applied to (iv) a Sephacryl-S300 HR column (320 ml) run with buffer B. The yield through steps iii and iv was >55%. (v) ATP-Sepharose (2 ml) (Pharmacia) run with buffer B containing Brij 35 (0.05%) and 2 mM $MnCl_2$. For elution, $MnCl_2$ was omitted from the buffer and 300 mM sodium acetate was added. Yield was 45%. (vi) Chromatography on Phenyl-Superose HR 5/5 as in step ii (60% yield). (vii) Rechromatography on ATP-Sepharose (1 ml) as in step v with a recovery of 30%. Final yield of adenylyl cyclase activity was 1%. The enzyme was assayed as described (*3*). For SDS–polyacrylamide gel electrophoresis, adenylyl cyclase eluting in 1 ml was precipitated with four volumes of methanol-acetone (1:1). Upon storage at −20°C, approximately 50% of enzyme activity was lost per month.

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The *Son of sevenless* Gene Product: A Putative Activator of Ras

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The *Son of sevenless* (*Sos*) gene functions in signaling pathways initiated by the sevenless and epidermal growth factor receptor tyrosine kinases. The *Sos* gene has now been isolated and sequenced. Its product is a 1595–amino acid protein similar to the CDC25 protein in *Saccharomyces cerevisiae*, a guanine nucleotide exchange factor that activates Ras. These results imply a role for the *ras* pathway in *Drosophila* neuronal development.

IN THE DEVELOPING *DROSOPHILA* EYE, a cluster of eight photoreceptor neurons develop autonomously in each facet or ommatidium (*1, 2*) with the central cell R8 being the first to express neuronal markers (*3*). R8 directly induces a neighboring cell to develop as the R7 neuron (*4*). This induction is mediated by the products of the *bride of sevenless* (*boss*) and *sevenless* (*sev*) genes (*4–8*). The *boss* gene product is a transmembrane protein that is localized to the R8 cell (*5*), and the *sevenless* gene encodes a tyrosine kinase receptor expressed on the membranes of many cells in the developing cluster (*9, 10*). Extensive genetic and molecular analysis of this system suggests that the boss protein on R8 binds to and activates the sevenless receptor on the R7 precursor cell, leading to its eventual differentiation into a neuron (*5*). In *sev* or *boss* mutants, R7 cells are missing from all facets of the eye.

Recent genetic analysis has shown that a gene called *Son of sevenless* (*Sos*) functions in a signal transduction pathway initiated by the sevenless receptor (*11*). This gene was first identified as a dominant mutant allele called Sos^{JC2}, which suppresses the sev^{E4} allele. This suppression is allele-specific, suggesting an interaction between the proteins encoded by these genes. Genetic analysis further demonstrated that Sos^{JC2} product is necessary autonomously in the R7 cell for this cell to develop in a sev^{E4} background. The function of the *Sos* gene product is not limited to the development of the R7 cell; in weak loss of function alleles, other photoreceptor cells are missing, and complete loss of function leads to recessive lethality. The *Sos* gene also functions downstream of the *Drosophila* epidermal growth factor receptor (*11*). We now describe the isolation and characterization of the *Sos* gene and show that it encodes the *Drosophila* homolog of CDC25, an activator of Ras in *Saccharomyces cerevisiae*.

The *Sos* gene maps to the 34D5 region of the second chromosome. This region has been saturated for mutations and a deficiency map has been reported (*12*). We have confirmed the mapping results (relevant loci and breakpoints are shown in Fig. 1A).

Starting with a clone in 35A, we initiated a chromosomal jump to 34D3 and followed it with a chromosomal walk in the region of the *Sos* gene. In all, about 150 kb of genomic fragments were cloned as cosmid and phage DNA. Genomic fragments were used in chromosomal in situ hybridization experiments to delimit the *Sos* region (see below). A molecular map of this region was constructed (Fig. 1B) and four transcription units were identified through cDNA clones isolated from a cDNA library from eye imaginal discs.

