Development 117, 1309-1319 (1993)
Printed in Great Britain © The Company of Biologists Limited 1993

The *Drosophila Ras2* and *Rop* gene pair: a dual homology with a yeast *Ras*-like gene and a suppressor of its loss-of-function phenotype

Adi Salzberg[1], Noa Cohen, Naomi Halachmi, Ziva Kimchie and Zeev Lev*

Department of Biology, Technion - Israel Institute of Technology, 32000 Haifa, Israel

[1]Present address: Howard Hughes Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030
*Author for correspondence

SUMMARY

The promoter of the *Drosophila melanogaster Ras2* gene is bidirectional, regulating an additional gene oriented in the opposite polarity. The two divergently transcribed genes are only 93 bases apart and deletion analysis proved that common *cis*-acting elements within this promoter region are required for the transcriptional activity of both genes. We cloned the gene paired with *Ras2* in the bidirectional promoter and isolated cDNAs corresponding to its mRNA. The *Ras opposite* (*Rop*) gene encodes for a 68×10^3 M_r protein which shares sequence homology with the members of a novel *Saccharomyces cerevisiae* gene family, including the *SLY1, SEC1* and *VPS33* (*SLP1*) genes, all of which are involved in vesicle trafficking among yeast cellular compartments. A highly conserved motif in this family is also found in β-COP, a coat protein isolated from rat Golgi-bound non-clathrin vesicles. Thus, the *Rop* protein may be a component of one of the vesicle trafficking pathways in *Drosophila* cells. The *Rop* gene expression during embryogenesis is restricted to the central nervous system (CNS) and the garland cells, a small group of nephrocytes that takes up waste materials from the haemolymph by endocytosis. *Ras2* is also expressed in the embryonic garland cells. In postembryonic stages, the two genes are co-expressed in the larval salivary glands and the central nervous system, and in the adult CNS and reproductive systems. Interestingly, the *S. cerevisiae SLY1-20* allele is a suppressor of the loss of the *YPT1* gene, a *ras*-like gene implicated in vesicle translocation, suggesting that the two genes may interact with one another. Since Sec1p and β-COP may also interact with small GTP-binding proteins of the *ras* superfamily, it is conceivable that the *Rop* and *Ras2* gene products are not just co-expressed in common tissues, but may also functionally interact with one another in these tissues.

Key words: *Drosophila*, *Ras* gene, bidirectional promoter, vesicle trafficking, garland cells, endocytosis

INTRODUCTION

Three *D. melanogaster Ras* genes, *Ras1*, *Ras2* and *Ras3*/*Rap1*, were cloned and characterized (reviewed in Lev, 1993). Analysis of their sequence homology to other *ras*-like genes indicated that they are related to different families in the *Ras* branch of the *ras* supergene family. *Ras1* belongs to the *Ras* family, which includes the three human transforming *ras* genes and the yeast *RAS* genes. *Ras2* belongs to another gene family, which contains the human non-transforming R-*ras* gene and the TC21 gene. *Ras3*/*Rap1* is most similar to the *Rap* family which contains the *rap1*/*Ki-rev* gene and other *rap* and *smg* genes (Chardin, 1991; Valencia et al., 1991).

Studies on the developmental regulation of the *Drosophila Ras* genes have shown that they are expressed constitutively during all developmental stages and are moderately abundant in poly(A) RNA (Lev et al., 1985; Mozer et al., 1985). However, additional studies bearing on their expression in *Drosophila* tissues have indicated that they are spatially regulated. Thus, all three genes are expressed in a relatively small number of tissues, including undifferentiated proliferating cells and terminally differentiated tissues (Segal and Shilo, 1986).

The *Ras1* gene participates in the signal transduction pathway determining the fate of the R7 photoreceptor in the *Drosophila* compound eye (Simon et al., 1991; Fortini et al., 1992; Gaul et al., 1992). *Ras3*/*Rap1* may act as an inhibitor of this process (Hariharan et al., 1991). The *Ras2* gene does not participate in this pathway (Fortini et al., 1992), but there are indications for its involvement in other proliferative pathways. Transgenic *Drosophila* strains containing the activated form of the *Ras2* protein have low fertility and show developmental disturbances in their bristles, wings and eyes (Bishop and Corces, 1988). However, as yet no specific role has been assigned to this gene.

During the isolation and characterization of the *Drosophila Ras2* promoter region, we found that, in addi-

tion to *Ras2*, another gene is transcribed in this region, but in the opposite polarity relative to the *Ras2* gene (Cohen et al., 1988). *Ras2* and the divergently transcribed gene, temporarily termed *CS1* and now termed *Ras opposite* (*Rop*), are merely 93 nucleotides apart. Deletion analysis has proved that certain *cis*-acting elements within this promoter are required for full transcriptional activity of both genes simultaneously (Lev et al., 1989). Thus, *Ras2* and *Rop* are regulated by a genuine bidirectional promoter.

Here, we report on the cloning and sequencing of the *Rop* gene and the pattern of its expression during *Drosophila* development. The *Rop* protein shows significant homology with the members of a novel *S. cerevisiae* gene family involved in vesicle trafficking among yeast cellular membranes (Aalto et al., 1992). The *Rop* and *Ras2* gene products are co-expressed in several embryonic, larval and adult tissues, including the garland cells, a group of nephrocytes implicated in the removal of toxic materials from the haemolymph by endocytosis (Aggarwal and King, 1967; Rizki, 1978).

MATERIALS AND METHODS

Drosophila strains

The OregonR wild-type strain was obtained from the CalTech *Drosophila* stock center. The *white* strain utilized for generating the transgenic lines was obtained from the collection of E. Liphschitz.

Isolation of *Rop* genomic and cDNA clones

The *Ras2*/*Rop* genomic region was isolated from a recombinant phage provided by B. Shilo. *Rop* cDNAs were isolated from a *D. melanogaster* embryonic cDNA library constructed by L. Kauvar and T. Kornberg, using DNA probes from genomic regions upstream of the *Ras2* transcription start site. *Rop*-positive clones were detected at a frequency of 1 in 15,000. Each part of the sequence was determined at least twice, in both directions.

Detection of the *Rop* protein

Full-length *Rop* cDNA was cloned in the vector pHSREM1 (Knipple and Marsella-Herrick, 1988) under regulation of the *Drosophila hsp70* promoter. Schneider S2 cells stably transformed with this construct were obtained by co-transfection with the pPC4 vector (Jokerst et al., 1989) followed by selection for α-amanitin resistance, as described (Rio and Rubin, 1985).

To prepare anti-Rop antibodies the *Rop* cDNA fragment coding for amino acids 51 to 492 was cloned in frame with the N terminus of the *E. coli trpE* protein in the expression vector pATH11 (a gift of A. Tzagoloff). The fused *trpE-Rop* protein produced in MH1 bacteria was isolated and used to raise rabbit anti-Rop antibodies.

Total proteins from S2 cells or *Drosophila* adult flies were solubilized in lysis buffer, electrophoresed in 9% polyacrylamide-SDS gel, blotted, reacted with polyclonal anti-Rop antibodies and the *Rop* protein was detected by autoradiography or by the ECL system (Amersham). To obtain heat-induced *Rop* protein, the S2 cells were heat-pulsed for 15 minutes at 37°C and, after a recovery period of 90 minutes, total proteins were extracted.

In situ hybridization

DNA labeled with DIG-dUTP (Bohringer) was used as a probe for in situ hybridization essentially according to the published protocol (Tautz and Pfeifle, 1989). The *Ras2* probe was a 760 bp *Eco*RI fragment from a *Ras2* cDNA clone, which contains the entire *Ras2* coding region. The *Rop* probe was a 1.65 kb *Eco*RI fragment from the coding region of a *Rop* cDNA clone. This fragment detects the two *Rop* transcripts.

Construction of *Ras2-lacZ* and *Rop-lacZ* transcription fusions

The *Ras2* promoter region was a 1030 bp *Kpn*I/*Dra*I fragment from pTZ18-1.9 (Cohen et al., 1988) and the *Rop* promoter region was a 1095 bp *Kpn*I/*Sty*I fragment from SK-*Rop*-CAT(NcoI) (S. Ezer and Z. L., unpublished results). They were inserted into the vector pW-ATG-lacZ1 (Kuhn et al., 1988), between the *Kpn*I and *Xba* sites. pW-ATG-lacZ1 was derived from the pW8 transducing vector (Klemenz et al., 1987). The *Ras2* promoter fragment in *Ras2-lacZ* spanned 852 bases upstream of the *Ras2* transcription start site and 78 bases downstream of this site. The *Rop* promoter fragment in *Rop-lacZ* consisted of 1047 bases upstream of the *Rop* transcription start site and 48 bases downstream of this site. Transgenic flies were obtained by injecting these constructs into *white* embryos as described (Spradling and Rubin, 1982; Spradling, 1986). The transformed lines were made homozygous and the chromosomes containing the transposons were determined by standard methods (Ashburner, 1989a). Additional lines were obtained by the jumping of transposons from the X chromosome to an autosome as described (Cooley et al., 1988).

