**Gene editing and effect of ANP32 proteins splicing variant for viral replication: A review**


## Abstract

Because of the expanding human population, we must produce more food while lessening the environmental impact of farming. Cattle productivity has been revolutionized by selective breeding and genomic selection. Currently, transgenic and genome-editing technologies have promising prospects for producing cattle that are healthier and more productive. The 220–291 amino acid proteins that make up the acidic leucine-rich nuclear phosphoprotein 32 kDa (ANP32) family have been evolutionarily conserved and are distinguished by a low-complexity acidic region (LCAR) at the C-terminus and an N-terminal leucine-rich repeat domain (LRR). Numerous physiological processes, such as chromatin remodeling, apoptosis, and nervous system development, are regulated by proteins belonging to the ANP32 family. Tumorigenesis and abnormal ANP32 expression are tightly associated. The ANP32 family proteins' ability to promote influenza virus replication and limit the spread of the virus across species has drawn a lot of attention to their role in viral infections in recent years. Additionally, the replication of non-segmented negative-strand RNA viruses (NNSVs) and HIV is intimately linked to ANP32 proteins. The broad physiological roles of the proteins of the ANP32 family and their involvement in virus replication are outlined in detail in this paper.



**Keywords:** ANP32, splicing variant, Influenza virus , viral replication


## Introduction

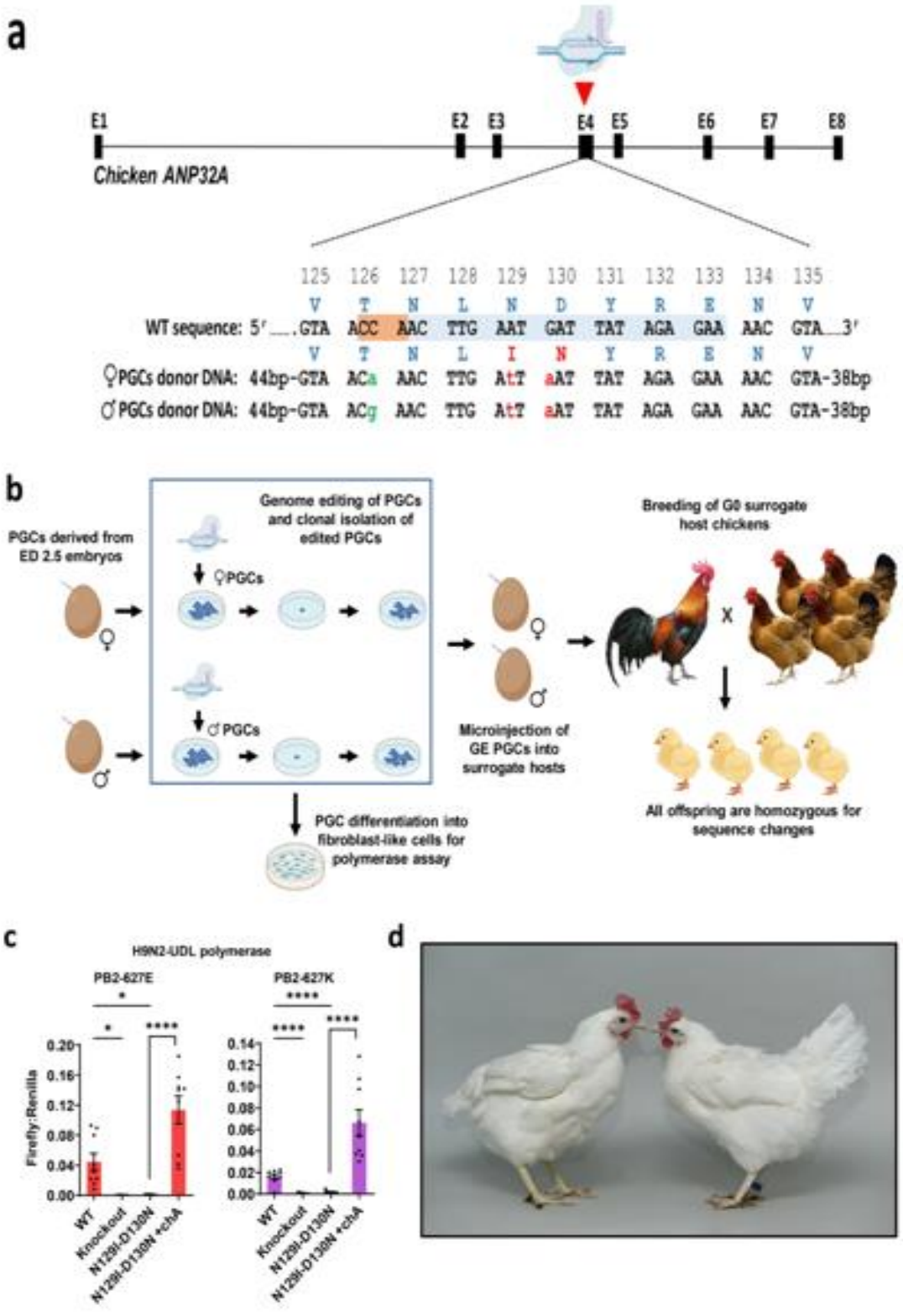
Recent developments in gene editing technology may make it possible to produce animals that are resistant to disease. This may slow the spread of bird flu, often known as avian influenza. My coworkers and I demonstrated the possibility of gene editing to shield hens against the dangers of avian flu in a recent study (Looi et al., 2018). The virus that causes this illness is constantly changing, and it evades several biosecurity precautions including proper sanitation, limiting bird movements, surveillance through appropriate testing, and the deliberate killing of diseased birds. A breakthrough in gene editing could stop the massive economic losses caused by avian flu outbreaks that occur now. It would also be a big step in managing a condition that can kill or seriously sicken people. Global avian flu outbreaks have resulted in losses up to billions of dollars. According to the US Department of Agriculture, bird flu could kill up to 50 million birds in 2022.

