DETERMINATION OF ASYMMETRIC CELL DIVISION IN THE DROSOPHILA LARVAL CENTRAL NERVOUS SYSTEM

A Senior Thesis

By

Stephanie Smith

1996-97 University Undergraduate Research Fellow

Texas A&M University

Group: BIOLOGY I

Determination of Asymmetric Cell Division in the Drosophila Larval Central Nervous System

Stephanie Smith
University Undergraduate Fellow, 1996-1997
Texas A&M University
Department of Biochemistry

APPROVED

Fellows Advisor _____

Honors Director _____

Determination of Asymmetric Cell Division in the Drosophila Larval Central Nervous System. Stephanie Smith (Suma Datta), Biochemistry, Texas A&M University.

Neuroblasts in the central nervous system of Drosophila have been increasingly utilized as a model for stem-cell study due to their clearly defined asymmetric division pattern and ease of genetic, cellular, and molecular analysis. This research involved looking at the control of asymmetric division in the different populations of neuroblasts throughout the larval instars using pros expression as a marker for asymmetric division. The pros protein is asymmetrically localized in the Ganglion mother cell and is necessary for establishing Ganglion mother cell fate. The results indicate that the role of pros in establishing GMC cell fate is implicated in the larva in addition to the embryo and that pros would be a good marker for most asymmetric division in the larval stages. However, pros is not an universal marker. Although it is expressed in the thoracic and central brain neuroblasts, it is not expressed in the asymmetrically dividing optic lobe neuroblasts, indicating that another mechanism for establishing cell fate in the optic lobe needs to be explored. Finally, staining with *trol* mutants and preliminary staining with *ana* mutants indicate that pros expression is coupled with the activation of cell division.

Determination of Asymmetric Cell Division in the Drosophila Larval Central Nervous System

BACKGROUND:

A great deal of emphasis has been placed on stem cells given the role they play in the development of multicellular organisms and the implications they have in many cancers. Although stem cells differ in cell character, rate of turnover, and their geometry of cell replacement, they share several fundamental properties. They are not terminally differentiated and they can divide without limit. Furthermore, when they divide, their daughter cells can either remain a stem cell or go on to become terminally differentiated (Alberts et al., 1989). Therefore, they replenish themselves in addition to giving rise to populations of differentiated cells (See Figure 1).

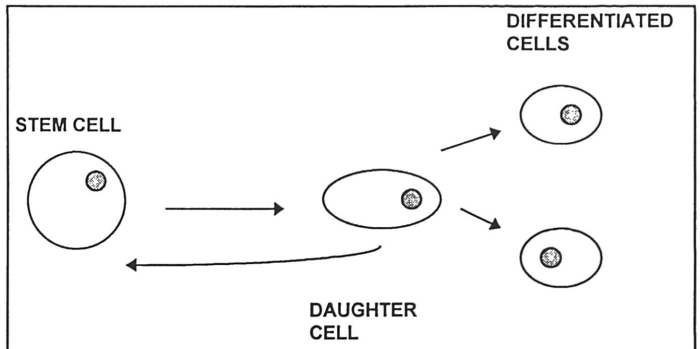

Figure 1: Daughter cells have the option of remaining a stem cell or becoming terminally differentiated.

It is a derangement in this process of differentiation that leads to tumor formation in tissues that require renewal by stem cells. If either a stem cell fails to produce one non-stem cell daughter with each division or a daughter cell fails to differentiate normally, a tumor can develop (Alberts et al., 1989). This renewal by stem cells is necessary when division is impossible given the terminal state of the cell which hinders mitosis or cytokinesis (Alberts et al., 1989). Examples of stem cells include germ-line,

bone marrow, and neuronal cells. Although stem cells are not terminally differentiated, they are determined. For instance, a muscle satellite cell will always be a source of skeletal muscle just as a spermatogonium will always be a source of spermatozoa (Alberts et al., 1989).

This renewal by stem cells raises several important questions. First of all, what determines when a stem cell should divide or remain quiescent? Second, when should a daughter cell stay a stem cell or decide to differentiate? Finally, how is differentiation of a daughter cell regulated after its commitment to differentiation? (Alberts et al., 1989) We are interested in addressing a certain aspect of the second question, known as asymmetric division, in Drosophila melanogater. For instance, if a daughter cell also becomes a stem cell, this is a symmetric division because two stem cells are produced when the mother cell divides; however, if the daughter cell begins to differentiate and take on a different cell fate than the mother cell, it has undergone an asymmetric division (See Figure 1).

