AIP and its interacting partners

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Abstract

Germline mutations in the aryl hydrocarbon receptor-interacting protein gene (AIP) predispose to young-onset pituitary tumours, most often to GH- or prolactin-secreting adenomas, and most of these patients belong to familial isolated pituitary adenoma families. The molecular pathway initiated by the loss-of-function AIP mutations leading to pituitary tumour formation is unknown. AIP, a co-chaperone of heat-shock protein 90 and various nuclear receptors, belongs to the family of tetratricopeptide repeat (TPR)-containing proteins. It has three antiparallel α-helix motifs (TPR domains) that mediate the interaction of AIP with most of its partners. In this review, we summarise the known interactions of AIP described so far. The identification of AIP partners and the understanding of how AIP interacts with these proteins might help to explain the specific phenotype of the families with heterozygous AIP mutations, to gain deeper insight into the pathological process of pituitary tumour formation and to identify novel drug targets.

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Introduction

Familial pituitary adenomas can occur in three diseases: multiple endocrine neoplasia type 1 (MEN1, MIM# 131100), the rare Carney complex (CNC, MIM# 160980) and the recently described familial isolated pituitary adenoma (FIPA, MIM# 102200). In the first two syndromes multiple endocrine organs are involved, whereas in FIPA tumours occur only in the pituitary gland (Chahal et al. 2010). A locus has previously been identified in a phenotypically relatively well-defined subgroup of FIPA families, but the gene causing the disease, namely the aryl hydrocarbon receptor-interacting protein gene (AIP, MIM# 605555), had not been identified until 2006 (Benlian et al. 1995, Gadelha et al. 2000, Soares et al. 2005, Vierimaa et al. 2006). About 30% of all FIPA families and 50% of acromegaly families have a mutation in the AIP gene (Chahal et al. 2010). Families with AIP mutations have a characteristic phenotype: childhood- or young-onset disease (the mean age at diagnosis is around 20 years), primarily GH- or prolactin-secreting adenomas (the majority of the patients have GH- or mixed GH- and prolactin-secreting adenomas, a minority have prolactinomas, whereas other pituitary adenoma types are rarely observed), and large and invasive pituitary tumours that do not respond well to somatostatin analogue treatment (Leontiou et al. 2008, Daly et al. 2010). Pituitary apoplexy can be a presenting feature in patients with AIP mutation (Chahal et al. 2011). Occasionally, patients with young-onset acromegaly or other childhood-onset pituitary adenomas carry germline mutation in the AIP gene without apparent family history (Cazabat et al. 2007, Georgitsi et al. 2008, Daly et al. 2010, Stratakis et al. 2010). The penetrance of the disease is low, probably around 30%, and there is a male preponderance (Cain et al. 2010, Daly et al. 2010). Three-quarters of AIP mutations lead to a truncated protein, and some of the missense mutations have also shown to result in loss of function of the protein suggesting, together with loss of heterozygosity data, that AIP functions as a tumour suppressor gene in the pituitary gland (Soares et al. 2005, Vierimaa et al. 2006, Leontiou et al. 2008, Igreja et al. 2010).

At first, AIP seems to be an unusual gene causing pituitary adenoma as it was previously only known as a co-chaperone of nuclear receptors or viral proteins (Kuzhandaivelu et al. 1996, Carver & Bradfield 1997, Ma & Whitlock 1997, Meyer et al. 1998, Kashuba et al. 2000). AIP, also known as X-associated protein-2 (XAP2; Kuzhandaivelu et al. 1996), Ah receptor-activated 9 (ARA9; Carver & Bradfield 1997) or FK506-binding protein 37 (FKBP37; Blatch et al. 2006), is a 37 kDa cytoplasmic protein. Structurally, it shares a significant degree of homology with immunophilins, such as FKBP52 (52 kDa FK506-binding protein), as it has a peptidyl-prolyl
cis–trans isomerase (PPIase)-like domain. Immunophilins are a huge family of ubiquitous and conserved proteins, which possess PPIase domains that bind immunosuppressant drugs of the FK506 or of the cyclosporin A groups (Galat & Metcalfe 1995). However, AIP does not function as an immunophilin. AIP lacks affinity for the immunosuppressant drugs FK506 and rapamycin and the PPIase-like domain displays no enzymatic activity (Carver et al. 1998, Laenger et al. 2009), so AIP cannot be considered a true immunophilin. These data are consistent with a weak homology between the PPIase domains of AIP and FKBP12 (only five of the 14 amino acids of the FK506-binding domain of FKBP12 are conserved in AIP (Carver et al. 1998)) and explain the different biochemical properties of AIP.

AIP belongs to the family of tetratricopeptide repeat (TPR) domain-containing proteins, such as the aryl hydrocarbon receptor-interacting protein-like 1 (AIPL1), protein phosphatase 5 (PP5), FK506-binding protein 51 (FKBP51), FKBP52, cyclophilin 40 (Cyp40), carboxyl terminus of Hsc70-interacting protein (CHIP), and heat-shock protein 70 (Hsp70)/Hsp90 organising protein (CHIP); and heat-shock protein 70 (Hsp70)/Hsp90 organising protein (Hop; D’Andrea & Regan 2003), and has three TPR motifs and a final α-7 helix at the C-terminus (Fig. 1). TPR domains are highly degenerate consensus sequences of 34 amino acids, often arranged in tandem repeats, formed by two α-helices forming an antiparallel amphipathic (having both hydrophilic and lipophilic properties) structure that mediates intra- and inter-molecular interactions in many proteins (Goebel & Yanagida 1991).

The AIP protein sequence is evolutionarily conserved among species. The protein sequence of human AIP is 100, 94 and 93% identical to chimpanzee, mouse and rat AIP respectively (Supplementary Table 1, see section on supplementary data given at the end of this article). Furthermore, AIP is located on a conserved synthetic block in the human, mouse and rat genome (Fig. 2). The fact that AIP is a highly conserved protein could be expected for two reasons: first, because AIP is associated with a human disease, and several studies have found that genes causing human disease are more conserved than non-disease genes (Lovell et al. 2009), and secondly, because AIP has been demonstrated to be essential in cardiac development and in maintaining productive erythropoiesis in mice (Lin et al. 2007, Kang et al. 2011) and previous studies in mammals and other eukaryotes showed that essential genes are usually located in highly conserved genomic regions (Lovell et al. 2009).

As expected from the presence of TPR motifs in the AIP protein, several proteins have been identified which interact with AIP. The identification of these molecules and the understanding of how AIP interacts with them could give us an insight into the pathological process of pituitary tumour formation and may lead to new therapeutic targets. The main focus of this review is thus to summarise the AIP-interacting partners described so far. The functional studies available about specific AIP amino acid mutations are also reviewed.

AIP-interacting proteins

Viral proteins

Hepatitis B virus X protein AIP was originally described as a protein associated with the X protein of the hepatitis B virus (HBV; Kuzhandaivelu et al. 1996), a small human DNA virus that causes acute and chronic hepatitis. Among the few genes contained in the genome of HBV, there is an open reading frame that encodes a 154 amino acid regulatory protein, termed X protein. This protein, which does not have a human homologue, activates the transcription of a wide variety of different genes through interaction with cellular factors (Koike 2009). In order to identify new proteins that may interact with X, the authors used the yeast two-hybrid (Y2H) method. Among the several potential cDNAs coding the protein X binding protein, six were found to be overlapping clones of a full-length cDNA encoding the same gene. The gene was named XAP2 because it was the second protein found to interact with the HBV X protein by this technique. The full-length AIP cDNA was subsequently isolated and in vitro translated in a rabbit reticulocyte lysate. The translated product corresponded perfectly with the native AIP from HeLa cells and had an apparent molecular mass of 36 kDa. AIP RNA expression was evaluated and detected in several different tissues and cell lines, but very low levels were found in the liver. The ubiquitous expression of AIP was subsequently confirmed in human and murine tissues at the mRNA and protein level (Kuzhandaivelu et al. 1996, Ma & Whitlock 1997, Carver et al. 1998, Meyer et al. 1998, 2000, Yano et al. 2003).

