UBIQUITIN MODIFICATION IN EUKARYOTES CATALYZED BY BACTERIAL (PATHOGEN) ENZYMES

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DEFINITION:

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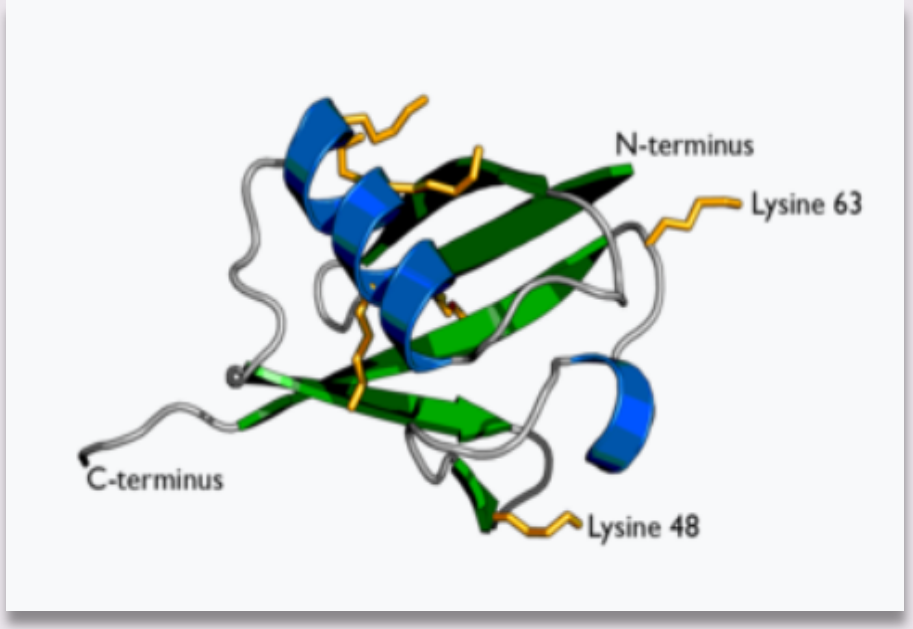
Ubiquitination (Ubiquitylation)

- Occurs *ubiquitously* in eukaryotic cells
- Reversible post-translational modification
- Covalent attachment of 1 or more ubiquitin proteins to substrate proteins
  - Ubiquitin is a small protein consisting of 76 amino acids
- Conjugation usually occurs on lysine residues (most often) or on the amino group of the substrate protein's N-terminus (less common)
  - Iso-peptide and peptide bond formation, respectively.

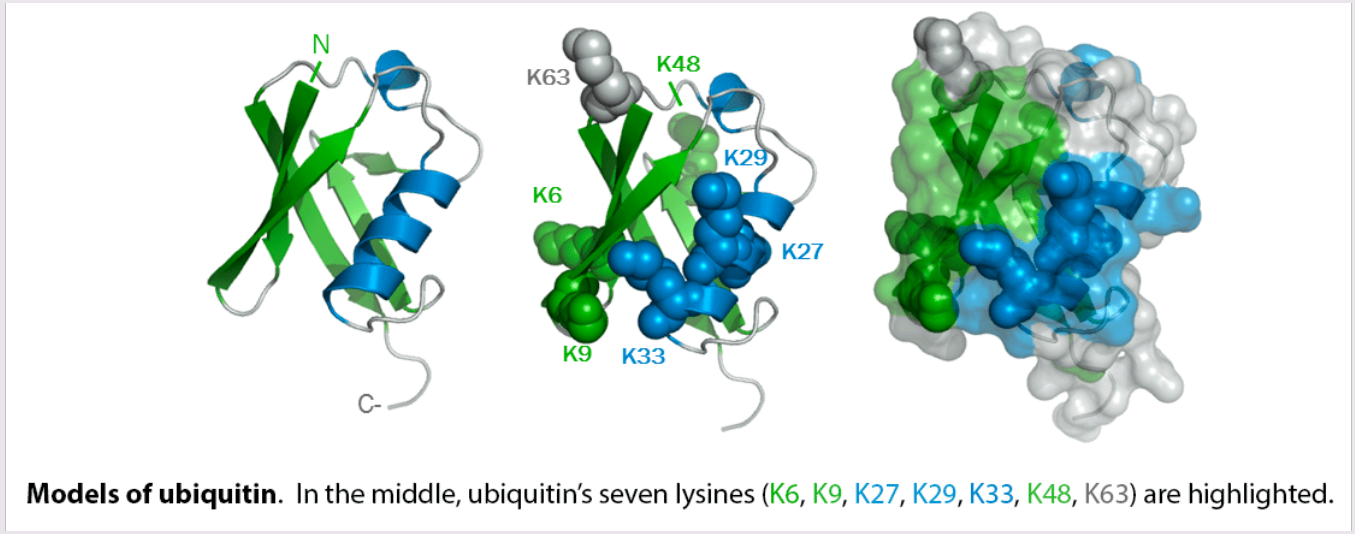


Ubiquitin Protein. Figure by Roger B. Dodd.

