Regulation of Insulin Receptor Gene Expression

CELL CYCLE-MEDIATED EFFECTS ON INSULIN RECEPTOR mRNA STABILITY*

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Posttranscriptional mechanisms play important roles in insulin receptor gene regulation; variability in cellular insulin receptor number and the growth arrest-mediated increases in insulin receptor mRNA are secondary to changes in insulin receptor mRNA stability. Therefore, further characterization of the pathways and kinetics of insulin receptor mRNA degradation were investigated. The insulin receptor mRNA in the insulin receptor-rich Hep G2 cells is more stable compared with the insulin receptor-sparse MCF-7 cells. Growth arrest results in a significant rise in insulin receptor mRNA in both cell lines. The increase in mRNA is caused by changes in mRNA stability. The half-life of the insulin receptor mRNA in growth-arrested cells is approximately three times that of proliferating cells. The insulin receptor gene contains four polyadenylation sites that produce four species of mRNA of 5.4, 6.9, 8.0, and 9.4 kilobases (kb). The mRNA species are not coordinately regulated. The ratio of the most abundant species (9.4/6.9) is significantly larger in growth-arrested cells compared with proliferating cells. By utilizing a specific cDNA probe for the 9.4-kb mRNA species, it was determined that the diminished 9.4/6.9 ratio in proliferating cells was caused by a more rapid rate of the 9.4-kb mRNA degradation. The kinetics of insulin receptor mRNA degradation were investigated. Insulin receptor mRNA levels were reduced to 56% of their baseline within 6 h when growth-arrested cells were stimulated to proliferate; protein inhibition with cycloheximide completely inhibited the decline in insulin receptor mRNA.

Insulin-induced pleiotropic cellular responses depend in part on the number of functional insulin receptors, a transmembrane protein with ligand-mediated tyrosine kinase activity. With the cloning of the cDNA for the insulin receptor and its 5' flanking region (1, 2), much attention has focused on mechanisms for regulating insulin receptor gene expression. The relatively long half-life of the insulin receptor mRNA (3-5) and the low abundance of cellular insulin receptor mRNA suggest a low transcription rate of the insulin receptor gene (6); conditions which are known to up- or downregulate insulin receptor number, such as insulin and cellular growth arrest, have little effect on transcription rates or insulin receptor promoter activity (7). Transcription is regulated, however. Glucocorticoids have been shown to increase transcription in pancreatic acinar cells (8) and IM-9 lymphocytes (9, 16); recently, binding of a glucocorticoid receptor to a consensus glucocorticoid regulatory element downstream from major transcriptional initiation sites on the insulin receptor promoter has been demonstrated (6). In addition to transcription, posttranscriptional mechanisms play important roles in gene expression; cytoplasmic insulin receptor mRNA stability is emerging as an important control point in the insulin receptor gene system. A recent study which examined cells with various numbers of insulin receptors demonstrated that the number of cellular insulin receptors and insulin receptor mRNA varies with the half-life of the insulin receptor mRNA (3); this posttranscriptional mechanism may account for differences in steady-state levels of insulin receptor mRNA and may account for the variability of insulin sensitivity in different cells.

Among the most intensively studied mRNAs for which instability is known to regulate gene expression are those whose products mediate growth control (11). This group includes proto-oncogenes (such as c-fos (12) and c-myc (13)) and lymphokines and cytokines (11). In addition, a number of mRNAs for DNA biosynthetic enzymes are regulated by posttranscriptional processes as cells enter the S phase of the cell cycle: among these enzymes are included thymidine kinase (14, 15), dihydrofolate reductase (16), and the histone proteins (17-19). Gene expression of the growth-mediating insulin receptor has been demonstrated to be dependent on the proliferative stage of the cell; insulin receptor mRNA levels increase approximately 7-fold in growth-arrested (Go) Hep G2 cells and are inhibited when cells are stimulated to grow (4, 7). In the present study we have investigated the regulation of the insulin receptor gene in various stages of cell proliferation with particular emphasis on the role of mRNA stability. Because posttranscriptional mechanisms may determine the variability of cellular insulin receptor number, we have compared the regulation of the insulin receptor gene in a cell line with abundant receptors (Hep G2) and a cell line with few receptors (MCF-7). We have found that growth arrest in both cell lines results in an increase in insulin receptor mRNA because of a specific increase in insulin receptor mRNA stability. In addition we have discovered that the largest insulin receptor mRNA species (9.4 kb)† is particularly responsive to cell cycle regulation.

