Epistasis, Suppressors and Chemical Genetics

Two genes, one pathway

Sweet pea (*Lathyrus odoratus*)

AaBb x AaBb

	AB	**Ab**	**aB**	**ab**
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

Two genes, one pathway = 9 : 7 phenotypic ratio

white flower color → (A gene product) → (B gene product) → pink flower color

Bateson and Punnett

Two genes, one pathway

Sweet pea (*Lathyrus odoratus*)

AaBb x AaBb

	AB	**Ab**	**aB**	**ab**
AB	AABB	AABb	AaBB	AaBb
Ab	AABb	AAbb	AaBb	Aabb
aB	AaBB	AaBb	aaBB	aaBb
ab	AaBb	Aabb	aaBb	aabb

Two genes, one pathway

white flower color → (A gene product) → blue flower color → (B gene product) → pink flower color

What is epistasis?

- Epistasis is hard to define!
  - Classically defined as suppression of the phenotype of one allele by another allele of a different gene (Bateson 1909)
  - Multiple genes, one pathway/phenotype
  - Can simply refer to interactions between alleles

- Epistasis analysis usually refers to the phenotypic analysis of double mutants compared to the singles
  - The epistatic gene/allele in a double mutant refers to the allele that gives a visible phenotype and “masks” the other.

- Epistasis can also refer to synthetic interactions between mutants
  - Synthetic lethality
  - Suppression

Assumptions in epistatic analysis

- There is a signal that affects phenotype. The experimenter can find out the state of the signal, independently of genotype or phenotype.
- The signal and the two genes under study are the sole determinants of phenotype under the conditions of the experiment.
- The signal and the two genes are either on or off; there are no intermediate levels of activity. (For instance, partial loss-of-function mutations should be avoided.)
- In the wild type the signal determines whether one of the genes (the upstream gene) is on or off; this in turn determines whether the second (downstream) gene is on or off.

Avery and Wasserman 1992

Genetic dissection of the secretory pathway

Cell, Vol. 21, 205–215, August 1980, Copyright © 1980 by MIT

Identification of 23 Complementation Groups Required for Post-translational Events in the Yeast Secretory Pathway

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Summary

Cells of a Saccharomyces cerevisiae mutant that is temperature-sensitive for secretion and cell surface growth become dense during incubation at the non-permissive temperature (37°C). This property allows the selection of additional secretory mutants by sedimentation of mutagenized cells on a Ludox density gradient. Colonies derived from dense cells are screened for conditional growth and secretion of invertase and acid phosphatase. The *sec* mutant strains that accumulate an abnormally large intracellular pool of invertase at 37°C (188 mutant clones) fall into 23 complementation groups, and the distribution of mutant alleles suggests that more complementation groups could be found. Bud emergence and incorporation of a plasma membrane sulfate permease activity stop quickly after a shift to 37°C. Many of the mutants are thermoreversible; upon return to the permissive temperature (25°C) the accumulated invertase is secreted. Electron microscopy of *sec* mutant cells reveals, with one exception, the temperature-dependent accumulation of membrane-enclosed secretory organelles. We suggest that these structures represent intermediates in a pathway in which secretion and plasma membrane assembly are colinear.

sis pathways, both in identifying intermediate structures and in providing biochemical assays for assembly steps (Wood and King, 1979). We believe that a similar approach may be useful in unraveling a eucaryotic morphogenesis pathway.

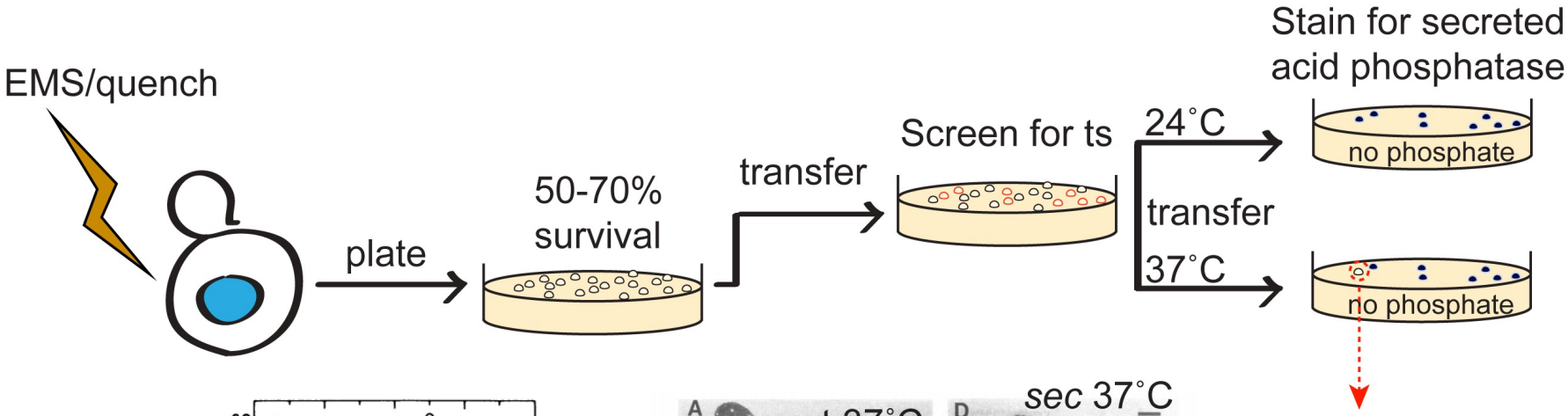
Yeast cell surface growth is restricted primarily to enlargement of the bud followed by cell division. Incorporation of new cell wall material, including secretion of the wall-bound enzymes invertase and acid phosphatase, is also restricted to the bud (Tkacz and Lampen, 1972, 1973; Field and Schekman, 1980). Membrane-enclosed vesicles have been implicated in secretion and bud growth (Moor, 1967; Matile et al., 1971). Our recent report of a conditional mutant blocked in secretion and cell surface growth, which accumulates membrane-enclosed vesicles containing a secretory enzyme (Novick and Schekman, 1979), supports such a role for vesicles.

In this report we describe a technique for the enrichment of conditional secretory and cell surface growth mutants. We have identified a large number of complementation groups that are required for the movement of at least two secretory enzymes and one plasma membrane permease through a series of distinct membrane-enclosed organelles in a pathway that leads to the cell surface.