Detection of β-galactosidase activity

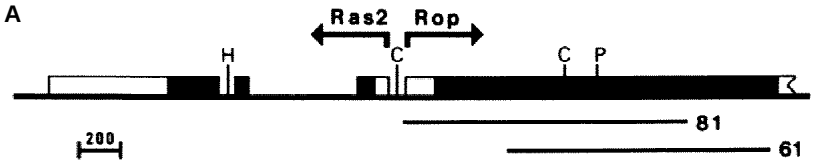
Histochemical staining for β-galactosidase activity was carried out essentially as described (Ashburner, 1989b). Staged embryos were collected and washed with water. After dechorionation in 50% bleaching solution, they were extensively washed with water and fixed for 15-20 minutes in fixing solution (7% formaldehyde in 100 mM Pipes, pH 6.9, 2 mM EGTA, 1 mM $MgSO_4$) mixed with 2 volumes of n-heptane. They were washed three times in washing solution (PBS [50 mM Na/Na_2PO_4, pH 7.4; 140 mM NaCl] containing 0.3% Triton X-100) and stained for 4 to 18 hours in staining solution (Na/Na_2PO_4, pH 7.2; 150 mM NaCl; 1 mM $MgCl_2$; 3.1 mM potassium ferricyanide; 3.1 mM potassium ferrocyanide; 0.3% Triton X-100) containing 0.2% X-gal, at 37°C. After three washes in washing solution, the embryos were incubated in PBS containing 70% glycerol and mounted for observation. Dissected larval salivary glands, brains, imaginal discs and adult testes were fixed for 15-20 minutes in 100 mM sodium cacodylate, pH 7.3, containing 0.75% glutaraldehyde. The organs were washed in PBS and stained as described above. Dissected ovaries were incubated for 10 minutes in devitellinization solution (16 mM K/K_2PO_4, pH 6.8; 75 mM KCl; 25 mM NaCl; 3.5 mM $MgCl_2$; 6% formaldehyde) mixed with six volumes of n-heptane. The ovaries were fixed and stained as described above for the embryos preparation.

RESULTS

The *Rop* gene and its product

The *Drosophila Rop* gene was initially identified as a distinct transcription unit located upstream of the *Ras2* gene and divergently transcribed in opposite polarity to the *Ras2* gene (Cohen et al., 1988, Fig. 1A). The transcription start sites of the *Rop* and *Ras2* transcription units were determined to be only 93 bases apart (Cohen et al., 1988). Furthermore, data obtained from sequencing *Ras2* cDNAs (Bishop and Corces, 1988; N. H., unpublished results) and *Rop* cDNAs (see below) revealed that they start, as pre-

dicted, from the start site determinations described above (Fig. 1B). Under stringent hybridization conditions *Rop* appeared as a single-copy gene (A. S., unpublished results), coding for two transcripts, 2.5 and 2.9 kb long (Cohen et al., 1988). These transcripts were initially assigned by others to *Ras2* (Mozer et al., 1985; Brock, 1987).



B

```
AGTTTGTACGTTTGCATCTGCATTTCCCCGTTTAAATCGACCAGTTGGCCAGCAAAATTATTTTATTTTCCTTCCTCGAC  -126
              Ras2 <------+1
AGCAGCAGCGTGACCGTTCTCGGTTAACTGAGAAATCCACACTCGCACTGACACGGGTGCGCGTCTCTGCTAGTCAGTAA   -46
                  11 <-+1------> Rop
TATCGATATCAACTATCGCTAGCCAGCAATCGACTTTCGCCCATCACTAAGGCTAAGTCATCAAACGAAAAAAATACAGA    35
                       <-81
AAAATTGGCCAAGGAATAGATAAAATTCTTTAAGTCAAGTTAGCCCACGCGAAAAAGCGAACGGAATAAGCTAAAAAGAC   115

ATATTTACACGCAGTGTGTTCATAATCGAGTTGCCTTTGCGCCCGTGACCAATAAAACGCAGTGAAAATGGCCTTGAAAG   195
                                                                M  A  L  K
TGCTGGTGGGCCAAAAGCTGATGAACGAGGTGGTGAAGTACAAGCCTCCACCACCCAAAAAGCAGGGCGTTACGTCCGCT   275
V  L  V  G  Q  K  L  M  N  E  V  V  K  Y  K  P  P  P  P  K  K  Q  G  V  T  S  A
GCGGGGGCGGGCGGCATGGAATGGCGGGTGCTGGTGGTGGACAAGCTGGGTATGCGCATGGTGTCCGCCTGCACCAAGAT   355
 A  G  A  G  G  M  E  W  R  V  L  V  V  D  K  L  G  M  R  M  V  S  A  C  T  K  M
GCACGAAATCAGCGCCGAGGGCATCACCCTGGTTGAGGACATCAACAAGAAGCGCGAGCCGCTGCCCACGATGGACGCGA   435
  H  E  I  S  A  E  G  I  T  L  V  E  D  I  N  K  K  R  E  P  L  P  T  M  D  A
TCTATCTGATTACGCCGTCGGATGAGTCAGTGCGCGGTCTGATACGAGACTTCGAGAACCCAGCGCGGCCCATGTATCGC   515
I  Y  L  I  T  P  S  D  E  S  V  R  G  L  I  R  D  F  E  N  P  A  R  P  M  Y  R
TATGCCCATGTCTTCTTCACCGAAGTTTGTCCCGAGGAGCTATTCAACGATCTGTGCAAGTCGTGCGCGGCCGGAAAGAT   595
 Y  A  H  V  F  F  T  E  V  C  P  E  E  L  F  N  D  L  C  K  S  C  A  A  G  K  I
CAAGACGCTGAAGGAGATCAACATTGCATTTTTGCCATACGAATGCCAGGTGTTCTCGCTGGACTCGCCGGACACATTCC   675
  K  T  L  K  E  I  N  I  A  F  L  P  Y  E  C  Q  V  F  S  L  D  S  P  D  T  F
AATGCCTCTACTCGCCGGCCTTTGCCTCCATTCGGAGCAAGCACATCGAGCGAATTGCCGAGCAAATAGCCACACTGTGC   755
Q  C  L  Y  S  P  A  F  A  S  I  R  S  K  H  I  E  R  I  A  E  Q  I  A  T  L  C
GCTACCCTTGGGGAGTACCCAAATGTGCGCTACCGCAGCGACTGGGACAGGAACATCGATTTGGCCGCGTCGGTGCAGCA   835
 A  T  L  G  E  Y  P  N  V  R  Y  R  S  D  W  D  R  N  I  D  L  A  A  S  V  Q  Q
GAAGCTGGACGCCTACAAGGCCGACGATGCCACCATGGGCGAGGGCCCCGAGAAGGCACGATCCCAGCTGCTAATCCTGG   915
  K  L  D  A  Y  K  A  D  D  A  T  M  G  E  G  P  E  K  A  R  S  Q  L  L  I  L
ATCGCGGCTTCGACTGCGTGTCGCCGCTGCTGCACGAGCTCACCCTGCAGGCCATGGCCTACGATTTGCTACCCATCGTC   995
D  R  G  F  D  C  V  S  P  L  L  H  E  L  T  L  Q  A  M  A  Y  D  L  L  P  I  V
AATGACGTGTATCGCTACACGCCCGGCCCCAACCAGCCGGATAAGGAGGTGCTGCTCGACGAGAACGACGACTTGTGGGT  1075
 N  D  V  Y  R  Y  T  P  G  P  N  Q  P  D  K  E  V  L  L  D  E  N  D  D  L  W  V
GGAGCTGCGTCACGAGCATATCGCTGTGGTCTCCACCCAGGTGACTCAGAACCTCAAGAAATTCACCGACTCTAAGCGCA  1155
  E  L  R  H  E  H  I  A  V  V  S  T  Q  V  T  Q  N  L  K  K  F  T  D  S  K  R
TGGGCTCGGCTGACAAGTCGTCGATGCGCGATCTCTCGCAGATGATCAAGAAGATGCCGCAGTACCAGAAGGAGCTGTCC  1235
M  G  S  A  D  K  S  S  M  R  D  L  S  Q  M  I  K  K  M  P  Q  Y  Q  K  E  L  S
AAGTACTCCACCCACTTGCATTTGGCCGAGGACTGCATGAAGTCGTACCAGAACTACGTGGACAAGCTGTGCCGCGTGGA  1315
 K  Y  S  T  H  L  H  L  A  E  D  C  M  K  S  Y  Q  N  Y  V  D  K  L  C  R  V  E
ACAGGATCTGGCCATGGGCACGGACGCCGAGGGCGAAAAGATCAAGGATCATATGCGCAACATTGTGCCCATCTTGCTGG  1395
  Q  D  L  A  M  G  T  D  A  E  G  E  K  I  K  D  H  M  R  N  I  V  P  I  L  L
ACGCCAATGTCTCGAACTACGACAAGGTTCGCATCATTGCCCTGTACGTCATGATCAAGAACGGAATTTCCGAGGAGAAC  1475
D  A  N  V  S  N  Y  D  K  V  R  I  I  A  L  Y  V  M  I  K  N  G  I  S  E  E  N
CTGACCAAGCTGTTTACGCATGCCCAGCTGTCGCCGAAGGATCAGGATATGGTGCGTAACCTTAGTTGCTTGGGCATCAA  1555
 L  T  K  L  F  T  H  A  Q  L  S  P  K  D  Q  D  M  V  R  N  L  S  C  L  G  I  N
TGTCATTGCAGATTCGCGCAAGAAGCAGTACTCGGTGCCGCGCAAGGAGCGCACCACAGAAAGCACCTACCAGATGTCCC  1635
  V  I  A  D  S  R  K  K  Q  Y  S  V  P  R  K  E  R  T  T  E  S  T  Y  Q  M  S
GCTGGACACCGGTGATCAAGGACATCATGGAGGATTGCATAGAGGATAAGCTCGATCTCCGCCACTTCCCCTTCCTCGAG  1715
R  W  T  P  V  I  K  D  I  M  E  D  C  I  E  D  K  L  D  L  R  H  F  P  F  L  E
GGCAGGGCCCAAAATACCAATTATCATGCCCCAACACTGGCACGCTACGGTCACTGGCACAAGGACAAGGGACAGGCGCA  1795
 G  R  A  Q  N  T  N  Y  H  A  P  T  L  A  R  Y  G  H  W  H  K  D  K  G  Q  A  Q
GGTAAAGAACGTGCCCAGGCTCATTGTCTTCATCGTGGGCGGCGTGAGTATGTCCGAGATGCGGTGCGCCTACGAGGTGA  1875
  V  K  N  V  P  R  L  I  V  F  I  V  G  G  V  S  M  S  E  M  R  C  A  Y  E  V
CCAATGCGGTTCGCAATTGGGAGGTCCTTGTTGGCTCTTCGCACATCCTGTCGCCCGAAATCTTCCTCAGCGATTTGGGC  1955
T  N  A  V  R  N  W  E  V  L  V  G  S  S  H  I  L  S  P  E  I  F  L  S  D  L  G
AGTCTCTCGAAGGAGGACTAAGGAACTTATTGGGTCATAATCTTGTTAATGGTTTTGTCACATTTGTCGTGTTTAAAAAT  2035
 S  L  S  K  E  D
AGTTTGTTATTCCTTACCGGAGTTCAAAAT
```

