Conformationally Sensitive Residues in Extracellular Loop 5 of the Na\(^+\)/Dicarboxylate Co-transporter*

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The Na\(^+\)/dicarboxylate co-transporter, NaDC-1, from the kidney and small intestine, transports three sodium ions together with one divalent anion substrate, such as succinate\(^2^-\). A previous study (Pajor, A. M. (2001) J. Biol. Chem. 276, 29961–29968), identified four amino acids, Ser-478, Ala-480, Ala-481, and Thr-482, near the extracellular end of transmembrane helix (TM) 9 that are likely to form part of the permeation pathway of the transporter. All four cysteine-substituted mutants were sensitive to inhibition by the membrane-impermeant reagent [2-(trimethylammonium)ethyl]-methanethiosulfonate (MTSET) and protected by substrate. In the present study, we continued the cysteine scan through extracellular loop 5 and TM10, from Thr-483 to Val-528. Most cysteine substitutions were well tolerated, although cysteine mutations of some residues, particularly within the TM, produced proteins that were not expressed on the plasma membrane. Six residues in the extracellular loop (Thr-483, Thr-484, Leu-485, Leu-487, Ile-489, and Met-493) were sensitive to chemical labeling by MTSET, depending on the conformational state of the protein. Transport inhibition by MTSET could be prevented by substrate regardless of temperature, suggesting that the likely mechanism of substrate protection is steric hindrance rather than large-scale conformational changes associated with translocation. We conclude that extracellular loop 5 in NaDC-1 appears to have a functional role, and it is likely to be located in or near the substrate translocation pore in the protein. Conformational changes in the protein affect the accessibility of the residues in extracellular loop 5 and provide further evidence of large-scale changes in the structure of NaDC-1 during the transport cycle.

The Na\(^+\)/dicarboxylate co-transporter, NaDC-1, is found on the apical membrane of the epithelial cells of the renal proximal tubule and the small intestine (1). NaDC-1 carries a broad range of divalent anion substrates including dicarboxylates, such as succinate and \(\alpha\)-ketoglutarate, and protonated tricarboxylates, such as citrate\(^2^-\). NaDC-1 is involved in regulating concentrations of citric acid cycle intermediates in kidney cells and in the urine. Consequently, NaDC-1 activity affects the development of kidney stones (2) and regulation of blood pressure (3). NaDC-1 has also been implicated in life span determination; the Drosophila homolog, Indy, is a longevity gene also expressed in the gastrointestinal tract (4). NaDC-1 belongs to the SCL13 gene family that includes sodium-coupled transporters for sulfate and di- and tricarboxylates in vertebrates (5) and the sodium-independent malate transporter from plant vacuoles (6).

The identification of the substrate and cation binding sites in NaDC-1 and the transmembrane helices (TMs)\(^3\) involved in forming the permeation pathway remains an area of active investigation. Our previous studies have shown that the carboxyl-terminal half of NaDC-1, TM7–TM11, contains residues that determine substrate specificity (7). Chimera studies suggest that TM10 and the adjacent loops contain amino acids that determine differences in \(K_m\) for citrate and sodium (8). Recently, we have found that TM9 is involved in the conformational changes seen during the transport cycle and likely forms part of the permeation pathway through the transporter (9).

The extracellular end of TM9 in NaDC-1 contains four conformationally sensitive residues, Ser-479, Ala-480, Ala-481, and Thr-482 (9). Cysteine substitutions of these amino acids resulted in proteins that were sensitive to inhibition by the membrane-impermeant methanethiosulfonate reagent [2-(trimethylammonium)ethyl]-methanethiosulfonate (MTSET). In the present study, the cysteine scan was continued through the extracellular loop between TM9 and TM10 (extracellular loop 5) and in TM10, from Thr-483 to Val-528, in order to identify conformationally sensitive residues. As described previously, the membrane-impermeant reagent MTSET was used to react with accessible thiolate anions (10), and the functional consequences of the labeling were measured.

