mlcD (Q7Z2B8) — Myosin-II / Cytokinesis Prediction Assessment

Gene: mlcD (UniProt Q7Z2B8, MLCD_DICDI), *Dictyostelium discoideum* (NCBITaxon:44689) **Focus:** computational_prediction — hypothesis `prediction-myosin-cytokinesis` **Terms tested:** GO:0000281 mitotic cytokinesis; GO:0030837 negative regulation of actin filament polymerization; GO:0043520 regulation of myosin II filament assembly; GO:0071976 protein localization to cell division site. **Reference context:** doi:10.64898/2026.03.19.712954

Executive Judgment

Verdict: REFUTED (over-annotation / paralog–class misassignment).

BioReason-Pro-SFT predicts myosin-II and cytokinesis regulatory roles for mlcD. Primary biochemistry and the curated UniProt/dictyBase record independently establish that **mlcD is the dedicated essential light chain of MyoD, a single-headed class I (unconventional) myosin** — not a component of the conventional myosin-II (mhcA) contractile machinery. All four predicted terms belong to the myosin-II system (heavy chain mhcA plus its regulatory light chain and essential light chain), whose bipolar filaments drive cytokinesis. Class I myosins are monomeric, membrane-associated motors that **do not self-assemble into filaments**, so mlcD cannot mediate or regulate myosin-II filament assembly or contractile-ring–based cytokinesis.

The most important caveat: I could not access the specific reference `doi:10.64898/2026.03.19.712954` programmatically, and I did not run a MyoD-null phenotype search successfully (PubMed queries for class-I phenotypes returned no hits in this environment). The refutation nonetheless rests on direct, unambiguous primary evidence (protein sequencing + complex purification) and on the curated functional record.

Evidence Matrix

Citation	Evidence type	Direction	Claim tested	Key finding	Context	Confid limitat
P 12826013 (De La Roche, Lee, Côté 2003)	Direct assay (protein sequencing, ESI-MS, ITC, complex purification)	**Refutes** seed	Is mlcD a myosin-II light chain?	MlcD is a 16 kDa 4-EF-hand protein co-purifying with **MyoD**, a long-tailed **class I** myosin; FLAG-MlcD complexes with MyoD but **not** MyoB/MyoC; low Ca^{2+} affinity (cannot sense physiological Ca^{2+})	*D. discoideum*, native + FLAG-tagged expression	High. Directly defines partner identity
P 21671662 (Crawley et al. 2011)	Interaction / biochemical mapping	**Refutes** seed	Light-chain assignments across Dictyostelium myosin-I	Each long-tailed myosin-I has a unique light chain: **MyoB–MlcB, MyoC–MlcC, MyoD–MlcD**; short-tailed MyoA/MyoE use calmodulin	*D. discoideum*	High. Confirm MyoD–pairing
UniProt Q7Z2B8 (MLCD_DICDI)	Database / curated record	**Refutes** seed	Protein identity, family, oligomeric state	Recommended name "**Myosin-ID light chain**"; FUNCTION "light chain for myosin-D"; SUBUNIT "Myosin I… **Inability to self-assemble into filaments**… Interacts with myoD; does not	Curated	High (review databa level, b backed the pri papers above).

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				interact with myoB or myoC"		
dictyBase GO (via UniProt xrefs)	Database (experimental IDA/IPI)	**Qualifies** / competing	What processes/ locations are experimentally supported?	Curated terms: myosin complex (IDA), myosin heavy chain binding→myoD (IPI), Ca^{2+} binding (IDA), **actin wave** (IDA), **macropinocytic cup cytoskeleton** (IDA). **None** of the 4 predicted myosin-II/ cytokinesis terms present	*D. discoideum*	High. Absenc predict terms i curated is inform
InterPro/ PANTHER/ Pfam (Q7Z2B8)	Structural/ evolutionary	**Qualifies**	Domain architecture	147 aa, four EF-hands (EF-hands 2–4 degenerate), CALM/Myosin/ TropC-like (IPR050230), calmodulin-like — consistent with an EF-hand myosin light chain, not a filament-assembly regulator	Sequence	High.
Iteration-2 NW/ BLOSUM62 (this study)	Computational (sequence identity)	**Qualifies/ refutes**	Which clade does MlcD belong to?	MlcD 45.6% id to calmodulin & 45.2% to MlcB (myosin-I LC) vs	*D. discoideum* paralogs	Mediur high. G alignm single

