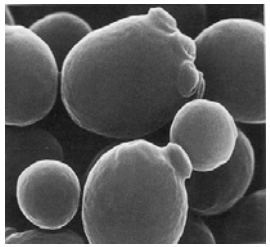
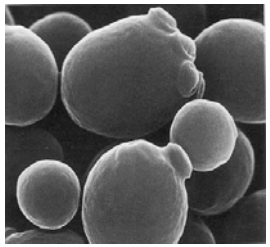


Molecular Cell Biology of the Yeast *Saccharomyces cerevisiae*

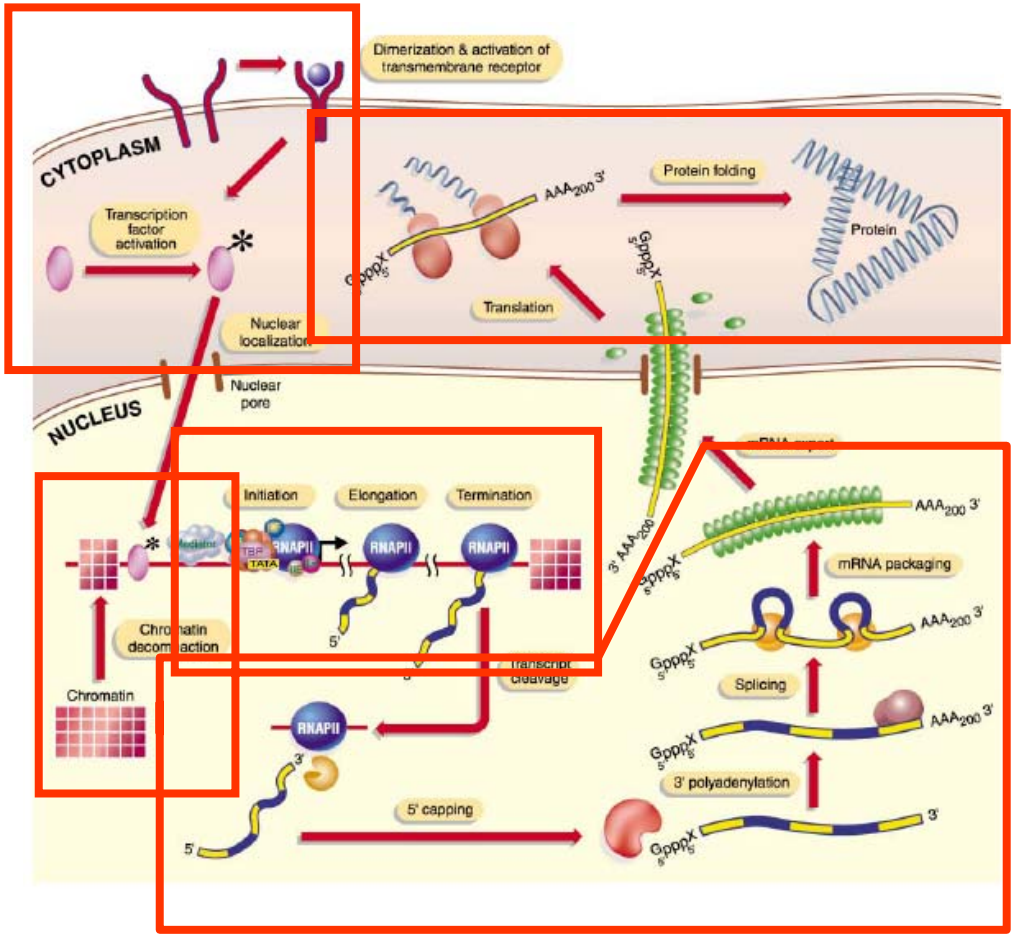
Lecture II: Transcriptional Regulation: Network Architecture, Molecular Mechanism, and Phenotypic Dynamics

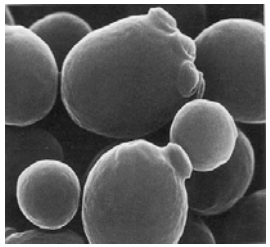
Zhang Yi,
National Institute of Biological Sciences, 20080518



Components of Gene Expression

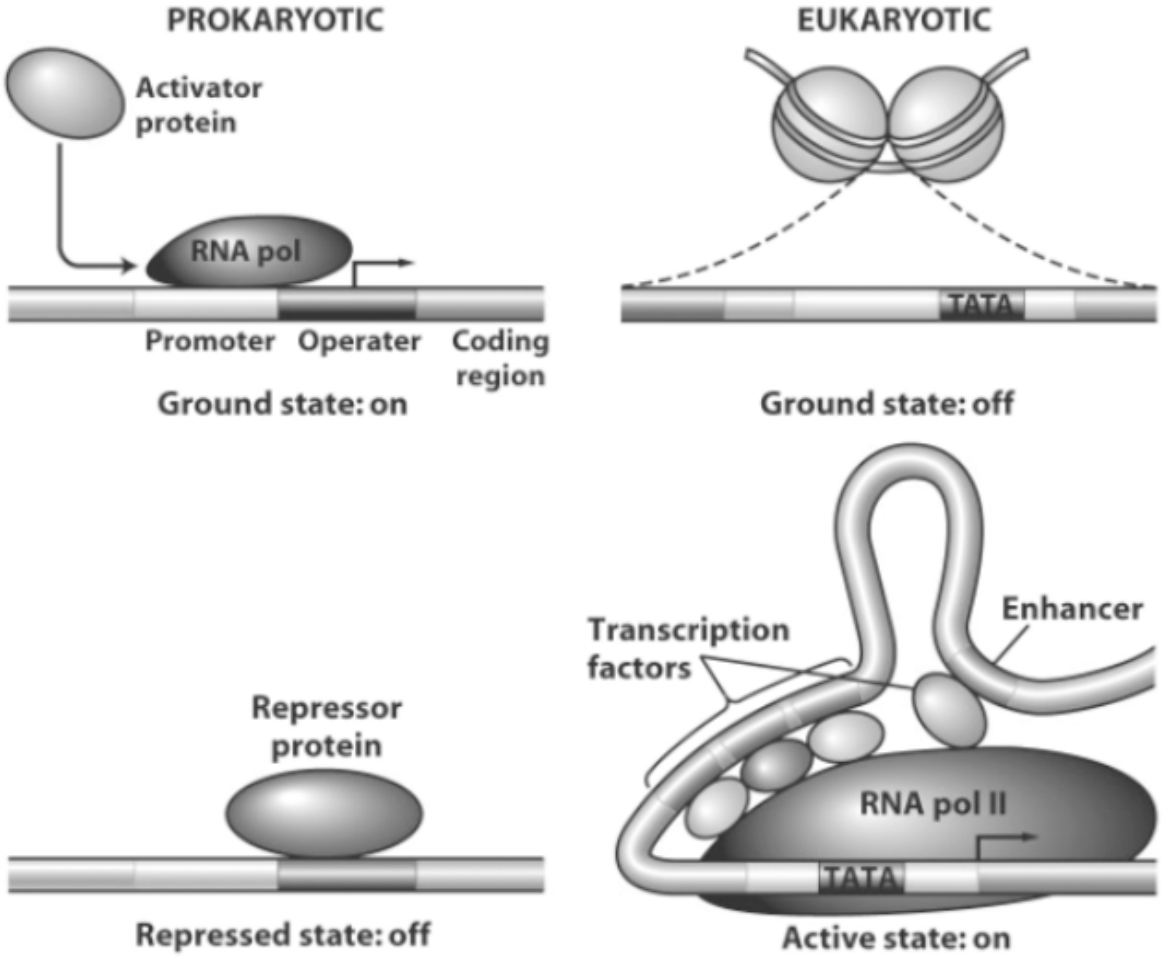
- Signalling Trigger
- Chromatin Dynamics
  - Histone Modification
  - Chromatin Remodelling
  - DNA editing
- Transcription Machinery Processing
  - Initiation
  - Elongation
  - Termination
- RNA Processing
  - Capping
  - Tailing
  - Splicing
  - Editing
  - Packaging
- Translational Regulation

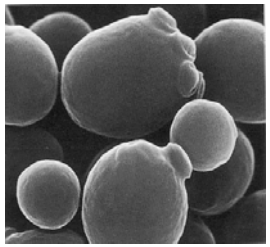




Transcription Overview

- Default to ON for **prokaryotes** and OFF for **eukaryotes**.

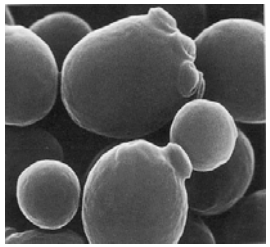




Ptashne and transcription factors

Young…

Old (and stubborn enough …)



Gal4 Structure

Table 2. Internal Deletion Mutants

GAL4 DERIVATIVES	GAL4 SEQUENCES N-Term.	GAL4 SEQUENCES C-Term.	β-gal ACTIVITY
WTGAL4 (pMA210)	1	881	1860
II-1 (ID5-1)	1-74	684-881	617
II-2 (ID6-1)	1-74	738-881	574
II-3 (ID6-3)	1-74	752-881	746
II-4 (ID5-6)	1-74	767-881	384
II-5 (ID5-3)	1-74	768-881	344
II-6 (ID5-1R)	1-74	684 848+13	1.0
II-7 (ID5-6R)	1-74	767-848+13	<1
II-8 (ID5-3S)	1-74	851-881	<1
II-9 (pMA236)	1-147	768-881	962
II-10 (pMA238)	1-147	768-848+2	1.0
II-11 (pMA237)	1-147	851-881	36

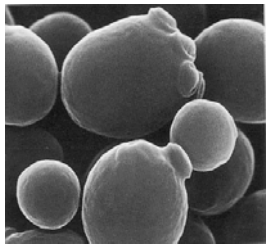
Table 3. Further Internal Deletion Mutants

GAL4 DERIVATIVES	GAL4 SEQUENCES N-Term.	GAL4 SEQUENCES C-Term.	β-gal ACTIVITY
WTGAL4 (pMA210)	1	881	1860
III-1 (CD8)	1-833	848-881	725
III-2 (CD16X)	1-823	851 881	664
III-3 (CD14XX)	1-792	848-881	467
III-4 (CD23X)	1-763	851-881	355
III-5 (CD20XX)	1-755	848-881	335
III-6 (CD19XX)	1-644	848-881	7.3
III-7 (CD56X)	1-614	851-881	<1
III-8 (CD38XX)	1-593	848-881	<1
III-9 (CD52XX)	1-478	848-881	<1
III-10 (CD59X)	1-238	851-881	534
III-11 (pMA242)	1-238	768-881	1406
NO GAL4 (pMA200)	—	—	<1

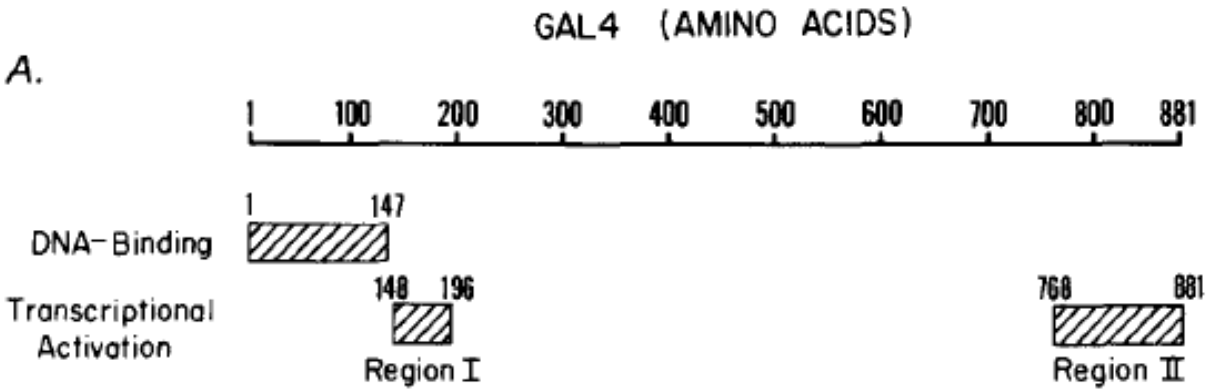
AMINO ACIDS

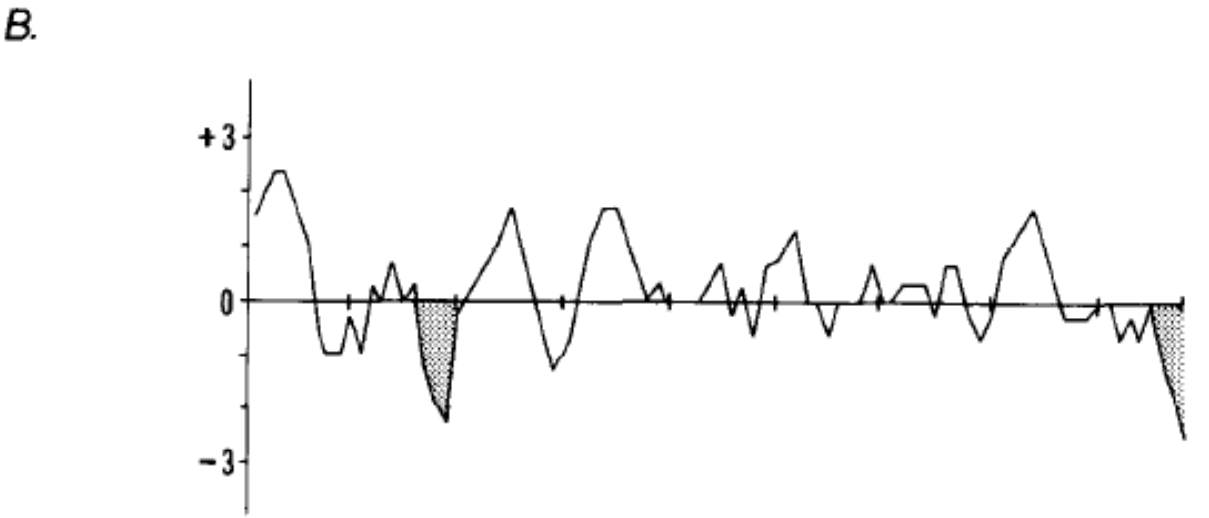
1 100 200 300 400 500 600 700 800 881

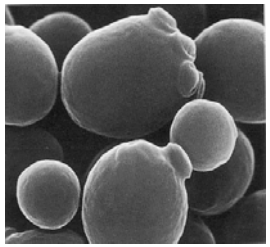
GAL4 DERIVATIVES	GAL4 SEQUENCES N-Term.	GAL4 SEQUENCES C-Term.	β-gal ACTIVITY
WTGAL4 (pMA210)	1	881	1860
I-1 (pMA230)	1	848+9	1584
I-2 (CD13)	1	844+9	867
I-3 (CD8XT)	1	833+2	337
I-4 (CD15XX)	1	823+13	994
I-5 (CD18)	1	809+8	413
I-6 (CD14XT)	1	792+2	281
I-7 (CD23XX)	1	763+13	7.1
I-8 (CD20XT)	1	755+3	2.6
I-9 (CD21X)	1	692+10	<1
I-10 (CD22X)	1	677+10	<1
I-11 (CD19XT)	1	644+2	<1
I-12 (CD56XX)	1	614+13	<1
I 13 (CD38XT)	1	593+2	<1
I-14 (CD52XT)	1	478+2	<1
I-15 (SD7)	1	396+0	<1
I-16 (SD8)	1	369+4	<1
I-17 (SD9)	1	342+0	<1
I-18 (SD19)	1	319+2	<1
I-19 (SD17)	1	288+2	<1
I-20 (SD22)	1	276+2	<1
I-21 (pMA246)	1	238+3	114
I-22 (SD16)	1	229+4	4.6
I-23 (SD15)	1	196+2	138
I-24 (pMA241)	1	147+3	<1
I-25 (pMA240)	1	74+9	<1
NO GAL4 (pMA200)	—	—	<1



