PAX6, Paired Domain Influences Sequence Recognition by the Homeodomain*

Rajnikant Mishra, Ivan P. Gorlov, Lian Y. Chao, Sanjaya Singh, and Grady F. Saunders‡

From the Department of Biochemistry and Molecular Biology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030

Received for publication, June 28, 2002, and in revised form, October 10, 2002
Published, JBC Papers in Press, October 17, 2002, DOI 10.1074/jbc.M206478200

PAX6 functions as a transcription factor and has two DNA-binding domains, a paired domain (PD) and a homeodomain (HD), joined by a glycine-rich linker and followed by a proline-serine-threonine-rich (PST) transactivation region at the C terminus. The mechanism of PAX6 function is not clearly understood, and few target genes in vertebrates have been identified. In this report we described the functional analyses of patient missense mutations from the paired domain region of PAX6 and a paired-domain-less isoform (PD-less) of Pax6 that lacks the paired domain and part of the glycine-rich linker. The PD-less was expressed in the brain, eyes, and pancreas of mouse. The level of expression of this isoform was relatively higher in brain. The mutation sites PAX6-L46R and -C52R were located in the PD of PAX6 on either end of the 5a-polypeptide insert of the alternatively spliced form of PAX6, PAX6-5a. Another PAX6 mutant V53L described in this report was adjacent to C52R. We created corresponding mutations in PAX6 and PAX6-5a, and evaluated their transcriptional activation and DNA binding properties. The PD mutants of PAX6 (L46R, C52R, and V53L) exhibited lower transactivation activities and variable DNA binding ability than wild-type PAX6 with PD DNA-binding consensus sequences. The mutated amino acids containing PAX6-5a isoforms showed unexpected transactivation properties with a reporter containing HD DNA-binding sequences. PAX6-5a-C52R, and -V53L showed lower transactivation activities, but PAX6-5a-L46R had greater transactivation ability than PAX6-5a. The PD-less isoform of Pax6 lost its transactivational ability but could bind to the HD DNA-binding sequences. Functional analysis of the PD-less isoform of Pax6 as well as findings related to missense mutations in the PD suggest that the PD of PAX6 is required for HD function.

PAX6 is considered to be the master control gene for morphogenesis of the eye. It is an evolutionarily conserved gene in both vertebrates and invertebrates (1–6). PAX6 functions as a transcription factor, and complete loss of PAX6 function leads to anophthalmia and nasal hypoplasia, as well as to central nervous system defects that cause postnatal death (2, 7, 8). It is required for lens placode formation, growth of the lens (9), correct placement of a single retina in the eye (10), formation of the iris, maintenance of the corneal epithelium, and fate of retinal progenitor cells (11). It has two DNA-binding domains, paired domain (PD)1 at the N terminus, a paired-like homeodomain (HD) linked by a glycine-rich domain and a transactivation region (PST), which is rich in proline, serine, and threonine amino acids at the C terminus of the PAX6 protein. Biochemical and crystallographic studies have shown that the PD actually consists of independent N-terminal (N subdomain) and C-terminal subdomains (C subdomain), which have significant physiological roles in target DNA recognitions (12–17). Crystal structure of the human PAX6 PD-DNA complex revealed specific roles for the linker region of the PD and C subdomain in DNA binding (18). Amino acid residues of PAX6 are highly conserved in almost all the PD, and various missense mutations have been reported (www.hgu.mrc.ac.uk/Soft-data/PAX6) in its domains (19–31). An alternatively spliced form of PAX6, known as PAX6-5a, contains an additional 14 amino acids in the PD (2), which disrupts the N subdomain and alters DNA binding properties (13, 32). Loss-of-function of PAX6-5a in mouse leads to iris-hypoplasia and also defects in the retina, lens, and cornea (33). The PDs of other genes of the Pax family, the Pax2, Pax3, and the binding sequence of Pax-5 (BSAP), and Pax6 proteins can recognize similar DNA sequences (12, 34–39). Our earlier studies showed that the transactivation function is distributed throughout the PST region (40). Although the involvement of PAX6 in ocular development is well known (41, 42), identification of target genes of PAX6 has been hindered by two factors. First, the PD of Pax6 can bind to a broad range of DNA sequences. Second, HDS in other proteins recognize similar sequences, as does the Pax6 PD (43).

