resistance of a single cell to ground and the resistance between a single cell and the rest of the cluster, respectively. R_c was constrained to values between 1 and 3 gigaohms because this is the range of resistances for embryonic neurons that are not dye-coupled.
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Direct Molecular Identification of the Mouse Pink-Eyed Unstable Mutation by Genome Scanning

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DNA sequences associated with the mouse pink-eyed unstable mutation were identified in the absence of closely linked molecular markers and without prior knowledge of the encoded gene product. This was accomplished by "genome scanning," a technique in which high-resolution Southern blots of genomic DNAs were hybridized to a dispersed and moderately repetitive DNA sequence. In this assay, pink-eyed unstable DNA was distinguished from the DNA of wild-type and revertant mice by enhanced hybridization to one of several hundred resolved fragments. The fragment showing enhanced hybridization in pink-eyed unstable DNA was cloned and found to lie within a DNA duplication that is located close to, or within, the pink-eyed dilution locus. The duplication associated with the mouse pink-eyed unstable mutation may mediate the high reversion frequency characteristic of this mutation.

THE PINK-EYED UNSTABLE MUTATION, p^{un}, is one of at least 13 alleles of the pink-eyed dilution locus, *p* (*1*), which, together with the albino locus, *c*, constitutes the first genetic linkage group that was identified in mammals (*2*). On a C57BL/6J background, mice carrying the wild-type *p* allele have intensely pigmented coats and eyes, whereas mice homozygous for p^{un} or most other recessive *p* alleles have greatly reduced pigmentation in both their coats and eyes. Although the p^{un} mutant allele apparently affects only pigmentation, several other mutant *p* alleles are associated with additional phenotypes, including neurological problems, male sterility, and cleft palate (*1*).

The p^{un} mutant allele has the distinction of displaying the highest rate of reversion reported in mammals, reverting at a frequency at least three to five orders of magnitude greater than other *p* alleles or recessive mutations at other coat color loci (*3*). Approximately 1.8% of the offspring of homozygous C57BL/6J p^{un}/p^{un} mice have patches of wild-type color in their coats and are mosaic revertants (*4*). Occasionally, an all-black mouse is produced from a cross between a p^{un} mosaic revertant and a non-revertant p^{un}/p^{un} mouse. These mice can be used to establish homozygous revertant lines (*5*).

The molecular basis of the high frequency of p^{un} reversion has remained an enigma until now, although several hypotheses have been advanced (*4, 6*). Only one mouse mutation with a detectable reversion frequency, *d* (dilute), has been examined in detail at the molecular level. The *d* mutation was caused by the insertion of a murine leukemia provirus in the *d* locus, and reversion from *d* to wild type is accompanied by loss of this retrovirus (*7*). However, because the reversion frequency of p^{un} is three orders of magnitude greater than that of *d*, the p^{un} mutation and reversion may be the result of a different process. Rates of genetic change of the same magnitude as the p^{un} reversion frequency are observed for DNA sequences repeated in tandem (*8, 9*), suggesting that the p^{un} mutation might similarly involve tandem repeats of DNA.

We have termed the method we used to identify sequence alterations associated with p^{un} DNA, "genome scanning." This method is similar to the DNA fingerprint assay (*9*) and, like DNA fingerprinting, is based on Southern hybridization (*10*) with the use of an interspersed repetitive DNA sequence as a hybridization probe. However, because our method uses a repetitive DNA probe of much higher copy number than that used in conventional DNA fingerprinting (~1000 copies per genome rather than ~60 copies per genome), a larger fraction of the genome can be scanned for sequence differences related to a mutant locus. In this type of approach, large net sequence differences (such as large duplications or deletions) have a greater chance of being detected than