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				only 30.8%/31.2% to myosin-II mlcR/ mlcE; reproduces published ~44% CaM benchmark		metho concor with primar data.
P 10423462 (Dai et al. 1999)	Mutant phenotype (micropipette aspiration)	**Qualifies** (class context)	What do Dictyostelium myosin-I motors do?	Amoeboid myosin-I's drive pseudopod formation, macropinocytosis; double mutants lose ~50% cortical tension; required for migration — NOT cytokinesis	*D. discoideum* myosin-I mutants	Mediu (about myosin class genera not My specifi

GO Curation Implications (leads — require curator verification)

- **Do NOT add** GO:0000281 (mitotic cytokinesis), GO:0030837 (neg. reg. actin filament polymerization), GO:0043520 (regulation of myosin II filament assembly), or GO:0071976 (protein localization to cell division site) to mlcD. These are conventional-myosin-II / contractile-ring functions and are **class-inappropriate** for a myosin-I light chain.
- **GO:0043520 specifically:** refuted. The correct effectors of Dictyostelium myosin-II filament assembly are the mhcA heavy chain (regulated by myosin heavy-chain kinases) and its **regulatory / essential light chains** — not MlcD. If any myosin light chain warrants GO:0043520, it is the myosin-II RLC/ELC paralog, not mlcD.
- **Retain / support instead (MF/CC):** MF `GO:0032036 myosin heavy chain binding` (IPI to myoD) and the calmodulin-like light-chain role; CC `GO:0016459 myosin complex`. Prefer these informative terms over generic "protein binding."
- **Class-appropriate BP/CC leads (verify against MyoD phenotype literature):** class-I myosin processes at the plasma membrane — macropinocytosis/endocytosis and cortical

actin dynamics (consistent with curated `macropinocytic cup cytoskeleton` and `actin wave` localizations).

Mechanistic Scope

Direct molecular function of the gene product: MlcD is a calmodulin-like EF-hand **essential light chain** that binds the IQ motifs in the neck of the class I myosin heavy chain **MyoD**, stabilizing its lever arm. Its Ca^{2+} affinity is low, so it is not a Ca^{2+} sensor; De La Roche et al. propose it confers Ca^{2+}-insensitive regulatory properties distinguishing MyoD from calmodulin-bearing myosin-I. The predicted terms describe **downstream cellular processes of a different motor system** (myosin-II contractile ring); they are neither the immediate activity of MlcD nor a documented phenotype of it.

Conflicts and Alternatives

- **Paralog/class confusion (most likely source of the prediction):** "mlc*" myosin light-chain genes form a paralog pool spanning classes. The myosin-II regulatory (mlcR) and essential (mlcE) light chains legitimately carry cytokinesis/filament-assembly context; a sequence/name-similarity or frequency-biased transfer plausibly propagated those terms onto mlcD.
- **Organism specificity:** In *Dictyostelium*, cytokinesis A depends on conventional myosin-II (mhcA); myosin-I isoforms function in motility, cortical tension, and endocytosis. This reinforces that mlcD's contribution, if any, is not contractile-ring cytokinesis.
- **No competing evidence found** that MlcD associates with mhcA or the contractile ring.

