Peer-reviewed version available at *Biomedicines* **2018**, *6*, 47; doi:10.3390/biomedicines6020047

Hypoxia and Chromatin, a focus on transcriptional repression mechanisms.

Michael Batie[1], Luis del Peso[2], and Sonia Rocha[1]*

1. Department of Biochemistry, Institute of Integrative Biology, University of Liverpool. Crown street. L697ZB Liverpool. United Kingdom.
2. Department of Biochemistry, Institute of Biomedical Research. Autonomous Madrid University. Arturo Duperier, 4. 28029 Madrid. Spain.

*Corresponding author: Sonia Rocha; srocha@liverpool.ac.uk

Abstract

Hypoxia, or reduced oxygen availability, has been studied extensively for its ability to activate specific genes. Hypoxia induced gene expression is mediated by the HIF transcription factors, although not exclusively so. Despite the great knowledge on the mechanisms by which hypoxia activates genes, much less is known about how hypoxia promotes gene repression. In this review, we discuss the potential mechanisms underlying hypoxia-induced transcriptional repression responses. We highlight HIF-dependent and independent mechanisms, but also the potential roles of dioxygenases with functions at the nucleosome and DNA level. Finally, we discuss recent evidence regarding the involvement of transcriptional repressor complexes in hypoxia.

Keywords: Hypoxia; Chromatin; Transcriptional repression; Repressor Complexes; JmjC, Histone methylation, HIF

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

Introduction

Hypoxia

Decreases in oxygen availability are generally termed as hypoxia. These can occur at the organism level, such as when climbing high mountains, or at the cellular level, when oxygen supply is reduced and/or metabolic activity is high [1-3]. Changes at the cellular level in response to hypoxia are paramount for cellular and organismic survival [4].

To achieve a cellular response to hypoxia, cells have evolved mechanisms that impinge at all levels of gene expression regulation [2, 5], as well as energy conservation processes. These involve blocks in translation and the cell cycle, and switches in metabolic processes such as moving from oxidative phosphorylation to glycolysis [2]. A major coordinator of the cellular response to hypoxia is the transcription factor family, Hypoxia Inducible Factor (HIF).

The HIF family is composed by three different heterodimers, encompassing HIF-1β (gene name Aryl Hydrocarbon Receptor Nuclear Translocator (ARNT), shared by all dimers), and HIF-1α; HIF-2α (gene name Endothelial PAS Domain Protein 1 EPAS1)) and HIF-3α. Oxygen sensitivity is conveyed to HIF via the action of dioxygenases, most specifically Prolyl Hydroxylase Domain-Containing Proteins (PHDs) and Factor Inhibiting HIF (FIH) (reviewed in [4]). Proline hydroxylation of HIF-α in their oxygen degradation domain create a high affinity binding site for the tumour suppressor protein von Hippel Lindau (VHL), which is part of the E3-ubiquitin ligase complex containing cullin-2, elongin B/C and Ring-Box 1 (RBX1) [6]. VHL-dependent ubiquitination signals HIF-α for proteasomal and autophagy mediated degradation [6, 7]. On the other hand, FIH-dependent hydroxylation of HIF-α, results in an impairment

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

of the recruitment of the key coactivator protein CBP/p300, which is required for a minimum of 40% of all HIF-dependent genes to be expressed [8-11].

HIF mediated gene expression is largely achieved by direct binding of HIFs to the Hypoxia Response Elements (HREs) present in the regulatory region of target genes [12-19]. However, HIF binding is limited to just a few hundred of the 3.1 million RCGTG motifs present in the human genome [20]. Therefore, chromatin accessibility is one of major determinants of HIF binding, although not the only one, which provides a potential explanation for the differential genome-wide HIF binding profile and gene expression patterns in response to hypoxia observed across cell lines.

As mentioned above, HIF's role in coordinating the cell's response to hypoxia is achieved by transcriptional regulation. For the majority of cases analysed, HIF is an activator of transcription, with very few cases of direct transcriptional repression being described (see below). To activate gene expression, HIF has to engage with chromatin, in order to access its DNA binding sites across the genome. Several studies have analysed genomic wide binding site for HIFs in a variety of cellular backgrounds, identifying binding to genes HIF is known to transactivate but also in genes who's expression is not altered in hypoxia [14]. As such, chromatin should be considered as an important player in the cellular response to hypoxia.

Chromatin

In mammalian cells, DNA is stored as chromatin. Chromatin is a complex and highly dynamic structure containing a mix of DNA and proteins. Chromatin is thus a fundamental regulator of cellular processes requiring access to DNA, including DNA-repair, DNA replication, and transcription. As mentioned before, gene transcriptional changes are a key part of the hypoxia response, and as such, delineating the crosstalk

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

between chromatin structure and transcription is essential in understanding the cellular response to low oxygen stress.

The structural repeating unit of chromatin is a nucleosome, which consist of 147 base pairs of DNA wrapped 1.65 times around a histone octamer (reviewed in [21, 22]). The histone octamer consists of two copies each of the four core histones (Histone (H) 2A, H2B, H3 and H4), which forms as a result of tetramer dimerization between a H3/H4 and a H2A/H2B tetramer (reviewed in [23]). Nucleosomes are linked by linker DNA and linker histone H1 and condense to higher order chromatin structures, eventually forming chromosomes (reviewed in [24]). There are two major functional states of mature chromatin, heterochromatin and euchromatin (reviewed in [25]). Heterochromatin is a highly compact state which constitutes a barrier to DNA binding and is associated with silenced loci. Conversely, euchromatin has a more open conformation and is associated with actively transcribing and poised loci. Further to these two major conformations, microscopy techniques and biochemical assays have shown that chromatin structure is more complex, with additional chromatin compaction states [26-28]. In *Drosophila* cells, five distinct chromatin states have been identified through DamID assays followed by Chromatin Immunoprecipitation (ChIP) and microarrays [27]. These five states differ in protein binding, histone modifications, biochemical characteristics and transcriptional activity. On the other hand, researchers using a live cell quantitative FLIM–FRET based assay for chromatin compaction, reported three chromatin states based on spatial characteristics [26]. This study also found altered variations in the relative signals of the three types of chromatin state in response to ATP depletion, Trichostatin A (TSA) treatment and different stages of the cell cycle, supporting previous work on chromatin compaction dynamics. More recently the aforementioned technique has been used to measure chromatin

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

compaction in the model organism *C. elegans,* finding heterogeneous chromatin compaction at the whole organism level with nanoscale spatial and temporal resolution [28]. These studies among others demonstrate the complexity of chromatin organization in metazoan organisms, indicating the existence of intricate control mechanisms.

There are various interrelated mechanisms by which chromatin structure is regulated, including Chromatin Remodeller Complex (CRC) functions (reviewed in [29]), post translational modifications to histones (reviewed in [30]), incorporation of histone variants (reviewed in [31]), DNA methylation (reviewed in [32]), action of non-coding RNAs (ncRNAs) (reviewed in [33]) and chromatin architectural proteins (reviewed in [24]) (Figure 1). These mechanisms dictate the chromatin landscape, which is a key determinant in the transcriptional output of the cell and thus cell fate decisions. Chromatin is responsive to numerous stimuli and developmental cues [34] and is often deregulated in disease (reviewed in [35]).

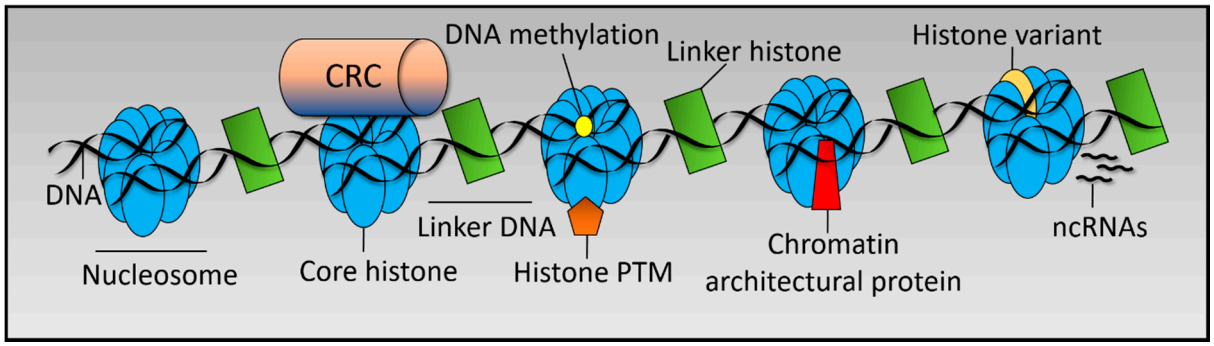

Figure 1. Chromatin structure. Simplified linear diagram of chromatin highlighting the main mechanisms by which chromatin structure is regulated. Chromatin Remodeller Complex (CRC), post translational modification (PTM), non-coding RNAs, (ncRNAs).

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

An emerging field is the study of chromatin structure in response to hypoxia, where some experimental evidence is now being published.

Hypoxia induced chromatin changes

Hypoxia has been shown to induce changes in chromatin structure, most notably histone methylation, acetylation, and DNA methylation. In this review, we will focus on methylation.

Less is known pertaining to chromatin compaction states in response to low oxygen stress. Through the use of Single Molecule Localisation Microscopy (SMLM) and *in situ* DNA digestion coupled with fluorescent microscopy, a rapid change in chromatin architecture and increase in chromatin compaction has been reported in human cardiomyocytes deprived of oxygen and nutrients [36]. Interestingly, the change in chromatin architecture was found be rapidly reversible in response to reoxygenation and replenishment of nutrients, demonstrating the dynamic capacity of chromatin to sense and respond to oxygen and metabolic changes [36]. Another study determined that A431 cancers cells treated with 0.1% oxygen for 48 hours have reduced sensitivity to Mononuclease digestion, suggesting increased heterochromatin composition [37]. Through the use of proteomics, this study also identified an increase in Heterochromatin Protein 1 Binding protein 3 (HP1BP3) in the chromatin bound fraction of cells treated to hypoxia. HP1BP3 has previously been shown to function in maintaining heterochromatin integrity, thus could be a player in inducing hypoxic chromatin compaction [37, 38].

Chromatin looping, which brings distal sequence regions together, represents additional mechanisms by which transcription is regulated by chromatin architecture

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

[39, 40]. Further to proximal promoter binding at HRE sites, HIF-1α and HIF-2α also bind to intergenic regions of the genome [12, 14, 41, 42] and there is evidence of HIF binding regulating distal gene expression through Promoter Enhancer Interactions (PEIs) [14]. Work from the Ratcliffe and Mole laboratories, utilising ChIP sequencing and Capture C in MCF7 cells treated to 0.5% for 16 hours, has revealed genome wide HIF binding-HIF regulated gene PEIs [41]. This study, and others, also elucidated that HIF promoter binding in hypoxia is predominantly at pre-established and primed, promoter enhancer loops [41, 43].

