Hypoxia-inducible Factor 1α (HIF-1α) Protein Is Rapidly Degraded by the Ubiquitin-Proteasome System under Normoxic Conditions

ITS STABILIZATION BY HYPOXIA DEPENDS ON REDOX-INDUCED CHANGES*

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The hypoxia-inducible factor 1 transcriptional activator complex (HIF-1) is involved in the activation of the erythropoietin and several other hypoxia-responsive genes. The HIF-1 complex is composed of two protein subunits: HIF-1α/ARNT (aryl hydrocarbon receptor nuclear translocator), which is constitutively expressed, and HIF-1α, which is not present in normal cells but induced under hypoxic conditions. The HIF-1α subunit is continuously synthesized and degraded under normoxic conditions, while it accumulates rapidly following exposure to low oxygen tensions. The involvement of the ubiquitin-proteasome system in the proteolytic destruction of HIF-1 in normoxia was studied by the use of specific inhibitors of the proteasome system. Lactacystin and MG-132 were found to protect the degradation of the HIF-1 complex in cells transferred from hypoxia to normoxia. The same inhibitors were able to induce HIF-1 complex formation when added to normoxic cells. Final confirmation of the involvement of the ubiquitin-proteasome system in the regulated degradation of HIF-1α was obtained by the use of ts20TG R cells, which contain a temperature-sensitive mutant of E1, the ubiquitin-activating enzyme. Exposure of ts20 cells, under normoxic conditions, to the non-permissive temperature induced a rapid and progressive accumulation of HIF-1. The effect of proteasome inhibitors on the normoxic induction of HIF-1 binding activity was mimicked by the thiol reducing agent N-(2-mercaptopropionyl)-glycine and by the oxygen radical scavenger 2-acetamidoacrylic acid. Furthermore, N-(2-mercaptopropionyl)-glycine induced gene expression as measured by the stimulation of a HIF-1-luciferase expression vector and by the induction of erythropoietin mRNA in normoxic Hep 3B cells. These last findings strongly suggest that the hypoxia induced changes in HIF-1α stability and subsequent gene activation are mediated by redox-induced changes.

Mammalian cells are able to sense oxygen tension and turn on a series of genes in response to the lack of oxygen. The best characterized of these hypoxia-regulated genes is the one coding for erythropoietin (Epo), the growth factor that regulates red cell production (reviewed in Ref. 1). The hypoxia response of the Epo gene is controlled by an enhancer element located in the 3'-flanking region of the gene (2–4). Transcriptional activation of the enhancer is mediated by a hypoxia-inducible DNA-binding protein complex termed HIF-1, which binds to the site-1 sequences of the enhancer (5, 6). Similar enhancer elements, also involving the binding of HIF-1, have been identified in other hypoxia-responsive genes, such as those coding for vascular endothelial growth factor (7), glucose transporter-1, and several glycolytic enzymes (8–10). All these genes also respond like Epo, to cobalt ions and iron chelators, suggesting a common mechanism for oxygen sensing and gene activation. The recent cloning of the protein components of the HIF-1 complex identified two subunits, HIF-1α and HIF-1β, which belong to the subfamily of basic helix-loop-helix transcription factors containing a PAS (PER-ARNT-SIM) motif (11). The HIF-1α subunit is a new member of the family, whereas HIF-1β corresponds to the known aryl hydrocarbon receptor nuclear translocator (ARNT) protein.

Hypoxia induces the formation of HIF-1 complex by a process that requires protein synthesis (5). The mechanisms by which cells sense the lack of oxygen and initiate the hypoxic response are currently unknown. However, significant indirect evidence suggests that redox-mediated processes are likely involved in this step. Treatment of cells with hydrogen peroxide greatly reduces HIF-1 formation and Epo mRNA expression in response to hypoxic stimulation (12, 13). Since oxygen radicals and superoxide formation are very dependent on oxygen availability, their reduced formation under hypoxic conditions could serve as the initial signal in oxygen sensing. Of the components of the HIF-1 complex, ARNT protein is constitutively expressed in all cells while HIF-1α is present only in hypoxic cells. Thus, HIF-1 complex formation appears to be determined primarily by the abundance of the HIF-1α subunit. The observation that hypoxia does not modify HIF-1α mRNA levels suggested that HIF-1α protein content is regulated at the level of its mRNA translation or by changes in its rate of degradation (14). Indeed, Huang et al. (15) recently reported that HIF-1α protein is highly unstable under normoxic conditions and that hypoxia significantly prolonged its half-life, thus allowing its accumulation and the formation of the complex. The mechanisms involved in the rapid degradation of HIF-1α under normoxic conditions and the signals involved in the stabilization process by hypoxia, are currently unknown. The results presented here

