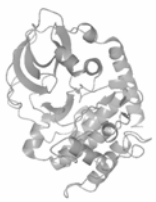
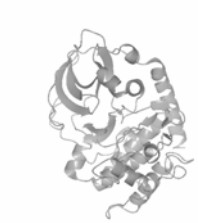


蛋白质翻译后修饰组学的研究进展

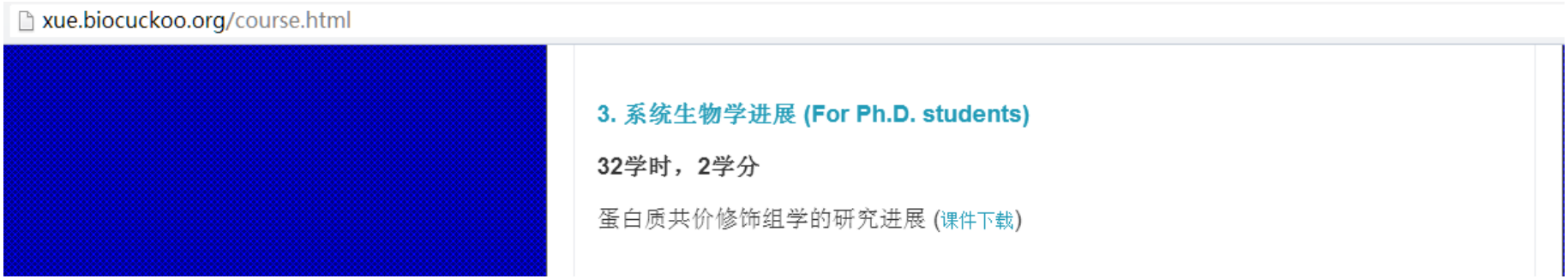
薛宇

xueyu@hust.edu.cn

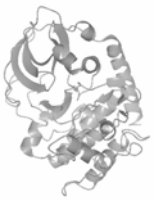
课件下载



- **http://xue.biocuckoo.org/course.html**

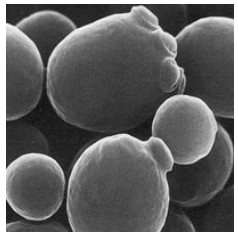


课程作业



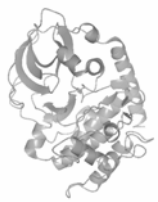
- 围绕你正在开展的研究工作，结合至少一种相关的系统生物学技术或方法，设计一个可行的研究项目，内容不少于3000字。内容包括：
  - ◆1）项目的立项依据，包括研究背景、研究意义、国内外研究现状及发展动态分析
  - ◆2）项目的研究内容、研究目标，以及拟解决的关键科学问题
  - ◆3）拟采取的研究方案及可行性分析
  - ◆4）本项目的特色与创新之处
- 电子版，裁剪后贴到答题本，姓名、学号
- 2019.10.29下午上课时交给唐大超同学

从酵母到人...



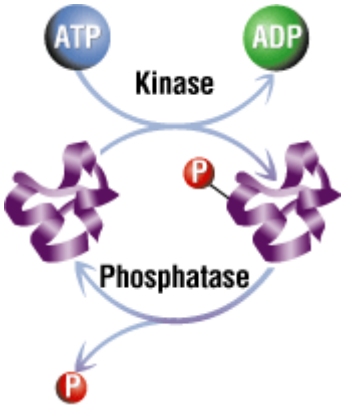

Genome	12Mb	97Mb	180Mb	3,038Mb
#Genes	6,000	19,000	14,000	21,000

基因数 vs. 生物多样性

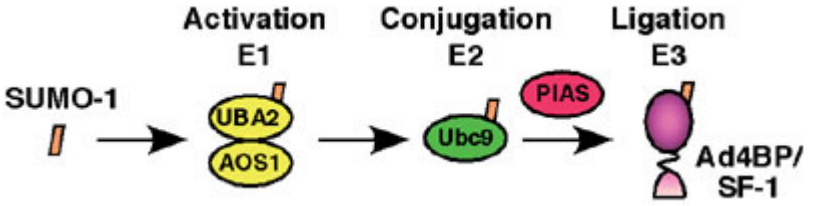


- 基因数 ? 生物学功能、调控复杂性和生物多样性
- 当前观点:
  - 蛋白质数量 -> 生物复杂性 & 多样性
  - ~1,000,000 protein isoforms
- 两种潜在的产生机制:
  - Post-transcriptional Modification (转录后修饰), eg., Alternative Splicing (可变剪切): 25,000 genes -> 10^5 transcripts
  - Post-translational Modification (翻译后修饰), eg., Phosphorylation: 10^5 transcripts -> 10^6 proteins

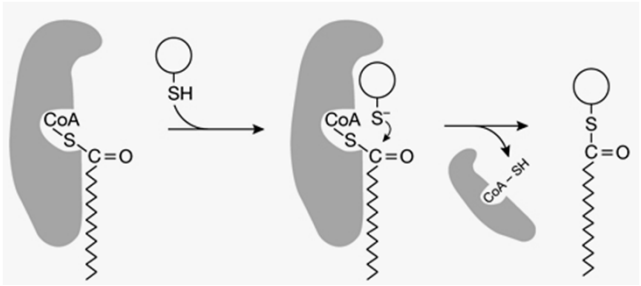
Post-translational Modification



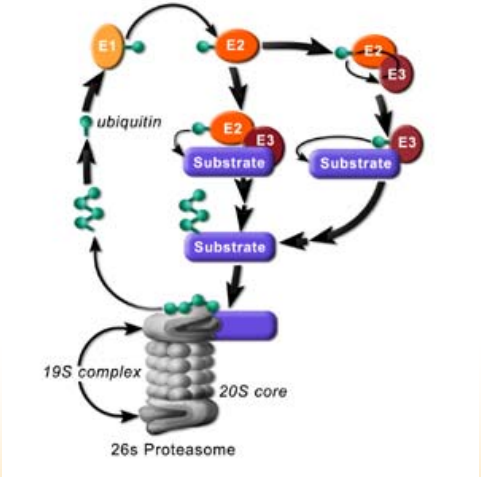
Phosphorylation



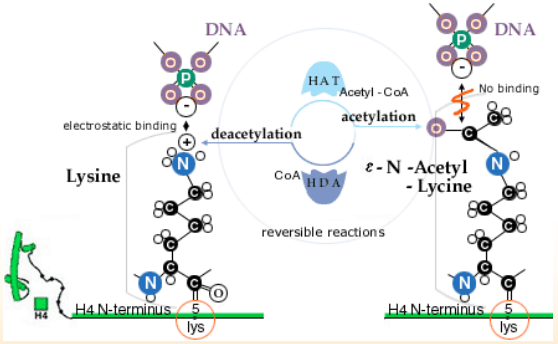
Sumoylation



Palmitoylation

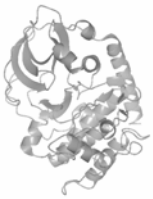


Ubiquitination



Acetylation

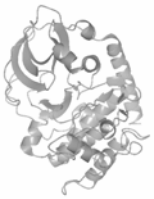
蛋白质共价/翻译后修饰



- **POST**: DNA转录为RNA，RNA翻译成蛋白质之后发生
- 生化反应：产生或破坏共价键
- 发生在氨基酸的主链或侧链上
- 通常由特定酶催化
- 可发生在15种非疏水的氨基酸上
- 目前已发现~650种PTMs

http://www.uniprot.org/docs/ptmlist

The first PTM substrate



- **Casein:**
  - **An abundant phosphoprotein found in milk**
  - **In 1883, the first protein shown to contain phosphate**
  - **Olof Hammarsten, Swedish biochemist**

Year	Event
1883	Casein is a phosphoprotein
1900	Vitellinic acid/phosvitin is a phosphoprotein
1932	Phosphoserine in vitellinic acid/phosvitin
1933	Phosphoserine in casein
1954	Casein is used as a model substrate for the detection of the first protein kinase activity
1960	Casein/phosvitin kinases are distinct from phosphorylase kinase
1969	Two distinct casein kinases, CK1 and CK2
1971	Complete amino acid sequence of α_{s1} casein is determined
1972	Casein kinase activity detected in Golgi fractions of lactating rat mammary gland
1988–1989	'Genuine/Golgi' casein kinase (G-CK) consensus is different from those of CK2 and CK1
1995	Casein kinase-1 and casein kinase-2 renamed protein kinase CK1 and CK2
1996	Specificity determinants for G-CK and the development of a synthetic peptide to detect G-CK activity with absolute specificity
2012	G-CK is identified as Fam20C

Olof Hammarsten (1841–1932)

Tagliabracci et al., Science, 2012, 336, 1150-1153
Tagliabracci et al., Trends Biochem Sci, 2013, 38, 121-130

蛋白质磷酸化

- 最重要、研究最广泛的一种翻译后修饰
- 可逆性：
  - Protein kinase: 磷酸化 - Writer
  - Phosphotase: 去磷酸化 - Eraser
  - Phospho-binding domain: 识别特定磷酸化位点 - Reader

Edmond H. Fischer

Edwin G. Krebs

The Nobel Prize in Physiology or Medicine 1992

"for their discoveries concerning reversible protein phosphorylation as a biological regulatory mechanism"

磷酸化的可逆性

- 1943，Cori & Green在兔骨骼肌中发现糖原磷酸化酶 (glycogen phosphorylase) *a* (有活性) 和 *b* (无活性)
  - 肌肉收缩：92%为*a*
  - 肌肉松弛：*b*
- 无AMP：*a* (活性为极值的60%~70%)；*b* (无活性)
- 1955，Fischer & Krebs
  - *a*和*b*是同一种分子
  - 生化反应：*b*利用ATP产生*a*
  - 该反应可逆
  - S15, Phosphorylase kinase, CAMK/PHK, 1975

Krebs & Fischer. JBC, 1955, 216, 113-120;
Fischer & Krebs. JBC, 1955, 216, 121-132.

CDK & Cyclin

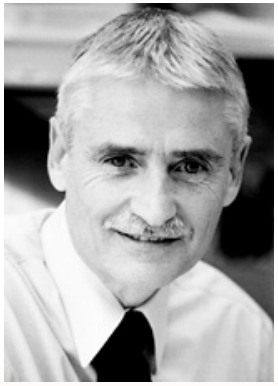
- 1970s, Hartwell等人发现一系列影响细胞周期的基因 (Cell division control, Cdc)
- 1986, Nurse等人发现Cdc2是一个蛋白激酶
  - ◆调控细胞的有丝分裂，34kd
  - ◆快速分裂的细胞蛋白质水平和磷酸化状态恒定
  - ◆停止分裂的细胞蛋白质水平下降，发生去磷酸化
- 1976，Brandhorst发现海胆受精前后蛋白质水平不变
  - ◆矛盾：抑制蛋白质合成，海胆不能分裂？
  - ◆1983，Hunt等人“猜测”并证实两个蛋白质在分裂过程中起作用，分裂后迅速降解 - Cyclin

Evans, et al. Cell, 1983, 33, 389-396

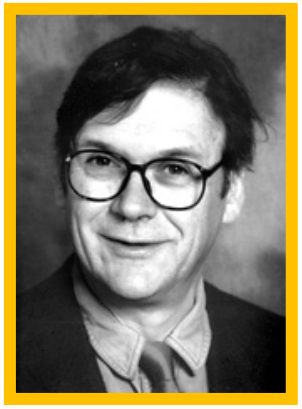
Cell cycle

- 1970, cdc1, *cdc2*, cdc3
- 1981, cdc2 is required
- 1984, cdc2/cdk1 activity is required
- How about Tim Hunt?

Marc W. Kirschner



Leland H. Hartwell



Tim Hunt

Paul M. Nurse

The Nobel Prize in Physiology or Medicine 2001

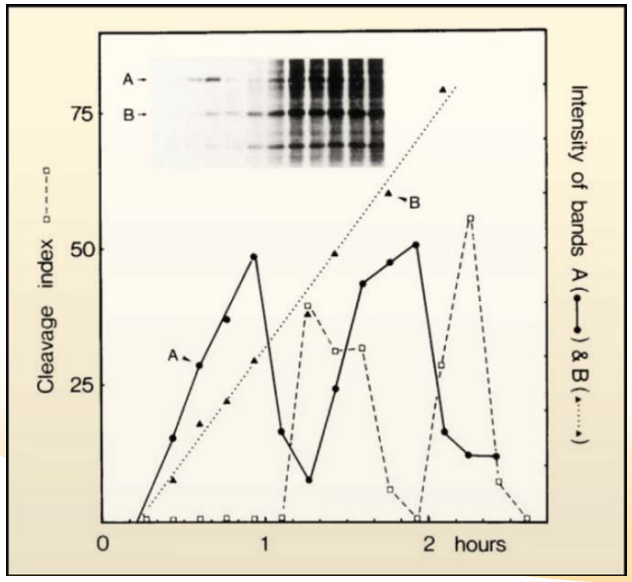
Hartwell et al., PNAS, 1970, 66:352-9
Nurse et al., Nature, 1981, 292:558-60
Newport et al., Cell, 1984, 37:731-42

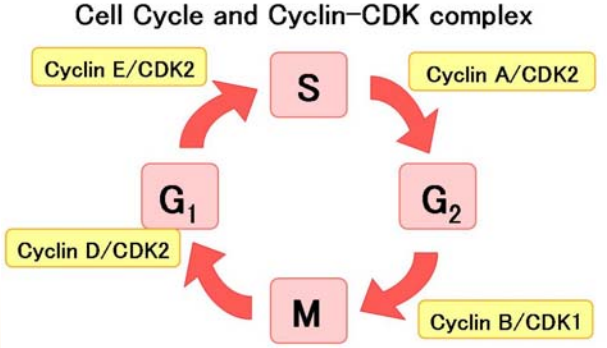
The Hunt for Cyclin

- July 22nd, 1982, Cyclin A & B
- 1989, Cyclins control cdks
- Why Tim Hunt?
  - *Sea urchin*
  - SDS-Page
  - Marc W. Kirschner

I was very lucky.

Marc W. Kirschner



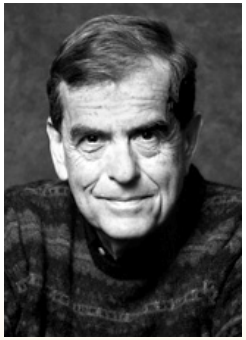


Evans et al., Cell, 1983, 33:389-96
Murray et al., Nature. 1989, 339:275-80
Murray et al., Nature, 1989, 339:280-6

Marc W. Kirschner

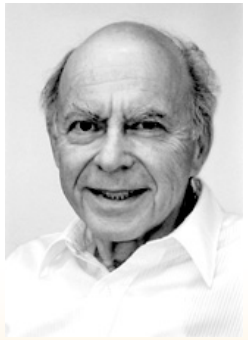
- 1984, the dynamic instability of microtubules
- 1991, Cyclin ubiquitination
- 2003, founding the Department of Systems Biology in Harvard

The Nobel Prize in Chemistry 2004

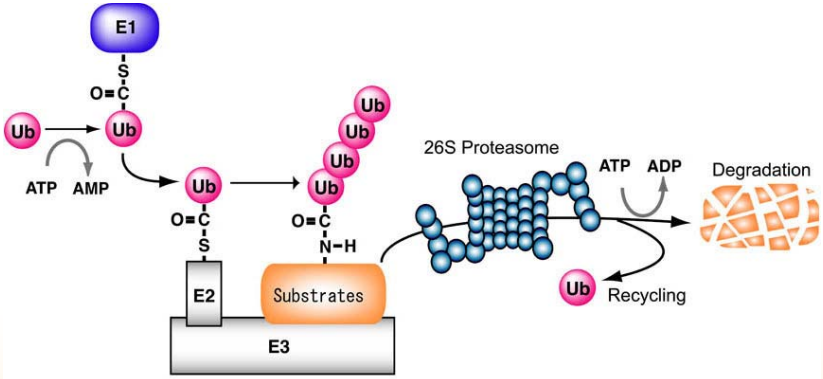


Aaron Ciechanover

Avram Hershko



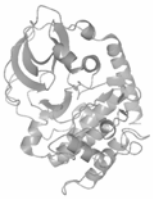
Irwin Rose



Mitchison et al., Nature, 1984, 312:237-42

Glotzer et al., Nature, 1991, 349:132-8

The discovery of protein kinase C



- The fundamental concepts of the intracellular signal transduction cascade
- PKC: activated in the lipid hydrolysis pathway
- Albert Lasker 1989: PKC in tumor promotion.
- 1995, the President of Kobe University

1932~2005

Yasutomi Nishizuka

西冢泰美

J. Biochem. 2010;148(2):125–130 doi:10.1093/jb/mvq066

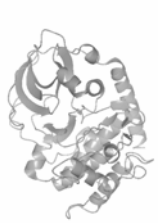
JB THE JOURNAL OF BIOCHEMISTRY

JB Reflections and Perspectives

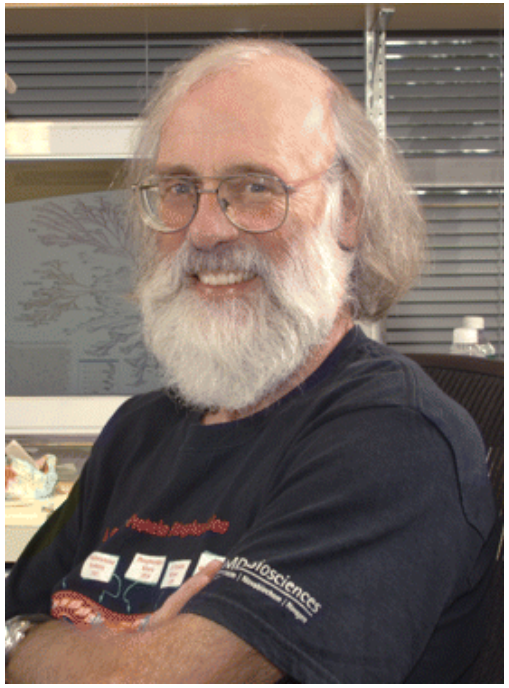
Yasutomi Nishizuka: Father of protein kinase C

Inoue et al., J Biol Chem, 1977, 252, 7610-6

Tyrosine Phosphorylation

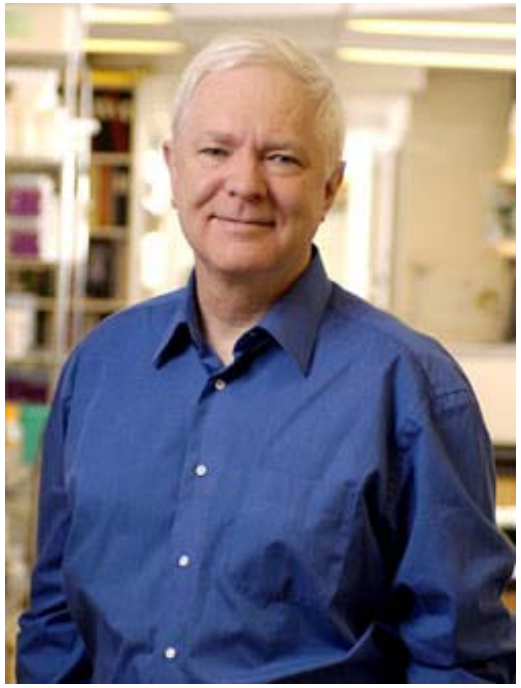


Tony Hunter: kinase king



Discovery of tyrosine kinase

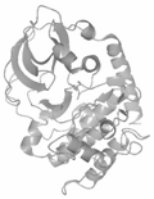
Tony Pawson



Discovery of SH2 domain that binds with phosphotyrosine

Hunter. J Cell Biol., 2008, 181:572-3

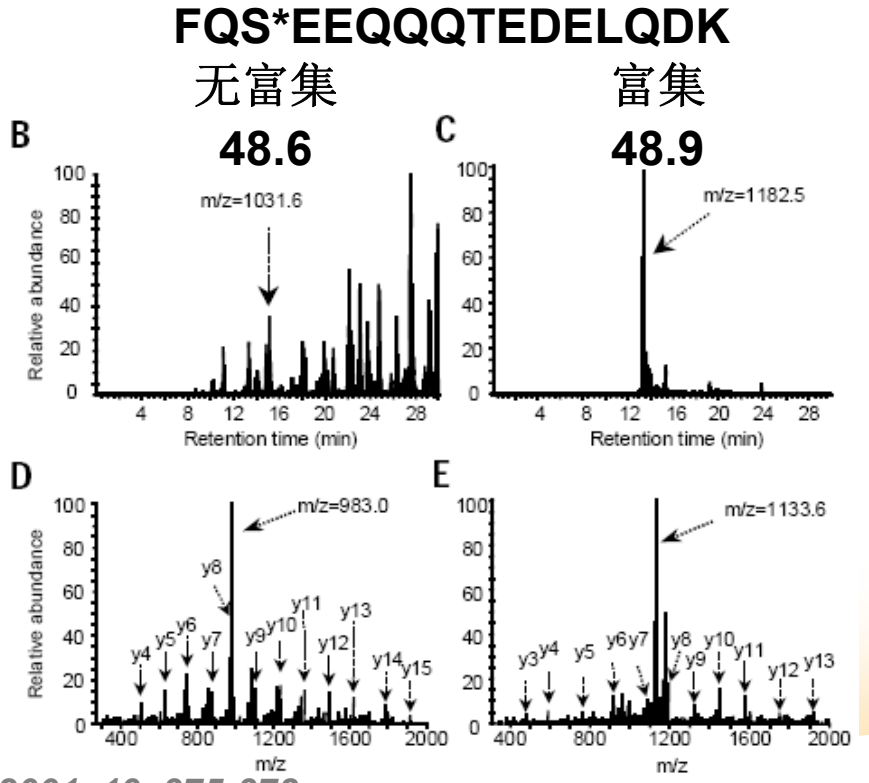
蛋白质磷酸化



- 人类基因组: ~520个蛋白激酶
- 功能重要性：
  - ~30% 的蛋白质发生磷酸化修饰
  - 参与所有的细胞过程
  - 激酶的变异与癌症和疾病密切相关
- Phosphoproteomics (磷酸化蛋白质组)
  - *a*. 高通量鉴定磷酸化底物、位点和模体
  - *b*. 当前进展: ~500K个磷酸化位点

磷酸肽的质谱鉴定

- **Phosphopeptide**
  - 磷酸肽的富集 – Immobilized Metal Ion Chromatographic (IMAC)
  - Liquid chromatography–tandem mass spectrometry (LC-MS/MS)
  - 根据序列数据库搜索磷酸化位点
- **分子量：**
  - H: 1.008
  - P: 30.97
  - Q: 16.00
  - H_3PQ_4: 97.994
- **肽段：带双电荷**
  - 质荷比*m*/*z*=m/2
  - H_3PQ_4: 48.997
  - Neutral loss: ±0.7

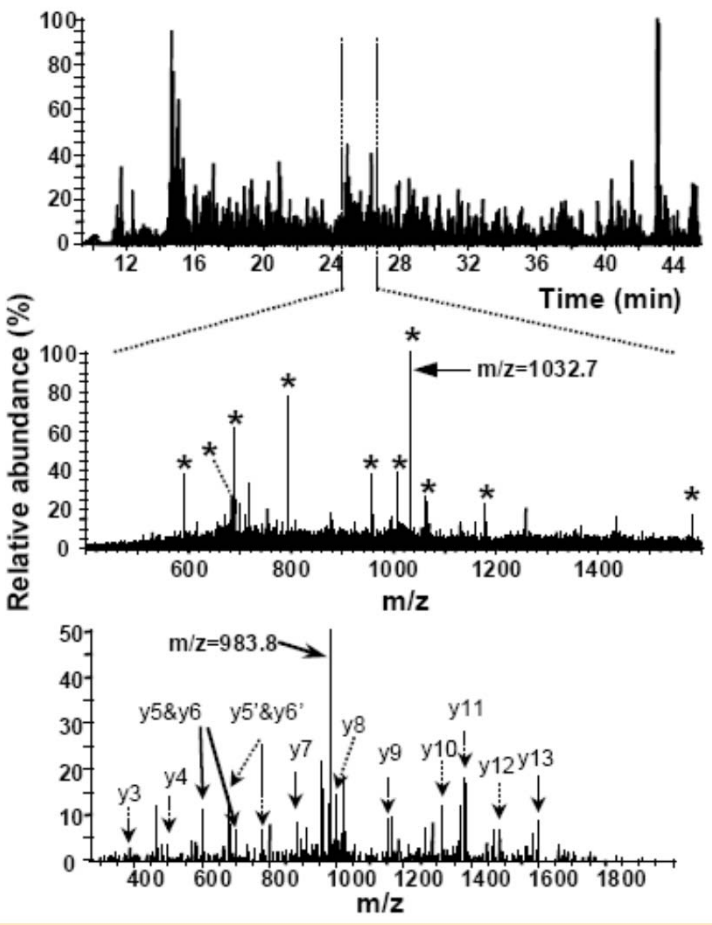


Zhou et al., Nat Biotechnol, 2001, 19, 375-378

芽殖酵母的磷酸化组分析

- 酵母的细胞裂解液
- Trypsin酶切：K/R↓-(not P)
- 磷酸肽富集、质谱分析
- 数据库搜索
  - ◆根据序列数据库，模拟酶切
  - ◆生成理论峰 + ~49
  - ◆比对实验峰与理论峰
- 局限：
  - ◆多位点磷酸肽难以确定位点
  - ◆Fe^{3+}：与磷酸肽的亲和度不高

TAGIQIVADDLT*VT*NPAR



48.9, 确切位点未知

Zhou et al., Nat Biotechnol, 2001, 19, 375-378

芽殖酵母的磷酸化组分析

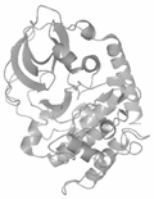
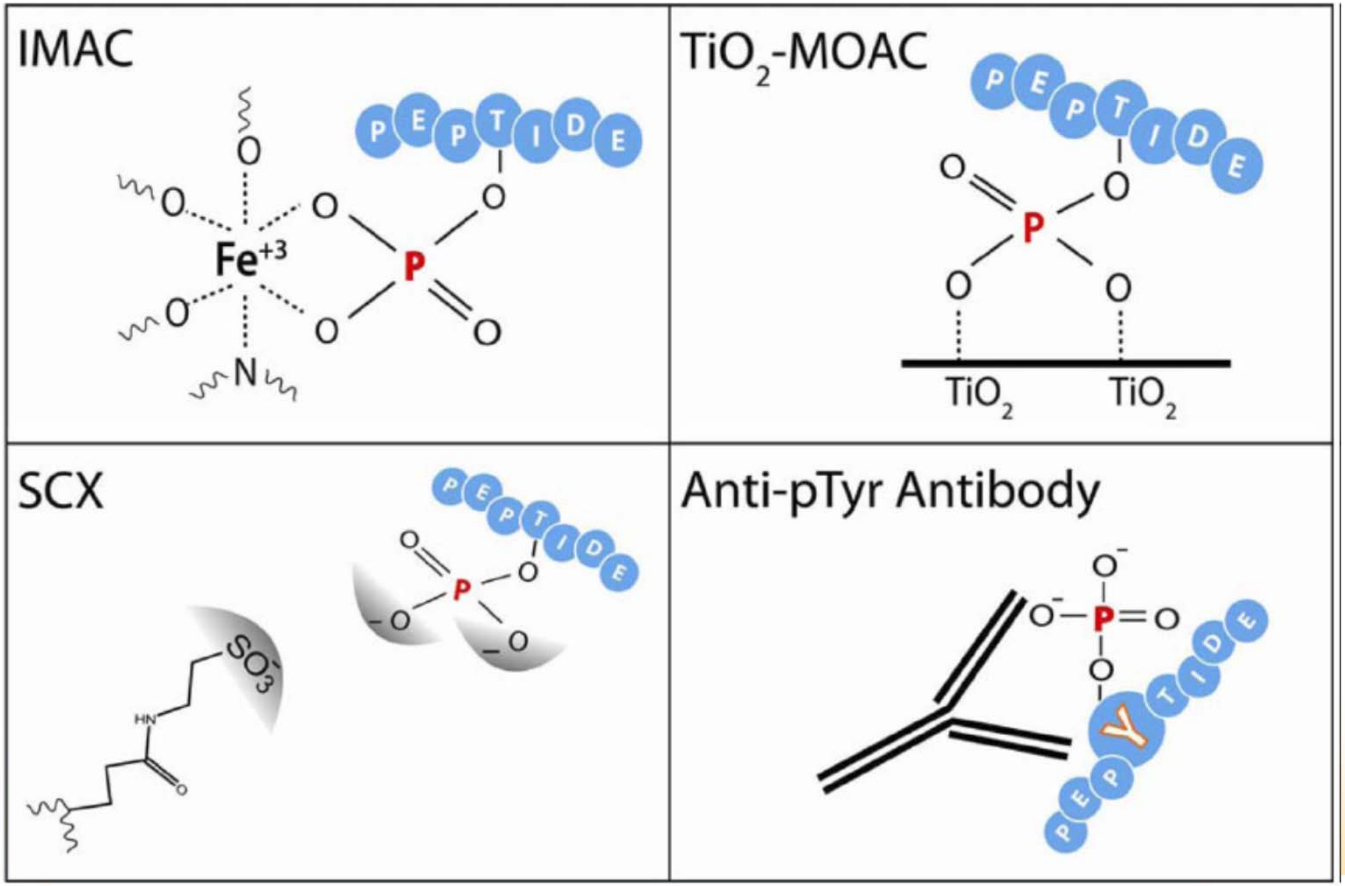


Table 1. Phosphopeptide profile from yeast grown with glucose as a carbon source

Entry name[a]	Protein name	Phosphopeptide identified[b]
ENO1_yeast & ENO2_yeast	Enolase	TAGIQIVADDLT*VT*NPAR[c]
		IGLDCAS*S*EFFK[c]
		SGET*EDT*FIADLVVGLR[c]
G3P1_yeast	Glyceraldehyde 3-phosphate dehydrogenase	LVSWYDNEYGYS*T*R[c]
		VIS*NASCTTNCLAPLAK
		VISNASCT*T*NCLAPLAK[c]
		TASGNIIPSST*GAAK
DCP1_yeast	Pyruvate decarboxylase isozyme 1	NPVILADACCS*R
		TPANAAVPAS*T*PLK[c]
KPY1_yeast	Pyruvate kinase 1	GVNLPGTDVDLPALS*EK
		GVNLPGT*DVDLPALSEK
PGK_yeast	Phosphoglycerate kinase	DVT*FLNDCVGPEVEAAVK
		VLENT*EIGDSIFDK
		EGIPAGWQGLDNGPES*R
		ASAPGS*VILLENLR
		ELPGVAFLS*EK
PGM1_yeast	Phosphoglycerate mutase 1	SFDVPPIDASSPFS*QK
		VYPDVLYT*S*K[c]
ALF_yeast	Aldolase	FAIPAINVT*S*S*S*T*AVAALEAAR[c]
G6PI_yeast	Glucose-6-phosphate isomerase	EANVT*GLR
HS75_yeast	Heat shock protein	SQIDEVVLVGGS*T*Rc
HS72_yeast	Heat shock protein	TTPSFVGFTDT*ER
RL11_yeast	60S ribosomal protein	VLEQLSGQTPVQS*K
R141_yeast	40S ribosomal protein	IEDVTPVPS*DS*T*Rc

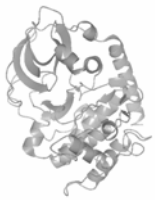
磷酸肽的富集

- IMAC & MOAC: 磷酸肽在后期分离出
- SCX: 磷酸肽在前期分离出



Grimsrud, et al., ACS Chem Biol, 2010, 5, 105-119

HeLa细胞核的磷酸化组分析

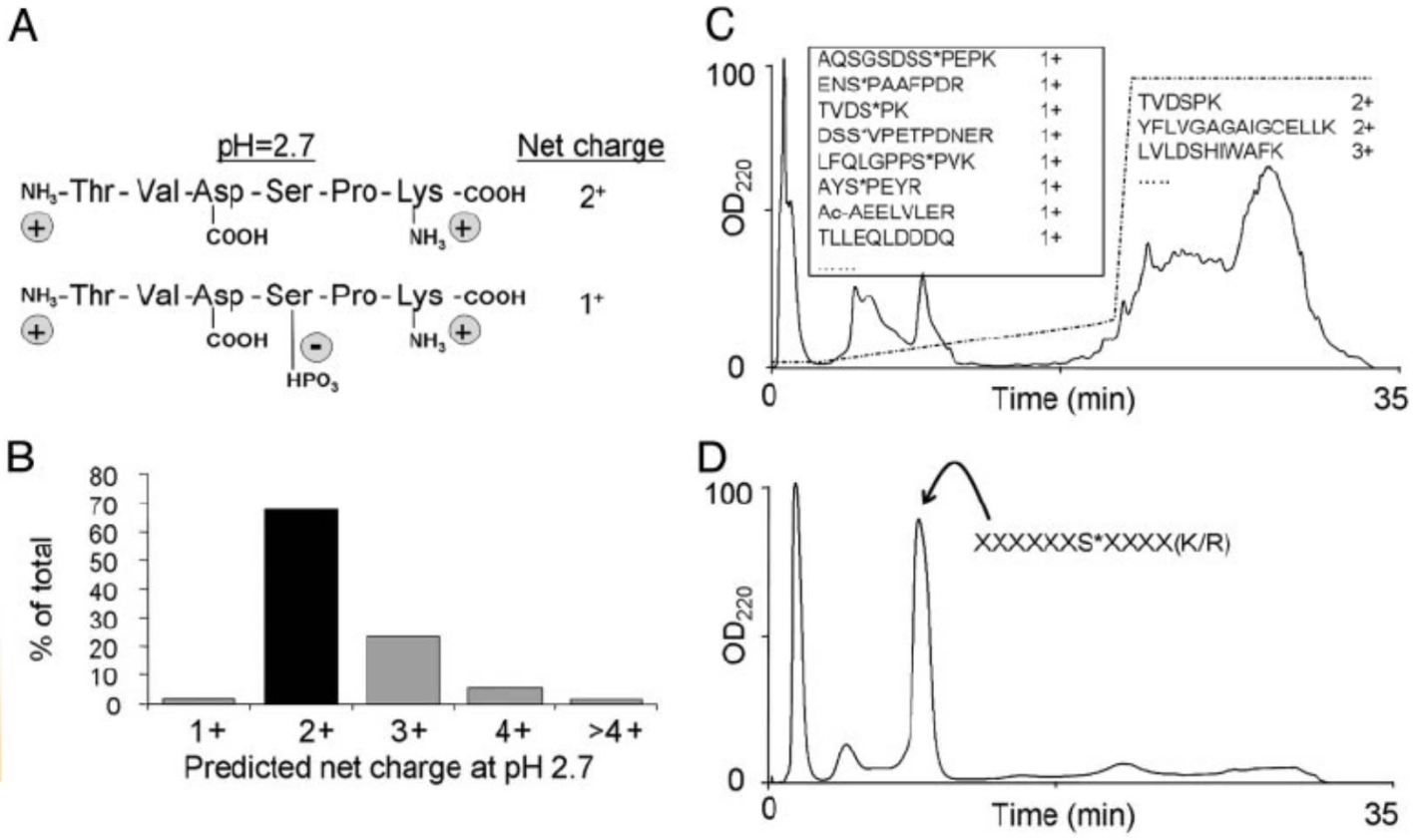


- HeLa细胞的核蛋白
  - SDS/PAGE胶，15*15*0.15cm
  - Buffer front为4cm的时候停止
  - 切成十个区域：0.4*15cm
- Strong cation exchange (SCX) 色谱分离
  - pH 2.7时，K, R, H和肽段N端带正电
  - Trypsin酶切：K/R↓-(not P)
  - 大多数肽段带双电荷 (+2)
  - 酸性环境中：磷酸根带负电
  - 磷酸肽带单电荷 (+1)

Beausoleil SA, et al. PNAS, 2004, 101:12130-12135

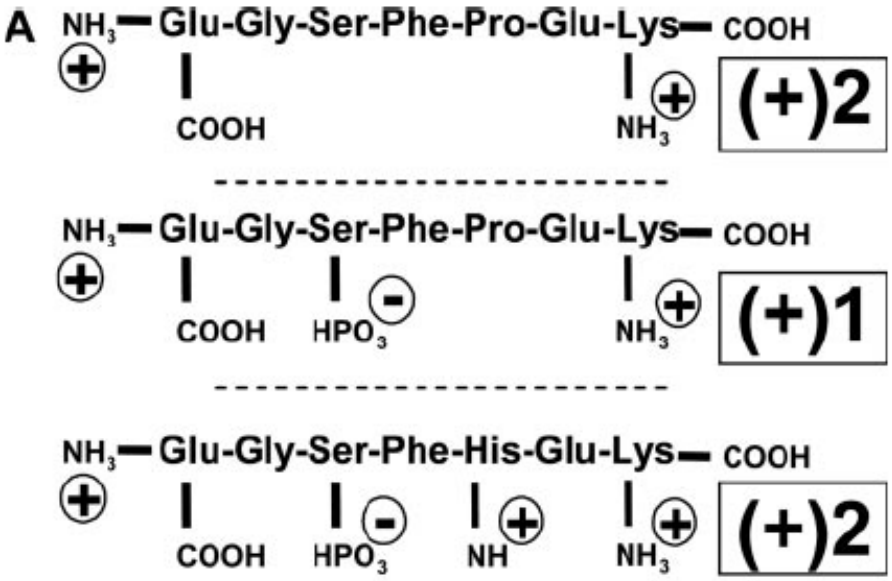
强阳离子交换色谱 (SCX)

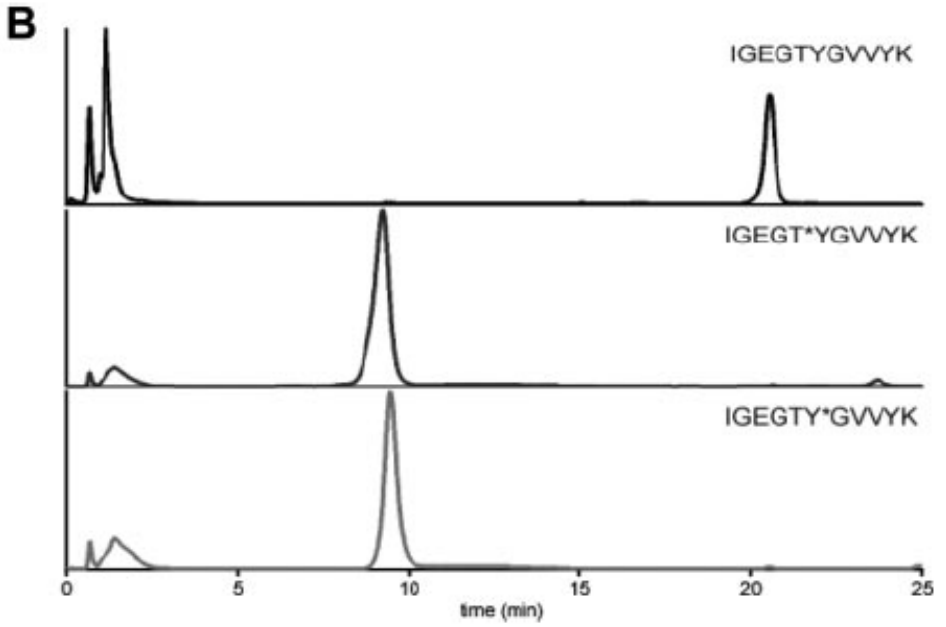
- 对序列数据库模拟酶切，估算带不同电荷的肽段分布
- 早期分离：2,000合成磷酸肽



强阳离子交换色谱 (SCX)

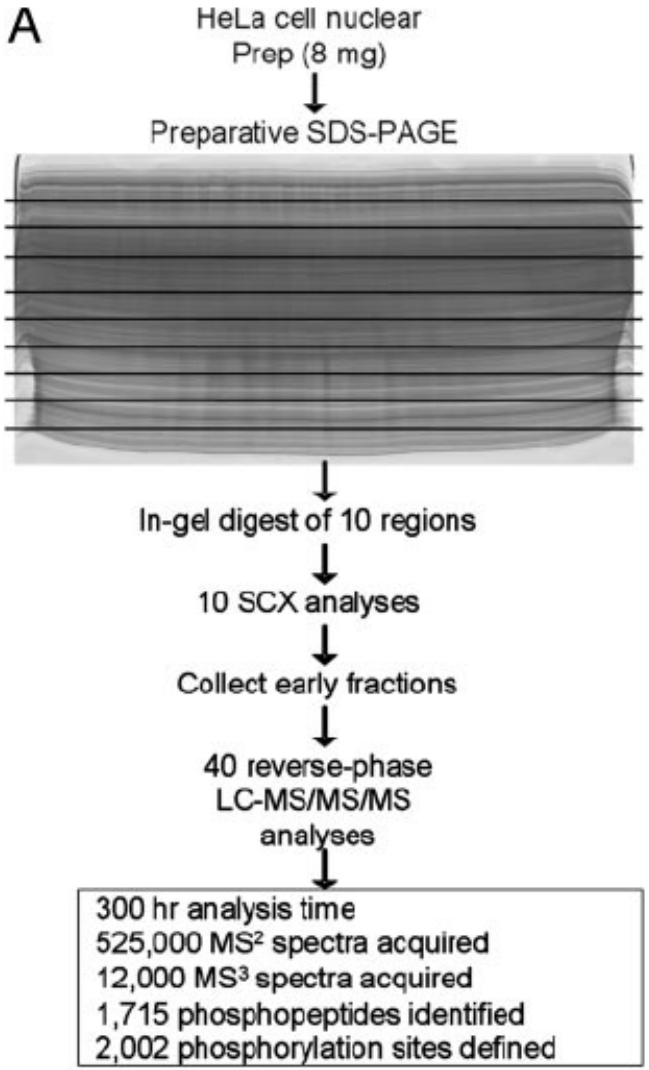
- 优点：磷酸肽带单正电荷，在早期被分离
- 缺点：磷酸肽包含H，则无法分离

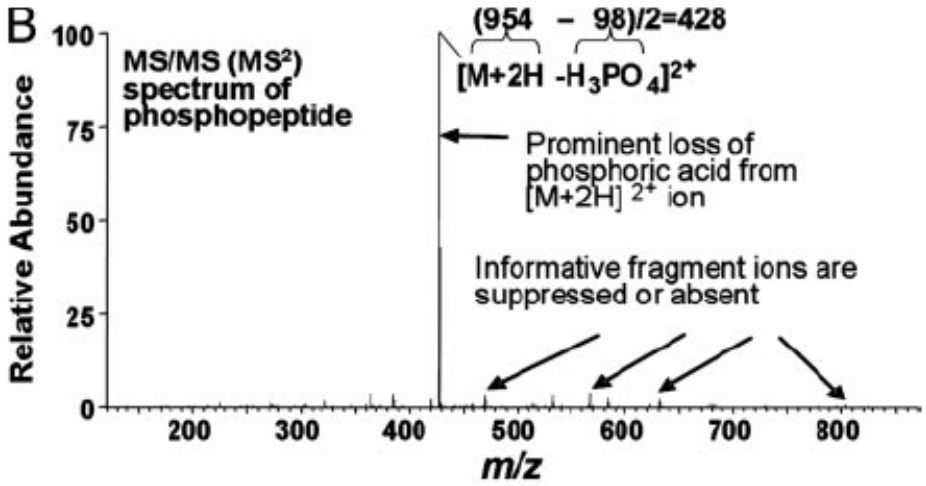


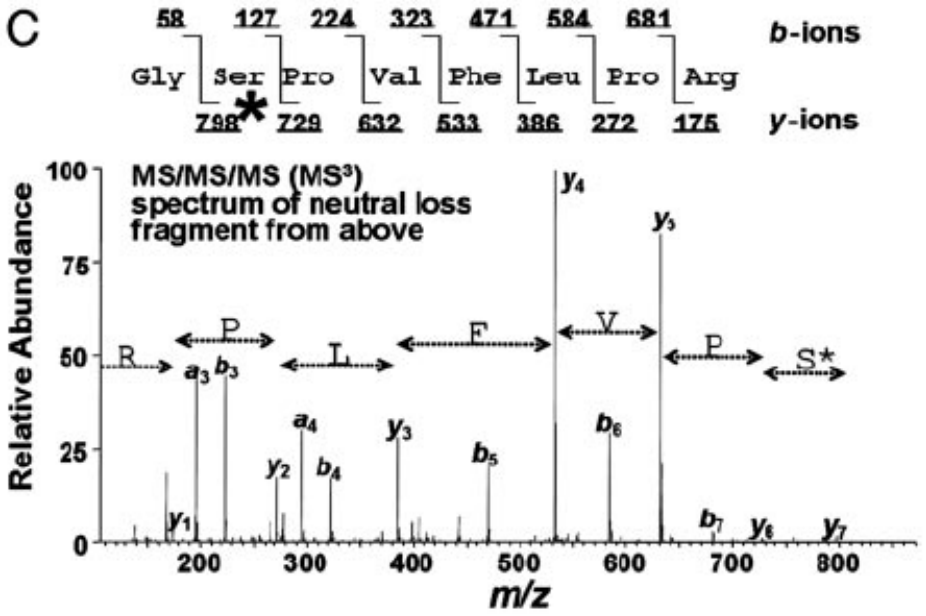


Ballif et al., Mol Cell Proteomics, 2004, 3:1093-1101

鉴定流程

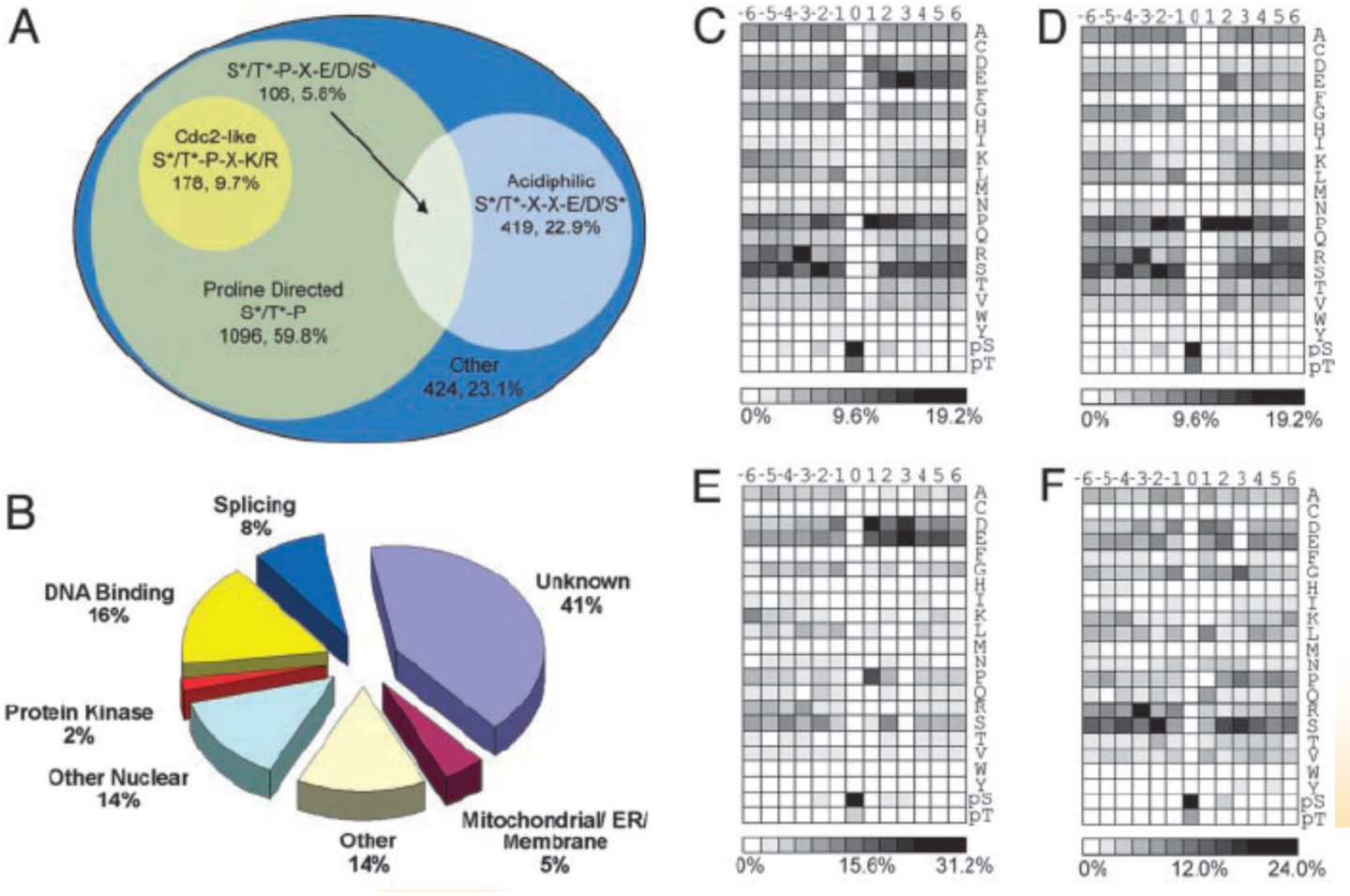

A
HeLa cell nuclear
Prep (8 mg)
Preparative SDS-PAGE
In-gel digest of 10 regions
10 SCX analyses
Collect early fractions
40 reverse-phase
LC-MS/MS/MS
analyses
300 hr analysis time
525,000 MS² spectra acquired
12,000 MS³ spectra acquired
1,715 phosphopeptides identified
2,002 phosphorylation sites defined


