Crystal Structures of γ-Glutamyltranspeptidase from *Escherichia coli*, a Key Enzyme in Glutathione Metabolism, and Its Reaction Intermediat

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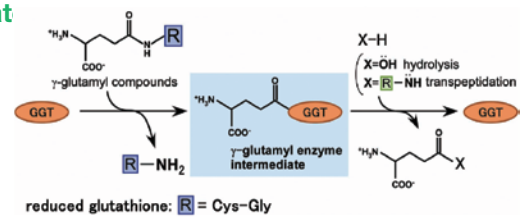
γ-Glutamyltranspeptidase (GGT) is a heterodimic enzyme that is generated from the precursor protein through posttranslational processing and catalyzes the hydrolysis of γ-glutamyl bonds in γ-glutamyl compounds such as glutathione and/or the transfer of the γ-glutamyl group to other amino acids and peptides (Scheme 1). Clinically, GGT is used in a blood test and, because GGT in serum is mainly derived from liver, high levels of GGT in the blood are indicative of hepatic or biliary tract-associated diseases. We have determined the crystal structure of GGT from *Escherichia coli* K-12 at 1.95 Å resolution (Fig. 1). GGT has a stacked αββα fold comprising the large and small subunits, similar to the folds seen in members of the N-terminal nucleophile hydrolase superfamily. The active site Thr-391, the N-terminal residue of the small subunit, is located in the groove, from which the pocket for γ-glutamyl moiety binding follows. We have further determined the structure of the γ-glutamyl-enzyme intermediate trapped by flash-cooling the GGT crystal soaked in glutathione solution and the structure of GGT in complex with L-glutamate (Fig. 2). These structures revealed how the γ-glutamyl moiety and L-glutamate are recognized by the enzyme. A water molecule (W2 in Fig. 2(a)) was seen on the carbonyl carbon of the γ-glutamyl-Thr-391 Oγ bond in the intermediate that is to be hydrolyzed. Notably the residues essential for GGT activity (Arg-114, Asp433, Ser-462, and Ser-463 in *E. coli* GGT) shown by site-directed mutagenesis of human GGT are all involved in the binding of the γ-glutamyl moiety. The structure of *E. coli* GGT presented here, together with sequence alignment of GGTs, may be applicable to interpret the biochemical and genetic data of other GGTs.



Scheme 1. Reactions catalyzed by GGT.

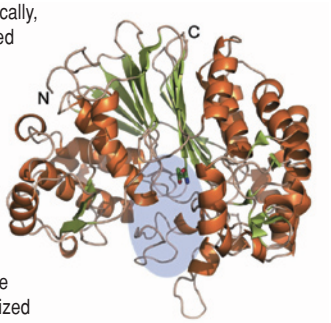


Fig. 1. Overall structure of *E. coli* GGT. α-helices are colored brown and β-strands are colored green. The active residue, Thr-391, is shown as a stick model and the substrate-binding pocket is shaded.

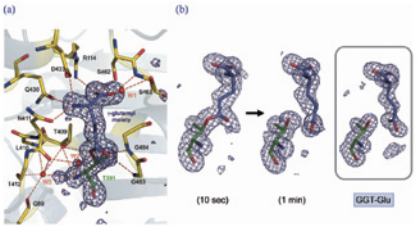


Fig. 2. a) Electron densities of γ-glutamyl moiety and Thr-391 in γ-glutamyl-enzyme intermediate. Ball and stick models are overlaid on the map. b) Hydrolysis of γ-glutamyl moiety. The bond between the γ-glutamyl moiety and Thr-391 in the intermediate was hydrolyzed in 1 min. The map on the right shows the structure of the GGT-Glu complex.

Complementation of Placental Defects and Embryonic Lethality by Trophoblast-Specific Lentiviral Gene Transfer

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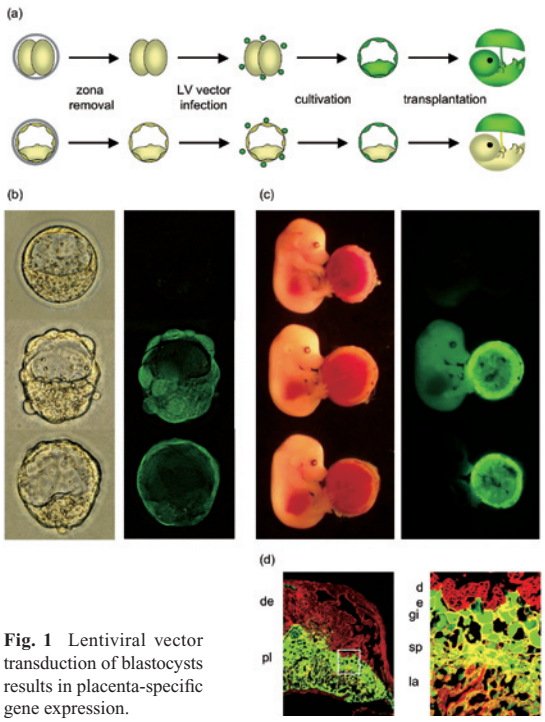

Fig. 1 Lentiviral vector transduction of blastocysts results in placenta-specific gene expression.

Placental dysfunction underlies many complications during pregnancy, and better understanding of gene function during placentation could have considerable clinical relevance. However, the lack of a facile method for placenta-specific gene manipulation has hampered investigation of placental organogenesis and the treatment of placental dysfunction. We have previously shown that transduction of fertilized mouse eggs with lentiviral vectors leads to transgene expression in both the fetus and the placenta. Here we report trophectoderm- and placenta-specific gene incorporation by lentiviral transduction of mouse blastocysts after removal of the zona pellucida (Fig. 1a-c). All of the placentas analyzed, but none of the fetuses, were transgenic. Histological analysis revealed uniform and ubiquitous transgene expression in all 3 major layers of the placenta; labyrinth, spongiotrophoblast, and giant cells (Fig. 1d). Application of this method substantially rescued mice deficient in Ets2, Mapk14 and Mapk1 from embryonic lethality caused by placental defects. Ectopic expression of Mapk11 also complemented Mapk14 deficiency during placentation (Fig. 2).

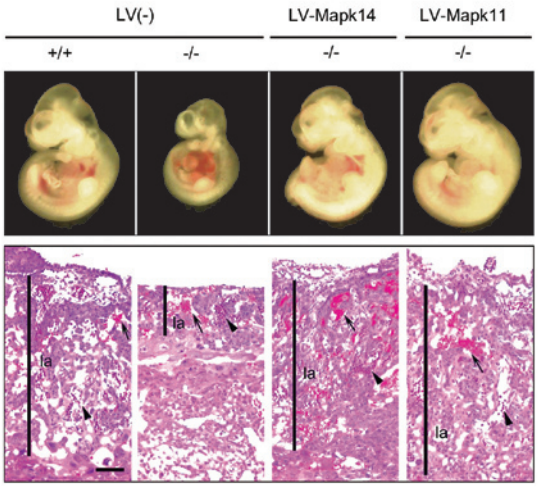

Fig. 2 Functional complementation of Mapk14 with Mapk11 in the Mapk14 deficient placenta.