possible that, like APP, these fragments are generated by BACE1 in the trans-Golgi network and might also serve to tether membrane vesicles to KLCs, a model that can now be tested by examining whether expression of APP carboxyl-terminal fragments in the neurons of APP-deficient mice can rescue axonal transport abnormalities in these animals.

Goldstein and colleagues observed that Aβ can be generated in membrane vesicles transported along the axons of peripheral nerves. This finding needs to be confirmed in brain neurons (*11*). In any event, these authors speculate that impaired APP transport leads to enhanced axonal generation and deposition of Aβ, resulting in disruption of neurotrophic signaling and neurodegeneration. Although attractive, there is limited evidence in humans, or in mouse models, to support this notion. Aβ deposits are rarely seen in the deep white matter of human brains, and dystrophic or swollen axons are present only in the vicinity of amyloid deposits. Furthermore, the accumulation of membrane vesicles within axons is not apparent in either young or aged transgenic mice that have 8- to 10-fold higher levels of human APP containing mutations that cause familial AD. A closer examination of axonal transport and axonal pathology in these animals is warranted.

Finally, the notion that APP is a kinesin-1 receptor overlooks the possibility that APP has a job at the synapse, and that γ-secretase–generated APP carboxyl-terminal fragments have transcriptional activity (*27*). A model that accommodates these alternative activities posits a dual role for APP: as a kinesin-1 receptor that facilitates the delivery of specific cargo proteins to specialized presynaptic sites, and as a receptor/ligand at nerve terminals. In this model, fusion of the transported vesicles with the presynaptic plasma membrane "exposes" the membrane-bound APP to specific ligands, resulting in activation of intracellular signaling events or the release of carboxyl terminal–truncated APP and Aβ into the synaptic cleft.

Stay tuned for more findings from this exciting foray into the biology of APP and APLPs in axonal transport and synaptic activity. These efforts will provide new insights into the molecular apparatus that regulates kinesin-1 selection of particular cargo vesicles and axonal trafficking. Such research will open the door to understanding how disruptions of these pathways might affect the initiation or progression of age-associated neurodegenerative diseases, such as AD.

References

1. W. P. Esler, M. S. Wolfe, *Science* **293**, 1449 (2001).
2. S. S. Sisodia *et al.*, *Trends Neurosci.* **24**, S2 (2001).
3. A. Kamal *et al.*, *Nature* **414**, 643 (2001).
4. N. Hirokawa, *Science* **279**, 519 (1998).
5. A. Kamal *et al.*, *Neuron* **28**, 449 (2000).
6. D. G. Cook *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9223 (1996).
7. W. G. Anneart *et al.*, *J. Cell Biol.* **147**, 277 (1999).
8. P. Cupers *et al.*, *J. Cell Biol.* **154**, 731 (2001).
9. E. H. Koo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 1561 (1990).
10. S. S. Sisodia *et al.*, *J. Neurosci.* **13**, 3136 (1993).
11. J. D. Buxbaum *et al.*, *J. Neurosci.* **18**, 9629 (1998).
12. A. Ferreira *et al.*, *J. Neurosci.* **13**, 3112 (1993).
13. C. Kaether *et al.*, *Mol. Biol. Cell* **11**, 1213 (2000).
14. Reviewed in M. P. Mattson, *Trends Neurosci.* **24**, 441 (2001).
15. C. S. von Koch *et al.*, *Neurobiol. Aging* **18**, 661 (1997).
16. S. Heber *et al.*, *J. Neurosci.* **20**, 7951 (2000).
17. S. Gunawardena, L. S. B. Goldstein, *Neuron* **32**, 389 (2001).
18. L. Torroja *et al.*, *Curr. Biol.* **9**, 489 (1999).
19. K. J. Verhey *et al.*, *J. Cell Biol.* **152**, 959 (2001).
20. A. B. Bowman *et al.*, *Cell* **103**, 583 (2000).
21. D. T. Byrd *et al.*, *Neuron* **32**, 787 (2001).
22. S. Matsuda *et al.*, *J. Neurosci.* **21**, 6597 (2001).
23. M. H. Scheinfeld *et al.*, *J. Biol. Chem.* published online 27 November 2001, 10.1074/jbc.M108357200.
24. K. Ijima *et al.*, *J. Neurochem.* **75**, 1085 (2000).
25. K. Ando *et al.*, *J. Neurosci.* **19**, 4421 (1999).
26. V. Muresan *et al.*, *Mol. Biol. Cell* **12**, 311a (2001).
27. X. Cao, T. C. Sudhof, *Science* **293**, 115 (2001).