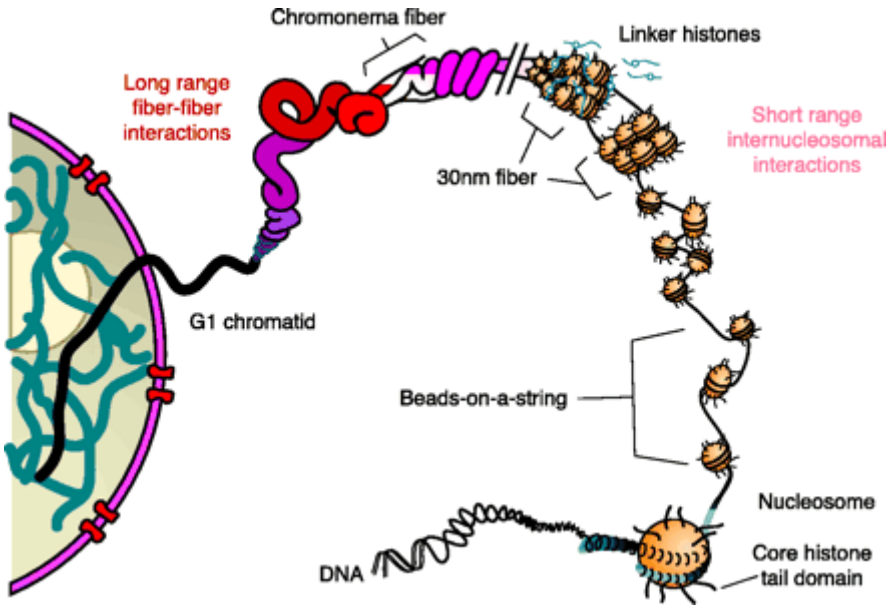
Gal4 Structure

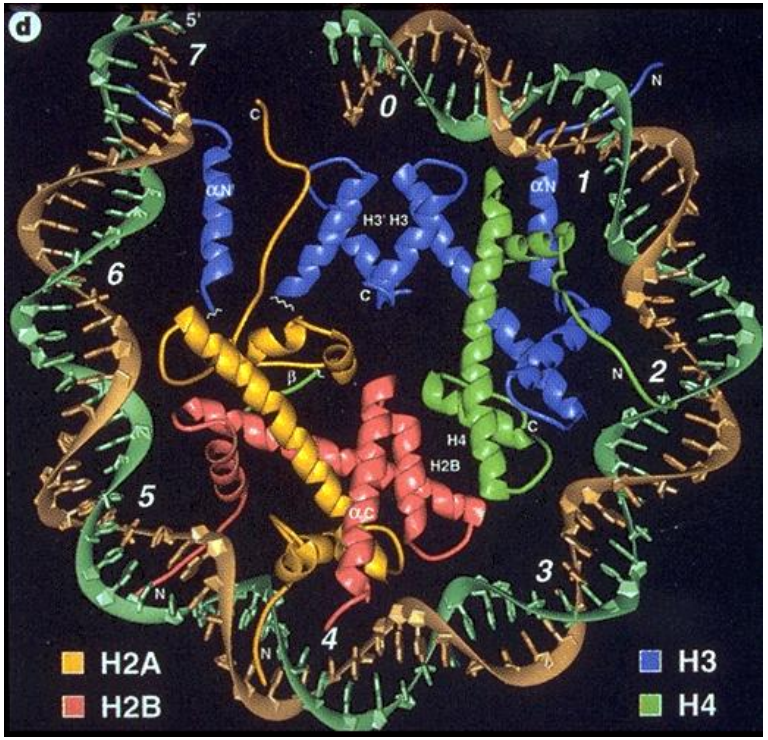


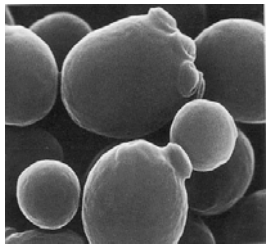




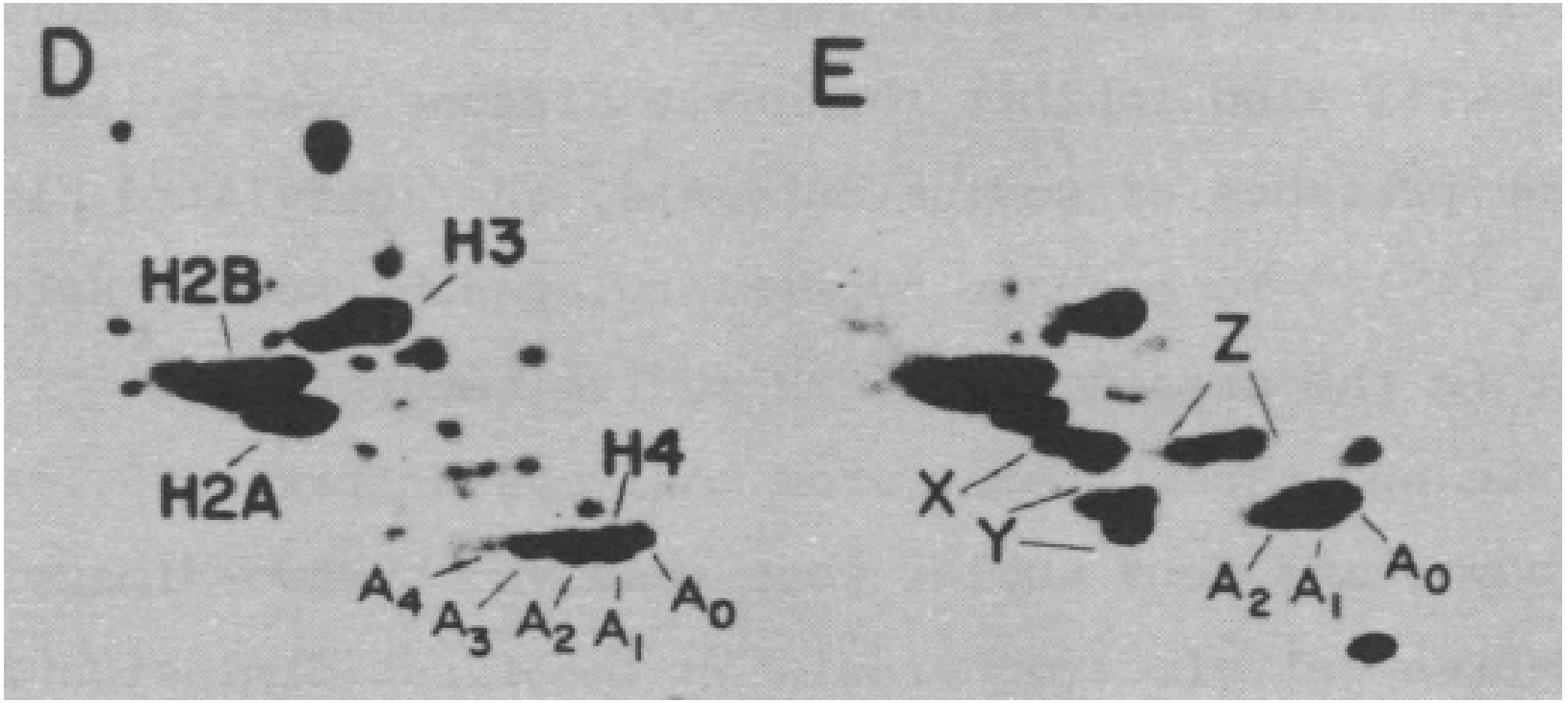
Chromatin Structure



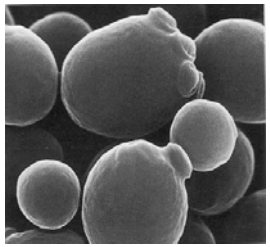




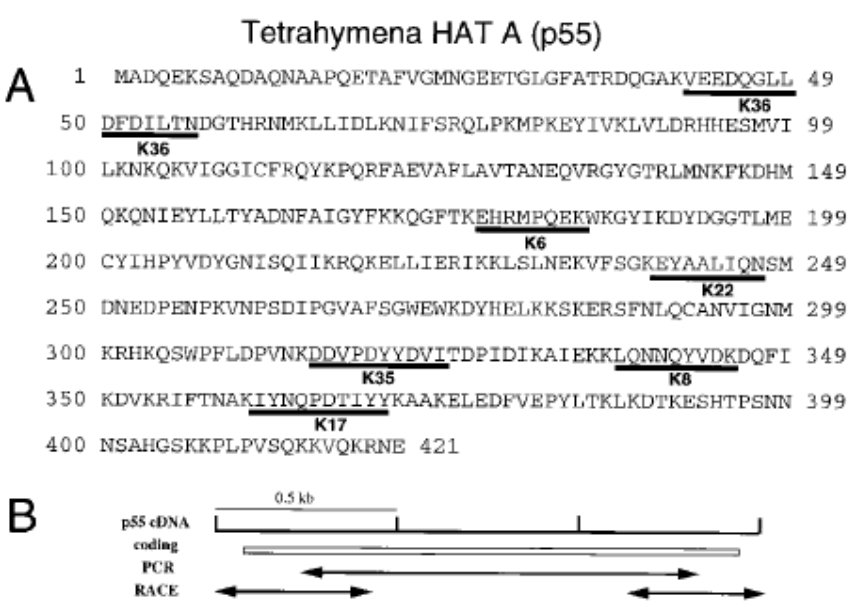
Histone Acetylation

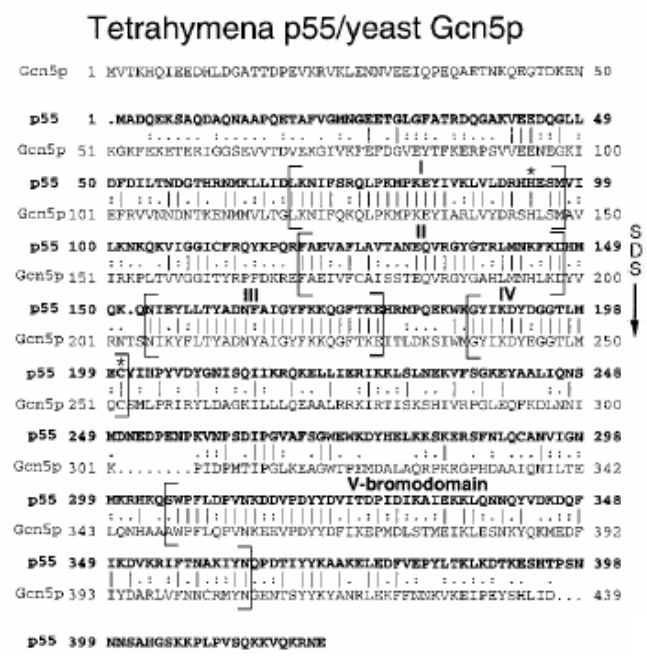


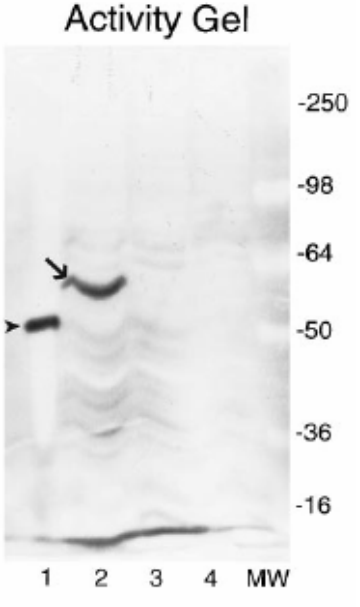
+100mM sodium butrylate in isolation

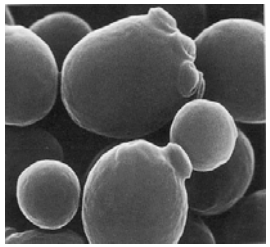


Biochemical Cloning of a Tetrahymena HAT with homology to yeast Gcn5p

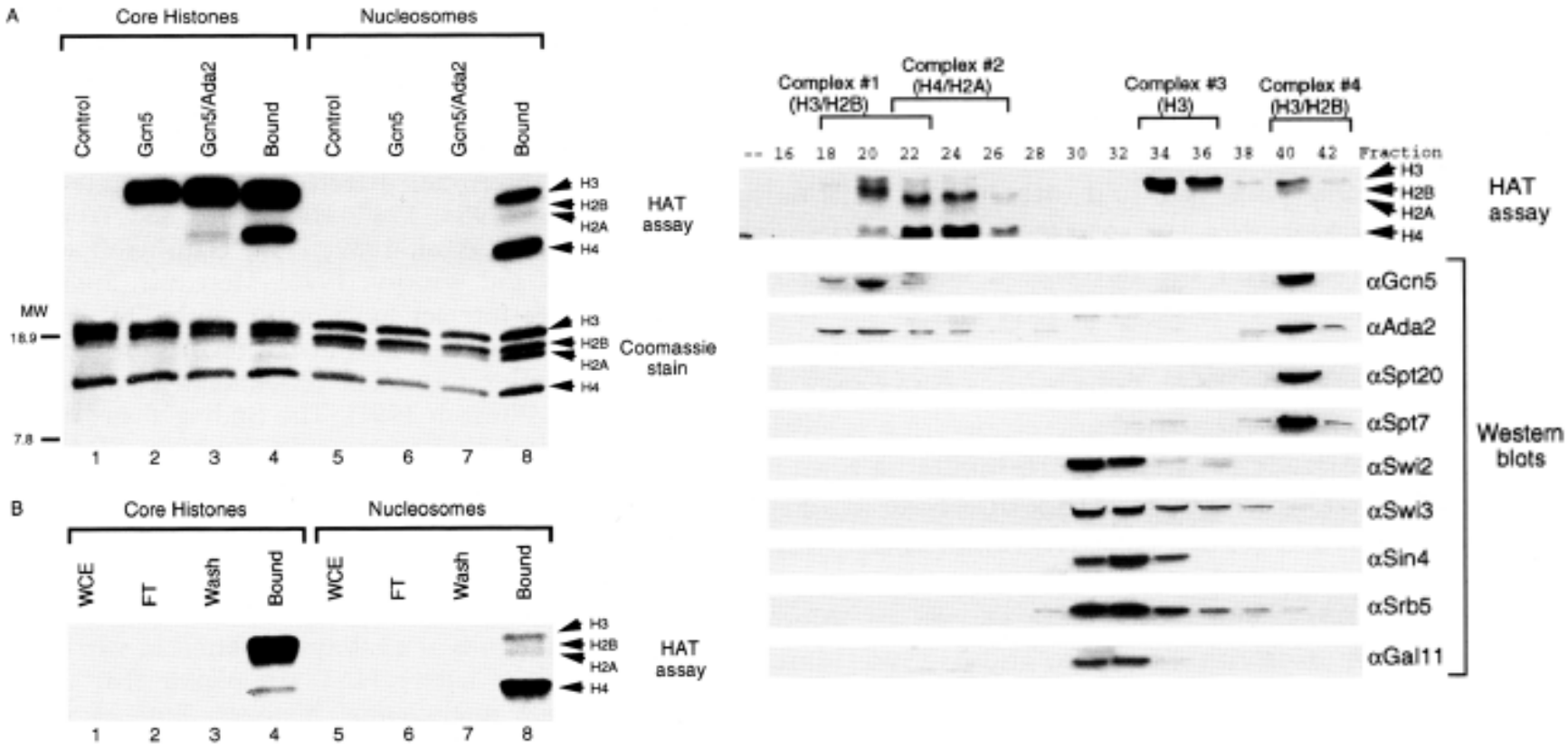


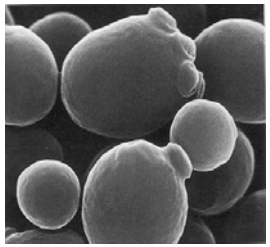




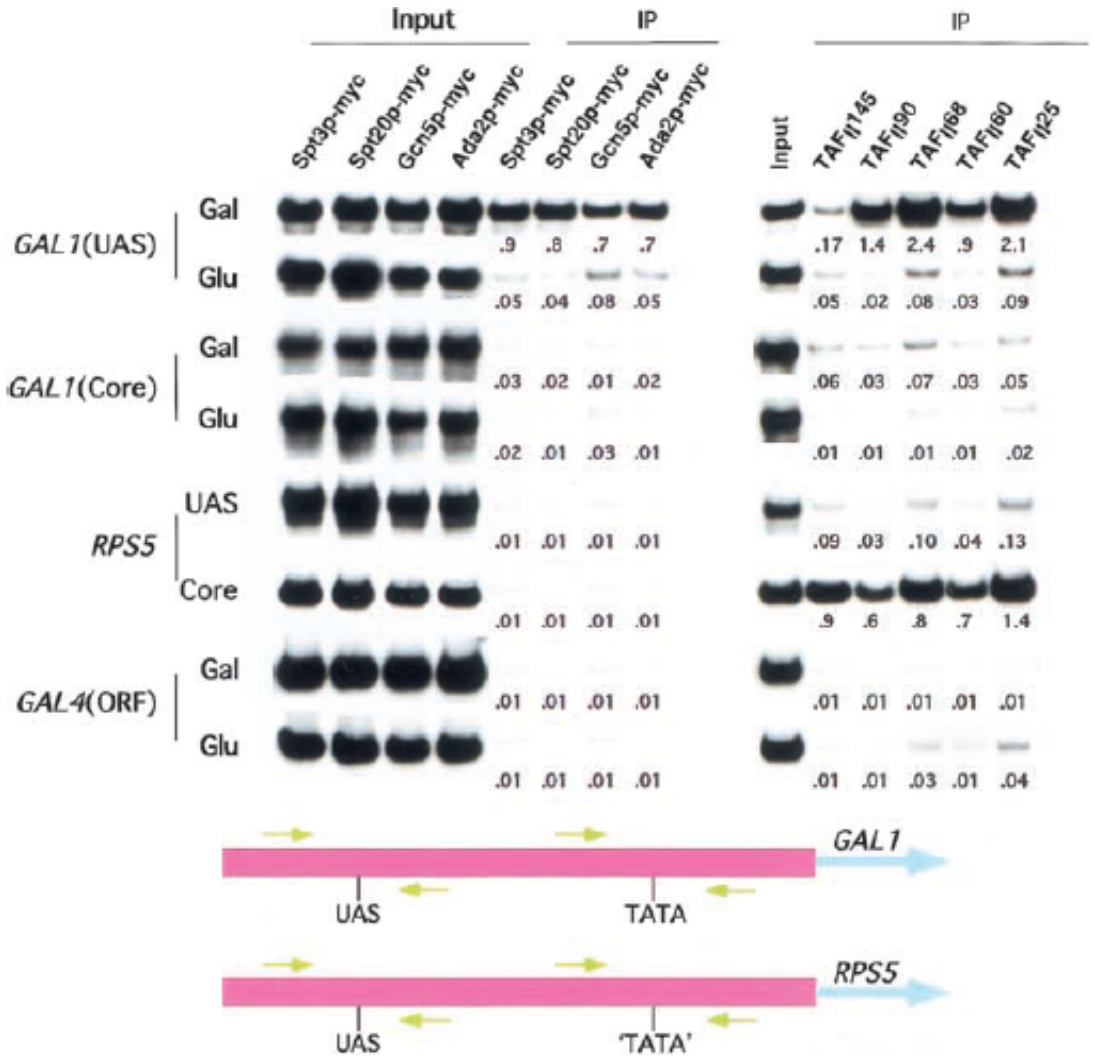


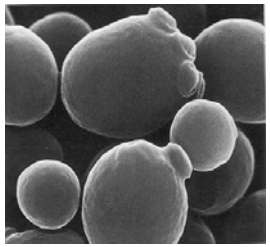
Gcn5 Functions in Ada/SAGA Complexes



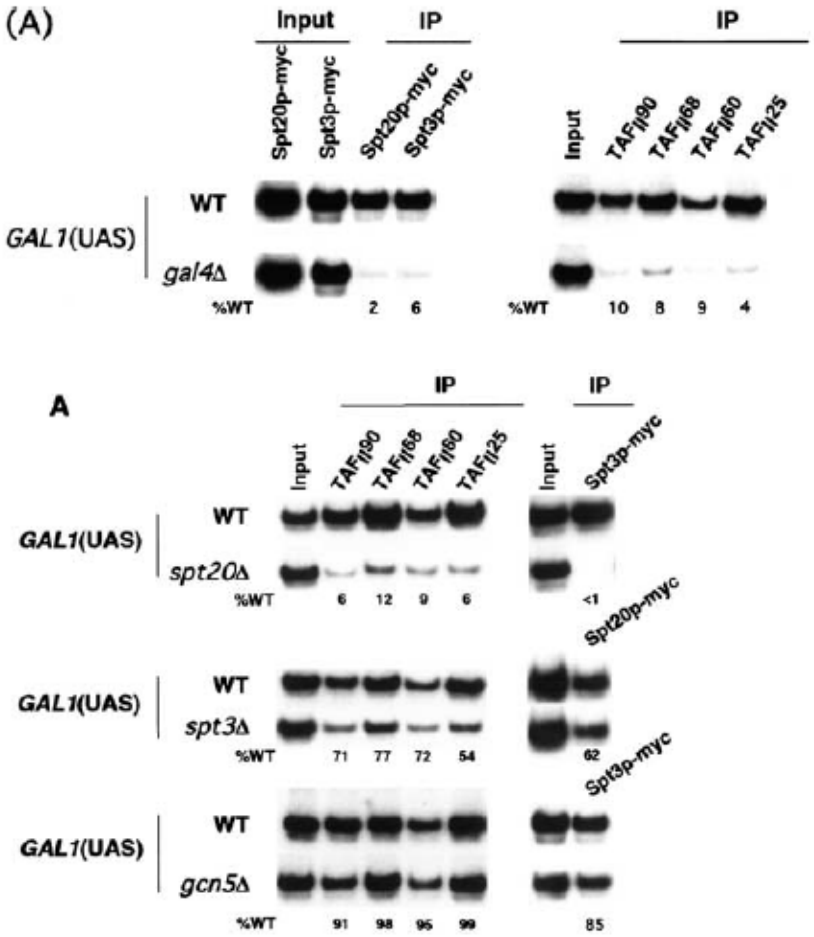


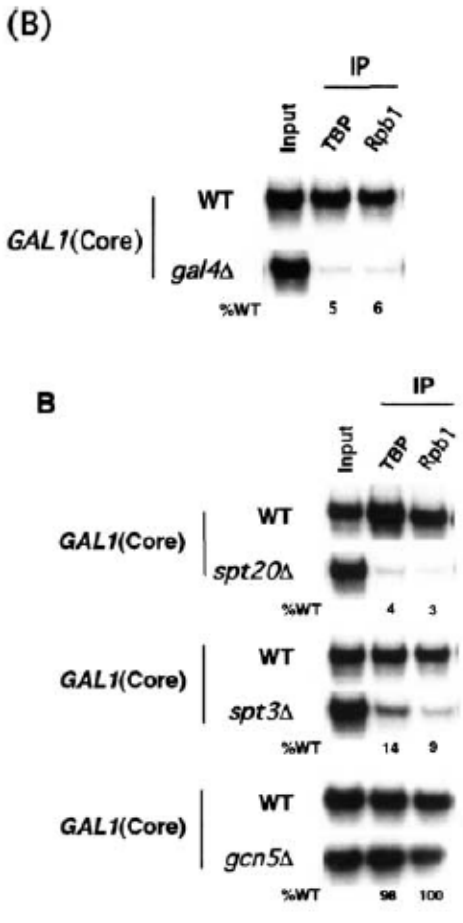
SAGA is Recruited to Gal4p Activated Promoter Regions in Galactose Induction

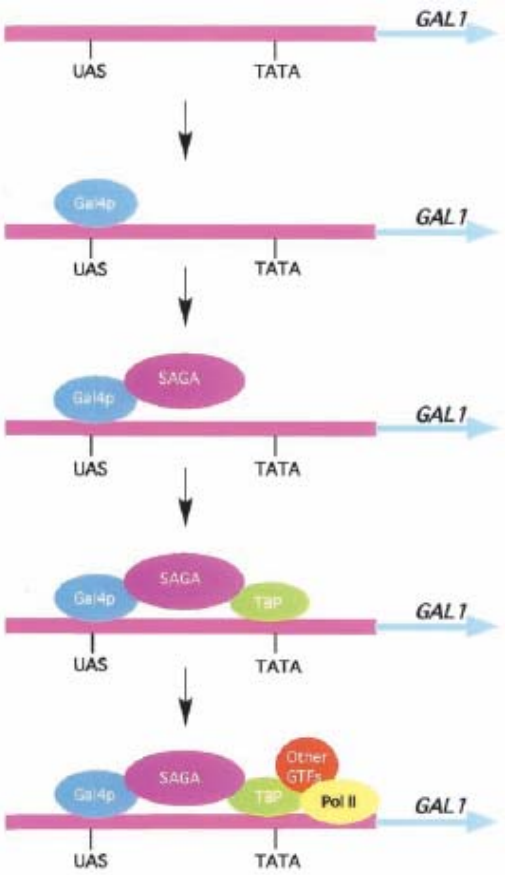


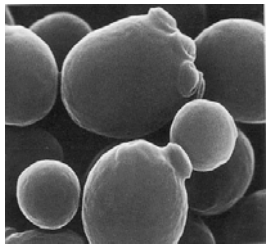


SAGA is Recruited by Gal4p and is Required for Transcriptional Activation

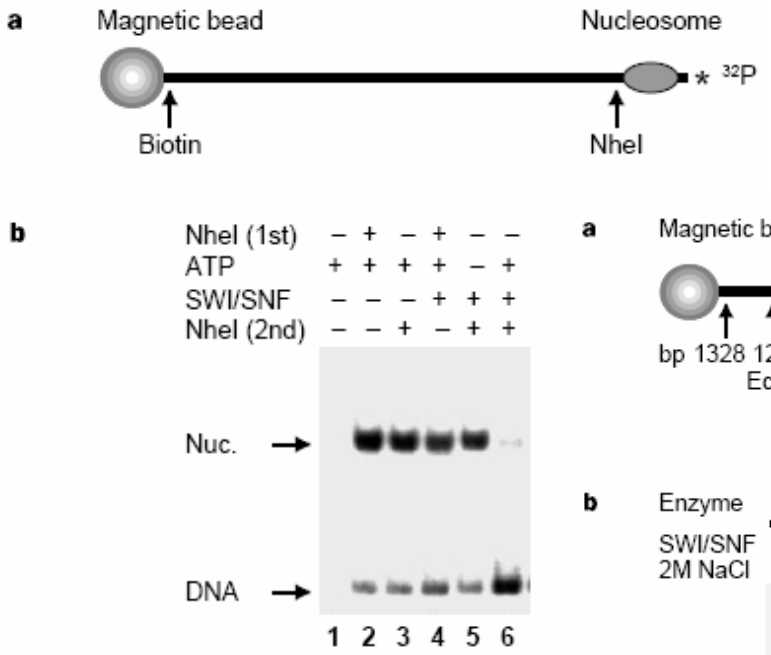


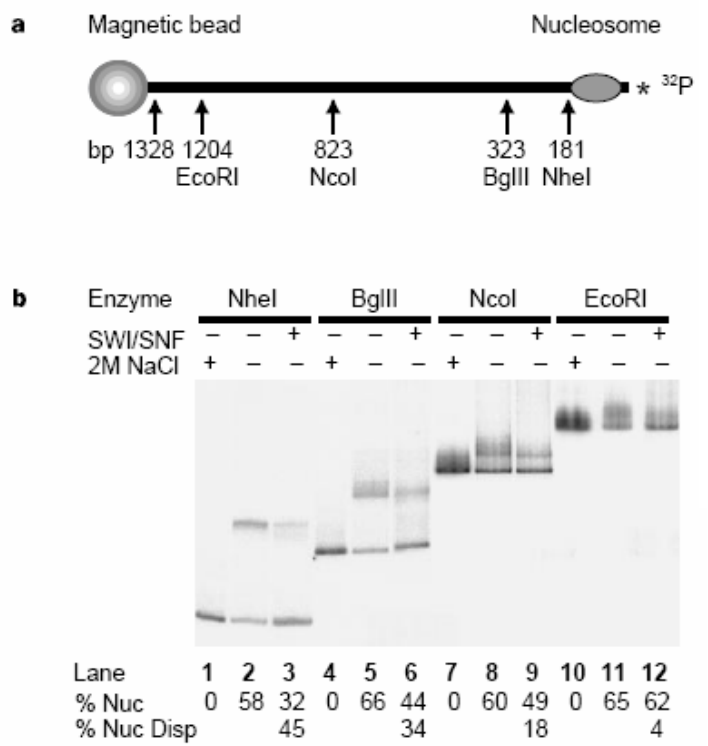


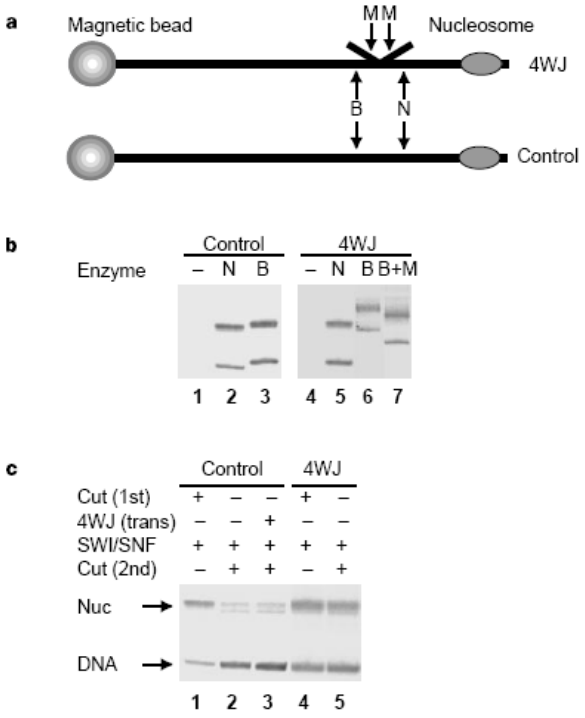


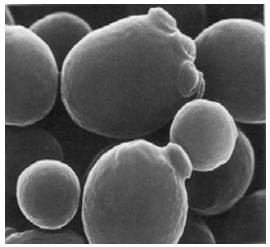


Swi/SNF is Required to Mobilize Nucleosome









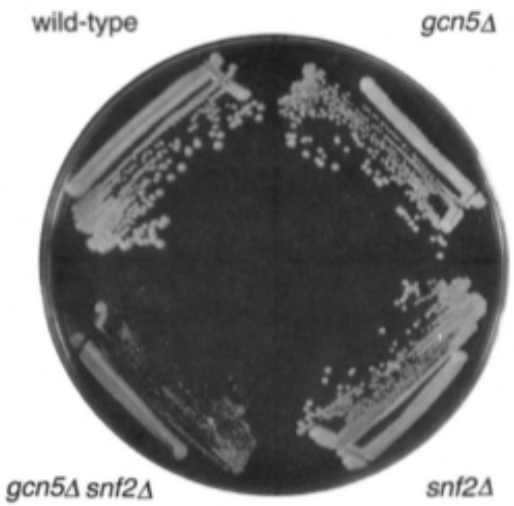
Genetic Interactions between SAGA and Swi/Snf Complexes

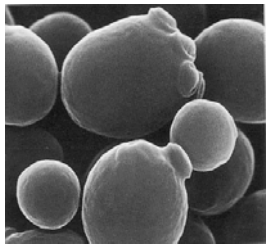
spt20 and _spt7_, but not _spt3_, or _spt8_ mutations are synthetically lethal with _snf/swi_ and _srb/mediator_ mutations

	Double mutant[a]	Phenotype[b]
A.	*spt20Δ snf2Δ*	Dead
	spt20Δ snf5Δ	Dead
	spt20Δ swi1Δ	Dead
	spt20Δ sin4Δ	Dead
	spt20Δ gal11Δ	Dead
	spt20Δ rgr1Δ2	Dead
	spt20Δ srb2Δ	Dead
	spt20Δ srb5Δ	Dead
B.	*spt7Δ snf2Δ*	Dead
	spt7Δ sin4Δ	Dead
	spt7Δ gal11Δ	Dead
C.	*spt3Δ snf2Δ*	Alive
	spt3Δ sin4Δ	Alive
	spt3Δ gal11Δ	Alive
	spt3Δ rgr1Δ2	Alive
	spt3Δ srb2Δ	Alive
D.	*spt8Δ snf2Δ*	Alive
	spt8Δ sin4Δ	Alive
	spt8Δ gal11Δ	Alive

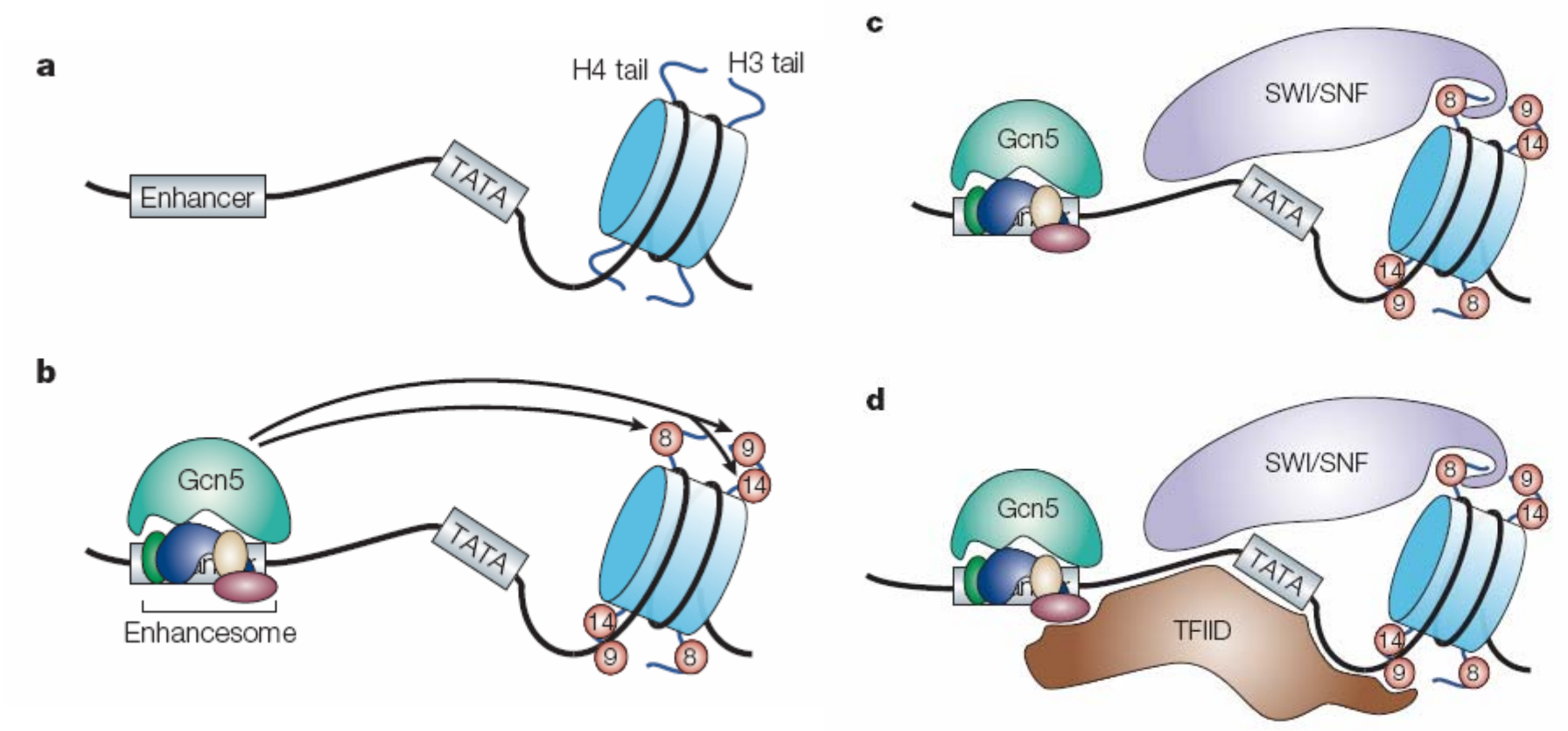
Genetic interactions between _gcn5Δ_, _srb_/mediator, _snf/swi_ and _spt_ mutants

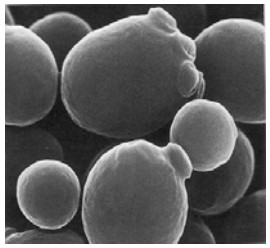
Double mutant[a]	Phenotype[b]
gcn5Δ sin4Δ	Alive
gcn5Δ srb2Δ	Alive
gcn5Δ snf5Δ	Alive, sick
gcn5Δ swi1Δ	Alive, sick
gcn5Δ snf2Δ	Alive, sick
gcn5Δ spt3Δ	Alive
gcn5Δ spt7Δ	Alive
gcn5Δ spt20Δ	Alive



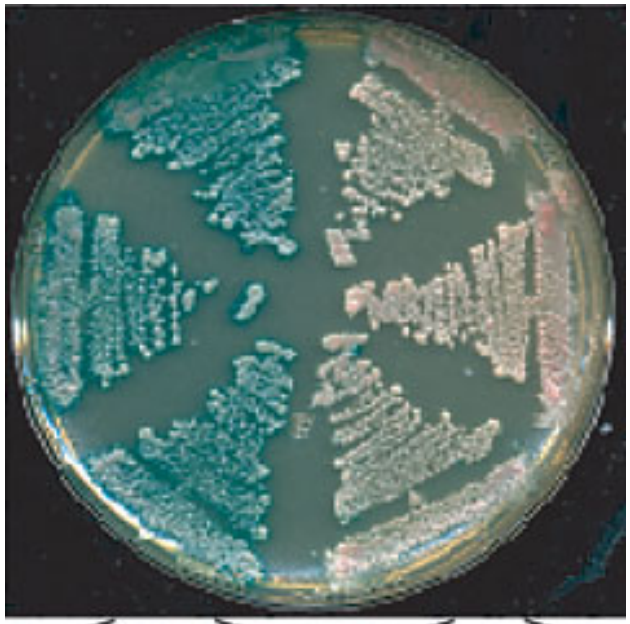


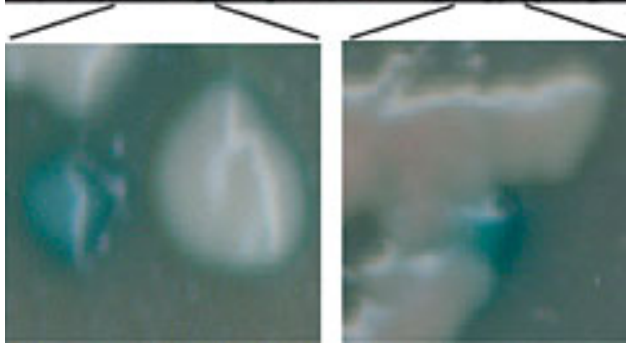
SAGA Functions Together with Swi/SNF Complex to Mobilize Acetylated Nucleosome

