Minireview

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Role of Protein Phosphatase Type 1 in Contractile Functions: Myosin Phosphatase*

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Protein phosphatase type 1 (PP1)[1] is involved in a wide range of cell activities (1), and even within the more restricted theme of contractile activity in muscle several processes may be considered. Important areas include regulation of ion channels (2), effect of phospholamban on Ca^{2+} uptake by the SR (3), and phosphorylation-dephosphorylation of myosin II.

Phosphorylation of myosin light chains (located in the head-neck junction of the myosin molecule) by the Ca^{2+}-calmodulin-dependent MLCK in all muscle types is established (4). Discovery of MLCK spurred numerous reports on the phosphatases involved. In smooth muscle, phosphorylation of myosin II increases actin-activated ATPase activity and is required for contraction (4). Much of the earlier work focused on smooth muscle myosin phosphatase (MP). An initial controversy was the type of catalytic subunit involved, *i.e.* PP1c, PP2Ac, etc. In smooth muscle the majority of MP activity is due to PP1c (5), and this finding was extended to include skeletal and cardiac muscle. Three genes encode PP1c: α, γ, and δ (also called β). Five PP1c isoforms are expressed, where α_1/α_2 and γ_1/γ_2 are generated by alternative splicing (1). To accommodate specific functions of the limited number of PP1c isoforms with the multiple roles of PP1c the concept of target subunits was developed. Over 50 potential target subunits have been identified (1) that in complex with PP1c may designate specific substrates, regulate activity, and direct distinct cell localization. This review describes one of the PP1 holoenzymes, namely the myosin phosphatase of muscle.

Smooth Muscle Myosin Phosphatase

In smooth muscle the role of MP is to dephosphorylate Ser-19 and to a lesser extent Thr-18 in P-LC20. (The PKC site, Thr-9, can also be dephosphorylated by MP.) The current model for smooth muscle MP is based on the gizzard holoenzyme (6, 7). The holoenzyme is a trimer consisting of: a catalytic subunit, PP1cδ; a target subunit of ~110 kDa; and a smaller subunit of ~20 kDa (M20). A scheme of the MP holoenzyme is shown in Fig. 1. The function of M20 is not known, and the critical properties of MP can be ascribed to the large subunit, *i.e.* binding of PP1c and the substrate, P-myosin. Thus, it is termed myosin phosphatase target subunit, MYPT1. (Other terms include M_{110}, myosin binding subunit (MBS), and M130/M133). Initially MYPT1 was cloned from chicken gizzard (M130/M133 (8)) and also from rat aorta (rat3 isoform (9)).

Structure of MYPT1

From the initial reports the basic features of the MYPT1 molecule were established. The molecule is hydrophilic, and no extensive hydrophobic patches are found. Plans of human and chicken MYPT1 are shown in Fig. 1. A striking feature of all MYPT isoforms is the presence of N-terminal ankyrin repeats. In MYPT1 each of the 7 or 8 repeats contains ~33 residues with 20 residues conserved. The 171–197 region is less homologous but shows similarity to ankyrin repeats and was so considered in chicken M130/M133 (8). It is suggested that the conserved sequence for ankyrin repeats is structure-based in that it forms a β-hairpin-helix-loop-helix ($\beta_2\alpha_2$) structure (10). Based on several solved structures it is known that both the β-hairpins and the surface of the ankyrin groove (helical bundle) can be involved in binding to target proteins (10). Many proteins interact with ankyrin repeats (11), and thus the proposed role for these repeats in MYPT1 is to act as an interactive protein platform.

