The FEBS Journal

FEBS PRESS science publishing by scientists

COMMENTARY

Genetic interaction between DNA replication and the Notch signaling pathway

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(Received 12 June 2018, accepted 12 June 2018)

doi:10.1111/febs.14584

The Notch pathway is a widely conserved cell signaling system found in most metazoans. In the nematode, *C. elegans*, GLP-1/Notch is required for developmental cell fate decisions in multiple contexts. In the germline, GLP-1/Notch signaling promotes proliferation of germ cells and controls the switch from mitosis to meiosis. An important aspect of cell proliferation is the replication of the genome. In this issue of *The FEBS Journal*, Yoon *et al.* [1] examined the requirements for components of Pol α-Primase complex, an essential component of DNA replication, in Notch-dependent proliferation in the germline.

Initiation of eukaryotic DNA replication requires synthesis of short RNA-DNA primers by the Polymerase α-Primase complex [2,3]. The complex comprises four polypeptides. The primase portion consists of a small catalytic subunit (PriS, PRI-1 in *C. elegans*) and a large regulatory subunit (PriL/PRI-2). The polymerase portion consists of a DNA polymerase α subunit (Pol α/POLA-1) and its B subunit (DIV-1). The primase synthesizes a short RNA of 7–12 nucleotides that is extended by Pol α as DNA to a length of 20–25 nucleotides. At the replication fork, the main polymerases Pol ε and Pol δ extend these primers on the leading and lagging strands, respectively (Fig. 1).

Gonad development in *C. elegans* is diagrammed in Fig. 2. The hermaphrodite gonad develops postembryonically from a small set of somatic and germline precursors. Over four larval stages, the two terminal Distal Tip Cells (DTCs) induce mitotic proliferation of the germ cells as the gonad grows in length (Fig. 2A). The DTCs, initially leading gonad formation by migrating away from each other, turn and migrate toward each other. The DTCs maintain a niche in which mitotic germline stem cells (GSCs) are maintained. GSCs exiting the niche enter meiosis to form spermatocytes, before the gonad changes to oogenesis for the remainder of the life span [4,5].

The conserved GLP-1/Notch pathway is the mechanism by which the DTCs control proliferation and maintenance of the mitotic pool of GSCs (Fig. 2C) [6].

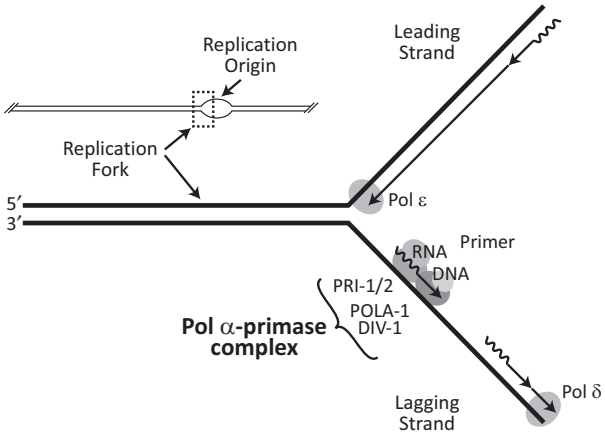

Fig. 1. Polymerase alpha-primase complex functions in replication. Short primers consisting of RNA (zig-zag line) and DNA (straight line) are synthesized by the complex. The 3′OH of the RNA/DNA primer is extended by Pol epsilon on the leading strand and Pol delta on the lagging strand. Redrawn from [3].

Abbreviations

FBF, *fem-3* binding factor; GLP, germline proliferation; PUF, Pumilio and FBF; RNAi, RNA interference.

