The EMBO Journal vol.12 no.3 pp.911-919, 1993

Genetic and molecular identification of a *Drosophila* histidine decarboxylase gene required in photoreceptor transmitter synthesis

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Communicated by M.Heisenberg

***Drosophila* mutants of a single complementation group with defective on-/off-transients of the electroretinogram (ERG) were found to be deficient in synthesis of the photoreceptor transmitter, histamine, in a gene-dosage dependent manner, suggesting that the gene identified by the mutants (*hdc*) might be the structural gene for *Drosophila* histidine decarboxylase (HDC). A rat HDC cDNA was used to isolate a *Drosophila* homolog which shows ~60% sequence identity with mammalian HDCs over a region of 476 amino acids. In RNA blots, the *Drosophila* homolog detects four transcripts that are expressed primarily in the eye and are severely reduced in *hdc* mutants. The cloned *Drosophila* cDNA hybridizes to the 46F region of the chromosome, to which *hdc* mutations have been mapped, and rescues the *hdc* mutant phenotype in transgenic flies generated by P element-mediated germline transformation. The results thus show that the *Drosophila* homolog corresponds to the histidine decarboxylase gene, identified by the *hdc* mutants, and that mutations in the gene disrupt photoreceptor synaptic transmission.**

Key words: Drosophila/histidine decarboxylase gene/mutant/photoreceptor/synaptic transmission

Introduction

A large number of *Drosophila* mutations that cause defects in the electroretinogram (ERG) (extracellularly recorded, light-evoked, mass response of the eye) have been isolated for the purpose of studying photoreceptor function (Pak *et al.*, 1969; Pak, 1975, 1979). One of the largest classes of mutations (over 30 complementation groups) in this collection consists of those that cause the on- and/or off-transient of the ERG to be defective while leaving the other components largely unaffected (see Figure 1A and B). The on-/off-transients of the ERG are thought to reflect activities of the postsynaptic neurons in the lamina in response to input from photoreceptors (e.g. Coombe, 1986). It has therefore been hypothesized that many of the transient-defective mutants are defective in synaptic transmission between the photoreceptors and their target neurons in the lamina (Pak, 1991).

Several lines of biochemical, immunocytochemical and physiological evidence suggest that histamine is the major transmitter used by invertebrate photoreceptors (Elias and Evans, 1983; Hardie, 1987, 1988; Nässel *et al.*, 1988, 1990; Pirvola *et al.*, 1988; Simmons and Hardie, 1988; Callaway and Stuart, 1989; Hardie, 1989; Schlemermeyer *et al.*, 1989; Battelle *et al.*, 1991; Pollack and Hofbauer, 1991). In *Drosophila* also, immunocytochemical and biochemical data have shown that histamine is a major transmitter used by all photoreceptors in the compound eye (Sarthy, 1991).

In both invertebrates and vertebrates, conversion of histidine to histamine is catalyzed by pyridoxal phosphate (PLP)-dependent histidine decarboxylase (HDC). A substantial body of information is available on mammalian HDCs. Recently, cDNAs encoding rat and human HDC have been cloned and sequenced (Joseph *et al.*, 1990; Yamauchi *et al.*, 1990). Little is known as yet about invertebrate HDCs. Neither HDC nor any gene encoding HDC has yet been isolated in invertebrates. Available evidence suggests, however, that invertebrate HDC has similar pharmacological properties to vertebrate HDC (*Aplysia*: Weinreich and Rubin, 1981; *Drosophila*: Sarthy, 1991; vertebrates: Kollonitsch *et al.*, 1978; Garbarg *et al.*, 1980).

The present work was undertaken in an attempt (i) to identify the gene encoding HDC, involved in the synthesis of photoreceptor transmitter, by screening a large collection of mutants defective in the on-/off-transients of ERG and (ii) to isolate the gene. If many of the transient-defective mutants are, indeed, defective in the photoreceptor synaptic process, as has been proposed (Pak, 1991), represented among the collection of mutants might be those that are defective in the synthetic enzyme for the photoreceptor transmitter. Thus, successful attainment of the above objectives would (i) provide direct support for the notion that many of the transient-defective mutants have defects in the genes encoding proteins directly involved in synaptic transmission, (ii) lend strong genetic support to the suggestion that histamine is a major transmitter used by *Drosophila* photoreceptors, (iii) identify mutants in the HDC structural gene and (iv) provide the first molecular data on an invertebrate HDC. In this report, we present evidence for the identification and cloning of the histidine decarboxylase gene of *Drosophila*.

Results

Identification of HDC-defective mutants

Thirty transient-defective mutants, each from a different complementation group, were rapidly screened by radiometric assay using isolated fly heads (Sarthy, 1991) to identify those that were defective in histamine synthesis. This screen identified one complementation group, consisting of four mutants (*P211*, *P217*, *P218* and *JK910*), which consistently showed very low levels (10-fold less than wild-type) of histamine synthesis (Sarthy *et al.*, 1991).

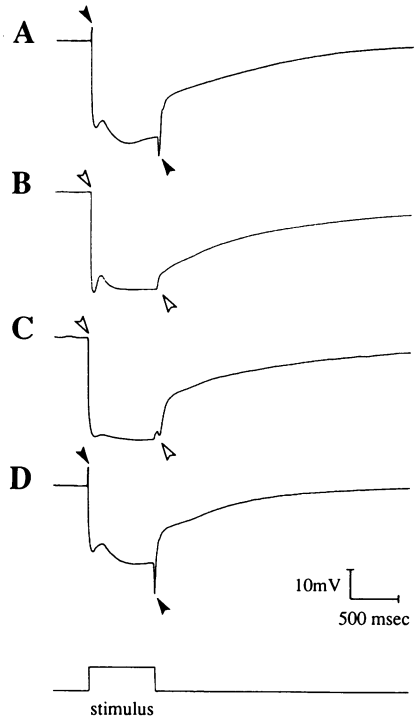

Fig. 1. Electroretinograms (ERGs) recorded from wild-type (**A**), hdc^{P211} mutant (**B**) and sibling transgenic flies (**C** and **D**), which carry the candidate HDC cDNA driven by the *hsp70* heat-shock gene promoter in a hdc^{P211} mutant background, before (C) and after (D) heat induction. The on-/off-transients are present in wild-type ERG (A, filled arrowhead) but absent in the hdc^{P211} ERG (B, open arrowhead). The transients are also absent in the transformant before heat induction (C, open arrowhead) but are fully restored in a sibling transformant after heat induction (D, filled arrowhead). The bottom trace indicates the white light stimulus delivered from a tungsten lamp attenuated by 2 log units.

