chains to that in β chains was almost 2; in contrast, the same ratio in unstable variants of the β chain has been consistently reported to be 1. This difference suggests that the mechanisms governing the rates of synthesis of α and β chains may not be similar. This dissimilarity may explain why there are free α chains present in red cells of unstable β chain variants (*13*), but apparently no free β chains or hemoglobin H in unstable α chain variants.

Although hemoglobin Ann Arbor is preferentially destroyed there are no classical inclusion bodies. This suggests that the degradation may be proteolytic. Moreover, a single amino acid substitution not only reduces the net synthesis of the variant chain, but decreases the synthesis of its complementary subunit, the β chain.

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7. Abbreviations: NKM, a solution of 0.153*M* NaCl, 0.005*M* KCl, and 0.005*M* $MgCl_2$; TKM, a solution of 0.01*M* tris(hydroxymethyl)-aminomethane (tris) hydrochloride, 1.5 m*M* $MgCl_2$, and 0.05*M* KCl at *p*H 7.4.
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Somatic Cell Genetic Assignment of Peptidase C and the Rh Linkage Group to Chromosome A-1 in Man

Abstract. *The segregation of the human peptidase-C phenotype in five different series of human-mouse hybrid clones was examined. The chromosome constitution of these hybrids was determined by quinacrine mustard fluorescence, Giemsa banding, and constitutive heterochromatin staining. That the clones could be classified without exception either as human peptidase C positive/A-1 positive (14 clones), or as peptidase C negative/A-1 negative (12 clones) indicates that peptidase C can be assigned to the human A-1 chromosome. Data from an extensive series of human-mouse clones used provide support for the syntenic association between peptidase C and phosphoglucomutase-1 and by inference a linkage of both to Rh factor group.*

We report data that supports the assignment of peptidase C (Pep C), and thus by inference, the rhesus (Rh) linkage group to chromosome A-1 in man. It is possible to establish gene-gene and gene-chromosome linkage relations by the analysis of somatic cell hybrids. Hybrids of human and mouse cells are useful for gene mapping in man because the human chromosomes are preferentially lost, the human phenotypes such as isozymes are constitutively expressed and can be readily detected, and the constitution of human chromosomes can be determined. Linkage relations are determined by correlation. If two phenotypes segregate concordantly (present or absent together in clonal populations of independent origin), then it can be presumed that they are syntenic (situated on the same chromosome). Gene assignment to a particular chromosome can be inferred if a particular human phenotype and chromosome segregate together. Details of the somatic cell genetic approach to linkage analysis have been reviewed (*1*, *2*).

We have used five different combinations of human-mouse hybrids in our studies. The first hybrid has been designated J and resulted from the fusion of mouse RAG cells, which are deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (*3*), with normal human leukocytes (*4*). The cells were selected in the HAT medium (*5*), and derivative subclones were isolated in HAT. The isolation and characterization of the J hybrid clones have been described (*4*). The second hybrid series isolated in HAT