Flanking the N-terminal edge of the first ankyrin repeat is the PP1c-binding motif, the "RV*X*F" (1) motif (residues 35–38). Some variation is allowed and a general consensus is (R/K)X_1(V/I)X_2(F/W), where X_1 may be absent or be residues other than large hydrophobes and X_2 is any residue except large hydrophobes, phosphoserine and probably aspartic acid (1). Residues flanking the motif, N-terminal basic residues, and C-terminal acidic residue(s) may contribute to binding (12). In human MYPT1 the pertinent sequence is ^{30}KRQKTKVKFDD. The RV*X*F motif is present in many target proteins and even occurs in proteins unlikely to bind PP1c (1). This motif interacts with PP1c in a hydrophobic groove involving residues Ile-169, Leu-243, Phe-257, Leu-289, Cys-291, and Phe-293. Important points are that the interaction site for RV*X*F on PP1c is within the invariant region for all PP1c isoforms (but not conserved in PP2A and PP2B) and that the site is distinct from the catalytic site. Peptides containing the RV*X*F motif may displace target subunits, but binding of the RV*X*F motif to PP1c does not directly influence activity (1, 13). In MYPT1 the RV*X*F motif (KVKF) acts as the primary interaction site (an anchoring site) for PP1cδ, but other interactions are involved (14). These include residues 1–22, the ankyrin repeats (possibly repeats 5–8, sequence 167–295 (7)), and a site within the sequence 304–511. Only the interaction with KVKF has high affinity, but the other secondary interactions are important in that they may modify PP1c properties. For example, interaction of PP1c or P-myosin with the N-terminal segment of MYPT1 could activate PP1c. These multiple and hierarchical interactions form a combinatorial control of PP1c (13), and considering these, it is likely that PP1c is pivoted via the RV*X*F motif and clasped by the N-terminal sequence of MYPT1 and the ankyrin repeats. Other structural features of MYPT1, including some phosphorylation sites, are shown in Fig. 1.

Interactions with MYPT1

A potentially important feature of MYPT1 as a target subunit is that it is a platform for multiple interactions. The binding of myosin to MYPT1 is an important but controversial point. One view is that P-myosin or P-LC20 binds to the ankyrin repeats, possibly repeats 6–8 (15). Interaction of the phosphorylated substrate with the catalytic site of PP1c is expected with an added contribution to binding by the ankyrin repeats. Dephosphorylated substrate binds less effectively, and in the presence of ATP only P-myosin or P-LC20 is bound (7). The opposing view is that dephosphorylated myosin binds to the C-terminal sequence of MYPT1 (excluding the C-terminal 72 residues (16) in the chicken isoforms). Binding to both the N-terminal and C-terminal regions of MYPT1

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[1] The abbreviations used are: PP1, protein phosphatase type 1; cGK, cGMP-dependent kinase; CPI-17, C-kinase-dependent phosphatase inhibitor of 17 kDa; ILK, integrin-linked kinase; LZ, leucine zipper motifs; MLCK, myosin light chain kinase; MP, myosin phosphatase; MYPT, myosin phosphatase target subunit; PKA, cAMP-dependent kinase; PKC, protein kinase C; P-LC20, phosphorylated 20-kDa myosin light chain; P-myosin, phosphorylated myosin; PP1c, catalytic subunit of PP1; ROK, Rho-associated kinase; TIMAP, transforming growth factor-β-inhibited membrane-associated protein; ATPγS, adenosine 5′-3-*O*-(thio)triphosphate.

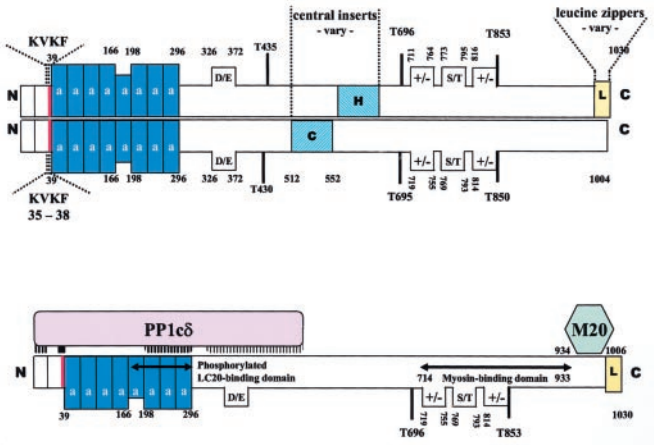

FIG. 1. **Plans of the human and chicken (M133) MYPT1 molecules (*upper*) and the human myosin phosphatase holoenzyme (*lower*).** Regions of MYPT1: *KVKF*, PP1c-binding motif; *a*, ankyrin repeat; *D*/*E*, acidic region; +/−, ionic region; *S*/*T*, Ser- and Thr-rich region; *L*, leucine zipper. Residue numbers are given and some phosphorylation sites are indicated (*bold*). For the holoenzyme, interactions of varying strengths between MYPT1 and PP1c are indicated (see text). Suggested binding sites for phosphorylated light chain and myosin are indicated by *arrows*.

