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Tropomyosin and caldesmon regulate cytokinesis speed and membrane stability during cell division

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Abstract

The contractile ring and the cell cortex generate force to divide the cell while maintaining symmetrical shape. This requires temporal and spatial regulation of the actin cytoskeleton at these areas. We force-expressed misregulated versions of actin-binding proteins, tropomyosin and caldesmon, into cells and analyzed their effects on cell division. Cells expressing proteins that increase actomyosin ATPase, such as human tropomyosin chimera (hTM5/3), significantly speed up division, whereas cells expressing proteins that inhibit actomyosin, such as caldesmon mutants defective in Ca^{2+}/calmodulin binding (CaD39-AB) and in cdk1 phosphorylation sites (CaD39-6F), divide slowly. hTM5 and hTM5/3-expressing cells lift one daughter cell off the substrate and twist. Furthermore, CaD39-AB- and CaD39-6F-expressing cells are sensitive to hypotonic swelling and show severe blebbing during division, whereas hTM5/3-expressing cells are resistant to hypotonic swelling and produce membrane bulges. These results support a model where Ca^{2+}/calmodulin and cdk1 dynamically control caldesmon inhibition of tropomyosin-activated actomyosin to regulate division speed and to suppress membrane blebs.

Keywords: Cytokinesis; Membrane bleb; Membrane bulge; Contractile ring; Cell cortex; Ca^{2+}/calmodulin; cdk1/cdc2; p21-Activated kinase

During cell division, cultured cells proceed through a characteristic series of morphological changes [1]. They partially detach from the substrate to round up, elongate during chromosome separation and pinch at the cell equator to produce two equivalent daughter cells. These shape changes are controlled by the actin cytoskeleton. Actin is organized into bundles at the cell equator during cytokinesis and into networks in the cell cortex. These assemblies are controlled by actin binding proteins to generate constriction force at the contractile ring while maintaining mechanical stability at the cell cortex.

It remains unclear how the actin cytoskeleton is regulated in time and space during cytokinesis. The classical contractile ring hypothesis [2–4] suggests that actin-activated myosin II contracts on the equatorial actin bundles to generate the force necessary to produce a furrow that cleaves the cell. This model requires little or no input from the cortical actin network. However, depolymerization of actin at the cell poles inhibits cytokinesis [5], suggesting a role for cortical actin in cytokinesis.

This has led to the hypothesis that the global actin network contributes to cytokinesis [5,6]. We test this hypothesis by force-expressing fragments, chimeras, or modified versions of tropomyosin (TM)[2] and caldesmon (CaD) in Chinese Hamster Ovary (CHO) cells in order to disturb normal actomyosin dynamics and/or the balance between cortical and equatorial actomyosin regulation. This strategy has several advantages. Since CaD and TM both bind actin and regulate myosin II ATPase activity, it is

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[2] *Abbreviations used:* hTM5/3, human tropomyosin chimera; TM, tropomyosin; CaD, caldesmon; CHO, Chinese Hamster Ovary; PAK, p21-activated kinase; FBS, fetal bovine serum; PBS, phosphate-buffered saline; DIAS, dynamic image analysis software; HMM, heavy meromyosin.

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possible to alter the activity of myosin or actin during cytokinesis. Minor modifications of TM or CaD can alter its localization to either the contractile ring or the membrane cortex to produce compartmentalized dominant negative effects. Further, stable force expression in the background of the endogenous TM or CaD allows the partial disruption of cytokinesis while maintaining cell viability.

TMs are actin-binding proteins encoded by four genes in mammals; α, β, γ, and δ [7,8]. Alternative splicing of exons results in more than 40 known isoforms [9]. Three isoforms, TM1, TM4, and TM5 are present in CHO cells [10]. When bound to actin, TM activates actomyosin ATPase [11,12] and antagonizes actin depolymerizing factors such as cofilin [13] and gelsolin [14,15]. TM isoforms are generally classified into two groups based on apparent molecular mass, high M_r isoforms, e.g. TM3, and low M_r isoforms, e.g. TM5. Different TM isoforms localize to different cellular compartments during interphase [9,16]. Here, we examine the behavior during cytokinesis of cells force-expressing human TM5 (hTM5), human TM3 (hTM3) and a chimera of these two TM isoforms, hTM5/3. These TMs stimulate actin-activated myosin ATPase to different extents [12]. Unlike hTM3 and hTM5, hTM5/3 binds to actin filaments in a KCl-independent manner [12]. All hTMs tested accelerate the speed of cell division, while hTM5 and more so hTM5/3 disrupts cytokinesis symmetry by forming large membrane bulges and causing daughter cells to lift and twist. Furthermore, cell lines that produce bulges are resistant to swelling in hypotonic solution.

