October 12, 2010

To:
Dr. Thomas Croxton,
NHLBI

Dear Dr. Croxton

Re: 1 R21 HL095948-01A1

Sometime ago, I had appealed the review of my R21 application (referenced above) and the decision not to fund my application based on those reviews (Appendix 1, pages 4-8).

The response to that is enclosed in Appendix 2, page 9.

I am sending a continuation of the appeal since the major premise and promise has been demonstrated by several publications (Appendices 3 and 4, pages 10 to 16). In other words, (i) my frontline thinking on the relevance to humans, (ii) utility of an approach that was new when the proposal was submitted (small RNA sequencing based discovery of novel viruses), and (iii) the need to build a model system with such unique advantages as was proposed by me (this is a continuing need), were all missed by that review board.

I had written that R21 for the study of "**Cross regulation of divergent host responses to viral and bacterial pathogens**".

The main premises were:

(i) Need for a simplified model system to study cross-regulation between RNA silencing and other arms of innate immunity, that I proposed exist in mammalian systems and is of importance to viral immunity (and multi-pathogen infections), despite no experimental evidence at that time. The use of model system was justified by the fact that one confounding layer (adaptive immunity) is removed from the analysis to uncover this proposed cross-regulation. Further, I had proposed using a multi-pathogen infection models using virus and bacterial pathogens infecting these model hosts. This was meant to address multi-factorial molecular processes rather than utilizing response to a single molecules causative of a process or in-depth study using host mutants in the dominant arms of the two diverse immune processes. And, I had chosen systems that were not grossly affected in physiology (in fact, in one case with clear evidence for lack of any discernible effect at an organismal scale).

(ii) Despite lack of experimental evidence and debate in the field over importance, I put forward a number of forward thinking theoretical analysis of fragments of data and extensions from other modes of host-pathogen evolution to suggest that suggested that RNA silencing definitely plays a role antiviral

immunity in mammals. Further, I posited that it can be uncovered and additional implications can be studied using the simple system I designed with intricate details and unique experimental advantages. The reports published (Appendices 3 and 4, pages 10-16) recently indicates the misinformed and not careful reading of the short "Background" section of the application, where I have clearly stacked all evidences and clearly written forward looking and accurate prediction of the existence of an undiscovered process. I had also clearly highlighted a major unmet need that I was addressing.

I should emphasize that to date no other model with such unique advantages exist. Being an R21 aimed at building the model system that caters such a major need, there were several variables and alternative paths as to specific results and experiments the system could lead to. But in each case the experimental system, specific advantage, and the assays (in many cases already well established) were clearly indicated. What need to be built for proposed study to succeed (when such tool/resource does not exist), and what the clearly defined back-ups were also clearly indicated. For example, a cursory look at Fig.1, Fig. 2 and Fig. 4 of "Research Design" section of the application is sufficient to understand the experimental design, and all the assays are already well established. Newer aspects were proposed to be developed along the way to make these assays high-throughput and automation amenable.

Among other things, two major scientifically misinformed criticisms were on (i) concerns on relevance to human, and (ii) the use and feasibility of small RNA sequencing as an approach to detect novel native viruses of *C. elegans*. (aim 2B in the revised application).

Here I provide evidences (i) addressing the first point above that covers the whole basis of the application and all aspects proposed, and relevance to humans (Appendix 3, pages 10 and 11) and (ii) several publications highlighting the utility and generality of small RNA sequencing based approach to identify viruses in various kingdoms, including mammals. Appendix 4, pages 12 to 16. Again the second aspect also caters to the question of relevance to humans, and if the application were funded the work from this grant would have been the first to show such feasibility and relevance across different kingdoms.

These studies distinctly prove the points I made as the rationale and basis for my proposal in the grant application. As I indicated in the revision and in the "appeal" document, the reviews were not sound with inadequate attention paid to the contents of to the application. This deprived me of an opportunity to be funded and potentially having made leading contributions to this important field of research and to have built a model with enormous utility to address practical and fundamental aspects, by now.