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1 The abbreviations used are: Epo, erythropoietin; HIF-1, hypoxia-inducible factor 1; ARNT, aryl hydrocarbon receptor nuclear translocator; NMPG, N-(2-mercaptopropionyl)-glycine; PAS, PER-ARNT-SIM; Ac, acetyl; Z, benzoyloxyacarbonyl; E1, Ub-activating enzyme.
indicate that the rapid degradation of HIF-1α under normoxic conditions is mediated by the ubiquitin-proteasome system and its stabilization is probably induced by redox-mediated changes.

MATERIALS AND METHODS

Cell Cultures—Hep 3B and B-1 cells were cultured in minimal essential medium (Life Technologies, Inc., Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT), penicillin (100 units/ml), and streptomycin (100 μg/ml) (Life Technologies, Inc.). Cells were maintained at 37 °C in an atmosphere of 5% CO₂. Hep 3B cells were obtained from the American Tissue Culture Collection. The B-1 cell line is a Hep 3B-derived cell line which was stably transfected with an expression vector containing luciferase cDNA under the control of a minimal Epo promoter (330-base pair SfI/NI-XhoII fragment) and the hypoxia responsive enhancer from the human Epo gene (150-base pair ApaI/PstI fragment). The response of these cells to hypoxia, cobalt, and desferrioxamine has been reported (16). For hypoxic stimulation cell cultures were flushed with a gas mixture containing 0.5% O₂, 5% CO₂ and balanced N₂ as already described (2). The BALB/c 3T3 and HIF-1 Apa human Epo gene (150-base pair these cells to hypoxia, cobalt, and desferrioxamine has been reported

RESULTS

Protease Inhibitors Decrease the Rate of Degradation of the HIF-1 Complex following Discontinuation of a Hypoxic Stimulation—Studies by Wang et al. (11) had shown that following discontinuation of hypoxia there is a rapid decay of HIF-1α protein and HIF-1 complex with a half-life of 5–10 min. To study the role of proteolytic degradation in this rapid decay we utilized a series of protease inhibitors with different enzyme specificities. Nuclear extracts were obtained from Hep 3B cells stimulated with hypoxia (0.5% O₂) for 4 h and then transferred to normoxia for an additional 30 min. Protease inhibitors were added during the last 15 min of the hypoxic period (except for lactacystin that it was added 1 h before ending the hypoxia) and continued while in normoxia. As shown in the gel shift assays in Fig. 1A, transfer from hypoxia to normoxia produced a rapid decay of the HIF-1 complex which was undetectable by the end of the 30 min in normoxia. Addition of the peptides N-Ac-Leu-Leu-norleucinal (calpain inhibitor I) and, to a much lesser extent, N-Ac-Leu-Leu-normethioninal (calpain inhibitor II) prevented this decay, whereas leupeptin, a lysosome inhibitor, and E-64d, a highly specific cysteine protease inhibitor, had no effect. The change from hypoxia to normoxia and the addition of inhibitors had no effect on the expression of SP1 binding activity (Fig. 1B). The protective effect of calpain inhibitor I, which also has activity against the proteasome, and the lack of effect of E-64d suggested that the ubiquitin-proteasome system was likely involved in HIF-1 degradation. The participation of the proteasome was confirmed using the proteasome-specific inhibitors lactacystin, and the peptide Z-Leu-Leu-Leu-II (MG-132), as shown in Fig. 2. Also shown in this figure is the lack of effect of the peptide Ac-Tyr-Val-Al-Asp-CMK, an inhibitor of interleukin 1β-converting enzyme proteases.

