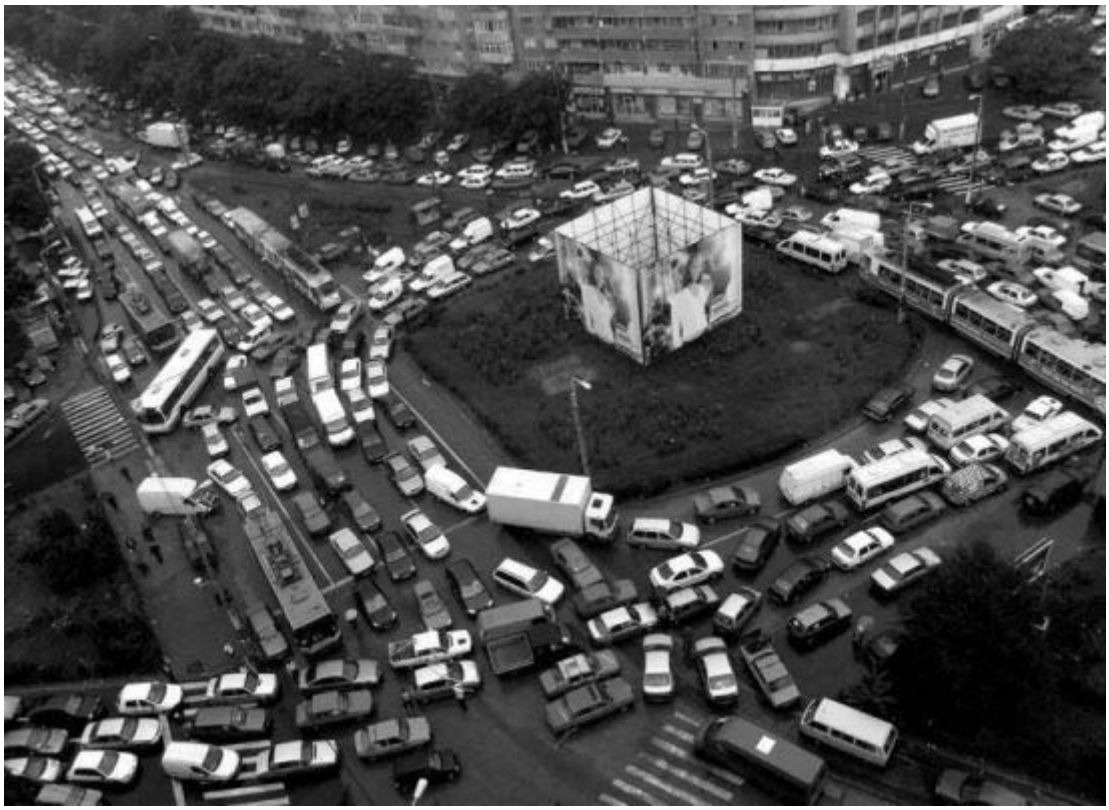
Endocytic control of tumor suppression in *Drosophila melanogaster*

"How a trafficking defect causes tumor formation"



By: Geert de Vreede
Master Thesis in Cancer, Genomics and Developmental Biology
Under the supervision of Catherine Rabouille

Contents

Abstract

Mutations in *Drosophila melanogaster* tumor suppressor genes are able to cause neoplastic overgrowth in various epithelial tissues. Screens in *Drosophila* showed that a multitude of components from the endocytic pathway act as tumor suppressors. This suggests that the process of endocytosis plays an essential role in suppressing neoplastic tumor formation in *Drosophila*. The question is how exactly the perturbation of endocytosis leads to the rise of epithelial neoplasms. Through assessing the endocytic genes that have a tumor suppressing function and highlighting the role of endocytosis in cell growth signaling, asymmetric cell division, and polarity, I will discuss how a defect in this pathway can be responsible for tumorigenesis. Understanding how different cellular processes are affected by aberrant endocytosis is pivotal to unravel the mechanisms behind *Drosophila* tumor formation and strengthens its use as a model for human cancer.

Introduction

Drosophila tumor suppressors

Modeling cancer processes is pivotal to aid our understanding of this complex disease. Cancer cell lines, mouse and zebrafish models prove to be valuable in answering questions about the biology behind these processes. The fruit fly *Drosophila melanogaster* also belongs to the arsenal of model systems. Being an invertebrate organism, at first glance it appears to be somewhat distant to its vertebrate counterparts to display the cancer processes that are considered as classical hallmarks. However, tumorous growths have been observed in both larval and adult tissue of *Drosophila* and even metastatic behavior can be seen. Although these fly tumors cannot be regarded as direct analogs of the human versions, the genes and biological processes involved are often similar. A big advantage of *Drosophila* is that the genetic wiring for a pathway is less complex than its mammal equivalent, since the fruit fly often uses one gene for a step in the cascade were mammals have evolved multiple subtypes for that gene which are, for example, tissue or timing specific. This simplicity makes a *Drosophila* model easier to understand, while still being informative about the situation in more complex organisms.

The first investigations in *Drosophila* tumors led to the identification of a selection of tumor suppressor genes (Bryant and Schubiger 1971; Gateff and Schneiderman 1969). Mutations in these genes caused tumorous growths in several *Drosophila* tissues, most notably the epithelial imaginal discs and neural tissue. The larval imaginal discs are paired clusters of epithelial cells which will develop into parts of the adult fly during pupation, for example the wing, eyes or halteres. Since the growth of these discs is under tight regulation, a defect in growth regulation is likely to affect this specific tissue. Not surprisingly, most tumor suppressor genes that were identified in the initial screens were causing imaginal disc tumors.

The fly imaginal discs tumors are subdivided into two separate classes: the 'hyperplastic' and 'neoplastic' tumors (Reviewed in Hariharan and Bilder 2006). Hyperplastic tumors show overproliferation of the epithelial cells, however the cells retain their characteristic shape, have ability to differentiate and maintain their organization in an epithelial monolayer. These tumors display no invasive properties when they are transplanted into wild type adults. The neoplastic mutant cells on the other hand, show a change in cell shape from polygonal to round, lose the capability to differentiate and have disrupted polarity. The normal cellular organizations level of an epithelial monlayer is lost by cells piling on top of each other. When these neoplastic tumors are transplanted into wild type adults, the cells have the capability to invade other tissues. Here we will focus more on the neoplastic tumors, since they share characteristics with human neoplasms. It must be noted though, that there is a clear distinction between human and fly tumor suppressor genes. The *Drosophila* genome encodes a multitude of homologs to known human tumor suppressors like for instance p53 and Rb (Sutcliffe, Korenjak, Brehm 2003), however the mutations in these genes do not generate tissue overgrowths in the fly. These genes are therefore not considered fly tumor suppressors.

Lethal Giant Larvae, Discs Large and Scribble

The first group of neoplastic tumor suppressor genes to be characterized were the junctional scaffold proteins *Lethal Giant Larvae (lgl)*¸ *Discs Large (dlg)* and *Scribble (scrib)*. Although these genes were discovered at different times (Bilder and Perrimon 2000; Stewart, Murphy, Fristrom 1972; Bridges and Brehme, 1944), they share a similar mutant phenotype, cellular localization and genetically

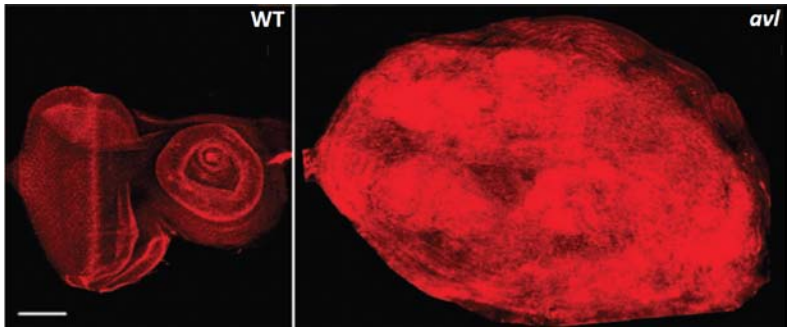


Figure 1:
Example of tumorous growth in an endocytic mutant. A wild type and avalanche (avl) mutant eye imaginal disc have been stained for actin. The avl mutant displays overgrowth and loss of organization. Figure adopted from Lu and Bilder, 2005. Scale bar: 100 µm.

interact with each other (Bilder, Li, Perrimon 2000). The three genes also have in common that their maternal product contribution is strong, which allows the homozygous mutant animals to develop into the third instar (L3) larval stadium. Wild type animals at this point in development form pupa, however the *lgl*, *dlg* and *scrib* mutants continue to grow and become 'giant larvae'. In these animals the imaginal epithelia and neural tissue do not cease to proliferate and form neoplastic tumors (Bilder, Li, Perrimon 2000).

