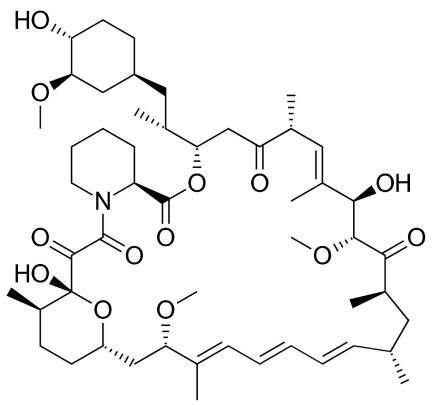
# Cell Biology Screens

# Target of rapamycin screen 1

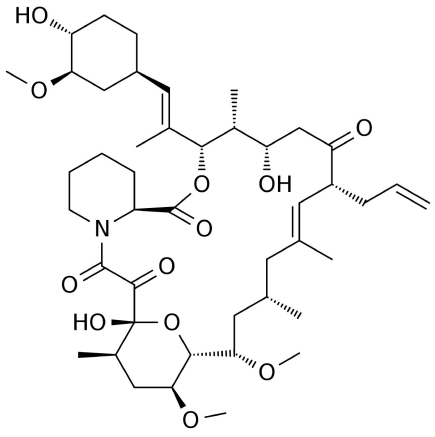
What are rapamycin/FK-506 and why are they important?

- Antifungal agents
  - Produced by *Streptomyces*
- Immunosuppressant functions
  - Inhibit T-cell and B-cell activation
  - Useful in organ transplants (esp kidney)

Rapamycin



FK-506



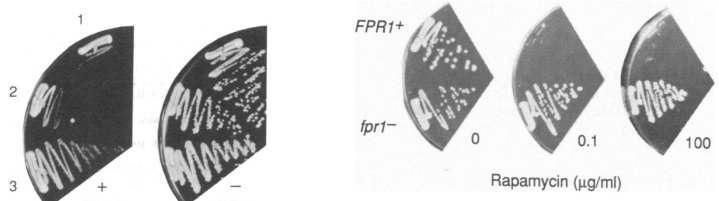
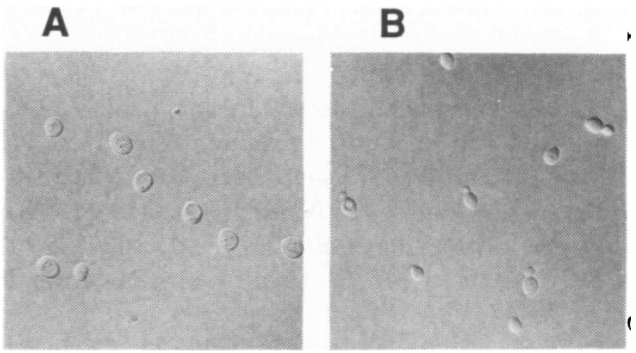



FIG. 5. Diploid FKBP-deficient strains are FK 506 resistant. The homozygous diploid parent strain JK9-3d **a**/α (*FPR1*/*FPR1*) (strain 1), the isogenic heterozygous diploid strain JHY1 bearing one disrupted *FPR1* allele (*FPR1*/*fpr1-1*) (strain 2), and the isogenic homozygous diploid JHY5 (JHY1-2a × JHY1-2c) in which both *FPR1* alleles are disrupted (*fpr1-1*/*fpr1-1*) (strain 3) were grown for 60 hr at 30°C on YPD medium with or without FK 506 at 50 μg/ml.




**Fig. 1.** Yeast strains that lack FKBP are resistant to rapamycin toxicity. Isogenic haploid strains that express (*FPR1*$^+$) or lack (*fpr1*$^-$) the FKBP proline isomerase were grown for 65 hours at 30°C on YEPD medium (*32*) containing rapamycin at 0, 0.1, or 100 μg/ml, prepared as described for FK506 medium (*16*). The *fpr1-1*, *fpr1-2*, and *fpr1-3* alleles (*16*) all conferred resistance to rapamycin. The strains are JK9-3dα (*FPR1*$^+$) and JH3-3b (*fpr1*$^-$) (*17*).




