Factors involved in regulating tissue-specific gene expression play a major role in cell differentiation. In adults, the pancreatic β-islet cell is the specific site of insulin gene expression. In rats, there are two nonallelic insulin genes, I and II (1), which are regulated coordinately in vivo (2, 3). The rat insulin I gene is believed to have been derived from the insulin II gene by a retrotransposition event (4). The 5′-flanking region of both rat insulin genes (an area spanning up to ~600 bp from the site of transcription initiation) has been demonstrated to confer β-cell-specific expression of reporter gene constructs by expression assays in cultured cells (5, 6) and in transgenic mice (7, 8). During development, the 5′-flanking region of both rat insulin genes also directs expression in the neural tube and neural crest cells as well as in the islet cell precursors of the evaginating pancreatic bud (7).

A high degree of sequence homology has been found between the two rat insulin promoter regions (4), and both have been shown by deletion analysis and site-directed mutagenesis to contain multiple cis-elements that are important for both the positive (9–11) and negative regulation of insulin gene transcription (12, 13). Seven different cis-elements, referred to as rat insulin promoter elements (RIPEs), were found by mutation analysis of the 5′-flanking region of the rat insulin II gene (9). One region, RIPE3 (−126 to −86), displayed the greatest loss of transcriptional activity when mutated. Placement of RIPE3 in either orientation upstream of the heterologous minimal ovalbumin promoter conferred islet cell-specific enhancer activity in transient transfection assays (14). When present in three copies, activated gene expression to the levels seen with the whole promoter. In addition, RIPE3 can be divided into two subregions, 3a and 3b. Both subregions are also active but at much lower levels than RIPE3.

Four distinct binding elements, 3a1, 3a2, 3b1, and 3b2, have been found in RIPE3 (15). Mutations in any one of these sites disrupts the binding of its corresponding factor and decreases enhancer activity in transient transfection assays. The 3a1 and 3b1 sites bind islet cell-specific factors, whereas the factors that bind 3a2 and 3b2 are ubiquitously distributed. The 3a1 site contains the sequence CANNTG, which is the consensus binding sequence for a group of DNA-binding proteins called basic-helix-loop-helix (bHLH) proteins. The 3a1 binding factor consists of a heterodimer between the pancreas- and brain-specific BETA2 (NeuroD) bHLH protein (16, 17) and the ubiquitous BETA1 or E12/E47 bHLH molecules (15, 18–21). The 3b2 binding factor is also composed of multimeric proteins, one of which is Rip1 (the hamster homologue of the transcription factor, glial factor-1, and the immunoglobulin switch region-binding protein, Smb-2) (22). Sequence comparison of the 3a2 and 3b1 binding sites shows that they are unique and may bind novel transcription factors.

In this study, we assessed the activity of the RIPE3 enhancer in transgenic mice to determine if this 41-bp enhancer is sufficient to confer proper temporal and tissue-specific activity in vivo. Mice were generated carrying a transgene consisting of three copies of RIPE3 linked to the chicken ovalbumin minimal promoter to direct transcription of the human growth hormone (hGH) gene. These mice were assessed by serum radioimmunoassay (RIA), RNase protection, and immunohistochemistry for their hGH expression pattern in adult and embryonic transgenic mice.

MATERIALS AND METHODS

Transgene and Probe Construction—The RIPE3/hGH (human growth hormone) transgene was constructed from the plasmid pKShGH (23), which contains the BomHI-EcoRI hGH genomic fragment. The BomHI site of pKShGH was digested to a blunt end with T4 polymerase and ligated with a HindIII linker. The hGH/EcoRI plasmid was then prepared by subcloning the HindIII-EcoRI fragment into pBluescript KS (Stratagene). A DNA fragment containing three copies of RIPE3

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linked to the chicken ovalbumin minimal promoter was isolated by blunt end digestion of the BglII site of RIPE3(+3)CAT plasmid (14) with T4 polymerase followed by digestion with HindIII. This fragment was then inserted into the HindII and HindIII sites of hGH-H/E.

