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Genetics

Platelet-derived growth factor receptor α-subunit gene (*Pdgfra*) is deleted in the mouse patch (*Ph*) mutation

(development/dominant spotting/linkage analysis/human homolog)

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ABSTRACT **Platelet-derived growth factor receptors are composed of two subunits (α and β) that associate with one another to form three functionally active dimeric receptor species. The two subunits are encoded by separate loci in humans and other species. In this study, we used conventional interspecific backcross mapping and an analysis of a deletional mutation to establish close linkage between the α-subunit gene (*Pdgfra*) and the dominant spotting (*W*) locus on mouse chromosome 5. Further, by analyzing the restriction fragment length polymorphisms in interspecific F_1 hybrids, we were able to demonstrate that the closely associated patch (*Ph*) locus carries a deletion in *Pdgfra*. This observation was confirmed by both DNA and RNA analysis of 10.5-day fetuses produced from crosses between *Ph* heterozygotes. Out of 16 fetuses analyzed, *Pdgfra* genomic sequences were absent and no mRNA for the receptor was detected in 6 fetuses that were developmentally abnormal (the presumptive *Ph* homozygotes). We also determined that the deletion associated with the *Ph* mutation does not extend into the coding sequences of the adjacent *Kit* gene, by analysis of the genomic DNA from both the interspecific F_1 hybrids and the presumptive *Ph* homozygotes. The absence of *Pdgfra* genomic sequences and the lack of detectable message associated with the *Ph* mutation should make this mutant a valuable asset for understanding the role of the receptor α subunit during mammalian development.**

A series of semidominant mutations that cause white spotting in the mouse are closely linked to one another on chromosome 5. Included in this cluster are the loci for dominant spotting (*W*), patch (*Ph*), and rump-white (*Rw*). Close genetic linkage between the *W* locus and the c-*kit* protooncogene (*Kit*), which encodes a transmembrane tyrosine kinase, was demonstrated by Chabot *et al.* (1). Geissler *et al.* (2) confirmed this association by demonstrating that *Kit* genomic sequences were altered in two *W* mutants (W^{44J} and W^{x}) and that the mRNA level was reduced in one of them (W^{44J}). Subsequent analysis of various *W* mutant alleles showed that the *W* locus encodes the *Kit* gene product (3–6). Furthermore, the severity of the phenotypic defects associated with the various mutant alleles could be correlated with the nature of the mutational event by assuming that *Kit* encodes a receptor that is functionally active as a dimeric molecule. Specifically, mutations that abolish tyrosine kinase activity (W^{37J}, W^{v}/W^{55J}, W^{41J}, and W^{42J}) have a more severe heterozygous phenotype than those mutants (*W* and W^{19H}) that result in loss of the receptor.

Patch heterozygotes (*Ph*/+) have a spotting phenotype that is similar to those of some of the *W* mutant alleles, although there is no evidence of a hematological defect (7). Presumptive homozygotes, produced in crosses between heterozygotes, display gross anatomical abnormalities and die midway through gestation (7). The *Ph* locus was shown to be distinct from the *W* locus by both complementation and recombinational linkage analysis (7). When *Ph* heterozygotes were crossed to viable dominant spotting (W^{v}) heterozygotes, no offspring with the characteristic black-eyed phenotype were observed that might have suggested allelism. However, approximately one-fourth of the offspring had a more extensive spotting phenotype than either *Ph* or W^{v} alone, suggesting that they were probably the double heterozygotes (i.e., *Ph* +/+ W^{v}). Hematological analysis of the double heterozygotes showed no enhancement of the anemia associated with the W^{v} mutation alone. Although the *Ph* locus is distinct from the *W* locus, it does fall within the deletion defined by the W^{19H} mutant allele, unlike the closely associated *Rw* locus (8).