Despite the increase in evidence for chromatin regulation in hypoxia, there is still a great deal of unknowns. The use of imaging and sequencing technologies to study chromatin spatial organization should be used to gain further insight into the dynamic interplay between hypoxia, chromatin and gene transcription. This would help elucidate how chromatin contributes to gene repression in hypoxia.

Histone methylation-focus on repression

Histone methylation is a dynamic and reversible post translational modification at lysine (K) and arginine (R) N terminal tails of histones. These modifications can provide binding sites for chromatin binding proteins and the histone methylation landscape is predictive of gene transcriptional state, transcription factor binding and chromatin compaction [44, 45]. H3K4, H3K9, H3K27, H3K36, are amongst the most common and well-studied histone methylation sites. H3K9 di-methylation (me2)/tri-methylation (me3) and H3K27me3 are linked to transcriptional repression and are key players in cell fate decisions and tissue specific transcriptional control (reviewed in [46]). H3K9me2/3 are markers of heterochromatin and are found at coding and non-coding regions (reviewed in [47]). Both modifications are associated with gene

silencing via crosstalk with DNA Methyl Transferases (DNMTs) and recruitment of other chromatin modifying protein such Heterochromatin protein 1 (HP1) which can which can regulate heterochromatin formation (reviewed in [48]). H3K27me3 is located primarily at gene promoters of open chromatin and is involved in gene repression through recruitment of Polycomb Repressive Complexes (PRCs) (reviewed in [49]). H3K27me3 also marks poised enhancers and co-occupies promoters with the active histone modification H3K4me3, termed bivalent promoters (reviewed in [50].)

Histone Methyl Transferases (HMTs) add methyl groups to histones, transferring a methyl group from S Adenosyl Methionine (SAM) (reviewed in [46]). Two families of enzymes remove histone methylations. LSDs target H3K4me1/2 and H3K9me1/2 through a Flavin Adenine Dinucleotide (FAD) dependent amine oxygenase reaction [51]. Jumonji C (JmjC) histone demethylases target a much broader range of histone targets (reviewed in [52, 53]). The later are molecular dioxygenases, requiring oxygen, iron and 2-oxoglutarate for demethylation. This oxygen dependency of JmjC histone demethylases provides an important link to chromatin structure and oxygen sensing (reviewed in [52, 53]). The writers and erasers of H3K9 and H3K27 methylation are shown in Table 1.

.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

Writer	Eraser	
HMTS	**JmjC histone demethylases**	**LSDs**
G9α (H3K9/H3K9me1/me2)	KDM3A (H3K9me1/me2)	LSD1
GL9 (H3K9/ H3K9me1/me2)	KDM3B (H3K9me1)	(H3K9me1/me2)
SUV39H1 (H3K9me1/me2)	KDM4A (H3K9me2/me3)	(H3K4me1/me2)
SUV39H2 (H3K9me1/me2)	KDM4B (H3K9me2/me3)	LSD2
SETDB1 (H3K9)	KDM4C (H3K9me2/me3)	(H3K4me1/me2)
PRDM2 (H3K9)	KDM4D (H3K9me2/me3)	
PRDM3 (H3K9)	KDM4E (H3K9me2/me3)	
PRDM6 (H3K9)	KDM6A (H3K27me2/me3)	
EZH2 (H3K27/H3K27me1/me2)	KDM6B (H3K27me2/me3)	
	KDM7A (H3K9me1/me2) (H3K27me1/me2)	
	PHF2 (H3K9me1/me2) (H3K27me1/me2)	
	PHF8 (H3K9me1/me2)	
	MINA (H3K9me3)	

Table 1. Writers and erasers of H3K9 and H3K27 methylation. Histone Methytransferases (HMTs), Jumonji C (JmjC) histone demethylases and Lysine Specific Demethylases (LSDs) targeting H3K9 and H3K27 are shown (targets are in brackets). Euchromatic Histone Lysine Methyltransferase 2 (EHMT2, G9α), G9α Like Protein 1 (GPL), Suppressor Of Variegation 3-9 Homolog (SUV39H), SET Domain Bifurcated 1 (SETDB1), PR/SET Domain (PRDM), Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2), Lysine Demethylase (KDM), PHD Finger Protein (PHF), Myc-Induced Nuclear Antigen (MINA).

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

Hypoxia induced increases in both active and repressive histone methylations have been shown in several human cancer lines, mouse embryonic fibroblasts and human tumour samples [54-59]. With regards to repressive modifications, H3K9me2 and H3K27me3 increased in Hepa 1-6 cells exposed to 0.2% oxygen for 48 hours [54]. H3K9me3 increases were also observed in RKO cells exposed to 2% and 0.1% oxygen, these changes were rescued upon reoxygenation [59]. Additionally, H3K9me2/me3 increases have were shown in mouse macrophages exposed to 1% oxygen for 24 hours [56]. Total H3K9me2/me3 levels were also elevated in A549 cells exposed to 0.5% oxygen, as well as site specific increases at the hypoxia repressed gene promoters, MutL Homolog 1 (MLH1) and Dihydrofolate Reductase 2 (DHFR2) [58]. Here researchers detected increased H3K9me3 after 90mins of 0.5% oxygen exposure, this is the only such study which has investigated histone methylation levels in response to acute hypoxia. Whilst the aforementioned studies investigated total levels of histone modifications and some site-specific changes, *Prickaerts et al* elucidated specific increases in H3K27me3 and H3K4me3 in response to hypoxia on a genome wide scale through the use of ChIP sequencing integrated with microarray analysis in MCF7 cells exposed to oxygen deprivation and reoxygenation [57]. Many of these changes were reversible upon reoxygenation and showed correlation with transcriptional changes in hypoxia. Interestingly, the researchers uncovered evidence for hypoxia acquired promoter bivalency modulating poised/active gene transcriptional control. Xenografts of human breast and lung cancer were also found to have increased H3K27me3 and H3K9me3 respectively [57, 59].

Mechanistically, hypoxic induction of histone methylation levels has been attributed to inhibition of JmjC histone demethylases [57, 58]. JmjC histone

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

demethylases are oxygen dependent enzymes and also have a catalytic fold similar to that of FIH [60, 61]. Whilst oxygen dependency of the majority of JmjC enzymes has not been established, *In vitro* histone peptide methylation assays and histone methylation assays from cell lysates have demonstrated the potential of some of these enzymes to function as bona fide oxygen sensors [57, 58, 62, 63]. Among these, the H3K27 demethylase KDM6B and the H3K9 demethylase KDM4E were inhibited over physiologically relevant oxygen concentrations, the latter displaying similar oxygen dependency kinetics to PHD2 [63]. Whilst the H3K9 demethylase KDM4A has a reported km for oxygen of 173 +/- 23μM, placing it between FIH and PHDs with regards to oxygen dependency [62]. Furthermore, increased H3K9me3 in U2OS cells overexpressing KDM4A was reduced in a stepwise fashion upon exposure to 5, 1 and 0.1% oxygen [62]. These studies demonstrate a role of JmjCs enzymes in functioning as cellular oxygen sensors.

Many JmjC histone demethylases are transcriptionally upregulated in hypoxia some of which are direct HIF target genes (reviewed in [53, 64]). It is speculated that upregulation of JmjC histone demethylases in response to low oxygen may be a feedback mechanism to help maintain to retain histone methylation status of the cell. However, it should be noted that there is evidence for JmjC histone demethylases remaining active in response to low oxygen stress and mediating the hypoxic response via histone demethylation [65, 66]. KDM4C and KDM3A were found to bind HIF-1α and function as an enhancer of HIF-1α transactivation activity in hypoxia via H3K9 demethyation at hypoxia responsive promoters. Thus, determining the dynamics of JmjC histone demethylase oxygen sensitivities, activities in different oxygen environments and cell backgrounds, and consequences this has on hypoxic gene transcription is needed. Non histone targets and histone methylation independent

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

functions of JmjC histone demethylases should also be considered when investigating their possible roles in hypoxia induced transcriptional repression (reviewed in [53]).

Pertaining to HMTs and histone methylation in hypoxia, G9α (gene name Euchromatic Histone Lysine Methyltransferase 2 (EHMT2)) protein levels are induced post transcriptionally in hypoxia [58]. G9α was recently identified as a PHD1 target for hydroxylation and is degraded by in a VHL dependent manner [67]. Moreover, via promoter H3K9me2, G9α mediates transcriptional repression at a subset of hypoxia repressed genes and there is growing evidence for chemotherapeutic benefit in targeting G9α through dysregulation of hypoxic gene expression [67-69]. SET Domain Bifurcated 1 (SETDB1) and Suppressor Of Variegation 3-9 Homolog (SUV39H) 2 protein levels are also stabilised is low oxygen stress and hypoxia induced, SETDB1 mediated H3K9me3 upregulation on the ATM And p53-Associated KZNF Protein (APAK) gene reduces its expression [70]. This triggers an increase in p53-dependent hypoxia-induced apoptosis and manipulation of this pathway deregulates cell viability in hypoxia [70]. Elevated H3K9me3 in hypoxia is also required for Ataxia telangiectasia mutated (ATM) activation in the absence of DNA damage, facilitating DNA replication in a low oxygen environment [59]. This study suggests a mechanism for H3K9me3 mediated activation of ATM in hypoxia involving transcriptional repression of ATM-specific phosphatases, including Protein Phosphatase 2 (PP2A). Developmental importance of H3K9 methylation and hypoxia is shown by SUV39H1 and SUV39H2, which are hypoxia inducible, and loss of their expression is associated with the epigenetic changes required during foetal lung development [71].

Given the recent evidence of hypoxia induced histone methylation changes, further analysis on how histone methylation contributes to the transcriptional repression observed in hypoxia is therefore necessary.

