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The role of the *Enhancer of split* complex during cell fate determination in *Drosophila*

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SUMMARY

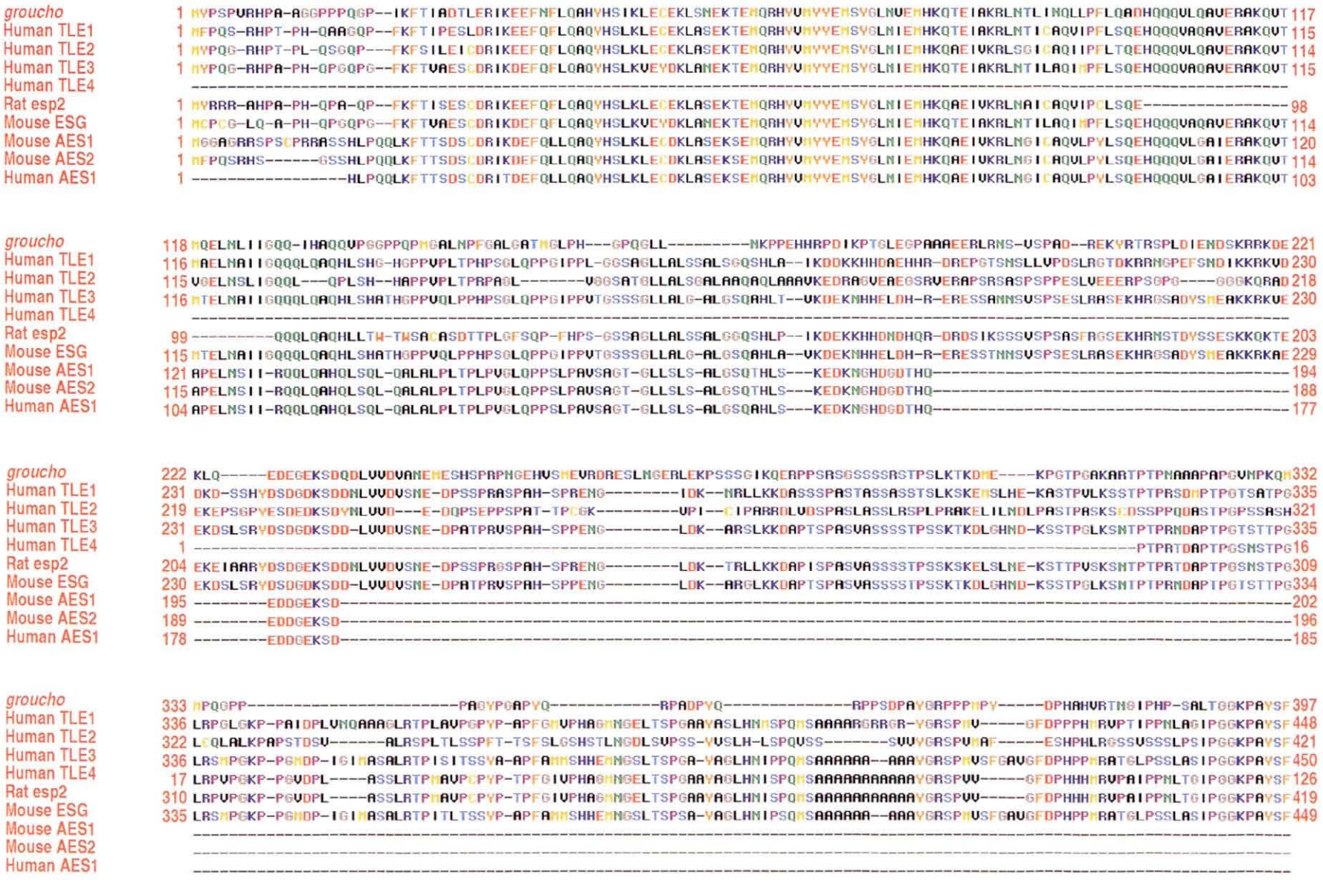
Molecular and genetic data predict that the *Enhancer of split* locus functions at the end of a pathway dictating appropriate cell fate determination in a number of developmental contexts. We have sought to dissect the role individual member genes of the complex play through a molecular analysis. Of the two principle class of genes, the first, members of the basic helix-loop-helix (bHLH) class of proteins are expressed in specific regions of the embryo in subtle, overlapping patterns in cells that will differentiate as epidermis. The second, *groucho*, a member of the WD40 class of proteins, is expressed more generally. Immunoprecipitation experiments do not implicate *groucho* in G protein mediated signal transduction, a known function of many WD40 type proteins. Instead, the nuclear localisation of the protein suggests a relationship to the bHLH members of the complex. Differences in expression of the bHLH genes between neurogenic mutants implies two pathways to their activation during epidermal determination.

Key words: *Drosophila*, cell fate determination,

INTRODUCTION

During development, cells rely upon extrinsic cues to adopt fates suitable to their surroundings. A group of genes in *Drosophila* appear to be called upon at many times and in many places to facilitate such decisions (Artavanis-Tsakonas and Simpson, 1991; Campos-Ortega and Knust, 1991; Hartley, 1990). They are called the neurogenic genes, since the primary defect in embryos lacking any of them is an inability to distinguish epidermal from neural precursors during neurogenesis (Lehmann et al., 1983; Poulson, 1937). Mutants of any of the neurogenic genes die as embryos, lack large regions of the epidermis, and have a concomitant enlarged nervous system. In addition to their role in neurogenesis, these genes appear to function in an analogous fashion in many other developing organs (Cagan and Ready, 1989; Hartenstein and Posakony, 1990; Ruohola et al., 1991; Corbin et al., 1991; Xu et al., 1992; Hartenstein et al., 1992). They are not a curiosity to *Drosophila* - for example, at least some of the genes appear to have been conserved between vertebrates and invertebrates (Coffman et al., 1990; Ellisen et al., 1991; Weinmaster et al., 1991; Del Amo et al., 1992; Stifani et al., 1992). However, it is in *Drosophila* that their function is best understood. From the ongoing molecular and genetic analysis of their function, it seems that two of the neurogenic genes, *Notch* and *Delta*, interact at the cell surface to provide necessary cues for cell fate determination, probably in the form of "lateral inhibition" of one cell type by another (Artavanis-Tsakonas and Simpson, 1991). Although it remains unproved, the data are consistent with a model whereby the *Delta* gene product acts as a ligand for a *Notch* gene product receptor (Heitzler and Simpson, 1991; Fehon et al., 1990). From standard epistasis tests using duplications and deficiencies, the *Enhancer of split* complex behaves as if it were downstream of *Notch* and *Delta* (de la Concha et al., 1988). The role of the remaining neurogenic genes, *mastermind*, *big brain* and *neuralized* remains relatively obscure when compared to the others. However, it has been proposed, at least in the case of determination of the adult sensilla, that the pathway mediated by the neurogenic genes serves to repress the expression of neural determinants (provided by the *Achaete-scute* complex of genes) in all cells of a "proneural cluster" with the exception of the single sensory mother cell (Artavanis-Tsakonas and Simpson, 1991). This acts to restrict fate to a single cell from within a field of equivalent pluripotential cells, thus allowing the remaining cells to adopt a different fate (Simpson, 1990). The molecular analysis of members of this pathway should test the merit of this model.

