Intracellular Protein Degradation and the Ubiquitin-Proteasome System

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Intracellular protein degradation

- **The basics**

  General features of intracellular protein degradation

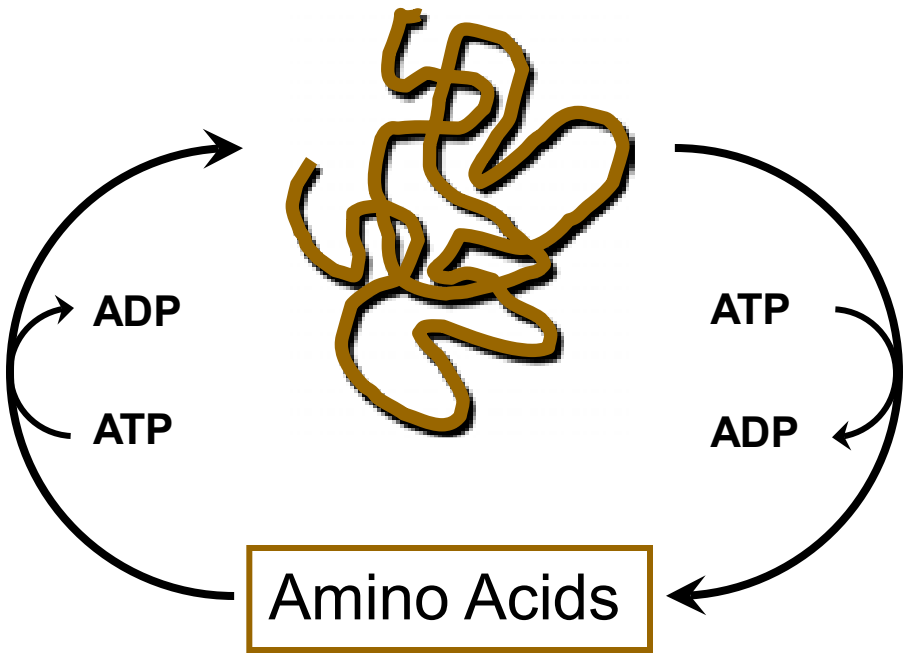
- **Biochemical mechanisms**

  The ubiquitin-proteasome pathway

- **Physiological and pathological examples**

  How the UPS does interesting and important things

Cellular proteins are in a state of continuous turnover

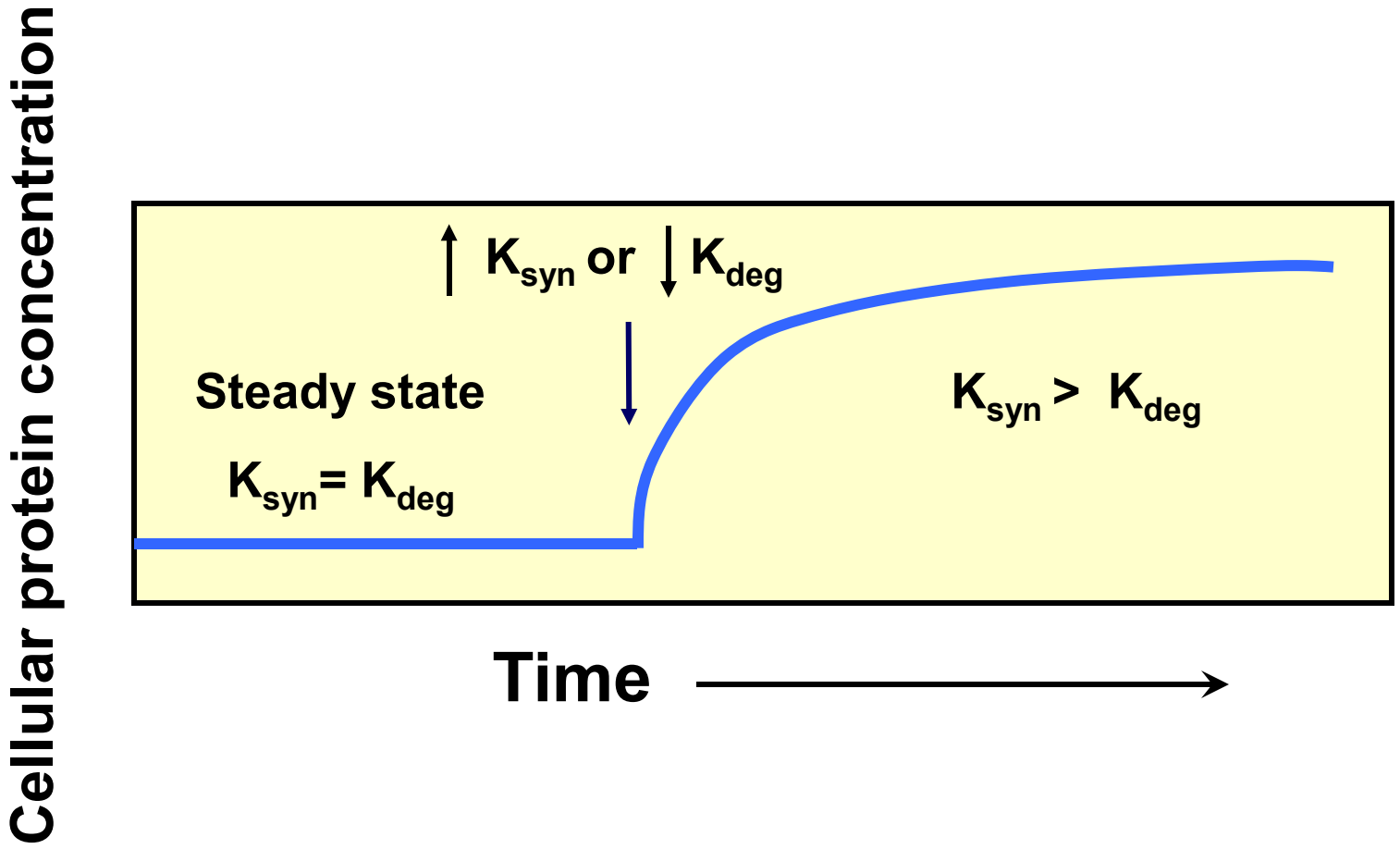


at steady state: $K_{syn} = K_{deg}$

$$T_{1/2} = \frac{\ln 2}{K_{deg}}$$

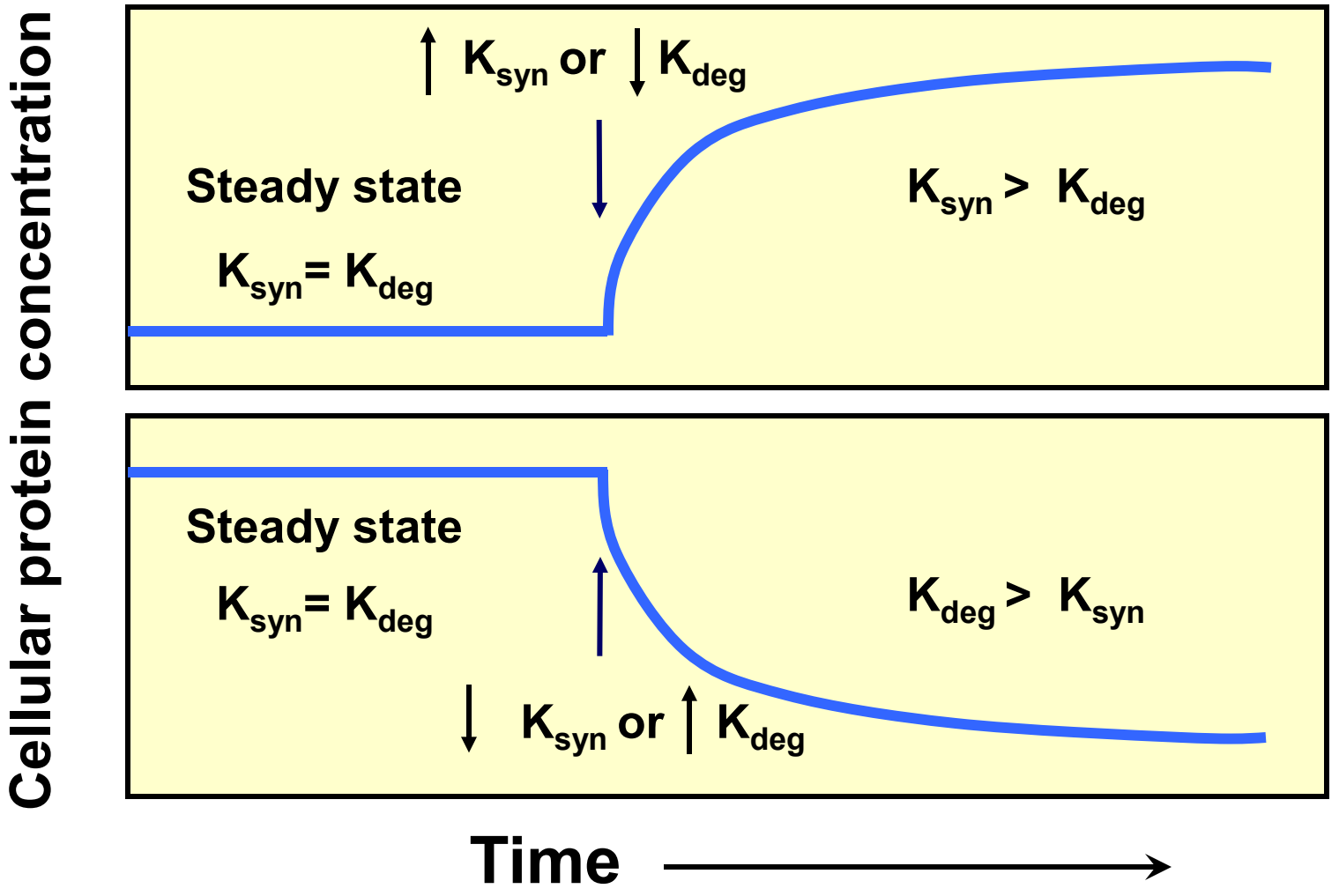
GND
PD01

The cellular concentration of a protein can be altered by changing relative rates of protein synthesis and degradation



GND
PD02

The cellular concentration of a protein can be altered by changing relative rates of protein synthesis and degradation



GND
PD03

Really important concepts

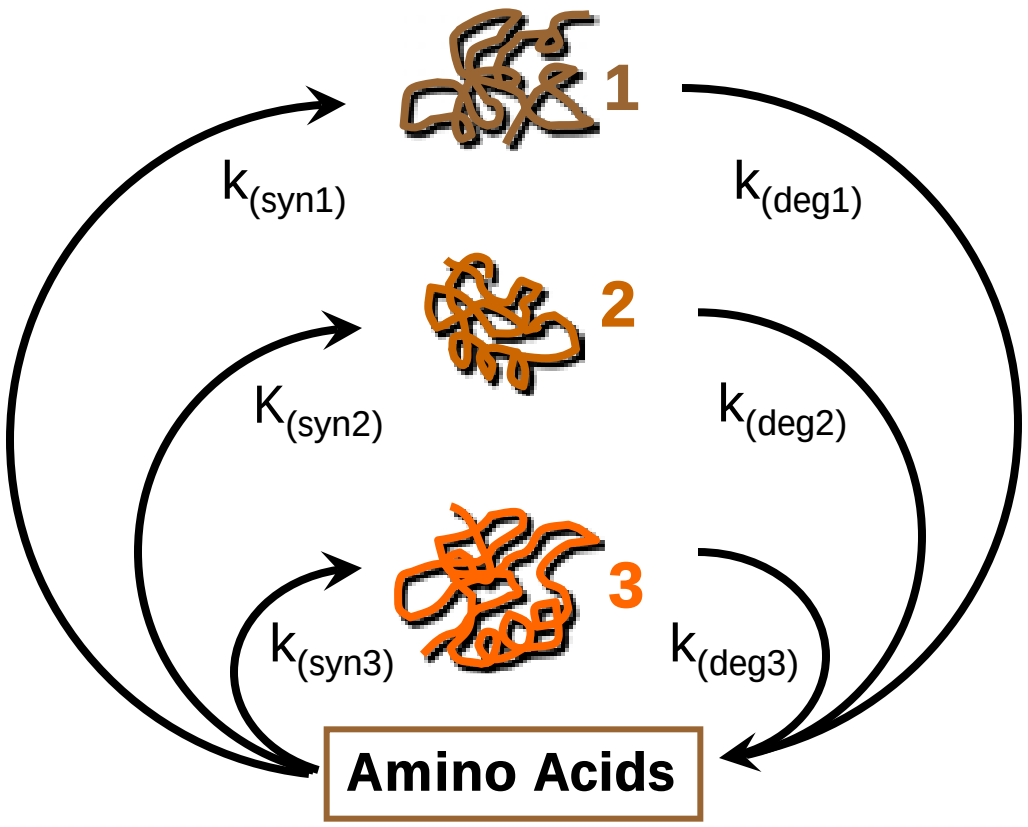
Intracellular protein degradation is a highly **regulated** process.

Rates of protein degradation increase or decrease under many physiological and pathological conditions.

Intracellular protein degradation is a **regulatory** process.

Protein degradation regulates cellular events by controlling levels of important proteins.

Cellular proteins have different half-lives



$T_{1/2}$ 1 = 1 hr

$T_{1/2}$ 2 = 10 hrs

$T_{1/2}$ 3 = 100 hrs

GND
PD05

Really important concept

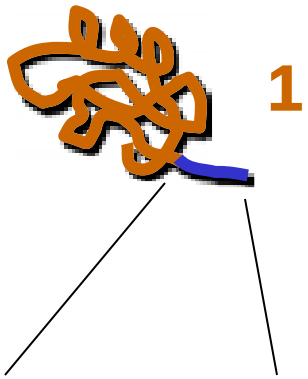
Structural features of proteins (degrons) determine half-lives.

GND
PD06

Really important concept

Structural features of proteins (degrons) determine half-lives.

$T_{1/2}$ 1 = 1 hr



AGREGTYFQRA

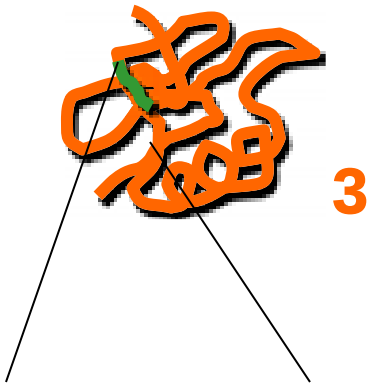
Degron 1

$T_{1/2}$ 2 = 1 hr

LVYQRESTLVAY

Degron 2

$T_{1/2}$ 3 = 1 hr



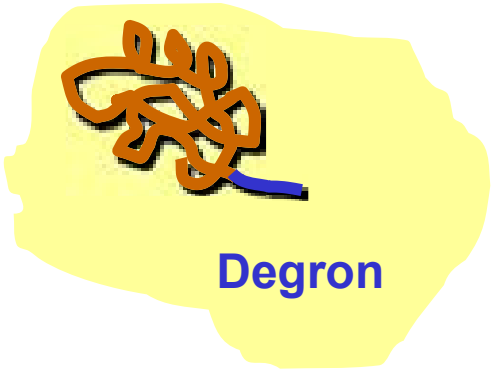
LGFERTSIGNAL

Degron 3

GND
PD07

Structural features of proteins (degrons) determine half-lives.