https://en.wikipedia.org/wiki/Ubiquitin

Detailed Chemistry

- Ubiquitin contains 7 lysine residues
  - *Lys6, Lys9, Lys11, Lys27, Lys29, Lys33, Lys48, Lys63*
- Conjugated to ε-amine group of a lysine residue in the substrate through its C-terminal glycine residue
- The attachment of ubiquitin to a substrate achieved through activity of a three enzyme cascade
  - *E1: ubiquitin-activating enzyme*
  - *E2: ubiquitin-conjugating enzyme*
  - *E3: ubiquitin ligase*
- ATP required for E1 to activate ubiquitin, then transferred to E2 through a thioester bond
- E3 catalyzes transfer of ubiquitin to the substrate



Models of ubiquitin. In the middle, ubiquitin's seven lysines (K6, K9, K27, K29, K33, K48, K63) are highlighted.

Figure from C4 Therapeutics: http://c4therapeutics.com/ubiquitin/

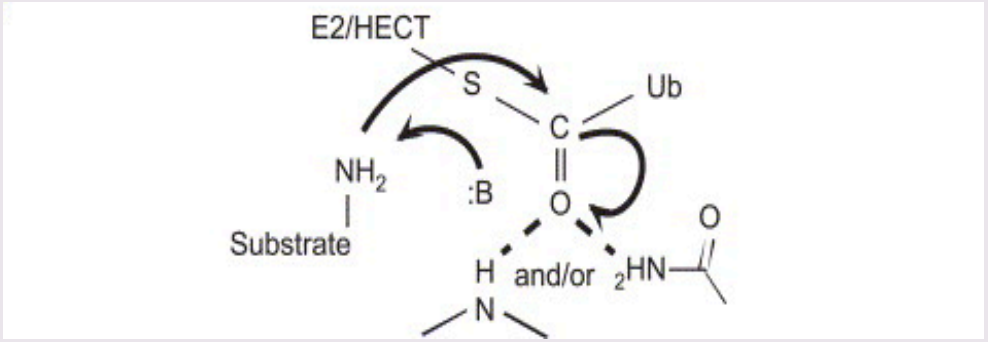


Figure from Pickart, C. M., and M. J. Eddins, 2004
doi: 10.1016/j.bbamcr.2004.09.019

Overview of attachment and removal of ubiquitin from target proteins.

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Ubiquitination Cascade in Eukaryotes (Canonical ubiquitination)

Activation via E1 ubiquitin-activating enzyme

- *Thioester linkage forms between ubiquitin and E1*
- *ATP dependent*
- *AMP and pyrophosphate released*

Conjugation via E2 ubiquitin-conjugating enzyme

- *E2 binds to both E1 and the activated ubiquitin molecule*

Ligation via E3 ubiquitin ligase

- *HECT domain*
  - Thioester intermediate
- *RING/U-box domain*
  - Direct transfer

Unconventional E3s (Non-Canonical Ubiquitination)

