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Epigenetic Regulation of Oxysterol Formation

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Keywords

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Abbreviations

24S-OHC: 24S-hydroxycholesterol; 5-Aza: 5-azacitidine; ASCOM: Activating signal cointegrator-2 containing coactivator complex; ATF3: Activating transcription factor-3; BAF: Brg-1 associated factors; Brg-1: Brahma-related gene-1; CBP: cAMP responsive element binding protein 1-binding protein; CH25H: Cholesterol 25-hydroxylase; CTCF: CCCTC-binding transcription factor; CYP27A1: sterol 27-hydroxylase; CYP39A1: 24-Hydroxycholesterol 7α-hydroxylase; CYP3A4: Cytochrome P450, family 3, subfamily A, polypeptide 4; CYP46A1: Cholesterol 24-hydroxylase; CYP7A1: Cholesterol 7α-hydroxylase; CYP7B1: Oxysterol 7α-hydroxylase; DMNT:DNA Methyltransferase; DZA: 5-aza-2'-deoxycitidine; ERK: Extracellular signal regulated kinase-1/mitogen activated protein kinase 3; FGF-1: Fibroblast growth factor-1; FXR: Farnesoid X receptor; G9a: Histone-lysine N-Methyltransferase; GABP: GA-binding protein; GROS: Genes responsible for oxysterol synthesis; H1: Histone protein H1; H2A: Histone protein H2A; H2B: Histone protein H2B; H3: Histone protein H3; H4: Histone protein H4; HD: Huntington's Disease;HDAC: Histone deacetylase (number indicates family member); HDACi: Histone deacetylase inhibitor; HNF1: Hepatocyte nuclear factor-1; HPRT: Hypoxanthine-guanine phosphoribosyltransferase; LRH-1: Liver receptor homolog-1; LDLD: Low-density lipoprotein receptor; LXR: Liver X receptor; MEK: Mitogen-activated protein kinase kinase-1; mSin3A: Transcriptional regulator, SIN3A; NHR: Nuclear hormone receptor; p300: Histone acetylase p300; PPARα: peroxisome proliferator-activated receptor-α PRMT1: Protein arginine methyltransferase-1; PXR: Pregnane X receptor; REST: Restrictive Elelement-1 silencing transcription factor; ROS: Reactive oxygen species; RUNX2: Runt-related transcription factor 2; RXR: Retinoid X receptor; SHP: Small heterodimer partner; SP: Specificity protein (number indicates family member); STAT1: Signal transducer and activator of transcription-1; TLR4: Toll like receptor-4; TSA: Trichostatin A.

Abstract

Oxysterols are oxygenated derivatives of cholesterol that may be formed by either enzymatic or non-enzymatic mechanisms. Expression of the genes responsible oxysterol synthesis (GROS) is known to be restricted across different tissues and cell types. Regulation of the transcription of GROS and the activity of their enzyme transcripts has been the subject of intense activity for many years. Recent studies have sought to decipher the mechanism(s) that underpin the restricted expression of the GROS. Available data indicates that epigenetic mechanisms have an important role to play in the control of the expression of GROS. In the current review we summarize the available evidence for the epigenetic regulation of these genes.

Introduction

Oxysterols are C27 derivatives of cholesterol that contain one or more oxygen function(s) in addition to the 3β-hydroxy group present in cholesterol. Oxysterols may be formed by two processes – non-enzymatic mechanisms mediated by reactive chemical species such as reactive oxygen species (ROS) [1] and enzymatic processes [2, 3]. With one exception (24S,25-epoxycholesterol) all oxysterols are formed either directly from cholesterol, or from oxysterols derived from cholesterol [4]. The enzymes regulating the formation of oxysterols (i.e. GROS) are not universally present in different tissues and cells, with the consequence that particular organs are enriched with particular oxysterols, typically in relation the relative expression of the appropriate GROS [5]. For example, the brain contains relatively high amounts of 24S-hydroxycholesterol (24S-OHC) and its generative enzyme, cholesterol 24-hydroxylase (CYP46A1), is highly expressed in CNS neurons [6].

Over the last two decades many investigators have explored the mechanisms by which different GROS are regulated at the transcriptional level [7]. These studies have highlighted a role for numerous different transcription factors, in particular the nuclear hormone receptor (NHR) family of proteins, as well as several different intracellular signaling pathways in the transcriptional regulation of GROS. While it is beyond the scope of the current review to detail these mechanisms, it should be emphasized that studies on the role of epigenetics in the regulation of GROS typically emerges from the limitations of classical mechanisms to explain certain regulatory features. For details of these studies the reader is directed to [7, 8] for recent reviews. In this paper we review the studies to date on the role of epigenetics in regulation of GROS and summarise the evidence for this (Table 1).