Chromatin remodellers in hypoxia-focus on repression

Hypoxia engagement with chromatin remodellers has been analysed mostly in the context of transcriptional activation. Four main families of chromatin remodeller can be found in mammalians. These are SWItch/Sucrose Non-Fermentable (SWI/SNF), Chromodomain Helicase DNA-binding CHD (CHD), Inositol-Requiring 80 and Imitation SWI families (Figure 2) [52, 53]. Many more sub-complexes exist, giving rise to increased complexity in function and regulation (reviewed in [72]).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

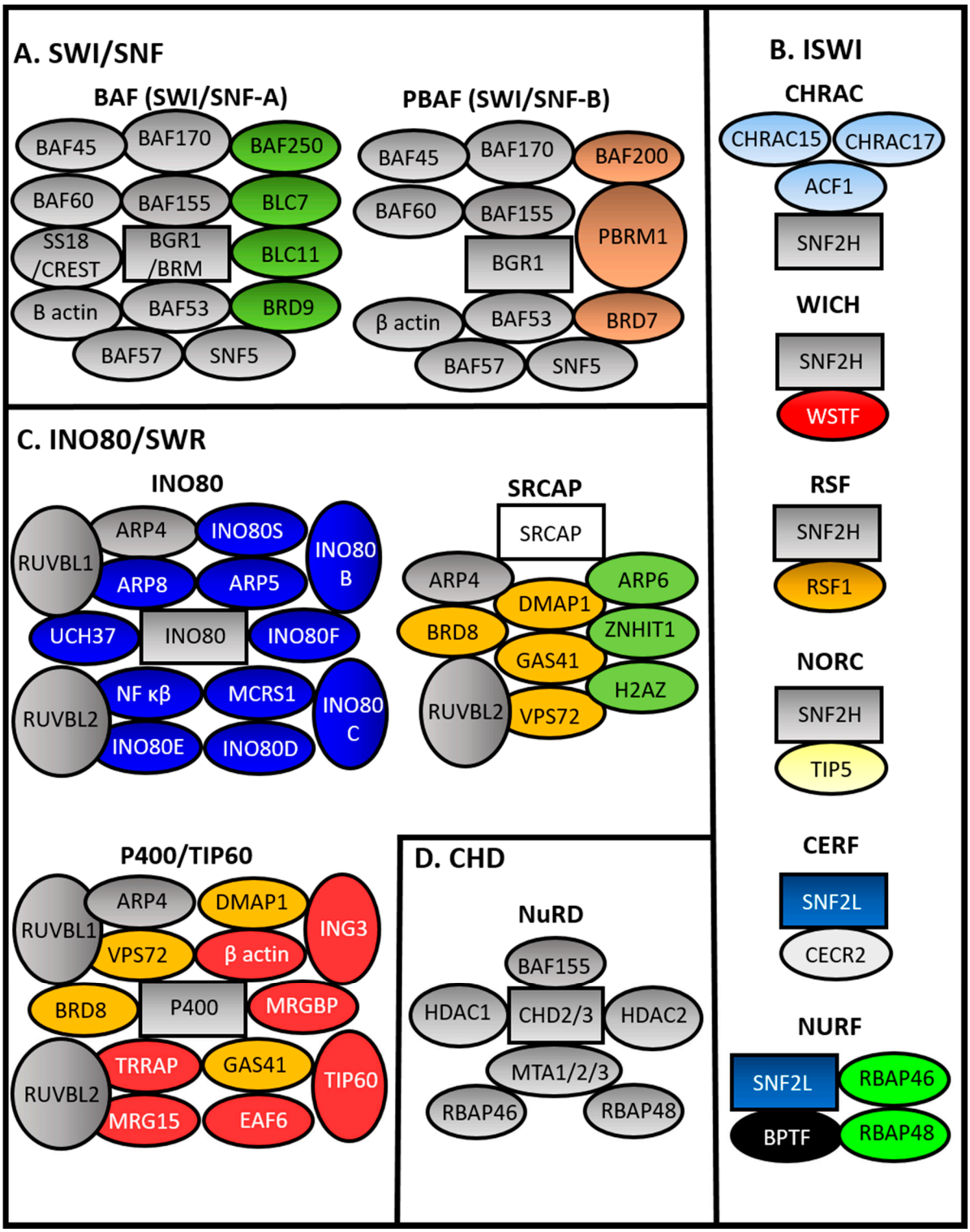

Figure 2. Mammalian Chromatin remodeller complexes. Chromatin Remodeller Complexes (CRCs) from the 4 subfamilies of CRCs based on ATPase domains are shown. A) SWItch/Sucrose Non-Fermentable (SWI/SNF), B) Imitation SWI (ISWI), C) Inositol-Requiring 80/ Sick With RSC (INO80/SWR), D) Chromodomain Helicase DNA-binding CHD (CHD). Rectangles represent ATPase domain. Brahma (BRM) BRM Related Gene 1 (BRG1), BRG1/BRM Associated Factor (BAF), Polybromo

Associated BAF Complex (PBAF), Snf2 Related CREBBP Activator Protein (SRCAP), Tat Interacting Protein 60 (TIP60), Nucleosome Remodeling Deacetylase (NuRD), Chromatin Accessibility Complex (CHRAC), Remodeling And Spacing Factor (RSF), Nucleolar Remodeling Complex, Cat Eye Syndrome Chromosome Region Candidate 2 (CERC2), CECR2 Containing Remodeling Factor (CERF), Nucleosome Remodeling Factor (NURF), B-Cell CLL/Lymphoma (BCL), Bromodomain Containing (BRD), Polybromo 1 (PBRM1), RuvB Like AAA ATPase (RUVBL)(RVB), Actin related protein (ARP), Ubiquitin C-Terminal Hydrolase L5 (UCH37 Microspherule Protein 1 (MCRS1), Nuclear Factor Kappa B Subunit (NF κβ), DNA Methyltransferase 1 Associated Protein 1 (DMAP1), Glioma-Amplified Sequence 41 (GAS41), Vacuolar Protein Sorting 72 (VPS72), Zinc Finger HIT-Type Containing 1 (ZNHIT1), H2A Histone Family Member Z (H2AZ), Transformation/Transcription Domain Associated Protein (TRRAP), MORF-Related Gene 15 Protein (MRG15), MRG Domain Binding Protein (MRGBP), Esa1 Associated Factor 6 (EAF6), Inhibitor Of Growth Family Member 3 (ING3), Histone Deacetylase, (HDAC), Metastasis Associated (MTA), Retinoblastoma-Binding Protein (RBAP), ATP-Dependent Chromatin Assembly Factor 1 (ACF1), Sucrose Nonfermenting Protein 2 Homolog (SNF2H), Sucrose Nonfermenting 2-Like Protein 1 (SNF2L), Williams Syndrome Transcription Factor (WSTF), Bromodomain PHD Finger Transcription Factor (BPTF).

With relevance to the hypoxia signalling pathway, the SWI/SNF family seems to be particular important, with high level of human mutations found in renal clear cell cancer, where VHL is also found highly mutated [73]. SWI/SNF complex (Figure 2A) can be subdivided into two sub-complexes called BRG1/BRM Associated Factor (BAF) and Polybromo Associated BAF Complex (PBAF). These are defined not only by their catalytic subunits (BRM Related Gene 1 (BRG1) or BRM) but also by differences in assessor factors such as BAF250/BAF250B (BAF), and Polybromo 1 (PBRM1) (PBAF) [74].

It is known that hypoxia engages and requires SWI/SNF for activation of HIF and its targets [53, 75]. However, there is no indication so far that SWI/SNF can be

involved in hypoxia mediated repression and has been associated only with transcriptional activation in hypoxia.

Perhaps the chromatin remodelling family most often associated with transcriptional repression is ISWI (Figure 2B). In mammalians, it is subdivided into several additional complexes depending on assessor partners for the two catalytic subunits Sucrose Nonfermenting Protein 2 Homolog (SNF2H) and Sucrose Nonfermenting 2-Like Protein 1 (SNF2L) (Figure 2B). ISWI is known to control nucleosome spacing and this has been shown to have a cooperative action with another family of remodellers called CHD [76]. ISWI also associates with additional factors and is important for the function of CTCF in establishing chromatin barriers [77].

ISWI has been shown to contribute to the cellular response to hypoxia by controlling levels of FIH, and hence some of the HIF-1 dependent targets [78]. In addition, it also controls the levels of FIH-independent targets suggesting a broader role in transcriptional regulation in hypoxia [78]. Interestingly, ISWI was identified as part of a complex containing the transcriptional corepressor C-terminal Binding Protein (CtBP) [79]. CtBP has been associated with the transcriptional response to hypoxia through a variety of studies [80-84]. It is thus possible that ISWI action in hypoxia, is connected to CtBP, however, this has not been formally addressed.

INO80 family (Figure 3C) comprises of a large family of remodellers with the catalytic subunits INO80 and Snf2 Related CREBBP Activator Protein (SRCAP), characterised by Rec-like helicase domains [85]. This family is known to be involved in nucleosome sliding and histone exchange [86]. Associations with the hypoxia response have been found, but whether the chromatin remodelling aspect of the complex is required is still not clear. Components of the Tip60 complex, more

specifically Pontin (gene name RuvB Like AAA ATPase (RUVBL)1) and Reptin (RUVBL2) have been associated with the modulation of the cellular response to hypoxia. In hypoxic conditions, Reptin is methylated at lysine 67 by the methyltransferase G9α and this modification allows its binding to HIF-1α and its recruitment to the promoters of hypoxia-responsive genes where it negatively affects their transcription [87]. Consequently, Reptin deletion results in enhanced induction of a subset of HIF targets, suggesting that the axis G9α/Reptin may work as a negative feed-back loop that acts to limit HIF activity. However, since these studies focused on the role of Reptin on genes upregulated by HIF, its potential role on gene repression under hypoxia remains unexplored. Similarly, methylation of Pontin by G9α induced by hypoxia, also potentiates HIF-mediated activation [88], however, no data was provided regarding the role of Pontin in hypoxia mediated repression in this study.

At present there are no studies concerning the role of CHD family in the response to hypoxia.

DNA methylation in hypoxia

One of the most studied aspects of chromatin changes and transcriptional repression mechanisms is DNA methylation. DNA methylation, classically occurs at CpG islands present at promoters, leading to inhibition of promoter activity via failing to recruit specific transcription factors or by active recruitment of transcriptional repressor complexes [89]. DNA methylation is set by DNMTs, of which three have been described in humans, DNMT1, DNMT3A, and DNMT3B [90, 91]. As with most processes in the cell, DNA methylation is a reversible state, through the involvement of specific enzymes called Ten-Eleven-Translocation (TET) [5, 90]. These enzymes remove DNA methylation by hydroxylation of 5-methylcytosine, followed by further

potential oxidation reactions. These additional modified bases are removed by thymine DNA glycosylase and base excision repair pathways [5].

DNA methylation in hypoxia has been recently studied due to the realisation that TET (TET1, TET2 and TET3) enzymes are dioxygenase enzymes, requiring oxygen, 2-oxoglutarate and iron for their catalytic activity [5]. A study using *in vitro* models, but also tumour hypoxia in patient samples, demonstrated that hypoxia does alter global levels of DNA methylation, irrespectively of proliferation or metabolism [92]. This study showed that hypoxia increased DNA methylation across promoters, in a manner that was dependent on TET enzymatic activity [92]. Since, TET enzymes require oxygen for their function, by default hypoxia should increase DNA methylation across the genome.

