Myostatin, also known as growth and differentiation factor 8, is a member of the transforming growth factor β superfamily that negatively regulates skeletal muscle mass (1). Recent experiments have shown that myostatin activity is detected in serum by a reporter gene assay only after activation by acid, suggesting that native myostatin circulates as a latent complex (2). We have used a monoclonal myostatin antibody, JA16, to isolate myostatin circulates as a latent complex (2). We have used a monoclonal myostatin antibody, JA16, to isolate native myostatin complex from normal mouse and human serum. Analysis by mass spectrometry and Western blot shows that circulating myostatin is bound to at least two major proteins, the myostatin propeptide and the follistatin-related gene (FLRG). The myostatin propeptide is known to bind and inhibit myostatin in vitro (3). Here we show that this interaction is relevant in vivo, with a majority (>70%) of myostatin in serum bound to its propeptide. Studies with recombinant V5-His-tagged FLRG protein confirm a direct interaction between mature myostatin and FLRG. Functional studies show that FLRG inhibits myostatin activity in a reporter gene assay. These experiments suggest that the myostatin propeptide and FLRG are major negative regulators of myostatin in vivo.

Myostatin is a member of the TGF-β family that is expressed nearly exclusively in skeletal muscle (1). Myostatin-deficient mice show a 2–3-fold increase in skeletal muscle mass when compared with their wild-type littermates, implying that myostatin acts as a negative regulator of muscle cells in vivo (1). This increase seems to be the result of an increase in both the number (hyperplasia) and thickness (hypertrophy) of muscle fibers (1), explained in part by the observation that myostatin inhibits proliferation of C2C12 myoblasts in culture (4, 5). The myostatin-null mice also show decreased fat accumulation (6, 7) but otherwise appear normal and healthy.

Like other TGF-β family members, myostatin is produced as a precursor protein that contains a signal sequence, an N-terminal propeptide domain, and a C-terminal domain that is the active ligand (1, 8, 9). Proteolytic processing between the propeptide domain and the C-terminal domain releases mature myostatin (1, 3, 10). Both unprocessed and mature active myostatin form disulfide-linked dimers (1).

In vitro, myostatin binds noncovalently to its propeptide after proteolytic processing, producing a biologically inactive complex that is prevented from binding to responsive cells (3, 10). Furthermore, overexpression of myostatin propeptide leads to an increase in muscle mass in transgenic animals (10, 11). The regulation of myostatin by its propeptide is highly similar to TGF-β, which also binds to its propeptide (often referred to as latency-associated peptide) to form the small latent complex (12–17).

The biological activities of other TGF-β family members, such as activin and BMP-2, are not regulated by their propeptide (18). However, a diverse array of inhibitory binding proteins performs similar roles for various members of the TGF-β superfamily (reviewed in Refs. 19 and 20). For example, activin and some bone morphogenetic proteins (BMPs) are inhibited by an interaction with the cysteine-rich glycoprotein follistatin (21–23). Interestingly, follistatin can block the activity of both BMP-11, a very close relative of myostatin, and myostatin itself (1, 10, 24). Like propeptide, overexpression of follistatin in the skeletal muscle of mice results in a double-muscle phenotype that is similar to myostatin-null animals (10). In addition, follistatin can inhibit myostatin activity in a transcription-based reporter assay (2). Thus, follistatin is capable of binding and inhibiting myostatin.

A number of proteins show homology to a 10-cysteine repeat in follistatin, including the highly similar follistatin-related gene (FLRG) (25, 26). Like follistatin, FLRG binds and inhibits activin and multiple BMPs in vitro (26–28). Transcription of FLRG is up-regulated by activin and TGF-β signaling through SMAD proteins, initiating a negative feedback loop that controls TGF-β signaling (29, 30). To date, however, FLRG has not been tied to a particular growth factor in vivo.