Results

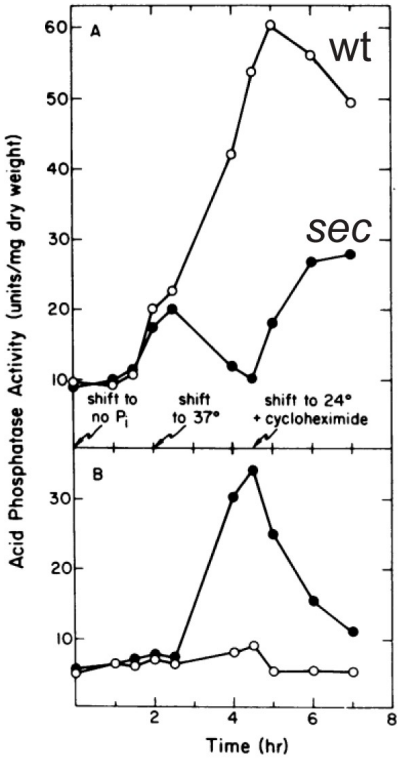
Secretory mutants are defined as those strains which fail to export active invertase and acid phosphatase, but continue to synthesize protein under restrictive growth conditions. In a previous report (Novick and Schekman, 1979) we described a screening proce-

The *sec* screen: Defining the secretory network



Secreted activity

Intracellular activity



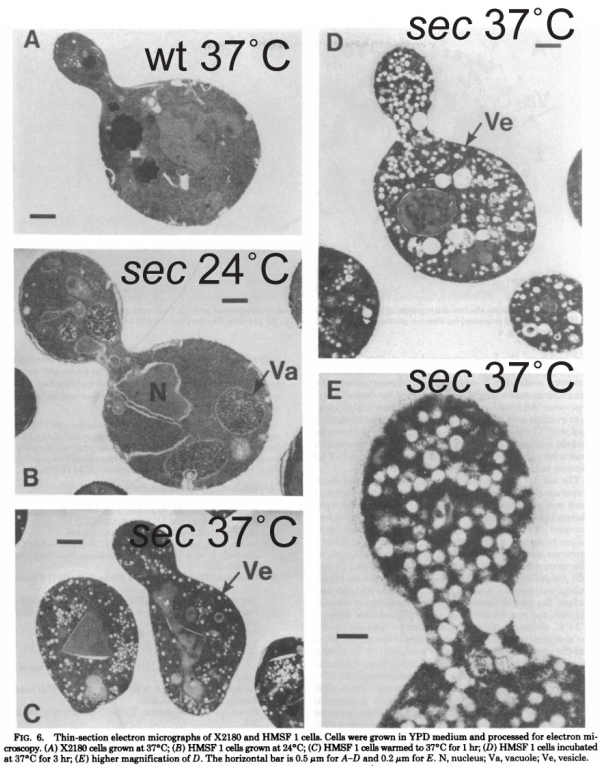
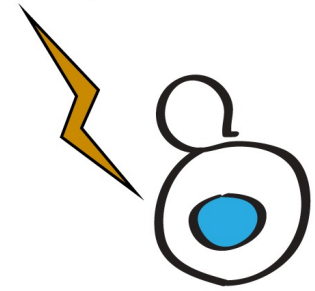


FIG. 6. Thin-section electron micrographs of X2180 and HMSF 1 cells. Cells were grown in YPD medium and processed for electron microscopy. (*A*) X2180 cells grown at 37°C; (*B*) HMSF 1 cells grown at 24°C; (*C*) HMSF 1 cells warmed to 37°C for 1 hr; (*D*) HMSF 1 cells incubated at 37°C for 3 hr; (*E*) higher magnification of *D*. The horizontal bar is 0.5 μm for *A–D* and 0.2 μm for *E*. N, nucleus; Va, vacuole; Ve, vesicle.

(Novick and Schekman, 1979)

Identification of 23 *sec* complementation groups

EMS/quench



Density gradient separation

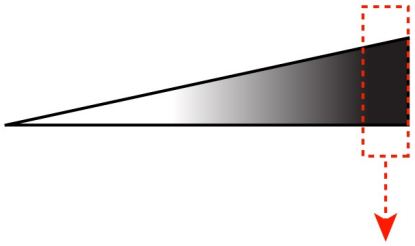


Table 1. Comparison of Screening Procedure with and without Density Enrichment

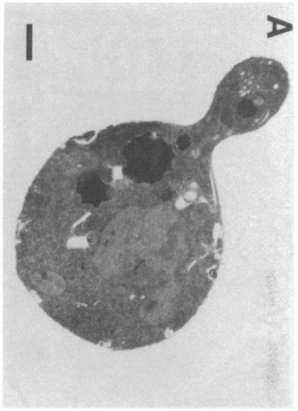
	Without Enrichment		With Enrichment	
Screening Stage	Colonies	%	Colonies	%
(1) Colonies tested	5,600	100	18,500	100
(2) TS mutants	291	5.2	2,830	15
(3) TS phosphatase secretion	63	1.1	980	5
(4) TS invertase secretion	16	.29	485	2.6
(5) TS invertase accumulation	2	.04	188	1.0

Table 2. Distribution of Mutants in the *sec*A Complementation Groups: EMS versus Nitrous Acid

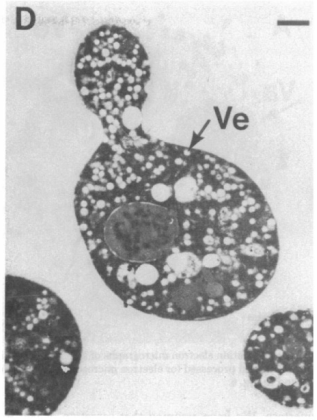
	EMS		Nitrous Acid	
sec	Isolates	%	Isolates	%
1	8	11	4	3
2	28	39	41	35
3	3	4	0	0
4	7	10	2	2
5	10	14	16	14
6	3	4	3	3
7	1	1	3	3
8	6	8	4	3
9	3	4	4	3
10	1	1	2	2
11	1	1	11	9
12	1	1	3	3
13			4	3
14			4	3
15			2	2
16			2	2
17			1	1
18			2	2
19			1	1
20			1	1
21			1	1
22			4	3
23			1	1

