The Amino-terminal Region of the Luteinizing Hormone/Choriogonadotropin Receptor Contacts Both Subunits of Human Choriogonadotropin

I. MUTATIONAL ANALYSIS*

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The luteinizing hormone/choriogonadotropin receptor is a seven-transmembrane receptor. Unlike most seven-transmembrane receptors, it is composed of two halves of equal size, the N-terminal extracellular exodomain and the C-terminal membrane-associated endodomain. The exodomain is exclusively responsible for high affinity hormone binding, whereas receptor activation occurs only in the endodomain. This mutually exclusive physical separation of the two functional domains sets the lutropin receptor and its subfamily of receptors apart from all other seven-transmembrane receptors. The mechanisms of hormone binding and receptor activation also appear to be different from those of other receptors in that binding occurs in at least two steps. However, the precise hormone contact sites in the exodomain are unknown. To determine the hormone/receptor contact sites, we have examined the receptor using progressive truncation from the C terminus, Ala scanning, immunofluorescence microscopy, and antibody binding. Progressive truncation from the C terminus of the receptor indicates several discrete regions that impact hormone binding. These regions are around the boundaries of exons 1–2, 4–5, 6–7, and 9–10. Ala scanning of the Asp17–Arg26 region near the exon 1–2 junction uncovered three alternating residues (Leu20, Cys22, and Gly24) crucial for hormone binding. Ala substitution for any one of these residues abolished hormone binding, although the resulting mutant receptors were successfully expressed on the cell surface. In contrast, Ala substitution for their flanking and intervening residues did not impair hormone binding. These results and the data in the accompanying article (Phang, T., Kundu, G., Hong, S., Ji, I., and Ji, T. (1998) J. Biol. Chem. 273, 13841–13847) indicate that this region directly contacts the hormone and suggest a novel mode of embracing the hormone.

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‡ The abbreviations used are: LH, luteinizing hormone; CG, choriogonadotropin; hCG, human CG; LH/CG-R, LH/CG receptor; PBS, phosphate-buffered saline.

The LH1/CG receptor belongs to a subfamily of glycoprotein hormone receptors within the seven-transmembrane receptor family. Unlike most seven-transmembrane receptors, it is composed of two equal halves: the 341-amino acid-long extracellular N-terminal exodomain and the 334-amino acid-long membrane-associated C-terminal endodomain, which includes seven transmembrane helices (1, 2). The exodomain binds the hormone with high affinity (3–7) without hormone action (5, 8). The exodomain-hCG complex is thought to make a secondary contact with the endodomain, thus generating a signal (9). Therefore, the high affinity interaction of the exodomain and hCG is the crucial first step leading to signal generation and hormone action. Despite the importance, only limited information is available concerning the precise hormone contact residues and sites in the exodomain. Roche et al. (10) found that three peptide mimics of the exodomain, peptide-(21–38), peptide-(102–115), and peptide-(253–266), attenuated 125I-hCG binding to membranes expressing the LH/CG receptor. More recently, Segaloff and co-workers (11) observed that receptors lacking any of several discrete exodomain sequences, the 11 N-terminal residues and leucine-rich motifs 1–6, were trapped in cells and failed to bind hCG.

In this work and the accompanying article (12), the exodomain was examined using several independent methods, including serial truncation from the C terminus, Ala scanning, peptide mimics of the receptor, photoaffinity labeling, affinity cross-linking, and immunofluorescence microscopy. Our results show that the Leu20-Pro28 sequence near the exon 1–2 junction contacts both the α- and β-subunits of hCG. In addition, another three sequences near the junctions of exons 4–5, 6–7, and 9–10 influence hormone binding.

EXPERIMENTAL PROCEDURES

Mutagenesis and Functional Expression of LH/CG Receptors—Mutant LH/CG receptor cDNAs were expressed in the pSELECT vector using the Altered Sites Mutagenesis system (Promega), sequenced, subcloned into pDNA3 (Invitrogen) as described (13), and sequenced again to verify mutation sequences. This procedure does not involve polymerase chain reaction. To produce truncated receptors, a stop codon was introduced at the 3’-end of the exons. Mutant and truncated LH/CG receptor constructs were transfected into human embryonic kidney 293 cells by the calcium phosphate method. Stable cell lines were established in minimal essential medium containing 10% horse serum and 500 μg/ml G418. These cells were used for hormone binding, cAMP production, antibody binding, and fluorescence microscopy. All assays were carried out in duplicate and repeated four to five times. Means ± S.D. were calculated.