Fig. 1. The *Drosophila Rop* nucleotide and deduced amino acid sequence. (A) The organization of the *Ras2*/*Rop* gene pair region. The arrows denote the transcripiton start site of each gene (Cohen et al., 1988). Thick line, intron; boxed line, exon; open, untranslated; filled, translated. The 3′-end of the *Rop* transcription unit has not been determined; 81, 61, the *Rop*81 and *Rop*61 cDNAs used for sequencing. (B) *Rop* genomic and cDNA nucleotide sequence, and the deduced amino acid sequence of the *Rop* protein. Thick arrows above the sequence, transcription start sites, determined by external primer extension and RNase protection (Cohen et al., 1988). Thin arrows below the sequence, most upstream 5′-ends of cloned cDNAs *Rop*81 and *Ras*11. The translation start codons of the *Rop* and *Ras2* proteins are underlined. The sequence of the promoter region contains a few corrections to the sequence published earlier (Cohen et al., 1988).

To learn about the identity of *Rop*, two overlapping *Rop* cDNA clones and the *Rop* genomic DNA region were isolated and sequenced in parallel (Fig. 1). The *Rop* translation start site is at position 183 and the ATG start codon is embedded in the sequence GAAAATG, which conforms in 6 out of 7 bases to the *Drosophila* translation start consensus sequence (Cavener, 1987). The open reading frame is 597 amino acids long, encoding for a protein with a predicted relative molecular mass of 67,800. The cDNA nucleotide sequence appears to be colinear with the genomic sequence along the 1950 bases sequenced, indicating that the translated portion of at least one *Rop* mRNA is transcribed from an intronless genomic DNA region. The *Rop* protein is slightly acidic (pI = 6.22) and is mostly hydrophilic, containing 26% charged amino acids. No transmembrane regions, conserved sites for nucleotide binding or enzymatic activity were found.

Homology of Rop with known proteins

A search of the SwissProt protein database revealed significant homology (FASTA score above 200) between the *Rop* protein and the products of a novel *S. cerevisiae* gene family including *SLY1*, *SEC1* and *VPS33*(*SLP1*). All of them are genes associated with protein trafficking among yeast cellular membranes (Aalto et al., 1992). The *SLY1* gene product may be functional in endoplasmic reticulum (ER)-to-Golgi transport (Ossig et al., 1991). The *SEC1* gene product is also involved in protein secretion, but in a different step. It is one of the ten genes identified by the *SEC* (for secretion) mutations needed for the final stage of protein secretion in which the secretory vesicles fuse with the cell membrane to release the secretory proteins to the cell exterior (Novick et al., 1980; Aalto et al., 1991). The product of the third gene, *VPS33*(*SLP1*), is involved in transport from the Golgi complex to the cell vacuole (Robinson et al., 1988; Wada et al., 1990).

Apparently *Rop* is the first member of this gene family to be identified in a multicellular organism. The highest homology in the family is among *Rop*, *SLY1* and *SEC1* (Fig. 2A). The *Rop* protein sequence appears to be even more similar to Sly1p and Sec1p than these two yeast proteins are to one another. There is 25% sequence identity (38% sequence similarity) between Rop and Sly1p, and 24% sequence identity (40% sequence similarity) between Rop and Sec1p. The homology of these proteins to VPS33p is slightly lower (not shown, but see Aalto et al, 1992). In some of the highly conserved regions the sequence identity reaches 50 to 70%. One of these regions is also present in β-COP (H. D. Schmitt, C. Dascher and M. Egerton, personal communication) a 110×10^3 M_r rat protein associated with non-clathrin-coated vesicles and the Golgi complex (Duden et al., 1991; Serafini et al., 1991).

Detection of the *Rop* protein

To identify the *Rop* gene product, anti-Rop antibodies were raised against a *trpE-Rop* fusion protein expressed in bacteria. The antibodies precipitated a single protein from metabolically labeled Schneider S2 tissue culture cells (Fig. 3A) with the predicted M_r of 68×10^3. They also recognized the expected 68×10^3 M_r heat-inducible protein in a heat-pulsed Schneider S2 cell line stably transformed with a *Hsp70-Rop* transcription fusion (Fig. 3B). The *Rop* protein was also detected in adult flies, with higher levels in males than in females (Fig. 3C).

Localization of the *Ras2* and *Rop* transcripts during embryogenesis

To learn about the temporal and spatial distribution of the *Ras2* and *Rop* transcripts during embryogenesis, we utilized DNA fragments labeled with digoxigenin as probes for the in situ hybridization analysis. Hybrids formed between the probe and the endogenous transcripts were detected by anti-digoxigenin antibodies coupled with alkaline phosphatase (Tautz and Pfeifle, 1989). The *Ras2*-specific probe was capable of detecting the two transcripts coded by the *Ras2* gene (Lev et al., 1985). The *Rop*-specific probe could detect the two transcripts coded by the *Rop* gene (Cohen et al., 1988). To test the specificity of the hybridization reaction, similar experiments were carried out using a DNA probe prepared from the bacterial plasmid pBR322. In addition, to exclude the possibility of cross-reactivity with other *Ras*-like genes, we repeated these experiments with *Ras1* and *Ras3* probes. Distinct, different patterns were obtained for each of the *Ras* genes. Although the presence of *Ras2* and *Rop* transcripts during early embryogenesis has been established by RNA-blotting experiments (Lev et al., 1985; Mozer et al., 1985; Segal and Shilo, 1986), until stage 11 of embryogenesis (5.3 to 7.3 hours, end of germ band extension; Campos-Ortega and Hartenstein, 1985), the signal was homogeneous and apparently with an intensity similar to the control reaction (not shown).

