Actin assembly by Fhod3 in the cardiac sarcomere

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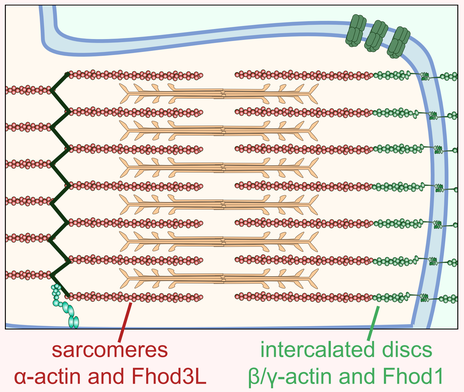
Background

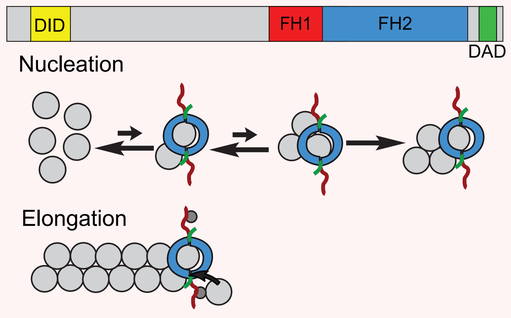
Cardiomyocytes assemble actin isoforms into distinct structures.



The major contractile structure in cardiac muscle, the sarcomere, consists of highly regular arrays of α-actin and cardiac myosin. The formin Fhod3 localizes to sarcomeres and is required for their assembly in cardiomyocytes.

Cardiomyocytes also express cytoplasmic β- and γ- actins. These isoforms are enriched at peripheral structures, such as costameres and intercalated discs, which link the sarcomere to the extracellular matrix and neighboring cells. The formin Fhod1 localizes to these structures in cardiomyocytes.

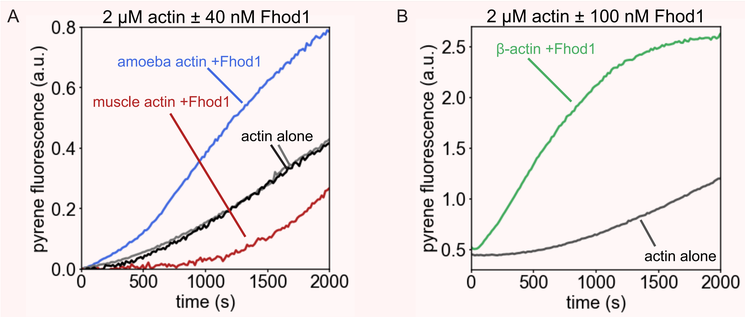
Fhod1 and Fhod3 belong to the formin class of actin nucleators/elongators.



Formins nucleate and processively elongate actin filaments through their formin homology (FH) domains. The FH2 domain homodimerizes to form a donut-shape, which is thought to nucleate actin filaments by stabilizing intermediate actin dimers and trimers. Formins processively elongate actin filaments through the combined action of their FH1 and FH2 domains. The FH2 domain binds tightly to the faster growing barbed end of the filament. While associated with the barbed end, the FH2 domain permits addition of actin monomers and processively steps after each addition. The FH1 domain contains polyproline tracks, which bind the actin monomer-binding protein profilin and thus recruit actin to the barbed end.

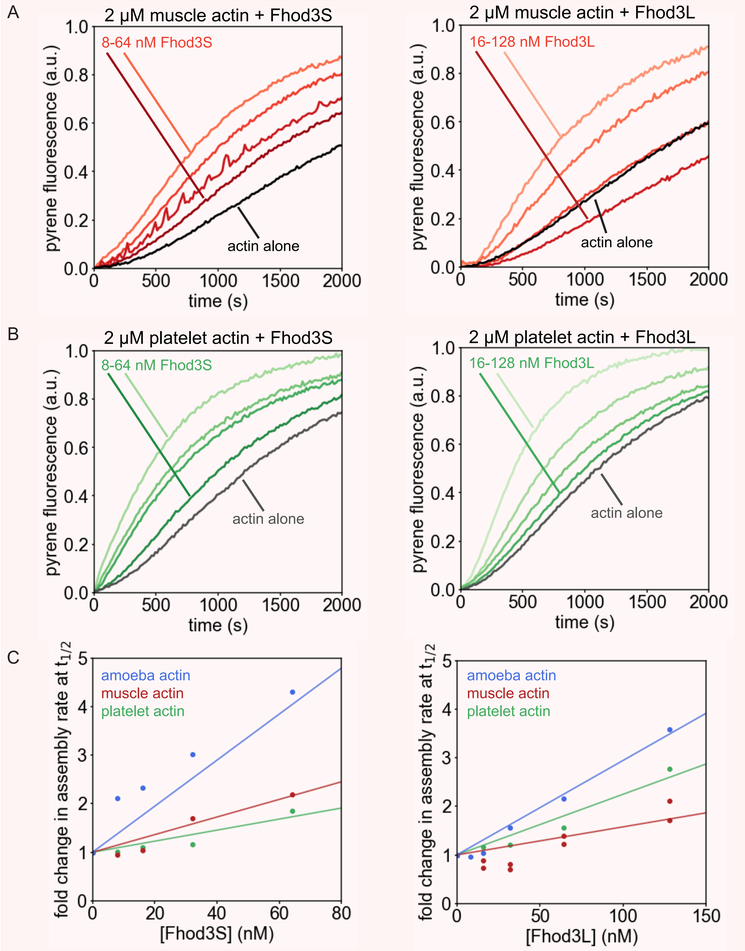
Fhod1 and Fhod3 were reported to neither nucleate nor elongate actin filaments in vitro. How do they assemble actin in cardiomyocytes?