Effect of Protease Inhibitors on HIF-1 Complex Induction in Normoxic Cell—The above results indicate that the rapid decay of HIF-1 complex observed after discontinuation of hypoxia could be prevented by the use of proteasome-specific inhibitors. We tested whether these inhibitors could also induce the formation of HIF-1 complex in normoxic cells. For this purpose Hep 3B cells were incubated under normoxic conditions for 6 h in the presence of protease inhibitors and their nuclear extracts assayed for HIF-1 activity by gel shift assays. As shown in Fig.

FIG. 1. Protease inhibitors decrease the rate of degradation of the HIF-1 complex. Hep 3B cells were exposed to hypoxia for 6 h followed by 30 min at normoxic conditions at the end of which nuclear extracts were obtained. Protease inhibitors were added 15 min (or 1 h for lactacystin) before the hypoxia was completed. A, nuclear extracts were assayed for HIF-1 binding using a labeled probe from the Epo gene. Lane 1, end of hypoxic (Hx) period. Lanes 2–6, end of the normoxic (Hx→N) incubation. Protease inhibitors added are: none (lane 2); leupeptin, 500 μg/ml (lane 3); E-64d, 200 μM (lane 4); calpain inhibitor I, 100 μg/ml (lane 5); calpain inhibitor II, 100 μg/ml (lane 6). B, nuclear extracts (as in A) assayed with an SP1-labeled probe.

![Image of gel shift assay](image-url)
3A, calpain inhibitor I, and to a lesser extent calpain inhibitor II, lactacystin, and MG 132, stimulated the formation of HIF-1 complex in normoxic cells, whereas leupeptin and E-64d had no effect (not shown). Again, these agents had no effect on SP1 (Fig. 3B). Confirmation of the presence of HIF-1α and ARNT proteins in the induced complexes was obtained by supershift assays utilizing specific antibodies against both protein subunits, as shown in Fig. 3C. Furthermore, Western blot analysis using anti-Gal-4 antibodies showed that lactacystin increased the level of Gal-4/HIF-1α fusion proteins in transfected normoxic cells.2

Effect of Redox Changes in HIF-1 Complex Formation and Gene Activation—The role of redox changes in HIF-1 induction was studied in normoxic Hep 3B cells exposed to the thiol-reductive agent N-(2-mercaptobenzoyl)-glycine (NMPG). As shown in Fig. 3A, lane 7, treatment of cells with 10 mM NMPG for 6 h induced HIF-1 complex, while no effect was observed in SP1. A possible effect of NMPG on the proteasome system was ruled out by the finding that NMPG did not affect the expression of p53 protein, whereas the proteasome inhibitors induced it (not shown). Supershifts shown in Fig. 3C confirmed the presence of HIF-1α and ARNT in the complex. To evaluate the effect of NMPG on gene activation we utilized a Hep 3B-derived cell line (B-1) stably transfected with a luciferase expression vector containing a minimal Epo promoter and a HIF-1-binding site. These cells have been shown to respond to hypoxia by increasing luciferase expression in a time-dependent manner (16). Exposure of B-1 cells to various concentrations of NMPG for 18 h showed a dose-dependent stimulation of luciferase expression, as shown in Fig. 4A. Similar results were found when the oxygen radical scavenger 2-acetamidoacrylic acid (AD-1) was used (Fig. 4B). A comparative effect between NMPG and hypoxia is shown in Fig. 4C, where B-1 cells were exposed to either NMPG at 10 mM or 0.5% O2 for 6 h. The stimulatory effect of NMPG was also mimicked by the addition of catalase, which dismutates H2O2 into water and molecular oxygen, thereby decreasing its oxidative potential. Further confirmation of the stimulatory effect of NMPG on gene expression was obtained by Northern blot analysis of RNA obtained from normoxic Hep 3B cells treated with NMPG for 6 h, as shown in Fig. 4D. Interestingly, the proteasome inhibitors did not stimulate Epo production. No changes in HIF-1α mRNA levels were found in untreated or hypoxia, proteasome inhibitors, and NMPG-treated Hep 3B cells (not shown).

