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Research Article

Myotonic dystrophy (DM), the most prevalent muscular disorder in adults, is caused by (CTG)n-repeat expansion in a gene encoding a protein kinase (DM protein kinase; DMPK) and involves changes in cytoarchitecture and ion homeostasis. To obtain clues to the normal biological role of DMPK in cellular ion homeostasis, we have compared the resting [Ca2+]i, the amplitude and shape of depolarization-induced Ca2+ transients, and the content of ATP-driven ion pumps in cultured skeletal muscle cells of wild-type and DMPK[-/-] knockout mice. In vitro-differentiated DMPK[-/-] myotubes exhibit a higher resting [Ca2+]i than do wild-type myotubes because of an altered open probability of voltage-dependent l-type Ca2+ and Na+ channels. The mutant myotubes exhibit smaller and slower Ca2+ responses upon triggering by acetylcholine or high external K+. In addition, we observed that these Ca2+ transients partially result from an influx of extracellular Ca2+ through the l-type Ca2+ channel. Neither the content nor the activity of Na+/K+ ATPase and sarcoplasmic reticulum Ca2+-ATPase are affected by DMPK absence. In conclusion, our data suggest that DMPK is involved in modulating the initial events of excitation-contraction coupling in skeletal muscle.

Myotonic Dystrophy Protein Kinase is Involved in the Modulation of the Ca^{2+} Homeostasis in Skeletal Muscle Cells

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Abstract

Myotonic dystrophy (DM), the most prevalent muscular disorder in adults, is caused by $(CTG)_n$-repeat expansion in a gene encoding a protein kinase (DM protein kinase; DMPK) and involves changes in cytoarchitecture and ion homeostasis. To obtain clues to the normal biological role of DMPK in cellular ion homeostasis, we have compared the resting $[Ca^{2+}]_i$, the amplitude and shape of depolarization-induced Ca^{2+} transients, and the content of ATP-driven ion pumps in cultured skeletal muscle cells of wild-type and DMPK[−/−] knockout mice.

In vitro–differentiated DMPK[−/−] myotubes exhibit a higher resting $[Ca^{2+}]_i$ than do wild-type myotubes because of an altered open probability of voltage-dependent L-type Ca^{2+} and Na^+ channels. The mutant myotubes exhibit smaller and slower Ca^{2+} responses upon triggering by acetylcholine or high external K^+. In addition, we observed that these Ca^{2+} transients partially result from an influx of extracellular Ca^{2+} through the L-type Ca^{2+} channel. Neither the content nor the activity of Na^+/K^+ ATPase and sarcoplasmic reticulum Ca^{2+}-ATPase are affected by DMPK absence.

In conclusion, our data suggest that DMPK is involved in modulating the initial events of excitation–contraction coupling in skeletal muscle. (*J. Clin. Invest.* 1997. 100:1440–1447.) Key words: excitation-contraction coupling • ion channels • ion pumps • knockout mouse • cultured myotubes

Introduction

Myotonic dystrophy (DM)[1] follows an autosomal dominant inheritance, and is characterized by a wide variety of symptoms, including increased excitability and delayed relaxation of muscle, muscle weakness and wasting, ocular cataract, sensorineural deafness, cardiac conduction defects, hypersomnia, testicular atrophy causing male sterility, and endocrine dysfunction (1). Clinical manifestation of this rather frequent disorder is caused by expansion of an unstable CTG-repeat in the 3′-untranslated region of a gene encoding the myotonic dystrophy protein kinase (DMPK), with the age of onset and the severity of the disease being correlated to the extent of expansion (2–4).

Contradictory results have been obtained with regard to the effects of abnormally long CTG repeats on DMPK mRNA and protein levels in patient tissues (5–8). In addition, the study of transgenic mouse models with altered DMPK levels (9, 10) has not provided the answer on the question whether over- or underexpression of this protein is involved in disease etiology. Homozygous DMPK[−/−] mice exhibit only minor changes in neck muscle fibres at older age, whereas animals carrying multiple copies of the DMPK transgene show hypertrophic cardiomyopathy and enhanced neonatal mortality as the only features.

Typically, muscle fibers and/or cultured skeletal muscle cells of DM patients exhibit a decreased resting membrane potential (11–13) and increased basal cytosolic Na^+ and Ca^{2+} concentrations (14–16). These features may be attributed to anomalies in the functioning of voltage-operated Na^+ channels (17, 18) and Ca^{2+} channels (15, 16) and/or to a reduced content of Na^+/K^+-ATPase and sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (19, 20). In addition, the persistence of an apamin-receptor, i.e. Ca^{2+}-activated K^+ channel, has been demonstrated (21).

Many of these ion channels and ion pumps are key players in the excitation–contraction (E-C) coupling mechanism of skeletal muscle in vivo, a cascade of events in which, sequentially, acetylcholine receptors (AChR) of the neuromuscular junction, sarcolemmal tetrodotoxin-sensitive voltage-operated Na^+ channels (TTXR), T-tubular dihydropyridine receptors (DHPR), and finally ryanodine receptors (RyR), i.e. Ca^{2+} release channels in the terminal cisternae of the SR, are involved (22). E-C coupling of vertebrate skeletal muscle is thought to occur by a mechanical coupling, in which intramembrane charge movements and associated conformational changes of the voltage-sensing DHPR activate the RyR without entry of external Ca^{2+} through DHPR (23–25). Before relaxation, the disturbed ion concentrations are restored to resting levels by ATP-driven ion pumps, i.e., Na^+/K^+-ATPase and SR Ca^{2+}-ATPase.

This whole process is subject to complex regulation in which local luminal and cytosolic Ca^{2+} and ATP levels are in-

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1. *Abbreviations used in this paper:* ACh(R), acetylcholine (receptor); DHPR, dihydropyridine receptor; DM, myotonic dystrophy; DMPK, myotonic dystrophy protein kinase; E-C, excitation-contraction; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; TTX(R), tetrodotoxin (receptor); τ_d, half-decay time; τ_i, half-increase time; 3-*O*-MFPase, 3-*O*-methylfluorescein phosphatase.

volved. It is also influenced by protein phosphorylation and ion channel–modulating proteins like FK506, triadin, or calsequestrin. Since dysregulation of ion fluxes is a likely determining factor in the abnormal cellular functions in DM, and since DMPK-mediated protein phosphorylation could play a role, we examined if and how DMPK deficiency affects the depolarization behavior and Ca^{2+} homeostasis in cultured skeletal muscle cells in the absence or presence of specific inhibitors of the voltage-operated ion channels or the SR Ca^{2+} release channel. We also determined the activity and content of SR Ca^{2+}-ATPase and Na^+/K^+-ATPase in cultured muscle cells and skeletal muscle of wild-type and DMPK[−/−] mice. Although the morphological and physiological effects of DMPK absence at the animal level are surprisingly mild, we demonstrate here, that DMPK deficiency has conspicuous consequences at the cellular level. Our finding of abnormally operating TTXRs and DHPRs gives evidence for a relation between DMPK activity and ion homeostasis in skeletal muscle.

