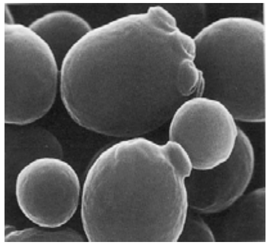
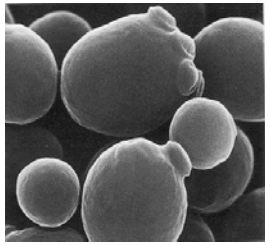


Molecular Cell Biology of the Yeast *Saccharomyces cerevisiae*

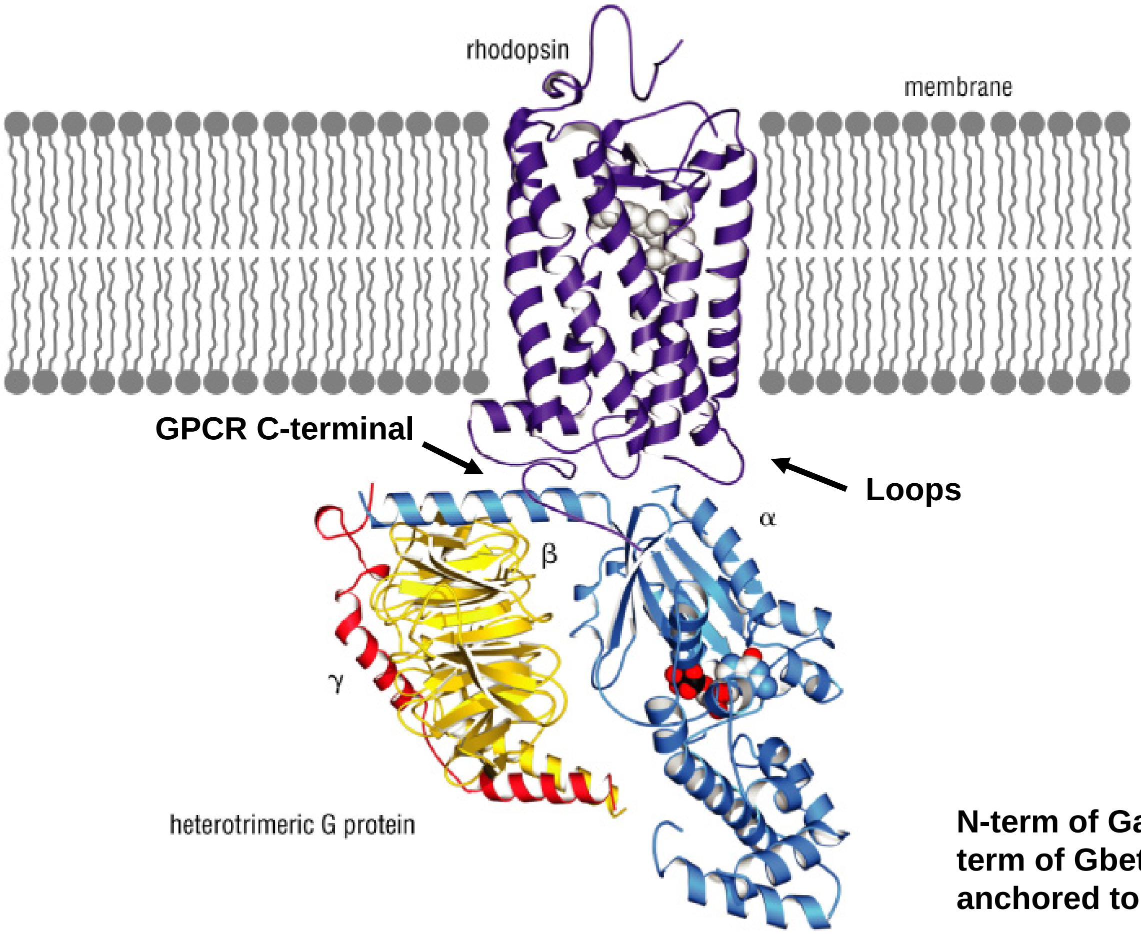
Lecture IV: A Primer on Signal Transduction

Zhang Yi,
National Institute of Biological Sciences, 20080720



Basic structure of GPCR

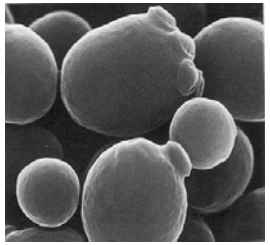
From **Protein Structure and Function** by Gregory A Petsko and Dagmar Ringe



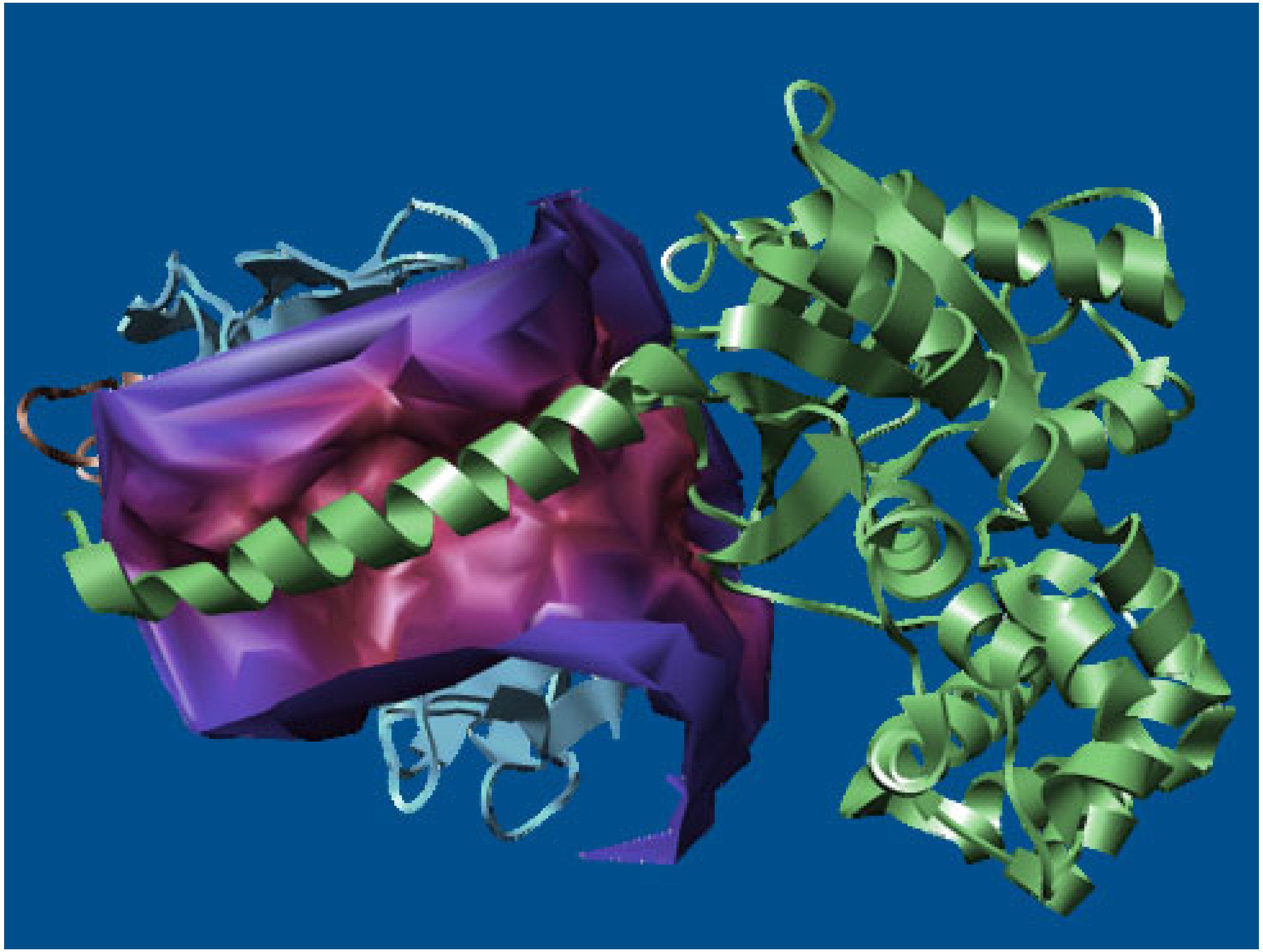
GPCR C-terminal

Loops

N-term of Galpha and C-term of Gbeta-gamma are anchored to membrane

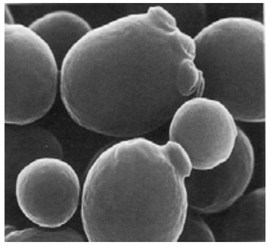


Heterotrimeric G protein



BetaGamma

Alpha



GTPase structure

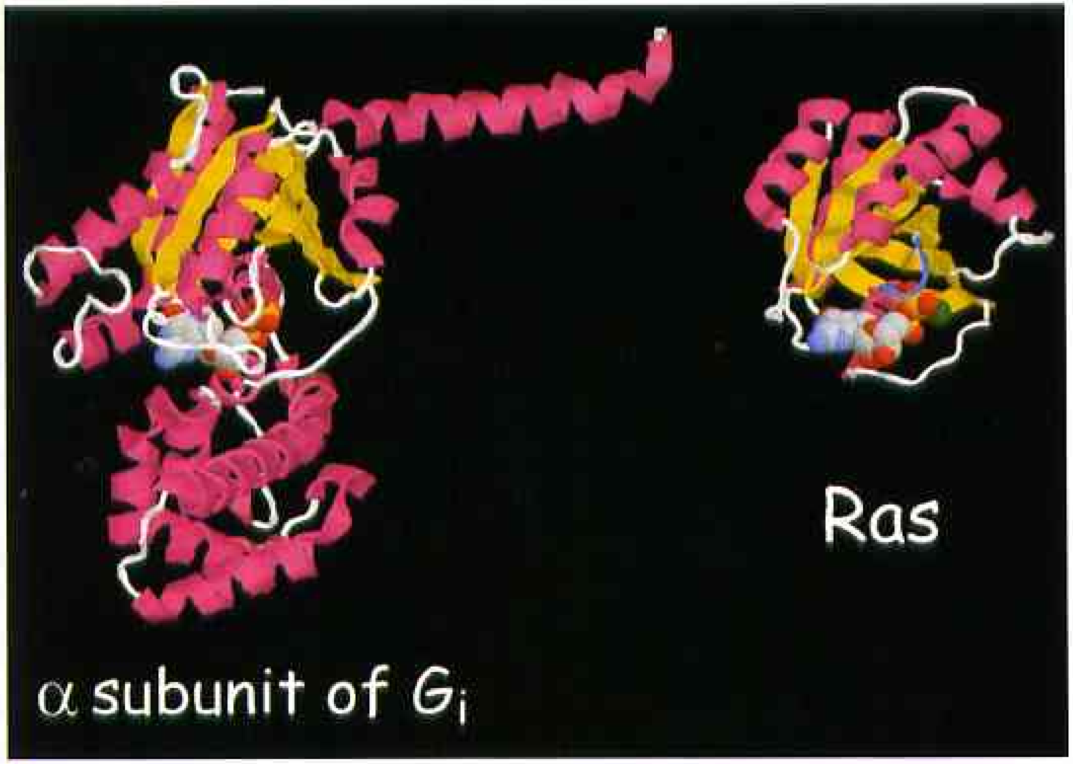
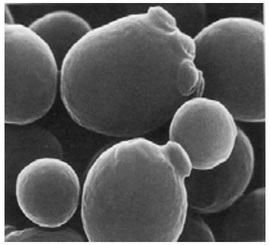
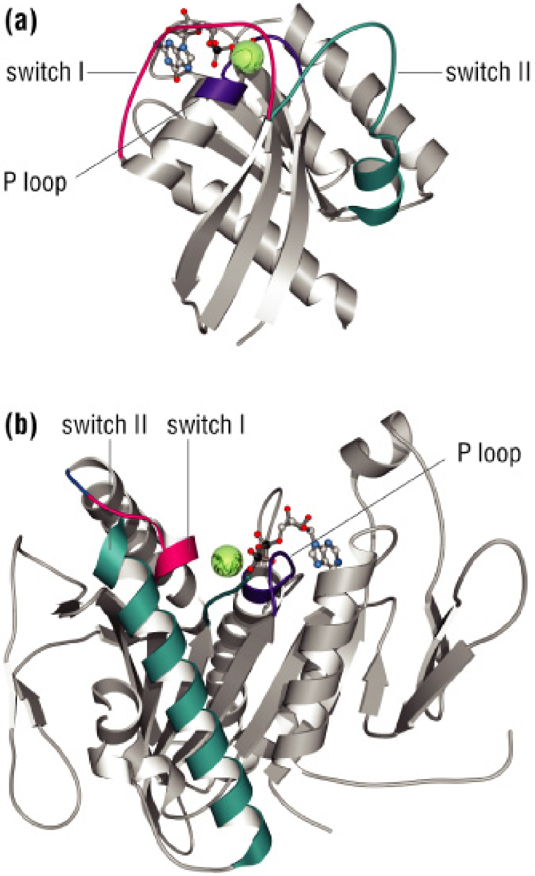


Figure 4.8 The α-subunits of G proteins and the monomeric GTPases exhibit structural similarities. α_i (left) has an rd domain (ras domain, upper half) that resembles the small GTPase Ras (right). The lower half of the α_i structure is the hd domain (helical domain). Each molecule has a bound GDP. (Mg^{2+} is only indicated in the RasGDP structure, green sphere.) Data source: α_i: 1gp2.pdb[58], RasGDP: 4q21.pdb.[125-128]

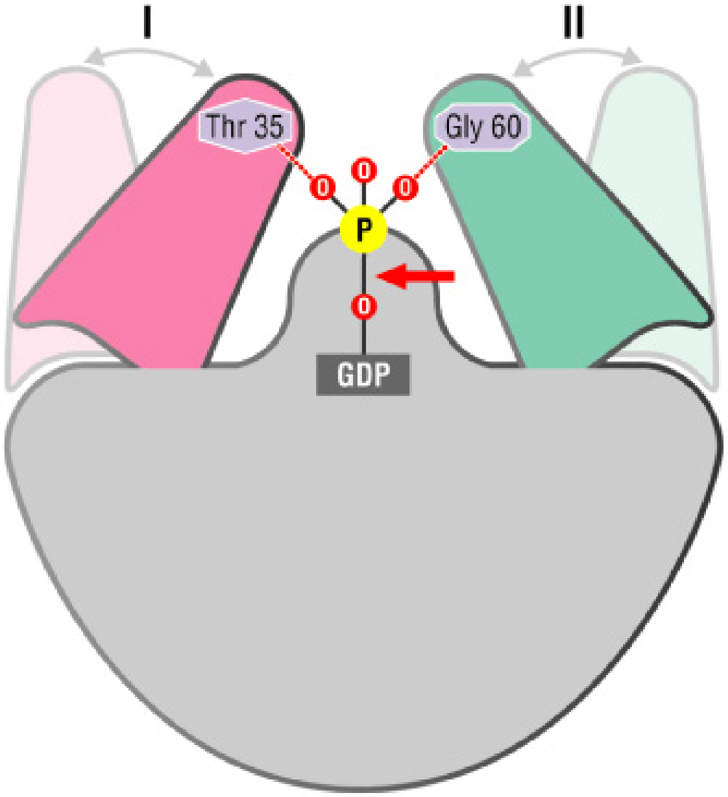


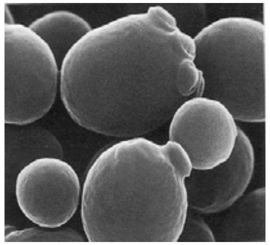
Compare GTPase to ATPase

From **Protein Structure and Function**
by Gregory A Petsko and Dagmar Ringe

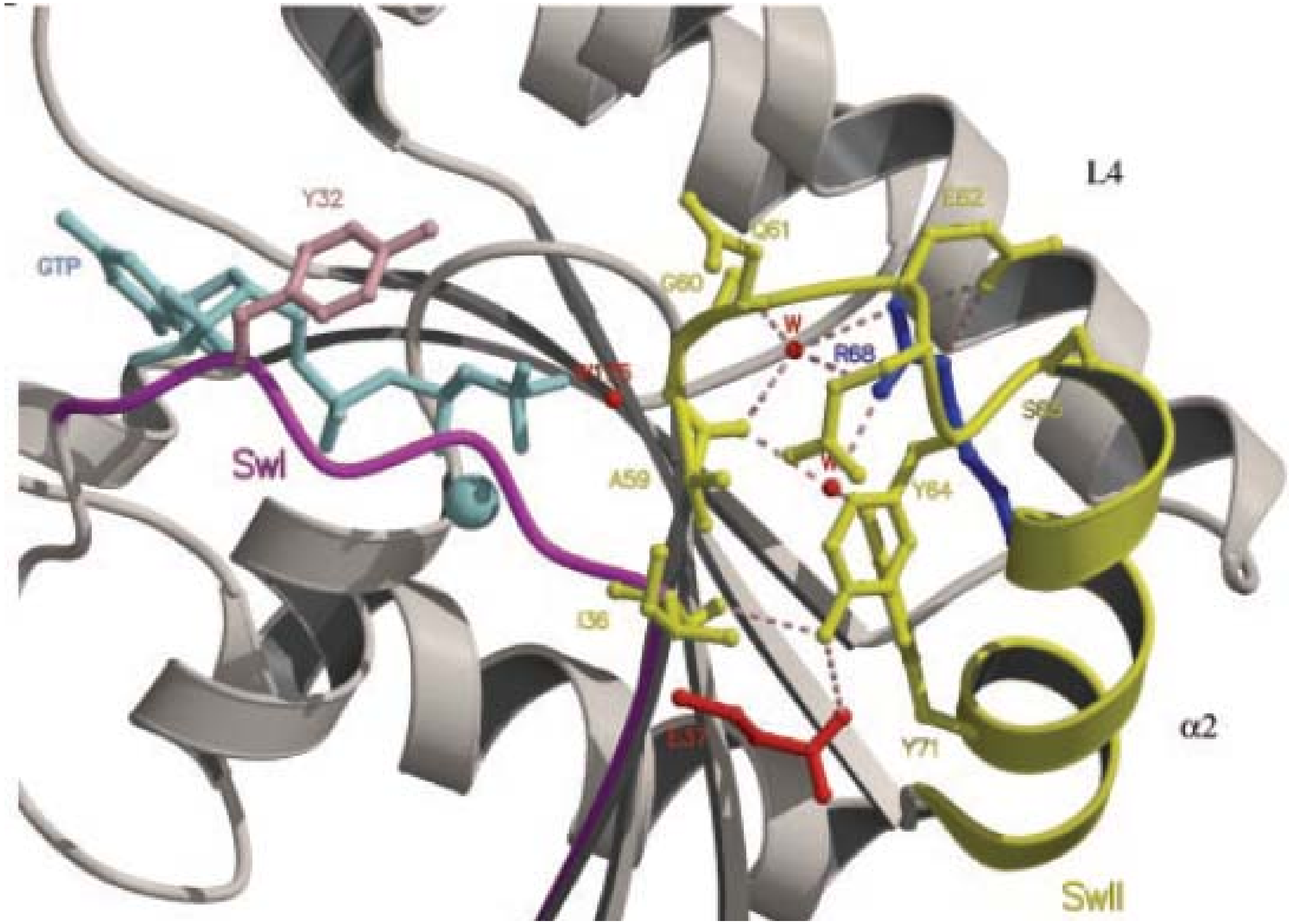


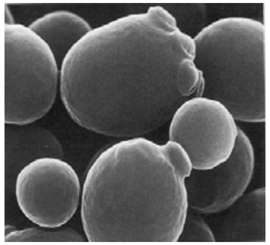
From **Protein Structure and Function**
by Gregory A Petsko and Dagmar Ringe



