Bacteriophage Ecology Group (BEG) News

Dedicated to the *ecology* and *evolutionary biology* of the parasites of unicellular organisms (UOPs)

July 1, 2004 issue (volume 21)

At this site you will find . . .

contents | BEG News (021) | top of page

Editorial

Declining Electron Microscopy

by Hans-Wolfgang Ackermann

Twenty years ago there were many electron microscopes and microscopists. My medical school alone had four Philips EM 300 microscopes. There is now only one, mine. Two others, though in perfect order, were dismantled. The fourth EM was sold to the U.S.A., which says a lot about the budget cuts that Canadian science has been subjected to. The general situation is that both electron microcopes (EM) and electron microscopists are endangered species.

A Huge Drop in Quality

It also used to be that phage papers included reasonably good micrographs. No longer. In the last 10 years standards have plunged and scores of terrible pictures, graced with improbable dimensions or no dimensions at all, have been published. Gone are such fine electron microscopists as D.E. Bradley in Canada, E. Kellenberger in Switzerland, or A.S. Tikhonenko in Russia, who led by example and kept standards up. Many (most?) phage pictures in the present literature are vastly inferior to those published in 1959 by Brenner and Horne, the fathers of negative staining (1).

This decline of EM is not seen in vertebrate and plant virology, where editorial standards are still high and poor electron micrographs and poor descriptions, as they are now frequent in phage papers, would probably be rejected. What happened? The reasons for the decline are manifold:

1. High costs of electron microscopes.
2. Run-away costs of EM service contracts.
3. Shifting of research interests to molecular biology (cloning, sequencing).
4. Retirement of experienced electron microscopists.
5. Disappearance of EM courses.
6. Contract research ('farming out').
7. Soft standards of journals: a reviewer system in disrepair.

'Farming out' of EM to other laboratories is due to the rarefaction of electron microscopes and microscopists, plus the perception of administrators that electron microscopy is a simple service. Nothing could be less true. While 'farming out' to a reputed research laboratory may be acceptable, this is not so in the case of commercial laboratories. It is just unlikely that an ordinary technician, ignorant of the project in question, lacking time and supervision, and trained in, say, pathology,

is able to produce good pictures of bacterial viruses. Yet these laboratories take good money for poor service. The problem is not confined to North America. I have seen terrible examples from Australia.

Since general standards have gone down, reviewers of periodicals, even reputed ones, now frequently tolerate poor pictures. Journals of the American Society of Microbiology are no exception. The situation is particularly bad in environmental microbiology journals.

Why Electron Microscopy?

EM provides instant identification of individual phages, phage families and, very often, genera and species. It cannot be replaced by molecular probes or genome sequencing. Probes will 'catch' part of a phage genome only, the sequencing and annotation of a phage genome may take a year or more, but EM identification may take as little as 1-2 minutes. The domain of EM is the description and identification of novel phages, classification, environmental research, and purity checks and identity controls of phages with practical applications (e.g., therapeutic or typing phages). EM cannot replace molecular biology and cannot be replaced by the latter. Both techniques are complementary. An EM is an expensive precision instrument that, when properly handled, produces data close to the molecular level.

EM pictures are easily archived, directly comparable, permanent documents. In contrast, scientists change institutes, countries, and jobs, retire, or die. It is even worse with phages because, as a rule, phages described more than five years ago can no longer be obtained. Thus, EM pictures are often the only permanent results of a scientist's activity and the only records of past observations. Therefore, good EM is a must.

The Disease

It is now common to see unsharp, astigmatic, low-contrast, or scratched pictures of impure phages, without scale markers or dimensions. Some phages are barely recognizable as such, especially in environmental papers, and some papers just mention 'head-tail phages' without dimensions and micrographs. It is surprising to see such a deterioration because the literature abounds in descriptions of basic electron microscopical techniques. Clearly, this literature is not consulted. Why? Because it is not on the Net?

Staining artifacts (e.g., capsid shrinkage after positive staining with uranyl acetate) are consistently ignored. Some people present capsular slime is as a 'tailed phage,' feel the need to fix phages with glutaraldehyde (useless), or stain with uranyl acetate for 15 minutes (instead of 30 seconds). More seriously, the 'Materials' sections of phage papers are generally silent on phage purification and magnification control. Together with poor pictures, this indicates to the insider that people examined crude lysates and confided into manufacturer indications on magnification (ignoring that the magnification of electron microscopes varies with the electrical current and must be controlled or adjusted).

This kind of 'science' is useless because it produces terrible data and does not help fellow scientists. Concretely, phage workers, who isolate new phages and want to compare their viruses to those of the literature, often face the problem that data from other laboratories cannot be interpreted. It follows that an individual who practices poor EM, and produces pictures which other scientists cannot use, does a disservice to the scientific community.

Diagnosis and Cure

The principal problems seem to be examination of crude or 'dirty' lysates, absence of magnification control, and poor contrast. The first can be addressed by simple washing in a buffer. Phages must be freed from proteins and sugars of the medium. No lengthy density gradient purification is necessary; it suffices to wash the phages 1-2 times in 0.1 M ammonium acetate (best) or phosphate buffer using, for reduction of time and g forces, a medium-sized centrifuge with a fixed-angle rotor; for example, 25,000 g for 1 hour are enough for all but the smallest phages. For magnification control, one must include into each film or cassette load 1-2 pictures of an internal standard. One may use T4 tails (length 113 nm). Then: please measure your phages. It takes a few minutes only. The phages must be so described so that other people can use your descriptions. This is for posterity! The contrast problem is one of basic photography and can be solved by selecting the right photographical papers and developers.

A deadly problem arose a year ago when Kodak Company (Rochester) changed abruptly its production line. Customers were not informed and no explanation was offered. Suddenly, the excellent Ektamatic paper, which lent itself to automatic processing, was no longer available. Kodak representatives could not be contacted or did not know their own products. Fortunately, fellow electron microscopists from the Armand-Frappier Institute near Montreal suggested to me the use of Kodak Polycontrast III RC paper in conjunction with the usual Dektol or the novel Polymax T developers.

My experience is that the paper provides excellent, if sometimes too strong contrast. It allows the salvage of underexposed or underdeveloped films, but grey shades may be lost. All development must be done manually because small table-top Kodak or Ilford processors can no longer be used. The development is followed by a stop bath, fixation and washing as usual, but drying and glazing in a machine are no longer necessary because the paper is resin-coated and naturally glossy, and dries in a short while. Caution: violet smears will develop if the the Dektol developer is not totally neutralized.

(1) Brenner, S. and Horne, R.W. 1959. A negative staining method for high resolution electron microscopy of viruses. Biochim. Biophys. Acta 34:103-110.

