Age-related changes in the mitotic and metabolic characteristics of muscle-derived cells

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Barani, Aude E., Anne-Cécile Durieux, Odile Sabido, and Damien Freyssenet. Age-related changes in the mitotic and metabolic characteristics of muscle-derived cells. J Appl Physiol 95: 2089–2098, 2003; 10.1152/japplphysiol.00437.2003.—Age-related sarcopenia could partly result from cumulative repeated episodes of incomplete repair and regeneration. We hypothesized that mitotic and metabolic events associated with satellite cell activation and proliferation could be altered with aging. Muscle-derived cells (mdc) were isolated from gastrocnemius and quadriceps muscles of young (3 wk old), adult (9 mo old), and old (24 mo old) Sprague-Dawley male rats (n = 10/group). The mdc from young growing rats started to proliferate earlier compared with adult and old animals. Cell cycle duration was significantly reduced with aging from 36.5 ± 3.2 to 28.0 ± 2.2 h. However, the proportion of noncycling (G0 phase) and cycling (G1 + S + G2 + M phases) cultured mdc was statistically unchanged among the three age groups. Significantly lower increase in c-met and proliferating cell nuclear antigen expression were observed in cultured mdc of old rats upon serum stimulation. Major changes in the expression of citrate synthase, lactate dehydrogenase, proteasome, caspase 3, plasminogen activators (PAs), and matrix metalloproteinase 2–9 (MMP2–9) were observed upon serum stimulation, but no age-related difference was noted. However, when measured on crushed muscle extracts, PAs and MMP2–9 enzyme activities were significantly decreased with aging. Our results show that cellular and biochemical events associated with the control of mdc activation and proliferation occur with aging. These alterations may participate in the accumulation of repeated episodes of incomplete repair and regeneration throughout the life span, thus contributing to the loss of skeletal muscle mass and function with aging.

Sarcopenia is a term that refers to a loss of skeletal muscle mass associated with a decrease in muscle strength and an increased fatigability. Sarcopenia is the common denominator of the aging process, responsible for a general and substantial decline in physical performance, which leads ultimately to physical disability. Muscle loss during aging may partly depend on how a muscle repairs itself after damage. This includes overt injury but also the daily small damages that may not be perceived via pain or alteration in function. The capacity of skeletal muscle to regenerate relies on satellite cells, a population of myogenic precursor cells located between the basal lamina and the sarcolemma of mature myofibers (38). As the process of aging occurs, the number of satellite cells gets reduced in rat skeletal muscle (1, 26) and regeneration is less successful (14, 48). Furthermore, the repeated participation of satellite cells in muscle regeneration throughout the life span results in a significant reduction in their cumulative mitogenic potential (15, 19). Finally, because of a decrease in myofiber compliance due to fibrosis, aging increases the sensitivity to contraction-induced injury and muscle injury (13, 24, 55). Altogether, these data suggest that age-related loss of skeletal muscle mass and function may partly result from cumulative repeated episodes of incomplete repair (14).

Growth factors and hormones released during injury represent essential cues for muscle precursor cell activation and proliferation. Among them, hepatocyte growth factor (HGF) appears to have major roles in the initial recruitment of quiescent satellite cells (3, 18, 25, 52). HGF is expressed by quiescent satellite cells and skeletal muscle fibers (30) and acts in a paracrine/autocrine manner through activation of its transmembrane tyrosine kinase receptor c-met. The prominent role of HGF/c-met in satellite cell activation and myoblast proliferation during muscle growth and muscle regeneration has been described (30, 40, 52) and suggests that alterations in HGF/c-met expression may have profound effects on the mitogenic behavior of satellite cells. Maintenance of c-met expression with aging may thus be critical to ensure a proper response of skeletal muscle to damaging insults.

Regulation of energetic metabolism is necessary for the execution of a number of regulatory and biosynthetic events that occur during skeletal muscle regeneration. Recently, we found that quiescent satellite cells and proliferating myoblasts had opposite glycolytic and

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mitochondrial protein expression patterns, suggesting that the metabolic status of these cells may be functionally linked to their mitogenic status (7). Similarly, previous studies documented the importance of a tight regulation of mitochondrial content and activity during myoblast proliferation/differentiation (21, 29). Maintenance of a coordinated expression of glycolytic and mitochondrial enzymes with aging may thus be critical to ensure a proper response of skeletal muscle to damaging insults.

