

## SHORT COMMUNICATION

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## An IQGAP-related gene is activated during tentacle formation in the simple metazoan *Hydra*

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**Abstract** Differentiation of body column epithelial cells into tentacle epithelial cells in *Hydra* is accompanied by changes in both cell shape and cell-cell contact. The molecular mechanism by which epithelial cells acquire tentacle cell characteristics is unknown. Here we report that expression of a *Hydra* homologue of the mammalian IQGAP1 protein is strongly upregulated during tentacle formation. Like mammalian IQGAP, *Hydra* IQGAP1 contains an N-terminal calponin-homology domain, IQ repeats and a conserved C terminus. In adult polyps a high level of *Hydra* IQGAP1 mRNA is detected at the basis of tentacles. Consistent with a role in tentacle formation, IQGAP1 expression is activated during head regeneration and budding at a time when tentacles are emerging. The observations support the previous hypothesis that IQGAP proteins are involved in cytoskeletal as well as cell-cell contact rearrangements.

**Key words** Cnidaria · IQGAP · *Hydra* · Cytoskeleton · Regeneration

### Introduction

In the freshwater polyp *Hydra*, cells proliferate continuously and are continuously displaced towards head and

foot region (Bosch, 1998). Position-dependent signals control the differentiation behavior of the cells. When epithelio-muscular cells are displaced towards the upper gastric region they immediately change their gene expression profile (Endl et al. 1999) and form multicellular complexes of nerve cells and nematocytes associated with the epithelial cells (Hobmayer et al. 1990). The differentiation of body column epithelial cells into tentacle epithelial cells is accompanied by drastic changes both in cell shape and adhesive contacts. The molecular mechanisms controlling this differentiation behavior are not understood.

IQGAP proteins are widely expressed modular proteins composed of various domains that bind multiple proteins and might be involved in several biological processes. The N-terminal region of IQGAP contains a calponin homology (CH) domain usually found in proteins that bind F-actin. Next to it, a domain named IQGAP repeats (IR) contains six copies of a unique amino acid consensus motif. To date, the IR domain is found only in this protein family. The function of the IR domain is unknown. C-terminal to the IR domain there is a WW domain as well as four IQ motifs which are implicated in binding calmodulin (Weissbach et al. 1994). The GAP related domain (GRD) of IQGAP is unable to stimulate GTPase activity and does not bind to Ras. Instead, IQGAP binds the Rho family members Cdc42 and Rac1 which have been implicated in reorganization of the actin cytoskeleton and diverse cellular functions including cell shape, cell motility and cytokinesis (Kuroda et al. 1996). The domain structure makes IQGAPs excellent candidates as signaling scaffolds and regulators of the cytoskeleton. After IQGAPs were described in mammals, several members of the IQGAP family were reported in simple organisms such as slime molds (reviewed in Chisholm 1997) and yeast (Epp and Chant 1997; Osman and Cerione 1998). In searching for a function for IQGAP proteins in multicellular organisms, IQGAP1 was recently found to play a crucial role in controlling cadherin-mediated cell-cell adhesion (Kuroda et al. 1998). In mammalian cells, IQGAP1 appears to induce the dissociation of  $\alpha$ -catenin from the E-cad-

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herin cell-adhesion complex by competing with  $\alpha$ -catenin's binding to  $\beta$ -catenin; this results in the loss of E-cadherin-mediated cell adhesion.

Since tentacle formation in the basal metazoa *Hydra* involves both modulation of adhesive contacts and extensive changes in cell shape, we investigated whether IQGAP-like proteins are associated with the transition of gastric epithelial cells into tentacle epithelial cells. Supporting the idea that the function of IQGAP proteins in metazoa is to control cell-cell and cytoskeletal rearrangements, we report here that in *Hydra* IQGAP1 is upregulated during the transition of body column epithelial cells into tentacle epithelial cells.

## Materials and methods

### Animals

*Hydra vulgaris* were cultured according to standard procedures at 18°C.

### Cloning the *Hydra* IQGAP cDNA

Coincidentally, a clone isolated in a differential display PCR screening approach contained a IQGAP-like sequence. On the basis of this sequence homology, a 126 bp cDNA fragment was isolated by PCR from *H. vulgaris* using primers HYGAP5 (5'-CAG CTG AAG AAA TCA ATG CTA TGA-3') and HYGAP3 (5'-CAG ATG TAG TGC AGC ATC ATT-3'). For isolation of a full-length cDNA clone,  $2 \times 10^5$  plaques of an *H. vulgaris* cDNA library (gift from Hans Bode, UC Irvine, USA) were screened with the PCR fragment. Ten positive clones were detected and isolated. Restriction maps and initial sequence analysis indicated that all ten clones were derived from the same gene. Although the largest clone contained an insert of about 4.5 kb with three of the five characteristic IQGAP domains, none of the clones contained the 5'- and the 3'-located calponin homology (CH) and GAP related (GRD) domains. Rescreening the library using 5'- and 3'-specific cDNA clones as probes resulted in isolation of a nearly full-length clone containing the five IQGAP-specific domains. Sequence comparison to IQGAP related genes indicated that about 600 bp at the 3' end were missing. All attempts to obtain this sequence by inverse PCR, 3' RACE techniques and by screening a genomic library have so far failed.

### Molecular biological procedures and expression analysis

Molecular biological procedures were done according to standard protocols and as described previously (Endl et al. 1999; Kumpfmüller et al. 1999). The nucleotide sequences were used to perform BLAST homology searches as described by Altschul et al. (1997). The expression pattern of the *Hydra* IQGAP1 gene in whole animals was determined using the whole-mount in situ hybridization procedure described previously (Endl et al. 1999; Kumpfmüller et al. 1999). Additionally, Northern blot analysis was used to compare the expression of the IQGAP1 gene at different time-points during head regeneration. Total RNA was isolated using the mRNA extraction kit from Pharmacia Biotech, fractionated on a 1.0% agarose / formaldehyde gel and transferred to a nylon membrane. Filters were hybridized at 50°C as described previously (Kumpfmüller et al. 1999).

