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Commentary

Coordinate regulation of Phase I and II xenobiotic metabolisms by the Ah receptor and Nrf2

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ABSTRACT

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor with important roles in metabolic adaptation, normal physiology and dioxin toxicology. Metabolic adaptation is based on coordinate regulation of a set of xenobiotic-metabolizing enzymes (XMEs), termed AhR battery. Coordination is achieved by AhR/Arnt-binding to XREs (xenobiotic response elements), identified in the 5′ upstream region of *AhR* target genes. The AhR battery encodes Phase I and II enzymes. Interestingly, these Phase II genes are linked to the *Nrf2* gene battery that encodes enzymes that are essential in protection against oxidative/electrophile stress. Nrf2 binds to AREs (antioxidant response elements) in the regulatory region of a large and distinct set of target genes. Functionally characterized response elements such as XREs and AREs in the regulatory region of target genes may provide a genetic basis to understand AhR- and Nrf2-induced genes. Linkage between AhR and Nrf2 batteries is probably achieved by multiple mechanisms, including *Nrf2* as a target gene of the AhR, indirect activation of Nrf2 via CYP1A1-generated reactive oxygen species, and direct cross-interaction of AhR/XRE and Nrf2/ARE signaling. Linkage appears to be species- and cell-dependent. However, mechanisms linking XRE- and ARE-controlled Phase II genes need further investigation. Tightened coupling between Phases I and II by AhR- and Nrf2-induced XMEs may greatly attenuate health risks posed by CYP1A1-generated toxic intermediates and reactive oxygen species. Better recognition of coordinate Phase I and II metabolisms may improve risk assessment of reactive toxic intermediates in the extrapolation to low level endo- and xenobiotic exposure.

1. Introduction

The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor of the bHLH/PAS (basic helix–loop–helix/Per–Arnt–Sim) family with important roles in metabolic adaptation, in normal physiology such as organ and vascular development and dioxin toxicology [1–3]. Metabolic adaptation is achieved by coordinate regulation of a set of

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Abbreviations: AhR, aryl hydrocarbon receptor; ARE, antioxidant response element; BaP, benzo[*a*]pyrene; BCRP, breast cancer resistance protein; GCS, glutamylcysteine synthetase; GSH, reduced glutathione; GST, glutathione *S*-transferase; MRP, multidrug resistance-associated protein; NQO, NAD(P)H:quinone oxidoreductase; Nrf2, nuclear factor erythroid 2-related factor 2; PAH, polycyclic aromatic hydrocarbon; ROS, reactive oxygen species; SULT, sulfotransferase; tBHQ, *tert*-butylhydroquinone; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; UGT, UDP-glucuronosyltransferase; XMEs, xenobiotic-metabolizing enzymes; XRE, xenobiotic response element

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xenobiotic-metabolizing enzymes (XMEs), termed AhR battery [4,5]. Coordination occurs by AhR/Arnt-binding to xenobiotic response elements (XREs, also termed dioxin response elements [DREs]), identified in the 5′ upstream region of *AhR* target genes [2,6,7]. The *AhR* gene battery is arguably one of the best-characterized examples of coordinately regulated genes in eukaryotes. The XRE sequence 5′-T/GnGCGTG-3′ is not symmetrical, suggesting that AhR and Arnt bind to different parts of the sequence. In vitro studies of the E-box sequence (5′-CACGTG-3′) indicated that Arnt binds to GTG; hence, AhR binds 5′ of this sequence [2,7]. Flanking sequences most likely influence AhR binding to particular target genes. It has to be noted that a number of XRE-controlled genes/proteins have been identified which are not involved in xenobiotic metabolism, but in cell proliferation and differentiation ([3,7,8] for references). The AhR battery discussed here is focused on Phase I XMEs (CYP1A1, 1A2 and 1B1) and on Phase II enzymes (NQO1, GSTA2, UGT1A1 and UGT1A6), with emphasis on UGTs which are often neglected in reviews. A schematic view of XME functions is illustrated in Fig. 1. Rodent and human conjugate transporters such as MRPs and BCRP may also be members of the *AhR* gene battery since their expression is increased by AhR agonists, but the responsible XREs still have to be elucidated [9,95,96]. Notably, two definitions of Phase I and II XMEs have emerged. For example, NQO1 (and other enzymes with similar regulation such as the aldehyde dehydrogenase ALDH3A1 [3–5], the latter not discussed here) is a Phase I enzyme on the basis of the catalyzed chemical reaction, but is often regarded as Phase II enzyme on the basis of common regulation by Nrf2 [10,11]. Among many coordinately regulated genes identified in microarray studies, characterization of functional XREs identifies the primary target genes of the AhR. In this context it is important to establish functionality since core XRE sequences may be present randomly in the genome.