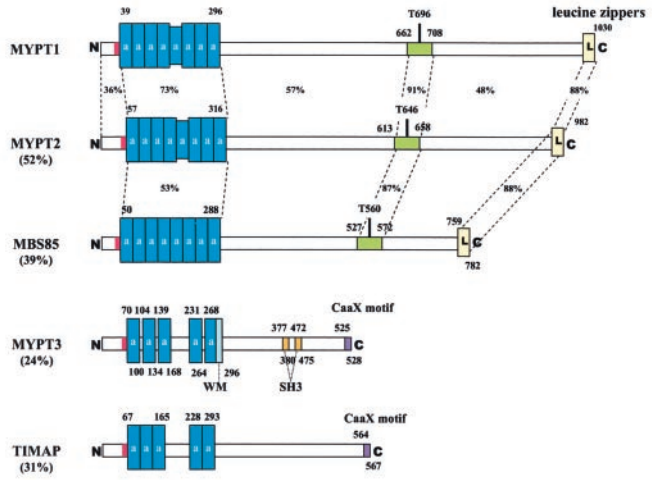

FIG. 2. **MYPT family.** Percentages in *parentheses* give amino acid identity to MYPT1. For MYPT1, MYPT2, and MBS85, percent identities for distinct regions and positions of suggested inhibitory phosphorylation sites (*bold*) are shown. Regions: PP1c-binding motif, *red area* at N-terminal edge of ankyrin (*a*) repeats; *WM*, Walker motif (nucleotide-binding site); *CaaX*, putative prenylation site. For MYPT3 two SH3 motifs shown. This figure was adapted from Ref. 20 with permission.

is feasible if P-LC20 (in the myosin S2 region) binds to the N-terminal sites and the rod portion of myosin binds to the C-terminal sites. It was suggested that phosphorylation of Thr-850 (chicken M133) by ROK reduced binding of MYPT1 to myosin (17). Adducin (α, β, γ (18)) and Tau and MAP2 (19) also bind to the ankyrin repeats and are phosphorylated and dephosphorylated by ROK and MP, respectively.

Multiple interactions with the ankyrin repeats are expected (11), but what is surprising is that the C-terminal half of MYPT1 also interacts with many molecules (20). M20 binds to the C-terminal region of MYPT1, residues 934–1006 of human MYPT1, in an interaction not involving LZ repeats (15, 16). M20 also binds to myosin but not PP1c (16). GTP-RhoA (but not inactive GDP-RhoA) binds to the C terminus and could represent an alternative docking site for GTP-RhoA in addition to the plasmalemma. Acidic phospholipids target residues 667–1004 of chicken M133 (does not contain LZ sequences) and inhibit PP1c activity (7). This binding/inhibition of MP activity is reversed on phosphorylation of MYPT1 by PKA. Moesin also binds to MYPT1 via the C-terminal part (18). Other interactions involve the LZ motifs in some isoforms of MYPT1 and include: 1) interaction of the LZ motifs of cGMP-dependent kinase (cGKIα) and MYPT1 (21); 2) binding of the PDZ2 domain of interleukin-16 precursor proteins to the C-terminal 30 residues of MYPT (22); and 3) interaction of a coiled-coil domain in RhoA-interacting protein (expressed in vascular smooth muscle) with the LZ motifs of MYPT1 (23).

The many interactions involving MYPT1 suggest several substrates and a possible targeting function in other macromolecular complexes and thus a much broader role in cell function than only dephosphorylation of P-myosin. This complexity may reflect the varied cell localizations observed with MYPT1 on filaments and membranes (20). In differentiated striated muscle cells MYPT may have a more restricted role.

