



泛素修飾系統

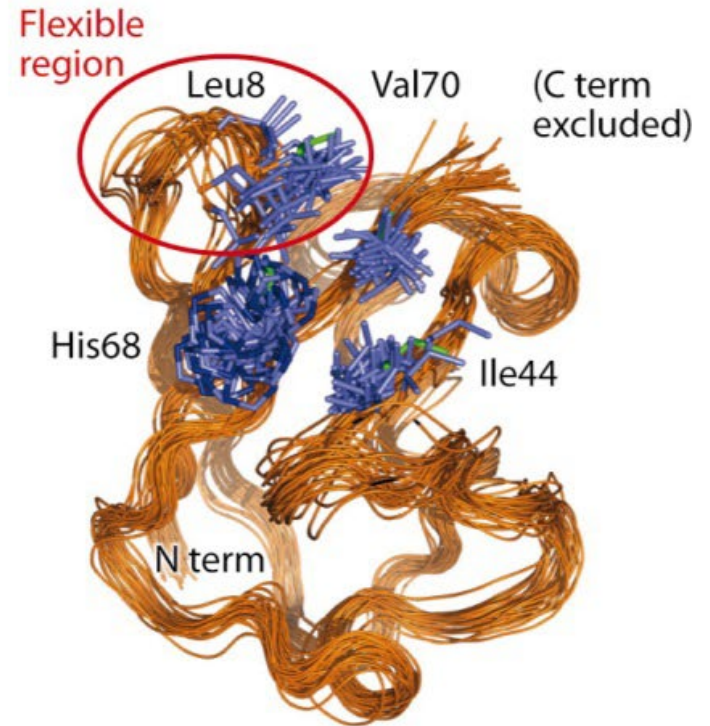
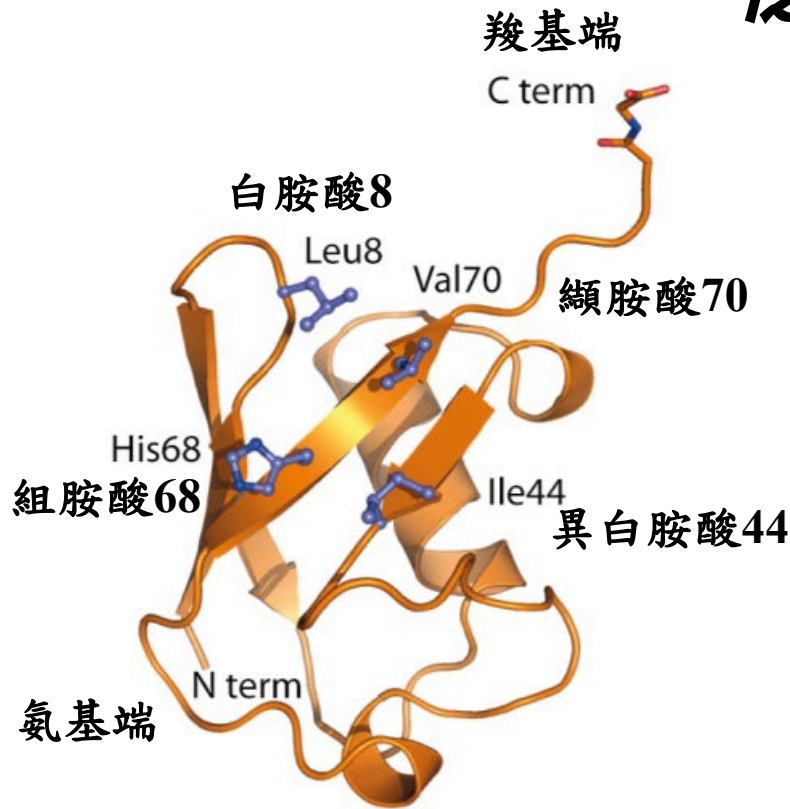
Ubiquitin Modification System

符宏勇 副研究員
中研院 植物暨微生物學研究所

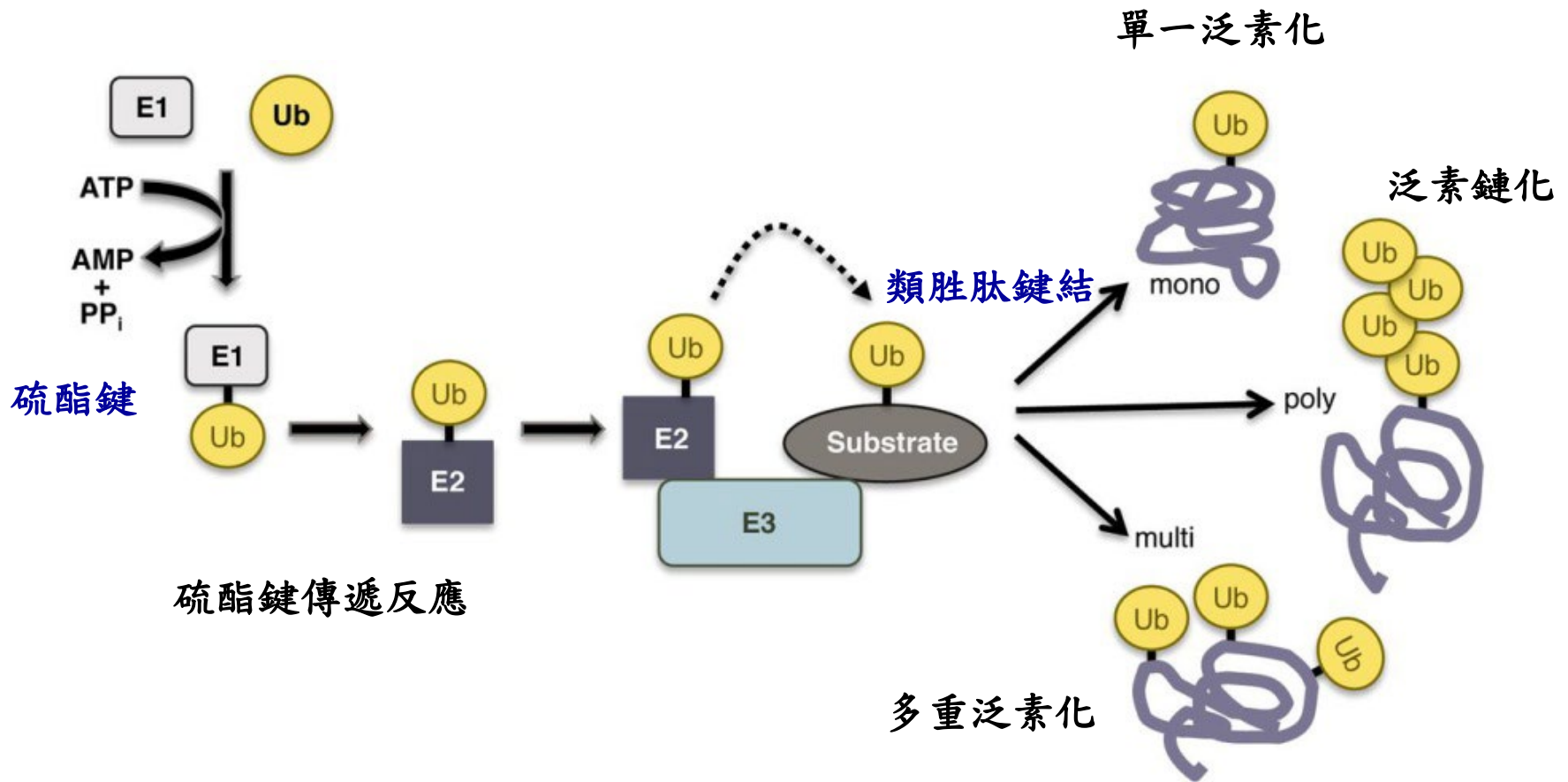
Jan 20, 2024

hongyong@gate.sinica.edu.tw; 02-27871183

泛素

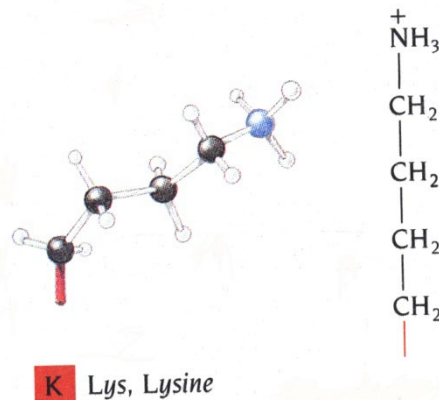
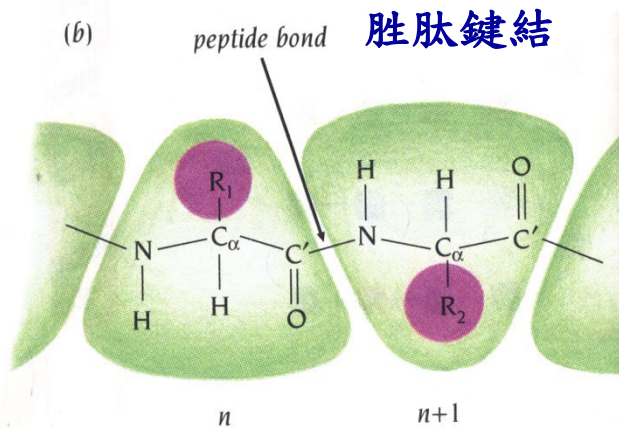
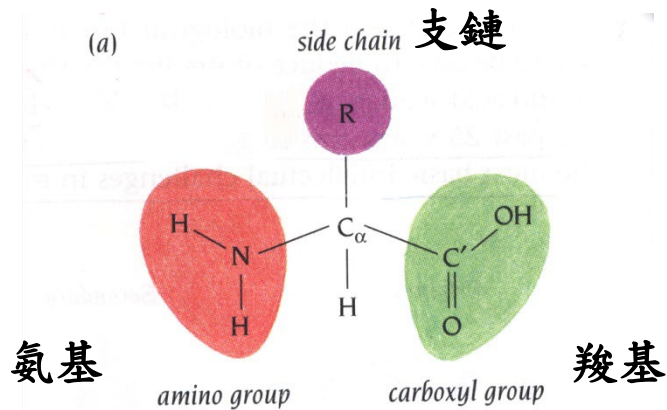


- 76個胺基酸、序列高度演化保守、穩定、橢圓狀、 β grasp (握)構型/5個 β sheets (片)握住1個 α helix (螺旋)
- 核心結構穩定、C-端六個胺基酸及 β 1- β 2連接環較具彈性



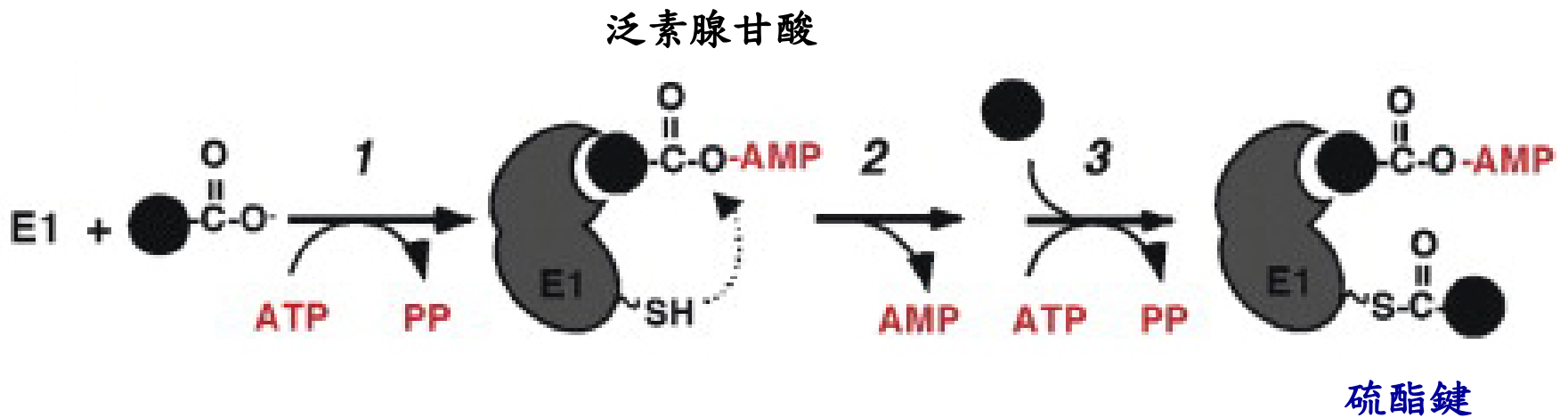
泛素化反應 (泛素與蛋白受質共價鍵結)
ubiquitination

類胜肽鍵結(Isopeptide bond)



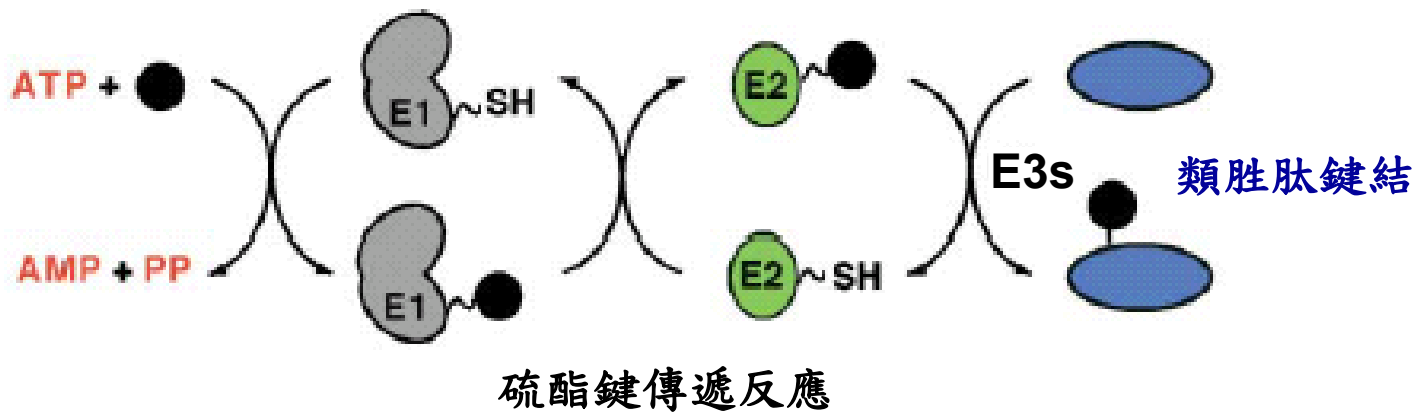
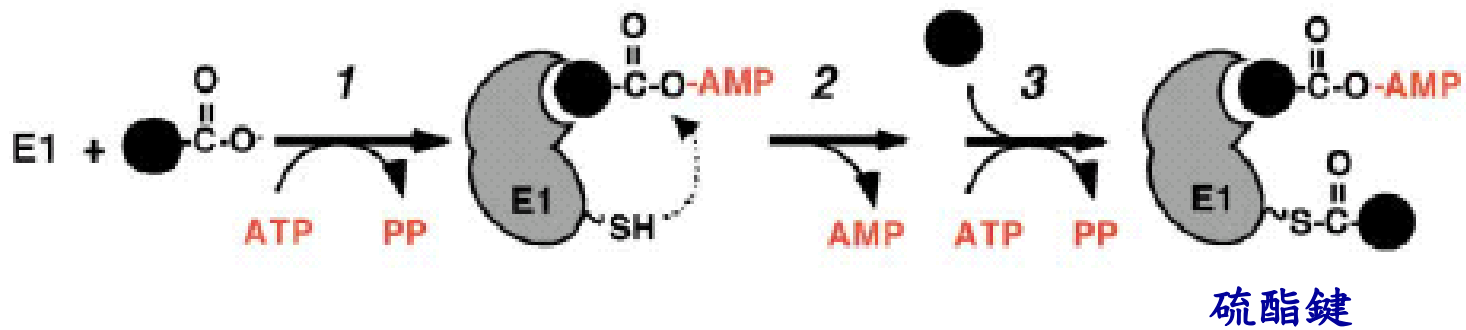
離氨酸

- 泛素-受質
- 泛素-組蛋白 (Histone 2A)
- 甲硫氨酸 Met/M
- 甘氨酸 Gly/G
- 離氨酸 Lys/K
- 半胱氨酸 Cys/C (-SH硫醇)



