Vol. 178, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1991 Pages 227-235

MAPPING THE HUMAN CAS2 GENE, THE HOMOLOGUE OF THE MOUSE BROWN (b) LOCUS, TO HUMAN CHROMOSOME 9p22-pter

Chaya D. Chintamaneni[1], Michele Ramsay[2], Mary-Anne Colman[2], Margaret F. Fox[3], Richard T. Pickard[1], and Byoung S. Kwon[1*]

[1]Department of Microbiology and Immunology and Walther Oncology Center, Indiana University School of Medicine, Indianapolis, IN 46202-5120

[2]MRC Human Ecogenetics Research Unit, Department of Human Genetics, School of Pathology, South African Institute for Medical Research and University of Witwatersrand, Johannesburg, South Africa

[3]MRC Human Biochemical Genetics Unit, The Galton Laboratory, University College London, Wolfson House, London

Received May 24, 1991

Melanin biosynthesis is a multistep process with the first step being the conversion of L-tyrosine to L-Dopa catalyzed by the enzyme tyrosinase. The enzymes which catalyze the other steps of melanogenesis are not known. One murine pigmentation gene, the brown (b) locus, when mutated, leads to a brown or hypopigmented coat. The b-locus protein has been shown to display catalase activity. The human b-locus, therefore, is designated as CAS2. We used the mouse b-locus cDNA to isolate the human homologue, which in turn, was used to map the CAS2 locus to a human chromosome. The potential CAS2 protein codes for 527 amino acids containing a putative signal sequence and transmembrane domain. The CAS2 protein has primary and probably secondary structures similar to human tyrosinase. The CAS2 was mapped to human Chromosome 9 by somatic cell hybridization and, more specifically, to 9p22-pter by *in situ* hybridization. The assignment of CAS2 on the human Chromosome 9 extends this region of known homology on mouse Chromosome 4.

Cutaneous melanin is found in the skin, hair follicles, and eyes and is synthesized and secreted by melanocytes. The melanin is synthesized in the organelles called melanosomes and is transferred to surrounding keratinocytes through the dendritic process. L-tyrosine is the initial substrate in the melanin biosynthetic pathway. The enzyme tyrosinase (monophenol, L-dopa; oxygen, oxidoreductase E.-C. 1.14.18.1) catalyzes the oxidation of L-tyrosine to L-dopa (3,4-dihydroxyphenylalanine) and the dehydrogenation of dopa to dopaquinone (1-3). Logan and Weatherhead (4) provided the first indirect evidence for post-tyrosinase inhibition of melanogenesis in the hair follicles of Siberian hamsters, suggesting a second regulatory site. In fact, recent work suggests that there are at least two sites where enzymes might be involved (5).

* Corresponding author.

0006-291X/91 $1.50

Control of melanization by additional factors could be significant for the understanding of tyrosinase-positive albinism, vitiligo, and variations in skin and hair pigmentation.

In recent years, two melanocyte-specific cDNA clones were isolated, and both were ascribed to tyrosinase (6-11). Based on deduced amino acid sequence, the two code for glycoproteins of similar size and have a membrane-spanning domain and conserved positions of cysteine and histidine (6). One of the clones was mapped to the *c* locus on mouse Chromosome 7 (6,9) and to human Chromosome 11 (q14-q21) (12), and the other was assigned to the brown (*b*) locus on mouse Chromosome 4 (13). The mutant *b* allele confers brown coat color, and the B^{lt} allele confers an almost white color to normally black mice (14). There is a difference of opinion as to whether or not the *b* locus protein is another tyrosinase, as maintained by Hearing and co-workers (15), or a different enzyme such as catalase (16). Houghton *et al.* showed that the mouse b-locus protein is identical to a known human melanosomal protein gp75 (17). We present the data for the mapping of the human b-locus gene to Chromosome 9 and study the sequence homology between the CAS2 protein and tyrosinase.

MATERIALS AND METHODS

cDNA Cloning and Sequencing of CAS2 Gene: The normal human melanocyte cDNA library prepared in λgt11 cloning vector (6) was screened for CAS2 cDNA (human b-protein cDNA) using mouse b-locus cDNA pMT4 (10) as a probe. The DNA restriction fragments subcloned into M13 vector (18) were sequenced by the dideoxy chain termination method (19) using Sequenase (United States Biochemical Corp., Cleveland, OH).