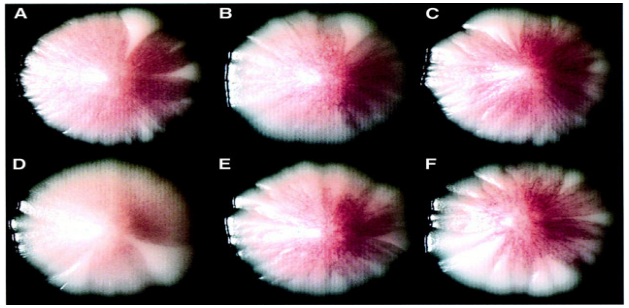


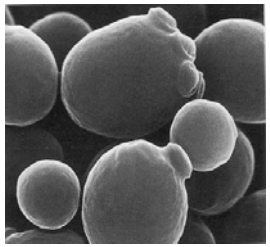


Phenotypic Variegation at the Level of Transcriptional Efficacy

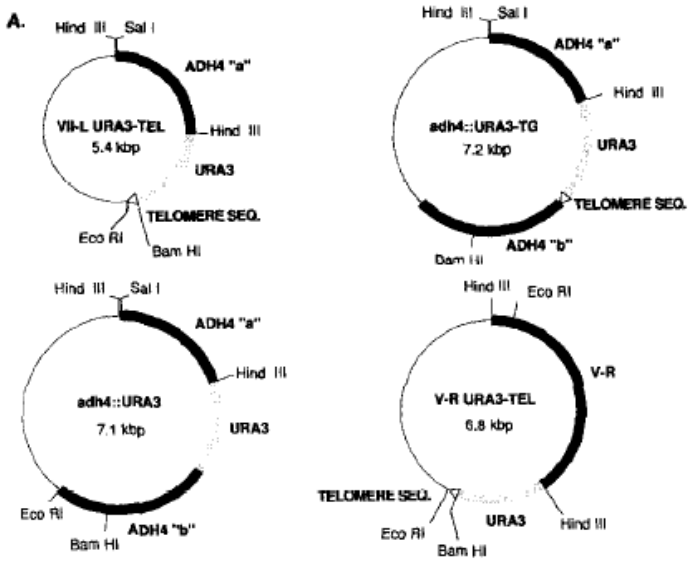


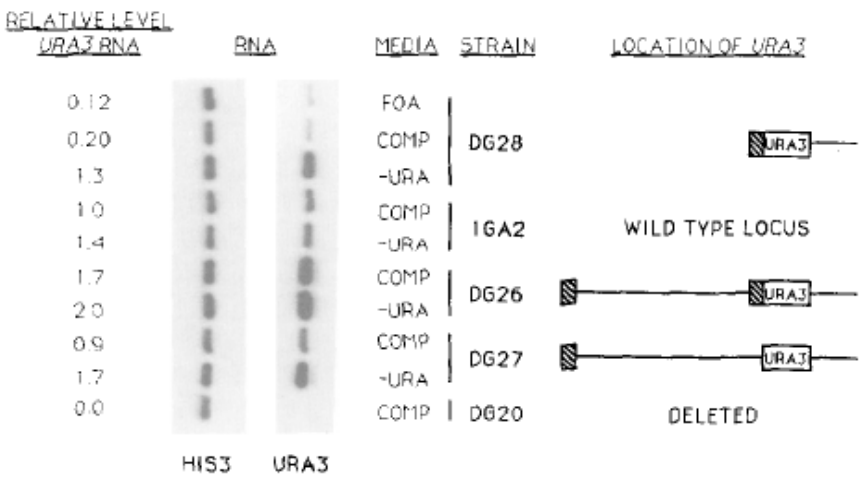


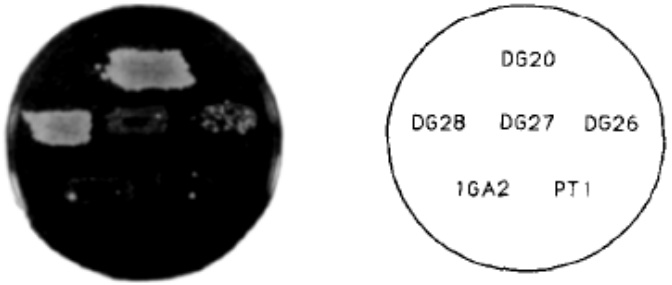




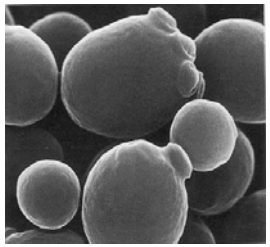
Telomeric Effect of Silencing



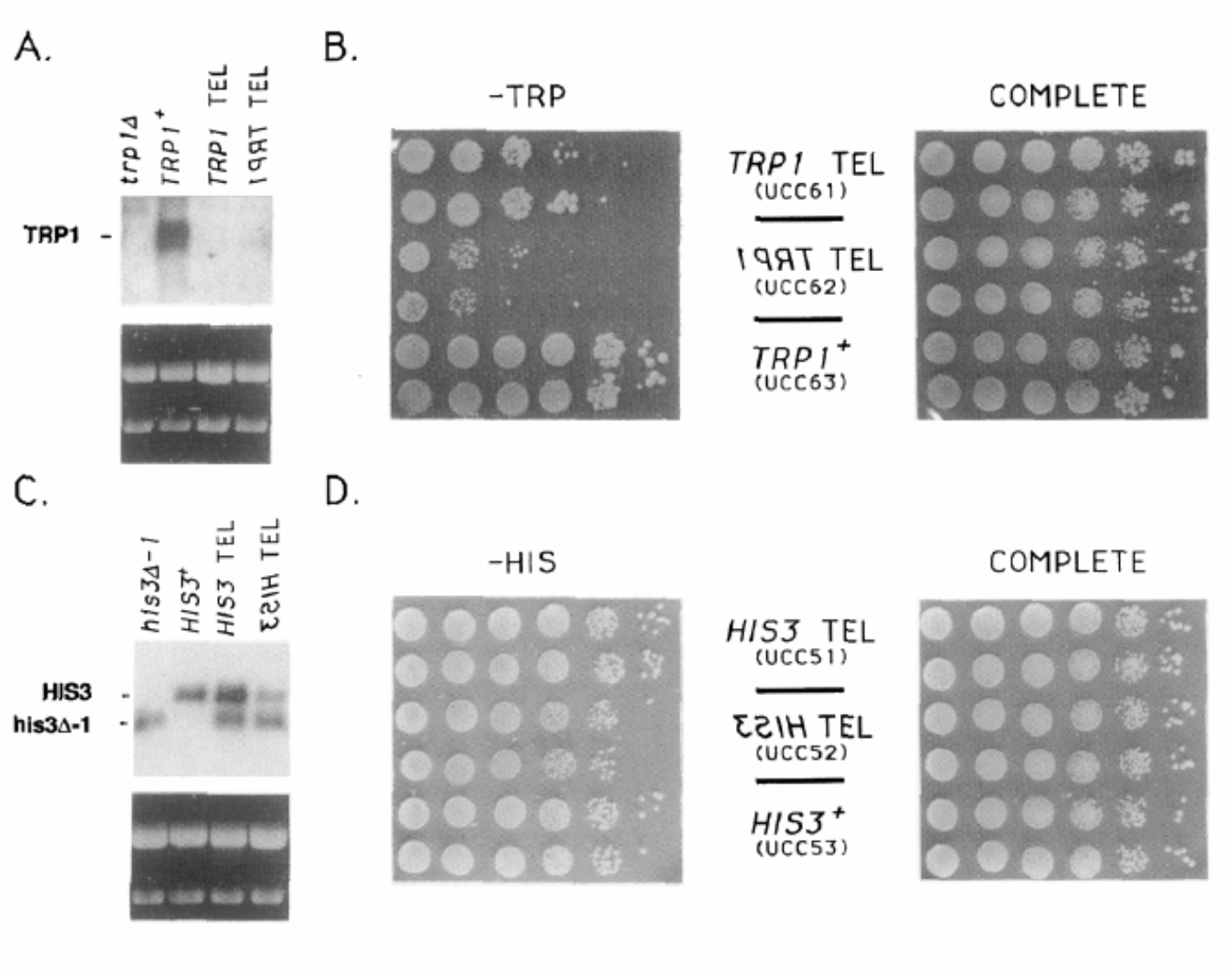


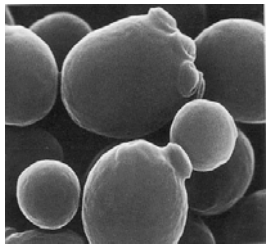


Gottschling et al 1990

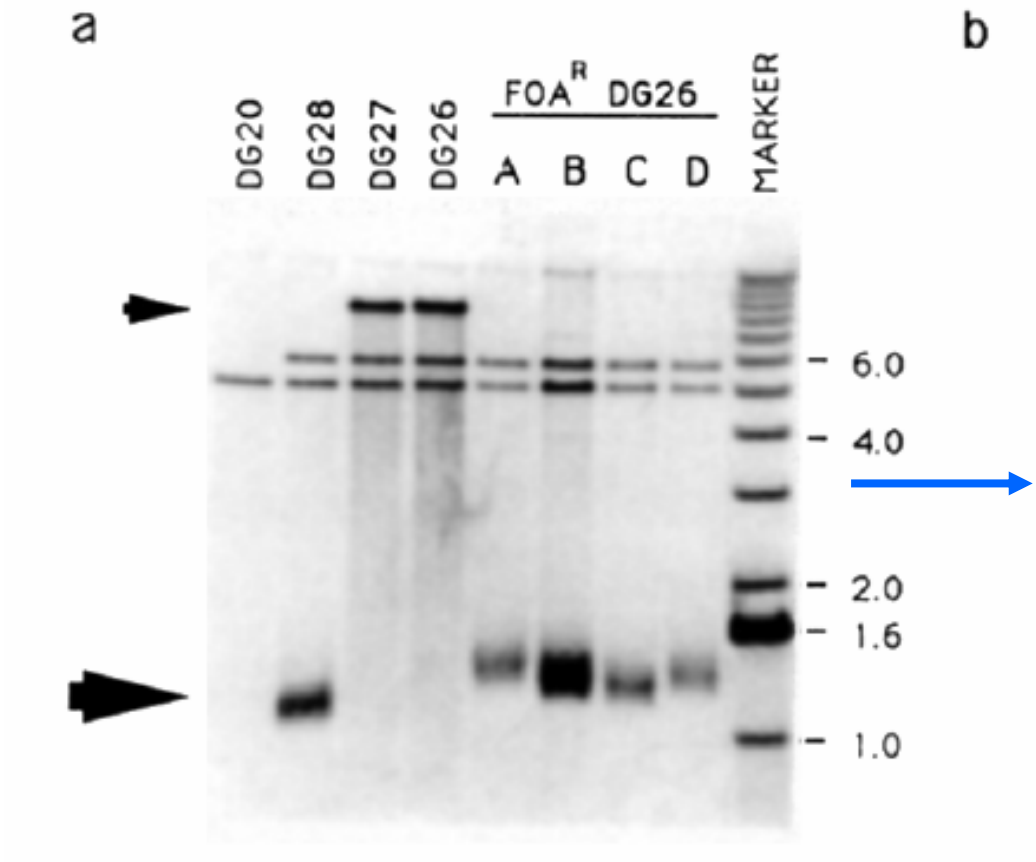


Telomeric Effect of Silencing



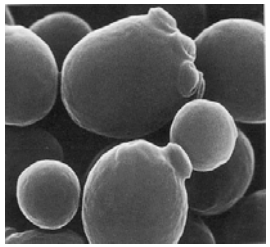


Telomeric Effect of Silencing

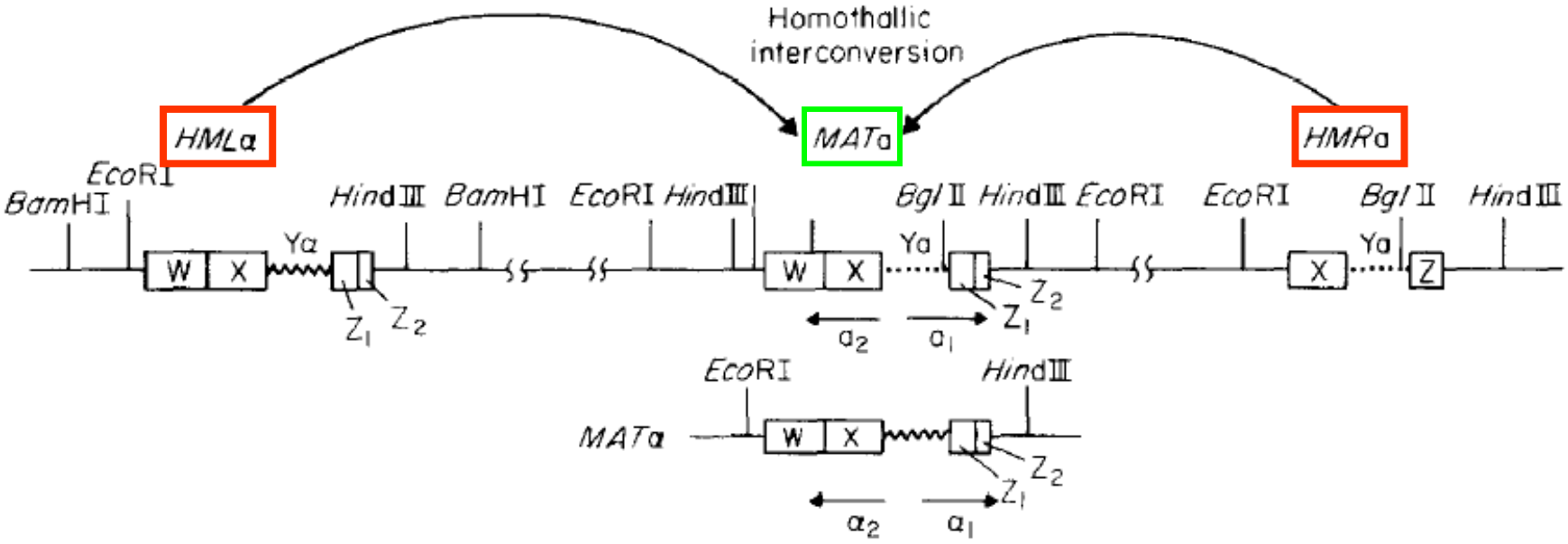


Southern
PstI cut genomic, URA3 probe

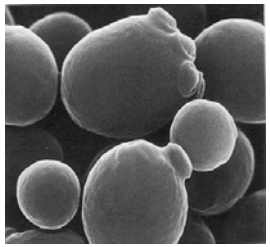
Northern



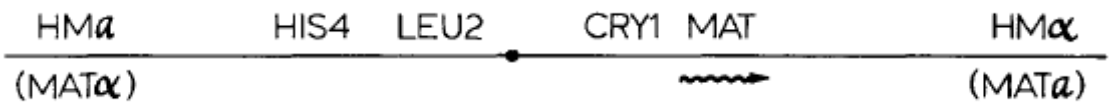
Mating Type Gene Loci



Feldman et al 1984



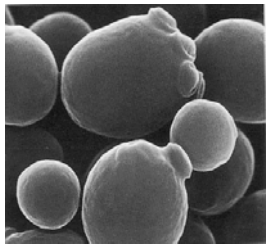
Mating Type Suppression by SIR



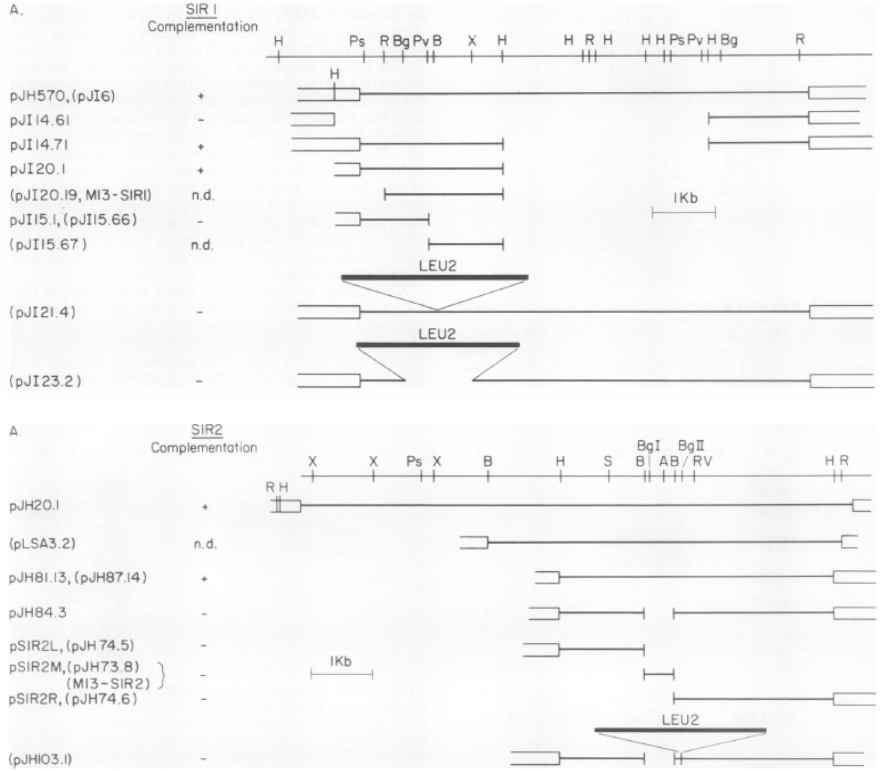
Suppression of the mating defect of matα1–5, matα2–4, *and* matα2–1 *by* sir1–1

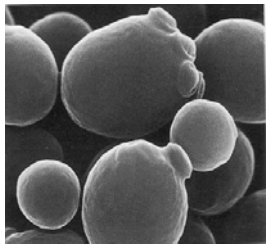
Diploid	Genotype	PD	Tetrad Types* NPD	T
XR202	*CRY1 matα1–2 / cry1–3 MAT***a** ; *SIR1 / sir1–1*	13	14	51
XS8E	*CRY1 matα1–2 / cry1–3 matα1–5* ; *SIR1 / sir1–1* ; *rme / csp1*	1	2	3
XR128E	*CRY1 matα2–4 / cry1–3 MAT***a** ; *SIR1 / sir1–1*	13	21	52
XS5F	*CRY1 matα2–4 / cry1–3 matα1–5* ; *SIR1 / sir1–1* ; *rme / csp1*	2	2	4
XJ111	*CRY1 matα2–4 / cry1–3 matα1–5* ; *SIR1 / sir1–1* ; *rme / csp1*	1	5	8
XJ104	*CRY1 matα2–1 / cry1–3 matα1–5* ; *SIR1 / sir1–1* ; *rme / csp1*	1	3	5
XJ110	*CRY1 matα2–1 / cry1–3 matα1–5* ; *SIR1 / sir1–1* ; *rme / csp1*	4	1	10

* For XR202 and XR128E, PD = 2 nm : 2 **a**; NPD = 2 α : 2 **a**; T = 1 α : 1 nm : 2 **a**. For other crosses, PD = 2 CryR α : 2 CryS nm; NPD = 2 CryR nm : 2 CryS α; T = 1 CryR α : 1 CryS α : 1 CryR nm : 1 CryS nm.

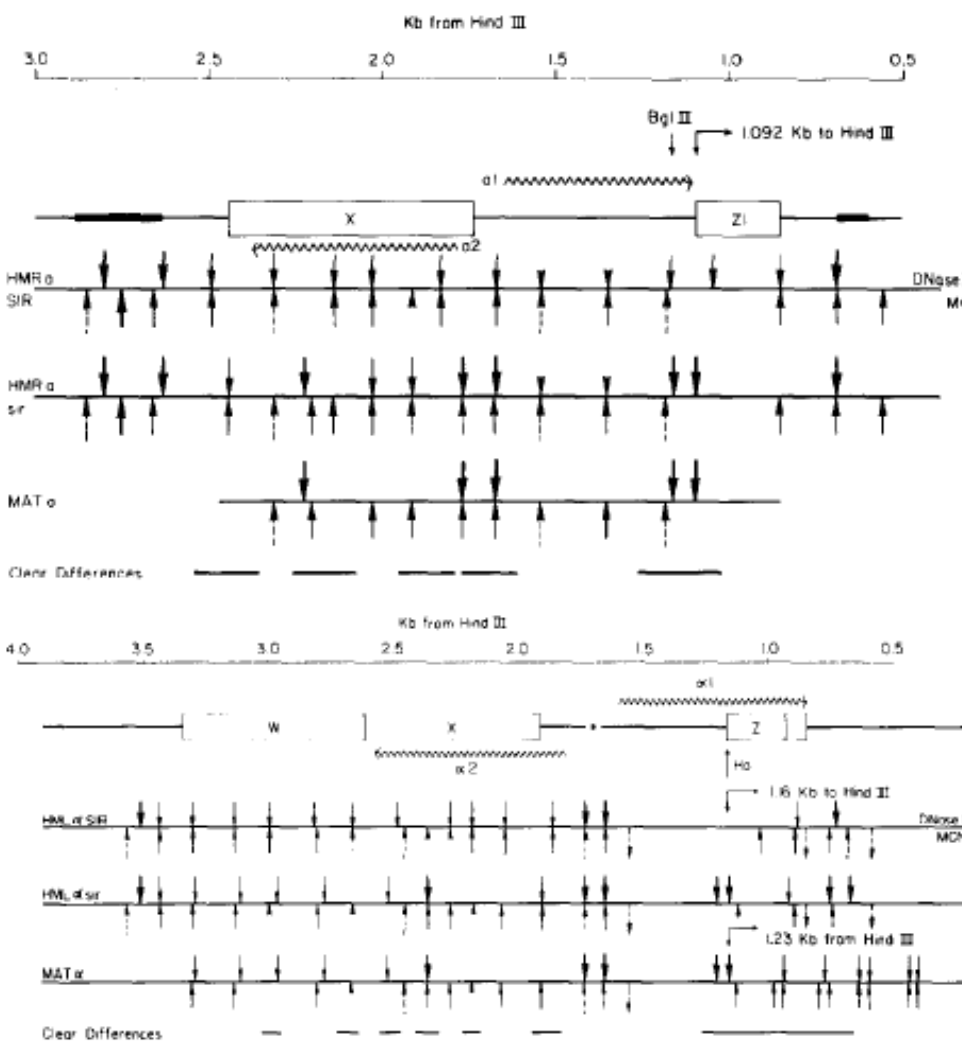


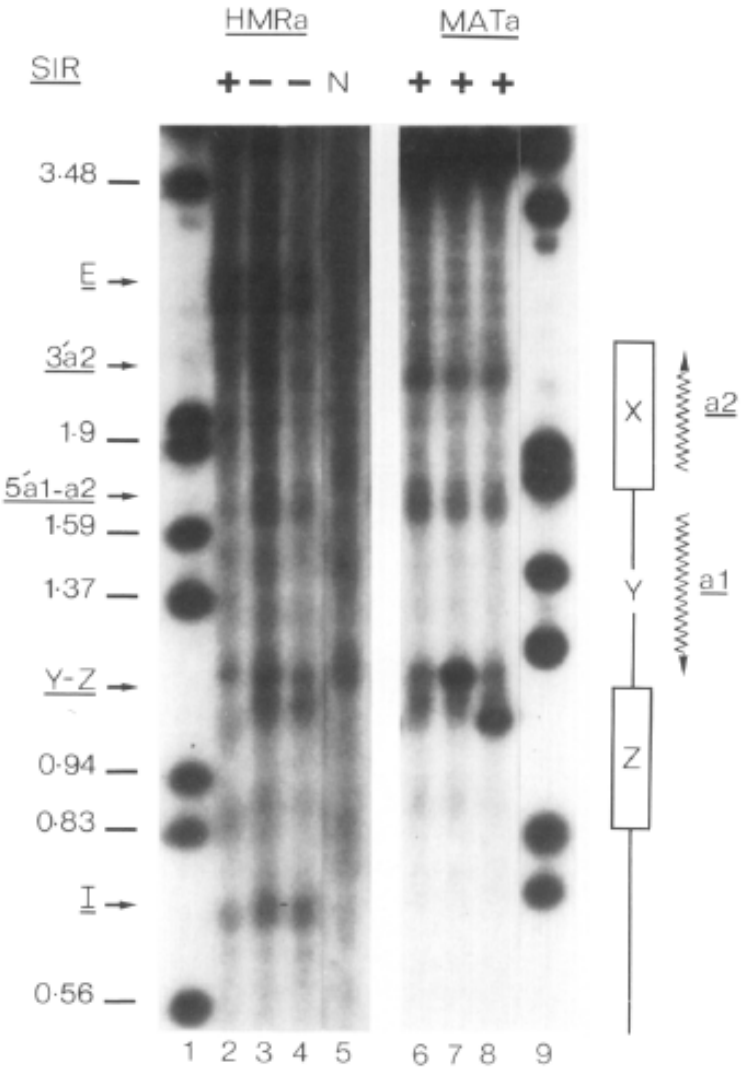
Cloning of SIR1-4

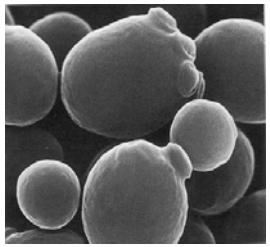




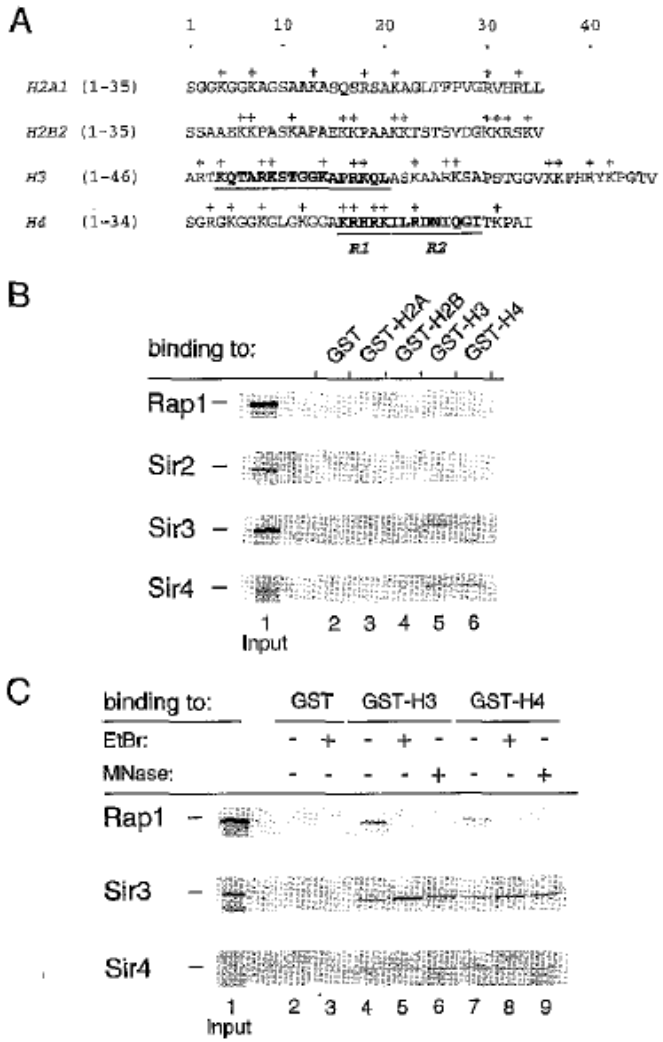
SIR Regulates Heterochromatin Structure at HML and MAT Loci

