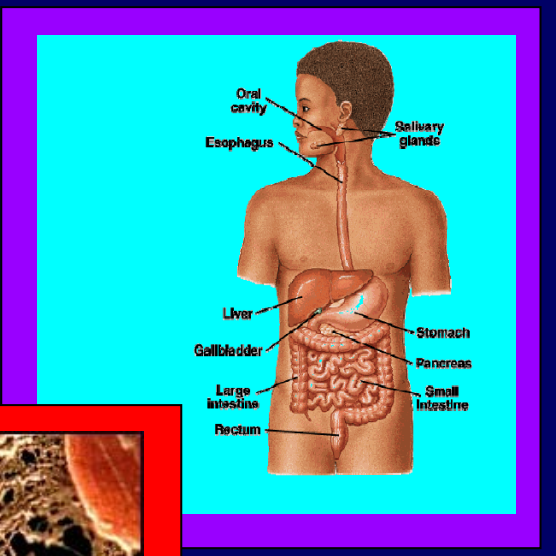
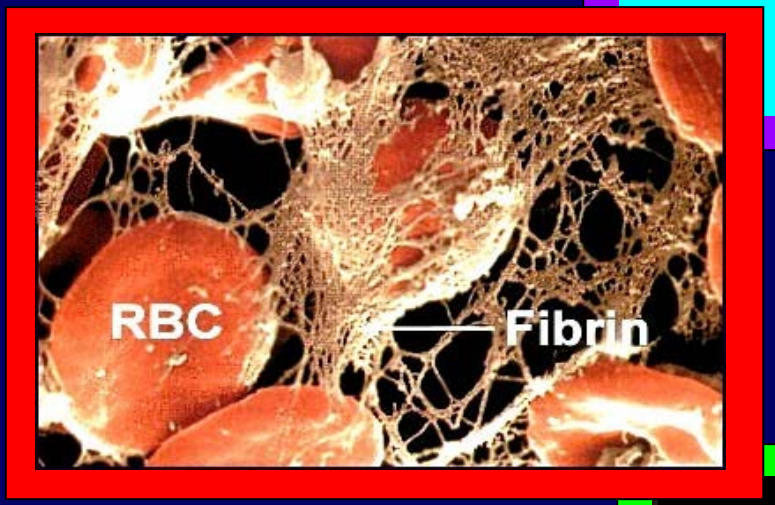
Topological Diversity of Proteolytic Systems

1. **Extracorporeal Extracellular - The gastrointestinal tract**



2. **Intracorporeal Extracellular - The blood coagulation system**



3. **Intracorporeal Intracellular - The Lysosome (organelle - membrane secluded) and the Ubiquitin system (free floating- cytosolic, nuclear, "ER")**

Intracellular Proteolysis is a thermodynamically paradoxical, energy (ATP)-requiring process,

Roles of Intracellular Protein Degradation

A. Quality Control

B. Control of processes

C. Differentiation and Morphogenesis

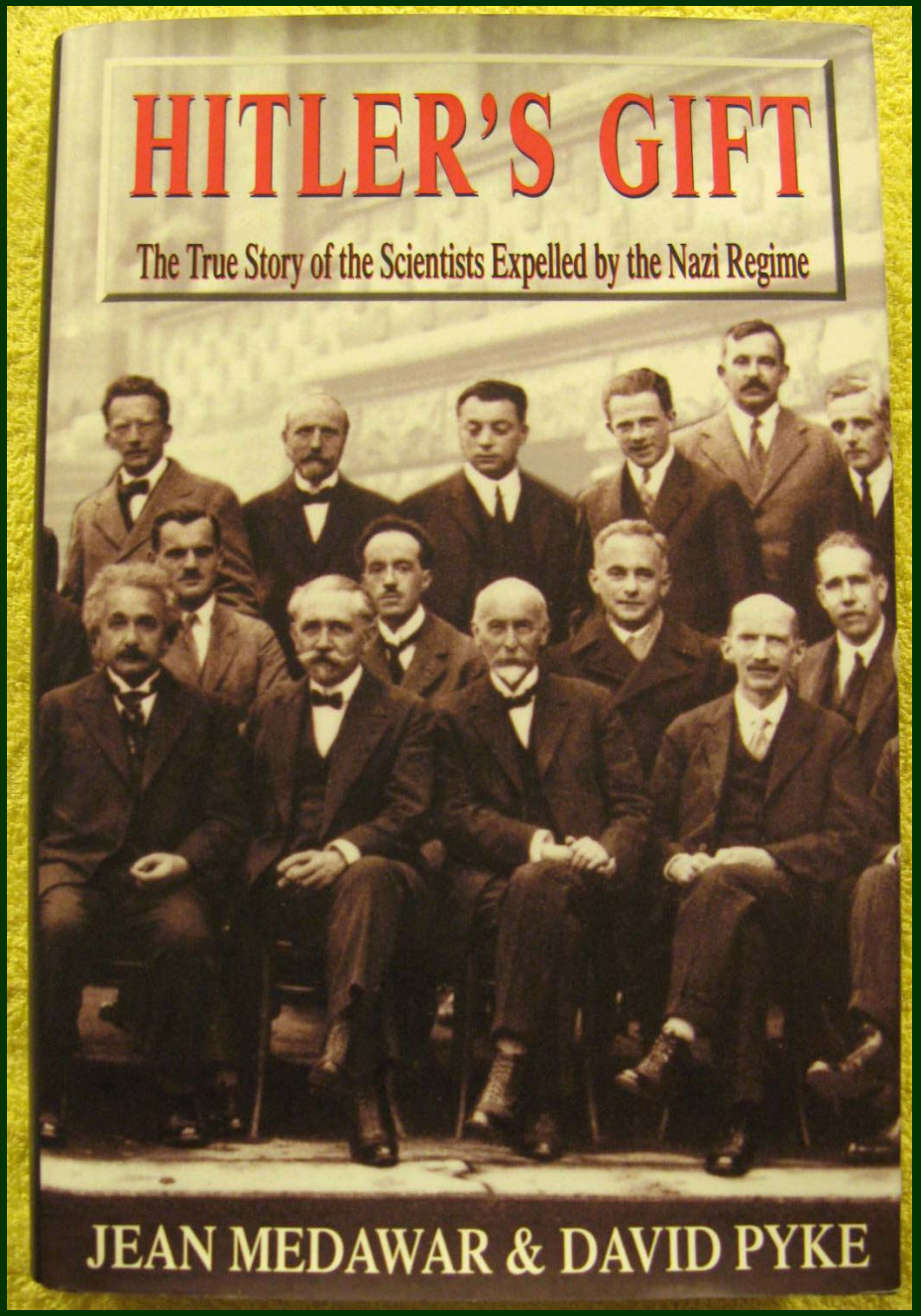
THE DYNAMIC STATE OF BODY CONSTITUENTS

BY

RUDOLF SCHOENHEIMER, M.D.

Late Associate Professor of Biological Chemistry, Columbia University

CAMBRIDGE, MASSACHUSETTS
HARVARD UNIVERSITY PRESS
1942


HITLER'S GIFT
The True Story of the Scientists Expelled by the Nazi Regime
JEAN MEDAWAR & DAVID PYKE

NOTED CHEMIST DEAD

Rudolf Schoenheimer
New York Times, 1939

SCIENCE PIONEER SUICIDE BY POISON

Tragic Death of Dr. Rudolf Schoenheimer in Yonkers on Sept. 11 Is Disclosed

HIS WORK AIDED MEDICINE

With 'Tagged Atoms' He Traced Food Through Body, Showing How Tissues Are Built

Special to THE NEW YORK TIMES.

YONKERS, N. Y., Sept. 21—Dr. Rudolf Schoenheimer, Associate Professor of Biological Chemistry at Columbia University and an outstanding research worker in the field of biochemistry, was found dead of poisoning at his home at 115 Wickes Avenue here on Sept. 11, it became known today. He was 42 years old.

Professor Schoenheimer's body was found by the police on the lawn in back of his house, where he had succumbed to a self-administered dose of a toxic acid, Dr. Amos O. Squire, medical examiner of Westchester County, said. He had committed suicide, according to the finding of the medical examiner, while suffering from mental depression.

Had Written Suicide Notes

Dr. Schoenheimer, who was separated from his wife, had been in ill health mentally for some time, according to the evidence gathered by the medical examiner. He had written suicide notes on Sept. 8, 9 and 10 in which he spoke of his intention to end his life because of personal troubles and his mental depression. The note of Sept. 10 directed the police to look for his body on the lawn where it was found.

The chemist, who was graduated from the University of Berlin with a doctorate in medicine in 1922, was formerly head of the Department of Pathological Chemistry at the University of Freiburg. He had engaged in research work in pathology, chemistry and biological chemistry at various German universities and was a pioneer in the field of "tagged atoms," in which atoms of heavy hydrogen, heavy nitrogen, as well as heavy carbon, oxygen and sulphur are introduced in the diet of animals to determine the uses the animal body makes of food constituents.

Experimented With Surgery

During the year 1930-31 he was Douglas Smith Fellow in the Department of Experimental Surgery at the University of Chicago. He came to the United States again in February, 1933, to engage in research work under the auspices of the Josiah Macy Jr. Foundation and in the following October was appointed to a visiting professorship at Columbia.

His work, together with that of others at Columbia, helped to establish that the living body is a chemical laboratory where varied and complex transformations of matter are taking place incessantly. He also succeeded in determining some of the transformations that take place. These researches attracted wide attention among his fellow-chemists.

New York Times vom Montag, den 22. 9.1941

"His work ... helped establish that the living body is a chemical laboratory where varied and complex transformations of matter are taking place incessantly."

Schoenheimer, R. (1942).
The Dynamic State of Body Constituents.
Harvard University Press, Cambridge, USA.

"*The simile of the combustion engine pictured the steady flow of fuel into a fixed system, and the conversion of this fuel into waste products. The new results imply that not only the fuel, but the structural materials are in a steady state of flux. The classical picture must thus be replaced by one which takes account of the dynamic state of body structure*".

Vol. 81, No. 4, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 28, 1978 Pages 1100-1105

A HEAT-STABLE POLYPEPTIDE COMPONENT OF AN ATP-DEPENDENT PROTEOLYTIC SYSTEM FROM RETICULOCYTES

Aharon Ciehanover, Yaacov Hod and Avram Hershko[1]

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

Received March 8,1978

SUMMARY: The degradation of denatured globin in reticulocyte lysates is markedly stimulated by ATP. This system has now been resolved into two components, designated fractions I and II, in the order of their elution from DEAE-cellulose. Fraction II has a neutral protease activity but is stimulated only slightly by ATP, whereas fraction I has no proteolytic activity but restores ATP-dependent proteolysis when combined with fraction II. The active principle of fraction I is remarkably heat-stable, but it is non-dialysable, precipitable with ammonium sulfate and it is destroyed by treatment with proteolytic enzymes. In gel filtration on Sephadex-G-75, it behaves as a single component with a molecular weight of approximately 9,000.