3 major *sec* mutant phenotypes

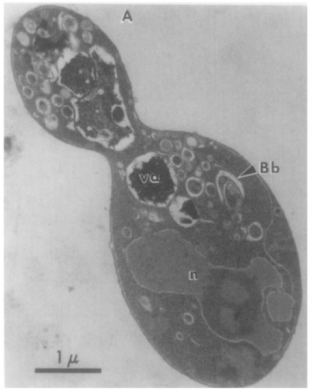
wt 37˚C



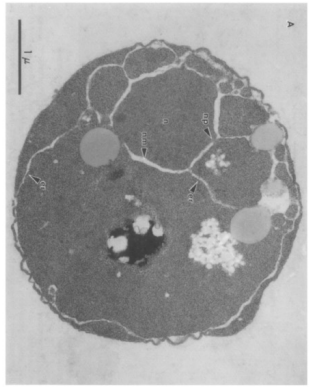
sec1 37˚C



sec7 37˚C



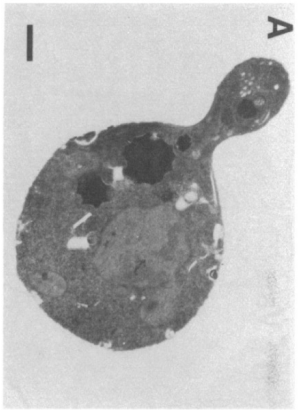
sec16 37˚C



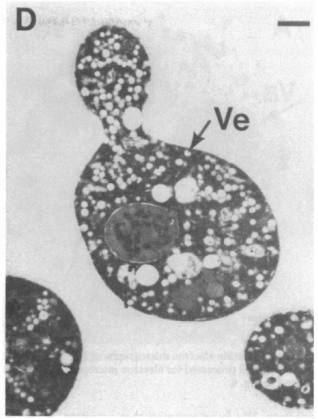
(Novick and Schekman 1979, Novick, Field and Schekman 1980)

Epistatic analysis: ordering the secretory pathway

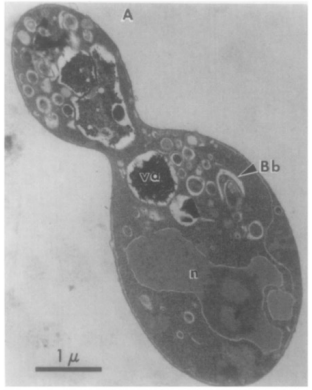
wt 37˚C



sec1 37˚C



sec7 37˚C



sec16 37˚C

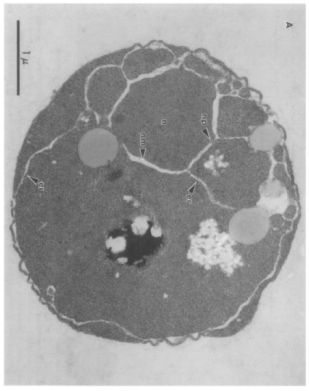


Table 1. Double-*sec*-Mutant Phenotypes[a]

	Single-Mutant Phenotype	Double-Mutant Phenotype with	
		sec7-1	*sec18-1*
Single-mutant phenotype		Bbs	ER and sv
sec1-1	ves	Bbs	ER and sv
sec2-56	ves and Bbs	Bbs	
sec3-2	ves	Bbs	
sec4-2	ves	Bbs	
sec5-24	ves	Bbs	
sec6-4	ves	Bbs	
sec7-1	Bbs		ER and sv
sec8-1	ves	Bbs	
sec9-4	ves and Bbs	Bbs	
sec10-2	ves	Bbs	
sec14-3	Bbs and ves	Bbs	
sec15-1	ves	Bbs	
sec19-1	ER and Bbs and ves and sv	Bbs and ER and sv	ER and sv
sec20-1	ER		ER and sv

[a] sv: small vesicles (40–60 nm). ves: vesicles (80–100 nm). Bbs: Berkeley bodies.

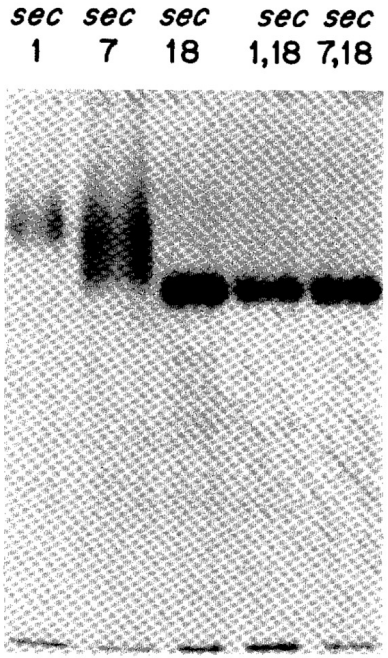


Figure 3. Immunoprecipitation of Invertase Accumulated in the Single and Double Mutants

(Lane 1) *sec1* (HMSF 1); (lane 2) *sec7* (HMSF 6); (lane 3) *sec18* (HMSF 176); (lane 4) *sec1*, *sec18* (SF 230-1); (lane 5) *sec7*, *sec18* (SF 231-1).

