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Research paper

DjRlc is required for the intestinal regeneration in planarian *Dugesia japonica*

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ABSTRACT

The myosin regulatory light chain (RLC) proteins play an important role in cellular processes, especially in muscle contraction. The planarian intestine is a fascinating system for studying the organogenesis during regeneration. In this paper, A homolog gene of Rlc, *DjRlc*, was identified and characterized in *Dugesia japonica*. The *DjRlc* sequence analysis revealed that it contains an opening reading frame encoding a putative protein of 175 amino acids with functionally domains that are highly conserved, including an EF-Hand motif and Ca2+ binding sites. Whole mount *in situ* hybridization showed that *DjRlc* is predominantly expressed in the intestine of intact and regenerating planarians. The cross sections of planarians revealed that the *DjRlc* distributes in the muscle of intact planarians. Knockdown of RNA interference of *DjRlc* by dsRNA-*DjRlc* affected the intestinal morphology, causing distinct defects in branching morphogenesis. These finding suggest that *DjRlc* is required for intestinal regeneration.

1. Introduction

Smooth muscle contraction is activated primarily by phosphorylation of myosin regulatory light chain (RLC)(He et al., 2008; He et al., 2011). The phosphorylation level of RLC is controlled by the balanced activities between myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) (Somlyo and Somlyo, 2003; Bitar, 2003). Myosin phosphatase targeting subunit 1 (MYPT1) phosphorylation may contribute to force generation by reorganization of the actin cytoskeleton (Perrino, 2016). RLC dephosphorylation by myosin light chain phosphatase containing a MYPT1 leads to myosin inactivation (Xia et al., 2005). Many cell functions are regulated by the MLCK-dependent RLC phosphorylation, including cell migration, adhesion, and epithelial barrier regulation (Xia et al., 2005;Mashukova et al., 2011; Shen et al., 2011; Su et al., 2013; Zha et al., 2016). Disruption of the balance between the coordinated activities of the MLCK and MLCP inhibits cell migration and adhesion (Hirano et al., 1997; Tóth et al., 2000; Velasco et al., 2002; Terrak et al., 2004). In addition, the phosphorylation of RLC is important for tumor cell proliferation and division, as breast cancer cell growth is blocked when the MLCK is inhibited pharmacologically (Bessard et al., 2006; Zhou et al., 2008; Yamashiro et al., 2008; Petecchia et al., 2010; Zhang et al., 2013). Pharmacological inhibitors of MLCK disrupt cell migration and adhesion that are associated with suppressed RLC phosphorylation (Hirano et al., 1997; Tóth et al., 2000; Mulder et al., 2005). Recent evidence suggests that RLC phosphorylation plays a central role in the regulation of the contractile response of gastrointestinal smooth muscles (Perrino, 2016). However, the relative contribution of RLC to the gastrointestinal smooth muscles is not fully understood.

Dugesia japonica, a freshwater planarian, can regenerate a complete individual from a head, trunk or tail fragment *via* activation of somatic pluripotent stem cells (named neoblasts). It has emerged as a powerful model system for studying the mechanism of development and regeneration (Newmark and Alvarado, 2002). The planarian has very simple internal structures, including the central nervous system, excretory system, digestive system and muscular system. The digestive system consists of a mouth, pharynx, and three intestinal branches. Planarians grow and regenerate organs by coordinating proliferation and differentiation of pluripotent stem cells for the remodeling of postmitotic tissues (Newmark and Alvarado, 2002; Wagner et al.,

Abbreviations: RLC, regulatory light chain; DjRlc, *Dugesia japonica* regulatory light chain; MLCK, myosin light chain kinase; MLCP, myosin light chain phosphatase; MYPT1, myosin phosphatase targeting subunit 1; NCBI, National Center for Biotechnology information; ORF, open reading frame; PCR, polymerase chain reaction; qRT-PCR, quantitative real-time PCR; RACE, rapid amplification of cDNA ends; PFA, paraformaldehyde; SSC, salinesodium citrate; RNAi, RNA interference; qRT-PCR, quantitative real-time PCR; UTR, untranslated region

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Table 1
Primers used in the experiments.