Cell cycle regulation of myoblasts is highly dependent on the proteasomal proteolysis of regulatory proteins such as cyclins and myoD (28, 53). Activation of plasminogen activators (PAs) and matrix metalloproteinases (MMPs) during skeletal muscle regeneration is also important for the expansion of proliferating cells and the activation/liberation of growth factors sequestrated in the extracellular matrix (ECM) (23, 35, 37). Furthermore, we recently described opposite and distinct expression patterns of these enzyme activities upon serum stimulation (7), suggesting that these adaptations may be crucial events involved in satellite cell activation and myoblast proliferation. Maintenance of proteinase expression with aging may thus be critical to ensure a proper response of skeletal muscle to damaging insults.

In the present study, we hypothesized that, if age-related loss of skeletal muscle mass and function may partly result from cumulative repeated episodes of incomplete repair, molecular events associated with the control of satellite cell activation and myoblast proliferation could be altered with aging.

Flow cytometry analysis of cultured mdc proliferation. Analysis of mdc proliferation of young and old rats was performed as previously described (7). Briefly, 7-day cultured mdc were harvested and labeled with PKH26 (Sigma, Saint Quentin Fallavier, France), a fluorescent reporter molecule, which incorporates into the cell membrane and is equally distributed to daughter cells after division. Cells (2 × 10^5 to 5 × 10^6 cells) were then allowed to proliferate. At different time points, 5 × 10^3 cells were acquired on a FACSStar™Fluos cell sorter (BD Biosciences, San Jose, CA) with the use of the 488-nm line of an Innova 90–5 W argon laser (Coherent, Palo Alto, CA) with a 585/42-nm band pass filter by using the Cell Quest program (BD Biosciences). Deconvolution of cell fluorescence was analyzed by using the Cell Proliferation Model program run on ModFit 2.0 (Verity Software House, Palo Alto, CA). Kinetic parameters of muscle cell proliferation (lag period and generation time) were calculated as previously described (7). Cell samples were also examined under a fluorescence microscope (Leica DMRB). Images were recorded with a video camera (Sony DXC 950P camera) and Visiolas 2000 software (Biocom, Les Ulis, France).

Isolation of muscle-derived cells. Animals were euthanized with an intraperitoneal injection of Nesodonal. Gastrocnemius and quadriceps muscles were removed, carefully cleaned from fat and connective tissues, and then weighed. Muscle-derived cells (mdc) from individual rats were isolated by pronase digestion (9, 27) with some modiﬁcations (7). Cells (3 × 10^4) were then either directly used for analyses (initial mdc) or subjected to pronase digestion (9, 27) with some modiﬁcations (7). Cells (3 × 10^4) were acquired at a stabilized flow rate (100 events/s) with a FACSStar™Fluos cell sorter using band pass filters of 530/30 and 675/40 nm for green (DNA) and red (RNA) ﬂuorescences. To exclude aggregated and multinucleated cells, pulse processing was activated. DNA green signals of AO were processed as area and width signals and then gated for double exclusion. Data were acquired by using the Cell Quest program. To assess the speciﬁcity of DNA and RNA signals, DNase and RNase control samples were systematically done. For each assay, L6E9 cells were used as a positive control of muscle cell proliferation.

Immunolabeling. Desmin (D33, Dako, Trappes, France) intracellular immunolabeling was performed on 7-day cultured mdc. Proliferating cell nuclear antigen (PCNA) (PC10, Neomarkers, Fremont, CA) and c-met (sc-162, Santa Cruz, CA) intracellular immunolabeling were performed on initial and 7-day cultured mdc. Fixation, permeabilization, and labeling were performed using IntraStain kit (Dako) according to the manufacturer’s instruction. Cells (10^4) were incubated for 45 min on ice with a primary antibody directed against desmin (1:20 dilution), or PCNA (1:20 dilution), or c-met (1:1). Cells were then incubated for 30 min on ice with the appropriate FITC-conjugated secondary antibody (1:20, 1:20, and 1:1 dilution for desmin, PCNA, and c-met, respectively). Cells were then suspended in PBS 2% formaldehyde and 5 × 10^3 cells were acquired on a FACSStarFluos cell sorter with a 530/30 nm band pass filter by using the Cell Quest program.

