contributions of the individual components are, how they may cooperate, and precisely how the complex is regulated by Ran-GTP and importins. Understanding the regulation of mitotic spindle function by Ran may lead to important new insights into the development of cancer.

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Sex Determination: Ways to Evolve a Hermaphrodite

Most species of the nematode genus *Caenorhabditis* reproduce through males and females; *C. elegans* and *C. briggsae*, however, produce self-fertile hermaphrodites instead of females. These transitions to hermaphroditism evolved convergently through distinct modifications of germline sex determination mechanisms.

Christian Braendle and Marie-Anne Félix

In the nematode *Caenorhabditis elegans*, sex determination mechanisms have been studied in detail and shown to involve a common molecular pathway in somatic and germline cells. Recent studies [1,2] have used this knowledge to ask how sex determination mechanisms have evolved among species closely related to *C. elegans*. The results have shed new light on how these mechanisms have been modified during evolution to produce alternative mating systems.

Convergent Evolution of Hermaphroditism

The genus *Caenorhabditis* currently encompasses eleven species in culture [3], nine of which produce females and males, while two, *C. elegans* and *C. briggsae*, produce self-fertile hermaphrodites and males. The phylogenetic relationships among these species suggest that hermaphroditism evolved twice independently from an ancestral male–female mating system [3–5] (Figure 1). In both species, the hermaphrodite anatomically resembles a female, which undergoes spermatogenesis for a brief period prior to oogenesis (Figure 2A). The hermaphrodite can then self-fertilize using its own sperm or mate with a male.

Sex determination in these nematodes is chromosomal: females and hermaphrodites carry two X chromosomes, while males carry one. In males, the germline is exclusively male, in females it is exclusively female, while in hermaphrodites, the first gametes differentiate as sperm and the later ones differentiate as oocytes. Evolution to hermaphroditism thus occurs through the modulation of germline sex determination in XX animals.

Conserved Determinants of Germline Sex

In *C. elegans*, a common pathway determines the sexual identity of both soma and gametes (Figure 2B). In males, low X dosage results in the expression of HER-1, a secreted protein, which inhibits TRA-2, a Patched-like receptor. A signal transduction pathway

through FEM-1, FEM-2 and FEM-3 then represses TRA-1, a Gli/Ci-like transcription factor. In the hermaphrodite germline, spermatogenesis is activated via a modification of this pathway at the level of *tra-2* mRNA; as in the male soma, *tra-2* repression then leads via the FEM proteins to inhibition of TRA-1. Downstream, the key germline-specific transcriptional target of TRA-1 is *fog-3*: inhibition of TRA-1 activates *fog-3* transcription and thereby spermatogenesis. The switch to oogenesis then operates at the level of *fem-3* translational repression [6].

In *C. briggsae* and *C. remanei*, the sex determination regulators are functionally conserved, despite rapid sequence evolution and several examples of protein coevolution [1,7]. In the germline, the role of *fog-3* appears conserved: the results of RNA interference (RNAi) experiments suggest that *fog-3* promotes spermatogenesis in males of all three species, *C. elegans*, *C. briggsae* and *C. remanei*, and in hermaphrodites of the first two species. Moreover, *fog-3* is expressed in the germline of *C. elegans* and *C. briggsae* hermaphrodites, but not in that of remanei females [8].

A common feature in the evolution of hermaphroditism of *C. elegans* and *C. briggsae*, therefore, is *fog-3* activation in the germline of XX animals. Moving up the pathway, *fog-3* expression is regulated in *C. elegans* by TRA-1, and TRA-1 binding sites in *fog-3* regulatory sequences are conserved in *C. briggsae* and *C. remanei* [1,8]. Further up the pathway, however, modulation of germline sex differs between *C. elegans* and *C. briggsae*.

Spermatogenesis in *C. briggsae*

In *C. elegans*, hermaphrodite spermatogenesis requires the inhibitory action of FEM-1, FEM-2 and FEM-3 on TRA-1 (Figure 2B): mutants for any of these FEM proteins are transformed into females (in both soma and germline). In *C. briggsae*, RNAi knockdown of FEM proteins did not produce this transformation [9,10], but the poor efficiency of RNAi required a better test. Hill *et al.* [2] have beautifully solved this problem by isolating deletion alleles of *Cb-fem-2* and *Cb-fem-3*, and screening for *Cb-tra-2* suppressors. This is the first published study in which targeted gene deletion and systematic mutagenesis have been applied to *C. briggsae*, a very promising result for future work using this species.

