Separating from the pack: Molecular mechanisms of Drosophila spermatid individualization

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Successful completion of gametogenesis is critical for perpetuation of the species. In addition to the inherent interest, studies of gamete development, in particular spermatogenesis, have yielded insight into diverse biological processes, including actin and microtubule organization, mitochondrial dynamics, plasma membrane remodeling, lipid signaling, apoptosis, and many others.

Mammalian sperm are formed from germline stem cells that reside near the basal surface of the seminiferous tubules. Spermatogonia produced from these stem cells undergo amplifying mitotic divisions with incomplete cytokinesis to eventually produce interconnected chains of spermatocytes that synchronously transition into meiosis. Cytokinesis of the meiotic divisions also is incomplete, such that cytoplasmic channels remain between sister spermatids after each division. This allows for the sharing of cytoplasm between sister spermatids, which synchronizes their development and protects them from the genetic effects of haploidy. Following meiosis, the haploid spermatids undergo spermiogenesis, the terminal differentiation process of gamete development, in particular spermatogenesis, have yielded insight into diverse biological processes, including actin and microtubule organization, mitochondrial dynamics, plasma membrane remodeling, lipid signaling, apoptosis, and many others.

Individualization is carried out by the individualization complex (IC), which first forms at the rostral end of the cyst, around the spermatid nuclei (Figure 1). The IC is composed of 64 actin cones, one for each germ nucleus of the cyst. Actin filaments form a meshwork at the leading edge of the cones and are organized into parallel bundles at the rear of the cones. The meshwork is formed by the Arp2/3 actin nucleating complex. The actin motor Myosin VI works with unknown binding partners to localize Arp2/3 and to stabilize the meshwork at the front of the cones. Other factors at the cone fronts include Actin Capsing Protein and Cortactin, and the membrane binding protein Amphiphysin. At the rear of the cones, the actin bundling proteins Quail/Villin, Chickadee/Profilin, and Singed/Fascin localize. As individualization proceeds, the actin cones of the IC move synchronously away from the nuclei toward the caudal end of the cyst, traversing the spermatid flagella at an average speed of 3 μm/minute and finishing the 1.8 mm journey in 10 hours. As it travels, the IC removes the cyst cytoplasmic contents and individualizes each spermatoozoon in its own plasma membrane. The cones accumulate actin during this process, especially at their front edges, and proper accumulation of actin filaments in the leading edge meshwork is required for cytoplasmic extrusion. Extruded cytoplasmic contents are collected in a cystic bulge that forms around the IC. When the IC and cystic bulge reach the end of the flagella, the actin cones and cytoplasmic contents find themselves in a waste bag, the contents of which are degraded. It is not yet known what generates the force for IC movement. Although Myosins V and VI are important for this process, motor activity does not seem to power migration of the sister spermatids, via intercellular bridges called ring canals. Following nuclear compaction and formation of the flagella, the interspermatid bridges are dissolved concurrently with cytoplasm removal in an actin-dependent process called spermatid individualization. Much has been discovered about this process in the 21st century. 

Figure 1

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The observation that actin polymerization is essential around the nuclei. Chickadee/Profilin is required for IC formation and are required for assembly of the cones focal adhesion protein Lasp localize to the actin cones during polymerization, many of which localize to the IC. Myosin V and the of actin binding proteins and regulators are required for individualization pathways that contribute to the process. As expected, a number of these factors according to function underlines several molecular pathways that contribute to the process. As expected, a number of actin binding proteins and regulators are required for individualization, many of which localize to the IC. Myosin V and the focal adhesion protein Lasp localize to the actin cones during their formation and are required for assembly of the cones around the nuclei. Chickadee/Profilin is required for IC movement and localizes to the rear of the cones. In its absence, the cones are short, lack rear bundles, and do not progress away from the nuclei. In contrast, ICs lacking leading edge proteins, such as Myosin VI or Arp3, have thin actin cones that are able to progress caudally, but these cones accumulate less actin, particularly in the front meshwork, do not remain in sync, and fail to successfully individualize the spermatids. Intriguingly, the actin regulator Rotund, a GTPase activating protein (GAP) for the signaling protein Rac, also plays a role in this process, suggesting that a signal cascade initiates IC formation or movement. The identity of such signal has yet to be discovered.