The X protein–AIP interaction was also demonstrated to occur in vitro by testing the ability of a glutathione S-transferase (GST)–X fusion protein to bind to a 35S-labelled AIP. Using different X mutants, the interaction with AIP was then shown to be mediated by X protein residues 13–26, a region highly conserved among all mammalian hepadnaviruses. Further evidence regarding this interaction shows similar cytoplasmic distributions of X protein and AIP with immunocytochemistry in X protein-transfected mammalian cells.

Overexpressing AIP resulted in inhibited X protein transcriptional activity, suggesting that AIP is an important negative regulator of the X protein and that their interaction may play a role in HBV pathology.

EBNA-3 EBV-immortalised lymphoblastoid cell lines express, among others, six nuclear antigens (EBNA 1–6) and three latent membrane proteins (LMP 1, 2a, 2b), whose concerted action is essential for the immortalisation and transformation of B-cells (Tomkinson et al. 1993). As the identification of cellular proteins that can interact with the transformation associated EBNAs was not yet complete, Kashuba et al. (2000) searched for proteins that can bind to the transcriptional regulator EBNA-3, which again does not have a human homologue, in a Y2H system. Among the several clones identified, one corresponded to the AIP protein.
Subsequently, this interaction was also confirmed in vitro using a GST pull-down assay.

Concordant to that shown for the aryl hydrocarbon receptor (AhR (MIM# 600253); see AIP–AhR–Hsp90 section), AIP was also found to translocate to the nucleus on expression of EBNA-3. The authors hypothesised that as AIP can bind to the transforming proteins of two evolutionarily distant viruses and also to the AhR, a phylogenetically ancient protein conserved in vertebrates and invertebrates (Hahn 2002), the AhR signal transduction pathway could be involved in virus-induced cell transformation. This hypothesis was further supported by a following demonstration from the same group that besides AIP, EBNA-3 can also directly interact with the AhR, with AIP enhancing the stability of the complex. As a result of this association, an enhanced transcription of AhR-responsive genes has been observed (Kashuba et al. 2006).

**AIP–AhR–Hsp90 complex**


**AIP–AhR** The AhR, a basic helix–loop–helix protein of the Per–ARNT–Sim (PAS) family of transcriptional regulators, is a cytoplasmic transcription factor that can be activated by a wide variety of structurally diverse exogenous ligands, the prototype of which is the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), as well as by some endogenous

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Figure 1  (A) Schematic structure of the AIP protein. Structurally, AIP is similar to immunophilins. AIP contains a PPlase-like domain at the N-terminus, which shows a weak identity to the low molecular weight FKBP12 (12 kDa FK506-binding protein) (Carver & Bradfield 1997), but does not show immunophilin activity (Carver et al. 1998, Laenger et al. 2009). The C-terminal part of the molecule contains three TPR domains that are conserved 34 amino structures consisting of two antiparallel α helices. There is a terminal α-7 helix that is crucial for protein–protein interaction. Numbers underneath the protein structure represent amino acids. Boxes with different shapes represent different domains. The location of the missense mutations investigated in functional binding assays is marked. PPlase-like, peptidyl-prolyl cis–trans isomerase-like domain; TPR, tetratricopeptide repeat domain. (B) Hypothetical structure of AIP based on the crystal structure of the related protein FKBP51 (Igreja et al. 2010). The PPlase-like domain, the three TPR motifs with three pairs of anti-parallel α-helices and the final extended α-helix, α-7, are highlighted. The amino acids subjected to site-directed mutagenesis analyses to study their role in the AhR complex are shown in red (Bell & Poland 2000, Meyer et al. 2000, Laenger et al. 2009).
compounds, such as the cAMP (Denison & Nagy 2003, Oesch-Bartlomowicz et al. 2005, Nguyen & Bradfield 2008). Ligand-free AhR in the cytoplasm binds to two molecules of hsp90 (Perdew 1988) and the co-chaperone proteins p23 (Nair et al. 1996) and AIP (Carver & Bradfield 1997, Ma & Whitlock 1997, Meyer et al. 1998). The interaction with hsp90 shapes the AhR's ligand-binding domain into a state competent for ligand binding, and it also negatively regulates AhR until ligand binding occurs (Beischlag et al. 2008). p23 is part of the AhR complex through interaction with hsp90 (Nair et al. 1996) and its presence is thought to stabilise the complex (Kazlauskas et al. 1999) and to favour its nuclear import (Kazlauskas et al. 2001). These functions are in line with the modulating role that p23 has been shown to exert in different steroid hormone receptors (Smith et al. 1995, Dittmar et al. 1997, Freeman et al. 2000). The presence of p23 in the AhR complex seems, however, not to be essential for the AhR physiology (Cox & Miller 2004, Flaveny et al. 2009).

Using the Y2H method with AhR as the bait, a human cDNA (termed at the time ARA9; Carver & Bradfield 1997) and a murine cDNA (termed AIP) were described (Ma & Whitlock 1997), both shown to be identical to XAP2. At the same time, since an uncharacterised protein of about 43 kDa was found to be part of the AhR–hsp90 complex (Chen & Perdew 1994), a third group decided to identify this protein. After purification from simian COS-1 cells, the molecular mass was reassigned from 43 to 38 kDa and the protein sequence was found to be 98% identical to human AIP (Meyer et al. 1998).

There is considerable controversy regarding the effect of AIP on AhR function. Some studies reported that AIP can enhance the transcriptional activity and expression levels of the AhR (Carver & Bradfield 1997, Ma & Whitlock 1997, Meyer et al. 1998, 2000, LaPres et al. 2000, Nukaya et al. 2010), whereas others described an inhibitory function (Hollingshead et al. 2004, Pollenz & Dougherty 2005, Pollenz et al. 2006). This variability is due to several factors, including species- and tissue-specific differences. Apart from these conflicting results, AIP was repeatedly shown to protect the AhR from ubiquitin–dependent degradation through the proteasome (Kazlauskas et al. 2000, Morales & Perdew 2007). Consistent with that, low levels or loss of AIP correlates with low expression of AhR (Jaffrain-Rea et al. 2009).

After ligand binding, AhR undergoes a conformational change that exposes a nuclear localisation sequence, resulting in translocation of the complex into the nucleus, where it forms a heterodimer with the aryl hydrocarbon receptor nuclear translocator 1 (ARNT, also known as HIF1B, MIM# 126110), which leads to the activation of AhR-sensitive genes. In mice, ligand binding leads to the dissociation of AIP from the complex as it enters the nucleus (Ma & Whitlock 1997), whereas in humans the association is maintained in the nucleus (Carver & Bradfield 1997, Ramadoss et al. 2004).

In addition to its well-established role as a transcription factor, the ligand-activated AhR has also been shown to modulate the functions of other transcription factors, such as the oestrogen receptor (ERα and ERβ) and the androgen receptor (AR). The crosstalk of AhR with ER and AR explicates via a direct association in the nucleus, which modulates oestrogen/androgen signalling both positively and negatively depending on the cellular context (Ohtake et al. 2009, Pongratz et al. 2009). Furthermore, a novel and unexpected role for AhR as a ligand-dependent E3 ubiquitin ligase has also recently been described: the ligand-bound
AhR promotes the ubiquitination and proteasomal degradation of ER and AR through the assembly of a ubiquitin ligase complex, referred to as CUL4B<sub>AhR</sub>. AIP, however, seemed unlikely to influence the CUL4B<sub>AhR</sub>-mediated ER degradation (Ohtake et al. 2007).

