Nuclear Exclusion of the HIV-1 Host Defense Factor APOBEC3G Requires a Novel Cytoplasmic Retention Signal and Is Not Dependent on RNA Binding*

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Human APOBEC3G (hA3G) is a host factor that defends against HIV-1 as well as other exogenous retroviruses and endogenous retroelements. To this end, hA3G is restricted to the cytoplasm of T lymphocytes where it interacts with viral RNA and proteins to assemble with viral particles causing a post-entry block during reverse transcription. hA3G also exhibits a mechanism to inhibit the reverse transcription of retroelements by RNA binding and sequestration into mRNA processing centers in the cytoplasm. We have determined that the molecular basis for this specialized property of hA3G is a novel cytoplasmic retention signal (CRS) that is necessary and sufficient to restrict wild-type hA3G and chimeric constructs to a cytoplasmic retention signal (CRS) that is necessary and sufficient to restrict wild-type hA3G and chimeric constructs to the cytoplasm. The CRS resides within amino acids 113–128 and is embedded within a basic flanking sequence and does not require RNA binding to retain hA3G in the cytoplasm. Paralogs of hA3G that have nuclear or cytoplasmic distributions differ from hA3G within the region encompassing the CRS motif with respect to charge and amino acid composition. We propose that the CRS enables hA3G to interact with cytoplasmic factors, and thereby enables hA3G to serve in host cell defense by restricting an antiviral sentinel to the cytoplasm. The CRS lies in a region involved in both Gag and Vif interactions; therefore, identification of this motif has important implications for the design of therapeutics that target HIV-1 while maintaining antiviral and cellular functions.

Cytidine deaminases of the APOBEC3 family (ApoB mRNA editing catalytic subunit) have one or more zinc-dependent deaminase (ZDD) signature motifs of the form (C/H)XEXXn-PCXXC that is characteristic of enzymes that use RNA or single-stranded DNA (ssDNA) as substrates for C to U or dC to dU deamination (1, 2). APOBEC-1, activation-induced deaminase (AID), and APOBEC3G are the most extensively characterized members of this family. In mammals APOBEC-1 carries out site-specific editing of apoB and NF1 mRNAs to produce nonsense codons that lead to truncated proteins with altered functional properties (3–5). Though not observed under physiological conditions, APOBEC-1 can carry out dC to dU ssDNA mutation when expressed under selection in an Escherichia coli-based DNA mutator assay (6). In contrast, the physiological function of AID in germinal center B cells is to carry out multiple dC to dU mutations on ssDNA regions within the variable region and switch regions of immunoglobulin genes as an essential mechanism for somatic hypermutation and class switch recombination, respectively (7, 8).

The activities of APOBEC-1 and AID are regulated through their tissue-specific and temporal expression during cell differentiation (4, 8–12). Both enzymes have cytoplasmic and nuclear distributions within cells, but the RNA and ssDNA editing activity of APOBEC-1 and AID are restricted to the cell nucleus (13–15). Each enzyme has a nuclear localization signal (NLS) and a nuclear export signal (NES) (16–19) and their interaction with cytoplasmic chaperones is essential for nuclear import and site-specific editing activities (15, 20–23). Overexpression of such enzymes and their production in cell types lacking regulatory chaperones results in promiscuous editing activity on RNA and DNA (6, 24, 25). This nonspecific activity can lead to cell transformation and cancer (26–32).

In contrast to APOBEC-1 and AID, the ssDNA deaminase human APOBEC3G (hA3G) has N- and C-terminal ZDD motifs that contribute to RNA binding and dC to dU conversion in the context of ssDNA, respectively (33–38). hA3G, along with other APOBEC3 paralogs, serves as a host defense factor against exogenous retroviruses and endogenous retroelements (39–43). hA3G is the most potent inhibitor of vif-deficient HIV-1 infectivity in the APOBEC family (44). Of particular interest is that hA3G is restricted to the cytoplasm of T lymphocytes where it has diffuse as well as punctuate distributions (40, 45–48). The localization of hA3G is functionally significant for each of its known activities. hA3G has an intrinsic ability to bind to RNA, and its cytoplasmic localization enables it to bind HIV-1 and retroviral/retroelement RNA (42, 43). Cytoplasmic localization also enables hA3G to block reverse transcription for both HIV-1 (49) and hepatitis B virus (50). hA3G also is bound to retroelement RNA as high molecular mass (HMM) complexes, and this has been posited to block their replication by sequestration within stress granules and p-bodies (42, 45, 46, 51). Several proteins co-purify with HMM

