The Ubiquitin Code

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MacMillan Research Group
Group Meeting
January 24th, 2023
Ubiquitinated proteins are brought to the proteasome and undergo proteolysis.
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
- 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
The Ubiquitin Code

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What is 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 can be conjugated to ubiquitin to form chains.
Ubiquitin Chains

C-terminus

OH

C-terminus
Ubiquitin Chains

Additional ubiquitin proteins can be conjugated to lysine on ubiquitin

Ubiquitin peptide sequence

MQIFVKTLTGTKITLEVEPSDTIENVKAKIQDEGIPPD
QQRLIFAGKQLEDGRTSDYNIKKESTLHLVLRLRGG
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

Ubiquitin peptide sequence

MQIFVKTLTGKITLEVEPSDTIENVKAKIQDKEGIPPD
QQRLIFAGKQLEDGRDYSNIVALIKESTLHLVLRLRGG

C-terminus
The History of Ubiquitin

“For the discovery of Ubiquitin-mediated protein degradation”

Advanced Information on the Nobel Prize in Chemistry 6 October 2004: nobelprize.org
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 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:

\[ \text{Ub} + \text{ATP} \rightleftharpoons \text{Ub} \sim \text{AMP} + \text{PP}_i \]

\[ \text{Ub} \sim \text{AMP} + \text{E-SH} \rightleftharpoons \text{E-S} \sim \text{Ub} + \text{AMP} \]

The History of Ubiquitin

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

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 (E₁), 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 E₂, is bound to the ubiquitin column when E₁ and ATP are present, and is eluted with a thiol compound at high concentration. The third enzyme, designated E₃, 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 ¹²⁵I-ubiquitin with proteins. All three affinity-purified enzymes are also required for the breakdown of ¹²⁵I-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

<table>
<thead>
<tr>
<th></th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<td>–</td>
</tr>
</tbody>
</table>

### 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

<table>
<thead>
<tr>
<th></th>
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<th>E2</th>
<th>E3</th>
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<tbody>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>E3</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Substrate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

SDS-PAGE using Radiolabeled Ub

Numerous Ub-bands observed with E3 addition

Full Mechanism of Substrate Ubiquitination

Deubiquitinases (DUBs)

- **Writers**: E2 enzyme / E3 ligase complex
  - E2 and E3
  - Ub

- **Readers**: Proteins with Ub-binding domains
  - Substrate
  - Ub

- **Erasers**: Deubiquitinases (DUBs)
  - Ub
  - Scissors
Deubiquitinases (DUBs)

Deubiquitinases can hydrolyze ubiquitin conjugates from proteins

Approximately 100 DUB genes in humans

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
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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

**Ubiquitin peptide sequence**
- MQIFVKTTLGTKITLEVEPSDTIENVKAKIQDKEGIPPD
- QQRLIFAGKQLEDGRTLSDYNIQKESTLHLVHLRLGG

All 8 Ub linkages have been detected in cells.
Type of ubiquitin attachment can determine function

The Ubiquitin Code: Linkage Type

<table>
<thead>
<tr>
<th>Linkage</th>
<th>Associated Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>K48</td>
<td>Degradation to the 26S Proteasome</td>
</tr>
<tr>
<td>K11</td>
<td>Can induce protein-interactions; DNA repair and transcription factor activation</td>
</tr>
<tr>
<td>M1</td>
<td>Mitophagy</td>
</tr>
<tr>
<td>K63</td>
<td>Innate Immunity from viral and bacterial infection</td>
</tr>
<tr>
<td>K6</td>
<td></td>
</tr>
<tr>
<td>K27</td>
<td>Signaling and neurodegenerative disorders</td>
</tr>
<tr>
<td>K29</td>
<td>Non-degradative</td>
</tr>
<tr>
<td>K33</td>
<td>Less Characterized</td>
</tr>
</tbody>
</table>

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
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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 leaves “di-gly” motif on lysine

