nucleoids, FtsZ in the double mutant often localized in multiple patches on top of normal-looking nucleoids. This suggested that NO was specifically suppressed and that Noc was important for NO.

But why was cell division inhibited in the double mutant, which removed two negative regulators? One potential model to explain this apparent paradox proposes that normal assembly of the Z ring requires molecular crowding (Rivas et al., 2001), which might tend to occur when most of the membrane surface is masked by NO and the Min system. When one system is missing, the other would still mask sufficient space. However, when both systems are absent, the limited amount of cellular FtsZ fails to assemble productively because there would be too many potential assembly sites. This effect was observed previously with an *ftsZ84 min* double mutant, in which a weakened FtsZ actually assembled less well in the absence of Min inhibition (Yu and Margolin, 2000). As with the *ftsZ84 min* mutant, overproduction of FtsZ in *noc min* double mutants resulted in partial suppression of the division inhibition phenotype, which supports the hypothesis.

If Noc is so important for nucleoid occlusion, then why did a *noc* null mutant lack detectable defects? To address this, Wu and Errington (2004) perturbed the cell cycle in order to uncover important roles for Noc in mediating NO. When cells were made longer by inhibiting later steps of cell division, Z rings, normally restricted to internucleoid spaces, assembled promiscuously on top of nucleoids near the center of these long cells. This supports the idea that Min-mediated inhibition of Z ring assembly extends a considerable distance from the cell poles but at some point is unable to block assembly on top of nucleoids in the absence of Noc. In the most dramatic demonstration of the importance of Noc, blocking reinitiation of chromosomal DNA replication caused division septa to cut nucleoids at high frequency in *noc* mutants, while they pinched to one side of nucleoids in Noc^+ cells.

How might Noc function to inhibit Z ring assembly? Noc is weakly homologous to the ParB/Spo0J family of proteins, and like these, Noc localizes to nucleoids. However, whereas ParB/Spo0J localize to polar foci near the partitioned chromosomal origin, Noc localizes to a more dispersed portion of the nucleoid. Interestingly, Noc did not localize to the middle of segregating nucleoids, which correspond to replication termini near the cell midpoint. A gradient of lower NO near the terminus, along with lower inhibition by the Min system at the center of a long predivisional cell, might be sufficient to permit assembly of the Z ring at the cell center at the correct time in the cell cycle. Perhaps assembly of the asymmetric Z ring on top of the filamentous nucleoid during *B. subtilis* sporulation is also mediated by a local decrease in Noc localization. Conversely, overproduction of Noc decreased the frequency of Z rings and partially inhibited cell division, possibly by localizing to lower affinity sites near the terminus or even away from the nucleoid.

The major challenges now will be to determine if Noc acts directly on FtsZ, how Noc blocks assembly of the Z ring, what other factors influence FtsZ positioning, and whether NO and Noc are widespread. Many prokaryotes lack Min homologs, and unless they have other proteins that perform a similar function, they may rely solely on NO. This idea makes sense if their nucleoids fill the cells, preventing significant DNA-free gaps at the cell poles. *Caulobacter crescentus,* for example, lacks a Min system, but Z rings tend to localize in areas of the cell with low DNA concentrations (Quardokus and Brun, 2002), suggesting that NO plays an important role. While NO may be universal, Noc is probably not, as it is poorly conserved at the sequence level. Clearly, the discovery of Noc delivers NO out of the realm of phenomenology and confirms the importance of NO. Its discovery also ushers in a new era for understanding the regulation of prokaryotic cellular organization.

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Selected Reading

Harry, E.J. (2001). Mol. Microbiol. *40*, 795–803.

Margolin, W. (2001). Curr. Opin. Microbiol. *4*, 647–652.

Marston, A.L., Thomaides, H.B., Edwards, D.H., Sharpe, M.E., and Errington, J. (1998). Genes Dev. *12*, 3419–3430.

Migocki, M.D., Freeman, M.K., Wake, R.G., and Harry, E.J. (2002). EMBO Rep. *3*, 1163–1167.