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§ The abbreviations used are: APOBEC, ApoB mRNA editing catalytic subunit; hA3G, human APOBEC3G; AID, activation-induced deaminase; CRS, cytoplasmic retention signal; NLS, nuclear localization signal; EGFP, enhanced green fluorescent protein; ZDD motif, zinc-dependent deaminase motif; HA, hemagglutinin.
complexes but most are indirectly associated with hA3G via an RNA bridge (42, 45, 46, 51). Recently, hA3G has been shown to enhance the stability and translation of cellular mRNAs that would be otherwise targeted for degradation by micro RNA (miRNA) by excluding them from p-bodies and enhancing their association with polysomes (52). Finally, HIV-1 Vif inhibits hA3G activity by shutting hA3G to proteasomal degradation (53–56). However, in the absence of Vif, hA3G is packaged within HIV-1 virions in the cytoplasm through interactions with RNA and the nucleocapsid (NC) portion of the HIV-1 Gag polyprotein (34, 57–64). Viral packaging provides spatially privileged access of hA3G to the reverse transcription complex and enables it to block viral replication as well as catalyze dC to dU hypermutations of single stranded newly replicated proviral DNA (33, 37, 38, 65–67). Cytoplasmic retention of a highly expressed protein such as hA3G could also be important as a means to prevent genotoxicity due to hA3G activity on chromosomal DNA as has been observed for uncontrolled APOBEC-1 and AID expression (26–32).

The amino acid motif or cellular chaperone responsible for cytoplasmic retention has yet to be identified. In this report we describe the identification of a novel cytoplasmic retention signal (CRS). Although hA3G has no NLS, we show that the CRS can act dominantly over an NLS from the SV40 large T antigen (68). The crucial components of the CRS map to a 16-amino acid region adjacent to the first ZDD motif (amino acids 113–128) that overlaps with critical residues within the domain of hA3G involved in both HIV-1 Gag and Vif interactions (69–72). We show that this region is both necessary and sufficient for cytoplasmic retention of reporter constructs that are otherwise nuclear. These findings explain why hA3G is restricted to the cytoplasm and are relevant in the rational design of novel HIV/AIDS therapeutics.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—Full-length hA3G and deletion mutants (amino acids 1–208, 1–194, 1–156, 1–143, 1–128, 1–111, 1–60, 60–384, 113–384, 130–384, 144–384, 209–384, and 113–128) were PCR-amplified and cloned with restriction sites EcoRV and Not1 into the pIRES-P vector (73) with N-terminal reporter constructs of either EGFP-HA or NLS-EGFP-HA. Amino acids PKKKRKV (NLS from SV40 large T-antigen) (68) were added to the N-terminal half (amino acids 1–208) and C-terminal half (amino acids 209–384) of hA3G within hA3G the N-terminal half (amino acids 1–208) and C-terminal half (amino acids 209–384) were PCR-amplified and cloned with restriction sites EcoRV and Not1 into the pIRES-P vector (73) with N-terminal reporter constructs (45, 47, 64).

**Transfections, Live Cell Imaging, and Western Blotting**—293T cells obtained from ATCC (Manassas, VA) were transfected using FuGENE® 6 according to the manufacturer’s protocol (Roche Applied Science). Twenty-four hours after 293T cells were transfected, 10 μM final concentration of Hoechst 33342 (Anaspec Inc., San Jose, CA) was added to the cell media, and cells were imaged by a QICIM-IR fast 12 bit monochrome camera viewed by Q capture software (Q-Imaging) through a ×40 Olympus objective with an Olympus IX 70 inverted fluorescence microscope and label-specific chrome filters. After imaging, cells were harvested in Reporter Lysis Buffer (Promega). Cell extracts were run on a 10.5% SDS-PAGE and transferred to nitrocellulose where proteins were detected by western blotting with antibodies: anti-HA (Covance) or anti-GFP (Roche Applied Science) and a goat anti-mouse secondary (eBioscience).

