and the parameter space in which their senses operate. For Warren *et al.* [3], duetting is the result of the successful detection and appropriate motor control that together generate frequency convergence. As an ultimate function, this behaviour serves recognition between sexes [3], and perhaps also between species in multispecies aggregations. For Cator *et al.* [2] the convergence in the frequency domain is seen as part of a courtship song, used to facilitate and maintain midair pair formation. Duetting is also hypothesised to be under sexual selection, a process by which females could acoustically assess a male's reproductive quality [2]. Equally, a male may be able to assess a female's reproductive status, or health, through aerial acoustic interactions. The newly reported acoustic interactions between two mosquitoes prompt yet another question. What is happening when hundreds of male mosquitoes engage in swarming behaviour? As mosquitoes are now known to be capable to entrain each other into frequency convergence, it may be timely to ask whether and how males acoustically interact when they swarm. The answer may reveal key information on the mechanisms of swarm function and cohesion, and generate valuable ideas on how to disrupt their formation.

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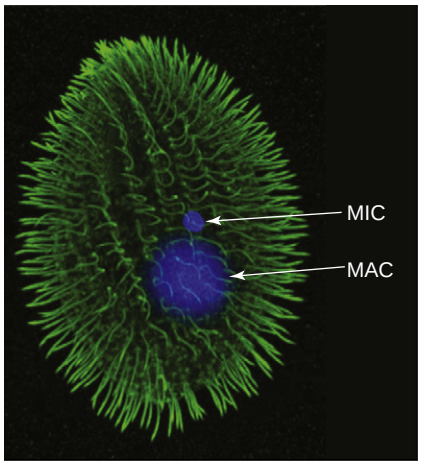
Nuclear Dimorphism: Two Peas in a Pod

The macro- and micronuclei of *Tetrahymena* reside in the same cytoplasm but are about as different as night and day. This extreme case of nuclear dimorphism can now be partially attributed to differences in the subunit compositions of their nuclear pore complexes.

David S. Goldfarb* and Martin A. Gorovsky

The dynamic compositions of the nucleus and cytoplasm depend in good measure on the selectivity of the nuclear transport apparatus, which is itself anything but static [1–3]. Multiple examples now demonstrate that changes in gene expression — for example, during the cell cycle, development, and in response to viral infection — often involve changing the composition of the nuclear transport apparatus [2,3]. Two recent studies conclude that the distinct properties of the two nuclei in *Tetrahymena*, the macronucleus (MAC) and micronucleus (MIC) (Figure 1), are determined, at least in part, by the distinct subunit compositions of their nuclear pore complexes (NPCs) [4,5].

Nuclear differentiation occurs at some point during the baroque nuclear machinations that characterize *Tetrahymena* conjugation, and nuclear transport differences between the two nuclei are apparent early in the process (Figure 2) ([6] and unpublished observations). During vegetative growth, the diploid MIC is transcriptionally silent and divides by a closed mitosis. During conjugation, it undergoes meiosis and forms zygotic nuclei that differentiate into MACs or MICs by a still mysterious process. MACs differentiate by a series of chromosomal rearrangements involving large scale DNA elimination, chromosome fragmentation, endoreplication, and gene amplification, resulting in a large nucleus containing ~45 copies of ~225 transcriptionally active chromosomes and ~9000 minichromosomes that encode the ribosomal RNAs [7]. MAC chromosomes assort randomly during division, and to prevent aneuploidy, their numbers are counted and adjusted in daughter cells. The MAC is degraded during conjugation and must be regenerated from post-zygotic MICs. Differences in the protein composition, morphology, and activities of insipient MACs and MICs appear



Current Biology

Figure 1. *Tetrahymena thermophila*.
Fluorescence micrograph of *Tetrahymena* showing anti-tubulin stained cilia and DAPI-stained macronucleus (MAC) and micronucleus (MIC). (Image courtesy of Wloga and Gaertig.)

towards the end of conjugation and are maintained throughout vegetative cell cycles (Figures 1 and 2). The basis for nuclear dimorphism in *Tetrahymena* has been a difficult nut to crack.

Recent studies indicate that the distinct properties of MACs and MICs can be partially explained by differences in their associated nuclear transport apparatuses [4,5]. Malone *et al.* [4] showed that several putative *Tetrahymena* nuclear transport receptors tend to localize preferentially to either MACs or MICs and provided the first evidence that the NPCs of the two nuclei contained different nucleoporins (nups). Iwamoto *et al.* [5], in a recent issue of *Current Biology*, set about exploring the hardware of MAC and MIC NPCs by a more extensive cataloging of the *Tetrahymena* orthologs of known nups and imaging the distribution of GFP-tagged versions of the proteins. Most of the 13 nups they tested localized to both MACs and MICs, but not so for the gene products of the four *Tetrahymena* NUP98 orthologs. Two of these (MacNup98A and MacNup98B) localized exclusively to the MAC, while the other two (MicNup98A and MicNup98B) localized exclusively to the MIC. The discovery that the nuclear transport apparatuses of MACs and MICs are different represents a significant breakthrough in the decades-long search for the basis of ciliate nuclear dimorphism.