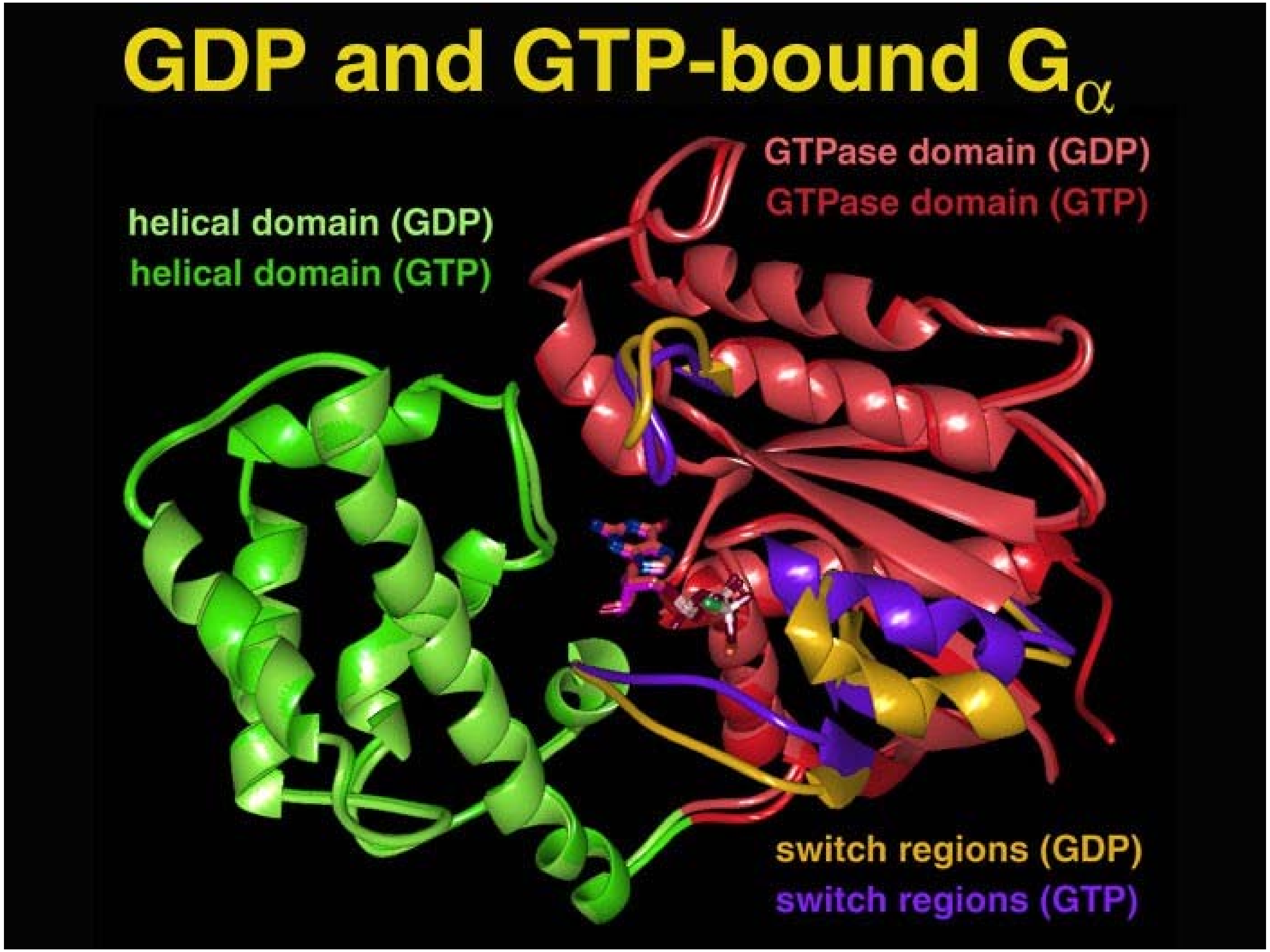


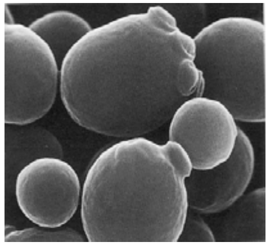
Ras switch domains binds to GTP



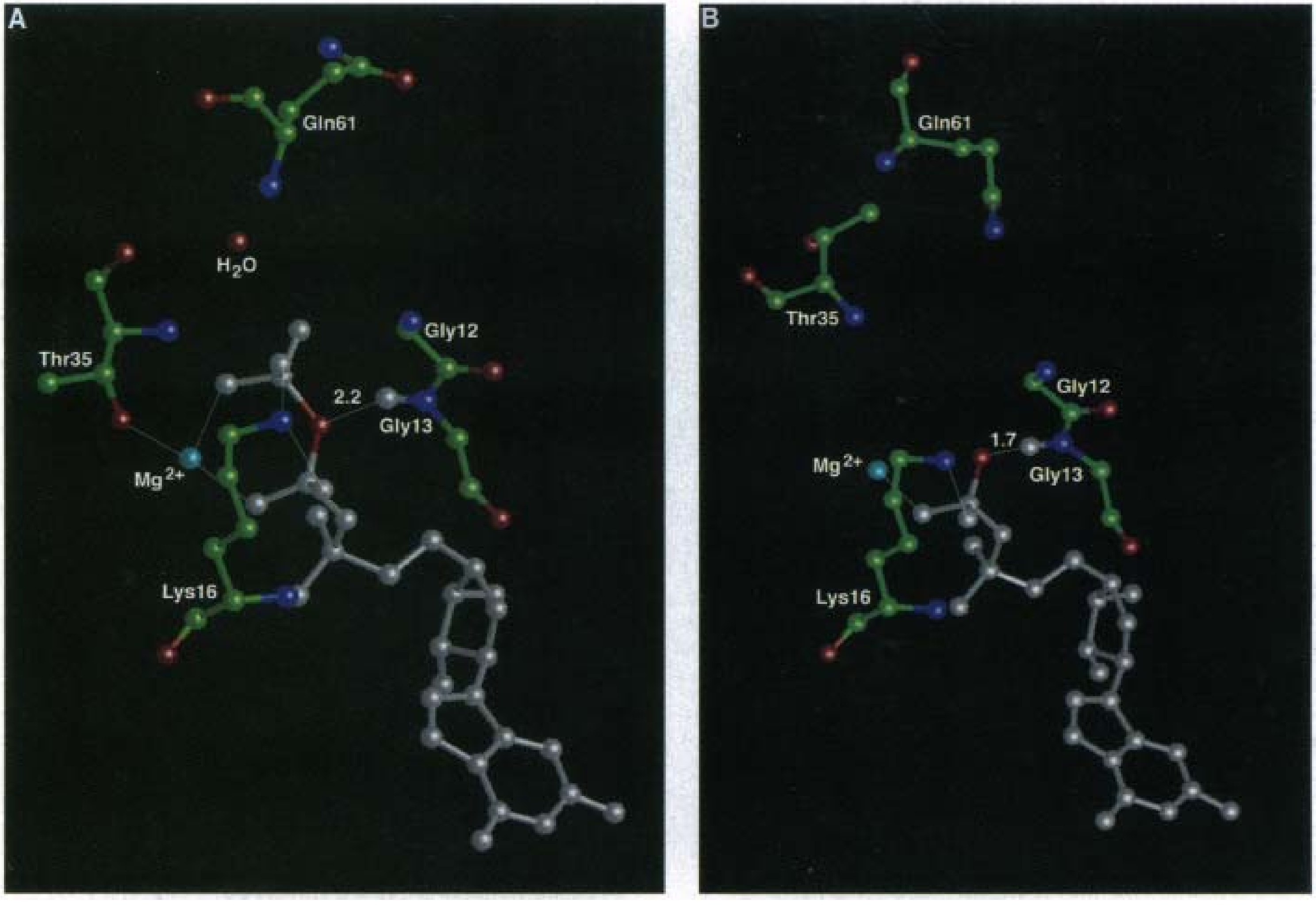


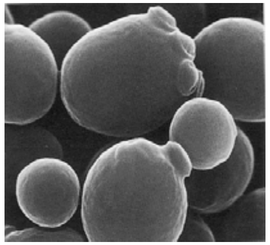
Switch region dynamics





Switch region Gly13 residue is important for GTP hydrolysis





G beta-gamma subunits

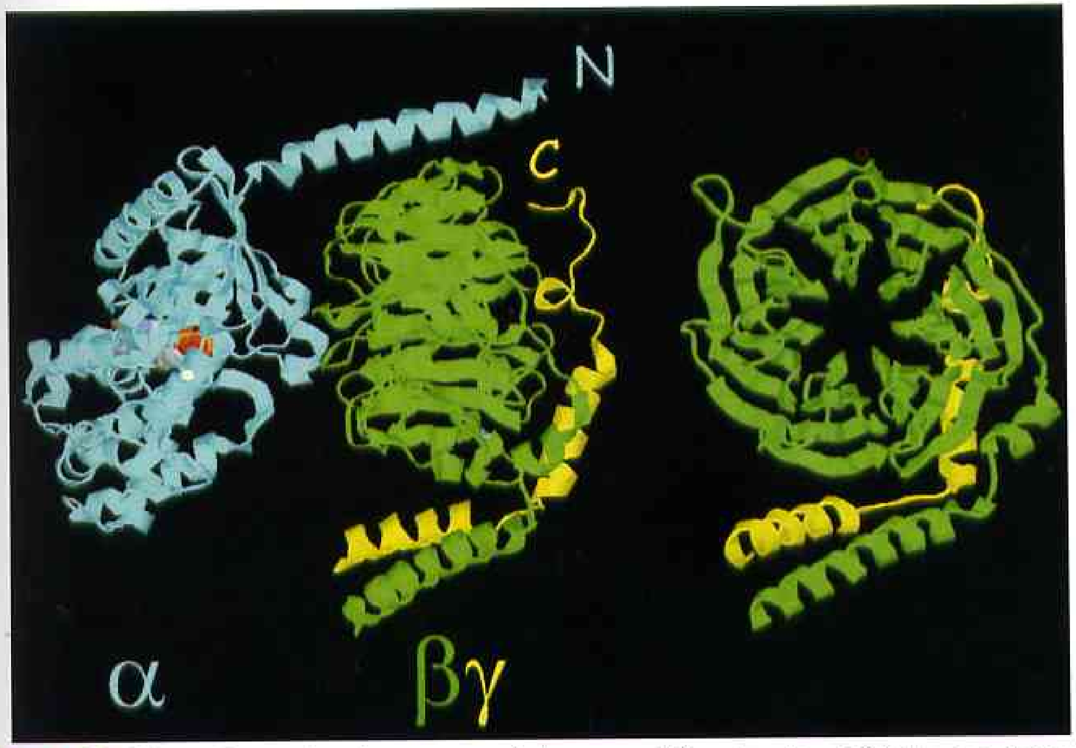
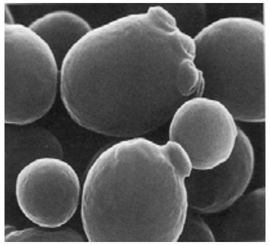
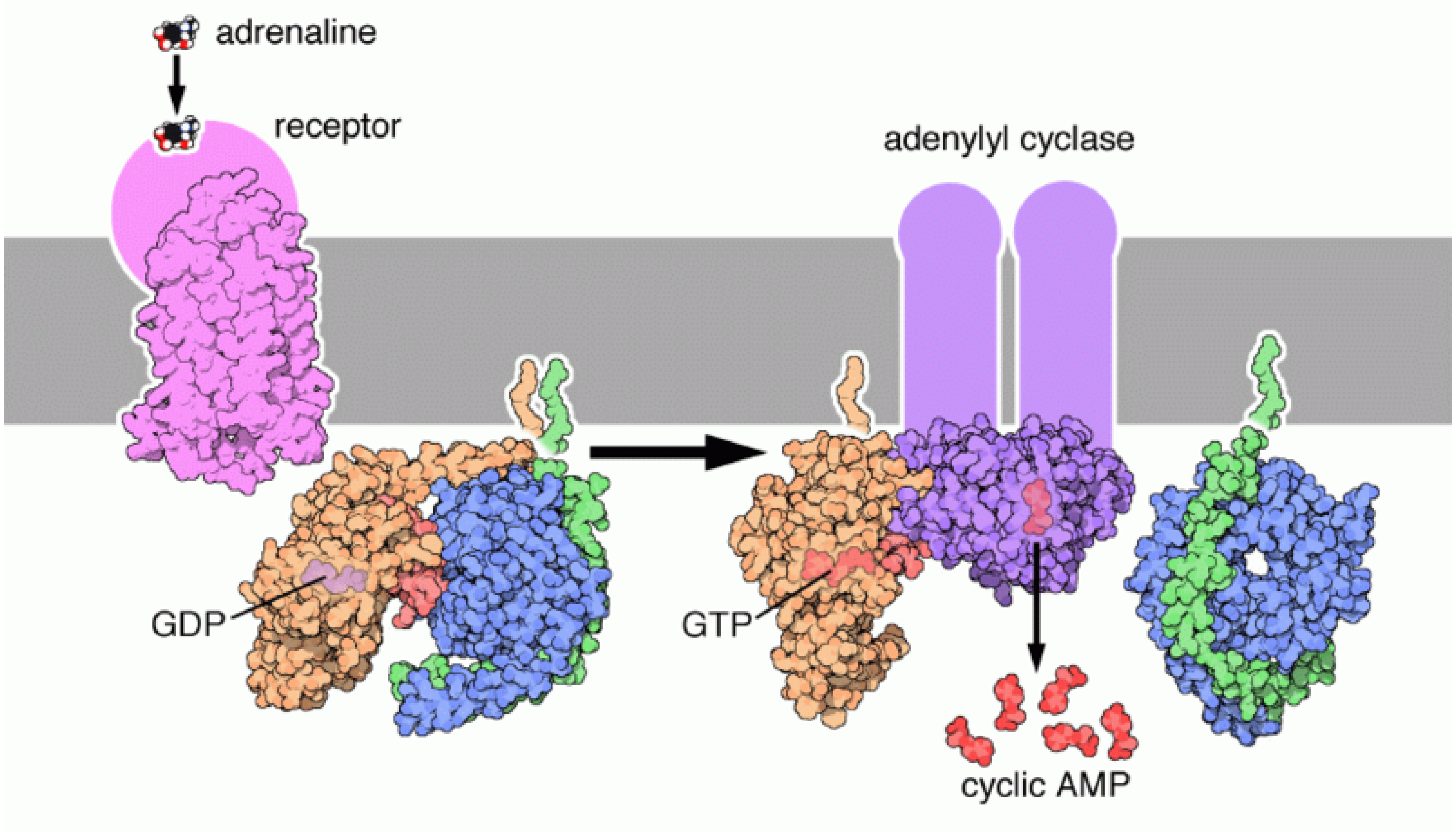
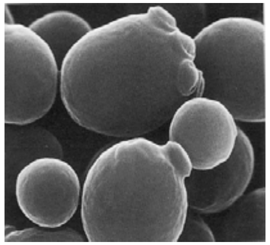


Figure 4.2 Three-dimensional structure of the α- and βγ-subunits of G_i. The α-subunit (left, cyan) has a molecule of GDP bound. The N-terminal helix is at top right. The βγ-subunits (β green, γ yellow) are in close apposition. The surface of the heterotrimeric structure that is in contact with the membrane is at the top of the figure. The hydrophobic attachments that are responsible are not shown. They involve the N-terminal of the α-subunit and the C-terminal of the γ-subunit. The separate βγ-subunit on the right has been rotated about a vertical axis to show the β propeller structure. (Data source: 1gp2.pdb[58]).