125I-hCG Binding and Intracellular cAMP Assay—Stable cells were assayed for 125I-hCG binding in the presence of 150,000 cpm of 125I-hCG (14) and increasing concentrations of unlabeled hCG. The Kd values were determined by Scatchard plots. hCG (batch CR 127) was supplied by the National Hormone and Pituitary Program. For intracellular cAMP assay, cells were washed twice with Dulbecco’s modified Eagle’s medium and incubated in the medium containing isobutylmethylxanthine (0.1 μM) for 15 min. Increasing concentrations of hCG were then added, and incubation was continued for 45 min at 37 °C. After removing the medium, the cells were rinsed once with fresh medium without isobutylmethylxanthine, lysed in 70% ethanol, freeze-thawed...
in liquid nitrogen, and scraped. After pelleting cell debris at 16,000 × g for 10 min at 4 °C, the supernatant was collected, dried under vacuum, and resuspended in 10 μl of CAMP assay buffer (Amersham Pharmacia Biotech). CAMP concentrations were determined with a 125I-CAMP assay kit (Amersham Pharmacia Biotech) following the manufacturer’s instructions and validated for use in our laboratory.

**Immunofluorescence Microscopy**—For fluorescence labeling of LH/CG receptors, the Flag epitope (15), Asp-Tyr-Lys-Asp-Asp-Asp-Lys (5’-GAG TAC AAG GAC GAT GAC GAT GAT AAG-3’), was inserted between the C terminus (Ser26) of the signal sequence and the N terminus (Arg27) of mature receptors. The Flag epitope (16) has successfully been used as a marker to identify, trace, and purify recombinant proteins without significantly impairing their biological activities (17, 18). For labeling intact cells, cells were cultured on coverslips equipped with Scanware analysis software. Entire experiments were completed in 1 day to prevent increasing background fluorescence.

**RESULTS**

**Progressive Truncation of the C Terminus**—The LH/CG receptor is encoded by 11 exons (19, 20). Exons 1–10 comprise most of the exodomain, whereas the endodomain is encoded in exon 11. As an initial step to define important regions for hCG binding, individual exons from 11 to 2 were progressively truncated from the C terminus (Fig. 1D). These truncated receptor fragments represent exons 1–10, 1–9, 1–8, 1–7, 1–6, 1–5, 1–4, 1–3, 1–2, and 1. None of the stably transfected cells bound hCG, and therefore, they were solubilized in Nonidet P-40 and assayed for hCG binding (Fig. 1, A and B). All of the expressed receptor fragments, 1–10, 1–9, 1–8, 1–7, 1–6, 1–5, 1–4, 1–3, 1–2, and 1, except for the exon 1 fragment, were capable of binding hCG, but were trapped in cells.

Interestingly, Kd values for the fragments increased stepwise in groups rather than continuously as the C terminus was progressively truncated (Fig. 1C). Truncation of exon 11 to produce the exon 1–10 fragment slightly reduced the Kd value compared with that of the wild type. In contrast, the exon 1–9 fragment, resulting from truncation of exons 10 and 11, showed

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**FIG. 1.** hCG binding to exodomain fragments. The 11 exons of the LH/CG receptor were progressively truncated from the C terminus to produce receptor fragments consisting of exons 1–10, 1–9, 1–8, 1–7, 1–6, 1–5, 1–4, 1–3, 1–2, and 1 (D). These exon fragments were stably expressed in 293 cells, solubilized in Nonidet P-40, and assayed for 125I-hCG binding in the presence of increasing concentrations of unlabeled hCG. The results are presented as displacement of 125I-hCG binding (A) and in a Scatchard plot (B). Experiments were repeated four to five times in duplicate, and means ± S.D. were calculated (C). Nontransfected cells did not show specific binding of hCG. WT, wild type; NS, not significant. Arrows indicate regions that influence hormone binding.
As the C terminus was successively truncated, the Kd values increased in several discrete steps. This result suggests that the ultimate effects of the truncated exons on hCG binding are not equal. There are four potentially important regions around the junctions between exons 1 and 2, 4 and 5, 6 and 7, and 9 and 10 (Fig. 1D). Among the four, the region near the exon 1–2 junction appears to be most crucial for hCG binding. Therefore, the region was further investigated by Ala scanning.