Recently, myostatin has been shown to circulate in serum as part of a latent complex (2). Myostatin activity, as measured by a reporter gene assay, is detected in serum only after activation by acid treatment (2). However, the myostatin-binding proteins that impart latency in vivo are currently unknown. To gain insight into the mechanism of myostatin regulation and to aid in the development of a therapeutic agent, we set out to define the composition of the circulating myostatin complex in serum. To accomplish this, we have isolated myostatin from normal mouse and human serum and analyzed co-purified proteins by mass spectrometry and Western blotting. This approach relies entirely on endogenous proteins at normal expression levels, allowing questions of in vivo binding specificity to be addressed.
**EXPERIMENTAL PROCEDURES**

**Antibodies and Purified Proteins**—The JA16 monoclonal antibody was generated in myostatin knockout mice by immunization with recombinant mature myostatin protein purified from Chinese hamster ovary cell conditioned medium (10) using standard procedures (1, 31). JA16 recognizes purified recombinant myostatin and the highly similar BMP-11 (24) as a single band on a nonreducing Western blot but does not recognize purified activin A (R & D Systems, Minneapolis, MN) (data not shown). In addition, JA16 inhibits the activity of myostatin and BMP-11, but not activin, in a (CAGA)12 reporter gene assay (data not shown) (3). The anti-myostatin polyclonal antibody, L8014, was produced in rabbit by immunization with the keyhole limpet hemocyanin-conjugated peptide MLYFNGKEQIIYG, corresponding to amino acids 350–362 of human myostatin. The polyclonal anti-myostatin peptidase antibody, L8825, was produced in rabbit by immunization with His6-tagged human myostatin propeptide protein. This protein was expressed in the CM of Chinese hamster ovary cells transfected with a mammalian expression plasmid encoding amino acids 1–266 of human myostatin in frame with a C-terminal His6 tag and purified by nickel-immobilized metal ion affinity chromatography. The FLRG monoclonal antibody was the kind gift of Kinihiro Tsuchida (26, 27).

**Production of JA16-conjugated Beads**—N-Hydroxysuccinimidyldiactivated beads (4% bead agarose, Sigma-H8065) were washed in 1 M LiCl and incubated for 4 h at 4°C with the anti-myostatin JA16 monoclonal antibody (3–4 μg/μl in 100 mM MOPS, pH 7.5) at a ratio to allow a final concentration of 10 mg of JA16/ml of resin. The remaining reactive groups were blocked by the addition of 100 μl of 1 M ethanolamine, pH 8/ml of resin for 1 h at 4°C. The beads were washed extensively with 100 mM MOPS, pH 7.5, and phosphate-buffered saline (PBS) and stored at 4°C in PBS until use. The control beads were prepared identically without JA16 antibody.

**Affinity Purification**—A total of 40 μl of packed JA16-conjugated or control beads was incubated with 15 ml of normal Balb/c mouse serum (Golden West Biologicals, Temecula, CA) or 30 ml of pooled normal human serum (ICN Biomedical, Aurora, OH) for 3 h at 4°C. The beads were washed twice in cold 1% Triton X-100 with PBS, 0.1% Triton X-100, phosphate-buffered saline and stored at 4°C. For each immunoprecipitation, 400 μl of serum or plasma was used for immunoprecipitation. After the beads were washed and dried, the beads were resuspended in 100 μl of PBS and incubated at 4°C for 30 min. The supernatant was collected and combined with 30 μl of 4× lithium dodecyl sulfate sample buffer (Invitrogen). 2 “Peptide elution,” 100 μl of 1 μg/ml JA16 peptide competition in PBS was added to the beads and again incubated at 4°C for 30 min. The supernatant was collected as before. The competing peptide (sequence: DFGLDSDEHSRSRSSRYPLTVDFEAFGWDOOH) was identified on its ability to prevent the binding of JA16 and myostatin using the (CAGA)12 reporter gene as a readout (data not shown). 3 “SDS elution,” 50 μl of 4× lithium dodecyl sulfate buffer (Invitrogen) and 100 μl of PBS were added to the beads and heated to 80°C for 10 min before transferring the supernatant to a fresh tube.

