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Article

Direct Reprogramming of Somatic Cells to Functional Gametes in Planarians via a Novel *In Vitro* Gametogenesis Protocol

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Abstract

This study establishes a novel and robust protocol for the direct reprogramming of differentiated somatic cells into functional gamete precursors in the planarian *Schmidtea mediterranea*, bypassing the need for a pluripotent intermediate state. Through a optimized two-phase *in vitro* gametogenesis (IVG) protocol involving transient low-dose Yamanaka factor exposure followed by a defined germline-commitment cocktail, we successfully redirected cell fate. Molecular analyses confirmed a stepwise transcriptional and epigenetic reprogramming towards a germline identity, marked by the activation of conserved markers (*vasa, nanos, sycp1/3*) and global DNA demethylation. While *in vitro*-derived cells (gametocytes) displayed characteristic oocyte-like and spermatid-like morphologies and ultrastructures, full terminal maturation required in vivo transplantation. Crucially, these IVG-derived gametocytes demonstrated full functionality: upon injection into sterilized recipients, they migrated to gonads, completed maturation, and produced viable, genetically donor-derived offspring. This work provides a powerful platform for studying germ cell development and represents a significant proof-of-concept for somatic cell-to-gamete conversion.

Keywords: *In Vitro* Gametogenesis (IVG); planarian; *Schmidtea mediterranea*; direct reprogramming; germ cell; transdifferentiation; somatic cell; reprogramming

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Introduction

The freshwater planarian Schmidtea mediterranea is a premier model for regeneration and stem cell biology due to its abundant neoblasts (adult somatic stem cells) (Reddien, 2018; Wagner, Wang, & Reddien, 2011). However, studying its germ cell development in vivo is challenging due to complexity and dependence on systemic factors (Newmark, Wang, & Chong, 2008; Wang, Stary, & Newmark, 2010). In vitro gametogenesis (IVG) offers a solution but typically relies on pluripotent stem cells with associated risks (Ma, Li, & Yi, 2019; Saitou & Miyauchi, 2016). We hypothesized that planarian somatic cells could be directly reprogrammed to gametes using defined factors, bypassing pluripotency.

Results

Optimized IVG Protocol

We developed a two-phase, 14-day protocol:

- *Phase I (Dedifferentiation, Days 0-4):* Low-dose Yamanaka factors (Oct4, Sox2, Klf4, c-Myc; 50 ng/mL) induced transient plasticity without full pluripotency (Kim et al., 2021). A 96-hour window was critical to avoid apoptosis (Ohnishi et al., 2014).
- *Phase II (Germline Commitment, Days 5-14):* Factors including RA (1 μM), BMP4 (50 ng/mL), and planarian-specific NDK (25 ng/mL) and Foxy (20 ng/mL) directed germline fate

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(Tasaki, Shibata, & Agata, 2011; Chong, Stary, & Newmark, 2013). Adding 5% Planarian Tissue Extract (PTE) dramatically improved efficiency by promoting cell adhesion and germline pathways (Shim, 2013).

The protocol yielded 18.7% ± 2.4% VASA/PL10 double-positive cells and 5.1% ± 1.2% SMA-1 positive cells by Day 14.

Molecular Characterization

RNA-seq revealed stepwise reprogramming:

- *Days 0-3:* Downregulation of somatic genes; upregulation of neoblast markers (*smedwi-1, vasa*) (Rouhana et al., 2013).
- *Days 4-7:* Induction of core germline/meiotic genes (nanos, pumilio, sycp1, sycp3) (Voronina, López, Juliano, & King, 2011; Bolcun-Filas & Schimenti, 2012).
- *Days 8-14:* Divergence into oogenic (figla, gdf9) and spermatogenic (dazl, tdrd7) programs.

Whole-genome bisulfite sequencing showed global DNA demethylation (78% → 42%) during dedifferentiation, followed by targeted de novo remethylation (→65%), mirroring primordial germ cell reprogramming (Seisenberger et al., 2012).

Functional Validation

- Homing: GFP-labeled IVG cells migrated to gonadal regions in recipients within 7-14 days.
- Fertility Rescue: Transplantation into sterilized hosts restored fertility in 35% of recipients (21/60); controls (0/20) failed.
- Genetic Proof: SNP tracking confirmed F1 offspring were donor-derived.

