BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **248,** 204–209 (1998)
ARTICLE NO. RC988915

Identification of a Receptor Tyrosine Kinase Involved in Germ Cell Differentiation in Planarians

Kazuya Ogawa,* Akihiko Wakayama,† Takahiro Kunisada,† Hidefumi Orii,* Kenji Watanabe,* and Kiyokazu Agata*,[1]

**Laboratory of Regeneration Biology, Department of Life Science, Faculty of Science, Himeji Institute of Technology, Harima Science Garden City, Akou, Hyogo 678-1297, Japan; and †Department of Immunology, School of Life Science, Faculty of Medicine, Tottori University, Nishi-machi 86, Yonago 683, Japan*

Received November 17, 1998

To investigate external signals involved in germ cell differentiation from somatic stem cells, we have tried to identify protein kinases whose expression is regulated during the process of sexualization of asexual-state planarians. It is known that in planarians germ cells differentiate from totipotent somatic stem cells called "neoblasts" during sexualization. As a first step, we have isolated twelve protein kinase genes from cDNAs of sexual-state planarians, including three non-receptor tyrosine kinases, three receptor-tyrosine kinases and three non-receptor serine/threonine kinases, and then analyzed their expression patterns during sexualization. One of them, the *DjPTK1* gene, is specifically expressed in germ cells of sexual-state planarians. *DjPTK1*-positive cells were also detected in the mesenchymal space during the process of sexualization, and it appears that these cells migrate to the dorsal side and then differentiate into spermatogonia/spermatocytes in testis. Sequence analysis indicated that the *DjPTK1* gene encodes a receptor protein tyrosine kinase belonging to the FGFR/PDGF family. These results suggest that a receptor tyrosine kinase system may be involved both at an early stage of germ cell differentiation and in a step of germ cell maturation in planarians.

Key Words: **protein tyrosine kinase; receptor; planarian; *Dugesia japonica;* germ cell.**

There are two major pathways for germ cell differentiation in the animal kingdom. In *Drosophila, C.elegans* and *Xenopus*, cells carrying cytoplasmic determinants localized in the posterior region of the cytoplasm of fertilized eggs during early cleavage are determined to differentiate into germ cells (1). On the other hand, such cytoplasmic determinants have not been identified in most animals including mouse and human. It has been believed that germ cells may differentiate from somatic stem cells in response to external signals during early embryogenesis in these animals. Existence of this pathway has been clearly demonstrated by production of chimeric mice combining embryonic stem (ES) cells and developing normal embryos (2). ES cells participate in differentiation of germ cells *in vivo* in such chimeric mice. However, nobody has succeeded in obtaining differentiation of germ cells from ES cells in *in vitro* culture conditions, since environmental factors regulating germ cell differentiation from somatic stem cells are still unknown.

To investigate the signal system controlling germ cell differentiation from somatic stem cells, our laboratory strain of planarian (*Dugesia japonica*, GI strain) may be one of the most suitable animals, since GI can be easily converted from the asexual state to the sexual state by changing their type of food. They usually propagate by transverse fission in the asexual state. However, if a sexual strain of planarian is freeze-thawed and fed to asexual-state GI, the asexual planarians start to produce germ cells, and form ovary, testis and copulatory organs, and then proliferate by sexual reproduction (3). The other important feature of planarians is that they have totipotent stem cells called "neoblasts" in their bodies (4). Neoblasts support the high regenerative ability of planarians. It has been suggested that germ cells of planarians may differentiate from neoblasts during sexualization (5). For these reasons we have started to analyze signal systems that change during sexualization in planarians in order to get some insight into germ cell differentiation from somatic stem cells.

[1] To whom correspondence should be addressed. Fax: +81-7915-8-0187. E-mail: agata@sci.himeji-tech.ac.jp.

The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GeneBank nucleotide sequence databases with the following Accession numbers: DjPTK1: AB014508; DjPTK2: AB014499; DjPTK3: AB014500; DjPTK4: AB014501; DjPTK5: AB014496; DjPTK6: AB014497; DjSTK1: AB014502; DjSTK2: AB014503; DjSTK3: AB014504; DjSTK4: AB014505; DjSTK5: AB014506; DjSTK6: AB014507.