B
MS/MS (MS²)
spectrum of
phosphopeptide
(954 – 98)/2=428
[M+2H -H3PO4]2+
Prominent loss of
phosphoric acid from
[M+2H] 2+ ion
Informative fragment ions are
suppressed or absent
Relative Abundance
m/z

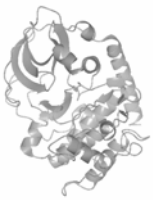

C
58 127 224 323 471 584 681
b-ions
Gly Ser Pro Val Phe Leu Pro Arg
798* 729 632 533 386 272 175
y-ions
MS/MS/MS (MS³)
spectrum of neutral loss
fragment from above
Relative Abundance
m/z

功能分析

- **2,002个磷酸化位点：**
  - **◆Pro-directed (AKT, PKA, and Clk2) or acidiphilic (CK2)**



EGF刺激的HeLa细胞

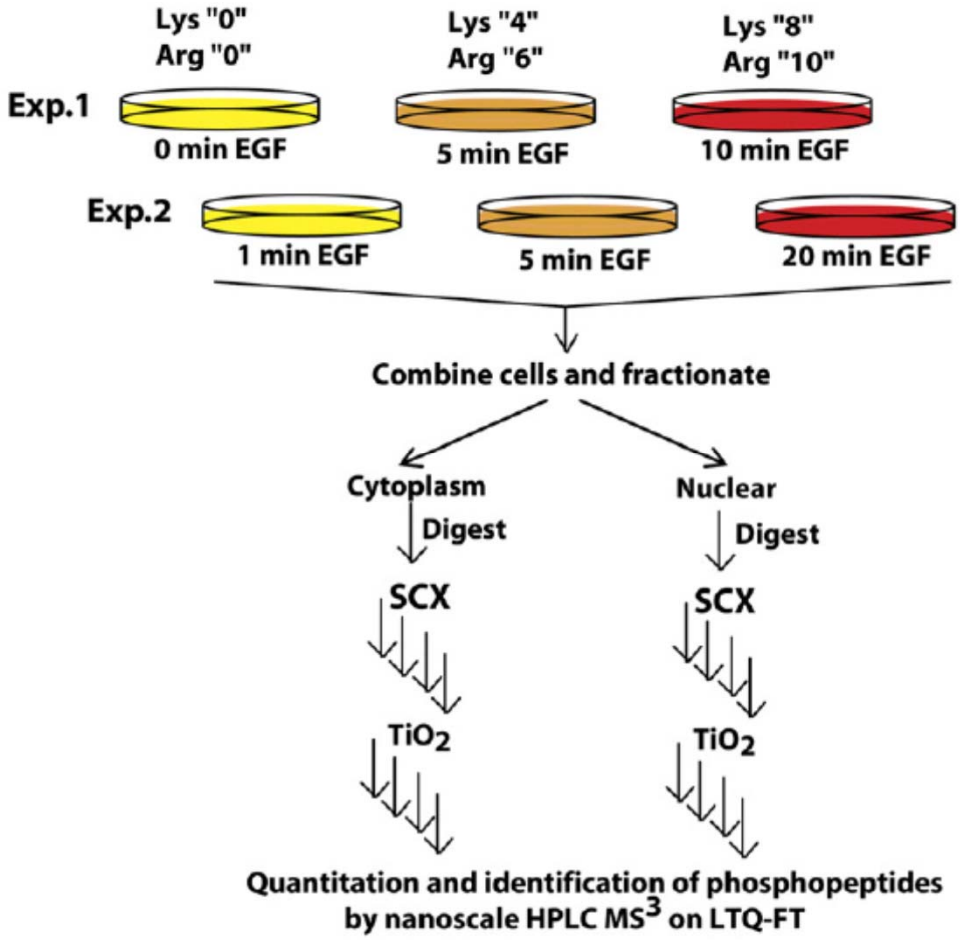


- 定量磷酸化蛋白质组学
  - Exp. 1: 0, 5min, 10min
  - Exp. 2: 1min, 5min, 20min
- SCX & TiO_2 (MOAC, metal oxide affinity chromatography)
  - 富集磷酸肽
- 6,600个磷酸化位点，2,244个蛋白质
- pY, pT, and pS 的比例：
  - 1.8%, 11.8%, and 86.4%

Olsen, et al., Cell, 2006, 127, 635-648

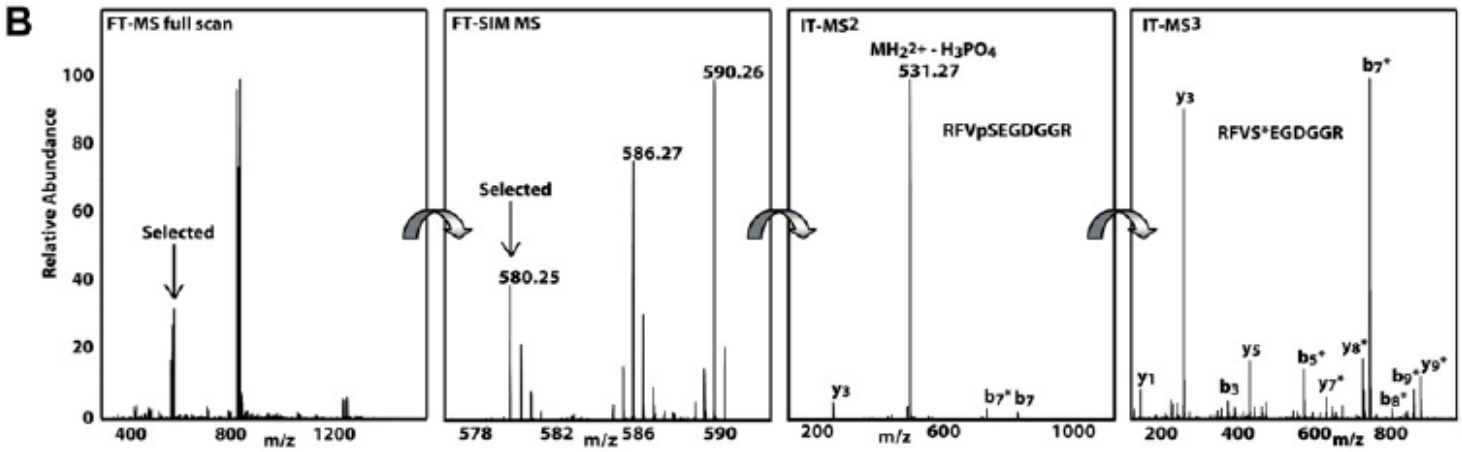
EGF刺激的HeLa细胞

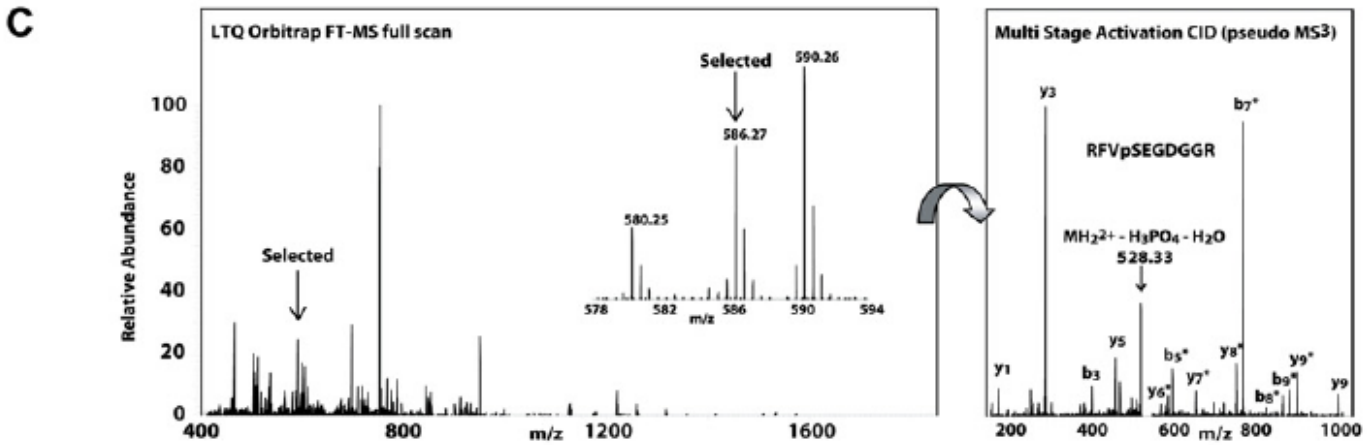
- **SILAC: Stable isotope labeling by amino acids in cell culture**



磷酸化位点的鉴定

- 根据分子量差异找到不同样本的同一个肽段
- 根据分子量最小的峰搜索相应磷酸肽

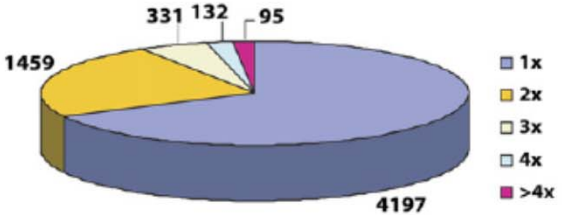




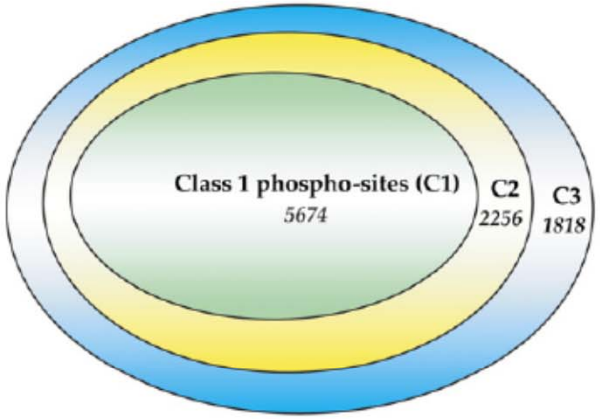
磷酸化的动态变化

- 大多数磷酸肽为单位点
- Class 1: 可信度高的结果
- 约15%的位点受EGF调控

A



B

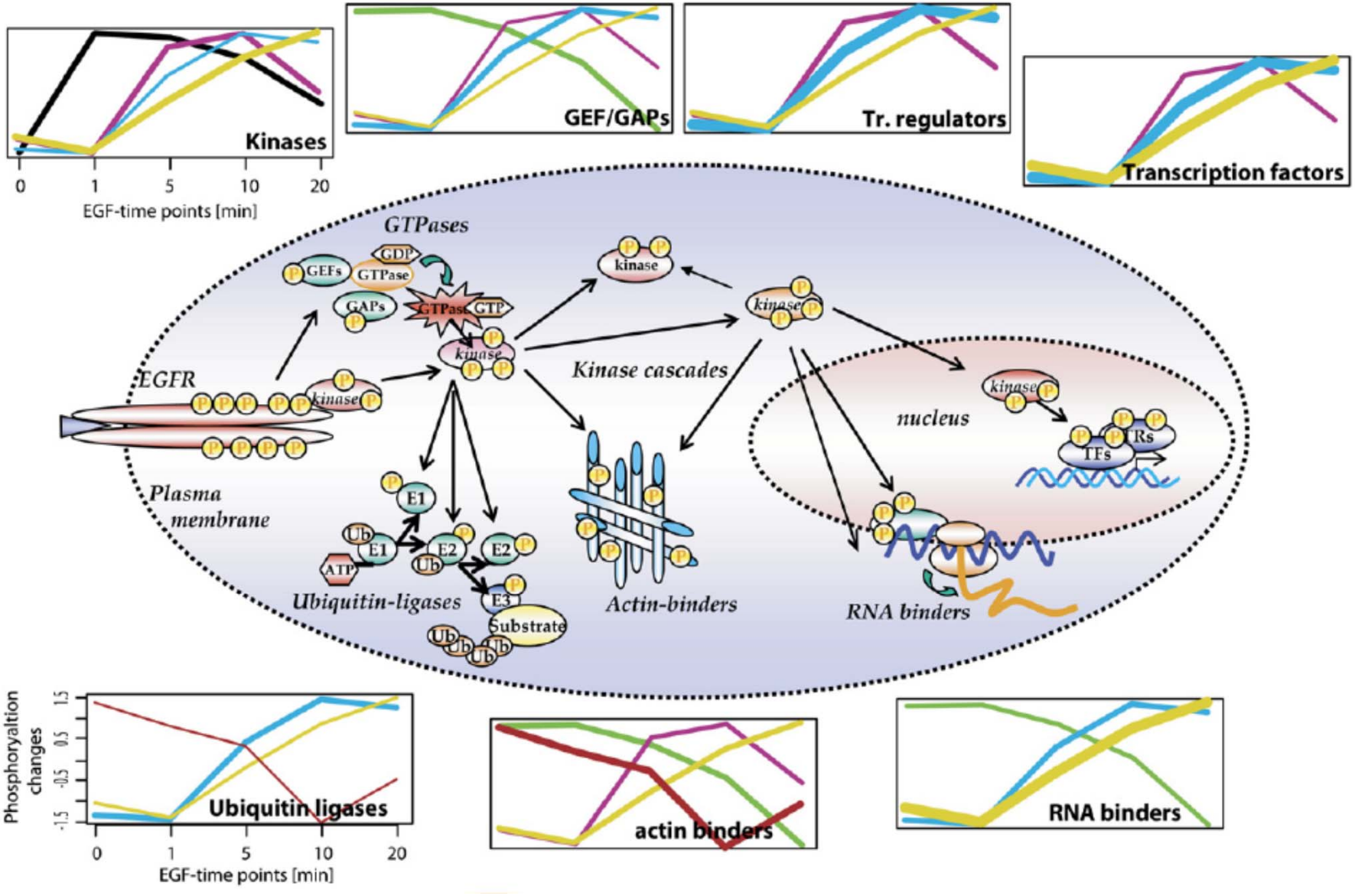


C

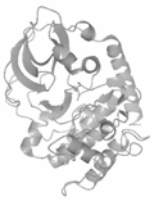
Distribution of phosphorylation sites by amino acid

Site	Class I	Percent	EGF-regulated	Percent
pSer	4901	86.4%	724	82.0%
pThr	670	11.8%	106	12.0%
pTyr	103	1.8%	53	6.0%

受调控磷酸化蛋白质分类



拟南芥磷酸化组分析

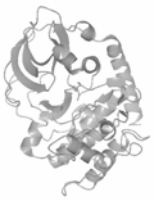


- 拟南芥的细胞裂解液：
  - ◆2,597个磷酸肽
  - ◆2,172个磷酸化位点
  - ◆1,346个蛋白质
  - ◆pS, pT, pY：85.0, 10.7, and 4.3%
- 磷酸肽富集：SCX + MOAC
- 拟南芥的酪氨酸激酶：与人类大致相当

Items	Number
Number of phosphopeptides[a]	2597
Number of phosphoproteins[b]	1346
Number of unique phosphorylation sites	2172
Phosphorylated residues (Ser:Thr:Tyr)	1847:231: 94 (85.0%) (10.6%) (4.3%)

Sugiyama, et al., MSB, 2008, 4, 193.

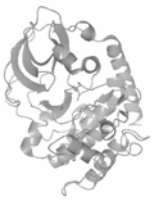
磷酸化位点的位置分布



- pS, pT不倾向分布在蛋白质功能结构域中
- pY基本无偏好
- 总体：磷酸化位点不倾向于发生在结构域内

	Number of proteins possessing Pfam domain	Number of phosphorylation sites		
		Pfam domain[a]		Total (%)
		ON (%)	OUT[b] (%)	
pS	1014	317 (19.1)	1340 (80.9)	1657 (100)
pT	195	74 (32.2)	156 (67.8)	230 (100)
pY	87	49 (48.5)	52 (51.5)	101 (100)
All	1118	440 (22.1)	1548 (77.9)	1988 (100)

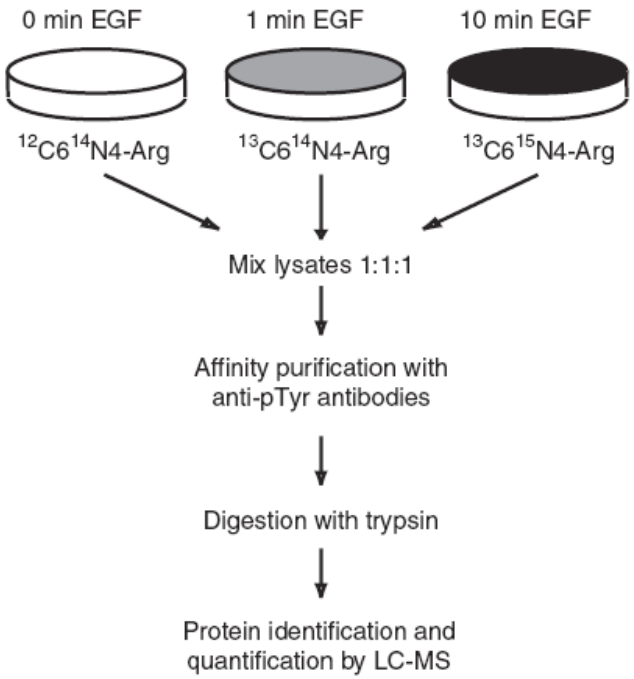
酪氨酸磷酸化组的定量分析

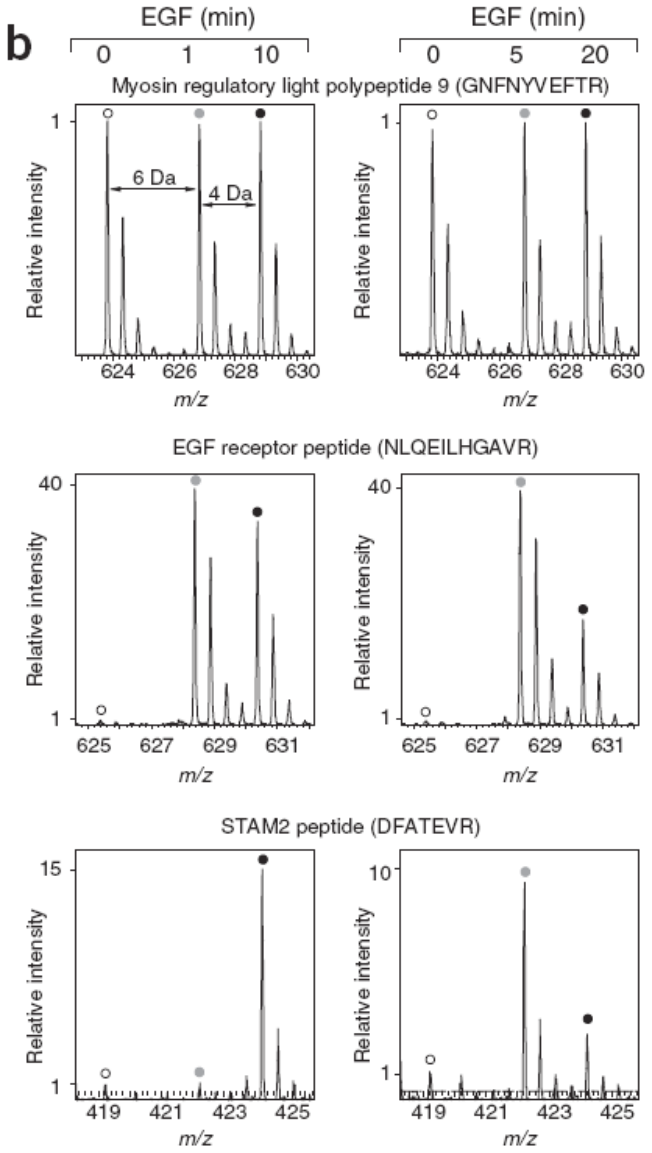


- IMAC & SCX
  - 对pS, pT的鉴定非常有效
  - 对pY的鉴定很差：pY的电性较弱，体积较大
- 酪氨酸磷酸化抗体：4G10, P-Tyr-100
- 生物学问题：EGF (表皮生长因子)的效应
  - 0, 1min, 5min, 10min, 20min
  - 同位素标记：SILAC
  - 样品混合，质谱鉴定

Blagoev, et al., Nat Biotechnol, 2004, 22, 1139-1145

酪氨酸磷酸化组分析



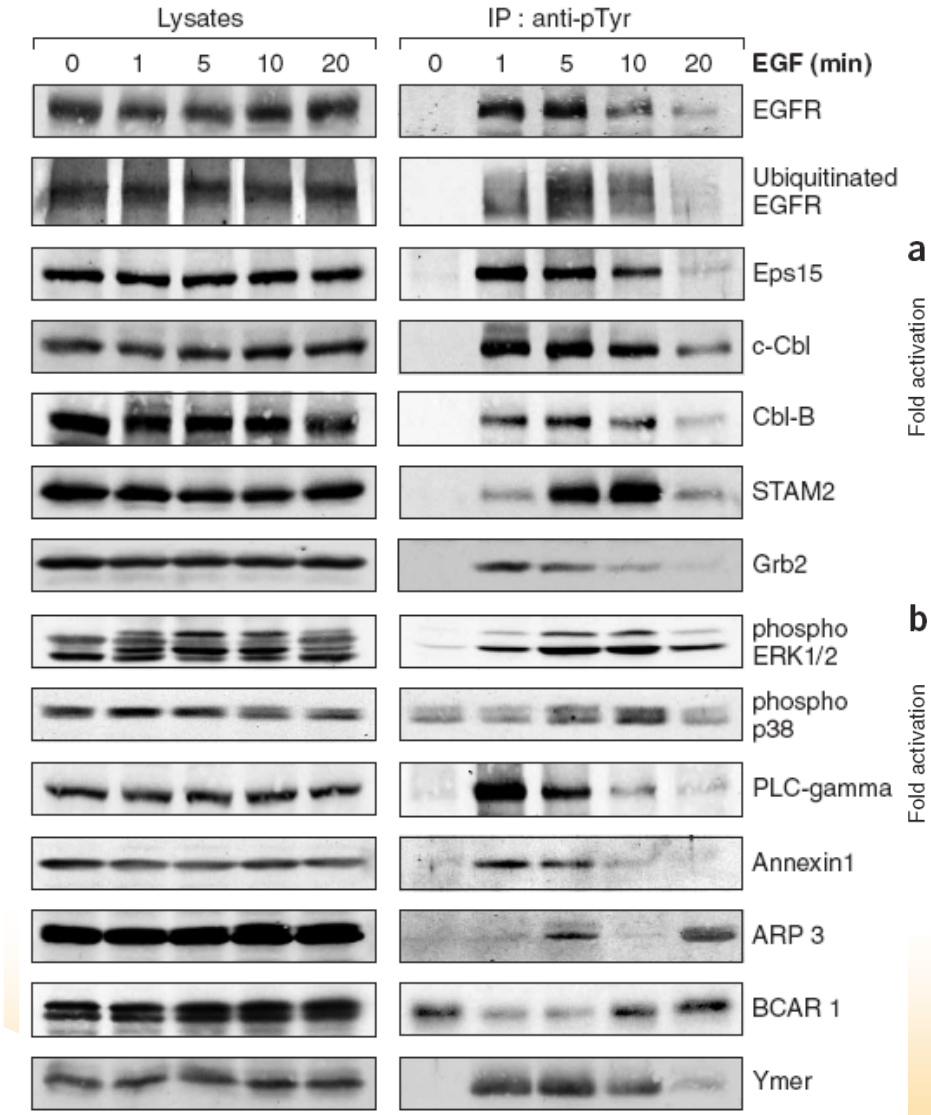


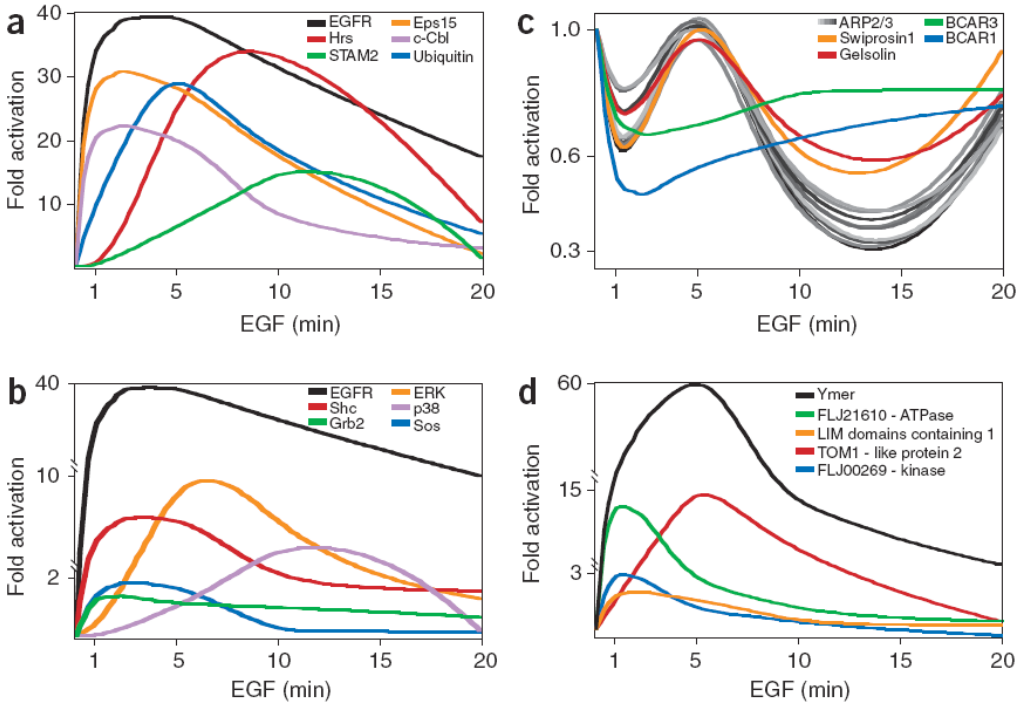
不变

升高->降低

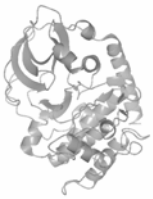
升高->降低

Western blot验证





DNA damage相关的磷酸化组

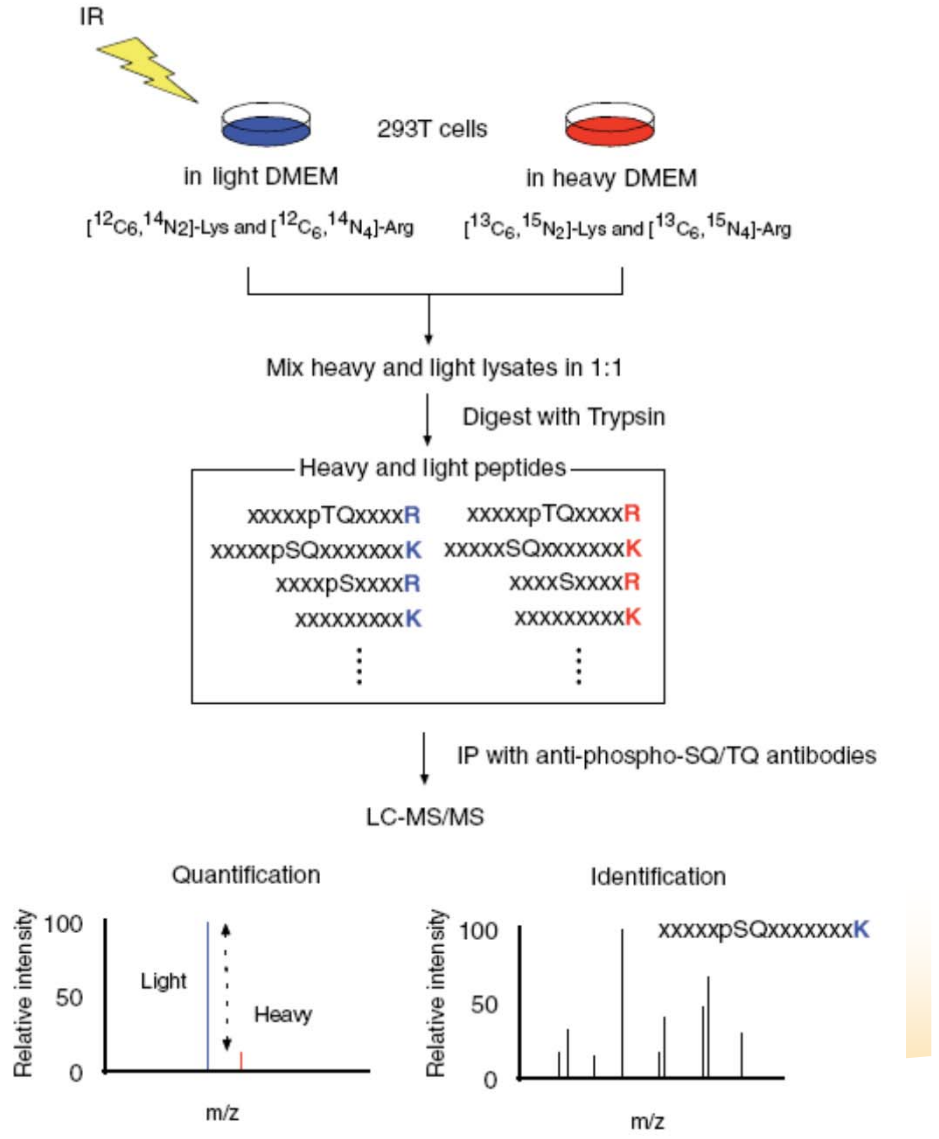


- DNA damage
  - Ionizing radiation (IR): 1hr
  - ATM和ATR激活：识别S/T-Q模体
- 富集磷酸肽：特异性抗体
- DNA damage response (DDR)通路
  - ~900个受调控的磷酸化位点
  - ~700个蛋白质

Matsuoka, et al., Science, 2007, 316, 1160-1166

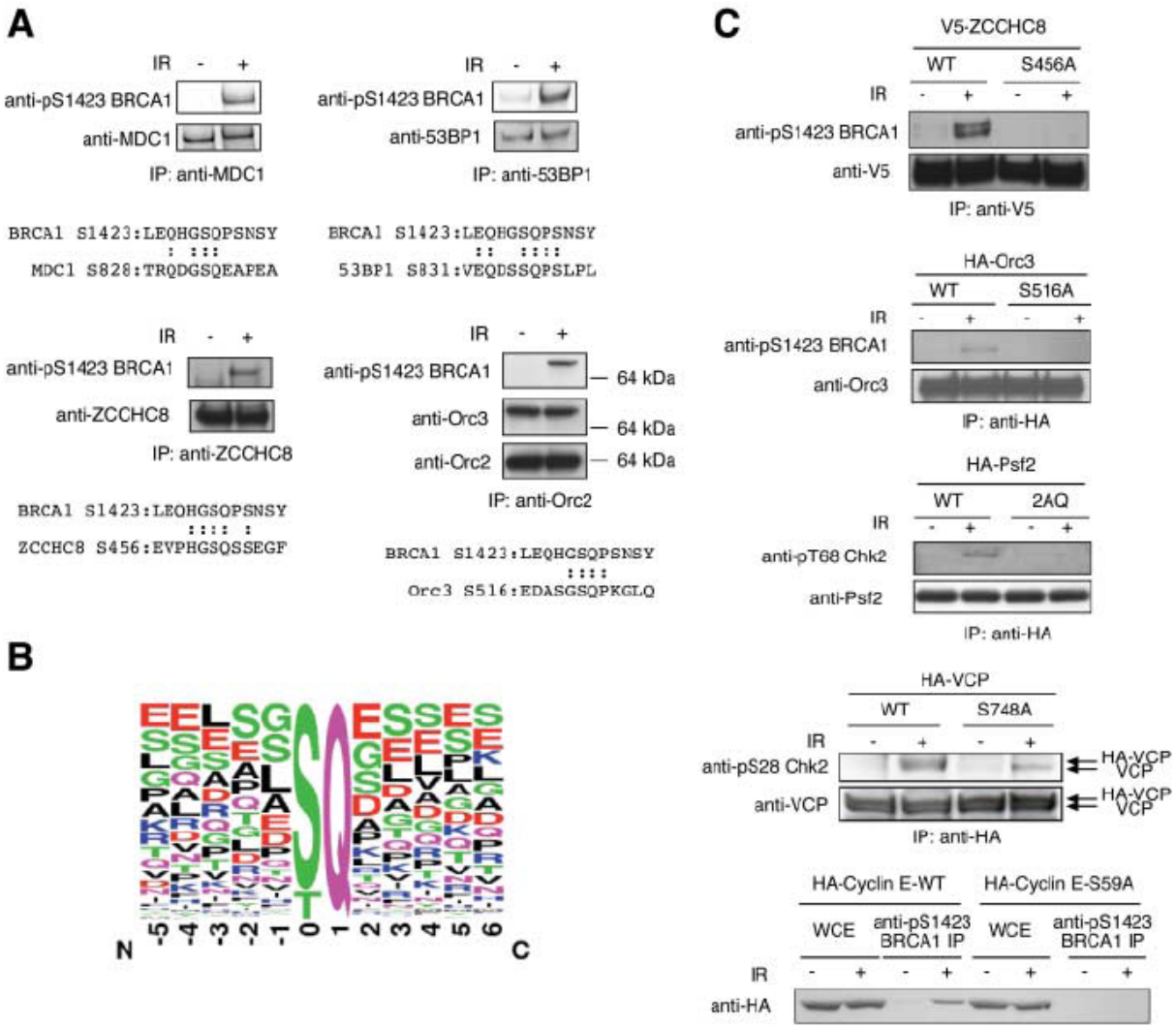
鉴定流程

- 实验组 (IR)与对照组
- SILAC标记
- 样品按照1：1合并
- 酶切，质谱鉴定
- 定量分析



蛋白质磷酸化的验证

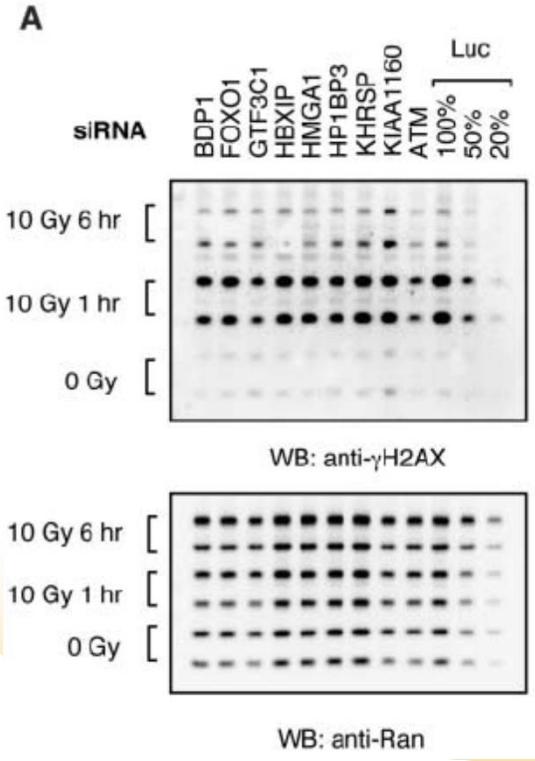
- 根据组学数据的结果，进行Western blot检测
- 序列模体分析 – ATM/ATR的潜在底物

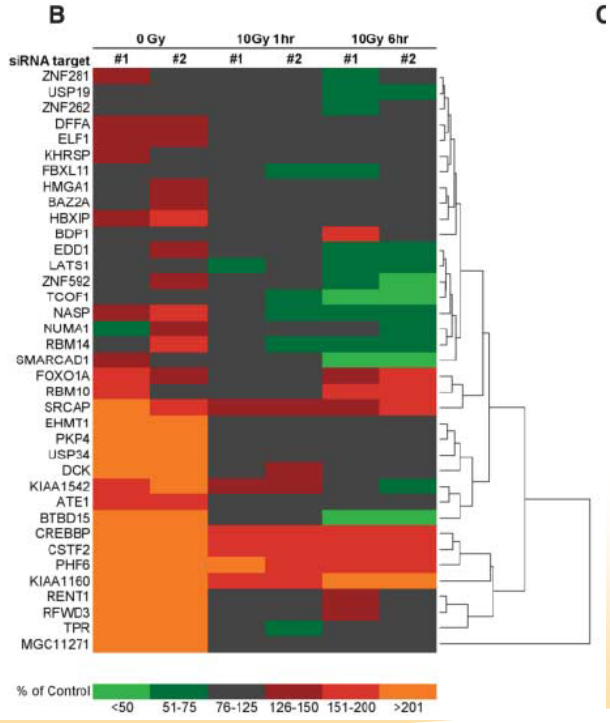


磷酸化蛋白质的功能分析

- γH2AX：DNA修复的关键蛋白质
  - 37个随机挑选的蛋白质，siRNA沉默
  - 对γH2AX表达的影响
  - 76-125%：影响不大

- 对细胞表型的影响
  - HR: 同源重组
  - G2-M checkpoint,
  - Intra-S phase checkpoint
  - γH2AX

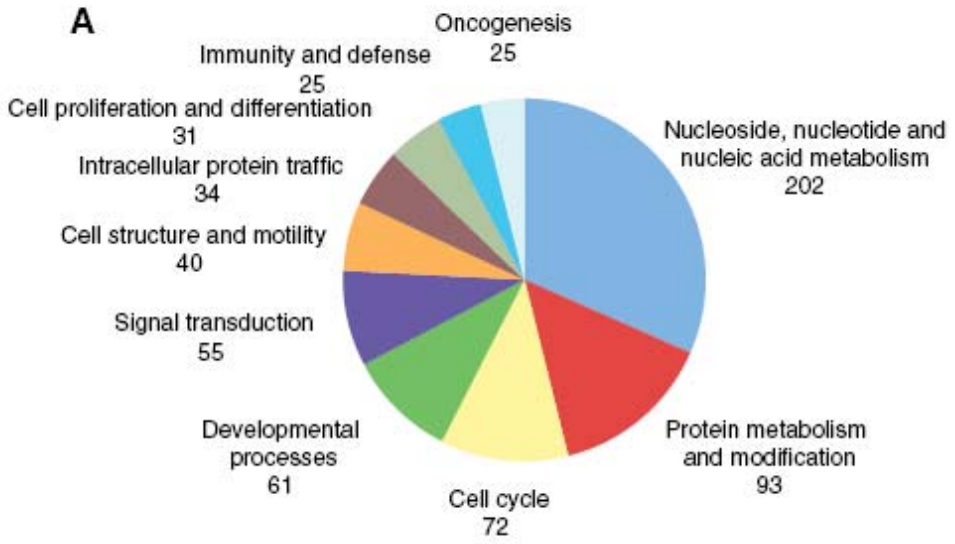


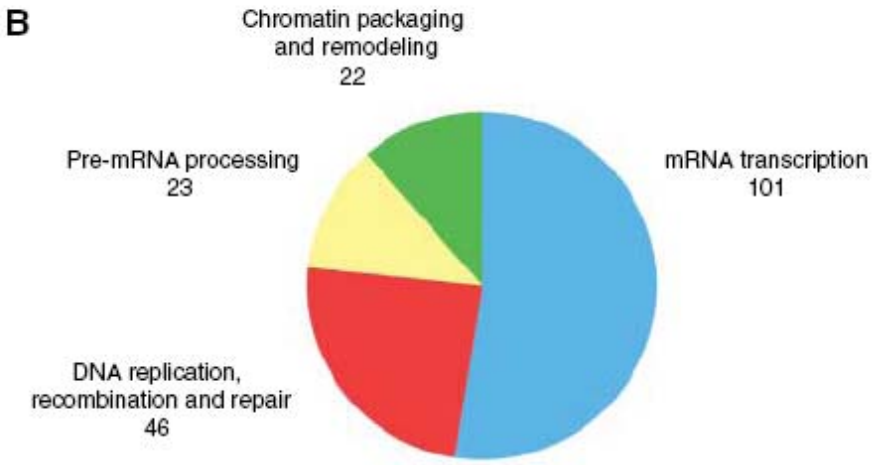


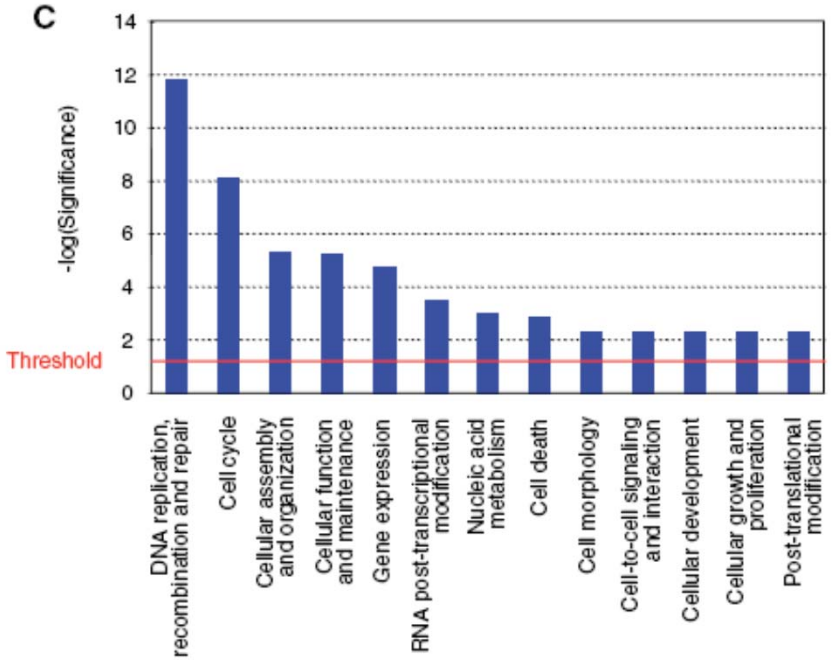
C

HR	G2/M	Intra-S	γH2AX
ATE1	ATE1	ATE1	ATE1
BAZ2A	BAZ2A	BAZ2A	BAZ2A
BDP1	BDP1	BDP1	BDP1
BTBD15	BTBD15	BTBD15	BTBD15
CREBBP	CREBBP	CREBBP	CREBBP
CSTF2	CSTF2	CSTF2	CSTF2
DCK	DCK	DCK	DCK
DFFA	DFFA	DFFA	DFFA
EDD1	EDD1	EDD1	EDD1
EHMT1	EHMT1	EHMT1	EHMT1
ELF	ELF	ELF	ELF
FBXL11	FBXL11	FBXL11	FBXL11
FOXO1A	FOXO1A	FOXO1A	FOXO1A
HBXIP	HBXIP	HBXIP	HBXIP
HMGA1	HMGA1	HMGA1	HMGA1
KHRSP	KHRSP	KHRSP	KHRSP
KIAA1160	KIAA1160	KIAA1160	KIAA1160
KIAA1542	KIAA1542	KIAA1542	KIAA1542
LATS1	LATS1	LATS1	LATS1
MGC11271	MGC11271	MGC11271	MGC11271
NASP	NASP	NASP	NASP
NUMA1	NUMA1	NUMA1	NUMA1
PHF6	PHF6	PHF6	PHF6
PKP4	PKP4	PKP4	PKP4
RBM10	RBM10	RBM10	RBM10
RBM14	RBM14	RBM14	RBM14
RENT	RENT1	RENT	RENT1
RFWD3	RFWD3	RFWD3	RFWD3
SMARCAD1	SMARCAD1	SMARCAD1	SMARCAD1
SRCAP	SRCAP	SRCAP	SRCAP
TCOF1	TCOF1	TCOF1	TCOF1
TPR	TPR	TPR	TPR
USP19	USP19	USP19	USP19
USP34	USP34	USP34	USP34
ZNF262	ZNF262	ZNF262	ZNF262
ZNF281	ZNF281	ZNF281	ZNF281
ZNF592	ZNF592	ZNF592	ZNF592