The *fem-2* and *fem-3* deletion mutants were identified by screening EMS-mutagenized worm pools using the polymerase chain reaction. The *Cb-fem-2* and *Cb-fem-3* mutants, and also *fem-2*; *fem-3* double mutants, were found to develop into self-fertile hermaphrodites, irrespective of whether they had one or two X chromosomes [2]. These genes are thus required for somatic male identity in *C. briggsae* as in *C. elegans*; but for hermaphrodite spermatogenesis they are essential in *C. elegans* yet dispensable in *C. briggsae*.

FEM-2 and FEM-3 are required for signal transduction from TRA-2 to TRA-1. In both *C. elegans* and *C. briggsae*, *tra-2* inactivation transforms XX animals into males (soma and germline). In an extensive screen for *Cb-tra-2* suppressors, Hill *et al.* [2] identified numerous mutations causing somatic feminization, including *Cb-fem-2* alleles. However, these mutants all developed into self-fertile hermaphrodites rather than females. This corroborates the conclusion that, in *C. briggsae*, unlike in *C. elegans*, male fate specification during hermaphrodite spermatogenesis does not require the same *tra-2* downstream genes, such as *fem-1/2/3*, as it does in the male soma.

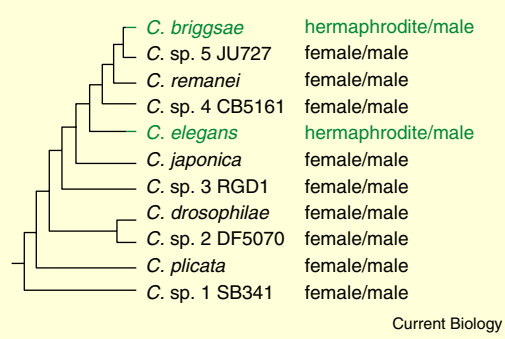

Figure 1. Convergent evolution of hermaphroditism in *C. elegans* and *C. briggsae*.

Molecular phylogeny of the genus *Caenorhabditis*. Hermaphroditism (green) appears to have evolved independently in *C. elegans* and *C. briggsae*.

fog-2 Is Unique to *C. elegans*

Upstream of *tra-2* in *C. elegans*, the specific regulator of hermaphrodite spermatogenesis is *fog-2*, which together with *gld-1* represses *tra-2* mRNA (Figure 2B). The *fog-2* gene is required for hermaphrodite spermatogenesis but, unlike *fog-3*, it is not required for male spermatogenesis [6].

Most sex determination factors identified in *C. elegans* have orthologues in *C. briggsae*, with one interesting exception: *fog-2*. This gene seems to have arisen in the course of a gene family expansion in the evolutionary branch leading to *C. elegans*. In *C. briggsae*, not only is *fog-2* absent but *gld-1* plays an opposite role: its inactivation suppresses oogenesis in XX animals [1].

Convergent Evolution

Taken together, these studies demonstrate that the convergent evolutionary transition to hermaphroditism in *C. briggsae* and *C. elegans* likely involved different modifications of the sex determination pathway.

In *C. elegans*, gene duplication and divergence generating *fog-2* may have been a key factor in the evolution of hermaphroditism, transforming germline fate through *tra-2* mRNA repression and *fem-2/3* activity.

In *C. briggsae*, the control of hermaphrodite spermatogenesis ultimately occurs through *fog-3* regulation, but without a *fog-2* orthologue and without requiring *fem-2* and *fem-3*. Regulation via the 3′ untranslated region of *tra-2* mRNA appears conserved in *C. briggsae* [11], although its role in hermaphrodite spermatogenesis was not tested. A direct action of TRA-2 on TRA-1 may regulate