Hershko et al., 1980

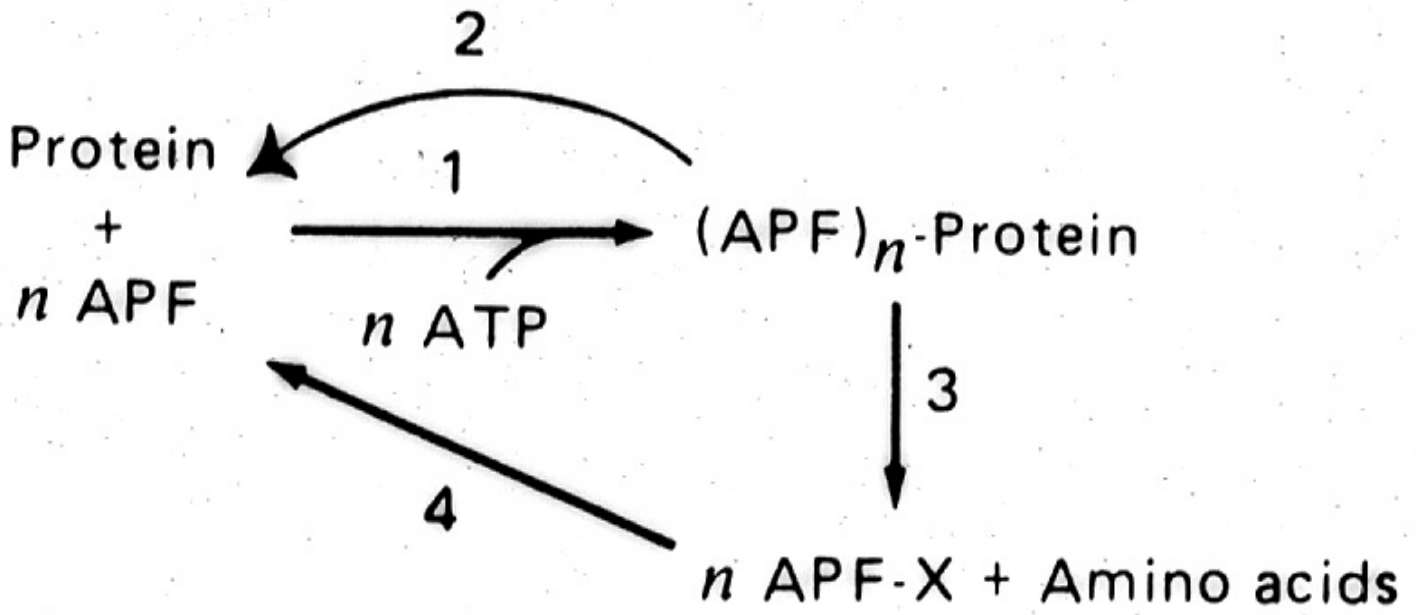
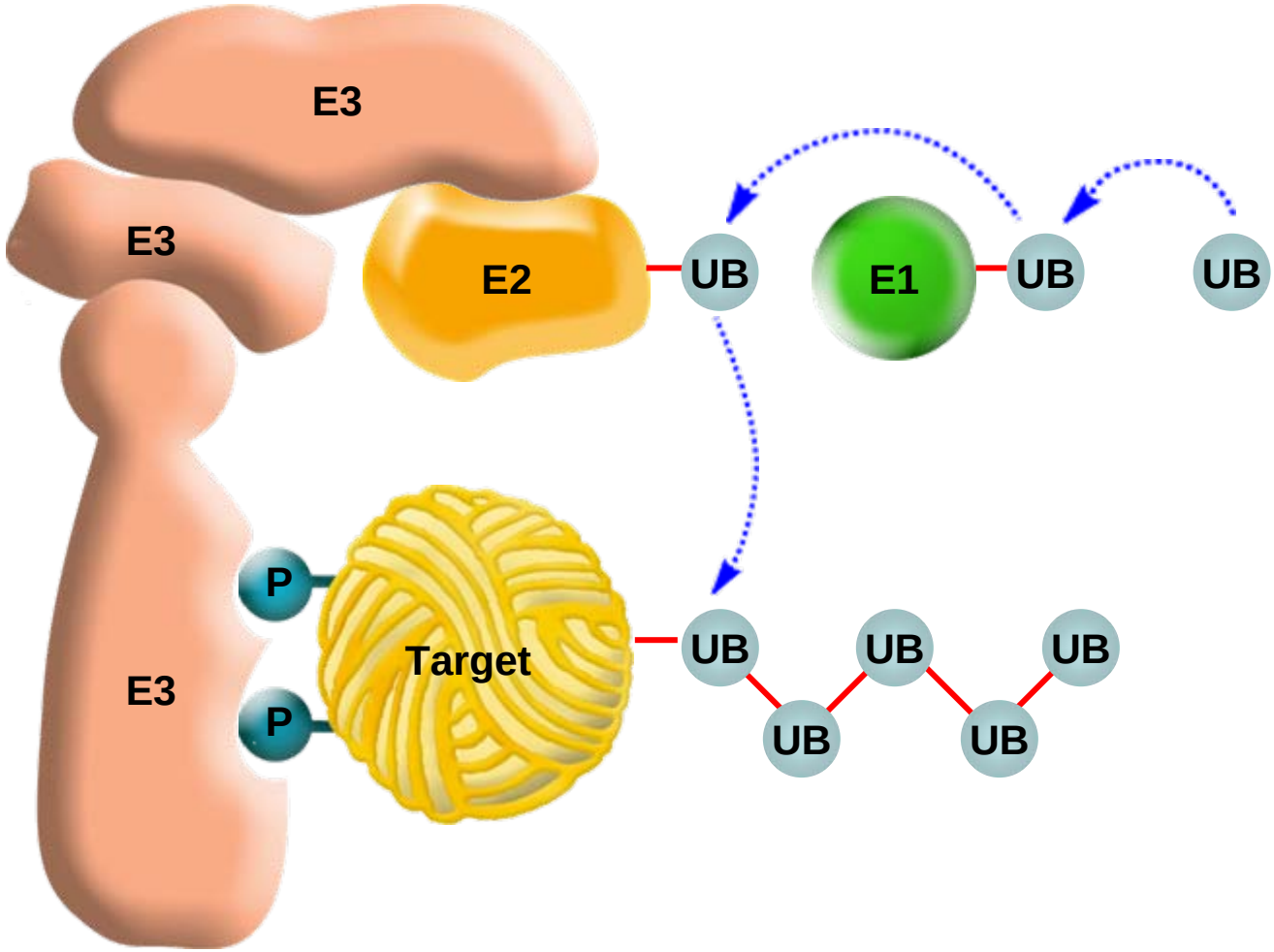