ATM/ATR底物的功能分析



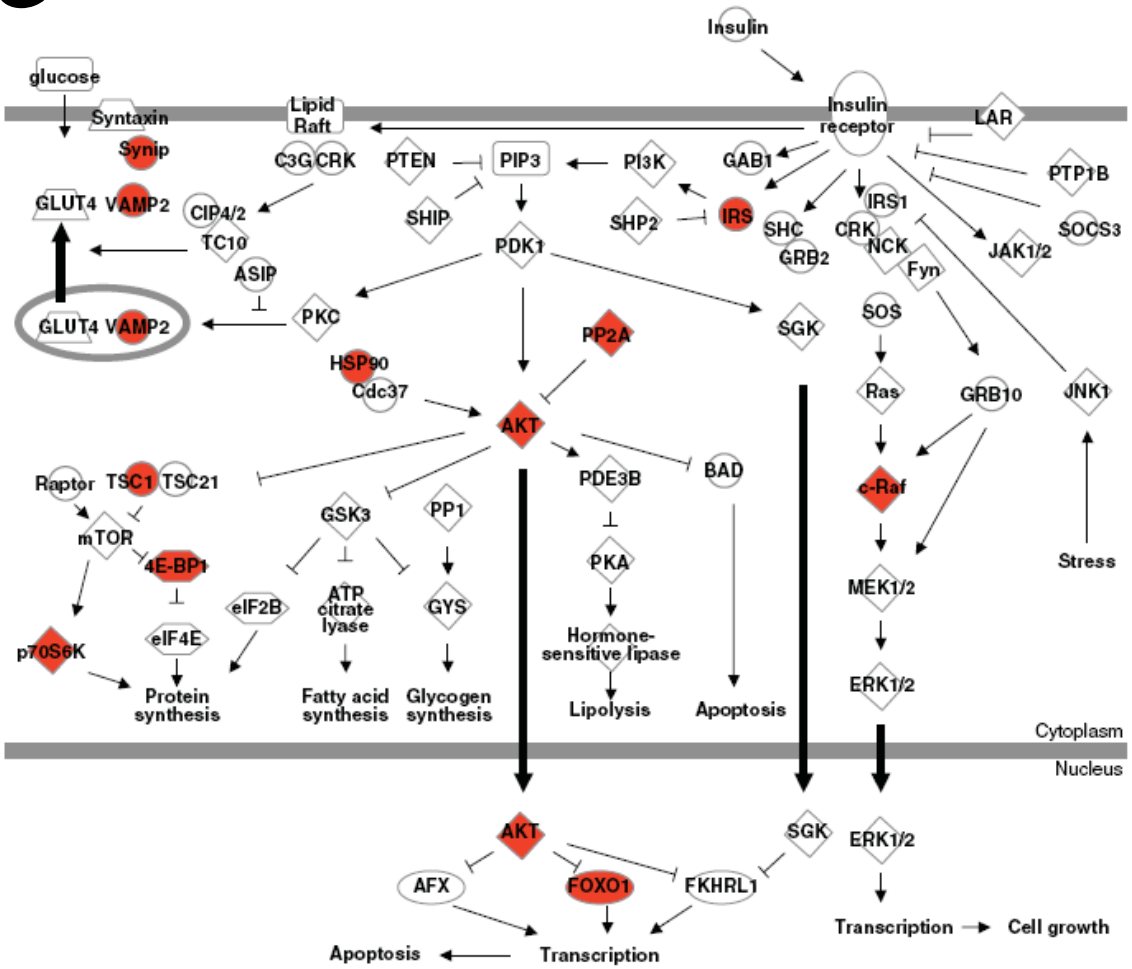




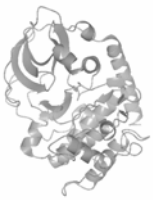
- 超几何分布或Fisher's exact test

网络与通路模拟

- AKT-insulin通路与DDR的潜在关系
- KEGG



小鼠肝脏的磷酸化组分析

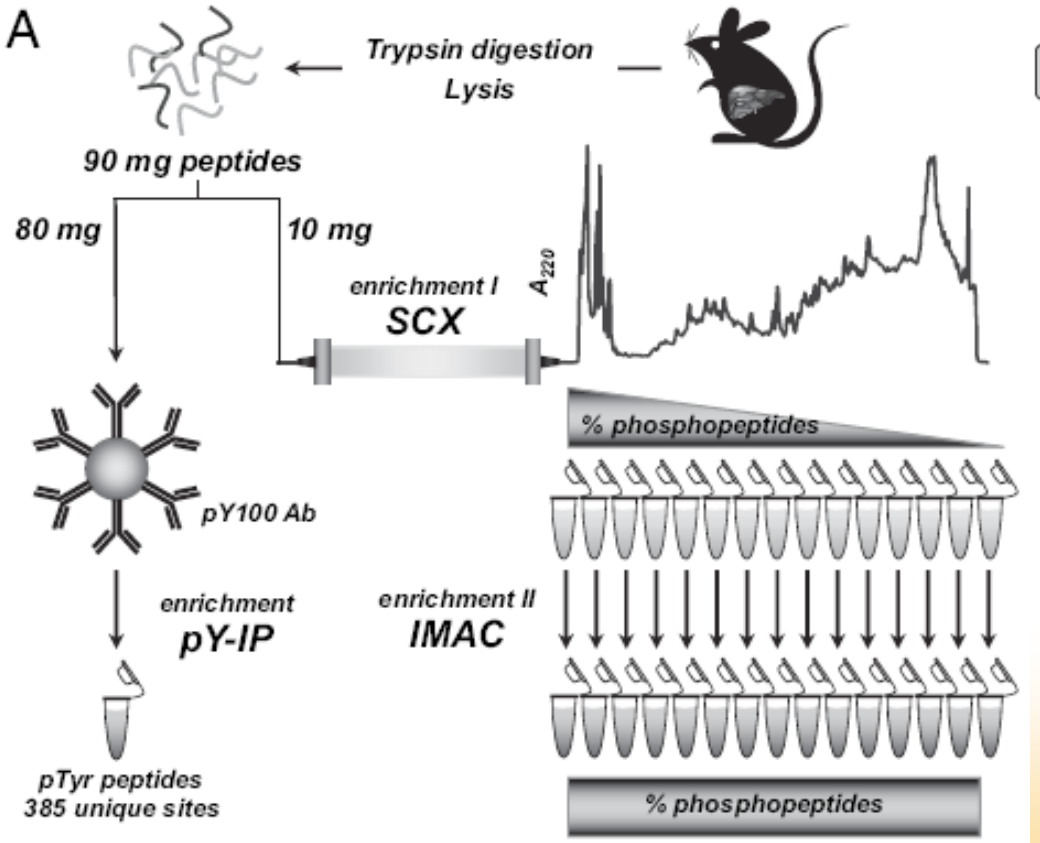


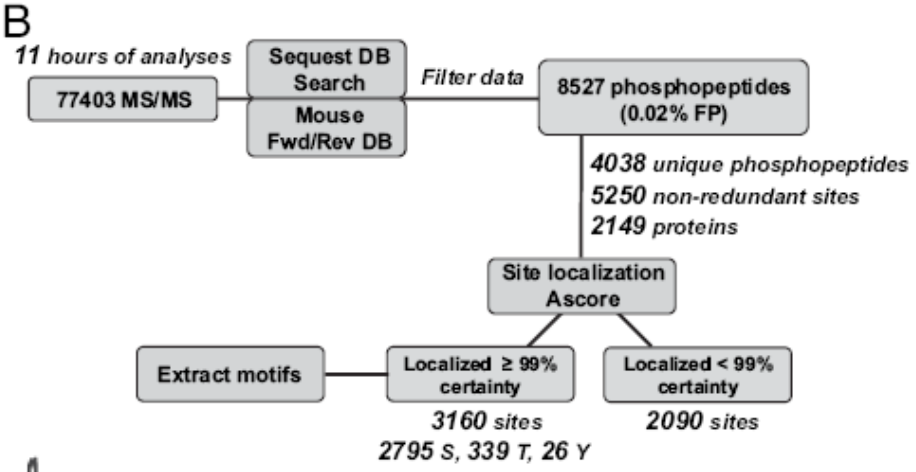
- 21天小鼠的肝脏
  - 90 mg肝脏蛋白质 trypsin酶切
  - 10mg: SCX & IMAC
  - 80mg: pY-IP
- 5,635 个磷酸化位点，2,328个蛋白质
- 磷酸化模体的发现
  - RRxs (PKA), LxRxxs (CaMK), and RxRxxs (AKT)
  - Casein kinase II:sxDxExE, sxxEE, sDxE, and sDxD
  - MAPK: PxsP and PxtP
  - “Dipolar”: Rxxsxx[DE]; R碱性，D/E酸性

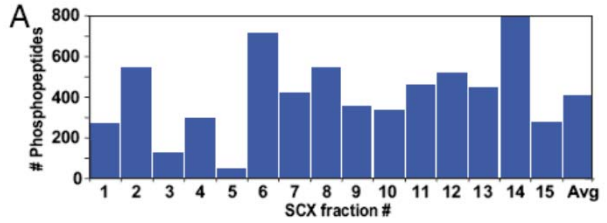
Villen, et al., PNAS, 2007, 104, 1488-1493

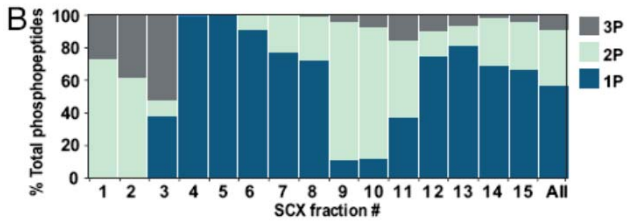
磷酸肽富集

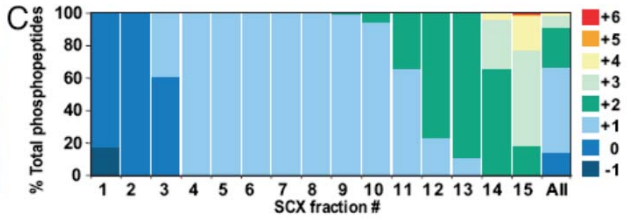
- **SCX + IMAC**
  - ◆15个SCX组分





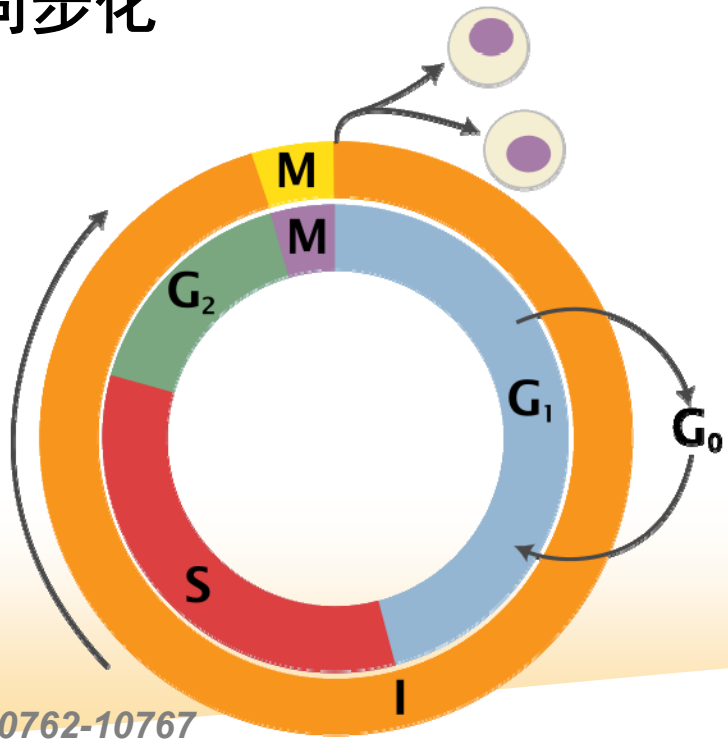






有丝分裂的定量磷酸化组

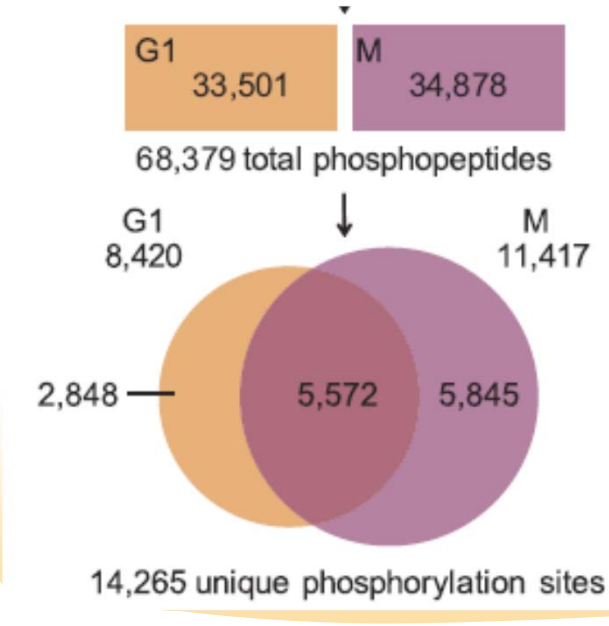
- 细胞有丝分裂：母细胞的物质均分到两个子细胞中
- CDKs激酶是调控有丝分裂的关键因子
- 细胞同步化：
  - ◆培养液中细胞处于各个时期：非同步化
  - ◆Hela cell cyle: 18-20hr
- Double thymidine (DT):
  - ◆2.5 mM, 19hr -> G_1/S phase
  - ◆Release, 9hr
  - ◆Thymidine, 16hr -> G_1/S
  - ◆0.2μg/ml nocodazole -> M

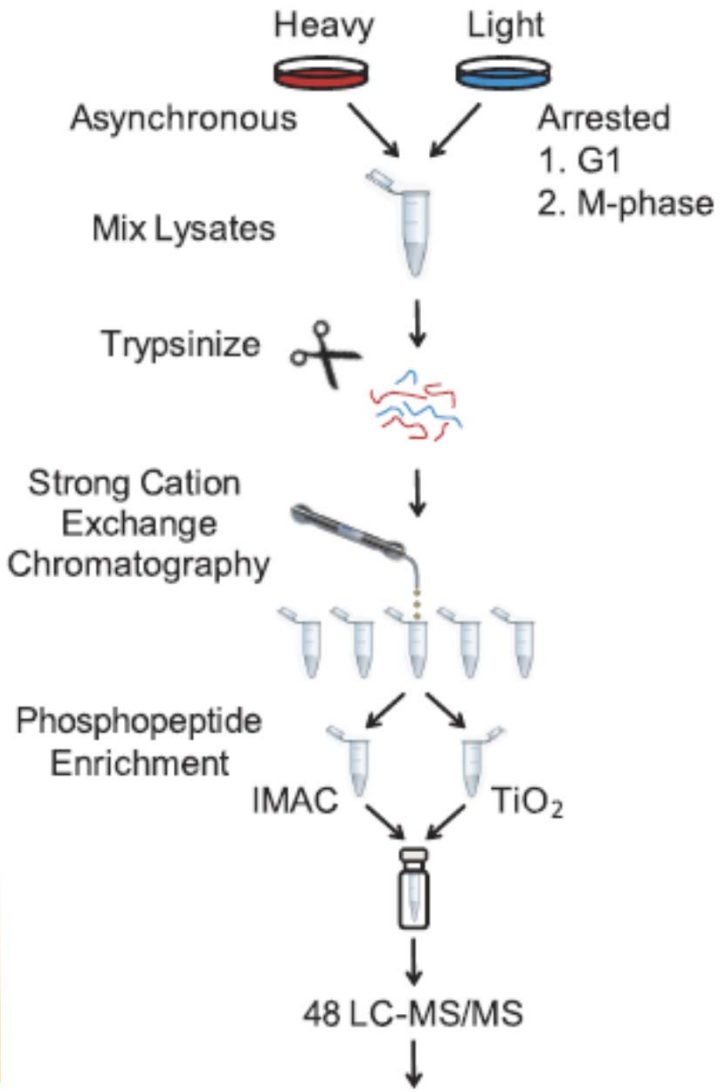


Dephoure, et al., PNAS, 2008, 105:10762-10767

磷酸化位点鉴定

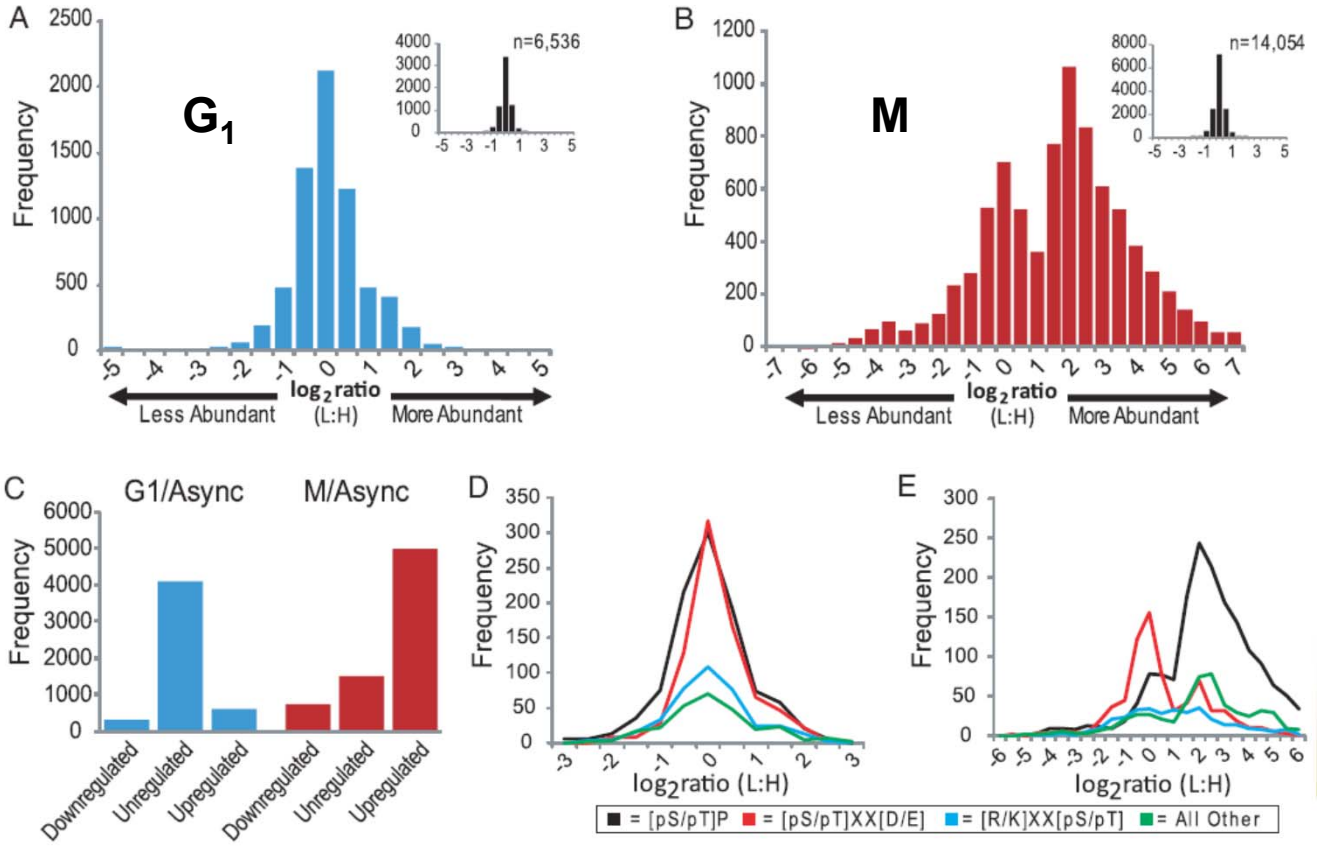
- SILAC标记
  - ◆重：非同步化细胞
  - ◆轻：G_1, M
- SCX + IMAC & MOAC
- 48个组分





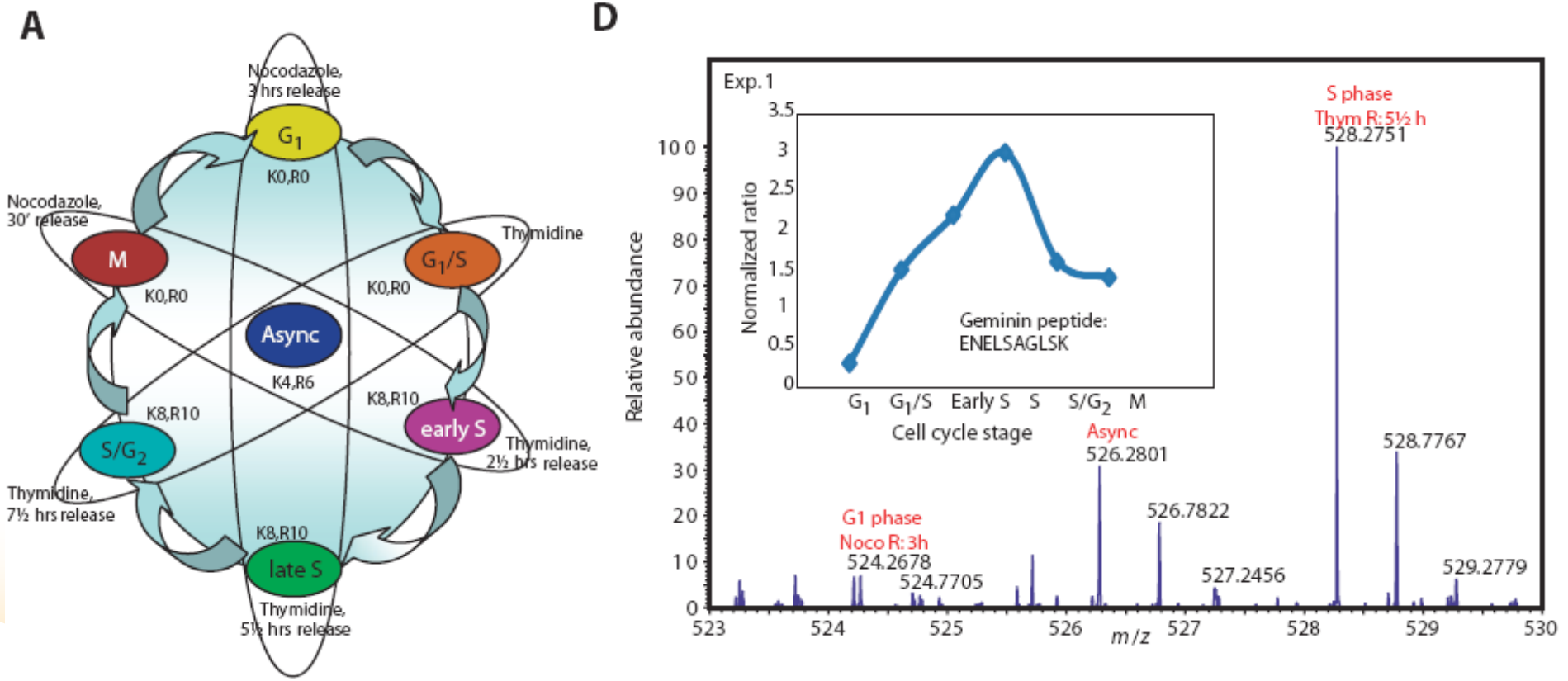
受调控的磷酸化位点

- 受调控 (≥ 2.5 fold) *vs*. 未受调控 (≤1.5 fold)
- M期磷酸化程度高
- [pS/pT]P模体显著 -> CDKs



细胞周期的磷酸化组分析

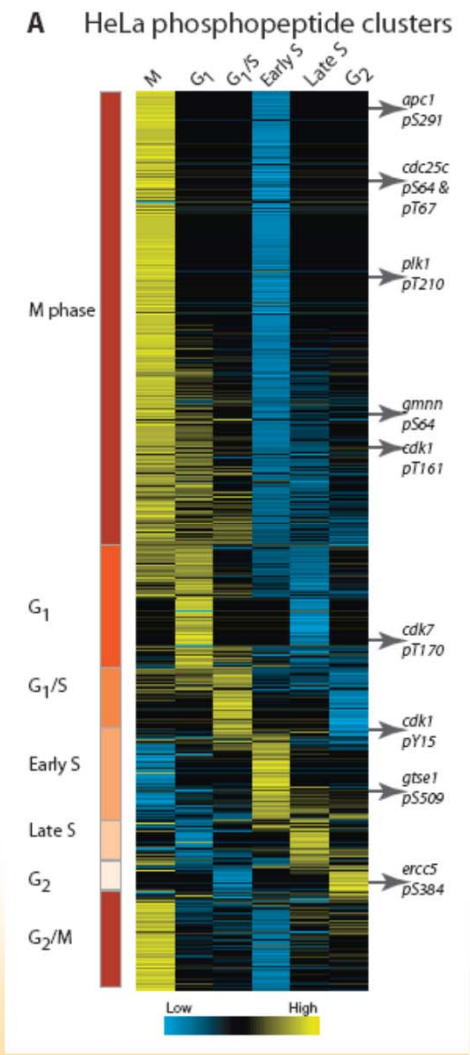
- 利用抑制剂将HeLa细胞阻断在细胞周期的各个时期
- SILAC + SCX + IMAC & MOAC: 6,027个蛋白质，20,443个位点

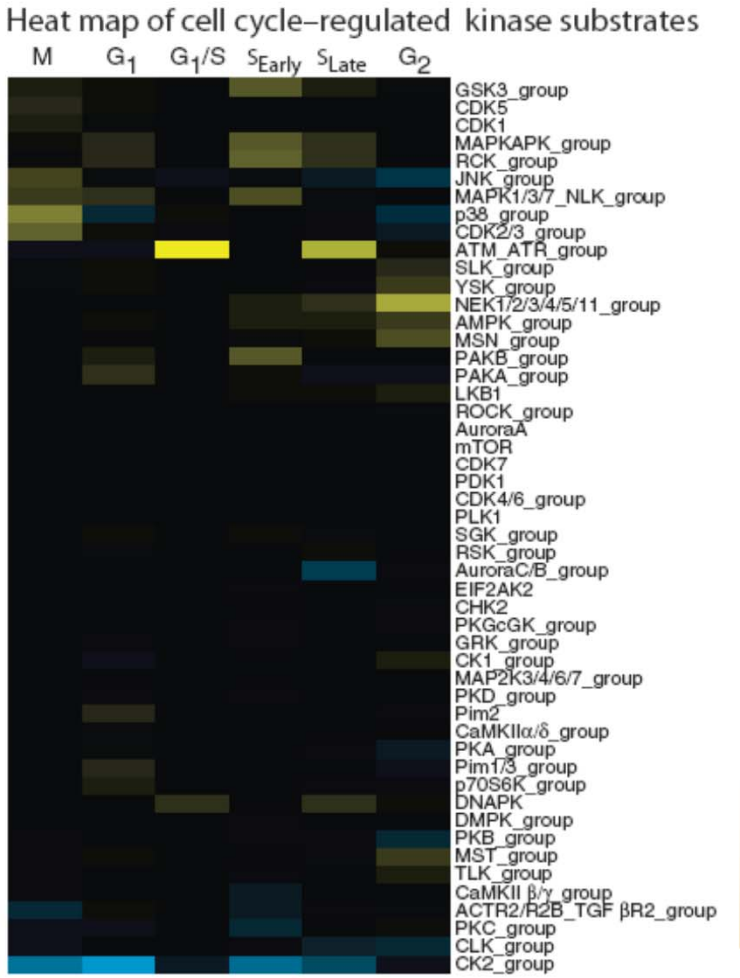


Olsen, et al., Science signaling, 2010, 3, ra3

细胞周期磷酸化组的分析

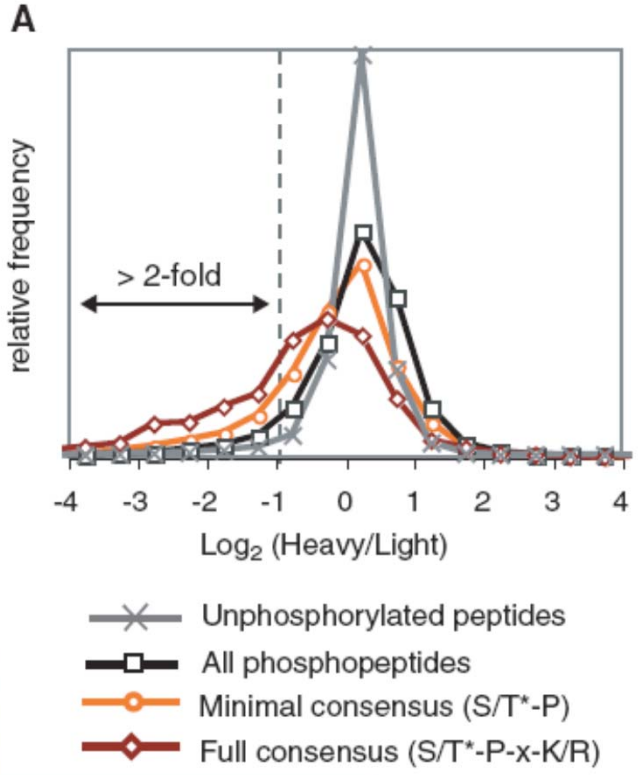
- >50%的蛋白质在有丝分裂期磷酸化水平最高
- 激酶-底物
  - M：CDK/MAPK
  - 其他：ATM/ATR





酵母Cdk1底物及位点的组学分析

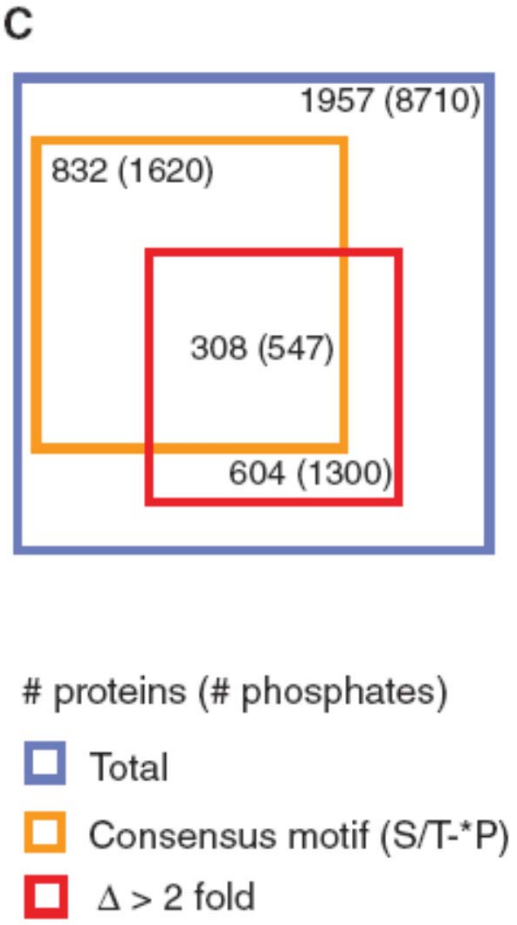
- Cdk1抑制剂：
  - Pyrimidine-based inhibitor 1-NM-PP1
- 酵母突变体：
  - *cdk1-as1; arg4Δ; lys1Δ* strain
  - 需要加入K & R才能生存
- SILAC标记：
  - Heavy: 1-NM-PP1
  - 抑制Cdk1活性
  - 与light比较
- 8,710个位点，1,957个蛋白质
  - >95% confidence



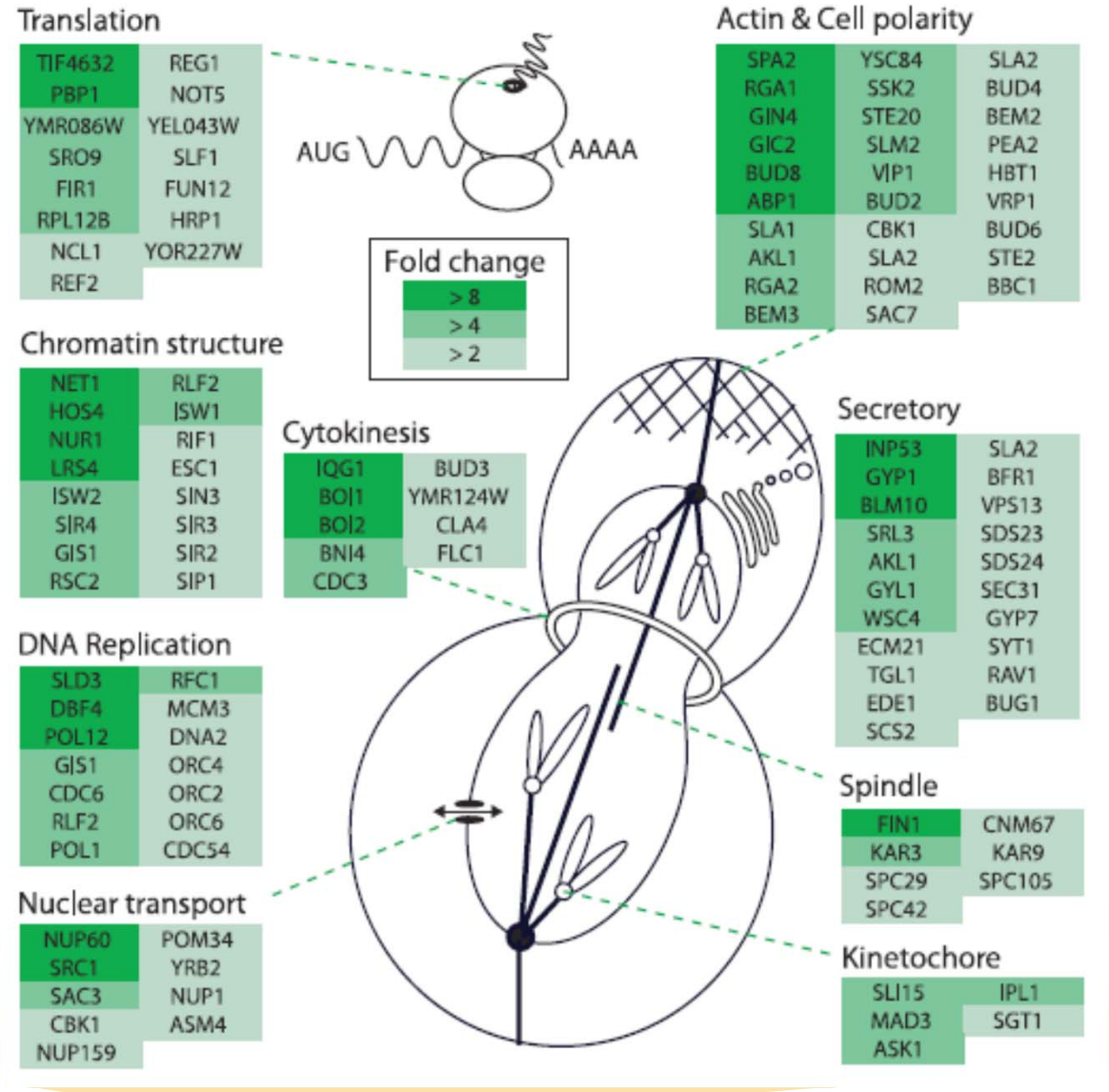
Holt, et al., Science, 2009, 325, 1682-1686

Cdk1的磷酸化位点

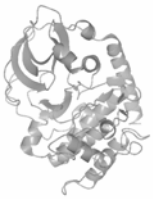
- CDK的motif：
  - ◆pS/pT-P
- 抑制其活性：
  - ◆激酶活性高：修饰的底物和位点多
  - ◆pS/pT-P蛋白质和位点数量下降
- 符合以上两个准则：
  - ◆308个蛋白质，547个位点



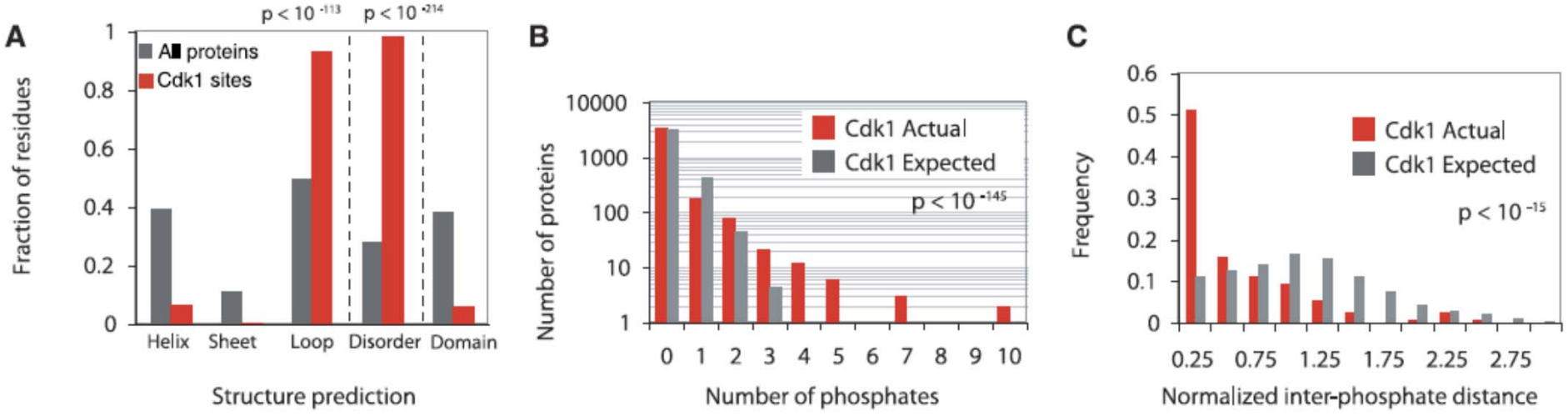
Cdk1底物的功能分析



Cdk1位点的结构和序列分析

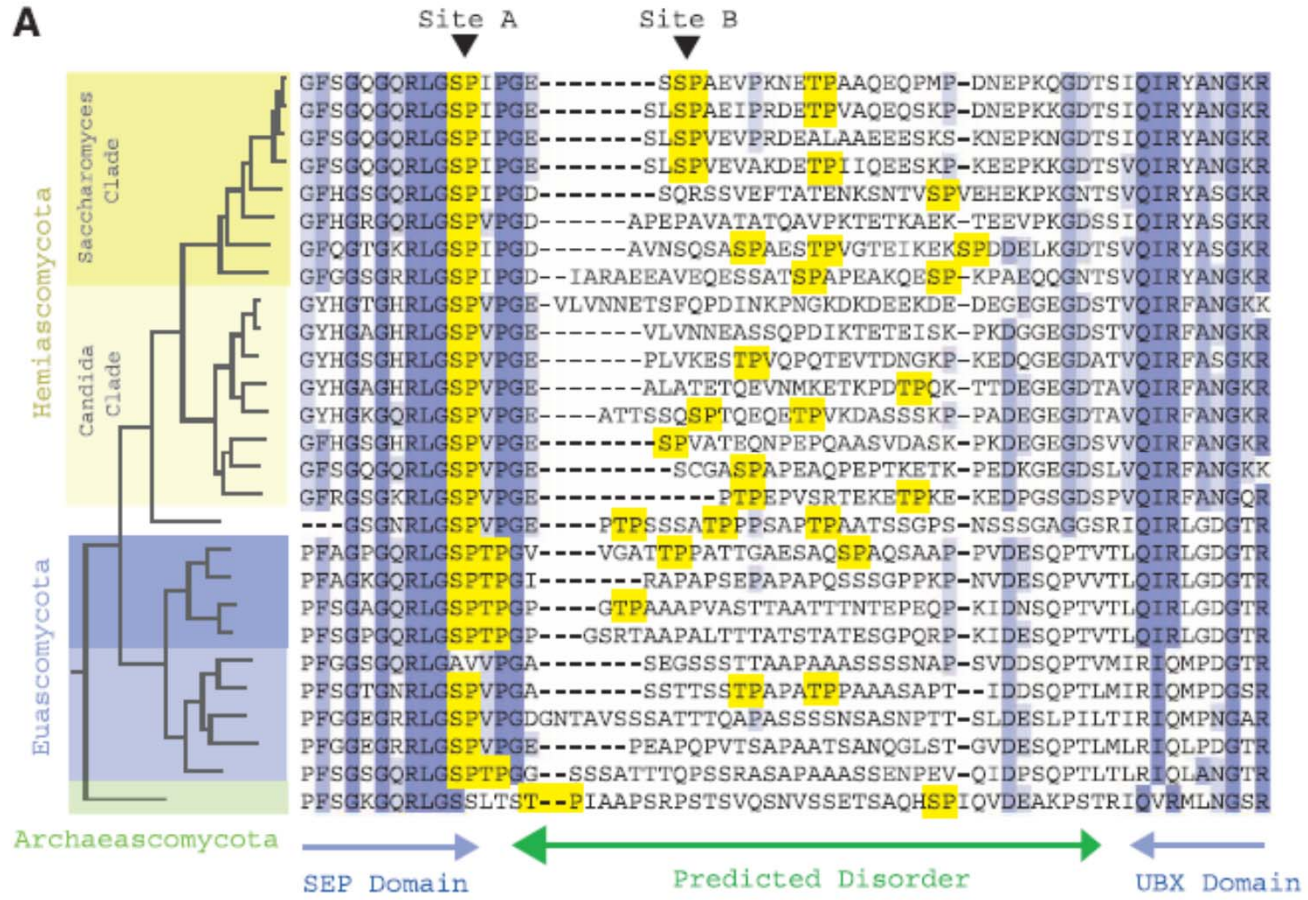


- Cdk1修饰位点的结构特征：
  - ◆主要发生在Loop和Disorder区域
- “成簇性”：
  - ◆单个底物包含多个Cdk1的位点
  - ◆位点之间距离较近



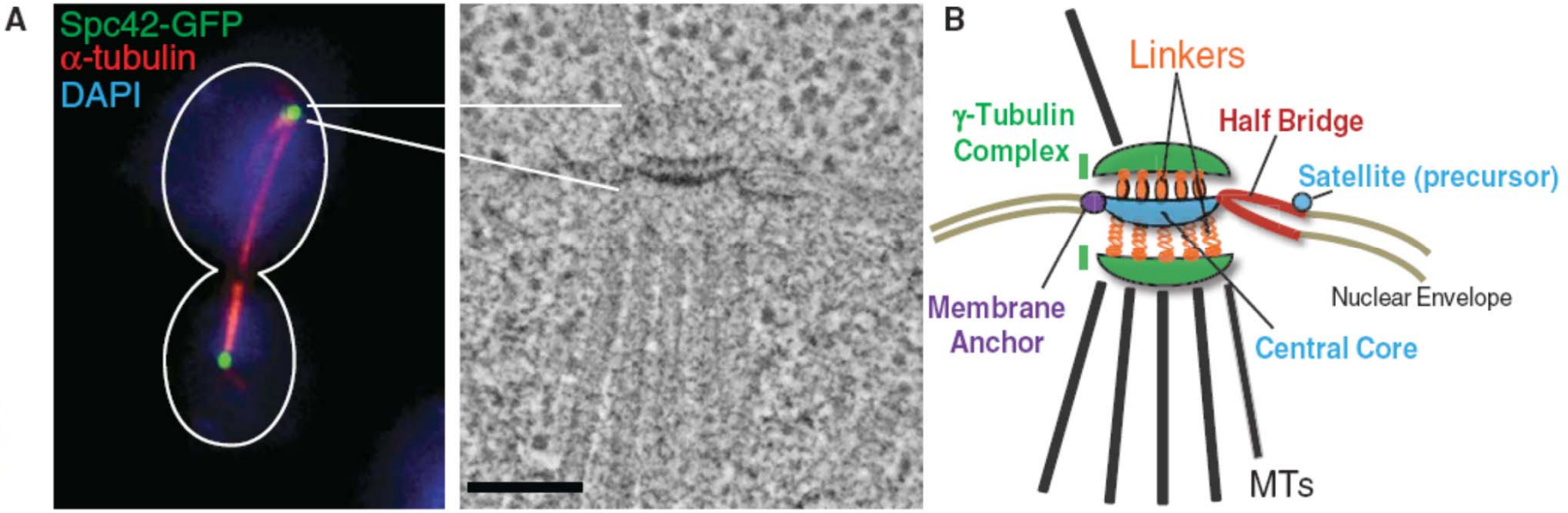
Cdk1位点的进化分析

- 大多数Cdk1位点不保守：进化速率较快
- “Site shift position”