MYPT Family

Several isoforms of MYPT1[2] are generated by cassette-type alternative splicing of single gene pre-mRNAs and involve the presence or absence of central inserts and the C-terminal LZ motifs. The M130/133 chicken gizzard MYPT1 isoforms differ by a 123-nucleotide insert (residues 512–552 of M133 (8)) arising from a single exon (24). In rat the situation is more complex, and 5 central insert isoforms are generated (24) resulting from differing expression of 2 exons. Control of this central region (in chicken) is exerted by a *cis*-enhancer complex close to the alternative exon 5′-splice site (25). In addition, isoforms are generated by cassette-type alternative splicing of a 3′-exon (31 nucleotides). Skipping of this exon codes for the LZ-positive MYPT1, and inclusion of the exon introduces a premature stop codon and codes for the LZ-negative MYPT1 (26). Thus many isoforms of MYPT1 can be generated and to some extent are expressed in a tissue-specific fashion (24, 26).

Recently several molecules similar to MYPT1 have been described (Fig. 2). Each has the N-terminal ankyrin repeats and the RV*X*F motif. The C-terminal half is more variable. As originally cloned (27) MYPT2 (human chromosome 1q32) contained 982 residues (110 kDa), and subsequently a second isoform (MYPT2B) was recognized from the genomic organization of the MYPT2 gene (28). These arise from alternative splicing of exon 24, each isoform reflecting the inclusion of either exon 24 (MYPT2B) or exon 25 (MYPT2A). Both contain C-terminal LZ motifs. A comparison of MYPT1 and MYPT2A is shown in Fig. 2. Overall identity is 52% but several areas are more conserved (Fig. 2). The N-terminal sequences (1–57 of MYPT2 and 1–38 of MYPT1) are distinct (36% identity) although both contain the PP1c-binding motif. MYPT1 may be considered a housekeeping gene and is expressed in most tissues but is higher in smooth muscle ([MYPT1] in rabbit portal vein is ~1.2 μM).[3] MYPT2 is more restricted with both A and B isoforms found in heart, skeletal muscle, and brain and predominantly MYPT2A in other tissues (28).

Another member of the MYPT family is MBS85 (29). This is widely distributed with higher levels in the heart. MBS85 binds specifically to the PP1cδ isoform and also binds and dephosphorylates P-myosin (or P-LC20). Phosphorylation of Thr-560 by myotonic dystrophy kinase-related Cdc42-binding kinase or ROK is required for its binding to PP1cδ and inhibition of phosphatase activity (29). MYPT3 has some similarity to MYPT1/2 (Fig. 2) but only in its N-terminal half (30). Other features are shown in Fig. 2. A putative regulatory site is not present. MYPT3 inhibited the activity of PP1cγ with phosphorylase *a* and P-myosin in contrast to activation of PP1cδ by MYPT1 with P-myosin. MYPT3 is widely distributed (mouse) and appears in high amounts in heart, brain, and kidney. TIMAP is similar to MYPT3 (~45% identity) and contains the "marker" N-terminal structure, conserved nuclear localization signal and a C-terminal C*AAX* box (31). It shows high expression in endothelial and hematopoietic cells. Analysis of genomic data bases indicates that several gene products homologous to MYPT3 and TIMAP exist (30, 31).

Each family member binds PP1c via the RV*X*F motif, and the binding of PP1c and substrate may be augmented by the ankyrin

[2] The Human Gene Nomenclature Committee has assigned the following: PPP1R12 A, B, or C for MYPT1, MYPT2, and MBS85, respectively; PPP1R16 A or B for MYPT3 and TIMAP, respectively; and PPP1R14A for CPI-17. Gene location is listed on www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl using PPP1R* as query.

[3] T. M. Butler, and M. J. Siegman, personal communication.

repeats. The C-terminal region is adapted for a given function, *e.g.* regulation or binding of other ligands or attachment to membranes (via the prenylated C terminus). However, the function(s) of most MYPT family members is not known. Even with MYPT1 and MYPT2 it is possible that substrates other than P-myosin are implicated.