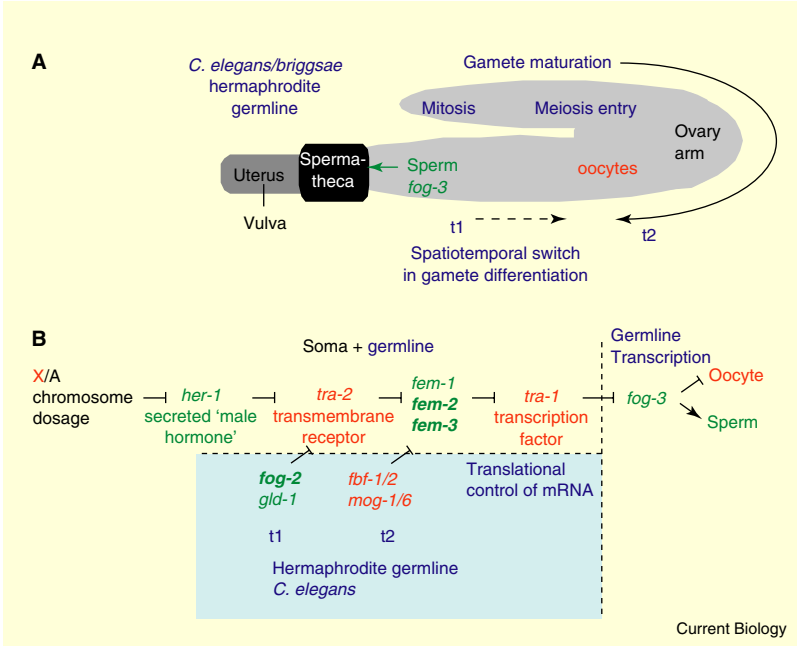

Figure 2. Germline sex determination in *C. elegans* versus *C. briggsae*.

(A) The temporal switch in hermaphrodite germline differentiation. In both species, *fog-3* is required for the onset of spermatogenesis. Sperm cells develop at the proximal end of the arm and then move to the spermatheca; oocytes mature from the distal end of the arm throughout adulthood (only one of the two gonadal arms is represented). In case of outcrossing, male sperm enter through the vulva and similarly reach the spermatheca. (B) Genetic pathway underlying somatic and germline sex determination. Genes whose products are present or activated in male-fated cells are in green, those active in female-fated cells are in red. Germline-specific regulatory components are in blue. Genes in bold letters are absent in the *C. briggsae* genome (*fog-2*) or do not participate to the regulation of hermaphrodite spermatogenesis in this species (*fem-2* and *fem-3*). Genes are named after their mutant phenotype in *C. elegans*: *Her*, hermaphrodization of XO animals; *Tra*, transformation of XX animals to males; *Fem*, feminization of XX and XO animals; *Fog*, feminization of the germline; *Mog*, masculinization of the germline.

fog-3 in *C. briggsae*, bypassing FEM-1/3. Interestingly, direct cross-regulator interactions between *tra-1* and *tra-2* are known in *C. elegans* [6]. The recent identification of feminized XX mutants in *C. briggsae* (Yiqing Guo and Ron Ellis, personal communication) will clarify the control of hermaphrodite spermatogenesis in this species.

One step towards understanding the transition to hermaphroditism will be to characterize sex determination in closely related male–female species, such as *C. remanei*. Remarkably, it is possible to use RNAi to create *C. remanei* females that produce sperm and oocytes, suggesting that the evolution of hermaphroditism could require few mutational steps (Chris Baldi, Soochin Cho and Ron Ellis, personal communication).

Moreover, it may be helpful to analyse sex determination mechanisms in wild isolates of a given species. There is evidence of genetic variation in the timing of the sperm-to-oocyte switch that determines sperm and thus hermaphrodite self-progeny number [12–14]. This suggests an additional, quantitative modulation of the germline sex determination mechanisms within species.

Evolution of Mating Systems

The current evidence suggests that switching between alternative mating systems is easy in *Caenorhabditis* species, mechanistically and evolutionarily. It may only take one or two mutations to shift between different reproductive modes, and these shifts seem largely limited to the germline with few pleiotropic effects. Moreover, mutation of different genes can cause transformation into the same reproductive phenotype. These factors may create the potential for rapid and frequent evolutionary transitions between mating systems in *Caenorhabditis* and other rhabditids.

Ultimately, what are the evolutionary forces favouring the adoption of a particular mating system and what are the consequences of its maintenance? Understanding the origin and maintenance of sexual reproduction (outcrossing) is a key topic in evolutionary biology, and *Caenorhabditis* species may be particularly well-suited to addressing this problem. A selfing mode of reproduction has in principle an advantage over sexual reproduction — the well-known two-fold cost of sex. In addition, the ability of an individual to self-fertilize obviates the need for a mating partner. This may be advantageous to organisms that colonize ephemeral habitats where population densities fluctuate dramatically, as observed for *Caenorhabditis* species [3,15]. But selfing rapidly increases levels of homozygosity — inbreeding — and novel deleterious mutations cannot be purged through sexual recombination. It is therefore crucial to know the relative costs and benefits of selfing versus outcrossing, in both the short and long term, to understand the evolution of alternative mating systems.

The hermaphroditic species *C. elegans* and *C. briggsae* are capable of producing functional males that allow outcrossing events. Several lines of evidence suggest that *C. elegans* and *C. briggsae* maintain a very low, yet detectable, outcrossing rate ([15,16] and Asher Cutter *et al.*, personal communication). Such partial outcrossing of a predominant selfer may combine the advantages of both selfing and outcrossing [17]. The further integration of evolutionary, ecological and developmental studies on different *Caenorhabditis* species presents a promising approach to clarifying the proximate and ultimate causes of mating system evolution.

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