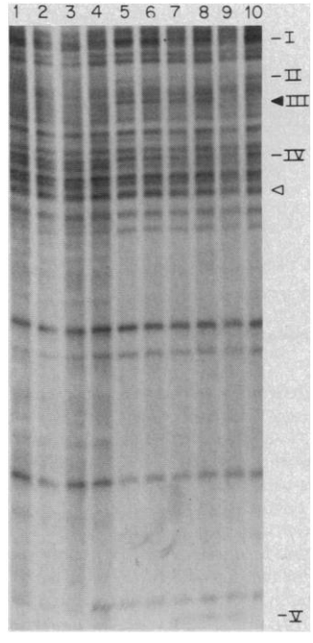

Fig. 1. Genome-scanning Southern blot of Apa I–digested DNA from individual mice homozygous for various *p* alleles hybridized to IAP DNA (*20*). An autoradiogram representing a 24-cm portion (0.88 kb to 3.4 kb) of a hybridized filter is shown (*21*). Lane 1, male revertant p^{un+1J}; lane 2, female revertant p^{un+1J}; lane 3, male revertant p^{un+2J}; lane 4, female revertant p^{un+2J}; lanes 5 and 6, female p^{un}; lanes 7 and 8, male p^{un}; lane 9, male wild type; lane 10, female wild type. Fragments enhanced in female DNA are indicated as bands I (3.3 kb), II (3.1 kb), and IV (2.6 kb). A fragment unique to male DNA is labeled V (0.9 kb). The fragment displaying enhanced hybridization in p^{un} DNA is band III (2.9 kb). Sizes of the indicated fragments were based on the migration of marker fragments, two of which are shown: closed triangle (comigrating with band III), 2.9 kb; open triangle, 2.4 kb.

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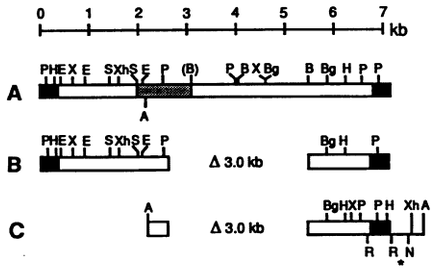

Fig. 2. Restriction map of IAP DNA elements and clone p28. (**A**) Full-length type I IAP element (*11*). The bold bars indicate the long terminal repeat (LTR) sequences of the IAP element. The fragment used as a probe in the genome-scanning Southern blots is the ~1-kb Sst I–Bam HI fragment indicated by stippling. Restriction sites common to most IAP elements are depicted at the top: B, Bam HI; Bg, Bgl II; E, Eco RI; H, Hind III; P, Pst I; S, Sst I; X, Xba I; and Xh, Xho I. The Bam HI site in parentheses is not common to most IAP elements. (**B**) Internally deleted type IΔ3 IAP element (*11*). The internal 3-kb deletion is common to this subset of IAP elements. (**C**) Clone p28. The p28 sequence shares IAP sequences common to a type IΔ3 IAP element and has 0.74 kb of non-IAP sequence. R and N, Rsa I and Nde I sites, respectively; the fragment bound by these two restriction sites is the 28RN fragment and is identified by an asterisk. A, Apa I site. Details of the isolation of p28 are presented in (*12*).

small sequence differences (such as point mutations).

Sequence differences between the DNAs from homozygous p^{un} and wild-type mice (of the same sex) are likely to be related to the p^{un} mutation. This is because the p^{un} mutation arose spontaneously in, and is maintained on, the same (coisogenic) inbred C57BL/6J background. To look for hybridization band variants associated with the p^{un} mutation, we chose a hybridization probe derived from the sequence family encoding the retroviral-like intracisternal A particles (IAPs); this family includes ~1000 to 2000 widely dispersed copies per haploid genome (*11*). We detected five prominent variant fragments by Southern blot analysis among the DNAs of individual coisogenic mice homozygous for p^{un}, wild-type, and revertant alleles, with an IAP DNA probe (Fig. 1). Four of these five variants were correlated with the sex of the mice, but not with a particular *p* allele, and appeared as bands displaying female-enhanced hybridization or male-specific hybridization. Presumably these fragments are located on the X and Y chromosomes, respectively. One variant, however, was correlated with the p^{un} allele: a 2.9-kb Apa I fragment displayed enhanced hybridization to the IAP probe in DNA from C57BL/6J p^{un}/p^{un} mice relative to DNAs from mice homozygous for wild-type or revertant alleles. A cloned isolate of the 2.9-kb fragment, p28 (*12*), was used in subsequent studies; a restriction map of p28 showing its overlap with the original IAP probe and IAP sequences is presented in Fig. 2.

