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Effects of Dimethyl Sulphoxide and Dexamethasone on mRNA Expression of Myogenesis- and Muscle Proteolytic System-related Genes in Mouse Myoblastic C2C12 Cells

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We examined the time course of mRNA expression of myogenic cell differentiation- and muscle proteolytic system-related genes in cultures of C2C12 cells during differentiation from myoblasts to myotubes. Furthermore, we treated C2C12 myotubes with dimethyl sulphoxide (DMSO) and dexamethasone (Dex), and examined changes in these mRNA levels. Myogenin (Myog), Atrogin1, forkhead box O1 (Foxo1) and Capn1 mRNA levels increased in C2C12 cells differentiating from myoblasts to myotubes, whereas Myf5 mRNA levels decreased. Although genes such as MRF4, Foxo3a, UbB, Capn1 and MuRF1 mRNAs in the myotubes were affected by DMSO exposure, mRNA levels of other genes were not markedly affected by exposure to 0.02% or 0.5% DMSO. Myf5, MRF4, Atrogin1, Foxo3 and MuRF1 mRNA levels were elevated by Dex at all time points, Cbl and Capn1 mRNA levels were significantly elevated by Dex at 8 h, and Myog mRNA levels were significantly elevated by Dex at 24 h. However, CtsH mRNA levels decreased significantly with Dex at 24 h. This study provides a useful database of gene profiles that are differentially expressed throughout myogenic cell differentiation and the muscle proteolytic system.

Key words: C2C12 myotubes, dexamethasone, dimethyl sulphoxide, muscle proteolytic system-related genes, myogenesis-related genes.

Abbreviations: Capn1, calpain 1; Capn2, calpain 2; Cbl, Casitas B-lineage lymphoma; CtsB, cathepsin B; CtsH, cathepsin H; Dex, dexamethasone; DMSO, dimethyl sulphoxide; Foxo1, forkhead box O1; Foxo3a, forkhead box O3a; HPRT1, hypoxanthine phosphoribosyltransferase 1; MRF, myogenic regulatory factor; MRF4, myogenic regulatory factor 4; MuRF1, muscle RING finger 1; Myf5, myogenic factor 5; Myog, myogenin; Myod1, myogenic differentiation 1; UbB, ubiquitin B; Ubr1, ubiquitin protein ligase E3 component n-recognin 1; Ubr2, Ubiquitin protein ligase E3 component n-recognin 2.

Skeletal muscle differentiation is characterized by the withdrawal of myoblasts from the cell cycle, activation of muscle-specific gene expression and cell fusion into multinucleated myotubes. The mouse myoblastic cell line C2C12 serves as a well-accepted model to investigate these events *in vitro* (*1*). Therefore, C2C12 cells are used as a model for studying muscle cell growth and differentiation. Myocyte differentiation is regulated by four myogenic regulatory factors (MRFs), viz. myogenin (Myog), MyoD, Myf5 and MRF4 (*2*, *3*).

Numerous studies regarding skeletal muscle protein degradation during disuse atrophy have focused on three primary proteolytic pathways, i.e. the cytosolic Ca^{2+}-dependent calpain pathway, the lysosomal protease pathway (cathepsins) and the ubiquitin-proteasome pathway. It has been reported that the three primary proteolytic pathways may work together during muscle proteolysis (*4*), and the *in vitro* study of these pathways has typically used cultures of C2C12 cells.

Glucocorticoids, such as dexamethasone (Dex), are used in studies of muscle atrophy in C2C12 cells (*5*–*7*). Atrogin-1 promotes skeletal muscle protein degradation in response to glucocorticoid (*8*, *9*) and contributes to muscle atrophy by targeting proteins for ubiquitination and proteasomal degradation (*10*). Overexpression of atrogin-1 in cultured C2C12 myotubes produces cell atrophy (*11*). Recently, the forkhead box O (Foxo) family of transcription factors was found to regulate skeletal muscle atrophy both *in vitro* and *in vivo* (*12*, *13*). Both Foxo1 and Foxo3a appear to up-regulate expression of muscle-specific ubiquitin ligases and to decrease muscle size (*8*, *9*). Based on these findings, we treated C2C12 myotubes with Dex in order to elucidate gene expression patterns during muscle atrophy.

In the present study, therefore, we evaluated the effects of exposure to DMSO and Dex on the mRNA levels of myogenic cell differentiation-related genes, such as Myog, Myod1, Myf5 and MRF4, and of muscle proteolytic system-related genes, such as Atrogin1, Cbl, Foxo1, Foxo3a, UbB, Ubr, Ubr2, Capn1, Capn2, CtsB, CtsH and MuRF1, in cultures of C2C12 cells using real-time RT–PCR. Generally, drugs such as Dex are

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dissolved in water-miscible organic solvents, such as dimethyl sulphoxide (DMSO), for *in vitro* studies. Therefore, we also examined the effects of exposure to DMSO on the expression of these genes. The nomenclature of these target genes is summarized in Table 1.

