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Research Article

Investigations into the polymorphisms at the *ECM38* locus of two widely used *Saccharomyces cerevisiae* S288C strains, YPH499 and BY4742

Chitranshu Kumar, Rakesh Sharma† and Anand Kumar Bachhawat*
Institute of Microbial Technology, Chandigarh 160 036, India

**Correspondence to:*
Anand Kumar Bachhawat, Institute of Microbial Technology, Sector 39-A, Chandigarh 160 036, India.
E-mail: akbachhawat@hotmail.com, anand@imtech.res.in

†*Present address: Department of Environmental Biology, University of Delhi, Delhi 110 007, India.*

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Abstract

The *ECM38* gene encodes the γ-glutamyl transpeptidase enzyme, an enzyme involved in glutathione turnover. The enzyme was found to be present in the S288C strain, BY4742, but absent in another widely used strain congenic to S288C, YPH499. Cloning and sequencing the genes from these yeasts indicated the presence of 11 single nucleotide polymorphisms in the coding region and eight single nucleotide polymorphisms in the promoter region of the *ECM38* gene of YPH499 (but none in that of BY4742). One of the SNPs in the *ECM38* ORF led to a G → D conversion in a region conserved in all γ-GT enzymes and was found to be responsible for the loss of activity in this strain. The presence of γ-GT activity in other YPH strains led us to trace the origins of the polymorphisms in YPH499. Our results indicated that among the progenitor strains, YPH1 and YPH2, YPH1 carried the polymorphisms seen in YPH499 and also lacked the γ-GT activity. The implications of these results for the use of these widely used S288C strains and the origin of these single nucleotide polymorphisms are presented. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: ***ECM38*; polymorphism; S288C; yeast; γ-glutamyl-transpeptidase**

Introduction

The *Saccharomyces cerevisiae* genome was sequenced using the S288C strain, which is widely used by yeast researchers. A direct derivative of this strain is BY4742 (Brachmann *et al*., 1998). Another widely used S288C strain is YPH499, which is also used worldwide and has been derived from YNN216, a strain congenic to S288C (Sikorski and Hieter, 1989).

In the present study we describe our attempts to understand and trace the origins of extensive polymorphisms at one of the genes (which we have isolated from YPH499) that is of interest to us. This gene, *ECM38*, encodes γ-glutamyl transpeptidase (γ-GT). Although deletion of this gene does not appear to confer any apparent growth defect to these cells in either YPD or minimal medium, the enzyme encoded by this gene plays a major role in glutathione turnover (Meister *et al*., 1981; Jaspers *et al*., 1985).

During our investigations on this enzyme, we found that YPH499 cells lacked γ-GT activity, while the strain BY4742 was found to have a functional enzyme. We have investigated this unexpected difference. The gene cloned from YPH499 revealed extensive polymorphisms. Since it is important to understand the nature and origin of these polymorphisms in general, as well as more particularly, in strains that are supposed to be isogenic, we initiated a detailed investigation into the polymorphisms of these genes. The results of these investigations are the subject of this report.

Materials and methods

Chemicals and reagents

All chemicals used were of analytical grade and were obtained from Sigma-Aldrich or Hi Media (India). Media components were either purchased

from Hi Media (India), or from Difco, USA. Restriction enzymes and Vent DNA polymerase were from New England Biolabs. Oligonucleotides were purchased from Biobasic Inc., Canada.

Yeast strains and growth conditions

The list of yeast strains used in this study is shown in Table 1. Yeast strains were routinely grown and maintained on YPD medium. The minimal medium (MM) with nutritional supplements has been described earlier (Kumar *et al*., 2003). Yeast chromosomal DNA isolation and yeast transformations were carried as described earlier (Kaiser *et al*., 1994).

