Acute Control of Insulin-like Growth Factor-I Gene Transcription by Growth Hormone through Stat5b*

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Many of the effects of growth hormone (GH) are mediated by insulin-like growth factor-I (IGF-I), a secreted peptide whose gene transcription is induced by GH by unknown mechanisms. Recent studies in mice have implicated Stat5b as part of a GH-regulated somatic growth pathway, because mice lacking this transcription factor show diminished growth rates and a decline in serum IGF-I levels. To test the role of Stat5b in GH-stimulated IGF-I gene expression, we have delivered a replication-defective adenovirus encoding enhanced green fluorescent protein was used to deliver the adenovirus to target distinct components of hormone-activated signaling pathways.

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EXPERIMENTAL PROCEDURES

**Materials**—Antibodies to Akt and Erk were from Cell Signaling Technology (Beverly, MA). Anti-Stat1 (anti-ISGF3 p91/p84) was from Transduction Laboratories (Lexington, KY). Anti-Stat3 was from Upstate Biotechnology (Lake Placid, NY), anti-SP1 was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), and anti-FLAG (M2) was from Sigma (St. Louis, MO). Alkaline phosphatase-conjugated goat anti-rabbit and anti-mouse IgG were from Southern Biotechnology Associates (Birmingham, AL). Goat anti-mouse-Alexa 488 conjugate was from Life Technologies Corporation (Burlington, ON).

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Molecular Probes (Eugene, OR). Recombinant rat GH was from the National Hormone and Pituitary Program, NIDDK, National Institutes of Health. Oligonucleotides were synthesized at the Oregon Health & Science University (OHSU) Core Facility. All other chemicals were reagent grade and were obtained from commercial suppliers.

Recombinant Plasmids—pEFGP-N3 was from Clontech, Palo Alto, CA; pShuttle and pAdEasy were from Quantum Biotechnologies, Montreal, Canada. The mouse GH receptor DNA was a gift from Dr. F. Talambides (University of California, Santa Cruz, CA). The 8xGHRE-TK Cat and TK Luc plasmids were gifts from Dr. Susan Berry (University of Minnesota, Minneapolis, MN). Reporter gene 8xGHRE-TK Luc was constructed by substituting the luciferase coding region from TK Luc for Cat by standard molecular biological methods. A CDNA encoding rat Stat5b was a gift from Dr. Christian Curter-Su (University of Michigan, Ann Arbor, MI). It was modified by addition of an NHL-terminal FLAG epitope tag by site-directed mutagenesis. Point mutations were introduced to create Stat5b<sup>DN</sup> (Y699F) and Stat5b<sup>CA</sup> (N642H) using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA). For each Stat5b recombinant plasmid, the coding region was verified by DNA sequencing.

Generation of Recombinant Adenoviruses—Adenoviruses encoding EGFP, Stat5b, and mutant Stat5b<sup>ws</sup> were prepared following procedures described by the provider of pAdEasy. The DNA inserts were checked by PCR and by restriction enzyme mapping, and the recombinant viruses were amplified in HEK293 cells. All viruses were purified over a discontinuous Ca<sup>2+</sup> gradient and titrated by optical density.

Reporter Gene Assays—Cos-7 cells were transfected with an expression plasmid for the mouse GH receptor and 8xGHRE-TK Luc reporter gene using Fugene-6 and a protocol from the supplier. After incubation for 16 h, cells were infected with either Ad-EGFP, or different Ad-Stat5b variants, and Ad-tTA, each at a multiplicity of infection of 100. Ad-tTA encodes a tetracycline-inhibited transcriptional activator that binds to response elements adjacent to the promoter-regulating gene encoding either EGFP or Stat5b or Stat5b<sup>ws</sup> in each recombinant adenovirus and is required for gene expression. 8 h later, after addition of serum-free media containing 1% bovine serum albumin, and recombinant rat GH (1 μg/ml final concentration) or vehicle, cells were incubated for 16 h, harvested, and STAT<sup>±</sup> cells were used for luciferase assays. For trans-infection experiments, COS-7 cells were transfected as described above, followed by infection with Ad-Stat5b<sup>WT</sup>, Ad-tTA, and either Ad-β galactosidase or Ad-Stat5b<sup>ws</sup> at a ratio of 1:10. This corresponded to a multiplicity of infection of 10 for Ad-Stat5b<sup>WT</sup>, 90 for Ad-β-galactosidase or Ad-Stat5b<sup>ws</sup>, and 100 for Ad-tTA. Subsequent steps were as outlined above.

