Tyrosine-dependent Basolateral Sorting Signals Are Distinct from Tyrosine-dependent Internalization Signals

(Received for publication, July 3, 1997, and in revised form, August 13, 1997)

Sasa Lin, Hussein Y. Naim‡, and Michael G. Roth§

From the Department of Biochemistry, University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-9038

Converting cysteine 543 to tyrosine in the influenza virus hemagglutinin (HA) introduces both a basolateral sorting signal and an internalization signal into the HA cytoplasmic domain. Another HA mutant, HA+8, contains eight additional amino acids at the end of the cytoplasmic domain that include a powerful internalization signal. HA+8 was also sorted efficiently to the basolateral surface of Madin-Darby canine kidney cells. The simplest explanation for the observation that multiple sorting phenotypes depend upon the same small amino acid sequence is that certain tyrosine-based internalization signals might also function as basolateral sorting signals. To test this hypothesis, second-site mutations were introduced into HA C543Y or HA+8 to determine if the internalization and basolateral sorting functions can be separated. For HA C543Y, the same sequence positions were important for both basolateral sorting and internalization, but the two functions responded differently to individual amino acid replacements, indicating that they were distinct. For HA+8, the basolateral sorting signal required the same tyrosine as the internalization signal, but did not share any other characteristics. Thus, even when basolateral sorting signals that depend on tyrosine overlap or are co-linear with internalizations signals, the two sorting processes are sensitive to different characteristics of the sequence.

The language of molecular recognition used to identify and segregate proteins to the locations where they must function is crucial to the organization of all cells. Some of the basic vocabulary of this process, the sorting signals used by the cell to classify proteins for transport, have recently been identified (1–3). However, this knowledge has not yet led to any unified hypothesis explaining how sorting is accomplished, and current understanding of the mechanisms controlling intracellular protein traffic is still poorly developed. For membrane proteins, the continuous line of Madin-Darby canine kidney (MDCK)1 cells have been an extremely useful model for investigating the regulation of intracellular protein transport. MDCK cells form monolayers of columnar epithelial cells in culture in which adjacent cells are joined by junctional complexes that physically separate the outer leaflet of the plasma membrane into two separate membrane domains (4, 5). The apical domain of MDCK cells contains different proteins and lipids from the basolateral domain and is also functionally distinct. Endocytosis is slower from the apical surface (6–8), and endocytic receptors are absent. In contrast, the basolateral domain contains receptors and transporters for nutrients. Cell adhesion proteins and extracellular matrix proteins are transported specifically to this surface.

Proteins and lipids bound for both surface membrane domains have been observed together in the Golgi complex and trans Golgi network, suggesting that the trans Golgi network might be the site for sorting apical and basolateral proteins (4). Peptide sequences have been identified in a number of proteins that are necessary and sufficient for delivery to the basolateral surface of MDCK cells. These fall into two groups functionally, those that overlap internalization signals (9–12) and those that do not (11, 13). The sorting signals currently identified are sufficiently diverse in primary sequence and predicted secondary structure that one cannot exclude the possibility that there are several parallel mechanisms for recognizing basolateral proteins, each using a separate class of sorting signals. A similar situation exists for apical proteins.

In addition to the possibility that parallel sorting mechanisms may operate to direct certain classes of proteins to the cell surface, there is good evidence that there is more than one pathway for travelling to certain membrane domains. Recently two reports have indicated that the sorting signals recognized during exocytosis are recognized again in endosomes after proteins are internalized (13, 14). Missorted proteins with defective basolateral sorting signals, or proteins with weak basolateral signals that were transported to the apical surface when overexpressed, were preferentially transcytosed to the correct surface domain rather than recycling back to the domain from which they were internalized. In contrast, proteins correctly targeted to the cell surface were recycled back to the correct membrane domain after internalization. The observation that sorting occurs in both the endocytic and exocytic pathways in MDCK cells raises the possibility that in this cell type, internalization signals function as a type of basolateral sorting signal, thus ensuring that endocytic receptors are efficiently localized to the basolateral domain.

