Nobel Prize 2004 – Chemistry: Ubiquitin-Mediated Proteolysis

Journal Club Presentation

Jelena Janjic *Dipl.Pharm.*

532 Salk Hall

12:40PM

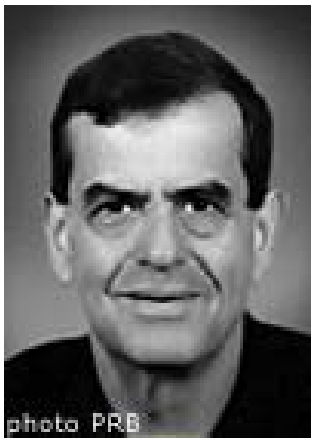
The Winners!

KUNGL. VETENSKAPSAKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

Press Release: The Nobel Prize in Chemistry 2004
6 October 2004
The Royal Swedish Academy of Sciences
has decided to award the Nobel Prize in Chemistry for 2004
"for the discovery of ubiquitin-mediated protein degradation" jointly to

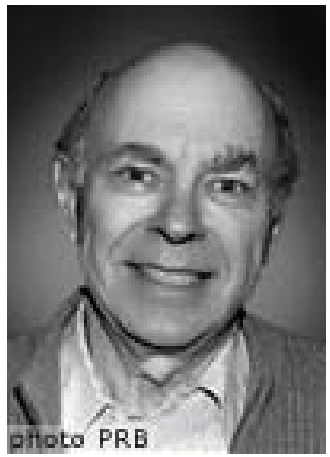
Aaron Ciechanover
Technion – Israel Institute of Technology, Haifa, Israel,
Avram Hershko
Technion – Israel Institute of Technology, Haifa, Israel and
Irwin Rose
University of California, Irvine, USA

The Winners!

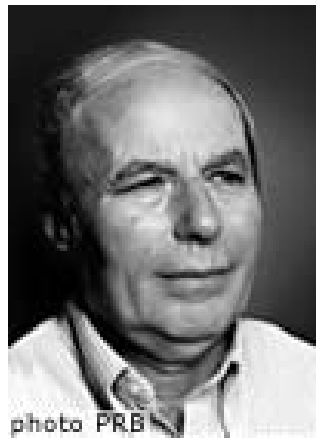


Aaron Ciechanover

Technion - Israel Institute of Technology



Irwin Rose



Avram Hershko

FOX CHASE CANCER CENTER

In the news

U.S. Department of Health and Human Services
NIH News
National Institutes of Health

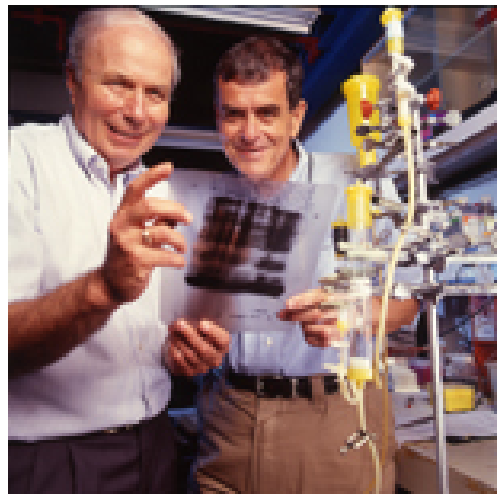
Press Statement from NIH Director Elias A. Zerhouni, M.D.
NIH Grantees Win 2004 Nobel Prize in Chemistry for Study of Protein Degradation Pathway

The 2004 Nobel Prize in chemistry is shared by two long-time NIH grantees, Irwin Rose, Ph.D., and Avram Hershko, M.D., Ph.D.

"This is a classic example of how basic research on the chemical mechanism underlying a biological process reveals a pathway essential to life. Understanding how cells maintain internal balance by regulating protein degradation is crucial for knowing how this balance is disrupted in disease."

From a PhD thesis to the Nobel Prize!

Prof. Ciechanover completed his Ph.D. thesis at the Faculty of Medicine, Technion- Israel Institute of Technology, under the supervision of Prof. Hershko where they discovered the ubiquitin system.



First paper published!

Ciechanover, A., Hod, Y., and Hershko, A.
A heat-stable polypeptide component of an ATPdependent proteolytic system from reticulocytes.
Biochem. Biophys. Res. Commun. **(1978)** 81, 1100-1105.

A major part of the work was done during a series of sabbatical leaves when Hershko and Ciechanover worked in Rose's laboratory at the Fox Chase Cancer Center in Philadelphia.

Prof. Ciechanover, Curriculum Vitae

Born in Haifa, Israel, 1947. In 1970 Received M.Sc.. degree in Medical Sciences (Summa Cum Laude), from the Faculty of Life Sciences and the Department of Biochemistry, "Hadassah" and the Hebrew University Medical School, Jerusalem. At the same school he completed his M.D. in 1974. Following a three years service as a military physician in the I.D.F., **Prof. Ciechanover completed his Ph.D. thesis at the Faculty of Medicine, Technion- Israel Institute of Technology, under the supervision of Prof. Hershko where they discovered the ubiquitin system.** Following a post-doctoral training at the M.I.T. (1981-1984), Prof. Ciechanover returned to the Technion and has been a member of the Faculty of Medicine ever since. Between 1994-2000 he served as the director of The Rappaport Family Institute for research in the Medical Sciences. Prof. Ciechanover published more than 90 original scientific papers on the ubiquitin system. He is a fellow of prestigious scientific associations such as the European Molecular Biology Organization (EMBO) and the Asia-Pacific International Molecular Biology Network, IMBN. He has been invited to give lectures in numerous prestigious international meetings. In 1999 he received the Jewish National Fund Distinguished Scientist Prize and Austria Ilse and Helmut Wachter Prize.

https://www.ats.org/northpacific/newsletters.php?id=67

Prof. Hershko, Curriculum Vitae

Prof Hershko was born in Karcag, Hungary, in 1937 and immigrated to Israel in 1950. In 1965 He completed his M.D. studies at the Hebrew University-Hadassah Medical School, Jerusalem (Cum Laude), and in 1969 received there his Ph.D. degree (Cum Summa Laude). Prof Hershko served as a physician in the I.D.F and was a lecturer in the Department of Biochemistry at the Hebrew University-Hadassah Medical School, Jerusalem. He joined the Technion at 1972 and from 1987 he is a member of The Rappaport Family Institute for research in the Medical Sciences. In 1998 he received the highest academic degree at the Technion - Research Professor. Prof. Hershko published more than 60 original scientific papers. He is a fellow of prestigious scientific associations, like EMBO and Israel National Academy of Sciences, and invited to lecture in many international meetings. He received many prestigious awards and prizes: The Weizmann Prize for Sciences, The Israel Prize in Biochemistry, The Gairdner Foundation International Award, The General Motors Cancer Research Foundation Award, and Austria Ilse and Helmut Wachter Prize.

https://www.ats.org/northpacific/newsletters.php?id=67

Irwin "Ernie" Rose, Ph.D.