According to the South African Poultry Association, outbreaks discovered in the first half of 2023 resulted in the destruction of over 7 million hens (Cousins et al., 2022).

Beyond the financial costs, outbreaks of avian flu can be harmful to people's health. Bird flu was thought to be a potential cause of a deadly pandemic that might affect humans, prior to the COVID-19 pandemic. This triggered global surveillance headed by the Food and Agricultural Organization of the United Nations, the World Health Organization, and the World Organization for Animal Health. The anxiety is justified because birds were the source of all three of the 20th century's flu pandemics, which included the 1918 outbreak that killed tens of millions of people. One of the main strategies for stopping bird flu epidemics in hens is vaccination. However, due to the avian flu virus's fast evolution, vaccinations are only partially effective. Over time, this reduces the effectiveness of current vaccinations. Additionally, the avian flu virus has several strains, yet a vaccination only works against that particular type. A vaccination must be compatible with the dominant strain that is producing the outbreak. Utilizing vaccines may also come with significant expenses and real distribution challenges (Nielsen et al., 2023).

**Creating resistance to avian influenza infection through gene editing**

To create ANP32A-GE (ANP32A$^{N129I\text{-}D130N}$) cells and chicks, we used CRISPR/Cas9 and a short single-stranded oligonucleotide (ssODN) template to induce a three-nucleotide base-pair alteration in exon 4 of ANP32A (Fig. 1a), causing a two-amino-acid substitution. Targeting the locus in vitro produced male and female chicken primordial germ cells (PGCs), and then grew single PGCs to establish clonal GE cell lines (Fig. 1b and Supplementary). Sanger sequencing of clonal cells revealed cells with biallelic modifications. We anticipated CRISPR/Cas9 off-target locations and discovered no off-target mutations in chosen GE clonal lines studied.



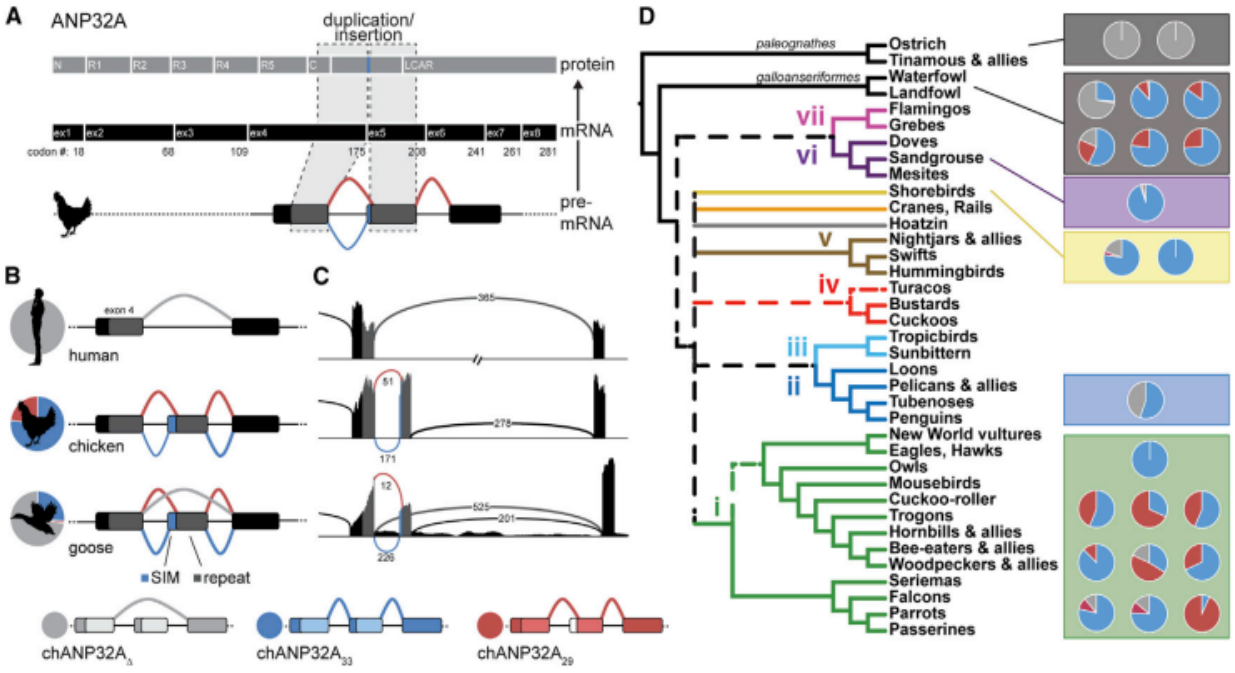

(Indoko et al., 2023)

