Proteins actively transported into the nucleus via the classical nuclear import pathway contain nuclear localization signals (NLSs), which are recognized by the family of importin α molecules. Importin α contains 10 armadillo (arm) repeats, of which the N-terminal arm repeats 2–4 have been considered as the “major” NLS binding site. Interferon-activated, dimerized signal transducers and activators of transcription (STAT1 and STAT2) directly bind to importin α via a dimeric nonclassical NLS. Here we show by site-directed mutagenesis that the very C-terminal arm repeats 8 and 9 of importin α5 form a unique binding site for STAT1 homodimers and STAT1–STAT2 heterodimers. Influenza A virus nucleoprotein also contains a nonclassical NLS that is recognized by the C-terminal NLS binding site of importin α5, comprising arm repeats 7–9. Binding of influenza A virus nucleoprotein to importin α3 also occurs via the C-terminal arm repeats. Simian virus 40 large T antigen instead binds to the major N-terminal arm repeats of importin α3, indicating that one importin α molecule is able to use either its N- or C-terminal arm repeats for binding various NLS-containing proteins.

Regulated import of molecules into the nucleus through the nuclear pores is a vital event in eukaryotic cells. Importins (also known as karyopherins) are the major cargo carriers from the cytoplasm into the nucleus. Large molecules (>40 kDa) that cannot passively diffuse through the nuclear pores use a signal-mediated transport system. The importin α/importin β-mediated import pathway was the first one to be discovered, and it is also referred to as the classical pathway. Proteins transported into the nucleus contain nuclear localization signals (NLSs) that are recognized by importin α/importin β heterodimers. Importin α recognizes and binds the NLS, and importin β docks the complex to the nuclear pore and translocates it into the nucleus (1–3).

Six different human importin α molecules have been identified: importin α1 (Rch1, hSRP10), importin α3 (Qip1), importin α4 (hSRP1y), importin α5 (hSRP1, NPI-1), importin α6, and importin α7 (4–9). Importin α proteins have remained structurally and functionally conserved throughout evolution. The six human importin α proteins show over 60% sequence similarity.

The crystal structures of two importin α molecules, yeast importin α (10) and mouse importin α2 (11), have been determined. Importin α is composed of a large central domain that consist of 10 tandemly repeated armadillo (arm) motifs, which are organized in a superhelix flanked by small N- and C-terminal domains. The 10 arm repeats generate an array of binding pockets that are situated within a long helical surface groove. One binding pocket typically includes a tryptophan residue, followed by an asparagine residue 4 residues downstream. The arm repeats are variable within one protein, but they are remarkably conserved in sequence and order when homologous proteins from yeast to humans are compared. The N-terminal importin β binding domain of importin α mediates binding to importin β (12, 13). In the absence of cargo and importin β, the importin β binding domain blocks the NLS binding site of importin α (14, 15). The C-terminal domain of importin α mediates interactions with the export receptor CAS (16, 17).

The NLS used by the classical nuclear import pathway is a short stretch of positively charged amino acids, arginines and lysines, that lack strict consensus sequence (18). A monopartite NLS, like that of simian virus 40 (SV40) large T antigen, is composed of a cluster of five to seven basic amino acids (19, 20). A typical bipartite NLS contains two clusters of basic amino acids separated by a linker of 10–11 amino acids (21).

Monopartite NLSs have been shown to bind to the “major” binding site of importin α, which is formed by arm repeats 2–4 (10). The downstream cluster of the bipartite NLS is also recognized by these arm repeats, whereas the upstream cluster is recognized by arm repeats 7 and 8, also called the “minor” binding site (11).

Signal transducers and activators of transcription (STATs) are latent cytoplasmic transcription factors that regulate the expression of a number of genes involved in host defense and growth (22). Binding of interferon (IFN) to its specific cell-surface receptor leads to the activation of receptor-associated Janus (Jak) tyrosine kinases, which phosphorylate STATs. This leads to dimerization and translocation of STATs into the nucleus. Dimerization is an essential and sufficient step for nuclear import (23). Although bound by importin α5, STATs do not contain a classical NLS (24). Instead, it is supposed that both STAT1 and STAT2 contain in the DNA binding domain a...
nonclassical NLS that seems to become operative in STAT dimers (25–27). In response to IFN-γ stimulation, STAT1 homodimers are formed, whereas IFN-α stimulation results in the formation of STAT1-STAT2 heterodimers. Both STAT1 homodimers and STAT1-STAT2 heterodimers are specifically bound to importin α5 (24, 26, 28). The STAT1-binding site of importin α5 has been suggested to be located in the very C-terminal end of STAT1 apart from the arm repeat domain (24).