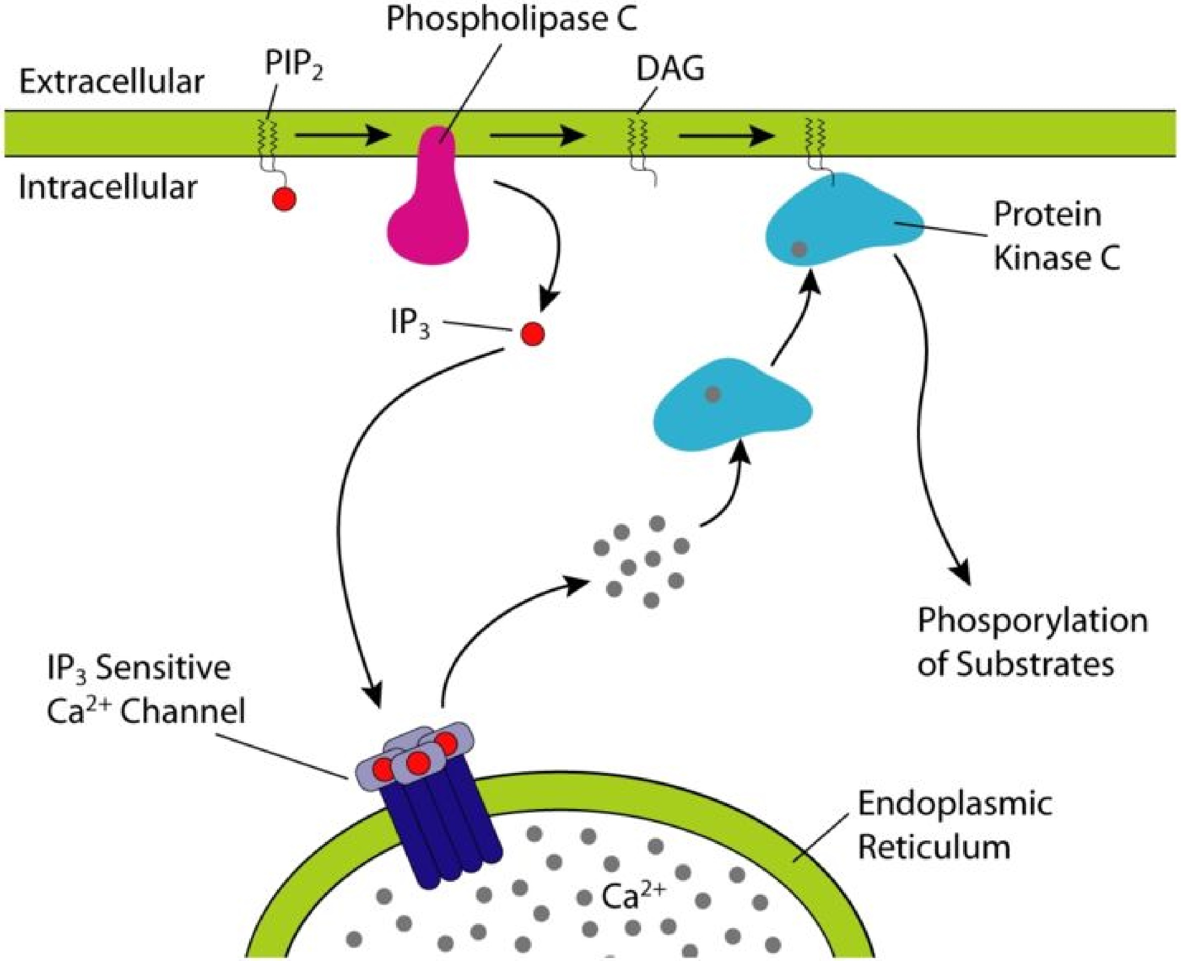


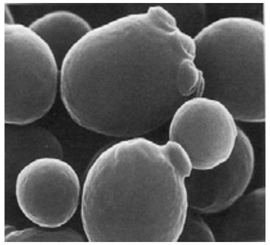
G alpha s/i mediated signal transduction through cAMP



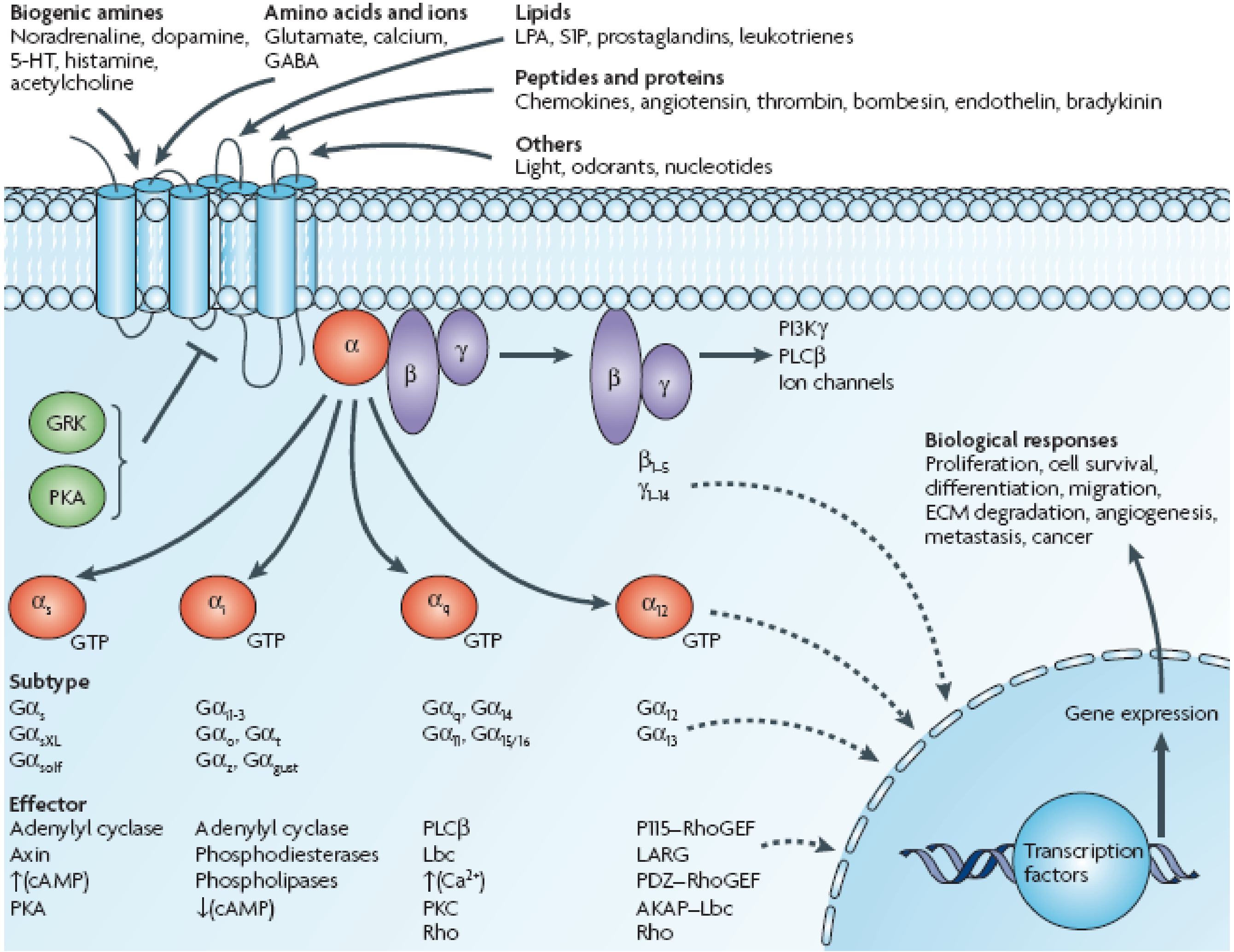


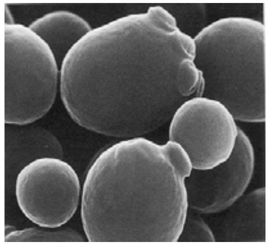
G alpha q mediated signal transduction through IP3/DAG/Ca