Protein 1: T ½ = 1 hr

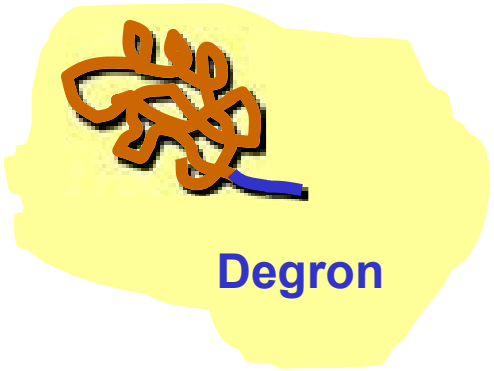


Protein 1: T ½ = >>1 hr

GND
PD08

Structural features of proteins (degrons) determine half-lives.

Protein 1: T ½ = 1 hr



c-jun

Protein 1: T ½ = >>1 hr

v-jun

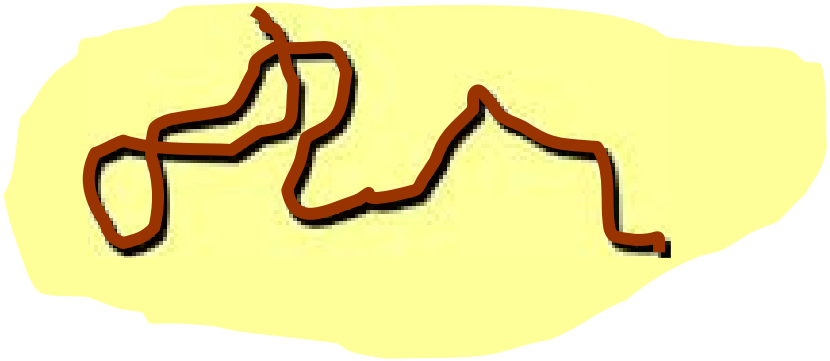
Physiological roles of intracellular protein degradation

- Quality control
- Antigen processing
- Regulation of cellular function
- Adaptation to physiological or pathological states

Cells selectively degrade proteins with abnormal structures

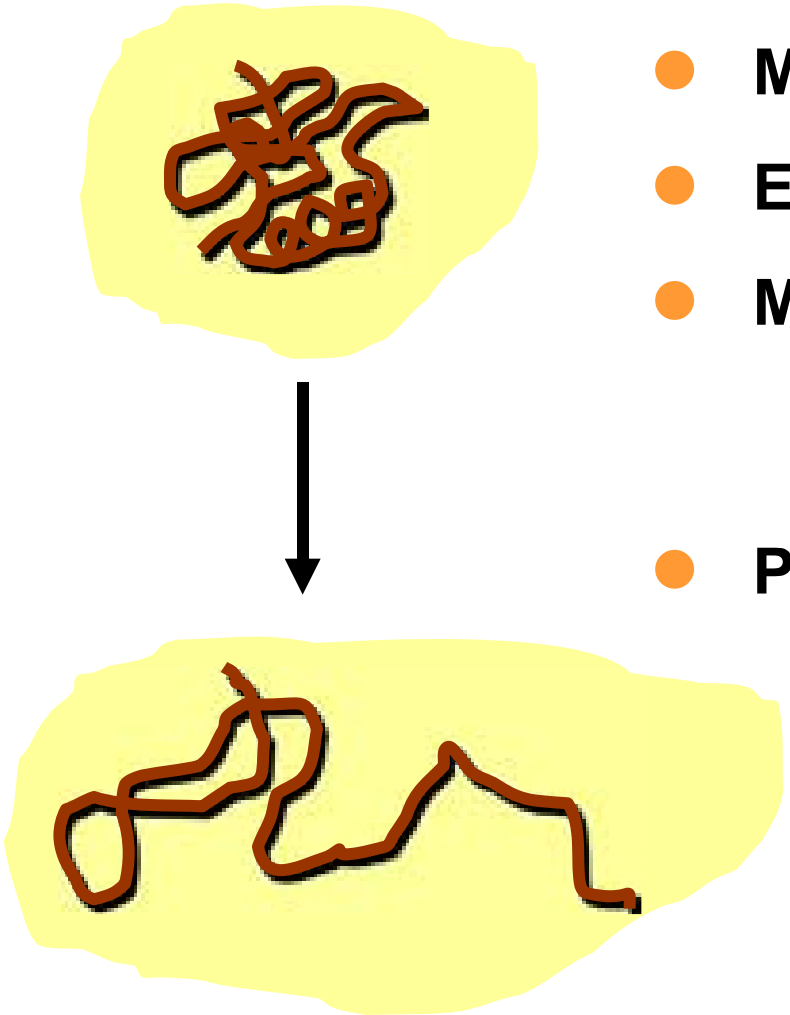
Normal Protein 1: T ½ = 10 hrs

Abnormal Protein 1: T ½ = 1 hr



GND
PD12

Cells selectively degrade proteins with abnormal structures



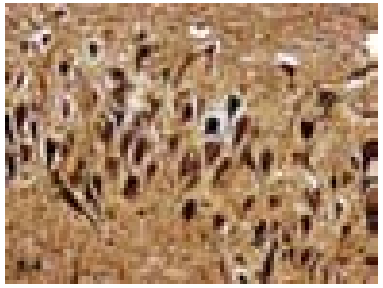
- Mutations
- Errors in translation
- Metabolic damage

  (e.g. oxidation or denaturation from high temperature, etc.)
- Proteins without partners

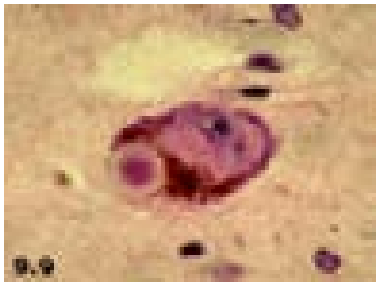
GND
PD13

Important clinical fact

Many neurological (and other) diseases may be caused by aggregation rather than degradation of proteins with abnormal structures.



Tau aggregates
(Azheimer's disease)



Lewy Bodies
(Parkinson's Disease)

- Alzheimer's disease (β–amyloid, tau)
- Huntington's disease (Huntingtin)
- Parkinson's disease (α-synuclein)
- ALS "Lou Gerhig's" disease (SOD)
- Spinal-Bulbar Muscular atrophy (androgen receptor)
- Prion diseases "Mad Cow disease" (prion)
- *et al, et al*

GND
PD14

Physiological roles of intracellular protein degradation

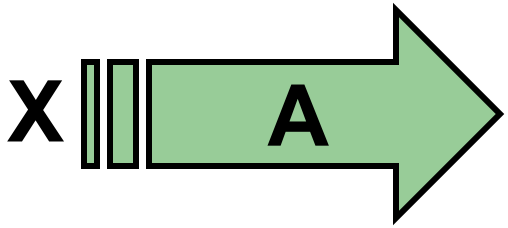
- Quality control
- Antigen processing
- **Regulation of cellular function**
- Adaptation to physiological or pathological states

Really important concept

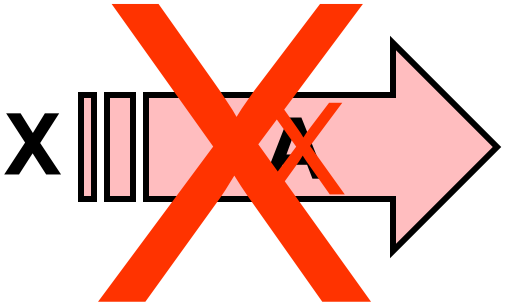
Protein degradation regulates processes positively and negatively via the conditional degradation of positive and negative effectors.

Negative regulation :

Process X depends on Protein A to proceed



Degrade Protein A to inhibit Process X



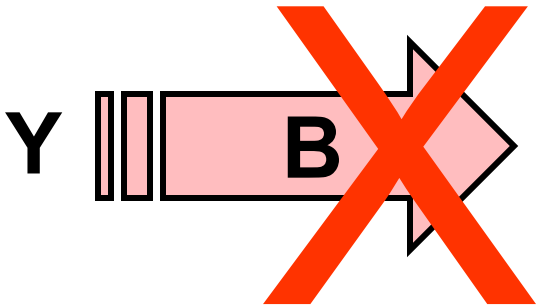
GND
PD18

Really important concept

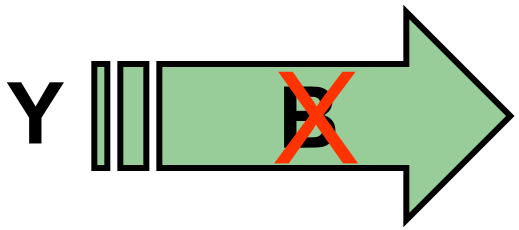
Protein degradation regulates processes positively and negatively via the conditional degradation of positive and negative effectors.

Positive regulation :

Process Y is normally inhibited by Protein B.



Degrade Protein B to promote Process Y.



GND
PD19

What is the advantage of having proteins with short half-lives?

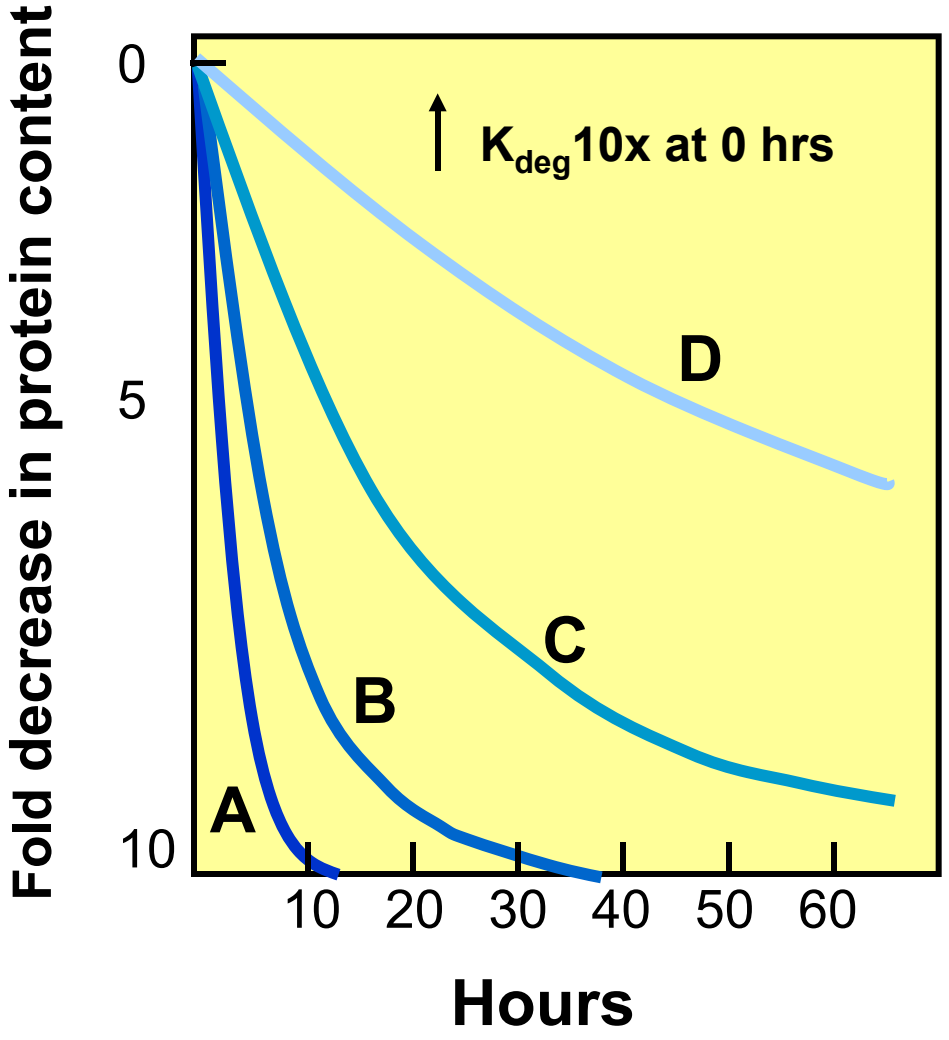
GND
PD22

Really important concept

Proteins with short half-lives can change their concentrations faster than proteins with long half-lives.

GND
PD22

Proteins with short half-lives can change their cellular concentrations rapidly



Protein	$T_{1/2}$ (hrs)
A	1.0
B	5.0
C	10.0
D	50.0

GND
PD20

Highly regulated proteins have the shortest constitutive half-lives

Protein	$T_{1/2}$	
Ornithine decarboxylase	10 mins	**Highly regulated**
Tyrosine amino transferase	1.5 hrs	
HMG CoA reductase	5 hrs	
Catalase	20 hrs	**Not highly regulated**
Cytochrome C	100 hrs	
Arginase	300 hrs	
Hemoglobin	∞	