Drosophila melanogaster has been increasingly utilized in the field of developmental neurobiology in the hopes that the knowledge gleaned from the fruit fly can be applied to higher organisms that are not conducive to such genetic analysis (Goodman and Doe, 1993). It is an attractive model system because of the powerful genetic and molecular tools such as monoclonal antibodies and enhancer trap lines that are associated with it. In

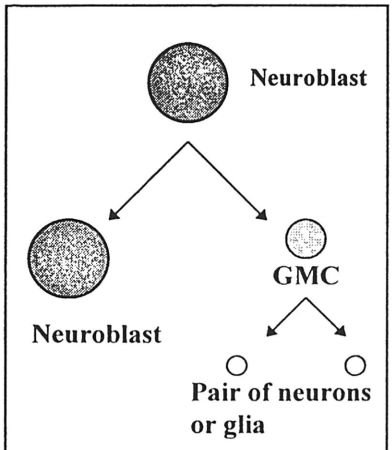

Figure 2: Asymmetric division of a neuroblast

addition, the life cycle of Drosophila has defined morphological events such as the transitions between the three larval stages (instars) which makes developmental analysis easier. We are interested in looking at the control of asymmetric division in the different populations of neuronal precursors called neuroblasts throughout these different larval instars.

The Drosophila central nervous system develops from these stem cells which arise from a special neurogenic region called the neuroectoderm (Goodman and Doe, 1993). The neuroblasts can either divide symmetrically to expand the population of neuroblasts or divide asymmetrically in which case a smaller ganglion mother cell buds off and eventually divides once to produce a pair of neurons or glia (Spana and Doe, 1995) (See Figure 2). Neuroblasts are a good model for stem cell study due to their clearly defined asymmetric division pattern and ease of analysis genetically, cellularly, amd molecularly (Lin and Schagut, 1997). Although asymmetric cell division has been well characterized in the Drosophila embryo (Goodman and Doe, 1993), it has not been well characterized in the larva. In addition, the larval brain contains the only set of neuroblasts known to switch from symmetric to asymmetric cell division. We hope to determine when and how these neuroblasts switch from symmetric to asymmetrical neuroblast division.

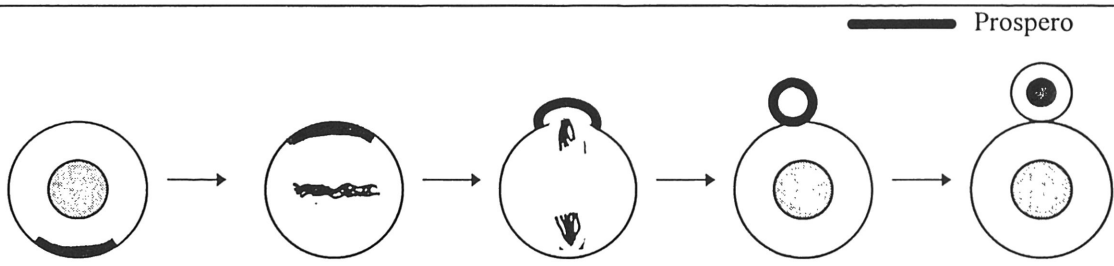

Figure 3: Illustration of the migration of the pros protein from the neuroblast to the nucleus of the ganglion mother cell in asymmetrically dividing neuroblasts (Spana and Doe, 1995)

To monitor the switch, we need a marker for either symmetric or asymmetric dividing cells. Several differences exist between neuroblasts and ganglion mother cells, namely size, gene expression profile, and cell lineage (Spana and Doe, 1995). These differences can be taken advantage of in order to label asymmetrically proliferating neuroblasts. One such potential marker is the *prospero* gene which "encodes a transcription factor necessary for proper ganglion mother cell gene expression" (Spana and Doe, 1995). It represses neuroblast specific genes while activating Ganglion Mother Cell specific genes (Doe and Spana, 1995). The prospero protein is synthesized in the neuroblast and first appears in a crescent on the apical side. It is then localized to a crescent on the basal side during metaphase and to the budding ganglion mother cell in anaphase. Finally, it is translocated to the nucleus in the ganglion mother cell following cytokinesis (Spana and Doe, 1995). (See Figure 3). This differential segregation of pros provides a mechanism for rapidly establishing Ganglion mother cell fate. Therefore, we should be able to use expression of the pros protein to follow asymmetrically dividing neuroblasts in the larval brain.