The primary defect causing this mutant phenotype is the loss of polarity. When assessing the mutant tissue for polarity markers, it becomes clear that the apical domain of the epithelial cells is expanded. Together with the fact that all three proteins associate with the cellular junctions just basolateral of the apical domain, this suggests that the mutants lose the ability to restrict apical determinants (Bilder, Li, Perrimon 2000). The apical domain of epithelial cells is controlled by the PAR and Crumbs protein complexes. Defining the baseloteral domain, *lgl, dlg* and *scrib* are considered the third major polarity module: the *scrib* module. The loss of the *scrib* polarity module triggers numerous defects including junctional instability, aberrant proliferation and failure to differentiate. However the molecular mechanisms through which these junctional scaffold proteins acted as tumor suppressors remained unclear. This instigated further research into the factors controlling *Drosophila* tumor suppression.

Tumor suppressor screens

To shed more light on the function of *Drosophila* neoplastic tumor suppressors, a number of screens were performed. The strong maternal product of the proteins in the *scrib* module allowed for a clear phenotype in the L3 larval stage. However to find more tumor suppressors, simply applying mutagenesis and screening for the 'giant larvae' phenotype would not suffice, since mutations in genes with a weaker maternal contribution or requirement in early development would not produce a phenotype. To circumvent this problem, screening based on mitotic recombination was utilized. Using the FLP/FRT system in an animal that is heterozygous mutant, it is possible to drive recombination in dividing cells, generating a wild type and a homozygous daughter cell after division (Tapon *et al* 2001)). This creates patches of mutant clones. Because the FLP/FRT system can be driven tissue specific, it is possible to create clones only within a tissue of interest, like the imaginal discs or the follicular epithelium. Through this method it is possible to screen for genes that cause phenotypes in larval and adult tissue while mutant animals would normally not survive up to those stages. The mutant clones can be examined for tumorous growth, epithelial disorganization and polarity disruption.

Endocytic genes involved in tumor suppression

Several screens for novel *Drosophila* tumor suppressors were conducted using mitotic recombination based techniques. Though these screens were performed by different institutes and were set up with an unbiased approach, remarkably the results they yielded all pointed to a specific group of genes that acted as tumor suppressors. The genes involved were all part of the endocytic trafficking machinery. Specifically, the genes found in the screens were *avalanche* (a syntaxin, Figure 1), *Rab5* (a small GTPase associated with early endosomes), *vps25* (a component of the ESCRT-II complex) and *erupted* (the *Drosophila* ortholog of mammalian tumor susceptibility gene 101, a component of the ESCRT-I complex) (Lu and Bilder 2005; Moberg *et al* 2005; Thompson *et al* 2005; Vaccari and Bilder 2005). In a later stage, the *Drosophila* dynamin *shibire*¸ the syntaxin binding protein *vps45,* the Rab5 effector *Rabenosyn* and several other ESCRT components were added to this list (Herz *et al* 2009; Morrison *et al* 2008; Vaccari *et al* 2008; Vaccari *et al* 2009). Not only are all these components involved in the endocytic process, they are also associated with different steps within the endocytic route. This indicates that the entire process of endocytic trafficking is pivotal for tumor suppression. Strengthening of this theory, soon after the first endocytic genes in *Drosophila* were connected to tumor suppression, a number of known *Drosophila* tumor suppressor genes were linked to endocytosis. This included the genes *lethal giant discs (lgd), merlin* and *expanded* (Childress *et al* 2006; Jaekel and Klein 2006; Maitra *et al* 2006).

The question arises through what mechanisms the endocytic trafficking pathway affects tumor suppression. The process of internalizing parts of the plasma membrane and trafficking them to internal compartments does not intuitively link to a tumor suppressive function. The aim of this thesis will be to assess the different cellular aspects that link endocytosis to tumor suppression. This will gain more insight on how endocytosis performs its tumor suppressive role and how blocking different parts of the endocytic pathway leads to the formation of neoplasms in *Drosophila melanogaster.*

Aim

The aim of this thesis is to answer the question as to how the endocytic trafficking pathway acts as a tumor suppression mechanism. I will discuss several cellular processes that have the potential to provide the key to connect endocytosis and tumorigenesis. Before that, I will give a brief overview of the process of endocytosis and the factors involved.

I. Endocytosis

Endocytosis entails the process through which the cell takes up molecules from the extracellular medium or residing at the plasma membrane into vesicles budding out from the plasma membrane. These molecules are transported into different cellular compartments of the endosomal/lysosomal system. The endocytic process involves a multitude of steps (Figure 2) and here I will briefly discuss them, with emphasis on those which involve *Drosophila* tumor suppressor genes.

1. Initial internalization

Endocytosis starts with the ingestion of plasma membrane (PM) parts leading to the formation of vesicles (Figure 2, Step 1). There are numerous known mechanisms for the initial internalization (Reviewed in Doherty and McMahon 2009), although the most common and well studied is the formation of clathrin-coated pits. Clathrin-coated vesicles transport components from the PM to endosomal compartments and are formed by clathrin combined with adaptor protein complexes. The structure of clathrin enables the formation of a basket around the piece of membrane that they enclose. The adaptor proteins bind both clathrin and transmembrane proteins, which can be, for instance, (activated) receptor proteins. There are different types of these adaptor protein complexes in the cell, although the main adaptor complex associated with vesicles budding from the PM is the AP2 complex (Reviewed in Hirst and Robinson 1998).

The pinching-off of the clathrin-coated bud is regulated by dynamin (*shibire* in *Drosophila*). Dynamin assembles around the neck of the bud, and promotes pinching-off by locally destabilizing the lipid bilayer (Damke *et al* 1994; Gammie *et al* 1995; Takei *et al* 1995). Interestingly, imaginal discs that are mutant for *shibire* develop neoplastic tumors (Vaccari *et al* 2008).

2. Early endosomes

The first compartment that vesicles budded from the PM encounter is the early endosome (Figure 2, Step 2). Early endosomes and the clathrin coated vesicles transported towards them are marked by the Rab5 GTPase. Rab5 ensures correct transport of the cargo by recruiting effectors that facilitate transport, membrane tethering and docking on both the sending and the receiving end (Reviewed in Grosshans, Ortiz, Novick 2006). Examples of those effectors are *rabenosyn* and *vps4*5. The exact endocytic function of Rabenosyn is unknown though it is needed to mediate the interaction between Rab5 and *vps45* (Morrison *et al* 2008). *Vps45* is a syntaxin binding protein. Syntaxins are part of the SNARE complexes that facilitate vesicle fusion (Reviewed in Jahn, Lang, Sudhof 2003). The *Drosophila* syntaxin *avalanche* specifically localizes to the early endosomes and physically interacts with *vps45* (Lu and Bilder 2005; Morrison *et al* 2008). *Drosophila* epithelial tissue mutant for either *Rab5, Rabenosyn, vps45* or *avalanche* displays neoplastic transformation and overgrowth (Lu and Bilder 2005; Morrison *et al* 2008).

