Membrane Fusion: HAP2 Protein on a Short Leash

Localized membrane fusion in the *Tetrahymena* conjugation junction generates pores that provide transient cytoplasmic continuity between the two partner cells. Without male gamete-specific fusion protein HAP2/GSC1, pores fail to form, fertilization is blocked, and pair stability is compromised.

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Sexual reproduction in eukaryotes depends on the ying/yang of meiosis and fertilization: meiosis to produce haploid nuclei and fertilization to fuse two such nuclei and restore the diploid state. In most species meiosis ultimately generates separate gamete cells to house the haploid pronuclei, and during fertilization the two gametes undergo cell–cell fusion to form the diploid zygote. As recently shown, fusion of the male and female gametes of many organisms depends on the broadly conserved membrane protein HAP2/GSC1 (HAP2p) [1–3]. To date, HAP2p has been shown to be required only in the male gamete, an asymmetry likely already well established in the last common ancestor of most if not all eukaryotes [4,5]. As reported by Cole *et al.* in this issue of *Current Biology* [6], the unicellular eukaryote *Tetrahymena* expresses a HAP2 family member also required for fertilization when cells have sex (conjugate). This ciliate, however, exhibits an interesting exception to the paradigm that two gamete cells fuse during fertilization, raising equally intriguing questions about the evolutionary diversity of HAP2 function.

Tetrahymena thermophila (and most other ciliates) are unicellular eukaryotes that accomplish meiosis and gametogenesis in the absence of cell division. Vegetative cells have a diploid germline nucleus (micronucleus) and a highly polyploidy somatic nucleus (macronucleus); only the former contributes DNA to the sexual progeny. To conjugate, *Tetrahymena* cells of different mating type pair. Meiotic division of the germline nucleus in each conjugant cell then generates four haploid meiotic nuclear products, only one of which survives. Gametogenesis is conceptually simple: the surviving nucleus in each conjugant divides mitotically to give rise to two haploid

nuclei: the migratory (male-equivalent) and the stationary (female-equivalent) gamete nuclei (Figure 1A). The male gamete nucleus of each conjugant then migrates through specialized, transient cell–cell fusion pores into the cytoplasm of the opposite conjugant, where it fuses with the resident female gamete nucleus to generate the diploid zygote nucleus (reviewed in [7]). Thus, *Tetrahymena* never forms a stable cell type possessing a single haploid nucleus, and thus forms neither male nor female gamete cells. So, what is a male-gamete-specific fusion protein doing in this organism?

Cole *et al.* elegantly answer this question by showing that *Tetrahymena* HAP2p has retained its core function in fusion (albeit partial and transient) of sexual cells. Preparation for mating ('co-stimulation'; [8]) requires direct contact with cells of a different mating type and causes the formation of a patch of specialized membrane on the cell surface ('smooth surface area'; [9]). The conjugal junction forms when the smooth surface patches of two cells adhere to one another. Roughly 200 pores are subsequently formed in the junction, which provide cytoplasmic connections between the two conjugants. These pores (Figure 2B–D in [6]) represent localized foci of membrane fusion, some of which eventually will greatly enlarge transiently to allow the reciprocal exchange of male gamete nuclei between conjugants (Figure 1A). Interestingly, in wild-type conjugating cells, *Tetrahymena* HAP2p localized to the junction. Significantly, when the *HAP2* gene was knocked out in both conjugants, the cells still paired but the conjugal junction failed to form pores. Furthermore, while gamete nuclei were generated normally in the absence of HAP2p, their reciprocal exchange was totally blocked. Thus, *Tetrahymena* conserves the HAP2p requirement for the male gamete nucleus to reach and fertilize the female gamete nucleus.

The ability to block so precisely a key step in conjugation has uncovered additional conserved features of interactions between sexually active cells that occur during the membrane fusion reaction and raises new questions about the regulation of events after membrane fusion. Pair formation in the absence of HAP2 reinforces the emerging theme that the membrane fusion reactions involved in fertilization occur in two distinct steps — membrane adhesion and membrane merger — carried out by distinct gene products [3,4]. Although free of the constraints that limit gamete fusion to just two cells, a similar paradigm is emerging in certain types of normal metazoan somatic cell–cell fusion (reviewed in [10]).