Molecular characterization of the many chromosomal rearrangements mapping to the region allowed us to discriminate between these cDNAs. Three deletions, Df(2L)*b*71ka, Df(2L)*b*84h1, and Df(2L)-*Sco*RV7, genetically uncover *Sos* and loci to its right (Fig. 1A). In situ hybridization

Table 1. Interaction of *Dras*-1 with *Sos* in flies homozygous for sev^{E4}. Df(3R)by62 and Df(3R)by10 overlap in the 85D11-F1 region and include the transcription unit for *Dras*-1 (*20*). One copy of either of these deletions eliminates the suppression of sev^{E4} by Sos^{JC2}. This is a specific effect in that over twenty deletions mapping outside the region [for example, Df(3R)D1BX12] have been tested in this assay and do not have any effect on the suppression. The ommatidia were screened by the optical technique called pseudopupil (*6*); *n* is the number of ommatidia screened. The $boss^1$ allele is a null allele at the *boss* locus.

Genotype	Ommatidia with R7 cells
sev^{E4}/sev^{E4}; Sos^+/Sos^+; +/+	0% (n = 2239)
sev^{E4}/sev^{E4}; Sos^{JC2}/Sos^+; +/+	16% (n = 2392)
sev^{E4}/sev^{E4}; Sos^{JC2}/Sos^+; $boss^1/boss^+$	0% (n = 2337)
sev^{E4}/sev^{E4}; Sos^{JC2}/Sos^+; Df(3R)by62/+	0% (n = 2175)
sev^{E4}/sev^{E4}; Sos^{JC2}/Sos^+; Df(3R)by10/+	0% (n = 2180)
sev^{E4}/sev^{E4}; Sos^{JC2}/Sos^+; Df(3R)D1BX12/+	17% (n = 898)

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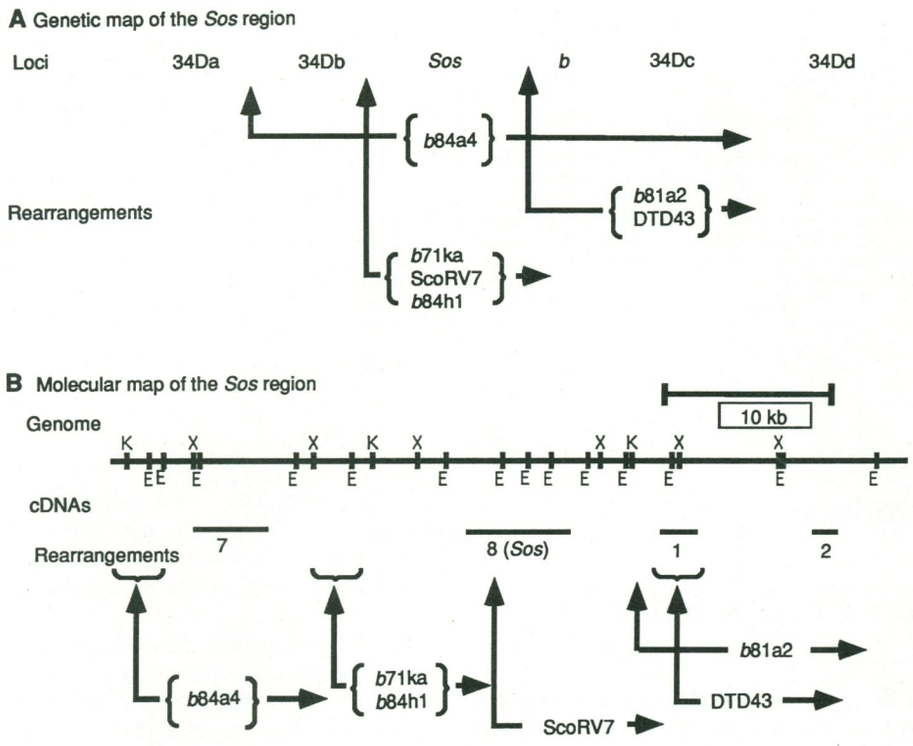
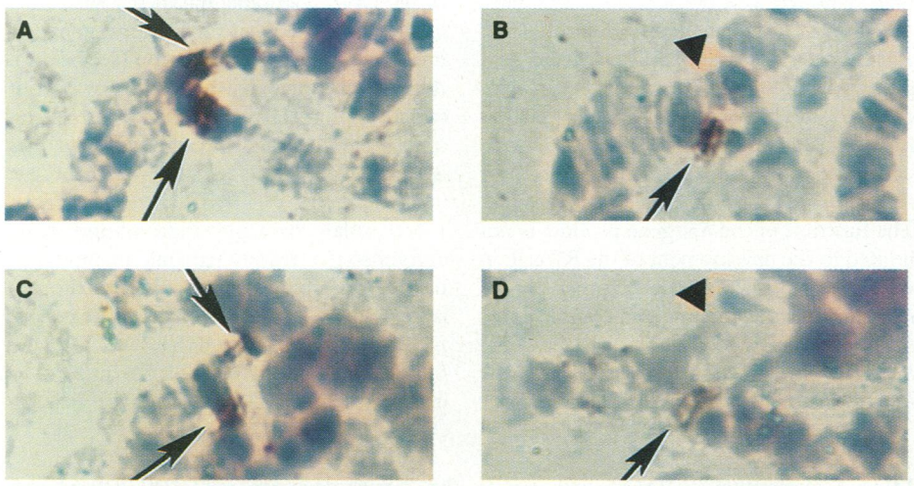