HYPOTHESIS:

Pros as a potential marker for asymmetric cell division

We know that a population of neuroblasts called the mushroom body neuroblasts (See Fig. 4) are asymmetrically dividing in the larval brain at 0-4 hours after hatching. Likewise, we know that the optic lobe neuroblasts (See Fig. 4) are undergoing symmetric cell division at 18-20 hours after hatching. Therefore, by looking at the pros staining patterns at these time points, we can determine whether the pros protein really is a good marker for asymmetric division in the larval central nervous system.

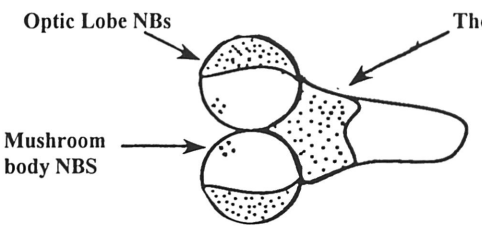

Figure 4: The different populations of neuroblasts in the larval brain.

Pros expression in proliferating neuroblasts of the larval brain

Several populations of neuroblasts are known to be proliferating in the larval brain (See Fig. 4). Assuming that pros expression can be used as an indication of asymmetric division, we can look at the type of proliferation that is occurring in these subsets of neuroblasts by dissecting and staining with pros at a series of carefully staged time points. For example, the optic lobe neuroblasts are known to be undergoing symmetric cell division at late first instar. We would like to determine when these neuroblasts make the switch from pool expanding symmetric division to asymmetric division. In addition, we

would also like to look at the type of divisions occurring in the thoracic neuroblasts and (if applicable) when the switch to asymmetric division occurs in these neuroblasts.

Pros expression in ana and trol mutants:

We work with two mutants in the lab called *ana* and $trol^{sd}$. Both are involved in regulation of the cell cycle. *Ana* acts as a repressor of premature proliferation of quiescent neuroblasts while *trol* bypasses ana and activates proliferation in quiescent neuroblasts. Therefore, *ana* mutants have a phenotype of early proliferation and *trol* mutants have a repressed phenotype. Looking at pros staining in these mutants, we can examine whether pros expression is tightly coupled to activation of cell division or is determined by reaching a certain developmental time point instead. Precedents for the regulation of the timing of gene expression by both mechanisms have been documented.

Pros expression in mud mutants

Furthermore, a mutation called *mud* exists which is proposed to result in extra mushroom body neuroblasts (Prokop and Technau, 1994). We can test that hypothesis by determining whether these extra neuroblasts really show asymmetric cell division (i.e. pros expression).

Numb expression in the larval brain

We have acquired the numb antibody and can now look at numb expression in the larval brain. Numb is a membrane-associated protein that is co-localized with prospero

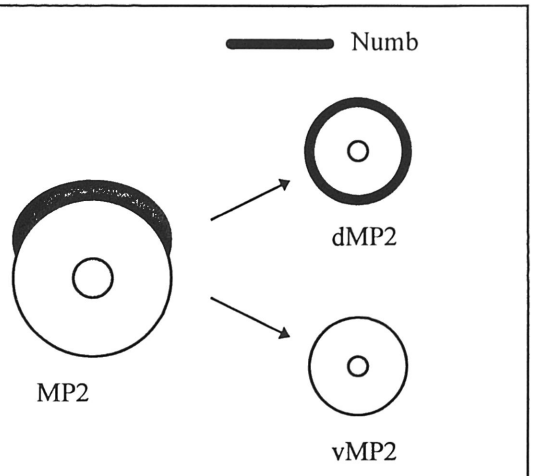

Figure 5: Segregation of numb in the dMP2 daughter cell.

in the neuroblast and segregated into the daughter Ganglion mother cell in asymmetric division (Knoblich, Jan and Jan, 1995). Although a loss of numb has no effect on Ganglion mother cell fate, it is implicated in the fate of the MP2 precursor daughter cells (Doe and Spana, 1995). Even though the MP2 precursor, found in the embryo, is morphologically indistinguishable from a neuroblast, it has a different cell division profile. MP2 divides once to produce a pair of neurons. Both are interneurons, but the daughter cell that receives numb will become the dMP2 and project its axon posteriorly while the daughter cell from which numb is excluded will become the vMP2 and project its axon anteriorly (See Figure 5). Asymmetric localization of numb has been shown to be sufficient for specifying dMP2 neuronal identity (Spana, Kopczynski, Goodman, and Doe, 1995)

The optic lobe neuroblasts are the only known population of neuroblasts that switch from symmetric to asymmetric division. Given numb's role in the MP2 precursor, it will be interesting to see if it is implicated in optic lobe neuroblast cell fate.

