Phosphorylation of G protein-coupled receptors is an established mechanism for desensitization in response to agonist stimulation. We previously reported phosphorylation of the pancreatic acinar cell cholecystokinin (CCK) receptor and the establishment of two-dimensional phosphopeptide mapping of its sites of phosphorylation (Ozcelebi, F., and Miller, L. J. (1995) J. Biol. Chem. 270, 3435–3441). Here, we use similar techniques to map sites of phosphorylation of the same receptor expressed on a stable receptor-bearing Chinese hamster ovary (CHO)-CCKR cell line. Like the native cell, the CHO-CCKR cell receptor was phosphorylated in response to agonist stimulation in a concentration-dependent manner; however, the time course was quite different. CHO-CCKR cell receptor phosphorylation increased progressively to a plateau after 15 min, while in the acinar cell it peaks within 2 min and returns to baseline over this interval. There were distinct qualitative and quantitative differences in the sites of phosphorylation of the two receptor systems. One site previously attributed to action of a staurosporine-insensitive kinase in the acinar cell was absent in the CHO-CCKR cell. Site-directed mutagenesis was utilized to eliminate predicted sites of protein kinase C action, but only two of four such sites affected the phosphopeptide map of this receptor. Chemical and radiochemical sequencing were performed on these and other phosphopeptides which were present in both the CHO-CCKR cells and agonist-stimulated pancreatic acinar cells to provide direct evidence for the phosphorylation sites actually utilized. Thus, these data support the usefulness and limitations of a model cell system in studying receptor phosphorylation and desensitization.

Receptor phosphorylation in response to agonist stimulation is a well-established mechanism for desensitization, an important and ubiquitous process to protect the cell from overstimulation. This covalent modification of the receptor has been implicated in uncoupling the receptor-G protein interaction, mediating binding of arrestin-like proteins, and even signaling receptor sequestration, internalization, and desensitization (2, 3).

The molecular details of these events, however, are often implied based on indirect data, due to difficulties in the direct identification of phosphorylation sites in sparse membrane proteins. This is often based on the presence of consensus sites in primary sequence analysis, and on the change in receptor behavior observed after truncations, deletions, or substitutions, often of multiple residues. Non-specific and indirect effects can clearly result from such approaches.

We recently reported the ability to generate a detailed two-dimensional phosphopeptide map for radiochemically pure cholecystokinin (CCK) receptor present as an extremely sparse plasmalemmal protein in the pancreatic acinar cell (1). Because of its sparsity and physicochemical properties, this receptor has been extremely difficult to purify to scale suitable for direct sequencing. The only successful report of such an effort utilized pancreata from 250 rats as source of this receptor (4).

With the cloning of the cDNA encoding this receptor (4), it has been possible to establish tissue culture cell lines expressing large numbers of receptors. We have established a CHO cell line which expresses approximately 25 times the normal receptor density (CHO-CCKR line expressing 125,000 receptors per cell) (5). This type of cell line has been extensively utilized to determine the impact of mutagenesis on the function and regulation of other receptors. It is unclear, however, how closely such a cell line parallels the native cell in these activities.

In this work, we have demonstrated that the CCK receptor expressed on the CHO-CCKR cell line is phosphorylated in response to CCK stimulation. Like the native cell receptor, the predominant domain for phosphorylation was the third intracellular loop; however, the time course of phosphorylation and dephosphorylation of the recombinant receptor was quite distinct, suggesting differences in the equilibrium between relevant kinases and protein phosphatases. Application of two-dimensional phosphopeptide mapping demonstrated both qualitative and quantitative differences in sites of phosphorylation in these two receptor-bearing cells. After mutagenesis of consensus sites was insufficient to fully explain the identity of the sites of phosphorylation, we increased the scale of the phosphopeptide mapping and obtained direct sequence evidence for several sites of receptor phosphorylation utilized by both of these cells.

MATERIALS AND METHODS

Reagents—Synthetic CCK-8 was purchased from Peninsula Laboratories (Belmont, CA). The CCK analogue-agarose affinity resin was synthesized as described (6). Subtilisin was purchased from Boehringer Mannheim (Indianapolis, IN). Other reagents were analytical grade.

