

Different Daughters: Regulation of Asymmetric Cell Division

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Development of a metazoan organism requires the tightly regulated coordination of cell proliferation, apoptosis, migration and differentiation. After the initial formation of the zygote, cell proliferation predominates as the organism forms a mass of cells from which the embryonic tissues can differentiate. Cell division was long considered to simply represent a mechanism of proliferation, with a parent cell dividing to form two identical daughters which, depending upon signals received, could either continue to proliferate or differentiate. It has also been known for many years, however, that cell division can in itself result in different progeny. Recently there has been a focus on the mechanisms that underlie this asymmetric division.

Single-celled organisms can divide asymmetrically

Saccharomyces cerevisiae, or budding yeast, is a single-celled eukaryotic organism that has been extensively utilised for genetic studies of cell division. The division of *S. cerevisiae* appears to be asymmetric due to the timing of cytokinesis. During mitosis the mother cell segregates a copy of the genome into a bud which undergoes cytokinesis and forms a new yeast cell before it has reached the size of the mother cell. This unequal division is due to placement of the mitotic spindle relative to the bud site, and hence segregation of unequal amounts of cytoplasm by cytokinesis into the mother and daughter cells (1). This in itself does not produce different progeny but another aspect of yeast biology indicates that cell division does produce an asymmetric outcome.

Yeast cell division can produce genetically different daughter cells

S. cerevisiae propagate as haploid cells but can produce diploids by fusion of two different mating-types, α and a . Yeast cells switch mating-type through a genetic recombination event that is dependent on the HO endonuclease, but this event is restricted to the mother cell after cell division. The restriction of competence to undergo mating-type switching is due to HO being transcribed only in the mother cell and not the daughter cell (1). This in turn is due to asymmetric inheritance of a nuclear factor, Ash1p, into the daughter nucleus prior to cytokinesis. Ash1p represses transcription of HO and thereby prevents mating-type switching. As may be expected, in *ash1* mutant cells both mother and daughter cells can undergo switching whereas neither can in cells that overexpress Ash1p (2). The mechanism of Ash1p localisation relies upon the highly polarised cytoskeleton of budding mother cells. Ash1p localisation requires polarised actin filaments and activity of a type V myosin motor, Myo4. Interestingly, it is the *ASH1* mRNA that is specifically localised to the distal tip of the growing bud in post anaphase cells (2). This results in the asymmetric cellular localisation of Ash1p in the daughter cell following cytokinesis.

Intrinsic factors can be specifically localised to produce asymmetric division

Similar examples of specific mRNA localisation resulting in different cell progeny have been identified in metazoan organisms. For example, the highly polarised microtubule cytoskeleton of the early syncytial *Drosophila melanogaster* embryo results in posterior localisation of *oskar* mRNA. The first cells to form in the syncytial embryo are the presumptive germ cells, or pole cells, at the posterior pole of the embryo. Oskar protein functions to recruit other mRNAs and proteins critical for germ cell development into the budding pole cells (3).

Intrinsic asymmetry associated with cell division has been intensively studied in another *Drosophila* cell type, the embryonic neuroblast. Neuroblasts divide to regenerate another neuroblast and a ganglion mother cell (GMC), which undergoes a subsequent division to produce two neurons. The neuroblast division is thus asymmetric and resembles a stem cell division as it regenerates a neuroblast and also produces a differentiated daughter (4). This division mode is transient, however, so embryonic neuroblasts are more properly referred to as progenitor cells.

Cortical localisation of asymmetric determinants specify ganglion mother cell fate

There are two key features of cell division that are associated with the intrinsic control of asymmetry. The first is the asymmetric localisation of cell fate determinants prior to cell division, and the second is a tight control of the orientation of the mitotic spindle to generate a plane of cell division that allows segregation of the asymmetric determinants to just one daughter cell.