The first indication of a specific signal was detected at stage 13 (9.3 to 10.3 hours, end of germ band retraction) where the garland cells were marked by the *Ras2* probe (Fig. 4A). The garland cells were clearly stained by the *Rop* probe as well (Fig. 4B). These nephrocytes are organized as a ring of cells around the oesophagus (Rizki, 1978; Fig. 5). They were implicated in the removal of toxic materials from the haemolymph by endocytosis (Aggarwal and King, 1967; Narita et al., 1989). The *Ras1* and *Ras3*/*Rap1* probes did not stain the garland cells. The *Rop* probe also marked cells in the central nervous system (Fig. 4B). We also observed staining with the *Rop* probe of cell clusters in the peripheral nerve system (not shown). In stage 14 (10.3 to 11.3 hours) the *Ras2* probe marked, in addition to the garland cells, a specific set of cells in the central nervous system (CNS; Fig. 4C,E). The *Rop* probe also detected the garland cells. A stronger signal in the CNS was observed, probably due to the the increase in the total number of cells found in this region (Fig. 4D,F). In stages 15 and 16 (11.3 to 13 hours and 13 to 16 hours, respectively), the *Rop* probe faintly stained all peripheral nerve cell clusters (data not shown, Fig. 4J,L). In stage 17 (16 hour embryos) the CNS is condensed towards the head. In this stage, there was a strong and general staining of the CNS by the *Rop* probe. Except for several clusters in the brain hemispheres, most other cells were stained. In the ventral nerve cord, the central cells stained stronger than the outer cells. Strong staining was also observed in the antennal-maxillary complex (Fig. 4N,O). This complex was also stained by the *Ras2*

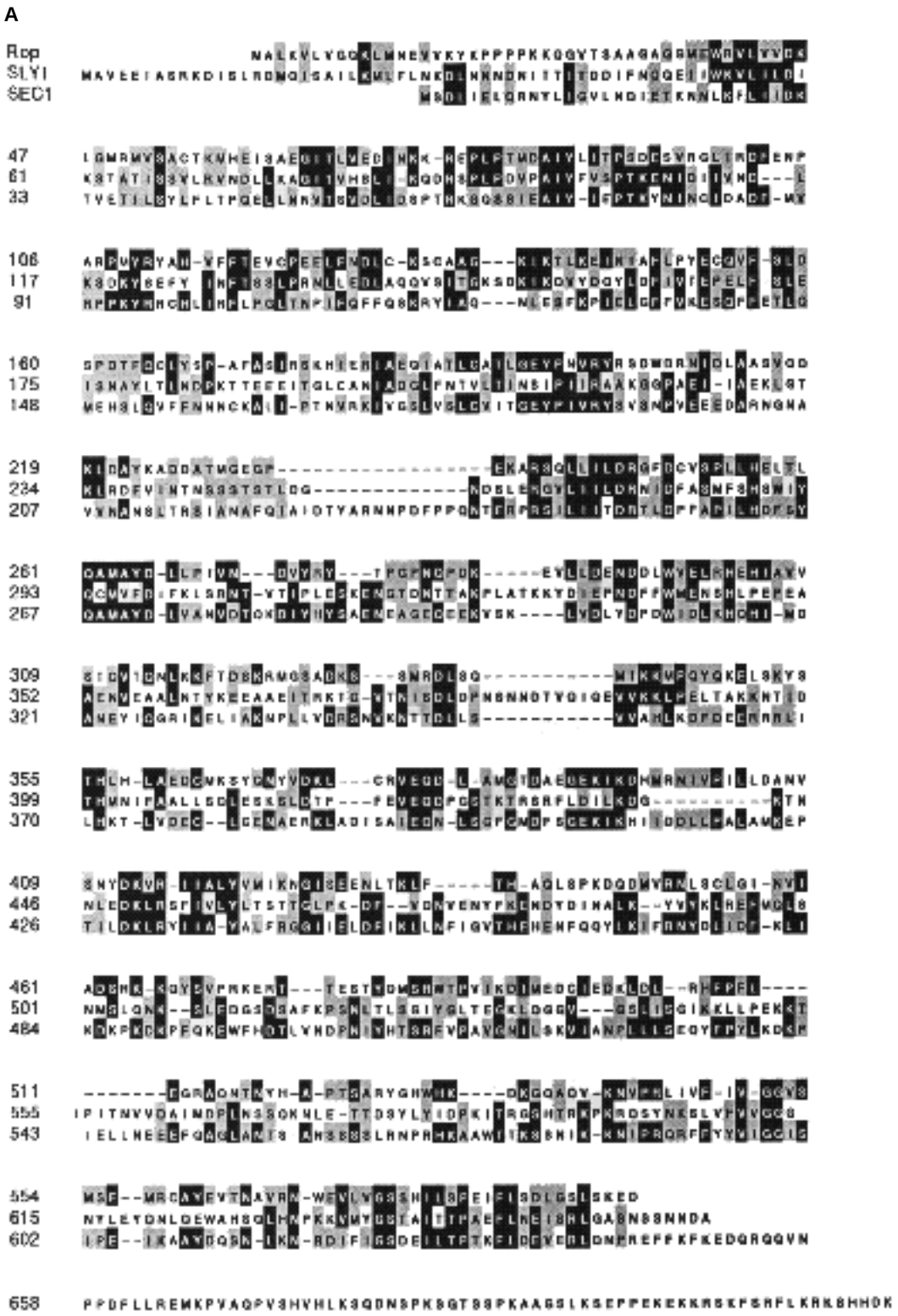


Fig. 2. Sequence alignments of the *Rop* protein. (A) Homology of Rop with the *S. cerevisiae* Sly1p and Sec1p proteins. Identical residues are boxed in black, similar residues are boxed in grey. The percentages of the identical/similar amino acids between these genes are 25/38, 24/40 and 21/34 for Rop and Sly1p, Rop and Sec1p, Sly1p and Sec1p, respectively.
(B) Alignment of a conserved motif among yeast, *Drosophila* and rat proteins. The following amino acids were considered similar: L,I,V,M; G,A,S,T,P; D,E,Q,N; K,R,H; W,Y,F.

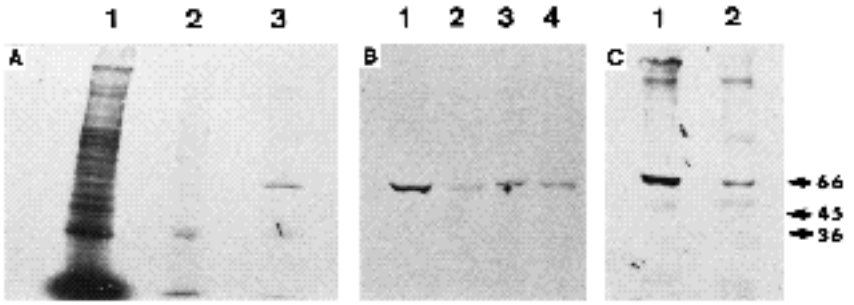


Fig. 3. The *Rop* gene product.
(A) Immunoprecipitation of the *Rop* protein from S2 cells grown with [^{35}S]methionine. 1, Total proteins; 2, immunoprecipitation with non-immune serum; 3, immunoprecipitation with anti-Rop serum. (B) Immunoblot of total proteins from Schneider S2 cell lines reacted with anti-Rop antibody. 1, 2, S2 cell line stably transformed with an *hsp70-Rop* transcription fusion; 3, 4, normal S2 cell line; 1, 3, with heat shock; 2, 4, without heat shock. (C) Detection of the *Rop* protein in adult flies. Immunoblot of total proteins from 1, males; 2, females, reacted with anti-Rop antibody.

(Fig. 4N,O). This complex was also stained by the *Ras2* probe, but to a lesser extent (Fig. 4M).

Activity of the *Ras2/Rop* bidirectional promoter in the larva and the adult fly

To learn about the expression of the two genes in postembryonic stages, the coding region of the bacterial *lacZ* gene was attached to the bidirectional promoter in either the *Ras2* orientation or the *Rop* orientation. The *lacZ* gene was provided by the vector pW-ATG-lacZ (Kuhn et al., 1988) derived from the pW8 transducing vector (Klemenz et al., 1987), which enables the transduction of foreign DNA into cells of the *Drosophila* germ line (Spradling, 1986). The maps of the resulting transcription fusions are shown in Fig. 6. In the *Ras2-lacZ* construct, the cloned fragment spanned 78 bases downstream to the *Ras2* transcription start site and 852 bases upstream to this site. In the *Rop-lacZ* construct, the cloned fragment contained 48 bases downstream to the