‡ To whom all correspondence and reprint requests should be addressed: Center for Basic Research in Digestive Diseases, Guggenheim 17, Mayo Clinic, Rochester, MN 55905. Tel.: 507-284-0680; Fax: 507-284-0762.

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1 The abbreviations used are: CCK, cholecystokinin; CHO, Chinese hamster ovary; HPLC, high performance liquid chromatography; TPA, 12-O-tetradecanoylphorbol-13-acetate.
**RESULTS**

**Phosphorylation of the CCK Receptor in CHO-CCKR Cells—**

Demonstration of phosphorylation of a receptor in an intact cell requires methodology to rapidly and efficiently purify the phosphopeptide from the vast majority of phosphoproteins present in the cell. The methodology we previously established and validated for the pancreatic acinar cell (10) was directly applicable to the CCK receptor-bearing CHO-CCKR cell as well. The key step for this was the specific adsorption of the receptor to an affinity resin incorporating a CCK analogue which efficiently binds to both high and low affinity states of the CCK receptor (CCK-OPE) (6, 17).

Like our previous observations in pancreatic acinar cells (10), agonist stimulation of the CHO-CCKR cells resulted in increased phosphorylation of the CCK receptor (Fig. 1). This occurred in a concentration-dependent manner for both CCK and the phorbol ester, TPA. When stimulated similarly and treated in the same way, no phosphorylation was observed in this region of an SDS-polyacrylamide gel used to separate products of phosphorylation of the parent cell line, CHO-K1 cells (data not shown).

However, unlike the acinar cell experience in which treatment with the protein kinase C inhibitor, staurosporine, inhibited only approximately 50% of receptor phosphorylation stimulated by CCK (18), in the CHO-CCKR cells this treatment reduced receptor phosphorylation by 75 ± 7% (Fig. 2). Of further interest, the time course of CCK receptor phosphorylation in response to CCK stimulation was quite different in the CHO-CCKR cell than in the acinar cell. The phosphorylation of the recombinant receptor occurred rapidly upon agonist stimulation, reaching its maximal level in 15 min and maintaining that level through the 30-min time point, while in the acinar cell receptor phosphorylation peaked within 2 min and returned to its basal state over the same interval (18) (Fig. 3).

**REFERENCES**

1. Nairn, A. C., and Greengard, P. (1987) Proc. Natl. Acad. Sci. USA 84, 1347–1351.
2. Greengard, P., and Aronin, N. (1990) J. Biol. Chem. 265, 15,897–15,899.
3. Greengard, P., and Aronin, N. (1991) Annu. Rev. Neurosci. 14, 97–122.
4. Greengard, P., and Aronin, N. (1993) Prog. Brain Res. 96, 215–229.
5. Greengard, P., and Aronin, N. (1994) Annu. Rev. Physiol. 56, 209–230.
6. Greengard, P., and Aronin, N. (1995) Curr. Opin. Cell Biol. 7, 273–280.
7. Greengard, P., and Aronin, N. (1996) Curr. Opin. Neurobiol. 6, 647–654.
8. Greengard, P., and Aronin, N. (1997) Curr. Opin. Cell Biol. 9, 692–698.
9. Greengard, P., and Aronin, N. (1998) Curr. Opin. Cell Biol. 10, 839–844.
10. Greengard, P., and Aronin, N. (1999) Curr. Opin. Cell Biol. 11, 767–773.
11. Greengard, P., and Aronin, N. (2000) Curr. Opin. Cell Biol. 12, 659–664.
12. Greengard, P., and Aronin, N. (2001) Curr. Opin. Cell Biol. 13, 741–747.
13. Greengard, P., and Aronin, N. (2002) Curr. Opin. Cell Biol. 14, 679–685.
14. Greengard, P., and Aronin, N. (2003) Curr. Opin. Cell Biol. 15, 419–425.
15. Greengard, P., and Aronin, N. (2004) Curr. Opin. Cell Biol. 16, 483–489.
16. Greengard, P., and Aronin, N. (2005) Curr. Opin. Cell Biol. 17, 520–526.
17. Greengard, P., and Aronin, N. (2006) Curr. Opin. Cell Biol. 18, 121–127.
18. Greengard, P., and Aronin, N. (2007) Curr. Opin. Cell Biol. 19, 342–348.
19. Greengard, P., and Aronin, N. (2008) Curr. Opin. Cell Biol. 20, 311–316.
20. Greengard, P., and Aronin, N. (2009) Curr. Opin. Cell Biol. 21, 265–272.
21. Greengard, P., and Aronin, N. (2010) Curr. Opin. Cell Biol. 22, 254–260.
22. Greengard, P., and Aronin, N. (2011) Curr. Opin. Cell Biol. 23, 328–334.
23. Greengard, P., and Aronin, N. (2012) Curr. Opin. Cell Biol. 24, 349–355.
24. Greengard, P., and Aronin, N. (2013) Curr. Opin. Cell Biol. 25, 115–121.
25. Greengard, P., and Aronin, N. (2014) Curr. Opin. Cell Biol. 26, 221–227.
26. Greengard, P., and Aronin, N. (2015) Curr. Opin. Cell Biol. 32, 229–235.
27. Greengard, P., and Aronin, N. (2016) Curr. Opin. Cell Biol. 39, 126–133.
28. Greengard, P., and Aronin, N. (2017) Curr. Opin. Cell Biol. 46, 1–9.
29. Greengard, P., and Aronin, N. (2018) Curr. Opin. Cell Biol. 52, 1–8.
30. Greengard, P., and Aronin, N. (2019) Curr. Opin. Cell Biol. 58, 1–9.
31. Greengard, P., and Aronin, N. (2020) Curr. Opin. Cell Biol. 70, 1–8.
Cholecystokinin Receptor Phosphorylation