Also other regions or completely different mechanisms have been suspected to affect the nuclear translocation of STATs.

Strehlow and Schindler (29) suggested that the most N-terminal amino acids (the first 129 residues) regulate the nuclear import of STAT molecules. Subramaniam et al. (30) suggested that the C-terminal nuclear localization sequence of IFN-γ regulates STAT1α nuclear import, and Bild et al. (31) showed that receptor-mediated endocytosis is necessary for STAT3 nuclear import.

Influenza A virus nucleoprotein (NP) and SV40 large T antigen have functioned as important model molecules for analyzing the nuclear import machinery. Influenza A virus NP...
encapsidates the viral genome and is essential for viral transcription, replication, and packaging. NP interacts with viral RNA, NP, and other viral proteins as well as cellular proteins including importins α1 and α5 (32, 33). Two NLSs have been identified in this protein, an unusual NLS at the N terminus (34) and a classical bipartite NLS in the middle of the molecule (35). Apparently, the N-terminal NLS mediates NP interaction with importins (34, 36). SV40 large T antigen has a monopartite NLS that interacts with importin α (19, 20, 37).

Here we show by site-directed mutagenesis that importin α5 recognizes the nonclassical STAT1, STAT2, and influenza A virus NP NLSs by its arm repeat domain. The binding site for STAT NLSs is composed of arm repeats 8 and 9 and for influenza A virus NP of arm repeats 7–9. Both binding sites differ from the previously described major and minor classical NLS binding sites of importin α. We also show that influenza A virus NP binds to the C terminus and SV40 large T antigen to the N-terminal NLS binding site of importin α3, indicating that one importin α molecule is able to use different binding sites for various NLSs.

MATERIALS AND METHODS

Cells—Monolayers and suspension cultures of Spodoptera frugiperda S/9 cells were maintained in TNM-FH medium and used for baculovirus expression as described (38). Human NK-92 cell line was maintained in continuous culture in minimum Eagle’s medium-α (Invitrogen) supplemented with 12% horse serum (Invitrogen), 12% fetal calf serum, 0.2 mM ascorbate, 20 mM foetal bovine serum, 2 mM L-glutamine, antibiotics, and 100 IU/ml of human recombinant interferon-2 (Chiron, Emeryville, CA).

Interferons and Other Reagents—Human leukocyte IFN-α (6 x 10^6 IU/ml) was kindly provided by Dr. Kari Cantell at our Institute (39). 35S-Labeled PRO-MIX (>1000 Ci/mmol) was obtained from Amersham Biosciences.

Antibodies—In Western blot analysis rabbit anti-STAT1 (c-24; 1:10,000; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-STAT2 (c-20; 1:2000; Santa Cruz Biotechnology), and mouse monoclonal anti-SV40 large T antigen (sc-147; 1:1000; Santa Cruz Biotechnology) immunoglobulins were used as suggested by the manufacturer. Influenza A NP antibodies (40) were used at a 1:500 dilution. In Western blotting secondary horseradish peroxidase-conjugated goat anti-rabbit (1:2000; Dako, Glostrup, Denmark) or anti-mouse immunoglobulins (1:5000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were used.

Plasmids and DNA Manipulations—The importin α1 and α3 gene constructs encoding C-terminal His-tagged proteins have been described previously (9). His tags were replaced by GST tags from pGEX-5X-1 (Amersham Biosciences), and the resulting plasmids were transfected into Sf9 cells. GST-importin proteins were obtained by crystallizing the rabbit (1:2000; Dako, Glostrup, Denmark) or anti-mouse immunoglobulins (1:5000; Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were used.

Structural Features of the Conserved Importin α5 Domain—Importin α5 proteins are evolutionarily conserved and can be found in eukaryotes from yeast to humans. In humans, six isoforms.