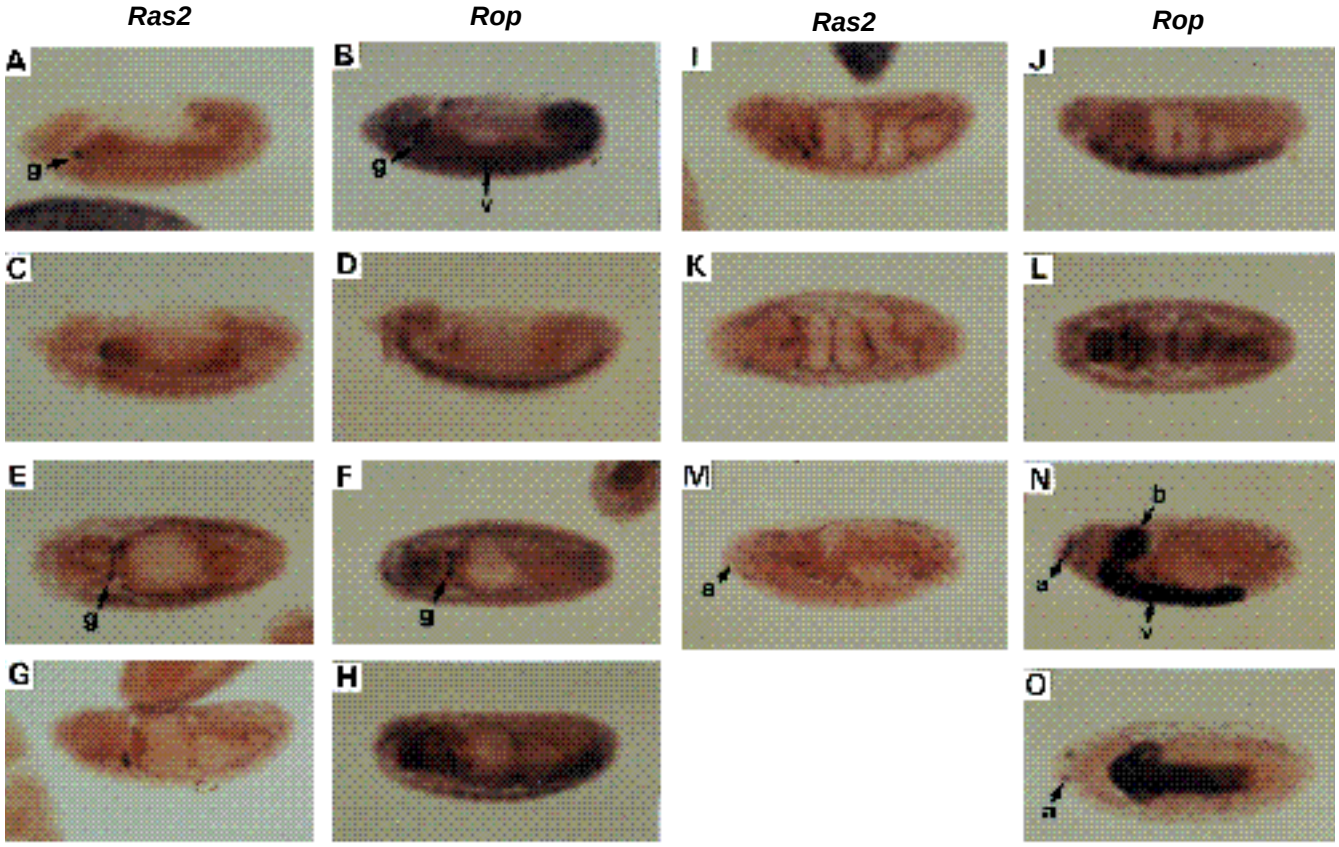


Fig. 4. Spatial distribution of *Ras2* (left column) and *Rop* (right column) transcripts during embryogenesis. In situ hybridization on whole-mount embryos with digoxigenin-labeled DNA probes. Orientation of embryos is anterior left, dorsal up. (A,B) Stage 13 and (C,D) stage 14. The *Ras2* transcripts are detected in the garland cells. *Rop* transcripts are detected in the garland cells and in the CNS. (E,F) Stage 14, a dorsal view. Abundant expression of *Ras2* and *Rop* in the garland cells, organized as a ring around the esophagus. (G,H) Stage 15, (I,J) stage 16 and (K,L) stage 16, dorsal view. The transcript localization is similar to the previous stages. (M,N) Stage 17 and (O) stage 17, dorsal view. *Ras2* transcripts are detected in the antennal-maxillary complex. *Rop* transcripts are located in the CNS and in the antennal-maxillary complex. a, antennal-maxillary complex; b, brain; g, garland cells; v, ventral nervous cord.

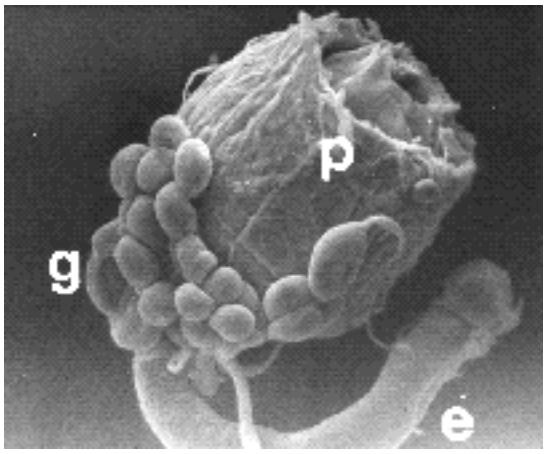


Fig. 5. Scanning electron micrograph of the garland cells. A third instar larva was dissected and a portion of the digestive system was isolated. e, Oesophagus; p, proventriculus; g, garland cells. Magnification ×350.

Rop transcription start site and 1047 bases upstream to this site. Since the length of the bidirectional promoter sequence between the two transcription start sites is 93 bases, the total length of the overlapping *Drosophila* sequences between these two constructs is 219 bases (Fig. 6).

Several transgenic lines were obtained and more lines were derived by transposing X-linked transposons to the autosomes as described by Cooley et al. (1988). In summary, six *Ras2-lacZ* lines and seven *Rop-lacZ* lines were established. The authenticity of the staining pattern may be distorted by the interaction of endogenous transcription elements in the vicinity of the transposon integration site with the transcription control elements of the *Ras2* and *Rop*

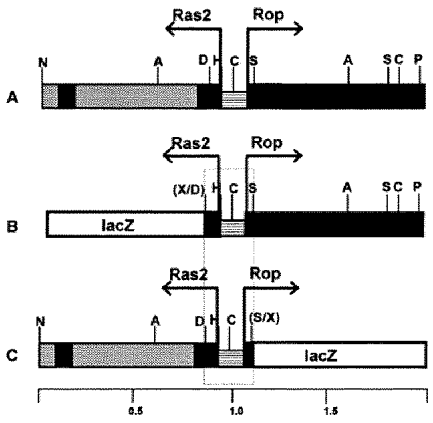


Fig. 6. Schematic maps of the *Ras2-lacZ* and *Rop-lacZ* transcription fusions. (A) The native sequences of the *Ras2*/*Rop* region. (B) The *Ras2-lacZ* construct. (C) The *Rop-lacZ* construct. Transcription start sites and gene polarity are marked with arrows. Filled bar, exon; grey bar, intron; striped bar, the bidirectional promoter region; empty bar, the *lacZ* reporter gene. The dashed box designates the *Drosophila* sequences common to the two constructs. Scale is in kb. Restriction sites: A, *Ava*I; C, *Cla*I; D, *Dra*I; H, *Hpa*I; N, *Hin*dIII; P, *Pst*I; S, *Sal*I; X, *Xba*I.

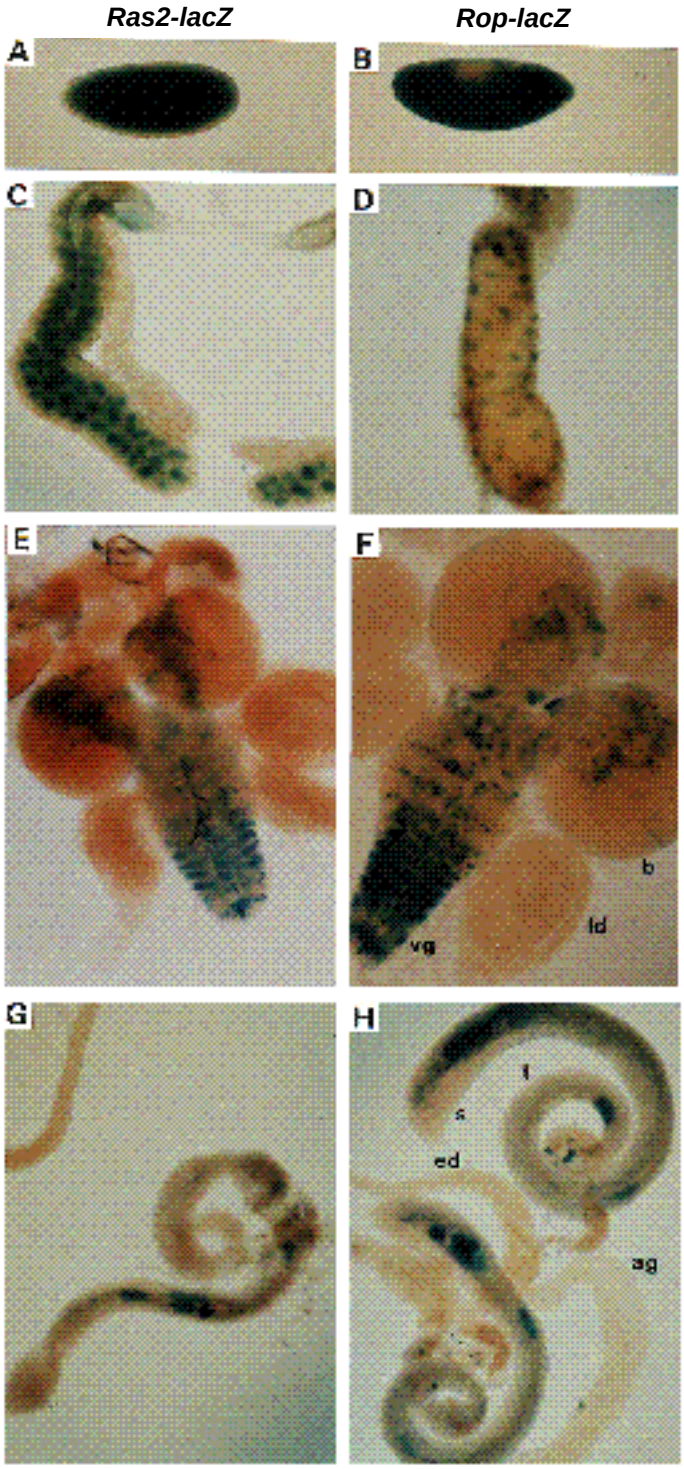


Fig. 7. Transcriptional activity of the *Ras2*/*Rop* bidirectional promoter in the larva and the adult fly. Similar patterns of β-galactosidase activity determined by X-gal staining of dissected organs were obtained in *Ras2-lacZ* (left column) and *Rop-lacZ* (right column) transgenic lines. (A,B) embryos; (C,D) salivary glands; (E,F) larval CNS; b, brain; ld, leg imaginal disc; vg, ventral ganglion; (G,H) testes; ag, accessory gland; ed, ejaculatory duct; s, spermatogonium; t, testis.

genes included in the transposon. Other position effects may be harmful and misleading as well. To compensate for possible interferences, several lines were analyzed simultaneously. Although some variation was observed regarding the signal intensity, the unique characteristics of the different staining patterns were the same in all cases.

