after only 1 h on glucose, FBPase protein is almost completely lost in wild-type and the *pra1*-deleted strain (part *a* in Fig. 1). From this experiment we conclude that catabolite degradation of FBPase is not dependent on vacuolar proteolysis and thus not localized to the vacuole.

The proteasome is a major, non-vacuolar proteinase, highly conserved in all eukaryotes[5]. This enzyme complex is localized in the cytoplasm and in the nucleus of cells[5], and is involved in the degradation of ubiquitinated and short-lived proteins *in vivo*[6]. When using the same derepression and inactivation protocol for FBPase as described in part *a of* Fig. 1, we found that mutants (*pre1-1* and *pre1-1 pre2-1*) (part *b*) defective in the chymotrypsin-like activity of the proteasome show a dramatic retardation of glucose-triggered FBPase degradation. In the double mutant (*pre1-1 pre2-1*) defective in two subunits of the proteasome necessary for the chymotrypsin-like activity, degradation of FBPase is nearly absent (part *b*). From these experiments we conclude that glucose-triggered degradation of FBPase is mediated by the proteasome.

Why do our results differ from those in ref. 4? We repeated the catabolite inactivation experiments with the wild-type and *pep4*-deleted strains (W303IB and W303IBF) used in ref. 4 under the same growth conditions. None of the experiments provided evidence for a proteinase yscA-dependent catabolite degradation of FBPase. However, when overexpressing FBPase on the multicopy plasmid, using immunofluorescence we found FBPase in the vacuole of *pra1 (pep4)*-deleted cells without glucose addition. Also after starvation for nitrogen, FBPase is found in the vacuole[7]. From this, we conclude that FBPase enters the vacuole under stress and starvation. The most plausible mechanism for this phenomenon at present is uptake of the enzyme into the vacuole via autophagocytosis[7].

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CHIANG AND SCHEKMAN REPLY — Because of the apparent contradiction between our results and those of Schork *et al.*, we performed the basic catabolite inactivation experiment using cells (chromosomal copy of FBPase gene only) grown in six different poor-carbon-source media followed by transfer to glucose medium (see Fig. 2). Cell samples were processed and FBPase detected by immunoblotting as before[4]. Condition A is the regimen we reported earlier[4]; the other regimens have since been found to

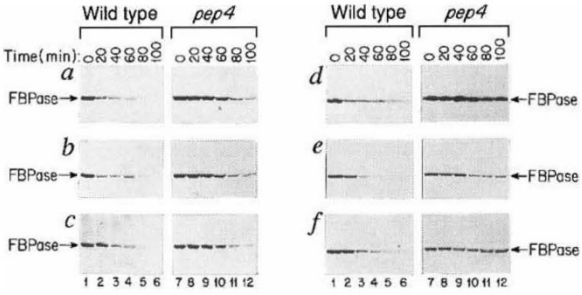

FIG. 2 Glucose-induced degradation of FBPase is dependent on the *PEP4* gene. Untransformed wild type cells (W3031B, *MATa, leu2–3,–112, ura3, ade2, his3, trp1*), and *pep4* (W303BF, *MATa, leu2-3,–112, ura3, ade2, his3, PEP4:TRP1*), were grown under various growth conditions and transferred to YP (1% Bacto-yeast extract, 2% Bacto-peptone) containing 2% glucose for 0, 20, 40, 60, 80 and 100 min. Cell extracts were obtained and FBPase examined on SDS-PAGE by immunoblotting with affinity-purified anti-FBPase antibodies[4]. *a*, YP containing 1% potassium acetate and 1% glucose for 48 h; *b*, 1% potassium acetate and 0.5% glucose for 48 h; *c*, 1% potassium acetate and 0.1% glucose for 48h; *d*, YPD (dextrose) for 24 h and adapted to YPO (2 % oleic acid) for additional 24 h; *e*, YP containing 2% pyruvate for 48 h; *f*, YP containing 2% galactose for 48 h.

be equally or even more effective in revealing the stabilization of FBPase in *pep4* mutant cells. Furthermore, we find by immunofluorescence microscopy that the alternative conditions of carbon source transfer confirm our conclusion concerning the transport of FBPase from the cytosol to the vacuole during catabolite inactivation in *pep4* mutant cells (H-L.C., in preparation).

Schork *et al.* conclude that FBPase is degraded in the cytosol because mutants defective in two subunits of the cytosolic proteosome fail to engage in catabolite-mediated degradation of FBPase. We agree that *pre1* and *pre2* mutations affect FBPase degration, but we feel it is premature to base the conclusion of cytosolic degradation of FBPase on the phenotype of two mutants known to be pleiotropic.

Ubiquitination of protein substrates which regulates proteosome-mediated protein degradation, is required in the biogenesis and function of certain cytoplasmic organelles. Ubiquitin conjugating enzymes have been localized to the endoplasmic reticulum, Golgi and peroxisome, and protein–ubiquitin conjugates are found in the yeast vacuole[8,9]. Note that a mammalian cell-cycle mutant defective in ubiquitin conjugation displays deficient autophagic degradation of protein substrates in the lysosome[10]. We have found that ubiquitin conjugation mutants (*ubc1, 4*,and *5*), as well as a variety of other mutants that affect membrane biogenesis (secretion, *sec62, 18, 7*; endocytosis, *end1, 3*; peroxisome assembly, *pas1, 2, 3*) are defective in catabolite-mediated FBPase degradation (ref. 4; our manuscript in preparation). These results are inconsistent with a cytosolic location of the FBPase degradation event, but instead suggest a ubiquitin-regulated autophagic process whereby FBPase is sequested in an autophagosome which is then degraded in the vacuole. This model accounts for our observation that FBPase is transported from the cytosol into the vacuole when *pep4* mutant cells are transferred into glucose medium[4].

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Invastion test

SIR — Butcher and Deng[1] state that I argued in my News and Views article[2] that high inbreeding would be required to prevent the invasion of SisterKillers. They go on to show that this assertion is wrong. I however made no statement of the required invasion conditions. I did argue that to test Haig's model[3] it would be useful to examine heavily inbred organisms as these are the least vulnerable to SisterKillers. I appreciate that Butcher and Deng analytically show that this statement is correct. Although Haig's proposal would be strengthened by a dynamic analysis, his argument is well founded, and Butcher and Deng are mistaken to say that my acceptance of its logic was premature.

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