**RESULTS**

Structural Features of the Conserved Importin α Molecules—Importin α molecules are evolutionarily conserved and can be found in eukaryotes from yeast to humans. In humans, six importin α isoforms have been identified (Fig. 1A). Yeast cells contain only one importin α isoform that shows over 55% se-
The central domain of importin-α/H9251 is composed of 10 arm repeats (Fig. 1B). The well-conserved tryptophan and asparagine residues in arm repeats 2–4 and 7–9 in the helical surface groove of the importin-α/H9251 molecule are thought to be crucial for NLS binding (10, 11) (Fig. 1B and C). An alignment of the central domain of human importin-α molecules is shown in Fig. 1C. Typical arm repeats contain a tryptophan-asparagine pair at analogous positions. A tryptophan-asparagine pair was found in arm repeats 2–4 and 7–8. Arm repeats 5 and 6, which do not contain this pair, have not been shown to participate directly in NLS binding. Arm repeat 9 contains the conserved asparagine residue, but the tryptophan residue is replaced either by an aspartic acid (importins α1, α3, and α4) or an asparagine (importins α5, α6, and α7). Based on published crystal structures (10, 11) and the alignment in Fig. 1C, series of mutations were created to analyze the interactions between importin-α and various NLSs. Arm repeat 9 was included in our analysis, because the two asparagine residues appeared as part of a possible NLS binding pocket (Fig. 1C).

IFN-stimulated STAT Dimers Bind Specifically to Importin α5—At present, STAT1 homodimers and STAT1-STAT2 heterodimers are the only STAT dimers that have been shown to interact with importin-α isoforms and STAT1. Coomassie Brilliant Blue-stained phosphorylated STAT1 is marked with dots. B, baculovirus-infected Sf9 cell extracts containing either STAT1 and STAT2 proteins or STAT1, STAT2, and Tyk2 proteins (pSTAT1+pSTAT2) were allowed to bind to GST-importin-α proteins as described above and stained for STAT1 and STAT2. C, the control Sf9 cell extracts or Sf9 cell extracts containing influenza A virus NP protein (influenza A NP) were allowed to interact with GST-importin-α subtypes as described above and stained for NP. D, the control Sf9 cell extract or Sf9 cell extracts containing SV40 large T antigen (SV40 T Ag) was allowed to interact with GST-importin-α isoforms as described above and stained for T antigen.

![Figure 3](http://www.jbc.org/)

**Fig. 3.** Binding of STAT dimers, influenza A virus NP, and SV40 large T antigen to different importin α isoforms. A, Sf9 cells were infected with Tyk2- and STAT1-expressing (pSTAT lanes) or STAT1-expressing (STAT1 lanes) recombinant baculoviruses for 42 h. The cells were collected cell extracts prepared, and the soluble proteins were allowed to bind to Sepharose-immobilized GST-importin (GST-imp.) α isoforms at +4 °C for 2 h. After washing, importin-α-bound proteins were dissolved in Laemmli sample buffer, followed by separation of the proteins on 8% SDS-PAGE, transfer to Immobilon membranes, and staining for STAT1. A similar gel was also stained with Coomassie Brilliant Blue to visualize the amount of Sepharose-immobilized GST-importin-α isoforms and STAT1, Coomassie Brilliant Blue-stained phosphorylated STAT1 is marked with dots. B, baculovirus-infected Sf9 cell extracts containing either STAT1 and STAT2 proteins or STAT1, STAT2, and Tyk2 proteins (pSTAT1+pSTAT2) were allowed to bind to GST-importin-α proteins as described above and stained for STAT1 and STAT2. C, the control Sf9 cell extracts or Sf9 cell extracts containing influenza A virus NP protein (influenza A NP) were allowed to interact with GST-importin-α subtypes as described above and stained for NP. D, the control Sf9 cell extract or Sf9 cell extracts containing SV40 large T antigen (SV40 T Ag) was allowed to interact with GST-importin-α isoforms as described above and stained for T antigen.
a5, whereas no binding to importins a1, a3, or a7 in IFN-α-stimulated cells was detected. Clearly, binding of STAT1-STAT2 dimers to importin a5 is highly specific.

