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2A: Transcriptional Repression

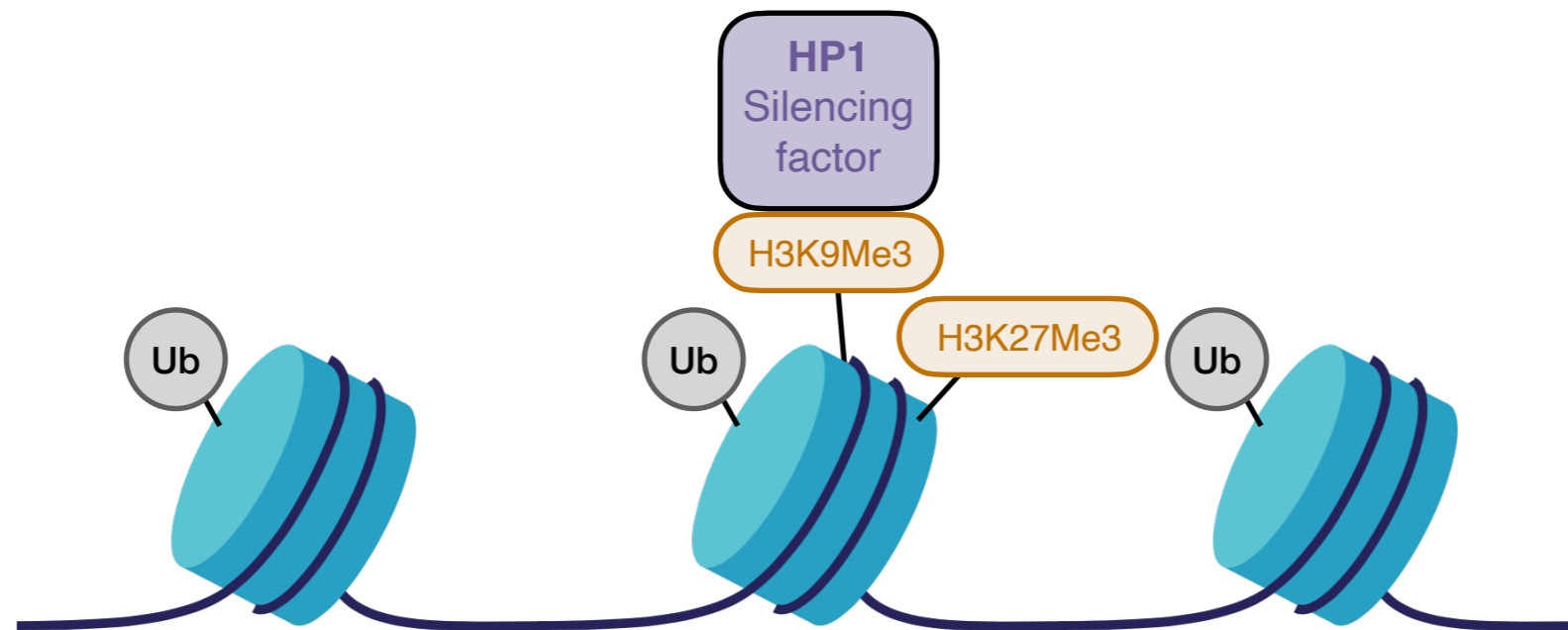


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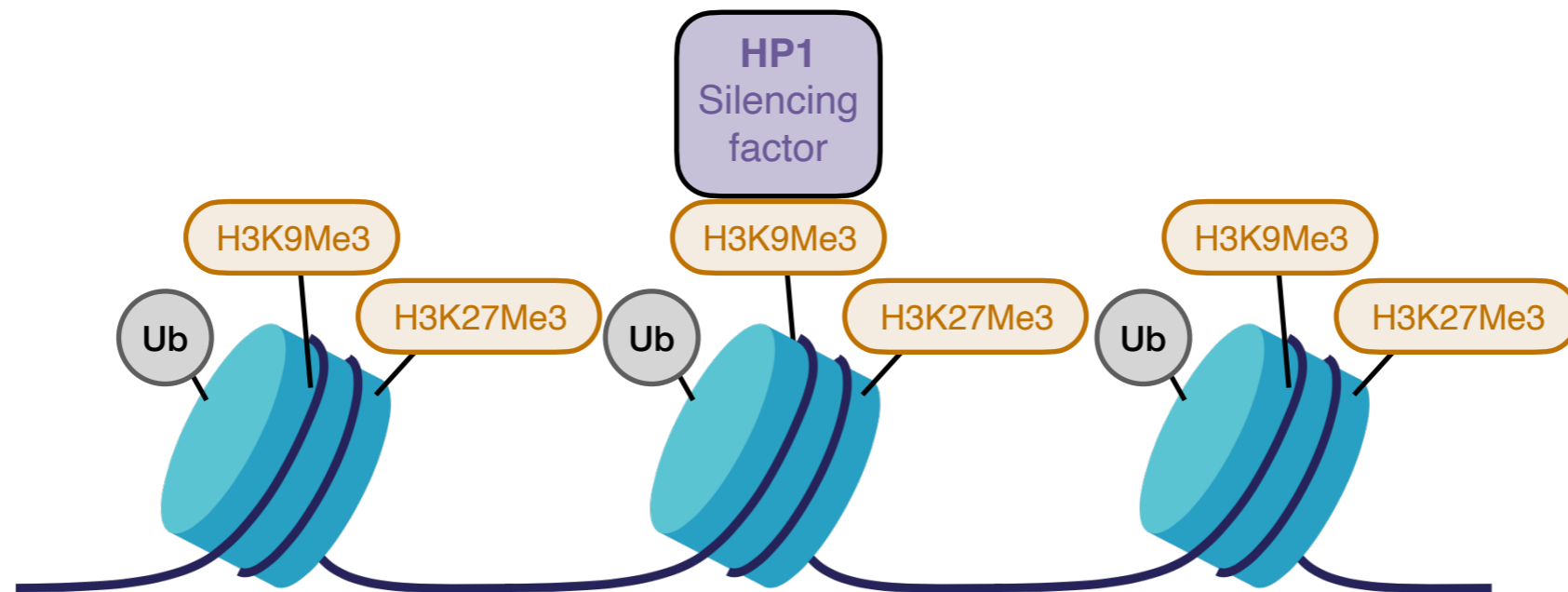


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- 5) H3K9Me3 recruits silencing factors such as HP1

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Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2A: Transcriptional Repression

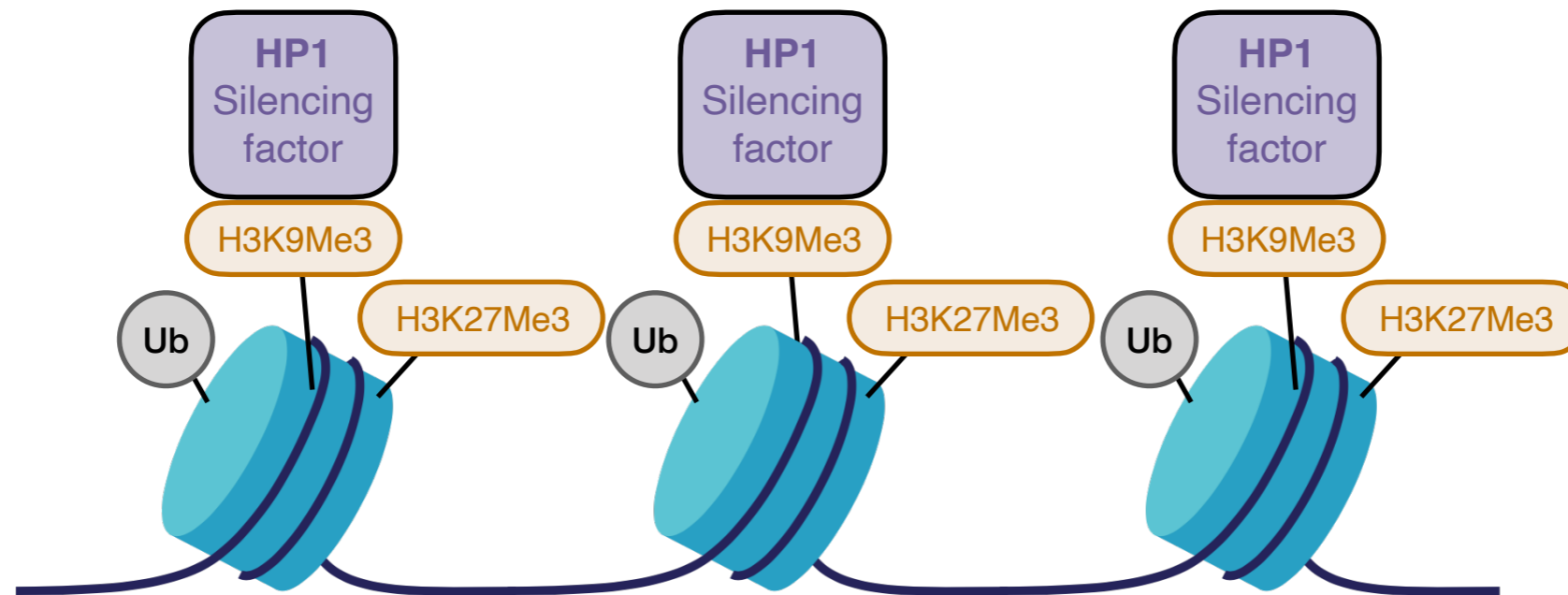


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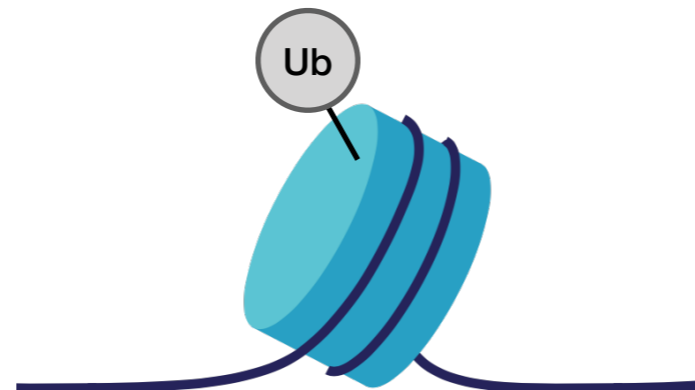