MATERIALS AND METHODS

All culture supplies were purchased from Sigma. Cycloheximide and actinomycin D were dissolved in 100% ethanol at stock concentrations of 1 and 2.5 mg/ml, respectively. All stock concentrations were made fresh with each experiment.

Cell Culture—Hep G2 and MCF-7 cells were plated at a density of approximately 5 x 10⁵ cells/60-mm dish or 5 x 10⁶ cells per 100-mm

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† The abbreviation used is: kb, kilobase(s).
dish in complete medium (Dulbecco's modified Eagle's medium, 10% fetal calf serum, 20 mM L-glutamine, and Pen/Strep) and incubated at 37 °C, 5% CO₂. To stimulate cell proliferation, the medium was aspirated and replaced daily with fresh complete medium. Failure to replace complete medium resulted in arrest of cell proliferation. The morphology of "starved" cells remained grossly normal, and they remained attached to the dish in monolayer and could be stimulated to proliferate with the addition of complete medium for up to 7 and 5 days in Hep G2 and MCF-7 cells, respectively. The cell number was determined by lifting the cells from the dish with trypsin/EDTA and counting an aliquot of the cell suspension with a hemocytometer.

Hybridization of 32P-Labeled cDNA Probes to RNA—Total RNA was extracted from each cell line with guanidium isothiocyanate and spun through a CsCl gradient as described previously (7). Poly(A) RNA was isolated from 1 mg of total RNA with the use of a biotinylated oligo(dT) primer as described by the supplier (PolyTract TM mRNA Isolation Systems, Promega). In Northern blot analyses, 10 μg of total RNA or 5 μg of poly(A) mRNA was loaded on to a 1.5% agarose, 0.66 M formaldehyde denaturing gel. The RNA was transferred to nitrocellulose by capillary action as described previously (7). In slot-blot analyses, 10 μg of total Hep G2 RNA and 20 μg of total MCF-7 RNA were transferred directly to nitrocellulose via a slot-blot apparatus as described by the supplier (Schleicher & Schuell). The nitrocellulose was air dried and baked at 80 °C for 2 h and hybridized to nick-translated, 32P-labeled cDNA probes as described previously (7). The membranes were dried under stringent conditions as described previously (7). The insulin receptor cDNA probe is a 3' fragment corresponding to base pairs 1100-1900 in exons 2-6 (available through American Type Tissue Culture). B-actin and histone 2B cDNA probes were generously supplied by Dr. Geoffrey Krystal (Medical College of Virginia, Richmond).