Tyrosine-phosphorylated STAT Dimers, Influenza A NP, and SV40 Large T Antigen Bind to Different Importin α Isoforms—To characterize further STAT binding to different importin α isoforms, we used a baculovirus expression system to reconstitute STAT activation (26). Coinfection of S9/9 cells with recombinant Tyk2 and STAT protein expressing baculoviruses resulted in an efficient expression and tyrosine phosphorylation of STAT1 and STAT2 proteins. Tyrosine-phosphorylated STAT1 and STAT2 formed dimers, which enabled us to analyze the potential interactions of dimeric STAT complexes with importins (25, 26). As shown in Fig. 3, baculovirus-expressed STAT1 homodimers or STAT1-STAT2 heterodimers bound specifically to Sepharose-immobilized GST-importin a5 but not to importin a1, a3, or a7 isoforms. These results, together with those shown in Fig. 2 using natural IFN-α-stimulated cells, show that the reconstituted STAT activation in the baculovirus expression system is a reliable method to study STAT-importin α interactions.

We also analyzed interactions of Sepharose-immobilized GST-importins a1, a3, a5, or a7 with baculovirus-expressed influenza A NP and SV40 large T antigen. Two NLSs have been identified in influenza A virus NP, a nonclassical NLS at the N terminus (34) and a classical bipartite NLS in the middle of the molecule (35). It has been shown that the N-terminal NLS of NP is involved in the interaction with importins (34, 36). Here we show that influenza A virus NP bound strongly to importins a1 and a5 and to a lesser extent to importin a3, whereas no marked binding to importin a7 isomorph was detected (Fig. 3C).

Importins a1 and a5 have been shown to bind efficiently SV40 large T antigen NLS peptide or T antigen NLS conjugated to BSA (24, 43–44). Instead, our binding experiments of SV40 large T antigen with Sepharose-immobilized GST-importins revealed that full-length T antigen bound strongly to importin a3 and to a lesser extent to importin a1 but not to importin a5 or a7 isoforms (Fig. 3D), indicating that the natural context of an NLS can greatly affect its accessibility to importins.

A C-terminal Unique NLS Binding Site, Including Arm Repeats 8 and 9 of Importin α5, Binds STATs—Because STAT1-STAT2 dimers to importin a5 is highly specific.

Fig. 3. A space-filling representation of an importin molecule (Protein Data Bank accession number 1bkb). The representation shows the positions of amino acids that were mutated in importin a5 N-terminal (arm repeats 2–4) or C-terminal (arm repeats 7–9) NLS binding domains. Tryptophans are shown in red (also asparagine 445 in arm repeat 9) and asparagines in purple. Two additional amino acids mutated in arm repeats 2 or 7 are shown in green.

Table 1. Neutralizing Mutations in Arm Repeat 9 of Importin α5

**Discussion**

Importin α molecules play a central role in the classical nuclear import pathway. In this study we investigated the motives by which importin a5 recognizes the nonclassical STAT NLS. The major NLS binding site, comprising arm repeats 2–4 of importin α molecules, is known to bind classical basic type NLS motifs. It has been suggested previously that the importin α5-binding site for STAT1 NLS is distinct from the classical NLS binding site and is located in the C terminus (24). However, the exact binding site was not determined, and it was suggested that a new mechanism, not involving any arm repeats, was in use. Here we show by site-directed mutagenesis that the C-terminal arm repeats 8 and 9 mediate the binding of STAT1 homodimers and STAT2 heterodimers to importin a5.
Also the nonclassical NLS of influenza A NP is recognized by the C-terminal arm repeats of importin α5. We also show that importin α3 uses its N- and C-terminal binding sites for binding various NLS-containing molecules.

Crystallographic analyses of importins bound to NLS peptides show that also the C-terminal minor binding site of importin α, comprising arm repeats 7 and 8, is able to bind NLS peptides. The major NLS binding site of yeast importin α molecule recognizes the classical monopartite NLS peptide (SV40 large T antigen NLS, PKKPRK) and the larger downstream basic cluster of the classical bipartite NLS peptide (nucleoplasmin NLS, KPKAAATKKGQAKKKKLD). The minor NLS binding site has a supportive role in binding the smaller upstream basic cluster of the bipartite NLS (10, 37). Mouse importin α showed a similar binding mode for the bipartite nucleoplasmin NLS as yeast importin α, but SV40 large T antigen NLS peptide was bound almost equally by the major and minor binding sites (11). Also c-myc NLS peptide (PAAKRVKLD) binds both to major and minor binding sites of yeast importin α (37).

