Myosin Diversity in the Marine Pennate Diatom *Phaeodactylum tricornutum*

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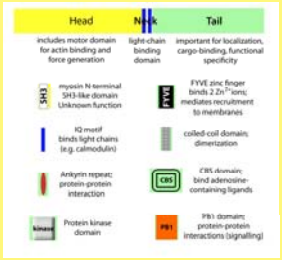
ABSTRACT

Myosins are actin-based molecular motors that participate in a wide range of cellular activities from vesicle transport to signal transduction to cell locomotion. As a prelude to exploring the roles of these mechanoenzymes in the biology of *Phaeodactylum*, we sought to inventory the myosins expressed in this cell. Taking advantage of sequence information available from the *P. tricornutum* genomic database (DOE Joint Genome Institute) and using a battery of degenerate myosin primers, PCR-based strategies have been employed to clone myosins from *Phaeodactylum* cDNA. As is typical of most organisms across the phylogenetic spectrum, *Phaeodactylum* expresses multiple myosins of different types. Comparison of sequences derived from the myosin head (motor) domain was used to assess the similarity of these diatom myosins to each other and to representatives of the established classes of myosins in the myosin superfamily. To date, ten myosins have been identified in *Phaeodactylum* and these segregate into three loose groups, none of which are closely akin to any of the established 24 classes currently defining the myosin superfamily. Even within the three groups of *Phaeodactylum* myosins, significant variations in myosin structure are evident. The diatom myosins range in size from less than 130kDa to greater than 240kDa and exhibit a great range of structural diversity in the three major myosin domains, the head, neck and tail. Analysis of myosin sequences using available algorithms for the prediction of protein domain architecture reveals putative domains common to many myosin classes (N-terminal SH3-like domains, IQ motifs, ankyrin repeats, coiled-coils) as well as some domains that are less common or not yet described in other types of myosins (FYVE zinc-binding domains, CBS domains, PB1 domains). This great diversity of myosins seen in *Phaeodactylum* will likely reflect the wide range of roles that these molecular motors will be discovered to play in the normal structure and function of diatoms.



The molecular architecture of a typical myosin

Myosins contain three primary domains:

1) The **head domain** includes the motor domain that is essential for binding to actin and generating the mechanochemical force used in contraction or other motor activity (e.g. vesicle transport). The N-terminal portion of the head domain may contain additional functional domains, such as the myosin N-terminal SH3-like domains found in some of the diatom myosins.

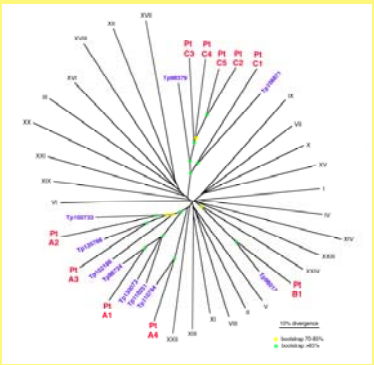
2) The **neck domain** contains one or more IQ motifs that serve as light chain binding domains. Calmodulin or other members of the calmodulin family of calcium-binding proteins typically serve as the myosin light chains and can modulate the mechanoenzymatic behavior of a given myosin.

3) The **tail domain** is the most variable domain among different myosins. The tail domain is thought to dictate how and where a particular myosin motor is used in the cell by mediating the interaction of the myosin motor with its target or other binding partners. These intermolecular interactions are made possible by the variety of structural/functional sub-domains that may be found in the tails of different myosins.

Where do the diatom myosins belong in the myosin superfamily?

The current organization of myosins into classes within a myosin superfamily is based upon phylogenetic analysis of the primary sequence of the myosin head domain, the most well-conserved domain among myosins of all types.

Using the full-length myosin head sequence from the *P. tricornutum* myosins, together with partial head sequences from myosins identified in the centric diatom *Thalassiosira pseudonana* (Montsant et al., 2007, J. Phycol. 43:585-604) and one representative from each of the established myosin classes (Foth et al., 2006, PNAS 103:3681-3686), phylogenetic analysis suggests that the diatom myosins associate into three main groups, largely distinct from the established classes of myosins.



I-XXIV are the established classes of the myosin superfamily

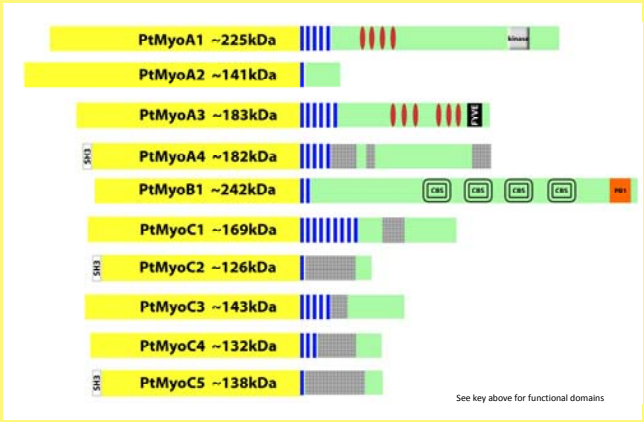
T. pseudonana myosins are indicated in purple

P. tricornutum myosins are indicated in red

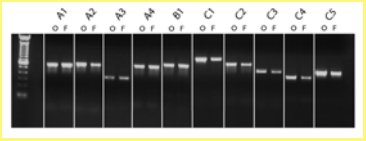
Cloning of *P. tricornutum* myosins

To identify the myosins expressed in the oval morphotype of *P. tricornutum* (derived from CCMP2559), total RNA was prepared from cell cultures, treated with DNAse to eliminate genomic DNA contamination, and then reverse transcribed into cDNA. Degenerate primers known to react with most known myosins, together with some specific primers derived from *P. tricornutum* genomic sequences, were used in a variety of PCR-based cloning strategies to obtain full-length coding sequence for 10 diatom myosins.

The myosins of *P. tricornutum*



Myosin expression in oval and fusiform morphotypes of *P. tricornutum*



P. tricornutum is an unusual diatom in that it can exist in one of three morphotypes, tri-radiate, fusiform and oval. Both the tri-radiate and fusiform morphotypes are planktonic while the oval morphotype is benthic and, possessing a raphe, moves through its environment by means of gliding locomotion. Given these two different life-styles, it is of interest to know if the expression of the different myosins varies among the morphotypes, perhaps reflecting the importance of one or more myosins in morphotype-specific behavior. Preliminary PCR results looking at the expression of myosin transcripts in oval (O) and fusiform (F) morphotypes suggest that all 10 myosins are expressed in both morphotypes. Whether significant variations in the levels of expression of the different myosins exist awaits a more quantitative analysis of the data.

Significance

Myosins play essential roles in the structure and function of most cells. In association with the actin cytoskeleton, this family of molecular motors participates in wide-ranging activities from contraction to locomotion to vesicle transport, as well as endo- and exocytosis, signal transduction and mRNA localization. Given the importance of all these cellular behaviors, and our relatively poor understanding of how these cellular processes occur in diatoms, the exploration of myosin function in diatoms is of great interest. With the recent development of techniques permitting the molecular manipulation of diatoms, we are now poised to begin dissecting the roles of the many intriguing myosins identified in *P. tricornutum*. This diatom expresses a great range of myosin types, some containing putative functional domains not yet described in any other myosin. The full characterization of these myosins will help to expand our understanding not only of myosin biology itself, but also the molecular cell biology of *P. tricornutum* and other diatoms.