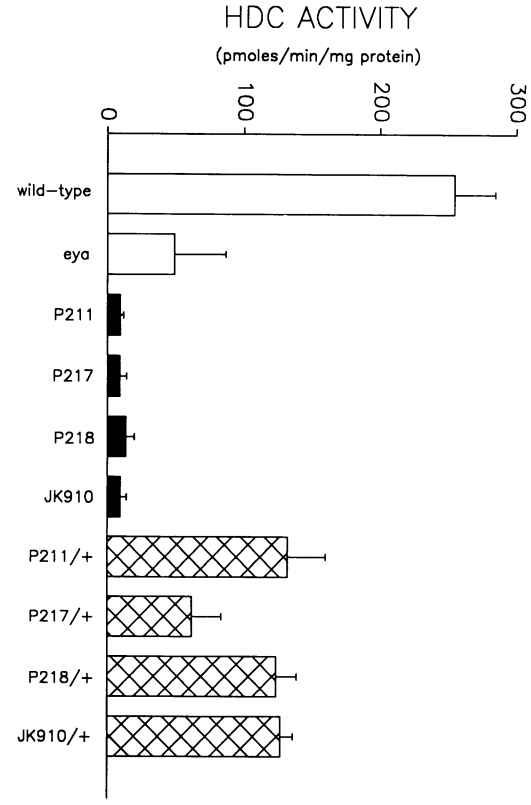

Fig. 2. HDC activity in four *hdc* allelic mutants. Histidine decarboxylase activity was assayed in head homogenates and expressed as pmol/min/mg protein. Error bar, S.D.; *eya*, *eyes absent*; unfilled bars, controls; filled bars, homozygous mutants; hatched bars, heterozygotes.

A quantitative, enzymatic assay was carried out on head extracts of all four mutants of this complementation group to determine the level of HDC activity in each (Sarthy, 1991). HDC activity was markedly lower in all four mutants than in wild-type (Figure 2). In *Drosophila*, the gene dosage effect, i.e. dependence of the amount or activity of a protein on the dosage of the gene, has been found to be a reliable indicator of structural genes (reviewed by O'Brien and MacIntrye, 1978). To obtain an indication of gene dosage effect, HDC activity was examined in flies heterozygous for each of the four mutant alleles. Flies heterozygous for three of the alleles (*P211*/+, *P218*/+ and *JK910*/+) displayed ~50% of the wild-type activity, while the remaining heterozygote, *P217*/+, showed ~25% of the wild-type activity (Figure 2). These results suggest that the gene corresponding to this complementation group does exhibit a gene dosage effect on HDC activity. Accordingly, the gene has been designated *hdc* (histidine decarboxylase).

ERG phenotype and map position

The *hdc* mutants are characterized by a defect in the on-/off-transients of the ERG. The transients are present in wild-type (Figure 1A) but missing in the hdc^{P211} (Figure 1B) and hdc^{JK910} (not shown) mutants. The remaining two *hdc* mutants, hdc^{P217} and hdc^{P218}, exhibit small on- and off-transients under very bright stimulus conditions (data not shown), suggesting that these two are less severely affected than the first two.

Using the ERG phenotype to detect the mutation, the *hdc* locus has been meiotically mapped ~3.6 cM to the right of *Bristle* (*B1*) (Lindsley and Grell, 1968) at map position 58.4 of chromosome 2. Deficiency mapping, which utilized the hdc^{P211} mutant and two deficiencies having distal breakpoints between 46E and 47A1 (Figure 3; see Materials and methods), placed the gene in the 46E-F region of the right arm of chromosome 2.

Isolation and identification of Drosophila HDC cDNA clones

Screening a *Drosophila* head cDNA library with a rat HDC cDNA clone (Joseph *et al.*, 1990) yielded 36 positively hybridizing clones falling into three non-overlapping groups (see Materials and methods). Chromosomal *in situ* hybridizations using the largest cDNA clone from each group showed that the three groups hybridized to 37C, 43E and 46F regions of polytene chromosomes, respectively. The clones mapping to 37C were not studied further because the Dopa decarboxylase gene, which is known to have homology with the probe (Joseph *et al.*, 1990) has been localized to this region (Hirsh and Davidson, 1981). Since deficiency mapping of *hdc* mutants placed the *hdc* gene at 46E-F, the clones mapping to 46F probably correspond to the *hdc* gene. The following RNA blot analyses were carried out to ascertain that this is the case.

Figure 4 shows an RNA blot of wild-type and mutant tissues probed with the largest cDNA from the 46F group. The probe recognized transcripts of 3.7, 3.2, 2.9 and 1.6 kb in wild-type heads. These transcripts were either missing

