Altered Turnover of Hypoxanthine Phosphoribosyltransferase in Erythroid Cells of Mice Expressing *Hprt a* and *Hprt b* Alleles

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ABSTRACT

We have previously shown that mice expressing *Hprt a* allele(s) have erythrocyte hypoxanthine phosphoribosyltransferase (HPRT) levels that are approximately 25-fold (*Mus musculus castaneus*) and 70-fold (*Mus spretus*) higher than in mice that express the *Hprt b* allele (*Mus musculus domesticus*; C57Bl/6J; C3H/HeHa), and that these differences in erythrocyte HPRT levels are due to differences in the turnover rates of the HPRT A and B proteins as reticulocytes mature to erythrocytes. We show here that: (1) the taxonomic subgroups of the genus *Mus* are essentially monomorphic for the occurrence of either the *Hprt a* or the *Hprt b* allele, with *Hprt a* being common in the aboriginal species (*M. spretus*, *Mus hortulanus* and *Mus abbotti*) and in several commensal species (*Mus musculus musculus*, *M. m. castaneus*, *Mus musculus molossinus*), while *Hprt b* is common in feral *M. m. domesticus* populations as well as in all inbred strains of mice tested; (2) in all these diverse *Mus* subgroups there is a strict association of *Hprt a* with high and *Hprt b* with low levels of erythrocyte HPRT; and, (3) the association between the occurrence of the *Hprt a* allele and elevated erythrocyte HPRT levels is retained following repeated backcrosses of wild-derived *Hprt a* allele(s) into the genetic background of inbred strains of mice with the *Hprt b* allele. Collectively, these observations indicate that the elevated and low levels of erythrocyte HPRT are specified by differences in the *Hprt a* and *b* structural genes. Since evidence indicates that *Hprt a* and *b* encode HPRT proteins which differ in primary structure, we infer that the structure of HPRT is an important factor in determining its sensitivity to turnover in mouse erythroid cells. *Hprt a* and *b* may provide a useful system of "normal" allelic gene products for identifying factors that participate in protein turnover during mouse reticulocyte maturation.

VARIATION in the structure of hypoxanthine phosphoribosyltransferase (HPRT) has been identified in several samples of feral mice trapped in various parts of the world and representing different taxonomic subgroups of the genus *Mus*. One identified HPRT structural variant, designated *Hprt a*, has been shown to be allelic with the *Hprt b* enzyme observed in inbred strains of mice (CHAPMAN, KRATZER and QUARANTILLO 1983).

Our initial investigations have shown that the purified HPRT A and B enzymes have similar maximal specific activities, and that the levels of HPRT activity are largely comparable in several tissues of *Hprt a* and *b* mice (*e.g.*, differing by a factor of 3-fold or less in homogenates of brain, kidney, liver and spleen tissues) (JOHNSON *et al.* 1985). In contrast, we have shown that mice expressing *Hprt a* allele(s) have erythrocyte HPRT activity levels that are approximately 25-fold (*Mus musculus castaneus*) and 70-fold (*Mus spretus*) higher than in mice that express the *Hprt b* allele (C57Bl/6J; C3H/HeHa) (JOHNSON *et al.* 1985). We have shown that these different levels of HPRT activity in erythrocytes of *Hprt a* and *b* mice are paralleled

by equivalent differences in the levels of HPRT immunoreactive protein (*i.e.*, CRM), and thus these marked differences in HPRT activity levels reflect corresponding differences in the levels of HPRT protein in erythrocytes of these mice (JOHNSON *et al.* 1985). Since erythroid cells of *Hprt a* and *b* mice have normal percentages of reticulocytes and comparable levels of another purine salvage enzyme, adenine phosphoribosyltransferase (APRT), the differences in the levels of HPRT in erythrocytes of *Hprt a* and *b* mice do not appear to result from abnormal erythroid cell development.