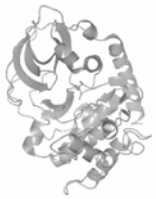
酵母中心体的磷酸化组分析

- 中心体：Centrosome, spindle pole
  - 与核膜连接，锚定
  - 发射微管，连接染色体
- 中心体复合物的获取：
  - IP：Mlp2-Protein A



Keck, et al., Science, 2011, 332, 1557-1561

鉴定结果



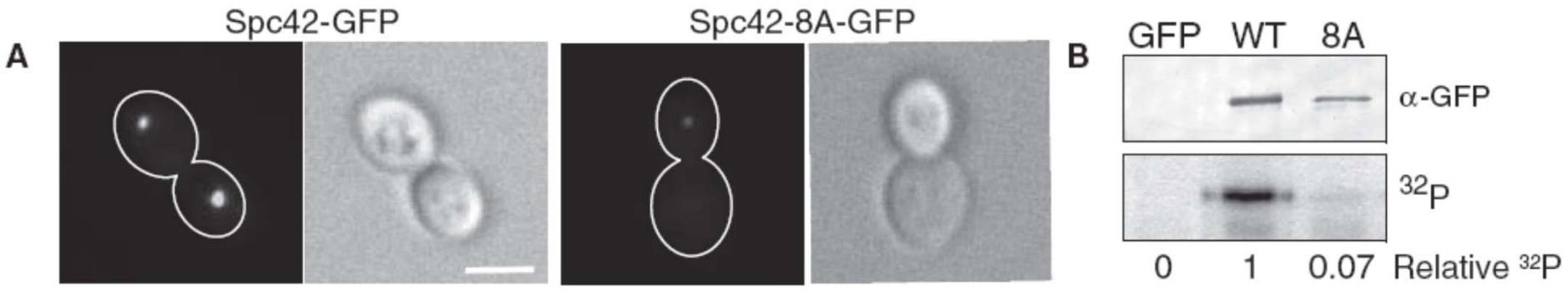
- 非同步化，G_1，M
- Spc42：32个磷酸化位点

Centrosome Proteins	Total Sites	S/T (P) Sites	Y Sites	Coverage	Cdk Kinase	Mps1 Kinase	Cdc5 Kinase	Human Homologs
γ-Tubulin Complex								
Tub4	8 (1)	1	2	85%	✓	-	-	TUBG1
Spc98	9	2	2	65%	✓	✓	-	TUBGCP3
Spc97	5 (1)	0	1	54%	-	-	-	TUBGCP2
Linkers								
Spc110	31	3	3	96%	✓	✓	-	Kendrin
Spc72	19 (2)	3	0	92%	-	-	✓	TACC*
Cmd1	7 (1)	0	0	91%	-	-	-	Calmodulin
Core and Satellite								
Nud1	52 (2)	5	1	91%	✓	-	✓	Centriolin
Spc42	31 (1)	6	3	96%	✓	✓	-	
Spc29	32 (2)	4	0	100%	✓	✓	-	
Cnm67	22	6	3	100%	✓	-	-	
Half Bridge								
Kar1	7 (1)	2	0	63%	-	-	-	
Sfi1	11	4	0	42%	✓	-	-	HSfi1
Cdc31	4	0	0	98%	-	✓	-	Centrin3
Mps3	0	0	0	7%	-	-	-	SUN domain*
Membrane Anchor								
Nbp1	27	6	5	90%	✓	-	-	
Bbp1	20	3	2	85%	✓	-	-	
Mps2	11	4	0	75%	✓	-	-	
Ndc1	1	0	0	24%	-	-	-	
TOTAL:	297 (11)	49	22					

Centrosome Proteins	Total Asynch	Unique Asynch	Total G1	Unique G1	Total Mitotic	Unique Mitotic	Shared G1/M
γ-Tubulin Complex							
Tub4	0	0	2	2	6	6	0
Spc98	3	3	3	2	4	3	1
Spc97	0	0	3	3	2	2	0
Linkers							
Spc110	16	6	14	9	16	11	5
Spc72	5	2	2	2	15	15	0
Cmd1	4	4	1	1	2	2	0
Core and Satellite							
Nud1	27	10	13	2	40	29	11
Spc42	25	1	20	3	27	10	17
Spc29	26	8	18	8	16	6	10
Cnm67	16	2	15	4	16	5	11
Half Bridge							
Kar1	6	3	0	0	4	4	0
Sfi1	7	4	2	2	5	5	0
Cdc31	3	2	1	0	2	1	1
Mps3	0	0	0	0	0	0	0
Membrane Anchor							
Nbp1	20	10	12	7	10	5	5
Bbp1	12	4	12	6	10	4	6
Mps2	9	6	4	3	2	1	1
Ndc1	1	0	0	0	1	1	0
TOTAL:	180	65	122	54	178	110	68

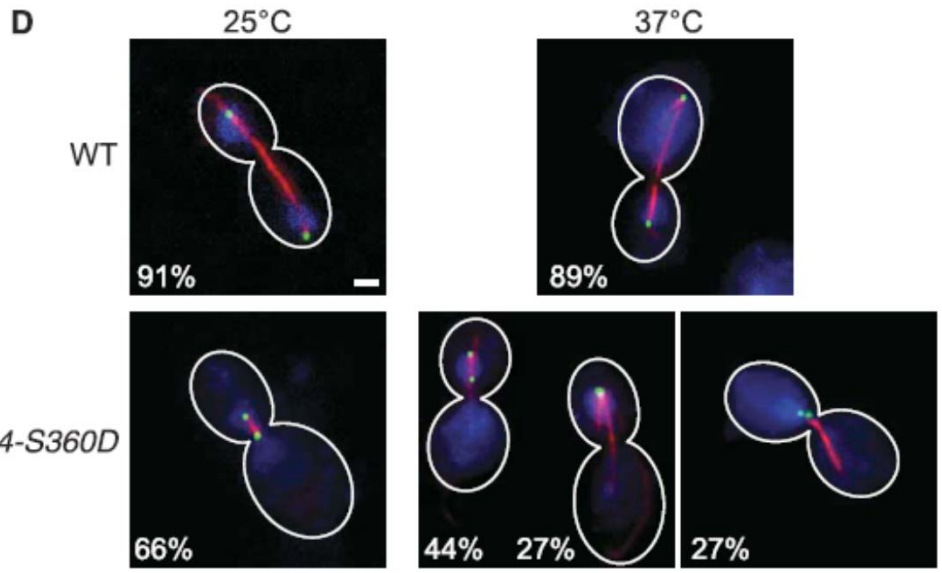
Spc42磷酸化的功能功能

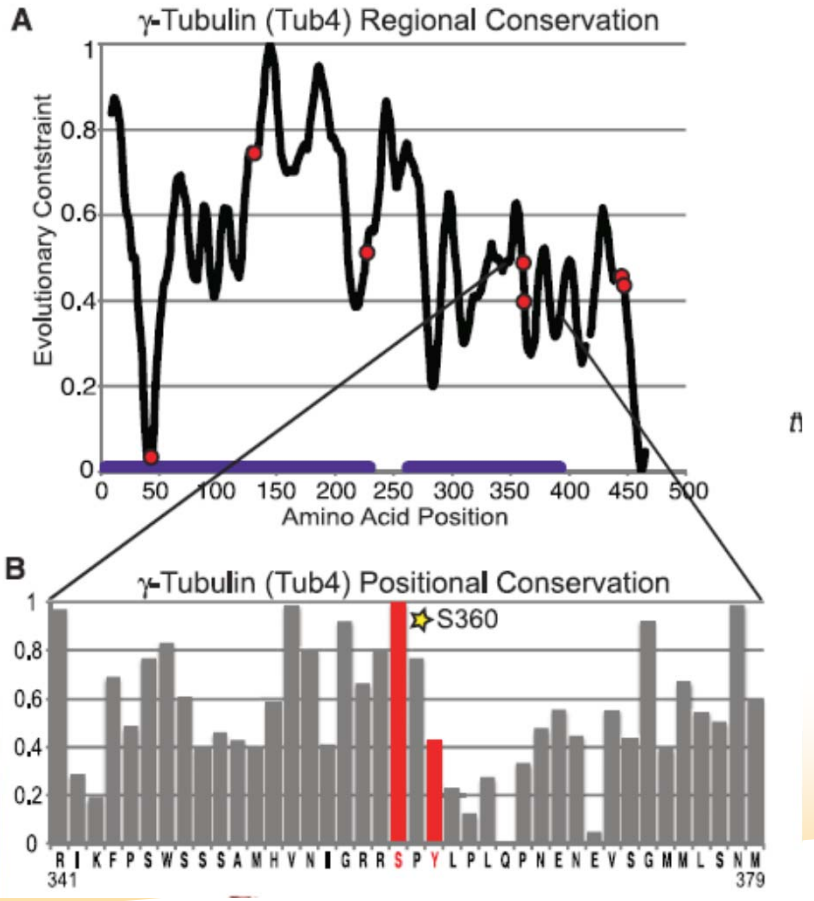
- 8个Cdk1修饰位点：8A
- 突变：削弱中心体的组装



Tub4磷酸化的功能

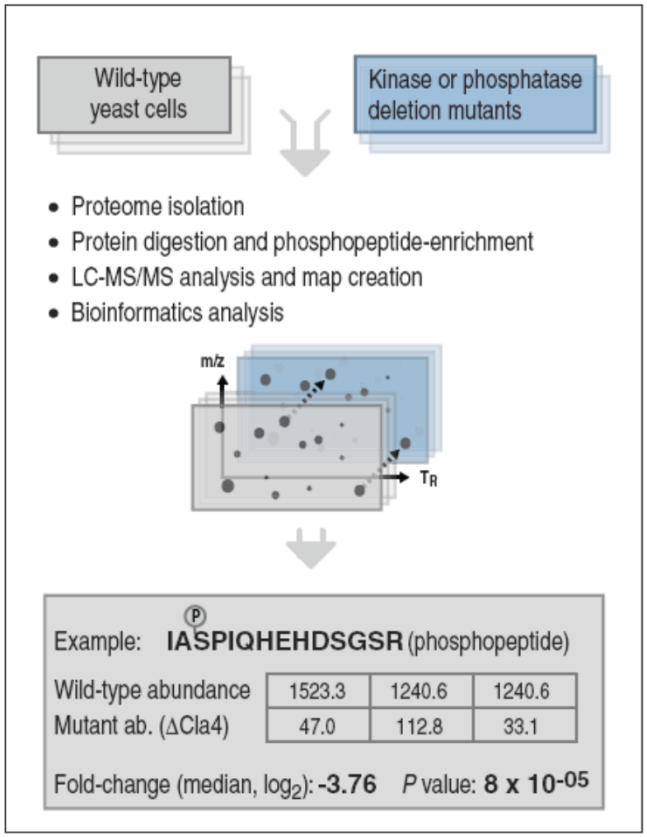
- S360是一个保守的潜在Cdk1磷酸化位点
- 模拟磷酸化S360D：
  - ◆有丝分裂延迟
  - ◆后期纺锤体延长的异常





酵母磷酸化组分析

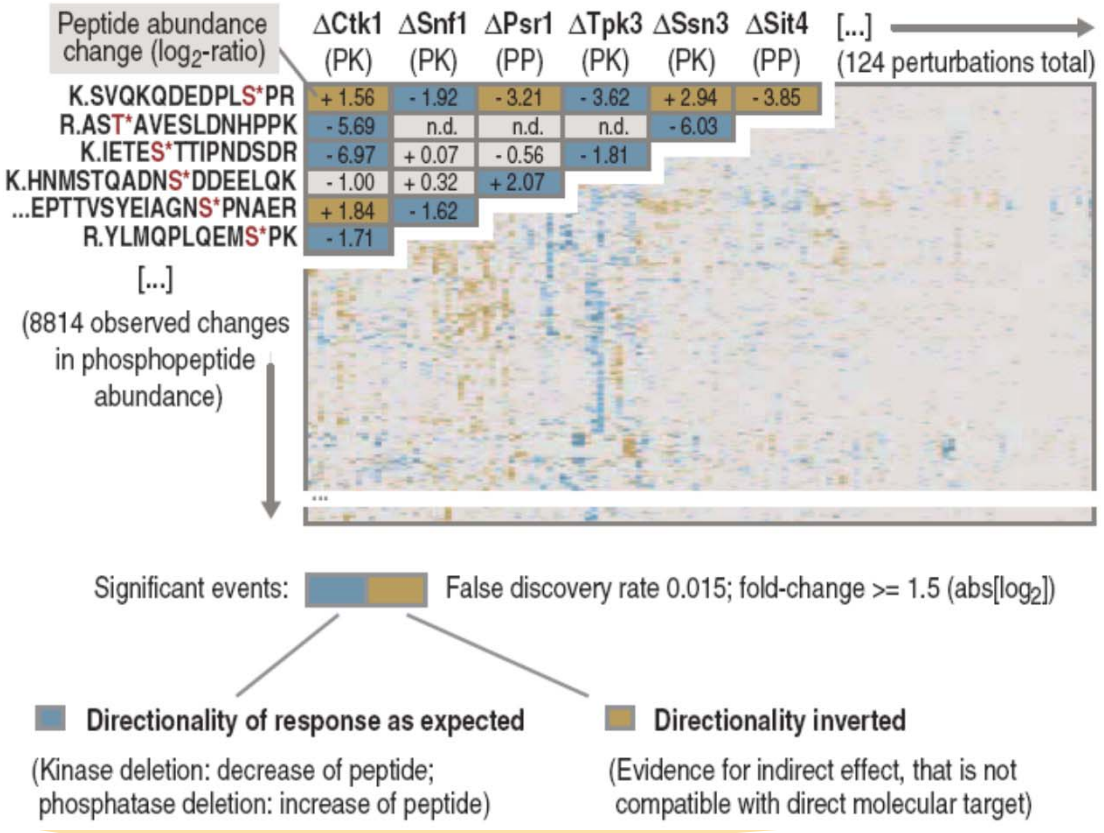
- 酵母的激酶与磷酸酶：161
- 突变体：缺少某个激酶或磷酸酶
  - 致死：37个 – 无法实验
  - 116个突变体
  - 8个激酶：抑制剂
- 实验内容与结果
  - 97个激酶，27个磷酸酶
  - >1,000个磷酸化蛋白质
  - 8,814个受调控的磷酸化



Bodenmiller, et al., Science signaling, 2010, 3, rs4

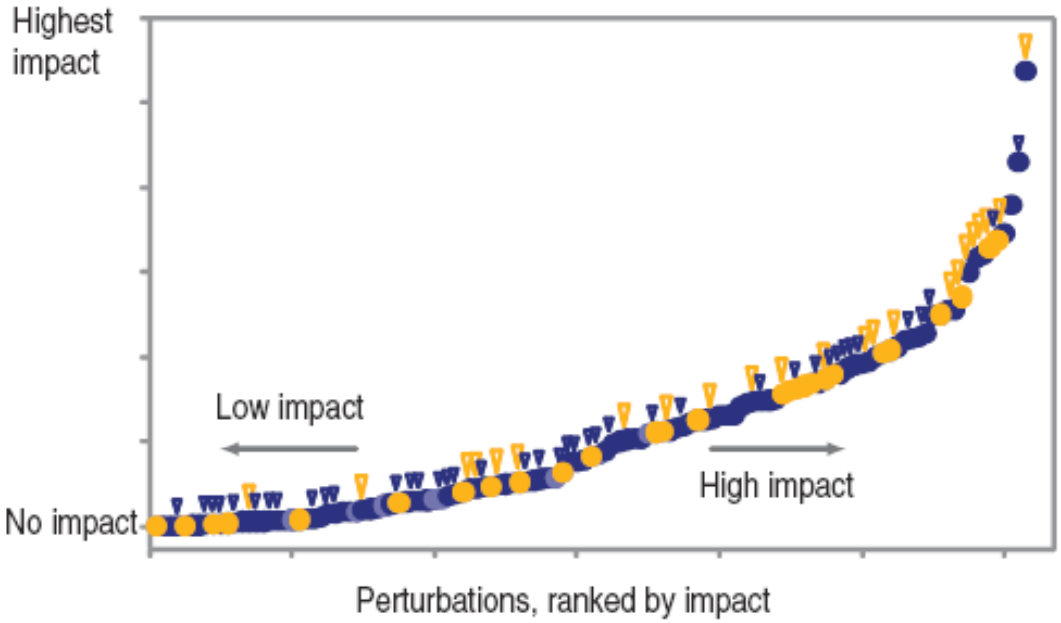
激酶、磷酸酶和底物

- 直接相互作用：
  - ◆无激酶/磷酸酶：肽段磷酸化下降/上升
- 间接关联：反之？



激酶、磷酸酶和底物

- 不同激酶/磷酸酶对细胞的影响不同
  - ◆影响大：有丝分裂相关
  - ◆影响小：MAPK通路，信号转导



High-impact kinases or phosphatases

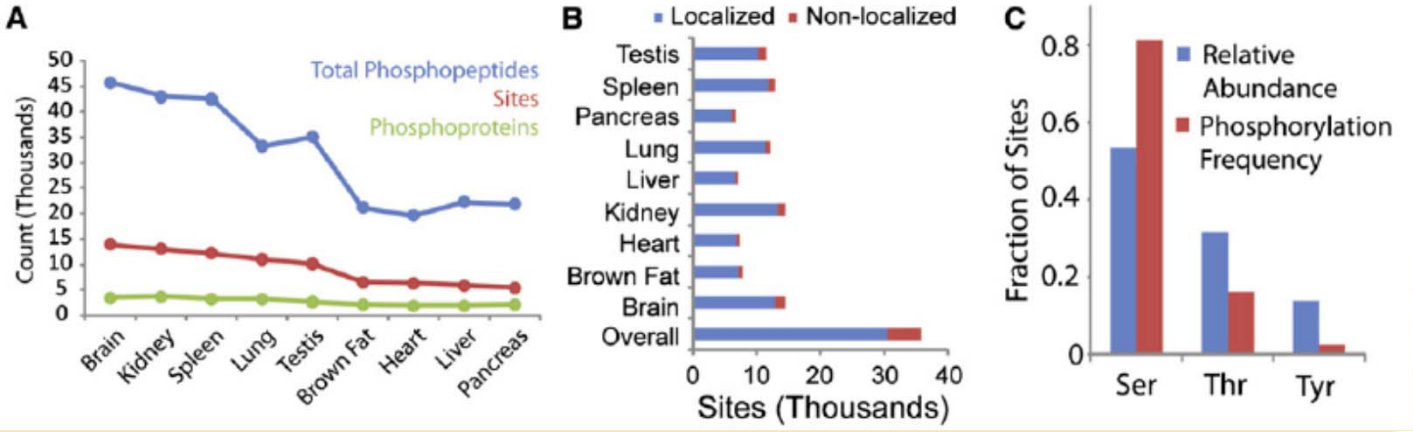
Bud32	Interphase of mitotic cell cycle
Sit4	G1/S transition of mitotic cell cycle
Pph21	Mitotic cell cycle
...	...

Low-impact kinases/phosphatases

Prr1	MAPKKK cascade
Mek1	Osmosensory signaling pathway
Rck2	MAPKKK cascade during osmolarity sensing
...	...

组织特异性的磷酸化组分析

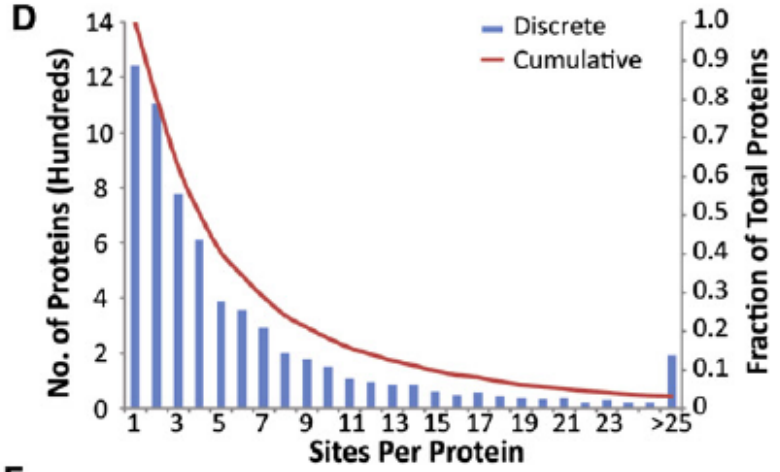
- 3-week-old male Swiss-Webster mice
  - Brain, brown fat, heart, liver, lung, kidney, pancreas (胰腺), spleen (脾), and testis
- SCX + IMAC
  - 284,000 phosphopeptide
  - 36,000 个磷酸化位点，6,296个蛋白质
  - 假阳性：肽段 (0.15%); 蛋白质(1.7%)

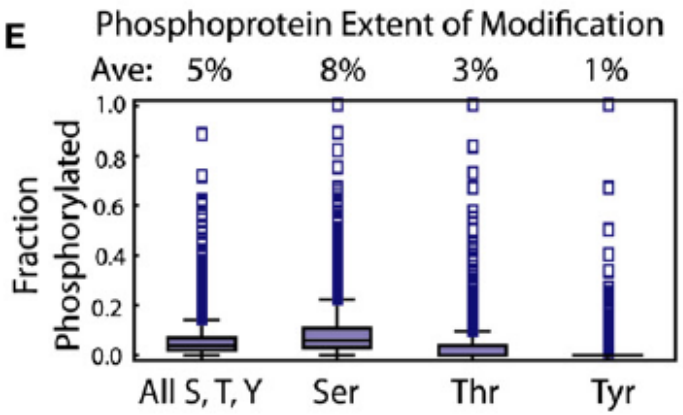


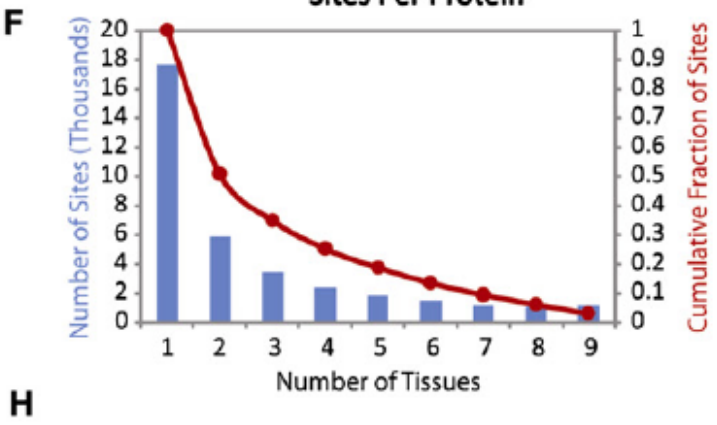
Huttlin, et al., Cell, 2010, 143, 1174-1189

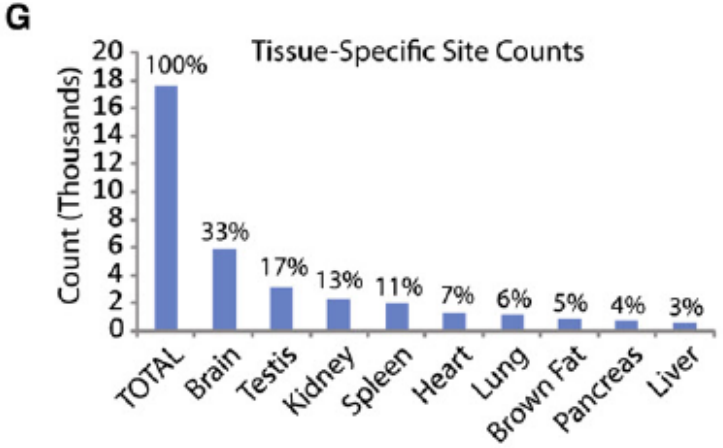
组织特异性磷酸化位点的分布

- 大多数磷酸化位点是组织特异性的
- 仅有少部分S/T/Y可被修饰：5%





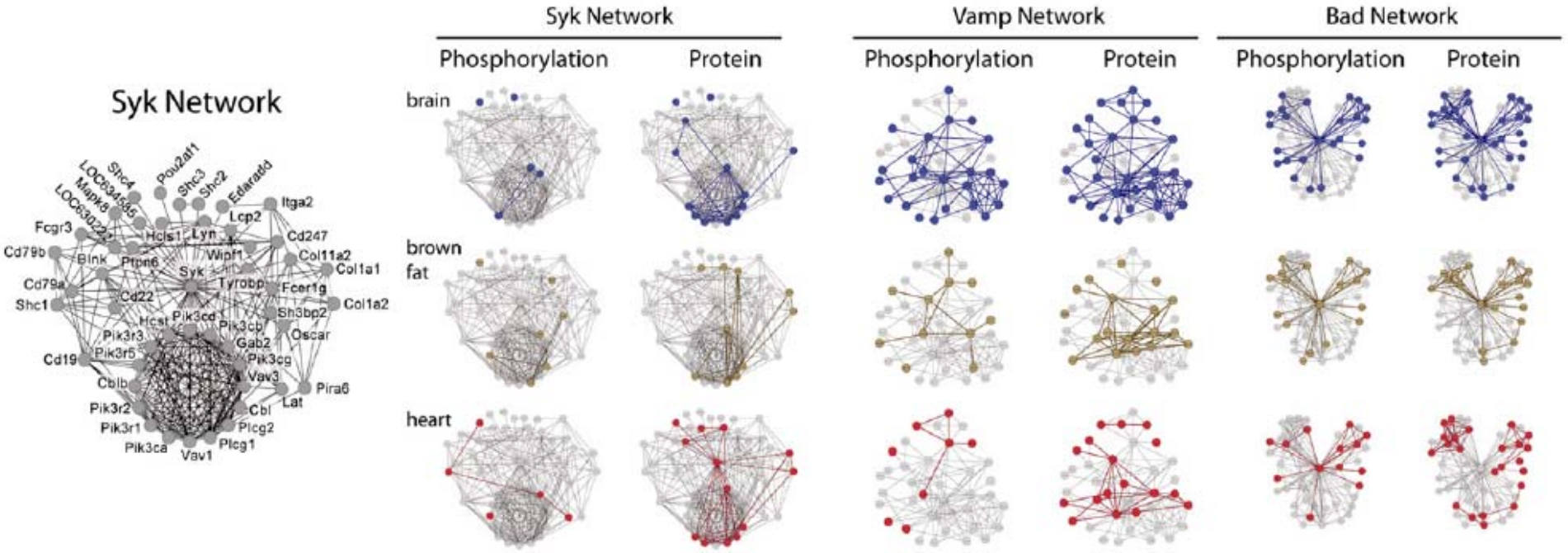




H

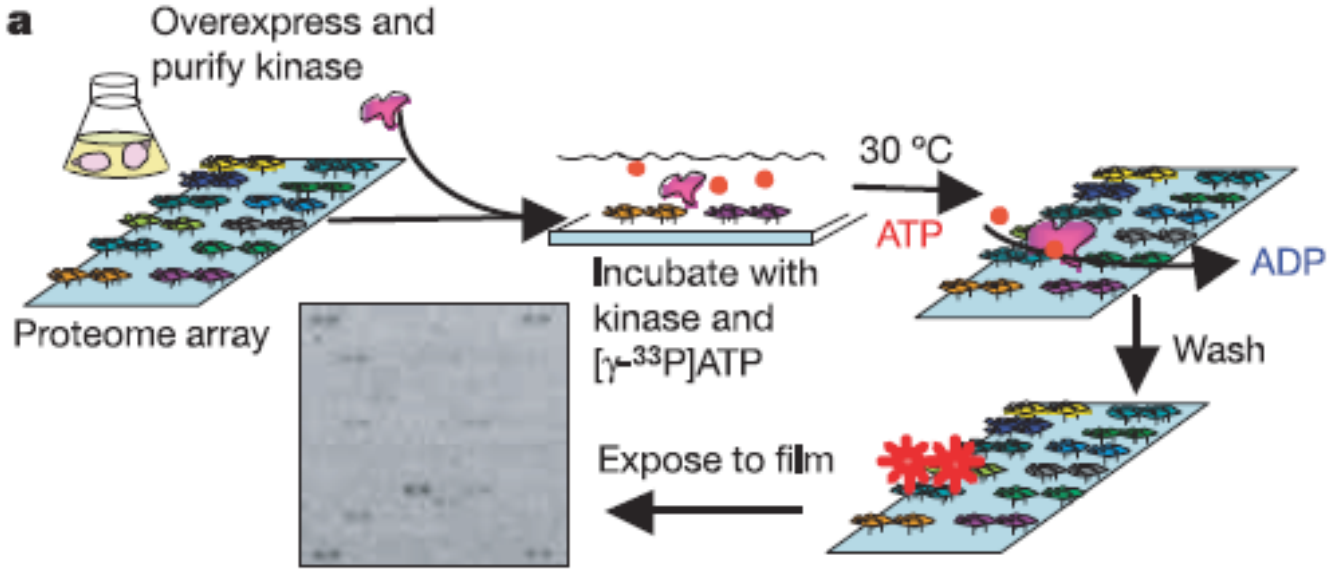
组织特异性的磷酸化网络

- 磷酸化网络：激酶-底物的关系
- 具有高度的组织特异性



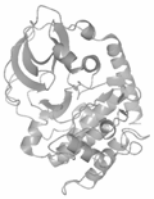
基于蛋白质芯片的磷酸化组分析

- 蛋白质芯片：
  - 芽殖酵母的蛋白质组：4,400个蛋白质
- 酵母的激酶：
  - 122个
  - 实验：82个可体外表达纯化

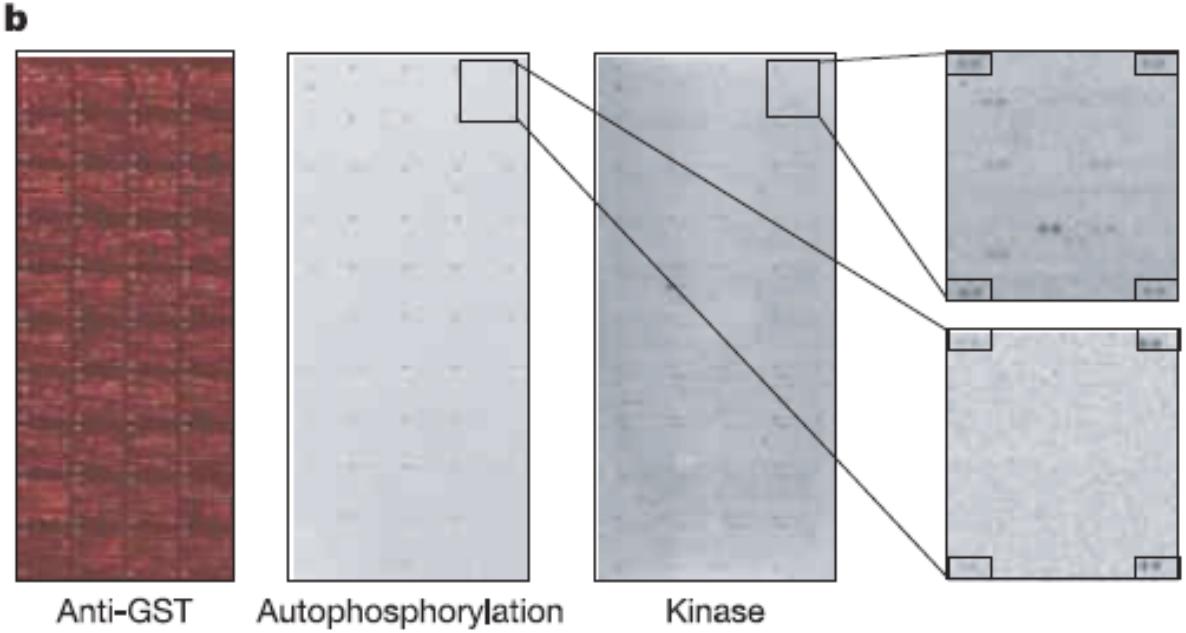


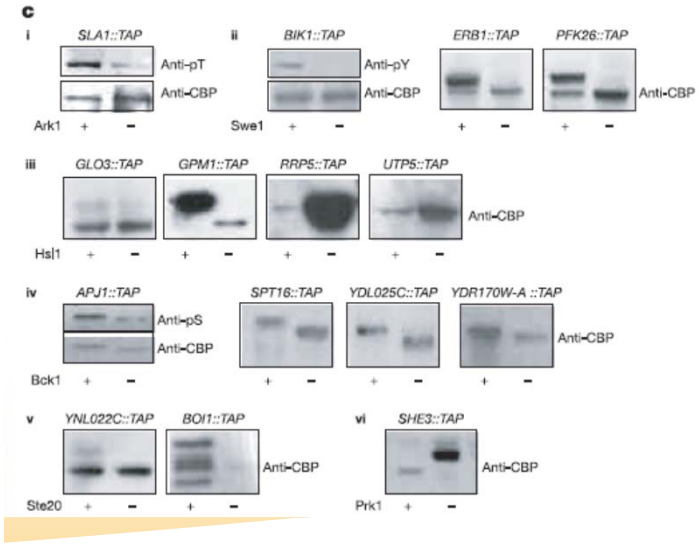
Ptacek, et al., Nature, 2005, 438, 679-684

芯片鉴定及体外验证



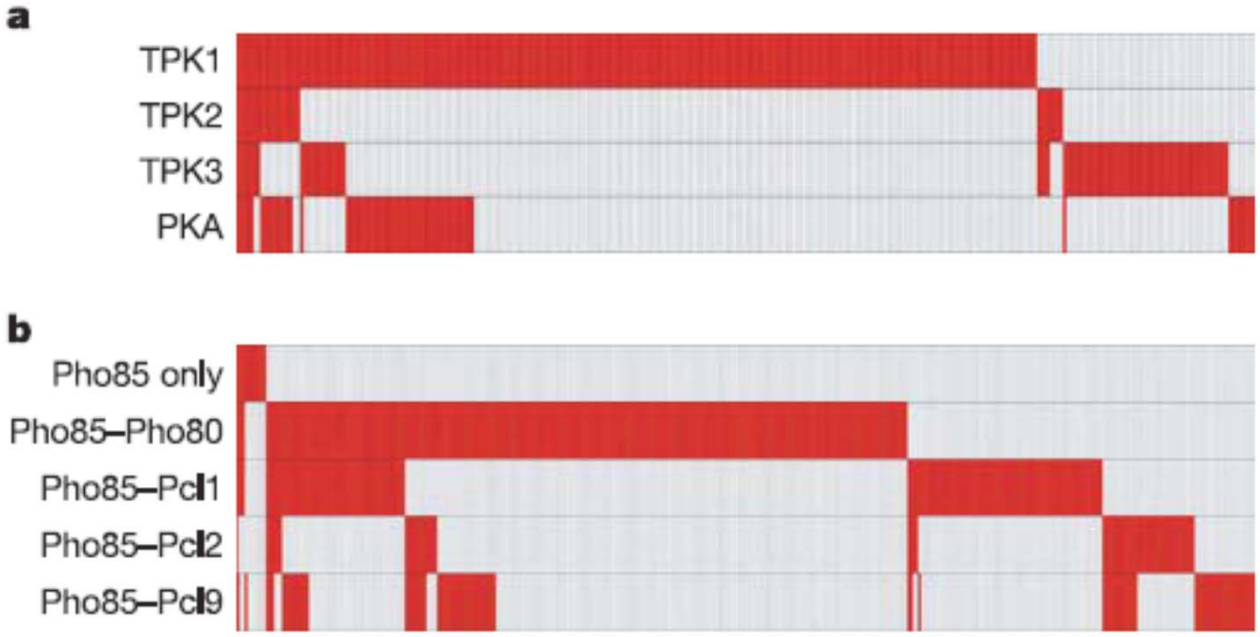
- 蛋白质芯片包含激酶：
  - 激酶的自磷酸化现象
  - 芯片上的激酶磷酸化其他底物
- 背景消除：消除自磷酸化的影响





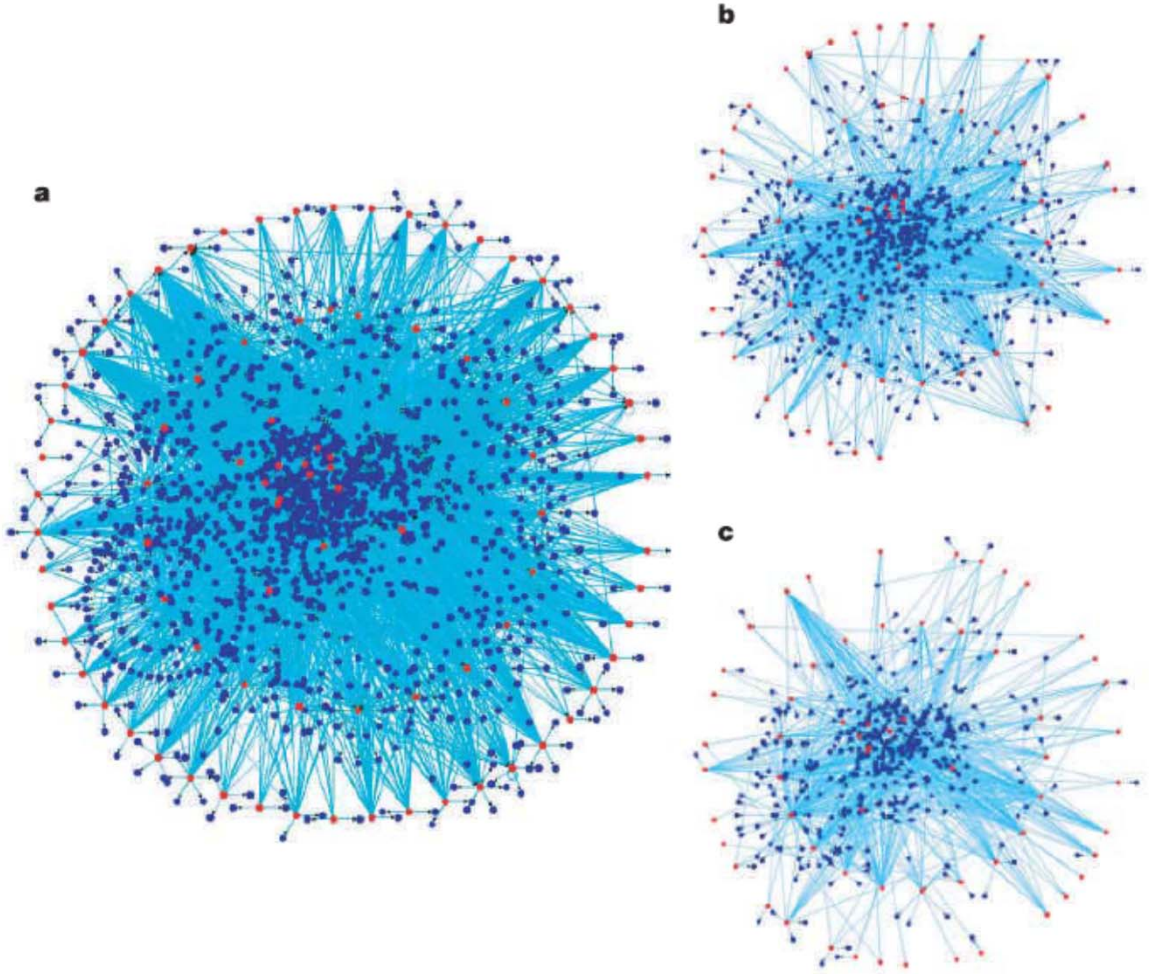
磷酸化底物的分析

- 同一亚家族激酶的底物不同
- 直系同源的底物相似
  - ◆86.6%的PKA底物为TPK1的底物
- Pho85-cyclin的复合构成不同：底物不同

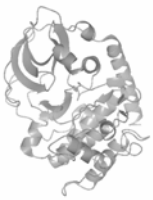


磷酸化调控网络

- 激酶-底物的关系
- 缺点：修饰位点未知；体外 *vs.* 体内



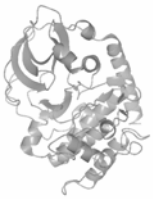
酵母激酶-磷酸酶互作网络



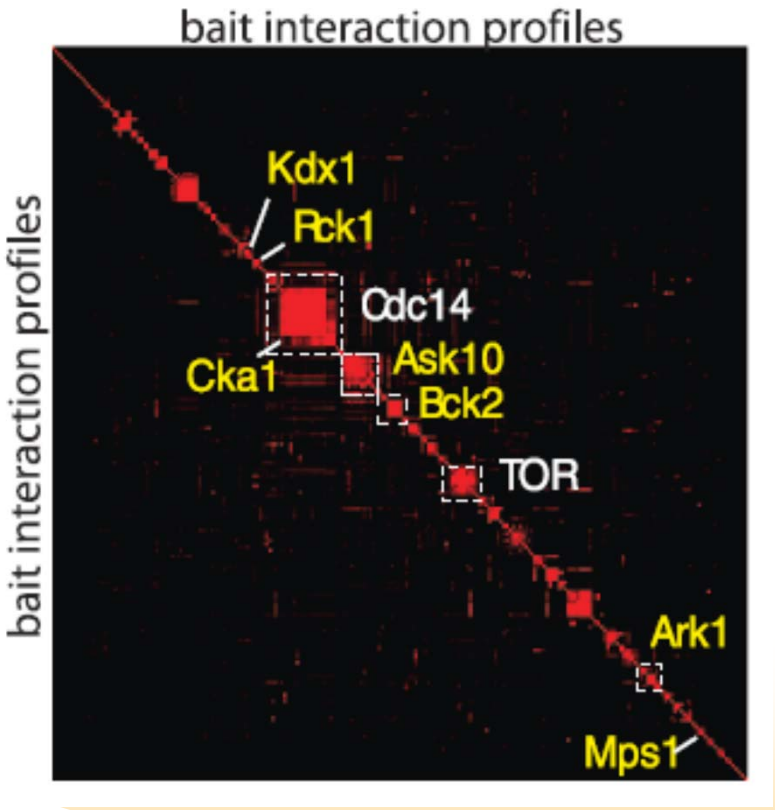
- 激酶与磷酸酶的相互作用
  - 130个蛋白质激酶
  - 24个脂类和代谢激酶
  - 47个激酶调控因子
  - 38个磷酸酶
  - 32个磷酸酶调控因子
  - 5个代谢磷酸酶
- IP: 磁性小球，检测两两直接的相互作用

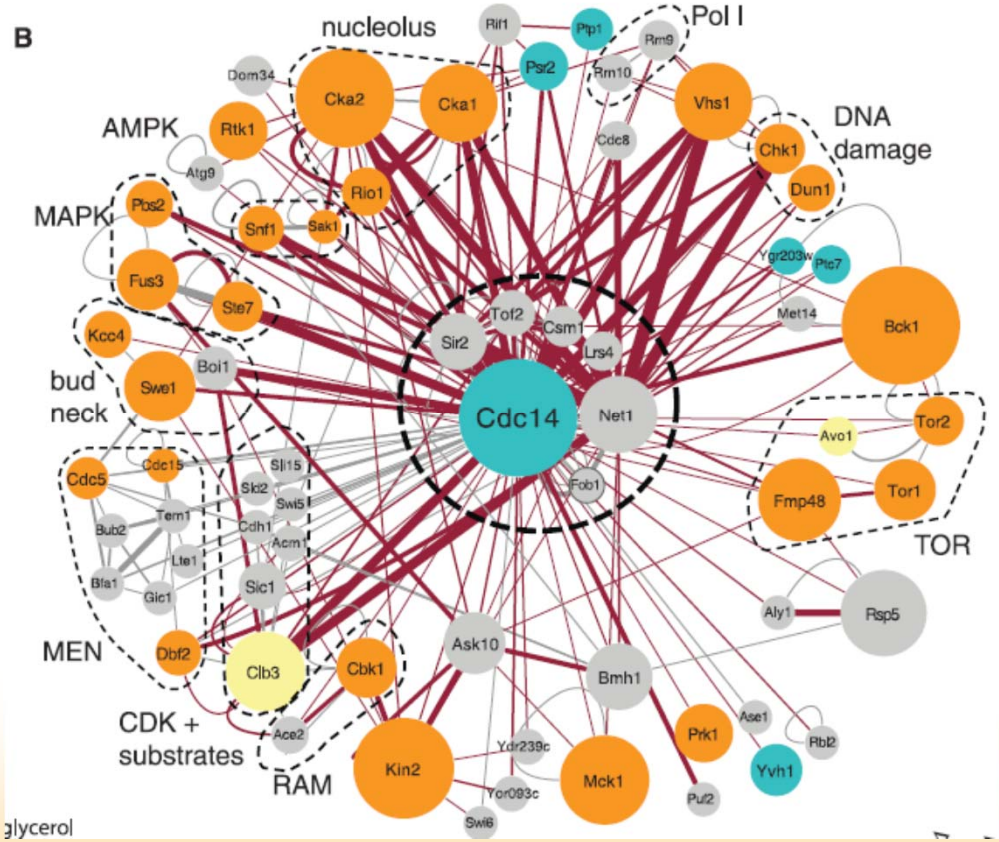
Breitkreutz, et al., Science, 2010, 328, 1043-1046