The *Enhancer of split* (*E(spl)*) locus consists of a number of genes some or all of which may have a function in cell fate determination. On the basis of mutant phenotype, two classes of genes are definitely implicated (Delidakis et al., 1991; Schrons et al., 1992). The first, and only gene that mutates to give a neurogenic phenotype, is allelic to a previously described gene *groucho* (Preiss et al., 1988). The *groucho* gene has been cloned and sequenced and the gene product has homology to a class of proteins defined by the presence of an approximately 40 amino acid repeat with characteristically located tryptophan and aspartate residues - the so called WD40 repeat (Hartley et al., 1988). Closely related genes in humans and mice have been identified


groucho
Human TLE1
Human TLE2
Human TLE3
Human TLE4
Rat esp2
Mouse ESG
Mouse AES1
Mouse AES2
Human AES1

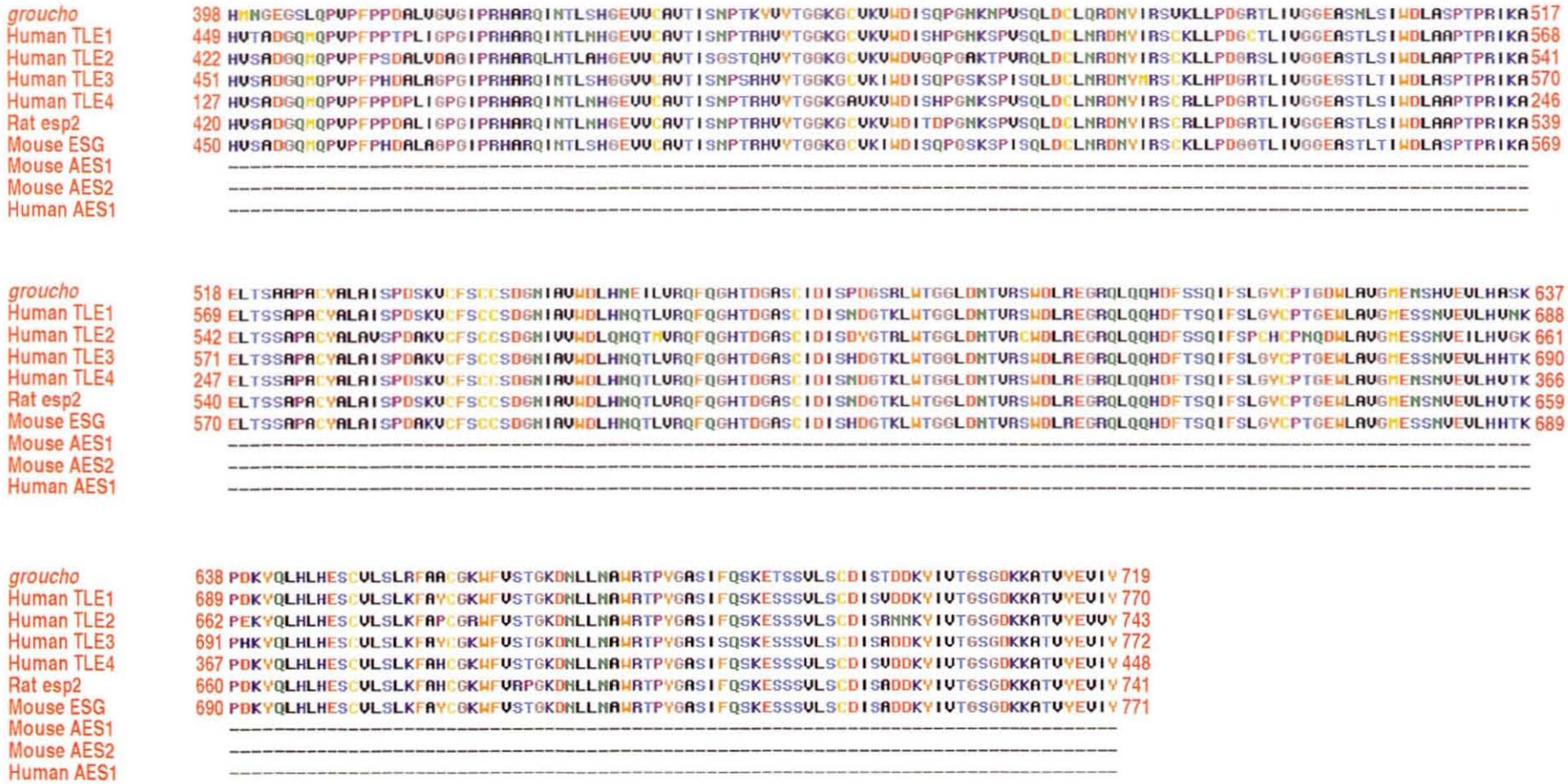


Fig. 1. The related amino acid sequences of *groucho* in flies, humans, rats and mice. A multiple alignment using ClustalV was made of the ten related sequences found in the GenBank non-redundant database. Amino acids are coloured using the SeqApp program where blue codes for basic residues, red for acidic, cyan for hydroxyl, green for amide, yellow for sulphur containing, black for hydrophobic residues, orange for aromatic, purple for proline and grey for glycine. Note as well as the full-length orthologues in humans (TLE 1,2 and 3) mice (Mouse ESG) and rats (Rat esp2), there are proteins highly related to just the N-terminal domain of groucho (Mouse AES 1 and 2 ; Human AES1) whilst the other sequences are homologous over the entire length of groucho. The source references are for Human TLEs (Stifani et al., 1992), Rat esp2. (Schmidt, C.J. and Sladek, T.E. (1993), unpublished), Mouse grg (Mallo, M., Franco del Amo, F. and Gridley, T. (1993), unpublished), Human and mouse AES sequences (Miyasaka, H., Choudhury, B.K., Hou, E.W. and Li, S.S.L (1993), unpublished).

(Stifani et al., 1992). Amongst the members of this class of proteins are the β subunits of guanine nucleotide regulatory proteins (G proteins). A prominent function for G proteins is in the transduction of cell surface receptor mediated signals (Bourne et al., 1991). It was therefore an attractive possibility to postulate the role of the *groucho* gene in signal transduction, particularly since point mutations of *groucho* interact genetically with specific point mutations of *Notch*, potentially the receptor in a lateral inhibition pathway (Xu et al., 1990). The second class of *Enhancer of split* genes number seven homologous members (Knust et al., 1992; Delidakis et al, 1992). This family is related to basic helix-loop-helix proteins which are known in many instances to be DNA sequence specific transcription factors (e.g. Pabo and Sauer, 1992). The relationship between *groucho* and the bHLH members of the *E(spl)* complex is implied from genetic interaction. Transformed *groucho* genes are able to improve the phenotype of deficiencies of the complex as long as at least one bHLH gene is present in the genome (Delidakis et al., 1991, Schrons et al., 1992). Thus, genetically, *groucho* acts as an activator of *E(spl)* bHLH function. We have been investigating the function of the genes of *E(spl)* in *Drosophila* development with a view to revealing the molecular mechanism of their action. Our data do not conform to a role for *groucho* in G protein mediated signal transduction. Instead, the localisation of the gene product points to a requirement in the nucleus. Thus, the interaction between *groucho* and *E(spl)* bHLH members suggested genetically, may be direct.

The expression of *Enhancer of split* bHLH genes is temporally and spatially complex (Godt et al., 1991; Knust et al., 1992). From various work, a part of this expression appears to be immediately succeeding or coincident with the separation of neural from epidermal precursor cells, specifically in the epidermal precursors. This expression is superficially normal in *Notch* mutants, but abnormal in *neuralized* mutants. This hints at two requirements for normal *Enhancer of split* function in dictating cell fates during embryogenesis.

MATERIALS AND METHODS

Antibody staining

Affinity purified-antibodies raised to pATH or pGEX fusions of *groucho* or *Notch* cDNA have been previously described (Fehon et al., 1990; Delidakis et al., 1991). Third instar larval discs and brains were fixed in 4% formaldehyde in PBS and stained with antibody overnight at 4°C in PBS/0.2% saponin/3% normal goat serum. Anti-IgG secondaries labelled with fluorescein were used to visualise staining. Preparations were imaged using the MRC 500 laser scanning confocal microscope.

Immunoprecipitation

Embryonic protein extracts were prepared from dechorionated embryos by homogenising in 300 mM NaCl, 50 mM Tris-HCl, 0.5% NP-40, 0.5% deoxycholate in the presence of proteinase inhibitors and immunopreciptiations performed as described in Fehon et al. (1990).

G protein experiments

Embryonic or *Drosophila* Kc cell protein extracts were prepared in 20 mM Tris-HCl, pH 8.0, 100 mM NaCl, and either 1 mM EDTA, 0.1 mM GDP or 10 mM $MgCl_2$, 0.3 mM GTP or 10 mM $MgCl_2$, 0.3 mM GTPγS. Extracts were incubated for 40 minutes at 30°C with 2 μg activated pertussis toxin in the presence of phosphatidyl choline and [^{32}P]NAD. The reactions were chased for 10 minutes with 14 mM NAD and immunoprecipitated as above with anti-groucho antibodies. In a parallel set of experiments, the same types of protein extract were incubated with ^{32}P-labelled 8-azido-GTP and irradiated with UV for 30 seconds. Then the different extracts were immunoprecipitated with anti-groucho antibodies.

