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REVIEW

Myosin phosphorylation and force potentiation in skeletal muscle: evidence from animal models

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Abstract The contractile performance of mammalian fast twitch skeletal muscle is history dependent. The effect of previous or ongoing contractile activity to *potentiate* force, i.e. increase isometric twitch force, is a fundamental property of fast skeletal muscle. The precise manifestation of force potentiation is dependent upon a variety of factors with two general types being identified; *staircase potentiation* referring to the progressive increase in isometric twitch force observed during low frequency stimulation while *posttetanic potentiation* refers to the step—like increase in isometric twitch force observed following a brief higher frequency (i.e. tetanic) stimulation. Classic studies established that the magnitude and duration of potentiation depends on a number of factors including muscle fiber type, species, temperature, sarcomere length and stimulation paradigm. In addition to isometric twitch force, more recent work has shown that potentiation also influences dynamic (i.e. concentric and/or isotonic) force, work and power at a range of stimulus frequencies in situ or in vitro, an effect that may translate to enhanced physiological function in vivo. Early studies performed on both intact and permeabilized models established that the primary mechanism for this modulation of performance was phosphorylation of myosin, a modification that increased the Ca^{2+} sensitivity of contraction. More recent work from a variety of muscle models indicates, however, the presence of a secondary mechanism for potentiation that may involve altered Ca^{2+} handling. The primary purpose of this review is to highlight these recent findings relative to the physiological utility of force potentiation in vivo.

Keywords Myosin regulatory light chains · Myosin light chain kinase · Isometric twitch · Concentric · Eccentric · Dynamic

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Introduction

In 1993 two review articles were published that addressed functional, mechanistic and biochemical features of myosin phosphorylation mediated force potentiation in vertebrate skeletal muscle (Grange et al. 1993; Sweeney et al. 1993). These comprehensive reviews provided important theoretical and practical information for future investigations. Work performed in the past 20 years has greatly expanded our knowledge concerning myosin phosphorylation and force potentiation. Thus, the purpose of this review is to highlight both functional and mechanistic aspects of force potentiation, focusing on mechanistic studies on animal muscle models. This article includes recent evidence from both wildtype and transgenic mouse as well as rat skeletal muscle models expanding the physiological utility of this mechanism for contraction. Existing cross-bridge models for the influence of myosin phosphorylation on mechanical

Table 1 Potentiation in isolated skeletal muscles in which RLC phosphorylation was not measured

Authors	Species	Muscle	Temp	Rate and duration	Main findings
Abbate et al. (2000)	Rat	MG	34	160 Hz, 1 s	Concentric power ↑ at 80–120 Hz
Bagust et al. (1974)	Cat	FDL, sol	NR	100 Hz, 300 ms	PTP ↓ as time to peak tension ↑
Brown and Loeb (1998)	Cat	CF	37	Various high freq	Pt:Po ↑ five fold
Brown and Loeb (1999)	Cat	CF	37	Various high freq	PTP ↓ as muscle length ↑
Brown and von Euler (1938)	Cat	Gastroc, sol, TA	37	High frequency, 2–20 s	Stimulus duration dependence for PTP
Buller et al. (1981)	Cat	Sol	37	100 Hz, 300–1,000 ms	↓ Pt:Po with ↑ stim number
Close and Hoh (1968a)	Rat	EDL, sol	20–35	Repetitive stimulation	EDL, PTP ↑ with temp; sol, no PTP
Close and Hoh (1968b)	Rat	EDL	35	20 and 300 Hz; various	PTP varies with pulse # at each frequency
Close and Hoh (1969)	Rat	EDL, sol	35	200 Hz, 1 s	Cross-innervation: PTP ↑ sol, ↓ EDL
Grange et al. (1998)	Mouse	EDL	25	5 Hz, 20 s	Work loop area ↑ 25–30 % by PTP
Guttman et al. (1937)	Frog	Gastroc	NR	10–30 Hz, 1–20 s	PTP coexists with fatigue
Krarup (1981a)	Rat	EDL	37–38	2–5 Hz; 125–167 Hz	Biphasic effects of duration and stimuli #
Krarup (1981b)	Rat	EDL	20–38	5 Hz, 50 s	SP ↑ as temp ↑
Krarup (1981c)	Rat	EDL	37–38	5 Hz, 50 s; 167 Hz, 1.5 s	Dantrolene ↑ SP and PTP
Krarup (1983)	Rat	EDL	37–38	3–10 Hz, 1.5–3 s	Potentiation ↓ in myasthenia gravis
MacIntosh and Gardiner (1987)	Rat	Gastroc	37	200 Hz, 1 s	Fat x caff ↑ PTP additively
MacIntosh and Willis (2000)	Rat	Gastroc	37	2–5 pulses at 20–80 Hz	PTP ↓ as force approaches Po
MacIntosh et al. (1988)	Rat	Gastroc[a]	37	10 Hz, 15 s	TTX ↓ SP by ~75 %
MacIntosh et al. (2008b)	Rat	Gastroc	37	200–400 Hz (2–4 pulses)	↑ Force, work and power at high frequencies
Ramsey and Street (1941)	Frog	Single fibers	22	Tetani 5–180 s	PTP ↑ at low P_t:P_o in isolated fibers
Rankin et al. (1988)	Rat	Sol, EDL	35–37	0.5 Hz 32 s; 100 Hz 500 ms	↑ Pot in ↓ fatigable EDL muscle
Rassier and MacIntosh (2002)	Mouse	EDL	22–35	10 Hz 10 s; 75 Hz 1.5 s	Pot ↓ long lengths at both temps
Rassier and MacIntosh (2000)	Rat	Gastroc	37	5 Hz, 20 s	Dant ↓ Pot but not length dependence
Rassier et al. (1998)	Rat	Gastroc	37	10 Hz, 10 s	Caff ↓ Pot and abolishes length dependence
Rassier and Herzog (2002)	Mouse	EDL	25	10 Hz, 10 s	↓ pH abolishes length dependence
Standaert (1964)	Cat	Sol, gastroc	37	400 Hz 10 s (s);200 Hz 10 s (g)	Fibre and motor unit dependence for PTP
Vergara et al. (1977)	Frog	Semitend	15	20 Hz, 5–200 s	Long lasting PTP with fatigue

Studies examining staircase or PTP in which RLC phosphorylation was not measured arranged alphabetically by first author. Columns (left to right) describe species, muscle(s) and experimental temperature (Temp) as well as the stimulation paradigm used to induce potentiation as frequency in Hz and duration (in ms or sec) with a summary of main findings

CF caudofemoris, *EDL* extensor digitorum longus, *FDL* flexor digitorum longus, *gastroc* gastrocnemius, *MG* medial gastrocnemius, *sol* soleus, *semitend* semitendinosus, *TA* tibialis anterior, *caff* caffeine, *Dant* dantrolene, *#* number, *Po* peak tetanic force, *Pt* peak twitch force, *pot* potentiation, *SP* staircase potentiation, *TTX* tetrodotoxin. (*NR* not reported). Note that due to space constraint not all studies could be included

[a] Tetrodotoxin muscle model used

function will be modified to account for these results. The genetic, enzymatic and regulatory aspects of RLC phosphorylation have been recently reviewed by Stull et al. (2011) and will not be detailed in this article.