Casein kinase might also be involved in transduction of the signal.

The microtubule cytoskeleton, as well as the actin cytoskeleton, seems to be important for individualization. Loss of the microtubule binding protein Abnormal Spindle (Asp) results in many spermatogenesis defects, including failed individualization. Mutations in components of the Dynein-Dynactin complex, including Cytoplasmic Dynein Intermediate Chain (CDIC) and two Drosophila Dynein Light Chains, DDLC1 and DLC90F, perturb synchronous movement of the actin cones, but they also perturb nuclear shaping and positioning. Mutations in two other genes implicated in cytoskeletal dynamics, yuri gagarin and merlin, disrupt both nuclei and ICs as well. Cytoplasmic microtubules adjacent to the nuclei are important for nuclear shaping, which in turn may be required for the IC to assemble properly. Alternatively, microtubules in the vicinity of the nuclei might play independent roles in nuclear shaping and in aligning the actin cones during their formation. Recently, the individualization mutant mulet was mapped to a tubulin-specific chaperone E-like protein (TBCEL), again pointing to a role for microtubules. Unlike the Dynein-Dynactin complex mutations, the mulet mutation disrupts IC translocation without affecting the nuclei. The TBCEL protein can block microtubule assembly by disrupting tubulin heterodimers, and in the mulet mutant, cytoplasmic microtubules persist aberrantly in individualizing cysts, suggesting that these microtubules interfere with IC progression. Altogether, these observations indicate that cytoplasmic microtubules are important for assembly of the IC around the nuclei but must be cleared in order for the IC to translocate. Experiments with microtubule depolymerizing or stabilizing drugs in cultured cysts suggest that cytoplasmic microtubules are not involved in IC movement per se, but it is not clear whether the progressing IC interacts with axonemal microtubules. When axonemal microtubules are not properly posttranslationally modified, individualization is affected. Furthermore, the putative axonemal Dynein Intermediate Chain Dic61B is required for individualization. However, other studies suggest that individualization can occur normally in the absence of certain axonemal components. Finally, DDLC1 plays a role in actin accumulation on the cones, which is independent of the Dynein-Dynactin motor and could result from its association with Myosin V.

The individualization process may require deposition of new membrane between the spermatids. Other processes that involve new plasma membrane deposition, such as cytokinesis and spermatid elongation, use vesicles to shuttle phospholipids from the Golgi. However, visualization of membranes with fluorescent dye shows little vesicle trafficking at the cystic bulge during IC progression. Despite this observation, a number of vesicle trafficking factors are required for individualization, including Auxulin, Clathrin Heavy Chain, Rab11, Shibire/Dynamin, Vps28, and the Vps54-like protein Scattered. The cystic bulge contains numerous membranous structures, and puncta within the cystic bulge stain positively for the endocytic adaptor α-adaptin. Because most of the cytoplasmic contents are removed by the individualization process, perhaps
### Table 1. Drosophila genes in spermatid individualization