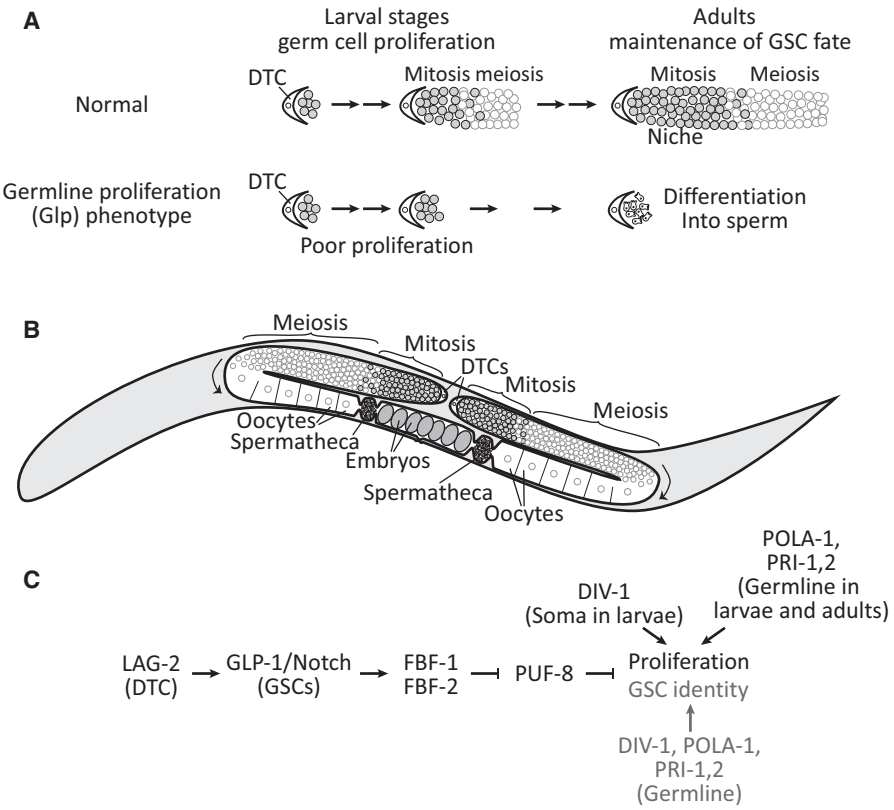

Fig. 2. Development and anatomy of the *Caenorhabditis elegans* hermaphrodite gonad and role of Notch signaling. (A) The distal tip cell (DTC) controls proliferation of germ cells in larval development and maintenance of the GSC niche in adults. Only one gonad arm is shown. (B) The fully formed gonad in an adult hermaphrodite. Adult *C. elegans* are about 1 mm long. (C) Genetic interaction of Pol α-primase components with the network that maintains GSC identity. Modified from [1,4].

The DTCs express the Delta-like ligand LAG-2, while downstream of Notch signaling received by the GSCs, a complex network of RNA-binding proteins, primarily those of the PUF family, regulates the choice between self-renewal and entry into meiosis [6]. The paralogous FBF-1 and FBF-2 regulators are a central pair of these PUF regulators [7]. If transduction of the Notch signal is prevented, as in a *glp-1(null)* background, a germline proliferation (Glp) defect, and sterility, result (Fig. 2A). Conversely, overactivation of Notch signaling results in a germline tumor [8].

The paper by Yoon *et al.* examined genetic interactions between DNA replication and the Notch pathway in maintenance of GSCs. Such an interaction might be expected because a central feature of cellular proliferation is replication of the genome. The authors use a *glp-1(bn18)* temperature sensitive strain grown at a permissive temperature of 20 °C, at which this strain exhibits only a mild Glp defect. Treatment with the S-phase inhibitor hydroxyurea caused a greatly enhanced Glp defect in *glp-1(bn18)*, consistent with a role for S phase progression in Notch-dependent GSC proliferation. The authors then used RNAi during larval development to target individual several S phase regulators, and, among some of the genes tested, found that RNAi of the regulatory polymerase alpha subunit gene *div-1* greatly enhanced the Glp phenotype of *glp-1(bn18)*. Curiously, RNAi of the other DNA Polymerase α-Primase complex components *pola-1*, *pri-1,* and *pri-2* individually enhanced the Glp phenotype only mildly.

By testing mutant backgrounds that are competent for either somatic or germline RNAi, it was found that RNAi of *div-1* in the soma, but not the germline, synergized with *glp-1(bn18)* in its germline proliferation phenotype. This is an unexpected result, as it would be predicted that loss of the polymerase α regulatory subunit would affect the cells in which it is directly required. A *div-1*::GFP reporter is expressed in many somatic tissues including the somatic gonad and DTCs, suggesting that it is plausible that nearby tissues influence Notch-dependent proliferation. However, *div-1(RNAi)* does not seem to affect development of the somatic gonad, nor does it affect

expression of a *lag-2*::GFP transgene in the DTCs, or expression of GLP-1 protein in the mitotic pool of germ cells. Hence, the mechanism by which somatic *div-1(RNAi)* affects germline proliferation is not clear.