First described in the literature over 100 years ago (see Lee 1907) force potentiation has long been recognized as a fundamental property of fast twitch skeletal muscle. Potentiation is generally expressed as an increase in isometric twitch force independent of change to peak tetanic force, thus increasing the twitch to tetanus ratio (e.g., Bagust et al. 1974; Ramsey and Street 1941). In contrast, slow twitch skeletal muscle displays a *posttetanic depression* of isometric twitch force (Buller et al. 1981; Close and Hoh 1969). A list of studies describing potentiation of fast twitch vertebrate striated muscle is compiled in Table 1. Potentiation is readily induced in most fast twitch skeletal muscles studied and as such may be a normal operating feature of these muscles (Brown and Loeb 1998). In the

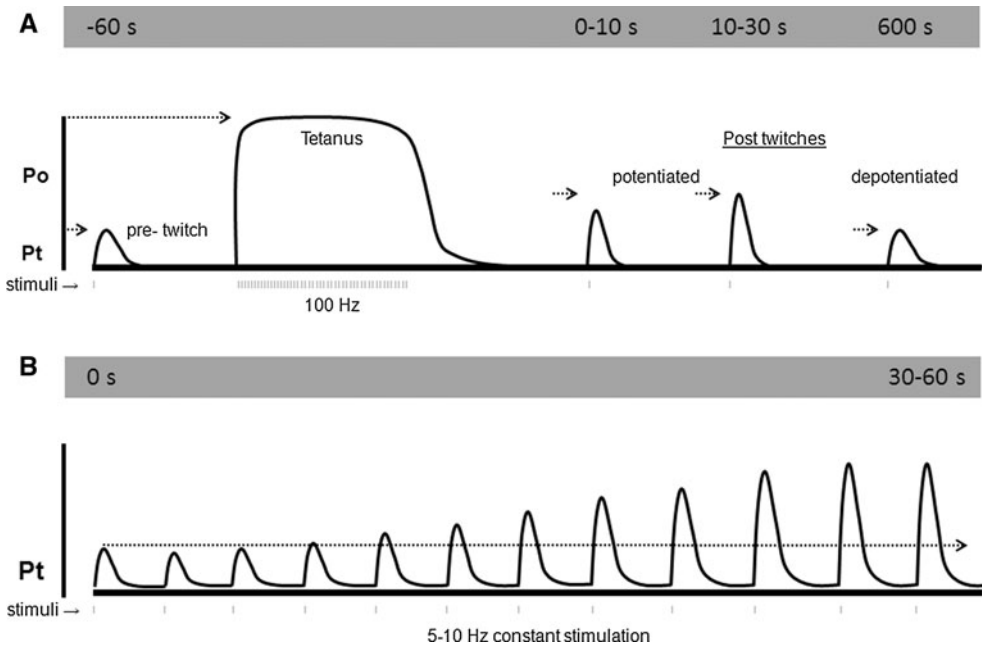

Fig. 1 Examples of potentiation in fast twitch skeletal muscle. Typical experimental paradigms producing posttetanic potentiation (PTP) (**a**) or staircase potentiation (**b**). In **a** a single control or unpotentiated isometric twitch is elicited under resting conditions prior to the delivery of a brief, but high frequency conditioning stimulation. The precise time course of PTP depends on the frequency and duration of stimulation; in mouse EDL muscle at 25 °C the peak tends to occur shortly after the conditioning stimulus before decaying over several minutes to pre-stimulus response. Twitch duration may or may not be abbreviated compared to the unpotentiated twitch. In **b** a series of evenly spaced isometric twitches is elicited at a fixed low frequency (typically 5–10 Hz). In most species, stimulation rates below 5 Hz do not produce staircase while stimulation rates above 10 Hz produce fused contractions. During staircase, an initial modest decrease in twitch force is often observed before progressively increasing. As for PTP, the precise magnitude of staircase observed depends on stimulation rate, species, temperature and muscle length. Note that in both the top and bottom panels the time scale is variable

laboratory setting, the precise manifestation of potentiation is highly paradigm dependent, however (Fig. 1). For example, *staircase potentiation* describes the progressive increase in isometric twitch force observed during a period of low frequency stimulation (e.g., Colomo and Rocchi 1965; Isaacson 1969).

During staircase potentiation, isometric twitch amplitude may initially decrease before increasing to a peak that is dependent upon both stimulus rate and number; upon the cessation of stimulation, twitch force dissipates slowly at first and then with a more rapid time course (Krarup 1981a). On the other hand, posttetanic potentiation (PTP) describes the acute increase in isometric twitch force observed following high (or sometimes low) frequency stimulation (e.g., Close and Hoh 1968b). Like staircase, the posttetanic response is also stimulus frequency and duration dependent, often displaying an initial increase to a peak shortly after the cessation of stimulation before dissipating, rapidly at first and more slowly thereafter, over the course of several minutes (e.g., Krarup 1981a). In addition to stimulus parameters, both staircase and PTP are highly sensitive to experimental factors such as temperature (high>low), muscle length (short>long) and contraction type (concentric>isometric). Even though a single mechanism for staircase and PTP is often assumed, the existence of divergent mechanisms now seems probable.

Although much has been learned regarding how potentiation benefits the mechanical performance of isolated muscles, a definitive teleological role in vivo has not yet been identified. Interesting in this regard is evidence suggesting that the potentiated state is the normal operating state of fast twitch skeletal muscle in vivo (Brown and Loeb 1998). In addition, work on isolated mouse fast twitch skeletal muscles shows that beta-adrenergic stimulation prolongs the potentiated state, an outcome that may indicate a role for potentiation during the fight or flight response of mammals (Decostre et al. 2000). Thus, much remains to be discovered regarding how potentiation integrates into overall organismal function when extra-muscular signaling is intact.

Many early studies attempted to identify an ionic origin for potentiation, with mostly equivocal results (e.g., Brown and von Euler 1938; Bernhard et al. 1941; Walker 1948). The demonstration by Ramsey and Street (1941) that potentiation is a property of isolated skeletal muscle fibers indicated a muscle origin for this phenomenon. Indeed, by the 1960s both staircase and PTP were considered to be intrinsic properties of skeletal muscle (e.g., Standaert 1964)

although the precise mechanism was still not known. Interestingly, the observation that the magnitude of PTP was inversely related to the twitch:tetanus ratio was seen as evidence that alterations to the "active state" were responsible (Close and Hoh 1968a; Ritchie and Wilkie 1955). It was not until the demonstration that the myosin II molecule of vertebrate striated muscle contained a phosphorylatable light chain subunit (Perrie et al. 1973) that a viable intracellular mechanism reconcilable with contractile data was available, however. Two small protein subunits, the essential light chain and regulatory light chain (RLC) wrap around the α-helical neck or light chain binding domain of the myosin heavy chain, providing mechanical stability (Rayment and Holden 1994; Trybus 1994). Although skeletal muscle contraction results from the Ca^{2+} —regulated formation of force-generating actomyosin complexes and the linking of myosin ATPase activity to structural changes in this complex (reviewed by Geeves and Holmes 2005; Vale and Milligan 2000), phosphorylation of the RLC subunit may modify these unitary reactions to enhance muscle cell force and shortening (Sweeney and Stull 1990).

Potentiation by RLC phosphorylation in rodent muscle

Work performed on isolated rat and mouse skeletal muscle demonstrates that isometric twitch potentiation is temporally correlated with RLC phosphate content following various stimulus regimes. A comprehensive list of studies examining the phosphorylation-potentiation relationship in different rodent muscle models is presented in Tables 2 and 3. In studies where multiple measurements of isometric twitch potentiation and RLC phosphorylation have been made, both non- linear (Klug et al. 1982) and linear relationships (Manning and Stull 1979; Moore et al. 1990; Palmer and Moore 1989; Vandenboom et al. 1995, 1997; Xeni et al. 2011) have been documented. However, when data from these respective studies are pooled according to species and temperature in Fig. 2, the resultant scatter plot depicting twitch potentiation versus RLC phosphorylation are decidedly linear. The robustness of these relationships is particularly striking given the inevitable differences in methodology between the different studies used to construct these plots. This analysis thus indicates that RLC phosphorylation has a direct and predictable influence on the isometric twitch force of unfatigued fast twitch rodent skeletal muscle, an effect that increases as muscle temperature approaches the physiological range. Because these results were obtained from mostly unfatigued muscle it is possible that more extreme stimulus regimes causing more severe fatigue will disrupt the linearity of these relationships, especially at high phosphorylation levels. In addition, experimental interventions that alter thin filament activation level may also alter the slope of any of these relationships (Krarup 1981b, c; MacIntosh and Gardiner 1987; MacIntosh and Kupsh 1987; Palmer and Moore 1989; Vandenboom and Houston 1996).

