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Ks1, an epithelial cell-specific gene, responds to early signals of head formation in *Hydra*

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SUMMARY

As a molecular marker for head specification in *Hydra*, we have cloned an epithelial cell-specific gene which responds to early signals of head formation. The gene, designated *ks1*, encodes a 217-amino acid protein lacking significant sequence similarity to any known protein. KS1 contains a N-terminal signal sequence and is rich in charged residues which are clustered in several domains. *ks1* is expressed in tentacle-specific epithelial cells (battery cells) as well as in a small fraction of ectodermal epithelial cells in the gastric region subjacent to the tentacles. Treatment with the protein kinase C activator 12-*O*-tetradecanoylphorbol-13-acetate (TPA) causes a rapid increase in the level of *ks1* mRNA in head-specific epithelial cells and also induces ectopic *ks1* expression in cells of the gastric region. Sequence elements in the 5′-flanking region of *ks1* that are related to TPA-responsive elements may mediate the TPA inducibility of *ks1* expression. The pattern of expression of *ks1* suggests that a ligand-activated diacylglycerol second messenger system is involved in head-specific differentiation.

Key words: hydra, regeneration, pattern formation, protein kinase C, phosphatidylinositol, TPA, TRE

INTRODUCTION

Knowledge of the mechanisms of position-dependent differentiation of specific cell types is central to understanding how embryos are formed. 25 years ago, Lewis Wolpert (1969) introduced the concept of positional information and proposed that cells within a developing system are informed of their position with respect to one or more reference points. The cells then acquire a positional value and interpret their positional value by differentiating in a particular way. One example of pattern formation considered in detail by Wolpert was development and regeneration in *Hydra* (Wolpert, 1969; Wolpert et al., 1974).

Development in hydra consists mainly of head and foot formation from tissue of the body column (see Bode and Bode, 1984). When head and/or foot are removed, they regenerate within two to three days. A head always regenerates at the apical end and a foot regenerates at the basal end. These and other observations have produced strong evidence for the existence of positionally restricted ‘morphogenetic’ molecules in hydra (see Bode and Bode, 1984; Summerbell et al., 1991). The naturally occurring morphogens have not yet been identified. Considerable progress, however, has been made recently towards the molecular analysis of axial patterning in hydra.

Several homeodomain genes have been identified as candidates for specification of positional information (for review see Shenk and Steele, 1993). The expression pattern of one of them, *cnox-2*, is consistent with a role in axial pattern formation (Shenk et al., 1993). Furthermore, several genes have been isolated that become activated in hydra in spatial and temporal patterns (Kurz et al., 1991; Lopez de Haro et al., 1994).

The signal transduction system used in axial patterning in hydra has recently begun to be examined. Treatment with diacylglycerol (DAG) or 12-*O*-tetradecanoylphorbol-13-acetate (TPA) was found to induce ectopic heads in the body column (reviewed in Müller, 1993). Prolonged exposure of polyps to lithium causes appearance of ectopic feet along the body column (Hassel et al., 1993). Coadministration of DAG with arachidonic acid, a subordinate signaling molecule in the phosphatidylinositol-protein kinase C (PI-PKC) system, causes a strong increase in the head formation potential (Müller et al., 1993). DAG and TPA are known to activate PKC (Nishizuka, 1986) while lithium blocks several enzymes in the PI-PKC system (Berridge et al., 1989). Thus, it appears likely that a PKC-mediated intracellular pathway initiated by a ligand-receptor interaction on the cell surface mediates head specification in hydra.

Candidate genes responding to such a signaling system should be (i) expressed in head-specific cells, (ii) induced by TPA, and (iii) contain TPA-responsive elements in their promoter. Here we report the cloning and characterization of a gene, *ks1*, which fulfills these criteria and thus supports the view that a ligand-activated PI-PKC system is involved in the conversion of hydra gastric tissue to head tissue.

MATERIAL AND METHODS

Animals

Polyps of *Hydra magnipapillata* (strain sf-1) and *Hydra vulgaris* were used in this study. Polyps were cultured according to standard procedure (Lenhoff and Brown, 1970) at 18˚C.

TPA treatment

TPA was dissolved in acetone at 10 mM and stored at –20˚C. Polyps were incubated in TPA at a final concentration of 30-50 nM (in hydra medium) for 20 minutes at 18˚C. After treatment the animals were transferred to hydra medium and cultured at 18˚C until they were used.

cDNA library construction and differential screening

1×10^5 amplified recombinants of a oligo(dT)-primed epithelial cell-specific cDNA library (Lopez de Haro et al., 1994) were screened with ^{32}P-labeled cDNA made from head and gastric tissue of epithelial polyps by the random primer labeling procedure. 19 clones were picked as head specific; 17 of these clones encoded a single gene, designated *ks1*. The remaining 2 clones encoded different genes and are described elsewhere (Lopez de Haro et al., 1994). Since none of the 17 *ks1* cDNA clones was full length, one of the clones (cLK7) was used to rescreen a *H. vulgaris* cDNA library. The cDNA library in lambda ZAPII was prepared commercially (Stratagene) from *Hydra vulgaris* whole polyp poly(A)+ RNA and was generously provided by Drs Michael Sarras (University of Kansas Medical Center) and Hans Bode (University of California, Irvine). Rescreening resulted in the isolation of a number of additional clones, one of which was cLK7-41. Sequence analysis of cLK7-41 indicated the presence of an open reading frame beginning at the 5′ most ATG triplet. cLK7-41, however, did not encode 3′ sequences present in the initially isolated *ks1* clones. To complete the sequence and to confirm the region of overlap between cLK7-41 and the initial cDNA clones, inverse PCR was carried out as described below.