In humans, heterozygous mutations in the PAX6 gene are responsible for several phenotypes, including aniridia, foveal hypoplasia, Peters’ anomaly, ectopia pupillae, and autosomal dominant keratitis (2, 19, 25, 26, 44, 45). Pax6 mutation in rodents results in small eye (46), which, as in humans, appears to be expressed as highly variable phenotypes. However, it is not clear how mutation of the PAX6 gene results in these various phenotypes, and why the phenotypes are of variable expressivities. Functional studies using PAX6 missense mutations with observed developmental defects are useful in understanding the mechanism of PAX6 function. Missense mutations in the DNA-binding domains of PAX6 may cause failure to properly recognize binding sites in the target genes, resulting in partial or complete loss of protein function (40, 47–49).

In this report, we describe the functional properties of three missense mutations in the PD domain of PAX6 that were identified in the DNA of patients with aniridia, and a PD-less

* This research was supported by National Institutes of Health Grants EY09675, EY10608, and CA 16672. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, Box 117, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030. Tel.: 713-792-2690; Fax: 713-791-9478; E-mail: gsaunders@odin.mdacc.tmc.edu.
isofrom of PAX6 from mouse. Since patients with aniridia also have the PAX6-5a isoform, and PAX6-5a was found to be important in maintaining the normal structure of eyes in adult mice (33), these mutations on either end of the 5a-polypeptide insert have been used to examine the role of the PAX-6a isoform. The results indicate that the functional properties of PAX6 and the PAX6-5a isoform are unique, and PD of PAX6 is required for the function of HD.

**EXPERIMENTAL PROCEDURES**

**Mutation Detection and Luciferase Reporter Constructs**—To identify mutations in the PAX6 gene, genomic DNA samples were isolated from peripheral blood lymphocytes of patients with aniridia. Each exon and its immediate flanking sequence was amplified by PCR with primer sets described previously (50). Gel analysis and sequence confirmation were performed as described earlier (26). The missense mutations in PAX6 and PAX6-5a were introduced by site-directed mutagenesis using the QuikChange kit (Stratagene). All constructs were assessed by automated sequencing to ensure that no random mutations were introduced during PCR. Construction of the CD19-2(A-ins)-luciferase and P2-luciferase reporter plasmids has been described previously (47). CD19-2(A-ins) (5'-TACTCGAGCTGGGACTGAGGTGCAATCTCTGACATCCGATCGGG-3') and P2 (5'-GGGCACTGAGGCGTGACCA TGTAATGATTAGCATCCGATCGGG-3') are consensus DNA-binding sequences of PAX6 and PAX6-5a.

**Cell Culture and Transfections**—NIH/3T3, a murine fibroblast cell line, was maintained in Dulbecco’s minimal essential medium supplemented with 10% fetal calf serum. Transfections were performed with plasmid DNA coated with the polycationic lipid LipofectAMINE (Invitrogen) according to the manufacturer’s instructions. Each well in a 12-well plate was transfected with 0.2 μg of P2-luciferase or CD19-2 (A-ins)-luciferase reporter plasmid, 0.3 μg of pRc-CMV effector plasmids (containing wild-type or mutant PAX6 and/or PAX6-5a cDNA), and 0.05 μg of pSV2β-gal plasmid (Promega Corp.) with the pRc-CMV construct containing wild-type or mutant PAX6 and/or PAX6-5a cDNA, and 0.05 μg of pSV2β-gal plasmid (Promega Corp.) as an internal control. An equivalent amount of vector plasmid pRc-CMV was used as a vector control. The luciferase activity of the reporter construct is shown as the mean ± S.D. of three separate experiments. A, differential transactivation to the luciferase reporter gene containing target DNA sequences CD19-2(A-ins) by PAX6 and PAX6-5a. B, differential transactivation by the PAX6, PAX6-5a, and mutants with the luciferase reporter. C, EMSA was performed to compare the DNA binding ability of wild-type and mutant proteins of the PAX6 and PAX6-5a. Double-stranded oligodeoxynucleotides (CD19-2(A-ins)) was end-labeled with [γ-32P]ATP. The binding reactions were carried out as described under “Experimental Procedures.”