Editorial Archive

- Lytic, Lysogenic, Temperate, Chronic, Virulent, Quoi? by Stephen T. Abedon
- Which Ecology are You? by Stephen T. Abedon
- *Science* NetWatch October 13, 2000
- The Best of Times, the Worst of Times by Ry Young
- Naming Bacteriophages by Hans-Wolfgang Ackermann and Stephen T. Abedon
- The Bacteriophage Rise by Stephen T. Abedon
- Mathematics for Microbiologists by Stephen T. Abedon
- Shipping Phages by Hans-Wolfgang Ackermann
- Calling a Phage a "Phage" by Stephen T. Abedon
- Phage or Phages by Hans-Wolfgang Ackermann
- The Phage Manifesto by Ry Young
- The Félix d'Hérelle Phage Center Changes Hands by Hans-Wolfgang Ackermann
- Phage T4 Meets Microbial Diversity by Jim D. Karam
- Phage T1: A lambdoid phage with attitude? by Andrew Kropinski
- ASM Conference on the New Phage Biology
- A Brief History of Phage Art
- Road Trip to Key Biscayne Florida

Editorials should be written on subjects relevant to The Bacteriophage Ecology Group as an organization, to *BEG News* (either the concept or a given issue of *BEG News*), or the science of Bacteriophage Ecology. While my assumption is that I will be writing the bulk of these editorials, I wish to encourage as many people as possible to seek to relieve me of this duty, as often as possible. Additionally, I welcome suggestions of topics that may be addressed. Please address all correspondences to microdude+@osu.edu or to "Editorials," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all submissions as Microsoft Word documents, if possible (I'll let you know if I have trouble converting other document formats), and in English.

contents | BEG News (021) | top of page

New BEG Members

Please welcome our newest members

name (home page links)	status	e-mail	address
Silvana Martin	---	smartin @immunotech-sa.com	Instituto de Investigaciones Biomédicas, Fundación Pablo Cassará, Saladillo 2452 CP 1440, Buenos Aires - Argentina
	interests:	Isolation and characterization of phages for treatment o chronic infections of *Pseudomonas aeruginosa* in cystic fibrosis patients. (contents | BEG members | top of page)	

The BEG members page can be found at www.phage.org/beg_members.htm. There are two ways of "joining" BEG. One, the "traditional" way, is to have your name listed on the web page and on the list server. The second, the "non-traditional" way, is to have your name only listed on the list server. The latter I refer to as "non-members" on that list. Members, e.g., individuals listed on the BEG members list page, should be limited to individuals who are actively involved in science (research, instruction, outreach, industry) and who can serve as a phage ecology resource to interested individuals. If you have an interest in phage ecology but no real expertise in the area, then you should join as a non-member. To join as a member, please contact BEG using the following link: microdude+@osu.edu . Include:

- your name
- your e-mail address
- your snail-mail address
- the URL of your home page (if you have one)
- a statement of whether or not you are the principal investigator
- a statement of your research interests (or phage ecology interests)
- a list of your phage ecology references, if any

Note that it is preferable that you include the full reference, including the abstract, if the reference is not already present in the BEG bibliography. Responsibility of members includes keeping the information listed on the BEG members page up to date including supplying on a reasonably timely basis the full references of your new phage ecology publications. Reprints can also be sent to *The Bacteriophage Ecology Group*, care of Stephen Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. To join BEG as a non-member, please contact BEG using the following link: http://mansfield.ohio-state.edu/mailman/listinfo/beg.

contents | BEG News (021) | top of page

Meetings

Please send photos, etc. from meetings, etc. for inclusion in this section.

Proposed BEG News Recap of ASM Conference on the New Phage Biology

If all goes according to plan then next month's BEG News will be a recap of August's phage meeting in Florida (see last quarter's editorial and phage image for links and details). To aid in putting together this issue, if you were a participant in this meeting:

- Please send Powerpoint versions of your posters or talks to microdude+@osu.edu . If you are concerned about sharing certain portions of the talks or posters then please consider editing your presentations before sending. Modification to a preferred format is also OK, e.g., such as PDF or some sort of locked Powerpoint format. We will take anything we can get, though will prefer the raw data" of the original Powerpoints.
- Please send photographs to microdude+@osu.edu . We prefer electronic versions, and higher resolution to lower, but well take anything we can get. It will be helpful if you can indicate who is found in each photo, but please avoid that step if it impedes your actually sending the photo.

Thanks!

The BEG Meetings link will continue. Reminders of upcoming meetings will be placed in this section of *BEG News*. If you know of any meetings that might be of interest to BEG members, or would like to recap a meeting that you've attended, then please send this information for posting to microdude+@osu.edu or to "BEG Meetings," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906.

