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Expression of Hailey–Hailey Disease Mutations in Yeast

To the Editor:

The yeast *Saccharomyces cerevisiae*, with its compact genome and tractable genetics, is a valuable model organism for the heterologous expression of mammalian proteins. In this letter, we show that phenotypic screening in yeast provides insight into the molecular basis of a human disease.

Hailey–Hailey disease (HHD), or benign familial pemphigus, is an autosomal dominant intraepidermal blistering skin disorder. HHD has been mapped to *ATP2C1* (Hu *et al*, 2000; Sudbrak *et al*, 2000), which encodes the *S*ecretory *P*athway *C*a^{2+}–*A*TPase 1 (hSPCA1). This protein shares 49% identity with Pmr1, the founding member of a family of Golgi-localized Ca^{2+} and Mn^{2+}-transporting pumps (Rudolph *et al*, 1989; Sorin *et al*, 1997). The phenotypes of *pmr1* mutants are similar to the ion homeostasis and cell surface abnormalities reported in HHD keratinocytes (Hu *et al*, 2000; Sudbrak *et al*, 2000). Defects in protein sorting, processing and glycosylation in *pmr1* mutants result from Ca^{2+} and Mn^{2+} deficiency within the secretory pathway (Antebi & Fink, 1992; Durr *et al*, 1998). Such defects can be assessed by hypersensitivity to cation chelators (BAPTA or EGTA) (Rudolph *et al*, 1989; Wei *et al*, 2000). In addition, *pmr1* mutants fail to clear calcium and manganese from the cytosol, thus their growth is inhibited by excess extracellular levels of these ions (Halachmi and Eilam, 1996; Wei *et al*, 2000). Quantitative growth assays in BAPTA- or Mn^{2+}- supplemented media have been very effective in screening Pmr1 site-directed point mutations. These approaches identified the critical role of membrane helices M4, M5, and M6 in ion binding, transport, and Ca^{2+}/Mn^{2+} selectivity (Mandal *et al*, 2000, 2003; Wei *et al*, 2000).

Strikingly, the distribution of 15 missense mutations identified in HHD patients (Hu *et al*, 2000; Sudbrak *et al*, 2000; Chao *et al*, 2002; Dobson-Stone *et al*, 2002; Yokota *et al*, 2002; Li *et al*, 2003) are clustered in the stretch of polypeptide including M4, M5, M6, and the intervening cytosolic loop that constitutes the nucleotide-binding and phosphorylation domains characteristic of P-type ion pump superfamily (Fig 1). In contrast, chain termination or nonsense mutations scatter along the entire length of hSPCA1 (not shown). Among the point mutations, D742Y and A304S most likely disrupt ion binding, as seen in a homology model of hSPCA1 based on the crystal structure of the related sarco/endoplasmic reticulum Ca^{2+}-ATPase from rabbit muscle in the high affinity calcium-binding conformation (Toyoshima *et al*, 2000; Ton and Rao, 2004). The carbonyl oxygen of D742 directly coordinates Ca^{2+} and has been shown to be indispensable for Pmr1 function, whereas the backbone carbonyl oxygen of A304 binds calcium in SERCA1. L318P, T709M, and P744R are likely to cause structural perturbations, since replacement of the equivalent residues with Ala in yeast Pmr1 could be tolerated without loss of function (Wei *et al*, 2000, Mandal *et al*, 2003). Two other substitutions of Ala and Gly residues with bulkier side chains (A746T, G309C) are also likely to destabilize protein structure based on their location in the homology model (Ton and Rao, 2004).