DNA methylation changes at particular loci may also be altered by hypoxia in an indirect manner via altered recruitment of DNMTs. For example, DNMT3 enzyme are targeted to loci marked with unmethylated H3K4, or H3K36me3 [90] and H3K9 methylation is strongly linked to DNA methylation via DNMT recruitment [48]. Since, JmjC containing histone demethylases are also oxygen sensitive induces H3K4me2/me3, H3K36me3 and H3K9me3 [53], it is possible to speculate that hypoxia would lead to decreased recruitment of DNMT3 at promoters marked H3K4me3, but would increase the methylation across gene bodies, marked with H3K36me3 and loci marked with H3K9me2/me3 (Figure 3). However, such studies have not been performed and as such further investigation is required for this to be formally demonstrated.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

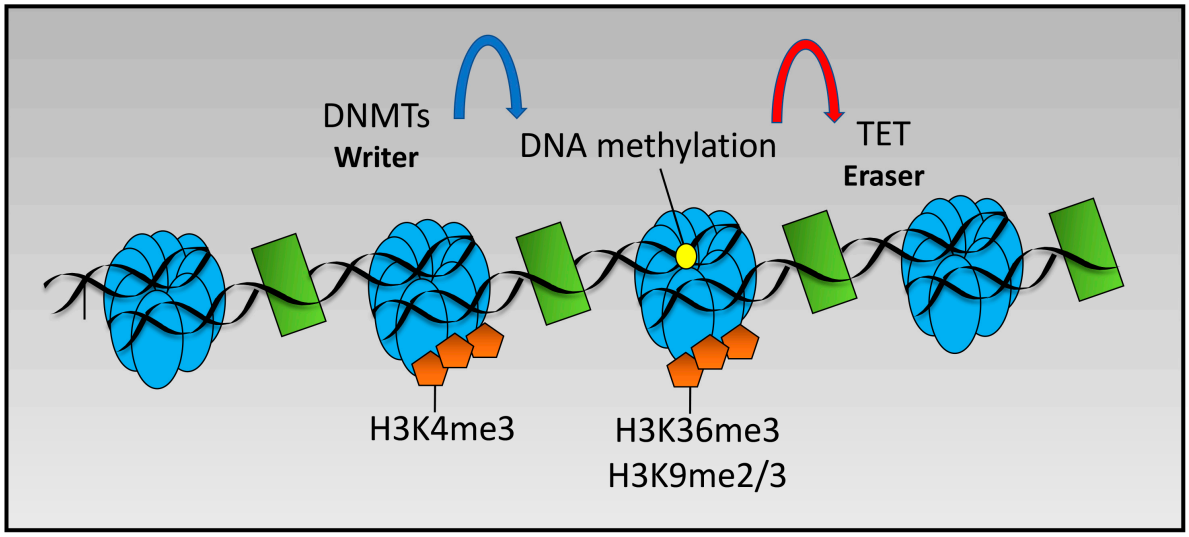

Figure 3. Dynamics of DNA methylation. DNA methylation is performed by DNMT enzymes and removed by Ten-Eleven-Translocation (TET) enzymes. DNMTs target areas of unmethylated H3K4 and trimethylated H3K36 or di/trimethylated H3K9.

HIF-dependent mechanisms of repression

As mentioned above, in contrast to gene upregulation, the mechanisms by which hypoxia leads to gene repression are not well understood. In some sporadic cases, direct HIF binding could mediate the transcriptional repression, as suggested for the regulation of Carbamoyl-Phosphate Synthetase 2, Aspartate Transcarbamylase, And Dihydroorotase (*CAD)* [93], Alpha Fetoprotein (*AFP)* [94], Adenosine Kinase (*AK)* [95], Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR)* [96] and APC, Adenomatous Polyposus Coli (*APC)* [97]genes. It has been proposed that HIF binding could displace transcriptional activators, such as MYC Proto-Oncogene, BHLH Transcription Factor (MYC), or recruit co-repressors that could account for down-regulation of gene expression [98]. However, these cases appear to be sporadic as genome-wide mapping of HIF-binding sites was unable to detect a significant association between gene repression and proximal HIF binding [12, 13, 15-19]. Interestingly, knock-down of HIF isoforms prevents the majority of the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

effects of hypoxia on both gene induction and repression [12, 99]. Thus, for the large majority of genes, hypoxia-triggered gene repression is likely to be indirectly mediated by HIF probably through trans-acting elements. In keeping with this hypothesis, HIF-1α directly regulates the expression of several sequence-specific repressors such as the MAX Interactor 1, Dimerization Protein (*MXI1)* gene, which encodes a repressor of *MYC*, thus leading to the repression of MYC targets such as PPAR-Gamma Coactivator 1-Beta (PGC-1β) [100]. Similarly, the transcriptional repressors Basic Helix-Loop-Helix Family Member E40 (*BHLHE40)* [101] and BTB Domain And CNC Homolog 1 (*BACH1)* [102] are a direct HIF targets. *BHLHE40* induction by hypoxia results in the repression of Peroxisome Proliferator Activated Receptor Gamma 2 (*PPARG2)* [103], Sterol Regulatory Element Binding Transcription Factor 1 (*SREBP1)* [104], Signal Transducer And Activator Of Transcription 1(*STAT1)* [105] and Melanogenesis Associated Transcription Factor (*MITF)* [106] genes, while induction of *BACH1* results in Heme Oxygenase 1 (*HMOX1)* repression [102]. Although these individual gene studies suggest a role for these repressors in the response to hypoxia, their relative contribution to the global transcriptional repression has only been analysed for MXI1 [107, 108]. These studies found that *MXI1* knockdown had very little effect on the hypoxic transcriptome, suggesting functional redundancy with other repressors of the Mad family. Alternatively, it could be that MXI1 is just one of many repressors downstream of HIF, each one acting on a small fraction of the genes repressed by hypoxia, additional studies are therefore required to fully answer this question.

Interestingly, BHLHE40 was shown to inhibit myogenic differentiation in response to hypoxia through the induction of Myogenin (*MYOG)* independently of HIF [109], suggesting that BHLHE40 has a major role in hypoxia-triggered gene repression

acting in pathways both HIF-dependent and independent. In addition to BHLHE40, the Repressor Element 1-Silencing Transcription Factor (REST) accumulates in the nucleus in response to hypoxia and acts as a key repressor of the hypoxic transcriptome in a HIF independent manner [110].

Finally, several studies have established a role of hypoxia and HIF in the regulation of specific microRNAs, particularly miR-210, which act to repress gene expression by inducing mRNA decay and/or inhibiting their translation [16, 111, 112]. Thus, at least part of the gene repression observed under hypoxia, could occur at the post-transcriptional level.

Transcriptional repression complexes in hypoxia

Sin3A-HDAC

Although it is known that HIF recruits Histone Deacetylases (HDACs) and, intriguingly, HDAC inhibitors prevent HIF-mediated transcription (reviewed in [113]), little attention has been paid to the role of these co-repressor complexes in the transcriptional response to hypoxia. The class I HDACs HDAC1, HDAC2 and HDAC3 are ubiquitously expressed nuclear enzymes that are components of multiprotein repressor complexes. These include SIN3 Transcription Regulator Family Member A (SIN3A), nucleosome-remodeling HDAC (NuRD) and CoREST; the two highly related HDAC3-containing complexes nuclear receptor co-repressor (NCoR or NCOR1) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT or NCOR2) [114]. However, to date the only the HDAC-containing co-repressor complex whose function in hypoxia has been analyzed at global scale is SIN3A.

The SIN3 protein is highly conserved from yeast to mammals. It is a central component of the SIN3 corepressor complex that participates in a wide variety of

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

processes including development, energy metabolism, cell growth and differentiation and as well as several pathological conditions including oncogenic transformation [115]. In mammals there are two SIN3 isoforms, SIN3A and SIN3B, encoded by separate genes, that are widely expressed and bind common as well as distinct transcriptional repressors and complexes (reviewed in [115]).

A recent bioinformatics approach found that the factor SIN3A was overrepresented in the proximity of genes whose transcription is repressed by hypoxia [108]. This study also identified enrichment for HDAC1, HDAC2 and Sap30, all components of the SIN3A co-repressor complex, and the sequence-specific repressors MXI1, Max, E2F4 and E2F6, known to interact with the SIN3A complex. In agreement with these computational predictions, knock-down of SIN3A significantly attenuated the repression of over 75% of the genes that were down-regulated in control cells, suggesting a major role for this co-repressor complex in this process [108]. However, several lines of evidence indicate that the function of SIN3A in the control of transcription is more complicated than previously anticipated. On the one hand, the genome-wide binding pattern of SIN3A showed a strong enrichment for this factor in active promoter regions, with SIN3A signal centered at the transcription start site (TSS) of actively transcribed genes and absent from genes with low or undetectable expression. On the other hand, SIN3A depletion not only affected gene repression, but also diminished the induction of about 47% of the genes upregulated by hypoxia. These results suggest that, beyond its function as co-repressor, SIN3A has wider role on transcriptional regulation. In agreement, SIN3A was initially described in yeast as a protein with dual functions as activator and repressor [116, 117] and recent studies are putting forward its role as an activator of specific genes [86], including targets of the aryl hydrocarbon receptor (ARNT), a transcription factor

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

related to HIF, known to mediate the transcriptional response to xenobiotics [118]. Moreover, the dual role of the Sin3A in transcriptional repression and activation in response to hypoxia is not unique to this repressor complex; it has been reported that knock-down the repressor HEXIM1 affects similar number of genes repressed and induced by hypoxia [119]. Furthermore, close analysis of results obtained by Casciello *et al* [67] indicate G9α also possess dual functions. In fact, emerging models propose that recruitment of both co-repressor and co-activator complexes is needed for gene induction in general [120]. As such further scrutiny is required for the understanding of how these complexes promote or repress transcription at particular target genes.

REST

Perhaps the transcription silencer mostly associated with hypoxia in recent years has been REST. Initially associated with neuronal development [121], its functions have now been extended to multiple cell types including cancer cells [122], cardiac [123] and beta cells [124].

REST mediates transcriptional repression via several mechanisms. Via its zinc fingers it is able to bind to repressor elements; via its N-terminus recruits SIN3A complex and via its C-terminus recruits CoREST, HDACs, LSD1, G9α and Methyl-CpG Binding Protein 2MeCP2 (reviewed in [124]). A role for CoREST and its associated histone demethylase LDS1, has been described for the repression of the gene *MLH1*, a key component of the DNA mismatch repair system, in response to hypoxia [125]. Interestingly, the CoREST complex is recruited by REST sequence-specific factor to repressed genes and, in addition to LSD1, CoREST complexes also interact with the G9α histone methylase. Thus, given the role of CoREST in *MLH1* down-regulation and the effects of REST and G9α in the repression of genes under

hypoxia described above, it would be interesting to investigate the effect the role of this co-repressor in the global response to hypoxia.

REST was shown to be repressed by an hypoxia induced micro-RNA, miR-106 b~25 cluster, in advance prostate cancer [126]. However, more recent studies have shown that REST is involved in transcriptional repression in hypoxic conditions [110, 127-129]. Cavadas et al [110] demonstrated that hypoxia can induce nuclear translocation of REST and importantly, REST is required for the repression of around 20% of hypoxia repressed genes in HEK293 cells. This data indicate the importance of this transcriptional repressor but also highlight that other mechanisms are important for transcriptional repression in hypoxia. Furthermore, it is tempting to speculate that REST's importance in contributing to transcriptional repression following hypoxia, might be dependent on the cell type. Given REST importance in neural and cardiac tissue (two tissue very sensitive to changes in oxygen supply), it is possible that REST contribution to the hypoxia response in these tissues could be even more prevalent. However, further studies are necessary to establish if this is the case.