FIG. 6. Proposed sequence of events in ATP-dependent protein breakdown. See the text. 1, APF-1-protein amide synthetase (acting on lysine ϵ-NH_2 groups). 2, Amidase that allows correction when $n = 1$ or 2. 3, Peptidases that act strongly on $(APF\text{-}1)_n$ derivatives, when $n > 1$ or 2. 4, Amidase for APF-1-X; X is lysine or a small peptide.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E2
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E1
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P
Target
E3
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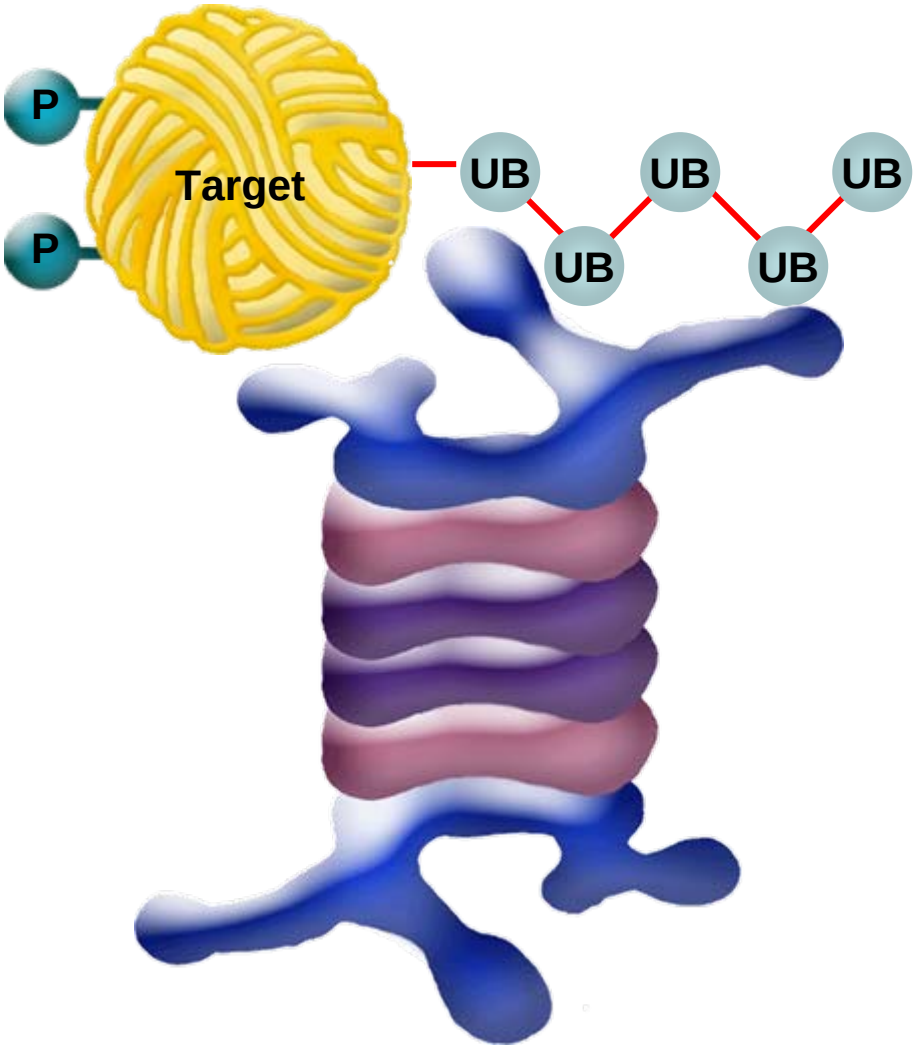
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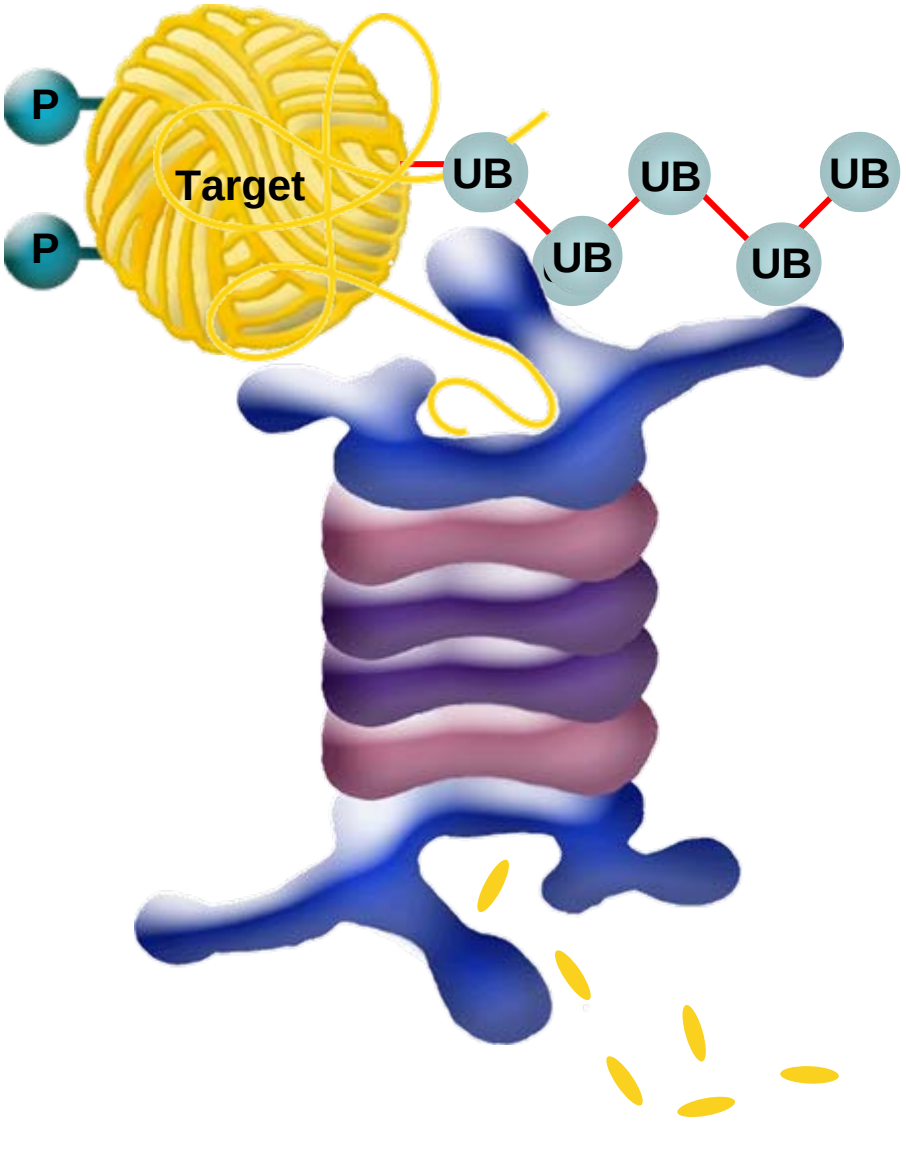
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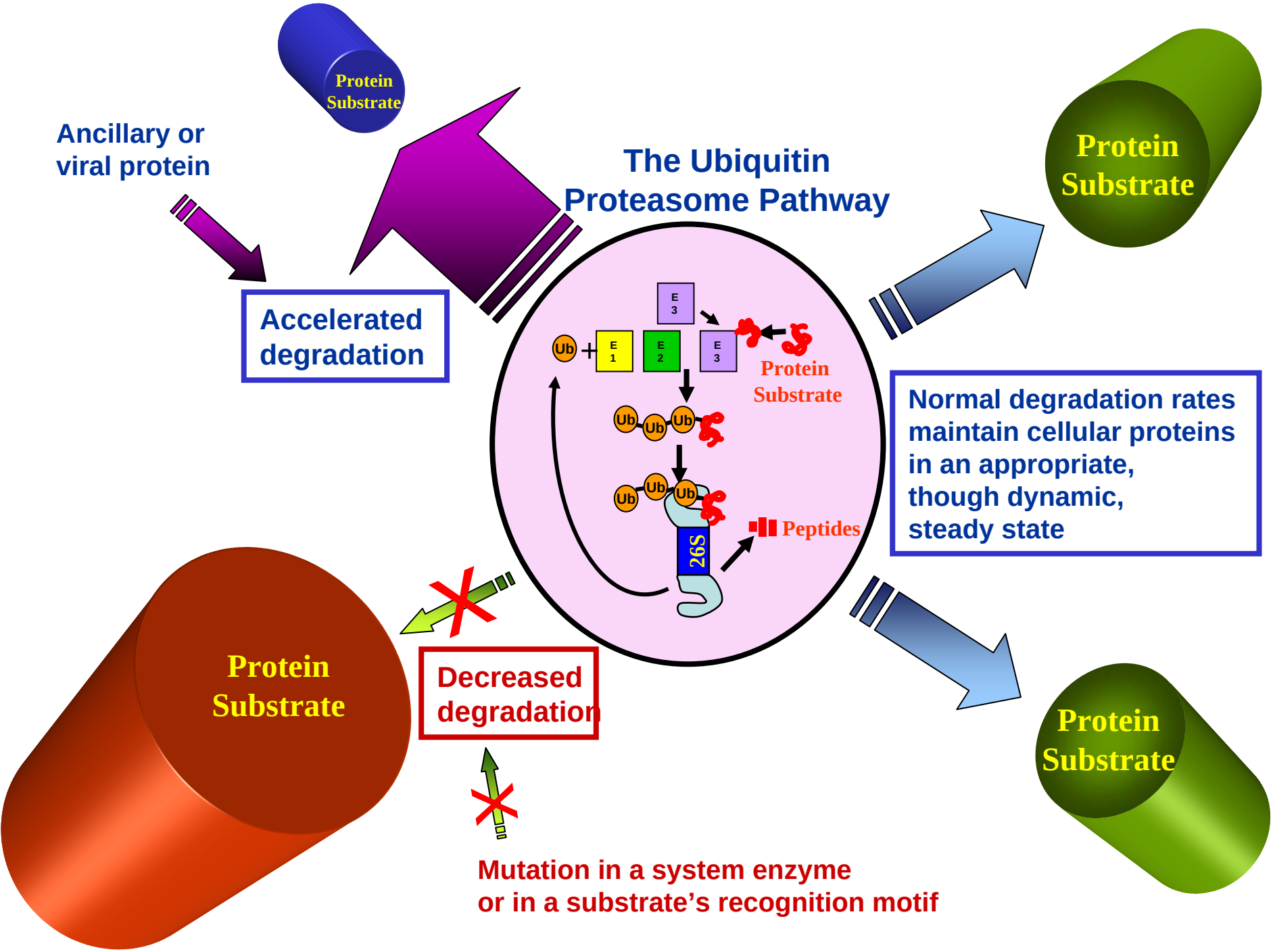
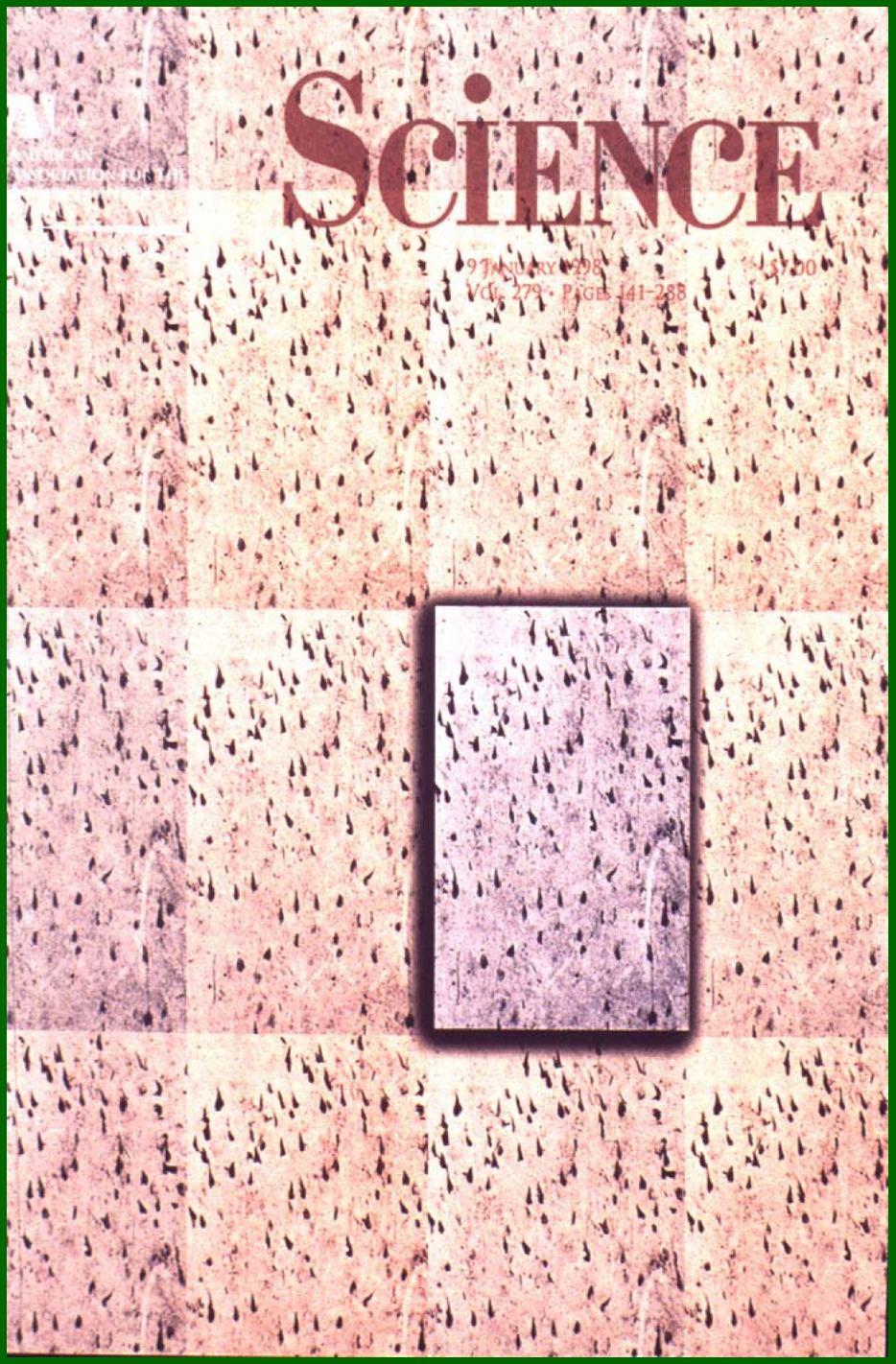

Protein Substrate
Ancillary or viral protein
The Ubiquitin Proteasome Pathway
Protein Substrate
Accelerated degradation
E 3
Ub
E 1
E 2
E 3
Protein Substrate
Ub
Ub
Ub
Ub
Ub
Ub
26S
Peptides
Normal degradation rates maintain cellular proteins in an appropriate, though dynamic, steady state
Protein Substrate
Protein Substrate
Decreased degradation
Mutation in a system enzyme or in a substrate's recognition motif