Methods

Materials. The acetoxymethyl ester of Fura-2 (Fura-2/AM) was purchased from Molecular Probes Europe (Leiden, The Netherlands); acetylcholine chloride (ACh), tetrodotoxin (TTX), ryanodine and ionomycin were from Sigma (St. Louis, MO, USA); and nifedipine from Bayer (Leverkusen, Germany). Sources of other materials were described previously (20, 26).

DMPK-deficient mice. Homozygous DMPK-deficient (DMPK[−/−]) mice were generated by targeted mutagenesis and genotyped by PCR analysis as described (9). DMPK[−/−] animals from the F3 generation (on a mixed C57BL/6 × 129/OLA 50-50% background) were used. Wild-type animals from the C57BL/6 inbred strain, and from a colony with a C57BL/6 × 129/OLA mixed background were taken as controls.

Cytosolic Ca^{2+} concentration measurement in cultured skeletal muscle cells. Hind leg muscles of 3–10-d-old wild-type and DMPK[−/−] mice were dissociated, and the isolated satellite cells were allowed to proliferate for 2 d, and were then cultured under differentiation-promoting conditions for 4 d on collagen-coated glass coverslips (ϕ 25 mm) in serum-containing media, as described for human muscle cells (26). The free cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) and the effects of depolarization induced by the addition of 20 μM ACh or 125 mM KCl on $[Ca^{2+}]_i$ were measured in single cells with Fura-2 using conventional fluorescence video microscopy (26). For calibration, Ca^{2+}-saturated or Ca^{2+}-free dye was set by 2 μM ionomycin in the presence of 1.8 mM Ca^{2+} (pH 7.5) or 20 mM EGTA (pH 8.0), respectively. Under these conditions the myotubes remain attached to the coverslip. Although the calibration parameters of wild-type and DMPK[−/−] skeletal muscle cells did not differ significantly, we used these parameters strictly separated for calculation of $[Ca^{2+}]_i$ to rule out cell-type–dependent artefacts (27). The half-increase (τ_i) and half-decay time (τ_d) of the depolarization-triggered Ca^{2+} responses were analyzed as described (26, 28).

SR Ca^{2+}-ATPase and Na^+/K^+-ATPase. The activity and concentration of SR Ca^{2+}-ATPase were examined in homogenates of cultured muscle cells (differentiated for 4 d) and hind leg skeletal muscle of adult mice by measuring Ca^{2+}-dependent ATP hydrolysis and steady-state phosphorylation, respectively (20). The activity of Na^+/K^+-ATPase was assayed as the K^+-dependent, ouabain-sensitive 3-*O*-methylfluorescein phosphatase (3-*O*-MFPase) activity and the content of Na^+/K^+-ATPase was derived from the binding capacity of [^{3}H]ouabain (20).

Other procedures. Creatine kinase (CK) activity of cultured muscle cells (differentiated for 4 d) was determined with the CK *N*-acetylcysteine-activated monotest (26). For determining the percentage CK muscle-specific isoenzyme MM (CK-MM), this isoenzyme was separated from the other CK isoenzymes by gel electrophoresis (29). The protein content was assayed according to Lowry et al. (30) with BSA as a standard.

Statistics. Data represent means±SD. Statistical analysis was performed by means of the unpaired Student's *t* test, and significance was set at $P < 0.05$.

Results

General characteristics of cultured wild-type and DMPK[−/−] skeletal muscle cells. Upon differentiation, in vitro skeletal muscle cells derived from wild-type and DMPK-deficient mice exhibit a similar morphological appearance. Typically, the diameter of these cells is ∼ 10–20 μm, whereas their length varies between 500 and 700 μm. Also, the total CK activity of wild-type (1.93±0.59 U/mg protein; $n = 11$) and mutant cells (1.84±0.84 U/mg protein; $n = 5$) and the percentage of CK-MM, a measure for the overall maturation grade, are the same for both cell types (wild-type: 33.8±10.1; $n = 11$, and DMPK[−/−]: 37.1±5.8; $n = 5$). Spontaneous contractions of wild-type and DMPK[−/−] myotubes are occasionally observed in culture, and occurred frequently upon agonist-induced depolarization in both cell types.

Ca^{2+} homeostasis of wild-type skeletal muscle cells. The resting $[Ca^{2+}]_i$ of differentiated wild-type mouse skeletal muscle cells in culture is 120 nM (Table I). Depolarization of the sarcolemma of these cells either by 20 μM ACh or by 125 mM KCl, induces Ca^{2+} transients with an amplitude ($[Ca^{2+}]_{i,max}$) of ∼ 0.8 μM (Fig. 1) and a τ_i and τ_d of ∼ 3 and 5 s, respectively. The provoked Ca^{2+} responses are completely prevented by inhibition of the SR Ca^{2+} release channel (RyR) using 10 μM ryanodine (Fig. 2, *B* and *C*). Thus, Ca^{2+} release from the SR is entirely responsible for the increase of the $[Ca^{2+}]_i$, indicating that the wild-type myotubes possess a skeletal muscle type of E-C coupling mechanism (28, 31). As anticipated, also nifedipine (5 μM), a blocker of DHPR, impedes the depolarization-induced Ca^{2+} transients (Fig. 3, *B* and *C*). Inhibition of TTXR by 5 μM tetrodotoxin only partially suppresses depolarization by ACh (Fig. 4 *B*). This inhibition is incomplete due to the coexistence of TTX-sensitive and TTX-resistant volt-

Table I. Ca^{2+} Transients Induced by ACh or KCl in Cultured Skeletal Muscle Cells From Wild-type and DMPK[−/−] Mice

Stimulus	Parameter	Wild-type	DMPK[−/−]
None	$[Ca^{2+}]_{i,basal}$	122±17	185±24*
		(170)	(63)
20 μM ACh	$[Ca^{2+}]_{i,max}$	775±131	511±124*
	τ_i	3.0±0.6	6.6±1.9*
	τ_d	4.6±0.8	8.4±2.1*
		(84)	(38)
125 mM KCl	$[Ca^{2+}]_{i,max}$	881±164	552±130*
	τ_i	3.2±0.7	6.1±1.7*
	τ_d	4.8±0.7	7.9±2.8*
		(86)	(25)

Values are means±SD of the number of muscle cells examined (listed between parentheses) from at least four individual cultures. The valves of $[Ca^{2+}]_i$ are expressed in nM, and the half-increase (τ_i) or half-decay time (τ_d) in s. Parameters of DMPK[−/−] and wild-type cells differ with $^*P < 0.01$.

