Cracking the ANP32 whips: Important functions, unequal requirement, and hints at disease implications

Patrick T. Reilly1), Yun Yu2), Ali Hamiche3) and Lishun Wang2)

The acidic (leucine-rich) nuclear phosphoprotein 32 kDa (ANP32) family is composed of small, evolutionarily conserved proteins characterized by an N-terminal leucine-rich repeat domain and a C-terminal low-complexity acidic region. The mammalian family members (ANP32A, ANP32B, and ANP32E) are ascribed physiologically diverse functions including chromatin modification and remodelling, apoptotic caspase modulation, protein phosphatase inhibition, as well as regulation of intracellular transport. In addition to reviewing the widespread literature on the topic, we present a concept of the ANP32s as having a whip-like structure. We also present hypotheses that ANP32C and other intronless sequences should not currently be considered bona fide family members, that their disparate necessity in development may be due to compensatory mechanisms, that their contrasting roles in cancer are likely context-dependent, along with an underlying hypothesis that ANP32s represent an important node of physiological regulation by virtue of their diverse biochemical activities.

Keywords:
- caspase regulation; chromatin regulation; intracellular transport; leucine-rich repeats; low-complexity acidic region; phosphatase inhibition

Introduction

Cell physiology is normally viewed as a number of discrete functional assignments with limited overlap. Cell proliferation, transcription, intracellular transport, and execution of apoptosis are generally studied separately. With the advent of systems biology, scientists are finding interplay between seemingly disparate pathways that are far more significant than originally expected. Therefore, recognition of regulatory nodes that can influence a range of cellular activities will be increasingly important for a holistic understanding of the cell.

As detailed below, there are reasons to believe that the acidic (leucine-rich) nuclear phosphoprotein 32 kDa (ANP32) family of proteins represent one of these critical regulatory nodes, having impacts on transcriptional regulation, protein phosphorylation, intracellular transport, and cell-death pathways. In this review, we aim to bring context to the expanding base of literature on the ANP32 family, a topic that can quickly become complicated given the diverse activities ascribed to the family, the varied gene nomenclature, the existence of annotated intronless sequences of questionable relevance, and indeterminate functional overlap between the family members.

A highly conserved structure of ANP32 proteins through evolution

Although the first cloning of an ANP32 family member was in 1994 [1], the extent of identifiable ANP32 family of proteins was first examined in 2005 [2]. These proteins mostly range in size from 220 to 290 amino acid residues in length and are characterized by an N-terminal leucine-rich repeat (LRR) domain [3] composed of four LRR motifs and a C-terminal low-complexity acidic region (LCAR) of approximately 100 amino acids.

Protein sequences resembling the ANP32 family, i.e. proteins containing both LRR and LCAR sequences, have been annotated in the animal and plant kingdom as well as in protists [2], but not in yeast or other fungi. This observation
suggests an early eukaryotic origin of the gene(s) for ANP32 and their loss specifically in fungi. The existence of the LRR and LCAR region on all known ANP32 family members, from the protist Plasmodium to Drosophila to the three vertebrate family members, suggests that both regions play critical roles for survival. Unfortunately, functional conservation in eukaryotes is unclear because, thus far, only animal ANP32 proteins have been studied: specifically, only the Drosophila member, termed Mapmodulin, and the mammalian family members.

The ANP32 structure gets whipped into shape

The structure of the LRR domain has been solved for family members ANP32A and ANP32B in three separate studies [4–6]. The resulting models wherein the LRR motifs generate canonical parallel beta-sheet structures are very similar in the different studies [6] and consistent with those proposed earlier [2], suggesting high confidence in the consensus model. A number of protein-protein interactions have been mapped to the LRR of the ANP32 members, including CRM1 [7], PP2Ac [8], Ataxin-1 [9], Histone H3-H4 [6], and Clip170 [4].

Although other LCAR-containing proteins are recognizable and the class was described as early as 1986 [10, 11], ANP32s possess the longest LCARs that we have identified so far. The vertebrate ANP32 LCARs are approximately 100 amino acids long and are composed of 60–75% either glutamatic or aspartic acid. Distantly related species have less acidic content in their ANP32 LCAR (36% for Caenorhabditis elegans to 51% in Plasmodium) but are still recognizable as acidic. Unfortunately, these regions are difficult to categorize precisely. Sequence homology searches do not function effectively, because of the low complexity of the proteins and the fact that such methods mask low-complexity regions [12]. Furthermore, the paucity of hydrophobic residues likely prevents tertiary structure in this region, allowing it to remain flexible in solution with a capacity for physical interaction with any positively charged surface. Conceptually, the ANP32 protein structure likely resembles a whip, where the LRR domain constitutes the handle and the LCAR is the thong. Due to the very high anionic content of the ANP32 LCARs, we expect that some ascribed functions will ultimately be viewed as artefacts resulting from non-specific cation binding.

