Hypoxia. Cross talk between oxygen sensing and the cell cycle machinery

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Semenza GL. Hypoxia. Cross talk between oxygen sensing and the cell cycle machinery. Am J Physiol Cell Physiol 301: C550–C552, 2011.—A fundamental physiological property of mammalian cells is the regulation of proliferation according to O2 availability. Progression through the cell cycle is inhibited under hypoxic conditions in many, but not all, cell types, and this G1 arrest is dependent on hypoxia-inducible factor (HIF) 1α. Components of the hexameric MCM helicase, which binds to replication origins before the onset of DNA synthesis, are present in large excess in mammalian cells relative to origins, suggesting that they may have additional functions. Screens for HIF-1α interacting proteins revealed that MCM7 binds to the amino-terminal PER-SIM-ARNT (PAS) domain of HIF-1α and stimulates prolyl hydroxylation-dependent ubiquitination and degradation of HIF-1α, whereas MCM3 binds to the carboxyl terminus of HIF-1α and enhances asparaginyl hydroxylation-dependent inhibition of HIF-1α transactivation domain function. Thus MCM proteins inhibit HIF activity via two distinct O2-dependent mechanisms. Under prolonged hypoxic conditions, MCM mRNA expression is inhibited in a HIF-1α-dependent manner. Thus HIF and MCM proteins act in a mutually antagonistic manner, providing a novel molecular mechanism for homeostatic regulation of cell proliferation based on the relative levels of these proteins.

hypoxia-inducible factor; MCM proteins

A fundamental biological process that is regulated by O2 availability is cell division. For most cell types, hypoxia inhibits cell proliferation, because an increase in cell number would only further increase O2 consumption, resulting in even more severe hypoxia. On the other hand, certain specialized cell types are particularly likely to undergo mitosis under hypoxic conditions. For example, a key response to tissue hypoxia is angiogenesis, which requires the proliferation of vascular endothelial cells. These considerations suggest the existence of O2-dependent mechanisms to control cell proliferation as well as opposing mechanisms by which such controls may be overcome. This review will focus on studies that have identified cross talk between systems controlling O2 homeostasis and cell division.

When primary mouse cells (either embryonic fibroblasts or B lymphocytes) were cultured for 24 h under hypoxic conditions of 0.5% O2 (P O2 = 3.5 mmHg), bromodeoxyuridine (BrdU) incorporation into DNA (a measure of progression through S phase of the cell cycle) was significantly decreased compared with duplicate plates of cells incubated under standard tissue culture conditions of 20% O2 (4). The effect of hypoxia was observed in both wild-type and p53-deficient cells. In contrast, BrdU incorporation was not inhibited when HIF-1α-deficient fibroblasts or B cells were cultured under hypoxic conditions (4). These results were consistent with previous studies demonstrating reduced BrdU incorporation when wild-type, but not HIF-1α-deficient, mouse embryonic stem cells were cultured under hypoxic conditions (1). Both studies reported that expression of mRNA encoding the cyclin-dependent kinase (CDK) inhibitor p21 was increased in response to hypoxia in wild-type, but not in HIF-1α-deficient, cells (1, 4).

Overexpression of HIF-1α was sufficient to increase p21 expression and block BrdU incorporation in human HCT116 cells cultured under nonhypoxic conditions, even when HIF-1α was mutated to inhibit DNA binding or transactivation function or when amino acid residues 401–826 of HIF-1α were deleted (12). C-Myc is known to promote cell proliferation and one mechanism by which it does so is to repress transcription of the CDKN1A gene (which encodes p21) by interacting with MIZ1 bound to the initiator element in the CDKN1A promoter (7, 20). Analysis of CDKN1A promoter occupancy by chromatin immunoprecipitation assays revealed binding of C-Myc, but not HIF-1α, in cells cultured under nonhypoxic conditions, whereas under hypoxic conditions C-Myc binding was decreased and HIF-1α binding was detected (12). C-Myc was shown to interact with the amino-terminal half of HIF-1α, which was proposed to displace C-Myc binding at the CDKN1A promoter, resulting in derepression of CDKN1A gene transcription (12). Whereas HIF-1α usually activates transcription by dimerizing with HIF-1β and binding to a hypoxia

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response element (HRE) (19), its effect on CDKN1A transcription is independent of HIF-1β and HRE binding. Forced overexpression of HIF-1α is sufficient to arrest the cell cycle even under nonhypoxic conditions (6).