Epigenetic Regulation of Gene Expression

The organisation of the genome in eukaryotes is based on nucleosomes, nucleoprotein complexes that consist of 147 bp of DNA wound around an octameric complex made up of two copies of the histone

proteins H2A, H2B, H3 and H4 [9]. Histone protein H1 binds to a 20 bp linker region that connects nucleosomes together. Further organisation of nucleosomes leads to the formation of solenoids, chromatin and chromosomes. While packaging the genome in this manner is crucial to permit it to fit into the cell nucleus, it also has profound effects on gene expression. Highly packed regions of the genome (i.e. heterochromatin) are transcriptionally silent while more open regions of the genome (i.e. euchromatin) are accessible to the basal transcriptional machinery and are thus active. Changes in the structural organisation of the genome are thus considered important determinants of gene expression [10].

Epigenetics is defined as 'the study of heritable changes in gene expression that occur independent of changes in the primary DNA sequence' [11]. These changes are mediated by alteration in the structural organisation of the genome, typically as a result of developmental, nutritional, microbial, toxic or pharmacological challenge, that influence the expression of genes in a cell, tissue and organ specific manner [12]. Such structural organisation is driven by post-translational modifications of histone proteins, DNA methylation and/or modification of nucleosome position along the genome which ultimately leads to a more open genomic organisation [12]. The heritability of these changes enables cells to manifest distinct identities even though they contain the same basal genomic sequence.

The N-terminal tail region of histone proteins can be modified post-transcriptionally by acetylation, methylation, phosphorylation, sumoylation, ubiquitylation and citrullination [13]. These modifications either directly alter the genomic structure, or facilitate recruitment (or occlusion) of other effector proteins, e.g. Histone Deacetylases (HDAC) [13]. In general, hyperacetylation of histone proteins is associated with gene activation and hypo/deacetlytation is associated with gene repression. In contrast, the effects of histone tail methylation are strongly linked to the position and type of methylation. For example, trimethylation of lysine 4 of histone H3 is a mark of

transcriptionally active regions, while trimethylation of lysine 9 of histone H3 is a mark of transcriptional repression[12].

DNA methylation is a result of the addition of a methyl group to cytosine present in a CpG unit by the action of either so-called de novo or maintenance DNA methyltransferases (DNMT) which establish methylation patterns during early development or cellular division, respectively [14]. As with histone post-translational modifications, DNA methylation can influence the recruitment or occlusion of other regulatory proteins or methyl-CpG binding proteins. These changes typically result in transcriptional repression.

The position of nucleosomes on a gene sequence also influences gene expression and nucleosome-free regions are often present at the putative promoter region where they are believed to provide a platform for assembly of the basal transcriptional machinery [9]. ATP dependent remodelling of nucleosome organisation can also regulate access to regulatory regions which may be wound around the histone octamer, thereby permitting (or preventing) regulation of gene expression. See [15] for a recent review of nucleosome remodelling).

CYP7A1

CYP7A1 encodes the cholesterol 7α-hydroxylase enzyme which is the initial and rate limiting step in the classical pathway for bile acid formation [2]. It is highly expressed in the hepatocytes of the liver but virtually absent form all other tissues [5]. The regulation of CYP7A1 expression has been extensively studied and sophisticated regulatory pathways have been characterised. In short, cholesterol stimulates while bile acids, the terminal products of the bile acid biosynthetic pathway, inhibit the expression of *CYP7A1*. The precise details of these regulatory loops are species-dependent, e.g. activation of CYP7A1 by the liver X receptor (LXR) occurs in the mouse but not in man (the reader is directed to [7] for a recent review of this area). Many other transcription factors and intracellular

signalling pathways have been implicated in the regulation of CYP7A1, at least in some species [7]. The regulatory mechanisms involved in the control of CYP7A1 defined a paradigm for the investigation of the transcriptional regulation of other GROS.

Feedback repression of CYP7A1 expression by bile acids occurs in an indirect mechanism, whereby the farnesoid X receptor (FXR), a nuclear hormone receptor which is activated by bile acids and which forms a heterodimer with the retinoid X receptor (RXR), binds to the promoter of the small heterodimer protein (SHP). Binding of the FXR:RXR heterodimer increases transcription of the SHP gene, which then interacts with the liver receptor homologue-1 (LRH1) at the CYP7A1 promoter, leading to transcriptional repression of the gene. Studies initiated in 2004 by Kemper *et al* ([16]and elegantly summarised by Smith in [17]) have characterised the mechanisms by which FXR and SHP lead to activation and repression, respectively, and have identified a crucial role for post-translational modifications of histone proteins and dynamic chromatin remodelling in these processes. In particular, SHP has emerged as a central coordinator of chromatin modifying factors and has been shown to interact with dozens of different nuclear receptors, transcription factors and co-regulators (See [18] for a review of these interactions).