- *HECT, RING/U-box, F-box Mimics*
- *NELs*
- XL-box
- Other

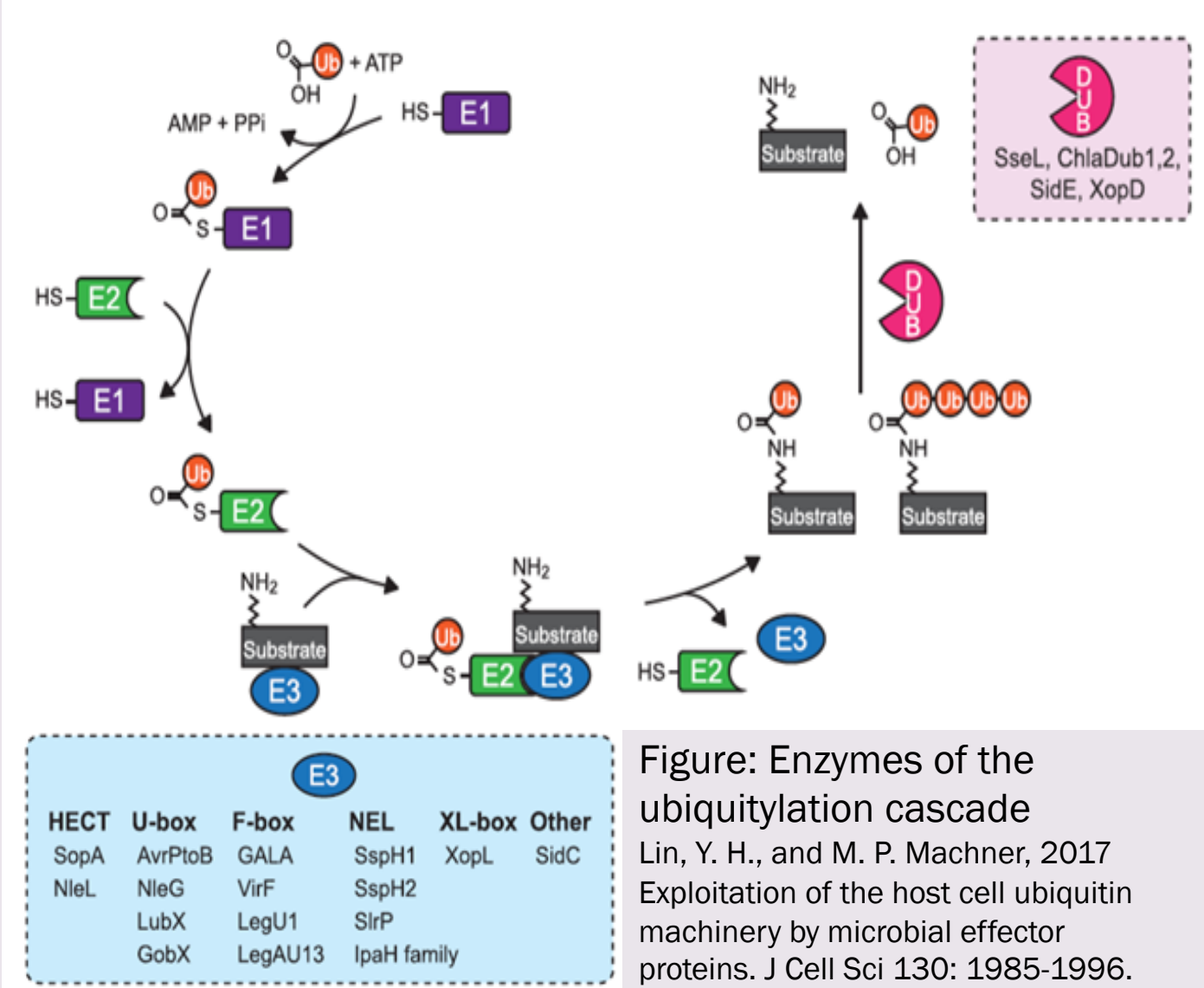

Figure: Enzymes of the ubiquitylation cascade

Lin, Y. H., and M. P. Machner, 2017 Exploitation of the host cell ubiquitin machinery by microbial effector proteins. J Cell Sci 130: 1985-1996.

Overview of attachment and removal of ubiquitin from target proteins.

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- Types of ubiquitination
  - Mono-ubiquitination
    - One ubiquitin to one protein substrate
  - Poly-ubiquitination
    - Chain forms off of single lysine residue
  - Multi-mono-ubiquitination
    - Multiple individual ubiquitins attached to one substrate protein
  - Type of ubiquitination determines fate of the substrate protein

- Deubiquitination
  - DUBS (Deubiquitinases)
  - Protease enzymes can cleave both isopeptide and peptide bonds
    - Cysteine Proteases
    - Metalloproteases

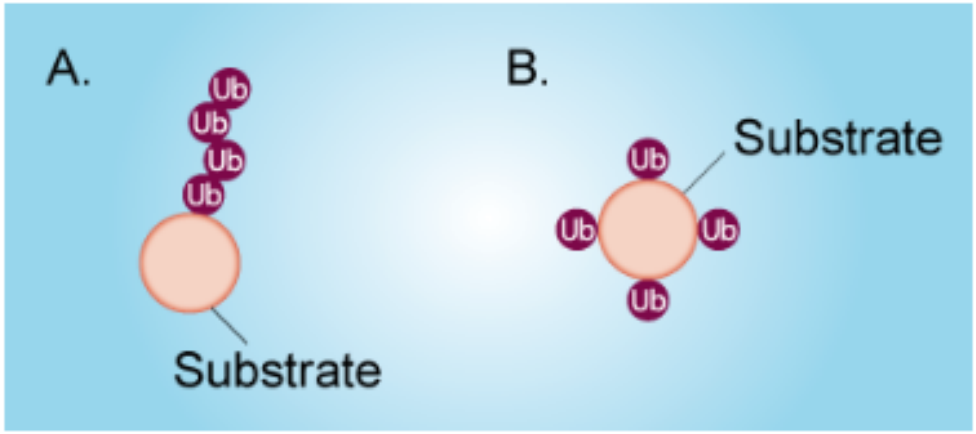

Figure 1. Poly-ubiquitination vs Multi-mono-ubiquitination

https://www.rndsystems.com/resources/protocols/distinguish-between-poly-ubiquitination-and-multi-mono-ubiquitination

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Overview of attachment and removal of ubiquitin from target proteins.

