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A chromosomal segment conserved since divergence of lineages leading to man and mouse: The gene order of aminoacylase-1, transferrin, and beta-galactosidase on mouse Chromosome 9

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Summary

Linkage crosses were used to determine the gene order of aminoacylase-1, beta-galactosidase, and transferrin on mouse Chromosome 9. The most likely gene order, which was more than 10^7 times more likely than the next most likely order, was transferrin, aminoacylase-1, beta-galactosidase. These results demonstrate that aminoacylase-1 and beta-galactosidase mark a chromosomal segment that has been conserved since divergence of lineages leading to man and mouse. The length of this segment is about 11 cM and the estimate of the true length of the conserved segment marked by these two genes is 33 cM. A second aminoacylase allozyme, which requires $MgCl_2$, is described.

1. Introduction

Comparison of the chromosomal location of homologous genes in different species provides a powerful means for studying genome organization and evolution (Ohno, 1973; Lalley *et al.* 1977, 1978; Womack, 1982; Nadeau & Taylor, 1984; Kao, 1985; O'Brien *et al.* 1985). Analysis of linkage maps has demonstrated that synteny for genes on the X-chromosome has been highly conserved during evolution and that many autosomal linkages have been conserved since divergence of lineages leading to species such as man and mouse. As these linkage maps become more precise, it should be possible not only to assess the degree of linkage conservation, but also to study the rates, patterns, and characteristics of chromosomal rearrangements that have disrupted linkages during evolution. This analysis will, of course, depend very heavily on the accuracy of the linkage maps; errors or uncertainties about linkage or gene order could seriously confound the analysis.

An example of uncertainty about gene order involves genes on mouse Chromosome 9 and human Chromosome 3. Aminoacylase-1 (*Acy-1*), transferrin (*Trf*), and beta-galactosidase (*Bgl*) are located on the distal portion of mouse Chromosome 9 (Chapman, 1973; Chapman & Frels, 1975; Lalley *et al.* 1982; Antonucci *et al.* 1984). The human homologues of *Acy-1*, ACY1, and *Bgl*, GLB1, are located on Chromosome 3p21 (Kit *et al.* 1980; Voss *et al.* 1980; Naylor *et al.* 1982) and 3p21-cen (Bruns *et al.* 1979; Shows *et al.* 1979; De Wit *et al.* 1979), respectively, whereas the human homologue of *Trf*, TF, is located on Chromosome 3q21-q26.1 (Huerre *et al.* 1984; Yang *et al.* 1984). These data demonstrate that synteny has been conserved. However, information about gene order is important for determining whether the chromosomal segment marked by these genes has been conserved since divergence of the lineages leading to man and mouse, i.e. is *Trf* located between *Acy-1* and *Bgl* and does it consequently interrupt a segment that otherwise appears to be conserved? It is here where the mouse data are weak. *Acy*-1 and *Trf* are both about 7–10 cM from *Bgl*, but they have not been mapped relative to each other (Lalley *et al.* 1982; Antonucci *et al.* 1984). Because recombination frequencies between these genes and *Bgl* are so similar, it is not possible to infer the most likely gene order (see Bishop [1985] for a discussion of this problem.) To determine the most likely order, three point crosses involving *Acy-1*, *Trf*, and *Mod-1* (malic enzyme -1) were made. *Mod-1* is a gene whose position is firmly established (Chapman, 1973, Chapman and Frels, 1975, Lalley *et al.* 1982; Antonucci *et al.* 1984). Analysis of data resulting from these linkage crosses showed that the most likely gene order was *Mod-1–Trf–Acy-1–Bgl* and demonstrated that the chromosomal segment marked by *Acy-1* and *Bgl* has been conserved since divergence of lineages leading to man and mouse.

2. Materials and Methods

(i) *Mice*

All mice were obtained from the production colonies of the Jackson Laboratory.