In brief, ligand binding to FXR triggers the recruitment of the histone acetylase p300 (EP300, more commonly known as p300) and results an increase in histone acetylation at lysine 9 and 14 of histone H3 [19] as well as direct acetylation of FXR. The modifications promote the recruitment of further effector proteins, including ATP dependent chromatin remodelling complex containing the Brahma-Related Gene 1 (Brg-1) and Brg-1 Associated Factors (BAF) which results in chromatin remodelling of the SHP promoter. In addition, the Activating Signal Cointegrator-2 containing coactivator Complex (ASCOM) is recruited and catalyses the trimethylation of lysine 4 of histone H3 [20]. The combined effect of these factors is to significantly activate the SHP gene. Subsequently the SHP protein interacts with LRH-1 at the CYP7A1 promoter where it recruits repressor proteins leading to sequential epigenetic changes at the CYP7A1 promoter. Recruitment of the murine homolog of yeast

ranscriptional regulator, SIN3A (mSin3a) and histone deacetlyases (HDAC) -1 and -2 led to the deacetylation of lysines 9 and 14 of histone H3 while histone-lysine N-methyltransferase (EHMT2, more commonly known as G9a protein) trimethylates lysine 9 of histone H3. Subsequently a Brm containing complex remodels the chromatin in an ATP-dependent manner. The combined effect of these modifications is to suppress the expression of CYP7A1 (See Figure 1).

In addition Knutson *et al* presented evident that HDAC3 may have a role in the regulation of CYP7A1 - liver specific knockout of HDAC3 in mice resulted in an increase in CYP7A1 mRNA with a concimmitant increase in histone tail acetlyation at the promoter of CYP7A1 [21]. SHP has been reported to interact with HDAC3[22] again underscoring the central role of this small protein in orchestrating formation of bile acids. Li et al [23] have also presented evidence that glucose can modify the chromatin structre at the CYP7A1 promoter facilitating expression of the mRNA.

Using the low density lipoprotein-receptor (Ldlr) deficient mouse Mitro *et al* investigated the effects of HDAC inhibition under *in vivo* conditions [24]. They reported that plasma cholesterol (including low density lipoprotein cholesterol) was decreased, while at the same time liver cholesterol, bile acids and fecal bile-acid excretion were all increased treatment with either of two different HDAC. At the transcriptional levels the mRNA level of *Cyp7a1* increased by approximately ten-fold. Detailed mechanistic studies revealed that HDAC inhibition prevented normal bile acid medicated suppression of *Cyp7a1* and that Hdac7 is critical for this process, via control of the assembly of a repressive complex. A more thorough discussion of CYP7A1 can be found in the review by Dr. de Fabiani in this issue.

CYP27A1

CYP27A1 encodes the enzyme sterol 27-hydroxylase which is the initial step in the alternate (or acidic) pathway for bile acid formation [2]. It is widely expressed enzyme and is found in many

different cells and tissues. However, hepatocytes, endothelial cells and monocyte-macrophage cells express particularly high levels of CYP27A1 [5, 25, 26]. CYP27A1 is capable of catalysing multiple hydroxylations of its substrates, leading to the production of both 27-hydroxycholesterol and its terminal oxidation product 3β-hydroxyl cholestenoic acid [27].

As far as I have been able to determine, there are only three publications reporting investigations of CYP27A1. In a 2005 study designed to investigate the influence of the DNMT inhibitor 5-azacytidine (5-Aza), Escher *et al* reported that treatment with 5-Aza had not effect on the levels of CYP27A1 in RAW macrophages [28]. Subsequently Chittur *et al* investigated the effects of a HDAC inhibitor on cholesterol homeostasis in HepG2 cells and reported that treatment with the inhibitor led to a reduction in CYP27A1 mRNA to 10-15% of the control levels [29]. This effect was time dependent – mRNA levels were reduced during the first 24 hours of treatment but the suppression was lost by 48 hours. In contrast, Shafaati *et al* reported that in SH-SY5Y neuroblastoma cells, the levels of CYP27A1 mRNA increase by a factor of five following treatment with the same HDAC inhibitor [30]. A possible reason for this discrepancy is that HepG2 cells are known to express a relatively large amount of CYP27A1 mRNA in comparison to SH-SY5Y cells and the basal chromatin architecture may and accessory proteins, including HDACs, are likely to be different and respond differently to the broad acting HDAC inhibitor used in these studies. Further studies will be essential to map the specific changes in response to HDAC inhibition and explore the potential for other epigenetic mechanism to regulate CYP27A1.

CYP46A1

CYP46A1 encodes cholesterol 24-hydroxylase an enzyme which is highly expressed in the neurons of the central nervous system but which is virtually absent from other tissues [6]. *CYP46A1* does not appear to be regulated in a similar manner to the other GROS and initial characterisation of the

CYP46A1 promoter revealed insensitivity to pathways known to regulate other GROS such as *CYP7A1* and *CYP27A1*[31].