As the name “ANP32” suggests, phosphorylation events on these proteins further increase their acidity. Upon the cloning of rat ANP32A, the researchers recognized a number of potential sites of phosphorylation by Casein kinase II (CKII), Calcium-Calmodulin dependent kinase II, and protein kinase C [13]. Phosphorylation by CKII has been verified [14]. These and/or other phosphorylation events are predicted to affect protein function [15, 16].

Based on the absence of oligomerization domains and the results of overexpression experiments on the founding member ANP32A, these proteins likely exist as monomers [17], although ANP32A isolated from Chinese hamster ovary cells is reported to form a homotrimer through its LRR domain [16]. No model currently exists to reconcile these potentially discrepant observations.

SET and other LCAR-containing proteins are related to ANP32s

Intriguingly, several other prominent LCAR-containing factors are multifunctional, including the cellular proteins Nucleophosmin, Nucleoplasm, high mobility group box (HMGB1, HMGB2, and SET, as well as the human herpesvirus 8 protein LANA. It is notable that each of these factors has been implicated in regulating chromatin structure in some way, suggesting a primary activity of the LCAR. Among the other multifunctional LCAR-containing proteins, the SET oncoprotein (a.k.a. TAF1β, I2PP2A, PHAPII) is of particular interest for ANP32 biology. Firstly, the ANP32 proteins and SET have been found in different biochemical isolations with shared characteristics [1, 18, 19] in spite of structural differences in their N-termini. In contrast to the LRR motifs in the N-termini of ANP32s, SET contains a nucleosome-assembly-protein domain. Secondly, SET and ANP32 proteins have been isolated together in higher order complexes [7, 20, 21]. This physical interaction and functional overlap suggest that SET and ANP32 proteins coevolved in particular regulatory pathway(s). Finally, a cell-permeable inhibitor of SET, likely targeting its LCAR [22–24], is being investigated as a cytotoxic anti-cancer agent [25, 26]. Simultaneous inhibition of ANP32 proteins by this candidate drug has not been excluded.

The intronless ANP32 loci: Expressed and functional?

Controversy exists about the extent of the ANP32 family in mammals. A previous article has suggested eight different ANP32 family members in humans [2], of which six different loci are currently annotated in Genbank. Three conserved family members exist in vertebrates, namely ANP32A (a.k.a. PHAPI, pp32, I1PP2A, LANP, HPPCn, Mapmodulin), ANP32B (a.k.a. SSP29, APRIL, PAL31, PHAPII), and ANP32E (a.k.a. CPDI, LANP-L, PHAPIII). These have all been isolated in multiple biochemical fractionations [7, 19, 27–29] and are well represented in transcriptomic and proteomic surveys. The controversy surrounds the nature of intronless loci, ANP32C, and ANP32D. Researchers alternately characterize these sequences as retrogenes – products of retrotransposition that support protein coding – as functional transcribed pseudogenes, or as inert pseudogenes.

Here we examine the evidence for ANP32C, also known as pp32r1, which is the most frequently described of these intronless sequences in the literature [30–35]. This open-reading frame has physiological effects when ectopically expressed [17, 30–33] but doubts surround its endogenous expression. On a genomic level, ANP32C is not conserved from rodent to human. Mouse and human sequences of ANP32C, present in introns of Ranbp17 and MARCH1, respectively, are more homologous with ANP32A sequences of their own species than with each other [2] suggesting separate and relatively recent origins. This would not preclude functional
s significance, but it indicates that these should be treated as non-orthologous sequences.

At the transcript level, ANP32C is reportedly expressed in a variety of cancers and cell lines [33–35]. Unfortunately, the intronless nature makes targeted reverse transcription-PCR very susceptible to DNA contamination and these reports of ANP32C expression do not overtly show the reverse transcriptase controls to assess this potential contamination. A significant tool for examining the human transcriptome is expressed sequence tag (EST) analysis, which has extensive coverage of neoplasias in which ANP32C expression is reported. ANP32C is currently represented by a total of four ESTs compared to 752 for ANP32A, 720 for ANP32B, and 490 for ANP32E. The EST count for ANP32C is also low compared to the functional transcribed pseudogene PTENP1, which is represented by 99 ESTs. With its presence within the MARCH1 gene, the ANP32C locus is almost certainly transcribed, at least as part of the intron, but the stability and functionality of the resulting RNA remains an outstanding question.