Small MP Subunit (M20)

M20 was cloned from chicken gizzard (9), and two splicing variants of 161 (M_{18}) and 186 (M_{21}) residues were detected (32). Only M_{21} contains C-terminal LZ motifs. Expression of each isoform is tissue-specific (32). Part of M20 arises from the MYPT2 gene (33), and two heart-specific isoforms of M20 arise from the MYPT2A and MYPT2B genes involving exons 14–25 (28). M20 was not detected in either brain or skeletal muscle (20). The function of M20 is not established. Suggested roles (20) include: modification of Ca^{2+} sensitivity in renal artery and cardiac myocytes; binding to the myosin dimer; and a role in microtubule dynamics. The binding of M20 to MYPT1 does not affect phosphatase activity (7).

Regulation of MP Activity

Early studies assumed that MP activity was unregulated, but more recently inhibition and activation have been documented. At fixed suboptimal [Ca^{2+}] inhibition of MP would increase [P-myosin] and activation would decrease [P-myosin], resulting in Ca^{2+} sensitization and Ca^{2+} desensitization, respectively.

More data are available for Ca^{2+} sensitization. Inhibition of MP was found to be linked to agonist stimulation via numerous trimeric G protein-coupled receptors (list given in Ref. 34), and RhoA and thus Rho-associated kinase, ROK, are important downstream links (7, 34). There are two isoforms of ROK (ROKα/ROCKII and ROKβ/ROCKI (18) with 32% identity). ROKα was isolated from chicken gizzard smooth muscle (35). There are several mechanisms proposed for inhibition of MP (7, 20), *i.e.* phosphorylation of MYPT1; phosphorylation of an inhibitory protein, CPI-17; dissociation of the holoenzyme; and translocation and subsequent dissociation of the holoenzyme. The first two are cited more frequently.

Initially it was found that incubation of permeabilized portal vein with ATPγS induced Ca^{2+} sensitization, inhibition of MP, and thiophosphorylation of MYPT1 (see Refs. 7 and 20). Subsequently, it was shown that phosphorylation of MYPT1 at Thr-695 (Thr-696 in human MYPT1) by an endogenous kinase inhibited gizzard MP activity. ROK was the first known kinase shown to phosphorylate MYPT1 (7, 20), and two major sites, Thr-696 and Thr-853 (20, 36), and several minor sites (36) were identified. Several kinases are now known to phosphorylate MYPT1 (20), many at the inhibitory site. The endogenous kinase was identified and termed MYPT1 kinase (previously ZIP-like kinase (37)). Some of these kinases (ROK, MYPT1 kinase, and ILK) can also directly phosphorylate LC20 at Ser-19 and have been implicated in Ca^{2+}-independent contractile events, particularly in non-muscle cells. The relevance of multiple kinases that phosphorylate the inhibitory site is not known but may represent the convergence of different pathways at MYPT1, *e.g.* Rac-1/myotonic dystrophy protein kinase, Rac or Cdc42/p21-activated kinase, and Ras/Raf-1. The molecular basis for inhibition of MP as a result of phosphorylation is not understood. The inhibition with several substrates is due largely to a decrease in V_{max}. Dissociation of MP following phosphorylation is unlikely (20). For intramolecular inhibition (autoinhibition) folding of the molecule is expected to allow interaction of Thr-696 with the N-terminally located PP1c. However, it should be pointed out that sequences around both Thr-696 and Thr-853 are similar to that around Ser-19 of LC20 (from +3 to −6). Intermolecular inhibition also could occur via formation of head-to-tail dimers of MYPT1 (20).