In situ hybridization

In situ hybridization to whole embryos was performed essentially as described in Tautz and Pfeifle (1989). Anti-sense RNA probes were labelled with digoxigenin as described by the manufacturer (Boehringer Mannheim). After staining, embryos were dehydrated through an ethanol series and mounted in methacrylate.

RESULTS

The members of the *Enhancer of split* complex

Genetic and molecular genetic criteria have been used to define members of the *Enhancer of split* complex (Delidakis et al., 1991; Schrons et al., 1992; Knust et al., 1992). In terms of sequence homology, two types of gene product have been implicated with respect to cell fate determination. Seven members of the complex (called m3, m5, m7, m8, mα, mβ and mγ) are related to the basic helix loop helix class of trans regulators (Delidakis and Artavanis-Tsakonas, 1992; Knust et al., 1992). Another member, variously called m9/10 or *groucho*, is a member of the WD40 class of proteins, whose largest individual members are the β subunits of signal transducing guanine nucleotide binding proteins. However, since the sequence of groucho was determined, a number of extremely homologous vertebrate sequences have been identified (Stifani et al., 1992; Schmidt and Sladek, unpublished; Mallo et al., unpublished; Miyasaka et al., unpublished). Fig. 1 shows the current members of this orthologous gene family, a subset of the WD40 domain proteins, found in flies, mice, rats and humans. The sequences are conserved essentially throughout the length of the proteins, but virtually identical in two areas, firstly within the N-terminal region and secondly the C-terminal halves of the proteins, which consist of the WD40 domain. The striking degree of homology within this family of related sequences implies functional conservation, although of these members, only groucho has a known function. The N-terminal sequence domain has no significant homology to any other protein sequences in the databases but the WD40 domain has a number of relatives. Fig. 2 shows some of these relatives with the salient residues of the primary sequence based on the BLOCKS v6.0/Prosite v10.0 database definition of consensus sequence for the WD40 repeat. These classes of proteins exhibit diverse functions and intracellular locations (see van der Voorn and Ploegh, 1992; Duronio et al., 1992 for reviews) ranging from the membrane related signal transduction of G proteins to the nuclear transcriptional apparatus of TUP1 (Keleher et al., 1992) and the *Drosophila* TFIID subunit dTAF$_{II}$80 (Dynlacht et al., 1993). In addition, since groucho has obvious vertebrate orthologues (see Fig. 1) the functional significance of the relatedness of WD40 domain proteins is not clear. It is supposed that this motif is involved in protein-

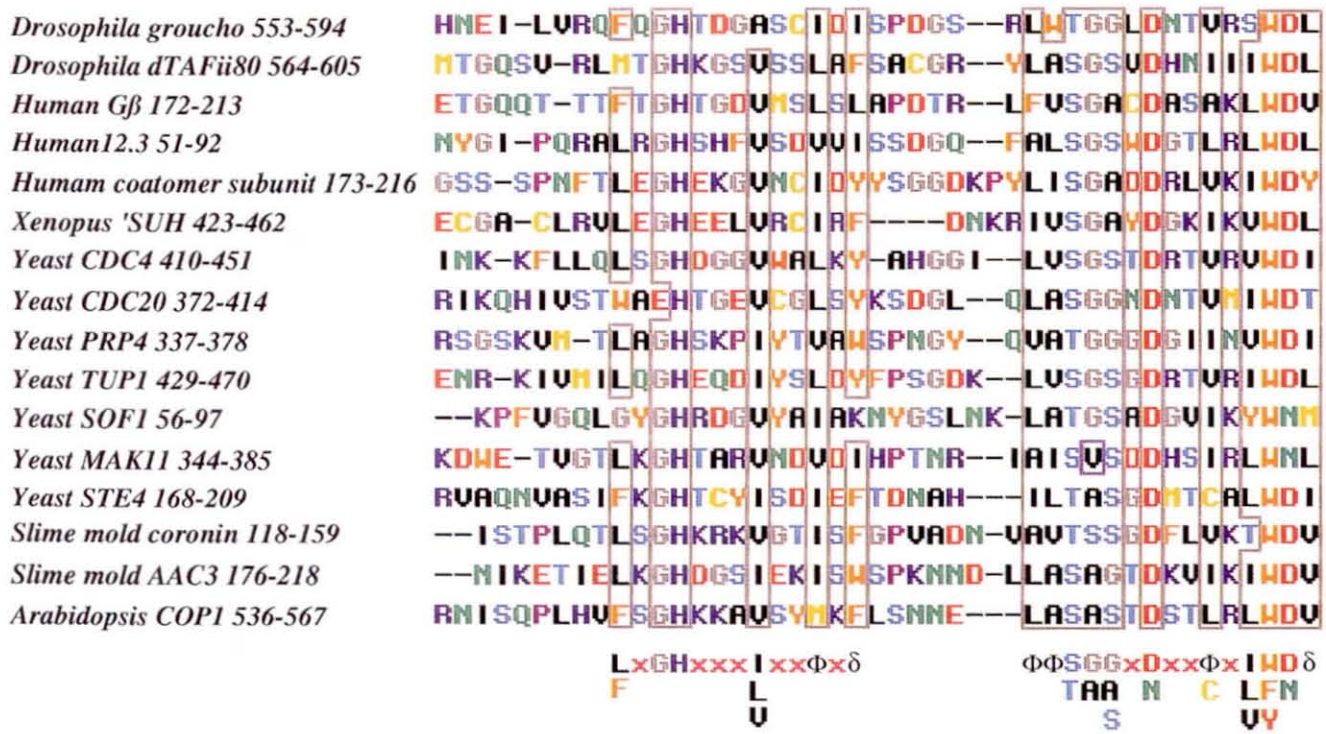


Fig. 2. The WD40 amino acid sequence domain. The single best fit to the consensus sequence (shown at the bottom) was found using the BLOCKS program of sixteen sequences exhibiting homology to this motif. Note that in each of these cases there are more than one of these repeats found in each of the sequences, varying from three to eight, and only the best is shown. Grey boxes indicate a fit to the consensus sequence. In addition to those proteins shown, there are a number of others not shown, either because they are identical (in the case of the large number of G protein β subunit sequences in the database), or they contain only a single repeat. Also a number of anonymous yeast ORFs are not shown. References: groucho (Hartley et al., 1988), *Drosophila* TFIID subunit (Dynlacht et al., 1993), Human G protein β subunit (Fong et al., 1986), Human/chicken 12.3 G protein related sequence (Guillemot et al., 1989), Human coatomer subunit (Harrison-Lavoie et al., 1993), *Xenopus* 'SUH, a suppressor of yeast CDC15 mutations (Spevak et al., 1993), Yeast CDC4, required early in the cell cycle and a component of the nucleoskeleton (Yochem and Byers, 1987), Yeast CDC20 a protein required for microtubule dependent processes (Sethi et al., 1991), Yeast PRP4 a component of the U4/U6 snRNP (Dalrymple et al., 1989), Yeast TUP1 part of a general repression mechanism (Zhang et al., 1991), Yeast SOF1 a component of the U3 snRNP (Jansen et al., 1993), Yeast MAK11 a membrane associated member of gene products required for M1 dsRNA replication (Icho and Wickner, 1988), Yeast STE4, required for response to mating pheromone and presumed to be a Gß subunit (Whiteway et al., 1989), Slime mold coronin a component of the actin/myosin complex (de Hostos et al., 1991), Slime mold AAC3 one of several developmentally regulated genes in *Dictyostelium* containing AAC nucleotide or *opa* repeats (Shaw et al., 1989), *Arabidopsis* COP1, a putative negative regulator of photomorphogenesis (Deng et al., 1992).

protein or protein-nucleic acid interactions and secondary structure predictions imply a series of β strands separated by turns (Duronio et al., 1992).