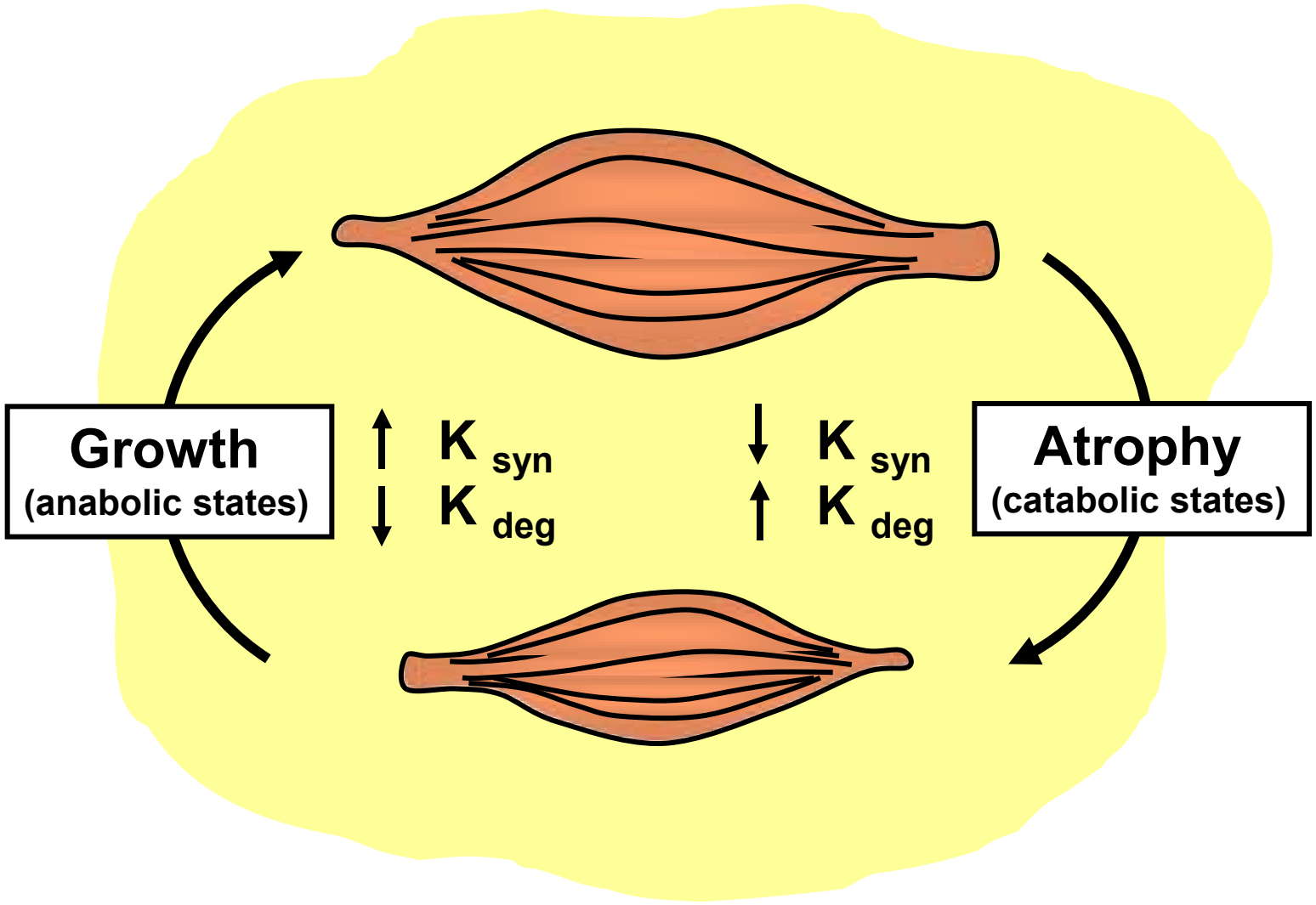
GND
PD23

Physiological roles of intracellular protein degradation

- Quality control
- Antigen processing
- Regulation of cellular function
- **Adaptation to physiological or pathological states**

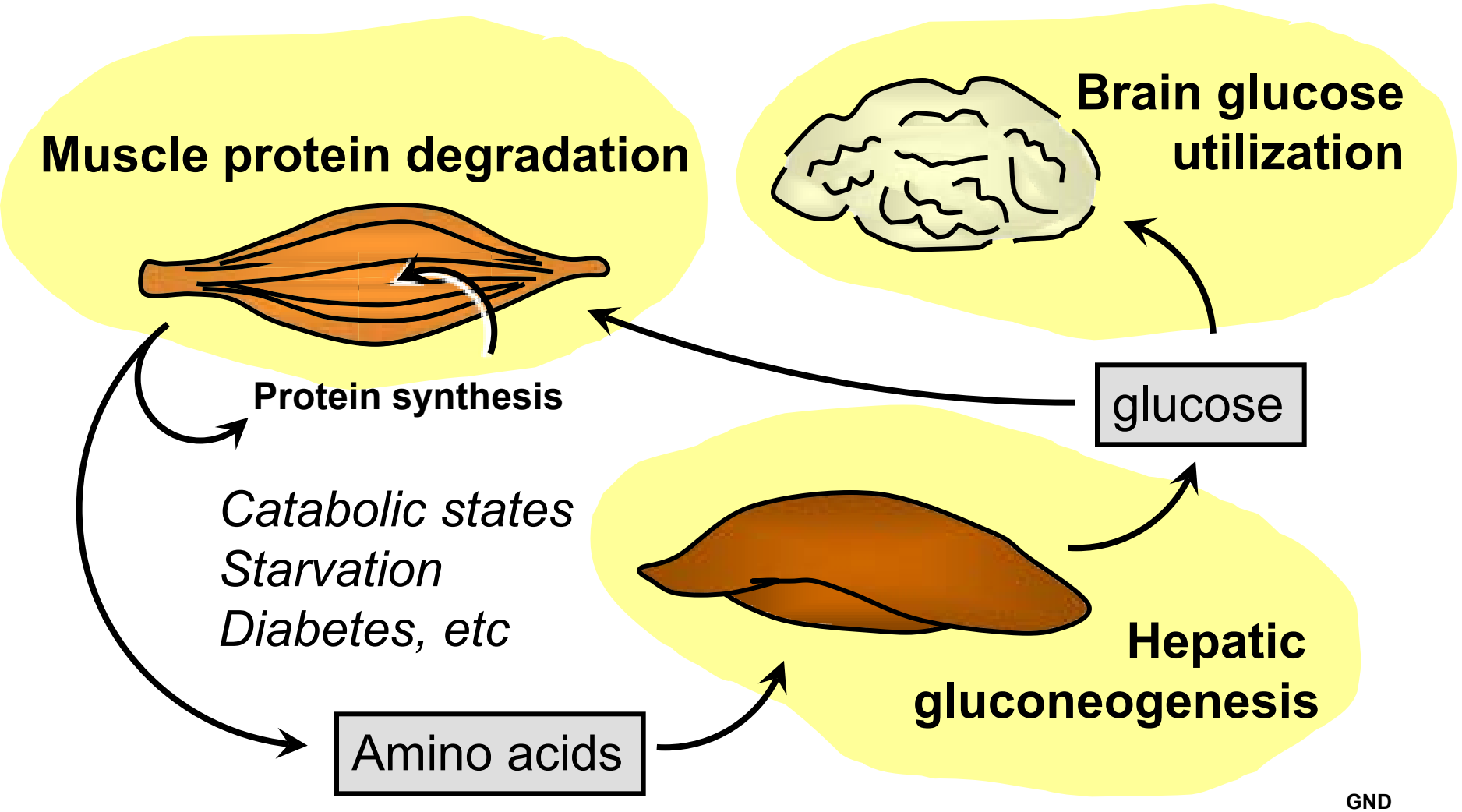
GND
PD24

Changes in relative rates of global protein synthesis and degradation determine tissue growth or atrophy



GND
PD26

Protein degradation allows organisms to adapt to certain physiological and pathological conditions



GND
PD27

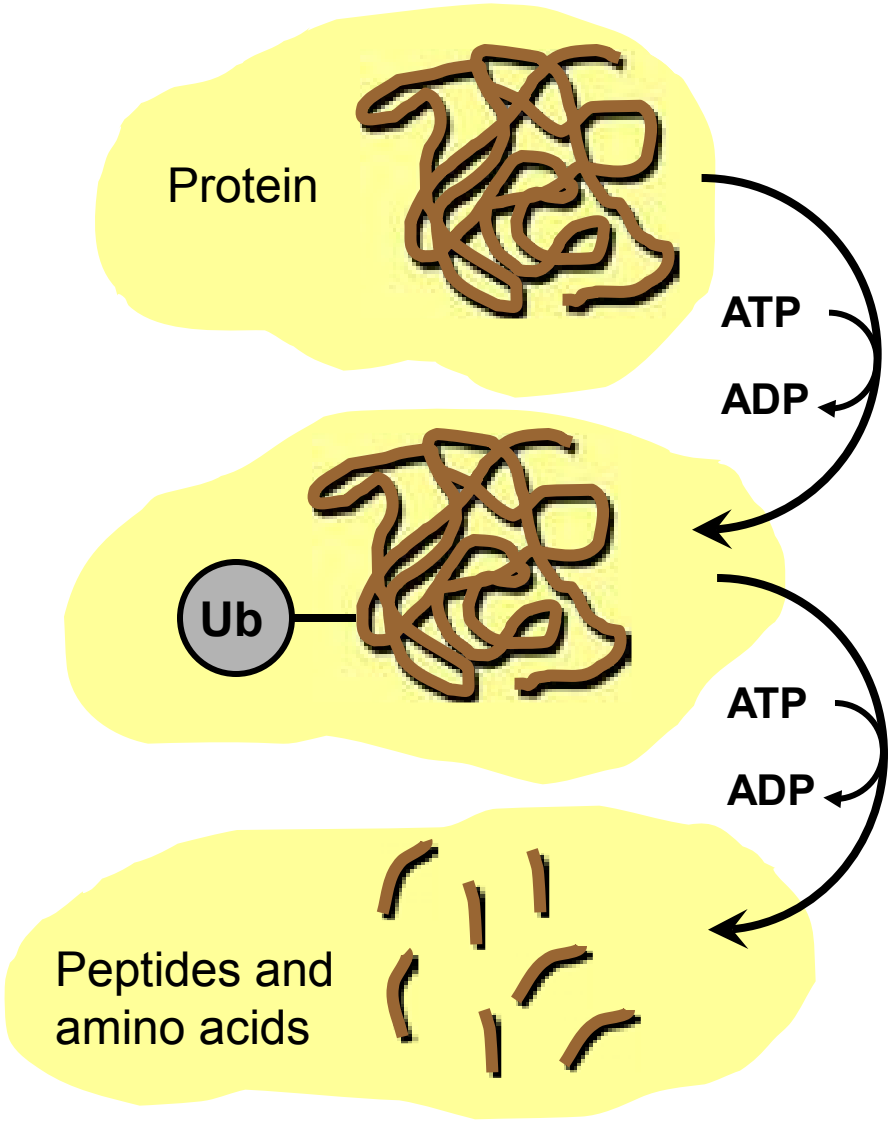
Biochemical mechanisms of intracellular protein degradation

Cells contain multiple systems for degrading constituent proteins

- **Ubiquitin-proteasome system**
- **Lysosomal system**
- **Intra-mitochondrial systems**
- **Calpains (Ca^{2+}-dependent proteases)**
- **Caspases**
- **Membrane proteases**
- ***et al***

GND
PD29

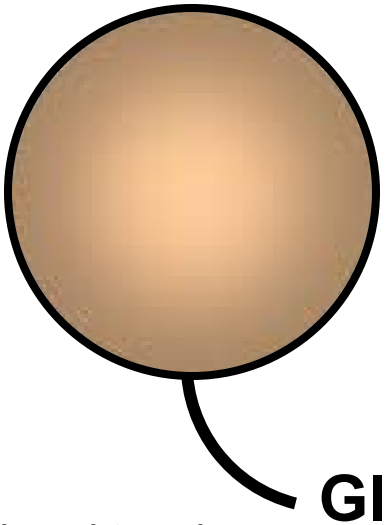
The ubiquitin-proteasome pathway for degradation of proteins has two phases



