Developmental Timing: *let-7* Function Conserved through Evolution

Expression of the heterochronic microRNA *let-7* is tightly correlated with the onset of adult development in many animals, suggesting that it functions as an evolutionarily conserved developmental timer. This hypothesis has now been confirmed by studies in *Drosophila*.

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The current excitement surrounding microRNAs (miRNAs) makes it difficult to remember that this field arose from elegant genetic studies of developmental timing. The two founding members of the miRNA family, *lin-4* and *let-7*, were discovered in genetic screens for heterochronic defects in the nematode *Caenorhabditis elegans* [1–3]. As core components of the heterochronic pathway, *lin-4* and *let-7* act in genetic switches that regulate progression through stage-specific developmental events [4,5]. For example, the dramatic up-regulation of *let-7* near the end of larval development results in reduced expression of key heterochronic proteins that promote larval-specific cell fates, thereby ensuring the successful transition into adulthood [4,5]. As might be expected, the timing of *let-7* expression is crucial. Precocious *let-7* expression leads to the premature onset of adult fates while the absence of *let-7* retards exit from the juvenile stage [3,6].

In a landmark paper, Pasquinelli *et al.* [2] reported that the *let-7* miRNA is not restricted to nematodes but rather is conserved throughout bilaterian animals. More importantly, its temporal expression pattern is also conserved, with *let-7* induction tightly coordinated with the progression from juvenile to adult fates in all species examined. These observations raised the exciting possibility that *let-7* acts as an evolutionarily conserved regulator of adult fates, a hypothesis that has waited nearly a decade for *in vivo* confirmation. Two recent papers, by Caygill and Johnston in *Current Biology* [7] and Sokol *et al.* [8], have addressed this topic, and defined key roles for *let-7* in controlling the juvenile-to-adult transition in several tissues of *Drosophila melanogaster*.

let-7 Regulates Developmental Timing in *Drosophila*

The *Drosophila let-7* miRNA is induced at the onset of metamorphosis and processed from an RNA precursor that contains two other conserved miRNAs, *miR-100* and *miR-125* [9–11]. Both research teams use gene targeting to specifically delete *let-7*, although this mutation also inactivates *miR-125* in the study by Caygill and Johnson [7,8]. For the purpose of simplicity, we will focus on phenotypes that have been characterized using both a loss of *let-7* function as well as specific, ectopic *let-7* expression.

Caygill and Johnston [7] found that their mutant displays widespread defects during metamorphosis, with surviving adults having small wings. They attribute this phenotype to a significant reduction in cell size, in spite of the presence of more cells than wild type. Like other cuticular structures in the adult fly, the wing develops from a population of progenitor cells in the imaginal discs. These disc cells proliferate throughout larval stages until cell divisions cease at the onset of metamorphosis in preparation for terminal differentiation. Since *C. elegans let-7* acts to temporally restrict specific larval cell divisions, Caygill and Johnston [7] asked if the extra wing cells result from a delayed exit from the cell cycle. Consistent with this hypothesis, the cells in mutant wing discs continue to divide 24 hrs after puparium formation, a time when divisions have largely ceased in wild-type cells. In the reciprocal experiment, ectopic expression of *let-7* during larval development causes wing disc cells to precociously exit the cell cycle. Thus, the onset of *let-7* expression at puparium formation temporally restricts the period during which wing disc cells undergo division.

This newfound role for *let-7* in the imaginal discs provides an exciting complement to studies of *let-7* in other systems. Prior to this discovery, our understanding of *let-7* function *in vivo* has primarily focused on its roles in the development of a specific *C. elegans* epidermal cell type referred to as ‘seam cells’. When *let-7* is absent, the seam cells continue to divide and fail to undergo terminal differentation [3]. Although significantly less is known about *let-7* in humans and mice, the observation that *let-7* is abundantly expressed in differentiated tissues and absent in progenitor cells and certain types of cancer cells suggests a role in regulating cell proliferation [12,13]. Future studies of *let-7* in the *Drosophila* wing disc, which is an ideal system for detailed characterization of gene function, will provide a new opportunity to understand how this miRNA controls the timing of cell divisions in higher animals.