While the damage caused by this misinformed review process on my career cannot be directly addressed, I seek your advise on the procedures the NIH and NHLBI has established as recourse to such failures in the system. With the current emphasis on streamlining and making the review process fair and to reduce the amount of information

that can be included in the application, example like this should definitely help in refining the process better.

I look forward to your reply and advise in this regard.

Sincerely,

Suresh

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28 September, 2009

Dear Dr. Susan Banks-Schlegal

After careful consideration of the fact that the reviews of my R21 application # 1 R21 HL095948-01A1 entitled “Cross regulation of divergent host responses to viral and bacterial pathogens” do not reflect the contents of the application, I have decided to appeal the review. Clarifying some concerns raised in review of the first submission explicitly in the resubmission did not seem to have had any positive effect.

Basically there seem to be an inherent bias against the use of some of the best suited facile experimental model organisms from non-mammalian phyla for the stated goals. In addition, and probably due to this, the reviewers seem to have ignored the details already provided in the application. I base this conclusion on the fact that the referees made numerous criticisms (many technically flawed) based on the above stated misconceptions of the proposed model system. I outline some of these aspects below. Since all the arguments highlighted here are directly taken from the application where there are references cited, I have not used citations in this “Appeal” document.

In this application I proposed to study the cross regulation of two diverse arms of the immune response using well established invertebrate model systems and two classes of pathogens (RNA viruses and bacteria). The primary responses to these two classes of pathogens are through the two divergent arms of the innate immune response. One of them, involves the machinery of RNA silencing (to viral pathogens), and the other (to bacterial pathogens) through the extensively studied innate immune response to pathogen-associated molecular patterns that activates the production of antimicrobial effectors and other host strategies. Activation of the latter arm involves conceptually conserved signaling modules and pattern recognition receptors across different kingdoms.

1. Mammalian/medical relevance not clear

In the application, I proposed that the cross regulation between these arms of immunity is sparsely studied and hence poorly understood. One primary reason for this (that is addressed through the major advantages of the models of this study) is the increased complexity resulting from the interaction between the innate and the classically defined adaptive immune components in mammalian systems. In contrast to mammalian systems, the different model systems that I propose to use have complementary advantages and facilitate the study of the existence of the hypothesized cross regulation that likely hasn’t been recognized due to the above mentioned layers of complexity. Since innate immunity is among the first lines of defense even before adaptive immunity is activated the relevance still holds despite removing that layer of complexity. A number of lines of evidence are provided to substantiate the likelihood and relevance of this

proposed cross regulation. Prominent among them being the facts that (i) host microbe interactions co-evolve to negate each others advantages, (ii) the fact that there is continually increasing evidence of host and viral encoded miRNA affecting the outcome of viral pathogenesis, and (iii) there is direct evidence that where mammalian viral components interfere with both the interferon response (well established mammalian antiviral response) as well as RNA silencing. Though a prominent role for RNAi is still a matter of debate in mammalian systems, the RNA silencing machinery (that includes RNAi, miRNA mediated processes and some aspects of epigenetic regulation) include some shared and other structurally related components in the different kingdoms. Besides emphasizing this aspect in several sections of the application with appropriate examples and references, I also pictorially depict this rationale in Fig. 1 where I represented the commonality by using the term “silencesome” and included the different aspects mentioned above to further emphasize this. In the case of bacterial pathogens it has recently been demonstrated that they harbor effectors (delivered to the host through type III secretion system into the host) that interfere with RNA silencing machinery. Thus the proposal to test cross regulation between these two arms and the premonition that it has not yet been uncovered in mammals due to the complexities of the mammalian immune response is within the norms of scientific reasoning.

