Furthermore, the pathway that detects free DNA ends and the pathway that detects the N/C ratio appear to be able to activate Chk1 synergistically (Conn et al., 2004).

Such a model of Chk1 activation will have significant implications for our understanding of the regulation of cell cycles at the MBT. In *Xenopus*, the MBT occurs after the 12th cell cycle, and developmental slowing of the cell cycle was shown to be dependent on the activity of Chk1 (Carter and Sible, 2003; Shimuta et al., 2002). Similarly in *Drosophila*, the homologs of ATR (*mei-41*) and Chk1 (*grapes*) are required to terminate the cleavage cell cycle at the MBT (Sibon et al., 1999, 1997). The results by Conn et al. raise the possibility that, at the MBT, the ATR/Chk1 pathway is activated in response to a predetermined N/C ratio (a developmental checkpoint) or to a large amount of free DNA ends (a cell cycle checkpoint), or more likely in response to both of these two signals. Clearly, additional studies will be needed to distinguish these possibilities. Furthermore, questions about the mechanism that is responsible for sensing the N/C ratio and the mechanism by which the N/C ratio is relayed to the regulation of ATR or Chk1 remain to be addressed. In this regard, it is of interest to note that the expression of *frühstart* (*frs*), a gene reported to be important for cell cycle arrest in early *Drosophila* development, is apparently also controlled by the N/C ratio (Grosshans et al., 2003). In haploid embryos, frs expression is delayed by one cell cycle. Nothing is known about the molecular function or regulation of this protein, but it is possible that the N/C sensor that activates *frs* expression could also be linked to Chk1 activation.

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Chewing the Fat: Regulating Autophagy in *Drosophila*

Autophagy is the major cellular process responsible for bulk cytoplasmic degradation. Two reports in this issue of *Developmental Cell* describe how both PI3 kinase and TOR signaling in *Drosophila* are critical for controlling autophagy in response to developmental and environmental cues.

Autophagy is the major mechanism by which organelles and long-lived proteins are degraded in eukaryotes. In this process, portions of the cytoplasm are sequestered in double membrane vesicles known as autophagosomes. These subsequently fuse with lysosomes, resulting in degradation of their cytoplasmic contents. Studies in yeast have yielded valuable insights into the control of autophagy (Levine and Klionsky, 2004). These single cells exhibit a simple lifestyle, growing when environmental nutrients are abundant and ceasing growth when nutrients become limiting. Autophagy is rapidly induced by nutrient starvation in yeast, providing a critical means for recycling nonessential macromolecules to sustain viability under suboptimal conditions. The conserved nutrient-responsive TOR signaling pathway appears to be the chief regulator of autophagy in this context. Moreover, key genetic studies in yeast have begun to unravel the molecular machinery of autophagy, contributing a framework for understanding how this process is regulated (Klionsky et al., 2003).

The induction of autophagy following nutrient starvation has also been well documented in mammals, both in cell culture and, recently, in vivo, using transgenic mice that express a fluorescent marker for autophagosomes (Levine and Klionsky, 2004). Multicellular organisms have also evolved to use autophagy to control other critical events such as cellular remodeling during development, programmed cell death, and the turnover of damaged organelles (Klionsky et al., 2003). Indeed, aberrant autophagy has been implicated in aging and cancer. Despite this, few studies have genetically analyzed the regulation and role of autophagy in metazoans. Two studies reported in this issue begin to tackle this topic using the power of *Drosophila* genetics.

The larval period in *Drosophila* is characterized by a tremendous increase in mass with growth occurring predominantly in larval-specific tissues such as the salivary gland, gut, epidermis, musculature, and fat body. This growth is driven by nutrition-dependent activation of the insulin/PI3K and TOR signaling pathways. Upon removal of dietary protein, these pathways are inactivated and growth is shut down (Britton and Edgar, 1998; Britton et al., 2002). By analyzing the larval fat body, both Scott et al. (2004) and Rusten et al. (2004) demonstrated that this cessation of growth is accompanied by marked increases in autophagy. Using a combination of electron and fluorescent microscopy, they observed