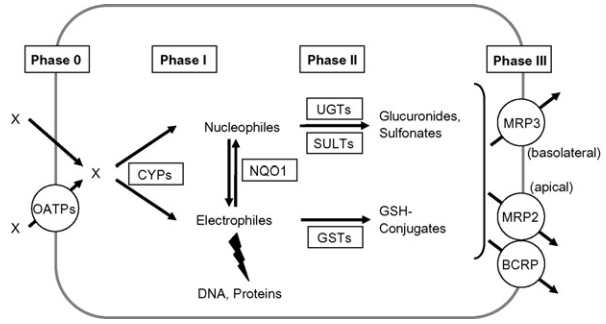

Fig. 1 – Overview of Phase I and II enzymes of xenobiotic metabolism in hepatocytes, and of functionally related transporters in Phases 0 and III of the biotransformation system. Abbreviated XMEs: CYPs, cytochromes P450; NQO1, NAD(P)H:quinone oxidoreductase 1; UGTs, UDP-glucuronosyltransferases; SULTs, sulfotransferases; GSTs, glutathione S-transferases. Phase 0 includes uptake transporters, including organic anion transport proteins (OATPs) and Phase III efflux transporters such as multidrug resistance-associated proteins (MRPs) and breast cancer resistance protein (BCRP) [9,95,96].

It has been recognized recently that Phase II genes of the *AhR* gene battery are linked to a second gene battery, termed *Nrf2* gene battery, which is involved in protection against oxidative stress [10–15]. The bZip transcription factor Nrf2 binds to antioxidant response elements (AREs) in the regulatory region of a large and distinct set of target genes, including the Phase II genes NQO1, GSTA2 and UGT1A6, discussed under Section 2.2. It also includes glutamyl-cysteine synthetase (GCS, the rate limiting enzyme in the synthesis of glutathione), heme oxygenase-1 and other proteins protecting against oxidative stress. An ARE consensus sequence (5′-TG/TAC/GnnnGC-3′) has been identified [13]; but so far no universally applicable consensus sequence can be derived [16,17]. Deficiency of both Nrf1 and Nrf2 results in early embryonic lethality due to oxidative stress [18]. Nrf2 and its function appear to be evolutionary conserved since a Nrf2-like protein (SKN-1) has been identified in *Caenorhabditis elegans* [19]. Linkage between AhR and Nrf2 batteries is probably achieved by multiple mechanisms in a species and cell-specific manner, discussed under Section 2.3: (i) *Nrf2* is a target gene of the AhR [20]. (ii) Nrf2 can be activated indirectly by reactive oxygen species generated by induced CYP1A1 [42,43]. (iii) In the case of NQO1, direct cross-interaction between AhR/XRE and Nrf2/ARE signaling has been proposed [14].

In the present commentary current knowledge about transcriptional regulation of Phase I and II XMEs by AhR/XRE and Nrf2/ARE signaling is reviewed and compared in rodents and humans. Functional consequences of coordinated Phase I and II enzyme regulations are discussed using detoxification of benzo[*a*]pyrene (BaP) quinones and catechol estrogens as examples.

2. AhR/XRE-induced xenobiotic-metabolizing enzymes (XMEs)

After brief comparison of the AhR battery in rodents and humans, linkage of AhR- and Nrf2-controlled Phase II genes is addressed. The discussion is based on selected Phase I and II genes with characterized functional XREs and/or AREs (Fig. 2). In addition, factors responsible for hormonal control and for tissue-specific expression are discussed to underline that the AhR exerts its functions in concert with many other factors.

2.1. *Phase I XMEs*

2.1.1. *CYP1A1*

CYP1A1 represents the best-characterized AhR-induced enzyme, and often serves as paradigm [6]. Studies with CYP1A1-null mice after oral BaP exposure indicated that it is the major CYP involved in bioactivation and detoxification of PAHs [21], as discussed under Section 3.3. Clusters of functional XREs have been characterized in the enhancer region of rodent and human CYP1A1 (Fig. 2) [6,22–25]. Presence of multiple XREs may allow strong dose-dependent responses upon exposure to AhR agonists. The human CYP1A locus on chromosome 15 includes both CYP1A1 and 1A2 genes in a head to head orientation. Transcription start sites are separated by approximately 20 kb of intervening DNA [26].

