IDENTIFICATION OF A GENE REGULATING THE TISSUE EXPRESSION OF A PHOSPHOGLUCOMUTASE LOCUS IN RAINBOW TROUT

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ABSTRACT

Nine percent of the rainbow trout (*Salmo gairdneri*) from a hatchery source have a greater than 100-fold increase in expression of a phosphoglucomutase (PGM) locus, *Pgm1*, in the liver but have normal expression of this locus in other tissues. The results of genetic crosses are consistent with a single regulatory gene with additive inheritance being responsible for the differences in the amount of PGM activity in the liver.——The allele responsible for the expression of *Pgm1* in the liver is apparently a recent mutation. This is supported by its restricted distribution in rainbow trout and the absence of liver *Pgm1* expression in closely related species. This genetic system is valuable for future analysis of the control of gene expression and in determining the relative evolutionary importance of genetic variation at structural and regulatory genes.

DIFFERENCES in gene regulation are a potentially important basis for evolutionary change in morphology and metabolism. A number of authors have suggested that changes in the regulation of enzyme loci may be of more evolutionary importance than structural differences in the enzymes coded by these loci (e.g., WALLACE 1963; WILSON 1976; AYALA and MCDONALD 1981). The genetic and epigenetic bases for differences in expression of enzyme loci have only begun to be explored. Evidence of intraspecific allelic variation at regulatory genes in eukaryotes is sparse. Nevertheless, data from several organisms (e.g., maize, Drosophila and mice) show that there are genes that control the timing and tissue-specificity of enzyme loci in eukaryotes (reviewed in PAIGEN 1979). The exact molecular and cellular bases for these differences in enzyme activity are not generally known (PAIGEN 1979; DAVIDSON and BRITTEN 1979). A series of molecular events results in the time of appearance and the tissue-specific amounts of functional enzymes; this process represents the 'regulation' of gene expression in its broadest sense.

Recent studies clearly show the existence of putative regulatory genes that are responsible for the tissue-specific expression of enzyme loci (PAIGEN 1979; MCCARRON *et al.* 1979; POWELL and LICHTENFELS 1979; DICKINSON 1980; WOODMAN and FREELING 1981). Some of these regulatory genes control only the structural gene that they are linked to, "*cis*" regulation. Other regulatory genes appear to produce diffusible effector molecules that result in "*trans*" regulation. Improved insight into the processes and effects of differences in tissue-specific expression of enzyme loci is crucial to our understanding of developmental and evolutionary mechanisms.

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We have initiated a search for intraspecific variation of the tissue-specific expression of enzyme loci in the rainbow trout (*Salmo gairdneri*). We have discovered a presumed regulatory gene responsible for differences in the tissue-specific expression of a phosphoglucomutase (PGM, E.C. 2.7.5.1) locus (ALLENDORF 1980). We propose here that the observed difference is caused by a regulatory polymorphism for the tissue-specific expression of the *Pgm1* locus.

METHODS

Tissue samples and mature fish for the experimental matings were obtained from the Arlee strain of rainbow trout held at the Jocko River State Trout Hatchery of the Montana Department of Fish, Wildlife, and Parks (MDFWP). The matings were performed as described in ALLENDORF and UTTER (1973). Samples of rainbow trout from the Badger Lake and Premier Lake strains were obtained from the Kootenay Trout Hatchery, Wardner, British Columbia. Samples of the Soap Lake, New Zealand, and Winthrop strains of rainbow trout held at the National Fisheries Center-Leetown of the United States Fish and Wildlife Service were analyzed by Steve Phelps while he was an employee there. Samples of the Post Creek strain were obtained from the Harriman Trout Company, St. Ignatius, Montana.

Sample preparation and electrophoresis in starch gels followed previously described methods (UTTER *et al.* 1974), using the stains described by ALLENDORF *et al.* (1977) and their buffer systems.