A role for *groucho* in signal transduction

Two lines of data suggested a role for groucho in signal transduction of cell fate determination. First, the sequence homology of the gene product to the β subunit of G proteins (Hartley et al., 1988) and second, the specific interaction between groucho and Notch point mutations (Xu et al., 1990). Notch is known to act at the cell surface (Fehon et al., 1991) and in an autonomous fashion (Hoppe and Greenspan, 1990; Heitzler and Simpson, 1991), as would be expected for a receptor. This specific interaction between point mutations of the *groucho* gene and *Notch* point mutations insinuated that the two gene products might physically combine. Previous immunochemical approaches had shown this to be the case for *Notch* and *Delta*, since antibodies specific to one can co-precipitate the other, and specific cellular aggregates form when the two are over-expressed in mixed cell cultures (Fehon et al., 1990). We have attempted to co-precipitate *Notch* and *groucho* protein from *Drosophila* embryos or Kc tissue culture cells using antibodies to Notch or to groucho. Under the conditions used, we were unable to detect each protein in its complementary immunoprecipitate (Fig. 3). This result does not exclude the potential interaction of Notch and groucho, but suggests that if such interaction exists, it is non-stoichiometric.

Mammalian G proteins have been previously assayed by their ability to bind guanosine nucleotide phosphate (Offermans et al., 1991), and by their sensitivity to ADP-ribosylation by cholera toxin and pertussis toxin (Gierschik, 1992). Both of these are features of the α subunit of G proteins, but using conditions which favour the heterotrimeric α/β/γ form (low Mg^{2+} and GDP), it is possible to immunoprecipitate all three subunits with an antibody to one. We have reacted total *Drosophila* cell extracts with pertussis toxin and [^{32}P]NAD and tested the ability of mon-

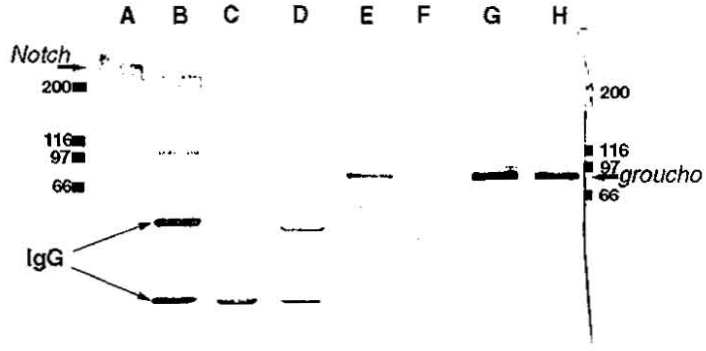


Fig. 3. Immunoprecipitations of *Notch* and *groucho* protein from embryonic extracts. Immunoprecipitates or complete protein extracts were subjected to SDS-PAGE and transferred to nitrocellulose. Blots were stained with monoclonal antibodies against Notch (lanes A-D) or rabbit polyclonal anti-groucho (lanes E-H). Lanes A,E: total protein extract from 0-14 hour embryos. Lanes B,F: immunoprecipitate using monoclonal anti-Notch. Lanes C,G: immunoprecipitate using monoclonal anti-groucho. Lanes D,H: immunoprecipitate using a second independently isolated monoclonal anti-groucho. These data demonstrate that *Notch* protein cannot be detected in anti-groucho immunoprecipitates (lane C,D) and similarly that *groucho* protein cannot be detected in anti-Notch immunoprecipitates.

oclonal anti-groucho antibodies to precipitate [^{32}P]ADP-protein under conditions which should favour the association (low Mg^{2+}, GDP) or dissociation (high Mg^{2+}, GTP or GTPγS) of G proteins. We found no specific labelling of proteins in the immunoprecipitate above background (data not shown), suggesting that the anti-*groucho* antibodies fail to co-immunopreciptiate pertussis toxin-sensitive α subunits. In a similar assay, we reacted total protein extracts with 8-azido-[^{32}P]GTP which binds covalently to protein on photoactivation, and immunoprecipitated the labelled extract. Once again, no specifically labelled protein was co-precipitated by anti-groucho antibodies (data not shown).

groucho expression in the nucleus

The immunoprecipitation experiments designed to investigate a relationship between groucho and G proteins did not provide any evidence for such a link. In addition, previous immunohistochemical work argued that *groucho* protein was almost exclusively in the nucleus, counter to what would be expected for G protein mediated signal transduction (Delidakis et al., 1991). Given the ubiquitous staining patterns within the embryo, we have looked at other developmental stages of the fly to ascertain whether it plays a purely embryonic role. In the imaginal discs of the third instar larvae (eye/antenna, wing, leg, haltere), all the nuclei appear to stain (for example, see Fig. 4). In addition, most, if not all, cells of the larval CNS express this antigen. We have also stained ovaries and the protein is found in the somatic and germ cell components - in the follicle cell nucleii and the polyploid giant nurse cell nucleii (Fig. 4). The nurse cell nuclear staining reveals a curious subnuclear organisation which does not seem to correspond to chromatin (as adjudged by counter-staining with DAPI). In addition, the oocyte nucleus stains quite strongly (Fig. 4).

One possibility remained that the nuclear localisation of groucho might represent an active uptake in response to Notch mediated cell surface signals. A corollary of this hypothesis might be that in neurogenic mutants where the cellular interaction mechanism is presumably blocked, the protein would no longer be sent to the nucleus. We have used our antibodies to stain mutants for the zygotic neurogenic genes Notch, big brain, mastermind, neuralized, and Delta. In none of these cases do we see absence of groucho protein, or abnormal distribution within the embryo, or within the cell (assayed by indirect immunofluorescence, see

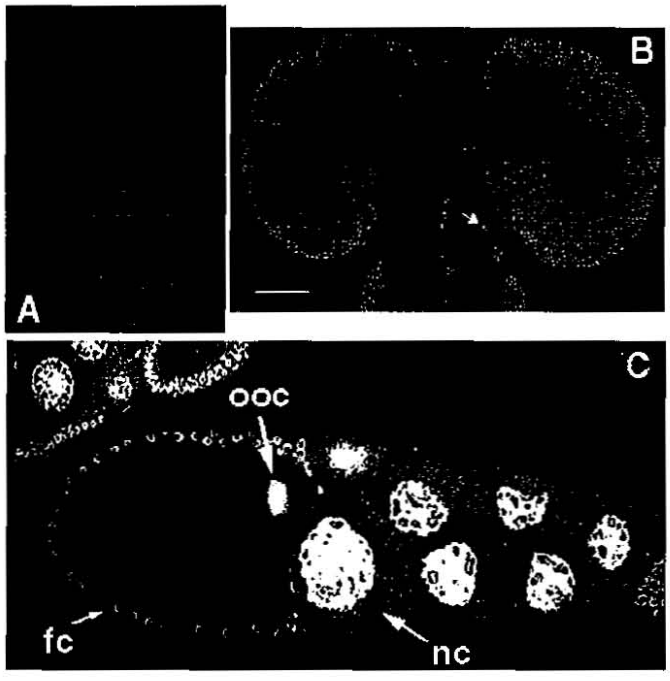
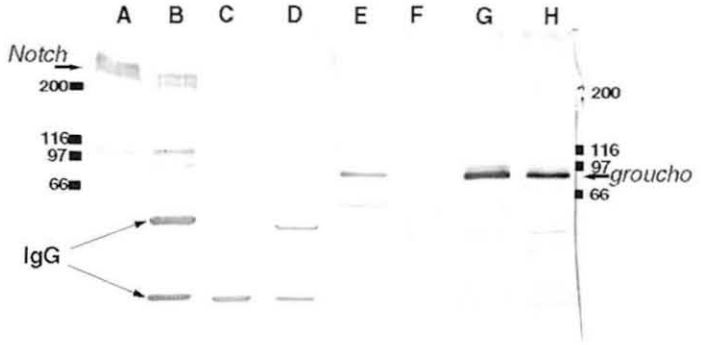


Fig. 4. *groucho* expression in post-embryonic tissues. Monoclonal anti-groucho was used to stain wing imaginal discs (A), the third instar larval CNS (B) and ovaries (C) and detected by laser scanning confocal microscopy. (A) Ubiquitous nuclear staining of the wing imaginal disc - absence of staining in the folds of the discs is due to the fact that some nucleii are out of the plane of optical section. (B) Staining of the larval CNS is also widespread. A larger staining cell, presumably a neuroblast, is shown by an arrowhead. (C) *groucho* expression in the ovary. The antibody stains both the follicle cells (fc) and the nurse cells (nc). The nuclear sub-cellular localisation is most apparent in the large, multi-nucleolate nurse cells and even in the smaller follicle cells an absence of staining in the nucleolus is evident. The oocyte nucleus (ooc) is also strongly stained in this stage 10 oocyte.