Western Blotting and EMSAs—Crude nuclear extracts were prepared from transfected NIH/3T3 cells as previously described (51). In vitro-transcribed and -translated proteins were generated using the transcription and translation (TnT)-coupled reticuloocyte lysate system (Promega Corp.) with the pRc-CMV construct containing wild-type or mutant PAX6 and/or PAX6-5a cDNA. The amount of PAX6 protein expressed or translated was quantitated by Western blot analysis with antibodies against the linker region of PAX6. The bands on the Western blots were quantitated using a Personal Densitometer Scanner 1.30 and Image Quant 3.3 software (Molecular Dynamics). Occasionally, quantitation of in vitro synthesized [35S]-labeled proteins were further confirmed by drying SDS-PAGE gels and analyzing by using a Storm 840 PhosphorImager (Molecular Dynamics) and Image Quant 5.0 software (Molecular Dynamics).

EMSA was performed to compare the DNA binding ability of the PAX6 and PAX6-5a isoforms proteins bearing patient mutations. Double-stranded oligodeoxynucleotides (CD19-2(A-ins)) (5'-TACTCGAGCTGGGACTGAGGTGCAATCTCTGACATCCGATCGGG-3'), P2 (5'-GGGCACTGAGGCGTGACCA TGTAATGATTAGCATCCGATCGGG-3'), P6CON (5'-GGGAATTCAGGAAAAATTTTTCGCGTTGATCACAGC TCGAG-3') (13, 34), Pax-5 (BSAP) Gel Shift oligonucleotides (sc-2589 from Santa Cruz Biotechnology) (5'-GAATGGGGCACTGAGGCGTGA CCACC-3') (38) and ZPE (ζ-protected element) having PD consensus

**FIG. 1.** A, the diagram of the PAX6 protein with missense mutations described in the report. B and C, Western blot analyses were performed to verify the amount of protein expressed by various expression constructs: in vitro translated (B), and nuclear extracts (C) (10 and 20 μl) from cells transfected with 1 μg of expression plasmids were resolved on 10% SDS-PAGE and immunoblotted using anti-PAX6 antibodies. The results showed that all the proteins are expressed and are stable.

**FIG. 2.** Analysis of PD function. NIH/3T3 cells were transiently transfected with 0.2 μg of CD19-2 (A-ins)-luciferase reporter plasmid, 0.3 μg of pRc-CMV effector plasmids (containing PAX6 and/or PAX6-5a cDNA) either wild-type or mutant, and 0.05 μg of pSV2β-gal plasmid (Promega Corp.) as an internal control. An equivalent amount of vector plasmid pRc-CMV was used as a vector control. The luciferase activity of the reporter construct is shown as the mean ± S.D. of three separate experiments. A, differential transactivation to the luciferase reporter gene containing target DNA sequences CD19-2(A-ins) by PAX6 and PAX6-5a. B, differential transactivation by the PAX6, PAX6-5a, and mutants with the luciferase reporter. C, EMSA was performed to compare the DNA binding ability of wild-type and mutant proteins of the PAX6 and PAX6-5a. Double-stranded oligodeoxynucleotides (CD19-2(A-ins)) was end-labeled with [γ-32P]ATP. The binding reactions were carried out as described under “Experimental Procedures.”
binding sequences (5'-TGCATCTTTTTAGGATGCATCATTGCTAAAC-CATCCGTGCAAATGCACTGC-3') (52) were end-labeled with [γ-32P]ATP. The binding reactions were carried out as described under "Experimental Procedures."

RESULTS

In this report, we describe the functional analyses of three missense mutations from aniridia patients detected in the PD region of PAX6 and a PD-less isoform of Pax6 from mouse. The results indicate that the functional properties of Pax6 and the Pax6-5a isoform are unique and PD of Pax6 is required for the function of HD.

Plasmid Constructs and Expression Analysis of the PD-less Isoform—

The expression vector with the PD-less isoform of Pax6 was constructed using the PCR approach. PCR products corresponding to the coding region of the PD-less isoform were cloned into a modified pcDNA3 vector (53) harboring the 5'-untranslated region of the herpes simplex virus-thymidine kinase gene. To amplify specifically the PD-less isoform we designed primers that locate the junction between exons 3 and 8: PD-lessF, 5'-CAAAACTCTTGACAGGAAGGAG-3' and reverse primer from exon 12: Pax612R, 5'-ACTTGGACGGGAACTGACAC-3'. These two primers allowed us specifically to amplify the PD-less isoform. RT-PCR products (~500 bp) were detected in the brain, eye, and pancreas. Identity of the band to the Pax6 sequence was confirmed by sequencing. The GenBank accession number of the PD-less isoform is AY064175.