Trypsin leaves ubiquitinated lysine

Ubiquitin Peptide Sequence

MQIFVKTGTGTKTITLEVEPSDTIENV KAKIQDKEGIPPDQRLIFAGKQLE DGRTLSDYNIQKESTLHLVLRLRGG

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

WT Ub → Cell lysate → Denaturing nickel chromatography → Cell lysate → 6xHis-Ub

Trypsin

LC-MS/MS

Set digly of lysine as a modification during database-search

110 ubiquitination sites on 72 proteins identified

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

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

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

Methods to Study Covalent Modifications by Ubiquitin

Ubisite antibody

LysC Protease
Cleaves after lysine

Ubiquitin sequence
MQIFVKTLTGKITLEVEPSDTIENV KAKIQDKEGIPPDQQRLIFAGKQLE
DGRTLSDYNIQKESTLHLVLRLRGG
digestion with LysC protease

Ubisite antibody
recognizes C-terminal region of ubiquitin

Methods to Study Covalent Modifications by Ubiquitin

**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

What type of ubiquitin chain is on a protein?

Methods to Study Covalent Modifications by Ubiquitin

Approximately 100 DUB genes in humans

Ubicrest

Erasers

Deubiquitinases (DUBs)

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)

Methods to Study Covalent Modifications by Ubiquitin

Example: K63-Ubiquitinated protein

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<tr>
<td>Lys33</td>
<td>OTUD2 (Lys11, Lys27, Lys29, Lys33)</td>
</tr>
<tr>
<td>Lys48</td>
<td>TRABID (Lys29, Lys33, Lys63)</td>
</tr>
<tr>
<td>Lys63</td>
<td>OTUB1 (Lys48)</td>
</tr>
<tr>
<td>Met1</td>
<td>OTUD1 (Lys63)</td>
</tr>
<tr>
<td></td>
<td>OTULIN (Met1)</td>
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Certain DUBs prefer deubiquitination of specific Ub linkages

Methods to Study Covalent Modifications by Ubiquitin

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**Ubicrest**

Disappearance of polyubiquitin smear is key result

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<td>OTUB1 (Lys48)</td>
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<tr>
<td>Met1</td>
<td>OTUD1 (Lys63)</td>
</tr>
<tr>
<td>Substrate-/</td>
<td>OTULIN (Met1)</td>
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Certain DUBs prefer deubiquitination of specific Ub linkages

Disappearance of polyubiquitin smear is key result
Methods to Study Covalent Modifications by Ubiquitin

**Ubicrest**

Disappearance of polyubiquitin smear is key result

Some deubiquitinases can behave nonspecifically to release longer chains

Methods to Study Covalent Modifications by Ubiquitin

TUBES (Tandem-Repeated Ubiquitin-Binding Entities)

Advantages: Protects ubiquitin sites from proteasomal degradation and deubiquitinase cleavage

Ubiquilin 1 promotes the delivery of ubiquitinated proteins to the proteasome

Ubiquitin-binding domain

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

Hjerpe, R. et al. EMBO Reports 2009, 10, 1250.
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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**

- Cyclin A
- Cyclin E
- Cyclin B
- Cyclin D

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

SKP / Cullin / F-box-Containing Complex

Anaphase Promoting Complex (APC/C)

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

Promotes progression through S and G2 phase

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

Anaphase Promoting Complex (APC/C)

Mitosis
Cell division

G2 Phase
Preparation for mitosis

S Phase
DNA-replication

G1 Phase
Cell growth

Promotes progression through Mitosis and G1 phase

Ubiquitin-Mediated Regulation: Control of the Cell-Cycle

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

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>K6</th>
<th>K11</th>
<th>K48</th>
<th>K63</th>
</tr>
</thead>
<tbody>
<tr>
<td>time (hr)</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

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

Anaphase Promoting Complex (APC/C)

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

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

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

K11-chains/α-tubulin/DNA

<table>
<thead>
<tr>
<th>prometaphase</th>
<th>metaphase</th>
<th>anaphase</th>
<th>early telophase</th>
<th>late telophase</th>
</tr>
</thead>
</table>