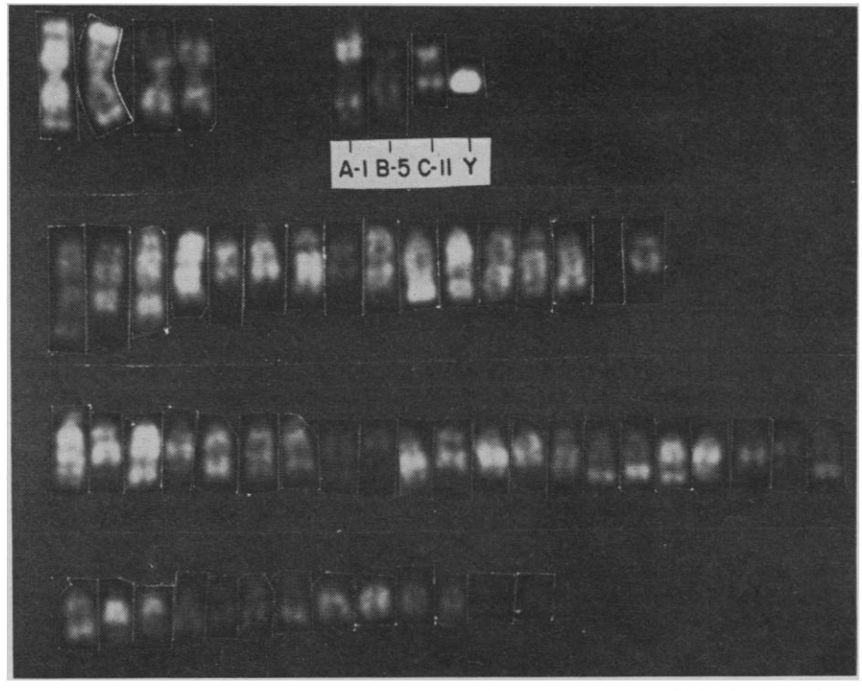

Fig. 1. Human-mouse clone retaining human chromosomes A-1, B-5, C-11, and Y. Unlabeled chromosomes are derived from the mouse RAG parent.

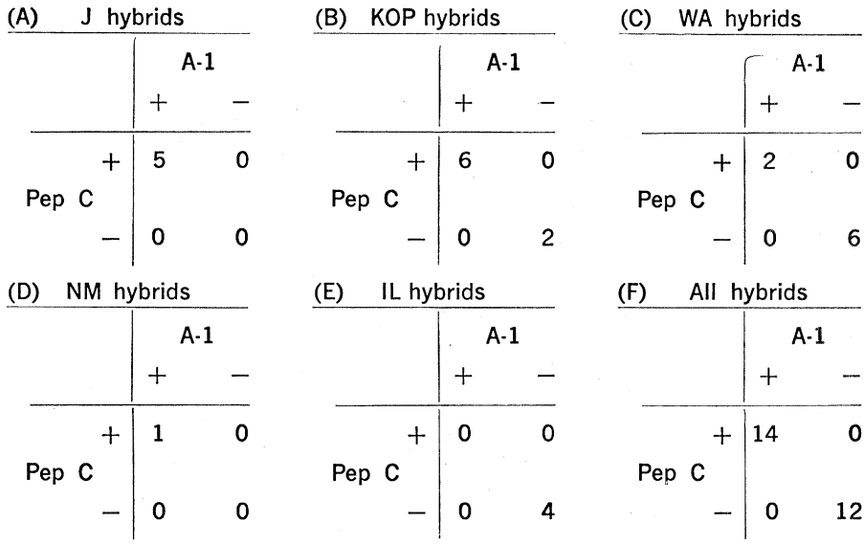

Fig. 2. Concordant segregation between A-1 and Pep C in 26 primary human-mouse clones of independent origins. (A) Jennifer clones, (B) KOP clones, (C) WA clones, (D) NM clones, (E) IL clones, and (F) combined data.

medium was formed between human fibroblasts termed KOP and mouse RAG cells (*6*). The KOP cells were obtained from members of a family carrying a translocation between the X chromosome and autosome D-14 (*7*). The KOP parental cells and somatic hybrids derived from it have been described (*8*). The third hybrid series is termed WA and results from the hybridization of a mouse L cell line deficient in adenine phosphoribosyltransferase (APRT) and the normal human fibroblastic cell strain WI-38. These hybrids were isolated in the Dulbecco Vogt modification of Eagle's medium supplemented with alanosine and adenine (AA medium). The AA medium has been described in connection with the selection for the human form of APRT in human-mouse hybrids (*9*). The fourth hybrid series, termed IL, resulted from the fusion of human neuroblastoma cells of strain IMR-32 with the mouse L cell line LM(TK$^-$). The fifth series, NM, was produced by hybridizing human primary skin fibroblasts, MRC-5, with mouse neuroblastoma cells of clone NA deficient in HGPRT. The IL and NM hybrids were isolated by single-sided selection in HAT medium. Fusion in all instances was stimulated by Sendai virus (*3*). Isolation and husbandry of hybrid clones have been described (*3*).