(Novick and Schekman 1979, Novick, Field and Schekman 1980, Novick, Ferro, and Schekman 1981)

The yeast secretory pathway

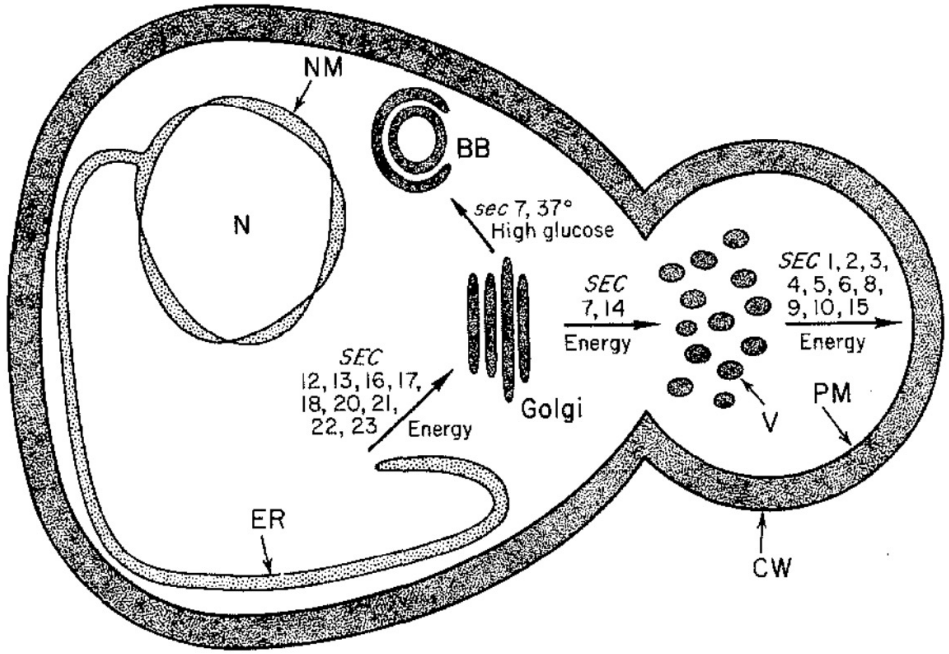
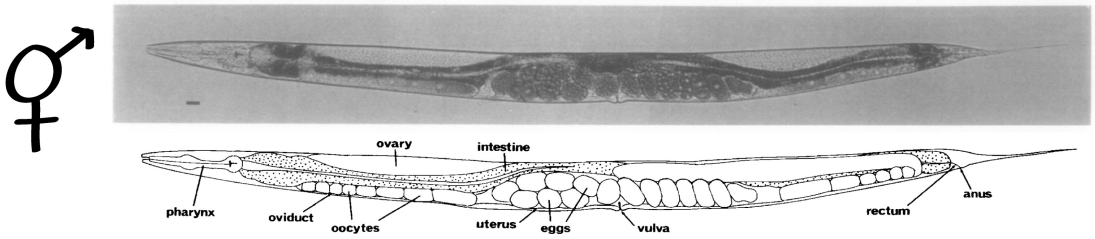


Figure 6. Yeast Secretory Pathway

N: nucleus. NM: nuclear membrane. ER: endoplasmic reticulum. *SEC*: wild-type gene product. *sec*: mutant gene product. V: vesicle. PM: plasma membrane. CW: cell wall. BB: Berkeley body.

Screen for genes involved in programmed cell death



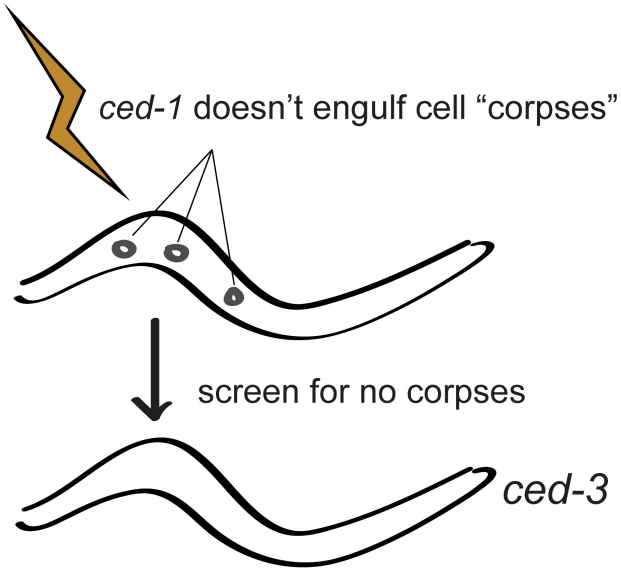
Sulston & Horvitz 1976

- Rapid life cycle
- Invariant lineage
    - Generation of 959 somatic nuclei is accompanied by generation and subsequent death of 131 cells

How to ID genes involved in programmed cell death?

Problem: Dying cells are rapidly engulfed in any developmental stage few if any dying cells can be seen. Direct observation in live animals is too slow

EMS *ced-1* individuals



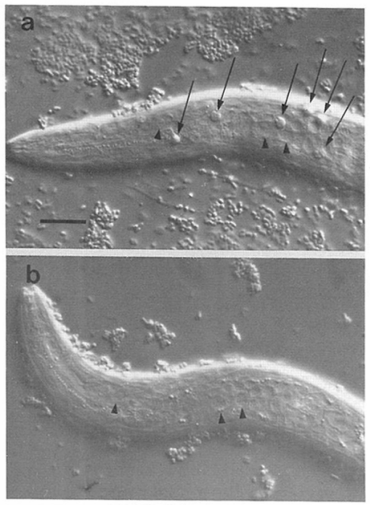


Figure 1. Absence of Cell Deaths in *ced-3* Animals

(a) Nomarski photomicrograph of a newly hatched *ced-1* larva. Arrows indicate dying cells. (b) Nomarski photomicrograph of a newly hatched *ced-1; ced-3* larva. Plane of focus is approximately that shown in (a). Arrowheads indicate several of the nuclei that can be seen in both (a) and (b). No cell deaths are seen in the *ced-1; ced-3* larva. Bar = 10 μ.

Ellis & Horvitz 1986

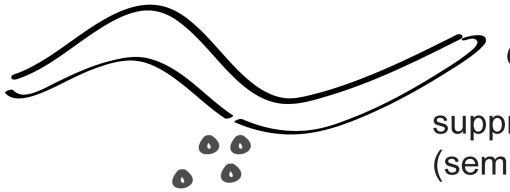
Suppressor screens can reveal additional genes in a pathway

egl-1 doesn't lay eggs

lacks two neurons that ennervate vulva

egl-1

are these neurons undergoing an inappropriate cell death program?



egl-1; ced-3

suppresses eggless phenotype (semi-dominant)

egl-1

screen eggless suppression

cross mutants to *ced-1* and look for lack of corpse accumulation

Identify several *ced-3* alleles as well as *ced-4* (recessive suppressor of *egl-1*)