0006-291X/98 $25.00

TABLE 1

Categorization of cDNA Clones

Gene	Class	No. of clones (/86)
DjPTK1	FGF receptor	19
DjPTK2	FGF receptor	1
DjPTK3	EGF receptor	1
DjPTK4	Abl	20
DjPTK5	Src	11
DjPTK6	Src	2
DjSTK1	MEKK/STE11	18
DjSTK2	KIN1/SNF/Nim1	9
DjSTK3	KIN1/SNF/Nim1	2
DjSTK4	KIN1/SNF/Nim1	1
DjSTK5	KIN1/SNF/Nim1	1
DjSTK6	ERK (MAP) kinase	1

As the first step, in order to survey a wide range of signal systems, we focused on protein kinase gene families, since it is well known that these genes are involved in a variety of signal systems (6, 7, 8), and ideal PCR primers have been designed to amplify protein kinase genes from various animals (9). Also, it has been indicated that receptor tyrosine kinases are involved in germ cell differentiation/maturation in mouse (10, 11). Here, we have isolated a set of protein kinase genes from planarian and analyzed their expression patterns during sexualization to identify genes involved in germ cell differentiation.

MATERIALS AND METHODS

Organisms. Asexual-state planarians (GI) proliferating as single worms of *Dugesia japonica*, derived from the Irima river in Gifu, Japan, are maintained on a diet of chicken liver in our laboratory. Clonal asexual-state planarians converted to the sexual state by feeding them freeze-thawed sexual-state planarians.

Cloning of protein kinase cDNAs. One microgram of total RNA of sexual-sate planarians was reversed-transcribed using a First-Strand cDNA Synthesis Kit (Pharmacia). PCR amplification of the cDNA using the degenerate oligonucleotide primers 5′-CGGATC-CAC(A/C)GNGA(C/T)(C/T)T3′ (PTKI; sense strand for IHRDL plus BamHI linker) and 5′-GGAATTCCA(A/T)AGGACCA(C/G)AC(A/G)TC-3′ (PTKII; antisense strand for DVWS(F/Y)G plus EcoRI linker) (9). The PCR reactions were carried out with annealing at 40°C. The products were cloned into PCR2.1. (Invitrogen) and sequenced using an automatic DNA sequencer (Shimazu Co.Ltd,). Searches for genes or proteins similar to the PCR products were conducted by tFASTA (DDBJ, Release 33).

Isolation of DjPTK1 cDNA. *DjPK1* cDNA was isolated by stepwise dilution screening (12) of 3.5×10^6 recombinant phage in λZAPII vector (Stratagene) from the cDNA library. The positive cDNA clone with the longest insert was recloned into pBluescript according to the manufacturer's protocol, and sequenced.

In situ hybridization. Digoxigenin-labeled RNA probe was prepared according to the manufacturer's protocol (Boehringer), with the *DjPTK1* cDNA as a template. Whole-mount *in situ* hybridization was performed as described by Umesono (13). For fixation, the relax-

Protein Tyrosine Kinase

```
DjPTK1   IHRDLRAANVLVDQYVEMKIADFGLTR-I-V-ENYYRKT---TDGRLPIKWMAPECLLDR-VYTVKSDVWSFG
DjPTK2   IHRDLRAANVLLSDHYVCKISDFGMSRQLPVNETYY-QH---VNGIIPLKWMAPEVLIQK-KYTIQADVWSFG
DjPTK3   IHRDLSARNILVGEHFEMKIADFGLTR-I-V-DYYYRKK---TDGILPVKWMAPEALLEK-KYTTKSDVWSFG
DjPTK4   IHRDLAARNCLVGQDNIVKVADFGLAR-CMERDDTYTAH---VGAKFPIKWTAPEGLAYN-QFSTKSDVWSFG
DJPTK5   IHRDLRAANILVDEDLSVKVADFGLAR---VTDDVYNAD---TGTKFPIKWTAPEAGMHR-RFSVKSDVWSFG
DjPTK6   IHRDLAARNILVGENNMCKVADFGLARMIRENSGTYEAK---EGTKFPIKWTAPEAAMIGR-FTIKSDVWSFG
```

Serine/Threonine Kinase

```
DjSTK1   IHRDLKSTNILLDNNLDIKISGFSLSKYLAGANSTIMSEGFMQSKPGTCNFMAPEVLIDQ-RITRKSDVWSFG
DjSTK2   IHRDLKAENMLLNSKMQIKIADFGFANNFDPKSKL-------STFCPSPPYAAPELFAGQRYVGPEVDVWSFG
DjSTK3   IHRDLKAENLLLDKDLTIKIADFGFSNHFSRQSKL-------NTFCPSPPYAAPELFQGRRYEGPEVDVWSFG
DjSTK4   IHRDLKAENLLLDKEMNIKIADFGFSNEFKPGDKL-------DTFCPSPPYAAPELFQGKKYDGPEVDVWSFG
DjPTK6   IHRDLAARNILVGENNMCKVADFGLARMIRENSGTYEAK---E.TKFPIKWTAPEAAMIGR-FTIKSDVWSFG
DjSTK5   IHRDLKPENLLLDDKLNIRIADFGMASLQPEGSML-------ETSCPSPHYACPEVIRGEKYDGRKADVWSFG
DjSTK6   IHRDLKPSNIGINTNLDLRILDFGLAEDTKDGM---------ANYVVTRWYRPLEVFYSS-EYTAAVDVWSFG
```

