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Racing against the clock: How flies regenerate just in time

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In this issue of *Developmental Cell*, Cohen et al. show that the *Drosophila* hindgut is a genetically tractable model for studying tissue regeneration. This tissue exhibits different regeneration strategies at different developmental times, demonstrating that the hindgut developmental clock, not tissue type, dictates the mode and capacity for regeneration.

Tissues need to respond to damage that occurs during development as well as throughout an adult organism's lifespan. Failure to respond to damage can result in a variety of developmental birth defects, organ failure, and death. Regenerative capabilities can vary widely among tissue types and at different developmental stages. For example, during the neonatal stage, damage to the mammalian heart results in additional cell divisions to restore tissue mass, while damage later in development does not lead to regeneration. This is in part because as the organism matures, cardiomyocytes transition from a mitotic cell cycle into a variant cell cycle, called "endoreduplication" ("endocycle"), in which cells enter S-phase, increase DNA content, and become polyploid, but do not undergo mitosis. The transition from a mitotic to a post-mitotic mode of tissue growth is termed the "mitotic-to-endocycle" (M-E) transition (Schaeffer et al., 2004). M-E transitions are conserved across many organisms in differentiating tissues, including the epidermis, specific types of muscle, and glandular epithelium (Gandarillas et al., 2018). Although endocycles in the mammalian heart do not restore function after damage, endocycles in other tissues can contribute to restoration of tissue function, perhaps most famously in the human liver. Transitions to post-mitotic states with poorer regeneration capability are often controlled by temporal hormonal signals, for example thyroid hormone triggering metamorphosis and limited regeneration in frogs, or ecdysone hormone, initiating metamorphosis and limiting regeneration in *Drosophila* (Halme et al., 2010). Delaying developmental transitions and tissue maturation can therefore prolong mitotic regenerative capacity in tissues, and this has been described in detail for the *Drosophila* wing (Colombani et al., 2012; Garelli et al., 2012).

Generally, developing damaged tissues can functionally regenerate via one of three main mechanisms: developmental delay of the organism while a tissue recovers, as in the *Drosophila* wing; an acceleration of the cell cycle to allow for additional cell divisions before a post-mitotic transition, as in the neonatal heart; and entry into variant cell cycles such as the endocycle for compensatory growth exemplified by the liver.

In this issue of *Developmental Cell*, Cohen and colleagues (2021) show that the *Drosophila* hindgut is a novel, genetically tractable model that can be used to study all three modes of regeneration and to examine key players that drive the M-E transition. Remarkably, the same tissue exhibits each of these modes of regeneration at different times during development, providing an example in which the developmental clock, rather than tissue type, dictates the mode and capacity for regeneration.

Cohen et al. find that damage to the *Drosophila* hindgut during early larval stages induces a developmental delay to allow for recovery, while damage in later larval stages and early metamorphosis induces an accelerated cell cycle

which allows for recovery without delay, before the transition to a post-mitotic state. Finally, damage to the adult tissue induces endocycling to allow for recovery after transition to a post-mitotic state (Cohen et al., 2018). The key to "racing against the clock" is that injury during later larval stages induces Jak/STAT signaling via the ligand Upd3, which accelerates the cell cycle by shortening the G1 phase. Cohen et al. then show that Dichaete (D), a sox-domain-containing transcription factor, and Fizzy-related (Fzr, Cdh1), an activator of the mitotic Cyclin/Cdk ubiquitin ligase APC/C, are factors that terminate the hindgut's mitotic capacity for regeneration. Termination is induced by a systemic pulse of ecdysone, the *Drosophila* steroid hormone that triggers metamorphosis, which activates Fzr expression through upstream regulatory elements. Thus, the damaged *Drosophila* hindgut is racing against the "hormone clock" to regenerate via mitotic cycles before ecdysone induces activation of Fzr and the transition to a postmitotic state.

In the *Drosophila* hindgut, Fzr is broadly expressed, yet must be tightly temporally controlled because it closes the window of opportunity for mitotic regeneration. An important picture is emerging about how rate-limiting cell cycle genes are temporally controlled during development. Although they often exhibit broad expression, they are not regulated like housekeeping genes. They can have complex, modular enhancers like many developmental patterning genes (Jones et al., 2000; Lehman et al., 1999), and the use of these enhancers can be temporally regulated at the level of chromatin accessibility to allow cell cycle modulation at the right times (Ma et al., 2019).

The findings by Cohen and colleagues (2021) raise additional unresolved questions. First, the data presented show the hindgut as a model in which developmental delays do not occur when damage takes place at a later developmental stage. But how does the amount of damage a tissue receives impact an organism's decision to undergo developmental delays? Would a larger amount of damage in the hindgut during this later developmental stage lead to developmental delay? Conversely, would a smaller amount of damage in the hindgut during early development lead to accelerated cell cycles without delay? Second, this manuscript shows that Upd3 plays a critical role in the acceleration of the cell cycle seen during late developmental regeneration. An important next step will be to elucidate the mechanism downstream of Upd3 activation. What is the target of Upd3 signaling and how does the activation of this target speed up G1? Third, this study finds that Dichaete has an important role in the M-E transition and identifies Dichaete binding motifs within *fzr*. Together, these data suggest that Dichaete is playing a role in *fzr* regulation, however, it remains unclear if this is a linear pathway. Is the loss of mitotic capacity due to Dichaete regulation of *fzr*, or does Dichaete have other targets that play a role in the M-E transition?

Cohen and colleagues present the *Drosophila* hindgut as a powerful system for studying multiple modes of regeneration. This is exciting because relatively little is known about how and why some tissues can functionally regenerate via endocycling while others cannot. Although it is known that most mammalian tissues transition from a developmental, mitotic stage to a post-mitotic adult stage, little is known about why post-mitotic regeneration is so limited in most mammalian tissues. Understanding ways to allow regeneration after the clock signals the post-mitotic transition will be an important step toward increasing regenerative capacity for adult tissues, and perhaps even one day, can help mend a broken heart.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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