Heitman et al., 1991

# Target of rapamycin screen 2:
# FK506 and rapamycin act through distinct mechanisms

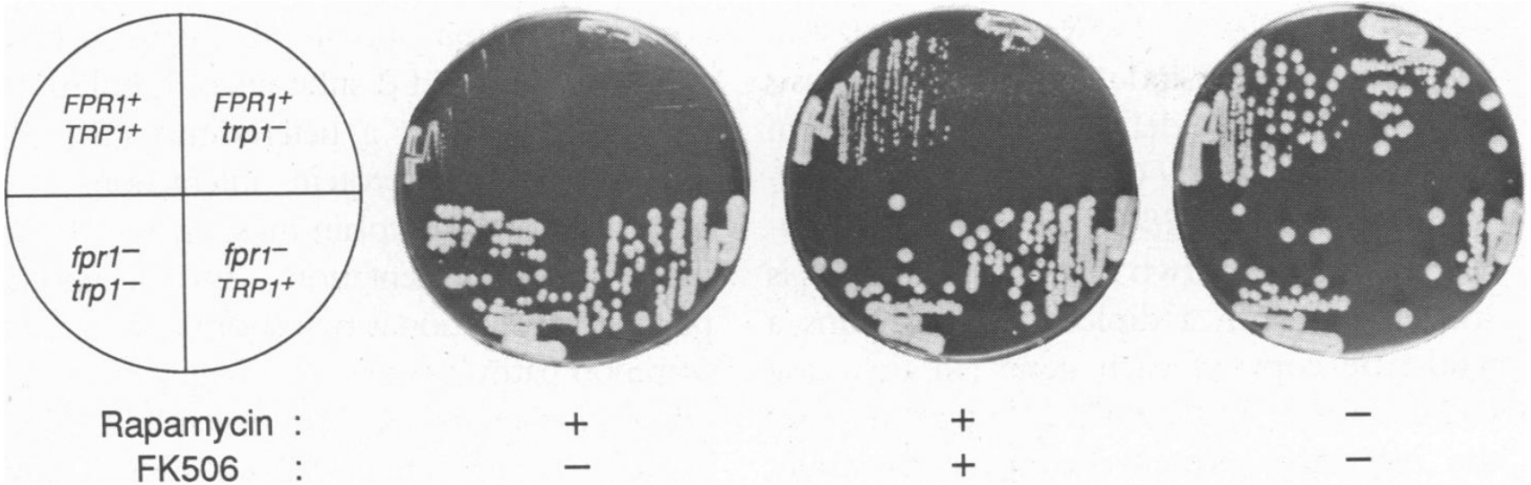
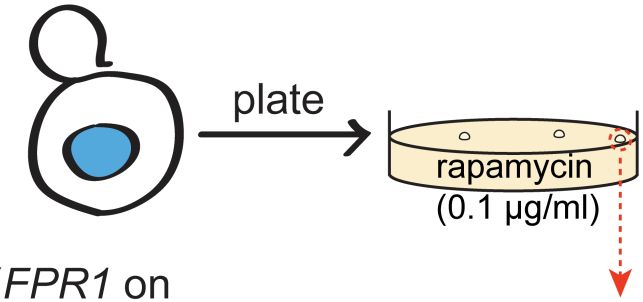



**Fig. 3.** Antagonism of rapamycin toxicity by FK506. Isogenic haploid derivatives of strain JK9-3d (*17*) were grown for 96 hours at 30°C on YEPD medium (*32*) that contained either rapamycin (0.1 μg/ml), rapamycin (0.1 μg/ml) plus FK506 (50 μg/ml), or neither drug (presence or absence indicated by + or −). Antagonism is seen with the *FPR1*$^+$ *TRP1*$^+$ strain JH6-1b (upper left quadrant); this is the only strain that is both sensitive to rapamycin (*FPR1*$^+$) and resistant to the toxic effects of FK506 (*TRP1*$^+$) (*16, 19*). All other strains are controls. The strains are JH6-1b (*FPR1*$^+$ *TRP1*$^+$), JK9-3dα (*FPR1*$^+$ *trp1*$^-$), JH10 (*fpr1*$^-$ *TRP1*$^+$), and JH3-3b (*fpr1*$^-$ *trp1*$^-$) (*17*).

