Endosymbiotic origins of sex

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Summary

Understanding how complex sexual reproduction arose, and why sexual organisms have been more successful than otherwise similar asexual organisms, is a long-standing problem in evolutionary biology. Within this problem, the potential role of endosymbionts or intracellular pathogens in mediating primitive genetic transfers is a continuing theme. In recent years, several remarkable activities of mitochondria have been observed in the germline cells of complex eukaryotes, and it has been found that bacterial endosymbionts related to mitochondria are capable of manipulating diverse aspects of metazoan gametogenesis. An attempt is made here to rationalize these observations with an endosymbiotic model for the evolutionary origins of sex. It is hypothesized that the contemporary life cycle of germline cells has descended from the life cycle of the endosymbiotic ancestor of the mitochondrion. Through an actin-based motility that drove it from one cell to another, the rickettsial ancestor of mitochondria may have functioned as a primitive transducing particle, the evolutionary progenitor of sperm. ***BioEssays*** **26:558–566, 2004.**

Introduction

Cytologists have long been aware of extraordinary mitochondrial behaviors in germ cells. The Balbiani body or its functional equivalent, which features dense aggregates of mitochondria,[1–3] is an outstanding morphogenetic feature of oogenesis. In the male germline, the fusion of mitochondria to form the mammalian sperm midpiece[4,5] or insect nebenkern[6,7] is highly coordinated and involves massive transformations of mitochondria. Mitochondrial dynamics in germ cells have generally been regarded as the consequence of two obvious biological requirements: (1) the necessity of transmitting healthy mitochondria to the next generation, and (2) the likely requirement for a rich supply of energy to support sperm motility and the extensive morphogenetic changes associated with gametogenesis. However, a number of observations in recent years may indicate that specialized mitochondrial activities in the life history of germ cells reflect a more fundamental morphogenetic role for this organelle in the cellular functions "unique to" germ cells. Several of the developmental and regulatory pathways specific to germ cells are observed to involve mitochondria in some puzzling and unexpected ways that have no obvious connection to energy metabolism or mitochondrial transmission.

Here I review some of these observations and attempt to rationalize them within the context of a model in which the specialized life cycle of germ cells, and therefore sexual reproduction in complex multicellular organisms, is descended in evolution from the life cycle of the microbial parasite/endosymbiont that eventually became the mitochondrion.[8]

A challenge to conventional wisdom about mitochondrial evolution is implicit in this model. It is commonly assumed that the endosymbiotic ancestor of mitochondria was passively engulfed by a phagocytic protoeukaryotic cell, in whose descendents it has remained ever since. Here I am promoting an alternative view of the ancestral mitochondrion, that of an active, motile, opportunistic pathogen that could remain quiescent within a cell lineage for many generations, then move to another host when circumstances required, much as a temperate phage can lie dormant within a host for long intervals between lytic cycles. Recent observations of extensive mitochondrial motility in yeast[9–12] support this notion of the active/motile mitochondrion. Moreover, I am suggesting that, rather than experiencing a single "capture" by a protoeukaryotic host cell,[13] mitochondria "moved in" over a long period of time, perhaps hundreds of millions of years, during which they were frequently exchanged between cells. If they sometimes carried genes from one cell to another, as I will suggest below, this traffic would constitute a massive network for primitive gene exchange between cells. Molecular evolutionary biologists increasingly hold the view that extensive horizontal gene exchange occurred between diverse ancient cells early in evolution.[14] In the model promoted here, gametogenesis pathways exhibit a wide variety of exceptional mitochondrial behaviors as a vestige of their evolutionary roots in the activities of this network.

I apologize in advance for the obvious shortcomings of this attempt. In particular, the cellular examples that I have drawn on come from model organisms nested high in the tree of life. If the mitochondrial phenomena highlighted here are truly vestiges of protoeukaryotic behavior, they should be observable in simpler organisms residing lower on the tree, such as the Porifera. The potential gap of a billion years between the appearance of eukaryotic cells and complex metazoans with anisogamous sex could be a major problem for this hypothesis. But it is my hope that the synthesis of a bit of cell

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biology with a little evolutionary biology attempted here, however crude, can stimulate discussion that will lead to a clearer view of the cellular origins of sex.