A senior member of Fox Chase Cancer Center's division of basic science since 1963, Rose was elected to the National Academy of Sciences in 1979. He retired from Fox Chase in 1995. In 1997, he accepted a special appointment as emeritus researcher at the University of California at Irvine, where he continues to have research responsibilities. Before joining Fox Chase, he served on the faculty of Yale Medical School's department of biochemistry from 1954 to 1963.

Born in Brooklyn, N.Y., on July 16, 1926, Rose grew up in Spokane, Wash. He studied at Washington State College and then served in the U.S. Navy as a radio technician near the end of World War II. He completed his undergraduate degree under the G.I. Bill of Rights in 1949 at the University of Chicago and went on to earn his Ph.D. in biochemistry there. Prior to his appointment at Yale, he held one-year postdoctoral fellowships in the department of medicine at what is now Case-Western Reserve University in Cleveland and in the department of pharmacology at New York University.

http://www.fccc.edu/news/2004/Nobel-Winner-Rose/

The Beginning…

Prof. Hershko in "Lessons from the discovery of the ubiquitin system"

"It seemed reasonable to assume that there was an ***unknown proteolytic system that uses energy to attain the high selectivity of intracellular protein degradation***. I also thought that the best way to identify such a system is that of **classical bio chemistry**: to reproduce ATP dependent protein degradation in a cell-free system, and then to fractionate such a system in order to isolate and characterize its enzyme components."

cell-free system: reticulocytes!

"My ***simplest working hypothesis*** at that time was that ATP might be required either to phosphorylate, and thus tag the protein substrate, or to phosphorylate and activate an unknown protease. In either case, **at least two enzyme components were expected**: a **protein kinase** that carries out the phosphorylation and a **protease** that eventually degrades the protein substrate."

Trends Biochem Sci. 1996;21(11):445-9.

Pay attention to unexpected!

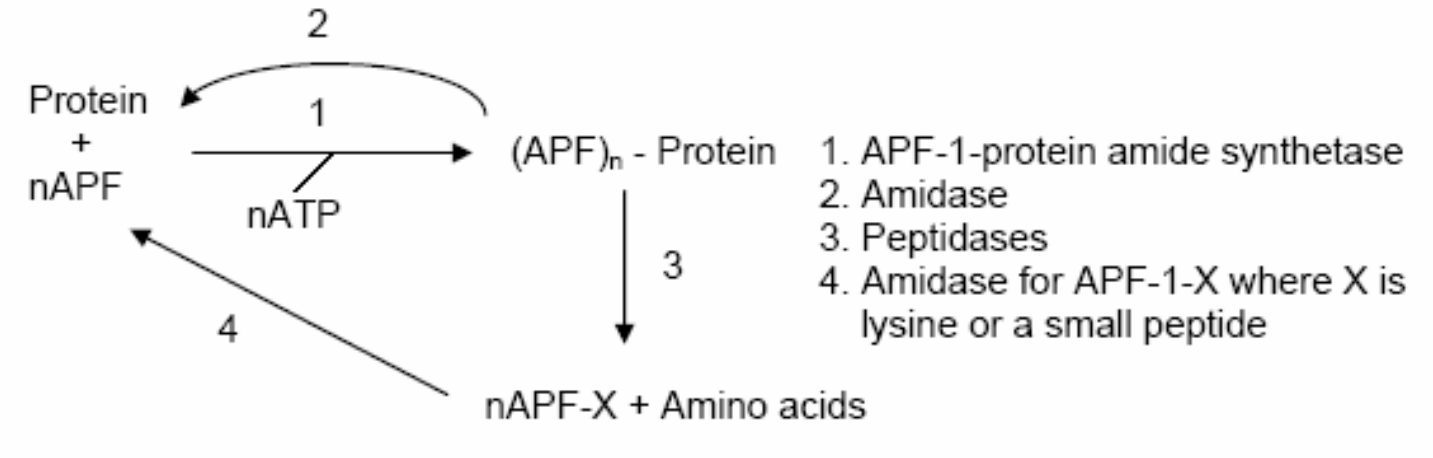
The first step came in 1978 when the reticulocyte lysate was passed over a DEAE cellulose column to remove the hemoglobin. Surprisingly, this ***separated the lysate into two fractions, each one individually inactive; but after recombination of the two fractions ATP-dependent proteolysis was reconstituted.***

A heat-stable protein, **APF-1** (active principle of fraction 1), 9kDa, was identified as the active component in the first fraction – this protein was later identified as **ubiquitin** by Wilkinson, Urban and Haas (1980).

Ubuiquitin “dream team” was born!

Prof. Hershko**:* “The purification of ubiquitin from Fraction 1 allowed the elucidation of the mode of its action in the proteolytic system. This work was done in part in ***collaboration with Irwin Rose, who hosted me in his laboratory at Fox Chase Cancer Center in Philadelphia for a sabbatical year in 1978 and many times afterwards.”

Trends Biochem Sci. 1996;21(11):445-9.



Proposed ATP-dependent protein degradation

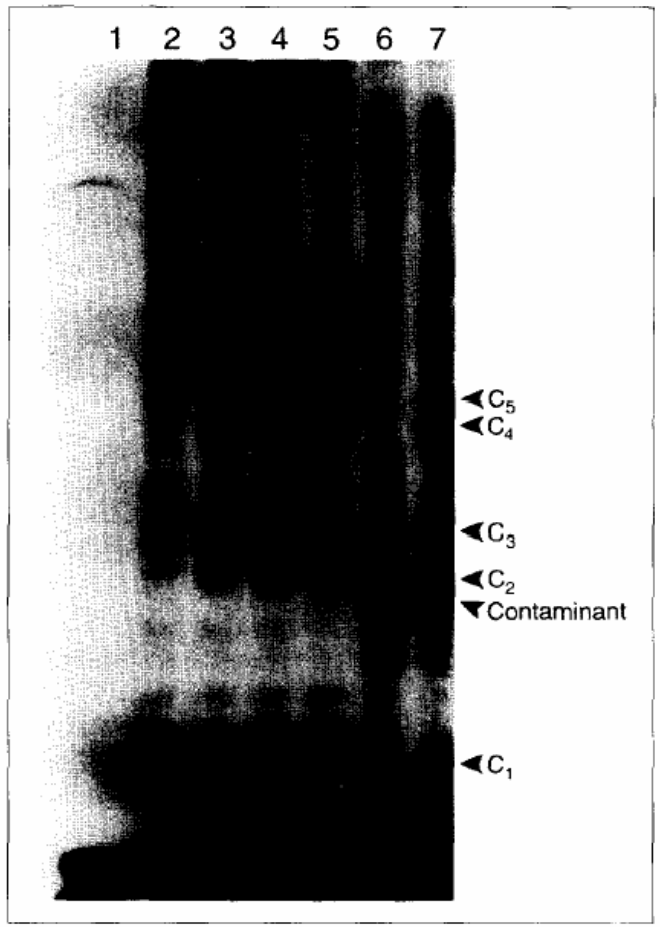
And then, an ugly gel made history…

Covalent ligation of ubiquitin to lysozyme.

