Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Fox Chase Cancer Center

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 – 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Maria Gill, M.B.A.

4. **Grant Contact Person's Telephone Number:** 215-728-2659

5. **Grant SAP Number:** 4100050895

6. **Project Number and Title of Research Project:** 5 - Reducing the Burden of Hepatocellular Carcinoma: Identification of New Targets against Hepatitis B virus (HBV) for Drug Development

7. **Start and End Date of Research Project:** 1/1/2010 – 6/30/2012

8. **Name of Principal Investigator for the Research Project:** Christoph Seeger, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $436,157

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Seeger, Christoph	Principal Investigator	15% Years 1-2	$64,069.48
Sohn, Ji	Technical Specialist	100% Years 2-3	$69,373.18
Goodman, Jason	Scientific Technician	100% Year 1	$21,963.17
Miller, Bruce	Scientific Technician	100% Year 2	$36,199.37

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No____X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes____X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Mechanism of CCC DNA synthesis in Hepatitis B virus	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	March 2012	$490,874	$474,810
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____X___ No_____

If yes, please describe your plans: Will apply for R01 application during 2014.

12. Future of Research Project. What are the future plans for this research project?

Investigations on hepatitis B virus CCC DNA formation.

13. **New Investigator Training and Development**. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No__X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. **Recruitment of Out-of–State Researchers**. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No_____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. **Impact on Research Capacity and Quality**. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No_____X_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No_____X____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No___X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. <u>Provide detailed results of the project.</u> Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a <u>DETAILED</u> report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

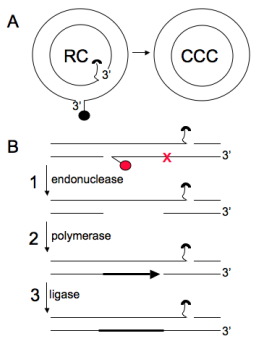

Figure 1. Model for covalently closed circular (CCC) DNA formation. A) Physical structure of relaxed circular (RC) and CCC) DNA. B) The figure depicts the three enzymatic steps required for the formation of CCC DNA. Filled circle; viral reverse transcriptase, half circle; capped RNA primer, X; cleavage site for endonuclease.

A critical step in the hepatitis B virus (HBV) DNA replication cycle is the conversion of relaxed circular (rc) DNA into covalently closed circular (ccc) DNA (Figure 1). Ccc DNA is the template for transcription of the viral RNAs. The exact mechanism for ccc DNA formation is still obscure and represents a major gap in our knowledge of the HBV life cycle. We hypothesize that rc to ccc DNA conversion could rely on cellular enzymes normally involved in base excision repair (BER) of DNA damage caused by UV or DNA adducts formed by certain chemicals. Required activities would include an endonuclease, a cellular DNA polymerase, and a ligase.

Identification of the polymerase required for ccc DNA synthesis: To investigate the mechanism of ccc DNA formation, we developed an *in vitro* system based on frog oocyte extracts, which permits the conversion of rc into ccc DNA. We found that ccc DNA formation depended on ATP and a DNA polymerization step that is inhibited by aphidicolin, but not ddCTP. This observation suggested that ccc DNA formation depends on either polymerase δ or ε, but not β, and on an ATP-dependent ligation reaction. However, we also found that in tissue culture cells, formation of ccc DNA was not inhibited in the presence of aphidicolin, ddCTP or a combination of the two inhibitors. Thus, our results were inconsistent with a dependence on DNA polymerase δ, ε or β in infected cells. Hence, while these results demonstrated that rc DNA can be converted to ccc DNA by the cellular DNA repair machinery under certain *in vitro* conditions, they also demonstrated that hepatitis B viruses are likely to use a different mechanism for ccc DNA formation in a natural infection, such as the one

used for excision of nucleotides that were damaged by UV or as a consequence of lipid peroxidation.

This so called nucleoside excision repair (NER) pathway requires enzymatic activities of endonucleases to excise the damaged DNA, polymerases to fill in the gaps created by the excision and DNA ligase to seal the ends of DNA. To test the hypothesis that HBV employs NER enzymes for ccc DNA synthesis, we used cell culture systems that are permissive for duck hepatitis B virus (DHBV) and HBV replication. To identify enzymes required for ccc DNA formation, we employed commercially available lentiviruses that express short hairpin RNAs (shRNAs) specific for the selected gene. shRNAs inhibit expression of selected proteins by degrading mRNA.

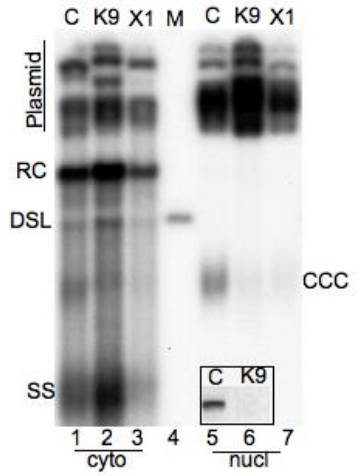

Figure 2. Inhibition of Duck Hepatitis B Virus (DHBV) covalently closed circular (CCC) DNA synthesis in 293T cells expressing short hairpin RNAs (shRNAs) against the DNA polymerase (K9) and the endonuclease (X1). The left panel shows cytoplasmic core DNA, the right panel nuclear CCC DNA The inset shows a western blot developed with Ab to the DNA polymerase.