Further, we have shown that the levels of HPRT in *Hprt b* mice are approximately 35-fold higher in reticulocyte-rich samples than in erythrocytes, and approach the levels of HPRT in reticulocyte-rich samples from *Hprt a* mice (JOHNSON *et al.* 1985). Since the levels of HPRT activity (and protein) are comparable in reticulocyte-rich samples of *Hprt a* and *b* mice, the marked differences in erythrocyte HPRT activity levels in *Hprt a* and *b* mice appear to result from differences in the extent to which the HPRT A and B proteins are degraded as reticulocytes mature to erythrocytes.

The objective of the present study was to determine if these differences in erythrocyte HPRT levels of *Hprt a* and *b* mice are due to differences in the *Hprt* structural genes or other genetic differences between *Hprt a* and *b* mice. In this paper we report the results of extensive surveys of both laboratory and wild-derived mice on the distributions of the *Hprt a* and *b* alleles in geographically and taxonomically diverse house mouse populations, and in closely related aboriginal *Mus* species. In order to further examine the association between the *Hprt a* and *b* alleles and erythrocyte HPRT activity levels, we also report the results of studies of the segregation of erythrocyte HPRT activity levels with the *Hprt a* and *b* structural gene alleles in matings in which the *Hprt a* allele(s) from wild-derived mice are repeatedly backcrossed to inbred strain genetic backgrounds.

MATERIALS AND METHODS

Laboratory strains: Established inbred strains of laboratory mice were obtained from the Jackson Laboratory and from the West Seneca Laboratories of Roswell Park. These strains are typically derived from mice which have been in the laboratory environment since the early 1980s and they appear to carry genetic elements characteristic of both the *musculus* and *domesticus* subspecies. We also sampled several inbred strains recently established from wild-trapped mice from diverse geographical locations, including Israel (Is/CamEi), the United Kingdom (SK/CamEi and FS/Ei), and the United States (SF/CamEi from San Francisco; MOR 2/CV from Ohio; and PAC from Philadelphia). These mice are generally characterized as *Mus muscalus domesticus* which is found in Western Europe and the Americas.

Wild-derived house mouse subspecies: We have examined representative samples of different *Mus musculus* subspecies, including *M. m. castaneus* from Thailand, *Mus musculus molossinus* from Japan, *Mus musculus musculus* from Northern and Eastern Europe and *M. m. domesticus* from Western Europe and the Mediterranean basin. *Mus musculus brevirostris* isolates are listed separately, although they belong to the same biochemical group as *M. m. domesticus* (BONHOMME et al. 1984). The locations from which each of these founder populations has been derived are indicated in Table 1. Most of these mice are maintained as outbred laboratory populations either at Roswell Park Memorial Institute or at Montpellier. The founder populations are not well documented in many cases but they are presumed to be relatively small. The mice were obtained from Drs. RICHARD SAGE (University of California, Berkeley), FRANÇOIS BONHOMME (Montpellier, France), and J. TØNNES NIELSEN (Aarhus, Denmark).

Aboriginal *Mus* species from Europe: Three aboriginal *Mus* species groups have been recently characterized which are geographically sympatric with house mice but do not readily interbreed in the wild. *M. spretus* (Mus 3) was trapped in field habitats in the Western Mediterranean areas while *Mus hortulanus* (Mus 4B) and *Mus abbotti* (Mus 4A) were trapped in Eastern European field habitats. Males of these species will interbreed with the other species and with established inbred strains under laboratory conditions. The hybrid females are generally fertile but the hybrid males are sterile (BONHOMME et al. 1984).

Congenic strains: Two parallel congenic strains have been constructed in which the *M. m. castaneus* and *M. spretus Hprt a* allele(s) have been introduced into the genetic backgrounds of inbred strains of mice which express the *Hprt b* allele. The *Hprt a* allele from *M. castaneus* was recombined with Pgk-1a allele in an existing congenic strain *C3H Pgk-1a* by selecting a recombinant backcross male who was *Hprt a, Pgk-1a*. This male was crossed with the *C3H Pgk-1a* congenic, and female progeny heterozygous for *Hprt a/b* were selected for breeding in the subsequent generation. We used *Hprt a/b* female progeny in one subline and alternate generations of males and females in a second congenic subline. The mice used in this study come from the female only congenic subline but subsequent studies indicate that the relative HPRT activities of hemolysates do not differ between the two sublines. The partial congenic involving *M. spretus* used only heterozygous females in each generation since F_1 males and a portion of backcross males are sterile. The stock used in this study was at the third backcross generation.