Fig. 5). We have not, however, performed the control of eliminating the maternal contribution provided by many of these genes. Since zygotic mutants result in abnormal cell fate determination, we expected the zygotic condition to be sufficient to see abnormal groucho localisation if this were relevant. Thus, nuclear localisation of groucho does not appear to require neurogenic function. An alternative



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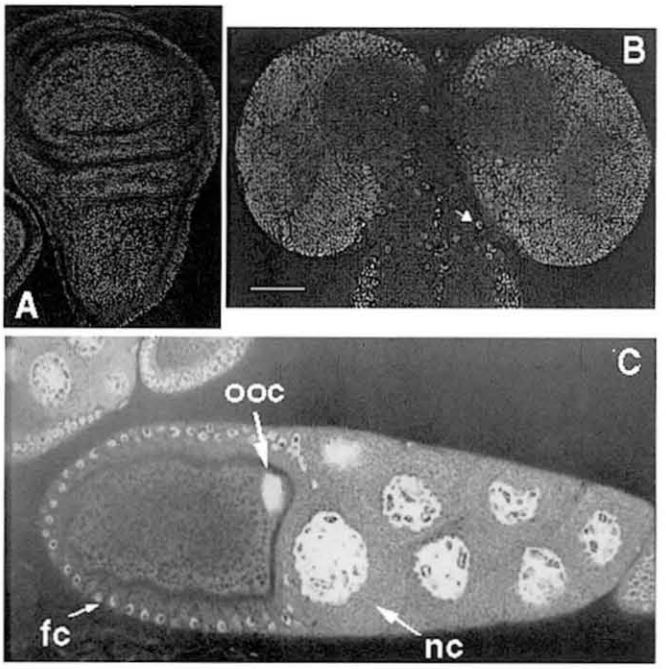

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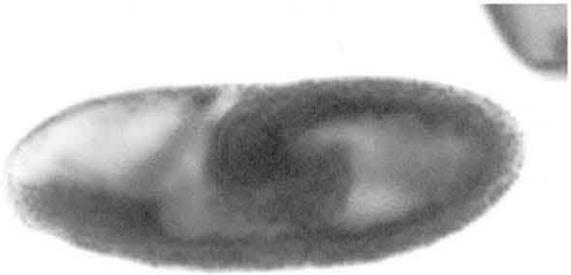

Fig. 5. *groucho* still localises to the nucleus in neurogenic mutants. A stage 8 (Campos-Ortega and Hartenstein, 1985) embryo homozygous for the *neuralized* mutation neu^{IF65}. Even with this preparation viewed with the light microscope and DIC optics, it is clear that *groucho* is still very much in the nucleii of all the cells of the embryo. Mutants were identified by the absence of expression of a β-galactosidase gene from the balancer chromosome.

hypothesis suggests that removal of the nuclear targets for the cell surface machinery would be necessary to see abnormal localisation of groucho protein. We and others have suggested that such targets could be represented by two sets of helix-loop-helix (bHLH) proteins; one set encoded by the Achaete-scute complex (AS-C) representing neural determinators and the other, the *E(spl)* bHLH genes, representing epidermal determinators (Hartley, 1990, Campos-Ortega and Knust, 1991). However, staining embryos deficient for either cluster of bHLH genes shows an apparently normal intracellular localisation of groucho protein (not shown). Thus, groucho nuclear localisation does not require AS-C gene function.

Enhancer of split bHLH gene expression

The examination of *Enhancer of split* bHLH gene expression has been illuminating as regards its function in cell fate determination at neurogenesis (Godt et al, 1991, Knust et al., 1992). Each of the seven genes has a similar, but not identical pattern of expression. We have examined *Enhancer of split* m5, m7 and m8 expression by whole mount in situ hybridisation (see for example, Fig. 6). In agreement with previous studies, we find an initial row of expression in cells of the cellular blastoderm that have been called mesectoderm since they abut both mesoderm and ectoderm. These cells also seem to express another bHLH gene product from the single minded gene, which appears to regulate cell fates of mesectodermal cells (Nambu et al., 1991). Since bHLH proteins can form heterodimers, it is possible that this coincidence is informative. In addition to mesectodermal expression, there is a dorsal wedge of expression in cells that may be the precursors of the amnioserosa. After gastrulation, the three genes are expressed in clusters of cells in the neurogenic ectoderm initially in medial and lateral rows and later in more intermediate cells. This expression pattern is extremely dynamic and superficially resembles topographical maps corresponding to neuroblast segregation from this region (Hartenstein and Campos-Ortega, 1984; Doe, 1992). Of the three genes, m5 is expressed in the largest number of cells and m8 in the smallest number. The m8 expression pattern appears not to be a subset of the m5 pattern however, since hybridising with probes to both genes labels more cells than one sees with a probe to m5 alone. Since the expression pattern is very dynamic, we cannot be sure whether the genes are expressed in ectoderm immediately before, during or immediately after neuroblast segregation. However, we have never seen labelling in the neuroblast cell layer, implying that the expression is specific to the epidermal precursors, which make up the bulk of the neurogenic ectoderm (Hartenstein and Campos-Ortega, 1984). In addition to expression in the ectoderm, we also see expression in the mesoderm cell layer, in a discrete number or in single cells analogous to that reported for other *Drosophila* bHLH genes, like the myoD homologue, *nautilus* (Michelson et al., 1990). This expression pattern is consistent with the requirement for neurogenic gene function during myogenesis (Corbin et al., 1991).

Enhancer of split bHLH expression in neurogenic mutants

Since the expression of *Enhancer of split* bHLH genes in the neurogenic ectoderm appears restricted to the epidermal precursors, we expected abnormal expression in neurogenic mutants which do not differentiate epidermis from this region. We examined m5 expression in embryos laid by *Notch* heterozygous mothers. Since we were unable to unambiguously identify homo- or hemizygous mutant embryos in our stainings, we looked over a number of embryos for obvious defects but found none. We therefore conclude that m5 expression is superficially normal in *Notch* mutants. In contrast, some embryos (approximately 1/4 as would be expected if they were the homozygous mutants) derived from heterozygous *neuralized* parents did show defects in m5 expression. In these embryos, whilst the early mesectodermal expression appeared superficially normal, the neurogenic ectoderm and later mesoderm expression was undetectable (see Fig. 7). From this, we deduce that *neuralized* is required for normal *Enhancer of split* m5 expression. In a more detailed analysis, Godt et al. (1991) have shown that *Enhancer of split* m4, m5, m7 and m8 expression starts off normal in *Notch* mutant embryos, but is less normal in *neuralized* mutants.