The first direct evidence of a causal relationship between RLC phosphorylation and isometric force potentiation was provided by studies utilizing permeabilized skeletal muscle fibers. A list of these studies is compiled in Table 4. Persechini et al. (1985) were the first to show that the addition of exogenous skeletal myosin light chain kinase (skMLCK) to the media bathing rabbit psoas fibers both phosphorylated the RLC and increased steady state tension at submaximal, but not maximal, Ca^{2+} activation. Subsequent studies by this and other goups have elegantly documented how the potentiation of steady state force by RLC phosphorylation appears to be inversely related to thin filament activation levels (e.g., Davis et al. 2002; Metzger et al. 1989; Patel et al. 1996, 1998; Sweeney and Kushmerick 1985; Stephenson and Stephenson 1993; Sweeney and Stull 1986, 1990; Szczesna et al. 2002). An important feature of many of these studies is the increase in the rate constant for steady state force development (i.e. the kTR) that occurs following RLC phosphorylation, an effect that may be the mechanical basis for twitch potentiation in intact muscle. These studies have thus provided important mechanistic links between skMLCK catalyzed phosphorylation of the RLC and isometric twitch force potentiation in intact skeletal muscle models. A model for the influence of RLC phosphorylation on cross-bridge kinetics, and interactions with Ca^{2+} activation of the thin filament, is detailed below.

RLC phosphorylation and cross-bridge structure

Skeletal muscle force is regulated by Ca^{2+} ion binding to regulatory proteins on the thin filament (Ebashi and Endo 1968). Although the ligand binding role of Ca^{2+} and regulatory protein conformation on the thin filament have been studied extensively (e.g., Lehman et al. 2009) there may be, in addition, thick filament structural constraints that participate in force regulation. For example, reconstructions of the thin filament of rested skeletal fibers from arthropods and mammals show that myosin head distribution is highly ordered with close proximity to the thick filament surface (Woodhead et al. 2005; Zhao et al. 2009).

Head to head interactions via highly conserved motifs present in both regulated and unregulated myosins may be responsible for this constraint, a profile that may minimize myosin head interactions with inactivated thin filaments (Jung et al. 2008). Interestingly, structural studies employing a variety of fiber types show that the addition of a negative charge to the RLC via phosphorylation disrupts

Table 2 Potentiation in isolated rat and rabbit skeletal muscle in which RLC phosphorylation was measured

Author(s)	Species	Muscle	Temp	Rate and duration	% RLC-P	Main findings
Abbate et al. (2001)	Rat	MG	34	160 Hz, 1 s	13–42–50	PTP ↓ economy of contraction
Barsotti and Butler (1984)	Rat	EDL	23	66 and 100 Hz, 1–10 s	5–73	RLC-P does not alter energy usage
Klug et al. (1982)	Rat	Gastroc	37	5 Hz, 5–20 s	19–63	PTP correlated with RLC-P
MacIntosh and Bryan (2002)	Rat	MG	37	80 Hz, 4–7 s	11–33–50	↑ Isotonic work (shortening)
MacIntosh et al. (2008a)[a]	Rat	Gastroc	37	5 Hz 21 s; 200 Hz 1–10 s	15–42 11–9[a]	SP without RLC-P
MacIntosh et al. (1993)	Rat	Gastroc	37	10 Hz 20 s	10–72; 3–19	Fatigue ↓ RLC-P
Manning and Stull (1982)	Rat	EDL sol	23–35	10–200 Hz	14–60	FT and temp dep for RLC-P and PTP
Manning and Stull (1979)	Rat	EDL	23	200 Hz, 1 s	10–75	PTP and RLC-P correlated
Moore and Stull (1984)	Rat	Sol, gastroc	37	1–100 Hz, 10 s	0–34 s; 10–62 g	PTP & skMLCK activity ↓ with ↑ OP
Moore et al. (1985)	Rabbit	Sol, plan	38	100 Hz, 15 s (s) 5 Hz, 20 s (p)	2–10 s;17–45 p	No PTP (sol); 58 % PTP (plan)
Rassier et al. (1999)	Rat	Gastroc, plan	37	10 Hz, 10 s	5–26	SP w/o ↑ RLC-P
Rassier et al. (1997)	Rat	Gastroc	37	10 Hz, 10 s	10–15; 35–40	Len Dep of SP not RLC-P dependent
Tubman et al. (1996a)	Rat	Gastroc	37	200 Hz, 0.5–2 s	55 c; 40 f	Fatigue ↓ RLC-P but not PTP
Tubman et al. (1996b)[b]	Rat	Gastroc	37	10 Hz, 10 s	5–9[b]; 21–57	No PTP and no RLC-P with atrophy
Tubman et al. (1997)[a]	Rat	Gastroc	37	200 Hz, 2 s	5–21[a]; 14–49	PTP and RLC-P ↓ with atrophy

Studies examining staircase or PTP in rat and rabbit muscles in which RLC phosphorylation was determined, arranged alphabetically by first author. Columns (left to right) describe species, muscle(s) experimental temperature (Temp), stimulation paradigm used to induce potentiation (as frequency in Hz and duration in ms or sec), the range (minimum and maximum) of RLC phosphorylation (as %) reported at rest and in response to stimulation and a summary of main findings

EDL extensor digitorum longus, *gastroc* gastrocnemius, *GM* medial gastrocnemius *plan* plantaris, *sol* soleus, *FT* fiber type, *len dep* length dependence, *OP* oxidative potential, *PTP* posttetanic potentiation, *skMLCK* skeletal myosin light chain kinase, *SP* staircase potentiation, *temp dep* temperature dependence

[a] Hemisection muscle model used; [b] tetrodotoxin muscle model used

these interactions to displace myosin heads away from the thick filament (Alamo et al. 2008; Brito et al. 2011; Craig et al. 1987; Hidalgo et al. 2001; Levine et al. 1991, 1996, 1998; Padrón et al. 1991; Ritz-Gold et al. 1980; Sweeney et al. 1994; Yang et al. 1998). It is proposed that these phosphorylation-induced changes to myosin head position on the thick filament surface increase the Ca^{2+} sensitivity of force development by promoting the formation of the actomyosin complex achieved by Ca^{2+} signalling alone (see below). These structure–function relations are consistent with findings from intact rat skeletal muscle showing that potentiation is greatest at short and minimized at long sarcomere lengths, respectively (e.g., Rassier et al. 1997, 1998; Rassier and MacIntosh 2000) (*c.f.* Moore and Persechini 1990). Finally, findings from experiments using in vitro motility assays showing that phosphorylation of the RLC does not improve intrinsic myosin motor function in isolation from the thin filament supports these arguments (Greenberg et al. 2009).