MATERIALS AND METHODS

Materials—We purchased the following materials: DMSO and Dex (Wako Pure Chemical Industries, Ltd; Osaka, Japan); C2C12 myoblast cells (American Type Culture Collection; Manassas, VA, USA); Dulbecco's modified Eagle's medium (DMEM) containing 1.0 g/l glucose and horse serum (Invitrogen; Carlsbad, CA, USA); L-glutamine, penicillin/streptomycin and fetal bovine serum (Sigma Aldrich; St Louis, MO, USA); the Rneasy Mini Kit and QIAshredder (Qiagen; Hilden, Germany); yeast tRNA (Life Technologies, Inc.; Rockville, MD, USA); and TaqMan One-Step RT-PCR Master Mix Reagents (Applied Biosystems; Foster City, CA, USA). All other chemicals used in this study were of reagent grade.

Cell Culture—C2C12 myoblast cells were maintained in 24-well plates under subconfluent conditions in growth media containing DMEM with 1.0 g/l glucose, L-glutamine, penicillin/streptomycin (100 μg/ml) and 10% fetal bovine serum. Confluent cells were differentiated by lowering the serum concentration to 2% horse serum. All cells were grown in a humidified 37°C incubator with 5% CO_2 and 95% air.

Experiments with Cell Cultures of C2C12 Myotubes—In these studies, DMEM with 1.0 g/l glucose, L-glutamine, penicillin/streptomycin (100 μg/ml) and 2% horse serum was used. C2C12 myotubes, which were differentiated for 72 h, were exposed to DMSO (0.02%, 0.1%, 0.5% and 2.5%) or Dex (0.0001, 0.001, 0.01, 0.1, 1, 10 and 100 μM). Dex was dissolved in DMSO at a final vehicle concentration of 0.1% (v/v), and controls were also exposed to 0.1% (v/v) DMSO. Total RNA was extracted from the C2C12 myotubes using QIAshredder and RNeasy Mini Kit.

Oligonucleotides—Pairs of forward and reverse primers and TaqMan probes for HPRT1 used in the RT–PCR sequences were as reported previously (*14*). The primer pairs and TaqMan probes for the target mRNAs were designed based on the mouse mRNA sequence (Table 2) using Primer Express software (Applied Biosystems). GenBank accession number and the position from the initiation codon are also shown in Table 2. Each primer and/or probe was subjected to an NCBI BLAST search to ensure that it was specific to the target mRNA transcript. The primers and TaqMan probes were synthesized by the Genosys Division of Sigma-Aldrich Japan K.K. (Ishikari, Hokkaido, Japan). The TaqMan probes contained 6-carboxyfluorescein (FAM) at the 5′-end and 6-carboxytetramethylrhodamine (TAMRA) at the 3′-end and were designed to hybridize to a sequence located between the PCR primers.

TaqMan RT–PCR Conditions—Total RNA was diluted to about 4 μg/ml with 50 μg/ml yeast tRNA. RT–PCR assay was performed in 50 μl of TaqMan One-Step RT–PCR Master Mix reagents containing 300 nM forward primer, 900 nM reverse primer, 200 nM TaqMan probe and about 20 ng of total RNA. The assay was performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems) under the same conditions as in our previous studies (*15*, *16*).

Statistical Analysis—Data analyses were performed with the ABI Prism Sequence Detection System software. The relative expression of each mRNA was calculated as the ΔC_t (value obtained by subtracting the C_t value of HPRT1 mRNA from the C_t value of target mRNA); this formula was also used in our previous studies (*15*, *17*). The amount of target mRNA relative to HPRT1 mRNA was expressed as $2^{-(\Delta Ct)}$. Data were expressed as the ratio of target mRNA to HPRT1 mRNA. Statistical analysis was performed using Student's *t*-test or Dunnett's test. *P*-values of < 0.05 were considered to be statistically significant.

Table 1. **Target genes evaluated in this study.**

Name	Abbreviation	Function	Trivial name(s)/synonym (s)
Myogenin	Myog	Cell differentiation	myo, MYF4
Myogenic differentiation 1	Myod1	Cell differentiation	MYF3, MyoD
Myogenic factor 5	Myf5	Cell differentiation	
Myogenic regulatory factor 4	MRF4	Cell differentiation	herculin, Myf6
Atrogin1		Ubiquitin cycle	MAFbx, Fbxo32
Casitas B-lineage lymphoma	Cbl	Cell surface receptor-linked signal transduction	Cbl-2, c-Cbl
Forkhead box O1	Foxo1	Insulin receptor signalling pathway	Afxh, FKHR, Fkhr1, Foxo1a
Forkhead box O3a	Foxo3a	Glucose homeostasis	Fkhr2, Foxo3, FKHRL1
Ubiquitin B	UbB		Ubb2
Ubiquitin protein ligase E3 component n-recognin 1	Ubr1	ubiquitin-dependent protein catabolism	
Ubiquitin protein ligase E3 component n-recognin 2	Ubr2	Ubiquitin-dependent protein catabolism	
Calpain 1	Capn1	Proteolysis	Capa-1, mu-calpin
Calpain 2	Capn2	Proteolysis	Capa-2, m-calpin, m-calpain
Cathepsin B	CtsB	Proteolysis	
Cathepsin H	CtsH	Proteolysis	
Muscle RING finger 1	MuRF1	Proteasomal ubiquitin-dependent protein catabolism	RF1, Trim63, Rnf28