Cloning the 3'-Flanking Region of the Insulin Receptor Gene—A 30-mer oligonucleotide corresponding to the 3'-flanking region (4755-4897) of the published sequence of the cDNA of the human insulin receptor (2) was synthesized and used to screen a genomic human placental library (Stratagene, a-Fix 11). Approximately 10⁶ plaques were screened, of which only one hybridized to the end-labeled synthesized oligonucleotide. The 20-kb insert was digested with restriction enzymes, and an 8-kb SstI/BamHI fragment was subcloned into a pGEM T7(+) vector (Promega). A restriction map was performed as shown in Fig. 1 and was consistent with the published map of the human insulin receptor 3'-flanking region of Tewari et al. (20). Transcription of the insulin receptor gene results in at least four insulin receptor mRNA species of 5.4, 6.9, 8.0, and 9.4 kb (20). The polyadenylation site for the 5.4-kb transcript (labeled P1 in Fig. 1) has been identified by Ulrich et al. (2) and is located 4962 base pairs from the translational initiation site of the α-subunit. Based on the above observations and the predicted sizes of the transcripts, 3' and 5' polyadenylation sites were identified at base locations of 6.9- (P2), 8.0- (P3), and 9.4- (P4) kb mRNAs are shown in Fig. 1. Therefore, we predicted that digestion of the 3'-flanking region with the restriction enzyme BglII would result in a 1.3-kb fragment that would hybridize to the 9.4-kb insulin receptor mRNA but not to the smaller insulin receptor mRNA species.

RESULTS

The effect of replacing complete medium (solid lines) on the proliferation of the insulin receptor-rich Hep G2 (closed circles) and insulin receptor-sparse MCF-7 (closed triangles) cells is shown in Fig. 2. Both cell lines grow rapidly and have similar doubling times (approximately 30 h). Failure to replace fresh medium (broken lines) results in growth arrest within 3-4 days in both cell lines. Cells that are growth-arrested for 5 days will proliferate normally when complete medium is replenished daily (compare days 3-6 with days 5-8, solid lines).

Insulin receptor mRNA increases approximately 7-fold in growth-arrested Hep G2 cells (4, 7). To determine whether this observation is tissue-specific, total RNA was extracted from the insulin receptor-sparse MCF-7 cells after 3-7 days of growth arrest, applied directly to nitrocellulose via a slot-blot apparatus, and hybridized to the 32P-labeled 5' insulin receptor cDNA and B-actin cDNA probes. As observed in Hep G2 cells, MCF-7 insulin receptor mRNA levels increase progressively from 3 to 6 days (see Fig. 3). This effect is genespecific; B-actin mRNA levels in the same time frame progressively decline. Densitometric analysis demonstrated that the MCF-7 insulin receptor mRNA level increased 5.3-fold from day 3 to day 6 (data not shown). On day 7 the insulin receptor mRNA levels decrease. However, the MCF-7 cells on day 7 were less viable; many cells were noted to have changed their morphology and were lifting from the tissue culture dish. All future experiments on growth-arrested MCF-7 cells were performed 5 days after plating the cells, which have normal morphology and proliferate normally in response to complete medium (see Fig. 2).

The mechanism for the rise in insulin receptor mRNA in growth-arrested Hep G2 and MCF-7 cells was investigated. Since regulation of mRNA stability plays a role in cell cycle-mediated changes in growth-related gene expression, the turnover of insulin receptor mRNA was examined in proliferating Hep G2 and MCF-7 cells. Approximately 5 × 10⁶ Hep G2 (circles) and MCF-7 (triangles) cells were plated on day 1 in complete medium (see "Materials and Methods") and incubated in 5% CO₂ at 37 °C. On day 3, in half of the cells, the medium was replaced with fresh medium daily (refed, solid lines). In the other half of the cells, the medium was not replaced (starved, dotted lines). On days 3-6, an aliquot of the cell suspension was counted by hemocytometer as described under "Materials and Methods." A second experiment is also included in this figure. Hep G2 and MCF-7 cells were starved for 5 days after plating. On the 5th day, the cells were incubated with fresh medium daily. Cell count was determined as above.
was extracted from the cells as described under “Materials and Methods.” 20 μg of RNA was applied directly to nitrocellulose via a slot-blot apparatus, baked, and hybridized to 32P-labeled 5' insulin receptor and B-actin cDNA probes. The nitrocellulose was washed under stringent conditions (0.1 x SSC, 65 °C). After drying, autoradiography was performed with XAR film. The insulin receptor (IR) and the actin (A) blots were exposed for 1 week and overnight, respectively.

