Potential role for Id myogenic repressors in apoptosis and attenuation of hypertrophy in muscles of aged rats

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Alway, Stephen E., Hans Degens, Gururaj Krishnamurthy, and Cheryl A. Smith. Potential role for Id myogenic repressors in apoptosis and attenuation of hypertrophy in muscles of aged rats. Am J Physiol Cell Physiol 283:C66–C76, 2002. First published February 13, 2002; 10.1152/ajpcell.00598.2001.—Aging attenuates the overload-induced increase in myogenic regulatory transcription factor (MRF) expression and the extent of muscle enlargement. To identify whether mRNA levels of repressors of the MRFs are greater in overloaded muscles from aged animals, overload was achieved in plantaris muscle of aged (33 mo; n = 14) and adult (9 mo; n = 17) rats. After 14 days, plantaris muscles in the overloaded limb were ~25% and 6% larger in adult and aged rats, respectively, compared with the contralateral limb. Hypertrophied muscles of adult rats had significantly greater levels of mRNA and protein levels for myogenin and MyoD compared with control muscles, but neither MRF increased with overload in muscles of aged rats. Muscles of aged rats had greater Id mRNA (150–700%) and protein repressor (200–6,000%) levels compared with adult rats. BAX and caspase 9 protein levels were 9,500% and 300% greater, respectively, in both control and hypertrophied muscles of aged rats compared with young adult rats. These data are consistent with the hypothesis that aging increases Id transcripts that activate apoptotic pathways involving BAX. This may contribute to sarcopenia by attenuating MRF protein levels in muscles of old animals.

sarcopenia; muscle atrophy; transcription factors; aging; MyoD; myogenin

MYOGENIC REPRESSOR PROTEINS (e.g., Id-2, Id-2, Id-3) are basic helix-loop-helix (bHLH) proteins that act as negative regulators of the myogenic regulatory transcription factor (MRF) family in a variety of ways. For example, Id repressors suppress the formation of MRF-E protein heterodimers (48), which bind to a CANNTG consensus or “E box” domain and transactivate downstream muscle genes such as myosin light chain (MLC), desmin, and creatine kinase (26), by sequestration of E proteins (51, 52). The action of MRFs might also be inhibited by the formation of MRF-Id heterodimers that are unable to bind to the E boxes and in this way prevent the initiation of transcription (7, 8, 17, 25).

Id proteins increase under conditions that impair neural transmission to the muscle such as denervation (22), and Id-1 is highest in fibers undergoing the greatest atrophy. Furthermore, Id proteins have been proposed to prevent muscle differentiation and the concomitant expression of muscle-specific genes (5, 17, 25, 40, 48). Moreover, recent observations have suggested that Id repressors may have dual functions in regulating cell function. For example, Id-2 has been shown to play a role in promoting apoptosis in nonmuscle cell lines (21, 38). However, the relationship between Id proteins and apoptosis in adult and aged muscles has not yet been investigated.

We found (2) elevated mRNA and protein levels of Id repressors in fast muscles of aged rats compared with young rats, suggesting that Id repressors are involved in sarcopenia during aging. Part of the mechanism responsible for the attenuated overload-induced hypertrophy in muscles of aged animals (30, 32) might be an elevated level of Id proteins. In line with this suggestion is the attenuated increase in MRF protein (2, 3) and mRNA (50) levels in response to changes in muscle loading in old age. In addition, the elevated Id repressor levels in old age may impair muscle growth via their activation of apoptotic pathways, counterbalancing the trophic stimulus. Therefore, we hypothesized that the elevated repressor levels in old age correlate with 1) an attenuated increase in MRF levels, 2) an increased level of markers of apoptosis, and 3) a consequently attenuated increase in muscle mass as a response to muscle overload. Because aging preferentially affects muscle fibers containing fast myosin, we chose to look at responses to an overload in the fast plantaris muscle.

