INTRODUCTION

Asymmetric cell division in which one progenitor divides to produce two progeny that adopt distinct cellular identities is a process that is central to the generation of cellular diversity. It is a general process utilised in a variety of developmental contexts (reviewed by Horvitz and Herskowitz, 1992; Guo and Kemphues, 1996; Shapiro and Losick, 1997; Jan and Jan, 1998).

The Drosophila CNS provides an appealing model for understanding the mechanism of asymmetric cell divisions. The segmented Drosophila embryonic CNS comprises repeats of a basic building block, the hemi-neuromere, which make up a diverse population of approx. 300 neurons (Bossing et al., 1996) and approx. 30 glia (Ito et al., 1995) differing in gene expression, morphology and function. The majority of these cells develop from approx. 30 neural progenitor cells, called neuroblasts (NBs) and glioblasts. NBs are derived from neuroectodermal cells that delaminate from the ventral neurogenic region of the embryo in 5 temporal waves (Doe, 1992). Each NB possesses a unique identity, gives rise to a unique lineage of neurons and/or glia and can be identified by its time of delamination, position, pattern of gene expression and neuronal/glia progeny. NBs undergo repeated asymmetric divisions, ‘budding off’ a series of ganglion mother cells (GMCs) from their basal/lateral cortex. Each GMC divides once to give rise to neurons.

NBs divide to produce progeny NB and GMC that are not only different in morphology but also show different capacities for cell division and different patterns of gene expression. The homeobox protein Prospero (Pros) acts as an intrinsic cell fate determinant for the NB asymmetric division. Pros is asymmetrically localised as a basal cortical crescent in mitotic NBs and preferentially segregates to the more basally located GMC daughter cell (Hirata et al., 1995; Knoblich et al., 1995; Spana and Doe, 1995).

pros plays an important general role in specifying the identity of GMCs. Pros localises to GMC nuclei where it functions to repress the expression of genes normally expressed in NBs (Vaessin et al., 1991) and also activate genes normally expressed in GMCs (Doe et al., 1991). Miranda is an adapter protein which colocalises with Pros and acts to facilitate its asymmetric localisation (Shen et al., 1997; Ikeshima-Kataoka et al., 1997; Schuldt et al., 1998).

Whereas NB asymmetric divisions might be mediated primarily via an intrinsic mechanism, in GMC divisions (Buescher et al., 1998; Wai et al., 1999; Lear et al., 1999), as well as progenitor cell divisions in other contexts, e.g. MP2 precursor (Spana et al., 1995; Spana and Doe, 1996; Skeath and Doe, 1998), sensory organ precursors (SOP) (Rhyu et al., 1994; Guo et al., 1996) and muscle progenitors (Ruiz-Gomez...
and Bate, 1997; Carmona et al., 1998), both cell-intrinsic and cell-extrinsic mechanisms converge to effect the distinct fates of the daughters. In GMC asymmetric division, Numb acts as an intrinsic cell fate determinant. Numb is asymmetrically localised in (at least some) dividing GMCs and preferentially partitioned into only one of the progeny where it acts to antagonise Notch signaling. Hence the two progeny neurons attain distinct identities because in the cell inheriting Numb, Notch signaling is off, whereas in its sibling which fails to inherit Numb, Notch signaling is on. Partner of Numb (Pon) is a protein that colocalises with Numb and functions to facilitate its asymmetric localisation (Lu et al., 1998).

The proteins Inscutable (Insc; Kraut and Campos-Ortega, 1996; Kraut et al., 1996), Partner of Inscutable (Pins; Yu et al., 2000; Schaefer et al., 2000) and Bazooka (Baz; Kuchinke et al., 1998; Schober et al., 1999; Wodarz et al., 1999) form a complex that is localised to the apical cortex of neural progenitors. They function both to coordinate and to mediate several aspects of neural progenitor asymmetric cell divisions including Pros/Miranda, Staufen/pros RNA and Pon/Numb asymmetric localisation in NBs (Shen et al., 1997; Ikeshima-Kataoka et al., 1997; Li et al., 1997; Broadus et al., 1998; Schuld et al., 1998; Lu et al., 1998) and orientation of the mitotic spindle along the apical/basal axis (Kraut et al., 1996; Kuhlenschmidt et al., 2000). In addition, insc and pins are required for the asymmetric localisation and segregation of Numb and for the resolution of distinct fates for the sibling neurons derived from (at least some) GMC divisions (Buescher et al., 1998; Yu et al., 2000). These observations suggest this apical complex of proteins may act by providing positional information necessary to coordinate and mediate the processes which ensure the correct execution of asymmetric cell divisions.