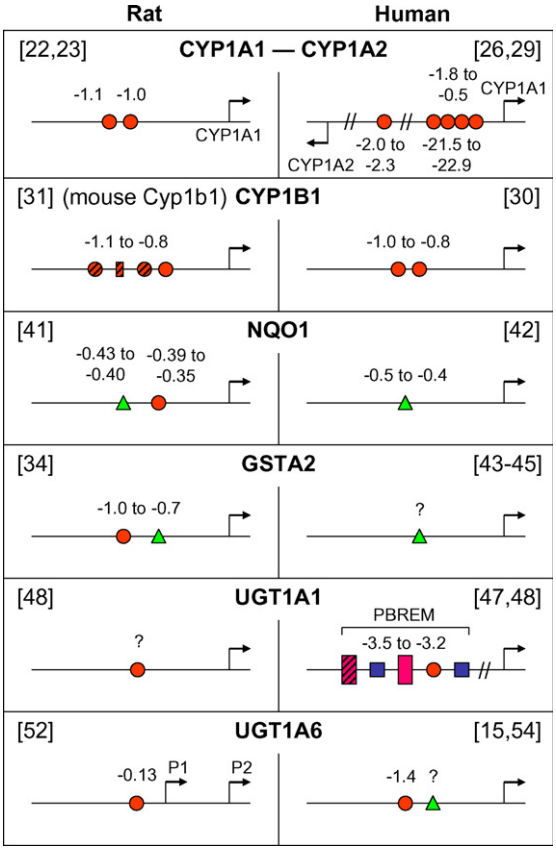

Fig. 2 – Simplified illustration of the position of characterized XREs (circles) and AREs (triangles) in the enhancer region of rat and human *AhR* target genes. The position is given in kb with respect to the transcription start site, and corresponding references are indicated in brackets. Positions are not drawn to scale; large distances (>2 kb) are indicated. Anomalous XREs surrounding an E-box found in mouse CYP1b1 are listed (hatched circles). In addition to the XRE identified in the regulatory region of UGT1A1, other motifs present in the phenobarbital response module (PBREM) are indicated: glucocorticoid response elements (GREs, squares) as well as binding sites for constitutive androstane receptor (CAR, indicated by the rectangle) and pregnane X receptor (PXR, indicated by the hatched rectangle). In the rat UGT1A6 gene, the position of the XRE is given with regard to the P1 promoter. Question marks indicate that XREs or AREs have not been identified but are likely to exist, based on induction studies. For details see text.

This orientation raised the possibility that the genes for CYP1A1 and 1A2 share the 5′-flanking region.

2.1.2. *CYP1A2*

CYP1A2 is expressed constitutively in human liver but is further inducible by AhR agonists [1,4,27]. It is involved in the metabolism of aromatic amines and of caffeine, the latter being widely used as an in vivo probe drug for this cytochrome [27,28]. It also catalyses bioactivation and detoxification of the human mycotoxin and carcinogen aflatoxin B1. Aflatoxin B1 in combination with endemic viral hepatitis constitutes a major public health problem in developing countries where high heat and humidity favors the growth of the mold, and where food storage is inadequate [27].

Recently, it was demonstrated in elegant studies using a dual vector containing the intergenic regulatory region that the XRE cluster near the human CYP1A1 transcriptional start site (at −0.5 to −1.8 kb, corresponding to the region −21.5 to −22.4 from the start site of CYP1A2; Fig. 2) works bidirectionally and is essential for expression of both CYP1A1 and 1A2 [26]. Negative control regions have also been identified. The role of an earlier characterized weak functional XRE at −2.3 kb from the transcriptional start site of CYP1A2 needs to be reevaluated [29].

2.1.3. *CYP1B1*

CYP1B1 is important for bioactivation of BaP in extrahepatic tissues such as fibroblasts and steroidogenic tissues in humans [30] and in the mouse [31]. In addition to its control by the AhR, the cytochrome is also controlled by other factors including cAMP. Clusters of evolutionary conserved XREs have been detected in the regulatory region of human CYP1B1 on chromosome 2 [30]. In the mouse, anomalous XREs surrounding an E-box in proximity of a regular XRE have been described which may modulate its function (illustrated in Fig. 2) [31].

2.2. *Phase II XMEs*

2.2.1. *NQO1*

NQO1 represents a multifunctional flavoprotein that detoxifies quinones by two-electron reduction to quinols (without generating reactive semiquinones) [40]. Quinols are either (i) autoxidized and undergo redox cycles with generation of ROS (including superoxide anion radical and hydrogen peroxide), or (ii) are conjugated by UGTs or SULTs, and are readily excreted via MRPs and BCRP. The enzyme also participates in reduction of endogenous quinones such as vitamin E quinones and ubiquinone. Interestingly, it may also exhibit functions in signaling pathways such as stabilization of the tumor suppressor p53 [40]. Regulation of human and rodent NQO1 by XREs and AREs has been intensely investigated. With rat NQO1 functional XREs and AREs have been identified in close proximity [14,41]. Human NQO1 is located on chromosome 16 and will be discussed under Section 2.3.

2.2.2. *GSTA2*

GSTs include seven classes of cytosolic GSTs as well as mitochondrial and microsomal GSTs, the latter also termed MAPEG proteins [32,33]. Only cytosolic human and rat GSTA2 are discussed here. Human GSTA isoforms, including GSTA2, form a cluster at chromosome 6p12 [35]. Many epoxide carcinogens are detoxified by GSTs; for example, the ultimate carcinogen BaP-7,8-diol-9,10-epoxide, although obviously not efficient enough [32]. It is expected that a number of other GSTs will be identified as members of the AhR and Nrf2 batteries [33]. One functional XRE and one ARE of rat GSTA2 have been characterized [34]. Human GSTA1/2 (two genes/enzymes, which are 95% similar in nucleotide and amino acid sequence) are induced by AhR agonists in human hepatocyte

cultures of some individuals, but XREs have not yet been characterized [44,45].

