Mutagenesis!
- Chemical
- X-ray
- Transposons

Selection

Phenotypic screening

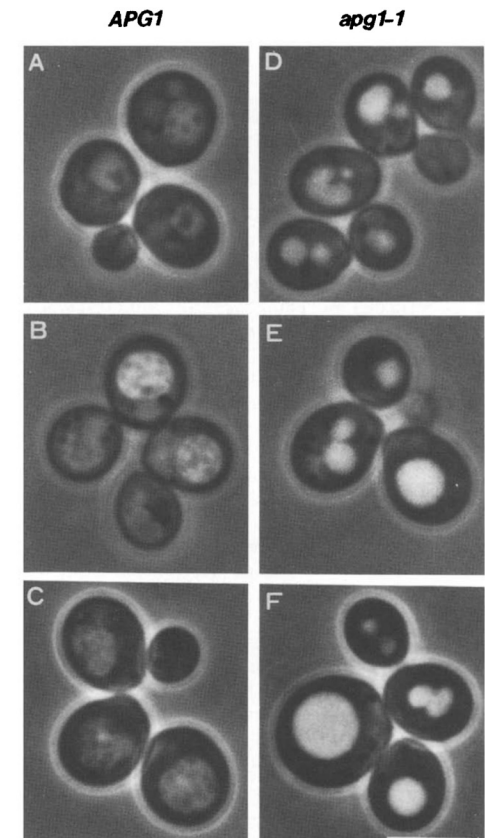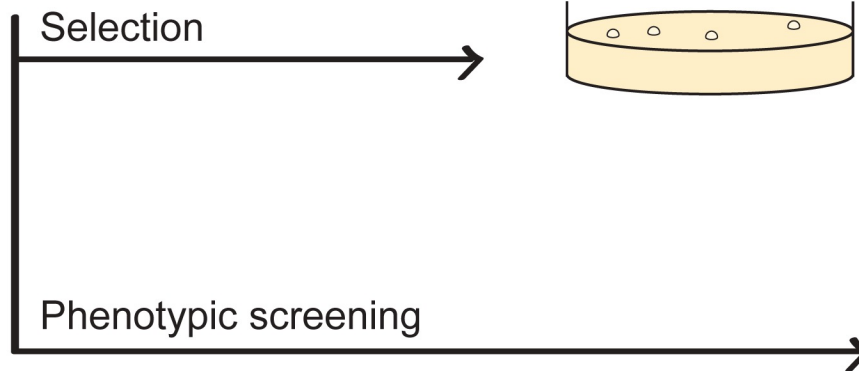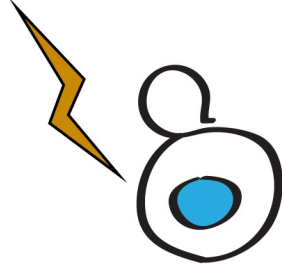APG1   apg1-1

A   D

B   E

C   F

Fig. 1. Morphological changes of vacuoles during nitrogen starvation. Phase contrast microscopic images of cells of X2180-1A (*APG1*, left), and MT14-1B (*apg1-1*, right) incubated in SD (-N) medium containing 1 mM PMSF for 2 h (A,D), 4 h (B,E), and 8 h (C,F). Bar = 5 μm.

(Tsukada and Ohsumi, 1993)

Types of mutant alleles

- Constitutive

- Conditional

  - Types of Conditional alleles

    - Temperature sensitive (ts)

    - pH sensitive

    - Drug sensitive

      - ATP-analog sensitive kinases

Features of yeast (for screening purposes)

- Small genome, sequenced, annotated

- Haploids and diploids can undergo mitosis and are stable
  - Ease of mating and sporulation
  - Easy complementation analysis
  - Tetrad analysis