PERSPECTIVES: TRANSCRIPTION

Oxygen Sensing Gets a Second Wind

Richard K. Bruick and Steven L. McKnight

Mammalian cells are able to sense prolonged decreases in oxygen concentration (hypoxia) through a conserved hypoxic response pathway. This pathway facilitates adaptation to hypoxia-induced physiological stress by regulating changes in gene expression, and is also critical for the execution of many physiological events, including formation of blood vessels during embryogenesis, and pathophysiological processes such as tumorigenesis. A family of hypoxia-inducible transcription factors (HIFs) lies at the heart of this adaptive pathway. HIF proteins are activated by a decrease in the concentration of molecular oxygen (O_2), which results in the induced expression of downstream target genes that mediate adaptation and survival of cells and the whole organism.

Enhanced online at www.sciencemag.org/cgi/content/full/295/5556/807

The authors are in the Department of Biochemistry, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard L3.124, Dallas, TX 75390–9152, USA. E-mail: bruick@biochem.swmed.edu, smckni@biochem.swmed.edu

Until recently, the means by which cells sense alterations in oxygen tension and subsequently induce changes in HIF activity remained obscure. The first inkling of an oxygen sensing pathway in higher organisms came last year with the discovery of a family of oxygen-dependent enzymes responsible for the modulation of HIF stability (*1*–*4*). A report by Lando *et al.* (*5*) on page 858 of this issue now identifies a second, oxygen-dependent, posttranslational modification of HIF that regulates the ability of HIF to recruit stimulatory transcriptional cofactors to its target genes.

The HIF transcription factors are composed of two subunits: the hypoxia-regulated α subunit, HIF-1α (or its paralogs HIF-2α and HIF-3α), and the oxygen-insensitive HIF-1β subunit (also known as the arylhydrocarbon receptor nuclear translocator, or ARNT). Under normal oxygen conditions (normoxia), the HIF-1α subunit, although expressed, is rapidly degraded such that almost no HIF protein accumulates (see the figure). Under hypoxic conditions, degradation of the α subunit is blocked, allowing HIF-1α to accumulate within the nucleus where, upon binding to HIF-1β, it recognizes HIF-responsive elements (HREs) within the promoters of hypoxia-responsive target genes. Degradation of HIF-1α under normoxic conditions is triggered by posttranslational hydroxylation of conserved proline residues within a polypeptide segment known as the oxygen-dependent degradation domain (ODD). The hydroxylated proline residues in this sequence are recognized by the product of the von Hippel–Lindau tumor suppressor gene (pVHL), a component of a ubiquitin ligase complex that tags the α subunit for degradation by the proteasome (see the figure) (*6*, *7*). This critical regulatory event is carried out by a family of iron (II)–dependent prolyl hydroxylase enzymes (*3*, *4*) that use O_2 as a substrate to catalyze hydroxylation of the target proline residues. Because O_2 appears to be rate limiting for prolyl hydroxylase activity (*3*), these enzymes may represent bona fide oxygen sensors that provide a direct link between O_2 concentration and components of the hypoxic response pathway.