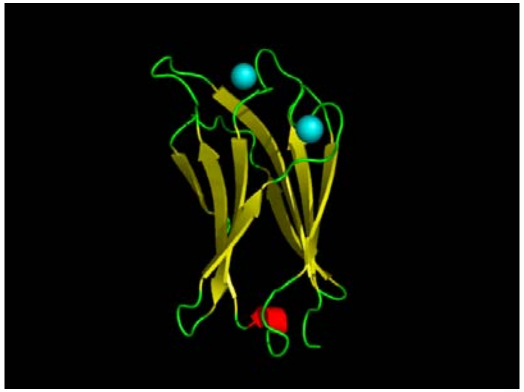


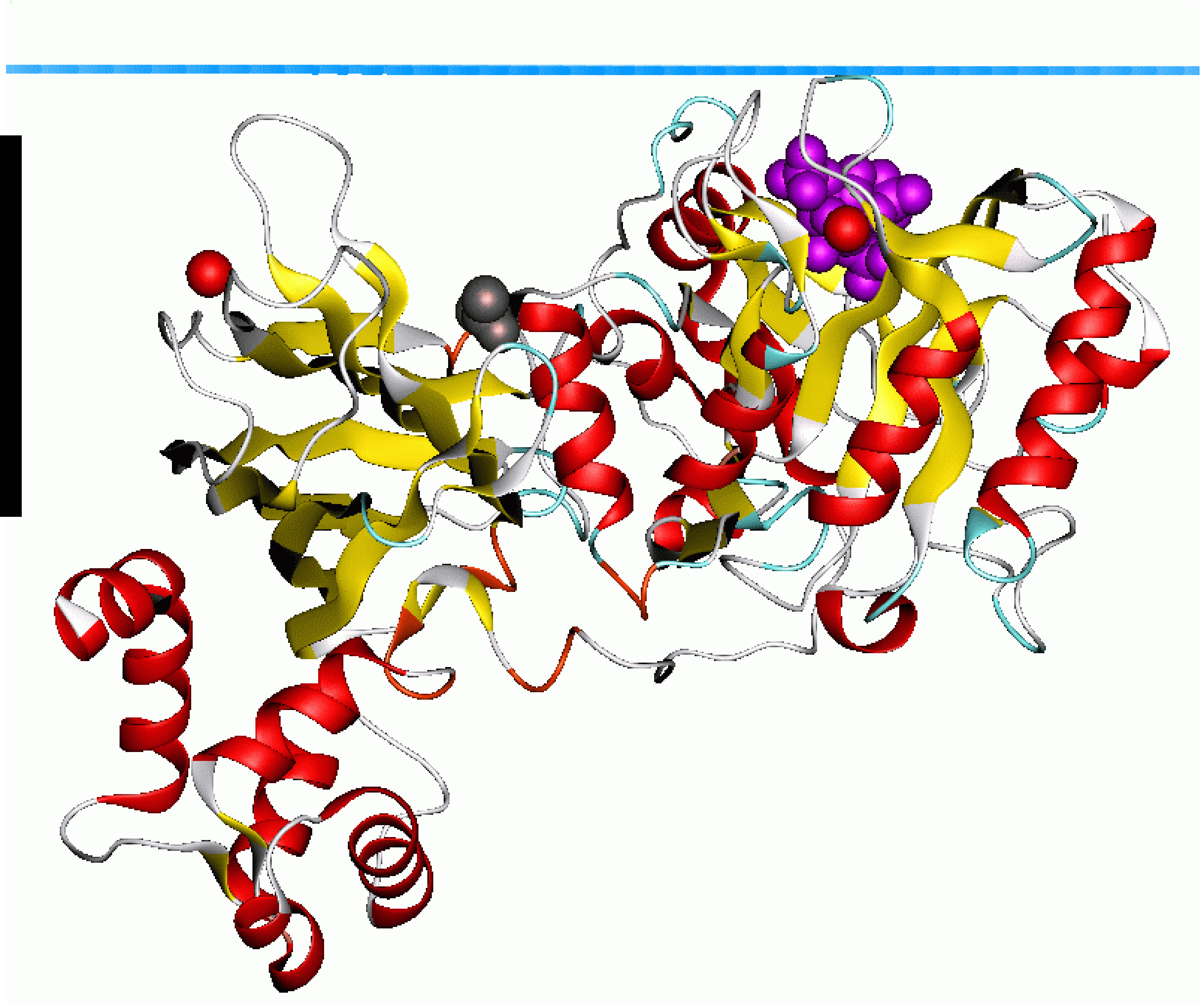
Signalling overview…

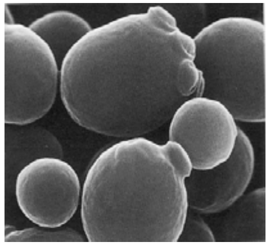




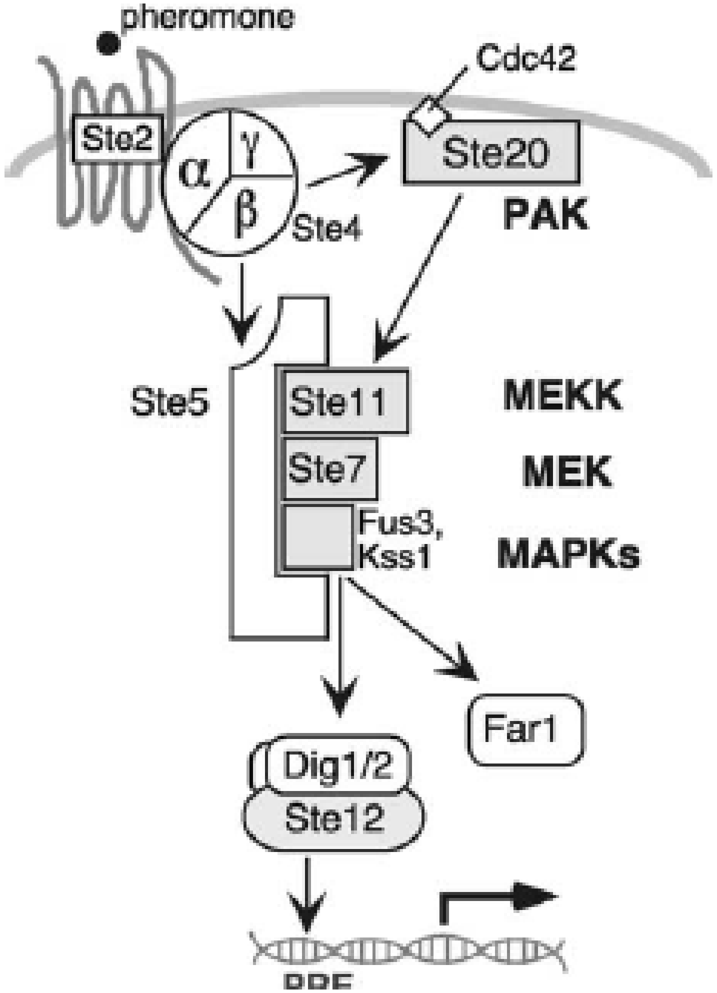
Activation of PLC

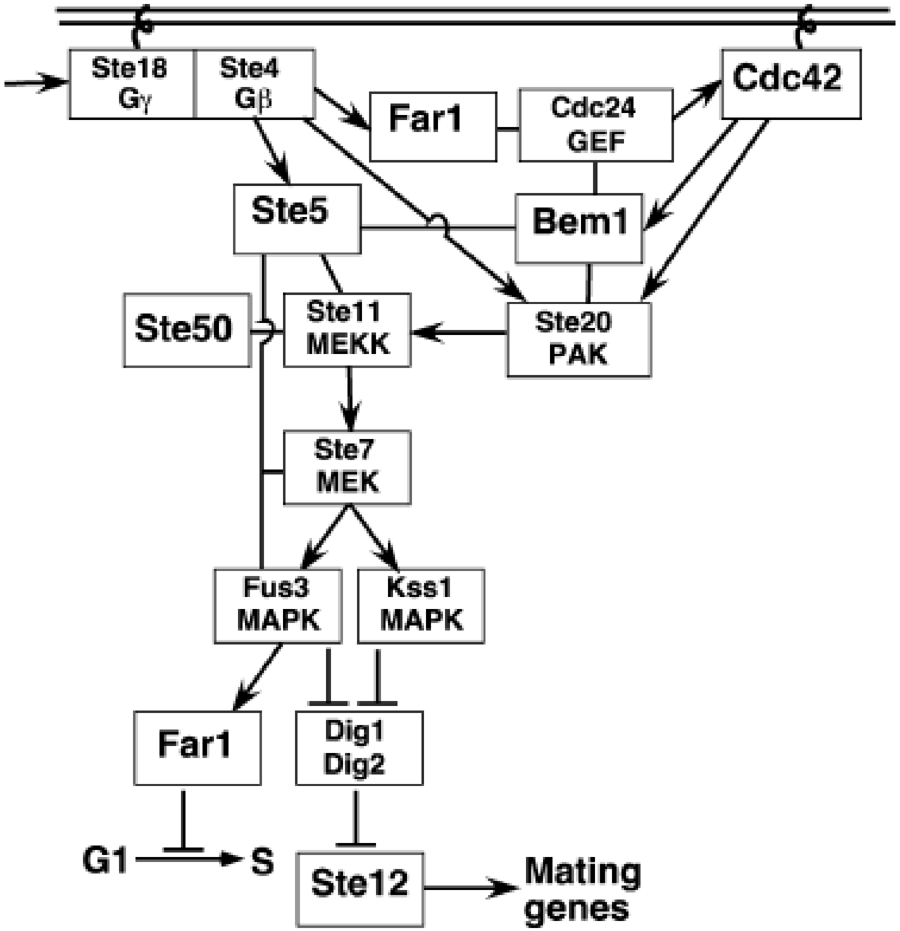




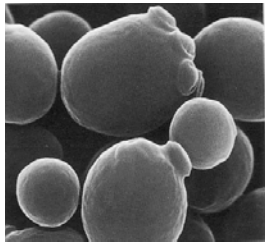


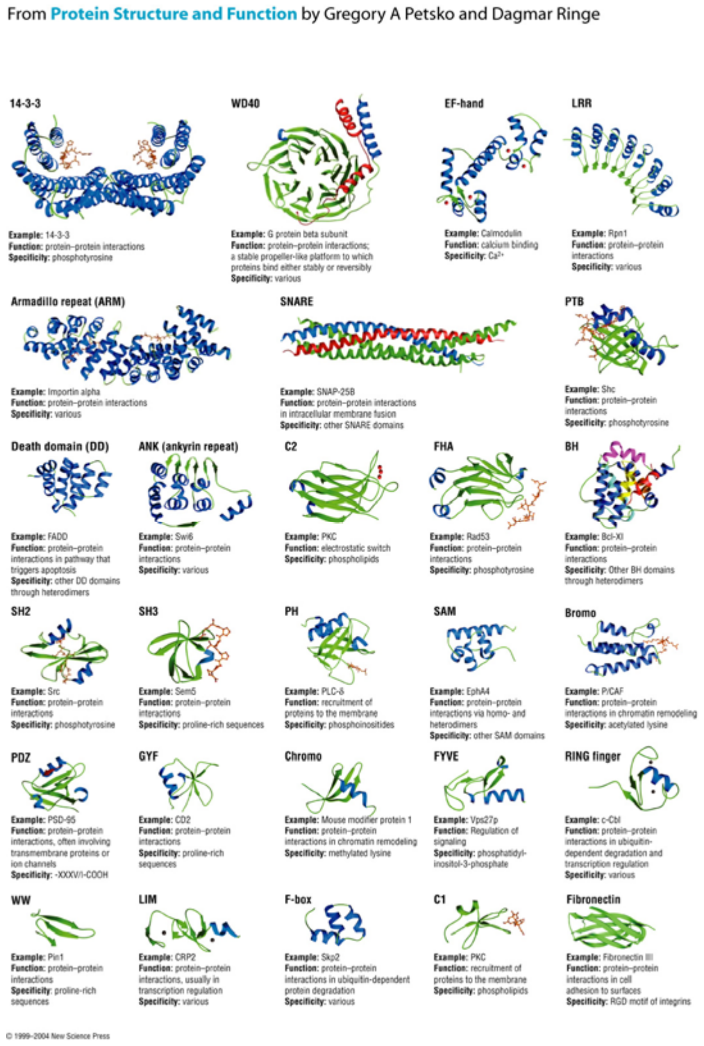
Yeast mating: in principle





Domains





From **Protein Structure and Function** by Gregory A Petsko and Dagmar Ringe

14-3-3
Example: 14-3-3
Function: protein–protein interactions
Specificity: phosphotyrosine

WD40
Example: G protein beta subunit
Function: protein–protein interactions; a stable propeller-like platform to which proteins bind either stably or reversibly
Specificity: various

EF-hand
Example: Calmodulin
Function: calcium binding
Specificity: Ca^{2+}

LRR
Example: Rpn1
Function: protein–protein interactions
Specificity: various

Armadillo repeat (ARM)
Example: Importin alpha
Function: protein–protein interactions
Specificity: various

SNARE
Example: SNAP-25B
Function: protein–protein interactions in intracellular membrane fusion
Specificity: other SNARE domains

PTB
Example: Shc
Function: protein–protein interactions
Specificity: phosphotyrosine

Death domain (DD)
Example: FADD
Function: protein–protein interactions in pathway that triggers apoptosis
Specificity: other DD domains through heterodimers

ANK (ankyrin repeat)
Example: Swi6
Function: protein–protein interactions
Specificity: various

C2
Example: PKC
Function: electrostatic switch
Specificity: phospholipids

FHA
Example: Rad53
Function: protein–protein interactions
Specificity: phosphotyrosine

BH
Example: Bcl-Xl
Function: protein–protein interactions
Specificity: Other BH domains through heterodimers

SH2
Example: Src
Function: protein–protein interactions
Specificity: phosphotyrosine

SH3
Example: Sem5
Function: protein–protein interactions
Specificity: proline-rich sequences

PH
Example: PLC-δ
Function: recruitment of proteins to the membrane
Specificity: phosphoinositides

SAM
Example: EphA4
Function: protein–protein interactions via homo- and heterodimers
Specificity: other SAM domains

Bromo
Example: P/CAF
Function: protein–protein interactions in chromatin remodeling
Specificity: acetylated lysine

PDZ
Example: PSD-95
Function: protein–protein interactions, often involving transmembrane proteins or ion channels
Specificity: -XXXV/I-COOH

GYF
Example: CD2
Function: protein–protein interactions
Specificity: proline-rich sequences

Chromo
Example: Mouse modifier protein 1
Function: protein–protein interactions in chromatin remodeling
Specificity: methylated lysine

FYVE
Example: Vps27p
Function: Regulation of signaling
Specificity: phosphatidyl-inositol-3-phosphate

RING finger
Example: c-Cbl
Function: protein–protein interactions in ubiquitin-dependent degradation and transcription regulation
Specificity: various

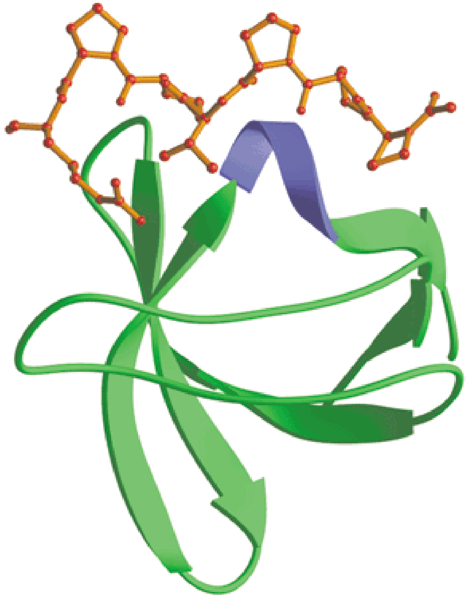
WW
Example: Pin1
Function: protein–protein interactions
Specificity: proline-rich sequences

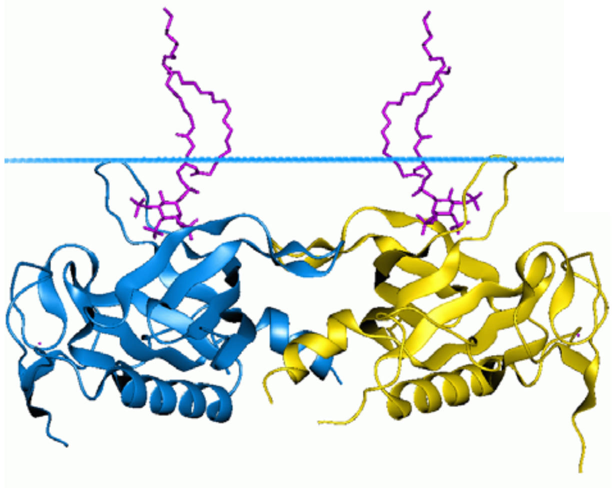
LIM
Example: CRP2
Function: protein–protein interactions, usually in transcription regulation
Specificity: various

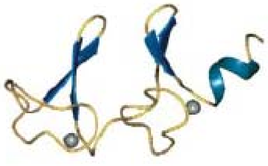
F-box
Example: Skp2
Function: protein–protein interactions in ubiquitin-dependent protein degradation
Specificity: various

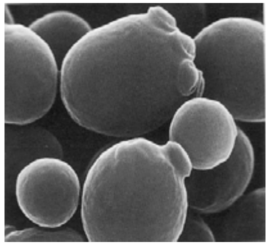
C1
Example: PKC
Function: recruitment of proteins to the membrane
Specificity: phospholipids

Fibronectin
Example: Fibronectin III
Function: protein–protein interactions in cell adhesion to surfaces
Specificity: RGD motif of integrins









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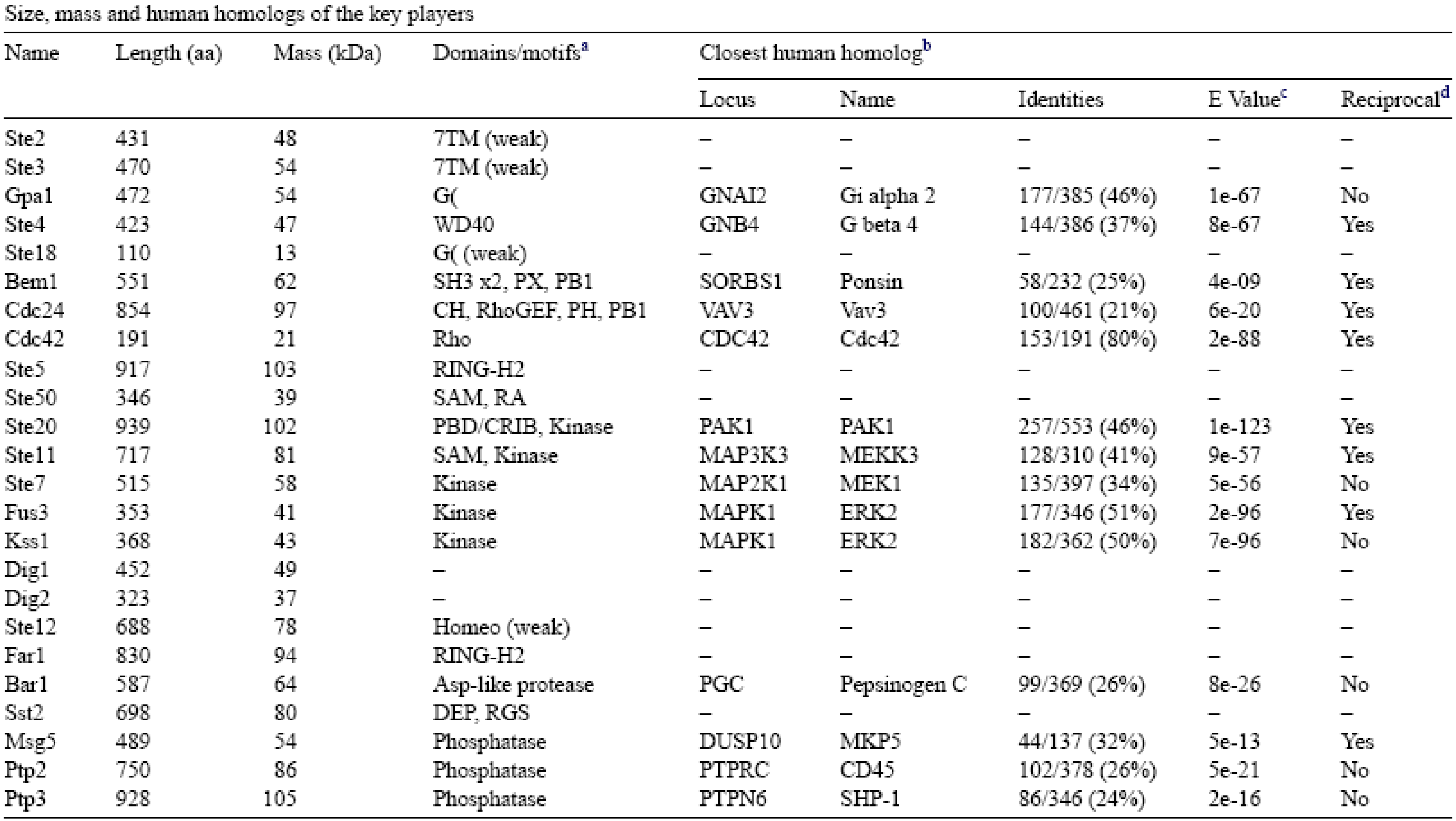
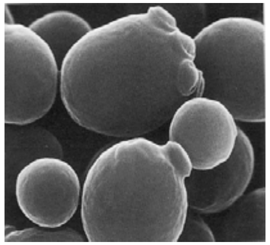


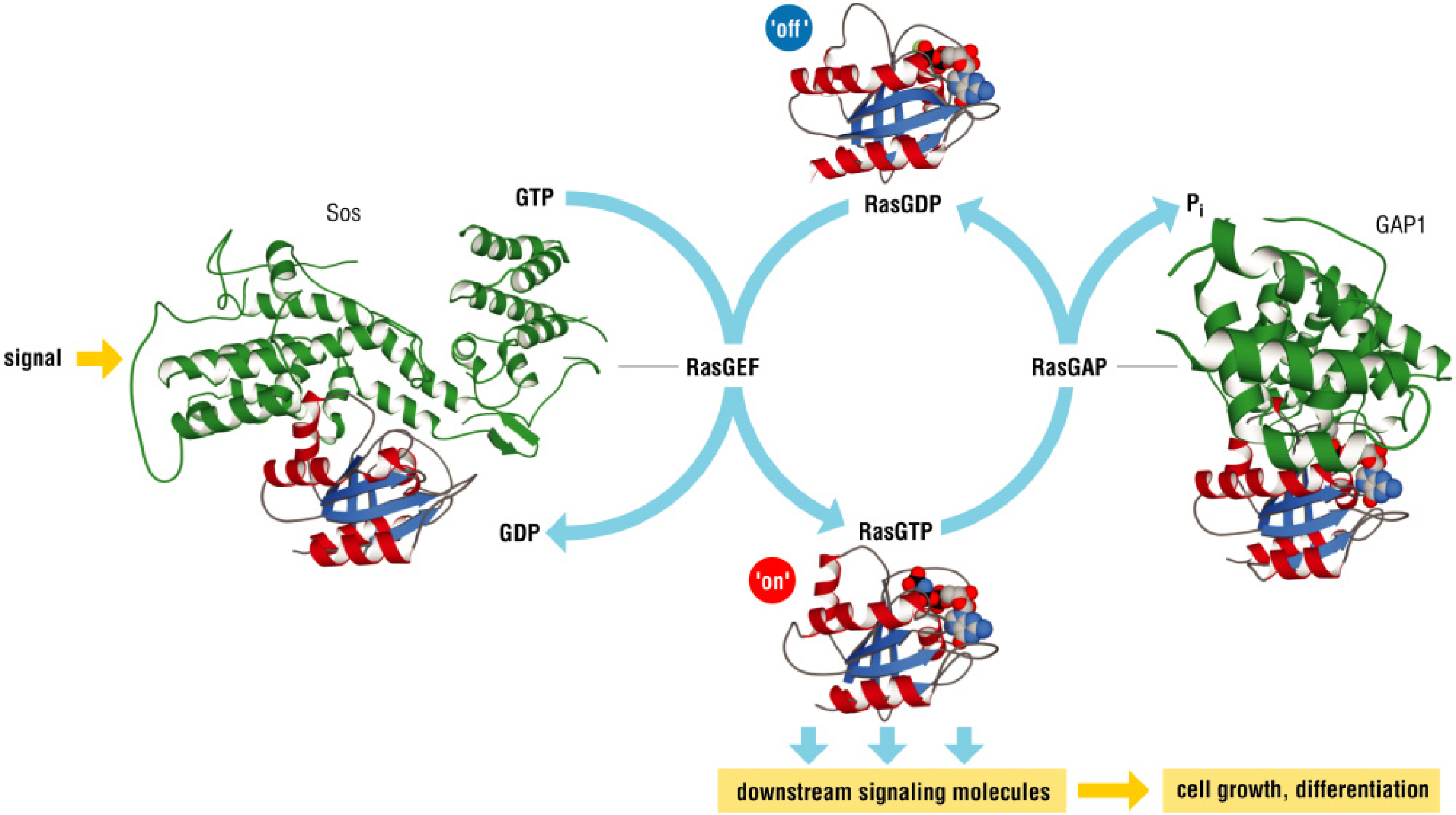
Size, mass and human homologs of the key players