The number of tissues and organs tested was limited to those that can be isolated by dissection and which do not posses high endogenous β-galactosidase activity. Embryos were stained homogeneously in almost all lines: this is probably due to the maternal contribution of the fusion-encoded β-galactosidase, which is a relatively stable enzyme (Fig. 7A,B). We tried to use transgenic males and wild-type females in order to overcome the masking of zygotic expression by the maternal contribution, but the signal was too weak to be detected either by X-Gal staining or by using anti-β-galactosidase antibodies. In the larval salivary glands, all cells were stained (Fig. 7C,D). A highly specific staining pattern was obtained in the larval CNS, which was identical in the *Ras2-lacZ* and *Rop-lacZ* lines tested (Fig. 7E,F). Specific cells within the ventral ganglion and the brain hemispheres were distinctly marked. Apparently the staining was limited to ganglion mother cells and larval nerve cells that had already terminated their differentiation and were not dividing anymore. The neuroblasts, which undergo many divisions in the brain hemispheres to form the adult optic centers, were not stained. The staining of the larval ventral ganglion was segmental. The three lines of the thoracic ganglions and the eight lines of the abdominal ganglions were clearly observed. Several types of imaginal discs, including the eye-antenna discs, the wing discs, the haltere discs and the genital discs, were also included in these preparations. However, none of them showed β-galactosidase activity originating at the transposons.

A specific staining pattern was also obtained in the adult testis. It was similar in the *Ras2-lacZ* and *Rop-lacZ* lines. The spermatogonia, which are the parental sperm cells, undergo four divisions which give rise to immature cysts containing 16 nuclei. There is no β-galactosidase expression in this stage. However, after a while the growing cysts get larger and transcription commences. At this stage, strong activity of β-galactosidase was observed in the maturing cysts, in all the *Ras2-lacZ* and *Rop-lacZ* lines tested (Fig. 7G,H). No activity was found in the accessory gland nor in the ejaculatory duct. In the female reproductive system, the X-gal staining was apparent in all cells, including the nurse cells, the follicle cells and the oocytes. Mature oocytes frequently stained more intensely (not shown).

DISCUSSION

Co-expression of the *Ras2* and *Rop* genes during early *Drosophila* development

The extent of the expression of the *Ras2* and *Rop* genes during *Drosophila* development was initially studied by poly(A)-RNA blotting analysis (Lev et al., 1985; Mozer et al., 1985). Apparently the two genes are expressed in unfertilized eggs, embryos, larvae, pupae and adult flies. The level of expression of the *Ras2* 1.8 kb transcript is fairly constant during all stages. A shorter transcript is evident in unfertilized eggs and early embryos. The expression of the *Rop* transcripts is less uniform. In unfertilized eggs and early embryos it is low, increasing in older embryos and young larvae. In mature larvae it is low again, increasing strongly in pupae and adult flies. In all stages, the 2.2 kb transcript is more prevalent than the longer, 2.6 kb transcript. However, studies utilizing in situ hybridization to tissue sections revealed that, except for the embryo, in which a uniform, homogeneous pattern of hybridization was found, *Ras2* expression in the larva and the adult fly was limited to a number of tissues and organs (Segal and Shilo, 1986).

Our results, using in situ hybridization to whole embryos, suggest that the transcript distribution of the *Ras2* gene is differentially regulated in the embryo as well (Fig. 4). The first indication for specific *Ras2* localization is seen during germ band retraction (stages 12-13) in the garland cells. *Rop* is also detected in the garland cells and, in addition, in the CNS. At this stage, the neuroblasts start to differentiate into neurons (Campos-Ortega and Hartenstein, 1985). There are no signs of *Rop* expression in the neurogenic region in the earlier stages of CNS development, namely stages 8-10, in which neuroblasts are segregating from the neuroectoderm. This timing suggests that *Rop* may have no role in the differentiation of the neuroblasts but rather in their function as neurons. During all later stages, the *Ras2*-specific staining is limited to a specific set of cells in the CNS (weak signal) and to the garland cells (strong signal). However, *Rop*-specific staining gets stronger as the number of cells in the CNS increases. Except for several clusters in the brain hemispheres whose nature is unknown, the staining seems to be strong and general in all cells of the CNS. In the peripheral nervous system, the staining is weak, but appears in all neurogenic clusters. Distinct co-expression of both genes in the antennal-maxillary complex is also evident. Staining of the CNS and the garland cells was also observed using anti-Rop antibodies (N. H., unpublished results).

Co-expression of the *Ras2* and *Rop* genes during postembryonic development

In previous in situ hybridization experiments, *Ras2* transcripts were detected in the larval imaginal discs, but not in the salivary glands (Segal and Shilo, 1986). Additionally, *Ras2* transcripts were detected in the cortex of the larval brain, but not in its interior. In adult flies, specific hybridization was found in the brain cortex, the thoracic and abdominal ganglia, the flight muscles and the ovaries.

We have used *Ras2*- and *Rop*-promoter fragments fused to a β-galactosidase reporter gene to detect *Ras2* and *Rop* expression in postembryonic stages (Fig. 7). Our results show that both genes are potentially co-expressed in the larval salivary glands and CNS, and in the adult CNS and reproductive systems. The utilization of a reporter gene to detect promoter activity is a widely used approach, providing unsurpassed resolution and sensitivity. However, in the fusion constructs, any putative control elements upstream of the promoter fragment and downstream of the fusion junction are missing. Consequently, a false signal may be obtained, or a real signal may be absent, due to the

lack of an essential negative or positive control element, respectively. For example, in situ hybridization of the imaginal discs with a *Ras2* probe indicated that this gene is expressed in these organs (Segal and Shilo, 1986). However, we were not able to detect this expression using the reporter gene approach (Fig. 7E,F).

It is possible that our negative results in this case are due to weak activity of the transposon-encoded β-galactosidase. Alternatively, a positive enhancer, specific for *Ras2* expression in the imaginal discs, may be missing in our *Ras2-lacZ* fusion. Nevertheless, in those cases where positive β-galactosidase activity was detected, it is clear that the *Ras2*- and *Rop*-promoter fragments tested have at least the potential to promote the identical tissue-specific patterns as illustrated in Fig. 7. It is very likely that this potential is realized, since anti-Rop antibodies detected *Rop* protein in extracts of larval and adult brains (A. S., unpublished results). Furthermore, using in situ hybridization with the *Rop* probe, the highly specific pattern of β-galactosidase activity obtained in the larval ventral ganglion and brain hemispheres was reproduced by the pattern of *Rop* transcript localization in these organs (N. C., unpublished results).

Why a bidirectional promoter?

The two promoter fragments examined in these studies are overlapping along a sequence of only 216 bases (Fig. 6), which contains the 93 bases of the bidirectional promoter region between the two start sites and 123 bases from the untranslated regions of the two genes. Since both promoter fragments give rise to the same specific patterns shown in Fig. 7, we are forced to conclude that these patterns are determined by the same set of control elements located within this short overlapping region. We have already shown that all the *cis*-acting elements required for the activity of the bidirectional promoter in both orientations, in Schneider 2 tissue culture cells (Lev et al., 1989) and in growing embryos (N. C., unpublished results) are located within the 93 base-pair region between the *Ras2* and *Rop* transcription start sites.

In general, eucaryotic genes are widely dispersed within the genome and pairs of closely located, divergently transcribed genes which are controlled by joint bidirectional promoters are infrequent. A bidirectional promoter may set strict limitations on the optimal evolution of the transcriptional control mechanism of each member of the gene pair. Any change in favor of the regulation of one gene must also be in favor of the control mechanism of the second gene, or at least not hamper it. Good reasons to keep this type of promoter organization after all are: (a) participation of the two gene products in the same regulatory or enzymatic pathway, e.g. the SV40 early and late genes (Gidoni et al., 1985) and the yeast GAL1 and GAL10 genes (Giniger et al., 1985); (b) participation in the same biological structures, e.g. a pair of H2A and H2B sea urchin late histones (Kemler and Busslinger, 1986), the human collagens α1(IV) and α2(IV) (Burbelo et al., 1988) and (c) co-expression of the two genes in one common tissue e.g. the *Drosophila* glue proteins *sgs7* and *sgs8* (Hofmann et al., 1991), the *Drosophila* yolk proteins *yp1* and *yp2* (Logan et al., 1989), the *B. mori* chorion proteins *2132* and *2574* and other chorion gene pairs (Iatrou and Tsitilou, 1983). Apparently, the results presented in Figs 4 and 7 suggest that the *Ras2* and *Rop* genes are actually co-expressed in several *Drosophila* tissues. Although other possibilities cannot be excluded, it is very likely that it is the simultaneous demand for the *Ras2* and *Rop* gene products in at least one of these tissues that has kept their bidirectional promoter in its unusual compact organization.