2. Rationale for use of multiple models and multi-pathogens not clear

It has been shown from numerous studies (including many leading contributions from Prof. Fred Ausubel’s laboratory that I am currently affiliated with) that the two invertebrate models have utility in highlighting different aspects of host-pathogen responses including innate immunity, and in many cases have direct utility in design and study of those aspects relevant to mammalian systems. In several instances, shared components of mammalian and agriculturally relevant pathogens have been unambiguously demonstrated. As to the question of rationale for using multiple pathogens (simultaneously or sequentially), I have explicitly stated that the use of viral and bacterial pathogens *per se* as opposed to known components or mutants when possible should highlight more aspects than the use of mutants in currently known dominant pathways. In accordance with the stated purpose of the RFA “Novel Approaches To Study Polymicrobial Diseases “ and of R21 applications, I propose exploration and development of model systems with unique advantages to the study of changes in outcome during multi-pathogen infections at the same site or at different sites with emphasis on cross regulation of the two divergent arms of immunity. The above mentioned studies address the question of relevance of these models in the study of diseases of mammalian systems. The previous studies that are still in progress deal with individual pathosystems, while this proposal is aiming to take it to the next higher level of complexity. Many aspects of biology have benefited enormously by the use of model systems, sometimes quite distant from mammalian biology (that the reviewer considers artificial). To cite an example from one of my personal contributions, I demonstrated during an earlier

postdoctoral experience by (artificially) expressing an avirulence gene product of a bacterial pathogen transgenically in plants that the site of action of such proteins is inside the (model) host plant cell even though the bacteria never invade the host. This paved way to the first published report of now what is considered a major demonstration of commonalities between plant and mammalian pathogens – the conservation of type III secretion systems.

Additionally, the understanding of the breadth of knowledge generated using these models and the thought process as to how to maximally exploit this understanding has led me to the different aspects of the intricate experimental design that I propose. In each case, it is clearly stated in the text as to what that advantage or knowledge is that can be exploited using that particular system and why that choice of system or design is specially suited is explicitly stated. This again points to the fact that a predetermined judgment against the use of these model systems for these kind of studies and that a lack of time and/or familiarity with these models played a major role in eliciting the negative responses from these reviewers. If the infection model involving *C.elegans* and TEV is a success then the unique advantage of having the ability to use same pathogen in two divergent hosts will be self-evident.

Questions from the referees such as: if a signal is found what relevance it would have to mammalian systems or and if a natural virus is found by deep sequencing of field isolates of *Caenorhabditis* sp. it may not be propagatable, further emphasize the flaws in the referees' reasoning. In the first case one wouldn't know until one finds a signal and tests if conceptually, structurally or modularly similar mammalian signals also exist. As to the second question, it is like questioning the deep sequencing of the gut microbiome or of a deep sea sampling of microbes – since most of the microbes cannot be cultured, what is the point? In fact, however, these deep sequencing projects have led to enormous interest and have shown great potential to develop novel and essential advances.

3. Lack of experimental detail, feasibility difficult to assess

In each case, the experimental design is depicted pictorially as to what is being tested and how. As to outcomes: (i) all of these are extensively studied models for single pathogen infections – thus the assays of infection are very well established, (ii) there is a section devoted to clearly how and what will be evaluated, even though many are well established protocols, (iii) in addition there are proposed new adaptations to these procedures that will be developed during the course of the proposal that would capitalize on newer developments in technologies. The overall goal to study the effect of pre- or concomitant infection of one pathogen on the other that uses these well established assays. In addition, even if the new model involving TEV - *C.elegans* is not successful, there are other backup viral infection systems from previous studies (incorporated into the experimental design) that will be modified to suit the goals

of this study. These alternate infection systems (proposed as backups) have to be modified because they are not in the right format to address the questions of this proposal – though they served to answer the questions those studies were addressing.

In every case I have stated what could pose difficulty and how it will be overcome or circumvented. Since it is an exploratory proposal testing the effect of one pathogen infection on the other under specific conditions, the exact phenotypic outcome cannot be predicted in advance in many cases.

