Apoptosis Induced by Withdrawal of Trophic Factors Is Mediated by p38 Mitogen-activated Protein Kinase*

(Received for publication, February 4, 1997, and in revised form, April 28, 1997)

Jennifer L. Kummer, Pravin K. Rao, and Kim A. Heidenreich‡

From the Department of Pharmacology, University of Colorado Health Sciences Center, Denver, Colorado 80262 and Denver Veteran Affairs Medical Center, Denver, Colorado 80220

p38 is a member of the mitogen-activated protein (MAP) kinase superfamily activated by stress signals and implicated in cellular processes involving inflammation and apoptosis. Unlike the extracellular signal-regulated kinases (ERKs), the Jun N-terminal kinases (JNKs), and the p38 MAP kinases, ERK1 and ERK2 are regulated by mitogens and growth factors via a Ras-dependent pathway involving sequential activation of a MAP kinase kinase kinase and a MAP kinase kinase (MEK) (4). JNKs are activated by proinflammatory cytokines, hyperosmolarity, heat shock, etodotoxin, and other cellular stresses (5–8). The activation cascades for JNK and p38 appear to be distinct. The MAP kinase kinase MEK4 activates JNK but not p38 in vivo (9), whereas MEK3 and MEK6 activate p38 and not JNK (10, 11).

The JNK and p38 kinases are activated by proinflammatory cytokines, hyperosmolarity, heat shock, etodotoxin, and other cellular stresses (5–8). The activation cascades for JNK and p38 appear to be distinct. The MAP kinase kinase MEK4 activates JNK but not p38 in vivo (9), whereas MEK3 and MEK6 activate p38 and not JNK (10, 11). p38 phosphorylates and activates the transcription factors, ATF-2 and MEF2C (11, 12), indicating a role for p38 in transcriptional regulation. p38 activates MAP-activated protein kinase 2, which, in turn, phosphorylates the small heat shock protein 27 (8). Other investigators have provided evidence that p38 regulates cytokine production (13), platelet aggregation (14), and neuronal apoptosis (15, 16). p38 was identified as the target for a group of pyridinyl imidazole compounds that block the production of interleukin-1 and tumor necrosis factor-α from monocytes stimulated with endotoxin (17). These compounds, such as SB202190, fail to block the activity of ERK1/ERK2, JNK, and a number of other protein kinases (17–19). A recent paper describing the crystal structure of p38 in complex with a pyridinyl imidazole inhibitor describes the structural basis for the specificity of these compounds (19).

Our laboratory recently reported that insulin inhibits the activity and tyrosine phosphorylation state of p38 MAP kinase in primary neuronal cultures for which insulin is a potent survival factor (20). These data taken together with recent findings by Xia et al. (15). and Ichijo et al. (16) suggested that insulin promotes neuronal survival by inhibiting an apoptotic pathway regulated by p38 MAP kinase. Here we report that inhibition of p38 MAP kinase by insulin or by a pyridinyl imidazole inhibitor blocks apoptosis induced by trophic factor withdrawal in a non-neuronal and neuronal cell line. These data provide direct evidence for a key role of p38 MAP kinase in cellular apoptosis.

**Experimental Procedures**

Materials—Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of Lilly. Rat-1 fibroblasts were obtained from Dr. Jerrold Olefsky (UCSD, San Diego, CA), and rat pheochromocytoma (PC12) cells were obtained from Dr. Gary Johnson (National Jewish Hospital, Denver, CO). Tissue culture media, fetal calf serum (FCS), and nerve growth factor (NGF, 2.5 S) were obtained from Life Technologies, Grand Island, NY. A number of other compounds were obtained from Calbiochem (San Diego, CA) and Sigma Chemical Co. (St. Louis, MO). PD169316 is a pyridinyl imidazole compound (6) developed by Dr. Jennifer Kummer at the University of Colorado Health Sciences Center. p38 is a member of the mitogen-activated protein (MAP) kinase superfamily activated by stress signals and implicated in cellular processes involving inflammation and apoptosis. Unlike the extracellular signal-regulated kinases (ERKs), the Jun N-terminal kinases (JNKs), and the p38 MAP kinases, ERK1 and ERK2 are regulated by mitogens and growth factors via a Ras-dependent pathway involving sequential activation of a MAP kinase kinase kinase and a MAP kinase kinase (MEK) (4). JNKs are activated by proinflammatory cytokines, hyperosmolarity, heat shock, etodotoxin, and other cellular stresses (5–8). The activation cascades for JNK and p38 appear to be distinct. The MAP kinase kinase MEK4 activates JNK but not p38 in vivo (9), whereas MEK3 and MEK6 activate p38 and not JNK (10, 11).