Heitman et al., 1991

# Target of rapamycin screen 3: rapamycin toxicity requires functional *TOR* genes



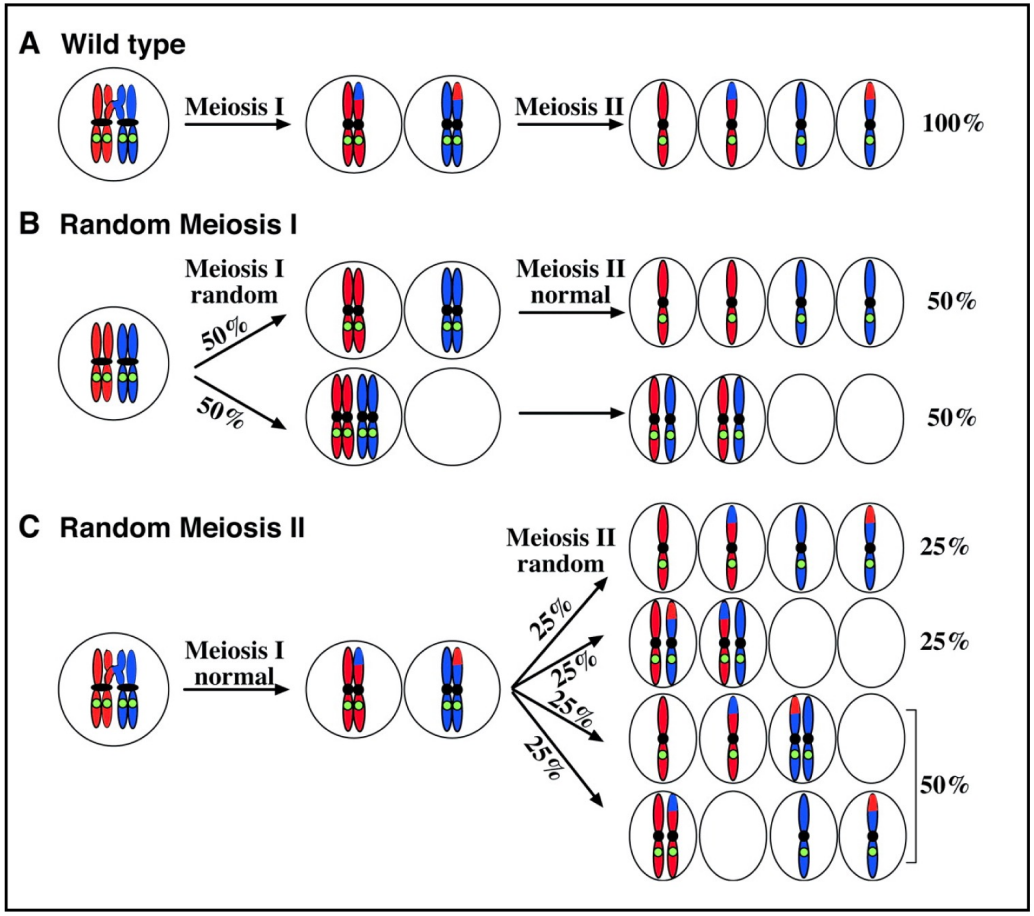

(*FPR1* on multicopy plasmid)