Roles of Intracellular Protein Degradation

A. Quality Control

B. Control of processes

C. Differentiation and Morphogenesis

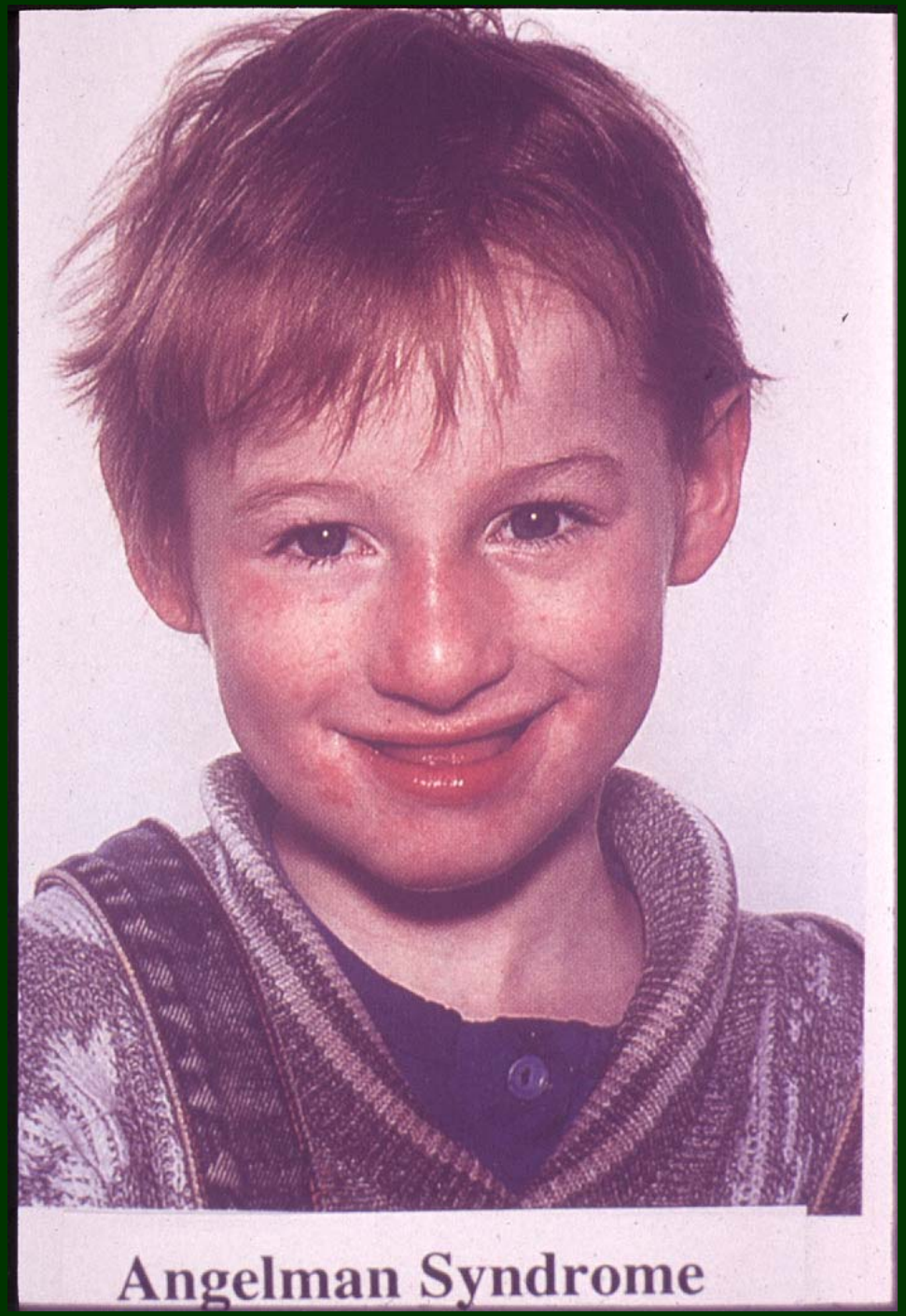

SCIENCE

Roles of Intracellular Protein Degradation

A. Quality Control

B. Control of processes

C. Differentiation and Morphogenesis



Angelman Syndrome

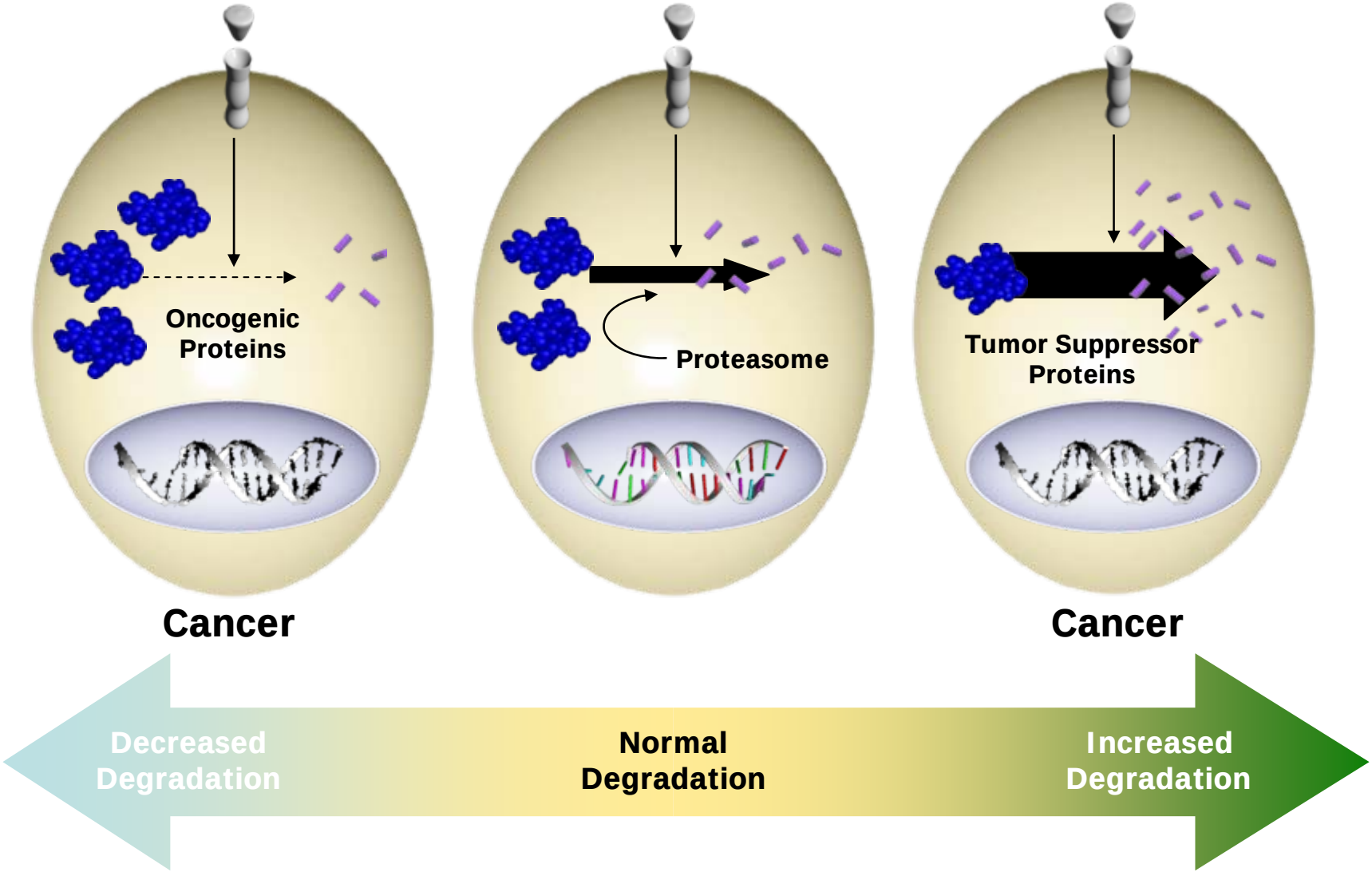
Roles of Intracellular Protein Degradation

A. Quality Control

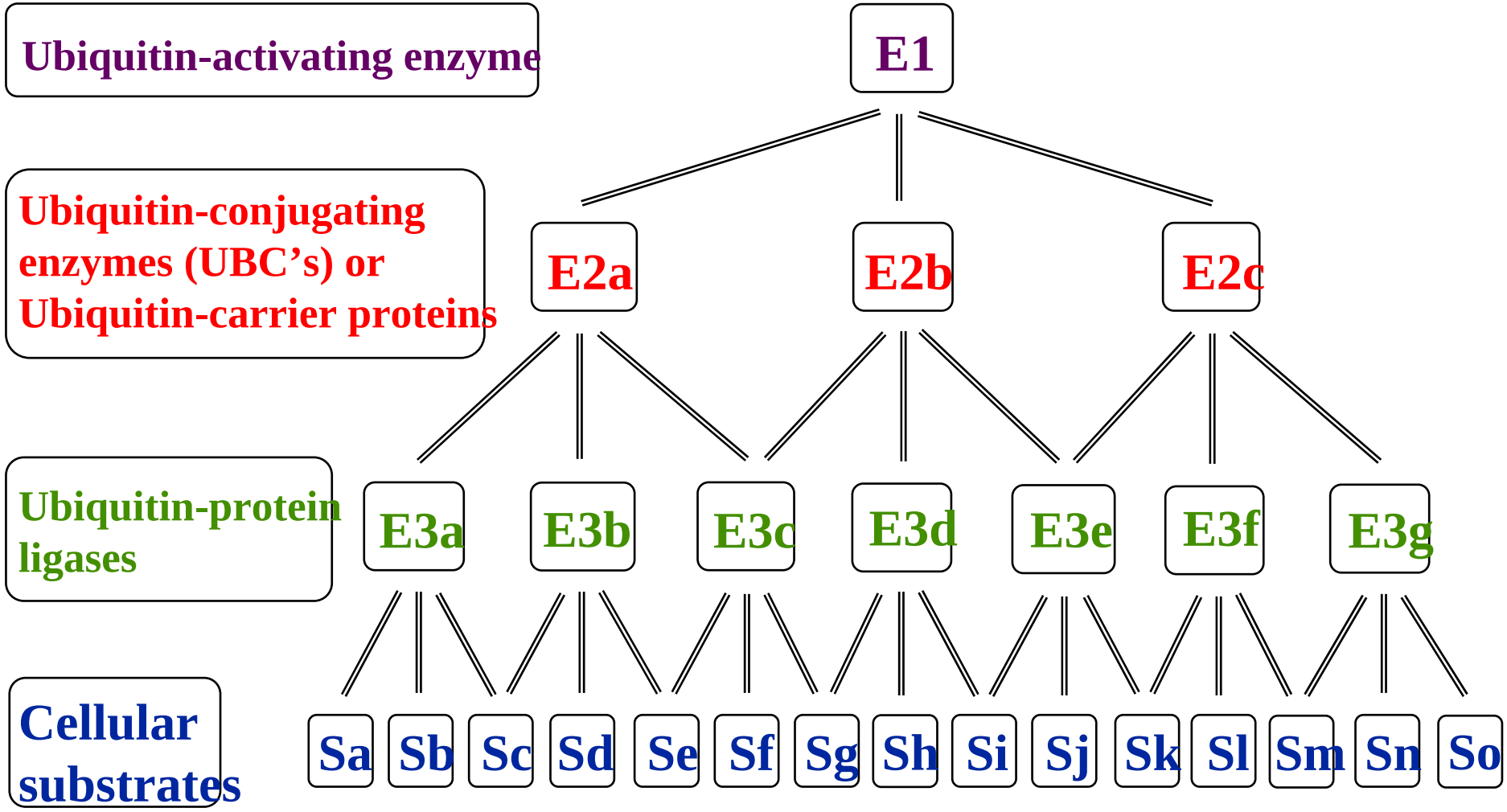
B. Control of processes

C. Differentiation and Morphogenesis

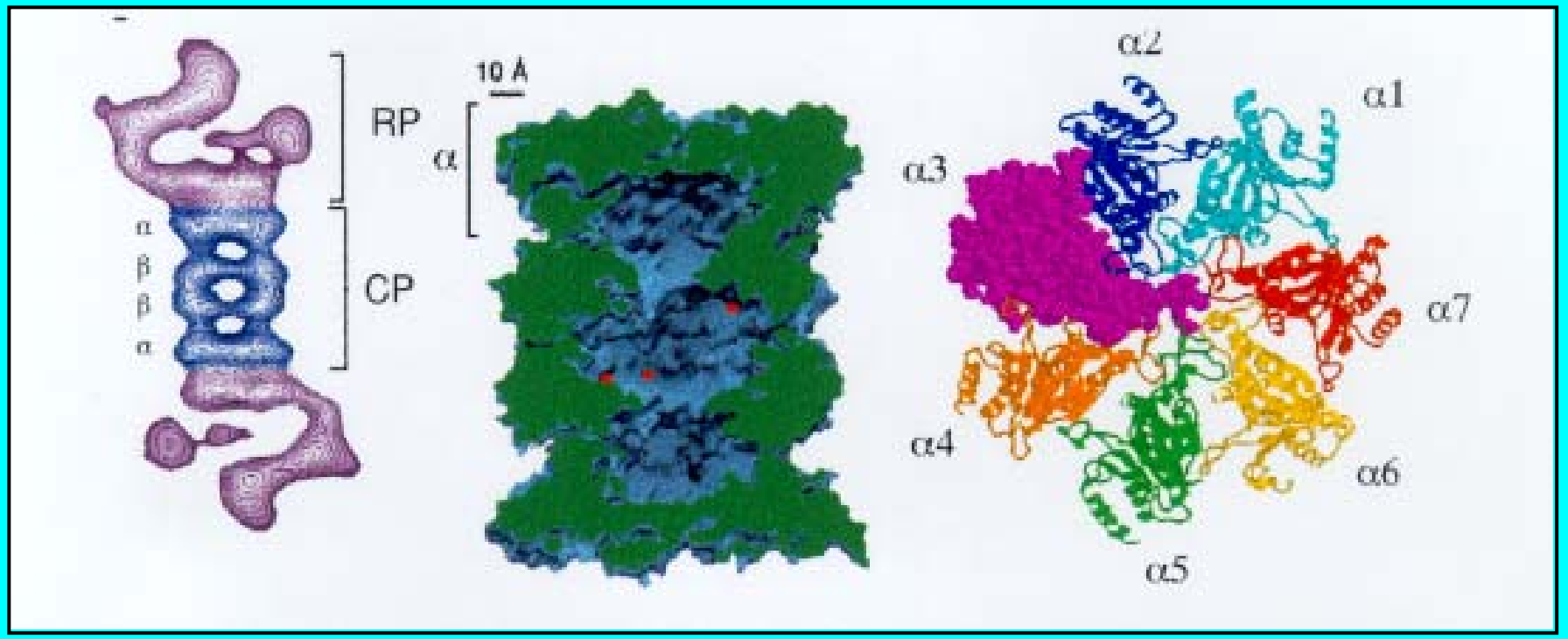
Aberrant Protein Degradation Leads to Disease