**Ala Scanning of the Asp17–Arg26 Sequence**—As a first step to identify important residues near the exon 1–2 junction, Asp17–Gly18-Ala19-Leu20-Arg21-Cys22-Pro23-Gly24-Pro25-Arg26 was Ala-scanned (Fig. 2). Ala substitution for Arg21, Pro23, Pro25, or Arg26 did not impair hCG binding to intact cells but increased the Kd values approximately 5–6-fold (Fig. 2, A and B). All of the mutant receptors that bound hCG on intact cells were capable of inducing cAMP production (Fig. 2C). Their EC50 values for cAMP induction were similar to the wild type value except the R26A mutant, which has a 2.4-fold higher EC50 value (Fig. 2, table). In contrast, Ala substitution for Asp17, Gly18, Leu20, Cys22, or Gly24 impaired hCG binding to intact cells. EC50 values for cAMP production are generally lower than Kd values of the corresponding receptors. This is thought to be due to the fact that receptors are activated before they are fully occupied by hormone. Sometimes, it happens when <5% of receptors are occupied. Furthermore, the maximum receptor activation is reached long before receptors are fully occupied.

**Hormone Binding of Solubilized Receptors**—To determine whether the non-binding mutants are defective in hCG binding or trapped in the cytoplasm, cells expressing the mutants were solubilized in Nonidet P-40 and assayed for hCG binding (Fig. 3). hCG bound to the D17A and G18A mutants. Their Kd values are comparable to those of the R21A, P23A, P25A, and R26A mutants, but ~5–8-fold higher than the wild type value. This result suggests that the D17A and G18A mutants are trapped in cells. In contrast to these binding-competent mutants, the L20A, C22A, and G24A mutants failed to bind hCG. Therefore, the L20A, C22A, and G24A mutants either were not synthesized or were synthesized but incapable of binding hormone. Even if they were expressed, our data shown in Figs. 2 and 3 could not pinpoint whether the mutants were located either on the cell surface or within the cells. To distinguish these possibilities, we utilized two independent immunological methods, immunofluorescence microscopy and 125I-antibody that should bind to receptors expressed on the cell surface and in cells.

**Immunofluorescence Microscopy**—For immunological studies, the Flag epitope (16) was inserted between the C terminus of the signal sequence and the N terminus of mature receptors. The resulting receptors are the Flag-wild type LH/CG receptor, Flag-LH/CG-RD17A, Flag-LH/CG-RG18A, Flag-LH/CG-RG19A, Flag-LH/CG-RP20A, Flag-LH/CG-RP22A, and Flag-LH/CG-RG23A. Cells were transfected with the plasmids encoding receptors carrying the Flag epitope. They were either examined intact or after treatment

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**Fig. 2.** Ala substitution for Asp17–Arg26. Residues from Asp17 to Arg26, except Ala19, of the LH/CG receptor were individually substituted with Ala, and the resulting mutant receptors were stably expressed in 293 cells. Intact cells were used for 125I-hCG binding in the presence of increasing concentrations of unlabeled hCG (A and B) and for cAMP production (C). Experiments were repeated four to five times in duplicate as described in the legend to Fig. 1. As a control to verify intended mutations and to identify unintended changes, if any, in the cDNA sequence, mutant receptor cDNAs were reverted to the wild type, and their sequences were confirmed. The revertant cDNAs were used to transfect cells and were assayed for hormone binding and cAMP induction. All revertants behaved the same as the wild type (data not shown). NS, not significant.