To investigate the nature of sorting signals controlling the traffic of membrane proteins to the basolateral surface in MDCK cells, we have analyzed the sequence requirements for two basolateral signals that overlap internalization signals. These sequences were studied in the context of HA, which normally lacks basolateral sorting signals and is transported to the apical surface. The cytoplasmic domain of HA is quite small, predicted to contain only 12 amino acids (15). Thus, when we observed that a single, randomly selected amino acid change of cysteine to tyrosine at position 543 created both an
internalization signal (16) and a basolateral sorting signal (9), one possible interpretation was that both sorting events might use a common signal in MDCK cells. A second explanation was that certain basolateral sorting signals containing tyrosine might only resemble internalization signals at the level of primary sequence, perhaps because one had evolved from the other. Both types of signal might be quite degenerate with respect to primary sequence, so that the number of sequences capable of functioning as each type of signal would be fairly large. Recently several proteins have been shown to contain basolateral sorting signals that overlap, but are distinct from, internalization signals (12, 14), whereas in Fc receptors the same residues appear necessary for both functions (17). Therefore, it seemed possible that by chance we might have constructed in HA C543Y a sequence that, while suboptimal for both internalization and basolateral sorting, nevertheless functioned for both. To investigate this possibility, we have made lines of polarized MDCK cells that express proteins with a series of mutations in the cytoplasmic domains of two HAs with different internalization signals and have determined their sorting and internalization properties.

EXPERIMENTAL PROCEDURES

Materials—All commercial chemicals were obtained from Sigma, except as noted. Polyclonal serum from a rabbit immunized with A/Japan/305 virus glycoproteins was used to immunoprecipitate HA.

Cell Lines—MDCK cells were routinely cultured in plastic dishes and were grown on Costar (Cambridge, MA) Transwell filter inserts for experiments, as described (9). Mutant HA proteins were constructed by megaprimer polymerase chain reaction on 500-base pair DNA fragments encoding the cytoplasmic terminus of HA as described previously (3, 18, 19). For each mutant, the region encoding HA was sequenced to verify the mutation and then the fragments were subcloned into the expression vector pcB6HA (20) and transfected into the MDCK subline D5 (21) according to published protocols (3, 22). Except where noted, cells were selected for resistance to G418 and used for experiments without subcloning, to control for possible effects of clonal variation.

Expression levels in uncloned populations were similar to those of clonal lines derived from them (3). Experiments employing HA C543Y were performed on one of two permanently expressing transfected cell lines described in a previous study (9). Cells were treated with 10 mM sodium butyrate for 15 h prior to all experiments except for those measuring the effect of varying sodium butyrate on protein expression. Experiments on HA mutants produced during an alanine scan of the HA+C8 sequence were performed with a minimum of three independently cloned cell lines. Although the expression level of the clones varied, none produced more protein under any condition that did the reference clonal cell lines expressing HA+C8. The sorting of HA+C8 to the basolateral surface (99%) did not change significantly when cells were treated with 10 mM sodium butyrate. Experiments investigating the effect of amino acid replacements at position 549 of HA+C8 were performed on uncloned populations of transfected MDCK D5 cells that expressed similar levels of HA+C8 as did clonal lines isolated during the alanine scan.

Polarity of Surface Arrival and Internalization Assays—Cells were washed four times with Dulbecco’s phosphate-buffered saline containing Ca$^{2+}$ and Mg$^{2+}$ at room temperature to remove excess cysteine and methionine, then were preincubated for 30 min at 37 °C in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) lacking these amino acids. Then the same medium was added to the top of the Transwell inserts and 0.15 ml of medium was added to the bottom compartment. The cells were incubated for 45 min at 37 °C and the medium containing trypsin was then replaced with medium containing 0.1 mg/ml soybean trypsin inhibitor, and the cells were incubated an additional 15–30 min on ice to inhibit any remaining trypsin activity.