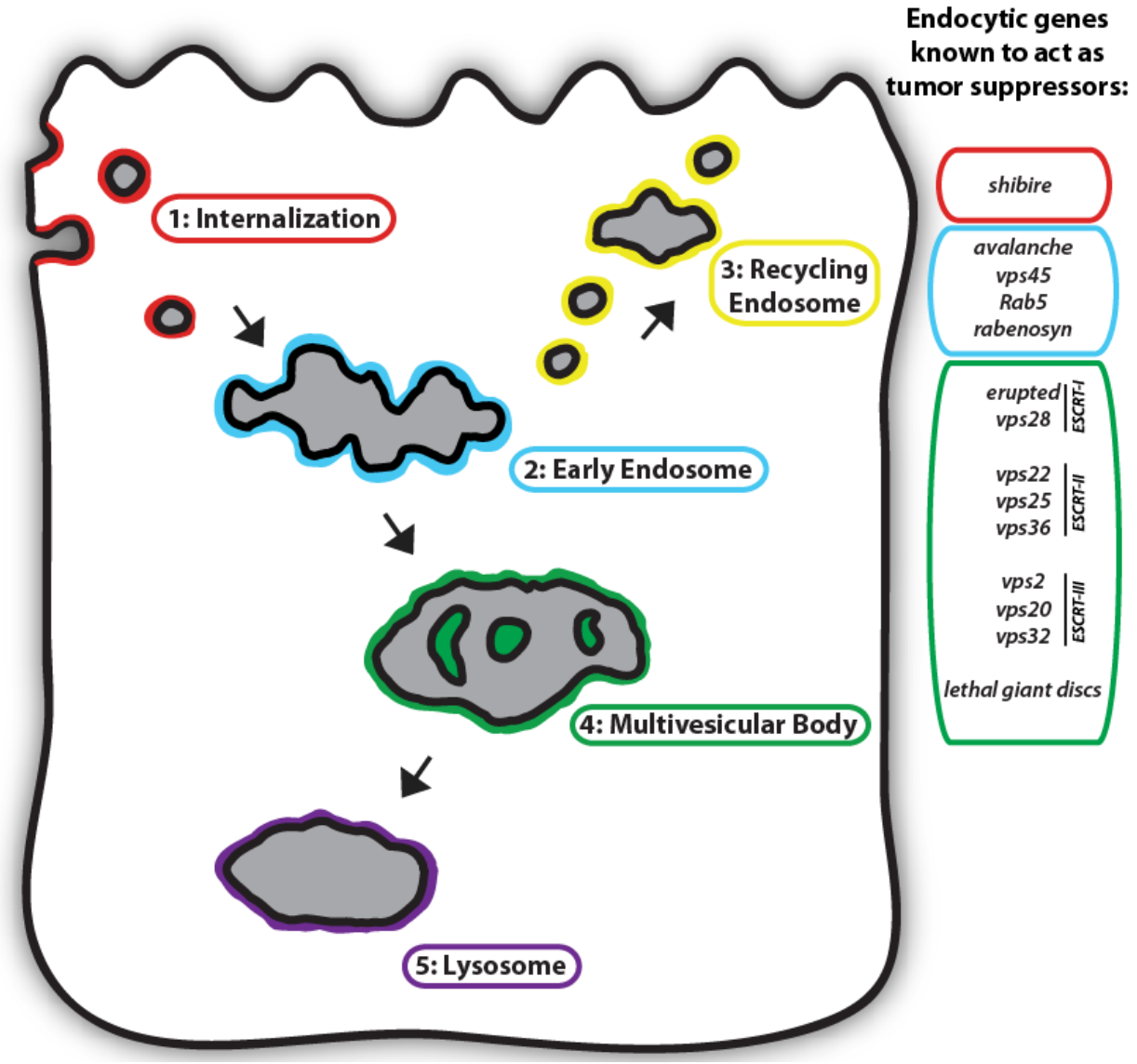


Figure 2:
A general overview of the process of endocytosis. The endocytic steps that are discussed in this thesis are shown. The endocytic genes that are known to act as tumor suppressors in Drosophila melanogaster are sorted on the specific endocytic process that they affect.

3. Recycling

From the early endosome endocytic cargo has two trafficking routes: it can be targeted to end up in the lysosome for degradation or it can be recycled (Figure 2, Step 3). In this sense the early endosome acts as a sorting station for incoming cargo**.** Recycling is essential for maintaining the homeostasis of transmembrane receptors. In the early endosome, most receptor proteins dissociate from their ligands and are sent back either to their original location or to a different site at the PM (transcytosis). Recycling is mediated through a specific type of endosome, the recycling endosome, marked by the GTPase Rab11 (Reviewed in Hsu and Prekeris 2010). Though recycling is an essential process in membrane trafficking, no components of the recycling machinery have been implicated directly in *Drosophila* tumor suppression.

4. Multivesicular body sorting

In the next step of endosomal maturation, the early endosomes form multivesicular bodies (MVBs, Figure 2, Step 4). The MVBs carry the proteins destined to be degraded in the lysosome or excreted through exosomal release (Reviewed in Huotari and Helenius 2011). Cargo that is going to be either secreted or degraded is sequestered into internal vesicles. Exosomal release is then achieved through fusion of the MVB with the plasma membrane. To facilitate degradation, the internal vesicles allow easy access for the digestive enzymes that break ubiquitinated cargo down. The sorting of this ubiquitinated cargo into these vesicles happens through a series of protein sorting units called the ESCRT (Endosomal Sorting Complex Required for Transport) complexes (Reviewed in Henne, Buchkovich, Emr 2011). Entry into the ESCRT-pathway is initiated by ESCRT-0, that binds cargo and membrane components, before handing it over to ESCRT-I and ESCRT-II respectively. ESCRT-I and II create an ESCRT-cargo-enriched zone and attract ESCRT-III, which does not directly bind ubiquitinated cargo. Instead, ESCRT-III mediates vesicle budding through a fairly unknown mechanism (Henne, Buchkovich, Emr 2011). In *Drosophila*, mutants for *erupted* and *Vps28* (ESCRT-1), *Vps22*, *Vps25* and *Vps36* (ESCRT-II) and *Vps2*, *Vps20* and *Vps32* (ESCRT-III) generate overgrowth and loss of polarity ((Herz *et al* 2009; Vaccari *et al* 2009). On top of that, the *Drosophila* tumor suppressor *Lethal Giant Discs (lgd)* is also implicated in endosomal sorting, though it is not part of an ESCRT-complex and its exact function is unknown ((Jaekel and Klein 2006). This large amount of endocytic sorting genes points to a pivotal role of this step in *Drosophila* tumor suppression.

5. Lysosomal degradation

Multivesicular bodies mature into lysosomes due to increasing acidification and fusion with preexisting lysosomes (Reviewed in Kornfeld and Mellman 1989), Figure 2, Step 5). Multiple cellular trafficking pathways end in the lysosome and it is a primary site for protein degradation. Surprisingly, no genes involved in these final steps of the endocytic process have been linked directly to tumor suppression in *Drosophila.*

II. Cell growth signaling pathways

One of the dangerous properties of tumor cells that sets them apart from regular cells is that they attain a self sufficiency in growth signaling. Normally growth control is tightly regulated and carefully orchestrated signaling cues instruct the cells when to enter or exit the cell cycle. Tumor cells acquire the ability to ignore the stop signals or constitutively activate signals that stimulate proliferation. *Drosophila* neoplastic tumors are no exception to this rule. The overproliferation seen in mutant tissues has often been attributed to the impairment of normal mitogenic signaling. Since endocytic mutants display the neoplastic tumor phenotype, the connection between endocytosis and the regulation of mitogenic signals is an obvious candidate for causing overgrowth.

In mammalian cells, it has been shown that the down-regulation of mitogenic signaling depends on endosomal trafficking to degrade activated receptors in the lysosome (Reviewed Seto, Bellen, Lloyd 2002). The importance of endocytic trafficking in cell signaling is conserved throughout organisms and has also been thoroughly investigated in fruit flies (Reviewed Kramer 2002; Gonzalez-Gaitan 2003). The next step is to assess if the neoplastic endocytic mutants display defects in mitogenic signaling. This indeed seems to be the case since disruption of several important growth signaling cascades have been linked to the endocytic mutant phenotype. Here I will discuss the different signaling pathways affecting the cell cycle and proliferation and how they are connected by endocytosis.

II.1 EGFR signaling

Receptor tyrosine kinase (RTK) signaling is a very general signaling pathway involved in a multitude of cellular processes. One of the more prominent members of the RTK family is the Epidermal Growth Factor Receptor (EGFR, Figure 3A). This is a cell surface receptor that is involved in controlling proliferation and its misregulation is often implicated in cancer (Reviewed Grandal and Madshus 2008).