Whereas HIF-1α inhibits C-Myc-mediated transcriptional repression of CDKN1A, HIF-2α has been reported to enhance C-Myc activity by promoting C-Myc:MAX heterodimerization, thereby promoting the transcription of C-Myc target genes encoding Cyclin D2 and other proteins required for cell division (5). In contrast, HIF-1 binds to an HRE in the MXI1 gene and activates its transcription, leading to the expression of an inhibitory subunit that competes with MAX for binding to C-Myc (2, 25). Because HIF-1α and HIF-2α are playing distinct roles, it is not clear whether the pro-proliferative effects of HIF-2α can necessarily overcome the anti-proliferative effects of HIF-1α.

Cyclin D2 and p21 are positive and negative regulators of CDKs, the activity of which is required for the transition from G1 to S phase. However, before the onset of DNA replication, prereplicative complexes assemble at origins of replication during G1. A principal component of the prereplicative complex is the MCM helicase, which is composed of six protein subunits that are designated MCM2–7. Loading of the MCM helicase onto an origin is referred to as replication licensing (17). The MCM proteins are present in 10- to 100-fold excess relative to origins, and the majority do not colocalize with sites of DNA synthesis, suggesting that they may have other functions (3). Screening for proteins that interact with the amino- and carboxy-terminal halves of HIF-1α identified MCM7 and MCM3, respectively (9). MCM7 enhanced the O2-dependent ubiquitination and degradation of HIF-1α by stabilizing HIF-1α-VHL-Elongin C complex formation, which was dependent on prolyl hydroxylation (Fig. 1). In contrast, MCM3 inhibited HIF-1α transactivation by a mechanism that was dependent on asparaginyl hydroxylation. Both MCM3 and MCM7 also bound to HIF-2α and inhibited its activity (9). Analysis of other MCM subunits revealed that MCM2 and MCM5 also inhibited HIF activity, whereas MCM4 and MCM6 had no effect, indicating that individual MCM subunits, rather than the intact MCM helicase, were functioning as negative regulators of HIF activity (9). These results may provide a resolution to the “MCM paradox” regarding the vast excess of MCM proteins relative to replication origins (10) by providing a novel role for these proteins that is independent of their function as a DNA helicase.

To determine whether alterations in the levels of MCM proteins affected HIF activity, we analyzed the effect of contact inhibition, which was first described in NIH-3T3 mouse fibroblasts four decades ago (8) and involves a decrease in MCM protein levels (22). Under hypoxic conditions, confluent (contact-inhibited) cells expressed higher levels of HIF-1α protein and transactivation function compared with nonconfluent (noncontact-inhibited) cells (9). Similarly, when quiescent serum-starved HCT116 cells were stimulated to re-enter the cell cycle by serum repletion, HIF transactivation activity declined in a MCM-dependent manner.

Remarkably, exposure of cells to prolonged hypoxia led to decreased MCM mRNA expression in a HIF-dependent manner, indicating that MCMs and HIFs function in a mutually antagonistic manner (9, 15). Taken together, these results have revealed the existence of a novel homeostatic mechanism to regulate cell cycle activity based on the balance between MCM and HIF proteins. Because inhibition of HIF activity by the MCMs occurs via O2-dependent hydroxylation, the ability of HIF-1α to inhibit cell cycle progression under conditions of severe hypoxia may be dominant over the ability of MCMs to promote cell cycle progression by inhibiting HIF-1α, since hydroxylation is inhibited under conditions of severe hypoxia (11).

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