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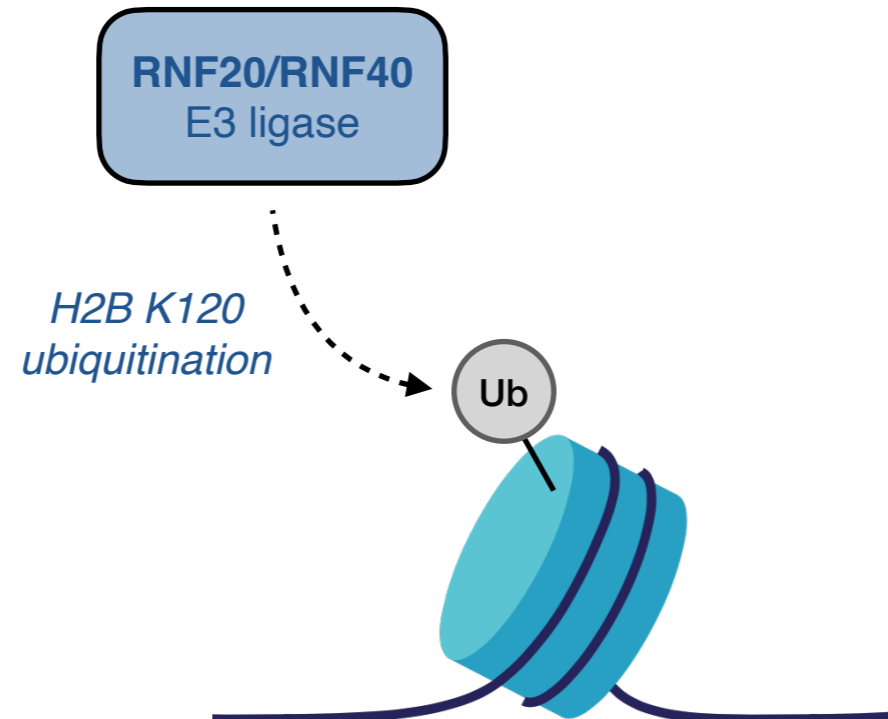
Ubiquitination of H2B: Transcriptional Elongation



Monoubiquitination of H2B promotes gene expression

Ubiquitin-Mediated Regulation: Transcription

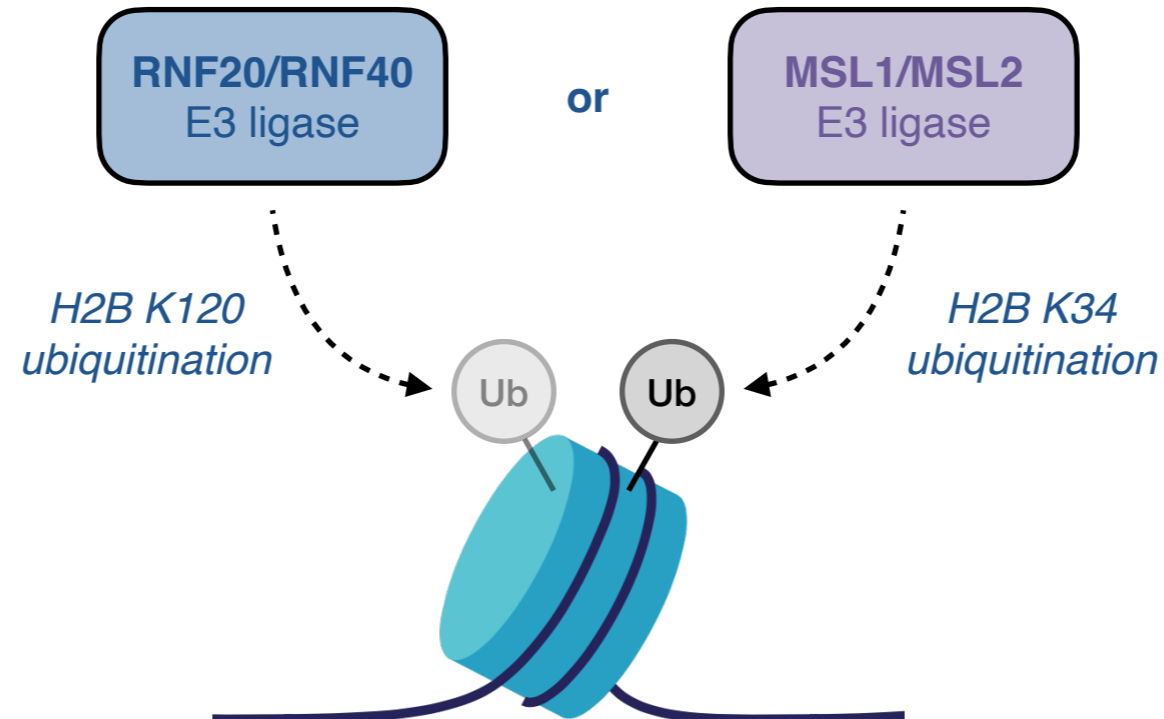
Ubiquitination of H2B: Transcriptional Elongation



Monoubiquitination of H2B promotes gene expression

Ubiquitin-Mediated Regulation: Transcription

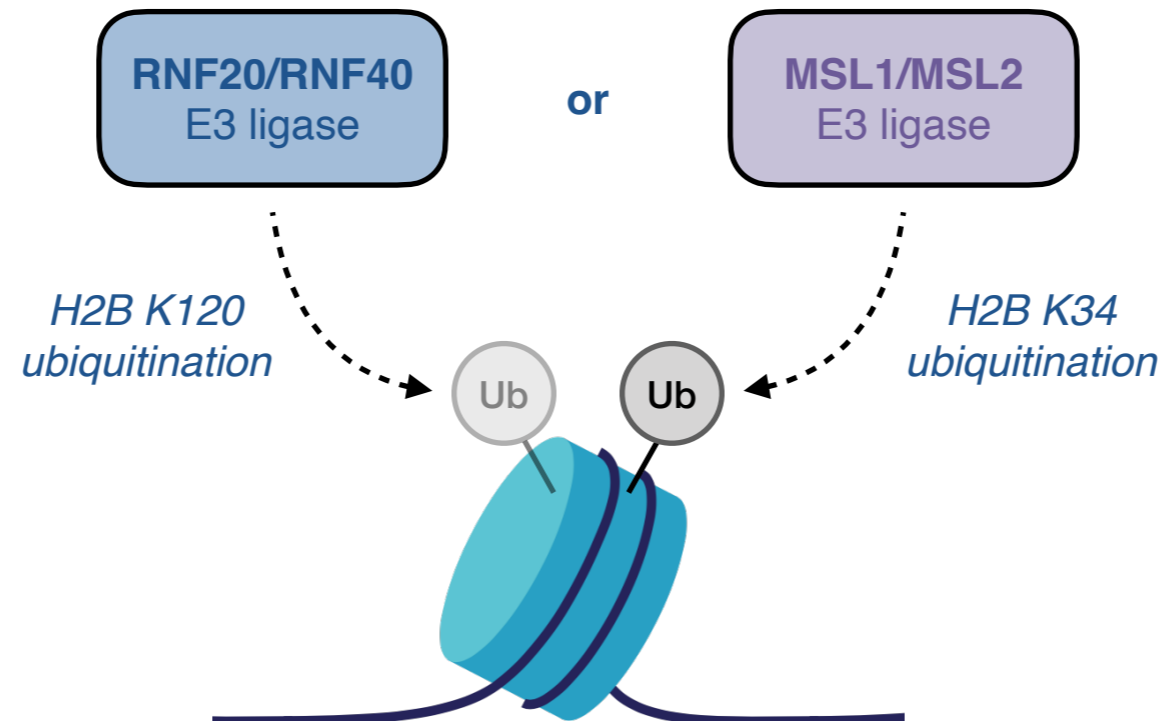
Ubiquitination of H2B: Transcriptional Elongation