Hybrid Cell lines: A panel of somatic cell lines was derived from fusion between diploid human cells and Chinese hamster cells. PK-87-9 has a Chinese hamster background with Chromosome 9 as its only human chromosome (20). Cell line 640-63a12 contains 9 cen-qter as its only human DNA (21) and is GAT^+, $AK1^+$, $AK3^-$, $ACO1^-$, and CHR^+.

Southern Blot Hybridization: DNA samples from the hybrid cell lines of PK-87-9, 640-63a12, normal human male and female genomic DNA, and rodent DNA were digested with Taq I and electrophoresed in a 0.8% agarose gel. The DNA fragments were transferred to a nylon membrane (Hybond-N, Amersham) (22). The probes used for mapping were the mouse b-protein cDNA pMT4 (10) and human b-protein cDNA CAS2 (our unpublished data). DNA probes were labeled by random primer method (23). Hybridization was done overnight at 42°C. The blots were washed twice in 2 X SSPE, 0.1% $NaDodSO_4$, at room temperature for 15 min each time; then twice in 1 X SSPE, 0.1% $NaDodSO_4$, at 65°C for 30 min each time; rinsed in 0.1 X SSPE at room temperature; and subjected to autoradiography overnight or longer at -70°C using intensifying screens.

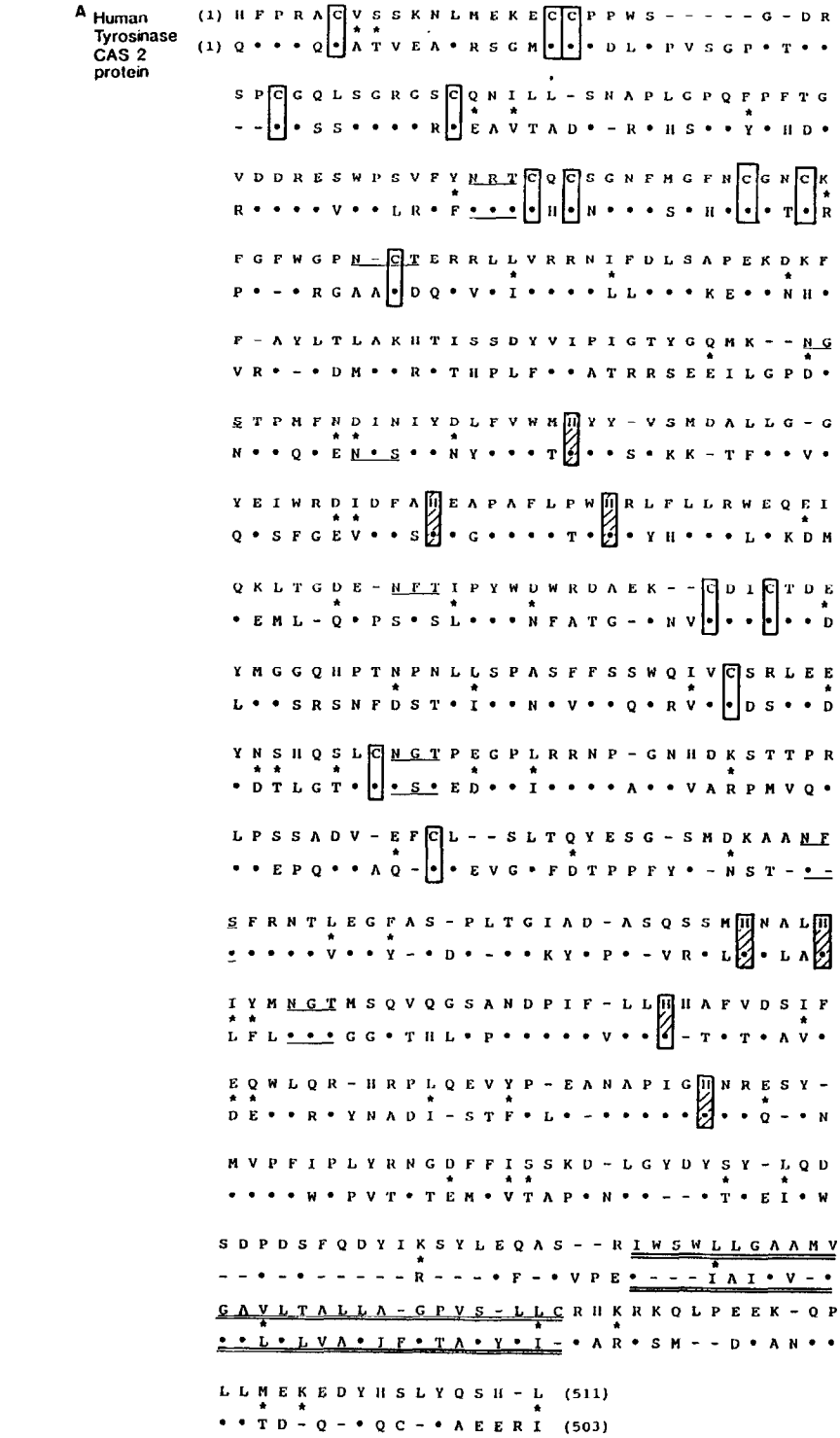
In situ hybridization: The *in situ* hybridization method was as previously described (24,25). The chromosome preparations were G-banded and photograped. After hybridization, the positions of the grains over the chromosomes were scored on prints of the previous metaphase spreads.