T. thermophila has seven mating types (genders, sexes). They constitute a self vs. non-self recognition system that prevents cells of the same mating type (and, by extension, genetically identical cells from the same vegetative clone) from mating with one another. *HAP2* expression was independent of the mating type of the conjugants, consistent with the functional equivalence of costimulated cells of every mating type [11] and their ability, when paired, to generate male gamete nuclei that migrate to the other conjugant. Intermediate fertility and pair stability were found in wild-type x HAP2 KO pairs. Cole *et al.* propose a model in which the junction "represents the equivalent of a male/female interface, and pore formation is driven on both sides of the junction by the presence of HAP2". In this model, gamete-cell asymmetry is not an intrinsic requirement of HAP2p function. As the authors point out, HAP2p may have lacked male-gamete specificity in the earliest eukaryotes, so that both interacting gametes could reciprocally initiate fusion. A deeper question remains: what challenge was met early on in eukaryotic evolution — and perhaps continues to be met — by restricting HAP2p function to just one (the male) gamete cell?

The behavior of junction pores raises additional interesting questions of membrane dynamics. Each of the
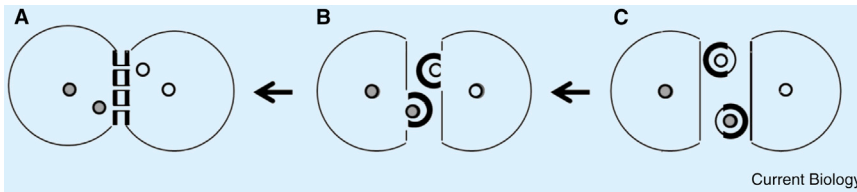
Figure 1. Model of the co-evolution of HAP2p localization with the loss of male gamete nucleus formation in *Tetrahymena*.

Evolutionary time runs from right to left (arrows). Shown are transverse sections of two conjugating cells after female and male gamete formation. The imaginary plane of section includes the four relevant gamete nuclei. Thick lines: HAP2p localization — known [6] (panel A) or putative (panels B and C). Small circles: haploid gamete nuclei; male are those closest to the center in each panel. White and grey gamete nuclei originate from the same conjugant, respectively. In every panel, male gamete nuclei move in a 'counterclockwise' direction, so that the zygote nucleus will be formed by fusion of a white and a grey nucleus. (A) Contemporary evolutionary stage, when male gamete nuclei are poised to be exchanged through a junction pore about to be enlarged. (B) Putative evolutionary intermediate in which each male gamete cell will fuse with the opposite female gamete cell just before completing the unequal gametogenic cell division that generates it. (C) Putative ciliate ancestral stage, prior to fusion of a large (female) and small (male) gamete cell generated by unequal gametogenic cell division.

pores in the wild-type junction resembles the initial stages of HAP2-dependent membrane fusion in other eukaryotes. Protrusions from one membrane into the lumen of the junction are seen in the presence or absence of HAP2p (Figure 2A and illustration in Figure S3 in [6]), which in wild-type pairs have been observed to fuse with the apposed membrane (Cole, unpublished). The difference in *Tetrahymena* is that subsequent pore expansion is sharply limited, so that both cells retain their integrity. What keeps the spread of membrane fusion under short leash in *Tetrahymena*? One possibility is the thick cytoskeletal layer lining the cytoplasmic face of the pore membrane [12]. How are pores reversibly enlarged to allow male gamete nucleus exchange while preventing fusion of the two conjugants? How is every pore undone to allow cell separation at the end of normal conjugation?

The *Tetrahymena* conjugal junction is a remarkable product of eukaryotic cell biology and evolution also from a mechanical standpoint. To allow male gamete nucleus exchange, the junction must retain its integrity for several hours while conjugating pairs actively swim, propelled by several hundred cilia. Forces generated by the tendency of the individual partners to rotate as they swim forward should place the conjugal junction under significant stress. What keeps pairs from falling apart? The authors show that in the absence of HAP2p, the partner cells are only loosely held together, so that pairs

are ultrasensitive to mechanical agitation and most of them spontaneously split earlier than their wild-type counterparts. This result implies that HAP2p-dependent pore formation significantly strengthens the mechanical coupling initiated by adhesion between the two cells. The dense cytoskeletal layer lining the cytoplasmic face of the junction and pore walls (Figure 2 in [6]) may cause this strengthening. Thus, the wall-reinforced HAP2p-dependent pores may 'rivet' the two wild-type conjugants to one another. How is the number of pores regulated to around 200? Since wild-type x HAP2 KO pairs showed intermediate physical stability and HAP2 transcript level, the number of pores may be limited by HAP2p amount.

Mating type specificity in *Tetrahymena* was recently found to reside in a pair of membrane-bound, mating type proteins, MTAp and MTBp [13]. Could mating type proteins of different mating type specificity bound to one another be the cell-adhesion molecules whose function precedes HAP2-dependent pore formation? The answer is not yet known but observations that pre-costimulated cells can immediately pair with cells of different but not of the same mating type [9] strongly suggest a requirement for mating-type-specific interactions in *Tetrahymena* heterotypic cell adhesion (pairing).