Studies in *Drosophila* may also help to unravel the role of *let-7* in other cell types. Sokol *et al.* [8] observed that *let-7* is expressed in a wide range of tissues, including the central nervous system, motor neurons, and muscle. Consistent with this expression pattern, both groups observe defects in neuromuscular development [7,8]. Sokol and colleagues [8] find that the dorsal internal oblique muscles (DIOMs), which are normally destroyed during adult maturation, persist in *let-7* mutant adults, while both groups show that the adult-specific dorsal muscles and their associated neuromuscular junctions appear immature when compared to wild-type controls.

The finding that *let-7* induces the destruction of the larval-specific DIOMs is intriguingly reminiscent of a recently described role for *let-7* in *C. elegans* male development, where a single cell known as the ‘linker cell’ dies at the larval-to-adult transition [14]. The death of this cell is dependent on the expression of *let-7* but

independent of the known apoptotic machinery. Does *Drosophila let-7* regulate DIOM destruction via a similar mechanism? If so, then the molecular pathways that govern this response may provide important new insights into our understanding of cell-death regulation. Moreover, the behavioral defects seen in *let-7* mutants and the widespread expression of *let-7* in the nervous system of both flies and humans suggests that further studies of fly *let-7* will provide insights into the regulation of neuronal maturation during adult development [3,7,8].

Discovery of a New *let-7* Target

The characterization of *let-7* in *Drosophila* provides, for the first time, an opportunity to identify its *in vivo* targets in an organism other than *C. elegans*. Caygill and Johnston [7] have taken advantage of this new resource by demonstrating that the BTB-zinc finger transcription factor Abrupt (Ab) is regulated by *let-7*. Several important characteristics of the *abrupt* gene define it as a likely direct target for *let-7* control. The 3'-untranslated region of the *abrupt* mRNA contains five *let-7* binding sites [15], and Ab protein is downregulated during metamorphosis in synchrony with the upregulation of *let-7*. Furthermore, Ab protein levels are intimately linked to *let-7* expression. While ectopic *let-7* expression in wing disc cells leads to precocious downregulation of Ab protein, the levels of Ab remain high in *let-7* mutants. Moreover, the retarded dorsal muscle and neuromuscular junction phenotype observed in *let-7* mutants is suppressed by a partial loss of *abrupt* function, suggesting that this phenotype is caused by Ab overexpression. Ab thus appears to be a critical regulator of developmental timing in *Drosophila*, inhibiting adult fates until it is down-regulated by *let-7* during terminal differentiation.

Integrating Heterochronic and Endocrine Timers

As we have discussed previously [16], studies in *C. elegans* and *Drosophila* have revealed two distinct aspects of developmental timing. Studies in *C. elegans* have focused primarily on roles for the heterochronic genes in assigning temporal identity to specific cells within the context of their defined lineages. In contrast, studies in *Drosophila* have focused on the role of systemic pulses of the steroid hormone 20-hydroxyecdysone (20E) in establishing temporal boundaries that define developmental transitions in the life cycle [16]. The discovery of heterochronic functions for *let-7* in *Drosophila* provides an exciting new opportunity to integrate these two pathways. The fly *let-7* miRNA is induced in late third instar larvae, in precise synchrony with known direct targets of 20E and its EcR receptor, as the hormone triggers puparium formation and the onset of adult differentiation [2,9,11]. Curiously, however, this induction appears to occur independently of either 20E or EcR, implying the existence of other systemic temporal regulators [9]. This could be provided by the precursor to 20E, ecdysone, which also peaks in late third instar larvae and appears to have distinct hormonal activity [17,18]. Interestingly, some *let-7* target genes also appear to be regulated by 20E. Both *abrupt* and *brat*, a *lin-41* homolog, are induced by 20E and potentially down-regulated by *let-7* [17,19]. Future studies of *let-7* regulation in *Drosophila* as well as the characterization of additional *let-7* targets should provide a new basis for understanding how the temporal identity conferred by heterochronic genes is integrated with the systemic temporal boundaries established by hormone signaling.

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