

## **PATTERNS OF TRANSLATION THAT LEAD TO RAPID DEVELOPMENT DURING SPERMIOGENESIS IN *Marsilea vestita***

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Spermiogenesis in the water fern *Marsilea vestita* is a rapid process that is activated by placing dry microspores into water. Populations of male gametophytes develop synchronously, and each gametophyte develops within the microspore wall. The microspore contains a single cell that initiates a series of nine successive mitotic division cycles to produce 39 cells - one prothallial cell, six sterile jacket cells and 32 spermatids. The cell division planes are precise. Both position and distinct compositional differences between spermatogenous cells and sterile cells underlie cell fate determination in the gametophyte. As the division phase nears completion, a novel cytoplasmic particle, known as a blepharoplast, forms in each spermatocyte, serves as the centrosome for the spindle of the last division, and then serves as a site for the *de novo* formation of basal bodies. During the next 5.5 h, each spermatid assembles a complex cytoskeleton that facilitates extensive elongation and coiling of the cell body and the nucleus. The basal bodies serve as templates for the formation of ciliary axonemes. The process reaches completion in ~11 h with the release of 32 spermatozooids from each gametophyte. Each spermatozoid is a coiled cell that possesses ~140 cilia.

For the past several years, we have been interested in the processes of basal body formation and cell fate determination in the male gametophytes of *M. vestita*. We have found that rapid development of the male gametophyte is controlled at a post transcriptional level; the dry microspore contains large quantities of stored proteins and stored mRNAs, and the translation of stored transcripts controls the rate and extent of development. For the assembly of the cytoskeleton and the ciliary apparatus, the fern uses a set of highly conserved genes that have been identified in fungal and animal cells. We have found that specific mRNAs (*e.g.*, centrin, cyclin A, cyclin B,  $\alpha$ -tubulin, P28, kinesin) are translated at specific times during development, and translation of these transcripts is restricted in distribution to the spermatogenous cells. We have developed RNAi strategies to disrupt individual mRNAs in the gametophytes, and have shown that centrin is an essential component in the blepharoplast; in the absence of centrin, basal body formation will not occur. We have disrupted the cell division cycles in the gametophyte using pharmacological probes and with dsRNAs derived from cyclin A and cyclin B. Even in the absence of cell divisions, centrin is made at its normal time, but blepharoplasts fail to form. In the absence of blepharoplasts, basal body assembly is completely blocked. We have begun to look at controlling factors for the patterns of mRNA and protein distributions during cell fate determination. In a screen of our gametophyte cDNA library, we found a homolog for mago nashi, a protein involved with axis determination and gonad formation in animals. The protein encoded by our *Mv-mago* cDNA is more than 70% identical with mago nashi proteins from a variety of animals. The destruction of stored *Mv-mago* mRNA in the gametophyte by RNAi treatment results in a change in the plane of cell divisions in the gametophyte. In addition, the *Mv-mago* protein is apparently involved in the control of spatial distributions of certain mRNAs and the

locations of translational activities in the gametophyte. Thus, this protein appears to play multiple roles in cell fate determination during spermiogenesis.