Table 2. **Primers and probes used for RT–PCR analysis.**

mRNA (GenBank No.)	Forward primer (position)	Reverse primer (position)	Probe (position)
Myog (NM_031189)	5′-AGAAGCGCAGGCTCAAGAAA-3′ (269–288)	5′-ATCTCCACTTTAGGCAGCCG-3′ (362–343)	5′-CCCTGAAGAGGAGCACCCTGCTCAA-3′ (308–332)
Myod1 (NM_010866)	5′-TGATGATCCGTGTTTCGACTC-3′ (90–110)	5′-ACCGCAGTAGAGAAGTGTGCGT-3′ (206–185)	5′-CAGACCTGCGCTTTTTTGAGGACCTG-3′ (113–138)
Myf5 (NM_008656)	5′-GCCAGTTCTCCCCTTCTGAGTA-3′ (20–41)	5′-TGGTCCCCAAACTCATCCTCT-3′ (92–72)	5′-TTCTATGAAGGCTCCTGTATCCCCTCACC-3′ (43–71)
MRF4 (NM_008657)	5′-ATTCTGCGGAGTGCCATCA-3′ (397–415)	5′-CCAGCTCCTGCATCTTCTCTT-3′ (481–461)	5′-CTACATTGAGCGTCTACAGGACCTGCTGCA-3′ (417–446)
Atrogin1 (AF441120)	5′-AGACCTGCATGTGCTCAGTGA-3′ (762–782)	5′-AACGCTTGCGAATCTGCCT-3′ (847–829)	5′-ACCGGCTACTGTGGAAGAGACTCTGCCAGTA-3′ (785–815)
Cbl (NM_007619)	5′-GGAGAAGTGCTGGAAGCTCAT-3′ (162–182)	5′-ATATAAGGCGGGCTGTTCTTG-3′ (245–225)	5′-CAAGGTGGTGCGGTTGTGTCAAAACC-3′ (186–211)
Foxo1 (NM_019739)	5′-ACAGCAAATCAAGTTACGGAGG-3′ (1322–1343)	5′-GGGATCAACCGGTGACATAAT-3′ (1437–1417)	5′-AACCAGTATAACTGTGCCCCAGGACTCTTGAAA-3′ (1348–1380)
Foxo3a (NM_019740)	5′-AAACGGCTCACTTTGTCCCA-3′ (523–542)	5′-CTGTCGCCCTTATCCTTGAAG-3′ (596–576)	5′-ATCTACGAGTGGATGGTGCGCTGTGT-3′ (544–569)
UbB (NM_011664)	5′-TGACACCATCGAGAACGTGA-3′ (60–79)	5′-GGGTTGACTCCTTCTGGATGT-3′ (199–179)	5′-CAAGATCCAGGATAAAGAGGGCATCCC-3′ (84–110)
Ubr1 (NM_009461)	5′-TGGGATGACACCGTGGAT-3′ (4264–4281)	5′-TGTGCGCCATGGTGATC-3′ (4351–4335)	5′-CAGCCGTCGCCACTTAGTTCTTCATATAACCAC-3′ (4285–4317)
Ubr2 (NM_146078)	5′-AGCCATTGGCTTTGCAACTAC-3′ (768–788)	5′-GCTGGTGTTCCTCACAATGAC-3′ (876–856)	5′-ATCGAGATGGCCGTAGTCTGTCCGATAT-3′ (794–822)
Capn1 (NM_007600)	5′-TCATGGATCGAGATGGCAAC-3′ (1784–1803)	5′-GTCAGGTAATTTCGGATGCGG-3′ (1862–1842)	5′-AAGCTGGGTCTGGTGGAGTTCAACATCCT-3′ (1807–1835)
Capn2 (NM_009794)	5′-GACATGCACACCATTGGCTT-3′ (1273–1292)	5′-TTTGCCGAGGTGGATGTTG-3′ (1350–1332)	5′-TATGAGGTTCCAGAGGAGCTAACAGGGCAGA-3′ (1300–1330)
CtsB (NM_007798)	5′-ACAATGGTCCAACTGCCCG-3′ (264–282)	5′-AAATGCCCAACAAGAGCCG-3′ (333–315)	5′-CCATTGGACACATTAGAGACCAGGGCTCC-3′ (284–312)
CtsH (NM_007801)	5′-TCATGGCTGCAAAGGAGGT-3′ (528–546)	5′-TGTCTTCTTCCATGATGCCC-3′ (604–585)	5′-CCAGCCAGGCCTTCGAGTACATCCTATA-3′ (551–578)
MuRF1 (NM_0010390048)	5′-TTCCTCTCAAGTGCCAAGCA-3′ (823–842)	5′-TCCATGTTCTCAAAGCCTTGC-3′ (917–897)	5′-CAAGAGCAATGTAGAAGCCTCCAAGGGC-3′ (849–876)

Positions of primers and probes were counted from the initiation codon.

RESULTS AND DISCUSSION

We evaluated changes in the mRNA levels of myogenic cell differentiation- and muscle proteolytic system-related genes during differentiation from C2C12 myoblasts to C2C12 myotubes, as well as the effects of DMSO and Dex on the mRNA levels of myogenic cell differentiation- and muscle proteolytic system-related genes in cultures of C2C12 myotubes. We made several interesting observations with regard to the mRNA levels of myogenic cell differentiation- and muscle proteolytic system-related genes.

