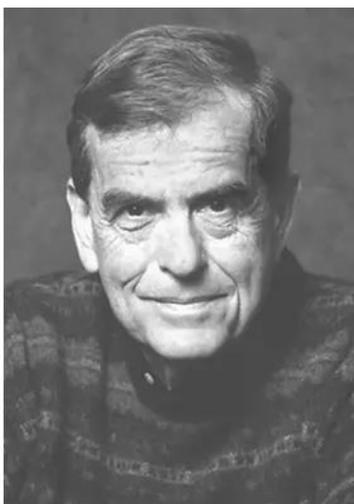


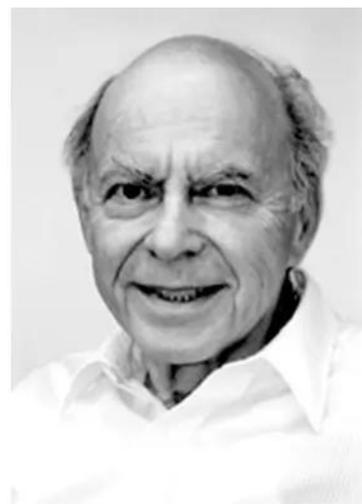
PROTAC简述 (一)



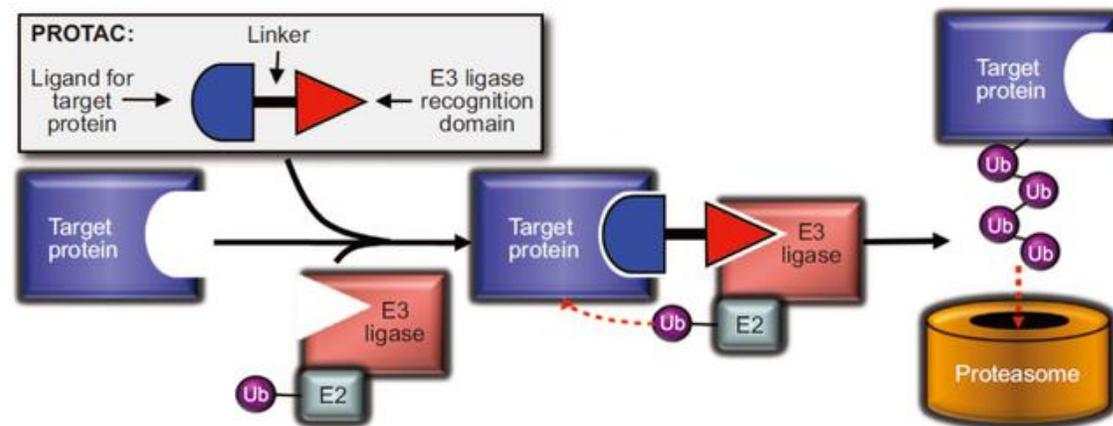
Aaron Ciechanover
(1947-)



Avram Hershko
(1937-)



Irwin Rose
(1926-2015)



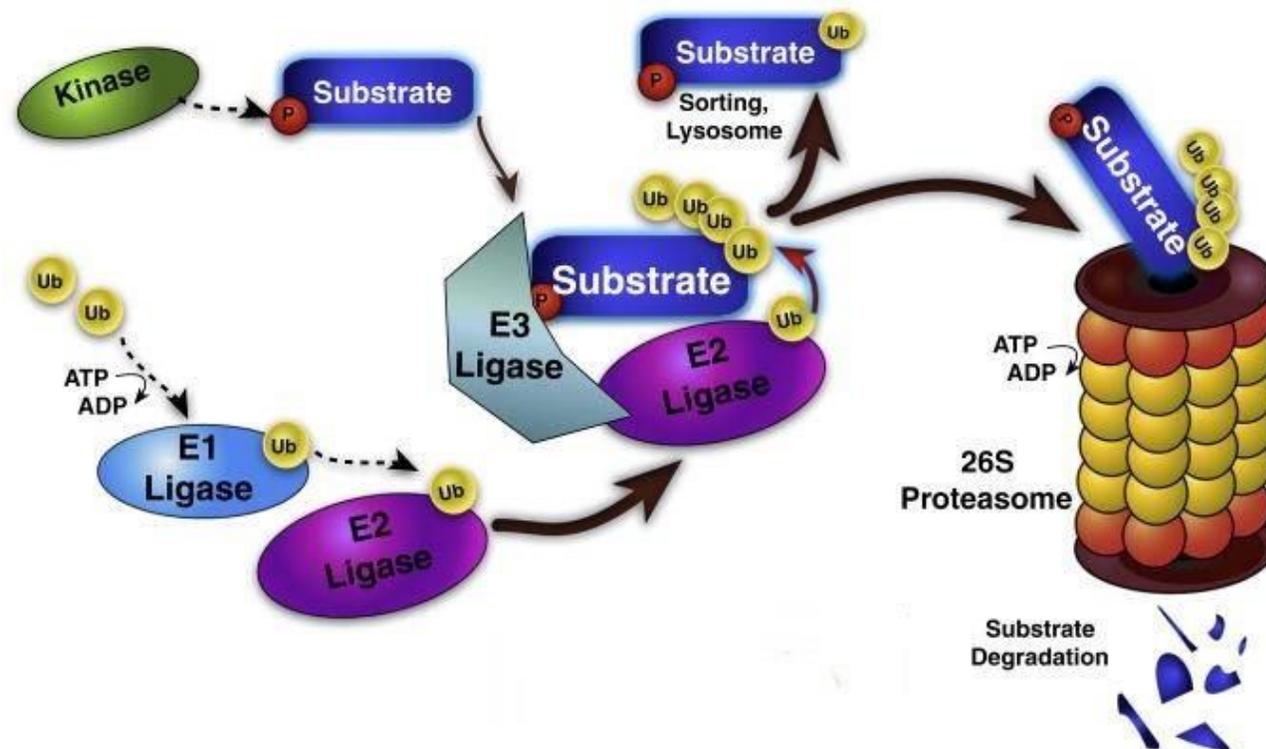
2004年诺贝尔化学奖得主

泛素——蛋白酶体系统的发现历程

PROTAC概念的提出

PROTAC的应用

PROTAC的机遇与挑战



泛素——蛋白酶体系统的发现历程



1948
芝加哥大学获得了学
士学位

1953
Severo Ochoa (1959年诺贝
尔生理学或医学奖获得者)
的指导下完成了博士后的工作

1975
在一次会议上, 遇见了以
色列科学家Avram
Hershko

1926

1952

1954

1977

Irwin Rose出生于纽
约布鲁克林的一个
犹太人家庭

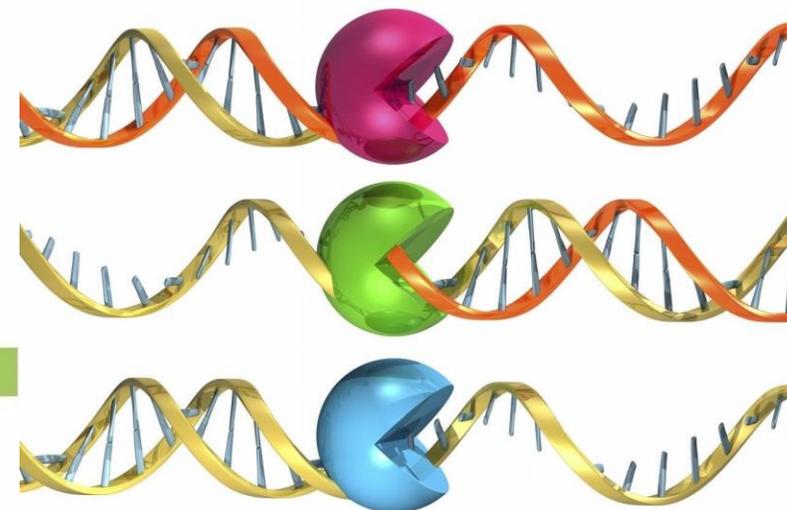
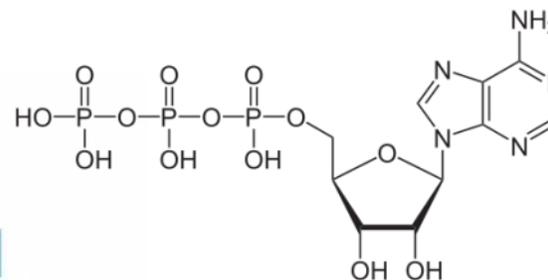
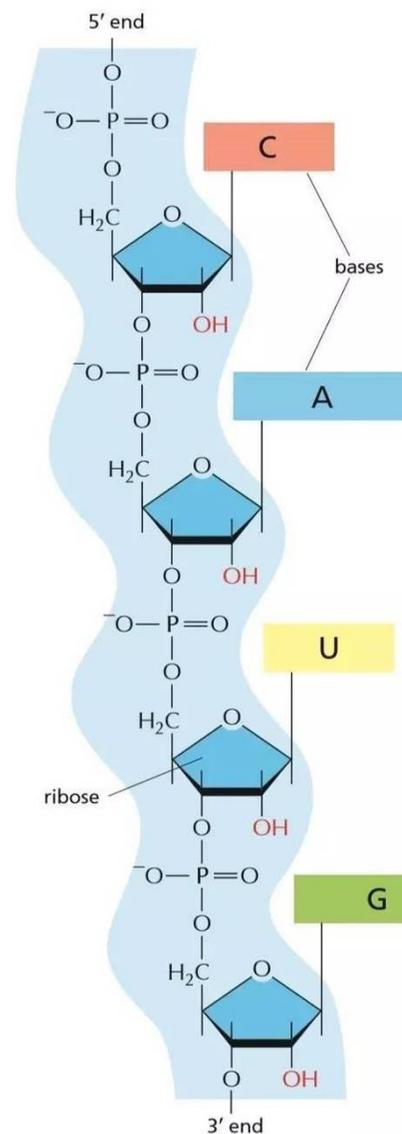
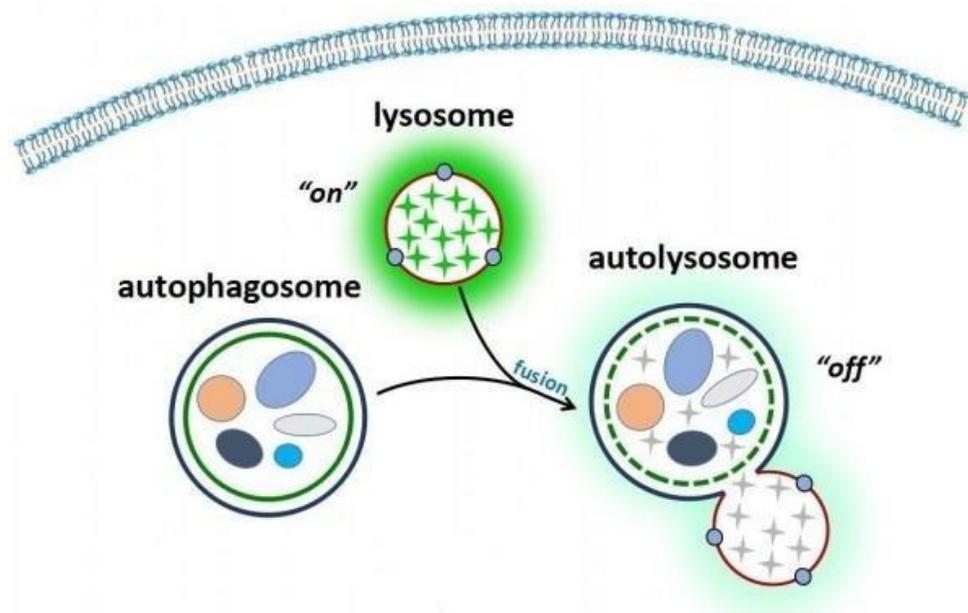
芝加哥大学获得了博士学位,
并对酶的催化机制产生了兴
趣

耶鲁大学药学院
生物化学讲师
与Melvin Simpson成为
同事

Irwin Rose
Avram Hershko
Aaron Ciechanover
于费城全面展开蛋白质降
解的工作

郭晓强.蛋白质泛素化发现者——罗斯[J]. 自然杂志,2016,38(05):387-390.

泛素——蛋白酶体系统的发现历程



√ 肽键的水解在热力学上是放热反应。

√ 已知的溶酶体降解蛋白途径不需要ATP。

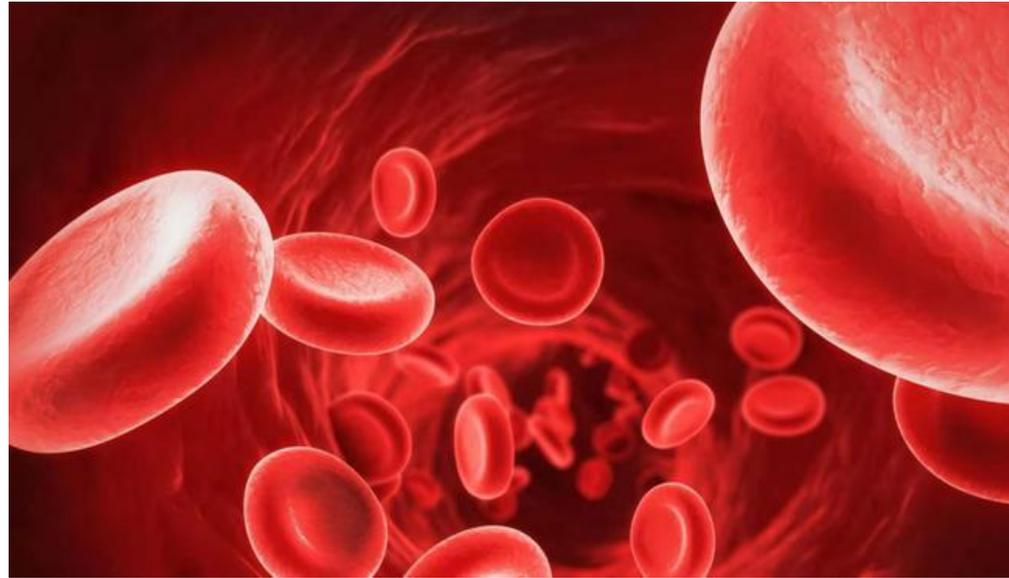
Neuron 2005, 45(3): 339-344.

1. 细胞内是否存在不依赖溶酶体的蛋白质降解途径？
2. 蛋白质降解是否都不需要能量？



Alfred L. Goldberg

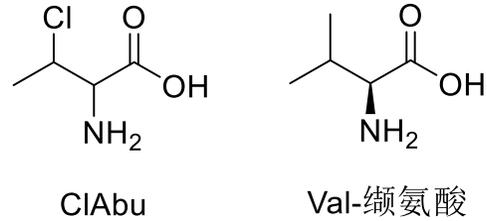
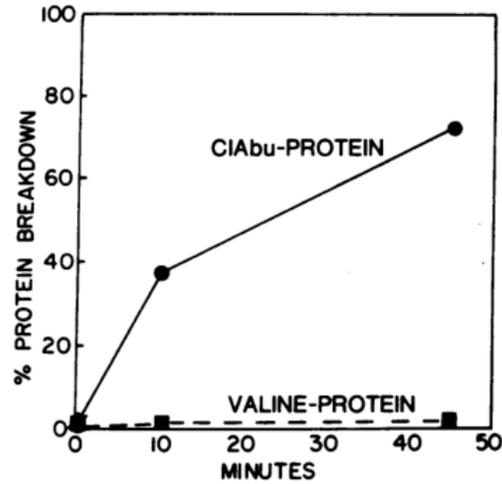
哈佛大学医学院



网织红细胞

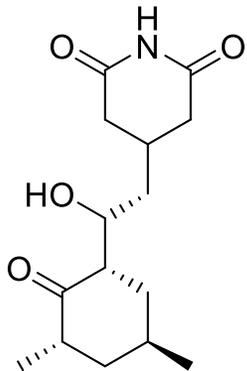
哺乳动物的
成熟红细胞
不含细胞器！

泛素——蛋白酶体系统的发现历程



在**无溶酶体**的情况下，
蛋白质也可以降解

reduced (Fig. 2). The inhibition of proteolysis by ATP depletion in these cells is not secondary to a requirement for protein synthesis, because the rate of degradation of ClAbu-containing protein was not affected by addition of 0.5 mM cycloheximide.



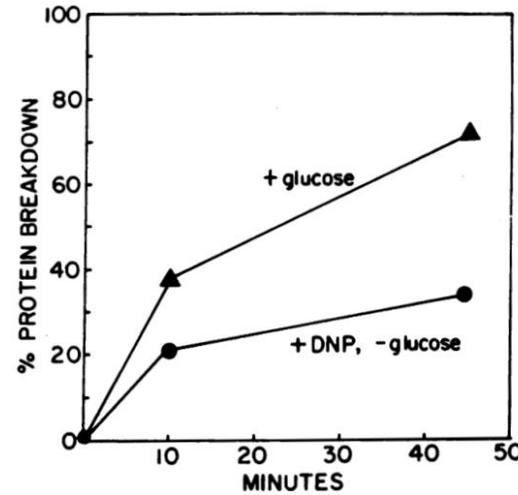
蛋白质含量的下降，是否是因为抑制了蛋白质合成？

放线菌酮

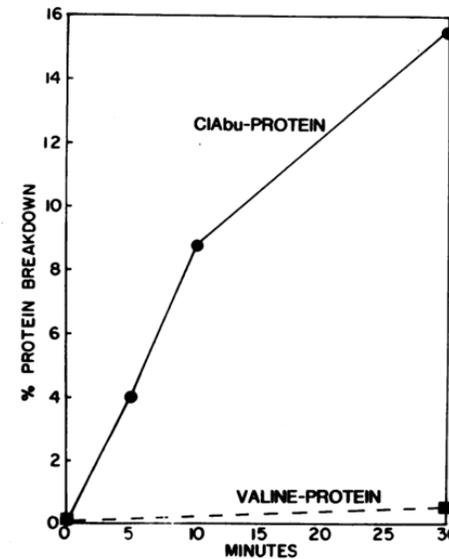
用途  百科

蛋白质生化合成抑制剂。

Proc Natl Acad Sci USA, 1977, 74(3): 54-58.