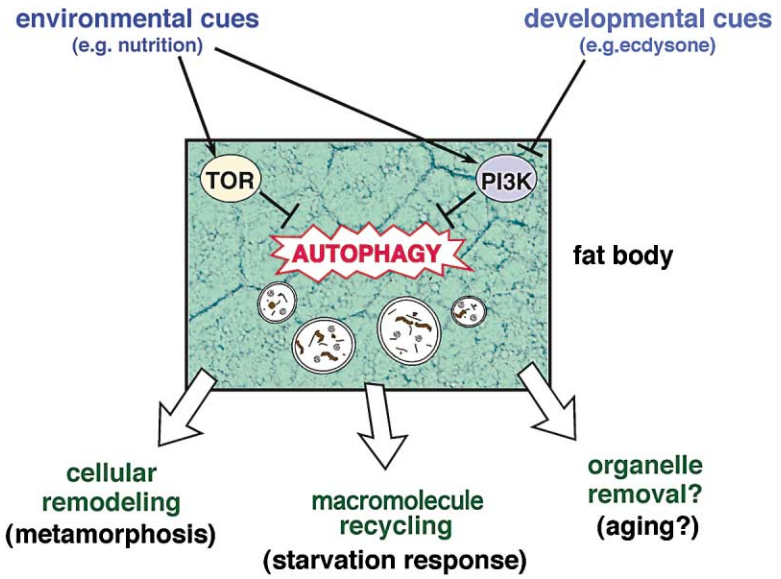

Figure 1. TOR and PI3K Signaling Mediate Autophagic Responses in the Fat Body of *Drosophila*

Both TOR and PI3K activity suppress autophagy in the fat body. In response to nutritional status or developmental signals, inhibition of TOR and/or PI3K activates the autophagic machinery, which subsequently can serve to promote metamorphosis or maintain nutritional homeostasis. Another possible consequence may be to protect against cellular aging. How TOR and PI3K engage the autophagic machinery remains unclear, as does how this signaling can produce such discrete outcomes.

that autophagosomes and lysosomes are rapidly assembled de novo following removal of dietary protein. Moreover, these groups showed that increased PI3K and TOR signaling could cell autonomously inhibit autophagy, whereas, even in the presence of abundant nutrients, larvae mutant for components in either signaling pathway had constitutively high levels of autophagy. Of the 20 yeast Atg genes dedicated to autophagy, Scott et al. identified *Drosophila* homologs for 11 (Klionsky et al., 2003). Using classic P-element mutants and RNAi, they showed that these genes were required for starvation-induced autophagy. These data provide evidence that the general mechanisms controlling autophagy are conserved from yeast to *Drosophila*.

One significant finding was that the larval fat body was the predominant organ that exhibited nutrient-dependent changes in autophagy. Other organs such as the salivary glands and gut had constitutive, nutrition-independent levels of autophagy. The fat body is thought to function as a sensor of nutritional status that controls organismal development and growth by secreting mitogens, growth factors, and nutrients (Britton and Edgar, 1998; Colombani et al., 2003). These are essential for supporting the development of other tissues, such as the nervous system and the imaginal discs, which eventually form the adult structures. These two new reports suggest that during periods of starvation, the fat body engages autophagy to allow it to maintain adequate haemolymph nutrient levels, which would ensure survival during unfavorable nutritional conditions. In fact, starved larvae can remain viable for an extended period and resume development upon return to favorable nutritional condition (Britton et al., 2002). In this respect, the fat body acts to buffer fluctuations in nutrition. Accordingly, Scott et al. demonstrated that blocking autophagy either in whole larvae or specifically within the fat body was extremely deleterious. Autophagy-incompetent larvae were unable to tolerate starvation and suffered reduced viability when nutrient deprived. Moreover, inhibiting autophagy in TOR mutants led to larvae arresting at a 6-fold smaller size. These findings suggest a critical role for autophagy in the ability of the fat body to act as a core regulator of organism homeostasis.

Drosophila larval tissues also exhibit marked autophagy at the end of larval life. This process is developmentally programmed and is responsible for triggering the removal of the larval organs, such as the fat body, during metamorphosis. Rusten et al. demonstrate that this autophagy is responsive to ecdysone signaling. Precocious activation of ecdysone signaling within the fat body early in development could induce autophagy, whereas inhibition of ecdysone blocked programmed autophagy. Interestingly, these ecdysone effects appeared to be mediated through the regulation of PI3K signaling. Using a marker for PI3 kinase activity, Rusten et al. showed that programmed autophagy is accompanied by a reduction in PI3K activity and could be blocked by constitutive expression of components of the PI3K pathway. Furthermore, alterations in ecdysone signaling inversely affected PI3K activity and, critically, the block of autophagy seen when ecdysone was inhibited could be restored by inhibiting PI3K signaling.

