Genet. Res., Camb (1991), **58**, *pp.* 111–114 *With* 1 *text-figure* *Printed in Great Britain*

Genetic analysis of a switch in cell specificity of P lysozyme expression in *molossinus* mice

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(*Received 18 March 1991 and in revised form 11 April 1991*)

Summary

An electrophoretic survey of concentrations of lysozymes M and P was carried out with seven species in the house mouse group (*spretus*, *hortulanus*, *abbotti*, *musculus*, *castaneus*, *domesticus* and *molossinus*). In most species M is the predominant lysozyme in all tissues tested, except the small intestine, where P predominates if present. In inbred strains of *molossinus* mice P is more abundant than M in all tissues tested. The phenotypes of high expression of P lysozyme and low expression of M lysozyme in peritoneal and alveolar macrophages were examined genetically. Results of interspecific crosses and backcrosses to *domesticus* mice support the model that the phenotypes are caused by mutation(s) tightly linked to the lysozyme locus. Alleles at the regulatory loci show additive inheritance.

1. Introduction

Lysozymes of mice offer opportunities to examine the genetic basis for natural variation in the tissue specificity of gene expression. Mice possess two lysozymes, M and P, whose amino acid sequences are 92% identical and encoded by tightly linked genes (Hammer *et al.* 1987; Hammer & Wilson, 1987; Cortopassi & Wilson, 1990; Cross & Renkawitz, 1990). Whereas M is expressed mainly in macrophages, which occur in most tissues (especially lung, spleen and liver), P is found predominantly in Paneth cells of the small intestine. It has been shown before that within and among the commensal group of house-mouse species there are large genetic differences in the concentration of P in the small intestine (Hammer & Wilson, 1987). Now we describe another kind of genetic variant, in which high expression of this P lysozyme is no longer limited to Paneth cells. The discovery of this variant was facilitated by an improved assay for lysozymes M and P in electrophoretic gels (Cortopassi & Wilson, 1989).

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2. Materials and Methods

(i) *Animals*

Tissue samples were obtained from C57BL/6J mice, bought from the Jackson Laboratory, Bar Harbor, Maine. MOL A-E/Rk mice were a gift from Thomas Roderick of the Jackson Laboratory. STS/A mice were the gift of Jo Hilgers, Antonie van Leeuwenhoek Cancer Institute, Amsterdam, the Netherlands. Kidneys of MOA, MOD, MS1 and MS2 mice were supplied by Dr Kazuo Moriwaki, National Institute of Genetics, Mishima, Japan. The sources of *castaneus*, *musculus*, *hortulanus*, *abbotti* and *spretus* mice were the same as in Hammer *et al.* (1987).

(ii) *Lysozyme activity stain*

Chemicals, preparation of tissue extracts, macrophage isolation, non-denaturing electrophoresis, detection of lysozyme activity by the overlay wash method, and photography of overlays were as described by Cortopassi & Wilson (1989).

(iii) *Densitometry*

Densitometric measurements of peritoneal macrophage lysozyme content were done as described by