Knowledge Gaps

1. **MyoD-null / MlcD-null cellular phenotype.** Partially addressed: MyoD-specific knockout papers were not retrievable here, but class-level phenotype data (Dai et al. 1999, P 10423462) show Dictyostelium myosin-I motors drive pseudopod formation, macropinocytosis, and

cortical tension — not cytokinesis. A positive class-I process annotation for mlcD (e.g., macropinocytosis, cortical dynamics) should still be grounded in MyoD/mlcD-specific phenotype data via dictyBase records.

2. **Reference doi:10.64898/2026.03.19.712954.** Not accessible programmatically here; its content (possibly the AIGR review itself or a benchmarking preprint) could add or contextualize evidence.
3. **Exact myosin-II light-chain paralog identities (mlcE vs mlcR).** Confirming which paralog rightly holds GO:0043520 would let the curator redirect the mis-transferred term rather than merely delete it.

Discriminating Tests

- **Co-IP / affinity purification:** MlcD pulls down MyoD but not mhcA (already supported for MyoB/MyoC exclusion; a direct mhcA test would formally close it).
- **Localization:** GFP-MlcD should track MyoD to the plasma membrane / macropinocytic cups / actin waves, **not** the cleavage furrow. Curated IDA localizations already favor the membrane pattern.
- **Genetic epistasis:** mlcD-null should phenocopy myoD-null (motility/endocytosis), not mhcA-null (multinucleate cytokinesis-defective) cells.
- **Sequence/orthology:** phylogenetic placement of MlcD with myosin-I light chains / calmodulin clade vs myosin-II RLC/ELC clade.

Curation Leads (require curator verification)

- **Action change:** Reject the four predicted terms for mlcD; flag as class/paralog over-annotation.
- **Candidate references to verify:**

  P 12826013

  (identity, MyoD specificity, Ca^{2+} properties);

P 21671662

(MyoD–MlcD light-chain assignment). Snippets provided in the recorded findings.

- **Candidate retained/added terms:** MF `GO:0032036 myosin heavy chain binding`; CC `GO:0016459 myosin complex`; consider class-I membrane/endocytic BP terms only if MyoD phenotype data support them.
- **Suggested question for curator:** Should GO:0043520 be redirected to the myosin-II light-chain paralog (mlcE/mlcR) rather than deleted outright?
- **Suggested experiment:** GFP-MlcD localization relative to the cleavage furrow vs macropinocytic cup.

Computed Provenance — Pairwise Sequence Identity (Iteration 2)

Global Needleman–Wunsch alignment (BLOSUM62, gap = −8), MlcD (Q7Z2B8) vs *D. discoideum* paralogs. The reproduction of the published ~44% MlcD–calmodulin identity (De La Roche et al. 2003,

P 12826013

) serves as a method-validity check.

Comparison (vs MlcD Q7Z2B8)	UniProt	% identity	Aligned cols	Class
Calmodulin (calA)	P02599	**45.6**	147	Ca^{2+} sensor / myosin-I LC clade
MlcB — Myosin-IB light chain	Q54GL7	45.2	73*	Class I myosin LC
mlcR — Myosin-II **regulatory** LC	P13833	**30.8**	146	Myosin-II LC
mlcE — Myosin-II **essential** LC	P09402	**31.2**	141	Myosin-II LC

*MlcB record is only 73 aa (partial), so its alignment spans fewer columns.

Interpretation: MlcD is ~14–15 percentage points more similar to the calmodulin-like / myosin-I light chains than to the myosin-II regulatory/essential light chains (mlcR/mlcE) that legitimately carry cytokinesis and myosin-II-filament-assembly annotations. This independently supports the class-I identity and the paralog-misassignment interpretation of the seed prediction. (Computed value 45.6% ≈ published 44% → analysis validated.)

Limitations

Findings rest primarily on two primary papers plus curated database records; I could not fetch the supplied DOI or MyoD-null phenotype papers in this environment. No sequence alignment/phylogeny was run here (identity was already unambiguous from the primary literature and UniProt). Database-level GO evidence is treated as orientation but is corroborated by the primary assays.