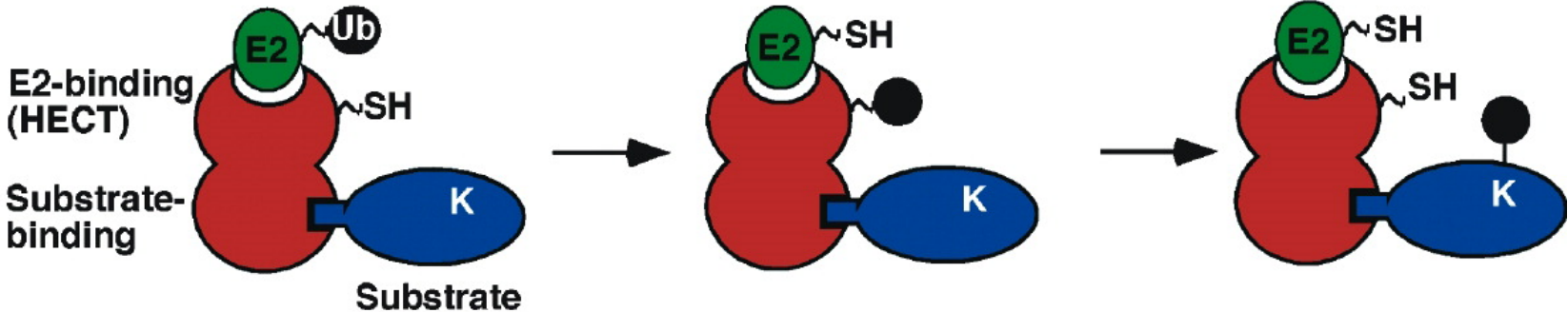
酵素1 (E1): 泛素活化反應

Pickart 2004, *Cell* 116:181-190

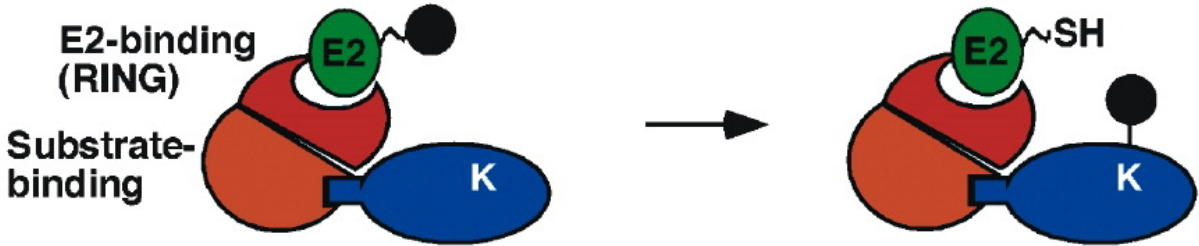


Pickart 2004, *Cell* 116:181-190

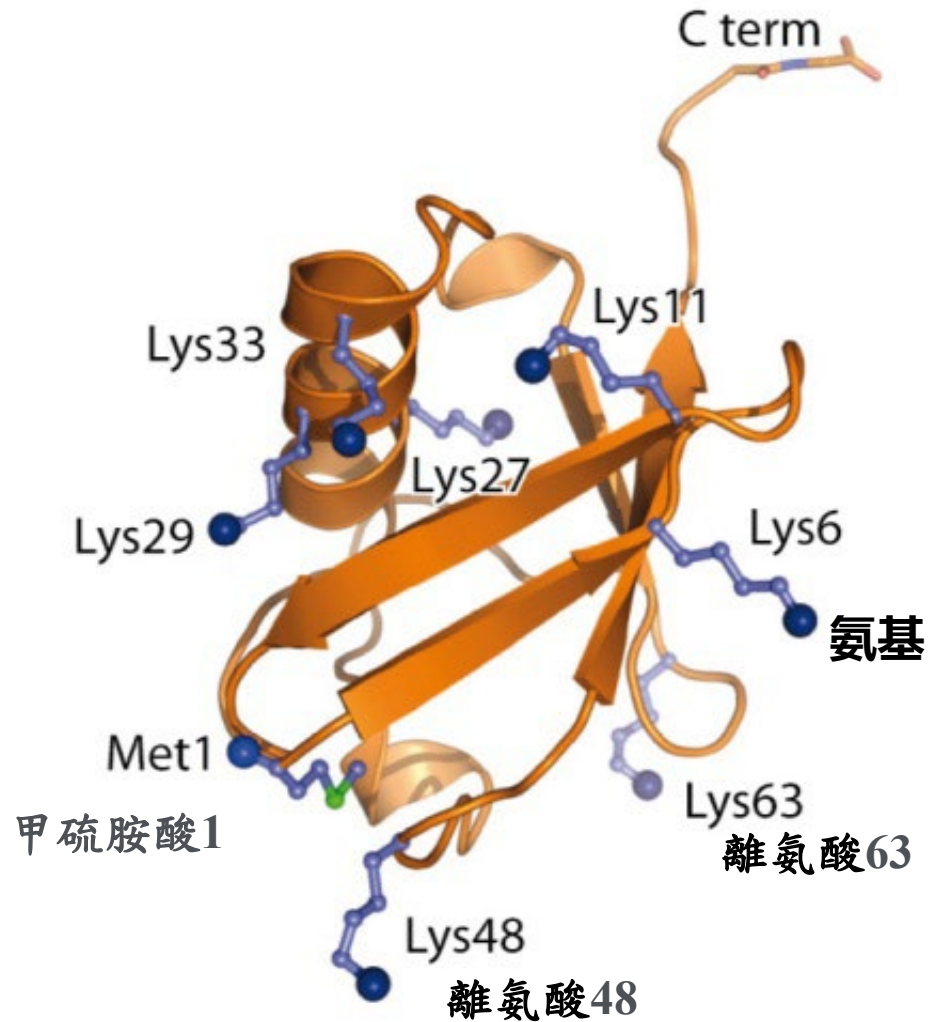
A. HECT domain E3s (Homologous to E6AP C-terminus)



B. RING domain E3s

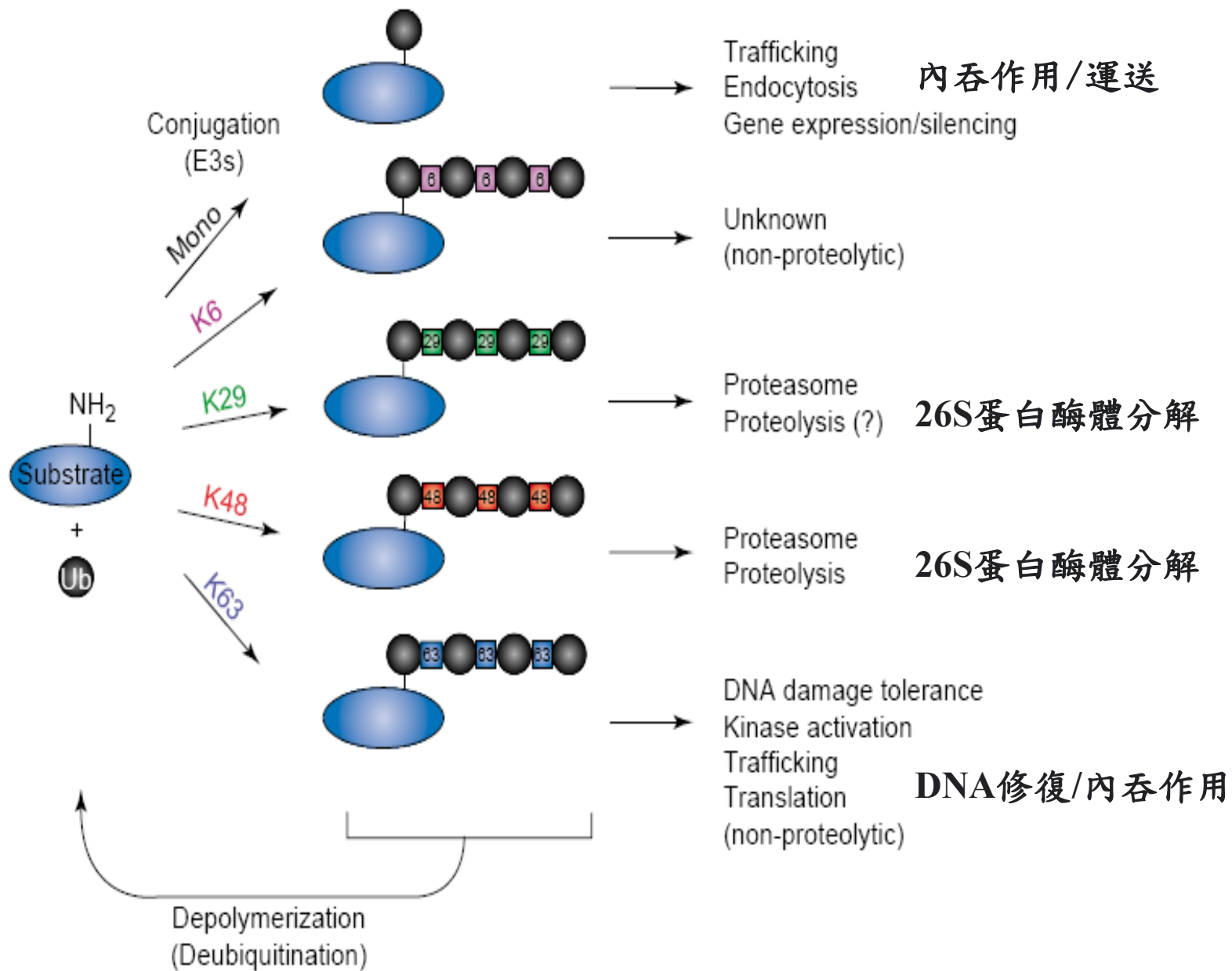


泛素密碼 (ubiquitin codes)

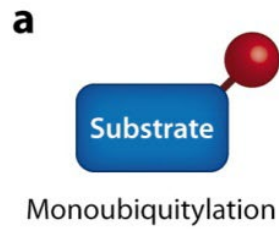


M1-
K6-
K11-
K27-
K29-
K33-
K48-
K63-

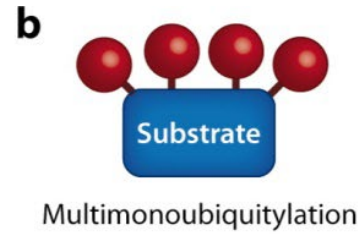
泛素藉甲硫胺酸(Met1)及七個離胺酸(Lys)形成不同泛素鏈



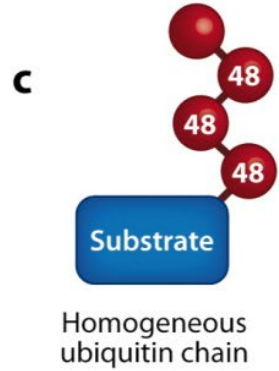
單一泛素化



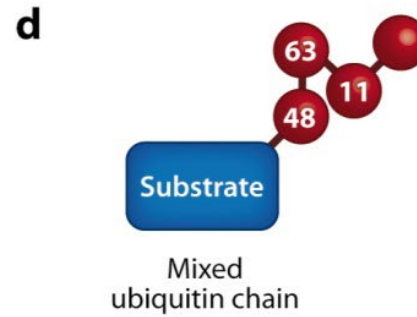
多重泛素化



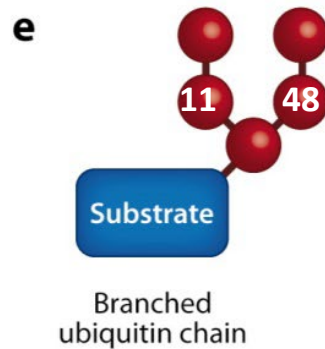
同鍵型泛素鏈化
(K48/11/63最多)



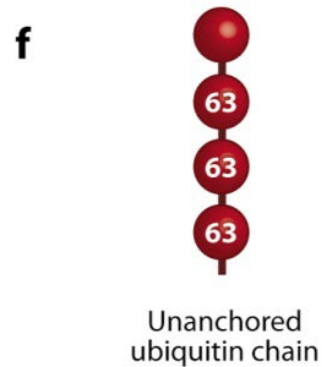
混合鍵型泛素鏈化




分叉鍵型泛素鏈化

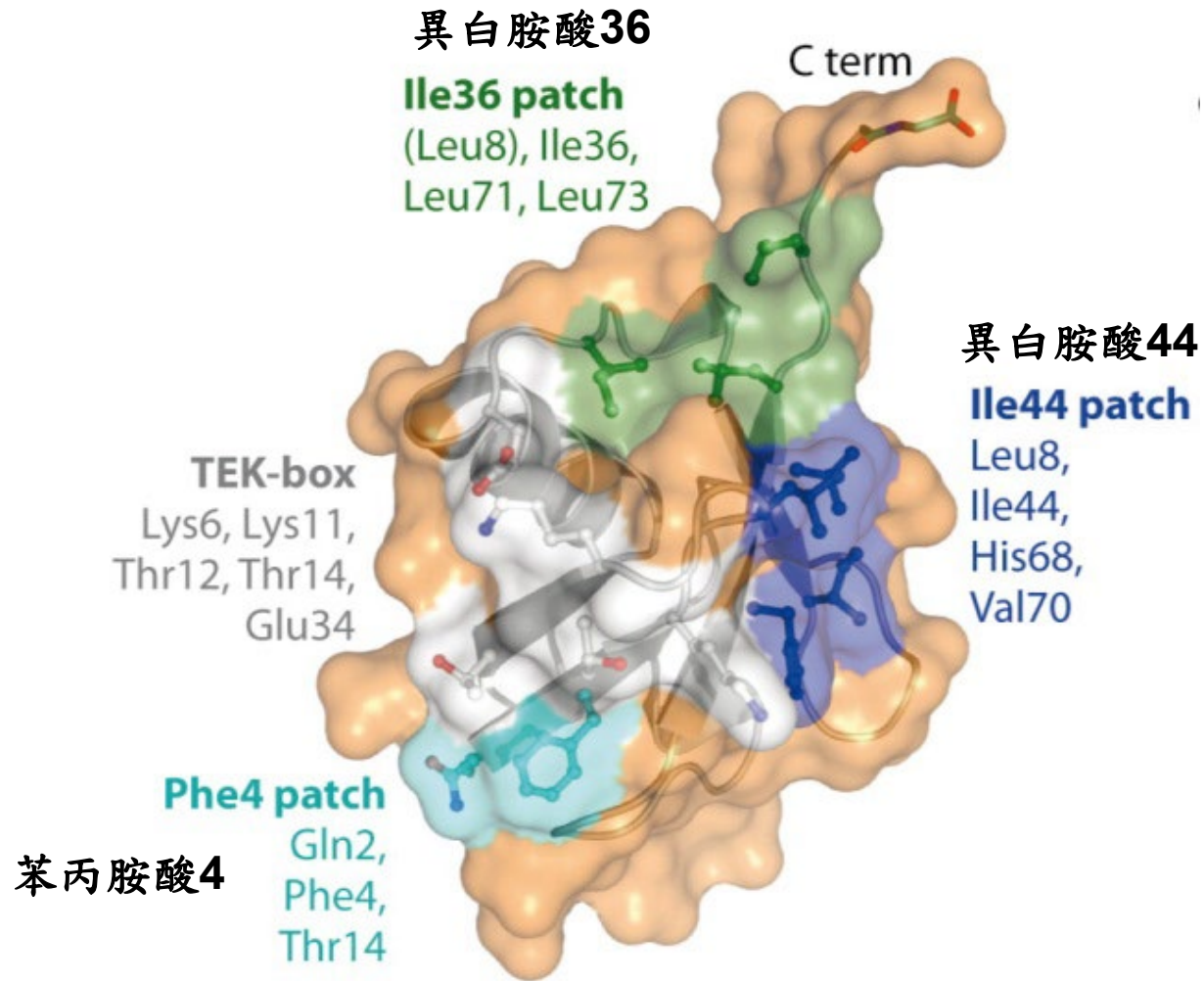


游離泛素鏈

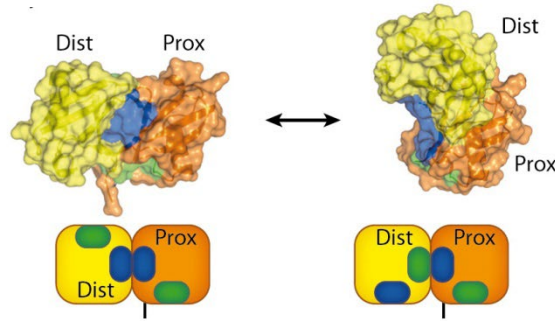


 Komander D, Rape M. 2012.
Annu. Rev. Biochem. 81:203–29

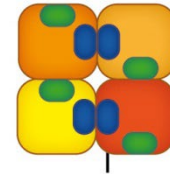
泛素交互作用介面



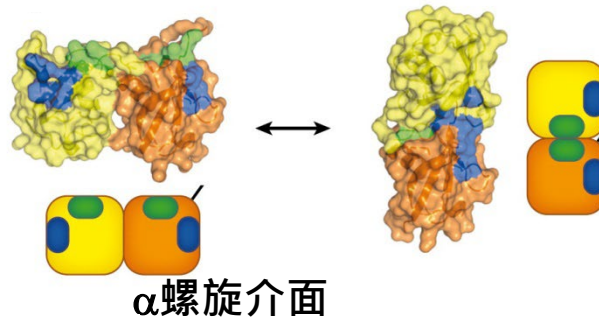
**離胺酸48鏈
K48-chain**



Lys48 tetraUb (Xtal, NMR)



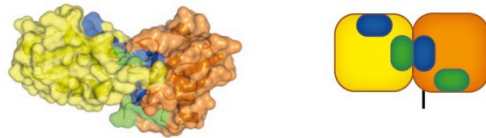
**離胺酸11鏈
K11-chain**



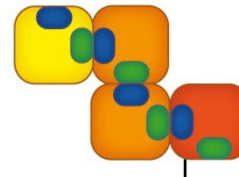
Lys11 octaUb (Xtal)



**離胺酸6鏈
K6-chain**



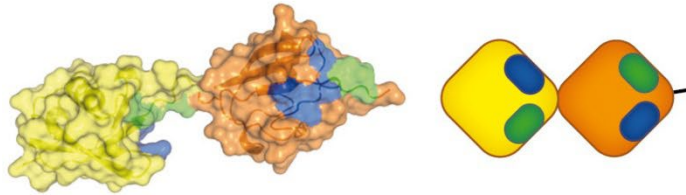
Lys6 tetraUb ?