The platelet-derived growth factor receptor (PDGFR) is functionally active as a homodimer or heterodimer of two different protein subunits identified as α and β. The genes (*PDGFRA* and *PDGFRB*) encoding these subunits map to human chromosomes 4 and 5, respectively (9–11). *In situ* hybridization analysis localized *PDGFRA* to the same region of human chromosome 4 as the c-*kit* protooncogene. Since this region of human chromosome 4 is homologous with mouse chromosome 5, it was probable that the mouse *Pdgfra* gene would also map to the same region as the *Kit*/*W* locus. We reasoned that if *Pdgfra* did map to this region, it might be related to other mutant loci in the region that affect mammalian development.

Here we show that *Pdgfra* is closely linked to *W*/*Kit* on mouse chromosome 5. We also provide evidence from Southern analysis of interspecific F_1 hybrids that the *Ph* mutation carries a deletion in the genomic sequences associated with *Pdgfra*. This observation is supported by DNA and RNA analysis of 10.5-day fetuses obtained from intercrossing *Ph* heterozygotes. Presumptive *Ph* homozygotes were shown to lack the *Pdgfra* genomic sequences. Furthermore, no *Pdgfra* mRNA expression was detected in these abnormal fetuses. *Kit* gene analysis of both the interspecific F_1 hybrids and the *Ph* homozygotes indicates that the deletion does not extend into these coding sequences.

METHODS

Mice. Laboratory stocks carrying either the sash (W^{sh}) allele at the dominant spotting locus (12), the W^{19H} deletional mutation (8), or the patch (*Ph*) mutant allele (7) were obtained from the Medical Research Council Radiobiology Unit (Chil-

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Abbreviations: PDGF, platelet-derived growth factor; PDGFR, PDGF receptor.

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ton, U.K.). Both the W^{sh} and W^{19H} mutations occurred on a C3H × 101 F_1 hybrid background; however, during the maintenance of the W^{sh} mutation it was transferred onto a C57BL/6J.*bg* congenic background. The *Ph* mutation occurred on a C57BL background and was transferred onto a CBA strain background but is now maintained on a C3H × 101 F_1 hybrid background. Wild-derived *Mus musculus* (PWK stock) and *Mus spretus* used to produce interspecific F_1 hybrids were originally obtained from trappings in Czechoslovakia and Spain, respectively. PWK is a partially inbred stock whereas the *M. spretus* stock is maintained as an outbred colony.

Mouse Crosses and Backcrosses. The interspecific backcross offspring used in the linkage analysis were as described by Chabot *et al.* (1). A laboratory stock homozygous for the sash and beige coat pigment mutations (W^{sh}/W^{sh} *bg/bg*) was crossed to the wild-derived PWK stock and the F_1 hybrids were backcrossed to the C57BL/6J.*bg* congenic strain. Interspecific F_1 hybrids were produced between (*i*) the W^{19H} mutant stock and *M. spretus* and (*ii*) *Ph* mutant heterozygotes and the wild-derived PWK stock. Patch heterozygotes were intercrossed (*Ph*/+ × *Ph*/+) to produce *Ph* homozygotes. Since the homozygotes die midway through gestation (7), the pregnant females were sacrificed 10 days after the observation of a vaginal plug (day 0.5 of gestation).

All live offspring were classified for their pigment gene phenotypes prior to dissection. Sash heterozygotes ($W^{sh}/+$) had a broad white band across the loins in the interspecific backcross offspring, characteristic of the phenotype described by Lyon and Glenister (12). Similarly, the spotted phenotype associated with the *Ph* heterozygotes (7) was unequivocal in the interspecific F_1 hybrids. However, the spotting phenotype associated with W^{19H} was less pronounced in the F_1 hybrids with *M. spretus*. Thus the W^{19H} genotype of presumptive heterozygotes displaying slight white spotting of the feet and tail was confirmed by the hemizygous presence of the *M. spretus Kit* allele, as described by Chabot *et al.* (1).