In *Drosophila* neuroblasts the cell fate determinants that specify GMC fate are Numb and Prospero. Numb is a cytoplasmic protein that functions to repress Notch signalling, while Prospero is a transcription factor that enters the GMC nucleus after cell division to activate transcription of GMC-specific genes and repress neuroblast-specific genes. The adaptor proteins Miranda, Partner of Numb (PON) and Staufén (Fig. 1A) facilitate segregation of the GMC determinants. The adaptor proteins localise the cell fate determinants in a cortical basal crescent during neuroblast division. Staufén is an RNA-binding protein and causes cortical localisation of *Prospero* mRNA, alongside PON-directed Prospero protein. Mutagenesis experiments identified another group of proteins important for GMC specification. In *inscuteable* mutants the crescent of GMC determinants forms but is not correctly segregated into the GMC. This is because the orientation of the plane of neuroblast division is randomised. The cell fate determinants must localise in a cortical crescent that overlies a mitotic spindle pole. Therefore for correct segregation the neuroblast must divide in a particular direction, orthogonal to the plane of the neuroepithelium (or perpendicular to the apical-basal axis).

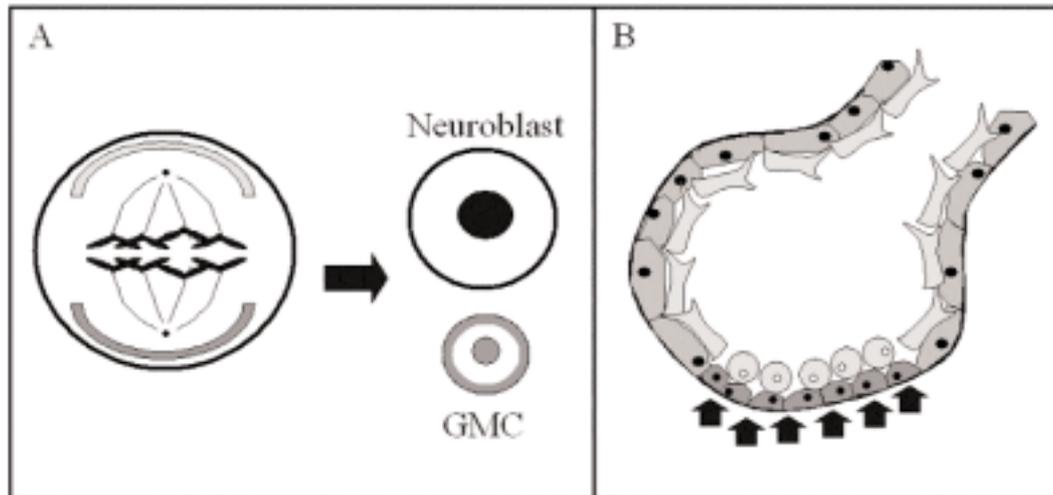


Fig. 1. Models of extrinsic and intrinsic regulation of asymmetric cell division.

A. Intrinsic control of cell division is observed in *Drosophila* neuroblasts. Neuroblast polarity and mitotic spindle orientation are organised by a complex of proteins containing Inscuteable, Partner of Inscuteable and $G_{\alpha i}$ localised in an apical cortical crescent (light grey). The cell fate determinants Prospero and Numb are localised along with Miranda, Partner of Numb (PON) and Staufien in a basal cortical crescent (dark grey). This ensures inheritance of Numb and Prospero into the GMC daughter cell.

B. Extrinsic control in the stem cell niche hypothesis suggests that cell-cell interactions or short-range signals (arrows) maintain cells as stem cells (round, light grey cells). Daughters that move away from the niche receive different signals and differentiate.

A correct division plane requires apical cortical localisation of Inscuteable, Partner of Inscuteable and the $G_{\alpha i}$ subunit of heterotrimeric G proteins (Fig. 1A). In turn, localisation of this complex requires a set of proteins (Bazooka, PAR-6, atypical protein kinase C) that confer apico-basal polarity to the neuroepithelium prior to neuroblast delamination, and hence polarity to the neuroblast (4).

Intrinsic control of asymmetry is conserved in mammalian neurogenesis

Neuroepithelial progenitor cells in the E13 mouse cortex undergo repeated asymmetric divisions to generate both neurons and glia. A mouse homologue of *Drosophila* Numb, m-Numb, is expressed in the progenitor cells and shows both cortical localisation and asymmetric segregation into daughter cells. Recent experiments in cultured progenitor cells isolated from the embryonic mouse cortex, and therefore removed from environmental cues, have shown that when progenitor cells divide asymmetrically to produce another progenitor and a neuron, m-Numb segregates into the neuronal daughter (5). Other experiments have shown that the situation is not as simple as first proposed. In both *Drosophila* and the mouse there is now evidence that the primary function of Numb may not be as a cell fate determinant but that the differential segregation of Numb causes daughter cells to respond differently to extrinsic cues (5).