The hGH-ex1 plasmid used for RNAse protection analysis was prepared from a plasmid similar to RIPE3/hGH except that it contained the m322 mutation of RIPE3 (15), which also mutated a BalII site. This allowed the plasmid to be digested with BalII, which cuts the hGH gene in the first intron, and with SmaI, which cuts in the polylinker 3' to the gene, eliminating most of the transcribed region. This was then religated to retain the mutated RIPE3 sequences, the ovalbumin promoter and transcription start site, the first exon of hGH, and 137 bp of the first intron.

Generation and Identification of Transgenic Mice—The DNA for microinjection was prepared as described (23). The procedure for the generation of transgenic mice is similar to that described by Hogan et al. (24). The embryos used for microinjection were obtained from FVB/SJL mice. After microinjection, the surviving embryos were transferred to pseudopregnant ICR recipient females (Harlan, Indianapolis, IN). Transgenic pups were identified from tail biopsies as described by Hogan et al. (24). The incorporation of the transgene was confirmed in the founder animals by Southern analysis and in the progeny by polymerase chain reaction of the tail DNA.

Timed matings were obtained by superovulating ICR female mice with one half of the recommended dosage of pregnant mare serum (2.5 IU) and chorionic gonadotropin hormone (2.5 IU) and allowing them to mate overnight with transgenic males. The first day after this mating was assumed to be gestational age 0.5 days. Transgenic embryos were assessed by polymerase chain reaction of yolk sac DNA. The primers used for transgene detection were CTTGGTTGTTCAACATTCAG, which is specific for the ovalbumin sequence (25), and TGGGCTTACATGGCGATACT, which is specific for the hGH sequence (26).

Serum hGH RIA was performed as per the manufacturer’s instructions (Nichols Institute, San Juan Capistrano, CA).

RNAse Protection Assay—In order to examine the expression of the transgene in the pancreas, we performed a RNAse protection assay. Total RNA was extracted from the pancreas of transgenic mice with RNAzol B (Cinna/Biotec Laboratories Inc., Houston, TX) and was reversed transcribed on ethanol-bromide-stained gels. The procedure involved the use of 32P-labeled riboprobes synthesized by PCR amplification from the 3' end of the transgene. The 32P-labeled riboprobes were hybridized to the RNA of the pancreas, and the protected bands were analyzed by autoradiography.

RESULTS

Generation of hGH-expressing Transgenic Mice—A 2.4-kilobase pair XhoI-EcoRI RIPE3/hGH fragment (Fig. 1) was used for the embryo microinjection. This DNA fragment contained three copies of RIPE3 linked to the chicken ovalbumin minimal promoter (−50 to +41) to drive transcription of the hGH sequences. The only known cis-element this promoter contains is a TATA box, and it directs very minimal basal expression on its own. This same enhancer-promoter combination has been shown in transient transfections to drive chloramphenicol acetyl transferase gene expression to levels comparable with those produced by the entire rat insulin II promoter (14). 49 mice survived to weaning, and 13 of these carried the transgene. Copy numbers ranged from 1 to >5 per diploidy genome.

Founder mice were bred to assess transgene expression and outbred to establish breeding lines. RIA was used to assess the levels of hGH in the serum of the transgenic mice. This assay is specific for hGH and does not cross-react with mouse GH. Of the 13 founder mice, 11 produced detectable levels of hGH in their serum (Fig. 2). The levels of the serum hGH ranged from 0.9 ng/ml to −500 ng/ml and were independent of the copy number of the transgene. These findings demonstrated that this 41-bp enhancer was capable of promoting transcription in vivo. The transgenic mice also exhibited a decreased fertility; thus, we were only able to generate transgenic lines from four of the founder mice (mouse 6691, 7146, 7151, and 7158).

RIPE3 Directs Pancreatic Islet-specific Expression—To assess RIPE3 activity in the pancreas, immunohistochemical analysis was performed in 10 of the lines of mice (mouse 7150 died before the analysis could be performed). All 10 founder mice and/or their offspring showed hGH staining in the pancreas. In addition, hGH expression was only found in islets and not in the acinar or ductal cells in every case (Fig. 3). These results indicate that the 41-bp RIPE3 enhancer directs transcription in the pancreas with a high degree of cell specificity.