Cloning and sequencing of *ECM38* gene from YPH499 (*ECM38**) and BY4742 (*ECM38*)

The γ-GT enzyme of yeast has very recently been shown to be encoded by the *ECM38* (*CIS2*) gene (Mehdi *et al*., 2001). *ECM38* was amplified using vent polymerase by PCR from YPH499 and BY4742 using primers CIS2-FOR, 5′-TAGCGTTCTAGACTTACAGTTATGCTGTTG-3′ and CIS2-REV, 5′-TTACCCCTCGAGTTAGTATACGGAGGAGATTCCTC-3′. The PCR products were purified from agarose gels, digested with *Xba*I and *Xho*I and ligated downstream of the TEF promoter in the centromeric yeast expression vector p416-TEF, having a URA3 selection marker (Mumberg *et al*., 1995). The *ECM38* gene from both the resultant clones was completely sequenced using the above-mentioned primers and internal primers to the gene. The *ECM38* PCR product from YPH499 was also directly sequenced (from an independent PCR reaction) to eliminate the possibility that the differences observed in *ECM38* gene sequence are not an artifact of PCR. The sequence of the PCR product was found to be identical to the cloned gene sequence. The *ECM38* gene cloned from YPH499 is represented in the manuscript as *ECM38** and that cloned from BY4742 as *ECM38*. Sequencing was carried out on an automated ABI sequencer.

Enzyme assays

γ-Glutamyl transpeptidase assay: crude extracts were prepared as described previously (Kumar *et al*., 2003) and γ-GT assayed according to Penninckx *et al*. (1980).

β-galactosidase reporter assays: *S. cerevisiae* YPH499 (ABC154) transformants containing the different reporter plasmids were induced for β-galactosidase as described previously (Kumar *et al*., 2003) and whole cell assays were performed and activity expressed as β-galactosidase units per OD_{600} cells (Guarente, 1983).

Plasmids and their construction

pAB1072 (2 µm *URA3* P_{ECM38}*–*lacZ*), containing 634 bases of the *ECM38** promoter fused in-frame to *lacZ* gene, was constructed by PCR amplification of 634 bases upstream of *ECM38** ORF from YPH499, using the primers *ECM38*-FUSX (5′-CCG TTACT CGAGCAC CTTCAT CGCCTACACTA GGAG-3′) and *ECM38*-FUSB (5′-ACACAAGGAT CCCAGCATAACTGTAAGT CTA GTACGC-3′). The PCR product was gel purified, digested with *Xho*I–*Bam*HI and cloned into the *Xho*I–*Bam*HI sites of pLG699Z (2 µm *URA3*–*lac*Z) (Guarente and Ptashne, 1981). Similarly, pAB1118 (2 µm *URA3* P_{ECM38}–*lacZ*) was constructed by amplifying 634 bases of the *ECM38* promoter from BY4742, followed by digestion with *Xho*I–*Bam*HI and cloning into the *Xho*I–*Bam*HI

Table 1. Strains used in this study

Strain number and (name)	Genotype	Source
ABC154 (YPH499)	*MAT***a** *ura3-52 leu2-Δ1 lys2-801 his-Δ200 trp1-Δ63 ade2-101*	K. Kuchler
ABC1193 (YPH499)	*MAT***a** *ura3-52 leu2-Δ1 lys2-801 his-Δ200 trp1-Δ63 ade2-101*	Stratagene
ABC734 (BY4742)	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	J. Boeke
ABC1234 (YNN214)	*MAT***a** *ura3-52 lys2-801 ade2-101*	P. Hieter
ABC1235 (YNN215)	*MATα ura3-52 lys2-801 ade2-101*	P. Hieter
ABC1236 (YNN216)	*MAT***a**/*α ura3-52 lys2-801 ade2-101*	P. Hieter
ABC156 (YPH98)	*MAT***a** *ura3-52 lys2-801 ade2-101 leu2-Δ1 trp1-Δ1*	M. Brendel
ABC1143 (YPH250)	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ1 his3Δ200 leu2-Δ1*	Y. Inoue

sites of pLG699Z. The sequences of both the cloned promoters and resultant in-frame fusions were confirmed by sequencing.

Results and discussion

The *ECM38* locus on chromosome 12 (*YLR299w*), which encodes the γ-glutamyl transpeptidase enzyme, reveals several single nucleotide polymorphisms in YPH499

We have recently initiated a study on γ-glutamyl transpeptidase and its role in glutathione turnover (Kumar *et al*., 2003). Despite several attempts to determine the levels of activity of this enzyme in the wild-type strain YPH499 under different conditions, we could not detect any activity in this strain under a variety of growth conditions, including conditions known to derepress the enzyme (Table 2). However, in another wild-type strain that was also S288C, BY4742, significant activity could be detected, suggesting that although these strains were both S288C, there were possibly differences in either the gene level or the regulatory level of this enzyme.