Fig. 3. Time course of insulin receptor RNA abundance in growth-arrested MCF-7 cells. MCF-7 cells were plated on day 1. Medium was not aspirated or supplemented. On days 3–7, total RNA was extracted from the cells as described under “Materials and Methods.” After 5 days, actinomycin D (25 μg/ml) was added to inhibit transcription. After 2, 4, and 6 h, total RNA was extracted and applied directly to nitrocellulose via a slot-blot apparatus. After baking, the blots were hybridized to 32P-labeled insulin receptor and B-actin cDNA probes. The blots were washed under stringent conditions and dried. Denaturation was performed from the autoradiograms of three separate experiments. The data represent the mean ± S.E. normalized as a percentage of the optical density of the time 0 slot (RNA extracted before the addition of actinomycin D).

Fig. 4. Stability of insulin receptor mRNA in proliferating and growth-arrested Hep G2 and MCF-7 cells. After plating, Hep G2 (left panel, circles) and MCF-7 (right panel, triangles) cells were incubated in the absence (open symbols) or presence (closed symbols) of fresh complete medium as described under “Materials and Methods.” After 5 days, actinomycin D (25 μg/ml) was added to inhibit transcription. After 2, 4, and 6 h, total RNA was extracted and applied directly to nitrocellulose via a slot-blot apparatus. After baking, the blots were hybridized to 32P-labeled insulin receptor (exons 2–6), B-actin, and histone 2B cDNA probes. The blots were washed under stringent conditions and dried. Denaturation was performed from the Northern blot analysis of the insulin receptor RNA in proliferating and growth-arrested Hep G2 cells (as will be shown below).

A comparison of the half-lives of the insulin receptor, actin, and histone 2B RNAs in proliferating and growth-arrested Hep G2 and MCF-7 cells is shown in Table I. The half-life of the insulin receptor RNA in proliferating MCF-7 cells is significantly reduced by approximately 2 h compared with the insulin receptor RNA half-life in proliferating Hep G2 cells. This finding may explain in part the diminished steady-state levels of insulin receptor mRNA in MCF-7 compared with Hep G2 cells and confirms a previous study (5). Growth arrest significantly prolongs the half-life of the insulin receptor RNA by approximately 3-fold in both cell lines. By contrast, the long half-life actin mRNA appears to be significantly reduced by growth arrest. Histone 2B mRNA is consistently less than 2 h and demonstrates that transcription was significantly inhibited by the conditions of the assay.

Four polyadenylation sites in the 3' flanking region of the insulin receptor gene are responsible for the production of at least four species of insulin receptor mRNA, approximately 5.4, 6.9, 8.0, and 9.4 kb in size (20). The functional significance of these multiple species is currently unknown. One possibility is that different mRNA species play a role in regulation of gene expression under various physiologic conditions. To investigate whether the insulin receptor mRNA species are differentially expressed in various stages of cell growth, a Northern blot analysis was performed on poly(A) RNA extracted from MCF-7 cells in proliferating and growth-arrested cells. As shown in the left lane of Fig. 5, growth-arrested MCF-7 cells contain abundant 9.4- and 6.9-kb mRNA species in contrast to the barely detectable 8.0- and 5.4-kb mRNA species. When cells are stimulated to proliferate (right lane), an overall decline in insulin receptor mRNA is observed. However, the decline in the levels of individual species varies considerably. The largest (9.4 kb) species declines more rapidly than the 6.9-kb species, resulting in a 9.4/6.9 ratio that is less in the proliferating cells compared with the growth-arrested cells. Densitometric analysis of this Northern blot reveals that the area under the total optical density curve in growth-arrested cells is reduced by 43% when cells are stimulated to proliferate. The densities of the individual mRNA species do not vary coordinately; the area under the 9.4-kb curve falls more rapidly than the area under the 6.9-kb curve.