RESULTS

Basolateral Sorting and Internalization Signals in HA C543Y Are Different but Overlap—The possibility of overlapping, distinct sorting signals in a protein can be directly tested by site-directed mutagenesis. If one signal serves for both internalization and basolateral sorting, any second-site mutations that change the activity of one of the two sorting events will have a similar effect on the other. Thus, we constructed a series of mutants designed to make the HA C543Y sequence more, or less, similar to that of a popular “consensus” for internalization signals, Y-X-X-large hydrophobic residue (2). The DNAs encoding these proteins were subcloned into the expression vector pcB6 (20) and lines of polarized MDCK cells that express HA wild-type (HA wt) or the HA mutants, HA C543Y, HA C543Y,C546F, HA C543Y,C546R, HA C543Y,C546S, HA C543Y,C546R, tail-less HA (HA+C8), or HA C543F, were made. These cells were grown on filters and the polarity of the cells, and the location of the HAs was determined by previously published assays (3, 9). The results of these experiments are shown in Table I. Both HA wt and HA+C8 were sorted mainly to the apical cell surface. HA C543Y and HA C543Y,C546F mutants were sorted efficiently to the basolateral domain, whereas HA C543Y,C546S and HA C543Y,C546R mutants were delivered to both apical (about 35–45%) and basolateral (about 55–55%) sides. A phenylalanine at position 543 instead of tyrosine did not function for basolateral sorting (or internalization) (24). When the results of basolateral sorting experiments were compared with internalization rates measured for these mutants at the basolateral surface of MDCK cells (Fig. 1), it is apparent that changes in internalization and basolateral sorting did not occur in parallel. Although arginine at position 546 contributed to a significantly better internalization signal than cysteine at the same position, it decreased basolateral sorting.

![Table I](http://www.jbc.org)
nine at position 546 increased basolateral sorting approximately 10% but increased internalization by 6-fold in comparison with Cys546. Thus, the basolateral sorting and internalization signals in HA C543Y have different sequence requirements.

In these experiments, the portion of the HA C543Y protein sorted to the basolateral surface was less than observed previously (9). A difference in the two sets of experiments is that, in the current set, cells were treated with sodium butyrate to increase protein expression. Treatment of MDCK cells with butyrate has been reported to decrease the fidelity of basolateral sorting of LDL receptors with mutations in their basolateral sorting signals in a manner inversely correlated with their expression level (25, 26). Without butyrate, HA C543Y was sorted to the basolateral surface was less than observed previously (9). A difference in the two sets of experiments is that, in the current set, cells were treated with sodium butyrate to increase protein expression. Treatment of MDCK cells with butyrate has been reported to decrease the fidelity of basolateral sorting of LDL receptors with mutations in their basolateral sorting signals in a manner inversely correlated with their expression level (25, 26). Without butyrate, HA C543Y was 94 ± 3% basolateral (n = 4) and with butyrate, 84 ± 3% basolateral (n = 6; the probability that these values are not significantly different is less than 0.002, by Student’s t test). Butyrate did not affect the overall polarity of the MDCK cells under the conditions of our experiments because basolateral expression of HA C543Y, C546F was more complete under any condition than was sorting of HA C543Y and was insensitive to treatment with butyrate (3).

HA+8 Is Sorted with High Fidelity to the Basolateral Surface in MDCK Cells and Is Also Rapidly Internalized—HA+8 is a mutant HA protein that contains eight amino acids added to the carboxyl terminus by a mutation at the normal translational stop codon that shifts the reading frame (19, 27). To determine if HA+8 would also be transported to the basolateral surface of MDCK cells, continuous, clonal MDCK cells expressing HA+8 were made, and the delivery of HA+8 to the cell surface of monolayers grown on filter inserts was measured by pulse-chase experiments. HA+8 was transported almost exclusively to the basolateral plasma membrane (Table II). Previously we had mapped the internalization signal in HA+8 to the sequence YKSF, in experiments in monkey fibroblasts (19). To determine if the basolateral sorting information in HA+8 was co-linear with the internalization signal, as was the case for HA C543Y, we made a series of second-site mutants in which the amino acids from position 546 to 555 were changed to either alanine or serine. Polarized MDCK cell lines were made expressing these proteins, and the amount of each protein residing at the basolateral surface after a pulse and chase was compared with the amount of terminally glycosylated protein residing inside the cells (Fig. 2). After an 80-min chase at 37 °C with trypsin present in the culture medium, the ratio of surface to internal protein for each of the mutant HA+8 proteins had reached a maximum value and did not change during the next 40 min of chase. Rates of degradation for these proteins were similar, ranging from 0.1%/min for HA+8 Y550S to 0.5%/min for HA+8 Y548S, which is 10–20-fold slower than the rates of internalization or recycling we have measured previously for other HA mutants in MDCK cells (8). These observations were similar to measurements of the traffic of HA+8 that we had made previously in monkey fibroblasts, where HA+8 and several of its mutants were internalized in minutes and remained cycling through an early endosome compartment for several hours (19). Thus, in monkey fibroblasts and presumably in