| Gene/protein | IC phenotype in mutant | EM$^a$ showing individualization defects? | Caspase staining? | Other spermatogenesis phenotypes | Reference | Molecular function | Cellular function |
|--------------|------------------------|------------------------------------------|-------------------|---------------------------------|-----------|--------------------|------------------|
| Ark/ Apaf-1  | Scattered migrating ICs, reduced cystic bulges and waste bags | + | Active caspase staining reduced | None reported | $^{58,59}$ | Apoptosis effector | Apoptosis |
| Dcp-1        | ND$^b$                  | + | ND | None reported | $^{58}$ | Caspase | Apoptosis |
| Dredd        | Scattered migrating ICs | + | No change$^c$ | None reported | $^{58}$ | Caspase | Apoptosis |
| Drice        | ND                      | + | No change | None reported | $^{61}$ | Caspase | Apoptosis |
| Driceless    | Normal ICs              | + | Active caspase staining eliminated | None reported | $^{58}$ | Apoptosis effector | Apoptosis |
| Dronc        | Scattered migrating ICs, reduced cystic bulges and waste bags | + | Active caspase staining reduced | None reported | $^{58,59}$ | Caspase | Apoptosis |
| Fadd         | Scattered migrating ICs | + | No change | None reported | $^{58}$ | Apoptosis effector | Apoptosis |
| Hid          | Scattered migrating ICs | + | No change | None reported | $^{58}$ | Apoptosis effector | Apoptosis |
| Tango7       | Scattered migrating ICs | ND | Active caspase staining eliminated | None reported | $^{60}$ | Apoptosis effector | Apoptosis |
| Cytochrome-c-d | No migrating ICs      | + | Active caspase staining eliminated | Axoneme microtubules are not properly polyglycylated, defective mitochondrial derivatives | $^{58,59,62}$ | Apoptosis effector, electron transport chain | Apoptosis, mitochondria |
| Bruce        | ICs do not form normally | ND | No change | Scattered misshapen nuclei | $^{62,63}$ | Inhibitor of apoptosis (IAP), ubiquitin conjugating enzyme (E2) | Apoptosis, ubiquitin-proteasome pathway |
| Arp3         | Thin migrating actin cones, less actin density on ICs, reduced cystic bulges | + | ND | Defective minor mitochondrial derivative | $^{25}$ | Actin meshwork nucleator | Cytoskeleton |
| Abnormal spindle | ND                    | + | ND | Meiosis failure, abnormal spindles, defective mitochondrial derivatives | $^{33}$ | Cytoskeleton regulator | Cytoskeleton |
| Bug22        | Scattered migrating ICs, reduced cystic bulges | + | No change | Defective mitochondrial derivatives, abnormal axoneme structure, | $^{42}$ | Ciliary protein | Cytoskeleton |

(continued on next page)
| Gene/protein | IC phenotype in mutant | EM$^a$ showing individualization defects? | Caspase staining? | Other spermatogenesis phenotypes | Reference | Molecular function | Cellular function |
|-------------|------------------------|--------------------------------------|-----------------|---------------------------------|-----------|--------------------|------------------|
| Drosophila genes in spermatid individualization (Continued) |
| CDIC/ Short wing | Scattered migrating ICs | ND | ND | Scattered nuclei | 34 | Microtubule motor | Cytoskeleton |
| Dynein intermediate chain 61B | Scattered migrating ICs | ND | ND | Defective mitochondrial derivatives, abnormal axoneme structure | 44 | Microtubule motor | Cytoskeleton |
| Dynein light chain 1/ Cut up | Scattered migrating ICs, less actin density on ICs | + | ND | Scattered nuclei, spermatid elongation abnormal | 34, 35 | Microtubule motor | Cytoskeleton |
| Dynein light chain 90F/ Tctex | Scattered migrating ICs | ND | ND | Scattered misshapen nuclei, defective basal body positioning | 36 | Microtubule motor | Cytoskeleton |
| Lasp | ICS do not form normally, less actin density on ICs | + | ND | Hub is displaced from apical tip, premature sperm coiling, cyst degeneration | 30 | Actin binding protein | Cytoskeleton |
| Merlin | Scattered migrating ICs | + | ND | Mild cytokinesis defects, cyst polarization defects, scattered misshapen nuclei, defective mitochondrial derivatives | 38 | Cytoskeleton regulator | Cytoskeleton |
| Mulet | Scattered migrating ICs | ND | ND | Cytoplasmic microtubules persist | 22, 40, 41 | Tubulin-specific chaperone E-like | Cytoskeleton |
| Myosin V | ICS do not form normally, scattered migrating ICs | ND | ND | None reported | 29 | Actin motor | Cytoskeleton |
| Myosin V/ Jaguar | Scattered migrating ICs, less actin density on ICs, reduced cystic bulges | + | No change | Mild nuclear scattering | 23, 28, 62 | Actin motor | Cytoskeleton |
| Profilin/ Chickadee | No migrating ICs, short actin cones | ND | ND | Cytokinesis defects | 25 | Actin bundling regulator | Cytoskeleton |