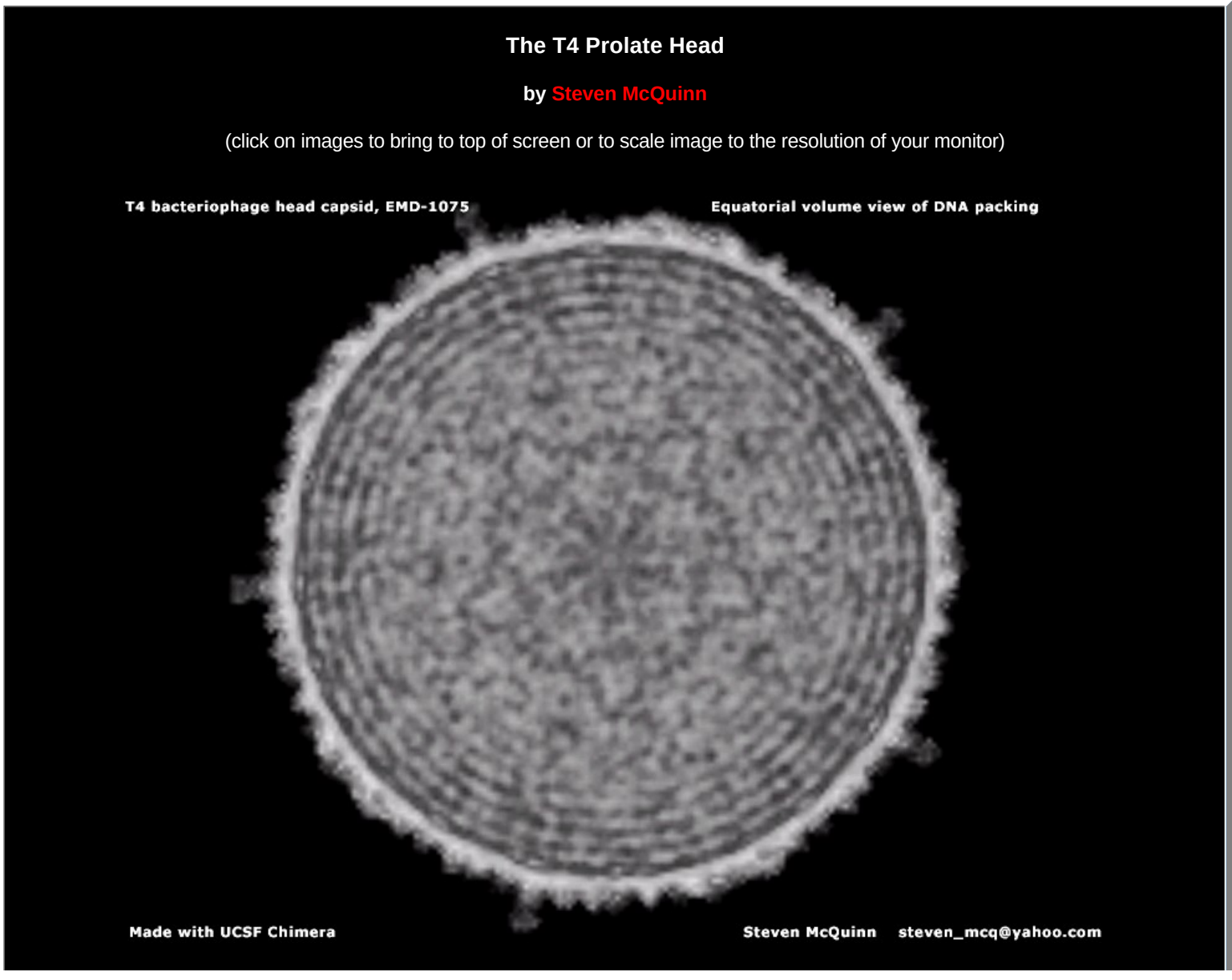
contents | BEG News (021) | top of page

Submissions

The T4 Prolate Head

by Steven McQuinn

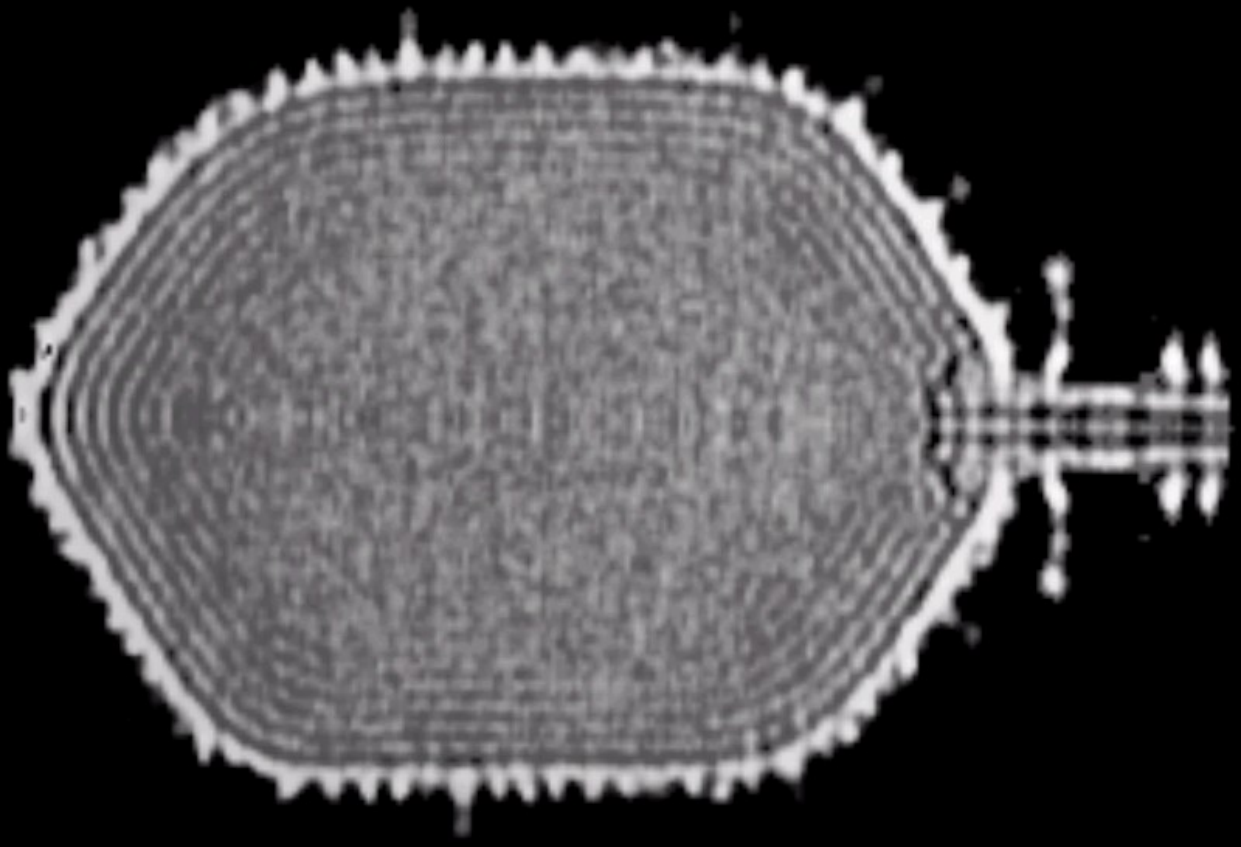
(click on images to bring to top of screen or to scale image to the resolution of your monitor)



Click here to view to view the above image scaled to your monitor.

T4 bacteriophage head capsid, EMD-1075

DNA packing shown by thin slice of volume



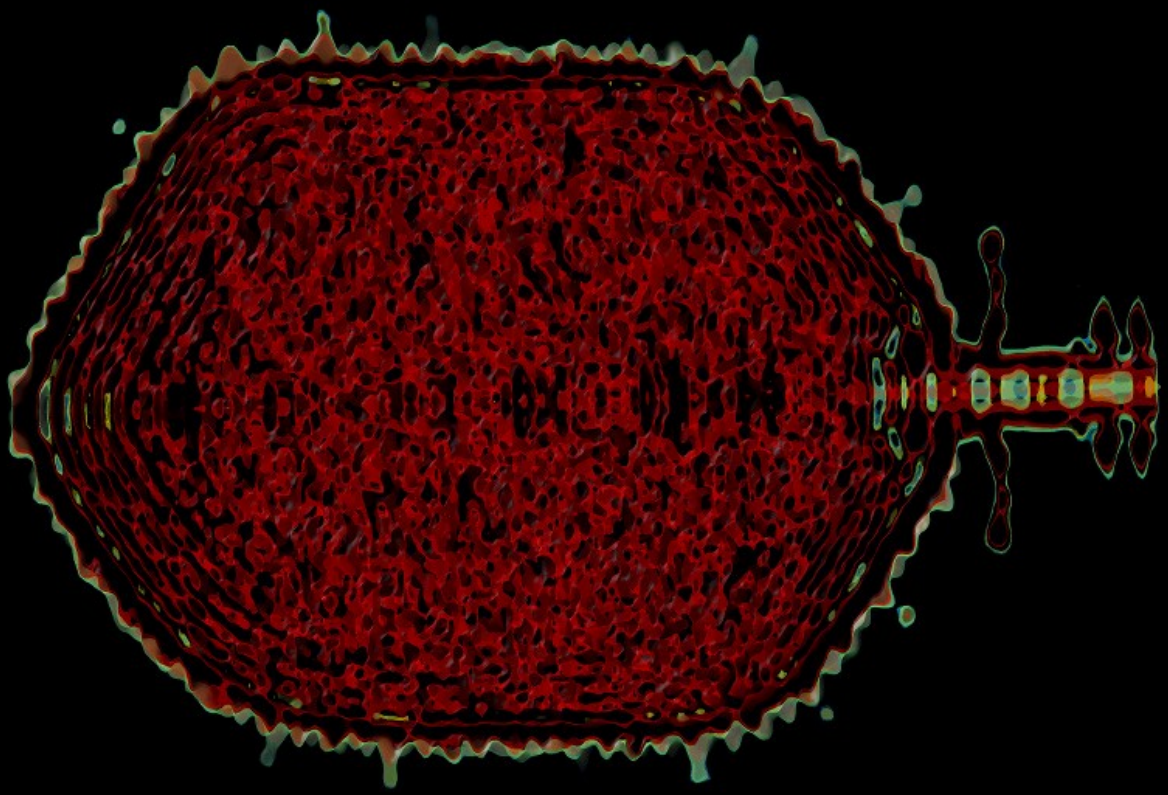
Made with UCSF Chimera

Steven McQuinn steven_mcq@yahoo.com

Click here to view to view the above image scaled to your monitor.