The putative role of the *Rop* gene

The sequence homology between Rop and Sly1p, Sec1p and Vps33p, the three yeast proteins involved in vesicle trafficking and the conserved motif that they share with β-COP, suggests that the *Rop* protein may also function in vesicle trafficking among membranes of *Drosophila* cells. It has been shown that both β-COP, which cycles between the cytosol and the Golgi, and Sly1p can be found in the cytosolic and membrane subcellular fractions (Duden et al., 1991; Donaldson et al., 1991; C. Dascher, personal communication). Likewise, the *Rop* protein can be either soluble or bound to membranes (A. S., unpublished results).

The tissue-specific expression of the *Rop* gene during embryogenesis in the CNS, a tissue that is highly active in membrane recycling, and particularly in the garland cells, is in concert with this suggestion. The garland cells, also termed wreath cells, surround the esophagus near its junction with the proventriculus (Fig. 5; Rizki, 1978). The cells are already differentiated in the embryo and they persist to the adult stage. Studies bearing on the ultrastructure of these cells reveal extensive invaginations of the plasma membrane, termed the labyrinthine channels, which are the origin of a number of coated pits and coated vesicles. Many vacuoles are found in this region. The garland cells are considered to be accessory cells to the open blood system and have been implicated in the removal of waste materials from the haemolymph by endocytosis (Aggarwal and King, 1967; Narita et al., 1989).

The putative role of the *Ras2* gene

Considering the relatively high expression of *Ras2* in the garland cells and its putative interaction with *Rop* (as discussed below), it is possible that *Ras2* is also involved in endocytic processes and/or other transport pathways mediated by vesicle trafficking. The role of yeast small GTPases in these pathways has been mentioned above. In vertebrates, a large number of ras-like proteins are involved in exocytosis and endocytosis (e.g. Chavrier et al., 1990). Two of these proteins, rab4 and rab5, have recently been implicated in the regulation of distinct early events in the endocytic pathway (Bucci et al., 1992; van der Sluijs et al., 1992).

Studies with the activated form of the *Ras2* protein suggest that it may also be associated with developmental processes. Expression of *Ras2* protein, in which the glycine at position 14 (which corresponds to glycine at position 12 of the human Ha-ras) was mutated in vitro into valine, resulted in a variety of phenotypic changes (Bishop and Corces, 1988). These changes were observed either in ectopic expression under the regulation of the *hsp70* gene promoter, or in normal expression under the regulation of the endogenous *Ras2* promoter. They include low fertility,

developmental disturbances in the wings and bristles of variable size and placement. A dual role for a small GTP-binding protein in vesicle transport and in the control of cell proliferation has already been documented. Microinjection of human *Ha-ras* protein into rat embryo fibroblasts resulted in increased membrane ruffling and fluid phase pinocytosis (Bar-Sagi and Feramisco, 1986).

Possible interaction between the *Ras2* and *Rop* proteins

Our finding that the *Rop* protein is homologous to the products of the *S. cerevisiae SLY1* and *SEC1* genes is very stimulating, since the *SLY1-20* allele was isolated in a screen designed to identify suppressors of the loss of the *YPT1* gene function (Dascher et al., 1991; Ossig et al., 1991). *YPT1* is a *ras*-like gene (Gallwitz et al., 1983) essential for ER-to-Golgi protein transport and possibly also participates in intra-Golgi vesicle movements (Schmitt et al., 1988; Segev et al., 1988). The *SLY1-20* allele, which allows *YPT1*-independent growth, contains a single point mutation in the wild-type *SLY1* gene (Dascher et al., 1991). The mutation is absolutely required to complement the loss of *YPT1*, since overexpression of the wild-type *SLY1* allele is not sufficient for complementation. A possible mechanism of the suppression is that *SLY1* encodes a protein acting downstream of *YPT1*. It is suggested that the mutated Sly1-20p protein has a specific conformation which the wild-type protein can achieve only through interaction with Ypt1p (Ossig et al., 1991). Thus, *SLY1* may interact, directly or indirectly, with *YPT1*.

Interestingly, *SEC1* and *SEC4*, a *ras*-related gene (Salminen and Novick, 1987), may be another yeast gene pair of a similar type, since both genes are involved in protein transport from the Golgi to the plasma membrane. However, in this case there is as yet no proof of genetic or biochemical interaction between them. Finally, β-COP also interacts with a member of the ras superfamily. Recently it has been shown that ARF, a *ras*-like ADP ribosylation factor, is required for the binding of β-COP to Golgi membranes (Donaldson et al., 1992). Thus, the Rop/Sly1p/Sec1p/β-COP sequence homology may represent not only common sites associated with transient binding to internal membranes but also common sites required for their interaction with small GTP-binding proteins.

As discussed above, the unusually close proximity of the *Ras2* and *Rop* genes and their joint regulative mechanism could be due solely to the need for their products in the same tissue. However, since Sly1p, Sec1p and β-COP, may interact with small GTP-binding proteins of the ras superfamily, it is conceivable that the *Rop* and *Ras2* proteins may also interact functionally with one another in these tissues.

We thank L. Kauvar and T. Kornberg for the cDNA library; B-Z. Shilo, A. Tzagoloff, M. Piovant and D. Knipple for DNA clones and vectors; W. Gehring and his colleagues for the most useful “little blue book”; and H. D. Schmitt, C. Dascher and M. Egerton for personal communications. We also thank K. Moses and M. Ashburner who taught us how to inject embryos. The travel to Cambridge was supported by an EMBO Short-term fellowship to Z. L. This work was supported by grants from the US-Israel Binational Science Foundation, the Basic Research Foundation administered by the Israel Academy of Sciences and Humanities, and the Israel Cancer Research Fund.

EMBL/GenBank/DDBJ nucleotide sequence databases accession numbers X67218, X67219.

REFERENCES

Aalto, M. K., Ruohonen, L., Hosono, K. and Keranen, S. (1991). Cloning and sequencing of the yeast Saccharomyces cerevisiae SEC1 gene localized on chromosome IV. *Yeast* **7**, 643-650.

Aalto, M. K., Keranen, S. and Ronne, H. (1992). A family of proteins involved in intracellular transport. *Cell* **68**, 181-182.

Aggarwal, S. K. and King, R. C. (1967). The ultrastructure of the wreath cells of Drosophila melanogaster larvae. *Protoplasma* **63**, 343-352.

Ashburner, M. (1989a). Drosophila: *A Laboratory Manual.* Cold Spring Harbor Laboratory Press.

Ashburner, M. (1989b). Drosophila: *A Laboratory handbook.* Cold Spring Harbor Laboratory Press.

Bar-Sagi, D. and Feramisco, J. R. (1986). Induction of membrane ruffling and fluid-phase pinocytosis in quiescent fibroblasts by ras proteins. *Science* **233**, 1061-1068.

Bishop, J. G. and Corces, V. G. (1988). Expression of an activated ras gene causes developmental abnormalities in transgenic Drosophila melanogaster. *Genes Dev.* **2**, 567-577.

Brock, H. W. (1987). Sequence and genomic structure of ras homologues Dmras85D and Dmras64B of Drosophila melanogaster. *Gene* **51**, 129-137.

Bucci, C., Parton, R. G., Mather, I. H., Stunnenberg, H., Simons, K., Hoflack, B. and Zerial, M. (1992). The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell* **70**, 715-728.

Burbelo, P. D., Martin, G. R. and Yamada, Y. (1988). Alpha 1(IV) and alpha 2(IV) collagen genes are regulated by a bidirectional promoter and a shared enhancer. *Proc. Natl. Acad. Sci. U. S. A.* **85**, 9679-9682.

Campos-Ortega, J. A and Hartenstein, V. (1985). *The Embryonic Development of* Drosophila melanogaster. Berlin: Springer Verlag.

Cavener, D. R. (1987). Comparison of the consensus sequence flanking translational start sites in Drosophila and vertebrates. *Nucleic Acids Res.* **15**, 1353-1361.

Chardin, P. (1991). Small GTP-binding proteins of the ras family: a conserved functional mechanism? *Cancer Cells* **3**, 117-126.

Chavrier, P., Parton, R. G., Hauri, H. P., Simons, K. and Zerial, M. (1990). Localization of low molecular weight GTP binding proteins to exocytic and endocytic compartments. *Cell* **62**, 317-329.