METHODS:

Pros as a potential marker for asymmetric division

We obtained the Mouse anti-pros antibody (courtesy of Chris Q. Doe) which was used to detect the pros protein. To look at pros staining in the mushroom body neuroblasts, wild-type larvae were collected in one hour windows, and their brains were dissected 2-3 hours after hatching in 1XPBS. The brains were fixed in enhancer trap fix (1 part Buffer B, 1 part formaldehyde, and 4 parts distilled water) for 30 minutes. They were washed three times in 1X PBS with Triton (PBST) and blocked in 10% NGS with PBST for 30 minutes to 1 hour at room temperature. The samples were incubated in a 1:4 to 1:5 dilution of the mouse anti-pros antibody in 10% NGS for 4 hours at room temperature or overnight at 4^0C. They were then washed three times for ten minutes each in PBST. The secondary Goat anti-mouse conjugated antibody was diluted 1:800 in 10% NGS/PBST and samples were incubated overnight at 4^0C. After incubation, they were rinsed in PBST for three ten minute washes. The samples were then developed with DAB and H_2O_2. Due to this reaction, a precipitate should have formed where the prospero protein was found. All pros staining in the following experiments was carried out according to a similar protocol. To look at the symmetrically dividing optic lobe neuroblasts, larvae were collected in one hour windows, and their brains were dissected 18 hours after they hatched. The brains were then stained for pros.

Pros staining at developmental time points

To obtain data for the developmental time points, wild-type larval brains of wild-type flies (Canton-S or Oregon R) were dissected in a series of carefully staged time points in the second and third larval instars (See Figure 6). Larvae were collected in one hour windows after hatching. Then, time points were collected at 6 hours after the first molt, 12 hours after the first molt, 2nd molt, 12 hours after the second molt, 24 hours after the second molt, and at climbing third instar. The larvae were checked in one hour windows for molting, and timed from the point of their respective molts. Their brains were then dissected and stained with pros

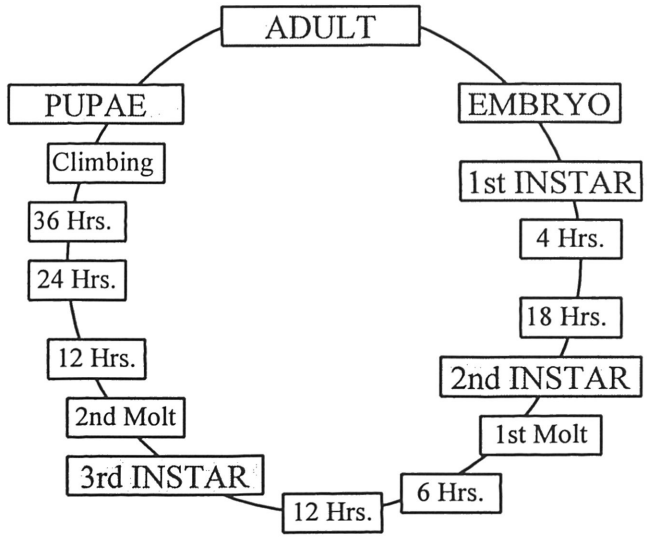

Figure 6: The life cycle of Drosophila and the developmental time points of interest.

Pros expression in trol^{sd}

Trol mutants were collected in one hour windows and allowed to develop to third instar. The brains were dissected and stained with pros. As a control, the wild type flies of the stock were dissected at the second molt.

Pros expression in ana

Ana homozygotes were dissected 25-26 hours after hatching and stained with pros. Canton S was used as a wild-type control at this time point.

Numb expression in Canton-S

The rabbit anti-numb antibody was diluted 1:1000 in 10% NGS/PBST, and the goat anti-rabbit HRP-conjugated secondary was diluted 1:3000 in 10% NGS/PBST. Climbing third instar Canton-S brains were dissected and stained with numb.

Results:

Pros is a good indicator of asymmetric division- first instar time point data

At four hours after hatching, wild-type brains showed pros staining in the mushroom body neuroblasts. However, at 18 hours after hatching, the brains showed no pros staining in the optic lobe neuroblasts (See Fig. 7). The mushroom body neuroblasts undergo asymmetric division throughout the larval stages of development, while the optic lobe neuroblasts undergo symmetric division in late first instar. Therefore, the data is consistent with pros's role in asymmetric division in the embryo and implies that 1) the role of pros in establishing an asymmetric cell fate is implicated in the larval stages and 2) pros is a good marker for asymmetric division in larval stages.