To identify genes involved in mediating the asymmetric GMC cell division, we screened for mutations that cause both sibling neurons to adopt identical fates. We focused on the early born cells of the well characterised NB4-2 lineage (see Fig. 1). With the adaptation of the Dil-labelling technique to Drosophila neural progenitors (Bosson and Technau, 1994), the complete terminal lineage of all NB lineages including NB4-2 has been elucidated (Bosson et al., 1996; Schmid et al., 1999). In addition, mutations in several genes have been shown to cause cell fate transformations involving the NB4-2 lineage (e.g. Chu-LaGraff and Doe, 1993; Yeo et al., 1995; Bhat et al., 1995; Buescher and Chia, 1997; Skeath and Doe, 1998). The first born GMC from this lineage, GMC4-2a, divides to produce progeny with distinct cellular identities, the RP2 motoneuron and its sibling cell. Here we report the molecular and phenotypic characterisation of the gene jumu (jumu) as well as the expression of the Jumu protein during embryonic development. Loss of jumu function causes the GMC4-2a division to produce sibling cells both of which adopt characteristics of the RP2 neuron. jumu encodes a 720 amino acid (aa) nuclear protein that contains a 118aa region with strong homology to the DNA binding domain of the winged-helix family of transcription factors (see Reth, 1994; McMahon, 1994). The Jumu protein is expressed in the developing embryonic CNS including GMC4-2a where it is dispensable for Insc apical localisation but necessary for the basal localisation of Pon/Numb. These results suggest that in addition to the correct formation of an apical complex, transcription mediated by molecules like Jumu is also required to facilitate the correct asymmetric localisation and segregation of cell fate determinants like Numb.

**MATERIALS AND METHODS**

**Generation of mutant alleles**

Excisions of P1683 (from Bloomington Stock Center P[ry+,
lacZ:Hsp70, Km[r], or1] ry506/TM3 ry85) were identified by the loss of the rosy+ marker. Excisions were induced by gamma-irradiating P1683 males with 4000 rads or by generating jump starter males carrying both P1683 and a transposable source. Lethal ry revertants from both transposable and gamma irradiation induced excisions were ultimately balanced over TM3 Sb Ubx-lacZ for ease of identifying mutant embryos.

**Molecular techniques**

Genomic DNA flanking the P1683 insertion was rescued by digesting the DNA with XhoI, self-ligating, transforming into bacteria and plating on LB-kanamycin plates (Mlodzik and Hiromi, 1991). Wild-type genomic clones were obtained and mapped using standard techniques. Genomic fragments were used as probes on northern blots and for screening embryonic cDNA libraries (Brown and Kafatos, 1988). Protein homology searches were carried out using the databases at the National Center of Biotechnology Information, National Institute of Health using Blastp and Blastn programmes.

**Antibody production**

A fusion protein containing a nonconserved portion of the deduced Jumu protein (residues 143-330) was produced in E. coli using the pQE-30 (6 x His) vector from Qiagen. The fusion protein was purified using Ni-NTA resin and used to immunise rabbits.

**Immunohistochemical staining of embryos**

Embryos were fixed and immunostained as previously described (Yang et al., 1997). Primary antibodies included mouse anti-Eve (1:40, gift from K. Zinn), rabbit anti-Eve (1:1000, gift from M. Frasch), mouse anti-Pros (1:5, gift from C. Q. Doe), mouse mAb22C10 (1:5, gift from S. Benzer), mouse anti-β-gal (1:500, Promega), rabbit anti-β-gal (1:1000, Cappel), mouse anti-Zfh1 (gift from Z. Lai) and rabbit anti-Jumu (1:4000). Histochemical detection was performed using Jackson Immunoresearch Inc HRP-conjugated secondary antibodies (1:150 dilution) and visualised using the glucose oxidase-DAB-nickel method as previously described (Yang et al., 1997). Embryos were mounted in 70% glycerol and examined using DIC optics in a Zeiss Axioshot. Fluorescence detection was performed using Jackson Immunoresearch Inc secondary anti-mouse or anti-rabbit conjugated to fluorescein (1:200) or Texas red (1:200). Embryos were mounted in Vector Shield and images collected on a Bio-Rad MRC600 confocal microscope.

