Toti"potent" repressors

Christopher M. Gallo* and Geraldine Seydoux

Summary

A fascinating property of germ cells is their ability to maintain totipotency throughout development. At fertilization, this totipotency is unleashed and the egg generates all the cell types needed to make a brand new organism. Occasionally, germ cells differentiate precociously in the embryo or in the gonads and form teratomas, tumors containing many differentiated somatic cell types. Until recently, the genetic basis for teratoma formation was not known. The unexpected discovery of a teratoma in a *C. elegans* double mutant points to translational control as a key mechanism to maintain totipotency in developing germ cells.
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Introduction

Totipotency is defined as the potential of a cell or lineage to give rise to any cell type that makes up the fully differentiated organism. In most animals, with the exception of those capable of regeneration, the germline is believed to be the only lineage to maintain totipotency through development. The germline develops from progenitors termed primordial germ cells (PGCs). PGCs typically are segregated as a small group of cells early in development before most adult structures are formed.[2] A key step in the formation of PGCs is thought to be mediated by transcriptional repressors that prevent the expression of somatic differentiation genes.[2] After specification, the PGCs migrate to the somatic gonads, proliferate and begin the complex process of meiosis and gametogenesis. Remarkably, during and after their migration to the gonads, PGCs and their descendents remain totipotent, as evidenced by their potential to form teratomas in mammals. Teratomas are rare, germ-cell-derived tumors that contain many differentiated somatic cell types, including representatives of all three germ layers.[3] The existence of teratomas suggests that germ cells normally have mechanisms in place to "restrain"

totipotency and prevent uncontrolled proliferation and differentiation. What are these mechanisms? A recent study[1] reports the first incidence of a teratoma in an invertebrate genetic model system and offers a rare insight into the genes that control totipotency.

GLD-1 and MEX-3, guardians of totipotency

C. elegans is an ideal model system to study germ cells: all stages of germline development from PGC formation to gametogenesis and fertilization can be examined directly in live animals. In the adult gonad of hermaphrodites, germ cells are arranged in an assembly-line fashion: mitotic cells at the most distal end, cells that have begun meiosis in the middle, and growing oocytes at the most-proximal end (Fig. 1A).[4] Genetic screens for regulators of germline development have identified many RNA-binding proteins that regulate stem-cell maintenance, meiotic entry, sex determination, gametogenesis, and embryonic development.[5] The study by Ciosk et al. now reports now that the combined loss of two of these RNA-binding proteins leads to the formation of teratomas.

GLD-1 is a member of the STAR KH-domain family of RNA-binding proteins conserved in metazoans (Fig. 1C).[6] GLD-1 functions as a translational repressor and several of its mRNA targets have been identified.[7−9] The primary function of GLD-1 is to control the transition from mitotic proliferation to meiosis.[10] GLD-1 protein levels are highest in the central meiotic region of the gonad, where germ cells initiate meiosis (Fig. 1A).[11] MEX-3 belongs to a second family of KH-domain proteins, characterized by having two KH domains (Fig. 1C).[12] Like GLD-1, MEX-3 has been predicted to function as a translational repressor. MEX-3 is expressed in a pattern complementary to that of GLD-1, with high levels in the mitotic region and in oocytes (Fig. 1A).[7,12,13] MEX-3 expression is dependent on GLD-1. In *gld-1* mutant animals, MEX-3 is expressed uniformly throughout the gonad,[7,13] raising the possibility that some of the *gld-1* phenotypes are due to ectopic expression of MEX-3.