Preparation of Tissues. High molecular weight genomic DNA was prepared from either freshly dissected or frozen kidneys according to standard protocols (13). In brief, proteins were digested with proteinase K (Sigma) and removed by phenol/chloroform extraction. The DNA was purified by ethanol precipitation. RNA from the 10.5-day fetuses was purified according to standard procedures (14). The fetus was dissected free of the yolk sac and homogenized in 4 M guanidinium isothiocyanate/20 mM sodium acetate/0.1 mM dithiothreitol/0.5% *N*-lauroylsarcosine. RNA was collected as a pellet from a 5.7 M CsCl gradient. The yolk sac was used for DNA extraction.

Southern Analysis. Approximately 5 μg of genomic DNA from each sample was digested with individual restriction endonucleases according to the manufacturer's recommendations (either New England Biolabs, Bethesda Research Laboratories, or Promega). The digested DNA was resolved in a 0.8% agarose gel (SeaKem, FMC) in 40 mM Tris acetate/2 mM EDTA. After denaturation, the DNA was transferred to a nitrocellulose membrane (Schleicher & Schuell). The blots were prehybridized, hybridized, and washed using the same conditions for each probe. Radioactively labeled probe was produced by the random primer method (15). Prehybridization and hybridization were performed at 65°C for 3 hr and ≈18 hr, respectively, in 0.1% Ficoll/0.1% polyvinylpyrrolidone/0.1% bovine serum albumin/600 mM NaCl/60 mM sodium citrate, pH 7/0.1% SDS/0.1% sodium pyrophosphate containing salmon sperm DNA at 100 μg/ml. Three washes of 30 min each were also conducted at 65°C in 150 mM NaCl/15 mM sodium citrate, pH 7/0.1% SDS. The 4.3-kilobase (kb) *Kit* cDNA used in the analysis was one of three clones described by Qiu *et al.* (16). Preliminary characterization of the clone indicates that it lacks ≈0.8 kb from the start of the open reading frame (unpublished data). The *Pdgfra* gene was identified with either a 1.5-kb cDNA encoding the second tyrosine kinase domain and ≈1.0 kb of untranslated message (the 3′ cDNA) or a 6.4-kb full-length cDNA (17).

RNA Analysis. RNA was assayed for specific gene expression by RNase protection assay using antisense transcripts synthesized *in vitro* in the presence of [α-^{32}P]CTP (18). Transcript templates for mouse PDGF α ligand (PDGF A chain) and PDGFR α chain were as described (17). Probe transcripts (10^6 cpm) were hybridized to each RNA sample for 16 hr and then digested with RNases A and T1 (Pharmacia or Boehringer Mannheim). Protected fragments were analyzed in a 6% polyacrylamide/8 M urea gel. The radioactivity in the protected bands was quantitated by exposing the dried gel to a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

RESULTS

***Pdgfra* Is Closely Linked to *W*.** Genomic DNA from the parental mouse stocks (C57BL, C3H, CBA, 101, and PWK) was analyzed for restriction fragment length polymorphism with the 3′ cDNA probe to the mouse *Pdgfra* gene. No variation was observed between the laboratory strains and the wild-derived PWK stock with the restriction endonucleases *Bam*HI, *Bgl* II, *Eco*RI, *Hin*dIII, and *Pvu* II. However, variation was observed with the restriction endonucleases *Eco*RV and *Pst* I. An *Eco*RV fragment of 23 kb identified the C57BL allele (and other laboratory strains tested), whereas a 20-kb fragment defined the PWK allele. This variation was used to follow the segregation of the *Pdgfra* gene in the interspecific backcross offspring segregating the sash (W^{sh}) allele at the *W* locus. A total of 107 interspecific backcross offspring were characterized for the segregation of alleles at the *Pdgfra* locus (Table 1). The laboratory allele for the *Pdgfra* gene cosegregated with the W^{sh} allele at the *W* locus (53/107). Similarly, the PWK allele for *Pdgfra* cosegregated with the wild-type allele at the *W* locus (54/107). No recombination was observed between *Pdgfra* and *W/Kit*. Thus, these two genes lie between 0 and 4 centimorgans apart at the 95% confidence interval. Nine recombinants were observed between the α-fetoprotein gene (*Afp*) and the *W* locus in the same backcross, placing the two loci an estimated 8.4 ± 2.7 centimorgans apart (Table 1). This estimate is consistent with earlier studies (19) and suggests that the lack of *Pdgfra* and *W/Kit* recombination is not due to a suppression of recombination.