As far as we have been able to determine, the earliest report on the regulation of *Cyp46a1* by HDAC inhibition is by Ballas et al in a study on the plasticity of neuronal gene chromatin during neurogenesis [32]. In this study they reported that HDAC inhibitor treatment led to an increase in the expression of *Cyp46a1* mRNA by approximately ten-fold in primary cultures of mouse cortical neurons. Later contemporaneous studies by Shafaati et al and Nunes et al reported that HDAC inhibition led to a profound upregulation of the mRNA expression of CYP46A1 (over 50-fold) in several different cell systems [30, 33]. In addition Shafaati *et al* also reported that treatment of mice with HDAC inhibitors modestly increased the expression of *Cyp46a1* in murine liver [30].

The group of Rodrigues *et al* have significantly advanced our understanding of the mechanisms underpinning the regulation of CYP46A1 and identified that specificity proteins (SP) are necessary for the TSA effect to occur – a decrease in SP3 binding at specific elements favours the loss of HDAC1 and HDAC2 from the CYP46A1 promoter and the subsequent recruitment of p300 and CREB-binding protein (CBP) [34]. The same group also reported that treatment with DMNT inhibitors led to the increase in mRNA expression of CYP46A1 and potentiated the effect of HDAC inhibition [34]. However, despite the presence of predicted CpG islands, there were no methylated bases in the proximal promoter of CYP46A1 and application of DMNT inhibitors led to effects in cells where no promoted methylation was observed. Again the increase was attributed to the alteration in the promoter occupancy of Sp1, Sp3 and Sp4, with a loss of Sp3 correlated with a loss of Sin3A and HDAC, thus facilitating remodelling of chromatin (Figure 2).

Very recently Nunes *et al* presented further evidence of a central role for SP proteins in the regulation of CYP46A1 [35]. Treatment of SH-SY5Y cells with inhibitors of mitogen activated kinase-kinase (MEK) or extracellular signal-regulated kinase-1 (ERK) potentiated the stimulatory effect of the HDAC

inhibitor trichostatin A (TSA). This is in line with data on *Cyp46a1* mRNA levels presented by Lu et al, in a rat astrocyte model, indicating increased mRNA levels following MEK inhibition, although there is high variability in these results [36]. In contrast treatment with the protein phosphatase inhibitor okadaic acid reduced the potency of the TSA effect. The authors provide evidence that phosphorylated ERK (pERK) is present at the promoter of CYP46A1 where it interacts with Sp3, at least during certain time points, and suggest that pERK phoshorylates Sp3 and triggers the recruitment of corepressor proteins.

It is notable that an oligomeric protein complex containing the oxysterol binding protein (OSBP) has been shown to act as a cholesterol sensitive protein phosphatase and is involved in regulating the amount of pERK in the cell [37]. This phosphatase activity was inhibited by decreased cholesterol or treatment with 25-hydroxycholesterol. This raises the intriguing possibility that increased 24S-hydroxycholesterol may inhibit the dephosphorylation of pERK, leading to downregulation of CYP46A1, while cholesterol facilitates the opposite, effectively promoting CYP46A1 expression and oxidative pathway for cholesterol removal.

While the involvement of the MEK-ERK signalling pathway in the regulation of CYP46A1 is intriguing, it prompts the question as to the identity of the signal which triggers this pathway. The study cited above of the effects of fibroblast growth factor-1 (FGF-1) on cholesterol homeostasis in rat astrocytes did not find evidence of any significant effects on *Cyp46a1* expression. However, it should be noted that astrocytes express at best trace amounts of *Cyp46a1* mRNA and it would be highly interesting to investigate the analogous situation in neuronal cell systems or the differentiated Ntera2 cell system reported by Milagre *et al* [38].

Computational analysis of the CYP46A1 gene revealed two candidate response elements for the RE-1 Silencing Transcription Factor (REST) a protein involved in restricting the expression of neuron specific genes to neurons [39] – one in intron 1 which is conserved in different species and a second in

intron 12 only present in primates (Figure 3). Two candidate sites for the CCCTC-binding factor (CTCF) were also identified, one in the proximal promoter region and the second in intron 1. CTCF can act as a boundary marker between active and repressive chromatin domains [40]. The marked differences in the expression of the CYP46A1 and neighbouring genes in the context of the location of the putative CTCF sites suggests that CTCF may function in this role for CYP46A1. In the context of REST, preliminary studies of SHSY5Y cells using a dominant negative REST construct results in a small induction in the mRNA levels of CYP46A1 (cf Figure 3; M Shafaati, S Meaney, unpublished). Although this effect is consistent with a role for the REST protein in the regulation of CYP46A1, the small size of this effect is likely to be aresult of the low level of basal expression of REST in this cell system used and warrants further exploration of these effects.