Substrate modification by ubiquitin

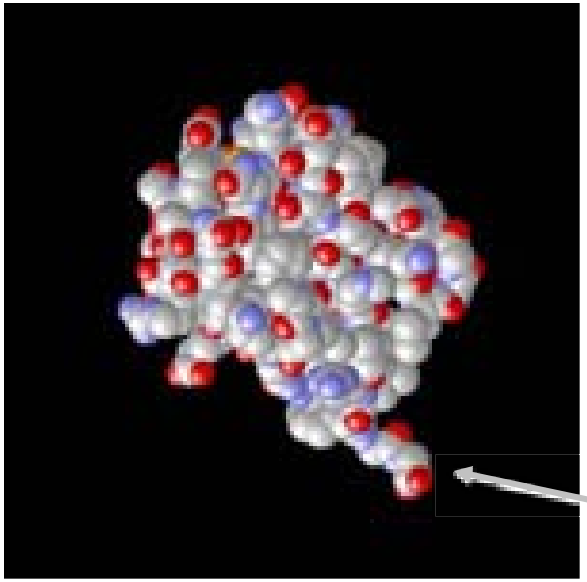
Substrate degradation by the proteasome

GND
PD35



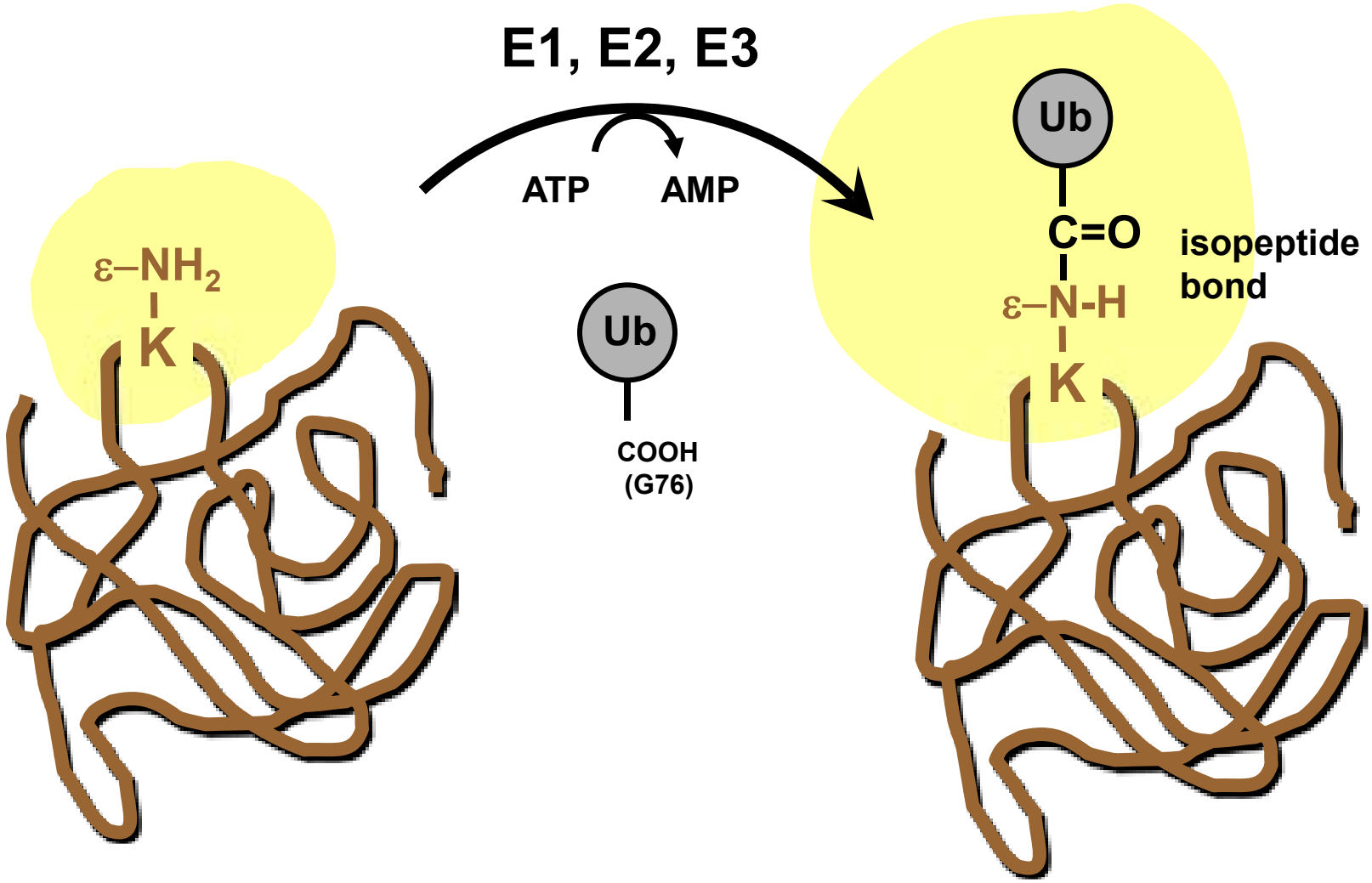
Ubiquitin

- 76 amino acids
- M*r* = 8,000
- highly conserved
- widely distributed



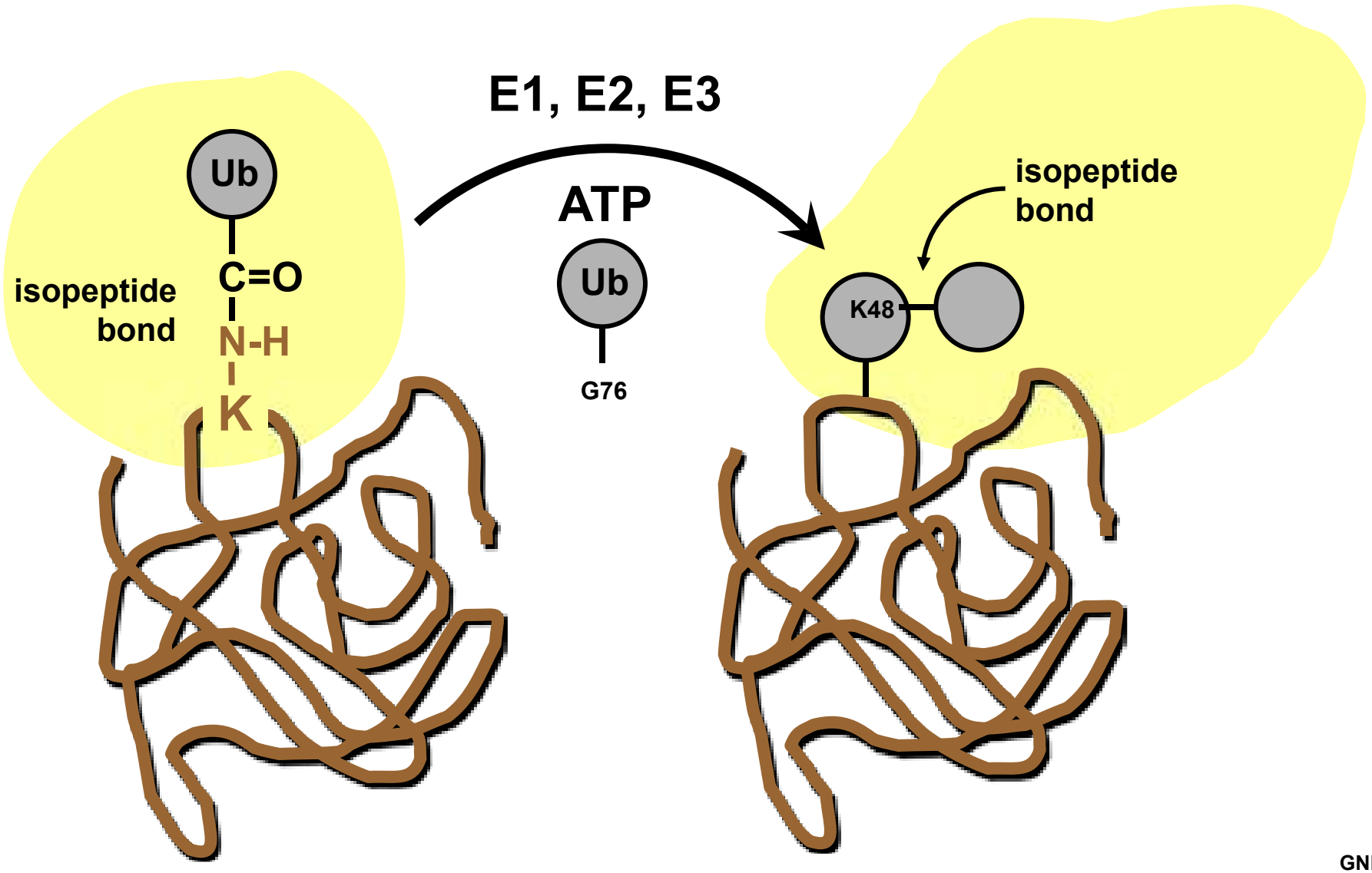
GND
PD36

Ubiquitin is covalently attached to free ε–amino groups of target proteins



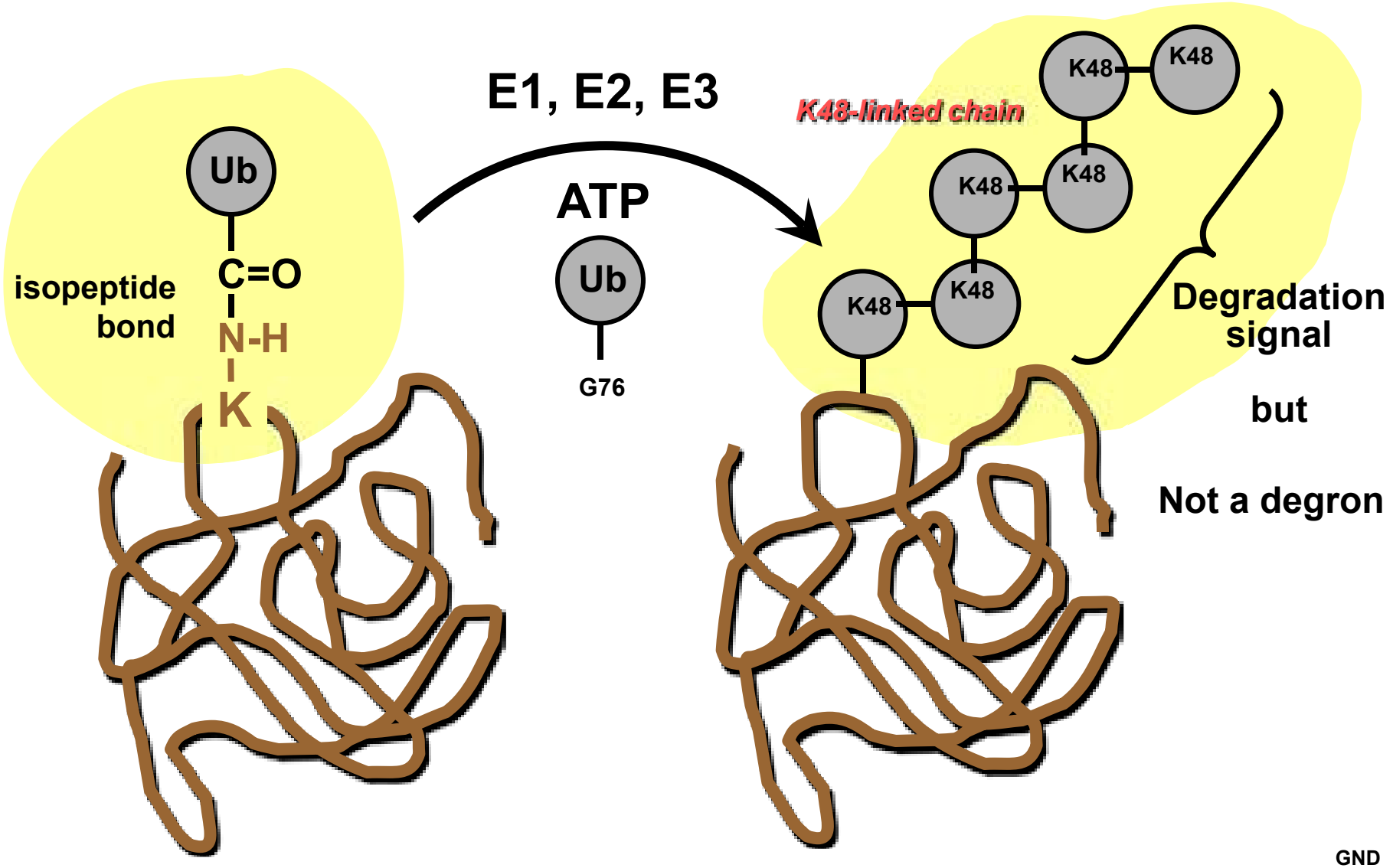
GND
PD37

A polyubiquitin chain is required to target proteins for degradation



GND
PD38

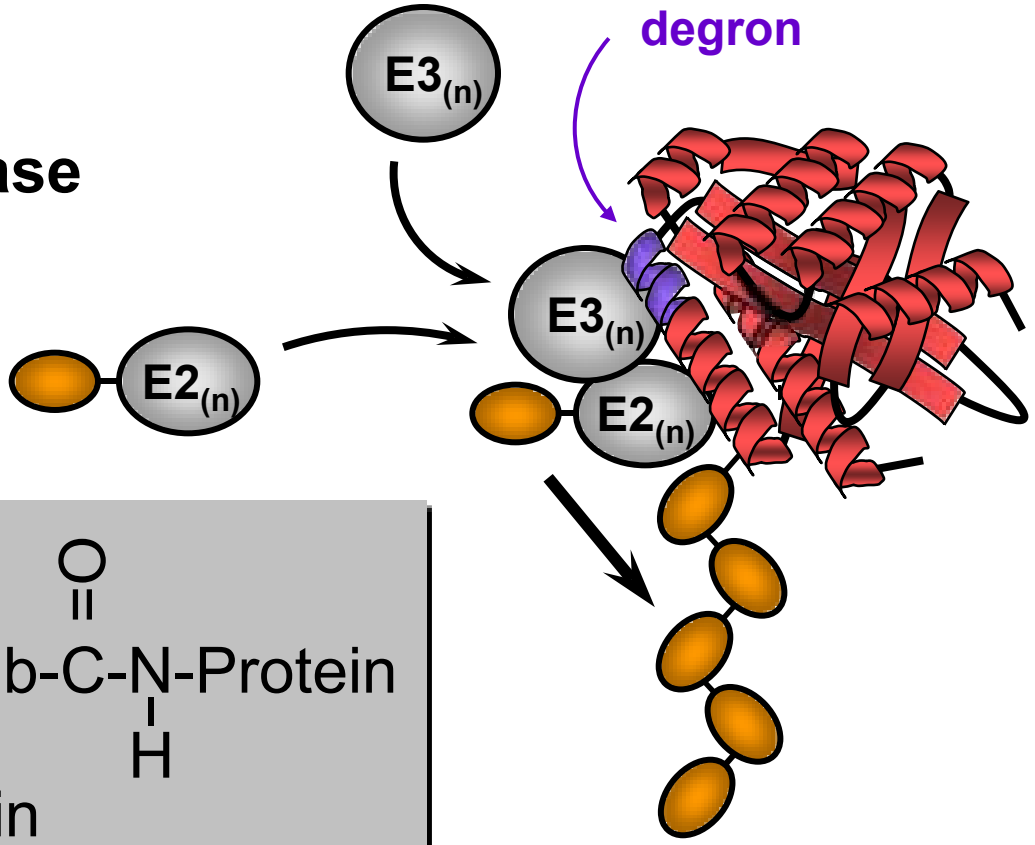
A polyubiquitin chain is required to target proteins for degradation