To map the p28 sequence, we isolated a unique sequence fragment of clone p28 (28RN; a 0.39-kb Rsa I–Nde I fragment of non-IAP sequence, identified by the asterisk in Fig. 2C). Restriction fragment length polymorphisms (RFLPs) detected by 28RN hybridization were used to map the p28 sequence in recombinant inbred (RI) strains (*13*). No crossovers were observed between 28RN and the *p* locus in the NX129 and SWXJ RI strains (0/20 strains) indicating close linkage (*14*). We also found that the 28RN sequence was missing from the DNA of mice homozygous for the radiation-induced mutant allele of the *p* locus, P^{6H} (*15*), further demonstrating the close linkage of 28RN with the *p* locus and identifying p^{6H} as a deletion mutation of *p* (Fig. 3).

The 28RN probe was able to detect two p^{un} hybridization band variants. The first variant was a hybridization intensity ratio of approximately 2:1, p^{un} DNA:wild-type DNA, with equivalent amounts of genomic DNA digested with a variety of restriction enzymes. The second variant detected by the 28RN probe was an additional Sst I fragment in p^{un} DNA that was not found in wild-type or revertant DNA (Fig. 4).

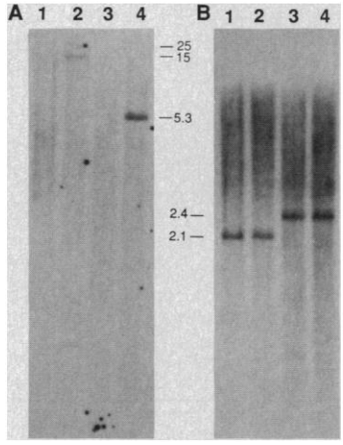

Fig. 3. Southern hybridization of 28RN and tyrosine hydroxylase probes to genomic DNA of mice that are homozygous for p^{un} or p^{6H} alleles. (**A**) 28RN probe hybridization to Sst I–digested DNAs (lanes 1 and 2) and Pst I–digested DNAs (lanes 3 and 4). Lanes 1 and 3, DNA from p^{6H}/p^{6H} mice; lanes 2 and 4, DNA from p^{un}/p^{un} mice. (**B**) Tyrosine hydroxylase probe (*22*) hybridization to the same (erased) blot shown in (A). Genomic DNAs were isolated, digested, separated by electrophoresis (gel bed length, 21 cm), and transferred to filters (*21*). The filters were hybridized and washed under relatively low stringency conditions (28RN probe, 61°C hybridization, washing with 2× SSC and 0.1% SDS at 59°C; pHR3.0 probe, 63°C hybridization, washing with 1× SSC and 0.1% SDS at 62°C) to ensure detection of all possible hybridization signals. Sizes of fragments are shown in kilobase pairs.

The occurrence of restriction fragments displaying a 2:1, p^{un} DNA:wild-type DNA, hybridization intensity ratio suggests a duplication of this region in p^{un} DNA. This duplication was confirmed by restriction analyses of the two Sst I fragments of p^{un} DNA; the same 2.9-kb Apa I fragment (p28) is found within both Sst I fragments. Both Sst I fragments share at least 8.8 kb of sequence (between common Sst I and Sca I sites) (Fig. 4C). However, because the Sst I fragments also contain nonoverlapping sequences, a boundary of the p^{un} duplication must exist within the region of nonoverlapping sequences (within the 6.2-kb Sca I–Sst I segment of the 15-kb Sst I fragment). The minimum length of the p^{un} duplication is 17.8 kb (8.8 kb of duplicated sequence shared by the Sst I fragments plus 9 kb of duplicated sequence not included in the Sst I fragments) (Fig. 4C).

IAP elements are associated with genomic sequence changes, including transpositions (*11*) and a DNA duplication in certain mouse strains at the renin locus (*16*). However, because the p28 IAP element was found within the same sequence milieus in p^{un}, wild-type, and revertant DNAs (Fig. 4), it must have been present at the *p* locus before the p^{un} duplication. This indicates that the p^{un} duplication did not arise from a simple or novel transposition of this IAP element. Rather, the IAP probe detected the p^{un}-enhanced fragment only because it was duplicated along with a much larger segment of DNA in the mutational process leading to the p^{un} mutation.