In adult mice, insulin is specifically expressed in the pancreatic β-cells, and the insulin promoter has been shown to direct transcription only in the β-cells (8). To assess which islet cell type RIPE3 is active, we stained adjacent sections from several pancreata for insulin as well as the α-cell marker, glucagon, and the δ-cell marker, somatostatin. Mouse 7143 revealed a similar staining pattern between insulin and hGH (Fig. 4, A and B), indicating that hGH expression occurred in β-cells. In contrast, the glucagon- (small arrows) and somatostatin- (large arrows) expressing α- and δ-cells did not appear to stain for hGH (Fig. 4, C, D, and E). Similar results were found in mouse 7155 as well (data not shown). Line 7151 (Fig. 4, F, G, H, I, and J) and mouse 4972 (data not shown) also showed hGH expression in the insulin-expressing β-cells (Fig. 4, F and G), but, unlike the other mice, the glucagon-staining α-cells also ap-

FIG. 1. RIPE3/hGH transgene construction. The transgene was constructed by linking three copies of the 41-bp RIPE3 enhancer to the TATA box containing chicken ovalbumin minimal promoter (cOV) coupled to the human growth hormone reporter gene. The transcriptional start site is indicated by a bent arrow.

FIG. 2. Serum hGH levels in RIPE3/hGH transgenic mice. RIA was used to measure the hGH levels. Results are reported in ng/ml. The numbers on the x axis represent the various transgenic lines.
peared to express hGH (Fig. 5, G and H, small arrows). In this line, the somatostatin-staining δ-cells did not appear to express the transgene (Fig. 5, I and J, large arrows), but to absolutely verify this possibility, double staining will be required. These results indicate that the RIPE3 enhancer contains sufficient cis-information to direct β-cell expression, and additional cis-elements may be required to confine the expression specifically to the β-cells.

**RIPE3 Tissue Expression Pattern**—To assess the tissue expression pattern of hGH, RNA was extracted from multiple tissues from 10 of the transgenic lines and was assessed by RNase protection. The hGH-ex1 plasmid was used to generate a 269-nt 32P-riboprobe and was predicted to protect a 77-nt fragment (Fig. 5A). Because the riboprobe also contained some intronic sequences, improperly spliced messages would also be detected. Fig. 5B depicts the RNase protection results from mouse line 7151. In this mouse, expression was primarily detected in the pancreas, thymus, and brain, and a faint band could also be detected in the small intestine and the spleen. In addition to the 77-nt band, several additional larger bands could be detected in the brain, which are likely due to altered splicing. A comparison of the hGH expression in all 10 founder mice and/or their offspring (Table I) revealed fairly consistent expression in the pancreas (7 of 10 mice) and brain (9 of 10 mice). Also, hGH expression was often detected in the thymus at relatively moderate to high levels (4 of 6 mice) and in the gonads at relatively low levels (3 of 5 mice), while in the other organs it was quite variable, ranging from no mice exhibiting liver hGH expression to half of the mice exhibiting heart and spleen expression. Curiously, although the pancreas from all of the lines of mice stained histochemically for hGH, three lines appeared negative by RNase protection. Although the nature of this discrepancy was not determined, it is probably due to dilution of the islet cell RNA by the more abundant acinar cell RNA and/or partial RNA degradation.

Due to the large number of mice expressing hGH mRNA in the brain, brain tissue sections from mouse 4967, 4972, 7143, 7153, and 7156 were assessed immunohistochemically. Surprisingly, these sections did not stain for hGH, although the message was detected by RNase protection (data not shown). This suggests that there is a low abundance of hGH protein in a large number of neuronal cells, while in the pancreas there is a larger abundance in a few cells, since the islets compose 1–2% of the pancreas.

**RIPE3 Controls Proper Temporal Expression in Embryonic Islet Precursor Cells**—During the initial evagination of the pancreatic bud at ~embryonic day 9.5, islet precursor cells exist that are believed to give rise to all of the different islet cell types. These precursor cells have been shown by immunohistochemistry to co-express glucagon and transgenes containing the insulin promoter (7) and, under the correct conditions, insulin (31). To determine if RIPE3 also directs expression during this time period, we assessed glucagon and hGH expression in embryonic day 9.5 RIPE3 transgenic embryos. Fig. 6A shows the glucagon-expressing islet precursor cells in the evaginating pancreatic bud. Immunostaining of adjacent sections for hGH (Fig. 6B, arrows) demonstrated that the transgene was also expressed in these precursor cells at this time point. Thus, the RIPE3 enhancer directs the same temporal expression pattern as the insulin genes during pancreas development.