In the first stage of this study, we examined the time course of mRNA expression of myogenic cell differentiation- and muscle proteolytic system-related genes in cultures of C2C12 cells that were differentiating from myoblasts to myotubes. This analysis was performed by quantitative real-time RT–PCR with primers and TaqMan probes. HPRT1 mRNA, which was stably expressed in our recent study using C2C12 cells (*14*) and is stably expressed under various conditions (*14*, *15*, *18*, *19*), was used as the internal control for the measurement of target mRNAs. The ratio of Myog mRNA levels to HPRT1 mRNA levels in C2C12 cells that were differentiating from myoblasts to myotubes increased from 0 to 48 h of culture, whereas the ratio of Myf5 mRNA levels to HPRT1 mRNA levels decreased from 24 to 120 h of culture (Fig. 1A). The ratio of Myod1 mRNA levels to HPRT1 mRNA levels remained constant and/or showed slight changes from 0 to 120 h of culture (Fig. 1A). The ratio of Atrogin1, Foxo1 and Capn1 mRNA levels to HPRT1 mRNA levels in differentiating C2C12 cells increased from 0 to 72 h of culture (Fig. 1B). The ratio of Cbl, Foxo3a, UbB, Ubr1, Ubr2, Capn2, CtsB and CtsH mRNA levels to HPRT1 mRNA levels remained constant and/or showed slight changes from 0 to 120 h of culture (Fig. 1B).

In the second stage of this study, we treated the C2C12 myotubes with DMSO and Dex, and examined the changes in expression of myogenic cell differentiation- and muscle proteolytic system-related genes. Myog, Myod1 and Myf5 mRNAs in the myotubes were significantly lower after exposure to 2.5% DMSO, as compared with controls (Fig. 2A). On the other hand, MRF4 mRNA in the myotubes was significantly higher after exposure to 2.5% DMSO, as compared with controls, and was also higher after exposure of 0.02–0.5% DMSO, as compared with controls at 4 and 8 h (Fig. 2A). DMSO substantially increased the mRNA levels of Foxo3a, and MuRF1 in a concentration-dependent manner at each time (Fig. 2B). UbB and Capn1 mRNA in the myotubes was higher after exposure to DMSO, as compared with controls at 4 and 8 h (Fig. 2B). The mRNA levels of other muscle proteolytic system-related genes were not affected by exposure to DMSO concentrations at 0.02% or 0.5% (Fig. 2B).

DMSO is usually the solvent of choice in these types of study due to its ability to dissolve a large number of organic chemicals. In our previous study (*14*), HPRT1 mRNA levels were unaffected by exposure to DMSO concentrations of $\leq 0.1\%$ in cultures of C2C12 myotubes. Therefore, we concluded that the use of HPRT1 mRNA as an internal control for evaluating mRNA expression