2.2.3. *UGT1A1 and UGT1A6*

Mammalian UDP-glycosyltransferases represent a large gene superfamily [36–38]. Enzymes of families 1 and 2 are most efficient at using UDP-glucuronic acid as the glycosyl donor. They are therefore also termed UDP-glucuronosyltransferases. So far no UGT family 2 members have been classified as members of the *AhR* gene battery. Therefore, only UGT1 family members are discussed. The human UGT1 locus on chromosome 2q37 spans approximately 200 kb and contains 13 individual promoters/first exons and a shared set of exons 2–5 [38]. The UGT1 locus encodes nine UGT1A enzymes that play a prominent role in endo- and xenobiotic metabolism. Individual genes/enzymes were named according to their upstream position, relative to exons 2–5. Interestingly, studies with transgenic mice expressing the entire human UGT1 locus revealed that all nine expressed hepatic and intestinal UGT1A members were induced by the AhR agonist TCDD [39]. In the case of UGT1A1 and UGT1A6 functional XREs have been identified.

2.2.3.1. UGT1A1. UGT1A1 is a major UGT enzyme expressed in liver and intestine. The isoform controls bilirubin homeostasis by forming bilirubin mono- and diglucuronides. It is also conjugating β-estradiol at the 3-OH position, catechol estrogens and many drugs, such as the irinotecan metabolite SN-38 [28]. Murine UGT1A1 is probably controlled by the AhR, based on the inheritance of bilirubin UGT activity in mouse strains expressing high- and low-affinity AhR [46], but functional XREs have not been identified. With human UGT1A1, one XRE has been characterized at −3.3 kb [47], within a 290 bp 'phenobarbital response enhancer module' (PBREM) which contains binding motifs for constitutive androstane receptor (CAR), pregnane X receptor (PXR) and two glucocorticoid response elements (GREs) (Fig. 2) [48].

2.2.3.2. UGT1A6. UGT1A6 is conjugating the neurotransmitter serotonin and a variety of planar phenols, such as 1-naphthol and paracetamol as well as planar phenolic metabolites of polycyclic aromatic hydrocarbons [49,50]. Murine UGT1A6 is a known AhR battery member [5]. Interestingly, in AhR-null mice both constitutive and TCDD-inducible UGT1A6 expression was abolished [51]. Rat UGT1A6 was the first enzyme to be characterized as 3-methylcholanthrene-inducible UGT, and one XRE was identified upstream of a second promoter [52]. Rat and murine UGT1A6 and UGT1A7 (the upstream adjacent gene on the UGT1A locus encodes an enzyme which is efficiently conjugating bulky phenols) appear to be coregulated by the AhR [53]. With the human UGT1A6, one XRE was characterized in proximity to a truncated ARE [54].

2.3. *Links between Phase II genes of AhR and Nrf2 batteries*

Studies of the Nrf2 promoter suggest that Nrf2 is an *AhR* target gene (Fig. 3) [20]. In the mouse Nrf2 promoter, three functional XREs and two AREs have been identified. The mouse promoter has a high degree of homology with the rat Nrf2 promoter. The human Nrf2 promoter also contains five copies of XRE-like elements in its 2 kb region, but its sequence and location is distinct from the rodent Nrf2 promoter. Notably, with regard to Nrf2 activation a second signal by ROS/electrophiles is required for stabilization of the rapidly degraded Nrf2 protein. This second signal disrupts the cytosolic Keap1-Nrf2 complex leading to nuclear translocation of Nrf2, its association with Maf proteins, binding of the heterodimers to AREs and induction of target genes [13]. The present commentary focuses on rodent and human AhR batteries and their linkage to Nrf2-induced Phase II enzymes. Therefore, well-studied enzymes common to AhR and Nrf2 batteries (NQO1, GSTA2 and UGT1A6) are subsequently discussed.

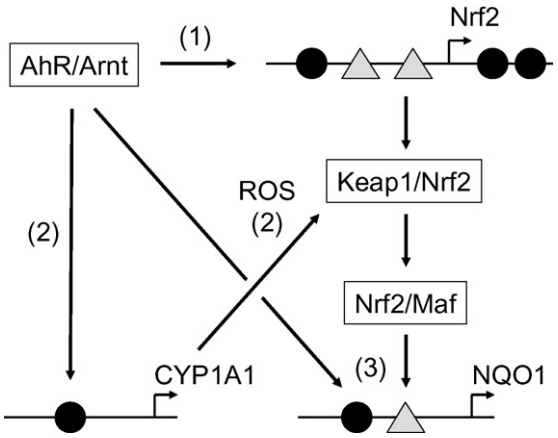

Fig. 3 – Proposed links between *AhR* and *Nrf2* gene batteries: (1) Nrf2 as an *AhR* target gene [20], (2) indirect activation of Nrf2 by CYP1A1-generated ROS/electrophiles [42,43], and (3) direct interaction of AhR/XRE and Nrf2/ARE signaling due to close proximity of the XRE and ARE in the regulatory region of NQO1 [14]. XREs (circles) and AREs (triangles) are indicated as in Fig. 2. XREs and AREs downstream and upstream of the Nrf2 transcription start site are taken from the mouse Nrf2 promoter [20].