Monoubiquitination of H2B promotes gene expression

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2B: Transcriptional Elongation

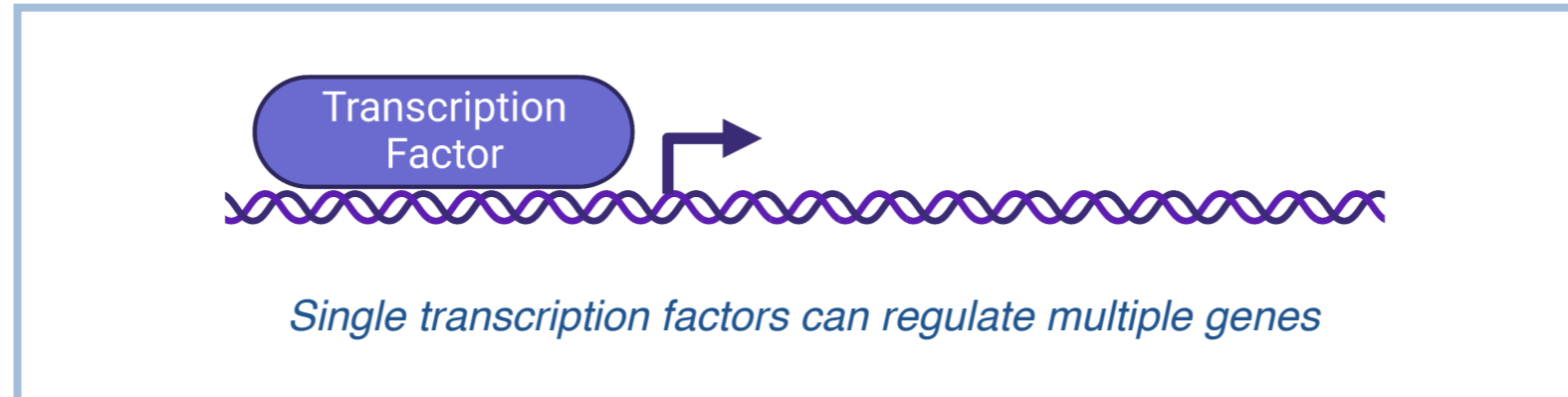


Monoubiquitinated H2B can attract over ninety effectors

Monoubiquitination of H2B promotes gene expression

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of Transcription Factors



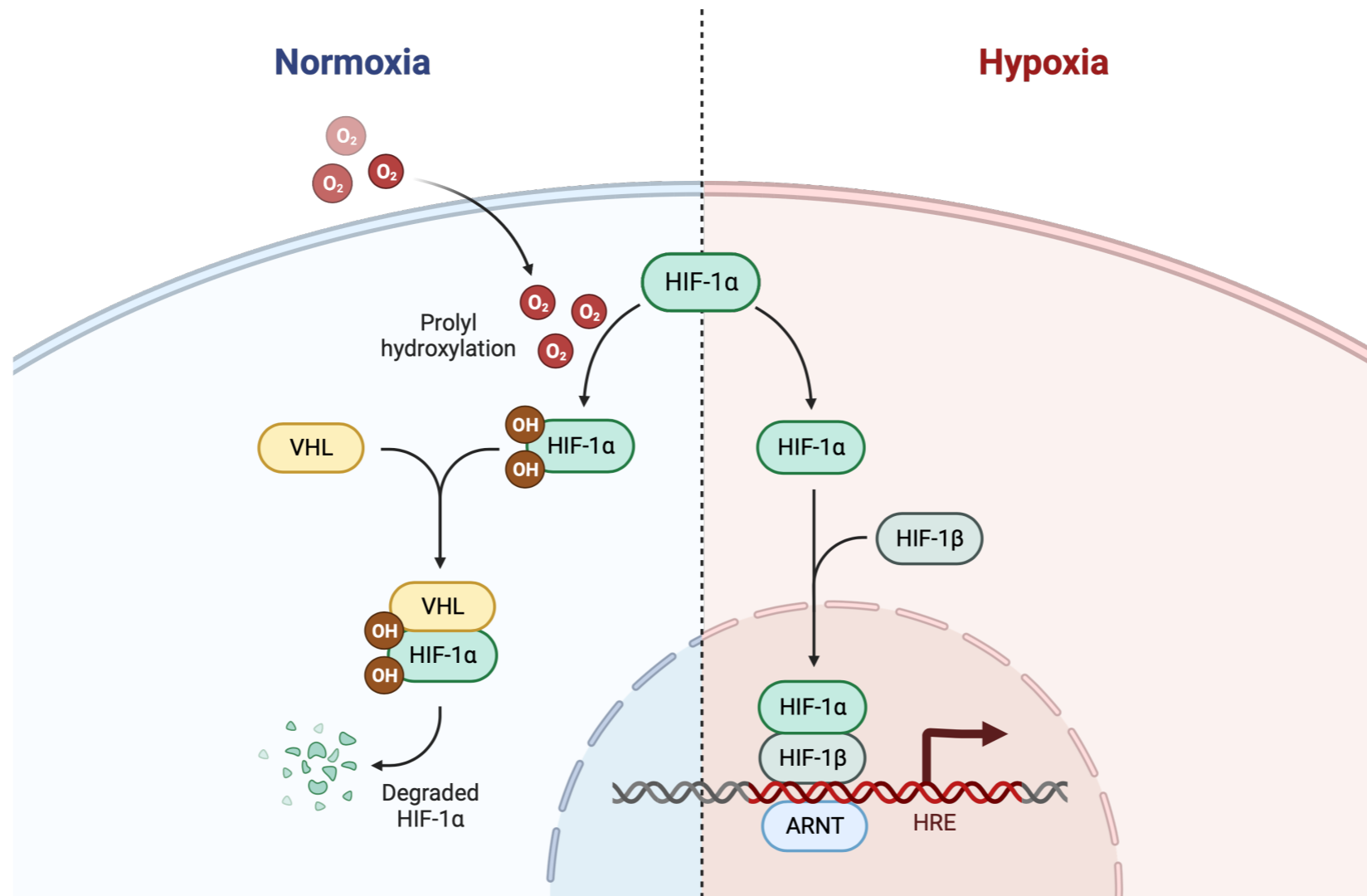
External stimuli can affect ubiquitin-mediated degradation of transcription factors

Stimulus	Substrate	E3 ligase
Hypoxia	HIF1 α	VHL
Oxidative stress	NRF2	KEAP1
DNA damage	P53	MDM2

Stimulus induces the inhibition of transcription factor ubiquitination

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of Transcription Factors: HIF-1 α