Assays of mouse whole blood samples: Typically, a 50 μl sample of mouse peripheral blood was collected from the retro-orbital sinus into a heparinized capillary. Isoelectric focusing analysis of HPRT was carried out as described by CHAPMAN, KRATZER and QUARANTILLO (1983), while all other assays, including determinations of HPRT and APRT activities, hemoglobin concentrations and reticulocyte percentages, were performed on the whole blood samples as previously described (JOHNSON et al. 1985). Assays of HPRT and APRT levels in whole blood samples directly estimate the levels of these enzymes in erythroid cells, as there is no detectable HPRT or APRT activity in the plasma fraction (data not shown). Reticulocyte percentages were based on counts of 1000 cells.

RESULTS

As indicated by the data summarized in Table 1, there is a strict association between the occurrence of the *Hprt a* and *b* alleles with elevated and low erythrocyte HPRT activity levels, respectively. The *M. m. domesticus* and *M. m. brevirostris* mice, from feral populations from Northern Africa, the Mediterranean basin, the United Kingdom and the United States (see footnote to Table 1) have the *Hprt b* allele and HPRT activity levels of approximately 80 μunits/mg Hb (range, 27–191 μunits/mg Hb) (Table 1A). In addition, all of the 37 different inbred strains of mice we have analysed are uniformly *Hprt b* type and have HPRT activity levels averaging 97 μunits/mg Hb (range, 30–234 μunits/mg Hb) (Table 1B), which do not differ from the HPRT activity levels obtained for the wild-derived *M. m. domesticus*. In contrast, all of the other species of mice that we have examined have *Hprt a* allele(s) and elevated levels of erythrocyte HPRT activity. The commensal species (including *M. m. musculus*, *M. m. castaneus* and *M. m. molossinus*) have erythrocyte HPRT activity levels that are approximately 30-fold higher than in *Hprt b* mice (average 3371 μunits/mg Hb; range 2453–4694 μunits/mg Hb), while the aboriginal species (including *M. spretus*, *M. hortulanus* and *M. abbotti*) have erythrocyte HPRT activity levels that are approximately 100-fold higher than in *Hprt b* mice (average 9263 μunits/mg

TABLE 1

HPRT activity levels in erythrocytes of feral derived stocks and in mice expressing *Hprt a* and *b* alleles