The major finding of this study is that MTSET-accessible residues are found in the loop between TM9 and TM10 and not in the transmembrane region. Eleven of the cysteine-substituted mutants were sensitive to MTSET, with either increased or decreased activity after chemical labeling. The most sensitive cysteine mutants were T483C, T484C, L485C, L487C, I489C, and M493C, all of which exhibited conformational differences in their sensitivity to MTSET labeling as well as substrate protection. All six cysteine-substituted mutants were most accessible to MTSET in the presence of sodium, the conformational state with the highest affinity for substrate. Because the conformational changes in accessibility of TM9 are also continued in the extracellular loop between TM9 and TM10, both TM9 and the loop are likely to contribute residues that line the permeation pathway in NaDC-1.

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1 The abbreviations used are: TM, transmembrane helix; MTSET, [2-(trimethylammonium)ethyl]-methanethiosulfonate; HRPE, human retinal pigment epithelium; PBS/CM, phosphate-buffered saline, pH 9, containing 1 mM Ca\(^{2+}\) and Mg\(^{2+}\); MTSEA, (2-aminoethyl)methanethiosulfonate.
Inhibitor that produces half-maximal inhibition, and differences between experiments done with [14C]succinate and [3H]suc- 
fection was modeled according to reversible non-competitive inhibition, transport inhibition, the concentration dependence of MTSET inhibi-
experiment contained four groups: vector controls with and without 
Control groups were preincubated in sodium buffer without MTSET. 
buffer except with choline chloride replacing NaCl), with or without 10 
SET (Toronto Research Chemicals), the MTSET was pre-weighed and 
uptakes in NaDC-1-transfected cells. Statistical analysis with one-way 

test was done using the Sigma Stat software (Eastman Kodak Co.). 

Labeling of Cysteine Mutants with MTSEA-biotin—HRPE cells were 
preincubated with sodium uptake buffer with or without 1 mM MTSET 
for 1 h at room temperature. The MTSET solution was washed 
away by three washes with 3 ml of PBS/CM, pH 7.5. For each experiment, a 
200 mM stock solution of MTSEA-biotin (Toronto Research Chemicals) 
was prepared in MeSO and kept cold and dark. The MTSEA-biotin was 
diluted to 2 mM with PBS/CM, pH 7.5, just before use and added to the cells for 30 min at room temperature with rocking. The cells were then 
three times with 3 ml of cold PBS/CM, pH 7.5. The remaining steps were 
identical to those in the Sulfo-NHS-LC biotinylation protocol. The 
cells were lysed and centrifuged, and biotinylated proteins were precipi-
ted with Immunopure Immobilized Streptavidin (Pierce). The eluted 
samples were used in Western blotting as described above.

RESULTS
Cysteine-scanning Mutagenesis of NaDC-1—The secondary 
structure model of the rabbit NaDC-1 is shown in Fig. 1A. There are 11 predicted transmembrane helices, and the car-
boxyl terminus, containing a conserved N-glycosylation site, is 
on the outside of the cell (17). Forty-three amino acids between 
Thr-483 and Val-528 (Fig. 1B), located in extracellular loop 5 
between transmembrane helices (TM) 9 and 10 and within 
TM10, were mutated to cysteine one at a time. The sequence 
alignment of this region with sequences from selected members of 
the SLC13 family is shown in Fig. 1B. The start of TM10 is 
predicted to be the conserved leucine residue at position 502.

As in our previous studies (9, 18, 19), the parental trans-
portor for this study was the C476S mutant of NaDC-1. Al-
though the wild-type NaDC-1 is insensitive to methanethiosul-
fonate reagents, the endogenous cysteine at position 476 mediates inhibition by another cysteine selective reagent, p-
chloromercuribenzenesulfonate. The C476S mutant still con-
tains 10 endogenous cysteines. As in any mutagenesis project, 
there is a possibility that the introduction of new cysteines 
could produce changes in protein conformation that expose the 
remaining endogenous cysteines, although this does not appear to 
be the case in NaDC-1. In the TM9 cysteine scan study, we 
found that there was no difference in MTSET sensitivity of 
cysteine-substituted mutants made in a background containing 
only three endogenous cysteines (3C mutant) or in the C476S 
mutant containing 10 endogenous cysteines (9). Because the 
replacement of endogenous cysteines can produce a reduction 
in protein expression, the risk of exposing endogenous cyste-
ines has to be balanced against having measurable transport 
activity. We found previously that there is a correlation be-
tween the number of endogenous cysteines in NaDC-1 and the 
amount of protein expressed at the plasma membrane, and the 
transport activity of the 3C mutant is only about 25% of that of 
the C476S mutant (11). It is possible that removing endogenous cysteines changes the structure of the transporter, which then 
results in decreased protein expression.
Transport Activity and Expression of Cysteine-substituted Mutants—The cysteine mutants of NaDC-1 were transiently transfected into the HRPE cell line derived from human retinal pigment epithelium. This cell line has low background dicarboxylate transport activity (20), and it is useful for plate transport assays because the cells are strongly attached to the plastic culture dishes.