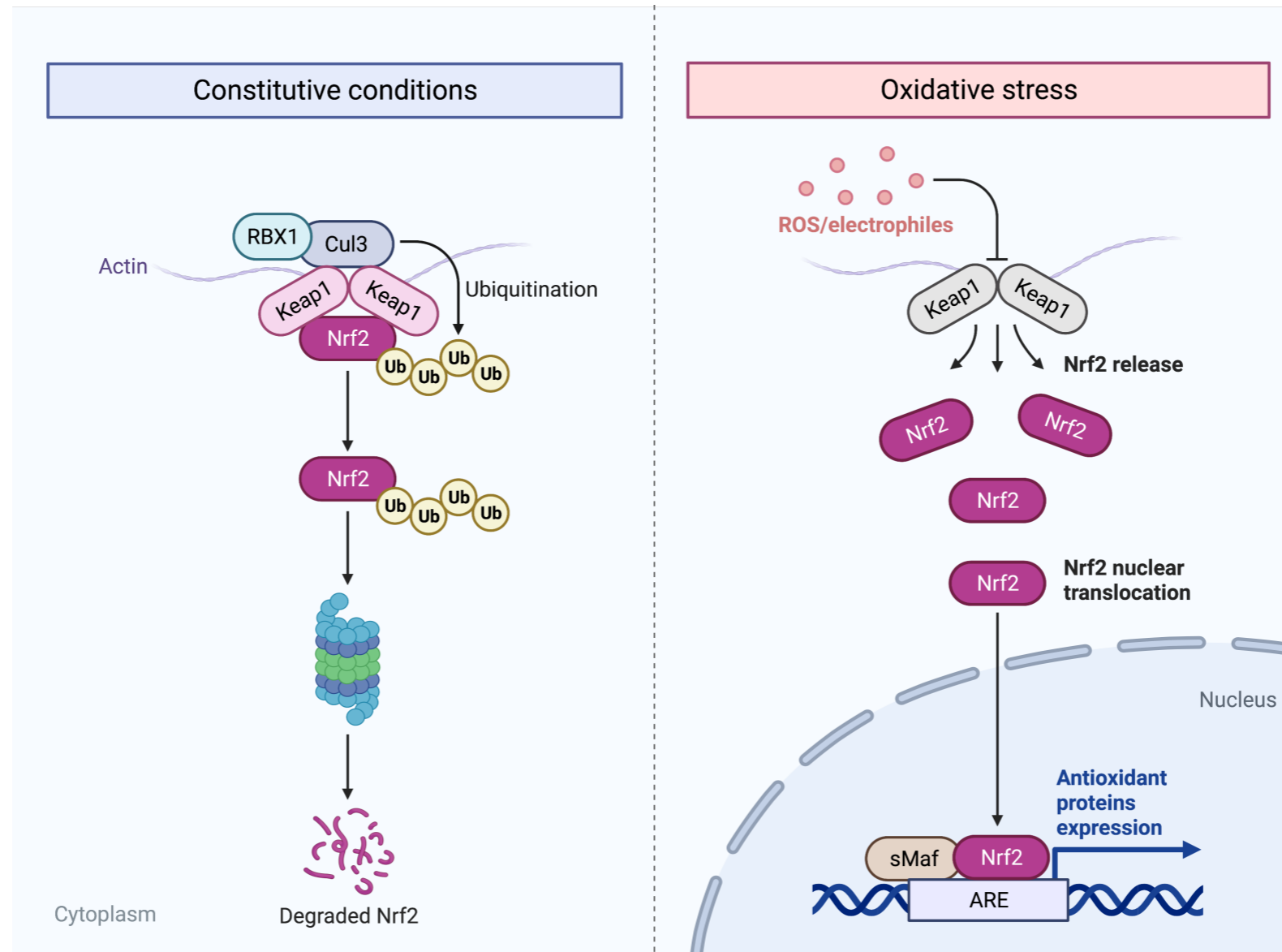


Hypoxic conditions reduce degradation of HIF1 α to promote transcription of genes that increase oxygen delivery

Overexpressed in many cancers to improve tumor vascularization

Ubiquitin-Mediated Regulation: Transcription

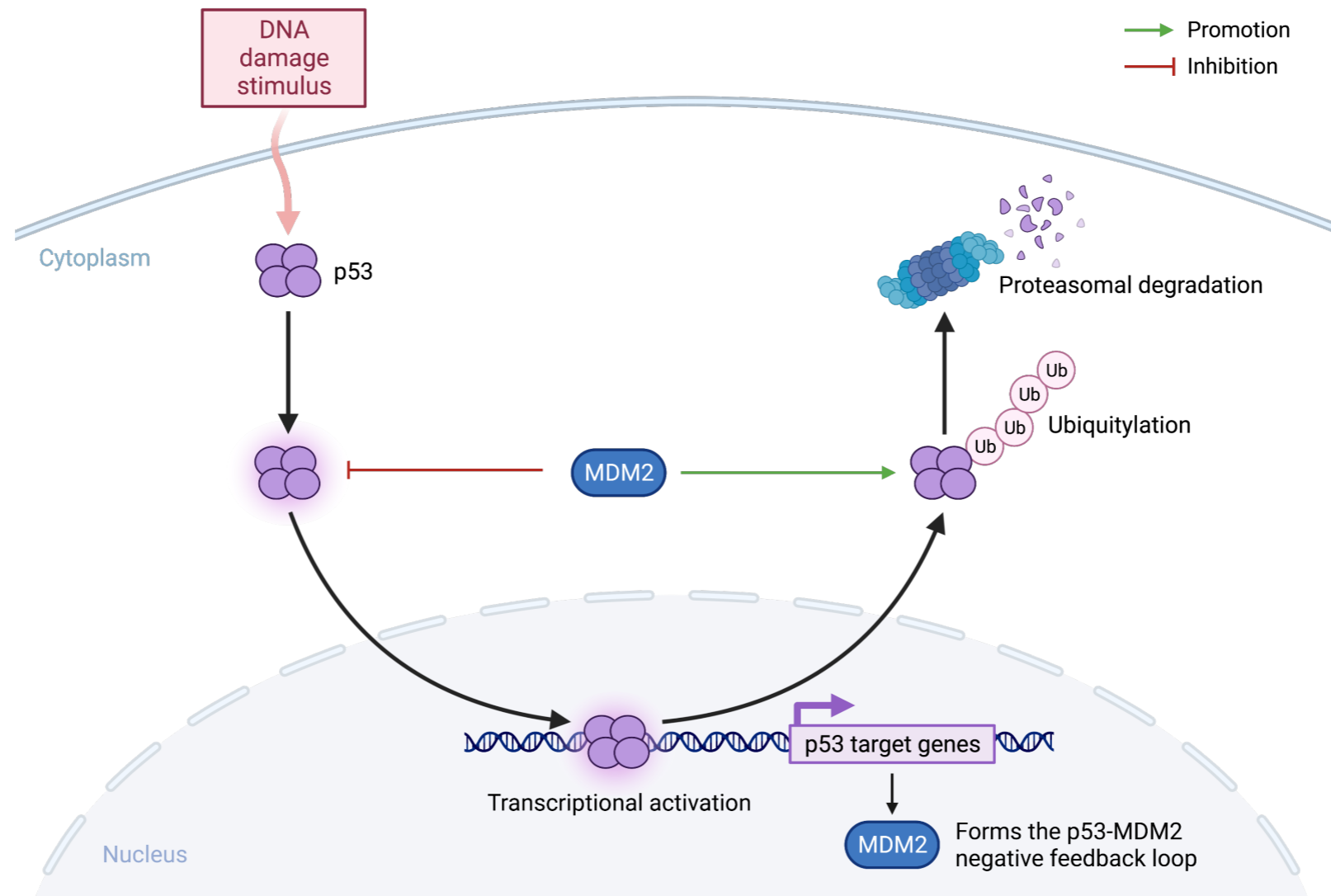
Ubiquitination of Transcription Factors: NRF2



Oxidative stress inhibits NRF2 degradation and leads to antioxidant protein expression

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of Transcription Factors: p53

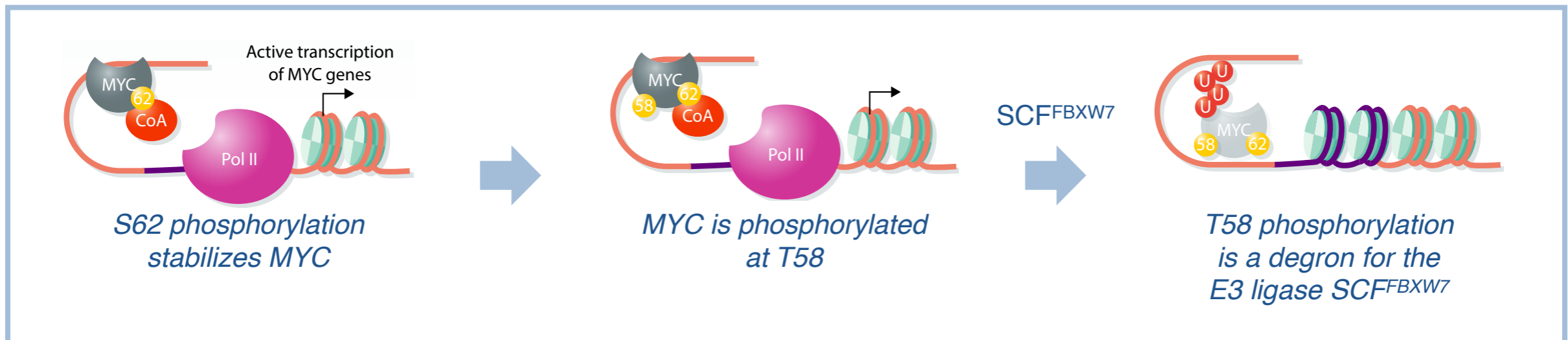
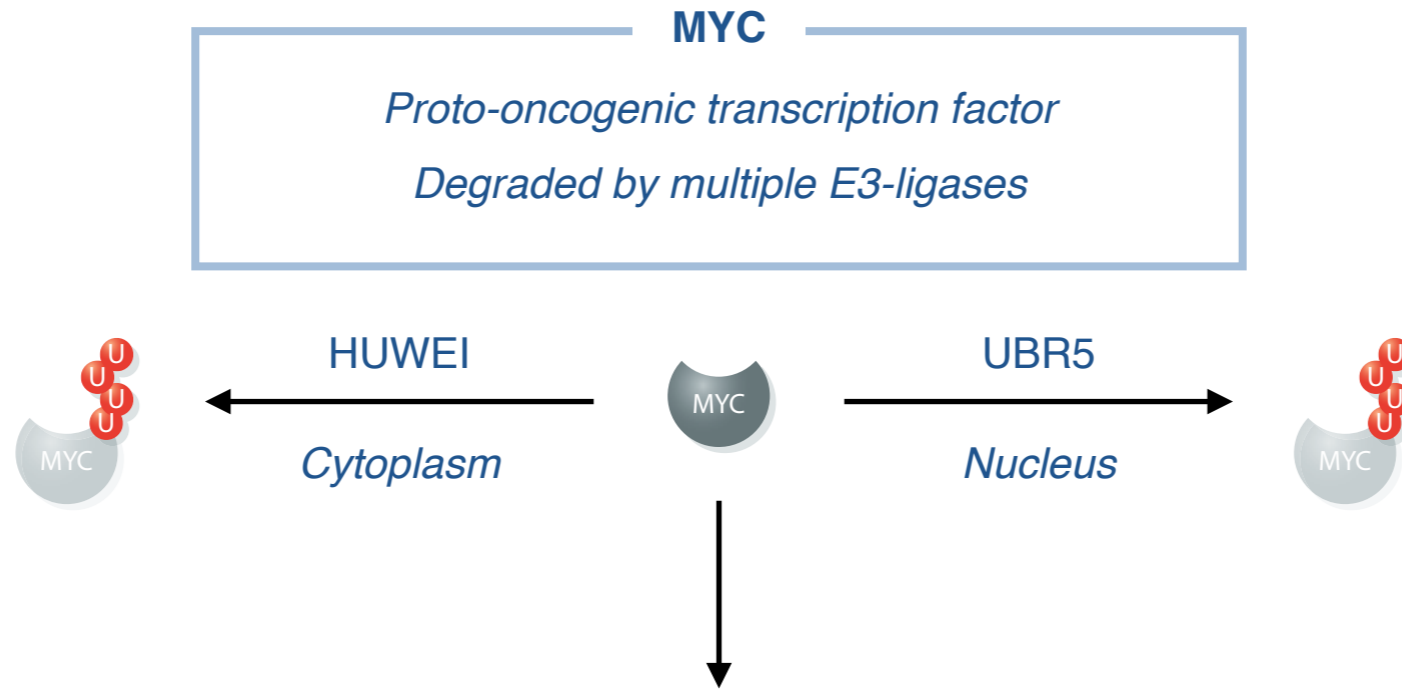