RESULTS

Sequence Homology Between Human Tyrosinase and CAS2: Human CAS2 cDNA was isolated from a normal human melanocyte cDNA library constructed in λgt 11 cloning vector (6) by screening with mouse b-protein cDNA pMT4 (10). Three positive clones were isolated

and sequenced. The sequence of the human b-protein cDNA was found to be essentially the same as the human b-protein sequence which was published recently by Cohen *et al.* (26). We analyzed the amino acid sequence of the human CAS2 protein deduced from the cDNA sequence and studied the amino acid homology between the two pigment cell specific protein tyrosinases and the human CAS2 protein (Fig. 1A). Mature human CAS2 protein is composed of 503 amino acids as reported by Cohen *et al.* (26), with a Mr of about 57,000; and tyrosinase is composed of 511 amino acids with a Mr of 58,000. The two proteins are similar to one another in many respects. Both are glycoproteins and have putative signal peptides of 24 amino acids in human CAS2 protein and 18 amino acids in tyrosinase. Both contain the transmembrane domain which indicates that the two proteins are membrane-bound. The two proteins are homologous in 45% of the amino acids. If we take into account discrepancies among chemically similar amino acids, the homology is about 57%. There are 5 glycosylation sites in human CAS2 protein compared with 7 in tyrosinase. The transmembrane domain is comprised of 27 amino acids in tyrosinase and of 24 amino acids in the b-protein toward the carboxy terminus. There are four regions of homology in the two proteins (Fig. 1B). Homologous domains I and III, which are both cysteine-rich regions, include residues 2-133 and 225-306 of tyrosinase and 2-137 and 231-317 of human CAS2 protein. The space between cysteine is similar, and the residues around the cysteines are especially similar in the two proteins. Homologous domains II and IV include residues 144-218 and 313-441 of tyrosinase and 150-225 and 325-446 of human CAS2 protein. In domain II, three histidines are aligned and the amino acid residues surrounding each histidine are similar in both proteins. In domain IV, there are six histidines in tyrosinase and five in CAS2 protein, and four histidines are aligned. Histidine 402 of tyrosinase and its surrounding seven amino acids are identical in the two proteins. The amino acid identity between the homologous domains of the two proteins is 50.4% in domains I and IV, 52% in domain II, and 50% in domain III.

Mapping of CAS2 Gene to Human Chromosome 9: The mouse b-locus gene was mapped to Chromosome 4 (13), but no data is available on the human counterpart. The chromosomal location of the sequence corresponding to the CAS2 gene was studied by Southern blot analysis of the DNA from Chinese hamster and human hybrid cell lines. Hybridization of the CAS2 cDNA or mouse b-protein cDNA pMT4 to the human genomic DNA digested with TaqI produced two bands, one of 10Kb and the other of 14Kb (Fig. 2A and 2B, respectively, lanes 1 and 2). Somatic cell hybrid line PK-87-9 which has human Chromosome 9 as its only human source also exhibited a similar band pattern (Fig. 2A and B, lane 3). The human syntenic region to mouse Chromosome 4 is human Chromosome 9 (27,28) The other hybrid cell line 640-63a12 which is reported to contain human Chromosome 9qcent-9qter did not hybridize to CAS2 or pMT4 (Fig 2A and B, lane 4).