**RNAi**

RNA interference (RNAi) experiments were carried out essentially as described in Kennerdell and Carthew (1998). The Saff-Xhol fragment (590 bp) encompassing the C-terminal region of Jumu was used for double-stranded RNA synthesis. approx. 50-100 pl of a 1 mg/ml dsRNA solution was injected. Injected embryos were incubated at 25°C and fixed at stage 14 or later.

**RESULTS**

**Identification and generation of jumu mutant alleles**

The anti-Even skipped antibody (anti-Eve) stains the nuclei of
Table 1. The frequency of RP2 duplication in jumu mutant alleles

<table>
<thead>
<tr>
<th>Allele</th>
<th>Expressivity</th>
<th>Mutant hemisegments/ hemisegments scored</th>
</tr>
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<tbody>
<tr>
<td>P1683 (homozygote)</td>
<td>4%</td>
<td>5/120</td>
</tr>
<tr>
<td>L40 (homozygote)</td>
<td>15%</td>
<td>61/420</td>
</tr>
<tr>
<td>L70 (homozygote)</td>
<td>29%</td>
<td>46/160</td>
</tr>
<tr>
<td>L40/B22-5</td>
<td>27%</td>
<td>255/940</td>
</tr>
<tr>
<td>L70/B22-5</td>
<td>23%</td>
<td>121/520</td>
</tr>
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*B22-5 homozygotes do not develop sufficiently to be scored.

approx. 20 neurons in each of the hemineuromeres of the CNS (see Fig. 2A): the EL cluster, the aCC and pCC neurons, the RP2 neuron and its sibling cell and the CQ neurons (Patel et al., 1989). Using anti-Eve and anti-β-gal, we screened 500 lethal single P-element enhancer trap lines from the Bloomington Stock Center focusing on mutations that affected RP2 cell number. We identified P1683 as an insertion at cytological position 86B which expresses β-gal in NB subsets and which exhibits an occasional duplication of the Eve+ RP2 neuron. In P1683 homozygous embryos, the expressivity of the RP2 duplication phenotype is low (see Table 1). Although P1683 is lethal, the P element is inserted in the large first intron of the transcription unit (see Fig. 3) and represents a weak hypomorphic allele. We mobilised the P element in order to ascertain that the phenotype was due to the P-element insertion and also to obtain stronger alleles. 200 ry− excisions were scored; about one-fifth of which were viable and several of the viable excisions examined reverted to a wild-type phenotype with no duplication of the Eve+ RP2 neuron. Hence, both the lethality and the RP2 duplication phenotype can be attributed to the P1683 insertion. Embryos from all of the excision lines that remained lethal were examined following anti-Eve staining. Twelve of these alleles showed an increase in the expressivity of the RP2 duplication phenotype compared to the P1683 homozygote. jumuL40 and jumuL70, which showed the strongest phenotypes were further analysed. jumuL40, which deletes sequences from the transcribed region of jumu (Fig. 3), increased the expressivity of the RP2 duplication phenotype four fold: jumuL70, which showed a seven-fold increase in expressivity appeared to be a total loss-of-function allele (see below and Table 1).

By analysing gamma irradiation induced ry− revertants from P1683, we identified a deficiency, Df(3)B22-5, which, on the basis of Southern analysis (not shown), removed the entire genomic region encompassing the P1683 insertion site shown in Fig. 3. This deficiency allowed us to show that jumuL40 was a strong hypomorphic allele since jumuL40/Df(3)B22-5 embryos showed a stronger phenotype than jumuL40 homozygotes; jumuL70 homozygotes on the other hand showed similar expressivity as jumuL70/Df(3)B22-5 embryos and it therefore represents an amorphic allele (see Table 1). The analyses of the jumu mutant phenotype described in this paper were carried out with either jumuL40/Df(3)B22-5 or jumuL70 homozygous embryos which show high penetrance (>95%) for the RP2 duplication phenotype.