2.3.1. *NQO1*

In the case of rat NQO1, functional XRE and ARE motifs have been characterized in close proximity (Fig. 2) [41]. As expected, transfection of XRE-containing plasmids of NQO1 was induced by TCDD (preferential AhR agonist), but not by tBHQ (preferential Nrf2 activator). In support of separate actions, ARE-containing plasmids were induced by tBHQ, but not by TCDD [41]. Functional cross-interaction between XRE and ARE signaling has been postulated on the basis of findings with AhR-, Arnt- and Nrf2-null mice: induction of NQO1 by TCDD has been shown to depend on the presence of AhR, Arnt and Nrf2. Hence, basal and inducible expression of NQO1 by either TCDD or tBHQ requires functional Nrf2 [14]. The close proximity of XRE and ARE may suggest a 'composite' response element or interaction between AhR and Nrf2 by an adaptor protein (Fig. 3). In studies of human NQO1, the identified ARE was found to be functional, whereas the identified XRE-like motif was not [42]. It was suggested that induction of NQO1 appears to occur by an indirect mechanism: TCDD-induced

CYP1A1 generates ROS, leading to activation of Nrf2. Generation of hydrogen peroxide and Nrf2 activation was demonstrated in HepG2 cells [43]. These NQO1 studies may serve as an example for multiple mechanisms linking AhR- and Nrf2-induced batteries. As illustrated in Fig. 3, three links are emphasized: (i) *Nrf2* as target gene of the *AhR* gene battery [20], (ii) indirect Nrf2 activation by ROS generated by CYP1A1 [42,43], and (iii) possible direct cross-talk between AhR and Nrf2 signaling [14]. These multiple mechanisms may be used in the species- and cell-specific manner.

2.3.2. *GSTA2*

In the case of rat GSTA2, one XRE and ARE was characterized in close proximity [34]. However, with human GSTA2 no functional XRE and ARE has been identified, although the enzyme is induced by TCDD and Nrf2-activating dithiolthiones [44,45]. Hence, multiple mechanisms may be operating, as described in the case of NQO1.

2.3.3. *UGT1A6*

Studies with Nrf2-null mice indicate that UGT1A6 is under the control of Nrf2, in addition to AhR control [10,13,55]. As already mentioned, rodent UGT1A6 and UGT1A7 (located next to UGT1A6 at the UGT1A locus) are coregulated by the AhR. Interestingly, murine UGT1A7 is also controlled by Nrf2 since its expression was reduced to about 50% in Nrf2-null mice [55]. Human UGT1A6 expression is induced by both TCDD and tBHQ in colon carcinoma Caco-2 cells [49,50,54]. An ARE-like element (ARE′) has been studied which was identified in proximity to the functional XRE. Unexpectedly, in cells expressing plasmids containing mutated ARE′ sequences, the XRE function was found to be impaired [54], suggesting cross-interaction between XRE and ARE′. However, mechanisms responsible for Nrf2 control of human UGT1A6 remain unclear. A role of the AhR/XRE in the Nrf2 response is strengthened since coordinate responses to TCDD and tBHQ were observed in rat hepatoma 5 L cells, in mutant 5 L cells without AhR and with recomplemented AhR [54].

It is noteworthy that AhR and Nrf2 signaling goes beyond regulation of xenobiotic metabolism. Functional XREs and AREs have been identified in the proto-oncogene c-Ha-ras [56], suggesting – as in the case of the AhR – a connection with signaling pathways responsible for cell proliferation. Expression of c-Ha-ras has been suggested to be important in TCDD-mediated atherogenesis in murine vascular smooth muscle cultures [56].

In conclusion, comparison of rodent and human Phase II enzyme expression (exemplified by NQO1, GSTA2 and UGT1A6) suggests that linkage between AhR and Nrf2 batteries may be different in rats and humans. In this context it should be noted that the roles of AhR and Nrf2 are often underestimated: while induction studies using primary human hepatocytes [57] or probe drugs [28] often reveal only moderate induction, investigations with AhR- and Nrf2-deficient mice demonstrate that these transcription factors control both inducible and basal expression [10,51]. It is understood that the AhR and Nrf2 operate together with hormonal and tissue-specific factors (discussed subsequently). Elucidation of AhR and Nrf2 batteries and their linkage in rodents and humans is of major interest, in particular with regard to human risk assessment and to current efforts for cancer chemoprevention using phytochemicals, characterized as selective or mixed AhR and Nrf2 activators [10,11,15], as further discussed under Section 3.3.