A
Human Tyrosinase
CAS 2 protein
(1) M F P R A C V S S K N L M E K E C C P P W S - - - - - G - D R
(1) Q • • • Q • A T V E A • R S G M • • • D L • P V S G P • T • •
S P C G Q L S G R G S C Q N I L L - S N A P L G P Q F P F T G
- - • • S S • • • • R • E A V T A D • - R • H S • • Y • H D •
V D D R E S W P S V F Y N R T C Q C S G N F M G F N C G N C K
R • • • • V • • L R • F • • • • H • N • • • S • H • • • T • R
F G F W G P N - C T E R R L L V R R N I F D L S A P E K D K F
P • - • R G A A • D Q • V • I • • • • L L • • • K E • • N H •
F - A Y L T L A K H T I S S D Y V I P I G T Y G Q M K - - N G
V R • - • D M • • R • T H P L F • • A T R R S E E I L G P D •
S T P M F N D I N I Y D L F V W M H Y Y - V S M D A L L G - G
N • • Q • E N • S • • N Y • • • T • • • S • K K - T F • • V •
Y E I W R D I D F A H E A P A F L P W H R L F L L R W E Q E I
Q • S F G E V • • S • G • • • • T • • Y H • • • L • K D M
Q K L T G D E - N F T I P Y W D W R D A E K - - C D I C T D E
• E M L - Q • P S • S L • • • N F A T G - • N V • • • • • D
Y M G G Q H P T N P N L L S P A S F F S S W Q I V C S R L E E
L • • S R S N F D S T • I • • N • V • • Q • R V • • D S • • D
Y N S H Q S L C N G T P E G P L R R N P - G N H D K S T T P R
• D T L G T • • • S • E D • • I • • • • A • • V A R P M V Q •
L P S S A D V - E F C L - - S L T Q Y E S G - S M D K A A N F
• • E P Q • • A Q - • • E V G • F D T P P F Y • - N S T - • -
S F R N T L E G F A S - P L T G I A D - A S Q S S M H N A L H
• • • • • V • • Y - • D • - • • K Y • P • - V R • L • • L A •
I Y M N G T M S Q V Q G S A N D P I F - L L H A F V D S I F
L F L • • • G G • T H L • P • • • • • V • • • - T • T • A V •
E Q W L Q R - H R P L Q E V Y P - E A N A P I G H N R E S Y -
D E • • R • Y N A D I - S T F • L • - • • • • • • • • Q - • N
M V P F I P L Y R N G D F F I S S K D - L G Y D Y S Y - L Q D
• • • • W • P V T • T E M • V T A P • N • • - - • T • E I • W
S D P D S F Q D Y I K S Y L E Q A S - - R I W S W L L G A A M V
- - • - • - - - - - R - - - • F - • V P E • - - - I A I • V - •
G A V L T A L L A - G P V S - L L C R H K R K Q L P E E K - Q P
• • L • L V A • I F • T A • Y • I - • A R • S M - - D • A N • •
L L M E K E D Y H S L Y Q S H - L (511)
• • T D - Q - • Q C - • A E E R I (503)
B
Human Tyrosinase
CAS 2 protein
NH2
COOH
TM
S 2
133 144
218 225
306 313
441 456 482 511
50.4%
52.0%
50.4%
50.0%
2
137 150
225 231
317 325
446 454 477 503
I
II
III
IV