In jumu embryos, the RP2 sibling cell takes on characteristics of the RP2 neuron

In stage 14 or older wild-type embryos stained with anti-Eve, the nucleus of one Eve+ RP2 neuron can be seen at its characteristic position in each hemisegment (Fig. 2Aa). In anti-Eve stained jumu mutant embryos of a similar age, many hemisegments exhibit two Eve+ nuclei, of unequal size, at the characteristic RP2 position (Fig. 2Ab). Since the two nuclei are usually tightly apposed we asked whether they represent two cells in close association or one binucleated cell; we double labelled mutant embryos with anti-Eve and a membrane marker anti-phosphotyrosine. Confocal micrographs indicate that the two Eve+ nuclei are associated with two cells with their nuclei of distinct size separated by cell membrane (Fig. 2Ac, arrowhead). We show, in addition, that the duplicated RP2s in jumu embryos are stained by two other antibodies, anti-Zfh1 (Lai et al., 1991b) (Fig. 2Ag) and mAb22C10 (Fig. 2Ad), which stain wild-type RP2 neurons. Hence on the basis of anti-Eve, anti-Zfh1 and mAb22C10 stainings, the duplicated cells both express markers consistent with a RP2 identity.

If jumu is exerting its effect on sibling cell fate choice at the level of the postmitotic neurons we would not expect to observe an alteration in the GMC4-2a cell identity in jumu mutant embryos. To see whether the GMC4-2a undergoes a cell fate transformation, we assessed the identity of mutant GMC4-2a cells using a variety of markers that label the wild-type GMC4-2a including, anti-Eve, anti-Ftz, anti-Pros and anti-Pdm1 (see Fig. 1). The expression of these markers in GMC4-2a is unaffected in the mutant (not shown). These results suggest
that in jumu mutant embryos the mutant GMC4-2a appears to retain a wild-type GMC4-2a identity.

In order to understand the origin of the extra RP2-like cell in jumu mutant embryos, we followed the appearance of Eve+ cells from the NB4-2 lineage. In the wild-type temporal series (Fig. 2B), the first born GMC, GMC4-2a, buds off from the dorsal/lateral cortex at late stage 10 and by mid-stage 11 becomes Eve+; GMC4-2a divides to produce the postmitotic RP2 and RP2sib, both of which express Eve at stage 11; RP2 retains Eve expression until the end of embryogenesis but its sibling cell extinguishes Eve expression such that by stage 13, Eve expression is maintained in the RP2 sibling cell. In mutants, many hemisegments become extinguished in the mutant RP2sib; both cells remain Eve+ at stage 13 and stage 15 (Fig. 2B), and until stage 17. These results (schematised below the panels in Fig. 2B) indicate that the extra RP2-like cell found in jumu embryos is derived from the RP2 sibling cell.

**Molecular characterization of jumu**

The P1683 insertion line was generated using an engineered P transposon which carried, in addition to the P-element ends, the lacZ reporter gene, the eye marker (rosy), a plasmid origin of replication and an antibiotic resistance selectable marker (Mlodzik and Hiromi, 1991). An approx. 5 kb XbaI fragment containing the 3’-end of the P-transposon along with approx. 3 kb of flanking genomic DNA was obtained by ‘plasmid rescue’. With the flanking DNA as probe, we isolated several recombinant phages from a wild-type genomic library (Tamkun et al., 1992) encompassing the P1683 insertion site (Fig. 3).

Using a series of genomic fragments as probes for northern blot filters, we have identified two developmentally regulated polyadenylated RNA species of 2.8 kb and 3.8 kb (data not shown). We isolated two classes of cDNA clones from embryonic cDNA libraries (Brown and Kafatos, 1988) which differed apparently in their respective sites of poly(A) addition. The respective sizes of the largest member of each cDNA class corresponded well with the 3.8 kb and 2.8 kb transcripts seen on northers; they encode identical open reading frames (ORF) and differed only in their 3’ termini. Our results suggest that alternative polyadenylation sites are utilised to generate the 3.8 kb and 2.8 kb transcripts; the locations of the P1683 insertion site and the cDNA clones relative to the genomic region, determined from cross hybridisation experiments and comparisons with the genomic sequence from the Berkeley Genome Project, are shown in Fig. 3.

