Projects in the Pilgrim Lab

In the Pilgrim laboratory, we work on developmental neurobiology, sex determination, and myosin assembly in muscle and embryo development. We work with the nematode Caenorhabditis elegans, and the zebrafish, which serves as a vertebrate model for medical research. Both systems allow us to use genetic tools to complement molecular and cellular biology approaches toward the study of the mechanisms controlling developmental and differentiation decisions. C. elegans and zebrafish offer several advantages as models:
- developmental pathways are highly conserved with humans, allowing medical research in a simpler, tractable system
- a rapid generation time makes genetic screens feasible, for the isolation of mutations affecting a particular developmental process
- reverse genetics (gene knockouts) are simple and rapid (RNAi in C. elegans, morpholinos in zebrafish). Gene products can be selectively removed one at a time, and we can examine the effect of their removal on development.
- mutations are already available in many genes affecting many developmental and biochemical processes, allowing genetic approaches for identifying and isolating interacting genes, or testing molecular therapies.
- animals are transparent, allowing us to study development in living animals, without dissection, and are amenable to the use of fluorescent reporters (e.g. GFP, and other coloured reporters).

Development of the nervous system

C. elegans has an invariant cell lineage, with similar locations and patterns of connectivity of neurons in every animal. Genes required in the nervous system of C. elegans have mutant phenotypes affecting behaviours such as response to touch, sensitivity to chemical attractants, contraction of muscles and feeding. The defect in unc-119 mutants lies in at least two aspects: chemosensation and movement. At the cell biological level, it appears that neuronal growth cones in unc-119 mutants are aberrant, resulting in neurite branching and axonal fasciculation defects. UNC-119 homologues exist in mammals, and these homologues are also neuronally expressed. When expressed in C. elegans, these homologues can rescue all aspects of the unc-119 mutant phenotype. Knockdowns of unc-119 homologues in mice or zebrafish result in nervous system and eye defects. Therefore, we have identified a new family of evolutionarily conserved neuronal proteins. Our recent work suggests that UNC-119 regulates neuronal cytoskeletal organization via two distinct pathways involving G-proteins. Since most of the other identified components of the C. elegans nervous system have direct human homologues, this work may also shed light on the processes and products involved in the development of the human nervous system. We are using genetic and molecular approaches to identify proteins with which UNC-119 interacts, in the neurons of C. elegans, zebrafish and humans.

A myosin regulatory protein in muscle development and cytokinesis

The Caenorhabditis elegans unc-45 gene is essential for normal thick filament development in body wall muscles, as unc-45 mutants are paralyzed. UNC-45 regulates muscle myosin assembly, but (surprisingly for a muscle protein) is also essential in the embryo, where non-muscle myosins such as NMY-2 are necessary for asymmetric cell division and cytokinesis. UNC-45 interacts with NMY-2, and UNC-45 and NMY-2 also colocalize in vivo, at the cortex of the cell in early embryos, and localization of UNC-45 is dependent on the presence of NMY-2. Disruption of either UNC-45 or NMY-2 leads to a complete failure in cytokinesis in the fertilized embryo. Rather than being a muscle-specific protein, this would argue that the UNC-45 protein may have a more general role as a myosin chaperone or assemblase.

We have shown that UNC-45 is an evolutionarily conserved class of myosin interacting proteins. Zebrafish UNC-45 is essential for trunk muscle development and also for cardiac muscle function. Knockdowns of muscle UNC-45 result in paralyzed embryos, with defects in thick filament assembly in skeletal muscle, and in heart
contraction. Since mammals have UNC-45 homologues, this implicates UNC-45 as a candidate gene for cardiomyopathies in humans, a set of muscle wasting and degenerative diseases.

Students in my lab are studying (i) the role of UNC-45 and non-muscle myosins in cell polarity and cytokinesis; (ii) using genetics and cell biology to identify and study the proteins that work in the same pathway as UNC-45 in vertebrates; (iii) analyzing the transcriptional control of UNC-45 in zebrafish muscle; and (iv) analyzing and characterizing the molecular interactions between UNC-45 and myosins in both vertebrates and C. elegans.

Why do genes controlling sex determination evolve so fast?

Sex is determined in C. elegans by the number of sex (X) chromosomes, and some mutations cause the animal to completely ignore the chromosomal signal. FEM-2 acts at an important branch point in the pathway for the regulation of sex determination. C. elegans are normally hermaphrodites (XX) or males (XO), but fem-2 mutants develop as females, regardless of the number of X chromosomes. FEM-2 is thought to signal between a cell surface receptor (TRA-2) and a nuclear transcription factor (TRA-1). We are presently undertaking a molecular, genetic and cell biological characterization of the fem-2 gene and the interaction of its product with other genes in the pathway.

One of the surprising features of sex determination in all species is that proteins evolve unusually quickly. To study this, we have begun to use genetics and molecular biology to investigate sex determination in the closely related nematode C. briggsae to test whether the observed sequence divergence between orthologues is underlying functional changes at the protein level. So far, we have shown conclusively that while functional conservation is present in the somatic sex determination pathway, the mechanism that regulates sexual fate in the hermaphrodite germline differs considerably between C. elegans and C. briggsae. In particular, while fem-2 is necessary for C. elegans hermaphrodite spermatogenesis, it does not play the same role in C. briggsae. The regulation of germline fate that ultimately produces similar hermaphroditic outcomes is accomplished through different molecular mechanisms in these two species. We are particularly interested in using this system to understand how developmental regulatory pathways evolve, and how this may lead to speciation.

We have a wealth of genetic and molecular resources for studying this process, and I am particularly interested in recruiting students who are keen to develop their skills in genetics and may be interested in a project that combines molecular and cell biology with a thread of evolutionary biology.

Recent Publications (2003-08)

Wohlgemuth et al. 2007. The myosin co-chaperone UNC-45 is required for skeletal and cardiac muscle function in zebrafish. Developmental Biology 303:483-492
Hill et al. fem-independent hermaphroditism in Caenorhabditis briggsae. Developmental Cell 10:531-538