Fig. 1. Genetic and molecular maps of the *Sos* region. (**A**) Genetic map showing mutant loci and genetic breakpoints of chromosomal rearrangements. Only the left breakpoints of the deletions are relevant to this study and are shown. (**B**) Molecular map of the *Sos* region showing relative positions of cDNAs and rearrangement breakpoints. Df(2L)*b*84a4 was used to jump from 35A to 34D and its breakpoint served as the left-most limit for the walk. The breakpoints of the deficiencies were determined by chromosomal in situ hybridization and Southern blot analysis (see Fig. 2). Brackets at the tips of arrows showing breakpoints indicate the Eco RI fragments in the genome to which these breakpoints map. E, Eco RI; K, Kpn I; X, Xba I.

experiments showed that the breakpoints of Df(2L)*b*71ka (Fig. 2, A and B) and Df(2L)*b*84h1 (*13*) map to the same genomic fragments to the right of cDNA 7 and left of cDNA 8 (Fig. 1B). Thus, these deficiencies, which are genetically *Sos*$^{-}$, also delete cDNA 8. Similarly, the breakpoint of Df(2L)*Sco*RV7 was mapped to the coding region of cDNA 8 by Southern (DNA) blot analysis (Fig. 2E). Because cDNA 7 is not included in any of these deletions, it is ruled out as a candidate for the *Sos* gene.

The deletions Df(2L)*b*81a2 and Df(2L)-DTD43 are genetically wild type for *Sos* but uncover loci immediately to the right of that gene. The breakpoint of Df(2L)*b*81a2 was mapped to a 2.8-kb Eco RI restriction fragment to the right of cDNA 8 but to the left of cDNAs 1 and 2 (Figs. 1B and 2F). Therefore, deletion Df(2L)*b*81a2 eliminates cDNAs 1 and 2. Because Df(2L)*b*81a2 is genetically wild type for *Sos*, cDNAs 1 and 2 do not encode *Sos*. These results are consistent with in situ hybridization experiments using Df(2L)DTD43 in which the breakpoint maps to the same genomic fragments as cDNA 1 (Figs. 1B, and 2, C and D). When taken together, the rearrangement breakpoints assign cDNA 8 to *Sos*.