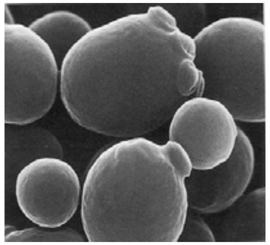
Name	Length (aa)	Mass (kDa)	Domains/motifs[a]	Closest human homolog[b]				
				Locus	Name	Identities	E Value[c]	Reciprocal[d]
Ste2	431	48	7TM (weak)	–	–	–	–	–
Ste3	470	54	7TM (weak)	–	–	–	–	–
Gpa1	472	54	G(	GNAI2	Gi alpha 2	177/385 (46%)	1e-67	No
Ste4	423	47	WD40	GNB4	G beta 4	144/386 (37%)	8e-67	Yes
Ste18	110	13	G((weak)	–	–	–	–	–
Bem1	551	62	SH3 x2, PX, PB1	SORBS1	Ponsin	58/232 (25%)	4e-09	Yes
Cdc24	854	97	CH, RhoGEF, PH, PB1	VAV3	Vav3	100/461 (21%)	6e-20	Yes
Cdc42	191	21	Rho	CDC42	Cdc42	153/191 (80%)	2e-88	Yes
Ste5	917	103	RING-H2	–	–	–	–	–
Ste50	346	39	SAM, RA	–	–	–	–	–
Ste20	939	102	PBD/CRIB, Kinase	PAK1	PAK1	257/553 (46%)	1e-123	Yes
Ste11	717	81	SAM, Kinase	MAP3K3	MEKK3	128/310 (41%)	9e-57	Yes
Ste7	515	58	Kinase	MAP2K1	MEK1	135/397 (34%)	5e-56	No
Fus3	353	41	Kinase	MAPK1	ERK2	177/346 (51%)	2e-96	Yes
Kss1	368	43	Kinase	MAPK1	ERK2	182/362 (50%)	7e-96	No
Dig1	452	49	–	–	–	–	–	–
Dig2	323	37	–	–	–	–	–	–
Ste12	688	78	Homeo (weak)	–	–	–	–	–
Far1	830	94	RING-H2	–	–	–	–	–
Bar1	587	64	Asp-like protease	PGC	Pepsinogen C	99/369 (26%)	8e-26	No
Sst2	698	80	DEP, RGS	–	–	–	–	–
Msg5	489	54	Phosphatase	DUSP10	MKP5	44/137 (32%)	5e-13	Yes
Ptp2	750	86	Phosphatase	PTPRC	CD45	102/378 (26%)	5e-21	No
Ptp3	928	105	Phosphatase	PTPN6	SHP-1	86/346 (24%)	2e-16	No



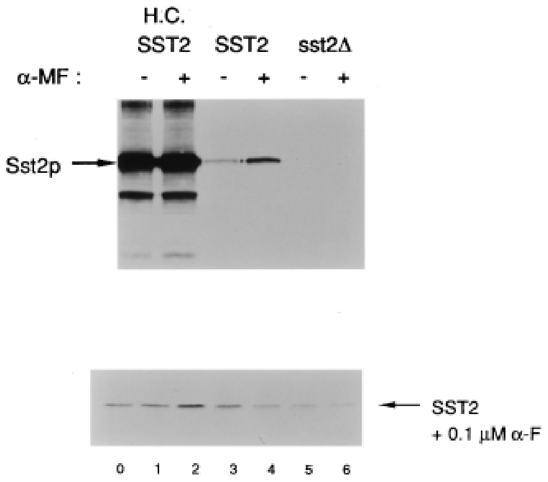
Concepts: GEF and GAP

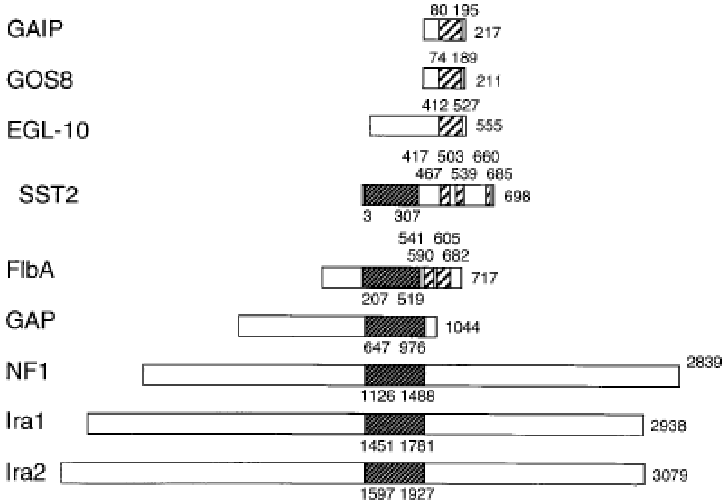
From **Protein Structure and Function** by Gregory A Petsko and Dagmar Ringe