The hallmarks of the p^{un} duplication detected by the 28RN probe (the twofold increase in hybridization intensity signals and the novel 15-kb Sst I fragment) were eliminated in three independently derived revertants of p^{un}; 28RN hybridization to revertant DNA and wild-type DNA were indistinguishable (Fig. 4B). Therefore, the loss of the duplication was coupled with the reversion of the p^{un} phenotype to wild-type phenotype. The p^{un} duplication may disrupt the coding sequences of the *p* locus. Alternatively, the p^{un} phenotype could be the result of a dosage effect, a position effect, or a disruption in regulatory sequences. In all cases, removal of one of the duplicated copies restores the linear array of genetic information at the *p* locus and leads to a reversion of phenotype. The future isolation of the coding sequences of this locus will be necessary to determine precisely how the p^{un} duplication results in the mutant phenotype.

The coupling of the loss of the p^{un} duplication with a reversion event that occurs at high frequency suggests a tandem head-to-tail orientation of the duplication. Tandem head-to-tail duplications (but not inverted duplications) allow for the loss of one of the duplicated copies by homologous, but unequal, crossing-over between the duplicated copies without the generation of chromosomal inversions. Examples of high-frequency genetic changes mediated by homologous, but unequal, crossing-over between head-to-tail repeated DNA sequences include the reversion of the *Drosophila Bar* mutation and changes in the unit number of tandem-repetitive minisatellite sequences in human and mouse DNA (detected as both meiotic and mitotic events) (*8, 9*). Extensive restriction map and sequence analyses of the p^{un} duplication are consistent with a tandem head-to-tail orientation of the duplication (*17*).

The aim of reverse genetics is the identification of a mutant gene starting from the mutant phenotype, "without reference to a specific protein or without any reagents or functional assays useful in its detection" (*18*). Most current reverse genetic schemes begin with the identification of molecular markers closely linked to the locus of interest, followed by chromosome jumping or linking procedures to get to the site of the mutation. We have directly identified the site of the p^{un} mutation in the absence of closely linked DNA markers and without chromosome jumping or linking procedures. An advantage exploited in our study was the availability of coisogenic inbred mouse strains; the only expected sequence differences between the DNAs we compared were those relating to either the sex of, or the *p* allele carried by, an individual mouse. We were able to detect female-enhanced fragments and the p^{un}-duplicated fragment because the method is sensitive enough to detect a 2:1 difference in hybridization signal strength. Similar analyses comparing coisogenic strains bearing wild-type and radiation-induced alleles (potential deletions) may reveal the site of other mutations.