(ii) *Tissue preparation*

Kidney samples (fresh or frozen) were mixed with an equal volume of distilled water and homogenized with a Kontes tissue grinder, and centrifuged for 15 min on an Eppendorf microfuge. The supernatant was used immediately for electrophoresis. Blood samples were collected from the retro-orbital sinus by using heparinized capillary tubes and then were centrifuged for 5 min on an Eppendorf microfuge. Plasma samples (fresh or frozen) were isolated and used for electrophoresis.

(iii) *Electrophoresis*

ACY-1. The electrophoresis buffer consisted of 3·0 g Trizma base (Sigma T-1503) and 14·4 g ammonia-free glycine (Sigma G-7126) per litre, pH 8·5. Titan III (Helena) or Sepraphore X (Gelman) cellulose acetate plates were used. A Helena Zip Zone sample applicator was used to apply 2 μl of tissue homogenate 1 cm from the cathodal end of the gel. Electrophoresis was conducted for 25–30 min at 180 V. The staining solution consisted of 10 mg *N*-formyl-L-methionine, 0·25 ml of 10 mg per ml rattlesnake venom (*C. adamenteus*; Sigma V-6875), 0·25 ml of 10 mg per ml horseradish peroxidase (Sigma P-8250), and 0·15 ml of 1% *O*-dianisidine dihydrochloride (Sigma, D-3252) in 5 ml 0·1 M phosphate buffer, pH 7·0 (Fisher, SO-B-108). To detect ACY-2 activity, 0·10 ml of $MgCl_2$ was added to the stain solution. The stain solution was applied as an agar overlay to the surface of the cellulose acetate plate.

MOD-1. Electrophoretic methods described by Eicher and Coleman (1977) and Eicher *et al.* (1980) were used for typing MOD-1.

TRF. Electrophoretic methods provided by E. M. Eicher (personal communication) were used to type TRF.

3. Results

The order of *Mod-1*, *Trf*, and *Acy-1*. Two crosses were used to study the gene order of *Mod-1*, *Trf*, and *Acy-1*. (C3H/HeJ × CBA/J) F_1 hybrid males were backcrossed either to CBA/J females (Cross 1) or to C3HeB/FeJ females (Cross 2). A total of 208 backcross progeny were typed in Cross 1 and 138 progeny in Cross 2 (Table 1). Data for the two crosses were pooled because heterogeneity between the sets of data was not detected ($\chi^2_3 = 5{\cdot}58$, $P > 0{\cdot}10$). Maximum likelihood methods were used to identify the most likely gene order and to calculate the support for the most likely order (Bishop, 1985). The log likelihood for the order *Mod-1–Trf–Acy-1* was −74·099, whereas the log likelihood was −65·484 for the next most likely order *Mod-1–Acy-1–Trf*. The difference in the log likelihoods for alternative orders provides an estimate of the support for the most likely order (Bishop, 1985). This difference between the most likely order and the next most likely order was 8·615; the gene order *Mod-1–Trf–Acy-1* was therefore more than 10^7 times more likely than the alternative order *Mod-1–Acy-1–Trf*. The order *Trf–Mod-1–Acy-1* was the least likely (data not shown). The combined data give the following recombination frequencies: *Mod-1*–0·087 ± 0·015–*Trf*–0·032 ± 0·009–*Acy-1* (Table 2, Fig. 1).

Table 1. *Recombination between* Acy-1, Trf, *and* Mod-1; *allelic combination inherited from the* F_1 *parent**

Allelic combination								
Mod-1			*Trf*			*Acy-1*	No. observed
Cross 1: CBA/J × (C3H/HeJ × CBA/J) F_1							
a			b			c	80
b			a			b	102
a	×	a				b	12
b	×	b				c	5
a			b	×	b		6
b			a	×	c		3
b	×	b		×	b		0
a	×	a		×	c		0
							208
Cross 2: C3HeB/FeJ × (C3H/HeJ × CBA/J) F_1							
a			b			c	53
b			a			b	71
a	×	a				b	4
b	×	b				c	8
b			a	×	c		1
a			b	×	b		0
a	×	a		×	c		1
b	×	b		×	b		0
							138

* × indicates the position of a cross-over.

