Chromosome Assignments of Genes in Man Using Mouse-Human Somatic Cell Hybrids: Mitochondrial Superoxide Dismutase (Indophenol Oxidase-B, Tetrameric) to Chromosome 6

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Summary. Evidence from mouse/human somatic cell hybrids is presented for the synteny of the genes for indophenol oxidase-B (tetrameric) and cytoplasmic malic enzyme (EC 1.1.1.40). Assignment of these two genes to chromosome 6 is further confirmed. The identification of indophenol oxidase-B (tetrameric) as mitochondrial superoxide dismutase is discussed.


Introduction

Current results have confirmed the potential of inter-specific somatic cell hybrids for genetic analysis. Rodent/human hybrids have proven particularly useful because human chromosomes are preferentially lost, the retained human chromosomes can be reliably identified, and many human phenotypes are expressed and can be detected in the hybrid cells.

We present here evidence from several series of mouse/human somatic cell hybrids for the synteny (location on the same chromosome) of indophenol oxidase-B (tetrameric form) (IPO-B) and of NADP-dependent cytoplasmic malic enzyme (ME 1) (EC 1.1.1.40). ME 1 has previously been assigned to chromosome 6 (Chen et al., 1973). Our data confirm the assignment of both IPO-B and ME 1 to chromosome 6.

It is now apparent that what has been called indophenol oxidase-B (tetrameric) or tetrameric tetrazolium oxidase is actually mitochondrial superoxide dismutase, an enzyme that catalyzes the reaction:

\[ \text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2 \]

We will refer to the cytosol, dimeric, form of superoxide dismutase as c-SOD or SOD 1, and the mitochondrial form as m-SOD or SOD 2.
Materials and Methods

Formation of mouse/human hybrids used in this study was facilitated by the use of betapropiolactone inactivated Sendai virus (Klebe et al., 1970). Selection of hybrid clones was mediated either by the hypoxanthine-aminopterin-thymidine (HAT) system of Littlefield (1964) or by a modification (Tischfield and Ruddle, 1973) of the alanosine-adenine (AA) system of Kusano et al. (1971).

Four different series of mouse/human hybrid clones which have been described in detail elsewhere were studied: (i) J hybrids (22 clones) (Ruddle et al., 1970) resulting from fusion of human peripheral leucocytes with a hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficient mouse cell line (RAG), (ii) JBA (1 clone) and JFA hybrids (4 clones) (Tischfield and Ruddle, 1973) derived respectively from the fusion of human leucocytes and skin fibroblasts obtained from a carrier of a 14/22 fusion product, with the mouse L-cell line A-9, which is spontaneously deficient for adenosine phosphoribosyltransferase (APRT) as well as being deficient for HGPRT, (iii) WA hybrids (11 clones) (Tischfield and Ruddle, 1973) derived from the fusion of A-9 with the human lung fibroblast cell line WI-38, and (iv) RK hybrids (23 clones) (Ricciuti and Ruddle, 1973) derived from the fusion of human fibroblasts (KOP-1 and KOP-2) which carry reciprocal X/14 translocations with RAG.

Extracts obtained from homogenized mass cultures of hybrid clones were analyzed by starch, acrylamide, or Cellogel electrophoresis (Brewer, 1970; Omenn and Cohen, 1971; Ruddle and Nichols, 1971; Tischfield et al., 1973) for the following enzymes: adenosine deaminase (EC 3.5.4.4), adenine phosphoribosyltransferase (EC 2.4.2.7), glutamate oxaloacetate transaminase (EC 2.6.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), glucose phosphate isomerase (EC 5.3.1.9), hypoxanthine-guanine phosphoribosyltransferase (EC 2.4.2.8), isocitrate dehydrogenase (EC 1.1.1.42), superoxide dismutase-1 (indophenol oxidase-A, dimeric form), superoxide dismutase-2 (indophenol oxidase-B, tetrameric form), lactate dehydrogenase-A (EC 1.1.1.27), lactate dehydrogenase-B (EC 1.1.1.27), malic enzyme [malate oxidoreductase (decarboxylating)] (EC 1.1.1.40), malate oxidoreductase (EC 1.1.1.37), mannose phosphate isomerase (EC 5.3.1.8), nucleoside phosphorylase (EC 2.4.2.1), peptidase-A, peptidase-B, peptidase-C, peptidase-D, phosphoglycerate kinase (EC 2.7.2.3) and phosphoglucomutase-1 (EC 2.7.5.1).

Air-dried chromosome preparations obtained by standard procedures (Moorhead et al., 1960) from colcemid treated cultures were analyzed utilizing the following staining methods (i) Constitutive heterochromatin (Chen and Ruddle, 1971), (ii) Giemsa banding (Sumner et al., 1971), and (iii) Quinacrine mustard (QM) fluorescence banding (Caspersson et al., 1970). Sequential staining of chromosomes with QM and constitutive heterochromatin techniques enhanced the reliability of chromosome identification. Metaphases suitable for analysis were photographed using a Zeiss Standard or Photomicroscope, H & W Control (ASA 80) or Kodak HC Copy film was used for QM fluorescence. HC Copy film was used for all other photomicrography.

Results

If in a series of independently derived hybrid clonal lines, two or more human phenotypes are always expressed or absent concordantly, we can tentatively conclude that the genes for these phenotypes are syntenic (located on the same chromosome). The alternate possibility of preferential joint retention or loss of two or more chromosomes, can be ruled out, and the syntenic genes can be assigned to a particular chromosome through cytogenetic analysis of the hybrid genome.

The data for the synteny of the genes for SOD2 and ME1 are presented in Table 1. In a total of 56 hybrid clonal lines, with no exceptions, the two phenotypes were always concordantly retained or absent.

Thirty-two of the hybrid lines were examined cytogenetically (Table 2). Chromosome 6 was found in all fourteen clonal lines that expressed SOD2. Chromosome 6 was absent in all but one of the clones that did not express SOD2. In this