Animal Studies—Male Sprague-Dawley rats, hypophysectomized by a transauricular route at age 7 weeks, were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed at the OHSU Animal Care Facility on a 12-h light/dark schedule with free access to food and water, and received care according to National Institutes of Health guidelines. All procedures were pre-approved by the OHSU Animal Care and Use Committee. Guercotiducins (cortisol phosphate, 400 μg/kg/day) and thyroxine (10 μg/kg/day) were replaced by daily oral gavage (g/kg/day) and were introduced into COS-7 cells transfected with the mouse GH receptor, and the 8xGHRE-TK Luc reporter gene. Cells were infected with different recombinant adenoviruses as indicated, and incubated with recombinant rat GH (1 μg/ml) or vehicle (10 μM NaHCO<sub>3</sub>) for 16 h before analysis of luciferase activity as described under “Experimental Procedures.” B, immunoblot using an antibody to the FLAG epitope tag showing equivalent expression of different Stat5b proteins after transduction of recombinant adenoviruses into COS-7 cells. C, dominant-negative Stat5b blocks gene activation by wild-type Stat5b. COS-7 cells were transfected as in A. Cells were infected with adenoviruses encoding wild-type Stat5b and β-galactosidase at a ratio of 1:10 (WT/Mock) or with wild-type Stat5b and dominant-negative Stat5b 5b at a ratio of 1:10 (WT/ΔN). After incubation with rat GH as in B, cells were harvested, and luciferase activity measured. Results for A and C represent the means ± S.E. of three independent experiments, each performed in duplicate, and were normalized for protein concentration.

washes, immunoreactive proteins were visualized by enhanced chemiluminescence followed by detection using a Molecular Imager FX and quantification using Quantity One software (Bio-Rad, Hercules, CA).

RNA Isolation and Analysis—Total liver and hepatic nuclear RNA was isolated as described previously (13). RNA concentration was determined spectrophotometrically at 260 nm, and its quality was assessed by agarose gel electrophoresis. RNA (5 μg) was reverse-transcribed in a final volume of 20 μl using an RT-PCR kit (Invitrogen, Carlsbad, CA), with either oligo-DT primers (for total RNA) or random hexamers (for nuclear RNA). Each PCR reaction contained 0.5 μl of cDNA. Primer sequences are listed in Table I. The linear range of product amplification was established in pilot studies for each primer pair, and the cycle number that reflected the approximate midpoint was used in final experiments. This varied from 18 to 25 cycles for total RNA...
Fig. 2. Infection of pituitary-deficient rats with recombinant adenoviruses. A, immunocytochemistry demonstrating quantitative infection of hepatocytes after in vivo infection with a recombinant adenovirus encoding dominant-negative Stat 5b. Hypophysectomized rats were infected as described under "Experimental Procedures." After 48 h, hepatocytes were isolated and cultured for 24 h as outlined under "Experimental Procedures." Cells then were fixed for immunocytochemistry and stained in the left panel with an antibody for the FLAG epitope tag. The right panel shows the same field of cells visualized by phase microscopy. In this experiment, 88% of cells expressed Stat5bDN. B, demonstration of adenoviral infection by immunoblotting for Stat5b using the FLAG epitope tag or for EGFP by direct immunofluorescence. Rats were infected with recombinant adenoviruses as indicated. After 48 h, animals received an intraperitoneal injection of rat GH at a dose of 1.5 mg/kg, or vehicle (saline). After an additional 30–120 min, animals were euthanized. Rat liver protein extracts were isolated and assessed for recombinant protein expression, as outlined under "Experimental Procedures."