The cell surface expression of the mutants was measured by biotinylation with a membrane-impermeant reagent, Sulfo-NHS-LC-biotin (Fig. 2). Single Western blots are shown in Fig. 2, and the results of scans of multiple blots are shown in Fig. 3. The protein expression of many of the mutant transporters was similar to that of the C476S parental transporter. The transport activity of the mutants is also shown in Fig. 3 for comparison with protein expression. The mutants were initially screened for functional activity in 24-well plates, and those that had activity less than ~30% of the activity of the C476S control were assayed in 6-well plates. Most of the mutants exhibited some measurable transport activity (Fig. 3). Eight of the mutants were either completely inactive or had activity that was less than 6% of the parental transporter activity: L485C and P488C (in the loop) and A515C, A521C, T522C, P523C, and P524C (in TM10). Most of the inactive mutants found in TM10 (S512C, T522C, and P523C) had little or no cell surface expression, suggesting that the mutations altered protein trafficking or stability. Interestingly, a few mutants exhibited measurable transport activity but greatly reduced cell surface expression, including L487C, H500C, and V530C, suggesting that these mutations might increase activity or decrease Km.

Sensitivity to MTSET—Thirty-nine mutants with measurable transport activity were screened for their sensitivity to a 1 mM concentration of MTSET. The parental C476S mutant (Fig. 4) is insensitive to MTSET (Fig. 4). Two mutants, A494C and L499C, were only moderately inhibited (about 30%) by pretreatment with 1 mM MTSET, suggesting that either they are not accessible to the MTSET or they are accessible to the MTSET, but the functional consequences of chemical modification are minimal. Six of the mutants (T483C, T484C, L485C, L487C, I489C, and M493C) were almost completely inactive after preincubation with 1 mM MTSET. These six mutants were then studied in more detail.

The substrate affinity of the cysteine-substituted mutants did not appear to be affected by the cysteine mutation. The C476S parental transporter had a Km for succinate of 399 μM, compared with 238 μM in our previous study (9). The Km for succinate in T483C was 410 ± 28 (n = 2); in L487C, the Km was 227 μM; and in M493C, the Km was 650 μM (data not shown). The remaining mutants, T484C, L485C, and I489C, were assayed in 6-well plates because of low activity (see Fig. 3). For those mutants, the signal above background at high substrate concentrations was too low for accurate kinetic measurements.

Effects of Cations and Substrate on MTSET Sensitivity—To determine whether the substituted cysteines are accessible to MTSET in different conformational states, the preincubation with MTSET was done in either sodium or choline buffer, with or without succinate. As shown previously, NaDC-1 has an ordered binding mechanism in which sodium binds first and triggers a conformational change that increases substrate affinity, then substrate binds, and translocation occurs (9).
Therefore, the transporter is likely to be in different conformational states when incubated in the presence or absence of sodium or substrate. As shown in Fig. 5, the Y503C mutant, located near the extracellular end of TM10, reacted with MTSET at the same rate, regardless of the preincubation conditions. Therefore, Y503C probably has similar accessibility to the extracellular medium in all conformational states of the transporter. In contrast, the other mutants that were inhibited by labeling with MTSET (T483C, T484C, L485C, I489C, and M493C) exhibited differences in apparent accessibility depending on the preincubation conditions (Fig. 5). Five of the mutants (T483C, T484C, L485C, I489C, and M493C) were most sensitive to inhibition by MTSET when the preincubation was done in sodium buffer, suggesting that the acces-
Conformationally Sensitive Residues in Loop 5 of NaDC-1

sibility of these substituted cysteines is greatest in the conformational state or states seen in the presence of sodium. The L487C mutant was equally sensitive to inhibition by MTSET in sodium or choline, suggesting that this residue is exposed in the conformational states seen in the absence as well as the presence of sodium. The six conformationally sensitive mutants also exhibited substrate protection because there was decreased inhibition by MTSET in the presence of sodium and 10 mM succinate (Fig. 5).