Quardokus, E.M., and Brun, Y.V. (2002). Mol. Microbiol. *45*, 605–616.

Raskin, D.M., and de Boer, P.A. (1999). Proc. Natl. Acad. Sci. USA *96*, 4971–4976.

Rivas, G., Fernandez, J.A., and Minton, A.P. (2001). Proc. Natl. Acad. Sci. USA *98*, 3150–3155.

Woldringh, C., Mulder, E., Valkenburg, J., Wientjes, F., Zaritsky, A., and Nanninga, N. (1990). Res. Microbiol. *141*, 39–49.

Wu, L.J., and Errington, J. (2004). Cell *117*, this issue, 915–925.

Yu, X.-C., and Margolin, W. (1999). Mol. Microbiol. *32*, 315–326.

Yu, X.-C., and Margolin, W. (2000). J. Bacteriol. *182*, 6203–6213.

Siah Proteins, HIF Prolyl Hydroxylases, and the Physiological Response to Hypoxia

New evidence suggests that at least two members of the family of hypoxia-inducible factor (HIF) prolyl hydroxylases that regulate HIF stability in response to oxygen (O_2) availability are also targeted for proteosome-dependent degradation by the E3 ubiquitin ligases Siah1a and Siah2. This preview examines cellular responses to O_2 deprivation (hypoxia) and the complexity of the regulation of the HIF O_2 sensing pathway in mammals.

Oxygen homeostasis is maintained in higher eukaryotes via highly developed respiratory and circulatory systems, as O_2 is central to the viability of most organisms. Conserved O_2 sensing pathways are present in all mammalian cells and tissues and critical for a variety of physiological and pathological processes (reviewed in Semenza [2000]). Many rapid intracellular responses to low O_2 (hypoxia) exist, but hypoxia-inducible transcription factors (HIFs) regulate a majority of the changes in gene

expression that occur when O_2 becomes limiting. HIFs are heterodimeric DNA binding proteins, composed of HIFα and HIFβ (ARNT) subunits, that enhance the expression of approximately 100 genes involved in the physiological response to hypoxia. HIF targets include genes involved in glucose transport, glycolysis, angiogenesis, vascular function, erythropoiesis, and cell proliferation and/ or survival. While ARNT is constitutively expressed, HIF1α is rapidly degraded at normoxia (21% O_2). Upon decreases in local PO_2 (≤6% O_2), HIF1α is stabilized, binds ARNT in the nucleus, and activates O_2-regulated gene expression. Therefore, the HIF O_2 sensing pathway is primarily regulated by the abundance of the α subunit.

The mechanism by which O_2 deprivation increases HIF1α stability was obscure until it was recognized that the von Hippel-Lindau tumor suppressor protein (pVHL) targets HIF1α for proteosome-mediated proteolysis (Maxwell et al., 1999). pVHL is a component of a multisubunit ubiquitin-protein ligase complex that tags HIF1α with polyubiquitin (see Figure 1). pVHL interaction with the α subunit is dependent upon hydroxylation of two conserved proline residues within the "oxygen-dependent degradation domain," or ODD (Huang et al., 1998; Ivan et al., 2001; Jaakkola et al., 2001; Yu et al., 2001). Based on this information, 3-4 HIF prolyl hydoxylases (HIF "PHDs" for *p*rolyl *h*ydroxylase *d*omain containing enzymes) were identified (reviewed in Bruick [2003]). HIF PHDs utilize molecular O_2 as a substrate in the hydroxylation of HIF1α; therefore, it has been suggested that they act as O_2 sensors. However, whether the PHDs act as bona fide O_2 sensors in all circumstances involving HIF and physiological hypoxia remains to be determined (Schumacker, 2002).