Iwamoto *et al.* [5] also noted that MacNup98A and MacNup98B contain conventional 'GLFG' repeats, but MicNup98A and MicNup98B do not and instead contain a novel set of 'NIFN' repeats. Nup98 is one of a family of phenylalanine glycine (FG)-repeat nups that populate the central translocation channel of the NPC and serve as binding sites for receptor–cargo complexes [8,9]. Specific nups are increasingly seen as regulatory targets, and Nup98 is one of several nups whose expression is altered in response to physiological cues [1].

To test the hypothesis that the GLFG and NIFN repeats confer 'MACness' and 'MICness', Iwamoto *et al.* [5] swapped these domains in MacNup98A and MicNup98A, and over-expressed the chimeras in cells that continued to express the native *Nup98* genes. The nucleus-specific localization of the chimeras was determined not by their GLFG or NIFN repeats, which are found in the amino-terminal domains, but by the carboxy-terminal domains. This result is consistent with the fact that the carboxyl terminus of *Saccharomyces cerevisiae* Nup98 is responsible for linking the protein to the NPC proper. The phenotypes of cells expressing the chimeras support the hypothesis that the different Nup98s direct the import of nucleus-specific cargo, but the results are not simple. MACs and MICs contain different linker histones [7]. Switching the GLFG and NIFN domains between MACs and MICs did not switch the localization of GFP-tagged linker histones. Instead, the misplaced domains inhibited the import of the native linker histones. Interestingly, the localization of the chimeras increased the volumes of their respective host nuclei, a phenotype that was previously associated with deletions of linker histone genes [10]. The inhibitory activity of the chimeras is best explained by positing that the carboxy-terminal domains also play an important role(s) in nucleus-specific transport that must be integrated with the amino-terminal GLFG/NIFN domain in order to function properly. Finally, the import of a GFP-tagged nuclear reporter that localizes normally to both nuclei was unaffected by expression of the chimeras, supporting the hypothesis that the GLFG and NIFN domains of the *Tetrahymena* Nup98s are involved in nucleus-specific transport pathways.

Nup98 is tethered to the NPC in part through Nup96 [11]. Nup98 and Nup96 are usually expressed as a polyprotein that is autoproteolytically cleaved to yield mature Nup96 and a pro-form of Nup98 that is proteolytically processed to produce mature Nup98. A pro-form of human Nup98 is also expressed from an alternatively spliced message that lacks the Nup96 open reading frame. In the case of the four *Tetrahymena* Nup98s, only MicNup98B is expressed as a fusion to Nup96. Iwamoto *et al.* [5] show that the lone Nup96 localizes to both nuclei, which likely rules it out as a key determinant in nucleus-specific targeting. Because there is a surfeit of *NUP98* genes relative to *NUP96* genes in both *Tetrahymena* and in mammalian cells, and if Nup96 is, in fact, the primary binding site for Nup98, then one wonders how an unequal stoichiometry is accommodated within the NPC. It may be relevant that the yeast NPC harbors Nup100 and Nup116, which are clearly homologous to Nup98, but which do not appear to bind the NPC via Nup96 [11]. The situation may be more complex, because, if *Tetrahymena* Nup98s are like mammalian Nup98 and associate reversibly with NPCs [12], then their nucleus-specific localizations must be established at a certain transition during nuclear differentiation and then actively maintained during vegetative growth and the cell cycle. It actually makes sense that the nups that distinguish MACs from MICs should be exchangeable, since MACs develop from MICs during conjugation, and the pre-existing MIC NPCs must therefore be converted to MAC NPCs. The fact that both MICs and MACs undergo closed divisions means that nup exchange can not occur during the break-down and reassembly of nuclear envelopes that occurs in the characteristic open divisions of most higher cells [9]. Therefore, one would predict that an early event in nuclear differentiation is a facilitated exchange at MIC NPCs of newly synthesized MacNup98A and MacNup98B for the pre-existing pool of MicNup98A and MicNup98B. This possibility can now be investigated using GFP-tagged MacNup98 and