- Ubiquitination catalyzed by bacterial effector proteins (Non-canonical)
  - Type 3 and Type 4 Secretion Systems (T3SS & T4SS)
  - E3 ligase mimics
    - HECT-type mimics
      - *SopA produced by Salmonella Typhimurium*
    - RING/U-box type mimics
      - LubX produced by *Legionella pneumophila*
    - F-box mimic
  - Novel E3 ligases (NELs)
    - IpaH family: *Salmonella*, *Shigella*, *Pseudomonas*, and *Yersinia* species.
    - SidC: *Legionella* species
  - Other bacterial E3 ligases
    - F-box
    - XL-box
  - Deubiquitinase (DUB) mimics
    - *Salmonella* and *Chlamydia trachomatis*

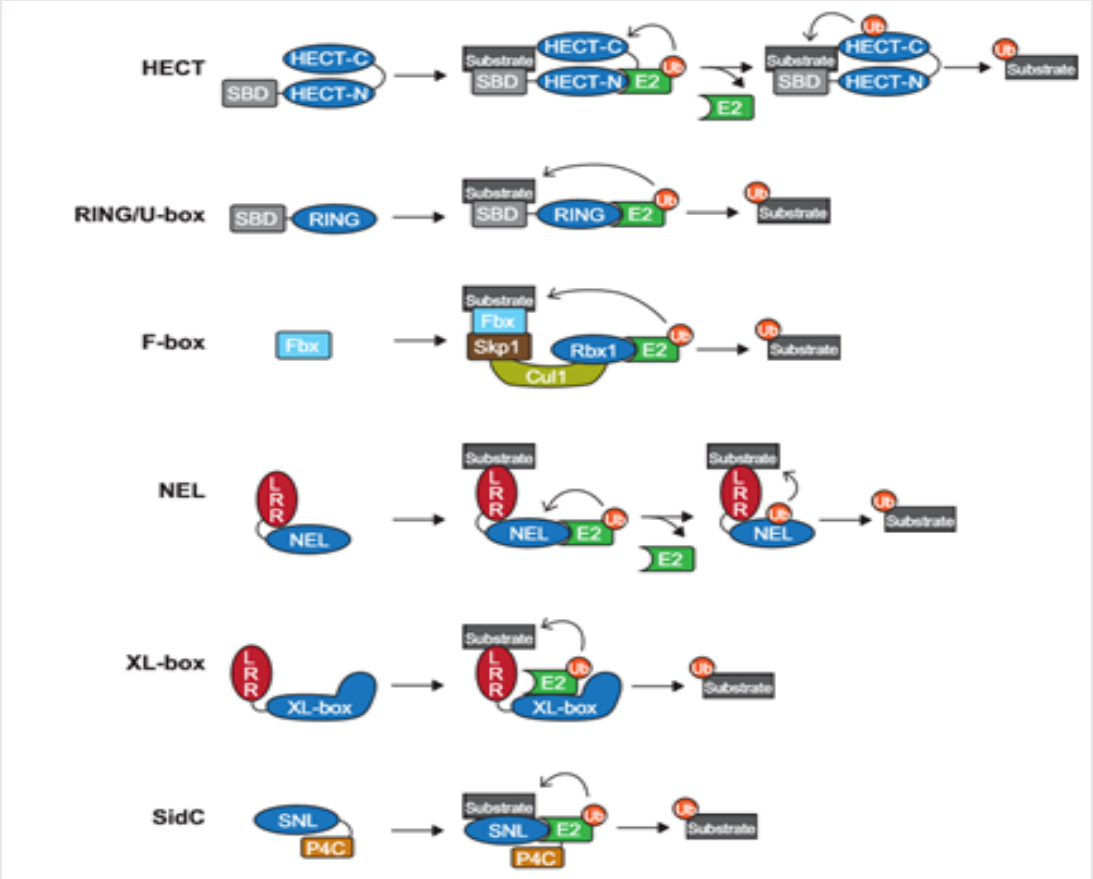


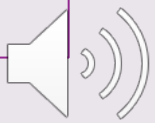
Figure: Categories of E3 bacterial ubiquitin ligases
Lin, Y. H., and M. P. Machner, 2017 Exploitation of the host cell ubiquitin machinery by microbial effector proteins. J Cell Sci 130: 1985-1996.

Details on protein factors of the ubiquitination pathway, including ubiquitin protein modifiers, enzymes catalyzing the addition and removal of the post-translational modification

- HECT-type E3 ligases
  - *SopA*
  - *NleL*
- RING/U-box-like E3 ligases
  - *AvrPtoB*
  - *NleG*
  - *LubX and GobX*
- F-box domain proteins
  - *Cul1 and Rbx1*
  - *LegU1*
  - *AnkB and ParvB*
- Novel E3 ligases (NELs)
  - *IpaH family*
  - *SspH1, SspH2, and SlrP*
  - *SidC and SdcA*
- Deubiquitinating enzymes (DUBs)
  - *SseL*
  - *ChlaDUB1 and ChlaDUB2*
  - *YopJ and YopP*
  - *TssM*

General distribution/function among the three domains of life.