"This experiment showed us that ubiquitin is ligated to lysozyme, a good substrate of the ATP-dependent proteolytic system, and that several molecules of ubiquitin are linked to one molecule of lysozyme.

All incubations contained Fraction 2 from reticulocytes. 1251-labeled ubiquitin was added in lanes 1-5, and 1251-labeled lysozyme in lanes 6-7. Lane 1, 1251-ubiquitin incubated without ATP; lane 2, with ATP; lanes 3-5, with ATP and increasing concentrations of unlabeled lysozyme. Lane 6, 1251-lysozyme incubated without ATP; lane 7, 1251-lysozyme incubated with ATP and unlabeled ubiquitin."

A. Hershko *et al.*. In: *Proc. Natl. Acad. Sci. U. S. A.* 77 (1980), pp. 1783–1786.

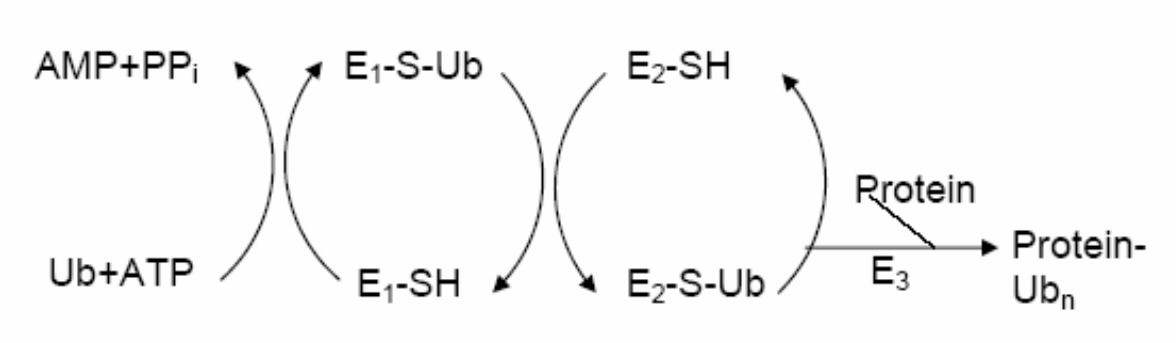


Trends Biochem Sci. 1996;21(11):445-9.

Read this paper!

1981-1983 the “dream team” put the multi-step ubiquitin-tagging hypothesis together

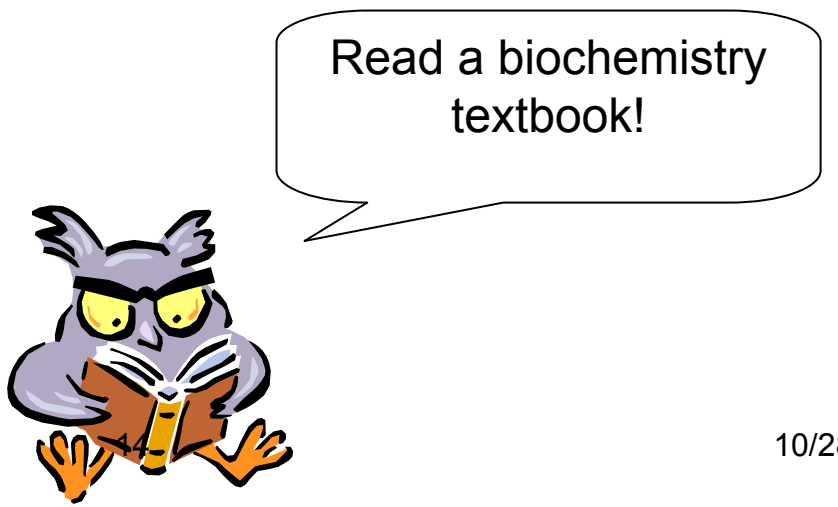
- **Ciechanover, Hershko and Rose** isolated and characterized three separate enzyme activities, ***E1, E2 and E3***.
- **Covalent affinity chromatography** was developed during the purification of E1



Imagine any serious protein work without this technique!

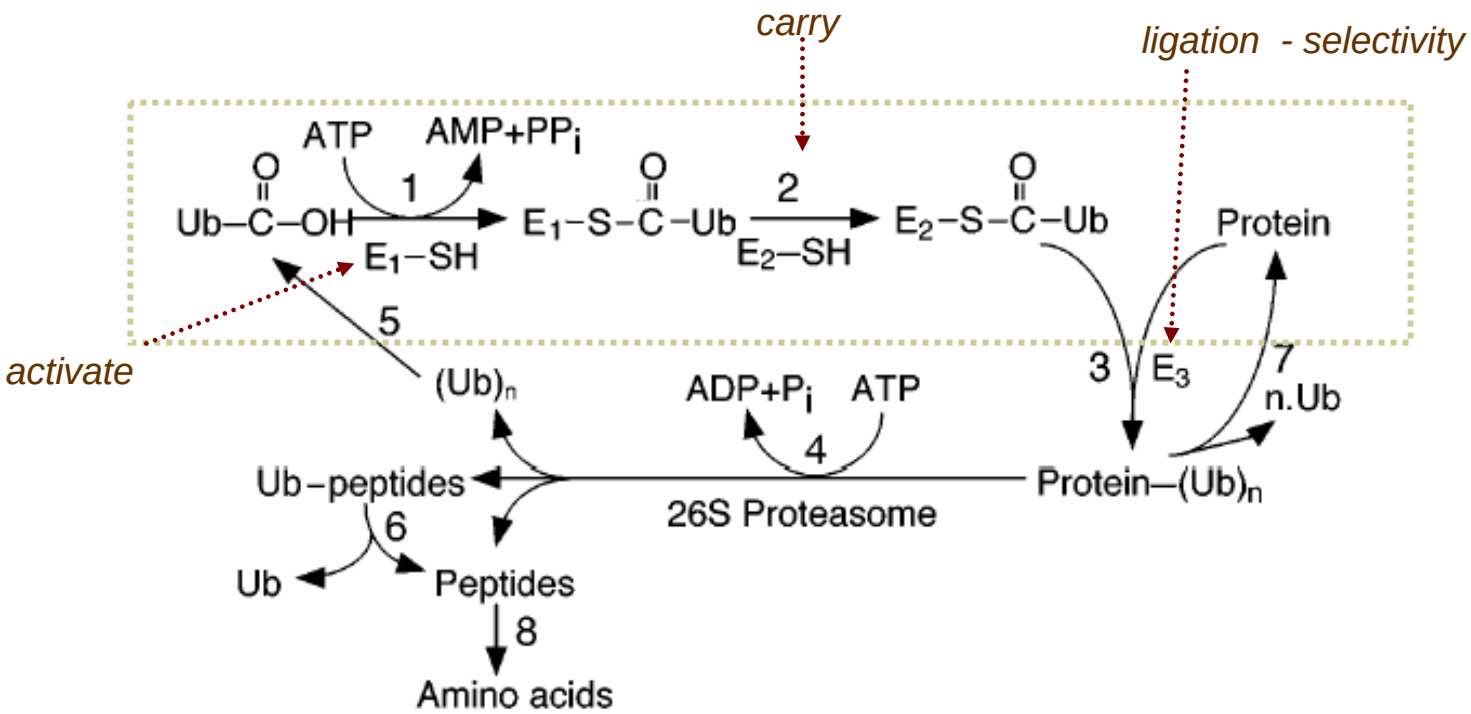
So, what is this ubiquitination business, then? And why is it so important?