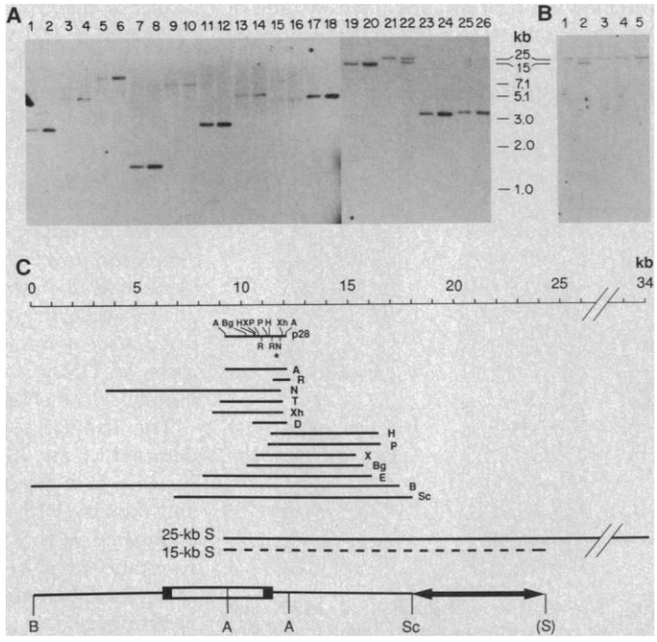

Fig. 4. Sequence variations detected by the 28RN probe. (**A**) Southern blot of C57BL/6JEi wild-type DNA (odd-numbered lanes) and C57BL/6JEi p^{un}/p^{un} DNA (even-numbered lanes) (gel bed length, 35 cm) (*21*). Lanes 1 and 2, Apa I; lanes 3 and 4, Bgl II; lanes 5 and 6, Bst EII; lanes 7 and 8, Dra I; lanes 9 and 10, Eco RI; lanes 11 and 12, Eco RV; lanes 13 and 14, Pst I; lanes 15 and 16, Hind III; lanes 17 and 18, Kpn I; lanes 19 and 20, Sca I; lanes 21 and 22, Sst I; lanes 23 and 24, Taq I; and lanes 25 and 26, Xho I. All restriction fragments shown (except Sst I) displayed a 2:1, p^{un} DNA:wild-type DNA, hybridization intensity ratio, as measured by computer-aided image analysis, relative to a control hybridization (*22*). (**B**) Southern blot of Sst I–digested C57BL/6JEi DNAs homozygous for wild-type (lane 1), p^{un} (lane 2), revertant p^{un+1J} (lane 3), revertant p^{un+2J} (lane 4), and revertant p^{un+3J} (lane 5) alleles (*5*). (**C**) Restriction map of the p28 region determined from analysis of Southern blots of doubly digested genomic DNA, the p28 restriction map, or direct sequence analysis. The first line under the arbitrary scale (marked in 1-kb increments) is a restriction map of p28. Shown in solid lines are the positions of genomic restriction fragments that hybridize to the 28RN probe (indicated by the asterisk). Restriction enzymes: A, Apa I; B, Bam HI; Bg, Bgl II; D, Dra I; E, Eco RI; H, Hind III; N, Nde I; P, Pst I; R, Rsa I; S, Sst I; Sc, Sca I; T, Taq I; X, Xba I; and Xh, Xho I. The 15-kb Sst I fragment (dotted line) is unique to p^{un} DNA. The minimum region of the duplication is indicated on the last line of the figure as the 17.8-kb fragment between the leftmost Bam HI site and the rightmost Sca I site. The region where p^{un} and wild-type DNA diverge is within the 6-kb region indicated by the bold double arrow (delineated by a common Sca I site and the mutant-specific Sst I site shown in parenthesis). The IAP element is indicated by the boxed region; filled black portions represent the IAP LTRs. The IAP sequences to the right of the internal Apa I site were confirmed by the analysis of the p28 clone; the IAP sequences to the left of the internal Apa I site were inferred by the presence of several conserved restriction sites (compare with Fig. 2).