The nomenclature is adapted from ALLENDORF and UTTER (1979) and PAIGEN (1979). Multiple structural enzyme loci in salmonids have been named with a sequential numerical designation, beginning with the least anodal form, *Pgm1*. The enzyme produced by this locus is referred to as PGM1. Alleles producing electrophoretically detectable allozymes are identified according to their relative electrophoretic mobilies. The genetic site responsible for differences in tissue expression of the PGM1 enzyme is designated a temporal regulatory locus, *Pgm1-t*. Alleles at this locus are assigned different alphabetical designations, *Pgm1-t(a)* and *Pgm1-t(b)*. Genotypes at this locus are represented by the appropriate alleles separated by a diagonal, *Pgm1-t(a/a)*.

PGM activity was measured using the assay of DAWSON and MITCHELL (1969), except that glucose-6-phosphate dehydrogenase was at a concentration of 1 unit/ml of assay mixture. One enzyme unit (U) of PGM catalyzed the reduction of 1 μmol of NADP/min at 25°. Specific activity is expressed as U/mg protein as determined by the method of LOWRY *et al.* (1951).

RESULTS

Rainbow trout PGM

Three major electrophoretic zones of PGM activity are found in rainbow trout (ROBERTS *et al.* 1969). Staining for PGM activity with and without the coenzyme glucose-1,6-diphosphate revealed that all three of these zones are dependent upon the presence of this coenzyme for activity. The most anodal zone of PGM activity is variable, but weak activity makes interpretation somewhat difficult, Results from controlled matings show that this zone of PGM activity is controlled by two loci, *Pgm-3,4* (R. F. LEARY and F. W. ALLENDORF, unpublished results). The middle zone of PGM activity in rainbow trout is produced by a single locus, *Pgm2*, having two reported electrophoretically detectable alleles (ROBERTS *et al.* 1969; UTTER and HODGINS 1972). *Pgm2* is clearly expressed in all tissues examined.

The most cathodal zone of PGM activity is generally represented by a single isozyme that is most strongly expressed in skeletal muscle and heart tissue, but is also present in eye, stomach, and brain. We have detected an electrophoretic mobility variant, *Pgm1(130)*, for this zone in rainbow trout collected from the Badger Lake and Premier Lake strains (Figure 1). This allelic variation indicates that this zone of activity results from a single structural locus, *Pgm1*. Both

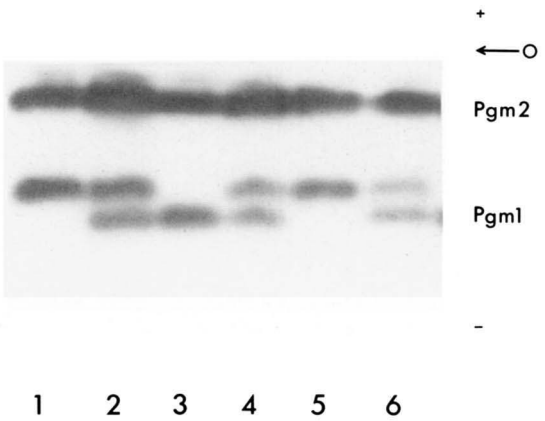

FIGURE 1.—PGM in skeletal muscle of rainbow trout from the Kootenay Hatchery showing electrophoretic variation at *Pgm1*: (*100/100*) 1 and 5; (*100/130*) 2, 4 and 6; (*130/130*) 3. The upper bands are products of *Pgm2*.

allozymes in heterozygotes have equal activity in all tissues. The homozygote *Pgm1(130/130)* has only the allozyme of the *(130)* allele in all tissues examined. There is, therefore, no indication of a second locus having a common allele that comigrates with *Pgm1(100)*, as has been found for many salmonid isozyme loci (BAILEY *et al.* 1970; ALLENDORF *et al.* 1975). The genotypes did not differ from expected Hardy-Weinberg proportions in either strain.

We have also detected a null allele at *Pgm1*. Fifteen out of 40 rainbow trout from a New Zealand strain possessed no PGM1 activity in any tissue. These fish were apparently homozygous for an allele producing no active enzyme (*i.e.*, null). The estimated frequency of this null allele in this strain is 0.61. We have not seen this allele in any other strain of rainbow trout. This allele could either be structural or regulatory; we have tentatively designated this null allele as *Pgm1(n)*.