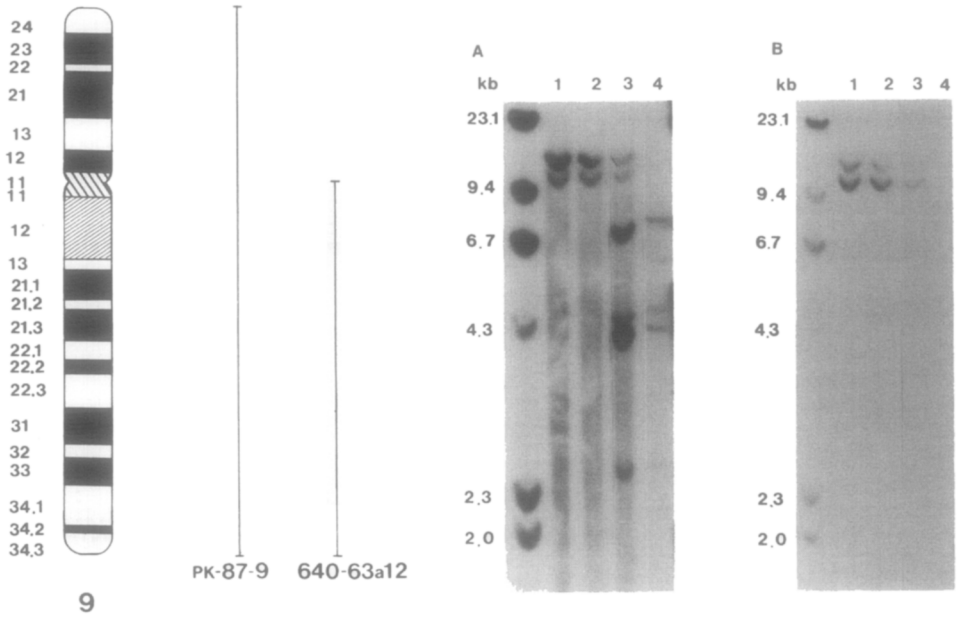


Figure 2. Chromosomal mapping of the human b protein locus. Hybridization of Taq 1 digested DNA from Chinese hamster and human hybrid cells and controls with human b-protein clone CAS2 (B) and with mouse b-protein clone pMT4 (A). Lane 1 and 2, control human male and female genomic DNA; lane 3, PK-87-9 hybrid containing human Chromosome 9; lane 4, 640-63a12 hybrid containing human Chromosome 9qcen-qter.

Regional localization of the CAS2 gene on Human Chromosome 9: In the *in situ* hybridization experiment, a total of 1422 grains were found to be present in 40 cells. Figure 3 shows an ideogram with the grain distribution over the human genome, where each dot represents an average of two grains. In the final analysis, 8.4% of the grains clustered around 9p21-pter, or more narrowly, 6.5% on 9p22-pter. This results indicate that CAS2 gene maps to human chromosome 9p22-pter.

Figure 1. (A) Primary structure of the deduced proteins of human tyrosinase and b-protein in the single letter amino acid code and their alignment in the homologous regions. The top line indicates the mature human tyrosinase, and the bottom line indicates the human b-protein. (•) Identical amino acids in these proteins. (*) Chemically similar amino acids found in both sequences. Residues in boxes are registered residues used for optimum alignment (cysteine or histidine). The potential glycosylation sites are underlined in regular print, and the putative transmembrane region is doubly underlined. (B) Diagram showing the regions of homology between human tyrosinase and CAS2 protein. The putative signal sequence (S), positions of cysteine residues (*), positions of possible copper ligands (H), and a putative transmembrane region (TM) are indicated. NH_2, amino-terminus; COOH, carboxyl terminus. The numbers of identical residues (expressed as percentage) in a given segment are indicated between the two proteins. The numbers along with human tyrosinase and CAS2 protein indicate the positions of amino acids in the putative mature proteins. The Roman numerals indicate the regions of homology.