Elevated MDM2 levels suppress P53 and hamper the cell's response to DNA-damage, increasing mutagenesis rates

MDM2 is amplified in a large number of hematological and solid tumors

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of Transcription Factors: MYC



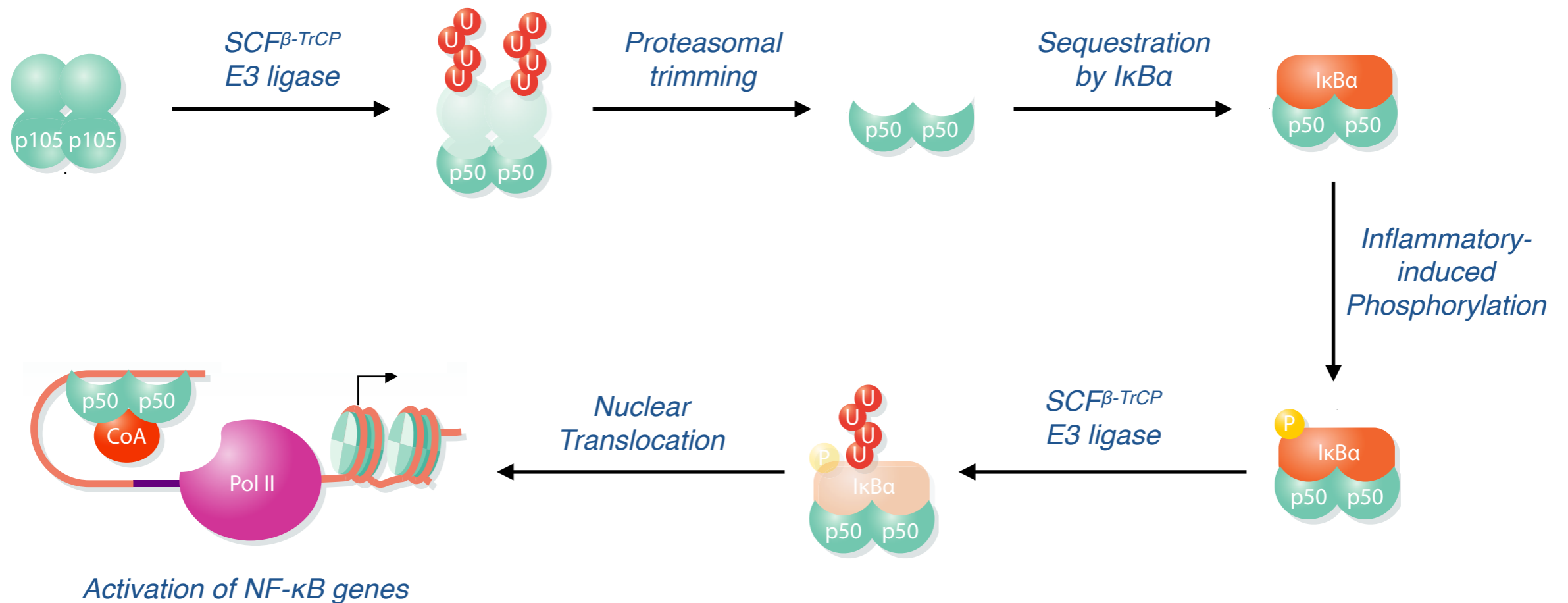
Mutation of T58 of MYC is observed in a high percentage of Burkitt's lymphoma patients

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of Transcription Factors: NF- κ B

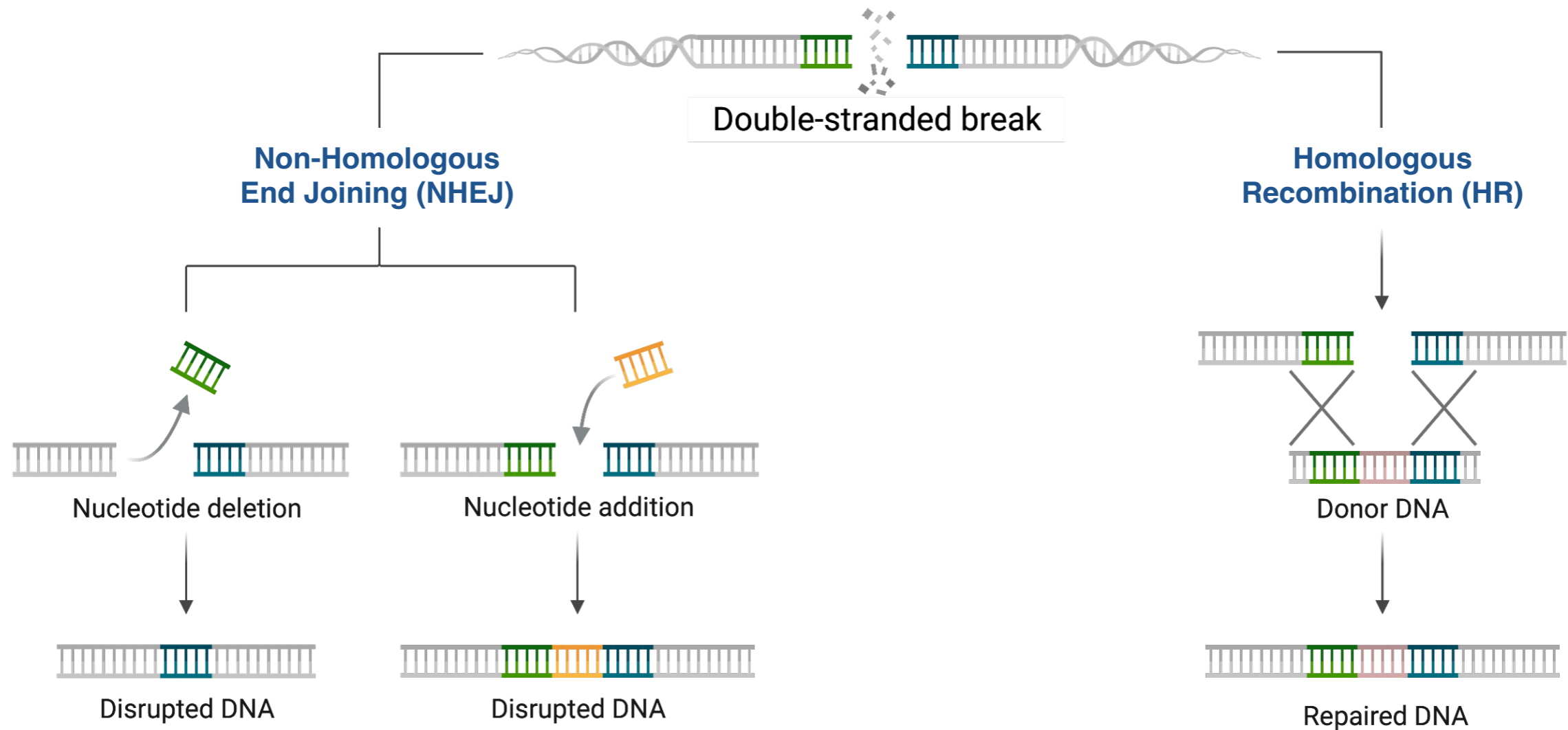
NF- κ B

Family of ROS and cytokine-responsive transcription factors
Aberrant activation is observed in many cancers



Ubiquitin-Mediated Regulation: DNA-Damage Response

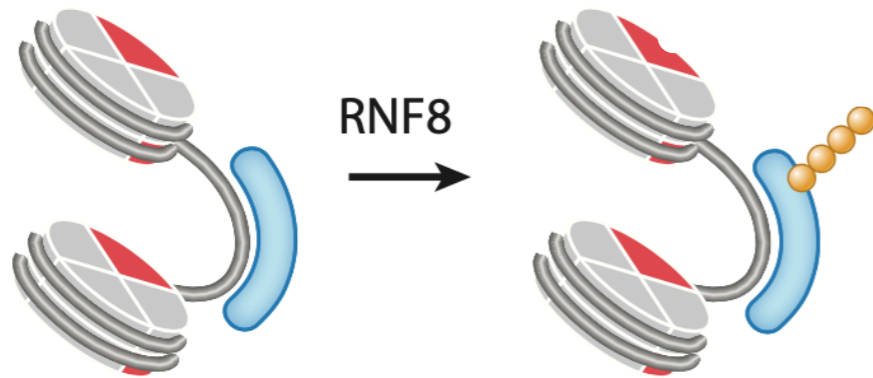
Repairing of Double-stranded DNA Breaks



Under Double-stranded breaks, how does the cell non-homologous end joining or homologous recombination?