### Orthology assignments

Amino acid sequences corresponding to the deduced IQGAP1 fragment from *H. vulgaris* were aligned to domains of other Ras-

GAP related proteins using the CLUSTAL W Multiple Sequence Alignment Program version 1.7 (Thompson et al 1994) available at the internet site of the Baylor College of Medicine (<http://dot.imgen.bcm.tmc.edu:9331/multi-align/Options/clustalw.html>). Phylogenetic analysis was performed using the TREE-CON for Windows (version 1.1) program (Van de Peer and De Wachter 1994). Branch lengths correspond to the number of amino acid substitutions per site.

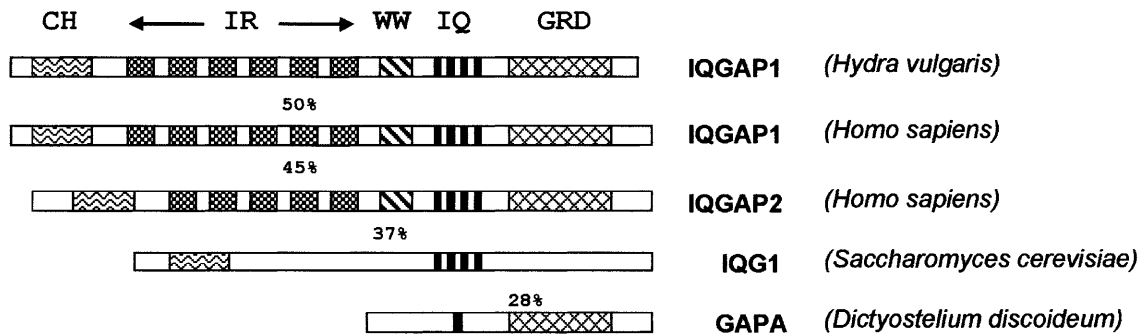
## Results and discussion

### Isolation and structure of *Hydra* IQGAP1

In a PCR-based differential screening (DD-PCR) approach designed to search for transcripts that are differentially expressed in polyps undergoing sexual reproduction, one of the non-differential PCR fragments cloned showed high sequence similarity to the recently reported human IQGAP1 gene. Cloning of the corresponding full-length cDNA was carried out in two steps. First, using sequence-specific primers against the coincidentally isolated PCR fragment, we isolated a short 126 bp cDNA fragment from *H. vulgaris* by PCR. This cDNA clone was then used to screen a *H. vulgaris* cDNA library (see Materials and methods). The largest cDNA clone obtained (deposited as GenBank accession number AF139186) contained a 4447 bp insert with an open reading frame (ORF) which is 1448 residues long. Since this cDNA clone contains all five domains which are characteristic for the IQGAP family of proteins, it was designated *Hydra* IQGAP1.

Alignment of the sequences of the *Hydra* IQGAP1 and the mammalian IQGAP proteins shows about 50% sequence identity at the amino acid level over the entire length of the protein chain (Fig. 1A), revealing a high degree of conservation through the 400 million years of evolution that separates *Hydra* and man. Similar to the mammalian IQGAP, the N-terminal region of the *Hydra* IQGAP1 protein contains a calponin homology (CH) domain followed by six unique repeats (IR) and a WW domain (Fig. 1A–C). At the C-terminal end of the protein there is a 234 amino-acid long GAP related domain domain (GRD) that is also present in *Dictyostelium* GAPA protein but absent in yeast IQG1. A phylogenetic tree based on the highly conserved GRD domain emphasizes the close relationship between *Hydra* IQGAP1 and its human homologues (Fig. 1F). The multidomain structure of *Hydra* IQGAP1 indicates that this protein is well suited for interaction with cytoplasmic targets and regulation of actin-dependent morphological changes.

In proteins from unicellular eukaryotes, several of the characteristic IQGAP features can be found. For example, the *Saccharomyces cerevisiae* IQG1 contains a calponin homology domain as well as the IQ repeats but lacks the GRD motif (Fig. 1A). *Dictyostelium discoideum* GAPA protein, in contrast, lacks the CH and IR motif but contains a GRD domain very similar to the corresponding motif in human IQGAP1. In contrast, the IQGAP1 protein described here in the basal metazoan