p38 phosphorylates and activates the transcription factors, ATF-2 and MEF2C (11, 12), indicating a role for p38 in transcriptional regulation. p38 activates MAP-activated protein kinase 2, which, in turn, phosphorylates the small heat shock protein 27 (8). Other investigators have provided evidence that p38 regulates cytokine production (13), platelet aggregation (14), and neuronal apoptosis (15, 16). p38 was identified as the target for a group of pyridinyl imidazole compounds that block the production of interleukin-1 and tumor necrosis factor-α from monocytes stimulated with endotoxin (17). These compounds, such as SB202190, fail to block the activity of ERK1/ERK2, JNK, and a number of other protein kinases (17–19). A recent paper describing the crystal structure of p38 in complex with a pyridinyl imidazole inhibitor describes the structural basis for the specificity of these compounds (19).

Our laboratory recently reported that insulin inhibits the activity and tyrosine phosphorylation state of p38 MAP kinase in primary neuronal cultures for which insulin is a potent survival factor (20). These data taken together with recent findings by Xia et al. (15). and Ichijo et al. (16) suggested that insulin promotes neuronal survival by inhibiting an apoptotic pathway regulated by p38 MAP kinase. Here we report that inhibition of p38 MAP kinase by insulin or by a pyridinyl imidazole inhibitor blocks apoptosis induced by trophic factor withdrawal in a non-neuronal and neuronal cell line. These data provide direct evidence for a key role of p38 MAP kinase in cellular apoptosis.

**Experimental Procedures**

**Materials**—Porcine monocomponent insulin was generously supplied by Dr. Ronald Chance of Lilly. Rat-1 fibroblasts were obtained from Dr. Jerrold Olefsky (UCSD, San Diego, CA), and rat pheochromocytoma (PC12) cells were obtained from Dr. Gary Johnson (National Jewish Hospital, Denver, CO). Tissue culture media, fetal calf serum (FCS), and nerve growth factor (NGF, 2.5 S) were obtained from Life Technologies, Grand Island, NY. A number of other compounds were obtained from Calbiochem (San Diego, CA) and Sigma Chemical Co. (St. Louis, MO). PD169316 is a pyridinyl imidazole compound (6) developed by Dr. Jennifer Kummer at the University of Colorado Health Sciences Center.

**Mammalian MAP**1 kinases are serine/threonine kinases that

---

*This work was supported by a Veterans Affairs Merit Review Grant. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed: Dept. of Pharmacology C-236, University of Colorado Health Science Center, 4200 East Ninth Ave., Denver, CO 80262. Tel.: 303-392-4820 (ext. 3891); Fax: 303-377-5686; E-mail: Kim.Heidenreich@UCHSC.edu.

1The abbreviations used are: MAP, mitogen-activated protein; ERK, extracellular signal-regulated kinase; JNK, Jun N-terminal kinase; MEK, MAP kinase kinase; FCS, fetal calf serum; NGF, nerve growth factor; PBS, phosphate-buffered saline; IGF, insulin-like growth factor.
p38 MAP Kinase and Apoptosis