Mouse stock/strain	HPRT allele	N	HPRT activity (μunits/mg Hb)	APRT activity (μunits/mg Hb)
A. Feral-Derived Stocks				
M. m. domesticus				
Azrou, Morocco	*b*	5	57 (42-86)	55 (42-70)
Egypt	*b*	6	46 (30-67)	42 (25-57)
Jerusalem	*b*	5	66 (31-119)	50 (33-83)
Is/CamEi (Israel)	*b*	4	84 (58-139)	102 (69-135)
Oretta, Italy	*b*	2	52;137	57;84
Corse, France	*b*	1 (M)	(M) 47	34
Annemesse, France	*b*	2	17;37	28;44
SK/CamEI [United Kingdom]	*b*	4	122 (108-141)	73 (62-84)
FS/Ei [United Kingdom]	*b*	4	85 (60-95)	38 (34-43)
California	*b*	6	70 (47-111)	50 (25-73)
SF/CamEi [San Francisco]	*b*	4	98 (88-103)	68 (56-86)
MOR 2/CV (Ohio)	*b*	4	90 (70-123)	54 (31-87)
PAC (Philadelphia)	*b*	4	68 (49–89)	83 (51-128)
Tahiti		1	206	94
Vlas, Bulgaria	*a*	2	3431;3648	27;39
M. m. brevirostris				
Oran, Algeria	*b*	1 (F)	45	52
Israel	*b*	2 (F)	41;73	70;88
Afula, Israel	*b*	2	49;74	45;49
Kefar-galim, Israel	*b*	2	46;49	50;55
Montpellier, France	*b*	2	52;67	35;62
Majorque, Spain	*b*	1 (F)	27	86
	b	1 (M)	191	151
Minorque, Spain	*b*	1 (M)	81	71
M. m. musculus				
Illmitz, Austria	*a*	2	4638;4750	21;22
Bania, Bulgaria	*a*	2	4229;4728	22;51
Kravero, Bulgaria	*a*	2	3848;3948	18;33
Sokolow, Bulgaria	*a*	1 (M)	4587	20
Czechoslovakia	*a*	4	2453 (1944-2945)	28 (16-40)
Czechoslovakia	*a*	4	2567 (2502-2688)	33 (27-36)
Brno, Czechoslovakia	*a*	4	4154 (3784-4849)	64 (60-68)
Yugoslavia	*a*	4	3152 (2543-3570)	50 (46-54)
Belgrade, Yugoslavia	*a*	4	3731 (3492-4163)	60 (52-69)
Warsaw, Poland	*a*	6	3030;3410	17;20
Denmark	*a*	6	2988 (2252-4589)	31 (23-38)
Skive, Denmark	*a*		2958 (2218-3486)	30 (26-38)
M. m. castaneus				
Djarkarta, Thailand	*a*	2	3504;3722	23;27
M. m. molossinus				
	a	4	3395 (2331-4172)	79 (62-98)
Hishima, Japan	*a*	1 (M)	4403	19
M. spretus				
Fondouls-djedid, Tunisia	*a*	2	4965;6497	23;27
Ibiza, Spain	*a*	2	8165;8754	25;26
Granada, Spain	*a*	2	8807;9286	37;42
Montpellier, France	*a*	2	8456;9818	49;52
M. hortulanus				
	a	4	10361 (9201-10927)	36 (27-56)
Pancevo, Yugoslavia	*a*	6	6 10050 (9214-12051)	18 (14-24)
Debeljica, Yugoslavia	*a*	6	6 8673 (7134-10777)	18 (14-21)

Hb; range 5736–10361 μunits/mg Hb) (Table 1A). The *Hprt a* allele (and elevated erythrocyte HPRT levels) was observed in one *M. m. domesticus* population isolated from Vlas, Bulgaria (Table 1). These mice

TABLE 1—*Continued*

Mouse stock/strain	HPRT allele	N	HPRT activity (μunits/mg Hb)	APRT activity (μunits/mg Hb)
M. abbotti				
Slantchev, Bulgaria	*a*	8	9735 (7674-11735)	16 (11-20)
B. Inbred Strains				
A/J	*b*	4	80 (56-98)	120 (93-157)
Au/SS	*b*	4	96 (86-108)	70 (45-101)
AKR/J	*b*	3	47 (43-54)	45 (40-51)
BALB/c CR	*b*	2 (M)	66;71	98;121
BDP/J	*b*	4	80 (73-87)	44 (27-61)
BuB/BnJ	*b*	3	99 (59-133)	55 (30-73)
C3H/HeJ	*b*	4	106 (94-117)	97 (66-134)
CBA/CaJ	*b*	4	90 (81-97)	54 (32-75)
C57/e/Ha	*b*	4	116 (79-186)	55 (33-90)
C57L/J	*b*	6	129 (107-174)	43 (31-56)
C58/J	*b*	3	113 (90-128)	49 (32-70)
DBA/2Ha	*b*	4	102 (89-124)	69 (37-106)
DBA/2J	*b*	4	78 (49-120)	48 (24-64)
Ha/ICR	*b*	4	145 (128-175)	141 (116-194)
I/St	*b*	4	142 (118-198)	84 (56-112)
LP/J	*b*	4	64 (30-134)	53 (30-83)
LTS/A	*b*	4	75 (65-84)	124 (87-151)
MA/MyJ	*b*	2 (M)	78;101	43;45
NB9[PAC]	*b*	3	63 (49-89)	94 (52-118)
NZB/BINJ	*b*	2 (M)	95;162	55;71
P/J	*b*	4	76 (62-99)	79 (45-123)
PL/J	*b*	4	119 (108-130)	124 (100-148)
RIIIS/J	*b*	4	30 (23-42)	35 (20-51)
RF/J	*b*	2 (M)	110;157	54;91
SB/Le	*b*	4	117 (110-122)	121 (116-123)
SEA/GnJ	*b*	4	82 (80-85)	64 (31-93)
SEC/1ReJ	*b*	4	65 (59-74)	108 (87-123)
SJL/J	*b*	2 (M)	200;268	72;84
	b	2 (F)	73;117	94;98
SM/J	*b*	4	98 (81-118)	117 (105-123)
ST/bJ	*b*	4	108 (70-176)	94 (53-127)
STS/A	*b*	4	69 (55-83)	114 (92-123)
SWR/J	*b*	9	144 (101-178)	113 (86-141)
T	*b*	4	81 (53-104)	72 (41-115)
WB/Re	*b*	4	97 (90-107)	83 (56-117)
O2O	*b*	4	95 (81-103)	69 (46-94)
101	*b*	4	83 (72-95)	72 (26-106)
129/J	*b*	2 (M)	46;58	25;34
	b	2 (F)	103;105	65;72