![Fig. 1. Features required for internalization and basolateral sorting can be altered independently in HA C543Y. Internalization rates and polarity of exocytosis were measured for HA proteins that had the mutations shown in the cytoplasmic domain. For each mutant HA, the percent of protein reaching the plasma membrane that arrived at the basolateral surface after a 1-h chase at 37 °C, and the percent internalized at the basolateral surface after 1 min are plotted.](http://www.jbc.org/)

**Table II**

Sequences of HA+8 and second-site mutants

The single letter code for the entire cytoplasmic sequence of HA or HA+8 is shown. Positions marked by . contain the amino acid found at that position in HA or HA+8. Residues changed in the second-site mutants are indicated. In the mutant HA+8 A541–545, Ser246 adjoins Tyr546. The percent of each protein at the basolateral (BL) surface is given, with S.D. where indicated. Values represent the means of a minimum of four experiments.

<table>
<thead>
<tr>
<th>Mutant protein</th>
<th>Cytoplasmic sequence</th>
<th>% BL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA wt</td>
<td>CSNGSLQCRIICYDYKSFYN</td>
<td>25</td>
</tr>
<tr>
<td>HA + 8</td>
<td>CSNGSLQCRIICYDYKSFYN</td>
<td>99</td>
</tr>
<tr>
<td>HA+8-C546A</td>
<td>. . . . . . . . . . . . . . .</td>
<td>94 ± 6</td>
</tr>
<tr>
<td>HA+8-I547A</td>
<td>. . . . . . . . . . . . . . .</td>
<td>87 ± 13</td>
</tr>
<tr>
<td>HA+8-I547R</td>
<td>. . . . . . . . . . . . . . .</td>
<td>92 ± 7</td>
</tr>
<tr>
<td>HA+8-Y548S</td>
<td>. . . . . . . . . . . . . . .</td>
<td>96 ± 5</td>
</tr>
<tr>
<td>HA+8-Y548S</td>
<td>. . . . . . . . . . . . . . .</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>HA+8-N555A</td>
<td>. . . . . . . . . . . . . . .</td>
<td>35 ± 4</td>
</tr>
<tr>
<td>HA+8-K551A</td>
<td>. . . . . . . . . . . . . . .</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>HA+8-S552A</td>
<td>. . . . . . . . . . . . . . .</td>
<td>92 ± 5</td>
</tr>
<tr>
<td>HA+8-F553A</td>
<td>. . . . . . . . . . . . . . .</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>HA+8-Y554S</td>
<td>. . . . . . . . . . . . . . .</td>
<td>95 ± 14</td>
</tr>
<tr>
<td>HA+8-N555A</td>
<td>. . . . . . . . . . . . . . .</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>HA+8-Y548S,F553A,Y554S</td>
<td>. . . . . . . . . . . . . . .</td>
<td>93 ± 4</td>
</tr>
<tr>
<td>HA+8-Y548S,Y554S</td>
<td>. . . . . . . . . . . . . . .</td>
<td>90 ± 12</td>
</tr>
<tr>
<td>HA+8-541–545</td>
<td>CSNGSLQCRIICYDYKSFYN</td>
<td>90 ± 9</td>
</tr>
</tbody>
</table>

* Numbers above the residue indicate the amino acid position.
MDCK cells, the internalization rate is the principle determinant of the fraction of HA+8 that is internal at steady state.

Examination of the ratio of the surface population to the internal population of each mutant protein after an 80-min chase revealed two characteristics of the HA+8 internalization signal in MDCK cells (Fig. 2). Change of any amino acid in the region from position 546 to 555 decreased the internal fraction of the protein. However, the effect of most mutations was consistent with a protein that was internalized as fast or faster than it was recycled back to the plasma membrane. Only mutations at positions Tyr550 and Phe553 resulted in essentially all of the protein remaining at the cell surface. Values shown are derived from measurements on three independently derived clonal cell lines. Error bars are standard deviations from three or more measurements.