在**缺少葡萄糖**供给的情况下，
错误合成的蛋白质降解速度明显**下降**



在**无细胞裂解物**条件下，
发现，ATP（内源）确实可以**加快**错误合成蛋白质的降解速度

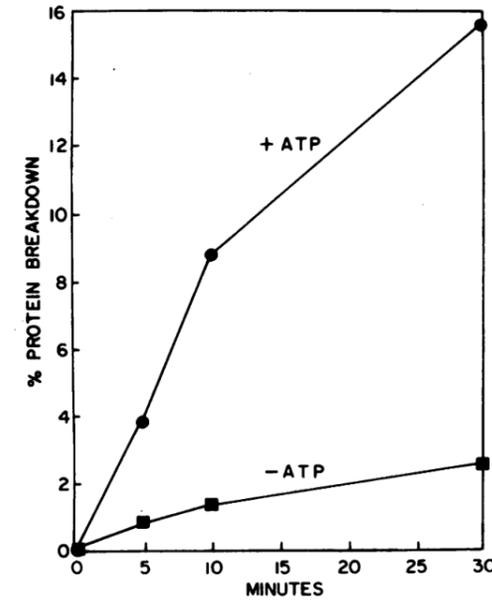
FIG. 3. Cell-free degradation of proteins containing ClAbu (●) or valine (■). Prior to lysis, reticulocytes were incubated in the presence of [¹⁴C]leucine and either valine or ClAbu. ATP (1 mM) was present in these incubations. Similar results were obtained with five independent preparations.

泛素——蛋白酶体系统的发现历程

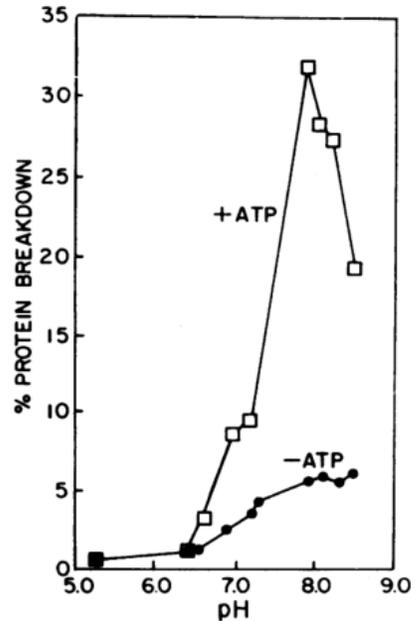
Table 1. Effects of nucleotides and ATP analogs on the hydrolysis of ClAbu-containing protein in cell-free extracts

Experiment	Nucleotide (1 mM)	% Stimulation
1	ATP	189
	ADP	21
	AMP	8
	cAMP	5
	cAMP + ATP	195
2	ATP	384
	α,β -methylene ATP	57
	β,γ -methylene ATP	13

验证是否是ATP的供能促进了蛋白质的降解。通过验证ATP的代谢产物或者类似物引起蛋白质降解均不明显。



ATP (外源) 的加入, 可以促进错误合成蛋白质的降解。



ATP (外源) 促进错误合成蛋白质的降解, 在弱碱性条件下效果最佳 (PH \approx 7.8)。

✓ ATP正常水解PH \approx 7.4

✓ 溶酶体最佳水解条件多为弱酸性

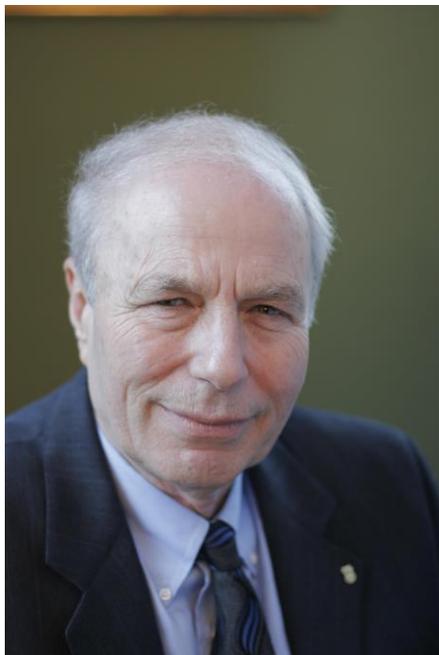
Table 2. Relative susceptibility of different polypeptides to degradation in reticulocytes and in cell-free extracts

Substrate	Reticulocytes (% protein breakdown/45 min)	Cell-free extracts (% protein breakdown/30 min)
Puromycyl-polypeptides	81	19
Protein containing ClAbu	70	18
Protein containing S-(β -aminoethyl)cysteine	16	3
Protein containing valine	3	1

无细胞裂解物的蛋白质降解情况与正常细胞无明显差异

泛素——蛋白酶体系统的发现历程

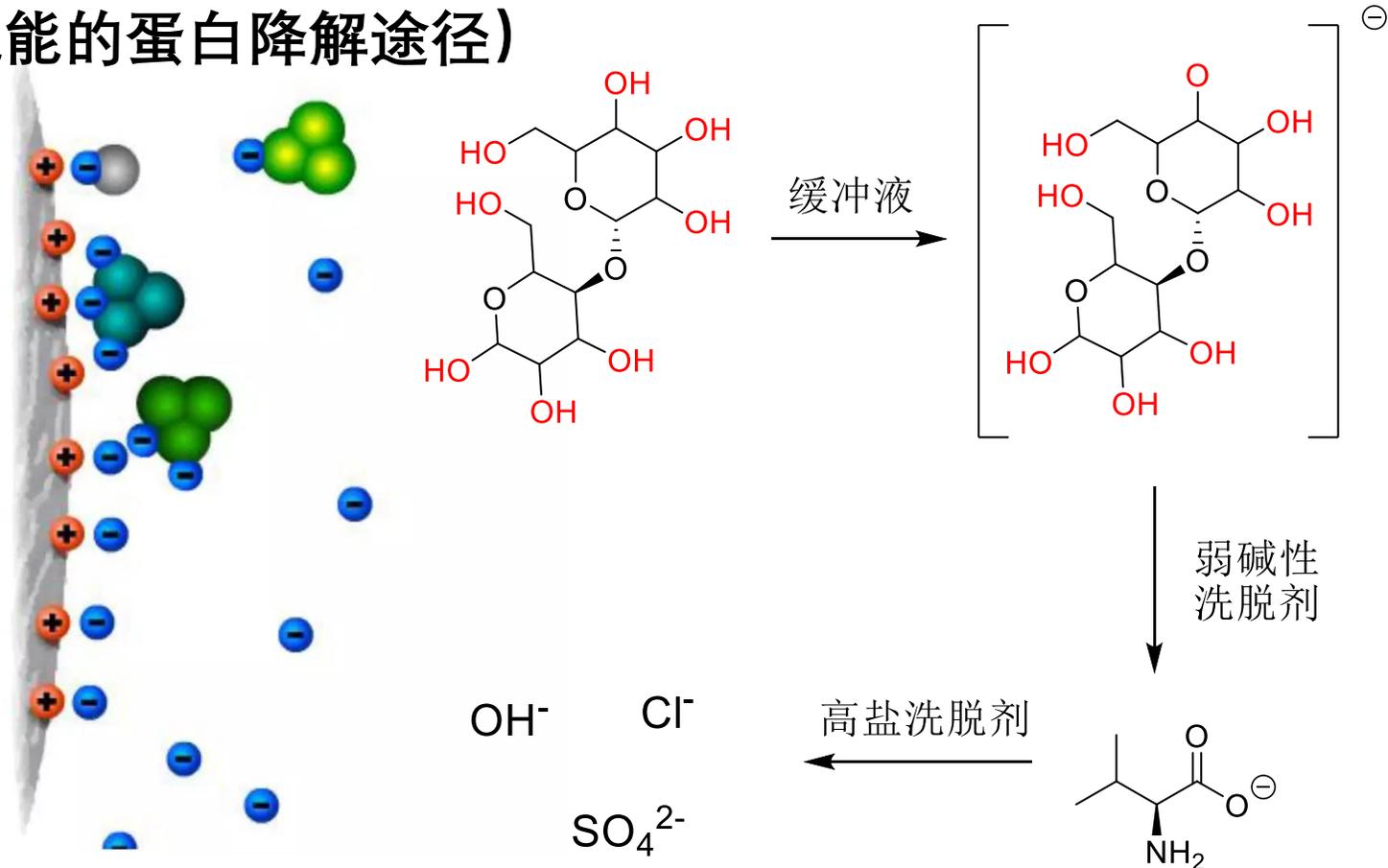
具体机制？（不依赖溶酶体、需要耗能的蛋白降解途径）



Avram Hershko



Iwrin Rose



DEAD纤维素离子交换树脂

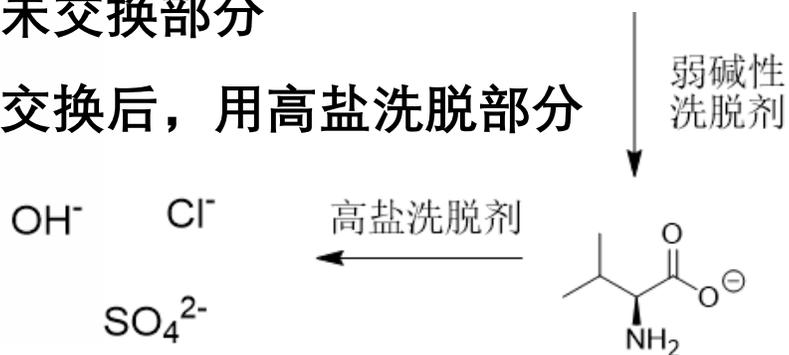
泛素——蛋白酶体系统的发现历程

TABLE 1: Resolution of the ATP-Dependent Cell-Free Proteolytic System Into Complementing Activities

Enzyme fraction	Degradation of [³ H]globin percent/h	
	-ATP	+ATP
lysate	1.5	10.0
fraction I	0	0
fraction II	1.5	2.7
fraction I and fraction II	1.6	10.6

Fraction I 未交换部分

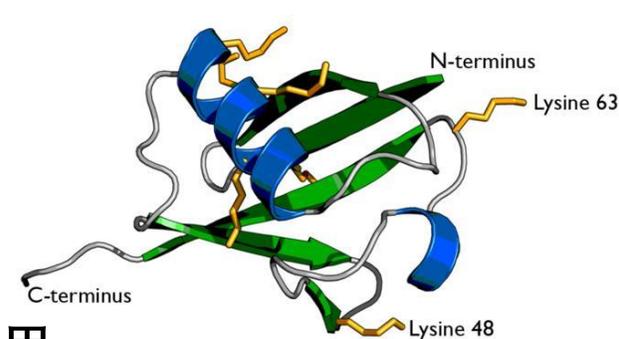
Fraction II 交换后, 用高盐洗脱部分



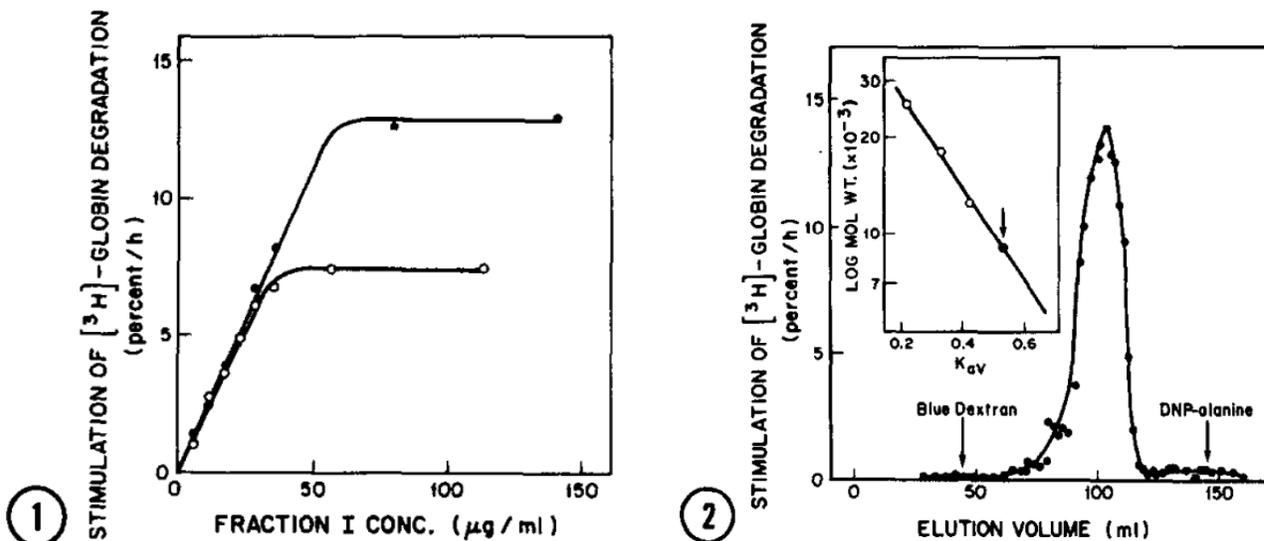
- √ 纯裂解物, 加入ATP, 蛋白降解能力增加。
- √ Fraction I 无论ATP存在与否都不能降解蛋白。
- √ Fraction II 单独可降解蛋白, ATP加入后, 降解能力增加。
- √ Fraction I & II 在加入ATP后, 降解能力提升至与裂解液几乎同一水平。

Fraction I 发挥**辅酶**作用

Fraction II 发挥**水解酶**作用



泛素——蛋白酶体系统的发现历程



√ Fraction I 促进 II 的蛋白降解能力。

6.2%/hr. Insert: Estimation of molecular weight. The Sephadex-G-75 column was calibrated with the following marker proteins (open circles): α -chymotrypsinogen (mol. wt. 25,000); myoglobin (mol. wt. 17,800); and cytochrome C (mol. wt. 12,100). The closed circle indicates the elution position of heat-stable polypeptide activity.

√ 只能用洗脱剂和洗脱时间来说明Fraction I 中分离的物质。

√ Fraction I 相对分子量 ≈ 9.0 kDa。8.0kDa以下认为是多肽。

Biochem. Biophys. Res. Commun. 1978, 81, 1100-1105.

TABLE 2: Properties of Fraction I

Treatment of fraction I	Increase of ATP-dependent proteolysis	
	$[^3\text{H}]$ globin degraded (percent/h)	% of control
Experiment 1		
Control	6.6	100
Heated 96°C, 15 min	6.3	95
Heated 96°C, 60 min	4.6	70
Dialyzed (20 h)	6.7	102
Pronase-treated	0	0
Chymotrypsin-treated	0	0
Experiment 2		
Control	4.7	100
$(\text{NH}_4)_2\text{SO}_4$ precipitate	4.1	87
$(\text{NH}_4)_2\text{SO}_4$ supernatant	0	0

√ Fraction I 热稳定性强 (很特殊的性质)。

√ Fraction I 是蛋白 (不能透过半透膜、蛋白酶可破坏其活性、盐析沉淀)。

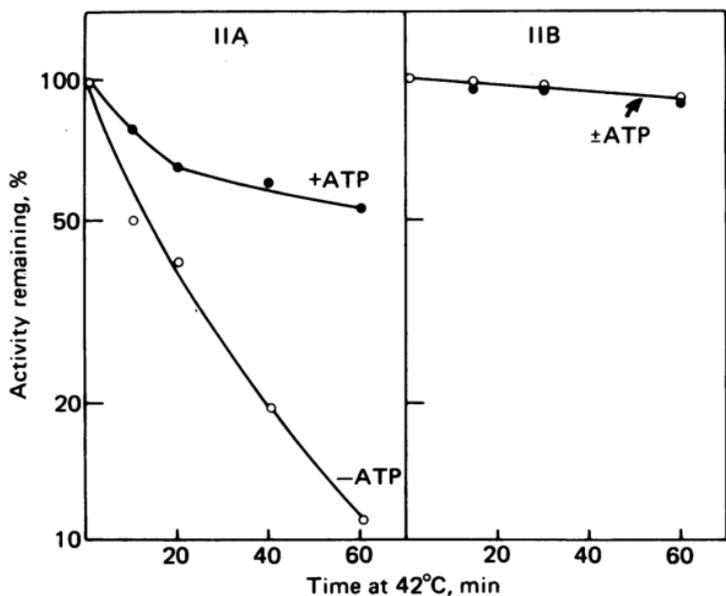
√ Fraction II 稳定性差 (42°C即可失活)。

泛素——蛋白酶体系统的发现历程

Fraction II 的组分?

Table 1. Separation of fraction II into complementing activities

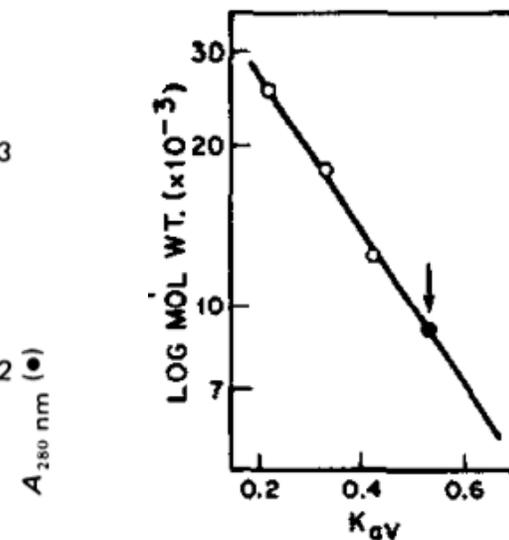
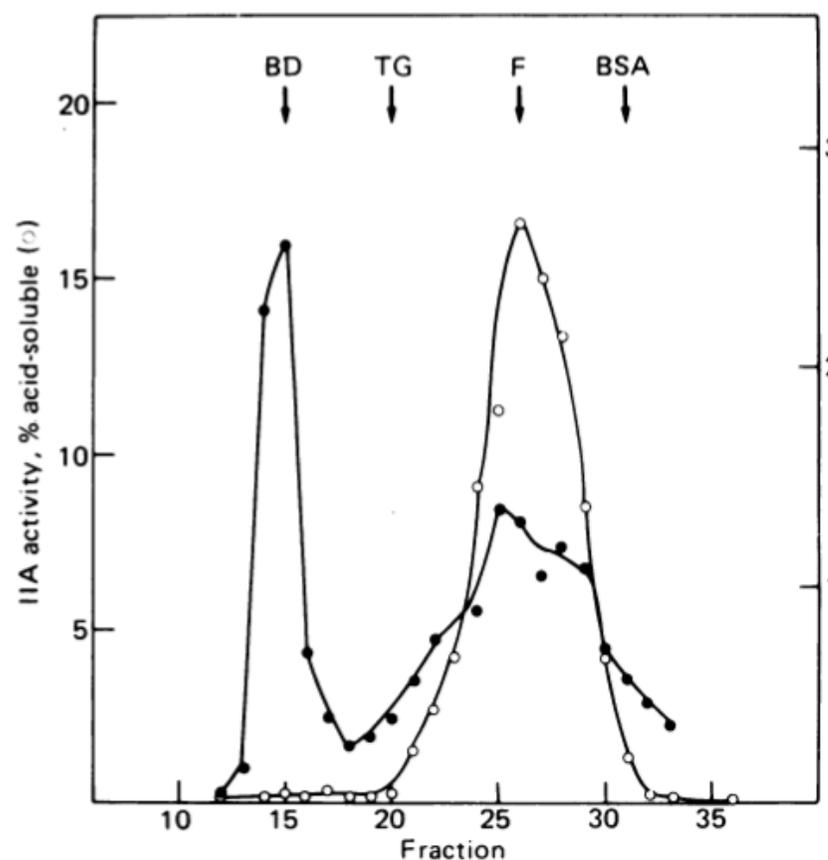
Fraction added			Degradation of ¹²⁵ I-albumin, %	
IIA, μl	IIB, μl	APF-1	-ATP	+ATP
10	—	+	0.3	0.7
—	10	+	1.3	0.7
2	10	+	—	11.7
10	5	+	—	13.0
10	10	+	0.2	21.1
10	10	—	—	1.4



Proc Natl Acad Sci USA. 1979, 76, 3107-3110.