Here we report that levels of Id repressors were greater in plantaris muscles of aged rats compared with muscles in young adult rats. Although Id levels increased with overload in muscles of young rats, there...
was no further increase in Id mRNA or protein levels in overloaded muscles of aged rats. The levels of the apoptotic markers BAX and caspase 9 are elevated in both control and overloaded muscles of aged rats, whereas the abundance of these markers of apoptosis was low or undetectable in control and hypertrophied muscles of young rats. These data suggest that an elevation of repressor transcripts in old age might activate apoptotic pathways involving BAX. We suggest that this mechanism contributes to sarcopenia in old age and, together with the attenuated change of MRF levels during hypertrophy, provides an explanation for the suppressed hypertrophic response in old age.

METHODS

Animals

Experiments were conducted on young adult (9 mo; n = 12) and aged (37 mo; n = 9) Fischer F344 × Brown Norway F1 hybrid male rats (FBN; Harlan, Indianapolis, IN). In addition, we examined young adult (6 mo; n = 5) and aged (26 mo; n = 5) Fischer F344 (F344) rats. The rats were housed separately in pathogen-free conditions at 20°C and aged (37 mo; n = 9) Fischer 344 (F344) rats. The rats were housed in the same PCR product.

RT-PCR Estimates of mRNA for Repressor and MRF Genes

Because RNA quantities were insufficient to run additional RPAs, semi-quantitative RT-PCR analysis was conducted as described in detail elsewhere (2). Briefly, total RNA was extracted from plantaris muscles treated with DNase I (Ambion) and reverse transcribed (RT) with oligo dT primers and procedures detailed in the Guide for the Care and Use of Laboratory Animals as published by the US Department of Health and Human Services and proclaimed in the Animal Welfare Act (PL89-544, PL91-979, and PL94-279).

RNase Protection Assays for MRF Genes

RNase protection assays (RPAs) were performed according to the directions from the manufacturer (Ambion, Austin, TX) with riboprobes and hybridization conditions previously described in detail (3, 30, 32). Briefly, 50 μg of total RNA from plantaris muscles was used for each RPA. Positive and negative control samples consisted of all riboprobes and either yeast mRNA or mouse liver mRNA. Full-length riboprobes (≥10% larger than protected fragments) were observed when RNase digestion was omitted (positive controls), whereas protected fragments were not observed when control samples were digested with RNase (negative controls). Century Marker Template (Ambion) was transcribed with biotin-14-CTP and used as size standards in the RPAs. Protected fragments, controls, and standards were electrophoresed on 5% acrylamide-8 M urea gels, electroblotted onto positively charged nylon membranes, and immobilized by ultraviolet cross-linking. Chemiluminescence was used to detect the signals (BrightStar BioDetect nonisotopic detection kit; Ambion) and made visible by exposure to film (BioMax MS-1; Eastman Kodak, Rochester, NY) for 2–4 h.

Induction of Hypertrophy of Plantaris Muscles

Rats were placed under general anesthesia of 2% isoflurane and 1.5 l oxygen/min. After reflex activity had disappeared, the tibial nerve was dissected free from the surrounding tissue just proximal to the cranial border of the gastrocnemius muscle. Care was taken to avoid disrupting blood vessels and damaging the tibial nerve. The branches of the tibial nerve that innervate the medial and lateral heads of the gastrocnemius muscle and the soleus muscle were transected as close to their entry point to the muscle belly as possible (19). The sectioned nerves were reflected proximally and sutured to a segment of the hamstring muscles with 4-0 suture silk to ensure that the nerve stump did not reinnervate the plantar flexor muscles. Innervation to the plantaris and deep toe flexor muscles was left intact so that the animals could ambulate normally around the cages, thereby overloading the plantaris muscle and inducing muscle growth. After experimental denervation, the hamstring muscle layers were closed with absorbable suture and the skin incision was closed with 9-mm wound clips. The contralateral limb served as the intra-animal control. Animals received 0.3 mg of buprenex subcutaneously as an analgesic at the end of surgery. The animals recovered quickly and were alert and walking within ~45 min after surgery. A subset of animals underwent sham surgeries on the control limb, in which the plantar flexor muscles and the branches of the tibial nerve innervating them were freed from the surrounding tissue but the nerves were not cut. Because the muscle weights and gene expression of sham-operated and unoperated control muscles did not differ, the control limb was not sham operated in most animals.