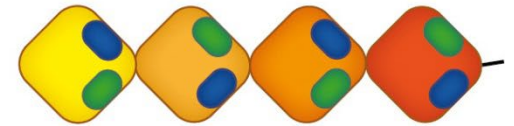
- Ile44 hydrophobic patch
- Ile36 hydrophobic patch
- / Free C terminus (substrate)

緊密式泛素鏈構形

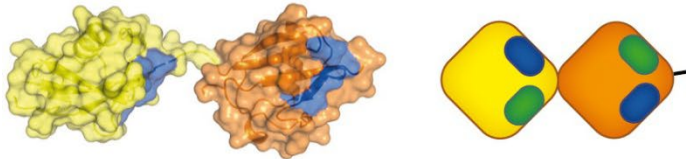
離胺酸63鏈
K63-chain



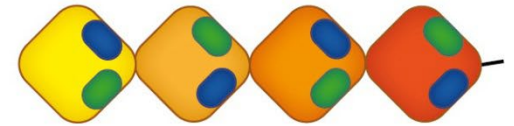
Lys63 tetraUb (Xtal, NMR)



甲硫胺酸1鏈
M1-chain



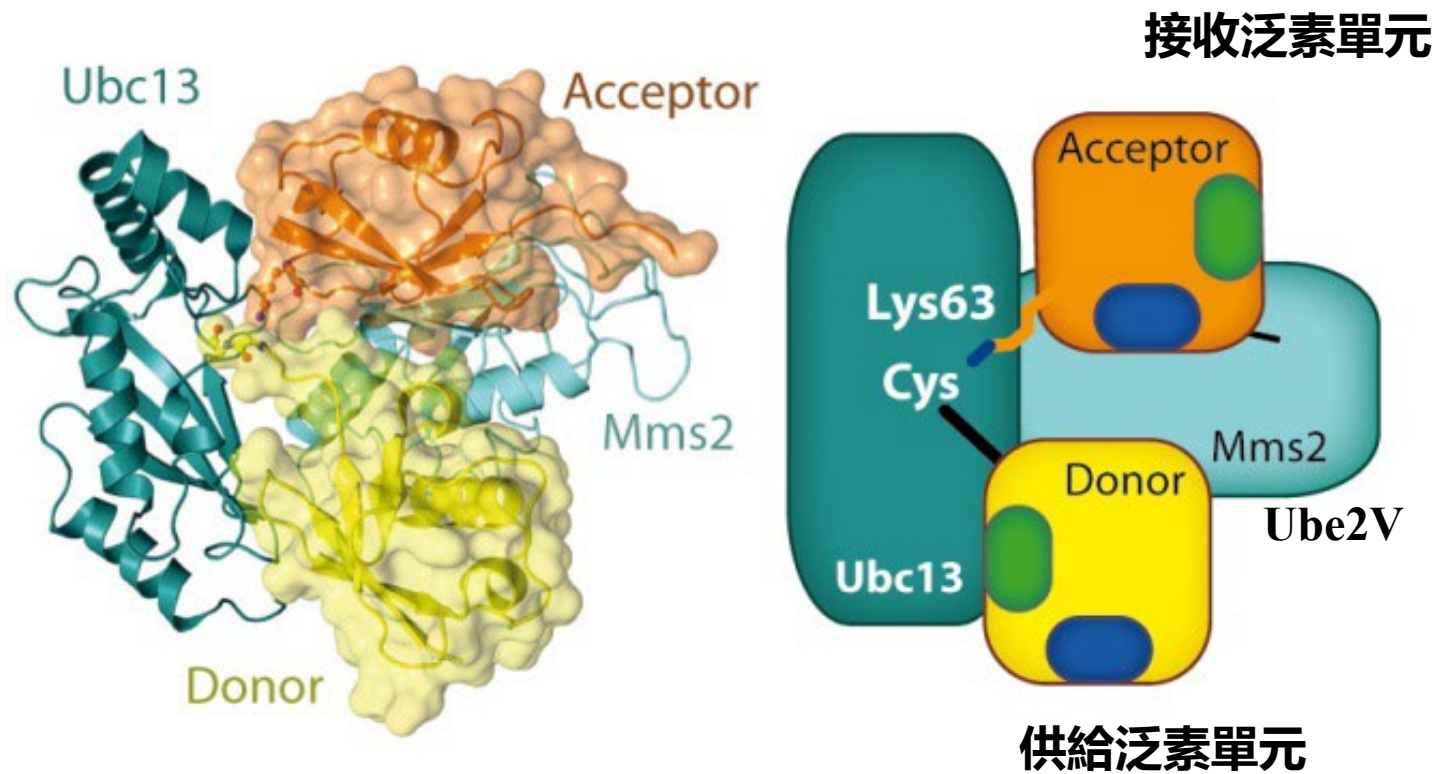
Met1 tetraUb ?



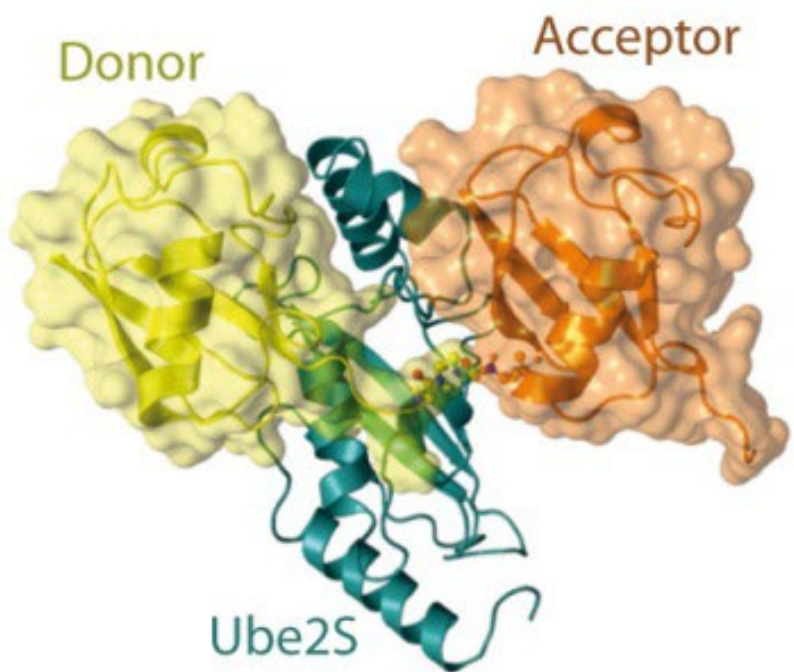
- Ile44 hydrophobic patch
- Ile36 hydrophobic patch
- Free C terminus (substrate)

開放式泛素鏈構形

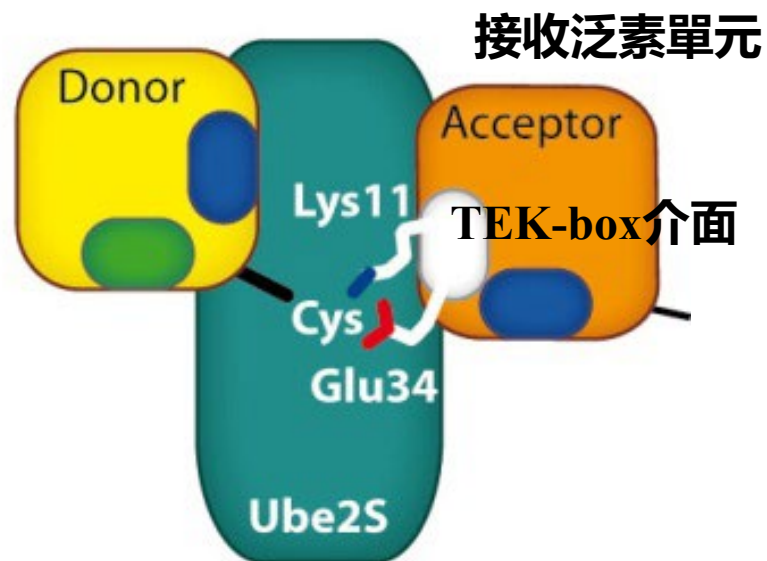
撰寫(合成)泛素密碼



離胺酸63鏈-專一-E2 Ube2N (Ubc13)



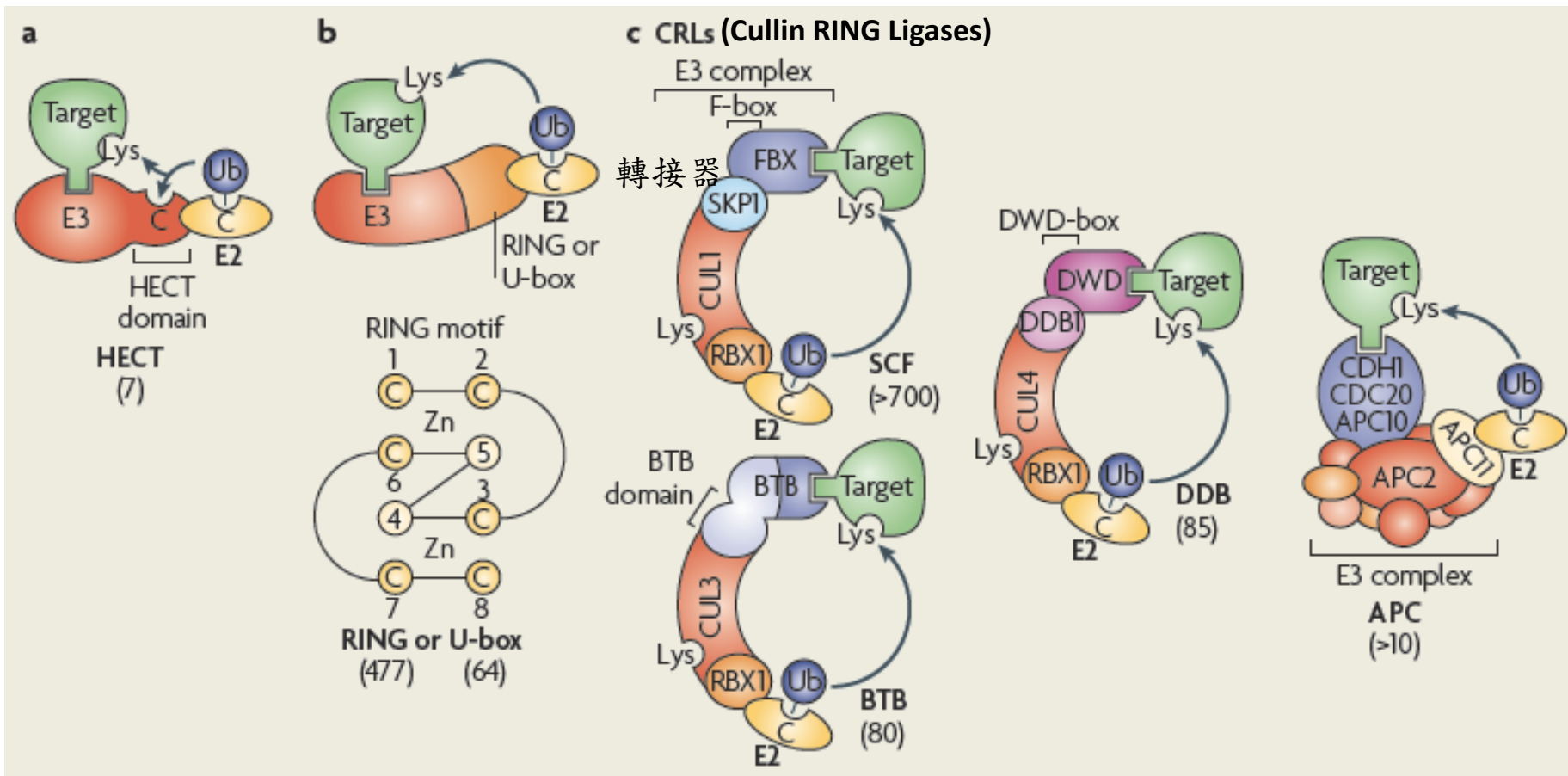
供給泛素單元



穀氨酸(Glu)34
受質輔助催化反應

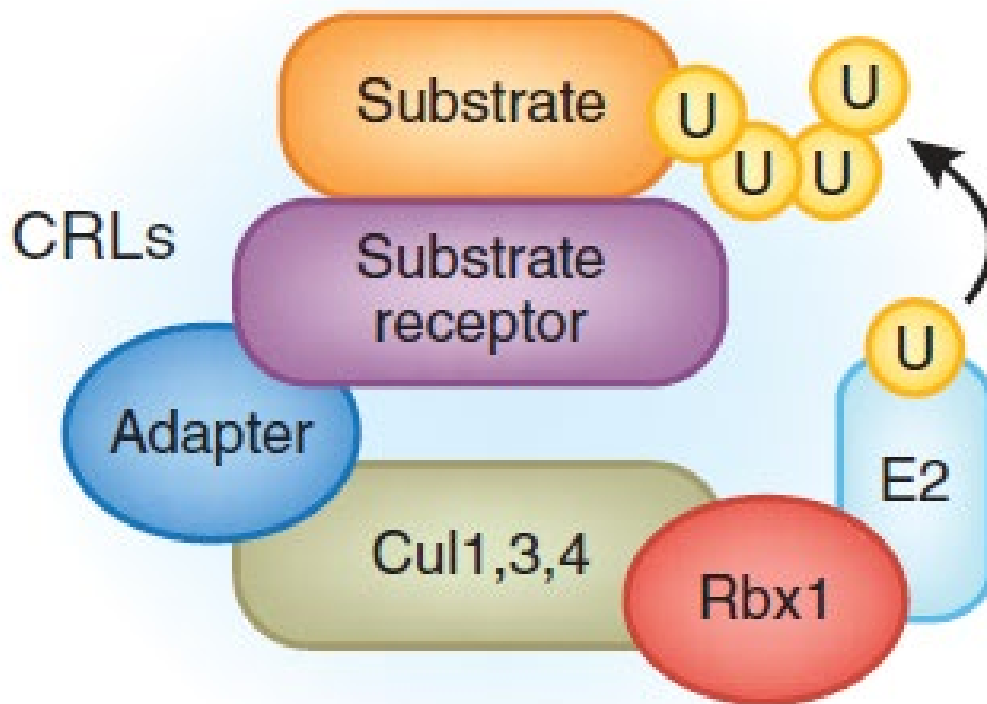
離胺酸11鏈-專一E2 Ube2S (AtUBC22)

Threonine, 蘇胺酸



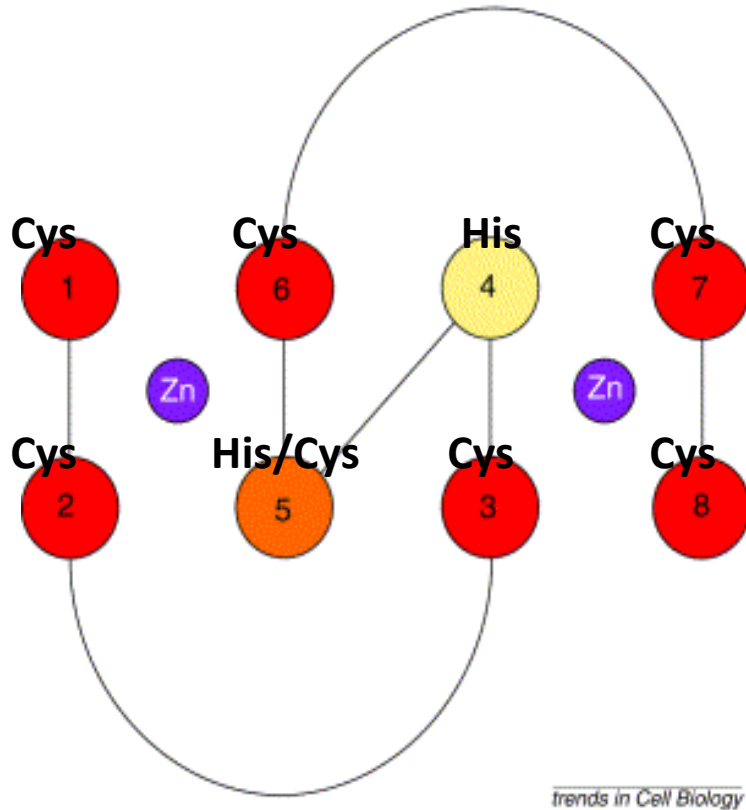
SKP1, S phase kinase-associated; BTB, bric-a-brac-tramtrack-broad; DDB, DNA damage-binding; DWD, DDB1-binding WD40 proteins