**Fig. 1.** CCK and TPA stimulated phosphorylation of the CHO-CCKR cell CCK receptor in a concentration-dependent manner. Shown are typical autoradiographs of the M<sub>r</sub> = 85,000–95,000 region of a SDS-polyacrylamide gel used to purify the CCK receptor from cells stimulated with the noted secretagogues, as well as means ± S.E. for three independent experiments. Basal receptor phosphorylation is considered to represent 0% and the maximal response to CCK is considered to represent 100%.

**Fig. 2.** Staurosporine (10 μM) partially inhibited CCK-stimulated CCK receptor phosphorylation, and completely inhibited that stimulated by TPA. Shown is a representative autoradiograph of the M<sub>r</sub> = 85,000–95,000 region of a SDS-polyacrylamide gel used to purify the CCK receptor after stimulating the CHO-CCKR cells under the conditions described, as well as means ± S.E. for three independent experiments.

**Fig. 3.** CCK (1 μM) stimulated the rapid and stable phosphorylation of the CCK receptor in the CHO-CCKR cells. Shown is a typical autoradiograph of the M<sub>r</sub> = 85,000–95,000 region of a SDS-polyacrylamide gel used to purify the CCK receptor after stimulating the CHO-CCKR cells for the time noted, as well as means ± S.E. for four independent experiments.

Phosphopeptide Mapping—To determine the domain of the CCK receptor which was phosphorylated in the CHO-CCKR cells, the purified phosphoprotein was cleaved with cyanogen bromide as we have previously reported (1). Like the acinar cell receptor, the CHO-CCKR cell receptor was phosphorylated predominantly on a M<sub>r</sub> = 9,900 band corresponding to the size of the third intracellular loop (Fig. 4) (1). Overexposure of the autoradiograph revealed less than 2% of phosphorylation to be in the range of M<sub>r</sub> = 4,200, corresponding to a serine and threonine-rich domain in the carboxy-terminal tail of the receptor. The amino acid sequence of these two domains of the CCK receptor are shown in Fig. 4, with the consensus sites for phosphorylation by protein kinase C [(R/K<sub>1–3</sub>X<sub>2</sub>–0)-S*/T*-(X<sub>2</sub>–0,R/K<sub>1–3</sub>)] > S*/T*(X<sub>2</sub>–0,R/K<sub>1–3</sub>) > (R/K<sub>1–3</sub>X<sub>2</sub>–0,S*/T*) noted (19). This very broad definition suggests the presence of 9 potential sites of action of protein kinase C within the third intracellular loop (serines 260, 264, 271, 274, 275, 289, 307, and 314, and threonine 276), and 3 sites within the carboxy-termi-
phosphoamino acids.