Isolation of genomic sequences by inverse PCR

To obtain the full-length sequence of *ks1* as well as 5′-flanking sequences, inverse PCR was carried out as described (Gellner et al., 1992) using two 20-nucleotide primers oriented such that primer-extension proceeded outward from the known sequence. Cleavage of *H. vulgaris* genomic DNA with *Hin*dIII resulted in a 3 kb fragment, which in Southern blots strongly hybridizes with cLK7-41. For inverse PCR, therefore, *Hin*dIII-digested and ligated DNA was used as template DNA. Oligonucleotide primers were synthesized complementary to nucleotides 84-103 and identical to nucleotides 297-319 (see Fig. 2). The expected 1.7 kb inverse PCR product was obtained and cloned into pBS– for further analysis. The relationship of this PCR-derived genomic clone, gIPCR1, to the cDNA clones is shown in Fig. 1.

Molecular techniques

Nucleic acid isolation, sequence analysis and RNA blot analysis were carried out following standard procedures (Sambrook et al., 1989). DNA sequences were analysed using the Hibio DNASIS/PROSIS program (Hitachi). All polymerase chain reactions (PCR) were carried out using the buffer conditions and Taq polymerase supplied by the manufacturer (Amersham). The 5′ boundary of the *ks1* transcript was determined by primer-extension following standard procedures. The 20-nucleotide IPCR5 primer was 5′ end-labeled, mixed with 9 μg of poly(A)+ RNA from head tissue, denatured and allowed to hybridize at 56˚C for 16 hours as described previously (Gellner et al., 1992). After primer elongation and RNase A treatment, the primer extension products were separated by 8.3 M urea/6% PAGE and visualized by autoradiography. EMBL database accession number for *ks1*, X78596.

In situ hybridization on macerates and whole mounts

In situ hybridization on whole mounts and macerates was carried out with digoxigenin-labeled DNA probes as described by Kurz et al. (1991) with the following modifications. Postfixed whole mounts were washed for 15 minutes in PBT, then for 20 minutes in 1/1 hybridization solution (HS)/PBT at room temperature and for 20 minutes in HS at room temperature. Afterwards whole mounts were prehybridized for 20 minutes in HS at 45˚C and hybridized as described.

RESULTS

Isolation and nucleotide sequence of *ks1*

We used a differential cDNA screening approach to isolate hydra genes expressed in head but not in gastric tissue. We focused our attention on epithelial cells, since this is the cell type responsible for morphogenesis in hydra. We isolated a number of clones encoding a tentacle-specific cDNA, which we have designated *ks1*. Since none of the cDNA clones was full length, we isolated additional clones from a cDNA library and a genomic clone by inverse PCR (see Materials and Methods) to complete the sequence.

The nucleotide sequence of the *ks1* gene was obtained from the combined results of the *H. vulgaris* cDNA and genomic clones shown in Fig. 1. The *ks1* sequence contains an open reading frame of 651 bp (Fig. 2). At the 5′ end, 392 nucleotides precede an ATG triplet. 15 bp upstream of the first in-frame methionine codon, there is an in-frame termination codon indicating that the first ATG triplet is indeed the initiator. The length of the transcript predicted from the combined length of the cDNA clones (906 bp) corresponds well to 0.9 kb mRNA detected on northern blots (see below) indicating that the full-length cDNA has been identified. Conceptional translation of the open reading frame predicts a 217 amino acid protein with a relative molecular mass of 25.4×10^3.

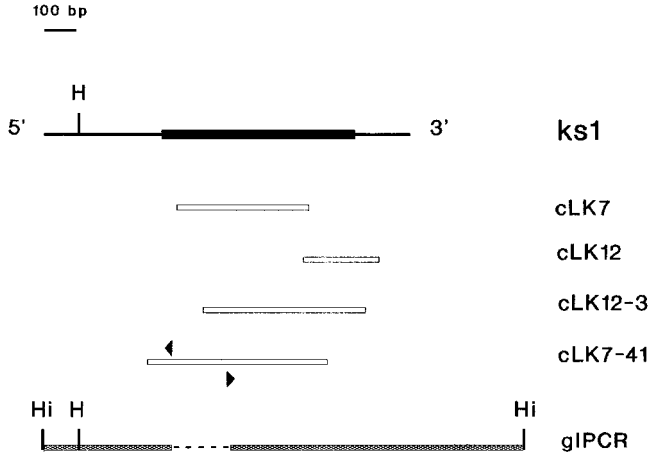

Fig. 1. Schematic representation of *ks1* clones and the *ks1* gene. Clones cLK7 and cLK12 were isolated by differential screening of a *H. magnipapillata* cDNA library. Subclone cLK12-3 was isolated from the same library by rescreening with cLK12. The nearly full-length cDNA clone cLK7-41 was isolated from a *H. vulgaris* cDNA library by rescreening with cDNA clone cLK7. The cDNA clones are represented by open bars. The genomic region amplified by inverse PCR (gIPCR) from *H. vulgaris* genomic DNA is represented as hatched bars. Filled arrowheads show position of primers used in inverse PCR. The solid bar in the *ks1* gene represents the coding region, the thin line indicates untranslated regions. H, *Hin*cII; Hi, *Hin*dIII.