**A****B****CH domain**

IQGAP1 (Hydra) 33-L<sup>W</sup>E<sup>W</sup>E<sup>W</sup>A<sup>W</sup>R<sup>W</sup>I<sup>W</sup>L<sup>W</sup>E<sup>W</sup>G<sup>W</sup>C<sup>W</sup>I<sup>W</sup>E<sup>W</sup>E<sup>W</sup>V<sup>W</sup>P<sup>W</sup>T<sup>W</sup>T<sup>W</sup>E<sup>W</sup>L<sup>W</sup>E<sup>W</sup>Q<sup>W</sup>L<sup>W</sup>T<sup>W</sup>N--G<sup>W</sup>V<sup>W</sup>I<sup>W</sup>L<sup>W</sup>A<sup>W</sup>L<sup>W</sup>A<sup>W</sup>H<sup>W</sup>F<sup>W</sup>A<sup>W</sup>P<sup>W</sup>N<sup>W</sup>V<sup>W</sup>L<sup>W</sup>R<sup>W</sup>K<sup>W</sup>I<sup>W</sup>F<sup>W</sup>D<sup>W</sup>K<sup>W</sup>L<sup>W</sup>T<sup>W</sup>R<sup>W</sup>Y<sup>W</sup>K<sup>W</sup>E<sup>W</sup>R<sup>W</sup>G<sup>W</sup>L<sup>W</sup>H<sup>W</sup>F<sup>W</sup>R<sup>W</sup>H<sup>W</sup>T<sup>W</sup>D<sup>W</sup>N<sup>W</sup>I<sup>W</sup>M<sup>W</sup>Y<sup>W</sup>F<sup>W</sup>D<sup>W</sup>R<sup>W</sup>A<sup>W</sup>M<sup>W</sup>E<sup>W</sup>I<sup>W</sup>G<sup>W</sup>L<sup>W</sup>P<sup>W</sup>K<sup>W</sup>I<sup>W</sup>F<sup>W</sup>Y<sup>W</sup>P<sup>W</sup>E<sup>W</sup>T<sup>W</sup>T<sup>W</sup>I<sup>W</sup>D<sup>W</sup>M<sup>W</sup>K<sup>W</sup>N<sup>W</sup>P<sup>W</sup>R<sup>W</sup>A<sup>W</sup>I<sup>W</sup>Y<sup>W</sup>C<sup>W</sup>I<sup>W</sup>H<sup>W</sup>A<sup>W</sup>L-141  
 IQGAP1 (Human) 47-L<sup>W</sup>E<sup>W</sup>E<sup>W</sup>A<sup>W</sup>K<sup>W</sup>R<sup>W</sup>W<sup>W</sup>E<sup>W</sup>A<sup>W</sup>C<sup>W</sup>L<sup>W</sup>G<sup>W</sup>E<sup>W</sup>D<sup>W</sup>L<sup>W</sup>P<sup>W</sup>T<sup>W</sup>T<sup>W</sup>E<sup>W</sup>L<sup>W</sup>E<sup>W</sup>G<sup>W</sup>L<sup>W</sup>R<sup>W</sup>N--G<sup>W</sup>V<sup>W</sup>Y<sup>W</sup>L<sup>W</sup>A<sup>W</sup>K<sup>W</sup>L<sup>W</sup>G<sup>W</sup>N<sup>W</sup>F<sup>W</sup>F<sup>W</sup>S<sup>W</sup>P<sup>W</sup>K<sup>W</sup>V<sup>W</sup>S<sup>W</sup>L<sup>W</sup>K<sup>W</sup>I<sup>W</sup>Y<sup>W</sup>D<sup>W</sup>R<sup>W</sup>E<sup>W</sup>Q<sup>W</sup>T<sup>W</sup>R<sup>W</sup>Y<sup>W</sup>K<sup>W</sup>A<sup>W</sup>T<sup>W</sup>G<sup>W</sup>L<sup>W</sup>H<sup>W</sup>F<sup>W</sup>R<sup>W</sup>H<sup>W</sup>T<sup>W</sup>D<sup>W</sup>N<sup>W</sup>I<sup>W</sup>Q<sup>W</sup>W<sup>W</sup>L<sup>W</sup>N<sup>W</sup>A<sup>W</sup>M<sup>W</sup>E<sup>W</sup>I<sup>W</sup>G<sup>W</sup>L<sup>W</sup>P<sup>W</sup>K<sup>W</sup>I<sup>W</sup>F<sup>W</sup>Y<sup>W</sup>P<sup>W</sup>E<sup>W</sup>T<sup>W</sup>T<sup>W</sup>I<sup>W</sup>D<sup>W</sup>R<sup>W</sup>K<sup>W</sup>N<sup>W</sup>M<sup>W</sup>P<sup>W</sup>R<sup>W</sup>C<sup>W</sup>I<sup>W</sup>Y<sup>W</sup>C<sup>W</sup>I<sup>W</sup>H<sup>W</sup>A<sup>W</sup>L-155  
 IQGAP2 (Human) 44-L<sup>W</sup>E<sup>W</sup>E<sup>W</sup>A<sup>W</sup>K<sup>W</sup>R<sup>W</sup>W<sup>W</sup>E<sup>W</sup>V<sup>W</sup>C<sup>W</sup>L<sup>W</sup>V<sup>W</sup>E<sup>W</sup>L<sup>W</sup>P<sup>W</sup>T<sup>W</sup>T<sup>W</sup>E<sup>W</sup>L<sup>W</sup>E<sup>W</sup>G<sup>W</sup>L<sup>W</sup>R<sup>W</sup>N--G<sup>W</sup>V<sup>W</sup>Y<sup>W</sup>L<sup>W</sup>A<sup>W</sup>K<sup>W</sup>L<sup>W</sup>A<sup>W</sup>K<sup>W</sup>F<sup>W</sup>A<sup>W</sup>P<sup>W</sup>K<sup>W</sup>M<sup>W</sup>V<sup>W</sup>S<sup>W</sup>E<sup>W</sup>K<sup>W</sup>I<sup>W</sup>Y<sup>W</sup>D<sup>W</sup>V<sup>W</sup>E<sup>W</sup>Q<sup>W</sup>T<sup>W</sup>R<sup>W</sup>Y<sup>W</sup>K<sup>W</sup>S<sup>W</sup>L<sup>W</sup>H<sup>W</sup>F<sup>W</sup>R<sup>W</sup>H<sup>W</sup>T<sup>W</sup>D<sup>W</sup>N<sup>W</sup>T<sup>W</sup>V<sup>W</sup>Q<sup>W</sup>W<sup>W</sup>L<sup>W</sup>R<sup>W</sup>A<sup>W</sup>M<sup>W</sup>E<sup>W</sup>S<sup>W</sup>I<sup>W</sup>G<sup>W</sup>L<sup>W</sup>P<sup>W</sup>K<sup>W</sup>I<sup>W</sup>F<sup>W</sup>Y<sup>W</sup>P<sup>W</sup>E<sup>W</sup>T<sup>W</sup>T<sup>W</sup>I<sup>W</sup>D<sup>W</sup>R<sup>W</sup>K<sup>W</sup>N<sup>W</sup>I<sup>W</sup>P<sup>W</sup>R<sup>W</sup>M<sup>W</sup>I<sup>W</sup>Y<sup>W</sup>C<sup>W</sup>I<sup>W</sup>H<sup>W</sup>A<sup>W</sup>L-152  
 IQG1 (Yeast) 111-V<sup>W</sup>S<sup>W</sup>E<sup>W</sup>V<sup>W</sup>K<sup>W</sup>I<sup>W</sup>W<sup>W</sup>E<sup>W</sup>A<sup>W</sup>V<sup>W</sup>I<sup>W</sup>E<sup>W</sup>A<sup>W</sup>L<sup>W</sup>P<sup>W</sup>S<sup>W</sup>E<sup>W</sup>I<sup>W</sup>E<sup>W</sup>L<sup>W</sup>C<sup>W</sup>V<sup>W</sup>G<sup>W</sup>D<sup>W</sup>S<sup>W</sup>L<sup>W</sup>R<sup>W</sup>N<sup>W</sup>G<sup>W</sup>V<sup>W</sup>L<sup>W</sup>A<sup>W</sup>K<sup>W</sup>L<sup>W</sup>T<sup>W</sup>Q<sup>W</sup>R<sup>W</sup>I<sup>W</sup>N<sup>W</sup>P<sup>W</sup>D<sup>W</sup>L<sup>W</sup>T<sup>W</sup>T<sup>W</sup>V<sup>W</sup>I<sup>W</sup>P<sup>W</sup>A<sup>W</sup>G<sup>W</sup>D<sup>W</sup>K<sup>W</sup>L<sup>W</sup>Q<sup>W</sup>N<sup>W</sup>I<sup>W</sup>A<sup>W</sup>F<sup>W</sup>G<sup>W</sup>L<sup>W</sup>V<sup>W</sup>E<sup>W</sup>H<sup>W</sup>V<sup>W</sup>G<sup>W</sup>V<sup>W</sup>D<sup>W</sup>S<sup>W</sup>F<sup>W</sup>R<sup>W</sup>E<sup>W</sup>L<sup>W</sup>Q-----D<sup>W</sup>L<sup>W</sup>Y<sup>W</sup>K<sup>W</sup>N<sup>W</sup>K<sup>W</sup>N<sup>W</sup>I<sup>W</sup>P<sup>W</sup>Q<sup>W</sup>V<sup>W</sup>F<sup>W</sup>E<sup>W</sup>T<sup>W</sup>L<sup>W</sup>H<sup>W</sup>I<sup>W</sup>L-213