2.4. *Inducibility of AhR target genes by hormones*

AhR target genes are also under hormonal control. Effects of dexamethasone on the expression of *AhR* gene battery members have been studied in rat primary hepatocyte cultures: Dexamethasone-potentiated induction of CYP1A1 and GSTA2 by AhR agonists and tBHQ, while it suppressed the induction of rat NQO1 [58]. Similarly, UGT1A1 [42] and UGT1A6 induction by AhR agonists [59] is modulated by dexamethasone. In the case of UGT1A1 two GREs (glucocorticoid response elements) have been identified in the PBREM (Fig. 2) [42]. Interestingly, studies with transgenic mice expressing the entire human UGT1 gene locus suggest that hormonal changes dominate maternal UGT1 expression during pregnancy and lactation [39].

2.5. *Factors responsible for tissue-specific XME expression, UGTs as example*

Xenobiotic metabolism by AhR and Nrf2 battery members occurs in a tissue-specific fashion which is essential for their function. For example, studies using CYP1A1-null mice clearly showed that expression of this enzyme in intestinal epithelial cells is mainly responsible for first-pass detoxification of orally administered BaP [60]. Similarly, studies of UGT1A7 polymorphisms and its tissue-specific expression suggest an important role in tobacco carcinogen detoxification in the aerodigestive tract [61,62]. Factors regulating tissue-specific expression are beginning to be elucidated. For example, it was found that Cdx2 (caudal homeodomain transcription factor) in cooperation with HNF1 is an important regulator of the UGT1A8 and UGT1A10 gene proximal promoters; Cdx2 is found exclusively in the small intestine and colon; it is absent in gastric epithelium and esophagus [63]. Recently, evidence was obtained that Wnt/β-catenin signaling regulates *AhR* gene expression in the perivenous zone of the liver [64]. AhR is preferentially expressed in perivenous hepatocytes [65] and may explain preferential perivenous expression of AhR battery members, such as CYP1A1 [4], GSTA2 [34] and UGT1A6 in rat liver [66].

3. Tightened coupling between Phase I and II metabolisms by AhR- and Nrf2, detoxification of benzo[*a*]pyrene quinones as example

Tight coupling of Phase I and II enzymes is expected in homeostatic control of endogenous ligands of the AhR, such as UV light generated indolocarbazole derivatives from tryptophane. This amino acid serves as a chromophore for UV light in the exposed skin. 6-Formylindolo[3,2-*b*]carbazole (FICZ) is formed in keratinocytes which binds to the AhR with higher affinity than TCDD [67,68]. However, and in contrast to TCDD, FICZ is rapidly metabolized by the AhR family members CYP1A1, CYP1A2 and CYP1B1 [68]. Rapid conjugation by

AhR-induced UGTs is to be expected [69], although Phase II metabolism of FICZ metabolites has not been studied.

Naturally occurring dietary AhR ligands including toxic contaminants such as BaP represented a challenge to the XME system for millions of years and may have shaped regulatory mechanisms in evolution. Excellent reviews have been published both on BaP metabolism and its biological effects as carcinogen, atherogen and teratogen [70–72]. BaP is an agonist of the AhR which is responsible for both bioactivation and detoxification of the carcinogen. The most conclusive evidence for functions of particular enzymes in BaP metabolism has been obtained in studies with transgenic animals or human polymorphisms, which are subsequently discussed. Thereafter, biliary and urinary BaP metabolites are described as an in vivo basis for discussion of the role of cellular enzymes in BaP bioactivation and detoxification.

3.1. *Studies with transgenic animals and human polymorphisms*

BaP is typically activated by CYP1A1 and CYP1B1 to reactive epoxides that bind covalently to DNA and protein. To examine the role of these enzymes, BaP was administered orally to CYP1A1-null and wild-type mice [60]. It was expected that the animals were protected from BaP toxicity in CYP1A1-null mice. However, damage to the bone marrow was much greater in CYP1A1-null mice. The reason turned out to be the loss of first-pass detoxification of BaP in the intestinal epithelium by CYP1A1: feeding of BaP for 5 days (125 mg/kg) led to approximately 25-fold higher blood levels of BaP in CYP1A1-defective mice. CYP1B1-null mice had BaP blood levels similar to wild-type mice. CYP1B1 appeared to be responsible for BaP bioactivation in bone marrow. The results established the predominant role of CYP1A1 in intestinal first-pass detoxification of orally administered BaP.

The protective role of UGTs was tested in mutant Gunn rats that express virtually no UGT1 enzymes due to a frame shift mutation in the shared region of all UGT1 enzymes. Covalent binding of BaP was approximately two-fold higher in Gunn rats compared to wild-type Wistar rats, supporting a protective role of UGT1 enzymes [73]. Furthermore, it was shown that low UGT activity in lymphocyte cultures from UGT-deficient human individuals was correlated with increased BaP cytotoxicity [74]. As mentioned before, epidemiologic studies of patients expressing polymorphic UGTs support their essential role in detoxification of BaP [61,62].