Table 2. γ-GT activity in different strains under different growth conditions and in strains harbouring pTEF–*ECM38** or pTEF–*ECM38*

Strains	Growth conditions	Specific activity of γ-GT[†]
ABC154 (YPH499)	Ammonium sulphate	nd
ABC154 (YPH499)	YPD	nd
ABC154 (YPH499)	Glutamate	nd
ABC 734 (BY4742)	Ammonium sulphate	nd
ABC 734 (BY4742)	YPD	9.0
ABC 734 (BY4742)	Glutamate	22.4
ABC1234 (YNN214)	YPD	nd
ABC1234 (YNN214)	Glutamate	nd
ABC1235 (YNN215)	YPD	14.0
ABC1235 (YNN215)	Glutamate	57.3
ABC156 (YPH98)	YPD	21.0
ABC156 (YPH98)	Glutamate	66.4
ABC1143 (YPH250)	YPD	17.0
ABC1143 (YPH250)	Glutamate	73.6
ABC154 (pTEF–*ECM38**)	Ammonium sulphate	nd
ABC154 (pTEF–*ECM38*)	Ammonium sulphate	144.2
ABC734 (pTEF–*ECM38*)	Ammonium sulphate	157.5

nd, below limit of detection.
*ECM38**, cloned gene from YPH499; *ECM38*, cloned gene from BY4742.
[†] The γ-GT activity (nmol product/min/mg protein) values reported here are from a single experiment.

To understand the basis for the differences in γ-GT activity seen in the two S288C strains, BY4742 and YPH499, the *ECM38* gene was amplified and cloned from both these strains. In addition, to eliminate errors due to PCR, the PCR product was also directly sequenced. Interestingly, although the sequence of the *ECM38* gene from BY4742 completely matched the published sequence, the sequence from YPH499 showed 11 mismatches (Table 3). These included nine mismatches that were silent (in that they did not lead to differences in amino acids), while two of the mismatches led to alterations in the amino acids (histidine to arginine at amino acid position 171 and glycine to aspartic acid at position 494). One of these two changes, (G → D), corresponded to a change in an amino acid that was a highly conserved residue present in γ-GT of a wide range of organisms (Figure 1B). This change was probably responsible for the lack of γ-GT activity in YPH499. Even overexpression of γ-GT from YPH499 (pTEF–*ECM38**) under a strong TEF promoter did not yield any activity (Table 2), while overexpression of *ECM38* from BY4742 (pTEF–*ECM38*) gave significant activity, indicating that the lack of γ-GT activity in YPH499 may be due to the variations in the coding region.

Table 3. Nucleotide polymorphisms in the *ECM38*/*ECM38** ORFs and their respective promoters

Distance from ATG	*ECM38* gene (BY4742)	*ECM38** gene (YPH499)	Amino acid change
−531	C	T	—
−527	A	G	—
−491	T	C	—
−485	G	C	—
−376	A	T	—
−360	A	—	—
−133	G	A	—
−44	A	G	—
93	C	G	G = G
381	G	A	L = L
512	**A**	**G**	**H = R**
780	A	G	T = T
1026	G	A	L = L
1038	T	C	I = I
1260	A	G	L = L
1386	C	T	Y = Y
1471	T	C	L = L
1481	**G**	**A**	**G = D**
1959	A	G	K = K

The two polymorphisms leading to amino acid changes are shown in bold.