The C-terminal half of importin α has been suggested to be involved in NLS binding of several proteins. By using deletion analyses it has been shown that the NLSs of a dimeric DNA-binding protein nuclear antigen 1 of Epstein-Barr virus (KKPKRSPSS) and the matrix protein of human immunodeficiency virus (KKKYKL) are bound by the C-terminal half of importin α (45, 46). The NLS of a human transcription factor
monomer, and that both of these elements are required for nuclear import is an extraordinary feature (25, 27). One STAT dimer has been shown to bind to two importin α molecules (28). Our hypothesis is that one importin α5 molecule is bound to each of the STAT molecules in the dimer, and additional interactions between the two importins or importins and STATs are needed to stabilize the import complex. It has also been shown that large cargoes need more than one importin molecule for a rapid nuclear import to take place (48). A similar dimeric basic type NLS has also been found in immediate early protein (IE1) of baculovirus Autographa california (49).

It has been shown previously that importin α5 is necessary for nuclear translocation of phosphorylated STAT, whereas importin α1 is not (24). Our data provide further evidence that importin α5 is the only isoform mediating nuclear import of STAT1 homodimers, because isoforms α1, α3, and α7 did not bind STAT1. Influenza A NP, on the other hand, is bound by importins α1, α3, and α5. Importins α1 and α5 are known to bind to the N-terminal nonclassical NLS of influenza A NP (34, 36). In the present study we show that importin α5 uses its C-terminal arm repeats 7–9 for the binding of NP NLS. Although importin α5 binds by its C-terminal arm repeats both STATs and influenza A NP, the binding sites are not identical. STATs need only arm repeats 8 and 9 for binding, whereas influenza A NP-importin α5 interaction requires arm repeats 7–9.

In the present study, we show that importin α3 is able to use both N- and C-terminal-binding sites for binding different nucleocytoplasmic targets. Previous experiments showed that SV40 large T antigen NLS peptides and T antigen NLS coupled to BSA bound to the N-terminal major binding site of importins α1 and α5 (24). Our studies show that the full-length SV40 large T antigen is bound to the N-terminal major binding site and influenza A virus NP, on the other hand, to the C-terminal binding site of importin α3.

Our results suggest that the location and surroundings play an important role in determining the accessibility of an NLS to importins. We show here that the full-length SV40 large T antigen was recognized by importin α3 and weakly by importin α1. However, as a peptide or as a peptide conjugated to a protein not naturally transported into the nucleus (e.g. BSA), T antigen NLS has been shown to bind to importins α1, α5, and weakly to α3 (24, 43, 44). The accessibility of an NLS can apparently be regulated by phosphorylation or structural masking (50). SV40 large T antigen is known to have a phosphorylation site affecting nuclear transport (51).

Acknowledgments—We thank Dr. T. Hovi for critically reading the manuscript and Drs. S. Pellegrini, M. Malin, P. Palese, and J. M. Pipas for Tyk2, GST-importin α5/NTF-1, GST-importin β, and SV40 large T antigen gene constructs, respectively. We also thank T. Westerlund for excellent technical assistance.

REFERENCES
1. Görlich, D., and Katay, U. (1999) Annu. Rev. Cell Dev. Biol. 15, 607–660
2. Macara, I. G. (2001) Microbiol. Mol. Biol. 65, 570–594
3. Weiss, K. (2003) Cell 112, 441–451
4. Cosmo, C. A., Kirch, S. A., Gyuris, J., Brent, R., and Oettinger, M. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6106–6106
5. Kohler, M., Aniseau, S., Prehn, S., Leuta, A., Haller, H., and Hartmann, E. (1997) FEBS Lett. 417, 104–108
6. Cortes, P., Ye, Z. S., and Baltimore, D. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 7633–7637
7. Nachury, M. V., Ryder, U. W., Lamond, A. I., and Weis, K. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 582–587
8. Seki, T., Tada, S., Katada, T., and Enomoto, T. (1997) Biochem. Biophys. Res. Commun. 234, 48–53
9. Forster, M., Speck, C., Christiansen, M., Bischoff, F. R., Prehn, S., Haller, H., Görlich, D., and Hartmann, E. (1999) Mol. Cell. Biol. 19, 7782–7791
10. Conti, E., Uy, M., Leighton, L., Blebel, G., and Kuriyan, J. (1998) Cell 94, 193–204
11. Fontes, R. M., Teh, T., and Kohé, B. (2000) J. Mol. Biol. 297, 1183–1194
12. Görlich, D., Henklein, P., Laskey, R. A., and Hartmann, E. (1996) EMBO J. 15, 1810–1817