***Pdgfra* Is Deleted in W^{19H}.** To further define the linkage of the *Pdgfra* locus with *W/Kit* on mouse chromosome 5, we decided to determine whether *Pdgfra* mapped within the 2- to 7-centimorgan deletion associated with the W^{19H} mutation. Specifically, we asked whether genetic variation defining

Table 1. Recombinational linkage analysis of *Pdgfra* relative to *W/Kit* and *Afp* on mouse chromosome 5

	W/Kit		*Pdgfra*		*Afp*	Total
Parental	L		L		L	47
classes	P		P		P	51
Recombinant	L		L	×	P	6
classes	P		P	×	L	3
	L	×	P		P	0
	P	×	L		L	0
					(Grand total)	107

Segregation of alleles at the α-fetoprotein (*Afp*) locus was determined by Southern analysis using the *Pst* I polymorphism previously identified (1). L, the laboratory-derived allele; P, the wild-derived (PWK) allele.

both *Pdgfra* alleles would be present (the heterozygous phenotype) or absent (the hemizygous phenotype) in W^{19H} mutant mice. The restriction fragment pattern of *Pdgfra* was examined in interspecific F_1 hybrid progeny from crosses between $W^{19H}/+$ mice and *M. spretus*. Variation in *Pdgfra* was observed between laboratory mice and *M. spretus* with the restriction endonucleases *Eco*RV and *Hin*dIII when the full-length *Pdgfra* cDNA was used as probe. The *M. spretus* allele ($+^S$) had *Eco*RV fragments of 26, 11, and 4.2 kb, whereas the *Pdgfra* allele common to laboratory strains ($+^L$) was defined by unique fragment sizes of 20 and 15 kb (Fig. 1). A 23-kb fragment was common to both alleles. The phenotypically wild-type F_1 hybrid ($+^L/+^S$) had the expected additive heterozygous pattern of fragment sizes that were inherited from both parents, whereas the spotted offspring ($W^{19H}/+^S$) displayed only the variant form that was specific to the *M. spretus* allele. These data show that the W^{19H} deletional mutation encompasses the *Pdgfra* gene in addition to *Kit* (1), the anonymous cDNA *D5SC25* (20), the *Ph* locus, and a recessive lethal mutation (8).

***Kit* Is Not Deleted in *Ph*.** The *Ph* locus is distinct from the *W/Kit* locus as determined by complementation studies and recombinational linkage analysis (7) but it is close enough to fall within the W^{19H} deletion (8). Given the speculation that the *Ph* mutant may also be a deficiency mutation (21), we asked whether there was an alteration in the *Kit* gene associated with the *Ph* mutation. The strategy used to demonstrate a deletion of the *Pdgfra* gene in the W^{19H} mutation was also employed in the analysis of the *Ph* mutation. In this case, interspecific F_1 hybrids were produced by mating *Ph*/+ heterozygous mice with the wild-derived PWK stock.