To test this hypothesis, Ciosk et al. constructed a strain carrying loss-of-function alleles in both *gld-1* and *mex-3*. To their surprise, instead of an amelioration of the *gld-1* phenotype, the *gld-1 mex-3* double mutant showed a brand new phenotype: the central region of the gonad was filled with cells whose nuclei looked like somatic nuclei. Using electron microscopy, immunofluoresence and a GFP transgene, they identified several cell types, including muscles, neurons and intestinal cells.[1] The first worm teratoma!
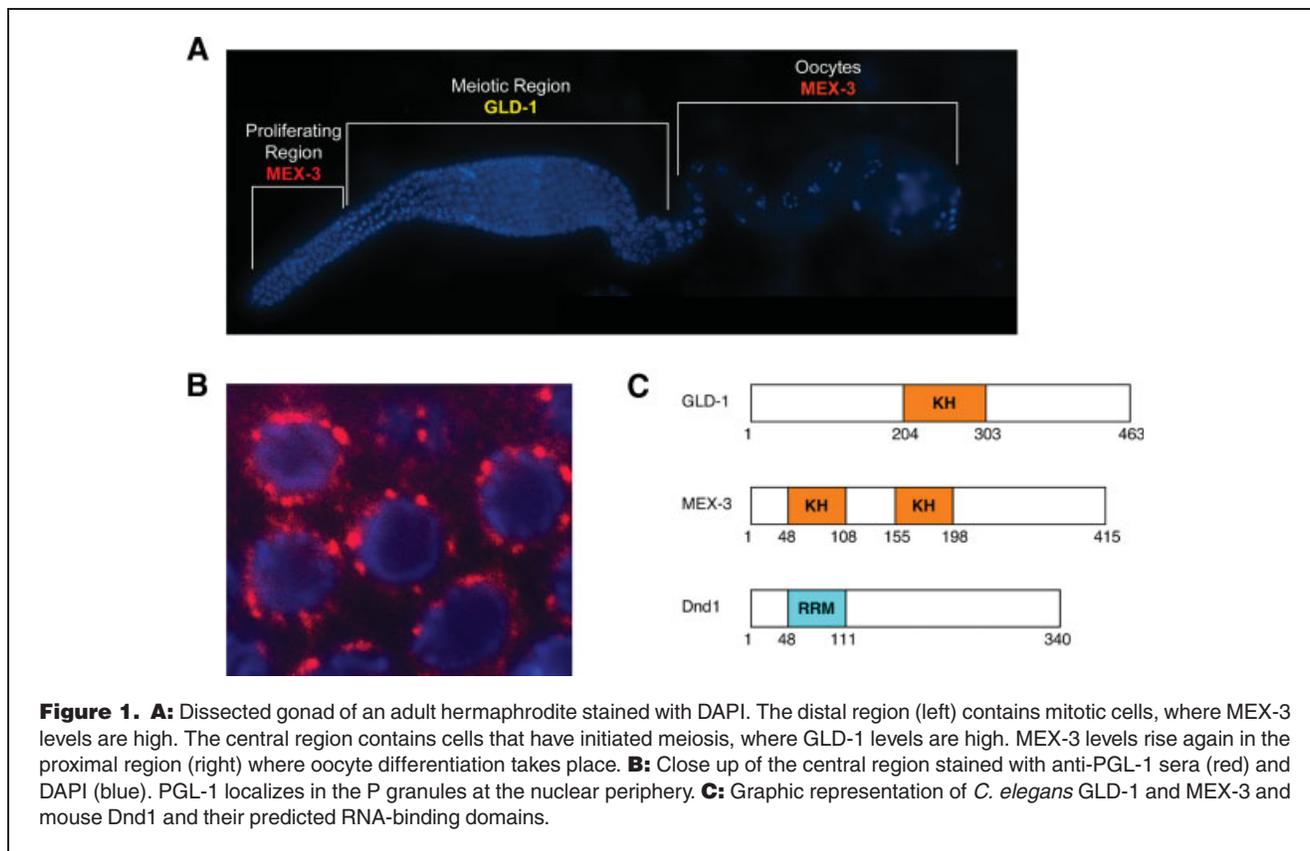
Figure 1. A: Dissected gonad of an adult hermaphrodite stained with DAPI. The distal region (left) contains mitotic cells, where MEX-3 levels are high. The central region contains cells that have initiated meiosis, where GLD-1 levels are high. MEX-3 levels rise again in the proximal region (right) where oocyte differentiation takes place. **B:** Close up of the central region stained with anti-PGL-1 sera (red) and DAPI (blue). PGL-1 localizes in the P granules at the nuclear periphery. **C:** Graphic representation of *C. elegans* GLD-1 and MEX-3 and mouse Dnd1 and their predicted RNA-binding domains.

The location of the teratoma in the central gonad suggested that these cells were derived from cells that had initiated meiosis. Consistent with this view, teratomas were not formed when *gld-1* and *mex-3* were depleted in a mutant that causes all germ cells to remain in mitosis. Interestingly, teratomas were only observed in germlines that had initiated oogenesis, no teratomas were observed in males or in mutant hermaphrodites that produce only sperm. The teratomas, however, did not appear to be derived from prematurely activated oocytes, as no oocytes were detected at any stage in the *gld-1 mex-3* double mutant.