Sixteen hours after the removal of serum from Rat-1 fibroblasts or NGF from differentiated PC12 cells, the cells were incubated in the absence or presence of insulin (50 ng/ml) for 15 min at 37 °C. After washing with 2 ml of ice-cold PBS, the cells were solubilized in 400 μl of ice-cold immunoprecipitation buffer containing 10 mM Tris·HCl, pH 7.4, 1% Triton X-100, 0.5% Nonidet P-40, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 0.2 mM sodium orthovanadate, and 0.2 mM phenylmethylsulfonyl fluoride. The cell lysates were centrifuged to remove insoluble material, and 200 μg of the supernatant protein (400 μl total volume) were incubated with 1 μg of anti-p38 antibodies for 1 h at 4 °C followed by incubation with 30 μl of Protein G Plus/Protein A-agarose for an additional hour. The immunocomplexes were pelleted and washed twice in immunoprecipitation buffer and then once in kinase wash buffer (50 mM β-glycerophosphate, 1 mM EDTA, 20 mM MgCl₂, 100 mM sodium orthovanadate). The protein kinase assay was initiated by the addition of 20 μl of 2X reaction buffer (50 mM β-glycerophosphate, 1 mM EGTA, 20 mM MgCl₂, 100 mM sodium orthovanadate, 0.1 mg/ml ATP-2 (N-terminal half), 50 μg/ml IP20, a peptide inhibitor of c-AMP dependent protein kinase, 200 μM ATP, and 0.9 mM CaCl₂ (10 mM PIP2)) to 20 μl of immune complex. The reaction was allowed to proceed for 10 min at 30 °C and then terminated by the addition of 2X Laemmli sample buffer and analyzed by SDS-polyacrylamide gel electrophoresis using 12% acrylamide gels. After electrophoresis, the gels were dried and subjected to phosphoimaging (Bio-Rad, GS-100).

Mono Q Chromatography—After treating the cells with or without insulin and various inhibitors, the cells were rinsed with 2 ml of PBS, pH 7.4, and solubilized in lysis buffer (pH 7.2) containing 50 mM β-glycerophosphate, 5 mM EGTA, 2 mM EDTA, 1 mM dithiothreitol, 2 mM sodium orthovanadate, 10 μM leupeptin, 5 μM aprotinin, and 1 mM phenylmethylsulfonyl fluoride. Cell extracts (3–5 mg of protein) were applied at a flow rate of 0.8 ml/min to a Mono Q column (Pharmacia FPLC HR5/5) equilibrated with Buffer A (50 mM β-glycerophosphate, 5 mM EGTA, 2 mM EDTA, 1 mM dithiothreitol, 2 mM sodium orthovanadate, pH 7.2). The column was developed at the same rate with a linear NaCl gradient (0–800 mM) in Buffer A. Fractions (200 μl) were collected and assayed for myelin basic protein phosphorytansferase activity as described previously (20).

Quantification of Apoptosis—Apoptosis was routinely measured by counting the number of cells with condensed or fragmented chromatin as described previously (22). Briefly, the cells were washed in PBS and then fixed in 1% paraformaldehyde for 2 min at room temperature followed by 70% ethanol in glycerine buffer for 10 min at −20 °C. Following fixation, the cells were rinsed 3 times in PBS and then incubated with the 33258 Hoechst stain (8 μg/ml) for 15 min at room temperature. The cells were rinsed 3 times in PBS and viewed under a fluorescent microscope. The amount of apoptosis was expressed as the percentage of the total number of attached cells that showed condensed chromatin in eight randomly chosen fields. Typically, at least 1000 cells per condition were counted. In initial experiments the number of cells that showed condensed chromatin was compared with the number of cells that stained for DNA fragmentation using the Tunel method (Apotag).

The values were found to be identical, although the Apotag method had higher backgrounds.

RESULTS AND DISCUSSION

To determine if insulin inhibits p38 MAP kinase activity in cells other than primary neurons and to ascertain the involve-
with previous data by Xia et al. (15). As was seen for Rat-1 cells, insulin markedly prevented apoptosis in PC12 cells. Thus, under conditions where apoptosis was very low, i.e. either in the presence of FCS or in the presence of NGF, p38 MAP kinase activity was barely detectable. When FCS or NGF was withdrawn, apoptosis increased and p38 MAP kinase activity was elevated. Insulin blocked apoptosis induced by trophic factor withdrawal in both Rat-1 fibroblasts and PC12 cells and inhibited p38 activity in the same cultures.