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-324          aagcttaaatttttttgaaaaatatttttcaaagcacaaaagagaataac -275
-274  aaaattaaaaagcagtaaattaagataaaactttaaattaaatttgaaggctaatgaca -216
-215  taatggttgactgctgcgttatactttttttatatcctatgtataatacatctacaaaa -157
-156  tgacctacttgcttcaaagacaccgaaaacatataaataacatcgtatttctacatagc  -98
 -97 atcatcacatctatttgtattacatccaagattcaggtatgaaatttgaaggttttctg  -39
 -38 ttttcattatcttaaagatttattaacataaatagttcgatgaatttctagattatct    21
                                                  M   K   L      3
  22  acttttatctgtgtttttttcggattacaagtaattcaaagcagaa ATG AAA CTA   77

   4   I   I   V   L   V   M   M   L   V   C   V   Y   S   M   S   18
  78  ATA ATT GTG CTT GTA ATG ATG TTG GTT TGT GTG TAC AGC ATG AGT  122
                      IPCR5
  19   I   E   K   N   I   P   K   N   H   E   V   P   A   K   K   33
 123  ATT GAA AAG AAT ATA CCT AAG AAT CAC GAA GTA CCA GCC AAA AAA  167

  34   Q   F   A   E   T   K   V   E   K   K   K   R   S   D   D   48
 168  CAA TTT GCA GAA ACT AAA GTT GAA AAA AAG AAA CGT AGC GAT GAT  212

  49   G   D   E   E   I   C   D   D   D   D   E   D   C   E   D   63
 213  GGT GAT GAG GAA ATA TGC GAT GAC GAT GAT GAA GAT TGC GAA GAC  257

  64   V   V   D   I   E   E   C   N   E   D   D   D   D   C   V   78
 258  GTA GTA GAT ATA GAA GAA TGC AAT GAA GAT GAT GAT GAT TGC GTA  302

  79   D   G   G   E   T   E   E   C   D   E   D   D   D   D   C   93
 303  GAT GGA GGA GAA ACA GAA GAA TGC GAT GAA GAT GAT GAT GAT TGT  347
          IPCR3
  94   Q   E   E   K   K   K   K   K   R   E   T   K   P   K   L  108
 348  CAG GAG GAA AAA AAG AAA AAG AAA AGG GAA ACT AAA CCT AAA TTA  392

 109   K   K   R   N   D   D   E   E   E   E   E   C   E   E   D  123
 393  AAG AAA CGT AAC GAT GAC GAA GAA GAG GAA GAA TGC GAA GAA GAT  437

 124   D   E   D   C   E   V   E   V   D   I   E   E   C   D   E  138
 438  GAC GAA GAT TGC GAA GTT GAA GTA GAT ATA GAA GAA TGC GAT GAA  482

 139   E   D   D   D   C   Y   D   E   D   K   K   K   K   K   E  153
 483  GAA GAC GAT GAT TGC TAT GAT GAA GAT AAG AAA AAG AAA AAG GAA  527

 154   N   K   L   K   K   E   S   K   K   K   N   S   K   K   T  168
 528  AAC AAA CTG AAA AAA GAA AGC AAG AAG AAA AAT TCA AAG AAA ACA  572

 169   V   P   K   N   A   K   K   S   S   K   R   S   T   S   T  183
 573  GTG CCA AAG AAT GCA AAG AAA AGT TCT AAA AGA TCA ACG AGT ACA  617

 184   K   K   T   S   Q   K   K   Q   Q   D   K   R   G   A   I  198
 618  AAA AAG ACC AGC CAA AAA AAG CAA CAA GAT AAA AGA GGC GCA ATT  662

 199   Q   K   N   L   K   I   K   E   A   N   F   K   K   K   F  213
 663  CAA AAA AAC CTC AAA ATA AAA GAA GCA AAC TTT AAA AAA AAA TTC  707

 214   N   L   N   F   *                                          218
 708  AAC CTG AAT TTT TAA gta atg tat ttt tgt aaa agt tac ggc gtt  752
 753  ttt taa tat tat gtt tat ttt ttg att gcg ttt ttt gta gct cta  797
 798  ctt ttt aaa gcc aag gta gag ttt tca gta taa cac aat att taa  842
 843  att att cgt taa atc tga tta act tta aga aac aca ata aaa aac  887
 888  ttt tct ttt aat ctc atc c                                  906
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Fig. 2. Sequence of the *ks1* gene. The sequence was obtained from the *H. vulgaris* cDNA and genomic clones shown in Fig 1. The complete sequence of the *ks1* gene, the amino acid sequence of the single large open reading frame, as well as 580 nucleotides of flanking sequence are presented. Double underlines indicate putative regulatory elements (see Fig. 3 for details). Single underlines indicate primers used in inverse PCR.