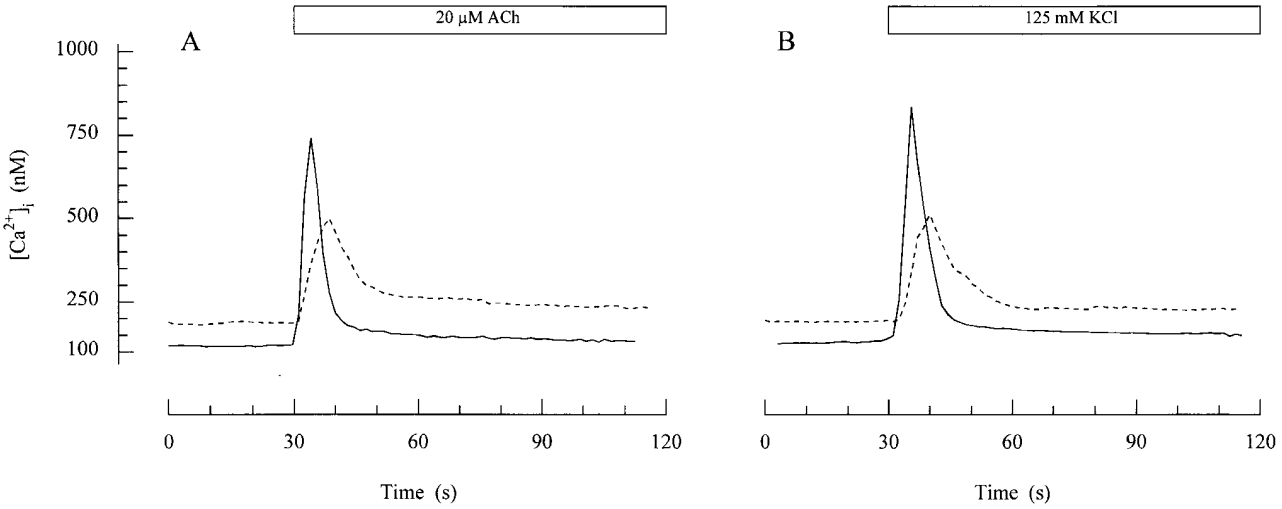

Figure 1. Depolarization-induced Ca^{2+} transients in cultured skeletal muscle cells derived from wild-type (*solid line*) and DMPK[−/−] mice (*dashed line*). Depolarization was generated by 20 μM ACh (*A*) or 125 mM KCl (*B*) as marked by the bars. Traces show the average $[Ca^{2+}]_i$ after superimposing all appropriate experiments as listed in Table I.

age-operated Na^+ channels in cultured skeletal muscle cells (28, 32). The K^+-triggered Ca^{2+} transients are not affected by tetrodotoxin (Fig. 4 *C*), as expected. It is important to note that neither of the three inhibitors have any effect on the resting $[Ca^{2+}]_i$ (Figs. 2 *A*, 3 *A*, and 4 *A*).

Depolarization of the cultured muscle cells in the absence of extracellular Ca^{2+} could not be performed, since the omission of external Ca^{2+} before depolarization provokes a Ca^{2+} transient (data not shown). As a result, the SR becomes at least partially depleted for Ca^{2+}, which in turn can affect the kinetics of the SR Ca^{2+} release channel (33). In addition, the DHPR may become inactivatable due to a loss in occupancy of its priming site (23, 25).

Ca^{2+} homeostasis of DMPK[−/−] skeletal muscle cells. Surprisingly, the resting $[Ca^{2+}]_i$ (185 nM) of DMPK[−/−] skeletal muscle cells in culture is significantly higher than in wild-type cells (Table I, Fig. 1). Ryanodine does not affect this elevated level (Fig. 2 *A*), but nifedipine and tetrodotoxin normalize the increased resting $[Ca^{2+}]_i$ completely or partially, respectively (Figs. 3 *A* and 4 *A*). This means that DHPRs and TTXRs are constitutively open in resting mutant skeletal muscle cells, unlike in wild-type cells. Strikingly, the amplitudes of the Ca^{2+} responses provoked by depolarization with ACh or KCl are reduced by about 40%, whereas τ_i and τ_d are increased 2- and 1.6-fold, respectively (Fig. 1). We observed that ryanodine reduces the depolarization-induced Ca^{2+} responses in DMPK[−/−] cells by only 90% (Fig. 2, *B* and *C*), whereas complete blocking is achieved with nifedipine (Fig. 3, *B* and *C*). This observation indicates that the Ca^{2+} transients in these cells partially result from an influx of extracellular Ca^{2+} through the DHPRs. Tetrodotoxin incompletely inhibits ACh-evoked Ca^{2+} transients like in wild-type cells (Fig. 4 *B*). After inhibition of TTXR by tetrodotoxin, when the basal $[Ca^{2+}]_i$ in DMPK[−/−] cells has become lowered, the KCl-generated Ca^{2+} responses are both higher and faster than in the absence of tetrodotoxin. The average amplitude of the Ca^{2+} transients raises ∼ 140% and τ_i and τ_d are reduced 1.5- and 1.6-fold, respectively, and become similar to the values in wild-type cells

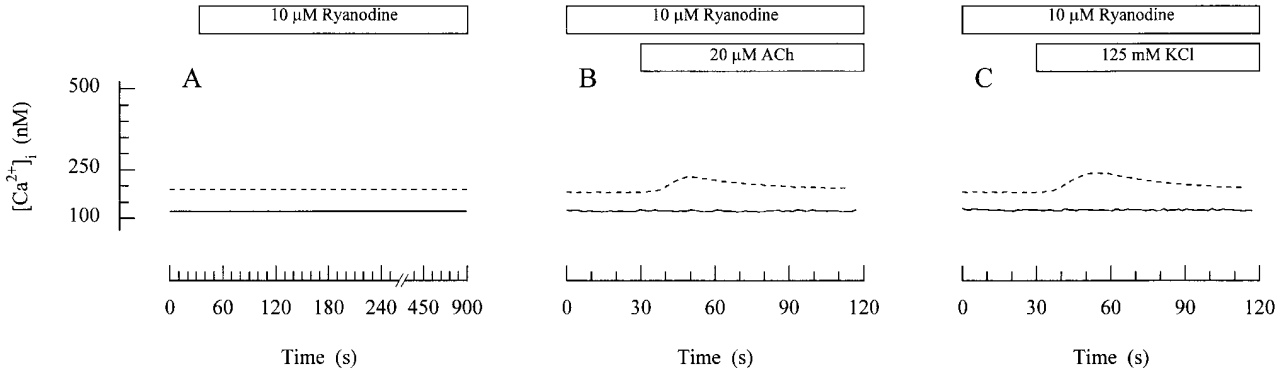

Figure 2. Effects of ryanodine on resting $[Ca^{2+}]_i$ (*A*) and depolarization-provoked Ca^{2+} responses (*B* and *C*) in cultured wild-type and DMPK[−/−] muscle cells. Muscle cells were incubated with 10 μM ryanodine for 30 min before depolarization (*B* and *C*). Traces show the mean $[Ca^{2+}]_i$ after superimposing all relevant experiments as given in Table II. Ryanodine does not normalize the higher resting $[Ca^{2+}]_i$ of DMPK[−/−] cells (*A*), and only partially inhibits depolarization-induced Ca^{2+} transients (*B* and *C*).