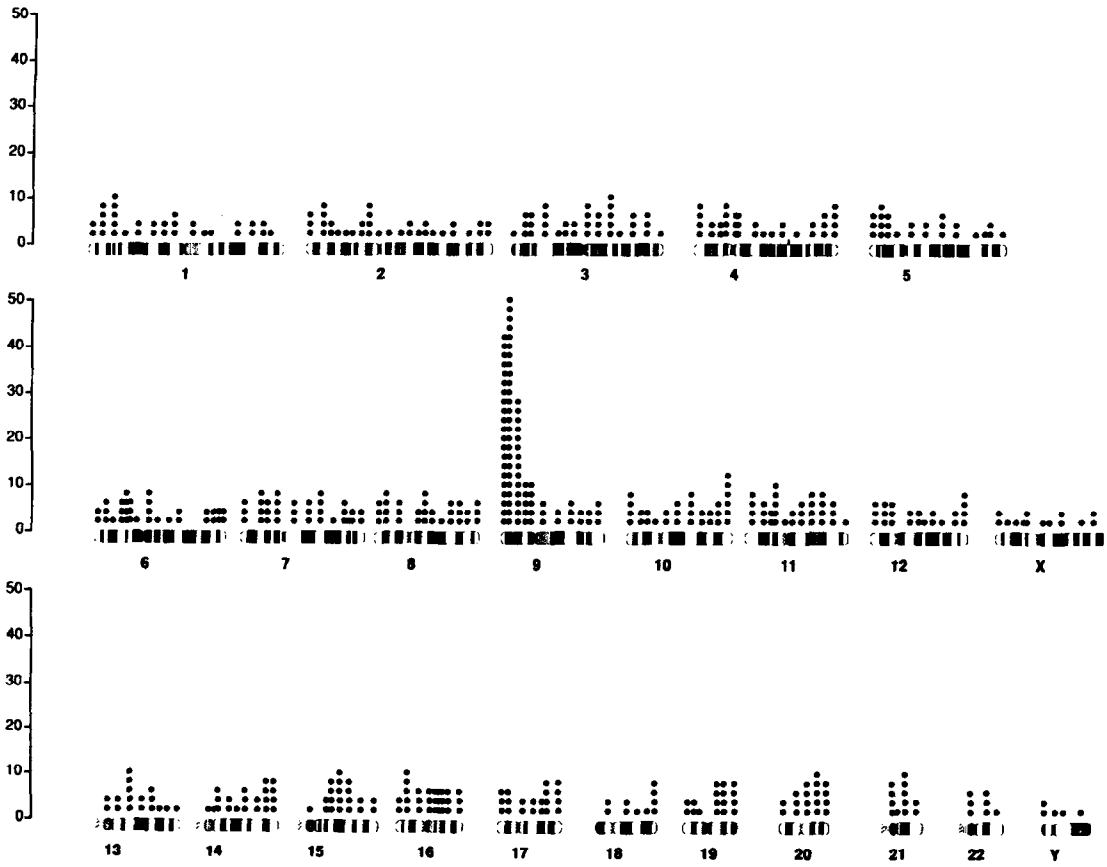


Figure 3. *In situ* hybridizatin with the CAS2 cDNA probe. Ideogram showing the grain distribution over the human genome, where each dot represents an average of 2 grains. A total of 1422 grains were found in 40 cells. The peak is seen at 9p22-pter.

DISCUSSION

The CAS2 gene is located on human chromosome 9 and has been mapped more precisely to 9p22-pter by *in situ* hybridization. Human chromosome 9 is known to contain a region of homology on mouse chromosome 4 (27,28). The mouse b locus maps to chomosome 4 and is flanked proxomally by the loci Lv, Aco-1 and Galt, and distally by Ifa, Ifb, Orm-1 and Orm-2 (27). All these loci are homologous with loci on human chromosome 9 and it was therefore likely that CAS2 would map to choromosome 9, though its regional assignment could not be predicted since the closest proximal marker, ALAD (homologous to mouse Lv) maps to 9q and the closest distal markers, IFNA and B, map to 9p (Fig. 4). The region of synteny between mouse 4 and human 9 is therefore split into several segments by the insertion of 9q loci into an area of 9p homology.

Southern blot hybridization of CAS2 to somatic cell hybrids are consistant with a position on 9p. There was hybridization to line PK-87-9 with chromosome 9 as its only human