Variation for the *Kit* locus identified with *Bgl* II (1) was used to follow the parental alleles in the interspecific F_1 hybrids between *Ph*/+ and PWK (Fig. 2*A*). Given the derivation of the *Ph* stock (see *Methods*), we needed to establish whether there were naturally occurring *Kit* polymorphisms between the inbred strains and, if so, which one segregated with the *Ph* mutation. The C3H *Bgl* II polymorphism for the *Kit* gene (Fig. 2*A*) was identical to that found in the CBA and C57BL inbred strains, with fragment sizes of 9.2, 7.4, 6.6, 4.8, 3.8, 2.3, 1.8, and 1.2 kb. The 101 polymorphism (defined by fragment sizes of 14, 4.8, 3.8, 2.3, 1.8, and 1.2 kb) was distinct from the C3H allele but similar to that present in the PWK stock (14, 5.0, 4.6, 3.8, 2.3, 2.2, 1.8, and 1.2 kb; Fig. 2*A*). The segregation of the C3H and 101 *Kit* polymorphisms was analyzed in the progeny of the *Ph* stock. It was determined that the *Ph* mutation cosegregated with the C3H *Kit* allele (data not shown). We therefore examined F_1 hybrids between the *Ph* mutant and the PWK stock to determine whether the *Kit* locus was either altered or deleted in the *Ph* mutant. Using the 4.3-kb *Kit* cDNA, we observed that *Ph*/PWK heterozygotes had fragments from both the C3H and PWK alleles (fragment sizes of 9.2, 7.4, 6.6, and 4.8 kb for the C3H allele and 14, 5.0, 4.6, and 2.2 kb for the PWK allele; Fig. 2*A*). This result indicates that the portion of the *Kit* gene identified with the cDNA probe is unaffected by the *Ph* mutation.

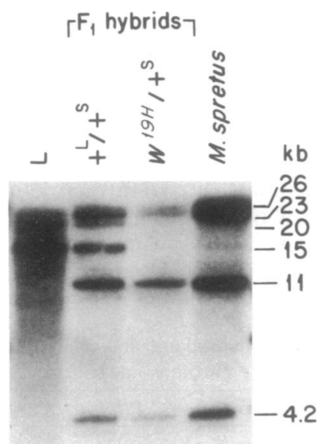

FIG. 1. Southern analysis of interspecific F_1 hybrids between the laboratory-derived W^{19H} mutant stock and the wild-derived *M. spretus* stock. *Eco*RV-digested DNA was hybridized with the full-length mouse *Pdgfra* cDNA (17). The *M. spretus* allele (S) is defined by fragment sizes of 26, 11, and 4.2 kb, whereas C57BL/6JRos, which defines the polymorphism present in the laboratory strains (L), is identified by fragments of 20 and 15 kb. The F_1 hybrid carrying the W^{19H} mutation lacks the 15-kb fragment, indicating that it carries a deletion in the laboratory-derived allele of *Pdgfra*. The 20-kb fragment is also absent.

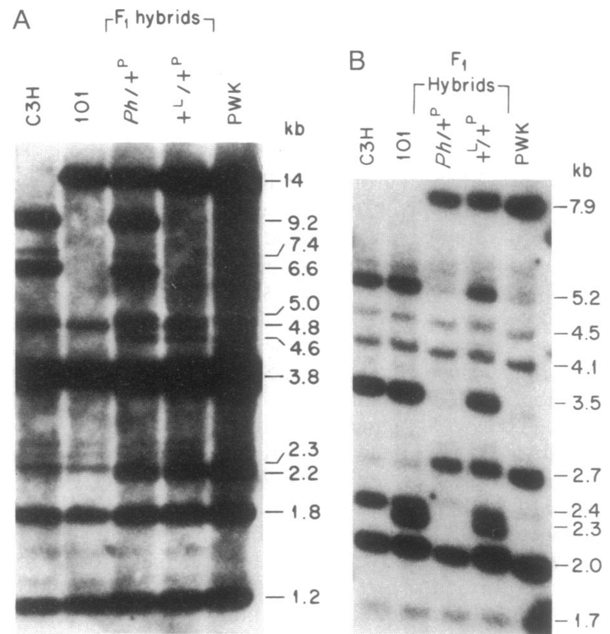

FIG. 2. Southern analysis of interspecific F_1 hybrids between the laboratory-derived *Ph* mutant stock and the wild-derived *M. musculus* (PWK) stock. (*A*) *Bgl* II digests hybridized with the mouse *Kit* cDNA (16). The F_1 hybrid heterozygous for the *Ph* mutation has the 9.2, 7.4, and 6.6-kb fragments characteristic of the C3H allele, consistent with the method of stock maintenance (see text). (*B*) *Pst* I digests hybridized with the mouse 3′ *Pdgfra* cDNA (17). Unlike *A*, the *Ph*/+ hybrid does not carry the allele-specific fragments of the inbred parental strain as represented by C3H and 101.