**Mass Spectrometry**—The samples were reduced with NuPage 10× reducing agent (Invitrogen) for 10 min at 80°C and alkylated with 110 μl iodoacetamide for 30 min at 22°C in the dark. The samples were run immediately on 10% NuPage Bis-Tris gels in an MES buffer system according to the manufacturer’s recommendations (Invitrogen) and silver-stained using a dinitrofluorobenzene-free system (32). The bands were excised and subjected to in-gel digestion with modified trypsin (Promega, Madison, WI) in a Digest Pro (Abimed, Langenfeld, Germany) or ProGest Investigator (Genomics Solutions, Ann Arbor, MI). The volume of digested samples was reduced by evaporation and supplemented with 1% acetic acid to a final volume of ~20 μl. The samples (5–10 μl) were loaded onto a 10-cm × 75-μm inner diameter C18 reverse-phase column packed in a Picofrit needle (New Objectives, Woburn, MA). MS/MS data was collected using an LCQ Deca or LCQ Deca XP (Finnigan, San Jose, CA) mass spectrometer and searched against the NCBI nonredundant data base using the Sequest program (Finnigan). All of the peptide sequences listed in this paper had Xcorr scores of >2.4 in the Sequest scoring system and were confirmed manually by examining their corresponding raw MS/MS spectra.

**Western Blots**—The proteins were transferred to a 0.45-μm nitrocellulose membrane (Invitrogen) and blocked with blocking buffer (5% nonfat dry milk in Tris-buffered saline (10 mM Tris-Cl, pH 7.5, 150 mM NaCl) at 4°C overnight. The blots were then probed with primary antibody diluted 1:2000 in blocking buffer for 1–3 h at room temperature, washed five times with Tris-buffered saline, probed with horseradish peroxidase-conjugated secondary antibody in blocking buffer, and washed as before. The signals were detected by autoradiography using the West Pico Substrate (Pierce).

**Immunoprecipitation from Conditioned Medium**—COS1 cells (~60% confluent) were transfected with plasmids for myostatin/propeptide (mFLRG-V5-His) or the empty vector using the FuGENE 6 reagent (Roche Molecular Biochemicals). CM was harvested 48 h post-transfection and centrifuged to remove cellular debris. For each immunoprecipitation, 400 μl of mock- or FLRG-transfected CM was combined with 1.2 μg of purified myostatin and/or propeptide protein. JA16 or anti-V5 (Sigma) antibody-conjugated beads (30 μl of packed volume) were incubated with the supplemented CM for 2 h, washed as above, and resuspended in 45 μl of 1× LDS buffer with dithiothreitol.

**Reporter Gene Assay**—A luciferase reporter construct, pGL3-(CAGA)12 (33), was transiently transfected into 2A04 cells. Multiple dilutions of CM from vector (mock)- or FLRG-transfected COS cells were incubated with 10 ng/ml myostatin for 30 min at 37°C and assayed as described previously (2, 3).

**RESULTS**

A Monoclonal Anti-myostatin Antibody, JA16, Successfully Isolates Myostatin from Normal Mouse Serum—To characterize the major components of the circulating myostatin complex in vivo, we first isolated myostatin and the endogenous myostatin-binding proteins from normal mouse serum by affinity purification with an agarose-conjugated anti-myostatin monoclonal antibody, JA16. Captured proteins were subjected to subsequent elution steps with PBS buffer alone (mock elution), a competing peptide, and SDS sample buffer. A reducing silver-stained gel of the proteins released in each of these elution steps is shown in Fig. 1. Two protein bands of ~12 and 36 kDa were specifically eluted with peptide from JA16-conjugated beads.
bears (Fig. 1, lanes labeled JA16) but not from unconjugated control beads (Fig. 1, lanes labeled 0). Because the molecular mass of reduced myostatin protein is 12 kDa, we speculated that the lower band was mature myostatin. To confirm this hypothesis, we excised the band from the silver-stained gel, digested it with trypsin, and obtained MS/MS spectra of the resulting peptides by liquid chromatography/MS/MS. MS/MS spectra corresponding to six tryptic peptides found in mature myostatin were identified from this excised gel slice (Table I). A representative MS/MS spectrum is shown in Fig. 2A. In contrast, no myostatin peptides were found in the corresponding region of the peptide-eluted sample from the negative control beads. A Western blot using L8014, a polyclonal antibody raised against a peptide epitope found in the mature region of myostatin, confirmed this identification (Fig. 2B). Although the JA16 antibody recognizes both myostatin and the highly related protein BMP-11 (Ref. 24 and see “Experimental Procedures”), extensive mass spectrometric analysis of the JA16-isolated samples revealed no evidence of BMP-11-derived peptides, suggesting that BMP-11 does not contaminate the JA16 serum immunoprecipitates. Furthermore, we did not detect peptides from any other TGF-β family member in these samples, confirming the selective purification of myostatin in these experiments.