**RNA UV Cross-linking**—A 448-nt section of apolipoprotein B mRNA was in vitro transcribed with 32P-radiolabeled ATP and CTP (NEN) using T7 polymerase (Promega) as a probe for nonspecific RNA binding activity. The radiolabeled RNA was gel-isolated and added to cell extracts made from 293T cells expressing EGFP or EGFP-V5-tagged hA3G, N terminus (amino acids 1–208), and C terminus (amino acids 209–384) for 30 min at 4 °C. The RNA was UV-cross-linked to the proteins in the cell extract using short wave UV light in quartz cuvettes at 4 °C for 7 min as described previously (74). Immediately after UV cross-linking, cell extracts were treated with a mix of RNases T1 and A for 1 h at 37 °C followed by preclearing and immunoprecipitations. The nitrocellulose-transferred protein was exposed to Biomax XAR film (Kodak) for 48 h to identify radiolabeled bands from nucleotides covalently cross-linked to EGFP, EGFP-hA3G, or half domains. The nitrocellulose was subsequently western blotted for GFP (Roche Applied Science) or V5 (Invitrogen) to overlay with the radiolabeled band and to verify that the immunoprecipitation was successful.

**RESULTS**

**The CRS Is within the N-terminal Half of hA3G**—Previously, we utilized indirect immunostaining of fixed HeLa cells to determine that hA3G was actively retained in the cytoplasm even when a strong NLS from SV40 was added onto the protein (48). Focusing on the cis-acting sequence determinants for the cytoplasmic localization of hA3G, a live cell assay system was established using transfection of chimeric proteins containing EGFP or NLS-EGFP. This reporter system enabled visualization of the subcellular distribution of hA3G and parts thereof. EGFP was selected because it has no subcellular localization determinants (diffuses freely throughout the cell), does not form multimers, and it, along with its derivatives (i.e. YFP and CFP), has been shown not to affect hA3G functionality or localization when attached to the N terminus of hA3G reporter constructs (45, 47, 64).

As expected, EGFP alone was distributed homogenously throughout both the cytoplasm and nucleus of 293T cells (Fig. 1A). The addition of the SV40 NLS to EGFP (NLS-EGFP) resulted in near quantitative nuclear localization of the chimeric protein (Fig. 1B). However, when hA3G was attached to the C terminus of EGFP or NLS-EGFP the chimeric proteins demonstrated a cytoplasmic localization (Fig. 1, C and D). These data corroborated our earlier study using fixed cells (48).

Each half of hA3G contains a consensus ZDD motif (1, 2) and have 62.8% similarity in pairwise amino acid sequence relatedness (Table 1). To begin to determine the position of the CRS within hA3G the N-terminal half (amino acids 1–208) and C-terminal half (amino acids 209–384) of hA3G were attached to NLS-EGFP (NLS-EGFP-NT and NLS-EGFP-CT, respectively) and expressed in 293T cells. Whereas NLS-EGFP-NT was cytoplasmic (Fig. 1E), NLS-EGFP-CT had a predominant nuclear localization (Fig. 1F). This established the N-terminal portion of hA3G as the region containing the CRS. Western
blotting with anti-GFP or anti-HA confirmed that all chimeric proteins were expressed and were of the expected size (Fig. 1G).

Cytoplasmic Retention Does Not Depend on RNA Binding—We analyzed whether RNA binding was a critical component for cytoplasmic retention of hA3G, because RNA binding is involved in hA3G-dependent sequesterization of retroelements and miRNAs in cytoplasmic mRNA processing centers (i.e. HMM complexes, polysomes, stress granules, and p-bodies) (42, 45, 46, 51, 52). To this end, we UV cross-linked radiolabeled RNA to EGFP-hA3G, EGFP-NT, EGFP-CT, and EGFP in cell extracts, followed by RNase digestion and immunoprecipitation (IP). If a protein is capable of robust RNA binding, radiolabeled nucleotides will remain covalently cross-linked to the protein following RNase digestion. hA3G is known to display RNA binding activity in a sequence-independent manner (46, 58). EGFP alone does not bind RNA. As predicted a radiolabeled band overlaid with the EGFP-hA3G IP band but not with the IP of EGFP alone (Fig. 1H, top and bottom rows). Surprisingly, when individual halves of hA3G were expressed neither was capable of cross-linking to the radiolabeled RNA (Fig. 1H, middle rows). The observation that the N-terminal half of hA3G is retained in the cytoplasm (Fig. 1E) but had low or no RNA binding activity suggests that RNA binding is not required for cytoplasmic retention of hA3G.