The analyses were typically of at least two males and two females of each strain or stock. We observed comparable levels of HPRT activity for males and females in virtually all strains and stocks of mice [with the exception of two inbred strains noted below] and the values for males and females have been combined. For those mice where only males or females were available for study, the sexes of the mice analyzed are specified (M/F). The specific activities of HPRT and APRT are reported individually for one or two mice, and as averages when three or more mice were analyzed, with the ranges of the values observed in parentheses. The cumulative results of this study are based on determinations made on 10 separate occasions. Two C57Bl/6J male samples were included for parallel analysis on each of these 10 occasions, from which we obtained a mean of 91 μunits/mg Hb and range of 65–123 μunits/mg Hb. The mean and range values for these 20 C57Bl/6J mice do not differ significantly from the means and ranges obtained from similar numbers of C57Bl/6J analyzed at the same time (see Table 2A and JOHNSON *et al.* 1985), and indicate that the average values reported for the other mice are not expected to deviate from their true means by more than 50% for even the smallest sample sizes. For the inbred strains SJL/J and 129/J, males and females may differ in the levels of HPRT by a factor of twofold. Additionally, several of the inbred strains show differences between males and females in the levels of APRT of approximately twofold. Finally, all individuals studied were 8 weeks of age or older in order to eliminate developmental changes in the levels of HPRT and APRT in erythroid cells of younger mice (BLAKELY 1980). The percentages of reticulocytes in whole blood smears were the following (the ranges and numbers of mice analyzed are presented in parentheses; and see MATERIALS AND METHODS): inbred strains, 1.1% (0–8.4%; N = 132); *M. m. domesticus*, 1.6% (0–7.7%; N = 56); *M. m. brevirostris* 1.2% (0.1–6.7%; N = 12); *M. m. musculus* 2.5% (0.5–7%; N = 42); *M. m. castaneus* 0.7% (0.2–1.7%; N = 8); *M. m. molossinus* 1.7% (0.4–3.7%; N = 5); *M. spretus* 2.8% (0.8–7%; N = 16); *M. hortulanus* 3.1% (1.6–4.5%; N = 16); *M. abbotti* 1.3% (0.3–4.1%; N = 8). N = number of individuals analyzed.

