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Roles of ANP32 proteins in cell biology and viral replication

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Abstract

The acidic leucine-rich nuclear phosphoprotein 32 kDa (ANP32) family consists of evolutionarily conserved proteins of 220–291 amino acids characterized by an N-terminal leucine-rich repeat domain (LRR) and a C-terminal low-complexity acidic region (LCAR). ANP32 family proteins regulate a variety of physiological functions, including chromatin remodeling, apoptosis and nervous system development. Abnormal ANP32 expression is closely related to tumorigenesis. In recent years, the role of ANP32 family proteins in viral infections has received considerable attention due to their activity supporting influenza virus replication and restriction of virus cross-species transmission. Moreover, ANP32 proteins are closely related to the replication of HIV and nonsegmented negative-strand RNA viruses (NNSVs). In this review, the general physiological functions of ANP32 family proteins, as well as their roles in virus replication, are summarized in detail.

Keywords: ANP32, ANP32A, Physiological process, Influenza virus, Replication, Cross-species transmission, HIV-1

Introduction

Proteins of ANP32 family are widely found in eukaryotes (except yeast) but not in bacteria or archaea (Matilla and Radrizzani 2005). The first identified member of ANP32 family, ANP32A, also known as PHAPI, pp32, I1PP2A, LANP, HPPCn and Mapmodulin, was discovered independently by different teams in the 1990s (Malek et al. 1990; Matsuoka et al. 1994). After that, other members of ANP32 family were discovered in succession. ANP32B is also known as SSP29, APRIL and PAL31. Other names for ANP32E include CPD1, LANP-L and PHAPIII (Mencinger et al. 1998; Radrizzani et al. 2001).

However, there is a dispute about the composition of ANP32 family genes in mammals. As described by Antoni Matilla and Martin Radrizzani in 2005, the human *ANP32* (*huANP32*) family contains eight members, *ANP32A* to *H*, while *ANP32C* and *ANP32D* (also named *pp32r1* and *pp32r2*) are annotated as pseudogenes generated by retrotransposition because their genes are intronless (Matilla and Radrizzani 2005). Interestingly, some studies have found that *ANP32C* is oncogenic and overexpressed in tumor cells but not in normal cells (Buddaseth et al. 2013, 2014; Kochevar et al. 2004; Brody et al. 1999; Kadkol et al. 1999). *ANP32D* can also function as an oncogene, a predictor of chronic mountain sickness, and is significantly upregulated during the progression of type 1 diabetes (Prashanth et al. 2021; Cole et al. 2014; Zhou et al. 2013). Other members of ANP32 family, including *ANP32F*, *ANP32G* and *ANP32H*, are only found in humans. They are poorly studied due to their limited species distribution and the lack of characteristic motif common to most members of the family (Matilla and Radrizzani 2005). The remaining members of ANP32 family, *ANP32A*, *ANP32B* and *ANP32E*, which are present in almost all animal species based on transcriptomic and proteomic evidence have received the most research interest because of their highly conserved structure and function (Malek et al. 1990; Costanzo et al.

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2006; Santa-Coloma 2003; Jiang et al. 2002; Brennan et al. 2000; Li et al. 1995; Jiang et al. 2003; Obri et al. 2014).

ANP32 proteins have a broad cellular distribution, as demonstrated by different studies, and they are predominantly found in nucleus (Pan et al. 2009; Kadkol et al. 1998), cytoplasm (Martinvalet et al. 2005; Ulitzur et al. 1997a), or shuttling between cytoplasm and nucleus (Fries et al. 2007; Hostetter et al. 2008). Interestingly, they have also been found to be located on the cell surface (Callebaut et al. 1998) and even secreted outside of cells (Chang et al. 2010). Based on the locations in which they have been found, ANP32 proteins are thought to be involved in a wide range of biological processes, including cell signaling and transduction, transcriptional regulation, intracellular transport differentiation, proliferation, apoptosis, neuronal development and cancer (Reilly et al. 2014).

Furthermore, numerous studies have demonstrated that ANP32 proteins are involved in the replication processes of a variety of viruses, such as the influenza virus (Bradel-Tretheway et al. 2011; Watanabe et al. 2014; Long et al. 2016, 2019b; Fodor and Te Velthuis 2020) and HIV-1 (Wang et al. 2019).

The structure of ANP32 proteins

The ANP32 proteins are conceptually considered to have a highly conserved "whip-like" structure, with the N-terminal LRRs forming the "handle" and the C-terminal LCAR domain forming the "thong" (Reilly et al. 2014). The LRR is a short motif comprising 20-29 residues and generally containing a conserved 11-residue sequence LxxLxLxxN/CxL (x represents any amino acid, and L can be replaced by valine, isoleucine, or phenylalanine, followed by a variable stretch of 9-18 residues, Kobe and Deisenhofer 1993). LRR is a protein recognition motif and is often present in tandem in a variety of proteins with diverse functions (Kobe and Kajava 2001).

Crystal structure analysis revealed that the N-terminus of human ANP32A (huANP32A) protein is composed of a linear array of five LRRs capped by a helix-loop-helix motif at its N-terminus (N-Cap, 1-17 aa) and a β-hairpin at its C-terminus (C-Cap, 142-152 aa). Meanwhile, LRR5 and C-Cap domains are also defined as LRRCT domains. Each LRR contains a β strand and an α helix connected by loops and forms the basic unit of the canonical curved structure, while N-Cap and C-Cap help to maintain the stability of this structure (Kobe and Deisenhofer 1993; Huyton and Wolberger 2007; Dao et al. 2015).