A 15.1-kb Kpn I fragment containing the



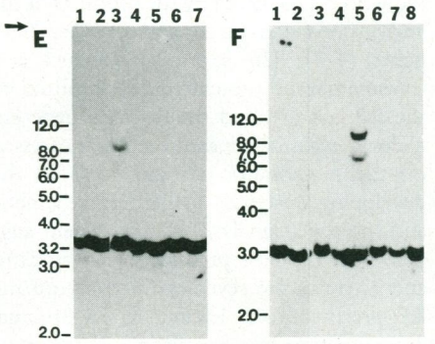


Fig. 2. Mapping of deficiency breakpoints by chromosomal in situ hybridization and DNA blot analysis. Breakpoints of Df(2L)*b*71ka and Df(2L)DTD43 were mapped by in situ hybridization. The analysis of the deficiency and wild-type chromosomes was facilitated by the presence of associated inversions that cause asynapsis in this region. (**A**) Chromosomes from Df(2L)*b*71ka/+ flies probed with cDNA 7. Signals (arrows) are present on both wild-type and deficiency homologues. (**B**) Chromosomes from Df(2L)*b*71ka/+ flies probed with cDNA 8. A signal is present on the wild-type homologue (arrow). The corresponding region in the deleted homologue (arrowhead) does not have a signal. (**C**) Chromosomes from Df(2L)DTD43/+ flies probed with the 3′ end of cDNA 8. Signals (arrows) are present on both wild-type and deficiency homologues. (**D**) Chromosomes from Df(2L)DTD43/+ flies probed with the 5.3-kb Eco RI fragment mapping to cDNA 2. A signal (arrow) is seen on the wild-type homologue only, indicating that this deficiency breaks to the left of this fragment. The arrowhead points to the deficiency chromosome, which lacks a signal. (**E**) Breakpoint of Df(2L)*Sco*RV7. Total genomic DNA was digested with Eco R1, transferred to a Zetaprobe blot, and probed with the 3.2-kb Eco RI genomic fragment mapping to cDNA 8. An 8-kb restriction fragment length polymorphism (RFLP) was seen with Df(2L)*Sco*RV7. The rest of the lines serve as controls for genetic background. Lane assignments are as follows: 1, *CyO/Star*; 2, zw^{E11}; 3, Df(2L)*Sco*RV7/*CyO*; 4, Df(2L)*b*84h1/*CyO*; 5, Df(2L)*b*81a2/SM5; 6, Df(2L)*b*83b22/SM5; 7, Df(2L)DTD43/*CyO*. *CyO* and SM5 are balancer chromosomes, zw^{E11} is a wild-type parental stock. Arrow marks the origin. (**F**) Breakpoint of Df(2L)*b*81a2. Total genomic DNA was digested with Eco R1. The 2.8-kb Eco R1 genomic fragment mapping to the right of cDNA 8 and left of cDNA 1 was used as a probe. Df(2L)*b*81a2 is the only one to show an RFLP. This is a complex breakpoint and gives rise to two new bands. The arrow in (E) marks the position of the origin. Lane assignments are as follows: 1, *CyO/Star*; 2, zw^{E11}; 3, Df(2L)*Sco*RV7/*CyO*; 4, Df(2L)*b*84h1/*CyO*; 5, Df(2L)*b*81a2/SM5; 6, Df(2L)*b*83b22/SM5; 7, Df(2L)*b*85b2/SM5; and 8, Df(2L)DTD43/*CyO*.

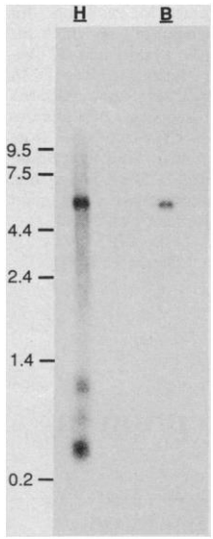


Fig. 3. Northern blot probed with *Sos* cDNA. Head (H) and body (B) polyadenylated RNA (10 μg each) was placed in the indicated lanes, transferred to a Zetaprobe blot, and probed with the *Sos* cDNA.

entire cDNA 8 (Fig. 1) was used in P-element–mediated transformation experiments (*14*) and was shown to rescue the lethal phenotype of the null allele Sos^{x122}. This formally proves that cDNA 8 corresponds to *Sos*.

On a Northern (RNA) blot (Fig. 3), the *Sos* cDNA hybridized to a 5.6-kb band of RNA from adult head and body. Three other small bands were seen in RNA from the head. These arise from the 5′ end of the gene and may result from alternative splicing. The *Sos* cDNA was hybridized to whole mount embryos and eye discs in situ. The *Sos* gene was widely expressed at low levels throughout the eye disc and the embryo (*15*).