For desmin immunocytochemistry, 7-day culture dishes were washed with PBS, ﬁxed with PBS 4% formaldehyde (10 min at 4°C), and permeabilized with a PBS-0.1% Triton X-100 solution (5 min at 4°C). Culture dishes were saturated for 1 h with PBS-1% BSA and then incubated for 1 h at 37°C with desmin antibody (1:100 dilution). A FITC-conjugated secondary antibody (1:20, 1:20, and 1:1 dilution for desmin, PCNA, and c-met, respectively). Cells were then suspended in PBS 2% formaldehyde and 5 × 10^3 cells were acquired on a FACSStarFluos cell sorter with a 530/30-nm band pass filter by using the Cell Quest program.

Protein extraction. Initial and cultured mdc of the three age groups were suspended (10^7 cells/ml) in a buffer consist-
aging of 20 mM HEPES and 2 mM EDTA, pH 7.2. Cells were then forced through a syringe needle (301/2 gauge), submitted to three freezing-thawing cycles, and centrifuged (12,000 g x 10 min). The resulting supernatant was stored at -80°C. Protein concentration was spectrophotometrically measured at 750 nm (Bio-Rad DC kit protein assay).

Crushed muscle extracts. Crushed muscle extracts were prepared from the extensor digitorum longus, plantaris, soleus, and tibialis anterior muscles of young and old rats, as previously described (11). Brieﬂy, muscles were gently crushed with forceps in cold PBS (1:4 wt/vol) and then incubated for 90 min at 4°C with gyratory shaking. The liquid phase containing released substances was collected by centrifugation (12,000 g at 750 nm (Bio-Rad DC kit protein assay).

Enzyme activities. Enzyme assays were performed on initial and cultured mdc of the three age groups. Citrate synthase activity (CS; EC 4.1.3.7), lactate dehydrogenase activity (LDH; EC 1.1.1.27), chymotrypsin-like activity of 20S proteasome (EC 3.4.25.1), PAs activity (EC 3.4.21.73 and EC 3.4.21.68), and MMP2–9 activity (EC 3.4.24.24 and EC 3.4.25.35) were fluorometrically measured as previously described (7). For caspase 3 activity, 30 µg of protein were added to 1 ml of assay buffer (10 mM HEPES, 2 mM EDTA, pH 7.4). The assay was started by the addition of 100 µM of Ac-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin (Bachem, I-1660). Enzyme activity was fluorometrically recorded at λex = 380 nm and λem = 460 nm on a SFM25 fluorimeter (Kontron Instruments).

PAs and MMP2–9 enzyme activities were also fluorometrically determined on crushed muscle extracts of young and old animals, as previously described (7). MMP2–9 enzyme activity was monitored for 24 h.

Statistical analyses. Values are means ± SE. Two-way ANOVA was used to evaluate the effects of cell type (initial vs. cultured mdc) and aging on PCNA expression, c-met expression, and enzyme activities. Post hoc comparisons were performed with Fisher’s protected least signiﬁcance difference test. One-way ANOVA was used to evaluate the effects of aging on body weight, muscle mass, muscle mass/body weight ratio, the number of satellite cells per gram of muscle, kinetic parameters of mdc proliferation, DNA content, and RNA content. Crushed muscle extract enzyme activities of young and old animals were compared with unpaired t-test. A P < 0.05 was considered signiﬁcant.

RESULTS

Aging induces skeletal muscle atrophy. Body weight was signiﬁcantly increased between young (54 ± 3 g), adult (546 ± 15 g), and old (638 ± 34 g) animals. Gastrocnemius and quadriceps muscle mass increased signiﬁcantly in adult compared with young rats, whereas muscle mass of old rats decreased signiﬁcantly compared with that of adult rats (Fig. 1). Expressed as a percentage of body weight, gastrocnemius and quadriceps muscle wet weight was decreased in old compared with adult (P < 0.01) and young (P < 0.05) animals. Taken together, these data were indicative of age-related atrophy in the gastrocnemius and quadriceps muscles of old rats.

Cell yield and myogenicity. Cell yield was statistically unchanged between adult (0.41 ± 0.03 x 10^6 cells/g of muscle) and old rats (0.58 ± 0.04 x 10^6 cells/g of muscle). By contrast, cell yield was signiﬁcantly decreased in both adult and old rats compared with young rats (3.23 ± 1.10 x 10^6 cells/g of muscle; both P < 0.01). Flow cytometry analysis of desmin expression on 7-day cultured mdc indicated that we harvested 71.1 ± 7.4, 85.2 ± 5.5, and 79.5 ± 7.0% of desmin-positive cells in young, adult, and old rats, respectively. No statistical difference was noted. Figure 2 is a representative fluorescence microscopy image of desmin expression in 7-day cultured mdc of adult rats.