The potential translation of ANP32C is also unclear. While its open-reading frame contains a Kozak translational initiation sequence, intact endogenous ANP32C protein has not yet been reported in biochemical isolations. Furthermore each of the peptides annotated for ANP32C in the Human Proteomic Project database portal, “PeptideAtlas” [36], has 100% identity with sequences in ANP32A including one peptide inappropriately described as unique to ANP32C. In contrast, a commercial antiserum was recently used to show ANP32C protein expression [32], suggesting that it is a retrogene. RNA inhibition (RNAi) validation of this antiserum will be essential to determine endogenous expression going forward.

Given what we feel is the lack of solid unbiased evidence for transcription and translation of ANP32C as well as other purported family members, we believe that only ANP32A, ANP32B, and ANP32E should be considered bona fide mammalian ANP32 family members at this time. While there may be means of parental gene regulation associated with these loci, we limit ourselves to the three unquestioned protein-coding ANP32 family members for the purpose of the functional review.

ANP32s: Here, there, and everywhere

The literature describes a startlingly diverse array of biochemical activities for the ANP32 family. Since cellular localization would preclude certain activities, there has been significant attention placed on determining where the ANP32 proteins are located. Unfortunately, not even the localization of these factors is generally accepted, because different reports conclude, variously, that ANP32 proteins are predominantly nuclear [17, 37–39], shuttling nuclear- cytoplasmic [7, 15, 40, 41], predominantly cytoplasmic [42–44], on the cell surface [45], or even secreted [46]. In the case of ANP32B, a phosphorylation event on Thr244 appears to regulate its nuclear export, because a phospho-site-deficient protein localized exclusively to the nucleus [15]. For ANP32A, induction of reactive oxygen species causes a cytoplasmic-to-nuclear translocation [42]. In contrast, a nuclear to cytoplasmic translocalization of ANP32A has been seen in the process of in vitro neuronal differentiation [47]; however, this effect was not seen in brain tissue [48] or cultured primary neurons [38]. A separate study suggests that apoptotic stimuli can induce translocation to cytoplasm [17], although this may be due to nuclear envelope breakdown.

Whereas some studies are more compelling than others, it is very plausible that these disparate findings with respect to localization reflect different model systems and reagents used. It is clear from these studies that no particular activity of the ANP32 proteins may be excluded based on protein localization.

ANP32 proteins regulate chromatin by various means

From the earliest classification of LCAR-containing proteins, it was evident that they are involved in regulating transcription and chromatin architecture [11]. The reported activities of ANP32 proteins in chromatin regulation are diagrammed in Fig. 1. The ANP32 proteins were first noted to function in transcriptional repression upon purification of ANP32A, a member of the inhibitor of histone acetyltransferase (INHAT) complex [21]. Further studies revealed that ANP32A blocks histone modification by binding to histone tails and sterically inhibiting acetylation. More specifically, ANP32A preferentially binds to unmodified histone H3 tails [49, 50]. After this discovery, a number of groups suggested that ANP32A likely modifies activated transcription due to its recruitment to promoters by DNA-binding transcription factors (TFs) [51–54]. In one study, ANP32A also facilitated estrogen receptor loading onto DNA [54]. ANP32B has also been found to bind TFs and modulate their activity [55]. In this study, Krüppel-like factor 5 (KLF5) binds to ANP32B and is recruited to specific regions of DNA to repress transcription in a promoter-specific manner. In addition to binding histones, recombinant ANP32B demonstrated plasmid-superoiling activity indicative of histone chaperone activity [55]. Further studies mapped a physical interaction between ANP32B LRR region and core histones H3-H4 [6]. ANP32E has recently also been shown to have histone chaperone activity. In this case, ANP32E is associated with the p400/Tip60 complex and specifically removes histone H2A.Z from DNA [29, 56]. Since H2A.Z is associated with transcriptional regulation based on its preferential localization to transcriptional start sites [57, 58] and its role in determining transcriptional responsiveness [59], recruitment of ANP32E can likely alter activated gene transcription. Although this is yet to be shown, deletion of ANP32E in mouse cells changes the profile of H2A.Z occupancy [29]. H2A.Z chaperone activity seems to be specific for ANP32E since the H2A.Z interaction maps to a discrete conserved sequence within the LCAR of ANP32E that is not found in ANP32A or ANP32B [29, 56]. Intriguingly, however, the Drosophila Protein Interaction Map identified variant H2A (Dmel(His2Av), the precursor of mammalian H2A.Z, as a Mapmodulin-interacting factor [60]. This suggests a conserved role for ANP32 proteins in regulating H2A.Z placement.
The revelation that ANP32 proteins have histone binding and chaperone activity is another example of shared functions with its binding partner SET. SET, also present in the INHAT complex, independently shows H3 tail binding [49] as well as histone chaperone activity [55, 61]. These findings suggest that ANP32A recruitment to promoters may likewise provide histone chaperone activity as a means of transcriptional repression.