RESULTS

Development of Recombinant Adenoviruses Encoding Rat Stat5b and Variants—To determine if Stat5b plays a role in the transcriptional regulation of IGF-I gene expression, we first constructed a series of recombinant adenoviruses encoding wild-type, dominant-negative, and constitutively active versions of the protein. Dominant-negative rat Stat5b (Ad-Stat5bDN) contained a substitution mutation of phenylalanine for tyrosine at amino acid 699. The mutation prevents phosphorylation at this residue, thus blocking the inducible dimerization of two Stat5b molecules and the subsequent nuclear import that occurs normally after hormone treatment (20, 21). Constitutively active Stat5b (Ad-Stat5bCA) contains a point mutation of histidine for asparagine at residue 642 that was modeled after an activating mutation in the analogous location of human Stat5b. As seen in Fig. 1A, this mutation of histidine for asparagine at residue 642 resulted in a large increase in luciferase activity in COS-7 cells that had been transfected a day earlier with expression plasmids for the mouse GH receptor and the hormone-responsive reporter gene, 8xGHRE-TK Luc. GH treatment caused a 7.5-fold increase in luciferase activity in COS-7 cells that had been transfected a day earlier with expression plasmids for the mouse GH receptor and the hormone-responsive reporter gene, 8xGHRE-TK Luc. GH caused a small further rise in luciferase activity in cells infected with Ad-Stat5bDN and Ad-Stat5bWT (Fig. 1A). As seen in Fig. 1B, Stat5b protein expression from all three recombinant adenoviruses was similar. To test the function of Ad-Stat5bDN, cells were co-infected with Ad-Stat5bWT, and either Ad-Stat5bDN or an adenovirus encoding β-galactosidase (Ad-β-galactosidase) at a ratio of 1:10 as indicated under "Experimental Procedures." Ad-Stat5bDN completely prevented induction of reporter gene expression by GH, whereas Ad-β-galactosidase was ineffective (Fig. 1C). Based on these results, we conclude that both Ad-Stat5bCA and Ad-Stat5bDN function appropriately.

Quantitative Liver Expression after in Vivo Infection with Ad-Stat5b Variants—Traditional analyses of gene activation and studies in transgenic mice have not yielded the molecular mechanisms by which GH regulates IGF-I gene expression (23–25), even though in vivo studies have demonstrated that GH exerts a rapid and potent stimulatory effect on IGF-I transcription in the liver (13). Thus to assess any potential role of Stat5b in GH-induced IGF-I gene expression, it was necessary to establish conditions in which quantitative in vivo hepatic
infection with recombinant adenoviruses could be achieved. At optimal doses of viruses (2 × 10^{10}pfu of Ad-Stat5b and 2 × 10^{8}pfu of Ad-tTA), we found that 80–90% of liver cells were infected in vivo, as demonstrated by immunocytochemistry of subsequently isolated hepatocytes (Fig. 2A). In the absence of co-infection with Ad-tTA, no Stat5b was produced (data not shown). As seen in Fig. 2B, hypophysectomized rats treated without GH or with hormone for up to 120 min expressed relatively comparable levels of FLAG-tagged Stat5b^{DN} or Stat5b^{CA}, which was absent in rats infected with Ad-EGFP. We were unable to determine the effects of infection with either Ad-Stat5b^{DN} or Ad-Stat5b^{CA} on endogenous Stat5b, because both native and recombinant proteins reacted with Stat5b antibodies.