Intrinsic or extrinsic control of asymmetry?

Genetic and cell biological experiments from *C. elegans* and *Drosophila* have indicated that intrinsic factors play an important role in cell division asymmetry (4). However, it has also been determined that extrinsic factors are crucial for determination of cell fate. For example, changes in growth factors in media can dramatically alter the fate of differentiating embryonic

stem (ES) cells. How then are we to explain the regulation of mammalian stem cell division? Models suggest that stem cells divide in an asymmetric manner to regenerate themselves and produce a daughter that is committed to differentiation, usually after a period of mitotic expansion. Stem cells can also divide symmetrically, however, as exemplified by growth *in vitro* of ES cell cultures. In this case, symmetric division of ES cells *in vitro* is dependent upon exogenous factors, e.g. LIF, suggesting that *in vivo*, stem cell division patterns may depend upon tight control of exposure to environmental cues (6). Such control could be exerted by the physical environment of stem cells, the so-called stem cell niche hypothesis.

The stem cell niche

Recent findings have shown that in many cases stem cells are capable of populating heterologous tissues and contributing to tissue regeneration. This has led to a questioning of the importance of intrinsic control mechanisms in stem cell division as intrinsic cell fate determinants associated with specialised cell types may be superseded by external cues from a new environment. The nature of stem cell regeneration and production of differentiated progeny may be primarily regulated by the neighbouring cells and matrix *in vivo*. This has led to the concept of the stem cell niche, a stable microenvironment where defined signals and cell-cell interactions control stem cell behaviour (Fig. 1B). Two candidate niches will be discussed here: the invertebrate *Drosophila* testis and the vertebrate endodermal intestinal crypt, along with methodologies for studying stem cell behaviour in each.

The *Drosophila* testis

Identification of stem cell niches requires tissues where stem cells and surrounding soma can be detected and effects of cell division observed. The *Drosophila* testis is one such

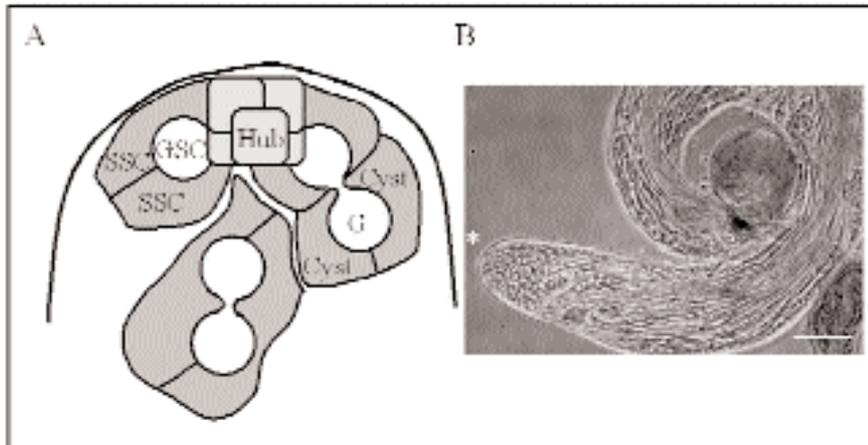


Fig. 2. The *Drosophila* testis stem cell niche.

A. The apical testis region showing close association of the germline stem cells (GSC) and somatic stem cells (SSC) with the hub cells. Stem cell division produces differentiated daughters – the germline spermatogonial cell (G) and cyst cells. **B.** Phase-contrast micrograph of an adult *Drosophila* testis. The apical region is marked with an asterisk. Scale bar is 100 μ m.