**DISCUSSION**

We have used transgenic mice to analyze the in vivo activity of the 41-bp insulin enhancer, RIPE3. All 10 of the hGH-expressing mice analyzed displayed islet-specific transgene expression in the pancreas. This islet expression was primarily found in the β-cells, although some mice also expressed the transgene in the α-cells as well. In developmental analysis, the transgene was found to be active at embryonic day 9.5, indicating that RIPE3 is important for regulating both the proper spatial and temporal expression of the insulin gene during embryogenesis.

Tissue-specific transcription factors play a vital role in differentiation. Assessing the in vivo activity of these transcription factors is often complicated by the need for cooperative interaction between multiple factors. Because of this, transgenic expression usually requires relatively large pieces of DNA with multiple cis-elements to obtain proper temporal and tissue-specific transgene expression. Thus, the significance of RIPE3 directing proper temporal and islet-specific activity in the pancreas is underscored by the fact that it is only 41 bp long.

Cooperativity still plays an integral role in RIPE3 activity and is required between the tissue-specific components of RIPE3a and RIPE3b (14). This is evident in mice harboring the transgene IEB2-IEB1-Ptk-CAT (which contains the rat insulin I gene’s equivalent of RIPE3a). This element alone was unable to induce expression in transgenic mice (32). The need for cooperative interaction may also explain why our mice only had low levels of transgene expression in the brain. Although the brain is one of the primary sites for BETA2 (NeuroD) expres-
**FIG. 4. Assessment of the hGH-staining islet cell type.** Immunohistochemical analysis is shown of adjacent pancreatic sections from mouse 7143 (A–E) and line 7151 (F–G) (magnification × 500). Small arrows, α-cells; large arrows, δ-cells. Antibodies used were anti-hGH (B, D, G, and J), anti-insulin (A and F), anti-glucagon (C and H), and antisomatostatin (E and I).
Our RIPE3/hGH transgenic mice exhibited decreased fertility. Similar problems were reported in mice harboring a transgene consisting of a glucokinase promoter linked to hGH (33). These mice expressed hGH in pancreatic islet cells and various neuroendocrine cells including pituitary corticotropes. This led to altered development of the pituitary gland in these mice, resulting in decreased fertility. Pituitary gland expression of hGH was not evaluated in our mice, but BETA2 has been found to be expressed in a corticotroph cell line, AT20 (17). Thus, it is possible that hGH was expressed in the corticotropes of our mice and affected fertility.

RNA analysis of multiple tissues from these transgenic mice combined with immunohistochemical results revealed that RIPE3 was active in the pancreas of all 10 hGH-expressing mice analyzed and was active in the brain in 9 of the 10 mice. In addition, a variable pattern of expression in other tissues was also detected. For the most part, this expression was fairly low and required the sensitivity of RNase protection to detect the message. Similar to the brain, immunohistochemical analysis of some of these tissues revealed that the amount of hGH protein in these tissues was too low for detection or that only a rare cell stained for hGH (data not shown) as opposed to the pancreas, which contains a small number of highly expressing cells. Low levels of expression in a tissue along with a variable number of mice expressing the transgene in that tissue suggest that this is the result of leaky expression due to the effects from the integration site, but in actuality we can not rule out the possibility that these tissues contain transcription factors that are able to induce low levels of RIPE3 activity. An exception to this may be the thymus, in which 4 of 6 mice expressed hGH. Immunohistochemical analysis on thymus sections from mice 4967 and 4972 revealed abundant protein levels as well. Currently, it is not clear what thymus cell type has the strong RIPE3 activity, and a more detailed analysis will be required to determine this. In comparison, the pancreatic islets in all 10 mice analyzed immunostained for hGH. This indicates significant levels of expression in the islets, although the RNA levels appeared low in the whole pancreas samples. This is probably due to a dilution effect, since the islets compose only ~1–2% of the pancreas.