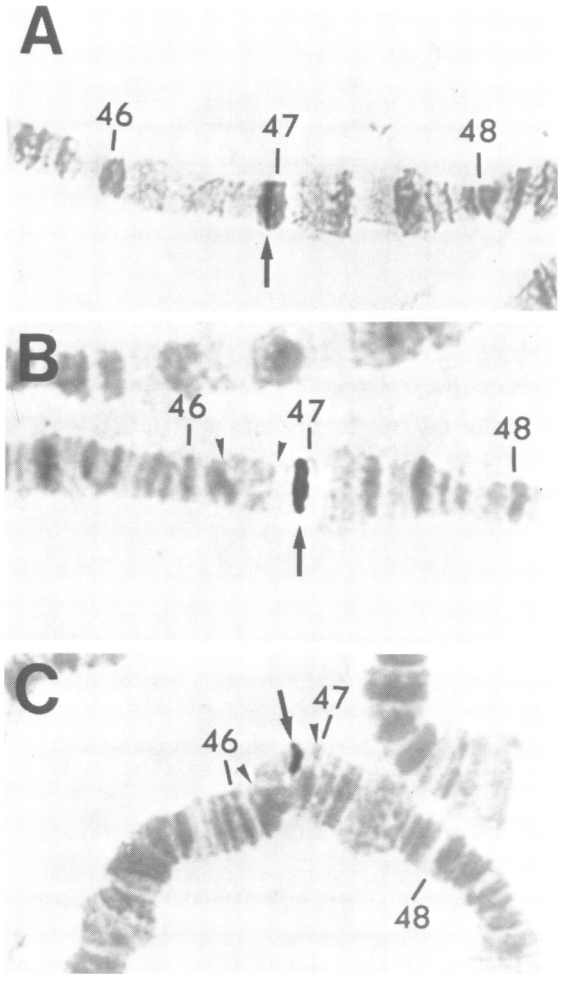

Fig. 3. *In situ* hybridization of *Drosophila* HDC cDNA to polytene chromosomes. The biotin-labeled HDC cDNA was hybridized to squashed salivary gland chromosomes of wild-type larvae (**A**) and of larvae carrying heterozygous deficiency chromosomes, *Df(2R)X3/+* (**B**) and *Df(2R)X1/+* (**C**). The cDNA hybridizes to the second chromosome at position 46F5-9 in all three cases (arrow). Note, however, that in the *Df(2R)X1/+* chromosome, only a half-width hybridization signal is present (C), while in the *Df(2R)X3/+* chromosome, the hybridization signal extends over the full width of the chromosome (B). The results show that the deficiency *Df(2R)X1* extends into and beyond the labeled bands, while the deficiency *Df(2R)X3* lies entirely outside of them. In both (B) and (C), the upper half of the chromosome is the wild-type homolog, and the bottom half, the deficiency-bearing homolog. Arrowheads in (B) and (C) indicate the extent of each deficiency. Numbers identify the beginning of divisions 46, 47 and 48 of the salivary gland chromosome map.

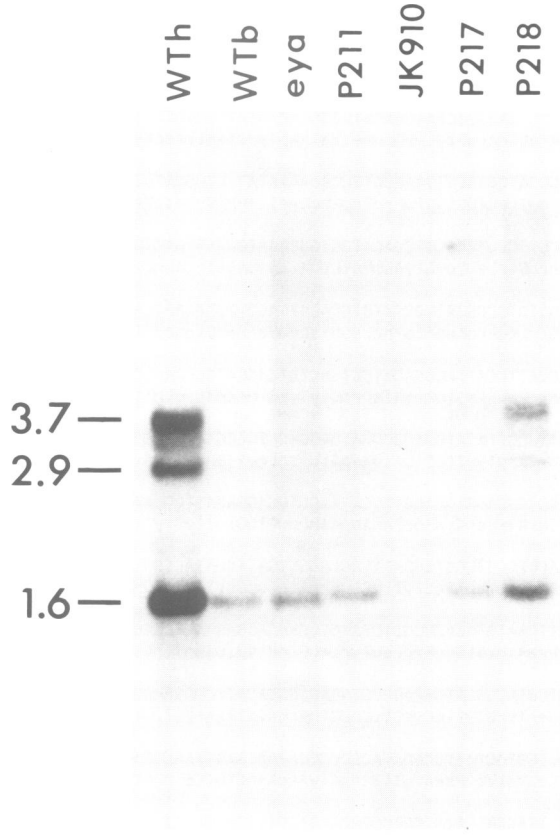

Fig. 4. Transcripts recognized by candidate *Drosophila* HDC cDNA in RNA blot experiments. The candidate HDC cDNA detects four transcripts, three major (3.7, 2.9 and 1.6 kb) and one minor (3.2 kb), that are present in wild-type heads (WTh) but either absent or greatly reduced in amount in wild-type bodies (WTb) and in heads of *eya* (*eyes absent*: Sved, 1986). The transcripts are also greatly reduced in the *hdc* mutants, particularly hdc^{P211} and hdc^{JK910}. Transcript size was determined using a 9.5−0.24 kb RNA ladder (Bethesda Research Laboratories). An actin probe (42A; Fryberg *et al.*, 1983) was used to be certain that equivalent amounts of poly(A)$^+$ RNA were loaded in all lanes (data not shown).

or greatly reduced in amount in wild-type bodies or heads of the mutant *eya*, which lack compound eyes (Sved, 1986), indicating that they are predominantly expressed in the eye. The transcripts were also missing or greatly reduced in all four mutants. The reduction was less severe in hdc^{P217} and hdc^{P218} than in hdc^{P211} or hdc^{JK910}, consistent with their ERG phenotypes. In contrast, probes from the other two groups of cDNA did not recognize any transcripts that showed either predominant expression in the eye or alterations in *hdc* mutants (data not shown).

It has been shown previously that ~90% of HDC activity in *Drosophila* is confined to the photoreceptors (Sarthy, 1991). Therefore, the fact that a cDNA from the 46F group identifies transcripts that are expressed predominantly in the eye is consistent with the idea that this group of cDNAs is derived from the *Drosophila hdc* gene. More importantly, *hdc* mutants show severe reductions in the amount of transcripts recognized by a probe from the 46F group, but not the other two groups, providing strong suggestive evidence that the 46F group of cDNAs, indeed, corresponds to the gene identified by *hdc* mutants.