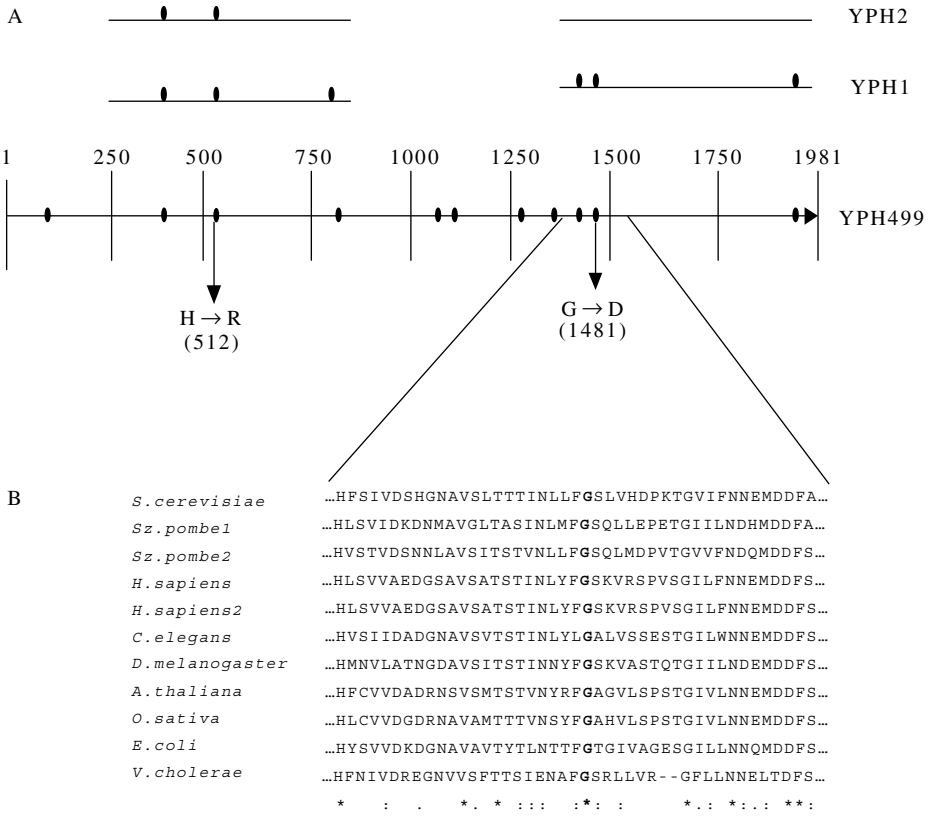

Figure 1. (A) Schematic representation of *ECM38* ORF showing the polymorphisms in YPH499, YPH1 and YPH2. The complete gene was sequenced from YPH499 and the positions with nucleotide polymorphisms are shown (♦). The regions sequenced in case of YPH1 and YPH2 are also shown schematically. The changes leading to amino acid changes are represented by arrows. (B) Multiple sequence alignment of a region of Ecm38p, highlighting the conserved glycine residue that is changed in the encoded protein of *ECM38**. The protein sequence shown here is amino acid position 472–514 of Ecm*38*p from *S. cerevisiae*. The conserved amino acid glycine (G) at position 494 is changed to aspartic acid (D), in the case of YPH499 and YPH1, and is shown in bold

To further eliminate the possibility of a regulatory mutation being responsible for the lack of activity, we cloned and sequenced the promoters of this gene from the two strains and examined whether polymorphisms also extended to the promoter region of these genes. The promoter sequence of the *ECM38* gene of BY4742 was identical to the published sequence; however, significant differences were seen in the promoter of *ECM38** gene from YPH499 (Table 3). A total of eight mismatches were observed. Despite these differences the promoter was still active, since β-gal fusions to this promoter were still functional, although the basal level activity was two-fold less than the equivalent promoter of *ECM38* gene from BY4742 (data not shown; Kumar *et al*., 2003). This further confirmed that the loss of activity in the enzyme in YPH499 was not due to regulatory mutations but was a result of alterations in the coding region of the gene.

Comparison of other gene sequences from YPH499 with those in the database — lack of polymorphisms in general

The extensive polymorphism in the *ECM38* gene of YPH499 as compared to BY4742 was surprising considering that YPH499 was a strain 'congenic' to S288C. We re-examined other genes that we had isolated from YPH499 to see whether this polymorphism was restricted to *ECM38* or whether it was also seen in other genes of this yeast. We have isolated and sequenced several genes from different chromosomes of YPH499, including two genes from chromosome 12. These include

TRX1 (*YLR043c*; Sharma *et al*., 2000) and *GTT2* (*YLL060c*; R. Kaur and A. K. Bachhawat, unpublished information). However, apart from one SNP seen in the coding region of one gene, *HGT1* (*YJL212c*; Bourbouloux *et al*., 2000), which was a silent change, and one in the promoter of *YCF1* (Sharma *et al*., 2002), no other differences could be seen that were as extensive as the differences seen in the *ECM38* gene.