PTKI PTKII

VI VII VIII IX

FIG. 1. Alignment of amino acid sequences of planarian protein kinases deduced from nucleotide sequences of PCR fragments. Shaded residues represent amino acids which are conserved. Roman numerals show highiy conserved subdomains.

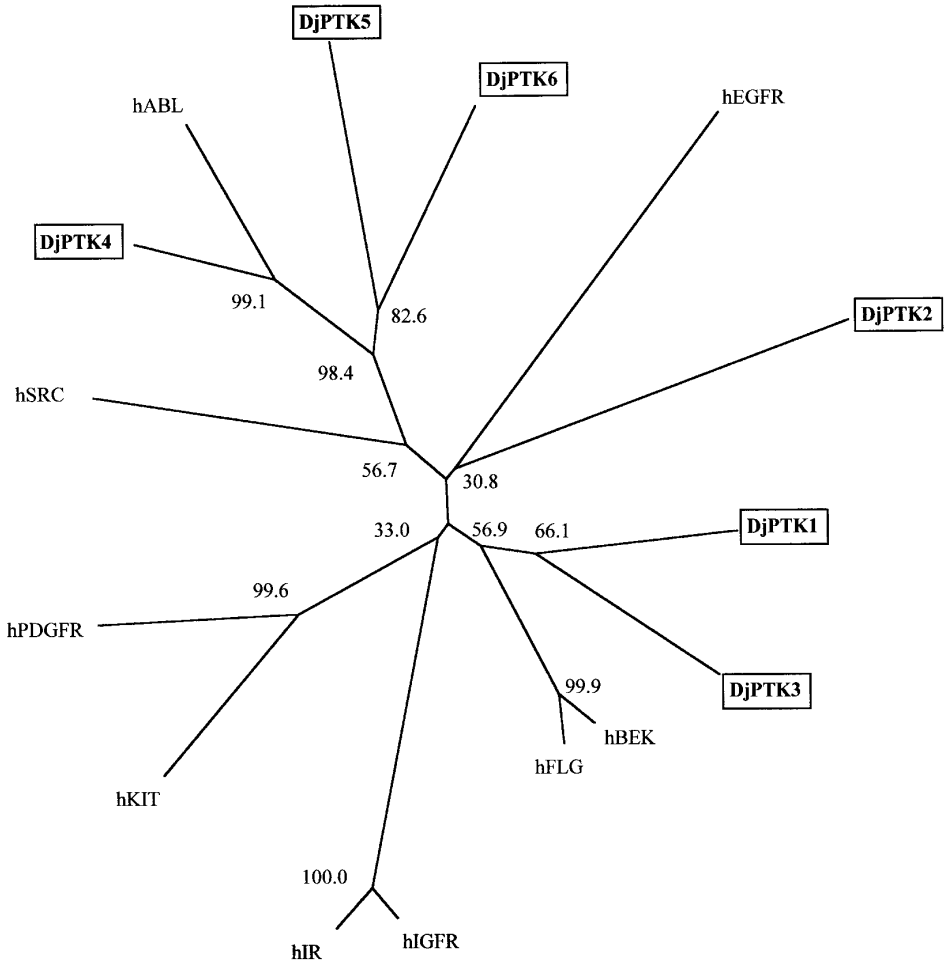

FIG. 2. Phylogenetic relationship among the six planarian protein tyrosine kinases and nine human protein tyrosine kinases. The tree was drawn by the NJ method. The number shows bootstrap value.

ant solution was 1% HNO_3, 2.25% formalin, 50μM $MgSO_4$ in modified Holtfleter solution. Fixed samples were embedded in paraffin and serially sectioned at 4μm. *In situ* hybridization of sections was performed as described by Agata (14). Cell nuclei were labeled with Hoechst No.33342 (Sigma).