**Fig. 3.** 125I-hCG binding to non-binding mutant receptors solubilized in Nonidet P-40. Cells transfected with plasmids encoding the non-binding receptors shown in Fig. 2 were solubilized in Nonidet P-40 and assayed for 125I-hCG binding in the presence of increasing concentrations of unlabeled hCG. NS, not significant.
with Triton X-100 to permeabilize the plasma membrane and to allow the antibody to enter the cytosol. The cells were treated sequentially with mouse anti-Flag and Texas Red-conjugated goat anti-mouse IgG monoclonal antibodies. Confocal laser fluorescence microscopy showed bright fluorescence of the Flag-wild type receptor on intact cells and in permeabilized cells (Fig. 4). Cells expressing the wild type receptor lacking the Flag tag did not show fluorescence, regardless of permeabilization. In addition, the cells expressing the Flag-wild type receptor did not show fluorescence when treated for fluorescence labeling without anti-Flag antibody. These controls demonstrate that the fluorescence staining is specific for the Flag epitope and that the Flag-wild type receptor is expressed both on the cell surface and within cells. Flag-LH/CG-R_{D17A}, Flag-LH/CG-R_{G18A}, Flag-LH/CG-R_{L20A}, Flag-LH/CG-R_{C22A}, and Flag-LH/CG-R_{G24A} were also observed on intact and permeabilized cells, indicating they were expressed on the cell surface and within cells. On the other hand, Flag-LH/CG-R_{D17A} and Flag-LH/CG-R_{G18A} were observed in permeabilized cells, but not intact cells, indicating that they were not transported to the cell surface.

**125I-Anti-Flag Antibody Binding to Intact Cells**—125I-Anti-Flag antibody was used to verify the surface expression of receptors. Fig. 5 shows that 125I-anti-Flag antibody bound to cells expressing the Flag-wild type receptor, Flag-LH/CG-R_{D17A}, Flag-LH/CG-R_{G18A}, Flag-LH/CG-R_{L20A}, Flag-LH/CG-R_{C22A}, and Flag-LH/CG-R_{G24A}, but not to those expressing Flag-LH/CG-R_{D17A} or Flag-LH/CG-R_{G18A}. The K_d values were 29, 96, 106, and 135 nM for the Flag-wild type receptor, Flag-LH/CG-R_{D17A}, Flag-LH/CG-R_{G18A}, Flag-LH/CG-R_{L20A}, Flag-LH/CG-R_{C22A}, and Flag-LH/CG-R_{G24A}, respectively. The numbers of binding sites on the cell surface (27,000 and 187,000 per cell) are significant, and therefore, these bindings are likely to be specific. Taken together, these and the fluorescence microscopy results demonstrate that the Flag-wild type receptor, Flag-LH/CG-R_{D17A}, Flag-LH/CG-R_{G18A}, Flag-LH/CG-R_{L20A}, Flag-LH/CG-R_{C22A}, and Flag-LH/CG-R_{G24A} were expressed on the cell surface, whereas Flag-LH/CG-R_{D17A} and Flag-LH/CG-R_{G18A} were trapped in the cells.

**Activities of Flag-tagged Receptors**—To test whether the Flag epitope might have interfered with normal processing so that the mutant receptors carrying the Flag epitope were fortuitously expressed on the cell surface, the activities of the Flag-LH/CG receptors (hCG binding, and cAMP induction) were determined. The Flag-wild type receptor and the wild type receptor on intact cells bound hCG with the same affinity (Fig. 6, A and B). In addition, the Flag-wild type receptor was capable of hCG-dependent cAMP induction, although the EC_{50} value for cAMP induction was ~3-fold higher than the value of the wild type receptor (Fig. 6C). These data show that the Flag-LH/CG receptors are active, although their potency is somewhat different compared with LH/CG receptors lacking the Flag epitope. With this in mind, we examined Flag-LH/CG-R_{D17A}, Flag-LH/CG-R_{G18A}, Flag-LH/CG-R_{L20A}, Flag-LH/CG-R_{C22A}, and Flag-LH/CG-R_{G24A}. They did not bind hCG or induce cAMP production (data not shown). These results, along with the results obtained from the non-Flag mutants, demonstrate that LH/CG-R_{D17A}, LH/CG-R_{G18A}, and LH/CG-R_{C22A} are expressed on the surface of intact cells, but are defective in hCG binding. In contrast, LH/CG-R_{D17A}, LH/CG-R_{G18A}, LH/CG-R_{L20A}, LH/CG-R_{C22A}, LH/CG-R_{G24A}, and LH/CG-R_{G25A} are capable of binding hCG.