LRR domain of ANP32 proteins also contains 2-3 nuclear export signals (NESs), typically with the sequence LxxLxxLxL (x represents any amino acid), which is important for the cytoplasmic distribution and subsequently the function of ANP32 proteins (Brennan et al. 2000). As the structural core of ANP32 proteins, LRRs are responsible for the interactions of ANP32 with other host proteins, including histone H3-H4 (Tochio et al. 2010), PP2Ac (Iwatsuki-Horimoto et al. 2008), ataxin-1, CRM1 (Brennan et al. 2000), hyperphosphorylated Rb (Adegbola and Pasternack 2005), Clip170 (de Chiara et al. 2007) and some components of the SET complex (Lieberman and Fan 2003). In addition, this domain is also vital for the interactions between ANP32 proteins and viral proteins, such as the polymerase complex of influenza viruses (Zhang et al. 2019, 2020a). Interestingly, one previous study suggested that LRR domain is essential for the formation of ANP32 homo/heteropolymers (Ulitzur et al. 1997b). Overexpression experiments have led some scientists to believe that ANP32 proteins exist naturally as monomers (Pan et al. 2009).

LCAR domain, the other important region at the C-terminus of ANP32 proteins, constitutes approximately 100 amino acids, mainly (60-75%) acidic glutamyl and aspartic acids (Reilly et al. 2014). It was identified in other proteins as early as 1986 (Kuehl et al. 1986), but its crystal structure is as yet unresolved because of its low complexity. It has a low isoelectric point and therefore is assumed to be flexible in solution, with a capacity for physical interaction with any positively charged surface (Matilla and Radrizzani 2005).

LCAR domain also contains a conserved basic KRKR nuclear localization signal (NLS), which mediates the interaction of ANP32 proteins with the nuclear transport receptor family of importins, including importin-α2, importin-α5 and importin-α7 (Matsubae et al. 2000; Yu et al. 2022). This acidic domain also plays a role in maintaining the function of ANP32 proteins through interactions with MAP1B (Opal et al. 2003), H2A.Z (Obri et al. 2014), p120E4F (Cvetanovic et al. 2007), and cytochrome c (Rivero-Rodríguez et al. 2021). Interestingly, most ANP32A/B/E proteins from different species possess the typical structure of huANP32A protein, while avian ANP32A proteins are found to have a unique insertion located between LRRCT and LCAR (Fig. 1).

ANP32 proteins involved in chromatin remodeling

ANP32A has been found to be a component of the inhibitor of histone acetyltransferase (INHAT) complex, which can inhibit histone acetylation activated by p300/CBP and PCAF (Seo et al. 2001, 2002), suggesting that ANP32 proteins might be involved in chromatin remodeling and transcription. Additional studies indicated that the main ANP32 proteins, including ANP32A, ANP32B and ANP32E, regulate chromatin by binding to histone proteins in various ways. ANP32A can bind to unmodified histone tails, with a preference for H3 tails, blocking the acetylation of histones and repressing gene transcription

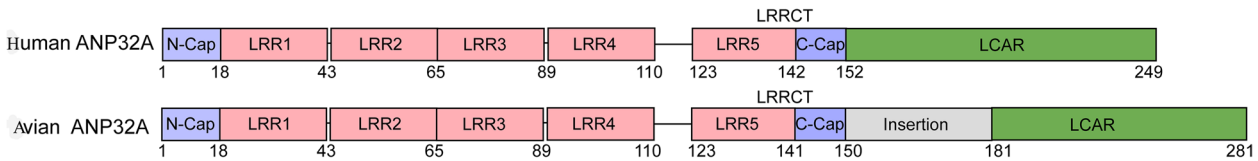

Fig. 1 Schematic structure of human ANP32A protein (NP_006296.1) and avian ANP32A (represented by chicken ANP32A (chANP32A), XP_413932.2)