CH25H

CH25H encodes cholesterol 25-hydroxyase which is responsible for the formation of 25-hydroxycholesterol. Data in the literature concerning the regulation of this gene are extremely limited. It has been reported that Ch25h expression is induced following treatment of murine bone marrow derived macrophages with lipopolysaccharide, in a Toll Like Receptor 4 dependent manner [41]. Recently, Park described a role for interferon-γ in the regulation of murine *Ch25h* expression in dendritic cells and macrophages, via the Signal Transducers and Activators of Transcription 1 (STAT1) pathway [42]. As it has been described that HDAC activity is required for STAT1 dependent gene activation [43], it is likely that other epigenetic processes are involved in the regulation of *Ch25h*. Very recently the first clear evidence for such an involvement has been presented by Gold *et al* [44]. Deletion of the Activating transcription factor 3 (*Atf3*) gene resulted in an increase in *Ch25h* promoter histone acetylation in bone marrow derived macrophages, leading to an increase in both the mRNA levels of CH25H as well as the enzymatic product, 25-hydroxycholesterol.

CYP3A4

CYP3A4 encodes an enzyme responsible for the metabolism of a wide variety of xenobiotics and pharmaceuticals. It has been estimated that CYP3A4 can metabolise about half of commonly used drugs and it is highly expressed in the liver. With regards to oxysterol formation, CYP3A4 has been reported to catalyse the formation of both 25-hydroxycholesterol and 4β-hydroxycholesterol [45, 46]. While it is well established that the nuclear hormone receptor pregnane X receptor (PXR) is a key regulator of CYP3A4 there is a paucity of data on the epigenetic regulation of CYP3A4. Studies by Sheen's group in 2004 revealed that HDAC inhibitors influence CYP3A4 promoter activity in hepatoma cells and suggested that HDAC inhibitors facilitated PXR dependent regulation of CYP3A4 expression [47, 48]. Recent studies by Xie *et al* highlighted a role for Protein Arginine Methyltransferase 1 (PRMT1), which methylates arginine 3 of histone H4, in the regulation of CYP3A4 [49]. Ligand binding to PXR resulted in a recruitment of PXR and PRMT1 to the upstream xenobiotic response enhancer module of CYP3A4, with concomitant induction of histone acetylation and methylation. Habano *et al* also presented data in support of a role of direct DNA metyylation in cellular models of the intestine – treatment with 5-aza-2'-deoxycytidine resulted in induction of CYP3A4 expression in some but not all of these cellular models [50].

CYP7B1

CYP7B1 encodes the oxysterol 7α-hydroxylase enzyme that catalyses the introduction of a 7α-hydroxyl group into 27-hydroxycholesterol and 25-hydroxycholesterol [14]. In similarity with CYP27A1 it is also widely expressed in different cells and tissues. CYP7B1 is also expressed in a sexually dimorphic manner with expression greater in males than in females [15].

In an elegant study, Leuenberger *et al* explored the mechanistic basis for the sexually dimorphic expression and provided evidence that methylation was critical for this to occur [51]. Post

translational sumoylation of the peroxisome proliferator-activated receptor-α (Pparα) increases interaction with GA-binding protein (Gabp) and leads to recruitment of HDAC and DNMT, ultimately leading to methylation of CpG residues within a key SP1 site and the trimethylation of lysine 9 of histone H3. These post translational modifications abrogate the binding of SP1 to the *Cyp7b1* proximal promoter which releases the mechanisms driving *Cyp7b1* expression.

Olsson *et al* showed that the promoter of human CYP7B1 is methylated and that this methylation is of critical importance for the down regulation of CYP7B1 in the human prostate [52]. They also showed that DNMT inhibitors could influence expression of CYP7B1 in prostate cell lines. Shafaati *et al* reported that in the SH-SY5Y cell system, CYP7B1 expression was suppressed by HDAC treatment [13]. Subsequently, the study of Leuenberger et al reported that HDAC inhibitor treatment led to an increase in CYP7B1 expression in HepG2 hepatoma cells and that silencing of HDAC1 and HDAC3 activated CYP7B1 in this cell system [51]. Thus while the available evidence is relatively limited, the consistent pattern of results in both the human prostate and mouse liver indicates that both methylation of DNA and/or proteins and histone acetylation are likely to be of importance for the regulation of the expression of CYP7B1.

CYP39A1

CYP39A1 encodes an oxysterol 7α-hydroxylase enzyme that appears to have a preference for 24S-hydroxycholesterol as a substrate [53]. It was originally cloned form the liver, although low levels have been detected in the eye [54, 55] and brain[56]. A notable feature is the sexually dimorphic, with females expressing a higher level of both the mRNA and the protein [53].