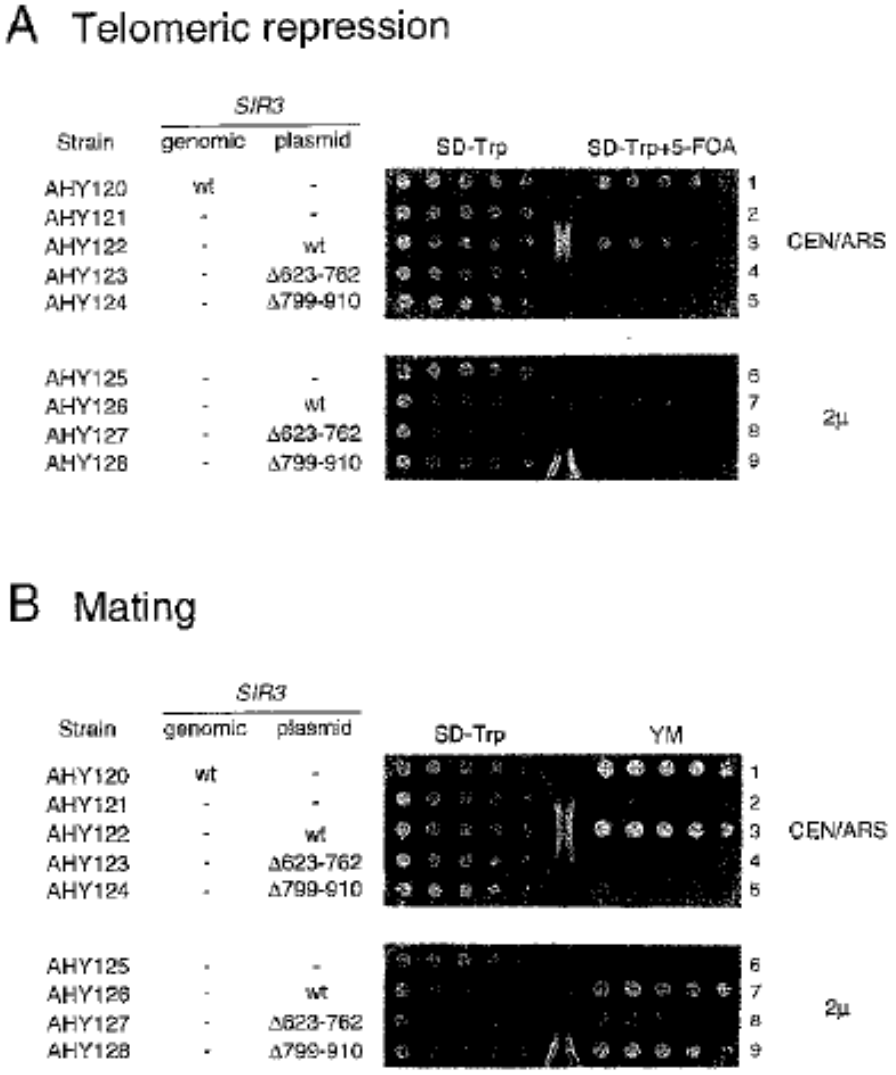


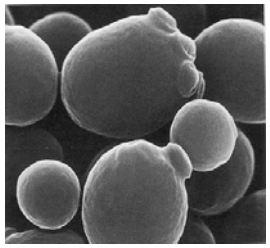




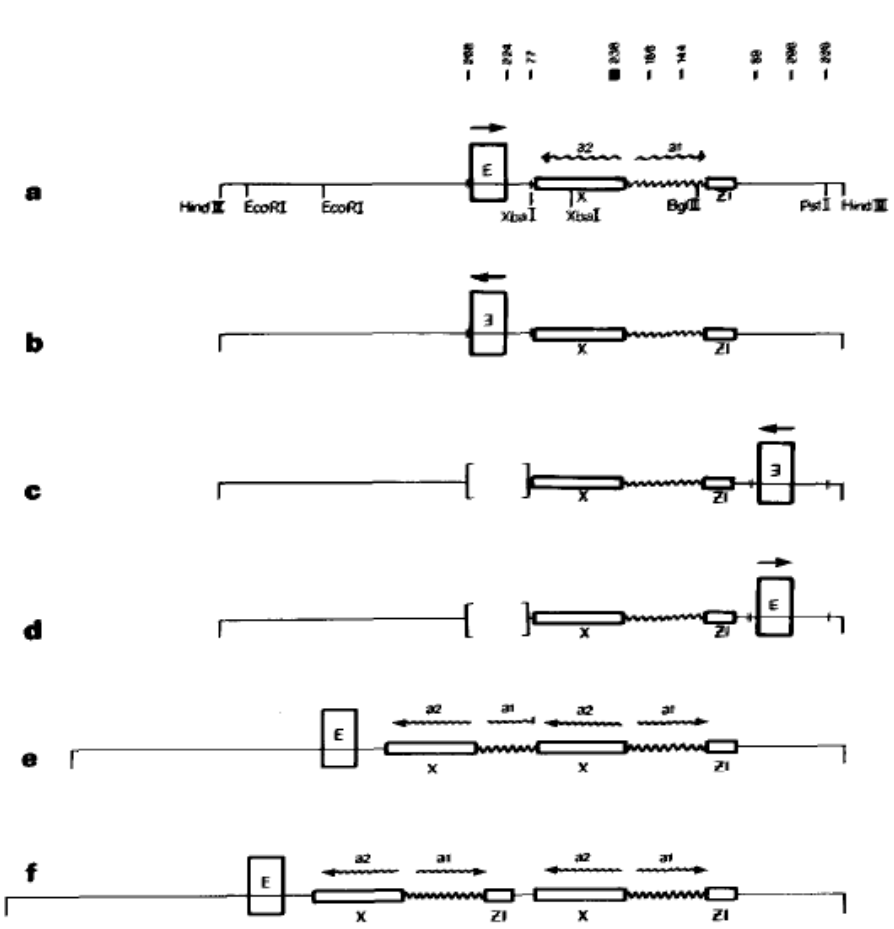
SIR3/4 Binds to H3/4 N-termini

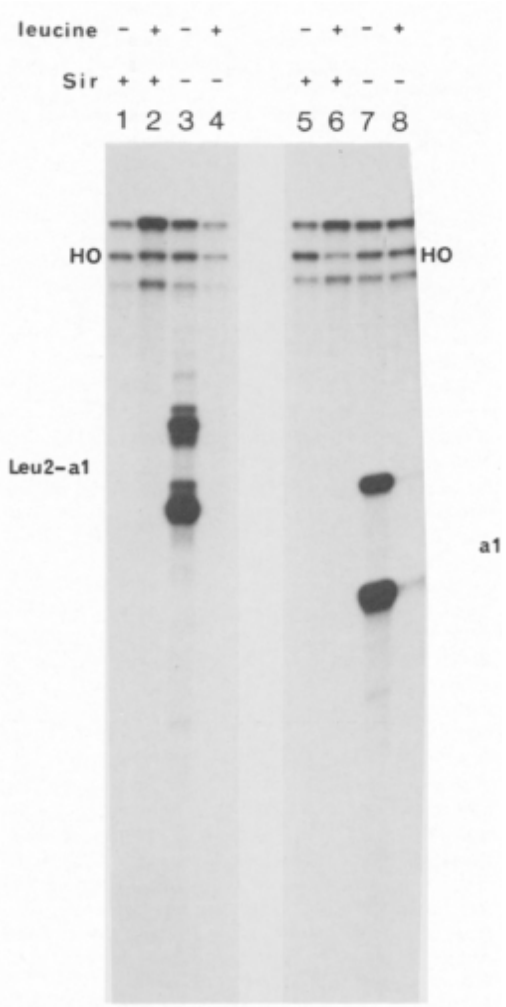


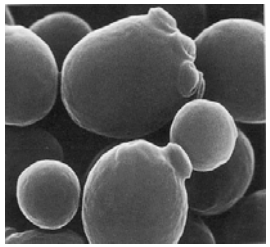




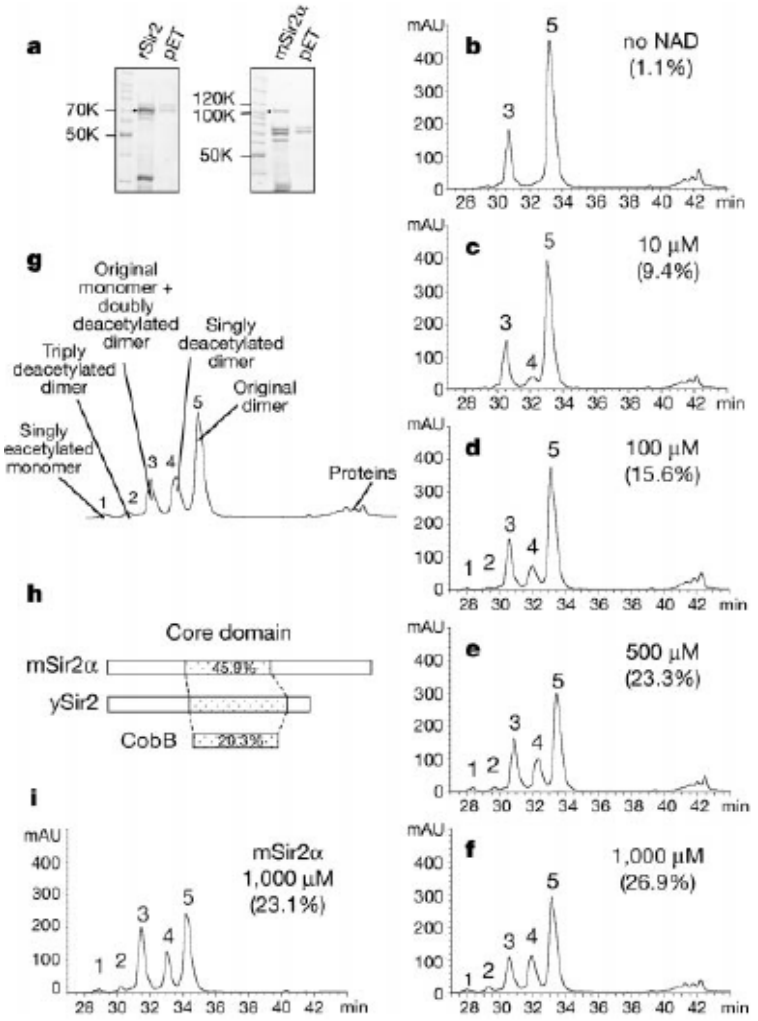
Chromatin Suppressor Binding Site for SIR Complexes

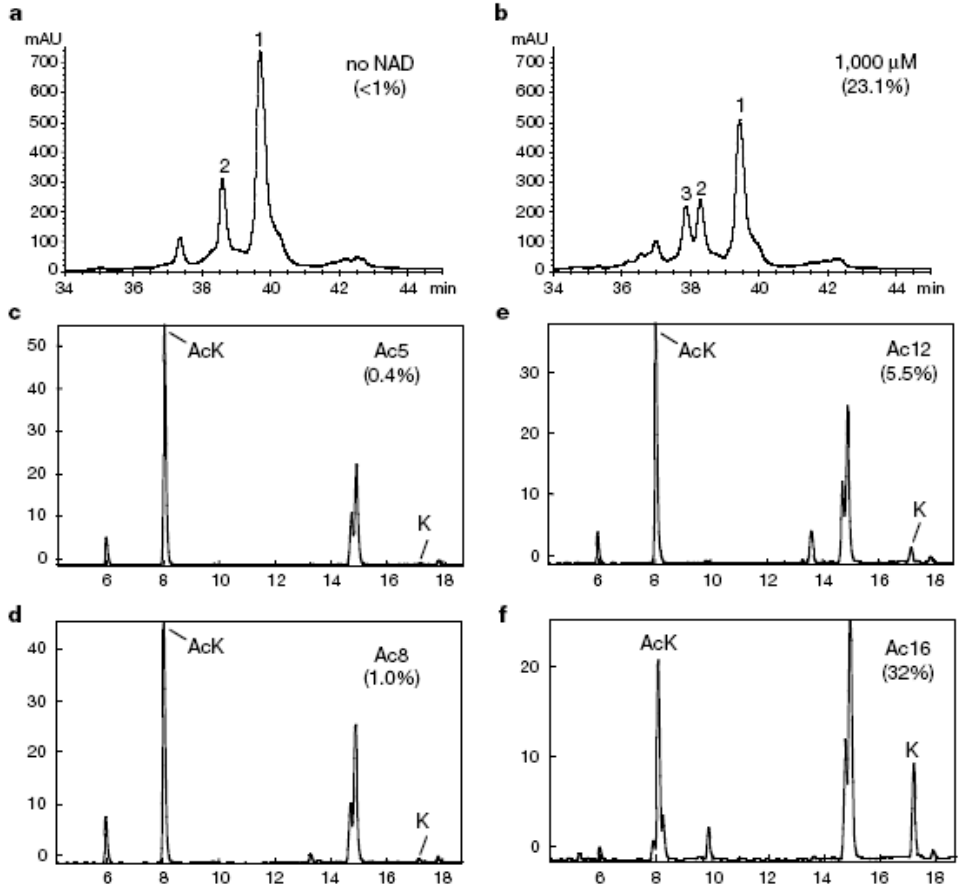




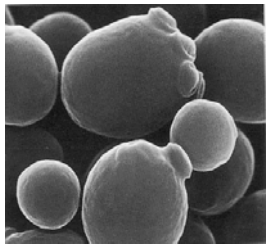


SIR2 Proteins are HDACs

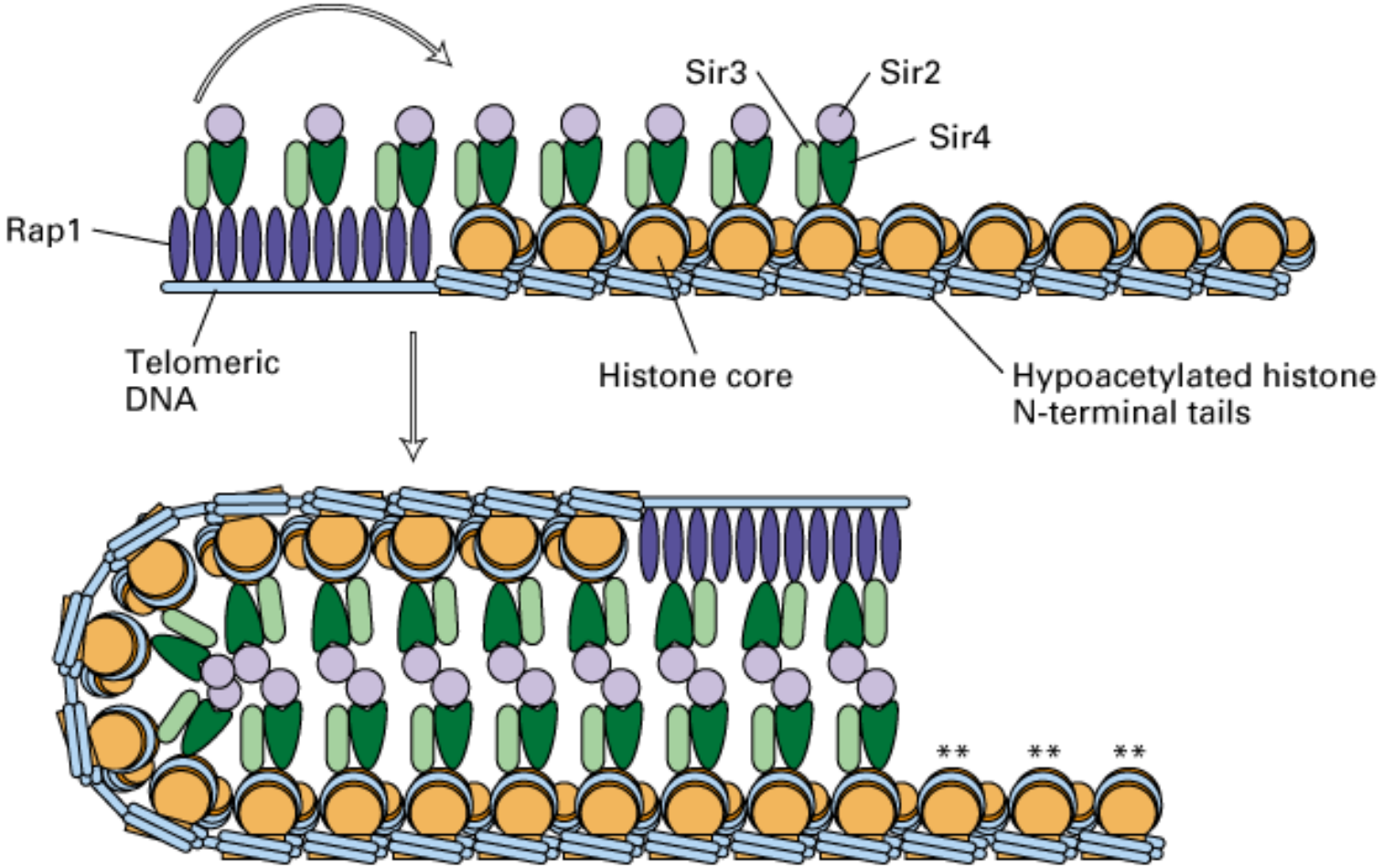


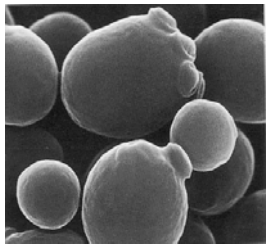


ySir2

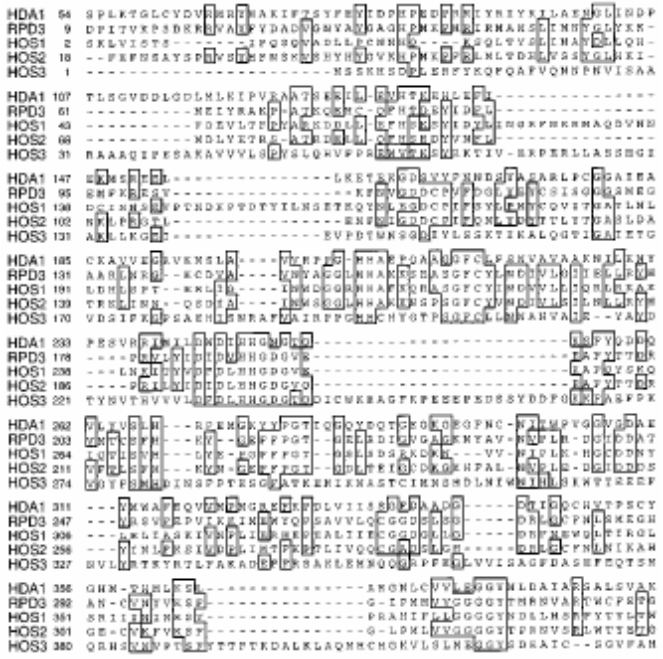


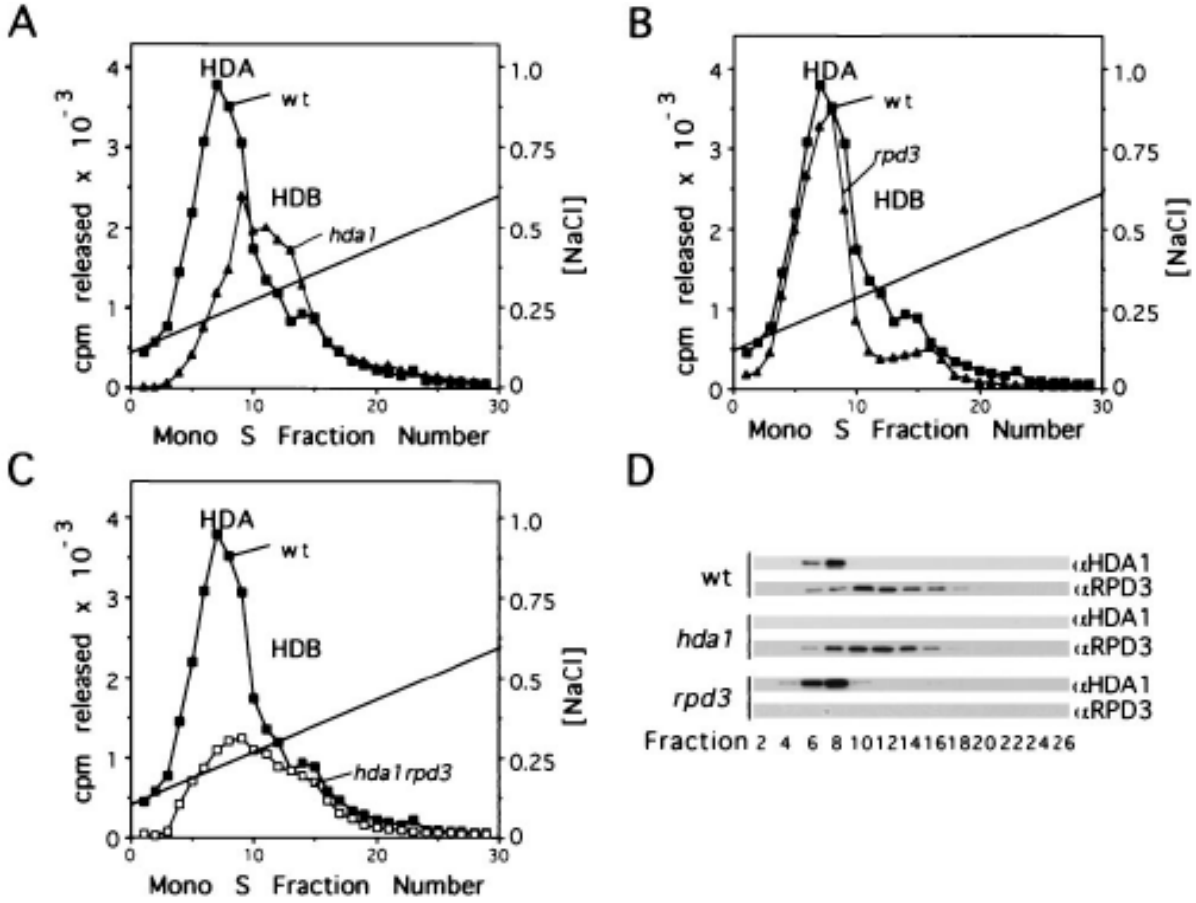
SIR2/3/4 Complex Formation During Heterochromatin Assembly

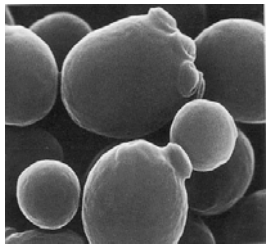




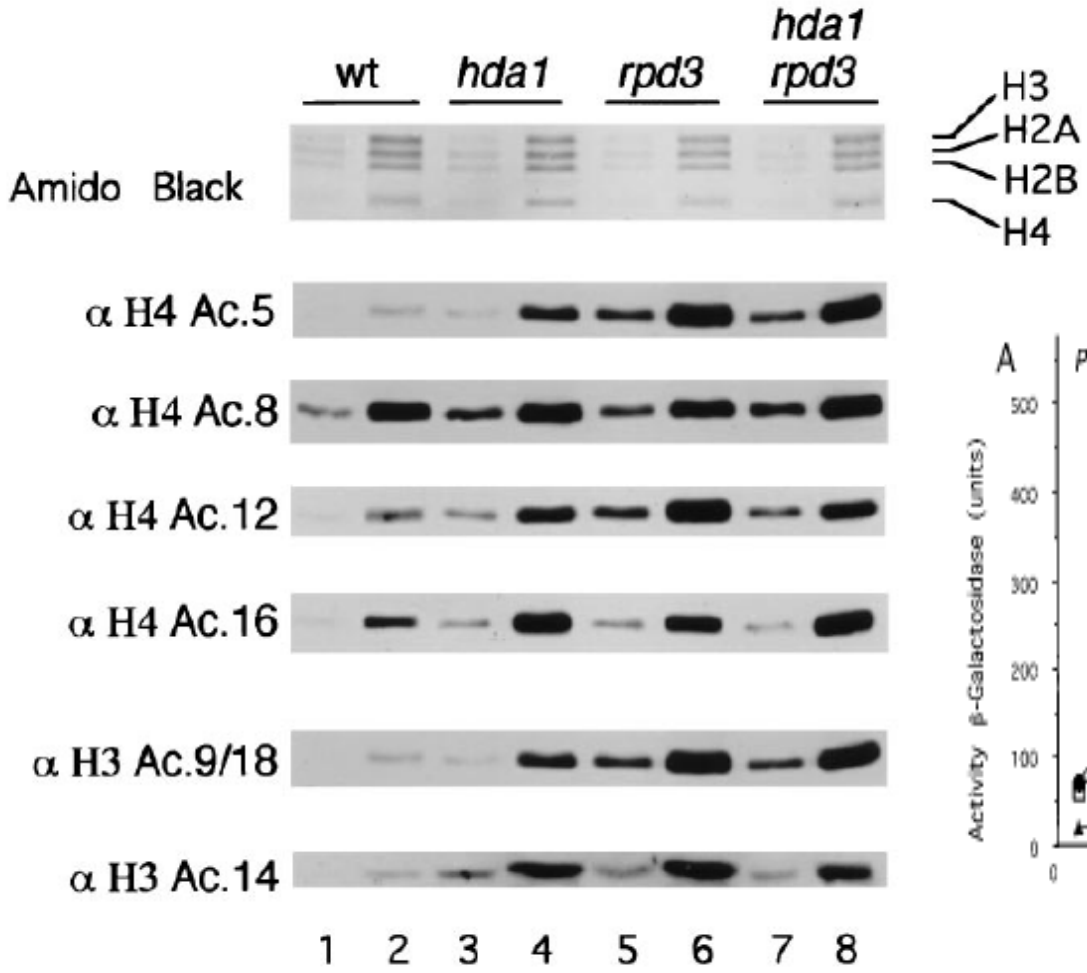
Biochemistry Assays Identified Rpd3/Hos2/Hda1 in different HDAC Complexes