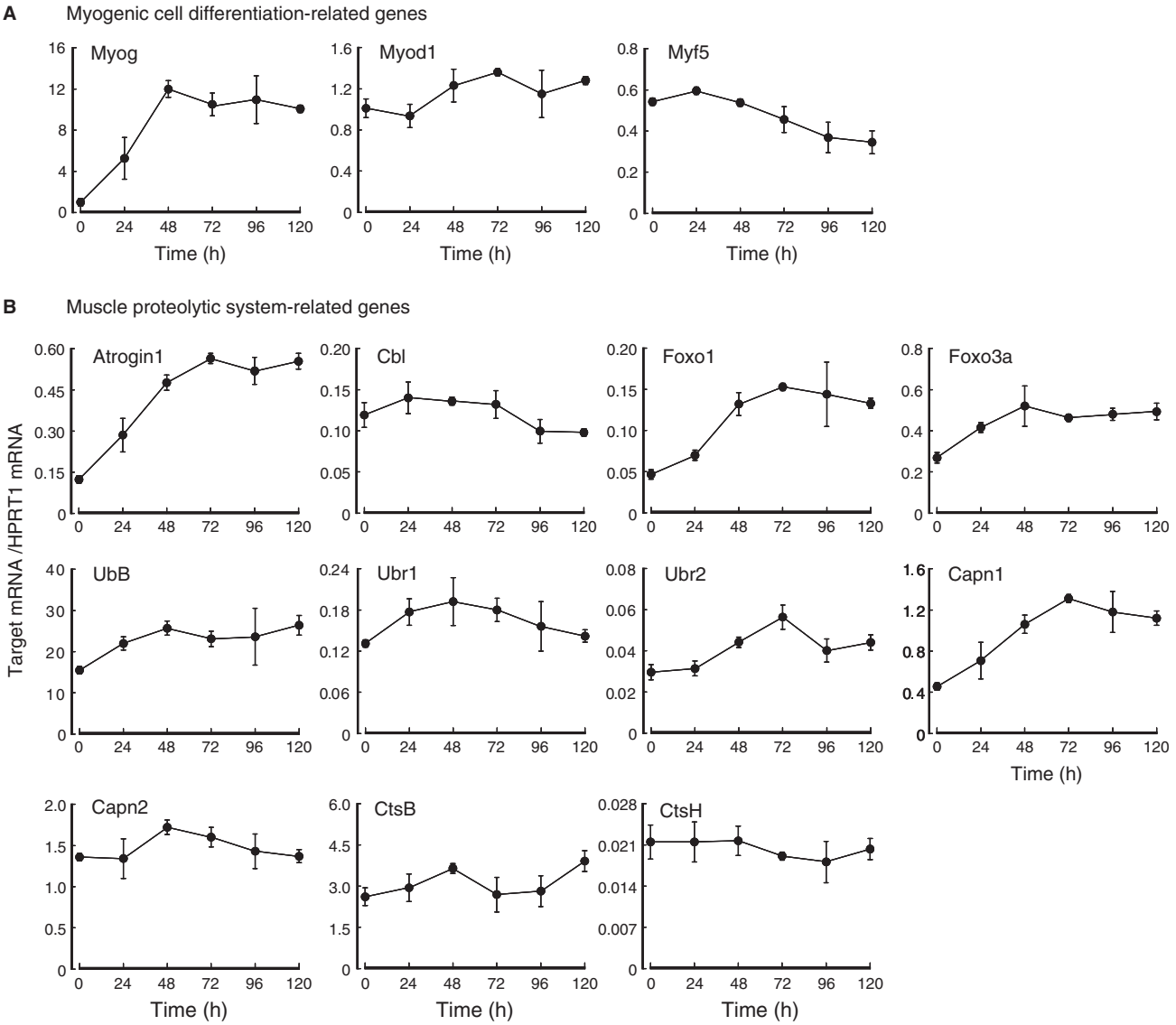

Fig. 1. **Changes in mRNA expression levels of myogenic cell differentiation-related genes (A) and muscle proteolytic system-related genes (B) in C2C12 cells during differentiation from myoblasts to myotubes.** Data are expressed as ratios of target mRNA levels to HPRT1 mRNA levels. Experiments (C2C12 cell cultures) were performed in triplicate, and data are means ± SD.

levels in C2C12 myotubes after Dex exposure is suitable at DMSO concentrations of 0.1%. Furthermore, in the present study, genes such as MRF4, Foxo3a, UbB, Capn1 and MuRF1 were affected by exposure to 0.1% DMSO (Fig. 2). However, we considered that the influence of DMSO on mRNA expression could be excluded in controls at 0.1% DMSO. Myf5 and MRF4 mRNA levels in the myotubes were of higher level after exposure to 1 μM Dex, as compared with controls, at 0.1% DMSO (Fig. 3A). Furthermore, Dex substantially increased the mRNA levels of Myf5 and MRF4 in a concentration-dependent manner (Fig. 4A). Myog mRNA levels at 24 h were significantly higher after exposure to 1 μM Dex, as compared with controls, at 0.1% DMSO (Fig. 3A). On the other hand, mRNA levels of Myod1 were largely unaffected by exposure to 1 μM Dex (Fig. 3A), and were unaffected by exposure to Dex at any concentration (0.0001–100 μM) for 4 h (Fig. 4A). Atrogin1, Foxo3a and MuRF1 mRNA levels in the myotubes were higher after exposure to 1 μM Dex, as compared with controls, at 0.1% DMSO (Fig. 3B). Furthermore, the Dex substantially increased the mRNA levels of Atrogin1, Foxo3a and MuRF1 in a concentration-dependent manner (Fig. 4B). Both Cbl and Capn1 mRNA levels at 8 h were significantly higher after exposure to 1 μM Dex, as compared with controls, at 0.1% DMSO (Fig. 3A). CtsH mRNA levels at 24 h were significantly lower after exposure to 1 μM Dex, as compared with controls, at 0.1% DMSO (Fig. 3B). On the other hand, mRNA levels of Cbl, Foxo1, UbB, Ubr1, Ubr2, Capn1, Capn2 and CtsB were not markedly affected by exposure to 1 μM Dex (Fig. 3B), and were unaffected by exposure to Dex at any concentration (0.0001–100 μM) for 4 h (Fig. 4B).