It is a way to simply “dump” some unwanted proteins, right?



Ubiquitin ligation pathway

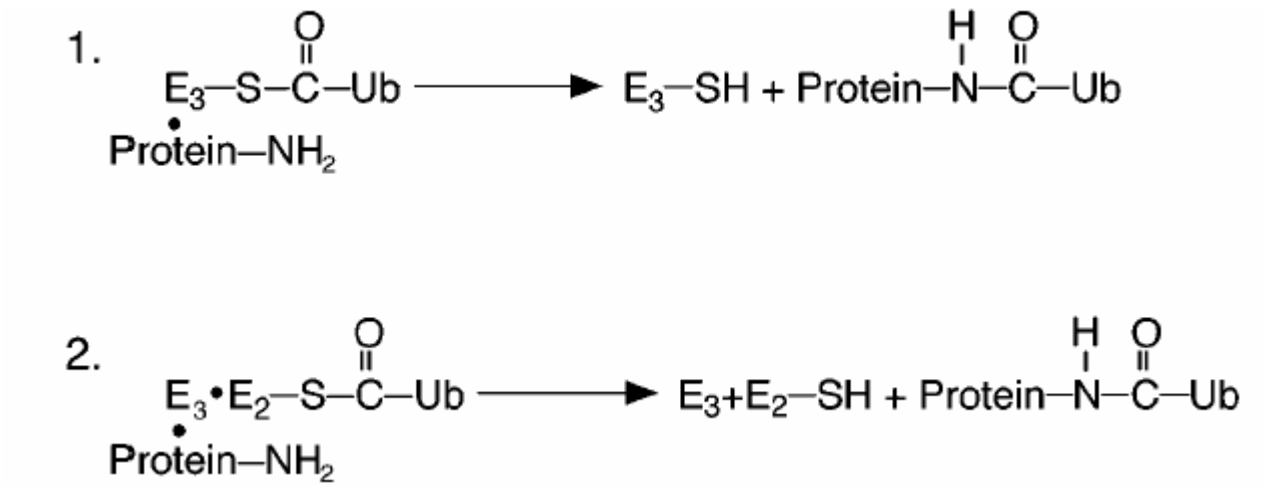
- **Step 1**: Ubiquitin activated by E1.
- **Step 2**: Activated ubiquitin transferred to an active site Cys residue of a ubiquitin-carrier protein, E2.
- **Step 3**: Ubiquitin is linked by its C-terminus in an amide isopeptide linkage to an ε-amino group of the substrate protein's Lys residues, cat. by E3



Annu. Rev. Biochem. 1998. 67:425–79

How the cell knows which protein to ubiquitinate? Where the specificity comes from?

Possible mechanisms of ubiquitin transfer by different types of **E3 enzymes**.

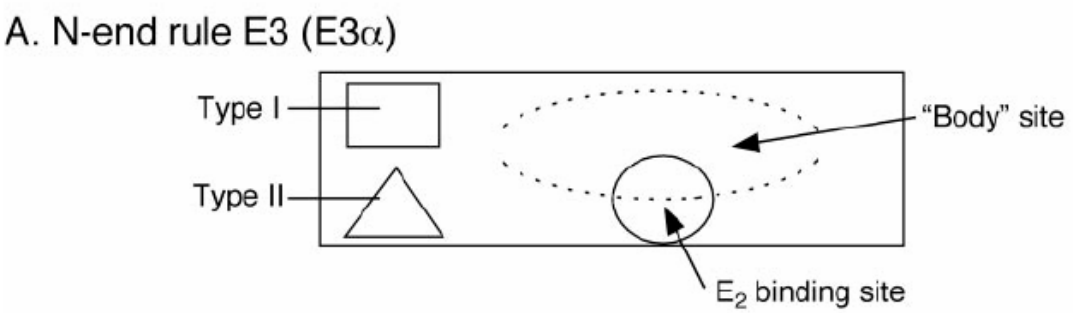


1. E3 accepts the activated ubiquitin from an E2 and binds it as a thiolester intermediate prior to transfer to protein
2. E3 helps to transfer ubiquitin directly from E2 to a protein, by tight binding of E2 and the protein substrate

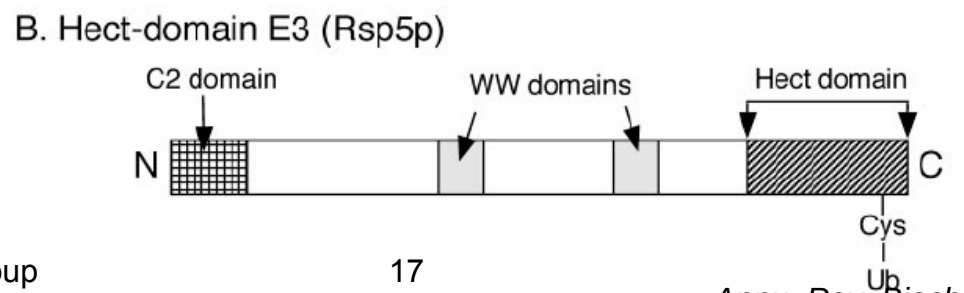
Annu. Rev. Biochem. 1998. 67:425–79

Enzyme E3 - four types

1. ***E3α***, 200kDa, binds N-end rule protein substrates that have basic (Type I) or bulky-hydrophobic (Type II) N-terminal amino acid residues



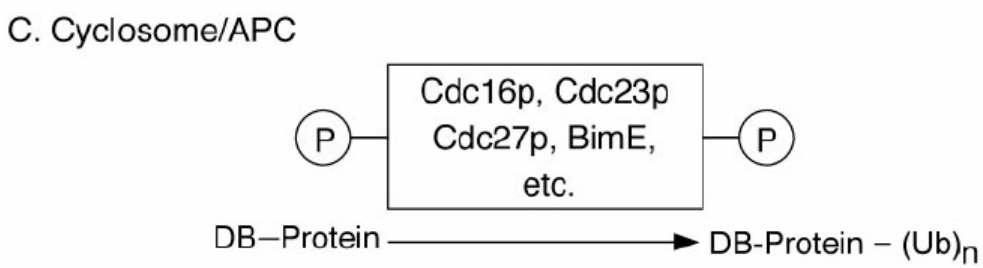
2. ***Hect-domain E3*** – E6-AP, 100-kDa cellular protein that was required, together with papillomavirus E6 oncoprotein, for the ubiquitinylation and degradation of p53 in reticulocyte lysates