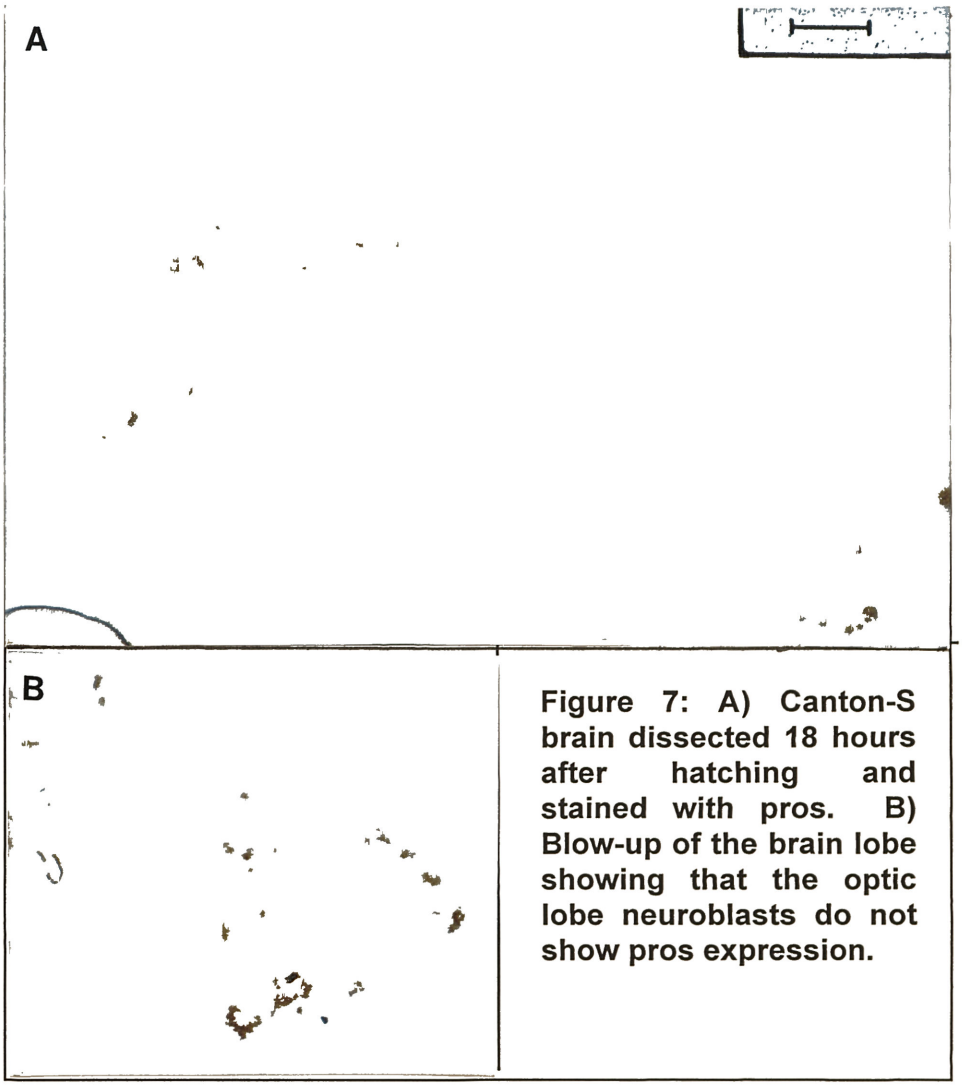

Figure 7: A) Canton-S brain dissected 18 hours after hatching and stained with pros. B) Blow-up of the brain lobe showing that the optic lobe neuroblasts do not show pros expression.