T4 bacteriophage head capsid, EMD-1075

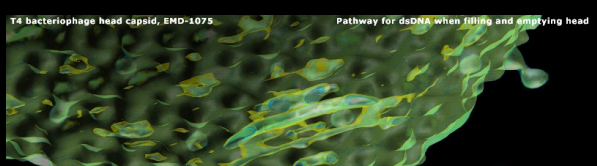
DNA packing shown by thin slice of isosurfaces

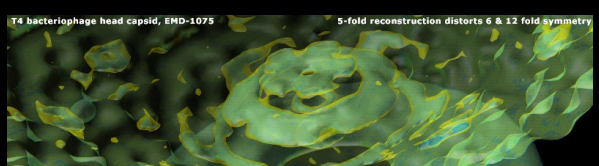


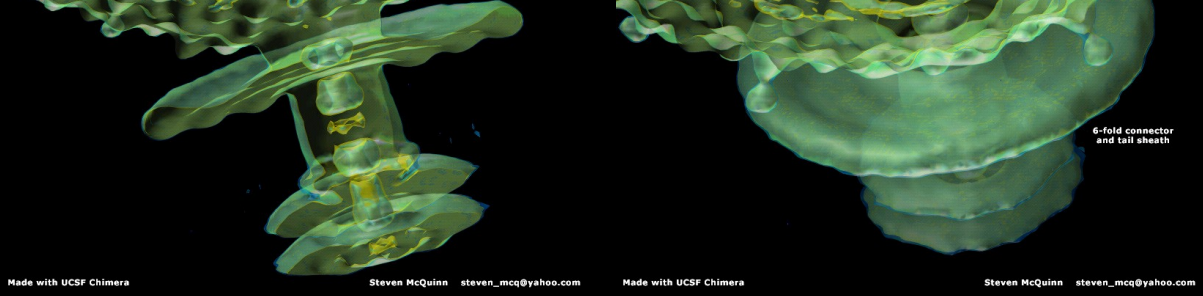
Made with UCSF Chimera

Steven McQuinn steven_mcq@yahoo.com

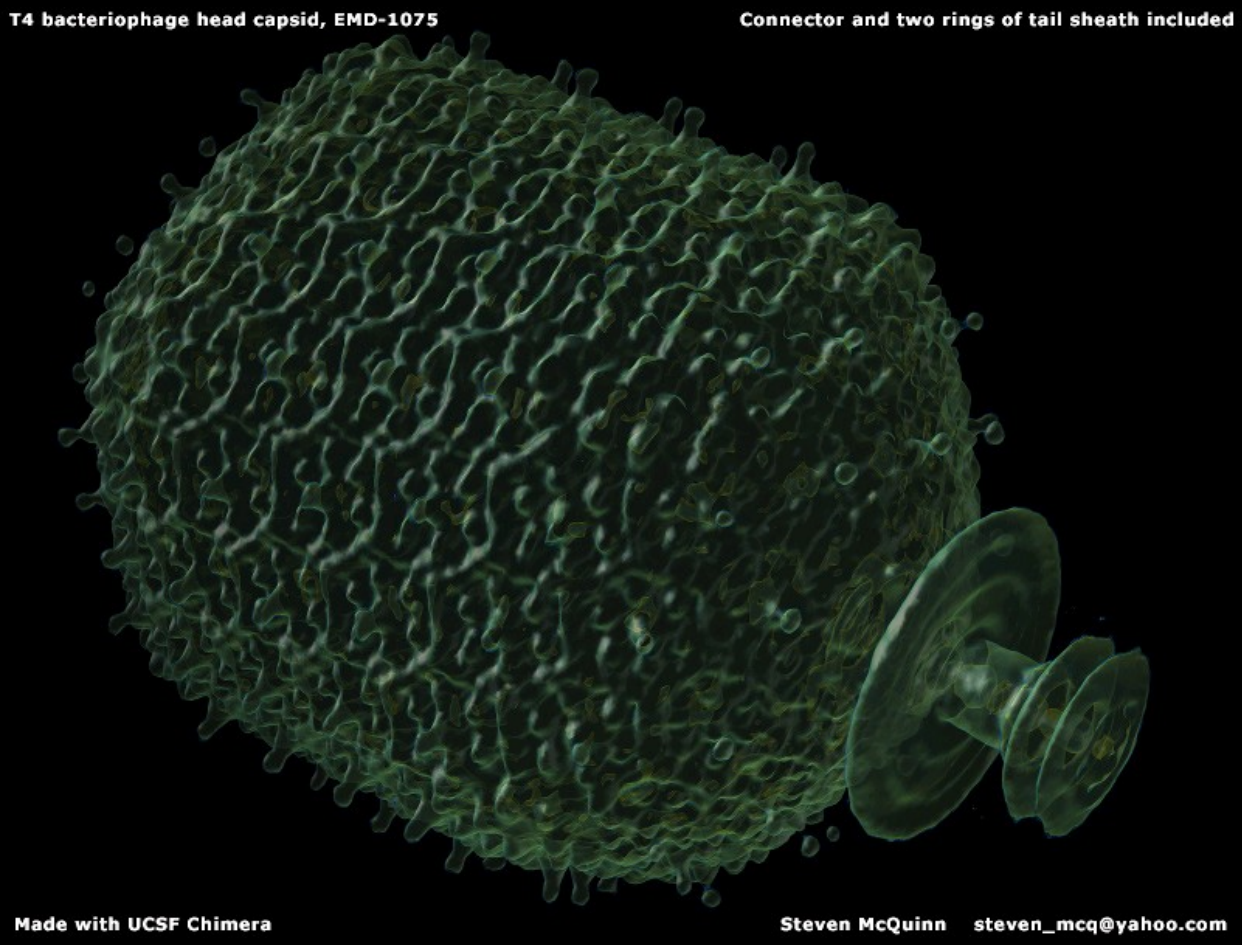
Click here to view to view the above image scaled to your monitor.







Above shows two views of tail connector proteins.
Click on images to for larger view.
Click here to view completed head in isolation.
Click on head image, below, to view image scaled to your monitor□s resolution.