The transcription initiation site was determined by primer extension using the IPCR5 primer complementary to nucleotide 84-103 of *ks1*. The labeled primer was hybridized to RNA from head tissue and extended by reverse transcriptase. The single extension product indicated that transcription initiates 68 bases upstream of the translational initiation codon (data not shown).

To identify sequences that potentially control *ks1* expression, we examined the 5′ flanking sequence for potential transcription factor binding sites. Comparison of this region with promoters of other genes revealed several segments of sequence conservation (Fig. 3). The sequence TATTAA (single underline in Fig. 3A) 17 bp upstream of the transcription start site is similar to TATA-like sequences in many eukaryotic promoters. At positions −62 to −70 the *ks1* gene contains the sequence 5′-AAGATTCAG-3′ (double underlined in Fig. 3A), which is similar to the TPA-responsive element (TRE, consensus sequence 5′-TGAGTCAG-3′, Angel et al., 1987). A sequence comparison between TREs in the control regions of TPA-inducible genes and the putative TRE in *ks1* is shown in Fig. 3B. The short conserved TRE sequence serves as the binding site for the transcription factor AP1 and is the only element required for TPA induction in a number of genes (Angel et al., 1987). At position −175 to −182, there is a sequence related to the binding site for the transcription factor

A

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-324 AAGCTTAAAT TTTTTTGAAA AATATTTTTC AAAGCACAAA AGAGAATAAC AAAATTAAAA AGCAGTAAAT -255

-254 TAAGATAAAA CTTTAAATTA AATTTGAAGG CTAATGACAT AATGGTTGAC TGCTGCGTTA TACTTTTTTT -185

-184 ATATCCTATG TATAATACAT CTACAAAATG ACCTACTTGC TTCAAAGACA CCGAAAACAT ATAAATAACA -115
       "U S F"
-114 TCGTATTTCT ACATAGCATC ATCACATCTA TTTGTATTAC ATCCAAGATT CAGGTATGAA ATTTGAAGGT  -45
                                              "T R E"
 -44 TTTCTGTTTT CATTATCTTA AAGATTTTAT TAACATAAAT AGTTCGATGA ATTTCTAGAT TATCTACTTT   26
                                                 +1
  27 TTATTAGACT GTGTTTTTTT CGGATTACAA GTAATTCAAA GCAGAAATG                          75
                                                    Met
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B

TPA inducible gene	TRE[1]
Hydra *ks1*	A A G A T T C A G
Human collagenase	A T G A G T C A G
Rat stromelysin	A T G A G T C A G
Human MTIIA	G T G A C T C A G
Interleukin 2	T T C A G T C A G

[1] For references see Angel et al.(1987)

C

TPA inducible gene	USF[1]
Heme oxygenase	G T C A T A T G A C
Hydra *ks1*	A T C C T A T G T A
	* * *

[1] See Muraosa and Shibahara (1993). The three nucleotides marke with a star are essential for protein binding.

Fig. 3. Analysis of the 5′ flanking sequence of *Hydra ks-1*. (A) Nucleotide sequence of the 5′ flanking region of the *ks-1* gene. Only the coding-strand sequence, numbered from the transcription start site (indicated by an asterisk), is shown. The putative binding sites for transcription factors USF/MLTF (MTE) and AP1 (TRE) are indicated by double underlines. A single underline shows the putative TATA sequence. (B) Sequence comparison of putative TREs in the control regions of TPA-inducible genes (Angel et al., 1987) and *Hydra ks1*. Sequences are aligned with respect to the invariant TCAG tetranucleotide stretch. Conserved nucleotides are enclosed in boxes. (C) Sequence comparison of the MTE sequence in the 5′ flanking region of TPA-inducible human heme oxygenase gene and hydra *ks1*. The two genes are aligned with respect to the three nucleotides (CNNNTG) shown to be crucial for protein binding (Muraosa Y. and S. Shibahara, 1993). Conserved nucleotides are enclosed in boxes.

MLTF/USF in the TPA-inducible heme oxygenase gene (Muraosa and Shibahara, 1993). As shown below, hydra *ks1* is responsive to TPA (see Fig. 9). Thus, it is the first TPA responsive gene known to contain both TRE and MLTF/USF sequences in the 5′ flanking region. At the 3′ terminus, a consensus polyadenylation sequence, AATAAA, is located at position 878-883 (Fig. 2).

The *ks-1* protein is composed of several structural domains

A search of various protein data banks with the KS1 amino acid sequence revealed no homology between KS1 and any other protein. KS1, however, contains several interesting structural features (Fig. 4). Hydropathy analysis of KS1 predicted a strongly hydrophilic character (Fig. 4A). The N-terminal 15 residues are strongly hydrophobic, indicative of a signal sequence for translocation across the endoplasmic reticulum. An acceptable cleavage site for a signal peptidase is present between Ser16 and Met17 (von Heijne, 1983). It seems likely, therefore, that KS1 encodes a secreted protein. No potential N-linked glycosylation sites are present in the KS1 sequence.