Fraction II 稳定性差是限制鉴定的主要原因。

重要突破: 在洗脱剂中加入ATP可以, 稳定Fraction II。



√ Fraction II A 的分子质量
≈45kDa, Fraction I ≈9kDa

√ Fraction I、II A、II B共同
发挥作用

泛素——蛋白酶体系统的发现历程

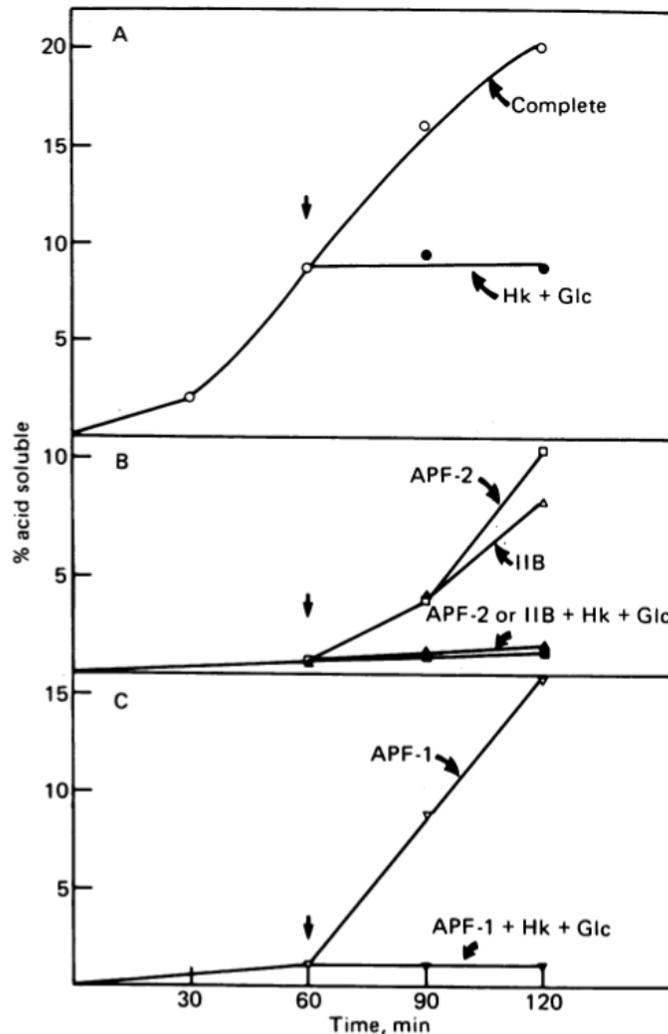
ATP是通过**稳定**Fraction II 发挥的作用，还是释放能量？ **(至关重要)**

Table 2. Nucleotide specificity of the stabilization of APF-2

Addition	Relative activity	
	Stimulation of protein breakdown	Stabilization of APF-2
ATP	1.00	1.00
ATP, -Mg ²⁺	0	0.98
ADP	0	0.56
ADP + creatine- <i>P</i> + kinase	1.57	—
AMP	0	0
AdoPP[NH]P	0	0.53
AdoPP[CH ₂]P	0	0.54
GTP	0	0
CTP	0	0
Creatine- <i>P</i>	0	0

√ ADP、AdoPP[NH]P、AdoPP[CH₂]P均有**稳定**APF-2的作用，但是**不具备**促进蛋白水解的功能。

Proc Natl Acad Sci USA. 1979, 76, 3107-3110.



√ 消耗ATP，会使蛋白质的降解速度显著下降。

√ APF-1、APF-2、APF- IIB均发挥重要作用。

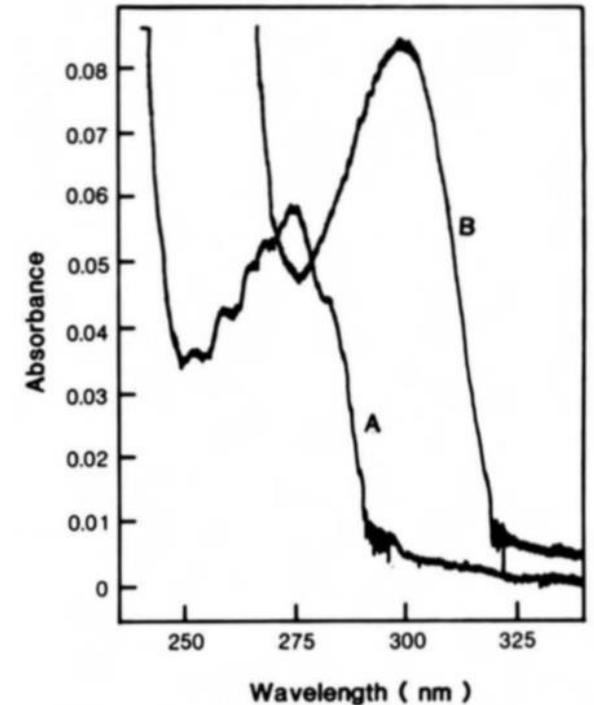
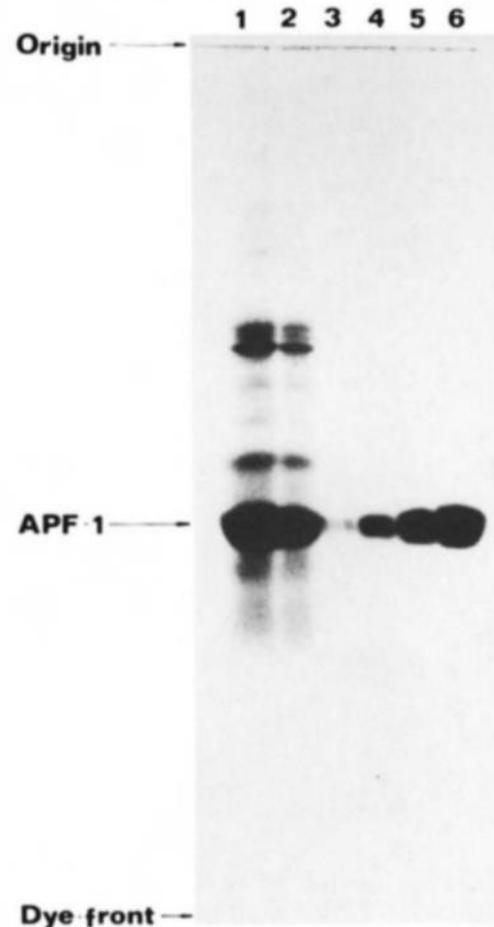
√ ATP既稳定APF-2的作用，又可以促进蛋白质降解。

泛素——蛋白酶体系统的发现历程

具体研究APF-1、APF-2、APF- II B的作用 (APF-1的纯化)

TABLE I
Purification of heat-stable polypeptide of the ATP-dependent proteolytic system from rabbit reticulocytes

Fraction	Total protein	Total activity	Spe- cific activity	Purifi- cation	Yield
	mg	units	units/mg	-fold	%
1. Fraction I ^a	43,100	33,900	0.78	1	100
2. First heat treatment	174	28,600	164	210	84
3. (NH ₄) ₂ SO ₄ precipita- tion, dialysis and sec- ond heat treatment	35.3	20,500	582	746	60
4. Sephadex G-75	8.1	8,000	990	1,270	24
5. CM-Sephadex	4.1	6,500	1,590	2,040	19



to us by Drs. Ciechanover and Hershko prior to publication, it was apparent that APF-1 was very similar to another small, heat-stable polypeptide termed ubiquitin.

√ APF-1纯化了2000倍。

√ Western blot 结果和紫外吸收结果，验证 APF-1的纯度，也进一步支持了APF-1是蛋白。

√ APF-1与之前报道过的泛素部分性质类似。（分子量、氨基酸种类和数量）

具体研究APF-1的作用

Ubiquitin was first isolated in the search for thymic polypeptide hormones. Such hormones were thought to exist since thymus extracts (12) were able to restore immunological competence to athymic animals. Several polypeptides have been

√ 泛素最初在胸腺中提取，发现与免疫功能相关。

yeasts, and bacteria (15, 19–22). Recent work demonstrates that the chromosomal protein A-24 consists of ubiquitin covalently bound through its COOH terminus to an ϵ -amino group of histone H2A (19, 20). This isopeptide linkage is

√ 泛素和A-24蛋白是共价结合 (-CO-NH-)
(对APF-1后续的研究颇有启示)。

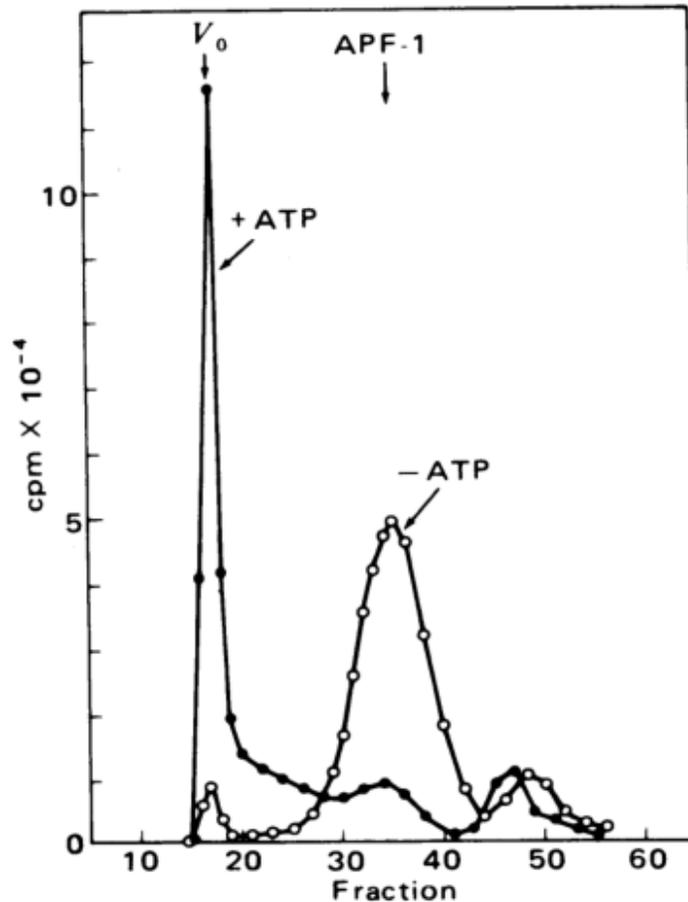
Proc Natl Acad Sci USA. 1966, 66, 1010-1017.

Proc Natl Acad Sci USA. 1977, 74, 864-868.

Proc Natl Acad Sci USA. 1980, 77, 1365-1368.

泛素——蛋白酶体系统的发现历程

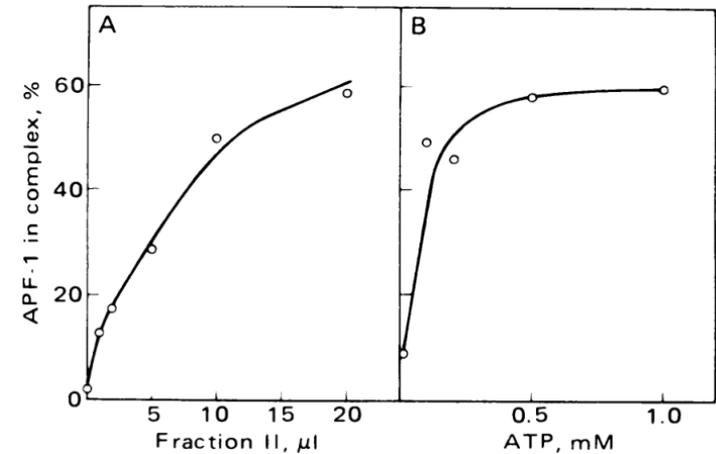
具体研究APF-1的作用



- √ 在没加入ATP的情况下，APF-1出现了与蛋白结合分子量增加的情况。
- √ 在加入ATP的后，APF-1又重新出现在了低质量区。



APF-1与蛋白可能是共价结合
(突破性进展)



√ APF-1与蛋白质的结合，同时需要ATP和Fraction II。

Table 1. Effects of nucleotides and inhibitors on the binding of APF-1

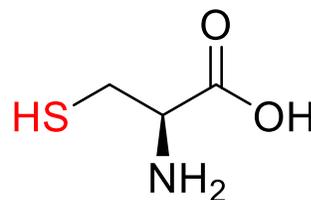
Addition		APF-1 bound, %
Experiment 1		
ATP		74.7
GTP		17.8
UTP		17.4
Experiment 2		
ATP		78.6
ATP plus hexokinase and glucose		14.1
ATP minus Mg ²⁺		14.0
ATP plus EDTA (5 mM)		6.6
ATP plus N-ethylmaleimide (5 mM)		18.3

泛素——蛋白酶体系统的发现历程

具体研究APF-1的作用

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ATP plus <i>N</i> -ethylmaleimide (5 mM)		18.3



半胱氨酸

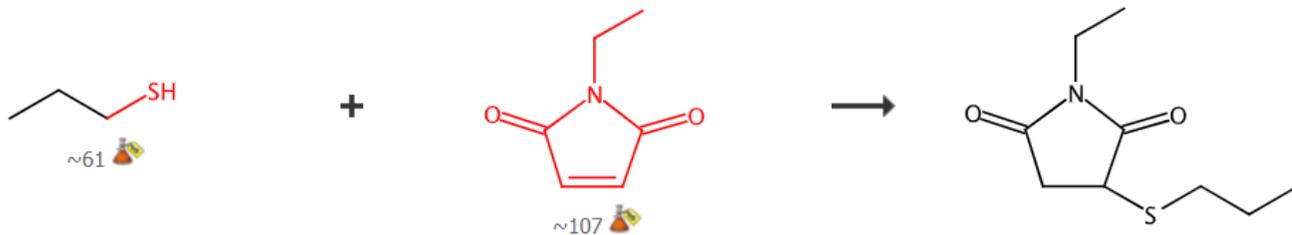


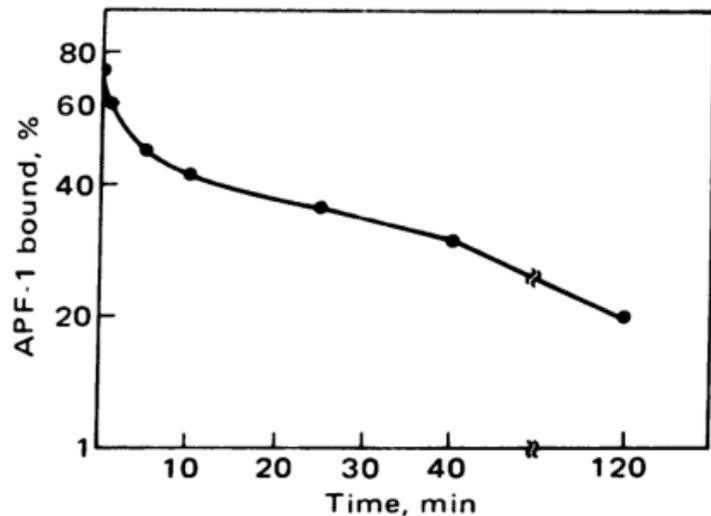
TABLE I
Amino acid analysis of APF-1 and ubiquitin

	Residues per molecule				Ratio APF-1/Ubiquitin
	APF-1		Ubiquitin		
	Literature ^a	Found ^b	Literature ^c	Found ^b	
Asp	7	8.51	7	8.22	1.04
Thr	7	7.46	7	7.80	0.96
Ser	3	3.50	3	3.29	1.06
Glu	12, 13	13.77	12	13.66	1.01
Pro	3	— ^d	3	— ^d	— ^d
Gly	6	6.20	4	5.83	1.06
Ala	2	2.60	2	2.53	1.03
½ Cys	0	— ^d	0	— ^d	— ^d
Val	4	3.71	4	4.08	0.91
Met	1	0.47	1	0.56	0.84
Ile	7	6.91	7	7.37	0.94
Leu	9	9.74	9	9.68	1.01
Tyr	1	0.92	1	0.98	0.94
Phe	2	1.83	2	2.01	0.91
Lys	7	6.77	7	7.13	0.95
His	1	1.10	1	1.02	1.08
Arg	4	3.69	4	4.35	0.85
Trp	0	— ^d	0	— ^d	— ^d
					0.971 ± .076

