Hypoxia-inducible factor 1: master regulator of O_2 homeostasis

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Hypoxia-inducible factor 1 (HIF-1) is a transcription factor that mediates essential homeostatic responses to reduced O_2 availability in mammals. Recent studies have provided insights into the O_2-dependent regulation of HIF-1 expression, target genes regulated by HIF-1, and the effects of HIF-1 deficiency on cellular physiology and embryonic development.

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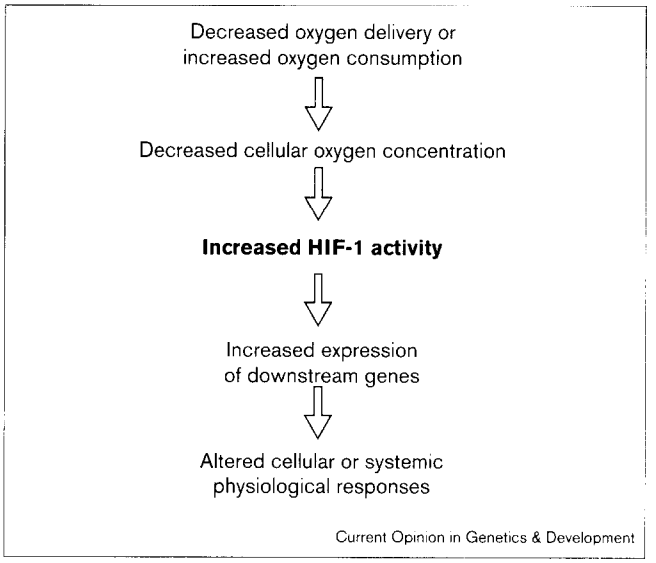
Abbreviations

ARNT	aryl hydrocarbon nuclear translocator
bHLH	basic helix-loop-helix
EPO	erythropoietin
HRE	hypoxia response element
TADs	transcriptional activation domains
VEGF	vascular endothelial growth factor

Figure 1



The central role of HIF-1 in O_2 homeostasis.

Introduction

Humans and other mammals have a constant and absolute requirement for O_2. In mammals, more O_2 is consumed than any other inorganic or organic substance and there is no substance which when withheld causes death faster. Prior to birth, humans develop complex respiratory and circulatory systems to ensure delivery of O_2 sufficient to meet the metabolic requirements of each of the ~10^{14} cells of the adult organism. In many disease states, these systems fail and hypoxia is a major factor contributing to the pathophysiology of heart attack, stroke, pulmonary hypertension, and other significant causes of mortality. Conversely, cancer cells adapt to hypoxia — utilizing these same physiological systems during the processes of tumor growth, invasion, and metastasis.

In this review, I summarize recent investigations which provide evidence that a single factor, HIF-1, plays a major role in essential adaptive responses to hypoxia. This transcriptional activator is at the center of complex pathways that bridge molecular biology, embryology, and cellular/systemic physiology (Figure 1). Recent efforts to delineate several of these pathways are described. Finally, because HIF-1 plays a central role in O_2 homeostasis, it is critical to determine the mechanisms by which HIF-1 activity is regulated by O_2 concentration and recent data addressing this issue are presented.

Isolation of HIF-1 coding sequences

Studies of the erythropoietin gene (*EPO*) led to the identification of a *cis*-acting hypoxia-response element (HRE) in the 3′-flanking region [1–3]. HIF-1 was identified as a hypoxia-inducible HRE-binding activity [4]. The HIF-1 binding site in the *EPO* HRE was used for purification of the HIF-1α and HIF-1β subunits by DNA affinity chromatography [5]. cDNA cloning revealed that both subunits were basic helix-loop-helix (bHLH)-PAS proteins: HIF-1α was a novel protein, whereas HIF-1β was identical to the aryl hydrocarbon nuclear translocator (ARNT) protein [6]. HIF-1 DNA-binding activity and HIF-1α protein expression were rapidly induced by hypoxia [6,7] and the magnitude of the response was inversely related to the cellular O_2 concentration [8]. Dimerization with HIF-1β induced a conformational change in HIF-1α, possibly mediated by the chaperone HSP90, which was required for high-affinity DNA binding [9•].