Fig. 3. EMSA for PD function. EMSA was performed to compare the DNA binding ability of the PAX6 and PAX6-5α wild-type and mutant proteins. Double-stranded oligodeoxynucleotides P6CON (A) (13, 34), BSAP (B) (38), and ZPE (C) (52) were end-labeled with [γ-32P]ATP. The binding reactions were carried out as described under "Experimental Procedures."
polarity and conformation of the proteins. The missense mutation L46R causes substitution of a positively charged amino acid, arginine, in place of nonpolar (i.e. hydrophobic) leucine. The patient has atrophy of both pupil and iris. This was a familial case where the mother had congenital cataracts and nystagmus, maternal grandmother had bilateral cataracts, and a misshaped pupil. In the case of missense mutation C52R there was substitution of a positively charged amino acid arginine in place of an uncharged and polar cysteine amino acid residue. The patient had aniridia and cataracts. This was also a familial case, and the mother had juvenile cataracts and glaucoma. 2 Another highly conserved amino acid residue of PAX6 valine, is replaced by a bulky leucine without a net charge difference in the case of the missense mutation PAX6-5a C52R. The patient had moderate photophobia (24) (Fig. 1A). The property of binding site recognition by PAX6 or PAX6-5a proteins was stable as analyzed by Western blot analyses (25) of in vitro translation (Fig. 1B) or in nuclear extracts (Fig. 1C) prepared after transfection with expression constructs of wild-type and mutant PAX6 and PAX6-5a cDNA.

Analysis of Transcriptional Activation and DNA Recognition Properties of PD Mutants—To assess the functional significance of the missense mutations in the different domains of PAX6, we compared the mutant and wild-type PAX6 proteins by transcriptional activation assays and DNA binding abilities. NIH/3T3 cells were co-transfected with PAX6 or mutant PAX6 expression plasmids with a luciferase reporter plasmid bearing two copies of either the PD-binding consensus sequences (CD19-2(A-ins)) or the HD-binding consensus sequences (P2). The property of binding site recognition by PAX6 or PAX6-5a was tested by EMSA with consensus binding sequences of PD (CD19-2A), P6CON, (13, 34), ZPE (52), and BSAP (38) or HD (P2) to compare the DNA binding ability of the PAX6 and PAX6-5a isoform proteins bearing patient mutations.

Analysis of PD Function—When the reporter plasmid containing the PD-binding consensus sequence (CD19-2(A-ins)) was used, wild-type PAX6 could activate the reporter but PAX6-5a had basal levels of activity (Fig. 2A). The ability of the PAX6-L46R, -C52R, and -V53L mutants to activate transcription of the reporter gene was significantly lower than that of the wild-type PAX6, and PAX6-5a or PAX6-5a mutants failed to activate the luciferase reporter with (CD19-2(A-ins)) (Fig. 2B). When CD19-2(A-ins) was used as a probe for EMSA, PAX6 mutants showed variable binding ability. PAX6-L46R and -C52R failed to bind, and PAX6-V53L showed about 50% lower binding ability than wild-type PAX6. PAX6-5a and mutants showed no binding to CD19-2(A-ins) (Fig. 2C). Unlike CD19-2(A-ins) PAX6-L46R showed 40% lower binding to P6CON, PAX6-C52R did not bind, and PAX6-V53L has similar binding ability compared with wild-type PAX6. PAX6-5a and mutants failed to bind P6CON (Fig. 3A). When the BSAP was used as a probe to test the DNA binding capability, PAX6 had binding ability, but the PAX6-5a and mutants failed to bind this consensus sequence. (Fig. 3B). Like CD19-2(A-ins) PAX6 could bind to ZPE, but mutants PAX6-L46R and -C52R failed to bind, and -V53L showed 50% lower binding than the wild-type PAX6 (Fig. 3C).