- Easy to grow
  - Freezable
  - 90 minute generation time
  - Cheap to maintain

- Highly efficient transformation and recombination

Complementation analysis

A) Trans-heterozygote for two mutations in different genes
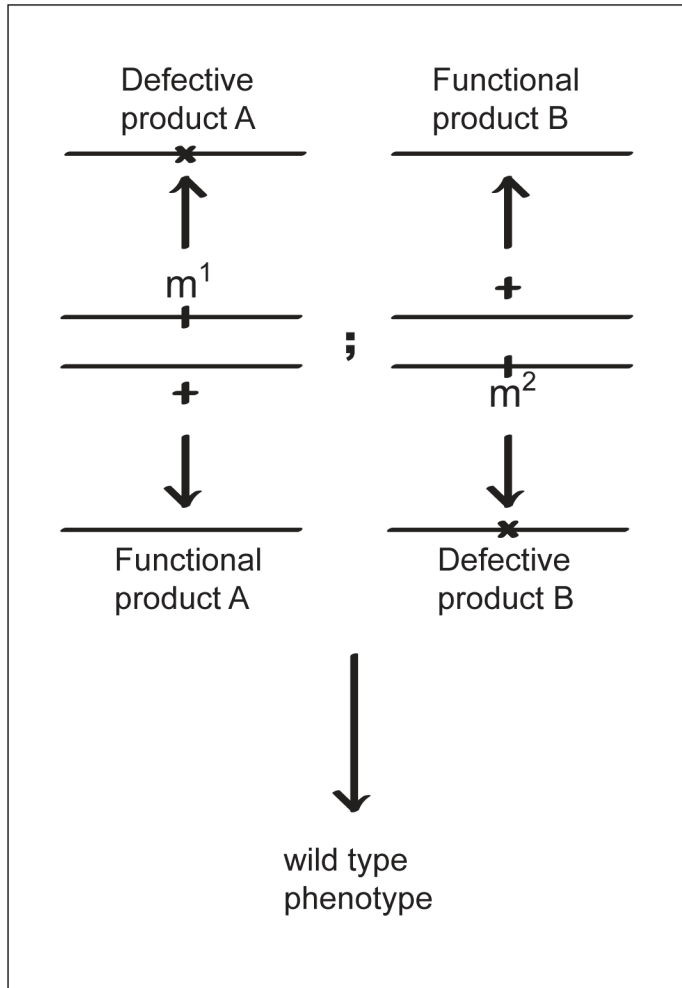
B) Trans-heterozygote for two mutations in the same gene
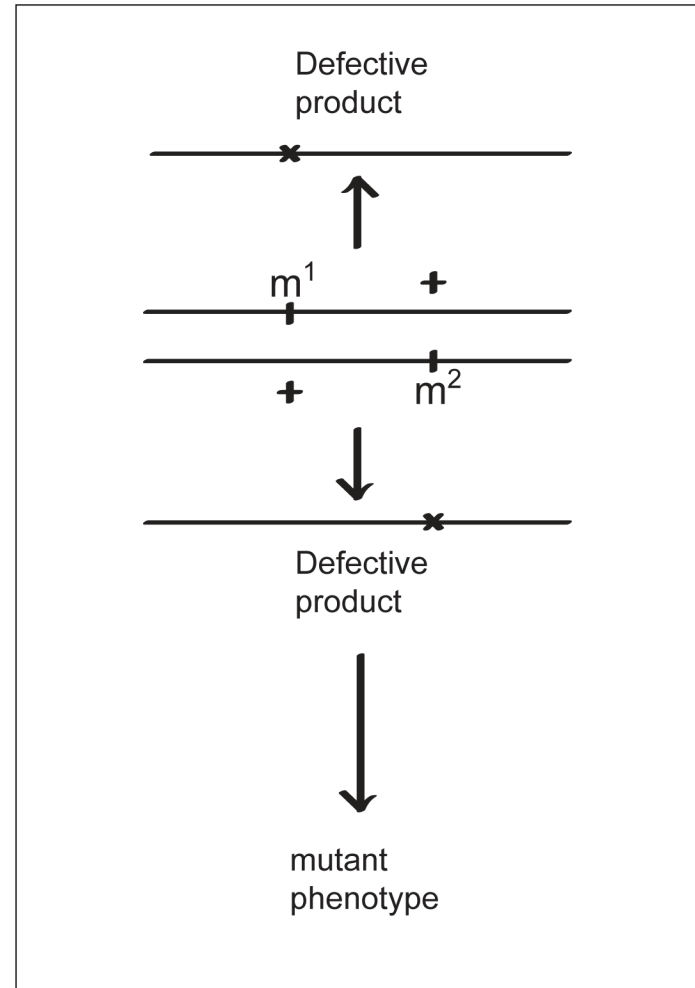
Complementation data 1 (*gal* screen)

Mutant number

	1	2	3	4	5	6	7	8
1	−							
2	+	−						
3	+	+	−					
4	+	−	+	−				
5	−	+	+	+	−			
6	+	+	−	+	+	−		
7	+	+	−	+	+	−	−	
8	+	−	+	−	+	+	+	−

Mutant number

Galactose utilization pathway

Table 2 *GAL* genes and regulators

Gene	Function	Approximate fold induction	Glucose repression	References
GAL1	galactokinase	1000x	severe	St. John and Davis (1979, 1981)
GAL2	galactose permease	1000x	severe	Nehlin et al. (1989); Szkutnicka et al. (1989)
GAL3	inducer function	3–4x	severe	Bajwa et al. (1988)
GAL4	transcriptional activator		modest	Laughon and Gesteland (1984)
GAL5	phosphoglucomutase	3x	severe	Oh and Hopper (1990)
GAL7	galactotransferase	1000x	severe	St. John and Davis (1979, 1981)
GAL10	UDP-Gal epimerase	1000x	severe	St. John and Davis (1979, 1981)
GAL11	transcriptional activator?			Himmelfarb et al. (1990)
GAL80	inhibits Gal4p	10x	none	Igarashi et al. (1987)
GAL82	glucose repression			Matsumoto et al. (1981, 1983a)
GAL83	glucose repression			Matsumoto et al. (1981, 1983a)
MEL1	α-galactosidase	100x	severe	Post-Beittenmiller et al. (1984)