Mouse and rat HIF-1α amino acid sequences share 95% identity with the human sequence, providing evolutionary evidence for the functional importance of HIF-1α [10•,11,12•,13]. Despite the conservation of amino acid sequence, only the mouse and rat *Hif1a* genes appear to contain an alternative first exon that encodes a HIF-1α isoform lacking the first 12 amino acids following the initiator methionine [10•,13,14•–16•]. The shorter isoform contains just 4 amino acids prior to the bHLH domain, which could potentially affect the affinity and/or specificity of dimerization and/or DNA binding. HIF-1α and HIF-1β mRNAs are expressed ubiquitously in human and rodent tissues [13,15•,17].

Table 1

Functional categorization of HIF-1 target genes.

Increased O_2 delivery
Erythropoiesis
Erythropoietin (EPO) [1–4,22]
Transferrin [51•]
Increased vasodilation or vascularization
Vascular endothelial growth factor (VEGF) [19••,36,52–54]
Inducible nitric oxide synthase (NOS2) [55,56•]
Heme oxygenase 1 (HO1) [57•]
Decreased O_2 consumption
Glycolysis/energy metabolism
Glucose transporters (GLUT1, GLUT3) [19••,33••,58]
Glycolytic enzymes (Figure 2) [18,19••,33••,59–61]
Adenylate kinase 3 [33••]

HIF-1 target genes identified to date

HREs containing functionally-essential HIF-1-binding sites were identified in genes encoding vascular endothelial growth factor (VEGF), glucose transporter 1 (GLUT1), and the glycolytic enzymes aldolase A, enolase 1 (ENO1), lactate dehydrogenase A, and phosphoglycerate kinase 1 (Table 1). HIF-1 has also been shown to activate transcription of genes encoding inducible nitric oxide synthase (i-NOS) and heme oxygenase 1 (HO1) — which are responsible for the synthesis of the vasoactive molecules NO and CO, respectively — and transferrin — which, like EPO, is essential for erythropoiesis. Each of these genes contains an HRE sequence of <100 bp that includes one or more HIF-1-binding sites containing the core sequence 5′-RCGTG-3′ [18].

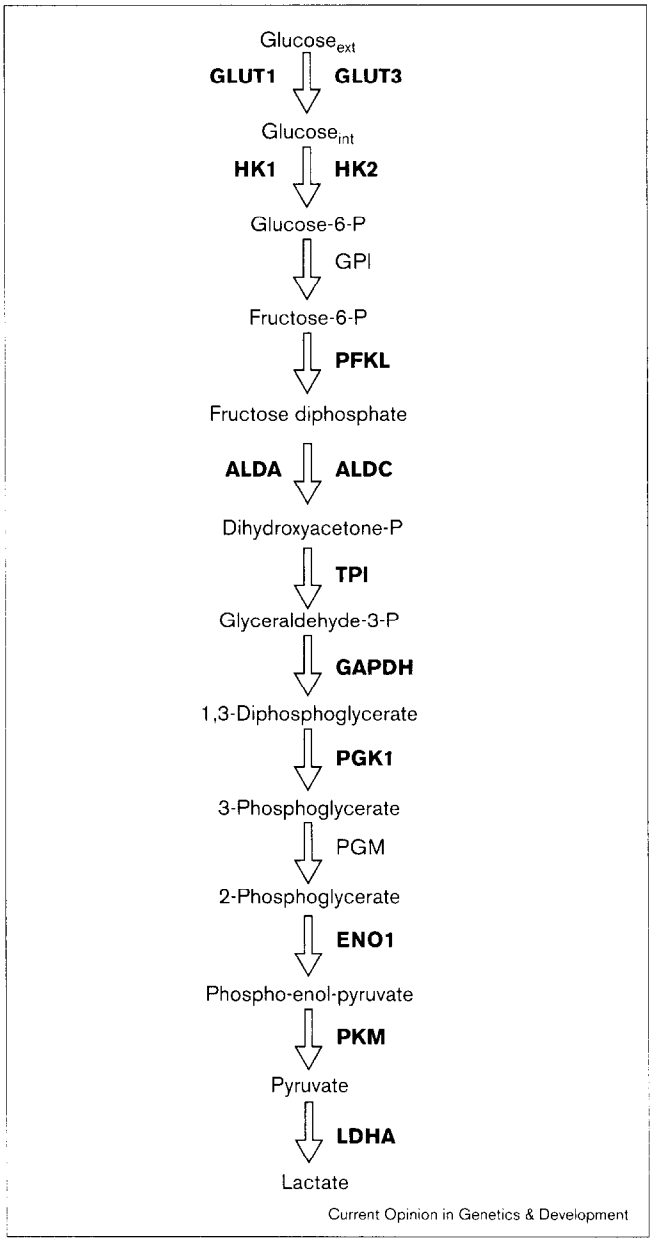
The recent analysis of embryonic stem cells that lack expression of HIF-1α [19••] or HIF-1β [20••] has confirmed the importance of HIF-1 for the expression of many of these genes and, in the case of the glycolytic pathway, has revealed that HIF-1 coordinately regulates transcription of almost every gene in the pathway (Figure 2). The known target genes demonstrate that HIF-1 facilitates both increased O_2 delivery, by promoting erythropoiesis, angiogenesis, and vasodilation, and decreased O_2 utilization, by participating in the transition from oxidative phosphorylation to glycolysis as a means of generating ATP. The genes listed in Table 1 represent only a fraction of all genes that are regulated by HIF-1. New techniques for global analysis of gene expression will probably enlarge this list by several orders of magnitude.