Click on head image, above, to view image scaled to your monitor□s resolution.
Click here to view to view PDF-based slide show of the above images.

Visualization with a Grain of Salt

Pretty pictures these may be, but somewhat misleading. Nothing wrong with the data downloaded from the European Macromolecular Database (EMD-1075). Reading the cited publication closely, however, will reveal that the T4 prolate head was resolved with exquisite accuracy using five-fold symmetry (bottom figure), whereas the tag-along connector parts have 12-fold and 6-fold symmetry, respectively (two previous figures). Therefore the apparent detail of the head/tail portal assembly so magnificently revealed in these portraits is bogus, a hot-butter blended smear of symmetries.

We can expect to see the portal revealed in all its macromolecular glory, eventually. The authors of the citation, a brilliant team of collaborators from Moscow and Purdue under Dr. Michael G. Rossmann□s lead, have many more cards left to play. For instance, they have determined the rotational position of the head relative to the tail within 2 degree accuracy, and they have imaged the structure of the tail sheath in both contracted and extended form. That data is not available yet, so my own efforts to fit pieces together will have to be based on guesswork and previous research, for now.

The T4 head capsid dataset, EMD-1075, consists of a cubic latticework of density values compiled from multiple T4 heads regarded as multiple perspectives of an identical shape. Thus, many 2D electron micrographs of flash-frozen T4 heads are converted into a 3D numerical description of a single particle.

The surface renderings you see here show two closely spaced, differently colored, semi-transparent 3D contours, or isosurfaces, that envelop all volumes at or above the selected threshold densities (third figure). The free molecular visualization software, UCSF Chimera,

provides many manipulations for teasing out structure from volume density data.

The thin slabs hinting at DNA packing structure in the head take slices from the center of the loaf and show them as fuzzy clouds of density, ironically called a solid rendering (top and second figures). Density brightness is additive by line of sight, accentuating differences along the selected slab and obscuring differences at angles to the slab. Thus, only the outer layers of DNA show their spacing clearly, while the inner layers tease us with apparent signs of symmetry, which can be misleading.

You can download both the data and Chimera using the links below and play with them, or do serious research with them, to your heart's content. I predict that such availability of macromolecular data sets and the software tools to examine them will give rise to a resurgence of sound amateur science, such as one sees today in astronomy. The experts should be flattered to have a following.

References

Citation: Fokine, A., Chipman, P.R., Leiman, P.G., Mesyanzhinov, V. V., Rao, V. B. and Rossmann, M.G. (2004). Molecular architecture of the prolate head of bacteriophages T4. Proceedings of the National Academy of Science, U.S.A. 101(16):6003-6008 (http://www.pnas.org/cgi/reprint/101/16/6003.pdf).

Software: Images produced using the UCSF Chimera package (NIH P41 RR-01081) (http://www.cgl.ucsf.edu/chimera). Huang, C.C., Couch, G.S., Pettersen, E.F., Ferrin, T.E. (1996). Chimera: An extensible molecular modeling application constructed using standard components. Pacific Symposium on Biocomputing 1:724.

Data: Electron density map: EMD-1075, 22.5 Å resolution (http://www.ebi.ac.uk/msd-srv/emsearch/atlas/1075_summary.html).

Submissions Archive

Submissions are non-editorial items describing or highlighting some aspect of bacteriophage ecology including news pieces, historical pieces, reviews, and write-ups of research. Peer review of submissions is possible and a desire for peer review should be indicated. Send all submissions to microdude+@osu.edu or to "Submissions", Bacteriophage Ecology Group News, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Please send all submissions as Microsoft Word documents, if possible (I'll let you know if I have trouble converting any other document formats), and in English.

contents | BEG News (021) | top of page

Phage Images

Phage Image Archive

- BEG Phage Images Page
- The Face of the Phage
- Bacteriophage T2
- SSV1-Type Phage
- Saline Lake Bacteriophage
- Coliphage LG1
- Bacteriophage HK97
- Phage T4 (art)
- Phage T4 on the pedestal outside of Barker Hall at Berkeley
- Electron micrograph of phage P22
- Thin section of T4 phages hitting a microcolony of *E. coli* K-12
- T4 phage v1
- T4 Tail Model
- Gingerbread phage
- T4 adsorbing en mass
- Lysis of *E.coli* O157
- Homologous Recombination - 2000 by Jake McKinlay
- X-Ray Structure of Bacteriophage HK97 by William R. Wikoff
- Balloon Phage T4 by Celeste O'Neil and Larry Goodridge
- Image from the 2004 ASM Conference on the New Phage Biology
- Siphovirus pin by Jutta Loeffler
- Vaudeville will never be dead for coliphages!!!

Please send any phage images that you would like to present in this section to "Phage Images," *The Bacteriophage Ecology Group*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906. Alternatively, you may scan the images yourself and send them as an attachment to microdude+@osu.edu . Please save all scans in gif or jpg formats and preferably with an image size (in terms of width, height, and kbytes) that will readily fit on a standard web page. No copyrighted material without permission, please!

contents | BEG News (021) | top of page

New Publications

New bacteriophage publications are listed below. Each quarter not-yet-listed publications from the previous two years will be presented along with their abstracts. The indicator "???" denotes, of course, that specific information is not yet in the BEG Bibliography. Please help in the compilation of the BEG Bibliography by supplying any updated information, correcting any mistakes, and, of course, e-mailing with the references to your bacteriophage ecology publications, as well as the references to any bacteriophage ecology publications that you know of but which are not yet in the bibliography or to point out references that are not appropriate for the bibliography (send to microdude+@osu.edu or to "BEG Bibliography," *Bacteriophage Ecology Group News*, care of Stephen T. Abedon, Department of Microbiology, The Ohio State University, 1680 University Dr., Mansfield, Ohio 44906). This list is also present with available abstracts at the end of *BEG News*.

1. Diversity and population structure of a near-shore marine-sediment viral community. Breitbart, M., Felts, B., Kelley, S., Mahaffy, J. M., Nulton, J., Salamon, P., Rohwer, F. (2004). *Proceedings of the Royal Society of London Series B Biological sciences* 271:565-574. [PRESS FOR ABSTRACT]

2. Phage community dynamics in hot springs. Breitbart, M., Wegley, L., Leeds, S., Schoenfeld, T., Rohwer, F. (2004). *Applied and Environmental Microbiology* 70:1633-1640. [PRESS FOR ABSTRACT]

3. Isolation and characterization of a generalized transducing phage for *Pseudomonas aeruginosa* strains PAO1 and PA14. Budzik, J. M., Rosche, W. A., Rietsch, A., O'Toole, G. A. (2004). *Journal of Bacteriology* 186:3270-3273. [PRESS FOR ABSTRACT]

4. Genome properties and the limits of adaptation in bacteriophages. Bull, J. J., Badgett, M. R., Springman, R., Molineux, I. J. (2004). *Evolution* 58:692-701. [PRESS FOR ABSTRACT]

5. Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection. Butkus, M. A., Labare, M. P., Starke, J. A., Moon, K., Talbot, M. (2004). *Applied and Environmental Microbiology* 70:2848-2853. [PRESS FOR ABSTRACT]