Concentration Dependence of MTSET Inhibition—The concentration dependence of MTSET inhibition for the six sensitive mutants, T483C, T484C, L485C, L487C, I489C, and M493C, was determined by measuring the succinate transport activity before and after a 10-min preincubation with increasing concentrations of MTSET (Fig. 6). Three of the mutants, T484C, L485C, and I489C, were very sensitive to inhibition by MTSET, with estimated IC$_{50}$ values of < 0.5 mM. M493C had an IC$_{50}$ of ~6 µM. T483C and L487C were relatively insensitive to MTSET with IC$_{50}$ values of 0.3 and 0.7 mM, respectively. For comparison, the pseudo first order rate constants ($k$) were estimated from the IC$_{50}$ values (note that Fig. 6 shows a single experiment; the $k$ values represent mean ± range of two experiments). The most reactive residues, I489C, T484C, and L487C, had the largest estimated $k$ values of about 180 min$^{-1}$ mM$^{-1}$. M493C had an intermediate value of 11 ± 0.3 min$^{-1}$ mM$^{-1}$. The least reactive residues were T483C (0.2 ± 0.04 min$^{-1}$ mM$^{-1}$) and L487C (0.1 ± 0.02 min$^{-1}$ mM$^{-1}$).

Effect of Temperature on MTSET Inhibition—There are several possible explanations to account for substrate protection of MTSET labeling. For example, the conformational change that occurs after substrate binding could occlude the substituted cysteine and make it inaccessible to MTSET. Alternately, substrate binding could physically prevent access of the MTSET to the substituted cysteine. Because large-scale conformational changes in transporters are reduced at low temperature, we compared the extent of substrate protection at room temperature and on ice. The remaining transport activity after MTSET labeling was measured at room temperature. As shown in Fig. 7, there was very little effect of temperature on MTSET inhibition in the absence of substrate. There was also no effect of temperature on substrate protection from MTSET in the T483C and M493C mutants. However, the T484C, L485C, L487C, and I489C mutants had a significant decrease in the effect of MTSET in the presence of substrate when the incubation was carried out on ice as compared with at room temperature (Fig. 7). The result of our experiment suggests that the rate of inactivation by MTSET in the presence of substrate is likely to be lower in the cold than at room temperature. If the major effect of substrate protection is a conformational change due to translocation, then we would expect to see a decrease in substrate protection in the cold, i.e. increased sensitivity to MTSET. For example, in a similar experiment with the Na$^+$/serotonin transporter, cold temperature abolished substrate protection of MTSEA binding at Cys-357 (21). Instead, T484C, L485C, L487C, and I489C exhibited increased substrate protection in the cold. Therefore, the major effect of substrate protection in these mutants is likely to be related to substrate binding, thereby producing steric hindrance of MTSET labeling.

Labeling of Substituted Cysteines with MTSEA-biotin—Most of the experiments in this study have examined the functional effects of chemical modification with MTSET. Residues that show no functional consequence of MTSET treatment could still be accessible to chemical labeling by this reagent. Therefore, we also examined whether some of the substituted cysteines could be labeled with a biotin derivative, MTSEA-biotin. Labeling was done after preincubation with or without MTSET. The parental transporter, C476S, and a cysteine mutant that was very sensitive to MTSET in our previous study, T482C (9), were used as controls in each experiment. As shown in Fig. 8, there is some residual labeling of C476S with MTSEA-biotin, but this is not affected by preincubation with MTSET. The C476S mutant contains 10 endogenous cysteines, but the results suggest that these cysteines are not accessible to MTSET. This result rules out the possibility that MTSET labels the endogenous cysteines in C476S without any functional consequences. The positive control mutant, T482C, was strongly labeled with MTSEA-biotin, and this was inhibited by preincubation in MTSET. M493C was sensitive to MTSET and also...
showed MTSET-blockable labeling by MTSEA-biotin. Interestingly, L499C exhibits MTSET-blockable labeling by MTSEA-biotin, and it is one of the residues that was activated after treatment with MTSET. Two residues that exhibit only partial inhibition by MTSET, Q495C and Y503C, were not strongly labeled by MTSEA-biotin despite expression on the plasma membrane that was ~50% of control (Sulfo-NHS-LC-biotin; Fig. 3). It appears that the sensitivity of MTSEA-biotin is lower than that of Sulfo-NHS-LC-biotin. Three residues that are insensitive to MTSET and predicted to be in transmembrane helix 10 (M505C, T509C, and A511C) also had little labeling by MTSEA-biotin compared with the C476S control and little or no difference with MTSET preincubation.