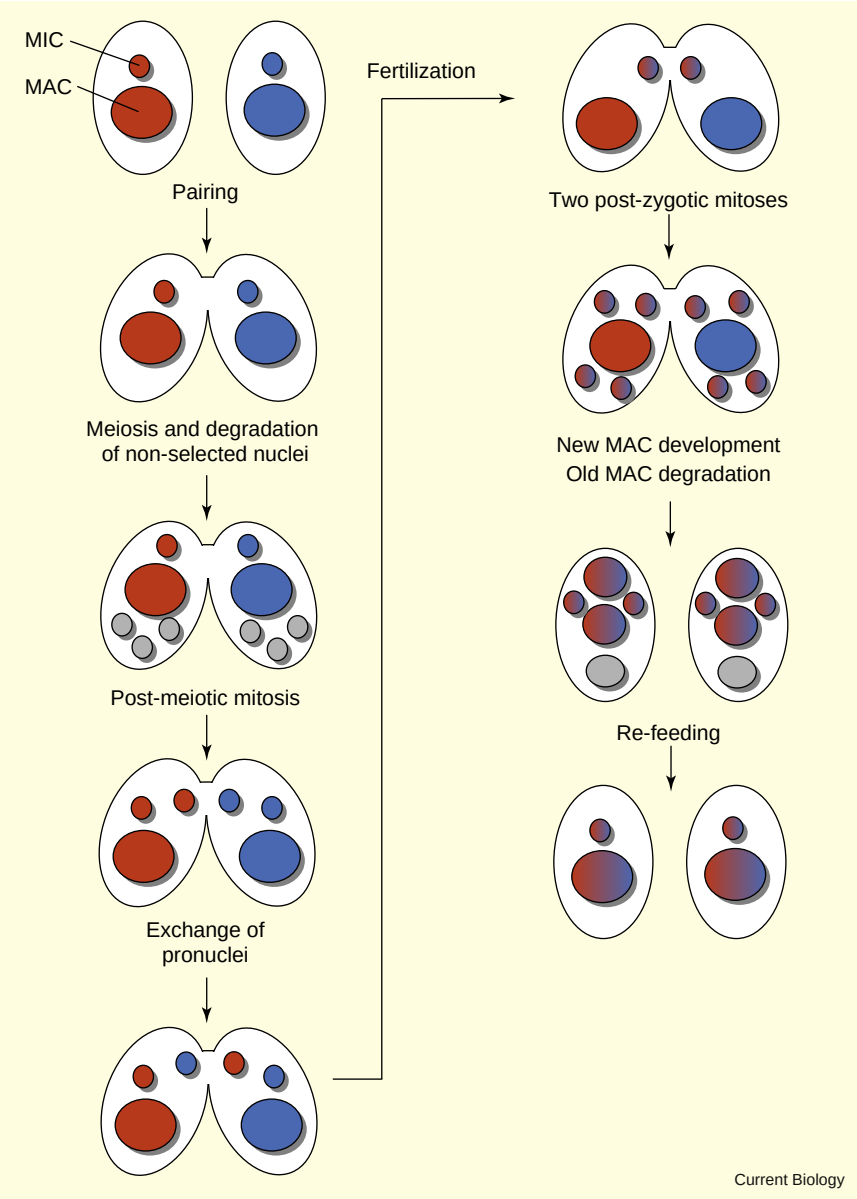


Figure 2. Nuclear dynamics during sexual reproduction of *Tetrahymena*.

Small circles represent micronuclei (MICs) and large circles represent macronuclei (MACs). Gray circles represent nuclei undergoing degradation. Nuclei colored both red and blue indicate mixed genotypes. (Adapted from [15,16].)

MicNup98 reporters. Finally, during vegetative growth MICs divide before MACs. It would be interesting to determine if MIC- and MAC-specific NUP98 gene expression is temporally coordinated with the replication of their cognate nuclei.

The Iwamoto study [5] still begs the issue of how the identities of the two nuclei are initially established. The exclusive targeting of Dorsal to ventral nuclei in syncytial *Drosophila* embryos is triggered by morphogen gradients [13]. The possible role of cytoplasmic determinants in *Tetrahymena* nuclear dimorphism was explored over half a century ago by David Nanney who, at that time, noted "…differentiation of (*Tetrahymena*) nuclei is directly related to their positions in the cytoplasm at a critical time. The conditions at the anterior end of the cell are such as to bring about the development of macronuclei; the conditions at the posterior end cause the development of micronuclei. That the nuclei developing as macronuclei are not different in their potentialities from those developing as micronuclei is shown by the fact that presumptive macronuclei may be induced to become micronuclei and presumptive micronuclei may be induced to become macronuclei by altering the positions of the nuclei in the cytoplasm" [14]. Could the localized determinants be components of the nuclear transport apparatus? The results of Iwamoto *et al.* [5] break exciting new ground in an old field, and, with these new reagents, provide hope that a final resolution is coming.