RLC phosphorylation and cross-bridge cycling kinetics

Huxley (1957) was the first to provide an analytical framework for understanding cross-bridge cycling in skeletal muscle. In his original scheme Huxley proposed that muscle force was regulated by the cyclic attachment and detachment of "side pieces" between the myofilaments. These rate constants, known as F and G, respectively, were considered intrinsic properties of independently operating force generators within the sarcomere; these structures were, in time, recognized as the actomyosin cross-bridge. Brenner and Eisenberg (1986) and Brenner (1988) advanced this two state model for cross-bridge action by incorporating the regulatory influence of Ca^{2+} binding to troponin C (TnC) on force. In their modern scheme, Ca^{2+} binding to TnC controls the rate constant describing the transition of cycling cross-bridges from non-force to force generating states (i.e. f_{app}). In contrast, the reverse rate constant describing the transition of cycling cross-bridges from force generating

Table 3 Potentiation in isolated mouse skeletal muscle in which RLC phosphorylation was measured

Author(s)	Muscle	Temp	Rate and duration of stim	% RLC-P	Main findings
Butler et al. (1983)	EDL	22	66 and 100 Hz, 0.5–2 s	9–50	XB cycling & RLC-P are independent
Caterini et al. (2011)	EDL	25	(3)100 Hz × 500 ms in 10 s	17–52	PTP is shortening speed dependent
Crow and Kushmerick (1982a)	EDL, sol	20	66 Hz 15 s	11–55 EDL; 11–11 sol	↓ Energy cost, ↑ RLC-P (EDL only)
Crow and Kushmerick (1982b)	EDL, sol	20	High frequency 3–15 s	22–51 EDL; 10 –12 sol	↑ RLC-P ∝ ↓ AM cycling (Vo)
Decostre et al. (2000)	EDL	20	125 Hz for 1 s	20–53	Adrenaline ↑ PTP duration
Gittings et al. (2011)[a]	EDL	25	(4×) 150 Hz 400 ms in 10 s	16–60 WT; 8–8 KO	↑ PTP in WT vs skMLCK KO during moderate fatigue; no change in Vo
Gittings et al. (2012)	EDL	25	(4×) 100 Hz 400 ms in 10 s	8–55	PTP is shortening speed dependent
Grange et al. (1995)	EDL	25	5 Hz 20 s	14–68	PTP ↑ force–velocity characteristics
Moore and Persechini (1990)	EDL	23	75 Hz 1.5 s	10–25 and 43–59	PTP ↑ with ↑ Length[a]
Moore et al. (1990)	EDL	25, 30, 35	5 Hz for 20 s	10–25–40–70	RLC-P and PTP are temp dependent
Palmer and Moore (1989)	EDL	22	110–150 Hz 350–1500 ms	20–45, 44–46, 18–34	Dantrolene ↑ PTP
Ryder et al. (2007)[b]	EDL, sol	30	10 Hz for 3 or 15 s	13–56–71 EDL; 8–17–62 sol	SkMLCK is limiting to RLC-P
Smith et al. (2010)[c]	EDL	25	20 Hz 10 s	3–74	PTP ↑ with age and *mdx* condition
Smith et al. (2013)	Lumbrical	37	20 Hz 2.5 s	0	PTP w/o RLC-P ∝ ↑ resting $[Ca^{2+}]$
Vandenboom and Houston (1996)	EDL	25	(3×) 100 Hz × 500 ms	15–56	PTP counteracts LFF
Vandenboom et al. (1993)	EDL	25	5 Hz 20 s	8–74	PTP ≤ 15 Hz
Vandenboom et al. (1995)	EDL	25	5 Hz 20 s	13–68	Increase in +dP/dt ∝ RLC-P
Vandenboom et al. (1997)	EDL	25	2.5–100 Hz, 10 s	10–82	PTP ∝ RLC-P, fatigue independent
Xeni et al. (2011)	EDL	25	2.5–100 Hz, 10 s	15–55	PTP of power graded to RLC-P
Zhi et al. (2005)[a]	EDL	30	150 Hz 2 s; 10 Hz 15 s	15–54 WT 5–9 KO	No RLC-P eliminates PTP and ↓ SP

Studies examining staircase or PTP in mouse skeletal muscles in which RLC phosphorylation was determined, arranged alphabetically by first author. Columns describe (left to right) muscle(s) used, experimental temperature (Temp), stimulation paradigm used to induce potentiation (as frequency in Hz and duration in ms or s, the range for RLC phosphorylation (as %) reported at rest and in response to stimulation and a summary of main findings)

EDL extensor digitorum longus, *gastroc* gastrocnemius, *GM* medial gastrocnemius, *plan* plantaris, *sol* soleus, *AM* actomyosin, *XB* cross-bridge, *LFF* low frequency fatigue, *PTP* posttetanic potentiation, $+dF/dt$ rate of isometric force development, *skMLCK* skeletal myosin light chain kinase, *SP* staircase potentiation, *Vo* unloaded shortening velocity, *WT* wild type, Ca^{2+} intracellular calcium concentration

[a] skMLCK *KO* knockout muscles used. [b] *TG* transgenic mice overexpressing skMLCK used. [c] mdx muscles used

back to non-force generating states (i.e. g_{app}) is mostly unregulated and not under the influence of Ca^{2+}. Thus, although only a fraction of the total available cross-bridge population may be involved, force is proportional to the fraction of cycling cross-bridges able to attain the force generating state (αFS). In turn, this term is determined by the balance between f_{app} and g_{app} according to the equation $\alpha FS = f_{app}/(f_{app} + g_{app})$. Within this scheme the influence of RLC phosphorylation on force may be understood by noting that RLC phosphorylation increases f_{app} at all Ca^{2+} activation levels with little or no effect on g_{app} (Sweeney et al. 1993). Thus, at low levels of myofilament activation where f_{app} is small relative to g_{app}, the RLC phosphorylation-mediated increases in f_{app} greatly enhance cross-bridge formation (i.e. increase αFS). Conversely, at high levels of myofilament activation where f_{app} is large relative to g_{app}, crossbridge formation is little affected as αFS is already near unitary (Sweeney and Stull 1990). Moreover, the absence of

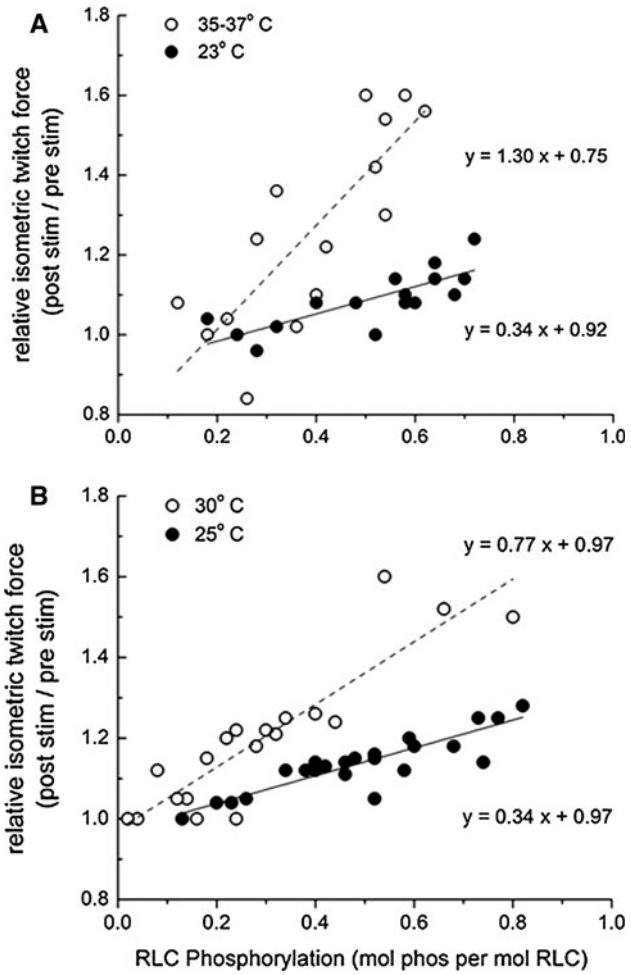

Fig. 2 Quantitative relationship between stimulation induced isometric twitch potentiation and RLC phosphorylation in rodent skeletal muscle. **a** Data from studies employing rat gastrocnemius and EDL muscle with separate linear fits to results obtained at 23 and 35–37 °C (r value = 0.79 and 0.82, respectively). **b** Data from studies employing mouse EDL muscle with separate linear fits to data obtained at 25 and 30 °C (r value = 0.89 and 0.90, respectively). Although the scatter of data is increased, there is a clear effect of increasing temperature on the slope of the RLC phosphorylation vs isometric twitch force potentiation relationship in both species. Note that in both panels we have pooled data from experiments in which the conditioning stimulus was fixed and the decay in RLC phosphorylation and twitch force over time was tracked and in which different conditioning stimuli were used and RLC phosphorylation and twitch force were assessed at a fixed time point. Data in panel A taken from Klug et al. 1982; Manning and Stull 1979, 1982; Moore and Stull 1984. Data in panel B taken from Moore et al. 1990; Palmer and Moore 1989; Vandenboom et al. 1995, 1997; Xeni et al. 2011. Although not included, data from Moore et al. (1990) for mouse EDL muscle at 35 °C also shows a linear response

any effect on g_{app} accounts for why unloaded shortening velocity of permeabilized skeletal fibers (Persechini et al. 1985; Sweeney and Stull 1990) or intact skeletal muscle (Butler et al. 1983; Decostre et al. 2000; Gittings et al. 2011; Palmer and Moore 1989) are not altered.