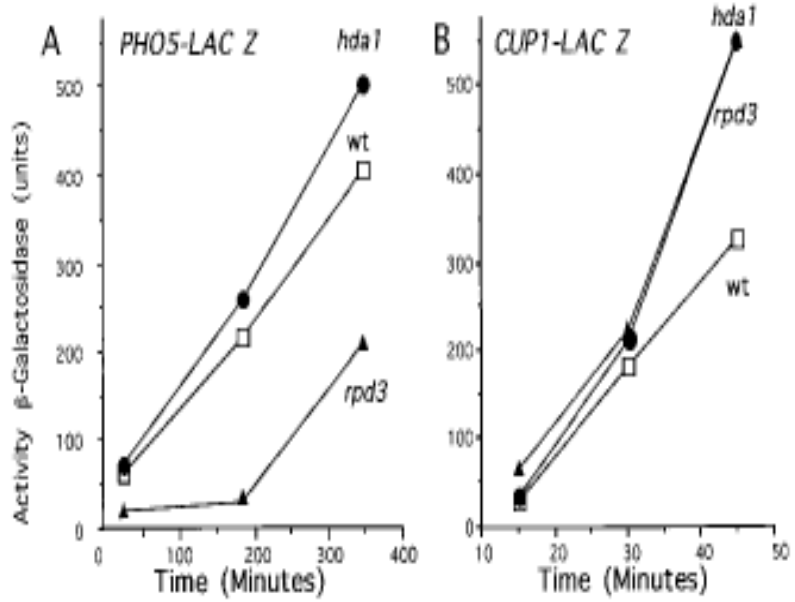


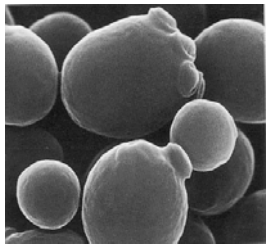




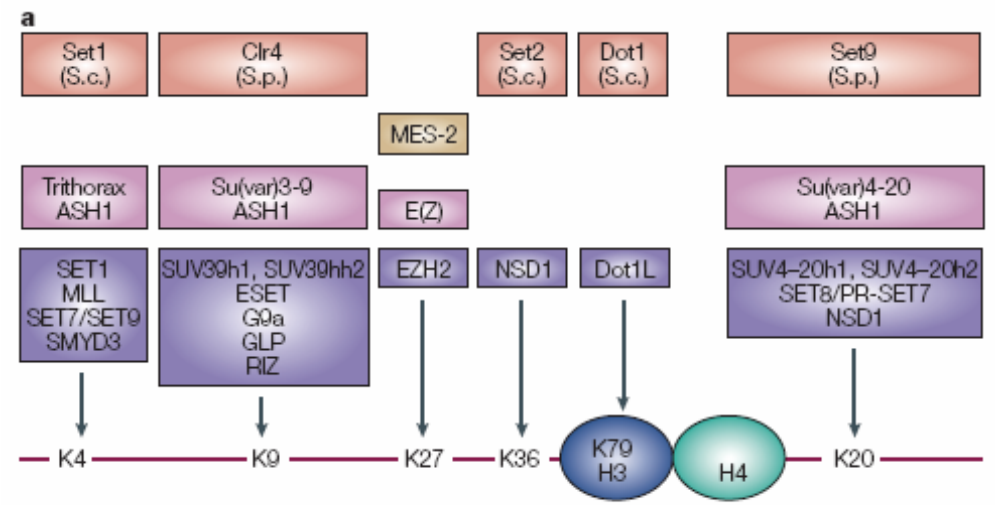
Differential Activity of HDACs

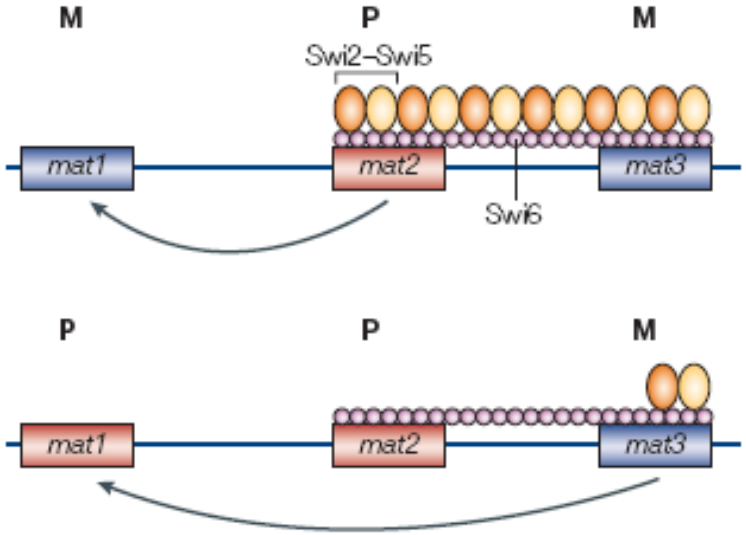


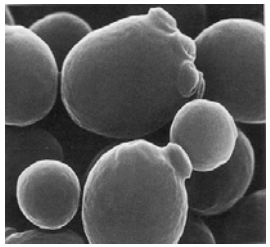




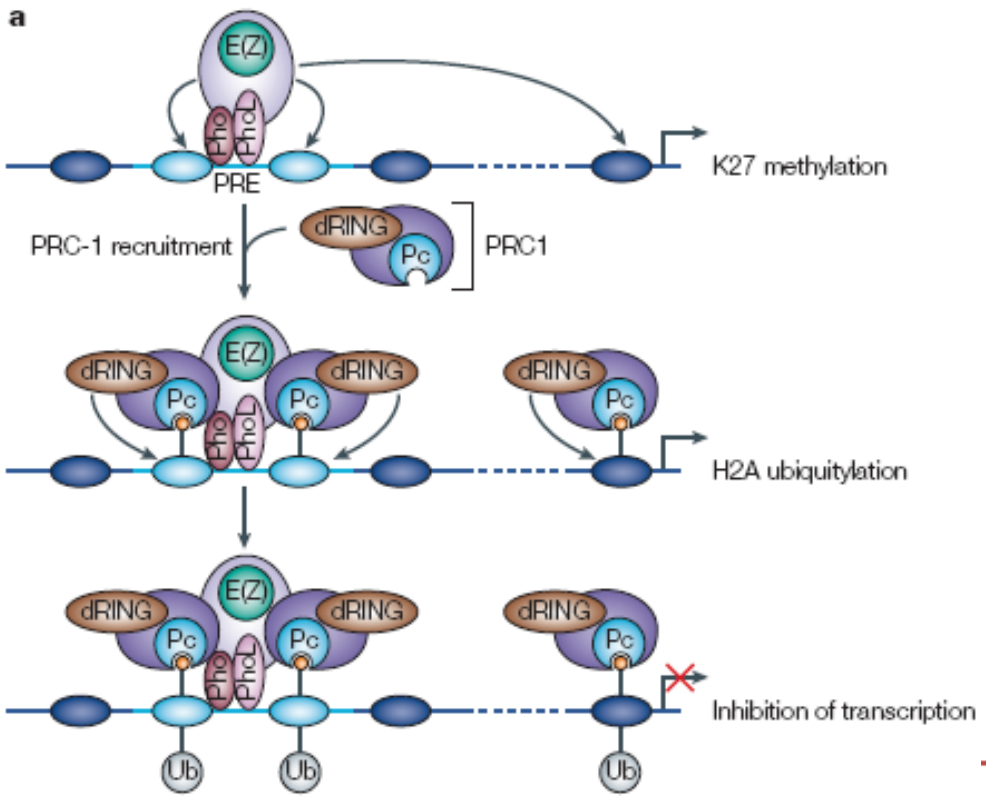
Histone Methylation in Mating Type Switch

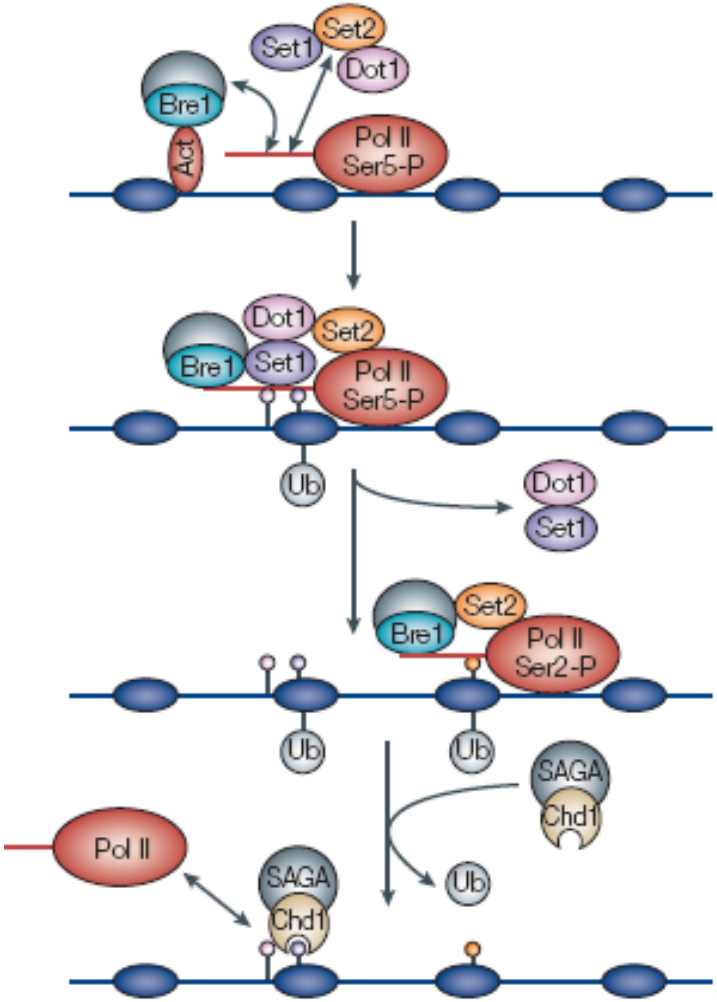


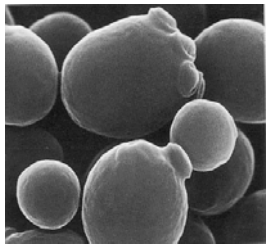




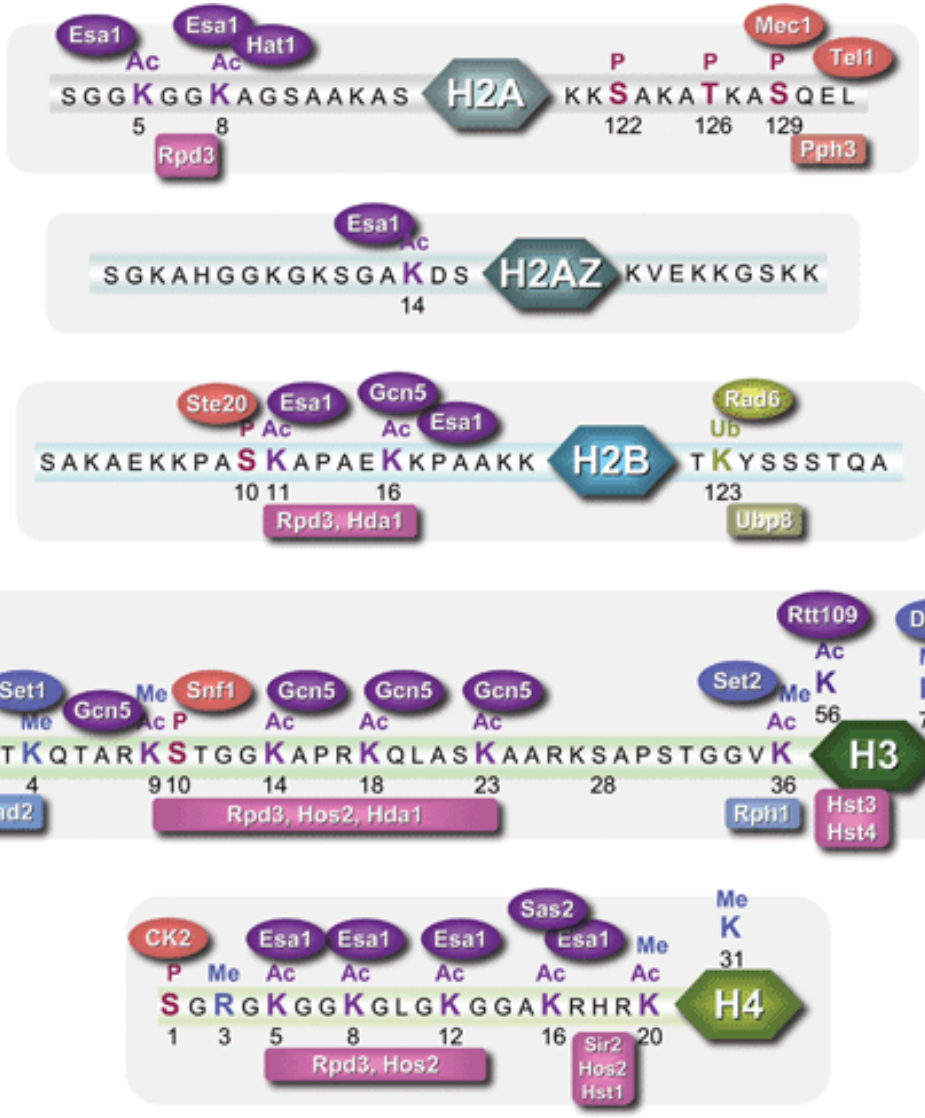
Histone Methylation in Transcription

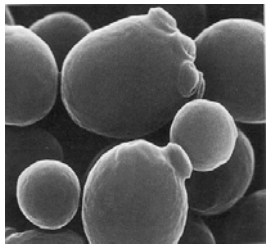




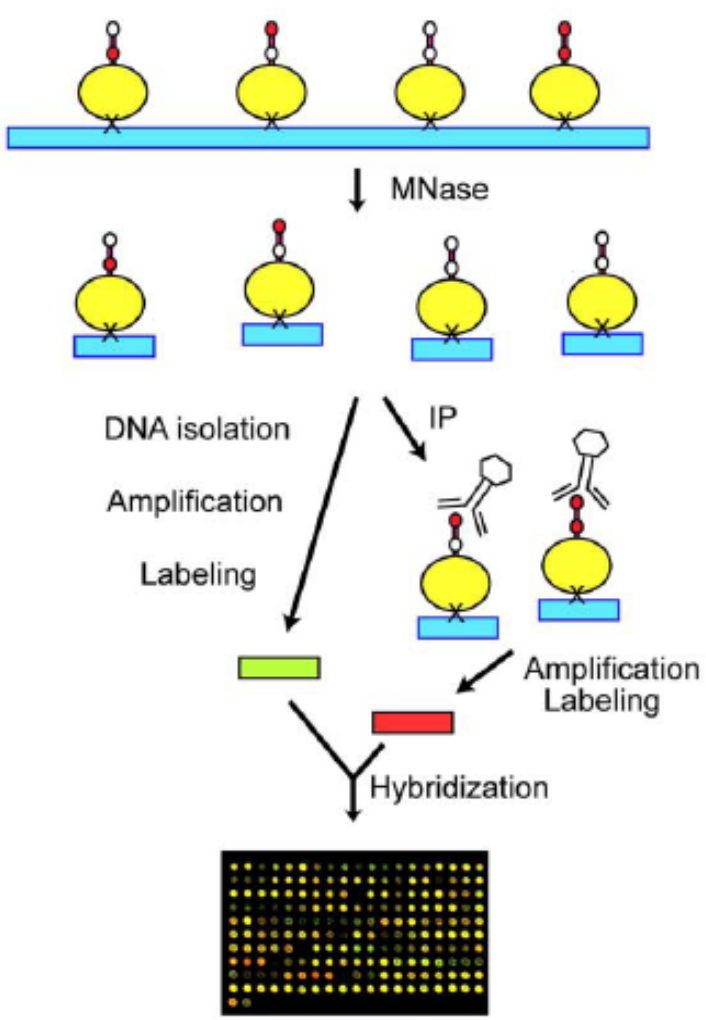


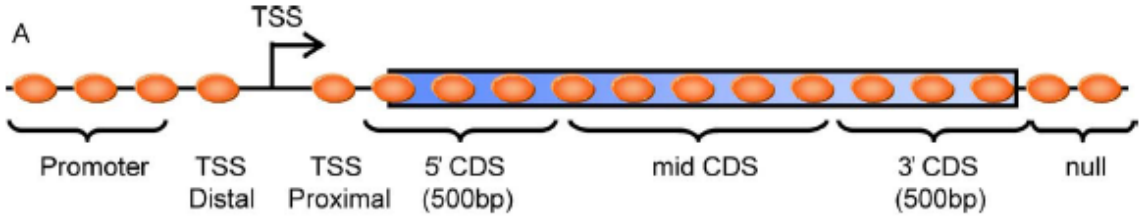
The Histone Code

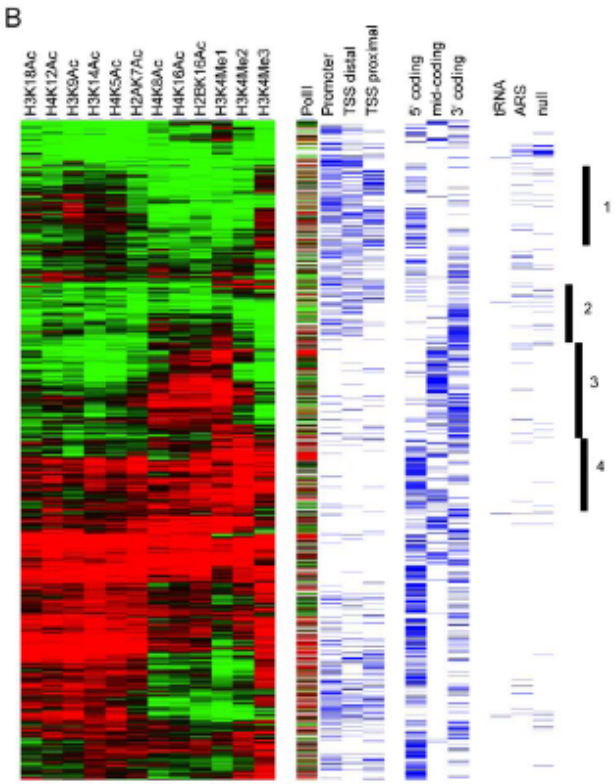


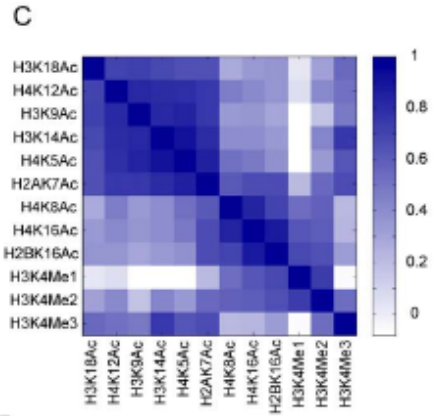


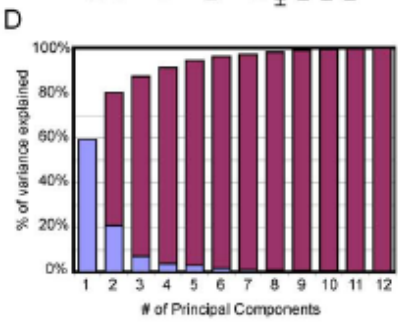
Genomic Histone Modification Distribution