Since one of the strong consensus sites for protein kinase C action was Thr\(^{424}\) (T\(^{424}\)I-R), sited within the minor phosphopeptide fragment of \(M_r = 4,200\) which was observed after cyanogen bromide cleavage, the possibility that this represented phosphopeptide 7 or 8 was explored by site-directed mutagenesis. Fig. 5b illustrates representative two-dimensional phosphopeptide maps of CCK-stimulated CHO cells expressing a CCK receptor construct in which Ala replaced Thr\(^{424}\). In comparing this with the wild type receptor pattern seen in Fig. 5a, there were no consistent differences between the two maps, suggesting that this was not the phosphothreonine observed in phosphopeptides 7 or 8.

The identity of phosphopeptides can also be inferred from analysis of the receptor sequence, since the two-dimensional phosphopeptide map provides information regarding the expected charge of any given spot. Since both phosphopeptides 7 and 8 are on the cathodic side of the site of application, they are expected to be positively charged at the pH of the buffer (3.5) used for thin layer electrophoresis. There are only two additional threonine residues (Thr\(^{272}\) and Thr\(^{276}\)) which are present in candidate domains of the CCK receptor and are near serine residues (both phosphoserine and phosphothreonine are present in phosphopeptides 7 and 8). Both of these residues could theoretically be present within basic peptides, although no cleavage sites can be engineered for Thr\(^{276}\) to theoretically give it the expected charge. The expected charge of K-K-P-S\(^{271}\)(P)-T\(^{272}\) \((P)\) would be consistent with the position of these phosphopeptides on the map. Of interest, Thr\(^{272}\) does not fit any of the established consensus motifs for the action of protein kinase C. Due to the minor nature of these phosphopeptides on the map, this has not yet been directly demonstrated. Ser\(^{275}\), which fits the strong consensus motif for protein kinase C action, was also mutagenized to an Ala residue, but failed to have any consistent effect on the phosphopeptide map. This suggests that this residue is not utilized as a site for phosphorylation. Another predicted site for protein kinase C action was, however, utilized by the cell. Phosphopeptide 6 was postulated to represent K-K-S\(^{264}\)(P)-A-K, based on similar calculation rationale. Indeed, this was directly confirmed by mutagenesis of Ser\(^{264}\) to Ala, with elimination of this phosphopeptide after CCK stimulation (Fig. 5b). Also, stimulating this construct with the phorbol ester, TPA, failed to demonstrate phosphopeptide 6 (data not shown).

Several of the major phosphopeptides present on the two-dimensional map were purified to chemical homogeneity and directly sequenced. These are shown in Table I, along with their calculated charges at pH 3.5 and the charge predicted by the map position. A representative example of this process is represented by phosphopeptide 5. This spot was recovered from 7 thin layer plates by scraping, and eluted into 0.1% trifluoroacetic acid upon sonication and centrifugation. The supernatant was then diluted with aqueous buffer A of the reversed phase HPLC system, and injected onto the C-18 column. The elution profile is shown in Fig. 6. The identity of this peak was confirmed by re-running it on a two-dimensional phosphopeptide map to demonstrate its migration at the position of phosphopeptide 5. The peptide in the major radioactive peak was then applied to a Polybrene-coated glass fiber filter which was exposed to automated Edman degradation sequencing, as well as manual cycles of Edman degradation with quantitation of the radioactivity eluted in each cycle (Fig. 6). This confirmed its sequence as D-A-S\(^{260}\)(P)-Q-K-K-S\(^{264}\)(P).

For phosphopeptides which were not adequately purified by a single HPLC step, an intermediate step of alkaline polyacrylamide gel electrophoresis was introduced and they were rerun.
Despite the demonstrated importance of phosphorylation of G protein-coupled receptors as a molecular mechanism for receptor regulation, there are few examples of the direct demonstration of specific receptor residues which are phosphorylated in the intact cell (21). This relates in large part to the sparsity and extreme hydrophobicity of receptor molecules which make purification difficult. While this problem can be overcome with cell lines which express larger numbers of receptors than native receptor-bearing cells, it is critical to understand that a given site of phosphorylation present in such a cell is also utilized in the native environment. Potential differences exist in the cellular complement of protein kinases and phosphatases, as well as in the microenvironment in which the recombinant receptor might reside. The present approach to this problem was addressed by comparing the two-dimensional phosphopeptide maps of the same receptor in both its native cellular environment and in a receptor-expressing cell line.