Mitochondrial dynamics in spermiogenesis

A number of bacteria, including *Listeria, Shigella* and *Rickettsia* of the spotted fever group (SFG), upon gaining entry into the cytoplasm of their cellular victims, hijack the host actin cytoskeleton for their own motility. The result is ''comet tail'' motility in which the microbe is propelled around the cytoplasm of the host cell on a treadmilling tail of actin fibers.[15] The impetus for the model is our observation that the motile actin structures of the *Drosophila* sperm individualization complex, following directly behind highly dynamic, dense-staining membranous bodies derived from mitochondria, are strikingly reminiscent of microbial actin-based motility[16] (Fig. 1). Because considerable evidence argues that mitochondria are descended via endosymbiosis from the *Rickettsiaciae*,[17,18] actin-based mitochondrial motility in spermiogenesis (Fig. 1) could be a vestige of the mitochondrion's rickettsial ancestry, descended from an ancestor shared by the SFG.

The molecular details of actin-based mitochondrial motility in spermiogenesis remain to be deciphered, leaving uncertainty as to whether it is truly an actin-treadmilling process that moves the individualization complex down the spermiogenic cyst, and whether it is truly descended from an ancient proto-mitochondrial microbe as opposed to a more recent product of convergent evolution. The recent work of Noguchi and Miller[19] indicates that the movement of the individualization complex is dependent on actin polymerization/depolymerization, but that the actin within the prominent investment cone immediately behind the mitochondrial whorls of the IC is not treadmilling as would be expected. The apparent similarity between the individualization complex in *Drosophila* spermiogenesis and actin-based microbial motility could turn out to be