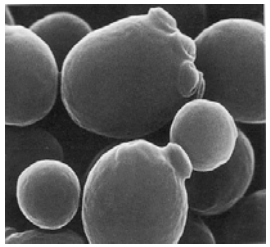




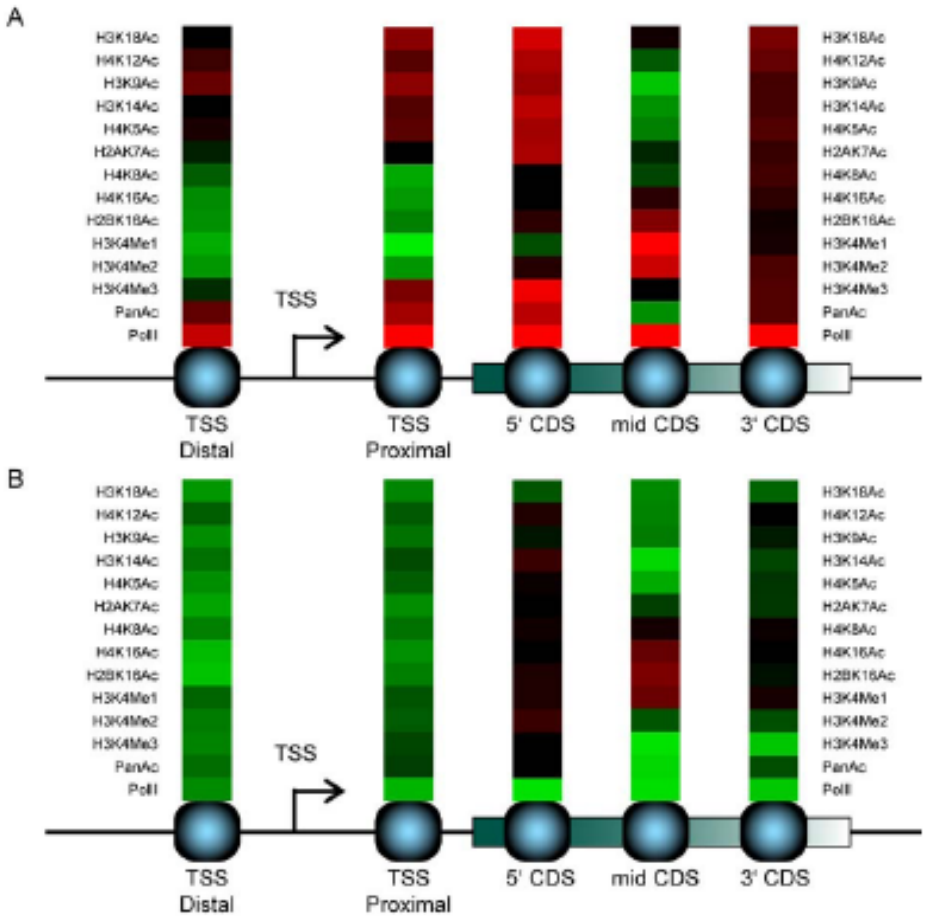


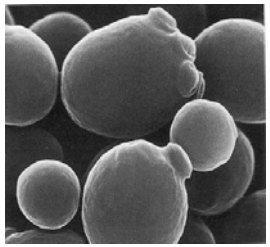




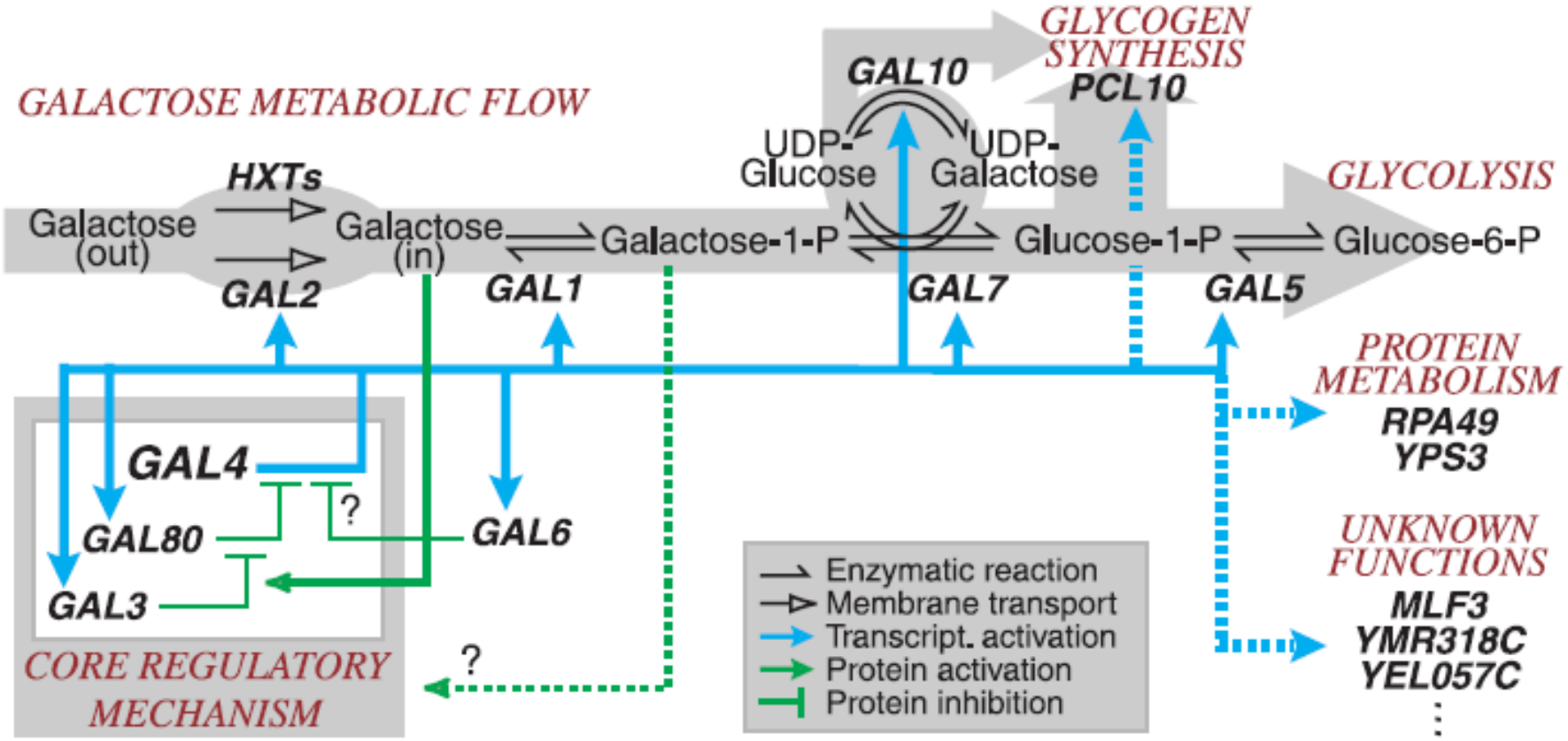


RNA Polymerase Type-dependent Histone Modification Pattern

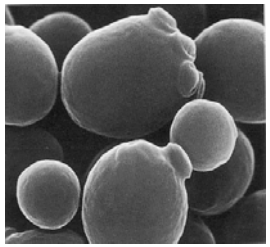




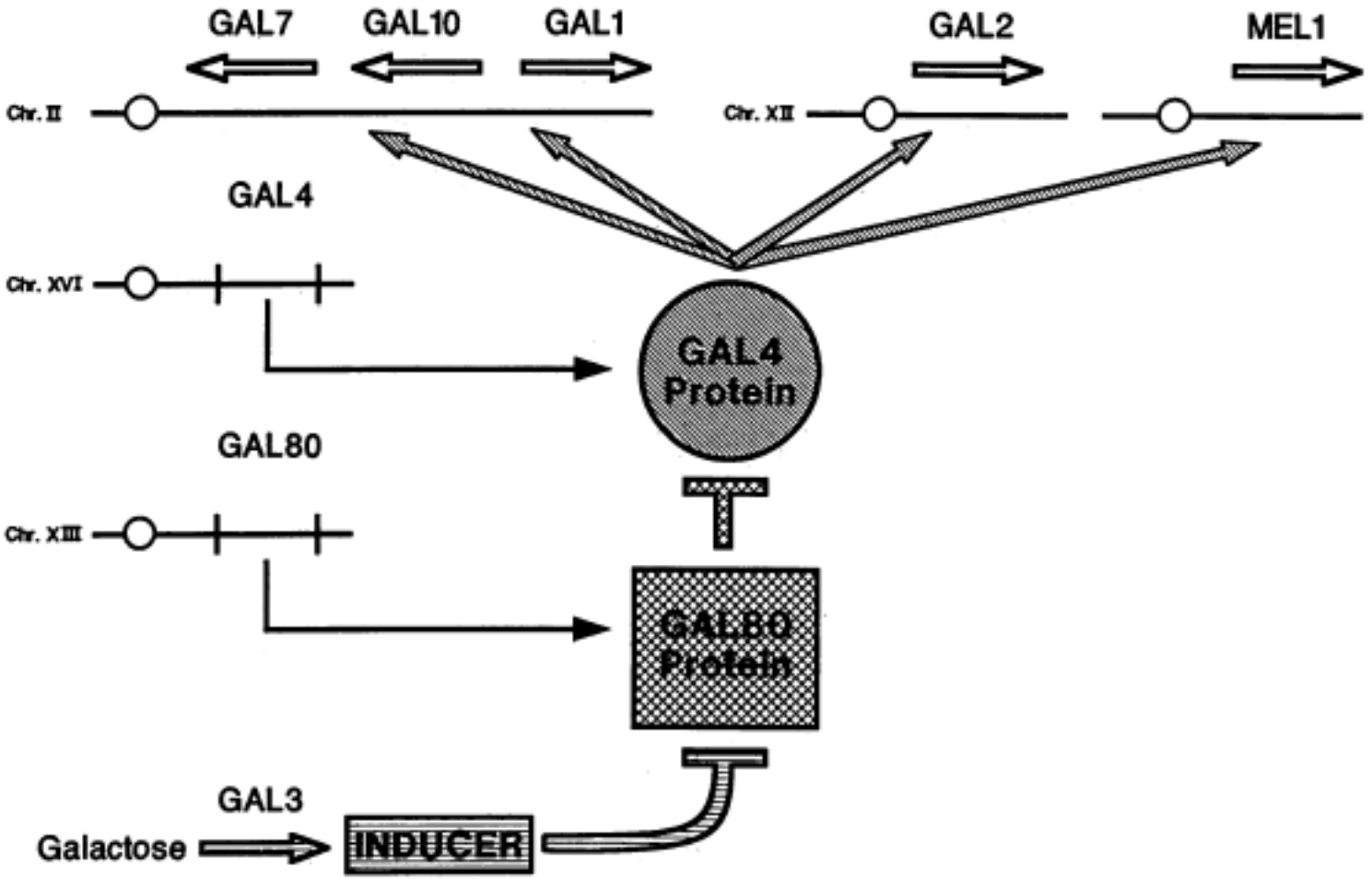
Galactose Response Circuit

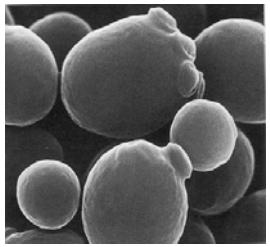


Ideker et al 2001

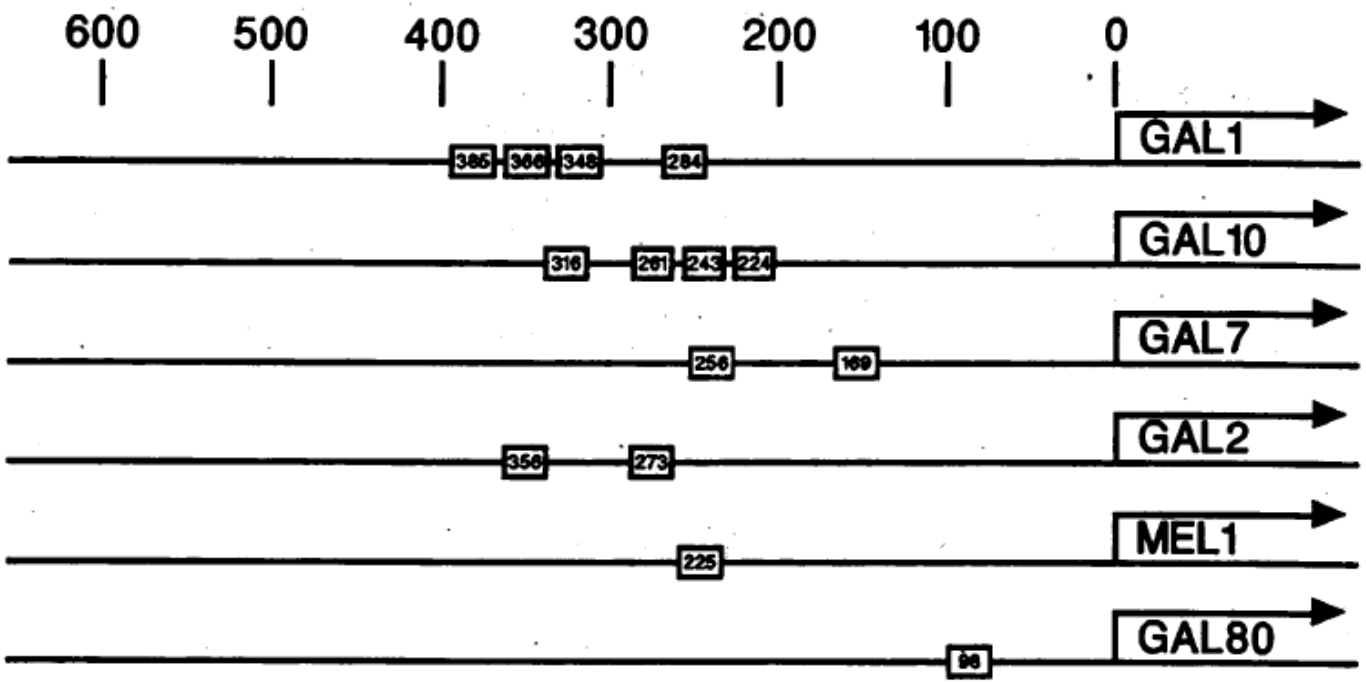


Transcription in Galactose Response Circuit



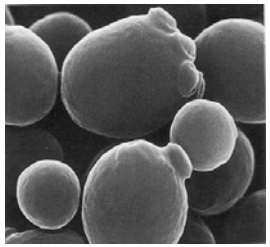


Gal4 Binds to a Consensus UAS

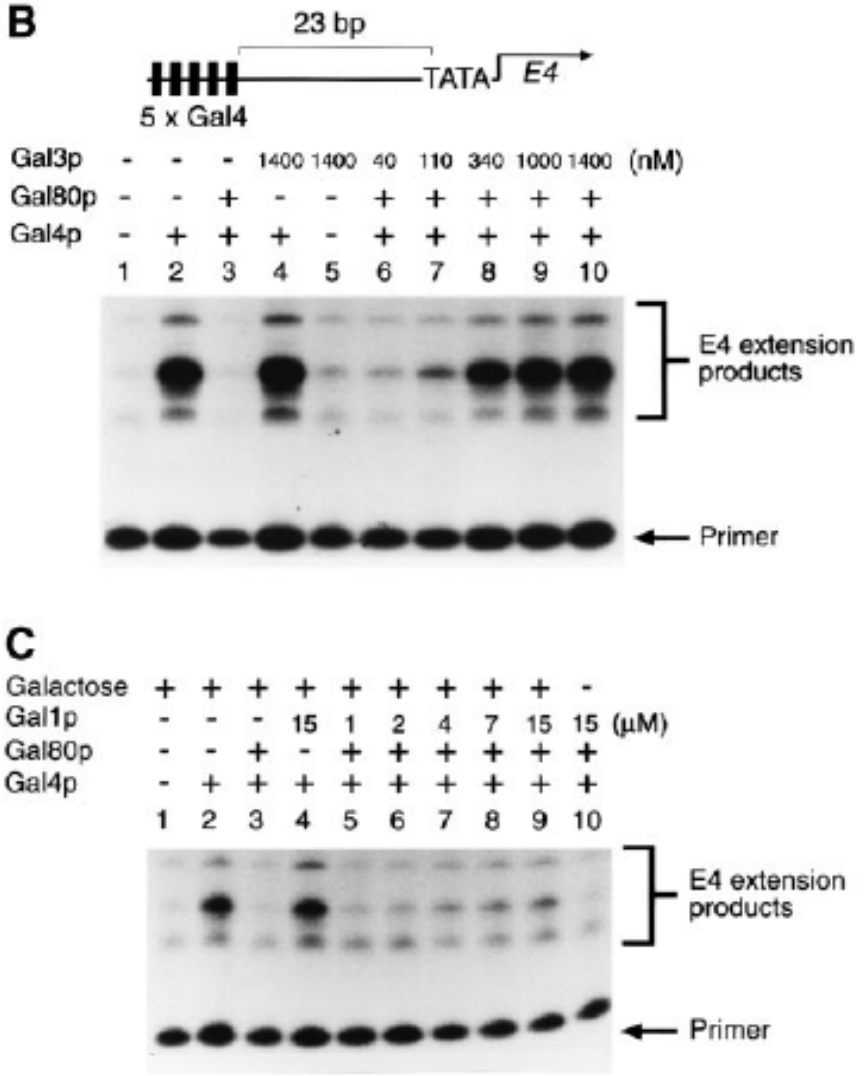


Consensus Binding Site

C	G	G	A	G/C	G	A	C	A/T	C	A	G	G/C	A	G	G	C
19	20	18	10	16	9	13	11	20								



Basic Mechanism in Gal3p-Gal80p-Gal4p Complex Function



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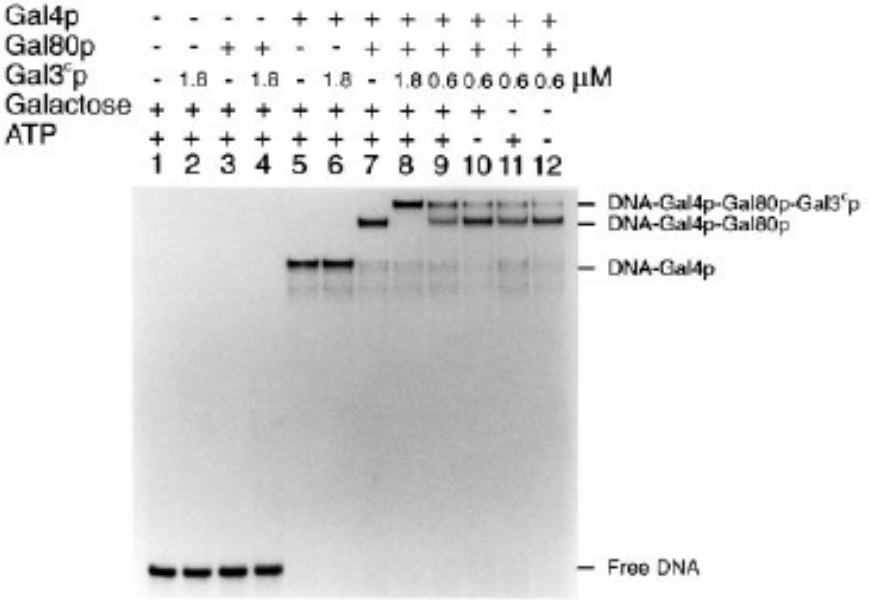
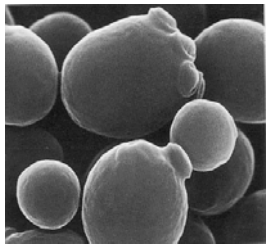
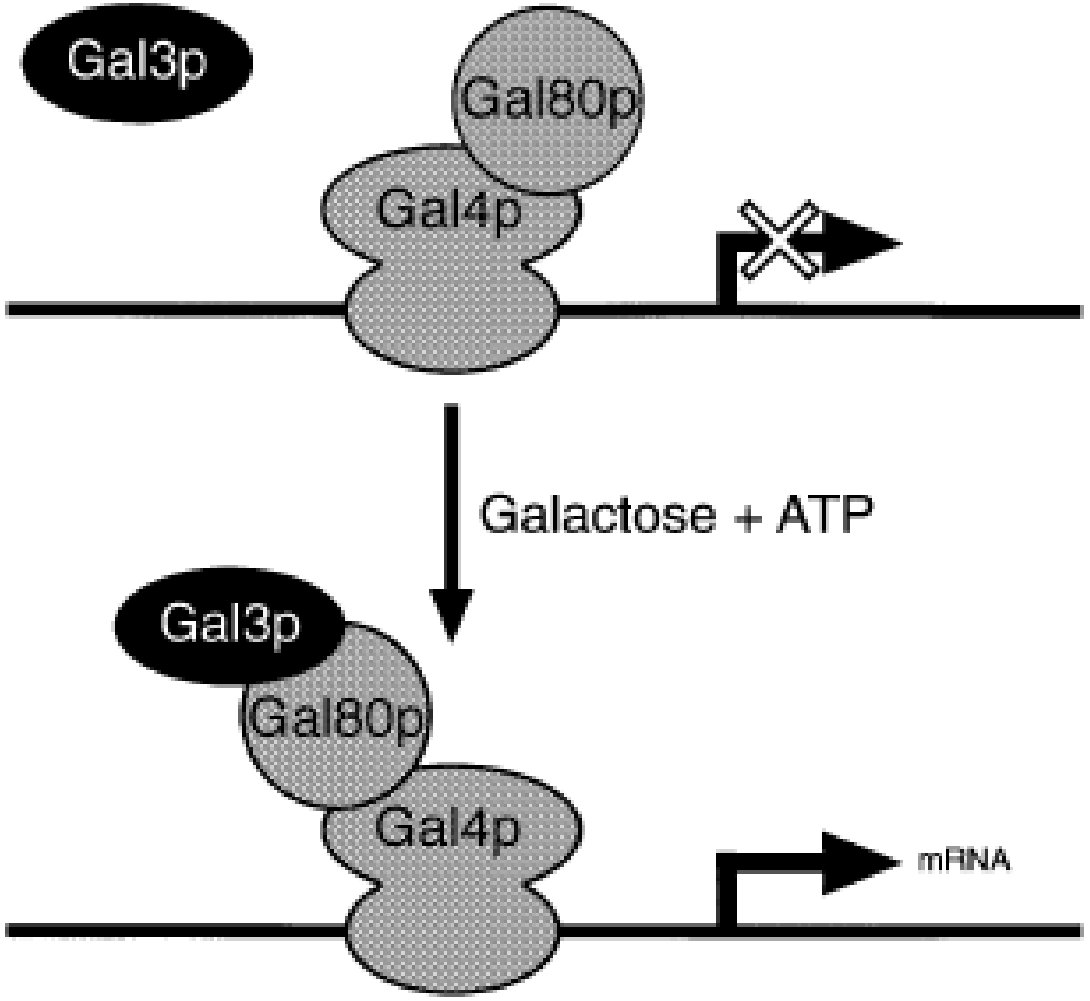
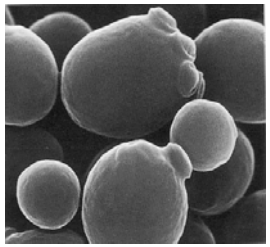
The yeast galactose genetic switch is mediated by the formation of a Gal4p–Gal80p–Gal3p complex



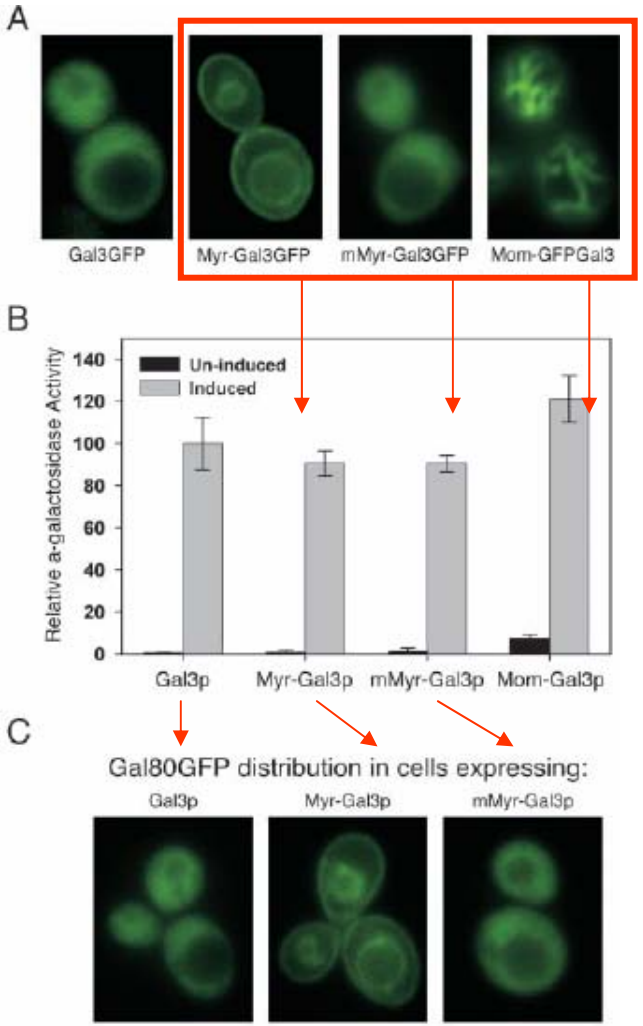


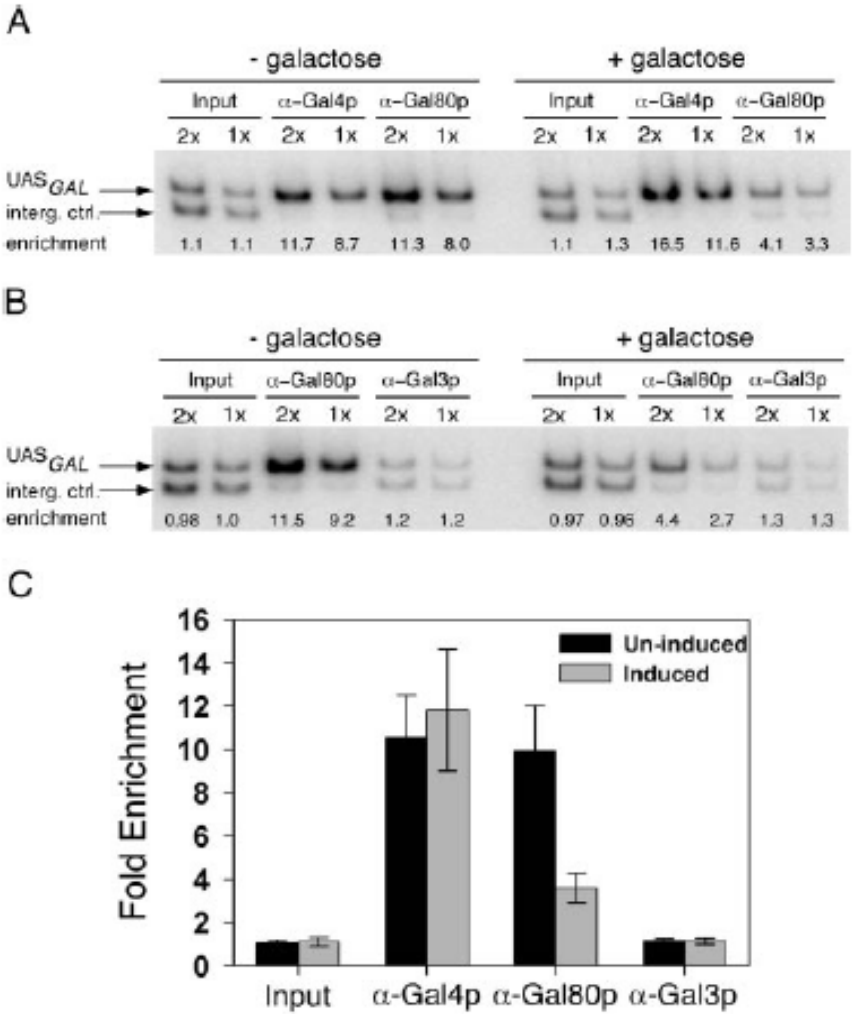
First Model of Gal3p-Gal80p-Gal4p Complex Function (*in vitro*)





Gal3p Functions in Cytoplasm to Sequester Shuttling Gal80p

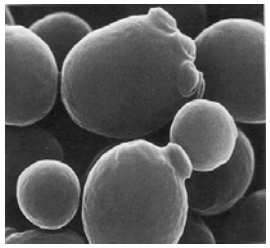




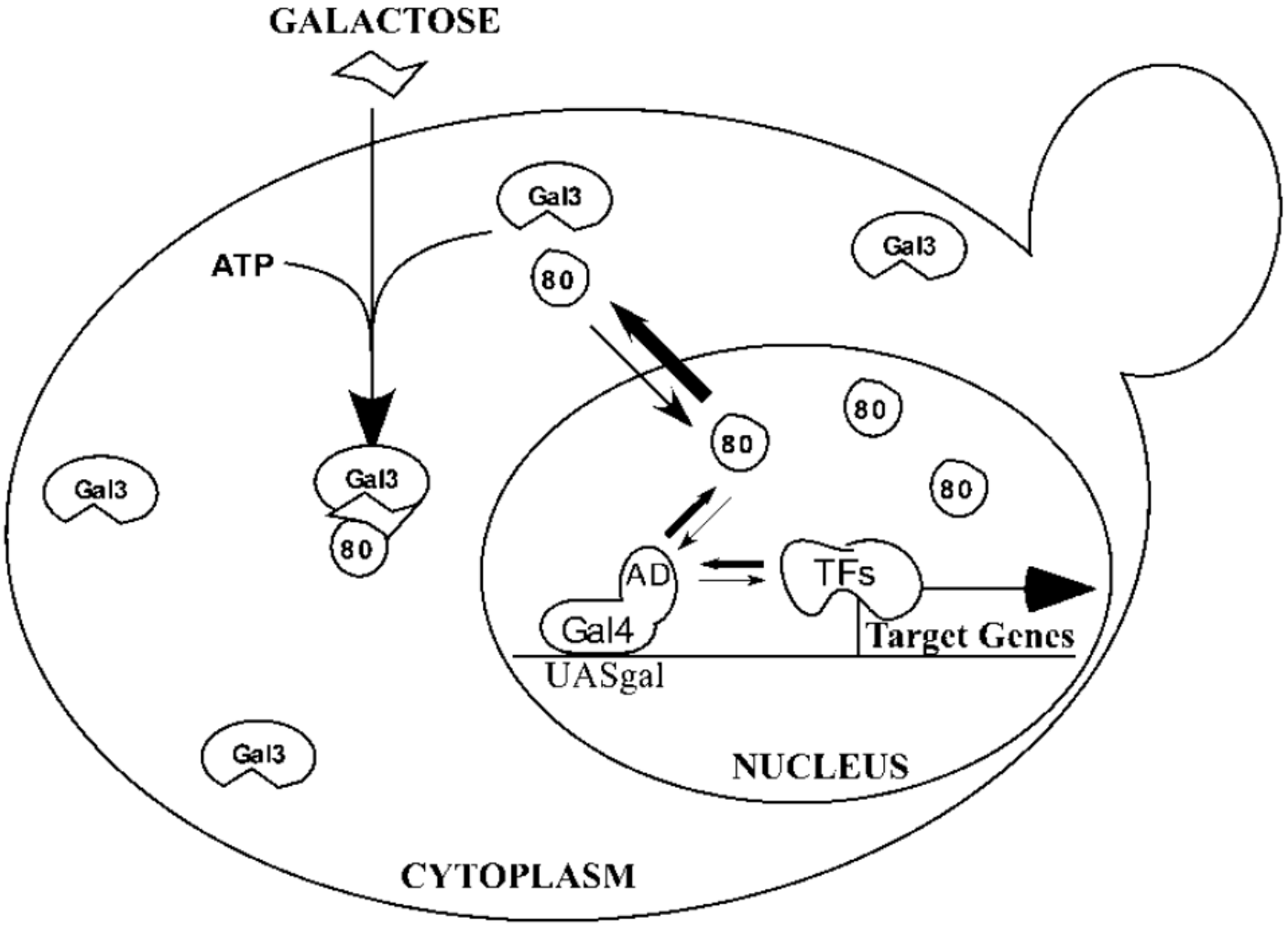
ChIP

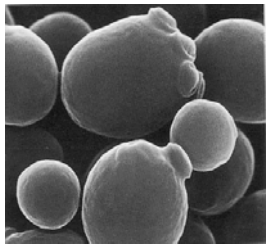
ChIP

Peng and Hopper 2002

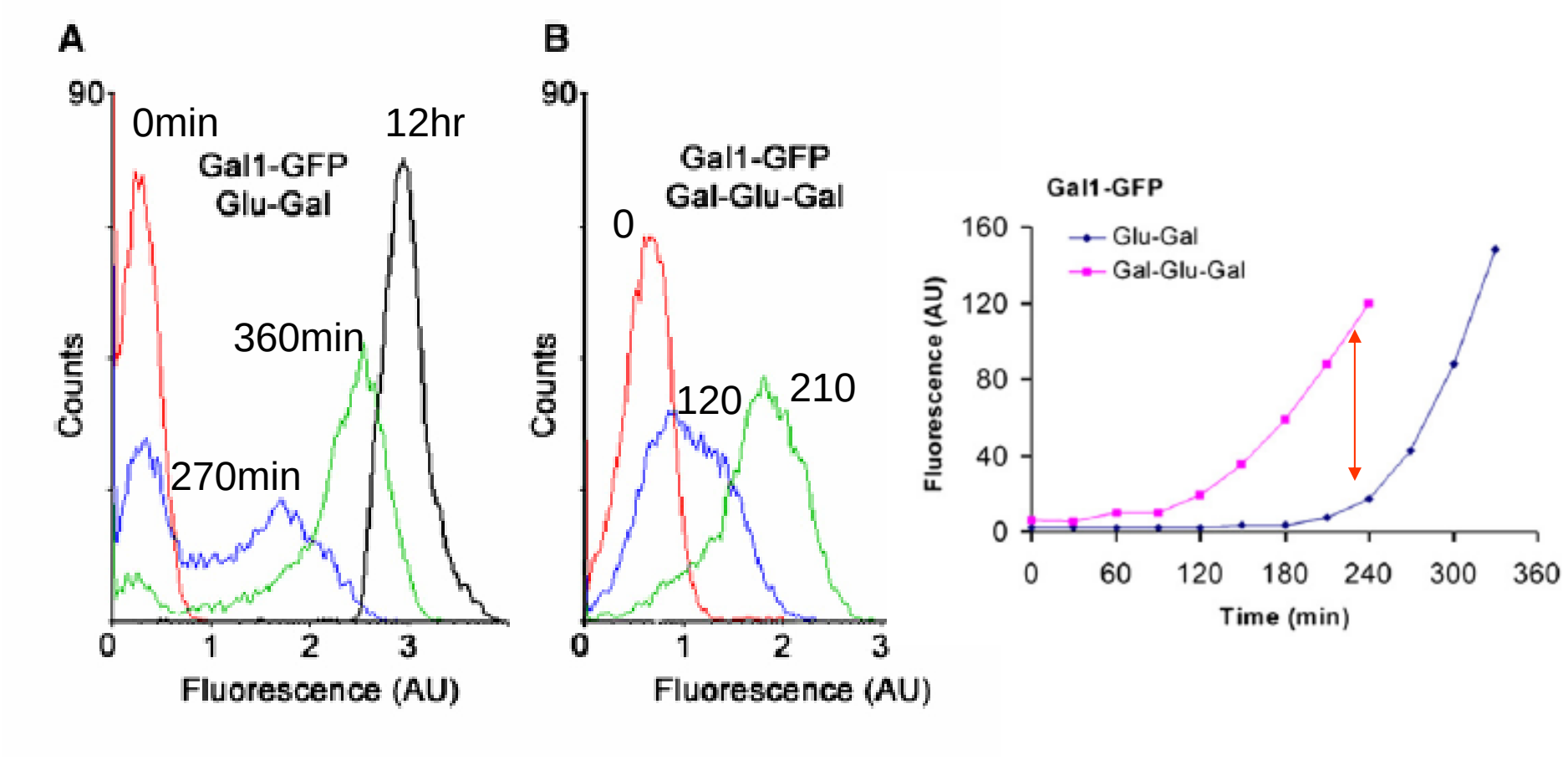


Second Model of Gal3p-Gal80p-Gal4p Complex Function (*in vivo*)