To begin to investigate how the combined loss of GLD-1 and MEX-3 could lead to germ cells trans differentiating into somatic cells, the authors examined the requirement for transcription factors known to initiate muscle development in embryos. Muscle development depends on PAL-1, a maternally expressed transcription factor related to Caudal, and on zygotically expressed transcription factors, including the MyoD homolog HLH-1.[14,15] *pal-1* mRNA is synthesized during oogenesis and kept translationally silent by GLD-1 and MEX-3 until the start of embryonic development.[7] *gld-1 mex-3* teratomas contained many HLH-1-positive nuclei, and depletion of PAL-1 by RNA interference caused a reduction in HLH-1-positive nuclei. These results suggest that somatic differentiation in the *gld-1 mex-3* teratoma depends on the activation of transcriptional cascades normally restricted to embryos. Ectopic expression of PAL-1 alone, however, is not sufficient to cause germ cells to transdifferentiate into muscle,[7] suggesting that other maternal factors are involved. Misexpression of maternal RNAs as a cause for teratoma formation is consistent with a requirement for female gametogenesis, since maternal RNA transcription is linked to oogenesis.

A characteristic feature of germ cells in most organisms is the presence of large ribonucleoprotein (RNP) granules on the cytoplasmic face of the nuclear envelope (Fig. 1B). In *C. elegans*, several developmentally regulated mRNAs, including translationally repressed maternal RNAs, localize to the germ granules (P granules).[16] Consistent with a role in mRNA regulation, P granules were recently shown to share components with processing bodies,[17–20] large cytoplasmic RNP granules implicated in mRNA silencing in yeast and mammalian cells.[21] In *gld-1 mex-3* double mutants, P granules are lost from the central region of the gonad. In several animals, this loss appeared to precede overt signs of *trans*-differentiation, suggesting a possible cause-and-effect relationship. Disassembly of P granules could in principle release maternal mRNAs for immediate translation, and thus represent a critical step in the progression from totipotent germ cell to differentiated somatic cell. How loss of GLD-1 and MEX-3 leads to P

granule disassembly, however, remains mysterious. GLD-1 and MEX-3 associate with P granules,[11,12] most obviously during embryonic stages, but are also present at high levels throughout the cytoplasm. It will be critical to determine how GLD-1 and MEX-3 contribute to P granule maintenance and whether loss of P granules is a cause or consequence of teratoma formation.

Totipotent repressors in mice

Regulation of gene expression at the post-transcriptional level is a common theme in germ cells. Conserved RNA-binding proteins have been implicated in germline development in several organisms. For example, homologs of the translational repressors Nanos and Pumilio regulate germline differentiation and PGC survival in various species.[22,23] So far, the individual loss of these regulators has not been reported to lead to teratoma formation, but a recent study suggests that, in mice, combining loss of a germ cell RNA-binding protein with other mutations can lead to teratomas.

The *Ter* mutation causes PGC loss in all mouse strains and acts as a strong genetic modifier in the 129-inbred background, leading to a nearly fully penetrant incidence of testicular germ cell tumors (TGCTs).[24] Recent cloning of the *Ter* locus revealed that it encodes the mouse homolog of the zebrafish gene *dead end*.[25] *dead end* encodes a maternal RNA expressed in PGCs that is essential for PGC migration and viability.[26] Like zebrafish *dead end*, mouse *Dnd1* is expressed in germ cells.[25] DND1 protein contains an RNA recognition motif (Fig. 1C), and is most related to RNA-editing enzymes that convert cytidine to uridine. The *Ter* mutation introduces a premature stop codon in the *Dnd1*-coding region.[25] The mutation(s) in the 129-inbred background that synergize with *Ter* to cause testicular tumors are not yet known, but it will be interesting to determine whether they also affect predicted RNA-binding proteins. The finding that loss of an RNA-binding protein essential for PGC development in mice and zebrafish can lead to tumors when combined with other mutations suggests that combinatorial control by RNA-binding proteins may be critical to prevent uncontrolled growth and differentiation in the mammalian germline, as it is in *C. elegans*. RNA regulation may turn out to be a common mechanism to control and maintain totipotency.