Kinetic parameters of mdc proliferation, DNA and RNA contents. A flow cytometry analysis of PKH26 membrane ﬂuorescence was used to determine the distribution of cultured mdc in different generations

Fig. 1. Gastrocnemius and quadriceps muscle mass and muscle weight to body weight ratio as a function of age. Results are means ± SE (n = 8). †††P < 0.001, signiﬁcantly different from adult and old rats. #P < 0.05, signiﬁcantly different from adult rats. *P < 0.05 and **P < 0.01, signiﬁcantly different from old rats.

Nuclei are stained with DAPI (blue). Magnification, ×200.
over time (7). Figure 3 illustrates the decrease in PKH26 membrane labeling following divisions. A lag period ($T_{\text{lag}}$) was systematically observed until cells started to proliferate (Table 1). $T_{\text{lag}}$ in cultured mdc of young animals was shorter compared with those obtained in adult ($P < 0.05$) and old ($P < 0.08$) animals. The generation time ($T_g$) was lower in young compared with old animals ($P < 0.06$). When a contrast analysis was used to compare the value obtained in old animals with the mean value of young and adult animals, the $T_g$ was significantly lower in cultured mdc of old animals compared with those of young and adult rats ($P = 0.05$).

Interestingly, the presence of quiescent cells with low DNA and RNA contents was systematically observed in cultured mdc of the three age groups. A representative profile is presented in Fig. 4A. As indicated by the DNA content analysis (Fig. 4B), the relative proportion of noncycling ($G_0$ phase) and cycling ($G_1 + S + G_2 + M$ phases) cultured mdc was statistically unchanged among the three age groups. The proportion of quiescent cultured mdc compared favorably with the relative proportion of cells with low RNA content (6.2 ± 1.1, 5.8 ± 1.0, and 4.8 ± 0.6% in young, adult, and old animals, respectively).

Aging downregulates c-met and PCNA expressions upon serum stimulation. The percentage of c-met-positive cells was statistically the same in initial mdc of the three age groups (Fig. 5A). The relative proportion of c-met-positive cells was significantly increased upon serum stimulation. However, the extent of this increase was significantly lower in old compared with young animals. Expressed as a relative increase, the stimulation of c-met expression in cultured mdc was significantly lower in old (131 ± 9%) compared with young (189 ± 5%) and adult (190 ± 4%) rats (both $P < 0.001$). Cell surface density of the receptor was statistically the same in initial mdc of three age groups (Fig. 5B) and was dramatically increased in response to serum exposure ($P < 0.001$). This increase was virtually the same in the three age groups.

As expected, a majority of initial mdc was PCNA negative in young (97.1 ± 0.6%), adult (94.7 ± 0.8%), and old (96.2 ± 0.9%) rats. By contrast, a majority of cultured mdc was PCNA positive in the three age groups (Fig. 6). Interestingly, the percentage of PCNA-positive cells in

Table 1. Effects of aging on the kinetic parameters of cultured mdc

<table>
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<tr>
<th>Parameters</th>
<th>$T_{\text{lag}}$, h</th>
<th>$T_g$, h</th>
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<tbody>
<tr>
<td>Young</td>
<td>22.2 ± 5.9</td>
<td>36.5 ± 3.2</td>
</tr>
<tr>
<td>Adult§</td>
<td>49.0 ± 6.5*</td>
<td>34.0 ± 2.8</td>
</tr>
<tr>
<td>Old †</td>
<td>40.0 ± 7.9†</td>
<td>28.0 ± 2.2‡</td>
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Values are means ± SE ($n = 6–8$ group). Cultured muscle-derived cells (mdc) were labeled with PKH26 and allowed to proliferate. Cells were then harvested at different time points and analyzed by flow cytometry. Deconvolution of the overall fluorescence patterns into peaks provided the number and relative abundance of each daughter generation. ModFit 2.0 analysis calculated lag time ($T_{\text{lag}}$) and generation time ($T_g$). *$P < 0.05$, significantly different from young rats. †$P < 0.08$, when compared with young rats. ‡$P < 0.06$, when compared with young rats. §These data were originally described in Ref. 7.