E3 ligases 主要種類

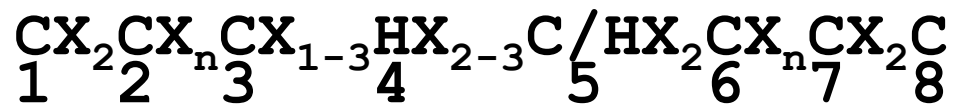


CRLs

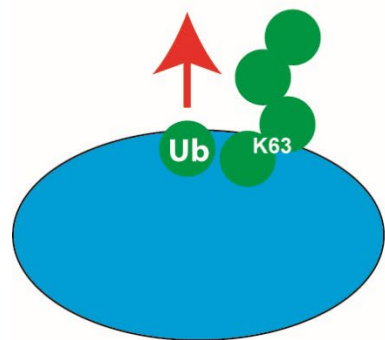
RING



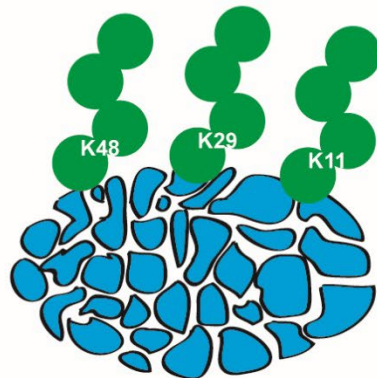
- RING (**R**eally **I**nteresting **N**ew **G**ene) finger, consists of 8 well conserved, spaced Cysteine and Histidine residues coordinating two zinc-ions in a **cross-brace configuration**
- RING-H2 and RING-HC
- spacing of zinc ligands, but not primary sequence, is conserved
- molecular scaffolds instead of chemical catalyst (for Ub-E2 binding) + E2 activation



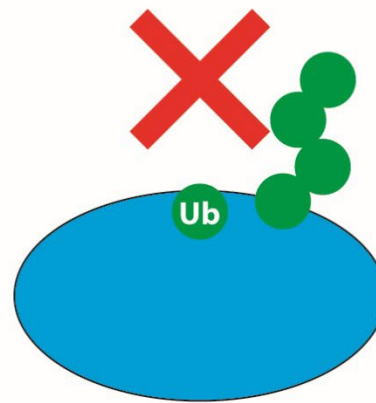
Cystine :半胱氨酸; Histidine: 組氨酸



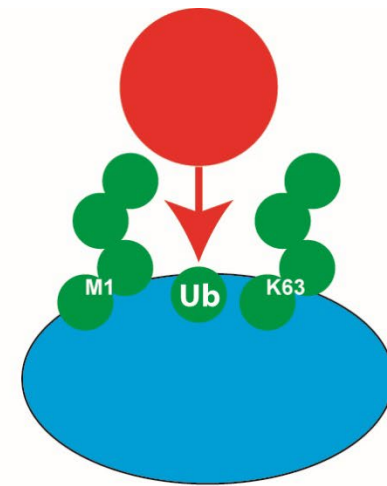
胞內移動



分解



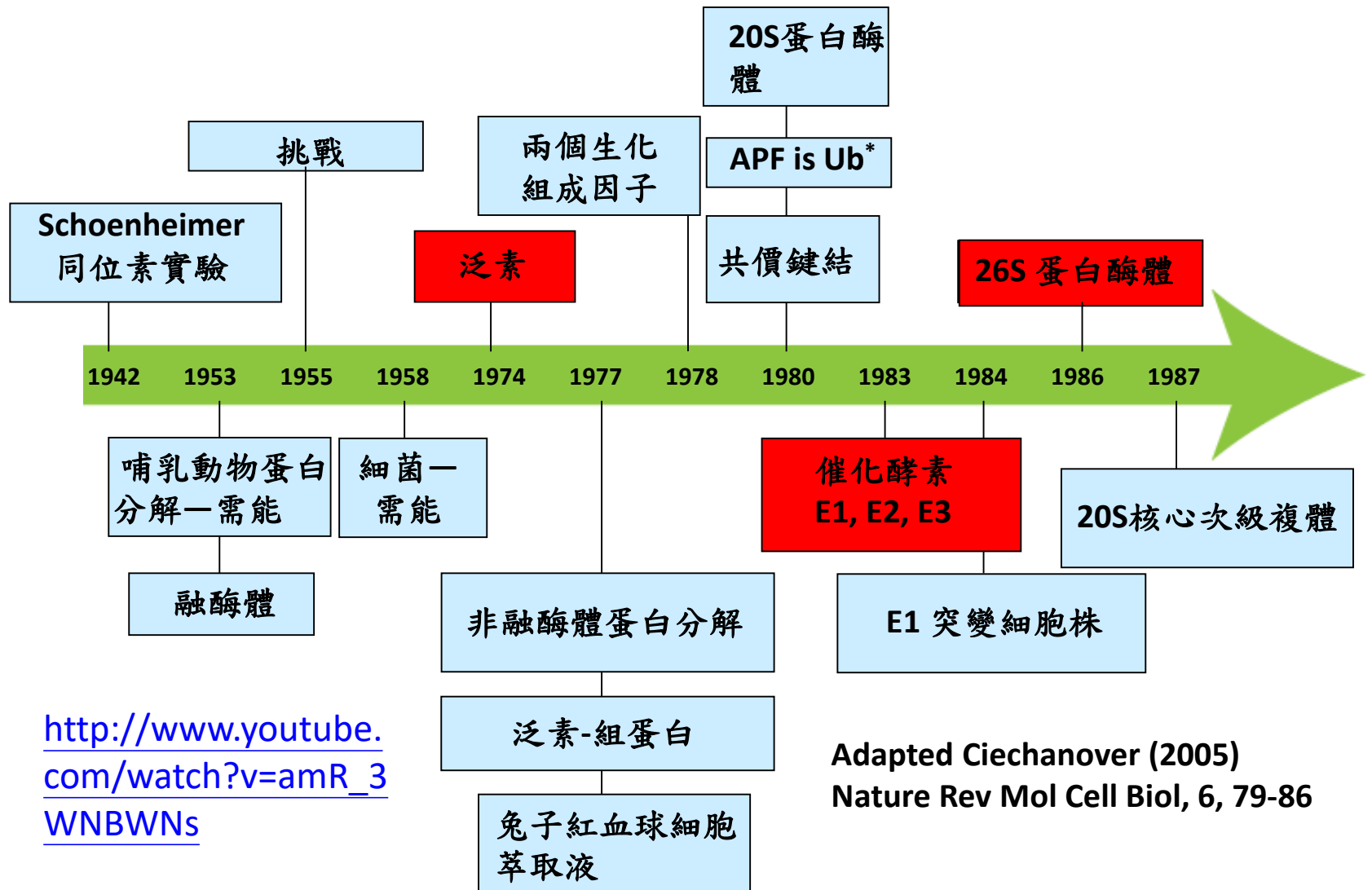
抑制修飾



招募訊號因子

泛素修飾後效應

發現泛素修飾系統之重要里程碑



http://www.youtube.com/watch?v=amR_3WNBWNs

Adapted Ciechanover (2005)
Nature Rev Mol Cell Biol, 6, 79-86

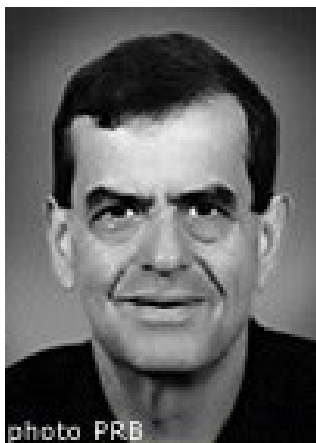
* ATP-dependent proteolysis factor₂₂



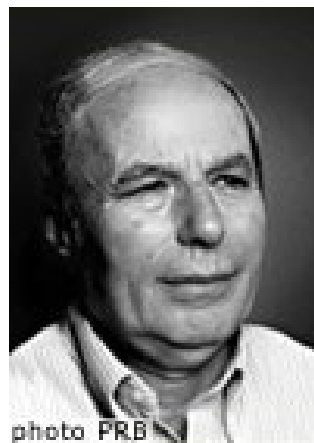
2004諾貝爾化學獎得主

"for the discovery of ubiquitin-mediated protein degradation"

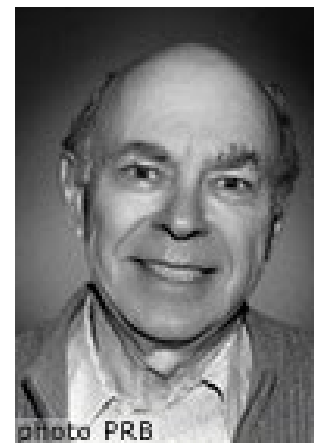
<http://nobelprize.org/chemistry/laureates/2004/chemadv04.pdf>



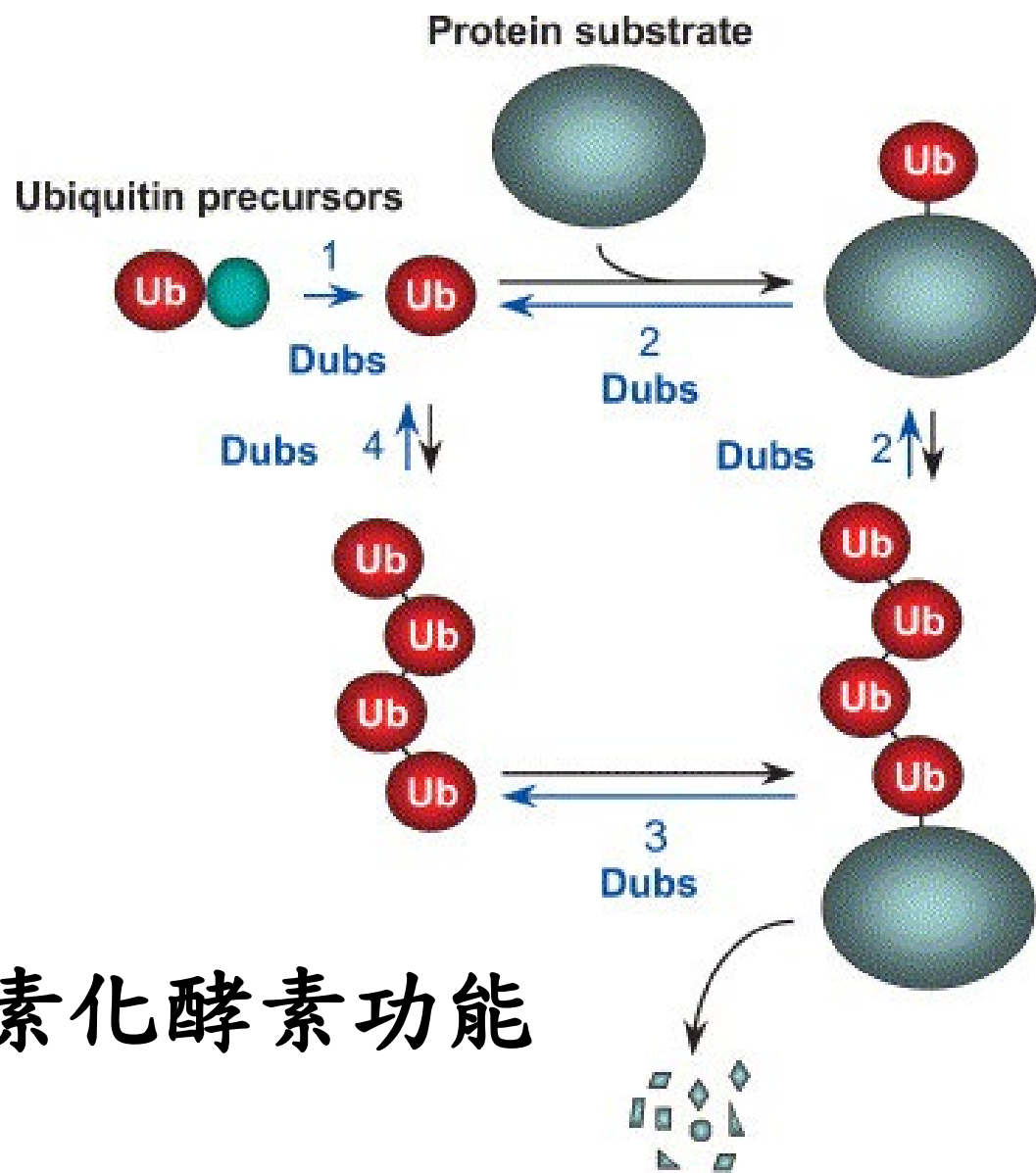
Aaron Ciechanover
Israel
Technion –
Israel Institute of
Technology
Haifa, Israel
b. 1947



Avram Hershko
Israel
Technion –
Israel Institute of
Technology
Haifa, Israel
b. 1937



Irwin Rose
USA
University of
California
Irvine, CA, USA
b. 1926



去泛素化酶功能

Seven DUB Classes

1. UBP/USP (27)

```

      *
HAUSP  214  VGLKNGCNTCYMNSLLQTLFF
ISOT   326  TGIKRLNGNSCYLNSVVOVLF
Ubp14  345  CGLINLNGNSCYLNSVVOVLF
Ubp3   460  RGLINLRANICFMSVLOVLY
Doa4   562  VGLKNGCNTCYMNSLLQTLFF
Ubp6   109  VGFKNMGNTCYLNSVVOVLF
Ubp10  362  RGLLNHCVTCYTNAAVQAMEH
Faf    1668 CGLKNAGATCYMNSVLOVLYM
UBP43  55   VGLKNGCNTCYMNSLLQVFFM
Consensus  GlkNlgtCymnsllQ lv
    
```

```

      *           *
HAUSP  445  PANYILHAAVLVHSG-DNHGGHYVVYLNPKG----DGKWKCFDDDVV
ISOT   775  PGKYOLFAPFISHMGTSTMCGHYVCHIKK-----EGRWVIYNDQKV
Ubp14  741  P--YALTAVICHKGNVSHSGHYVVFIRKLVAD--KWKVLYNDEKL
Ubp3   841  DRRYKLTGVVYHGGVSSDGGHYTADVYHS---EHNKWYRIDVNI
Doa4   861  PFKYELYGVAACHFG-TLYGGHYTAYVKKG--L--KKGWLYFDDTKY
Ubp6   427  SCVYNLIGVITHOGANSESGHYQAFIRDEL--ENKWKYFNDDKV
Ubp10  671  EVKYQLLSVVVHGRSLSSGHYIAHCKQP----DGSNATYDDEYI
Faf    1967 TTKYELTGIIVVHSG-QASGGHYFSYILSKNPANGKQWYKFDGGEV
UBP43  299  GGQYELFAVIAHVG-MADSGHYCVYIRNAV---DGKWFCEFNDSNI
Consensus P kY L avi H G s GHY yik d k w f d d v
    
```

2. UCH (3)

```

      *
Yuh1   79  VIWFKQSVKNACGLYAILHSLSNQ
UCH-L1 79  VYFMKQTIENS CGTIGLTHAVANNQ
UCH-L3 84  VYFMKQTIENS CGTIGLTHAVANNQ
UCH37  77  VYFAKQVINNACATQAVSVLNLNCTH
Consensus vyfmKQti NaCgtiaihalaNnqd
    
```

```

      *           *
Yuh1   165  LHWITVVEENCGIFELDGRNLSCFLYLCNS
UCH-L1 160  FHFILFNNDGHLYELDGR-MPPFVNHGAS
UCH-L3 168  LHFIALVHVDGHLYELDGR-KPPFVNHGAS
UCH37  163  FHFVSVVFNGLRYELDGL-REGPIDLGAC
Consensus Hfityv v GhlyELDGr p Pin Gas
    
```