Mapping the CRS with NLS-EGFP—Fig. 2 shows the predicted secondary structure of hA3G based on sequence alignment with the known crystal structures of APOBEC-2 and other cytidine deaminases (75–76) as well as the solution structure of hA3G (77) and exon junctions. Deletion constructs of the N-terminal half of hA3G were prepared using predicted halves (amino acids 1–208 and 209–384, respectively) are shown as bar diagrams below their respective images. The relative location of the ZDD motif (black) is shown within each protein construct. A key for symbols used for NLS-EGFP, EGFP, and the HA tag is shown at the bottom of the panels. Full-length hA3G (amino acids 1–384) and either the N- or C-terminal
loop regions of hA3G as break points to avoid disrupting predicted secondary structural elements. All hA3G deletions constructs were prepared as NLS-EGFP chimeric proteins and transfected into 293T cells and validated for their expression by western blotting.

Deletion of 14 residues from the C terminus of the N-terminal half (amino acids 1–194) did not affect the cytoplasmic retention of the chimeric protein (Fig. 3A). However, deletion of predicted α-helix five (α5) located between two exon junctions (Fig. 2) in the construct containing amino acids 1–156 resulted in partial distribution of the chimeric protein to the nucleus (Fig. 3B). This suggested that the CRS had been weakened and that the NLS function was partially able to compete with the CRS. Further deletion (to amino acids 1–143 and 1–128) did not alter the cytoplasmic and nuclear distribution of the chimeric proteins (Fig. 3, C and D). Deletion of the predicted β-strand 4 (β4) (amino acids 1–111) or further (amino acids 1–60) resulted in a prominent nuclear accumulation of the chimeric protein as expected from the SV40 NLS containing reporter (Fig. 3 E and F). Expression of the appropriate molecular mass for each construct was confirmed by western blotting (Fig. 3K). These data suggested that the CRS mapped to a region including the predicted β4 within the N terminus of hA3G.

To confirm these data, progressively smaller constructs from the N terminus of hA3G were made as NLS-EGFP chimeric proteins and transfected into 293T cells and validated for their expression by western blotting. The SV40 NLS was used to rapidly focus on the occurrence and position of a CRS within hA3G sequence. In the absence of an NLS or CRS, EGFP has a homogeneous distribution determined by diffusion. To further define the boundaries of the hA3G CRS, deletion constructs were made as EGFP chimeras lacking the SV40 NLS with the anticipation that the hA3G CRS would restrict diffusion of these proteins to the cytoplasm. As expected, all deletion constructs that contained β4 (amino acids 1–156, 1–143, 1–128) had robust cytoplasmic localization (Fig. 4, A–C). Alternatively, constructs lacking this region (amino acids 1–60, 1–111) distributed to both the cytoplasm and nucleus (Fig. 4, D and E), similar to that observed with EGFP alone (Fig. 1A) suggesting the loss of CRS function. The data corroborate our findings with NLS-EGFP chimeric deletion constructs of hA3G suggesting that the region including the predicted β4 is essential for cytoplasmic retention.

The hA3G CRS Is Autonomously Capable of Cytoplasmic Retention of Reporters—We next tested the ability of the region including the predicted β4 (amino acids 113–128) to act autonomously as a CRS when attached to the C terminus of either, NLS-EGFP or EGFP. This motif alone was only partially dominant over the SV40 NLS and these chimeric proteins displayed both cytoplasmic and nuclear localization (Fig. 5A) compared with robust nuclear retention with NLS-EGFP alone (Fig. 1B). On the other hand, this motif was sufficient for robust cytoplasmic retention of EGFP (Fig. 5B) compared with EGFP alone. As with the constructs in Fig. 1 and 3, a western blot with anti-GFP confirmed that the EGFP reporter constructs had the expected size (Fig. 5C). The fact that this small region retained EGFP in the cytoplasm reveals that indeed the CRS motif was responsible for cytoplasmic retention and not the relative size of the chimeric proteins, because larger constructs lacking this motif were capable of diffusing to the nucleus (Fig. 4, D and E). These data demonstrated that the region containing residues KVTLTIFVARLYYFWD was a novel CRS motif that functions to restrict hA3G to the cytoplasm.