W L G L L P V N L G D I L

**C****WW domain**

IQGAP1 (Hydra) 666-D<sup>W</sup>S<sup>W</sup>S<sup>W</sup>W<sup>W</sup>V<sup>W</sup>G<sup>W</sup>T<sup>W</sup>E<sup>W</sup>T<sup>W</sup>R<sup>W</sup>V<sup>W</sup>P<sup>W</sup>Q<sup>W</sup>Y<sup>W</sup>T<sup>W</sup>Y<sup>W</sup>L<sup>W</sup>N<sup>W</sup>V<sup>W</sup>S<sup>W</sup>S<sup>W</sup>K<sup>W</sup>E<sup>W</sup>F<sup>W</sup>R<sup>W</sup>W<sup>W</sup>S<sup>W</sup>L<sup>W</sup>P<sup>W</sup>N<sup>W</sup>F<sup>W</sup>Q<sup>W</sup>F<sup>W</sup>G-703  
 IQGAP1 (Human) 679-G<sup>W</sup>D<sup>W</sup>N<sup>W</sup>S<sup>W</sup>K<sup>W</sup>W<sup>W</sup>K<sup>W</sup>H<sup>W</sup>W<sup>W</sup>V<sup>W</sup>K<sup>W</sup>G<sup>W</sup>Y<sup>W</sup>Y<sup>W</sup>H<sup>W</sup>N<sup>W</sup>L<sup>W</sup>E<sup>W</sup>T<sup>W</sup>Q<sup>W</sup>E<sup>W</sup>G<sup>W</sup>W<sup>W</sup>D<sup>W</sup>E<sup>W</sup>P<sup>W</sup>N<sup>W</sup>F<sup>W</sup>V<sup>W</sup>Q<sup>W</sup>N-716  
 IQGAP2 (Human) 594-V<sup>W</sup>S<sup>W</sup>D<sup>W</sup>G<sup>W</sup>S<sup>W</sup>W<sup>W</sup>L<sup>W</sup>K<sup>W</sup>L<sup>W</sup>N<sup>W</sup>L<sup>W</sup>H<sup>W</sup>K<sup>W</sup>Y<sup>W</sup>D<sup>W</sup>Y<sup>W</sup>Y<sup>W</sup>N<sup>W</sup>T<sup>W</sup>D<sup>W</sup>S<sup>W</sup>K<sup>W</sup>E<sup>W</sup>S<sup>W</sup>W<sup>W</sup>V<sup>W</sup>T<sup>W</sup>P<sup>W</sup>E<sup>W</sup>S<sup>W</sup>C<sup>W</sup>F<sup>W</sup>Y<sup>W</sup>K-631  
 DYSTROPHIN (Human) 3055-T<sup>W</sup>S<sup>W</sup>V<sup>W</sup>Q<sup>W</sup>G<sup>W</sup>P<sup>W</sup>W<sup>W</sup>E<sup>W</sup>R<sup>W</sup>A<sup>W</sup>I<sup>W</sup>S<sup>W</sup>P<sup>W</sup>N<sup>W</sup>K<sup>W</sup>V<sup>W</sup>P<sup>W</sup>Y<sup>W</sup>I<sup>W</sup>N<sup>W</sup>H<sup>W</sup>E<sup>W</sup>T<sup>W</sup>Q<sup>W</sup>T<sup>W</sup>T<sup>W</sup>C<sup>W</sup>W<sup>W</sup>D<sup>W</sup>H<sup>W</sup>P<sup>W</sup>K<sup>W</sup>M<sup>W</sup>T<sup>W</sup>E<sup>W</sup>L<sup>W</sup>Y-3092  
 C38D4.5 (C.elegans) 96-R<sup>W</sup>D<sup>W</sup>L<sup>W</sup>L<sup>W</sup>N<sup>W</sup>G<sup>W</sup>W<sup>W</sup>F<sup>W</sup>E<sup>W</sup>Y<sup>W</sup>E<sup>W</sup>T<sup>W</sup>D<sup>W</sup>V<sup>W</sup>G<sup>W</sup>R<sup>W</sup>T<sup>W</sup>F<sup>W</sup>F<sup>W</sup>F<sup>W</sup>N<sup>W</sup>K<sup>W</sup>E<sup>W</sup>T<sup>W</sup>G<sup>W</sup>K<sup>W</sup>S<sup>W</sup>Q<sup>W</sup>I<sup>W</sup>P<sup>W</sup>P<sup>W</sup>R<sup>W</sup>F<sup>W</sup>I<sup>W</sup>R<sup>W</sup>T<sup>W</sup>P-133