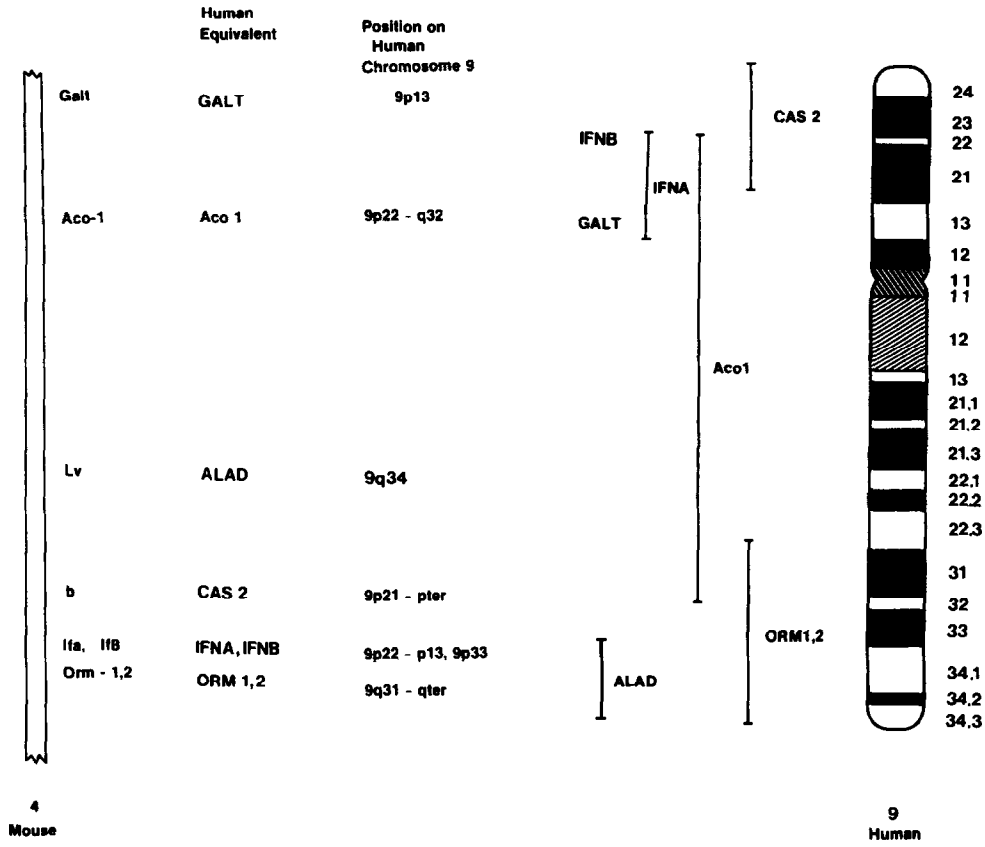


Figure 4. Homologous genes on mouse chromosome 4 and human chromosome 9 (Searle et al 1989). The mouse b locus is flanked by Lv, for which the human homologue, ALAD, maps to 9q34, and by Ifa and b which have human homologues, IFNA and B, which map to 9p22--13 and 9-33, respectively.

component, and a lack of hybridization to line 640-63a12 which has 9q as its only human component (Fig. 2). *In situ* hybridization was performed and maps CAS2 to 9p22-pter.

The location of the tyrosinase gene on human Chromosome 11 (12) and the CAS2 gene on Chromosome 9 indicates that these two genes are indeed different and probably involved in different functions. Human b-protein is expressed specifically in melanocyte (our unpublished data, 10) showing expression patterns similar to tyrosinase (6).

Although the two genes are located on different chromosomes, there is an extensive homology between the two proteins (57% identity). The conserved regions of histidine and their surrounding amino acids in the two proteins suggest that the b-locus protein is probably a copper-binding protein similar to tyrosinase. Histidines serve as site for the binding of copper in tyrosinase (29). However, histidines are also ligands for iron in heme. An invariant sequence of Leu-His is commom between plant peroxidases and the proximal heme-binding site of globin (30) which suggests that this segment may act as a ligand for heme. This invariant

sequence is also present in tyrosinase (residues 190-193) and CAS2 protein (197-200). These two melanocyte specific proteins either bind both copper and heme similarly to cytochrome C oxidase (31) or share homology through a potential porphyrin binding site. The precise role of b-protein/CAS2 protein in the biosynthesis of melanin is not known. Halaban and Moellmann (16) demonstrated that the b-protein is a catalase and is identical to human melanosomal glycoprotein gp75. They suggested that the b-protein or catalase B is probably involved in scavenging the H_2O_2 produced during the oxidation of melanin precursors like dopa and 5,6 dihydroxyindole (4).

ACKNOWLEDGMENTS

We thank Cynthia Jackson and Carole Jones for providing the cell lines PK-87-9 and 640-63a12, respectively; Isabel Marques for providing the hybrid lines; Sue Chamberline, Jacquis Shaw, and Sue Povey for growing the cells and isolating the DNA; Dr. Shibahara for providing pMT4 cDNA clone; Yvonne Kobayashi for excellent technical assistance; and Susan Fordyce for editing and typing the manuscript. This work was supported by grants from the NIH AR-40248, Schering Corp, and the March of Dimes Birth Defects Foundation.