Comparison of different YPH strains for γ-GT activity

Since the YPH499 strain did not contain polymorphisms in other locations, it was of interest for us to know whether the extensive polymorphisms in the *ECM38* locus were seen only in YPH499, or whether they were present in other YPH strains constructed at the same time (Sikorski and Hieter, 1989). This would indicate whether the lack of γ-GT activity in YPH499 was a result of laboratory-derived mutations. We had in our collection two other strains (YPH98 and YPH250) that were constructed at the same time. We examined these strains for γ-GT activity and observed that they had significant γ-GT activity, unlike YPH499 (Table 2).

The presence of activity in YPH98 and YPH250 suggested that there was a specific lack of γ-GT activity only in YPH499. The YPH499 strain that we had obtained was a gift from Dr Kuchler and the possibility that the polymorphisms were laboratory-derived needed to be examined more carefully. We therefore procured a fresh slant of YPH499 from Stratagene. Examination of this strain revealed that this strain also lacks γ-GT activity, similar to the strain that we were working with. We sequenced the relevant regions of the gene (Figure 1A) to see whether the same polymorphisms were seen in this strain. Our results indicated that the YPH499 from Stratagene also had the same polymorphisms at the *ECM38* locus that we had seen with the strain we were working with (and had been obtained from a different source).

Examination of YPH1 (YNN214) and YPH2 (YNN215), progenitor strains of YPH499 for γ-GT activity and the polymorphisms seen in YPH499

The finding that YPH499 original stocks also had the polymorphisms in the *ECM38* gene that we had identified, and the corresponding lack of γ-GT enzymatic activity, and the finding that other YPH strains, YPH98 and YPH250, constructed and described at the same time (Sikorski and Hieter, 1989), had activity, were surprising. The results suggested that either the polymorphisms were introduced very early on after the construction of these strains, or were existing in the progenitor strains. We re-examined the construction of these strains and this is shown schematically in Figure 2. The progenitor strains are YPH1 (YNN214) and YPH2 (YNN215). In the final construction of YPH499, one of the parents of the parent diploid was YPH1. This suggested that YPH1 might in fact carry the polymorphisms that we were observing. To confirm this we first examined γ-GT activity in the parent strains, YPH1 and YPH2. Activity was seen in YPH2 but not in YPH1. When we sequenced the relevant regions of the gene from the two strains we also found that YPH1 showed identical polymorphisms to YPH499, including the two that led to change in amino acid composition (Figure 1A). YPH2, on the other hand, showed only two variations in total, one of which led to a amino acid change (H $\rightarrow$ R), the other being silent. The other amino acid residue (G $\rightarrow$ D), which was conserved in a wide variety of organisms, was unchanged in YPH2 (Figure 1A, B). Our results thus demonstrated that the parent strain, YPH1 (YNN214) was the bearer of the polymorphisms in YPH499 and was also responsible for the consequent lack of γ-GT activity seen in these strains. Further, the lack of γ-GT activity in YPH499 and YPH1 can be attributed to the conserved glycine residue which is changed to aspartic acid in these strains (Figure 1B). The glycine residue is not only conserved in the γ-GT enzyme from prokaryotes to eukaryotes but is also present in what is considered as the γ-GT signature sequence (Mehdi *et al*., 2001). The lack of this SNP leading to the G $\rightarrow$ D change in YPH2 also explains why certain YPH strains constructed at that time (such as YPH98 and YPH250) have a functional γ-GT enzyme. Subsequent information gathered on these strains has revealed that YNN214 and YNN215 are sister spores from the cross of MC39.5A $\times$ MC50.9C. The genotype of MC39.5A is *lys2-801* GAL^+ $SUC2^+$ and is a certified S288C strain. The genotype of M50.9C is *ura3-52 his4-539 ade2-101* GAL^+ $SUC2^+$, and is the product of the 10th backcross of *ura3-52* (from FL1200, a non-S288C