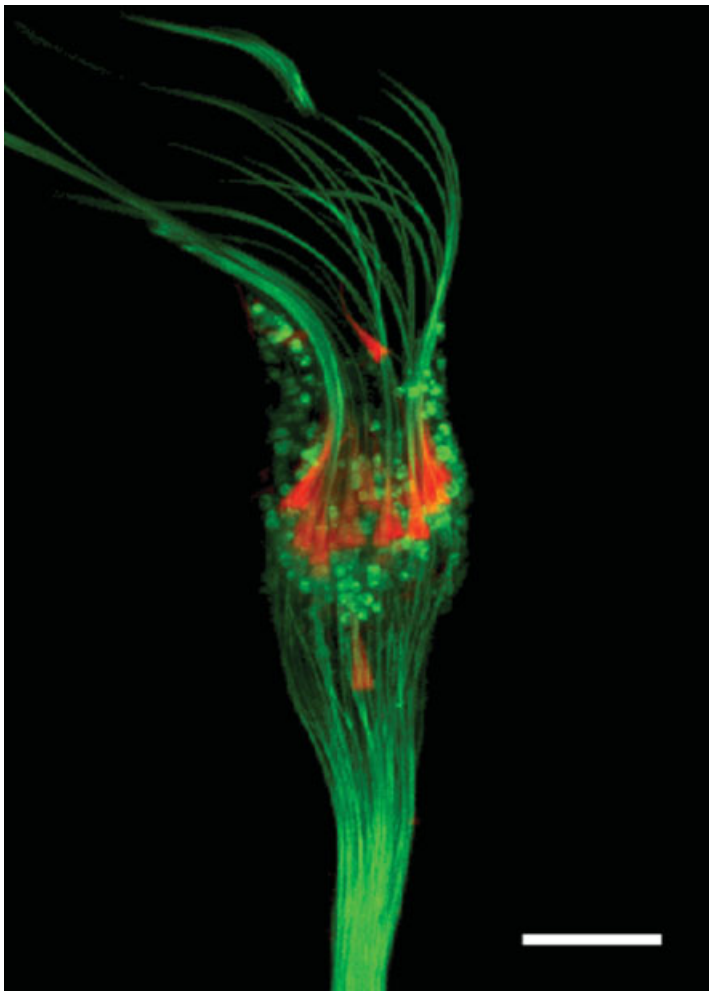

Figure 1. Individualization complex moving down a spermiogenic cyst of *Drosophila*. Green, *don juan*-GFP, which marks mitochondria; red, rhodamine phalloidin, which marks actin individualization cones. Note the swarm of GFP-labeled bodies that presumably emerge from the mitochondrial derivatives along the flagellar axoneme in association with the ''comet tail'' like actin structures. Preparation from wild-type flies expressing *dj*-GFP as in Ref. 16. Bar, 10 microns.

a case of convergent evolution, or indeed not a case of treadmilling actin motility at all. While this potential homology was the inspiration for the development of the model, the principle could just as easily apply to any similarly motile symbiont. However, the observations of frequent nuclear transit by SFG *Rickettsia*, relatives of mitochondria, and the growing evidence that mitochondrial-based mechanisms are deeply embedded in a number of gametogenic pathways (reviewed below) would seem to make a rickettsial proto-mitochondrion the parsimonious suspect for an endosymbiotic catalyst of sex.

The model

The life cycle of SFG *rickettsia* includes several features favorable for the evolution of a sexual life cycle in an ancient protoeukaryotic host. First, the actin-based motility of SFG rickettsia provides them with a ready mechanism for movement between cells. The actin "comet tail" can drive the bacterium from one cell to a neighboring cell[20,21] (Fig. 2). What is required to get the beginnings of sex from these travels of an actin-driven protomitochondrion? The endosymbiont need only carry some genetic material from one cell to another. The life cycle of the SFG *rickettsiae* may provide a ready means for this: SFG *rickettsia* are often seen within the nuclei of infected cells. Indeed, recent work[22] documents *R. rickettsiae* thrashing about on their actin tails within nuclei of infected cells. Therefore, there is ample opportunity for these bacteria to come in contact with genetic material of the host cell during this nuclear phase of the SFG life cycle. With a gene or two ingested or perhaps simply entrapped in or spooled onto its comet tail, the passage of such a bacterium to another cell through the mechanism described above constitutes an opportunity for the transfer of genes between the two host cells (Fig. 3). Proof of principle is suggested by recent reports of functional gene transfer from intracellular bacteria to mammalian cells.[23,24]

The relative ease with which mutations affecting fertility can be induced in contemporary animals and plants is an indication of the highly derived and constrained status of complex reproductive pathways. But meiosis, gametogenesis and fertilization must have coevolved from analogous functions that were far less constrained and very flexibly linked. Driving the endosymbiont through the nucleus, the cytoplasm and, finally, into another cell, the actin-based motility mechanism could provide this simple linkage between a primitive gene segregation event, cytoplasmic morphogenesis and cell–cell interactions. These nuclear, cytoplasmic and intercellular transit phases of a motile rickettsial life cycle, linked through the actin-based motility mechanism, are proposed to be the simple precursors of meiosis, gametogenesis and fertilization, respectively (Fig. 4). Assuming that there is, or has been during some significant period of evolution, a significant adaptive advantage of sexual gene transfer over asexual reproduction,[25,26] this advantage would in turn select for