One striking feature of the predicted KS1 protein is the large number of charged residues that are clustered into highly acidic and basic domains (Fig. 4B). Beginning at residue 32, 7 out of the next 15 amino acids are lysine and arginine. This basic domain (B1 in Fig. 4B) is followed by a acidic domain (A1 in Fig. 4B) consisting of 66% aspartic acid and glutamic acid residues. This acidic domain is immediately followed by a 16 amino acid basic region (B2), including a stretch of 6 consecutive lysine and arginine residues. The 35 amino acid domain A2 following this basic domain shows remarkable amino acid similarity to the first acidic domain A1. The carboxy terminal domain (B3) contains 42% lysine or arginine residues.

Analysis of the deduced amino acid sequence revealed two regions of internal sequence similarity comprising residues 31-96 and 97-146. The repeated sequences are aligned in Fig. 4C. Structural-prediction algorithms (Chou-Fasman) predict a helix-turn-helix structure for these repeats.

Position-dependent expression of *ks1*

To determine the expression pattern of *ks1*, we analyzed northern blots of RNA from head, upper one third and lower two thirds of *H. vulgaris* polyps (Fig. 5). In head tissue a single, abundant 0.9 kb transcript was detected. The same transcript was also found in the upper third of the body column, although at a much lower level (lane 2 in Fig. 5). No *ks1* transcripts were detected in the lower two thirds.

To determine the cell-type specificity of *ks1* expression, we performed in situ hybridizations on macerated cells (Fig. 6). Fig. 6A shows that, in macerates hybridized with clone cLK12, tentacle-specific ectodermal epithelial cells (battery cells) were heavily labelled. The occurrence of *ks1* transcripts in the upper third of the gastric region, which includes the 'tentacle formation zone' (Fig. 5, lane 2), suggests that there is a population of cells in this region not yet differentiated to tentacle-specific cells but already expressing *ks1*. To test this possibility, we performed in situ hybridization on macerates from the upper third of the gastric region. Fig. 6B shows that in this region *ks1* hybridizes to a population of epithelial cells which are morphologically indistinguishable from gastric epithelial cells. Cell counts indicated that about 10% of the ectodermal epithelial cells in the 'tentacle formation zone' region are *ks1* positive. These cells appear to represent body column cells determined to differentiate to tentacle epithelial cells. Thus, *ks1* expression appears to be initiated early in head specification.

ks1 expression during head regeneration

Activation of *ks1* expression during head regeneration was studied by in situ hybridization on whole mounts (Fig. 7). Animals were decapitated directly below the tentacles and hybridized to *ks1* 1, 2 and 4 days later. *ks1* expression could be detected in ectodermal epithelial cells by 2 days when tentacles were beginning to evaginate (Fig. 7C,D). When regenerating heads at this stage were viewed from above (Fig. 7D), staining could also be seen in the most apical region of the hypostome (arrow in Fig. 7D). In mature heads (Fig. 7E,F) staining was restricted to the tentacles: staining appeared to end abruptly at the base of the tentacle; no staining was found between the tentacles. In whole mounts, it was not possible to detect *ks1*-positive cells in the upper third of the gastric region. Thus, the whole-mount in situ procedure appears to be too

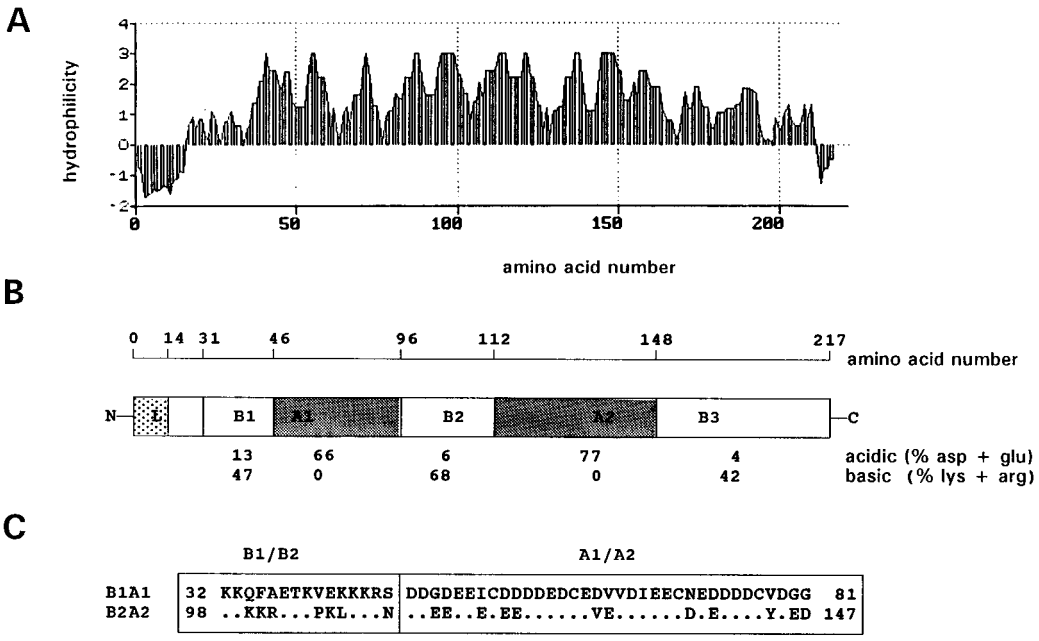

Fig. 4. Analysis of the KS1 amino acid sequence. (A) Hydropathy plot of the predicted KS1 product. (B) Domain structure of KS1. Cross-hatched areas, acidic domain; open boxes, basic domains; stippled, signal peptide. (C) Internal sequence similarity in two stretches of KS1 sequence. Dots represent identical amino acids.

