

[ScienceWatch Home](#)
[Interviews](#)
[Featured Interviews](#)
[Author Commentaries](#)
[Institutional Interviews](#)
[Journal Interviews](#)
[Podcasts](#)
[Analyses](#)
[Featured Analyses](#)
[What's Hot In...](#)
[Special Topics](#)
[Data & Rankings](#)
[Sci-Bytes](#)
[Fast Breaking Papers](#)
[New Hot Papers](#)
[Emerging Research Fronts](#)
[Fast Moving Fronts](#)
[Research Front Maps](#)
[Current Classics](#)
[Top Topics](#)
[Rising Stars](#)
[New Entrants](#)
[Country Profiles](#)
[About Science Watch](#)
[Methodology](#)
[Archives](#)
[Contact Us](#)
[RSS Feeds](#)
[Inside This Month...](#)

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2008 : October 2008 - Fast Breaking Papers : Yasumasa Iwatani & Judith G. Levin

FAST BREAKING PAPERS - 2008

October 2008


Yasumasa Iwatani & Judith G. Levin talk with *ScienceWatch.com* and answer a few questions about this month's Fast Breaking Paper in the field of Biology & Biochemistry. The authors have also sent along a PowerPoint presentation of their work.



Article Title: Deaminase-independent inhibition of HIV-1 reverse transcription by APOBEC3G

Authors: Iwatani, Y; Chan, DSB; Wang, F; Maynard, KS; Sugiura, W; Gronenborn, AM; Rouzina, I; Williams, MC; Musier-Forsyth, K; Levin, JG

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(addresses have been truncated)

SW: Why do you think your paper is highly cited?

The human protein APOBEC3G (A3G), a cytidine deaminase, was identified as a host defense factor that blocks HIV-1 reverse transcription and virus replication in the absence of the viral protein Vif. A3G is currently under active investigation by a large group of researchers because it has potential application in AIDS therapy. For this reason, work in the field is focused on elucidating the mechanism of A3G's antiviral activity and papers that contribute new insights into this process receive a lot of attention.

Most of the studies reported in the literature have been performed primarily with cell-based systems and/or with unfractionated enzyme derived from viral lysates. Since it is difficult to dissect individual events that occur during the course of virus infection in cells, we chose a more biochemical approach to clarify the effect of A3G on reverse transcription at the molecular level.

To perform these experiments, we took advantage of defined biochemical assay systems that we developed over the years for studies on viral DNA synthesis and used purified proteins: A3G (see below); HIV-1 reverse transcriptase (RT); and HIV-1 nucleocapsid protein (NC), which we and others have shown to increase the efficiency and specificity of reverse transcription.

Our A3G paper was the first to show that A3G inhibits all RT-catalyzed DNA elongation reactions, but not RNase H activity or NC's ability to



Coauthor
Judith G. Levin

promote annealing. These results could be explained by critical differences in the nucleic acid binding properties of A3G, RT, and NC, as measured by single-molecule DNA stretching and fluorescence anisotropy. Figure 1 shows how A3G binding to single-stranded nucleic acid acts as a roadblock to RT-catalyzed DNA polymerization during reverse transcription.

SW: Does it describe a new discovery, methodology, or synthesis of knowledge?

This work describes a new discovery. Since A3G's deaminase activity was not required for inhibition of polymerization reactions in our system, our findings provide a novel mechanism for deaminase-independent A3G-mediated antiviral activity that has also been observed in infected cells. This type of mechanism might also explain how A3G and other members of the human APOBEC3 family inhibit replication of retrotransposons, Hepatitis B virus, and Adeno-associated virus, without a requirement for deamination.

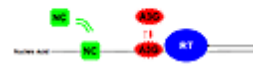
SW: Would you summarize the significance of your paper in layman's terms?

All animals, including humans, have cellular defense factors, which fight viral and bacterial infections that are harmful to the host. Often these defense factors are not enough and drugs (or vaccination) must be employed to obtain a favorable outcome. This is also the case for HIV-1 infection.

In 2002, scientists in the United Kingdom identified a human protein, called APOBEC3G (A3G), which can block HIV replication under certain conditions. Since A3G is a potential inhibitor that could be used in AIDS therapy, this protein is the subject of intense investigation in the field. A3G is a "cytidine deaminase," which means it is an enzyme that converts cytidine residues (one of the four bases in DNA) into uridines (not a normal constituent of DNA).