Regulation of HIF-1 activity by O_2 concentration

Whereas HIF-1β is present in excess and is a common subunit for multiple transcription factors (see below), HIF-1α is unique to HIF-1 and the level of its expression is the primary determinant of HIF-1 DNA-binding and transcriptional activity [8,18,21]. Induction of HIF-1α thus appears to be a critical step in the hypoxic response and occurs via increased stability of the protein and increased activity of its transcriptional activation domains (TADs) (Figure 3).

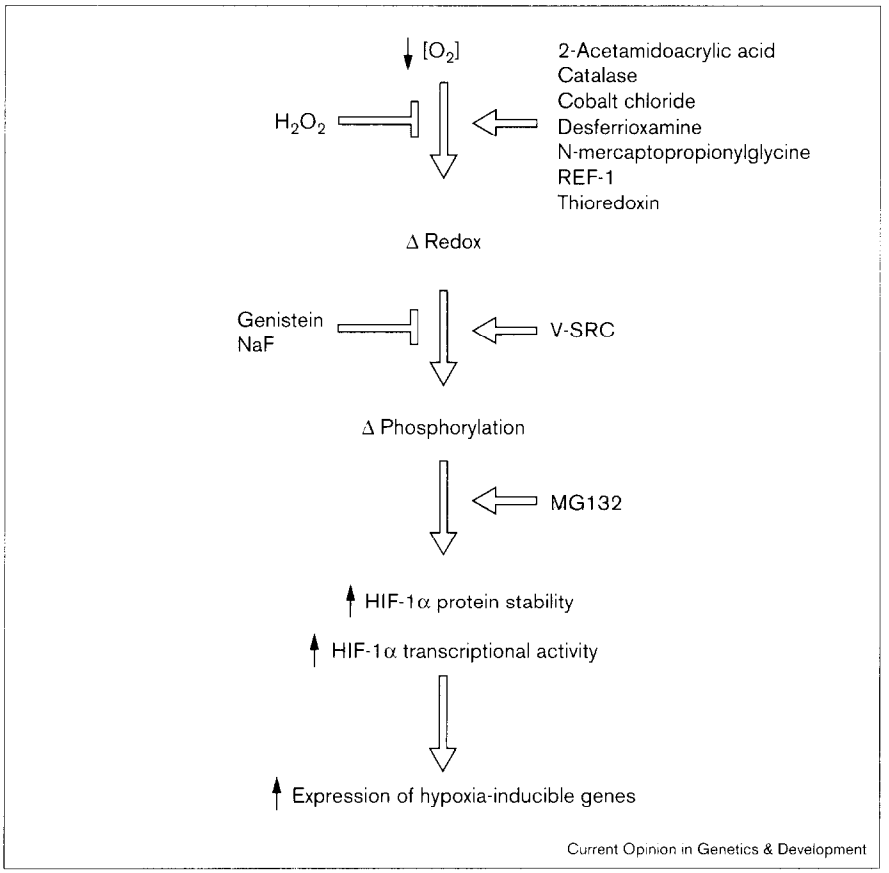
When cells exposed to hypoxia were reoxygenated, HIF-1α was rapidly degraded with a half-life of <5 min [6]. The carboxy-terminal sequence of HIF-1α contains two