**DISCUSSION**

The transport cycle of secondary active transporters such as the Na+/dicarboxylate co-transporter, NaDC-1, involves several conformational changes induced by sodium and substrate binding and a large-scale conformational change to allow translocation from one side of the membrane to the other. Our previous study identified TM9 in NaDC-1 as a potential component of the substrate permeability pathway and a participant in the conformational changes that occur during transport (9). Four amino acids at the extracellular face of TM9 (Ser-478, Ala-480, Ala-481, and Thr-482) were found to show differences in accessibility to the membrane-impermeant cysteine reagent, MTSET, during the transport cycle. In the present study, we have continued the cysteine scan from Thr-483 to Val-528, including extracellular loop 5 and TM10, and examined the sensitivity of these mutants to MTSET. The main findings are that extracellular loop 5 is also likely to participate in the conformational changes during the transport cycle of NaDC-1 because residues in this loop exhibit differences in MTSET accessibility with different conformational states produced by

![MTSET inhibition of succinate transport by cysteine-substituted mutants](image-url)
the presence or absence of sodium and substrate. In contrast, cysteine mutations within TM10 were insensitive to inhibition by MTSET.

Most of the cysteine substitutions made in this study were well tolerated, with many mutants retaining at least 50% of the wild-type activity and protein expression. Some conserved residues, particularly within the putative TM10, were sensitive to substitution with cysteine, and the resulting proteins were not well expressed on the plasma membrane. The alignment of sequences from representative members of the SLC13 family (Fig. 1B) shows several residues that are conserved in all members of the family, including homologs from Drosophila and plants. The highly conserved residues seemed to correlate well with the greatest decrease in transport activity. For example, cysteine mutants of two highly conserved residues, Ala-515 and Phe-516, were expressed at the plasma membrane but had very low activity, suggesting that these residues may have functional roles.

Eleven of the 43 cysteine-substituted amino acids in NaDC-1 were functionally affected by chemical modification with MTSET, verifying that these residues are either located on the outside of the cell or in an aqueous pore accessible from the outside. MTSET is a water-soluble reagent that does not cross the plasma membrane, and it labels cysteines that are accessible from the outside of the cell. In our secondary structure model (Fig. 9), ten of the MTSET-sensitive residues are found in extracellular loop 5 between TM9 and TM10, and one residue, Tyr-503, is located at the extracellular end of TM10. In general, there was good correlation between the functional effects of MTSET and the accessibility to labeling by MTSEA biotinylation, with a few exceptions. For example, the Q495C and Y503C mutants are both accessible to MTSET, although the inhibition was modest (at most 40%). These residues were not labeled by MTSEA-biotin. This might indicate that there are constraints on the size of the molecules that can reach these residues. Alternately, there could be differences in assay sensitivity between the Sulfo-NHS-LC biotinylations, which label all extracellular lysines, and MTSEA biotinylations, which would label a single cysteine. The addition of more biotin molecules to the protein may result in more effective precipitation with streptavidin beads.

The MTSET accessibility of five of the mutants in this study (T483C, T484C, L485C, I489C, and M493C) seems to parallel the accessibility of the substrate binding site to the outside of the cell. As illustrated in Fig. 10, the transport cycle of NaDC-1 involves ordered binding, with the three sodium ions binding first, triggering a conformational change that increases affinity for succinate, followed by substrate binding (22). The binding of substrate is then followed by a conformational change to reorient the substrate and cation binding sites to the inside of the cell, after which the substrate and cations are released. At present, we have little information on the order of substrate release on the inside of the cell, but it appears that at least one of the sodium ions is released last (22). Similar to the exposure...
Conformationally Sensitive Residues in Loop 5 of NaDC-1

In conclusion, we have found that the fifth extracellular loop in NaDC-1, located between TM9 and TM10, participates in the conformational changes during the transport cycle. Six amino acids in the loop are sensitive to inhibition by MTSET, and they exhibit differences in accessibility in different conformational states of the transporter. The accessibility of five of these residues (T483C, T484C, L485C, I489C, and M493C) appears to follow the accessibility of the substrate binding site. Substrate protection was seen in all MTSET-sensitive mutants, which appears to be due to a steric hindrance of MTSET binding rather than inaccessibility of the substituted cysteine as a consequence of large-scale conformational change. Therefore, in addition to TM9 identified in our previous study, the extracellular loop adjacent to it also participates in conformational changes seen during the transport cycle. Our findings suggest that extracellular loop 5 either transmits structural information to other parts of the protein during conformational changes or that residues in the loop are located within the large central water-filled cavity that contains the substrate binding site.