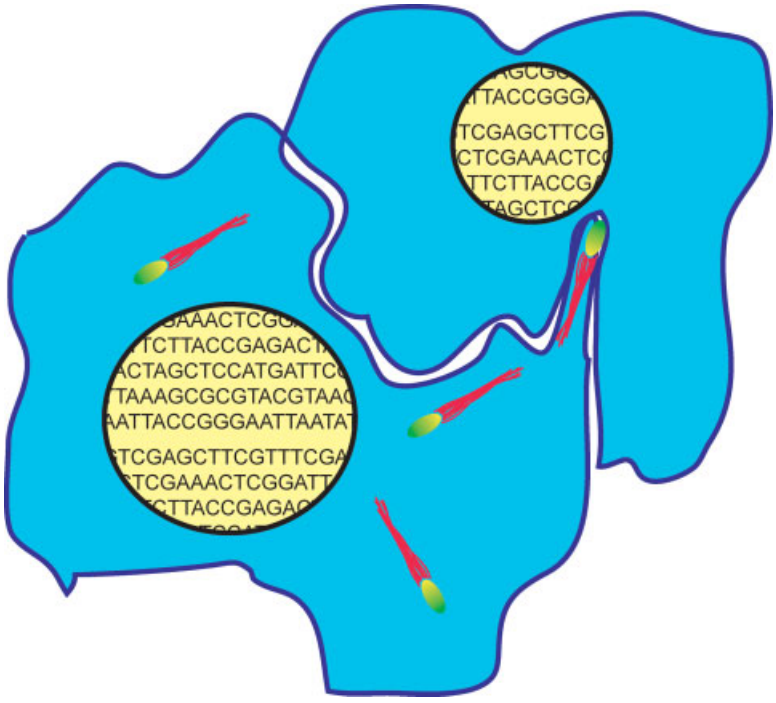

Figure 2. Horizontal endosymbiont transmission through actin-based motility. In a large number of cases where actin-based motility of microbes has been observed, bacteria are often reported to gain sufficient momentum to exit through the plasma membrane of one cell and break through into the cytoplasm of a neighboring cell.

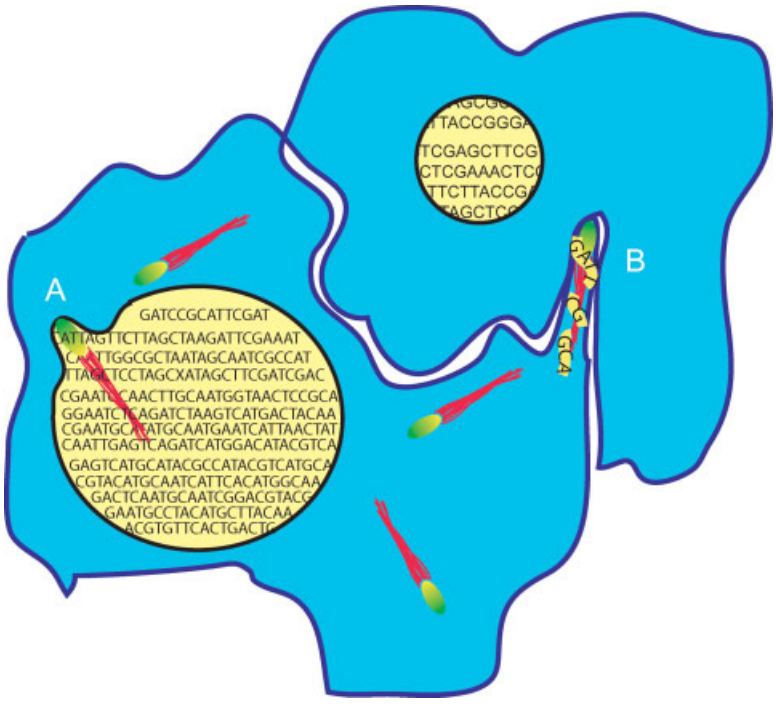

Figure 3. Endosymbiont-mediated horizontal gene transfer. A protomitochondrial endosymbiont like the SFG *Rickettsia* might carry DNA encountered during its passage through the host cell nucleus (A) with it to subsequently infected cells (B). A primitive infection process prefigures fertilization.

those lines in which the "sloppy" transfer of genes was made increasingly efficient. For example, cells that evolved more efficient receptors for protomitochondria or for increasing contact with infected cells would be more likely to be the recipients of genes carried by the endosymbiont, thereby enjoying a potential adaptive advantage over competing cells. The "cytoplasmic phase" of the infectious cycle would experience selection for increased efficiency in the assembly