Sequence analysis of the HDC cDNA

The largest of the 19 cDNAs in the 46F group was subcloned into M13mp18 and sequenced completely in both strands. The results (Figure 5) showed that the 3.3 kb cDNA contains a single, long open reading frame (ORF), which would encode a protein of 847 amino acids. The first methionine codon in the ORF, proposed as the translation start site, is preceded by the sequence CACC, which is the best match to the *Drosophila* consensus sequence flanking the translation start site (Cavener, 1987), found in the 5′ region of the ORF. The cDNA ends in 45 consecutive A residues at its 3′ end, and there is a non-consensus polyadenylation signal, ATTAAA (double-underlined in Figure 5; Sheets *et al.*, 1990) at nucleotide position 3238, 25 bases upstream of the poly(A)$^+$ tract. No potential signal peptide sequence is present in the deduced protein (von Heijne, 1985), and

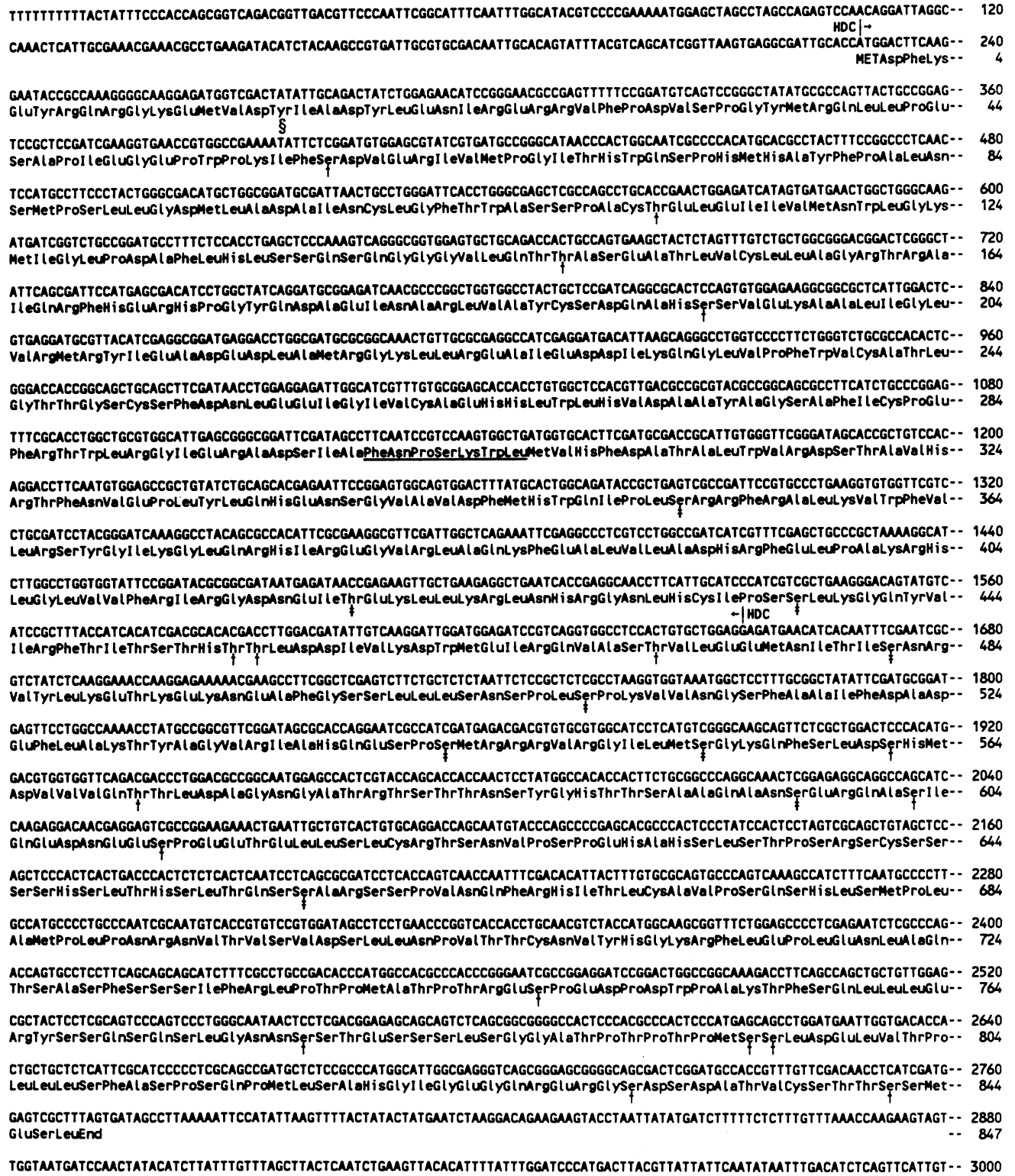

Fig. 5. Nucleotide sequence and deduced amino acid sequence of the largest *Drosophila* HDC cDNA. The sequence of the cDNA is shown with the predicted translation product sequence below it. The predicted coding sequence begins at nucleotide 229 and ends at nucleotide 2769. There are several in-frame termination codons both 5′ and 3′ to this ORF. A putative polyadenylation signal (Sheets *et al.*, 1990) is double-underlined. Shown below the amino acid sequence are potential phosphorylation sites for tyrosine kinase (Arg-2Xaa-Glu-3Xaa-Tyr), indicated by §; protein kinase C (Ser/Thr-Xaa-Arg/Lys), indicated by ‡; and casein kinase II (Ser/Thr-2Xaa-Asp/Glu), indicated by †.

hydropathy analysis revealed no hydrophobic regions of sufficient length to serve as membrane-spanning regions (Kyte and Doolittle, 1982).

Searches through the NBRF and SwissProt databases using the FASTA program (Lipman and Pearson, 1985) revealed that the N-terminal 476 amino acids of the deduced

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          1          §                                                    66
Dro-HDC   MDFK-EY---RQRGKEMVDYIADYLENIRERRVFPDVSPGYMRQLLPESAPIEGEPWPKIFSDVERIVMP
Hum-HDC   MMEPEEY---.E..R.....CQ..STV.....T...Q...L.AQ......EDPDS.DS..G.I...I..
Rat-HDC   MMEPSEYH..QA.........CQ..STV...Q.T.N.K...L.AQI.S...E.PDS.DS..G.I.Q.I..