Given the fact that I have published evidence for experience with all aspects of the experimental design, the feasibility of testing the effect of one pathogen on other should not be a question. A reviewer questioned if I have experience with viruses. I have worked with TEV, and other viruses (including TuMV) and their interactions with hosts including a high-throughput Arabidopsis mutant screen for two years in the laboratory of James Carrington (whose lab has carried out bulk of the published work on molecular aspects of TEV over the years). In addition I have established the system in my current location, which accounts to over four years of experience with that system. The relevant information is in the Biosketch and in the publication list. In the case of worm-bacteria interactions, I do not have publications yet, but I have been doing these assays on and off over the last two and half years at the Ausubel laboratory. Evidence to this effect can be inferred from preliminary experiments included in the application. People from various parts of the world come to this laboratory for a couple of weeks to a couple of months to learn worm pathogen assays and go back and establish the system in their own laboratories, evidence that this is not a daunting task.

Another example of the referees' failure to read the proposal carefully is their questioning whether TEV-GUS infects *C. elegans* -- a question that is being addressed by the proposal. The rate of whole or significant parts of the worm showing GUS activity (controls don't under these conditions) is 5-10%. 'Mutants not named in figure legends' is a factually incorrect statement. The only relevant mutant to be named in any of the figures is *rde-1* in Fig. 5 and it is mentioned. Adding specific mutant names in the other figures that depict appropriate mutant genotypes to be included in the experimental design do not serve any purpose at this stage of the proposal. The only study related to the proposal that was published between the two submissions of this application came to a conclusion preinfection with TuMV makes the host more susceptible to a bacterial challenge. I explicitly state that TuMV causes extensive phenotypes, though not apparent at that time point used in that study (an example can be seen in my published article that has both these viral infections) and that TEV is unlike TuMV. The reviewer again questioned that no visual symptom does not mean extensive physiological compromise. A lot of evidence including my extensive qualitative study clearly indicate infection of Arabidopsis with TEV does not have any such drastic effect (including subsequent growth and development or qualitative yield of progeny seeds). Such non-drastic changes in host physiology prevailing during

subsequent challenge is likely to be more relevant than otherwise, and makes this a very attractive model.

I would appreciate appropriate remedial action in this issue and getting my application funded.

Sincerely,

Suresh

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DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Heart, Lung, and
Blood Institute
Bethesda, Maryland 20892

December 20, 2009

Suresh Gopalan, Ph.D.
Department of Molecular Biology
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Re: 1 R21 HL095948-01A1

Dear Dr. Gopalan:

The information that you sent in your letter of September 28, 2009 regarding your grant application referenced above has been considered by the National Heart, Lung, and Blood Institute staff and we concur with the recommendations of the initial review group. This was based on concerns outlined in the summary statement. Notably, lack of responsiveness to the prior review and clear rationale for the planned experiments; lack of detail about experimental methods, data processing and interpretation; uncertainty as to whether the results would be interpretable; and concern that findings would not translate into humans.

Although you responded to some of these issues in your letter, we believe there is a fundamental difference in scientific judgment and not evidence of a flawed review.

If you have any questions, please do not hesitate to contact Thomas Croxton, M.D., Branch Chief, Airway Biology and Disease Program (croxtont@nhlbi.nih.gov).

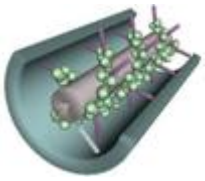
Sincerely,

James Kiley, Ph.D.
Director
Division of Lung Diseases

People Also Have Antiviral 'Plant Defences'

User Rating : / 0

Tuesday, 28 September 2010 05:15



In addition to known antiviral agents such as antibodies and interferons, people also seem to have a similar immune system to that previously identified in plants. This is the result of research carried out by Esther Schnettler at Wageningen University. Together with the group of Professor Ben Berkhout of the Academic Medical Centre (AMC) in Amsterdam, Schnettler discovered that a protein used by plant viruses to bypass plant resistance can also impair the defence against HIV viruses in people. Schnettler's findings may open up new opportunities for improving health.

Plants defend themselves against viruses by attacking, deactivating and breaking down genetic material in a process called RNA silencing. Viruses try to bypass this defence by producing proteins that block it. Schnettler researched the functioning of these silencing suppressor proteins in plants, recognising that the improvement of plant defences would enable more sustainable cultivation by reducing the need for chemical pesticides to combat insects and pathogens.