The ratio of the 9.4-kb to total insulin receptor RNA is approximately 0.43 in growth-arrested cells and declines to 0.18 in proliferating cells. By contrast, the ratio of the 6.9-kb to total RNA increases from 0.56 to 0.81 when growth-arrested cells are stimulated to proliferate. The ratio of the 9.4- to 6.9-kb insulin receptor RNA declines from approximately 0.76 to 0.22 in growth-arrested and proliferating cells, respectively. Similar qualitative results were observed in the Northern blot analysis of the insulin receptor RNA in proliferating and growth-arrested Hep G2 cells (as will be shown below).

### Table I

|                  | IR (h) | Actin (h) | H2B (h) |
|------------------|--------|-----------|---------|
| Hep G2           |        |           |         |
| Proliferating†   | 6.6 ± 0.7 | >24       |         |
| Growth-arrested† | 19.4 ± 1.9* | 20 ± 2.4 | <2      |
| MCF-7            |        |           |         |
| Proliferating†   | 4.5 ± 0.9** | >24       | <2      |
| Growth-arrested† | 12.0 ± 1.5*** | 13 ± 2.5 | <2      |

Summary of data performed in experiments described in the legend of Fig. 4. All data are the mean ± S.E. from three separate experiments. *b versus a, p < 0.05 (Student's t test). **c versus a, p < 0.05. ***d versus a, p < 0.05.
The change in the ratio of the 9.4- to 6.9-kb insulin receptor mRNA species in proliferating and growth-arrested cells could be caused by either differential selection of the polyadenylation sites in the various stages of cell growth or variation in the stability of individual mRNA species. To examine the latter possibility, the 3'-flanking region of the insulin receptor gene was cloned, and a cDNA probe was selected that hybridized to the 9.4-kb insulin receptor mRNA but not to the other mRNA species. As shown in Fig. 6, a radiolabeled, EgrIII-digested fragment of 1.3 kb in the 3'-flanking region (see Fig. 1) of the insulin receptor gene hybridized to the 9.4-kb insulin receptor mRNA species in proliferating (lane 3) and growth-arrested (lane 4) MCF-7 cells. A 5' insulin receptor probe hybridized to all four insulin receptor mRNA species in the same blot (lanes 1 and 2). The half-life of the 9.4-kb species was then compared with the half-life of total insulin receptor mRNA in growth-arrested and proliferating Hep G2 cells. The half-life of the 9.4-kb species in growth-arrested cells was identical to that of total insulin receptor RNA (approximately 20 h, data not shown). By contrast, the stability of the 9.4-kb species (t_1/2 = 4 h) is less than that of total insulin receptor mRNA (t_1/2 = 6.5 h) in proliferating cells as shown in Fig. 7. Because total insulin receptor RNA includes the 9.4-kb species, this turnover analysis actually underestimates the differences between individual species. Therefore, differential expression of individual insulin receptor mRNA species results in part from the cell proliferation induced variability of individual mRNA stability.