Using this approach, we have obtained strong experimental evidence for a role of the Y-DNA polymerase, pol κ in the formation of ccc DNA (Figure 2). Inhibition of expression of the polymerase with several shRNAs led to a significant reduction in ccc DNA formation. To validate these observations, we set out to create a HEK293T cell line that is deficient in the expression of pol κ. To accomplish this goal we purchased a zinc finger nuclease (ZFN) that specifically cleaves and inactivates the human pol κ gene. With the help of this reagent, we were successful in isolating two cell clones (293P1, 293P2) with mutations in both alleles of the pol κ gene. Surprisingly, we found that that ccc DNA could still accumulate in the mutant cell line. These results indicated that other polymerases could substitute for polκ in polκ-deficient cells or that the shRNA caused an off-target effect that led to inhibition of ccc DNA synthesis by another mechanism. For example, the off-target effect could have inhibited the retrograde transport of core particles into the cell nucleus, which occurs prior to the formation of ccc DNA.

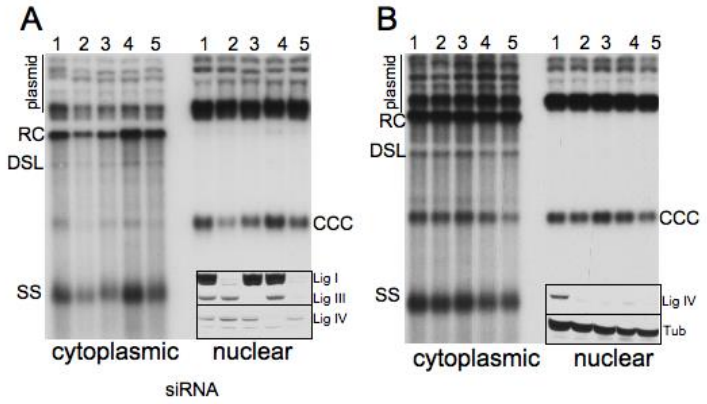

Figure 3. Duck Hepatitis B Virus (DHBV) covalently closed circular (CCC) DNA synthesis in 293T cells. A) Cells were transfected with short interfering RNA (siRNAs) against DNA ligases I,II and IV. The left panel shows cytoplasmic core DNA (relaxed circular (RC), double stranded linear (DSL), single stranded (SS)), the right panel nuclear CCC DNA The inset shows a western blot developed with Ab to each of the three DNA ligases. B) Same as A, except that 293T cells were infected with a lentivirus vector expressing short hairpin RNAs (shRNAs) against DNA ligase IV prior to transfection with DHBV DNA.

We continued our efforts to identify additional polymerases that play a role in ccc DNA synthesis. We continued to focus on Y-DNA polymerases, because we confirmed that ccc DNA synthesis is resistant to aphidicolin, an inhibitor of B-family polymerases (i.e. polymerases delta, epsilon, zeta). Moreover, we found that ccc DNA synthesis was resistant to treatment of cells with hydroxyurea, which is known to inhibit ribonucleotide reductase leading to a lower concentration of the nucleotide pool, which inhibits the enzymatic activity of B-, but not Y-family DNA polymerases. We also investigated a potential role of the Y DNA polymerases, eta and iota that could substitute for polκ in our mutant cell lines. However, we could not attribute a role in ccc DNA synthesis to these enzymes.

Identification of the exonuclease and DNA ligase required for ccc DNA synthesis. Using the same approach as described for the polymerase, we initiated experiments aimed at identification of the relevant endonuclease and DNA ligase required for the repair process. While we have obtained evidence for a role of an endonuclease in ccc DNA formation, our efforts to identify the relevant DNA ligase have not yielded firm results. The mammalian genome encodes only three known DNA ligases, DNA ligase I, III and IV. We have been able to inhibit expression of each of the three ligases with shRNA and Small interfering RNA (siRNAs), but could not demonstrate inhibition of ccc DNA synthesis, even when we suppressed two ligases simultaneously (Figure 3). These results were unexpected. It is conceivable that suppression of one or all ligases was not complete. Moreover, it is known that DNA ligases I and III can substitute for each other.