Schnettler also studied whether the silencing suppressor proteins that allow plant viruses to bypass plant defences could also have an influence on our immunity systems. We know that antibodies can detect the protein shells of viruses, which allow them to be broken down. Our bodies also protect themselves against viruses by releasing interferons that give a sign to cells to die, preventing the viruses within those cells from multiplying or spreading.

In cooperation with a group of scientists from the AMC, Schnettler found that HIV mutants which are unable to produce a specific protein (making it almost impossible for them to multiply) can start multiplying up to wild type virus titer levels when a silencing suppressor protein from a plant virus is added. This seems to suggest that people also have the defence against viruses used by plants against intruders and which detects and deactivates the genetic material of the HIV virus.

"The research has helped us to understand that the process of RNA silencing seems to be a widely occurring antiviral defence," says Schnettler. "Our findings could offer new opportunities for developing antiviral medication. This is not yet certain, however, as the RNA silencing process in the human body has (additional) other functions that must not be impaired by medicines."

Sources: Wageningen University and Research Centre, AlphaGalileo Foundation.

EMBO Rep. 2009 Mar;10(3):258-63. Epub 2009 Feb 13.

The NS3 protein of rice hoja blanca virus complements the RNAi suppressor function of HIV-1 Tat.

Schnettler E, de Vries W, Hemmes H, Haasnoot J, Kormelink R, Goldbach R, Berkhout B.

Laboratory of Virology, Wageningen University, Binnenhaven 11, 6709 PD Wageningen, The Netherlands.

Abstract

The question of whether RNA interference (RNAi) acts as an antiviral mechanism in mammalian cells remains controversial. The antiviral interferon (IFN) response cannot easily be distinguished from a possible antiviral RNAi pathway owing to the involvement of double-stranded RNA (dsRNA) as a common inducer molecule. The non-structural protein 3 (NS3) protein of rice hoja blanca virus (RHBV) is an RNA silencing suppressor (RSS) that exclusively binds to small dsRNA molecules. Here, we show that this plant viral RSS lacks IFN antagonistic activity, yet it is able to substitute the RSS function of the Tat protein of human immunodeficiency virus type 1. An NS3 mutant that is deficient in RNA binding and its associated RSS activity is inactive in this complementation assay. This cross-kingdom suppression of RNAi in mammalian cells by a plant viral RSS indicates the significance of the antiviral RNAi response in mammalian cells and the usefulness of well-defined RSS proteins.

PMID: 19218918 [PubMed - indexed for MEDLINE]PMCID: PMC2658557

Cell Host & Microbe Volume 8, Issue 1, 22 July 2010, Pages 12-15

Viral Suppressors of RNA-Based Viral Immunity: Host Targets

Qingfa Wu[1, 2], Xianbing Wang[1, 2] and Shou-Wei Ding[1, 2],

Does VSR Activity Play a Role in Mammalian Viral Infection?

Many mammalian viruses encode a VSR (Li and Ding, 2006). However, it is not entirely clear if the VSR activity has a specific role in mammalian viral infection because rescue of VSR-deficient mutant viruses in mammalian host cells defective in RNA silencing is yet to be demonstrated. Using an indirect approach, two recent studies ([Qian et al., 2009] and [Schnettler et al., 2009]) investigated the role of VSR during infection of human immunodeficiency virus (HIV). HIV was reported to encode a putative VSR, Tat (Bennasser et al., 2005), but this conclusion has been debated by a subsequent study carried out by another lab (Lin and Cullen, 2007). A known function of Tat is to enhance transcription of HIV RNA from the integrated proviral DNA by binding to an internal stem-loop structural element of HIV RNA. However, HIV gene expression in infected cells requires a transcriptional enhancer-independent activity of Tat, and this activity could be substituted by two distinct plant VSRs: the tombusviral P19 and the NS3 protein of rice hoja blanca virus ([Qian et al., 2009] and [Schnettler et al., 2009]). NS3 has the same affinity for siRNA duplexes as P19, and neither sequesters long dsRNA, which in vertebrates is recognized as a pathogen-associated molecular pattern and activator of multiple innate immunity pathways. Notably, NS3/P19 mutants defective in siRNA binding were also unable to rescue HIV gene expression in infected mammalian cells ([Qian et al., 2009] and [Schnettler et al., 2009]). These findings suggest that HIV infection requires suppression of small RNA-directed gene silencing, which is consistent with previous observations that knockdown of Dicer enhances virus accumulation in mammalian host cells ([Matskevich and Moelling, 2007], [Otsuka et al., 2007] and [Triboulet et al., 2007]).