Acy-2. During the development of the electrophoretic assay for ACY-1, staining solutions were prepared with and without $MgCl_2$. When $MgCl_2$ was included in the solution, an additional band of ACY activity was observed (Fig. 2). This band was not detected when the substrate for ACY, *N*-formyl-L-methionine, or when $MgCl_2$ was deleted from the staining solution. The gene encoding this ACY enzyme, which is characterized by its requirement for Mg, was designated *Acy*-2. An extensive strain survey of inbred and wild strains, including two strains of *Mus spretus*, was conducted, but electrophoretic polymorphisms were not detected. A list of the strains tested will be provided on request.

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Table 2. Summary of linkage and recombination data

Interval	Cross 1	Cross 2	Combined
Mod-1–Trf	17/208 = 0·082	13/138 = 0·094	30/346 = 0·087±0·015
Acy-1–Trf	9/208 = 0·043	2/138 = 0·014	11/346 = 0·032±0·009
Mod-1–Acy-1	26/208 = 0·125	13/138 = 0·094	39/346 = 0·113±0·017

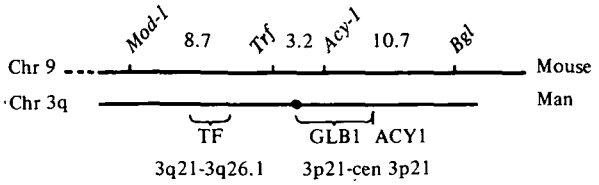

Fig. 1. Linkage map of mouse Chromosome 9 showing the relative positions of *Mod-1*, *Trf*, *Acy-1*, and *Bgl*. Also given is the most likely location of the latter three genes on human Chromosome 3.

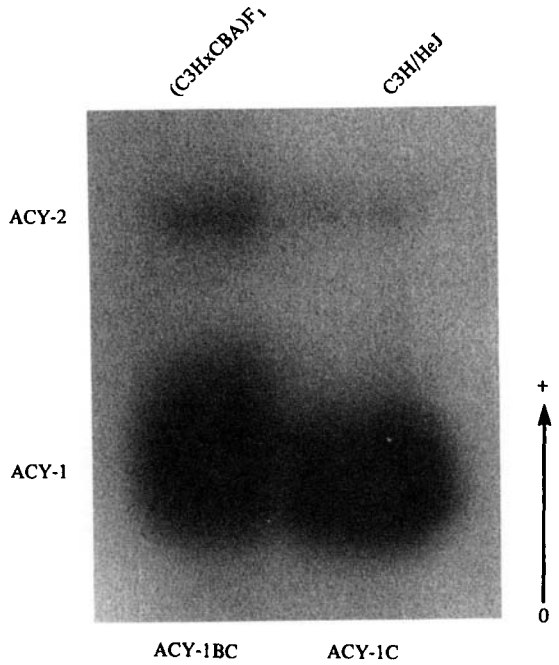

Fig. 2. ACY-1 and ACY-2 allozymes in kidney samples from C3H/HeJ and (C3H × CBA) F_1 mice.

4. Discussion

The recombination frequencies presented here are generally consistent with results presented previously. The map distance of 11·3±1·7 cM between *Mod-1* and *Acy-1* is very similar to the distance of 10·9±2·6 cM reported by Antonucci *et al.* (1984) and the distance of 8·7±1·5 cM between *Mod-1* and *Trf* is similar to the distance of 4·6 cM (the standard error was not given) reported by Chapman & Frels (1975). *Acy-1* and *Trf*, however, have not been included together in any previously published linkage study and their relative positions could not be inferred reliably because of contradictions between published results. For example, *Trf* is located between *Mod-1* and *Bgl* and is 4·6 cM from *Mod-1* and 7·7 cM from *Bgl* (Chapman & Frels, 1975). However, Lalley *et al.* (1982) reported that *Acy-1* is 10·7±6·2 cM proximal to *Bgl*, which suggests it is proximal to *Trf*. By contrast, Antonucci *et al.* (1984) reported that *Acy-1* is 10·9±2·6 cM distal to *Mod-1*, which would place it distal to *Trf*. Results presented here resolve this contradiction and show that *Acy-1* is distal to *Trf*.