***Pdgfra* Gene Sequences Are Deleted in *Ph*.** The C3H *Pst* I restriction pattern for the *Pdgfra* gene, as identified with the 3′ cDNA probe used in the mapping studies (Fig. 2*B*), was identical to that found in the CBA and C57BL inbred strains. The 101 inbred strain had almost the same pattern as C3H except for the presence of a unique 2.3-kb fragment. The PWK allele (Fig. 2*B*) had two unique fragments of 7.9 and 2.7 kb and lacked the 5.2-, 3.5-, and 2.4-kb fragments that were present in the C3H pattern. The same *Ph*/+ × PWK interspecific F_1 hybrids used above were also used to determine whether the *Ph* mutant carried a deletion in the *Pdgfra* gene. The F_1 hybrid with the wild-type phenotype ($+^L/+^P$) displayed a banding pattern indicating that both parental *Pdgfra* alleles were present (Fig. 2*B*, lane 4), whereas the progeny with the spotted phenotype ($Ph/+^P$) showed only the PWK polymorphism (lane 3). The 3′ cDNA probe for *Pdgfra* identified ≈23 kb of the genomic sequence with a 50% difference between the two alleles. To further define the extent of the deletion as it related to *Pdgfra*, we performed the same analysis using a full-length cDNA. This probe identified 45 kb of the *Pdgfra* genomic sequence in the C3H

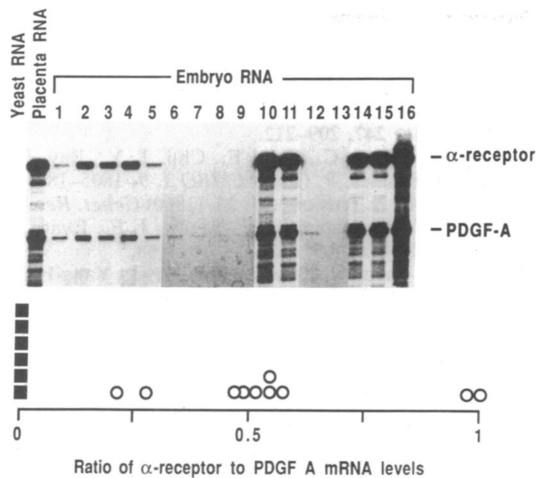

FIG. 3. Analysis of RNA from individual 10.5-day fetuses from a *Ph*/+ × *Ph*/+ cross. (*Upper*) RNase protection analysis using radiolabeled hybridization probes specific for the PDGFR α subunit (α-receptor) and PDGF A subunit. Each lane represents RNA from one fetus. As controls, 20 μg of RNA from yeast or 15-day mouse placenta was included. Lanes corresponding to fetuses 6–9, 12, and 13 and yeast RNA are darker exposures of the same autoradiograph. (*Lower*) Relative expression of the PDGFR α-subunit mRNA in individual fetuses. The radioactivity of the protected bands (*Upper*) was quantitated by a PhosphorImager and the levels of the PDGFR α bands were normalized to those of the PDGF A bands for each sample. The highest ratio was arbitrarily set to 1. Open circles indicate fetuses containing *Pdgfra* sequences (+/+ and *Ph*/+); filled squares indicate fetuses lacking *Pdgfra* sequences (*Ph*/*Ph*).