Ellis & Horvitz 1986

Working out pathways from epistasis data

nuc-1 accumulates DNA in cells programmed to die (nuclease)
ced-1 and *ced-2* fail to engulf and clear corpses
ced-3 and *ced-4* do not initiate programmed cell death
egl-1 activates inappropriate cell death

ced-1; ced-2 double mutants are identical
ced-1; *nuc-1* double mutants have *ced-1* phenotype
ced-3 and *ced-4* mutants are epistatic to *ced-1* and *ced-2*
However, *ced-3* and *ced-4* are also epistatic to *egl-1*

egl-1 ⊣ *ced-9* ⊣

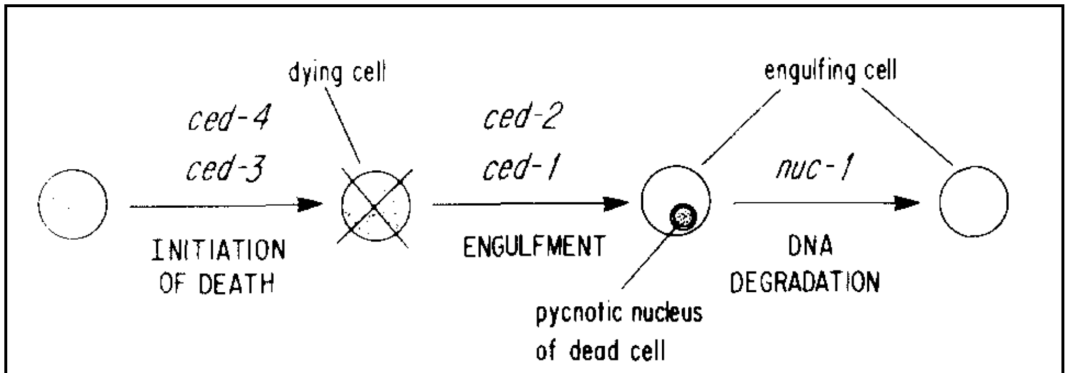


Figure 10. A Proposed Genetic Pathway for Programmed Cell Death in C. elegans

Ellis & Horvitz 1986

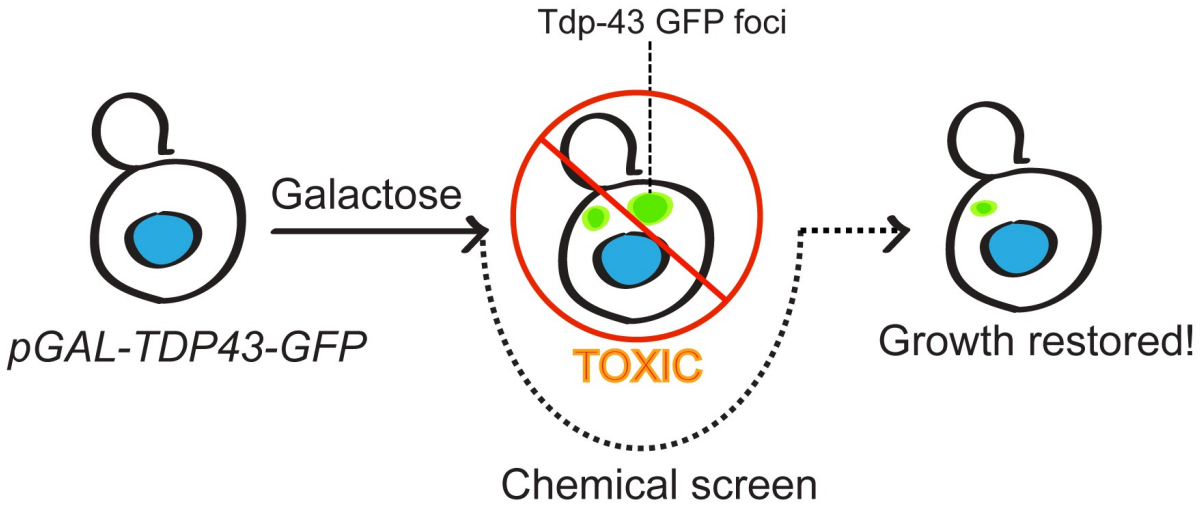
Epistasis analysis general rules

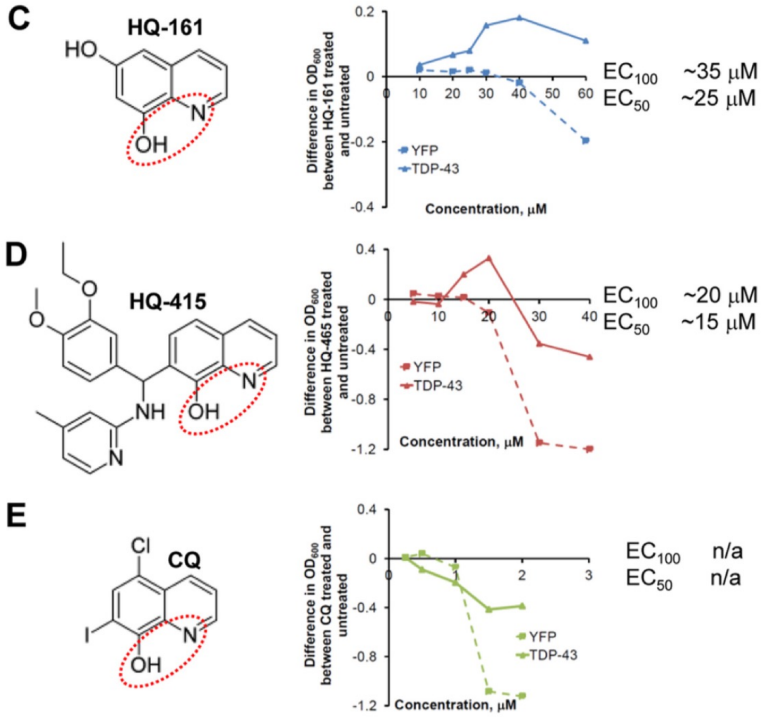
- The epistatic mutation is the one whose phenotype is displayed in the doubly mutant animal; the mutation whose phenotype is not displayed is hypostatic to the other.