**AIP–Hsp90** Hsp90 is a highly abundant molecular chaperone, which associates as a dimer with a set of highly different client proteins. Hsp90 is required to maintain signalling proteins in an active conformation that can be rapidly triggered by ligands. Hsp90 functions as the core component of a dynamic set of multiprotein complexes, involving a set of co-chaperones. Structurally, hsp90 can be divided into five domains: a highly conserved N-terminal domain involved in nucleotide and drug binding, a charged domain, a middle domain with ATPase activity involved in client protein binding, a second charged domain and a C-terminal domain involved in dimerisation and binding of TPR-containing proteins (mediated by a conserved EEVD motif) (Pearl & Prodromou 2006).

The direct but moderate association of AIP with hsp90 has been demonstrated in different studies (Carver et al. 1998, Meyer & Perdew 1999, Bell & Poland 2000, Kazlauskas et al. 2002, Yano et al. 2003, Laenger et al. 2009, Schulke et al. 2010). Hyperacetylation of hsp90 was found to lead to the loss of complex formation with AhR, p23 and AIP (Kekatpure et al. 2009). Discordant findings arose about the role exerted by hsp90 in assisting the AIP–AhR interaction. A study demonstrated that AhR, in order to bind AIP, needs to fold into the mature ligand-binding conformation with the help of hsp90 (Bell & Poland 2000). This requirement of hsp90 was instead proved to be not essential in another report (Meyer & Perdew 1999).

**AIP–Hsc70** In the absence of AhR, AIP was shown to interact – with higher affinity – with another heat-shock protein, the heat-shock cognate 70 (hsc70, MIM# 600816), rather than to hsp90 (Yano et al. 2003). Hsc70 is a constitutively expressed co-chaperone protein that is involved, as hsp90, in protein folding and in mitochondrial protein import (discussed later in Translocase of the outer membrane of mitochondria 20 (TOMM20) and mitochondrial preproteins section) (Young et al. 2003), but it also functions as an ATPase in the disassembly of clathrin-coated vesicles during transport of membrane components through the cell (Alberts et al. 2002). Hsc70 is a member of the hsp70 family. Although human hsc70 shares 85% sequence identity with human hsp70, they play different cellular functions (Gething & Sambrook 1992, Goldfarb et al. 2006). Consistent with this, AIP was found to be unable to bind hsp70 (Schulke et al. 2010).

**AIP interactions with other proteins of the AhR signalling pathway** Apart from AhR and hsp90, it was also investigated whether AIP binds to two other proteins involved in the AhR pathway, ARNT and p23.

ARNT was thought to be a good candidate because it belongs to the same family of transcription factors of AhR and both share a similar modular structure. However, the results from three different studies demonstrated that AIP is excluded from the AhR–ARNT heterocomplex in vitro and in vivo (Carver & Bradfield 1997, Ma & Whitlock 1997, Meyer & Perdew 1999). These findings agree with the AhR mapping evidence that AIP and ARNT have been shown to contact, at least in part, the same or an adjacent binding site on the AhR (Meyer & Perdew 1999). Even if AIP does not interact with ARNT, two recent studies showed that the expression of ARNT protein is significantly reduced in AIP-mutated pituitary tumours, suggesting that loss of AIP leads to an imbalance in the AhR–ARNT complex formation (Heliovaara et al. 2009, Raitila et al. 2010).

P23 was then demonstrated to contact AIP in co-immunoprecipitation (co-IP) experiments (Hollingshead et al. 2004), but only indirectly via hsp90, as previously demonstrated for AhR. (Nair et al. 1996). AIP was also shown to be able to displace p23 from the AhR complex, an effect only specific for the AhR complex and not for hsp90 alone (Hollingshead et al. 2004).

**AIP self-association** An interesting finding was the evidence that AIP can exist in multimeric complexes of at least two molecules even without requiring AhR or hsp90 (Hollingshead et al. 2004). The self-association of AIP is very likely mediated by the TPR domain, as demonstrated for other TPR-containing proteins (Das et al. 1998, Taylor et al. 2001, Nyarko et al. 2007). This suggests that AIP can homodimerise without the association of other auxiliary proteins or at least others than AhR or hsp90 and also that more than one molecule of AIP could be present in the AhR complex. However, two previous studies, using different stoichiometric approaches to examine the AhR complex subunits composition, showed a AhR:hsp90:AIP ratio of 1:2:1 (Chen & Perdew 1994, Petrulis et al. 2000). If these ratios are correct, the authors suggested that the multimeric complexes of AIP act as a reservoir able to regulate the amount of available monomeric AIP that can be included into the AhR complex (Hollingshead et al. 2004) or maybe in the other complexes with which AIP has been shown to interact. Furthermore, the TPR-mediated self-association of AIP might be a mechanism to specifically regulate its biological functions, as reported for the TPR-containing proteins PP5 and Sgt1 (Yang et al. 2005, Nyarko et al. 2007). For example, the phosphatase activity of PP5 is suppressed by an autoinhibited conformation maintained by the TPR domain–catalytic domain interaction (Yang et al. 2005).

**Domains mediating AIP–AhR–Hsp90 interaction and functional studies** Different studies contributed to define the domains involved in AIP–AhR–Hsp90 interaction and also, more specifically, the AIP residues essential for AhR and hsp90 binding.
Mapping experiments using AIP deletion mutants showed that the C-terminal half of AIP (residues 154–330), which contains the three TPR motifs, was necessary for binding both the AhR and the hsp90 (Fig. 3; Carver et al. 1998, Meyer & Perdew 1999, Kazlauskas et al. 2000). An indispensable role in mediating AhR binding was shown for the α-helical C-terminus (α-7) of murine AIP: deletion of the last five amino acids abolishes almost completely AhR binding, without affecting AIP–hsp90 interaction (Bell & Poland 2000). However, another study presented evidences suggesting that the human final α-7 helix is more likely to bind hsp90 than AhR (Kazlauskas et al. 2002). These contrasting results might be explained by species-specific differences. There are controversial results regarding the role played by the N-terminus of AIP. Some reports demonstrated that this region did not interact with either AhR or hsp90 (Carver et al. 1998, Meyer & Perdew 1999, Kazlauskas et al. 2000), whereas Kazlauskas et al. (2002) showed that the N-terminus contains an additional site of interaction with the AhR complex. Moreover, the N-terminal part of AIP was shown to confer stability to the complex and to be essential in the regulation of the subcellular localisation of AhR (Kazlauskas et al. 2002). Taken together, these results suggest that despite the C-terminal region of AIP being capable of interacting alone with the AhR complex, this interaction is not functional. This hypothesis is further supported by the finding that an AIP mutant lacking the first 17 amino acids, even if it did not loose the ability to bind hsp90, was expressed at lower levels compared with the wild type (WT) protein, maybe as a result of a higher turnover in cells (Meyer & Perdew 1999).