CPI-17 inhibits PP1. It is composed of 147 residues (17 kDa) and expressed in smooth muscle and brain (38). In human aorta a splicing variant is expressed that lacks sequence 68–94, encoded by exon 2 of the 4 exons (20). Unlike other inhibitors of PP1, *e.g.* inhibitors 1 and 2, CPI-17 inhibits both the catalytic subunit and PP1 holoenzymes. Phosphorylation at Thr-38 enhances inhibitory potency about 1000-fold, and the first kinase implicated was PKC (α and δ isoforms). Subsequently several kinases, notably ROK, were found to phosphorylate Thr-38. Residues 35–120 are required for recognition of MP and Tyr-41 is necessary to reduce dephosphorylation of Thr-38 by MP (39). The solution NMR structure (40) indicates that phosphorylation of Thr-38 induces a conformational change that promotes specific recognition of MP by CPI-17. Histamine stimulation of smooth muscle fibers caused phosphorylation of Thr-38 that was reduced by both ROK and PKC inhibitors, suggesting input from two signaling pathways (41).

Whether CPI-17 or MYPT1 is dominant in regulation of MP is controversial and perhaps depends on the tissue/cell involved. General considerations are that MLCK and MP are lower in tonic than phasic smooth muscle and that CPI-17 is higher in vascular compared with visceral muscle (42). Several reports have suggested that CPI-17 plays a dominant role in Ca^{2+} sensitization in various smooth muscles (42–44). Also, in NO-mediated vasorelaxation a transient decrease in CPI-17 phosphorylation (Thr-38) correlated to a transient activation of MP activity (45).

Two other phosphorylation-dependent inhibitors of PP1 are the phosphatase holoenzyme inhibitors (PHI-1 and PHI-2 (46)) and kinase-enhanced protein phosphatase type 1 inhibitor (KEPI (47)). PHI-2 and KEPI are high in cardiac muscle. CPI-17 has sequence similarity to PHI-1 and also to the C-terminal domain of Lim kinase-2 (48).

Lipid messengers should be considered in inhibition of MP and Ca^{2+} sensitization. Arachidonic acid was proposed to dissociate the MP subunits (20, 34) with inhibition reflecting the reduced activity of isolated PP1c. An alternative explanation is that arachidonic acid activates ROK, independent of RhoA, and this phosphorylates either MYPT1 or CPI-17 (20). Sphingosine 1-phosphate, sphingosylphosphorylcholine, and lysophosphatidic acid also activate the ROK pathway (34).

Because phosphorylation of myosin II is critical for smooth muscle function defects in the balance of phosphorylation may lead to disorders of smooth muscle. Several implicate inhibition of MP via the RhoA/ROK pathway, and the ROK inhibitors, Y-27632 and HA-1077, have been widely used. Examples include hypertension, coronary spasm, cerebral vasospasm, vasoplastic angina, and bronchial asthma (20, 34).

Less is known about activation of MP activity. Usually this is associated with relaxation of smooth muscle caused by increases in cAMP and cGMP. An exception is the activation of MP by mitosis-specific phosphorylation of MYPT1 at Thr-435 and/or Ser-432 (in human isoforms) thought to reflect an increase in binding to P-myosin (49). The mechanism underlying activation of MP (initially shown in permeabilized smooth muscle preparations (34)) by PKA and cGK is not clear, but in general, this opposes the RhoA/ROK pathway. Direct phosphorylation of MYPT1 by either kinase does not activate MP (20), and thus it is assumed that additional components are involved. One suggestion is that PKA and cGK inactivate the RhoA/ROK pathway. Under *in vitro* conditions RhoA is phosphorylated by PKA and cGK at Ser-188 and was reported to inactivate RhoA (20, 34). A problem with this proposal is that phosphorylation of RhoA under *in vivo* conditions was difficult to detect although phosphorylation of non-prenylated RhoA was detected in cells (50). The mechanism of RhoA inactivation may be because of enhanced interaction of phosphorylated RhoA with GDI (50) and/or phosphorylation of the switch I region of Gα_{13} and reduced downstream signaling by Gα_{13} (51). Any decrease in RhoA activation would not effect a net activation of MP but would reduce the extent of inhibition. Activation of MP by cGKIα is suggested to require interaction of the LZs of MYPT1 and cGKIα (21), and only those smooth muscles expressing the LZ-positive MYPT1 isoforms show cGMP-dependent Ca^{2+} desensitization (26).