Overall, our experiments have not yet yielded the desired results. The fact that we have been successful in identifying an shRNA that can suppress ccc DNA formation demonstrated that our experimental approach was in principle correct. However, there are reasons to believe that shRNAs and siRNAs have certain limitations that hampered progress for our research plan. First, it is plausible that several polymerases can participate in the conversion of rc to ccc DNA. Second, it is possible that very low amounts of a polymerase or ligase are sufficient for ccc DNA formation. As a consequence of the information gained from our studies, we have changed our strategy and are using gene knockout technology in lieu of shRNA to identify the genes required for HBV ccc DNA formation.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes

__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in

more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1.None				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____ No_____

If yes, please describe your plans:

After completion of ongoing research we plan to submit a manuscript.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Seeger, Christoph, Ph.D.	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Biocenter, University of Basel, Switzerland	M.S.	06/79	Microbiology
Biocenter, University of Basel, Switzerland	Ph.D.	06/82	Virology
University of California at San Francisco, School of Medicine, San Francisco, CA	Postdoctoral Fellow	06/86	

POSITIONS

Postdoctoral Fellow, Department of Microbiology and Immunology, UCSF	1982-1986
Assistant Professor of Virology, Department of Microbiology, College of Veterinary Medicine, Cornell University, Ithaca, NY	1986-1990
Assistant Professor, Fox Chase Cancer Center, Philadelphia, PA	1990-1993
Associate Professor (with tenure), Fox Chase Cancer Center, Philadelphia, PA	1993-1999
Professor, Fox Chase Cancer Center, Philadelphia, PA	1999-pres.

OTHER EXPERIENCE AND PROFESSIONAL MEMBERSHIPS

Co-Organizer, Molecular Biology of Hepatitis B Viruses	1990
Editorial Board Member, *Virology*	1992-1998
Editorial Board Member, *Journal of Virology*	1994-2006
Permanent Member, Virology B Study Section	2003-2007
Member, American Association for the Advancement of Science	
Member, American Society for Virology	
Member, American Society for Microbiology	

HONORS – SEEGER, CHRISTOPH, Ph.D.

Postdoctoral Fellowship, Roche	1982
Postdoctoral Fellowship, Swiss National Science Foundation	1983
American Society for Virology State of the Art Lecture	1997
Fellow, American Academy of Microbiology	2000
Fellow, American Association for the Advancement of Science	2003

SELECTED PEER-REVIEWED PUBLICATIONS

1. **Seeger, C.**, Ganem, D., Varmus, H.E. Biochemical and genetic evidence for the hepatitis B virus replication strategy. *Science* **232**:477-487, 1986.
2. Wang, G.-H., **Seeger, C.** The reverse transcriptase of hepatitis B virus acts as a protein-primer for viral DNA synthesis. *Cell* **71**:663-670, 1992.
3. Wang, G.-H., **Seeger, C.** A novel mechanism for reverse transcription in hepatitis viruses. *J. Virol.* **67**:6507-6512, 1993.
4. Zoulim, F., **Seeger, C.** Woodchuck hepatitis virus X gene is required for viral infection *in vivo*. *J. Virol.* **68**:2026-2030, 1994.
5. Wang, G.-H., Zoulim, F., Leber, E., Kitson, J., **Seeger, C.** Role of RNA in enzymatic activity of the reverse transcriptase of hepatitis B viruses. *J. Virol.* **68**:8437-8442, 1994.
6. **Seeger, C.**, Leber, E.H., Wiens, L.K., Hu, J. Mutagenesis of a hepatitis B virus reverse transcriptase yields temperature sensitive virus. *Virology* **222**:430-439, 1996.
7. Hu, J., **Seeger, C.** Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **93**:1060-1064, 1996.
8. Hu, J., **Seeger, C.** Hepadnavirus assembly and reverse transcription require a multi-component chaperone complex which is incorporated into nucleocapsids. *EMBO J.* **16**:59-68, 1997.
9. Guo, J.-T., Zhou, H., Liu, C., Aldrich, C., Saputelli, J., Whitaker, R.A., Barrasa, M.I., Mason, W.S., **Seeger, C.** Apoptosis and regeneration of hepatocytes during recovery from transient hepadnavirus infection. *J. Virol.* **74**:1495-1505, 2000.
10. Barrasa, M.I., Guo, J.-T., Saputelli, J., Mason, W.S., **Seeger, C.** Does a cdc2 kinase-like recognition motif on the core protein of hepadnaviruses regulate assembly and disintegration of capsids? *J. Virol.* **75**:2024-2028, 2001.
11. Guo, J.-T., Hayashi, J., **Seeger, C.** West Nile virus inhibits the signal transduction pathway of interferon alpha. *J. Virol.* **79**:1343-1350, 2005.
12. Evans, J.D., **Seeger, C**. Differential effects of mutations in NS4B on West Nile virus replication and inhibition of interferon signaling. *J. Virol*. **81**:11809-11816, 2007.
13. Stiffler, J.D., Nguyen, M., Sohn, J.A., Liu, C., Kaplan, D., **Seeger, C.** Focal distribution of hepatitis C virus RNA in infected livers. *PLoS ONE* **4**:e6661, 2009. PMCID: PMC2722721
14. Sohn, J.A., Litwin, S., **Seeger, C.** Mechanism for CCC DNA synthesis in hepadnaviruses. *PLoS ONE* **4**: e8093, 2009. PMCID: PMC2778999
15. Evans, J.D., Crown, R.A., Sohn, J.A., **Seeger, C.** West Nile Virus infection induces depletion of IFNAR1 protein levels. *Viral Immunol.* **24**:253-263, 2011. PMCID: PMC3154464