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Short communication

Dissecting the function of Cullin-RING ubiquitin ligase complex genes in planarian regeneration

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ABSTRACT

The ubiquitin system plays a role in nearly every aspect of eukaryotic cell biology. The enzymes responsible for transferring ubiquitin onto specific substrates are the E3 ubiquitin ligases, a large and diverse family of proteins, for which biological roles and target substrates remain largely undefined. Studies using model organisms indicate that ubiquitin signaling mediates key steps in developmental processes and tissue regeneration. Here, we used the freshwater planarian, *Schmidtea mediterranea*, to investigate the role of Cullin-RING ubiquitin ligase (CRL) complexes in stem cell regulation during regeneration. We identified six *S. mediterranea cullin* genes, and used RNAi to uncover roles for homologs of Cullin-1, −3 and −4 in planarian regeneration. The *cullin-1* RNAi phenotype included defects in blastema formation, organ regeneration, lesions, and lysis. To further investigate the function of *cullin-1*-mediated cellular processes in planarians, we examined genes encoding the adaptor protein Skp1 and F-box substrate-recognition proteins that are predicted to partner with Cullin-1. RNAi against *skp1* resulted in phenotypes similar to *cullin-1* RNAi, and an RNAi screen of the F-box genes identified 19 genes that recapitulated aspects of *cullin-1* RNAi, including ones that in mammals are involved in stem cell regulation and cancer biology. Our data provides evidence that CRLs play discrete roles in regenerative processes and provide a platform to investigate how CRLs regulate stem cells *in vivo*.

1. Introduction

Planarians have emerged as an important model organism to examine gene function in stem cell-based tissue regeneration (Elliott and Sánchez Alvarado, 2012; Roberts-Galbraith and Newmark, 2015; Ross et al., 2017). These animals can restore lost or damaged tissues from a population of adult pluripotent stem cells, termed neoblasts (Baguñà, 2012; Rink, 2013; Wagner et al., 2011; Zhu and Pearson, 2016). Recent studies have provided insights into the molecular mechanisms that regulate regeneration in planarians at the genetic level (Elliott and Sánchez Alvarado, 2012; Roberts-Galbraith and Newmark, 2015; Wurtzel et al., 2015). However, the dynamic regulation of proteins during regeneration remains an open area of investigation. An essential cellular pathway in protein regulation is the ubiquitin system, in which cells utilize the highly conserved small ubiquitin polypeptide as a post-translational modification of other proteins, which can lead to degradation of target proteins (Ciechanover et al., 1984, 1980; Finley et al., 1984; Hershko et al., 1980). The ubiquitin-system plays a crucial role in diverse cellular processes, including DNA repair, transcription, synaptic plasticity, and regulation of the cell cycle, wherein ubiquitin-mediated proteolysis is a key regulatory step (Bennett and Harper, 2008; Dhananjayan et al., 2005; Finley et al., 2004; Glickman and Ciechanover, 2002; Hershko and Ciechanover, 1998; Hershko et al., 2000; Kawabe and Brose, 2011; Nakayama and Nakayama, 2005; Pickart, 2004; Varshavsky, 2005).

Ubiquitin is directed onto specific substrate proteins by E3 ubiquitin ligases (Ardley and Robinson, 2005; Dikic and Robertson, 2012; Glickman and Ciechanover, 2002; Hershko and Ciechanover, 1998), a large class of enzymes (over 600 predicted genes in humans; Li et al., 2008) for which many of the target specificity and function remain poorly understood. Identification of the biological roles of the E3s has been facilitated by siRNA screens using human cells *in vitro*, and by genetic screens in model organisms, such as *Drosophila* and *C. elegans* (Williamson et al., 2013). Specific roles for ubiquitin ligases have been demonstrated in embryonic stem cell fate determination (Werner et al., 2017; Xu et al., 2009), eye development (Ou et al., 2003), and neural development (Boix-Perales et al., 2007; Bury et al., 2008; J. Chen et al., 2012; D'Arca et al., 2010; Hoeck et al., 2010; Sobieszczuk et al., 2010;