**DISCUSSION**

We have tested the hypothesis that hA3G is actively retained in the cytoplasm through its own cytoplasmic retention signal (CRS) by evaluating the subcellular distribution of hA3G domains expressed as EGFP and NLS-EGFP chimeric reporters. We demonstrated that the sequence KVTLTIFVARLYYFWD (amino acids 113–128) within the N-terminal portion of hA3G is a novel CRS that restricts hA3G to the cytoplasm and is capable of autonomous function within the context of chimeric reporters. While hA3G binds to cellular RNAs that serves to bridge interactions with numerous ribonucleoproteins (42, 45, 46, 51, 52), our data suggest that nuclear exclusion of hA3G via the CRS is not determined by RNA binding interactions with the CRS.

An important consideration in the experimental design was that regions of hA3G were selected for evaluation based on secondary structure domain boundaries predicted from the known crystal structure of APOBEC-2 (75) and other cytidine deaminases (76) as well as the solution structure of APOBEC3G (77). In the absence of an atomic resolution structure for hA3G, this is the most directed approach for deletion mapping that has
reasonable expectations for maintaining functional domain integrity such that folded, soluble regions of hA3G can be expressed. An additional strength in the experimental design lies in the ability to evaluate the subcellular distribution determinants of hA3G in living cells through the use of an EGFP fluorescent reporter. When expressed alone, EGFP is uniformly and diffusely distributed in the nucleus and cytoplasm as it lacks a subcellular localization determinant. The addition of a strong nuclear localization signal from the SV40 large T antigen (68) resulted in quantitative retention of NLS-EGFP in the nucleus. Our initial analyses showed that hA3G (48) and APOBEC-1 (16) acted dominantly over the SV40 NLS. It was also shown that unlike APOBEC-1 or AID (16–19), hA3G does not traffic between the cytoplasm and nucleus (48). This enabled a simple experimental design wherein chimeric NLS-E GF P reporters containing domains of hA3G with CRS activity could be positively identified by their ability to induce cytoplasmic localization of an otherwise nuclear restricted NLS-EGFP reporter.

The data showed that a stretch of 16 amino acids was a powerful cytoplasmic determinant capable of excluding reporters lacking an NLS entirely from the nucleus, and markedly reducing the ability of NLS-EGFP to be retained in the nucleus. The data also showed that within the native sequence context of the N terminus of hA3G, the CRS activity was dominant over the SV40 NLS. This characteristic suggested that elements surrounding the CRS, such as secondary structure or charge, promoted or enhanced the function of the CRS.

The N-terminal half of hA3G has a distinctly basic sequence with a theoretical isoelectric point (pI) of 9.4 (Table 1). APOBEC3F (hA3F) is also localized exclusively in the cytoplasm (40, 45) and the homologous CRS region in the N terminus lies within a basic sequence (pI 8.9).

**FIGURE 3.** Deletion mapping of the hA3G CRS with NLS-EGFP. A–J, EGFP fluorescence (EGFP, left) and the live cell nuclear staining with Hoechst (right) are shown for 293T cells transfected with the reporter construct listed to the left of each set of images. The observed localization is given on the right as cytoplasmic (C), nuclear (N), or cytoplasmic and nuclear (C/N). The first and last amino acid of each deletion construct were designed based on predicted loop regions and/or exon junctions shown in Fig. 2 and are shown above each bar diagram. The key for symbols used for NLS-EGFP and the HA tag are also shown (bottom). K, western blots (WB) with anti-GFP detecting the protein constructs indicated above each lane by their boundary amino acid positions. The migration of molecular mass standards is indicated to the right.
The other APOBEC3 paralogs hA3B, hA3C, and hA3A all have predominantly nuclear localizations in transfected cells (40) and have close to neutral theoretical pI in their homologous regions. Also, the C-terminal half of the two ZDD-containing family members (hA3G, hA3F, and hA3B) are all distinctly acidic (Table 1). This suggests that in the native context the hA3G CRS is most effective in a basic environment.