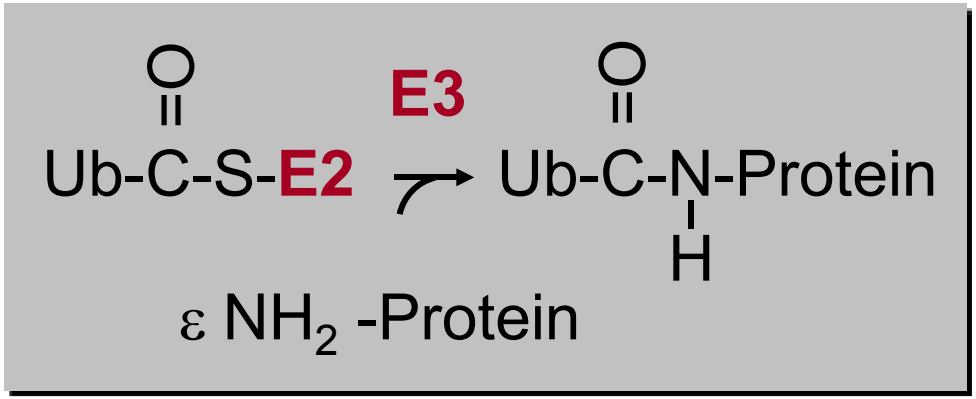


GND
PD39

Transfer of activated ubiquitin to to a protein substrate

E3 ubiquitin ligase

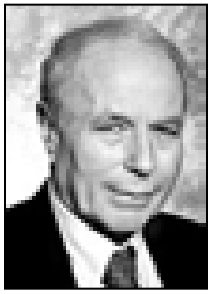




Isopeptide bond

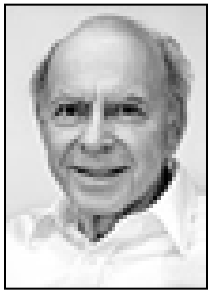
GND
PD43

2004 Nobel Prize for discovery of ubiquitin conjugation

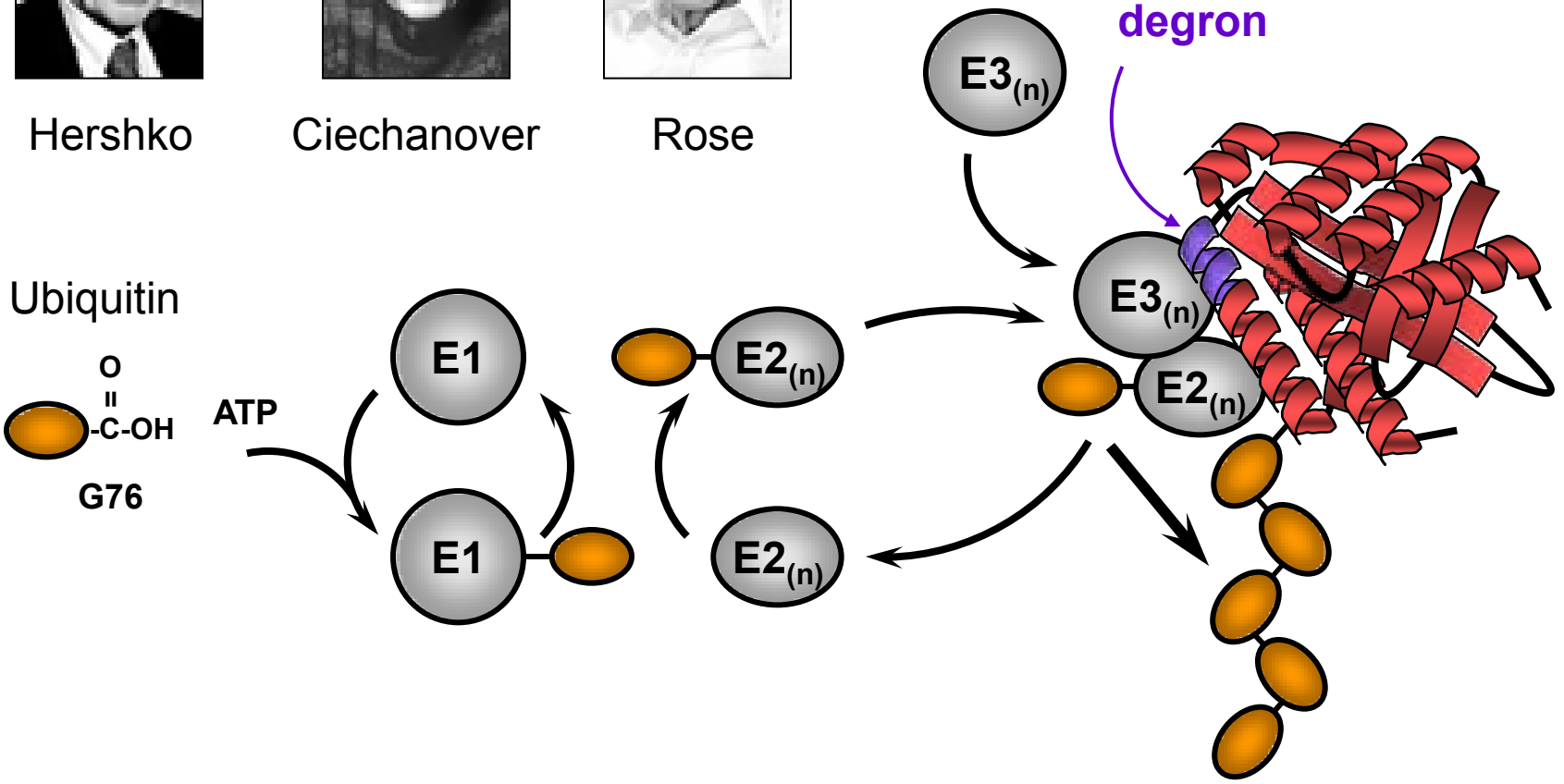


Hershko

Ciechanover



Rose



GND
PD44

Really important concept

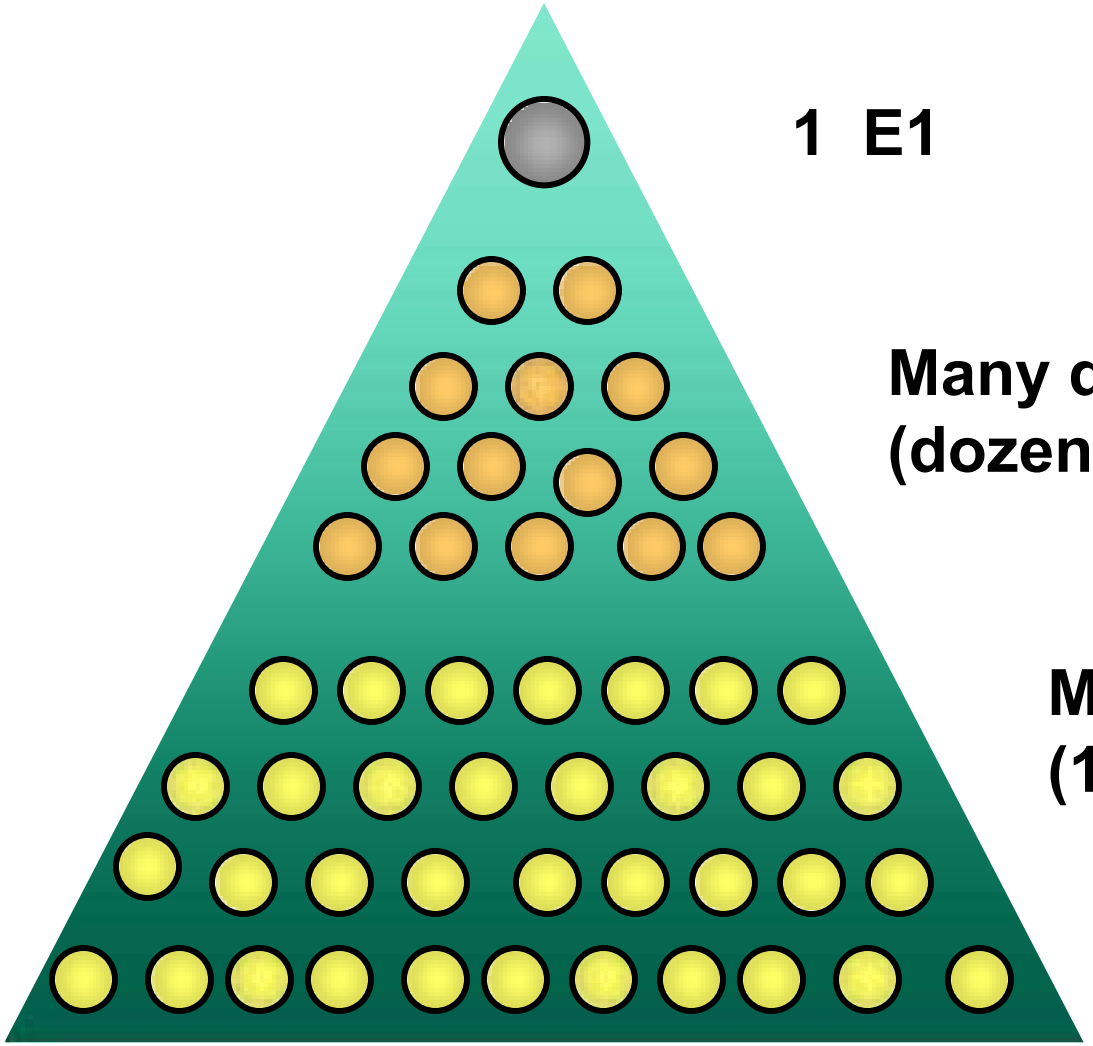
Proteins are selected for ubiquitylation through the recognition of specific degrons by E3s.

Different E3s recognize different degrons of different substrate proteins.

THIS PROVIDES SPECIFICITY.

GND
PD45

Specificity for ubiquitination is achieved with multiple conjugating enzymes



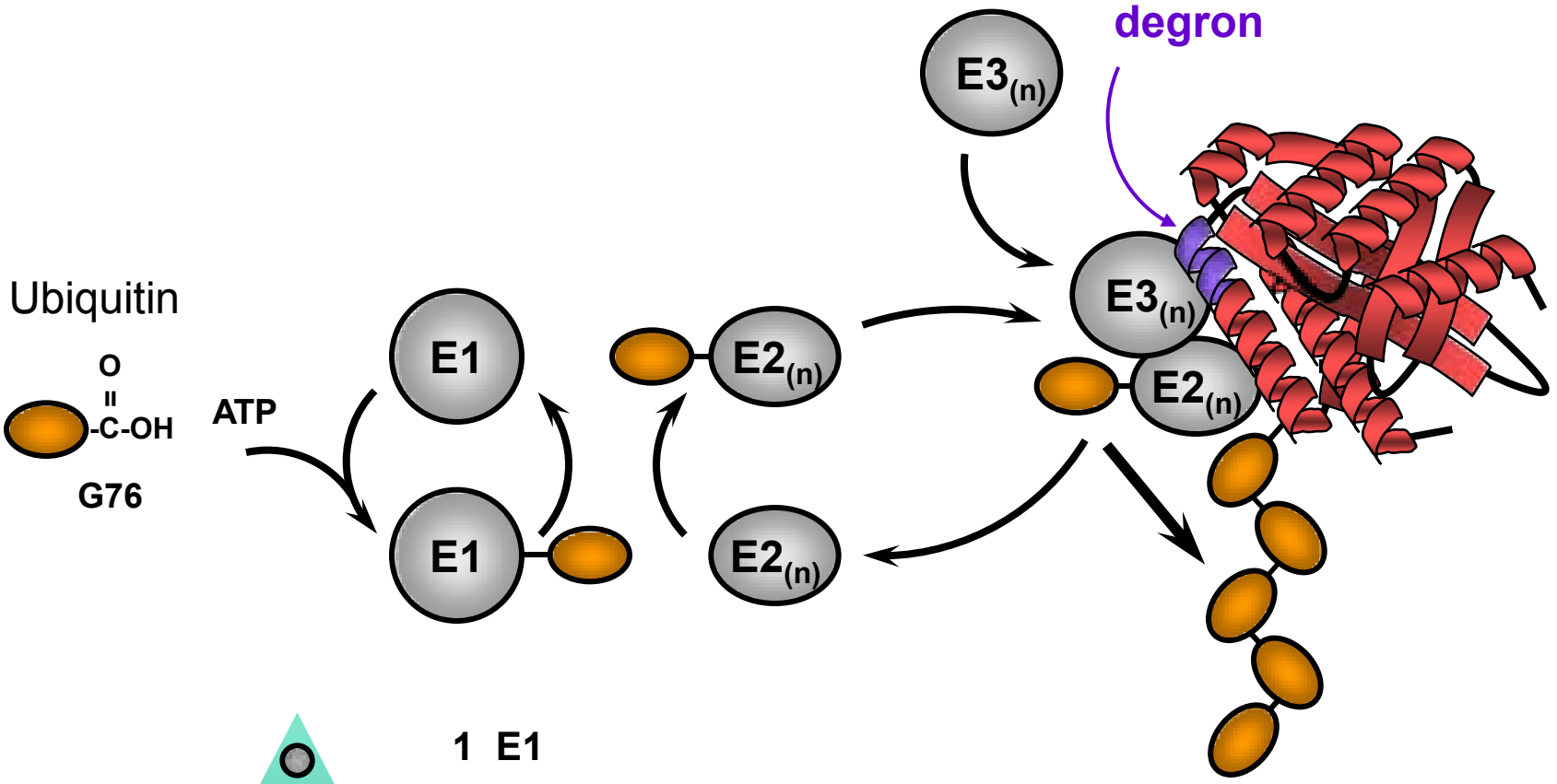
1 E1

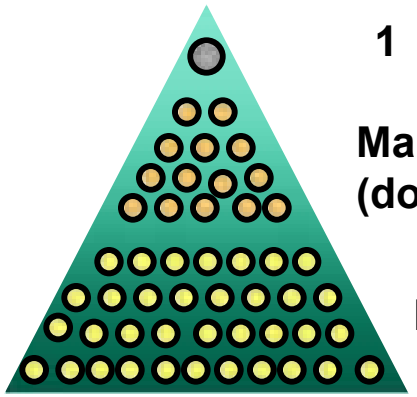
Many different E2s (dozens)

Many different E3s (100s-1000s !!)

GND
PD49

Specificity for ubiquitination is achieved with multiple conjugating enzymes





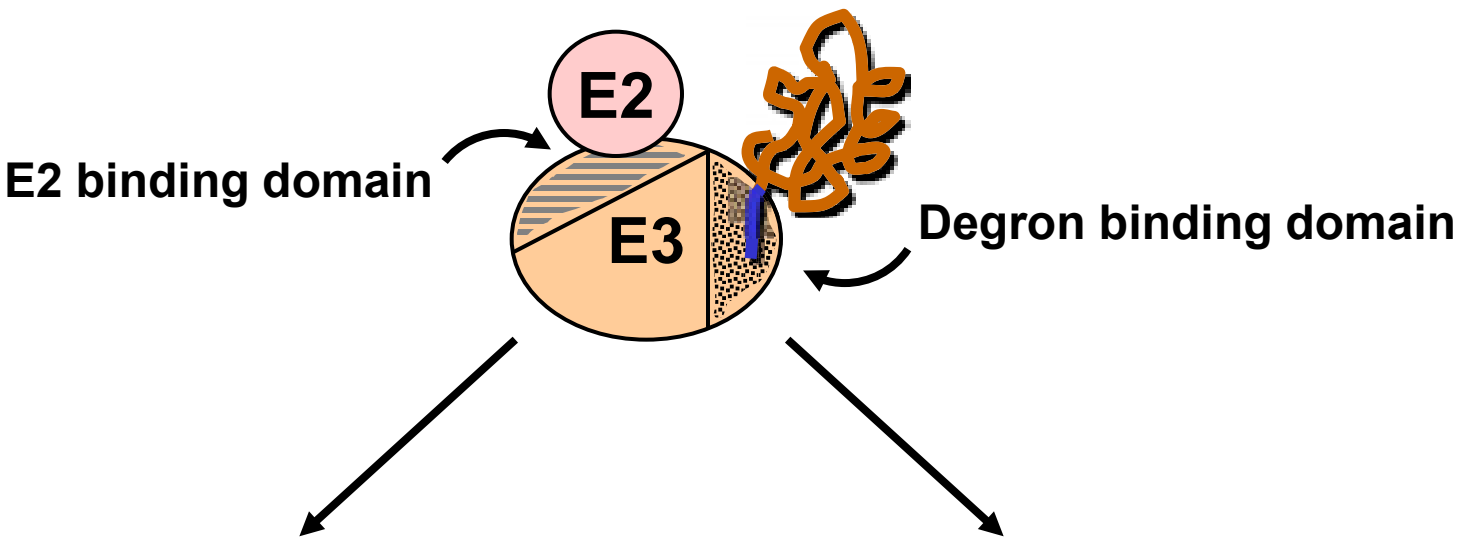
1 E1

Many different E2s (dozens)

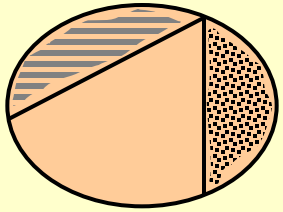
Many different E3s (100s-1000s !!)