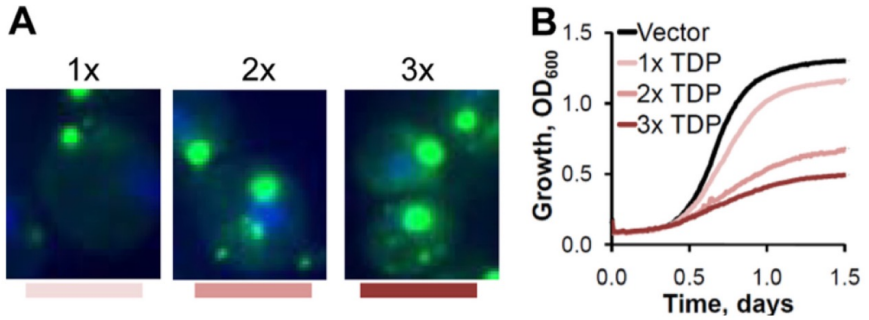
- In a chemical synthesis pathway- the epistatic mutation defines the upstream gene.

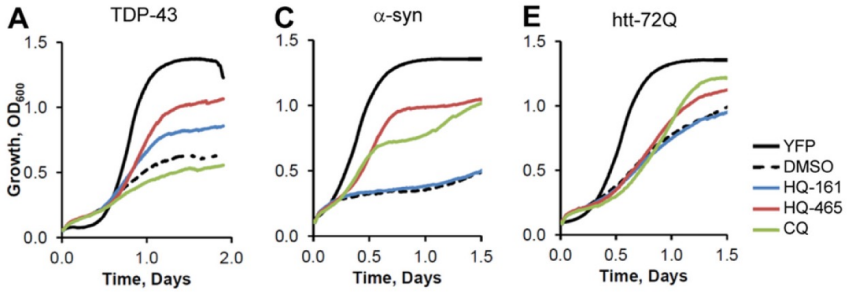
- In an on/off switch pathway with a binary output, the epistatic mutation defines the downstream gene.
  - The two mutations must act oppositely in order for the analysis to be interpretable

Chemical screening in yeast



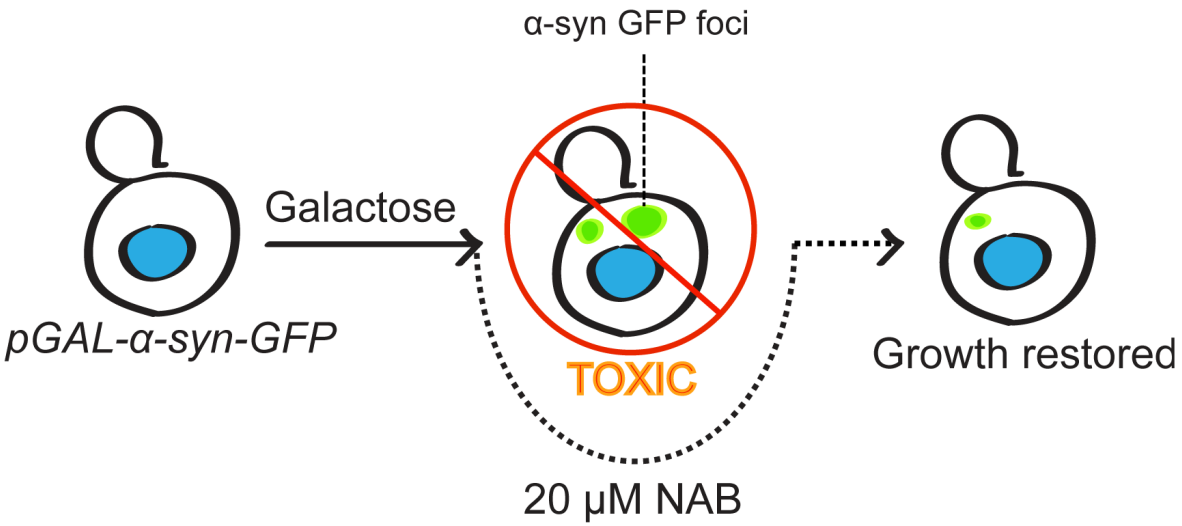


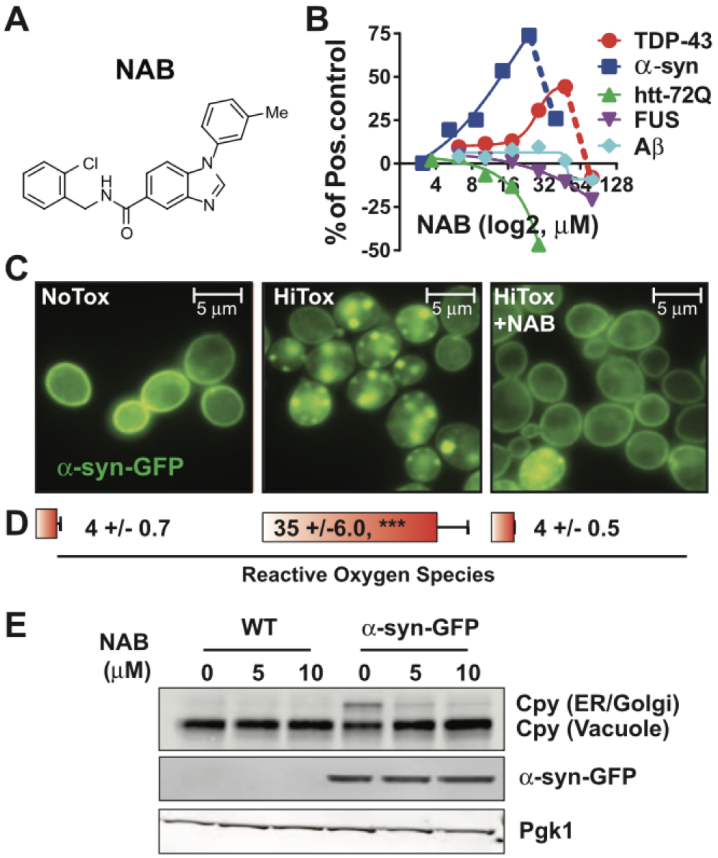




(Tardiff et al., 2012)