Cohen, N., Salzberg, A. and Lev, Z. (1988). A bidirectional promoter is regulating the Drosophila ras2 gene. *Oncogene* **3**, 137-142.

Cooley, L., Kelley, R. and Spradling, A. C. (1988). Insertional mutagenesis of the Drosophila genome with single P elements. *Science* **239**, 1121-1128.

Dascher, C., Ossig, R., Gallwitz, D. and Schmitt, H. D. (1991). Identification and structure of four yeast genes (SLY) that are able to suppress the functional loss of YPT1, a member of the RAS superfamily. *Mol. Cell Biol.* **11**, 872-885.

Donaldson, J. G., Kahn, R. A., Lippincott Schwartz, J. and Klausner, R. D. (1991). Binding of ARF and beta-COP to Golgi membranes: possible regulation by a trimeric G protein. *Science* **254**, 1197-1199.

Donaldson, J. G., Cassel, D., Kahn, R. A. and Klansner, R. D. (1992). A small GTP binding protein, ARF, is required for non clatherin coat protein binding to Golgi membranes. *Proc. Natl. Acad. Sci. USA* **89**, 6408-6412.

Duden, R., Griffiths, G., Frank, R., Argos, P. and Kreis, T. E. (1991). b-COP, a 110 kd protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to b-adaptin. *Cell* **64**, 649-665.

Fortini, M. E., Simon, M. A. and Rubin, G. M. (1992). Signalling by the Sevenless protein tyrosine kinase Is mimicked by Ras1 Activation. *Nature* **355**, 559-561.

Gallwitz, D., Donath, C. and Sander, C. (1983). A yeast gene encoding a protein homologous to the human c-has/bas proto-oncogene product. *Nature* **306**, 704-707.

Gaul, U., Mardon, G. and Rubin, G. M. (1992). A putative Ras GTPase activating protein acts as a negative regulator of signaling by the sevenless receptor tyrosine kinase. *Cell* **68**, 1007-1019.

Gidoni, D., Kadonaga, J. T., Barrera-Saldana, H., Takahashi, K.,

Chambon, P. and Tjian, R. (1985). Bidirectional SV40 transcription mediated by tandem Sp1 binding interactions. *Science* **230**, 511-517.

Giniger, E., Varnum, S. M. and Ptashne, M. (1985). Specific DNA binding of GAL4, A positive regulatory protein of yeast. *Cell* **40**, 767-774.

Hariharan, I. K., Carthew, R. W. and Rubin, G. M. (1991). The Drosophila Roughened mutation: Activation of a rap homolog disrupts eye development and interferes with cell determination. *Cell* **67**, 717-722.

Hofmann, A., Garfinkel, M. D. and Meyerowitz, E. M. (1991). *cis*-Acting sequences required for expression of the divergently transcribed Drosophila melanogaster Sgs-7 and Sgs-8 glue protein genes. *Mol. Cell. Biol.* **11**, 2971-2979.

Iatrou, K. and Tsitilou, S. J. (1983). Coordinately expressed chorion genes of Bombyx mori: is developmental specificity determined by secondary structure recognition? *EMBO J.* **2**, 1431-1440.

Jokerst, R. S., Weeks, J. R., Zehring, W. A. and Greenleaf, A. L. (1989). Analysis of the gene encoding the largest subunit of RNA polymerase II in Drosophila. *Mol. Gen. Genet.* **215**, 266-275.

Kemler, I. and Busslinger, M. (1986). Characterization of two nonallelic pairs of late histone H2A and H2B genes of the sea urchin: Differential regulation in the embryo and tissue-specific expression in the adult. *Mol. Cell Biol.* **6**, 3746-3754.

Klemenz, R., Weber, U. and Gehring, W. J. (1987). The white gene as a marker in a new P-element vector for gene transfer in Drosophila. *Nucleic Acids Res.* **15**, 3947-3959.

Knipple, D. C. and Marsella-Herrick, P. (1988). Versatile plasmid vectors for the construction, analysis and heat-inducible expression of hybrid genes in eukaryotic cells. *Nucleic Acids Res.* **16**, 7748.

Kuhn, R., Schafer, U. and Schafer, M. (1988). pW-ATG-lac, P-element vectors for lacZ transcriptional gene fusions in Drosophila. *Nucleic Acids Res.* **16**, 4163.

Lev, Z., Kimchie, Z., Hessel, R. and Segev, O. (1985). Expression of ras cellular oncogenes during development of Drosophila melanogaster. *Mol. Cell Biol.* **5**, 1540-1542.

Lev, Z., Segev, O., Cohen, N., Salzberg, A. and Shemer, R. (1989). Structure of the Drosophila Ras2 bidirectional promoter. In *ras Oncogenes* (ed. D. Spandidos) pp. 75-81. New York: Plenum.

Lev, Z. (1993). Ras genes in D. melanogaster. In *The* ras *Superfamily of GTPases* (ed. J. C. Lacal and F. McCormick). Boca Raton: CRC Press.

Logan, S. K., Garabedian, M. J. and Wensink, P. C. (1989). DNA regions that regulate the ovarian transcriptional specificity of Drosophila yolk protein genes. *Genes Dev.* **3**, 1453-1461.

Mozer, B., Marlor, R., Parkhurst, S. and Corces, V. (1985). Characterization and developmental expression of a Drosophila ras oncogene. *Mol. Cell Biol.* **5**, 885-889.

Narita, K., Tsuruhara, T., Koenig, J. H. and Ikeda, K. (1989). Membrane pinch-off and reinsertion observed in living cells of Drosophila. *J. Cell. Physiol.* **141**, 383-370.

Novick, P. J., Field, C. and Schekman, R. (1980). Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. *Cell* **21**, 205-215.

Ossig, R., Dascher, C., Trepte, H. H., Schmitt, H. D. and Gallwitz, D. (1991). The yeast SLY gene products, suppressors of defects in the essential GTP-binding Ypt1 protein, may act in endoplasmic reticulum-to-Golgi transport. *Mol. Cell Biol.* **11**, 2980-2993.

Rio, D. C. and Rubin, G. M. (1985). Transformation of cultured Drosophila melanogaster cells with a dominant selectable marker. *Mol. Cell. Biol.* **5**, 1833-1838.

Rizki, T. M. (1978). The circulatory system and associated cells and tissues. In The genetics and biology of Drosophila, vol. 2b (ed. M. Ashburner and T. R. F. Wright), pp. 397-452. London: Academic Press.

Robinson, J. S., Klionsky, D. J., Banta, L. M. and Emr, S. D. (1988). Protein sorting in Saccharomyces cerevisiae: isolation of mutants defective in the delivery and processing of multiple vacuolar hydrolases. *Mol. Cell Biol.* **8**, 4936-4948.

Rubin, G. M. and Spradling, A. C. (1982). Genetic transformation of Drosophila with transposable element vectors. *Science* **218**, 348-353.

Salminen, A. and Novick, P. J. (1987). A ras-like protein is required for a post-Golgi event in yeast secretion. *Cell* **49**, 527-538.

Schmitt, H. D., Puzicha, M. and Gallwitz, D. (1988). Study of a temperature-sensitive mutant of the ras-related YPT1 gene product in yeast suggests a role in the regulation of intracellular calcium. *Cell* **53**, 635-647.

Segal, D. and Shilo, B. Z. (1986). Tissue localization of Drosophila melanogaster ras transcripts during development. *Mol. Cell Biol.* **6**, 2241-2248.

Segev, N., Mulholland, J. and Botstein, D. (1988). The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell* **52**, 915-924.

Serafini, T., Stenbeck, G., Brecht, A., Lottspeich, F., Orci, L., Rothman, J. E. and Wieland, F. T. (1991). A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein beta-adaptin. *Nature* **349**, 215-220.

Simon, M. A., Bowtell, D. D. L., Dodson, G. S., Laverty, T. R. and Rubin, G. M. (1991). Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. *Cell* **67**, 701-716.

Spradling, A. C. (1986). P element mediated transformation. In Drosophila: *A Practical Approach* (ed. D. B. Roberts), pp. 175-198. Oxford: IRL Press.

Spradling, A. C. and Rubin, G. M. (1982). Transposition of cloned P elements into Drosophila germ line chromosomes. *Science* **218**, 341-347.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in Drosophila embryos reveals translational control of the segmentation gene hunchback. *Chromosoma* **98**, 81-85.

Valencia, A., Chardin, P., Wittinghofer, A. and Sander, C. (1991). The ras protein family: Evolutionary tree and role of conserved amino acids. *Biochemistry* **30**, 4637-4648.

Van der Sluijs, P., Hull, M., Webster, P., Male, P., Goud, B. and Mellman, I. (1992). The small GTP-binding protein rab4 contrls an early sorting event on the endocytic pathway. *Cell* **70**, 729-740.

Wada, Y., Kitamoto, K., Kanbe, T., Tanaka, K. and Anraku, Y. (1990). The SLP1 gene of Saccharomyces cerevisiae is essential for vacuolar morphogenesis and function. *Mol. Cell Biol.* **10**, 2214-2223.

(Accepted 6 January 1993)