Cdc14的相互作用网络

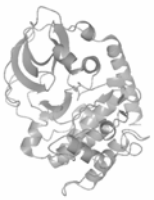


- CDKs、DNA损伤等





磷酸化组学数据分析



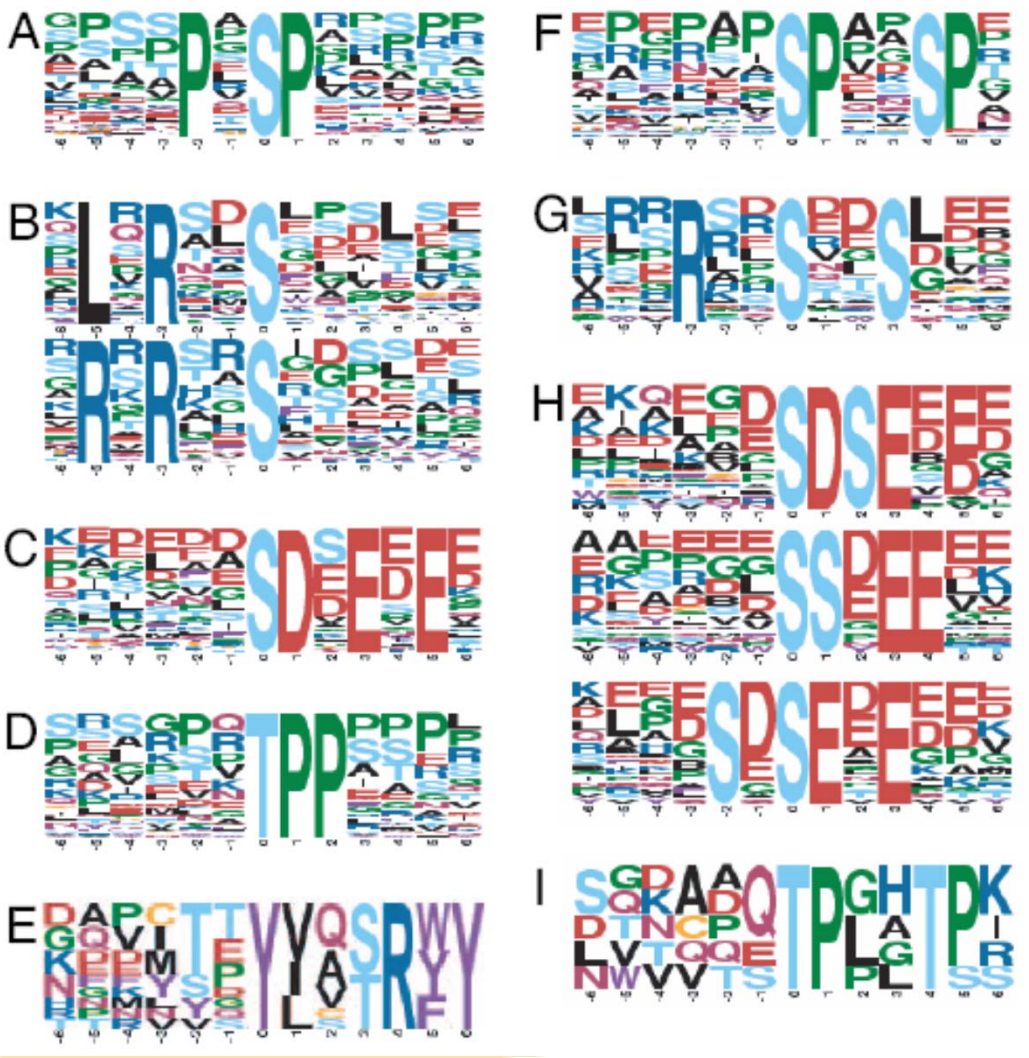
- 磷酸化位点预测与模体发现
  - 激酶识别底物的序列模体：Motif-X, GPS
- 磷酸化网络的构建
  - 激酶-底物的磷酸化网络：NetworkIN, iGPS

Xue, et al., Curr Protein Pept Sci, 2010, 11, 485-496

Ren et al., Curr Protein Pept Sci. 2011, 12:591-601

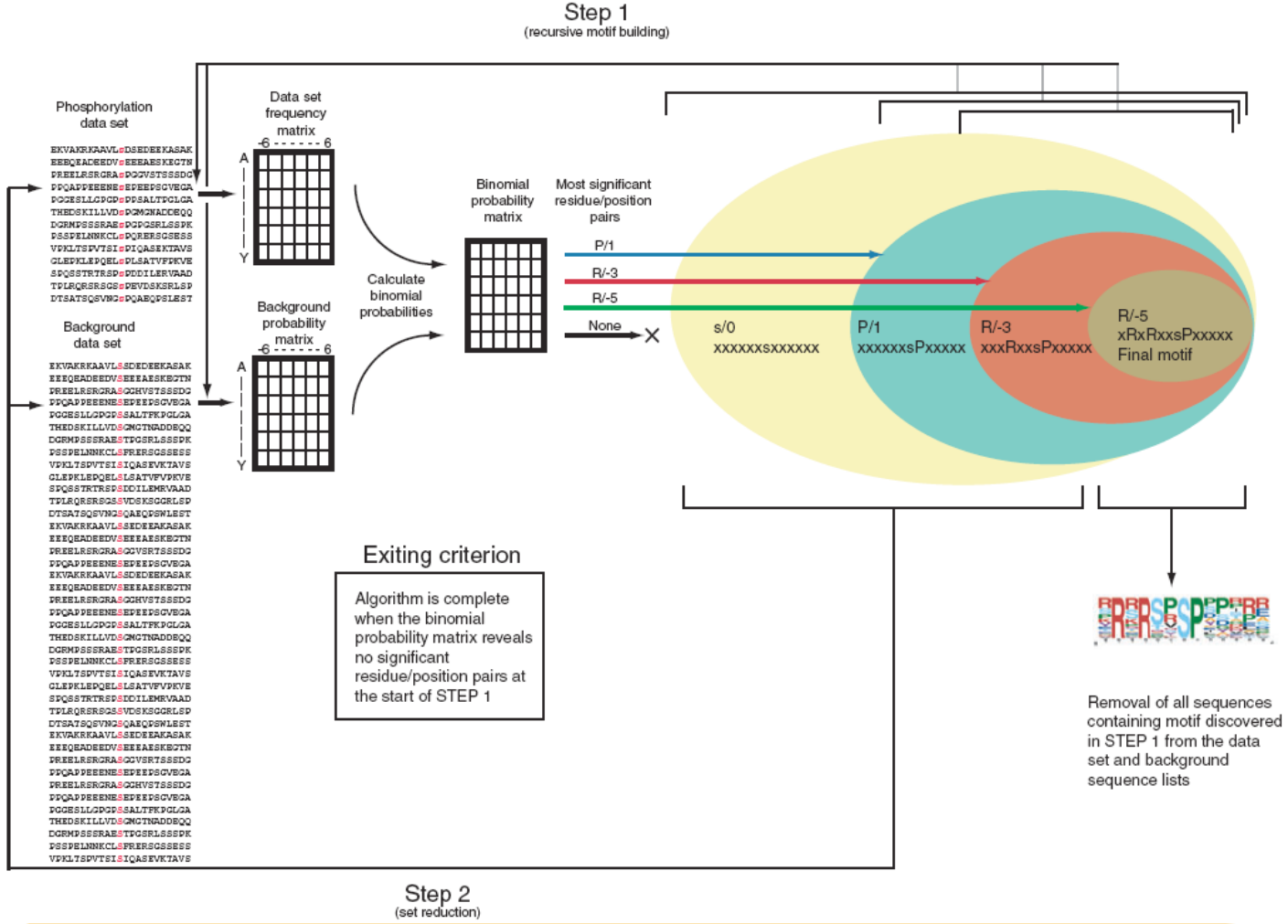
磷酸化模体的发现

- **Motif-X**



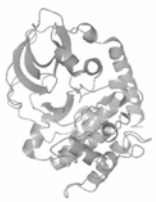
Motif-X

- 磷酸化蛋白质组数据：Phosphorylation motif



Schwartz, et al., Nat Biotechnol, 2005, 23, 1391-1398

算法流程



- 数据
  - 前景：实验鉴定的磷酸化位点
  - 背景：非磷酸化位点数据
- 位点简并：20->11
  - A=AG, D=DE, F=FY, K=KR, I=ILMV, Q=QN, S=ST, C=C, H=H, P=P, W=W
- 二项分布计算模体的统计显著性
- 相似模体的合并

计算结果

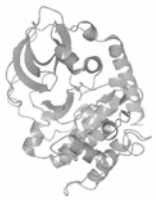


Table 1 S-centered motifs extracted from an *in silico* generated protein data set containing the motifs RxSxxL, RxSxxP, TVxSxE, DxxSQxN and KSxxxI

Motif*	Score**	"S"–centered data set (*Matches*/Size)		Background data set (*Matches*/Size)	
....R.S..L...	32.00	*199*	9,774	*758*	111,506
....R.S..P...	28.82	*192*	9,575	*547*	110,748
...TV.S.E....	40.85	*137*	9,383	*154*	110,201
...D..SQ.N...	40.46	*128*	9,246	*135*	110,047
.....KS...I..	24.15	*158*	9,118	*413*	109,912

* $P < 10^{-6}$, *occurrences* ≥ 20. ** Score = $\sum$-log(P).

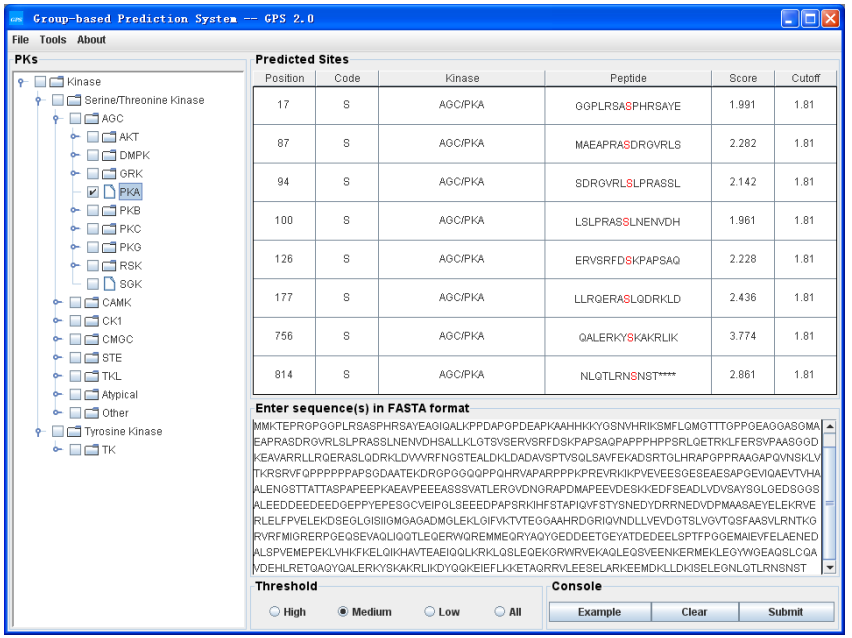
Table 2 Motifs extracted from an experimentally validated data set of ATM, Casein II, CaMK II, and MAPK kinase substrates

Motif*	Kinase	Score**	Phospho data set (*Matches*/Size)		Background data set (*Matches*/Size)	
......sD.E...	Casein II	29.83	*33*	298	*5,574*	1,279,892
......s..E...	Casein II	16.00	*70*	265	*77,819*	1,274,318
......s..D...	Casein II	16.00	*51*	195	*61,044*	1,196,499
......sQ.....	ATM	16.00	*38*	144	*51,451*	1,135,455
......sP.....	MAPK	9.33	*29*	106	*80,073*	1,084,005
...R..s.....	CaMK II	8.78	*21*	77	*57,969*	1,003,932

* $P < 10^{-6}$, *occurrences* ≥ 20. ** Score = $\sum$-log(P).

激酶特异性磷酸化位点的预测

- **Group-based Prediction System (GPS)**
  - 基于分组的预测系统
- **GPS 2.0：**
  - **408**个人类激酶
- **原理: 磷酸化位点的序列模体提供识别特异性**
  - ***In vitro***: 合理
  - ***In vivo***: 不足够




Supplemental Material can be found at:
http://www.mcponline.org/cgi/content/full/M700574-MCP200/DC1

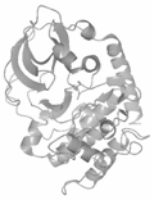
Research

GPS 2.0, a Tool to Predict Kinase-specific Phosphorylation Sites in Hierarchy*

Yu Xue‡§, Jian Ren‡§, Xinjiao Gao‡, Changjiang Jin‡, Longping Wen‡‖, and Xuebiao Yao‡**‡‡¶

Xue et al., MCP, 2008, 7:1598–1608

GPS的打分策略



- 1. 给定两条肽段：

  AQE<u>S</u>ILR (Phos)

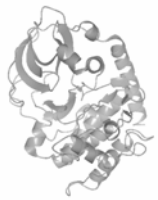
  IQESLIR (Unknown)

- 2. 相似性分值：
  - -1+5+5+6+2+2+5=24
- 3. 计算流程：
  - (1) 给定肽段与训练集中每一个已知磷酸化位点分别比较，计算相似性分值
  - (2) 置零： <0
  - (3) 最终分值: 平均

BLOSUM62 (partial)

	A	R	N	S	T	Q	E	G	H	I	L
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2
N	-2	0	6	1	-3	0	0	0	1	-3	-3
S	-2	-2	1	6	-3	0	2	-1	-1	-3	-4
T	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1
Q	-1	1	0	0	-3	5	2	-2	0	-3	-2
E	-1	0	0	2	-4	2	5	-2	0	-3	-3
G	0	-2	0	-1	-3	-2	-2	6	-2	-4	-4
H	-2	0	1	-1	-3	0	0	-2	8	-3	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4

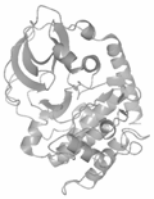
磷酸化的linear motif atlas



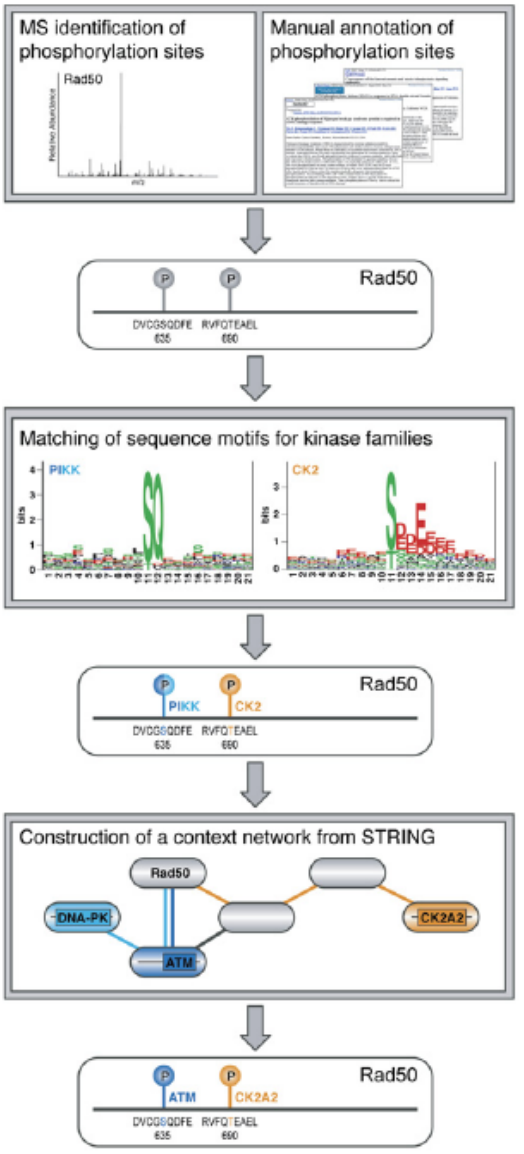
- NetPhorest
  - 文献检索已知的线性模体：磷酸化、磷酸化结合
  - 建立PSSM矩阵
  - 机器学习算法
- 179个激酶
- 104个磷酸化绑定结构域
  - Src homology 2 (SH2)
  - Phosphotyrosine binding (PTB)
  - BRCA1 C-terminal (BRCT)
  - WW
  - 14–3–3
- http://netphorest.info

Miller, et al., Science signaling, 2008, 1, ra2

磷酸化调控网络的模拟



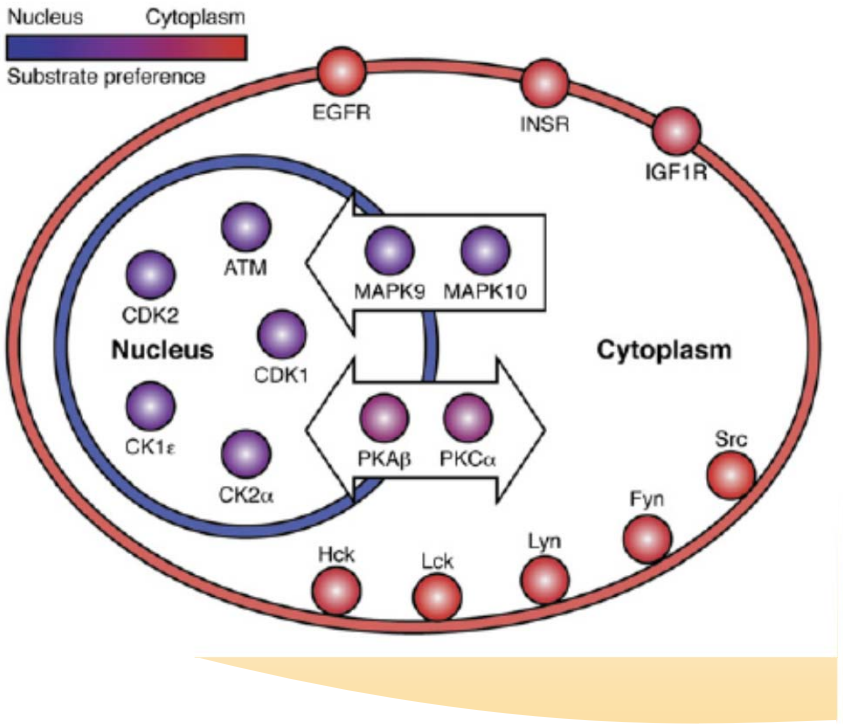
- 基本假设
  - 相似激酶识别相似模体
  - Contextual filters: 蛋白质的相互作用、共表达、共定位等
- 人类磷酸化调控网络
  - 3,978个蛋白质, 73个激酶, 22,224对激酶-底关系
- 实验验证
  - CDK1修饰53BP1
  - ATM修饰Rad50
  - GSK-3修饰BCLAF1

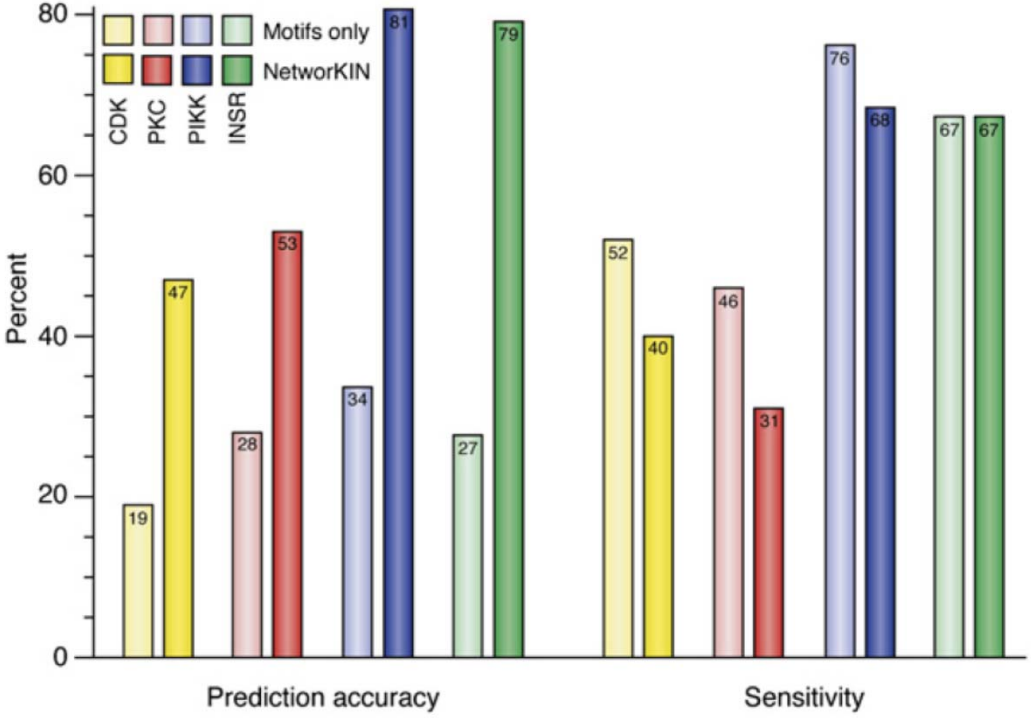


Linding, et al., Cell, 2007, 129, 1415-1426

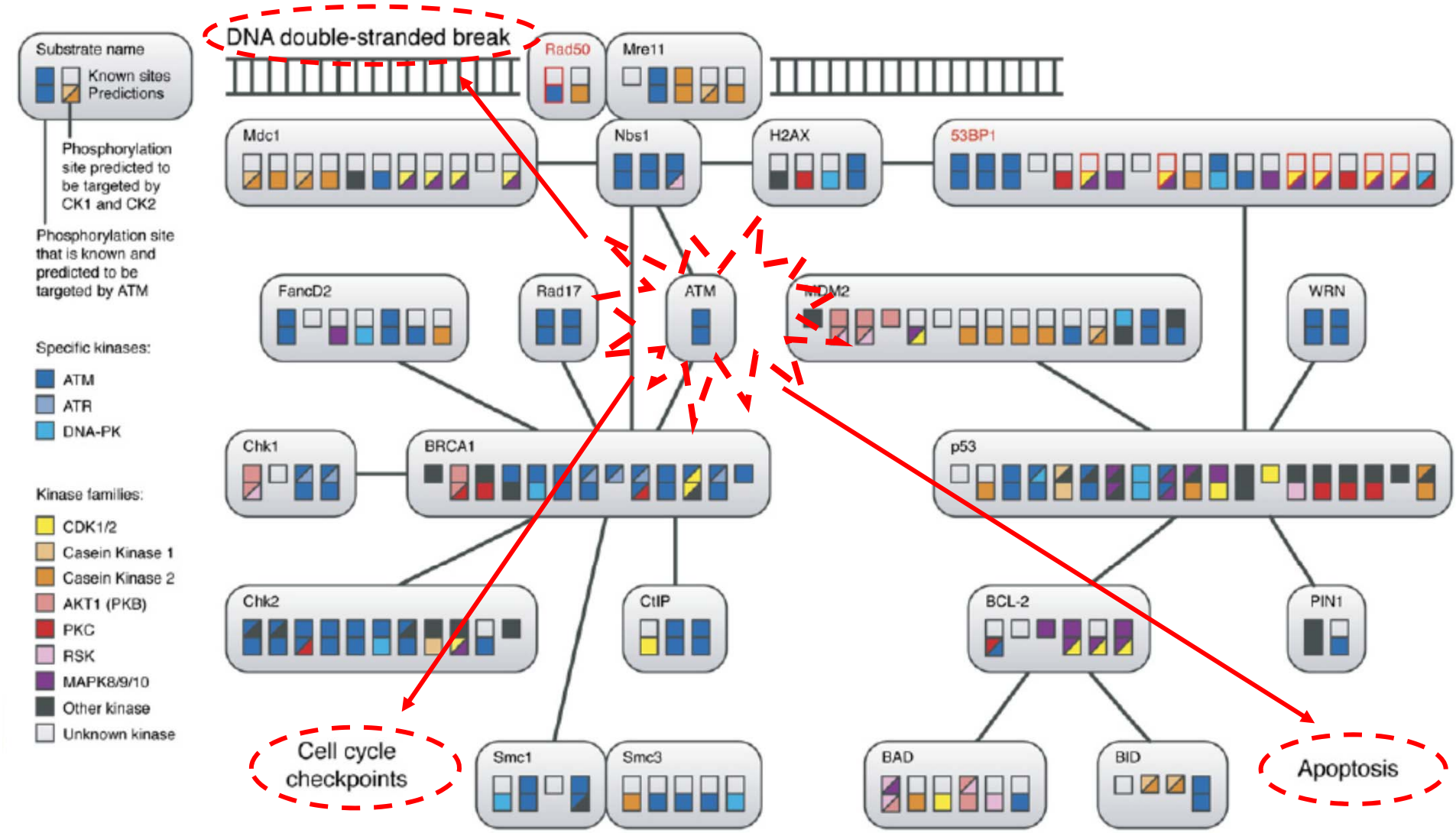
Contextual filters

- 根据蛋白质相互作用网络，计算离底物最近的激酶
- 预测灵敏度和精度都大幅提高
- 进一步考虑细胞共定位信息

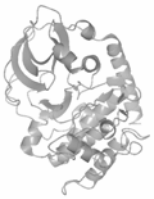




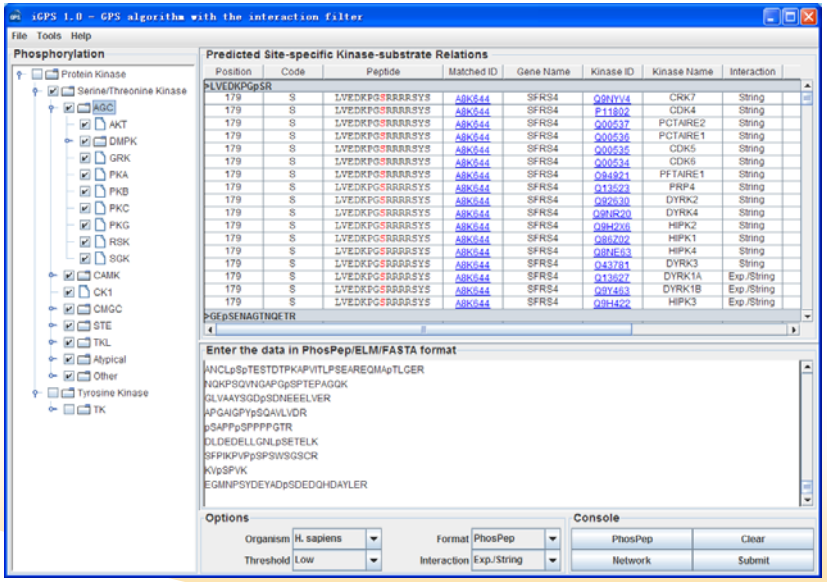
DNA损伤应答网络


Substrate name
Known sites
Predictions
Phosphorylation site predicted to be targeted by CK1 and CK2
Phosphorylation site that is known and predicted to be targeted by ATM
Specific kinases:
ATM
ATR
DNA-PK
Kinase families:
CDK1/2
Casein Kinase 1
Casein Kinase 2
AKT1 (PKB)
PKC
RSK
MAPK8/9/10
Other kinase
Unknown kinase
DNA double-stranded break
Rad50
Mre11
Mdc1
Nbs1
H2AX
53BP1
FancD2
Rad17
ATM
MDM2
WRN
Chk1
BRCA1
p53
Chk2
CtIP
BCL-2
PIN1
Cell cycle checkpoints
Smc1
Smc3
BAD
BID
Apoptosis

iGPS: '*in vivo*' GPS



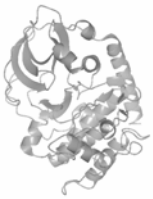
- "吻别模型"：激酶-底物之间的直接相互作用
- iGPS：
  - GPS 2.0
  - 蛋白质相互作用信息
  - 磷酸化蛋白质组学数据



PK clusters	NetworKIN				iGPS			
	Ac	*Sn*	*Sp*	*MCC*	*Ac*	*Sn*	*Sp*	*MCC*
NetworKIN 1.1								
AGC/AKT	**98.87%**	**58.89%**	**99.44%**	**0.5865**	**98.81%**	**52.22%**	**99.47%**	**0.5445**
AGC/PKA	92.24%	32.20%	93.28%	0.1277	92.67%	57.56%	93.28%	0.2481
Atypical/PIKK/ATM	**97.50%**	**77.42%**	**97.83%**	**0.5204**	**97.47%**	**75.27%**	**97.83%**	**0.5080**
CAMK/CAMK2	98.43%	5.85%	99.91%	0.1671	98.51%	10.64%	99.91%	0.2580
CMGC/MAPK	93.45%	65.34%	94.06%	0.3312	93.60%	71.12%	94.09%	0.3614
CMGC/CDK/CDC2[a]	94.58%	46.67%	95.69%	0.2825	94.75%	54.04%	95.69%	0.3268
Other/CK2	**90.17%**	**55.49%**	**91.14%**	**0.2511**	**90.25%**	**54.85%**	**91.24%**	**0.2495**
TK/EGFR	85.26%	12.16%	96.97%	0.1554	91.42%	56.76%	96.97%	0.6059
TK/Src	90.04%	23.97%	95.40%	0.2141	90.69%	32.64%	95.40%	0.2956
NetworKIN 2.0 beta								
AGC/AKT	98.55%	42.78%	99.33%	0.4436	98.71%	54.44%	99.33%	0.5334
AGC/PKA	96.02%	32.44%	97.11%	0.2110	96.31%	50.24%	97.11%	0.3244
Atypical/PIKK/ATM	93.90%	91.40%	93.94%	0.4064	94.60%	90.32%	94.67%	0.4246
CAMK/CAMK2	91.18%	27.13%	92.20%	0.0881	91.56%	49.47%	92.23%	0.1866
CMGC/MAPK	96.80%	1.59%	98.87%	0.0063	97.43%	31.08%	98.87%	0.3280
CMGC/CDK/CDC2	97.29%	0.35%	99.54%	-0.0023	97.46%	7.72%	99.54%	0.1377
Other/AUR/AUR-A	91.61%	32.73%	92.66%	0.1244	92.15%	54.55%	92.82%	0.2291
Other/CK2	**80.06%**	**75.32%**	**80.19%**	**0.2202**	**79.65%**	**60.34%**	**80.19%**	**0.1619**
TK/ABL	89.00%	13.33%	96.72%	0.1449	90.54%	30.00%	96.72%	0.3323
TK/EGFR	84.70%	12.16%	96.32%	0.1362	91.42%	60.81%	96.32%	0.6162
TK/Src	90.88%	2.07%	98.09%	0.0029	92.18%	19.42%	98.09%	0.2611
TK/Syk	86.67%	2.08%	99.68%	0.0806	89.17%	20.83%	99.68%	0.4051

Song et al., MCP, 2012, 11:1070-83

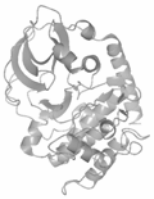
真核生物磷酸化调控网络



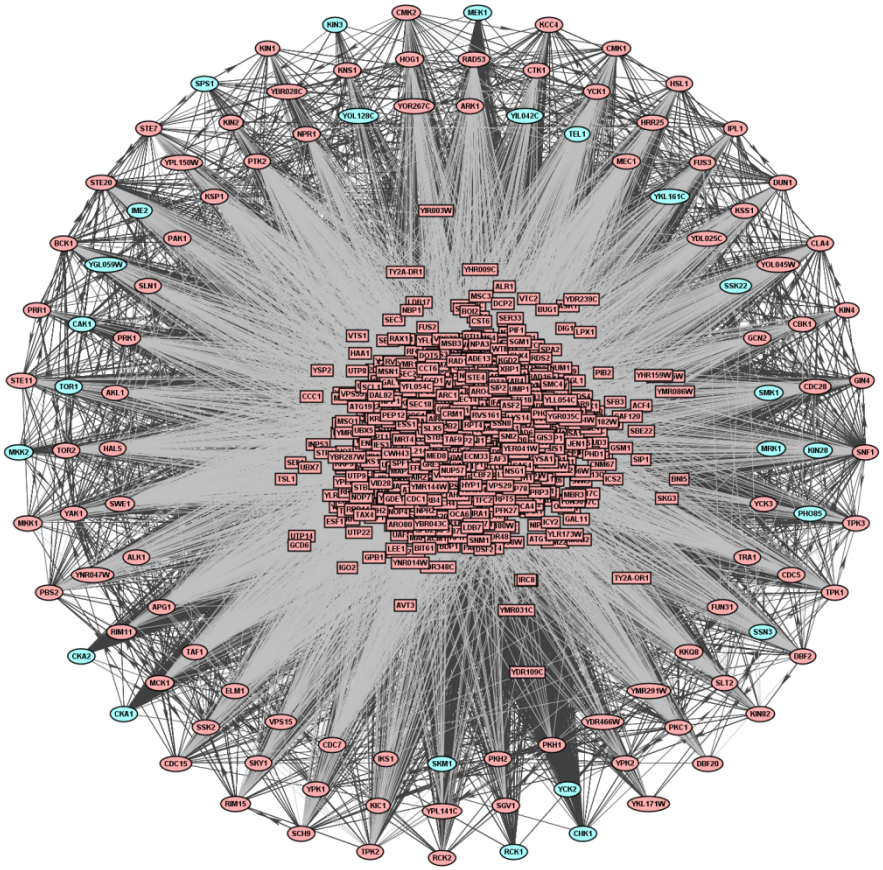
- 五个模式生物:
  - ◆9,247个蛋白质
  - ◆1,079个激酶
  - ◆186,922 对激酶-底物关系
- 引入蛋白质相互作用信息
  - ◆降低>20倍的假阳性

Organism	String & Exp. PPI					No PPI				
	PK	Sub.[a]	Site	ssKSR	Ave[b]	PK	Sub.	Site	ssKSR	Ave
S. cerevisiae	91	1,598	7,041	20,909	3.0	91	2,658	12,889	145,409	11.3
C. elegans	110	272	544	867	1.6	302	2,153	5,112	107,738	21.1
D. melanogaster	140	888	2,697	6,191	2.3	172	3,896	13,656	236,780	17.3
M. musculus	358	2,349	11,191	45,032	4.0	415	8,219	43,131	1,588,383	36.8
H. sapiens	380	4,140	22,817	113,923	5.0	407	9,452	52,909	1,922,988	36.3
Total	1,079	9,247	44,290	186,922	4.2	1,387	26,378	127,697	4,001,298	31.3

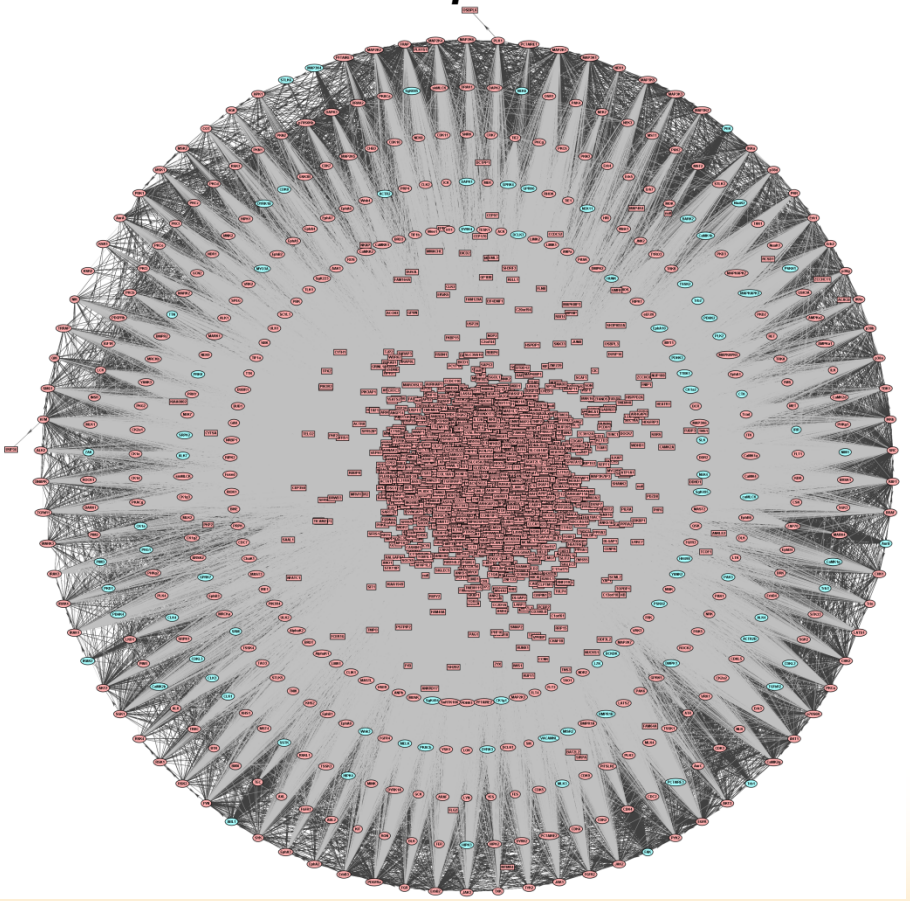
真核生物磷酸化调控网络



S. cerevisiae



H. sapiens



Neuronal autophagy

- In Alzheimer's & Parkinson's diseases
  - Proteins accumulate in central nervous system
  - Defective autophagy in patient brains
- Enhanced autophagy
  - Neuroprotective by promoting the clearance of disease-associated aggregates
- Small-molecule autophagy enhancers
  - *Uncaria rhynchophylla* (Gouteng,钩藤)

草部·钩藤

作者：李时珍

气味

甘、微寒、无毒。

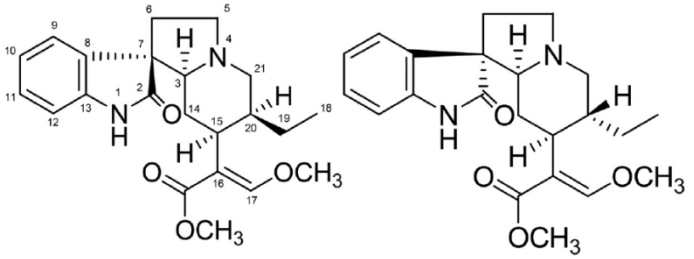
主治

小儿惊热。用钩藤一两、硝石半两，甘草（炙）一分，共研为末。每服半钱，温水服，一天服三次。此方名"延龄散"。

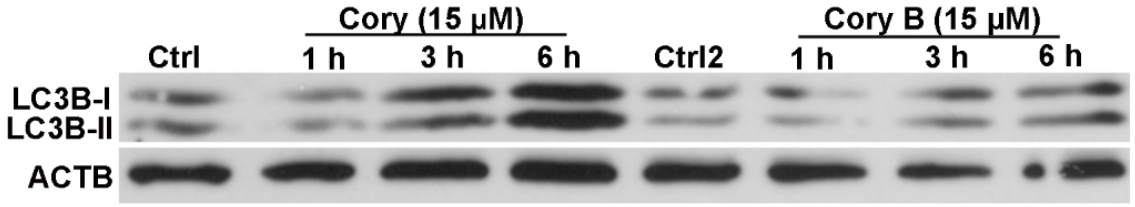
班疹。用钩藤的钩子、紫草茸，等分为末。每服三分或半钱，温酒送下。

Neuroprotective alkaloids in Gouteng

- Corynoxine (柯诺辛碱) & corynoxine B (柯诺辛B)
  - Same molecular formula, different conformation
  - Induce autophagy in different way
- Question:
  - Find key regulators in neuronal autophagy
  - Distinguish two compounds



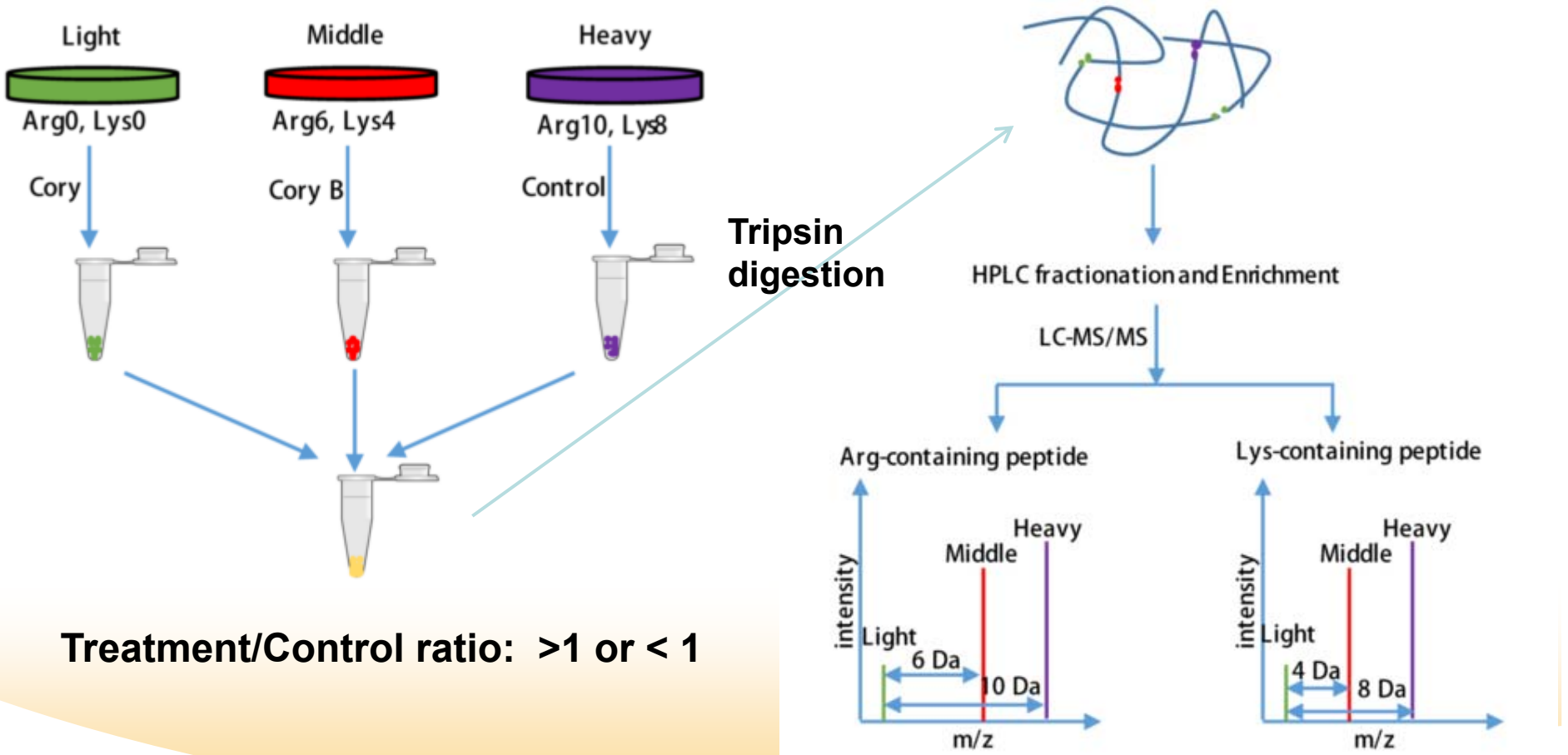
Corynoxine B Corynoxine



Lu et al., Autophagy, 2012, 8, 98-108
Chen et al., J Neuroimmune Pharmacol., 2014, 9, 380-7