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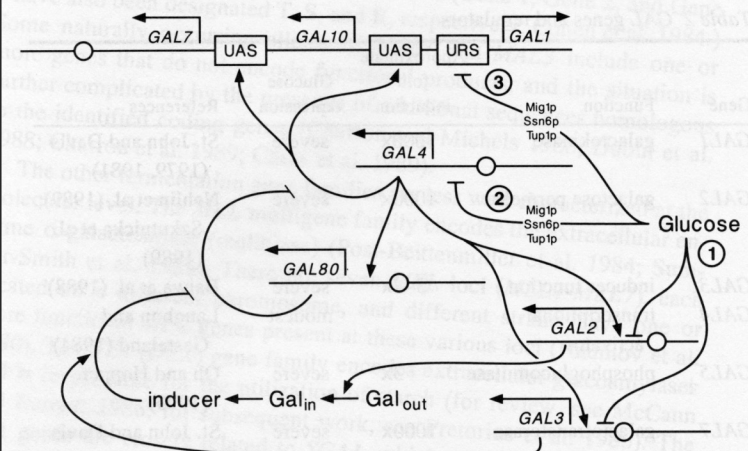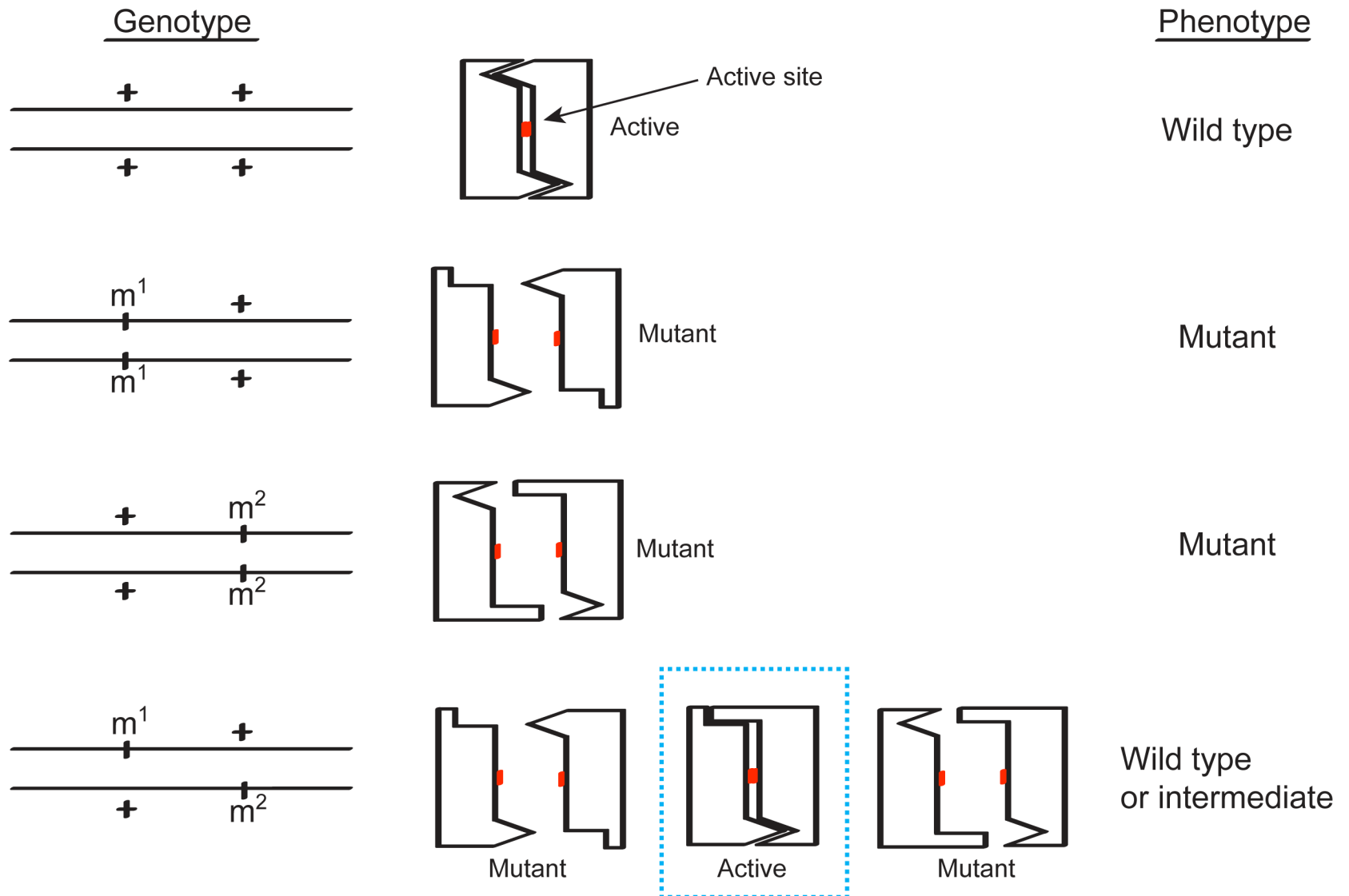
Figure 1 Model of the *GAL* gene regulatory mechanism. Arrows denote stimulation; bars denote inhibition. The three routes of glucose repression are numbered, as described in the text. The UAS elements in the *GAL1*, *GAL7*, and *GAL10* promoters are Gal4p-binding sites; the URS element in the *GAL1* promoter confers glucose repression via Mig1p, Ssn6p, and Tup1p (see text for details).