Fourteen days after surgery, control and overloaded plantaris muscles were removed, quickly weighed, frozen in liquid nitrogen, and then stored at −80°C. Animals were euthanized with an overdose of pentobarbital sodium. All experiments carried approval from the institutional animal use and care committee of West Virginia University School of Medicine. Animal care standards were followed by adhering to the recommendations for the care of laboratory animals as advocated by the American Association for Accreditation of Laboratory Animal Care (AAALAC) and following the policies and procedures detailed in the Guide for the Care and Use of Laboratory Animals as published by the US Department of Health and Human Services and proclaimed in the Animal Welfare Act (PL89-544, PL91-979, and PL94-279).

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RT-PCR Estimates of mRNA for Repressor and MRF Genes

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The cDNA from all muscle samples were amplified simultaneously for a given gene. After amplification, 20 μl of each reaction was electrophoresed on 1.5% agarose gels. Gels were stained with ethidium bromide. PCR signals were quantified in arbitrary units as optical density × band area, with Kodak image analysis software (Eastman Kodak). Although the MRF and repressor signal ratios normalized to cyclophilin differed from the same gene normalized to 18S under these conditions, the relative aging-associated and overload-induced differences between muscles of aged and young adult rats were similar when the RT-PCR signals were normalized.
to either cyclophilin or 18S (2). PCR signals were normalized to the cyclophilin signal for the same PCR product to provide a semiquantitative estimate of gene expression (2).

Western Blot Analyses

Western blotting was conducted as reported previously (2) with only minor modifications. Briefly, muscle samples were minced on ice and homogenized in ice-cold T-PER tissue protein extraction buffer (Pierce, Rockford, IL) containing protease inhibitors (Pierce, Rockford, IL) and Triton X-100. Lysates were incubated in a 10% polyacrylamide gel and separated by routine SDS-PAGE largely as described previously (20, 43). Brieﬂy, the gels were blotted to polyvinylidene ﬂuoride (PVDF) membranes (Bio-Rad, Hercules, CA) and stained with Ponceau S (Sigma) to conﬁrm similar loading and transfers in each lane. As a second approach to verify equal loading between the lanes, the membranes were reprobed with antibodies against myogenin, MyoD, Id-1, Id-2, Id-3, BAX, and Bel-2 (Santa Cruz Biotechnology, Santa Cruz, CA, or PharMingen, San Diego, CA), at a concentration of 1–3 µg/ml. The signals were developed by chemiluminescence (Boehringer-Roche, Indianapolis, IN), and the membranes were then exposed to X-ray ﬁlm (BioMax MS-1; Eastman Kodak). The resulting bands were quantiﬁed as optical density × band area with Kodak imaging software (Eastman Kodak) and expressed in arbitrary units.

Myosin Heavy Chain Composition

Because aging may affect plantaris myosin heavy chain (MHC) expression, as a consequence of an altered MyoD expression, we determined the MHC protein composition in a subset of plantaris muscles from young adult (n = 4) and aged (n = 4) FBN rats. The MHC isoforms were separated by SDS-PAGE largely as described previously (20, 43). Briefly, a 10-µm-thick section of the plantaris was homogenized and sonicated in a Tris buffer containing protease inhibitors and the total protein content was determined with bichoninic acid reagents (Pierce). The samples were diluted in a SDS sample buffer. Approximately 0.2 µg of protein was loaded on the gel. The acrylamide-bisacrylamide (37.5:1) concentrations of the gels were 4% (wt/vol) and 7% (wt/vol) in the stacking and separating gels, respectively. The samples were run for 25 h at 120 V, 15°C (20, 43). MHC bands were visualized by silver staining and identiﬁed as types I, IIb, IIA, and IIX/d in order of decreasing migration rate.