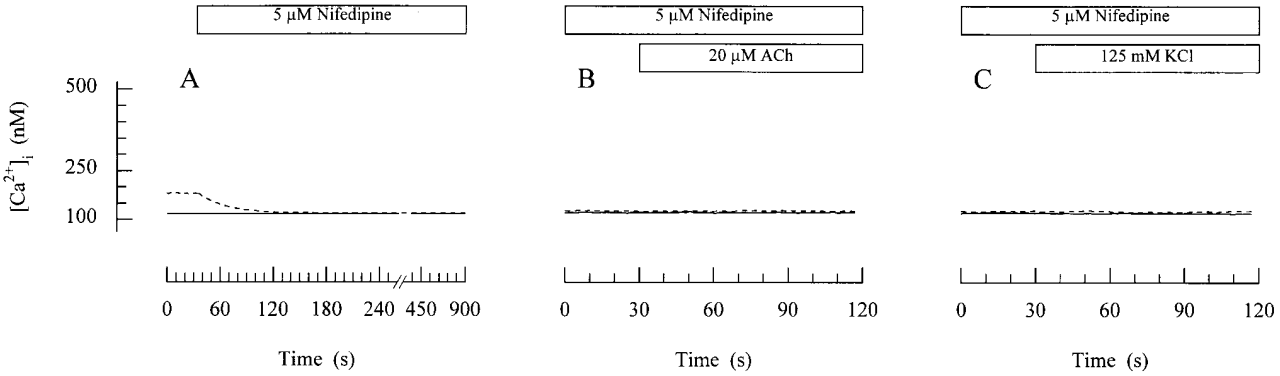

Figure 3. Effects of nifedipine on resting $[Ca^{2+}]_i$ (*A*) and depolarization-generated Ca^{2+} transients (*B* and *C*) in cultured wild-type and DMPK[−/−] muscle cells. Muscle cells were incubated with 5 μM nifedipine for 30 min before depolarization (*B* and *C*). For other details, see legend to Fig. 2. Nifedipine completely normalizes the higher resting $[Ca^{2+}]_i$ of DMPK[−/−] cells (*A*).

(compare Figs. 1 *B* and 4 *C*). Since these latter observations suggest a $[Ca^{2+}]_i$-regulated fine-tuning of depolarization-induced Ca^{2+} responses, we investigated whether there was any relation between $[Ca^{2+}]_i$ and any of the parameters that describe the shape and the magnitude of the Ca^{2+} transients in both cell types. The calculations clearly demonstrate that the resting $[Ca^{2+}]_i$ inversely correlates with $[Ca^{2+}]_{i,max}$ (Fig. 5 *A*), and exhibits a high correlation with τ_i (Fig. 5 *B*). In addition, an inverse correlation exists between $[Ca^{2+}]_{i,max}$ and τ_d (Fig. 5 *C*).

The statistical evaluations of the depolarization-induced Ca^{2+} transients of both wild-type and mutant skeletal muscle cells in the absence or presence of ion channel–specific inhibitors are listed in Tables I and II, respectively, and sustain the described phenomena.

SR Ca^{2+}-ATPase and Na^+/K^+-ATPase. The here observed Ca^{2+} homeostasis of cultured DMPK[−/−] mouse muscle cells has the same characteristics as in cultured skeletal muscle cells derived from DM patients (15, 16). Since muscle and cultured muscle cells of patients have been shown to exhibit a lowered activity of both SR Ca^{2+}-ATPase and Na^+/K^+-ATPase due to a reduction of their content (20), we also examined these ATP-driven ion pumps in our wild-type and mutant mice. As for human (20), the activity and the concentration of SR Ca^{2+}-ATPase as well as the K^+-dependent, ouabain-sensitive 3-*O*-MFPase activity and the ouabain-binding capacity, i.e., the number of Na^+/K^+-ATPase molecules, are lower in cultured mouse muscle cells than in mouse whole hind limb muscle, but the molecular activities do not differ (Table III). Furthermore, pairwise comparison between the two types of cultured muscle cells or skeletal muscle of wild-type and DMPK[−/−] mice do not reveal any difference in the activity or content of SR Ca^{2+}-ATPase and Na^+/K^+-ATPase (Table III).

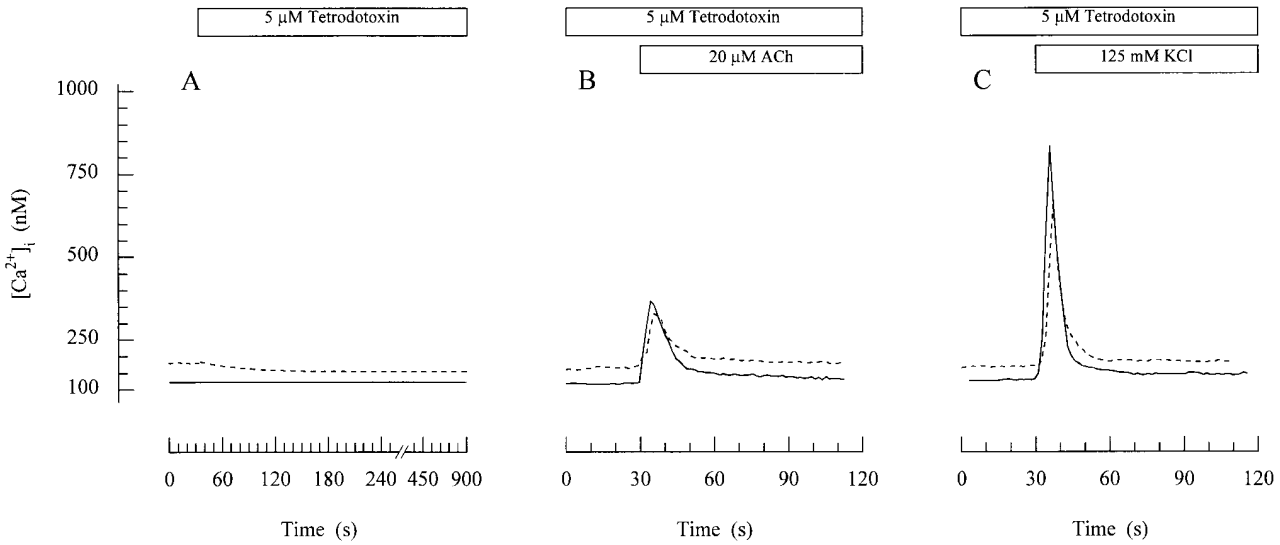

Figure 4. Effects of tetrodotoxin on resting $[Ca^{2+}]_i$ (*A*) and depolarization-elicited Ca^{2+} responses (*B* and *C*) in cultured wild-type and DMPK[−/−] muscle cells. Muscle cells were preincubated with 5 μM tetrodotoxin for 30 min before depolarization (*B* and *C*). For other details, see legend to Fig. 2. Tetrodotoxin partially normalizes the higher resting $[Ca^{2+}]_i$ of DMPK[−/−] cells (*A*).

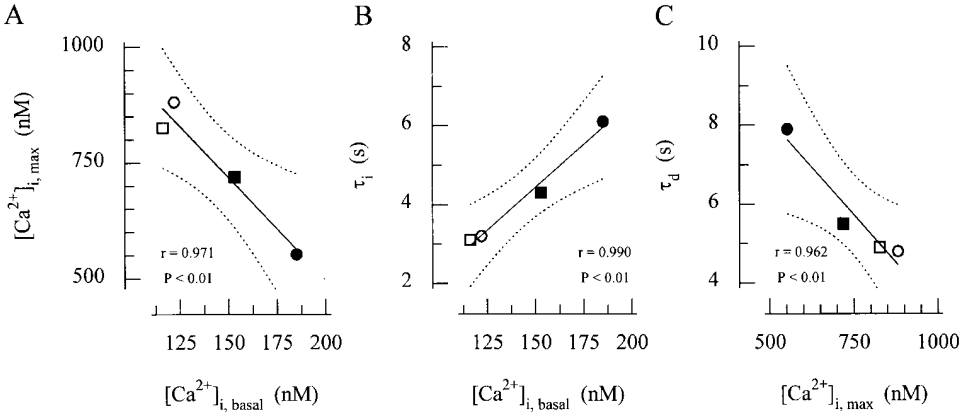

Figure 5. $[Ca^{2+}]_i$-regulated fine-tuning of Ca^{2+} responses in cultured wild-type (*open symbols*) and DMPK[−/−] skeletal muscle cells (*solid symbols*). The relation is shown for the mean values of $[Ca^{2+}]_{i,rest}$ and $[Ca^{2+}]_{i,max}$ (*A*) or τ_i (*B*), and for $[Ca^{2+}]_{i,max}$ and τ_d (*C*). Data originate from Ca^{2+} transients induced by 125 mM KCl in the absence (○, ●) and presence of tetrodotoxin (□, ■). The *solid lines* represent the regression curves, and the *dotted lines* mark the 99% confidence intervals.