6. Thermal and chemical resistance of *Lactobacillus casei* and *Lactobacillus paracasei* bacteriophages. Capra, M. L., Quiberoni, A., Reinheimer, J. A. (2004). *Letters in Applied Microbiology* 38:499-504. [PRESS FOR ABSTRACT]

7. The chromosome of *Shigella flexneri* bacteriophage Sf6: complete nucleotide sequence, genetic mosaicism, and DNA packaging. Casjens, S., Winn-Stapley, D. A., Gilcrease, E. B., Morona, R., Kuhlewein, C., Chua, J. E. H., Manning, P. A., Inwood, W., Clark, A. J. (2004). *Journal of Molecular Biology* 339:379-394. [PRESS FOR ABSTRACT]

8. Cyanophage diversity, inferred from g20 gene analyses, in the largest natural lake in France, Lake Bourget. Dorigo, U., Jacquet, S., Humbert, J. F. (2004). *Applied and Environmental Microbiology* 70:1017-1022. [PRESS FOR ABSTRACT]

9. Identification of peptide sequences that induce the transport of phage across the gastrointestinal mucosal barrier. Duerr, D. M., White, S. J., Schluesener, H. J. (2004). *Journal of Virological Methods* 116:177-180. [PRESS FOR ABSTRACT]

10. Resuscitation of a defective prophage in *Salmonella* cocultures. Figueroa-Bossi, N., Bossi, L. (2004). *Journal of Bacteriology* 186:4038-4041. [PRESS FOR ABSTRACT]

11. Isolation and characterization of the bacteriophage WO from *Wolbachia*, an arthropod endosymbiont. Fujii, Y., Kubo, T., Ishikawa, H., Sasaki, T. (2004). *Biochemical & Biophysical Research Communications* 317:1183-1188. [PRESS FOR ABSTRACT]

12. Diversity, distribution and specificity of WO phage infection in *Wolbachia* of four insect species. Gavotte, L., Vavre, F., Henri, H., Ravallec, M., Stouthamer, R., Bouletreau, M. (2004). *Insect Molecular Biology* 13:147-153. [PRESS FOR ABSTRACT]

13. Hot new virus, deep connections. Hendrix, R. W. (2004). *Proceedings of the National Academy of Sciences, USA* 101:7495-7496. [PRESS FOR ABSTRACT]

14. Isolation of phages infecting Actinoplanes SN223 and characterization of two of these viruses. Jarling, M., Bartkowiak, K., Robenek, H., Pape, H., Meinhardt, F. (2004). *Applied Microbiology and Biotechnology* 64:250-254. [PRESS FOR ABSTRACT]

15. The persistence and removal of enteric pathogens in constructed wetlands. Karim, M. R., Manshadi, F. D., Karpiscak, M. M., Gerba, C. P. (2004). *Water Research* 38:1831-1837. [PRESS FOR ABSTRACT]

16. Bacteriophages that infect the cellulolytic ruminal bacterium *Ruminococcus albus* AR67. Klieve, A. V., Bain, P. A., Yokoyama, M. T., Ouwerkerk, D., Forster, R. J., Turner, A. F. (2004). *Letters in Applied Microbiology* 38:333-338. [PRESS FOR ABSTRACT]

17. Group I intron homing in *Bacillus* phages SPO1 and SP82: a gene conversion event initiated by a nicking homing endonuclease. Landthaler, M., Lau, N. C., Shub, D. A. (2004). *Journal of Bacteriology* 186:4307-4314. [PRESS FOR ABSTRACT]

18. Involvement of colicin in the limited protection of the colicin producing cells against bacteriophage. Lin, Y. H., Liao, C. C., Liang, P. H., Yuan, H. S., Chak, K. F. (2004). *Biochemical & Biophysical Research Communications* 318:81-87. [PRESS FOR ABSTRACT]

19. Standardised evaluation of the performance of a simple membrane filtration-elution method to concentrate bacteriophages from drinking water. Mendez, J., Audicana, A., Isern, A., Llaneza, J., Moreno, B., Tarancon, M. L., Jofre, J., Lucena, F. (2004). *Journal of Virological Methods* 117:19-25. [PRESS FOR ABSTRACT]

20. Factors influencing the replication of somatic coliphages in the water environment. Muniesa, M., Jofre, J. (2004). *Antonie van Leeuwenhoek* 86:65-76. [PRESS FOR ABSTRACT]

21. Detection of enteric viruses in shellfish from the Norwegian coast. Myrmel, M., Berg, E. M. M., Rimstad, E., Grinde, B. (2004). *Applied and Environmental Microbiology* 70:2678-2684. [PRESS FOR ABSTRACT]

22. Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. Noble, R. T., Lee, I. M., Schiff, K. C. (2004). *Journal of Applied Microbiology* 96:464-472. [PRESS FOR ABSTRACT]

23. Testing for viral penetration of non-latex surgical and examination gloves: a comparison of three methods. O'Connell, K. P., El Masri, M., Broyles, J. B., Korniewicz, D. M. (2004). *Clinical microbiology and infection* 10:322-326. [PRESS FOR ABSTRACT]

24. Characterization of three *Lactobacillus delbrueckii* subsp. *bulgaricus* phages and the physicochemical analysis of phage adsorption. Quiberoni, A., Guglielmotti, D., Binetti, A., Reinheimer, J. (2004). *Journal of Applied Microbiology* 96:340-351. [PRESS FOR ABSTRACT]

25. [Combined use of active chlorine and coagulants for drinking water purification and disinfection]. Rakhmanin, I. A., Zholdakova, Z. I., Poliakova, E. E., Kir'ianova, L. F., Miasnikov, I. N., Tul'skaia, E. A., Artemova, T. Z., Ivanova, L. V., Dmitrieva, R. A., Doskina, T. V. (2004). *Gigiena i Sanitariia* 6-9. [PRESS FOR ABSTRACT]

26. The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. Rice, G., Tang, L., Stedman, K., Roberto, F., Spuhler, J., Gillitzer, E., Johnson, J. E., Douglas, T., Young, M. (2004). *Proceedings of the National Academy of Sciences, USA* 101:7716-7720. [PRESS FOR ABSTRACT]

27. Distribution, sequence homology, and homing of group I introns among T-even-like bacteriophages: evidence for recent transfer of old introns. Sandegren, L., Sjoberg, B. M. (2004). *The Journal of biological chemistry* 279:22218-22227. [PRESS FOR ABSTRACT]

28. Newly isolated *Vibrio cholerae* non-O1, non-O139 phages. Sarkar, B. L., Ghosh, A. N., Sen, A., Rodrigues, D. P. (2004). *Emerging Infectious Diseases* 10:754-756. [PRESS FOR ABSTRACT]

29. Phage offer a real alternative. Schoolnik, G. K., Summers, W. C., Watson, J. D. (2004). *Nature biotechnology* 22:505-506. [PRESS FOR ABSTRACT]

30. [Bacteriophages as antibacterial agents]. Shasha, S. M., Sharon, N., Inbar, M. (2004). *Harefuah* 143:121-5, 166. [PRESS FOR ABSTRACT]