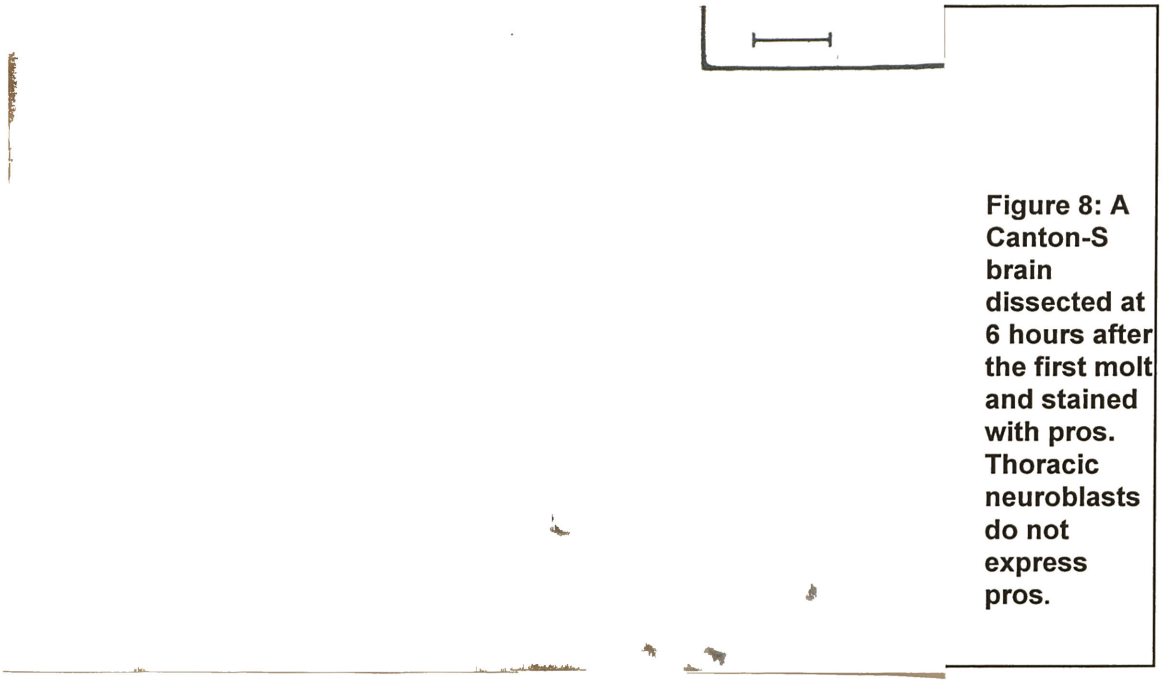

Figure 8: A Canton-S brain dissected at 6 hours after the first molt and stained with pros. Thoracic neuroblasts do not express pros.

Pros expression in second instar

At six hours after the first molt, the thoracic neuroblasts have not started showing pros expression yet (See Fig. 8). At twelve hours after the first molt, the thoracic neuroblasts seem to begin proliferating.

Pros is not a universal marker- Third instar time points

At the second molt, the thoracic neuroblasts and central brain neuroblasts were shown to be actively expressing pros, indicating that the neuroblasts were undergoing asymmetric division as expected (See Fig. 9). The optic lobe neuroblasts at this time point showed no pros staining. Interestingly, in late third instar (second molt plus 24 hours, second molt plus 36 hours, and climbing third) the central brain and thoracic

neuroblasts labeled as expected; however, the optic lobe neuroblasts still did not show pros staining (see Figure 10) even though they are known to be undergoing asymmetric division in late third due to innervation from the eye-imaginal disc triggering a round of asymmetric division in the lamina of the optic lobe (See Figure 11). Therefore, a crescent of pros staining should have been seen at some point in late third instar. Overall, while pros is a good marker for some populations of neuroblasts, it is not a universal marker for

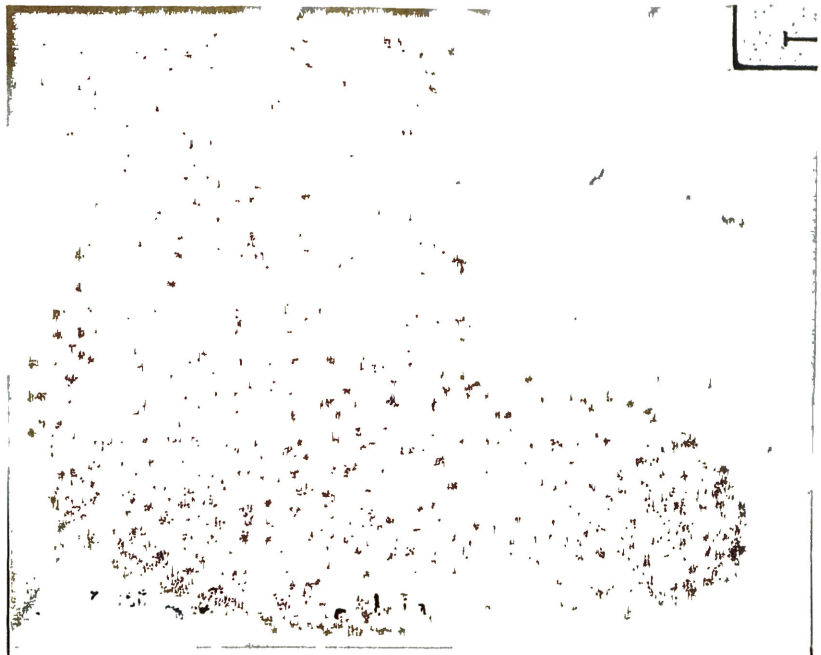

Figure 9: Canton- S brain dissected at the second molt and stained with pros. Thoracic and central brain neuroblasts are actively dividing.