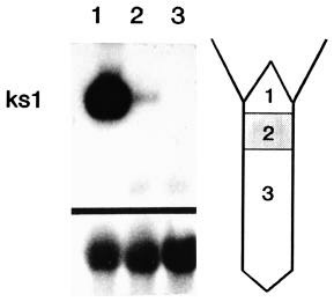

Fig. 5. Spatial expression pattern of *ks1* in hydra. 15 µg of total RNA was isolated from the region indicated and hybridized with a ^{32}P-labeled *ks1* cDNA probe. A ribosomal DNA fragment was used as control to demonstrate equal loading of RNA (Bosch et al., 1989).

insensitive to detect the *ks1* expressing epithelial cells observed in macerates of the tentacle formation zone (Fig. 6B).

ks1 expression in TPA-treated polyps

Treatment of hydra with the PKC activators DAG or TPA results in appearance of ectopic head structures in the body column (Müller, 1989, 1993). To investigate whether TPA caused changes in the pattern of *ks1* expression, we exposed decapitated polyps to TPA for 20 minutes and allowed heads to regenerate for 1, 4 and 24 hours. Thereafter regenerating polyps were cut in the middle and the resulting upper and lower body column segments were assayed for *ks1* expression using northern blotting. The results in Fig. 8 demonstrate that treatment with TPA alters *ks1* expression significantly.

In the upper portions of TPA-treated polyps, the level of *ks1* transcripts increased dramatically within 1 hour compared to untreated animals. In situ hybridization of macerated tissue from these animals indicated that there was no corresponding increase in the proportion of *ks1*-expressing cells compared to untreated animals. Thus, the increase in *ks1* transcripts appears to represent an activation of transcription in *ks1*-expressing cells. In the lower portions of TPA-treated animals, there were no rapid changes in *ks1* expression. There was, however, an increase in *ks1* expression beginning 4 hours after TPA treatment. Cells in this region do not normally express *ks1* (Fig. 5) and hence this expression represents ectopic activation of a positionally dependent gene.

The results in Fig. 8 also indicate that *ks1* transcription is activated during normal head regeneration. The increase in *ks1* transcripts is detectable beginning by 4 hours of regeneration and thus is delayed compared to the rapid effect of TPA (see Discussion).

DISCUSSION

Patterning in hydra has been studied extensively at the tissue level using grafting techniques and monoclonal antibodies (for reviews see Bode and Bode, 1984; Müller, 1993). No probes have been available, however, for examining the patterning process at the molecular level. In this report, we present the molecular cloning of such a probe.

The head-specific *ks1* gene encodes a novel protein

The sequence of the *ks1* gene product has no similarity to other proteins in various databases. KS1 is predicted to contain a