The kinetics of insulin receptor mRNA turnover and the effects of inhibition of transcription and protein synthesis on insulin receptor mRNA degradation were investigated. Hep G2 and MCF-7 cells were plated and observed for 5 days without replacing fresh medium. On day 5, the medium from these growth-arrested cells was aspirated and replaced with fresh complete medium in the absence and presence of actinomycin D (25 µg/ml) or cycloheximide (1 µg/ml). Total RNA was extracted after 2 and 6 h and hybridized to the 5' insulin receptor and B-actin cDNA probes. The results are shown in Figs. 8-10. In Hep G2 cells (Fig. 8, left panel, and Fig. 9), the insulin receptor mRNA level falls to approximately 56% of base line (time 0) 6 h after replacing fresh medium (Fig. 9, closed circles). The time at which 50% of the base-line level of mRNA decays is similar to the half-life of the insulin receptor RNA in proliferating Hep G2 cells (see Table I). Addition of the transcription inhibitor actinomycin D (open circles) has little effect on the rate of fall of insulin receptor mRNA levels, suggesting that the methods in this assay are not sensitive enough to detect the effects of the low rates of insulin receptor RNA transcription on total insulin receptor mRNA levels. Protein synthesis inhibition with cycloheximide (open triangles) completely inhibits the decline in insulin receptor mRNA. These results are specific for the insulin mRNA.
FIG. 8. Kinetics of insulin receptor RNA turnover: the effects of transcription and protein synthesis inhibitors. Hep G2 cells were plated, and fresh complete medium was not replaced. On day 5, the medium was aspirated and replaced with fresh complete medium to stimulate cell proliferation in the absence and presence of actinomycin D (A, 25 μg/ml) or cycloheximide (Cx, 1 μg/ml). After 2 and 6 h, total RNA was extracted. 10 μg of total Hep G2 cell (left panel) and 20 μg of total MCF-7 cell (right panel) RNA was applied to nitrocellulose by a slot-blot apparatus. The RNA was hybridized to a 5' insulin receptor cDNA probe (IR) and to B-actin (A) as described under "Materials and Methods."

FIG. 9. Densitometric analysis of insulin receptor RNA kinetics in Hep G2 cells. The experiment is identical to that described in Fig. 8. Densitometry was performed, and each data point is a percentage of the optical density of the control RNA (time 0) for each probe. The results represent the mean of two separate experiments. No data point deviated by more than 15%.

FIG. 10. Densitometric analysis of insulin receptor RNA kinetics in MCF-7 cells. The experiment is identical to the described in Figs. 8 and 9 except that MCF-7 cells were analyzed rather than Hep G2 cells.

receptor mRNA. As shown in Fig. 9 (right panel) the level of B-actin mRNA (closed circles) rises rapidly when growth-arrested Hep G2 cells are stimulated to grow with fresh medium. This rise is secondary to increased rates of transcription since inhibition of transcription with actinomycin D (open circles) prevents the growth-stimulated rise in B-actin mRNA levels. Cycloheximide has very little effect of B-actin mRNA levels (open triangles). The effect of growth stimulation in the absence and presence of transcription and protein synthesis inhibition on insulin receptor mRNA levels in MCF-7 cells is shown in Fig. 10. The results are very similar to those observed in Hep G2 cells.

Since cycloheximide inhibits the proliferation-induced de-
lives are similarly affected by cell proliferation, growth arrest, and protein synthesis inhibition.

In the present study, we have characterized some of the mechanisms responsible for insulin receptor gene expression in proliferating and growth-arrested cells. We propose that in proliferating cells, a low rate of gene transcription combined with a relatively rapid rate (compared with growth-arrested cells) of insulin receptor mRNA degradation results in low steady-state levels of insulin receptor mRNA. When proliferating cells are deprived of fresh complete medium, factors responsible for cell proliferation are being depleted or factors inhibiting cell growth are being produced which result in cellular growth arrest; these conditions also affect cellular mechanisms responsible for gene-specific mRNA degradation. Insulin receptor mRNA half-life is markedly prolonged in growth arrest. This increased stability can fully account for the rise in steady-state levels of insulin receptor mRNA. When growth-arrested cells are refed fresh medium and begin to proliferate, cellular mechanisms for insulin receptor mRNA degradation are stimulated without a compensatory increase in insulin receptor gene transcription (see Figs. 8–10), and the insulin receptor mRNA levels fall markedly. Proliferation-stimulated mRNA degradation is mediated, in part, by a labile protein since the protein synthesis inhibitor cycloheximide inhibits the fall in insulin receptor mRNA levels. It is unlikely that the mechanism of the cycloheximide-mediated inhibition of the decline in insulin receptor mRNA levels is caused by effects on insulin receptor gene transcription. We base this conclusion on two observations. First, the rate of insulin receptor gene transcription is undetectable as growth-arrested cells are stimulated to proliferate (see Figs. 8–10). Second, analyses of the activity of the insulin receptor promoter have failed to detect any inhibitory regulatory elements (6, 7, 20, 22–25). Other investigators have postulated that the cycloheximide-mediated inhibition of mRNA degradation is secondary to the requirement that mRNA degradation depends upon ongoing gene translation (12). The present study does not differentiate between these two possible theories.