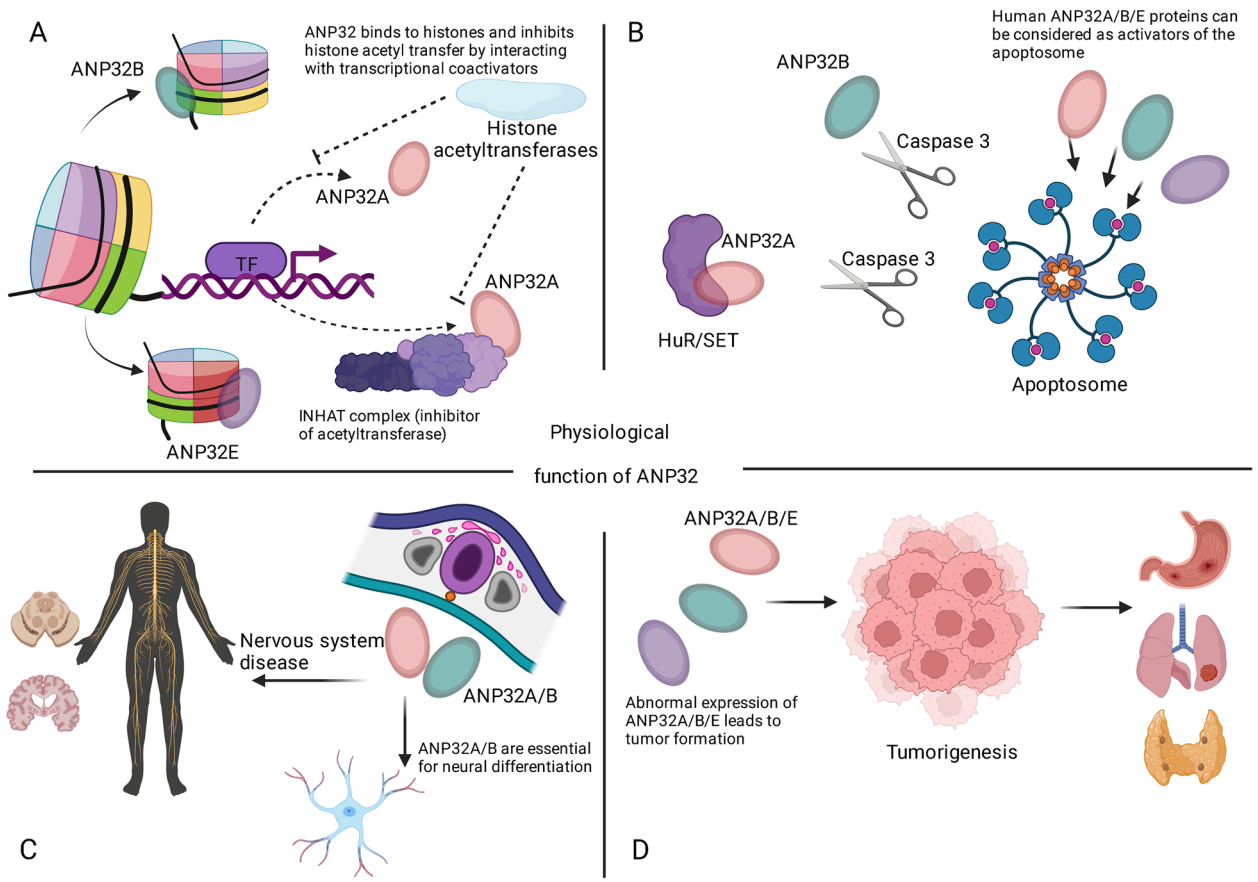

Fig. 2 ANP32 family proteins involve in various physiological functions. **A** Chromatin remodeling; **B** Apoptosis; **C** Regulation of neural development; **D** Malignant tumorigenesis

(Schneider et al. 2004). It can also form complexes with different DNA-binding transcription factors, including ER alpha (Loven et al. 2004), p120E4F (Cvetanovic et al. 2007), LHX3 (Hunter et al. 2013), and STAT1–STAT2 heterodimer (Kadota and Nagata 2011), subsequently triggering the modification of activated gene transcription (Fig. 2A).

ANP32B is recruited to the promoter regions of DNA through its interaction with the DNA-binding transcription factor Kruppel-like Factor 5 (KLF5), where it subsequently represses KLF5-downstream gene transcription (Munemasa et al. 2008). This process relies on the binding of ANP32B to the histone dimers H3–H4 and H2A-H2B (Tochio et al. 2010). It has also been reported that ANP32B plays a role in the DNA damage response (DDR) by inhibiting the activity of protein phosphatase 2A (PP2A). A recent study demonstrated that the inhibition of PP2A by ANP32B can be impaired by cytochrome c, which can compete with histones for binding sites on the LCAR domain of ANP32B. The interaction between ANP32B and cytochrome c induces a conformational change in ANP32B, resulting in the release of ANP32B

from the ANP32B:PP2A complex (Rivero-Rodríguez et al. 2021).

ANP32E, as a primary member of ANP32 family, can also function as a histone chaperone, preferentially associate with H2A.Z-H2B dimers, and help to remove H2A.Z from the nucleosomes (Obri et al. 2014; Mao et al. 2014). A recent study further confirmed that the ANP32E-mediated inhibition of PP2A is essential for nucleosomal inclusion of H2A.Z and subsequent regulation of gene expression (Shin et al. 2018). Additionally, ANP32E can effectively remove H2A.Z-H2B from DNA double-strand break (DSB) sites and can promote the normal progression of the DNA damage response (Gursoy-Yuzugullu et al. 2015). Crystal structure of the complex of ANP32E and H2A.Z-H2B revealed that specific binding of ANP32E leads to a significant extension of the αC helix of H2A.Z (Mao et al. 2014). This conformational change not only enhances the specific recognition of H2A.Z by ANP32E but also destroys the formation of H2A.Z universal docking site, which normally mediates the interaction of H2A.Z with other proteins. Interestingly, the key motif responsible for ANP32E mediation of the interaction with H2A.Z was mapped to residues 214-224 of LCAR of ANP32E, which are conserved in ANP32E across different species but absent in ANP32A and ANP32B. Numerous recent studies have focused on the association between ANP32E and H2A.Z-H2B dimers and have demonstrated that this interaction has multiple functions in different areas, such as altering the genome-wide state of chromatin (Murphy et al. 2020; Tachiwana et al. 2021), regulating memory formation and regulating dendritic morphology (Stefanelli et al. 2021).

ANP32 proteins are involved in the process of apoptosis

Apoptosis is closely related to apoptosome formation, in which the key component is apoptotic protease-activating factor 1 (Apaf-1) (Jiang and Wang 2000; Acehan et al. 2002). The apoptosome recruits and activates the caspase pathway, and the active caspases cleave multiple intracellular substrates. cells respond to this apoptotic stimulus with cell death (Li et al. 1997; Rodriguez and Lazebnik 1999; Thornberry and Lazebnik 1998). Some research results have suggested that ANP32A/B/E proteins may be involved in promoting the formation and activity of apoptosomes (Jiang et al. 2003; Hoffarth et al. 2008; Hill et al. 2004; Fan et al. 2006; Schafer et al. 2006), and ANP32A/B/E proteins can be considered as activators of apoptosomes.