Accumulation of HIF-1 Complex in a Mutant Cell Line Defective in the Ubiquitin Pathway—Polyubiquitination of proteins is the first step in the degradation of proteins by the proteasome system. Ubiquitin, a small basic protein of 76 amino acids found in all eukaryotic cells, can be covalently linked to proteins in an ATP-dependent process. Ubiquitin-activating enzyme (E1) catalyzes this first step, resulting in the formation of an E1-bound ubiquitin adenylate. To further confirm the involvement of the ubiquitin-proteasome in the proteolytic degradation of HIF-1α under normoxic conditions we utilized a BALB/c 3T3-derived cell line, ts20, containing a temperature-sensitive mutant of E1 (17). Cells cultured under the permissive temperature (35°C) maintain a functional ubiquitination pathway, whereas the shift to the non-permissive temperature (39°C) inactivates ubiquitination. For these experiments, ts20 cells were cultured under normoxic conditions at 35 and 39°C for 6 and 20 h and nuclear extracts were obtained and evaluated for HIF-1 complex formation by gel shift assays. As shown in Fig. 5A, ts20 cells at 35°C do not express HIF-1, whereas the
shift to the non-permissive temperature causes a progressive accumulation of the complex. No effects were observed on SP1 binding activity. Supershift assays against HIF-1α and ARNT (Fig. 5B) confirmed the identity of the complex as HIF-1. Similar experiments conducted with the parental 3T3 cell line showed no induction of HIF-1 with the temperature shifts (not shown).

DISCUSSION

Hypoxia responses of the Epo and other genes are mediated by the binding of a hypoxia-inducible complex (HIF-1) to a hypoxia-responsive enhancer. This process requires ongoing protein synthesis (5) and is also dependent on some as yet undetermined phosphorylation step, since it is abolished by several kinase inhibitors (16, 20). The protein components of this complex were recently cloned and characterized as belonging to the PAS family of the basic helix-loop-helix group of transcription factors (11). One of the protein components, HIF-1α, is the already known ARNT, the dimerization partner of the aryl hydrocarbon receptor protein. ARNT protein is constitutively expressed in normal cells and its level is not affected by hypoxic conditions. On the contrary, the other component, HIF-1β, a new member of that family, is not expressed in normoxic cells, but accumulates rapidly under hypoxic conditions. Since HIF-1α mRNA is constitutively present in normoxic cells, the lack of HIF-1α protein is the consequence of either a lack of translation of the mRNA or the result of a rapid degradation of the protein. A recent report by Huang et al. (15) indicates that the half-life of the HIF-1α protein is extremely short in normoxic conditions and is markedly prolonged during hypoxic stimulation. The mechanisms responsible for the rapid degradation of the protein were not known.

Control of gene expression by regulated proteolysis of transcription factors has been recently described to be an important and frequent mechanism of regulating gene transcription (reviewed in Ref. 21). Although all transcription factors are eventually degraded as part of the natural turnover of proteins, the stability of some factors is exquisitely controlled. This type of control can operate at a very rapid rate and has the advantage, over other post-translational modifications, of its irreversible nature. Eukaryotic cells depend mainly on the lysosome and the proteasome systems for the degradation of intracellular proteins. Proteins destined for proteosomal degradation are usually modified by the addition of multiple copies of ubiquitin,
a 76-amino acid basic polypeptide, to specific lysine residues (reviewed in Ref. 22). The ubiquitination process requires several enzymatic steps, the first one utilizing E1, an activating enzyme that produces a high energy thiol ester intermediate. To study the role of regulated proteolysis in the regulation of HIF-1α, we utilized initially a series of inhibitors with different proteolytic enzyme specificities. The cells were maximally stimulated by hypoxia and the effect of the inhibitors on the rate of degradation of the HIF-1 complex was evaluated by gel shift analysis. These experiments indicated that controlled proteolysis was indeed involved in HIF-1 formation and that it was mediated by the proteasome system, since it was markedly affected by lactacystin, a highly specific inhibitor of the 20 S proteasome (23). No significant effect was observed with the use of lysosome inhibitors or with inhibitors of interleukin 1β-converting enzyme proteases, the proteolytic system regulating apoptosis. The lack of involvement of this last pathway was further confirmed by the use of a stably transfected cell line overexpressing the anti-apoptotic baculovirus P35 protein (24), which showed no constitutive HIF-1 activation (not shown).