Figure 1: Breeding plan for the homozygous ANP32A$^{N129I-D130N}$ chicken

ANP32A editing strategy:- Two nucleotide alterations (red letters) introduce missense mutations at asparagine (N) position 129 (N129I) and aspartic acid (D) position 130 (D130N). The third nucleotide alteration (green letters) is a synonymous mutation in the gRNA PAM that acts as a marker for allelic contribution from both male and female surrogate hosts. B. Male and female PGC cultures were created using the blood of individual chick embryos. The PGCs were altered, and clonal lines of GE PGCs were grown and studied. GE PGCs were transformed into fibroblast-like cells for IAV polymerase tests. To produce GE chicks, GE PGCs were combined with B/B dimerization chemical (to cause cell death in host embryo germ cells) and injected into iCaspase9 host embryos, which were then incubated until hatching. After hatching, the surrogate hosts were brought to sexual maturity and mated directly. All children from the surrogate hosts' eggs were biallelic for the edit and carried the parent-specific PAM nucleotide alteration. c, The activity of reconstituted IAV polymerase was measured in fibroblast-like cells generated from $ANP32A^{knockout}$ (Knockout), $ANP32A^{N129I\text{-}D130N}$ (N129I-D130N), and wild-type (WT) PGCs. Cells were transfected with avian IAV polymerase (PB2/627E - black bars) or human-adapted isoforms (PB2/627K - grey bars), Firefly minigenome reporter, and Renilla reporter control plasmids, and incubated at 37°C for 48 hours. Wild-type chicken ANP32A (chA) cDNA was co-expressed with minigenome plasmids to restore polymerase activity in ANP32AN129I-D130N cells. Firefly activity was normalized to Renilla and plotted as mean ± SEM across three different experiments (n = 3), each with three technical duplicates.

**ANP32A alternative splicing variant**

Differential splicing generates three predominant isoforms of avian ANP32A that have different effects on avian influenza polymerase activity. Because species-specific changes in ANP32A alter its function during infection, we investigated ANP32A expression in a variety of avian species. Most avian ANP32A encodes a partial duplication and insertion of exon 4, repeating a piece of the leucine-rich repeat capping motif in the produced protein.




(Baker *et al.,* 2018)

Figure 2: the splicing variants of ANP32A

In birds, three main splice versions are made when ANP32A exon 4 is copied and inserted. (A) A diagram showing how the chANP32A protein and exonic (ex) mRNA are organized, showing that some domains are repeated. C, N is the N-cap and R1–R5 are the leucine-rich regions 1–5. Exon numbering is based on chANP32A33. (B) There is no gene duplication (light gray) in human ANP32A transcripts. Schematics of chicken and goose transcripts illustrate splicing upstream to capture the SIM's coding sequence (blue), splicing downstream to exclude the SIM (red), and, in certain cases, bypassing the repeating exon to produce a mammalian-like transcript (light gray). The pie charts represent the relative abundance of each splice isoform in the RNA-seq data. SIM, SUMO-interacting motif. (C) Sashimi plots of ANP32A comparable to the instances in (B) are colored similarly. The abundance of each splice variant is shown on the lines that correspond to the intron-spanning reads. (D) ANP32A splicing patterns in various bird species placed on the Aves consensus phylogeny (dashed lines denote branches from two of three consensus trees).

**Gene editing to improve animal welfare**

Unlike immunizations, gene editing effectively halts the virus in its tracks by targeting a protein or proteins within birds that are essential for all forms of avian flu. The practice of precisely

altering a single gene in an animal to bring traits like enhanced productivity, illness resistance, and qualities that improve animal welfare is known as gene editing. An advantageous genetic alteration induced through gene editing in one species might already exist in a different animal naturally. For instance, a genetic alteration present in naturally hornless cattle was introduced into dairy cow using gene editing to render them hornless. This is significant because many dairy cattle have horns, which makes dehorning calves—a painful procedure that lowers the possibility of harm to both the animal and the farmer—necessary (Looi et al., 2018).

It's critical to distinguish genetic modification—which involves transferring a gene from one species to another—from gene editing. This distinction is required for regulatory purposes, particularly since the development of the earlier genetic modification technology has been hampered by strict rules in many nations. We made a single gene edit using the potent molecular scissors CRISPR/Cas9 in order to create the gene-edited hens in our work. In hens, we specifically targeted the ANP32A protein. These chickens with altered genes hatched at the same time as regular chickens, but they did not experience any noticeable negative effects on their health or general wellbeing until they reached adulthood. We gave the gene-edited hens a tiny amount of the avian flu virus to see if they would be resistant. Surprisingly, all 10 of these birds showed total resistance, and there was no spread to other hens (Mackelprang, R., and Lemaux, P. G. 2020).