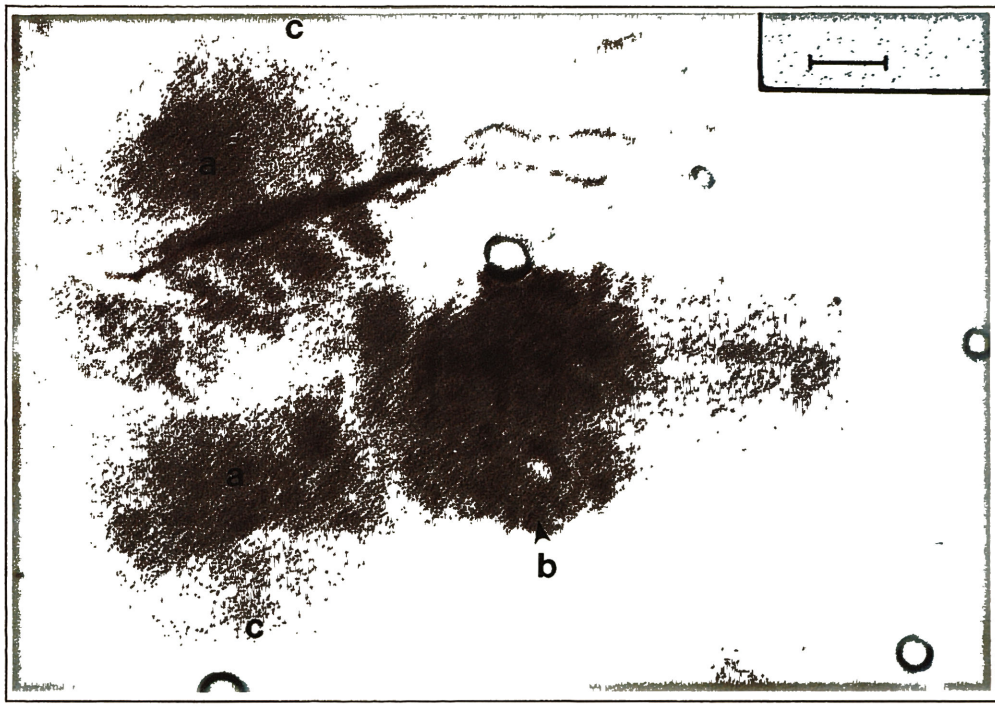

Figure 10: Canton-S brain dissected at 36 hours after the second molt. It is representa tive of late third instar in that the central brain nbs (a) and thoracic nbs (b) are proliferating, but there is no proliferation in the optic lobe.[c]

asymmetric cell division.

That pros does not seem to be a universal marker raised an interesting question. In the embryo, all asymmetrically dividing neuroblasts and Ganglion Mother Cells showed pros, and the localization of pros at mitosis was shown to be the mechanism for rapidly establishing asymmetric cell fate. So, if pros is not responsible for cell fate in the optic lobe neuroblasts, then what is? The results indicate that a new pathway for turning on gene expression in the optic lobe neuroblasts needs to be explored. One such possibility could be numb. Consequently, it will be interesting to see if numb stains the lamina of the optic lobes.