**Myostatin Propeptide and FLRG Bind Myostatin in Normal Mouse Serum**—Once we had confirmed that our affinity purification technique successfully isolated native myostatin from serum, we proceeded to identify myostatin-binding proteins. Mass spectrometric analysis of the 36-kDa silver-stained band identified two co-migrating proteins that are specific to the JA16 immunopurified sample: myostatin propeptide and FLRG. The peptides identified from each of these proteins are shown in Table I. High quality MS/MS spectra were found for six unique peptides from propeptide and three unique peptides from FLRG (Table I and Fig. 3, A and C). Furthermore, the presence of both of these proteins was confirmed by Western blotting with antibodies specific to propeptide (L8825) and FLRG, respectively (Fig. 3, B and D). Thus, circulating myostatin is bound to its propeptide and FLRG in vivo.

**Follistatin** itself is present in serum and has been shown to interact with myostatin in *vitro* and increase muscle mass when overexpressed in *vitro* (2, 10, 34, 35). In addition, the
JA16 antibody can recognize and immunoprecipitate a complex between recombinant purified myostatin and follistatin. The entire gel region spanning from 6 to 100 kDa was excised into 13 bands, and each band was digested with trypsin and analyzed by mass spectrometry as before. We did not detect follistatin in the JA16-purified samples from serum in any of the four repetitions of this experiment. In contrast, FLRG-derived peptides were identified in every repetition. This finding suggests that in normal serum, myostatin activity is regulated by FLRG rather than follistatin.

The Majority of Circulating Myostatin Is Bound to Its Propeptide—In an effort to determine the amount of myostatin in serum that is bound to its propeptide, we used purified recombinant myostatin and propeptide protein (3) as standards to quantitate Western blots. Myostatin was purified from mouse serum using JA16-conjugated beads, and bound proteins were eluted with SDS in a single step. A portion of the eluted proteins was subjected to Western blotting alongside known amounts of purified myostatin (Fig. 4A) or propeptide (Fig. 4B). The blots then were probed with anti-myostatin L8014 (Fig. 4A) or anti-propeptide L8825 (Fig. 4B) polyclonal antibodies. Although it is difficult to determine the precise amount of protein in the JA16 immunoprecipitate by this method, the propeptide band contains 2–3-fold more protein mass than mature myostatin. Because the molecular mass of propeptide (36 kDa) is three times that of mature myostatin (12 kDa), we estimate that more than 70% of mature myostatin is bound to propeptide in these samples. This finding implies that the majority of circulating myostatin is bound to propeptide and suggests that propeptide may play an important role in the regulation of myostatin activity in vivo.

FLRG Binds Directly to Mature Myostatin, Not Propeptide—To confirm the interaction between FLRG and myostatin, the mouse FLRG coding sequence (26) was cloned by PCR from first strand heart cDNA. A mammalian expression vector encoding mouse FLRG with a C-terminal V5-His tag was transiently transfected into COS1 cells. Secreted FLRG-V5-His protein was detected in the conditioned medium by Western blot using an anti-V5 antibody (data not shown). This conditioned

![Fig. 3. The myostatin propeptide and FLRG bind to circulating myostatin isolated from normal mouse serum. A and B, representative MS/MS spectra from one of the peptides derived from myostatin propeptide (A) and FLRG (B) found in the 36-kDa band. C, a Western blot of affinity-purified myostatin complex probed with a polyclonal antibody that specifically recognizes the propeptide region of myostatin confirms the mass spectrometric identification of this protein in the myostatin complex. D, a Western blot of affinity-purified myostatin complex probed with a monoclonal antibody to FLRG.](image-url)