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| Gene/protein | Other spermatogenesis phenotype | IC phenotype in mutant | EM showing individualization defect? | Caspase staining? | Other spermatogenesis phenotypes | Reference | Molecular function | Cellular function | Organization | Mitochondria | Lipid metabolism | Mitochondria | Lipid metabolism | Mitochondria | Mitochondria | Mitochondria |
|--------------|-------------------------------|-----------------------|--------------------------------------|------------------|---------------------------------|-----------|-------------------|-----------------|-------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Tubulin, Tubulin Ligase-like 3B | + | Scattered migrating ICs | ND | + | None reported | 39 | Glycylase | Cytoskeleton | | | | | | | | |
| Yuri gagarin | | Yg do not form normally | | + | Ectopic caspases behind IC | None reported | 37 | Cytoskeleton regulator | Cytoskeleton | | | | | | | | |
| Noa | | Noa | | + | None reported | 90 | Fatty acid elongase | Lipid metabolism | | | | | | | | |
| NPC1 | | NPC1 | | + | Ectopic caspases behind IC | None reported | 57 | ER resident, OSBP binding | Lipid metabolism | | | | | | | | |
| Osb and Nes | | Osb and Nes | | + | None reported | 57 | Lysophospholipid acyltransferase | Lipid metabolism | | | | | | | | |
| Pxt | | Pxt | | + | None reported | 91 | Cyclooxygenase | Lipid metabolism | | | | | | | | |
| Hsp60B | | Hsp60B | | + | None reported | 91 | Heat shock protein, chaperone | Lipid metabolism | | | | | | | | |
| Mitoferrin | | Mitoferrin | | + | None reported | 49 | Mitochondrial carrier protein | Lipid metabolism | | | | | | | | |
| Parkin | | Parkin | | + | None reported | 49 | Ubiquitin ligase (E3) | Lipid metabolism | | | | | | | | |

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| Gene/protein | IC phenotype in mutant | EM<sup>a</sup> showing individualization defects? | Caspase staining? | Other spermatogenesis phenotypes | Reference | Molecular function | Cellular function |
|--------------|------------------------|-----------------------------------------------|------------------|---------------------------------|-----------|-------------------|------------------|
| Pink1        | ND                     | +                                            | ND               | Defective mitochondrial derivatives | 66-68     | Kinase            | Mitochondria      |
| Tafazzin     | ND                     | +                                            | ND               | None reported                    | 70        | Cardiolipin transacylase | Mitochondria      |
| Topo II<sub>a</sub> | Scattered migrating ICs | ND                                           | ND               | Scattered nuclei, germine stem cell loss | 71 | Topoisomerase | Mitochondria      |
| Ago2         | Scattered migrating ICs | ND                                           | ND               | Scattered nuclei                 | 81        | RISC complex       | RNAi pathway      |
| Blanks/ Lump | Scattered migrating ICs, arrested ICs | ND                                           | ND               | Scattered nuclei                 | 82,83     | dsRNA binding     | RNAi pathway      |
| Dcr2         | Scattered migrating ICs | ND                                           | ND               | Scattered nuclei                 | 81        | dsRNA processing | RNAi pathway      |
| HpRNA1       | Scattered migrating ICs | ND                                           | ND               | Mild nuclear scattering          | 81        | Non-coding hairpin RNA | RNAi pathway      |
| Crossbronx   | Scattered ICs          | ND                                           | ND               | Scattered nuclei                 | 22        | Ubiquitin conjugating enzyme (E2) | Ubiquitin-proteasome pathway |
| Cullin-3     | ND                     | ND                                           | Active caspase staining eliminated | None reported | 64 | Ubiquitin ligase complex | Ubiquitin-proteasome pathway |
| Klh10        | ND                     | ND                                           | Active caspase staining eliminated | None reported | 64 | Ubiquitin ligase complex | Ubiquitin-proteasome pathway |
| Nutcracker   | ICS do not form normally | +                                            | Active caspase staining eliminated | None reported | 92 | Ubiquitin ligase complex | Ubiquitin-proteasome pathway |
| Prosa6T      | Scattered migrating ICs | ND                                           | Active caspase staining reduced | Scattered misshapen nuclei       | 93        | Proteasome core subunit | Ubiquitin-proteasome pathway |
| Purity of essence | Reduced cystic bulges, less actin density on ICs | ND                                           | No change         | Mild nuclear scattering          | 22, 62    | Ubiquitin ligase (E3) | Ubiquitin-proteasome pathway |
| Roc1b        | ND                     | ND                                           | Active caspase staining reduced | None reported | 64 | Ubiquitin ligase complex | Ubiquitin-proteasome pathway |
| Scotti       | Scattered migrating ICs, arrested ICs, reduced cystic bulges | ND                                           | Elevated active caspase staining | None reported | 63 | Ubiquitin ligase inhibitor | Ubiquitin-proteasome pathway |
| Auxilin      | +                      | ND                                           |                  |                                  | 50        | Clathrin regulator | Vesicle transport |
Table 1. Drosophila genes in spermatid individualization (Continued)