We took a bolder approach and injected the gene-edited chickens with 1,000 times the low dose of the virus, a high and unusual dosage. Five of the ten gene-edited chickens who were vaccinated contracted the infection this time. Additionally, we discovered that the bird flu virus could modify itself to utilize the altered ANP32A protein along with two related proteins, ANP32B and ANP32E. However, we showed in cell tests that the virus could be totally suppressed by altering all three proteins at the same time. The goal of ongoing research is to determine the precise set of gene edits required to produce the next generation of hens that have been gene edited to offer total and irreversible protection against bird flu. One of the most important tools for stopping and managing fatal animal diseases is gene editing. Government laws that are supportive of the advancement of gene editing for the purpose of improving animal health and welfare will be necessary. The potential for disease-resistant animals to safeguard public health and global food security is a strong argument in favor of continuing along this novel biotechnological path (Sid, H., and Schusser, B. 2018).

**Genetic improvement impact on animal breeding**

Even though many domesticated animals have been raised for millennia, carefully planned selective breeding techniques have significantly increased output. Animal production is now faster, less expensive, healthier, and more effective thanks to genetic advancements, all while having a smaller negative environmental impact. Using selective breeding, for instance, between the 1960s and 2005, pig litter sizes increased by 50%, lean pork meat increased by 37%, and lean pork meat per kg of feed intake doubled; in chickens, the number of days required to gain 2 kg of mass decreased to 40, the percentage of breast meat increased from 12 to 20%, the feed conversion ratio decreased, the number of eggs per year increased by 30%, and the number of eggs per ton of feed increased by 80%; and lastly, in cattle, milk production increased by 67%. (Gratacap et al., 2019).

These revolutionary gains in food production, however disproportionately felt in affluent nations, are remarkable accomplishments in a matter of decades. Genomic selection is now commonly incorporated into pedigree-based breeding programs for key livestock and aquaculture species, which has revolutionized selective breeding and food production. Utilizing genome-wide genetic marker data, genomic prediction equations are used in genomics selection to determine an individual's genomic breeding values (GEBVs). This genomic prediction equation is applied to selection candidates, who frequently only have marker genotype information, after being computed using a "training" or "reference" population of animals with both genotype and phenotype. It has been estimated that the rates of genetic increase in salmon, pigs, chickens, and cattle range from 20 to 30% (Tait-Burkard et al., 2018).

Community-driven per-competitive research in functional genomics and animal genomics has expedited advances in genomics. Following their sequencing, the main genomes of farm animals are being functionally annotated to the same level as the human genome. The most contiguous complex genomes ever sequenced are now found in the genomes of several farm animals. Genomic tools and new, less expensive sequencing technologies, which are based on these efforts, have been, and will continue to be, important advances in modern animal breeding and increased productivity of farmed animals. The genetic variety that already exists in the species or population of interest and the additional variants that result from denovo mutations limit the use of selective breeding. By generating new advantageous alleles or introducing known desirable alleles from different breeds or species, transgenic and genome-editing technologies provide fresh avenues for

genetic improvement without the linkage drag that comes with classical introgression. Here is a summary of some of the ways that genome editing and genetic modification can improve the health and productivity of farm animals (Rexroad et al., 2019).

**ANP32 family proteins supports the replication of influenza virus**

Based on the genetic variations in the nucleoprotein (NP) and matrix protein (M1) and their antigenicity, influenza viruses can be classified into four groups. The influenza A (IAV), influenza B (IBV), influenza C(ICV), and influenza D (IDV) viruses are among the four groups. Human pathogens include IAV, IBV, and ICV. Of these, IAV and IBV are the main culprits behind influenza outbreaks in humans. Pandemics of influenza represent a serious threat to both the security of public health worldwide and economic growth (Zhai et al. 2017).

The viral ribonucleoprotein complex (vRNP) is formed when influenza virus RNA-dependent RNA polymerase (RdRp), a heterotrimer made up of Polymerase basic protein 1 (PB1), Polymerase basic protein 2 (PB2), and Polymerase acidic protein (PA), assembles with viral RNA. The transcription and replication of the viral genome are carried out by vRNP. During the replication of the influenza virus, RdRp interacts with a number of host components. ANP32A/B are essential for maintaining viral polymerase activity and establishing the interspecies restriction of influenza viruses. It is known that they participate in the creation of the influenza virus RdRp complex. Furthermore, during influenza virus genome replication, LCAR, a functionally significant domain of the ANP32 family, may directly interact with NP and recruit NP into developing RNAs (Wang et al. 2022).

The influenza RdRp complex may attach to the ANP32A and ANP32B proteins, according to research in proteomics and whole genome sequencing. In 2015, it was first shown that the ANP32A and ANP32B proteins regulate the replication of influenza viruses. Researchers showed that ANP32A and ANP32B proteins specifically support the complementary RNA (cRNA) binding to viral RNA (vRNA) step in viral replication in an in vitro viral RNA synthesis system. They also found that a double knockdown of ANP32A and ANP32B can significantly reduce influenza virus replication (Sugiyama et al. 2015). A restriction on IAV interspecies transmission was then discovered by a number of experiments, which showed that chANP32A specifically supports avian IAV RdRp, whereas human ANP32A or ANP32B did not support this. (Baker et al. 2018).