          67                                                 †              136
Dro-HDC   GITHWQSPHMHAYFPALNSMPSLLGDMLADAINCLGFTWASSPACTELEIIVMNWLGKMIGLPDAFLHLS
Hum-HDC   .VV.........Y...T.............................MN..D..A..L...EH...HH
Rat-HDC   .VV.........Y...T.W...........................MNI.D..A..L....F...HH

          137        †                                                 †     206
Dro-HDC   SQSQGGGVLQTTASEATLVCLLAGRTRAIQRFHERHPGYQDAEINARLVAYCSDQAHSSVEKAALIGLVR
Hum-HDC   PS........Q.V..S..IA...A.KNK.LEMKTSE.DADESCL.......A..........G..S..K
Rat-HDC   PS........R.V..S..IA...A.KNK.LEMKAHE.NADESSL.......A..........G..S..K

          207                                                              276
Dro-HDC   MRYIEADEDLAMRGKLLREAIEDDIKQGLVPFWVCATLGTTGSCSFDNLEEIGIVCAEHHLWLHVDAAYA
Hum-HDC   .KFLPV.DNFSL..EA.QK...E.KQR....VF.........V.A..C.S.L.PI..REG....I.....
Rat-HDC   IKFLPV.DNFSL..EA.QK...E.KQ.....VF.........V.A..K.S.L.PI..REG..........

          277                                                              346
Dro-HDC   GSAFICPEFRTWLRGIERADSIAFNPSKWLMVHFDATALWVRDSTAVHRTFNVEPLYLQHENSGVAVDFM
Hum-HDC   .T..L.....GF.K...Y...FT______M.....C.GF..K.KYKLQQ..S.N.I..R.A.....T...
Rat-HDC   .T..LRP.L.GF.K...Y...FT______M.....C.GF..K.KYKLQQ..S.N.I..R.A.....T...

          347   ‡                                                          416
Dro-HDC   HWQIPLSRRFRALKVWFVLRSYGIKGLQRHIREGVRLAQKFEALVLADHRFELPAKRHLGLVVFRIRGDN
Hum-HDC   ..........SV.L...I..F.V.N..A.V.H.TEM.KY..S..RN.PS..I...........LK.P.
Rat-HDC   ..........SI.L...I..F.V.N..A.V.H.TDM.KY..S..RS.PV..I..E.........LK.P.

          417                                        ††              476
Dro-HDC   EITEKLLKRLNHRGNLHCIPSSLKGQYVIRFTITSTHTTLDDIVKDWMEIRQVASTVLEE
Hum-HDC   CL..NV..EIAKA.R.FL..ATIQDKLI....V..QF..R...LR..NL..DA.TLI.SQ  62% IDENTITY
Rat-HDC   CL..SV..EIAKT.QVFL..ATIQDKLI....V..QF..K...LR..NL..EA.NL..SQ  60% IDENTITY
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Fig. 6. Alignment of the deduced *Drosophila* (Dro-HDC), human (Hum-HDC) and rat HDC (Rat-HDC) amino acid sequences. The N-terminal 476 residues of the *Drosophila* protein are aligned with the corresponding regions of the mammalian sequences. Identical amino acids are indicated by dots, and spaces inserted for optimal alignment are indicated by dashes. The pyridoxal phosphate-binding site is underlined. Indicated above the *Drosophila* sequence are potential tyrosine protein kinase (§), protein kinase C (‡) and casein kinase II (†) phosphorylation sites shared by all three eukaryotic HDCs. The amino acid identity shared between the *Drosophila* HDC and each of the two mammalian HDCs is given at bottom right.

Drosophila protein is 62% and 60% identical to the corresponding regions of human (Yamauchi *et al.*, 1990) and rat HDC (Joseph *et al.*, 1990), respectively (Figure 6). If conservative substitutions are allowed, the sequence similarity among these three proteins in this region is 90%. Within this region of homology is a sequence that matches the conserved sequence surrounding the PLP-binding site found in human (Yamauchi *et al.*, 1990) and rat HDCs (Joseph *et al.*, 1990; Figure 5 and 6, underlined) and other PLP-dependent enzymes (Morino and Nagashima, 1984). Several potential phosphorylation sites (one tyrosine kinase site, one protein kinase C site and five casein kinase II sites) are also conserved among all three eukaryotic HDCs sequenced to date (Figure 6).

The remaining C-terminal region of the *Drosophila* protein (containing 371 amino acids) is ~184 amino acids longer than the corresponding regions of the rat or human HDC proteins. No significant sequence similarities were found between this region of the putative *Drosophila* HDC protein and proteins contained in the databases, including the C-terminal regions of mammalian HDCs. This region also contains several potential phosphorylation sites (Figure 5).

P-element-mediated transformation rescue of the hdc^{P211} mutant

To establish definitively that the cDNA cloned does, indeed, correspond to the gene identified by the *hdc* mutants, the cDNA, driven by the heat-inducible promoter of the heat-shock gene *hsp70*, was introduced into the germline of the *hdc^{P211}* mutant by P-element-mediated transformation (Lis *et al.*, 1983; Schneuwly *et al.*, 1987; Materials and methods). ERG traces shown in Figure 1C and D were obtained from sibling transformants carrying the insert in the identical chromosomal position, but one was obtained without heat induction (Figure 1C), while the other was obtained after exposing the transformant to 37°C for 30 min once daily for 4 days (Figure 1D). The mutant phenotype is retained in the uninduced transformant (Figure 1C), but the ERG phenotype of the sibling transformant after heat induction is indistinguishable from that of wild-type (Figure 1A and D), unequivocally demonstrating that the cDNA completely rescues the mutant phenotype, when its expression is induced. Several independently derived transformants, with inserts in different chromosomes, all gave the same results.