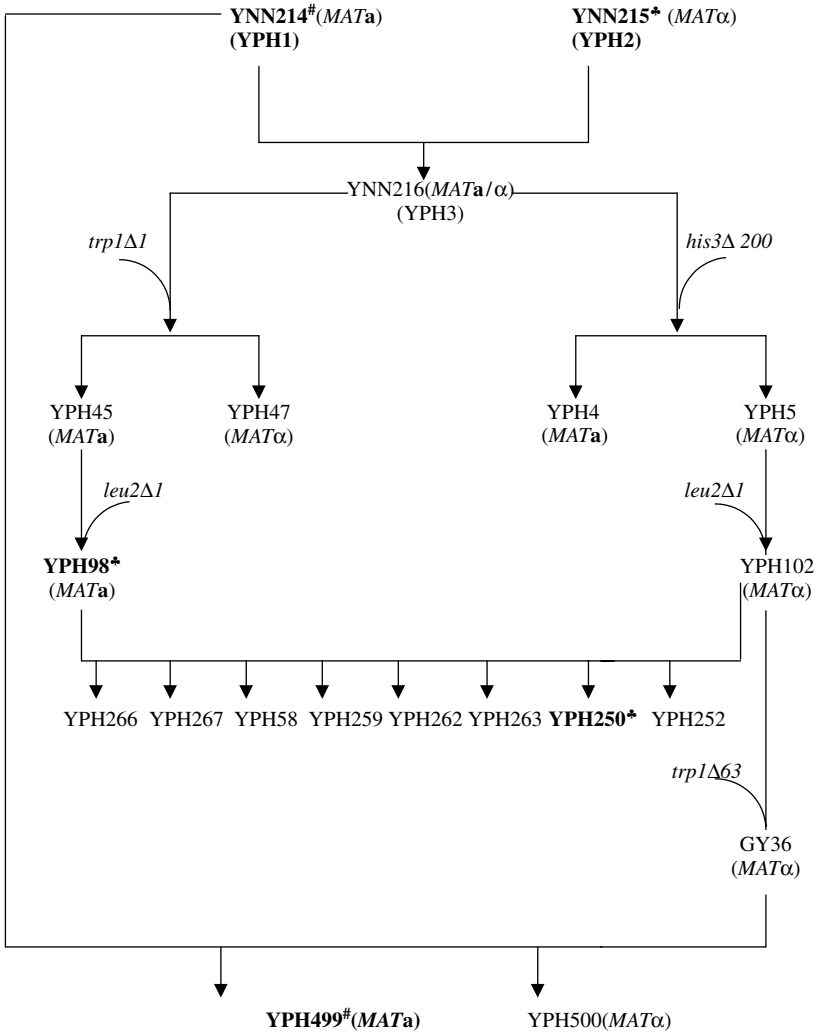

Figure 2. Schematic diagram showing the genealogy of YPH499 and the γ-GT activity of selected strains. The construction of the different YPH strains based on the description provided earlier (Sikorski and Hieter, 1989) has been shown schematically, highlighting the strains where γ-GT activity was examined. #, absence of activity; ♣, presence of activity

strain that was obtained from F. Lacroute) with S288C.

The source of the polymorphism in YNN214 could either be laboratory-derived or, more likely, as mentioned above, it could be the residual genotype of the non-S288C strain from where the *ura3-52* allele was introduced, and the source of the polymorphism is therefore of extraneous origin. While the method of back-crossing should theoretically make the strains essentially identical to the parent, the possibility that some regions might be cold spots for recombination needs to be taken into account, or alternatively might just have escaped recombination. Examination of the physical/genetic map ratio page of SGD for chromosome XII indicates that the ECM38 region is not in a region of unusual recombinogenicity.

The findings of this study are important, as they highlight an important difference in the genotype of two very widely used S288C strains, YPH499 and BY4742, and this should be taken into account by all users of these strains. This is especially so because γ-GT is involved, directly or indirectly, in several cellular processes (Lussier *et al*., 1997; Manning *et al*., 1997), in addition to being involved in glutathione turnover.

Finally, while the *ECM38** gene from YPH499 was non-functional, the promoter still revealed residual activity as well as regulation similar to the *ECM38* gene. Considering also that the reading frame has not accumulated any stop codons or frameshift mutations, the ORF is not strictly a pseudogene (or a disabled ORF). Furthermore the *ECM38** ORF has 11 polymorphisms. Of these, nine were silent, suggesting that the coding region was not changed. Only two of these changes led to changes in amino acids. Another possibility that has to be seriously considered, therefore, considering that this protein does not contain any stop codons (and yet has no γ-GT activity), is that the protein has evolved to a slightly different function whose activity was not being measured by our assay. However, these are possibilities that must await further investigation.

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