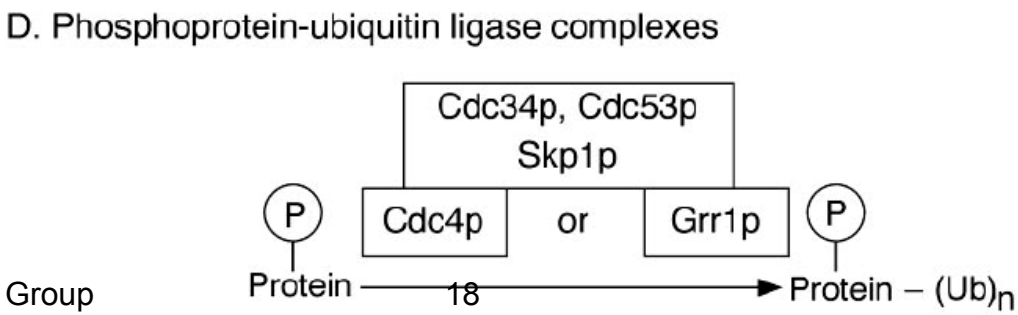
Annu. Rev. Biochem. 1998. 67:425–79

Enzyme E3 - four types

3. **Cyclosome** or anaphase promoting complex (**APC**) - ubiquitin ligase activity specific for cell-cycle regulatory proteins that contain a 9AA degenerate motif (destruction box). Its **substrates** are **mitotic cyclins**, some **anaphase inhibitors**, **and spindle-associated proteins**, all of which are degraded at the end of mitosis.

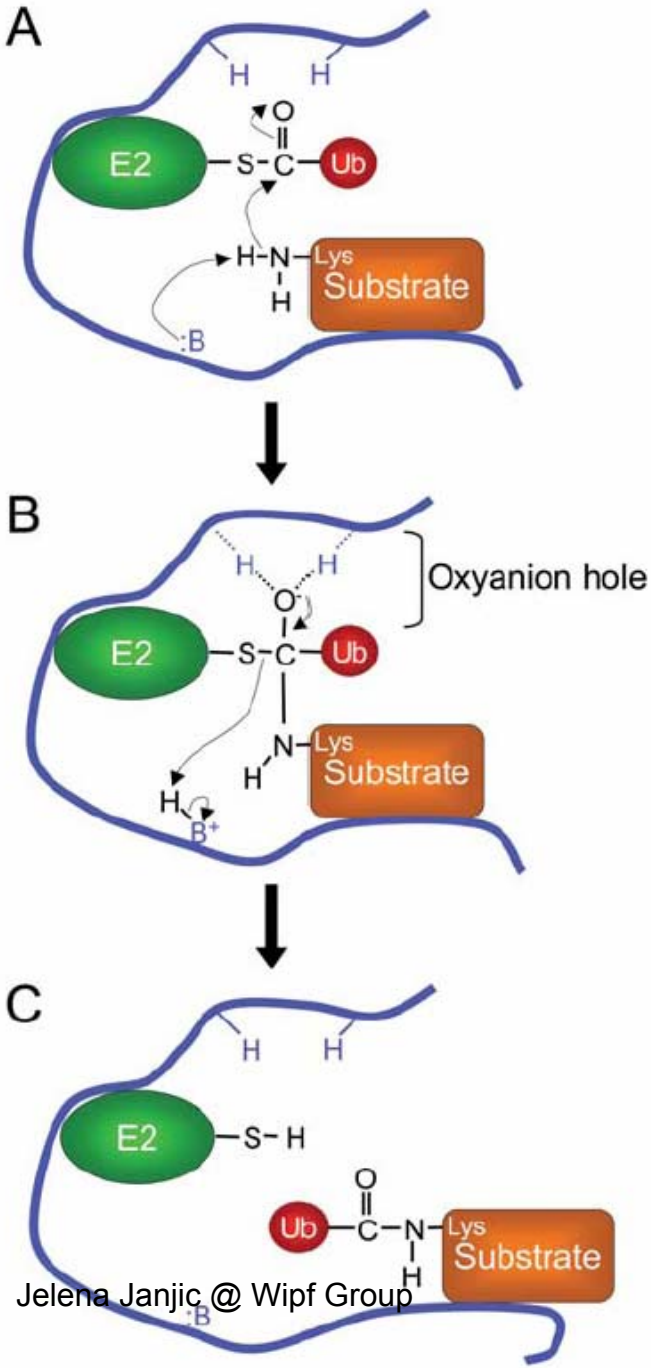


4. Phosphoprotein-ubiquitin ligase complexes (**PULCs**) - degradation of some other cell-cycle regulators, such as the Sic1p Cdk inhibitor or the G1 cyclin Cln2p



Annu. Rev. Biochem. 1998. 67:425–79

Proposed mechanism for ubiquitin transfer



The E3 ligase (shown in blue) has several possible roles:

Binds both E2 and substrate and position them in optimal orientations.

E3 may provide a general base to deprotonate the acceptor lysine (A). The acceptor lysine is shown here as a substrate lysine; alternatively, it could reside on another ubiquitin (Ub).

E3 may stabilize the negative charge on the oxygen using an oxyanion hole (B).

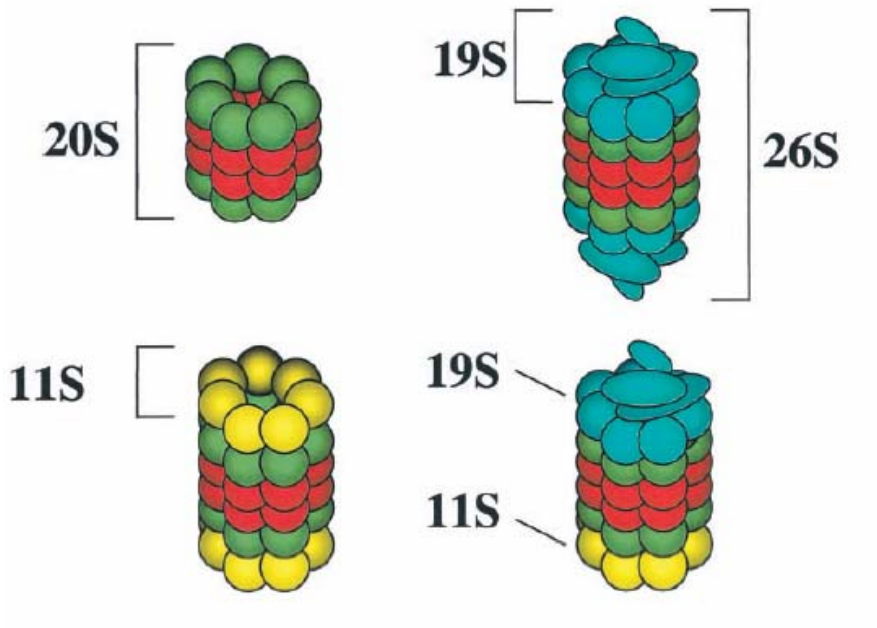
The E3 may reposition the substrate so Lys48 of ubiquitin is in the active site and prepared for reaction with a recharged E2 (C).