![Fig. 4. The myostatin propeptide is bound to the majority of circulating myostatin in vivo.](image-url)
Mouse and Human—activity. infected cells had no effect. Thus, FLRG inhibits myostatin 6). In contrast, COS1 conditioned medium from mock trans- myostatin activity in a concentration-dependent manner (Fig. 5). We found that FLRG in conditioned medium potently inhibited assay has been shown to reflect myostatin activity (2, 3). We
struct in A204 rhabdomyosarcoma cells. This reporter gene panels and/or propeptide and immunoprecipitated (IP) with both JA16 (left panels) and anti-V5 (right panels). These samples were subjected to Western blotting with polyclonal antibodies that recognize the V5 tag (top panels), myostatin (middle panels), or propeptide (bottom panels).

medium was supplemented with purified mature myostatin and/or propeptide protein, and the presence of the FLRG-myostatin complex was confirmed by immunoprecipitation with both JA16 and a monoclonal anti-V5 antibody (Fig. 5). We found that FLRG bound directly to mature myostatin but not to propeptide. However, propeptide is detected in anti-V5 (anti-FLRG) immunoprecipitates when myostatin is present, suggesting that myostatin can bind simultaneously to both FLRG and propeptide. Because native myostatin is a homodimer (1), it is possible that one myostatin molecule in a given dimer is bound to FLRG, whereas the other molecule is bound to propeptide. Thus, it remains unclear whether a single myostatin protein can bind simultaneously to both FLRG and propeptide.

**FLRG Inhibits Myostatin Activity**—To determine the functional role of FLRG, we looked at the ability of conditioned medium from FLRG-V5-His-transfected COS1 cells to modulate myostatin-induced activation of a (CAGA)_{12} reporter construct in A204 rhabdomyosarcoma cells. This reporter gene assay has been shown to reflect myostatin activity (2, 3). We found that FLRG in conditioned medium potently inhibited myostatin activity in a concentration-dependent manner (Fig. 6). In contrast, COS1 conditioned medium from mock transfected cells had no effect. Thus, FLRG inhibits myostatin activity.

In Vivo Myostatin-binding Proteins Are Conserved between Mouse and Human—Acid activation of mouse serum reveals myostatin activity in a reporter gene assay, providing an estimate of the myostatin concentration at 80 ng/ml (2). In contrast, identically treated human serum does not have detectable myostatin activity in this assay.\(^6\) This finding suggests that the concentration of myostatin in human serum is considerably lower than that found in mouse serum.

Because myostatin has potential as a therapeutic target, we were interested in determining the composition of the circulating myostatin complex in humans. This knowledge would determine the validity of the mouse model and in myostatin studies. Thus, we repeated the JA16-based affinity purification of myostatin from human serum. Because of the low level of myostatin, bands corresponding to mature myostatin and myostatin propeptide/FLRG were not detected by silver stain (Fig. 7A). However, mass spectrometric analysis of the 12-kDa region of the gel identified peptides derived from myostatin (Table I). In addition, Western blotting with the polyclonal myostatin antibody L8014 revealed the presence of mature myostatin in the JA16-purified samples (Fig. 7B).

Unfortunately, the antibodies against propeptide and FLRG were not sufficiently sensitive to detect these proteins in JA16 immunoprecipitations from human serum by Western blot. Thus, we took advantage of the high sensitivity of mass spectrometry to identify proteins that co-purified with mature myostatin. The 36-kDa gel region was excised from the lanes containing the peptide-eluted product from both negative control and JA16-conjugated beads. These gel slices were analyzed by mass spectrometry as before. As with the mouse serum, both propeptide and FLRG were identified in this region of the gel. The peptides found from each of these proteins are listed in Table I. Because of the high degree of conservation between human and mouse myostatin (36), the peptides identified in human serum from the propeptide were identical to ones that had been detected in mouse. In FLRG, peptides unique to the human FLRG sequence were identified. No peptides corresponding to these proteins were found in the negative control sample. This result suggests that the in vivo myostatin complex is conserved between mouse and human, validating the mouse as a model for human disease studies involving myostatin.