To formally demonstrate that this transcription unit is associated with the phenotype, RNAi experiments were performed (Fire et al., 1998; Kennerdell and Carthew, 1998). Injection of double stranded jumu (see methods) RNAi induced RP2 duplication phenotypes, with incomplete expressivity (22%; 86/398 hemisegments), similar to those observed in the
mammals the Hepatocyte Nuclear Factor3 family (Lai et al., 1991a) and the Winged-Helix-Nude protein (Nehls et al., 1994). Alignment of the winged-helix domains (Fig. 4B) showed that the highest homology (68% identity; 85% similarity) is with the Winged-Helix-Nude protein (Nehls et al., 1994), which is associated with the gene that is mutated in nude mice. These results suggest that Jumu effects asymmetric sibling cell fate choice through its actions as a transcription regulator.

### Jumu is expressed in neural progenitors including NB4-2 and GMC4-2a

Antiserum was raised against a Jumu fusion protein (see Methods). This polyclonal antibody (anti-Jumu) specifically recognises the Jumu protein as evidenced by the fact that it fails to stain mutant embryos (e.g. jumu<sup>L70</sup> homozygotes); in addition, the general embryonic staining pattern of anti-Jumu (Fig. 5) appears to parallel the expression pattern of the lacZ reporter gene in P1683 (not shown). Consistent with its homology to the winged-helix family of transcription factors, Jumu protein shows nuclear localisation. Protein expression is first detected in the nuclei of syncitial embryos (Fig. 5A) and appears to be present in all nuclei during cellular blastoderm (Fig. 5B). During germ band extension, and in the germ band extended embryo, nuclear expression is seen throughout the ectoderm and in the CNS primordia (Fig. 5C). During germ band retraction the ectodermal expression fades and Jumu expression is seen predominantly in the brain lobes, in the segmented CNS and elements of the PNS (Fig. 5D). The CNS expression persists into late embryogenesis (Fig. 5E,F).

We examined in greater detail the Jumu protein expression pattern in the segmented CNS. The NB expression pattern of Jumu is highly dynamic and is shown following SHI (Fig. 6A) and SHII (Fig. 6B) NB segregation. To elucidate how Jumu might act to generate the duplicated RP2 neurons, we assessed the expression of Jumu protein within the NB4-2 lineage. Pros is expressed in the nuclei of many GMCs in the developing CNS, including GMC4-2a and can be used as a general GMC marker. At stage 10 (following SIII NB segregation), anti-Pros staining shows that there is a Pros+ cell dorsal to NB4-2 (Fig. 6B,E) at a time when GMC4-2a is not yet expressing Jumu protein (not shown); however, no Jumu+ NB4-2 are seen prior to the formation of the Pros+ GMC4-2a; therefore, Jumu is expressed in NB4-2 only after its first division. Later, during mid-stage 11, GMC4-2a expresses Eve just prior to its division (note that unlike Pros which is present in GMC4-2a when it is born, Eve expression commences only late in the GMC4-2a cell cycle); double labelling experiments with anti-Eve and anti-Jumu demonstrate that GMC4-2a also expresses Jumu at this time (Fig. 6F). Late in stage 11, GMC4-2a divides to produce the Eve+ postmitotic RP2 and RP2 sibling cell; double labelling experiments indicate that both of these cells are also positive for Jumu shortly after their births (Fig. 6G). However,
Jumu protein does not persist for long in the postmitotic neurons and can no longer be detected by the beginning of stage 12 (Fig. 6H).

These data indicate that within the NB4-2 lineage, Jumu accumulates only following the first NB cell division, in the nuclei of both NB4-2a and GMC4-2a. Jumu expression in NB4-2a precedes its expression in GMC4-2a (since we never see a Jumu+ GMC4-2a in conjunction with a Jumu− NB4-2a). Furthermore, Jumu protein is present in the nuclei of the postmitotic RP2 and its sibling cell, although its presence in these cells is short lived. A schematic summary of Jumu expression pattern in the early NB4-2 lineage is shown (Fig. 6I).