Experimental procedure

- **N2a: a mouse neuroblastoma cell line**

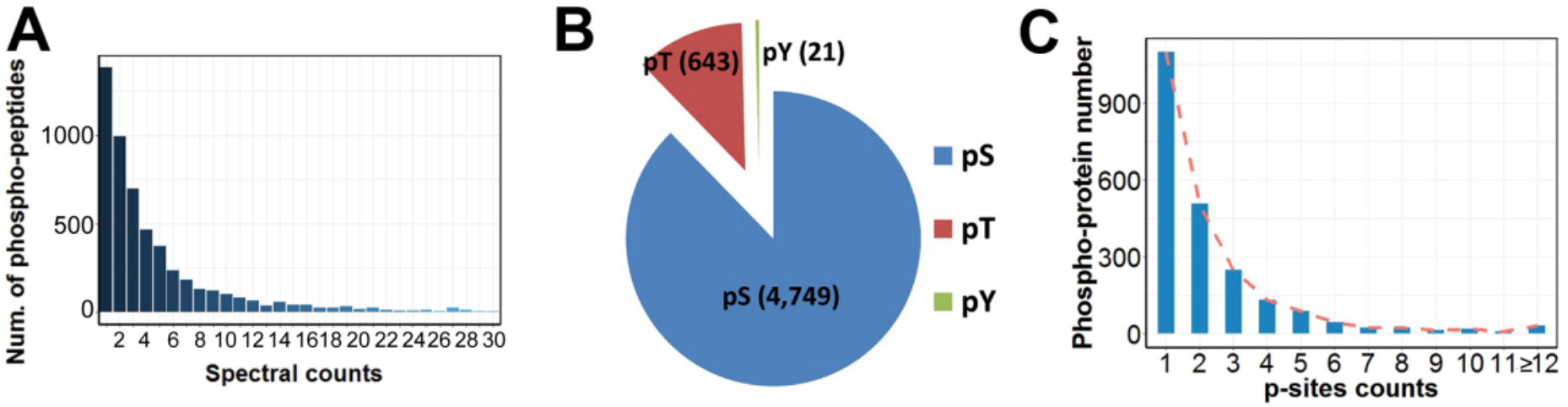


Treatment/Control ratio: >1 or < 1

Chen et al., Autophagy, 2017, 13, 1969-1980

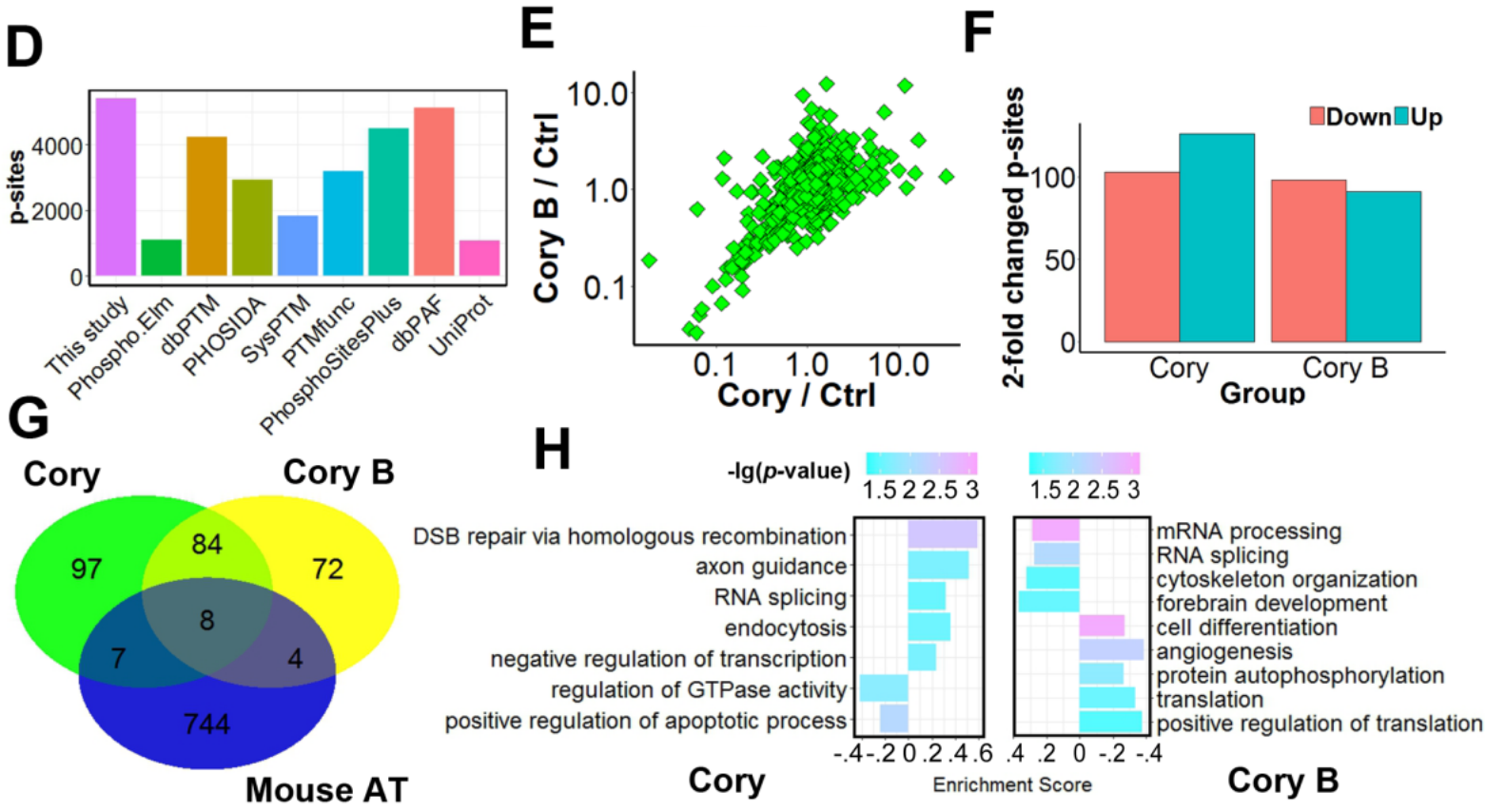
Phosphoproteomics profiling

- Quantified 5,328 unique phosphopeptides
  - 74.0% phosphopeptides (3,943;) with > 1 spectrum
  - Average spectral counts: 5.5
- 2,233 phosphoproteins with 5,413 p-sites



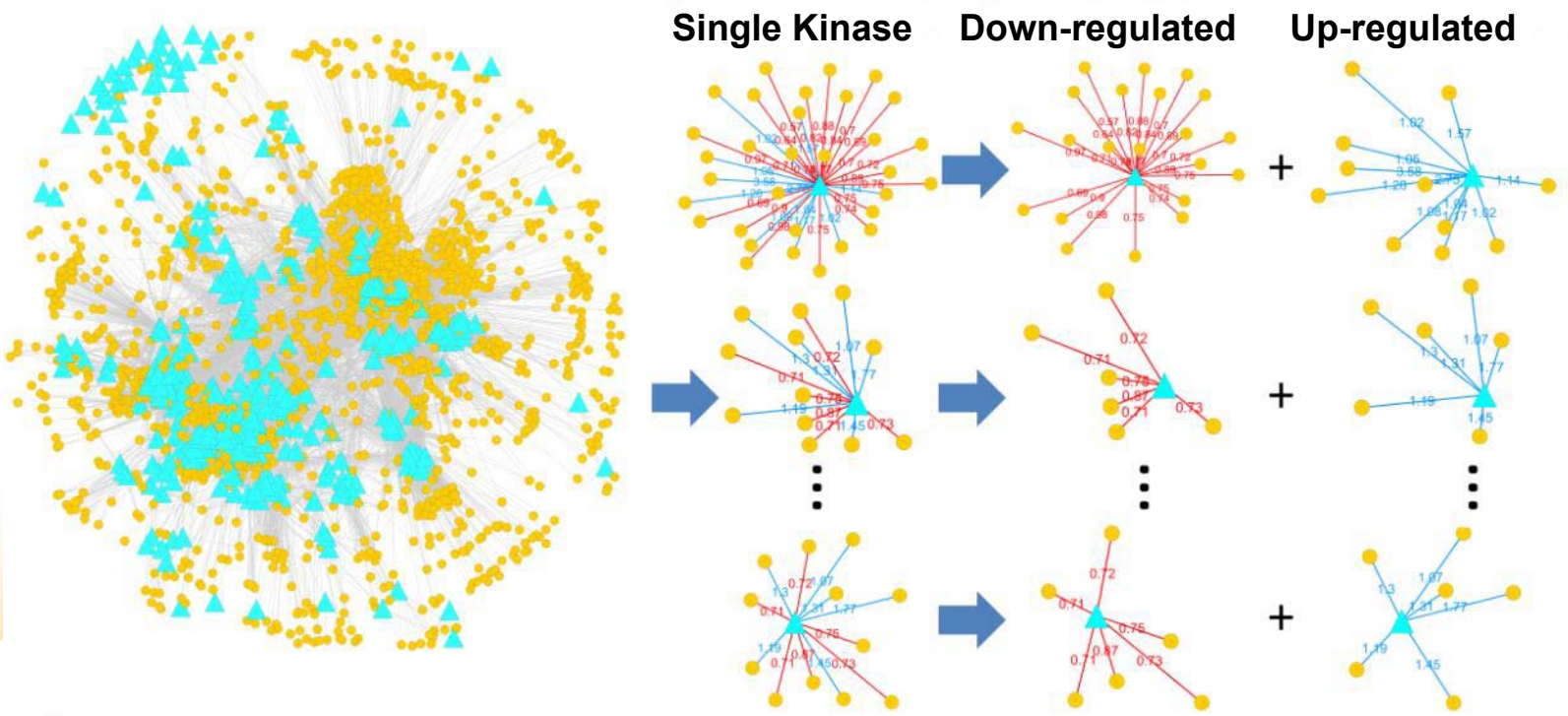
Phosphoproteomics profiling

- ~95.1% p-sites reported previously
- Cory & Cory B regulate different proteins & pathways

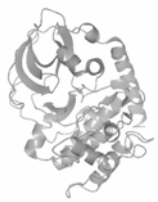


Neuronal autophagy phosphorylation network

- GPS: Cory- & Cory B-regulated phosphorylation networks
- Single kinase network
  - Down-regulated network & up-regulated network



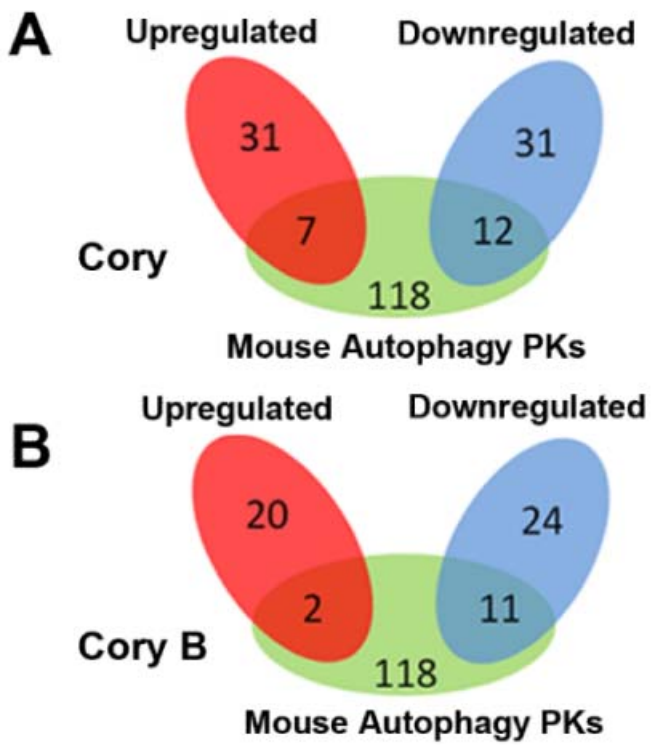
iKAP algorithm

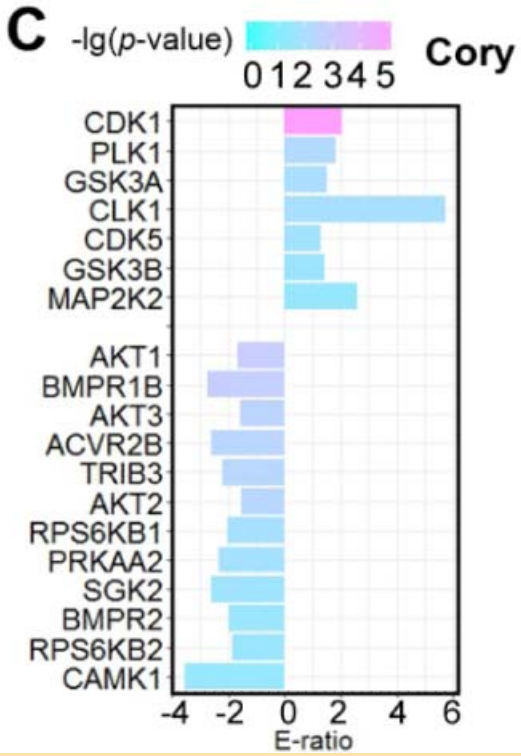


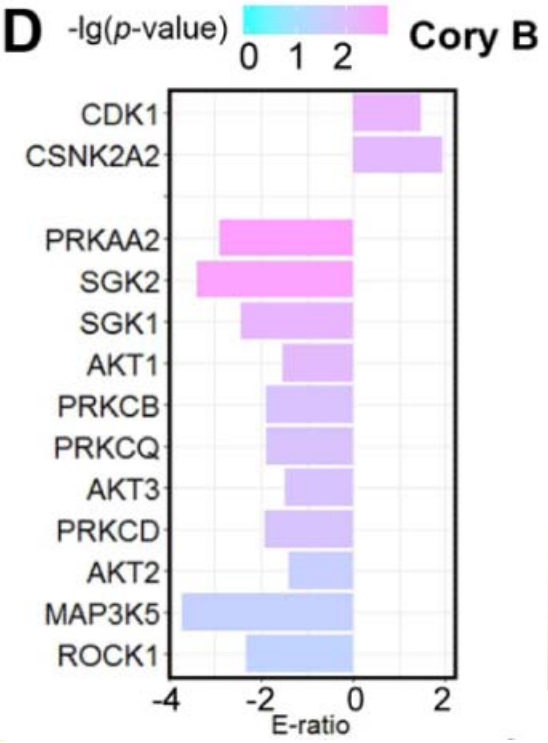
- For Kinase *i*, statistically test whether it prefer to be involved in up-regulated (high activity) or down-regulated (low activity) networks
  - *KA*: kinase activity; *KS*: Treatment/Control (T/C) ratio
- Up-regulated network
  - T/C >1, $KA_{up}(i) = \sum_{j=1}^{m} int(KS_{ij})$
- Down-regulated network
  - T/C < 1, $KA_{Down}(i) = \sum_{j=1}^{n} int(\frac{1}{KS_{ij}})$
- Yates' chi-squared test
  - $KA_{up} = \sum_{i=1}^{k} KA_{up}(i)$, $KA_{down} = \sum_{i=1}^{l} KA_{down}(i)$

Differentially activated kinases

- **THANATOS filter**
  - **Up-regulated: 7 (Cory) & 2 (Cory B)**
  - **Down-regulated: 12 (Cory) & 11 (Cory B)**

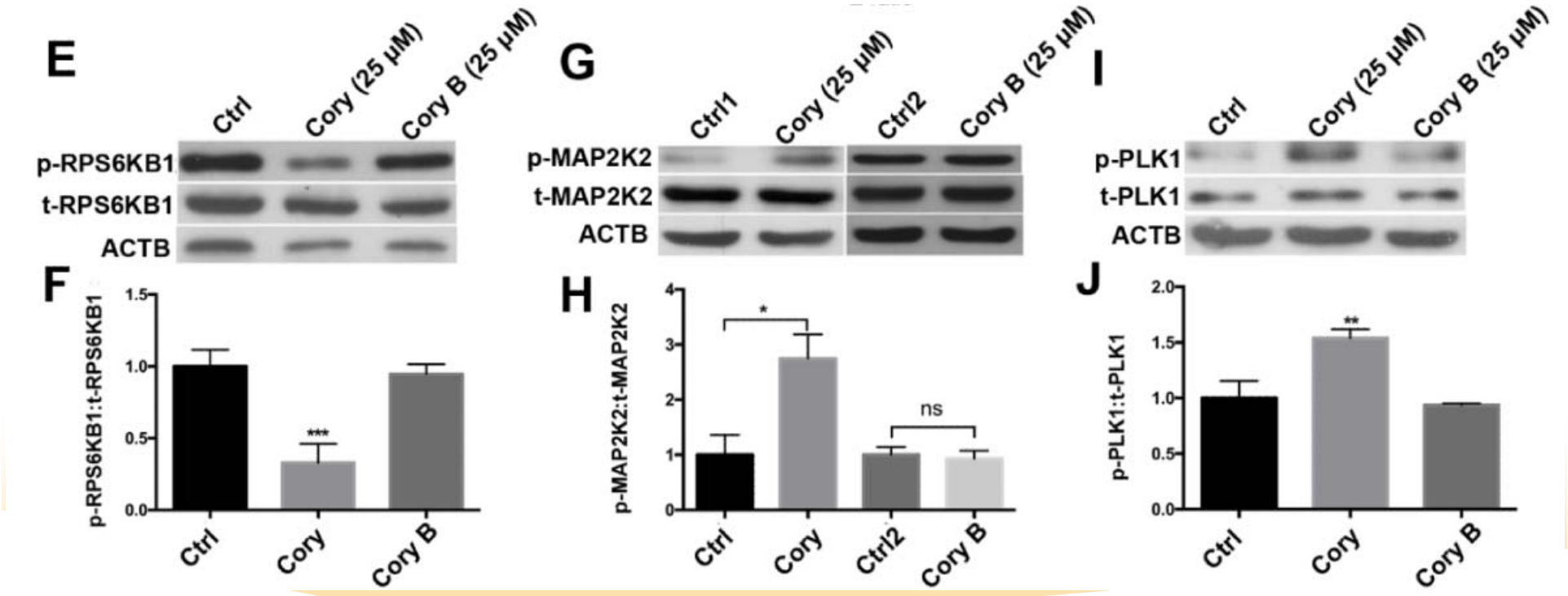






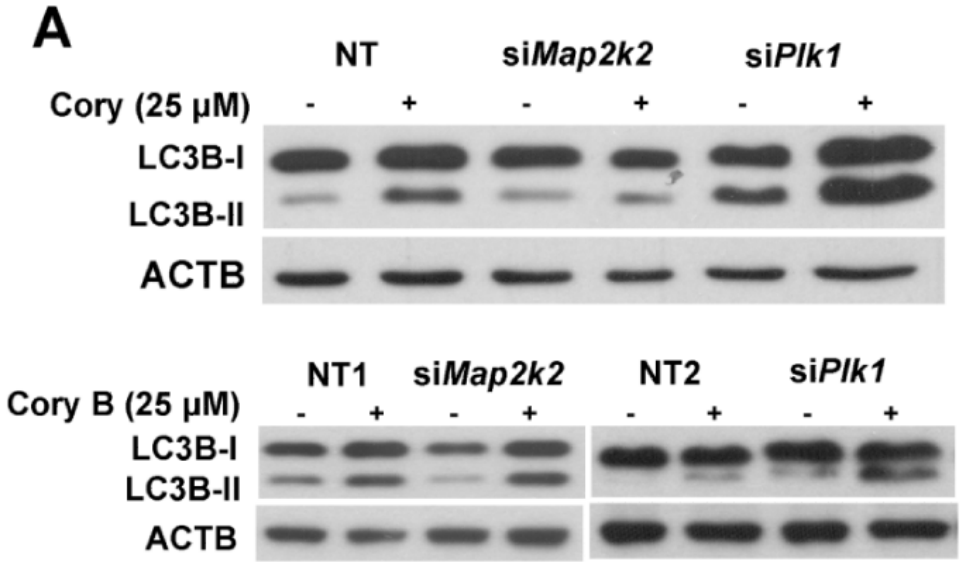
Cory, but not Cory B

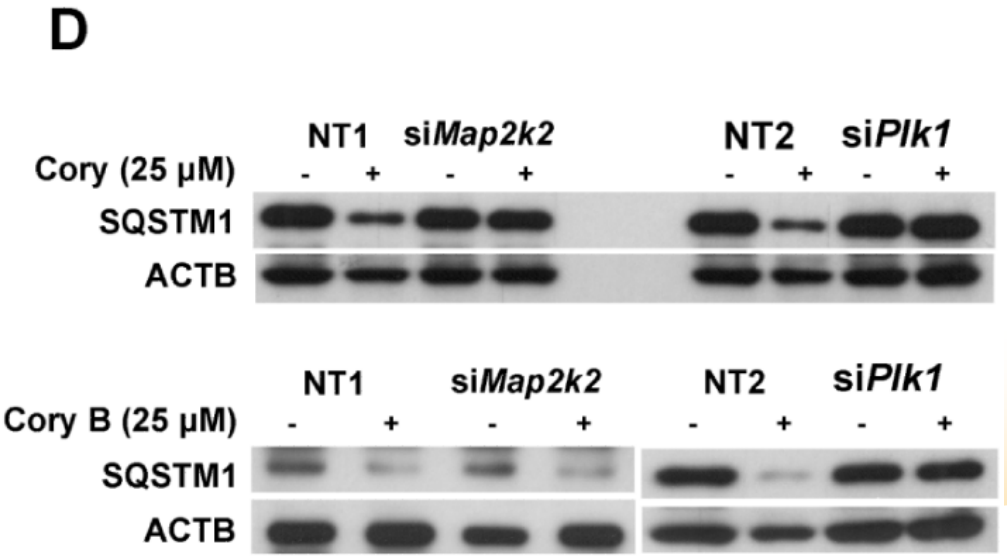
- **Kinase activity-associated p-sites**
  - **Down-regulates RPS6KB1 (p70S6K)**
  - **Up-regulates MAP2K2 (MEK2) & PLK1**



MAP2K2 & PLK1

- Silencing MAP2K2 but not PLK1 decreases LC3B-II
- Silencing MEK2 & PLK1 both increase SQSTM1/p62

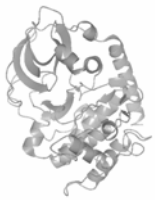




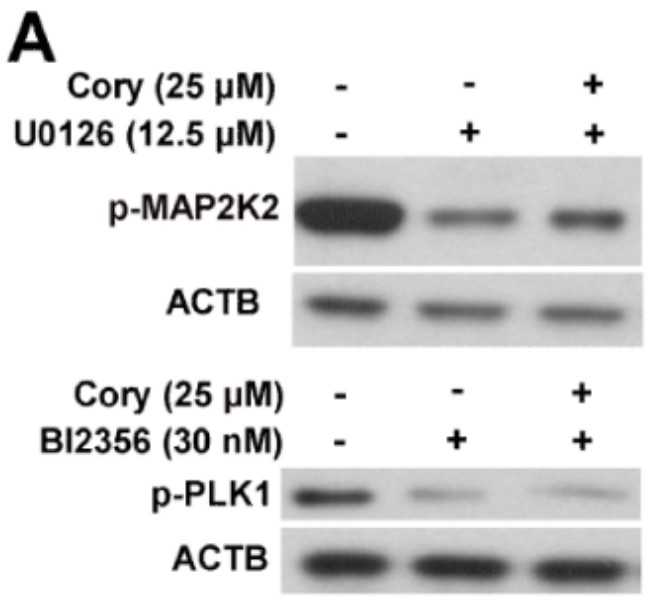
LC3B-II ↑: autophagy inhibition & activation

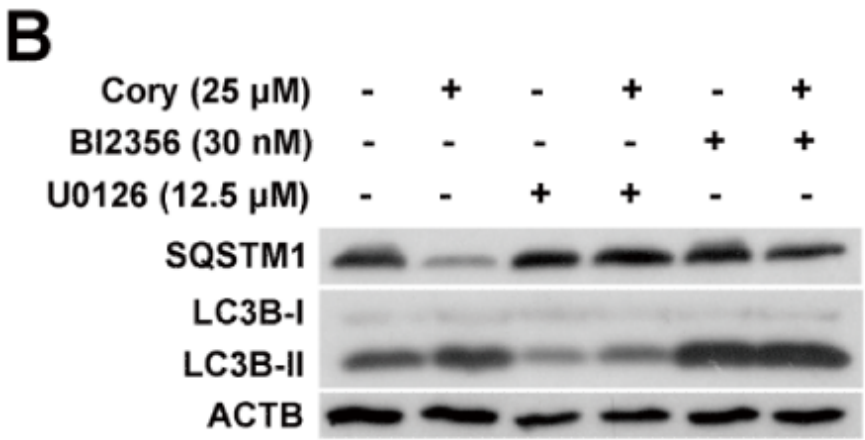
SQSTM1↓: autophagic flux ↑

MAP2K2 & PLK1 Inhibition



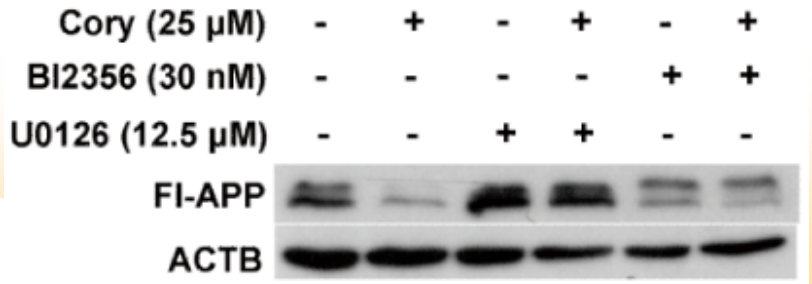
- Inhibitors: U0126 (MAP2K2) & BI2356 (PLK1)
- SQSTM1 is not decreased
- LC3B-II decreases in MAP2K2 inhibition, but increases in PLK1 inhibition

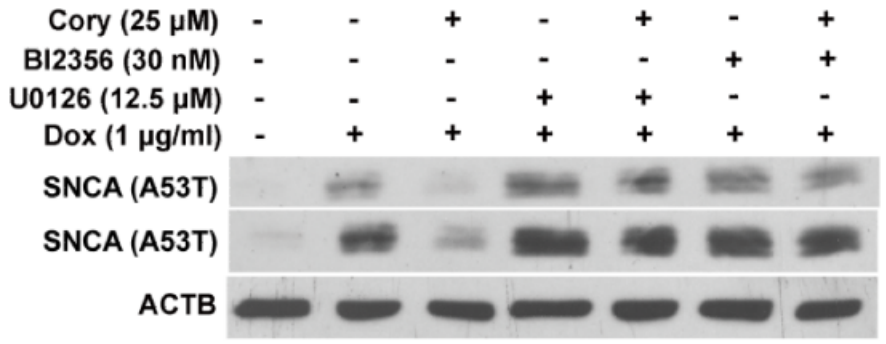




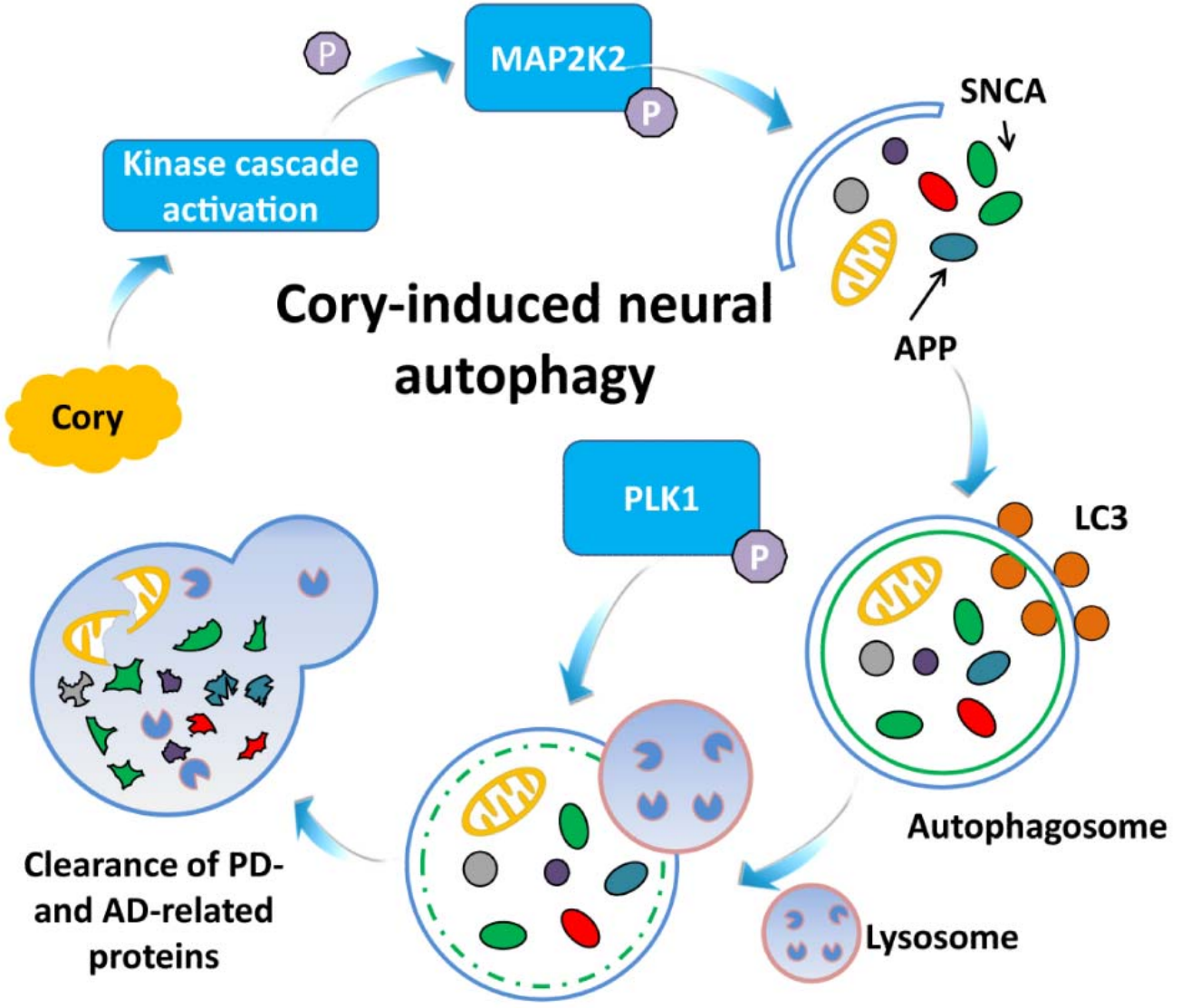
In neuronal autophagy

- Alzheimer's disease: APP (β-amyloid precursor protein)
- Parkinson’s disease: α-synuclein (α-syn)
- The inhibition of MEK2 or PLK1 diminishes the clearance of disease-associated proteins by Cory
- The activation of MEK2 & PLK1: neuroprotective

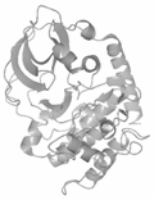




MAP2K2 and PLk1 in In neuronal autophagy



蛋白质泛素化



- 最复杂的共价修饰
- 可逆生化反应：
  - ◆泛素化修饰：E1-E2-E3
  - ◆泛素链延长：E4 – 多聚泛素化链
  - ◆去泛素化：DUB
  - ◆泛素化绑定：Ubiquitin-binding

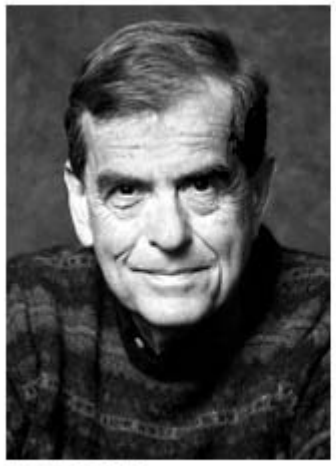


Photo: D. Porges

Aaron Ciechanover

Photo: D. Porges

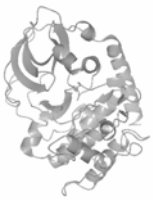
Avram Hershko

Irwin Rose

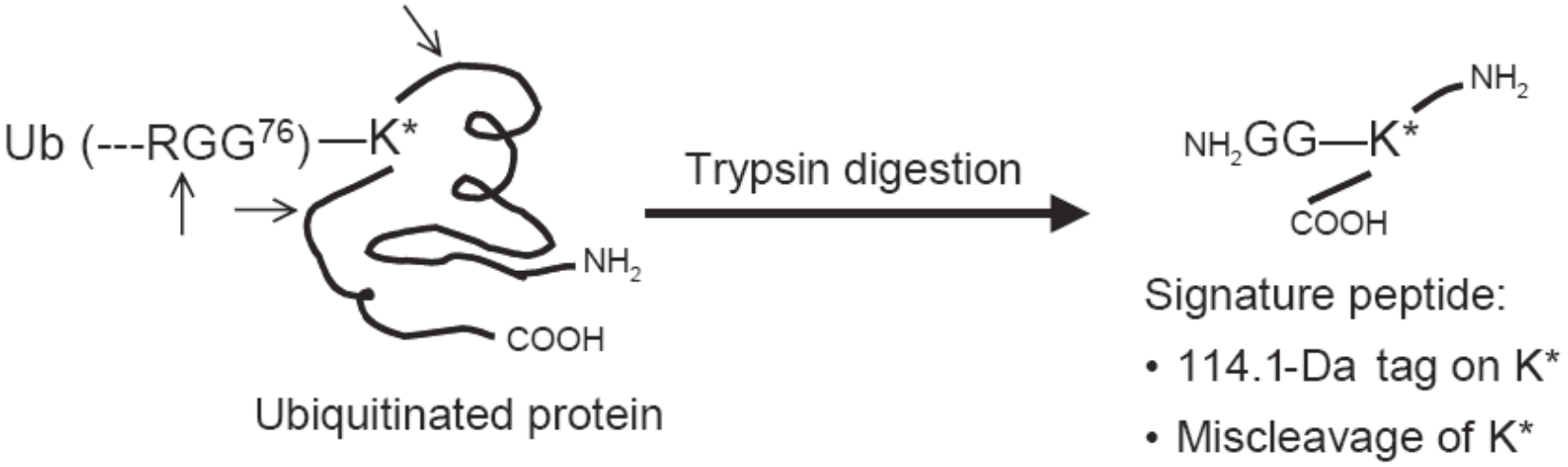
The Nobel Prize in Chemistry 2004

“for the discovery of ubiquitin-mediated protein degradation”

酵母泛素化蛋白质组学研究

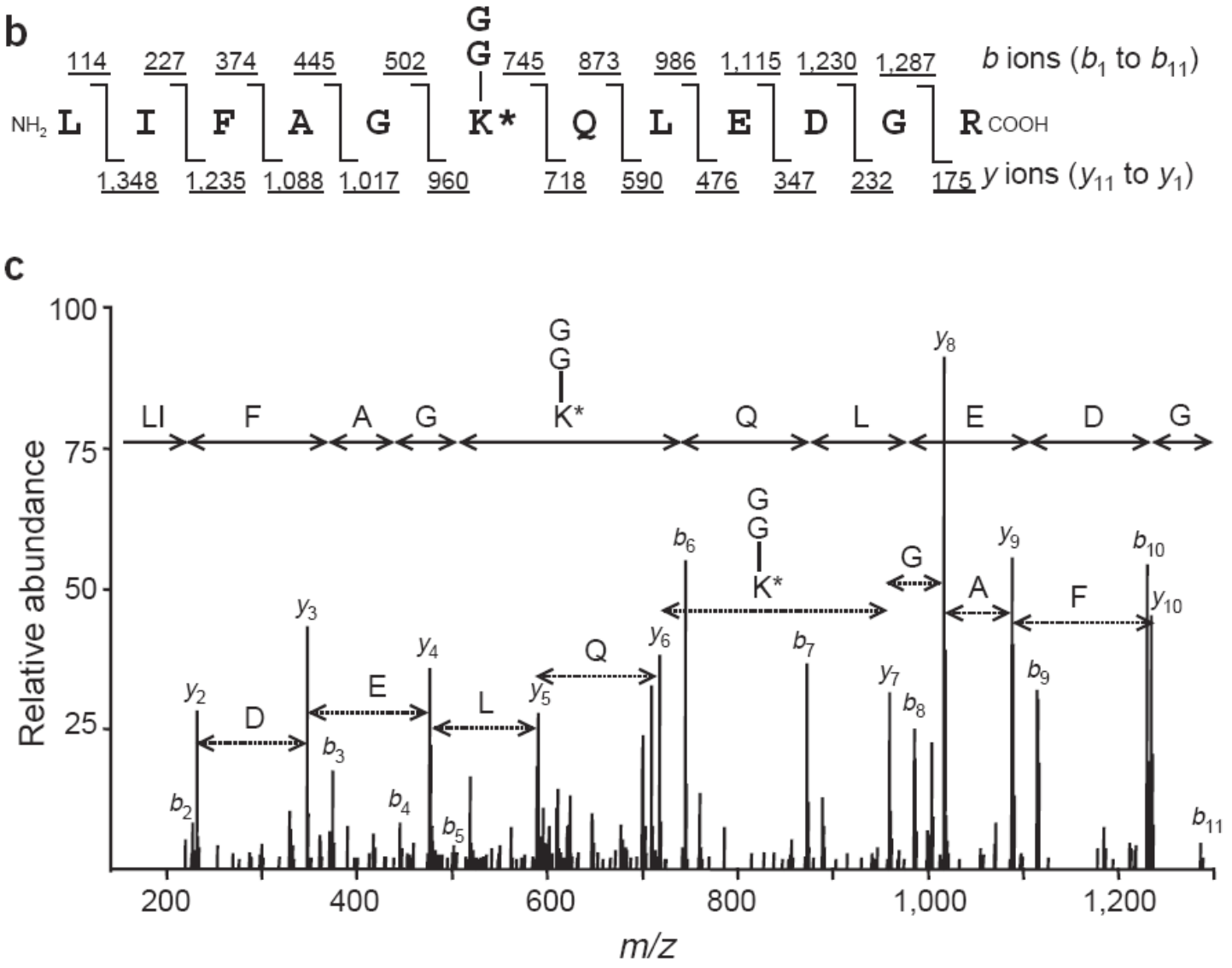


- 泛素蛋白质：~8kDa
  - 共价连接：-GG
  - 非共价的相互作用：尿素变性
- 110个位点，72个底物



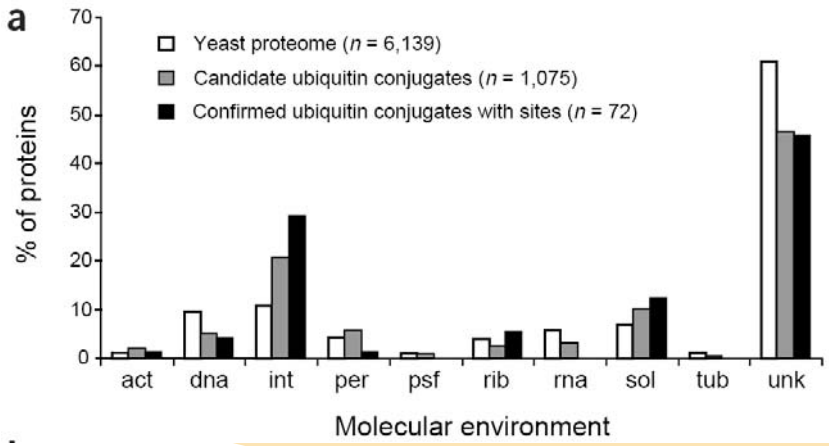
Peng, et al., Nat Biotechnol, 2003, 21, 921-926

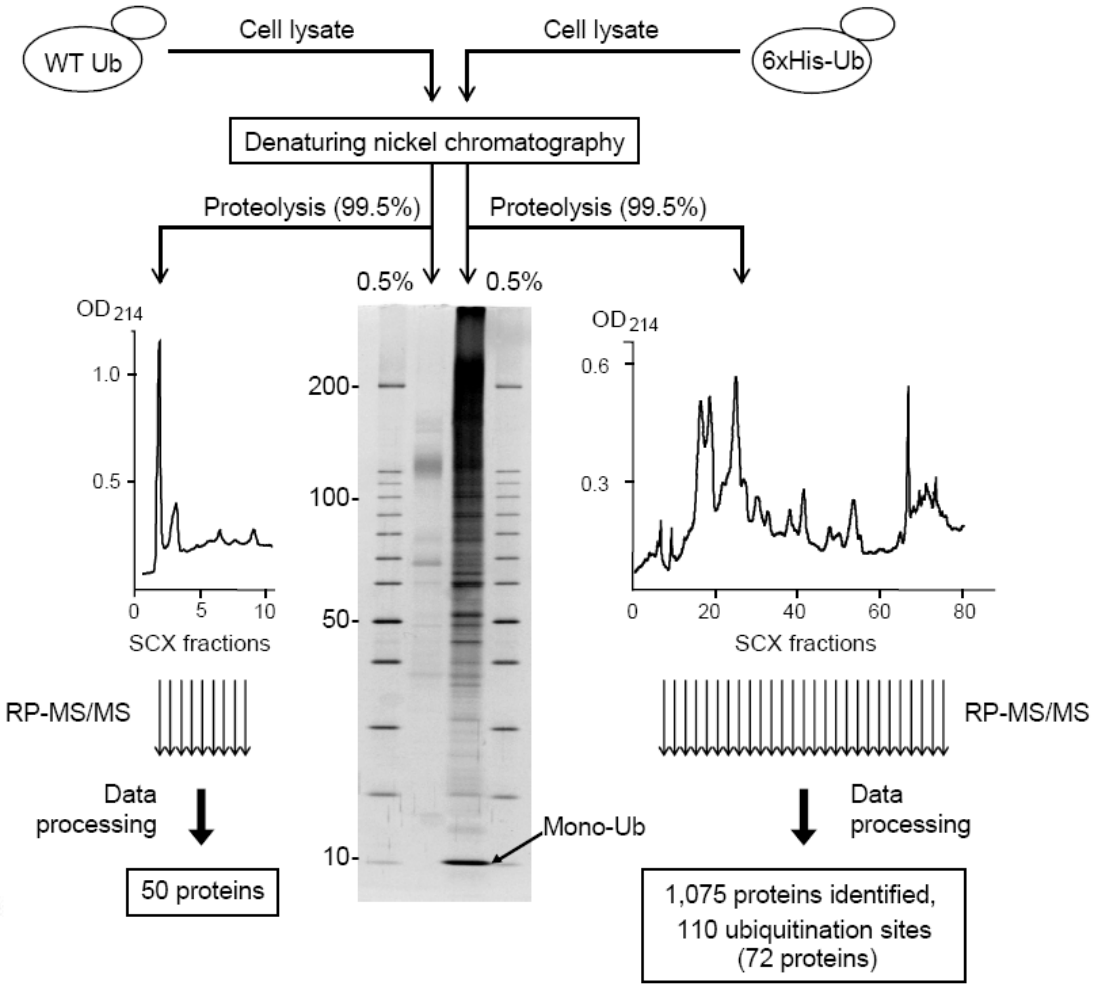
-GG：114.1Da


b
114 227 374 445 502 GG 745 873 986 1,115 1,230 1,287
b ions (b_1 to b_{11})
NH2 L I F A G K* Q L E D G R COOH
1,348 1,235 1,088 1,017 960 718 590 476 347 232 175
y ions (y_{11} to y_1)
c
Relative abundance
100
75
50
25
LI F A G GG K* Q L E D G
y_8
b_6
y_9
b_{10}
y_{10}
y_3
y_4
y_6
b_7
y_7
b_8
b_9
y_2
y_5
b_2
b_3
b_4
b_5
b_{11}
D E L Q G A F
200 400 600 800 1,000 1,200
m/z

酵母泛素化蛋白质组

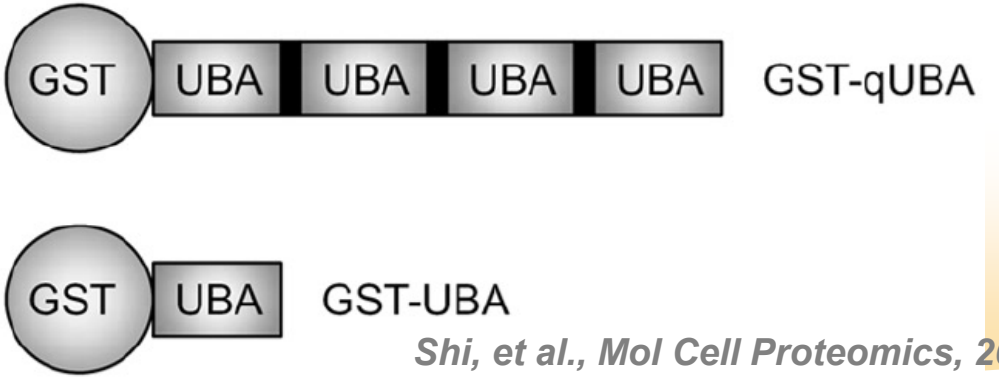
- 对比实验：
  - ◆细胞内的泛素
  - ◆6xHis-tag泛素

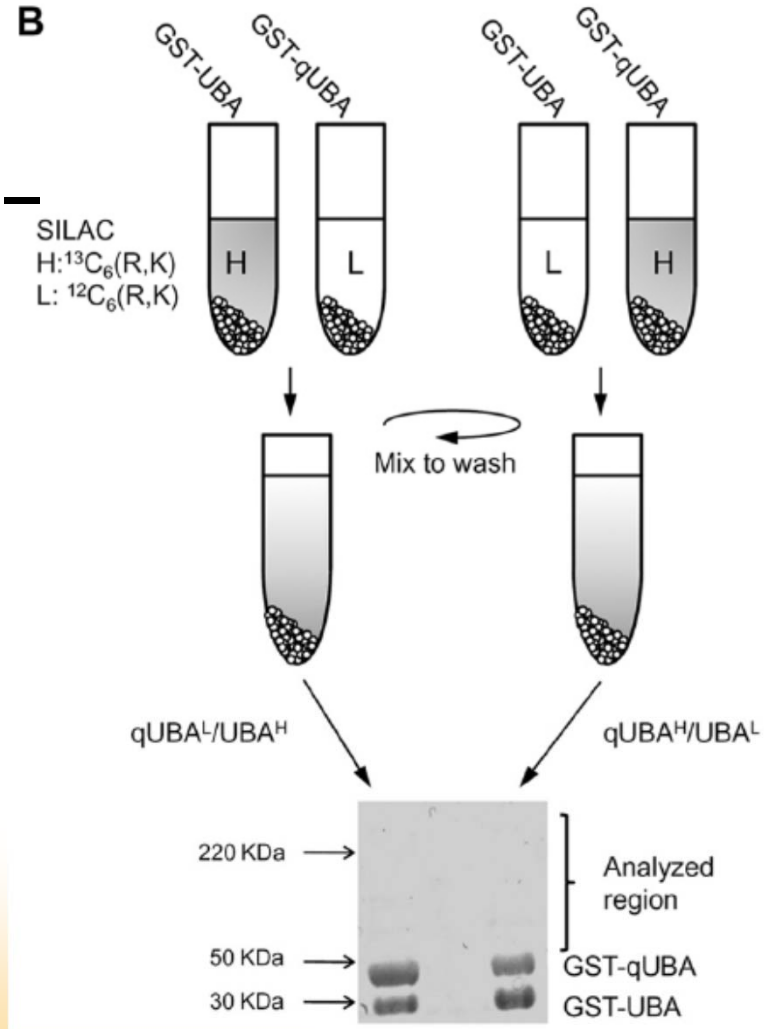




人类泛素化蛋白质组

- 人类293T细胞株
- IP实验
  - ◆UBQLN1的四个泛素绑定结构域 – 结合多聚泛素化链
  - ◆单个泛素绑定结构域 – 单泛素化
  - ◆GST tag
- 294个位点，223个底物
  - ◆14.7%：线粒体蛋白