TABLE 2

HPRT levels in erythrocytes of *Hprt a* and *Hprt b* mice

Mouse (stock/strain)	N	HPRT allele	HPRT activity (μunits/mg Hb)	APRT activity (μunits/mg Hb)	Reticulocytes (%)
A. Inbred Strains and Stocks					
C57Bl/6J	4	b	87 (81–99)	40 (38–42)	1 (0.8–1.4)
C3H/HeHa	4	b	92 (82–103)	101 (98–109)	1.4 (0.4–3.1)
M. m. castaneus	4	a	2404 (2327–2462)	48 (35–60)	1 (0.3–1.7)
M. spretus	4	a	6178 (4873–7076)	42 (23–69)	4.4 (1.1–7.0)
B. Reciprocal Crosses-F₁ Males					
C3H [F] × *M. m. castaneus* (M)	5	b	73 (67–80)	27 (24–31)	1.7 (1.4–2.1)
M. m. castaneus (F) × C3H (M)	5	a	2451 (2312–2569)	19 (16–22)	1.9 (1.2–2.5)
C. Backcrosses of HPRT A Alleles into Inbred Strains					
C3H-Hprt a (*M. m. castaneus*)	4	a	2614 (2231–3251)	47 (37–67)	0.9 (0.5–1.4)
C57-Hprt a (*M. spretus*)	5	a	9067 (8471–10283)	22 (16–26)	2.6 (0.3–4.8)

The determinations of HPRT and APRT activities, isoelectric focusing of HPRT, hemoglobin concentrations and reticulocyte percentages have been described (CHAPMAN, KATZER and QUARANTILLO 1983; JOHNSON *et al.* 1985). The values for HPRT and APRT specific activities as well as reticulocyte percentages are reported as averages, with the range of values observed for all samples in parentheses. A, Comparison of the levels of HPRT in erythrocytes of inbred strains of mice expressing the *Hprt b* allele (C57Bl/6J and C3H/HeHa) and in two wild-derived stocks of mice expressing *Hprt a* allele(s) (*M. m. castaneus* and *M. spretus*). B, F₁ male progeny of reciprocal crosses between C3H/HeHa (*Hprt b*) and *M. m. castaneus* (*Hprt a*). C, Male progeny of backcrossing the *Hprt a* allele of *M. m. castaneus* into the C3H/HeHa genetic background (eight backcross generations) and of the *Hprt a* allele of *M. spretus* into the C57Bl/6J genetic background (three backcross generations). N = number of mice analyzed.

appear to result from hybridization between *M. m. domesticus* and *M. m. musculus* populations, and this subject will be considered in the discussion.

It should be noted that the difference in erythrocyte HPRT levels of *Hprt a* and *b* mice is not a result of abnormal erythroid cell development since *Hprt a* and *b* mice have similar levels of APRT, another purine salvage enzyme (Table 1), and normal percentages of reticulocytes (footnote to Table 1).

Since the HPRT structural gene is on the *X* chromosome of mice (CHAPMAN and SHOWS 1976; HASHMI and MILLER 1976; FRANCKE *et al.* 1977; CHAPMAN, KRATZER and QUARANTILLO 1983), one can examine the association of erythrocytre HPRT levels with the occurrence of the *Hprt a* and *b* alleles in male progeny of reciprocal crosses of the parental stocks. An example of a reciprocal cross obtained between *M. m. castaneus* (*Hprt a*) and C3H/HeHa (*Hprt b*) is shown in Table 2B. Males receiving their *X* chromosome from the *Hprt a* mother have approximately 30-fold higher levels of HPRT activity in their erythrocytes than do males inheriting the *Hprt b* allele. Since these F₁ male mice are presumably identical at autosomal loci, this indicates that differences in HPRT levels in erythrocytes of *Hprt a* and *b* mice are specified by genetic differences in the *X* chromosome. (*M. spretus* females do not mate with inbred strains of mice, and therefore those reciprocal cross progeny were not obtained.)

Further tests of the association between erythrocyte HPRT levels and the *Hprt a* and *b* alleles were obtained by analysis of mice in which the wild-derived *Hprt a* allele(s) of *M. m. castaneus* and *M. spretus* have been repeatedly backcrossed into the genetic back-

ground of inbred strains of mice. We have observed a strict association between elevated levels of HPRT activity in erythrocytes of mice expressing the *Hprt a* allele(s) through eight successive backcrosses of the *M. m. castaneus Hprt a* allele into C3H/HeHa and in three successive backcrosses of the *M. spretus Hprt a* allele into C57Bl/6J mice (Table 2C). Since differences between high and low levels of erythrocyte HPRT activity are apparent using *in situ* HPRT activity assays following separation of the HPRT A and B proteins by isoelectric focusing, we have used this assay for additional backcross studies. No recombinants were observed between the HPRT A and B proteins and high and low red cell HPRT activity levels in more than 160 additional backcross progeny.