Telokin, an independent protein derived from the smooth muscle MLCK gene, contains the C-terminal domain of MLCK (20) and also has been implicated in cyclic nucleotide-dependent relaxation of smooth muscle (34). It is expressed at relatively high levels (70–80 μM, *i.e.* about the same concentration as myosin II) only in phasic smooth muscle. Under *in vivo* conditions telokin is phosphorylated by PKA and cGK at Ser-13 (34). Telokin also is phosphorylated at Ser-19, a mitogen-activated protein kinase site, but the *in vivo* role for this is not known (34). The mechanism by which telokin or phosphorylated telokin activates MP is not established.

Myosin Phosphatase in Striated Muscle

In striated muscle the target for MP is Ser-15 on the P-regulatory light chain. The idea that MP in skeletal muscle requires a

different regulatory subunit than that in smooth muscle was proposed by Cohen and co-workers (6). This was identified as MYPT2 based on tissue distribution (27), matching of fragments isolated from skeletal muscle (33, 52), and the detection of the full-length MYPT2 in rat muscle (53). In cardiac muscle, MYPT2 also is the major targeting subunit of MP (27). In the myoblast cell line, C2C12, a transition from MYPT1 to MYPT2 occurs as the non-differentiated cells develop a sarcomeric phenotype (53). The function of myosin phosphorylation in striated muscle is not as pronounced as in smooth muscle. In general, phosphorylation of striated muscle myosin increases force at submaximal [Ca^{2+}], *i.e.* an increase in Ca^{2+} sensitivity, and is most pronounced in fast-twitch muscle. It is proposed that phosphorylation of the regulatory light chains moves the myosin head closer to the thin filament and increases the transition from non-force- to force-generating states (54). This may be a general mechanism in all muscle types. Myosin phosphorylation (in fast-twitch muscle) is slower than the twitch contraction but considerably faster than calculated phosphatase rates (approximately 1 s^{-1} compared with 0.007 s^{-1} (54)). In cardiac muscle the rates of myosin phosphorylation and dephosphorylation are lower (1–4% of fast-twitch muscle (54)), and effects due to myosin phosphorylation thus are more difficult to detect. However, in rat hearts a positive correlation was found between myosin phosphorylation and left ventricular pressure development (54). Transgenic mice expressing a non-phosphorylatable regulatory light chain showed loss of Ca^{2+} sensitivity and longer term structural changes (55).

A more recent possibility is that the level of myosin phosphorylation may be important in differentiation or sarcomere organization. In C2C12 cells partial inhibition of non-muscle MLCK caused a decrease in the numbers of larger myotubules (53), and in cardiac myocytes MLCK mediates agonist-induced sarcomere organization during the early hypertrophic response (56). In view of the importance of maintaining a certain level of myosin phosphorylation it is likely that striated muscle MP is regulated, although there are no data to indicate this.

Perspectives

Although knowledge on MP is progressing, notably acceptance of the basic molecular structure, *i.e.* PP1cδ plus MYPT1 (in smooth muscle) and MYPT2 (striated muscle), several questions are unanswered. Details of MP regulation in smooth muscle are important to establish, both for a molecular appreciation of contractile functions and to facilitate pharmaceutical intervention for many disorders of smooth muscle function. In striated muscle there are no data on regulation of MP. Several components (proteins/lipids) bind to MYPT1, and the possibility is raised that MP is not dedicated to P-myosin but has alternative substrates and functions. Another intriguing area for future research is to establish the roles of the other members of the MYPT family.

Note Added in Proof—The crystal structure of PP1cδ and an N-terminal fragment of MYPT1 (residues 1–299) was reported recently (Terrak, M., Kerff, F., Langsetmo, K., Tao, T., and Dominguez, R. (2004) *Nature* **429,** 780–784). This illustrates the critical interaction of the RV*X*F motif and PP1cδ and also secondary interactions involving the N-terminal sequence (residues 1–34) and ankyrin repeats 1, 5, 6, and 7.

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