It is usually assumed that synteny of two or more homologous markers in different species represents linkage homology (Nadeau & Taylor, 1984). This is a reasonable assumption until contrary evidence for any particular segment is obtained. Several exceptions have been identified, however (Nadeau & Taylor, 1984; Nadeau *et al.* 1986). These exceptions demonstrate that caution must be exercised when comparing linkage maps to identify synteny and linkage homologies.

Identification of chromosomal segments that have been conserved since divergence of lineage leading to man and mouse may be confounded by uncertainty about the relative position of genes within the putatively conserved segment. Results presented here resolve the uncertainty about one of these conserved segments and demonstrate that the conserved segment includes at least *Acy-1* and *Bgl* and, depending on the relative position of ACY1 and GLB1 in man, may include *Trf* as well (Fig. 1). The length of the conserved segment marked by *Acy-1* and *Bgl* is 10·7±6·2 cM (Lalley *et al.* 1982) and the estimate of the true length of the conserved segment is 33 cM (eq. 2; Nadeau & Taylor, 1984). If GLB1 is shown to be distal to ACY1 in man, then the conserved segment includes *Trf* and these estimates become 13·9 cM (*Trf*–3·2 cM–*Acy-1*–10·7 cM–*Bgl*) and 28 cM, respectively. However, it seems more likely that GLB1 will be proximal to ACY1 in man (Fig. 1) and therefore the conserved segment minimally includes *Acy*-1 and *Bgl* but not *Trf*.

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References

Antonucci, T. K., von Deimling, O. H., Rosenblum, B. B., Skow, L. C. & Meissler, M. H. (1984). Conserved linkage within a 4 cM region of mouse chromosome 9 and human chromosome 11. *Genetics* **107**, 463–475.

Bishop, D. T. (1985). The information content of phase-known matings for ordering genetic loci. *Genetic Epidemiology* **2**, 349–361.

Bruns, G. A. P., Leary, A. C., Regina, V. M. & Gerald, P. S. (1977). Lyosomal beta-D-galactosidase in man–hamster somatic cell hybrids. *Human Gene Mapping* **4**, 177–181.

Chapman, V. (1973). Personal communication. *Mouse Newsletter* **49**, 46.

Chapman, V. & Frels, W. (1975). Personal communication. *Mouse Newsletter* **53**, 61.

Eicher, E. M. & Coleman, D. L. (1977). Influence of gene duplication and X-inactivation on mouse mitochondrial malic enzyme activity and electrophoretic patterns *Genetics* **85**, 647–658.

Eicher, E. M., Taylor, B. A., Leighton, S. C. & Womack, J. E. (1980). A serum protein polymorphism determinant on chromosome 9 of *Mus musculus*. *Molecular and General Genetics* **177**, 571–576.

Huerre, C., Uzan, G., Greschik, K. H., Weil, D., Levin, M., Hors-Cayla, M. C., Boue, J., Kahn, A & Junien, C. (1984). The structural gene for transferrin TF maps to 3q21-3qter. *Annales de Genetique* **27**, 5–10.

Kao, F.-T. (1985). Human genome structure. *International Review of Cytology* **96**, 51–88.

Kit, S., Hazen, M., Qavi, H., Dubbs, D. R. & Pathak, S. (1980). Integration site(s) of herpes simplex virus type 1 thymidine kinase gene and regional assignment of the gene for aminoacylase-1 in human chromosomes. *Cytogentics and Cell Genetics* **26**, 93–103.