Perspectives/Conclusions

As mentioned above, although much is known regarding hypoxia induced gene expression, less is so for hypoxia induced gene repression (Figure 4). With the discovery of a variety of dioxygenases impinging on several aspects of gene regulation such as histone and DNA methylation, there is now a suggestion for these to be involved in transcriptional repression in response to reduced oxygen, However, further studies are needed to address this. Similarly, some repressor complexes have been associated with the regulation of the hypoxia response. However, these are still just the tip of the iceberg. Whilst H3K27me3 is levels are induced by hypoxia [54, 57], and

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 March 2018 doi:10.20944/preprints201803.0004.v1

Peer-reviewed version available at Biomedicines 2018, 6, 47; doi:10.3390/biomedicines6020047

H327me3 is associated with Polycomb repressive complexes (PRCs) [49], there is no evidence for activation of gene silencing mechanisms involving PRCs in response to hypoxia. This is also extended to chromatin remodellers associated with repression of transcription, where much more work is needed to explain how hypoxia is able to repress as many genes as it induces.

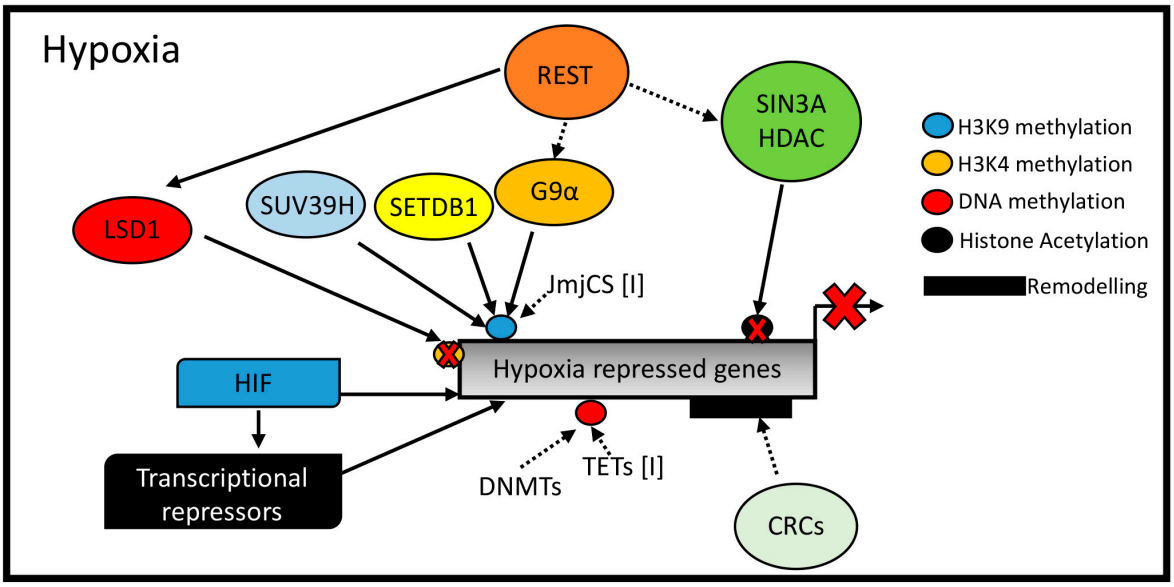

Figure 4. Mechanisms of hypoxic gene repression in the context of chromatin structure. Hypoxia Inducible Factor (HIF), Repressor Element 1-Silencing Transcription Factor (REST), SIN3 Transcription Regulator Family Member A (SIN3A), Histone Deacetylase, (HDAC), G9α Like Protein 1 (GPL), Suppressor Of Variegation 3-9 Homolog (SUV39H), SET Domain Bifurcated 1 (SETDB1), Chromatin Remodeller Complex (CRC), Jumoni C (JmjC), DNA Methyl Transferase (DNMT), Ten-Eleven-Translocation (TET), Lysine Specific Demethylase 1 (LSD1).

Acknowledgments

This work was supported by CRUK (C99667/A12918), Wellcome Trust (097945/C/11/Z; 206293/Z/17/Z) to SR and the Spanish Ministry of Science, MICINN (SAF2014-53819-R and SAF2017-88771-R) to LdelP.

Author Contributions

Michael Batie, Luis del Peso and Sonia Rocha researched the literature and wrote the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Rocha, S. "Gene Regulation under Low Oxygen: Holding Your Breath for Transcription." *Trends Biochem Sci* 32, no. 8 (2007): 389-97.
2. Kenneth, N. S., and S. Rocha. "Regulation of Gene Expression by Hypoxia." *The Biochemical journal* 414, no. 1 (2008): 19-29.
3. Xie, H., and M. C. Simon. "Oxygen Availability and Metabolic Reprogramming in Cancer." *J Biol Chem* 292, no. 41 (2017): 16825-32.
4. Kaelin, W. G., Jr., and P. J. Ratcliffe. "Oxygen Sensing by Metazoans: The Central Role of the Hif Hydroxylase Pathway." *Mol Cell* 30, no. 4 (2008): 393-402.
5. Koivunen, P., and T. Laukka. "The Tet Enzymes." *Cell Mol Life Sci* (2017).
6. Robinson, C. M., and M. Ohh. "The Multifaceted Von Hippel-Lindau Tumour Suppressor Protein." *FEBS Lett* 588, no. 16 (2014): 2704-11.
7. Bremm, A., S. Moniz, J. Mader, S. Rocha, and D. Komander. "Cezanne (Otud7b) Regulates Hif-1alpha Homeostasis in a Proteasome-Independent Manner." *EMBO Rep* 15, no. 12 (2014): 1268-77.
8. Mahon, P. C., K. Hirota, and G. L. Semenza. "Fih-1: A Novel Protein That Interacts with Hif-1alpha and Vhl to Mediate Repression of Hif-1 Transcriptional Activity." *Genes Dev* 15, no. 20 (2001): 2675-86.
9. Lando, D., D. J. Peet, J. J. Gorman, D. A. Whelan, M. L. Whitelaw, and R. K. Bruick. "Fih-1 Is an Asparaginyl Hydroxylase Enzyme That Regulates the Transcriptional Activity of Hypoxia-Inducible Factor." *Genes Dev* 16, no. 12 (2002): 1466-71.
10. Arany, Z., L. E. Huang, R. Eckner, S. Bhattacharya, C. Jiang, M. A. Goldberg, H. F. Bunn, and D. M. Livingston. "An Essential Role for P300/Cbp in the Cellular Response to Hypoxia." *Proc Natl Acad Sci U S A* 93, no. 23 (1996): 12969-73.
11. Kasper, L. H., F. Boussouar, K. Boyd, W. Xu, M. Biesen, J. Rehg, T. A. Baudino, J. L. Cleveland, and P. K. Brindle. "Two Transactivation Mechanisms Cooperate for the Bulk of Hif-1-Responsive Gene Expression." *EMBO J* 24, no. 22 (2005): 3846-58.
12. Mole, D. R., C. Blancher, R. R. Copley, P. J. Pollard, J. M. Gleadle, J. Ragoussis, and P. J. Ratcliffe. "Genome-Wide Association of Hypoxia-Inducible Factor (Hif)-1alpha and Hif-

2alpha DNA Binding with Expression Profiling of Hypoxia-Inducible Transcripts." *The Journal of biological chemistry* 284, no. 25 (2009): 16767-75.

13. Xia, X., M. E. Lemieux, W. Li, J. S. Carroll, M. Brown, X. S. Liu, and A. L. Kung. "Integrative Analysis of Hif Binding and Transactivation Reveals Its Role in Maintaining Histone Methylation Homeostasis." *Proc Natl Acad Sci U S A* 106, no. 11 (2009): 4260-5.
14. Schodel, J., S. Oikonomopoulos, J. Ragoussis, C. W. Pugh, P. J. Ratcliffe, and D. R. Mole. "High-Resolution Genome-Wide Mapping of Hif-Binding Sites by Chip-Seq." *Blood* 117, no. 23 (2011): e207-17.
15. Xia, X., and A. L. Kung. "Preferential Binding of Hif-1 to Transcriptionally Active Loci Determines Cell-Type Specific Response to Hypoxia." *Genome biology* 10, no. 10 (2009): R113.
16. Choudhry, H., J. Schodel, S. Oikonomopoulos, C. Camps, S. Grampp, A. L. Harris, P. J. Ratcliffe, J. Ragoussis, and D. R. Mole. "Extensive Regulation of the Non-Coding Transcriptome by Hypoxia: Role of Hif in Releasing Paused Rnapol2." *EMBO Rep* 15, no. 1 (2014): 70-6.
17. Ortiz-Barahona, A., D. Villar, N. Pescador, J. Amigo, and L. del Peso. "Genome-Wide Identification of Hypoxia-Inducible Factor Binding Sites and Target Genes by a Probabilistic Model Integrating Transcription-Profiling Data and in Silico Binding Site Prediction." *Nucleic Acids Res* 38, no. 7 (2010): 2332-45.
18. Salama, R., N. Masson, P. Simpson, L. K. Sciesielski, M. Sun, Y. M. Tian, P. J. Ratcliffe, and D. R. Mole. "Heterogeneous Effects of Direct Hypoxia Pathway Activation in Kidney Cancer." *PLoS One* 10, no. 8 (2015): e0134645.
19. Schodel, J., C. Bardella, L. K. Sciesielski, J. M. Brown, C. W. Pugh, V. Buckle, I. P. Tomlinson, P. J. Ratcliffe, and D. R. Mole. "Common Genetic Variants at the 11q13.3 Renal Cancer Susceptibility Locus Influence Binding of Hif to an Enhancer of Cyclin D1 Expression." *Nat Genet* 44, no. 4 (2012): 420-5, S1-2.
20. Roche, O., M. L. Deguiz, M. Tiana, C. Galiana-Ribote, D. Martinez-Alcazar, C. Rey-Serra, B. Ranz-Ribeiro, R. Casitas, R. Galera, I. Fernandez-Navarro, S. Sanchez-Cuellar, V. Bernard, J. Ancochea, W. W. Wasserman, F. Garcia-Rio, B. Jimenez, and L. Del Peso. "Identification of Non-Coding Genetic Variants in Samples from Hypoxemic Respiratory Disease Patients That Affect the Transcriptional Response to Hypoxia." *Nucleic Acids Res* 44, no. 19 (2016): 9315-30.
21. Owen-Hughes, T., and T. Gkikopoulos. "Making Sense of Transcribing Chromatin." *Curr Opin Cell Biol* 24, no. 3 (2012): 296-304.
22. Kornberg, R. D. "Chromatin Structure: A Repeating Unit of Histones and DNA." *Science* 184, no. 4139 (1974): 868-71.
23. Woodcock, C. L., and R. P. Ghosh. "Chromatin Higher-Order Structure and Dynamics." *Cold Spring Harbor perspectives in biology* 2, no. 5 (2010): a000596.
24. Luger, K., M. L. Dechassa, and D. J. Tremethick. "New Insights into Nucleosome and Chromatin Structure: An Ordered State or a Disordered Affair?" *Nature reviews. Molecular cell biology* 13, no. 7 (2012): 436-47.
25. Li, B., M. Carey, and J. L. Workman. "The Role of Chromatin During Transcription." *Cell* 128, no. 4 (2007): 707-19.
26. Lleres, D., J. James, S. Swift, D. G. Norman, and A. I. Lamond. "Quantitative Analysis of Chromatin Compaction in Living Cells Using Flim-Fret." *J Cell Biol* 187, no. 4 (2009): 481-96.