Tissue-specific expression differences

Although most rainbow trout have no detectable PGM1 activity in liver tissue, others have a large amount of activity (Figure 2). This difference is consistent in fish from the earliest age that we can remove livers (approximately 60 days when the fish are 20–25 mm long) until sexual maturity at 2–4 years of age. There are no apparent differences between these types in the expression of *Pgm1* in other tissues (skeletal muscle, eye, heart, brain, stomach and pyloric cecum). The phenotype showing expression of *Pgm1* in the liver was present in 39 out of 432 fish (0.090) examined from the Arlee strain of rainbow trout.

The serial dilution technique of KLEBE (1975) was used with ten individuals of each phenotype to determine how much more PGM1 activity is present in the variant phenotype. No PGM1 activity was detected in the livers of individuals having the common phenotype. PGM1 enzyme activity disappeared in the variant phenotypes on the seventh or eighth one-half dilution. Thus, there is at least 100 times more PGM1 enzyme activity in the liver of individuals having the uncommon phenotype.

A series of genetic crosses were made to test if these different phenotypes have a simple genetic basis. The presence of PGM1 enzyme activity in the liver

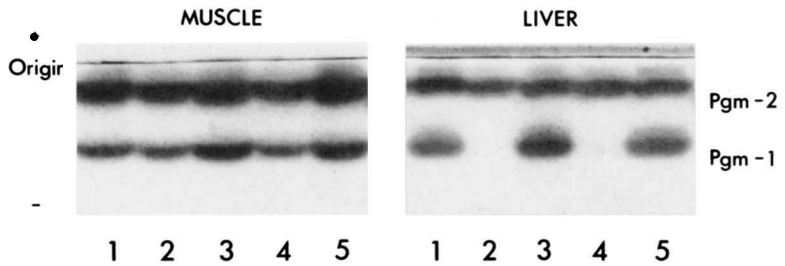

FIGURE 2.—PGM in skeletal muscle and liver showing differential tissue expression of *Pgm1* in five parents from Table 1. Two phenotypes can be seen: *Pgm1-t(a/a)* 2 and 4; *Pgm1-t(a/b)* 1, 3 and 5.

is inherited as a simple Mendelian dominant trait (Table 1). Two of the families (G4 and H6) show an excess of progeny having PGM1 activity in the liver. However, the magnitude of the excess in either family is not statistically significant when correction is made for the 15 independent chi-square tests that we have performed (COOPER 1968). We have designated the allele associated with PGM1 activity in the liver as *Pgm1-t(b)*, and the common allele as *Pgm1-t(a)*.

The parents having PGM1 enzyme activity in the liver are apparently all *Pgm1-t(a/b)* heterozygotes on the basis of the observed 1:1 segregation ratios. The estimated frequency of the *t(b)* allele, assuming binomial genotypic proportions, is 1 minus the square root of the frequency of the recessive *t(a/a)* genotype. The frequency of the *t(b)* allele in this hatchery stock is estimated to be 0.046. The expected frequency of the *t(a/b)* and *t(b/b)* genotypes are 0.088 and 0.002, respectively. Thus, we expect only 1 out of every 41 fish having PGM1 activity in the liver to be homozygous *t(b/b)* in this strain.

The families (H3 and H6) produced by mating two heterozygotes make it possible to test whether the amounts of PGM1 enzyme in the liver show additive or dominant inheritance. We tested these alternatives by measuring the total amounts of specific activity for PGM in the liver of progeny from five different families (Table 2 and Figure 3). These measurements also included the activity of PGM produced by the other three PGM loci. Three families (H1, H2, and G4) were segregating 1:1 [*t(a/a):t(a/b)*] for the presence of PGM1 in the liver. Progeny having PGM1 activity in the liver [*t(a/b)*] had an average of 48% increase in total liver PGM activity. The dominant model predicts that fish having PGM1 activity in the liver [*t(a/b)* and *t(b/b)*] from a family segregating 3:1 should show the same increase. The additive model, however, predicts that the average increase in activity should be 33% greater than this because of the presence of the additional genotypic class [*t(b/b)*] consisting of one-third of the fish having twice the average increase in activity.

These results fit the additive prediction. Fish having liver PGM1 activity in family H6 show an average increase in activity of 66%; this enhanced increase results from a group of fish [putative *t(b/b)* homozygotes] showing a much higher increase in activity than seen in the families segregating 1:1 (Figure 3). The ten highest specific activities among the 54 *t(b)* fish in families H1, H2 and H6 are all from H6 ($P < 0.01$). The differences in liver PGM1 activity are thus apparently controlled by a regulatory gene resulting in additive inheritance of the amount of liver *Pgm1* expression.