DISCUSSION

Our results show that the *Hprt b* allele occurs in all inbred strains of mice we have analyzed and in all but one of the feral populations of *M. m. domesticus* and *M. m. brevirostris* tested (Table 1). All of the other mice we examined, which belong to *M. m. musculus*, *M. m. castaneus*, or *M. m. molossinus* subspecies or to the aboriginal species *M. spretus*, *M. hortulanus* and *M. abbotti* express the *Hprt a* allele. *Hprt a* and *Hprt b* are therefore presumed to be the common alleles in these respective species and subspecies of the genus *Mus*. Further, although *Hprt a* and *b* mice differ by as much as 100-fold in erythrocyte HPRT levels (Table 1), *Hprt a* and *b* are presumed to be "normal" alleles with no obvious deleterious phenotypic effect. In particular, erythroid cell development appears normal in both types of mice, as erythrocytes of *Hprt a* and *b* mice have comparable levels of another purine salvage

enzyme, APRT, and normal percentages of reticulocytes (Table 1).

The one population of *M. m. domesticus* mice that were trapped in Vlas, Bulgaria contain the *Hprt a* allele (and elevated HPRT activity levels) and represent the single exception to the essentially monomorphic distribution of *Hprt a* and *b* alleles in these *Mus* subspecies and species (Table 1). Previous studies by others have demonstrated that *M. m. domesticus* and *M. m. musculus* interbreed along a narrow hybridization zone which extends through Bulgaria (HUNT and SELANDER 1973; THALER, BONHOMME and BRITTON-DAVIDIAN 1981; BOURSOT *et al.* 1984), and these mice likely represent an example of penetration of the *M. m. musculus Hprt a* containing *X* chromosome to an *M. m. domesticus* population.

A striking feature of the *Hprt a* and *Hprt b* distribution in *Mus* is the apparent association of the *Hprt b* allele in *M. m. domesticus* samples which include all of the historical laboratory strains and the finding of *Hprt a* in nearly all other house mouse species groups studied. Our sampling of feral populations is relatively limited and the use of laboratory populations of wild-derived mice for making inferences about allelic distributions in natural populations should be done with considerable caution. Nevertheless, the *Hprt a* allele was observed in a broadly dispersed sampling of *M. musculus* from Northern Denmark and Czechoslovakia. Moreover, we have not observed any samples of wild-derived mice that were polymorphic for the *Hprt* allelic forms. Whether these conditions prevail in feral mice, especially those near hybrid zones such as Vlas, Bulgaria, remains to be demonstrated. Additional studies of these allelic forms in feral mice are necessary before we can speculate about the evolution of this unusual allelic distribution, but the initial data do suggest that these alleles may be useful markers for following the dispersion of *X* chromosomes across the hybrid zones between house mouse subspecies.

We have observed a strict concordance between high and low erythrocyte HPRT levels with the *Hprt a* and *b* alleles in these population studies as well as in the progeny of crosses in which the wild-derived *Hprt a* allele(s) of *M. m. castaneus* and *M. spretus* were repeatedly backcrossed into the genetic background of inbred strains (RESULTS and Table 2C). Statistically, our backcross studies would have greater than a 95% probability of detecting recombination between the *Hprt* structural gene and a second gene that alters the levels of the enzyme in erythroid cells if it were more than 2 cM distant from the *Hprt* structural gene locus. We do not have detailed information on the homology between the *M. m. domesticus* and *M. m. castaneus X* chromosomes, but these two subspecies show either relatively normal recombination for autosomal genes in various genetic tests (CHAPMAN, NICHOLS and RUD-