case. As in most animals, *Drosophila* adult males produce sperm originating from germline stem cells throughout their lifetime. In adult *Drosophila* males some 5-9 germline stem cells can be found in the apical region of the testis surrounding a group of tightly packed somatic cells known as the hub (7). Hub cells secrete a ligand, Unpaired, that acts through a cytokine family receptor, Domeless, to stimulate JAK-STAT signalling in the germline stem cells. STAT activation has been shown to be required for germline stem cell survival, and ectopic stimulation of the pathway can lead to stem cell overproliferation (8). A second type of stem cell is also found in the apical testis. Each germline stem cell is closely associated with a pair of somatic stem cells. When a germline stem cell divides the somatic stem cells also divide. This produces a germline stem cell daughter committed to differentiation (a spermatogonial cell) surrounded by a pair of cyst cells, the progeny of the somatic division (Fig. 2). One role of the cyst cells is to limit the proliferative capacity of the spermatogonial cells (9). The signals that regulate somatic stem cell proliferation are not known but at least some signals are known to originate in the germ cells (10). Signalling molecules that regulate germline and somatic stem cells may include Hedgehog, Wingless, Notch, EGF receptor - all of which are known to be expressed in the apical testis (8,9 and G. Hime, unpublished observations). The presence of these factors suggests that a combination of signals regulate stem cell proliferation and differentiation in the testis stem cell niche.

Genetic modulation of stem cell proliferation

The genetic tools available in *Drosophila* make the testis stem cell niche an excellent model for discovering the signals that control stem cell division. Expression profiling by enhancer trapping and microarray analysis has identified many genes that are expressed in the testis. One of the problems associated with genetic analyses of stem cell niches is that they exist in adult organs or late forming organs in the embryo. This means that mutation of many genes that may play a role in controlling stem cell division will result in embryonic lethality well before formation of the stem cell niche. The identification of these genes has been assisted by the recently developed methods of ectopic functional screening. The bipartite GAL4-UAS expression system has

allowed tissue specific expression of transgenes via the yeast transcriptional activator, GAL4 (11). Insertion of the GAL4 recognition site, UAS, onto transposable elements has allowed generation of libraries of genetic strains each with the UAS element adjacent to a different gene (12). By mating a strain carrying a testis specific GAL4 to a UAS strain genes can be rapidly screened by ectopic expression in the testis.

The mammalian intestinal crypt

Adult male mammals also produce sperm throughout their lifetime but a number of other organs are continuously regenerated in the adult animal. These include hair, blood, skin and the lining of the intestinal tract. In the intestine, regeneration occurs in small invaginations of the epithelium known as intestinal crypts (Fig. 3). These crypts bear some similarity to the *Drosophila* testis but also differ in important ways. The stem cells reside within the polarised epithelial monolayer but cannot be identified morphologically or by any set of molecular markers. The intestinal stem cells are multipotent, giving rise to columnar epithelial cells, mucin-secreting goblet cells, enteroendocrine cells and Paneth cells (13,14). Lineage tracing experiments suggest that a small number of stem cells reside in the basal epithelium of the crypt (15). As cells proliferate and differentiate they move up the crypt walls until they are finally shed into the lumen. Many questions remain as to how intestinal stem cell division is regulated. Planar signals may be received by the stem cells from other cells in the epithelium or signals may be sent from the underlying stroma (14). As for the *Drosophila* testis, the intestinal crypts form late in development and regenerate throughout adult life. Thus, conditional knockout or highly targeted transgenesis experiments must be employed to study stem cell regeneration and differentiation *in vivo*.

Introduction of genes into cultured gut by electroporation

Low voltage electroporation is a recent development that allows genetic modulation of vertebrate tissues without the time and expense involved in producing transgenic animals (16). Traditional methods of electroporation of cultured cells result in high levels of cell death. This is obviously unacceptable for insertion of genes into complex living tissues. New methods of electroporation utilise low voltages and multiple square pulse waves to allow the introduction