Fig. 3. PKH26 fluorescence decreases as cultured mdc divide. A representative image of membrane labeling fluorescence of cultured mdc immediately at the end of the staining procedure (A) and after 3 (B), 5 (C), and 7 days (D) in proliferation medium. Magnification, ×630.

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old rats was significantly lower than those reported for young and adult animals. In initial mdc, enzyme activity of the mitochondrial matrix enzyme CS was significantly lower in adult compared with young and old rats, whereas LDH enzyme activity was virtually the same in the three age groups. Percentage of noncycling cells was determined with G0 gate. The remaining population corresponded to cycling cells. Results are means ± SE (n = 5–9/group).

**Proteasome and caspase 3 enzyme activities.** Proteasome enzyme activity was significantly lower in initial mdc of old rats compared with young rats (P < 0.001; Table 3). Proteasome enzyme activity was significantly increased in response to serum stimulation. No age-related difference was observed. Caspase 3 enzyme activity was about sixfold lower in crushed

related difference was observed. Caspase 3 enzyme activity was virtually the same in initial mdc of the three age groups.

**PAs and MMP2–9 enzyme activities.** PAs enzyme activity was the same in initial mdc of the three age groups (Table 4). Serum stimulation decreased by about fivefold PAs enzyme activity in cells of young, adult, and old rats (P < 0.001). No age-related difference was observed. MMP2–9 enzyme activity measured in initial mdc was virtually the same in the three age groups. Serum exposure significantly increased MMP2–9 enzyme activity in mdc of young, adult, and old rats (P < 0.001). No age-related difference was noted.

When measured on crushed muscle extracts, major age-related differences were observed (Fig. 7). PAs enzyme activity was about sixfold lower in crushed

old rats was significantly lower than those reported for young (P < 0.05) and adult animals (P < 0.01).

**CS and LDH enzyme activities.** In initial mdc, enzyme activity of the mitochondrial matrix enzyme CS was significantly lower in adult compared with young and old rats, whereas LDH enzyme activity was virtually the same in the three age groups (Table 2). CS enzyme activity was strongly reduced in response to serum stimulation (P < 0.001), whereas LDH enzyme activity was dramatically increased by about 10-fold (P < 0.001). No age-related difference was noted.

**Proteasome and caspase 3 enzyme activities.** Proteasome enzyme activity was significantly lower in initial mdc of old rats compared with young rats (P < 0.001; Table 3). Proteasome enzyme activity was significantly increased in response to serum stimulation. No age-related difference was observed. Caspase 3 enzyme activity was virtually the same in initial mdc of the three age groups. Caspase 3 enzyme activity was significantly increased to the same extent in the three age groups upon serum stimulation.
The number of PCNA-positive cells was lower in cultured mdc of young, adult, and old rats. The fluorescence intensity of PCNA-positive cells was the same in the 3 age groups. Isotypic control corresponds to PCNA-negative cells. Fluorescence microscopy indicated that a large majority of cultured mdc were myogenic. These cells isolated from skeletal muscle. Analysis of desmin expression in 7-day cultures by flow cytometry and immunofluorescence microscopy indicated that a large majority of cultured mdc were myogenic. These observations are in agreement with previously reported studies (2, 7, 27, 44).

We first determined whether aging altered the capacity of mdc to proliferate in response to mitogenic signals. Our data indicate that the first division was delayed by ~24 h in cultured mdc of adult and old animals compared with young animals. Mdc from young growing animals may thus attach more easily to the culture dish and start to proliferate earlier. The mean cell cycle time reported in the present study is in agreement with the one reported by Schultz (50) in rat skeletal muscle in vivo but differs notably from the 12–22 h previously reported in single rat fiber culture (9, 10) or in mdc from newborn mice (45). Such differences can be attributed to the method used to obtain mdc and to measure $T_g$, to the culture conditions, as well as the animal species, and the age of the animals. By contrast to previous studies reporting a decrease in the proliferation potential (51) or no change (20) in