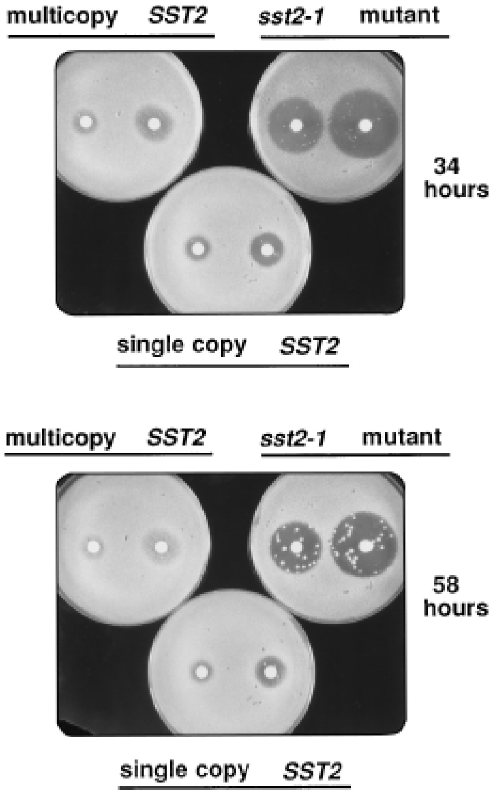


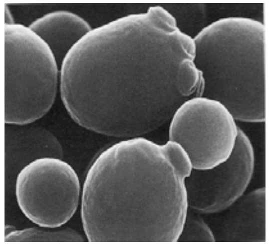


Sst2 as a GAP for attenuating GPCR signalling

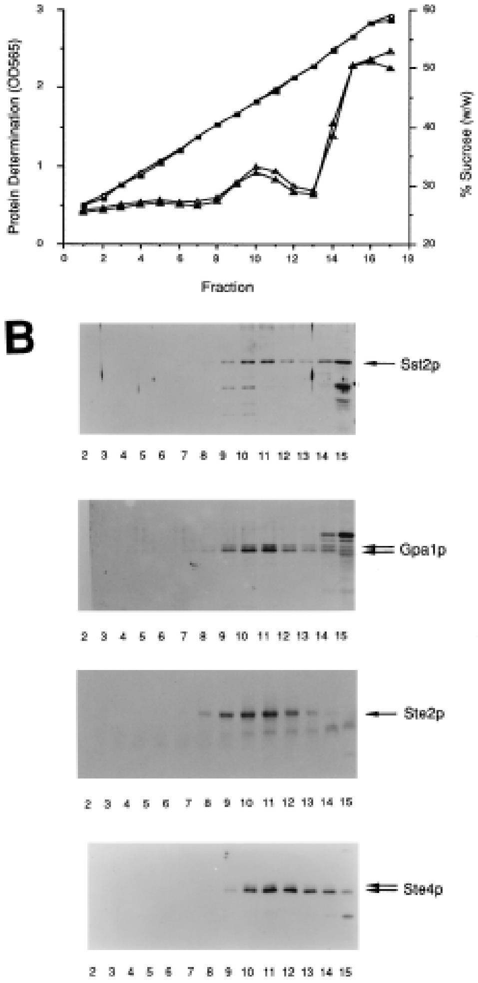


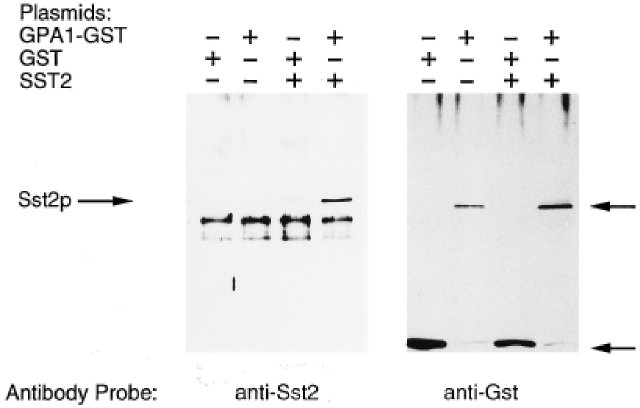


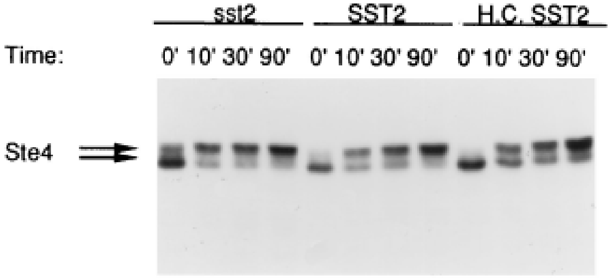


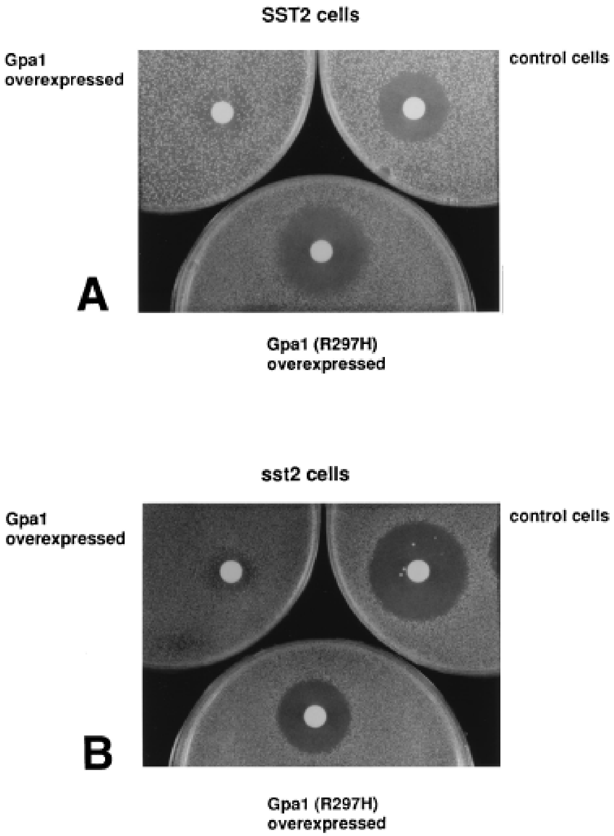


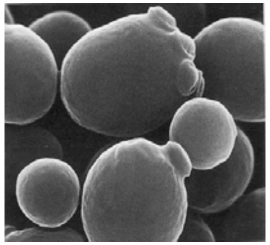
Purification of Sst2 Complex



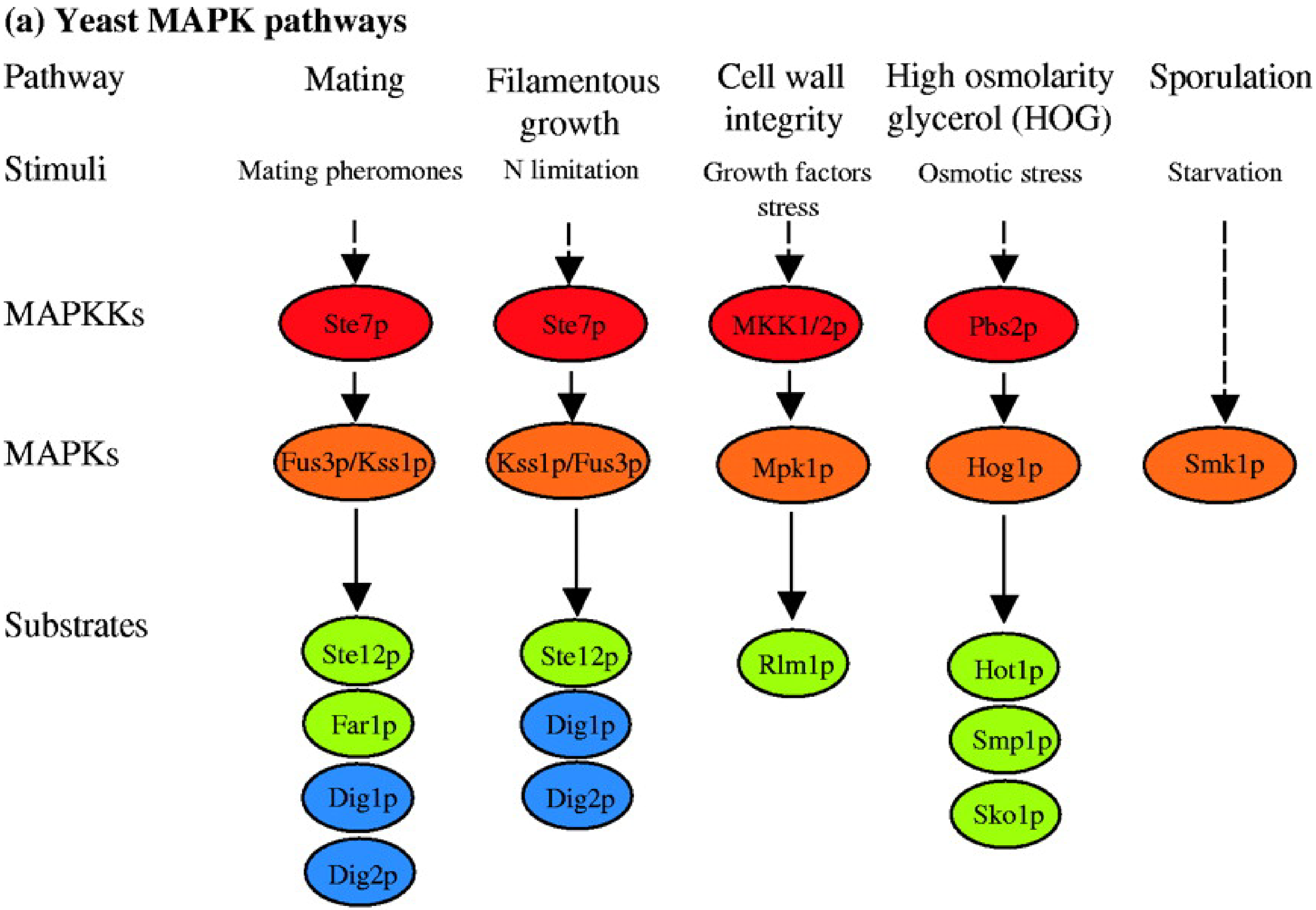


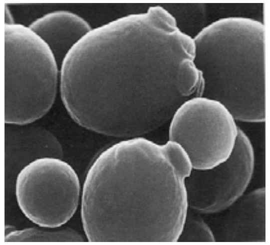




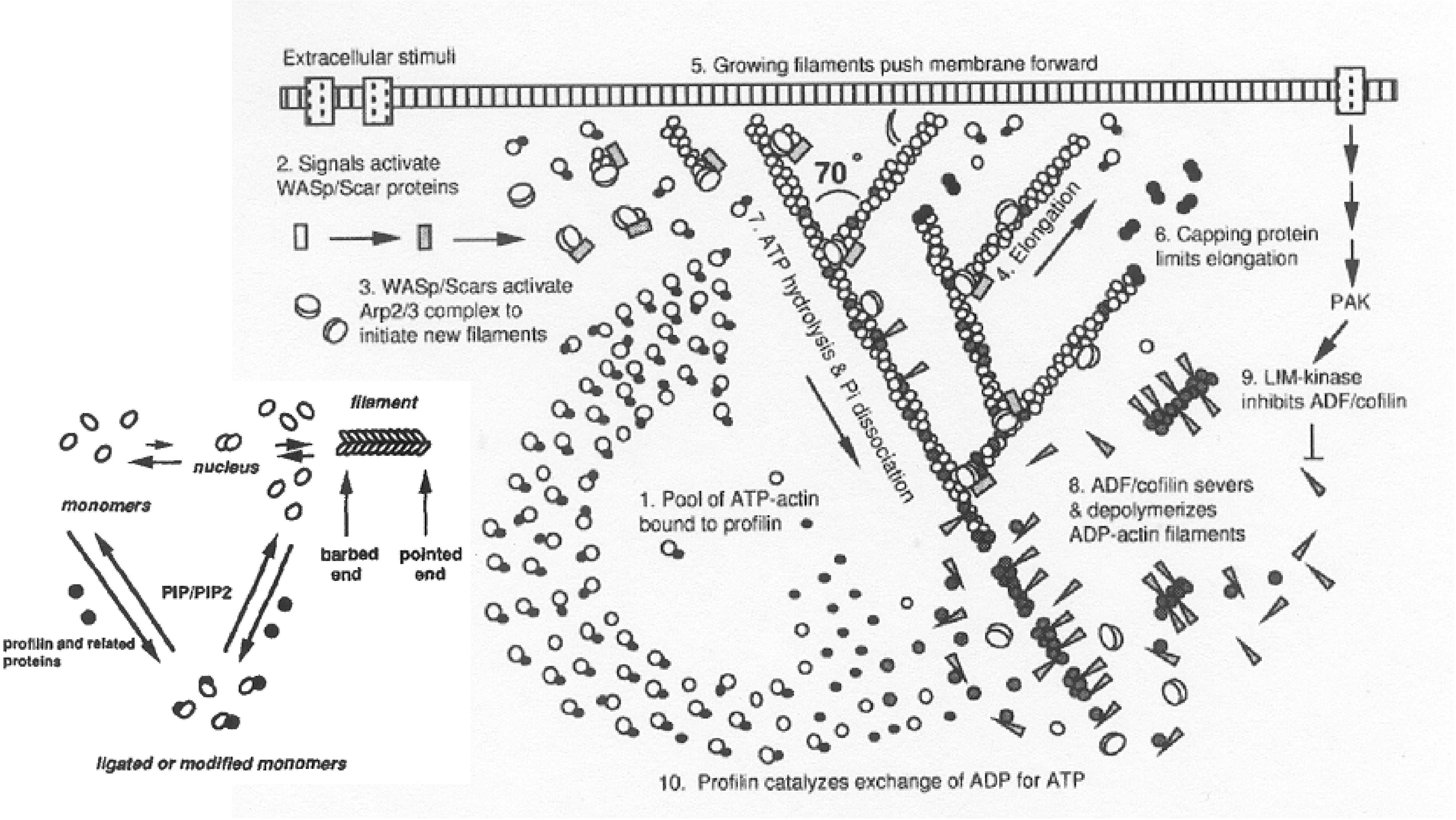


MAPK cascade

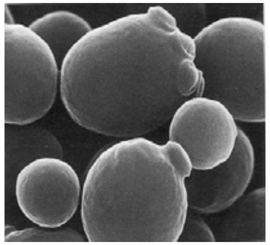


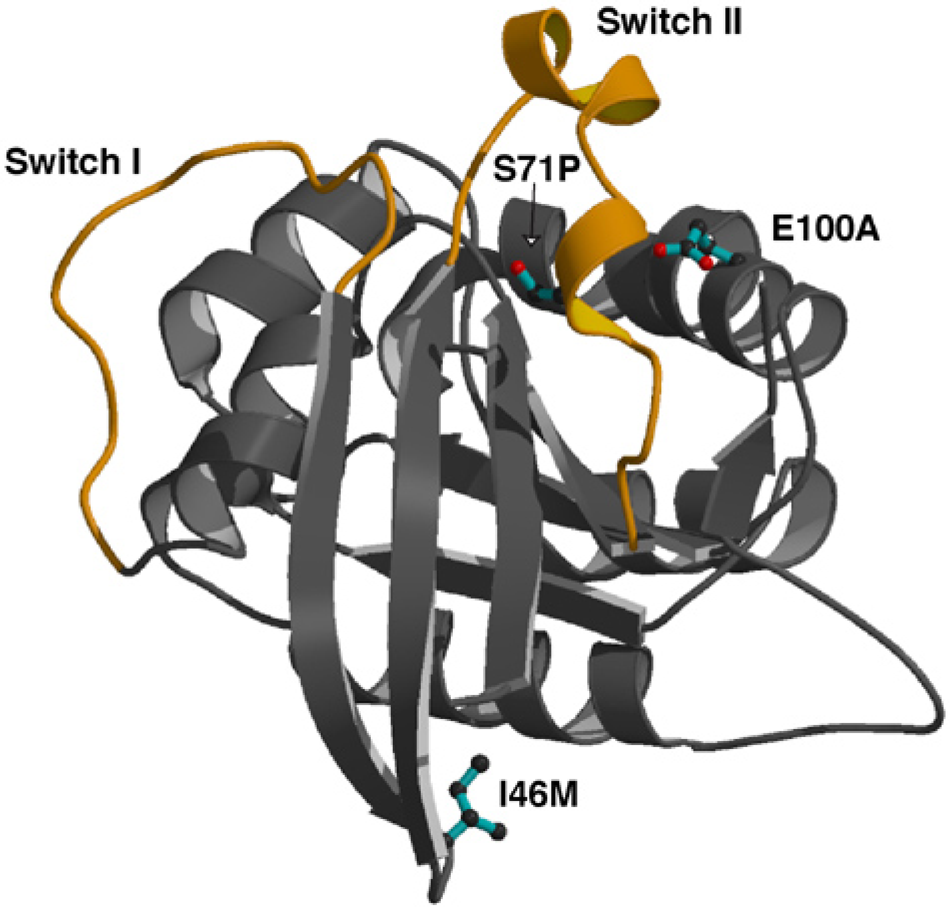


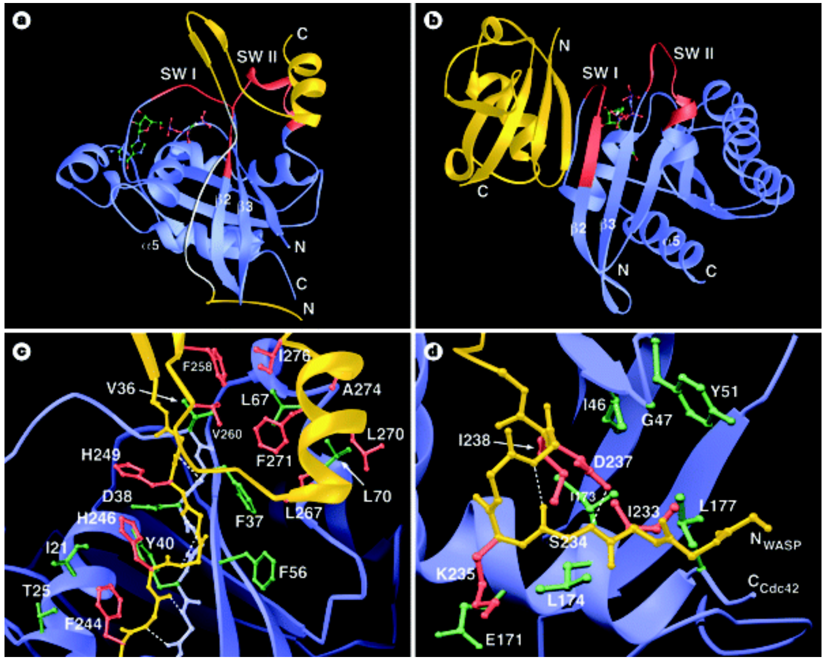
Actin dynamics

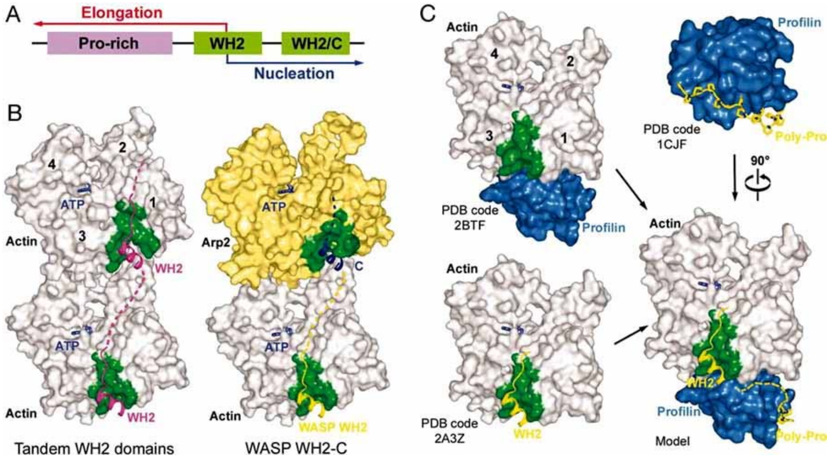


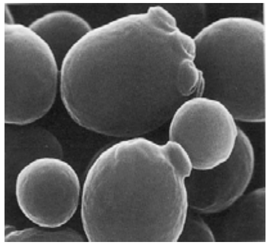
Cdc42 as actin controller



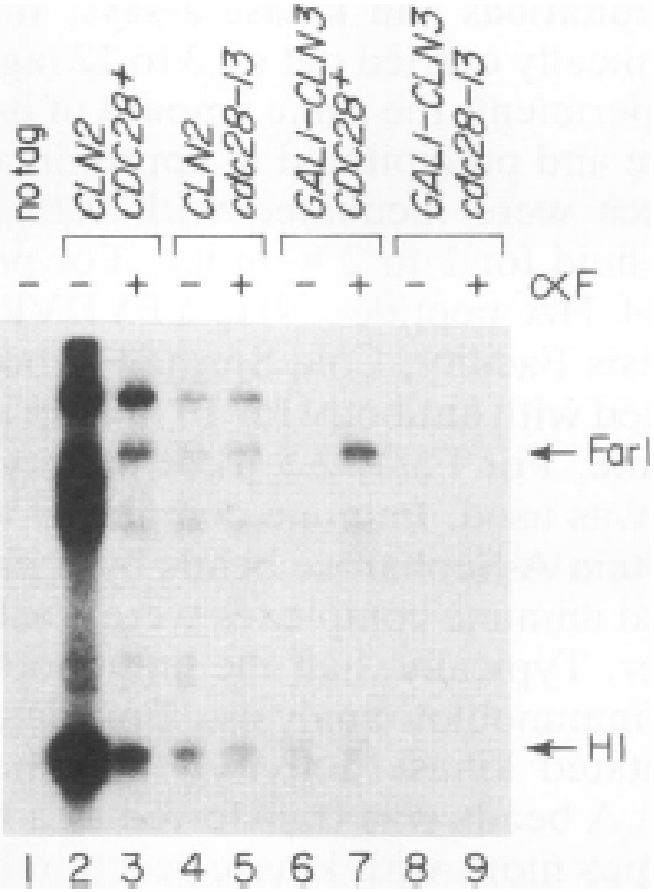


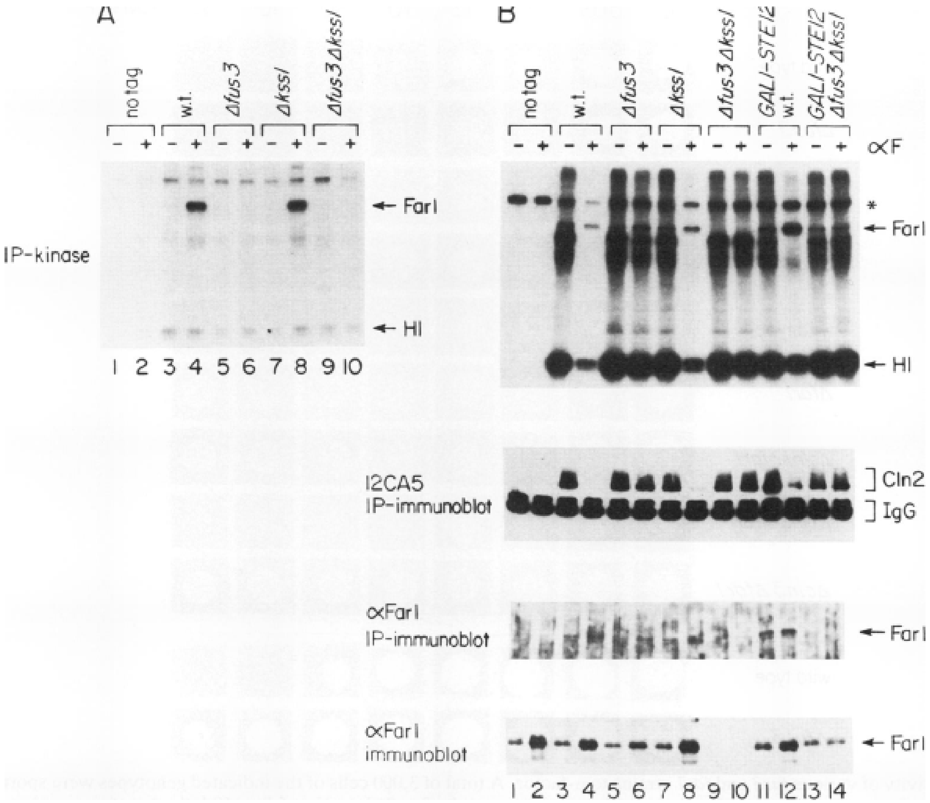


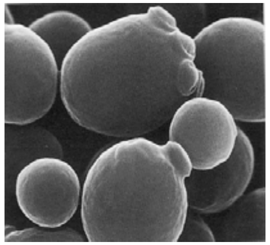




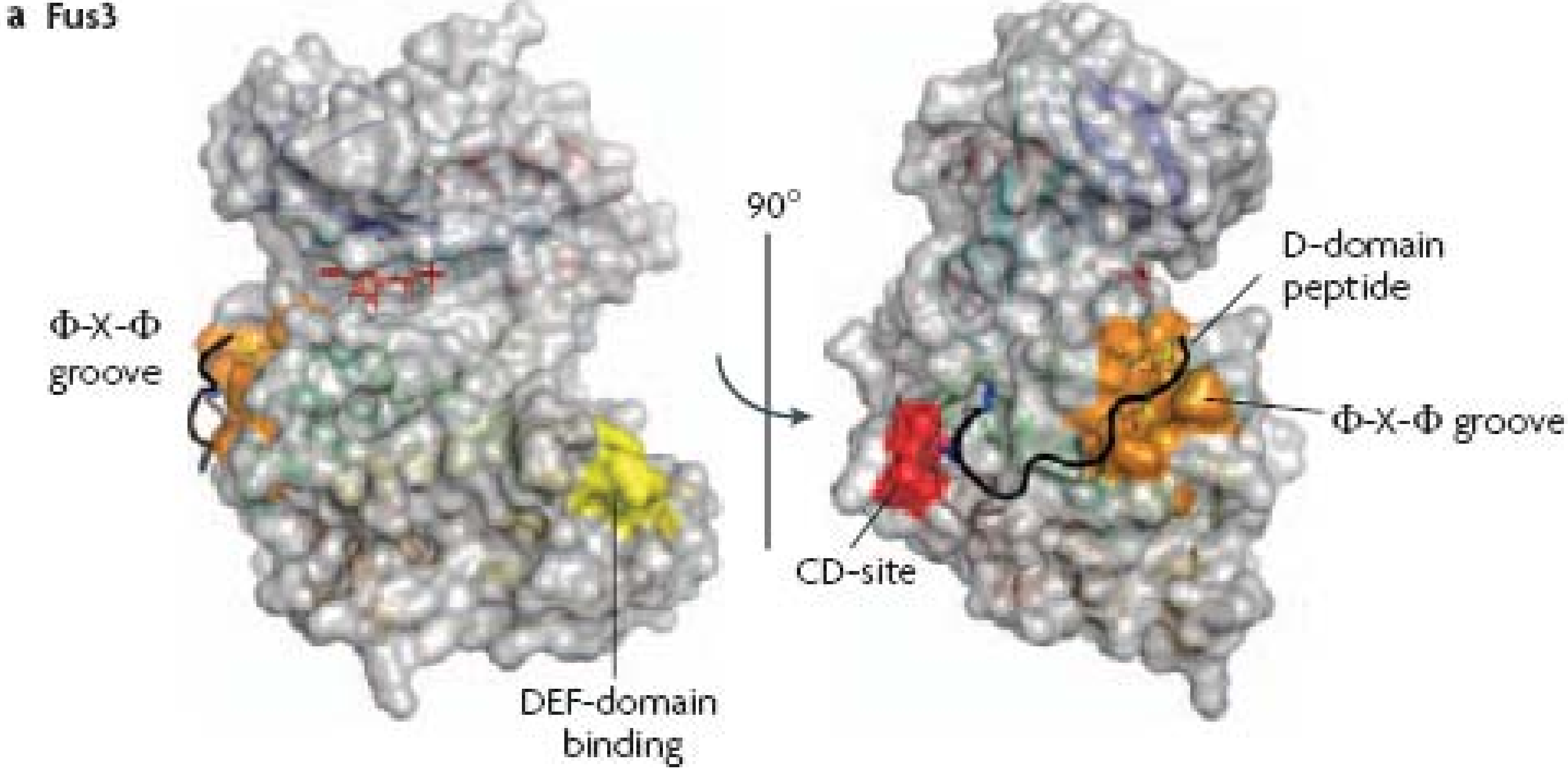
Far1 associates with Fus1 and inhibits CDK complexes