At the low concentrations of insulin used in these studies, the effect of insulin in preventing apoptosis is likely mediated by the insulin receptors on these cell lines, although we cannot entirely rule out the involvement of IGF-I receptors (24, 25). Although early studies suggested that insulin’s effects on cell survival were mediated by the IGF-I receptor, it is now generally believed that the insulin receptor itself can mediate growth effects in a number of different cell types. This is particularly evident in the nervous system, where insulin receptors lack acute metabolic functions but have potent survival and growth effects (26).

To better understand the relationship of p38 MAP kinase activity and apoptosis, we examined the effect of a pyridyl imidazole inhibitor of p38 MAP kinase inhibitor on apoptosis induced by serum withdrawal in fibroblasts or by NGF withdrawal in PC12 cells. The p38 MAP kinase inhibitor used for these studies, PD169316, is a member of the class of pyridyl imidazole compounds such as SB202190 and SB203580, which are selective inhibitors of p38 MAP kinase (17–19). To confirm the in vivo specificity of the p38 inhibitor in our studies, PC12 cells were treated with or without the inhibitor (10 μM) at the time of NGF withdrawal. After 16 h, the cells were solubilized, and cell lysates were fractionated by ion exchange chromatography. The cell fractions were then assayed for MAP kinase activity using myelin basic protein (MBP) as the substrate.

Experiments were then carried out to examine the effect of the p38 inhibitor on apoptosis in PC12 cells (Fig. 4A) and Rat-1 fibroblasts (Fig. 4B). As previously observed, withdrawal of NGF from differentiated PC12 cells increased the number of apoptotic PC12 cells by about 13-fold. Treatment of PC12 cells with a maximally effective concentration of PD169316 (10 μM) at the time of serum withdrawal blocked apoptosis induced by serum withdrawal by 83%. Its effect on blocking apoptosis was similar to the effects of insulin. Under these conditions where maximal concentrations of insulin and PD169316 were used, there was no additivity between the two compounds. In contrast to the p38 MAP kinase inhibitor, the MEK inhibitor, an upstream inhibitor of ERK, only partially blocked apoptosis by 38%. Interestingly, the MEK inhibitor did not influence insulin’s ability to inhibit apoptosis, indicating that the ERK pathway is not necessary for the inhibition of apoptosis by insulin. Thus, under these conditions, blockade of p38 MAP kinase alone prevents apoptosis induced by NGF withdrawal. In Rat-1 fibroblasts (Fig. 4B) a similar picture was observed. Withdrawal of serum induced apoptosis and this increase in apoptosis was attenuated 70% by the p38 inhibitor and 85% by insulin. The MEK inhibitor did not block apoptosis, unlike the

**Fig. 2.** Insulin blocks apoptosis induced by trophic factor withdrawal in Rat-1 fibroblasts and differentiated PC12 cells. Apoptosis was determined by counting the number of cells with condensed and/or fragmented chromatin after staining the cells with the DNA stain 33258 Hoescht. At least 1000 cells per condition were counted. Data represent the mean ± S.E.M. of three experiments.

**Fig. 3.** Mono Q chromatography of differentiated PC12 cell lysates. Cell extracts from differentiated PC12 cells in the presence of NGF (○), after 16 h of NGF withdrawal (●), and after NGF withdrawal in the presence of 10 μg/ml p38 inhibitor circles (□) were applied to a Mono Q column and developed with a linear NaCl gradient (0–800 mM). Fractions were assayed for MAP kinase activity using myelin basic protein (MBP) as the substrate.
Figure 4. PD169316 blocks apoptosis induced by trophic factor withdrawal in differentiated PC12 cells (A) and Rat-1 fibroblasts (B). The effect of various inhibitors on apoptosis was measured by adding the inhibitor to the cells at the time of trophic factor withdrawal. Apoptosis was determined by counting the number of cells with condensed and/or fragmented chromatin after staining the cells with the DNA stain 33258 Hoescht. At least 1000 cells per condition were counted. Data in A represent the mean ± S.E.M. of three experiments. Data in B are the average of two experiments. SS, serum-starved.