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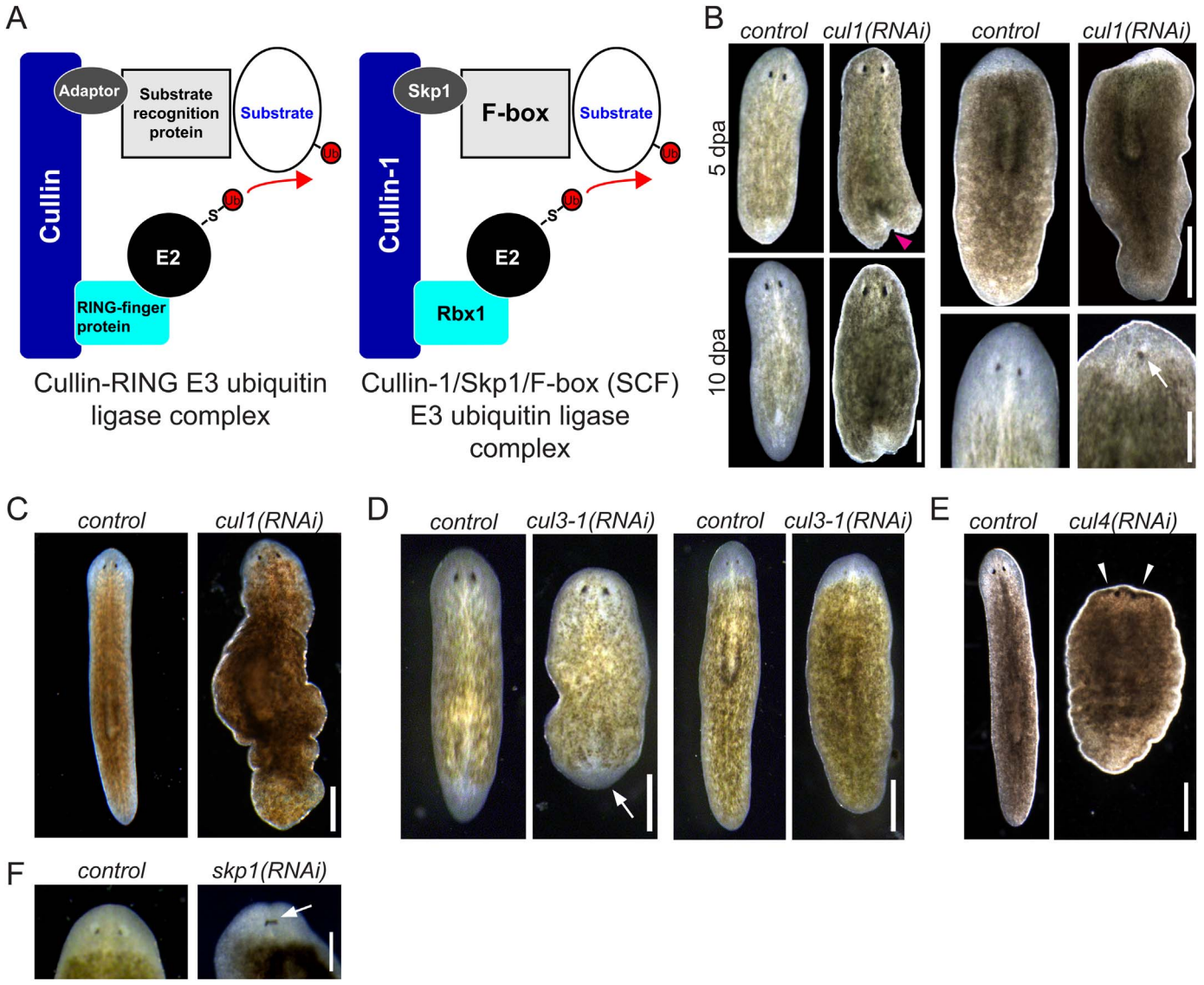