Figure 2



Coordinate regulation of genes encoding glucose transporters and glycolytic enzymes by HIF-1. Genes encoding enzymes shown in bold are regulated by HIF-1 as determined by analysis of mRNA expression in HIF-1α-deficient cells [19••, 33••]. GLUT1 and GLUT3, glucose transporters 1 and 3; HK1 and HK2, hexokinase 1 and 2; GPI, glucosephosphate isomerase; PFKL, phosphofructokinase L; ALDA and ALDC, aldolase A and C; TPI, triosephosphate isomerase; GAPDH, glyceraldehydephosphate dehydrogenase; PGK1, phosphoglycerate kinase 1; PGM, phosphoglucomutase; ENO1, enolase 1; PKM, pyruvate kinase M; LDHA, lactate dehydrogenase A.

Figure 3



Hypoxia signal transduction. Reduction of cellular O_2 concentration is associated with redox changes that lead to altered phosphorylation of HIF-1α, which increases its stability and transcriptional activity, resulting in the induction of downstream gene expression. Putative inducers (horizontal arrows) and inhibitors (blocked arrows) of different steps in the proposed pathway are indicated.

20 amino acid sequences (499–518 and 581–600) that each contain 15 PEST (proline, glutamate, serine, threonine) residues, which have been implicated in protein instability; the function of these sequences, however, remains unproven. Truncation of HIF-1α at amino acid 390 resulted in a constitutively stable protein [22]. These results suggest that the HIF-1α half-life is modulated by cellular O_2 concentration and that this regulation requires sequences carboxy-terminal to amino acid 390.

Exposure of cells to the proteasome inhibitors lactacystin or MG132 resulted in detectable levels of HIF-1 DNA-binding activity under non-hypoxic conditions and a decreased rate of HIF-1 degradation under post-hypoxic conditions [23••]. Incubation of ts20 cells, containing a temperature-sensitive mutation in the E1 ubiquitin-activating enzyme, at the non-permissive temperature resulted in expression of HIF-1 activity under non-hypoxic conditions [23••]. These results suggest that rates of ubiquitination and proteasomal degradation of HIF-1 may be modulated by cellular O_2 concentration.

In addition to mediating O_2-dependent modulation of protein half-life, the carboxy-terminal half of HIF-1α is also required for hypoxia-inducible transcriptional activity [22]. Analysis of chimeric proteins containing the GAL4 DNA-binding domain and HIF-1α carboxy-terminal sequences revealed the presence of two powerful TADs (amino acids 531–575 and 786–826) separated by a domain (amino acids 576–785) that inhibited TAD function under non-hypoxic conditions [24••,25••]. Transcriptional activity mediated by the TADs was hypoxia-inducible in the absence of changes in steady-state levels of the chimeric proteins. Both protein stability and TAD function were also induced by treatment of cells with cobalt chloride or desferrioxamine, which also induce the expression of HIF-1-regulated downstream genes.

How do cells sense O_2 concentration and transduce this signal to HIF-1α? Induction of HIF-1α can be blocked by the tyrosine kinase inhibitors genistein and herbimycin A and by the serine-threonine phosphatase inhibitor sodium fluoride [26], implying the existence of a kinase/phosphatase cascade. Changes in cellular redox state have also been implicated in hypoxia signal transduction leading to HIF-1 expression [21,23••,27]. HIF-1 activity was induced by exposure of non-hypoxic cells to the thiol reducing agent N-(2-mercaptopropionyl)-glycine (NMPG) or the oxygen radical scavenger 2-acetamidoacrylic acid [23••]. Both NMPG and catalase, which catalyzes the degradation of H_2O_2 to H_2O and O_2, also induced expression of a reporter gene containing the *EPO* HRE [23••].

Over-expression of either thioredoxin or REF-1 (redox factor-1) also increased transcription of an HRE-containing reporter gene [21]. It is not known whether the stimulatory effect of redox-modulating factors occurred upstream or downstream of HIF-1α protein expression.