31. *Burkholderia cenocepacia* phage BcepMu and a family of Mu-like phages encoding potential pathogenesis factors. Summer, E. J., Gonzalez, C. F., Carlisle, T., Mebane, L. M., Cass, A. M., Savva, C. G., LiPuma, J. J., Young, R. (2004). *Journal of Molecular Biology* 340:49-65. [PRESS FOR ABSTRACT]

32. The interaction of phage and biofilms. Sutherelandl, I. W., Hughes, K. A., Skillman, L. C., Tait, K. (2004). *FEMS Microbiology Letters* 232:1-6. [PRESS FOR ABSTRACT]

33. Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. Tanji, Y., Shimada, T., Yoichi, M., Miyanaga, K., Hori, K., Unno, H. (2004). *Applied Microbiology and Biotechnology* 64:270-274. [PRESS FOR ABSTRACT]

34. Old dogma, new tricks--21st Century phage therapy. Thiel, K. (2004). *Nature biotechnology* 22:31-36. [PRESS FOR ABSTRACT]

35. Bead-based electrochemical immunoassay for bacteriophage MS2. Thomas, J. H., Kim, S. K., Hesketh, P. J., Halsall, H. B., Heineman, W. R. (2004). *Analytical Chemistry* 76:2700-2707. [PRESS FOR ABSTRACT]

36. The role of prophage-like elements in the diversity of *Salmonella enterica* serovars. Thomson, N., Baker, S., Pickard, D., Fookes, M., Anjum, M., Hamlin, N., Wain, J., House, D., Bhutta, Z., Chan, K., Falkow, S., Parkhill, J., Woodward, M., Ivens, A., Dougan, G. (2004). *Journal of Molecular Biology* 339:279-300. [PRESS FOR ABSTRACT]

37. Evaluating microbial purification during soil treatment of wastewater with multicomponent tracer and surrogate tests. Van Cuyk, S., Siegrist, R. L., Lowe, K., Harvey, R. W. (2004). *Journal of Environmental Quality* 33:316-329. [PRESS FOR ABSTRACT]

38. Models of phage growth and their applicability to phage therapy. Weld, R. J., Butts, C., Heinemann, J. A. (2004). *Journal of Theoretical Biology* 227:1-11. [PRESS FOR ABSTRACT]

39. Genome function--a virus-world view. Yin, J. (2004). *Advances in experimental medicine and biology* 547:31-46. [PRESS FOR ABSTRACT]

40. Bacteriophage observations and evolution. Ackermann, H.-W. (2003). *Research in Microbiology* 154:245-251. [PRESS FOR ABSTRACT]

41. Evolution of phage with chemically ambiguous proteomes. Bacher, J. M., Bull, J. J., Ellington, A. D. (2003). *BMC evolutionary biology [electronic resource]* 3:24. [PRESS FOR ABSTRACT]

42. Do viruses form lineages across different domains of life? Bamford, D. H. (2003). *Research in Microbiology* 154:231-236. [PRESS FOR ABSTRACT]

43. Isolation and characterization of marine psychrophilic phage-host systems from Arctic sea ice. Borriss, M., Helmke, E., Hanschke, R., Schweder, T. (2003). *Extremophiles : life under extreme conditions* 7:377-384. [PRESS FOR ABSTRACT]

44. Prophage insertion sites. Campbell, A. (2003). *Research in Microbiology* 154:277-282. [PRESS FOR ABSTRACT]

45. The diversity and evolution of the T4-type bacteriophages. Desplats, C., Krisch, H. M. (2003). *Research in Microbiology* 154:259-267. [PRESS FOR ABSTRACT]

46. Haloarchaeal viruses: how diverse are they? Dyall-Smith, M., Tang, S.-L., Bath, C. (2003). *Research in Microbiology* 154:309-313. [PRESS FOR ABSTRACT]

47. Effects of pH and temperature on the survival of coliphages MS2 and Qβ. Feng, Y. Y., Ong, S. L., Hu, J. Y., Tan, X. L., Ng, W. J. (2003). *Journal of Industrial Microbiology & Biotechnology* 30:549-552. [PRESS FOR ABSTRACT]

48. The role played by viruses in the evolution of their hosts: a view based on informational protein phylogenies. Filée, J., Forterre, P., Laurent, J. (2003). *Research in Microbiology* 154:237-243. [PRESS FOR ABSTRACT]

49. The great virus comeback—from an evolutionary perspective. Forterre, P. (2003). *Research in Microbiology* 154:223-225. [PRESS FOR ABSTRACT]

50. Observation of virus-like particles in high temperature enrichment cultures from deep-sea hydrothermal vents. Geslin, C., Le Romancer, M., Gaillard, M., Erauso, G., Prieur, D. (2003). *Research in Microbiology* 154:303-307. [PRESS FOR ABSTRACT]

51. New insights into the possible role of bacteriophages in transplantation. Gorski, A., Nowaczyk, M., Weber-Dabrowska, B., Kniotek, M., Boratynski, J., Ahmed, A., Dabrowska, K., Wierzbicki, P., Switala-Jelen, K., Opolski, A. (2003). *Transplantation Proceedings* 35:2372-2373. [PRESS FOR ABSTRACT]

52. Bacteriophages with tails: chasing their origins and evolution. Hendrix, R. W., Hatfull, G. F., Smith, M. C. M. (2003). *Research in Microbiology* 154:253-257. [PRESS FOR ABSTRACT]

53. [Bacteriophage therapy: Stalin's forgotten medicine]. Kaulen, H. (2003). *Deutsche medizinische Wochenschrift*

128:307. [no abstract]

54. The view from Les Treilles on the origins, evolution and diversity of viruses. Krisch, H. M. (2003). *Research in Microbiology* 154:227-229. [PRESS FOR ABSTRACT]

55. *Myoviridae* bacteriophages of *Pseudomonas aeruginosa*: a long and complex evolutionary pathway. Krylov, V., Pleteneva, E., Bourkaltseva, M., Shaburova, O., Volckaert, G., Sykilinda, N., Kurochkina, L., Mesyanzhinov, V. (2003). *Research in Microbiology* 154:269-275. [PRESS FOR ABSTRACT]

56. Bacteriophage therapy: an alternative to conventional antibiotics. Mathur, M. D., Vidhani, S., Mehndiratta, P. L. (2003). *The Journal of the Association of Physicians of India* 51:593-596. [PRESS FOR ABSTRACT]

57. [Evaluation of relations between plasmids and phage host range among clinical isolates of *Enterobacter cloacae*]. Nieradko, J., Kurlenda, J. (2003). *Medycyna Doswiadczalna i Mikrobiologia* 55:343-349. [PRESS FOR ABSTRACT]

58. Evolutionary insights from studies on viruses from hot habitats. Prangishvili, D. (2003). *Research in Microbiology* 154:289-294. [PRESS FOR ABSTRACT]