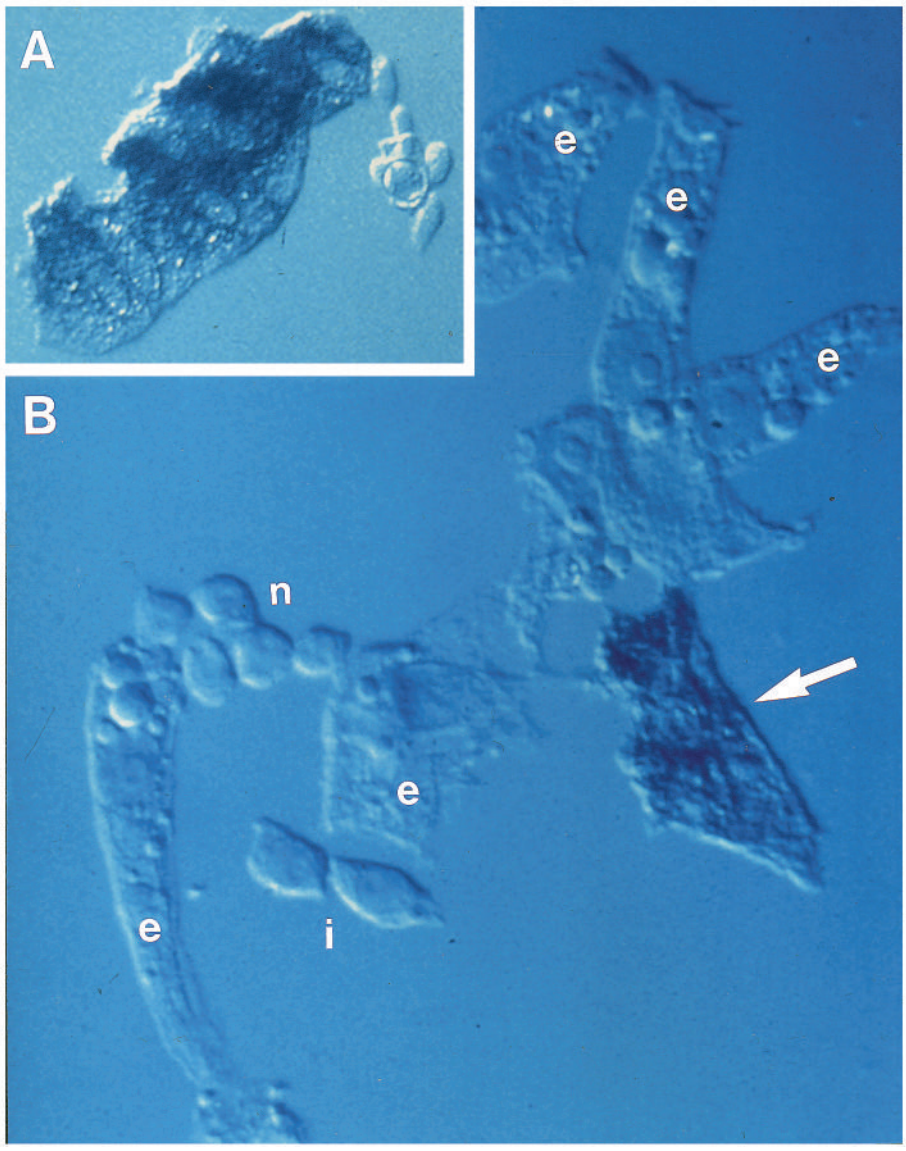

Fig. 6. Expression of *ks1* in macerated cells. (A) Macerates from whole polyps showing heavily stained tentacle-specific epithelial cell. (B) Macerates from the upper gastric region (region 2 in Fig. 5) showing *ks1*-expressing ectodermal epithelial cell (arrow). e, epithelial cell; i, interstitial cell; n, nematoblasts. *ks1* clone cLK7 labeled with digoxigenin-dUTP was used as probe.

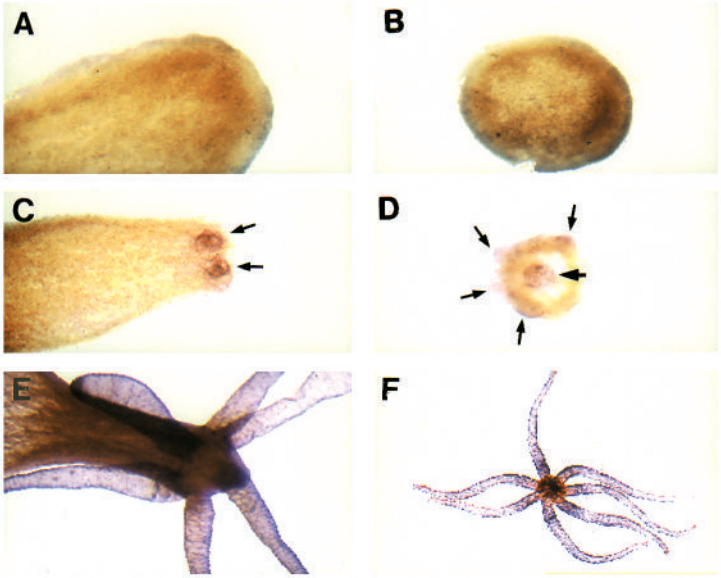

Fig. 7. Activation of expression of *ks1* during head regeneration. A/B, 1 day after decapitation. C/D, 2 days after decapitation. Filled arrow points to staining in hypostomal zone. E/F, 4 days after decapitation. *ks1* expression is indicated by the purple-blue staining along the entire length of the tentacles. The body column (E) and hypostome (F, center) are not stained and appear brownish. A, C and E are from side. B, D and F are from above. Staining is restricted to tentacle epithelial cells.

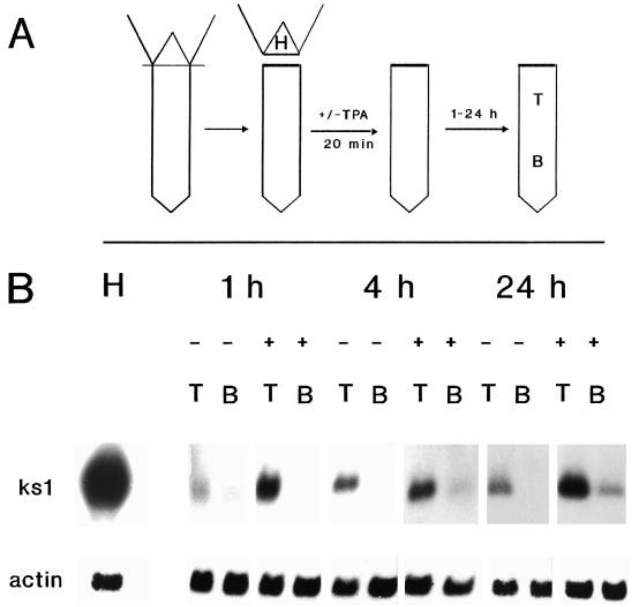

Fig. 8. Effect of TPA on expression of *ks1* in regenerating polyps. (A) Experimental procedure. (B) Northern blot using 15 μg of total RNA and a *ks1* cDNA probe. A hydra actin cDNA kindly provided by Hans Bode was used as control to demonstrate equal loading of RNA. T, upper gastric region; B, lower gastric region. –, untreated polyps. +, polyps exposed to 30 nM TPA for 20 minutes.