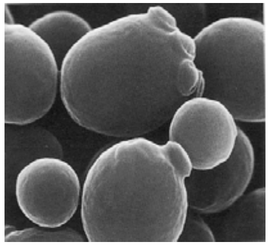




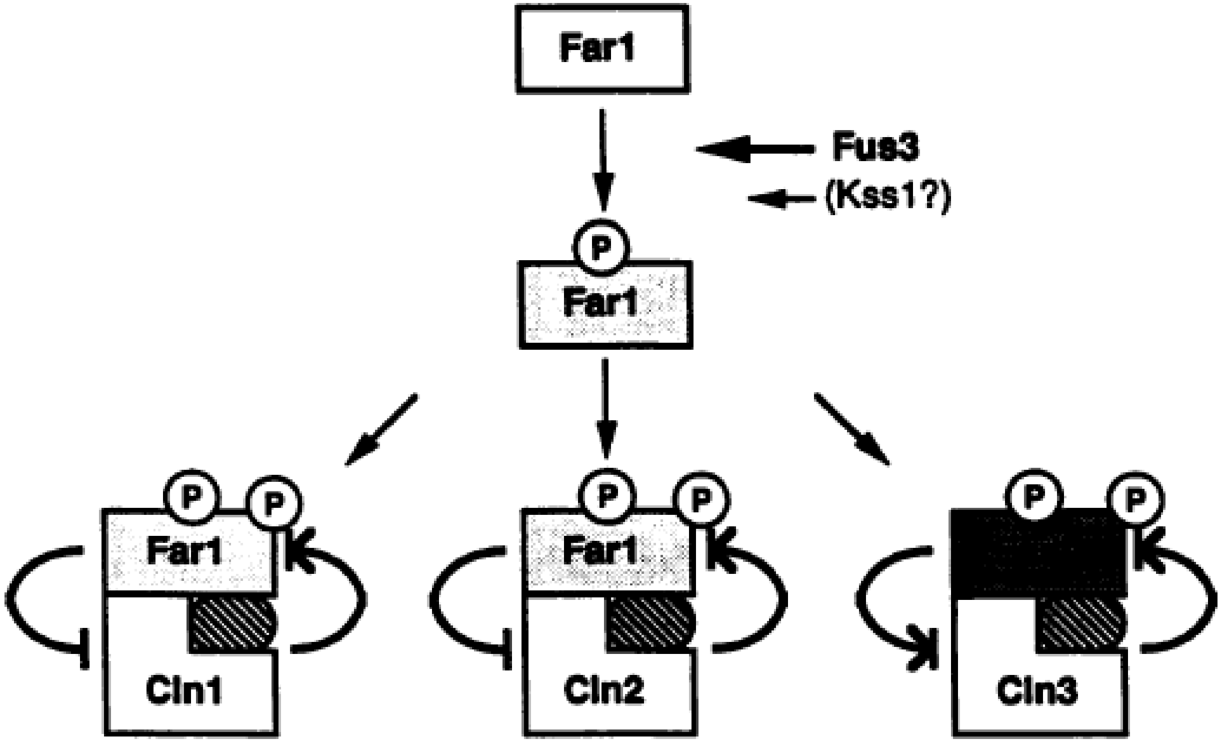


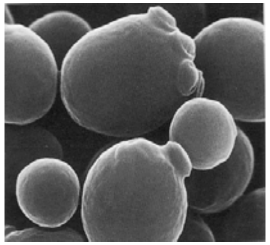
Far1 priming phosphorylation by Fus3



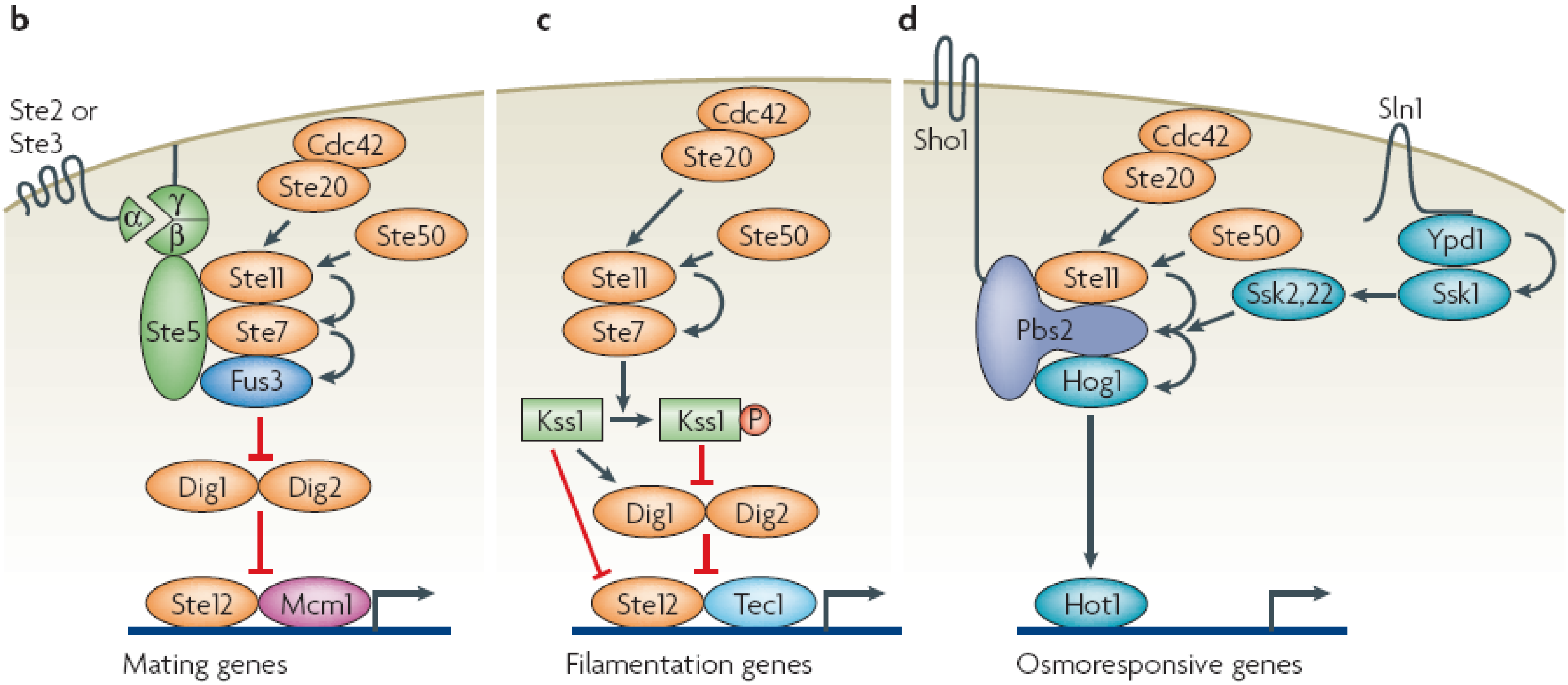


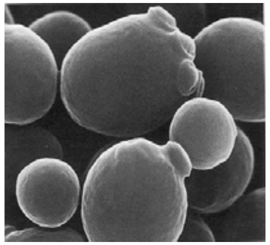
Multiple phosphorylation cascade in single protein signalling



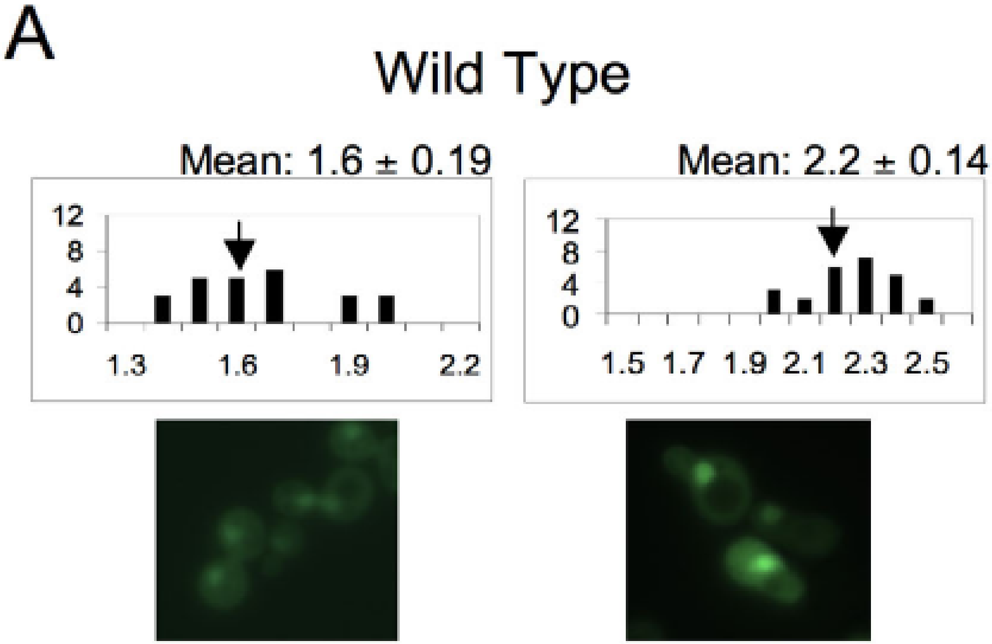


Multiple phosphorylation cascade in multiple protein signalling needs scaffolding protein

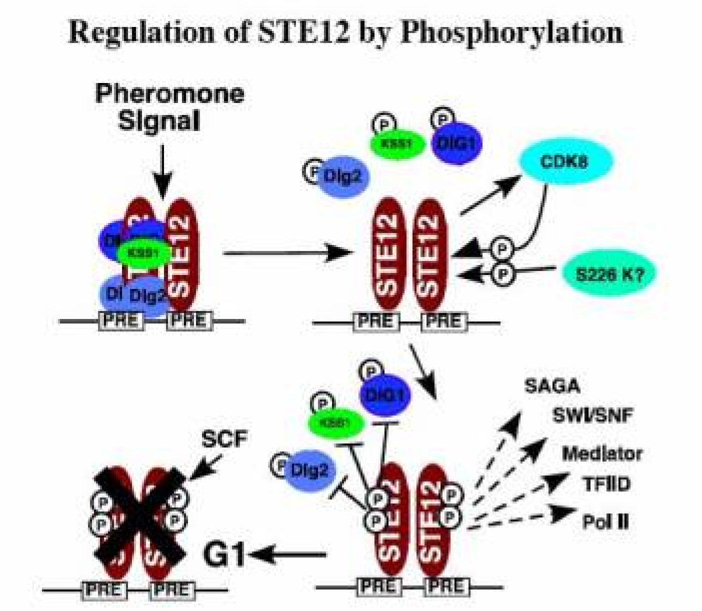


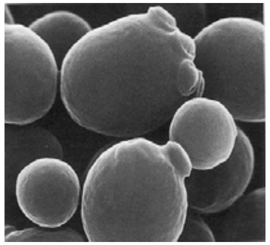


Nuclear translocalization of MAPK triggers downstream TF phosphorylation and activation

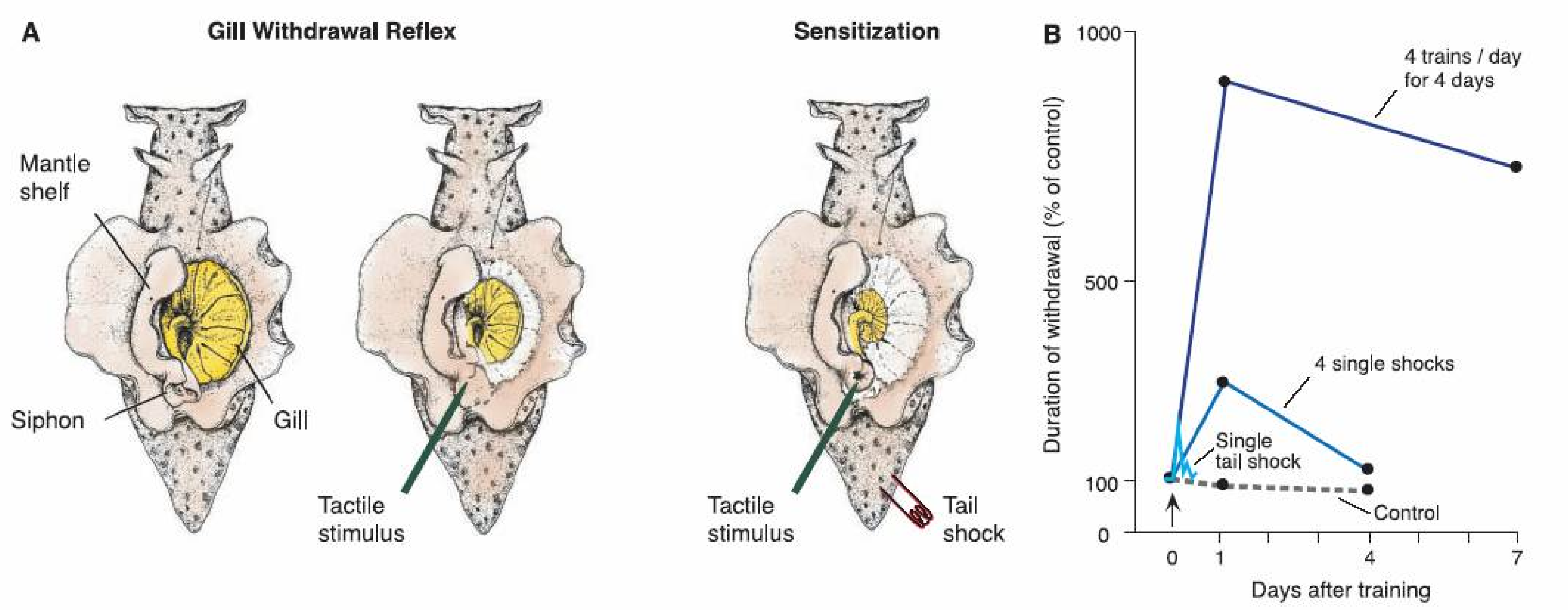


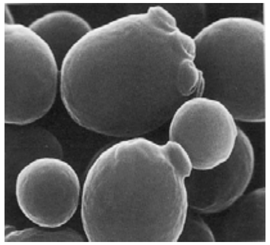
FUS3::GFP



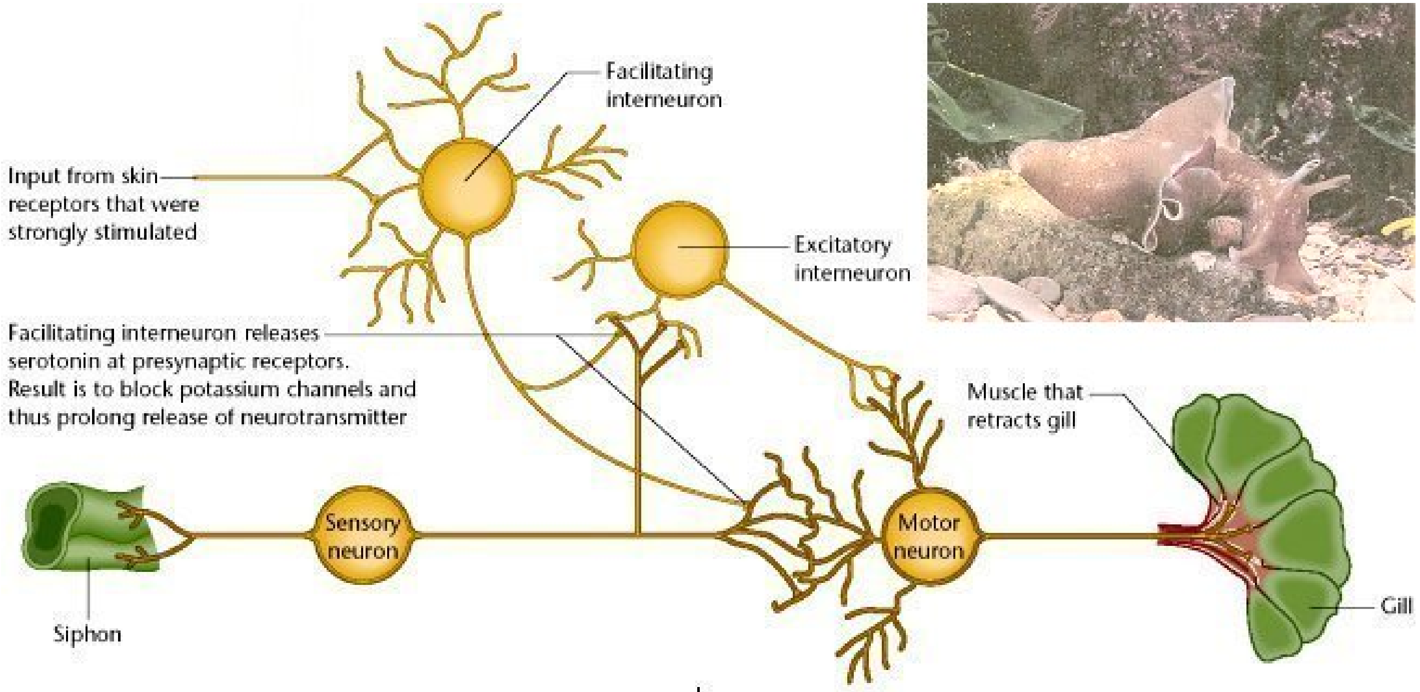


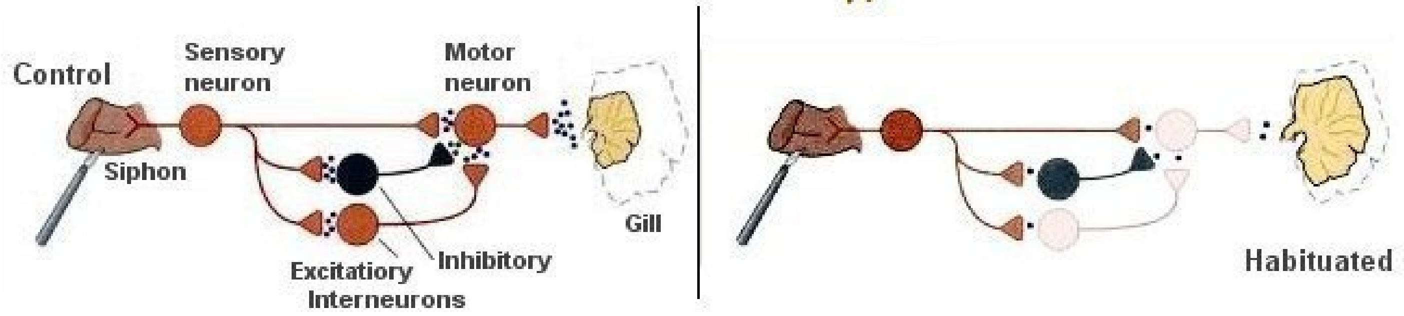
Aplysia

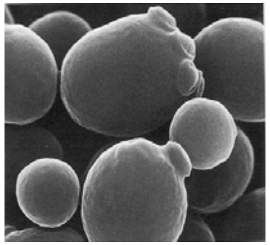

A
Gill Withdrawal Reflex
Mantle shelf
Siphon
Gill
Tactile stimulus
Sensitization
Tactile stimulus
Tail shock
B
1000
500
100
0
Duration of withdrawal (% of control)
4 trains / day for 4 days
4 single shocks
Single tail shock
Control
0
1
4
7
Days after training