- cross to wild type
- complementation analysis

| Allele | Growth on rapamycin (µg/ml) | | | | FKBP sequence |
|---|---|---|---|---|---|
| | 0 | 0.1 | 1 | 10 | |
| *fpr1-4* | + | + | + | − | M1I |
| *fpr1-5* | + | + | + | − | P23R |
| *fpr1-6* | + | + | +/− | − | H32P |
| *fpr1-7* | + | + | n.d. | n.d. | G35D |
| *fpr1-8* | + | + | + | + | G65D |
| *fpr1-9* | + | + | −/+ | − | G65C |
| *fpr1-10* | + | + | + | −/+ | G65V |
| *fpr1-11* | + | + | +/− | − | L81S |
| *fpr1-12* | + | + | + | + | E109ochre (UAA) |
| *tor1-1* | + | + | + | + | WT |
| *TOR1-2* | + | +/− | +/− | −/+ | WT |
| *tor2-1* | + | + | + | + | n.d. |
| *fpr1-3* | + | + | + | + | *fpr1::URA3-3* |
| *FPR1* | + | − | − | − | WT |

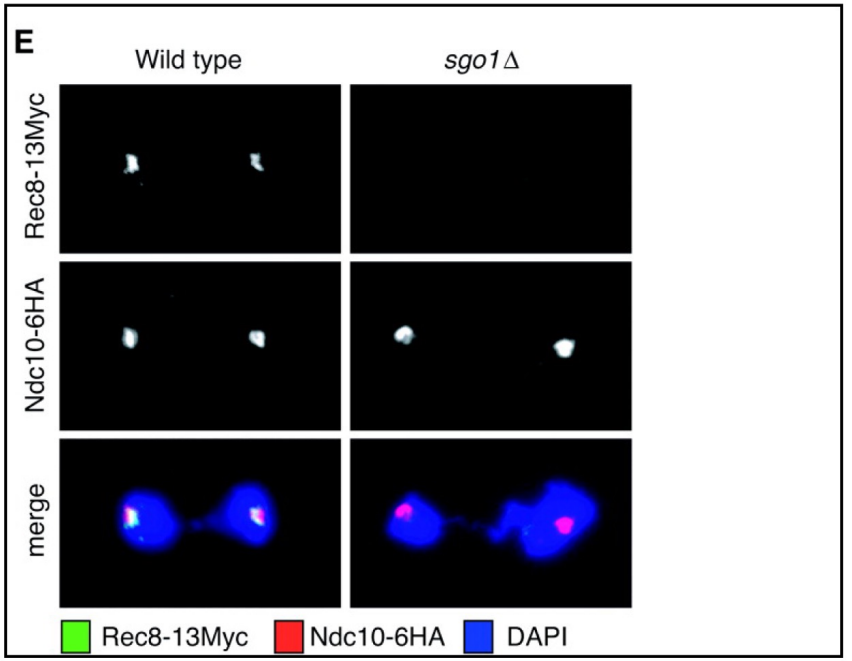
| diploid | rap phenotype |
|---|---|
| *fpr1-3* / *FPR1* | sensitive |
| *fpr1-6* / *fpr1-3* | ~ resistant |
| *fpr1-12* / *fpr1-3* | resistant |
| *tor1-1* / *fpr1-3* | sensitive |
| *TOR1-2* / *fpr1-3* | resistant |
| *TOR1-2* / *FPR1* | resistant |
| *tor1-1* / *TOR1-1* | resistant (segregates 4R:0S) |
| *tor1-1* / *tor2-1* | resistant (non-allelic non complementation) |
| *tor2-1* / *fpr1-12* | resistant (non-allelic non complementation) |