In the reciprocal mapping analyses, the boundaries of the AhR protein segment interacting with AIP were defined to be approximately between amino acids 380 and 419, which encompass the C-terminal portion of the PAS domain (PAS-B; Carver et al. 1998, Meyer & Perdew 1999, Kazlauskas et al. 2000). This interaction seems to be mediated by nonpolar or hydrophobic amino acids (Hollingshead et al. 2004). It was also established that AIP, like other immunophilins found in hsp90 complexes, binds to the C-terminal segment of hsp90 (residues 629–732), whereas the AhR binds to the middle region (residues 272–617) (Meyer & Perdew 1999, Bell & Poland 2000). Hsp90 was found to interact with two spatially distinct motifs of the AhR, the PAS-B and the bHLH domains (Antonsson et al. 1995). The domains involved in the AIP–hsc70 interaction were not experimentally determined (Yano et al. 2003).

Mutational analyses of some TPR domains indicate that they perform essential functions (Chen et al. 1996, Blom et al. 2004) and the interaction with hsp90 through TPR domains has been shown to be conserved in plants and animals (Owens-Grillo et al. 1996). It is, therefore, very likely that this is a basic protein interaction critical to the function of AIP. Some studies addressed this issue analysing how mutations in

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**Figure 3** Location of key interacting domains of the AIP–AhR–Hsp90 complex. The size of domains is drawn to linear scale proportional to amino acid number. bHLH, basic helix-loop-helix; PAS, Per–ARNT–Sim homology domain; Q, glutamin-rich domain.

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the TPR domain of AIP affect AhR and hsp90 binding, as well as PDE4A5 and RET (rearranged during transfection; described in PDE4A5 and RET section).

Site-directed mutagenesis studies in the binding groove of the TPR domain of PP5, an immunophilin co-chaperone protein present in the glucocorticoid receptor (GR, MIM# 138040) complex (Silverstein et al. 1997), confirmed the prediction made from the previously discovered three-dimensional structure of the protein (Das et al. 1998) that basic amino acid residues in this region are important for hsp90 binding (Russell et al. 1999). As these residues are conserved in the TPR domains of other hsp90-interacting proteins, such as FKBP51, FKBP52 and AIP, they are likely to be of functional significance in order to mediate efficient interactions with their binding partners (Fig. 4). Based mainly on this assumption, point mutations in the third TPR motif of AIP were introduced in different studies (Bell & Poland 2000, Meyer et al. 2000, Laenger et al. 2009). These studies confirmed the importance of the TPR domain and the specific conserved amino acids in the AIP–AhR and AIP–hsp90 interactions.

Figure 4 Representation of the second TPR motif of AIP. A TPR motif is composed of a pair of antiparallel helices, A and B. Consensus amino acids are located at positions 4, 7, 8 and 11 in helix A and at positions 20, 24, 27 and 32 in helix B. Residues 8 and 20 are located at the position of closest contact between the A and the B helices of a TPR, whereas residue 27 on helix B is located at the interface of three helices (A, B and the A helix of the next TPR motif) within a three-helix bundle (Das et al. 1998). Orange lines connect interacting amino acids in A and B helices (modified from Jarymowycz et al. (2008)). Patients with familial isolated pituitary adenoma have been identified with mutations affecting these crucial amino acids, such as the cytosine (C) at position 8 (p.C238Y (Leontiou et al. 2008)), the lysine (K) at position 11 (p.K241E; Daly et al. 2007) and the isoleucine (I) at position 27 (p.I257V (Montanana et al. 2009)).
have a similar half-life in the cell line used, suggesting that AIP is stable even if it is not associated with any proteins.

Both the p.K266A and the p.R271A mutants were shown to be unable to bind hsp90 (Bell & Poland 2000, Laenger et al. 2009). The p.K266A mutant also showed a 75–80% reduced AhR binding, whereas the 271A–AhR binding was normal (Bell & Poland 2000).

As stated previously, in addition to the TPR domain, the five C-terminal amino acids of AIP are also important for AhR (and probably hsp90) binding. Another proof of their crucial role was given by an alanine-scanning mutagenesis experiment, which demonstrated that the replacement of any of the last four amino acids with alanine ablates the binding to the AhR (Bell & Poland 2000).

Cytoskeletal proteins

After ligand-dependent activation in the cytoplasm, signalling proteins that affect gene transcription, such as the AhR or the GR, move to their sites of action within the nucleus. Lots of evidences point out the essential role of the hsp90-binding immunophilins in mediating various phases of nuclear receptor movements. For instance, FKBP52, which is associated with several steroid receptor heterocomplexes, has been shown to interact with tubulin cytoskeletal networks. This interaction takes place through the PPIase domain of FKBP52 and the cytoplasmic dynein, the motor protein that processes along the microtubules in a retrograde direction towards the nucleus (Pratt et al. 2004). It is thus conceivable to suppose that AIP could also associate with actin or tubulin filaments in order to regulate the cytoplasmic localisation of the AhR.

Three studies addressed this matter. In one, it was shown by a co-immunoadsorption assay, that the PPIase-like domain of AIP does not bind, or binds only very weakly, the cytoplasmic dynein (Galigniana et al. 2002). In another study, it was demonstrated that the well-documented AIP-mediated cytoplasmic retention of the AhR (Kazlauskas et al. 2000, 2002, LaPres et al. 2000, Petroulis et al. 2000, Kashuba et al. 2006) involves the anchoring of the complex to actin filaments (Berg & Pongratz 2002). By co-IP experiments, this interaction was proved to involve a direct binding of AIP to actin and also to take place only in the non-activated AhR complex, because TCDD treatment induces the release of the complex from actin (Berg & Pongratz 2002). However, it should be noted that the AIP–actin interaction was subsequently rejected by another group (Petroulis et al. 2003). The only difference between the two studies was the cell line used for the experiments. In absence of a conclusive demonstration, this interaction cannot be considered certain at the moment.

Phosphodiesterases

The cAMP functions as an intracellular second messenger in several signalling pathways. cAMP often acts as a promoter of both differentiation and apoptosis; however, in certain tissues such as thyroid and pituitary somatotroph cells, cAMP stimulates cell proliferation (Stork & Schmitt 2002). An altered cAMP pathway could result in hypertrophy and hyperplasia of the pituitary, which could lead to the development of pituitary adenomas. For instance, abnormally high cAMP levels have been linked to CNC and McCune–Albright syndrome (MAS, MIM# 174800) and have been identified in 30–40% of sporadic GH-secreting adenomas. The underlying cause of these high cAMP levels is constitutively activating mutations in the stimulatory G protein (Gαs, MIM# 139320) in MAS and sporadic somatotropinomas and inactivating mutations in the protein kinase A regulatory subunit-1-alpha (PRKAR1A) in CNC (Boikos & Stratakis 2007).

cAMP is generated in the cytoplasm by activation of adenyl cyclase, but it is inactivated by phosphodiesterases (PDEs). PDEs are a huge family of enzymes that catalyse the hydrolysis of cAMP and cGMP, generating the corresponding nucleotides 5’AMP and 5’GMP. The PDE superfamily can be subdivided into 11 subfamilies that differ by structure, enzymatic properties, sensitivity to different inhibitors and specific expression profiles. Each subfamily comprises from one to four distinct genes and each gene, in turn, generates several transcripts (Bender & Beavo 2006). This multiplicity of PDE isoforms (currently more than 50 different PDE proteins with tissue-specific subtypes have been identified) ensures the compartmentalisation, fine-tuning and crosstalk of the cAMP and cGMP pathways (Zaccolo & Movsesian 2007). All the PDEs consist of a modular architecture, with variable regulatory domains located at the N-terminus and a highly conserved catalytic region at the C-terminus (Conti & Beavo 2007).

