Possible Assignment of the Glyoxalase I (GLO) Gene to Chromosome 6 Using Man-Mouse Somatic Cell Hybrids*

K. Bender and K.-H. Grzeschik
Institute für Humangenetik der Universitäten Freiburg and Münster

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Summary. A correlation between the expression or absence of human glyoxalase I and chromosome 6 (as well its markers ME1, IPO-B, and PGM3) was observed in man-mouse somatic cell hybrids. This segregation pattern indicates that the GLO gene is situated on chromosome 6.


Glyoxalase I (GLO, EC 4.4.1.5) catalyzes the irreversible conversion of glutathione and methyl-glyoxal to S-lactoylglutathione.

Kämpf et al. (1975a) have detected the GLO gene to be polymorphic in man with 2 common alleles. This polymorphism can be demonstrated in red cells and fibroblasts. Here we report on results raised in man-mouse somatic cell hybrids providing strong evidence for synteny of the GLO and the PGM3 genes as well as for their association with chromosome 6.

Material and Methods

Cell Culture. Human-mouse somatic cell hybrids between RAG or A9 mouse cells, both deficient in hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT, EC 2.4.2.8) (Klebe et al., 1970; Littlefield, 1966) and 5 different diploid human fibroblastic lines were obtained, maintained in culture and processed for segregation analysis of the expression of human enzyme markers and the presence of human chromosomes as described previously (Grzeschik, 1973a).

Electrophoresis of Enzyme Markers. Lysates obtained from homogenized mass cultures of hybrid clones and parental cells (Meera Khan, 1971) were analyzed by Cellogel electrophoresis (Chemetron, Milan) (Meera Khan, 1971; Grzeschik, 1973b; Van Someren et al., 1974) for the following enzymes:

Glucose-6-phosphate-dehydrogenase (G6PD, EC 1.1.1.49), 3-phosphoglycerate-kinase (PGK, EC 2.7.2.3), phosphopyruvate hydratases (ENO1, ENO2, EC 4.2.1.11), phosphoglucomutase-1 (PGM1, EC 2.7.5.1), adenylate kinases 1 and 2 (AK1, AK2, EC 2.7.4.3), fumarate hydratase (FH, EC 4.2.1.2), NAD dependent soluble malate dehydrogenase (MDH3, EC 1.1.1.37), NADP dependent soluble malic enzyme (ME-1, EC 1.1.1.40), mitochondrial

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and soluble superoxide dismutases (IPO-A and IPO-B, EC 1.15.1.1), glutathione reductase (GSR, EC 1.6.4.2), soluble glutamate oxaloacetate transaminase (GOTs, EC 2.6.1.1), lactate dehydrogenases A and B (LDH-A, LDH-B, EC 1.1.1.27), peptidases A, B, and C (PEP-A, PEP-B, PEP-C, EC 3.4.11), peptidase D (PEP-D, EC 3.4.13.9), nucleoside phosphorylase (NP, EC 2.4.2.1), mannosephosphate isomerase (MPI, EC 5.3.1.8), pyruvate kinase (M2) (PKM2, EC 2.7.1.40), glucosephosphate isomerase (GPI, EC 5.3.1.9), adenosine deaminase (ADA, EC 3.5.4.4), β-glucuronidase (GUS, EC 3.2.1.31).

Esterase-D (Es-D, EC 3.1.1.1) and phosphoglucomutase-3 (PGM3, EC 2.7.5.1) were separated on starch gel (Bender and Frank, 1974; Lamm, 1969). For the separation of GLO from mouse and man the cells were lysed in 0.005 M phosphate buffer, pH 6.4, followed by sonication and deep freezing. The isozymes were separated by starch gel electrophoresis (5 hrs, 14 V/cm) using a discontinuous phosphate-buffer system, pH 7.4 (for details see Bender and Frank, 1974). Under these conditions the GLO bands migrate about 5 cm, the PGM bands about 4 cm towards the anode. One side of the sliced gel was stained for PGM according to the method of Lamm (1969), the other for GLO as proposed by Kömpf et al. (1975a):

The sliced gel was covered on both sides with Whatman No. 3 filter paper and moistened with the following staining solution:

- 0.65 ml glyoxal (35%, Roth, Karlsruhe)
- 60 mg GSH (Boehringer, Mannheim)
- 6 mg MTT (Serva, Heidelberg)
- 12.5 ml 0.2 M phosphate-buffer pH 6.8

After wrapping in cellophane the gel was incubated for 30–40 min at 37°C. Thereafter the filter papers were discarded and the gel was transferred (in the light at room temperature) into 0.1 M Tris HCl-buffer, pH 9.0, containing 0.06 M dichloroindophenol (DCIP, Serva, Heidelberg). The GLO bands became visible after 10–30 min as colorless fractions against the dark background.

Chromosome Studies. Metaphase preparations of hybrid clones were obtained as described previously (Grzeschik, 1973a) and analyzed by the Q-banding technique of Caspersson et al. (1970), since we consider it to be more reliable and informative than Giemsa-banding. At least 30 metaphases of each clone were analyzed. A chromosome was considered to be present, if it was found in at least 20% of the metaphases. The identification of human D-group chromosomes was not always easy. Therefore, to ascertain the presence of these chromosomes and the origin of small centric fragments in cases of doubt, the cell cultures were treated with "33258 Hoechst" before chromosome preparation. As described previously the centromeric regions of mouse chromosomes are elongated by this treatment, whereas the human chromosomes are not altered (Kim and Grzeschik, 1974). This difference allows to distinguish murine and human chromosomes clearly.

Results and Discussion

As it can be seen from Fig. 1 the mouse cell line A9 exhibits a single fraction of GLO activity in about the same position as the human GLO 1-1 phenotype. If the human fusion partner has the phenotype GLO 2-2, both parental strains express clearly distinguishable GLO patterns.

The phenotypes of 3 of the resulting hybrid clones are presented in Fig. 1: clone 4 (with chromosomes 6, 13, 14, 21, 3p—) comprises mouse and man type GLO; clone 5 (with chromosomes 2, 7, 13, 14, 18, 21, 22, 3p—) and clone 9 (with chromosomes 2, 14, 18, 22, 3p—) lack the human GLO. The chromosomal constitutions of the clones therefore indicates the GLO gene to be on chromosome 6.

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