**Assessing Signal Transduction Pathways Regulated by GH in Hypophysectomized Rats Expressing Stat5b Variants**—Fig. 3 shows results of time-course experiments designed to evaluate intracellular signal transduction pathways that are usually activated by GH (1). As seen in Fig. 3A, Erk phosphorylation is acutely stimulated by *in vivo* GH treatment, as evidenced in rats infected with Ad-EGFP by detection of phosphorylated Erks 1 and 2 beginning at 30 min after hormone injection and continuing for up to 120 min, with a peak response of 3-fold above baseline at 30 min. An analogous time course of induction of Erk phosphorylation was observed in rats receiving either either Ad-Stat5b^{DN} or Ad-Stat5b^{CA} (2-fold above time 0 at 30 min for Ad-Stat5b^{DN} and 3-fold for Ad-Stat5b^{CA}). Akt also becomes rapidly phosphorylated after GH (1), as seen in Fig. 3B, and was induced for up to 120 min after GH in hypophysectomized rats infected with any of the three recombinant viruses, although a slightly higher level of basal phosphorylation was noted in animals expressing Ad-Stat5b^{CA}. This latter result is consistent with a prior observation in cultured cells overexpressing Stat5a^{CA} (26). At 30 min after GH in rats infected with any of the recombinant adenoviruses, levels of pAkt were nearly 7-fold above baseline values.

In addition to Stat5, the nuclear expression and activity of Stat1 and 3 is acutely stimulated by *in vivo* GH treatment (3–5). As shown in Fig. 3C, nuclear accumulation of these latter transcription factors was rapidly induced by GH in rats expressing each recombinant virus, although in animals infected with Ad-Stat5b^{DN}, basal nuclear expression of each protein was increased slightly in the absence of GH. Nevertheless, at 30 min after GH, nuclear Stat1 was 2-fold above basal levels in rats infected with each of the recombinant adenoviruses; nuclear Stat3 was 3-fold higher than baseline, except in animals infected with Ad-Stat5b^{DN}, where it was 2-fold higher. No change in abundance was seen for the constitutively nuclear transcription factor Sp1, confirming that equal amounts of nuclear protein were examined at each time point. Taken together, results from these experiments show that forced expression of recombinant adenoviruses for Stat5b or EGFP did not impede the acute biological effects of GH at the levels of cytoplasmic or nuclear signaling.

The activity of Stat5 was assessed in rats infected with recombinant adenoviruses by gel mobility shift assays, using hepatic nuclear protein extracts isolated before or after GH treatment, and a double-stranded DNA oligonucleotide containing the GH/prolactin response element from the β-casein gene. As seen in Fig. 3D, in Ad-EGFP-infected pituitary-deficient rats, GH stimulated sustained nuclear expression and DNA binding activity of Stat5. GH-induced binding toward the β-casein GH/prolactin DNA response element was markedly reduced after forced expression of Stat5b^{DN} with a detectable gel shift being seen only at a single time point, 30 min after GH, where Stat5b^{DN} protein expression was slightly diminished compared with other animals (see Fig. 2B). In contrast, DNA binding activity was greatly enhanced even in the absence of GH in rats infected with Ad-Stat5b^{CA}. Taken together with the results in Fig. 3 (A–C), the observations in Fig. 3D demonstrate the effectiveness and specificity of *in vivo* delivered Stat5b^{DN} and Stat5b^{CA}, respectively, in repressing or activating Stat5 function in the liver.

**Hormonal Regulation of Gene Expression**—We next examined the effects of Stat5b^{DN} and Stat5b^{CA} on GH-regulated hepatic gene expression. RNA was isolated at various times after GH injection and used as template for measurement of mRNA abundance by semi-quantitative RT-PCR (for primers see Table I). Spi 2.1 is a GH-stimulated liver-specific gene whose expression is dependent on active Stat5 (16, 18). As shown in Fig. 4A, GH induced Spi 2.1 mRNA within 120 min of hormone treatment in Ad-EGFP-infected rats but not in Ad-
The experiments reported in this manuscript document the feasibility of selectively targeting one component of a complex set of hormone-regulated signal transduction pathways in ex-
perimental animals (in this case activation of Stat5b by GH) through use of an adenovirus-mediated gene delivery system. This approach offers a potentially rapid and simple alternative to technically more complicated and slower transgenic strategies to knock out or overexpress specific genes in selected tissues in adult rodents.