Complementation data 2 (*ade* screen)

	1	2	3	4	5	6	7	8	9	10
1	−	+	−	+	−	+	+	+	±	+
2		−	+	+	+	−	+	−	+	−
3			−	+	−	+	+	+	±	+
4				−	+	+	−	+	+	+
5					−	+	+	+	−	+
6						−	+	−	+	−
7							−	+	+	+
8								−	+	−
9									−	+
10										−

Cross

1 x 2	10 cM
2 x 3	10 cM
4 x 5	50 cM
5 x 6	10 cM
7 x 8	50 cM
8 x 9	10 cM
9 x 10	10 cM

Intragenic complementation

Genotype

Phenotype

+ +
+ +
Active site → Active
Wild type

m^1 +
m^1 +
Mutant
Mutant

+ m^2
+ m^2
Mutant
Mutant

m^1 +
+ m^2
Mutant Active Mutant
Wild type or intermediate

Adapted from: Snyder et al. *General Genetics* 1985

Non-allelic non-complementation
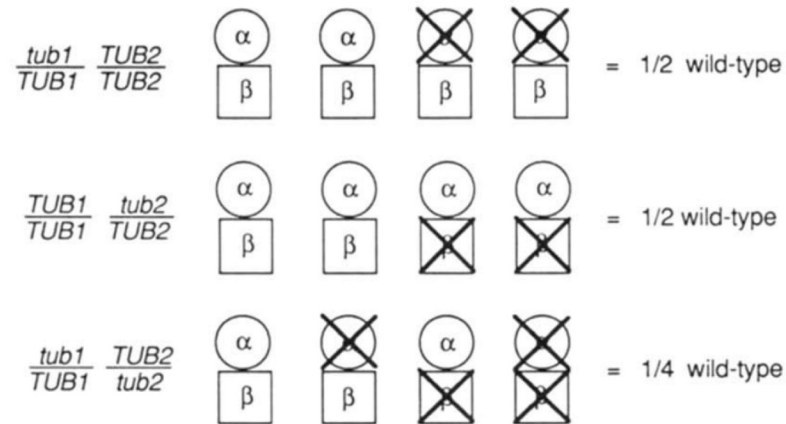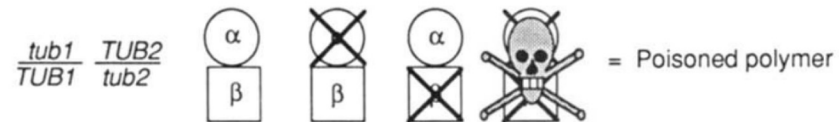
FIGURE 3.—Models for the mechanism of unlinked noncomple-mentation.

Yeast complementation 3

Complementation matrix (rows 1–10, columns 1–10):

	1	2	3	4	5	6	7	8	9	10
1	−	+	+	+	+	+	−	−	+	+
2		−	−	+	+	−	−	+	+	−
3			−	+	+	−	−	+	+	−
4				−	+	+	−	+	+	+
5					−	+	−	±	−	+
6						−	−	+	+	−
7							−	−	−	−
8								−	+	+
9									−	+
10										−

Tetrads

Cross	4^+0^-	3^+1^-	2^+2^-
1 x 10	42	8	0
1 x 4	32	16	2
1 x 9	8	34	8
1 x 7	50	0	0
7 x 8	50	0	0
2 x 4	30	20	0
2 x 5	8	34	8
4 x 9	7	34	9
4 x 6	35	14	1