- Even though the ubiquitin system is present in eukaryotes, it is absent in prokaryotes and archaea
- Some bacterial pathogens of eukaryotes have evolved mechanisms that hijack the ubiquitin system of the host
- These hijacking mechanisms are present in some plant and mammalian bacterial pathogens
- The enzymes of pathogenic bacteria involved are effector proteins secreted through type III and IV secretion systems
- The main focus of these Ub modifications for the presentation is the mammalian pathogens such *Shigella, Salmonella, Legionella, E. coli*, and *Yersinia*



Type III Effector Proteins and Their Role in Ubiquitin Modification in Eukaryotes

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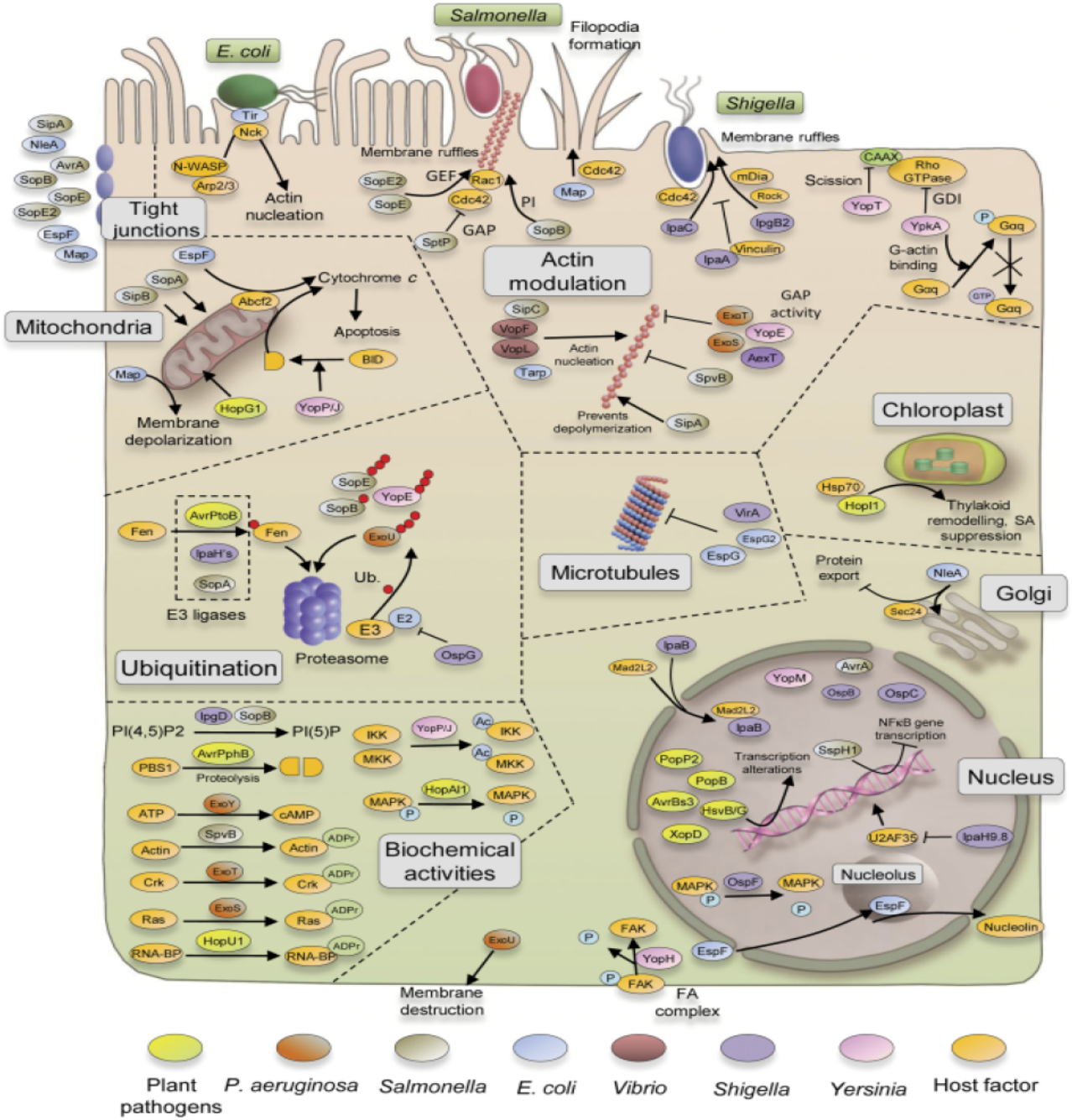