**DISCUSSION**

In this paper, we show that the majority of endogenous myostatin circulates as a latent complex with propeptide and FLRG. Both of these proteins act independently as negative regulators, most likely by preventing the association of myostatin with its receptor (3, 10). It remains unclear why two different inhibitory proteins bind to myostatin. However, based on similarities to TGF-β and activin, a model of the regulation of myostatin activity by its propeptide and FLRG can be proposed. TGF-β superfamily members are produced as a single polypeptide chain that undergoes proteolytic cleavage to form two polypeptide chains, the N-terminal propeptide domain and

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\(^6\) M. V. Davies, unpublished observations.
FLRG binds to myostatin in vivo. The resulting active myostatin initiates signaling, eventually triggering a negative feedback loop that leads to secretion of FLRG and the inhibition of active myostatin.

The apparent absence of follistatin in circulating myostatin complexes is intriguing. It is known that follistatin is present in serum and can bind to myostatin (2, 10, 34, 35). Furthermore, transgenic mice overexpressing follistatin under a skeletal muscle-specific promoter show a double muscle phenotype consistent with the negative regulation of myostatin (10).

Lastly, the JA16 antibody is capable of immunoprecipitating a myostatin-follistatin complex (data not shown). Thus, it was surprising that we did not find any evidence of follistatin bound to myostatin in vivo after extensive mass spectrometric analysis of the entire gel lane from multiple JA16 immunoprecipitations. Although we cannot eliminate the possibility that follistatin binds to myostatin in serum at a level that is below our ability to detect, FLRG has been identified in every JA16 immunoprecipitation from serum that we have analyzed by mass spectrometry, and follistatin has always been absent.

A human hepatoma cell line, HepG2, up-regulates both follistatin and FLRG in response to activin (29). One way to explain the absence of follistatin-bound myostatin in serum would be if myostatin-responsive cells such as muscle produce only FLRG, not follistatin, in response to myostatin signaling. In this case, free myostatin would bind preferentially to FLRG, because the local concentration of FLRG would be higher than follistatin. Because both follistatin and FLRG bind to activin and BMPs in an essentially irreversible manner (40), FLRG would remain bound to myostatin despite the presence of follistatin in serum. In contrast, when follistatin is overexpressed in skeletal muscle using transgenic technology (10), follistatin is present in high concentrations at the site of myostatin action and thus can bind myostatin as soon as it becomes activated, explaining the increased muscle mass in these animals.

It is also possible that follistatin does inhibit myostatin in vivo but that this interaction is limited to the muscle tissue. One of the major differences between follistatin and FLRG is that follistatin contains a heparin-binding sequence that FLRG does not (26, 41). Because the heparin-binding sequence mediates an interaction between follistatin and cell surface proteoglycans (42, 43), follistatin produced in muscle may remain associated with the extracellular matrix of the cells that secrete it. Thus, myostatin that is bound to follistatin may be sequestered in the muscle and therefore absent in serum.

In this paper, we have isolated myostatin from normal serum and analyzed the composition of the latent complex. In both mouse and human serum, myostatin circulates as a latent complex with the myostatin propeptide and FLRG. Previous works has shown that the myostatin-propeptide complex is inactive and incapable of binding to its receptor (3, 10, 11). Here we show that the majority of myostatin in serum is bound to its propeptide.