RESULTS AND DISCUSSION

We cloned PCR fragments from cDNA of sexual-state planarians using kinase domain-specific degenerate primers (PTK1 and PTK2) (9). One hundred and fifty clones in PCR2.1 plasmid were randomly selected and categorized into twelve groups by sequence and restriction fragment analyses (Table. 1). The amino acid sequences deduced from the nucleotide sequences of the twelve genes are aligned in Fig. 1. Since all of them contained amino acid residues conserved in domains VI-IX of the protein kinase family (15), they might encode protein kinases of the planarian *Dugesia japonica*. These twelve protein kinase genes were classified into three families by homology search (tFASTA search) and molecular phylogenic analysis (Table. 1, Fig. 2): three receptor protein tyrosine kinase genes (*DjPTK1-3*), three non-receptor protein tyrosine kinase genes (*DjPTK4-6*), and six non-receptor serine/threonine kinase genes (*DjSTK1-6*).

To identify signal molecules involved in germ-cell differentiation, we compared the expression pattern of these genes in the asexual state with that in the sexual state by whole mount *in situ* hybridization analysis. One of these genes, *DjPTK1*, showed a very interesting expression pattern during sexualization. Fig. 3B shows whole mount views of both sexual and asexual planarians. Expression of the *DjPTK1* gene was observed in both testis and ovary in the sexual-state planarian (right panel), but not in the asexual planarian (left panel). The cells expressing the *DjPTK1* gene were precisely localized in transverse sections of sexual planarians. The *DjPTK1*-expressing cells were detected in a layer containing spermatogonia/spermatocytes in testis, but not in spermatids after meiosis nor in sperm (Fig. 3C and D). The most informative view (Fig. 3E)