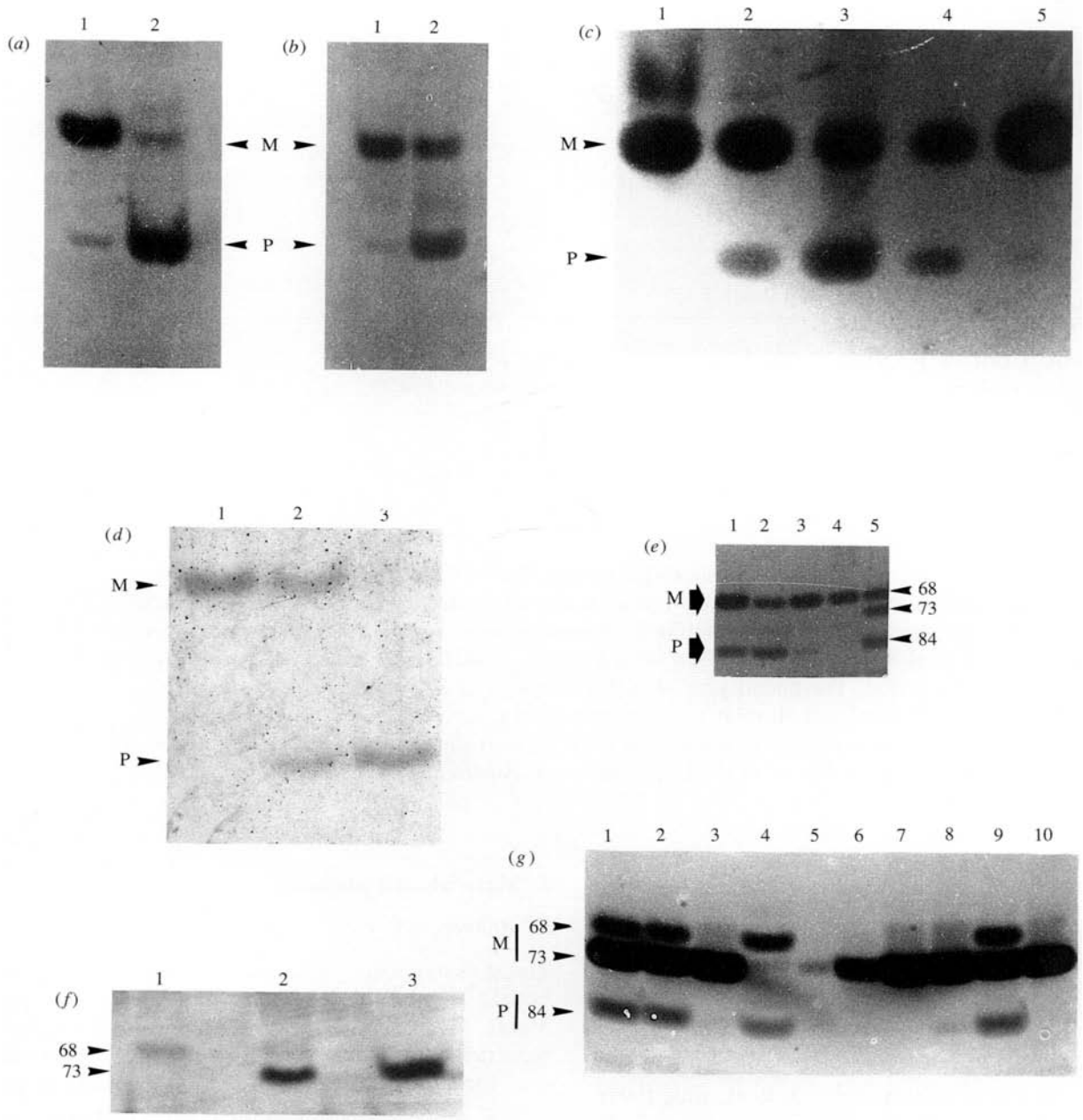

Fig. 1. Electrophoretic separation and detection of lysozyme M and P in tissue extracts from *domesticus* and *molossinus* mice. Panels (*a–c*), (*e*) and (*g*) show results obtained with the overlay-wash method of detecting lysozyme activity, whereas panels (*d*) and (*f*) are based on the Coomassie method of detecting protein. The strains of mice used were C57BL/6J (*domesticus*) in panels (*a–e*), STS/A (*domesticus*) in panels (*f*) and (*g*), MOL D/Rk (*molossinus*) in panels (*b–g*), and three wild *molossinus* in panel (*e*). **Panel (*a*).** Lysozyme activity in *domesticus* lung (lane 1) and small intestine (lane 2) [This panel is based on Fig. 3 in Cortopassi & Wilson (1989)]. **Panel (*b*).** Lysozyme activity in lung from *domesticus* (lane 1) and *molossinus* (lane 2). **Panel (*c*).** Lysozyme activity in peritoneal macrophages from *domesticus* (lanes 1 and 5), *molossinus* (lane 3), and their F1 hybrids (lanes 2 and 4). **Panel (*d*).** Lysozyme protein in lungs from *domesticus* (lane 1), *molossinus* (lane 3) and their F1 hybrid (lane 2). **Panel (*e*).** Lysozyme activity in kidney from three wild *molossinus* (lanes 1, 3 and 5), an inbred *molossinus* (lane 2) and an inbred *domesticus* (lane 4): the A haplotype is present in lane 5, the B haplotype is present in lanes 1, 3 and 4 and the C haplotype is present in lanes 1, 2 and 5. **Panel (*f*).** Lysozyme protein in lung from *molossinus* (lane 1), *domesticus* (lane 3) and their hybrid (lane 2). **Panel (*g*).** Lysozyme activity in lung from backcross mice and controls. Lanes 1–3 and 7–10 are the results of a backcross of (STS/A × MOL D) F1 to STS/A, lane 4, *molossinus*, lanes 5 and 6, two dilutions of STS/A.

Cortopassi & Wilson (1989), using hen eggwhite lysozyme as an activity standard, and were found to be in the range of 1–30 ng lysozyme per peritoneum.