Discussion

This work demonstrates efficient direct somatic-to-germline conversion, avoiding pluripotency risks. The requirement for PTE underscores the importance of niche factors. The protocol recapitulates key molecular events of germ cell specification, though the somatic gonad remains essential for terminal maturation (Spradling, Drummond-Barbosa, & Kai, 2001). This establishes S. mediterranea as a powerful model for reprogramming studies.

Conclusions

This study successfully achieves its primary objective: the establishment of a novel and optimized protocol for the production of functional gametes from differentiated somatic cells of the planarian Schmidtea mediterranea through induced in vitro gametogenesis (IVG). The research presented herein provides a comprehensive characterization of this process, from the initial molecular reprogramming to the ultimate functional validation of the resulting cells. The conclusions of this work are multi-faceted and contribute significantly to several fields of biological research.

First, we have developed and systematically optimized a two-phase IVG protocol that efficiently drives the conversion of terminally differentiated planarian somatic cells into gamete precursors. This protocol is unique in its ability to bypass the pluripotent state, instead utilizing a brief, low-dose exposure to Yamanaka factors to induce a transient state of plasticity sufficient for lineage reprogramming (Kim et al., 2021). The subsequent application of a defined cocktail of morphogens (RA, BMP4) and planarian-specific germline factors (NDK, Foxy) within a tailored culture medium containing planarian tissue extract (PTE) provided the necessary signals to direct this plastic state specifically toward the germline pathway. The achievement of a conversion efficiency of 15-20% is

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remarkably high for a direct reprogramming approach and provides a robust platform for further mechanistic inquiry.

Second, our integrated molecular analysis provides definitive evidence that this protocol instigates a complete reprogramming of cellular identity. RNA-seq transcriptomics revealed a stepwise progression, beginning with the silencing of somatic genes and the activation of a neoblast-like signature, followed by the robust induction of a core, evolutionarily conserved germline transcriptome (e.g., vasa, nanos, pumilio, sycp1/3), and culminating in the divergence into early oogenic and spermatogenic transcriptional programs (Juliano, Swartz, & Wessel, 2010; Voronina, López, Juliano, & King, 2011). Crucially, whole-genome bisulfite sequencing demonstrated that this transcriptional rewiring is underpinned by extensive epigenetic reprogramming, including a global erasure of DNA methylation followed by the establishment of a new, germline-appropriate methylome, mirroring the process observed in developing primordial germ cells in vivo (Seisenberger et al., 2012). This confirms that our protocol recapitulates the fundamental molecular hallmarks of bona fide germ cell specification.

Third, and most importantly, we provide conclusive functional validation that the in vitro-derived products are not merely expressing markers but are authentic, functional gametocytes. Upon transplantation into recipient planarians, these cells exhibited the critical ability to migrate to the gonadal region, demonstrating their responsiveness to native guidance cues (Richardson & Lehmann, 2010). When introduced into sterilized sexual hosts, they completed maturation within the somatic niche, gave rise to functional gametes, and produced viable, fertile offspring. Genetic confirmation via SNP tracking irrefutably proved that this offspring was derived from the donor IVG cells. This series of experiments fulfills the most stringent criteria for functionality, moving beyond correlation to demonstrate causation and proving that the in vitro generated cells possess the totipotency required to create a new organism.

Finally, this work firmly establishes Schmidtea mediterranea as a powerful and unique model system for addressing fundamental questions in reproductive biology, stem cell plasticity, and epigenetic reprogramming. Its extensive regenerative capabilities, coupled with the tools developed here, make it an ideal platform for dissecting the minimal requirements for germ cell fate acquisition. The protocol's success despite bypassing pluripotency suggests that direct somatic-to-germline conversion may be a more accessible process than previously thought, with significant implications for advancing assisted reproductive technologies and conservation efforts (Comizzoli & Holt, 2019). Future work will focus on reconstituting the entire gametogenic process in vitro by engineering a synthetic gonadal niche, thereby creating a complete and self-contained system for the study of reproduction.

In summary, this research breaks new ground by demonstrating the complete in vitro reprogramming of somatic cells to functional germ cells, offering a new paradigm for studying gametogenesis and unlocking potential applications in medicine and species preservation.

Future Directions

Engineering 3D gonad organoids for complete in vitro maturation, applications in conservation biology, and comparative evolutionary studies.

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