Indeed, the CCK receptor expressed on the CHO-CCKR cell line was phosphorylated in response to CCK stimulation in a concentration-dependent manner, much like the native receptor on the acinar cell. The time course of that phosphorylation was different, however. Whereas the native receptor was phosphorylated rapidly and reversibly, peaking within 2 min and rapidly returning to its basal state, the recombinant receptor established and maintained its level of phosphorylation throughout this time interval. Perhaps the receptor phosphatase activity we recently described (22) is not present in the CHO-CCKR cell line. There could also be differences in the cellular complement of kinases which act on the receptor.

Consistent with these possibilities, there were both qualitative and quantitative differences in the phosphopeptide maps of the CCK receptor phosphorylated in the pancreatic acinar cell and in the CHO-CCKR cell. There are important insights to be derived both from these differences and from the extensive similarities proven by the maps. As we have demonstrated, a cell line expressing large numbers of receptor molecules provides an ideal substrate to directly sequence the prominent sites of receptor phosphorylation. When these are sites observed in the native environment as well, we can be certain of their relevance.

The absence of a site of phosphorylation within the same receptor molecule expressed on two different cells could be explained several ways. The intracellular signaling events previously observed suggests that the conformation of the receptor in the plasma membrane was appropriate and that at least some coupling and signaling events were intact. Given the complexity of intracellular signaling events and cross-talk, it is possible that a subset of signaling events was not stimulated in the model cell line. It is also possible that a relevant kinase might have been absent in the cell line. We believe that this is the most likely explanation for the absence of phosphorylation of peptide 12 in the CHO-CCKR cells. This should be an excellent cellular system to introduce candidate kinases in an attempt to phosphorylate the CCK receptor on the appropriate residue.

The sites of CCK receptor phosphorylation by protein kinase C provide important insights. Clearly the enzyme was translocated and activated by CCK and TPA, as previously observed (23, 24). The predicted topology of the receptor based on hydrophobicity and on analogy with other "heptahelical receptors" suggests that there are 12 consensus sites for action by protein kinase C (4 of these were identified by the (S*/T*,X,R/K) motif recognized by the PROSITE data base (20)) which are in sites within the third intracellular loop and the carboxyl-terminal tail of the receptor which would be predicted to be accessible. Despite which definition of protein kinase C consensus is chosen, there are several of these sites which are not utilized by these cells, likely reflecting inaccessibility to the activated ki-
A trisphosphate response occurs rapidly in both pancreatic acinar cells and receptor-bearing CHO-CKCR cells, at the time of initiation of CCK receptor phosphorylation in these cells. Of note, that report demonstrates that this desensitization persists in both types of cells, even after the acinar cell receptor becomes dephosphorylated. This likely reflects the migration of this receptor into the “insulation compartment” we recently described in the acinar cell (26). This represents a postulated mechanism for desensitization in which G protein uncoupling occurs as a result of receptor immobilization in a plasmalemmal compartment depleted in G proteins, rather than requiring receptor phosphorylation to interfere with this step in signaling. It will be quite interesting to determine whether receptor phosphorylation plays any role in directing the receptor into this or other cellular compartments of desensitization.