**Table 3. Proteasome and caspase 3 enzyme activities in initial and cultured mdc of young, adult, and old rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial mdc</th>
<th>Cultured mdc</th>
<th>Initial mdc</th>
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<tr>
<td>Young</td>
<td>315.2 ± 20.0</td>
<td>520.3 ± 63.0†</td>
<td>1.58 ± 0.27</td>
<td>10.12 ± 1.11†</td>
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<tr>
<td>Adult</td>
<td>260.1 ± 27.0</td>
<td>617.4 ± 77.7†</td>
<td>1.61 ± 0.09</td>
<td>9.72 ± 0.56†</td>
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<tr>
<td>Old</td>
<td>208.0 ± 11.8*</td>
<td>490.1 ± 69.2†</td>
<td>1.52 ± 0.23</td>
<td>10.93 ± 1.16†</td>
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</table>

Values are means ± SE (n = 4–6/group). Proteasomes and caspase 3 enzyme activities were fluorometrically measured and expressed as pmol-min⁻¹-mg⁻¹ of protein. *P < 0.001, significantly different from young rats. †P < 0.001, significantly different from initial mdc.

**Table 4. PAs and MMP2–9 enzyme activities in initial and cultured mdc of young, adult, and old rats**

- **PAs**
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<th>Parameters</th>
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<th>Cultured mdc</th>
<th>Initial mdc</th>
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<tr>
<td>Young</td>
<td>921.8 ± 47.1</td>
<td>159.4 ± 15.5*</td>
<td>7.3 ± 1.4</td>
<td>12.8 ± 1.6*</td>
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<tr>
<td>Adult</td>
<td>886.4 ± 50.6</td>
<td>152.4 ± 10.5*</td>
<td>7.4 ± 1.7</td>
<td>16.3 ± 1.0*</td>
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<tr>
<td>Old</td>
<td>797.0 ± 50.7</td>
<td>156.7 ± 14.1*</td>
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- **MMP2–9**
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Values are means ± SE (n = 4–7/group). Plasminogen activators (PAs) and matrix metalloproteinase 2 and 9 (MMP2–9) enzyme activities were fluorometrically measured and expressed as pmol-min⁻¹-mg⁻¹ of protein and relative light U-min⁻¹-mg⁻¹ of protein, respectively. *P < 0.05, significantly different from initial mdc.

**DISCUSSION**

Satellite cells have been originally defined from their histological situation between the basement membrane and the sarcolemma (38). Once isolated, the origin of these cells cannot be ascertained because committed myogenic cells within mature skeletal muscle constitute distinct cell populations (8, 39, 46). For these reasons, we refer in the present study to mdc for such cells isolated from skeletal muscle. Analysis of desmin expression in 7-day cultures by flow cytometry and immunofluorescence microscopy indicated that a large majority of cultured mdc were myogenic. These observations are in agreement with previous reports (2, 7, 27, 44).

We first determined whether aging altered the capacity of mdc to proliferate in response to mitogenic signals. Our data indicate that the first division was delayed by ~24 h in cultured mdc of adult and old animals compared with young animals. Mdc from young growing animals may thus attach more easily to the culture dish and start to proliferate earlier. The mean cell cycle time reported in the present study is in agreement with the one reported by Schultz (50) in rat skeletal muscle in vivo but differs notably from the 12–22 h previously reported in single rat fiber culture (9, 10) or in mdc from newborn mice (45). Such differences can be attributed to the method used to obtain mdc and to measure $T_g$, to the culture conditions, as well as the animal species, and the age of the animals. By contrast to previous studies reporting a decrease in the proliferation potential (51) or no change (20) in

**Table 2. CS and LDH enzyme activities in initial and cultured mdc of young, adult, and old rats**

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<th>Cultured mdc</th>
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<tr>
<td>Young</td>
<td>593.9 ± 99.5†</td>
<td>48.9 ± 6.5‡</td>
<td>107.4 ± 25.6</td>
<td>984.2 ± 20.4‡</td>
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<tr>
<td>Adult</td>
<td>158.4 ± 32.0*</td>
<td>54.0 ± 6.5‡</td>
<td>78.9 ± 10.3</td>
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<td>53.5 ± 4.2‡</td>
<td>79.8 ± 8.0</td>
<td>929.1 ± 45.2*</td>
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Values are means ± SE (n = 5–7/group). Lactate dehydrogenase (LDH) and citrate synthase (CS) enzyme activities were fluorometrically measured and expressed as pmol-min⁻¹-mg⁻¹ of protein. *P < 0.01 and †P < 0.001, significantly different from adult rats. ‡P < 0.01, significantly different from initial mdc.