W Y W

**D****IR repeats****Hydra IQGAP1**

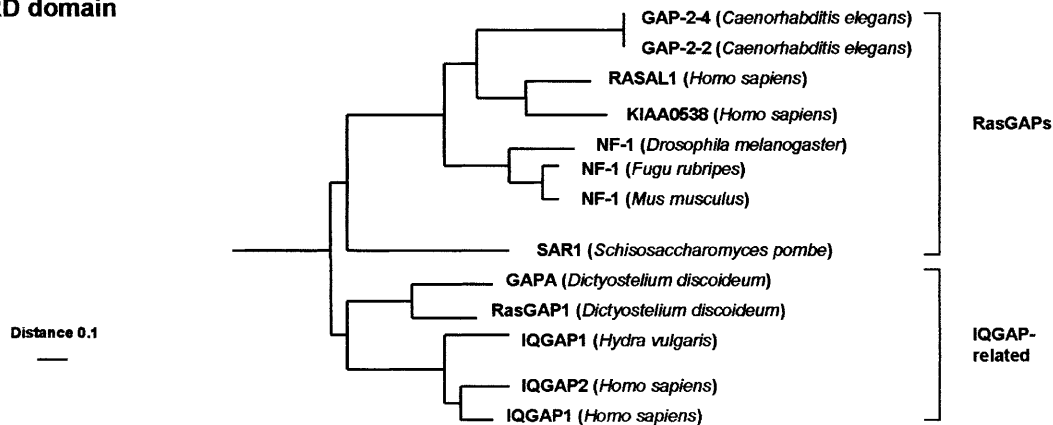
1 202-ALHAAI<sup>L</sup>IAI<sup>L</sup>NEALET<sup>L</sup>NNP<sup>L</sup>PAET<sup>L</sup>MKAL<sup>L</sup>QNP<sup>L</sup>DAR<sup>L</sup>LSD<sup>L</sup>LT<sup>L</sup>SL<sup>L</sup>SPDY<sup>L</sup>HS<sup>L</sup>IL<sup>L</sup>YES<sup>L</sup>KK<sup>L</sup>Q<sup>L</sup>LA<sup>L</sup>Q<sup>L</sup>YE-260  
 2 289-NL<sup>L</sup>KNC<sup>L</sup>IEE<sup>L</sup>I<sup>L</sup>NR<sup>L</sup>AID<sup>L</sup>Q<sup>L</sup>TR<sup>L</sup>GAL<sup>L</sup>F<sup>L</sup>NQ<sup>L</sup>SS<sup>L</sup>PM<sup>L</sup>AT<sup>L</sup>LR<sup>L</sup>FK<sup>L</sup>LE<sup>L</sup>NS<sup>L</sup>AW<sup>L</sup>YL<sup>L</sup>Q<sup>L</sup>N<sup>L</sup>G<sup>L</sup>DE<sup>L</sup>KT<sup>L</sup>NK<sup>L</sup>AK<sup>L</sup>REG-347  
 3 374-NM<sup>L</sup>EGAV<sup>L</sup>IQ<sup>L</sup>IN<sup>L</sup>RL<sup>L</sup>LD<sup>L</sup>K<sup>L</sup>GS<sup>L</sup>PE<sup>L</sup>LM<sup>L</sup>V<sup>L</sup>WL<sup>L</sup>QR<sup>L</sup>PE<sup>L</sup>GL<sup>L</sup>LV<sup>L</sup>VD<sup>L</sup>KS<sup>L</sup>RP<sup>L</sup>N<sup>L</sup>Y<sup>L</sup>MD<sup>L</sup>N<sup>L</sup>L<sup>L</sup>K<sup>L</sup>L<sup>L</sup>KE<sup>L</sup>H<sup>L</sup>Q<sup>L</sup>SD<sup>L</sup>LT-432  
 4 441-K<sup>L</sup>LL<sup>L</sup>AV<sup>L</sup>SA<sup>L</sup>V<sup>L</sup>NQ<sup>L</sup>AID<sup>L</sup>M<sup>L</sup>N<sup>L</sup>NE<sup>L</sup>N<sup>L</sup>K<sup>L</sup>V<sup>L</sup>ST<sup>L</sup>LEN<sup>L</sup>P<sup>L</sup>DAR<sup>L</sup>LNG<sup>L</sup>V<sup>L</sup>DE<sup>L</sup>SL<sup>L</sup>VS<sup>L</sup>RY<sup>L</sup>L<sup>L</sup>SH<sup>L</sup>V<sup>L</sup>AV<sup>L</sup>VE<sup>L</sup>KK<sup>L</sup>RE<sup>L</sup>DE<sup>L</sup>VG-499  
 5 526-D<sup>L</sup>LL<sup>L</sup>AA<sup>L</sup>L<sup>L</sup>SK<sup>L</sup>I<sup>L</sup>NE<sup>L</sup>A<sup>L</sup>IS<sup>L</sup>AN<sup>L</sup>PH<sup>L</sup>I<sup>L</sup>IL<sup>L</sup>K<sup>L</sup>AL<sup>L</sup>EL<sup>L</sup>P<sup>L</sup>TAK<sup>L</sup>L<sup>L</sup>T<sup>L</sup>N<sup>L</sup>VR<sup>L</sup>P<sup>L</sup>K<sup>L</sup>NA<sup>L</sup>E<sup>L</sup>LY<sup>L</sup>TI<sup>L</sup>IL<sup>L</sup>K<sup>L</sup>Q<sup>L</sup>AK<sup>L</sup>LE<sup>L</sup>K<sup>L</sup>I<sup>L</sup>Q<sup>L</sup>H<sup>L</sup>SG-584  
 6 611-RL<sup>L</sup>ANG<sup>L</sup>I<sup>L</sup>NV<sup>L</sup>NE<sup>L</sup>AID<sup>L</sup>N<sup>L</sup>ADS<sup>L</sup>AD<sup>L</sup>LL<sup>L</sup>L<sup>L</sup>ALK<sup>L</sup>SK<sup>L</sup>S<sup>L</sup>I<sup>L</sup>AL<sup>L</sup>RS<sup>L</sup>IT<sup>L</sup>P<sup>L</sup>E<sup>L</sup>CT<sup>L</sup>EQ<sup>L</sup>Y<sup>L</sup>HT<sup>L</sup>EL<sup>L</sup>FK<sup>L</sup>AK<sup>L</sup>G<sup>L</sup>Q<sup>L</sup>ND<sup>L</sup>SS<sup>L</sup>W-669