TABLE 1

Inheritance of differences in Pgm1 *expression in the liver of rainbow trout*

Family	Parental *Pgm1-t* genotypes		Progeny *Pgm1-t* liver phenotypes		Expected ratio[a]	Chi-square (1 d.f.)
	Female	Male	Absent (a/a)	Present (a/b or b/b)		
9 families	a/a	a/a	218	0	1:0	—
G1	a/b	a/a	26	37	1:1	2.00
G2	a/b	a/a	17	14	1:1	0.29
G4	a/b	a/a	145	184	1:1	4.62
G7	a/b	a/a	8	9	1:1	0.06
G10	a/b	a/a	21	31	1:1	1.92
G11	a/b	a/a	9	9	1:1	0.00
G13	a/b	a/a	7	3	1:1	1.60
H1	a/a	a/b	259	261	1:1	0.01
H2	a/a	a/b	263	246	1:1	0.57
H4	a/b	a/a	24	21	1:1	0.10
H8	a/a	a/b	95	79	1:1	1.47
H15	a/a	a/b	27	19	1:1	1.39
H16	a/a	a/b	43	42	1:1	0.02
H3	a/b	a/b	29	87	1:3	0.00
H6	a/b	a/b	42	182	1:3	4.67

[a] Assuming the *t(b)* allele is dominant resulting in the presence of PGM1 activity in the liver.

The observed intermediate expression in heterozygotes is consistent with *cis*-regulation resulting in two independently regulated chromosomes. *trans*-Acting regulatory elements in prokaryotes are either recessive or dominant (PAIGEN 1979). However, *trans*-acting eukaryotic genes resulting in intermediate enzyme levels in heterozygotes have been identified (PAIGEN 1979). We have not yet been able to perform an unambiguous *cis-trans* test.

cis-Regulation would suggest close linkage between the regulatory and structural regions. We cannot test this until we can examine the progeny of a heterozygote at both the structural and regulatory genes, Pgm1(100/130)-t(a/b). Because both the structural and regulatory variation are rare, we have not yet found a population containing both variants. We are thus making efforts to produce double heterozygotes by crossing two different strains to test for *cis-trans* regulation. When the hybrid fish are sexually mature, several years later, we will be able to test for linkage.

Population distribution of Pgm1-t(b)

The *Pgm1-t(b)* allele is apparently a recent mutation. All other species closely related to rainbow trout that we have examined do not show expression of *Pgm1* in liver tissue: coastal cutthroat trout, *Salmo clarki clarki*; westslope cutthroat trout, *S. c. lewisi*; coho salmon, *Oncorhynchus kisutch*; chinook salmon, *O. tshawytscha*; and chum salmon, *O. keta*. The absence of liver *Pgm1* expression is thus most likely the primitive condition.

In addition, the *Pgm1-t(b)* allele is rare in rainbow trout. We have only found it in 4 out of some 30 strains of rainbow trout that we have screened (Table 3). It has also been reported in one other strain of rainbow trout held at Pennsylvania State University (MAY, WRIGHT and JOHNSON 1982). The relationships between different domestic strains of rainbow trout are generally unknown

TABLE 2

PGM specific activity in livers of progeny without, t(a/a), *and with,* t(a/b or b/b), Pgm1 *expression in the liver*

Family	Age (days)	PGM activity (U/mg protein)[a]		% increase
		t(a/a)	*t(a/b)* or *t(b/b)*	
G4	169	9.5 ± 2.4 (18)	14.5 ± 2.3 (27)	52.6
H1	93	12.5 ± 0.9 (7)	19.6 ± 2.1 (13)	56.8
H2	93	13.2 ± 1.6 (9)	17.9 ± 1.7 (11)	35.6
H6	93	12.8 ± 2.8 (25)	21.2 ± 2.5 (30)	65.6
H7	93	13.9 ± 2.8 (14)	—	—

[a] Values represent the mean and standard deviation and the number of individuals tested.