Structures of E3s indicate that there are no E3 residues close to the catalytic cysteine of the E2. Catalytic groups may originate in the E2 or other proteins, or ubiquitin transfer may occur spontaneously due to the highly labile thioester bond.

Reviewed in great detail in Biochem. J. (2004) **379**, 513–525

Where does the ubiquitin ligated protein go?

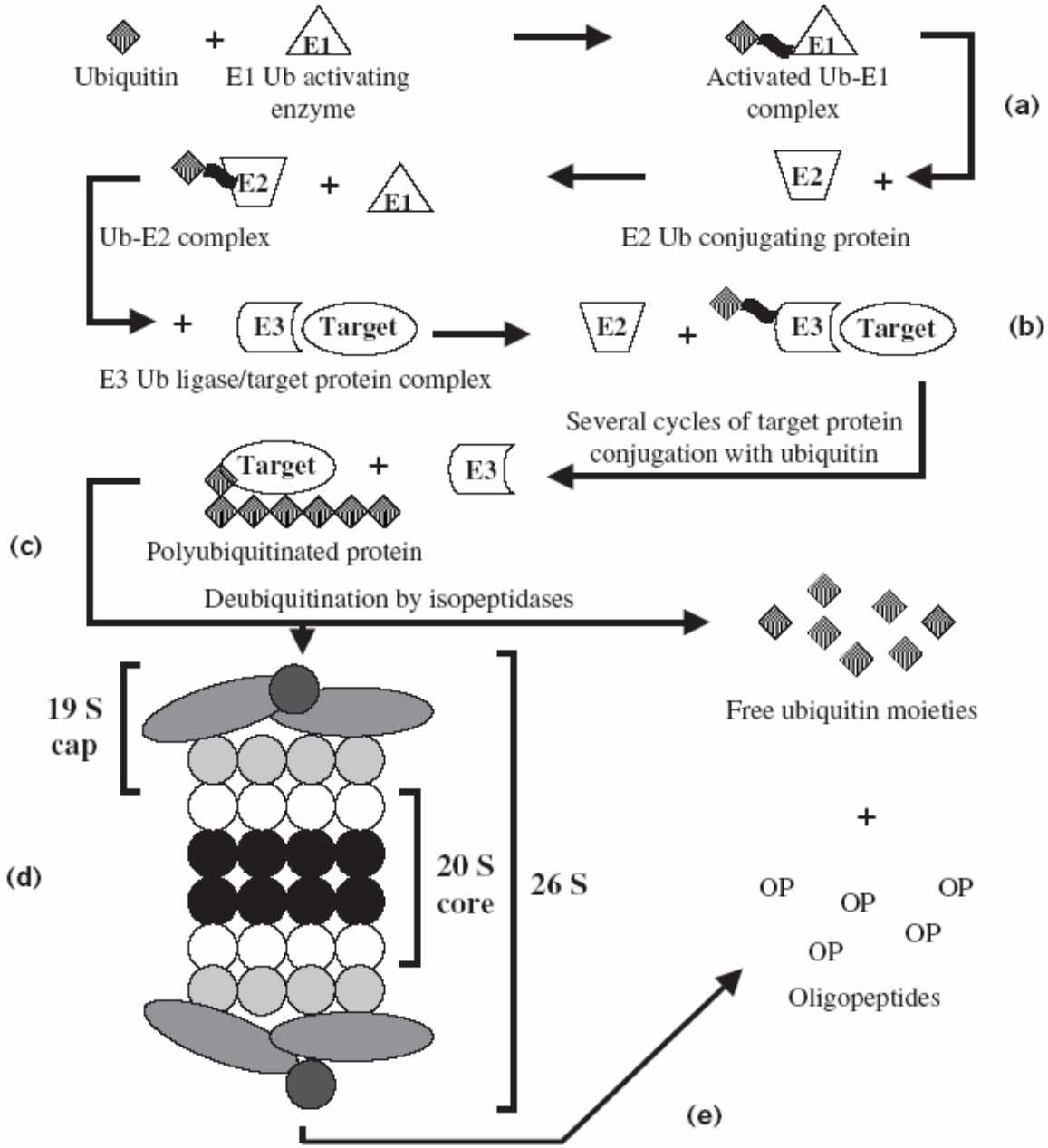
- The 20S and 26S Proteasome Complexes



Clin.Can.Res. 2003. 9: 6316-6325

UPP

General Mechanism!



Breast Can Res. 2003, 5:1-7

Protein degradation through the ubiquitin (Ub)-proteasome pathway (UPP)

Why is this process so important?