1. Human Fhod1 preferentially nucleates non-muscle actin isoforms.



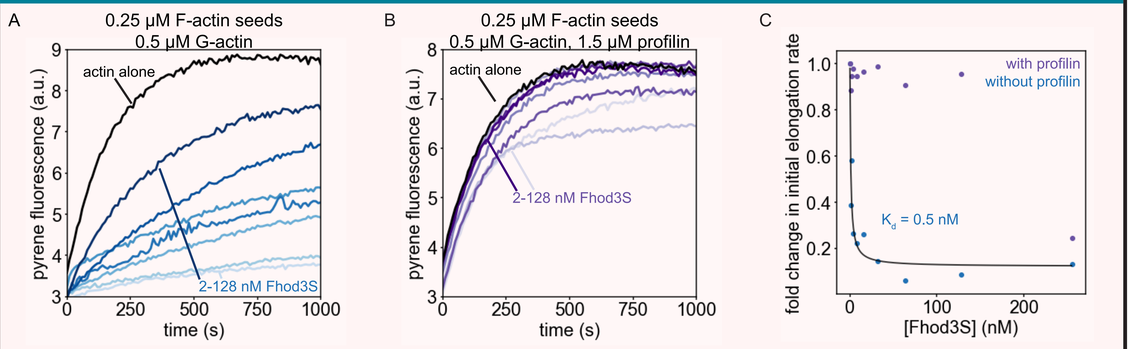
A, pyrene actin assembly assay. Human Fhod1 nucleates *Acanthamoeba* actin (blue), but not α-actin from rabbit skeletal muscle (red). Data adapted from Patel et al., 2018. B, pyrene actin assembly assay. Human Fhod1 also nucleates human β-actin (green) recombinantly expressed in *Pichia pastoris*.

2. Human Fhod3 nucleates both muscle and non-muscle actin isoforms.



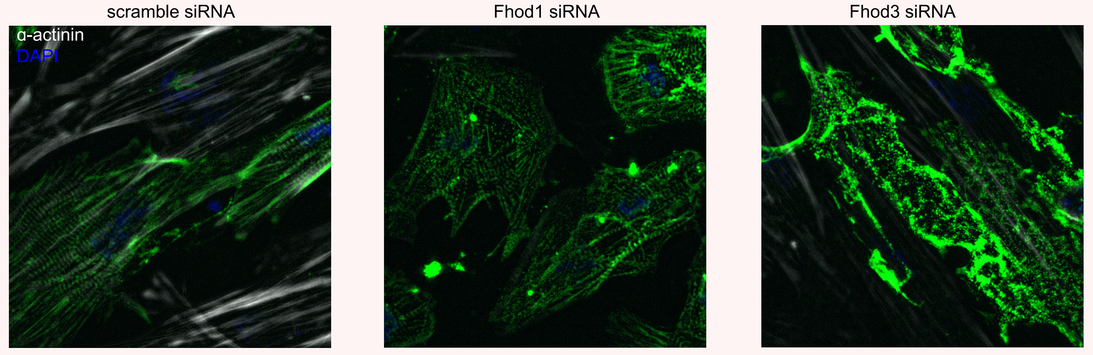
A, pyrene actin assembly assays. Human Fhod3 (both the muscle-specific Fhod3L isoform and the non-muscle Fhod3S isoform) nucleate actin from rabbit skeletal actin. B, pyrene actin assembly assays. Fhod3 splice isoforms nucleate human platelet actin (80% β-actin, 20% γ-actin). C, quantification of actin assembly rates at time to half-polymerization from A and B. Fhod3 polymerizes *Acanthamoeba* actin faster than it does mammalian actins.

3. Human Fhod3 binds barbed ends but does not accelerate elongation.



A, pyrene seeded elongation assay. Human Fhod3S inhibits the elongation of *Acanthamoeba* actin in a dose-dependent manner in the absence of profilin. B, pyrene seeded elongation assay with *S. pombe* profilin. Human Fhod3S permits, but does not accelerate, the elongation of Acanthamoeba actin in the presence of profilin. C, quantification of rates from A and B. Fhod3S binds barbed ends of *Acanthamoeba* actin with an affinity of ~0.5 nM.

4. Fhod3 is required for sarcomere assembly in hESC-derived cardiomyocytes.



Human embryonic stem cell-derived cardiomyocytes were transfected with scramble, Fhod1, or Fhod3 siRNA, then fixed 21 days post-differentiation and stained for α-actinin. Depletion of Fhod3, but not Fhod1, led to a complete loss of sarcomeres, consistent with previous work in other model systems.

Future Directions

1. Systematically measure the nucleation and elongation rates of Fhod1, Fhod3L, and Fhod3S with each actin and profilin isoform.

2. Map the sites in Fhod1 and actin that confer isoform specificity.

3. Generate mutants and chimeras that alter nucleation, elongation rate, and isoform-specificity of Fhod1 and Fhod3.

4. Test ability of mutants to assemble actin in human cardiomyocytes.

Acknowledgements

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