Ubiquitin-Mediated Regulation: DNA-Damage Response

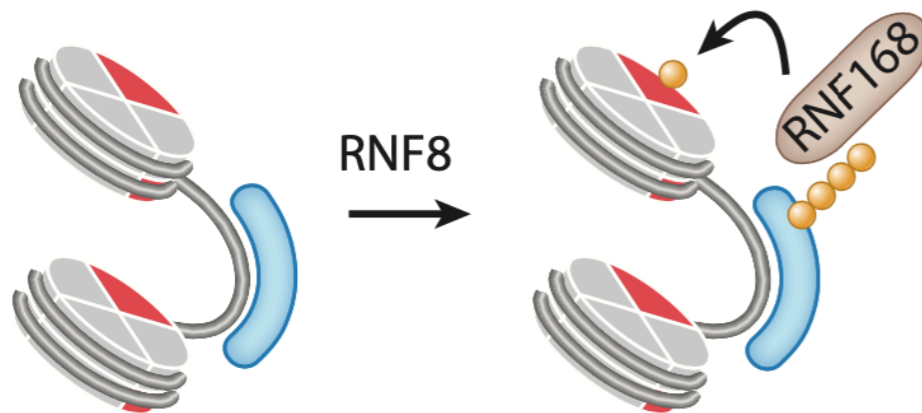
Non-Homologous End Joining (NHEJ)



1) RNF8 decorates H1 linker with K63-linked ubiquitin chains

Ubiquitin-Mediated Regulation: DNA-Damage Response

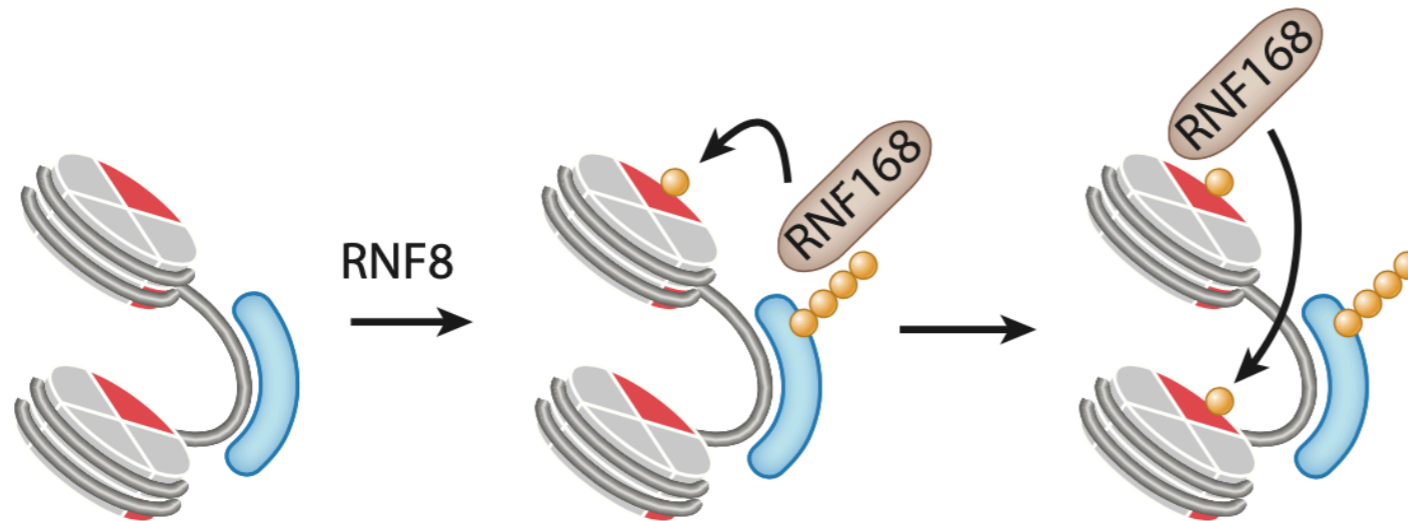
Non-Homologous End Joining (NHEJ)



- 1) RNF8 decorates H1 linker with K63-linked ubiquitin chains
- 2) RNF168 binds to the K63-chains and mono-ubiquitinates K13 and K15 of H2A

Ubiquitin-Mediated Regulation: DNA-Damage Response

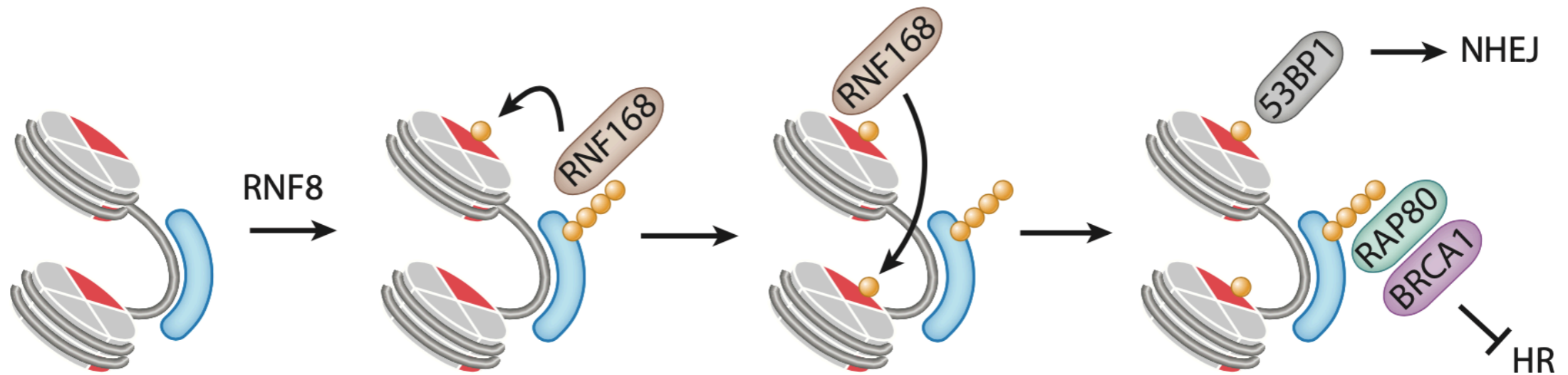
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Ubiquitin-Mediated Regulation: DNA-Damage Response