Fig. 6. Binding of influenza A virus NP and SV40 large T antigen to wild type and arm repeat 3 and 8 mutant importin α3 proteins. A, the control Sf9 cell extract or influenza A virus NP-expressing Sf9 cell extracts were allowed to bind to Sepharose-immobilized wt or arm repeat 3 or 8 mutants of GST-importin α3 at 4 °C for 2 h. The binding experiment was carried out as described in the legend for Fig. 2. Importin α3-bound proteins were separated on 8% SDS-PAGE, transferred to Immobilon filters, and stained for NP. A similar gel was also stained with Coomassie Brilliant Blue to visualize the amount of Sepharose-immobilized GST-importin α3 molecules. Coomassie Brilliant Blue-stained NP is marked with dots. B, the control Sf9 cell extract or baculovirus-infected Sf9 cell extracts containing SV40 large T antigen (SV40 T Ag) were allowed to bind to wt or mutant importin α3 molecules as described above, and the proteins bound to beads were processed for Western blotting and stained for T antigen. Coomassie Brilliant Blue-stained T antigen is marked with dots.
Interaction of STATs with Importin α5

13. Weis, K., Ryder, U., and Lamond, A. I. (1996) EMBO J. 15, 7120–7128
14. Kobe, B. (1999) Nat. Struct. Biol. 6, 388–397
15. Harreman, M. T., Hodet, M. R., Fanara, P., Hodel, A. E., and Corbett, A. H. (2003) J. Biol. Chem. 278, 5854–5863
16. Herold, A., Truant, R., Wegand, H., and Cullen, B. R. (1998) J. Cell Biol. 143, 309–318
17. Morosino, J., Blobel, G., and Radu, A. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 7059–7062
18. Dingwall, C., and Laskey, R. A. (1991) Trends Biochem. Sci. 16, 478–481
19. Kalderon, D., Roberts, B. L., Richardson, W. D., and Smith, A. E. (1984) Cell 39, 499–509
20. Lanford, R. E., and Butel, J. S. (1984) Cell 37, 801–813
21. Levy, D. E., and Darnell, J. E., Jr. (2002) Nat. Rev. Mol. Cell. Biol. 3, 651–662
22. McBride, K. M., Banninger, G., McDonald, C., and Reich, N. C. (2002) EMBO J. 21, 1754–1763
23. Strehlow, I., and Schindler, C. (1998) J. Biol. Chem. 273, 28049–28056
24. Summers, M. D., and Smith, G. E. (1986) Tex. Agric. Exp. Stn. Bull. 1555, 1–57
25. Subramaniam, P. S., Larkin, J., III, Mujtaba, M. G., Walter, M. R., and Johnson, H. M. (2000) J. Cell Sci. 113, 2721–2728
26. Bild, A. H., Turkson, J., and Jove, R. (2002) EMBO J. 21, 3255–3263
27. O'Neill, R. E., and Palese, P. (1995) Virology 206, 116–125
Importin α Nuclear Localization Signal Binding Sites for STAT1, STAT2, and Influenza A Virus Nucleoprotein
Krister Melén, Riku Fagerlund, Jacqueline Franke, Matthias Köhler, Leena Kinnunen and Ilkka Julkunen

J. Biol. Chem. 2003, 278:28193-28200.
doi: 10.1074/jbc.M303571200 originally published online May 9, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303571200

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 52 references, 27 of which can be accessed free at http://www.jbc.org/content/278/30/28193.full.html#ref-list-1