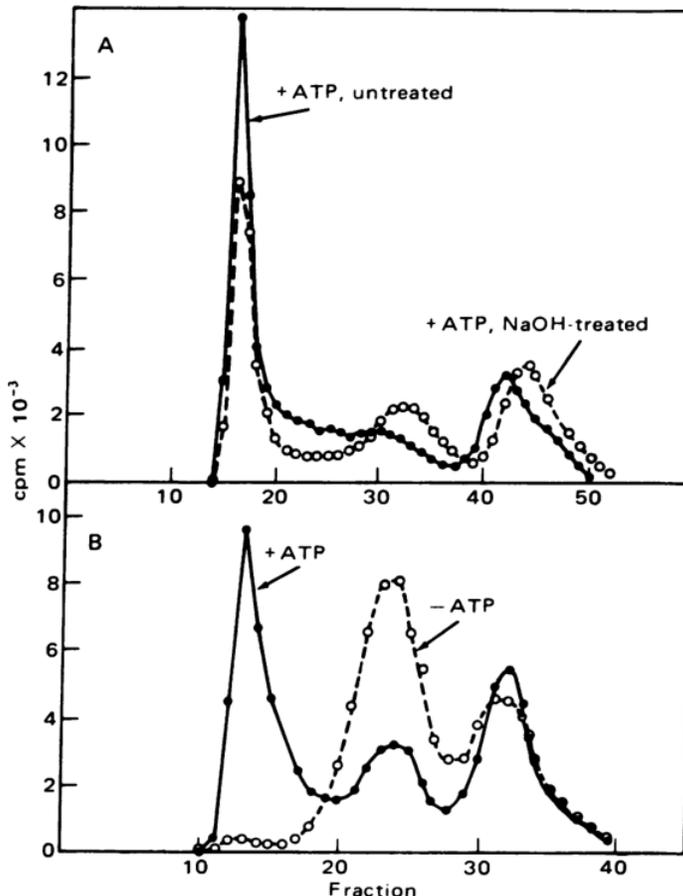
✓ APF-1与蛋白结合可能与巯基有关 (突破性进展)

✓ 蛋白质上的巯基与APF-1发生了反应 (突破性进展)

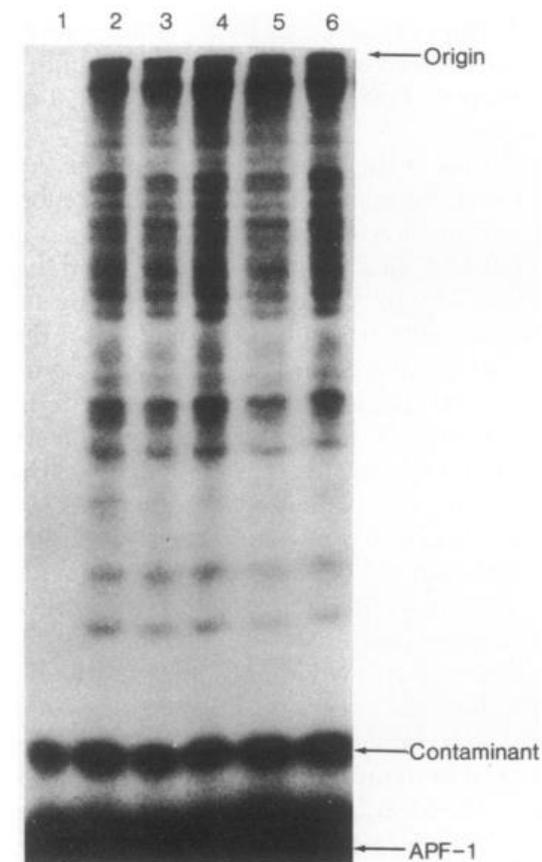
泛素——蛋白酶体系统的发现历程



√ 降解速度变化明显，最初 $t_{1/2} \approx 1\text{min}$ ，最合理的解释是 APF-1 与多种蛋白结合。



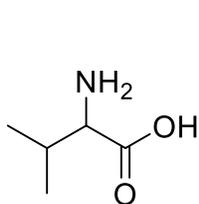
√ 碱性不会影响结合，大概率不是氢键的作用。



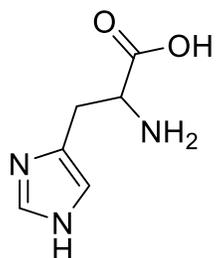
√ 100°C加热后，APF-1仍然与多种蛋白结合。（最合理的解释就是共价结合）

泛素——蛋白酶体系统的发现历程

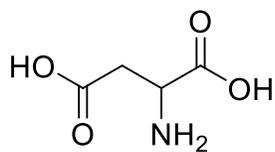
—SH



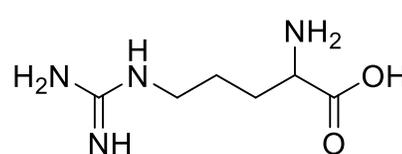
缬氨酸



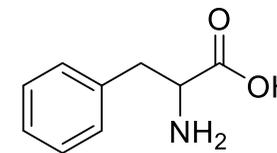
组氨酸



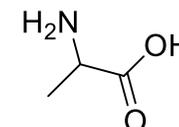
天冬氨酸



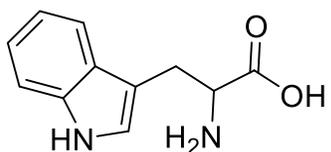
精氨酸



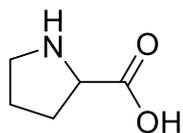
苯丙氨酸



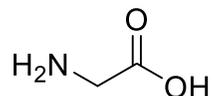
丙氨酸



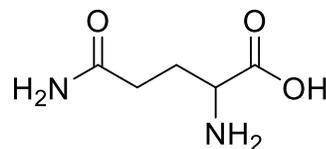
色氨酸



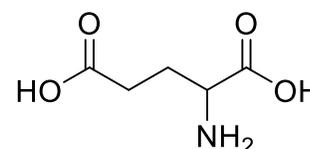
脯氨酸



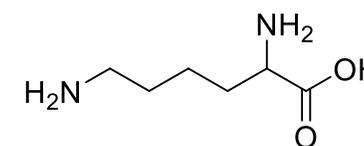
甘氨酸



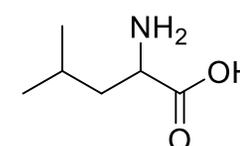
谷氨酰胺



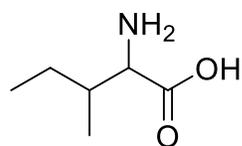
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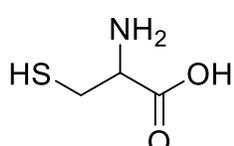
赖氨酸



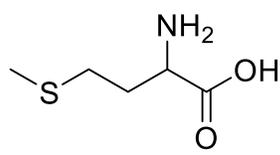
亮氨酸



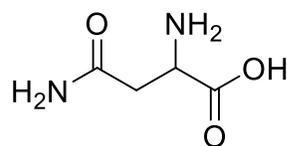
异亮氨酸



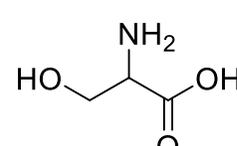
半胱氨酸



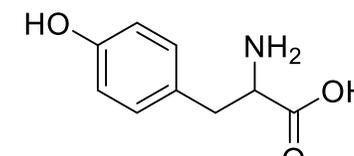
甲硫氨酸



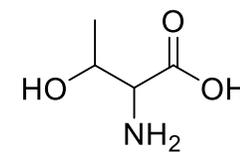
天门冬氨酸



丝氨酸

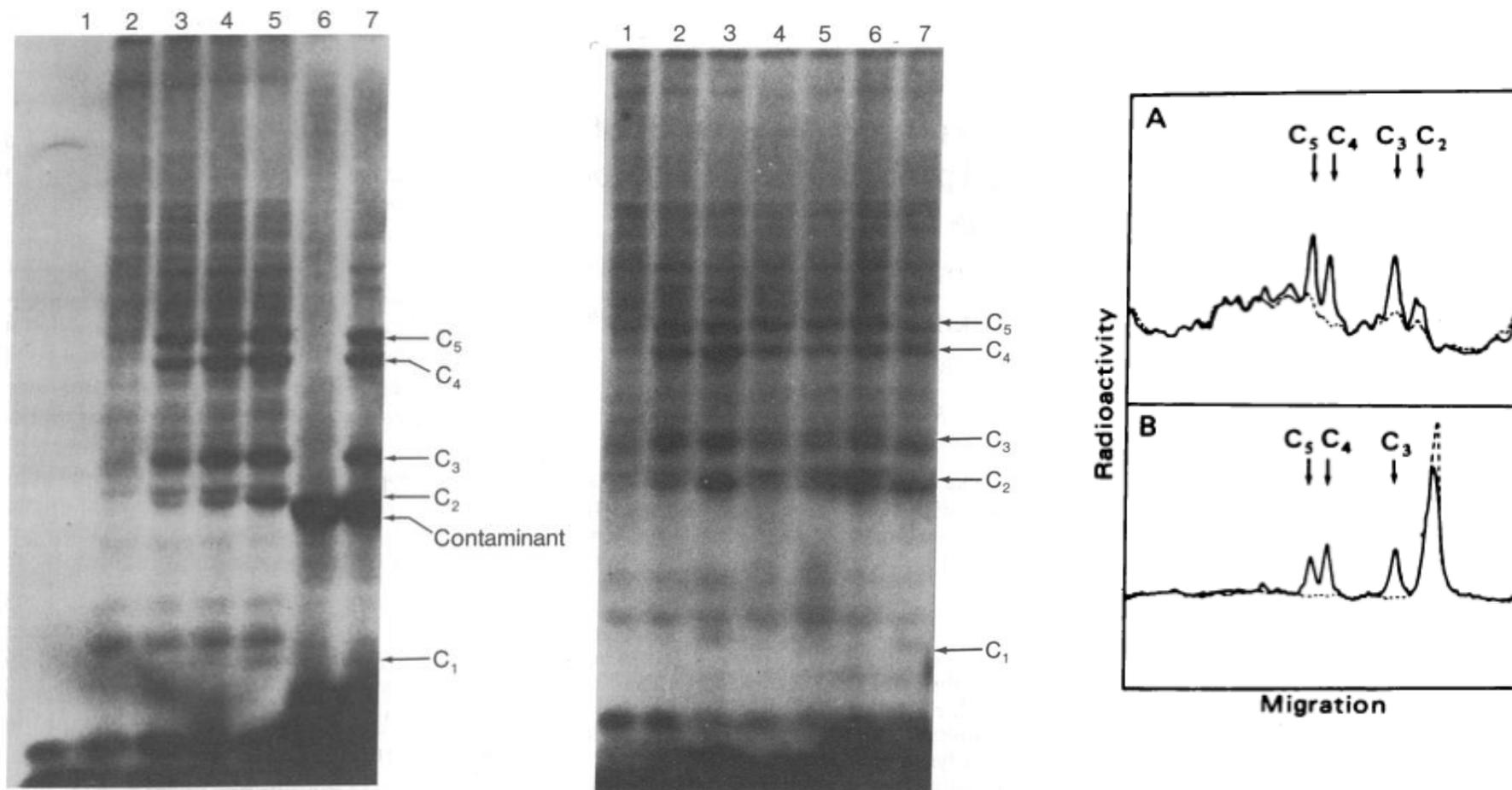


酪氨酸



苏氨酸

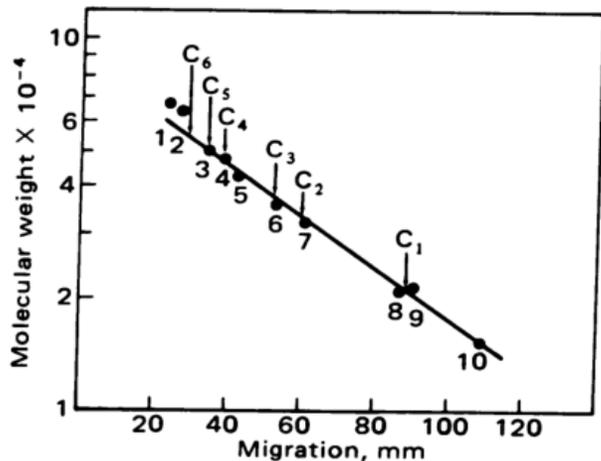
APF-1共价结合的具体方式研究



√ 同一蛋白与APF-1结合，可产生**多个**条带。

最可能的解释是，单个蛋白与多个APF-1结合？

泛素——蛋白酶体系统的发现历程



√ 用已知的纯品蛋白，
制定标准曲线。

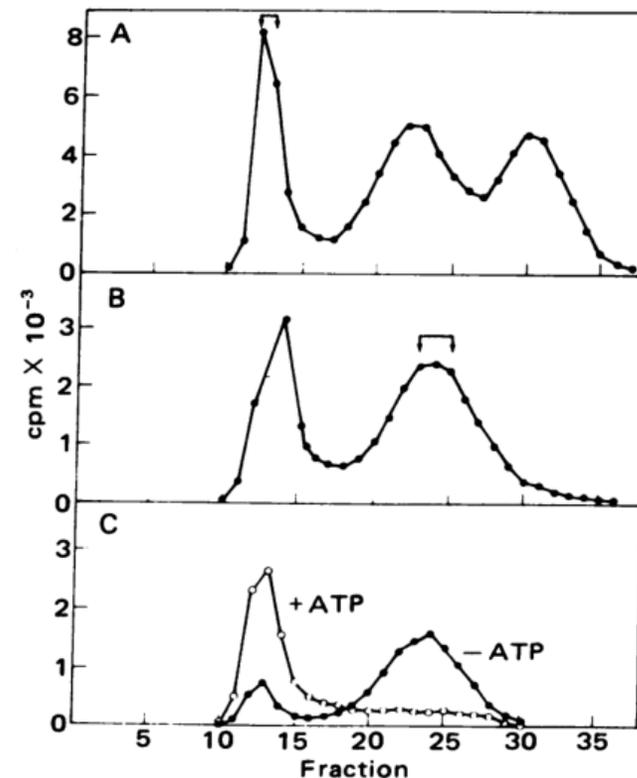
√ 测量C1~5的分子量。

Table 2. Estimation of relative radioactivities and molecular weights of APF-1-lysozyme compounds

Com- pound	Radioactivity relative to C ₃ *			Molecular weight	
	¹²⁵ I-		Ratio (A/B)	Ob- served [†]	Calcu- lated [‡]
	APF-1 (A)	lysozyme (B)			
C ₁				21,000	22,500
C ₂				34,000	30,500
C ₃	1.00	1.00	1.00	38,000	38,500
C ₄	1.11	0.87	1.28	47,000	46,500
C ₅	1.11	0.63	1.76	50,000	54,500

√ 根据分子量的差异 (APF-1≈8000)
和辐射强度变化的比例，确定了一分
子蛋白可以与多个APF-1结合。

APF-1是可以催化循环的？还是一次
性催化？



√ 催化蛋白降解一次的APF-1，仍然
可以继续与蛋白结合。

√ 结合后，仍然具有催化活性。

泛素——蛋白酶体系统的发现历程

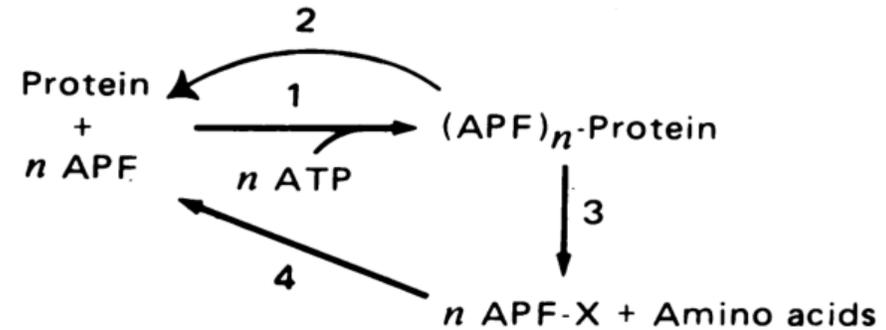
Table 3. Covalent APF-1 adducts with polylysine

Additions	¹²⁵ I-APF-1 retained on CM-cellulose, %
None	1.0
ATP (2 mM)	2.4
Polylysine (1 mg/ml)	3.2
ATP + polylysine	10.0

√ 在PH9.0的条件下，抚育20h仍然稳定，让作者认为是形成了酰胺键。

√ 多聚赖氨酸作为底物，也观察到了结合现象。作者认为是蛋白质中的赖氨酸参与形成了酰胺键。

Proc Natl Acad Sci USA, 1980, 77, 1783-1786.