Analysis of HD Function—Both PAX6 and PAX6-5a could transactivate the reporter containing P2, and the level of activity by the PAX6-5a was about 50% of the wild-type PAX6 (Fig. 4A). Some of the missense mutations in the PD and PST domains also affect HD functions. Activation of a reporter containing the HD-binding sequences (P2) by PAX6 mutants V53L was significantly lower than that of wild-type PAX6. Interestingly mutants C52R and V53L of the PAX6-5a isoform lost their transactivation abilities when used to activate the reporter containing P2, whereas PAX6-5a-L46R exhibited greater transactivation ability than normal PAX6-5a (Fig. 4B). When P2 was used as a probe, PAX6 and PAX6-5a mutant proteins had similar HD binding capabilities (Fig. 4C).

Analysis of the PD-less Isoform of PAX6—A new alternatively spliced isoform of PAX6 (PD-less) (AY064175) was isolated from mouse brain cDNA. This isoform of PAX6 lacks the promoter 1-specific exon as well as common exons 2, 4–7 and the first 21 nucleotides of exon 8 (Fig. 5A). This isoform resulted from the use of a cryptic acceptor splice site in exon 8 as well as the normal donor/acceptor sites in exon 2(P1) and the normal donor site in exon 3. Conceptual translation of the sequence generated a 221-amino acid protein lacking the paired domain and part of the glycine-rich linker (Fig. 5B). The PD-less isoform of PAX6 was found to be expressed in brain, eyes, and pancreas of mouse. The level of expression of this isoform was relatively

---

2 Chao, L. Y., Mishra, R., Strong, L. C., and Saunders, G. F. (2002) Hum. Mutat., in press.
higher in brain (Fig. 5C). Translation of this expression vector in cultured cells show that the PD-less isoform is stable (Fig. 5, D and E).

To further verify that both PD- and HD of the Pax6 act cooperatively in the induction of gene expression, we performed the functional analysis of the PD-less isoform of the Pax6. If HD and PD of Pax6 act independently, then one can expect that the removal of the paired domain should not affect the activities of the HD. However, the PD-less isoform of Pax6 fails to activate the reporter containing HD DNA-binding sequences (P2) (Fig. 5F). The PD-less isoform of Pax6 could bind to P2 (Fig. 5G) but failed to bind PD DNA-binding consensus sequences P6CON, CD19-2(A-ins) and ZPE compared with normal Pax6 (Fig. 6, A–C).

**DISCUSSION**

The DNA-binding domains of PAX6 are highly conserved among animal species. The diverse functions of Pax6 appear to originate from both the complex regulatory mechanisms controlling the tissue-specific transcription and splicing as well as its ability to participate in multiple molecular interactions (54). Missense mutations in the DNA-binding domains of PAX6 may result in failure to properly recognize the binding sites in normal target genes, thus causing partial or complete loss-of-function. The loss-of-function may also be due to impaired interaction between DNA-binding domains or failure of cofactor-mediated functions. In order to understand the mechanism of PAX6 function we performed functional analysis of missense mutations found in the PD region of PAX6 and alternatively spliced form of PAX6, PAX6-5a, of aniridia patients (Fig. 1). We also performed functional analyses of a PD-less isoform of Pax6 (AY064175), which was found to be expressed in the brain, eyes, and pancreas of mice. The level of expression of PD-less isoform was relatively higher in brain (Fig. 5C).

Missense mutations described in this paper (PAX6 and PAX6-5a) -L46R, -C52R, and -V53L) are found in the DNA-binding region of PD and located on either end of the 5a-polypeptide insert in the PAX6-5a isoform. The expressed proteins were found to be stable (Fig. 1, A–C). Among the mutations in the N subdomain of PD, L46R is present in the /H9251 2-helix, and C52R and V53L are located in /H9251 3-helix. This N subdomain uses a helix-turn-helix (HTH) unit to dock against the major groove at one end of the binding site (18). As compared with wild-type PAX6, the PAX6-5a isoform could not transactivate the luciferase reporter gene containing target DNA-binding consensus sequences CD19-2(A-ins) for PD (Fig. 2A). PAX6 mutants PAX6-L46R, -C52R, and -V53L showed significantly lower transactivation ability than wild-type PAX6 (Fig. 2B) with luciferase reporter containing CD19-2(A-ins). EMSA results with CD19-2(A-ins) showed that PAX6-L46R and -C52R lost their binding abilities, and -V53L showed significantly lower binding ability than wild-type PAX6 (Fig. 2C). PAX6-5a and its respective mutants did not show transactivation or binding ability with the PD target sequences as insertion of the 5a-polypeptide disrupts its PD binding capability (13, 32). These mutants showed variable DNA binding abilities.
with other Pax6-binding consensus sequences like P6CON, BSAP, and ZPE (Fig. 3, A–C).