At the site of TGF-β signaling, it is thought that serine proteases such as plasmin and cathepsin D cleave the propeptide moiety, thus allowing the release of active TGF-β (37, 38). Because propeptide is irreversibly removed during the activation process, it is no longer possible to turn off active TGF-β using this protein. Interestingly, transcription of FLRG has been shown to be up-regulated upon signaling by both TGF-β and activin (29, 30). This occurs through binding of activated Smad proteins to a Smad-binding element in the FLRG promoter and results in the increased production of secreted FLRG and the eventual inhibition of activin signaling (29). This negative feedback loop almost certainly occurs with myostatin signaling, because activin, TGF-β, and myostatin all signal through Smad2 and Smad3 proteins (39). Here we show that FLRG binds to myostatin in vivo and inhibits its activity. We propose that this FLRG binding likely occurs after myostatin has bound to its receptor and initiated signaling. In this model, propeptide binds to myostatin as it is secreted, providing a pool of latent growth factor. At the site of action, the propeptide is removed by proteolysis. The resulting active myostatin initiates signaling, eventually triggering a negative feedback loop that leads to secretion of FLRG and the inhibition of active myostatin.

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REFERENCES
1. McPherron, A. C., Lawler, A. M., and Lee, S. J. (1997) Nature 387, 83–90
2. Zimmers, T. A., Davies, M. V., Koniaris, L. G., Haynes, P., Esquela, A. F., Tomkinson, K. N., McPherron, A. C., Wolfman, N. M., and Lee, S. J. (2002) Science 296, 1486–1488
3. Thies, R. S., Chen, T., Davies, M. V., Tomkinson, K. N., Pearson, A. A., Shakesy, Q. A., and Wolfman, N. M. (2001) Growth Factors 18, 251–259
4. Thomas, M., Langley, B., Berry, C., Sharma, M., Kirk, S., Bass, J., and...
Kambadur, R. (2000) J. Biol. Chem. 275, 40235–40243
5. Taylor, W. E., Bhasin, S., Artaza, J., Byhower, F., Azam, M., Willard, D. H., Jr., Kull, F. C., Jr., and Gonzalez-Cadavid, N. (2001) Am. J. Physiol. 280, E221–E228
6. McPherron, A. C., and Lee, S. J. (2002) J. Clin. Invest. 109, 595–601
7. Lin, J., Arnold, H. B., Della-Fera, M. A., Axzin, M. J., Hartzell, D. L., and Baile, C. A. (2002) Biochem. Biophys. Res. Comm. 291, 701–706
8. Derynck, R., Jarrett, J. A., Chen, E. Y., Eaton, D. H., Bell, J. R., Assoian, R. K., Roberts, A. B., Sporn, M. B., and Goeddel, D. V. (1985) Nature 316, 701–705
9. Wozney, J. M., Rosen, Y., Celeste, A. J., Mitsoke, L. M., Whitters, M. J., Kriz, B. W., Hewick, R. M., and Wang, E. A. (1988) Science 240, 1528–1534
10. Lee, S. J., and McPherron, A. C. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 9306–9311
11. Yang, J., Ratovitski, T., Brady, J. P., Solomon, M. B., Wells, K. D., and Wall, R. J. (2001) Mol. Reprod. Dev. 60, 351–361
12. Miyazono, K., Hellen, U., Wernstedt, C., and Heldin, C. H. (1988) J. Biol. Chem. 263, 6407–6415
13. Wakefield, L. M., Smith, D. M., Flanders, K. C., and Sporn, M. B. (1988) J. Biol. Chem. 263, 7646–7654
14. Gentry, L. E., and Nash, B. W. (1990) Biochemistry 29, 6851–6857
15. Brown, F. D., Wakefield, L. M., Levinson, A. D., and Sporn, M. B. (1990) Growth Factors 3, 35–43