REFERENCES

1. Pajor, A. M. (2000) J. Membr. Biol. 175, 1–8
2. Pajor, A. M. (1999) Semin. Nephrol. 19, 195–200
3. He, W., Miao, F. J. P., Lin, D. C. H., Schwaner, R. T., Wang, Z., Gao, J., Chen, J. L., Tian, H., and Ling, L. (2004) Nature 429, 188–193
4. Regina, B., Reenan, R. A., Nilsen, S. P., and Helfand, S. L. (2000) Science 290, 2137–2140
5. Markovich, D., and Murer, H. (2004) Pflugers Arch. 447, 594–602
6. Emmerlich, V., Linka, N., Reinhold, T., Hurth, M. A., Traub, M., Martinova, E., and Neuhaus, H. E. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 11122–11126
7. Pajor, A. M., Sun, N., Bai, L., Markovich, D., and Sule, P. (1998) Biochim. Biophys. Acta 1370, 98–106
8. Kahn, E. S., and Pajor, A. M. (1999) Biochemistry 38, 6151–6156
9. Pajor, A. M. (2001) J. Biol. Chem. 276, 29961–29968
10. Danielsen, M. A., Bass, R. B., and Falke, J. J. (1997) J. Biol. Chem. 272, 32873–32888
11. Pajor, A. M., Krajewski, S. J., Sun, N., and Gangula, R. (1999) Biochem. J. 344, 205–209
12. Kuncel, T. A. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 488–492
13. Segel, I. H. (1975) Enzyme Kinetics, John Wiley and Sons, New York
14. Pajor, A. M., Sun, N., and Valmente, H. G. (1998) Biochem. J. 331, 257–264
15. Chen, J.-G., Liu-Chen, S., and Rudnick, G. (1997) Biochemistry 36, 1479–1486
16. Li, H., and Pajor, A. M. (2000) Am. J. Physiol. Cell Physiol. 280, C1188–C1196
17. Zhang, F. P., and Pajor, A. M. (2001) Biochim. Biophys. Acta 1511, 80–89
18. Seal, R. P., and Amara, S. G. (1998) Neuron 21, 1487–1498
19. Yao, X., and Pajor, A. M. (2002) Biochemistry 41, 1083–1090
20. Wang, H., Fei, Y.-J., Kokuda, R., Yang-Feng, T., Devoe, L. D., Leibach, F. H., Prasad, P. D., and Ganapathy, M. E. (2000) Am. J. Physiol. Cell Physiol. 278, C1019–C1030
21. Androusslis-Theotokis, A., Ghassemi, F., and Rudnick, G. (2001) J. Biol. Chem. 276, 45933–45938
22. Yao, X., and Pajor, A. M. (2000) Am. J. Physiol. Renal Fluid Electrolyte Physiol. 279, F54–F64
23. Hamasaki, N., Aikawa, Y., and Tanigawa, T. (1998) Biochim. Biophys. Acta 1370, 98–106
24. Zomot, E., and Kanner, B. (2003) J. Biol. Chem. 278, 42950–42958
25. Dambrosia, J. M., and Pajor, A. M. (1997) Biochemistry 36, 1322–1328
26. Stephen, M. M., Chen, M. A., Penado, K. M. Y., and Rudnick, G. (1997) Biochemistry 36, 1322–1328
27. Gruenwald, M., Menaker, D., and Kanner, B. I. (2002) J. Biol. Chem. 277, 26074–26080
28. Leighton, B. H., Seal, R. P., Shimamoto, K., and Amara, S. G. (2002) J. Biol. Chem. 277, 29647–29655
29. Henry, L. K., Adams, E. M., Han, Q., and Blakely, R. D. (2003) J. Biol. Chem. 278, 37052–37063