6. MINDY (3)

7. ZUP1(2)

3. OTU-related (12)

```

      *
Otubain 1  80  SYIRKTRPDGNCFYRAFGFSHL--
Otubain 2  40  TAIRKTKGDGNCFYRALGYSYL--
Cezanne  198  LEPPLATTGDGNCLEHAASLGMWGF
A20      92  LVALKTNNGDGNCLMHATSQYMWGV
VCIP135  207  LIPVHVVDGDGHCLVHAVSRALVGR
Consensus  ll lkt gDnClIhA s mlg
    
```

```

      *
Otubain 1  249  FPEGSEPKVYLYRPGHYDILYK
Otubain 2  208  FPEAATPSVYLYKTSHYNIIYA
Cezanne  363  SP-----LVLAVDQAHFSALVS
A20      246  YP-----IVLGYDSHHEVPLVT
VCIP135  348  SS-----GRNHVHPLVVG
Consensus  fp v l y Hy Lv
    
```

4. Ataxin-3/Josephin (MJD/2)

```

      *
Ataxin-3 (H.sapiens) 2  ESIFHEKQEGSLCAQHCLNLLQGE
Ataxin-3 (C.elegans) 8  NSIFFEHQEAALCAQHCLNLLQDA
Ataxin-3 (A.thaliana) 8  GMLYHEVQESNLCAVHCYNTVLLQGV
Consensus  sifhEkQeG LCAqHclN lLQg
    
```

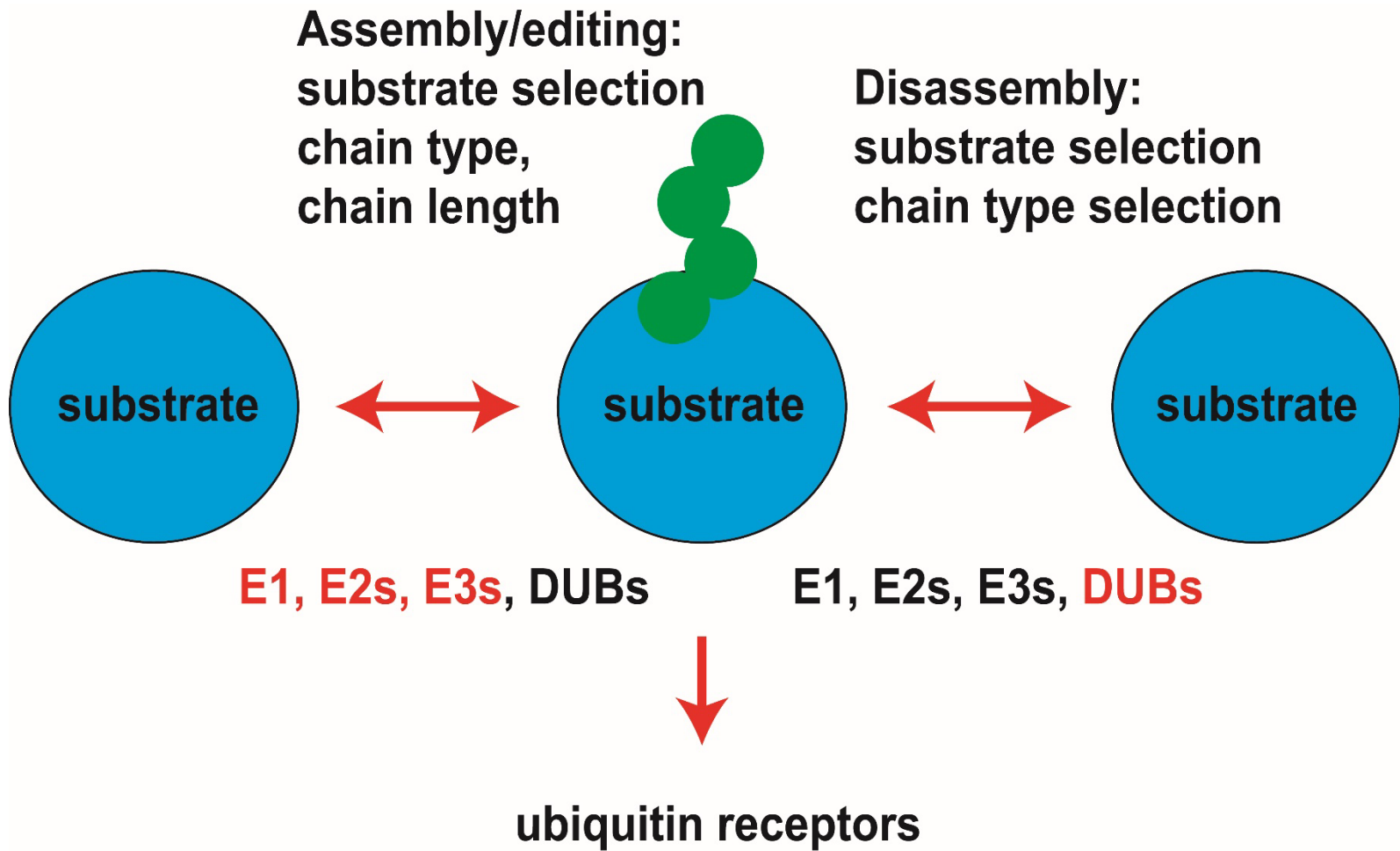
```

      *
Ataxin-3 (H.sapiens) 108  NERSFICNYEHWFVTRKLGK
Ataxin-3 (C.elegans) 106  TARAVICNLEHWFVTRKFGN
Ataxin-3 (A.thaliana) 115  LESAFICNLEHWFVTRKLVNG
Consensus  erafICnlkeHWF vRklg
    
```

5. MPN+/JAMM (5)

```

      * *           *
Rpn11  74  --TGR-DMVVGWYHSHG--GCWSS-VDVNTKS----NSRAVAVVVD--
POH1   101  -QTGRPEMVGWYHSHPGEGCWLSGVDINTQDSFEALSERAVAVVVDPI
Rri1   166  DYKCAKLNVGWYHSHPGYDCWLSNIDIQDLDNQRFDQPYAVAVVVDPI
Csn5   126  -QVGRLENAGWYHSHPGYGCWLSGIDVSTQMLNQDFQEPFVAVVVDPT
Consensus  qtGr e vVGWYHSHpgygCWlSgvDvnTq lnq fqe aVAVVvDpi
    
```



泛素化訊號

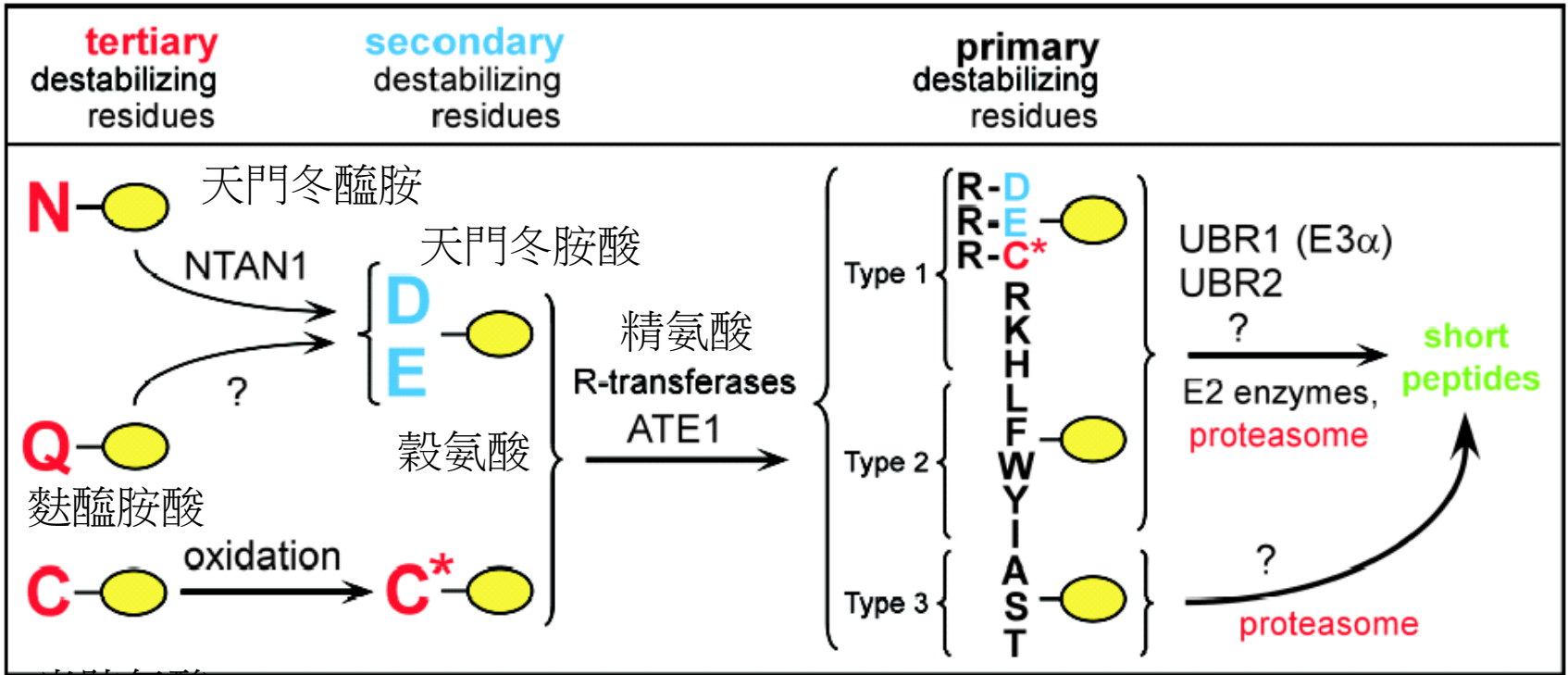
- destruction boxes, **RXALGXIXN**
 - short region of primary sequence
 - mitotic cyclins and APC substrates
- N-端胺基酸(N 端規則N-end rule)
- 轉譯後修飾(磷酸化)
- 小分子結合

N-end rule in *Saccharomyces cerevisiae*

Residue at ^[L]_{SEP}N terminus Half-life of ^[L]_{SEP}X-βgal

Arg	2 min
Lys	3 min
Phe	3 min
Leu	3 min
Trp	3 min
His	3 min
Asp	3 min
Asn	3 min
Tyr	10 min
Gln	10 min
Ile	30 min
Glu	30 min
Cys	>20 hr
Ala	>20 hr
Ser	>20 hr
Thr	>20 hr
Gly	>20 hr
Val	>20 hr
Pro	ND
Met	>20 hr

N-end rule



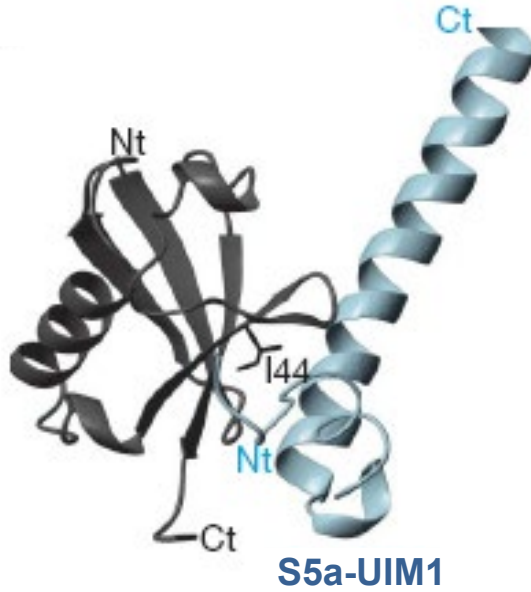
Varshavsky, 2003 Nature Cell Biol 5, 373

解讀泛素密碼

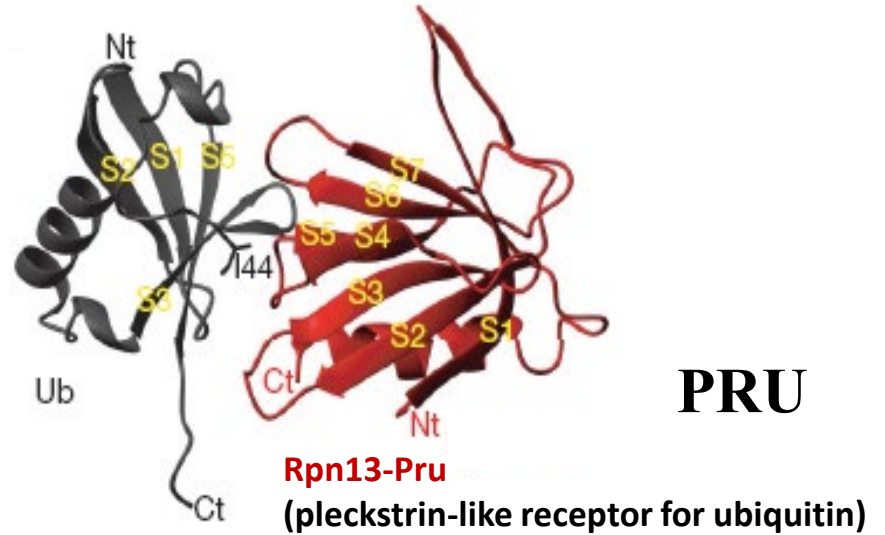
- 含泛素結合結構(UBD)之蛋白係解讀密碼成特定泛素效應之重要因子
- 泛素受體