The *Sos* cDNA was sequenced on both strands (GenBank accession number M83931) and the conceptual translation product is shown in Fig. 4A. The COOH-terminal end of protein encoded by *Sos* is similar to the catalytic domain of CDC25 of *S. cerevisiae* (Fig. 4B). The amino acid sequences of the most similar segment of the two proteins show 45% identity and 70% similarity. Motifs within this region that are conserved between the structurally similar yeast genes CDC25, SCD25, BUD5, and ste6 (*16*) are also conserved in the *Drosophila* sequence.

The product of the CDC25 gene in *S. cerevisiae* is required for the exchange of guanine nucleotides bound to Ras (*17, 18*). The activation of Ras is caused by its conversion from a GDP-bound form to a GTP-bound form. For both CDC25 and SCD25, the COOH-terminal end is necessary and sufficient for this activation (*19*). The strong similarity of sequences between *Sos* and CDC25 at the catalytic domain suggests that the Sos protein may activate *Drosophila* Ras in an analogous manner.

If the Sos protein is an activator of Ras, loss of function of the *ras* gene itself should show genetic interactions with mutations at the *Sos* locus. Point mutations in the *ras* genes are not available, but we demonstrated such an interaction with two overlapping deficiencies that uncover the *Dras*-1 locus of *Drosophila* (*20*). In a sev^{E4} background, one copy of the dominant allele Sos^{JC2} rescued R7 cells in 16% of all ommatidia (Table 1). The sev^{E4} allele may encode a weak tyrosine kinase and Sos^{JC2}, a hyperactive product (*11*). The assay used to determine *Dras* interactions is based on our finding that mutations in genes closely associated with *sev* in the pathway have a strong effect on the suppression of sev^{E4} by Sos^{JC2}. For example, introduction of one mutant copy of *boss* eliminates the suppression effect of Sos^{JC2}. One explanation of this result is that a partially active kinase encoded by the sev^{E4} allele is even weaker when the amount of boss, the ligand, is reduced and an overactive Sos^{JC2} protein can no longer compensate for the lower kinase activity. Loss of one mutant copy of *Dras*-1 entirely eliminates the suppression of sev^{E4} by Sos^{JC2} (Table 1). This provides additional support to the notion that the Sos protein interacts with Ras and implicates Ras in the developmental pathway of the R7 neuron.