L A I N L P L Y L K K

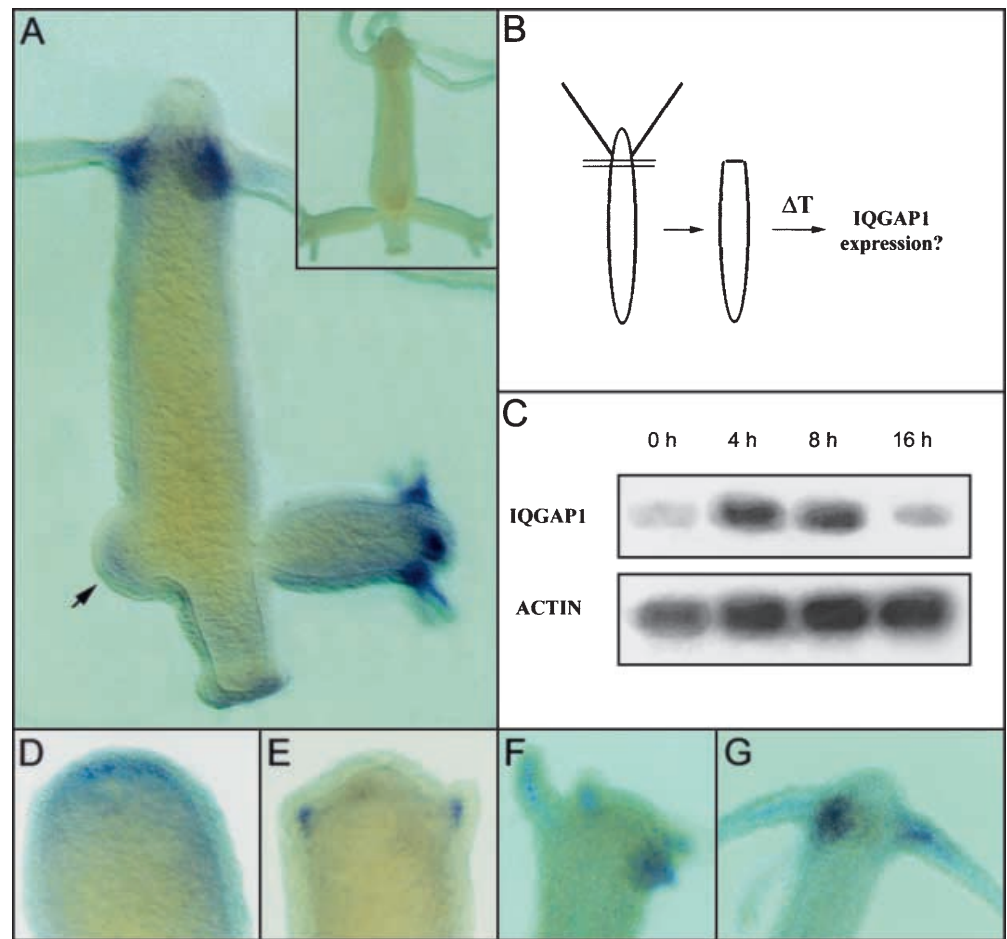
**Human IQGAP1**

1 216-ALHAAVIA<sup>L</sup>I<sup>L</sup>NEA<sup>L</sup>ID<sup>L</sup>RR<sup>L</sup>I<sup>L</sup>PAD<sup>L</sup>T<sup>L</sup>FA<sup>L</sup>L<sup>L</sup>K<sup>L</sup>NP<sup>L</sup>NAM<sup>L</sup>L<sup>L</sup>V<sup>L</sup>N<sup>L</sup>LE<sup>L</sup>E<sup>L</sup>P<sup>L</sup>AST<sup>L</sup>Y<sup>L</sup>QD<sup>L</sup>IL<sup>L</sup>Y<sup>L</sup>QAK<sup>L</sup>D<sup>L</sup>K<sup>L</sup>M<sup>L</sup>T<sup>L</sup>NAK-274  
 2 304-N<sup>L</sup>T<sup>L</sup>F<sup>L</sup>SAL<sup>L</sup>ANI<sup>L</sup>D<sup>L</sup>LA<sup>L</sup>EQ<sup>L</sup>GDAL<sup>L</sup>AL<sup>L</sup>F<sup>L</sup>RAL<sup>L</sup>Q<sup>L</sup>SPAL<sup>L</sup>GL<sup>L</sup>R<sup>L</sup>GL<sup>L</sup>QQ<sup>L</sup>NS<sup>L</sup>D<sup>L</sup>W<sup>L</sup>Y<sup>L</sup>L<sup>L</sup>Q<sup>L</sup>LL<sup>L</sup>SD<sup>L</sup>K<sup>L</sup>Q<sup>L</sup>K<sup>L</sup>R<sup>L</sup>Q<sup>L</sup>SG-362  
 3 387-RR<sup>L</sup>LA<sup>L</sup>AV<sup>L</sup>ALI<sup>L</sup>NA<sup>L</sup>A<sup>L</sup>IQ<sup>L</sup>G<sup>L</sup>VA<sup>L</sup>E<sup>L</sup>KT<sup>L</sup>V<sup>L</sup>LE<sup>L</sup>LM<sup>L</sup>N<sup>L</sup>PE<sup>L</sup>A<sup>L</sup>QL<sup>L</sup>P<sup>L</sup>Q<sup>L</sup>V<sup>L</sup>Y<sup>L</sup>FA<sup>L</sup>AD<sup>L</sup>LY<sup>L</sup>Q<sup>L</sup>EL<sup>L</sup>AT<sup>L</sup>L<sup>L</sup>Q<sup>L</sup>R<sup>L</sup>Q<sup>L</sup>SP<sup>L</sup>EH<sup>L</sup>NL-445  
 4 455-ER<sup>L</sup>LS<sup>L</sup>VAL<sup>L</sup>IN<sup>L</sup>RA<sup>L</sup>LES<sup>L</sup>GD<sup>L</sup>V<sup>L</sup>N<sup>L</sup>T<sup>L</sup>V<sup>L</sup>WK<sup>L</sup>Q<sup>L</sup>SS<sup>L</sup>SV<sup>L</sup>T<sup>L</sup>GL<sup>L</sup>T<sup>L</sup>NI<sup>L</sup>EE<sup>L</sup>ENC<sup>L</sup>Q<sup>L</sup>RY<sup>L</sup>L<sup>L</sup>DE<sup>L</sup>LM<sup>L</sup>K<sup>L</sup>L<sup>L</sup>QA<sup>L</sup>HA<sup>L</sup>ENN-513  