ANP32 proteins as regulators of cell death pathways

Thus far, only a single biochemical study has isolated all three of the mammalian ANP32 family members [27]. In this work, the focus was their capacity to aid activation of the apoptosome, the initiator caspase complex containing procaspase 9, APAF-1, and cytochrome c [27]. This and a subsequent study [62] found that ANP32 proteins allowed apoptosome activation at physiological levels of dATP. This finding is now supported by several different studies [17, 63–66], while one study showed that the ANP32A LCAR also promotes caspase-3 activation directly [63]. Two different groups have posited that ANP32A is part of a regulated positive feedback mechanism in caspase activation, whereby...
complexed ANP32A is sequestered from caspases until the appropriate cell death stimulus is applied [67, 68]. In one model, Granzyme A stimulates release of ANP32A concurrent with release of the Granzyme A-activated DNase, NM23H1 [69], hence suggesting that it may have a role in caspase-independent cell death. Strangely, however, the different groups provide evidence for ANP32A sequestration in different complexes (Fig. 2). We are sceptical of this model since pro-survival sequestration would imply that no free pool of ANP32A would exist to perform transcriptional control or other noted activities.

In contrast, although ANP32B could activate the apoptosome [27], its depletion by RNAi in cells induces high rates of apoptosis [70–72]. This pro-survival activity correlates with ANP32B’s reported inhibition of caspase 3 [72], the converse of the activity reported for ANP32A. Since we also found that ANP32B is a caspase substrate [71], we propose that ANP32B may be part of a separate positive feedback network for effector-caspase activation (Fig. 2).

**ANP32s control phosphatase activity**

ANP32A was isolated together with SET as an inhibitor of protein phosphatase 2A (PP2A) [19, 73], and each has been reported to bind to protein phosphatase 1 (PP1) independently to affect activity [74]. One study has found that ANP32A binding to the signalling lipid molecule, sphingosine, abrogates its PP2A interaction, suggesting a physiological regulation of this activity [75]. Although a shared activity with SET would imply involvement of the LCAR, the interaction between ANP32A and PP2A requires the LRR region [8], which is not present on SET. Functionally, the interaction with PP2A is suggested to impact cell proliferation [76], inflammation [75], and neurodegenerative disorders [8, 77, 78], although these potential consequences invite further in vivo study. Intriguingly, in separate studies ANP32E was able to inhibit PP2A [79], whereas ANP32B was not [72], suggesting either a functional divergence or, more likely, differences in in vitro assay conditions.

**ANP32 proteins mediate intracellular transport**

Regulating transport within the cell is another activity that ANP32 proteins are reported to possess. As adaptors between the nuclear-export factor CRM1 and the mRNA-binding protein HuR, ANP32A, and ANP32B have been implicated in...
expediting transport of mRNA strands containing adenosine-rich elements [7]. This ANP32 function has been suggested as a means of control of both cellular [7, 15] and viral mRNAs [80–82].

In addition to mRNA shuttling, the ANP32 proteins are reported to regulate transport of factors within the cytoplasm. This conclusion comes from the initial characterization of the ANP32A in Chinese hamster ovary cells as a microtubule-associated protein (MAP) interacting factor [16, 44, 83]. Following on this finding, an ANP32A-MAP1B interaction was demonstrated in mammals and shown to impact neuritogenesis [47].

**ANP32A: Moonlighting outside the cell**

In addition to the role of the ANP32 proteins inside the cell, two groups have suggested a role for ANP32A in the extracellular space. A study in 1998 suggested that ANP32A could be present on the surface of intact peripheral blood mononucleocytes, potentially acting as an HIV receptor by virtue of its ability to bind an HIV gp120 mimic peptide [45].