| Gene/protein          | IC phenotype in mutant | EM\(^a\) showing individualization defects? | Caspase staining? | Other spermatogenesis phenotypes                                                                 | Reference | Molecular function          | Cellular function |
|-----------------------|------------------------|---------------------------------------------|-------------------|---------------------------------------------------------------------------------------------------|-----------|-----------------------------|------------------|
| Clathrin heavy chain  | IC formation delayed, scattered migrating ICs | +                                           | ND                | Mild nuclear scattering, abnormal spermatid plasma membranes, mild cytokinesis defects            | 22,50     | Vesicle coat                | Vesicle transport |
| Rab11                 | ICs do not form normally, less actin density on ICs | ND                                          | ND                | None reported                                                                                     | 24,34     | GTPase, endocytosis         | Vesicle transport |
| Scattered             | Scattered migrating ICs | ND                                          | ND                | Scattered misshapen nuclei                                                                       | 22        | Vps54-like, Golgi Associated Retrograde Protein (GARP) complex | Vesicle transport |
| Vps28                 | Scattered migrating ICs | ND                                          | ND                | None reported                                                                                     | 51        | ESCRT-I complex             | Vesicle transport |
| Asunder               | Scattered migrating ICs | ND                                          | ND                | Cytokinesis defects, meiosis failure, abnormal spindles, defective basal body positioning, scattered misshapen nuclei | 94        | Integrator complex          | RNA metabolism    |
| eIF4E-3               | ICs do not form normally | ND                                          | ND                | Cytokinesis defects, abnormal meiotic segregation, scattered misshapen nuclei                      | 86        | Translation initiation factor | RNA metabolism    |
| GLD2                  | ICs do not form normally | ND                                          | ND                | Scattered nuclei, abnormal chromatin compaction, defective basal body positioning                  | 88        | Cytoplasmic poly(A) polymerase | RNA metabolism    |
| Novel spermatogenesis regulator | Scattered migrating ICs, reduced cystic bulges | +                                           | ND                | Coiling defects, abnormal axoneme structure                                                       | 87        | RNA binding protein         | RNA metabolism    |
| Orb2                  | ICs do not form normally, scattered migrating ICs | ND                                          | ND                | Meiosis failure, scattered misshapen nuclei, defective nebenkerne, spermatid elongation            | 85        | Cytoplasmic Polyadenylation Element Binding protein | RNA metabolism    |