How did *Tetrahymena* HAP2p evolve to be localized at the conjugal junction? This can be glimpsed by considering

that ciliate ancestors of *Tetrahymena* are likely to have produced gamete cells, as do other alveolates (dinoflagellates and apicomplexans). Furthermore, some primitive (Karyorelictid) ciliates generate male and female gamete cells. The smaller (male) gametes migrate across the small extracellular space between the two conjugants (converted into female gametes) and reciprocally fuse with the other conjugant cell, followed by zygote nucleus formation in each recipient [14]. If *Tetrahymena*'s ciliate ancestors generated gamete cells in the same way, one can imagine an evolutionary sequence (Figure 1) in which the contemporary specialized junction region is the functional remnant of the male gamete cell surface and HAP2p function has essentially retained its conserved male-gamete specificity.

In summary, this stimulating report strengthens the idea that HAP2p is *directly and primarily* involved in membrane fusion, functions after membrane adhesion, and is part of an ancient gamete cell–cell fusion mechanism. This work uncovers the evolutionary adaptability of this gamete fusion protein, which retains its male gamete-related function in an organism that has dispensed with the production of male gamete cells. This 'experiment of evolution' broadens our view of HAP2p versatility by demonstrating its competence for gamete membrane fusion when present in either participating membrane — and opens the possibility that it so functioned before it became male gamete-specific. This contribution should spur progress by providing a new, versatile experimental system for understanding mechanisms of cell–cell fusion and its control — an area of biology still poorly understood.

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Neuroscience: Retinal Projectome Reveals Organizing Principles of the Visual System

A new study using zebrafish genetics and whole-brain imaging has identified more than 50 retinal ganglion cell morphologies and produced the first comprehensive map of connectivity between retina and its target visual centers.

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When a predator fish attacks a prey fish, different features of the predator's image and motion, such as its boundaries, color, approach and lateral motion, are extracted separately by different types of ganglion cells, the output neurons in the prey's retina. Each ganglion cell type consists of a mosaic of ganglion cells covering the retinal surface. The extracted features are sent in parallel to distinct visual centers by ganglion cell axons. The brain of the prey interprets the visual scene by integrating messages from the different ganglion cell types and then plans and executes a motor output that provides a potential escape from the predator. Similarly, the predator uses its own set of ganglion cell types and extracted features to track and catch its prey.

General consensus among researchers has been that the vertebrate retina has about 20 distinct types of retinal ganglion cells and, therefore, they extract 20 different features from the visual scene. In this issue of Current Biology,

Robles et al. [1] present the first complete connectivity map between the retina and central brain regions of zebrafish. When both dendritic morphology and central projections are taken into account, the data suggest that more than 50 ganglion cell morphologies exist. This new result further emphasizes the large number of parallel computations that are performed at the front end of the visual system.

In attempts to identify retinal ganglion cell morphologies, researchers in the field have relied mainly on three different experimental approaches. The first approach is random sparse labeling of ganglion cells using fluorescent or other dyes, and reconstruction of dendritic morphology [2–4]. The second is serial electron microscopy to reconstruct the fine structure of neurons [5]. One limitation of these two approaches is that they cannot look at axonal projections. The third approach is genetic labeling of specific cell types [6–12], which allows researchers to relate dendritic morphology, axonal projection and physiology of identified ganglion cell types. The number of available markers is far from complete,

however, and we still lack systematic approaches for identifying such markers. To date, all existing classifications have been based on dendritic and somatic morphology.

Robles et al. [1] mapped the connectivity between the retina and the central projection targets and classified ganglion cells based on the combination of dendritic morphology and axonal projection patterns. Taking advantage of the advanced genetics available in zebrafish as well as the fact that the larvae are transparent, they were able to image the entire retinal projection pathway using confocal microscopy. Ganglion cells were labeled sparsely, less than 1% at a time, which allowed the characterization of dendritic morphology and axonal projection patterns. Their work provides at least three key insights into the organizing principles of the vertebrate visual system.

The first insight concerns the structural diversity of ganglion cells. Robles et al. [1] identified 20 stereotyped axonal projection classes based on the 18 projection sites they found (Figure 1). The projection sites consist of nine sublaminar divisions within the tectum (homologous to the mammalian superior colliculus) and nine extratectal arborizing fields. Combining the projection patterns with distinct dendritic morphologies, more than 50 ganglion cell morphologies were identified. Importantly, the authors found that ganglion cells with the same dendritic morphology could be further categorized into multiple structural types based on the axon projection pattern. This echoes a