Table 1

| Treatment          | Amount of Apoptosis (%) of Attached Cells |
|--------------------|------------------------------------------|
| Control            | 0.0 ± 0.1                                |
| PD169316 + NGF      | 14.0 ± 1.2                               |
| PD169316 + Insulin  | 12.0 ± 0.8                               |
| PD169316 + Insulin + PD169316 | 0.0 ± 0.1 |

These experiments provide direct evidence that p38 MAP kinase plays a critical role in cellular apoptosis in both a non-neuronal transformed cell line and a more differentiated neuronal cell line. When trophic factors are removed, apoptosis increases and p38 activity is elevated in both cell lines. Selective blockade of p38 MAP kinase in both types of cells prevents apoptosis. These data complement previous findings by Xia et al. (15) that overexpression of constitutively active MEK3, an upstream regulator of p38 MAP kinase, promotes apoptosis, whereas overexpression of a dominant-negative MEK3 prevents apoptosis induced by NGF withdrawal in PC12 cells. Furthermore, at the time of preparing this paper, it was reported that ASK1, a mammalian MEK kinase that activates JNK and p38 signaling pathways, induces apoptosis in lung epithelial cell lines (16). An important distinction between the two previous reports and the present study is that the previous reports could not distinguish between JNK effects and p38 effects because overexpression of the upstream regulators blocks both pathways, whereas the inhibitor used in our studies is specific for p38. p38 has also been implicated in Fas-induced apoptosis in Jurkat cells (27). Fas activation of p38 required the action of interleukin-converting enzyme-like pro- teases, whereas the activation of p38 by sorbitol or etoposide did not (27), suggesting that there are multiple pathways for activating p38 by different apoptotic stimuli.

The data are less straightforward concerning the role of ERK in neuronal survival. In the studies by Xia et al. (15), overexpression of a constitutively active MEK1, the upstream regulator of ERK, prevented apoptosis in PC12 cells induced by NGF withdrawal. This led the authors to suggest that cell fate is controlled by the balance of activity in the ERK pathway, which promotes proliferation, and the stress pathways that activate programmed cell death. The current study suggests that blockade of the p38 pathway alone is sufficient to prevent apoptosis.

Regarding insulin’s ability to support cell survival, one could propose that the major way insulin supports cell survival is to inhibit apoptosis by negatively regulating p38 MAP kinase. ERK does not appear to play a major role in neuronal survival in response to insulin. Previous studies have shown that ERK is not stimulated by insulin in primary neuronal cultures (20), and the present study shows that blockade of the ERK pathway does not influence insulin’s ability to attenuate apoptosis. The latter data are analogous to recent findings by Creedon et al. (28) showing that the ERK activation is not required for the actions of cAMP or NGF on neuronal survival. However, it remains possible that in addition to inhibiting an apoptotic pathway, insulin stimulates a survival pathway. Recent studies by Greenberg et al. (29) indicate that insulin and IGFs support survival of primary cerebellar neurons by activation of a serine-threonine protein kinase Akt (also known as PKB-α or RAC-α). Akt is a widely expressed kinase that appears to be activated by a phosphatidylinositol 3-kinase-dependent mechanism. It is likely that the sum of the activities of multiple signaling pathways is critical for determining cell fate and that the contribution of one pathway versus another is cell-specific.

Acknowledgments—We thank Dr. Lynn Heasley for the recombinant glutathione S-transferase-ATF-2 and Dr. Alan Saltiel for the MEK and p38 inhibitors.