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| Gene/protein | IC phenotype in mutant | EM<sup>a</sup> showing individualization defects? | Caspase staining? | Other spermatogenesis phenotypes | Reference | Molecular function | Cellular function |
|--------------|------------------------|-----------------------------------------------|-----------------|---------------------------------|-----------|-------------------|------------------|
| Polypyrimidine tract-binding protein/hnRNP1/ Hephaestus | Scattered migrating ICs | ND | No change | Scattered nuclei, swollen testis tips | 62,89 | RNA binding protein | RNA metabolism |
| Dud | Scattered migrating ICs | ND | ND | Mild nuclear scattering, lacking nebenkerne | 22 | ND |
| Gilgamesh | Scattered migrating ICs | + | ND | Scattered nuclei | 32 | Casein kinase γ1 | Signaling |
| Gudu | Scattered migrating ICs | ND | ND | None reported | 95 | ND |
| Long island expressway | Scattered migrating ICs | ND | ND | Scattered nuclei | 22 | ND |
| Mozzarella | ICs do not form normally | ND | ND | Scattered misshapen nuclei | 22 | ND |
| Nanking | ICs do not form normally | ND | ND | Mild nuclear scattering, defective nebenkerne | 22 | ND |
| Rae1 | ICs do not form normally | ND | ND | Nuclear herniation, abnormal chromatin compaction, abnormal spindles, meiosis failure, defective nebenkerne, scattered misshapen nuclei | 96 | WD40 protein |
| Thousand points of light | Scattered migrating ICs | ND | ND | Scattered misshapen nuclei | 22 | ND |

<sup>a</sup>EM, electron micrograph.

<sup>b</sup>ND, no data.

<sup>c</sup>No change indicates that active caspases are still seen, but in many of these mutants, cystic bulges are not normal.
vesicle trafficking prior to IC progression segregates cellular components destined for degradation from those that will remain in the mature spermatozoa. Vesicles within the cystic bulge could provide specific lipids for incorporation into sperm membranes as well (see below). Shibire mutants show additional defects in actin accumulation on the ICs, suggesting that Shibire/Dynamin plays a role in IC assembly as well as translocation.24,34 Dynamin could anchor the plasma membrane to the IC, possibly in concert (or in parallel) with Amphiphysin, Cortactin, and Myosin VI.24,26,53 Alternatively, Dynamin could play a role in actin deposition independent of the membrane, perhaps with DDLC1.34,54 Some of the other vesicle trafficking mutants show nuclear defects, suggesting that they may also be required for IC assembly.22,50 Thus, it is not clear whether vesicle trafficking plays a direct role in IC movement.

Several lipid metabolism factors are required for individualization. In the absence of the sterol trafficking proteins OSBP, Fan, and NPC1, the actin cones do not migrate synchronously.55,56 Using filipin dye, sterols can be visualized in puncta within the cystic bulge, suggesting that trafficking of specific lipids occurs during this process.55 In mammals, the molecular composition of the sperm plasma membrane changes during maturation, and proper composition is required for fertility.13,14 Furthermore, failure to remove the cytoplasm can lead to peroxidation of membrane lipids and infertility.13,14 Perhaps a similar process occurs during Drosophila individualization, wherein the molecular composition of the sperm membranes is determined during migration of the IC. In this case, membranes and vesicles within the cystic bulge may act as a depot for the lipids. Specific lipids might also tether the IC to the membrane. In addition to cholesterol, phospholipid metabolism pathways contribute to individualization. In the absence of the lysophospholipid acyltransferases Oys and Nes or the cyclooxygenase Pxt, the actin cones do not migrate properly, suggesting that prostaglandin-like lipids generated from membrane phospholipids are important for this process.57,98 When phospholipid levels are genetically manipulated, no effect is seen, indicating that specific molecules, rather than bulk phospholipids, are critical.98 Whether these lipids play structural or signaling roles remains to be determined.

The discarded cytoplasm undergoes an apoptosis-like program during the process of individualization. Numerous apoptotic proteins are required for individualization to proceed correctly, including the apoptosis effectors Tango7, Fadd, and Hid and the apoptosome component Ark/Apa1.56,60 These proteins activate the pathway via initiator caspases Dronc and Dredd and effector caspases Drice and Dcp-1.58,59,61 The spermatid apoptosis program seems to be limited by the inhibitor of apoptosis (IAP) Bruce, and Bruce in turn is localized by the ubiquitin-proteasome system.62-64 Ubiquitylation of Bruce by the Khhl-10/Cullin-3 ubiquitin ligase complex at the rostral end of the cyst reduces Bruce levels, either by degradation or redistribution, which permits apoptosis initiation there.63,64 At the caudal end of the cyst, the ubiquitin ligase inhibitor Scotti protects Bruce by preventing its ubiquitylation, thereby preventing apoptosis initiation.63 Thus, by this mechanism, the apoptosis pathway is limited to the region of the cystic bulge, which begins at the rostral end. However, the spermatid nuclei also reside at the rostral end, and it is not known how they are protected from apoptotic degradation. Several mutants that disrupt movement of the IC have no effect on apoptosis initiation, suggesting that activation of this program is independent of other individualization events.62 However, mutation of apoptosis components disrupts migration of the IC, indicating that faulty apoptosis can disturb the entire process.