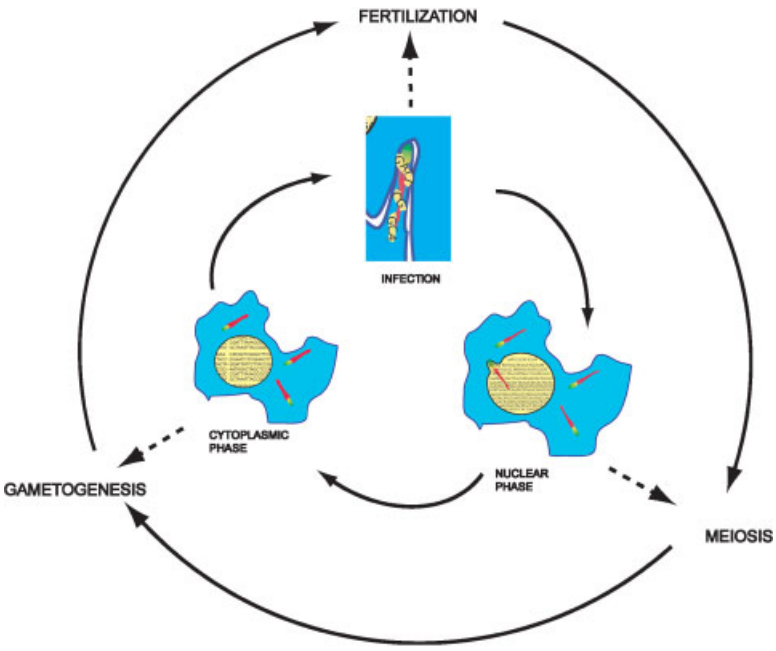

Figure 4. Radiational evolution of the germ-cell life cycle from an endosymbiont life cycle. The sloppy linkage between the three phases of the microbial life cycle allows their coevolution into the highly constrained life cycle of modern germ cells.

of protospermic genetic projectiles from endosymbionts. Similarly, endosymbionts carrying more genes from donor cells might confer a selective advantage on the cells that they subsequently infected, and therefore also on their progeny because of the greater number of recombinant genotypes that were made possible by their larger genetic payloads.

Because increased efficiencies in "protospermiogenesis" and "sperm reception" would have been determined by new mutations in the genomes of the host and/or endosymbiont genomes, a virtuous cycle or hypercycle could result, in which these two classes of mutation would be rapidly conjoined within the same cells. For example, a mutation that increased the efficiency with which new genes were broadcast through endosymbiont spread to other cells would tend to circulate with higher frequency in the population than its alleles. Moreover, the cells most likely to capture this gene in the genetic marketplace would be those carrying mutations increasing their efficiency of "protosperm reception". In the next generation of cells, these two mutations would be more likely to be inherited together than other mutations occurring randomly throughout the cell population, and the progeny cells would therefore have a greater potential for both increased transmission and reception of genetic information than either of their "parent" cells. The enlarged spectrum of genotypes and phenotypes sampled by these "supersexual" individuals would include variations in those biological functions directly required for survival (e.g., general metabolism, resistance to environmental stresses) and also an increased variability in the sexual processes themselves.

In a sense, the resulting self-reinforcing cycle can be thought of as the original instance of runaway sexual selection, or, since it is a virtuous cycle driving the evolution of sex itself, runaway sexual autoselection. The primitive cellular equivalent of male "display" would be gene transmission, while the primordial form of "female choice" would be gene reception. In a population of primitive cells exchanging genes through protomitochondrial transmission, mutations for increased display (more, faster, longer lived protosperm with bigger genetic payloads) would have been rapidly accumulated by individuals with mutations for better reception (choice), and their progeny would tend to inherit more of each of these than the progeny of other cells. To put it another way, cells with greater broadcasting capacity and cells with increased reception capability (sensitivity, discrimination between endosymbionts bearing genetic gifts and freeloaders, mechanisms for extracting and editing genes delivered by protosperm) will tend to exchange genes with each other more frequently than other cells, giving them the potential to evolve (and speciate?) much more rapidly over multiple generations. Thus, the self-reinforcing cycle of display and choice originally remarked upon by Darwin[27] and modeled by Fisher as "runaway sexual selection"[28] may have played a central role in driving the evolution of sex itself.