Results from skMLCK knockout models

Perhaps the most compelling evidence from intact skeletal muscles for a causative relationship between RLC phosphorylation and isometric twitch force potentiation comes from experiments using muscles from mice devoid of the skMLCK enzyme and which, as a consequence, do not display stimulation-induced elevations in RLC phosphorylation. In this regard, Zhi et al. (2005) showed that brief tetanic stimulation of EDL muscles from wildtype mice increased both RLC phosphorylation and twitch force by ~4 and 1.8-fold, respectively, but did not elevate either of these parameters in EDL muscles from skMLCK deficient or skMLCK$^{-/-}$ mice. Examples of posttetanic responses from wildtype and skMLCK$^{-/-}$ muscles is shown in Fig. 3. Interestingly, however, low frequency repetitive stimulation of skMLCK$^{-/-}$ muscles still produces a significant staircase potentiation, albeit attenuated by ~50 % compared to wildtype muscles. Indeed, this remnant potentiation in skMLCK$^{-/-}$ muscles accords with the presence of potentiation in muscle disease or atrophy models (MacIntosh et al. 2008a; Rassier et al. 1999). Interestingly, Ryder et al. (2007) showed that overexpression of skMLCK in mouse EDL muscle enhanced the rate of RLC phosphorylation and staircase potentiation relative to wildtype muscles. The absence of PTP but continued presence of staircase potentiation in this genotype suggests that although skMLCK catalyzed phosphorylation of the RLC is the primary mechanism for PTP, additional mechanisms may contribute to staircase potentiation (Zhi et al. 2005; Gittings et al. 2011).

The influence of RLC phosphorylation on skeletal muscle mechanics may apply to both force development and relaxation. For example, evidence from skMLCK$^{-/-}$ muscles suggests that, in addition to increases in the rate and/or extent of force development, RLC phosphorylation may influence force relaxation kinetics as well. Gittings et al. (2011) compared high frequency tetanic contractions of EDL muscles from skMLCK$^{-/-}$ and wildtype mice following a stimulus protocol that elevated RLC phosphorylation in wildtype muscles only. They found that, despite no changes in peak tetanic force in either genotype, the relaxation rate of wildtype muscles was significantly slowed relative to skMLCK$^{-/-}$ muscles (in vitro, 25° C). This outcome corroborates findings from both intact cat (Brown and Loeb 1999) and permeabilized rabbit psoas skeletal fibers (Patel et al. 1998) but the functional significance of this effect is unknown. Moreover, although an increased rate of relaxation of potentiated twitch contractions is often observed, this effect may not be attributable to RLC phosphorylation as skMLCK$^{-/-}$ muscles also show this effect (Gittings et al. 2011).

An interesting corollary to the results from skMLCK$^{-/-}$ mice are results from insect flight muscle in which

Table 4 Studies using permeabilized skeletal muscle fibers or in vitro motility assay

Author(s)	Species and muscle	Temp	[skMLCK]	% RLC-P	Main findings
Childers and MCDonald (2004)	Rat psoas	NR	0.5 μM	NR	↑ Eccentric-induced injury w/skMLCK
Davis et al. (2002)	Rabbit soleus; psoas	5, 20	0.1 μM	0–100	RLC-P ↑ Pi release during XB cycling
Franks-Skiba et al. (2007)	Rabbit psoas	10,30	NR	10–60	RLC-P ↓ Vo at high temp (+vanadate)
Greenberg et al. (2009)	Rabbit fast muscle	24, 35	100 μg	0–100	↓ Myosin cycling velocity with RLC-P
Greenberg et al. (2010)	Rabbit fast muscle	35	100 μg	0–100	↓ Vo with RLC-P (unfatigued conditions); ↑ Vo with RLC-P (fatigued conditions)
Karatzaferi et al. (2008)	Rabbit psoas	10, 30	Endogenous	10–50	RLC-P ↓ Vo as temp, $[P_i]$ and $[H^+]$ ↑
Levine et al. (1996)	Rabbit psoas	23–25	0.13 mg/ml	<10, variable	RLC-P disorders myosin head orientation
Levine et al. (1998)	Rabbit psoas	25	0.15 μM	NR	RLC maintains XB orientation in relaxation
Metzger et al. (1989)	Rabbit psoas, Rat VL	15	0.5 μM	10–80	RLC-P ↑Ca^{2+} sensitivity of k_{tr}
Miller et al. (2011)	Drosophila	NR	NR	NR	Non-P RLC mutation reduces power output
Patel et al. (1998)	Rabbit psoas	15	0.5 μM	0–85	RLC-P ↓ relaxation rate from Fmax
Persechini et al. (1985)	Rabbit psoas	25	0.12 μM	10–80	↑ Ca^{2+} sensitivity with RLC-P, no Δ in Vo
Stephenson and Stephenson (1993)	Rat EDL	22	Endogenous	50	Endogenous RLC-P ↑ Ca^{2+} sensitivity
Stewart et al. (2009)	Rabbit psoas	10, 30	300 μg/ml	0–100	RLC-P ↓ Vo all temps(+blebbistatin)
Sweeney and Kushmerick (1985)	Rabbit psoas	12	3 μM	<10–60	No Δ to Po and Vmax with RLC-P
Sweeney and Stull (1986)	Rabbit psoas, ventricle	NR	0.15 μM	5–10, 60–75	RLC-P alters AM function (cardiac & skeletal)
Sweeney and Stull (1990)	Rabbit psoas	15	0.15 μM	<10– > 75	RLC-P ↑ αFS at low activation
Sweeney et al. (1994)	Sea scallop, turkey gizzard	15, 30	0.13 mg/ml	0– > 80	Charge replacement mimics RLC-P effects
Szczesna et al. (2002)	Rabbit psoas	22	0.5 μM	0–100	RLC-P ↑ Ca^{2+} sensitivity
Yang et al. (1998)	Rabbit psoas	23	0.15 μM	<10–75	Pot dependent on interfilament spacing

Studies examining potentiation of steady-state tension in permeabilized skeletal muscle fibers, arranged alphabetically by first author. Columns (left to right) describe species and muscle(s), experimental temperature (Temp), the concentration of skMLCK used, the range of RLC phosphorylation reported (as %) reported before and after addition of enzyme and a summary of main findings

EDL extensor digitorum longus, *VL* vastus lateralis. *AM* actomyosin, *XB* cross-bridge, *αFS* fraction of cycling cross-bridges in the force generating state, *Pi* inorganic phosphate, H^+ hydrogen, *Fmax* maximal Ca^{2+} activated force, *Vmax* maximal velocity of shortening, *Po* peak tetanic tension, *Pot* potentiation, k_{tr} rate constant for isometric force development, *skMLCK* skeletal myosin light chain kinase, *Vo* unloaded shortening velocity. (*NR* not reported)

mutations to the serine residue have rendered the RLC unphosphorylatable. In these experiments, the power and flight characteristics of *Drosophila melanogaster* were attenuated related to wildtype flight characteristics (Dickinson et al. 1997; Miller et al. 2011; Tohtong et al. 1995).