strain compared with 32 kb for the PWK stock. Two additional fragments specific to the PWK allele were observed at 4.9 and 2.5 kb, whereas the C3H allele was further defined by fragment sizes of 5.5, 4.8, 4.2, 2.6, 1.9, and 0.9 kb. Approximately 70% of the sequences identifying the C3H allele were specific to that allele when compared with the PWK allele. The $Ph/+^{P}$ F_1 hybrid had the additional PWK fragments as well as some fragments specific to the C3H allele (i.e., 4.2, 1.9, and 0.9 kb). The other additional C3H-specific fragments were absent. The presence of C3H-specific fragments indicates that the deletion terminates within *Pdgfra*. However, our data do not establish whether these fragments are specific to the *Pdgfra* locus or whether they identify elements at either the 3′ or 5′ end of the gene. Nonetheless, these observations indicate that most of the unique genomic sequences specific to C3H are absent in the *Ph* mutations and that the deletion terminates within *Pdgfra*.

Fetal Tissues from *Ph*/+ Intercrosses Have Reduced *Pdgfra* mRNA in Heterozygotes and Show Loss of Both *Pdgfra* mRNA and *Pdgfra* Genomic Sequences in *Ph* Homozygotes. To separately confirm the deletion of *Pdgfra* in the *Ph* mutation, we analyzed 10.5-day fetal tissue produced in crosses between *Ph* heterozygotes. If *Pdgfra* were deleted in the *Ph* mutation, we would expect approximately one-fourth of the conceptuses to be developmentally abnormal and to lack *Pdgfra* DNA and transcript. A total of 16 conceptuses were observed in two pregnancies with no indication that there were any dead or resorbing fetuses; thus all classes were likely to be present in this analysis. Of the 16 fetuses examined, 7 were identified visually as potential *Ph* homozygotes based on their retarded development (7).

DNA extracted from the yolk sac of each fetus was digested with *Bgl* II or *Pst* I and analyzed with the probes for *Kit* and *Pdgfra*, respectively. All 16 conceptuses were positive for *Kit* sequences that were the same as those observed in adult tissues of the laboratory strains. By contrast, only 8 of 13 conceptuses had detectable *Pdgfra* sequences in Southern analysis with the 3′ cDNA probe (technical difficulties precluded the analysis of the remainder). These results are consistent with the deletion of *Pdgfra* that was indicated in the interspecific *Ph*/PWK hybrids.

The levels of *Pdgfra* RNA were determined by RNase protection assay and normalized by using the internal ratio of PDGFR α-subunit mRNA to PDGF A-subunit mRNA (Fig. 3). In this instance, PDGF A mRNA was used as a constant high-level transcript of a gene that was genetically unlinked to the receptor (17). The levels of total RNA were very low in 6 of 16 fetuses, which corresponded to 6 of the 7 fetuses that had been visually scored as potential *Ph* homozygotes. Very low levels of PDGF A mRNA were observed in 3 of these fetal samples (nos. 6, 7, and 12), but no evidence of PDGFR α message was detected in any of these samples. Ten fetuses had measurable PDGFR α and PDGF A mRNA levels that could be grouped on the basis of the ratio of PDGFR α to PDGF A. Two fetuses had a ratio of 1.0 and the remainder (8/16) had a ratio of ≈0.5 or less. Overall, these results are consistent with an F_2 segregation of 6 *Ph*/*Ph*, 8 *Ph*/+, and 2 +/+ in the 16 fetuses, which does not differ from the expected ratio of 1:2:1.

DISCUSSION

The results demonstrate the following. (*i*) The gene encoding the α subunit of the PDGFR (*Pdgfra*) is linked to the *W*/*Kit* locus on mouse chromosome 5, thus confirming speculation that *Pdgfra* might fall within the region of conserved linkage between mouse chromosome 5 and human chromosome 4. (*ii*) *Pdgfra* sequences are deleted in the W^{19H} mutation. (*iii*) The *Ph* mutant is a deletional mutation that encompasses *Pdgfra* but not the closely associated *Kit* gene. (*iv*) Additional analysis, using the full-length cDNA, indicates that the *Ph* deletion starts within *Pdgfra* itself. (*v*) No PDGFR α-subunit mRNA was detected in 10.5-day fetal tissue from *Ph* homozygotes. Although the *Ph* mutation carries a deletion in *Pdgfra*, it remains to be determined whether the phenotypic defects associated with both heterozygote and homozygote can be attributed to the loss of this gene.