The self-reinforcing aspect of sexual evolution, in which increased efficiency of gene exchange provides adaptive advantages to sexual species, further selecting for sexual exchange, seems to be generally appreciated in evolutionary textbooks and in the literature of sexual evolution.[25,29,30] However, it is generally held that anisogamous sexual systems, based on the fusion of gametes of disparate sizes, is descended from presumably "primitive" isogamous systems with gametes of equal size,[31] such as those of *Chlamydomonas* or yeast. A distinctive feature of models by which sex evolved with sexual dimorphism from the beginning,[32] as proposed here, is that dimorphism provides the opportunity for reciprocal display and choice, the combination of which is required for the runaway element in sexual selection. Interestingly, runaway sexual selection is often credited with driving unfavorable or nonadaptive traits to fixation;[33,34] perhaps the infamous "2-fold cost of sex" dilemma, (i.e. the assumption that sexual reproduction is twice as costly as asexual reproduction and should therefore have been selected against in evolution) can be resolved by an argument that sexual reproduction has been fixed in sexual populations via the runaway process.

Essentially, "infected" and "uninfected" states may have constituted a simple form of primitive sex determination, providing the necessary dimorphism required for the virtuous cycle of true runaway sexual selection to take hold. Dimorphism from the beginning of sex allows for a true runaway process.

Germline cysts as genetic buffers

The selection for increasing frequencies of gene transfer by a protomitochondrial endosymbiont is at odds with the obvious need for genomic stability of the host organism. The "recombinational" events being selected for in this primitive sexual hypercycle would have been unidirectional movement of genetic material from one cell to another, with potential losses of genes by donor cells and genetic surpluses in recipients. This would be expected to result in increasing imbalances in genetic content among cells participating in sexual transfer, with some cells losing many genes while others enjoyed (or suffered from) genetic surpluses. Increasingly large duplications and deficiencies, at least in the short run, would presumably result in reduced viability of the cells carrying them. Yet the reductional division and reciprocal recombinational mechanisms of meiosis cannot have sprung full-blown from primitive cells in a single stroke. A potential solution is that meiosis evolved among the cells of a syncytial organism akin to acellular slime molds.[35] In this case, the communal cytoplasm of the cyst could act as a genetic buffer, by which the overexpression of genes from cells with too many copies of some genes would be effectively diluted to a survivable level by distribution through many cellular equivalents of cytoplasm. Reciprocally, those nuclei

that had lost critical genetic material as a result of a protomitochondrial visit would enjoy the opportunity for complementation by gene products produced in neighboring nuclei. By this thinking, the genetic buffering of germline cysts has provided a permissive environment for the gradual evolution of sloppy, unregulated and unidirectional gene transfer mechanisms into the highly regulated mechanisms of contemporary meiosis. The prevalence of germline syncytia[36] might have been fixed through the requirement for this genetic buffering to support the evolutionary experimentation in gene segregation that eventually gave us reductional meiosis.

Vestiges

The highly reduced size of the mitochondrial genome has resulted from the loss of most mitochondrial genes or their movement to the nucleus of the host cell.[37,38] While it might be relatively easy for genes to have been moved from one cellular compartment to another in evolution, the morphogenetic pathways controlled by many nuclear genes of mitochondrial origin with germline-specific functions, in many cases, would still be physically "wired" through the mitochondria. In recent years, several puzzling observations suggesting mitochondrial involvement in fundamental germ-cell processes have been made. Their significance appears to have gone largely unappreciated because there has been no comprehensive theory that coherently rationalized them. I suggest that they may be best understood as vestiges of the endosymbiotic origins of germ-cell pathways.