In a series of papers since 2008, ANP32A has also been reported to act as a growth factor for hepatocytes [41, 46, 84–86] both in vivo and in vitro. These studies demonstrate that ANP32A is increased in hepatocytes in order to reduce cellular oxidative stress in response to both carbon tetrachloride and ethanol. Since provision of recombinant ANP32A in media in vitro and intravenously in vivo is sufficient to provide this protective activity [84], the activity is presumed to be extracellular. Although a pathway involving sphingosine kinase has been implicated [46, 84, 86], the hepatocyte receptor for ANP32A has yet to be identified.

Taken together, this unusual array of activities suggests that the ANP32 proteins may constitute a critical regulatory node in cell physiology that should affect viability and normal development. Differential affinities, expression patterns, or controlled complex formation may allow dynamic functions of the ANP32s across time, allowing them to change roles between regulating gene expression, cell signalling, and cell death, depending on changes in cell physiology.

**Unequal requirement for ANP32 proteins in development**

Gene expression analyses of ANP32 family members suggest that they are upregulated in proliferating tissues and downregulated in the process of terminal differentiation. This appears to be true for each of the mammalian family members to differing degrees. Indeed, both ANP32A and ANP32B were initially cloned based on their abundance in proliferating tissues [28, 39], while ANP32E was cloned from the most postnatal cerebellum [79], when tissue is most proliferative. Furthermore, ANP32A and ANP32B expression are associated with proliferative neoangiogenesis [87] and macrophage activation [70], respectively. In addition to licensing proliferation, ANP32 expression may enforce self-renewal capacity as has been hypothesized for a number of systems [88–92].

In vitro models also suggest that ANP32A can modulate neuritogenesis either positively [47, 78] or negatively [90, 93]. To determine their role in development, ANP32 genes have been examined through animal loss-of-function models. Whereas their conservation and reported functions would suggest catastrophic consequences, the resulting phenotypes have so far been quite varied. For two Mapmodulin loss-of-function models, one RNAi and one P-element insertion, the flies are viable in spite of Mapmodulin being the only known Drosophila ANP32 family member [94, 95]. On the other hand, in C. elegans ANP32 loss of function, i.e. RNAi of T19H12.2 is embryonic lethal [96]. No loss-of-function analysis is reported for the other C. elegans ANP32 family member, F33H2.3.

Five mutant-mouse strains carrying Anp32 loss-of-function alleles have so far been generated. Surprisingly, mice deficient in the most prominent family member, Anp32a, demonstrated no apparent phenotype in two separate studies [97, 98]. Likewise, two alleles of Anp32e are published [98, 99] and, apart from a disputed, subtle, neurological phenotype in the gene-trapped mutant [99, 100], the mice were apparently normal. On the other hand, the single targeted allele of Anp32b had a severe, albeit complex, phenotype. Anp32b-deficient mice demonstrated a strain-dependent penetrance of perinatal lethality with surviving mice in a genetically mixed background showing growth defects, premature aging, and a wide array of pathologies [101]. These mice also demonstrate a role for Anp32a in mouse development that only becomes apparent in the context of Anp32b deficiency [101], strongly suggesting a functional overlap. The same genetic interaction could not be established between Anp32b and Anp32e [101]. Intriguingly, the requirement of a particular Anp32 mouse gene seems inversely related to the size of its 3’ untranslated region (UTR). We speculate that 3’UTR regulation may affect the efficiency of compensation by alternate ANP32s. In this model, Anp32b is the most important by virtue of regulatory or context-dependent impediments to Anp32a and Anp32e translation. The physiological contexts in which Anp32a and/or Anp32e play an important role still await discovery. Certainly their conservation among vertebrates implies such importance.

**ANP32 proteins in human pathogenesis**

Whereas the functions ascribed to this protein family would lead one to believe that ANP32s are critically important, no human pathogenic mutations have yet been identified. ANP32A’s interaction with [9] and regulation by [78] mutated, pathogenic Ataxin-1 protein as well as its ability to regulate phosphorylation of the Alzheimer’s disease-related protein tau [8] suggest a possible activity in neurodegenerative disorders. In contrast, the functional significance of the physical interaction with ATXN-1 has not been addressed [78], the ANP32A-deficient mice have not demonstrated aberrant behaviour ([97], Reilly unpublished), and a polymorphism analysis suggests that ANP32A is not genetically associated with Alzheimer’s disease [102].