3.2. *BaP metabolites excreted in bile and urine*

Exposure to BaP, whether by inhalation, oral or dermal exposure, results in ready distribution of the toxin throughout the organism. BaP is highly lipophilic and can be taken up by cells through the plasma membrane and accumulates in endoplasmic reticulum membranes and mitochondria. Following intraperitoneal administration, approximately 68% of a BaP dose is excreted into bile within 6 h: 34% as glucuronides and 9% as sulfonates [75]. Biliary excreted conjugates were analyzed in detail in studies following intratracheal instillation. It was found that 37% of the conjugates were present as BaP-3,6-quinol diglucuronide, 33% as monoglucuronides and sulfonates, 20% as thioether conjugates and 9% as unconjugated metabolites [76]. A detailed analytical study of urinary metabolites revealed 40% GSH conjugates (among them GSH conjugates derived from BaP-7,8-diol-9,10-epoxide), several diglucuronide and disulfate conjugates and mixed glucuronide–sulfate conjugates [77]. These studies clearly demonstrate the importance of Phase II in overall BaP metabolism.

3.3. *Roles of Phase I and II enzymes in bioactivation and detoxification of BaP*

Metabolism of BaP is known to be complex (Fig. 4). BaP is first oxidized by CYP1A1 and CYP1B1 to a number of epoxides (partially detoxified by GSTs) and phenolic intermediates (detoxified in part by UGTS and SULTs). Two examples of initiators and promoters of BaP carcinogenesis are emphasized: (i) BaP-7,8-diol-9,10-epoxide (formed from BaP-7,8-diol) represents a major ultimate carcinogen forming DNA adducts which are poorly repaired. These adducts lead to mutagenic lesions and are probably responsible for initiation of carcinogenesis in target tissues such as bronchial epithelium. (ii) BaP-3,6-quinone (formed from various phenols) represents a major cytotoxic tumor promoter [78]. Quinones undergo redox cycles with generation of ROS leading to oxidative stress. Quinone–quinol redox cycles are efficiently prevented by the action of AhR- and Nrf2-induced NQO1 which transfers two electrons, thereby bypassing the semiquinone step. Autoxidation of the resulting quinol is prevented by conjugation with glucuronic acid or sulfate. BP-3,6-quinol is conjugated by AhR- and Nrf2-induced UGT1A6 and UGT1A7 in rodents [79–81] and UGT1A6

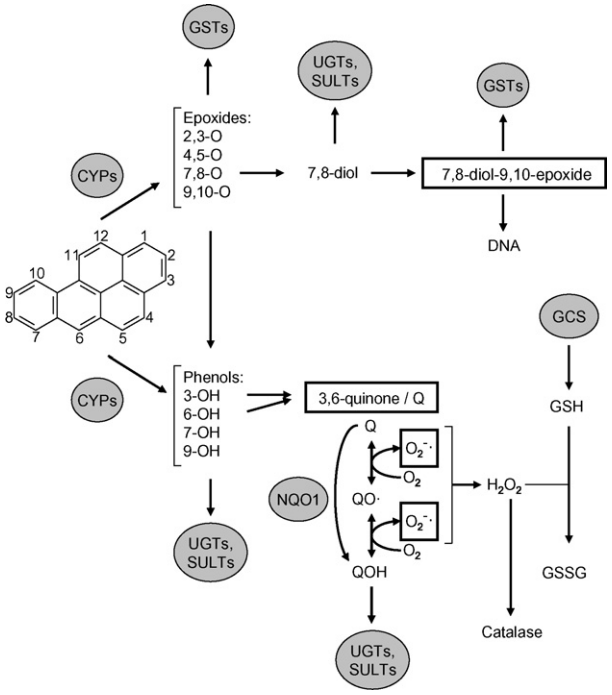

Fig. 4 – Schematic illustration of the roles of AhR and Nrf2 battery members (ovals) in bioactivation of benzo[*a*]pyrene (BaP) and in detoxification of BaP quinones with resulting oxidative stress. Ultimate carcinogens and cytotoxins are indicated by rectangles. Details are referred to in the text.

and UGT1A9 in humans [82,83]. SULTs often compete with UGTs for the same substrates. However, SULTs are not upregulated by the AhR; in fact, SULT2A1 is known to be downregulated [84]. Most importantly, oxidative stress due to quinone–quinol redox cycles is prevented by GSH, which prevents generation of hydrogen peroxide by GSH peroxidases [85,94]. GSH is replenished by Nrf2-induced GCS [12,13,16]. Hence, detoxification of BaP quinones demonstrates the usefulness of coordinate induction of Phase I and II enzymes by the *AhR* gene battery and the linked *Nrf2* gene battery. Tight coupling of Phases I and II prevents the accumulation of reactive semiquinones and oxidative stress, shifting the delicate balance between potential BaP bioactivation and detoxification in favor of detoxification, at least at low level exposure (Fig. 4).