PDE4A5 The cAMP-specific PDE4 group forms the largest PDE subfamily, which is the main enzyme responsible for cAMP degradation (Lugnier 2006). PDE4s can be distinguished from other PDE subfamilies by sequence identity in the catalytic region and by the presence of specific regions, located at the N-terminal portion of the proteins, called upstream conserved regions 1 and 2 (UCR1 and UCR2; Bolger et al. 2003, de Oliveira & Smolenski 2009). At least 35 splice variants are encoded by four genes (PDE4A4–D; Lugnier 2006). Of these, the highly conserved PDE4A4/5 isoform (PDE4A5 is the rat homologue of the human PDE4A4) is characterised by an extended N-terminal region involved in subcellular targeting (Bolger et al. 2003). PDE4A4/5 is expressed in a wide variety of tissues, including the pituitary (Mackenzie et al. 2008, Lennox et al. 2011), and both membrane and cytosolic localisation has been detected (Huston et al. 2000). PDE4A5 was demonstrated to interact with the SH3 domains of SRC family tyrosyl kinases (O’Connell et al. 1996, Beard et al. 2002), with AKAP3 (Bajpai et al. 2006) and with AIP (Bolger et al. 2003). AIP was initially identified as a direct binding partner of PDE4A5 by a Y2H screening of a rat brain cDNA library. The interaction was subsequently confirmed by a GST pull-down assay and
was also demonstrated in mammalian cells. In addition, the ability of AIP to bind PDE4A5 was found to be unaffected by the concomitant binding of LYN, a member of the tyrosyl kinase family.

The interaction was proved to be highly specific, because other immunophilins or TPR-containing proteins such as the AIP homologue AIPL1, FKBP51 and FKBP52 were unable to bind PDE4A5 and do not involve other PDE4 isoforms. The domains mediating the interaction were mapped in the TPR region of AIP and in both the unique N-terminal region (amino acids 11–42) and the UCR2 domain (only the PDE4-conserved EELD motif) of PDE4A5. The binding of AIP to PDE4A5 was demonstrated to lead to three distinct functional consequences. First is a reversible, dose-dependent inhibition of PDE4A5 catalytic activity by \( \sim 60\% \), which is directly mediated by the TPR domain of AIP. A second outcome, also leading to a decreased enzymatic activity, is the attenuation of PDE4A5 phosphorylation by PKA. The third consequence is an increased sensitivity of PDE4A5 to rolipram, the PDE4-specific inhibitor.

The impact of several missense and nonsense AIP mutations on PDE4A5 binding was studied by a Y2H \( \beta \)-galactosidase assay (Bolger et al. 2003, Leontiou et al. 2008, Igreja et al. 2010). All four naturally occurring truncating mutations investigated (p.R81X, p.Q164X, p.Q217X and p.R304X) (Leontiou et al. 2008, Igreja et al. 2010). The 12 missense mutations analysed were selected based on the evolutionarily conserved structurally critical amino acids located within the consensus TPR domain (Das et al. 1998, Russell et al. 1999, Scheufele et al. 2000) (p.N236A, p.K266A and p.R271A) (Bolger et al. 2003) or because they were described in patients or controls (p.R16H, p.V49M, p.K103R, p.C238Y, p.K241E, p.I257V, p.R271W, p.A299V and p.R304Q) (Leontiou et al. 2008, Igreja et al. 2010). Regarding p.N236A, p.K266A and p.R271A, it was shown that only the latter significantly attenuates the interaction of AIP with PDE4A5 and also its ability to inhibit PDE4A5, whereas the others exhibit normal binding and do not compromise the inhibitory capacity of AIP (Bolger et al. 2003). The other nine missense variants investigated can be divided into two groups: the first group comprises mutations with \( \beta \)-galactosidase assay activity values more than fivefold different from WT (p.K103R, p.C238Y, p.K241E and p.R271W), suggesting that they lead to a complete loss of PDE4A5–AIP binding (Leontiou et al. 2008, Igreja et al. 2010), whereas the other group has normal (p.V49M) or less than threefold different \( \beta \)-galactosidase assay activity values from WT (p.R16H, p.I257V, p.A299V and p.R304Q). The low impact of the p.R16H variant on PDE4A5 binding (Igreja et al. 2010), together with its normal interaction with the RET protein (described in RET section; Georgitsi et al. 2007), suggest that if this is a pathogenic variant and not a rare polymorphism, then based on the \textit{in vitro} studies, a PDE4A5– and RET-independent mechanism may take place. Notably, the p.R304Q variant has very strong clinical data suggesting that it is a disease-causing variant, but the in \textit{vivo} \( \beta \)-galactosidase assay was not showing strong abnormality (Igreja et al. 2010). Clearly, further data are needed to understand whether PDE4A5 binding correlates with the tumorigenic properties of AIP.

Combining together the results from these and other binding studies (Bolger et al. 2003, Laenger et al. 2009, Igreja et al. 2010), the essential role played by the R271 residue in mediating the binding of AIP with hsp90 and PDE4A5 emerges. This amino acid is likely to participate in an electrostatic interaction with the EEVD and EELD motifs of hsp90 and PDE4A5 respectively (Bolger et al. 2003), and its mutation completely disrupts these interactions. It is also interesting to note that the p.R271W human mutation affects a CpG site and is the second most common mutational hotspot described in the \textit{AIP} gene (Igreja et al. 2010).

**PDE2A3** Three isoforms of PDE2A (PDE2A1, PDE2A2 and PDE2A3), generated from a single gene by alternative splicing, have been cloned from several different species. These three isoforms are identical except for the N-terminal regions, which are responsible for their different subcellular localisation. The human variant (PDE2A3) encodes a membrane-associated protein of 941 amino acids. PDE2A functions as a homodimer and each monomer is formed by an N-terminal domain, two tandem GAF domains (GAF-A and -B) and a catalytic C-terminal domain. PDE2A is able to hydrolyse both cAMP and cGMP, but in the presence of cGMP, which binds to the allosteric GAF-B domain, the enzyme is activated and increases its affinity for cAMP, resulting in a greater hydrolysing capacity for cAMP than for cGMP (Bender & Beavo 2006). This enzyme thus contributes to the crosstalk between these two second messenger pathways (Zaccollo & Movsesian 2007). PDE2A is strongly expressed in the brain with a moderate presence in peripheral tissues such as the adrenal gland and heart and skeletal muscle (Rosman et al. 1997, Sadhu et al. 1999, Stephenson et al. 2009, Lin et al. 2010). In addition, PDE2A expression has been observed in rat (Velardez et al. 2000, Stephenson et al. 2009) and human pituitary (Lennox et al. 2011).

de Oliveira et al. (2007) identified an interaction between the human PDE2A and the AIP by a Y2H screening of a human brain cDNA library. The interaction was subsequently confirmed by GST pull-down and co-IP experiments both in cell lines and in brain tissue lysates. The two proteins were found to colocalise in the cytosol, with the occasional involvement of the plasma membrane. The regions that mediate the interaction were mapped in the GAF-B domain of PDE2A and the C-terminal half (amino acids 170–330) of AIP. In addition, preliminary results suggested that AIP was able to interact with PDE2A and hsp90 at the same time. Different from what was previously shown for PDE4A5, the enzymatic activity of PDE2A was unaffected by AIP binding. PDE2A, due to its binding to AIP, is located in the vicinity of AhR and is able to lower the local cAMP concentration in the compartment where the AhR complex is located, therefore the TCDD- and especially the forskolin-induced nuclear
translocation of AhR is 40% (with TCDD) or 55% (with forskolin) lower in PDE2A-transfected cells compared with control cells. Furthermore, this inhibition was demonstrated to correlate with a reduction of the AhR function, as reported by a reporter gene assay.

At present, it is unknown if both PDE4A5 and PDE2A can simultaneously associate via AIP to the AhR complex (de Oliveira & Smolenski 2009).