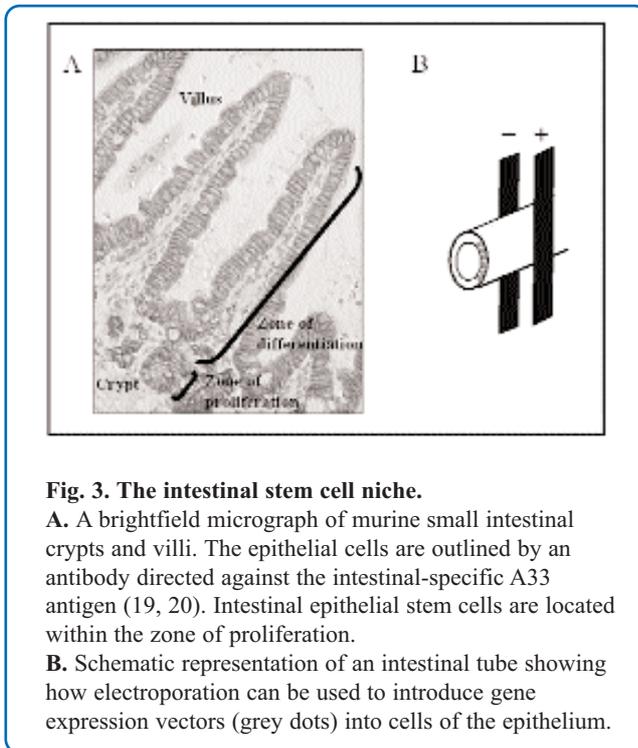


Fig. 3. The intestinal stem cell niche.

A. A brightfield micrograph of murine small intestinal crypts and villi. The epithelial cells are outlined by an antibody directed against the intestinal-specific A33 antigen (19, 20). Intestinal epithelial stem cells are located within the zone of proliferation.

B. Schematic representation of an intestinal tube showing how electroporation can be used to introduce gene expression vectors (grey dots) into cells of the epithelium.

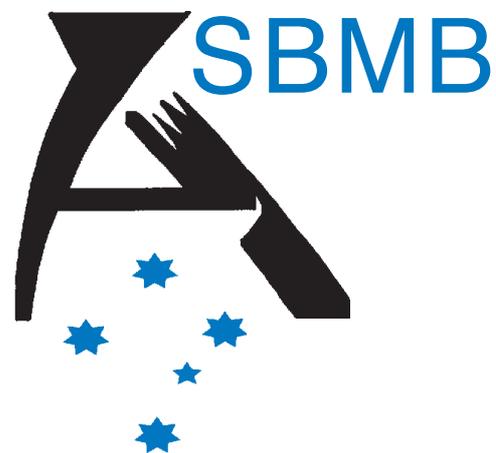
and expression of DNA constructs without significant cell death. This method has been used successfully *in vivo* in both mouse and chick embryos to introduce genes into the neural tube (16,17). Tissues with lumens, such as neural tube and gut are particularly amenable for electroporation as the DNA can be injected into the lumen and a voltage differential placed across the tissue. The voltage differential allows the directional entry of DNA into the cell layer lining one side of the tube, with the other serving as an untransfected control (**Fig. 3**). Combination of electroporation with methods for organ culture of embryonic murine gut has facilitated gene transfer into a tissue with otherwise difficult accessibility. Furthermore, identification of physiologically relevant regulators of intestinal epithelial stem cell proliferation and differentiation are more likely to be uncovered in a system that maintains the *in vivo* relationship of cells and surrounding tissues. Long-term study of cell regeneration can also be accomplished by transplantation of tissues that express transgenes back into adult mice for growth under the kidney capsule (18). Thus, short and long term consequences of altering gene expression in the intestinal epithelium can be observed (H. Abud and J.K. Heath, unpublished observations).

Do stem cells undergo asymmetric division?

Although much excitement has been generated about the possible therapeutic uses of stem cells we still have a lot to learn about how they behave *in vivo*. We do not know if different stem cells divide asymmetrically – possibly mammalian neuroblasts have some form of intrinsic regulation – or whether the stem cell niche directs the fate of daughter cells. A combination of vertebrate and invertebrate genetics and cell biology should provide clues to how these complex but vital forms of cell division and differentiation are regulated.