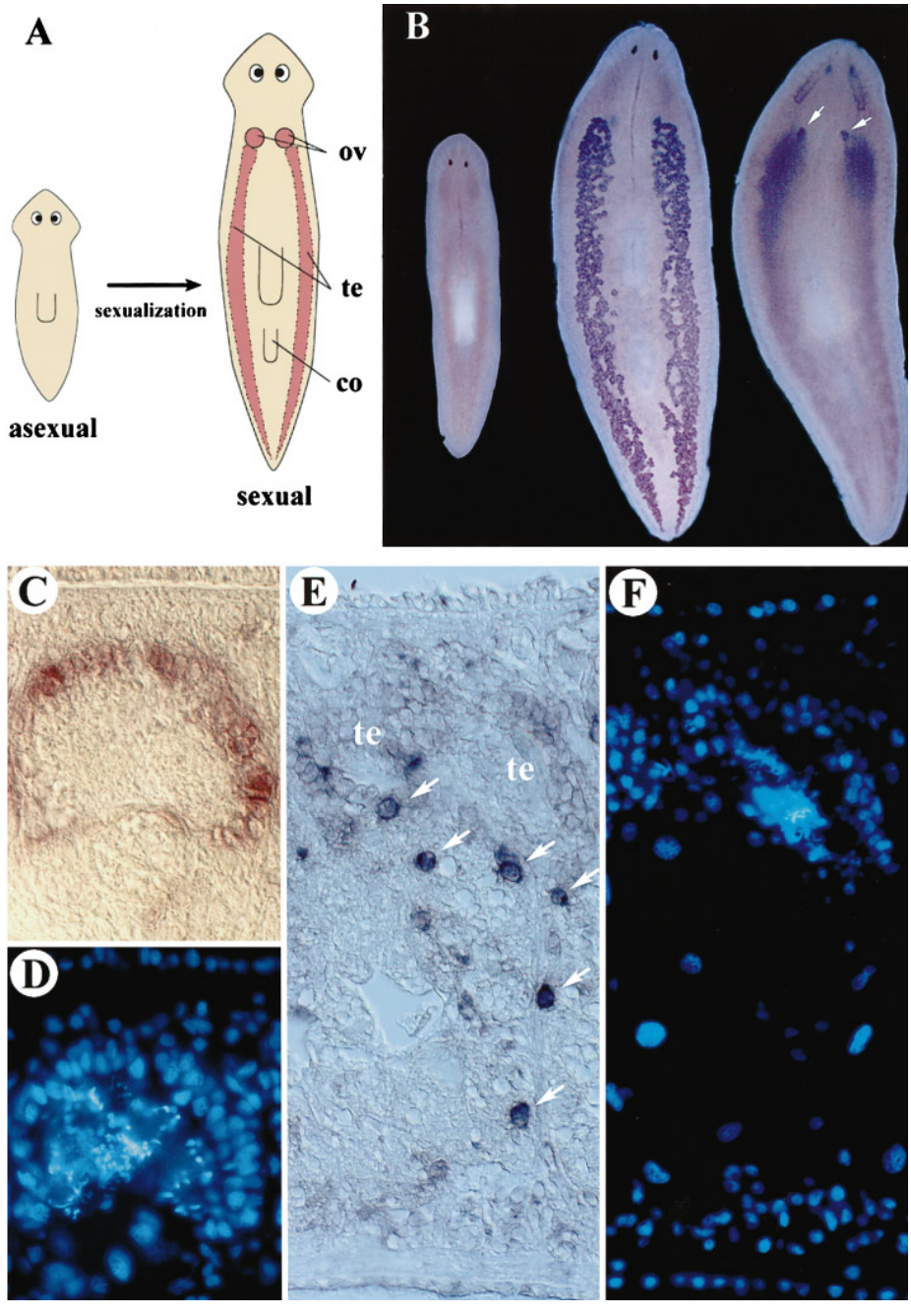

FIG. 3. Expression of DjPTK1. (A) Illustration of planarain sexualization. (B) Whole mount *in situ* hybridization of *DjPTK1*. Left: a dorsal view of an asexual planarian, middle: a dorsal view of a sexual planarian, right: a ventral view of a sexual palnarian. Expression is seen in testis and ovary (white arrows). (C) A transverse section of a testis. Expression is seen in the layer containing spermatogonia/spermatocytes. (D) A counter nuclear staining of Fig. 3C using Hoechst 33342 shows mature testis. (E) A parasagittal section of sexual palanarian in the process of sexualization. *DjPTK1*-positive cells were detected in the mesenchymal space (white arrows). te, testis. (F) A counter nuclear staining of Fig. 3E using Hoechst 33342 shows immature testis.

was a transverse section of a sexual planarian in the process of sexualization. Fig. 3F shows a counter nuclear staining of Fig. 3E using Hoechst 33342. Cells strongly expressing DjPTK1 were detected in the mesenchymal space of the body. The positive cells appear in the ventral mesenchymal space, appear to migrate to the dorsal side through the interspace between the intestines, and then differentiate into spermatogonia/spermatocytes on the dorsal side. These results suggested that *DjPTK1* may be involved in both an early stage of germ cell differentiation and the process of germ-cell maturation.