Fig. 1. Planarians possess multiple Cullin genes with distinct roles in regeneration and tissue homeostasis. A) Diagram summarizing the general organization of Cullin-RING E3 ubiquitin ligase complexes or the prototypical Skp1/Cullin-1/F-box (SCF) E3 ubiquitin ligase complex. E3 ubiquitin ligases transfer ubiquitin to the substrate by forming an isopeptide bond. B) Animals were fed dsRNA 6 times over 3 weeks against *gfp* (*controls*; n = 22) or *cul1* (n = 22), amputated pre-pharyngeally and allowed to regenerate for 10 days. Magenta arrowhead in head regenerate at 5 dpa indicates indented blastema in *cul1(RNAi)* planarian. White arrow marks abnormal regeneration in a single eye spot in the head of a *cul1(RNAi)* trunk regenerate. C) Animals were fed dsRNA over 6–8 weeks against *gfp* (*controls*; n = 38) or *cul1* (n =38). All *cul1(RNAi)* worms showed loss of mobility, formed lesions, and subsequently lysed. D) Animals were fed dsRNA 5 times over 3 weeks against *gfp* (*control*; n = 30) or *cul3-1* (n = 21), amputated pre-pharyngeally, and allowed to regenerate for 10 days. *cul3-1(RNAi)* planarians showed delayed regeneration and differentiation when compared to the controls. White arrow marks the small blastema observed in head regenerates. E) Animals were fed dsRNA 5 times over 3 weeks against *gfp* (*control*; n = 30) or *cul4* (n = 21), amputated pre-pharyngeally, and allowed to regenerate for 10 days. F) Animals were fed dsRNA 6 times over 3 weeks against *gfp* (*control*; n = 50) or *skp1* (n = 45), amputated pre-pharyngeally, and allowed to regenerate for 10 days. White arrow denotes abnormal eye regeneration. Scale bars = 500 μm.

Voigt and Papalopulu, 2006; Zhao et al., 2008; Zhu et al., 2005). Ubiquitin system components regulate regeneration in nematodes, flies, and mice, and are specifically upregulated during regeneration in sea cucumbers and axolotls (Hindi and Kumar, 2016; Pasten et al., 2012; Rao et al., 2009; Tian and Wu, 2013).

We are utilizing planarians as a model system to investigate the roles of E3 ubiquitin ligases in tissue regeneration. Previously, our lab demonstrated that members of the HECT E3 ligase gene family, which directly catalyze ubiquitin transfer onto a substrate via a ubiquitin-HECT complex intermediate (Metzger et al., 2012), are required for diverse aspects of regeneration in the planarian *Schmidtea mediterranea* (Henderson et al., 2015). In contrast to the HECT family, most E3 enzymes do not directly bind and transfer ubiquitin but rather coordinate the transfer of ubiquitin from an E2 onto a substrate, often through multimeric complexes, including the Cullin-RING ligase (CRL) family (Sarikas et al., 2011). Cullin proteins act as molecular scaffolds that organize the binding of other elements to form an E3 complex that requires a substrate recognition subunit (Fig. 1A). These recognition subunits confer target specificity for ubiquitylation and their differential utilization allows modularity within CRL classes, thereby enabling function in multiple aspects of cellular biology.

In this study, we analyzed CRL function in tissue regeneration by inhibiting genes encoding major components of these complexes in planarians. First, we identified and performed RNAi against *cullin* genes present in *S. mediterranea* and found that homologs of Cullin-1, -3 and -4 are involved in regulating tissue homeostasis and regeneration. Robust *cullin-1* RNAi phenotypes included lesions, lysis, and defects in blastema formation, organ regeneration, and homeostatic tissue maintenance. Cullin-1 is a core component of the canonical CRL, the SCF (Skp1/Cullin-1/F-box)-E3 ubiquitin ligase complex (Fig. 1A)