GND
PD44

Features and general structures of E3s

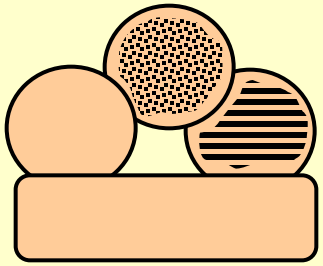


Single protein E3s



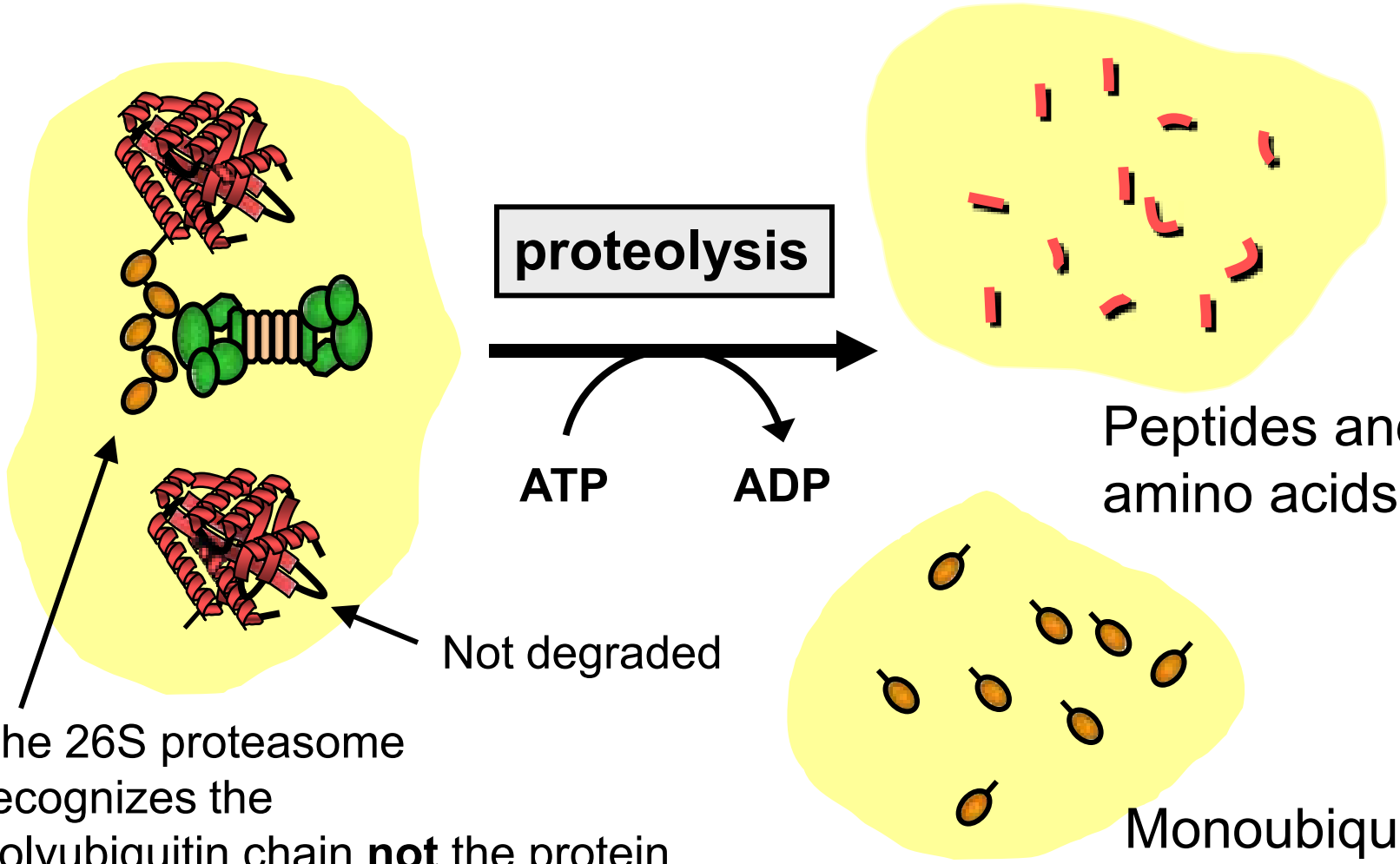
12s (e.g. MDM2)

Multisubunit E3s

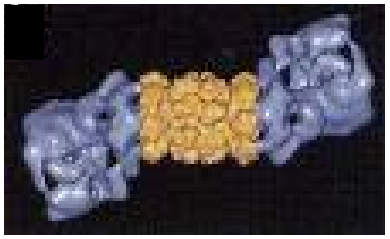


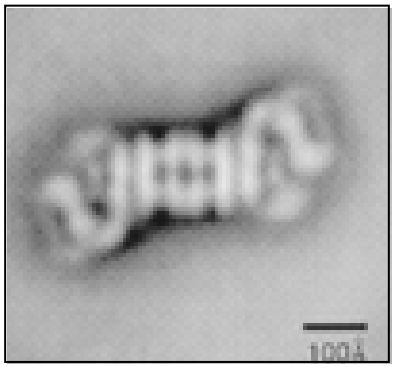
100s-1000s!! (e.g. SCF, APC)

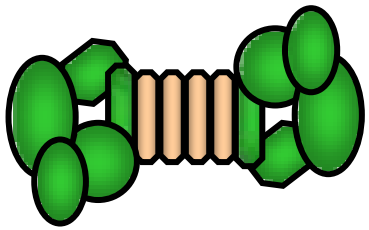
Ubiquitylated proteins are degraded by the 26S proteasome



The 26S proteasome: an energy-dependent molecular machine for degradation of ubiquitinated proteins

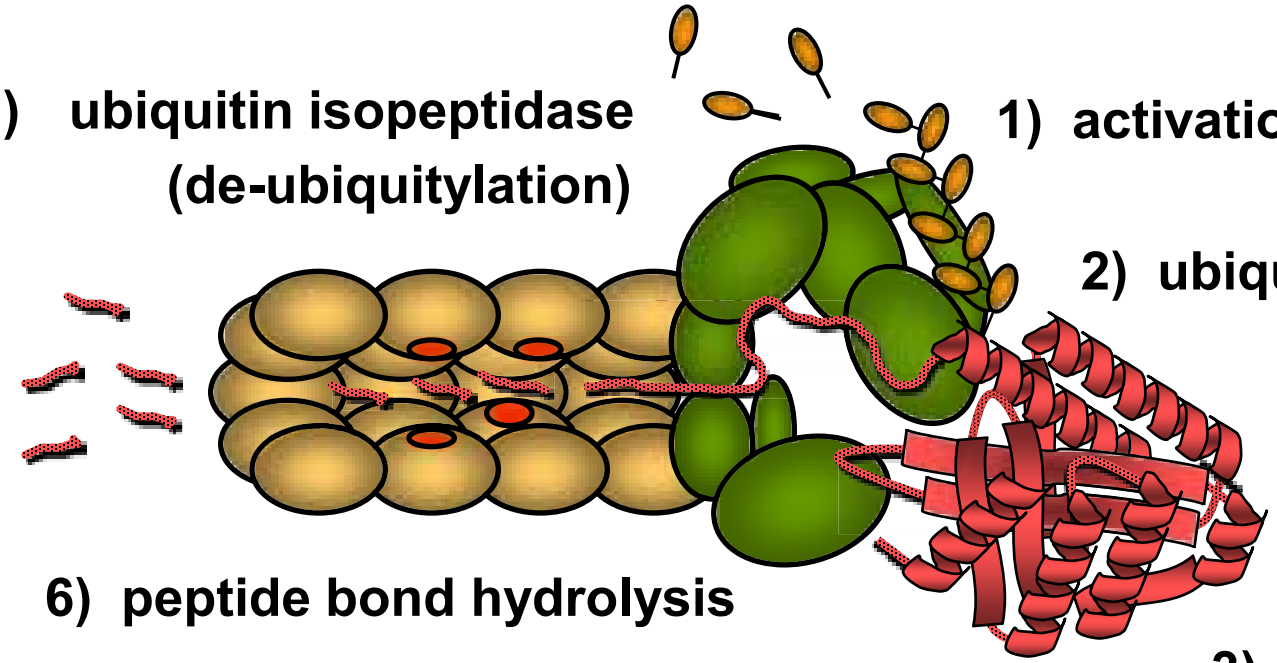






- Mr = 2,400,000
- 64 subunits
- 32 gene products

Model for how the 26S proteasome degrades ubiquitylated proteins



7) ubiquitin isopeptidase (de-ubiquitylation)

1) activation by gating

2) ubiquitin chain binding

6) peptide bond hydrolysis

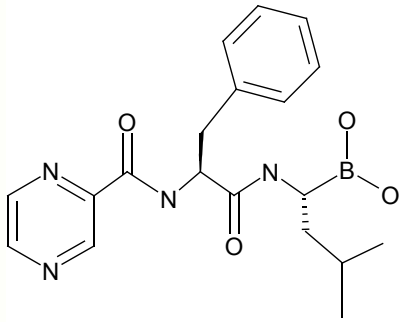
3) ATP hydrolysis

4) substrate unfolding

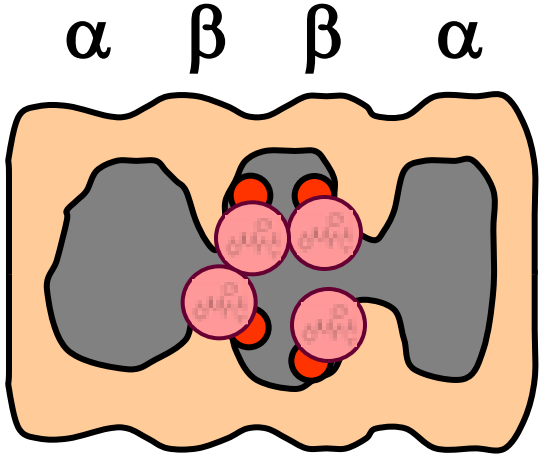
5) translocation of unfolded polypeptide chain

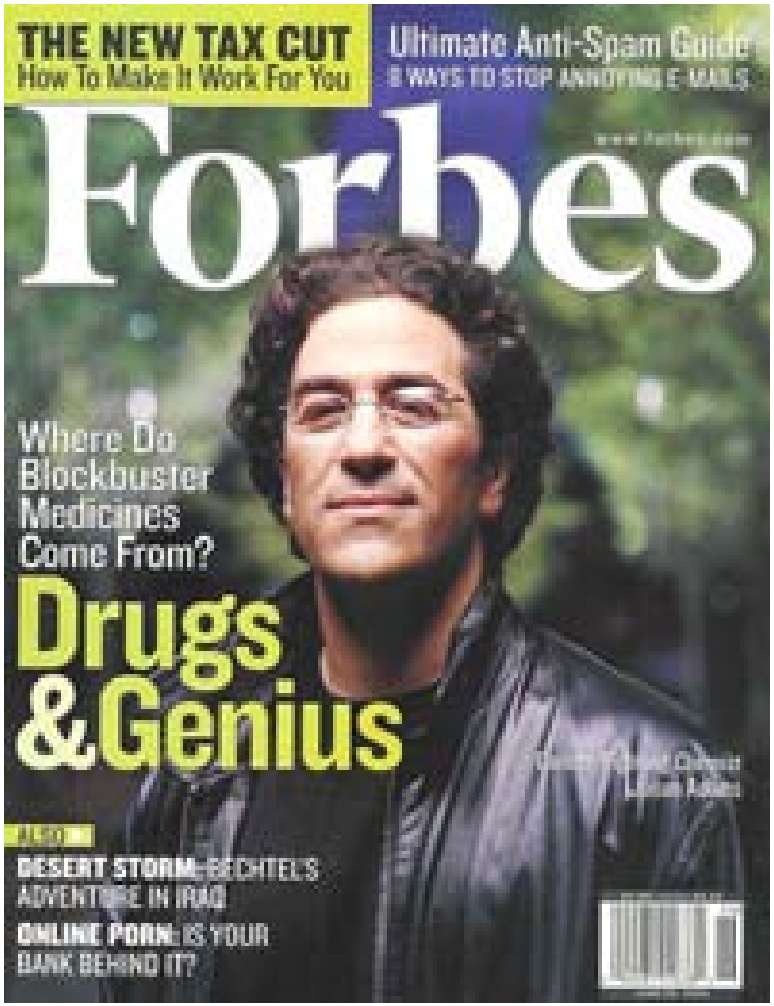
GND
PD63

Proteasome-inhibitor drugs are used to treat cancer



PS341 (Velcade ™)