ANP32A chelates the SET and HuR proteins to stimulate a positive feedback pathway leading to apoptosis (Fig. 2B). Moreover, RNAi-induced decreases in ANP32A protein expression in macrophages can induce rapid apoptosis (Shen et al. 2009). ANP32B plays the opposite role. ANP32B may be a substrate of Caspase-3 and functions as a negative regulator of apoptosis, and a noncleavable mutant ANP32B (D163A) has been demonstrated (Sun et al. 2006; Shen et al. 2010; Li et al. 2015). ANP32B regulates BCL2-associated agonist of cell death (Bad) phosphorylation, as well as the expression of BCL2 antagonist/killer (Bak) and BCL2 associated X (Bax), thereby regulating hepatoma cell apoptosis (Ohno et al. 2017). Therefore, ANP32 family proteins play diverse roles and functions in different cells. The specific mechanism of their regulation of apoptosis remains to be elucidated.

ANP32 proteins can be involved in the regulation of neural development

Initially, it was believed that the functions of different members of ANP32 family were highly redundant because knockout of ANP32A or ANP32E alone has no significant effect on the developmental phenotype of mice (Opal et al. 2004; Vaesen et al. 1994). ANP32B-deficient mice survived despite multiple systemic abnormalities during the embryonic period; however, ANP32A and ANP32B double-knockout mice did not survive (Reilly et al. 2011). This may be because ANP32A and ANP32B are both important but during different periods of mouse embryonic development (Matsuoka et al. 1994; Mutai et al. 2000), indicating that the functions of ANP32 family members in mouse development are not completely redundant. The cellular localization of ANP32A is related to neuronal differentiation, and the protein migrates from nucleus to cytoplasm during neuronal axon formation (Opal et al. 2004). ANP32A can participate in the regulation of neuronal differentiation and it regulates the expression of the neurofilament light chain by combining with the promoter of the neurofilament light chain gene. It also regulates expression level of histone acetylation, thereby affects the generation of neuronal axons (Kular et al. 2009) (Fig. 2C).

ANP32A is related to the occurrence of nervous system diseases. ANP32A expression levels are elevated in the brain tissue of patients with Alzheimer's disease, while the activity of PP2A enzyme decreases and tau protein is hyperphosphorylated. It can bind to the catalytic subunit of PP2A and thus inhibit the activity of the enzyme (Chen et al. 2008). At the same time, overexpression of ANP32A can cause hyperphosphorylation of tau protein (Tsujio et al. 2005). In both immature and mature neuronal cells, ANP32A can act as an important component of the INHAT complex, which interacts with retinoblastoma protein (RB) and causes gene inhibition, protecting neurons from excitotoxicity caused by N-methyl-D-aspartate (NMDA). In addition, ANP32A is associated with the pathogenesis of spinocerebellar ataxia type 1 (SCA1)

(Sanchez et al. 2013). However, ANP32B and ANP32E are rarely reported in neurological disease research and deserve further study.

Malignant tumorigenesis and ANP32 proteins

Tumor formation requires alterations in many key factors, including genetic mutations and epigenetic changes (DNA methylation, histone modification, or modification of noncoding RNA) in cancer cells. Tumorigenesis is usually closely related to gene mutations, which can lead to changes in the structure or quantity of the encoded proteins, resulting in the loss of gene function (Stratton et al. 2009; Graham and Sottoriva 2017; Vogelstein et al. 2013; Alexandrov et al. 2015; Xue et al. 2009; Genomes Project, C, et al. 2010; Laird et al. 2004; Lengauer et al. 1998).

Abnormal expression of ANP32 family proteins is associated with tumorigenesis, suggesting that ANP32 is an important regulator of this process. ANP32A in nucleus is able to significantly inhibit the ability of various oncogenes to transform rat embryonic fibroblasts (Chen et al. 1996). ANP32A has a sequence that functions as a tumor suppressor gene (Brody et al. 1999). The expression of ANP32A is low in prostate cancer in humans, and reducing its expression increases the tumorigenicity of NIH3T3 cells induced by resistance to audiogenic seizures (Ras), whereas ANP32B and ANP32E are highly expressed in prostate cancer tissues (Kadkol et al. 1999; Bai et al. 2001). However, laser capture microdissection and two-dimensional differential gel electrophoresis analysis revealed that ANP32A protein is highly expressed in colorectal cancer tissues. In addition, ANP32A is also highly expressed in ovarian cancer and liver cancer tissues (Shi et al. 2011). ANP32A is also a predictive gene marker for non-small cell lung cancer and hepatocellular carcinoma after treatment (Hoffarth et al. 2008; Zhu et al. 2010).

Unlike ANP32A, ANP32B may act as a tumor-promoting protein expressed at low levels in liver cancer tissues and it exerts an antiapoptotic effect. It is also associated with the prognosis of breast cancer (Ohno et al. 2017; Reilly et al. 2011). Furthermore, *in vivo* assays have demonstrated that ANP32C is tumorigenic (Kochevar et al. 2004).