Many other ubiquitin-proteasome pathway components have been identified that participate in individualization (see Table 1). Their targets are not currently known. There seems to be large-scale degradation of cellular components following cytoplasm extrusion.21 Therefore, it is not clear if the individualization defects observed in these mutants are due to the persistence of specific targets or to a general failure of protein degradation.

Bruce removal alone may not be sufficient to initiate apoptosis. Similarly to mammalian apoptosis pathways, the mitochondria also play a role in apoptosis initiation in spermatids, via Cytochrome c-d.58,59,62 Intriguingly, mutations that disrupt the mitochondria prevent proper individualization, including those in the genes pink1, parkin, mitoferrin, mitochondrially-targeted topoisomerase IIIα, and the cardiolipin transacylase gene tafazzin.65-72 It has yet to be determined if this effect is mediated by Cytochrome c-d.

Spermiogenesis, in particular spermatid individualization, appears to be easily disrupted. Mutagenesis screens have discovered many genes that block spermatogenesis at this late step.73-75,97 This may be because the process is complex, requiring many factors, as detailed above. Another hypothesis, not mutually exclusive, is that this step represents a checkpoint for the removal of improperly differentiated spermatids.76 Support for this idea is found in flies experiencing meiotic drive, e.g. heterozygotes for a Segregation Distorter (SD) second chromosome that prevents formation of viable sperm carrying the other, normal second chromosome by interfering with proper chromatin condensation.77,79 In heterozygous cysts, in which half of the 64 sister spermatids carry the SD chromosome and half carry the normal homolog, the spermatids carrying the normal homolog are blocked at the individualization step, while their sisters are properly individualized and released from the testis.73,78 Individualization also is very sensitive to temperature, suggesting that cellular stress can halt the process.98 Other cell stressors have not been tested, but Wolbachia infection has been seen to induce mild individualization defects in some cases.80 Recently, it was found that genetic perturbation of the RNAi pathway causes individualization phenotypes.81-83 RNAi pathway mutations also perturb cytoskeletal reorganization of the oocyte in a checkpoint-mediated process.84 This seems to be a way for the oocyte to abort development when chromosomal integrity is disturbed by unregulated transposon activity. Perhaps a similar mechanism operates in spermatogenesis. Some, but not all, mutants that disrupt individualization show other spermatogenesis phenotypes; thus their effects on individualization may be indirect.

In conclusion, genetic studies have identified numerous genes required for individualization of the differentiated spermatzoa, the final step of spermatogenesis. Many of these genes have been
characterized molecularly, and they have highlighted important mechanisms at play during this process, including actin and microtubule dynamics, plasma membrane reorganization, and apoptotic elimination of the cytoplasmic contents. Many questions still persist, including: What are the signals that initiate individualization? How is the membrane reorganized, structurally and molecularly? How is membrane reorganization coordinated with IC movement? What propels IC movement? How are cytoplasmic components correctly partitioned into the cystic bulge? What protects the nucleus from the apoptosis pathway? Do all mutations that perturb individualization do so directly? How is gene expression coordinated at this developmental stage? It is likely that many factors are regulated post-transcriptionally, as the spermatid nuclei are highly condensed by this time, and indeed, RNA metabolism proteins play important roles in this process.

Furthermore, several genes necessary for individualization have not been characterized molecularly yet, and many genes acting in the process likely will be discovered. Future studies will elucidate a more coherent model that will undoubtedly reveal interesting molecular mechanisms and shed light on human fertility and infertility as well.

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