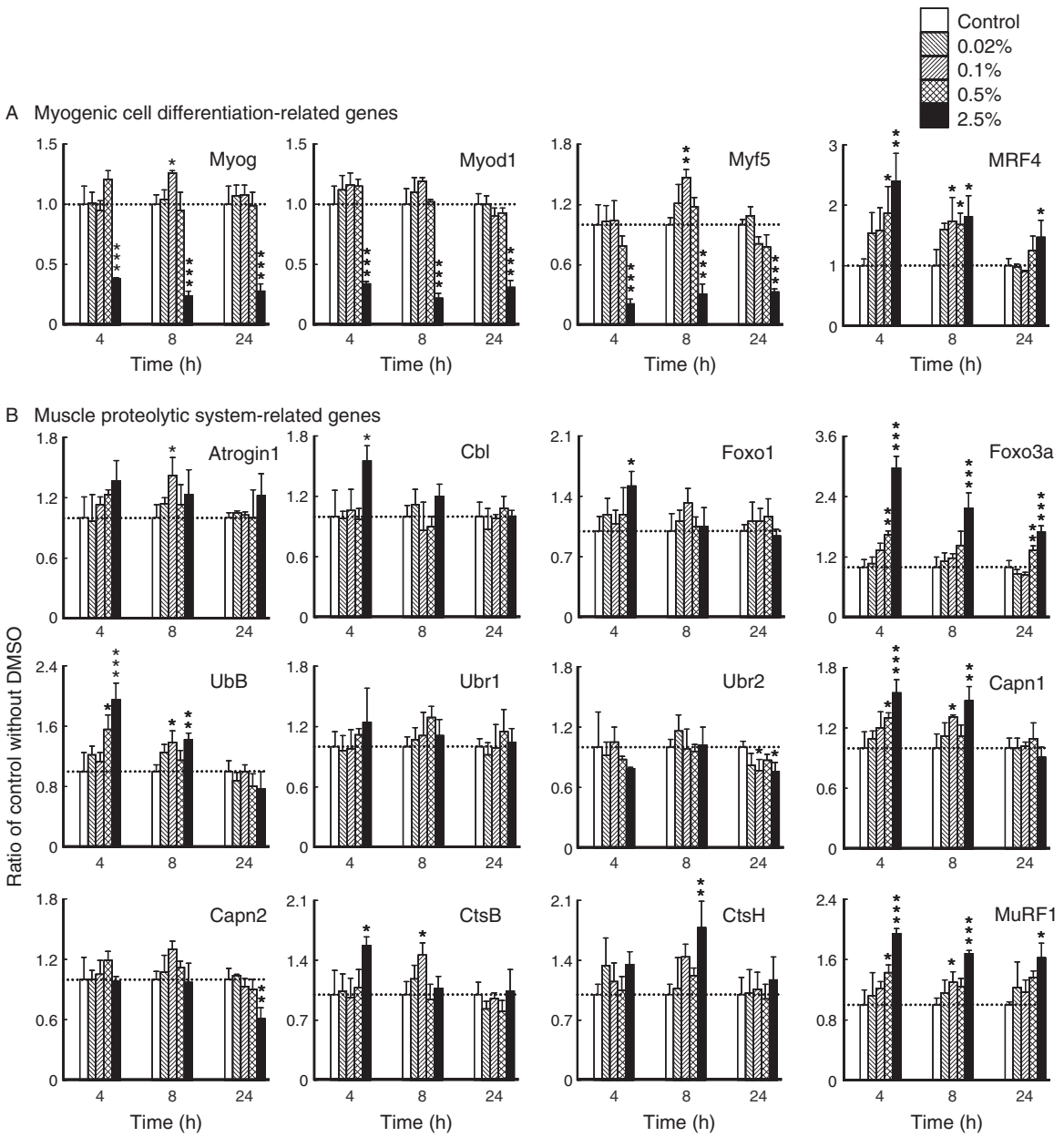

Fig. 2. **Effects of DMSO on mRNA expression of myogenic cell differentiation-related genes (A) and muscle proteolytic system-related genes (B) in cultures of C2C12 myotubes.** C2C12 myotubes were treated with DMSO (0.02%, 0.1%, 0.5% or 2.5%) for 4, 8 or 24 h. Data are expressed as ratios of target mRNA to HPRT1 mRNA. Experiments (C2C12 myotube cultures) were performed in triplicate, and data are means ± SD. The dotted line is the value in controls without DMSO, which was assigned a value of 1. Statistical analysis was performed using Dunnett's test. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ versus controls without DMSO.

In most common procedures, a differentiation period of 96 h is used, during which C2C12 cells are incubated in 2% horse serum before experiments (*20*). The modified procedure shortens the differentiation period to 72 h (*9*, *21*). We also studied the effects of DMSO and Dex in C2C12 myotubes that are differentiated for 72 h, as the expression levels of myogenic cell differentiation-related genes, such as Myog, and muscle proteolytic system-related genes, such as Atrogin1, Foxo1, Foxo3a and Capn1 mRNA, become constant prior to 72 h of incubation in 2% horse serum (Fig. 1).

Both Foxo1 and Foxo3a transcription factors up-regulate expression of the ubiquitin ligase, atrogin-1 and MuRF1, and are necessary for increased protein degradation during muscle atrophy (*8*, *9*). The demonstration of a common transcriptional programme for a variety of atrophic diseases in muscle has identified novel therapeutic targets, such as Atrogin1 and MuRF1. In the present study, Foxo3a, Atrogin1 and MuRF1 mRNA expression in C2C12 myotubes were markedly up-regulated in a concentration-dependent manner by exposure to Dex; however, the expression levels of some