The CRS residues KVTLTFVARLYYFWD are highly conserved among non-human primate APOBEC3Gs. The motif in chimpanzees is identical to humans and the only difference in African green monkeys and macaques is D128K, which has been shown by multiple labs to be an essential amino acid for HIV-1 or SIV Vif interaction (69–71). Moreover, there is a considerable amount of conservation of the motif in other APOBEC3 paralogs with the major differences being at residues Lys-113, Thr-115, and Val-120 (Table 1). Possible reasons for nuclear localization of some APOBEC3 family members could be that they are lacking key residues for cytoplasmic retention, the motif is buried and not surface-exposed or the charge environment around the motif (Table 1) prohibits its ability to function as a CRS. On the other hand, the fact that this region has been shown to contain important residues for both HIV-1 Gag (amino acids 124–127) (72) and Vif (Asp-128) (69–71) interactions suggests that this motif is surface exposed in hA3G. The hA3G molecular envelope restoration demonstrated that it dimerized through interactions within the C terminus and has an elongated conformation wherein the CRS is predicted to be exposed to solvent (77).

APOBEC family members such as APOBEC-1 and AID exert their activities in the cell nucleus but their entry into the nucleus is regulated through tissue-specific and temporal expression (4, 8–12) and through interactions with cytoplasmic proteins as molecular chaperones (15, 20–23). Given that dysregulation of APOBEC-1 and AID results in promiscuous editing activity that leads to cancer (26–32), the presence of CRS in hA3G that lacks an NLS may have been selected because this combination in hA3G (and perhaps hA3F) ensures that sufficient protein to saturate binding to retro(viral/element) RNAs can be expressed while ensuring that hA3G cannot diffuse into the nucleus and induce genomic hypermutations. It is of interest in this regard that the nuclear paralog hA3B mRNA is expressed at low to no levels in normal tissue types but highly expressed in cancer cell lines (1, 78).

Identification of the hA3G CRS is significant because the well studied antiviral activity of hA3G is a cytoplasmic phenomenon (40, 45–48). The block to HIV-1 reverse transcription, hypermutation of ssDNA replicating HIV-1 proviral DNA and assembly with HIV-1 virions all occur in the cytoplasm (33, 34, 37, 57, 64–66). In fact, the ability of hA3G to sequester endogenous retroelement RNAs in the cytoplasm has been proposed as a mechanism to inhibit their reverse transcription and genomic re-integration of retrotransposable intermediates (42).
and although we show that robust RNA binding activity is not required for cytoplasmic retention perhaps a specific RNA (i.e. miRNA or retroelement) requires the CRS motif to bind hA3G and sequester it in the cytoplasm.

HIV-1 Vif interacts with hA3G in the cytoplasm to induce its ubiquitination and degradation (53–56). In fact, Wichroski et al. (47) showed that Vif and a degradation-deficient mutant thereof (C114S) were predominantly nuclear in the absence of hA3G, but when co-expressed with hA3G were redistributed to the cytoplasm. This provides further evidence for the dominance of the CRS within hA3G and supports the coexistence of hA3G CRS function and Vif binding.

The identification of the hA3G CRS has important implications for the development of anti-HIV-1 therapeutics that target the interaction of Vif and hA3G. The regions of hA3G that are involved in Vif binding and Gag binding overlap with the CRS (69–72). Viral packaging is central to the antiviral mechanism of action for hA3G and therefore therapeutics must be selected that block the interaction of Vif and hA3G, while maintaining hA3G CRS function and viral packaging. Alanine-scan mutagenesis studies of this region by Huthoff and Malim (72) showed that amino acids 124–127 within the CRS are crucial for viral packaging of hA3G while amino acids 128–130 at the end of the CRS and just downstream are critical for interaction with Vif. This suggests that therapeutics targeting the hA3G region just downstream of the CRS are more likely to prevent Vif interaction and thwart HIV infectivity while therapeutics that target the CRS that contains residues that are critical for viral packaging could be detrimental to both the cellular and antiviral activities of hA3G. In fact Huthoff and Malim (72) suggest that mutagenesis within this region of hA3G and analysis of Gag and RNA binding do not fully explain why mutations in this region do not package efficiently into the virus, and they suggest that the motif could participate in determining subcellular localization as we show here.

Ongoing analyses will determine which amino acids within the CRS are crucial for cytoplasmic localization and cellular and antiviral functions. While RNA bridges link hA3G to other proteins, the lack of an RNA requirement for cytoplasmic localization suggests that hA3G binds directly to one or more cytoplasmic proteins that confer cytoplasmic retention of hA3G. Identification of hA3G CRS-interacting proteins is crucial to our understanding of the regulation of hA3G interactions and functions.

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