Nuclear receptors

Several studies demonstrated the involvement of AIP in various nuclear receptor signalling pathways.

Oestrogen receptor α ERα and ERβ mediate the biological effects of oestrogens both in reproductive and in nonreproductive organs in both sexes. After ligand binding, the ERs sequentially dimerise, translocate into the nucleus, associate with several coregulator proteins, and regulate the transcriptional activity of oestrogen target genes (Nilsson & Gustafsson 2011). ERα and ERβ display distinct or even opposite effects: in tumour cells, ERα was shown to promote cell proliferation (Zeng et al. 2008), whereas ERβ stimulates apoptosis (Cheng et al. 2004). The physiological actions of oestrogens are thus the result of a balance between ERα and ERβ signalling (Heldring et al. 2007).

It has recently been shown that AIP is involved in ERα transcriptional activation by interacting with the co-activator TIF-2 (Pongratz et al. 2009), which is structurally related to members of the bHLH–PAS family (Voegel et al. 2009). In particular, AIP negatively regulates the protein levels of TIF-2 both in the presence and in the absence of ligand, thus exerting a negative effect on ERα transcriptional activity. We could hypothesise that AIP may have a role in preventing ERα-dependent tumour growth.

Glucocorticoid receptor

The GR is a member of the nuclear receptor superfamily. Like the AhR, it has been demonstrated to exist in a multiprotein heterocomplex containing two molecules of hsp90 and other co-chaperone proteins, such as p23, PP5 and FKBP52 (Grad & Picard 2007). Moreover, the GR signalling pathway shares some mechanistic similarities with the AhR pathway. Both the receptors reside in the cytoplasm in the absence of the respective ligands and, on their binding, they undergo conformational changes, which lead to nuclear translocation. Inside the nucleus, the GR homodimerises and the dimer binds to the recognised enhancer elements, activating the transcription of the target genes (Heitzer et al. 2007). The similar mechanism of action with the AhR and especially the presence of TPR-containing proteins as GR regulators prompted different groups to test whether AIP is part of the GR complex.

The first studies by Carver et al. (1998), conducted by a Y2H screening (Carver & Bradfield 1997) and by co-IP experiments in yeast, demonstrated that AIP does not interact with the GR complex. However, subsequently, co-IP assays in a mammalian cell line showed that this interaction occurs via hsp90 (Laenger et al. 2009). The effect of AIP on the GR signalling is inhibitory due to a delayed nuclear accumulation of GR after ligand binding, with subsequent decrease of GR’s transcriptional activity (Laenger et al. 2009, Schulkel et al. 2010). A similar situation has occurred with FKBP51, which was found not to be associated with GR in yeast but was associated in mammalian cells. A possible explanation could be the lack of other TPR domain protein partners of the receptor resulting in suboptimal activity in yeast (Laenger et al. 2009).

Apart from ER and GR, some evidences suggest the potential of AIP to interact with other steroid hormone receptors. In particular, AIP has recently been shown to strongly inhibit the transcriptional activity of the receptors for progesterone (PR) and androgen (AR) (Schulkel et al. 2010).

Peroxisome proliferator-activated receptor α The peroxisome proliferator-activated receptor α (PPARα, MIM# 170998) is a soluble transcription factor belonging, as the GR, to the nuclear receptor superfamily. PPARα can be activated by different lipophilic compounds, and, in turn, it associates with the retinoid X receptor α (RXRα). This heterodimer activates the transcription of several genes encoding enzymes involved in the lipid and lipoprotein metabolism (Yoon 2009).

By co-IP experiments, using an antibody highly specific for the PPARα isoform and therefore not binding to the other PPARs subtypes PPARβ and PPARγ, the mouse PPARα was found to form a complex with AIP and hsp90 in the liver cytosol. However, as PPARα is predominantly nuclear, the authors hypothesised that this complex could exist as well in the nucleus (Sumanasekera et al. 2003). Similar to the inhibition exerted on the transcriptional activity of the HBV X protein and GR (Kuzhandaiavelu et al. 1996, Laenger et al. 2009), AIP was found to repress PPARα activity when overexpressed (Sumanasekera et al. 2003). However, the normally low expression levels of AIP in the liver, in contrast to the high PPARα expression, suggest that the inhibitory effect of AIP might be very weak or not explicited at all in physiological conditions.

Thyroid hormone receptors β1 Thyroid hormone receptors (TRs) mediate the genomic actions of the thyroid hormone triiodothyronine (T3). TRs are nuclear receptors derived from two genes, THRA and THRB. The THRB (MIM# 190160) gene encodes three isoforms, β1, β2 and B3 (Cheng et al. 2010). TRβ1 is involved in the negative feedback of T3 on TRH production in the paraventricular nucleus (PVN) but also mediates the T3-dependent activation of TRH transcription. As the mechanism by which TRβ1 exerts this activating role was unknown, Froidevaux et al. (2006) decided to look for TRβ1-interacting proteins. Using a Y2H assay to screen a mouse PVN cDNA, AIP was identified as a new TRβ1 partner. AIP and TRβ1

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colocalise in the same neurons in the PVN, thus giving to this interaction a functional significance in the regulation of TRH activation. The AIP–TRβ1 interaction was demonstrated to be very specific both in yeast and in a mammalian cell line, as no other TR isoforms interacted with AIP. However, the strength of the complex in yeast was T3 dependent, whereas in mammals the interaction (although weaker) was still happening in the absence of T3. AIP binds to TRβ1 via the TPR domain, because its deletion causes the loss of the interaction. AIP, however, does not change the subcellular localisation of TRβ1, regardless of the presence of the ligand T3. An in vitro small inhibitory RNA (siRNA) experiment demonstrated that the stability of the TRβ1 receptor was compromised by AIP knockdown. AIP binding to TRβ1 is independent of the presence of T3 in mammals. In vivo siRNA studies show that AIP is indispensable for the T3-independent TRβ1 activation of TRH transcription but is not necessary for the T3-dependent repression.

**Transmembrane receptors**

**Rearranged during transfection** The rearranged during transfection (RET) (MIM# 164761) proto-oncogene encodes a transmembrane tyrosine kinase receptor involved in the development, maturation and survival-controlling functions of epithelial, neuronal and several neuroendocrine cells (Lai et al. 2007). RET has been shown to be expressed in two major splicing isoforms that differ at the C-terminal end: a long isoform of 1114 amino acids, termed RET51, and a short isoform, 1072 amino acids long, named RET9 (Tahira et al. 1990). Structurally, RET can be divided into three domains: a large extracellular domain that includes a cadherin-like and a cysteine-rich region, a transmembrane domain, and an intracellular tyrosine kinase domain (Lai et al. 2007). In the presence of the ligand RET activates various signal transduction pathways that ultimately promote survival, growth and extension/migration of cells, whereas in its absence, it releases an intracellular fragment that induces apoptosis (Canibano et al. 2007). RET activating mutations have been associated with the following disorders: MEN2A (MIM# 171400) proto-oncogene encodes a cell-surface glycoprotein, which is required for normal cellular proliferation, survival, adhesion, migration and differentiation (Harari et al. 2007). EGFR interacts with a wide range of proteins, among which AIP was reported (Deribe et al. 2009). AIP was identified in a large-scale MYTH screen, a split-ubiquitin-based membrane Y2H assay, which allows the systematic analysis of full-length membrane protein interactions in a cellular environment (Snider et al. 2010). However, this interaction was not subjected to further confirmations employing different techniques and thus, given the high rate of false positives in Y2H analysis (Fields 2005), it cannot be considered certain. It would be important to confirm this interaction in the pituitary because EGFR inhibition has been shown to control pituitary tumour growth and hormone secretion (Vlotides et al. 2008).