DLE, 1974; CHAPMAN and RUDDLE 1972) or elevated levels of recombination in the H-2 region (FISCHER-LINDAHL, HAUSMANN and CHAPMAN 1983). The *X* chromosome mapping studies of Hprt-mdx-Pgk-1 place the mdx locus in the same general map region reported by other studies involving markers common to laboratory mice (BULFIELD *et al.* 1983). Collectively, these findings suggest that we have no reason to expect a lack of homology between the *Hprt a* and *Hprt b X* chromosomes which would lead to linkage disequilibrium between the *Hprt* locus and a possible second gene responsible for red cell HPRT activity. Thus, although our results from the population and backcross studies can not specifically exclude the possibility that high and low erythrocyte HPRT activity levels result from genetic differences between *Hprt a* and *b* mice that are closely linked to the *Hprt* structural gene locus in the mouse *X* chromosome, we presume that the differences in erythrocyte HPRT levels result from differences between the *Hprt a* and *b* structural genes themselves. Since the HPRT A and B proteins have different net charges (CHAPMAN, KRATZER and QUARANTILLO 1983; JOHNSON *et al.* 1985), we infer that the *Hprt a* and *b* alleles encode HPRT A and B proteins which differ in amino acid sequence, and that differences in the structures of the HPRT A and B proteins are responsible for differences in the rates of turnover of these proteins in mouse erythroid cells.

The levels of HPRT activity in reticulocyte-rich samples of *M. spretus* are 2–3-fold higher than in reticulocyte-rich samples of *M. m. castaneus* (JOHNSON *et al.* 1985), and thus the persistence of 2–3-fold higher HPRT levels in erythrocytes of *M. spretus* compared to *M. m. castaneus* (Tables 1 and 2) may be attributable to higher levels of expression of HPRT in nucleated erythroid cell precursors (*e.g.*, erythroblasts) of *M. spretus*. Alternatively, these two mouse species are estimated to have diverged approximately two million years ago (FERRIS *et al.* 1983a, b; BONHOMME *et al.* 1984), and there may be differences in the amino acid sequences of these two *Hprt a* proteins. The amino acid sequence of the HPRT B protein has been deduced from nucleotide sequencing (KONECKI *et al.* 1982; MELTON *et al.* 1984), and we are currently determining the nucleotide sequences of cDNAs encoding the HPRT A enzymes of *M. m. castaneus* and *M. spretus*. The results of these sequencing studies will clarify structural differences between the HPRT A and B proteins.

Although the factors involved in effecting turnover of individual proteins in mammalian somatic cells are presently unknown, the inherent susceptibility of a protein to degradation has been identified as an important determinant of its turnover rate (SCHIMKE and DOYLE 1970; GOLDBERG and DICE 1974). Evidence of the importance of protein structure in turn-

over in erythroid cells is vividly demonstrated by the observations that structurally abnormal proteins are degraded much more rapidly than their normal allelic gene products (RABINOVITZ and FISHER 1964; RIEDER, ZINKHAM and HOLTZMANN 1965; ETLINGER and GOLDBERG 1977; MORELLI et al. 1978). Our observations on HPRT suggest that differences in the structures of "normal" proteins are important in determining their rates of turnover in mouse erythroid cells. Protein degradative activities (or systems) have previously been demonstrated in reticulocytes (BOTBOL and SCORNIK 1979; MULLER et al.1980; BOCHES and GOLDBERG 1982) and a number of factors involved in protein degradation in reticulocyte lysates have been characterized (ETLINGER and GOLDBERG 1977; HERSHKO et al. 1983, 1984; TANAKA, WAXMAN and GOLDBERG 1984; RAPOPORT, DUBIEL and MULLER 1985; CIECHANOVER et al. 1985). A major objective of these investigations is to determine the in vivo function of the factors identified in studies in reticulocyte lysates (e.g. ATP-dependent proteolytic system; ubiquitin). The Hprt a and b allelic gene products, which turnover at considerably different rates, may therefore prove useful in defining aspects of "normal" protein structure that correlate with sensitivity to be degraded in mouse reticulocytes as well as for identifying factors that participate in that process.

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