Stimulus frequency and contraction type dependence for potentiation

Studies performed prior to the 1990s tended to study potentiation using single pulse, twitch contractions. Although single pulse contractions have been shown to represent ~30 % of all motor unit discharge events in hindlimb muscles of freely moving rats (Hennig and Lømo 1987), an influence of potentiation on higher frequency, multiple pulse (i.e. tetanic) contractions would greatly extend its physiological utility. The first to systematically study the stimulus frequency dependence for isometric force potentiation was Vandenboom et al. (1993) using a mouse EDL in vitro (25 °C) muscle model. These investigators showed that although a low frequency conditioning stimulus that elevated RLC phosphorylation to near maximal levels increased the rate of isometric force development at all frequencies between 1 and 200 Hz, the potentiation of peak force was restricted to frequencies below 20 Hz. Indeed, peak tetanic forces measured at higher frequencies were decreased rather than increased. It is important to point out that any threshold that is identified for peak force potentiation must be highly model dependent. As an example of this, more recent work from rat skeletal muscle in situ (35 °C) showed that potentiation of

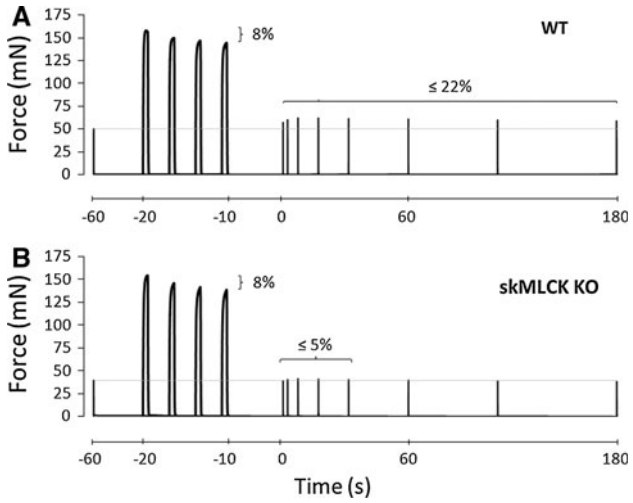

Fig. 3 Genotype dependence of potentiation in mouse fast skeletal muscle Comparison of posttetanic potentiation (PTP) of EDL muscles from and wildtype (WT) (**a**) and skeletal myosin light chain kinase (skMLCK) knockout (KO) (**b**) muscles (in vitro 25 °C). The influence of a tetanic conditioning stimulus (*four*-volleys of 100 Hz stimulation, each lasting 400 ms, in a 20 s time window) on isometric twitch force is shown. Note that although still present, the potentiation of twitch force was much greater in WT than skMLCK KO muscles. For example, in the WT muscle the post-twitches were potentiated by up to 22 % relative to the pre-twitch and were potentiated for more than 180 s. In the skMLCK KO muscle, post-twitches were potentiated by only 5 % relative to the pre-twitch and this potentiation lasted for only 30 s. Twitch time course was similar for each genotype regardless of differences in peak force. The time interval between tetani has been compressed for clarity. In both genotypes peak tetanic force was decreased similarly from the first to last tetanic volley. Previously unpublished data

peak isometric force may in fact be observed at high stimulus frequencies (MacIntosh and Bryan 2002; MacIntosh and Willis 2000). This apparent difference may be related to the tetanus duration used to examine high frequency force; the enhanced +dF/dt that is a general characteristic of potentiated rodent muscle (Grange et al. 1995) may be able to enhance the peak force that is attained during brief, but not necessarily long, duration tetani.

Another vital mechanical factor that modulates the ability of RLC phosphorylation to potentiate muscle function is that of contraction type, i.e. isometric versus concentric. A host of studies performed on rat and mouse skeletal muscle demonstrates that the same stimulus regimes that potentiates isometric function also potentiate dynamic function (Abbate et al. 2000; Caterini et al. 2011; Gittings et al. 2012; Grange et al. 1995, 1998; MacIntosh et al. 2008b; Xeni et al. 2011). Evidence from these studies indicates that muscle shortening during concentric force development may actually sensitize the contractile apparatus to potentiation; as a result, dynamic force levels observed during shortening are potentiated to a much greater extent than might be predicted based on isometric responses alone. Representative force records depicting the potentiation of concentric forces during tetanic stimulation of wildtype and skMLCK$^{-/-}$ muscles are shown in Fig. 4. The first study showing this effect was Grange et al. (1995) who used mouse EDL muscles (in vitro, 25 °C) to show that work and power during after—loaded twitch contractions were enhanced more than isometric twitch force levels (see also Grange et al. 1998). Many years later, the sensitizing influence of muscle shortening was confirmed by Xeni et al. (2011) who showed that concentric twitch force was potentiated more than isometric twitch force when RLC phosphorylation levels were similar. Subsequent work from our laboratory has shown that the influence of muscle shortening speed on potentiation is progressive, i.e. twitch forces during fast, moderate and slow shortening were increased more than isometric twitches for the same increase in RLC phosphorylation (Caterini et al. 2011) an effect that also applies to higher frequency forces observed during partially fused tetani (Gittings et al. 2012). A speed-dependent increase in potentiation has also been found in rat gastrocnemius muscle studied in situ, suggesting that this response is a general feature of muscle function rather than just a species specific response (Abbate et al. 2000).

Work on intact frog skeletal fibers by Piazzesi et al. (2007) examining the influence of filament sliding on cross-bridge cycling kinetics (i.e. f_{app} and/or g_{app}) may be able to account for the speed dependence for potentiation at the sarcomeric level. For example, their experiments show that while filament sliding may increase both f_{app} and g_{app} relative to isometric, the increase in g_{app} much exceeds the increase in f_{app} at both moderate and high, but not slow, speeds of shortening. Thus, the effect of shortening to decrease the number of attached cross-bridges (i.e. αFS) is consistent with a speed dependence for concentric force potentiation. Indeed, the relationship between f_{app} and g_{app} shown in Fig. 4d of Piazzesi et al. (2007) may be able to account for why although very slow shortening does not greatly increase the potentiation of concentric compared to isometric force, moderate speeds of shortening do greatly increase concentric compared to isometric force at most activation levels (i.e. Gittings et al. 2012).

Potentiation and locomotion

Although potentiation of isometric contractions may be physiologically relevant, the stimulus frequency and shortening speed dependence for potentiation described above has important ramifications for skeletal muscle function in vivo. During locomotion, for example, skeletal muscle must generate concentric work and absorb eccentric work on a cyclic basis (reviewed by Josephson 1993).

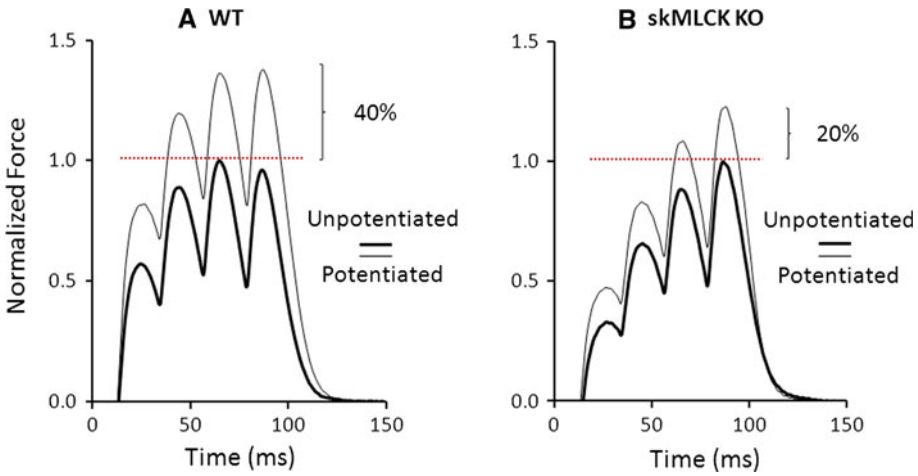

Fig. 4 Influence of muscle shortening on potentiation in mouse muscle. Comparison of concentric force potentiation of EDL muscles from WT (**a**) and skMLCK knockout (**b**) mice. Each panel shows concentric force during shortening at 25 % Vmax while stimulated at 45 Hz for 100 ms before (*thick trace*) and after (*thin trace*) a tetanic conditioning stimulus (4 volleys of 100 Hz stimulation each lasting 400 ms). This maneuver increased mean concentric force by ~40 and by 20 % in the WT and skMLCK knockout muscles, respectively. In each panel, the *horizontal line* depicts peak force level during the tetanus before the conditioning stimulus. Previously unpublished data