Tissue distribution of hdc gene expression

Tissue *in situ* hybridizations were performed on sections of wild-type and mutant (*hdc^{P211}*) flies using both antisense and sense riboprobes. The sense-strand probe (negative control) hybridized mostly to the cuticle in both wild-type and mutant tissue sections (data not shown). The antisense strand also displayed nonspecific hybridization to cuticle but showed, in addition, specific hybridization to the retina in wild-type sections, but not mutant sections (Figure 7). These results are consistent with the RNA blot data, which suggest that *hdc* RNA is localized primarily to the photoreceptors in the wild-type compound eye. In addition to the retina,

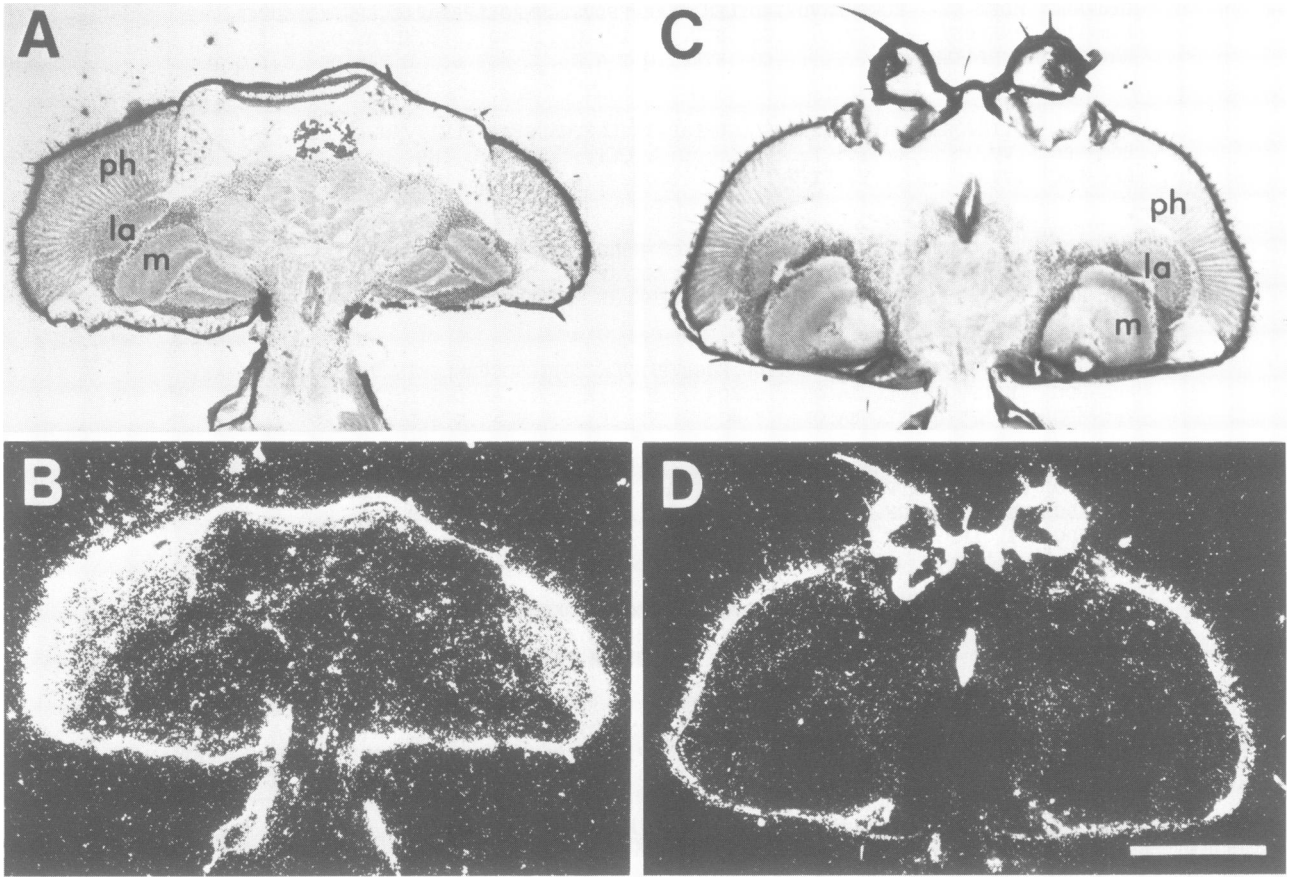

Fig. 7. Tissue distribution of HDC mRNA. ^{35}S-labeled HDC antisense RNA probe was hybridized to 10 μm horizontal sections of fly heads. Plates **A** and **B** are, respectively, brightfield and darkfield micrographs of a section from a wild-type head. The antisense probe hybridizes specifically to the photoreceptor cell layer. In other sections from wild-type flies, hybridization of the probe has also been detected in small, discrete cortical regions of the central brain and thoracic ganglia (data not shown). Plates **C** and **D** are, respectively, brightfield and darkfield micrographs of a section from an hdc^{P211} mutant. No significant hybridization above background is detected in the photoreceptor layer (D), nor was there any hybridization of discrete regions of the brain and thoracic ganglia in this mutant (not shown). ph, photoreceptor layer; la, lamina; m, medulla; scale bar: 100 μm.

hdc RNA was also observed in discrete regions of the central brain and thoracic ganglia of wild-type but not of the hdc^{P211} mutant (data not shown).

Discussion

Screening a large collection of ERG on-/off-transient-defective *Drosophila* mutants has identified a complementation group consisting of mutants with severely reduced HDC activity. Probing a *Drosophila* head cDNA library with a rat HDC cDNA has yielded a group of overlapping cDNA clones with the following properties: (i) the cDNAs map to the same chromosomal position as the HDC-defective mutants identified in the biochemical screen; (ii) probes derived from the cDNAs recognize transcripts that are expressed primarily in the eye and are missing or less abundant in the HDC-defective mutants; (iii) conceptual translation of the ORF contained in the largest of the cDNAs would generate a protein product with strong homology to rat and human HDCs; (iv) the largest cDNA, when introduced into the genome of the HDC defective mutant, hdc^{P211}, by P-element-mediated germline transformation, rescues the mutant phenotype. These results, taken together, demonstrate that the cDNAs isolated are derived from the gene that corresponds to the mutant complementation group identified in the screen and that this gene, named *hdc*, is the structural gene for histidine decarboxylase, expressed largely, though not exclusively, in the eye of *Drosophila.*

Histamine immunocytochemistry performed on a number of invertebrates by previous workers has demonstrated the presence of histamine-like immunoreactivity in photoreceptor endings (Nässel *et al.*, 1988, 1990; Pirvola *et al.*, 1988; Callaway *et al.*, 1989; Schlemermeyer *et al.*, 1989; Battelle *et al.*, 1991; Homberg and Hildebrand, 1991; Pollack and Hofbauer, 1991; Sarthy, 1991). Some of the studies have shown, in addition, histamine-like immunoreactivity in a small number of neurons in the brain (Nässel *et al.*, 1990; Pollack and Hofbauer, 1991) and thoracic and abdominal ganglia (Nässel *et al.*, 1990). Thus, the tissue distributions of *hdc* RNA found in this study are consistent with these immunohistochemical studies.