Are the HIF PHDs also regulated by O_2 availability? Decreased O_2 pressure (1%) leads to elevated levels of PHD2 and PHD3 mRNA, possibly in a HIF-dependent manner (Berra et al., 2003). However, a recent paper by Nakayama and colleagues (Nakayama et al., 2004 [this issue of *Cell*]) clearly demonstrates that the half-life of at least two HIF PHD proteins, PHD1 and PHD3, is regulated by low O_2. Like HIF1α, these enzymes are also degraded via the proteosome. However, in direct contrast to HIF1α, the stability of PHD1 and PHD3 *decreases* in hypoxic cells. Nakayama et al. determined that two mammalian homologs, Siah1a and Siah2, of the *Drosophila seven in absentia* RING finger protein target PHD1 and PHD3 for proteosome-mediated turnover under hypoxic conditions. Siah proteins possess E3 ubiquitin ligase activity implicated in the degradation of diverse proteins such as β-catenin and N-CoR. In searching for novel targets of Siah2, the authors performed mass spectrometry of Siah2-associated proteins and identified the PHDs as new substrates of Siah2 and Siah1a. Overexpression of Siah2 and Siah1a results in decreased levels of PHD1 and PHD3 in human 293T cells. Furthermore, PHD3 is more stable in *Siah2*$^{-/-}$ mouse embryo fibroblasts (MEFs) at both 21% O_2 and 1% O_2, while MEFs lacking both Siah2 and Siah1a exhibit extremely high levels of PHD3. These results provide genetic evidence in support of the role of Siah proteins in the regulation of PHD stability. As might be anticipated, HIF1α abundance is diminished in hypoxic *Siah2*$^{-/-}$ MEFs as compared to wild-type and barely detectable in hypoxic *Siah1a*$^{-/-}$*Siah2*$^{-/-}$ MEFs. This alteration in HIF1α stability in *Siah2*$^{-/-}$ cells is clearly dependent on PHD3, as exogenous expression of PHD3 in *Siah2*$^{+/+}$ cells decreases HIF1α to levels observed in *Siah2*$^{-/-}$ MEFs. Importantly, Siah2 mRNA levels are increased by hypoxia but most likely this is due to factors other than HIF1α. In aggregate, these results suggest that Siah2 transcription is stimulated by hypoxia, leading to PHD degradation and enhanced HIF1α stability (see Figure 1). Therefore, one important aspect of Siah activity is to positively regulate the HIF O_2 sensing pathway.

This clearly has implications for the physiological response to hypoxia. Perhaps the most exciting result described by Nakayama et al. is that viable *Siah2*$^{-/-}$ mice exhibit impaired hematopoietic and respiratory responses to continuous treatment with hypoxia (10% O_2) for 1–2 weeks. Whereas wild-type animals respond to O_2 deprivation by elevating red blood cell production (polycythemia), *Siah2*$^{-/-}$ mice display an attenuated increase in RBCs similar to that previously observed for *HIF1α*$^{+/-}$ mice. Siah2 also appears to be necessary for ventilatory changes (hyperpnea) in response to acute

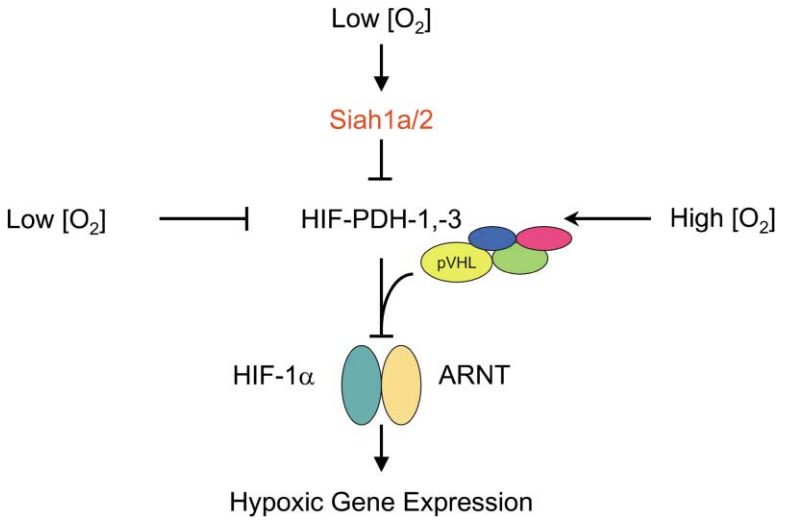

Figure 1. HIF Hydroxylation Allows Recognition by pVHL in Conjunction with Elongin B, Elongin C, Cul2, and Rbx1 and Subsequent HIF1α Ubiquitination and Degradation