4. Roles of AhR battery in detoxification of *o*-quinones, catechol estrogens as example

The *AhR* gene battery has also implications in preventing toxic redox cycles between catechol estrogens and *o*-quinones. Estradiol is hydroxylated by CYPs at many positions [86]. Whereas CYP1A1 mostly hydroxylates at C2, CYP1B1 is the major enzyme catalyzing C4 hydroxylation [87]. 4-Hydroxylated catechol estrogens are recognized as potent carcinogens due to high affinity for estrogen receptors and to ROS formation by redox cycling with *o*-quinones [87]. C2- and C4-hydroxylation is markedly increased in smoking premenopausal women [88]. Moreover, it has been shown that catechol estrogen formation is enhanced by administration of the AhR agonist indole-3-carbinol [89]. A number of UGTs are known to be involved in the inactivation of catechol estrogens. UGT1A1 and 1A3 are mostly conjugating at C2 whereas UGT1A9 and 2B7 are mostly conjugating at C4 [90]. Since these enzymes are expressed in estrogen sensitive target tissues such as breast, ovary, and prostate, it is conceivable that UGTs contribute to inactivation of catechol estrogens. In support of this hypothesis, epidemiologic studies suggest higher breast cancer risk in African Americans with mis-sense polymorphisms of UGT1A1 [91]. In particular, coordinate induction of CYP1A1 and UGT1A1 by the AhR battery may shift estrogen oxidation to the less reactive C2-hydroxylated catechol estrogen coupled with efficient glucuronidation. Coupling between AhR and Nrf2 batteries may be important to prevent oxidative stress, as discussed in the case of BaP quinone detoxification.

5. Conclusions

Coordinate induction of Phase I and II XMEs by the AhR and Nrf2 may greatly attenuate the accumulation of reactive intermediates generated by Phase I enzymes. These reactive intermediates, in particular reactive oxygen species, are known to modulate cell signaling and cell death in many ways [92]. In case of the discussed *AhR* gene battery, coordination is achieved by common DNA binding domains (XREs) for the ligand-activated AhR in the regulatory region of target genes. Note that the XRE core sequence GCGTG would be expected to occur every 512 bp by random chance alone (i.e. 4^5 within the +/− strands). However, XREs are not randomly distributed in the genome. Within well-characterized mouse genes, 85% of XREs have been found in the immediate vicinity (<2 kb) of promoters [93]. Microarray analysis of AhR- [8] and Nrf2-induced genes [12] revealed a large variety of coordinately induced genes. Focus on functionally characterized response elements, such as XREs, may provide a robust genetic basis to identify the primary responsive target genes of transcription factors. The *AhR* gene battery encodes both Phase I enzymes (CYP1A1, 1A2 and 1B1) and Phase II enzymes (including NQO1, GSTA2 and UGT1A6). In this context it should be noted that induction experiments underestimate the role of the AhR. For example, investigation of AhR-null mice suggests that the AhR is involved in both basal and inducible enzyme expression [51]. In addition, studies with transgenic mice expressing the entire human UGT1 locus suggest that all nine expressed UGT enzymes may be under the control of the AhR [39].

Difficulties arise in the discussion of the human AhR battery. In case of human Phase II genes, the putative XRE of NQO1 was found to be non-functional [42], and in case of human GSTs no functional XREs have been characterized so far [33]. However, NQO1 and GSTs are also members of the *Nrf2* gene battery. In experiments using transcription factor-deficient mouse strains, it was demonstrated that TCDD-mediated induction of NQO1 requires AhR, Arnt and Nrf2 [14], suggesting a linkage between AhR and Nrf2 batteries. To explain the above observations in human and mouse models, multiple mechanisms of cross-talk have been proposed: (i) Nrf2 was shown to be a target gene of the AhR [20]. (ii) In experiments with HepG2 cells Nrf2 was shown to be activated indirectly by hydrogen peroxide generated by AhR-induced CYP1A1 [43]. (iii) The close vicinity of XRE and ARE in the rat and mouse NQO1 gene led to the proposal of a direct interaction between AhR and Nrf2 signaling [14]. However, these proposals still need to be substantiated and warrant further investigation.

As exemplified by detoxification of BaP quinones (Fig. 4) and of catechol estrogens, coupling of AhR and Nrf2 batteries may greatly reduce generation of reactive oxygen species. For example, GSH regeneration (which is needed to detoxify large amounts of generated hydrogen peroxide) is stimulated by Nrf2-induced GCS, which is not a member of the AhR battery. Of course, protection is only achieved at low level exposure, and threshold levels depend on the species and on the exposed tissue. Nevertheless, recognition of coordinate induction of XMEs by AhR and Nrf2 batteries may improve risk assessment of reactive toxic intermediates, particularly in the extrapolation to low level endo- and xenobiotic exposure.

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