Keil et al. (1995) have never shown the presence of any human GALT protein in their yeast system. Thus, the molecular bases of the phenotypes observed in the yeast expression system is unclear at this time. No reports on expression of the common N314D mutation in yeast have been published. However, Elsas et al. (1993) reported that "an evaluation of the N314D mutation in a yeast expression system is in progress." In fact, Dr. Elsas reported at a public session of this Society's annual meeting in 1993 that N314D in yeast encodes near normal activity. This finding is inconsistent with activity data for the Duarte variant that depresses GALT activity significantly in humans. It seems that the yeast expression system, while paralleling patient data in some cases, has not been proved yet to model human biochemical phenotypes faithfully. Clearly, this system can be used very elegantly as shown by Fridovich-Keil et al. (1995). It is also now well documented that the *cos* cell system can—on occasion—overestimate GALT (and other) activities (e.g., Ashino et al. [1995]). Fridovich-Keil et al. (1995) offer some possible explanations for this situation. It is, therefore, suggested that the two expression systems, mammalian and yeast, be used in parallel for all structure/function studies of the GALT enzyme, since both have their own strengths and weaknesses.

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Reply to Reichardt

To the Editor:

We thank Dr. Reichardt for carefully scrutinizing table 2 in Elsas et al. (1995) for attribution of mutations. He is concerned about the S135L mutation, which we attributed to Reichardt and Woo (1991) instead of Reichardt et al. (1992*b*). In Reichardt et al. (1992*b*), he describes the S135L as a "polymorphism" that "encodes almost normal activity" (pp. 5430–5431). Typographical errors did inadvertently attribute the R333G and K334R mutations to Reichardt (1992*a*) rather than to Leslie et al. (1992) and the L195P to Leslie et al. (1992) rather than to Reichardt et al. (1992*a*). In this dynamic field, it is sometimes difficult to acknowledge description of a given "mutation." For example, although N314D was identified in 1991 (Reichardt and Woo), it was first recognized as associated with the Duarte biochemical phenotype in 1992 (Leslie et al.), and its prevalence was defined in 1994 (Elsas et al.). Lin et al. (1994) confirmed its association with the Duarte phenotype and found four N314D alleles in 95 biochemically defined G-alleles, (Lin et al. [1994], table 1, p. 168). Lin et al. found no "additional nucleotide substitutions in the entire [galactose-1-phosphate uridyl transferase] GALT region" (p. 169). The article by Elsas et al. (1995) suggests that he missed non-Q188R G-alleles associated with the generally prevalent N314D allele (5.9% in the general population).

Reichardt requests clarification of our speculation that the E203K mutation may complement the N314D

mutation when *in cis*. The proband's sister III-2 has normal red-blood-cell (RBC) GALT activity, but her GALT genome includes three mutations. She is homozygous for N314D, and the paternally derived allele also contains the E203K mutation. Since E203K alone reduces GALT activity by 50% (Elsas et al. 1995) and the N314D homozygote has 50% reduction in activity, our interpretation of her normal RBC GALT activity in the presence of these three mutations is that the oppositely charged amino acid substitutions stabilize the dimeric active enzyme and enable catalysis of uridyl transfer. I-1 and II-1 have "near-normal" activity as well, despite one normal allele and the presence of the E203K-N314D codon changes *in cis* on the other allele, which should produce >50% reduction in GALT activity. We are eager to test these hypotheses in controlled experiments using expression systems as described by Fridovich-Keil et al. (1995). We thank Dr. Reichardt for his continued interest in our work and hope that this answers his questions.

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Reply to Reichardt

To the Editor:

In his letter Dr. Reichardt raises a number of issues concerning two articles recently published in the *Journal* (Elsas et al. 1995; Fridovich-Keil et al. 1995). I have addressed each of the issues relating to Fridovich-Keil et al., below:

1. Dr. Reichardt suggests that we have misattributed to him the "assumption" that nonconserved residues (within the galactose-1-phosphate uridyl transferase [GALT] sequence) are likely to be "unimportant" for function. In fact, our article described this idea as a "commonly invoked assumption" (Fridovich-Keil et al. 1995, p. 645). With regard to the R148W substitution, the Reichardt et al. article (1992*a*, p. 599) states that "the mutated arginine is not conserved. . . . However, this mutation results in an unstable polypeptide and, therefore, the mutated arginine 148 is probably not enzymatically important." We certainly apologize for any misunderstandings resulting from our interpretation, or possible misinterpretation of these words.

2. Dr. Reichardt's second point is that Fridovich-Keil et al. (1995) "disregard" his mammalian expression data "when they are at odds with" our yeast system. We have at no point disregarded his data, and in fact went out of our way to cite his work and to offer logical explanations that might account for the differences observed using the two systems. We agree with Dr. Reichardt that the most important comparisons, however, are not between the two model systems (COS cells vs. yeast), but between each of these systems and patient cells. For both of the mutations described in Fridovich-Keil et al. (1995) that have also been modeled in the COS cell system, Q188R and S135L, the activity data derived from the yeast system more closely parallel what is seen in patient cells than do the COS cell data. To summarize, Q188R-GALT expressed in yeast encodes null activity (Fridovich-Keil and Jinks-Robertson 1993), and S135L-GALT encodes ~5% wild-type GALT activity (Fridovich-Keil et al. 1995). According to Dr.