**G proteins**

G proteins are heterotrimeric GTP-binding proteins formed by α, β and γ subunits (Gα, Gβ and Gγ), which mediate receptor-stimulated signalling pathways. Gα subunits are typically divided into four families, Gα15, Gα12/13, Gαq/11, and Gα12/13. Gα13, similar to AIP, is ubiquitously expressed and is an essential gene, because its deletion is embryonic lethal in mice (Worzfeld et al. 2008).

Using Y2H screening of a mouse fetal brain cDNA, Nakata et al. (2009), looking for new Gα13-interacting proteins, found that AIP is a binding partner of Gα13. This interaction was confirmed in vitro by a GST pull-down assay and was shown to involve the whole AIP protein sequence. It was also determined that the interaction is independent of the GTP/GDP binding status of the α subunit and that it does not involve the β and γ subunits. The other three types of α subunit were also tested for their ability to interact with AIP, and it was found that the Gα15 subunit also binds AIP, although weaker than Gα13, whereas Gαq and Gα11 do not bind AIP. It is interesting to note that another TRP protein, PP5, also binds Gα13/Gα15 (Yamaguchi et al. 2002).

Gα13 activation was then demonstrated to disturb the AIP–AhR interaction through the destabilisation of the AhR

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**Epidermal growth factor receptor** The epidermal growth factor receptor (EGFR, MIM# 131550) is a transmembrane glycoprotein, which is required for normal cellular proliferation, survival, adhesion, migration and differentiation (Harari et al. 2007). EGFR interacts with a wide range of proteins, among which AIP was reported (Deribe et al. 2009). AIP was identified in a large-scale MYTH screen, a split-ubiquitin-based membrane Y2H assay, which allows the systematic analysis of full-length membrane protein interactions in a cellular environment (Snider et al. 2010). However, this interaction was not subjected to further confirmations employing different techniques and thus, given the high rate of false positives in Y2H analysis (Fields 2005), it cannot be considered certain. It would be important to confirm this interaction in the pituitary because EGFR inhibition has been shown to control pituitary tumour growth and hormone secretion (Vlotides et al. 2008).

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Gα13 activation was then demonstrated to disturb the AIP–AhR interaction through the destabilisation of the AhR
protein via the ubiquitin–proteasome pathway and was also shown to inhibit the ligand-mediated transcriptional activation of the AhR independently from RhoA binding (RhoA is a small GTP-binding protein that is activated after the interaction with Gα13). Gαq, despite its less tight interaction with AIP, was also found to exert a strong inhibitory effect on the AhR (Nakata et al. 2009). The downstream signalling pathways regulated by Gα13, apart from the activation of RhoA, are not well characterised (Worzfeld et al. 2008). Because the Gα13-mediated inhibition of AhR explicates in a RhoA-independent manner, what happens to Gα13 signalling is at present unknown. However, it is interesting to note that there are some evidences reporting the involvement of the Gα13 pathway in the regulation of cAMP responses (Jiang et al. 2007, 2008). Specifically, the synergistic regulation of cAMP synthesis by the Gα13 and Gα13 pathways was shown to be mediated by a specific isoform of adenyl cyclase, termed AC7 (Jiang et al. 2008).

The AhR dissociation from AIP induced by Gα13 was shown to cause the translocation of the AhR into the nucleus in a ligand-independent manner. However, the AhR that moves into the nucleus in this way does not form an active complex with ARNT, as also seen for the cAMP-mediated nuclear translocation of the AhR (Oesch-Bartlomowicz et al. 2005). This divergence suggests that the AhR adopts a unique structure in the cytoplasm and that AhR does not undergo the same conformational change when moving to the nucleus with every nuclear transport inducer (such as TCDD, Gα13, or cAMP).

**TOMM20 and mitochondrial preproteins**

TOMM20 is an import receptor that, along with TOMM70 and other proteins, is part of the TOMM protein complex. The TOMM protein complex is involved in the recognition and translocation of mitochondrial preproteins synthesised in the cytoplasm. To be able to cross the mitochondrial membranes, the preproteins are maintained in the cytosol in an unfolded translocation–competent conformation by different molecular chaperones (Baker et al. 2007). Among them, hsp90 and hsc70 have been shown to mediate the import of TOMM70-dependent preproteins (Young et al. 2003), and AIP was demonstrated to interact with TOMM20 (Yano et al. 2003). In humans, TOMM20 can be structurally subdivided into five regions: a transmembrane segment at the N-terminus, a linker segment, a TPR motif, a Q-rich region and a conserved COOH-terminal acidic segment (Abé et al. 2000). This latter region is not required for preprotein binding but is specifically involved in an electrostatic interaction with the TPR motifs of AIP (Yano et al. 2003). In particular, an essential role has been found to be played by the very last five amino acids of TOMM20 (EDDVE), a segment similar to those present in PDE4A5 (EELD, involved in AIP binding) (Bolger et al. 2003) and hsp90 and hsc70 (EEVD, involved in the interaction with various TPRs) (Smith 2004).

In the same study (Yano et al. 2003), AIP was also shown to interact with several mitochondrial preproteins, for example preornithine transcarbamylase. Different from the AIP–TOMM20 interaction, both the PPlase-like and the TPR regions of AIP are required to bind the internal import signals of the preproteins. AIP was then demonstrated to maintain the loosely folded state of preproteins and to promote their transfer into mitochondria. This finding was confirmed by the demonstration that AIP forms a ternary complex with TOMM20 and the preprotein and by the fact that AIP interacts with preproteins less strongly than with TOMM20. Altogether, these results suggest the following scenario: mitochondrial preproteins may form a large complex in the cytosol with hsc70 and AIP, with both proteins contributing to maintain their unfolded conformation; once the complex has reached the outer membrane of the mitochondria, AIP binds to TOMM20 and promotes the transfer of the preprotein to the import receptor.

![AIP interactome](http://ophid.utoronto.ca/navigator; Brown et al. 2009). Nodes correspond to proteins and lines to physical protein–protein interactions. Solid lines signify confirmed interactions, whereas dashed lines represent uncertain associations. Node colours discriminate between different classes of proteins.
Table 1  Interacting partners of aryl hydrocarbon receptor-interacting protein (AIP). The techniques used to identify the various interactions, the functions of the different AIP partners, the effect of AIP on the partner’s activity/stability and the organisms/cell types where the interactions have been proved are reported.