The Hierarchical Structure of the Ubiquitin-Conjugating Machinery



Structure of the Yeast Proteasome and CP

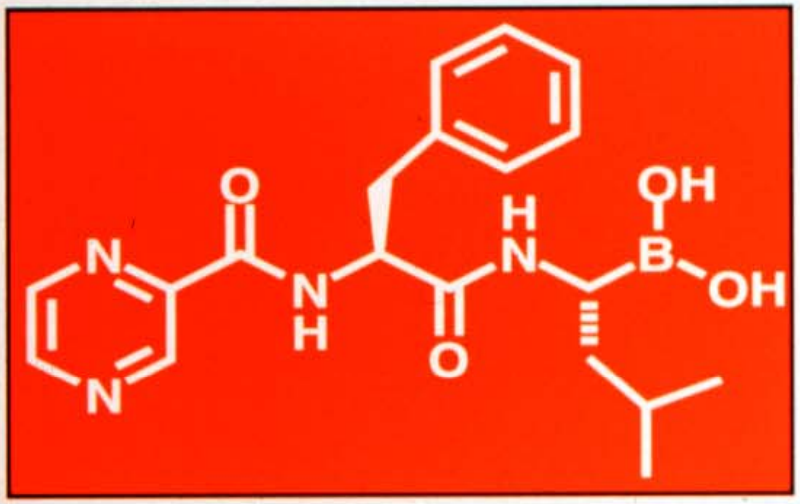


Proteasome

CP

Top view of CP

PS-341: *In Vitro* Activity



Velcade, Bortezomib

Lyophilized Product (GMP)

Ki=0.6 nM (Proteasome Selective)

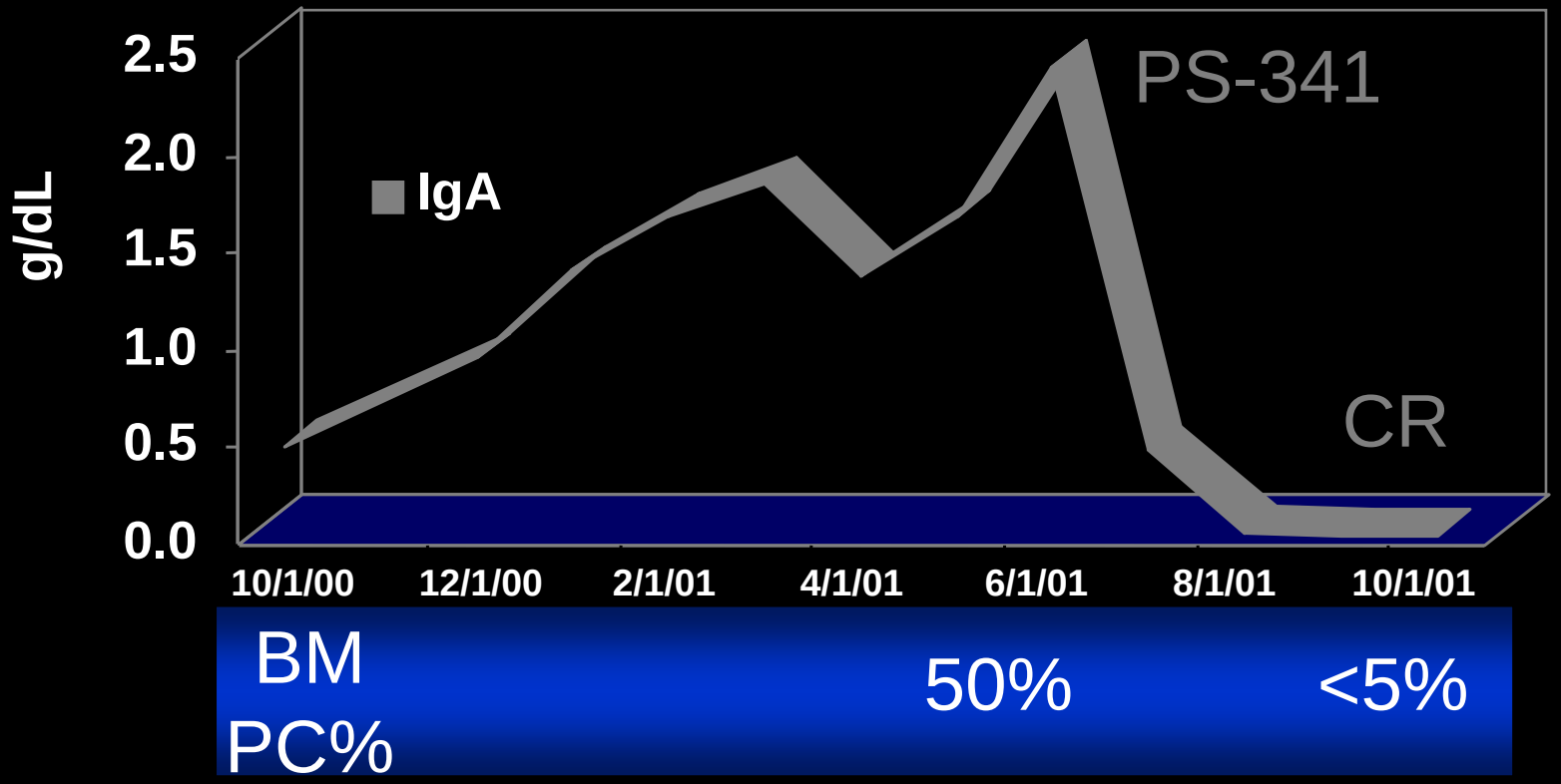
***In vitro* cytotoxicity involves multiple mechanisms of action**

Inhibition of NF-kB
Stabilization of cell-cycle regulatory proteins (p27, p53)
Induction of apoptosis
Weak MDR substrate
Overcomes Bcl-2 cytoprotection

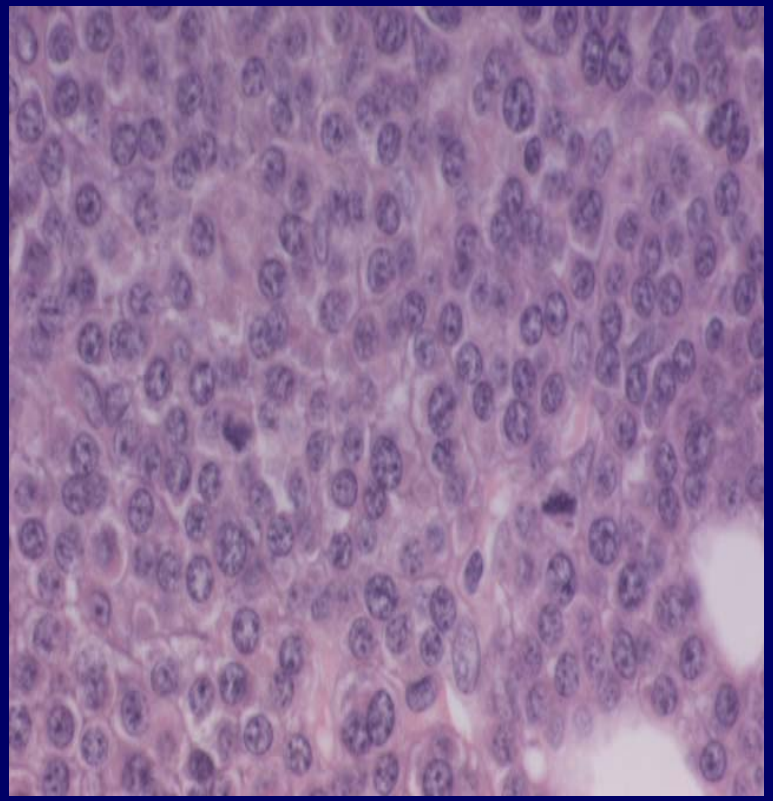
VELCADE® (bortezomib) FOR INJECTION

Complete Response to PS-341

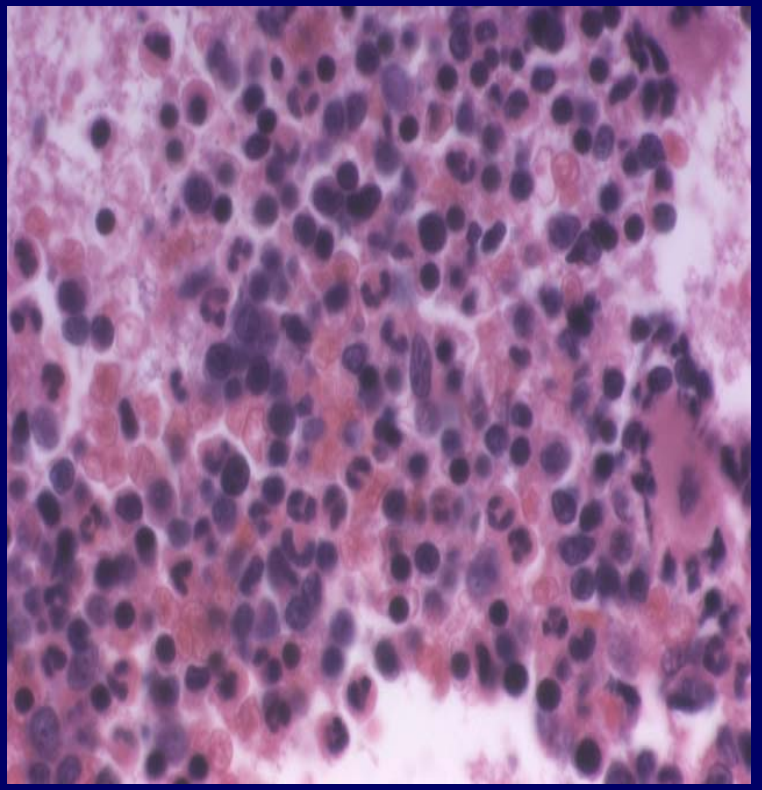
- 3 prior transplants, Ch 13 abnormal
- MP, VAD, Mel/PBSC x 3, dexamethasone
- Gemzar, DCEP, dendritic vaccine
- Thalidomide, DCEAP, Doxil/Navelbine