These results suggest that O_2 concentration is sensed by conversion to reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radical, possibly via the action of a flavoprotein NADPH oxidoreductase [28•,29•]. Diphenylene iodonium (DPI), an inhibitor of flavoproteins, blocked the induction of HIF-1 and downstream genes in hypoxic cells [30], which is the opposite of what is predicted on the basis of the model of O_2-concentration-dependent production of ROS by NADPH oxidase. Furthermore, H_2O_2 production in menadione-treated cells increased exponentially between ~4% and ~11% O_2 [31], whereas induction of HIF-1 was exponential between 6% and 0.5% O_2 [8]. Alternative hypotheses have been proposed, including the hypothesis that the mitochondrial O_2 acceptor cytochrome oxidase functions as a cellular O_2 sensor [32•]. No single model of O_2 sensing is consistent with all of the experimental data regarding inducers and inhibitors of HIF-1. It is possible that O_2 is sensed by mammalian cells via multiple mechanisms, some of which may even be cell-type-specific. A genetic approach to this problem is necessary and a system for selecting cells with mutations in hypoxia signal transduction has recently been described [33••].

Involvement of HIF-1 in (patho)physiology

When near-term fetal sheep were subjected to chronic anemia by daily isovolemic hemorrhage, the hematocrit and arterial O_2 content decreased 3-fold within one week [34]. Concomitantly, the fetuses underwent remarkable physiological adaptation: cardiac output increased by 50% and the heart:body weight ratio increased by 30%. To maintain myocardial oxygenation under these circumstances, myocardial blood flow increased 5-fold [34]. Capillary morphometry revealed signficantly increased capillary density and minimal capillary diameter and decreased intercapillary distance in the hearts of anemic fetuses [35••]. In the same samples, VEGF protein and mRNA were increased 4.5- and 3.2-fold respectively, and HIF-1α protein expression was increased 3.8-fold. There was thus a striking correlation between increased myocardial vascularization and expression of VEGF and HIF-1α. The physiological pathway described above (increased O_2 consumption→hypoxia→HIF-1→VEGF→vascularization) is clearly adaptive for the organism as, in its absence, cardiac output could not increase sufficiently to compensate for decreased blood O_2-carrying capacity and/or fatal myocardial ischemia would ensue.

Adaptation of cancer cells to hypoxia is also critical for tumor progression — a process that is decidedly not in the organism's best interests. ARNT (HIF-1β)-deficient mouse C4 hepatoma cells demonstrated markedly reduced induction of VEGF mRNA in response to hypoxic culture conditions [36–38]. When injected into nude mice, the mutant C4 cells formed tumors at a significantly reduced rate relative to wild-type Hepa1 cells, whereas partially revertant RB13 cells with intermediate levels of HIF-1 expression demonstrated an intermediate rate of tumor growth [39••,40••]. Capillary density was increased in tumors derived from Hepa1 compared to C4 cells and necrotic areas of Hepa1 tumors were surrounded by high levels of VEGF and GLUT1 mRNA expression which was not detected in corresponding areas of C4 tumors [40••].

HIF-1α expression, HIF-1 DNA-binding activity, and transcriptional activation of HIF-1 regulated genes such as *VEGF* and *ENO1* were also induced in non-hypoxic cells transformed by V-SRC, the oncogene of Rous sarcoma virus, thus establishing another link between HIF-1 and tumor progression [39••]. Constitutive expression of HIF-1 may contribute to the aerobic glycolysis that is one of the first signs of V-SRC-mediated cellular transformation. Cells transformed by overexpression of H-RAS also demonstrated increased *VEGF* promoter activity that was dependent upon an intact HIF-1 binding site [41•].

Involvement of HIF-1 in embryogenesis

The essential role of HIF-1 in embryonic development has been demonstrated by the analysis of mice that lack expression of HIF-1α [19••] or ARNT/HIF-1β [20••,42••]. *Arnt*$^{-/-}$ mice manifested developmental delay, neural tube defects, abnormal vascularization of the yolk sac, embryo, and placenta, and died by embryonic day (E) 10.5 [20••,42••]. Given the essential role of HIF-1 in activating *VEGF* transcription in hypoxic cells [36], these data suggested that HIF-1 deficiency was responsible for the observed pathology. However, ARNT dimerizes with several other mammalian bHLH-PAS transcription factors in addition to HIF-1α, including the aryl hydrocarbon receptor [43], singleminded-2 [44], and HIF-2α (also known as EPAS1, HLF, HRF, and MOP2) [45•–48•]. Loss of activity of one or more of these factors could therefore contribute to the observed phenotype. Furthermore, the *Arnt2* gene encodes a protein with a high degree of sequence similarity to ARNT which is expressed primarily within the central nervous system [49]. If ARNT2 can dimerize with HIF-1α, then ARNT-deficient embryos may have manifested only a partial deficiency of HIF-1.