Acknowledgments

References

1. Ciosk R, DePalma M, Priess JR. 2006. Translational regulators maintain totipotency in the Caenorhabditis elegans germline. Science 311:851–853.
2. Saitou M, Payer B, O'Carroll D, Ohinata Y, Surani MA. 2005. Blimp1 and the emergence of the germ line during development in the mouse. Cell Cycle 4:1736–1740.
3. Ulbright TM. 2004. Gonadal teratomas: a review and speculation. Adv Anat Pathol 11:10–23.
4. Albert Hubbard EJ, Greenstein D. 2005. Introduction to the germ line WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.18.1, http://www.wormbook.org.
5. Ellis R, Schedl T. 2006. Sex determination in the germ line WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.82.1, http://www.wormbook.org.
6. Vernet C, Artzt K. 1997. STAR, a gene family involved in signal transduction and activation of RNA. Trends in Genetics 13:479.
7. Mootz D, Ho DM, Hunter CP. 2004. The STAR/Maxi-KH domain protein GLD-1 mediates a developmental switch in the translational control of C. elegans PAL-1. Development 131:3263–3272.
8. Jan E, Motzny CK, Graves LE, Goodwin EB. 1999. The STAR protein, GLD-1, is a translational regulator of sexual identity in Caenorhabditis elegans. Embo J 18:258–269.
9. Lee M-H, Schedl T. 2001. Identification of in vivo mRNA targets of GLD-1, a maxi-KH motif containing protein required for C. elegans germ cell development. Genes Dev 15:2408–2420.
10. Kimble J, Crittenden SL. 2005. Germline proliferation and its control WormBook, ed. The C. elegans Research Community, WormBook, doi/10.1895/wormbook.1.13.1, http://www.wormbook.org.
11. Jones AR, Francis R, Schedl T. 1996. GLD-1, a cytoplasmic protein essential for oocyte differentiation, shows stage- and sex-specific expression during Caenorhabditis elegans germline development. Dev Biol 180:165–183.
12. Draper BW, Mello CC, Bowerman B, Hardin J, Priess JR. 1996. MEX-3 is a KH domain protein that regulates blastomere identity in early C. elegans embryos. Cell 87:205–216.
13. Ciosk R, DePalma M, Priess JR. 2004. ATX-2, the C. elegans ortholog of ataxin 2, functions in translational regulation in the germline. Development 131:4831–4841.
14. Baugh LR, Hill AA, Claggett JM, Hill-Harfe K, Wen JC et al. 2005. The homeodomain protein PAL-1 specifies a lineage-specific regulatory network in the C. elegans embryo. Development 132:1843–1854.
15. Fukushige T, Krause M. 2005. The myogenic potency of HLH-1 reveals wide-spread developmental plasticity in early C. elegans embryos. Development 132:1795–1805.
16. Schisa JA, Pitt JN, Priess JR. 2001. Analysis of RNA associated with P granules in germ cells of C. elegans adults. Development 128:1287–1298.
17. Boag PR, Nakamura A, Blackwell TK. 2005. A conserved RNA-protein complex component involved in physiological germline apoptosis regulation in C. elegans. Development 132:4975–4986.
18. Squirrell JM, Eggers ZT, Luedke N, Saari B, Grimson A et al. 2006. CAR-1, a protein that localizes with the mRNA decapping component DCAP-1, is required for cytokinesis and ER organization in Caenorhabditis elegans embryos. Mol Biol Cell 17:336–344.
19. Audhya A, Hyndman F, McLeod IX, Maddox AS, Yates JR 3rd et al. 2005. A complex containing the Sm protein CAR-1 and the RNA helicase CGH-1 is required for embryonic cytokinesis in Caenorhabditis elegans. J Cell Biol 171:267–279.
20. Lall S, Piano F, Davis RE. 2005. Caenorhabditis elegans decapping proteins: localization and functional analysis of Dcp1, Dcp2, and DcpS during embryogenesis. Mol Biol Cell 16:5880–5890.
21. Anderson P, Kedersha N. 2006. RNA granules. J. Cell Biol. %R 10.1083/jcb.200512082 172:803–808.
22. Moor CHd, Meijer H, Lissenden S. 2005. Mechanisms of translational control by the 3′ UTR in development and differentiation. Seminars in Cell & Developmental Biology 16:49.
23. Parisi M, Lin HH. 2000. Translational repression: A duet of Nanos and Pumilio. Current Biology 10:R81.
24. Matin A, Nadeau JH. 2005. Search for testicular cancer gene hits dead-end. Cell Cycle 4:1136–1138.
25. Youngren KK, Coveney D, Peng X, Bhattacharya C, Schmidt LS et al. 2005. The Ter mutation in the dead end gene causes germ cell loss and testicular germ cell tumours. Nature 435:360–364.
26. Weidinger G, Stebler J, Slanchev K, Dumstrei K, Wise C et al. 2003. dead end, a novel vertebrate germ plasm component, is required for zebrafish primordial germ cell migration and survival. Curr Biol 13:1429–1434.