Modulation of protein stability is just one means by which HIF activity is induced by hypoxia. In addition to the ODD domain, the α subunits of all three HIF isoforms contain two transactivation domains responsible for recruiting transcriptional coactivators essential for gene expression. One of the HIF transactivation domains overlaps the ODD, and regulation of its activity is likely to be a by-product of protein

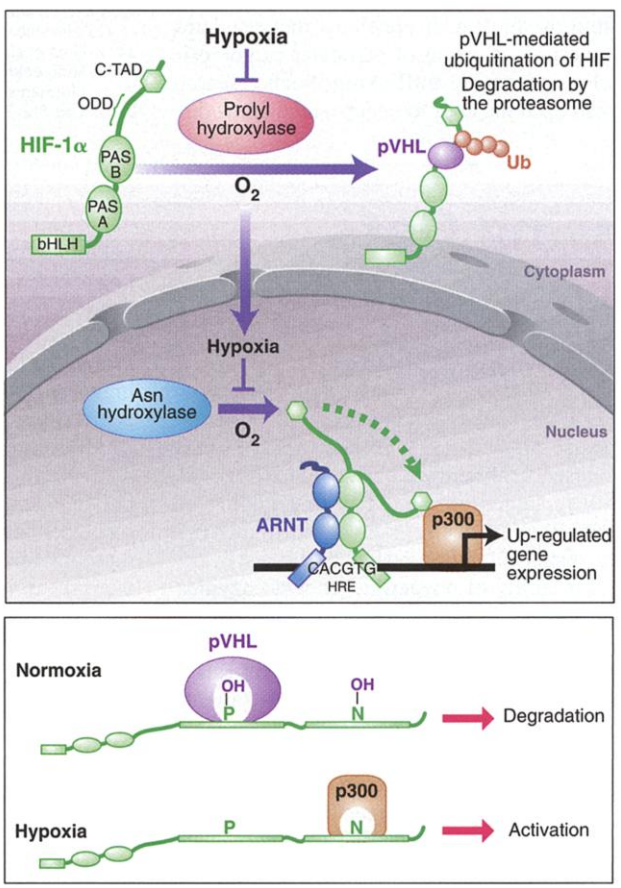
stability. The second carboxyl-terminal transactivation domain (C-TAD) operates independently of the ODD and is able to recruit coactivator complexes such as p300/CBP only under hypoxic conditions (*8*–*10*). Remarkably, the regulatory switch controlling the activity of the C-TAD also involves an oxygen-dependent hydroxylation event, in this case targeted to a conserved asparagine residue.

To examine oxygen-dependent regulation of the C-TAD, Lando and colleagues removed this domain from the α subunit, decoupling it from regulation of protein stability mediated through the ODD. When linked to the Gal4 DNA-binding domain, C-TAD stability is unaffected by hypoxia, yet remains able to stimulate transcription in response to hypoxia (*8*, *9*, *11*–*13*). Mass spectrometry revealed that under normoxic conditions, the inactive C-TAD had a mass 16 daltons greater than predicted, reminiscent of ODD hydroxylation. Following a shift to hypoxic conditions, the mass increase was lost, coinciding with increased C-TAD activity. Parsimony would have predicted that the HIF prolyl hydroxylase enzymes would execute this regulatory step. However, mass spectrometry analysis revealed that a conserved asparagine residue, rather than a proline residue, is the target for hydroxylation in the C-TAD. Mutation of the key asparagine residue to alanine resulted in loss of oxygen-dependent hydroxylation and led to constitutive C-TAD activity. Together, these data nicely fit a model in which the asparagine residue within the C-TAD is hydroxylated under normoxic conditions by a putative asparagine hydroxylase (see the figure). Furthermore, Lando and colleagues provide clear evidence that hydroxylation of the conserved asparagine residue blocks interaction of the C-TAD with the p300/CBP transcriptional coactivators. Under hypoxic conditions, asparagine hydroxylation is blocked, thereby derepressing the system and facilitating coactivator recruitment.

To date, the enzyme responsible for hydroxylation of the conserved C-TAD asparagine residue has not been identified. Well-characterized prolyl and lysyl hydroxylases are iron-binding enzymes that use 2-oxoglutarate as a cosubstrate. Lando and colleagues report that asparagine hydroxylation of the C-TAD can be abrogated by addition of iron chelators or competitive inhibitors of 2-oxoglutarate, indicating that this enzyme may also fall within the larger family of 2-oxoglutarate–dependent dioxygenases. Although it is tempting to believe that both the prolyl and asparaginyl hydroxylases will serve as direct oxygen sensors, further studies are required to assess whether these enzymes are sensitive to changes in O_2 concentration capable of inducing a hypoxic response in vivo.