# Phenotypic screens vs. selection screens



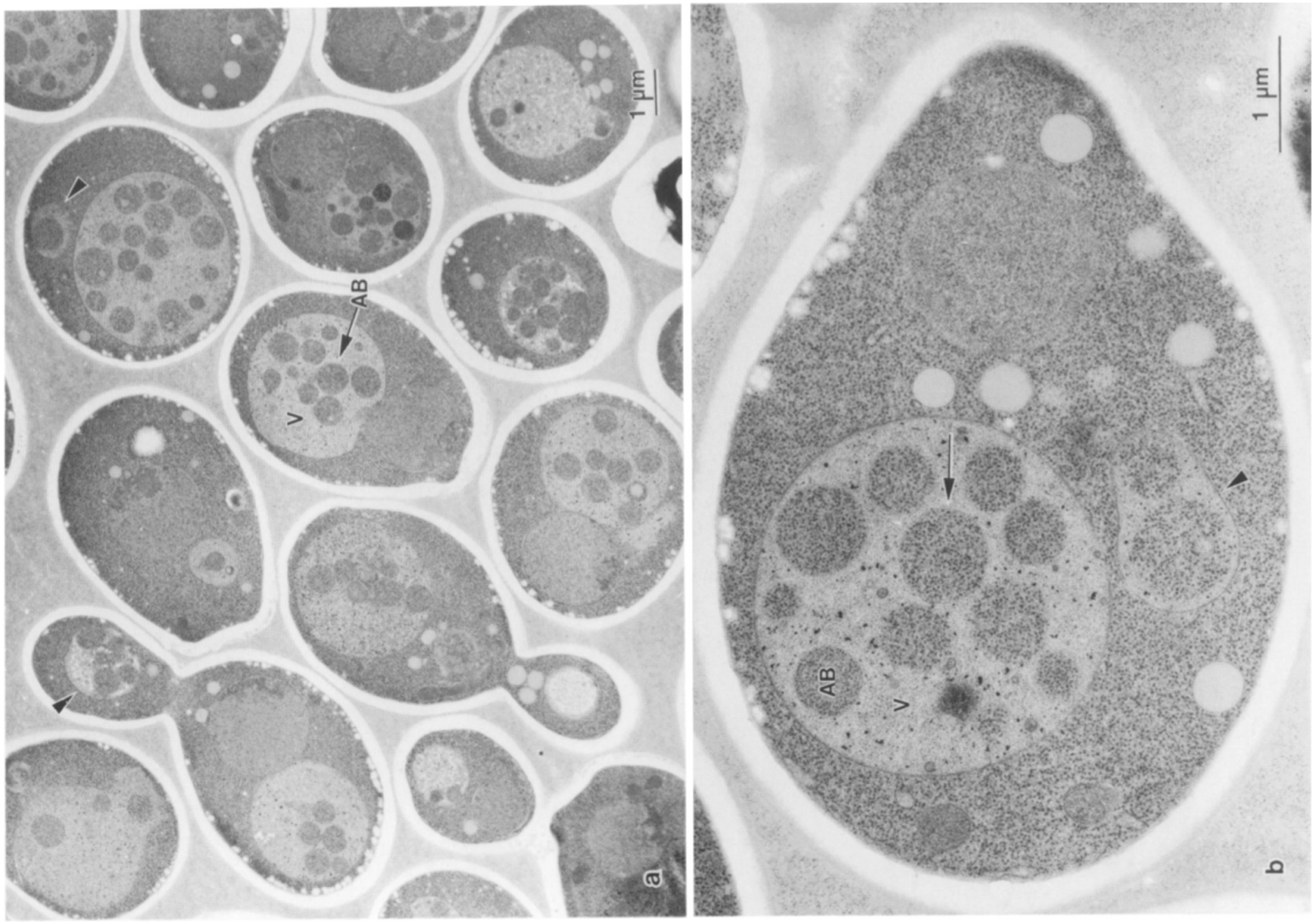

A

| Genotype |  |  |  | other |
|---|---|---|---|---|
| Wild Type | 99.5% | 0.5% | | |
| *sgo1*Δ | 21.6% | 43.1% | 20.1% | 15.2% |
| *iml3*Δ | 60.5% | 29.0% | 10.5% | |
| *chl4*Δ | 61.6% | 29.3% | 8.6% | 0.5% |