Hybrid clones of independent origin were examined for the human forms of the following 22 enzymes by means of starch-gel and acrylamide-gel electrophoresis and inference based on drug selection: adenosine deaminase (E.C. 3.5.4.4), adenine phosphoribosyltransferase (E.C. 2.4.2.7), glutamate oxaloacetate transaminase (E.C. 2.6.1.1), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), glucose phosphate isomerase (E.C. 5.3.1.9), hypoxanthine-guanine phosphoribosyltransferase (E.C. 2.4.2.8), isocitrate dehydrogenase (E.C. 1.1.1.42), indolephenol oxidase, lactate dehydrogenase-A (E.C. 1.1.1.27), lactate dehydrogenase-B (E.C. 1.1.1.27), malate oxidoreductase decarboxylating (E.C. 1.1.1.40), malate oxidoreductase (E.C. 1.1.1.37), mannose phosphate isomerase (E.C. 5.3.1.8), nucleoside phosphorylase (E.C. 2.4.2.1), peptidase-A, peptidase-B, peptidase-C, peptidase-D, phosphoglycerate kinase (E.C. 2.7.2.3), phosphoglucomutase-1 (E.C. 2.7.5.1.), and thymidine kinase (E.C. 2.7.1.21) (*10, 11*). The hybrid clones were characterized in terms of their human chromosomal constitution by quinacrine mustard staining (*12*), Giemsa banding staining (*13*), and centromeric, constitutive heterochromatin staining (*14*). Each of these staining procedures permits us to identify the human number 1 chromosome in comparison to other human and murine chromosomes (Fig. 1). Karyotype analysis was performed for each of the clones by the construction of ideograms. Presence or absence of the human number 1 chromosome was determined by an examination of 5 to 30 karyotypes per clone. It was possible to identify other human chromosomes with the exception of the D group chromosomes, which mimic the mouse acrocentrics. However, we have assigned the gene nucleoside phosphorylase (NP) (*15*) to human chromosome 14 (*8*), and this allows us to infer the presence or absence of D14 by isozyme analysis.

Tabulation of the segregation of Pep C and the human number 1 chromosome shows positive correlation between the enzyme and the chromosome (Fig. 2). The data also showed negative correlation between Pep C and human chromosomes 2, 3, 4, 5, 6, 7, 8, 9, 12, 16, 17, 19, 20, 21, and 22. Genes have been assigned to 6, 7, 10, 11, 12, X, 14, and 17, which are not syntenic with Pep C (*16*); and this removes these chromosomes from consideration. We do not at present have negative or positive correlations between Pep C and chromosomes 13, 15, and 18. However, the positive correlations between Pep C and chromosome 1 makes assignment of Pep C to these chromosomes unlikely. One clone in the KOP series possessed a deleted A-1 chromosome. Approximately one-half the distal short arm was lost. If this portion of the chromosome were truly absent and not translocated to some other part of the human or mouse genomes, then we can presume that the locus for Pep C is restricted to the proximal short arm or long arm portions of the A-1 chromosome.

The Rh linkage group is the largest of the human linkage groups. Family studies performed by Lawler (*17*) have

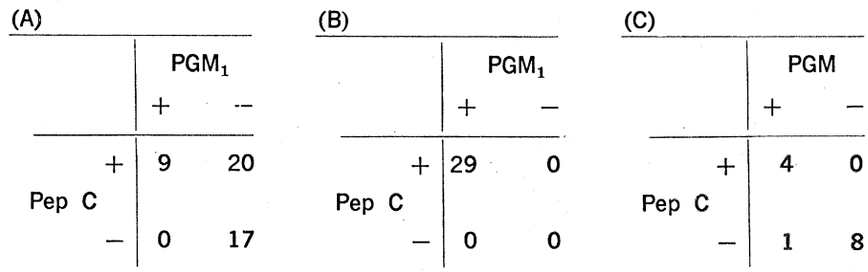

Fig. 3. Segregation between PGM$_1$ and Pep C. (A) Clones derived from human cells heterozygous for PGM$_1$. (B) Clones derived from human cells homozygous for PGM$_1$. (C) Data from Santachiara *et al.* (*21*) based on human-mouse hybrids. Two clones that were ambiguous regarding human PGM$_1$ activity and Pep C$^-$ are excluded.