Exogenous gene transduction by sperm

Most biologists consider that the highly evolved processes of meiosis, gametogenesis and fertilization must be carefully regulated to ensure the integrity of genomes. The highly condensed sperm nucleus is inert, with the function of delivering exactly one haploid genome to the egg, and very little else. The idea of its descent from a form of generalized transducing particle, as suggested here, may therefore be a bit difficult to swallow. However, significant evidence of the capacity of sperm to take up exogenous genetic material and deliver it to eggs has been accumulated over the past fifteen years, from a number of different laboratories.[39] This is readily rationalized as a vestige of ancient protospermic transductional activity.

Gametogenesis

The most striking and well-documented example of mitochondrial participation in gametogenesis is the apparently general requirement for mitochondrial rRNA in the germ plasm of many organisms. The work of Kobayashi and colleagues has documented that ribosomal RNAs encoded by the mitochondrial genome are transported out of the mitochondria into the cytoplasm of developing oocytes, where they are an essential component of the germ plasm, the maternal, cytoplasmic determinant of germ-cell fate.[40–44] The germ plasm is arguably among the most-primitive, highly conserved specialized component in germ cells, possibly the defining substance of "germcellness". That mitochondria are so centrally involved in germ plasm construction is an indicator that they may have been participants in the earliest evolutionary events that gave rise to sex. More recently, the presence of mitochondrial rRNA in sperm nuclei has also been reported.[45,46]

Fertilization/syngamy

In some cases, the connection of specialized sexual pathways to mitochondria may simply be vestigial remnants of functions that formerly were provided by incipient mitochondria, but in which mitochondria no longer figure prominently. For example, I have suggested that the endosymbiont, by virtue of its actin-based motility, played a central role in the precursor to fertilization. Although extensive polymerization of actin, at least in some cases to apparently assist in penetration of the egg,[47,48] remains a general feature of sperm capacitation and/or the acrosome reaction,[49] the participation of mitochondria in the process would seem to have been de-emphasized in evolution. However, the recent observation that basonuclin, a component of the acrosome, also traffics through the mitochondria might be seen as a vestigial connection between mitochondria and the mechanics of fertilization. Similarly, the murine *Tep22* gene encodes a testis-specific gene product found only in the acrosome of early spermatids, then later in the mitochondrial midpiece.[50] These observations are suggestive of a common organellar lineage of the acrosome and mitochondria.

Meiosis

In *Drosophila*, there is a report of recessive mutations disrupting meiosis in one gene (Des-I; AKA *infertile crescent*) encoding a spermatocyte mitochondrial protein.[51] However, aside from the classical observations of mitochondrial association with the meiotic spindle in yeast,[52] functional evidence for regulation of meiosis through a mitochondrial mechanism seems to be lacking in the literature.

Sex determination

Another "curiosity" of germ cells that might be rationalized as a vestige of the endosymbiotic origins of sex is the predominance of plant self-incompatibility mechanisms wired through the mitochondrial genome, in the phenomenon known as cytoplasmic male sterility (CMS).[53] In CMS, pollen formation of monoecious plants is specifically blocked by a variety of mitochondrial mutations. Since the female parts of these individuals have normal fertility, cytoplasmic male sterility is somehow linked with sex determination. The argument is sometimes made that CMS works through mitochondria because mitochondria are maternally inherited, and that CMS

mechanisms are a selfish means of increasing mitochondrial transmission, by eliminating the resource drain of male gametogenesis in monoecious plants. Indeed, any maternally inherited organelle could be expected to promote, whenever possible, female gamete production in host cells at the expense of male gamete production. Yet, although chloroplasts are usually maternally inherited, CMS is without exception associated with changes in the mitochondrial genome.[54] I would argue that this results from the fact that gametogenic mechanisms are rooted in mitochondrial biology.