Researchers discovered in 2019 that ANP32A/B are critical elements in IAV replication. The roles of huANP32A and B in facilitating human influenza virus RNA replication are identical in ANP32B and huANP32A double knockout cells (DKO), and the huANP32A/B proteins serve as the molecular foundation for the regular operation of influenza virus RdRp in host cells. Hence, HuANP32A/B are essential host factors for IAV replication and play a critical role in sustaining the influenza virus's polymerase activity in a variety of species, including humans, horses, pigs, and dogs. Furthermore, Carrique et al. (2020) state that the polymerase activity of IBV and ICV depends on the huANP32A/B proteins.

Research has long been focused on the molecular mechanism by which huANP32A/B facilitates influenza virus replication. It has been discovered that ANP32A/B's association with RdRp directly influences the latter's ability to assist influenza virus polymerase. ANP32A and ANP32B proteins may modify the PB2-PA dimer structure to bind to viral RdRp in an RNA-independent way; the presence of vRNA or cRNA may greatly increase this association (Baker et al. 2018).

Using fluorescence complementation and IP technology, the association between ANP32 protein and RdRp in the nucleus was shown, and it was confirmed that the deletion of these two proteins led to problems in cRNP to vRNP replication. Furthermore, it is thought that the huANP32A/B LCAR domain is necessary for the efficient binding of the influenza virus RdRp. Due to their role in facilitating the connection between the ANP32 protein and RdRp, sites 129/130 (found at the LRRCT domain) of huANP32A/B are essential for viral RNA replication (Zhang et al. 2019). Using this characteristic, scientists discovered that a single nucleotide variant (SNV) in huANP32B can result in a D130A substitution, which has a dominant negative influence on the generation of viral RdRp dimers. Moreover, mutations in huANP32A's Asp149 (D149) and Asp152 (D152) lessen the protein's interaction with RdRp and have an impact on how polymerase activity is regulated. Furthermore, viral polymerase activity is maintained in part by KPNA6, a significant host component involved in the interaction of ANP32A/B with RdRp. However, because of a mutation affecting amino acids 129/130 at the critical binding location, avian ANP32B has inherently lost its ability to sustain viral RNA replication. Avian ANP32A protein is a possible therapeutic target since only it can sustain influenza virus polymerase activity in an efficient manner. ANP32D, ANP32E, and HuANP32C all impede the influenza A virus's ability to replicate RNA. Compared to huANP32E, HuANP32A/B substantially promotes IBV replication;

however, because chANP32A has an insertion of 33 particular amino acids, it has weaker support for IBV polymerase activity than huANP32A. Because of their unique amino acid changes at positions 129–130, chANP32B/E and huANP32B/E have lesser activity than each other (Zhang et al. 2020b).

**ANP32 family proteins involvement in cross-species transmission of influenza virus and driving virus evolution**.

The primary defenses against the interspecies spread of the avian influenza virus to mammals are the influenza virus proteins HA and RdRp (Long et al. 2019). By attaching to various sialic acid (SA) receptor types in host cells, HA controls whether the influenza virus can penetrate those cells. Human influenza virus HA predominantly attaches to α2-6-linked SA, but avian influenza virus HA preferentially attaches to α2-3-linked SA. The key to the influenza virus successfully infecting the host is its effective reproduction via polymerase activity once it has entered the host cell. The limited ability of RdRp from avian influenza viruses to reproduce in mammalian cells restricts the spread of these viruses to mammals. Nonetheless, the avian influenza virus's RdRp component has a large number of adaptive point mutations that enable the virus to pass through the host restriction barrier. According to Long et al. (2019), the most common mutation occurs at position 627 of avian influenza RdRp PB2, where lysine (627K) replaces glutamine (627E). The molecular mechanism underlying the interspecies transmission of the avian influenza virus is further elucidated by the structural and functional distinctions between the ANP32A proteins found in birds and mammals. The 33 amino acid insertion in avian ANP32A allows avian ANP32A to support avian influenza polymerase activity (Long et al. 2019), validating the presence of a positive correlation factor supported by PB2-627E RdRp in bird cells and a restriction in putatively restricted cells. HuANP32A protein is a crucial barrier to limit the transmission of avian influenza viruses to mammals.

This clarifies why avian IAV polymerases' efficient activity is not supported by huANP32A and huANP32B homologs, whereas the function of human-adapted influenza polymerases is. A functional SIM site (VLSLV) surrounded by acidic residues plays a crucial role in SIM dependent SUMO interactions, and a specific Simulation domain in the 33 amino acid insertion of chANP32A has a considerable effect on polymerase activity compared with huANP32 (Domingues and Hale 2017). The capacity of chANP32A to bind to RdRp and increase 627E polymerase activity in