Our observations surprisingly identify Stat5b as a key agent regulating the acute actions of GH in stimulating IGF-I gene transcription in the liver, the major site of growth factor production in vivo (8). We find that dominant-negative Stat5b completely prevents GH-stimulated IGF-I gene transcription, whereas active Stat5b effectively substitutes for GH, leading to robust IGF-I gene expression even in the absence of hormone. Although our results establish that Stat5b is an essential component of an acutely regulated signal transduction pathway that extends from the transmembrane GH receptor at the cell surface to the IGF-I gene in the nucleus, they do not show that this transcription factor is critical for normal somatic growth. In fact, published studies demonstrating the modest growth impairment and only ~50% decrease in serum IGF-I levels in male mice lacking Stat5b (6, 7) indicate that other factors in addition to Stat5b may play collaborative roles in regulating normal somatic growth and IGF-I gene and protein expression in response to GH. In mice without Stat5b since conception, these factors may compensate for the defect in signaling by this transcription factor, such that little growth impairment is seen in female Stat5b-deficient mice and modest defects observed in males.

The IGF-I gene is more complicated than would have been predicted from its simple protein sequence. In rodents the 6 exon gene is transcribed from tandem promoters, each with its own unique leader exon, into several RNA precursor molecules that undergo both alternative processing and differential polyadenylation to yield a multiplicity of mature mRNA species (29). A similarly complex pattern of mRNA production has been observed for other mammalian IGF-I genes, including the human gene (29). Previous studies have demonstrated that GH exerts its primary effects on IGF-I gene transcription but have not established molecular mechanisms or identified the contributing transcription factors. In pituitary-deficient rats, GH acutely induces IGF-I mRNAs regulated by both promoters, leading to a sustained accumulation of multiple IGF-I transcripts (13). As documented by run-on assays using rat hepatic nuclei, GH can stimulate a robust increase in IGF-I gene transcription within 30 min of systemic administration of hormone that can last for at least 6 h (13). Other studies have demonstrated that the induction of IGF-I gene transcription by GH is part of a primary hormonal response that does not require continuous protein synthesis (14).

Despite accumulating evidence showing that the activity of both IGF-I gene promoters is stimulated by GH, little understanding of regulation of promoter function has been achieved to date. Although basal control regions have been identified for each promoter in several mammalian species (25), no GH-response elements have been mapped. The only physiologically significant regulatory domain that has been characterized has been a binding site in IGF-I promoter 1 for the transcription factor C/EBPβ that appears responsible for regulation through parathyroid hormone, cAMP, and protein kinase A in osteoblasts (30). Studies using cultured cells generally have been unsuccessful in even showing a significant response of transfected IGF-I promoter-reporter genes to GH (23), and a single experiment using transgenic mice failed to even detect IGF-I transgene expression in the liver, although it did show regulation by GH in the brain (24). Although our results do not identify a GH-response region within the IGF-I gene, they may be interpreted to indicate that this putative region is likely to contain a binding site for Stat5b, and thus offer a rational framework for defining such an element. Alternatively, our data are also consistent with a model in which Stat5b acutely activates another transcription factor that is responsible for induction of IGF-I gene expression by GH. Further studies will be needed to distinguish between these alternatives and to determine the biochemical mechanisms by which Stat5b mediates GH-stimulated IGF-I gene transcription.

Stat5b is normally activated by direct phosphorylation by the GH receptor-associated tyrosine-protein kinase, Jak2 (1, 2), leading to its dimerization and subsequent nuclear translocation (20, 21). Many studies have established that these events occur rapidly in the liver and in liver-derived cell lines, as well as in other cell types (5, 14, 20). Nuclear accumulation of Stat5b has been detected within 5–15 min after a single in vivo hormonal pulse, with specific DNA binding activity being measurable soon thereafter (5). The activation of Stats 1 and 3 by GH occurs with similar kinetics (5). Sustained GH treatment causes the initiation of counter-regulatory measures that dampen subsequent biological responses, and in terms of Stat5b expression its dephosphorylation by nuclear phosphatases and subsequent export to the cytoplasm (31).