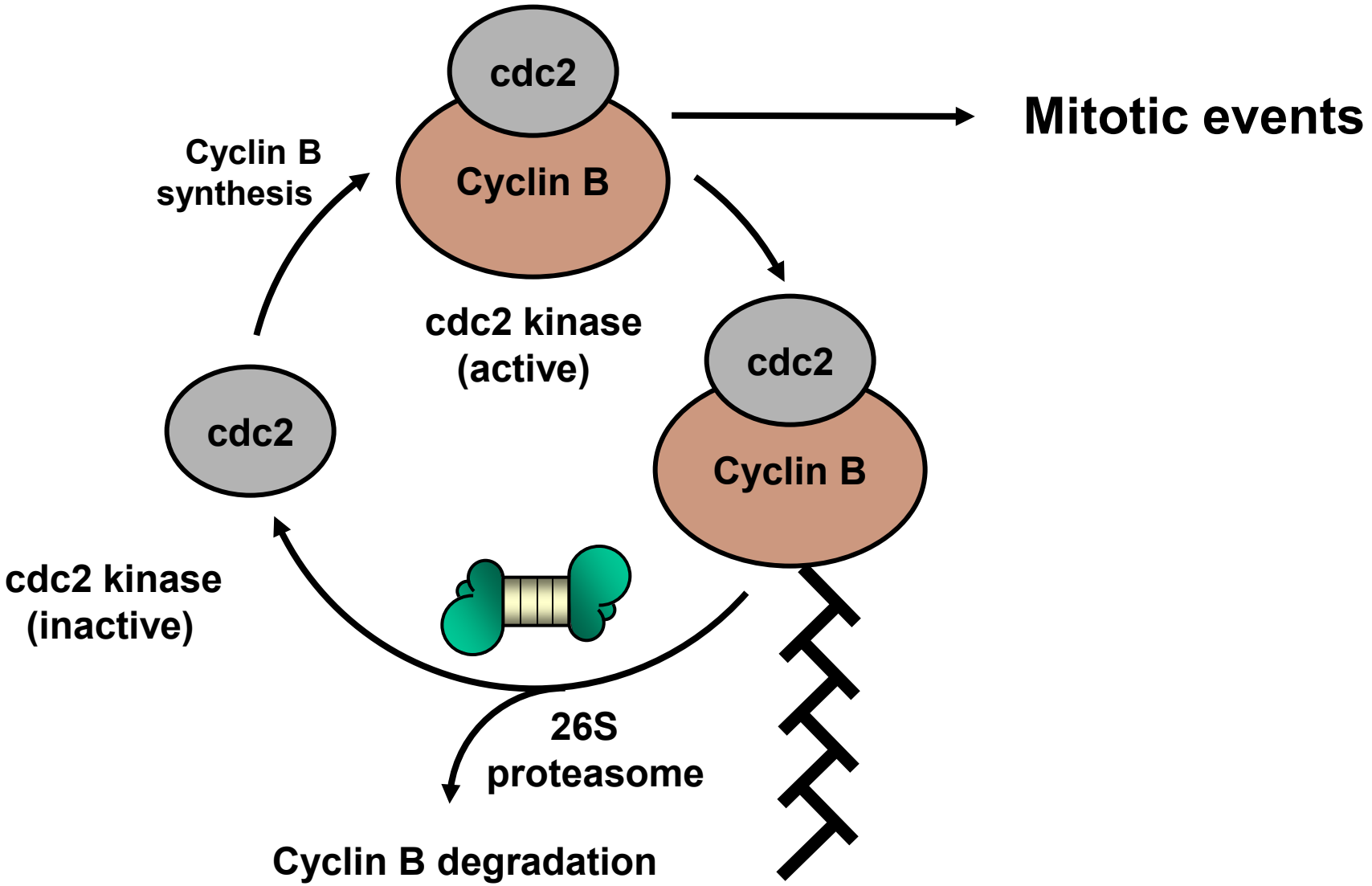


GND
V01

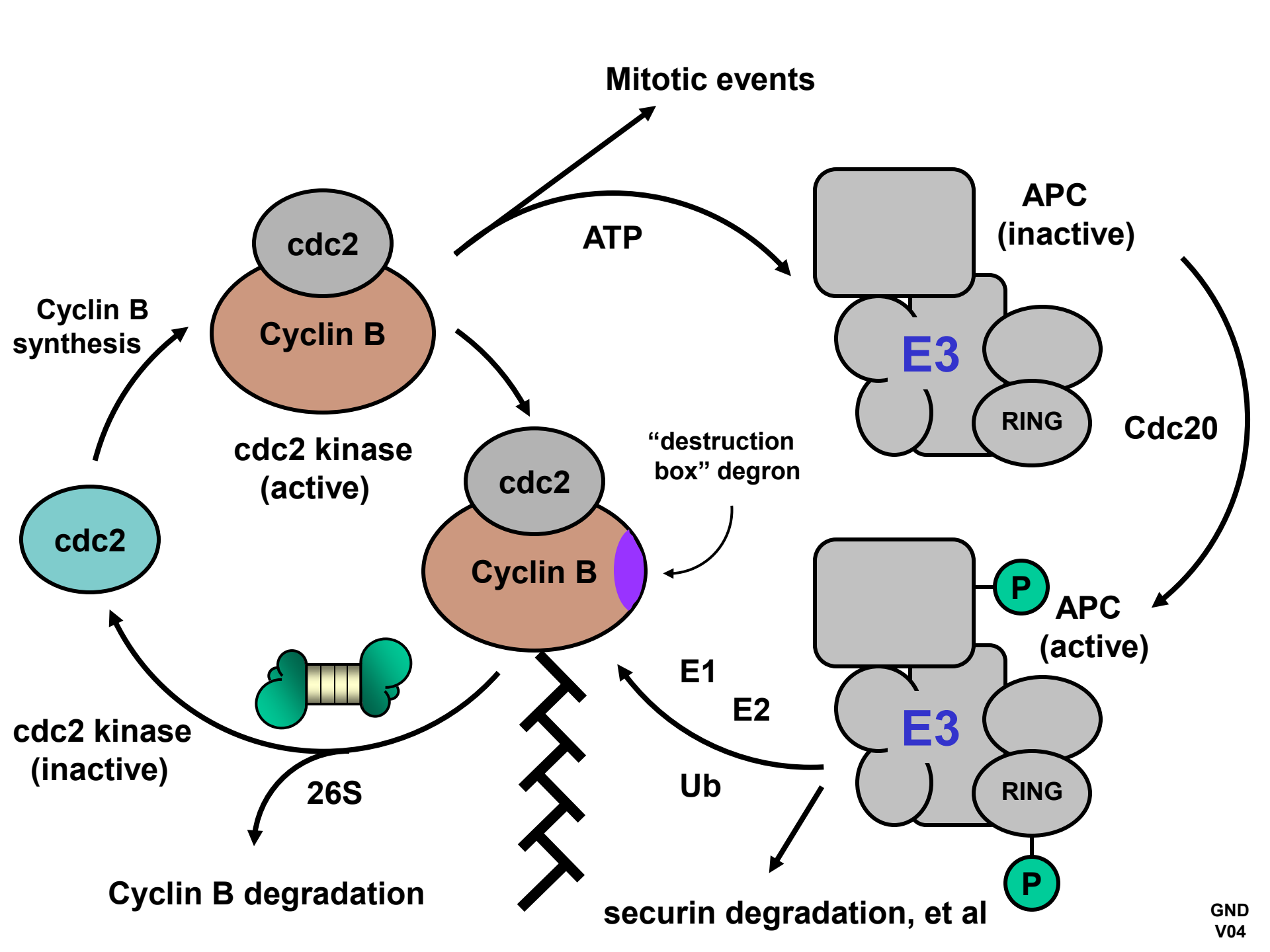
Ubiquitin/proteasome-dependent protein degradation in action

GND
PD64

Regulation of the cell cycle by protein degradation



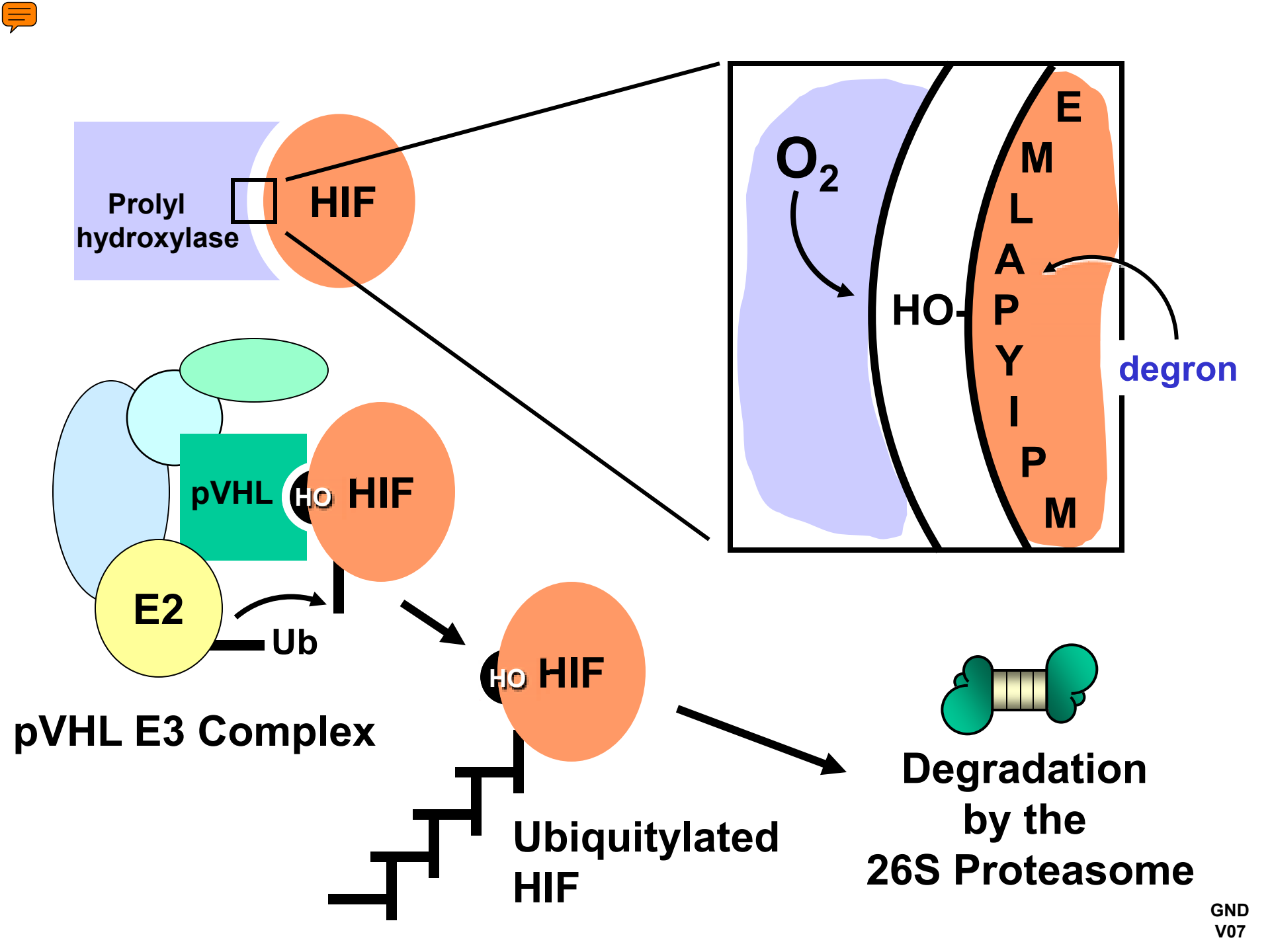
GND
V03


Mitotic events
ATP
APC
(inactive)
cdc2
Cyclin B
Cyclin B
synthesis
cdc2 kinase
(active)
E3
RING
Cdc20
"destruction
box" degron
cdc2
Cyclin B
cdc2
P
APC
(active)
E1
E2
E3
RING
Ub
cdc2 kinase
(inactive)
26S
P
Cyclin B degradation
securin degradation, et al
GND
V04

Regulation of transcription by protein degradation

- Hypoxia Inducible Factor (HIF) is a transcription factor for genes required for response to hypoxia (low O_2).
- Under normoxic conditions, HIF concentrations are low, and HIF is a short-lived protein, constitutively degraded by the UPS.
- Hypoxia increases HIF concentrations by decreasing HIF degradation.

GND
V05


Prolyl hydroxylase
HIF
O_2
HO
E
M
L
A
P
Y
I
P
M
degron
pVHL
HO
HIF
E2
Ub
pVHL E3 Complex
HO
HIF
Ubiquitylated HIF
Degradation by the 26S Proteasome

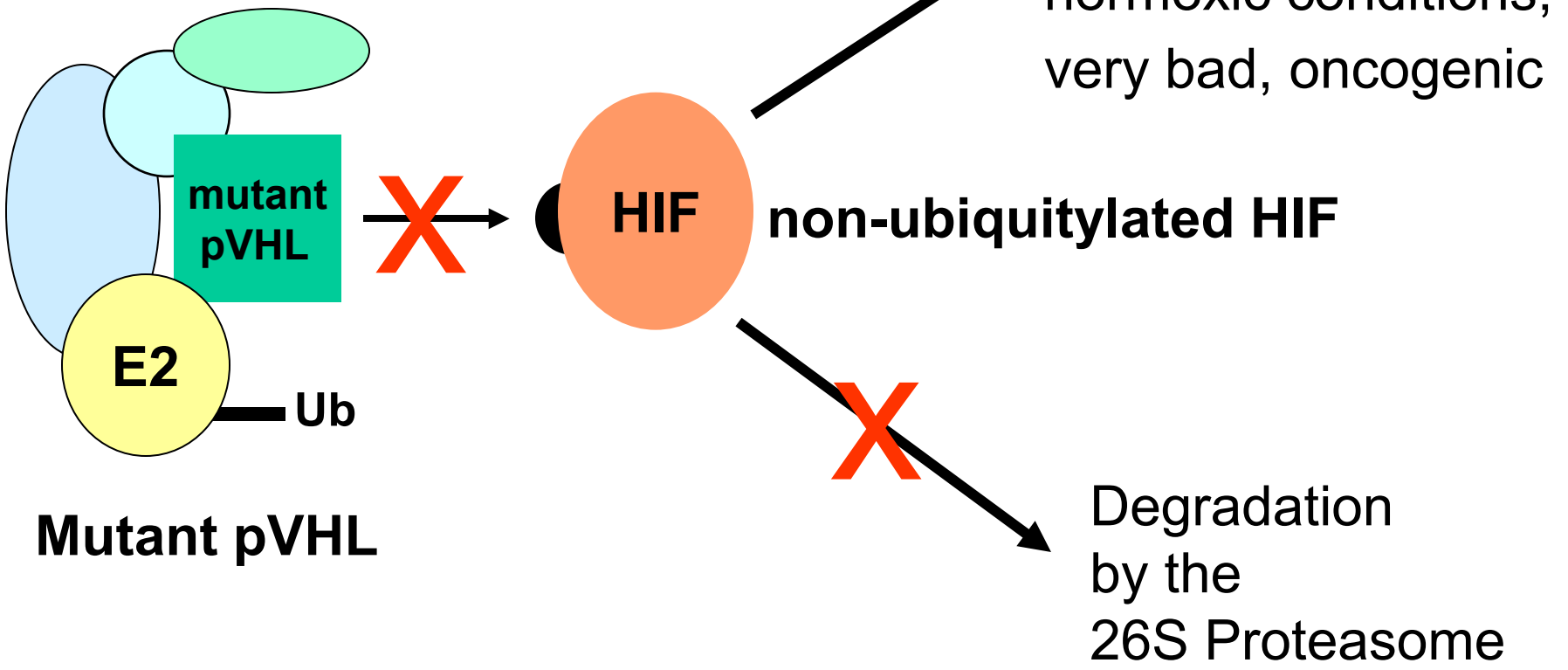
GND
V07

Abnormal degradation of HIF promotes onogenesis

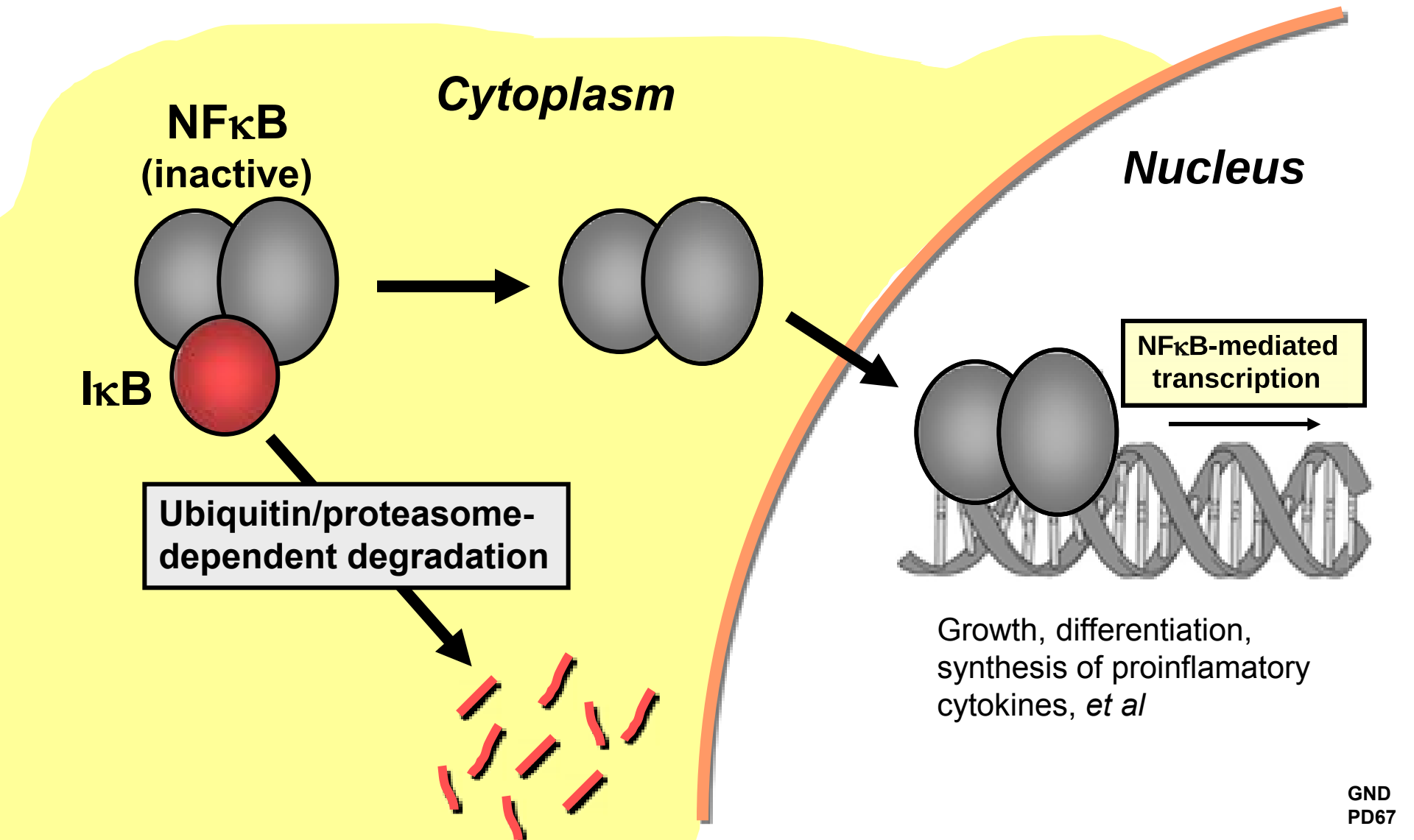
- Hypoxia Inducible Factor (HIF) is a transcription factor for genes required for response to hypoxia (low O_2).
- Under normoxic conditions, HIF concentrations are low, and HIF is a short-lived protein.
- Hypoxia increases HIF concentrations by decreasing HIF degradation.
- Many cancers are associated with increased transcription of genes controlled by HIF.
- Many of these cancers are associated with mutations of the von Hippel Lindau tumor suppressor protein.