ANP32E is generally highly expressed in malignant tumors, including mesothelioma (Pippa et al. 2020), gastric cancer (Kwon et al. 2017), lung adenocarcinoma (Wang et al. 2020), thyroid cancer (Huang et al. 2020), and follicular lymphoma (Björck et al. 2005). ANP32E is overexpressed in thyroid cancer (THCA) cells. Overexpression of ANP32E upregulated MMP9 and MMP13 (metastasis markers) expression, further revealing that ANP32E promotes THCA cell proliferation and migration by enhancing the protein kinase B/mammalian target of rapamycin/hexokinase 2 (AKT/mTOR/HK2) signaling pathway-mediated glycolysis (Huang et al. 2020). Recent studies have found that ANP32 family proteins are highly expressed in certain malignant tumors, suggesting that they have potential as therapeutic targets for cancer. The expression of ANP32 family proteins ANP32A, ANP32B and ANP32E was found to be elevated in medulloblastoma (MB). The occurrence of MB is driven by Hedgehog (Hh) signaling (SHH-MB). Other studies suggested that ANP32 protein can act as a positive regulator of mammalian Hh signaling to combat malignancy by activating GLI transcription factors (Hupfer et al. 2021). ANP32A, ANP32B and ANP32E are all upregulated in hepatocellular carcinoma (HCC) tissues, and the high expression of ANP32 family members is associated with advanced cancer (Liu et al. 2022). These studies have demonstrated that the abnormal expression of ANP32 family proteins is closely related to the occurrence of tumors (Fig. 2D), but the effects of overexpression of ANP32 family proteins in different tumors are inconsistent, and any underlying patterns remain obscure.

ANP32 family proteins support the replication of influenza virus

Influenza viruses can be divided into four categories according to their antigenicity and the genetic differences in the nucleoprotein (NP) and matrix protein (M1). The four groups include influenza A virus (IAV), influenza B virus (IBV), influenza C virus (ICV), and influenza D virus (IDV). IAV, IBV and ICV are pathogenic in humans, and IAV and IBV are the major causative agents of human influenza epidemics. Influenza pandemics pose a huge threat to global public health security and economic development (Taubenberger and Kash 2010; Webster et al. 1978; Belser et al. 2016; Zhai et al. 2017; Ritchey et al. 1976). Influenza viral RNA-dependent RNA polymerase (RdRp), a heterotrimer composed of Polymerase basic protein 1 (PB1), Polymerase basic protein 2 (PB2) and Polymerase acidic protein (PA), assembles with viral RNA and NP to form the viral ribonucleoprotein complex (vRNP). vRNP is responsible for the transcription and replication of the viral genome (Fodor and Te Velthuis 2020; Fodor et al. 1994). RdRp interacts with various host factors during influenza virus replication (Staller and Barclay 2021; Bradel-Tretheway et al. 2011; Watanabe et al. 2014). ANP32A/B are known to be involved in the formation of the influenza virus RdRp complex and are crucial in supporting viral polymerase activity and in determining the interspecies restriction of influenza viruses (Peacock et al. 2019). In addition, LCAR, a functionally important domain of ANP32 family, is able to directly interact with NP and recruit NP into nascent RNAs

during influenza virus genome replication (Wang et al. 2022).

Proteomics and whole genome sequencing works have suggested that ANP32A and ANP32B proteins may bind to the influenza RdRp complex (Bradel-Tretheway et al. 2011; Watanabe et al. 2014). The involvement of ANP32A and ANP32B proteins in the regulation of influenza virus replication was first described in 2015. In an *in vitro* viral RNA synthesis system, researchers demonstrated that ANP32A and ANP32B proteins specifically support the complementary RNA (cRNA) binding to viral RNA (vRNA) step in viral replication, and a double knockdown of ANP32A and ANP32B can significantly reduce the replication of influenza virus (Sugiyama et al. 2015).

Subsequently, several studies revealed that chANP32A has specific support for avian IAV RdRp, which was not supported by human ANP32A or ANP32B, posing a restriction for IAV interspecies transmission (Long et al. 2016; Domingues and Hale 2017; Baker et al. 2018). In 2019, researchers determined that ANP32A/B are decisive factors involved in IAV replication. In huANP32A and ANP32B double knockout cells (DKO), huANP32A and B have similar functions in supporting human influenza virus RNA replication, and huANP32A/B proteins are molecular basis for the normal function of influenza virus RdRp in host cells. HuANP32A/B are therefore necessary host factors for IAV replication and have a decisive role in supporting the polymerase activity of influenza viruses in different species, including humans, horses, pigs and dogs (Zhang et al. 2019; Staller et al. 2019). In addition, huANP32A/B proteins are required to support the polymerase activity of IBV and ICV (Staller et al. 2019; Zhang et al. 2020b; Carrique et al. 2020).

The molecular mechanism by which huANP32A/B supports influenza virus replication has long been the focus of research. The interaction between ANP32A/B and RdRp has been found to directly affect its activity in supporting influenza virus polymerase. It is possible that ANP32A and ANP32B proteins bind to viral RdRp in an RNA-independent manner through modification of the PB2-PA dimer structure, and this interaction could be significantly enhanced by the presence of vRNA or cRNA (Baker et al. 2018). The interaction between ANP32 protein and RdRp in nucleus was demonstrated with fluorescence complementation and IP technology, and it has been verified that the deletion of these two proteins resulted in defects in cRNP to vRNP replication. Moreover, LCAR domain of huANP32A/B is believed to be essential for effective binding to influenza virus RdRp (Zhang et al. 2019; Staller et al. 2019; Mistry et al. 2020).

Sites 129/130 (located at the LRRCT domain) of huANP32A/B are critical for viral RNA replication by mediating the interaction between ANP32 protein and RdRp (Mistry et al. 2020; Staller et al. 2019; Zhang et al. 2019). Taking advantage of this property, researchers found that a single nucleotide variant (SNV) in huANP32B can lead to a D130A substitution, which affects the production of viral RdRp dimers through a dominant negative effect (Staller et al. 2021). At the same time, mutationS of Asp149 (D149) and Asp152 (D152) of huANP32A also reduce its interaction with RdRp and affects its regulation of polymerase activity (Park et al. 2021). Additionally, KPNA6, an important host factor involved in the interaction of ANP32A/B with RdRp, plays an important role in maintaining viral polymerase activity (Yu et al. 2022).