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Alterations of the in vitro kinetic behavior of cultured mdc with aging may reflect differences in the expression of metabolic enzymes in satellite cells and/or preserve the population of satellite cells in skeletal muscle (1, 7, 8). Surprisingly, no age-related difference was noted in the relative proportion of these cells in response to muscle injury. This may lead to a decrease in the number of satellite cells that could be involved in subsequent muscle regeneration (15, 48) and ultimately contribute to the accumulation of repeated episodes of incomplete repair.

Cultured mdc were also characterized by the presence of noncycling cells. Such cells may have remained quiescent and/or had returned to quiescence after a few divisions. In vivo, these cells may contribute to renew and/or preserve the population of satellite cells in skeletal muscle (1, 7, 8). Surprisingly, no age-related difference was noted in the relative proportion of these cells. As suggested above, a shorter $T_g$ with aging may exhaust the replicative potential of mdc. Therefore, one could expect an increase in the proportion of noncycling cells in cultured mdc of old animals. Our results rather suggest that replicative senescence has not been reached in cultured mdc of old animals and that the capacity to maintain the pool of satellite cells following muscle injury was still preserved in old rats.

Differences in the kinetic behavior of cultured mdc with aging may reflect differences in the expression patterns of factors involved in satellite cell activation, proliferation, and migration. HGF appears to be an important mediator of these processes by its mitogenic, mitogenic, and morphogenic actions (3, 25, 52). In the present study, approximately one-half of the whole cell population expressed c-met in the three age groups at the end of the isolation procedure. Considering that HGF stimulates the precocious entry of quiescent satellite cells into the cell cycle (3, 52), our data suggest that c-met-positive cells could be activated earlier than c-met-negative cells upon serum stimulation. The c-met expression (relative proportion of positive cells and cell surface density) was significantly increased in response to serum stimulation in the three age groups, further illustrating the importance of HGF/c-met signaling in mdc activation and proliferation (7). Importantly, the increase in the relative proportion of c-met expressing cells was lower in cultured mdc of old animals. Considering that HGF is essential for satellite cell activation (3, 25, 40, 52), a decreased proportion of c-met-positive cells with aging could potentially impair or delay the onset of muscle repair/regeneration. However, it should be noted that the cell surface density of c-met was preserved with aging. Binding of HGF to its receptor c-met triggers the synthesis of multiple cell cycle regulatory proteins, such as cyclin D1 and PCNA (3). PCNA, a cofactor of the DNA polymerase δ mainly synthesized in S phase, was used as a marker of cell proliferation. In skeletal muscle, PCNA expression indicates the occurrence of a regeneration process (21). The proportion of initial mdc expressing PCNA was low (<6%) and was statistically the same in the three age groups. If skeletal muscle from old animals has an increased need for activated satellite cells, one may suspect to get an increased proportion of PCNA-positive cells at the end of the isolation procedure. However, this would only occur if skeletal muscle of old animals were severely injured. By contrast, if discrete episodes of incomplete repair and regeneration occur with aging, the increase in activated satellite cells may not be necessarily detected. Another important finding of the present study was the lower relative increase in the proportion of PCNA-positive cells upon serum stimulation in mdc of old rats. This result contrasts with the increased proliferation rate observed for these cells but could be explained by a lower protein synthesis rate and/or by an increase rate of protein degradation, suggesting a reduction in half-life of PCNA with aging. Furthermore, a number of studies suggested that PCNA expression could be linked to downstream signaling events triggered by HGF (3, 4). Therefore, the concomitant lower increase in the proportion of PCNA-positive cells could be related to the decreased proportion of c-met-positive cells. A c-met/PCNA costaining would be necessary to answer this question.

A number of reports suggested that a tight regulation of glycolytic and mitochondrial oxidative metabolisms may be functionally linked to the mitotic status of muscle cells and may contribute to the regulation of mdc proliferation and differentiation (21, 29). In the present study, initial and cultured mdc had opposite glycolytic and mitochondrial oxidative potentials, as determined by LDH and CS enzyme activities. In agreement with our previous report (7), these results indicate a shift toward an increase in the relative contribution of glycolytic metabolism for energy production in cultured mdc. This argues strongly in favor of a metabolic control of mdc proliferation. A lower CS enzyme activity was observed in initial mdc of adult rats but not with aging.
rats. It has been proposed that satellite cell mitochondria are partly synthesized in early G1, just before the exit from the cell cycle (7, 34). A decreased recruitment of satellite cells would lengthen the duration of the quiescent state of these cells and thus determine a lower renewal of the mitochondrial population leading to a decrease in mitochondrial content. Because satellite cells are frequently recruited in young growing rats and old rats, because of muscle growth (41, 49, 50) and increased muscle susceptibility to injury (13, 24, 55), respectively, the decreased mitochondrial fraction in initial mdc of adult rats could result from a lower recruitment of satellite cells in adult skeletal muscle. Despite the lower CS enzyme activity in initial mdc of adult rats, no age-related difference was observed in initial and cultured mdc, suggesting that the metabolic control of mdc proliferation was not altered with aging.