RIPE3-directed transgene expression in β-islet cells though some of the mice also displayed α-cell expression. This suggests that RIPE3 contains sufficient cis-information for β-cell-specific expression, but additional information is needed to confine the expression to only these islet cells. In addition, it indicates

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**FIG. 6.** Embryonic activity of the RIPE3 enhancer. Immunohistochemical analysis of adjacent sections from embryonic day 9.5 embryo from line 7151 (magnification × 500). Arrows mark islet precursor cells. Antibodies used were anti-glucagon (A) and anti-hGH (B).

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**TABLE I**

| Line  | Pancreas | Spleen | Stomach | Small intestine | Liver | Kidney | Heart | Lung | Thymus | Genads | Muscle | Brain |
|-------|----------|--------|---------|-----------------|-------|--------|-------|------|--------|--------|--------|-------|
| 4967  | +        | ++     | 0       | 0               | 0     | +      | +     | 0    | +      | 0      | +      | +     |
| 4972  | +        | +      | +       | 0               | 0     | 0      | +     | ++   | +      | +      | +      |       |
| 6691  | 0        | 0      | ++      | 0               | 0     | ND     | 0     | 0    | ND     | ND     | 0      | +     |
| 7143  | +        | 0      | 0       | 0               | 0     | 0      | 0     | 0    | ND     | ND     | 0      | +     |
| 7144  | 0        | 0      | 0       | 0               | 0     | 0      | +     | +    | ND     | ND     | 0      |       |
| 7146  | 0        | 0      | 0       | 0               | 0     | 0      | 0     | 0    | ND     | ND     | 0      | +     |
| 7151  | +        | +      | 0       | 0               | 0     | 0      | 0     | 0    | ND     | ND     | 0      | +     |
| 7155  | +        | +      | ND      | +               | 0     | ++     | ++   | +    | 0      | 0      | 0      | +     |
| 7156  | +        | +      | 0       | 0               | 0     | 0      | 0     | ND   | ND     | ND     | 0      | +     |
| 7158  | +        | 0      | ND      | 0               | 0     | 0      | +     | 0    | +      | +      | +      |       |
activity of RIPE3 binding factors in α-cells. In agreement with this, BETA2, the tissue-specific RIPE3α binding factor, is expressed in the αTC cell line (16), but whether BETA2 as well as the 3b1 binding factor is active in α-cells has yet to be determined. It is also possible that α-cells contain other factors that are capable of activating the RIPE3 enhancer.

Evidence suggests that during pancreas development the different islet cell types arise from common precursor islet cells (7, 31). These putative cells express both the glucagon and insulin genes and are first detected by immunocytochemistry (7, 31). These putative cells express both the glucagon and insulin genes and are first detected by immunocytochemistry (7, 31). As these cells differentiate, transcriptional modulation of islet specific genes occurs. Cells destined to become β-cells continue to express insulin but lose glucagon expression. Conversely, cells destined to become α-cells negatively regulate insulin gene expression while continuing to express glucagon. Thus, regulation of the insulin gene during islet development is at least a two-step process requiring both positive and negative factors. RIPE3 appears to contain the cis-information required for the first positive regulatory step, since transgene expression was detected at embryonic day 9.5. Interestingly, RIPE3 may not have enough information for the second negative regulatory step, since some mice also expressed the transgene in adult α-cells. These results indicate the need to assess additional cis-elements, possibly silencing elements, for the fine tuning of insulin gene expression to the β-cell and may be underscoring the important role negative acting factors play in development.