Endocytosis plays an essential role in the down-regulation of RTK signaling in *Drosophila* and this is also the case for the EGFR (Jekely *et al* 2005; Lloyd *et al* 2002). After internalization, the EGFR is ubiquitinated, targeting it to the lysosome for degradation. Any defect in this endocytic route could result in the accumulation of activated EGFR and therefore sustaining the mitogenic signals, resulting in aberrant proliferation. This mechanism of an endocytic defect leading to failure to down-regulate an activated receptor provides the ideal theoretical blueprint of how any cell signaling pathway is hyper-activated in neoplastic endocytic mutants. Of course, this theory needs practical evidence to back it up.

For EGFR signaling, this evidence comes from investigating ESCRT mutants. As mentioned earlier, a considerable number of ESCRT mutants develop neoplastic tumors. In tissue mutant for ESCRT-I, II or III components, EGFR accumulation has been shown to occur in the endosomes (Vaccari *et al* 2009). By staining for Capicua, an EGFR signaling component whose nuclear expression levels are downregulated upon signaling, and assessing genetic interactions between EGFR and ESCRT pathway mutants, it was concluded that the impairment of ESCRT components prevents down-regulation of EGFR signaling. These results suggest that a defect in endocytosis can promote constitutive EGFR signaling which contributes to continued proliferation.

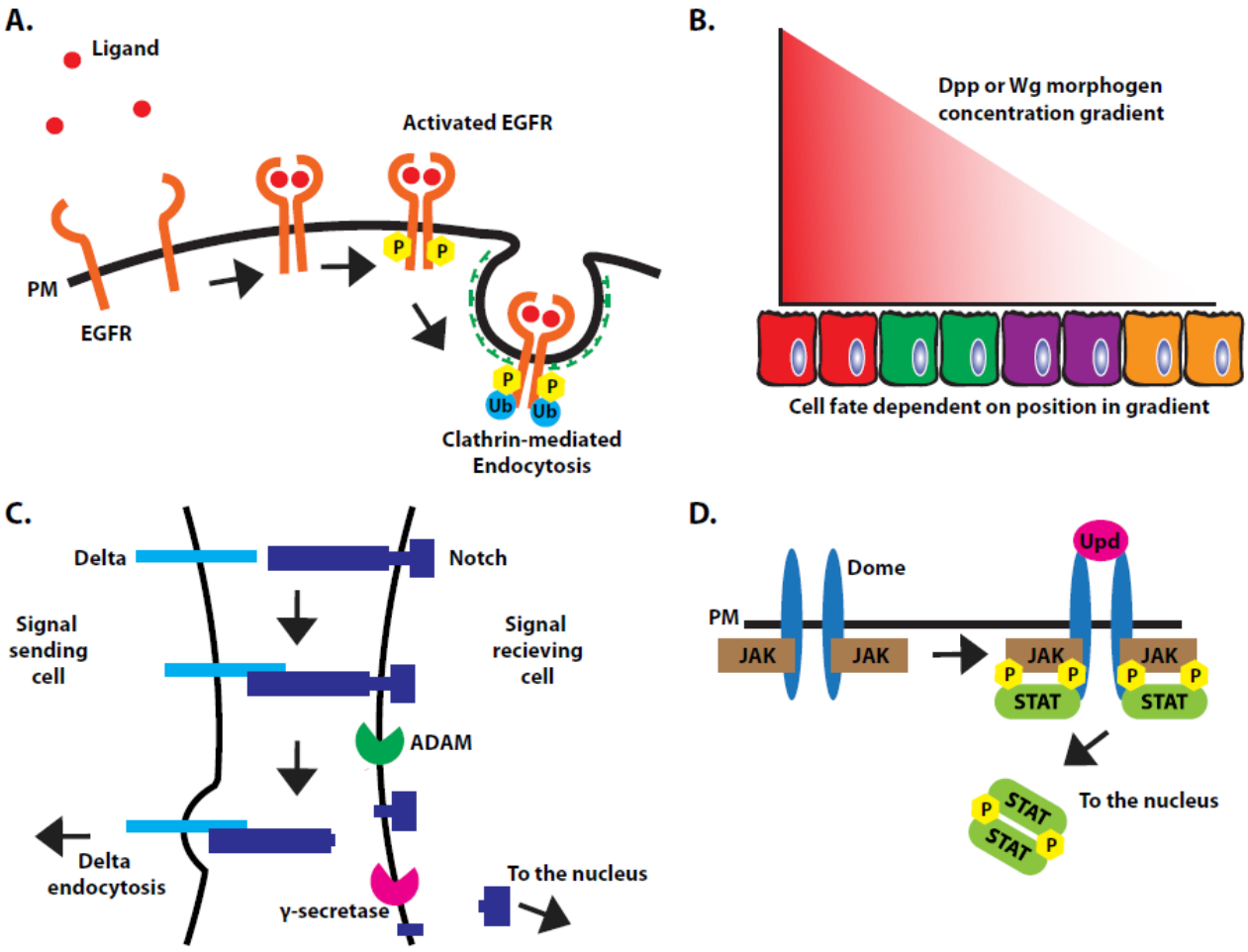


Figure 3:
The different cell growth signaling pathways mentioned in this chapter. ***A:*** *The EGFR receptor forms dimers upon activation by its ligand. Activated receptors are internalized through clathrin-mediated endocytosis. Ubiquitination of the receptor targets it for degradation in the lysosome.* ***B:*** *Dpp and Wg signaling control cellular development by setting up a concentration gradient. The relative position of a cell within this gradient determines cell fate.* ***C:*** *Notch signaling is triggered by contact between Delta from the signal sending cell and Notch from the signal receiving cell. Delta/Notch contact triggers cleavage of the extracellular domain of Notch by ADAM proteases and subsequent cleavage of the intracellular domain by γ-secretase. Endocytosis of Delta contributes to Notch signaling.* ***D:*** *Jak/STAT signaling is triggered by binding of the Unpaired (Upd) ligand to the Dome receptor. JAK activity mediates STAT recruitment and also drives STAT dimerization. STAT dimers activate transcription in the nucleus*.

II.2 Signaling induced by the morphogens Dpp and Wg

Another signaling pathway implicated in endocytic nTSGs is Dpp (Decapentaplegic) signaling. Dpp is primarily known for its role in creating a patterning morphogen gradient that determines cell fate (Figure 3B). However, it also plays a role in cell proliferation ((Schwank and Basler 2010; Wartlick *et al* 2011). Recent evidence shows that dpp concentrations and gradient scale with wing discs growth and the increase of dpp signal directly stimulates cell division (Wartlick *et al* 2011).

A link between Dpp signaling and endocytosis has been found in mutants for *vps25*, an ESCRT-II component. Clones of mutant cells in the epithelial imaginal discs show the accumulation of the activated Dpp receptor *thickveins* in endosomes (Thompson *et al* 2005). The upregulation of Dpp signaling is also confirmed by using a Dpp reporter in *vps25* mutant clones, and the fact that *vps25*

clones phenocopy clones that express an activated form of the Dpp receptor (Thompson *et al* 2005). This again displays a mechanism where defective endocytosis results in a traffic jam of activated signaling molecules. The *vps25* mutants develop neoplastic tumors, and Dpp signaling is clearly upregulated in these mutants, however there is no direct evidence that this signaling directly causes the overgrowth.

Another link between Dpp signaling and endocytosis is seen in the role of the so-called Sara endosomes in Dpp signaling. The Sara endosomes are a subpopulation multivesicular endosomes defined by the PI(3)P binding protein Sara (Gillooly, Raiborg, Stenmark 2003; Tsukazaki *et al* 1998). This category of endosomes is needed to ensure the even distribution of the dpp signal molecule in daughters of dividing wing discs epithelial cells (Bokel *et al* 2006). This seems to be a system to ensure "signaling level memory" in the daughter cells to ensure robust response to the dpp gradient. Although Sara mutants show signaling defects, again there is no sign of overproliferation directly induced by dpp (Bokel *et al* 2006).