The role of proteasomal inhibition in the induction of HIF-1 was then studied in normoxic cells. These experiments clearly demonstrated that proteasomal inhibition induced HIF-1 complex formation in a manner similar to hypoxic stimulation. Further confirmation of the involvement of the ubiquitin-proteasome system in the regulation of HIF-1 protein levels and HIF-1 complex formation was obtained through the use of a cell line (ts20TG<sup>3</sup>) containing a temperature-sensitive mutant of the E1 ubiquitin-activating enzyme. These cell lines were originally developed in H. L. Ozer’s laboratory and utilized to demonstrate the proteasomal degradation of p53 (17). When ts20 cells were cultured under normoxic conditions at the permissive temperature no HIF-1 was detected, whereas a shift to 39 °C produced a rapid accumulation of the complex. The HIF-1α protein contain several PEST-like domains, which, in other proteins have been implicated in proteasomal degradation (25). However, the actual sequences that determine HIF-1α ubiquitination have not yet been determined. It is of interest to note that proteasome inhibitors, although they clearly induced HIF-1 complex formation in normoxic cells, did not activate gene expression. However, this phenomenon is likely due to a nonspecific toxic effect of these inhibitors since lactacystin produced both a decrease in basal expression of the Epo-promoter driven luciferase reporter gene construct and inhibited the stimulation of its expression by cobalt, desferrioxamine, and hypoxia (data not shown). It is not yet clear if HIF-1 complex formation is necessary and sufficient for transcriptional activation. Results previously reported by Semenza et al. (26) showed that overexpression of HIF-1α in normoxic conditions is sufficient to activate transcription of enolase 1 through its hypoxia response element.

The mechanisms of oxygen sensing and the mechanisms by which hypoxia induces stabilization of the HIF-1α protein are currently unknown. The finding by several investigators of an inhibitory effect of H<sub>2</sub>O<sub>2</sub> on HIF-1 formation and Epo gene expression suggested that redox changes are likely to be involved in oxygen sensing and/or signal transduction. Furthermore, Huang et al. (15) recently reported that overexpression of the thiol reducing proteins thioredoxin and REF-1 potentiated hypoxia induced gene activation. The dramatic effect observed with the reducing thiol agent NMPG on HIF-1 formation and Epo gene activation in normoxic cells provides a strong indication that redox changes are, indeed, involved in the hypoxic response. The source of the radicals that mediate these signals and the mechanism of action of the reducing agents, are not yet known. A direct redox effect on HIF-1α protein, although possible, seems unlikely, since the normally reducing intracellular environment maintain cytoplasmic proteins already in their reduced states. Alternatively, redox changes could indirectly modify HIF-1α by affecting the activity of redox-sensitive kinases. Preliminary data<sup>3</sup> have shown that the induction of HIF-1 by NMPG could be inhibited by genistein. Mounting evidence suggest that free radicals are central participants in multiple intracellular signal events. The participation of free radicals in hypoxia-treated cells is strongly supported by the fact that 2-acetamidoacrylic acid increased the luciferase activity in B1-treated cells under normoxic conditions. 2-Acetamidoacrylic acid as well as other N-substituted dehydroalaines have been described to react with and scavenge both superoxide and hydroxyl radicals (27). It was recently reported that antioxidants activate c-fos via a Ras-dependent pathway (28). Several redox-sensitive kinases have been described and in the case of p21<sup>mm</sup>, the mechanism for activation appears to depend on the redox status of Cys<sup>116</sup> (29). Phosphorylation dependent changes of the degradation rate of c-Jun by the proteasome system was recently reported to be dependent on the action of stress-activated mitogen-activated kinases (30). Since protein phosphorylation is a necessary step in HIF-1 activation, the role of redox-dependent kinases in HIF-1α stabilization is a distinct possibility.