demonstrated a close linkage between Rh and elliptocytosis (EL_1). Family analysis by Weitkamp *et al.* (*18*) has shown a loose linkage (24 centiMorgans) between 6-phosphogluconate dehydrogenase (6PGD) and Rh. Using hybrids of Chinese hamster and human cells, Westerveld and Meera-Khan (*19*) have demonstrated a syntenic relation between 6PGD and phosphoglucomutase 1 (PGM_1) in man. We have not been able to study this linkage in human-mouse hybrids since the human and murine forms of 6PGD have similar electrophoretic properties and cannot be distinguished reliably (*10*). Van Cong *et al.* (*20*) have reported evidence based on human-mouse cell hybrids which supports the syntenic association of Pep C and PGM_1. We have also examined the segregation of these traits in human-mouse cell hybrids. Our data have shown three types of clones in terms of human phenotypic expression: PGM_1^+/Pep C^+; PGM_1^-/Pep C^-; and PGM_1^-/Pep C^+. We have not observed the type PGM_1^+/Pep C^- in 46 primary clones of independent origin examined for these characters (Fig. 3). These clones were obtained from crosses with human parental cells WI-38, MRC-5, and KOP, which are heterozygous for the PGM_1 trait and in which one human allozyme cannot be distinguished from the homologous murine isozymic form of PGM_1. Furthermore, in a series of 21 secondary clones from a fusion of peripheral human leukocytes and mouse cells of the RAG cell (J hybrids) line, only PGM_1^+/Pep C^+ clones were obtained. The human parent in this cross is homozygous for PGM_1 and differs from the mouse isozyme. Therefore, there is good agreement between our data and those of Van Cong *et al.*, which support synteny for human PGM_1 and Pep C. Data reported by Santachiara *et al.* (*21*) also generally support the syntenic association of PGM_1 and Pep C (Fig. 3). The combined data from family studies and somatic cell genetic studies thus support an Rh linkage group of the following composition: Rh, EL_1, 6PGD, Pep C, and PGM_1. Renwick (*22*) in a reexamination of family data has provided evidence that PGM_1 is on the side of Rh remote from 6PGD and about 30 centiMorgans from Rh. The distance between 6PGD and Rh has been confirmed subsequently by Cook *et al.* (*23*).

Two reports have presented views different from those advanced here and deserve consideration. Conover and Hirschhorn (*24*), using data obtained from somatic cell hybrids, reported a tentative linkage of PGM_1 to a C group chromosome. Their study was based on human parental cells that were heterozygous for PGM_1. Moreover, their work antedated modern methods of chromosome identification. Palmer and Schroder (*25*), using the segregation of a familial variant of chromosome C-9, reported a possible linkage of Rh to C-9. Our experiments provide evidence against linkage to C-9 since clones positive for Pep C do not possess recognizable C-9 chromosomes.

Family studies have suggested weak linkage associations between the A-1 chromosome or A-1 linked markers and members of the Rh linkage group. Jacobs *et al.* (*26*) have reported data suggesting a loose linkage between a translocation breakpoint near the end of the long arm of A-1 and Rh. Lamm *et al.* (*27*) reported data consistent with loose linkage between the Duffy marker Fy and PGM_1. Renwick and Izatt (*28*) have independent family data that is consistent with that of Lamm and co-workers. Kimberling has reported a possible linkage of auriculo-osteodysplasia (AOD) to Rh and Fy (*29*). We have learned from Bootsma and co-workers and Hamerton and co-workers working independently that studies with human/Chinese hamster somatic cell hybrids provide presumptive evidence for the assignment of 6PGD and PGM_1 to the A-1 chromosome (*30*). These reports provide independent evidence which supports the assignment of the Rh linkage group to chromosome A-1.

Several genes have been assigned to human chromosome A-1 as a result of family analysis. Uncoiler-1 (Un-1) represents a heritable structural modification of the centric heterochromatin of A-1. A number of studies [Renwick (*31*)] provide good evidence for a close linkage between Fy and Un-1, and between Fy and Cae, a locus controlling total nuclear cataract. Hill *et al.* (*32*) and Kamaryt *et al.* (*33*) and Merritt (*34*) have provided evidence for the linkage of the salivary and pancreatic amylase loci Amy-1 and Amy-2 to Fy. Our data thus provide support for the localization of the following genes on the human A-1 chromosomes: Fy, Cae, Amy-1, Amy-2, PGM_1, EL_1, Rh, 6PGD, Pep C, and possibly AOD. The order of these loci remains to be determined. However, our data showing that Pep C is probably not located on the distal half of the short arm, taken with that of Jacobs *et al.* indicating that Rh is possibly located toward the end of the long arm, would suggest location of the majority of the loci on the long arm of A-1.

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