主要泛素結合結構

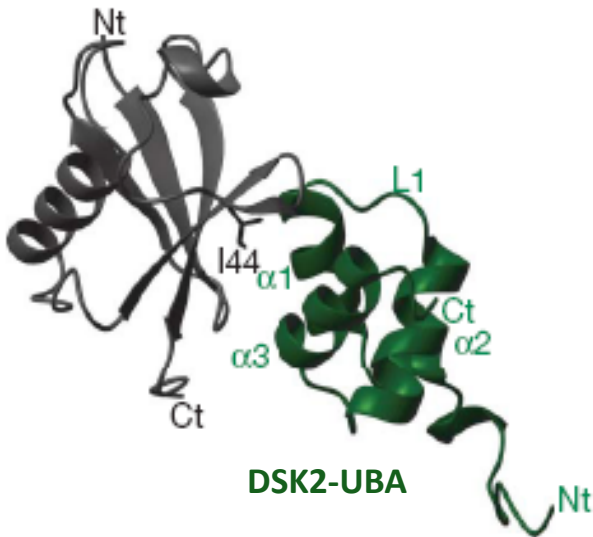
UIM



PRU

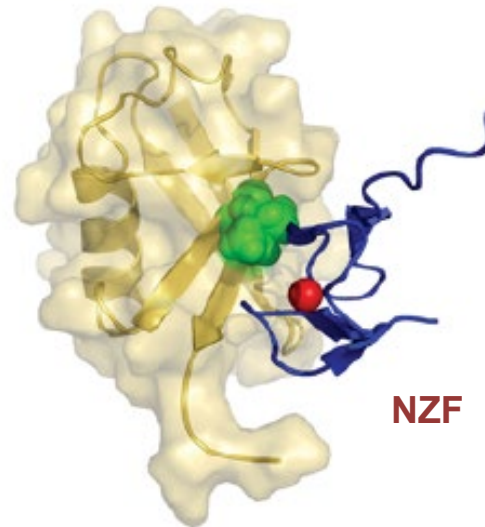


UBA

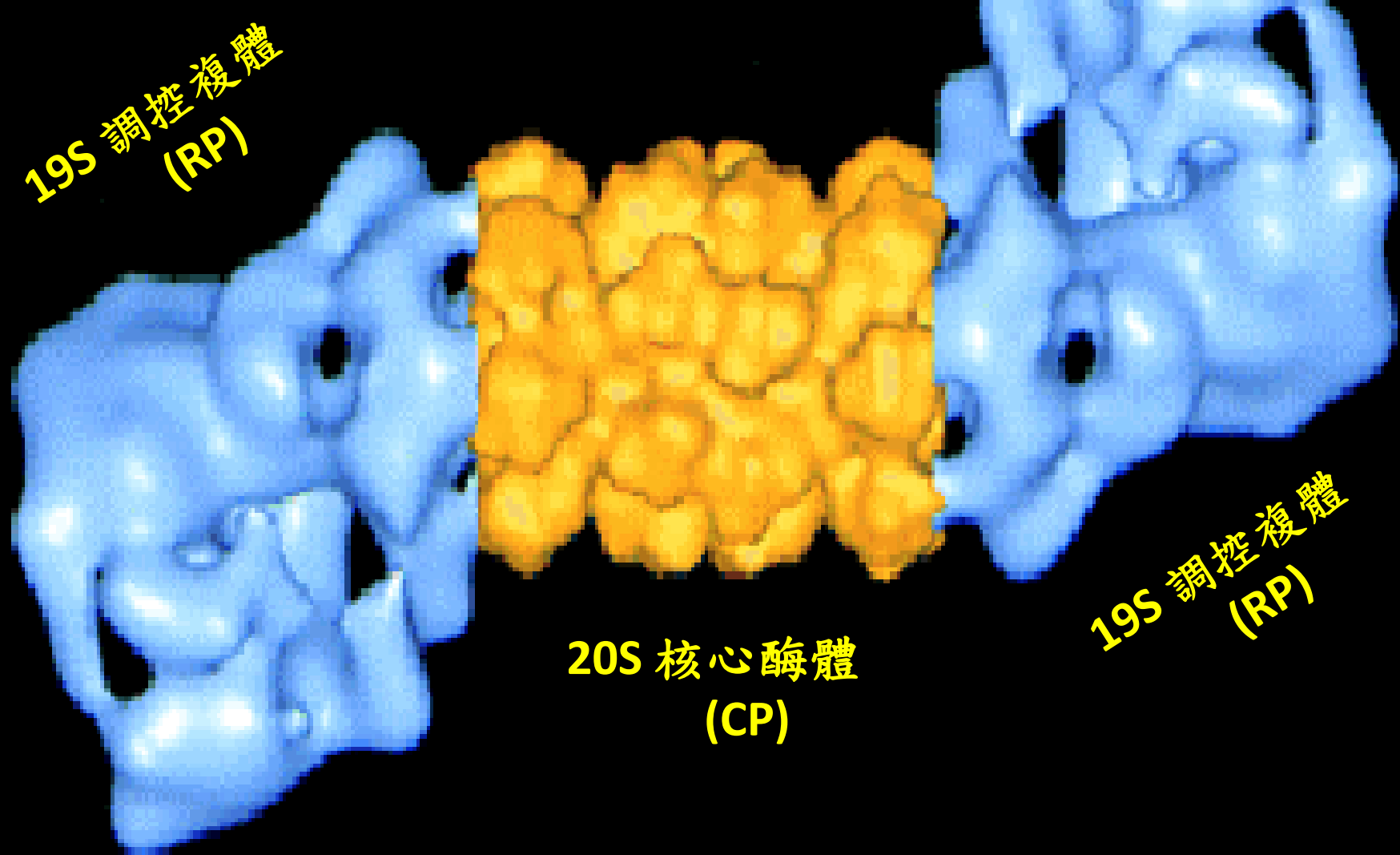


NZF

NZF



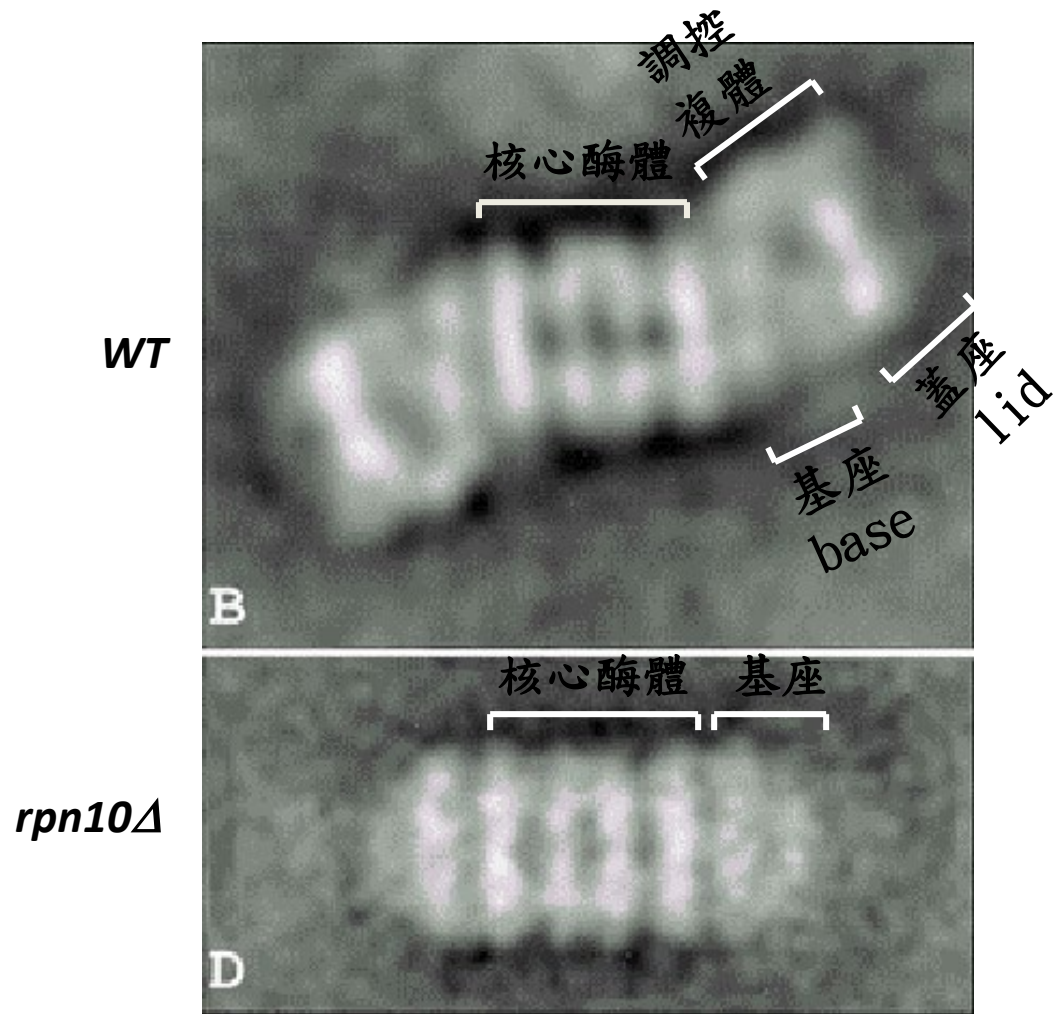
26S 蛋白酶體 (Proteasome)



19S 調控複體
(RP)

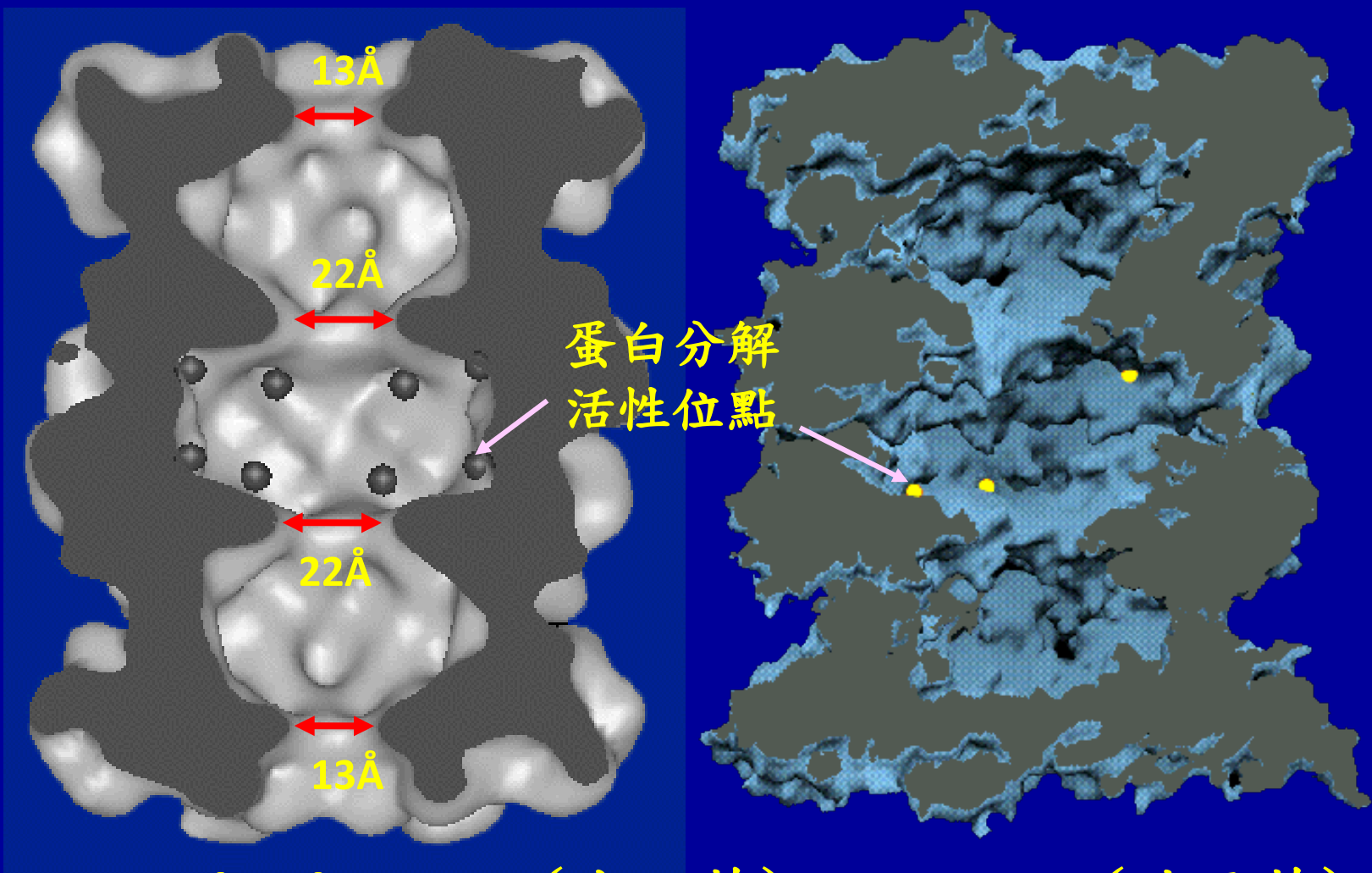
20S 核心酶體
(CP)

19S 調控複體
(RP)



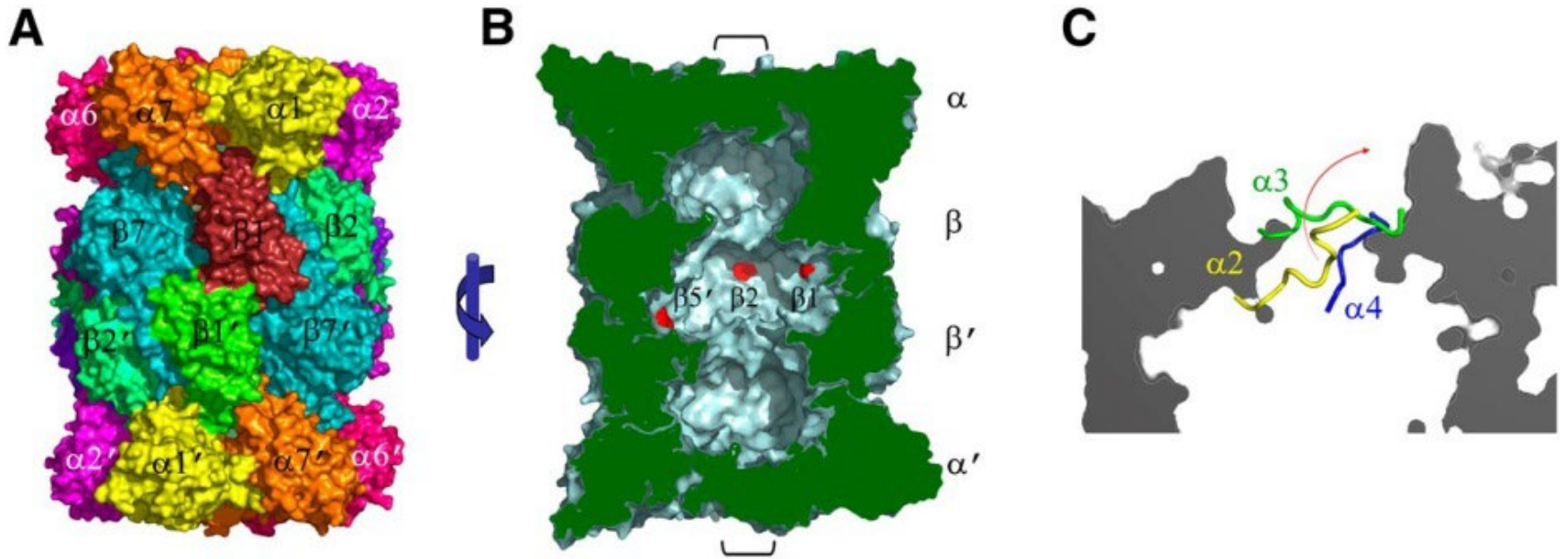
Glickman et al., Cell, 1998, 94, 615-623

20S 核心酶體結晶結構

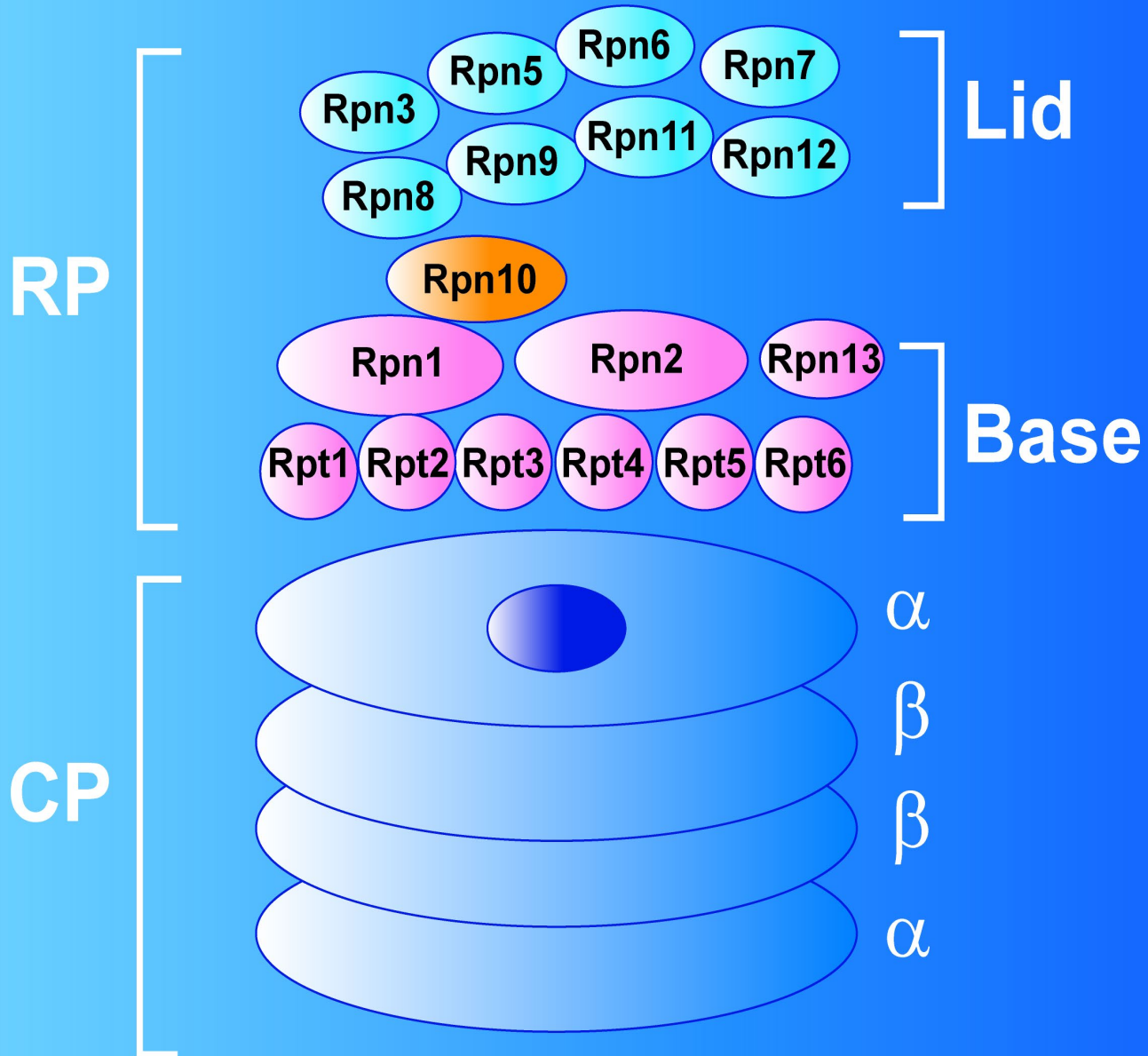


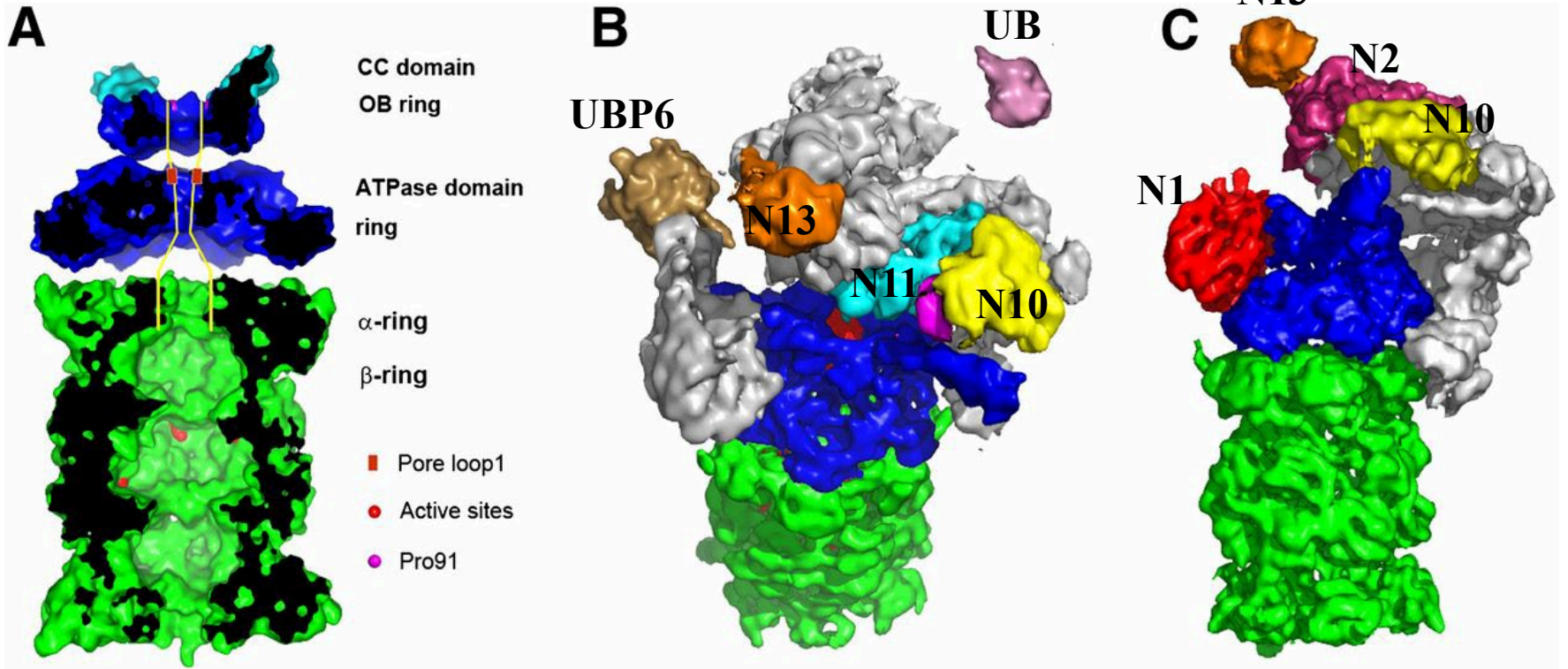
Archaeobacteria (古細菌)