DISCUSSION

The *Enhancer of split* complex is pivotal in directing cells to adopt a fate suitable to their environment. From DNA sequence homology to the β subunits of G proteins, it seemed attractive to speculate that one member of the gene complex, *groucho*, might play a role in signal transduction of signals mediated by the transmembrane *Notch* protein (Hartley et al., 1988). We have been unable to demonstrate such a role using immunoprecipitation to look for G protein activity either as a substrate for pertussis toxin catalysed ADP ribosylation or in GTP binding using a photoactivatable analogue. Furthermore, we were unable to co-immunoprecipitate groucho and Notch, indicating that these proteins are not stably associated with each other, as might be expected if groucho were involved in directly mediating Notch action in the cytoplasm. It is important to note that these data do not eliminate the possibility of groucho

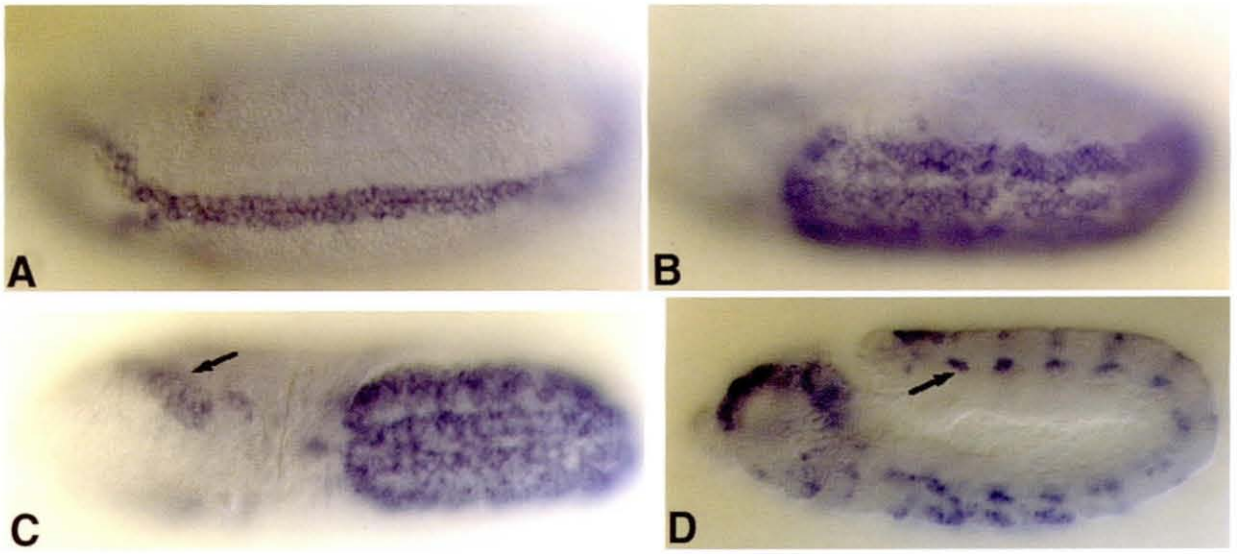

Fig. 6. Expression of *Enhancer of split* m5 in the embryo. (A) An embryo just after gastrulation at stage 6-7 viewed from below. This whole-mount in situ hybridization shows *E(spl)*m5 expression in two rows of cells abutting the ventral midline, the so-called mesectoderm, which gives rise to midline structures in both the CNS and the epidermis. In addition, out of the plane of focus, there is a wedge of expression on the dorsal surface of the embryo. (B) A later, stage 9 embryo where neurogenesis is commencing, viewed from the ventral surface. *E(spl)*m5 expression is throughout the germ band, predominantly in three rows. This pattern corresponds to the sites from which neuroblasts are segregating into the embryonic interior. As such it is very dynamic and later, interstitial cells start to hybridize, at the time when neuroblasts are born from this different part of the germ band ectoderm. (C) A similarly staged embryo viewed from the dorsal surface showing the three rowed pattern in the abdominal segments of the germ band and, in addition, expression in the procephalic neurogenic region (arrowed) which gives rise to the brain. (D) An older, stage 11 embryo still showing residual m5 expression in the epidermal precursors on the ventral side, and much expression in the procephalic region. Note the complete absence of labelling of the neuroepithelium in the germ band which is organised immediately below the outer cell layer. In addition, conspicuous staining is seen in the mesoderm (arrowed) in discrete cells repeated in each segment.

directly transducing information from the cell surface. Nevertheless, taken with the observation that the protein is almost exclusively nuclear when judged by immunohistochemistry and laser scanning confocal microscopy, such a model seems unlikely. Similarly, selective nuclear import (such as the dorsal nuclear uptake gradient which determines dorsal/ventral polarity; St. Johnston and Nüsslein-Volhard, 1992) does not seem to be a likely possibility for *groucho* mediated signal transduction, since none of the neurogenic mutants affect its nuclear localisation. On the other hand, some sort of intimacy between *Notch* and *groucho* seems likely, given the nature of genetic interactions between them (Xu et al., 1990). Nuclear function is not a precedent for proteins that share the WD40 motif. In yeast, a number of WD40 proteins have been described whose function is in the nucleus - CDC4, PRP4 and TUP1 which appear to be involved in the nucleoskeleton (Choi et al., 1990), pre-mRNA splicing (Rosenberg et al., 1991) and transcriptional repression (Keleher et al., 1992), respectively. TUP1 is particularly interesting as a model for *groucho* function. It appears to act as a general transcriptional repressor, required by a variety of DNA sequence specific *trans*-regulators, to repress expression from promoters adjacent to their binding site (Keleher et al., 1992). One can imagine that *groucho* might similarly co-ordinate the transregulatory functions of *Enhancer of split* bHLH proteins, given the nature of genetic interaction between them, perhaps to inhibit neural potential indirectly, or *Achaete-scute* directly. Consistent with this

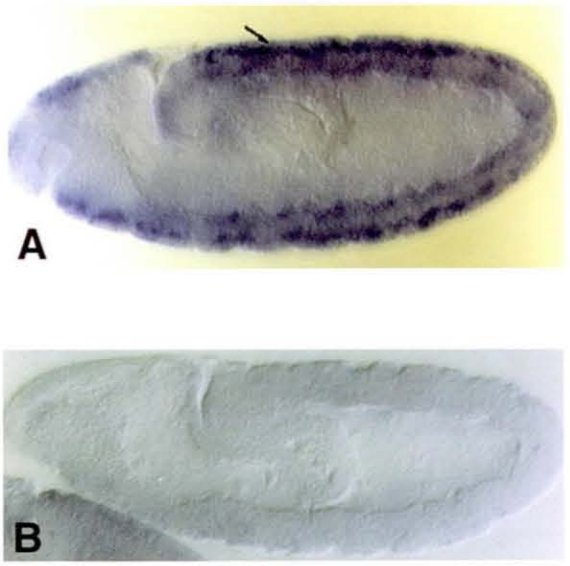

Fig. 7. *Enhancer of split* m5 expression is absent in the germ band of *neuralized* embryos. (A) A wild-type embryo at stage 9 hybridised to *E(spl)*m5. The arrow indicates the expression throughout the germ band, specifically in the outer cell layer which gives rise to the epdermis. (B) An embryo derived from a cross between *neuralized*- heterozygotes at a slightly earlier part of stage 9. No staining is observed, suggesting that *neuralized* is required for *E(spl)*m5 expression.

model is the observed derepression of *Achaete-scute* expression in *Enhancer of split* deficient embryos (Cabrera, 1990, Skeath and Carroll, 1992) and the observation that *E(spl)*m8 is capable of binding oligonucleotides corresponding to the "E box" found upstream of *Achaete-scute* and other bHLH genes (cited in Tietze et al., 1992).

The function of the *Enhancer of split* bHLH genes in cell fate determination is strongly suggested by their complex expression patterns. Initially expressed in ventral domains and dorsal domains probably corresponding to mesectoderm and amnioserosa respectively, they are later activated in clusters of cells in the neurogenic ectoderm, apparently specifically in the epidermal precursors. This expression is virtually coincident with or immediately succeeds neuroblast segregation. Thus it is exactly what one would expect if these genes are expressed in response to induction by the nascent neuroblasts, as the means of inhibiting the neural potential of these cells (lateral inhibition) and/or promoting their epidermal potential. However, in some of the neurogenic mutants, this expression is at least initially normal, despite the fact that no epidermal precursors arise in such mutants. Thus, the initial presence of *E(spl)* bHLH transcript *per se* is insufficient to allow epidermal determination. Yet these genes appear to act at the end of a pathway leading to epidermal determination (de la Concha et al., 1988). Therefore, it seems likely that the function of *Notch*, *Delta* and *mastermind* is to post-transcriptionally activate these bHLH genes, either through translation, or post-translational modification. In contrast, *E(spl)*bHLH expression in the neurogenic ectoderm of *neuralized* mutants appears to be undetectable. Hence, the function of *neuralized* appears to be to activate directly the expression of these genes, and the neurogenic phenotype of *neuralized* mutants would result from an inability to activate them. This model is consistent with the observation that the *neuralized* gene product has homology to known transcription factors (Price et al., 1993), and the known genetic interactions between *neuralized* and $E(spl)^{D}$, and allele of the m8 bHLH gene (Knust et al., 1987). Since *neuralized* is expressed in a wider territory than any individual *E(spl)*bHLH gene (Boulianne et al., 1991; Price et al., 1993), it would have to activate transcription of these genes in co-operation with other, as yet unknown, factors. Given this scenario there are two levels to neurogenic gene function - firstly, activation of transcription of *Enhancer of split* bHLH genes in the cells that must respond to them (in this case the epidermal precursors) and a second post-transcriptional activation step necessary for their ability to function in dictating epidermal fate. This second step requires *Notch*, *Delta* and *mastermind* and presumably the ability of the cells to interact. One mystery following from this idea is why cells need to bother with cellular interactions mediated by *Notch* and *Delta* if they already express epidermal determinants (in the form of *E(spl)*bHLH proteins) without requiring either *Notch* or *Delta*. Presumably this reflects an error checking necessity of cells to test they are doing the right thing in determining their own and/or surrounding cells' fate by "reading their environment" prior to activating gene expression programs appropriate to their position.