As documented in this manuscript, the genes for both IGF-I and SpI 2.1 comprise part of an acute response to GH that is dependent on Stat5b. GH also rapidly activates other genes in vivo through additional signal transduction pathways. As seen in this report, the early-response gene, c-fos, is induced with kinetics similar to those of IGF-I, but as expected is unmodified by dominant-negative or constitutively active Stat5b, because previous studies have demonstrated that c-fos gene transcription is stimulated by GH through pathways involving Stats 1 and 3, and the Erk-activated Elk-1 transcription factor (28, 32, 33).

In addition to altering gene activity through acute regulation of several signal transduction pathways, the pattern of GH secretion and action in vivo also influences gene expression. In male rodents, GH is secreted in a pulsatile fashion, whereas in females it is more continuous (34). These sexually dimorphic patterns of GH secretion reversibly modify the transcriptional activity of hepatic genes encoding several cytochrome P450 2C sterol hydroxylases (35) and rodent major urinary proteins (36). To date the molecular mechanisms underlying pattern-specific gene regulation by GH remain unknown, and the role of Stat5b has been undefined, although its activity in the nucleus is lower in models of continuous GH exposure than pulsatile treatment (37).

IGF-I plays a multifaceted role in human physiology. It is essential for normal developmental and somatic growth during childhood (38) and is a key agent in maintaining tissue integrity during aging (39). However, IGF-I also has been linked by epidemiological studies in humans and by experimental manipulations in animals to several cancers (40). Thus, full understanding of the molecular mechanisms by which GH and other trophic agents control IGF-I gene expression is essential for implementing rational therapeutic decisions designed to regulate this critical growth factor.

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REFERENCES
1. Herrington, J., and Carter-Su, C. (2001) Trends Endocrinol. Metab. 12, 252–257
2. Smit, L. S., Meyer, D. J., Billestrup, N., Norstedt, G., Schwartz, J., and Carter-Su, C. (1996) Mol. Endocrinol. 10, 519–533
3. Gronowski, A. M., and Rotwein, P. (1994) J. Biol. Chem. 269, 7874–7878
4. Gronowski, A. M., Zhong, Z., Wen, Z., Thomas, M. J., Darnell, J. E., Jr., and Rotwein, P. (1995) Mol. Endocrinol. 9, 171–177
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5. Ram, P. A., Park, S. H., Choi, H. K., and Waxman, D. J. (1996) J. Biol. Chem. 271, 5929–5940

6. Udy, G. B., Towers, R. P., Snell, R. G., Wilkins, R. J., Park, S. H., Ram, P. A., Waxman, D. J., and Davey, H. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 7239–7244

7. Teglund, S., McKay, C., Schuetz, E., van Deursen, J. M., Stravopodis, D., Wang, D., Brown, M., Bodner, S., Grosveld, G., and Ihle, J. N. (1998) Cell 93, 841–850

8. Daughaday, W. H., and Rotwein, P. (1989) Endocrinology 115, 8–91

9. Liu, J. P., Baker, J., Perkins, A. S., Robertson, E. J., and Efstratiadis, A. (1993) Cell 75, 59–72

10. D’Ercole, A. J., Stiles, A. D., and Underwood, L. E. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 935–939

11. Hyner, M. A., Van Wyk, J. J., Brooks, P. J., D’Ercole, A. J., Jansen, M., and Lund, P. K. (1987) Mol. Endocrinol. 1, 233–242

12. Murphy, L. J., Bell, G. I., Duckworth, M. L., and Friesen, H. G. (1987) Endocrinology 121, 684–691

13. Bichell, D. T., Kikuchi, K., and Rotwein, P. (1992) Mol. Endocrinol. 6, 1899–1908

14. Gronowski, A. M., Le Stunff, C., and Rotwein, P. (1996) Endocrinology 137, 55–64

15. Davey, H. W., Xie, T., McLachlan, M. J., Wilkins, R. J., Waxman, D. J., and Grattan, D. R. (2001) Endocrinology 142, 3836–3841