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REFERENCES

1. Ratcliffe, P. J., Ebert, B. L., Firth, J. D., Gleadle, J. M., Maxwell, P. H., Nagaog, M., O’Rourke, J. F., Pugh, C. W., and Wood, S. M. (1997) Kidney Int. 51, 514–526
2. Beck, L., Ramirez, S., Weimann, R., and Caro, J. (1991) J. Biol. Chem. 266, 15563–15566
3. Semenza, G. L., Nijmekt, M. K., Chi, S. M., and Antonarakis, S. E. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5680–5684
4. Pugh, C. W., Tan, C. C., Jones, R. W., and Ratcliffe, P. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10553–10557
5. Semenza, G. L., and Wang, G. L. (1992) Mol. Cell. Biol. 12, 5447–5454
6. Beck, L., Weimann, R., and Caro, J. (1993) Blood 82, 704–711
7. Levy, A. P., Levy, N. S., Wegner, S., and Goldberg, M. A. (1995) J. Biol. Chem. 270, 13333–13340
8. Firth, J. D., Ebert, B. L., Pugh, C. W., and Ratcliffe, P. J. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6496–6500
9. Semenza, G. L., Roth, P. H., Fang, H.-M., and Wang, G. L. (1994) J. Biol. Chem. 269, 23757–23763
10. Ebert, B. L., Firth, J. D., and Ratcliffe, P. J. (1995) J. Biol. Chem. 270, 29083–29089
11. Wang, G. L., Jiang, B.-H., Rue, E. A., and Semenza, G. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5510–5514
12. Pandrey, J., Frede, S., and Jelkmann, W. (1994) Biochem. J. 303, 507–510
13. Wang, G. L., Jiang, B.-H., and Semenza, G. L. (1995) Biochem. Biophys. Res. Commun. 212, 550–556
14. Saleeda, S., Beck, I., and Caro, J. (1996) Arch. Biochem. Biophys. 334, 389–394
15. Huang, L. E., Arroyo, Z., Livingston, D. M., and Bunn, H. F. (1996) J. Biol. Chem. 271, 32255–32259
16. Saleeda, S., Beck, I., Srinivas, V., and Caro, J. (1997) Kidney Int. 51, 556–559
17. Chowdary, D. R., Bemody, J. J., Jha, K. K., and Ozer, H. L. (1994) Mol. Cell. Biol. 14, 1997–2003
18. Omura, S., Fujimoto, T., Toguro, K., Matsuzaki, K., Moriguchi, R., Tanaka, H., and Sasaki, Y. (1991) J. Antibiot. (Tokyo) 44, 113–116
19. Chomczynski, P.; and Sacchi, N. (1987) Anal. Biochem. 162, 156–160
20. Wang, G. L., Jiang, B.-H., and Semenza, G. L. (1995) Biochem. Biophys. Res. Commun. 216, 669–675
21. Pahl, H. L., and Baeuerle, P. A. (1996) Curr. Opin. Cell Biol. 8, 340–347
22. Hilt, W., and Wolf, D. H. (1996) Trends Biochem. Sci. 21, 96–102
23. Dick, I. R., Cruikshank, A. A., Destree, A. T., Grenier, L., McCormack, T. A.,

3 S. Salceda and J. Caro, unpublished data.
Melandri, F. D., Nunes, S. L., Palombella, V. J., Parent, L. A., Plamondon, L., and Stein, R. L. (1997) *J. Biol. Chem.* **272**, 182–188

24. Robertson, N. M., Zangrilli, J., Fernandez-Alnemri, T., Friesen, P. D., Litwack, G., and Alnemri, E. S. (1997) *Cancer Res.* **57**, 43–47

25. Rechsteiner, M., and Rogers, S. W. (1996) *Trends Biochem. Sci.* **21**, 267–271

26. Semenza, G. L., Jiang, B.-H., Leung, S. W., Passantino, R.; Concordet, J.-P., Maire, P., and Giallongo, A. (1996) *J. Biol. Chem.* **271**, 32529–32537

27. Sipe, H. J., Buc-Calderon, P., Roberfroid, M., and Mason, R. P. (1993) *Chem.-Biol. Interactions* **86**, 93–102

28. Muller, J. M., Cahill, M. A., Rupec, R. A., Bacuerle, P. A., and Nordheim, A. (1997) *Eur. J. Biochem.* **244**, 45–52

29. Lander, H. M., Hajjar, D. P., Hempstead, B. L., Mirza, U. A., Chait, B. T., Campbell, S., and Quilliam, L. A. (1997) *J. Biol. Chem.* **272**, 4323–4326

30. Musti, A. M., Treier, M., and Bohmann, D. (1997) *Science* **275**, 400–402