References

- Herskowitz, I. (1988) *Microbiol. Rev.* **52**, 536-553
- Chang, F., and Drubin, D.G. (1996) *Curr. Biol.* **6**, 651-654
- Riechmann, V., and Ephrussi, A. (2001) *Curr. Opin. Genet. Dev.* **11**, 374-383
- Knoblich, J.A. (2001) *Nature Rev. Mol. Cell Biol.* **2**, 11-20
- Shen, Q., Zhong, W., Jan, Y.N., and Temple, S. (2002) *Development* **129**, 4843-4853
- Williams, R.L., Hilton, D.J., Pease, S., Willson, T.A., Stewart, C.L., Gearing, D.P., Wagner, E.F., Metcalf, D., Nicola, N.A., and Gough, N.M. (1988) *Nature* **336**, 684-687
- Fuller, M.T. (1993) in *The Development of Drosophila melanogaster*, M. Bate, and A. Martinez Arias, editors. Cold Spring Harbor Laboratory Press, New York. Vol 1, 71-147
- Kiger, A.A., Jones, D.L., Schulz, C., Rogers, M.B., and Fuller, M.T. (2001) *Science* **294**, 2542-2545
- Kiger, A.A., White-Cooper, H., and Fuller, M.T. (2000) *Nature* **407**, 750-754
- Gonczy, P., and DiNardo, S. (1996) *Development* **122**, 2437-2447
- Brand, A.H., and Perrimon, N. (1993) *Development* **118**, 401-415
- Toba, G., Ohsako, T., Miyata, N., Ohtsuka, T., Seong, K.H., and Aigaki, T. (1999) *Genetics* **151**, 725-737
- Montgomery, R.K., Mulberg, A.E., and Grand, R.J. (1999) *Gastroenterology* **116**, 702-731
- Clatworthy, J.P., and Subramanian, V. (2001) *Mech. Dev.* **101**, 3-9
- Bjerknes, M., and Cheng, H. (1999) *Gastroenterology* **116**, 7-14
- Itasaki, N., Bel-Vialar, S., and Krumlauf, R. (1999) *Nature Cell Biology* **1**, E203-E207
- Saito, T., and Nakatsuji, N. (2001) *Developmental Biology* **240**, 237-246
- Zorbas, M., Sicurella, C., Bertonecello, I., Venter, D., Ellis, S., Mucenski, M.L., and Ramsay, R.G. (1999) *Oncogene* **18**, 5821-5830
- Johnstone, C.N., Tebbutt, N.C., Abud, H.E., White, S.J., Stenvers, K.L., Hall, N.E., Cody, S.H., Whitehead, R.H., Catimel, B., Nice, E.C., Burgess, A.W., and Heath, J.K. (2000) *Am. J. Physiol.* **279**, G500-G510
- Abud, H.E., Johnstone, C.N., Tebbutt, N.C., and Heath J.K. (2000) *Mech. Dev.* **98**, 111-114