Discussion

The focus of interest in DM research has slowly drifted from the behavior of the instable $(CTG)_n$ repeat to the actual function(s) of individual genes in the mutant chromosome 19q area. Three candidate genes for the DM locus have now been characterized: DMR-N9, DMPK, and DMAHP (34, 35). As the expanding repeat in DM actually interrupts the gene for DMPK, most efforts in explaining disease etiology have been directed towards the involvement of the product(s) of this single gene. The results presented in this paper provide novel evidence that there is a specific relationship between DMPK activity and Ca^{2+} homeostasis in skeletal muscle cells. Although results from DMPK-cDNA transfection studies in BC_3H1 cells pointed to a function of DMPK in the myogenic pathway (36), we conclude from study of the close-to-natural context presented here, that the sole absence of DMPK does not appear to interfere with the growth and differentiation profiles of muscle-derived satellite cells in vitro. Neither the morphological appearance, nor the maturation grade, or the ability to contract, differed between wild-type and mutant cells. Moreover, our observations are in accordance with the absence of any gross morphological and structural modifications in vivo in mice that lack or overexpress the DMPK gene (9, 10).

Table II. Effects of E-C Coupling Inhibitors on ACh- and KCl-induced Ca^{2+} Transients in Cultured Skeletal Muscle Cells From Wild-type and DMPK[−/−] Mice

Inhibitor	Stimulus	Parameter	Wild-type	DMPK [−/−]
Ryanodine (10 μM)	ACh	$[Ca^{2+}]_{i,basal}$	119±21	182±38*
		$[Ca^{2+}]_{i,max}$	119±21	251±31*
			(40)	(21)
	KCl	$[Ca^{2+}]_{i,basal}$	120±24	179±39*
		$[Ca^{2+}]_{i,max}$	120±24	248±31*
			(28)	(17)
Nifedipine (5 μM)	ACh	$[Ca^{2+}]_{i,basal}$	120±23	127±33
		$[Ca^{2+}]_{i,max}$	120±23	127±33
			(42)	(19)
	KCl	$[Ca^{2+}]_{i,basal}$	121±20	125±29
		$[Ca^{2+}]_{i,max}$	121±20	125±29
			(52)	(32)
Tetrodotoxin (5 μM)	ACh	$[Ca^{2+}]_{i,basal}$	120±23	155±36*
		$[Ca^{2+}]_{i,max}$	378±56	351±67
			(29)	(23)
	KCl	$[Ca^{2+}]_{i,basal}$	116±25	153±40*
		$[Ca^{2+}]_{i,max}$	826±196	719±234‡
			(48)	(45)

Values expressed in nM, are means±SD of the number of muscle cells (listed between parentheses) from at least three individual cultures. Concentrations of ACh and KCl are 20 μM and 125 mM, respectively. Parameters of DMPK[−/−] and wild-type cells differ with $^*P < 0.01$ and $^‡P < 0.05$. In the presence of tetrodotoxin τ_i of the KCl-evoked Ca^{2+} responses in wild-type and mutant cells are 3.1±0.9 s and 4.2±1.3 s ($P < 0.01$), whereas τ_d are 4.9±1.1 s and 5.5±1.5 s ($P < 0.05$), respectively.

The behavior of Ca^{2+} homeostasis in cultured muscle cells depends on DMPK activity. Estimates for resting $[Ca^{2+}]_i$ values of mouse skeletal muscle cells in culture clearly depend on the cell origin, the Ca^{2+} indicator used for assaying, and the calibration procedure applied. All we can say is that our value for the resting $[Ca^{2+}]_i$ in cultured muscle cells of wild-type mice, based on the in vivo calibration of Fura-2, falls well within the range of values published for cultured mouse myotubes (37) and myofibers in vivo (38). Since inhibition of the SR Ca^{2+} release channel by ryanodine effectively blocks depolarization-induced Ca^{2+} efflux in cultured wild-type cells (Fig. 2, *B* and *C*), these cells, like those of rats and humans, must possess a skeletal muscle type of E-C coupling mechanism, rather than a cardiac muscle type, in which Ca^{2+} release is provoked by an influx of external Ca^{2+} (28, 31). Moreover, it suggests that the contribution of a Ca^{2+} influx through the ion-unselective AChR (39), the dihydropyridine-insensitive fast-activated voltage-operated T-type Ca^{2+} channel (40), and/or the slow-activated voltage-dependent L-type Ca^{2+} channel, i.e., DHPR (41), during depolarization is negligibly small, and/or is ineffective to induce Ca^{2+} release from the SR. This does not rule out that Ca^{2+} entry via the DHPR may play a role in long-lasting depolarizations in wild-type cells, but usually this process is slower than the charge movement in DHPR and the associated activation of the RyR (41).

The situation in mutant myotubes is clearly different in that absence of DMPK augments the resting $[Ca^{2+}]_i$, and has dampening effects on the release and sequestration of Ca^{2+} upon depolarization. Application of inhibitors showed that the phenomenon of higher resting $[Ca^{2+}]_i$ and the altered excitability are somehow linked to the mode of action of voltage-dependent Ca^{2+} and Na^+ channels. It is unlikely that a recently described Ca^{2+}-specific leak channel is involved, as the situation

Table III. SR Ca^{2+}-ATPase and Na^+/K^+-ATPase in Muscle and Cultured Muscle Cells of Wild-type and DMPK[−/−] Mice

Parameters	Muscle		Cultured muscle cells	
	Wild-type	DMPK[−/−]	Wild-type	DMPK[−/−]
SR Ca^{2+}-ATPase activity (mU/mg protein)	76.2±7.4 (5)	75.9±4.5 (3)	13.1±2.3 (8)	13.3±1.8 (4)
SR Ca^{2+}-ATPase content (pmol/mg protein)	94.9±8.0 (5)	94.7±5.8 (3)	16.4±2.8 (8)	16.5±2.0 (4)
Molecular activity (min^{-1})	802±14 (5)	801±9 (3)	800±8 (8)	803±13 (4)
Na^+/K^+-ATPase activity (mU/mg protein)	0.57±0.06 (5)	0.56±0.08 (3)	0.93±0.09 (7)	0.95±0.13 (4)
Na^+/K^+-ATPase content (pmol/mg protein)	6.3±0.6 (5)	6.2±0.2 (3)	10.1±0.7 (5)	10.4±1.6 (4)
Molecular activity (min^{-1})	91±7 (5)	89±11 (3)	92±8 (5)	91±8 (4)