**The Internalization Signal in HA+8 Is Not Co-linear with the Basolateral Targeting Signal**—The delivery of HA+8 and second site mutants to the basolateral surface was measured by pulse-chase experiments. Mutations at only two positions had any significant effect on basolateral sorting (Fig. 3, Table II). Mutation D549A created a protein that was delivered equally to the apical and basolateral surfaces, and mutation Y550S produced a protein that was sorted to the apical surface almost as well as the HA protein. The observation that the F553A mutation that prevented significant internalization had no effect on basolateral sorting indicated that in HA+8, as HA C543Y, the two signals were different. To determine if the two signals in HA+8 were co-linear, as they were in HA C543Y, we determined the sorting pattern of a mutant, HA5, in which the HA+8 sequence was truncated after position Lys551 (Table II). HA+4 was still sorted efficiently to the basolateral surface, but was essentially not internalized (Fig. 4). Thus, the basolateral sorting signal in HA+8 was not co-linear with the internalization signal, although both signals shared a requirement for Tyr550.

**The Basolateral Targeting Signal in HA+8 Requires Tyro-**
sine 550 and Is Sensitive to Hydrophobic Residues in the Pre-
ceding Position—A basolateral sorting signal having the pat-
tern DY had not been previously reported. The HA+8 sequence
has an isoleucine encoded by the wild type HA sequence at
position 547. To investigate the possibility that the basolateral
sorting signal in HA+8 might have the pattern Ile547-X-X-
Tyr550. Ile547 was mutated to arginine. This mutation made
very little effect on the basolateral sorting of HA+8 (Table II).

To determine if some other nearby residue to the amino-termi-
nal side of Tyr550 contributed to basolateral sorting, amino
acids between position 541 and 545 were deleted, and the
sorting of this mutant, HA+8 Δ541-545, was determined.
HA+8 Δ541-545 was still predominately basolateral (77%),
although it was not sorted as well as HA+8 (99%) or HA+4
(90%).

To investigate the role of Asp549, this position was subjected
to degenerate mutagenesis, and 13 mutant proteins were ex-
pressed in MDCK cells. The fraction of each protein sorted to
the basolateral surface was determined (Fig. 5). Replacement
of Asp549 with hydrophobic residues (Ala, Val, Leu, Phe, Cys)
resulted in proteins that were delivered equally to apical and
basolateral surfaces. Replacement of Asp549 with residues of
opposite charge (Arg, His), polar residues (Asn, Ser, Tyr), or
residues that promote turn formation (Gly, Pro) had no effect.

Thus, the aspartic acid side chain at position 549 was not
required for the functioning of the basolateral sorting signal
that included Tyr550.

DISCUSSION

For HA C543Y, as described previously for the LDL receptor
(25) and human lysosomal acid phosphatase (12), the baso-
lateral sorting signal overlaps an internalization signal but is
distinct. The basolateral signal in the HA C543Y protein, ty-
rosine-X-X-cysteine, proved to be a degenerate form of a signal
with the primary sequence pattern “tyrosine-X-X-large hy-
drophobic residue,” since substitution of cysteine by phenylalanine
made a much better signal by the criteria that it was insensi-
tive to a 10-fold increase in protein expression. In primary
sequence, the HA C543Y basolateral sorting signal resembles
those identified in rat lgp120 (28) and the vesicular stomatitis
G protein (3). This pattern is observed in a large number of
signals for different transport pathways (see Ref. 3 for a list),
suggesting that sorting of membrane proteins along many
pathways may be controlled by distinct, but related, cytosolic
sorting receptors that bind different subsets of related sorting
signals. In principle, this recognition system would resemble
the selectivity achieved by proteins containing SH2 domains
that recognize different subsets of tetrapeptide sequences con-
taining a phosphorylated tyrosine (29, 30). However, other
basolateral sorting signals do not depend upon a Y-X-X-hy-
drophobic motif (12, 13, 17, 25, 31), suggesting that there may be
parallel mechanisms for sorting different classes of proteins to
the same membrane domain.