A random subset of six HHD missense mutations shown in Fig 1 was introduced into hSPCA1 and expressed in a yeast mutant lacking endogenous Ca^{2+}-ATPases. We had shown that heterologous expression of *ATP2C1* in yeast completely restored mutant phenotypes resulting from disruption of *PMR1* (Ton *et al*, 2002). Here, HHD mutants L341P, C411R, T570I, T709M, and D742Y fail to correct BAPTA or Mn^{2+} hypersensitivity of *pmr1*Δ (Fig 2), indicative of loss of Ca^{2+} and Mn^{2+} transport function, respectively. Indeed, Fairclough *et al* (2003) reported a loss of Ca^{2+} and Mn^{2+}-dependent phosphoenzyme intermediate formation, and low protein expression level for four of these mutants (L341P, C411R, T570I, and D742Y) expressed in COS-1 cells, whereas the effects of mutant T709M have not been reported before. The same study, however, failed to discern any defects in enzyme catalysis, expression level or Golgi localization of mutant P201L. Consistent with these results, in the yeast model, P201L, like wild-type hSPCA1, fully restores cellular phenotypes of *pmr1*Δ. Could P201L be a benign polymorphism, despite its absence from 100 control sequences (Sudbrak *et al*, 2000; Fairclough *et al*, 2003)? If so, the underlying cause of HHD in patients might be undetected mutations in introns or promoter regions. These findings underscore the importance of confirming the effects of amino acid substitutions with functional assays.

It has been hypothesized that the autosomal dominant mode of inheritance of HHD is due to haploinsufficiency of SPCA1 rather than dominant negative effects of the mutants (Hu *et al*, 2000); however, there has not been direct experimental evidence supporting this speculation. We attempted to distinguish these possibilities by expressing the six HHD mutations, shown in Fig 2, in yeast carrying wild-type Pmr1. Given the high level of homology between Pmr1 and hSPCA1, any dominant negative effect of the mutants would have been detectable by changes in phenotype. Yeast expressing both Pmr1 and HHD mutants, however, showed no hypersensitivity to BAPTA or Mn^{2+} toxicity (Fig S1). Thus, at least in yeast, HHD mutants appear to exert no

Abbreviation: HHD, Hailey–Hailey disease

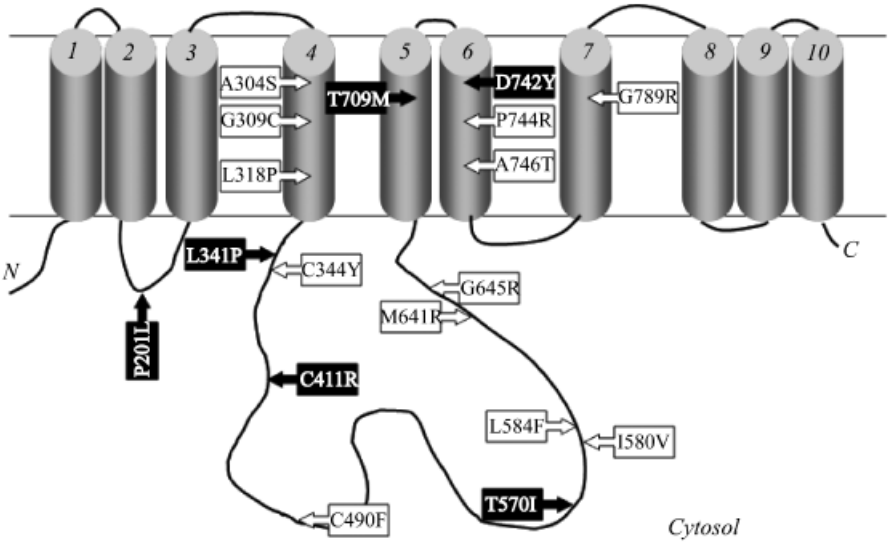

Figure 1
Missense HHD mutations cluster in functionally critical regions of hSPCA1. Fifteen missense mutations found in patients with HHD mainly cluster in membrane helices M4, M5, and M6 which are involved in Ca^{2+}/Mn^{2+}-binding, and in the large cytoplasmic loop comprising the ATP-binding and phosphorylation domains. Note that residue numbers are based on the long splice variant of hSPCA1 (Ikeda *et al*, 2001). Mutants expressed in yeast are shown in black.