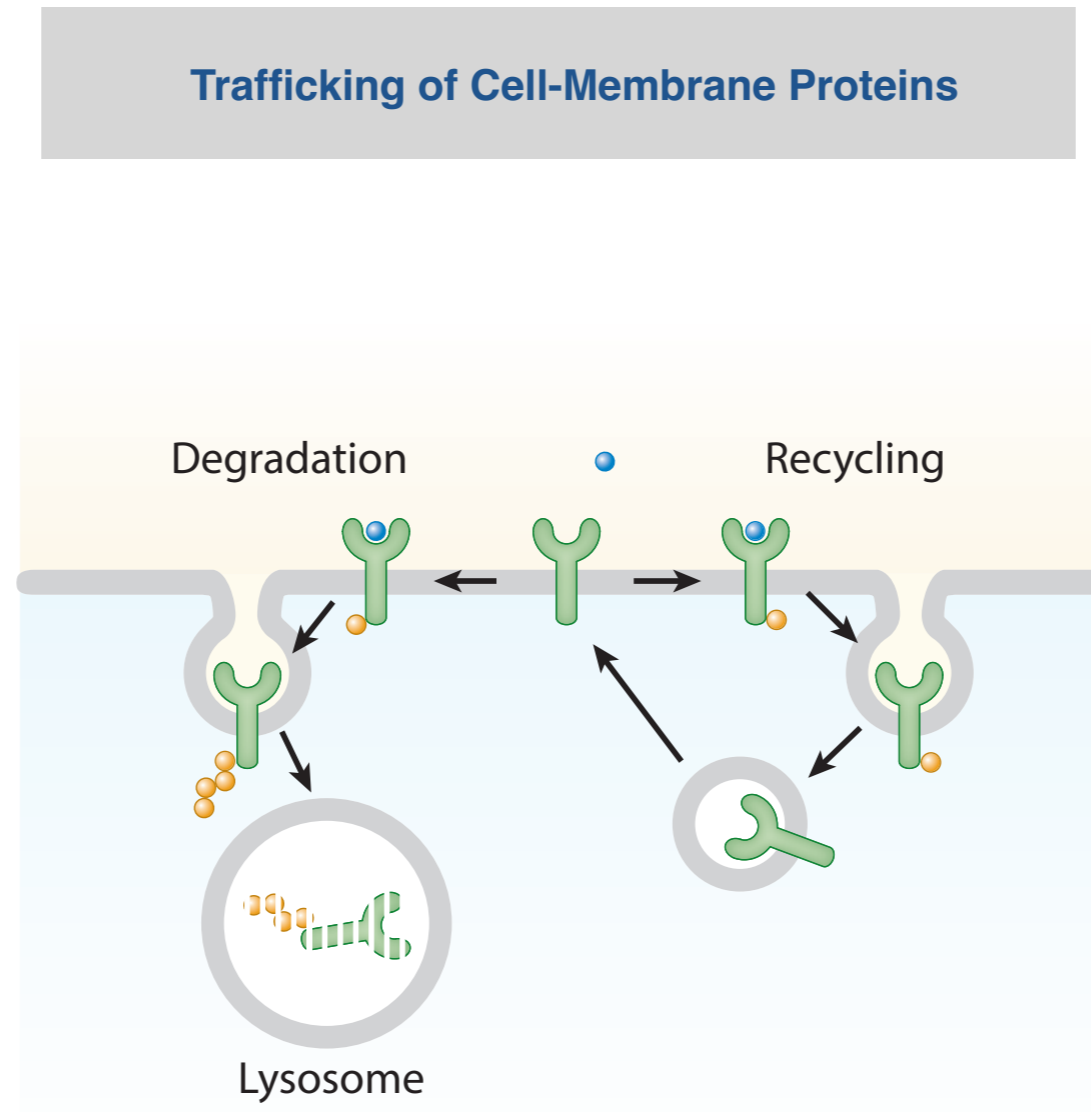
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- 4) 53BP1 attracts proteins that displace HR effectors; RAP80/BRCA1 suppresses BRCA1-mediated HR

Ubiquitin-Mediated Regulation: Protein-Trafficking

Trafficking of Cell-Membrane Proteins

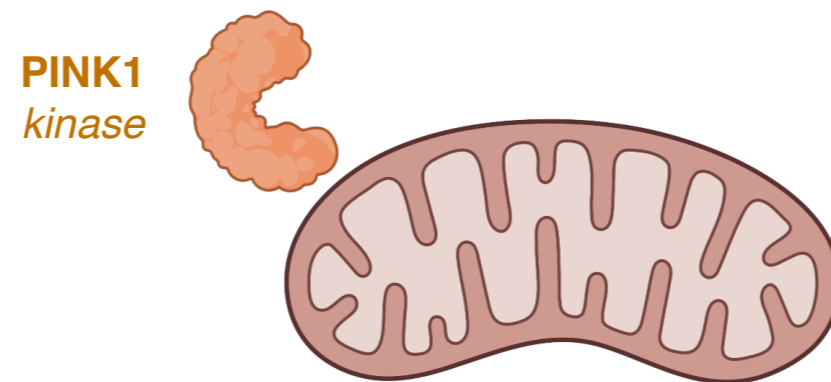


Ubiquitination trafficks membrane proteins to the lysosome or recycles them back to the cell-membrane

Ubiquitin-Mediated Regulation: Protein-Trafficking

Mitophagy

Elimination of defective mitochondria

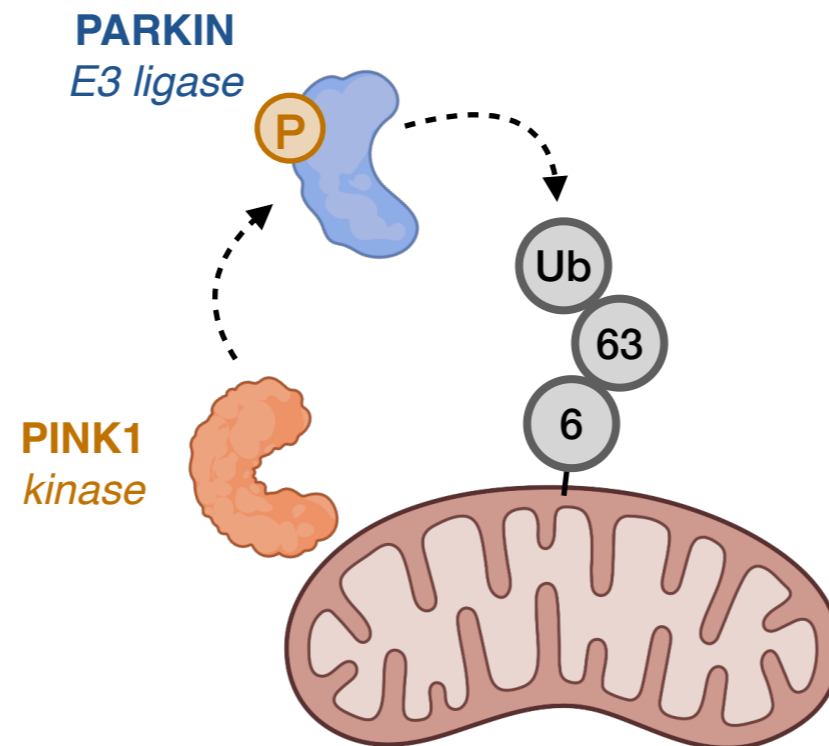


- 1) PINK1 kinase accumulates on outer membrane of damaged mitochondrion

Ubiquitin-Mediated Regulation: Protein-Trafficking

Mitophagy

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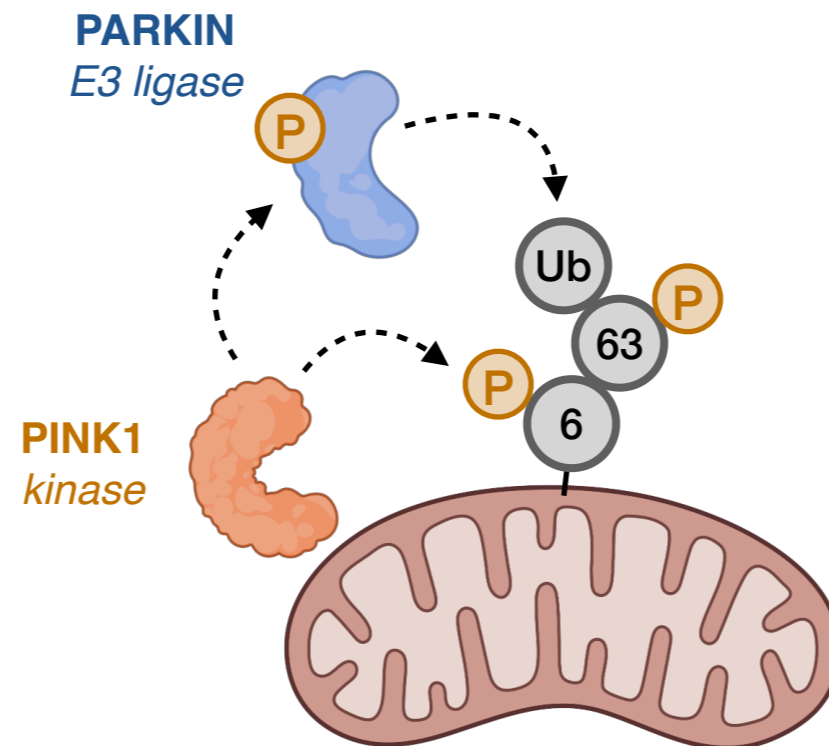


- 1) PINK1 kinase accumulates on outer membrane of damaged mitochondrion
- 2) PINK1 phosphorylates the E3 ligase PARKIN; PARKIN ubiquitinates the outer mitochondrial membrane (K6 and K63)