Non-muscle CaD binds actin, myosin, and TM and inhibits TM-actin-activated myosin ATPase [17]. CaD incorporates into actin-rich regions of interphase cells [18] where it stabilizes actin filaments [19,20]. Both immunofluorescent microscopy and isolation of TM-enriched microfilaments [21,22] have demonstrated that CaD becomes largely dissociated from actin filaments during cell division. The principal functional motifs of CaD, including those responsible for actin-binding, TM-binding, Ca^{2+}/calmodulin (Ca^{2+}/CaM)-binding and inhibition of actomyosin ATPase activity, lie in the C-terminal region (in human fibroblast, termed CaD39) [23–26]. Actomyosin ATPase inhibition by CaD is released by Ca^{2+}/CaM [27] and phosphorylation. Kinases that phosphorylate CaD in vitro include p21-activated kinase (PAK) [28], cdk1 [29], PKC [30], ERK [31], and p38 MAPK [32]. We previously reported that CHO cell lines expressing a CaD39 mutant lacking all six cdk1 phosphorylation sites (CaD39-6F) are able to complete cytokinesis, but they divide slowly and form excessive blebs during division [33]. Here, we report the behavior of cells expressing CaD39 mutations at both Ca^{2+}/CaM sites (CaD39-AB), mutations at all six cdk1 sites (CaD39-6F), mutations altering two major PAK sites to mimic the non-phosphorylated state (CaD39-PAKA) or the constitutively phosphorylated state (CaD39-PAKE). Cell lines expressing CaD39-AB and CaD39-6F, which inhibit myosin ATPase, slow cytokinesis. CaD39-AB slows cell elongation prior to invagination while CaD39-6F slows invagination. Cells expressing CaD39-PAKE, which fails to inhibit myosin ATPase in vitro, divide faster than cells expressing CaD39-PAKA, which inhibits myosin ATPase. CaD39-AB expressing cells, known to have focal adhesion defects, exhibit abnormal lifting during division. Cells expressing CaD39-AB or CaD39-6F form membrane blebs during cytokinesis and are sensitive to hypotonic solution. Together, the findings from this study suggest that actomyosin contraction contributes to cell division speed and suppression of membrane blebs, and that TM and CaD play opposing roles in the regulation of actin-activated myosin during cytokinesis.

Materials and methods

Plasmid DNA constructs

Plasmids containing human TM isoforms, hTM3 and hTM5, and the chimeric mutant hTM5/3 (Fig. 1) were reported previously [10]. Splicing through overlap extension mutagenesis [12] was used on the carboxyl-terminal fragment of human fibroblast CaD, CaD39, [19,22,34] to change the following residues (Fig. 2): (i) the cdk1/cdc2 responsive serine/threonine sites to alanines ($T^{383}A$; $S^{469}A$; $T^{475}A$; $T^{498}A$; $S^{504}A$; $S^{534}A$) to produce mutant CaD39-6F [33]; (ii) two tryptophan residues required for Ca^{2+}/CaM-

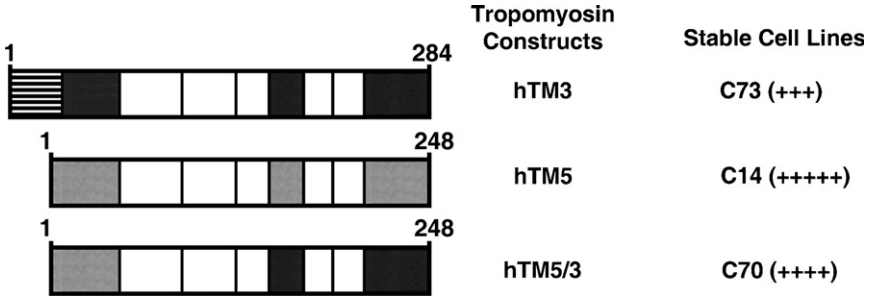

Fig. 1. Diagram of human fibroblast tropomyosin and its chimera and their corresponding expressing cell lines. High M_r isoform hTM3, encoded by the αTM gene, consists of 284 amino acids, whereas low M_r isoform hTM5, encoded by the γTM gene, consists of 248 amino acids. The open boxes represent five highly conserved exons among all TM isoforms, including hTM3 and hTM5. The boxes shaded black/striped/grey represent unique exons for hTM3 and hTM5. hTM5/3 was engineered to encode the amino terminus of hTM5 and carboxyl terminus of hTM3. CHO cell lines C73, C14, and C70 express stable levels of exogenous proteins hTM3, hTM5, and hTM5/3, respectively. The number of + symbols represents relative expression level. hTM3, hTM5, and hTM3/5 express 2.3×, 0.75×, and 2.8× times endogenous TM4 expression [10].