Hypoxia (1%–10% O_2) induces the expression of Siah1a and Siah2, resulting in proteosome-mediated degradation of HIF PHD1 and -3. Decreased PHD enzymatic activity enhances HIF1α stability and hypoxic gene transcription. HIF PHD catalysis of HIF ODD prolyl hydroxylation is inhibited by O_2 depletion; however, HIF-PHD2 and -3 mRNAs are also stimulated by hypoxia.

hypoxia. These findings provide critical physiological evidence that Siah2 modulates the HIF pathway in vivo and nicely support the biochemical results obtained in cell culture.

The current studies present an additional layer of complexity in the regulation of HIF hydroxylation and hypoxic gene induction. They also underscore differences between PHD1, PHD2, and PHD3. These enzymes display distinct subcellular locations and tissue distributions. Furthermore, while PHD1 does not appear to be transcriptionally induced by hypoxia, PHD2 and PHD3 mRNA levels increase under low O_2. Specific "silencing" of PHD2 (and not PHD1 or PHD3) is sufficient to stabilize and activate HIF1α in a variety of normoxic human cell lines (Berra et al., 2003). It has therefore been suggested that HIF PHDs have disparate functions in vivo, with PHD2 providing low steady state levels of HIF1α observed at 21% O_2. In contrast, elevated levels of Siah1a/2 under hypoxia should ultimately lead to decreased levels of PHD1 and PHD3 and increased HIF1α protein. Thus, PHD2 limits HIF1α expression under normoxia, whereas PHD1 and PHD3 could regulate HIF1α availability under hypoxia, especially moderate O_2 deprivation (5%–10% O_2). Exciting areas of investigation for the future include the identification of additional substrates for both the Siah and HIF PHD proteins.

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Selected Reading

Berra, E., Benizri, E., Ginouves, A., Volmat, V., Roux, D., and Pouyssegur, J. (2003). EMBO J. *22*, 4082–4090.

Bruick, R.K. (2003). Genes Dev. *17*, 2614–2623.

Huang, L.E., Gu, J., Schau, M., and Bunn, H.F. (1998). Proc. Natl. Acad. Sci. USA *95*, 7987–7992.

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J.M., Lane, W.S., and Kaelin, W.G., Jr. (2001). Science *292*, 464–468.

Jaakkola, P., Mole, D.R., Tian, Y.M., Wilson, M.I., Gielbert, J., Gaskell, S.J., Kriegsheim, A., Hebestreit, H.F., Mukherji, M., Schofield, C.J., et al. (2001). Science *292*, 468–472.

Maxwell, P.H., Wiesener, M.S., Chang, G.W., Clifford, S.C., Vaux, E.C., Cockman, M.E., Wykoff, C.C., Pugh, C.W., Maher, E.R., and Ratcliffe, P.J. (1999). Nature *399*, 271–275.

Nakayama, K., Few, I.J., Hagensen, M., Skals, M., Habelhah, H., Bhoumik, A., Kadoya, T., Erdjument-Bromage, H., Tempst, P., Frappell, P.B., et al. (2004). Cell *117*, this issue, 941–952.

Schumacker, P.T. (2002). Am. J. Physiol. Lung Cell. Mol. Physiol. *283*, L918–L921.

Semenza, G.L. (2000). Genes Dev. *14*, 1983–1991.

Yu, F., White, S.B., Zhao, Q., and Lee, F.S. (2001). Proc. Natl. Acad. Sci. USA *98*, 9630–9635.