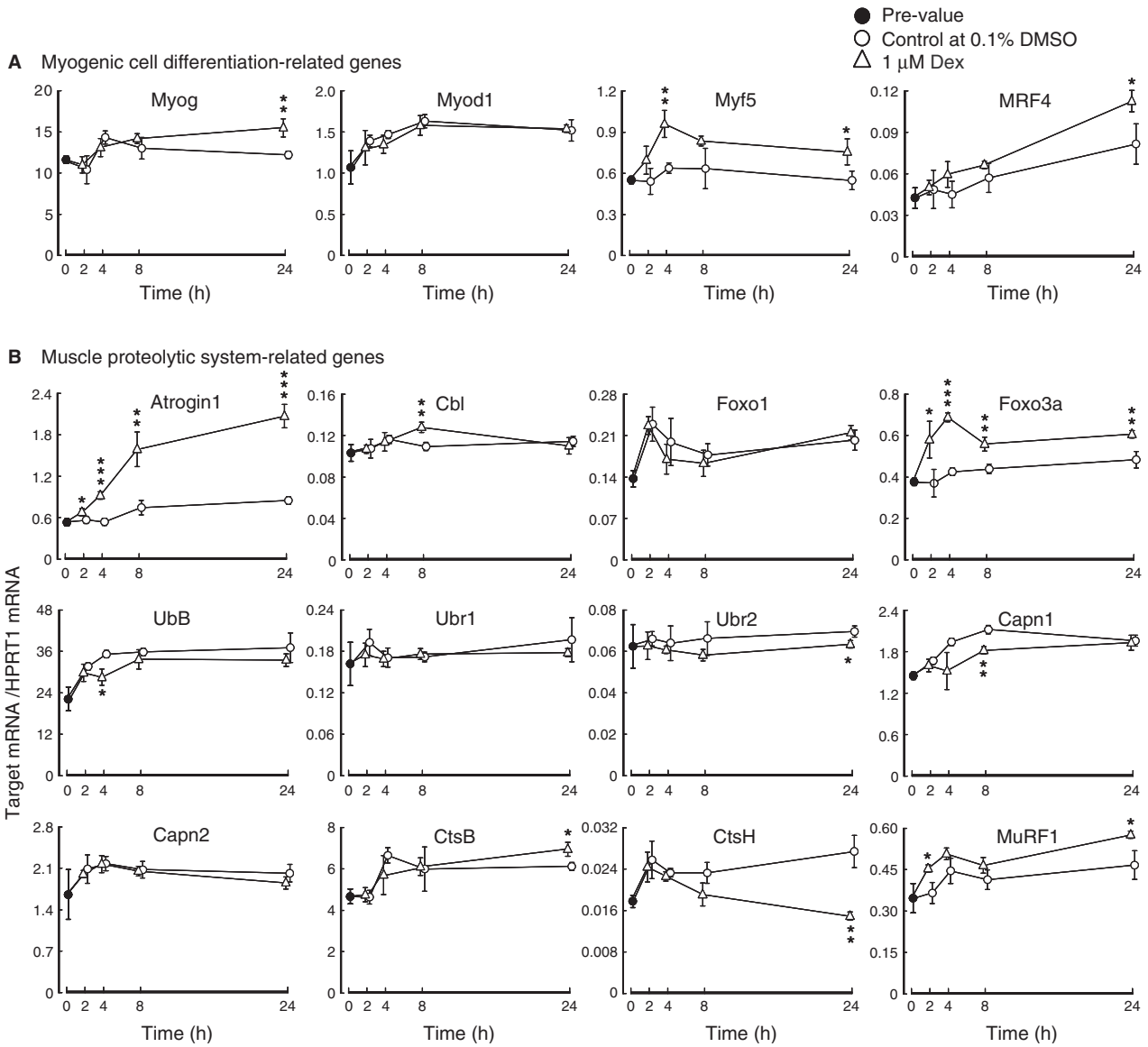

Fig. 3. **Effects of Dex on mRNA expression of myogenic cell differentiation-related genes (A) and muscle proteolytic system-related genes (B) in cultures of C2C12 myotubes.** C2C12 myotubes were treated with1 μM Dex for 4, 8 or 24 h. Data are expressed as ratios of target mRNA to HPRT1 mRNA. Experiments (C2C12 myotube cultures) were performed in triplicate, and data are means ± SD. Statistical analysis was performed using Student's *t*-test. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ versus controls at 0.1% DMSO.

muscle proteolytic system-related genes, such as Foxo1, were unaffected by exposure to Dex (Fig. 4). Both the level of transcription and the level of protein translation may thus be involved in regulating muscle proteolytic system-related genes. A better understanding of the atrophy process will require thorough analysis of the importance of the responses of these target genes.

Muscle atrophy (increased muscle proteolysis) occurs as a serious side-effect of therapeutic corticosteroid hormone treatment (*22–24*), while hypercortisolism plays a major role in muscular atrophy in Cushing's disease (*25*, *26*). Indeed, Dex treatment induces dephosphorylation of Foxo1 and Foxo3a, resulting in up-regulated expression of atrogens, such as Atrogen1 and MuRF1, *in vivo* as well as *in vitro* (*8*, *9*). Foxo1 and Foxo3a mRNA in particular are up-regulated during fasting and Dex treatment (*27*, *28*). It has been reported that active Foxo proteins can activate the Atrogin1 and MuRF1 genes (*8*, *9*), and these studies also used C2C12 cells. Therefore, the present study examining the expression profiles of mRNA under various experimental conditions in C2C12 cells may be useful for researchers investigating such muscle atrophy.