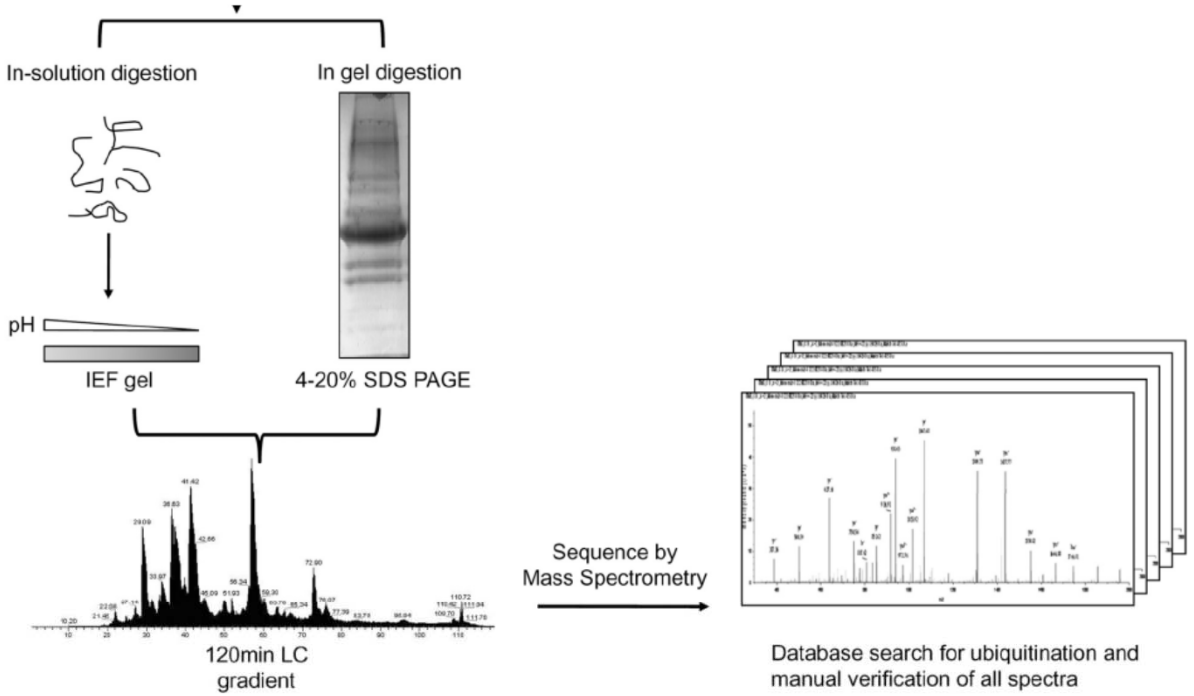


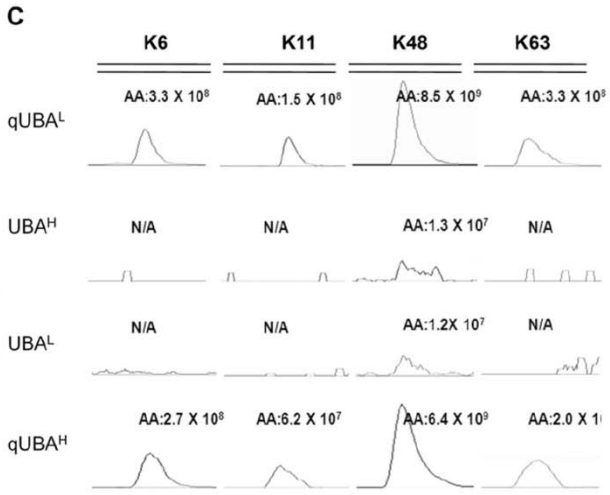


Shi, et al., Mol Cell Proteomics, 2011, 10, M110 002089

泛素化的定量分析

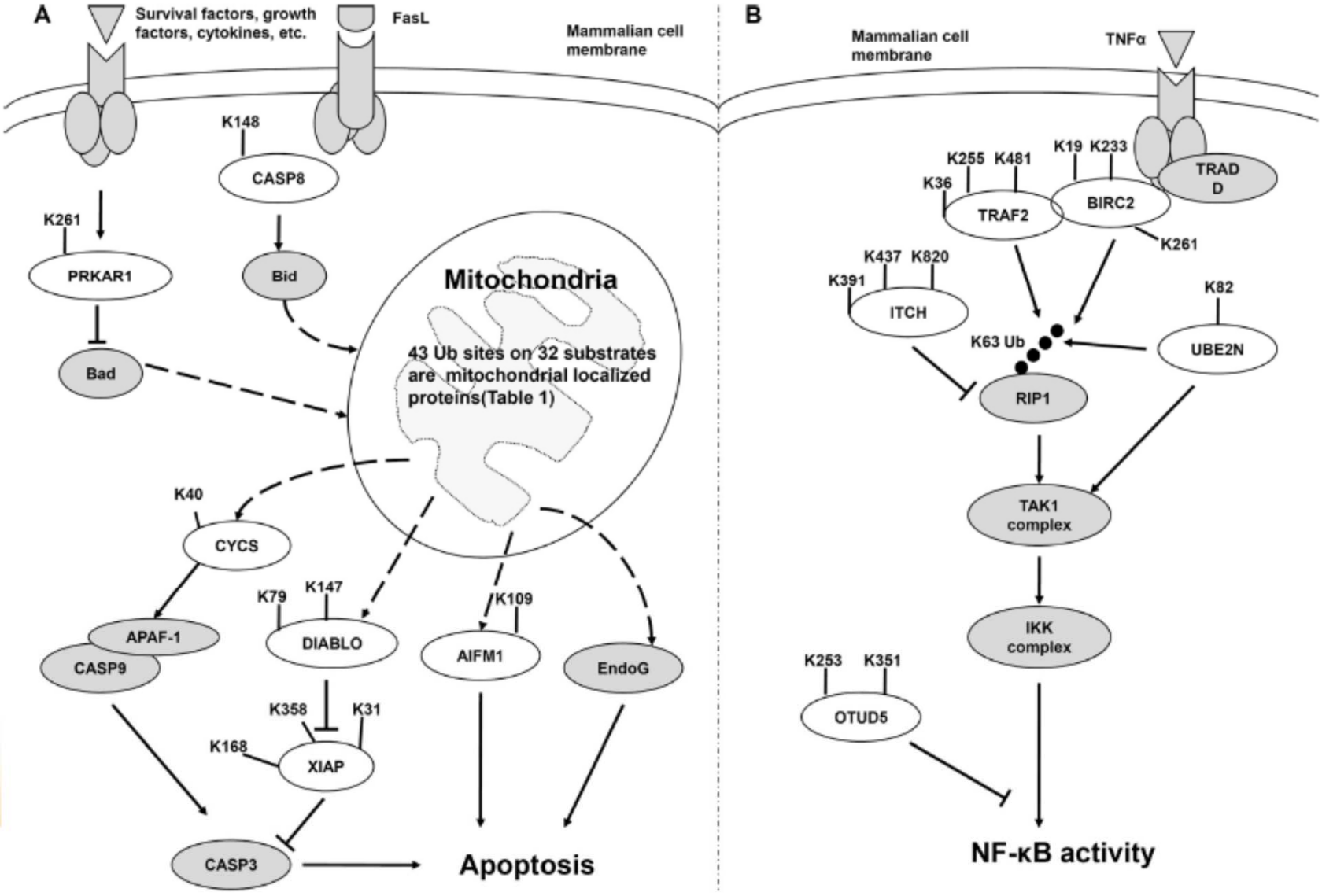
- 以多聚泛素化为主





Poly-Ub linkages	Sequence	m/z (H)	m/z(L)	Charge	ratio (qUBA/UBA)
K6	MQIFVk(GG)TLTGK	464.607	460.594	+3	>1000
K11	TLTGk(GG)TITLEVEPSDTIENVK	805.438	801.425	+3	>1000
K48	LIFAGk(GG)QLEDGR	491.611	487.598	+3	~500
K63	TLSDYNIQk(GG)ESTLHLVLR	752.748	748.735	+3	>1000

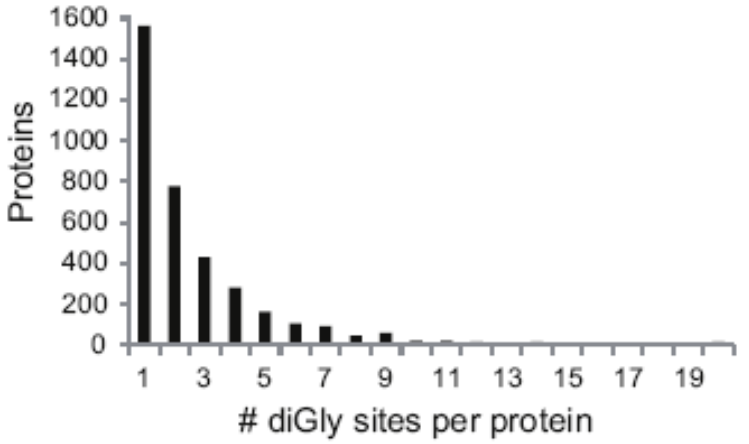
NF-κB通路的泛素化


A
Survival factors, growth factors, cytokines, etc.
FasL
Mammalian cell membrane
K148
CASP8
K261
PRKAR1
Bid
Bad
Mitochondria
43 Ub sites on 32 substrates are mitochondrial localized proteins(Table 1)
K40
CYCS
APAF-1
CASP9
K79
K147
DIABLO
K109
AIFM1
EndoG
K358
K31
K168
XIAP
CASP3
Apoptosis
B
Mammalian cell membrane
TNFα
K255
K481
K36
TRAF2
K19
K233
BIRC2
TRAD D
K261
K437
K820
K391
ITCH
K82
UBE2N
K63 Ub
RIP1
TAK1 complex
IKK complex
K253
K351
OTUD5
NF-κB activity

人类泛素化蛋白质组 (2)

- HCT116 and 293T细胞株
- 肽段抗体：
  - ◆CXXXXXXK(GG)XXXXXX
- LTQ Orbitrap
- Btz (Bortezomib): 蛋白酶体抑制剂

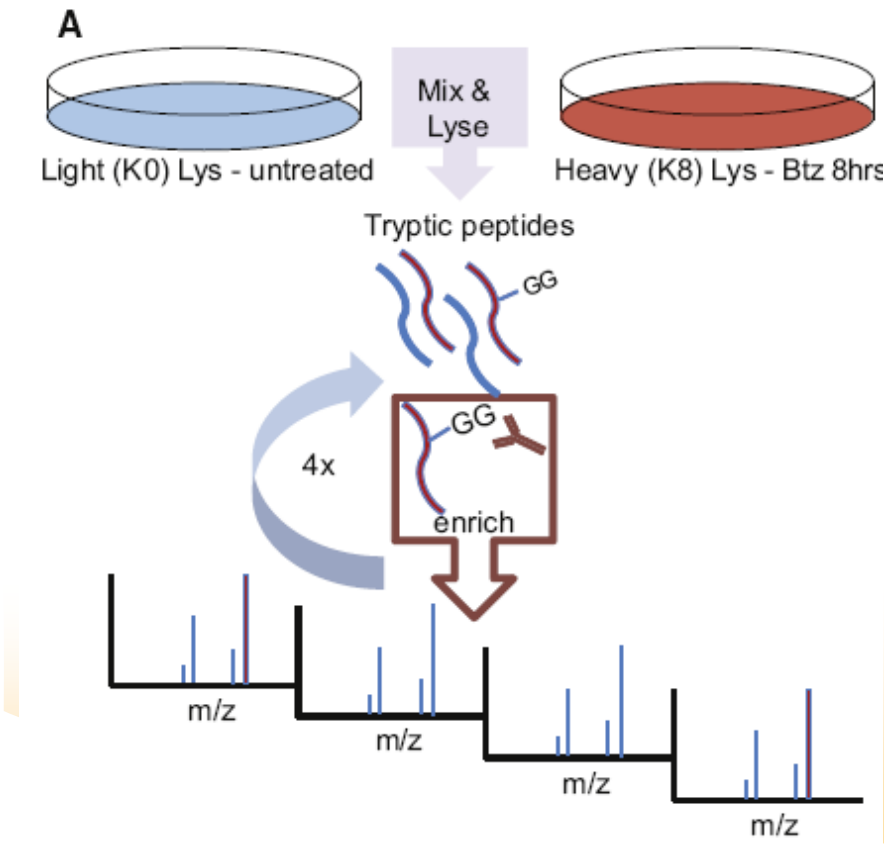
	Identified	Quantified
# Sites	10634	7640
Site FDR	0.5%	0.2%
# Proteins	3662	2879
Protein FDR	1.1%	0.6%

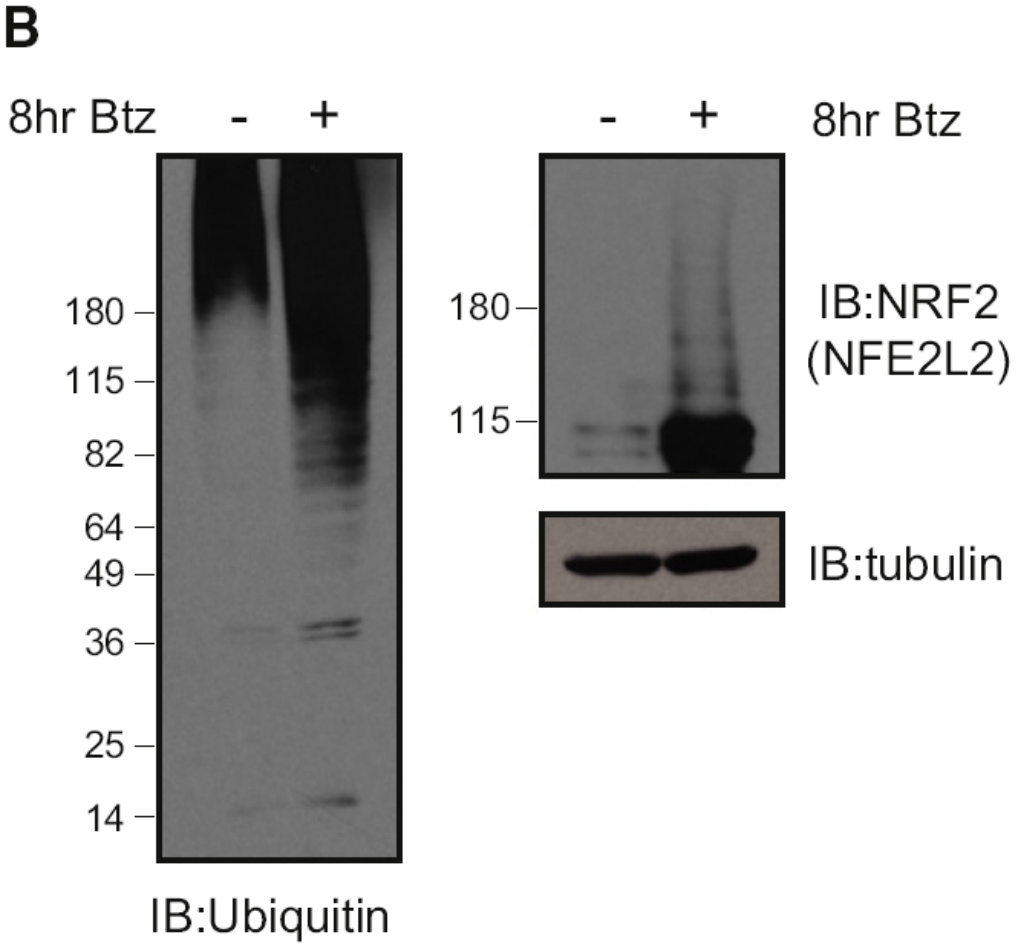


Kim, et al., Mol Cell, 2011, 44, 325-340

泛素化蛋白质组的定量分析

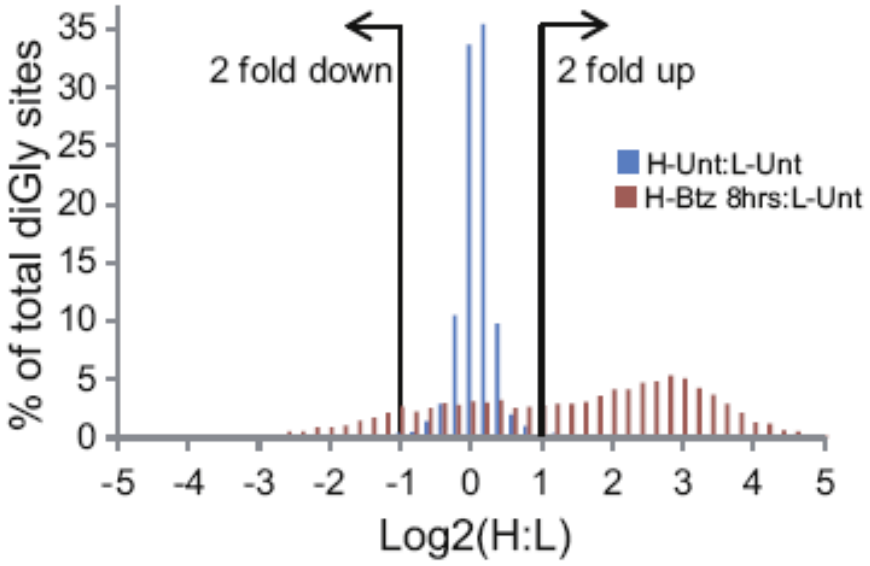
- **DiGly Proteomic Enrichment Strategy**
- **加入蛋白酶体抑制剂：泛素化程度升高**

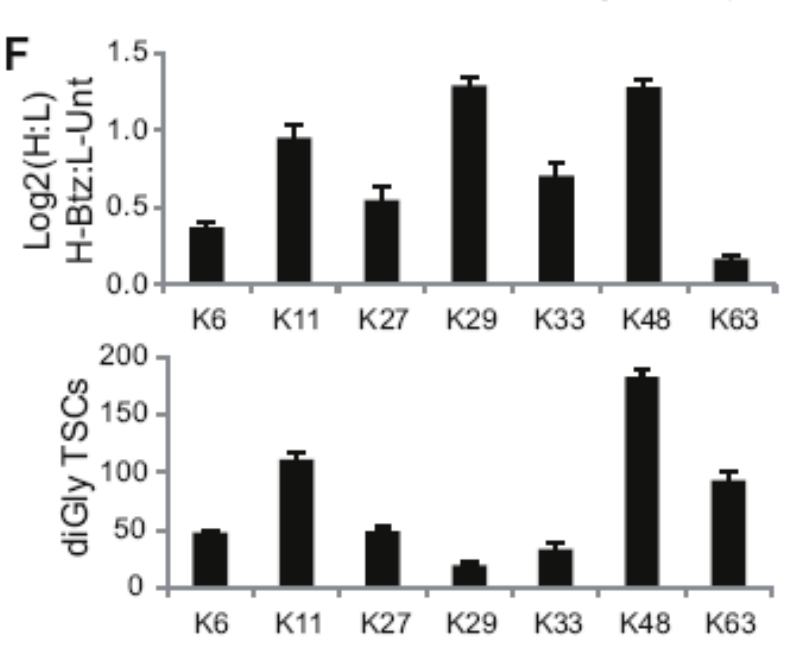




蛋白酶体抑制剂的作用

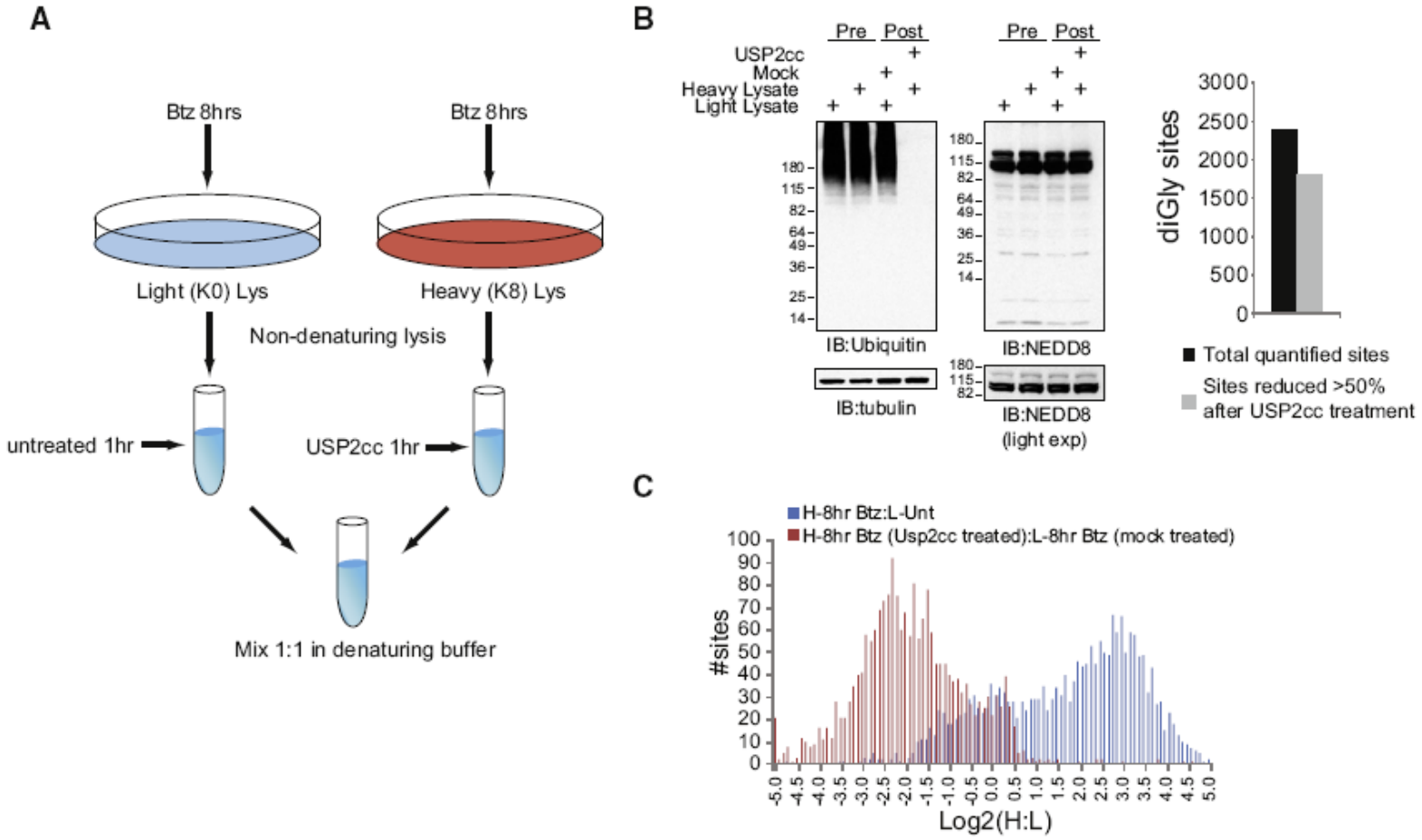
- 多数蛋白质的泛素化水平升高
  - 多聚泛素化水平升高
  - 单泛素化水平降低





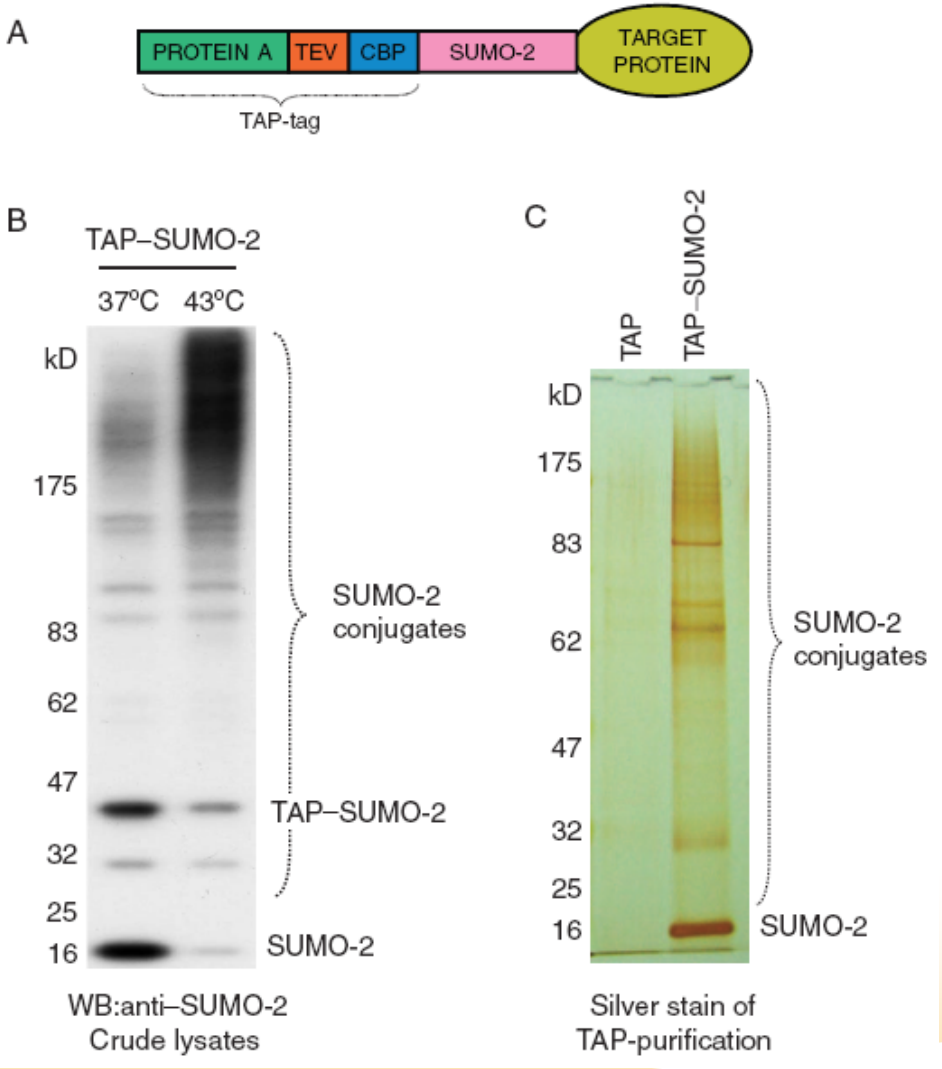
去泛素化酶的影响

- **USP2cc: 催化结构域**
  - ◆泛素化水平显著下降



SUMO化蛋白质组研究

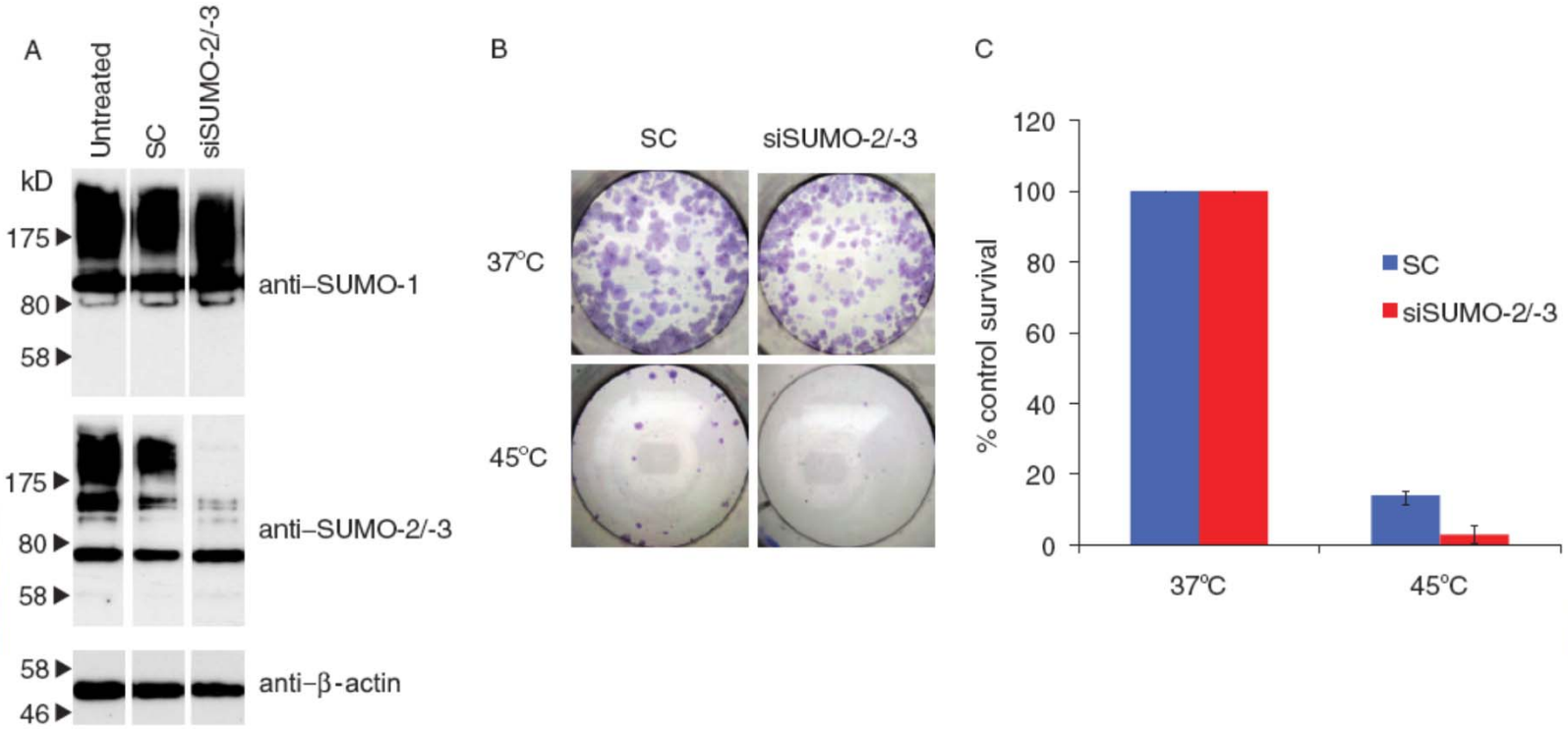
- SUMO
  - ◆泛素超家族成员
  - ◆单SUMO化
  - ◆SUMO-2/3多聚链
- 热休克：SUMO-2/3
  - ◆U2OS细胞
  - ◆45°C，30min
  - ◆37°C，10-14days
  - ◆SUMO化修饰增强



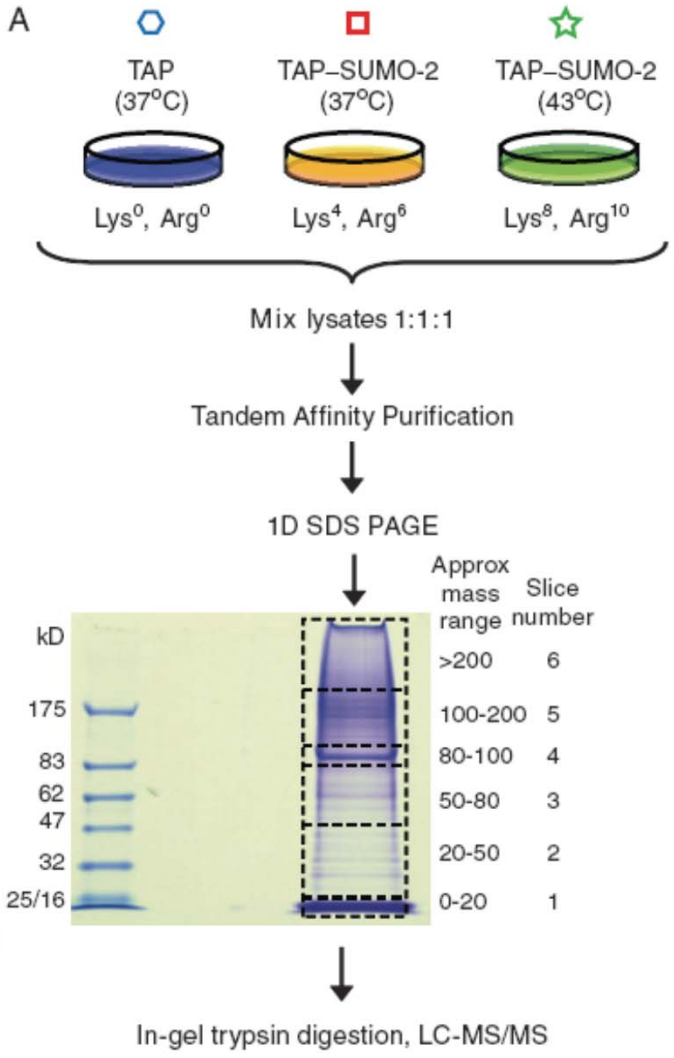
Golebiowski, et al., Science signaling, 2009, 2, ra24

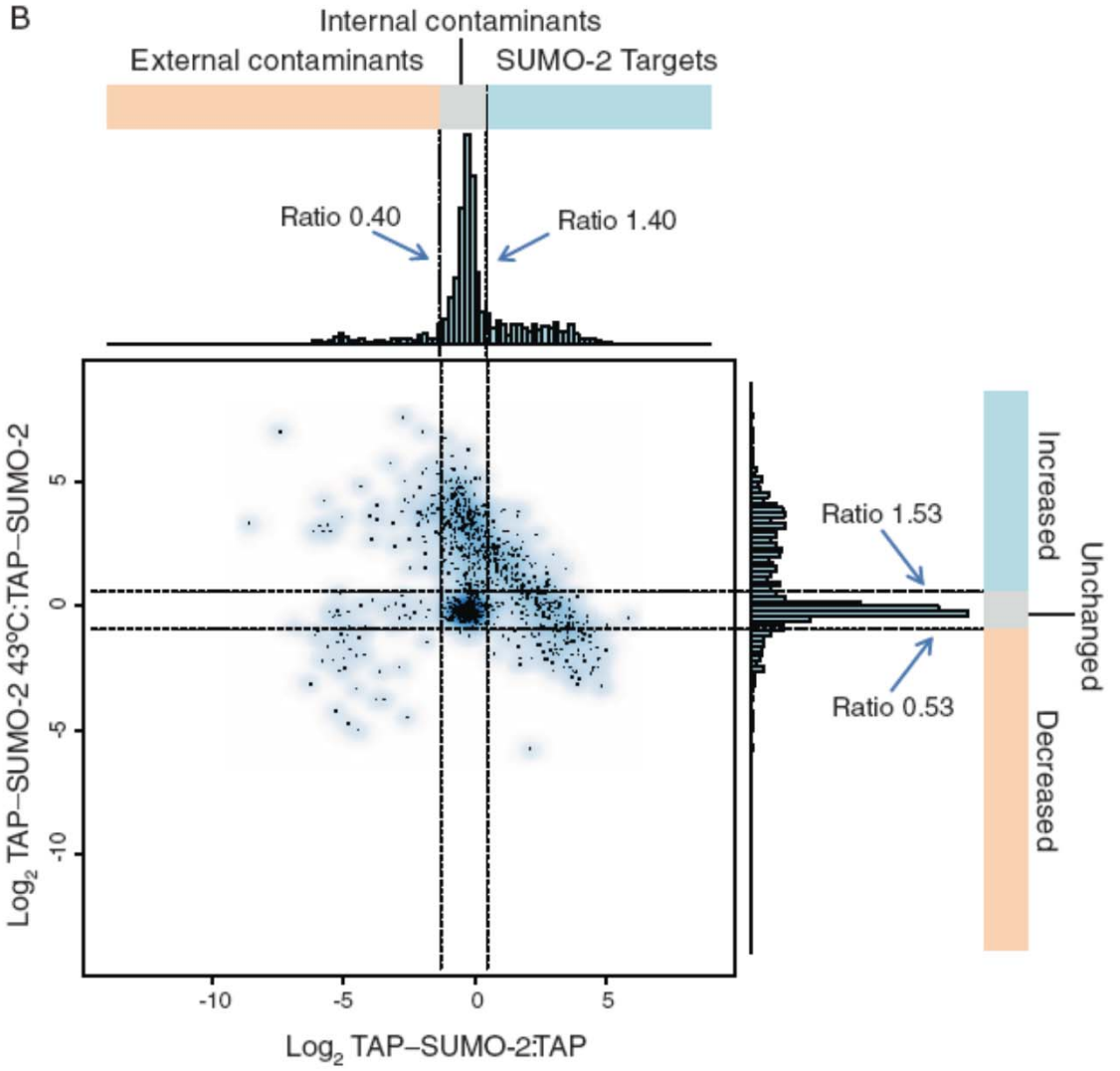
热休克：SUMO-2/3

- SUMO-2/3的特异siRNA
  - ◆不沉默SUMO-1
  - ◆热休克情况下提高细胞的生存率

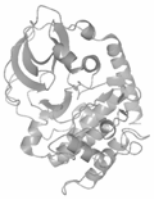


SUMO化蛋白质组鉴定





SUMO化蛋白质组鉴定



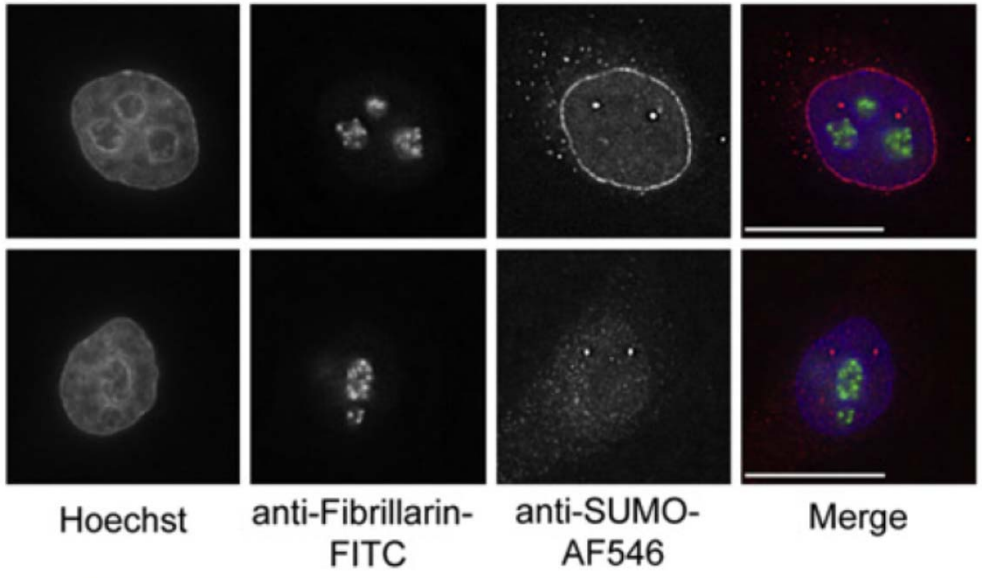
- 766个潜在的SUMO化底物
- 生物信息学分析
  - 可信度较高

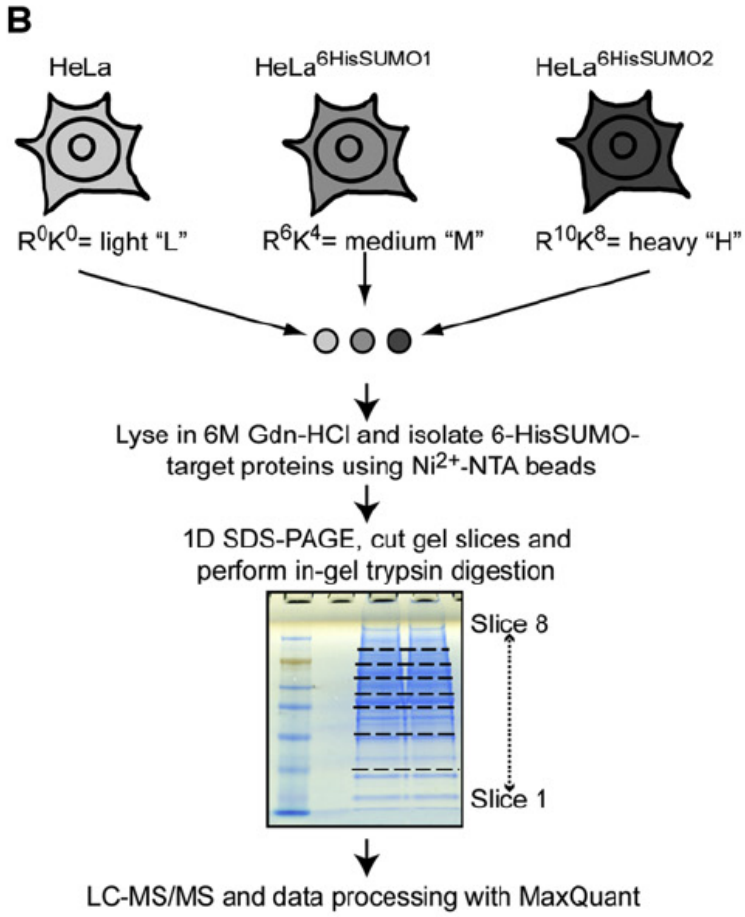
Table 1. Predicted frequency of SUMO consensus sites of proteins identified by different studies. SUMO consensus sites of the form ψKxE as predicted by SUMOsp2.0 (*52*) with a "High" threshold.