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5. To isolate revertant p^{un} alleles, we mated individual p^{un}/p^{un} mosaic mice displaying ≥50% wild-type pigmentation to nonmosaic p^{un}/p^{un} sibs. Fully pigmented offspring from these matings were mated to a p^{un}/p^{un} sib and the resulting pigmented offspring were intercrossed to obtain homozygous revertants. The three revertant alleles described here (p^{un+1J}, p^{un+2J}, and p^{un+3J}) were produced from independent crosses. Homozygous revertant mice are phenotypically indistinguishable from wild-type mice. Revertant alleles are stable over many generations. All mice used in this study were obtained from, or produced at, The Jackson Laboratory, Bar Harbor, ME.
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12. A partial genomic library of Apa I fragments of 2.9 ± 0.1 kb was prepared in a vector derived from pUC18 and pUC19 (provided by J. Stone) that was engineered to accept Apa I fragments. Of the 52 clones isolated from this library that hybridized to the IAP DNA probe, 12 contained insert fragments indistinguishable in size from the p^{un}-enhanced fragment. Five of the 12 clones were identical independent isolates; the remaining seven clones were apparently diverse isolates. Only the cloned sequence common to the five identical isolates matched the IAP sequences and restriction sites of the p^{un}-enhanced fragment. The p28 clone was one of these five isolates.
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20. A 1-kb fragment of IAP DNA (Sst I–Bam HI) from clone IAP 81 [J. A. Moshier, R. A. Morgan, R. C. C. Huang, in *Interrelationships Among Aging, Cancer and Differentiation*, B. Pullman, Ed. (Reidel, Dordrecht, Holland, 1985), pp. 101–116] that was subcloned into phage M13 and termed p815/B1.0 was provided by R. C. C. Huang. The fragment was labeled with ^{32}P by the random primer method (*19*) to a specific activity of 1×10^8 to 5×10^8 cpm/μg.
21. High molecular weight genomic DNA was isolated from individual adult mice as follows: minced liver (0.4 to 0.5 g) was homogenized in a tight-fitting glass-Teflon homogenizer with 5 ml of ice-cold Iso-Hi-pH buffer [0.14 M NaCl, 0.01 M tris (pH 8.4), 1.5 mM $MgCl_2$] [U. Lindberg and J. E. Darnell, *Proc. Natl. Acad. Sci. U.S.A.* **65**, 1089 (1970)] containing 0.1% NP-40 and then centrifuged at 3000 rpm in an HB-4 rotor at 4°C for 5 min. The supernatant and loose material over the pellet were removed by aspiration. The pellet was homogenized in 0.8 ml of Iso-Hi-pH buffer containing 0.1% NP-40 at 0°C. While vortex-mixing the resuspended nuclear pellet, we added an equal volume of ice-cold Iso-Hi-pH buffer containing 0.1% NP-40, 2% SDS, and proteinase K (1 mg/ml) (the latter solution is a slurry; SDS precipitates at this temperature). The resulting mixture was incubated at 37°C for 2 to 3 hours. The nucleic acids were gently extracted twice with 25:24:1 phenol:chloroform:isoamyl alcohol, followed by gentle extraction with 24:1 chloroform:isoamyl alcohol. The aqueous phase was dialyzed (Spectra/pore 2, Spectrum Medical Industries) first against 3 liters of 20 mM tris (pH 7.5) and 10 mM EDTA and then against 3 liters of 5 mM tris (pH 7.5) and 1 mM EDTA (with three changes of the latter dialyzing solution) at room temperature. The DNA samples were stored at 4°C for no longer than 2 weeks; storage for longer periods at 4°C or freezing of the DNA resulted in high backgrounds on genome-scanning Southern blots, presumably because of a reduction in size of the DNA. (Preparation of genomic DNA in agarose plugs, as in pulsed-field gel electrophoresis applications, may offer an acceptable alternative.) Digestion of genomic DNAs was performed with restriction endonucleases (Bethesda Research Labs., New England Biolabs), and the digested samples were separated by electrophoresis through either 0.6% or 0.8% agarose gels (SeaKem GTG, FMC, Rockland, ME) in TBE buffer [90 mM tris-borate and 1 mM EDTA (pH 8.3)]; the buffer was circulated (anode to cathode) at a rate of 250 ml/hour. The bed dimensions of the horizontal gel apparatus (Dan-Kar, Reading, MA) were 20 cm (width) by 60 cm (length). For some experiments, the gels were cast in wedge shape (by propping up the box at the cathode end before the gel was poured; for example, gel thickness at cathode end of 5 mm, gel thickness at anode end of 1 cm, buffer depth over cathode end of 4 mm, and buffer depth over anode end of 4 mm). Electrophoretic separation was at 185 V for 20 to 30 hours. The DNA in the gels was transferred to Gene Screen Plus membranes (Du Pont). The filters were placed in sealed plastic bags, incubated at room temperature with 4× SSPE [1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$ (pH 7.7), and 1 mM EDTA] for 30 min and then at 65°C in 0.5 M $NaPO_4$ (pH 7.1), 2 mM EDTA, 7% SDS, and 0.1% sodium pyrophosphate [G. M. Church and W. Gilbert, *Proc. Natl. Acad. Sci U.S.A.* **81**, 1991 (1984)] for 4 to 8 hours, and hybridized at 65°C in a fresh sample of the latter buffer containing 2×10^6 to 4×10^6 cpm of DNA probe per milliliter for 24 to 36 hours. The filters were then sequentially washed (twice; 20 min per wash) in 800 ml of the following: 2× SSC (standard saline citrate) at room temperature; 2× SSC containing 0.1% SDS at 65°C; 1× SSC containing 0.1% SDS at 65°C; 0.5× SSC containing 0.1% SDS at 65°C; and 0.2× SSC containing 0.1% SDS at 65°C. The filters were blotted dry, wrapped in plastic wrap, and exposed for 24 to 36 hours to XAR-5 film (Kodak) with a Lightning Plus intensifying screen (Du Pont) at −70°C.
22. A plasmid clone, pHR3.0, containing 3 kb of the rat tyrosine hydroxylase gene {previously mapped to the distal region of mouse chromosome 7 [M. H. Brilliant, M. M. Neimann, E. M. Eicher, *J. Neurogenet.* **4**, 259 (1987)]; provided by D. M. Chikaraishi and E. J. Lewis} was labeled with ^{32}P by nick-translation [P. W. J. Rigby, M. Diekman, C. Rhodes, P. J. Berg, *J. Mol. Biol.* **113**, 237 (1977)] to a specific activity of $>10^8$ cpm/μg. The 28RN probe (Fig. 2) was labeled with ^{32}P by the random primer method (*19*) to a specific activity of 1×10^8 to 5×10^8 cpm/μg.
23. We thank S. A. Deveau, B. K. Lee, and L. L. Washburn for technical assistance; and E. H. Birkenmeier, E. L. Kuff, K. K. Lueders, R. W. Melvold, B. Mintz, A. M. Skalka, J. C. Stone, and H. G. Wolfe for helpful discussions. Supported by NIH grants GM43840, CA06927, and RR05539, by an appropriation from the Commonwealth of Pennsylvania (The Institute for Cancer Research), by NIH grants CA34196, GM20919, and RR01183, and by American Cancer Society grant 1N155 (The Jackson Laboratory).