The deduced *Drosophila* HDC protein may be divided into two major domains on the basis of sequence similarity to other amino acid decarboxylases: the N-terminal domain, consisting of the first 476 amino acids, with strong sequence homology with the two known mammalian HDCs (Figure 6) and the remaining C-terminal domain with no homology. As Joseph *et al.* (1990) have noted, the N-terminal domain is also homologous to *Drosophila* and rat Dopa

decarboxylase (DDC) sequences (Eveleth *et al.*, 1986; Morgan *et al.*, 1986; Tanaka *et al.*, 1989).

Availability of mutants in a cloned gene is important in providing clues to the *in vivo* function of the protein encoded by the gene. Although cDNA cloning of HDCs was first reported for mammals (Joseph *et al.*, 1990; Yamauchi *et al.*, 1990), no mutants had previously been isolated in any HDC genes. Preliminary immunocytochemical results suggest that histamine-like immunoreactivity in photoreceptors and brain neurons are differentially affected by *hdc* alleles of *Drosophila* (Buchner *et al.*, 1991). Thus, future investigations of these *hdc* mutants may be important in shedding light on the role of histaminergic neurons in the brain.

Many lines of evidence support the view that the on- and off-transients of the fly ERG correspond to responses of laminar interneurons to synaptic input from the majority class of photoreceptor cells, R1−6 (e.g. Coombe, 1986; reviewed by Pak, 1991; Buchner, 1991). The present work has provided evidence that the *hdc* gene, identified by mutants severely defective in HDC activity, encodes HDC expressed in the eye. Since *hdc* mutants are characterized by the absence of the on-/off-transients in the ERG, it follows that mutations in the histidine decarboxylase gene which block the synthesis of histamine, also block the generation of the postsynaptic responses in the laminar interneurons. These results provide strong genetic support to a growing body of evidence suggesting that histamine is the major transmitter released by photoreceptors of many invertebrates (Elias and Evans, 1983; Hardie, 1987, 1988, 1989; Simmons and Hardie, 1988; Nässel *et al.*, 1988; Pirvola *et al.*, 1988; Callaway and Stuart, 1989; Callaway *et al.*, 1989; Schlemermeyer *et al.*, 1989; Battelle *et al.*, 1991; Pollack and Hofbauer, 1991; Sarthy, 1991).

Chemical mutagenesis to generate *Drosophila* mutants that are defective in the ERG has yielded many mutants, falling into over 30 complementation groups, that are defective in the on- and off-transients of the ERG (reviewed by Pak, 1975, 1979, 1991). Since the ERG transients arise from the laminar neurons postsynaptic to R1−6 photoreceptors, it has long been felt that many of these mutants may be defective in synaptic transmission between the photoreceptors and their target neurons. The reason for the large number of complementation groups identified by this class of mutants presumably reflects the fact that synaptic transmission is a complex process, requiring many proteins, such as those involved in transmitter synthesis, its uptake into vesicles, its release, various postsynaptic events, etc. The present work provides the first direct evidence that one of the complementation groups identified by ERG transient-defective mutants does, indeed, correspond to a gene encoding a protein involved in synaptic transmission. Availability of mutants that are defective in various steps of synaptic transmission should allow step-by-step molecular genetic dissection of this important biological process.

Materials and methods

Fly strains and mapping

Isolation of mutants defective in the ERG has been described elsewhere (Pak, 1975, 1979). Three of the *hdc* mutants (*P211*, *P217* and *P218*) were isolated in the Pak laboratory by chemical mutagenesis. The fourth mutant (*JK910*) was obtained from Dr J.Merriam of UCLA and later identified as being allelic to the other three.

Two deficiency strains, *Df(2R)X1(46C-47A1)/CyO Adh* and *Df(2R)X3(46C-46E1,2)/CyO Adh*, obtained from Drs M.O'Brien and P.Taghert, Washington University Medical School, St Louis, were crossed separately with the hdc^{P211} mutant, and the F_1 progeny collected and aged for 1 week. ERGs were obtained from 1 week old $hdc^{P211}/Df(2R)X1$, $hdc^{P211}/Df(2R)X3$, and $hdc^{P211}/CyO\ Adh$ flies. Only the $hdc^{P211}/Df(2R)X1$ fly exhibited a transient-defective phenotype (data not shown). The results localized the *hdc* locus in the 46E;46F interval, defined by the distal breakpoints of the two deficiencies (Figure 3).

Histamine synthesis

An assay using isolated heads was used to screen for mutants that were unable to synthesize and accumulate histamine from exogenously supplied [^{3}H]L-histidine, described by Sarthy (1991).

Histidine decarboxylase activity assay

HDC enzyme activity was determined from head extracts from both wild-type and mutant flies as described by Sarthy (1991).