Patient 004 : Marrow Biopsies

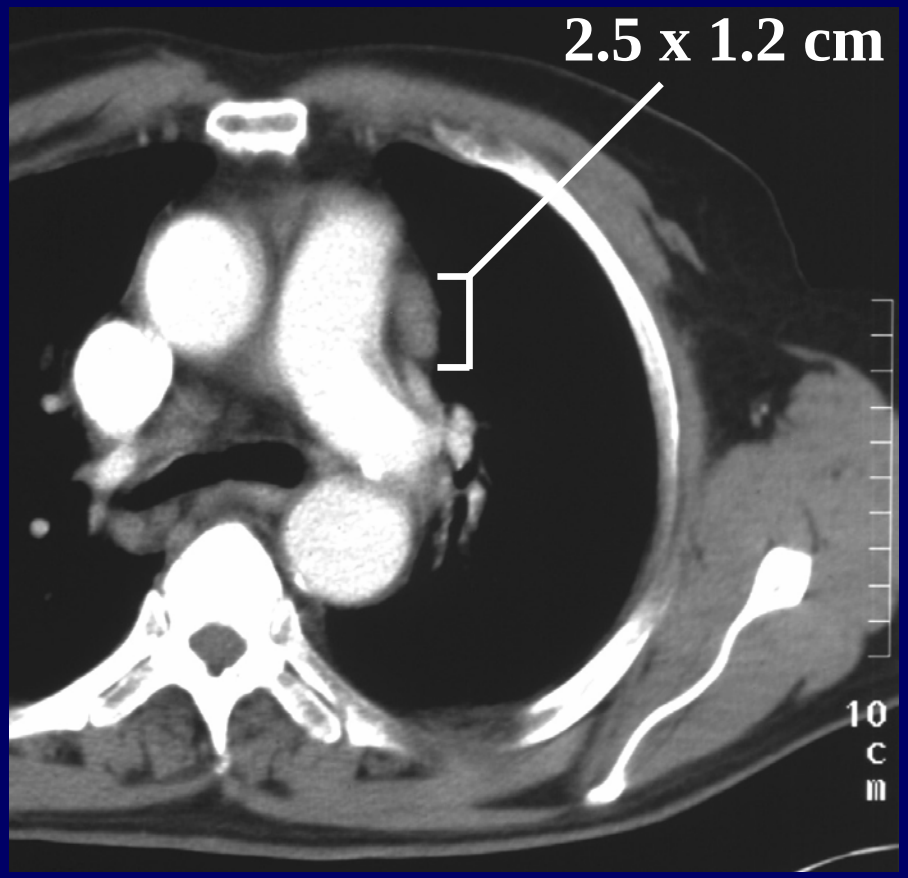


- Pre-PS-341: 41% plasma cells

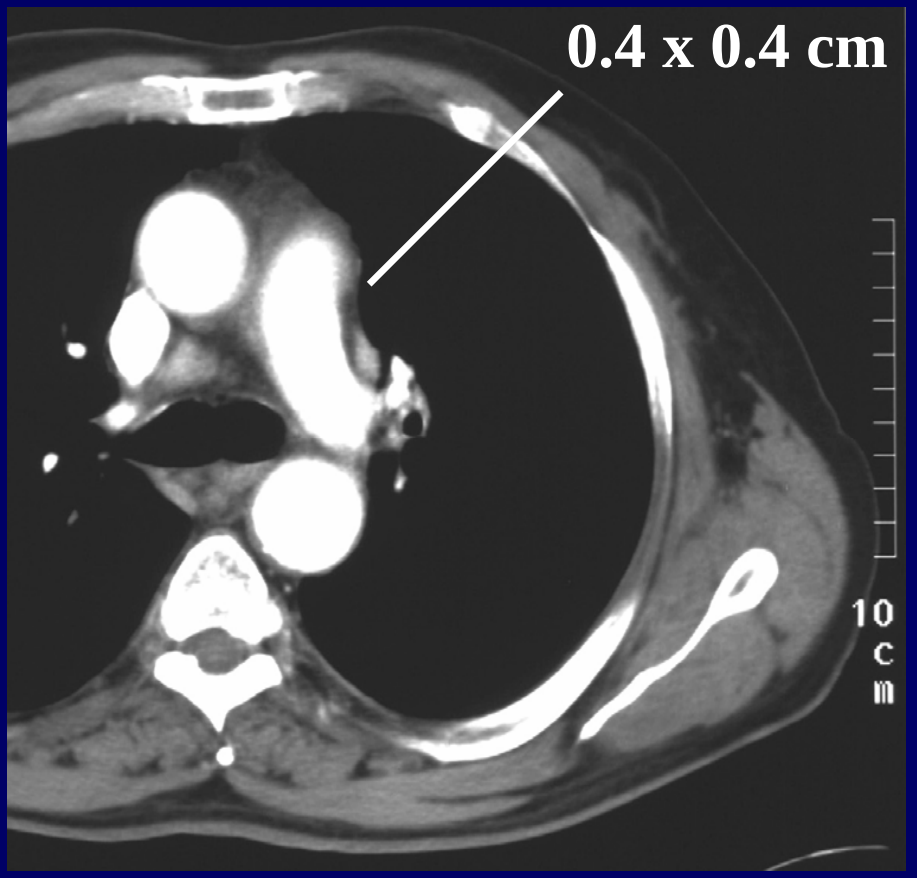


- Post-PS-341: 1%

Patient 010: Refractory Follicular NHL



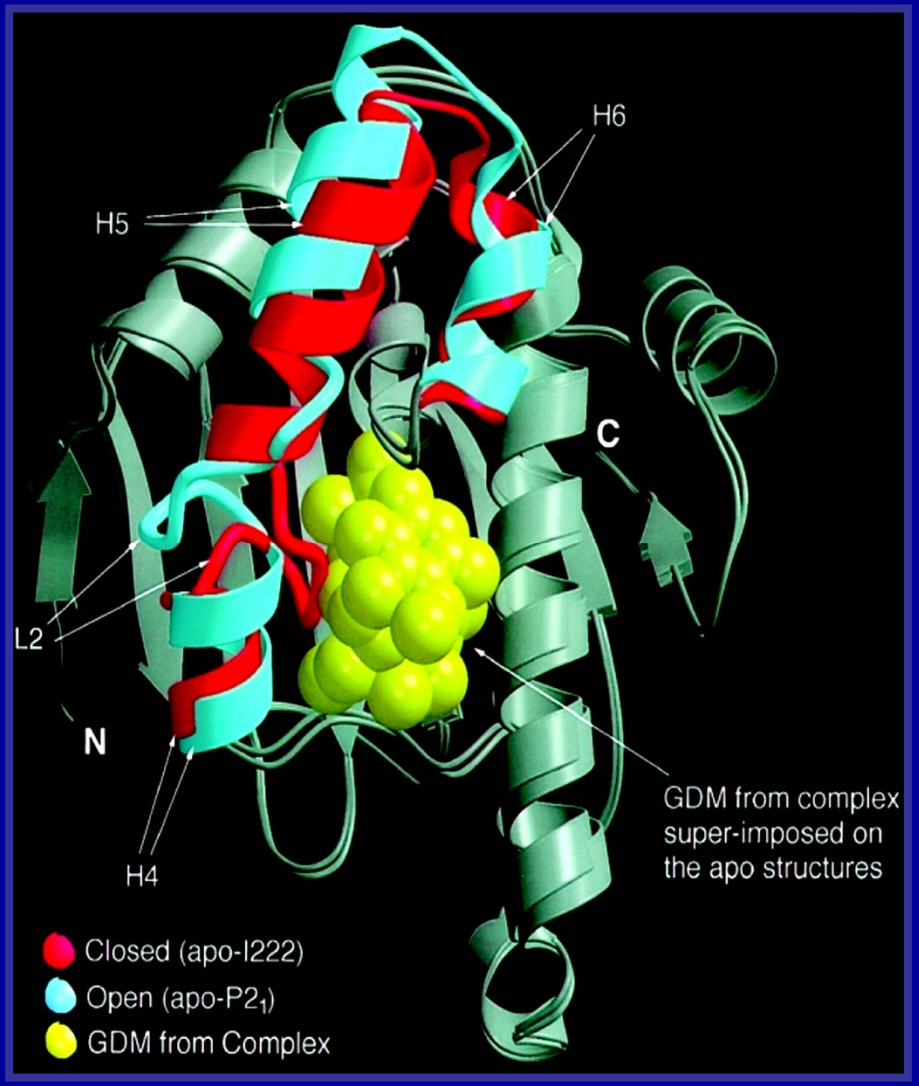
- Pre-treatment



- After cycle 1 of PS-341

Hsp90 Acts as an Evolutionary Capacitor

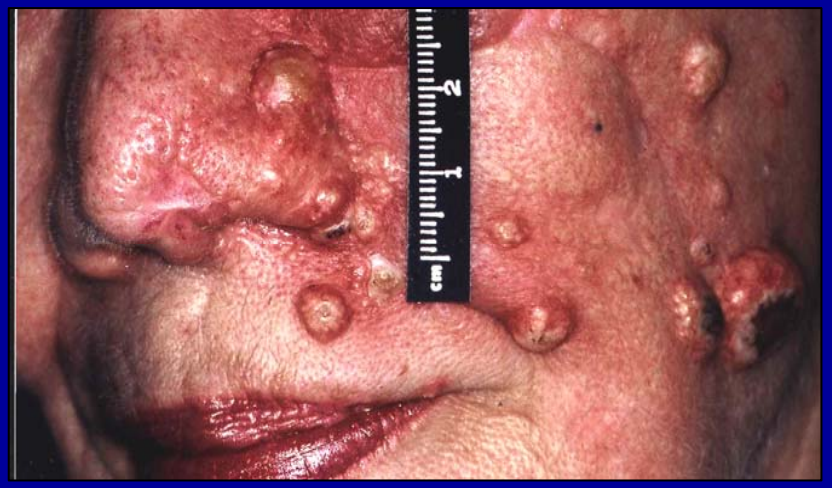
- **Hsp90 selects major hubs of signaling networks (e.g., Raf1 and ER).**
- ***Hsp90 acts as an evolutionary capacitor of mutations (S. Lindquist, Nature 396, 336-342; 1998)***
- **Geldanamycin and 17-AAG inhibit Hsp90 and target clients to proteasome degradation.**



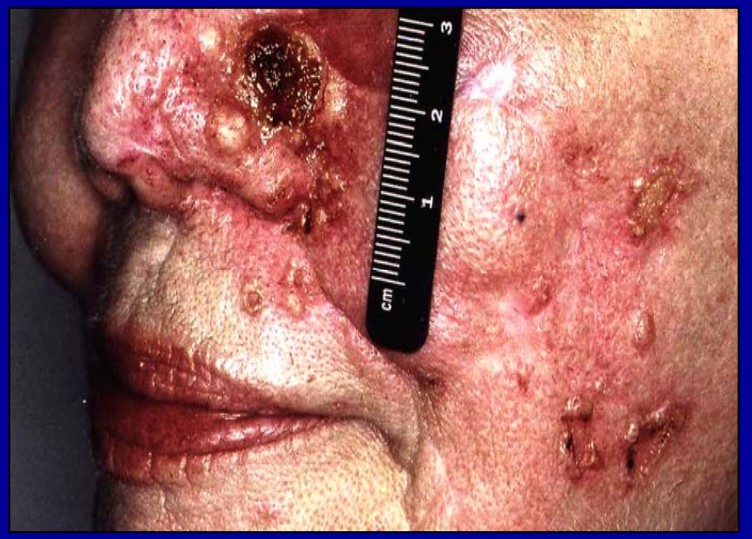
Response to CI-1033

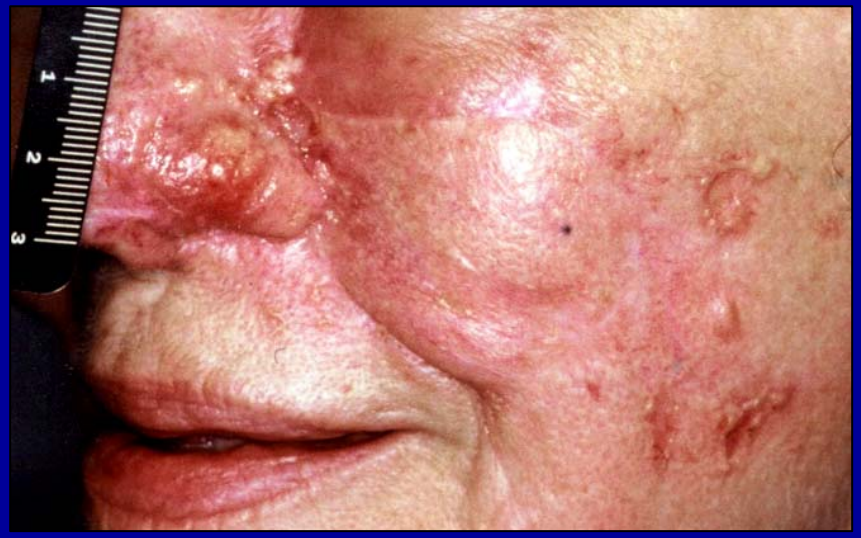
(450 mg)