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Determination of Primary Motoneuron Identity in Developing Zebrafish Embryos

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The developmental determination of primary motoneurons was investigated by transplanting identified motoneurons in embryonic zebrafish to new spinal cord positions. Some cells moved from the new positions in which they were placed back to their original positions, thus it was difficult to evaluate whether they were determined. Among cells that remained in their new positions, those transplanted about 1 hour before axogenesis developed axonal trajectories that were appropriate for their original soma positions, whereas those transplanted 2 to 3 hours before axogenesis developed morphologies appropriate for their new soma positions. These results suggest that motoneuronal identity is determined before axogenesis.

NERVOUS SYSTEMS CONSIST OF many different types of neurons that have characteristic shapes and patterns of synaptic connectivity. In some organisms, including many invertebrates (*1*) and the embryos of some fishes (*2*), certain neurons can be uniquely identified on the basis of morphological criteria such as soma position and axonal trajectory. When do the individual identities of these neurons become determined (*3*) so that their developmental potentials are restricted and are independent of their positions? To address this question, I transplanted identified motoneurons from their normal spinal cord positions in donor embryonic zebrafish to new spinal cord positions in host embryos (*4*). Transplanted motoneurons were considered undetermined if their axonal trajectories were appropriate for their new soma positions and determined if their trajectories were appropriate for their original soma positions. These studies suggest that the identities of individual primary motoneurons are determined before axogenesis.

The segmentally arranged axial muscles of the zebrafish trunk are innervated by a segmentally repeated set of three or four primary motoneurons (*5, 6*). Even before axogenesis, each motoneuron within this set is individually identifiable by the axial position of its soma within the spinal cord. Moreover, the growth cone of each motoneuron selects a different pathway as it extends directly to the muscle territory appropriate for its adult function (Fig. 1). Thus, within each set of motoneurons, each cell develops a unique morphology.

Previous studies have shown that individual primary motoneurons can be precisely identified and ablated with laser irradiation before axogenesis (*5, 7, 8*). To verify that individual primary motoneurons could be identified for transplantation with the same precision, I removed one or more specific primary motoneurons from trunk segments 6 to 10 of 16- to 18-h (hours after fertilization at 28.5°C) embryos. In trunk segments, CaP and MiP primary motoneurons typically undergo axogenesis around 17 h and 18 to 19 h, respectively; therefore, these experiments were performed ~1 hour before axogenesis. Embryos were allowed to develop until 25 h and were then fixed, and the remaining primary motoneurons were labeled with the zn-1 monoclonal antibody (*7*). In every case, the primary motoneurons that had been removed were absent (Fig. 1) (*9*). In all but one instance, all of the other primary motoneurons in the spinal segment were present at 25 h.

To investigate whether the individual identities of primary motoneurons were determined before axogenesis, I transplanted identified motoneurons isochronically from the CaP and MiP positions ~1 hour before axogenesis (Figs. 1 and 2) (*10*). No attempt

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