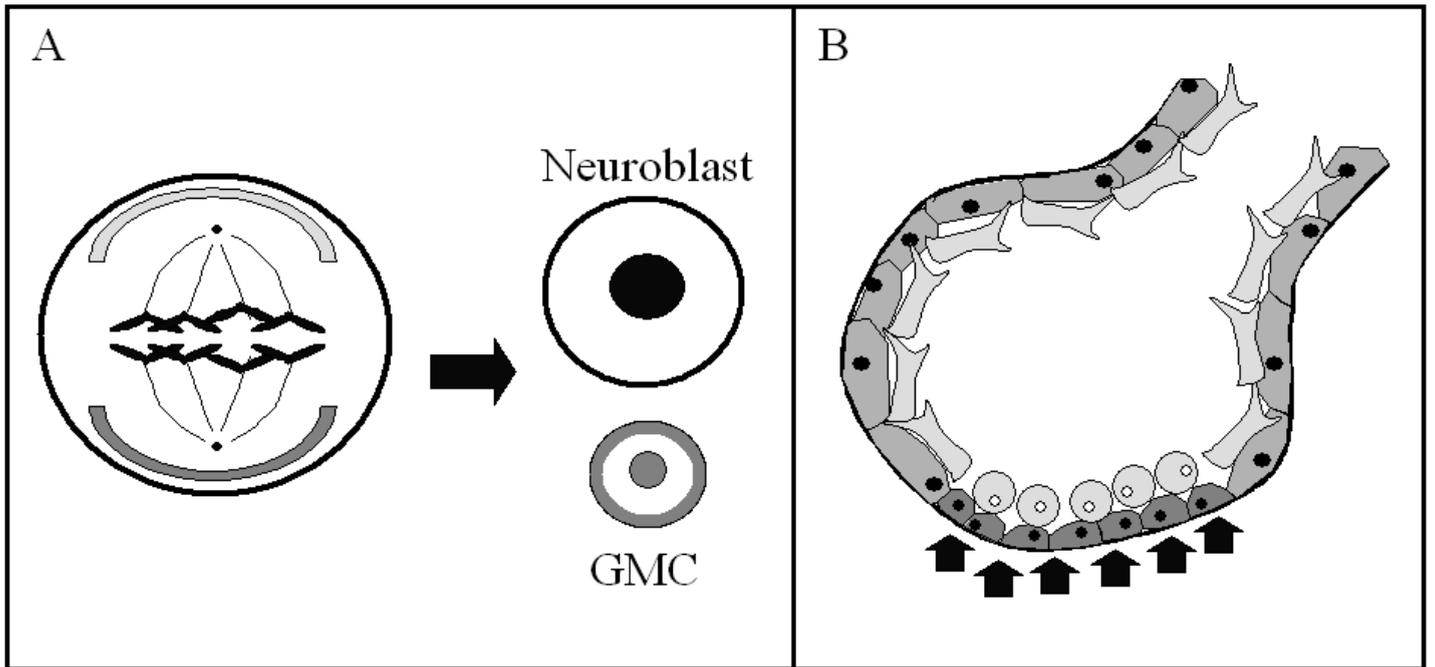


Fig. 1

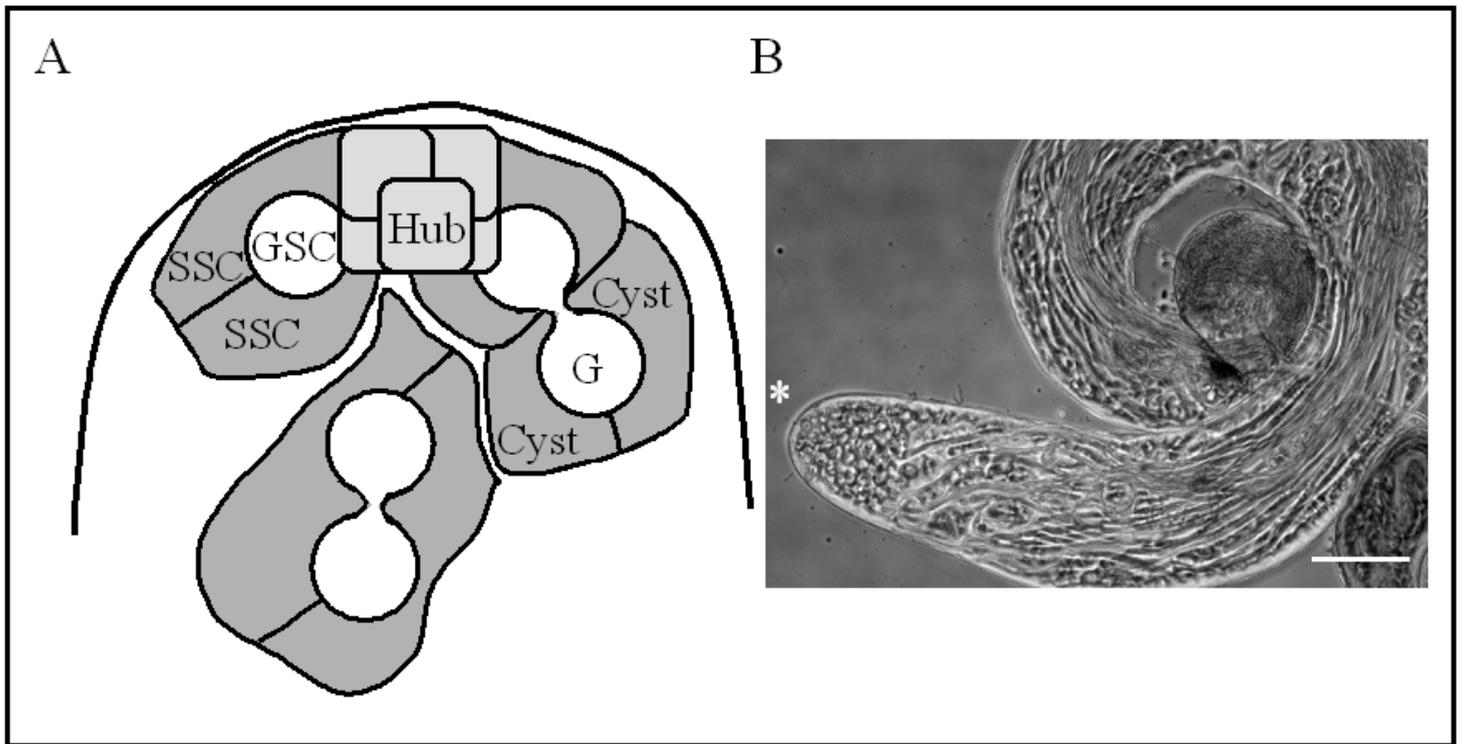