Baseline



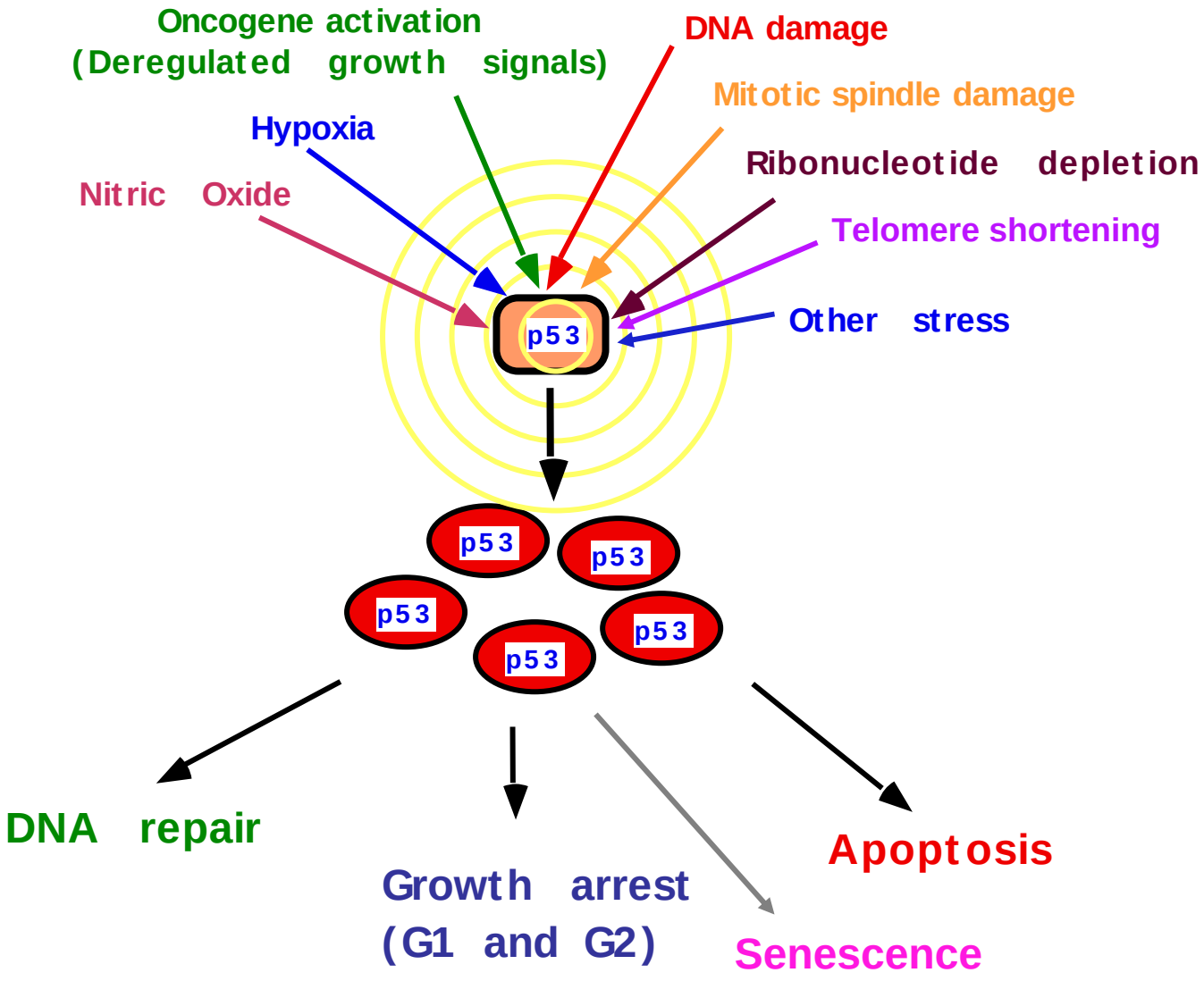
Day 47 (s/p 2 cycles)

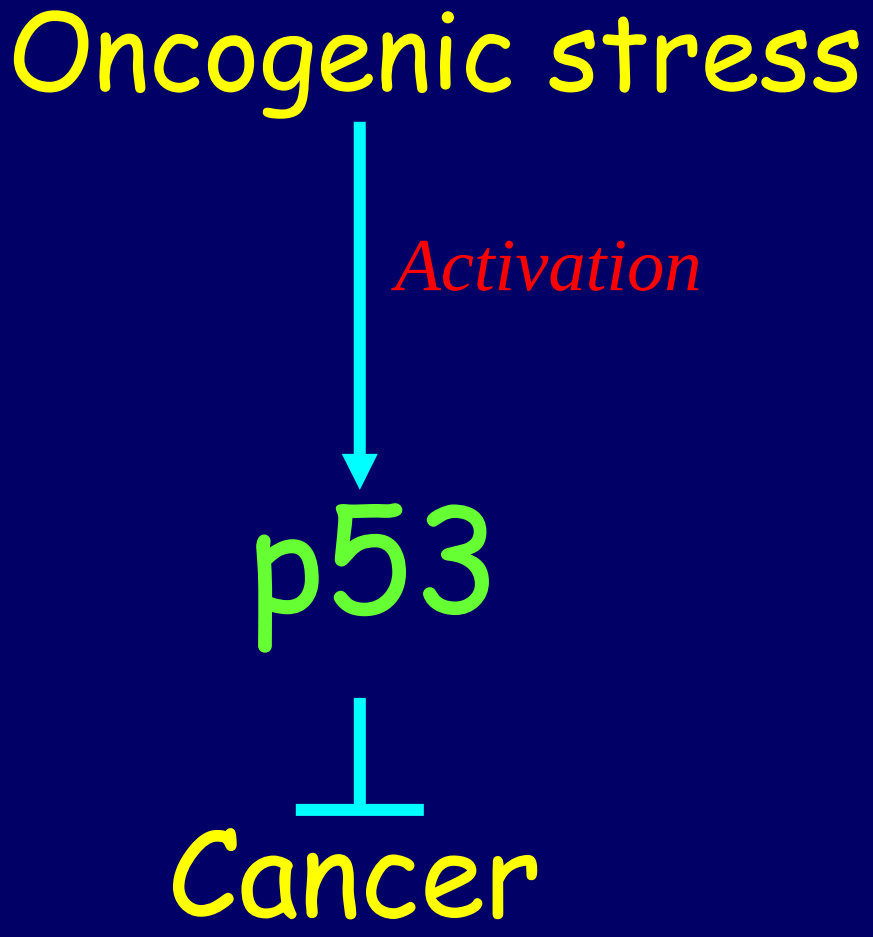




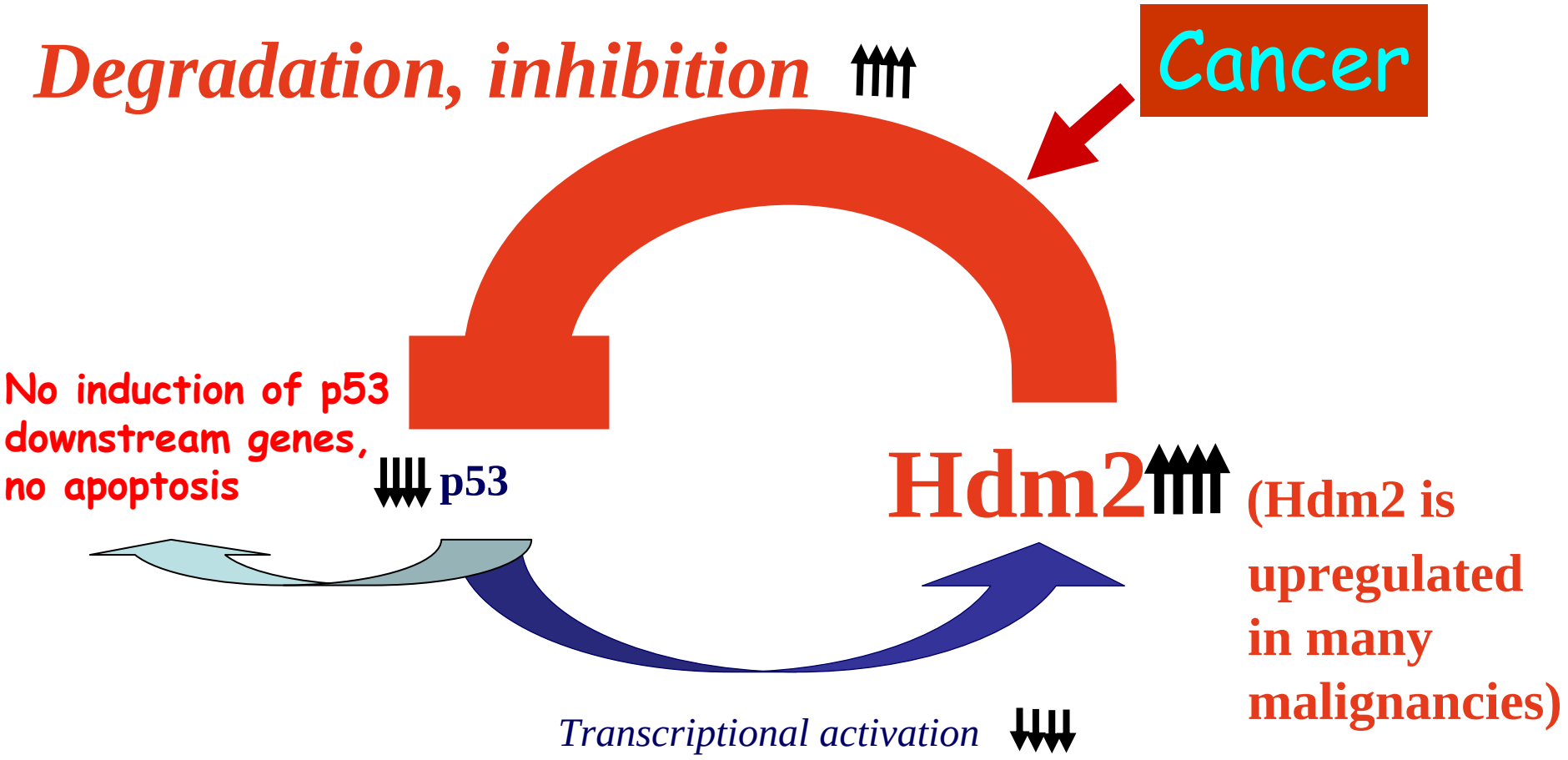
Day 81 (s/p 4 cycles)