However, avian ANP32B has naturally lost its support for viral RNA replication due to the mutation of amino acids 129/130 at the key binding site. Only avian ANP32A can effectively support influenza virus polymerase activity, which makes avian ANP32A protein a potential target for anti-influenza drugs (Zhang et al. 2019; Long et al. 2019a; Baker and Mehle 2019). HuANP32C, ANP32D and ANP32E have inhibitory effects on the replication of influenza A viral RNA (Park et al. 2020).

HuANP32A/B strongly supports IBV replication compared to huANP32E; chANP32A has weak support for IBV polymerase activity compared to huANP32A due to an insertion of 33 specific amino acids. The lower activity of chANP32B/E compared to huANP32B/E is due to their specific amino acid substitutions at 129-130 (Zhang et al. 2020b). The roles of ANP32 family proteins in influenza virus replication are summarized in Fig. 3.

The role of ANP32 family proteins in the cross-species transmission of influenza virus and its role in driving virus evolution

The influenza virus proteins HA and RdRp are the main barriers to interspecies transmission of avian influenza virus to mammals (Long et al. 2019b; Cauldwell et al. 2014; Long et al. 2016). HA determines whether the influenza virus can enter host cells by binding to different types of sialic acid (SA) receptors in host cells. HA from human influenza viruses preferentially binds α2-6-linked SA, while avian influenza virus HA preferentially binds α2-3-linked SA (Rogers and Paulson 1983; Rogers et al. 1983).

Once the influenza virus enters the host cell, its efficient replication *via* polymerase activity is the key factor for its successful infection of the host. RdRp from avian influenza viruses can hardly replicate in mammalian cells, limiting the transmission of avian influenza viruses to mammals. However, there are many adaptive point mutations on the RdRp subunit of the avian influenza virus that allow the virus to escape the host restriction barrier. The most typical mutation is at position 627

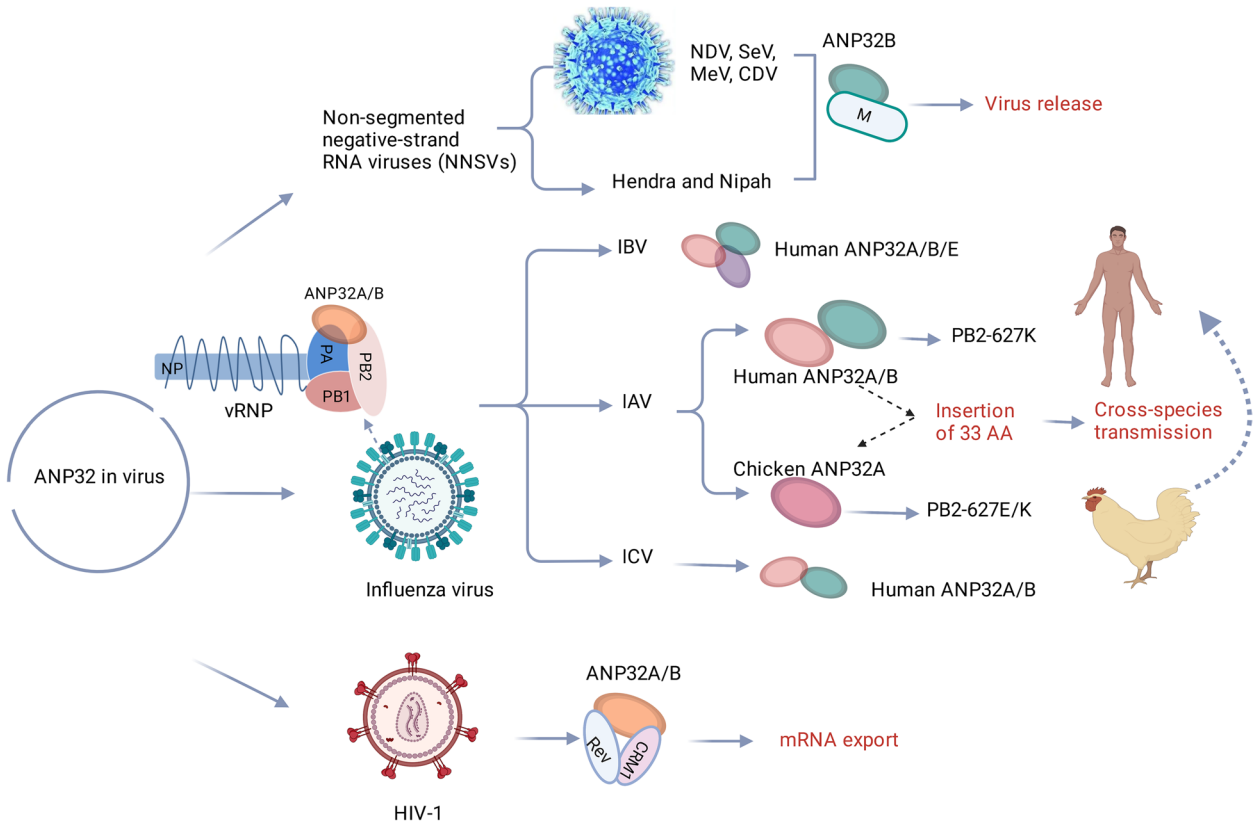

Fig. 3 The roles of ANP32 family proteins in different viral life cycles. Newcastle disease virus (NDV), Sendai virus (SeV), measles (MeV) and canine distemper virus (CDV), Viral ribonucleoprotein complex (vRNP), Polymerase basic protein 1 (PB1), Polymerase basic protein 2 (PB2), and Polymerase acidic protein (PA), nucleoprotein (NP), Influenza A virus (IAV), Influenza B virus (IBV), Influenza C virus (ICV), Human immunodeficiency virus-1 (HIV-1), Regulator of expression of virion protein (Rev), Chromosome region maintenance 1 (CRM1)