 5 537-ER<sup>L</sup>ILA<sup>L</sup>IG<sup>L</sup>L<sup>L</sup>NE<sup>L</sup>AL<sup>L</sup>KE<sup>L</sup>G<sup>L</sup>DA<sup>L</sup>Q<sup>L</sup>KT<sup>L</sup>L<sup>L</sup>QAL<sup>L</sup>IP<sup>L</sup>AA<sup>L</sup>K<sup>L</sup>LE<sup>L</sup>GV<sup>L</sup>LA<sup>L</sup>E<sup>L</sup>V<sup>L</sup>A<sup>L</sup>Q<sup>L</sup>HY<sup>L</sup>QD<sup>L</sup>TL<sup>L</sup>IR<sup>L</sup>AK<sup>L</sup>RE<sup>L</sup>KA<sup>L</sup>E<sup>L</sup>IQ-595  
 6 622-K<sup>L</sup>FAL<sup>L</sup>G<sup>L</sup>IF<sup>L</sup>AI<sup>L</sup>NE<sup>L</sup>AV<sup>L</sup>ES<sup>L</sup>GD<sup>L</sup>V<sup>L</sup>G<sup>L</sup>KT<sup>L</sup>LS<sup>L</sup>AL<sup>L</sup>RS<sup>L</sup>P<sup>L</sup>D<sup>L</sup>V<sup>L</sup>LY<sup>L</sup>G<sup>L</sup>VI<sup>L</sup>PE<sup>L</sup>CG<sup>L</sup>ET<sup>L</sup>Y<sup>L</sup>HS<sup>L</sup>DL<sup>L</sup>AE<sup>L</sup>AK<sup>L</sup>KL<sup>L</sup>AV<sup>L</sup>GD-680

L A I N L P L Y L K K

**F****GRD domain**

**Fig. 2A–G** Expression pattern of *Hydra* IQGAP1 in *Hydra vulgaris*. **A** digoxigenin-labeled RNA probe corresponding to the IQGAP1 coding region was used as the probe for the in situ hybridization experiments. **A** Whole-mount in situ hybridization reveals that IQGAP1 is expressed in adult polyps in a small group of endodermal cells at the base of tentacles. In young buds (*arrow*) IQGAP1 is expressed in endodermal cells at the apical tip of the protruding tissue. *Inset* Sense control. **B** Outline of experimental procedure to examine IQGAP1 expression during head formation. **C** Confirmation of upregulation of IQGAP1 expression during tentacle formation by Northern blot analysis using *H. vulgaris* mRNA extracted at various times after decapitation. A *Hydra* actin probe was used as a loading control. **D–G** IQGAP1 expression at 7, 16, 48 and 96 h of head regeneration, respectively



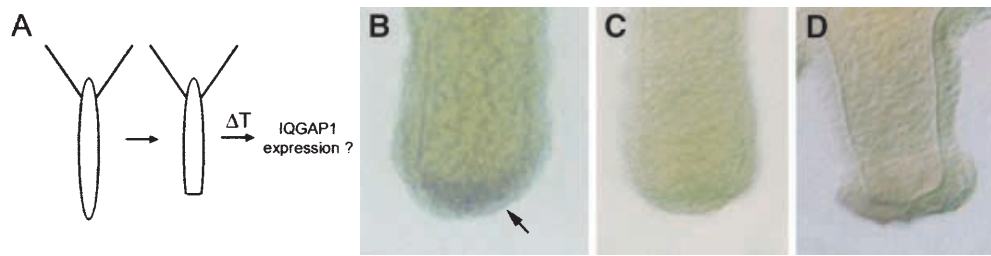
*Hydra* contains all the multidomain features known from mammalian IQGAP and, therefore, may have evolved from a combination of two or more protein modules already present in ancestral protist cells.