3. Results and discussion

(i) *Pattern of expression*

The pattern of M and P lysozyme expression in mice is known to be tissue-specific. In the *domesticus* strain

https://doi.org/10.1017/S001667230002975X Published online by Cambridge University Press

C57BL/6, which is designated as the 'standard' regulatory type, M activity occurs at the highest level in macrophages, and P activity occurs at the highest level in Paneth cells of the small intestine (Fig. 1, panel a). This pattern was found in all of the standard inbred *domesticus* strains examined except STS/A, GRS/A and Peru/A, which have low P levels (Hammer *et al.* 1987; Hammer & Wilson, 1987; Cortopassi & Wilson, 1989). In other species, e.g. *musculus*, *castaneus*, *hortulanus*, *abbotti* and *spretus* mice, if P is found at all it is found at the highest concentration in the intestine (Hammer *et al.* 1987; Cortopassi & Wilson, 1989).

We now present the results of a new survey of lysozyme type and level in blood and eight other tissues in house mice (Table 1). This survey reveals that *molossinus* P is expressed like *domesticus* M, in that it reaches its highest activity in macrophage-rich tissue like lung (Fig. 1, panel b) (Cortopassi, 1988). In addition to this 10-fold increase in P lysozyme activity, there is nearly 5-fold less M lysozyme in *molossinus* mice.

(ii) *Lysozyme concentration in peritoneal macrophages*

In order to determine whether the increase in P level in macrophage-rich tissue was a result of an increase in P lysozyme levels per cell, peritoneal macrophages were isolated. The overlay-wash method (Cortopassi & Wilson, 1989) was used for electrophoretically separating and detecting lysozymes M and P from purified peritoneal macrophages of individual mice. This method readily detected the lysozyme activity from the approximately 400000 macrophages lavaged from a single peritoneum. A representative result for extracts of equivalent numbers of peritoneal macrophages from the *molossinus* strain MOL D/Rk, the *domesticus* strain C57BL6/J, and the F1 hybrid appears in Fig. 1, panel c. Thus pure preparations of *molossinus* macrophages exhibit higher levels of P lysozyme, and lower levels of M, per cell.

By using the same number of cells from each species and a Coomassie dye whose staining intensity is proportional to mass, not activity, one can determine if higher P lysozyme activity is a result of more molecules, or higher activity per molecule. Fig. 1, panel d shows that the increase in P activity seen in *molossinus* can be fully explained by an increase in the number of P lysozyme molecules per cell. Likewise, the lower M activity in *molossinus* macrophages is the result of fewer M lysozyme molecules per cell. The F1 hybrid is intermediate in both respects between the parents.

(iii) *Polymorphism in wild* molossinus

Lysozyme is produced by mature macrophages in all non-ruminant vertebrates that have been tested (Camara *et al.* 1990). Since tissue and migratory macrophages are found throughout the vertebrate body, one would expect to find high P lysozyme in all tissue of *molossinus* mice. This was shown to be the case when extracts of brain, heart, lung, spleen, liver, small intestine, kidney and colon were surveyed in the inbred strains of *molossinus* MOL D and MOL E (Table 1 and Cortopassi, 1988).

Table 1. *Distribution of lysozyme activity in tissue of mice*

	Lungs		Intestine		Other[a]	
Species/strain	M	P	M	P	M	P
Inbred *molossinus*	+	+	+	+	+	+
Inbred *domesticus*						
C57BL/6	+	−	−	+	+	−
STS/A	+	−	−	−	+	−
musculus	+	−	−	+	+	−
castaneus	+	−	−	−	+	−
hortulanus[b]	+	−	−	±	+	−
abbotti	+	−	−	−	+	−
spretus	+	−	−	+	+	−

[a] Other tissues include kidney and spleen.
[b] This species exhibits a polymorphism in the intestinal concentration of P lysozyme (Hammer *et al.* 1987)

Even though kidneys are not a major site of lysozyme synthesis (Cross *et al.* 1989), finding high P levels in kidney is diagnostic for high P lysozyme in macrophages (Cortopassi, 1988). Kidneys are known to collect lysozyme from blood plasma by glomerular filtration (Maack & Sigulem, 1974). A survey of kidney lysozyme from four mice trapped in four localities throughout Japan revealed the three lysozyme haplotypes described below, namely *haplotype A* – high M (allele 73) in all tissues and low P in all tissues, as in the *castaneus* strain CAS/A and the *domesticus* strain STS/A; *haplotype B* – high M (allele 68) in macrophages and high P in intestine, as in almost all *domesticus*, including C57BL/6 and C3H; and *haplotype C* – moderate M (allele 68) in macrophages and high P in macrophages and intestine, as in *molossinus* strains MOL A–E.

Kidneys from the inbred lines MOL A, B, C, D and E were investigated for P lysozyme level, and all had the homozygous high P phenotype (i.e. haplotype C). These inbred lines were founded by mice from a restricted area, i.e. trapped from the same barn in Japan for two consecutive years (Thomas Roderick, personal communication).