**DISCUSSION**

Truncation at several discrete regions of the exodomain, near the boundaries of exons 1–2, 4–5, 6–7, and 9–10, noticeably influences hormone binding. It is unclear whether these regions represent hormone contact sites or whether their truncation has allosteric effects on the global structure of the exodomain and thus indirectly impacts hormone binding. In the sequence near the exon 1–2 junction, three alternate residues (Leu^{20}, Cys^{22}, and Gly^{25}) are important for hormone binding. It has been speculated that a primary hCG-binding site is the putative crescent structure (21–23) that is composed of Leu-rich motifs (24). However, the three residues are upstream (but not part) of the Leu-rich motifs and crescent structure of the exodomain. Therefore, this upstream region near the N terminus of the receptor appears to be at least equally important for hormone binding as the crescent structure. It will be interesting to see whether this upstream sequence is a hormone contact site and, if so, how it interacts with the hormone.
Role of Leu<sup>20</sup>, Cys<sup>22</sup>, and Gly<sup>24</sup>—The data in this work suggest two general possible roles for the region covering the three residues. It may contact hCG or be important for the exodomain to assume a structure necessary for hormone binding without directly interacting with the hormone. The latter possibility could be a result of misfolding of the L20A, C22A, or G24A mutant. However, the results of our specific photoaffinity labeling of both hCG α- and β-subunits by a peptide mimic of the receptor region indicate the direct interaction of the receptor region with hCG (12). Besides, several observations described in this study are consistent with the interaction of the receptor region with hCG. The effect of Ala substitutions for the three alternate residues on hormone binding are remarkably consistent with, yet strikingly different from, the effects of Ala substitutions for the intervening and flanking residues. For example, Ala substitutions for the flanking and intervening residues of Leu<sup>20</sup>, Cys<sup>22</sup>, and Gly<sup>24</sup> reduced the hormone binding affinity approximately 5–8-fold, but never abrogated hCG binding. Therefore, the flanking and intervening residues appear to be important for hormone binding, but are not as crucial for binding. Therefore, the flanking and intervening residues appear to be important for hormone binding, but are not as crucial for binding. Therefore, the flanking and intervening residues appear to be important for hormone binding, but are not as crucial for binding. Therefore, the flanking and intervening residues appear to be important for hormone binding, but are not as crucial for binding. Therefore, the flanking and intervening residues appear to be important for hormone binding, but are not as crucial for binding. Therefore, the flanking and intervening residues appear to be important for hormone binding, but are not as crucial for binding. Therefore, the flanking and intervening residues appear to be important for hormone binding, but are not as crucial for binding.

In addition to the three residues, our data and the peptide mimic (10) and deletion (11) studies predict the importance of Arg<sup>21</sup>–Pro<sup>38</sup>, substitution for Cys<sup>22</sup> (25), or deletion of residues 212–341 extending two general possible roles for the region covering the three residues. The alternate sequence of Leu<sup>20</sup>, Cys<sup>22</sup>, and Gly<sup>24</sup> suggests a β-like structure, orienting the three residues on one side where hCG might contact. Our results are consistent with other reports that the presence of the peptide mimic of receptor Arg<sup>21</sup>–Pro<sup>38</sup> (10), substitution for Cys<sup>22</sup> (25), or deletion of exon 1 (11) interferes with hormone binding.

Some of the mutant receptors such as R21A, P23A, P25A, L20A, C22A, and G24A were expressed at significantly higher levels compared with expression of the wild type receptor. It is unclear whether the higher expression levels are caused by better plasmid preparation, better transfection, more efficient translation and/or processing, or a reduced degradation rate. We have experienced that the expression level is dependent on the transfection efficiency, which, in turn, is affected by the quality and amount of plasmid preparation as well as the cell condition. If the transfection efficiency is equal, it is possible that mutations could impact on the expression level. Similarly, substitutions for a crucial amino acid can diversely influence the machinery for the surface expression depending on the side chain of the amino acid (26).