GND
V08

Mutant pVHL fails to promote HIF ubiquitination and degradation

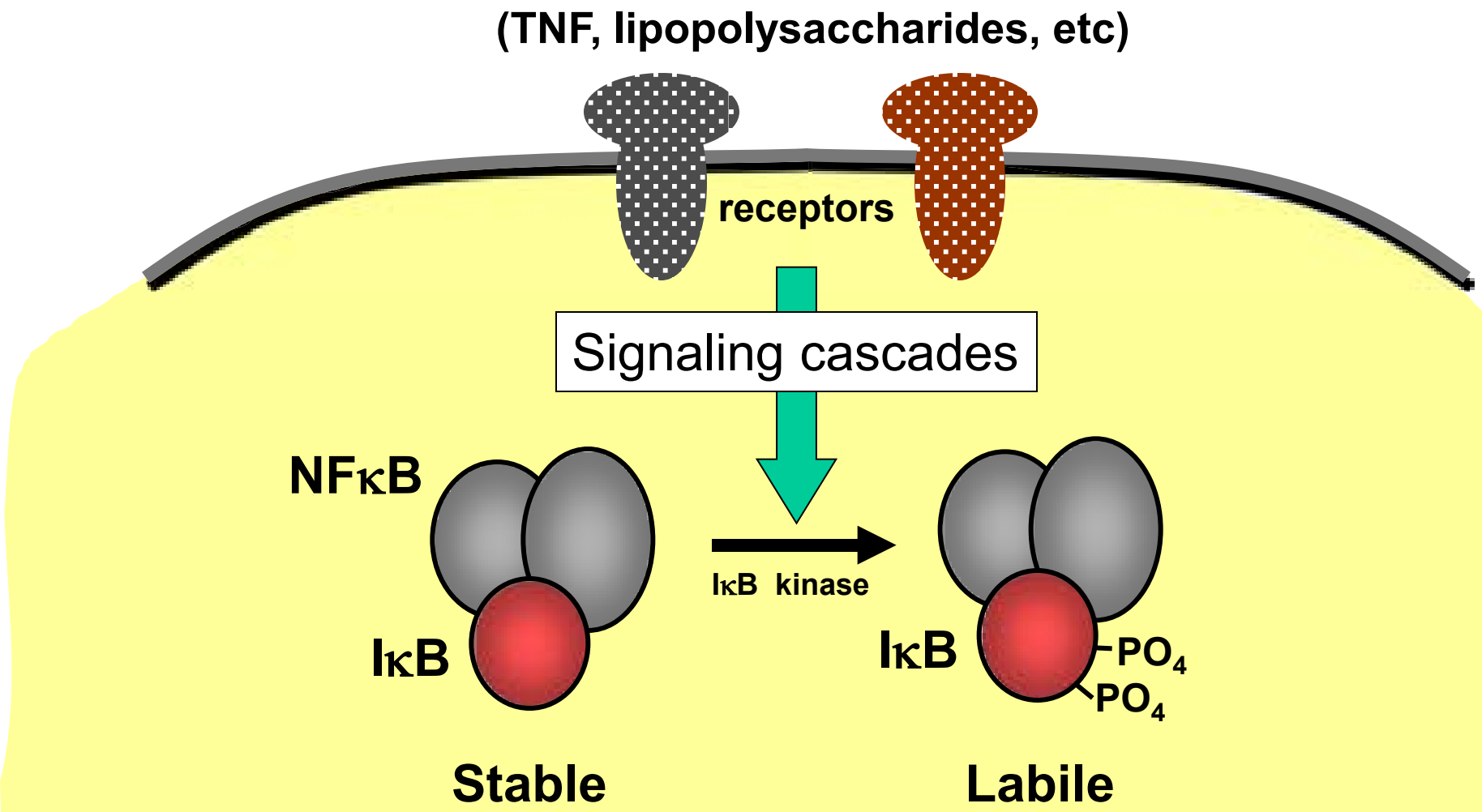


Regulation of transcription by ubiquitin/proteasome-dependent protein degradation



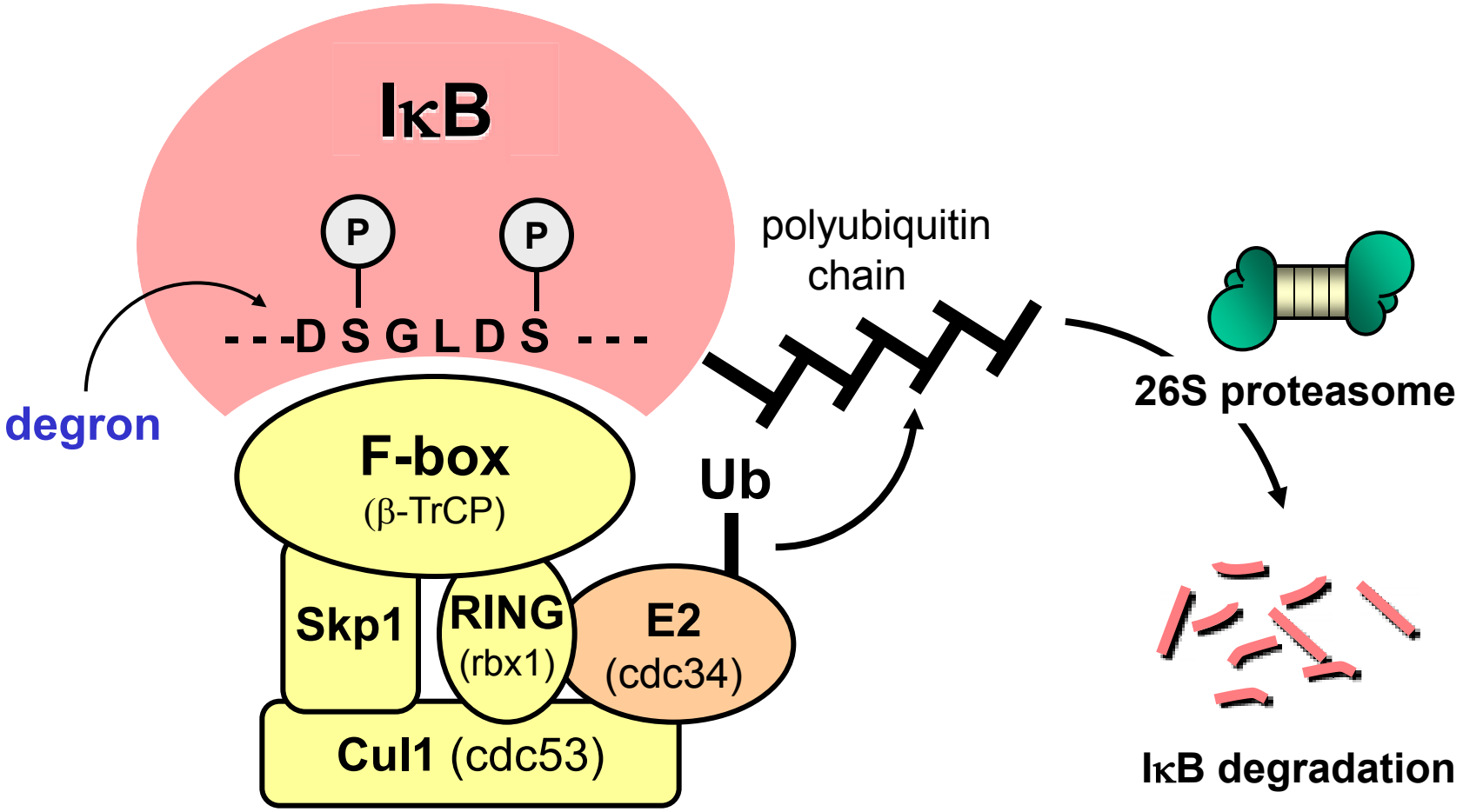
GND
PD67

Signal-dependent degradation of IκB



GND
PD68

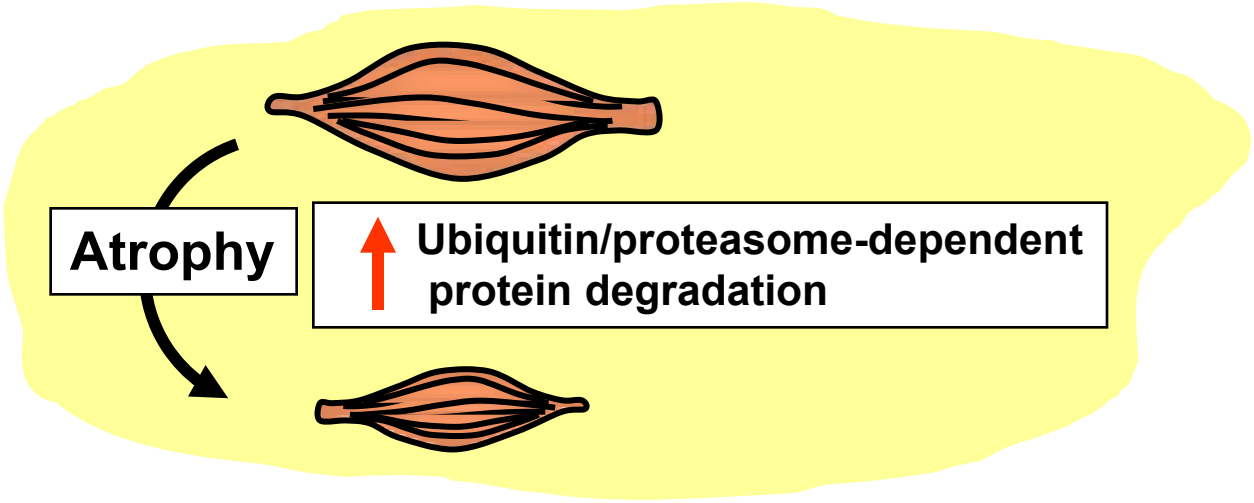
The degron of IκB is recognized by an SCF-type E3 complex



GND
PD69

Many muscle wasting conditions and diseases result from abnormally high rates of protein degradation

- **Inactivity or decreased load**
  immobilization (casts), denervation, zero gravity, bed rest
- **Myopathies**
  Muscular dystrophies
- **Endocrinopathies**
  thyrotoxicosis, diabetes, glucocorticoid excess, *et al*
- **Aging (sarcopenia)**
- **Nutritional state**
  starvation, malnutrition
- **Sepsis**
- **Cancer**
- **AIDS**
- ***et al, et al***



GND
PD70

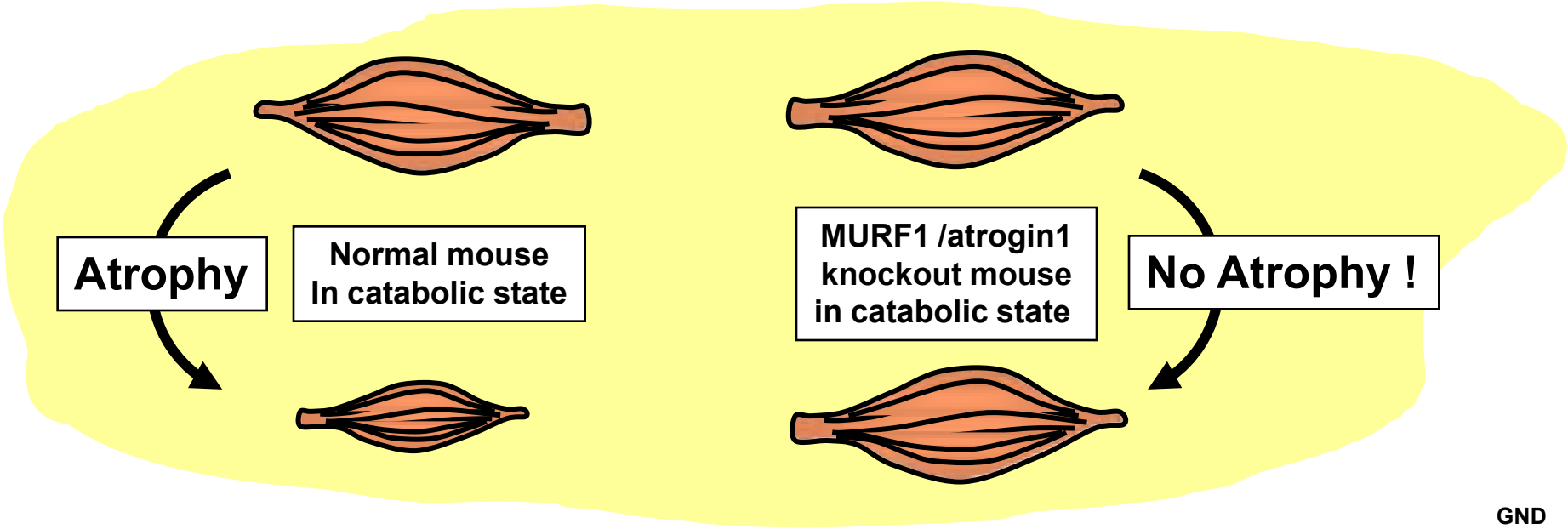
Proteins whose expression is altered in most muscle wasting conditions (*aka* atrogenes)

26S proteasome	2-4 fold increase
Ubiquitin	2-4 fold increase
Certain E2s	2-4 fold increase
Muscle-specific E3s:	
Atrogin 1 (F Box)	>10 fold increase
MURF 1 (Ring)	>10 fold increase

GND
PD71

Proteins whose expression is altered in most muscle wasting conditions (*aka* atrogenes)

26S proteasome	2-4 fold increase
Ubiquitin	2-4 fold increase
Certain E2s	2-4 fold increase
Muscle-specific E3s:	
Atrogin 1 (F Box)	>10 fold increase
MURF 1 (Ring)	>10 fold increase



GND
PD72