The mutated amino acids of PAX6 described here are highly conserved within the PD and are present in the DNA-binding region of the PD. The missense mutation L46R causes substitution of a positively charged amino acid, arginine, in place of nonpolar leucine, and C52R leads to substitution of a positively charged arginine in place of an uncharged and polar cysteine amino acid residue. These two mutations impaired both DNA binding and transactivation abilities of PAX6. In the case of the V53L missense mutation (24) valine was replaced by the bulky amino acid residue leucine, but the net charge remained the same. The PAX6 mutants L46R, and C52R might have lost their activity as they were unable to bind the DNA, while the V to L substitution at codon 53 retained binding ability but turned the protein into a transcriptional repressor or perhaps lost the conformation used to recruit the all co-factors used for transactivation. The variable properties of these PD mutants led to analysis of their HD function.

The PAX6-5a isoform had about 50% lower transactivation ability compared with the wild-type PAX6 (Fig. 4A) with the luciferase reporter containing HD-binding consensus sequences (P2). The missense mutations in the PD of PAX6 and PAX6-5a showed some unusual transactivation properties (Fig. 4B) with the P2-containing luciferase reporter. These mutants have almost identical HD DNA binding abilities (Fig. 4C) to that of wild-type PAX6 and PAX6-5a, but they show variable transactivation properties. The variable functional properties of PAX6 mutant may be one of the factors for variable phenotype of aniridia patients. PAX6 PD mutant V53L had significantly lower transactivation ability than wild-type PAX6. Thus PD mutant V53L affects both PD and HD functions. Interestingly, PAX6-5a-L46R showed a significantly higher transactivation ability than wild-type PAX6-5a. However, it behaves like wild-type PAX6 as far as HD-related transactivation is concerned. Furthermore, PAX6-5a-C52R, and -V53L mutants showed significantly lower transactivation activities.

The PD-less isoform of PAX6 fails to activate the reporter containing HD DNA-binding sequences (P2) (Fig. 5F) despite its binding ability to the P2 probe (Fig. 5G). It failed to bind the PD DNA-binding consensus sequences P6CON, CD19-2A-ins, and ZPE as compared with normal Pax6 (Fig. 6, A–C). These results indicate that the HD can bind to DNA but requires the PD for its transactivation function. Additionally, it may have some special regulatory role in the brain, eyes and pancreas as evident from its expression pattern (Fig. 5C). A similar paired-less isoform was identified earlier in two phylogenically distinct species, quail (55) and Caenorhabditis elegans (56), and these findings suggest some normal function for the PD-less isoform (57). If HD and PD of Pax6 act independently, then one can expect that the removal of the paired domain should not affect the activities of the HD.

These findings, at the level of in vitro DNA binding and transactivation assays, indicate a complex state of regulation of PAX6 function. The PD mutant V53L affects HD function. PAX6-5a mutants (L46R, C52R, and V53L) also alter activity with HD. These findings support our previous ideas of PD-dependent HD function (51, 58) and are in agreement with a possible cooperative interaction between the PD and HD of PAX6 (59). Recently intradomain and interdomain interactions in PAX6 have also been reported (15, 42) that support the concept that the PD and HD of PAX6 physically interact. Two possibilities of how the PD can influence the function of the HD are the following. Gain-of-function suggests that normally the PD does not interact with the HD and mutations in the PD result in misfolding of the PAX6 protein such that the PD interferes with HD function. Alternatively, loss-of-function suggests that PD and HD of PAX6 physically interact, and mutations within the PD affect the function of HD. Although the different domains of PAX6 are well delineated, their functions appear to be mutually dependent, and the PD of PAX6 is required for HD function.

Acknowledgments—We thank Lindsey Middleton and Dr. Louise Strong for clinical material. We also thank Drs. R. Mass and M. Busslinger for the recombinant vectors. The DNA sequencing work was done by the core sequencing facility at the M. D. Anderson Cancer Center.

REFERENCES

1. Ton, C. C., Hirvonen, H., Miwa, H., Weil, M. M., Monaghan, P., Jordan, T., van Heyningen, V., Hastie, N. D., Meijers-Heijboer, H., Drechsler, M., Royer-