(KINCAID 1981). It appears, however, that all strains of rainbow trout found to have the *Pgm1-t(b)* allele have possibly shared a common source. The Post Creek strain was apparently derived from the Arlee strain some 30 years ago and is similar to the Arlee strain at seven other polymorphic loci (unpublished data). Both the Winthrop and Pennsylvania strains that contain the *Pgm1-t(b)* allele were at least partially derived from eggs from the Ennis National Fish Hatchery, Ennis, Montana approximately 10 years ago (KINCAID 1981; J. E. WRIGHT, personal communication). The Ennis hatchery received eggs from the Arlee strain before that time. The *Pgm1-t(b)* allele is apparently a recent mutation, possibly occurring in the Arlee strain, or a recent ancestral stock.

DISCUSSION

An important question is whether this difference in tissue-specific expression results from genetic variation at a distinct regulatory locus. It is conceivable that a mutation in the *Pgm1* structural gene could produce the phenotypic differences detected. For example, the phenotype with *Pgm1* expression in the liver could possibly result from an enzyme having increased stability in the liver. We have not been able to detect any structural allelic differences in PGM1 enzymes using heat inactivation or a variety of electrophoretic conditions. In addition, we mixed homogenized muscle and liver extracts together to see if the PGM1 enzyme in *t(a/a)* fish was degraded at a faster rate. There were no detectable differences in stability of the PGM1 enzymes under these *in vitro* conditions. These results, when considered along with the large difference in liver PGM1 activity between segregating phenotypes, support the conclusion that the observed difference in tissue-specific expression of *Pgm1* reflects an increase in the number of PGM1 molecules produced. Further support comes from studies that show differences in tissue expression of enzyme loci are caused by differential rates of protein synthesis (LEBHERZ 1975; NADAL-GINARD 1978; PAIGEN *et al.* 1979; DICKINSON 1980; BROWN 1981; RABINOW and DICKINSON 1981; KENNEY and LEE 1982).

We began our search for allelic differences in tissue specific expression of isozymes in the rainbow trout because we felt that this species is a likely