Jumu is required for PON/Numb but not Insc asymmetric localisation in GMC4-2a

What might be the underlying mechanism responsible for the RP2 duplication phenotype associated with the jumu loss-of-function mutants? Several observations suggest that Jumu may be acting at the level of the GMC4-2a cell division. Jumu is not expressed in NB4-2 prior to its first divisions making it unlikely that it would be acting at the level of the NB4-2 cell divisions. Moreover, it is only transiently detected in the postmitotic RP2 and RP2sib and it is not asymmetrically segregated to one of these cells, it therefore seems unlikely that jumu would be acting at the level of the postmitotic neurons. Since nuclear Jumu can be detected in GMC4-2a, we examined the possibility that

Fig. 4. jumu encodes a winged-helix family protein. (A) The DNA and deduced protein sequence of jumu is shown. The putative winged-helix DNA binding domain is in bold and underlined. The polyadenylation sites AATAAA (boxed) are shown. (B) Alignment of the Jumu winged-helix domain with other members of the winged-helix protein family. The highest identity (68%) is with the winged-helix domain of the Winged Helix Nude (Whn) protein. Abbreviations: mmfkhn1, Whn protein; A42826, T-cell leukemia virus enhancer factor; hilfhuman, Human T-cell leukemia virus enhancer factor; fd1 drome, Fork Head domain protein FD1. Consensus residues are boxed and shaded.

Fig. 5. The general embryonic expression pattern of Jumu. (A) A preblastoderm syncitial embryo; (B) a stage 5 embryo; (C) a late stage 10 embryo; (D) a stage 12 embryo; (A-D) Lateral views. (E) Ventral view of a stage 15 embryo; (F) dorsal view of a stage 15 embryo. Anterior is towards the left. Embryos were stained with anti-Jumu.
jumu may be required for the asymmetric localisation of the cell fate determinant Numb during the GMC4-2a cell division. Since Numb always colocalises with Pon, which acts to facilitate its localisation (Lu et al., 1998), we used an anti-Pon antibody to illustrate the localisation of Numb. Examination of late prophase to metaphase GMC4-2a cells triple labelled with anti-Eve, DNA stain and anti-Pon indicated that Pon localisation is defective in dividing jumu GMC4-2a cells. In essentially all of the dividing wild-type GMC4-2a cells (19/19), Pon and Numb always form basal cortical crescents (Fig. 7C) and a previously study suggested that the more basal progeny, which preferentially inherits Numb, becomes the RP2 neuron (Buescher et al., 1998). However, in jumu mutant embryos, many dividing GMC4-2a cells fail to localise Pon as a basal crescent; about 36% (10/28) show either cortical Pon distribution or misplaced crescents (Fig. 7D,F). The frequency of the Pon mislocalisation roughly coincides with the frequency of hemisegments showing the RP2 duplication phenotype (29%; Table 1). Although similar conclusions can be drawn using anti-Numb, the quality of the images are inferior due to technical reasons (data not shown). These data are therefore consistent with the notion that the duplication of RP2 neurons in jumu embryos arises as a result of the symmetric segregation of Numb to both the postmitotic RP2 neuron and its sibling cell (arrows in G) express Eve and Jumu (yellow); however, Jumu expression is quickly extinguished and these cells (large and small arrows in H) retain only Eve expression by early stage 12 (red).

DISCUSSION

We focused on RP2 and RP2sib, sibling neurons derived from the first born GMC, GMC4-2a, from the NB4-2 lineage. We
RP2sib, N signalling cannot be switched off due to the absence of antagonises N signalling in this cell, enabling it to adopt the GMC4-2a cell division, the more basal progeny preferentially is oriented along the apical/basal axis such that following the apical cortex and its apical localisation is required for its expression pattern and its predicted sequence, it seems presumably due to carryover from the GMC4-2 mother. Given this expression pattern and its predicted sequence, it seems likely that the requirement for \textit{jumu} in the specification of distinct cellular identities for RP2 and RP2sib is likely to be through its actions as a transcription regulator in GMC4-2a.

We and others have shown that both intrinsic and extrinsic mechanisms are involved in mediating the distinct fates of RP2 and RP2sib (Buescher et al., 1998; Skeath and Doe, 1998; Lear et al., 1999). During the GMC4-2a division Insc is localised to the apical cortex and its apical localisation is required for Numb localisation becomes defective, resulting in cortical distribution or misplaced non-basal crescents. In these dividing GMCs where Numb is mislocalised both daughter cells can inherit Numb leading to the formation of an extra RP2 at the expense of the RP2sib. The expressivity of the RP2 duplication phenotype is never complete in embryos homozygous for an antigen-minus allele of \textit{jumu} nor in RNAi embryos, suggesting that other functions involved in the same process can partially compensate for the loss of \textit{jumu} function.