REFERENCES

1. Cobb, M. H., and Goldsmith, E. H. (1995) J. Biol. Chem. 270, 14833–14836
2. Cano, E., and Mahadevan, L. C. (1995) Trends Biochem. Sci. 20, 117–122
3. Kyriakis, J. M., and Avruch, J. (1996) J. Biol. Chem. 271, 24313–24316
4. Egan, S. E., and Weinberg, R. A. (1993) Nature 365, 781–783
5. Kyriakis, J. M., Banerjee, P., Nikolakaki, E., Dax, P., Rouhi, F. A., Ahmad, M. F., Avruch, J., and Woodgett, J. R. (1994) Nature 369, 156–160
6. Derijard, B., Hibi, M., Wu, I.-H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. (1994) Cell 76, 1025–1037
7. Han, J., Lee, J.-D., Bibbs, L., and Ulevitch, R. J. (1994) Science 265, 808–811
8. Roux, J., Cohen, P., Trigon, S., Morange, M., Alonso-Llamas, A., Zamanillo, D., Hunt, T., and Nehedra, A. R. (1994) Cell 76, 1027–1037
9. Derijard, B., Raingeaud, J., Barrett, T., Wu, I.-H., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) Science 267, 682–685
10. Han, J., Lee, J.-D., Jiang, Y., Li, Z., Feng, L., and Ulevitch, R. J. (1996) J. Biol. Chem. 271, 2886–2891
11. Raingeaud, J., Whitmarsh, A. J., Barrett, T., Derijard, B., and Davis, R. J.
12. Han, J., Jiang, Y., Li, Z., Kravchenko, V. V., and Ulevitch, R. J. (1997) *Nature* 386, 296–299
13. Shapiro, L., and Dinarello, C. A. (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 12230–12234
14. Kramer, R. M., Roberts, E. F., Strifler, B. A., and Johnstone, E. M. (1995) *J. Biol. Chem.* 270, 27395–27398
15. Xia, Z., Dickens, M., Raingeaud, J., Davis, R. J., and Greenberg, M. E. (1995) *Science* 270, 1326–1331
16. Ichijo, H., Nishida, E., Irie, K., ten Dyke, P., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K., and Gotoh, Y. (1997) *Science* 275, 90–94
17. Lee, J. C., Laydon, J. T., McDonell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumersthal, M., Heys, J. R., Landvatter, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L., and Young, P. R. (1994) *Nature* 372, 739–746
18. Cuenda, A., Rouse, J., Doza, Y. N., Meier, R., Cohern, P., Gallagher, T. F., Young, P. R., and Lee, J. C. (1995) *FEBS Lett.* 364, 229–233
19. Tong, L., Pav, S., White, D. M., Rogers, S., Crane, K. M., Cywin, C. L., Brown, M. L., and Pargellis, C. A. (1997) *Nat. Struct. Biol.* 4, 311–316
20. Heidenreich, K. A., and Kummer, J. L. (1996) *J. Biol. Chem.* 271, 9891–9894
21. Green, L. A., and Tischler, A. S. (1976) *Proc. Natl. Acad. Sci. U. S. A.* 73, 2424–2428
22. Zawada, W. M., Kirschman, D. L., Cohen, J. J., Heidenreich, K. A., and Freed, C. R. (1996) *Experimental Neurology* 140, 60–67
23. Sasaoka, T., Draznin, B., Leitner, W. J., and Olefsky, J. M. (1994) *J. Biol. Chem.* 269, 10734–10738
24. McClain, D. A., Maegawa, H., Lee, J., Dull, T. J., Ulrich, A., and Olefsky, J. M. (1987) *J. Biol. Chem.* 262, 14663–14671
25. Ohmichi, M., Pang, L., Ribon, V., and Saltiel, A. R. (1993) *Endocrinology* 133, 46–56
26. Heidenreich, K. A., de Vellis, G., and Gilmore, P. R. (1988) *J. Neurochem.* 51, 878–887
27. Juo, P., Kuo, C. J., Reynolds, S. E., Konz, R. F., Raingeaud, J., Davis, R. J., Biemann, H-P., and Blenis, J. (1997) *Mol. Cell. Biol.* 17, 24–35
28. Creedon, D. J., Johnson, E. M., and Lawrence, J. C. (1996) *J. Biol. Chem.* 271, 20713–20718
29. Dudek, H., Datta, S. R., Franke, T. F., Birnbaum, M. J., Yao, R., Cooper, G. M., Segal, R. A., Kaplan, D. R., and Greenberg, M. E. (1997) *Science* 275, 661–668

**p38 MAP Kinase and Apoptosis**
Apoptosis Induced by Withdrawal of Trophic Factors Is Mediated by p38 Mitogen-activated Protein Kinase
Jennifer L. Kummer, Pravin K. Rao and Kim A. Heidenreich

J. Biol. Chem. 1997, 272:20490-20494.
doi: 10.1074/jbc.272.33.20490

Access the most updated version of this article at http://www.jbc.org/content/272/33/20490

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 29 references, 17 of which can be accessed free at http://www.jbc.org/content/272/33/20490.full.html#ref-list-1