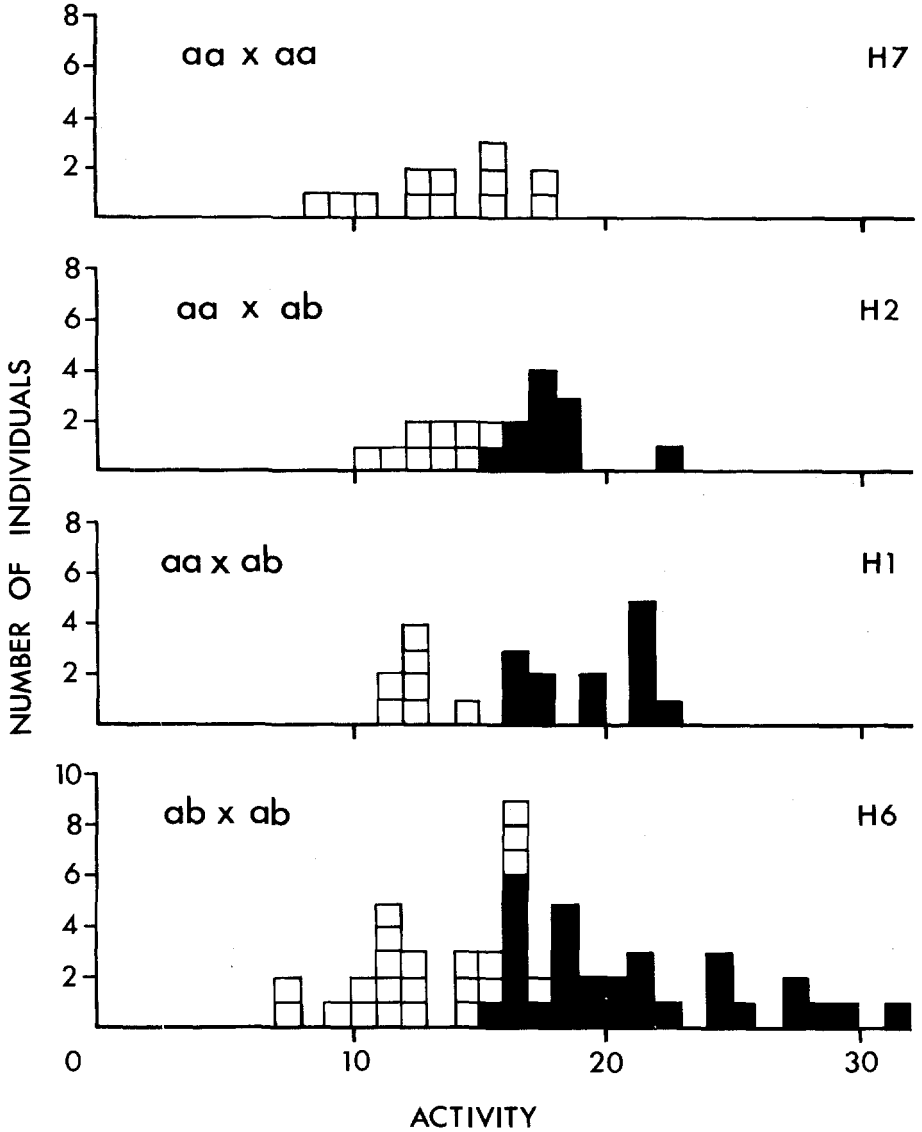

FIGURE 3.—Distribution of PGM activities (U/mg protein) in liver extracts from four families. Open squares indicate phenotypes lacking liver *Pgm1*; solid squares indicate phenotypes having liver *Pgm1* activity.

candidate for having such variation. Fishes of the family Salmonidae (trout, salmon, char, whitefish, and grayling) underwent a tetraploid event an estimated 100 million years ago (OHNO 1974; LIM and BAILEY 1977). Multilocus isozyme loci in the salmonids and the catostomids, another member of the ancient tetraploid family of fish (FERRIS and WHITT 1979), show considerable regulatory divergence between the duplicated loci produced by polyploidy.

Some duplicated isozyme loci in salmonids show no structural or regulatory divergence, and apparently have still not established complete disomic inheritance (WRIGHT *et al.* 1980; MAY, WRIGHT and JOHNSON 1982), a prerequisite for divergence of duplicated loci. Differences between chromosomes in the time of

TABLE 3

Distribution of Pgm1-t(b) *allele in populations of rainbow trout*

Strain	Liver *Pgm1* phenotype		*Pgm1-t(b)* allelic frequency[a]
	Absent	Present	
Arlee	393	39	0.090
Post Creek	25	15	0.209
Soap Lake	35	5	0.065
Winthrop	37	3	0.038

We have not detected any PGM1 activity in the livers of trout from approximately 30 other strains that we have examined.

[a] Estimated by taking the square-root of the recessive phenotype.

establishing disomic inheritance in salmonids provide many pairs of loci at different stages of divergence. We therefore expect that some pairs of duplicated loci are polymorphic for the type of tissue-specific regulatory differences that are eventually established between duplicated genes. The relatively large amount of structural variation at isozyme loci (ALLENDORF and UTTER 1979) and widespread culture in hatcheries make the rainbow trout the preferred salmonid species to search for regulatory polymorphisms.

The *Pgm1* system is promising for study of the molecular basis and evolutionary significance of a regulatory difference. The tremendous elevation of PGM1 activity is a clear qualitative difference that is easier to study than the more subtle differences in enzyme levels characteristic of the temporal gene differences described previously. In addition, previous studies of temporal genes have made use of differences between inbred lines or species. The *Pgm1* variation is intraspecific and exists as a polymorphism within a population of rainbow trout. Thus, study of *Pgm1* is not hindered by any reproductive barriers between species. Possible effects on fitness can also be explored without the large amounts of linkage disequilibrium generated by crossing strains or species.

Intraspecific regulatory variants are needed to test the relative evolutionary significance of genetic variation at structural and regulatory genes. It seems likely that large differences in enzyme amounts produced by regulatory genes may have a greater effect on the fitness of an organism than small differences in the physical and chemical properties of those enzymes. Preliminary data reveal that fish having PGM1 activity in the liver grow at a faster rate during one period of their development. The further analysis of this regulatory polymorphism should elucidate mechanisms important to both developmental and evolutionary genetics.

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