There are several more direct connections between mitochondria and sex-determination mechanisms. In mammals, steroidogenesis critical for fetal sexual differentiation has been reported to be dependent on the rate of mitochondrial protein import.[55] Perhaps most incredibly, in the blue mussel, *Mytilis*, male germline cells contain only mitochondria of paternal origin, while female germline cells contain mitochondria of maternal origin; the simplest explanation is that paternal and maternal mitochondria somehow compete for a compartment (presumably the mussel equivalent of germ plasm) in the zygote, and that the sex of the ensuing individual is somehow determined by the "winner".[56,57] The mussel case may in fact be the exemplar of the larger idea being suggested here. Perhaps the mitochondrion is not quite completely domesticated in germ cells, where its evolutionarily primitive behavior may be intrinsic to germ-cell identity.

The *Wolbachia* connection

In recent years, cell and evolutionary biologists have been converging in studies of endosymbionts with special talents for manipulating the reproductive pathways of animal hosts, primarily among the arthropods. Foremost among these microbial endosymbionts is *Wolbachia pipientis*, a member of a *Rickettsiaciae* family.[58,59] *Wolbachia* is capable of inducing parthenogenesis in the females of some species.[58,60] It can somehow imprint the sperm of infected males in some species such that their union with an egg is inviable if the egg is uninfected but viable if the egg is infected with the same strain of *Wolbachia*, in a phenomenon known as cytoplasmic incompatibility (CI).[61–63] In still other cases, *Wolbachia* is capable of altering the sex ratio in populations carrying it, either through manipulation of sex-determination pathways or by outright male killing. These multiple mechanisms of germ-cell manipulation clearly indicate a capacity by *Wolbachia* for interfering with several distinct germline processes, such as nuclear reorganization/condensation in spermiogenesis (CI) and syngamy (parthenogenesis). The allele-specificity of *Wolbachia*-induced suppression of female-sterile *sex-lethal* mutations in *Drosophila*[64] demonstrates the fine-tuned nature of these interactions and further expands the spectrum of reproductive functions functionally connected to Rickettsial endosymbionts. This multiplicity of entry points into germline pathways through which *Wolbachia* can act might be more readily understood in light of *Wolbachia*'s close relatedness to mitochondria. If the specialized pathways of reproductive cells involve mitochondria in ways that have not been fully appreciated up to now, a relative of mitochondria should be ideally equipped with the molecular machinery for interfering with critical mitochondrial germline functions to its own purposes. If the mitochondrion is a major protagonist in germ-cell biology, however underappreciated, who would be better equipped to impersonate it, co-opting its reproductive functions, than a close relative like *Wolbachia*?

Predictions

Several major predictions can be made from this hypothesis. First, if actin-based mitochondrial motility in spermiogenesis is descended from the extremely primitive experiments in sexuality, then it should be observable in spermiogenesis of more basal multicellular organisms. Second, as a deeper understanding of the germ-cell life cycle is developed, additional mitochondrial functions in specialized germ-cell pathways will be uncovered. Third, members of the SFG *rickettsia*, when tested, will be found to have a significant capacity to act as vectors for the transfer of genes between host cells. Finally, as the mechanisms by which *Wolbachia* manipulates host reproductive pathways come to be understood, they will be found to function through an interaction with specialized mitochondrial pathways of the germline.

One commonly cited model for the adaptive advantages of sex is a "Red Queen" model in which the exchange of genes between cells gave them survival advantages against the many parasites and disease organisms that preyed upon them in the environment.[65] Here we have suggested that one of these parasites, the endosymbiotic ancestor of mitochondria, was used as a vector to carry genes for its taming between its primitive victims. There have been previous suggestions of "infectious" or endosymbiotic origins of sex.[65–67] Motivated by the increasing number and variety of germline skills displayed by mitochondria and their rickettsial relatives, I have attempted to flesh out a crude mechanism by which the endosymbiotic ancestor of mitochondria could have given rise to the higher sexual pathways as we know them. No doubt some of these conjectures will be proved wrong in the future. But if we are to understand the cellular origins of sexual mechanisms, we must attempt to model simple gene transfer mechanisms that may have provided circumstances permissive for the evolution of the highly complex and constrained sexual pathways now being elucidated in remarkable detail by cell, developmental and molecular biologists.

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