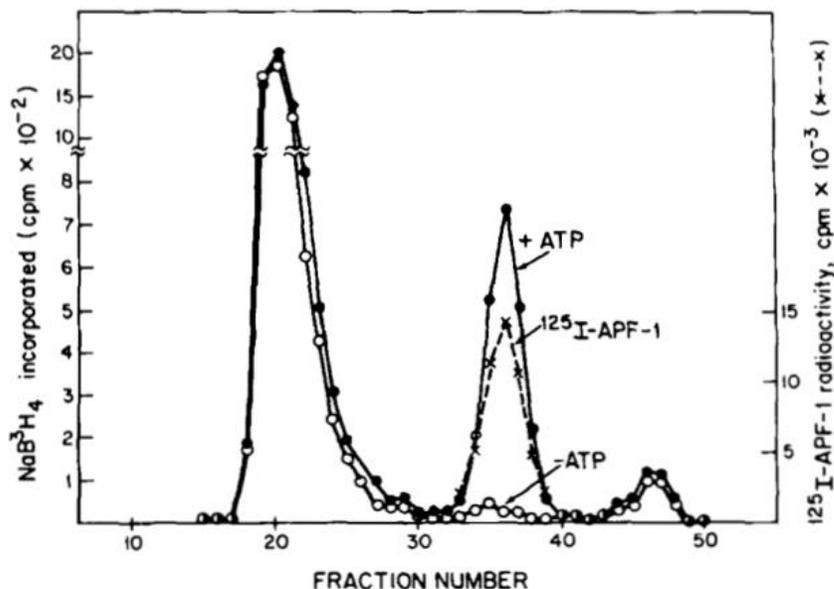
√ 最后提出了APF-1催化的简化机理图，后续实验也进一步证明了此假说的合理性



APF-1与蛋白结合时，具体发生了什么化学反应？
此前的研究认为是蛋白质的巯基参与了反应，但是此时发现赖氨酸中的氨基也参与了反应？

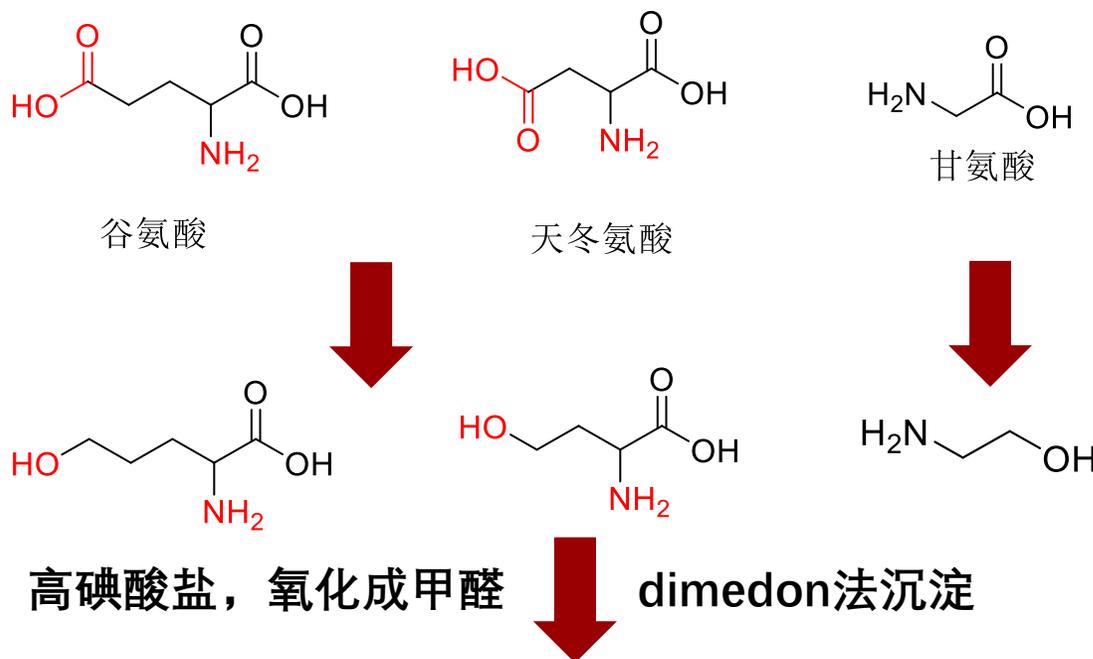
泛素——蛋白酶体系统的发现历程

APF-1与待降解蛋白的结合是氨基，那么
巯基与APF-1的反应？



√ 硼氢化钠可以降低辐射强度，说明可以部分还原硫酯。

既然Fraction II 部分中的蛋白是提供巯基，APF-1提供的是羧基，那么羧基的来源？（天冬氨酸or谷氨酸或者是末端COOH）

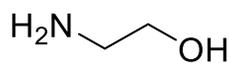
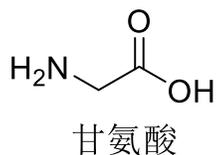
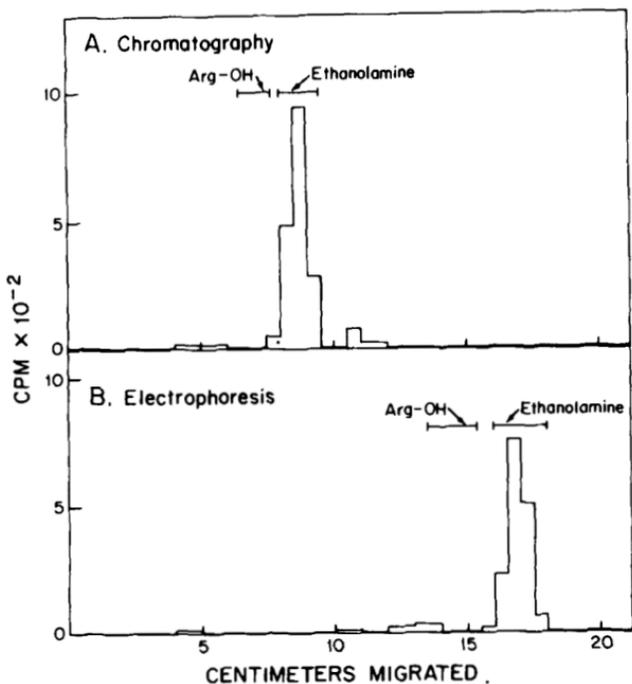
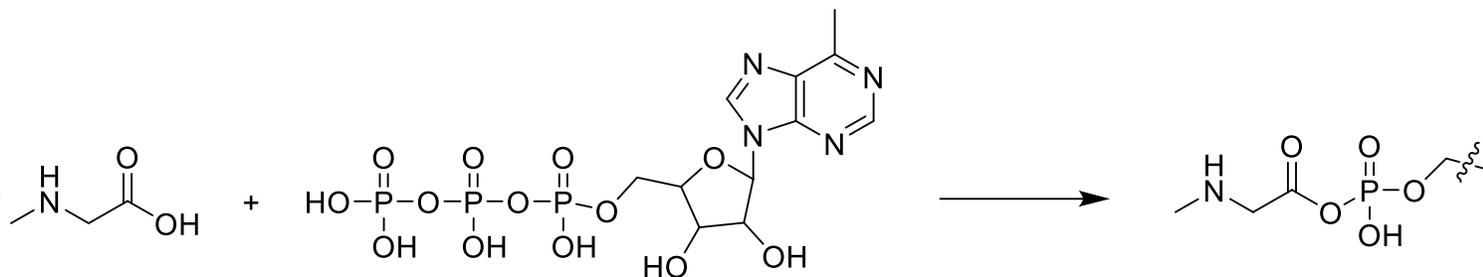


√ 鉴定出是甘氨酸的羧基参与共价结合

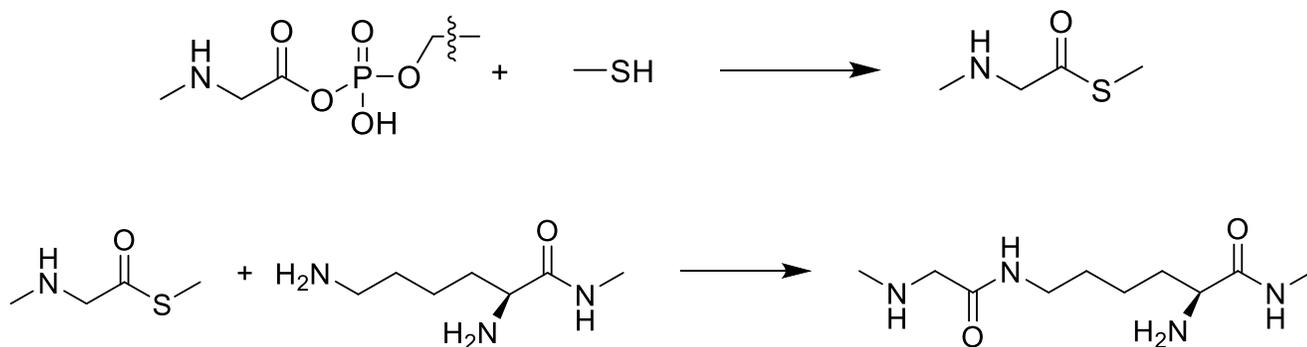
泛素——蛋白酶体系统的发现历程

Treatment	Dimedon phase	
	Supernatant	Precipitate
	<i>cpm</i>	
With periodate	97	1535
Without periodate	1768	19

√ 放射性集中于沉淀中,甘氨酸共价结合。

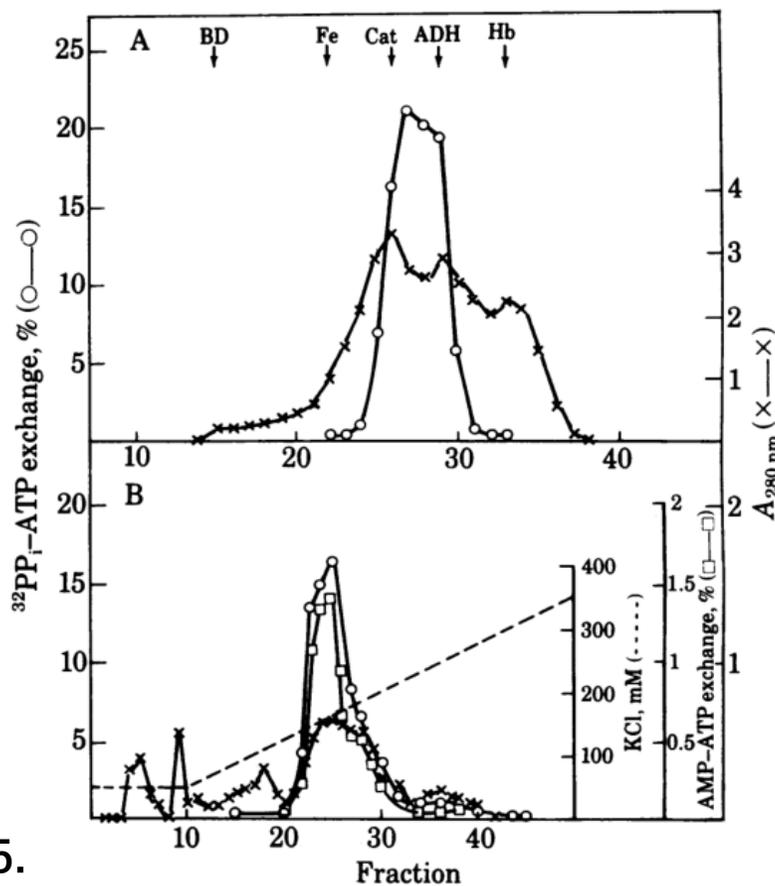
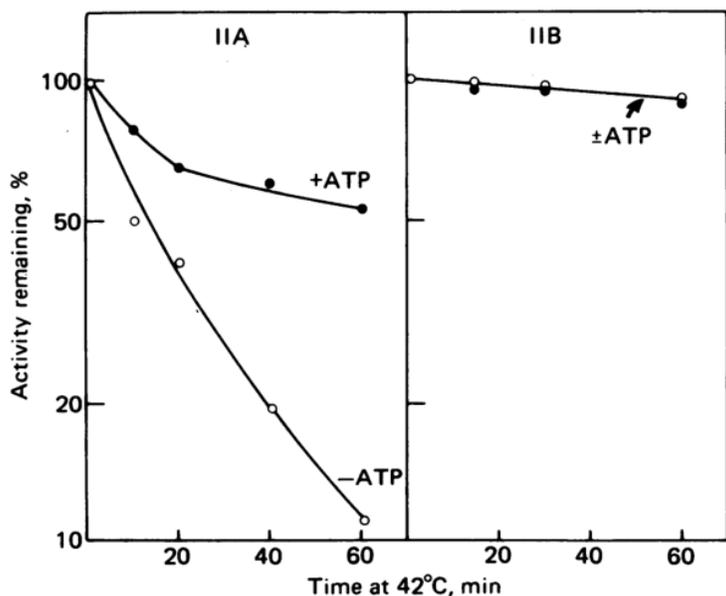
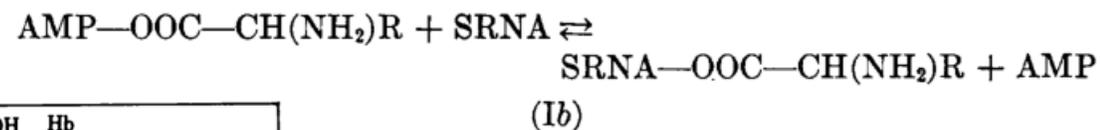
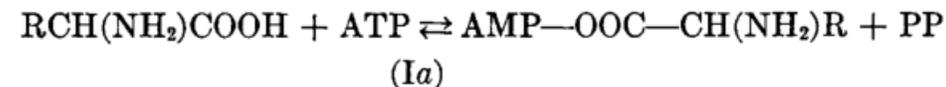
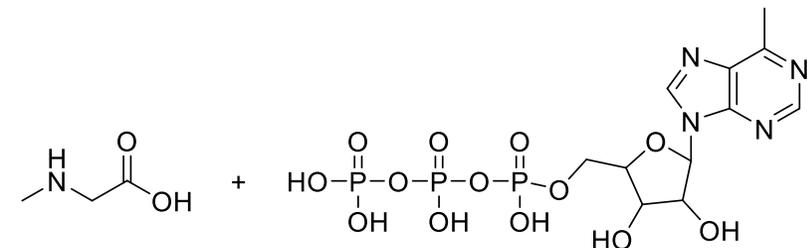


√ 乙醇胺和蛋白还原
水解后的产物出峰重合。



证明推测?

泛素——蛋白酶体系统的发现历程



✓ APF-1可以促进ATP-PPi、ATP-AMP的交换，依赖Fraction II B。

✓ ATP-PPi、ATP-AMP交换的程度是一致的，推测可能是由同一种酶催化。

✓ APF-1, Fraction II A & B
三者共同发挥作用。

Proc Natl Acad Sci USA, 1981, 78, 761-765.

泛素——蛋白酶体系统的发现历程

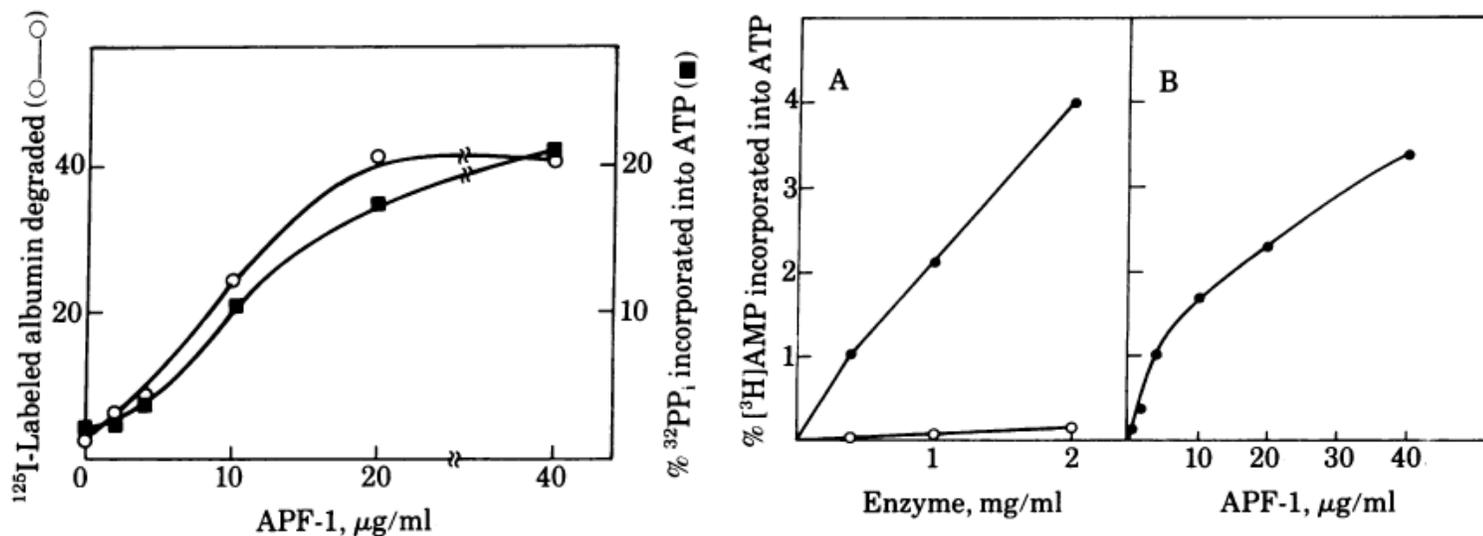
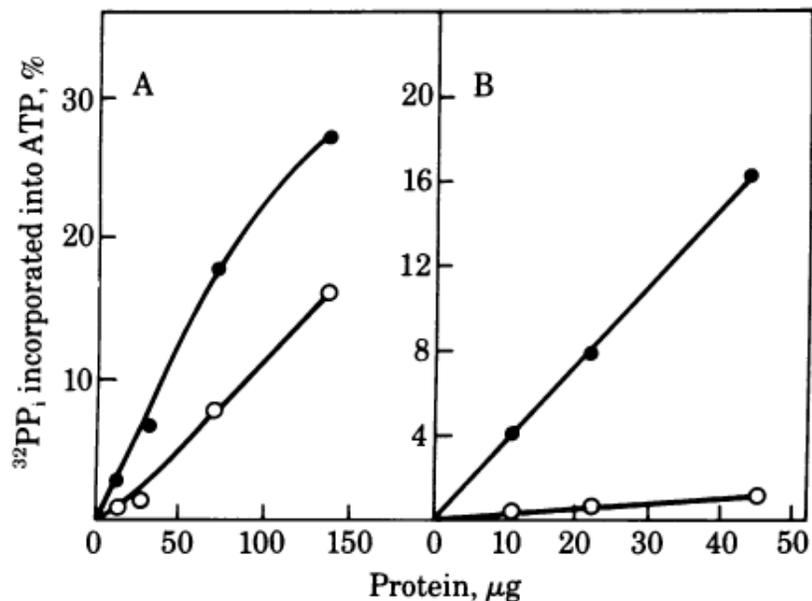
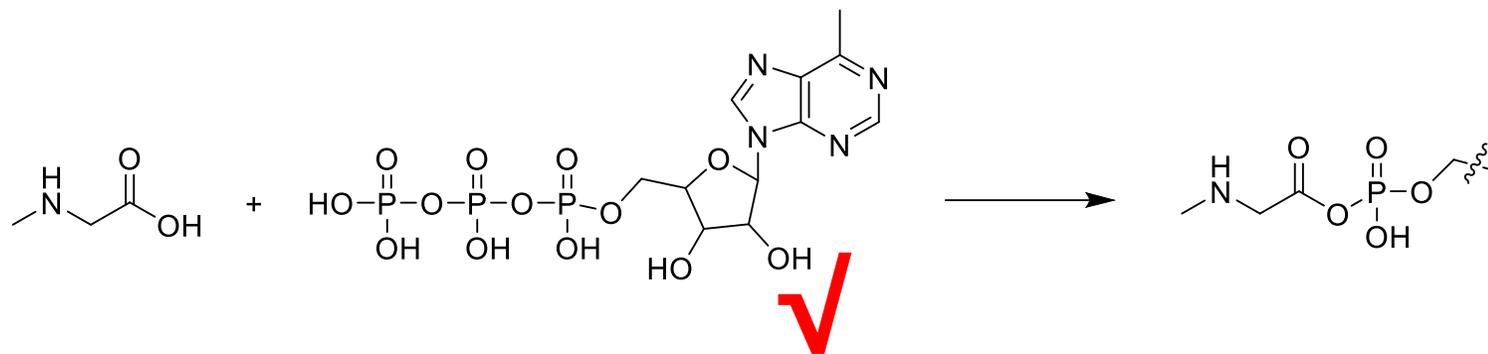


FIG. 2. Stimulation of ATP-PP_i exchange by ATP. (A) ³²PP_i-ATP exchange with crude fraction II. (B) Exchange with the partially purified enzyme after Sepharose-6B step (see Fig. 1A). ○, Without APF-1; ●, with 24 μg of partially purified APF-1 (final CM-Sephadex step omitted).