<table>
<thead>
<tr>
<th>Partner</th>
<th>Y2H</th>
<th>co-IP</th>
<th>Pull-down</th>
<th>Other</th>
<th>Confirmed interaction</th>
<th>Function</th>
<th>AIP effect on the partner’s activity/stability</th>
<th>Organism and/or cell type(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBV X</td>
<td>✔</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>Y</td>
<td>Transcriptional activator</td>
<td>↓</td>
<td>Human lymphoma, HeLa cells</td>
</tr>
<tr>
<td>EBNA-3</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Immortalization and transformation of B-cells</td>
<td>NE</td>
<td>EBV-immortalized lymphoblastoid cells, human lymphocytes and fetal brain</td>
</tr>
<tr>
<td>AhR</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>Y</td>
<td>Adaptive and toxic responses, development</td>
<td>↑↓</td>
<td>HeLa, Hepa1c1c7, COS-1, B-cells</td>
</tr>
<tr>
<td>Hsp90</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>Y</td>
<td>Protein folding, mitochondrial protein import</td>
<td>NE</td>
<td>HeLa, HEK, COS-1, COS-7, bacterial cells</td>
</tr>
<tr>
<td>Hsc70</td>
<td></td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Protein folding, mitochondrial protein import, disassembly of clathrin-coated vesicles</td>
<td>NE</td>
<td>HeLa cells</td>
</tr>
<tr>
<td>Actin</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>Cytoskeletal component</td>
<td>NE</td>
<td>COS-7 cells</td>
</tr>
<tr>
<td>PDE4A5</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>Y</td>
<td>cAMP degradation</td>
<td>↓</td>
<td>Rat brain, COS-7 cells</td>
</tr>
<tr>
<td>PDE2A3</td>
<td></td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>Y</td>
<td>cAMP and cGMP degradation</td>
<td>=</td>
<td>Human brain, COS-1, HeLa cells</td>
</tr>
<tr>
<td>TIF-2</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Activates ERα</td>
<td>↓</td>
<td>HC11 cells</td>
</tr>
<tr>
<td>PPARγ</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Regulation of energy homeostasis</td>
<td>↓↓</td>
<td>Mouse liver</td>
</tr>
<tr>
<td>TRβ1</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Activation of TRH transcription</td>
<td>↑</td>
<td>Mouse PVN of the hypothalamus</td>
</tr>
<tr>
<td>RET</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Development, maturation, survival</td>
<td>NE</td>
<td>Human fetal brain</td>
</tr>
<tr>
<td>EGFR</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>Cellular proliferation, survival, adhesion, migration, differentiation</td>
<td>NE</td>
<td>Human fetal brain</td>
</tr>
<tr>
<td>Gα13</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Mediates receptor-stimulated signalling pathways</td>
<td>NE</td>
<td>HEK293T, Hepa1c1c7, COS-7 cells</td>
</tr>
<tr>
<td>Gαq</td>
<td></td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Mediates receptor-stimulated signalling pathways</td>
<td>NE</td>
<td>HEK293T, Hepa1c1c7 cells</td>
</tr>
<tr>
<td>TOMM20</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>Y</td>
<td>Mitochondrial import receptor</td>
<td>NE</td>
<td>Human fetal liver, COS-7, HeLa cells</td>
</tr>
<tr>
<td>Survivin</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td>Y</td>
<td>Suppression of apoptosis</td>
<td>↑</td>
<td>HeLa, MCF-7, Raji cells</td>
</tr>
<tr>
<td>TNNI3K</td>
<td>✔</td>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>Promotes cardiomyogenesis, enhances cardiac performance, protects the myocardium from ischemic injury</td>
<td>NE</td>
<td>Human heart</td>
</tr>
</tbody>
</table>

Y, yes; N, no; NE, not evaluated; ↑, increase; ↓, decrease; =, no effect.
Survivin

Survivin is a member of the inhibitor of apoptosis (IAP) gene family, which includes evolutionarily conserved members that suppress apoptosis by preventing the maturation and/or the proteolytic activity of its effector enzymes, the caspases (Wei et al. 2008). Survivin, apart from inhibiting apoptosis, is also implicated in other essential cellular functions like the control of cell division and the stress response (Fortugno et al. 2003, Dohi et al. 2004, Yang et al. 2004). For instance, during harmful environmental stimuli, a significant release of survivin takes place from the mitochondria to the cytosol (Dohi et al. 2004) and survivin form complexes with hsp90 (Fortugno et al. 2003). Structurally, survivin is a 142 amino acid-long protein that exists as a functional homodimer. The dimerisation is mediated by the N-terminal baculovirus IAP repeat (BIR) domain, which is also involved in the interaction with other proteins, i.e. the caspases and hsp90 (Chantalat et al. 2000, Verdecia et al. 2000, Fortugno et al. 2003). The survivin–AIP interaction, discovered in a proteomics screening by Kang & Altieri (2006) is instead mediated by the C-terminal α-helical coiled-coil region, with a key role played by the last residue of the protein. The AIP region involved in the binding was mapped between residues 170 and 330, thus comprising the three TPR motifs. The interaction was demonstrated to be direct and to happen in pull-down and co-IP experiments. The association of AIP with survivin was shown to stabilise survivin protein levels independently from hsp90 binding (Fortugno et al. 2003). Loss of both proteins leads to proteasomal degradation of survivin, resulting in enhanced apoptosis and, only for hsp90, also to cell cycle arrest. The reported association of AIP with TOMM20 (Yano et al. 2003) leaves room to speculate that AIP could stabilise survivin in the cytoplasm and facilitate its displacement into the mitochondria pool, increasing the reserves of survivin in this compartment readily for subsequent events that involve apoptosis (Kang & Altieri 2006). This hypothesis has recently been experimentally confirmed by the same group (Kang et al. 2011). However, because several evidences suggest that AIP acts as a tumour suppressor gene (Leontiou et al. 2008), it is not clear why it stabilises survivin, thereby elevating the cellular anti-apoptotic threshold. To further increase the level of complexity of the system, the RET protein aboliishes the binding of AIP to survivin (Vargiolu et al. 2009), probably promoting apoptosis, a role already documented for RET (Bordeaux et al. 2000, Canibano et al. 2007). It is thus conceivable that when AIP and RET are co-expressed in the same tissue, RET acts to counteract the AIP-mediated stabilisation of survivin, in order to lower the anti-apoptotic threshold.

TNNI3K

Zhao et al. (2003) cloned a new cardiac-specific kinase gene named TNNI3K (cardiac troponin I-interacting kinase), belonging to the MAPKK family. In this study, TNNI3K was found to interact with several proteins including AIP, by a Y2H screening. The interaction with AIP was not further investigated with other techniques. In the light of the recent findings that AIP is essential in cardiac development (Lin et al. 2007) and TNNI3K plays an important role in the cardiac system (Lai et al. 2008), it will be of great interest to study more deeply this interaction.

Conclusions

Twenty interactions have been described for AIP. Fourteen proteins have been shown to directly interact with AIP: two viral proteins (HBV X and EBNA-3), two chaperone proteins (hsp90 and hsc70), two PDEs (PDE4A5 and PDE2A3), three nuclear (AhR, PPARα and TRβ1) and a transmembrane (RET) receptors, two G proteins (Gα13 and Gαq), an IAP (survivin) and a mitochondrial import receptor (TOMM20); three non-confirmed interactions with a cytoskeletal protein (actin), a growth factor receptor (EGFR) and a cardiac-specific kinase (TNNI3K) have also been described. The association of AIP with the GR and the co-chaperone protein p23 was proved to be mediated by hsp90, whereas the AIP–ERα interaction involves the ERα co-activator TIF-2 (Fig. 5 and Table 1).

Currently, it is uncertain which of the AIP interactions play a role in putitary tumorigenesis. The AhR–cAMP–PDE pathway seems to be a very attractive candidate as AhR activation has well-known tumorigenic effects (Dietrich & Kaina 2010) and PDEs are involved in the tight control of cellular cAMP levels (Zaccolo & Movsesian 2007), which are known to be involved in Gαi mutation- and CNC-related somatotroph tumorigenesis (Boikos & Stratakis 2007). Specially, the loss of PDE2A3 is postulated to lead to increased cAMP levels, which could result in hypertrophy and hyperplasia of the pituitary, possibly involving down-regulation of ARNT (Heliovaa et al. 2009). Also the interaction between RET and AIP might have a role in putitary adenoma tumorigenesis as the loss of RET results in somatotroph hyperplasia (Canibano et al. 2007). However, neither AIP nor RET mutations were shown to affect their interaction in vitro (Vargiolu et al. 2009). Further studies are thus indispensable to identify the exact mechanism by which AIP causes putitary tumorigenesis as this might ultimately lead to the design of novel strategies to cure the disease.
Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/JOE-11-0054.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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