Yoon *et al*. also examined the ongoing requirement for Polymerase α-Primase complex components in adults, by performing the same RNAi treatments in *glp-1(bn18)* animals starting in the last larval stage (L4) after developmental proliferation of the germ cells has occurred. The authors quantified Glp defects by using antibody staining to detect markers for mitotic and meiotic cells. Here, RNAi depletion of *pola-1, pri-1,* and *pri-2* enhanced the Glp mitotic proliferation phenotype of *glp-1(bn18)*, but, notably, *div-1(RNAi)* did not, showing that the requirement for DIV-1 in proliferation of germline cells is restricted to larval development. Use of a *ppw-1* mutant background, which is deficient in germline RNAi, prevented the enhanced Glp phenotype of RNAi of *pola-1, pri-1,* and *pri-2*, confirming a cell-autonomous requirement for these components within the adult germline.

Finally, the authors use an unusual assay to test for requirement for the polymerase alpha-primase subunits in PUF-mediate GSC maintenance. They first demonstrate that although *fbf-1 fbf-2* double mutants have a Glp phenotype as previously reported [9], simultaneous loss of the related gene *puf-8* can restore the population of mitotically dividing cells. The authors interpret this as meaning that PUF-8 normally antagonizes FBF-1/2 promotion of GSC identity, however, given the repressive nature of PUF factors it is possible to interpret the genetic interaction as FBF-1,2 repressing PUF-8, which in turn represses GSC identity as shown in Fig. 2C [10]. Using the *puf-8; fbf-1 fbf-2* triple mutant and including a genetic background that permits only germline-specific RNAi, Yoon *et al*. find that RNAi of *pola-1*, *div-1*, *pri-1,* or *pri-2* dramatically abrogates mitotic divisions. The authors conclude that all four polymerase α-primase complex components are likely to contribute at some level to maintenance of GSC identity.

The work by Yoon *et al*. raises many interesting questions. Prior work in the *C. elegans* embryo found that mutants in *div-1* result in specification defects due to delays in cell cycles in early embryonic lineages [11]. A later work showed that these delays were due to activation of a DNA replication checkpoint that relies on the kinase CHK-1, because inactivation of *chk-1* in a *div-1* mutant background restored cell divisions at the expense of causing chromosomal structure defects [12]. Curiously, Yoon *et al*. found that RNAi of *chk-1* produced as strong a Glp phenotype in *glp-1(bn18)* as RNAi of *div-1*, suggesting that the checkpoint is required for normal mitotic cell cycle progression in the germline. Similarly, the mechanism behind apparent cell nonautonomous contribution of DIV-1 to Notch-dependent germline proliferation remains a mystery, and suggests that at some level, somatic cells that can support DNA replication send signals to the germline.

Another question is how proliferation of cells could proceed without the activity of primase, which is a prerequisite for replication. Pol δ and Pol ε are unable to synthesize DNA *de novo* or from a 3′OH end provided by an RNA, hence DNA replication cannot proceed without a primase-polymerase. It seems likely that residual primase-polymerase activity remains in the individual knockdowns, as the experiments in Yoon *et al*. relied on RNA interference by bacterial feeding, which may not eliminate all activity. Yoon *et al*. report that they confirmed elimination of DIV-1 in *div-1(RNAi)* by western blotting and immunohistochemistry, however, the results need to be replicated with genetic elimination of *div-1*, for example, using a conditional allele [11]. There is evidence from the study that RNAi of *pri-1* and *pri-2* may not be complete. A prior work by Fox *et al*. found that RNAi of *pri-1* did not enhance the Glp phenotype of *glp-1(bn18)* [13]. However, in this work Yoon *et al*. attribute the results of Fox *et al*. to their shorter RNAi treatment, and show that simultaneous knockdown of both *pri-1* and *pri-2* was sufficient to produce the enhanced Glp defect under the shorter RNAi conditions used by Fox *et al*.; the increased severity of a *pri-1; pri-2* double knockdown further suggests that neither individual RNAi treatment completely abrogates function of these genes. Alternatively, there may be unknown unique functions for these subunits.