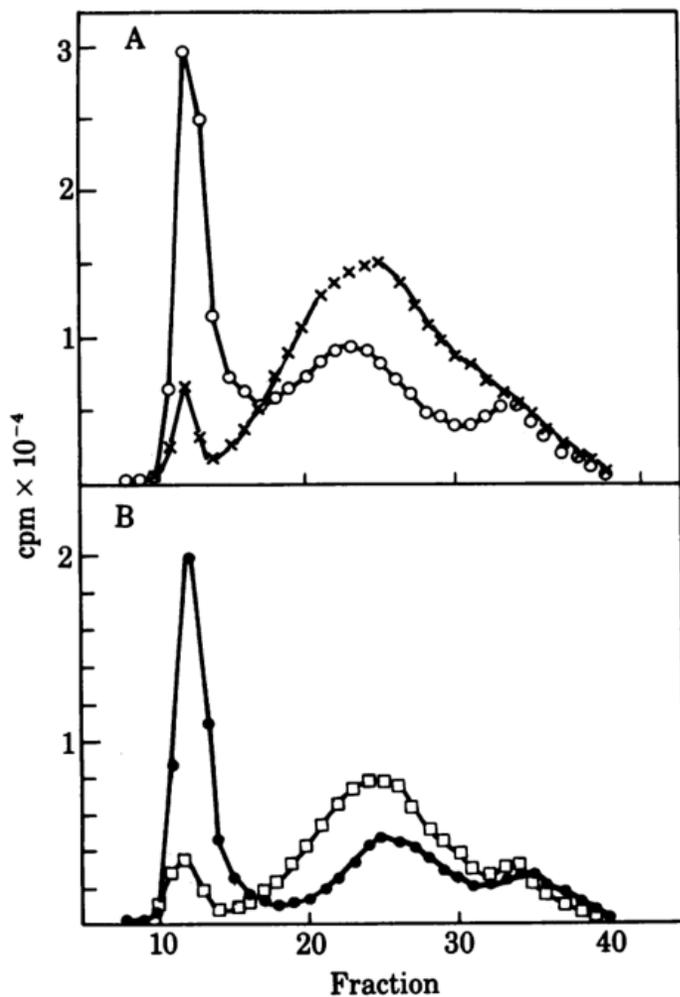
√ ATP-PP_i、AMP的交换程度与蛋白质降解程度呈正相关。

√ APF-1促进ATP-PP_i的交换。

√ Fraction II B粗提取物有内源促进ATP-PP_i的交换的物质。



泛素——蛋白酶体系统的发现历程

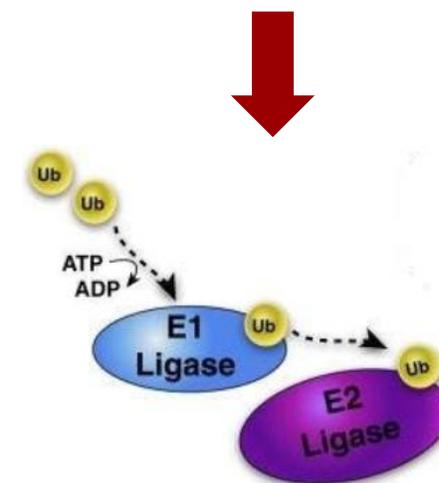
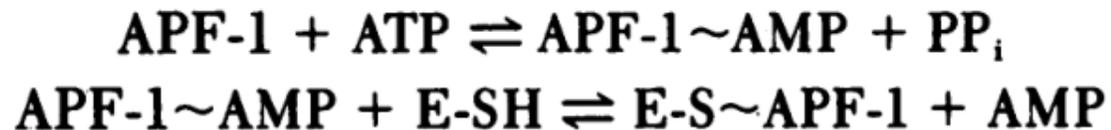


√ 共价键能被硼氢化钠、羟胺切断。
(间接支撑了硫酯键的假设)

√ APF-1与Fraction II B结合形成的共价键不耐碱性。
(间接支撑了硫酯键的假设)

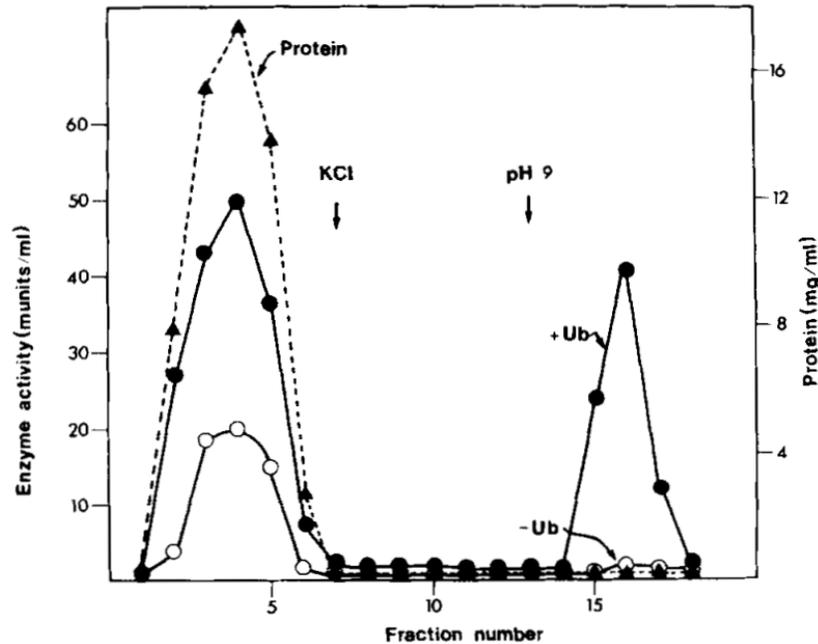
Table 2. Stability of bond between APF-1 and enzyme

Treatment	% APF-1 bound
None	47
Hydroxylamine (1 M), pH 6.1	10
Sodium borohydride (10 mM)	11
Mercuric acetate (3%)	8

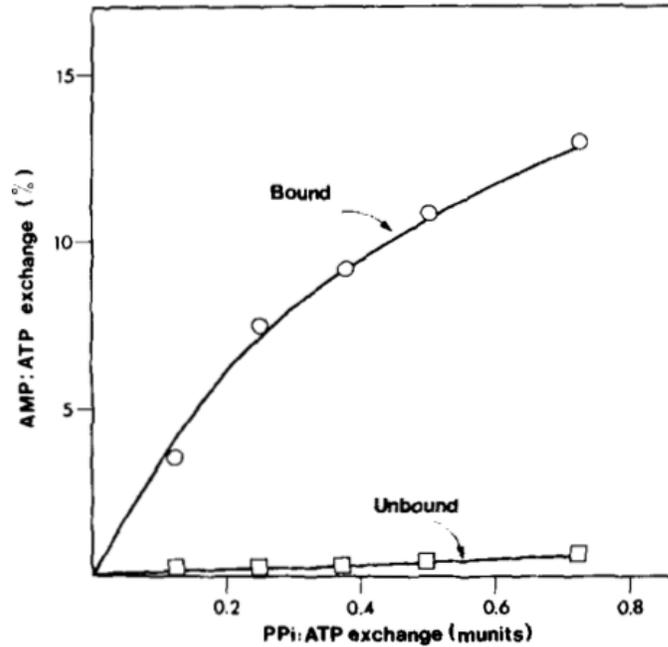


泛素——蛋白酶体系统的发现历程

APF-1的作用基本阐释完全，Fraction II A 和 B ？



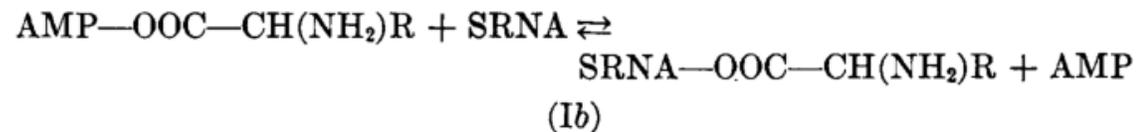
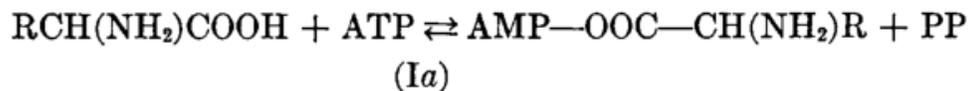
√ 泛素 (APF-1) 柱可以结合掉E-SH。



√ 与泛素柱结合的酶可以同时促进 I a 和 I b 过程。但是不能结合的酶只能促进 I a 过程。

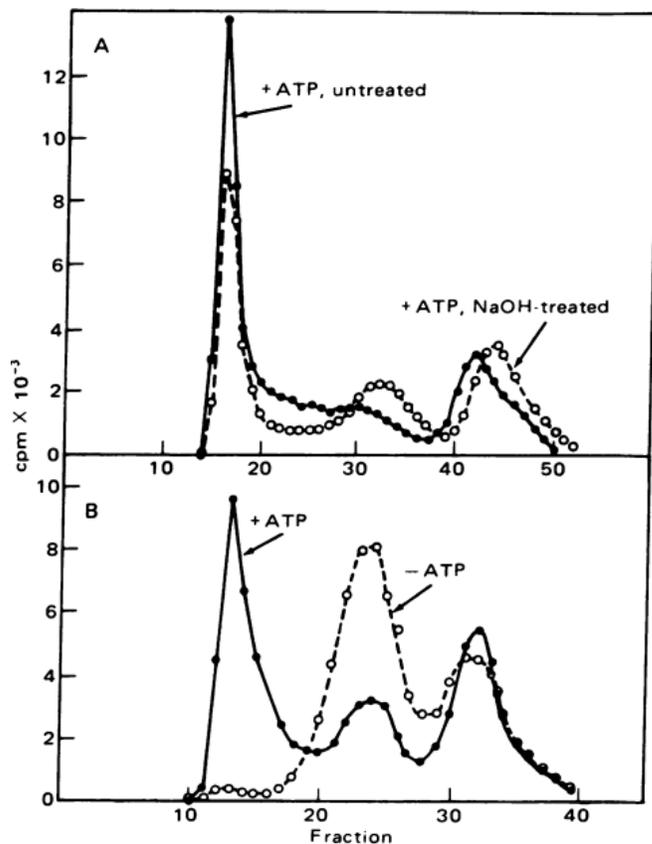


是否为两种不相关的酶？



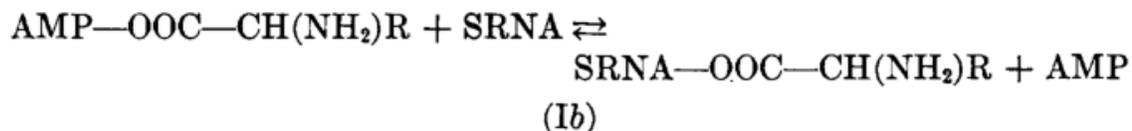
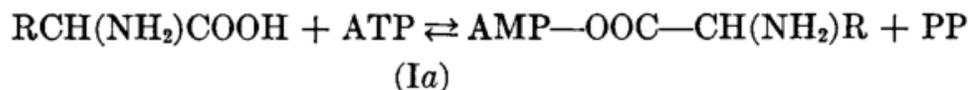
泛素——蛋白酶体系统的发现历程

由于洗脱的位置几乎相同，更可能是细微的结构差异，影响催化活性。

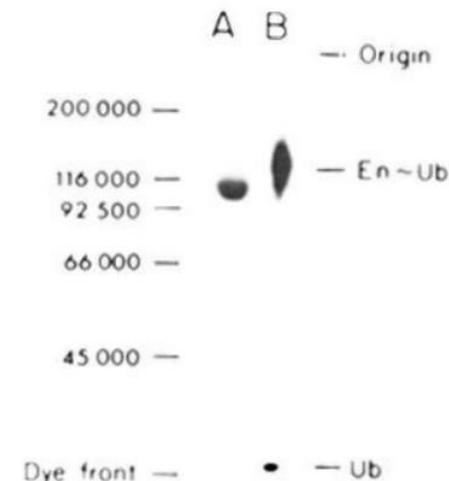


21 ml of fraction II were chromatographed on a 6-ml column of ubiquitin-Sepharose, as described under "Materials and Methods."

Fraction	Total protein mg	Total activity milliunits	Specific activity milliunits/mg	Purification -fold	Yield %
1. Fraction II	455.7	669.4	1.5	1	100
2. AMP-PP _i eluate	0.95	245.1	258.0	172	36.6

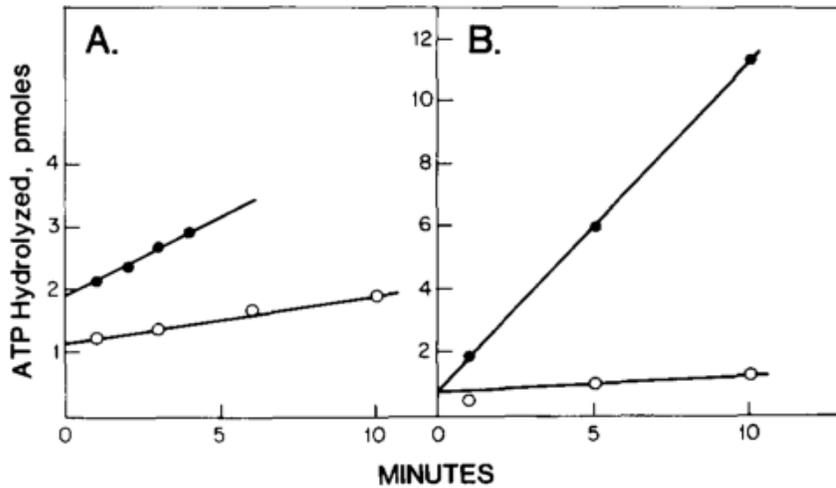
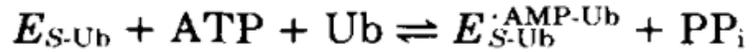
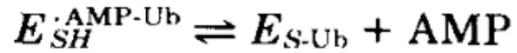
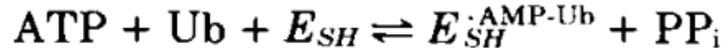


Addition	³² PP _i incorporated into ATP %
None	0.7
Ubiquitin (5 μg)	16.7
Glycine (10 mM)	0.4
Glycyl-glycine (10 mM)	0.3
Mixture of 20 amino acids (1 mM each)	0.4
Bovine serum albumin (30 μg)	0.9
Lysozyme (30 μg)	0.1
Reticulocyte fraction II, heated (20 μg of protein)	5.9
Reticulocyte fraction II, heated (20 μg), plus anti-ubiquitin serum (5 μl)	0.5

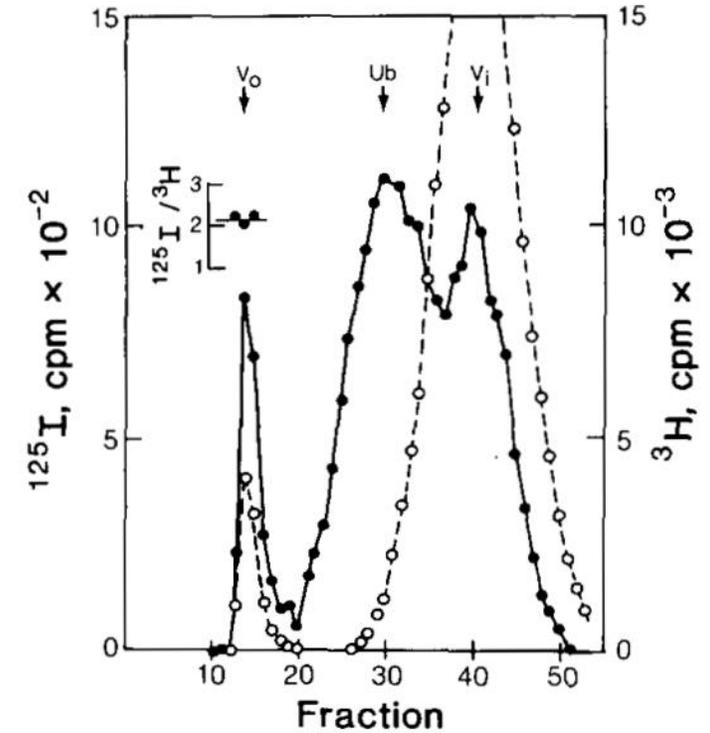
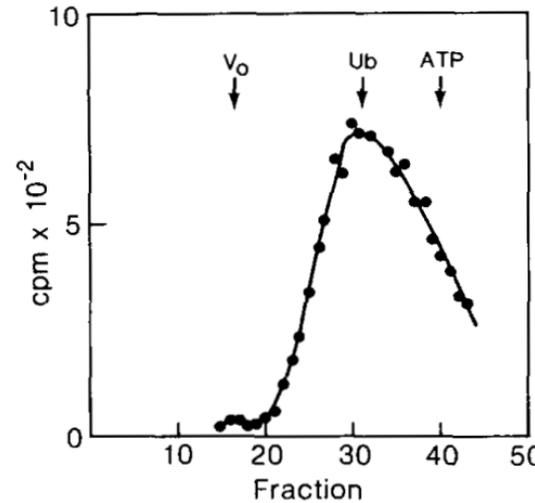


确定分离出的是不是已知酶
当时已知的酶都不能促进
PP_i和ATP的交换，大概率是
一种全新的功能酶，而且与
泛素的功能相关。

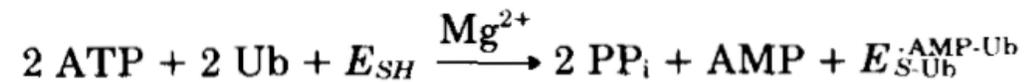
泛素——蛋白酶体系统的发现历程



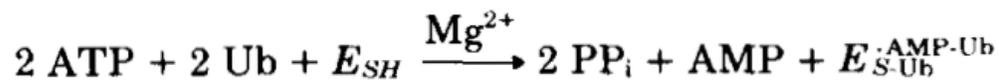
	Product		
	-Enzyme	+Enzyme	Δ
	<i>pmol</i>		
Experiment 1			
³² P _i extractable as Mo ²⁺ complex	0.51	1.17	0.66
³ H acid precipitated	0.03	0.39	0.36
Experiment 2			
³ H acid precipitated, 0 min	0.06	0.33	0.27
30 min		0.30	0.24