Proteasomal proteolysis exerts important functions beyond that of muscle protein degradation during muscle atrophy and catabolic states, such as the control of muscle cell proliferation through the selective degradation of short-lived cell cycle regulatory proteins and muscle transcription factors (28, 53). In the present study, proteasome enzyme activity was tightly related to the mitotic status of mdc, further suggesting a potential role for a proteasomal-dependent proteolysis in the regulation of muscle cell activation and proliferation (7). In agreement with this hypothesis, we previously observed a tight correlation between proteasome enzyme activity and PCNA expression during skeletal muscle regeneration (22). Furthermore, the proteasomal-dependent proteolysis of regulatory molecules, such as MyoD and cyclin E, is necessary for the progression through G1-S phase (33, 53). In this context, the lower proteasome enzyme activity observed in initial mdc of old rats may delay in vivo the reentry of satellite cells in late G1/S phase. As recently described for proteasome, a number of reports suggest that caspases are also involved in the control of cell cycle progression (for a review, see Ref. 36). This was also suggested in the present study because caspase 3 enzyme activity was significantly increased upon serum stimulation in the three age groups, whereas no sign of cell death was evident in the culture dish (data not shown). These data support the idea that, under non-apoptotic conditions, activation of pathways leading to caspase activation results in cell cycle progression. This hypothesis is also in agreement with a recent study (17) showing that TNF-α-mediated caspase activation inhibits myoblast differentiation in absence of cell death.

The broad spectrum of actions performed by PAs and MMP2–9, such as growth factor activation (37, 42), ECM remodeling (12, 32, 35), and myoblast migration (23, 43, 54), suggests that changes in these enzyme activities with aging may contribute to incomplete episodes of regeneration. In the present study, opposite expression patterns of PAs and MMP2–9 enzyme activities were observed upon serum stimulation. These expression patterns were similar in the three age groups. The decrease in PAs enzyme activities upon serum stimulation probably does not reflect an overall decrease in extracellular proteinase enzyme activity but rather suggests that other proteinases, such as MMP2–9, are activated. This is in agreement with data showing that PAs are able to initiate a proteolytic cascade leading to MMP2–9 activation (6, 31). Importantly, PAs and MMP2–9 are secreted proteases whose biological functions mainly depend on their extracellular concentrations. One may therefore suspect that measurement of enzyme activities from mdc protein extracts does not necessarily reflect their extracellular enzyme activities. Crushing mimics muscle injury processes, and factors present in crushed muscle extracts are likely to be the factors released during normal tissue injury. These factors originate mostly from skeletal muscle fibers (11, 16). When measured in crushed muscle extracts, PAs and MMP2–9 enzyme activities of old animals were strongly decreased. This may have important physiological implications for the regenerative response of skeletal muscle. Indeed, as the process of aging occurs, a reduced turnover of ECM proteins concomitant to the appearance of posttranslational modifications of ECM proteins is observed (5). Together with a decrease in PAs and MMP2–9 enzyme activities, this may functionally alter muscle regeneration by slowing down myoblast migration and decreasing growth factor biodisponibility (31, 32, 42). Particularly, the proteolytic activation of HGF by PAs could be reduced (37), thus potentially contributing to impair the mitogenic behavior of satellite cells. Furthermore, the ability of activated HGF to transactivate urokinase-type PA (uPA) promoter could also be reduced (47), leading to a decreased uPA expression. Overall, this may reduce HGF-induced ECM degradation and uPA synthesis during muscle regeneration.

In conclusion, our results show that cellular and biochemical events associated with the control of mdc activation and mdc proliferation are altered with aging. We propose that these alterations could be of physiological relevance during the life span and may contribute to the accumulation of repeated episodes of incomplete repair and regeneration of skeletal muscle, thus contributing to the loss of muscle mass and function with aging.

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