leader sequence and several distinct domains of charged amino acid residues (Fig. 4C). The first cluster of basic and acidic amino acids (residues 32-96) shows significant sequence similarity to the second cluster of basic and acidic amino acids (residues 97-147) and may be due to sequence duplication. Several other proteins with highly acidic domains have been described and include nuclear-localized proteins such as nucleolin and cytosolic proteins such as the yeast sec7 protein. In all these proteins, the acidic domains are thought to serve as structural motifs for interaction with lipids or other proteins (Achstetter et al., 1988). Hydra KS1 appears to be a secreted protein and could be adsorbed on the cell surface by its highly charged amino acid domains. It may be a component of the carbohydrate-rich glycocalyx that covers the surface of hydra polyps.

A monoclonal antibody, CP8, which specifically recognizes ectodermal epithelial cells in the head (Javois et al., 1986), has been shown to detect a protein localized in granules near the apical surface of ectodermal epithelial cells. Since KS1 contains a leader sequence, it could encode a secretory protein and be located, at least transiently, in cytosolic granules. It is intriguing to speculate that the *ks1* gene product is the CP8 antigen.

Position-dependent expression of *ks1*

The upper gastric region subjacent to tentacles has previously been defined as the 'tentacle formation zone' (Hobmayer et al., 1990); epithelial cells in this region become committed to battery cell differentiation and are displaced into the tentacles. *ks1* is expressed at high levels in battery cells as well as in a subpopulation of ectodermal epithelial cells in the tentacle formation zone. *ks1*-expressing cells in this region are morphologically indistinguishable from *ks1*-negative cells. Thus, *ks1* is a very early marker for head-specific differentiation.

During head regeneration, at a stage when tentacles are just beginning to evaginate (C and D in Fig. 7), *ks1* expression is observed at the most apical tip and in the tentacle buds. At later stages, no expression of *ks1* is detected in the tip whereas the tentacles are strongly stained. This pattern of *ks1* expression is strikingly similar to that found previously with the head-specific monoclonal antibody TS-19 (Bode et al., 1988). During head regeneration, TS-19 label first appears at the most apical tip. At later stages, it spreads to the evaginating tentacles and disappears from the apex.

The transient appearance of *ks1* and TS19 in the most apical region of regenerating tips is consistent with the view (Bode et al., 1988) that the presumptive hypostome area passes through a stage of 'tentacleness' before final differentiation. This pattern of expression of *ks1* and TS-19 can be explained by a model proposed recently by Hans Meinhardt (1993). According to Meinhardt's model, tentacle formation is activated at regenerating tips; hypostome formation then locally inhibits tentacle formation and displaces it to the sub-hypostomal region. *ks1* could be a suitable molecular probe to dissect the mechanism behind this patterning process.

Hydra head-specification appears to involve a receptor-activated protein kinase C second messenger pathway

The northern analysis in Fig. 8 indicates a rapid and a delayed effect of TPA on *ks1* expression. The rapid effect appears to be due to direct activation of the second messenger system in cells already expressing *ks1* since TPA treatment simply increases the amount of *ks1* transcript but does not increase the number of positive cells. This suggests that *ks1* expression is

regulated by a ligand-activated PKC second messenger pathway. The observation that *ks1* expression is also increased by 4 hours of regeneration in tissue untreated with TPA suggests that the endogenous ligand begins to be produced by this time.

By comparison, the delayed effect, which leads to generation of ectopic *ks1*-positive cells in the lower body column and requires at least 4 hours to become detectable, appears to be an indirect effect of TPA treatment. We imagine that TPA activates a signaling system for head formation in the body column and that this signal then induces ectopic formation of *ks1*-positive cells.

Two potential *cis*-regulatory elements are present in the 5′ flanking sequences of *ks1* and might serve to transmit the TPA signal(s) to the transcriptional machinery. *ks1* is the first gene in hydra found to be sensitive to TPA; its structure and expression pattern fulfill a prediction that followed from Müller's results (Müller 1989, 1993). Whether other head-specific genes in hydra also have TPA-responsive sequence elements in their 5′ flanking sequences remains to be elucidated. It is interesting to note, however, that *Cnox-2*, a Hydra homeobox gene thought to be involved in axial patterning, also seems to be regulated by a signal transduction pathway involving PKC (Shenk et al., 1993).

Similar observations have been made in several other developing organisms. PKC has been suggested to participate in controlling differentiation along an embryonic axis in *Xenopus* (Otte et al., 1988), in determining cell fate in *Dictyostelium* (Ginsburg and Kimmel, 1989; Peters et al., 1989), and in establishing cell fate in sea urchin embryos (Livingston and Wilt, 1992).

Regardless of the mechanisms by which PKC modulates *ks1* expression in *Hydra*, the results presented in this paper support the hypothesis that signaling mediated by ligand/receptor interactions at the plasma membrane is important in instructing hydra cells to differentiate according to their position in the embryo. Studying *ks1* expression may now allow us to trace the mechanism necessary for transcriptional activation back to events at the cell surface.

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