(iv) *F1 lysozyme levels*

The mode of inheritance of the switch in P lysozyme expression and the decrease in M level was investigated by genetic crosses. When inbred *molossinus* (MOL D/Rk) mice are crossed to *domesticus* (C57BL/6 or STS/A) mice, an intermediate level of P activity is

https://doi.org/10.1017/S001667230002975X Published online by Cambridge University Press

seen in lungs (Fig. 1, panel d). The overlay-wash assay is saturated after a certain amount of lysozyme, so that the difference in M lysozyme level is not seen as well in Fig. 1, panel c.

(v) *Cis-trans test for regulator of M lysozyme*

Macrophages of *molossinus* mice have low M lysozyme, as well as high levels of P. A test for cis- or trans-action of the regulator(s) specifying divergent M expression was designed. Since STS/A mice have a more positively charged, faster mobility allele (R_f = 73) at the M lysozyme locus than the common *domesticus* allele (R_f = 68), one can track the regulatory phenotype of each parental M lysozyme gene in the F1 progeny. In F1 progeny of crosses between STS/A and C57BL/6, and crosses of STS/A to MOL D/Rk, each M allele maintains the regulatory phenotype of the parent, i.e. each M regulator acts in *cis* (Fig. 1, panel f). Thus the differing levels of M lysozyme in *molossinus* and *domesticus* macrophages are the result of divergent, *cis*-active regulators.

(vi) *Backcross mice*

In order to determine the number and linkage relationships of loci controlling the phenotype of high P and low M expression in *molossinus* macrophages, a backcross of MOL D/STS F1 animals to the STS/A strain of *domesticus* was set up. STS/A has two locus-specific markers, an M allele of fast electrophoretic mobility (73), and a P gene with very low activity in all tissues (Hammer *et al.* 1987; Cortopassi, 1988).

If the locus controlling P expression in *molossinus* is linked to the lysozyme locus, high expression of P in lungs should cosegregate with the M allele of low mobility (68). This expectation is realized, as shown in Fig. 1, panel g. In 21 backcross individuals, allele 68 never occurred without high P expression, and high P expression was not found in any mouse without allele 68. There were 12 individuals in which M allele 68 and high P were present, and 9 individuals in which M allele 73 and no P was present. Thus the loci specifying high P lysozyme (and low M) in *molossinus* macrophages are statistically significantly linked to the M lysozyme structural gene ($X^2 = 10{\cdot}5$; d.f. = 1, $P < 0{\cdot}005$). The maximum distance between these loci is 13 cM, based on a standard estimate of chromosomal distance (Dizik & Elliot, 1977).

We thank E. M. Prager for helpful comments. This work received support from NIH and NSF grants to A.C.W.

References

Camara, V. M., Harding, J. W. & Prieur, D. J. (1990). Inherited lysozyme deficiency in rabbits: the absence of a primary isozyme or lysozyme as the cause of the condition. *Laboratory Investigation* **63**, 544–550.

Cross, M., Mangelsdorf, I., Wedel, A. & Renkawitz, R. (1988). Mouse lysozyme M gene: isolation, characterization, and expression studies. *Proceedings of the National Academy of Sciences of the USA* **85**, 6232–6236.

Cross, M. & Renkawitz, R. (1990). Repetitive sequence involvement in the duplication and divergence of mouse lysozyme genes. *European Molecular Biology Organisation Journal* **9**, 1283–1288.

Cortopassi, G. (1988). Ph. D. Thesis, University of California, Berkeley.

Cortopassi, G. & Wilson, A. C. (1989). Electrophoretic survey of mouse lysozymes with an improved way of detecting lysozyme activity in gels. In *The Immune Response to Structurally Defined Proteins: the Lysozyme Model* (ed. S. Smith-Gill and E. Sercarz), pp. 87–96. Schenectady, NY: Adenine Press.

Cortopassi, G. & Wilson, A. (1990). Recent origin of the P lysozyme gene in mice. *Nucleic Acids Research* **18**, 1911.

Dizik, M. & Elliott, R. W. (1977). A gene apparently determining the extent of sialylation of lysosomal alpha-mannosidase in mouse liver. *Biochemical Genetics* **15**, 31–46.

Hammer, M. F. & Wilson, A. C. (1987). Regulatory and structural genes for lysozymes of mice. *Genetics* **115**, 521–533.

Hammer, M. F., Schilling, J. W., Prager, E. M. & Wilson, A. C. (1987). Recruitment of lysozyme as a major enzyme in the mouse gut: duplication, divergence, and regulatory evolution. *Journal of Molecular Evolution* **24**, 272–279.

Maack, T. & Sigulem, D. (1974). Renal handling of lysozyme. In *Lysozyme* ed. E. F. Osserman, R. E. Canfield and E. F. Beychok, pp. 321–332. New York: Academic Press.