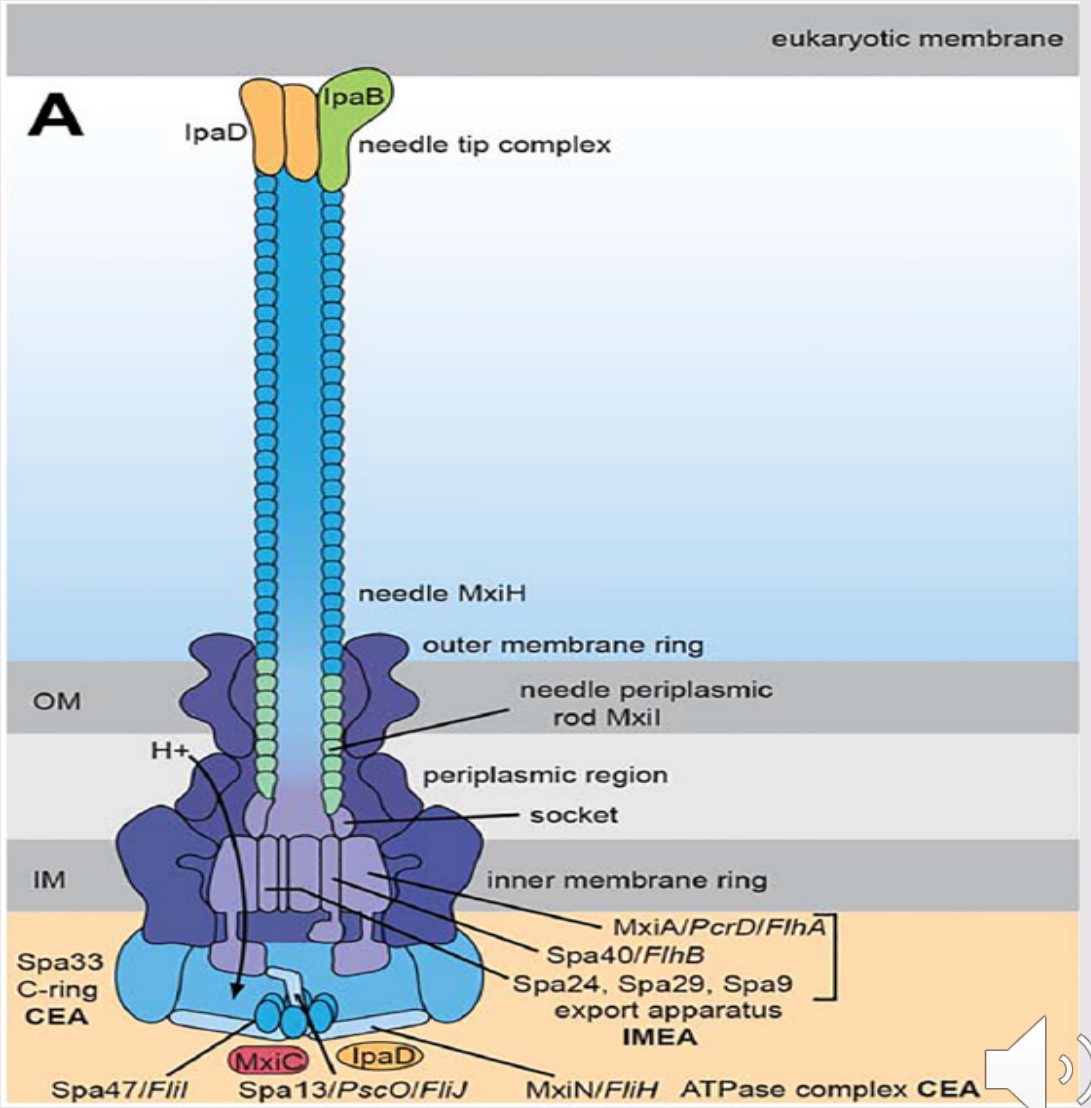

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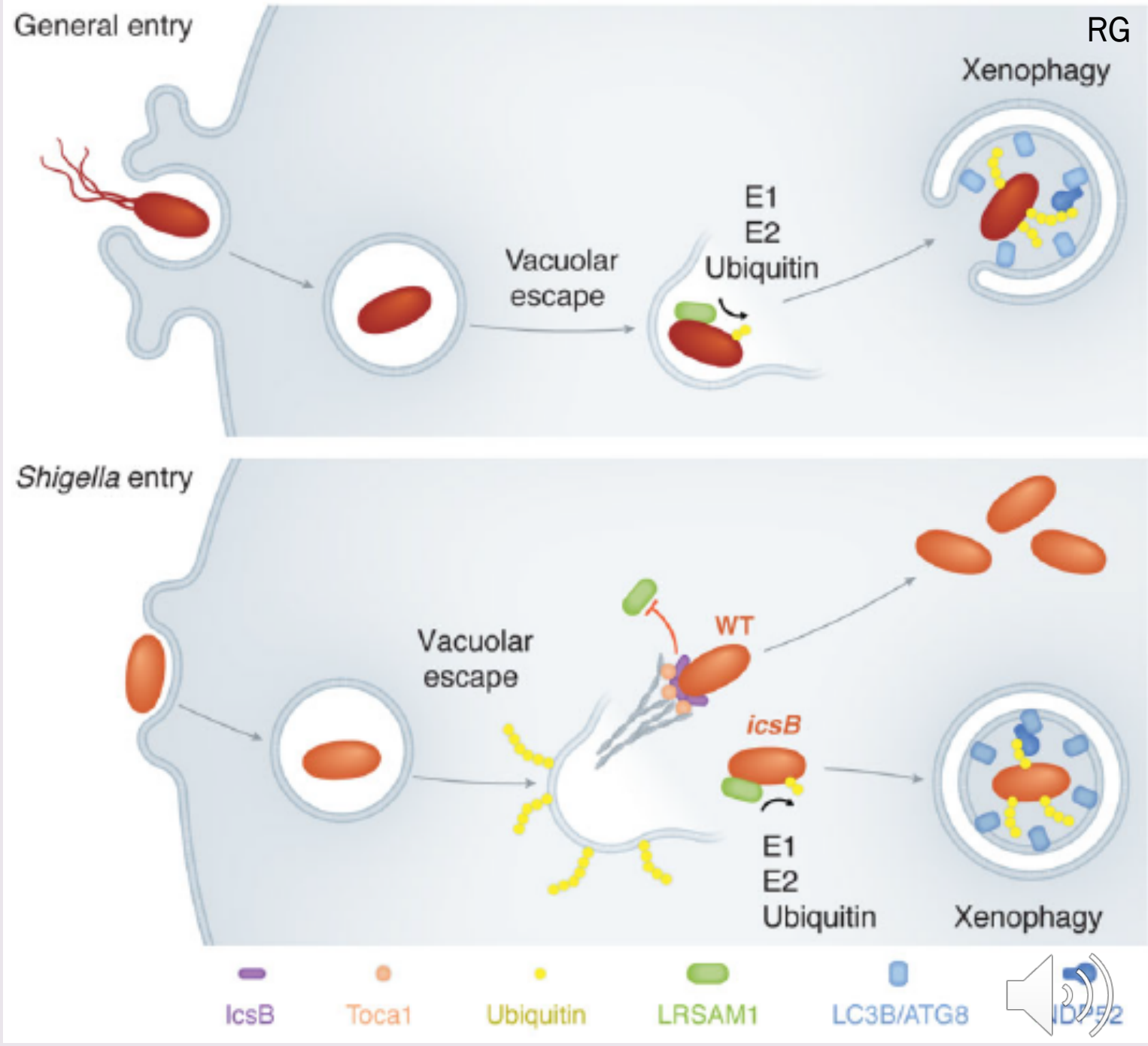
Shen DK, Blocker AJ. 2016. MxiA, MxiC and IpaD Regulate Substrate Selection and Secretion Mode in the T3SS of *Shigella flexneri*. PLoS One 11: e0155141.

BIOLOGICAL FUNCTION OF UBIQUITIN MODIFICATION, IN EUKARYOTES, BY BACTERIAL PATHOGEN ENZYMES

- Why must bacterial pathogens use the ubiquitin system of the host they infect?

- The ultimate purpose of any bacterial infection is survival and replication of the bacterium.
- Effector enzymes such as SopA, NleL, SidE, IpaH9.8, etc., help bacteria to establish an infection, survive inside cells, replicate, and spread to tissues.
- The mimicry effectors are results of plausible horizontal gene transfer through time.
- Effectors contribute to the different infection characteristics of pathogens



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Studying Ubiquitin Modification for Therapeutic Approaches

- Inhibition of NELs could possibly lead to new antibiotics
  - *Benefit of minimal effects on host since NELs are not found in eukaryotic cells*
- Possibility of less resistance compared to current antibiotics
- Antiviral strategies through host based therapeutics aiming at augmenting cellular processes to fight damage and infection by using molecules to
  - Augmenting translation
  - Augmenting autophagy
  - Augmenting interferon response

Methods used to detect and map the sites of post-translational modification

- Steps in proteomic analysis
  - *Isolation and/or Digestion*
  - *Enrichment*
  - *Analysis*
  - *Verification/Bioinformatics*
  - *Additional: Separation*
- Detection sensitivity depends on four factors:
  - *Yield of affinity enrichment*
  - *Level of contamination from irrelevant peptides*
  - *Sensitivity of the system*
  - *Complexity of the peptide mixture*
- New methods