Wingless (Wg) signaling, like Dpp signaling, is involved in numerous developmental processes (Figure 3B). Its misregulation can result in aberrant cell fate decisions and tumorigenesis (Reviewed in Cadigan and Nusse 1997). Although a direct analysis of Wg signaling in endocytic tumors have not been performed, several indirect connections exist. In *Drosophila* S2 cells the role of endocytosis in Wg was assessed by knocking down endocytic genes (Seto and Bellen 2006). RNAi against the early endocytic components *shibire* (dynamin) and *Rab5* conferred a reduction in Wg signaling. Both *shibire* and *Rab5* mutants display the neoplastic tumor phenotype (Lu and Bilder 2005; Vaccari *et al* 2008). Since the analysis of Wg signaling was done in cell culture, alterations of Wg signals in endocytic tumor tissue remain to be speculative. Still, the results point to a role for endocytosis in regulating Wg signaling, so an assessment of Wg components in endocytic mutant tissue would be highly informative.

II.3 Notch signaling

Out of the many different mitogenic signaling pathways, Notch signaling has received the most attention in endocytic tumors (Figure 3C). The attraction to Notch comes from the observation that ectopically activating Notch signaling in eye imaginal discs is sufficient to induce overgrowth (Chao *et al* 2004). This makes Notch a prime candidate to be hyperactivated in the endocytic mutants and therefore contribute to the overproliferation.

However, in reality the situation is not that straightforward. There appears to be a difference in Notch signaling behavior in different endocytic mutants. When regarding mutants in the earlier steps of endocytosis that regulate the entry of Notch into the early endosome, Notch signaling does not show upregulation (Lu and Bilder 2005; Vaccari *et al* 2008; Morrison *et al* 2008). This is seen in tissue mutant for *shibire*, *avalanche*, *vps45, rabenosyn* and *Rab5*. Notch signaling reporters in these tissues do not display elevation of signaling levels, though the levels of Notch itself are higher, accumulating at the cell surface (Lu and Bilder 2005; Vaccari *et al* 2008; Morrison *et al* 2008). These findings indicate that the activation of Notch signaling does not play a substantial role in overproliferation in early endocytic mutants.

A completely different behavior of Notch signaling is seen in mutants for later stages of endocytosis. A comprehensive list of mutants for MVB sorting components displays Notch accumulation in the endosomes and elevation of Notch signaling reporters. These components include the ESCRT genes Erupted and Vps28 (ESCRT-I), Vps22 and Vps25 (ESCRT-II) and Vps2, Vps20 and Vps32 (ESCRT-III), and the conserved C2-domain gene Lethal Giant Discs (Childress *et al* 2006;

Moberg *et al* 2005; Thompson *et al* 2005; Vaccari and Bilder 2005; Vaccari *et al* 2008; Vaccari *et al* 2009). The retention of activated receptors in ESCRT mutants is reminiscent of the EGFR signaling defects seen in the same mutants. This suggests that Notch signaling is able to stimulate overproliferation in these mutants. The question remains why Notch signaling is active in these mutants and not in mutants for earlier endocytic steps.

The answer may lie in the finding that in these mutants Notch activation happens independently of the presence of a ligand (Childress *et al* 2006; Jaekel and Klein 2006; Vaccari *et al* 2008). The mechanism behind ligand-independent Notch activation remains is still unresolved. One of the present theories is that the Notch receptor population that is built up in the endosomes is activated there by the enzyme that cleaves Notch, γ-secretase. This cleaving event activates Notch signaling and since the conditions in the endosomes are ideal for γ-secretase activity, the endocytic defects of *vps25, erupted* and *lgd* mutants put Notch in an optimal position to be aberrantly activated (Childress *et al* 2006; Jaekel and Klein 2006; Vaccari *et al* 2008). The internalization of Notch and therefore the endosomal accumulation in this case would not occur due to receptor activation at the plasma membrane, but would be part of the regular homeostasis of inactivated receptor, which is normally degraded in the lysosome. This would explain why Notch signaling is not activated in the early endocytic mutants, since the receptor never reaches the endosomes.

II.4 Jak/STAT signaling

Strongly connected to the Notch signaling defects seen in the endocytic mutants are observations of Jak/STAT signaling alterations (Figure 3D). Several studies have indicated that Unpaired (Upd) is ectopically activated in *Drosophila* endocytic tumors (Herz *et al* 2006; Moberg *et al* 2005; Vaccari and Bilder 2005)(Tsai and Sun 2004). Upd is the ligand for Jak/STAT signaling, and overexpressing Upd is able to drive non-autonomous overproliferation (Tsai and Sun 2004). Upd is also a target of Notch signaling and therefore one of reasons aberrant Notch signaling has aberrant mitogenic potential (Herz *et al* 2006; Moberg *et al* 2005; Vaccari and Bilder 2005). Since the activation of Jak/STAT in the endocytic tumors is Notch signaling dependent, mutants for early endocytic steps do not display Upd upregulation (Chao *et al* 2004; Lu and Bilder 2005). The attenuation of the Jak/STAT signal depends on degradation in the lysosome, which indicates that an accumulation of activated Jak/STAT components in the endosomes is again responsible for constitutive mitogenic signaling (Devergne, Ghiglione, Noselli 2007).

Overall it can be concluded that mitogenic signaling pathways are disrupted in the endocytic mutants mainly by the failure to degrade activated signaling components that in turn contribute to continuous proliferation causing tissue overgrowth. However, the research in Notch signaling shows that an endocytic defect does not always result in signaling activation and that groups of endocytic mutants can display completely different signaling phenotypes. The mutants for early endocytic components do not show any Notch signaling upregulation, yet they still overproliferate. This indicates that mitogenic signaling cannot be the sole contributor to the tumorous growth and that other processes are also involved in the generation of the endocytic tumor phenotype.

III. Asymmetric cell division

Interestingly, in the larvae mutant for the classical Drosophila tumor suppressor genes dlg, lgl or scrib, the imaginal discs are not the only tissues displaying overgrowth. The effects of the mutations are also clearly seen in the larval nervous system. The larval brain is significantly enlarged and elongated by the extensive overproliferation (Bilder and Perrimon 2000; Gateff 1978; Woods and Bryant 1989). Histological and fine structure analysis of the neural tumors showed that cells that have overproliferated are neural stem cells, the neuroblasts, that do not differentiate into adult neurons. These observations are in line with the recurring theme in cancer biology that the cells that fail to differentiate retain their stem cell-like properties and continue to divide.

Under normal circumstances, a stem cell maintains tissue homeostasis by dividing asymmetrically, therefore renewing itself while also creating a daughter cell that commits to differentiation. However, when tumor suppressor genes are mutated, stem cells lose their ability to suppress self-renewal and divide symmetrically, and instead of differentiating they continue to proliferate. This mechanism is believed to drive tumorigenesis when asymmetric division is impaired (Reviewed in Wodarz and Gonzalez 2006).

Different *Drosophila* cell types are extensively used as a model for asymmetric stem cell division. It has been shown that endocytosis plays a big part in coordinating the asymmetry of the daughter cells (Reviewed in Shen and Temple 2002; Coumailleau and Gonzalez-Gaitan 2008; Furthauer and Gonzalez-Gaitan 2009). The misregulation of asymmetric division forms an important contribution to tumorigenesis and it is therefore an attractive model for how the tumors in endocytic mutants originate. Here I will discuss how endocytosis affects asymmetric division and how this can lead to tumor formation.

III.1 Asymmetric endocytosis controls Notch signaling

A prime example and a well-studied system for asymmetric cell division are the sensory organ precursor (SOP) cells in *Drosophila*. A SOP cell divides asymmetrically to form the sensory bristle, a four cell mechanosensory structure (Figure 4). The fact that one mitotic cell creates several completely different cell types indicates that the asymmetric cell division in the SOP cells is tightly regulated.

The first division of a SOP cell creates the daughter cells pIIa and pIIb. PIIa divides to form the socket and hair cells, while pIIb forms the sheath cell and a neuron. The fate decision of the SOP division is driven by Notch signaling (Guo, Jan, Jan 1996). As previously mentioned, Notch signaling has been extensively connected to the endocytic tumor suppressor genes through its aberrant activation in tumor tissue. In SOP cell division it is associated with fate specification because of its asymmetric activation. Notch signaling is only activated in pIIa. This has dubbed pIIa as the 'signal receiving cell' and pIIb as the 'signal sending cell'. The asymmetric activation of Notch signaling in these cells is strongly dependent on asymmetric endocytosis (Reviewed in Le Borgne 2006).