Chemical screening in yeast



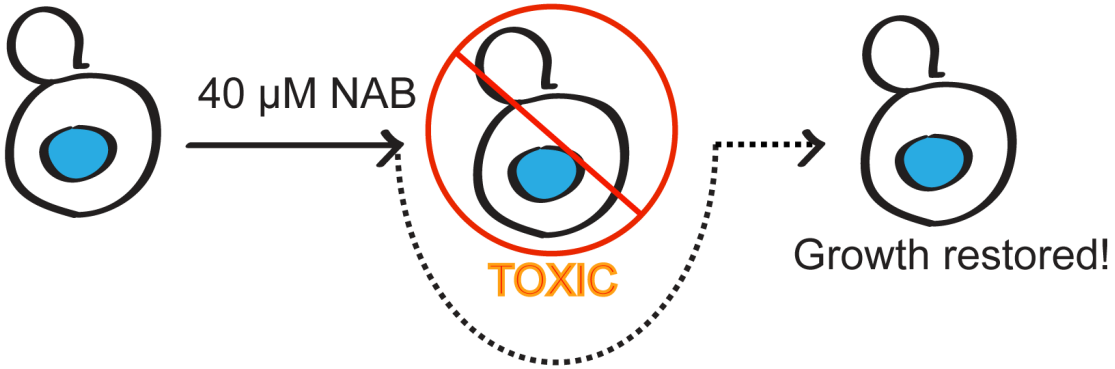


Also works in worm and cell culture Parkinson's models

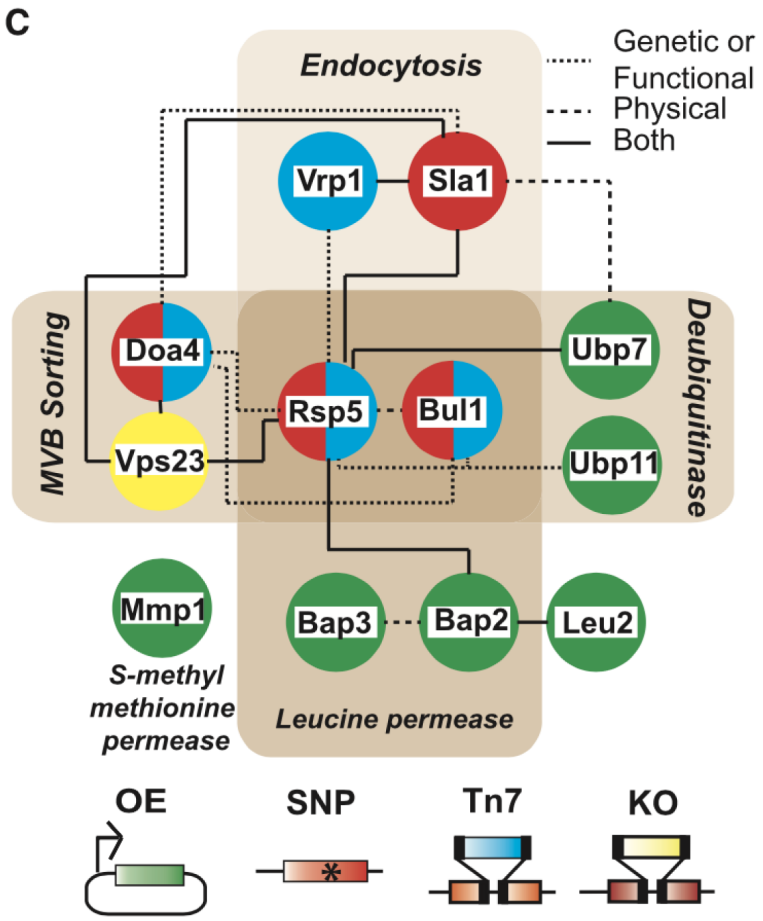
(Tardiff et al., 2013)

Genetic suppression of a drug-induced phenotype

Genetically probe drug interactions



- Overexpression library (~5,800 genes)
- Random transposon insertions (~300,000 lines)
- Spontaneous point mutations (~2,000,000 cells)



(Tardiff et al., 2013)

The search for downstream effectors of RAS

Cell, Vol. 50, 277–287, July 17, 1987, Copyright © 1987 by Cell Press

Three Different Genes in S. cerevisiae Encode the Catalytic Subunits of the cAMP-Dependent Protein Kinase

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Summary

We have isolated three genes (*TPK1*, *TPK2*, and *TPK3*) from the yeast S. cerevisiae that encode the catalytic subunits of the cAMP-dependent protein kinase. Gene disruption experiments demonstrated that no two of the three genes are essential by themselves but at least one *TPK* gene is required for a cell to grow normally. Comparison of the predicted amino acid sequences of the *TPK* genes indicates conserved and variable domains. The carboxy-terminal 320 amino acid residues have more than 75% homology to each other and more than 50% homology to the bovine catalytic subunit. The amino-terminal regions show no homology to each other and are heterogeneous in length. The *TPK1* gene carried on a multicopy plasmid can suppress both a temperature-sensitive *ras2* gene and adenylate cyclase gene.

(Toda et al., 1987), and the *PDE1* and *PDE2* genes, which encode cAMP phosphodiesterases (Sass et al., 1986; Nikawa et al., unpublished data). In this paper we present the nucleotide sequence of the genes for the cAMP-dependent protein kinase catalytic subunits, which are encoded by three similar but distinct genes (*TPK1*, *TPK2*, and *TPK3*). We also present biochemical and genetic analyses of the cAMP-dependent protein kinase system in yeast.