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Bacterial Development: Evidence for Very Short Umbilical Cords

Higher eukaryotes have channels, such as gap junctions and plasmodesmata, that allow intercellular communication. Recent studies on endospore formation in *Bacillus subtilis* suggest that an analogous structure may exist in prokaryotes.

Lee Kroos

Mothers of placental mammals nourish their fetuses and remove waste through umbilical cords. An analogous role might be played by gap-junction-like channels during bacterial endospore formation, based on the work of Camp and Losick [1]. The channels connect two cell types that form when *Bacillus subtilis* is starved (Figure 1, top). The larger cell is called the mother cell because it has long been known to provide proteins essential for the development of the smaller cell, the forespore, into a mature spore. For example, the mother cell synthesizes about 60 proteins that assemble on the surface of the forespore and protect the mature spore from environmental insults after it is released by programmed cell death of the mother cell. However, the results of Camp and Losick [1] suggest a more intimate dependence of forespore development on the mother cell than previously suspected. Specifically, channels are proposed to allow exchange of metabolites between the two cells and this appears to be crucial for gene expression in the forespore. If this feeding-tube model is correct and the channels function like gap junctions of eukaryotic cells or, anthropomorphically, like very short umbilical cords, it would provide a new paradigm for bacterial intercellular communication.

The existence of three communication pathways between the *B. subtilis* mother cell and forespore has been known for nearly 20 years [2]. These pathways govern the activation of sigma factors that direct transcription of particular sets of genes in each cell type (Figure 1). Two of the pathways involve secretion of one or more signaling proteins from the forespore under σ^F or σ^G control, leading to activation of membrane-embedded proteases that cleave membrane-associated precursors of σ^E or σ^K, respectively, releasing the active sigma factors into the mother cell [3,4]. Though unusual, the two forespore-to-mother-cell signal transduction pathways are relatively well understood. In contrast, the lone mother-cell-to-forespore communication pathway linking σ^E activity to activation of σ^G has defied understanding.

Earlier work by Camp and Losick [5] began to shed light on the mysterious σ^E-to-σ^G pathway. They used elegant genetic approaches to identify SpoIIIAH (AH) and SpoIIQ (Q) as the minimal components of the pathway. AH is made under σ^E control in the mother cell and is targeted to the mother cell membrane that engulfs the forespore (Figure 1, middle). A clue that the σ^E-to-σ^G pathway might involve channels came from the finding that AH's large extracellular domain is similar to proteins that form multimeric rings and are components of type III secretion systems or flagella [5,6]. Moreover, AH's extracellular domain had been shown to interact with Q's large extracellular domain in the space between the mother cell and forespore membranes, after Q is made under σ^F control in the forespore and targeted to the forespore membrane [7,8]. Meisner *et al.* [6] provided evidence for AH–Q channels by cleverly showing that biotin ligase made in the forespore could biotinylate the extracellular domains of Q and AH. Interestingly, although biotin ligase could apparently enter the channels from the forespore, it could not enter from the mother cell, suggesting the channels have a smaller diameter or are gated on the AH end.

Obviously, one would like to know exactly what goes through the channels to permit activation of σ^G in the forespore. As a step toward determining the nature of the signal, Camp and Losick [1], in their recent work, asked whether the channels are specifically required only for σ^G-dependent gene expression in the forespore or are generally required for transcription and/or translation in the forespore. They took advantage of the observation that a σ^F-dependent gene fails to be shut off in cells lacking σ^G. The resulting persistent expression of this gene late in development was observed to depend on the channels, suggesting that the channels are generally required for gene expression in the forespore during the later stages of development. However, σ^F and σ^G are quite similar proteins, leaving open the possibility that the channels might convey a factor specific to the activation of these transcriptional regulators, rather than a factor or factors generally required for transcription and/or translation. Therefore, Camp and Losick [1] engineered *B. subtilis* to express a gene encoding phage T7 RNA polymerase (RNAP), a heterologous enzyme, under the control of a σ^F-dependent promoter and measured expression of *lacZ* fused to a T7-RNAP-dependent promoter. Since T7 RNAP is a single-subunit enzyme that does not require a sigma factor, concern about competition between sigma factors for binding to core RNAP was eliminated. Strikingly, they found that expression of the T7-RNAP-dependent *lacZ* reporter in the forespore requires the channels. This result strongly suggests that the channels are generally required for transcription and/or translation in the