27. Filion, G. J., J. G. van Bemmel, U. Braunschweig, W. Talhout, J. Kind, L. D. Ward, W. Brugman, I. J. de Castro, R. M. Kerkhoven, H. J. Bussemaker, and B. van Steensel. "Systematic Protein Location Mapping Reveals Five Principal Chromatin Types in Drosophila Cells." *Cell* 143, no. 2 (2010): 212-24.
28. Lleres, D., A. P. Bailly, A. Perrin, D. G. Norman, D. P. Xirodimas, and R. Feil. "Quantitative Flim-Fret Microscopy to Monitor Nanoscale Chromatin Compaction in Vivo Reveals Structural Roles of Condensin Complexes." *Cell Rep* 18, no. 7 (2017): 1791-803.
29. Narlikar, G. J., R. Sundaramoorthy, and T. Owen-Hughes. "Mechanisms and Functions of Atp-Dependent Chromatin-Remodeling Enzymes." *Cell* 154, no. 3 (2013): 490-503.
30. Kouzarides, T. "Chromatin Modifications and Their Function." *Cell* 128, no. 4 (2007): 693-705.
31. Weber, C. M., and S. Henikoff. "Histone Variants: Dynamic Punctuation in Transcription." *Genes & development* 28, no. 7 (2014): 672-82.
32. Wade, P. A. "Methyl Cpg-Binding Proteins and Transcriptional Repression." *BioEssays : news and reviews in molecular, cellular and developmental biology* 23, no. 12 (2001): 1131-7.
33. Peschansky, V. J., and C. Wahlestedt. "Non-Coding Rnas as Direct and Indirect Modulators of Epigenetic Regulation." *Epigenetics* 9, no. 1 (2014): 3-12.
34. Dixon, J. R., I. Jung, S. Selvaraj, Y. Shen, J. E. Antosiewicz-Bourget, A. Y. Lee, Z. Ye, A. Kim, N. Rajagopal, W. Xie, Y. Diao, J. Liang, H. Zhao, V. V. Lobanenkov, J. R. Ecker, J. A. Thomson, and B. Ren. "Chromatin Architecture Reorganization During Stem Cell Differentiation." *Nature* 518, no. 7539 (2015): 331-6.
35. Mirabella, A. C., B. M. Foster, and T. Bartke. "Chromatin Deregulation in Disease." *Chromosoma* 125, no. 1 (2016): 75-93.
36. Kirmes, I., A. Szczurek, K. Prakash, I. Charapitsa, C. Heiser, M. Musheev, F. Schock, K. Fornalczyk, D. Ma, U. Birk, C. Cremer, and G. Reid. "A Transient Ischemic Environment Induces Reversible Compaction of Chromatin." *Genome Biol* 16 (2015): 246.
37. Dutta, B., R. Yan, S. K. Lim, J. P. Tam, and S. K. Sze. "Quantitative Profiling of Chromatome Dynamics Reveals a Novel Role for Hp1bp3 in Hypoxia-Induced Oncogenesis." *Mol Cell Proteomics* 13, no. 12 (2014): 3236-49.
38. Dutta, B., Y. Ren, P. Hao, K. H. Sim, E. Cheow, S. Adav, J. P. Tam, and S. K. Sze. "Profiling of the Chromatin-Associated Proteome Identifies Hp1bp3 as a Novel Regulator of Cell Cycle Progression." *Mol Cell Proteomics* 13, no. 9 (2014): 2183-97.
39. Kadauke, S., and G. A. Blobel. "Chromatin Loops in Gene Regulation." *Biochim Biophys Acta* 1789, no. 1 (2009): 17-25.
40. Mora, A., G. K. Sandve, O. S. Gabrielsen, and R. Eskeland. "In the Loop: Promoter-Enhancer Interactions and Bioinformatics." *Brief Bioinform* 17, no. 6 (2016): 980-95.
41. Platt, J. L., R. Salama, J. Smythies, H. Choudhry, J. O. Davies, J. R. Hughes, P. J. Ratcliffe, and D. R. Mole. "Capture-C Reveals Preformed Chromatin Interactions between Hif-Binding Sites and Distant Promoters." *EMBO reports* 17, no. 10 (2016): 1410-21.
42. Greenald, D., J. Jeyakani, B. Pelster, I. Sealy, S. Mathavan, and F. J. van Eeden. "Genome-Wide Mapping of Hif-1alpha Binding Sites in Zebrafish." *BMC Genomics* 16 (2015): 923.
43. Galbraith, M. D., M. A. Allen, C. L. Bensard, X. Wang, M. K. Schwinn, B. Qin, H. W. Long, D. L. Daniels, W. C. Hahn, R. D. Dowell, and J. M. Espinosa. "Hif1a Employs Cdk8-

Mediator to Stimulate Rnapii Elongation in Response to Hypoxia." *Cell* 153, no. 6 (2013): 1327-39.

44. Dong, X., M. C. Greven, A. Kundaje, S. Djebali, J. B. Brown, C. Cheng, T. R. Gingeras, M. Gerstein, R. Guigo, E. Birney, and Z. Weng. "Modeling Gene Expression Using Chromatin Features in Various Cellular Contexts." *Genome Biol* 13, no. 9 (2012): R53.
45. Benveniste, D., H. J. Sonntag, G. Sanguinetti, and D. Sproul. "Transcription Factor Binding Predicts Histone Modifications in Human Cell Lines." *Proc Natl Acad Sci U S A* 111, no. 37 (2014): 13367-72.
46. Hyun, K., J. Jeon, K. Park, and J. Kim. "Writing, Erasing and Reading Histone Lysine Methylations." *Exp Mol Med* 49, no. 4 (2017): e324.
47. Saksouk, N., E. Simboeck, and J. Dejardin. "Constitutive Heterochromatin Formation and Transcription in Mammals." *Epigenetics Chromatin* 8 (2015): 3.
48. Du, J., L. M. Johnson, S. E. Jacobsen, and D. J. Patel. "DNA Methylation Pathways and Their Crosstalk with Histone Methylation." *Nat Rev Mol Cell Biol* 16, no. 9 (2015): 519-32.
49. van Kruijsbergen, I., S. Hontelez, and G. J. Veenstra. "Recruiting Polycomb to Chromatin." *Int J Biochem Cell Biol* 67 (2015): 177-87.
50. Voigt, P., W. W. Tee, and D. Reinberg. "A Double Take on Bivalent Promoters." *Genes & development* 27, no. 12 (2013): 1318-38.
51. Kooistra, S. M., and K. Helin. "Molecular Mechanisms and Potential Functions of Histone Demethylases." *Nature reviews. Molecular cell biology* 13, no. 5 (2012): 297-311.
52. Melvin, A., and S. Rocha. "Chromatin as an Oxygen Sensor and Active Player in the Hypoxia Response." *Cellular signalling* 24, no. 1 (2012): 35-43.
53. Shmakova, A., M. Batie, J. Druker, and S. Rocha. "Chromatin and Oxygen Sensing in the Context of Jmjc Histone Demethylases." *The Biochemical journal* 462, no. 3 (2014): 385-95.
54. Johnson, A. B., N. Denko, and M. C. Barton. "Hypoxia Induces a Novel Signature of Chromatin Modifications and Global Repression of Transcription." *Mutation research* 640, no. 1-2 (2008): 174-9.
55. Zhou, X., H. Sun, H. Chen, J. Zavadil, T. Kluz, A. Arita, and M. Costa. "Hypoxia Induces Trimethylated H3 Lysine 4 by Inhibition of Jarid1a Demethylase." *Cancer research* 70, no. 10 (2010): 4214-21.
56. Tausendschon, M., N. Dehne, and B. Brune. "Hypoxia Causes Epigenetic Gene Regulation in Macrophages by Attenuating Jumonji Histone Demethylase Activity." *Cytokine* 53, no. 2 (2011): 256-62.
57. Prickaerts, P., M. E. Adriaens, T. V. Beucken, E. Koch, L. Dubois, V. E. Dahlmans, C. Gits, C. T. Evelo, M. Chan-Seng-Yue, B. G. Wouters, and J. W. Voncken. "Hypoxia Increases Genome-Wide Bivalent Epigenetic Marking by Specific Gain of H3k27me3." *Epigenetics & chromatin* 9 (2016): 46.
58. Chen, H., Y. Yan, T. L. Davidson, Y. Shinkai, and M. Costa. "Hypoxic Stress Induces Dimethylated Histone H3 Lysine 9 through Histone Methyltransferase G9a in Mammalian Cells." *Cancer research* 66, no. 18 (2006): 9009-16.
59. Olcina, M. M., I. P. Foskolou, S. Anbalagan, J. M. Senra, I. M. Pires, Y. Jiang, A. J. Ryan, and E. M. Hammond. "Replication Stress and Chromatin Context Link Atm Activation to a Role in DNA Replication." *Mol Cell* 52, no. 5 (2013): 758-66.