A

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1    MFSGPSGHAH TISYGGGIGL GTGGGGGSGG 811  IEHHLSVPND EITLLTLHPL ELARQLTLLE
31   SGSGSQGGGG GIGIGGGGVA GLQDCDGYDF 841  FEMYKNVKPS ELVGSPWTKK DKEVKSPNLL
61   TKCENAARWR GLFTPSLKKV LEQVHPRVTA 871  KIMKHTTNVT RWIEKSITEA ENYEERLAIM
91   KEDALLYVEK LCLRLLAMLC AKPLPHSVQD 901  QRAIEVMMVM LELNNFNGIL SIVAAMGTAS
121  VEEKVNKSFP APIDQWALNE AKEVINSKKR 931  VYRLRWTFQG LPERYRKFLE ECRELSDDHL
151  KSVLPTEKVH TLLQKDVLQY KIDSSVSAFL 961  KKYQERLRSI NPPCVPFFGR YLTNILHLEE
181  VAVLEYISAD ILKMAGDYVI KIAHCEITKE 991  GNPDLLANTE LINFSKRRKV AEIIGEIQQY
211  DIEVVMNADR VLMDMLNQSE ATSCPVPCHF 1021 QNQPYCLNEE STIRQFFEQL DPFNGLSDKQ
241  PRSASATYEE TVKELIHDEK QYQRDLHMII 1051 MSDYLYNESL RIEPRGCKTV PKFPRKWPHI
271  RVFREELVKI VSDPRELEPI FSNIMDIYEV 1081 PLKSPGIKPR RQNQTNSSSK LSNSTSSVAA
301  TVTLLGSLED VIEMSQEQSA PCVGSCFEEL 1111 AAAASSTATS IATASAPSLH ASSIMDAPTA
331  AEAEEFDVYK KYAYDVTSQA SRDALNNLLS 1141 AAANAGSGTL AGEQSPQHNP HAFSVFAPVI
361  KPGASSLTTA GHGFRDAVKY YLPKLLLVPI 1171 IPERNTSSWS GTPQHTRTDQ NNGEVSVPAP
391  CHAFVYFDYI KHLKDLSSSQ DDIESFEQVQ 1201 HLPKKPGAHV WANNNSTLAS ASAMDVVFSP
421  GLLHPLHCDL EKVMASLSKE RQVPVSGRVR 1231 ALPEHLPPQS LPDSNPFASD TEAPPSPLPK
451  RQLAIERTRE LQMKVEHWED KDVGQNCNEF 1261 LVVSPRHETG NRSPFHGRMQ NSPTHSTAST
481  IREDSLSKLG SGKRIWSERK VFLFDGLMVL 1291 VTLTGMSTSG GEEFCAGGFY FNSAHQGQPG
511  CKANTKKQTP SAGATAYDYR LKEKYFMRRV 1321 AVPISPHVNV PMATNMEYRA VPPPLPPRRK
541  DINDRPDSDD LKNSFELAPR MQPPIVLTAK 1351 ERTESCADMA QKRQAPDAPT LPPRDGELSP
571  NAQHKHDWMA DLLMVITKSM LDRHLDSILQ 1381 PPIPPRLNHS TGISYLRQSH GKSKEFVGNS
601  DIERKHPLRM PSPEIYKFAV PDSGDNIVLE 1411 SLLLPNTSSI MIRRNSAIEK RAAATSQPNQ
631  ERESAGVPMI KGATLCKLIE RLTYHIYADP 1441 AAAGPISTTL VTVSQAVATD EVLPLPISPA
661  TFVRTFLTTY RYFCSPQQLL QLLVERFNIP 1471 ASSSTTTSPL TPAMSPMSPN IPSHPVESTS
691  DPSLVYQDTG TAGAGGMGGV GGDKEHKNSH 1501 SSYAHQLRMR QQQQQQTHPA IYSQHHQHHA
721  REDWKRYRKE YVQPVQFRVL NVLRHWVDHH 1531 THLPHHPHQH HSNPTQSRSS PKEFFPIATS
751  FYDFEKDPML LEKLLNFLEH VNGKSMRKWV 1561 LEGTPKLPPK PSLSANFYNN PDKGTMFLYP
781  DSVLKIVQRK NEQEKSNKKI VYAYGHDPPP 1591 STNEE*
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B

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Sos      912 ELNNFNGILSIVAAMGTASVYRLRWTFQGLPERYRKFLEECREL..SDDH 1074
             |||||.:: .||.|: ...:|||:.|:: :..  :.:|.: .:|  |. :
CDC25   1494 ELNNFSSMTAIVSALYSSPIYRLKKTWDLVSTESKDLLKNLNNLMDSKRN 1543

Sos      960 LKKYQERLRSI.NPPCVPFFGRYLTNILHLEEGNPDLLAN.TELINFSKR 1122
             : ||.| |||: :.:|||||| ||.::     ||||:| | |::|||||
CDC25   1544 FVKYRELLRSVTDVACVPFFGVYLSDLTFTFVGNPDFLHNSTNIINFSKR 1593