Our data indicate that the failure to localise Numb is the primary defect responsible for the failure to resolve distinct RP2/RP2sib cell fates. The localisation of known asymmetric components is not affected in mutant NBs. Moreover, the localisation of Insc in GMC4-2a (and other GMCs) remains apical in mutant embryos. So the effect of loss of \textit{jumu} does not affect protein localisation in a general way but rather appears to be specific to Pon/Numb. Moreover, other aspects of the GMC4-2a division appear to occur normally in \textit{jumu} mutants. We have previously shown that the RP2 and RP2sib nuclei are distinct in size; furthermore, in most mutants that cause an RP2sib > RP2 cell fate transformation, including \textit{jumu}, the duplicated RP2 neurons exhibit distinct nuclear size differences. The only example in which the nuclei of the sibling neurons adopt equivalent size are in \textit{insc} and \textit{pins} embryos (Buescher et al., 1998; Yu et al., 2000). Hence the generation of different sized sibling nuclei, which requires \textit{insc} function, is not affected by the loss of \textit{jumu}. Similarly, the orientation of the GMC4-2a cell division is also not affected in \textit{jumu} mutants. These results indicate that \textit{jumu} acts downstream of Insc, or in a parallel pathway, to mediate Pon/Numb localisation but is not required for other aspects of the GMC4-2a division.

\textit{jumu} mutant embryos also exhibit an additional unique phenotype. In wild-type embryos, RP2 and RP2sib clearly separate from each other. In all of the known mutants which fail to resolve distinct sibling cell fates and cause RP2 duplication, e.g. \textit{insc}, \textit{sanpodo}, \textit{N. mastermind}, the two RP2 neurons separate from one another. In \textit{jumu} embryos, although there is clearly cell membrane between the nuclei of the duplicated RP2 neurons, these cells invariably fail to separate following cytokinesis. The phenotype is reminiscent of a number of mutations in yeast which show similar defects in cell separation (reviewed by Gould and Simanis, 1997). The gene associated with one of these mutations, \textit{sep1}, encodes a putative winged-helix family DNA binding domain. We show that the Jumu protein is expressed in the NB4-2 lineage only after the delaminated NB has divided to generate NB4-2a and GMC4-2a. Jumu is not asymmetrically localised, being present in the nucleus of GMC4-2a and NB4-2a. Jumu is only transiently detectable in the nuclei of newly born RP2 and RP2sib, presumably due to carryover from the GMC4-2 mother. Given its expression pattern and its predicted sequence, it seems likely that the requirement for \textit{jumu} in the specification of distinct cellular identities for RP2 and RP2sib is likely to be through its actions as a transcription regulator in GMC4-2a.

We speculate that the \textit{jumu} neuronal cell fate phenotype and

![Fig. 7](image_url)
the cell separation phenotype may be related by a common mechanism. The occurrence of the two defects appears to show a complete correlation; in jumtl mutant embryos the RP2 and RP2sib neurons in the hemisegments that undergo normal sibling cell fate resolution always undergo separation; whereas the duplicated RP2 neurons in the hemisegments that fail to resolve distinct sibling cell fates, also fail to separate. Little is known about the separation of sibling cells. However, it seems likely that cytoskeletal and membrane components must play a role in the separation of postmitotic sibling neurons. Similarly, the localisation of components of asymmetric cell division is likely to be dependent on components of cell cortex. Therefore it is possible that a transcription regulator like Jumu might mediate the expression of cortical/membrane components necessary for both processes.

In summary, the winged-helix family of transcription factors have been shown to play important roles in the development of a multitude of embryonic structures as well as in axial patterning (see reviews by Reth, 1994; McMahon, 1994). We demonstrate here that Jumu, a new member of this family, is also involved in mediating the distinct cellular identities of two sibling neurons during the development of the Drosophila embryonic CNS. Although Jumu is not itself asymmetrically localised, we propose that as a transcription regulator, it plays a permissive role for the asymmetric localisation of Numb, during the GMC4-2a progenitor cell division. We speculate that jumu may be required to express cortical/membrane components which are required for Numb asymmetric localisation in the progenitor and separation of the two daughter cells. Our results suggest that the asymmetric localisation and segregation of Numb in a dividing neural progenitor (e.g. GMC4-2a) require not only an apical complex of proteins, to define apical/basal polarity, but also de novo transcription mediated by molecules like Jumu.

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