Values are means±SD of the number of muscles or muscle cell cultures (given between parentheses). The activity and content of SR Ca^{2+}-ATPase were determined by Ca^{2+}-dependent ATP hydrolysis and steady-state phosphorylation, respectively. The K^+-dependent, ouabain-sensitive hydrolysis of 3-*O*-MFP and the binding capacity of ouabain were used as a measure for the activity and content of Na^+/K^+-ATPase, respectively.

is completely normalized by nifedipine, which fails to inhibit this leak channel (42). Our mutant myotubes lack a protein kinase that is closely related to the subfamilies of cAMP-dependent protein kinases and protein kinases C (2, 43). Hence, it is most likely that the phosphorylation status of the Ca^{2+} and/or Na^+ channels or any other protein that mediates the clustering of ion channels in the sarcolemma is modified in DMPK[−/−] myotubes. In turn, this may change the gating properties of these channels and/or influence the distribution and regulation of voltage-dependent SR Ca^{2+} release events (44). Importantly, phosphorylation of both the α_1 and β subunits of the DHPR is known to occur. Phosphorylation of the α_1 subunit leads to an increase of the Ca^{2+} current (45), and we would expect absence of phosphorylation to reduce this current, a feature that is inconsistent with the observed high $[Ca^{2+}]_i$ in resting DMPK[−/−] cells. Perhaps more relevant is that recombinant-DMPK can use the β-subunit as a substrate in vitro (46), although the precise effects of phosphorylation of this subunit, which regulates the α_1-subunit activity (47), are unknown. Similarly interesting is that phosphorylation of the α subunit of the TTX-sensitive Na^+ channel in cultured mouse and rat muscle cells by protein kinase C and in transfected oocytes by DMPK leads to a reduction of the Na^+ current (48, 49). A persistent influx of Na^+ as a consequence of DMPK absence may lead to mild long-lasting depolarization of the sarcolemma, and results in a more frequent opening of the L-type Ca^{2+} channel. We cannot, however, exclude the possibility that DMPK-mediated phosphorylation primarily influences the gating by affecting the sarcolemmal and T-tubular architecture, or is involved in the (in)activation of voltage-gated ion channels that set the resting membrane potential, like K^+ and Cl^- channels. Delayed rectifier (50), inward rectifier (51), as well as Ca^{2+}-activated K^+ channels (52) can be phosphorylated by cAMP-dependent protein kinase and/or protein kinase C in heart, smooth muscles, and neurons. Activation of protein kinase C reduces Cl^--conductance and leads to myotonia (53). It is difficult to discriminate as ion channel regulation by phosphorylation is extremely complex (51, 54). Thus, although our findings do not provide direct clues for any of these channels being a direct target for DMPK, they illustrate a unique property of DMPK, namely that it exerts a modulating function on the initial events of the E-C coupling in skeletal muscle. The localization of DMPK isoforms at sites of dense channel clustering (55–57) is consistent with the fact that these processes occur at or close to the sarcolemma.

Relevance for disease etiology? Since $[Ca^{2+}]_i$ is a crucial regulator of many physiological processes, elevated $[Ca^{2+}]_i$ could potentially have profound consequences in vivo. Can we extrapolate our in vitro findings and relate abnormal Ca^{2+} homeostasis to disease manifestation in DM patients? We realize that our knockout mouse and cell models do not provide a direct answer to this question, as the effects of CTG expansion on DMPK expression still have not been clearly resolved. The mouse myotubes, however, do provide the ideal test system to study the normal biological significance of DMPK against a well-defined, constant genetic background. Earlier we discussed the apparent lack of an overt phenotype in our animal model (9) in terms of possible adaptation, compensation, or threshold effects, which in mice, with a relatively low muscle workload due to small body weight, often obscure the manifestation of myopathic features (58). Here we study the system in isolation, outside the context of the entire tissue, and it is perhaps more relevant that the elevated resting $[Ca^{2+}]_i$, and the effects on release and sequestration of Ca^{2+} coupled to abnormal regulation of TTX-sensitive Na^+ channels and voltage-operated L-type Ca^{2+} channels match the data obtained with myotubes derived from DM patients (15–18). There are also differences in that abnormal SR Ca^{2+}-ATPase and Na^+/K^+-ATPase levels were not found in mouse DMPK[−/−] cells (Table III, 20). It is possible that Ca^{2+}-dependent gene expression critical for these changes may have a differential threshold sensitivity in man and mouse, and almost certainly there are differential effects due to the completely distinct mutation type in the human and mouse situation. For example, for DM patients it is conceivable that large expansions of the $(CTG)_n$ repeat affect other genes in the immediate vicinity, like the downstream-located DMAHP gene which specifies a transcription factor implemented in ion-channel expression regulation (35, 59).

Thus, although the cell model is clearly too simplistic, our finding of similar disturbances in Ca^{2+} homeostasis in muscle cells of both DM patients and DMPK[−/−] mice lends indirect support to the contention that the DM mutation results in a reduction or redistribution of DMPK mRNA and protein isoforms, in line with the molecular explanations provided by others (8, 60, 61). In conclusion, our study provides new insight in the relation between DMPK activity and Ca^{2+} responsive-

ness in skeletal muscle. The suggestion that DMPK modulates the movement of gating charge and/or the activity of voltage-gated ion channels opens up new possibilities for studies into the molecular etiology of DM.

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References

1. Harper, P.S. 1989. Myotonic Dystrophy (2nd ed.). W.B. Saunders Co. Ltd., London. 384 pp.

2. Brook, J.D., M.E. McCurrach, H.G. Harley, A.J. Buckler, D. Church, H. Aburatani, K. Hunter, V.P. Stanton, J.P. Thirion, T. Hudson, et al. 1992. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell.* 68: 799–808.

3. Mahadevan, M., C. Tsilfidis, L. Sabourin, G. Shutler, C. Amemiya, G. Jansen, C. Neville, M. Narang, J. Barceló, K. O'Hoy, S. Leblond, et al. 1992. Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. *Science (Wash. DC).* 255:1253–1255.

4. Fu, Y.-H., A. Pizzuti, R.G. Fenwick, Jr., J. King, S. Rajnarayan, P.W. Dunne, J. Dubel, G.A. Nasser, T. Ashizawa, P. de Jong, et al. 1992. An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science (Wash. DC).* 255:1256–1258.

5. Fu, Y.-H., D.L. Friedman, S. Richards, J.A. Pearlman, R.A. Gibbs, A. Pizzuti, T. Ashizawa, M.B. Perryman, G. Scarlato, R.G. Fenwick, Jr., and C.T. Caskey. 1993. Decreased expression of myotonin-protein kinase messenger RNA and protein in adult form of myotonic dystrophy. *Science (Wash. DC).* 260:235–238.

6. Hofmann-Radvanyi, H., C. Lavedan, J.-P. Rabes, D. Savoy, C. Duros, K. Johnson, and C. Junien. 1993. Myotonic dystrophy: absence of CTG enlarged transcript in congenital forms, and low expression of the normal allele. *Hum. Mol. Genet.* 2:1263–1266.