60. Elkins, J. M., K. S. Hewitson, L. A. McNeill, J. F. Seibel, I. Schlemminger, C. W. Pugh, P. J. Ratcliffe, and C. J. Schofield. "Structure of Factor-Inhibiting Hypoxia-Inducible Factor (Hif) Reveals Mechanism of Oxidative Modification of Hif-1 Alpha." *The Journal of biological chemistry* 278, no. 3 (2003): 1802-6.
61. Trewick, S. C., P. J. McLaughlin, and R. C. Allshire. "Methylation: Lost in Hydroxylation?" *EMBO reports* 6, no. 4 (2005): 315-20.
62. Hancock, R. L., N. Masson, K. Dunne, E. Flashman, and A. Kawamura. "The Activity of Jmjc Histone Lysine Demethylase Kdm4a Is Highly Sensitive to Oxygen Concentrations." *ACS chemical biology* 12, no. 4 (2017): 1011-19.
63. Sanchez-Fernandez, E. M., H. Tarhonskaya, K. Al-Qahtani, R. J. Hopkinson, J. S. McCullagh, C. J. Schofield, and E. Flashman. "Investigations on the Oxygen Dependence of a 2-Oxoglutarate Histone Demethylase." *The Biochemical journal* 449, no. 2 (2013): 491-6.
64. Batie, M., J. Druker, L. D'Ignazio, and S. Rocha. "Kdm2 Family Members Are Regulated by Hif-1 in Hypoxia." *Cells* 6, no. 1 (2017).
65. Luo, W., R. Chang, J. Zhong, A. Pandey, and G. L. Semenza. "Histone Demethylase Jmjd2c Is a Coactivator for Hypoxia-Inducible Factor 1 That Is Required for Breast Cancer Progression." *Proceedings of the National Academy of Sciences of the United States of America* 109, no. 49 (2012): E3367-76.
66. Krieg, A. J., E. B. Rankin, D. Chan, O. Razorenova, S. Fernandez, and A. J. Giaccia. "Regulation of the Histone Demethylase Jmjd1a by Hypoxia-Inducible Factor 1 Alpha Enhances Hypoxic Gene Expression and Tumor Growth." *Molecular and cellular biology* 30, no. 1 (2010): 344-53.
67. Casciello, F., F. Al-Ejeh, G. Kelly, D. J. Brennan, S. F. Ngiow, A. Young, T. Stoll, K. Windloch, M. M. Hill, M. J. Smyth, F. Gannon, and J. S. Lee. "G9a Drives Hypoxia-Mediated Gene Repression for Breast Cancer Cell Survival and Tumorigenesis." *Proc Natl Acad Sci U S A* 114, no. 27 (2017): 7077-82.
68. Ueda, J., J. C. Ho, K. L. Lee, S. Kitajima, H. Yang, W. Sun, N. Fukuhara, N. Zaiden, S. L. Chan, M. Tachibana, Y. Shinkai, H. Kato, and L. Poellinger. "The Hypoxia-Inducible Epigenetic Regulators Jmjd1a and G9a Provide a Mechanistic Link between Angiogenesis and Tumor Growth." *Mol Cell Biol* 34, no. 19 (2014): 3702-20.
69. Ho, J. C., L. N. Abdullah, Q. Y. Pang, S. Jha, E. K. Chow, H. Yang, H. Kato, L. Poellinger, J. Ueda, and K. L. Lee. "Inhibition of the H3k9 Methyltransferase G9a Attenuates Oncogenicity and Activates the Hypoxia Signaling Pathway." *PLoS One* 12, no. 11 (2017): e0188051.
70. Olcina, M. M., K. B. Leszczynska, J. M. Senra, N. F. Isa, H. Harada, and E. M. Hammond. "H3k9me3 Facilitates Hypoxia-Induced P53-Dependent Apoptosis through Repression of Apak." *Oncogene* 35, no. 6 (2016): 793-9.
71. Benlhabib, H., and C. R. Mendelson. "Epigenetic Regulation of Surfactant Protein a Gene (Sp-a) Expression in Fetal Lung Reveals a Critical Role for Suv39h Methyltransferases During Development and Hypoxia." *Mol Cell Biol* 31, no. 10 (2011): 1949-58.
72. Flaus, A., and T. Owen-Hughes. "Mechanisms for Atp-Dependent Chromatin Remodelling: The Means to the End." *The FEBS journal* 278, no. 19 (2011): 3579-95.
73. Larkin, J., X. Y. Goh, M. Vetter, L. Pickering, and C. Swanton. "Epigenetic Regulation in Rcc: Opportunities for Therapeutic Intervention?" *Nat Rev Urol* 9, no. 3 (2012): 147-55.

74. Pulice, J. L., and C. Kadoch. "Composition and Function of Mammalian Swi/Snf Chromatin Remodeling Complexes in Human Disease." *Cold Spring Harb Symp Quant Biol* 81 (2016): 53-60.
75. Kenneth, N. S., S. Mudie, P. van Uden, and S. Rocha. "Swi/Snf Regulates the Cellular Response to Hypoxia." *The Journal of biological chemistry* 284, no. 7 (2009): 4123-31.
76. Gkikopoulos, T., P. Schofield, V. Singh, M. Pinskaya, J. Mellor, M. Smolle, J. L. Workman, G. J. Barton, and T. Owen-Hughes. "A Role for Snf2-Related Nucleosome-Spacing Enzymes in Genome-Wide Nucleosome Organization." *Science* 333, no. 6050 (2011): 1758-60.
77. Wiechens, N., V. Singh, T. Gkikopoulos, P. Schofield, S. Rocha, and T. Owen-Hughes. "The Chromatin Remodelling Enzymes Snf2h and Snf2l Position Nucleosomes Adjacent to Ctcf and Other Transcription Factors." *PLoS Genet* 12, no. 3 (2016): e1005940.
78. Melvin, A., S. Mudie, and S. Rocha. "The Chromatin Remodeler Iswi Regulates the Cellular Response to Hypoxia: Role of Fih." *Mol Biol Cell* 22, no. 21 (2011): 4171-81.
79. Emelyanov, A. V., E. Vershilova, M. A. Ignatyeva, D. K. Pokrovsky, X. Lu, A. Y. Konev, and D. V. Fyodorov. "Identification and Characterization of Torc, a Novel Iswi-Containing Atp-Dependent Chromatin Assembly Complex." *Genes Dev* 26, no. 6 (2012): 603-14.
80. Mirnezami, A. H., S. J. Campbell, M. Darley, J. N. Primrose, P. W. Johnson, and J. P. Blaydes. "Hdm2 Recruits a Hypoxia-Sensitive Corepressor to Negatively Regulate P53-Dependent Transcription." *Curr Biol* 13, no. 14 (2003): 1234-9.
81. Zhang, Q., S. Y. Wang, A. C. Nottke, J. V. Rocheleau, D. W. Piston, and R. H. Goodman. "Redox Sensor Ctbp Mediates Hypoxia-Induced Tumor Cell Migration." *Proc Natl Acad Sci U S A* 103, no. 24 (2006): 9029-33.
82. Wu, X., M. S. Chang, S. A. Mitsialis, and S. Kourembanas. "Hypoxia Regulates Bone Morphogenetic Protein Signaling through C-Terminal-Binding Protein 1." *Circ Res* 99, no. 3 (2006): 240-7.
83. Dorman, K., Z. Shen, C. Yang, S. Ezzat, and S. L. Asa. "Ctbp1 Interacts with Ikaros and Modulates Pituitary Tumor Cell Survival and Response to Hypoxia." *Mol Endocrinol* 26, no. 3 (2012): 447-57.
84. Dias, J. M., S. Ilkhanizadeh, E. Karaca, J. K. Duckworth, V. Lundin, M. G. Rosenfeld, J. Ericson, O. Hermanson, and A. I. Teixeira. "Ctbps Sense Microenvironmental Oxygen Levels to Regulate Neural Stem Cell State." *Cell Rep* 8, no. 3 (2014): 665-70.
85. Tyagi, M., N. Imam, K. Verma, and A. K. Patel. "Chromatin Remodelers: We Are the Drivers!!" *Nucleus* 7, no. 4 (2016): 388-404.
86. Kliewe, F., M. Engelhardt, R. Aref, and H. J. Schuller. "Promoter Recruitment of Corepressors Sin3 and Cyc8 by Activator Proteins of the Yeast Saccharomyces Cerevisiae." *Curr Genet* 63, no. 4 (2017): 739-50.
87. Lee, J. S., Y. Kim, I. S. Kim, B. Kim, H. J. Choi, J. M. Lee, H. J. Shin, J. H. Kim, J. Y. Kim, S. B. Seo, H. Lee, O. Binda, O. Gozani, G. L. Semenza, M. Kim, K. I. Kim, D. Hwang, and S. H. Baek. "Negative Regulation of Hypoxic Responses Via Induced Reptin Methylation." *Mol Cell* 39, no. 1 (2010): 71-85.
88. Lee, J. S., Y. Kim, J. Bhin, H. J. Shin, H. J. Nam, S. H. Lee, J. B. Yoon, O. Binda, O. Gozani, D. Hwang, and S. H. Baek. "Hypoxia-Induced Methylation of a Pontin Chromatin Remodeling Factor." *Proc Natl Acad Sci U S A* 108, no. 33 (2011): 13510-5.
89. Schubeler, D. "Function and Information Content of DNA Methylation." *Nature* 517, no. 7534 (2015): 321-6.

90. Ravichandran, M., R. Z. Jurkowska, and T. P. Jurkowski. "Target Specificity of Mammalian DNA Methylation and Demethylation Machinery." *Org Biomol Chem* (2017).
91. Lyko, F. "The DNA Methyltransferase Family: A Versatile Toolkit for Epigenetic Regulation." *Nat Rev Genet* 19, no. 2 (2018): 81-92.
92. Thienpont, B., J. Steinbacher, H. Zhao, F. D'Anna, A. Kuchnio, A. Ploumakis, B. Ghesquiere, L. Van Dyck, B. Boeckx, L. Schoonjans, E. Hermans, F. Amant, V. N. Kristensen, K. P. Koh, M. Mazzone, M. L. Coleman, T. Carell, P. Carmeliet, and D. Lambrechts. "Tumour Hypoxia Causes DNA Hypermethylation by Reducing Tet Activity." *Nature* 537, no. 7618 (2016): 63-68.
93. Chen, K. F., Y. Y. Lai, H. S. Sun, and S. J. Tsai. "Transcriptional Repression of Human Cad Gene by Hypoxia Inducible Factor-1alpha." *Nucleic Acids Res* 33, no. 16 (2005): 5190-8.
94. Mazure, N. M., C. Chauvet, B. Bois-Joyeux, M. A. Bernard, H. Nacer-Cherif, and J. L. Danan. "Repression of Alpha-Fetoprotein Gene Expression under Hypoxic Conditions in Human Hepatoma Cells: Characterization of a Negative Hypoxia Response Element That Mediates Opposite Effects of Hypoxia Inducible Factor-1 and C-Myc." *Cancer Res* 62, no. 4 (2002): 1158-65.
95. Morote-Garcia, J. C., P. Rosenberger, J. Kuhlicke, and H. K. Eltzschig. "Hif-1-Dependent Repression of Adenosine Kinase Attenuates Hypoxia-Induced Vascular Leak." *Blood* 111, no. 12 (2008): 5571-80.
96. Zheng, W., J. Kuhlicke, K. Jackel, H. K. Eltzschig, A. Singh, M. Sjoblom, B. Riederer, C. Weinhold, U. Seidler, S. P. Colgan, and J. Karhausen. "Hypoxia Inducible Factor-1 (Hif-1)-Mediated Repression of Cystic Fibrosis Transmembrane Conductance Regulator (Cftr) in the Intestinal Epithelium." *FASEB J* 23, no. 1 (2009): 204-13.
97. Newton, I. P., N. S. Kenneth, P. L. Appleton, I. Nathke, and S. Rocha. "Adenomatous Polyposis Coli and Hypoxia-Inducible Factor-1{Alpha} Have an Antagonistic Connection." *Mol Biol Cell* 21, no. 21 (2010): 3630-8.
98. Keith, B., R. S. Johnson, and M. C. Simon. "Hif1alpha and Hif2alpha: Sibling Rivalry in Hypoxic Tumour Growth and Progression." *Nat Rev Cancer* 12, no. 1 (2011): 9-22.
99. Elvidge, G. P., L. Glenny, R. J. Appelhoff, P. J. Ratcliffe, J. Ragoussis, and J. M. Gleadle. "Concordant Regulation of Gene Expression by Hypoxia and 2-Oxoglutarate-Dependent Dioxygenase Inhibition: The Role of Hif-1alpha, Hif-2alpha, and Other Pathways." *J Biol Chem* 281, no. 22 (2006): 15215-26.
100. Zhang, H., P. Gao, R. Fukuda, G. Kumar, B. Krishnamachary, K. I. Zeller, C. V. Dang, and G. L. Semenza. "Hif-1 Inhibits Mitochondrial Biogenesis and Cellular Respiration in Vhl-Deficient Renal Cell Carcinoma by Repression of C-Myc Activity." *Cancer Cell* 11, no. 5 (2007): 407-20.
101. Miyazaki, K., T. Kawamoto, K. Tanimoto, M. Nishiyama, H. Honda, and Y. Kato. "Identification of Functional Hypoxia Response Elements in the Promoter Region of the Dec1 and Dec2 Genes." *J Biol Chem* 277, no. 49 (2002): 47014-21.
102. Kitamuro, T., K. Takahashi, K. Ogawa, R. Udono-Fujimori, K. Takeda, K. Furuyama, M. Nakayama, J. Sun, H. Fujita, W. Hida, T. Hattori, K. Shirato, K. Igarashi, and S. Shibahara. "Bach1 Functions as a Hypoxia-Inducible Repressor for the Heme Oxygenase-1 Gene in Human Cells." *J Biol Chem* 278, no. 11 (2003): 9125-33.