Courtesy of Ralph Zinner

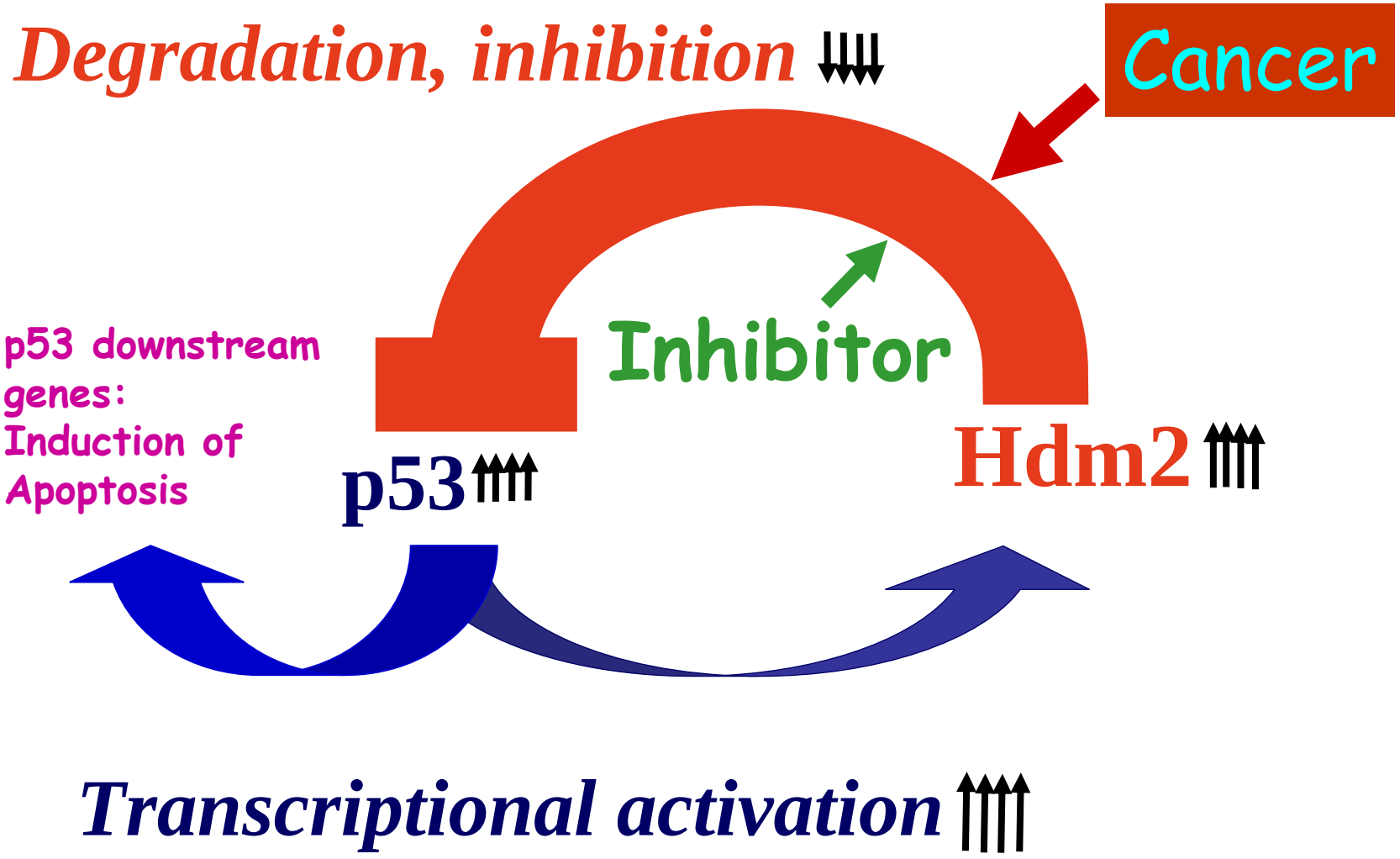

Oncogene activation
(Deregulated growth signals)
DNA damage
Mitotic spindle damage
Hypoxia
Ribonucleotide depletion
Nitric Oxide
Telomere shortening
Other stress
p53
p53
p53
p53
p53
p53
DNA repair
Growth arrest
(G1 and G2)
Senescence
Apoptosis


Oncogenic stress
Activation
p53
Cancer

The p53-Hdm2 feed-back loop in Cancer



The p53-Hdm2 feed-back loop repaired



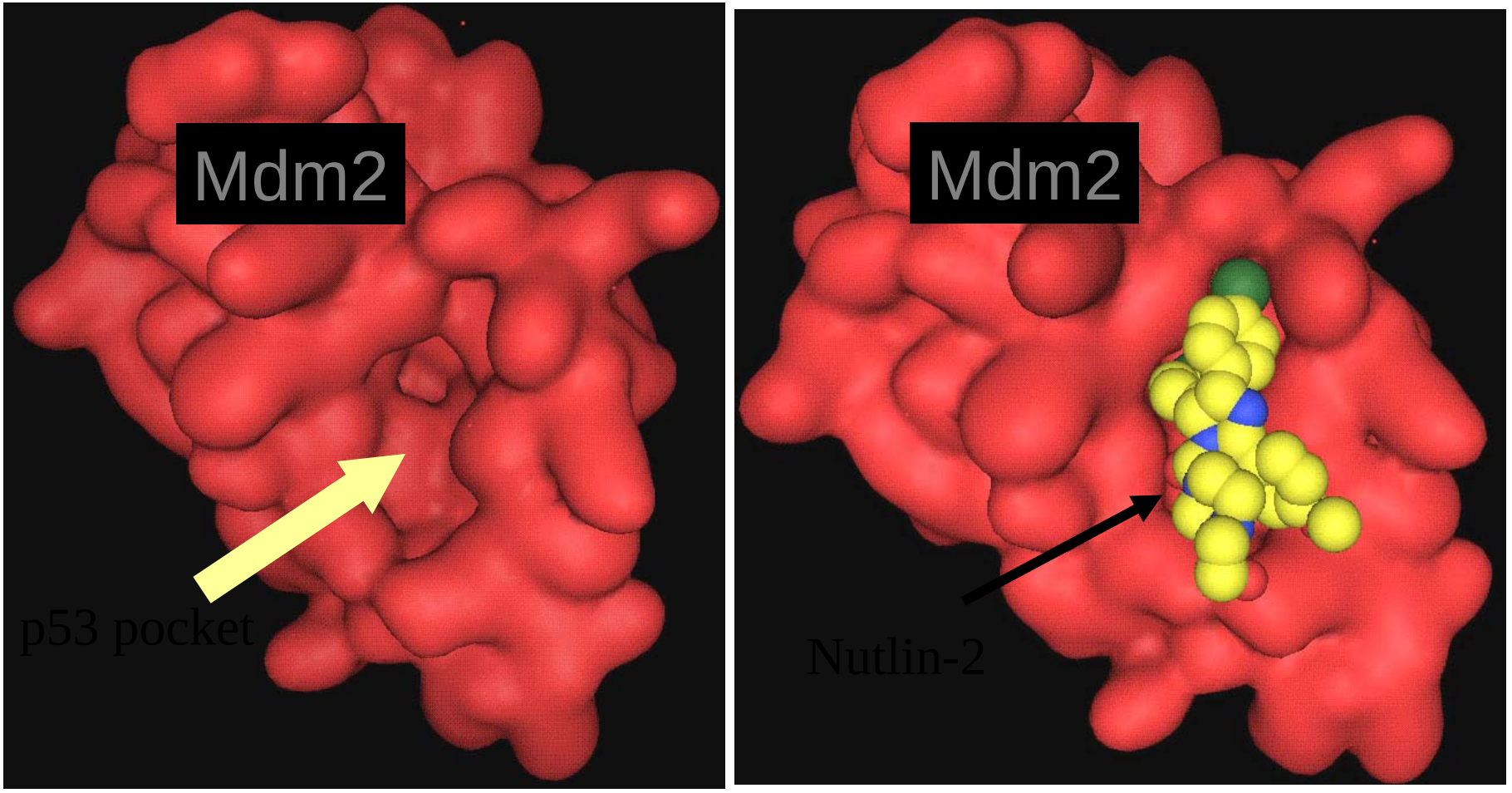
Nutlin

Disrupts Mdm2-p53 binding

(Roche Corp.)

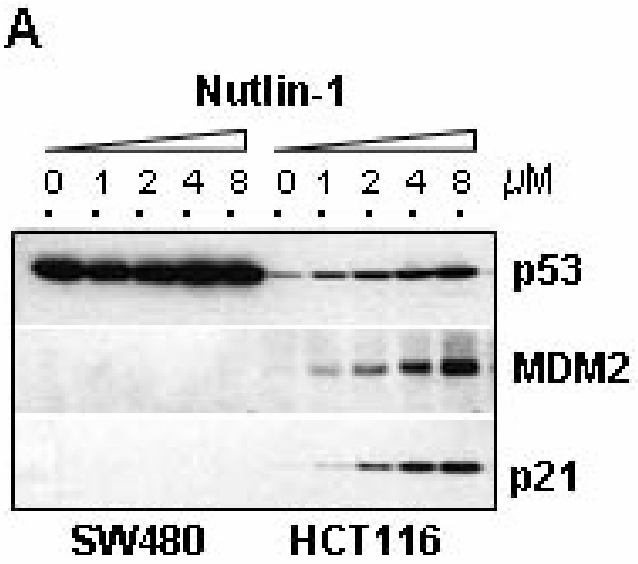
Nutlin 2, Nutlin 3

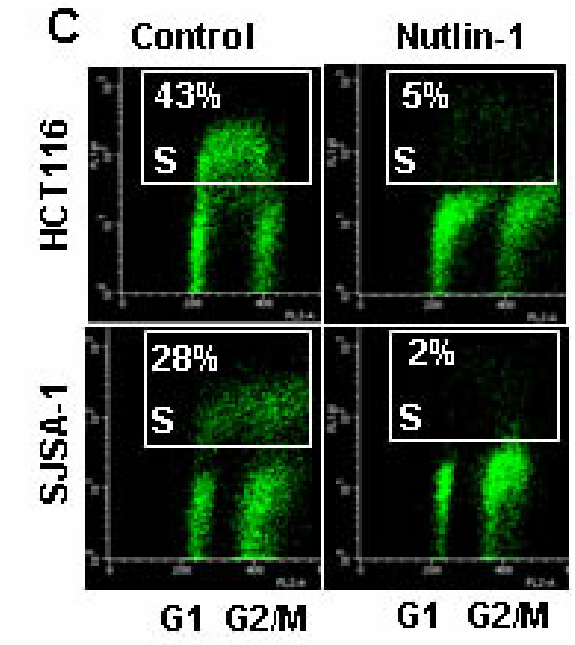
Nutlin-2: a drug that binds within the p53-binding pocket of Mdm2 and blocks p53 binding and inactivation



Courtesy of L. Vassiliev, Roche

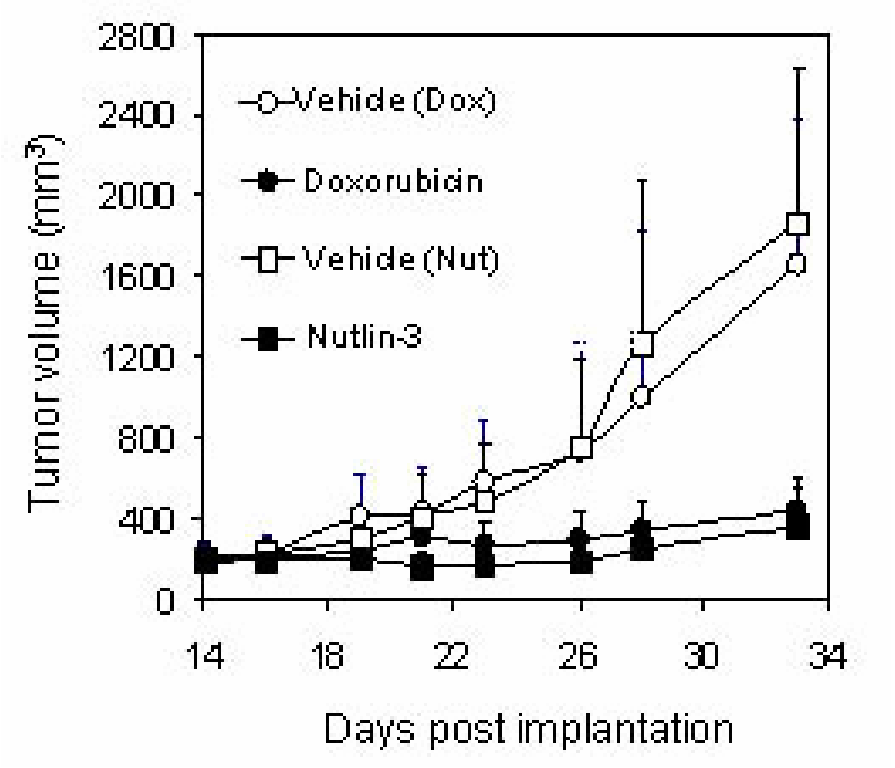
Effect of Nutlin-1 on the expression of p53 and on cell cycle.





Vassilev et al., Science 2004

Effect of Nutlin-3 (P.O.)
and Doxorubicin (I.V.)
on SJSA-1 human cancer xenograft in nude mice



Vassilev et al., Science 2004