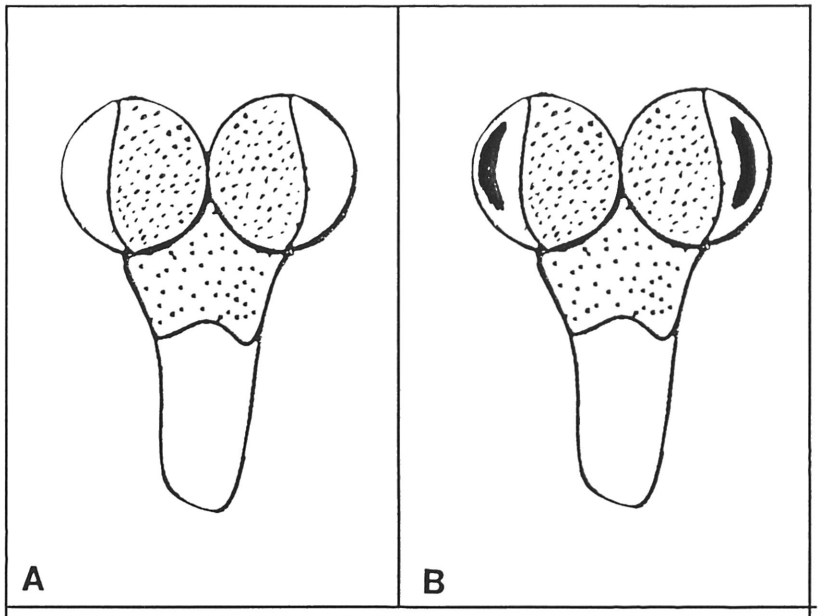

Figure 11: A) Cartoon drawing of actual staining pattern of pros in late third instar. B) Expected staining pattern of pros in late third instar when axons innervate the lamina triggering a round of asymmetric division.

Pros expression in trol^{sd}, ana, and mud

Third instar trol homozygotes do not show pros staining while the control brains show active proliferation and pros staining in the thoracic region. Prior to this, pros staining has only been looked at in the embryo where no neuroblasts are known to be quiescent. In trol mutants, the thoracic neuroblasts are present but are prevented from dividing. Therefore, when division is halted, there is no pros expression which indicates

that pros expression is tightly coupled to cell division. Furthermore, the results indicate that pros and trol may have a common pathway.

Preliminary results indicate that *ana* mutant brains at 26 hours after hatching begin to show pros staining in the thoracic neuroblasts before wild type brains of the same time point. This result is complimentary to those of the trol experiments and gives further indication that pros expression is coupled to cell division since premature proliferation of neuroblasts results in early pros staining.

The experiments with mud are in progress. The results at 4 hours after hatching were inconclusive, so the brains of the mud stocks will be dissected at 2 hours after hatching instead. Again, it will be interesting to see if the proposed extra mushroom body neuroblasts show pros staining.

Conclusions:

Prior to this, prospero expression has only been looked at in the embryo. These experiments implicate that pros also plays a role in asymmetric cell division in the larval central nervous system and would be a good marker for most asymmetric division in the larval stages. However, pros is not a universal marker. Although it is expressed in the thoracic and central brain neuroblasts, it is not expressed in the asymmetrically dividing optic lobe neuroblasts, indicating that another mechanism for establishing cell fate in the optic lobe needs to be explored. Furthermore, staining with *trol* mutants and preliminary staining with *ana* mutants indicate that pros expression is tightly coupled with cell division.

References

Alberts, Bruce; Bray, Dennis; Lewis, Julian; Ratf, Martin; Roberts, Keith; Watson, James D. The Molecular Biology of the Cell, 2nd ed. New York: Garland Publishing Inc., 1989.

Doe, C. Q. and Spana, E. P. (1995). A collection of cortical crescents: Asymmetric protein localization in CNS precursor cells. *Neuron*. 15, 991-995.

Goodman, C.S. and Doe, C.Q. (1993). Embryonic development of the *Drosophila* Central Nervous System. In The Development of Drosophila (eds. M. Bate and A. Martinez-Arias), pp 1331-1206. Cold Spring Harbor, NY: Cold Spring Harbor Press.

Knoblich, J.A., Jan, L.Y., and Jan, Y.N. (1995). Asymmetric segregation of Numb and Prospero during cell division. *Nature*. 377, 624-626.

Lin, H. and Schagat, T. (1997). Neuroblasts: a model for the asymmetric division of stem cells. *Trends in Genetics*. 13, 33-39.

Prokop, A. and Technau, G. M. (1994). Normal function of the *mushroom body* defect gene of *Drosophila* is required for the regulation of the number and proliferation of neuroblasts. Developmental Biology. 161, 321-337.

Spana E.P. and Doe, C.Q. (1995). The prospero transcription factor is asymmetrically localized to the cell cortex during neuroblast mitosis in *Drosophila*. *Development* 121, 3187-3195.

Spana, E. P. Kopczynski, C. Goodman, C. S., and Doe, C. Q. (1995). Asymmetric localization of numb autonomously determines sibling neuron identity in the *Drosophila* CNS. *Develpoment.* 121, 3489-3494.