How is asymmetric endocytosis initially set up and how does it control Notch signaling? When looking at the distribution of endocytic compartments prior to SOP division, the early and late endosomes are still symmetrically divided throughout the cell (Emery et al 2005). There is however an asymmetric distribution of two types of endosomes: recycling endosomes and Sara endosomes (Coumailleau *et al* 2009; Emery *et al* 2005). The asymmetric recycling endosomes, marked by Rab11, are used in a recycling mechanism that activates Delta signaling in the pIIb cell (Emery *et al* 2005).

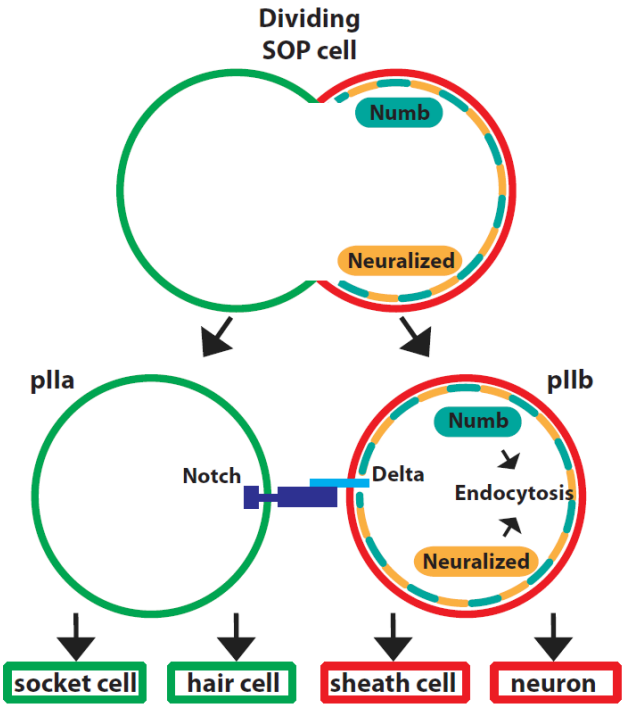


Figure 4:
SOP cell division in Drosophila melanogaster. Before the first SOP division, Numb and Neuralized localize to the anterior cortex of the cell. This causes Numb and Neuralized to be distributed into the pIIb cell after the first division. Numb and Neuralized drive endocytic processes that downregulate Notch and stimulate Delta activity in the pIIb cell, thereby regulating cell fate.

The Sara endosomes, also known for their role in dpp signaling, carry activated Notch and distribute this to the pIIa cell (Coumailleau *et al* 2009). This is not the only way Notch signaling is asymmetrically controlled. Before the division of the SOP cell there is also the polarized distribution of the cell fate determinants Neuralized and Numb (Le Borgne and Schweisguth 2003; Roegiers *et al* 2001). Both these two asymmetrically distributed cell fate determinants use endocytosis to perform their tasks.

III.2 Delta activation by Neutralized-controlled endocytosis

Neuralized is a membrane associated E3 ubiquitin ligase that interacts with the Notch ligand Delta (Le Borgne and Schweisguth 2003). The ubiquitination of Delta in pIIb promotes its asymmetric endocytosis which in turn promotes Notch signaling activation (Le Borgne and Schweisguth 2003). Since Delta-Notch signaling is mediated by physical contact at the plasma membrane, it seems counterintuitive that endocytosis is needed for activation.

Several models have been proposed that explain this phenomenon (Coumailleau and Gonzalez-Gaitan 2008). The first and most straightforward model suggests that a mechanical force is needed to activate Notch signaling. When Delta and Notch bind, endocytosis provides a pulling force on Delta, triggering a conformational change in the Notch receptor stimulating the cleavage event needed to activate Notch signaling. A second model states that the Delta ligand needs to be internalized through endocytosis to undergo an Epsin-dependent recycling pathway that enables post-translational modification. In contrast with the first model, this proposed mechanism is backed up by several observations (Emery *et al* 2005; Wang and Struhl 2004; Wang and Struhl 2005). Epsin, which is a membrane protein involved in creating membrane curvature and important in endocytosis, is required to direct Delta in a recycling pathway and Epsin inactivation disturbs cell fate specification in the SOP cells (Wang and Struhl 2004; Wang and Struhl 2005). As previously mentioned, in the signal sending cell pIIb Delta passes through Rab11 positive endosomes, which are recycling endosomes, and these endosomes are needed to mediate Notch signaling and fate specification (Emery *et al* 2005). The only gap in this theory is that it is unknown what the post-translational modification of Delta entails. A third model comes from the observation that Delta, upon endocytosis, is targeted to the internal vesicles of multi-vesicular bodies (MVBs)(Mishra-Gorur

et al 2002). Although this is usually a destination for proteins that will be degraded, it can as well be a first step for secretion through exosomes. This suggests that asymmetric endocytosis of Delta is needed to promote exosomal release in a later state.

III.3 Numb driving asymmetric endocytosis

Numb is a protein associated with cell fate specification and is also involved in endocytosis (Santolini *et al* 2000). It interacts with several endocytic components, most notably α-adaptin, a subunit of the AP2 complex (Santolini *et al* 2000). AP2 is a major component of clathrin coated-pits and a key player in receptor mediated endocytosis. Before the SOP cell division Numb is asymmetrically distributed by PAR polarity proteins (Betschinger, Mechtler, Knoblich 2003). This places Numb at the anterior cortex of the SOP cell which will target Numb for pIIb after division. Asymmetric Numb also triggers asymmetric distribution of the AP2 complex, because of its binding to α-adaptin. This drives higher endocytic activity in the signal sending pIIb cell (Berdnik *et al* 2002). This polarized receptor-mediated endocytosis is seen as the driving force behind Notch receptor down-regulation in pIIb, and therefore fate specification.

III. 4 Numb and tumor formation

Although this all highlights the importance of endocytosis in asymmetric cell division, it does not show a direct link to tumorigenesis. The SOP system shows that defects in endocytosis can lead to cell fate errors that set the basis for tumorigenic potential, however it is not possible to actually induce tumor-like growth in this system. The missing link was provided in a different cell type that was closely related to the SOP cells, the *Drosophila* neuroblasts. Again Numb plays a key role.

In a series of transplantation assays, tissue mutant for various genes that are important in asymmetric cell division was inserted in adult *Drosophila* hosts (Caussinus and Gonzalez 2005). The mutations were generated in larval neuroblasts. These types of transplantation experiments are often used to assay the tumorigenic potential of a mutation, since the tissue can be propagated for extended periods through consecutive transplants. One of the genes tested was Numb. It appeared that neuroblast tissue mutant for the asymmetric cell division genes generated large tumor masses, growing over 100 times of its original size (Caussinus and Gonzalez 2005). These tumors have the ability to invade other tissues and eventually kill the host animal. In addition, the tumors acquire malignant properties like genome instability, centrosome alterations and immortality (Caussinus and Gonzalez 2005). The traits displayed here are very reminiscent of the classical neoplastic tumors and those seen in the endocytic mutants. In this respect Numb, as an endocytic factor, can be categorized in the large group of endocytic tumor suppressors.

This proves that asymmetric cell division defects have the potential to develop tumors, and the involvement of Numb shows that endocytosis can be linked to that process. However, like with the mitogenic signaling pathways connected to endocytosis, the tumorigenic potential of aberrant asymmetric division is mostly driven by the effects of Notch signaling. As previously mentioned, it is unlikely that altered Notch signaling alone is responsible for the endocytic tumor phenotype, so there are likely other factors at play. More importantly, with exception of the Numb mutant neuroblast tumors, there is practically no direct experimental evidence of asymmetric cell division defects in mutant tissues of the known endocytic tumor suppressors. It could be that this is something that has not been thourougly investigated, but it could also indicate that the role of asymmetric cell division in these mutants is marginal. It is therefore interesting to take a look at the processes connecting endocytosis and tumorigenesis that have no link to Notch signaling.