Figure 1:

Roadblock Mechanism for Deaminase-Independent Inhibition of HIV-1 Reverse Transcription by A3G



Viewing options and description [below](#).

There is strong evidence indicating that deaminase activity is crucial for A3G's antiviral activity. However, a portion of the antiviral activity can also be deaminase-independent. We investigated the mechanism for A3G's inhibitory effect using purified proteins and a cell-free system to study the relationship between A3G and viral DNA synthesis. The data showed that inhibition was deaminase-independent in this case.

Based on the binding properties of the key proteins in our system, we concluded that when A3G binds to single-stranded DNA or RNA templates, it acts as a roadblock to hinder polymerization reactions that occur during reverse transcription (see Figure 1). This is the first study to

propose a mechanism for the deaminase-independent antiviral activity of A3G.

SW: How did you become involved in this research, and were there any problems along the way?

Our group has been engaged in research on reverse transcription for many years. We initiated the A3G project because of our strong interest in host factors that influence this process. Moreover, we were intrigued by early work showing that in the absence of the viral protein Vif, a cellular factor inhibits HIV-1 reverse transcription. This factor later turned out to be human A3G.

We decided to take advantage of the reconstituted systems that we had developed for our studies on HIV-1 reverse transcription to determine the mechanism of A3G inhibition. To do this, we had to first produce large amounts of catalytically active, highly purified A3G. Our decision to express and purify A3G was an enormous challenge, since we knew that many people had tried to do this and failed. We also endured fruitless attempts to obtain functional protein from *E. coli*.

The key to success turned out to be the use of a baculovirus expression system and the total commitment and determination of Dr. Yasumasa Iwatani, then a postdoctoral fellow in the laboratory, who performed this work. The molecular characterization of the human A3G protein is described in Iwatani *et al.*, "Biochemical Activities of Highly Purified, Catalytically Active Human APOBEC3G: Correlation with Antiviral Effect," *J. Virol.* 80:5992-6002, 2006.

SW: Where do you see your research leading in the future?

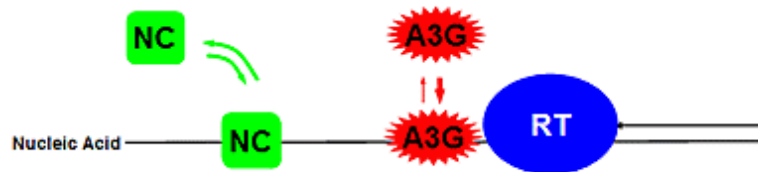
In our work on A3G thus far, we have demonstrated that the availability of a pure protein (uncontaminated by either host or viral proteins) has made it possible to perform a rigorous analysis of A3G's molecular properties, which complements *in vivo* assays that measure antiviral activity. The success of this approach has encouraged us to pursue additional biochemical studies of A3G, in conjunction with cell-based mutational analysis and structure-function studies.

SW: Do you foresee any social or political implications for your research?

The global AIDS pandemic continues to endanger the health of people worldwide and it is now estimated that 40 million people are currently infected with HIV. Research on A3G is critical for developing new antiviral strategies to combat this devastating disease. To achieve this goal, it is urgent that we obtain a more detailed understanding of A3G's antiviral activity and identify new targets for drug discovery and design. It is also vital to have sufficient funding to ensure the success of these efforts.

Figure 1:

Roadblock Mechanism for Deaminase-Independent Inhibition of HIV-1 Reverse Transcription by A3G



- Unlike A3G, RT has poor binding affinity to single-stranded nucleic acids. Thus, RT cannot readily displace A3G once it is bound to the single-stranded RNA or DNA template and RT-catalyzed DNA elongation is blocked.
- NC binding to nucleic acids exhibits a high on-off rate and NC function is not affected by A3G.

A3G, APOBEC3G
RT, reverse transcriptase
NC, nucleocapsid protein

Iwatani et al. *Nucleic Acids Res.* 35:7096 (2007)

NOTE: This figure is available in an animated PowerPoint file. When viewing, use "Slide Show" option.

[back]

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back to top

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