Because concentric forces are potentiated in a speed dependent manner it seems possible to suggest that faster speeds of locomotion benefit in two ways: i.e., motive forces produced by agonists may be increased at each stimulus frequency and/or the stimulus frequency range over which motive forces produced by agonists is greatly increased relative to that observed during wholly isometric contractions. This effect would both increase work and power output of potentiated muscles under a wide variety of activation envelopes and also decrease the neural input required to achieve a given submaximal level of muscle work and power (as demonstrated in human skeletal muscles during isometric contractions by Klein et al. 2001; Inglis et al. 2011). On the other hand, because eccentric forces are largely unaffected, the forces required by antagonists may be largely unchanged during cyclic muscle activity typical of locomotion. Interesting in this regard are the results of Childers and McDonald (2004) who used permeabilized rabbit psoas skeletal fibers to show that RLC phosphorylation exacerbated damage caused by active lengthening despite the fact that eccentric forces were not increased.

Fiber phenotype and potentiation

The fiber type dependence for potentiation was first demonstrated by elegant studies showing reduced PTP in muscles exposed to chronic stimulation (Close and Hoh 1969). This fundamental difference is accounted for, at least in part, on the basis of differences in skMLCK and myosin light chain phosphatase (MLCP), the enzyme responsible for dephosphorylating the RLC. In general, skMLCK content is highest in fast-glycolytic and lowest in slow-oxidative fiber types of rodents; on the other hand, MLCP content is highest in slow-oxidative and lowest in fast- glycolytic fibers (Stull et al. 2011). Moreover, chronic stimulation of rabbit tibialis anterior muscle reduces skMLCK expression in a time dependent manner that anticipates attendant changes in myosin heavy chain isoform expression, indicating that the skMLCK enzyme is part of the fast, but not slow, muscle genetic program (Klug et al. 1986, 1992). Phenotypical differences in contractile response to stimulation, highlighting differences of fast and slow muscles from mouse from our laboratory, are shown in Fig. 5.

Myosin heavy chain isoform expression may not singularly predict the capacity for potentiation. For example, although prolonged, high frequency stimulation may produce moderate elevations in RLC phosphorylation, little or no twitch potentiation is observed in rat soleus muscle (Manning and Stull 1982; Moore and Stull 1984). Consistent with this, Ryder et al. (2007) showed that overexpression of skMLCK in soleus muscle did not lead to twitch force potentiation despite stimulation induced phosphorylation of both slow and fast RLCs. The reason for the lack of twitch potentiation with substantial RLC phosphorylation in soleus muscles from transgenic mice is not clear. C57BL/6 mouse EDL muscle contains 70 % type IIb fibers (Gorselink et al. 2002). In contrast, the mouse soleus muscle contains as little as 6 % type IIb fibers with 59 % type IIa and 35 % type I fibers (Totsuka et al. 2003). Thus, the potentiation of contraction is correlated to type IIb fibers. Potentially, the sarcomeric interfilament spacing

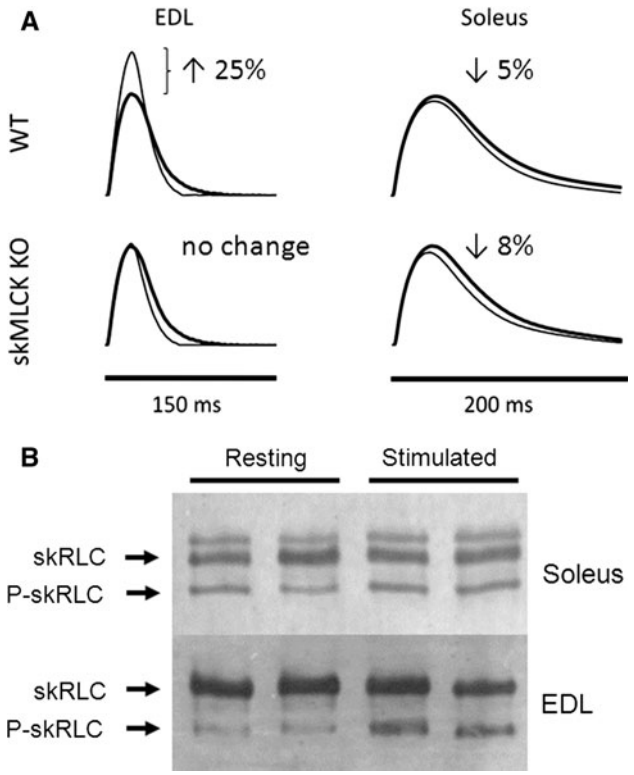

Fig. 5 Phenotype dependence of potentiation in mouse fast and slow skeletal muscle. **a** Comparison of posttetanic responses of EDL (*left*) and soleus (*right*) muscles. (*Top panels*) The effect of a brief tetanic conditioning stimulus (*four volleys* of 100 Hz stimulation, each lasting 400 ms, in a 20 s time window) to cause posttetanic potentiation of EDL and posttetanic depression of soleus muscles from wildtype mice. (*Bottom panels*) The effect of the same conditioning stimulus on EDL and soleus muscles from skMLCK KO mice. In this case PTP of EDL is not evident but PTD of soleus is still present. **b** *Western blots* showing phosphorylation of RLC in wildtype EDL and soleus muscles before and after a tetanic conditioning stimulus. Note that in these examples, the RLC phosphate content of wildtype (WT) EDL muscles was increased significantly from rest by stimulation and PTP was evident. In contrast, the RLC phosphate content of WT soleus muscles was similar before and after stimulation and PTP was absent. The RLC phosphate content of skMLCK KO muscles was not determined in these experiments. *Horizontal arrows* at blots depict unphosphorylated (skRLC) and phosphorylated blots (skRLCP), respectively. Previously unpublished data

of type I and type IIa fibers may be sufficiently small for optimal force development even if RLC is not phosphorylated. It may also be possible to hypothesize that myosin light chain (MLC) isoform expression provides additional regulation. Up to five distinct MLC isoforms are present in mammalian skeletal muscle with the expression of slow (MLC1s, MLC2s) or fast (MLCf, MLC1f, MLC2f, MLC3f) isoforms generally mirroring myosin heavy chain profile (Bicer and Reiser 2004; Gonsalez et al. 2002; Schiaffino and Reggiani 1996, 2011). Interestingly, although it is unclear if alterations to MLC expression can occur independent of myosin heavy chain expression, skeletal muscle plasticity includes the MLC isoform. For example, the slow to fast phenotype transformation of rat muscle shown with hindlimb suspension or clenbuterol administration includes a change in the relative expression of slow to fast MLC isoforms (Bozzo et al. 2003). Moreover, the complementary, reverse, pattern of change to MLC and myosin heavy chain isoform was observed when a fast to slow phenotype transformation was induced (Bozzo et al. 2005). Similar results have been presented by Stevens et al. (2000, 2004). These studies suggest that, although the slow MLC isoforms may still be phosphorylatable, alterations to MLC isoform may participate in the fiber type dependence for RLC phosphorylation—mediated force potentiation. The mechanistic details for this regulation are unknown but it may be possible that RLC isoform composition differentially influences head to head interactions and displacements in the absence and presence of serine phosphorylation, respectively.

Alternate Ca^{2+} based mechanism for potentiation?