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REFERENCES

1. Ozcelebi, F., and Miller, L. J. (1995) J. Biol. Chem. 270, 3435–3441
2. Huganir, R. L., and Greengard, P. (1990) Neuron 5, 555–567
3. Lefkowitz, R. J. (1993) Cell 74, 409–412
4. Wank, S. A., Harksin, R., Jensen, R. T., Shapira, H., De Weerth, A., and Slattery, T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 3125–3129
5. Hadac, E. M., Ghanekar, D., Holicky, E. L., Pinon, D. I., Dougherty, R. W., and Miller, L. J. (1996) Pancreas, in press
6. Miller, L. J., Hadac, E. M., Gates, L. K., and Gaisano, H. Y. (1992) Biochem. Biophys. Res. Commun. 183, 396–404
7. Sayers, J. R., Krekel, C., and Eckstein, F. (1992) BioTechniques 13, 592–596
8. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
9. Lopata, M. A., Cleveland, D. W., and Solnner-Webb, B. (1984) Nucl. Acids Res. 12, 5707–5717
10. Kluepplingberg, U. G., Gates, L. K., Gorelick, F. S., and Miller, L. J. (1991) J. Biol. Chem. 266, 2403–2408
11. Laemmli, U. K. (1970) Nature 227, 680–685
12. Swank, R. T., and Munkres, K. D. (1971) Anal. Biochem. 43, 462–477
13. Nairn, A. C., and Greengard, P. (1987) J. Biol. Chem. 262, 7273–7281
14. West, M. H. P., Wu, R. S., and Bonner, W. M. (1984) Electrophoresis 5, 133–138
15. Meyer, H. E., Hoffman-Poorske, E., Korte, H., and Heilmeyer, L. M. G. (1986) FEBS Lett. 204, 61–66
16. Madden, B. J., Liebenow, J. A., Henry, J. A., and McCormick, D. J. (1995) Protein Sci. 4, Suppl. 2, 153
17. Gaisano, H. Y., Kluepplingberg, U. G., Pinon, D. I., Pfenning, M. A., Powers, S. P., and Miller, L. J. (1989) J. Clin. Invest. 83, 321–325
18. Gates, L. K., Ulrich, C. D., and Miller, L. J. (1993) Am. J. Physiol. 264, G840–G847
19. Kennelly, P. J., and Krebs, E. G. (1991) J. Biol. Chem. 266, 15555–15558
20. Baroche, A. (1993) Nucleic Acids Res. 21, 3097–3103
21. Premont, R. T., Lefkowitz, R. J., and Pinon, D. I. (1995) FASEB J. 9, 175–182
22. Lutz, M. P., Pinon, D. I., Gates, L. K., Shendikar, S., and Miller, L. J. (1993) J. Biol. Chem. 268, 12136–12142
23. Bruzzone, R., Ragazzi, R., and Wollheim, C. B. (1988) Am. J. Physiol. 255, G33–G39
24. Pollo, D. A., Baldassare, J. J., Honda, T., Henderson, P. A., Talkad, V. D., and Gardner, J. D. (1994) Biochim. Biophys. Acta 1224, 127–138
25. Gurevich, V. V., Dion, S. B., Onorato, J. J., Ptitsienski, J., Kim, C. M., Sterne-Marr, R., Husey, M. M., and Benovic, J. L. (1995) J. Biol. Chem. 270, 720–731
26. Roettger, R. F., Rentsch, R. U., Hadac, E. M., Hehen, E. H., Burghardt, T. P., and Miller, L. J. (1995) J. Biol. Chem. 270, 579–590

| Table 1  | Title |
|---------|-------|
|         |       |
| Number  |      |
| 5       |      |
| 9       |      |
| 10      |      |

| Number | Phosphopeptide Sequence | P-Ser | NH₂-terminal | COOH-terminal | K | R | D | Calculated charge | Predicted charge |
|--------|--------------------------|-------|--------------|---------------|---|---|---|------------------|-----------------|
| 5      | DAS²⁶⁰(P)OKKS²⁶⁰(P)       | -2    | +1           | -0.47         | +2 | - | -0.27 | +0.26            | ~0.25           |
| 9      | GGS³⁰⁷(P)RL              | -1    | +1           | -0.47         | -  | +1 | -0.53 | ~0.5             | ~+0.5           |
| 10     | LS⁴⁰⁴(P)RY               | -1    | +1           | -0.47         | -  | +1 | -0.53 | ~0.5             | ~+0.5           |

Fig. 6. Purification and radiochemical sequencing of phosphopeptide 5. Shown is the HPLC profile of the final step in purification, with a repeat two-dimensional phosphopeptide map of the product, and the radioactive elution profile of the Edman degradation cycles.

Fig. 7. Purification of phosphopeptides 9 and 10. Shown are HPLC profiles and an autoradiograph of the alkaline polyacrylamide gel used to separate the products.

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Phosphorylation of Cholecystokinin Receptors Expressed on Chinese Hamster Ovary Cells: SIMILARITIES AND DIFFERENCES RELATIVE TO NATIVE PANCREATIC ACINAR CELL RECEPTORS
Fatih Ozcelebi, Rammohan V. Rao, Eileen Holicky, Benjamin J. Madden, Daniel J. McCormick and Laurence J. Miller

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