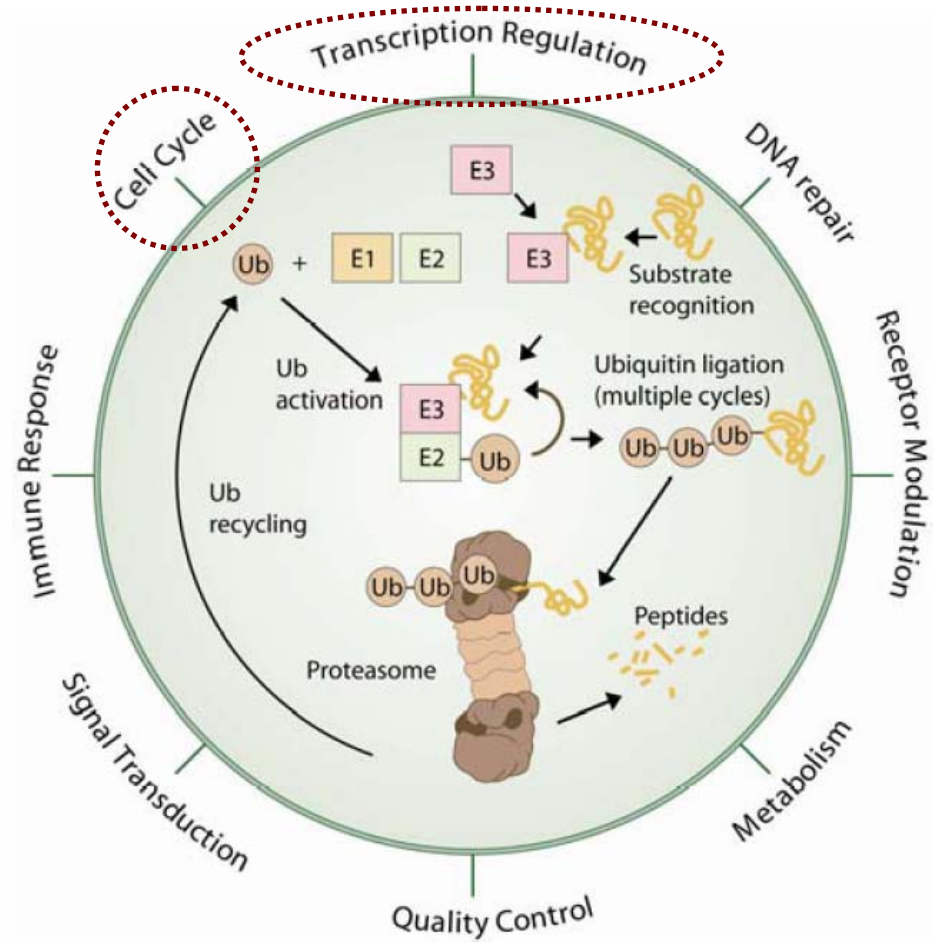
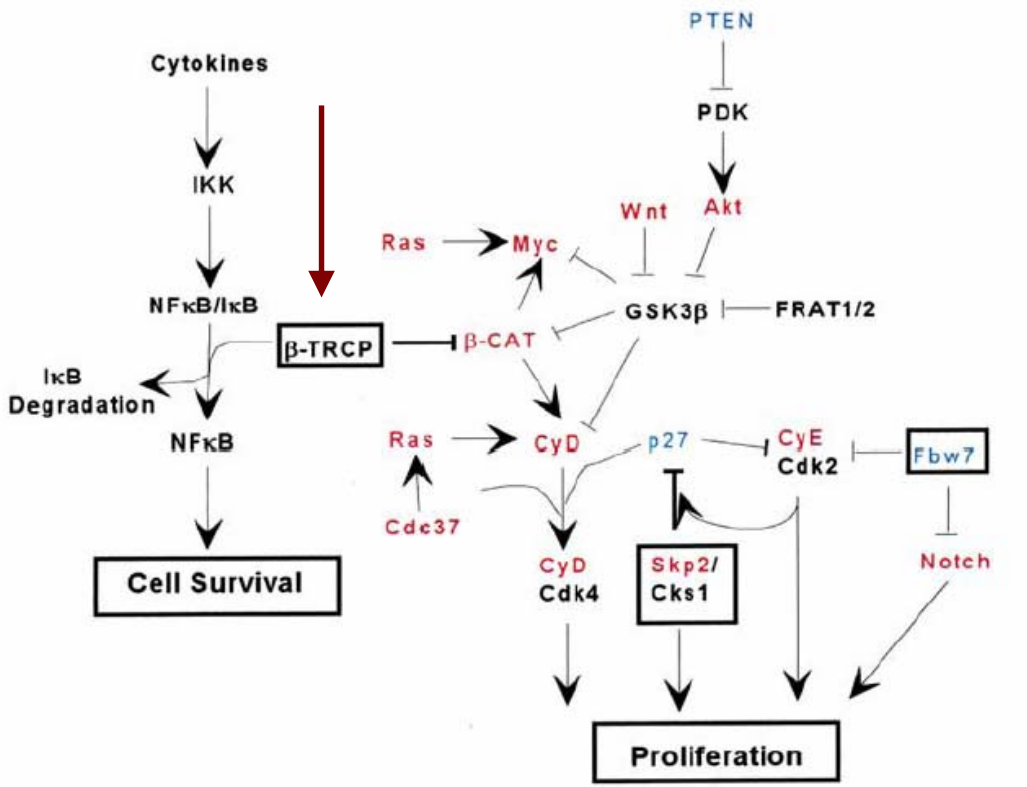


Fig. 1 Ubiquitin-mediated proteolysis and its many biological functions

Cell cycle regulation by UPP

Pathways regulating proliferative decisions in G1.

- Proliferation is a balance between cell cycle progression and apoptosis.
- These two pathways are connected by the SCF$^{\beta TRCP}$ E3 which functions to maintain low levels of the oncoprotein β-catenin and is also required for destruction of the NFκB inhibitor IκB in response to anti-apoptotic cytokines.



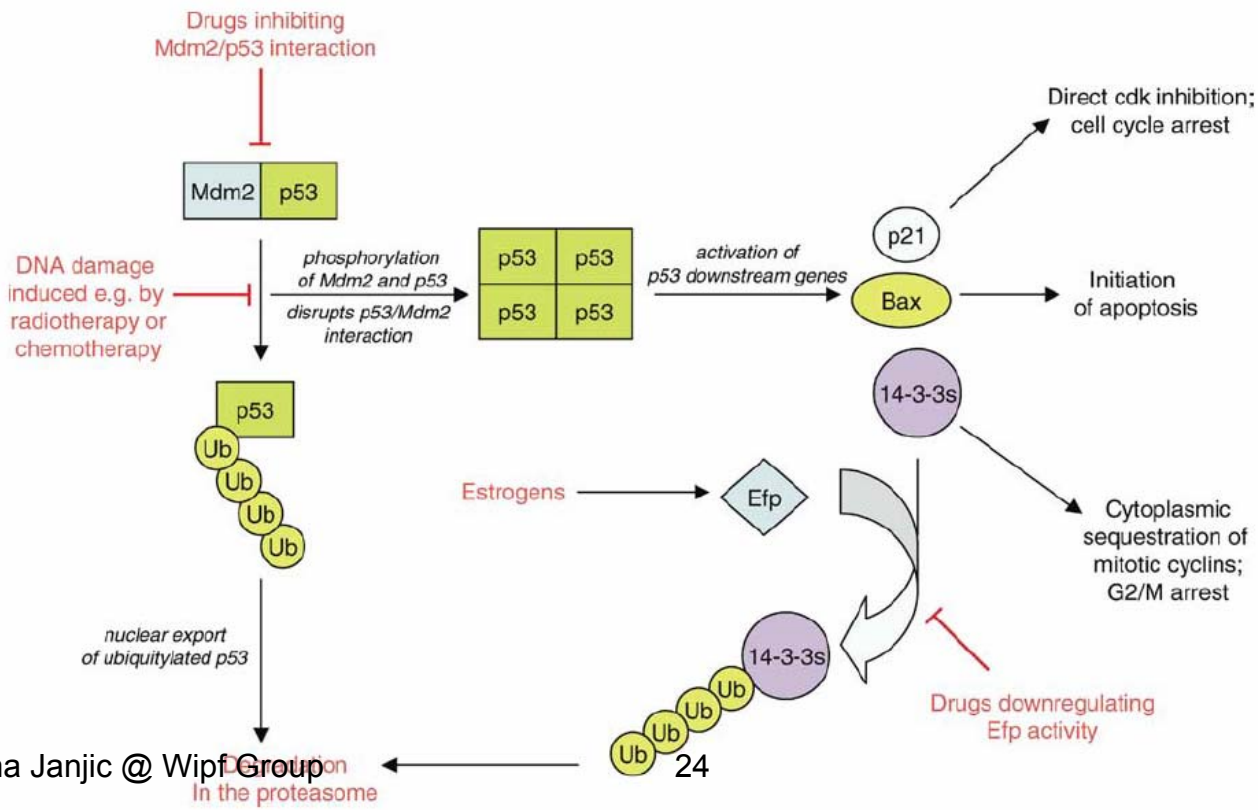
Red, oncoproteins.
Blue, known or candidate tumor suppressors.