A

```
   1  TTGGATCTTCTTCCGCGCAAAGTAAACGGATCATGTTACAGCAGTCGACTCTACCTTTACAATGTCGAAATCAAAGATCAAGGATTTTAT
   1  L  D  L  L  P  R  K  V  N  G  S  C  Y  S  S  R  L  Y  L  Y  N  V  E  I  K  D  Q  G  F  Y
  91  TCAGTAGAAGCAACTTTGCGTATGAAAAACGGATCGAGTCATGGCCTTAATTTCACATATGAATTGAAAATCAATATTGCCAACATACTT
  31  S  V  E  A  T  L  R  M  K  N  G  S  S  H  G  L  N  F  T  Y  E  L  K  I  N  I  A  N  I  L
 181  GATCCCGATAAAACCAACAGTATCCTTTCTACTCCACAAATCTCTTTCAATCTTAACTCAAGAGTTTGTATCAATGATAGATTTGATTGG
  61  D  P  D  K  T  N  S  I  L  S  T  P  Q  I  S  F  N  L  N  S  R  V  C  I  N  D  R  F  D  W
 271  ATATGCAAAGTTACTCCTGTAGTATCATATTATGTGACGATTTACAAAAACAACTCAAATCCTAATAACATCACTGTTCTGGCTGAATCA
  91  I  C  K  V  T  P  V  V  S  Y  Y  V  T  I  Y  K  N  N  S  N  P  N  N  I  T  V  L  A  E  S
 361  GAAGTATTACAAATGAATATTGATGGAAATAGTGGTCAAGGGTTTTATCTAAAGAGTAGTACGGTAAATTATTCTGTCAATTCGGTGCAA
 121  E  V  L  Q  M  N  I  D  G  N  S  G  Q  G  F  Y  L  K  S  S  T  V  N  Y  S  V  N  S  V  Q
 451  AGGGAGCATGCCGGAGTGTATGCCTGTAGAATAATAAATTTCAAAGACTATAGCTCTGATCATCAAAATCGTCATGAACCGGAAGTGTTG
 151  R  E  H  A  G  V  Y  A  C  R  I  I  N  F  K  D  Y  S  S  D  H  Q  N  R  H  E  P  E  V  L
 541  ATGAGATTAACAGTGAAGGATTGTGTGGGAAACTCATATTTCACAATAATATGGTACAGTATCAGTGTCGGCATTATCATTTTGGTCGTT
 181  M  R  L  T  V  K  D  C  V  G  N  S  Y  F  T  I  I  W  Y  S  I  S  V  G  I  I  I  L  V  V
 631  ATTTCCTTTTTAATTATTCGATTGTATAACAAGTATTCCAATGGTTATATAGTAAAAACTGTAATTGTTCAACATCCCAATAAACTATAT
 211  I  S  F  L  I  I  R  L  Y  N  K  Y  S  N  G  Y  I  V  K  T  V  I  V  Q  H  P  N  K  L  Y
 721  GTTCCACATGATACTTGCTTCCctCTCCTGATGCCCGATATCACCATAAAAACTATCCATAAACACATCAATAGTTCAGAAGATTCTCTA
 241  V  P  H  D  T  C  F  P  L  L  M  P  D  I  T  I  K  T  I  H  K  H  I  N  S  S  E  D  S  L
 821  CTACAACAAAAACATTTCACTAATAACTCAAATATTCCTTTTTCCCAGAAAATATCAAAATATTTCAGAAAATCTTTTATTTTCAGTTAC
 271  L  Q  Q  K  H  F  T  N  N  S  N  I  P  F  S  Q  K  I  S  K  Y  F  R  K  S  F  I  F  S  Y
 901  CGCCATGTTGATGTTTCAAGCTCTAATCTTGATTCTCCACTTGGAGTAATTTCCAATACAGAAACTAACAAATTGACTTCAAATTCTTTG
 301  R  H  V  D  V  S  S  S  N  L  D  S  P  L  G  V  I  S  N  T  E  T  N  K  L  T  S  N  S  L
 991  ACTGTTGAAACTCAACGGCCACAGTTAATTTTACAAAATGATGCTAATACTAAATATATATTACCATCTAATATTGGCTGGATATTTTCC
 331  T  V  E  T  Q  R  P  Q  L  I  L  Q  N  D  A  N  T  K  Y  I  L  P  S  N  I  G  W  I  F  S
1081  AGAGATAGTTTAATAATTGGATCAAAAATTGGAGAAGGCGCCTTTGGTATTGTGTACTCAGCTTTGGTCAAATCTTTCTCCGAAAATTCA
 361  R  D  S  L  I  I  G  S  K  I  G  E  G  A  F  G  I  V  Y  S  A  L  V  K  S  F  S  E  N  S
1171  GCTAGTGTAGAAGTAGCAATTAAAACTTTACACACGTCATTTGGAGATCAAGACGTCATAAACCTAATTCAGGAATTAGAAATGATGAAA
 391  A  S  V  E  V  A  I  K  T  L  H  T  S  F  G  D  Q  D  V  I  N  L  I  Q  E  L  E  M  M  K
1261  ATAATTGGTCGGCACCGTCATATAATATCATTATACGGAGCCTGCATCGACAACGGTCATCCCTATATGGTGATTGAATTAGCAAAGCAT
 421  I  I  G  R  H  R  H  I  I  S  L  Y  G  A  C  I  D  N  G  H  P  Y  M  V  I  E  L  A  K  H
1351  GGTAACTTGAGAGACTTTCTTAGGGCACAACGTAGCCAATCTAAAGTTGGAGAAATACAAAATAGTGGAGGTCTAGTAACACGATTAACA
 451  G  N  L  R  D  F  L  R  A  Q  R  S  Q  S  K  V  G  E  I  Q  N  S  G  G  L  V  T  R  L  T
1441  GTTACTGATTTTTTACGATTCAGTATAGAGATAGCAGAAGGAATGGAATATTTGTCATCACGAAAGATAATCCACAGAGATTTGGCAGCA
 481  V  T  D  F  L  R  F  S  I  E  I  A  E  G  M  E  Y  L  S  S  R  K  I  I  H  R  D  L  A  A
1531  AGAAATGTATTAGTTGATCAATATGTTGAAATGAAAATAGCAGATTTTGGTCTGACAAGAATTGTGGAAAATTATTATCGTAAAACTACT
 511  R  N  V  L  V  D  Q  Y  V  E  M  K  I  A  D  F  G  L  T  R  I  V  E  N  Y  Y  R  K  T  T
1621  GACGGACGTCTGCCTATTAAATGGATGGCTCCTGAATGTCTACTGGATCGAGTATATACAGTCAAAAGTGACGTGTGGTCCTATGGAATA
 541  D  G  R  L  P  I  K  W  M  A  P  E  C  L  L  D  R  V  Y  T  V  K  S  D  V  W  S  Y  G  I
1711  GTATTATGGGAAATATTCACTATGGGACAAACTCCTTATCCGACAATTCAATCAGATGGAATGCACCAAGCACTACGAAATGGAATCCGC
 571  V  L  W  E  I  F  T  M  G  Q  T  P  Y  P  T  I  Q  S  D  G  M  H  Q  A  L  R  N  G  I  R
1801  AACGAAAAACCAGCGTTAGCGTCTGATGAGATGTATCGACTGATGCTCACAATTTGGAATGATGATCCTCTTGAAAGGCACACTTTTAGT
 601  N  E  K  P  A  L  A  S  D  E  M  Y  R  L  M  L  T  I  W  N  D  D  P  L  E  R  H  T  F  S
1891  GAAATAATTGATAAATTGACCCATATTCAATTGTCCAATGGTGGATCATCTCCTAAACGGGATTATCTGGAGATAAGTAGTAATCAATGT
 631  E  I  I  D  K  L  T  H  I  Q  L  S  N  G  G  S  S  P  K  R  D  Y  L  E  I  S  S  N  Q  C
1981  TATTCTACAACAATAGTATAGTGAATCAATTCCCACAATTGTGCCAACCATATCGTCTTTCATTGTCACTCAAATTGGTAATGATTGACA
 661  Y  S  T  T  I  V  *
2071  CCAACTCAATCTGCTATTTCCAGTGGTAGTTAGCCTTGCTCTTGATCCTCTATGCCTTGTACAAACAGATTATTGTAACATTATTCGCTC
2161  CAATGTATTTATCGTATCATTTGTACATATCCTGATCATCCACGATAATTTATATCAATATATTGATACACCATTTACTATTTGCCACTG
2251  ATTCAACCTTTTATAATAAAATTTTTCATTTGTTTTAAAAAAAAAAAAAAAAAAA
```