mammalian cells is both markedly reduced by SIM site mutation. It is yet unclear, nevertheless, if chANP32A self-simulation plays a role in limiting interspecies transmission. Differentially splicing avian ANP32A can produce three distinct variants. Variants 1, 2, and 3 of the splice variants are as follows: variation 1, chANP32A (33), contains a complete 33 amino acid insertion; variant 2, chANP32A (29), has a 4 amino acid deletion at the SIM site; and variant 3, chANP32A (0/-33), is a splice variant that resembles the huANP32A protein. These three variations have the following effects on the PB2-627E polymerase's activity: chANP32A (33) > chANP32A (29) > chANP32A (0/-33). The impact of the prominent SIM motif's 33 amino acid insertion on RdRp is further supported by this result (Baker et al. 2018). Researchers later predicted that IAV RdRp would adapt to the ANP32Asplice site, suggesting that the ratio of divergent splicing variants may influence influenza virus adaptation and act as a catalyst for the virus's transition from birds to mammals (Domingues et al. 2019).Furthermore, the roles of murine and swine ANP32A in cross-species transmission have been examined in previous research. The swine ANP32A protein has the ability to particularly boost the polymerase activity of the avian influenza virus, hence promoting virus replication, in contrast to other mammalian ANP32A/B proteins. According to Zhang et al. (2020), swine ANP32A protein may therefore be a significant host factor and may be the cause of pigs' role as influenza "mixers." The influenza A and B viruses' polymerase activity is minimally supported by the murine ANP32A protein. The stimulation of influenza A and B virus replication in mice by ANP32B may be connected to its immunomodulatory activity, as the murine ANP32B protein is more capable of supporting influenza A and B virus replication than other species of ANP32B proteins (Beck et al. 2020). However, there are very few reports on the functions of ANP32 family proteins from other species in the cross-species spread of influenza viruses. The ANP32 family of proteins is linked to species-specific restrictions on influenza virus replication because of the ways that IAV and IBV employ these proteins differently. The substantial support that chANP32A has for IAV replication in birds and mammals is a result of a 33 amino acid insertion; however, this also reduces its support for IBV replication. IAV or IBV polymerase activity cannot be supported by ChANP32B because it is a naturally inactive molecule. Because of its 129E mutation, ChANP32E has minimal support for IBV RdRp function but does not support IAV RdRp function. This clarifies why birds hardly ever contract influenza B virus spontaneously (Zhang et al. 2020).

**Molecular basis of influenza virus replication by ANP32A protein**

ANP32A/B proteins attach to the RdRp triple subunit complex firmly, which supports influenza virus polymerase activity. ANP32A/B can bind to the PB2-627 domain alone (Baker et al. 2018) or directly interact with PB2 through ANP32A's Glu189 and Glu196 to increase viral RNA production, despite the fact that it cannot connect to the RdRp single subunit. According to Nilsson et al. (2017), the PB2-627 domain (aa535-667 of PB2) is essential for the cross-species spread of avian influenza. Thus, in order to uncover the molecular mechanisms behind viral replication, it is imperative to investigate the molecular underpinnings of the structures of ANP32A/B and PB2-627 as well as analyze the structures of ANP32A/B and influenza virus RdRp. The structure of avian-derived RdRp was discovered by using structural analysis to compare the stability of the complex produced by chANP32A and chicken-adapted 627-NLS (627-NLS(E)) with huANP32A and human-adapted 627-NLS (627-NLS(K)). Without the E627K mutation, avian RdRp is unable to operate in human cells (Camacho-Zarco *et al.* 2020). Researchers simultaneously examined the structure of ICV RdRp complexes with huANP32A and chANP32A using cryo-electron microscopy (cryo-EM). The N-terminal LRRs of ANP32A were observed to bridge the asymmetric dimer formation of ICV RdRp in these two structures. A possible mechanism by which the PB2 (E627K) mutation can facilitate effective replication of avian viral RNAs in mammalian hosts is suggested by the C-terminal LCAR of ANP32A, which was placed between the two PB2 627 domains of the asymmetric IAV RdRp dimer (Carrique et al. 2020). Though it is unknown if ANP32 gets packed into versions through this close connection with RdRp, structural research demonstrated that ANP32/B's strong binding to RdRp is the basis for regulating influenza virus replication.

**Discussions and Conclusion**

With the help of novel alleles intended to be advantageous, variation found in non-domesticated species, and variation found in other populations and species, transgenic and genome-editing technologies can yield greater benefits in a shorter time. One possible example of how an allele exclusive to the wild warthog population—which has coevolved with the disease for many thousands of years—has been inserted into domesticated pigs by genome editing is resilience to ASFV. While the phenotypic of the altered pigs remains unknown, the idea of transferring advantageous traits from a wild population to their domesticated counterparts makes sense.

Precise, accurate genome-editing methods differ greatly from trans-genesis methods. The legal and regulatory framework for animals with altered genomes is still being developed. Huge progress has been achieved, though, and the PRRS-resistant pigs raised at Missouri and Roslin in particular have a high chance of eliminating or greatly reducing this terrible illness. Beyond these straightforward illustrations, many interesting features are complicated, meaning that numerous alleles, each with a negligible impact, control them. Editing many alleles at once and frequently integrating editing techniques into commercial breeding program operations would be necessary to create substantial influence from genome editing by utilizing current genetic variation for a complicated characteristic. Genome editing may be able to improve cattle, even with complicated features, according to simulations. This may be achieved either enhancing the frequency of advantageous alleles or eliminating harmful alleles as part of a breeding program controlled by gnomic selection. Now that genome editing is accurate and fast, and assuming that the regulatory pathways can be identified, there has to be a renewed emphasis on identifying the editing targets. Numerous methods, including genetics, genomics, large-scale CRISPR-based functional screens, host-pathogen interactions, virology, bacteriology, and serendipity, were used to identify the target genes in the aforementioned examples. Even though the latter cannot be anticipated, it is evident that all other methods, when combined into a comprehensive, coordinated global research program, have the ability to pinpoint targets that will have a significant positive impact on the livestock industry and our species' capacity to produce enough food in an environmentally sustainable manner on a transnational scale.