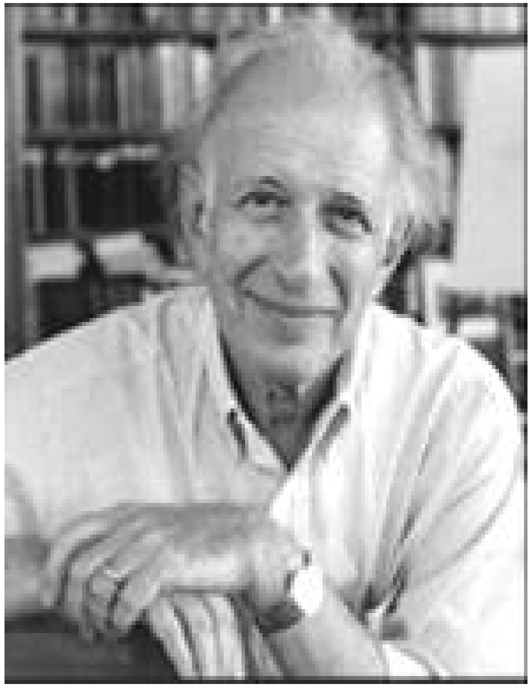
Neuroscience: Learning and memory as a matter of association

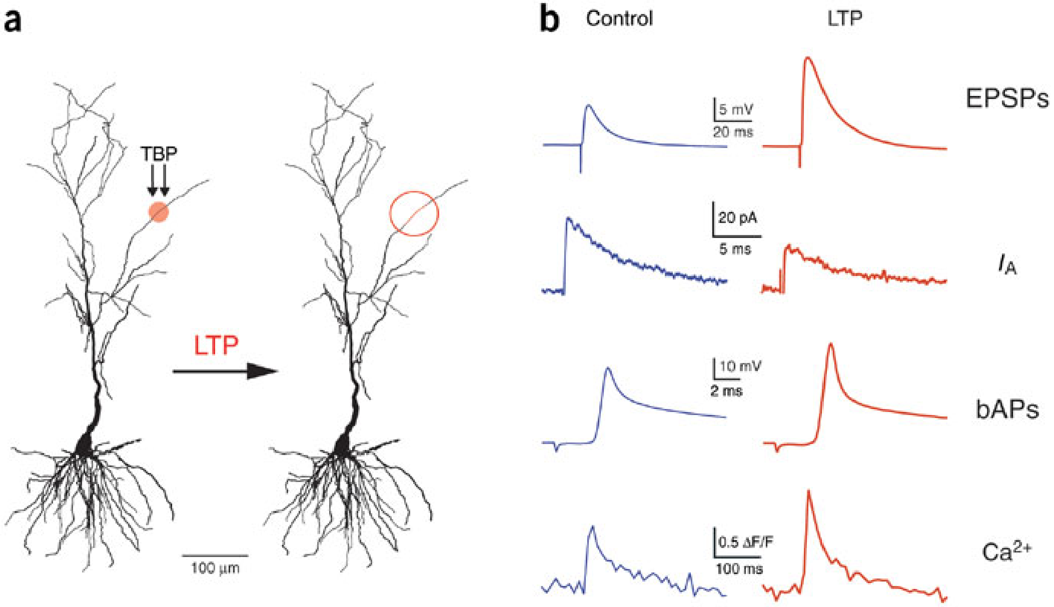


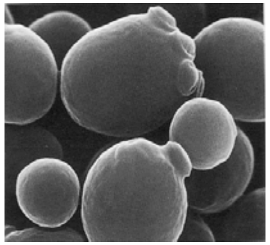




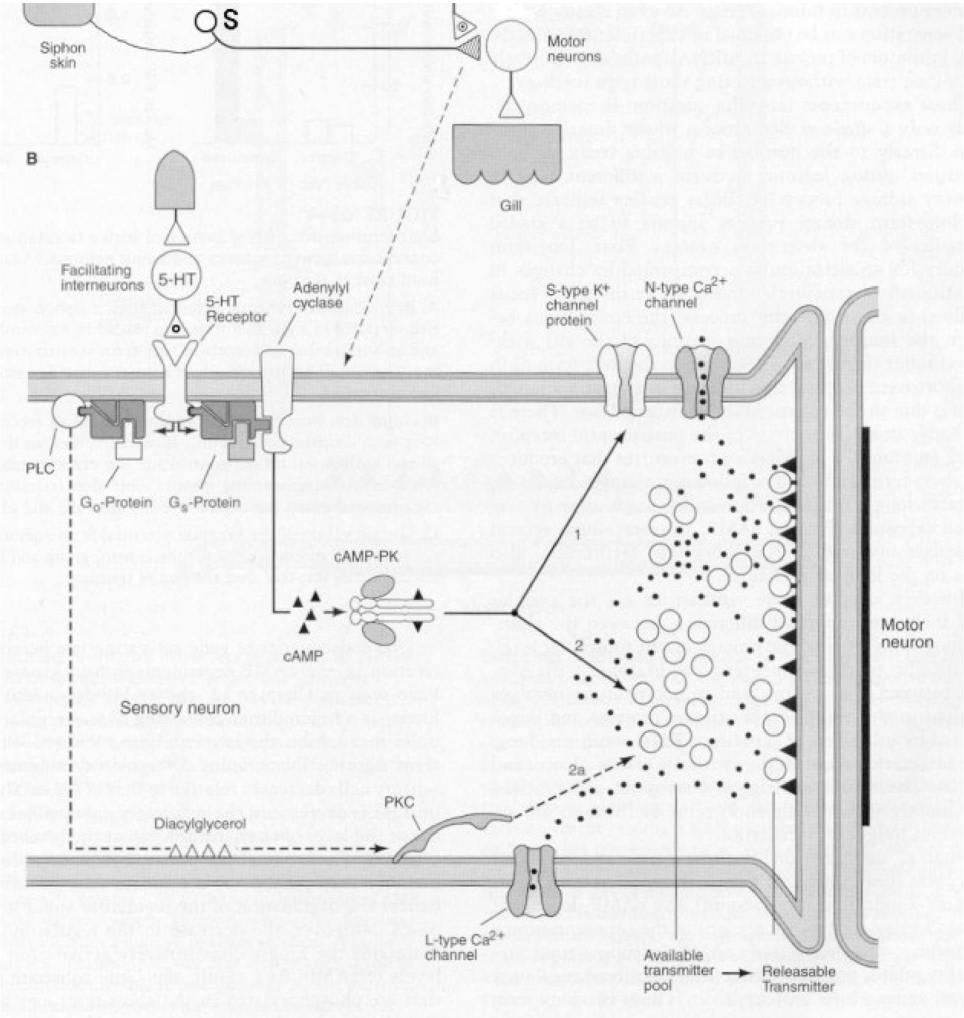
Mechanism of L&M: LTP

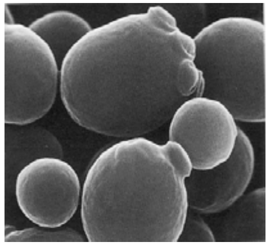




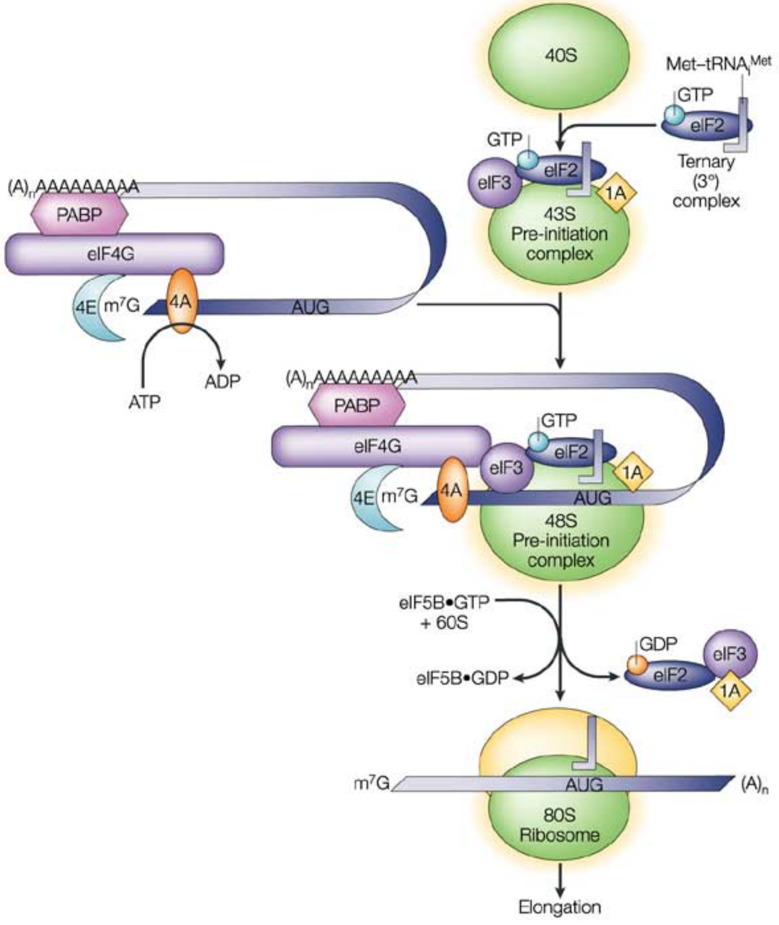


Molecular mechanism of L&M: reinforcement





Local translational regulation



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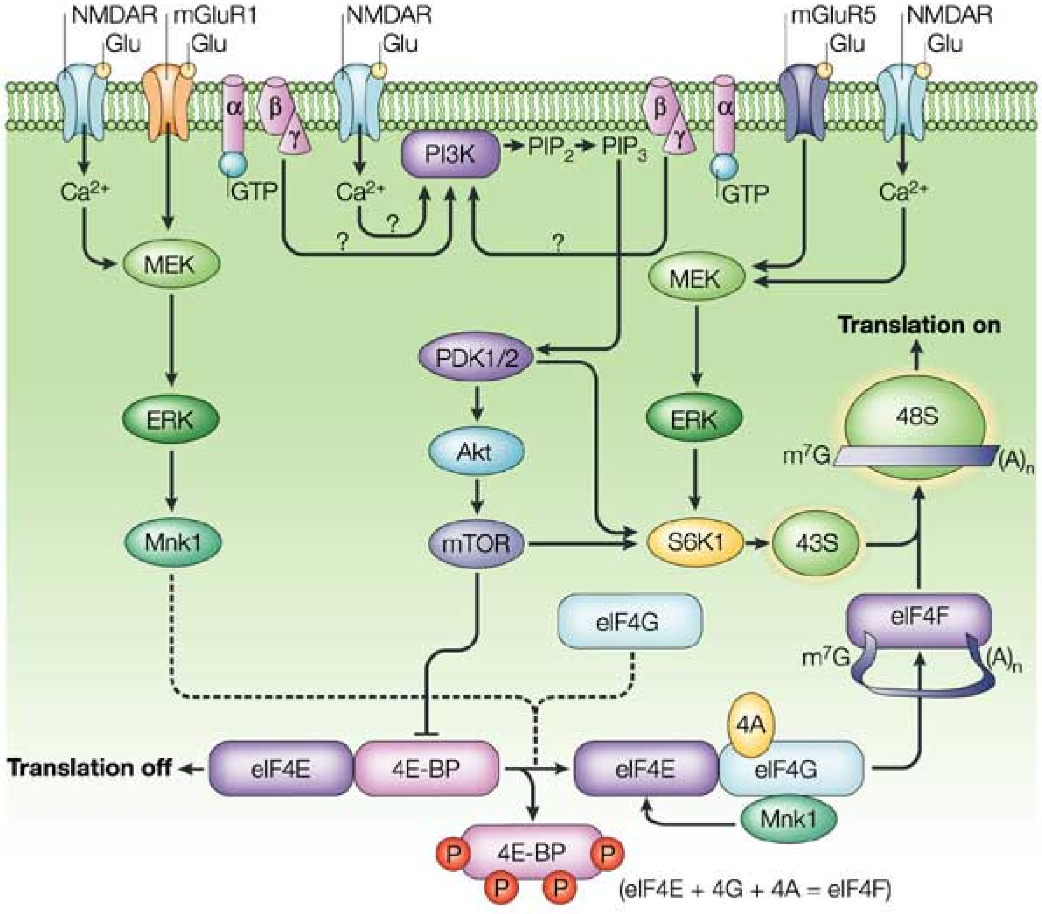

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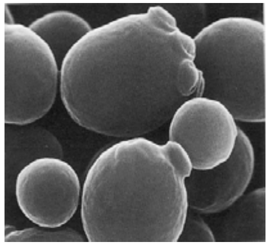
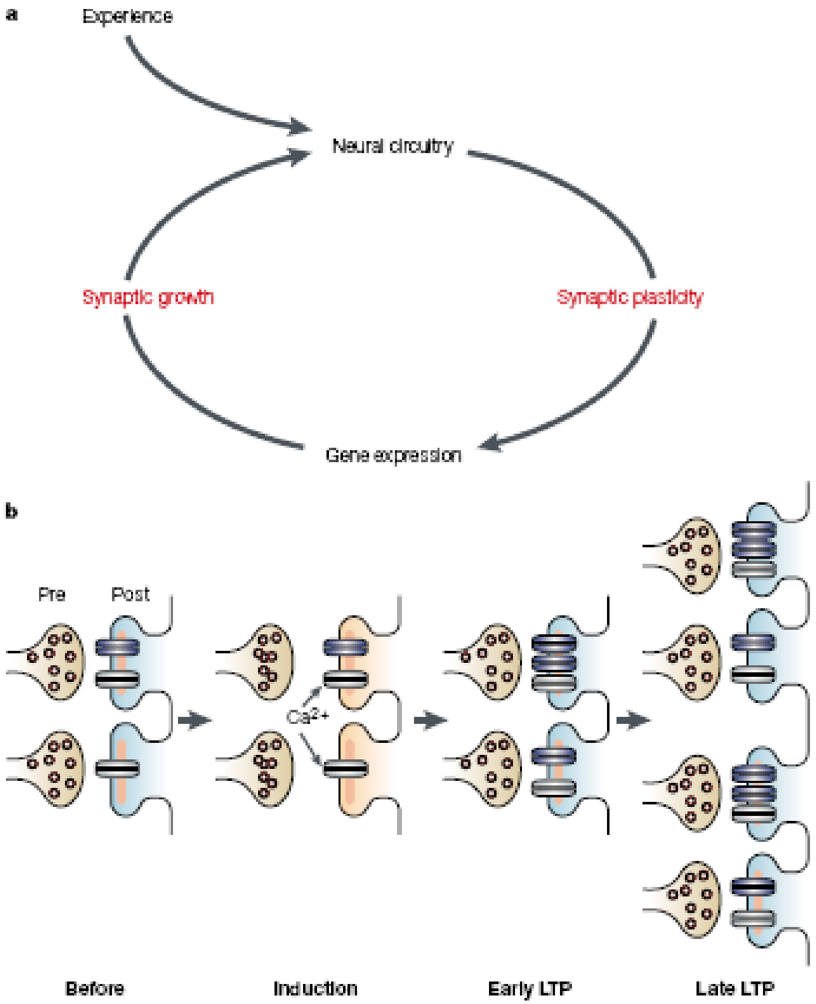
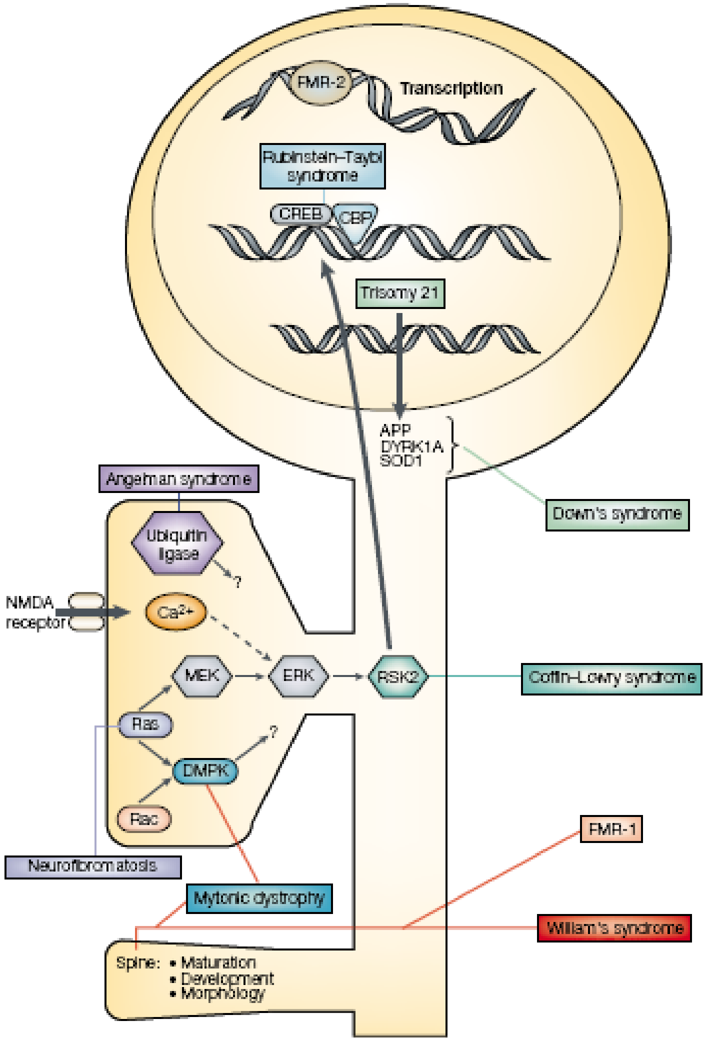
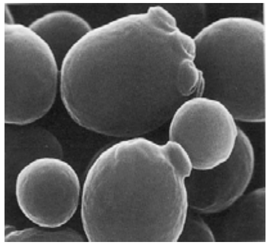


Late LTP and local-global events







Activation of CREB by nuclear translocation of MAPK