The phenotype of the *Ph* heterozygote is predominantly characterized by a variably sized white spot on the ventral surface, which may extend into the dorsal region to form a band (7). A spotting phenotype is also a prominent feature of mutant alleles at the closely linked *W*/*Kit* locus. Since *Ph* is a deletional mutation, can the spotting phenotype be attributed to a disruption of the *Kit* gene? Analysis of *Kit* genomic sequences in the interspecific F_1 hybrids between *Ph* and PWK indicates that the deletion does not extend into *Kit*. However, the *Kit* probe used in this analysis lacks sequences to the 5′ end of the gene; thus, a disruption in this region might go undetected. In their analysis of the *W* mutants, Geissler *et al.* (2) provided evidence that the 3.8-kb *Bgl* II fragment may identify the 5′ end of *Kit*. Although this fragment is common to both alleles in our analysis (Fig. 2*A*), there is no evidence of a gene dosage effect that might suggest a disruption in this region of the gene. However, more compelling evidence for functional *Kit* sequences was provided by Grüneberg and Truslove (7) in their initial characterization of the *Ph* mutant. In complementation analysis with W^{v}, the double heterozygotes had some pigmentation on the head and shoulders, not the black-eyed white phenotype of *W* homozygotes and mutant heterozygotes. There was no enhancement of the macrocytic anemia associated with the *W* mutant. Furthermore, the double heterozygotes were fertile. From their linkage studies, Grüneberg and Truslove (7) observed recombinants between *Ph* and W^{v} with the wild-type phenotype. Taken together, these observations indicate that there are functional *Kit* sequences on the *Ph* chromosome.

It is possible that the linkage relationship between *Pdgfra* and *Kit* may be analogous to the situation observed between the PDGFR β-subunit gene (*PDGFRB*) and the colony-stimulating factor 1 receptor gene (*CSF1R*) on human chromosome 5. *PDGFRB* and *CSF1R* appear to be separated by only 0.5 kb (22). If this analogy holds for *Pdgfra* and *Kit* on mouse chromosome 5, the deletion associated with the *Ph* mutation could introduce novel elements that disrupt normal *Kit* gene function. Thus the spotting phenotype observed in the *Ph* heterozygote may be a consequence of inappropriate *Kit* expression at critical times during fetal development. However, we have examined *Kit* expression in the brain of *Ph* heterozygotes and observed no marked decrease in *Kit* mRNA by Northern analyses (data not shown).

If the *Ph* phenotype is due to a deficiency in *Pdgfra*, it provides another example of a mutation in a different member of the same tyrosine kinase family that produces a spotting phenotype. In most instances, *Ph* complements alleles at the closely linked *W* locus and the associated rump-white (*Rw*) locus (7, 23). Although *Ph* heterozygotes have no measurable effects on hematopoiesis, some *Ph*/*W* combinations show an enhancement of the hematological defects associated with *W* gene mutations (24). This interaction with *W*, which is specific to the patch-extended (Ph^e) allele, has also been shown to include the germ cells (25). Thus, given the close association of the genes on the chromosome, and the similarities between the proteins encoded by the two genes, it is still possible that these receptors act in concert to affect the regulation of cell growth or differentiation.

The gross anatomical abnormalities observed in the *Ph* homozygote are consistent with previous observations indicating that PDGF A chain is expressed at high levels during early embryogenesis (26, 27). Furthermore, expression of PDGFR α chain, which binds PDGF A (28), is detectable at high levels in the developing fetus between the days 6 and 8 of gestation (17), at a time shortly before the death of *Ph* homozygotes (7). Thus, the *Ph* mutation should prove valuable for defining the role of the PDGFR α polypeptide during mammalian development.

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