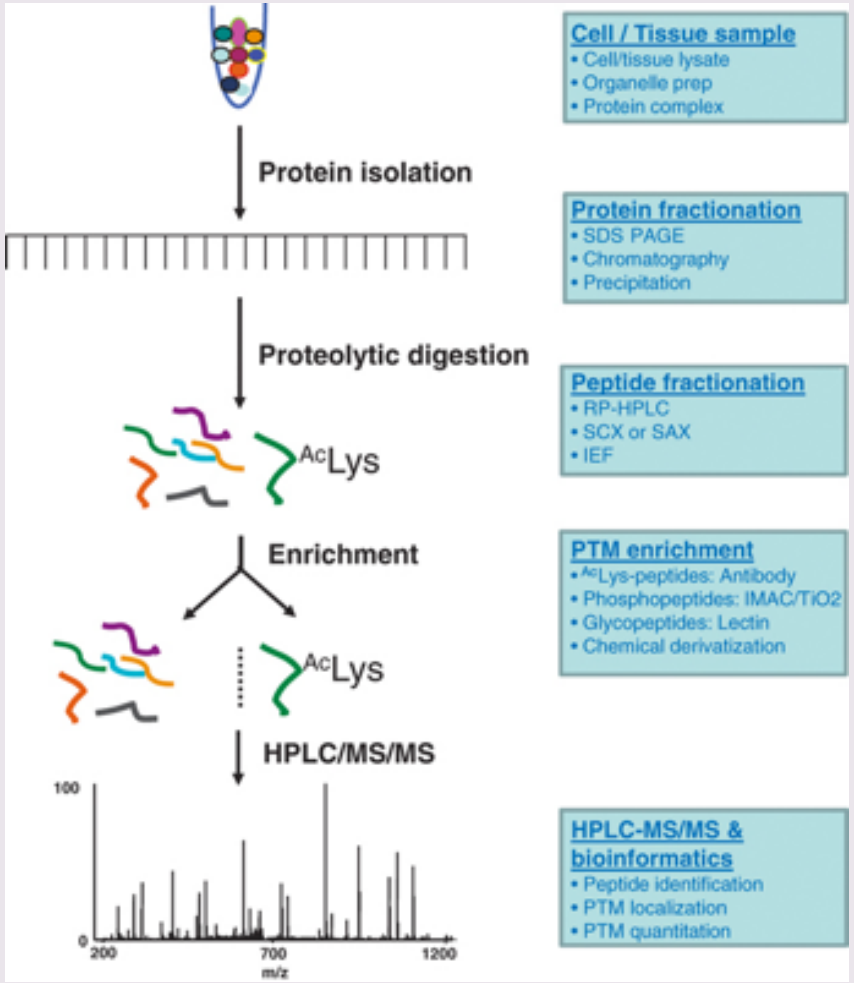

Figure from Zhao, Y. and Jensen, O. N. (2009) doi: 10.1002/pmic.200900398

Quotes by Scientific Leaders in the Field

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“Bacteria such as Shigella must escape innate immune defenses of their infected host. As part of this immunosuppressive strategy, they express several ubiquitin ligases that transfer ubiquitin molecules taken from infected cells to key proteins involved in innate immune signaling, thus neutralizing their function.”

Dr. Philippe J Sansonetti, Pasteur Institute, France

“Subversion of the host ubiquitin system through the expression of E3 effectors is a wily means of achieving a replicative niche. The study of these effectors is important as they hold promise as novel antibiotic targets. It is also likely that it will teach us more about the native ubiquitin system which certainly has many secrets yet to be revealed.”

Dr. Satpal Virdee, University of Dundee, Scotland

“Back in 2007 when I "cracked the nut" on IpaH function, I had no idea how rapidly this area would develop. It has been exciting to see all of the new bacterially encoded E3 ligases (BELs) that have come along since then. One disappointment has been how slow going the identification of IpaH substrates has been. My own lab as well as number of monster labs have gone after them but so far only a few (that I believe) have been identified. My guess is that we are missing something, I suspect that IpaHs will end up being something like StUbls that only recognize their substrates once they have been modified. Then we’ll see a quantum leap in ID of substrates. These BELs continue to surprise us as the recent Sde story (and all the nasty protein chemistry that goes along with it) from the labs of Dikic and Isberg have shown us.”

Dr. John Rohde, Dalhousie University, Canada

“The revelation of bacterial factors that target nearly every aspect of the host ubiquitin regulatory system - from Ub conjugation, ligases, and enzymes that remove Ub - demonstrates how precisely evolution has honed these virulence systems to exquisitely alter host cell biological processes.”

Dr. Erec Stebbins, DKFZ (German Cancer Research Center), Germany

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