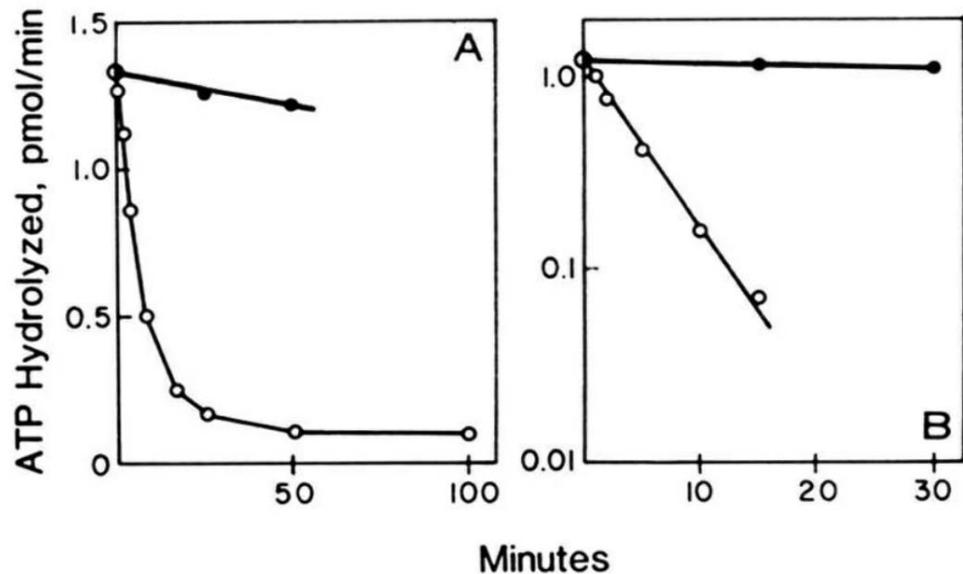
- √ 待降解酶加入，会促进ATP水解。
- √ 二硫苏糖醇加入，也会促进ATP水解。



泛素——蛋白酶体系统的发现历程



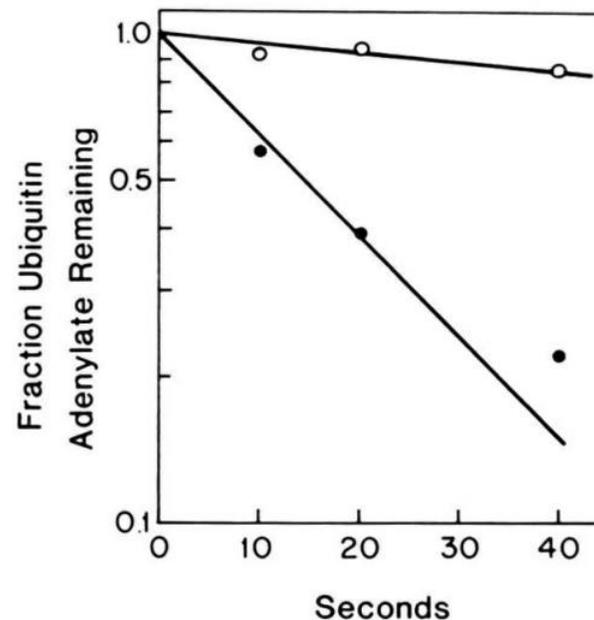
E是否有两个修饰位点？



√ 巯基的修饰位点只有一个。

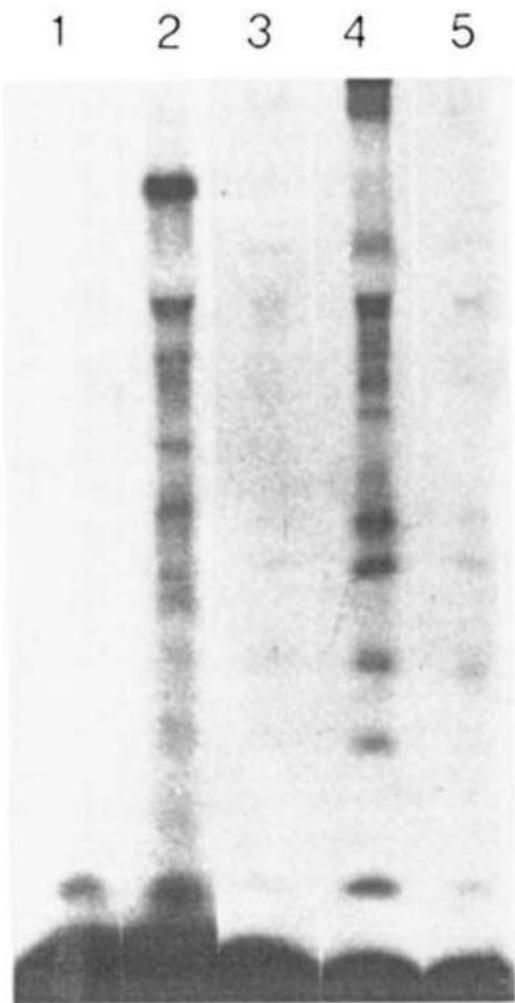
	Native enzyme	Modified enzyme
Experiment 1		
ATP:PP _i exchange (pmol/min)	49	42
ATP:AMP exchange (pmol/min)	7.4	0.4
Experiment 2		
³ H acid precipitated (pmol)	0.34	0.32
Experiment 3		
Enzyme-bound ¹²⁵ I-ubiquitin (pmol)		0.31
Enzyme-bound [³ H]AMP (pmol)		0.29

√ 碘乙酰胺封闭巯基之后，AMP交换被抑制。



√ 碘乙酰胺可以提高，泛素-腺苷酸的稳定性，说明硫酯的生成是通过泛素-腺苷酸中间体形成的。

泛素——蛋白酶体系统的发现历程



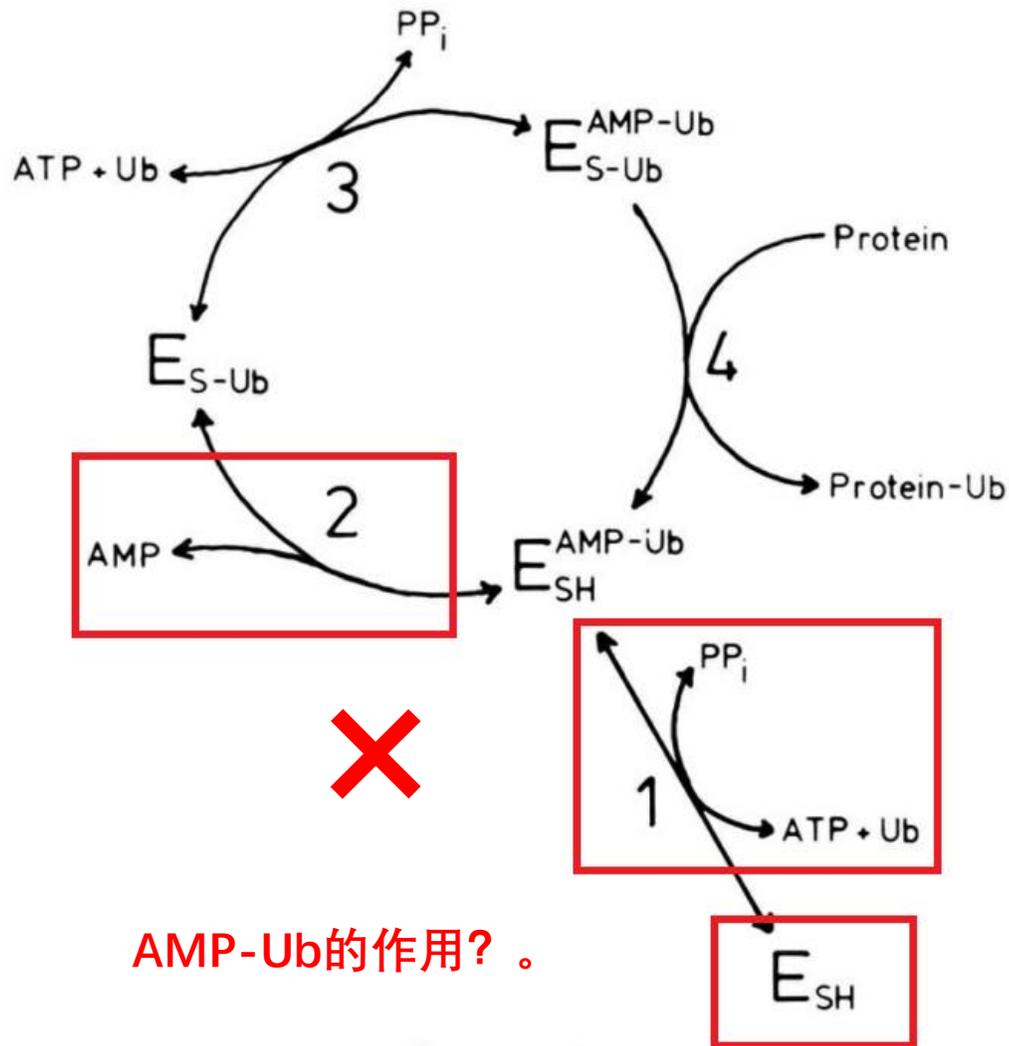
√ 证明和待降解蛋白质结合确实需要活化酶的参与 (1&2)。

√ 对2的定量分析, 发现1.5pmol泛素硫酸酯和0.04pmol的泛素腺苷酸酯最后形成了0.16pmol的异肽键 (说明泛素硫酸酯是蛋白质偶联的供体)。

√ 封锁巯基, 明显抑制与待降解蛋白的结合。

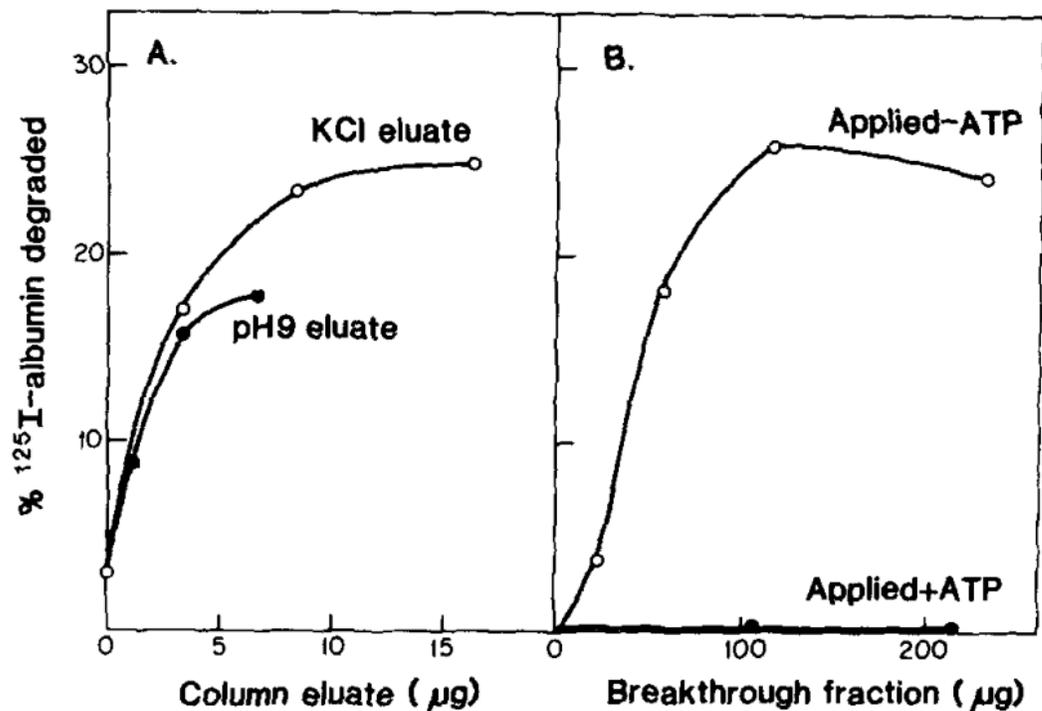
← ¹²⁵I-Ubiquitin

The Journal of Biological Chemistry, 1982, 257, 2543-2548.

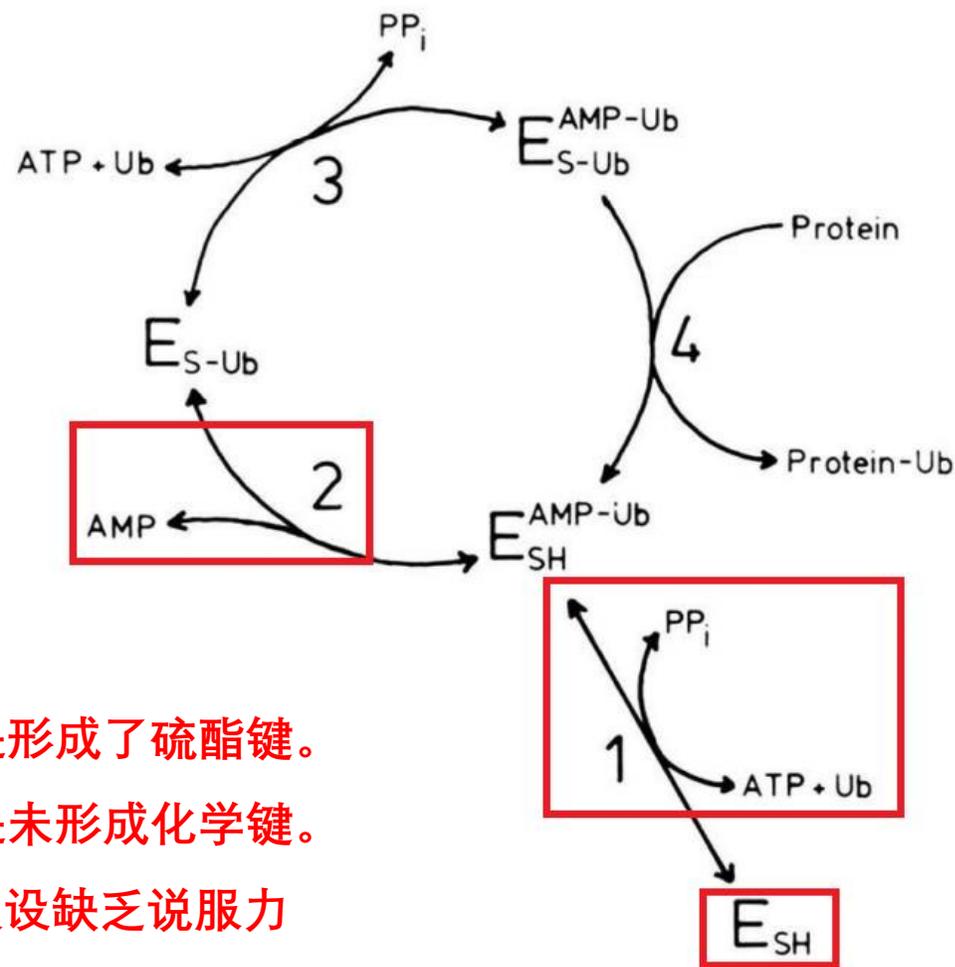


SCHEME 1

泛素——蛋白酶体系统的发现历程



√ 改善洗脱方式，尽可能将活性部分（3部分）依次洗脱，E1、E2（二硫苏糖醇洗脱）、E3（高盐洗脱部分或者高PH）。



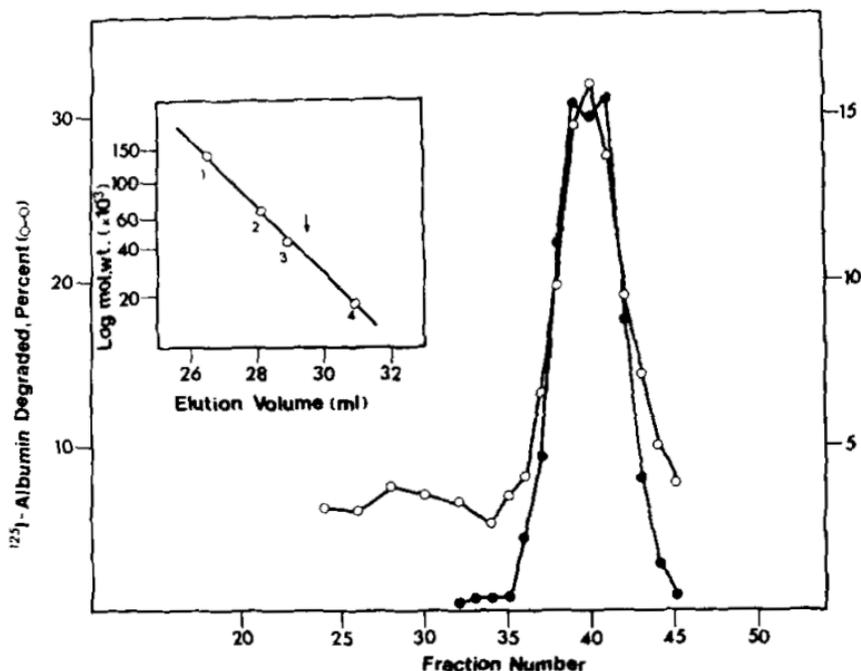
E2应该是形成了硫酯键。
E3应该是未形成化学键。
之前的假设缺乏说服力

SCHEME 1

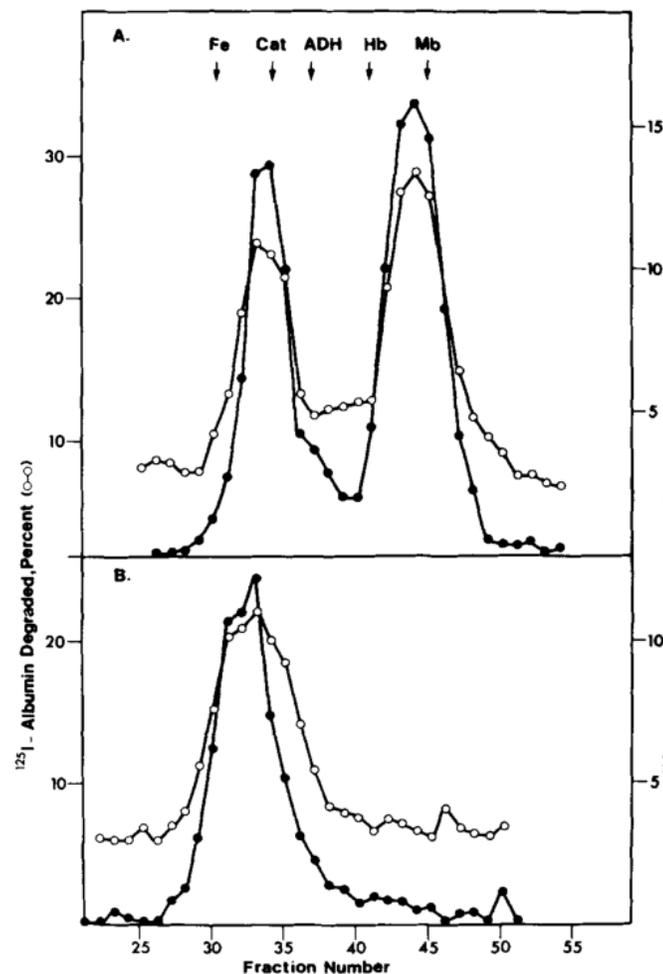
泛素——蛋白酶体系统的发现历程

Additions	¹²⁵ I-Albumin degraded %
Experiment 1	
KCl eluate	5.4
KCl eluate + E ₁	4.7
KCl eluate + DTT eluate	23.9
pH 9 eluate	4.7
pH 9 eluate + E ₁	11.0
Experiment 2	
KCl eluate + E ₁	4.2
KCl eluate + E ₂	5.8
KCl eluate + E ₁ + E ₂	24.4