Name	Forward primer(5′-3′)	Reverse primer(5′-3′)
PCR		
Rlc	ATGGCATCTACAAAGACAAG	AAGATTAGGATTATTCAGA
RACE		
Rlc5	CCAGTCATTTTTTCACCGAATAAAGTTAG	
Rlc3	TGACTTCAATGGGTGATCGG	
Real-timePCR		
Rlc-RT	CTTCACTTGGTAAAGATGCC	TCTCGAAGATGTTCTTCAG
β-Actin	ACACCGTACCAATCTATG	GTGAAACTGTAACCTCG

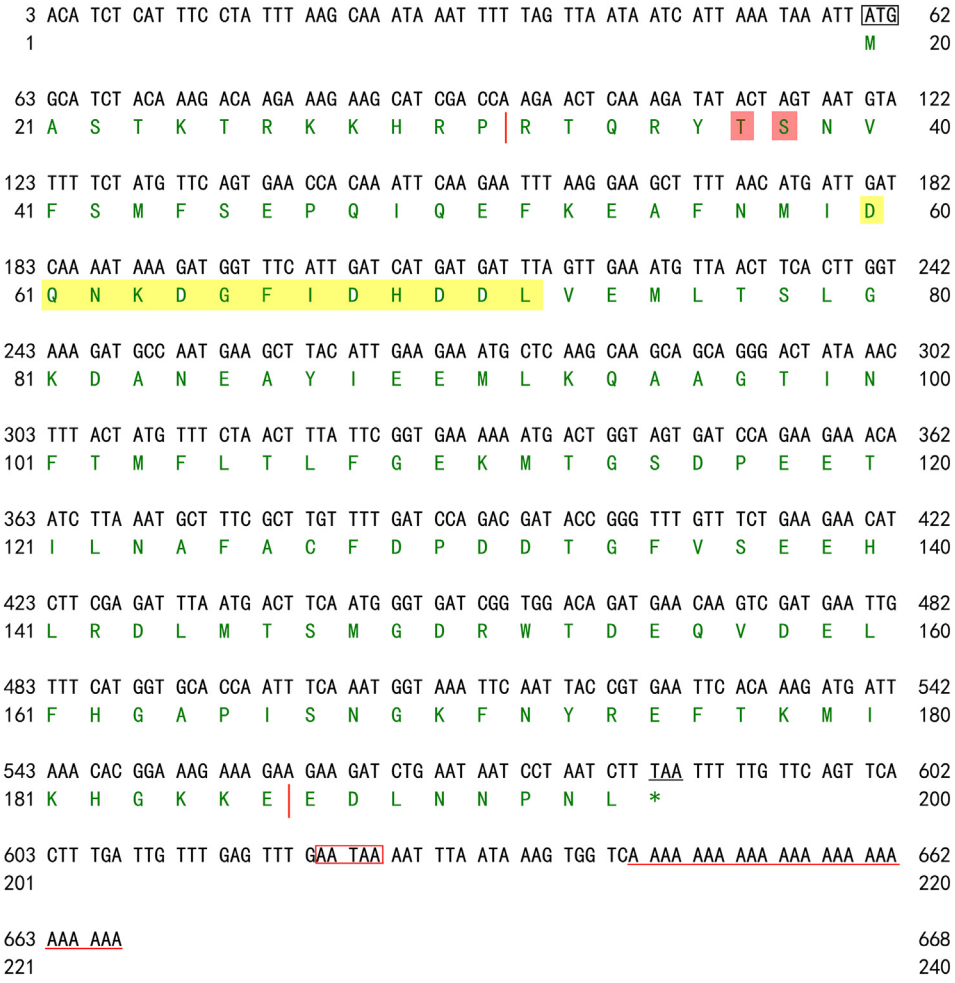

Fig. 1. The DjRlc cDNA sequence. The amino acids of the longest open reading frame(ORF) are in green. The initiation codon ATG is in black box. Termination codon is labeled with star, and the tailing signal is in red box. The EF-Hand motif is between the red vertical lines. The phosphorylation site is painted in red solid box. The Ca^{2+} binding site is painted in yellow solid box. The polyadenylation signal is red underlined. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2011). After amputation, neoblasts proliferate and differentiate, regenerating a variety of organs, including the intestine (Forsthoefel et al., 2011). Therefore, the planarian intestine provides an attractive model system in which to examine mechanisms of regeneration and organgenesis. Using RNA interference (Forsthoefel et al., 2012) identified cytoskeletal regulators required for intestinal branching morphogenesis. In this paper, we identified an Rlc gene homolog in planarian *D. japonica* and studied its expression patterns using whole mount *in situ* hybridization, and characterized a loss-of-function phenotype in the regenerating animals.

2. Materials and methods

2.1. Animals

The planarians *D. japonica* were catched at the foot of the mountain fountain in Quanhetou, Boshan, Shandong, China, and were cultured in clean Lushan spring water. The planarians were starved for at least one week before the experiments.

2.2. Rlc cDNA cloning

A set of degenerate PCR (polymerase chain reaction) and RACE