CANCER TREATMENT REVIEWS 2003; 29 (Suppl. 1): 49–57

Mdm2 and p53

- Drugs inhibiting interaction between p53 and its ubiquitin ligase, Mdm2, are proposed to stabilize p53 and therefore enhance p53-dependent tumor response to chemo- and radiotherapy.

- Downregulation of Efp, a ubiquitin ligase for a p53-regulated cell cycle inhibitory protein (14-3-3r), could possibly prevent the switch to estrogen resistance in breast cancer cells and sensitize estrogen-independent tumors to tamoxifen.



CANCER TREATMENT REVIEWS 2003; 29 (Suppl. 1): 49–57

Proteasome inhibition to overcome chemotherapy resistance

Table 1 Molecular targets of proteasome inhibitors

Target	Sequelae of proteasome inhibition	Contribution to antitumor effect
NFκB[a]	Stabilization of IκB, which inhibits nuclear translocation of NFκB	Decreases NFκB-dependent transcription of genes important in tumor cell survival, proliferation, invasion and metastasis, and angiogenesis
p53	Accumulation of p53 protein by inhibition of proteasome-mediated p53 degradation	Increases p53-dependent transcription of cell cycle inhibitors (p21) and proapoptotic factors (Bax)
p21 and p27	Accumulation of both p21 and p27, and increased transcription of p21 through accumulation of p53	Induces G_1/S cell cycle arrest and apoptosis
Bax	Accumulation of Bax by inhibition of proteasome-mediated Bax degradation and through increased p53-mediated transcription	Increases Bax interaction with Bcl-2 and Bcl-x_L, promoting release of mitochondrial cytochrome *c* and apoptosis
p44/42 MAPK	Transcriptional activation of the MKP-1 phosphatase, leading to p44/42 dephosphorylation and inactivation	Down regulates p44/42-dependent cell proliferation and survival signals, and possibly angiogenesis
tBid (62)	Accumulation through decreased proteasomal degradation	tBid induces conformational changes in Bak, promoting mitochondrial release of cytochrome *c*
Smac/Diablo (63)	Accumulation through decreased proteasomal degradation	Smac and Diablo bind to and inhibit members of the XIAP protein family

[a] NFκB, nuclear factor κB; IκB, inhibitor of nuclear factor κB; MAPK, mitogen-activated protein kinase; Diablo, direct IAP binding protein with low pI; Smac, Second mitochondria-derived activator of caspase; XIAP, X-chromosome-linked inhibitor of apoptosis.

Down-regulation of NFκB likely plays an important role in the ability of proteasome inhibitors to abrogate drug resistance.

Drugs that influence ubiquitin-proteasome activity

Drug class	Action and mechanism
Chemotherapeutic agents	
Aclarubicin	Inhibits the chymotrypsin-like proteolytic activity of the proteasome
All-*trans* retinoic acid	May accelerate PML fusion protein degradation through the proteasome
Arsenic trioxide	Inhibits ubiquitination and degradation of IκB through effects on the IκB kinase
Camptothecin	Stimulate ubiquitination and degradation of topoisomerase 1
Geldanamycin	Inhibits HSP90 ATPase, stimulating proteasomal degradation of client proteins
PS-341/LDP-341/MLN-341	Inhibits the chymotrypsin-like activity of the proteasome
Vinblastine, Vincristine	Inhibit the chymotrypsin-like, trypsin-like- and peptidyl-glutamyl peptide hydrolyzing proteasome activities
Immunosuppressive agents	
Cyclosporine A	Uncompetitive inhibitor of the proteasomal chymotrypsin-like activity
Rapamycin	Inhibits proteasome function by inhibiting the proteasome activator PA28
Miscellaneous agents	
Fulvestrant	Stimulates proteasome-dependent proteolysis of ERα
Tannic acid	Inhibits the chymotrypsin-like activity of the proteasome
Lovastatin	Mechanism unknown, but appears structurally similar to the proteasome inhibitor lactacystin
Anti-retroviral drugs	Inhibit the chymotrypsin-like and trypsin-like proteasome activities

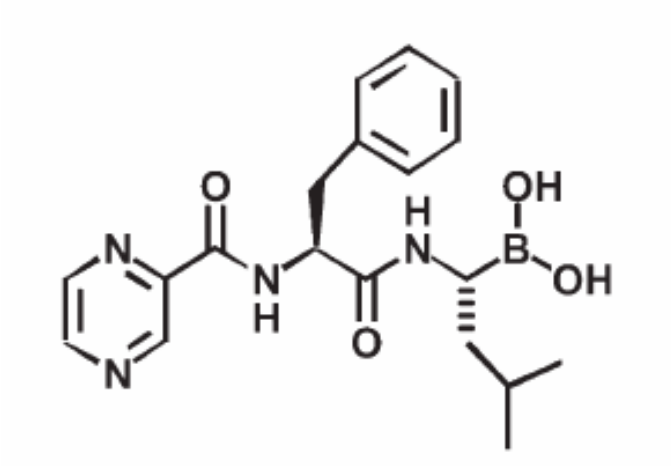
ER, estrogen receptor; HSP, heat shock protein; PML, promyelocytic leukemia.

Breast Cancer Res 2003, **5**:1-7

UPP targeting drugs…

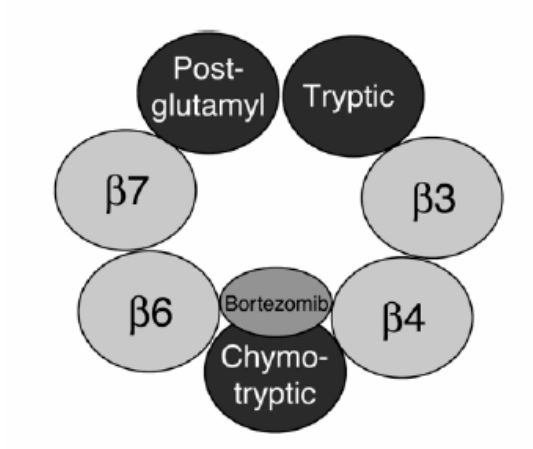
Peptide boronic acid analogs

- **Bortezomib (PS-341)**



In general, proteasome inhibition tends to induce apoptosis in proliferating cells while being protective in some but not all quiescent cells

- Mechanism



Cross-sectional view of the bortezomib binding site in the proteasome.

Because peptide boronic acids inhibit serine proteases such as chymotrypsin by mimicking substrate binding at the active site it was postulated that they might inhibit the proteasome by binding to the chymotrypsin-like site in the 20S core (Adams et al., 1998).

Bortezomib (PS-341)

- In May 2003, the US Food and Drug Administration approved bortezomib for the treatment of patients with multiple myeloma who have received at least 2 prior therapies and who have demonstrated disease progression on their last therapy.
- In addition, bortezomib was approved in April 2004 by the CPMP for use in the European Union.

Conclusions

- UPP holds many drug targeting sites that can be explored for new therapies development from cancer to antibiotics …