√ 保证分离纯化方式没有问题。



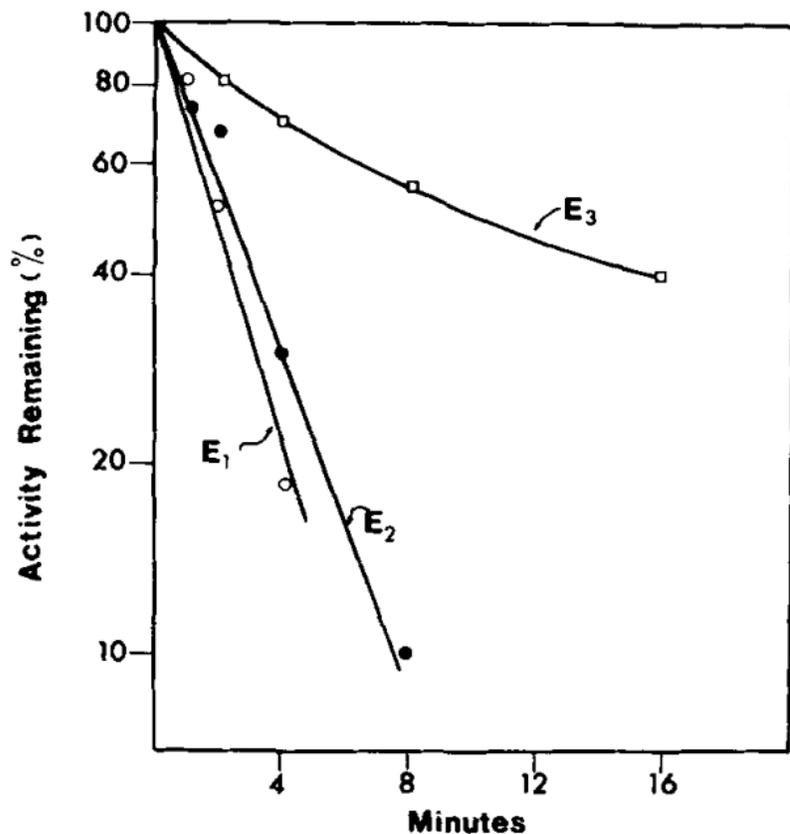
√ 证明了E2也与泛素发生了共价结合。
(通过质量数差异, 证明, 确是E2而不是E1)



√ 有一部分E2的质量发生了明显增加
(35kDa→250kDa)
(可能是与某种蛋白共价结合了, 但是还保留了活性)

有待后续解释

泛素——蛋白酶体系统的发现历程



√ 封闭巯基，E1、E2活性均下降（趋势接近）。加之此前的研究认为E2也与泛素形成了共价键，推测E2的共价键也是巯基。

Additions	¹²⁵ I-Ubiquitin conjugated pmol
Experiment 1	
<i>E</i> ₁	0.7
<i>E</i> ₂	0.7
<i>E</i> ₃	1.3
<i>E</i> ₁ + <i>E</i> ₂	2.4
<i>E</i> ₂ + <i>E</i> ₃	4.3
<i>E</i> ₁ + <i>E</i> ₃	5.3
<i>E</i> ₁ + <i>E</i> ₂ + <i>E</i> ₃	12.1
Experiment 2	
<i>E</i> ₃ , iodoacetamide-treated	0.3
<i>E</i> ₃ , iodoacetamide-treated + <i>E</i> ₁	1.0
<i>E</i> ₃ , iodoacetamide-treated + <i>E</i> ₂	1.2
<i>E</i> ₃ , iodoacetamide-treated + <i>E</i> ₁ + <i>E</i> ₂	7.1

√ 蛋白质与泛素共价结合，E1、E2、E3均需要。

√ 提前封闭E3巯基对蛋白和泛素的共价集合影响有限。（E3可能不发生共价结合）

泛素——蛋白酶体系统的发现历程

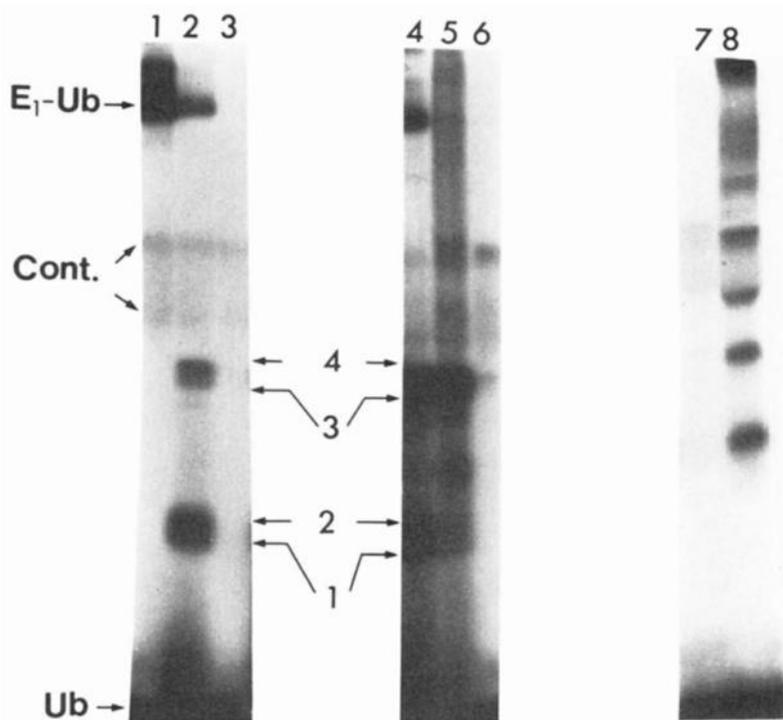


FIG. 6. Transfer of activated ubiquitin from E_1 to E_2 and to conjugate formation in the presence of E_3 . All incubations con-

- √ 泛素从E1、传递到E2，与ATP无关。
- √ ATP只在E1和泛素结合时候，发挥作用。
- √ E2与泛素结合后，在E3作用下，将泛素转移到蛋白。

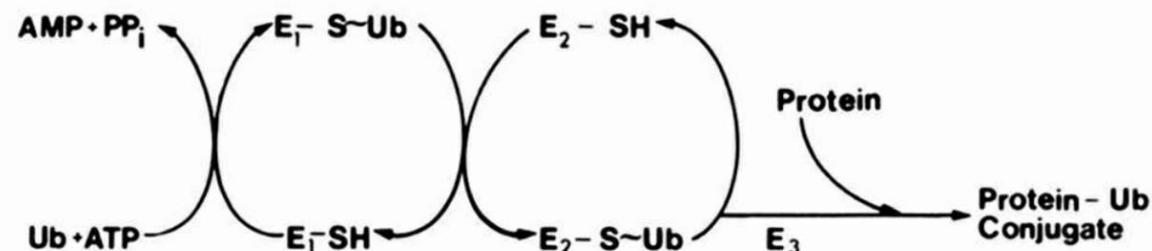


FIG. 7. Proposed sequence of events in the ubiquitin-protein ligase system. See the text. *Ub*, ubiquitin.

Nobel Prize in Chemistry 2004



Aaron Ciechanover



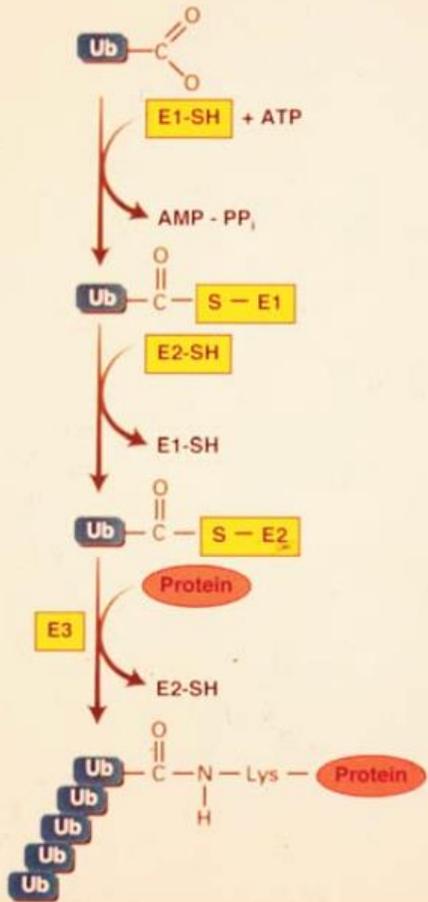
Avram Hershko



Irwin Rose

泛素——蛋白酶体系统的发现历程

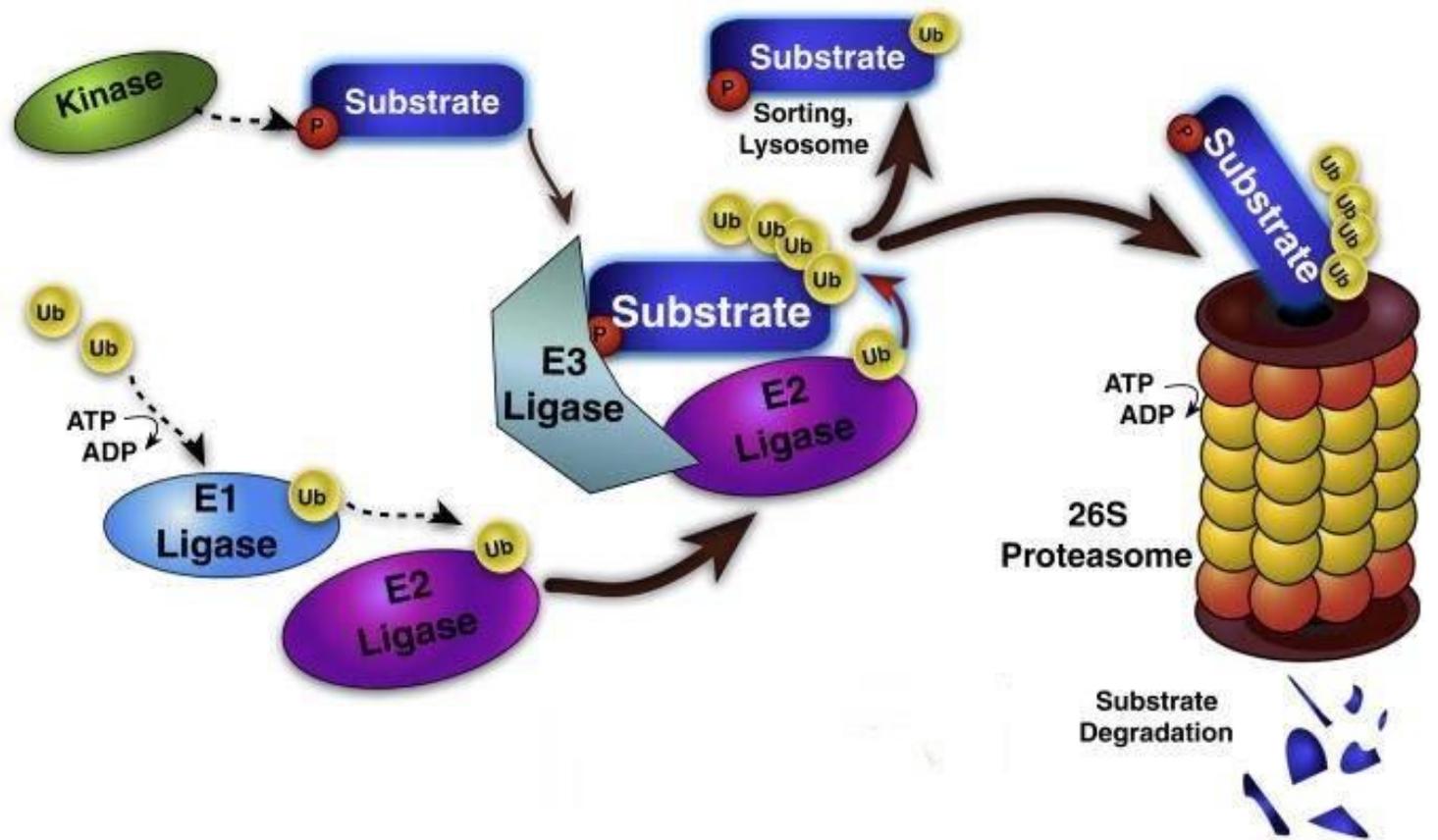
UBIQUITIN CONJUGATION TO PROTEIN SUBSTRATES



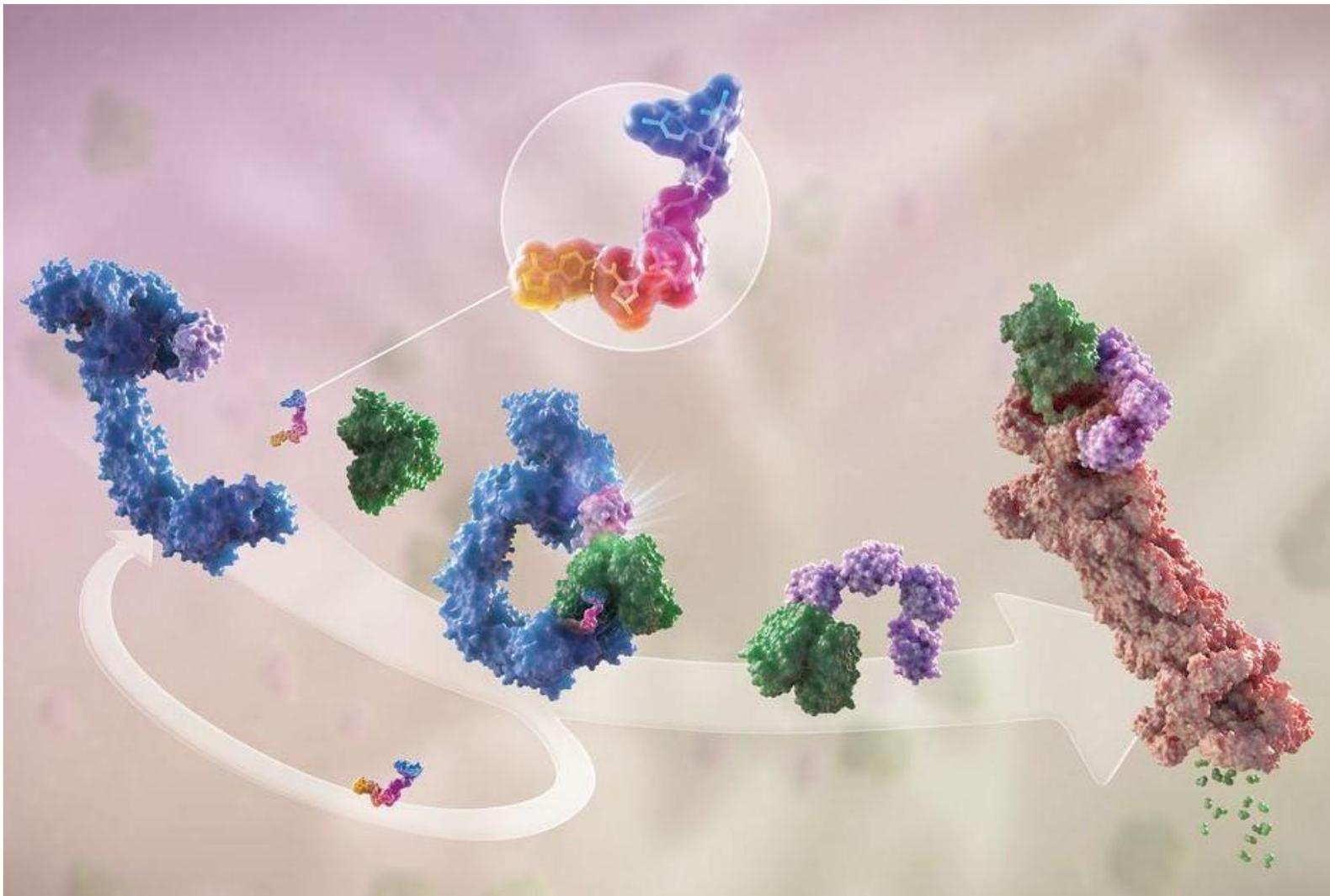
Ub-activating Enzyme (E1)
one/cell
(homologs exist for activating Ub-like proteins)

Ub-carrier proteins (E2s)
20-40 in mammals
(homologs exist for Ub-like proteins)

Ub-Ligases (E3s)
(500-1000 in mammals, specific for substrates and E2s, many monomeric, many large complexes)



生物化学  化学生物学



目标蛋白



药物分子



E3 (泛素连接酶)



泛素



蛋白酶体



感谢!