These new reports point to a general role for both PI3K and TOR signaling in the control of autophagy independent from their functions as regulators of cell growth in *Drosophila* (Figure 1). They also raise a number of key questions. For example, how does TOR regulate autophagy? A key finding was that the induction of autophagy occurred extremely rapidly (within an hour) following removal of dietary protein. Indeed, autophagy may be one of the most immediate cellular responses to starvation. Given the signaling pathways involved, a plausible scenario is that inactivation of TOR kinase activity rapidly alters the phosphorylation of key regulator(s) of autophagy. Perhaps the best-characterized downstream target of TOR is S6 kinase, but Scott et al. clearly show that it does not mediate TOR's effects on autophagy. Work in yeast has implicated Atg13 as a phosphorylation-dependent effector of TOR (Levine and Klionsky, 2004); however, no Atg13 homologs were identified in *Drosophila*. Interestingly, transcriptional induction of several of the *Drosophila* Atg homologs has been

reported in response to starvation (Zinke et al., 2002), suggesting that TOR may function to suppress the levels of the autophagy machinery. Nevertheless, the identity of the TOR effectors remains elusive. Similarly, it remains to be determined how PI3K signaling can regulate autophagy. Compared to the time course of autophagy induction, starvation-induced decreases in PI3K signaling occur significantly later. This suggests that PI3K may function indirectly to control autophagy, possibly by regulating and maintaining nutrient import.

The control of autophagy by PI3K and TOR may also have implications for the regulation of other processes. For example, in *C. elegans*, reductions in insulin/PI3K signaling lead to an extension of life span. Recent work has suggested that this phenomenon requires autophagy (Levine and Klionsky, 2004). Life span is also regulated by insulin/PI3 kinase and TOR signaling in *Drosophila* and, intriguingly, extension of life span is in part induced by reduced insulin signaling in the adult fat body (Giannakou et al., 2004; Hwangbo et al., 2004). Could the general effect of PI3K and TOR signaling on autophagy in the fat body be important in this context? One speculative possibility is that enhanced autophagy resulting from reduced PI3K or TOR signaling may allow for more efficient removal of damaged organelles, especially mitochondria. This would limit production of damaging free radicals and reactive oxygen species, which have been implicated in aging. The availability of *Drosophila* genetics will undoubtedly open the door to a more detailed understanding of the role of autophagy in metazoans.

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Signaling Mucins in the (S)limelight

Mucins may be the ugly ducklings of molecular biology. Their large size, repetitive nature, and unglamorous biological activities have not favored their study. However, integral membrane mucins have conserved intracellular C termini that may influence intracellular signaling. In a recent issue of *Genes & Development*, Cullen et al. show that the C terminus of membrane mucin-like Msb2 activates a CDC42/MAPK cascade to control filamentous growth of baker's yeast.

Mammalian mucins are large, highly O-glycosylated proteins that are secreted by a variety of epithelia (Agrawal et al., 1998; Carraway et al., 2003). They typically contain large numbers of repeat regions with a high serine-threonine content. The O-glycosylation of these repeat regions shapes mucins into rigid, extended conformations. The best-known class of mucins embodies the gel-forming constituents of viscous epithelial secretions. This class is thus largely responsible for the well-known, if not so attractive, physicochemical properties of mucus. Mucins that exemplify this class are MUC2, MUC5a and -b, and MUC6. Gel-forming mucins form extended polymers through disulfide-linkage of their N- and C termini. Other mucins, such as MUC7, are smaller and soluble, and occur in less viscous secretions such as saliva. Members of a third class of mucins share the property of being anchored in the epithelial cell membrane through a classical transmembrane domain. Prototypic mucins of this class are MUC1 and MUC4. Mucins play a key role in the protection and lubrication of vulnerable epithelial surfaces, be it as a membrane-bound shield or as a secreted constituent of the overlying mucin gel.

The class of membrane mucins has long been suspected to initiate or modulate intracellular signals (Carraway et al., 2003). The prototypic members MUC1 and MUC4 were originally discovered as carcinoma-associated molecular markers. Typically, they are overexpressed or differentially glycosylated by carcinomas, as compared to normal epithelial tissue. Upon cloning, MUC1 and -4 were found to be expressed by a variety of simple and squamous epithelia, including the airway, eye, the gastrointestinal and female reproductive tracts and the mammary gland. In simple epithelia, the two membrane mucins are sorted to the apical surface. Subsequent studies suggested that altered expression of the MUC genes supported tumor progression and metastasis (e.g., Komatsu et al., 2001). At least in part, these effects appeared to relate to the anti-adhesive and anti-immune recognition properties of these mucins (Agrawal et al., 1998). However, a more active role was