Standard procedures

Standard procedures were used to perform phage and plasmid DNA purification, Southern hybridizations and subcloning of DNA fragments (Sambrook *et al.*, 1989). DNA probes for library screening, Southern and RNA blot hybridizations were made using the random-prime radiolabelling method (Feinberg and Vogelstein, 1983, 1984), with [α-^{32}P]dCTP as label.

cDNA library screening

A rat liver HDC cDNA (Joseph *et al.*, 1990) was used to screen a λgt11 *Drosophila* head cDNA library (Itoh *et al.*, 1985) under low stringency hybridization conditions (Yoon *et al.*, 1989). Approximately 240 000 p.f.u. were screened to yield 36 positively hybridizing clones. High stringency Southern blot analyses were performed on *Eco*RI digests of each clone using several of the other clones as probes to determine mutual cross-hybridization patterns. From this analysis, three non-overlapping groups of cDNA clones were identified. *In situ* hybridizations of the largest purified insert from each cDNA class to polytene chromosomes were performed (see below) to determine their chromosomal locations.

Chromosomal in situ hybridizations

Polytene chromosomes from late third-instar larvae were squashed according to Gall and Pardue (1971). DNA probes were labeled with Bio-16-dUTP (ENZO Biochem) by random-prime labeling (Feinberg and Vogelstein, 1983, 1984), and hybridized to chromosomes as described by Engels *et al.* (1986). Chromosomal hybridization of labeled DNA was detected using the DETEK-1-HRP system (ENZO Biochem), with the exception that diaminobenzidine (Sigma; 0.5 mg/ml) was used as the substrate.

RNA blot analysis

Total RNA was isolated from separated fly heads or bodies (Oliver and Philips, 1970) as described by Montell *et al.* (1985). The poly(A)$^+$ RNA was separated from total RNA using oligo(dT)−cellulose columns (Pharmacia), and the amount of poly(A)$^+$ RNA obtained was determined spectrophotometrically. RNA blotting and hybridization of the [^{32}P]dCTP-labeled probe were done as described earlier (Shortridge *et al.*, 1991). Filters were then exposed to film (Kodak X-OMAT AR) at −70°C for several days.

DNA sequencing and analysis

The HDC cDNA clone was subcloned into M13mp18, and a nested set of deletions were made using the Cyclone Deletion System (IBI). The sequence of both strands was determined (Sanger *et al.*, 1977) using the Sequenase 2.0 DNA sequencing kit (United States Biochemical). Regions of sequence compression were resolved using the dITP sequencing mixtures supplied (United States Biochemical).

Identification of the open reading frame and generation of a deduced amino acid sequence was carried out using a microcomputer program (Mount and Conrad, 1986). The GCG analysis programs, Version 7.0 (Devereux *et al.*, 1984), were used to perform searches in the NBRA and SwissProt databases using FASTA (Lipman and Pearson, 1985). Searches for putative functional protein domains and phosphorylation sites were carried out using MOTIFS (Devereux, 1991). Identification of hydrophobic/hydrophilic regions was performed using PEPPLOT (Gribskov *et al.*, 1986), and multiple sequence alignments were performed using PILEUP (Devereux, 1991).

P-element-mediated germline transformation

P-element-mediated germline transformation was carried out as described in the literature (Rubin and Spradling, 1982; Spradling, 1986). The transformation vector, pCaSpeR-hs, is a modified form of pCaSpeR (Pirrotta, 1988) containing the promoter of the heat-shock gene *hsp70* to allow heat-induced,

ubiquitous expression of insert cDNAs (Lis *et al.*, 1983; Schneuwly *et al.*, 1987; Thummel *et al.*, 1988). The final plasmid construct, p[CaSpeR-hshdc], used for transformation was obtained by inserting the 3.3 kb HDC cDNA (Figure 5) into the *Eco*RI site of pCaSpeR-hs in proper orientation. Several independent lines of transformants were isolated, exposed to 37°C for 30 min once daily for up to 4 days to induce the *hsp70* promoter, and the phenotypes determined by ERG recording at room temperature.

Tissue in situ hybridizations

^{35}S-labeled riboprobes were synthesized *in vitro* (Stratagene) using a linearized pGEMblue plasmid (Promega) that contained the 5′-most 700 bp of the cDNA (see Figure 5) with the T7 (sense) and SP6 (antisense) DNA-dependent RNA polymerase promoters flanking the insert. The level of incorporation was similar for both sense and antisense strands.

Frozen tissue sections (~10 μm) were collected and treated as described by Hafen and Levine (1986). ^{35}S-labeled riboprobes were hybridized to the pretreated tissue sections for 18 h at 43°C in a humidified chamber. Approximately 5×10^6 c.p.m. of probe was used per slide. The hybridization solution was the same as that described by Hafen and Levine (1986) except that 10 mM dithiothreitol (DTT; Sigma) and 10 mM vanadyl-ribonucleoside complex (Sigma) were added. Sections were washed as recommended by Hafen and Levine (1986) except that the temperature of washing was increased to 50°C and 10 mM DTT (Sigma) was added to all wash solutions. After washing, the slides were treated with RNase A (Calbiochem; 40 μg/ml in 2.5 M NaCl, 5 mM EDTA, 10 mM DTT 50 mM Tris–Cl, pH 8.0) for 30 min at 37°C to eliminate nonspecific hybridization. Autoradiography was performed essentially as described by Angerer *et al.* (1987) using Kodak NBT-2 autoradiography emulsion.

Acknowledgements

We thank Dr D.R.Joseph, University of North Carolina, Chapel Hill, for providing the rat histidine decarboxylase cDNA clone; Dr P.Salvaterra of Beckman Research Institute, City of Hope, for providing us with a *Drosophila* head cDNA library; Dr C.Thummel, Howard Hughes Medical Institute, University of Utah Medical Center, for providing the transformation vector; Drs M.O'Brien and P.Taghert, Washington University School of Medicine, for providing deficiency strains; Dr J.Merriam of UCLA for providing us with the *hdc*^{JK910} mutant, and John Bowman for performing ERG recordings. Sequence analysis was performed using facilities of the ACLCB facility at Purdue Univ. (AI27713). This work supported by NIH grants, EY00033 (W.L.P.) and EY03664 (P.V.S.). M.G.B. was a recipient of NIH-NRSA Individual Postdoctoral Fellowship EY06214.

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Received on November 2, 1992

Note added in proof

The nucleotide sequence data reported here will appear in the EMBL, GenBank and DDBJ nucleotide sequence databases under the accession number X70644.