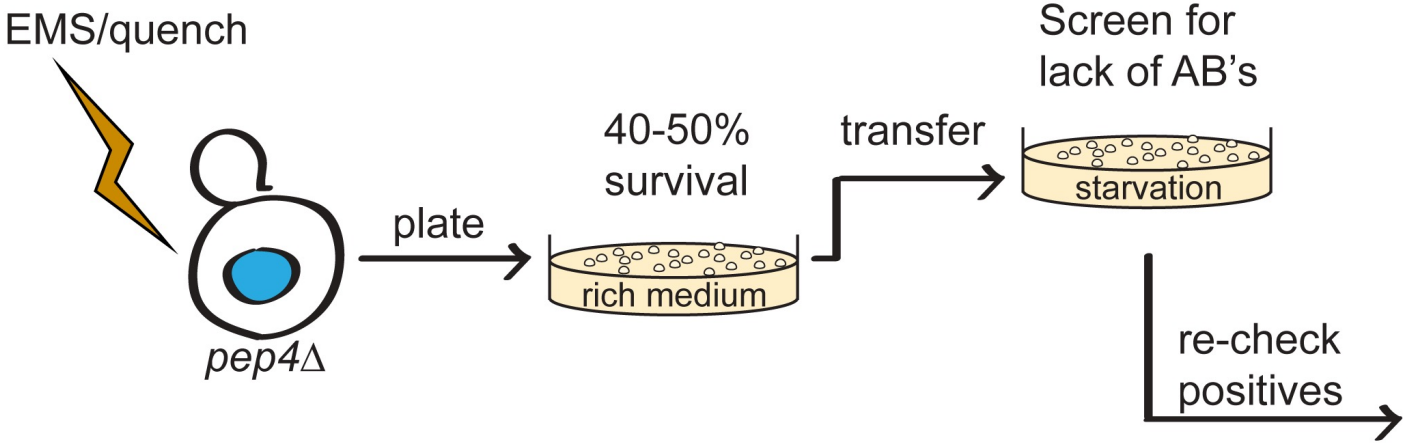

(Marston et al., 2004)

# Autophagy screen 1:
# protease-deficient cells accumulate autophagic bodies




(Takeshige et al., 1992)

# Autophagy screen 2:
# Isolation of the first autophagy mutant



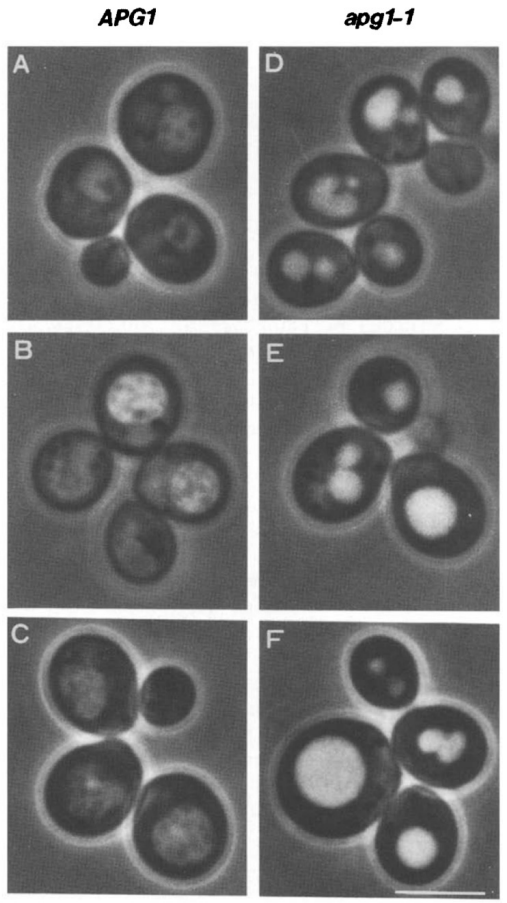




Fig. 1. Morphological changes of vacuoles during nitrogen starvation. Phase contrast microscopic images of cells of X2180-1A (*APG1*, left), and MT14-1B (*apg1-1*, right) incubated in SD (-N) medium containing 1 mM PMSF for 2 h (A,D), 4 h (B,E), and 8 h (C,F). Bar = 5 μm.