The degradation rate of total Hep G2 cell insulin receptor mRNA has previously been measured by two other laboratories. The half-life measurements of the insulin receptor mRNA in the present study concur with the studies by Tewari et al. (3); however, the measurements as reported by Hatada et al. (4) are much longer. The reasons for this discrepancy are not clear. To the best of our knowledge, half-life measurements of individual Hep G2 mRNA species have not been calculated previously. However, Goldstein et al. (5) have demonstrated that in Fao cells, the largest insulin receptor mRNA species (9.6 kb) had a similar rate of degradation as compared with the most abundant mRNA species (7.4 kb). It is not clear from the methods of this study whether these cells were in a growth-arrested versus a proliferative stage. We have found that the proportion of individual insulin receptor mRNA species varies according to cell growth. In quiescent cells, the 9.4-kb species comprises a larger proportion of the total insulin receptor mRNA compared with proliferating cells. The mechanism was also investigated; in proliferating cells the 9.4-kb species has a more rapid rate of degradation compared with the other insulin receptor species. The human insulin receptor gene contains four polyadenylation sites in the 3'-flanking region which results in the production of the four mRNA species of 5.4, 6.9, 8.0, and 9.4 kb. Although previously cloned, the 3'-flanking region has not been sequenced, and therefore consensus structural features that determine RNA stability (11, 26, 28), such as stem-loop secondary structures (29), (A+T)-rich instability regions (30), or variation in the size of the poly(A) tail (31), have not been examined to explain potential differences in stability of individual mRNA species. Although we have observed differences in stability, we cannot rule out the possibility that polyadenylation selection may also play a role in the differential expression of insulin receptor mRNA in proliferating cells. Selection of specific polyadenylation sites in response to cell growth has been observed in other gene systems. For example, upstream polyadenylation sites in the dihydrofolate reductase gene seem to be favored in proliferating cells (16). In contrast, the usage of the two polyadenylation sites in the c-myc gene is not altered during the growth induction of quiescent cells (32). Interestingly, Goldstein et al. (5) did observe variation in the expression of individual insulin receptor mRNA species in rat tissue. They found that the 9.6/7.4 mRNA ratio in rat brain, kidney, liver, and muscle was 2.69, 1.18, 1.56, and 0.99, respectively. The largest ratio occurs in a tissue (brain) known to be relatively quiescent. Whether this relationship is observed in other quiescent human cellular tissue remains to be determined.

Several of the most intensively studied mRNAs for which instability plays a role in regulating gene expression are those whose products mediate cell proliferation. mRNA for c-fos, c-myc, for dihydrofolate reductase are all induced when quiescent, growth-arrested cells are stimulated to proliferate with replacement of serum or specific growth factors. These fluctuations result from changes both in transcription and in mRNA stability. Although the insulin receptor also mediates cell proliferation and shares many structural and functional properties of oncogenes such as tyrosine kinase activity, cellular growth arrest stimulates while proliferation inhibits gene expression. Although the growth-mediated regulation of insulin receptor gene expression appears to run counter to other growth-related genes, inhibition of gene expression with cell proliferation is not unique to the insulin receptor. Interestingly, insulin mRNA levels of rat insulinoma cell lines increased 6–8-fold as cells entered a transient state of growth arrest when they were cultured in a serum-free medium (27). Perhaps increased stability of insulin and insulin receptor mRNA evolved to maintain essential cellular processes such as nutrient transport and intermediary metabolism in quiescent cells in contrast to their growth-promoting effects.

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