This study defined the expression profiles of mRNA under various experimental conditions in C2C12 cells, which are used in *in vitro* models of muscle wasting. The expression profiles of these16 genes are distinctly up- or down-regulated in catabolic states. We considered that most changes in the expression levels of these mRNAs after exposure to DMSO or Dex are likely to reflect

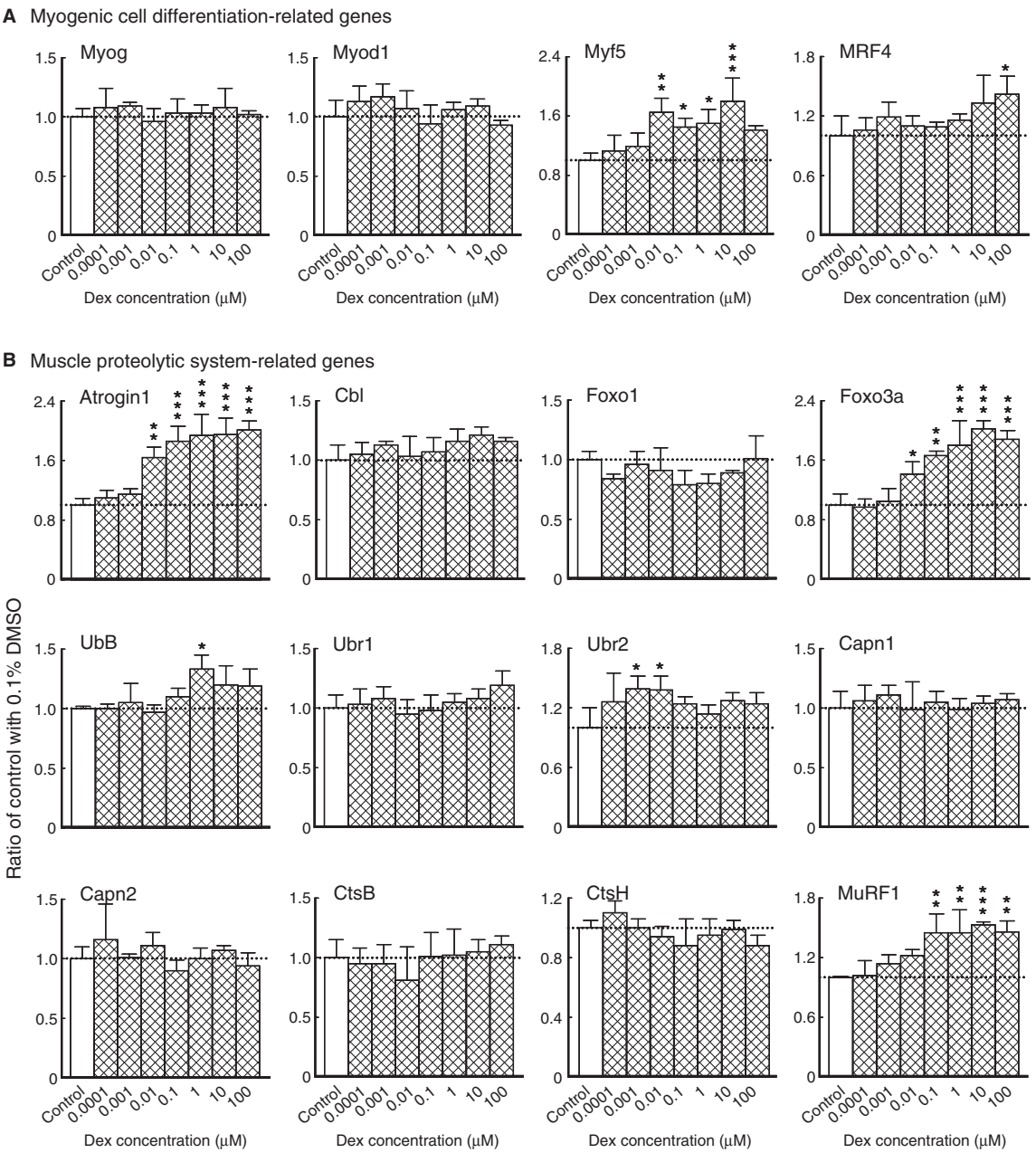

Fig. 4. **Effects of exposure to Dex on mRNA expression of myogenic cell differentiation-related genes (A) and muscle proteolytic system-related genes (B) in cultures of C2C12 myotubes.** C2C12 myotubes were treated with Dex (0.0001, 0.001, 0.01, 0.1, 1, 10 or 100 μM) for 4 h. Data are expressed as ratios of target mRNA to HPRT1 mRNA. Experiments (C2C12 myotube cultures) were performed in triplicate, and data are means ± SD. The dotted line is the value in controls at 0.1% DMSO, which was assigned a value of 1. Statistical analysis was performed using Dunnett's test. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ versus controls at 0.1% DMSO.

transcriptional changes, although differences in mRNA degradation rates or mRNA stability may also contribute to the changes described here. This analysis has thus provided valid information on the study of the atrophy process using C2C12 cells. A number of new and unexpected features in C2C12 myotubes after exposure to DMSO or Dex were identified in the present study.

In conclusion, this study provides a useful database of gene profiles that are differentially expressed throughout myogenic cell differentiation and the muscle proteolytic system in cultures of C2C12 cells. Furthermore, the results of the present study may also be useful for studying of atrophy in skeletal muscle.

CONFLICT OF INTEREST

None declared.

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