Yeast (酵母菌)



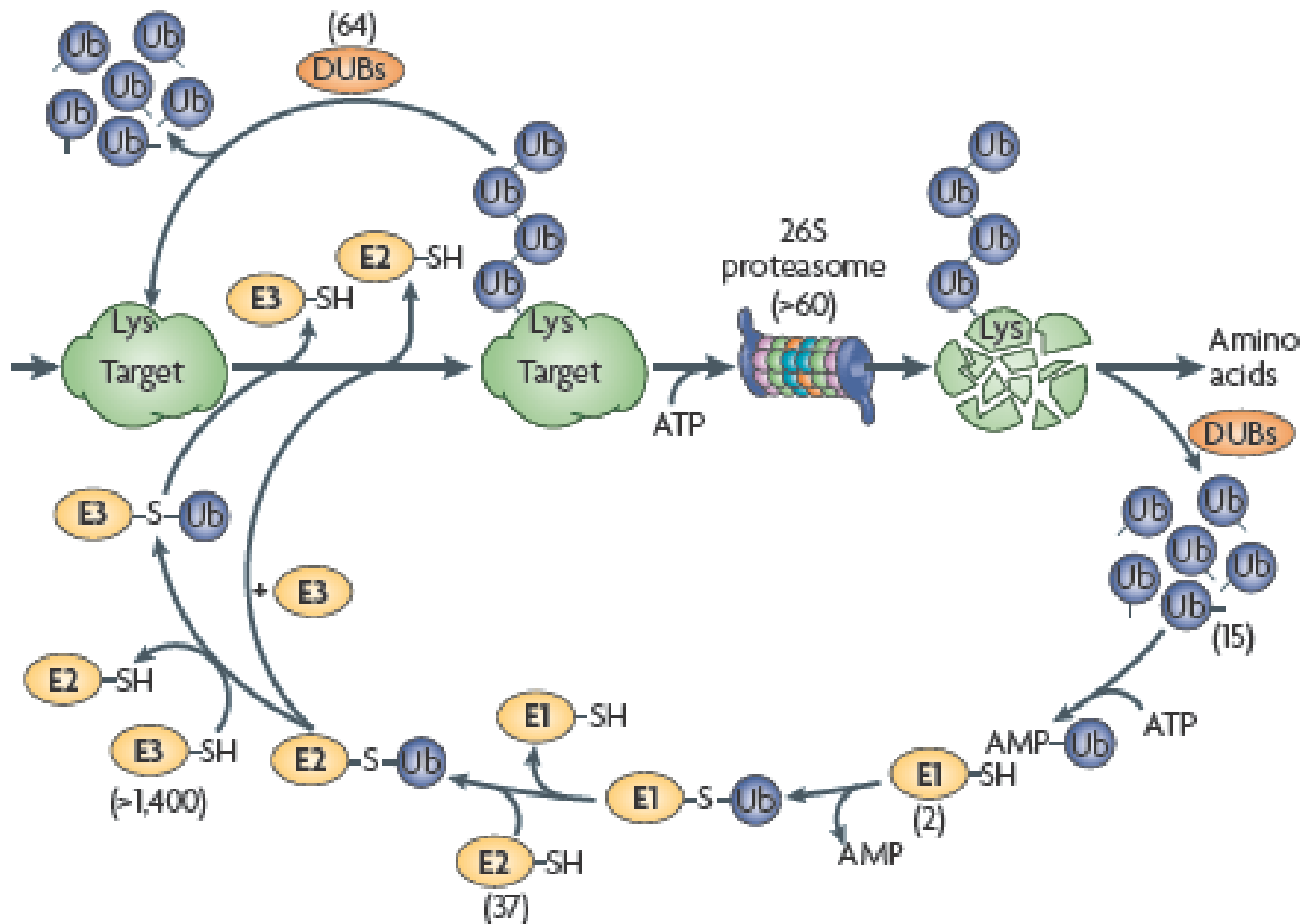
20S Core Particle (CP)



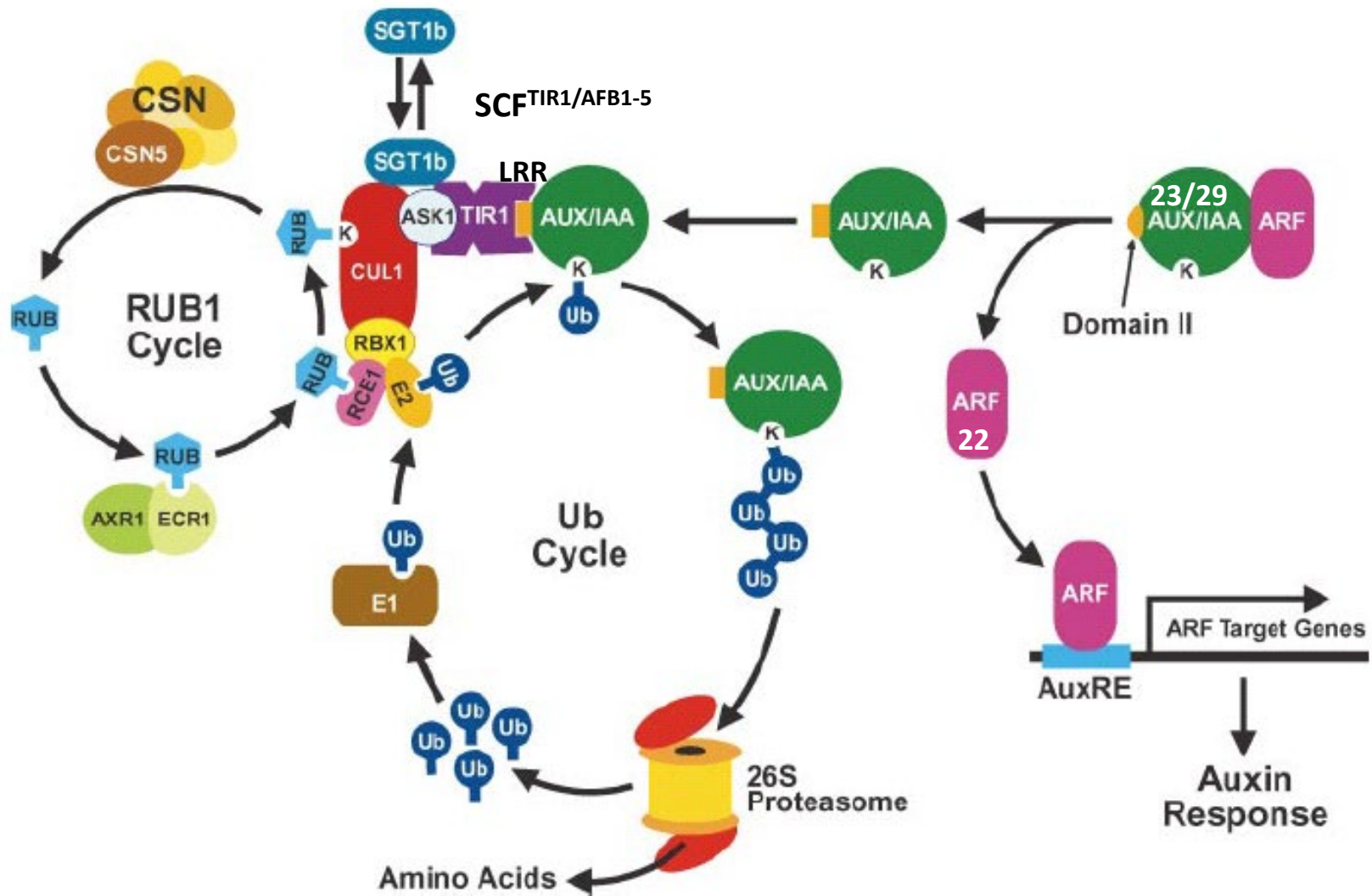


26S Proteasome

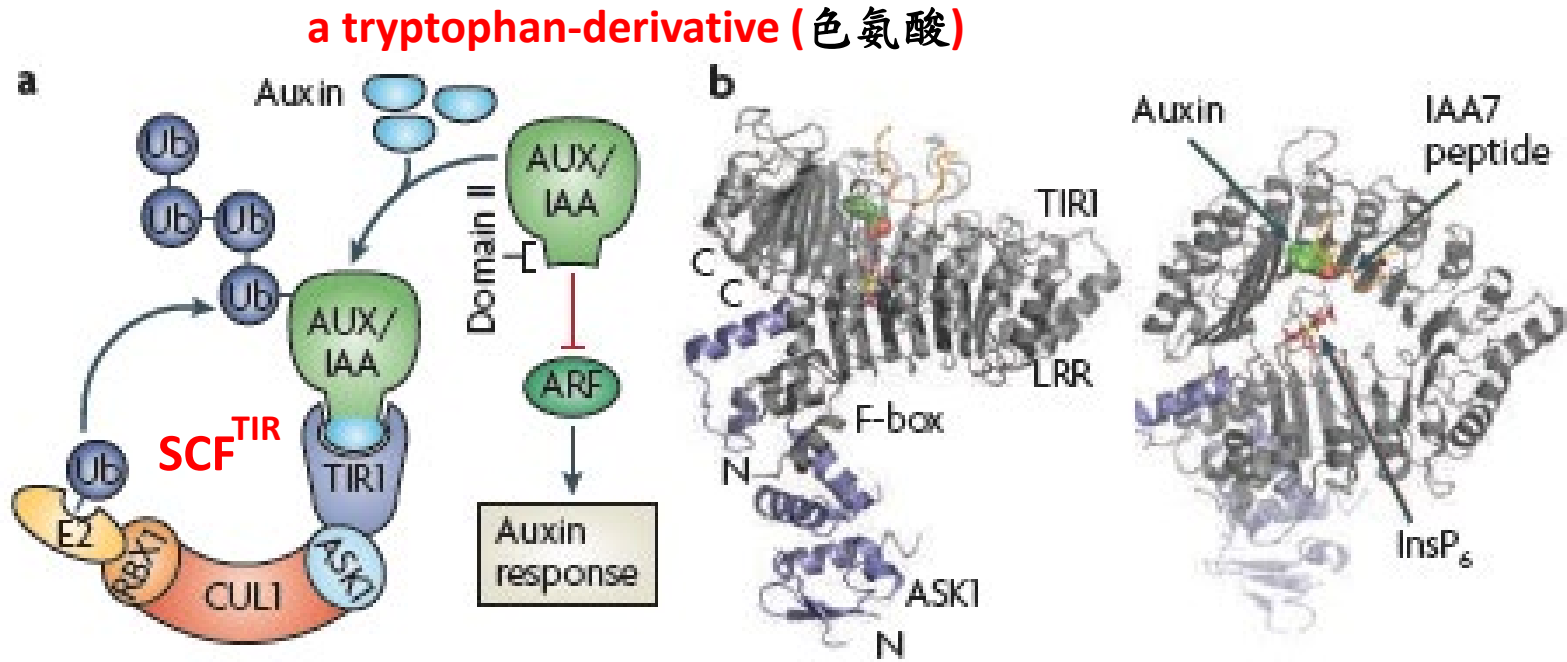
[http://www.nature.com/nature/journal/v482/
n7384/abs/nature10774.html#supplementary-
information](http://www.nature.com/nature/journal/v482/n7384/abs/nature10774.html#supplementary-information) **Supplementary Movie 1**



阿拉伯芥泛素蛋白酶體系統



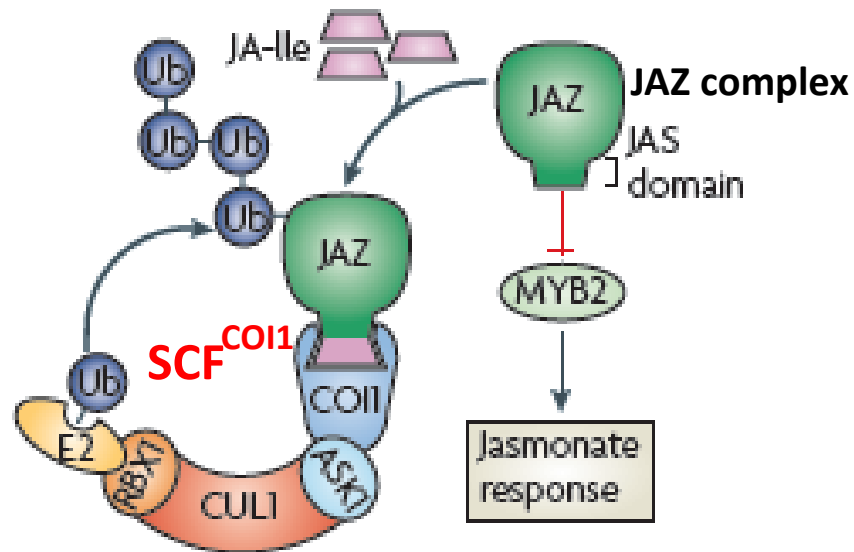
AFB, auxin signaling F-box protein; ARF, auxin response transcription factor; AUX/IAA, auxin indole-3 acetic acid repressors; TIR1, TRANSPORT INHIBITOR RESPONSE 1



Auxin enhances TIR1-IAA7 interaction

TIR1 and AUX/IAA form an auxin coreceptor

- Dharmasiri et al., 2005 *Nature* 435, 441
- Kepinski and Leyser, 2005 *Nature* 435, 446
- Villalobos et al., 2012, *Nature Chem Biol*, 9, 477



Degradation of JA response repressor by UPS

- SCF^{COI1} (COI1, CORONATINE INSENSITIVE 1)
- target: JAZ repressor (JA-ZIM domain repressor proteins)
- both LRR of COI1 and JA-Ile is required to recognize JAZ

CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETIOLATED/FUSCA (COP/DET/FUS) loci and derived complexes