IV. Cell polarity and endocytosis

The hyperproliferation of cells is one of the most obvious properties shared by mammalian and fly malignant tumors, but it is not the only one. Epithelial cells show strong polarized separation of the cell membrane domains into apical and basolateral domains, and placement of robust cellular junctions that connect the cells (Figure 5). A closer look at *Drosophila* endocytic neoplastic tumors reveals that this polarized epithelial architecture is lost. This trait is also seen in human malignant epithelial tumors (Tervonen *et al* 2011; Wodarz and Nathke 2007). The classical neoplastic tumor suppressor genes *dlg, lgl* and *scrib* are in fact mostly known for their critical role in regulating polarity. These junction-associated scaffolding proteins act as a single module, establishing and maintaining the basolateral identity of epithelial cells (Bilder, Li, Perrimon 2000). Since the phenotype of endocytic mutants copies that of these polarity regulators, it is obvious that endocytosis is involved in polarity regulation. The question rises how the process of endocytosis influences polarity regulation and the polarity defect can lead to tumor formation. That is what will be addressed in this chapter.

IV.1 Par and Cdc42: a link between vesicle traffic and polarity

The similarities between polarity and endocytic mutants indicates that endocytosis might play an important role in polarity regulation, either as a downstream effector of polarity regulators, or as a mechanism to set up polarity.

There are three major polarity regulating modules: the two apical PAR and Crumbs modules, and the basolateral Scrib module formed by the classical neoplastic tumor suppressors (Reviewed in Assemat *et al* 2008, Figure 5). The proteins in these modules are mostly cytoplasmic scaffolding proteins with multiple protein-protein interaction domains. Components of the PAR complex have been implicated in endocytosis regulation on multiple occasions. In *Drosophila* embryonic epithelial cells, the core PAR proteins Bazooka, Par6 and aPKC act together with the polarity regulating GTPase Cdc42 to control endocytosis (Harris and Tepass 2008). Impairing the function of these proteins results in a general endocytic trafficking defect since a multitude of cargo types is not being endocytosed. Cdc42 acts upstream of the Par components, which affect trafficking by interacting with an unknown endocytic component (Harris and Tepass 2008). Interestingly, uptake assays indicate that endocytic activity is increased in the polarity mutants, suggesting that Cdc42 and the Par proteins inhibit endocytosis. This is remarkable since similar research in the *Drosophila* notum epithelium shows that the same polarity regulators are needed here to promote dynamin-dependent endocytosis (Georgiou *et al* 2008). Again polarity components are upstream of endocytosis, but in this case with an opposite effect. It seems that the regulation of endocytosis by polarity proteins is very tissue-specific. However, the effect on the process that is controlled by these mechanisms is the same in both situations: disturbing Cdc42/Par regulated endocytosis leads to junctional instability (Georgiou *et al* 2008; Harris and Tepass 2008). The cell junctions are intimately connected to both polarity and endocytosis.

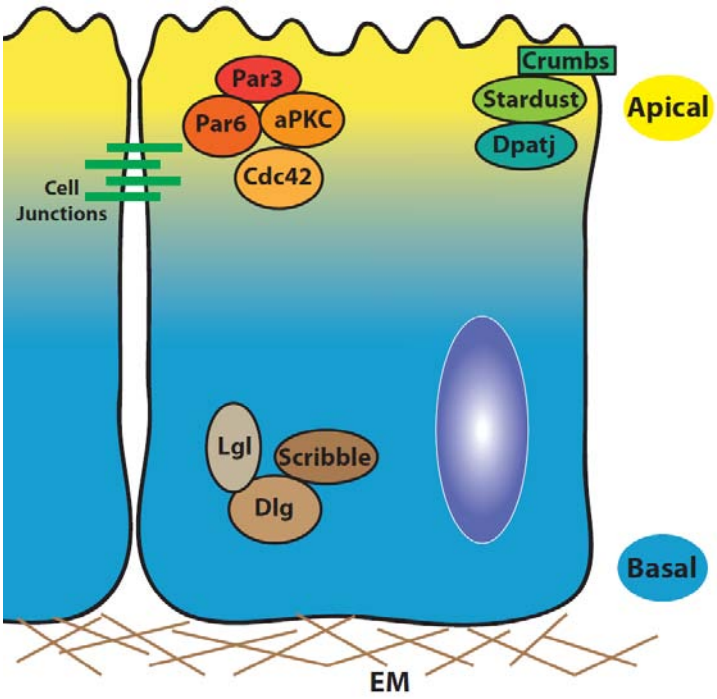


Figure 5:
The major polarity regulating modules in a typical Drosophila epithelial cell. The apical region is defined by the PAR and Crumbs modules. The Scribble polarity module regulates basolateral identity. Cell junctions are strongly developed in epithelial cells. They mediate tight adhesion and communication between the cells.

IV.2 Cell junctions

When looking for a link between polarity and endocytosis, the cellular junctions are an obvious candidate. Cell junctions connect epithelial cells, holding them together and also propagating communication between cells. These junctions are strongly polarized and polarity proteins localize to these structures. The integrity of the cell junctions is dependent on polarity proteins, since a knock down of polarity components results in a destabilization of the cellular junctions (Bilder, Schober, Perrimon 2003; Desai *et al* 2009; Harris and Peifer 2004). As mentioned before, this effect on the junctions is mediated by endocytosis (Georgiou *et al* 2008; Harris and Tepass 2008). However, it is not just polarity proteins setting up the junctions, the interaction also goes the other way around: cells without the junctional connections fail to establish correct polarity (Desai *et al* 2009; Qin *et al* 2005). Because of these interdependent interactions it is impossible to separate the junctions from polarity mechanisms.

A central role in polarity regulated junctional establishment and maintenance is played by E-cadherin. This calcium dependent cell adhesion molecule is a junctional component that also interacts with polarity proteins (Desai *et al* 2009; Harris and Peifer 2004). It is believed that E-cadherin-mediated cell-cell contact provides an initial polarity cue that drives polarity proteins to subsequently stabilize the junctions (Desai *et al* 2009; Navarro *et al* 2005). Interestingly, E-cadherin levels are dependent on endocytic trafficking processes (Georgiou *et al* 2008; Le, Yap, Stow 1999; Roeth *et al* 2009). The pools of E-cadherin at the plasma membrane are not static, but are constantly being endocytosed and recycled (Le, Yap, Stow 1999). This explains why endocytic defects can result in junctional destabilization. Furthermore, this destabilization has another consequence related to the endocytic tumors. A weakening of the cellular junctions also affects contact-mediated inhibition of proliferation. This refers to the fact that cells with stable connections, laying in a polarized tissue sheet, are restricted from proliferation by signaling that is regulated by their intimate contact (Reviewed in Fagotto and Gumbiner 1996). Loss of this contact inhibition is associated with proliferative potential and tumor growth.

Although this provides a mechanism through which endocytosis affects proliferation through junctional instability, the scientific evidence in *Drosophila* to back this theory up is still indirect. The role of endocytosis regulating E-cadherin in *Drosophila* has been recognized, but contact mediated inhibition is still a process primarily investigated in mammalian (tumor) cell culture. It would be interesting to see if this inhibition plays any part in *Drosophila*, since that would definitely provide a link to the endocytic tumors.

IV.3 Crumbs and apical identity

From the last two paragraphs it seems that polarity regulating proteins are generally upstream of endocytic components. However, endocytosis also plays a role in properly localizing polarity proteins. In the initial experiments investigating endocytic *Drosophila* tumors, the localization of polarity determinants in the mutant cells was assessed to detect polarity perturbation. It was found that the polarity disruption seen was driven by the mislocalization of apical determinants like aPKC and Crumbs (Lu and Bilder 2005; Moberg *et al* 2005; Vaccari and Bilder 2005). These polarity components are normally restricted to the apical periphery of the cell, above the junctions, where they act through downstream effectors to confer apical identity. In the endocytic mutants these components are not restricted to their apical domains anymore, leading to an apicalization of the cell membrane (Lu and Bilder 2005; Moberg *et al* 2005; Vaccari and Bilder 2005). This indicates that the interaction between polarity and endocytosis is more of a reciprocal mechanism where endocytic components are not just a downstream effector of polarity proteins, but endocytosis is also essential for keeping apical determinants in the right place. When looking for an apical polarity component that could be regulated by endocytosis, one gene is a likely candidate since it encodes a transmembrane protein: Crumbs.