Data set	Number of proteins*	Number of predicted consensus sites	Number of proteins without consensus	Average consensus sites per protein
Published SUMO substrates	264	517	49	2.0
TAP–SUMO-2 proteome	759	1,681	195	2.2
Human proteome†	43,964	28,078	28,571	0.6
TAP–SUMO-2 internal rejects	594	458	312	0.8

核仁SUMO化蛋白质组

- **SUMO化主要集中于细胞核内**
  - **离心分离细胞核，去膜**

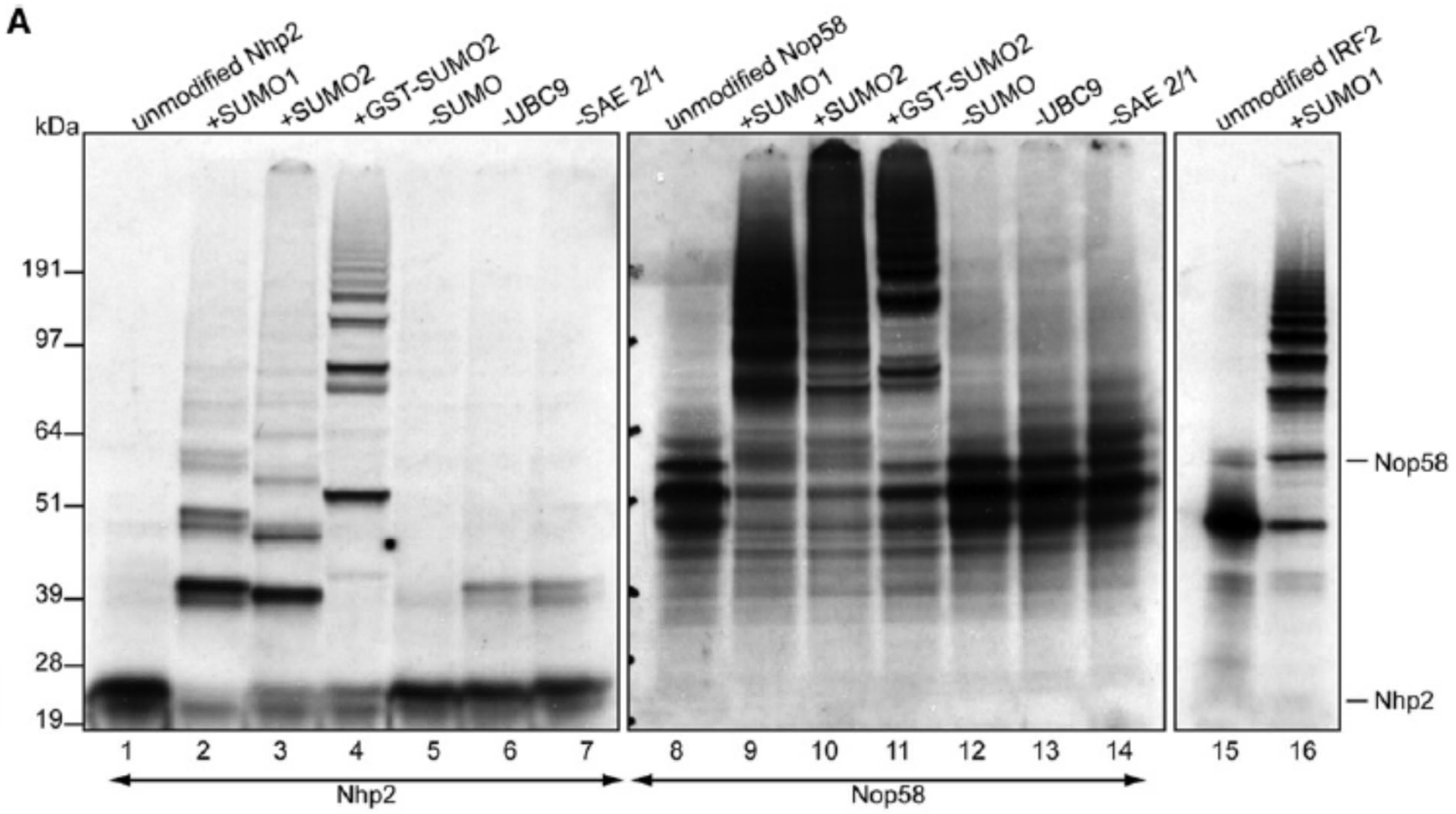




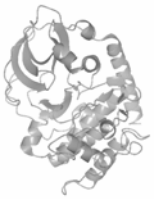
Westman, et al., Mol Cell, 2010, 39, 618-631

Nhp2 and Nop58

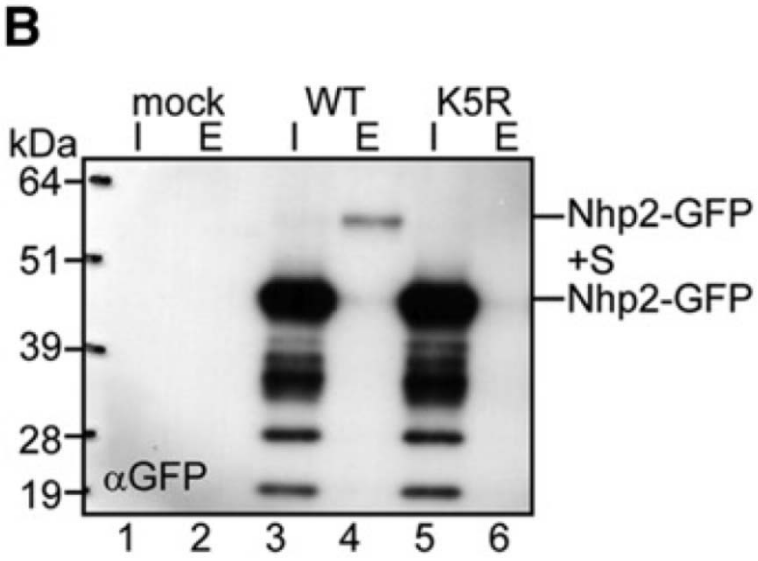
- 新的SUMO化底物

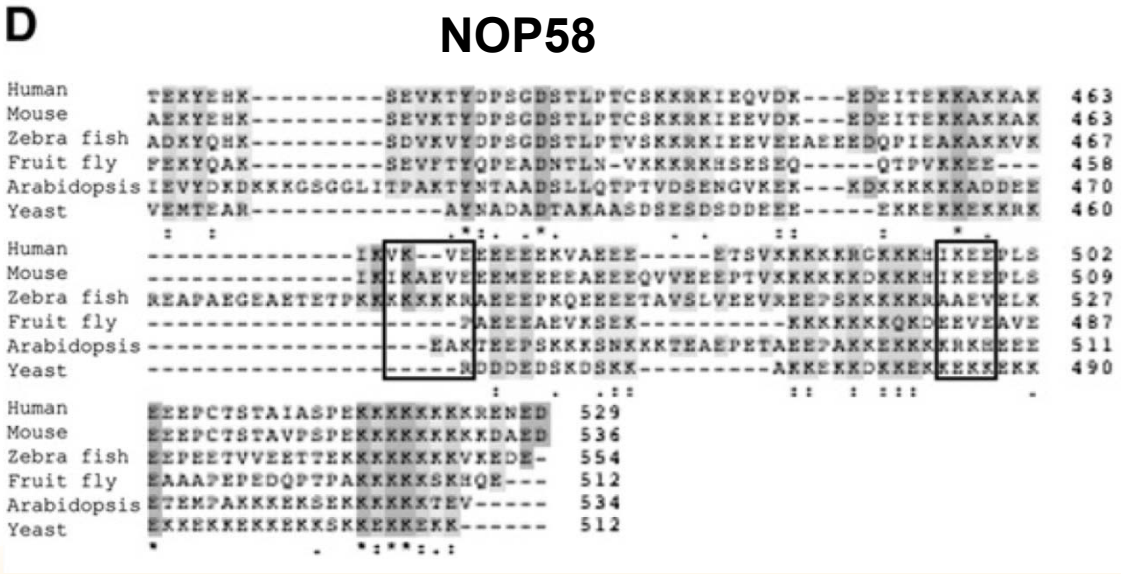


Nhp2 and Nop58



- 生物信息学预测：
  - Nhp2: K5
  - Nop58: K390, K415, K467, and K497

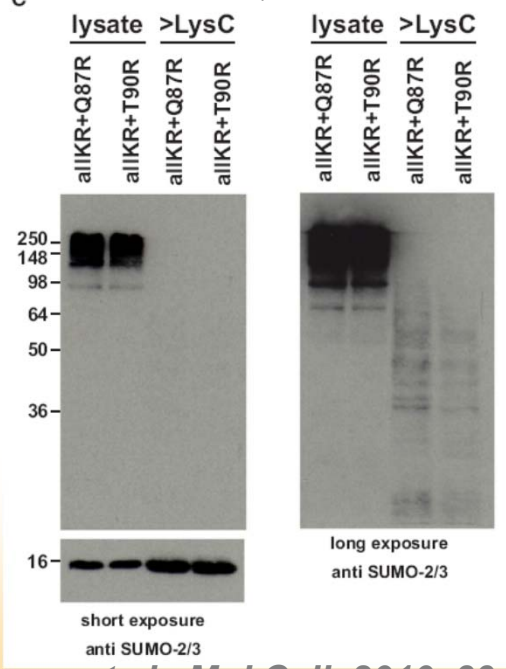


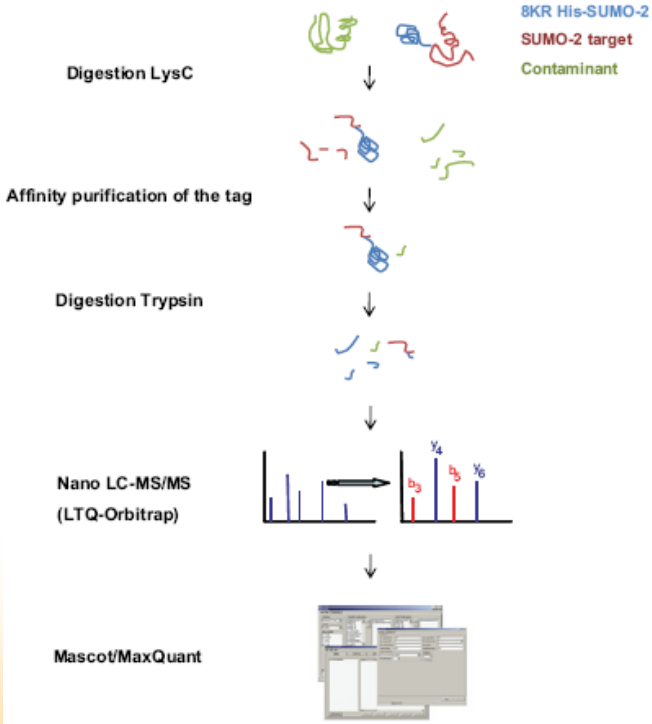


SUMO-2修饰的蛋白质组分析

- DiGly Proteomic Enrichment Strategy
  - ◆应用于泛素化分析
  - ◆构建突变体: SUMO-2
- Lys-C: 内切酶，识别K↓

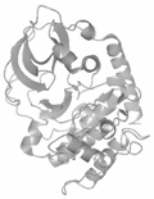
Ubiquitin	LHLVLRLRGG
SMT3	IEAHREQIGG
SUMO2 wild-type	IDVFQQQTGG
SUMO2-Q87R	IDVFRQQTGG
SUMO2-T90R	IDVFQQQRGG



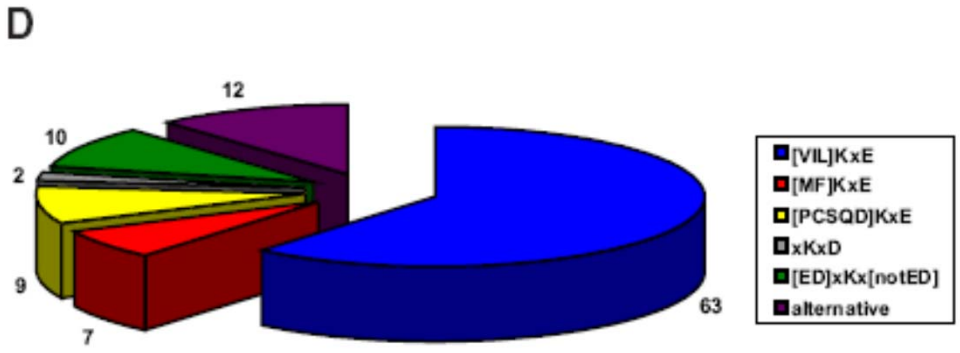


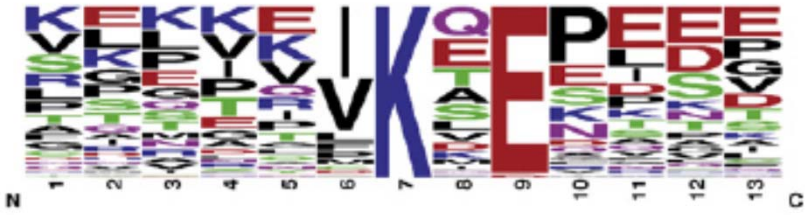
Westman, et al., Mol Cell, 2010, 39, 618-631

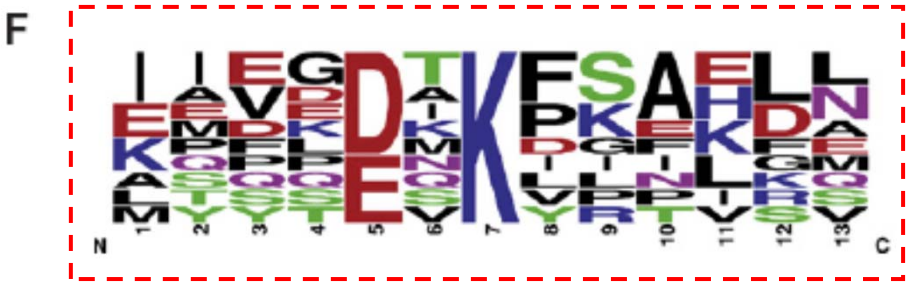
SUMO-2修饰的蛋白质组分析



- 多数位点符合Phi-K-X-E模体
- “Inverted motif”

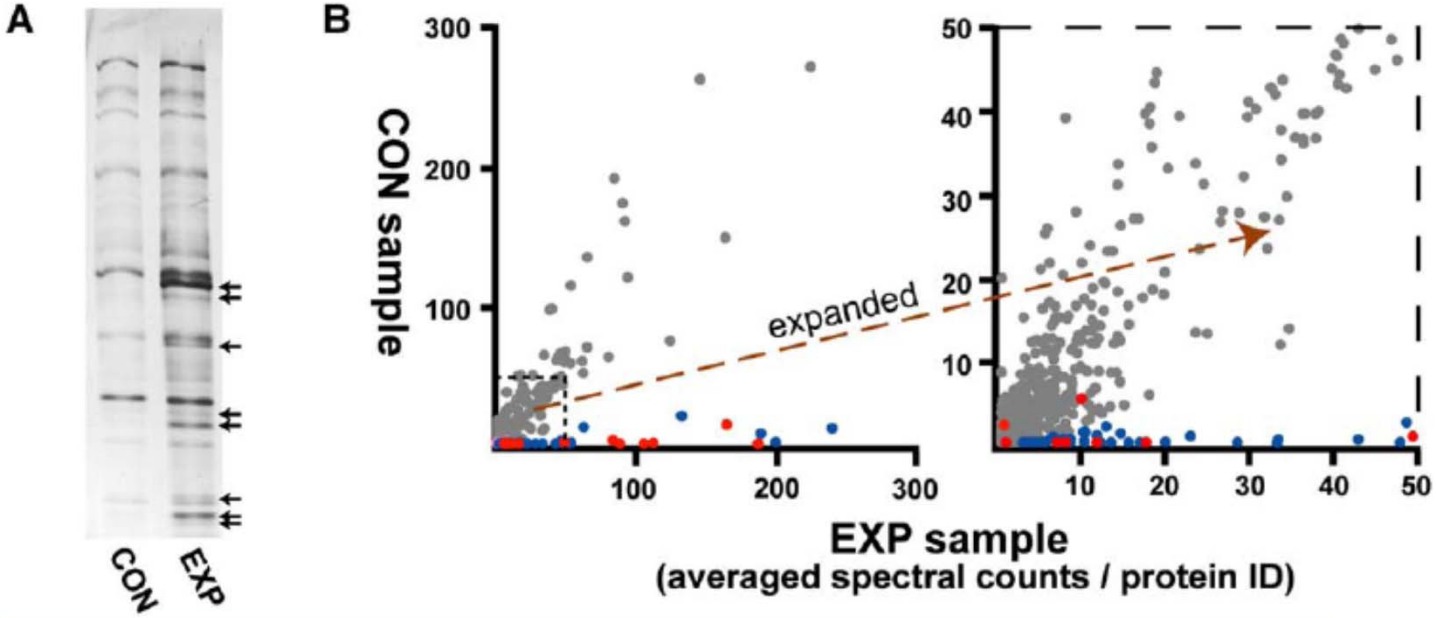






酵母棕榈酰化蛋白质组分析

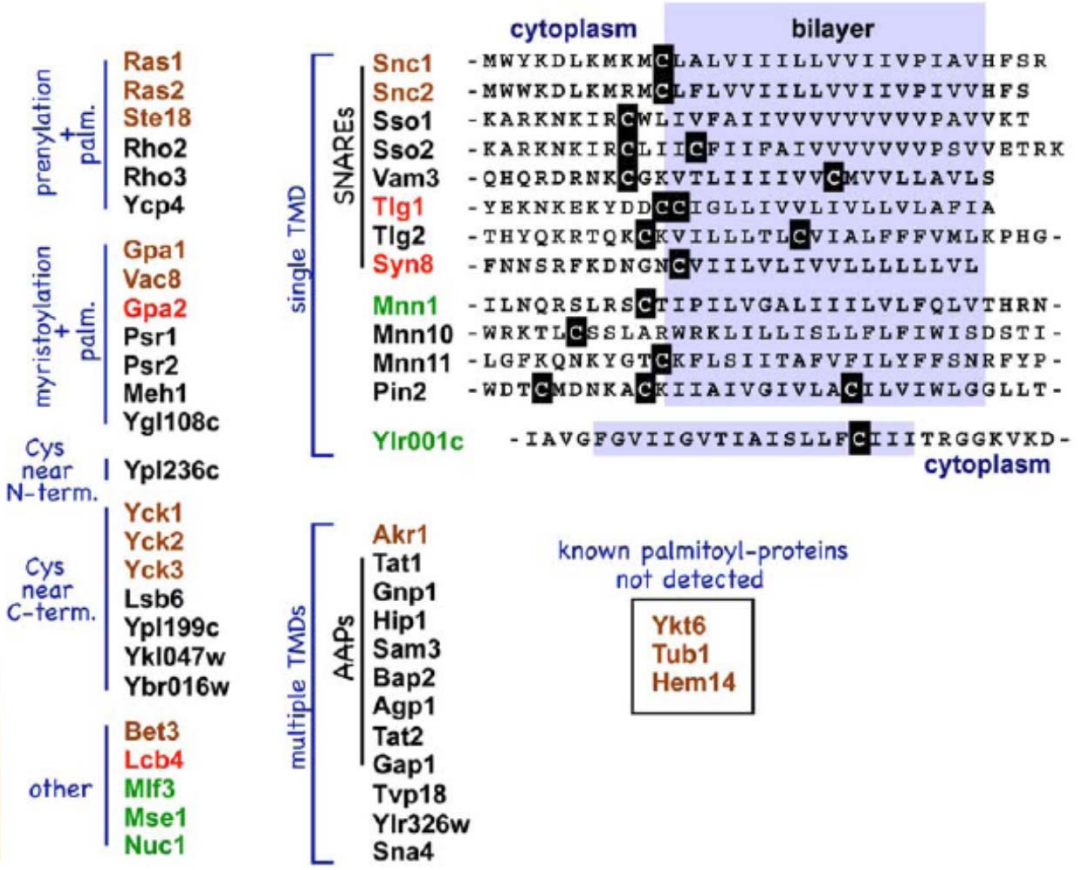
- 棕榈酰化蛋白质的分离与纯化
  - Acyl-biotinyl exchange (ABE)
  - N-ethyl maleimide (马来酰亚胺) (NEM) 保护硫醇
  - Hydroxylamine (羟胺)切断硫酯键
  - 连接半胱氨酸，羟胺后连接生物素/维生素H



Roth, et al., Cell, 2006, 125, 1003-1013

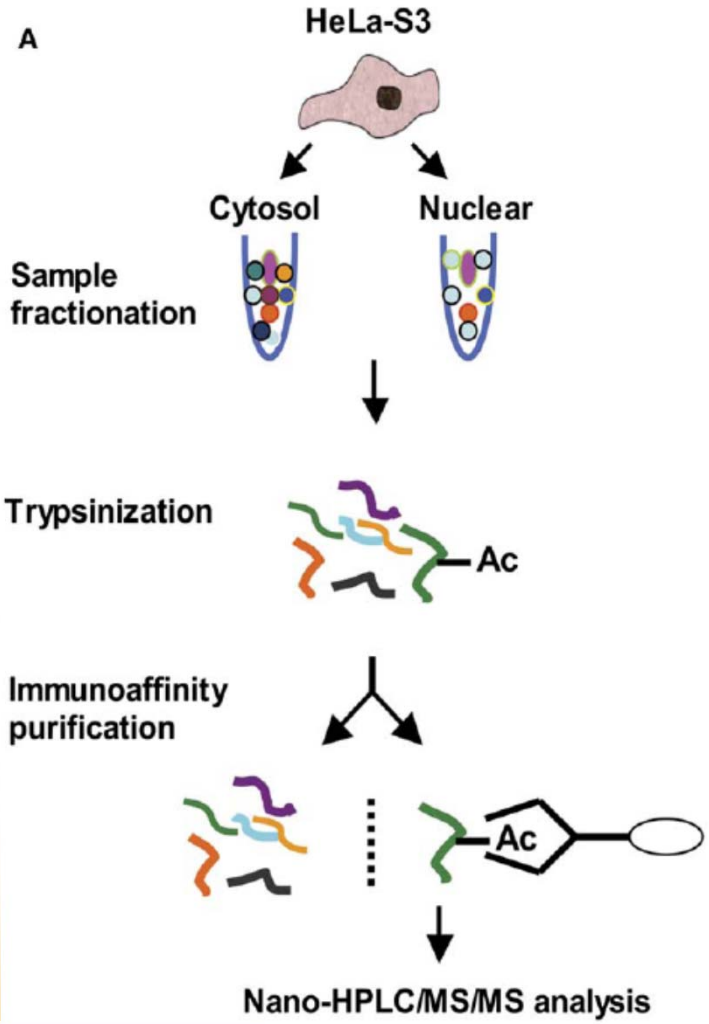
酵母的棕榈酰化蛋白质组

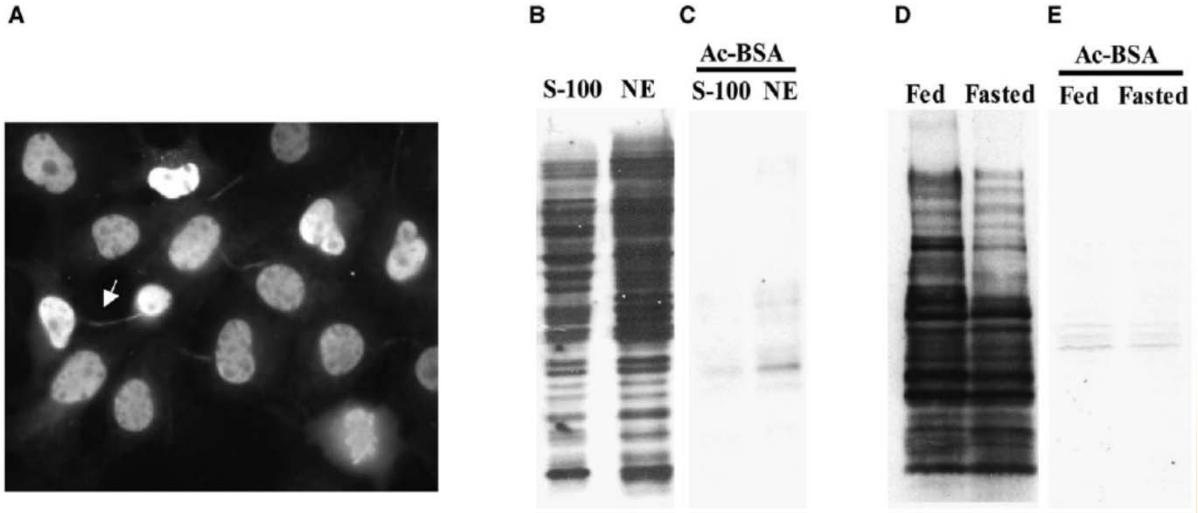
- 47个底物
- 修饰倾向于发生在胞内区



哺乳动物的乙酰化组研究

- HeLa细胞和小鼠线粒体：
  - ◆388个乙酰化位点
- 乙酰化抗体：IP

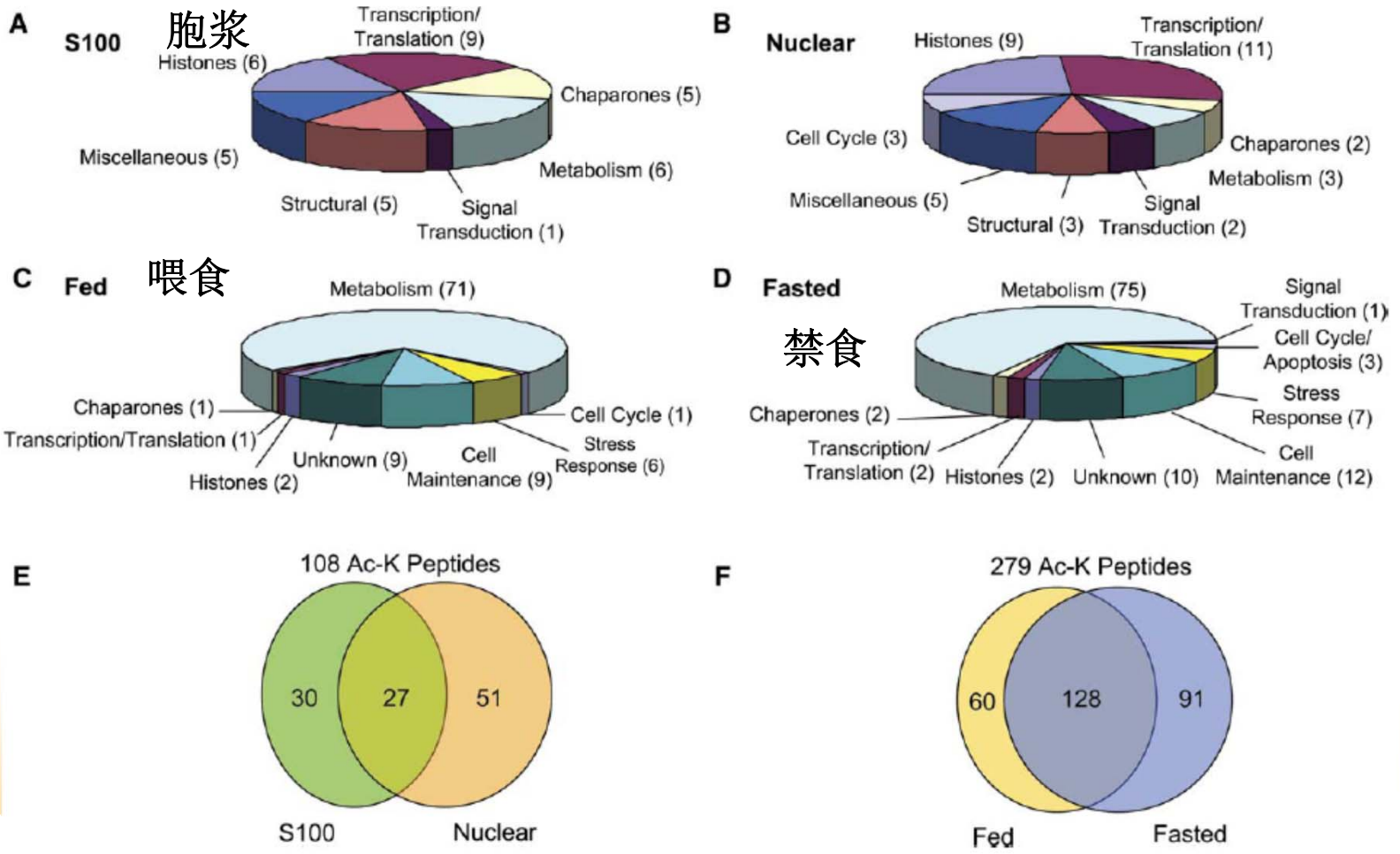




Kim, et al., Mol Cell, 2006, 23, 607-618

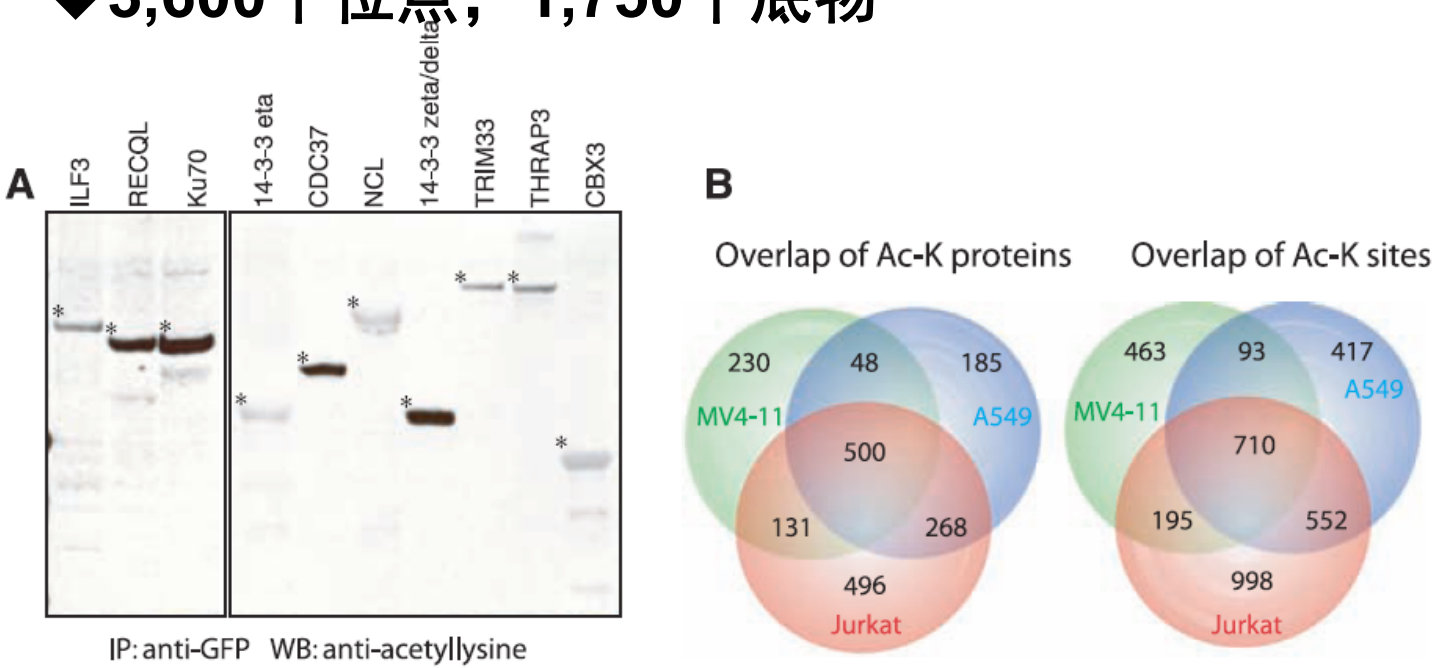
乙酰化底物分析

- 小鼠肝脏线粒体：与代谢相关



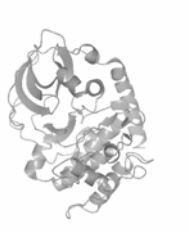
基于蛋白质芯片的乙酰化分析

- 去乙酰酶抑制剂
  - suberoylanilide hydroxamic acid (伏立诺他)
  - MS-275
- Leukemia cell lines
  - 3,600个位点，1,750个底物

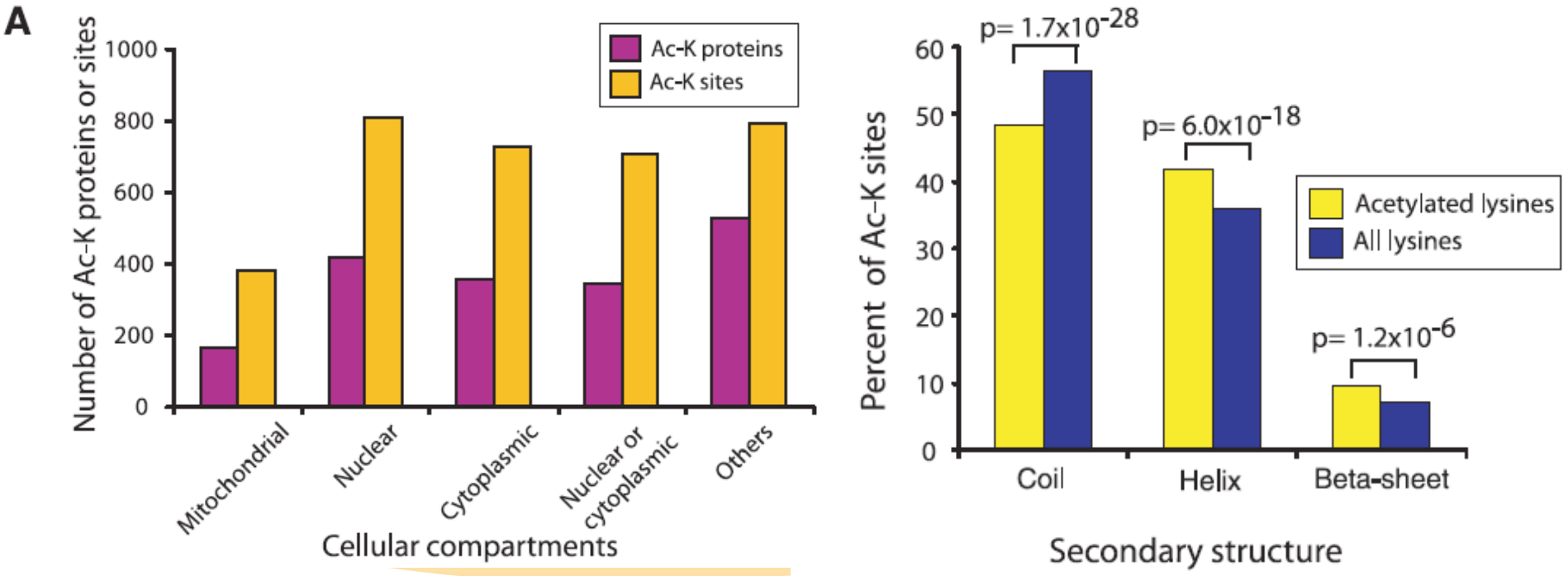


Choudhary, et al., Science, 2009, 325, 834-840

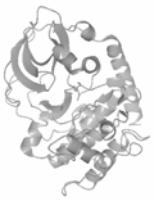
乙酰化底物和位点



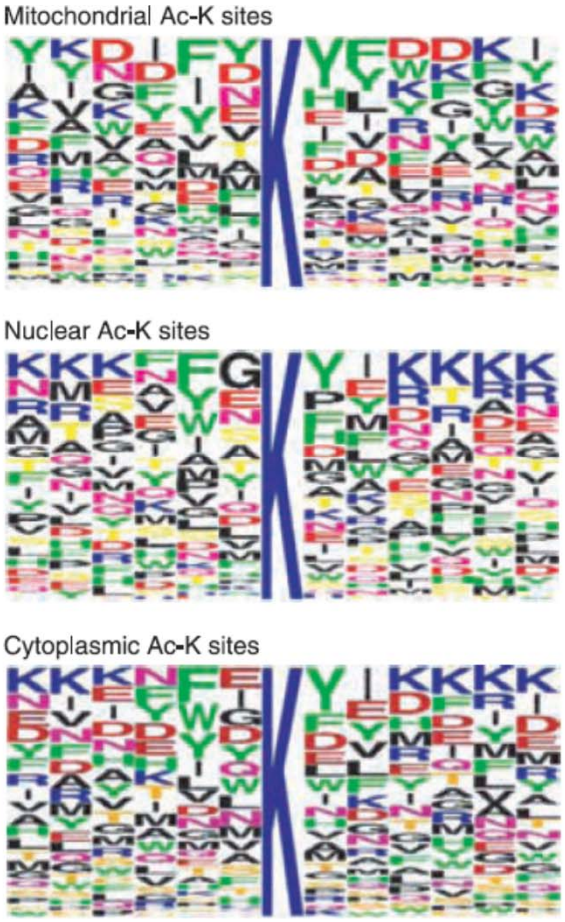
- 乙酰化蛋白质在核内较多
- 位点倾向于Helix和Beta-sheet
  - ◆Ordered region

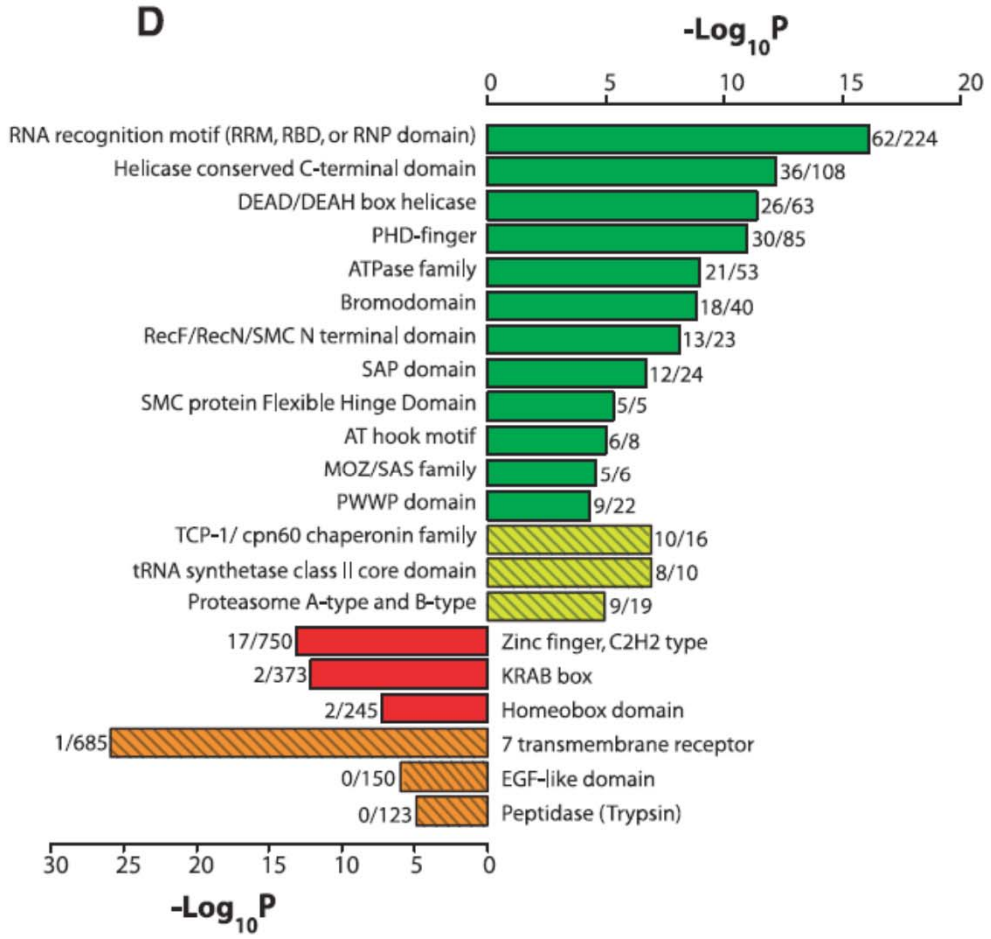


序列特征与结构域

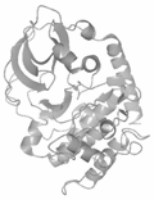


- 不倾向于发生在转录因子上





功能分析



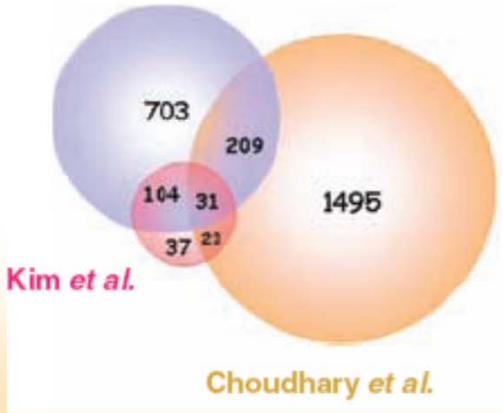
- 与DNA修复和RNA有关

Cellular functional categories and protein classes	Number of acetylated proteins	Number of acetylation sites
DNA replication	52	98
DNA damage and repair	72	167
Chromatin remodeling	26	46
Cell cycle	132	243
RNA transcription	31	71
RNA splicing	109	206
Nuclear hormone signaling	9	22
Nuclear transport	17	41
Cytoskeleton reorganization	50	137
Nucleotide exchange factors	55	92
Endocytosis and vesicular trafficking	39	62
DNA/RNA helicases	46	105
Ubiquitin ligases and deubiquitylases	46	70
Protein kinases	47	71
Acetyltransferases and deacetylases	21	61
Methyltransferases and demethylases	12	34
Transcription factors	29	40
Histones	15	61
Adaptor proteins	14	40
Chaperones	40	127
Ribosomal proteins	75	136

乙酰化 vs. 细胞代谢

- 人类肝脏样本
  - ◆1,300个乙酰肽，1,047个底物
- 与之前工作的重叠率
  - ◆小鼠肝脏195个，重叠135个(70%)
  - ◆白血病细胞株1,750，重叠240
  - ◆表明不同组织间区别较大

A

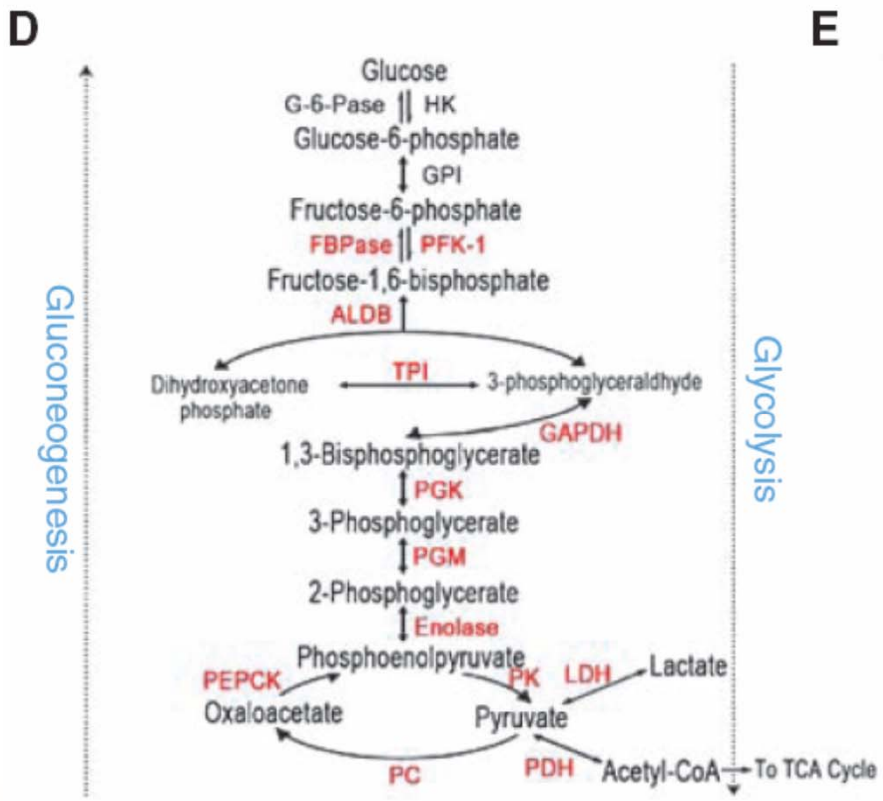


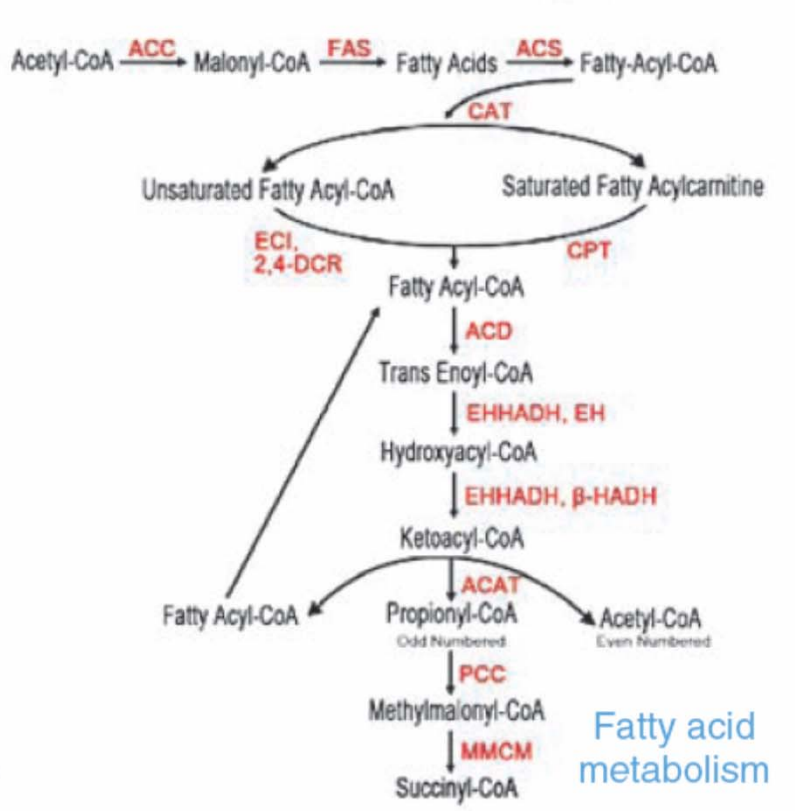
B

Pathway	Fisher's Exact Test
Fatty acid metabolism	4.02×10^{-14}
Citrate cycle (TCA)	1.13×10^{-9}
Urea cycle & metabolism of amino groups	2.40×10^{-8}
Glycolysis / glucogenesis	4.43×10^{-8}

Zhao, et al., Science, 2010, 327, 1000-1004

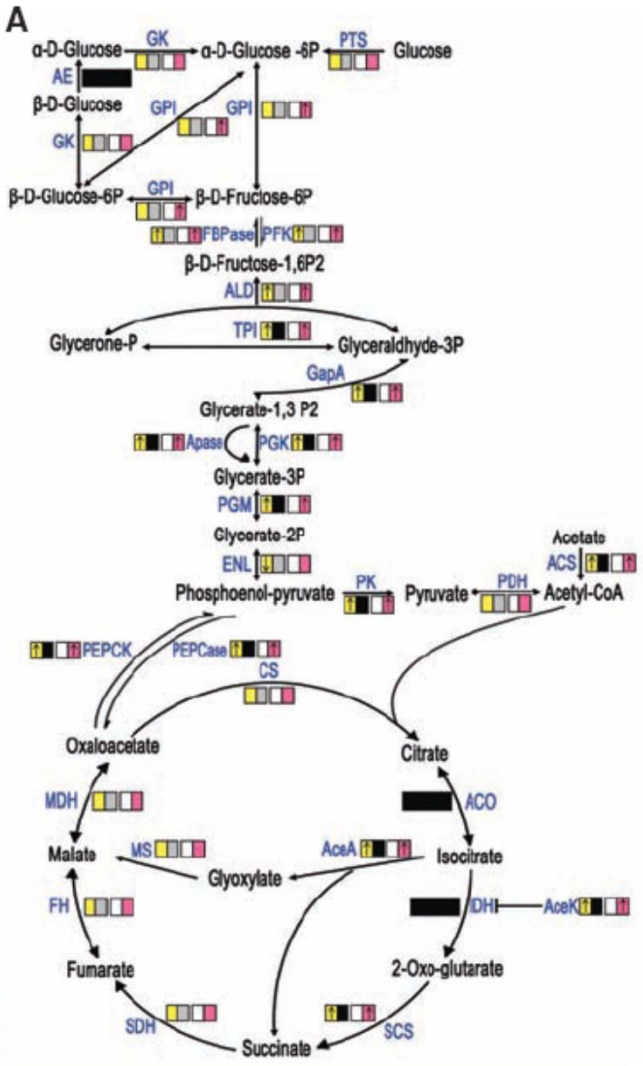
乙酰化 vs. 代谢酶


D
Gluconeogenesis
Glycolysis
Glucose
G-6-Pase
HK
Glucose-6-phosphate
GPI
Fructose-6-phosphate
FBPase
PFK-1
Fructose-1,6-bisphosphate
ALDB
Dihydroxyacetone phosphate
TPI
3-phosphoglyceraldhyde
GAPDH
1,3-Bisphosphoglycerate
PGK
3-Phosphoglycerate
PGM
2-Phosphoglycerate
Enolase
Phosphoenolpyruvate
PEPCK
PK
LDH
Lactate
Oxaloacetate
Pyruvate
PC
PDH
Acetyl-CoA
To TCA Cycle


E
Acetyl-CoA
ACC
Malonyl-CoA
FAS
Fatty Acids
ACS
Fatty-Acyl-CoA
CAT
Unsaturated Fatty Acyl-CoA
Saturated Fatty Acylcarnitine
ECI, 2,4-DCR
CPT
Fatty Acyl-CoA
ACD
Trans Enoyl-CoA
EHHADH, EH
Hydroxyacyl-CoA
EHHADH, β-HADH
Ketoacyl-CoA
ACAT
Fatty Acyl-CoA
Propionyl-CoA
Odd Numbered
Acetyl-CoA
Even Numbered
PCC
Methylmalonyl-CoA
MMCM
Succinyl-CoA
Fatty acid metabolism

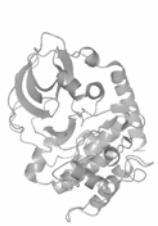
沙门氏菌的乙酰化组研究

- 乙酰化的保守性
  - ◆从原核到真核
- 235个乙酰化肽，191个底物
  - ◆50%的底物参与代谢通路
  - ◆90%代谢相关的酶乙酰化



Wang, et al., Science, 2010, 327, 1004-1007

总结



- 蛋白质共价修饰的组学研究
  - 磷酸化：SCX, IMAC, MOAC, 酪氨酸抗体；上万
  - 泛素化：DiGly；上万
  - SUMO化：突变体的DiGly；数百
  - 棕榈酰化：ABE；几十
  - 乙酰化：抗体；数千
- “干”、“湿”结合
  - 组学数据的注释和分析
  - 算法与数据库的设计