Assessment of Caspase 9

Caspase 9 was measured with a commercial colorimetric caspase 9 apoptosis assay kit (bioWorld, Dublin, OH), with the free dye of 7-amino-4-trifluromethyl-coumarin (AFC) as a standard, according to the procedures outlined by the manufacturer. Briefly, 10 mg of tissue was homogenized in the lysis buffer supplied with the kit. Lysates were incubated in 50 µM of the AFC-conjugated substrate at 22°C and read at 405 nm with a Dynex MRX plate reader controlled through PC software (Revelation; Dynatech Laboratories). All data were read in duplicate and averaged for each muscle. Samples were incubated in ﬂuoromethyl ketone as a negative control.

Statistical Analyses

The data were examined with a two-way ANOVA (age × experimental condition) with SPSS software (version 10.0). The within-subject variable was experimental induction of hypertrophy, and the between-animal variable was age. Bonferroni post hoc analyses were conducted when signiﬁcant age effects were found. Signiﬁcance level was set at Ρ < 0.05. Data are presented as means ± SE.

RESULTS

Body Mass and Muscle Characteristics

The body weight of aged rats was ~ 30% greater than that of young adult rats. However, control muscle weight was ~50% less in aged rats compared with young adult rats. In FBN rats, overload increased plantaris weight from 476 ± 24 to 584 ± 26 mg in young adults whereas in aged rats the hypertrophy was attenuated (plantaris weight increased from 282 ± 24 to 300 ± 25 mg). Hypertrophy was negligible in three aged rats. The age-related and overload-induced changes in muscle weight exhibited a similar pattern in F344 rats (Fig. 1).

mRNA and Protein Expression of MRFs

mRNA levels of MRFs. At both young and old age, the relative changes in muscle mass and gene expression induced by overload were similar in FBN and F344 rats. Therefore, data from FBN and F344 rats were combined per age group (young adult, n = 17; aged, n = 14) for further analysis. RPs demonstrated that both myogenin and MyoD were increased with hypertrophy in plantaris from young adult rats but not in aged rats (Fig. 2).

In line with our previous observations (2, 3) we observed that changes in gene expression were similar when the signals from PCR products where normalized to either 18S or cyclophilin (data not shown). The data reported in the current study are those normalized to cyclophilin.

![Fig. 1. Relative increase in plantaris muscle mass after 14 days of denervation of its synergistic muscles. Plantaris muscle weight is expressed as a % change relative to the intra-animal control muscle. * P < 0.05, young adult vs. aged rats of same species. Data are presented as means ± SE.](http://ajpcell.physiology.org/content/10.22033.3.3)
In confirmation of the RPA data, semiquantitative RT-PCR indicated that myogenin and MyoD levels in hypertrophied muscles from young adult rats were elevated by ~1,700% and 625%, respectively, whereas no increase in either MyoD or myogenin was observed in overloaded muscles of aged rats (Fig. 3). MRF4 mRNA levels, however, were similar in control muscles from aged and young adult rats, and overload increased MRF4 mRNA levels by ~200%, irrespective of age.

Myogenin and MyoD protein levels. The rats of both strains were pooled into a young adult (n = 17) and an aged (n = 14) group. Equal loading and transfer was confirmed as described in METHODS (see, for example, Ponceau S staining in Fig. 4). With the appropriate antibodies, immunoreactive bands of ~34 and ~35 kDa were detected, corresponding to the molecular mass of rat myogenin and MyoD, respectively. Their expression was higher in aged than in young control plantaris muscles. In young adult animals, hypertrophy induced a ~1,100 and 430% increase in myogenin and MyoD, respectively. In aged rats, however, hypertrophy failed to induce an increase in MyoD and myogenin protein levels (Fig. 4).

mRNA levels of desmin, MLC, and CS. Because MRFs regulate the transcription of desmin and MLC, we determined the mRNA levels of these muscle-specific genes in plantaris muscles. In young adult rats, hypertrophy resulted in a 120% and 200% increase in mRNA levels for desmin and MLC, respectively (Fig. 5). In aged rats, only MLC was increased by ~115% during hypertrophy, whereas desmin expression was not significantly altered (Fig. 5). CS mRNA level was estimated by semiquantitative RT-PCR and found to be greater in muscles from aged rats compared with muscles from young adult rats. Overload did not significantly affect the CS mRNA levels in young adult rat muscles but tended to reduce the CS mRNA level in muscles from aged rats, which tended (P = 0.07) to have lower levels of CS than control muscles (Fig. 5).