B

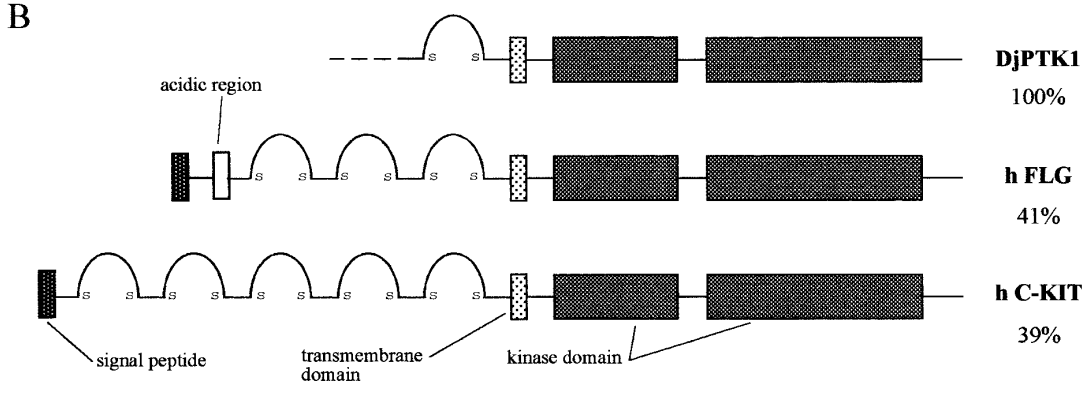

FIG. 4. (A) Nucleotide and deduced amino acid sequence of *DjPTK1*. Conserved Cys residues of the Ig-domain are circled. The shaded box indicates the predicted transmembrane region. The tyrosine kinase catalytic sequence is boxed. The conserved Tyr residue in the C-terminal tail is indicated by a triangle. (B) Diagram showing structures of DjPTK1, human FGF-R (FLG) and C-KIT. Percentages indicate similarities betwwen kinase domains.

To characterize the *DjPTK1* gene, the longest *DjPTK1* cDNA clone was isolated from cDNA library containing 3.5×10^6 independent clones in λZAPII vector by stepwise dilution screening (12). It contained a 2305 bp insert with an open reading frame (ORF) containing 666 amino acids (Fig. 4A). Unfortunately, the presumptive initial methionine contained in the putative signal peptide was not found. However, this clone contained a truncated extracellular region, a transmembrane region and an entire intracellular region. In the intracellular region, a relatively long juxtamembrane domain (amino acid positions 217-358), split tyrosine kinase catalytic sequence (359-636), and a short carboxyl terminal tail (637-666) containing a tripeptide sequence, Tyr-Leu-Glu, which might provide a tyrosine phosphorylation site (16), were found. One immunoglobulin-like domain (Ig-domain) containing two Cys residues (17) was found in the 192 amino acid residues of the putative extracellular region. These cDNA analyses confirmed that the *DjPTK1* gene encoded a receptor protein tyrosine kinase similar to FGFRs and *c-kit.* The overall sequence homology between the kinase domain of DjPTK1 and that of FGFRs or c-kits from other animals was estimated to be about 40% (41% homologous to human *flg*, 39% to human *c-kit;* Fig.4B) (18, 19).