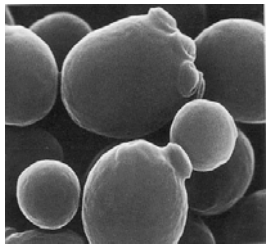




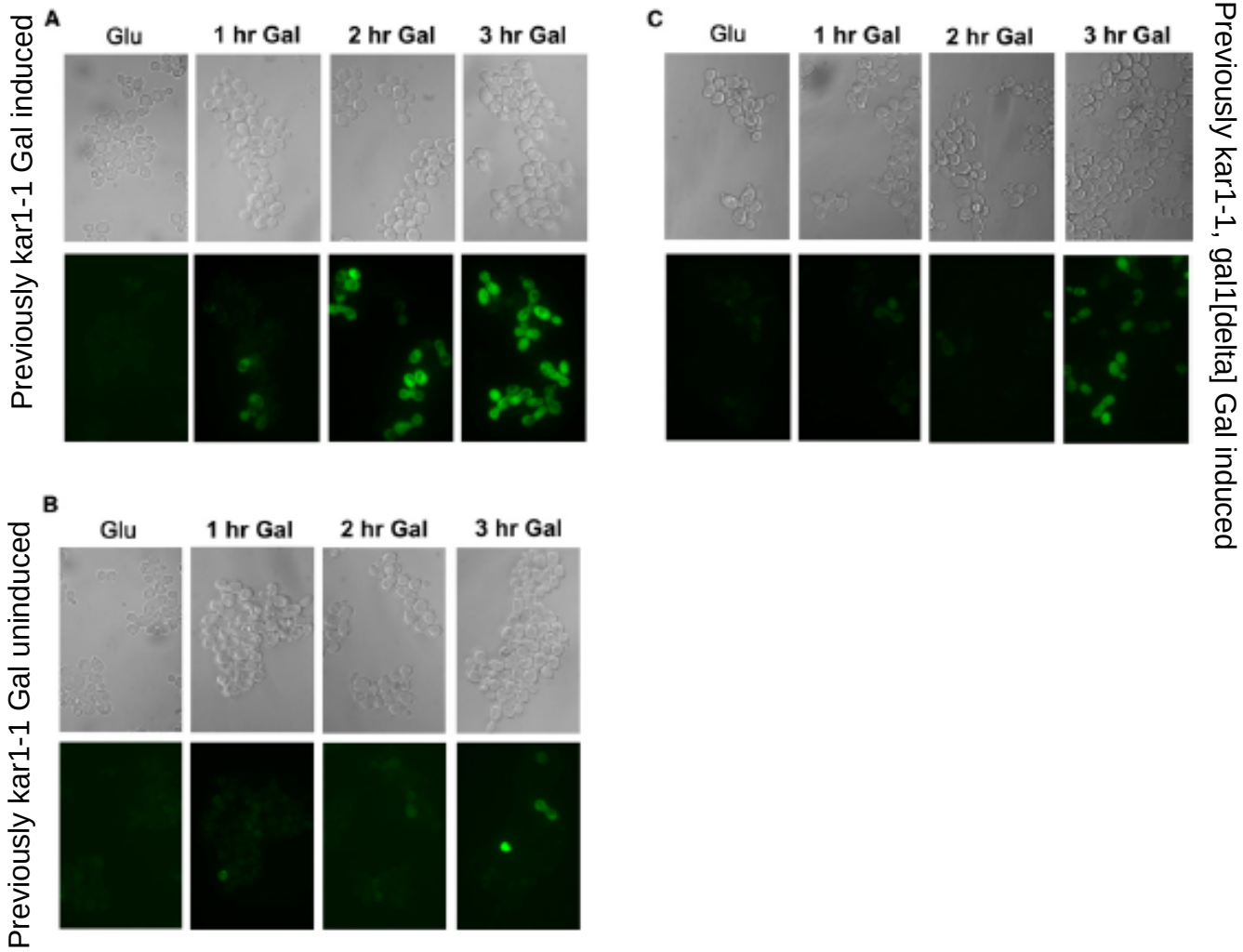
Transcriptional Memory in Galactose Response Circuit

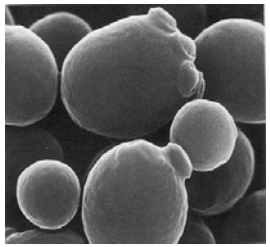


Zacharioudakis et al 2007

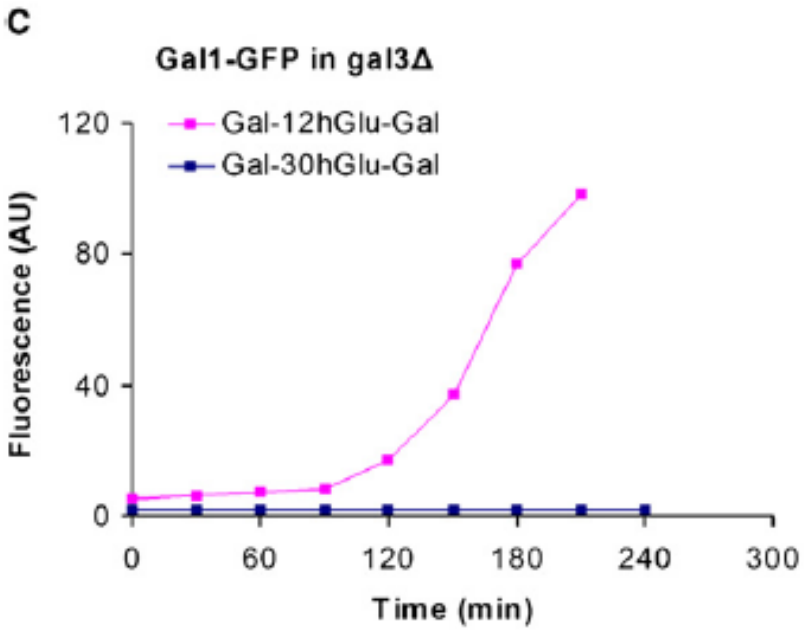


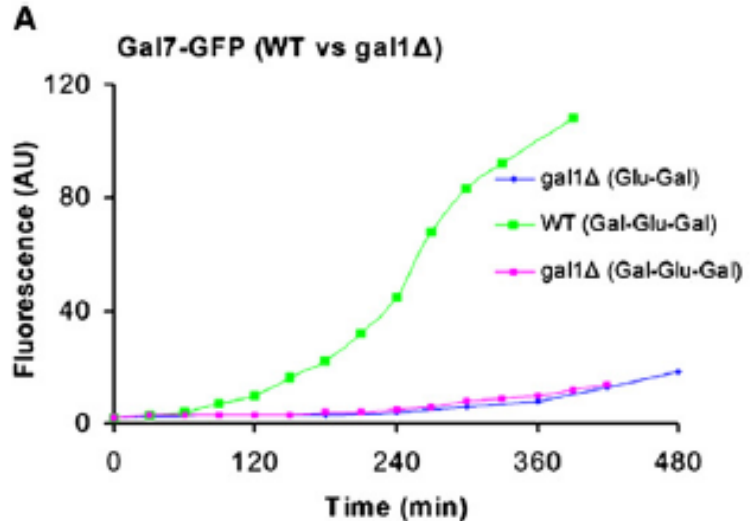
Transcriptional Memory is Mediated by Cytoplasmic Gal1p

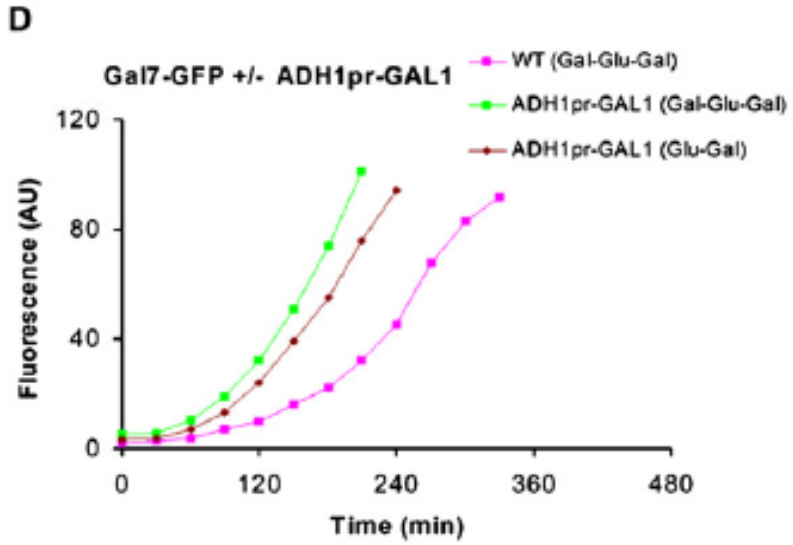


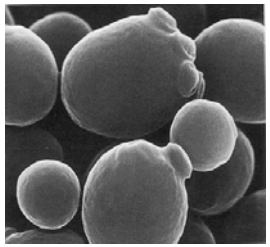


Cytoplasmic Gal1p is necessary and sufficient for memory

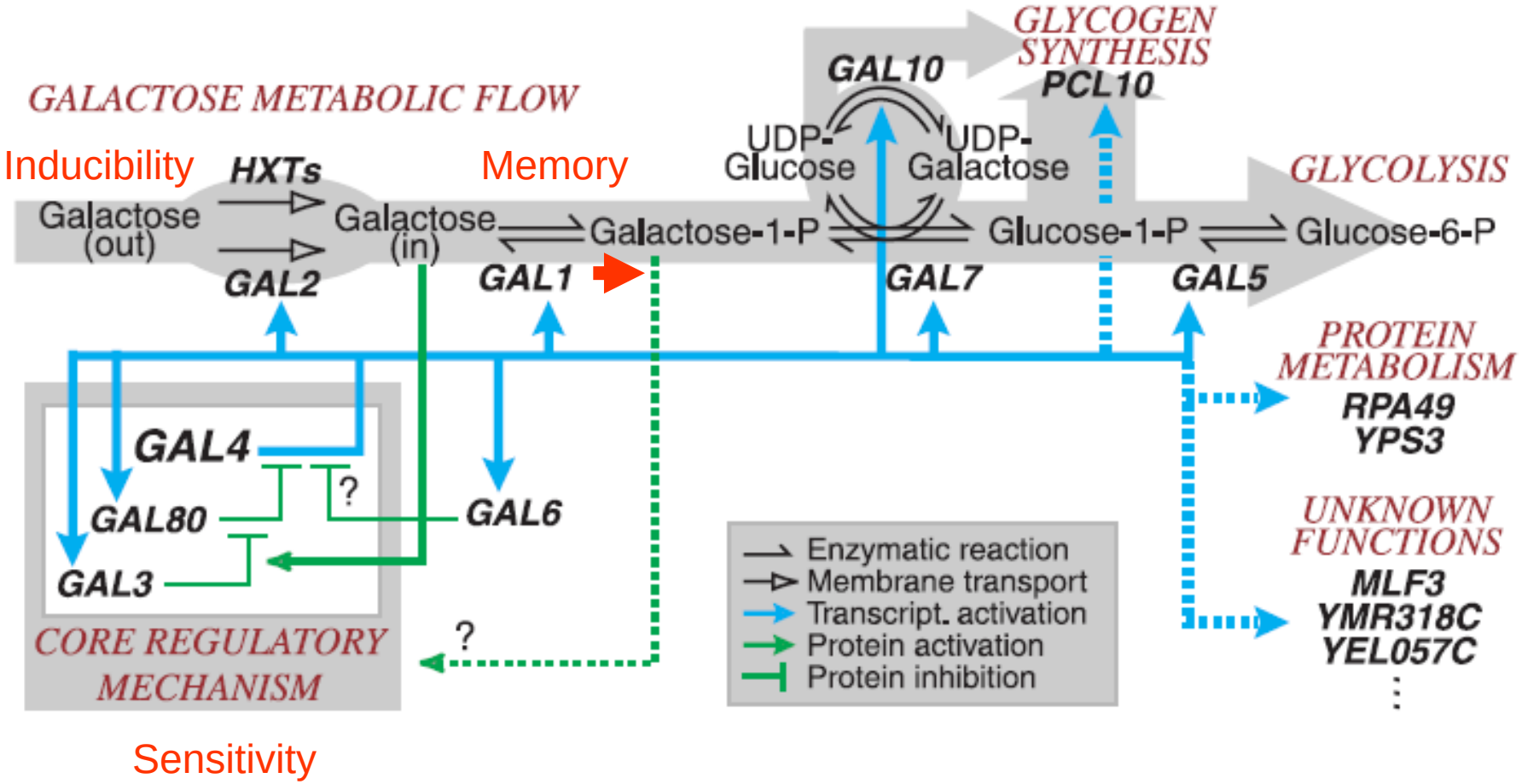


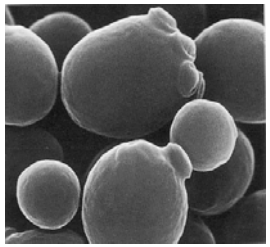




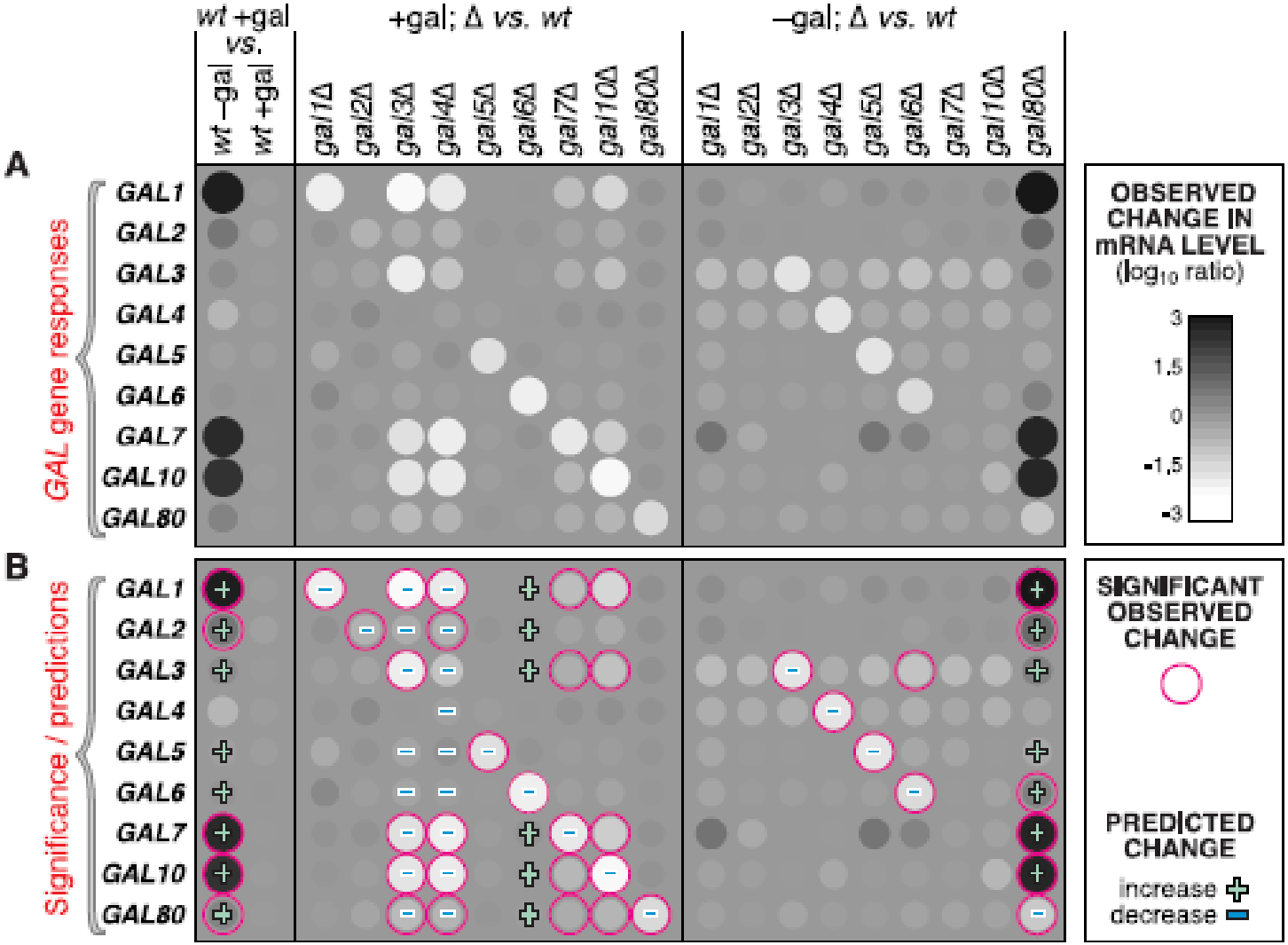


Revisit the Circuit

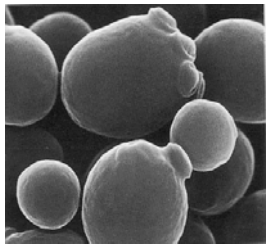




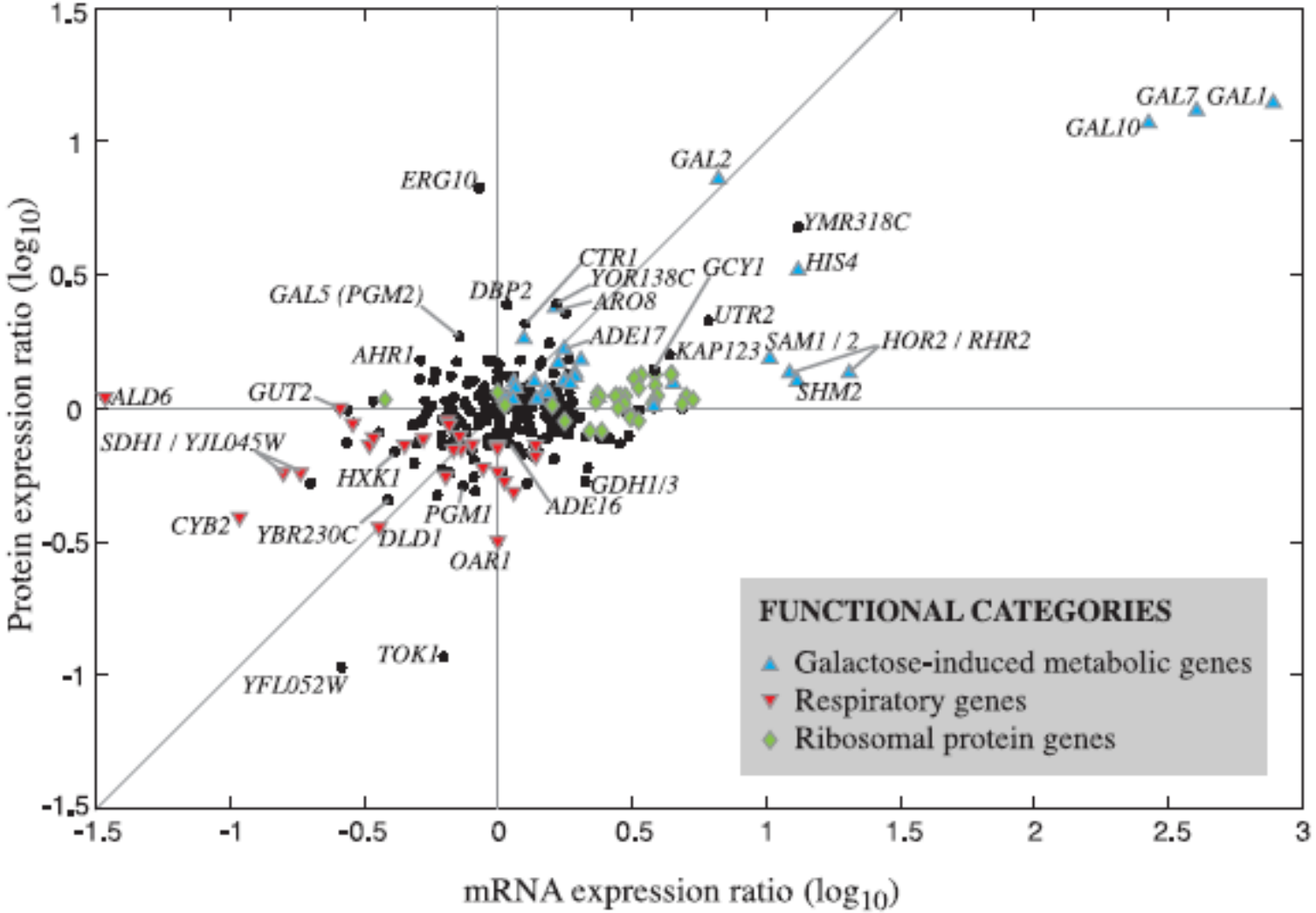
Genomic mRNA Response to Circuit Perturbation

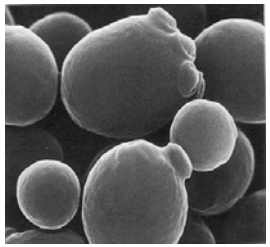


Ideker et al 2001

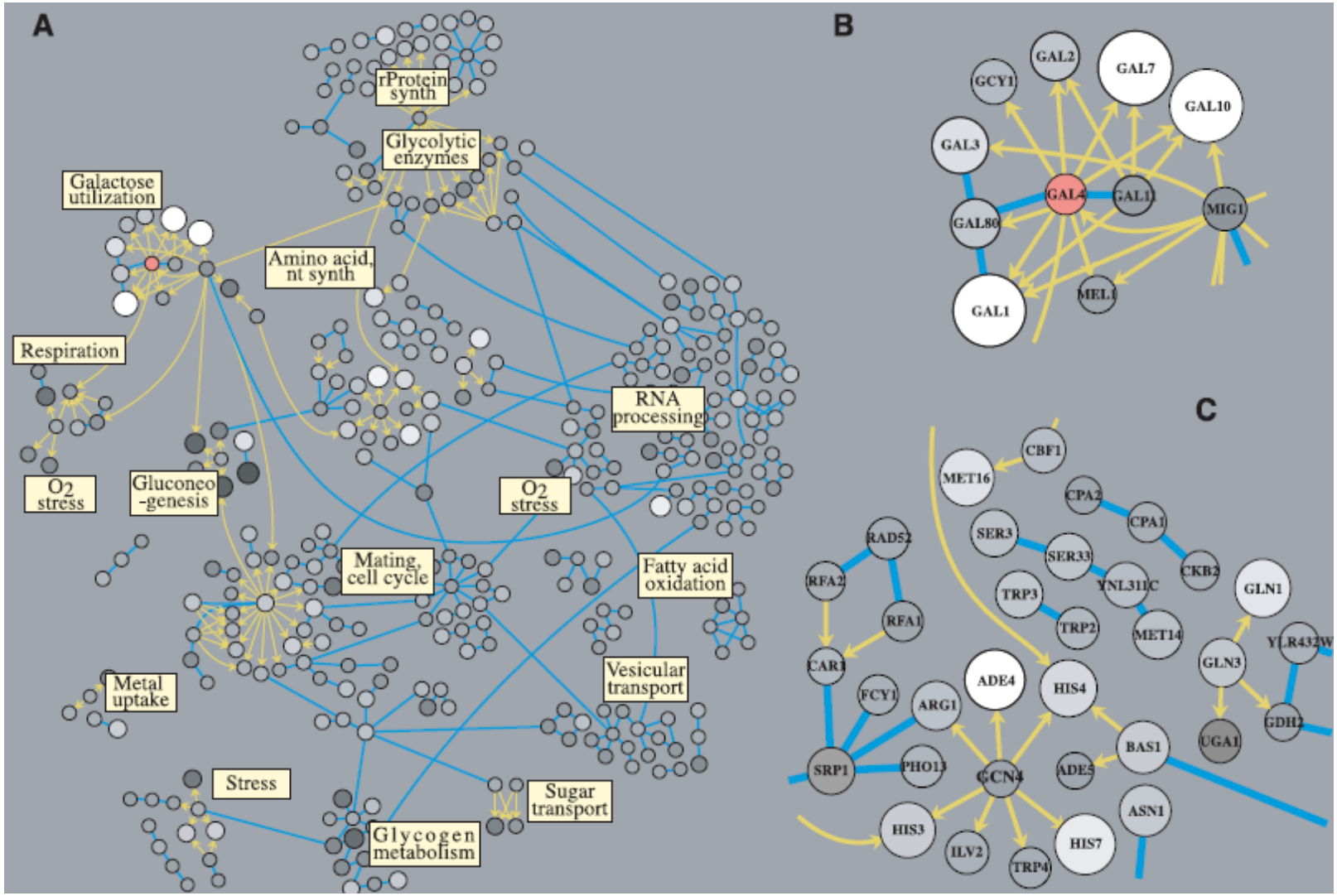


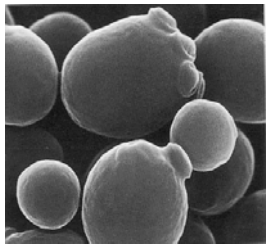
Protein-mRNA Discrepancy in Systematic Response