Future studies will be necessary to determine if Tat and other mammalian VSRs target RNA silencing induced by small RNAs of either viral or host origin. It is known that mammalian viral infection can induce production of virus-derived miRNAs and alter the expression profile of cellular miRNAs (Skalsky and Cullen, 2010). Moreover, although early studies based on standard RNA sequencing protocols were not successful, a recent survey in a wide range of mammalian host systems by deep sequencing has identified low abundant virus-derived small RNAs from several distinct RNA viruses (Parameswaran et al., 2010). Notably, the newly cloned viral small RNAs contain a subpopulation with features of siRNAs similar to those detected in plant and invertebrate hosts, including approximately equal positive and negative strand ratios, pairs of siRNA duplexes with one or two unpaired nucleotides at the 3′ ends, and association with AGO proteins in vivo (Parameswaran et al., 2010). These studies provide experimental systems for future rigorous exploration of the role of mammalian VSRs and virus-derived small RNAs in the RNA-based virus immunity. VSR mechanisms and host targets identified from studies in invertebrate and plant systems could provide useful guiding principles for future mammalian studies.

Proc Natl Acad Sci U S A. 2010 Jan 26;107(4):1606-11. Epub 2010 Jan 4.

Virus discovery by deep sequencing and assembly of virus-derived small silencing RNAs.

Wu Q, Luo Y, Lu R, Lau N, Lai EC, Li WX, Ding SW.

Department of Plant Pathology and Microbiology, Institute for Integrative Genome Biology, University of California, Riverside, CA 92521, USA.

Comment in:

- Cell Host Microbe. 2010 Feb 18;7(2):87-9.

Abstract

In response to infection, invertebrates process replicating viral RNA genomes into siRNAs of discrete sizes to guide virus clearance by RNA interference. Here, we show that viral siRNAs sequenced from fruit fly, mosquito, and nematode cells were all overlapping in sequence, suggesting a possibility of using siRNAs for viral genome assembly and virus discovery. To test this idea, we examined contigs assembled from published small RNA libraries and discovered five previously undescribed viruses from cultured Drosophila cells and adult mosquitoes, including three with a positive-strand RNA genome and two with a dsRNA genome. Notably, four of the identified viruses exhibited only low sequence similarities to known viruses, such that none could be assigned into an existing virus genus. We also report detection of virus-derived PIWI-interacting RNAs (piRNAs) in Drosophila melanogaster that have not been previously described in any other host species and demonstrate viral genome assembly from viral piRNAs in the absence of viral siRNAs. Thus, this study provides a powerful culture-independent approach for virus discovery in invertebrates by assembling viral genomes directly from host immune response products without prior virus enrichment or amplification. We propose that invertebrate viruses discovered by this approach may include previously undescribed human and vertebrate viral pathogens that are transmitted by arthropod vectors.

PLoS Pathog. 2009 Feb;5(2):e1000286. Epub 2009 Feb 6.

An RIG-I-Like RNA helicase mediates antiviral RNAi downstream of viral siRNA biogenesis in Caenorhabditis elegans.

Lu R, Yigit E, Li WX, Ding SW.

Department of Plant Pathology & Microbiology, University of California, Riverside, CA, USA.