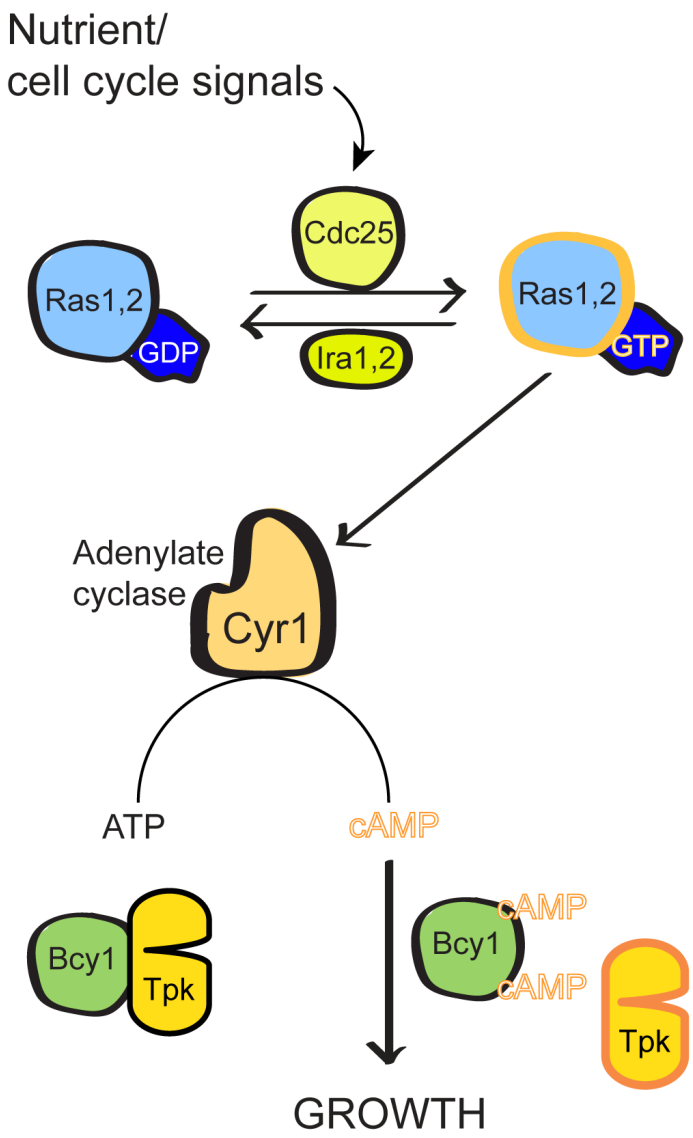
Results

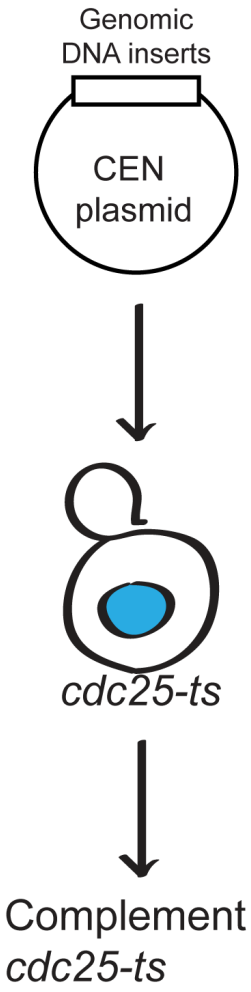
Isolation of the *TPK1* Gene

We transformed a temperature-sensitive *cdc25* strain, TT25-6 (see Table 1), with pooled DNA from a S. cerevisiae genomic library that had been constructed in the centromere-containing *URA3* vector YCp50 (kindly provided by M. Rose and G. Fink). Transformants were directly incubated at 35°C on synthetic plates lacking uracil. Colonies that could grow at 35°C were picked and plasmid segregation analysis was performed. Transformants whose growth at 35°C was plasmid-dependent were grown, and their plasmids were recovered in E. coli. Two different suppressor plasmids were obtained. One of these plasmids was shown to be allelic to the *CDC25* locus by an integrative mapping method (Broek et al., 1987). The other sup-

Overexpression suppressor screens/subcloning

Ras signaling cascade





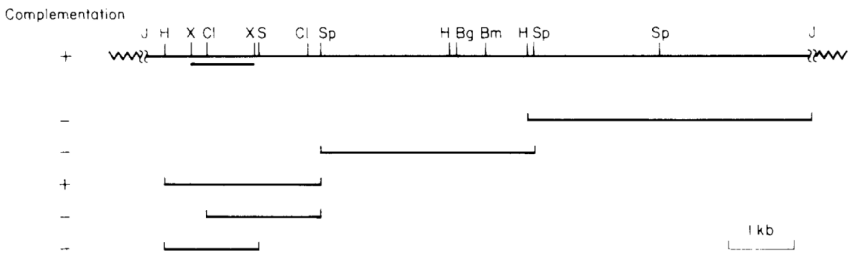


Figure 1. Restriction Map and Subcloning Analysis of *TPK1*

Structure and subcloning results of suppressor sequence *TPK1* are shown. Each fragment indicated in the figure was inserted into YEp13 (Broach et al., 1979) or YEp213 (Sherman et al., 1982). The resultant subclones were transformed into TT25-6 (temperature-sensitive *cdc25* strain; see Table 1) and suppression of temperature sensitivity was examined. "J" represents a junction between an insert yeast DNA and the vector. Abbreviations used are as follows: Bg, BglII; Bm, BamHI; Cl, ClaI; S, SalI; Sp, SphI; H, HindIII; X, XbaI. Only the 2.4 kb HindIII–SphI fragment (the left-most fragment in the figure) was mapped with ClaI, SalI, and XbaI. The 1.0 kb XbaI fragment that was used as a probe for genomic Southern hybridization is underlined (see the text and Figure 2).

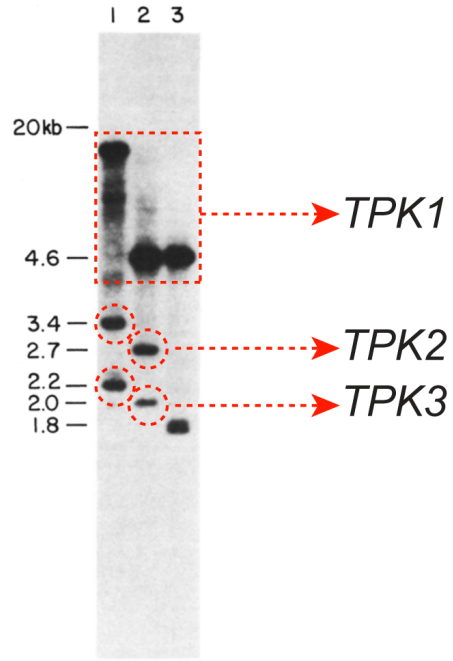


Figure 2. Genomic Southern Hybridization with *TPK1* Probe

(Toda et al., 1987)