Crumbs is part of one of the two major apical polarity complexes. The activity of Crumbs is sufficient to confer an apical identity in the plasma membrane and its function is mostly driven by the membrane-bound cytoplasmic part of the protein (Wodarz *et al* 1995). Staining for Crumbs in epithelial tissues of endocytic mutants reveals that there is an accumulation of the polarity protein at the cell membrane when early endocytic components are perturbed (Lu and Bilder 2005; Morrison *et al* 2008). In the ESCRT-I mutant *erupte* a buildup of Crumbs protein in sub-apical aggregates is seen, suggesting it piles up in the endosomes (Moberg *et al* 2005). This clearly indicates that endocytosis is needed for correct functioning of Crumbs. The accumulation of Crumbs at the plasma membrane explains the apicalization phenotype leading to a polarity defect, however it is less straightforward how the endosomal aggregation confers a disturbance to polarity. Perhaps the polarity effector is normally degraded and the endosomal aggregates in the endocytic mutants aberrantly drive polarity signaling. Although that remains speculative, the fact stands that endocytosis controls the correct Crumbs protein homeostasis.

Is this polarity defect sufficient to lead to tumorigenesis? Overexpression of Crumbs in epithelial tissues results in excessive growth and induces polarity defects similar to those in the endocytic mutants, which strengthens the idea that Crumbs mistrafficking is a major factor in causing endocytic tumors.

Discussion

The goal of this thesis was to assess the different mechanisms through which endocytosis has a tumor suppressive function in *Drosophila*. There are many direct and indirect links between loss of endocytic function and tumorigenesis. The role that endocytosis normally fulfills in cell growth signaling, asymmetric cell division and polarity regulation indicates that in endoytic mutants tumor formation is kick-started by disturbing these processes.

It is unlikely that the tumorigenic potential of endocytic mutants arises from the disturbance of a single cellular mechanism. Although endocytic defects in cell signaling and asymmetric division points to Notch signaling as a key player in tumorigenesis, a defect in Notch signaling is not sufficient to explain the neoplastic endocytic tumors. Indeed, the buildup of Notch cargo in endosomes makes it plausible that aberrant Notch signaling drives overproliferation. However, two observations indicate that. The first is that in a subset of endocytic mutants the Notch signaling pathway is not activated while tumorous overgrowth and neoplastic transformation still occurs. These are mostly mutants for early endocytic components like *shibire* and *avalanche*, although it is also seen in tissue mutant for ESCRT-II component *Vps36* (Herz *et al* 2009). This means Notch signaling is not required to promote neoplastic transformation. The second is that even when a Notch accumulates in endosomes due to an endocytic defect, this does not always produce tumors. This is the case in *hrs* mutants (Jekely and Rorth 2003). *Hrs* encodes a component of the ESCRT-0 complex, and it is needed to downregulate several types of signaling receptors. Epithelial cells mutant for *hrs* accumulate ubiquitinated receptors in endosomal compartments, including Notch (Jekely and Rorth 2003). This does however not induce overgrowth.

Still, the role of Notch activation in *Drosophila* tumorigenesis cannot be considered marginal. An interesting perspective is presented by the observations in mosaic mutant tissues. Mosaic tissues contain patches of cells that are mutant (clones) and patches that are wild-type. When an epithelial tissue is predominantly mutant for an ESCRT-I, II or III component, it results in neoplastic tumor formation (Herz *et al* 2006; Herz *et al* 2009; Moberg *et al* 2005; Thompson *et al* 2005; Vaccari and Bilder 2005). This neoplastic transformation is independent of Notch signaling. In contrast, in mosaic tissues, the ESCRT mutant cells drive non-automous overproliferation in the adjacent wild-type tissue. This non-automous effect is Notch dependent. The overgrowth seen in these mosaics differs from neoplastic mutants since the cellular polarity is not lost and the cells stop proliferating at some point (Herz *et al* 2009). The non-autonomous overgrowth is therefore hyperplastic rather than neoplastic. Since Notch signaling activation is needed for the formation of these hyperplastic tumors, it is likely that it also affects proliferation in the endocytic neoplastic tumors. The tumor chararistics that are specific for neoplasms, like the loss of polarity, have to originate from other defects.

Another interesting feature in the mosaic tissues is the apoptotic response. In tissue that is completely mutant for *vps25* (ESCRT-II), there is no activation of apoptosis. In contrast, mosaic mutant tissue does initiate programmed cell death (Herz *et al* 2006). Furthermore, in the adjacent wild-type tissue, the apoptotic response is repressed, though that is shown not to be the reason that this tissue overgrows. Blocking *hippo* signaling pathway in the *vps25* mutant tissue blocks cell death and results in dramatic overgrowth (Herz *et al* 2006). The Hippo pathway is a tissue growth regulatory pathway that controls proliferation and cell death. Hippo components function as hyperplastic tumor suppressors and have also shown to interact with polarity components (Reviewed in Genevet and Tapon 2011). Hippo activation is seen in *vps25* mutant tissue, but its role in other endocytic mutant has not been assayed. It would therefore be interesting to see if this pathway is

involved in endocytic tumor suppression, since it also provides a link to growth and polarity pathways.

As previously mentioned, mutants for the endocytic component *hrs* do not develop neoplastic tumors. This touches the intriguing point that a number of genes with an endocytic function do not appear to have a tumor suppressive role. Especially those involved in late steps of endocytosis are absent in tumor suppressor screens and do not produce tumors upon deactivation. This group includes *deep orange* (a homolog of Vps18), *light* (Vps41), *carnation* (Vps33) and *fab1* (a PtdIns(3)P 5-kinase) (Rieder and Emr 1997; Rusten *et al* 2006; Sevrioukov *et al* 1999; Warner *et al* 1998)(Rieder and Emr 1997). An explanation that has been given for this is that at late endocytic stages the cargo destined to be degraded is already unable to exert its function, although the final step still has to occur. For example, in cells mutant for *fab1*, Notch, Dpp and Wg receptors accumulate in late endosomes, though signaling for all these pathways is unaffected (Rusten *et al* 2006). This suggests that signal termination occurs during an earlier endocytic stage, therefore the build-up of endocytic cargo does not contribute to tumor formation.

Cooperation between aberrant cell growth signaling and a defect in cell polarity is a plausible mechanism for driving endocytic tumorigenesis. Combining cell growth signaling activation with a polarity defect has been shown to be a potent driver of tumorigenesis (Brumby and Richardson 2003). Using the FLP/FRT system, clones homozygous mutant for polarity regulator *scribble* were generated in *Drosophila* eye tissue, combined with either constitutively activated Ras or Notch. Activated Ras or Notch triggers moderate clonal overgrowth and clones that are only mutant for *scrib* die due to an apoptotic response. However, the combination of both defects causes extensive tumor growth and the cells even acquire metastatic properties (Brumby and Richardson 2003). This cooperative mechanism also works when Ras activation and *scrib* lesions occur in separate, adjacent cells (Wu, Pastor-Pareja, Xu 2010). This points to a non-autonomous effect. Indeed it has been found that the *scrib* mutant cells upregulate mitogenic Jak/STAT signaling in adjacent cells through Upd activation (Wu, Pastor-Pareja, Xu 2010). Jak/STAT activation has been observed in the endocytic mutants as well, so a cooperative mechanism could play a role in these tumors as well. It would be interesting to assess these cooperative mechanisms for endocytic lesions, for instance by using the FLP/FRT system to generate endocytic lesions adjacent to a polarity mutation or constitutive mitogenic activation.

In research the question always arises to what extent the knowledge gained from *Drosophila* tumor biology translates to human cancers. In general the process of tumor formation in both organisms is very different, since the generated fly tumors arise from a single mutation while the origin of human malignant tumors is based on the accumulation of several genetic lesions. In addition, few of the endocytic components discussed here have been implicated directly in causing human neoplasms. However, when considering the many different defects that endocytic mutants display in cellular processes, there are significant parallels. Malignant mammalian tumors show loss of polarity, defects in cell growth signaling, and the derailment of asymmetric division of stem cells is a hot topic in cancer biology. It is the heterogeneity seen in the endocytic tumors that makes it a fitting model for human malignancies. Dissecting the different pathways that are affected by loss of endocytic function will provide invaluable knowledge for understanding the tumor phenotype.

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