MHC protein expression. Because the expression of MHC isoforms is under the control of MRFs, we also evaluated the MHC protein composition of the plantaris muscles. In aged rats, a significant increase in type I MHC and a decrease in type IIb MHC was found. Overload significantly lowered type I MHC content in muscles from young adult rats but did not induce a significant change in MHC composition in muscles of aged rats (Fig. 6).

mRNA and Protein Expression of Repressor Genes

mRNA levels of Id. As for the MRFs, the RT-PCR signals were normalized to cyclophilin. As we showed previously (2), aging was accompanied by a ~700%, 150%, and 180% increase in Id-1, Id-2, and Id-3 mRNA levels in control plantaris muscles, respectively (Fig. 7). Although overload induced ~125% increase in Id-1 levels in plantaris muscles of young adult rats, their Id-1 levels were still ~200% less than in either control or overloaded muscles of aged rats. Id-1 levels, however, did not change with overload in the muscles of
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Fig. 4. A: expression of myogenin. Representative immunoblot of control (C) and overloaded (O) plantaris muscle from young adult (n = 17; YC, YO) and aged (n = 14; YC, YO) rats is shown. Data are presented as means ± SE. *P < 0.05, control vs. overloaded muscles from rats of the same age; **P < 0.05, adult vs. aged data from the same experimental condition. B: expression of MyoD as determined by Western blotting (YC, YO (n = 17); AC, AO (n = 14)) and a representative immunoblot of plantaris muscles that were incubated with anti-MyoD are shown. Symbols and conditions are as in A. C: membranes were stained with Ponceau S red after the proteins were electroblotted to the membranes. The area of the stained blot corresponding to the predicted location of myogenin and MyoD (35–36 kDa) is shown to indicate that loading and transfer of the proteins were similar for all lanes. OD, optical density.

Aged rats (Fig. 7). Id-2 was greater in overloaded muscles of young adult rats (~85%), but the increase was attenuated in hypertrophied muscles of aged rats (~30%). Overload did not alter the levels of Id-3 mRNA in either young adult or aged rats.

mRNA levels of Mist1 and Twist. The levels of Mist1 mRNA did not change significantly with age or overload. Although Twist levels were similar in control and overloaded muscle samples of aged rats and control samples of young adult rats, Twist levels in overloaded muscle in samples of young adult muscles were only ~45% of control levels (Fig. 7).

Id protein levels. Consistent with the mRNA data, Id-1, Id-2, and Id-3 protein levels were ~1,300%, 200%, and 6,100% greater in muscles of aged rats compared with young adult rats (Fig. 8). In young adult rats, Id-1 and Id-2 protein levels increased by ~480% and 95%, respectively, with overload compared with the intramuscular control muscle, whereas no such change was observed in the aged rats. Id-3 protein levels were unchanged in young adult rats after overload but were decreased by ~50% in overloaded muscles of aged rats (Fig. 8).

Markers of apoptosis. Immunoblotting indicated that the level of BAX protein, a proapoptotic gene marker, was ~9,500% greater in control muscles of aged rats compared with young adult rats. The level of BAX protein was not significantly changed by overload in either age group (Fig. 9). Caspase 9 was ~300% greater in muscles of aged rats compared with muscles of young adult rats. Similar to BAX, caspase 9 did not change significantly with overload in muscles of either aged or young adult rats. Protein levels of Bcl-2, as estimated from immunoblot analysis, were ~500% greater in muscles of young adult rats compared with aged rats. Moreover, Bcl-2 protein levels were very low and frequently below detection levels in muscles from aged rats.