of avian influenza RdRp PB2, changing from glutamine (627E) to lysine (627K) (Long et al. 2019b; Cauldwell et al. 2014; Subbarao et al. 1993; Mehle and Doudna 2009). The structural and functional differences between avian and mammalian ANP32A proteins further reveal the molecular mechanism of the interspecies transmission of avian influenza virus.

HuANP32A protein is an important barrier to limit the transmission of avian influenza viruses to mammals, and the 33 amino acid insertion in avian ANP32A allows avian ANP32A to support avian influenza polymerase activity (Long et al. 2016), thus validating the presence of a positive correlation factor supported by PB2-627E RdRp in bird cells and a restriction in putatively restricted cells (Moncorge et al. 2010; Mehle and Doudna 2008). This further explains why both huANP32A and huANP32B homologs support the function of human-adapted influenza polymerases but do not support the efficient activity of avian IAV polymerases. Compared with huANP32, a specific SUMOylation domain in the 33 amino acid insertion of chANP32A has a significant effect on polymerase activity, and a functional SIM site (VLSLV) surrounded by acidic residues plays an important role in SIM-dependent SUMO interactions (Domingues and Hale 2017). SIM site mutation significantly reduces the ability of chANP32A to enhance 627E polymerase activity in mammalian cells and reduces the ability of chANP32A to bind to RdRp. However, whether chANP32A self-sumoylation is involved in the restriction of interspecies transmission remains to be elucidated.

Avian ANP32A can be differentially spliced, resulting in three different variants. Variant 1, chANP32A(33), is a splice variant containing a complete 33 amino acid insertion; variant 2, chANP32A(29), is a splice variant with a deletion of 4 amino acids at the SIM site; and variant 3, chANP32A(0/-33), is a splice variant similar to the huANP32A protein. The effects of these three variants on the activity of the PB2-627E polymerase are chANP32A(33) > chANP32A(29) > chANP32A(0/-33). This result also supports the effect of the 33 amino acid insertion of the dominant SIM motif on RdRp (Baker et al. 2018). Adaptation of IAV RdRp to the ANP32A

splice site was subsequently predicted by researchers, indicating that the ratio of different splicing variants may affect the adaptation of influenza virus cross-species transmission, providing a force driving the crossing of the species barrier from birds to mammals (Domingues et al. 2019).

In addition, other studies have also investigated the roles of swine and murine ANP32A in cross-species transmission. Compared with other mammalian ANP32A/B proteins, the swine ANP32A protein is able to specifically support the polymerase activity of avian influenza virus and thus promote virus replication. Swine ANP32A protein may therefore be an important host factor and could be responsible for pigs being influenza "mixers" (Zhang et al. 2020a; Peacock et al. 2020). Murine ANP32A protein hardly supports the polymerase activity of the influenza A and B viruses. However, compared with other species of ANP32B proteins, the murine ANP32B protein is better able to support the replication of influenza A and B viruses (Liang et al. 2019), and the promotion of IAV replication by ANP32B in mice may be related to its immunomodulatory function (Beck et al. 2020). But, the roles of ANP32 family proteins from other species in the cross-species transmission of influenza virus are only rarely reported.

Due to differences in the ways that IAV and IBV use ANP32 family proteins, this protein family is associated with species-specific limitations of influenza virus replication. The 33 amino acid insertion in chANP32A contributes to its strong support for avian and mammalian IAV replication, but this impairs its support for IBV replication. chANP32B is a naturally inactive molecule and cannot support either IAV or IBV polymerase activity. chANP32E does not support IAV RdRp function but has weak support for that of IBV due to its 129E mutation. This further explains why birds are rarely naturally infected with influenza B virus (Zhang et al. 2020b; Baker and Mehle 2019). ANP32 family proteins affect the cross-species transmission of influenza viruses, as shown in Fig. 3.

Molecular structural basis of the support of influenza virus replication by ANP32A protein

ANP32A/B proteins play a role in supporting influenza virus polymerase activity by tightly binding to the RdRp triple subunit complex. Although ANP32A/B cannot bind to the RdRp single subunit, it can bind to the PB2-627 domain alone (Baker et al. 2018) or can directly interact with PB2 through Glu189 and Glu196 of ANP32A and promote viral RNA synthesis (Wei et al. 2019). The PB2-627 domain (aa 535-667 of PB2) is key for the cross-species transmission of avian influenza (Carrique et al. 2020; Nilsson et al. 2017). Therefore, exploration of the molecular basis of structures of ANP32A/B and PB2-627 and analysis of structures of ANP32A/B and influenza virus RdRp are crucial to reveal the molecular mechanisms underlying viral replication.