7. Sabourin, L.A., M.S. Mahadevan, M. Narang, D.S.C. Lee, L.C. Surh, and R.G. Korneluk. 1993. Effect of the myotonic dystrophy (DM) mutation on mRNA levels of the DM gene. *Nat. Genet.* 4:233–238.

8. Krahe, R., T. Ashizawa, C. Abbruzzese, E. Roeder, P. Carango, M. Giacanelli, V.L. Funanage, and M.J. Siciliano. 1995. Effect of myotonic dystrophy trinucleotide repeat expansion on DMPK transcription and processing. *Genomics.* 28:1–14.

9. Jansen, G., P.J.T.A. Groenen, D. Bächner, P.H.K. Jap, M. Coerwinkel, F. Oerlemans, W. van den Broek, B. Gohlsch, D. Pette, J.J. Plomp, et al. 1996. Abnormal myotonic dystrophy protein kinase levels produce only mild myopathy in mice. *Nat. Genet.* 13:316–324.

10. Reddy, S., D.B.J. Smith, M.M. Rich, J.M. Leferovich, P. Reilley, B.M. Davis, K. Tran, H. Rayburn, R. Brondon, D. Cros, et al. 1996. Mice lacking the myotonic dystrophy protein kinase develop a late onset progressive myopathy. *Nat. Genet.* 13:325–335.

11. Gruener, R., L.Z. Stern, D. Markovitz, and C. Gerdes. 1979. Electrophysiologic properties of intercostal muscle fibers in human neuromuscular diseases. *Muscle Nerve.* 2:165–172.

12. Merickel, M., R. Gray, P. Chauvin, and S. Appel. 1981. Cultured muscle from myotonic muscular dystrophy patients: altered membrane electrical properties. *Proc. Natl. Acad. Sci. USA.* 78:648–652.

13. Kobayashi, T., V. Askanas, K. Saito, W.K. Engel, and K. Ishikawa. 1990. Abnormalities of aneural and innervated cultured muscle fibers from patients with myotonic atrophy (dystrophy). *Arch. Neurol.* 47:893–896.

14. Edstrom, L., and R. Wroblewski. 1989. Intracellular elemental composition of single muscle fibres in muscular dystrophy and dystrophia myotonica. *Acta Neurol. Scand.* 80:419–424.

15. Jacobs, A.E.M., A.A.G.M. Benders, A. Oosterhof, J.H. Veerkamp, P. van Mier, R.A. Wevers, and E.M.G. Joosten. 1990. The calcium homeostasis and the membrane potential of cultured muscle cells from patients with myotonic dystrophy. *Biochim. Biophys. Acta.* 1096:14–19.

16. Benders, A.A.G.M., R.A. Wevers, and J.H. Veerkamp. 1996. Ion transport in human skeletal muscle cells: disturbances in myotonic dystrophy and Brody's disease. *Acta Physiol. Scand.* 156:355–367.

17. Rüdel, R., J.P. Ruppersberg, and W. Spittelmeister. 1989. Abnormalities of the fast sodium current in myotonic dystrophy, recessive generalized myotonia, and adynamia episodica. *Muscle Nerve.* 12:281–287.

18. Franke, Ch., H. Hatt, P.A. Iaizzo, and F. Lehmann-Horn. 1990. Characteristics of Na^+ channels and Cl^- conductance in resealed muscle fibre segments from patients with myotonic dystrophy. *J. Physiol. Lond.* 425:391–405.

19. Desnuelle, C., A. Lombet, G. Serratrice, and M. Lazdunski. 1982. Sodium channel and sodium pump in normal and pathological muscles from patients with myotonic muscular dystrophy and lower motor neuron impairment. *J. Clin. Invest.* 69:358–367.

20. Benders, A.A.G.M., J.A.H. Timmermans, A. Oosterhof, H.J. Ter Laak, T.H. van Kuppevelt, R.A. Wevers, and J.H. Veerkamp. 1993. Deficiency of Na^+/K^+-ATPase and sarcoplasmic reticulum Ca^{2+}-ATPase in skeletal muscle and cultured muscle cells of myotonic dystrophy patients. *Biochem. J.* 293:269–274.

21. Renaud, J.F., C. Desnuelle, H. Schmid-Antomarchi, M. Hugues, G. Serratrice, and M. Lazdunski. 1986. Expression of apamin receptor in muscles of patients with myotonic muscular dystrophy. *Nature (Lond.).* 319:678–680.

22. Dulhunty, A.F. 1992. The voltage-activation of contraction in skeletal muscle. *Prog. Biophys. Mol. Biol.* 57:181–223.

23. Ríos, E., G. Pizarro, and E. Stefani. 1992. Charge movement and the nature of signal transduction in skeletal muscle excitation-contraction coupling. *Annu. Rev. Physiol.* 54:109–133.

24. Schneider, M.F. 1994. Control of calcium release in functioning skeletal muscle fibres. *Annu. Rev. Physiol.* 56:463–484.

25. Melzer, W., A. Hermann-Frank, and H. C. Lüttgau. 1995. The role of Ca^{2+} ions in excitation-contraction coupling of skeletal muscle fibers. *Biochim. Biophys. Acta.* 1241:59–116.

26. Benders, A.A.G.M., J.H. Veerkamp, A. Oosterhof, P.J.H. Jongen, R.J.M. Bindels, L.M.E. Smit, H.F.M. Busch, and R.A. Wevers. 1994. Ca^{2+} homeostasis in Brody's disease: a study in skeletal muscle and cultured muscle cells and the effects of dantrolene and verapamil. *J. Clin. Invest.* 94:741–748.

27. Gailly, Ph., B. Boland, B. Himpens, R. Casteels, and J.M. Gillis. 1993. Critical evaluation of cytosolic calcium determination in resting muscle fibres from normal and dystrophic (mdx) mice. *Cell Calcium.* 14:473–483.

28. Benders, A.A.G.M., A. Oosterhof, R.A. Wevers, and J.H. Veerkamp. 1997. Excitation-contraction coupling of cultured human skeletal muscle cells and the relation between basal cytosolic Ca^{2+} and excitability. *Cell Calcium.* 21: 81–91.

29. Martinuzzi, A., V. Askanas, T. Kobayashi, and W.K. Engel. 1986. Expression of muscle-gene-specific isozymes of phosphorylase and creatine kinase in innervated cultured human muscle. *J. Cell Biol.* 103:1423–1429.

30. Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem.* 193:265–275.

31. Cognard, C., M. Rivet-Bastide, B. Constantin, and G. Ryamond. 1992. Progressive predominance of 'skeletal' versus 'cardiac' types of excitation-contraction coupling during in vitro skeletal myogenesis. *Pflügers Arch. Eur. J. Physiol.* 422:207–209.

32. Sherman, S.J., J.C. Lawrence, D.J. Messner, K. Jacoby, and W.A. Catterall. 1983. Tetrodotoxin-sensitive sodium channels in rat muscle cells developing in vitro. *J. Biol. Chem.* 258:2488–2495.

33. Hidalgo, C., and P. Donoso. 1995. Luminal calcium regulation of calcium release from sarcoplasmic reticulum. *Biosci. Rep.* 15:387–397.