103. Yun, Z., H. L. Maecker, R. S. Johnson, and A. J. Giaccia. "Inhibition of Ppar Gamma 2 Gene Expression by the Hif-1-Regulated Gene Dec1/Stra13: A Mechanism for Regulation of Adipogenesis by Hypoxia." *Dev Cell* 2, no. 3 (2002): 331-41.
104. Choi, S. M., H. J. Cho, H. Cho, K. H. Kim, J. B. Kim, and H. Park. "Stra13/Dec1 and Dec2 Inhibit Sterol Regulatory Element Binding Protein-1c in a Hypoxia-Inducible Factor-Dependent Mechanism." *Nucleic Acids Res* 36, no. 20 (2008): 6372-85.
105. Ivanov, S. V., K. Salnikow, A. V. Ivanova, L. Bai, and M. I. Lerman. "Hypoxic Repression of Stat1 and Its Downstream Genes by a Pvhl/Hif-1 Target Dec1/Stra13." *Oncogene* 26, no. 6 (2007): 802-12.
106. Feige, E., S. Yokoyama, C. Levy, M. Khaled, V. Igras, R. J. Lin, S. Lee, H. R. Widlund, S. R. Granter, A. L. Kung, and D. E. Fisher. "Hypoxia-Induced Transcriptional Repression of the Melanoma-Associated Oncogene Mitf." *Proc Natl Acad Sci U S A* 108, no. 43 (2011): E924-33.
107. Lofstedt, T., E. Fredlund, R. Noguera, S. Navarro, L. Holmquist-Mengelbier, S. Beckman, S. Pahlman, and H. Axelson. "Hif-1alpha Induces Mxi1 by Alternate Promoter Usage in Human Neuroblastoma Cells." *Exp Cell Res* 315, no. 11 (2009): 1924-36.
108. Tiana, M., B. Acosta-Iborra, L. Puente-Santamaria, P. Hernansanz-Agustin, R. Worsley-Hunt, N. Masson, F. Garcia-Rio, D. Mole, P. Ratcliffe, W. W. Wasserman, B. Jimenez, and L. Del Peso. "The Sin3a Histone Deacetylase Complex Is Required for a Complete Transcriptional Response to Hypoxia." *Nucleic Acids Res* 46, no. 1 (2018): 120-33.
109. Wang, C., W. Liu, Z. Liu, L. Chen, X. Liu, and S. Kuang. "Hypoxia Inhibits Myogenic Differentiation through P53 Protein-Dependent Induction of Bhlhe40 Protein." *J Biol Chem* 290, no. 50 (2015): 29707-16.
110. Cavadas, M. A., M. Mesnieres, B. Crifo, M. C. Manresa, A. C. Selfridge, C. E. Keogh, Z. Fabian, C. C. Scholz, K. A. Nolan, L. M. Rocha, M. M. Tambuwala, S. Brown, A. Wdowicz, D. Corbett, K. J. Murphy, C. Godson, E. P. Cummins, C. T. Taylor, and A. Cheong. "Rest Is a Hypoxia-Responsive Transcriptional Repressor." *Sci Rep* 6 (2016): 31355.
111. Kulshreshtha, R., M. Ferracin, S. E. Wojcik, R. Garzon, H. Alder, F. J. Agosto-Perez, R. Davuluri, C. G. Liu, C. M. Croce, M. Negrini, G. A. Calin, and M. Ivan. "A Microrna Signature of Hypoxia." *Mol Cell Biol* 27, no. 5 (2007): 1859-67.
112. Camps, C., F. M. Buffa, S. Colella, J. Moore, C. Sotiriou, H. Sheldon, A. L. Harris, J. M. Gleadle, and J. Ragoussis. "Hsa-Mir-210 Is Induced by Hypoxia and Is an Independent Prognostic Factor in Breast Cancer." *Clin Cancer Res* 14, no. 5 (2008): 1340-8.
113. Perez-Perri, J. I., J. M. Acevedo, and P. Wappner. "Epigenetics: New Questions on the Response to Hypoxia." *Int J Mol Sci* 12, no. 7 (2011): 4705-21.
114. Kelly, R. D., and S. M. Cowley. "The Physiological Roles of Histone Deacetylase (Hdac) 1 and 2: Complex Co-Stars with Multiple Leading Parts." *Biochem Soc Trans* 41, no. 3 (2013): 741-9.
115. Kadamb, R., S. Mittal, N. Bansal, H. Batra, and D. Saluja. "Sin3: Insight into Its Transcription Regulatory Functions." *Eur J Cell Biol* 92, no. 8-9 (2013): 237-46.
116. Vidal, M., and R. F. Gaber. "Rpd3 Encodes a Second Factor Required to Achieve Maximum Positive and Negative Transcriptional States in Saccharomyces Cerevisiae." *Mol Cell Biol* 11, no. 12 (1991): 6317-27.
117. Vidal, M., R. Strich, R. E. Esposito, and R. F. Gaber. "Rpd1 (Sin3/Ume4) Is Required for Maximal Activation and Repression of Diverse Yeast Genes." *Mol Cell Biol* 11, no. 12 (1991): 6306-16.

118. Solaimani, P., F. Wang, and O. Hankinson. "Sin3a, Generally Regarded as a Transcriptional Repressor, Is Required for Induction of Gene Transcription by the Aryl Hydrocarbon Receptor." *J Biol Chem* 289, no. 48 (2014): 33655-62.
119. Safronova, O. S., K. Nakahama, and I. Morita. "Acute Hypoxia Affects P-Tefb through Hdac3 and Hexim1-Dependent Mechanism to Promote Gene-Specific Transcriptional Repression." *Nucleic Acids Res* 42, no. 14 (2014): 8954-69.
120. Perissi, V., K. Jepsen, C. K. Glass, and M. G. Rosenfeld. "Deconstructing Repression: Evolving Models of Co-Repressor Action." *Nat Rev Genet* 11, no. 2 (2010): 109-23.
121. Qureshi, I. A., S. Gokhan, and M. F. Mehler. "Rest and Corest Are Transcriptional and Epigenetic Regulators of Seminal Neural Fate Decisions." *Cell Cycle* 9, no. 22 (2010): 4477-86.
122. Cavadas, M. A. S., A. Cheong, and C. T. Taylor. "The Regulation of Transcriptional Repression in Hypoxia." *Exp Cell Res* 356, no. 2 (2017): 173-81.
123. Zhang, D., Y. Wang, P. Lu, P. Wang, X. Yuan, J. Yan, C. Cai, C. P. Chang, D. Zheng, B. Wu, and B. Zhou. "Rest Regulates the Cell Cycle for Cardiac Development and Regeneration." *Nat Commun* 8, no. 1 (2017): 1979.
124. Martin, D., and A. Grapin-Botton. "The Importance of Rest for Development and Function of Beta Cells." *Front Cell Dev Biol* 5 (2017): 12.
125. Lu, Y., N. Wajapeyee, M. S. Turker, and P. M. Glazer. "Silencing of the DNA Mismatch Repair Gene Mlh1 Induced by Hypoxic Stress in a Pathway Dependent on the Histone Demethylase Lsd1." *Cell Rep* 8, no. 2 (2014): 501-13.
126. Liang, J., P. Tong, W. Zhao, Y. Li, L. Zhang, Y. Xia, and Y. Yu. "The Rest Gene Signature Predicts Drug Sensitivity in Neuroblastoma Cell Lines and Is Significantly Associated with Neuroblastoma Tumor Stage." *Int J Mol Sci* 15, no. 7 (2014): 11220-33.
127. Formisano, L., N. Guida, G. Laudati, L. Mascolo, G. Di Renzo, and L. M. Canzoniero. "Ms-275 Inhibits Aroclor 1254-Induced Sh-Sy5y Neuronal Cell Toxicity by Preventing the Formation of the Hdac3/Rest Complex on the Synapsin-1 Promoter." *J Pharmacol Exp Ther* 352, no. 2 (2015): 236-43.
128. Cavadas, M. A., M. Mesnieres, B. Crifo, M. C. Manresa, A. C. Selfridge, C. C. Scholz, E. P. Cummins, A. Cheong, and C. T. Taylor. "Rest Mediates Resolution of Hif-Dependent Gene Expression in Prolonged Hypoxia." *Sci Rep* 5 (2015): 17851.
129. Lin, T. P., Y. T. Chang, S. Y. Lee, M. Campbell, T. C. Wang, S. H. Shen, H. J. Chung, Y. H. Chang, A. W. Chiu, C. C. Pan, C. H. Lin, C. Y. Chu, H. J. Kung, C. Y. Cheng, and P. C. Chang. "Rest Reduction Is Essential for Hypoxia-Induced Neuroendocrine Differentiation of Prostate Cancer Cells by Activating Autophagy Signaling." *Oncotarget* 7, no. 18 (2016): 26137-51.