Relationship of Id protein levels and markers of apoptosis. When the control muscles of both age groups were collapsed and treated as a single group, Id-1, Id-2, and Id-3 protein levels correlated positively with caspase 9 (r = 0.99; P < 0.001) and BAX (r = 0.98; P < 0.001) protein levels (see Fig. 10 for an example). This relationship existed because muscles in aged animals showed a marked increase in these Id repressors and apoptotic markers compared with muscles from young adult animals. The relationship between BCl2 and Id protein levels was negative but less clear (r = -0.44; P = 0.07). There were no significant correlations between caspase 9 and Id protein levels within age groups or conditions, and the overload-associated changes in Id-1 or Id-2 were not associated with changes in these markers of apoptosis.

DISCUSSION

Main Findings

There is substantial evidence that MRFs are important regulators of muscle-specific gene expression during conditions of loading or unloading (2, 34). We observed that the hypertrophy-induced increase in the expression of MyoD and myogenin in the fast plantaris muscles is blunted in old age. This finding is consistent with the suggestion that aging attenuates the general transcriptional response associated with muscle protein accumulation during muscle loading (1, 28, 32, 50).

In this study we provide evidence that Id mRNA and protein levels are elevated in overloaded muscles of young adults, whereas in old age the already elevated basal repressor mRNA and protein levels are not in-
creased further during overload (14). Furthermore, the high levels of Id-1 and Id-2 in muscles from aged rats correlated with greater levels of BAX and caspase 9 and low levels of Bcl-2, suggesting that repressor proteins may be involved in apoptosis that apparently occurs in muscles from aged animals.

**Aging and Overload**

MyoD levels are typically higher in fast muscles (24, 53) containing a high percentage of type IIb MHC (47), whereas muscles with a high type I MHC content are associated with relatively high myogenin levels (24). However, MyoD mRNA and protein levels were higher, yet type IIb MHC expression was lower, in control muscles from aged rats compared with young adult rats. Furthermore, MyoD levels increased in overloaded muscles of young rats but type IIb MHC did not change relative to control muscles. In addition, myogenin levels were greater but type I MHC expression was lower in overloaded muscles of young adult rats compared with aged rats. Therefore, it seems unlikely that MRFs play a dominant role in regulating MHC expression in overloaded muscles of aged rats.

We and others (10) have observed that overload-induced hypertrophy is attenuated in old age. Potentially, this could be related to a reduced activity level in old age, thereby decreasing the overload stimulus compared with that in young active rats and consequently attenuating muscle growth. Although we did not evaluate this systematically, we did not observe obvious differences in the activity levels between young adult and aged rats. Even if the activity level was reduced in old age, it is unlikely that this would completely explain the attenuated increase in muscle mass, because it has been found that increased activity does not enhance muscle mass or prevent the age-associated decline in muscle mass in aged rats (11). Moreover, the hypertrophic response to stretch overload, which is largely independent of muscle activity (4, 23), was also suppressed in old age (13, 32).
Differences in activity level between young and aged rats might also affect the expression of MRFs. However, the increase in MRF levels was attenuated in old age both in response to resistance exercise (50) and in response to an activity-independent stretch-induced overload (13, 32). Finally, we have found that the expression of myogenin and MyoD in plantaris muscles from young adult rats was unaltered 24 or 48 h after exhaustive treadmill exercise (unpublished data). Together, these data suggest that differences in activity levels alone cannot explain the attenuation of protein accumulation and MRF expression in aged rats.