Other possibilities for replication with limiting primase activity are pathways that are activated at stalled replication forks, such as recombination, which could provide 3′OH DNA ends on a lagging strand without the need for primase [14]. There may also be as-yet-unidentified proteins that might provide this function. A recently discovered human protein, PrimPol, has both primase and polymerase activities in a single protein [15], although neither *C. elegans* nor *Drosophila* have apparent PrimPol orthologs. While an alternative primase-polymerase activity cannot be ruled out, it seems unlikely that complete elimination of this important complex in *C. elegans* would support any proliferation [3].

Regardless of the mechanisms, Yoon *et al*. have uncovered some interesting questions for future exploration in the relationship between DNA replication components and Notch-dependent germline proliferation in *C. elegans*. With a well-defined system in hand, these questions can be tackled in future work.

Acknowledgments

MM thanks Judith Kimble (UW-Madison, WI) and Connie Nugent (UC Riverside, CA) for helpful consultation.

References

1 Yoon DS, Cha DS, Alfhili MA, Keiper BD & Lee MH (2018) Subunits of the DNA polymerase alpha-primase complex promote Notch-mediated proliferation with discrete and shared functions in *C. elegans* germline. *FEBS J*, https://doi.org/10.1111/febs.14512.

2 Muzi-Falconi M, Giannattasio M, Foiani M & Plevani P (2003) The DNA polymerase alpha-primase complex: multiple functions and interactions. *Sci World J* **3**, 21–33.

3 Pellegrini L (2012) The Pol alpha-primase complex. *Subcell Biochem* **62**, 157–169.

4 Kimble J & Crittenden SL (2005) Germline proliferation and its control. In *The C. elegans Research Community* (WormBook, ed). WormBook. https:doi.org/10.1895/wormbook.1.13.1. Available at http://www.wormbook.org.

5 Hubbard EJ & Greenstein D (2005) Introduction to the germ line. In *The C. elegans Research Community* (WormBook, ed). WormBook, http://doi.org/10.1895/wormbook.1.18.1. Available at http://www.wormbook.org.

6 Byrd DT & Kimble J (2009) Scratching the niche that controls Caenorhabditis elegans germline stem cells. *Semin Cell Dev Biol* **20**, 1107–1113.

7 Lamont LB, Crittenden SL, Bernstein D, Wickens M & Kimble J (2004) FBF-1 and FBF-2 regulate the size of the mitotic region in the *C. elegans* germline. *Dev Cell* **7**, 697–707.

8 Berry LW, Westlund B & Schedl T (1997) Germ-line tumor formation caused by activation of glp-1, a Caenorhabditis elegans member of the Notch family of receptors. *Development* **124**, 925–936.

9 Crittenden SL, Bernstein DS, Bachorik JL, Thompson BE, Gallegos M, Petcherski AG, Moulder G, Barstead R, Wickens M & Kimble J (2002) A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. *Nature* **417**, 660–663.

10 Racher H & Hansen D (2012) PUF-8, a pumilio homolog, inhibits the proliferative fate in the Caenorhabditis elegans germline. *G3(Bethesda)* **2**, 1197–1205.

11 Encalada SE, Martin PR, Phillips JB, Lyczak R, Hamill DR, Swan KA & Bowerman B (2000) DNA replication defects delay cell division and disrupt cell polarity in early Caenorhabditis elegans embryos. *Dev Biol* **228**, 225–238.

12 Brauchle M, Baumer K & Gonczy P (2003) Differential activation of the DNA replication checkpoint contributes to asynchrony of cell division in C. elegans embryos. *Curr Biol* **13**, 819–827.

13 Fox PM, Vought VE, Hanazawa M, Lee MH, Maine EM, Schedl T & Cyclin E (2011) CDK-2 regulate proliferative cell fate and cell cycle progression in the *C. elegans* germline. *Development* **138**, 2223–2234.

14 Michel B, Flores MJ, Viguera E, Grompone G, Seigneur M & Bidnenko V (2001) Rescue of arrested replication forks by homologous recombination. *Proc Natl Acad Sci USA* **98**, 8181–8188.

15 Rudd SG, Bianchi J & Doherty AJ (2014) PrimPol-A new polymerase on the block. *Mol Cell Oncol* **1**, e960754.