These results suggest that a receptor tyrosine kinase system may be involved in an early stage of germ cell differentiation in planarians. However, we need further analyses to determine whether the *DjPTK1* gene is involved in differentiation of germ cells from totipotent stem cells, or whether it is involved only in differentiation/proliferation of cells already committed to become germ cells, like *c-kit* in mouse (20). Although we have not yet elucidated the exact function of *DjPTK1*, our laboratory strain of planarian may provide us a unique approach for investigating signal systems involved in germ cell differentiation from somatic cells. The findings should yield new insights for understanding germ cell differentiation. Signal systems using receptor tyrosine kinase commonly function in early stages of germ cell differentiation of invertebrates as well as vertebrates. This suggests that such systems may have been established at an early stage of evolution and have been well conserved during diversification of the animal kingdom.

ACKNOWLEDGMENTS

We thank Kentaro Kato, Norito Shibata, and Akira Tasaki for technical advices. This work was supported by Special Coordination Funds for Promoting Science and Technology to K.A. and Grant-in-Aid for Scientific Research on Priority Areas to K.A. and K.W.

REFERENCES

1. Beams, H. W., and Kessel, R. G. (1974) *Int. Rev. Cytol.* **39,** 413–476.
2. Bradley, A., Evans, M., Kaufman, M. H., and Robertson, E. (1984) *Nature* **309,** 255–256.
3. Sakurai, T (1981) *Annot. Zool. Japan.* **54**,103–112.
4. Morita, M., and Best, J. B. (1984) *J Exp Zool.* **229,** 425–436.
5. Baguna, J., Salo, E., and Auladell, C. (1989) *Development* **107,** 77–86.
6. Fleischman, R. A. (1993) *Trends Genet* **9,** 285–290.
7. Loveland, K. L., and Schlatt, S. (1997) *J Endocrinol* **153,** 337–344.
8. Linnekin, D., Mou, S., Deberry, C. S.,Weiler, S. R., Ruscetti, F. W., and Longo, D. L. (1997) *Leuk Lymphoma* **27,** 439–444.
9. Wilks, A. F. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1603–1607.
10. Manova, K., Nocka, K., Besmer, P., and Bachvarova, R. F. (1990) *Development* **110,** 1057–1069.
11. Asakai, S., Song, S. Y., Ito, N., Yamakuni, T., Tamura, K., and Okamoto, R. (1994) *Mol. Cell. Endocrinol.* **104,** 75–80.
12. Watanabe, K., Sakai, F., and Orii, H. (1997) *Analytical Biochemistry* **252,** 213–214.
13. Umesono, Y., Watanabe, K., and Agata, K. (1997) *Develop. Growth Differ.* **39,** 723–727.
14. Agata, K., Soejima, Y., Kato, K., Kobayashi, C., Umesono, Y., and Watanabe, K. (1998) *Zool. Sci.* **15,** 433–440.
15. Hanks, S. K., Quinn, A. M., and Hunter, T. (1988) *Science* **241,** 42–52.
16. Mohammadi, M., Honegger, A., Rotin, D., Fischer, R., Bellot, F., Li, W., Dionne, C. A., Jaya, M., Rubinstein, M., and Schlessinger, J. (1991) *Mol. Cell. Biol.* **11,** 5068–5078.
17. Williams, A. F., and Barclay, A. N. (1998) *Ann. Rev. Immnol.* **6,** 381–405.
18. Ruta, M., Burgess, W., Givol, D., Epstein, J., Neiger, N., Kaplow, J., Crumley, G., Dionne, C., Jaye, M., and Barclay, A. N. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8722–8726.
19. Yarden, Y., Kuang, W. J., Yang-Feng, T., Coussens, L., Munemitsu, S., Dull, T. J., Chen, E., Schlessinger, J., Francke, U., and Ullrich, A. (1987) *EMBO J.* **6,** 3341–3351.
20. Yoshinaga, K., Nishikawa, S., Ogawa, S., Kunisada, T., Fujimoto, T., and Nishikawa, S. (1991) *Development* **113,** 689–699.