The primary members of the ANP32 protein family, ANP32A, ANP32B, and ANP32E, have been shown to be crucial for RNA virus replication. According to Zhang et al. (2019), ANP32A and ANP32B play a critical role in promoting influenza virus replication. The structures of the ANP32 protein family members are similar because they share similar motifs. More information is needed to understand how different viruses can exploit members of the ANP32 family to their advantage as well as the various functions that these members play in viral replication. Further research is required to determine whether the ANP32 family proteins can be bundled into versions of the virus as backpack members to speed up replication in the early stages of infection or under unfavorable circumstances to aid in adaptation. These proteins are also essential for the replication of influenza viruses. With the exception of influenza and retroviruses, which replicate their genomes in the nucleus, the majority of RNA viruses reproduce their genomes in the cytoplasm of the cell. It's

fascinating that ANP32A/B, which are parts of the nuclear transport complex in the CRM1-dependent pathway, have been shown to facilitate the export of partially spliced or unsliced viral mRNA, hence promoting HIV-1 replication (Wang et al. 2019). ANP32 proteins most likely have several functions in the nucleus. There is currently no proof linking ANP32 proteins to DNA virus replication, despite the fact that DNA viruses replicate their genomes in the nucleus. Confirming whether ANP32 family proteins are involved in DNA viruses will require more research, but doing so could improve our understanding of the process behind DNA virus replication and identify potential therapeutic targets. The biological activity of proteins depends on posttranslational modification, or PTM. ANP32A exhibits inhibitory effect against protein phosphatase 2A (pp2A). It has been shown that casein kinase II is capable of phosphorylating ANP32 proteins, and that this mechanism is crucial to the apoptotic process. Concurrently, ANP32 was found to be a component of inhibitor of acetyltransferase (INHAT), which interacts with a number of transcriptional coactivators, such as p300/CBP and PCAF, to bind to histones and limit histone acetyl transfer. Nevertheless, there hasn't been any information released on ANP32's acetylation or the viral proteins that ANP32 acetylates thus far. Although the importance of a Simulation domain found in chANP32A in facilitating the spread of avian influenza viruses has been shown (Domingues and Hale 2017), it is unclear if the Simulation of ANP32A or viral proteins plays a part. To confirm the impact of PTMs on the functionality of ANP32 proteins and their connections with viral proteins, more focus should be placed on PTMs, such as phosphorylation, acetylation, or ubiquitination of ANP32 proteins and their associated viral proteins.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the

name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**References**


1. Baker, S.F., M.P. Ledwith, and A. Mehle. 2018. Differential splicing of ANP32A in birds alters its ability to stimulate RNA synthesis by restricted influenza polymerase. Cell Reports 24: 2581–2588.
2. Baker, S.F., and A. Mehle. 2019. ANP32B, or not to be, that is the question for influenza virus. Elife 8: e48084.
3. Beck, S., M. Zickler, V. Pinho dos Reis, T. Günther, A. Grundhof, P.T. Reilly, T.W. Mak, S. Stanelle-Bertram, and G. Gabriel. 2020. ANP32B deficiency protects mice from lethal influenza A virus challenge by dampening the host immune response. Frontiers in Immunology 11: e450.
4. Camacho-Zarco, A.R., S. Kalayil, D. Maurin, N. Salvi, E. Delaforge, S. Milles, M.R. Jensen, D.J. Hart, S. Cusack, and M. Blackledge. 2020. Molecular basis of host-adaptation interactions between influenza virus polymerase PB2 subunit and ANP32A. Nature Communications 11: 3656.
5. Carrique, L., H. Fan, A.P. Walker, J.R. Keown, J. Sharps, E. Staller, W.S. Barclay, E. Fodor, and J.M. Grimes. 2020. Host ANP32A mediates the assembly of the influenza virus replicate. Nature 587: 638–643.
6. Cousins, T., Pentecost, M., & Van Helden, L. (2022). Containment and conversion: Urban livelihoods and the circulation of value amid South Africa's avian influenza outbreak. American Ethnologist, 49(3), 413-426.
7. Domingues, P., D. Eletto, C. Magnus, H.L. Turkington, S. Schmutz, O. Zagordi, M. Lenk, M. Beer, S. Stertz, and B.G. Hale. 2019. Profiling host ANP32A splicing landscapes to predict influenza A virus polymerase adaptation. Nature Communications 10: 3396.