Studies in mice suggest that most mutations would be tolerated during developmental processes, but the
evolutionary sequence conservation suggests that this is not likely the case. It is a curious paradox that may be related to the controlled conditions of laboratory animals. Indeed, there is reason to believe that ANP32 proteins function in the life cycles of a wide variety of viruses, including DNA viruses adenovirus [82, 103] and adeno-associated virus [104] as well as retroviruses, Foamy virus [81], Nipah virus [80], and HIV [105]. Challenge with such pathogens is typically excluded from laboratory animal colonies.

Contrasting data on the role of ANP32s in cancer

The most prominent association between ANP32s and human pathology is in the case of cancer. Firstly, the activities described above can each be related to cancer. Cell death control, regulated protein phosphatase activity, and epigenetic gene regulation each have a clear and demonstrated role in cancer progression. Indeed, many of the factors with which ANP32s physically interact are established players in human cancer including SET, KLF5, pRB [38, 106], NM23H1 [20], and Axin-1 [107]. Potential regulation of any of these factors would imply that ANP32s are important regulators of tumorigenesis.

Secondly, and consistent with this hypothesis, significant direct genetic and epigenetic data exist to suggest that ANP32s are dysregulated in an array of cancers. The results, however, do not identify ANP32s as consistently tumor promoting or tumor suppressive, thus suggesting context dependency. ANP32A is regularly referenced as a tumor suppressor based on early studies that showed it could inhibit cell transformation [17, 108–110] as well as its apparent reduced expression in prostate [33] and breast cancer [34]. Additionally, ANP32A was also shown to be a positive prognostic marker in non-small-cell lung cancer [65], and reducing its expression increased ras-induced tumorigenicity of NIH3T3 cells [108]. These data contrast with other studies that show expression of ANP32A is increased in cancers including prostate [37], colorectal [111], ovarian [112], and liver [113]. Furthermore, it is a negative prognostic marker in hepatocellular carcinoma [41], where reducing its expression also reduces xenograft growth [41]. ANP32E similarly shows enhanced expression in gastric cancer [114], and is a negative prognostic marker in myeloma [115]. However, it is also reported as a positive predictor of follicular lymphoma treatment response [116]. For ANP32B, results in breast cancer prognosis suggest that it is a tumor-promoting gene [101], whereas it is also ranked among the highest hits in a tumor-suppressor-rich genome-wide search for recessive cancer genes [117]. Certainly the different functions of the ANP32 proteins provide plenty of opportunity to rationalize the genetic and epigenetic evidence. Currently, however, there is no clear theme for ANP32 expression in cancer. Taking their proliferation-related expression together with their roles in activating caspase-mediated cell death, we hypothesize that these proteins are a “double-edged sword” in cancer progression. Within a genetic context of defective apoptotic pathways they may provide proliferative advantage by selective gene expression, whereas in cancer cells with intact apoptotic cell-death pathways their overexpression would drive tumor reduction. This paradigm is now increasingly evident in cancer genetics regarding other factors.
Conclusions and outlook

Figure 3 provides an overview of the homology, functions, and loss-of-function phenotypes of mammalian ANP32s. With the rationale presented, we contend that there are only three real ANP32 genes in vertebrates, namely ANP32A, ANP32B, and ANP32E. We believe that any intronless sequences should be considered pseudogenes until compelling evidence of expression is presented. We also contend that, with some exceptions, the listed functions are shared among the family members. The field still awaits a systematic examination to clarify which, if any, of these activities are exclusive for particular members. With the compelling evidence for the described functions, however, we propose that the ANP32 proteins likely act as important regulators in the cell, providing molecular crosstalk between gene-expression, cell-survival, and cell-signalling pathways. The severity of their loss, however, appears to be gene- and context-dependent. For example, although RNAi against ANP32B induces apoptosis in tissue culture, mice lacking this gene can grow to adulthood. Is the cell able to compensate for individual loss, particularly slow loss, with alternative ANP32 protein usage, as suggested by a theory [118] that redundant genes prevent developmental error? Regardless of the interchangeability of the ANP32 proteins, these factors are clearly providing a regulatory role in the cell, which we believe can be exploited for medical benefit in the future. LCAR regions are already proving potential targets for peptide inhibition. With clearer understanding of the genuine human ANP32 proteins, we can focus on the best strategies and circumstances to fine-tune these multifunctional factors for desired physiological outcome.

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