34. Jansen, G., D. Bachner, M. Coerwinkel, N. Wormskamp, H. Hameister, and B. Wieringa. 1995. Structural organization and developmental expression pattern of the DMR-N9 gene immediately upstream of the myotonic dystrophy locus. *Hum. Mol. Genet.* 4:843–852.

35. Boucher, C.A., S.K. King, N. Carey, R. Krahe, C.L. Winchester, S. Rahman, T. Creavin, P. Meghji, M.E.S. Bailey, F.L. Chartier, et al. 1995. A novel homeodomain-encoding gene is associated with a large CpG island interrupted by the myotonic dystrophy unstable (CTG)n repeat. *Hum. Mol. Genet.* 4:1919–1925.

36. Bush, E.W., C.S. Taft, G.E. Meixell, and M.B. Perrymann. 1996. Overexpression of myotonic dystrophy kinase in BC_3H1 cells induces the skeletal muscle phenotype. *J. Biol. Chem.* 271:548–552.

37. Bakker, A.J., S.I. Head, D.A. Williams, and D.G. Stephenson. 1993. Ca^{2+} levels in myotubes grown from the skeletal muscle of dystrophic (mdx) and normal mice. *J. Physiol.* 460:1–13.

38. Williams, D.A., S.I. Head, A.J. Bakker, and D.G. Stephenson. 1990. Resting calcium concentration in isolated skeletal muscle fibres of dystrophic mice. *J. Physiol.* 428:243–256.

39. Decker, E.R., and J.A. Dani. 1990. Calcium permeability of the nicotinic acetylcholine receptor: the single-channel calcium influx is significant. *J. Neurosci.* 10:3413–3420.

40. García, J., and K.G. Beam. 1994. Calcium transients associated with the T type calcium current in myotubes. *J. Gen. Physiol.* 104:1113–1128.

41. García, J., T. Tanabe, and K.G. Beam. 1994. Relationship of calcium transients to calcium currents and charge movements in myotubes expressing skeletal and cardiac dihydro-pyridine receptors. *J. Gen. Physiol.* 104:125–147.

42. Woodward Hopf, F., P. Reddy, J. Hong, and R.A. Steinhardt. 1996. A capacitative calcium current in cultured skeletal muscle cells is mediated by the calcium-specific leak channel and inhibited by dihydropyridine compounds. *J. Biol. Chem.* 271:22358–22367.

43. Jansen, G., M. Mahadevan, C. Amemiya, N. Wormskamp, B. Segers, W. Hendriks, K. O'Hoy, S. Baird, L. Sabourin, G. Lennon, et al. 1992. Character-

ization of the myotonic dystrophy region predicts multiple protein isoform-encoding mRNAs. *Nat. Genet.* 1:261–266.

44. Klein, MG., H. Cheng, L.F. Santana, Y.H. Jiang, WJ. Lederer, and M.F. Schneider. 1996. Two mechanisms of quantized calcium release in skeletal muscle. *Nature (Lond.).* 379:455–458.

45. McDonald, T.F., S. Pelzer, W. Trautwein, and D.J. Pelzer. 1994. Regulation and modulation of calcium channels in cardiac, skeletal and smooth muscle cells. *Physiol. Rev.* 74:365–507.

46. Timchenko, L., W. Nastainczyk, T. Schneider, B. Patel, F. Hofmann, and C.T. Caskey. 1995. Full-length myotonin protein kinase (72 kDa) displays serine kinase activity. *Proc. Natl. Acad. Sci. USA.* 92:5366–5370.

47. Lacerda, A.E., H.S. Kim, P. Ruth, E. Perez-Reyes, and V. Flockerzi. 1991. Normalization of current kinetics by interaction between the α_1 and β subunits of the skeletal muscle dihydropyridine-sensitive Ca^{2+} channel. *Nature (Lond.).* 352:527–530.

48. Numann, R., S.D. Hauschka, W.A. Catterall, and T. Scheuer. 1994. Modulation of skeletal muscle sodium channels in satellite cell line by protein kinase C. *J. Neurosci.* 14:4226–4236.

49. Mounsey, J.P., P. Xu, J.E. John III, L.T. Horne, J. Gilbert, A.D. Roses, and J.R. Moorman. 1995. Modulation of skeletal muscle sodium channels by human myotonin protein kinase. *J. Clin. Invest.* 95:2379–2384.

50. Perozo, E., D.S. Jong, and F. Bezanilla. 1991. Single channel studies of the phosphorylation of K^+ channels in the squid giant axon. II Nonstationary conditions. *J. Gen. Physiol.* 98:19–34.

51. Fakler, B., U. Brandle, E. Glowatzki, H.P. Zenner, and J.P. Ruppersberg. 1994. Kir2.1 inward rectifier K^+ channels are regulated independently by protein kinases and ATP hydrolysis. *Neuron.* 13:1413–1420.

52. Savaria, D., C. Lanoue, A. Cadieux, and E. Rousseau. 1992. Large conducting potassium channel reconstituted from airway smooth muscle. *Am. J. Physiol.* 262:L327–L336.

53. Brinkmeier, H., and H. Jockusch. 1987. Activators of protein kinase C induce myotonia by lowering chloride conductance in muscle. *Biochem. Biophys. Res. Commun.* 148:1383–1389.

54. Levitan, I.B. 1994. Modulation of ion channels by protein phosphorylation and dephosphorylation. *Annu. Rev. Physiol.* 56:193–212.

55. Van der Ven, P.F., G. Jansen, T.H. van Kuppevelt, M.B. Perryman, M. Lupa, P.W. Dunne, H.J. ter Laak, P.H. Jap, J.H. Veerkamp, H.F. Epstein, and B. Wieringa. 1993. Myotonic dystrophy kinase is a component of neuromuscular junctions. *Hum. Mol. Genet.* 2:1889–1894.

56. Dunne, P.W., L. Ma, D.L. Casey, Y. Harati, and H.F. Epstein. 1996. Localization of myotonic dystrophy protein kinase in skeletal muscle and its relation with disease. *Cell Motil. Cytoskeleton.* 33:52–63.

57. Waring, J.D., R. Haq, K. Tamai, L.A. Sabourin, J.-E. Ikeda, and R.G. Korneluk. 1996. Investigation of myotonic dystrophy kinase isoform translocation and membrane association. *J. Biol. Chem.* 271:15187–15193.

58. Pette, D., and G. Vrbova. 1992. Adaptation of mammalian skeletal muscle fibers to chronic stimulation. *Rev. Physiol. Biochem. Pharmacol.* 120:115–202.

59. Thornton, C.A., J.P. Wymer, and R.T. Moxley. 1996. Expression of the DMAHP gene is suppressed in *cis* by the myotonic dystrophy (MTD) CTG repeat expansion. *Am. J. Hum. Genet.* 59(Suppl.):A33.

60. Wang, J., E. Pegoraro, E. Menegazzo, M. Gennarelli, R.C. Hoop, C. Angelini, and E.P. Hoffman. 1995. Myotonic dystrophy: evidence for a possible dominant-negative RNA mutation. *Hum. Mol. Genet.* 4:599–606.

61. Taneja, K.L., M. McCurrach, M. Schalling, D. Housman, and R.H. Singer. 1995. Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. *J. Cell Biol.* 128:995–1002.