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REFERENCES

Artavanis-Tsakonas, S. and Simpson, P. (1991). Choosing a cell fate: a view from the *Notch* locus. *Trends Genet.* **7**, 403-408.

Boulianne, G., de la Concha, A., Campos-Ortega, J.A., Jan, L.Y. and Jan, Y.N. (1991). The *Drosophila* neurogenic gene *neuralized* encodes a novel protein and is expressed in precursors of larval and adult neurons. *EMBO J.* **10**, 2975-2983.

Bourne, H., Sanders, D.A. and McCormick, F. (1991). The GTPase superfamily: conserved structure and molecular mechanism. *Nature* **349**, 117-27.

Cabrera, C.V. (1990). Lateral inhibition and cell fate during neurogenesis in *Drosophila*: the interactions between *scute*, *Notch*, *Delta*. *Development* **109**, 733-742.

Cagan, R.L. and Ready, D.F. (1989). *Notch* is required for successive cell decisions in the developing *Drosophila* retina. *Genes Dev.* **3**, 1099-1112.

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

Campos-Ortega, J.A. and Knust, E. (1991). Molecular analysis of a cellular decision during embryonic development of *Drosophila melanogaster*: epidermogenesis or neurogenesis. *Eur. J. Biochem.* **190**, 1-10.

Choi, W.J., Clark, M.W., Chen, J.X. and Jong, A.Y. (1990). The CDC gene product is associated with the yeast nuclear skeleton. *Biochem. Biophys. Res. Comm.* **172**, 1324-1330.

Coffman, C., Harris, W. and Kintner, C. (1990). *Xotch*, the *Xenopus* homolog of *Drosophila Notch*. *Science* **249**, 1438-1441.

Corbin, V., Michelson, A.M., Abmayr, S.M., Neel, B., Alcamo, E., Maniatis, T. and Young, M.W. (1991). A role for the *Drosophila* neurogenic genes in mesoderm differentiation. *Cell* **67**, 311-323.

Dalrymple, M.A., Peterson-Bjorn, S., Friesen, J.D. and Beggs, J.D. (1989). The product of the *PRP4* gene of *S. cerevisiae* shows homology to β subunits of G proteins. *Cell* **58**, 811-812.

de Hostos, E.L., Bradtke, B., Lottspeich, F., Guggenheim, R. and Gerisch, G. (1991) Coronin, an actin binding protein of *Dictyostelium discoideum* localised to cell surface projections, has sequence similarities to G protein beta subunits. *EMBO J.* **10**, 4097-4104

de la Concha, A., Dietrich, U., Weigel, D. and Campos-Ortega, J.A. (1988). Functional interactions of neurogenic genes of *Drosophila melanogaster*. *Genetics* **118**, 499-508.

Del Amo, F.F., Smith, D.E., Swiatek, P.J., Gendron-Maguire, M., Greenspan, R.J., McMahon, A.P. and Gridley, T. (1992). Expression pattern of *Motch*, a mouse homolog of *Drosophila Notch*, suggests an important role in early postimplantation mouse development. *Development* **115**, 737-744.

Delidakis, C., Preiss, A., Hartley, D.A. and Artavanis-Tsakonas, S. (1991). Two genetically and molecularly distinct functions involved in early neurogenesis reside within the Enhancer of split locus of Drosophila melanogaster. *Genetics* **129**, 803-823.

Delidakis, C. and Artavanis-Tsakonas, S. (1992). The *Enhancer of split* locus of *Drosophila* encodes 7 independent helix loop helix proteins. *Proc. Natl. Acad. Sci. USA* **89**, 8731-8735.

Deng, X.-W., Matsui, M., Wei, N., Wagner, D., Chu, A. M., Feldmann, K.A. and Quail, P.H. (1992). *COP1* an *Arabidopsis* regulatory gene, encodes a protein with both a zinc-binding motif and a Gβ homologous domain. *Cell* **71**, 791-801.

Doe, C.Q. (1992). Molecular markers for identified neuroblasts and ganglion mother cells in the *Drosophila* central nervous system. *Development* **116**, 855-863.

Duronio, R.J., Gordon, J.I. and Boguski, M.S. (1992). Comparative analysis of the β Transducin family with identification of several new members including *PWP1*, a nonessential gene of *Saccharomyces cerevisiae* that is divergently transcribed from *NMT1*. *Proteins* **13**, 41-56.

Dynlacht, B.D., Weinzierl, R.O.J., Admon, A. and Tijan, R. (1993). The dTAF$_{II}$80 subunit of *Drosophila* TFIID contains β-transducin repeats. *Nature* **363**, 176-179.

Ellisen, L.W., Bird, J., West, D.C., Soreng, A.L., Reynolds, T.C., Smith, S.D. and Sklar, J. (1991). *TAN-1*, the human homolog of the *Drosophila Notch* gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649-661.

Fehon, R.G., Kooh, P.J., Rebay, I., Regan, C.L., Xu, T., Muscavitch, M.A.T. and Artavanis-Tsakonas, S. (1990). Molecular interactions between the protein products of the neurogenic loci *Notch* and *Delta*, two EGF-homologous genes in *Drosophila*. *Cell* **61**, 523-534.

Fehon, R.G., Johansen, K., Rebay, I. and Artavanis-Tsakonas, S. (1991). Complex cellular and subcellular regulation of *Notch* expression during embryonic and imaginal development of *Drosophila*: implications for *Notch* function. *J. Cell Biol.* **113**, 657-669.

Fong, H.K.W., Hurley, J.B., Hopkins, R.S., Miake-Lye, R., Johnson, M.S., Doolittle, R.F. and Simon, M.I. (1986) Repetitive segmental structure and the transducin β subunit: Homology with the *CDC4* gene and identification of related mRNAs. *Proc. Natl. Acad. Sci. USA* **83**, 2162-2166.

Gierschik, P. (1992). ADP-ribosylation of signal transducing guanine nucleotide-binding proteins by pertussis toxin. *Curr. Topics Microbiol. Immunol.* **175**, 69-96.

Godt, D., Schrons, H., Guth, S. and Campos-Ortega, J.A. (1991). The distribution of transcripts of neurogenic genes in neurogenic mutants of *Drosophila melanogaster*. *J. Neurogenetics* **7**, 241-252.

Guillemot, F., Billault, A. and Auffray, C. (1989). Physical linkage of a guanine nucleotide-binding protein-related gene to the chicken major histocompatibility complex. *Proc. Natl. Acad. Sci. USA* **86**, 4594-4598.

Harrison-Lavoie, K.J., Lewis, V.A., Hynes, G.M., Collison, K.S., Nutland, E. and Willison, K.R. (1993). A 102 kd subunit of a Golgi-associated particle has homology to B subunits of trimeric G proteins. *EMBO J.* **12**, 2847-2853.

Hartenstein, V. and Campos-Ortega, J.A. (1984). Early neurogenesis in wild-type *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **193**, 308-325.