(Tsukada and Ohsumi, 1993)

# Autophagy screen 3:
# Characteristics of autophagy mutants

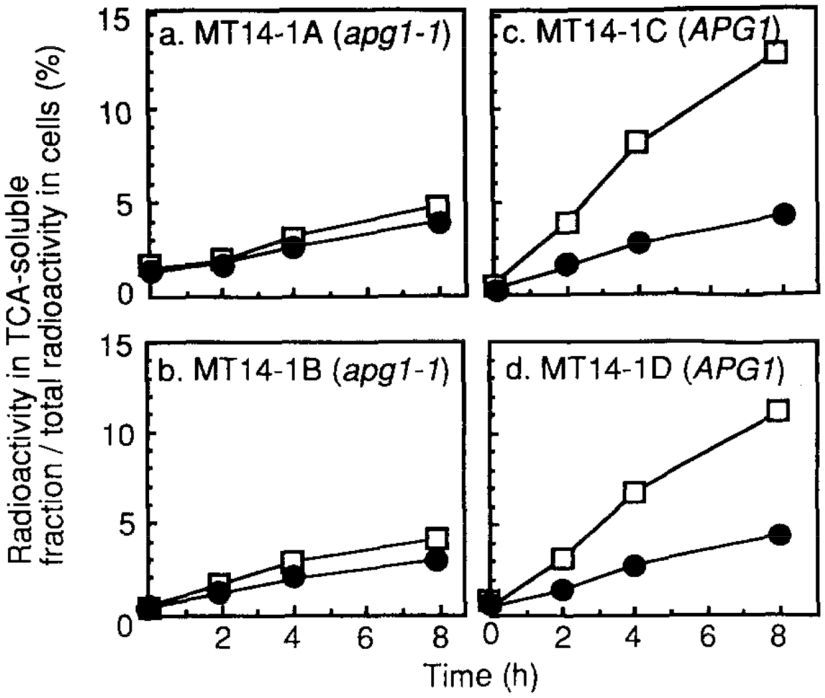



Fig. 2. Protein degradation in cells in nitrogen starvation medium. Radioactivities in the TCA-soluble fraction and in total cells were measured as described in section 2. With (●) and without 1 mM PMSF (□). (a) MT14-1A (*apg1-1*), (b) MT14-1B (*apg1-1*), (c) MT14- 1C (*APG1*), and (d) MT14-1D (*APG1*) are segregants from X2180-1A/ MT13-8C (*APG1/apg1-1*).

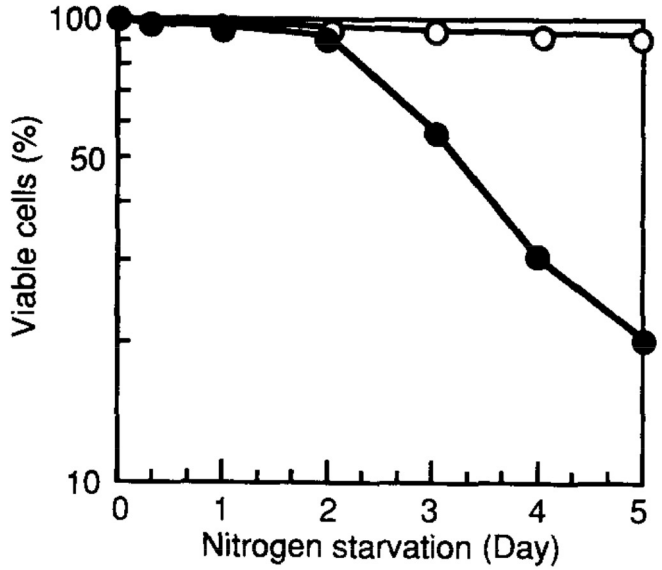
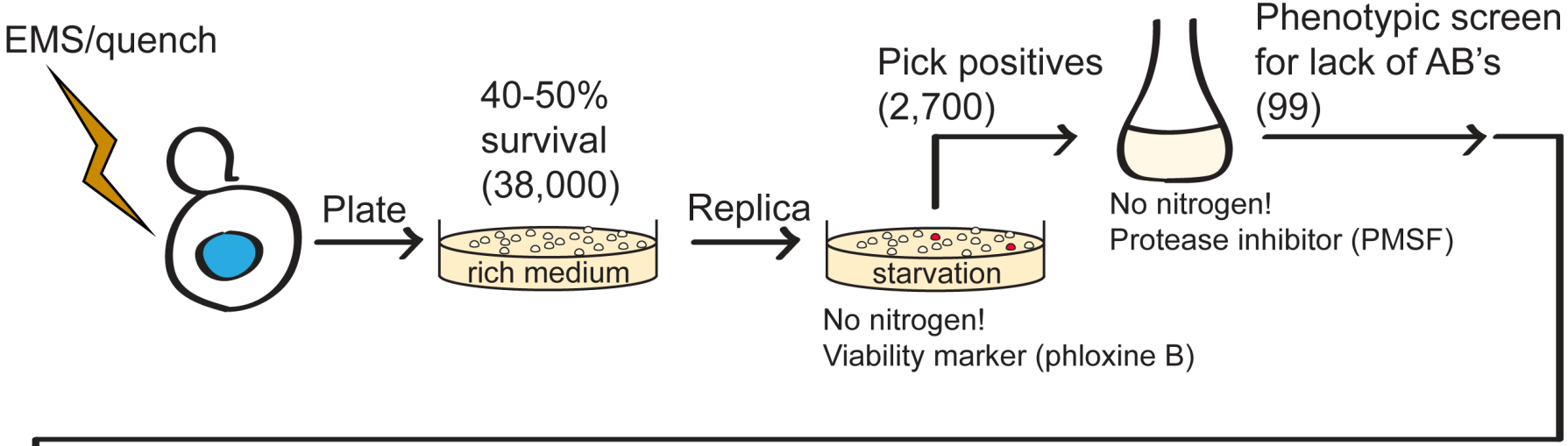