In light of these issues, it was not surprising that HIF-1α and ARNT (HIF-1β) deficiency resulted in overlapping but distinct embryonic phenotypes. *Hif1a*$^{-/-}$ embryos suffered developmental arrest at E8.5–8.75 and died at E10.5 [19••]. Analysis of viable embryos at E9.75–E10.0 revealed failure of neural tube closure with massive death of cephalic mesenchymal cells and the appearance of markedly enlarged/dilated vascular structures. Vascularization and development at E8.5–8.75 appeared normal, whereas one

day later the entire vascular system of the branchial/cephalic region was disrupted. In contrast to the demise of the cephalic vasculature and mesenchyme, the presumptive myocardium of *Hif1a*$^{-/-}$ embryos was strikingly hyperplastic, resulting in obliteration of the heart lumen. Neither the cephalic nor myocardial defects were observed in *Arnt*$^{-/-}$ embryos. Yolk sac vascularization and placental development in the growth-arrested *Hifa*$^{-/-}$ embryos and stage-matched *Hifa*$^{+/+}$ embryos were not significantly different. The analysis of *Hif1a*$^{-/-}$ embryos demonstrated that HIF-1 is required for the establishment of the embryonic cardiovascular system. Taken together with the fetal sheep studies described above, these results indicate that HIF-1α is required both for the formation of key physiological systems during embryonic development and for their subsequent utilization and thus represents a master regulator of O_2 homeostasis.

Conclusion

HIF-1 plays essential roles in embryonic development, cellular and systemic physiology. Manipulation of HIF-1 activity — either pharmacologically or by gene therapy — may represent a novel approach to the treatment of cancer, cerebral and myocardial ischemia. In cancer cells, inhibition of HIF-1 activity (e.g. by expression of a virally-transduced dominant-negative form of HIF-1α [22,36]) may prevent tumor adaptation to hypoxic conditions. Anti-HIF-1α therapy might be particularly efficacious when administered in concert with anti-angiogenic therapy. Conversely, expression of wild-type HIF-1α in ischemic tissue may stimulate angiogenesis, as has been demonstrated for gene therapy with VEGF and other angiogenic factors [50] but may also promote survival of ischemic cells during the period when vascularization is in progress. Research in this field thus holds out the prospect of understanding fundamental aspects of development and physiology while, at the same time, providing novel therapeutic approaches to the most common causes of mortality in the western world.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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The bHLH-PAS domain (amino acids 1–407) of ARNT (HIF-1β) dimerizes with HIF-1α but the resulting heterodimer has low DNA-binding activity. Similarly, the first 245 amino acid region of HIF-1α, encompassing the bHLH domain and half of the PAS domain, dimerizes to HIF-1β but the resulting dimer has low DNA-binding activity [22]. Although physiological regulation of HIF-1 activity at the level of dimerization or DNA binding has not been demonstrated, these papers [9•,22] suggest that the potential for such regulation exists.

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Transactivation functions of HIF-1α are shown to be regulated by hypoxia in a complex, cell-type-specific manner independent of the regulation of HIF-1α protein levels. Two minimal, independently-functioning TADs, spanning amino acids 531–575 and 786–826, are identified in the context of fusion proteins containing an amino-terminal GAL4 DNA-binding domain. The data suggest that hypoxia results in a derepression of TAD function.

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Minimal TADs spanning amino acids 549–582 and 775–826 are identified in the context of fusion proteins containing an amino-terminal GAL4 DNA-binding domain and carboxy-terminal ARNT transactivation domain (the latter representing a potentially confounding factor). In contrast to [24••], expression of several fusion proteins is regulated by O_2 concentration, suggesting that ARNT sequences may have contributed to this phenomenon.

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