To date there have been very few direct studies of this gene and the data on transcriptional regulation is very limited. Carbamazepine appears to induce *Cyp39a1* and it is likely that a PXR dependent

mechanism is involved [57]. Evidence has also been presented that *Hnf-1α* and Runt-related transcription factor 2 (*Runx2*) (which is known to be involved in maintenance of epigenetic bookmarking in the context of bone cell development) may have a role in the expression of this gene [58]. Shafaati *et al* have described that HDACi treatment led to an increase of *Cyp39a1* in the brain with a decrease in hepatic expression [30]. In addition, preliminary data suggests that epigenetic mechanisms may have a role in the observed sexual dimorphism of *Cyp39a1* (unpublished observations).

Applications of epigenetic regulation of GROS for sterol biology

Epigenetics has been widely applied in both cell biology and cancer, and there have been many advances in this area. In the case of cholesterol and lipid biochemistry there is much less known. However, the available evidence indicates that epigenetic mechanisms may have the potential to drive significant advances in our understanding of sterol related or dependent biological processes. For example, it has been reported that mutant huntingtin is able to modify the sterol content in the brains of patients with Huntington's Disease (HD) as well as HD model systems, both in vivo and in vitro. Epigentic mechanisms can contribute to the explanation for these phenomenon –the huntingtin protein is known to sequester the REST protein in the cytoplasm, preventing it from entering the nucleus where it normally plays a role in suppressing many genes [59]. Preliminary evidence indicates that CYP46A1 is one such gene as transfection with dnREST increases the expression and there are candidate REST binding sites in CYP46A1. In the case of mutant HD, it has been described that it is increased in the cytoplasm, thus leading to a decreased capacity to sequester REST in the cytoplasm, allowing REST to enter the nucleus at higher than normal levels, enhancing its repressive effect and thus reducing the expression of CYP46A1 and its oxysterol product. This is consistent with the cell models that have been described [60].

Formation of oxysterols from cholesterol represents an accepted mechanism for the removal of cholesterol from peripheral tissues which is active throughout the body but particularly so in macrophages and endothelial cells [25, 26]. Critically, while cholesterol can be exchanged between different lipoproteins, effectively 'short circuiting' lipoprotein dependent reverse cholesterol transport, the products of the oxidative pathway are on a short 'one-way-trip' out of the body. This pathway thus functions as a one-way reverse cholesterol transport pathway [25]. Epigenetic regulators of GROS may have potential as anti-atherosclerotic agents whereby these pathways are enhanced following transcriptional therapy with epigenetic regulators. Theoretically, reduction in CYP7B1 and induction of CYP27A1 would optimize this pathway, and evidence has been presented that HDACi may shift the expression of these genes in this manner, at least under *in vitro* conditions [30]. Epigenetic regulation of the oxidative pathway for cholesterol removal may thus represent a novel anti-atherosclerotic therapy.

Concluding Remarks

The exploration of the ways in which the cell, tissue and organ specific patterns of GROS are established and maintained is of profound interest to the oxysterol research community. The recent reports of the involvement in oxysterols in a myriad of biological processes, from neurodegeneration to the immune response, is likely to increase interest in this area. Studies on the epigenetic regulation of GROS is an emerging area which is ripe for exploration and the discovery of novel biology and it has the potential to answer questions about sterol biology which have remain. The next few years promise to be an exciting time in this emerging field.

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Table

Table 1: Summary of literature on epigenetic regulation of GROS.

Gene	Evidence for epigenetic regulation	CTCF	RE-1
CYP7A1	Li 2012; Li 2010; Kim 2009; Knutson 2008; Fang 2008; Gilardi 2007; Mitro 2007; Gobinet 2005; Kemper 2004;	✓	X
CYP7B1	Shafaati 2009; Leuenberger 2007; Olsson 2007;	✓	✓
CYP27A1	Shafaati 2009; Chittur 2008; Escher 2005;	✓	X
CYP46A1	Milagre 2012; Milagre 2010; Nunes 2010; Shafaati 2009;Ballas 2005	✓	✓
CYP39A1	Shafaati 2009; Tepyluk 2008;	✓	X
CYP3A4	Kim 2004; Ahn 2004; Xie 2009;	✓	X
CH25H	Gold 2012; Park 2012;	✓	X

Note that the columns for CTCF and RE-1 sites are based on bioinformatics analysis and data mining and have not been experimentally verified for these genes.

Figure Legends:

Figure 1. Mechanism of epigenetic regulation of cholesterol 7α-hydroxylase (CYP7A1). Binding of bile acids to FXR at the SHP promoter results in activation recruitment of histone acetylases and acetylation of histone proteins and FXR. Subsequent recruitment of Brg-1 containing chromatin remodelling factors and ASCOM lead to significant activation of the SHP gene. SHP then interacts with the LH1 protein at the CYP7A1 promoter and triggers the recruitment of co-repressors that lead to a series of post-translational modifications and chromatin remodelling which ultimately suppress the expression of CYP7A1. For full details see text.