REFERENCES

1. Lerner, A.B., Fitzpatrick, T.B., Calkins, E., and Summerson, W.H. (1949) J. Biol. Chem. 178:185-195.
2. Pomerantz, S.H. (1966) J. Biol. Chem. 241:161-168.
3. Shimao, K. (1962) Biochem. Biophys. Acta. 62:205-215.
4. Logan, A. and Weatherhead, B. (1980) J. Invest. Dermatol. 74:47-50.
5. Korner, A.M. and Pawelek, J.M. (1980) J. Invest. Dermatol. 75:192-195.
6. Kwon, B.S., Haq, A.K., Pomerantz, S.H., and Halaban, R. (1987) Proc. Natl. Acad. Sci. USA 84:7473-7477.
7. Ruppert, S., Muller, G., Kwon, B., and Schutz, G. (1988) EMBO J. 7:2715-2722.
8. Kwon, B.S., Wakulchik, M., Haq, A.K., Halaban, R. and Kestler, D. (1988) Biochem. Biophys. Res. Commun. 153:1301-1309.
9. Kwon, B.S., Haq, A.K., Wakulchik, M., Kestler, D., Barton, D.E., Francke, U., Lamoreux, M., Whitney, J.B.,III, and Halaban, R. (1989) J. Invest. Dermatol. 93:589-594.
10. Shibahara, S. Tomita, Y., Sakakura, T., Nager, C., Bhabatosh, C. and Muller, R. (1986) Nucleic Acids Res. 14:2413-2427.
11. Yamamoto, H., Takeuchi, S., Kuko, T., Sato, C., and Takeuchi, T. (1989) Jpn. J. Genet. 64:12-135.
12. Barton, D.E., Kwon, B.S., and Francke, U. (1988) Genomics 3:17-24.
13. Jackson, I. (1988) Proc. Natl. Acad. Sci. USA 85:4392-4396.
14. Silvers, S.K. (1979) In: The Coat Colors of Mice: A Model for Mammalian Gene Action and Interaction. Springer, New York.
15. Jimenez, M., Maloy, M.L., and Hearing V.J. (1989) J. Biol. Chem. 264:3397-3403.
16. Halaban, R. and Moellman, G. (1990) Proc. Natl. Acd. Sci. USA 87:4809-4813.
17. Vijayasaradhi, S., Bouchard, B., and Houghton, A. (1990) J. Exp. Med. 171:1375-1380.
18. Messing, J., Crea, R., and Seeburg, P.H. (1981) Nucleic Acids Res. 9:309-322.
19. Sanger, F., Nickles, S., and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 75:5463-5467.
20. Warburton, D., Gersen, S., Yu, M-T., Jackson, C., Handelin, B. and Housman, D. (1990) Genomics 6:358-366.

21. Jones, C. and Kao, F.T. (1984) Cytogenet. Cell Genet. 37:499-500.
22. Southern, E.M. (1975) J. Mol. Biol. 87:503-517.
23. Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132:6-13.
24. Abbott, C.L., Povey, S., Jeremiah, S., Murad, Z., Di Scipio, R., and Fey, G. (1989) Genomics 4:606-609.
25. Boyd, Y., Buckle, V.J., Munro, E.A., Choo, K.H., Migeon, B.R., and Craig, I.W. (1984) Ann.Hum. Genet. 48:145-152.
26. Cohen, T., Muller, R.M., Tomita, Y. and Shibahara, S. (1990) Nucl. Acids Res. 18 (9):2807.
27. Searle, A.G., Peters, J., Lyon, M.F., Hall, J.G., Evans, E.P., Edwards, J.H., and Buckle, E.J. (1989) Ann. Hum. Genet. 53:89-140.
28. Nadeau, J.H. (1989) Trends in Genet. 5:82-86.
29. Lerch, K. (1988) In: Advances in Pigment Cell Research. Bagnard, T., (ed.). Liss, New York, pp. 85-98.
30. Welinder, K.G. (1985) Eur. J. Biochem. 151:497-504.
31. Steffens, G.C.M., Biewald, R., and Buse, G. (1987) Eur.J.Biochem. 164:295-300.