16. Yoon, J. B., Berry, S. A., Seelig, S., and Towle, H. C. (1990) J. Biol. Chem. 265, 19947–19954

17. Thomas, M. J., Gronowski, A. M., Berry, S. A., Bergad, P. L., and Rotwein, P. (1995) Mol. Cell. Biol. 15, 12–18

18. Bergad, P. L., Shih, H. M., Towle, H. C., Schwarzenberg, S. J., and Berry, S. A. (1995) J. Biol. Chem. 270, 24903–24910

19. Jones, W. K., Yu, L., Lai, K. Y., Cift, S. M., Brown, T. L., and Rosen, J. M. (1985) J. Biol. Chem. 260, 7042–7050

20. Gouilleux, F., Wakao, H., Mundt, M., and Greener, B. (1994) EMBO J. 13, 4361–4369

21. Levy, D. E., and Darnell, J. E., Jr. (2002) Nat. Rev. Mol. Cell. Biol. 3, 651–662

22. Ariyoshi, K., Nosaka, T., Yamada, K., Onishi, M., Oka, Y., Miyajima, A., and Kitamura, T. (2000) J. Biol. Chem. 275, 24407–24413

23. Benbassa, C., Shoba, L. N., Newman, M., Adamo, M. L., Frank, S. J., and Lowe, W. L., Jr. (1999) Endocrinology 146, 3973–3981

24. Ye, P., Umayahara, Y., Ritter, D., Bunting, T., Auman, H., Rotwein, P., and D’Ercole, A. J. (1997) Endocrinology 138, 5466–5475

25. Adamo, M. L. (1995) Diabetes Rev. 3, 2–7

26. Santos, S. C., Lacremizie, V., Bouchaert, I., Monni, R., Bernard, O., Gisselbrecht, S., and Gouilleux, F. (2001) Oncogene 20, 2080–2090

27. Gronowski, A. M., and Rotwein, P. (1995) Endocrinology 136, 4741–4748

28. Chen, C., Clarken, R. W., Xie, Y., Hume, D. A., and Waters, M. J. (1995) Endocrinology 136, 4505–4516

29. Rotwein, P. (1999) in The IGF System (Rosenfeld, R. G., and Roberts, C. T., Jr., eds) pp. 19–35, Humana Press, Totowa, NJ

30. Umayahara, Y., Billiard, J., Ji, C., Centrella, M., McCarthy, T. L., and Rotwein, P. (1999) J. Biol. Chem. 274, 10609–10617

31. Gebert, C. A., Park, S. H., and Waxman, D. J. (1999) Mol. Endocrinol. 13, 38–56

32. Hodge, C., Liu, J., Stofega, M., Guan, K., Carter-Su, C., and Schwartz, J. (1998) J. Biol. Chem. 273, 31327–31336

33. Meyer, D. J., Stephenson, E. W., Johnson, L., Cochran, B. H., and Schwartz, J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 6721–6725

34. Jansson, J. O., Eden, S., and Isaksson, O. (1985) Endocrinology 116, 126–130

35. Legraverend, C., Mode, A., Westin, S., Strom, A., Eguchi, H., Zaphireopoulus, P. G., and Gustafsson, J. A. (1992) Mol. Endocrinol. 6, 259–266

36. Norstedt, G., and Palmeter, R. (1984) Cell 36, 805–812

37. Gebert, C. A., Park, S. H., and Waxman, D. J. (1997) Mol. Endocrinol. 11, 400–414

38. Woods, K. A., Camacho-Hubner, C., Savage, M. O., and Clark, A. J. (1996) N. Engl. J. Med. 335, 1363–1367

39. Musaro, A., McCullagh, K., Paul, A., Houghton, L., Dobrowolsky, G., Molinaro, M., Barton, B. R., Sweeney, H. L., and Rosenthal, N. (2001) Nat. Genet. 27, 195–200

40. Purvesberger, G., and Senn, H. J. (2002) Lancet Oncol. 3, 298–302