Figure 2. Mechanism of epigenetic regulation of cholesterol 24-hydroxylase. CYP46A1 transcription is very responsive to treatment with the HDACi TSA. Decreased SP3 presence at the promoter favours the loss of HDAC1 and HDAC2 (presumably in a complex with mSin3a) from the CYP46A1 promoter and the subsequent recruitment of p300 and CBP). DAC treatment also leads to the loss of SP3, despite no observable effects on DNA methylation, in contrast to the situation with CYP7B1. pERK has also been shown to interact with SP3 at the promoter, leading to suppression of CYP46A1 expression and interfering with the stimulatory effect of TSA. For full details see text.

Figure 3. Potential mechanisms for restricted expression of CYP46A1. A) Location of the potential CTCF and RE-1 sites flanking exon 1 of CYP46A1. In this genomic region, expression of CYP46A1 is approximately 50-fold greater than its neighbouring genes, which is consistent with the presence of insulator elements. B) Multi-species alignment of the putative intron-1 RE-1 site. Note the binding site is in the reverse orientation and capital letters indicate complete identity across all species and the black box indicates the core region of the RE-1 element. C) Transfection of SH-SY5Y cells with a dominant negative REST construct (p73-REST) results in an increase in the mRNA levels of CYP46A1 (normalized to HPRT). Although this effect is consistent with a role for the REST protein in the

regulation of CYP46A1, the small size of this effect may be a result of the low level of expression of REST in this cell system.