8. Domingues, P., and B.G. Hale. 2017. Functional insights into ANP32A-dependent influenza A virus polymerase host restriction. Cell Reports 20: 2538–2546.
9. Fodor, E., D.C. Pritlove, and G.G. Brownlee. 1994. The influenza virus panhandle is involved in the initiation of transcription. Journal of Virology 68: 4092–4096.
10. Fodor, E., and A.J.W. Te Velthuis. 2020. Structure and function of the influenza virus transcription and replication machinery. Cold Spring Harbor Perspectives in Medicine 10. https://doi.org/10.1101/ cshperspect.a038398.
11. Gratacap, R. L., Wargelius, A., Edvardsen, R. B., and Houston, R. D. (2019). Potential of genome editing to improve aquaculture breeding and production. Trends in Genetics, 35(9), 672-684.
12. Idoko-Akoh, A., Goldhill, D. H., Sheppard, C. M., Bialy, D., Quantrill, J. L., Sukhova, K. & McGrew, M. J. (2023). Creating resistance to avian influenza infection through genome editing of the ANP32 gene family. *Nature communications*, *14*(1), 6136.
13. Long, J.S., A. Idoko-Akoh, B. Mistry, D. Goldhill, E. Staller, J. Schreyer, C. Ross, S. Goodbourn, H. Shelton, M.A. Skinner, H. Sang, M.J. McGrew, and W. Barclay. 2019a. Species specific differences in use of ANP32 proteins by influenza A virus. Elife 8: e45066.
14. Long, J.S., B. Mistry, S.M. Haslam, and W.S. Barclay. 2019b. Host and viral determinants of influenza A virus species specificity. Nature Reviews Microbiology 17: 67–81.
15. Looi, F. Y., Baker, M. L., Townson, T., Richard, M., Novak, B., Doran, T. J., Short, K. R. 2018. Creating disease resistant chickens: a viable solution to avian influenza. Viruses, 10(10), 561.
16. Mackelprang, R., and Lemaux, P. G. (2020). Genetic engineering and editing of plants: an analysis of new and persisting questions. Annual Review of Plant Biology, 71, 659-687.
17. Nilsson, B.E., A.J.W. Te Velthuis, and E. Fodor. 2017. Role of the PB2 627 domain in influenza A virus polymerase function. Journal of Virology 91: e7.
18. Nielsen, S. S., Alvarez, J., Bicout, D. J., Calistri, P., Canali, E., Viltrop, A. 2023. Vaccination of poultry against highly pathogenic avian influenza–part 1. Available vaccines and vaccination strategies. EFSA Journal, 21(10), e08271.
19. Sugiyama, K., A. Kawaguchi, M. Okuwaki, and K. Nagata. 2015. pp32 and APRIL are host cell-derived regulators of influenza virus RNA synthesis from cRNA. Elife 4: e08939.

20. Sid, H., and Schusser, B. (2018). Applications of gene editing in chickens: a new era is on the horizon. Frontiers in genetics, 9, 456.
21. Rexroad, C., Vallet, J., Matukumalli, L. K., Reecy, J., Bickhart, D., Blackburn, H., Wells, K. (2019). Genome to phenome: improving animal health, production, and well-being–a new USDA blueprint for animal genome research 2018–2027. Frontiers in genetics, 10, 327.
22. Tait-Burkard, C., Doeschl-Wilson, A., McGrew, M. J., Archibald, A. L., Sang, H. M., Houston, R. D., and Watson, M. 2018. Livestock 2.0–genome editing for fitter, healthier, and more productive farmed animals. Genome biology, 19, 1-11.
23. Wang, F., C.M. Sheppard, B. Mistry, E. Staller, W.S. Barclay, J.M. Grimes, E. Fodor, and H. Fan. 2022. The C-terminal LCAR of host ANP32 proteins interacts with the influenza A virus nucleoprotein to promote the replication of the viral RNA genome. Nucleic Acids Research 50: 5713–5725.
24. Wang, L., J. Li, Y. Li, and L.B. Pang. 2020. Hsa-let-7c exerts an anti-tumor function by negatively regulating ANP32E in lung adenocarcinoma. Tissue and Cell 65: 101372. Wang, Y., H. Zhang, L. Na, C. Du, Z. Zhang, Y.H. Zheng, and X. Wang. 2019. ANP32A and ANP32B are key factors in the Rev-dependent CRM1 pathway for nuclear export of HIV-1 unsliced mRNA. Journal of Biological Chemistry 294: 15346–15357.
25. Zhai, S.L., H. Zhang, S.N. Chen, X. Zhou, T. Lin, R. Liu, D.H. Lv, X.H. Wen, W.K. Wei, D. Wang, and F. Li. 2017. Influenza D virus in animal species in Guangdong province, southern China. Emerging Infectious Diseases 23: 1392–1396.
26. Zhang, H., H. Li, W. Wang, Y. Wang, G.Z. Han, H. Chen, and X. Wang. 2020a. A unique feature of swine ANP32A provides susceptibility to avian influenza virus infection in pigs. PLoS Pathogens 16: e1008330.
27. Zhang, H., Z. Zhang, Y. Wang, M. Wang, X. Wang, X. Zhang, S. Ji, C. Du, H. Chen, and X. Wang. 2019. Fundamental contribution and host range determination of ANP32A and ANP32B in influenza a virus polymerase activity. Journal of Virology 93: e13.
28. Zhang, Z., H. Zhang, L. Xu, X. Guo, W. Wang, Y. Ji, C. Lin, Y. Wang, and X. Wang. 2020b. Selective usage of ANP32 proteins by influenza B virus polymerase: implications in determination of host range. PLoS Pathogens 16: e1008989.