Fig. 2

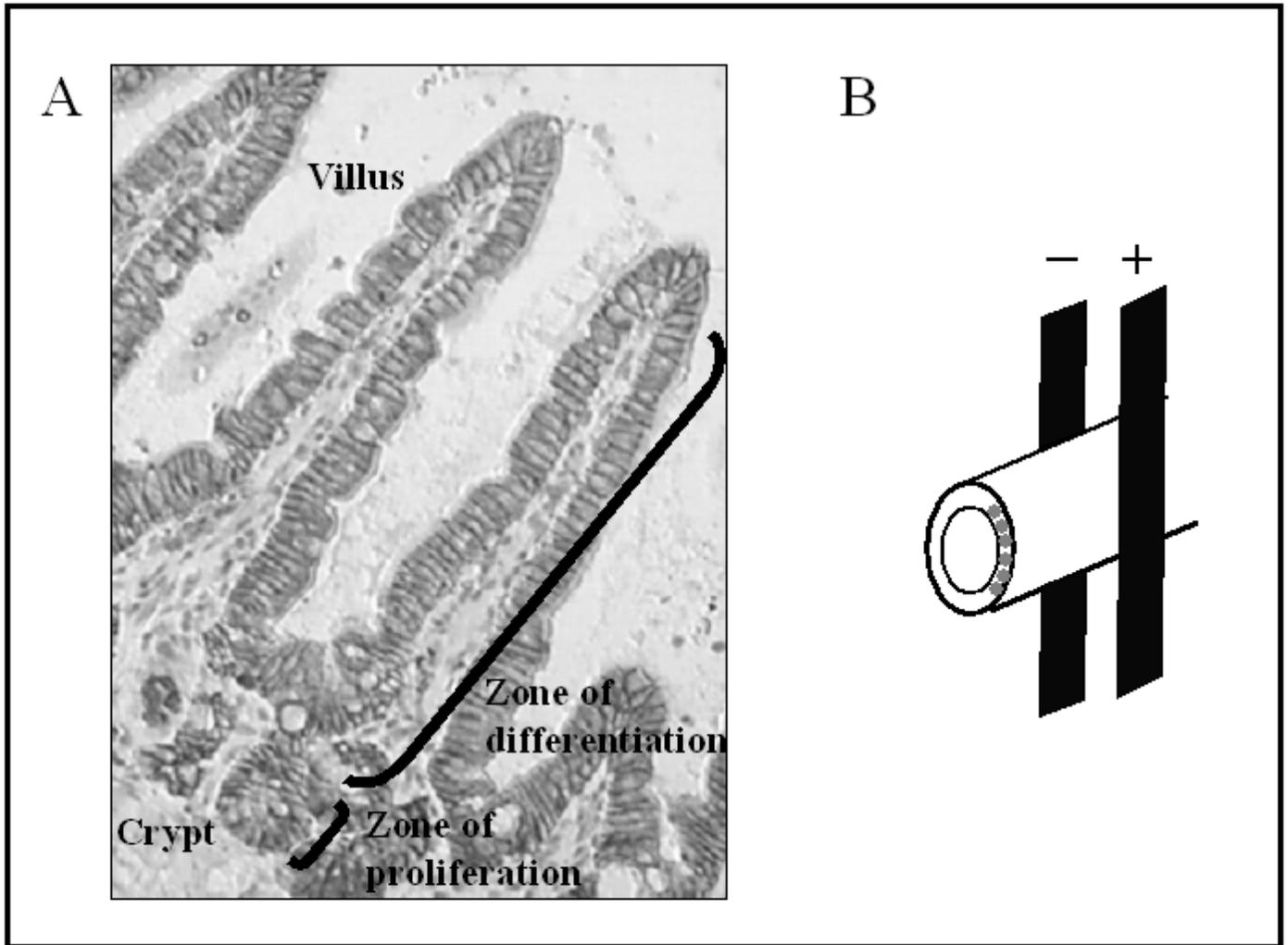


Fig. 3