Hartenstein, V. and Posakony, J.W. (1990). A dual function of the *Notch* gene in *Drosophila* sensillum development. *Dev. Biol.* **142**, 13-30.

Hartenstein, A., Rugendoff, A., Tepass, U. and Hartenstein, V. (1992). The function of the neurogenic genes during epithelial development in the *Drosophila* embryo. *Development* **116**, 1203-1220.

Hartley, D.A., Preiss, A. and Artavanis-Tsakonas, S. (1988) A deduced gene product from the *Drosophila* neurogenic locus, *Enhancer of split*, shows homology to mammalian G protein β subunit. *Cell* **55**, 785-795.

Hartley, D.A. (1990). Early neurogenesis. *Sem. Cell Biol.* **1**, 185-196.

Heitzler, P. and Simpson, P. (1991). The choice of cell fate in the epidermis of *Drosophila*. *Cell* **64**, 1083-1092.

Hoppe, P.E. and Greenspan, R.J. (1990). The *Notch* locus of *Drosophila* is required in epidermal cells for epidermal development. *Development* **109**, 875-885.

Icho, T, and Wickner, R.B. (1988). The MAK11 protein is essential for cell growth and replication of M double-stranded RNA and is apparently a membrane-associated protein. *J. Biol. Chem.* **263**, 1467-1475.

Jansen, R., Tollervey, D., Hurt, E.C. (1993). A U3 snRNP protein with homology to splicing factor PRP4 and G beta domains is required for ribosomal RNA processing *EMBO J.* **12**, 2549-2558.

Keleher, C.A., Redd, M.J., Schultz, J., Carlson, M. and Johnson, A.D. (1992). *Ssn6-Tup1* is a general repressor of transcription in yeast. *Cell* **68**, 709-719.

Knust, E., Bremer. K.A., Vaessin, H., Ziemer, A., Tepass, U. and Campos-Ortega, J.A. (1987). The *Enhancer of split* locus and neurogenesis in *Drosophila melanogaster*. *Dev. Biol.* **122**, 262-273.

Knust, E., Schrons, H., Grawe, F. and Campos-Ortega, J.A. (1992). 7 genes of the *Enhancer of split* complex of *Drosophila melanogaster* encode helix-loop-helix proteins. *Genetics* **132**, 505-518.

Lehmann, R., Jiménez, F., Dietrich, U. and Campos-Ortega J.A. (1983). On the phenotype and development of mutants of early neurogenesis in *Drosophila melanogaster*. *Roux's Arch. Dev. Biol.* **192**, 62-74.

Michelson, A.M., Abmayr, S.M., Bate, M., Martinez-Arias, A. and Maniatis, T. (1990). Expression of a MyoD family member preFig.s muscle pattern in *Drosophila* embryos. *Genes Dev.* **4**, 2086-2097

Nambu, J.R., Lewis, J.O., Wharton, K.A. and Crews, S.T. (1991). The *Drosophila single-minded* gene encodes a helix-loop-helix protein that acts as a master regulator of CNS midline development. *Cell* **67**, 1157-1167.

Offermans, S., Schultz, G. and Rosenthal, W. (1991). Identification of receptor activated G proteins with photoreactive GTP analog (alpha-P^{32})GTP azidoanilide. *Meth. Enzymol.* **195**, 286-301.

Pabo, C.O. and Sauer, R.T. (1992). Transcription factors - structural families and principles of DNA recognition. *Ann. Rev. Biochem.* **61**, 1053-1095.

Poulson, D.F. (1937). Chromosomal deficiencies and embryonic development of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **23**, 133-137.

Preiss A., Hartley, D.A. and Artavanis-Tsakonas, S. (1988). The molecular genetics of *E(spl)*, a gene required for early embryonic neural development in *Drosophila melanogaster*. *EMBO J.* **7**, 3917-3927.

Price, B.D., Chang, Z., Smith, R., Bockheim, S and Laughon, A. (1993). The *Drosophila neuralized* gene encodes a C_3H_4 zinc finger. *EMBO J.* **12**, 2411-2418.

Rosenberg, G.H., Alahari, S.K. and Kaufer, N.F. (1991). PRP4 from *Schizosaccharomyces pombe*, a mutant deficient in pre-messenger RNA splicing isolated using genes containing artificial introns. *Mol. Gen. Genet.* **226**, 305-309.

Ruohola, H., Bremer K.A., Baker, D., Swedlow, J.R., Jan, L.Y. and Jan, Y.N. (1991). Role of neurogenic genes in establishment of follicle cell fate and oocyte polarity during oogenesis in *Drosophila*. *Cell* **66**, 433-439.

St. Johnston, D. and Nüsslein-Volhard, C. (1992). The origin of pattern in the *Drosophila* embryo. *Cell* **68**, 201-219.

Schrons, H., Knust, E. and Campos-Ortega, J.A. (1992). The *Enhancer of split* complex and adjacent genes in the 96F region of *Drosophila melanogaster* are required for segregation of neural and epidermal progenitor cells. *Genetics* **132**, 481-503.

Sethi, N., Monteagudo, C., Koshland, D. and Burke, D.J. (1991). The *CDC20* gene of *Saccharomyces cerevisiae*, a ß transducin homologue, is required for a subset of microtubule dependent cellular processes. *Mol. Cell Biol.* **11**, 5592-5602.

Shaw, D.R., Richter, H., Giorda, R., Ohmachi, T. and Ennis, H.L. (1989). Nucleotide sequences of *Dictyostelium discoideum* developmentally regulated cDNAs rich in (AAC) imply proteins that contain clusters of asparagine, glutamine, or threonine. *Mol. Gen. Genet.* **218**, 453-459.

Skeath, J.B. and Carroll, S.B. (1992). Regulation of proneural gene expression and cell fate during neuroblast segregation in the *Drosophila* embryo. *Development* **114**, 939-946.

Spevak, W., Keiper, B.D., Stratowa, C. and Castanon, M.J. (1993). *Saccharomyces cerevisiae* CDC15 mutants arrested at a late stage in anaphase are rescued by Xenopus cDNAs encoding N-ras or a protein with β-transducin repeats. *Mol. Cell Biol.* (In Press).

Stifani, S., Blaumueller, C.M., Redhead, N.J., Hill, R.E. and Artavanis-Tsakonas, S. (1992). Human homologs of a *Drosophila Enhancer of split* gene product define a novel family of nuclear proteins. *Nature Genetics* **2**, 119-127.

Simpson, P. (1990). Lateral inhibition and the development of the sensory bristles of the adult peripheral nervous system of *Drosophila*. *Development* **109**, 509-519.

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

Tietze, K., Oellers, N. and Knust, E. (1992). *Enhancer of split*^{D}, a dominant mutation of *Drosophila* and its use in the study of functional domains of a helix loop helix protein. *Proc. Natl. Acad. Sci. USA* **89**, 6152-6156.

Weinmaster, G., Roberts, V.J. and Lemke, G. (1991). A homolog of *Drosophila Notch* expressed during mammalian development. *Development* **113**, 199-205.

Whiteway, M., Hougan, L., Dignard, D., Thomas, D.Y., Bell, L., Saari, G.C., Grant, F.J., O'Hara, P. and Mackay, V.L. (1989). The *STE4* and *STE18* genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. *Cell* **56**, 467-477.

Yochem, J. and Byers, B. (1987). Structural comparison of the yeast cell division cycle gene *CDC4* and a related pseudogene. *J. Mol. Biol.* **195**, 233-245.

Xu, T., Rebay, I., Fleming, R.J., Scottgale, T.N. and Artavanis-Tsakonas, S. (1990). The *Notch* locus and the genetic circuitry involved in early *Drosophila* neurogenesis. *Genes Dev.* **4**, 464-475.

Xu, T., Caron, L.A., Fehon, R.G. and Artavanis-Tsakonas, S. (1992). The involvement of the *Notch* locus in *Drosophila* oogenesis. *Development* **115**, 913-922.

Zhang, M., Rosenblum-Vos, L.S., Lowry, C.V., Aboyake, K. and Zitomer, R.S. (1991). A yeast protein with homology to the β subunit of G proteins is involved in control of heme-regulated and catabolite-repressed genes. *Gene* **97**, 153-161.