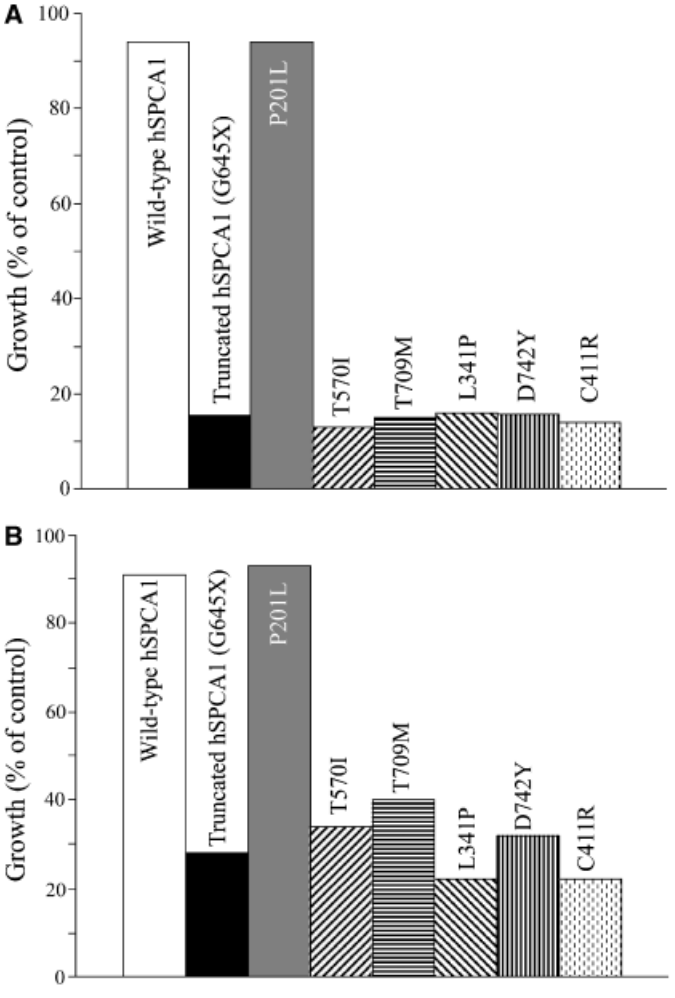

Figure 2
Phenotype screening of HHD mutants in yeast. A yeast strain lacking endogenous calcium pumps (K616; *pmr1Δpmc1Δcnb1Δ*) was transformed with plasmid bearing wild-type, mutant, or truncated (G645X) hSPCA1 as described in Ton *et al* (2002), then grown overnight at 30°C in synthetic drop-out media supplemented with 0.5 mM BAPTA (*A*) or 8 mM $MnCl_2$ (*B*). Growth ($A_{600\ nm}$) was measured and expressed as percent of control (no BAPTA or $MnCl_2$). Data shown are averages of duplicates and representative of 3 different experiments. G645X was a spontaneous mutant that arose during mutagenesis, and was used as a representative of hSPCA1 truncation mutations.

dominant negative effects. It should be noted that dominant negative mutations in the related H^+ pump, Pma1, could be readily detected by growth toxicity due to expression of mutants in wild-type yeast (Harris *et al*, 1994). Our results are consistent with hSPCA1 haploinsufficiency as the basis for HHD.

In summary, we show that *S. cerevisiae* is a convenient and facile model for the study of HHD. Mutations can be rapidly screened in yeast for loss-of-function phenotypes related to the disease. Our studies in yeast thus lend support to findings in mammalian cells regarding the molecular basis of HHD.

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Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/JID/JID23437/JID23437sm.htm

Figure S1
Hailey–Hailey Disease mutants in wild-type host do not impair BAPTA and Mn^{2+} resistance. Wild-type yeast (K601) was transformed with a multicopy plasmid carrying each of the Hailey–Hailey mutants. Cells were grown at 30°C in media lacking uracil but supplemented with 0.5 mM BAPTA (*A*) or 4 mM $MnCl_2$ (*B*). After 24 h, growth was measured as optical density at 600 nm. Data are averages of triplicates. All strains, including truncated hSPCA1 (G645X), grew as well or nearly as well as wild-type ($p > 0.05$: Student's two-tailed *t* test).

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