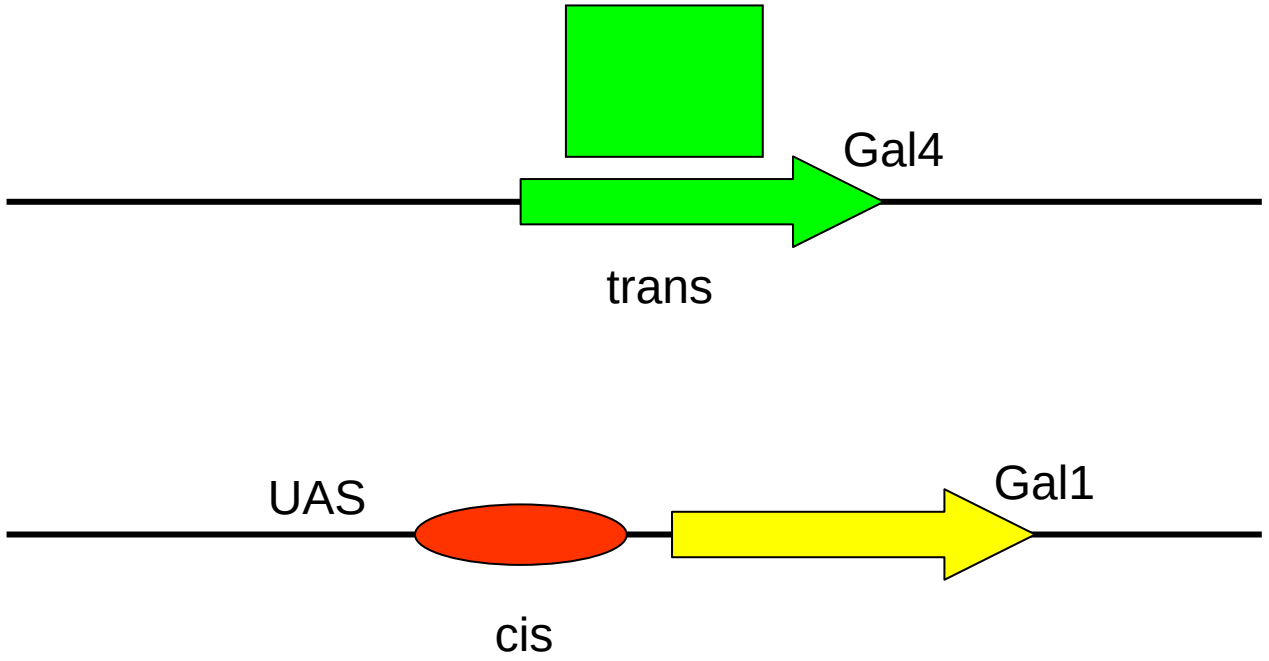


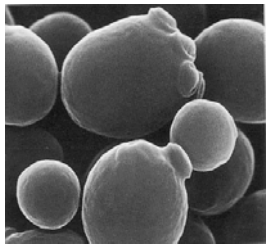
Systematic Response: The Genome is a Network



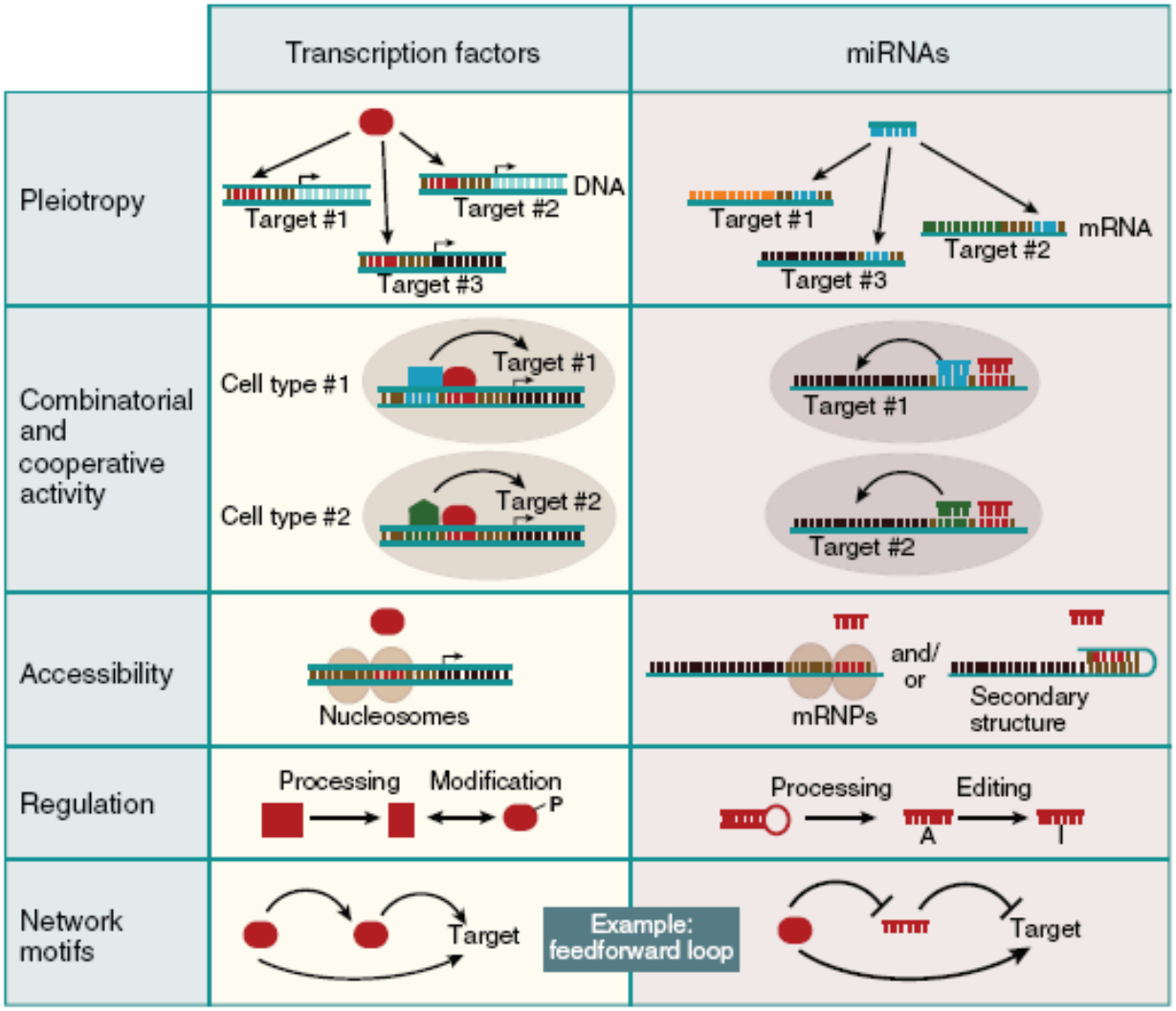


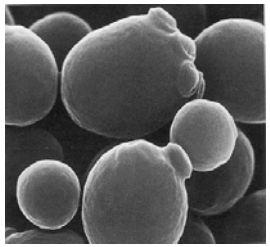
Cis- and Trans-acting Elements



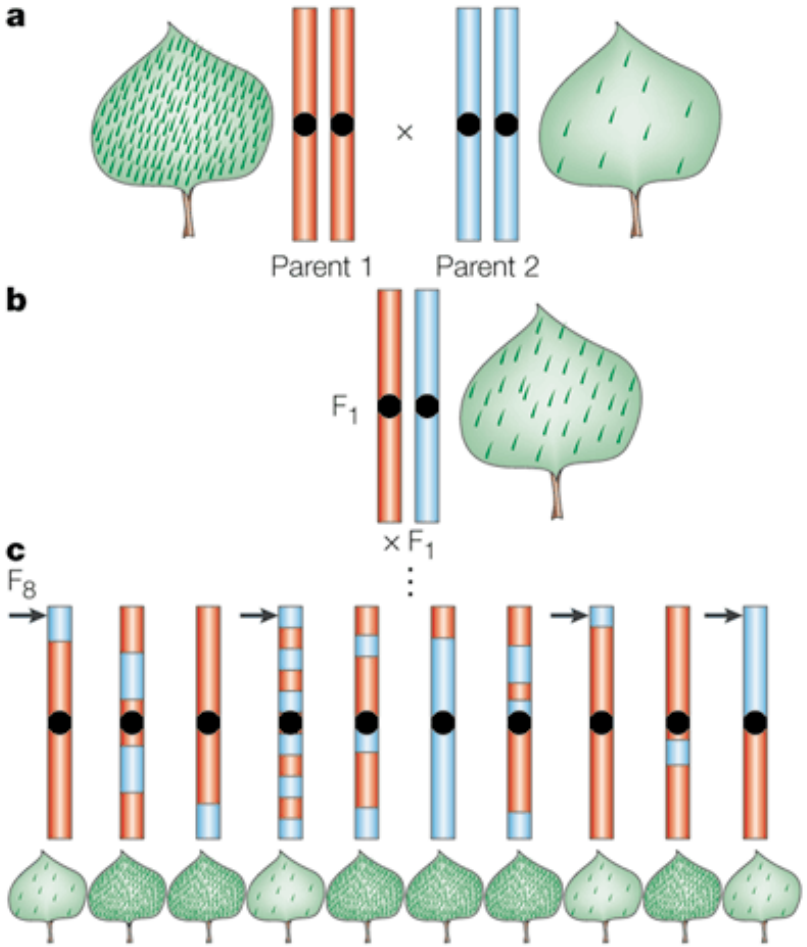


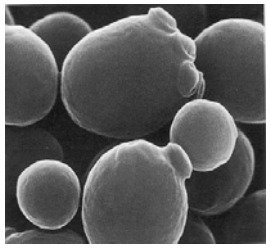
Cis- and Trans- mechanisms in Network Architecture



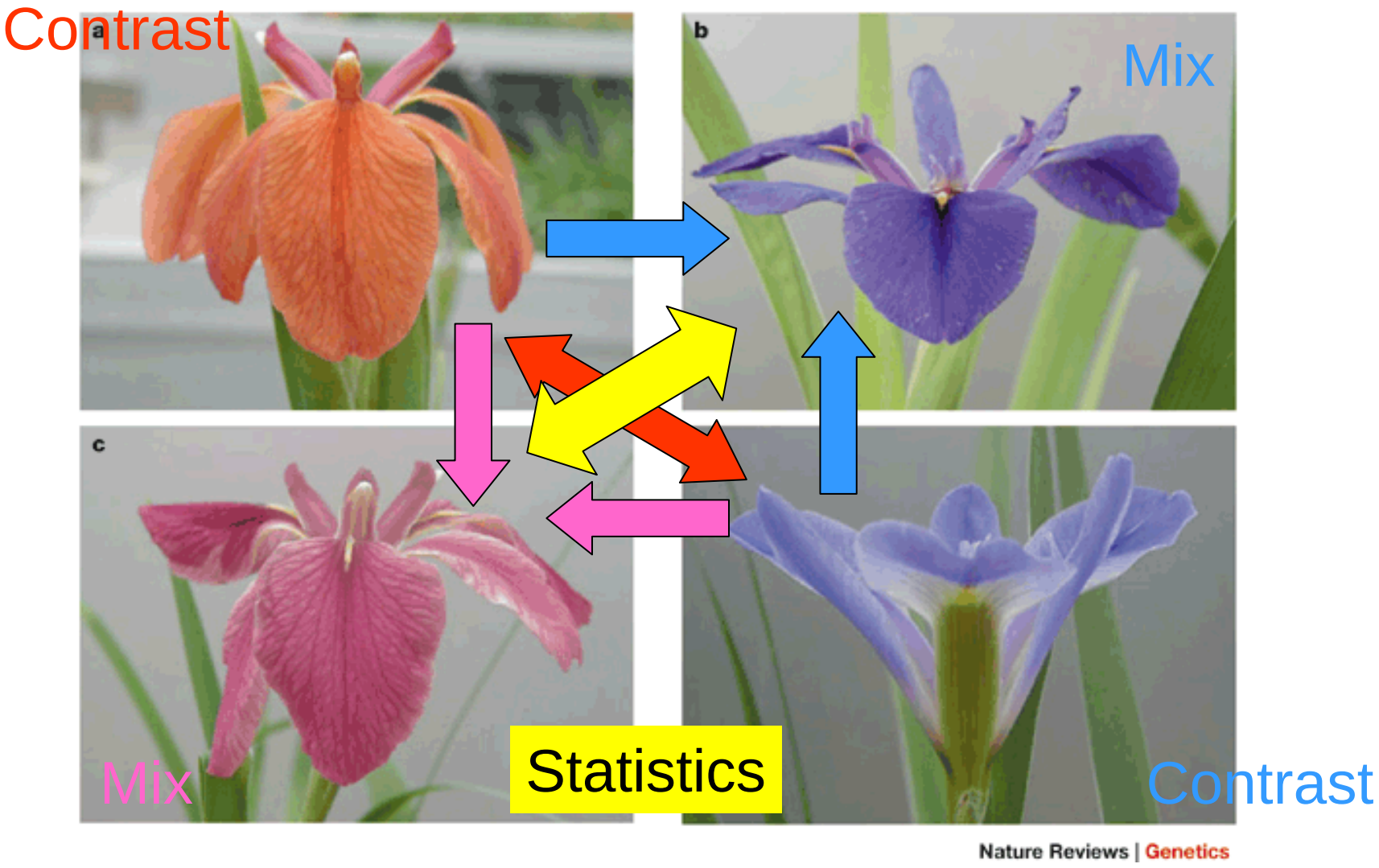


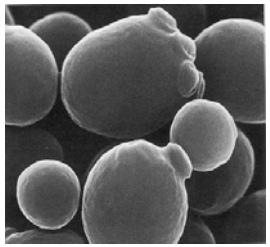
Forward Genomics: Quantitative Trait Loci



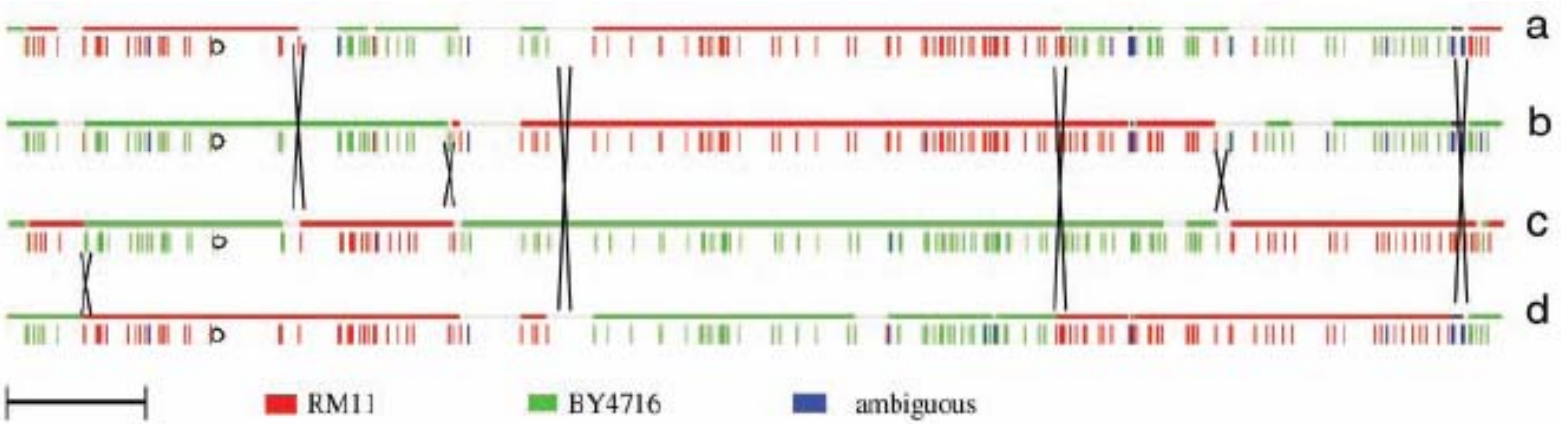


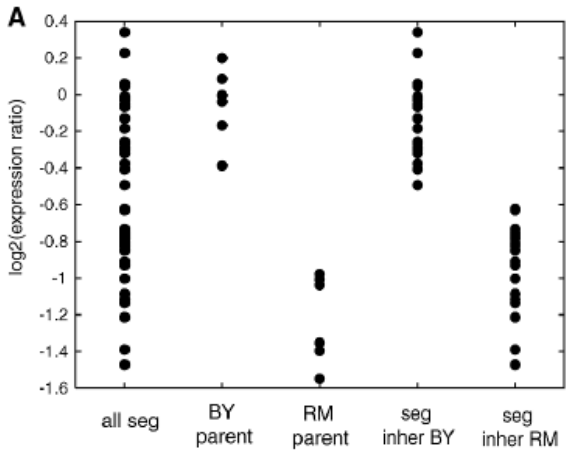
Quantitative Trait Loci Principles

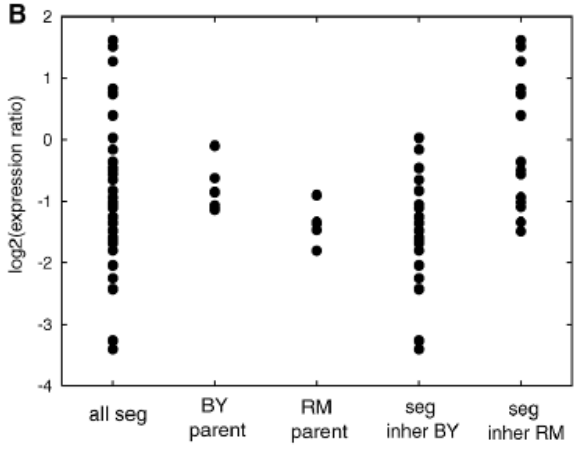


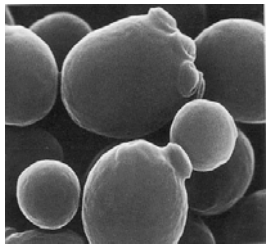


QTL in Yeast
Using Expression as a Phenotype



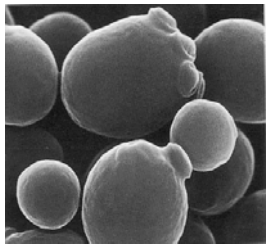




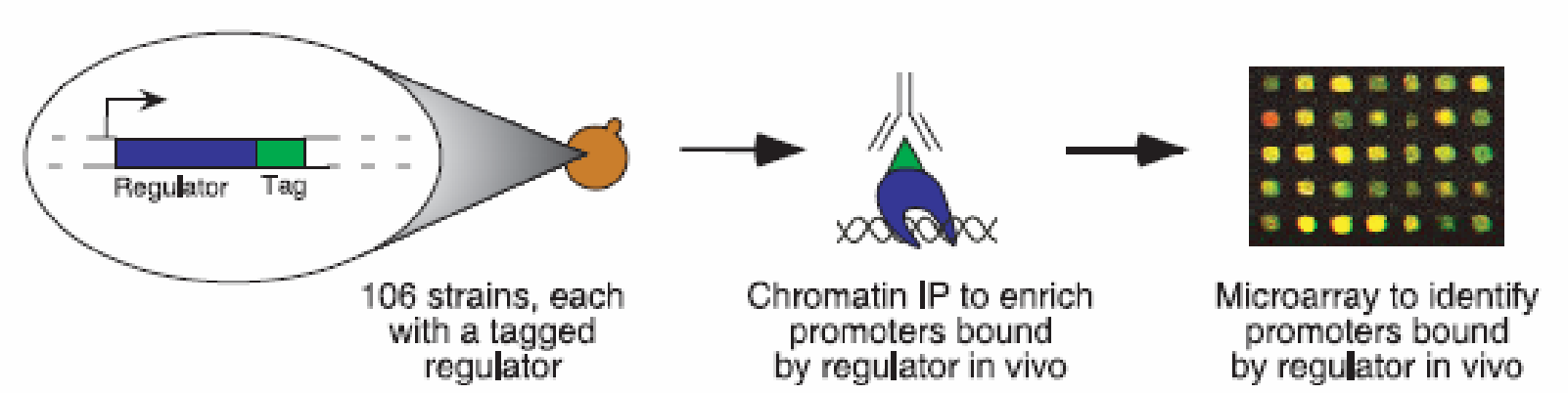


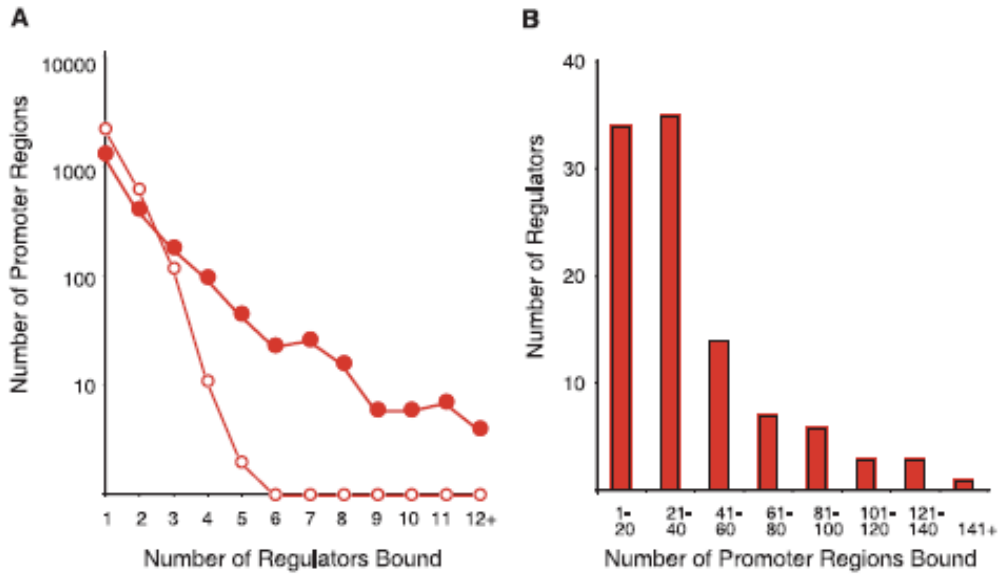
Trans- acting elements in QTL

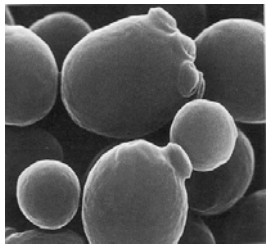
Group	Number of messages	Common function	Linkage bin	Putative regulator
1	18	Budding, daughter cell separation	II:550000	*CST13*
2	21	Leucine biosynthesis	III:90000	*LEU2*
3	28	Mating	III:190000	*MAT*
4	7	Uracil biosynthesis	V:110000	*URA3*
5	28	Heme, fatty acid metabolism	XII:670000	*HAP1*
6	16	Subtelomerically encoded helicases	XII:1030000	*SIR3*
7	94	Mitochondrial	XIV:490000	Unknown
8	19	Msn2/4-dependent induction in acid	XV:170000	Unknown



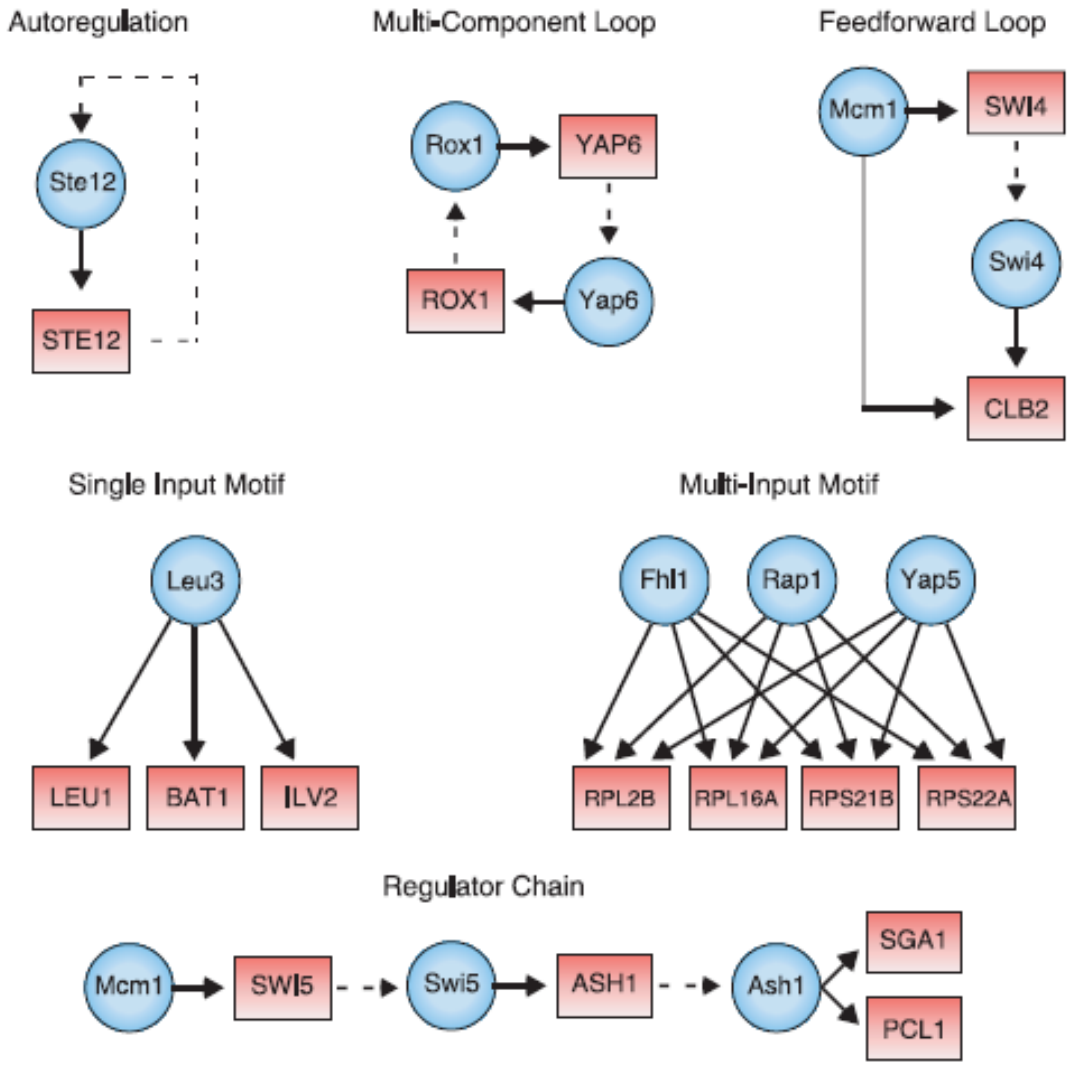
Reverse Genomics: Direct Visualizing by ChIP-on-Chip

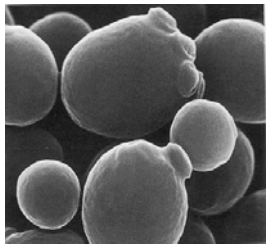






Uncovered Network Motifs





Recovered Network in Cell Cycle