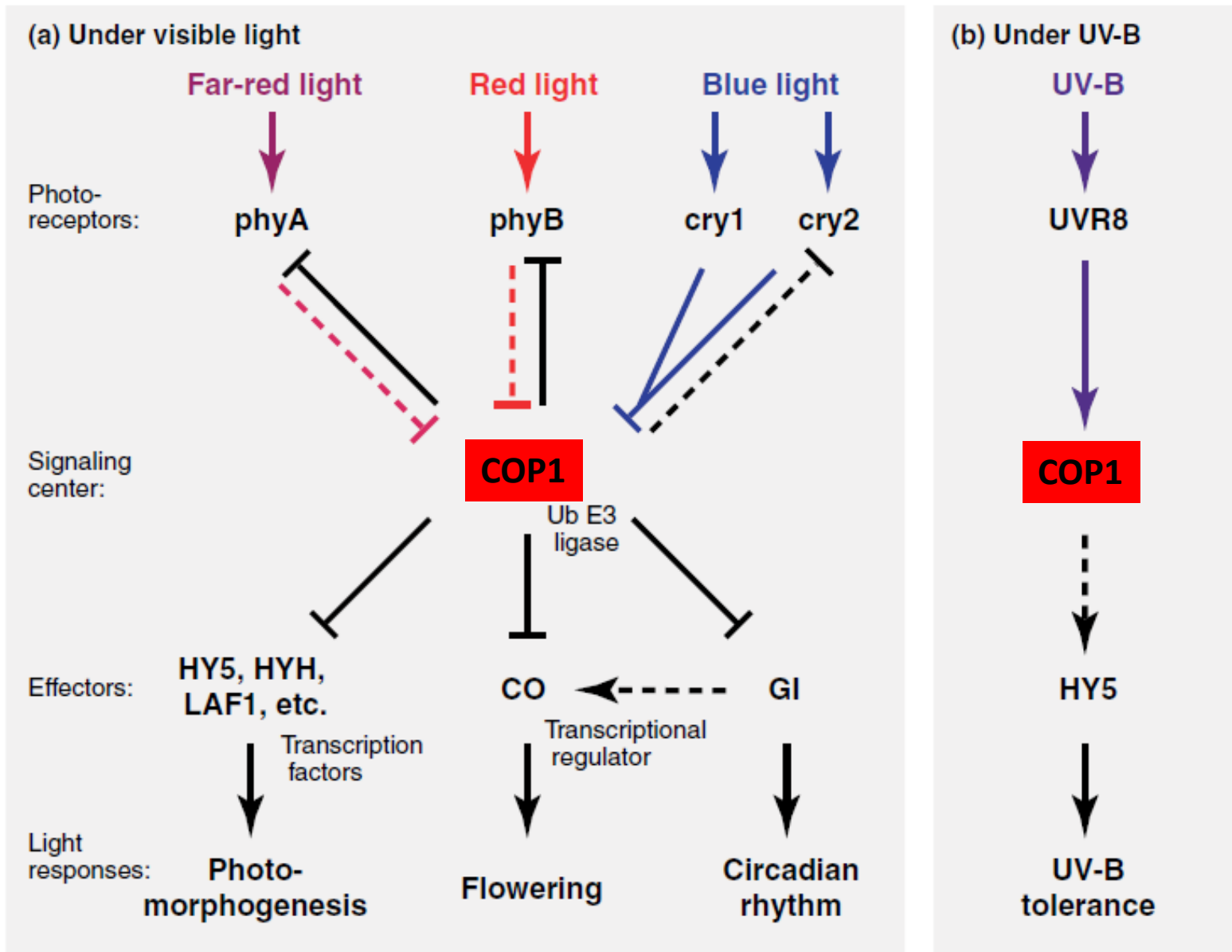
Table 1. Components of the three COP/DET/FUS complexes and the core CUL4–DDB1 E3 ubiquitin ligase in *Arabidopsis*

Symbol	AGI code	Full name	Phenotype of dark-grown mutant seedlings	Refs
COP1–SPA complex^a (SPA, SUPPRESSOR OF PHYA-105)				
COP1	AT2G32950	CONSTITUTIVE PHOTOMORPHOGENIC 1	<i>cop</i> phenotype (light-grown like) ^b	[9,93]
SPA1	AT2G46340	SUPPRESSOR OF PHYA-105 1	<i>cop</i> phenotype in the quadruple <i>spa1 spa2 spa3 spa4</i> mutant; milder <i>cop</i> phenotype in the triple and double mutants	[72]
SPA2	AT4G11110	SPA1-RELATED 2		
SPA3	AT3G15354	SPA1-RELATED 3		
SPA4	AT1G53090	SPA1-RELATED 4		
COP10–DET1–DDB1 (CDD) complex (DDB1, DAMAGED DNA BINDING PROTEIN 1)				
COP10	AT3G13550	CONSTITUTIVE PHOTOMORPHOGENIC 10	<i>cop</i> phenotype	[82,94]
DET1	AT4G10180	DE-ETIOLATED 1	<i>cop</i> phenotype	[81,95]
DDB1a	AT4G05420	DAMAGED DNA BINDING PROTEIN 1A	Wild-type phenotype, but enhances <i>cop</i> phenotype of <i>det1</i>	[8]
DDB1b	AT4G21100	DAMAGED DNA BINDING PROTEIN 1B	Embryonic lethal	
COP9 signalsome (CSN)				
CSN1	AT3G61140	COP9 SIGNALOSOME SUBUNIT 1	<i>cop</i> phenotype	[2]
CSN2	AT2G26990	COP9 SIGNALOSOME SUBUNIT 2	<i>cop</i> phenotype	
CSN3	AT5G14250	COP9 SIGNALOSOME SUBUNIT 3	<i>cop</i> phenotype	
CSN4	AT5G42970	COP9 SIGNALOSOME SUBUNIT 4	<i>cop</i> phenotype	
CSN5a	AT1G22920	COP9 SIGNALOSOME SUBUNIT 5a	<i>cop</i> phenotype in the <i>csn5a csn5b</i> double mutant	[2,96]
CSN5b	AT1G71230	COP9 SIGNALOSOME SUBUNIT 5b		
CSN6a	AT5G56280	COP9 SIGNALOSOME SUBUNIT 6a	<i>cop</i> phenotype in the <i>csn6a csn6b</i> double mutant	[2,96]
CSN6b	AT4G26430	COP9 SIGNALOSOME SUBUNIT 6b		
CSN7	AT1G02090	COP9 SIGNALOSOME SUBUNIT 1	<i>cop</i> phenotype	[2]
CSN8	AT4G14110	COP9 SIGNALOSOME SUBUNIT 1	<i>cop</i> phenotype	
Core CUL4–DDB1 ligase^c				
CUL4	AT5G46210	CULLIN4	<i>cop</i> phenotype in knockdown mutants	[79,85]
RBX1a	AT5G20570	RING-BOX 1a	Not tested in loss of function mutant; weak de-etiolation in overexpression lines	[97]
RBX1b	AT3G42830	RING-BOX 1b	Not tested	

^aThe COP1–SPA complex is likely to be a tetramer of two COP1 s and two SPA proteins.

^b*cop* phenotype: mutants undergo constitutive photomorphogenesis (*cop*) in darkness, which includes the development of a short hypocotyl and open cotyledons.

^cFor its component DDB1, please refer to the DDB1a/b entries under ‘CDD complex’.



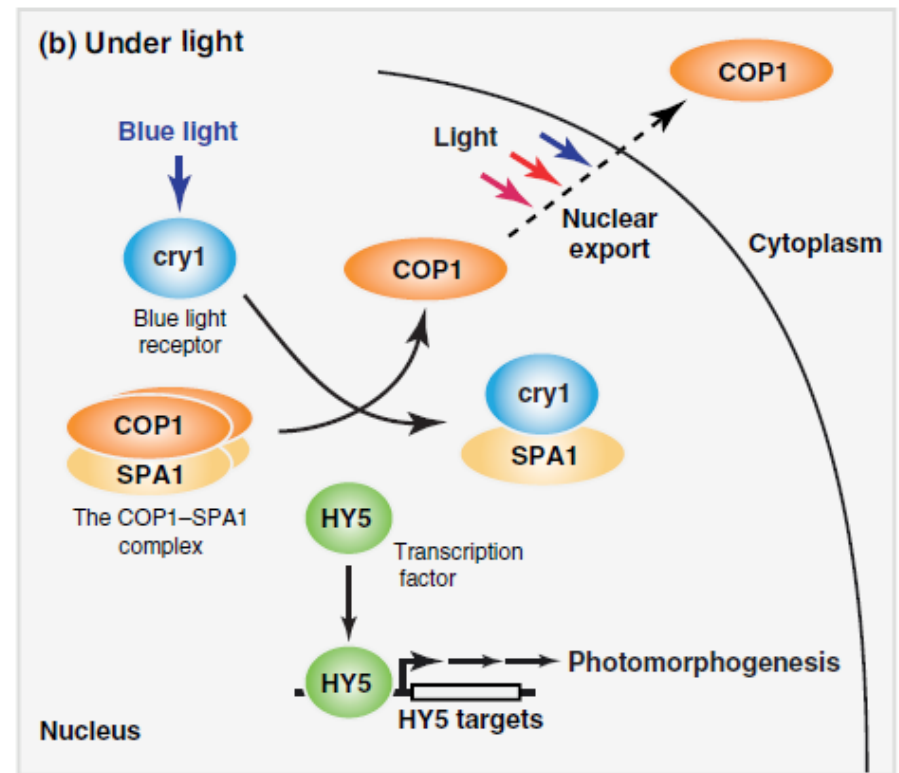
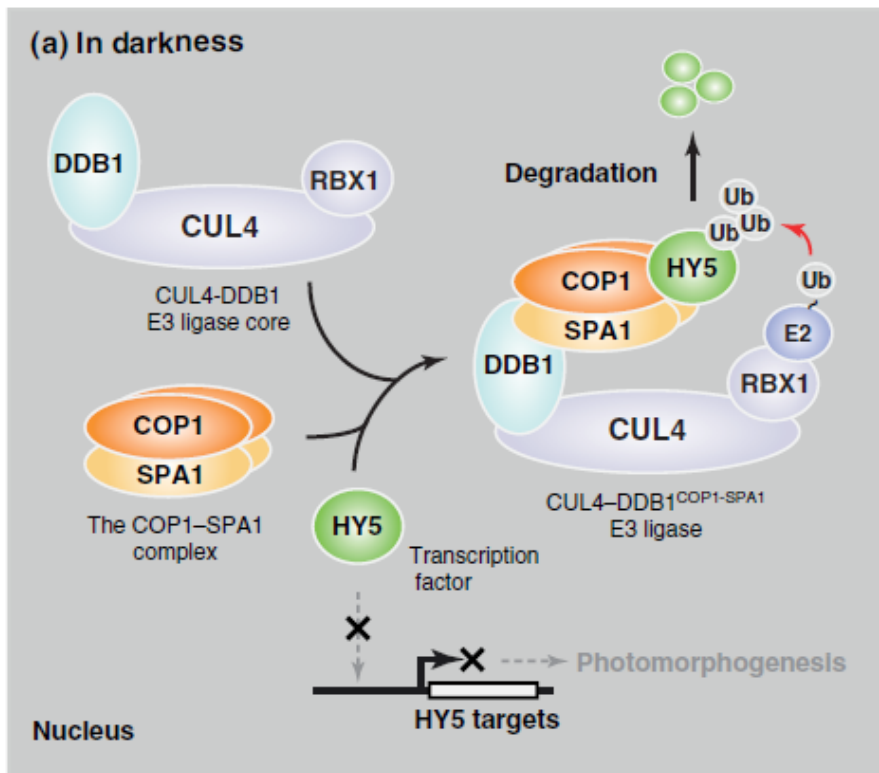
Stomatal opening and development, shade avoidance response, Plant defense, cold acclimation etc ...

COP1 targets various transcription factors critical for photomorphogenesis in the dark for ubiquitylation

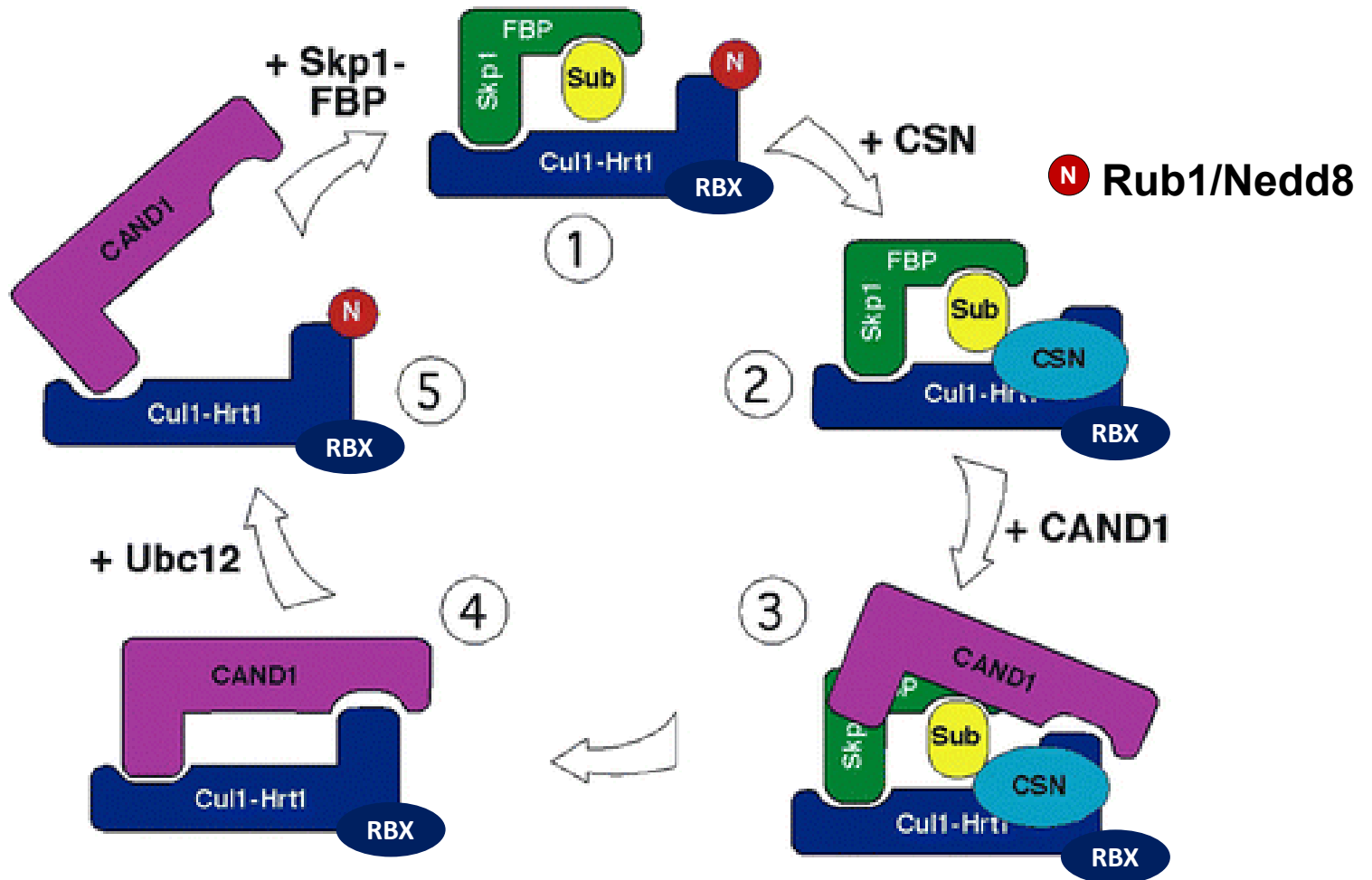
Table 2. Targets of the COP1 E3 ubiquitin ligase

Substrate	AGI code	Protein identity	Processes involved	Interaction with COP1
HY5	AT5G11260	bZIP transcription factor	Photomorphogenesis, light signaling	+
HYH	AT3G17609	bZIP transcription factor	Photomorphogenesis, light signaling	+
LAF1	AT4G25560	MYB transcription factor	Photomorphogenesis, light signaling	+
HFR1	AT1G02340	bHLH transcription factor	Photomorphogenesis, light signaling	+
BBX24/STO	AT1G06040	B-box Zinc Finger protein	Photomorphogenesis, light signaling	+
BBX4/COL3	AT2G24790	B-box Zinc Finger protein	Photomorphogenesis, light signaling	+
BBX22/LZF1/STH3	AT1G78600	B-box Zinc Finger protein	Photomorphogenesis, light signaling	Through HY5?
GATA2	AT2G45050	GATA transcription factor	Photomorphogenesis, light and brassinosteroid crosstalk	+
phyA	AT1G09570	Phytochrome	Light perception	+
phyB	AT2G18790	Phytochrome	Light perception	+
CO/BBX1	AT5G15840	B-box Zinc Finger protein	Flowering	+
GI	AT1G22770	Unknown protein	Circadian rhythm and flowering	Through ELF3
SCAR1	AT2G34150	SCAR family member	Root growth	+
HRT	AT5G43470	R protein	Plant defense	+

HY5, ELONGATED HYPOCOTYL 5; LAF1, LONG AFTER FAR-RED LIGHT, HFR1, LONG HYPOCOTYL IN FAR RED; BBXs, B-box Zinc Finger proteins; GATA2, a brassinosteroid (BR)-regulated GATA transcription factor



CRL assembly and disassembly is driven by neddylation/denannylation



CAND1, CULLIN-ASSOCIATED AND NEDDYLATION-DISSOCIATED 1

Cope and Deshaies 2003, Cell 114: 663-671