Sos     1008 RKVAEIIGEI 1132
              |:|:|::||
CDC25   1594 TKIANIVEEI 1603
```

Fig. 4. Sequence analysis of *Sos*. (**A**) Conceptual translation product of the *Sos* cDNA. The complete Sos protein is predicted to have 1595 amino acids. (**B**) Comparison of Sos and CDC25 peptide sequence. The COOH-terminal region of CDC25 is aligned against the COOH-terminal end of Sos. Lines indicate identity and dots indicate similarity.

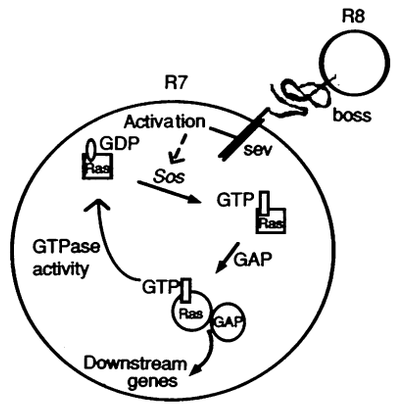

Fig. 5. A model for cell fate determination in the R7 neuron. The boss protein from the R8 cell binds to the sevenless receptor on the R7 precursor. This results in the activation of Sos by the sevenless tyrosine kinase. Activated Sos then facilitates guanine nucleotide exchange and the consequent activation of Ras. A multistep cascade follows, leading to the adoption of a neuronal fate.

We propose the following model for the role of *Sos* in R7 development (Fig. 5). The *Sos* gene product may be activated by the tyrosine kinase encoded by *sev*. This facilitates the activation of Ras. Ras, in turn, acts upon as yet unidentified substrates and leads to a commitment to the R7 cell fate. A GAP-like molecule has not yet been found in this pathway but is proposed in this model by analogy to other Ras pathways. The data presented here do not rule out the possibility that sevenless also acts through a molecule other than Sos.

Previous work in mammalian systems suggests the Ras pathway might operate downstream of receptor tyrosine kinases. A dominant-negative *ras* gene, for example, was able to interfere with oncogenic transformation by several tyrosine kinase–encoding oncogenes (*21*). Furthermore, genetic analysis has demonstrated a function for Ras in *Caenorhabditis elegans* vulval development. A *ras* gene, *let-60*, has been shown to act downstream of a receptor tyrosine kinase encoded by the *let-23* gene (*22, 23*). Results presented here implicate Ras in *Drosophila* neuronal development as well. Thus the Ras pathway may be a general component of many developmental systems.

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Linguistic Experience Alters Phonetic Perception in Infants by 6 Months of Age

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Linguistic experience affects phonetic perception. However, the critical period during which experience affects perception and the mechanism responsible for these effects are unknown. This study of 6-month-old infants from two countries, the United States and Sweden, shows that exposure to a specific language in the first half year of life alters infants' phonetic perception.

AT THE BEGINNING OF LIFE, HUMAN infants exhibit a similar pattern of phonetic perception regardless of the language environment in which they are born (*1*). They discern differences between the phonetic units of many different languages, including languages they have never heard, indicating that the perception of human speech is strongly influenced by innate factors. However, by adulthood, linguistic experience has had a profound effect on speech perception. Exposure to a specific language results in a reduction in the ability to perceive differences between speech sounds that do not differentiate between words in one's native language (*2, 3*). Adult native speakers of Japanese, for example, have great difficulty in discriminating between words containing English /r/ and /l/, phonetic segments that belong to the same underlying category in Japanese (*2*). Adults thus exhibit a pattern of phonetic perception that is specific to their native language, whereas infants initially demonstrate a pattern of phonetic perception that is universal. At what point in development does linguistic experience alter phonetic perception, and what is the nature of the change brought about by experience with a particular language?

Previous studies suggested that the effects of linguistic experience on phonetic perception occur at about 1 year of age (*3*), coinciding with the age at which children begin to acquire word meanings (*4*). It was thus proposed that the change from a language-universal pattern of phonetic perception to one that is language-specific was brought about by the emergence of a milestone in the child's linguistic development, namely, the child's understanding that phonetic units are used contrastively to specify different word meanings (*3*).

We show here that by 6 months of age, well before the acquisition of language (*4*), infants' phonetic perception has been altered by exposure to a specific language. Infants in America and Sweden were tested with both native- and foreign-language vowel sounds. Infants from both countries exhibited a language-specific pattern of phonetic perception. Thus, linguistic experience alters phonetic perception at an unexpectedly early age, and this has implications for theories of speech perception and the development of language.

The present test focused on phonetic "prototypes," speech sounds that are identified by adult speakers of a given language as ideal representatives of a given phonetic category. Experiments with adults have shown that phonetic prototypes function like "perceptual magnets" in speech perception (*5*). The magnet effect causes other

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