Abstract

Dicer ribonucleases of plants and invertebrate animals including Caenorhabditis elegans recognize and process a viral RNA trigger into virus-derived small interfering RNAs (siRNAs) to guide specific viral immunity by Argonaute-dependent RNA interference (RNAi). C. elegans also encodes three Dicer-related helicase (drh) genes closely related to the RIG-I-like RNA helicase receptors which initiate broad-spectrum innate immunity against RNA viruses in mammals. Here we developed a transgenic C. elegans strain that expressed intense green fluorescence from a chromosomally integrated flock house virus replicon only after knockdown or knockout of a gene required for antiviral RNAi. Use of the reporter nematode strain in a feeding RNAi screen identified drh-1 as an essential component of the antiviral RNAi pathway. However, RNAi induced by either exogenous dsRNA or the viral replicon was enhanced in drh-2 mutant nematodes, whereas exogenous RNAi was essentially unaltered in drh-1 mutant nematodes, indicating that exogenous and antiviral RNAi pathways are genetically distinct. Genetic epistatic analysis shows that drh-1 acts downstream of virus sensing and viral siRNA biogenesis to mediate specific antiviral RNAi. Notably, we found that two members of the substantially expanded subfamily of Argonautes specific to C. elegans control parallel antiviral RNAi pathways. These findings demonstrate both conserved and unique strategies of C. elegans in antiviral defense.

Virology. 2009 May 25;388(1):1-7. Epub 2009 Apr 23.

Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses.

Kreuze JF, Perez A, Untiveros M, Quispe D, Fuentes S, Barker I, Simon R.

Germplasm Enhancement and Crop Improved Division, Applied Biotechnology Laboratory, International Potato Center, Apartado, Lima 12, Peru. j.kreuze@cgiar.org

Abstract

We report the first identification of novel viruses, and sequence of an entire viral genome, by a single step of high-throughput parallel sequencing of small RNAs from diseased, as well as symptomless plants. Contigs were assembled from sequenced total siRNA from plants using small sequence assembly software and could positively identify RNA, ssDNA and dsDNA reverse transcribing viruses and in one case spanned the entire genome. The results present a novel approach which cannot only identify known viral pathogens, occurring at extremely low titers, but also novel viruses, without the necessity of any prior knowledge.

PMID: 19394993 [PubMed - indexed for MEDLINE]

PLoS Pathog. 2010 Feb 12;6(2):e1000764.

Six RNA viruses and forty-one hosts: viral small RNAs and modulation of small RNA repertoires in vertebrate and invertebrate systems.

Parameswaran P, Sklan E, Wilkins C, Burgon T, Samuel MA, Lu R, Ansel KM, Heissmeyer V, Einav S, Jackson W, Doukas T, Paranjape S, Polacek C, dos Santos FB, Jalili R, Babrzadeh F, Gharizadeh B, Grimm D, Kay M, Koike S, Sarnow P, Ronaghi M, Ding SW, Harris E, Chow M, Diamond MS, Kirkegaard K, Glenn JS, Fire AZ.

Department of Microbiology & Immunology, Stanford University School of Medicine, Stanford, California, United States of America.

Abstract

We have used multiplexed high-throughput sequencing to characterize changes in small RNA populations that occur during viral infection in animal cells. Small RNA-based mechanisms such as RNA interference (RNAi) have been shown in plant and invertebrate systems to play a key role in host responses to viral infection. Although homologs of the key RNAi effector pathways are present in mammalian cells, and can launch an RNAi-mediated degradation of experimentally targeted mRNAs, any role for such responses in mammalian host-virus interactions remains to be characterized. Six different viruses were examined in 41 experimentally susceptible and resistant host systems. We identified virus-derived small RNAs (vsRNAs) from all six viruses, with total abundance varying from "vanishingly rare" (less than 0.1% of cellular small RNA) to highly abundant (comparable to abundant micro-RNAs "miRNAs"). In addition to the appearance of vsRNAs during infection, we saw a number of specific changes in host miRNA profiles. For several infection models investigated in more detail, the RNAi and Interferon pathways modulated the abundance of vsRNAs. We also found evidence for populations of vsRNAs that exist as duplexed siRNAs with zero to three nucleotide 3' overhangs. Using populations of cells carrying a Hepatitis C replicon, we observed strand-selective loading of siRNAs onto Argonaute complexes. These experiments define vsRNAs as one possible component of the interplay between animal viruses and their hosts.