#### Expression of *Hydra* IQGAP1 during budding and head regeneration

Expression of *Hydra* IQGAP1 was examined by in situ hybridization on whole mounts of *H. vulgaris*. As shown in Fig. 2A, in intact polyps *Hydra* IQGAP1 transcripts

are restricted to the base of the tentacles. No expression can be seen in body column tissue nor in hypostome or mature tentacles. No staining was observed on whole mounts using an IQGAP1 sense probe (Fig. 2A, inset). To gain information about the putative role of this gene during patterning processes, IQGAP1 expression was studied as the head developed during budding and regeneration. During budding, IQGAP1 transcripts are first observed in a small area of the endoderm at the apical tip (arrow in Fig. 2A). In the final stages of budding (right bud in polyp shown in Fig. 2A), the level of IQGAP expression is high at the base of the tentacles, similar to the adult polyp. To examine more directly the role of IQGAP in tentacle formation, we examined the IQGAP1 expression level during head regeneration. Animals were bisected just below the head and allowed to regenerate. Periodically thereafter, samples were analyzed by both whole-mount in situ hybridization and Northern blot analysis (Fig. 2D–G). IQGAP1 expression is first detectable 4–7 h after decapitation in the endoderm of the apical tip of the regenerate (Fig. 2D). By 16 h, IQGAP1 expression is exclusively localized to the endoderm of the base of the regenerating tentacle (Fig. 2E) and remains there during later stages of tentacle formation (Fig. 2F, G). Thus, both the extensive cellular rearrangements at the apical tip during the first hours of head formation

◀ **Fig. 1** **A** Domain structure of IQGAP-related proteins. **B** Sequence of the calponin homology domain (CH) of *Hydra* IQGAP1 is compared with CH domains found in human IQGAP1 and IQGAP2 as well as in IQG1p, a yeast homologue of the mammalian IQGAPs. **C** Sequence of the WW domain of *Hydra* IQGAP1 is compared with WW domains found in human IQGAP1 and IQGAP2, Dystrophin, and *C. elegans* C38D4.5. **D** Sequence alignment of the IQGAP repeats of *Hydra* IQGAP1 compared with IQGAP repeats found in human IQGAP1. **E** Sequence alignment of the IQ motifs of *Hydra* IQGAP1 compared with IQ motif found in human IQGAP1. **F** Phylogenetic analysis of the GRD domain in IQGAP related proteins. The TREECON program was used to reconstruct the phylogenetic tree of CLUSTAL aligned protein sequences comprising the entire GRD domain. See Materials and methods for detail.



**Fig. 3A–D** Expression pattern of IQGAP1 in *H. vulgaris* during foot formation. **A** Outline of experimental procedure. **B** In tissue undergoing foot regeneration for 7 h, whole-mount in situ hybridization reveals only weak IQGAP1 expression (arrow) in a small group of endodermal cells. **C** Sense control. **D** No IQGAP1 expression can be detected by whole-mount in situ hybridization in mature foot tissue

(Bosch 1998), as well as the transformation of gastric epithelial cells into tentacle epithelial cells, are accompanied by an increase in IQGAP1 transcripts. This temporally and spatially restricted expression pattern was confirmed by Northern blot analysis. Fig. 2C indicates that a high level of IQGAP1 transcripts is detectable in regenerating polyps at 4 and 8 h after decapitation. Due to the localized expression in only a few endodermal cells at the base of the tentacle (see Fig. 2D–G), the IQGAP1 transcript level in Northern blots (Fig. 2C) appears to be decreased in older regenerates. To confirm that activation of transcription of IQGAP1 is specifically linked to tentacle formation, its expression was also examined during foot formation by whole-mount in situ hybridization. Fig. 3 indicates that only a weak signal could be observed during the first 7 h of foot regeneration (Fig. 3B), with no transcripts detectable in fully differentiated foot tissue (Fig. 3D). The results suggest that *Hydra* IQGAP1 is involved in tentacle morphogenesis, possibly controlling transdifferentiation of body column epithelial cells into tentacle epithelial cells.

The precise mechanism and the nature of the transcription factors controlling the temporally and spatially restricted expression pattern of IQGAP1 in endodermal cells of the regenerating apical tip remains to be determined. For two reasons, however, it is tempting to speculate that the *Brachyury* related transcription factor *HyBral* is involved. *HyBral* is expressed at high levels both in the same tissue region and at roughly the same time as IQGAP1 during apical regeneration in endodermal cells (Technau and Bode 1999). *HyBral* expression seems to slightly precede expression of IQGAP1 with the first transcripts detectable 3 h after decapitation and a maximum transcript level at 4–6 h. If *HyBral* functions as a transcriptional activator of IQGAP1, control of head-specific IQGAP1 expression would be totally different from control of expression of the head-specific gene *ks1*, which was shown recently to be regulated by absence of inhibitory factors rather than by activators (Endl et al. 1999).

Before body column epithelial cells differentiate into tentacle epithelial cells, they must modulate their adhesive contacts to neighboring cells as well as to the under-

lying substrate. Is activation of transcription of IQGAP1 involved in these changes? Although much remains to be done to understand the activation of localized IQGAP1 expression during tentacle cell formation, an interesting observation has been made recently by linking the WNT-mediated signalling pathway to head regeneration processes in *Hydra*. After cloning of several components of the pathway in *Hydra* (Hobmayer et al. 1996), it has been reported that WNT,  $\beta$ -catenin (as a major component of the E-cadherin adhesion complex), and transcription factor TCF (which mediates signalling by WNT proteins), are all rapidly activated during apical regeneration (Rentzsch, Hobmayer and Holstein, pers. comm.). This raises the intriguing possibility that IQGAP1 is a target gene for TCF and that changes in cell-cell adhesion modulate expression of IQGAP1 and thus cell fate.

In sum, the observation that a complete IQGAP-encoding gene, with all the multidomain features known from mammalian IQGAP, is first detected in the basal eumetazoan *Hydra* and activated in tissue undergoing extensive cellular rearrangement, supports the hypothesis that IQGAP family members are not only regulators of the cytoskeleton but also play a major role in changes in cell-cell contact in multicellular organisms.

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