Involvement of Id Proteins in Regulation of Proliferation

There is mounting evidence that Id proteins have an important role in the regulation of cell proliferation. Id gene expression is enhanced in response to mitogenic stimuli (6, 17) and is associated with the induction of DNA synthesis (41). Furthermore, Id gene expression is typically high during cellular proliferation and before differentiation in keratinocytes (36) and tumor and other cell lines (16, 27, 49). Overexpression of Id inhibits differentiation of cells of muscle lineage (7, 9, 17, 25, 29). Moreover, the observation that Id-2 knockout mice die at birth with an apparent lack of muscle tissue suggests that Id-2 is involved in the regulation of satellite cell proliferation during muscle growth (29). Because satellite cell proliferation provides additional nuclei that enable the muscle to increase its mass (35), it is possible that the elevation of Id-1 and Id-2 protein levels in muscles from aged rats is an apparently unsuccessful attempt to stimulate satellite cell proliferation to enhance fiber hypertrophy and thus to counterbalance sarcopenia. The attenuated overload-induced hypertrophy in old age and the absence of an increase in Id expression strongly suggest that Id repressor expression in aged control muscles is already maximal. Further work is needed to confirm that

![Fig. 7. Semiquantitative analysis of mRNA levels of Id-1 (A), Id-2 (B), Id-3 (C), Mist1 (D), and Twist (D) repressors in control (C) and overloaded (O) plantaris muscles of young adult (n = 17; YC, YO) and aged (n = 14; AC, AO) rats by RT-PCR amplification. Representative PCR gels are shown as insets in each panel. The expression of each PCR product was normalized to the cyclophilin signal of the corresponding RT product. Values are expressed (in arbitrary units) as means ± SE of the OD of the PCR product × the area of the PCR product. *P < 0.05, control vs. overloaded muscles from rats of the same age; **P < 0.05, young adult vs. aged data from the same experimental condition.]
the expression of Id repressors has reached a maximum in old age. However, the data suggest that the inability to further upregulate Id expression in old age contributes to the attenuated hypertrophic response.

Potential Involvement of Id Proteins in Apoptosis of Skeletal Muscle

In an apparent paradoxical role to proliferation, Id-1 has been shown to increase during denervation, which leads to muscle atrophy (12, 22). This paradox is also apparent in our data, because the overload-induced hypertrophy at young age and the age-related muscle atrophy were both accompanied by an elevated Id expression. Thus the data in the literature and our data point to a dual role for the Id gene.

Both Id-1 and Id-2 protein levels and markers for apoptosis (i.e., BAX and caspase 9) were higher in muscles of the aged compared with the young adult
rats (Fig. 10). Although these results do not prove a causative role for Id-1 and Id-2 in apoptosis in skeletal muscle of aged animals, they have been shown to at least partly play such a role in muscle damage and disease (18, 42, 45, 46). Moreover, clear evidence for a role of Id-1 and Id-2 in apoptosis in other cells was obtained by Florio and colleagues (21). However, further studies are required to determine whether Id-1 and Id-2 play a role in regulating apoptosis in skeletal muscle of aged animals via activation of BAX/Bcl-2 apoptotic pathways. In addition, because elevated Id-1 levels are associated with denervation (22), further studies are needed to determine whether the denervation-reinnervation process that occurs during aging regulates muscle Id protein levels in aging.

In contrast to the other Id proteins, Id-3 did not increase with overload in muscles of young rats and even decreased with overload in aged rat muscle. The discrepancy between this decrease and the unchanged BAX and caspase levels during hypertrophy in old age suggests that Id-3 plays no role in apoptosis. Nevertheless, overexpression of Id-3 has been shown to induce apoptosis in fibroblasts and other cell types (37, 39). In addition, in vitro studies raise the possibility that apoptosis induced by withdrawal of growth factors may induce apoptosis of satellite cells (33).
In summary, although Id levels increased in muscles of young adult rats with overload, there was no evidence that apoptosis increased. This suggests that Id may primarily function in pathways regulating satellite cell proliferation and growth in young adult muscles, because hypertrophy is dependent on satellite cell activation and fusion (44). Nevertheless, Id may have a dual role in pathways of both growth and apoptosis in muscles of aged rats. A dual role of genes in both growth and apoptosis is not unique to Id. For example, p53 and c-Myc activate progress through the cell cycle and E2F-1 transactivates genes required for progression into S phase, but those genes can also be uncoupled from these functions during apoptosis (15). Additional experiments are needed to determine whether Id has a direct role in apoptosis of aging skeletal muscle and sarcopenia.

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