Results from skMLCK$^{-/-}$ muscles indicates the presence of a secondary mechanism for potentiation that is highly stimulus regime dependent. In principal there are several mechanisms that may be able to account for this RLC phosphorylation-independent increase in isometric twitch force; clearly, the contribution of these mechanisms to potentiation may be obscured by RLC phosphorylation influences in wildtype muscles. Recent work using mouse lumbrical has provided evidence that stimulation-induced alterations to resting Ca^{2+} homeostasis can account for isometric twitch potentiation in the absence of stimulation-induced elevations in RLC phosphorylation (Smith et al. 2013). Indeed, the mouse lumbrical thus appears to be a unique fast-muscle in that it does not appear to contain the enzymatic apparatus for RLC phosphorylation (Ryder et al. 2007; Smith et al. 2013). In these experiments, stimulation did not produce any apparent change to the amplitude or kinetics of the intracellular $[Ca^{2+}]_i$ transient itself but a short lived (20−30 s) increase in resting myoplasmic $[Ca^{2+}]_i$ was observed, an increase that correlated temporally with the relatively small and short lived isometric twitch potentiation that was observed. The short lived nature of the increase in $[Ca^{2+}]_i$ observed in these experiments could explain why staircase potentiation is less affected than PTP by the absence of RLC phosphorylation (MacIntosh et al. 2008a; Rassier et al. 1999). Although the mechanism of action remains unknown, stimulation induced increases in resting $[Ca^{2+}]_i$ could enhance force by increasing the Ca^{2+} occupancy of troponin C or other Ca^{2+} buffers (e.g., parvalbumin) prior to twitch stimulation (e.g.,

Barclay 1992). On the other hand, the effect of increased resting $[Ca^{2+}]_i$ could be more complex and be mediated at the level of the thick filament, perhaps by increasing the population of weakly-bound cross-bridges, a necessary precursor to the attainment of the strongly-bound, force generating state in some cross-bridge models (Kraft et al. 1999). Clearly, more work is needed to establish how muscle force is potentiated in the absence of RLC as well as the relative contribution of secondary mechanisms to potentiation in muscles with RLC phosphorylation.

Fatigue and potentiation

Twitch force potentiation and fatigue have been demonstrated to exist in a variety of single fiber and whole skeletal muscle preparations (Gordon et al. 1990; MacIntosh and Gardiner 1987; MacIntosh and Kupsh 1987; MacIntosh et al. 1993; Rankin et al. 1988; Tubman et al. 1996a; Vandenboom and Houston 1996; Vergara et al. 1977). This may suggest that RLC phosphorylation offsets fatigue or that certain metabolites arising during repetitive stimulation are able to potentiate twitch force. Interestingly, the relative depression in twitch force observed during the early, but not late, stages of repetitive stimulation of mouse EDL muscle is greater in $skMLCK^{-/-}$ than in wildtype muscles (Gittings et al. 2011). Although this result seems to corroborate a role for RLC during moderate, but not severe fatigue, it does not eliminate the contribution of other intracellular mechanisms. As an example, Barclay (1992) used mouse EDL muscles (25 °C) to show that the enhanced rate of force development observed simultaneous with fatigue could be attributed to increases in intracellular levels for inorganic phosphate ([Pi]). Increased [Pi] may influence cross-bridge kinetics in such a way that the rate of rise of isometric force is increased coincident with fatigue (e.g., Hibberd et al. 1985). Although plausible, this mechanism is difficult to isolate experimentally from other mechanisms, including RLC phosphorylation, in fatigued wildtype muscle.

Cooke and colleagues have performed a series of studies examining the complex interactions that may occur between metabolic changes during fatigue and RLC phosphorylation (Cooke 2007). It could be noted that, in unfatigued skeletal muscle, RLC phosphorylation is expected to influence pre—but not post power—stroke steps in the cross-bridge cycle (i.e. Sweeney and Stull 1990). Work by Franks-Skiba et al. (2007) and Karatzaferi et al. (2008) both suggest, however, that when the metabolites $[P_i]$ and/or $[H^+]$ are elevated to levels that mimic those observed in fatigued skeletal muscle, the rate of release of ADP following the power stroke is delayed in phosphorylated cross-bridges (*c.f.*, Stewart et al. 2009). This mechanism is in fact supported by studies on individual myosin molecules using the in vitro motility assay technique under non-fatigue conditions (Greenberg et al. 2010). It is of interest that these results could account for both the augmented twitch force and/or the slowed shortening velocity sometimes observed during and attributed to fatigue. Interestingly, no differences in either peak tetanic force or unloaded shortening velocity that are expected from these interactions were noted between skMLCK knockout and wildtype mouse muscles during severe fatigue at 25 °C (Gittings et al. 2011). A possible explanation for this discrepancy is that the interaction between fatigue related changes in metabolites and RLC phosphorylation in permeabilized fibers were found to be highly temperature dependent, with little interaction noted below 30 °C. Further work on intact muscle at higher temperatures (e.g., 30 °C) is perhaps needed to clarify this issue.

RLC phosphorylation and metabolism

The physiological ability of the RLC phosphorylation mechanism to modulate skeletal muscle function in vivo may hinge upon its influences on the metabolic cost or efficiency of muscle contraction. For example, if RLC phosphorylation (or other mechanisms) increases the energetic cost of contraction, potentiated contractions may prove to be unsustainable metabolically. Unfortunately, studies in this area have been equivocal, however. For example, early studies were interpreted to suggest that phosphorylation of the RLC decreased energetic demand during sustained or repetitive contractions of mouse EDL muscle in vitro (Crow and Kushmerick 1982a, b). Subsequent work also using a mouse muscle model questioned this outcome (Barsotti and Butler 1984; Butler et al. 1983). More recently, Abbate et al. (2001) have re-examined the issue by studying the energetic cost of potentiated contractions in rat muscle. These authors reported that high energy phosphate turnover was increased to a greater extent than was force, work and power, thus decreasing muscle economy of potentiated versus unpotentiated contractions. Critically, the decreased economy was attributed to differences in force, not RLC phosphorylation, implying that potentiated contractions may not be metabolically sustainable (Abbate et al. 2001). Although this result would seem to have resounding implications for the physiological utility of RLC phosphorylation to modulate repetitive type contractions, the robustness of this decrease to different contractile conditions (i.e. temperature, species, fiber type, stimulation rate, shortening speed etc.) is as yet unknown.

Potentiation in diseased muscle

Most work examining potentiation has been performed using non-diseased, healthy animal skeletal muscles. Exceptions to this trend suggest that certain peripheral diseases may influence potentiation, however. For example, Krarup (1983) showed that both staircase and PTP were attenuated in a neuropathy rat muscle model of chronic *myasthenia gravis*. In addition, potentiation has been shown to be reduced in parallel with disuse and/or atrophy of rat fast twitch muscle (MacIntosh et al. 1988; Tubman et al. 1996b, 1997). Another example is work by Smith et al. (2010) who showed that a high frequency conditioning stimulus caused greater PTP in EDL muscles from *mdx* mice than in EDL muscles from age matched controls, despite similar RLC phosphorylation at both ages and muscle types (see also Hoekman 1977). Thus, more information regarding interactions between potentiation and peripheral diseases such as sarcopenia and/or cachexia is required before a full understanding of how this fundamental response is altered in disease is achieved.

Summary

The different fiber types comprising skeletal muscles display a range of metabolic and mechanical properties geared to optimize function. Prominent among these differences is that of force potentiation, a history dependent enhancement of dynamic force, work and power at physiological activation rates in mammalian fast twitch muscle. Evidence from a variety of sources points to stimulation-induced elevations in RLC phosphate incorporation by skMLCK as the primary mechanism for potentiation, although stimulation-induced elevations in resting $[Ca^{2+}]_i$ may provide a secondary mechanism in some muscle fibers. Evidence from skMLCK knockout and disuse models indicates that staircase potentiation observed during prolonged low frequency stimulation may be due to both RLC phosphorylation and alterations in resting $[Ca^{2+}]_i$ while PTP observed after brief or high frequency stimulation is predominantly due to RLC phosphorylation. Further investigations are required to resolve the relative contribution of these respective mechanisms to potentiation phenomena.

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