Ubiquitin-Mediated Regulation: Protein-Trafficking

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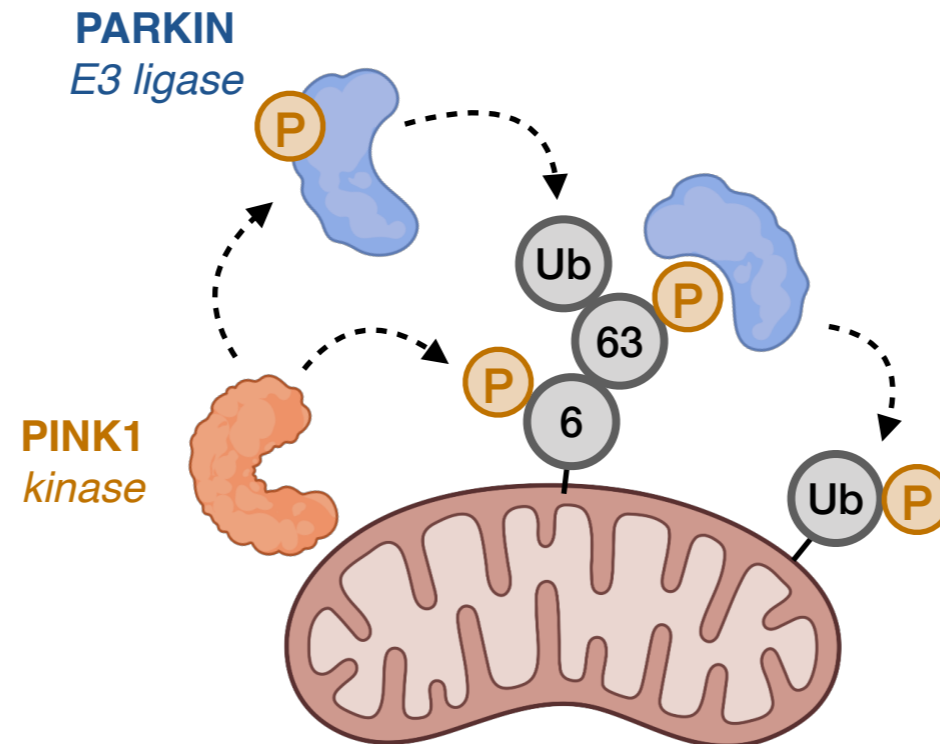


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Ubiquitin-Mediated Regulation: Protein-Trafficking

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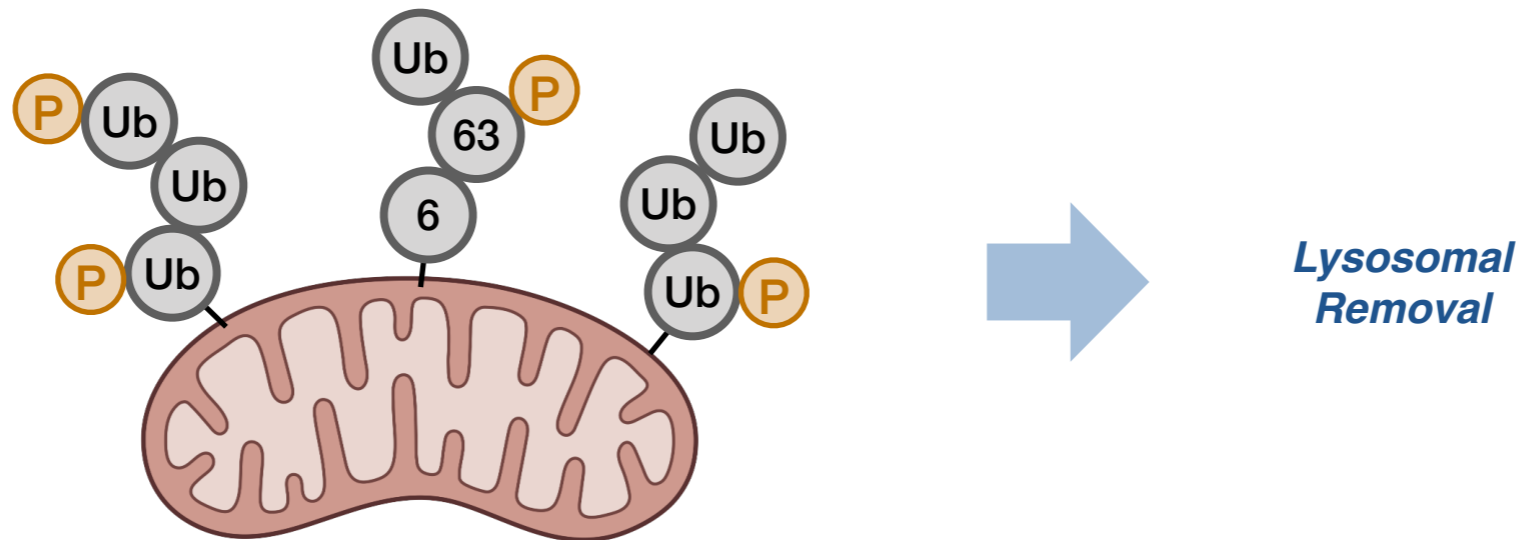


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Ubiquitin-Mediated Regulation: Protein-Trafficking

Mitophagy

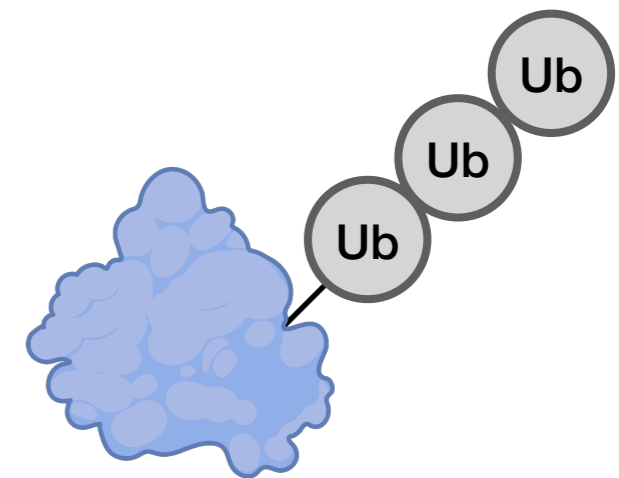
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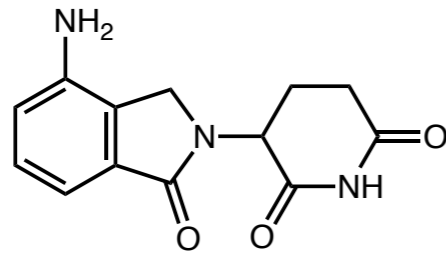
The Ubiquitin Code

- The History of Ubiquitin and How Substrates are Ubiquitinated
- The Ubiquitin Code
- Methods to Study Covalent Modifications by Ubiquitin
- How the Cell uses Ubiquitin for Regulation
 - Cell cycle / The APC/C
 - Transcription
 - DNA damage response
 - Protein-localization
- Therapeutic Outlook

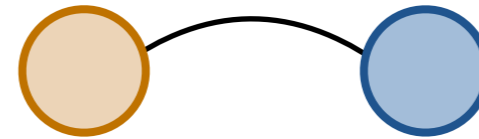


Therapeutic Outlook

Targeted Protein Degradation



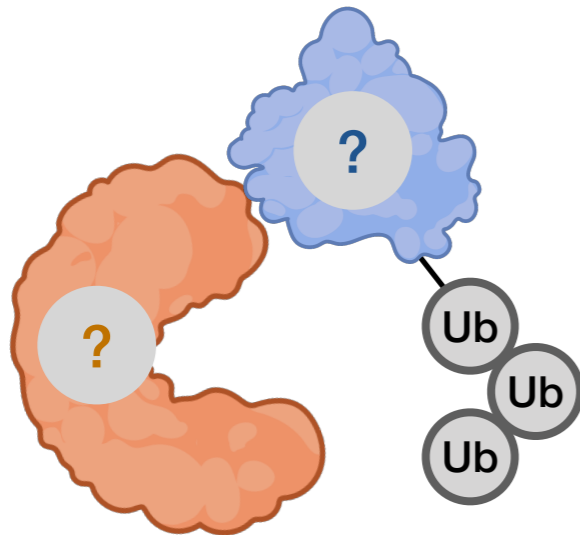
CeLMODs



PROTACs

New E3 ligases beyond CRBN and VHL for Targeted Protein Degradation

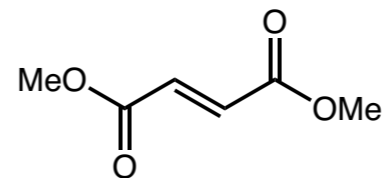
E3 ligase / Substrate Discovery



What E3 ligase is responsible for degrading a protein of interest?

What substrates are targeted by endogenous E3 ligases?

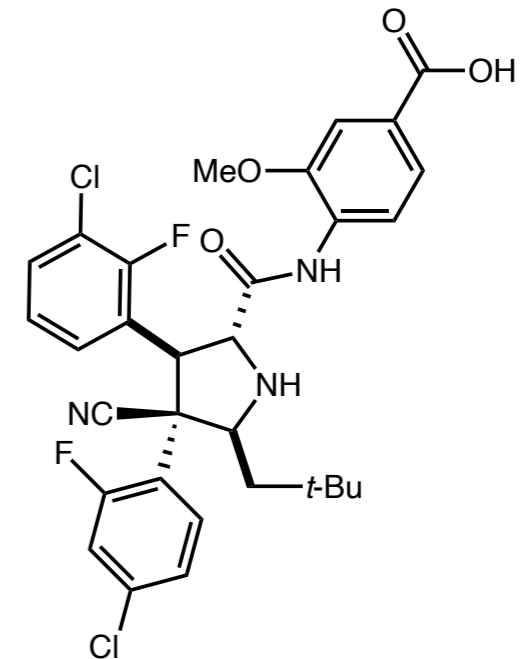
Small-molecule Inhibitors of E3 Ligases



Dimethyl fumarate

NRF2 activator

\$2.4 billion in 2021



Idasanutlin

MDM2 inhibitor

Currently in Phase II Clinical Trials

Questions?