Discovery of this second oxygen-dependent switch raises the question of why HIF activity is subject to multiple independent levels of regulation. The work described by Lando and co-workers demonstrates that both hydroxylase switches must be flipped to fully induce HIF. It is possible that multiple levels of regulation allow for graded responses to subtle changes in O_2 concentration. Alternatively, dependence upon two independent regulatory events may help to ensure that the hypoxic response pathway is tightly controlled. The products of well-characterized HIF target genes are known to promote increased vascularization and glycolytic metabolism, both of which are essential for solid tumor formation. Indeed, constitutive activation of HIF has been correlated with the progression of a variety of human tumors (*14*). Likewise, prolonged HIF induction leads to the expression of genes affecting the balance between cell death and survival and is required for promoting cell death pending failure to adapt to a hypoxic environment (*15*).

Do these two hydroxylase enzymes complete the story of oxygen-dependent HIF regulation, or might there be additional levels of HIF transcription factor modulation? By themselves, these prolyl and asparaginyl hydroxylase enzymes may not account for the effects of some hypoxia "mimics" such as carbon monoxide. Moreover, the secondary consequences of changes in oxygen concentration, such as alteration of intracellular redox potentials or the amount of reactive oxygen species, may influence HIF induction (*16*). These additional signals might regulate the prolyl and asparaginyl hydroxylase enzymes directly or influence HIF through unique pathways. Regardless of these potential complexities, the past year has been mighty good to HIF. The time is ripe for detailed enzymological studies of the relevant prolyl and asparaginyl hydroxylases, and the door now has been opened to the discovery of small-molecule agonists and antagonists of the hypoxic response pathway.



Regulation of the HIF-1 transcription factor. Under normoxic conditions, the ODD of HIF-1α is modified by a HIF–prolyl hydroxylase, triggering HIF-1α recognition by pVHL and subsequent degradation by the proteasome. Similarly, an asparaginyl hydroxylase modifies the C-TAD of HIF-1α, blocking its interaction with the transcriptional coactivator p300. Hypoxia blocks both prolyl hydroxylation and asparaginyl hydroxylation, allowing HIF-1α to accumulate and bind to p300, thereby promoting the transcription of downstream HIF-1 target genes, thus enabling cells and the whole organism to adapt to hypoxia.

References

1. M. Ivan *et al.*, *Science* **292**, 464 (2001).
2. P. Jaakkola *et al.*, *Science* **292**, 468 (2001).
3. A. C. R. Epstein *et al.*, *Cell* **107**, 43 (2001).
4. R. K. Bruick, S. L. McKnight, *Science* **294**, 1337 (2001).
5. D. Lando, D. J. Peet, D. A. Whelan, J. J. Gorman, M. L. Whitelaw, *Science* **295**, 858 (2002).
6. L. E. Huang, J. Gu, M. Schau, H. F. Bunn, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 7987 (1998).
7. S. Salceda, J. Caro, *J. Biol. Chem.* **272**, 22642 (1997).
8. M. Ema *et al.*, *EMBO J.* **18**, 1905 (1999).
9. J. Gu, J. Milligan, L. E. Huang, *J. Biol. Chem.* **276**, 3550 (2001).
10. A. L. Kung, S. Wang, J. M. Klco, W. G. Kaelin, D. M. Livingston, *Nature Med.* **6**, 1335 (2000).
11. P. Carrero *et al.*, *Mol. Cell. Biol.* **20**, 402 (2000).
12. B. H. Jiang, J. Z. Zheng, S. W. Leung, R. Roe, G. L. Semenza, *J. Biol. Chem.* **272**, 19253 (1997).
13. J. F. O'Rourke, Y. M. Tian, P. J. Ratcliffe, C. W. Pugh, *J. Biol. Chem.* **274**, 2060 (1999).
14. Reviewed in P. H. Maxwell, C. W. Pugh, P. J. Ratcliffe, *Curr. Opin. Genet. Dev.* **11**, 293 (2001).
15. P. Carmeliet *et al.*, *Nature* **394**, 485 (1998).
16. Reviewed in G. L. Semenza, *Curr. Opin. Cell Biol.* **13**, 167 (2001).