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REFERENCES
1. Ullrich, A., Shine, J., Chirgwin, J., Picket, R., Tischer, E., Rutter, W. J., and Goodman, H. M. (1977) Science 196, 1313–1319
2. Giddings, S. J., and Carnaghi, L. R. (1988) J. Biol. Chem. 263, 3845–3849
3. Deltour, L., Leboucq, P., Blume, N., Madsen, O., Dubois, P., Jamir, J., and Buechini, D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 527–531
4. Soares, M. B., Schon, E., Henderson, A., Karathanasis, K., Cate, R., Zeitlin, S., Chirgwin, J., and Efstratiadis, A. (1985) Mol. Cell. Biol. 5, 2090–2103
5. Walker, M. D., Edlund, T., Boulet, A. M., and Rutter, W. T. (1983) Nature 306, 557–561
6. Episkopou, V., Murphy, A. J. M., and Efstratiadis, A. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 4657–4661
7. Alpert, S., Hanahan, D., and Teitelman, G. (1988) Cell 53, 295–308
8. Hanahan, D. (1985) Nature 315, 115–122
9. Crowe, D. T., and Tsai, M.-J. (1989) Mol. Cell. Biol. 9, 1784–1789
10. Edlund, T., Walker, M. D., Barr, P. J., and Rutter, W. J. (1985) Science 230, 912–916
11. Karlsson, D., Edlund, T., Moss, J. B., Rutter, W. J., and Walker, M. D. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 8819–8823
12. Nir, U., Walker, M. D., and Rutter, W. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 3180–3184
13. Cordle, S. R., Whelan, J., Henderson, E., Masuoka, H., Wei, P. A., and Stein, R. (1991) Mol. Cell. Biol. 11, 2881–2886
14. Huang, Y. P., Gu, Y. Z., and Tsai, M.-J. (1990) Mol. Cell. Biol. 10, 1784–1788
15. Shieh, S.-Y., and Tsai, M.-J. (1991) J. Biol. Chem. 266, 16708–16714
16. Naya, F., Stellrecht, C. M. M., and Tsai, M.-J. (1995) Genes & Dev. 9, 1009–1019
17. Lee, J. E., Hollenberg, S. M., Snider, L., Turner, D. L., Lipnick, N., and Weintraub, H. (1995) Science 268, 836–844
18. Aronheim, A., Ohlsson, H., Park, C. W., Edlund, T., and Walker, M. D. (1991) Nucleic Acids Res. 19, 3893–3899
19. Cordle, S. R., Henderson, E., Masuoka, H., Wei, P. A., and Stein, R. (1991) Mol. Cell. Biol. 11, 1734–1738
20. German, M. S., Blaner, M. A., Nelson, C., Moss, L. G., and Rutter, W. J. (1991) Mol. Endocrinol. 5, 292–299
21. Peyton, M., Moss, L. G., and Tsai, M.-J. (1994) J. Biol. Chem. 269, 25396–25401
22. Shieh, S.-Y., Stellrecht, C. M. M., and Tsai, M.-J. (1995) J. Biol. Chem. 270, 21503–21508
23. Ray, M. K., Magdalenas, S. W., Finegold, M. J., and DeMayo, F. J. (1995) J. Biol. Chem. 270, 2689–2694
24. Hogan, B. L. M., Constantini, F., and Lacy, E. (1988) Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
25. Bradshaw, M. S., Tsai, M.-J., and O'Malley, B. W. (1988) Mol. Endocrinol. 2, 1286–1293
26. DeNoto, F. M., Moore, D. D., and Goodman, H. M. (1981) Nucleic Acids Res. 9, 3719–3728
27. Peyton, M., Stellrecht, C. M. M., Naya, F., Huang, H.-P., Samora, P. J., and Tsai, M.-J. (1996) Mol. Cell. Biol. 16, 626–633
28. Bordignon, M., Sacconanno, F., and Nordstrom, J. L. (1994) Biotechniques 16, 428–430
29. Wilkinson, D. G., and Green, J. (1990) in Postimplantation Mouse Embryos: A Practical Approach (Rickwood, D., and Cockcroft, D. L., eds) pp. 155–171, IRL Press, Oxford
30. Sepulveda, A. R., Finegold, M. J., Smith, B., Slagle, B. L., DeMayo, F. J., Shen, R. F., Wong, S. L., and Butel, J. L. (1989) Cancer Res. 49, 6180–6117
31. Teitelman, G., Alpert, S., Pelak, J. M., Martinez, A., and Hanahan, D. (1993) Development 118, 1031–1039
32. Dandoy-Dron, F., Monthioux, E., Jami, J., and Bucchini, D. (1991) Mol. Cell. Biol. 11, 266–270
33. Jetton, T. L., Liang, Y., Pettepher, C. C., Zimmerman, E. C., Cox, F. G., Horvath, K., Matochinsky, P. M., and Magnussen, M. A. (1994) J. Biol. Chem. 269, 3641–3654