Importance of Asp<sup>17</sup> and Gly<sup>18</sup> in Targeting—When either Asp<sup>17</sup> and Gly<sup>18</sup> was substituted with Ala, the corresponding mutant receptors were trapped within cells and could not be detected on the cell surface. This total lack of their surface expression implies the importance of these residues in targeting the receptor to the plasma membrane. Furthermore, the targeting machinery is extremely sensitive to a change in the structure of this sequence. At least in the case of the D17A and G18A substitutions, the targeting mechanism appears to be more sensitive than hormone binding is. Therefore, targeting to the cell surface could be used as an indicator of structural changes of the receptor including mutant receptors.

REFERENCES
1. McFarland, K., Sprengel, R., Phillips, H., Kohler, M., Rosemblit, N., Nikolaics, K., Segaloff, D., and Seeburg, P. (1998) Science 284, 489–499
2. Loosfelt, H., Mirrahi, M., Atger, M., Salesse, R., Thi, M., Jolivet, A., Guichon-Mantel, A., Sar, S., Jallal, B., Garnier, J., and Milgrom, E. (1998) Science 285, 525–528
3. Tsai-Morris, C. H., Buzcko, E., Wang, W., and Dufau, M. L. (1999) J. Biol. Chem. 274, 13841–13847
4. Xie, Y. B., Wang, H., and Segaloff, D. L. (1999) J. Biol. Chem. 274, 21411–21414
5. Ji, I., and Ji, T. H. (1991) Endocrinology 128, 2648–2650
6. Seetharamaiah, G. S., Kurosky, A., Desai, R. K., Dallas, J. S., and Prabhakar, B. S. (1994) Endocrinology 134, 549–554
7. Davis, D., Liu, X., and Segaloff, D. (1995) Mol. Endocrinol. 9, 159–170
8. Remy, J. J., Bozon, V., Couture, L., Goze, B., Salesse, R., and Garnier, J. (1995) Biochem. Biophys. Res. Commun. 203, 1023–1030
9. Ji, T. H., Murdoch, W., and Ji, I. (1995) Endocrine 3, 187–194
10. Roche, P. C., Ryan, R. J., and McCormick, D. J. (1992) Endocrinology 131, 268–274
11. Thomas, D., Rozell, T., Liu, X., and Segaloff, D. (1996) Mol. Endocrinol. 10, 760–768
12. Phang, T., Kundu, G., Hong, S., Ji, I., and Ji, T. H. (1998) J. Biol. Chem. 273, 13841–13847
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13. Ji, I., and Ji, T. H. (1993) J. Biol. Chem. 268, 20851–20854
14. Ji, I., and Ji, T. H. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 5465–5469
15. Prikett, K., Amberg, D., and Hopp, T. (1989) BioTechniques 7, 580–589
16. Hopp, T., Prikett, K., Price, V., Libby, R., March, C., Cerretti, P., Urdal, D., and Conlon, P. (1988) Bio/Technology 6, 1205–1210
17. Guan, X.-M., Kobilka, T. S., and Kobilka, B. K. (1992) J. Biol. Chem. 267, 21995–21998
18. Chiang, C., and Roeder, R. (1993) Pept. Res. 6, 62–64
19. Koo, Y. B., Ji, I., Slaughter, R. G., and Ji, T. H. (1991) Endocrinology 128, 2297–2308
20. Tsai-Morris, C. H., Buczko, E., Wang, W., Xie, X. Z., and Dufau, M. L. (1991) J. Biol. Chem. 266, 11355–11359
21. Jiang, X., Dreno, M., Buckler, D., Cheng, S., Ythier, A., Wu, H., Hendrickson, W., and Tayar, N. (1995) Structure 3, 1341–1353
22. Couture, L., Naharissa, H., Grebert, D., Remy, J. J., Pajot-Augy, E., Bozon, V., Haertle, T., and Salesse, R. (1996) J. Mol. Endocrinol. 16, 15–25
23. Bhowmick, N., Huang, J., Puett, D., Isaacs, N. W., and Lapthorn, A. J. (1996) Mol. Endocrinol. 10, 1147–1159
24. Kobe, B., Deisenhofer, J. (1994) Trends Biochem. Sci. 19, 415–421
25. Zhang, R., Buczko, E., and Dufau, M. L. (1996) J. Biol. Chem. 271, 5755–5760
26. Ryu, K.-S., Gilchrist, R. L., Ji, I., Kim, S.-J., and Ji, T. H. (1996) J. Biol. Chem. 271, 7301–7304