16. Bottinger, E. P., Factor, V. M., Tsang, M. L., Weatherbee, J. A., Kopp, J. B., Qian, S. W., Wakefield, L. M., Roberts, A. B., Thorpeirlson, S. S., and Sporn, M. B. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 5877–5882
17. Glezues, P. E., Munger, J. S., Nunes, I., Harpel, J. G., Mazziere, R., Noguera, I., and Boklin, D. B. (1997) Stem Cells 15, 193–197
18. Gray, A. M., and Mason, A. J. (1990) Science 247, 1328–1330
19. Smith, W. C. (1999) Trends Genet. 15, 3–5
20. Massague, J., and Chen, Y. G. (2000) Genes Dev. 14, 627–644
21. Nakamura, T., Tahino, K., Eto, Y., Shihai, H., Titani, K., and Sugino, H. (1990) Science 247, 836–838
22. Yamashita, H., ten Dijke, P., Huybreecck, D., Sampath, T. K., Andries, M., Smith, J. C., Heldin, C. H., and Miyazono, K. (1999) J. Cell Biol. 144, 217–226
23. Fainsod, A., Deissler, K., Yelin, B., Marem, K., Epstein, M., Piklemer, G., Steinbeisser, H., and Blum, M. (1997) Mech. Dev. 63, 39–50
24. Gamer, L. W., Wilm, N. M., Celeste, A. J., Hattersley, G., Hewick, R., and Rosen, V. (1999) Dev. Biol. 208, 222–232
25. Hayette, S., Gadoux, M., Martel, S., Bertrand, S., Tiguad, I., Magaud, J. P., and Rimokh, R. (1998) Oncogene 16, 2949–2954
26. Tsichinda, K., Arakawa, K. Y., Kuramoto, Y., Yamakawa, N., Hasegawa, Y., and Sugino, H. (2000) J. Biol. Chem. 275, 40788–40796
27. Tsichinda, K., Mateuzaki, T., Yamakawa, N., Liu, Z., and Sugino, H. (2001) Mol. Cell. Endocrinol. 180, 25–31
28. Schneyer, A. L., Tortoriello, D., Sidis, Y., Keutmann, H., Matsuaki, T., and Holmes, W. (2001) Mol. Cell. Endocrinol. 180, 33–38
29. Bartholin, L., Maguer-Satta, V., Hayette, S., Martel, S., Gadoux, M., Corbo, L., Magaud, J. P., and Rimokh, R. (2002) Oncogene 21, 2227–2235
30. Bartholin, L., Maguer-Satta, V., Hayette, S., Martel, S., Gadoux, M., Bertrand, S., Corbo, L., Lamadon, C., Morera, A. M., Magaud, J. P., and Rimokh, R. (2001) Oncogene 20, 5409–5419
31. Oi, V. T., and Herzenberger, L. A. (1980) in Selected Methods in Cellular Immunology (Mishell, B. B., Shiigi, S. M., Henry, C., and Mishell, R. I., eds) pp. 351–372, W. H. Freeman, San Francisco
32. Shevchenko, A., Wilm, M., Vorm, O., and Mann, M. (1996) Anal. Chem. 68, 850–858
33. Dennler, S., Ihoh, S., Vivien, D., ten Dijke, P., Huet, S., and Gauthier, J. M. (1988) EMBO J. 17, 3581–3590
34. Krummen, L. A., Woodruff, T. K., DeGuzman, G., Cox, E. T., Baly, D. L., Mann, E., Garg, S., Wong, W. L., Coosum, P., and Mather, J. P. (1993) Endocrinology 132, 431–443
35. Schneyer, A. L., O’Neil, D. A., and Crowley, W. F., Jr. (1992) J. Clin. Endocrinol. Metab. 74, 1320–1324
36. McPherron, A. C., and Lee, S. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12457–12461
37. Lyons, R. M., Kesk-Oija, J., and Moses, H. L. (1988) J. Cell Biol. 106, 1659–1665
38. Lyons, R. M., Gentry, L. E., Purckho, A. F., and Moses, H. L. (1990) J. Cell Biol. 110, 1361–1367
39. Hu, P. P., Datto, M. B., and Wang, X. F. (1998) Endocr. Rev. 19, 349–363
40. Schneyer, A. L., Rausilio, D. A., Blasi, P. M., and Crowley, W. F., Jr. (1994) Endocrinology 135, 667–674
41. Uno, N., Ling, N., Ying, S. Y., Esch, F., Shimazaki, S., and Guillemin, R. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8282–8286
42. Nakamura, T., Sugino, K., Titani, K., and Sugino, H. (1991) J. Biol. Chem. 266, 19432–19437
43. Sugino, K., Kurosawa, N., Nakamura, T., Takio, K., Shimazaki, S., Ling, N., Titani, K., and Sugino, H. (1999) J. Biol. Chem. 268, 15579–15587