Structural analysis was used to compare the stability of the complex formed by chANP32A and chicken-adapted 627-NLS (627-NLS(E)) with huANP32A and human-adapted 627-NLS (627-NLS(K)) and it revealed the structure of avian-derived RdRp. Avian RdRp cannot function in human cells without the E627K mutation (Camacho-Zarco et al. 2020). At the same time, researchers used cryo-electron microscopy (cryo-EM) to observe the structure of ICV RdRp complexes with huANP32A and chANP32A. In these two structures, ICV RdRp was seen to form asymmetric dimers, with the N-terminal LRRs of ANP32A bridging the dimer formation. The C-terminal LCAR of ANP32A was inserted between the two PB2 627 domains of the asymmetric IAV RdRp dimer, suggesting a putative mechanism by which the PB2 (E627K) mutation can enable efficient replication of avian viral RNAs in mammalian hosts (Carrique et al. 2020). Structural analysis showed that the strong binding of ANP32/B to RdRp is the basis for its regulation of influenza virus replication, but to date, whether ANP32 is packaged into virions through this tight interaction with RdRp remains unclear.

The role of ANP32 in the replication of other viruses

ANP32 family proteins also play important roles in the life cycles of other viruses. Most nonsegmented negative-strand RNA viruses (NNSVs) replicate in cytoplasm, but NNSV proteins typically function in nucleus. ANP32B functions as M protein of the Hendra and Nipah viruses targeting nucleus. It accelerates viral release by directly interacting with M protein to promote viral replication (Bauer et al. 2014). In addition, M proteins of Newcastle disease virus (NDV, an avian influenza virus), Sendai virus (SeV, a respiratory virus), measles (MeV, a measles virus) and canine distemper virus (CDV, a measles virus) were able to interact with ANP32B (Gunther et al. 2020). However, the molecular mechanism of ANP32B involvement in NNSV replication has not been elucidated and needs further study. Moreover, ANP32A and ANP32B mediate the unspliced or partially spliced export of HIV-1 virus mRNA through interactions with Rev and CRM1 (Wang et al. 2019) (Fig. 3).

Discussion and future directions

In this paper, we summarized the functions of ANP32 family proteins in cellular physiological metabolism, including chromatin remodeling, apoptosis, neurodevelopment and tumorogenesis. Studies demonstrated that ANP32 family proteins can have dissimilar or even contradictory roles in different situations. For example, ANP32A is a tumor suppressor gene in some cancers and a pro-oncogene in others. Many cancers are important problems in human life and health, but the roles of ANP32 family proteins in these cancers have not been fully elucidated. Further study of the roles and specific mechanisms of ANP32 family members in the occurrence of human cancers is necessary to help prevent cancer and provide potential therapeutic targets.

To date, ANP32 family proteins, or at least the main members ANP32A, ANP32B and ANP32E, have been confirmed to play an important role in RNA virus replication. ANP32A and ANP32B are decisive in supporting the replication of influenza viruses (Zhang et al. 2019; Staller et al. 2019), while for NNSVs, only ANP32B plays a role in promoting virus release by interacting with viral matrix proteins (Bauer et al. 2014). Considering that the members of ANP32 protein family have similar structures formed by the same motifs, the mechanisms by which ANP32 family members are hijacked and utilized by different viruses and the different roles of ANP32 family members in viral replication need to be clarified in detail. Moreover, ANP32 family proteins are vital for the replication of influenza viruses, and further clarification is needed to confirm whether they can be packaged into virions as members of the backpack to help virus replicate quickly in the early stages of infection or under adverse conditions to facilitate its adaptation.

The genome replication of most RNA viruses take place in the cell cytoplasm with the exception of influenza virus and retrovirus, which replicate their genome in nucleus. It is very interesting that ANP32A/B, as components of the nuclear transport complex in the CRM1-dependent pathway, have been confirmed to promote the replication of HIV-1 by mediating the export of unspliced or partially spliced viral mRNA (Wang et al. 2019). It is likely that ANP32 proteins play multiple roles in nucleus. DNA viruses replicate their genomes in nucleus, however, there is no evidence so far for the connection between ANP32 proteins and DNA virus replication. Further study is needed to confirm whether ANP32 family proteins play a role in DNA viruses, which may help us to understand the mechanism of DNA virus replication better and to provide therapeutic targets.

Posttranslational modification (PTM) of proteins is crucial for their biological function. ANP32A has protein phosphatase 2A (pp2A) inhibitory activity (Li et al. 1996). It was demonstrated that ANP32 proteins can be phosphorylated by casein kinase II (Hong et al. 2004) and that the phosphorylation of ANP32 proteins plays an important role in the apoptosis process (Adegbola and Pasternack 2005). At the same time, ANP32 was identified as a subunit of inhibitor of acetyltransferase (INHAT), which binds to histones and inhibits histone acetyl transfer through interactions with several transcriptional coactivators, including p300/CBP and PCAF (Seo et al. 2001, 2002). However, to date, there has been no report on the acetylation of ANP32 or on viral proteins acetylated by ANP32. It has been demonstrated that a SUMOylation domain located in chANP32A plays an important role in supporting the viral replication of avian influenza viruses (Domingues and Hale 2017), but it is unknown whether the SUMOylation of ANP32A or viral proteins is involved in this process. Therefore, further attention should be given to PTMs, such as phosphorylation, acetylation, or ubiquitylation of ANP32 proteins and their interacting viral proteins to confirm the influence of PTMs on the function of ANP32 proteins and their interactions with viral proteins. This field is an attractive direction for future research.

Authors' contributions

All authors reviewed and approved the final version of this manuscript. The authors contributed to the conception (XJW, MMY, HLZ), manuscript writing (MMY, HLZ, YXQ), and manuscript revision (XJW).

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Competing interests

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