Lalley, P. A., Francke, U. & Minna, J. D. (1978). Homologous genes for enolase, phosphogluconate dehydrogenase, phosphoglucomutase, and adenylate kinase are syntenic on mouse Chromosome 4 and human Chromosome 1p. *Proceedings of the National Academy of Sciences USA* **75**, 2382–2386.

Lalley, P. A., Minna, J. D. & Francke, U. (1977). Conservation of autosomal gene synteny groups in mouse and man. *Nature* **274**, 160–162.

Lalley, P. A., Naylor, S. L., Elliot, R. W. & Shows, T. B. (1982). Assignment of *Acy-1* to mouse chromosome 9: evidence for homologous linkage groups in man and mouse. *Cytogentics and Cell Genetics* **32**, 293.

Nadeau, J. H., Berger, F. G., Kelley, K. A., Pitha, P. M., Sidman, C. L. & Worrall, N. (1986). Rearrangement of loci located on homologous chromosomal segments in mouse and man: the location of alpha- and beta-interferon, alpha-1 acid glycoprotein-1 and -2, and aminolevulinate dehydratase on mouse Chromosome 4. *Genetics* (in press).

Nadeau, J. H. & Taylor, B. A. (1984). Lengths of chromosomal segments conserved since divergence of man and mouse. *Proceedings of the National Academy of Sciences USA* **81**, 814–818.

Naylor, S. L., Elliot, R. W., Brown, J. A. & Shows, T. B. (1982). Mapping of aminoacylase-1 and beta-galactosidase-A to homologous regions of human chromosome 3 and mouse chromosome 9 suggests location of additional genes. *American Journal of Human Genetics* **34**, 235–244.

Naylor, S. L. & Sakaguchi, A. Y. (1986). Chromosome mapping of serum protein genes. In *Protides of the Biological Fluids* (ed. H. Peeters), pp. 71–76. New York: Pergamon Press.

Naylor, S. L., Shows, T. B. & Klebe, R. J. (1979). Bioautographic visualization of aminoacylase-1: Assignment of the structural gene *ACY-1* to chromosome 3 in man. *Somatic Cell Genetics* **5**, 11–21.

O'Brien, S. J., Seuanez, H. & Womack, J. A. (1985). On the evolution of genome organization in mammals. In *Molecular Evolutionary Genetics* (ed. R. J. MacIntyre), pp. 519–589. New York; Plenum Press.

Ohno, S. (1973). Ancient linkage groups and frozen accidents. *Nature* **244**, 259–262.

Shows, T. B., Scrafford-Wolff, L. R., Brown, J. A. & Meisler, M. H. (1979). G_{M_1}-gangliosidosis: chromosome 3 assignment of the beta-galactosidase-A gene (B-GAL_A). *Somatic Cell Genetics* **5**, 147–158.

Voss, R., Lerer, I., Povey, S., Solomon, E. & Bobrow, M. (1980). Confirmation and further regional assignment of aminoacylase-1 (*ACY-1*) on human chromosome 3 using a simplified detection method. *Annals of Human Genetics* **44**, 1–9.

De Wit, J., Hoeksema, H. L., Bootsma, D. & Westerveld, A. (1979). Assignment of structural beta-galactosidase loci to human chromosomes 3 and 22. *Human Gene Mapping* **5**, 217.

Womack, J. A. (1982). Linkage of mammalian allozyme loci: A comparative approach. *allozymes: Current Topic in Biological and Medical Research* **6**, 207–246.

Yang, F., Lum, J. B., McGill, J. R., Moore, C. M., Naylor, S. L., van Bragt, P. H., Baldwin, W. D. & Bowman, B. H. (1984). Human transferrin: cDNA characterization and chromosomal localization. *Proceedings of the National Academy of Sciences USA* **81**, 2752–2756.