Mutants of the LDL receptor containing only the membrane
proximal of its two cytoplasmic basolateral sorting signals are
sorted less completely to the basolateral surface when their
expression level is raised. This basolateral signal overlaps the
LDL receptor internalization signal. Mutants containing only
the membrane-distal signal, which does not function for inter-
nalization, are sorted properly regardless of the level of expres-
sion, establishing a criteria for judging a sorting signal to be
“weak” or “strong” (25). The HA C543Y signal resembled the
LDL proximal signal in that HA C543Y was expressed more
apically when expression levels were elevated, although HA
C543Y remains significantly more basolateral than its LDL
counterpart at similar levels of high expression (1–2 × 105
molecules/cell). However, basolateral signals that are co-linear
with internalization signals are not inherently weaker than
those that are not. Mutation of cysteine 543 to phenylalanine
created both a better internalization signal and a strong baso-
lateral sorting signal.

![Figure 4](http://www.jbc.org/)

**Fig. 4.** Truncating the HA+8 sequence after Lys551 abolishes
internalization but has little effect on basolateral expression of
the mutant HA+4. Internalization was measured as described for
Fig. 2 and basolateral expression as described for Fig. 3.

![Figure 5](http://www.jbc.org/)

**Fig. 5.** Replacement of Asp549 with 13 other residues indicates that
hydrophobic amino acids are not allowed at that position, but the aspar-
tic acid side chain is not required. Basolateral expression was measured on
uncloned stably transfected cell populations. Error bars indicate the S.D. values
of four or more measurements.
Although the HA+8 protein contains an internalization signal of the pattern Y-XX-(large hydrophobic), this is not the basolateral sorting signal. The only amino acid absolutely required for basolateral sorting of HA+8 was Tyr550. Position 549 was important, but Asp549 was not essential, since it could be replaced by many different amino acids having different charges and sizes. Both Garnier-Robeson and Chou-Fasman algorithms predict that Tyr550 is located in a turn. Amino acids that functioned at position 549 had no effect on this prediction, whereas the nonfunctional class were all predicted to either prevent (Chou-Fasman) or change (Garnier-Robeson) the position of the turn so that it no longer contained Tyr550. A turn has been also proposed as an essential component of the basolateral sorting signal of the polyimmunoglobulin receptor, but does not appear to be the only essential characteristic (32). It is possible that a key component of the HA+8 basolateral sorting signal is the presentation of the tyrosine by secondary structure. If the proposal is correct that tyrosine-based internalization signals contain tyrosine at the first position of a type 1 β-turn (2), then Tyr550 should be in such a turn, since it functions extremely well for internalization. However, the requirements for function of the basolateral sorting signal containing Tyr550 are different than those required for internalization. Thus, there is either additional information required for basolateral sorting compared with internalization (information which we have been unable to detect), or the polypeptide chain around Tyr550 may be flexible and capable of assuming more than one conformation. Binding to a sorting receptor might or might not require the chain to adopt a particular conformation before the initial interaction, but would presumably lock the chain into the proper conformation once it is bound. This idea of induced fit is attractive, as it explains how two distinct signals can co-exist in the same space without interfering with each other.

Our work does not eliminate the possibility that there is a second amino acid side chain important for the functioning of the basolateral sorting signal in HA+8, that can be substituted by alanine, or in some cases, serine. However, if this residue lies beyond position 551, it contributes much less to the signal than does Tyr550, since truncation of the sequence at this position in HA+4 results in a protein that is still predominantly basolateral. This is a very different result than we observed for the vesicular stomatitis G protein, which also has a strong basolateral sorting signal that requires tyrosine, but does not function as an internalization signal. The basolateral sorting signal in G protein is Y-X-X-I, and alanine does not replace the isoleucine (3). Thus, the basolateral sorting signal in HA+8 does not resemble any previously described basolateral sorting signal. Either there is more than one class of tyrosine-dependent basolateral sorting signal (implying that these signals do not interact with the same receptor binding site), or basolateral sorting signals such as the one in HA+8 and vesicular stomatitis virus G protein share a structural context around the tyrosine that is important, rather than primary sequence context. Resolution of this question will require identification of the protein(s) that bind these signals.

REFERENCES
Tyrosine-dependent Basolateral Sorting Signals Are Distinct from
Tyrosine-dependent Internalization Signals
Sasa Lin, Hussein Y. Naim and Michael G. Roth

doi: 10.1074/jbc.272.42.26300

Access the most updated version of this article at http://www.jbc.org/content/272/42/26300

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 32 references, 21 of which can be accessed free at
http://www.jbc.org/content/272/42/26300.full.html#ref-list-1