Fig. 3. Loss of viability of the *apg1* mutant in nitrogen starvation medium. Exponentially growing cells of X2180-1A (*APG1*, ○), and MT14-1B (*apg1-1*, ●) were transferred to SD (-N) medium. At the indicated times, viability was determined as described in section 2. 400–600 cells were counted at each time point. Data are averages of 3 determinations.

(Tsukada and Ohsumi, 1993)

# Autophagy screen 4:
# *E unum pluribus*: Isolation of 15 autophagy mutants




15 genes

Table I

Distributions of *apg* mutations in 15 complementation groups

| | |
|---|---|
| *apg1* | 15 |
| *apg2* | 13 |
| *apg3* | 10 |
| *apg4* | 9 |
| *apg5* | 5 |
| *apg6* | 4 |
| *apg7* | 4 |
| *apg8* | 4 |
| *apg9* | 4 |
| *apg10* | 3 |
| *apg11* | 2 |
| *apg12* | 1 |
| *apg13* | 1 |
| *apg14* | 1 |
| *apg15* | 1 |
| Total | 77 |

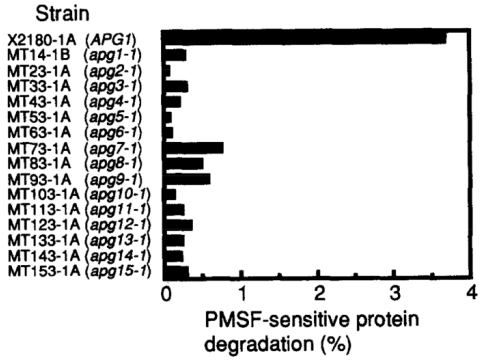



Fig. 4. PMSF-sensitive protein degradation of *apg* mutants in nitrogen starvation medium. PMSF-sensitive protein degradations in cells incubated in SD (-N) medium for 8 h are shown. PMSF-sensitive protein degradation was determined as described for Fig. 2. Total protein degradation in each *apg* mutants ranged to 28–36% of that in wild-type cells (see also Fig. 2). The strains used were representatives of each *apg* mutation.

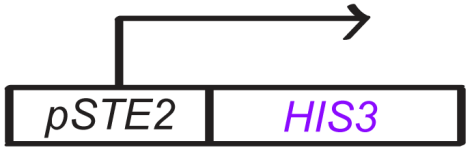
(Tsukada and Ohsumi, 1993)

# Using the yeast deletion collections

Gene disruption cassette


Each strain (~4,500 total) contains one gene disruption cassette

Useful selection cassettes




Allows for selection of particular mating types


Allows for selection of haploids/diploids

# Plasmid Shuffle/rescue