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Figure 1

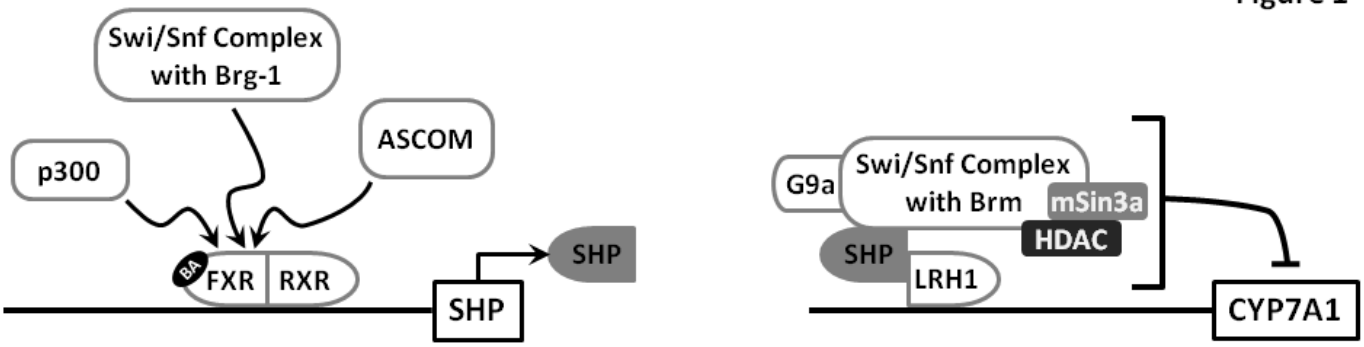
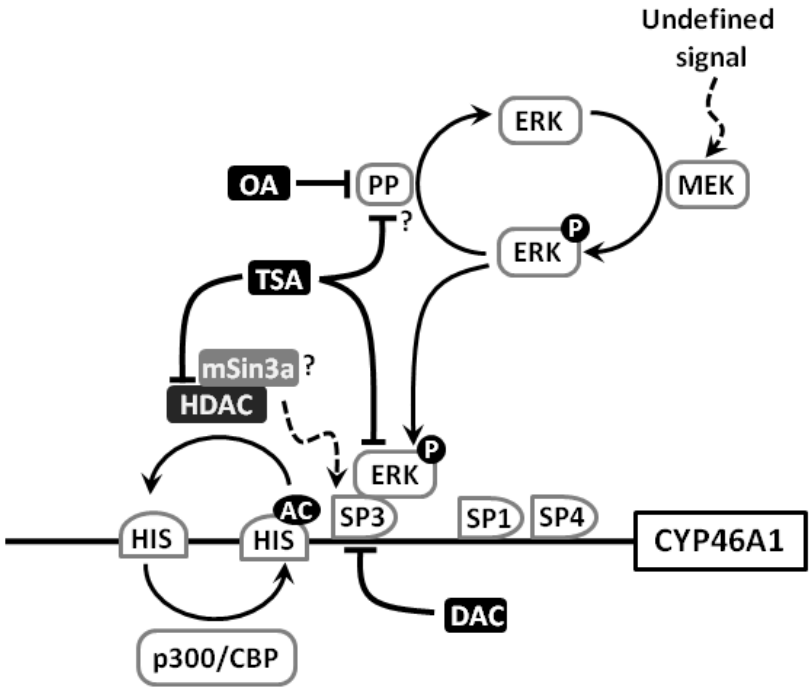
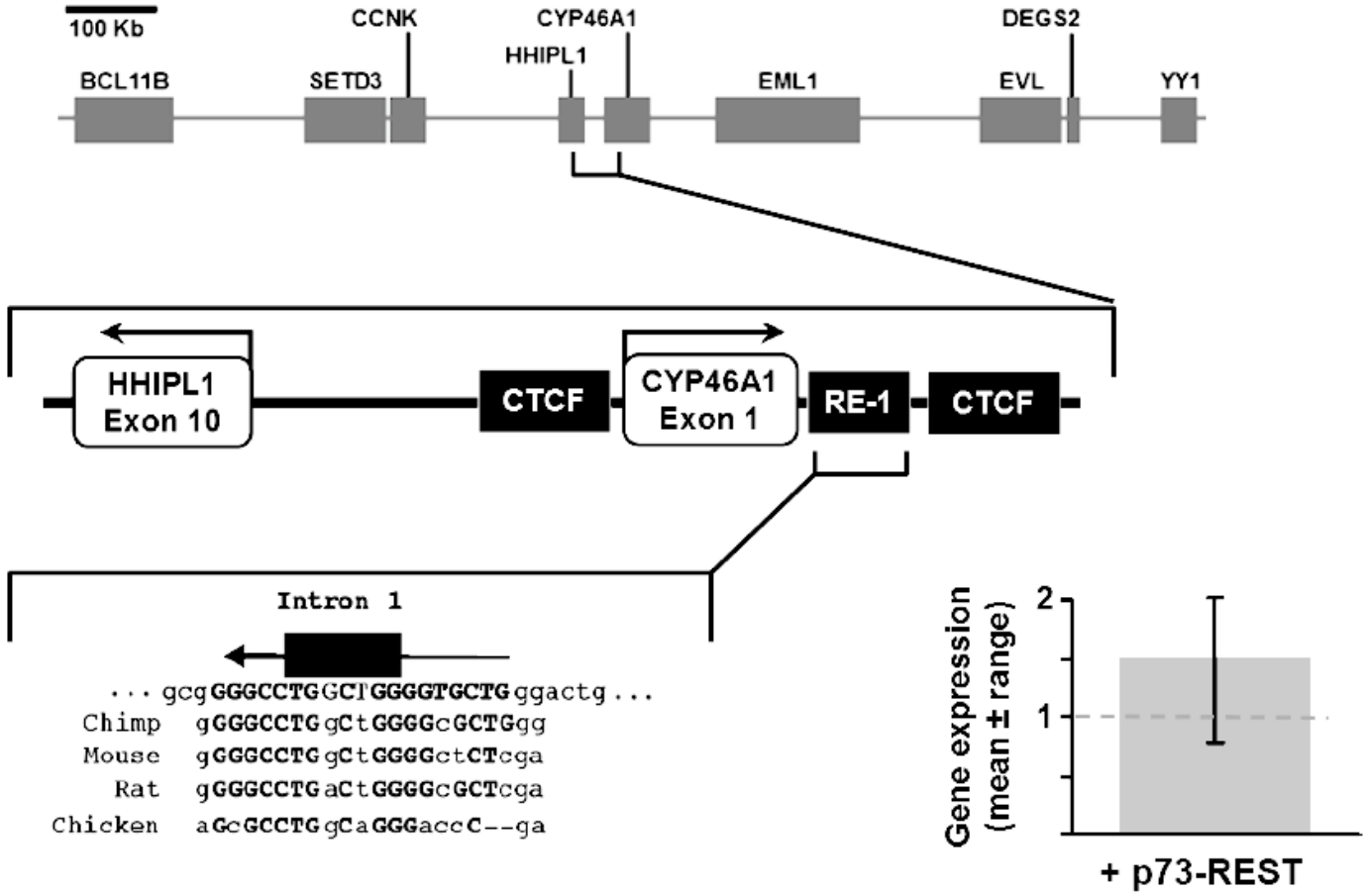


Figure 2

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SETD3
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CYP46A1
Exon 1
RE-1
CTCF
Intron 1
... gcgGGGCCTGGCTGGGGTGCTGggactg ...
Chimp gGGGCCTGgCtGGGGcGCTGgg
Mouse gGGGCCTGgCtGGGGctCTcga
Rat gGGGCCTGaCtGGGGcGCTcga
Chicken aGcGCCTGgCaGGGaccC--ga
Gene expression
(mean ± range)
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1
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