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

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

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

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

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

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

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

Ira L. Goldknope,‡ 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

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Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2A: Transcriptional Repression

1) PRC1 ubiquitinites K27 on H2A
2) PRC2 recognizes monoubiquitinated H3 and tri-methylates K27 on H3
3) PRC1 binds to H3K27Me3 and ubiquitinitates adjacent H2A histones

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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*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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4) SETDB1 is recruited to H3K27Me3 and tri-methylates K9 on H3

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5) H3K9Me3 recruits silencing factors such as HP1

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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3) PRC1 binds to H3K27Me3 and ubiquitinates adjacent H2A histones
4) SETD2B1 is recruited to H3K27Me3 and tri-methylates K9 on H3
5) H3K9Me3 recruits silencing factors such as HP1

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
3) PRC1 binds to H3K27Me3 and ubiquitinates adjacent H2A histones
4) SETDB1 is recruited to H3K27Me3 and tri-methylates K9 on H3
5) H3K9Me3 recruits silencing factors such as HP1

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

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of H2B: Transcriptional Elongation

Monoubiquitination of H2B promotes gene expression

Mark, K. G.; Rape, M. EMBO Reports 2021, 22, e51078.
**Ubiquitin-Mediated Regulation: Transcription**

Monoubiquitination of H2B promotes gene expression

Monoubiquitination of H2B promotes gene expression

Ubiquitin-Mediated Regulation: Transcription

Monoubiquitination of H2B promotes gene expression

Monoubiquitinated H2B can attract over ninety effectors

**Ubiquitin-Mediated Regulation: Transcription**

*Single transcription factors can regulate multiple genes*

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**External stimuli can affect ubiquitin-mediated degradation of transcription factors**

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Substrate</th>
<th>E3 ligase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoxia</td>
<td>HIF1α</td>
<td>VHL</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>NRF2</td>
<td>KEAP1</td>
</tr>
<tr>
<td>DNA damage</td>
<td>P53</td>
<td>MDM2</td>
</tr>
</tbody>
</table>

*Stimulus induces the inhibition of transcription factor ubiquitiation*

Ubiquitin-Mediated Regulation: Transcription

Ubiquitination of Transcription Factors: HIF-1α

Hypoxic conditions reduce degradation of HIF1α 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

Mark, K. G.; Rape, M. EMBO Reports 2021, 22, e51078.
**Ubiquitin-Mediated Regulation: Transcription**

**Ubiquitination of Transcription Factors: MYC**

**MYC**

*Proto-oncogenic transcription factor*

*Degraded by multiple E3-ligases*

- **HUWEI**
  - Cytoplasm
- **UBR5**
  - Nucleus

**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-κB

**NF-κB**

*Family of ROS and cytokine-responsive transcription factors*

*Aberrant activation is observed in many cancers*

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**SCFβ-TrCP E3 ligase**

**Proteasomal trimming**

**Sequestration by IκBα**

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**Activation of NF-κB genes**

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Mark, K. G.; Rape, M. *EMBO Reports* 2021, 22, e51078.
Ubiquitin-Mediated Regulation: DNA-Damage Response

Repairing of Double-stranded DNA Breaks

Non-Homologous End Joining (NHEJ)
- Nucleotide deletion
  - Disrupted DNA
- Nucleotide addition
  - Disrupted DNA

Homologous Recombination (HR)
- Donor DNA
  - Repaired DNA

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

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**Ubiquitin-Mediated Regulation: DNA-Damage Response**

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Ubiquitin-Mediated Regulation: DNA-Damage Response

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3) RNF168 itself binds mono-ubiquitinated H2A$^{K15}$ and spreads this signal to adjacent histones
4) 53BP1 attracts proteins that displace HR effectors; RAP80/BRCA1 suppresses BRCA1-mediated HR

Ubiquitin-Mediated Regulation: Protein-Trafficking

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

1) PINK1 kinase accumulates on outer membrane of damaged mitochondrion

Ubiquitin-Mediated Regulation: Protein-Trafficking

Elimination of defective mitochondria

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)

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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**

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?