59. Ecological aspects of circulation of the phytopathogevic bacteria' phages in biocenoses. Semchuk, L. I., Andriychuk, O. M., Romashev, S. A., Ignatenko, T. O., Yatskovska, L. I. (2003). *Ecological Bulletin* Special release:498-501. [no abstract]

60. Relationships between fuselloviruses infecting the extremely thermophilic archaeon *Sulfolobus*: SSV1 and SSV2. Stedman, K. M., She, Q., Phan, H., Arnold, H. P., Hoz, I., Garrett, R. A., Zillig, W. (2003). *Research in Microbiology* 154:295-302. [PRESS FOR ABSTRACT]

61. Sequences and replication of genomes of the archaeal rudiviruses SIRV1 and SIRV2: Relationships to the archaeal lipothrixvirus SIFV and some eukaryal viruses. Peng, X., Blum, H., She, Q., Mallok, S., Brügger, K., Garrett, R. A., Prangishvili, D. (2001). *Virology* 291:226-234. [PRESS FOR ABSTRACT]

contents | BEG News (021) | top of page

New Publications with Abstracts

For your convenience, a list of new publications without associated abstracts (but with links to abstracts) is found above. The list presented below is identical to the above list except that abstracts are included.

1. **Diversity and population structure of a near-shore marine-sediment viral community. Breitbart, M., Felts, B., Kelley, S., Mahaffy, J. M., Nulton, J., Salamon, P., Rohwer, F. (2004). *Proceedings of the Royal Society of London Series B Biological sciences* 271:565-574.** Viruses, most of which are phage, are extremely abundant in marine sediments, yet almost nothing is known about their identity or diversity. We present the metagenomic analysis of an uncultured near-shore marine-sediment viral community. Three-quarters of the sequences in the sample were not related to anything previously reported. Among the sequences that could be identified, the majority belonged to double-stranded DNA phage. Temperate phage were more common than lytic phage, suggesting that lysogeny may be an important lifestyle for sediment viruses. Comparisons between the sediment sample and previously sequenced seawater viral communities showed that certain phage phylogenetic groups were abundant in all marine viral communities, while other phage groups were under-represented or absent. This 'marineness' suggests that marine phage are derived from a common set of ancestors. Several independent mathematical models, based on the distribution of overlapping shotgun sequence fragments from the library, were used to show that the diversity of the viral community was extremely high, with at least 10^4 viral genotypes per kilogram of sediment and a Shannon index greater than 9 nats. Based on these observations we propose that marine-sediment viral communities are one of the largest unexplored reservoirs of sequence space on the planet.

2. **Phage community dynamics in hot springs. Breitbart, M., Wegley, L., Leeds, S., Schoenfeld, T., Rohwer, F. (2004). *Applied and Environmental Microbiology* 70:1633-1640.** In extreme thermal environments such as hot springs, phages are the only known microbial predators. Here we present the first study of prokaryotic and phage community dynamics in these environments. Phages were abundant in hot springs, reaching concentrations of a million viruses per milliliter. Hot spring phage particles were resistant to shifts to lower temperatures, possibly facilitating DNA transfer out of these extreme environments. The phages were actively produced, with a population turnover time of 1 to 2 days. Phage-mediated microbial mortality was significant, making phage lysis an important component of hot spring microbial food webs. Together, these results show that phages exert an important influence on microbial community structure and energy flow in extreme thermal environments.

3. **Isolation and characterization of a generalized transducing phage for *Pseudomonas aeruginosa* strains PAO1 and PA14. Budzik, J. M., Rosche, W. A., Rietsch, A., O'Toole, G. A. (2004). *Journal of Bacteriology* 186:3270-3273.** A temperate, type IV pilus-dependent, double-stranded DNA bacteriophage named DMS3 was isolated from a clinical strain of *Pseudomonas aeruginosa*. A clear-plaque variant of this bacteriophage was isolated. DMS3 is capable of mediating generalized transduction within and between *P. aeruginosa* strains PA14 and PAO1, thus providing a useful tool for the genetic analysis of *P. aeruginosa*.

4. **Genome properties and the limits of adaptation in bacteriophages. Bull, J. J., Badgett, M. R., Springman, R., Molineux, I. J. (2004). *Evolution* 58:692-701.** Eight bacteriophages were adapted for rapid growth under similar conditions to compare their evolved, endpoint fitnesses. Four pairs of related phages were used, including two RNA phages with small genomes (MS2 and Qβ) two single-stranded DNA phages with small genomes (ϕX174 and G4), two T-odd phages with medium-sized, double-stranded DNA genomes (T7 and T3), and two T-even phages with large, double-stranded DNA genomes (T6 and RB69). Fitness was measured as absolute growth rate per hour under the same conditions used for adaptation. T7 and T3 achieved the highest fitnesses, able to increase by 13 billionfold and three-quarters billionfold per hour, respectively. In contrast, the RNA phages achieved low fitness maxima, with growth rates approximately 400-fold and 4000-fold per hour. The highest fitness limits were not attributable to high mutation rates or small genome size, even though both traits are expected to enhance adaptation for fast growth. We suggest that major differences in fitness limits stem from different "global" constraints, determined by the organization and composition of the phage genome affecting whether and how it overcomes potentially rate-limiting host processes, such as transcription, translation, and replication. Adsorption rates were also measured on the evolved phages. No consistent pattern of adsorption rate and fitness was observed across the four different types of phages, but within each pair of related phages, higher adsorption was associated with higher fitness. Different adsorption rate limits within pairs may stem from "local" constraints-sequence differences leading to different local optima in the sequence space.

5. **Use of aqueous silver to enhance inactivation of coliphage MS-2 by UV disinfection. Butkus, M. A., Labare, M. P., Starke, J. A., Moon, K., Talbot, M. (2004). *Applied and Environmental Microbiology* 70:2848-2853.** A synergistic effect between silver and UV radiation has been observed that can appreciably enhance the effectiveness of UV radiation for inactivation of viruses. At a fluence of ca. 40 mJ/cm^2, the synergistic effect between silver and UV was observed at silver concentrations as low as 10 microg/liter ($P < 0.0615$). At the same fluence, an MS-2 inactivation of ca. 3.5 logs (99.97%) was achieved at a silver concentration of 0.1 mg/liter, a significant improvement ($P < 0.0001$) over the ca. 1.8-log (98.42%) inactivation of MS-2 at ca. 40 mJ/cm^2 in the absence of silver. Modified Chick-Watson kinetics were used to model the synergistic effect of silver and UV radiation. For an MS-2 inactivation of 4 logs (99.99%), the coefficient of dilution (n) was determined to be 0.31, which suggests that changes in fluence have a greater influence on inactivation than does a proportionate change in silver concentration.

6. **Thermal and chemical resistance of *Lactobacillus casei* and *Lactobacillus paracasei* bacteriophages. Capra, M. L., Quiberoni, A., Reinheimer, J. A. (2004). *Letters in Applied Microbiology* 38:499-504.** AIMS: The survival of two collection *Lactobacillus casei* and *L. paracasei* bacteriophages when subjected to thermal and chemical treatments was investigated. METHODS AND RESULTS: Thermal resistance was evaluated by heating phage suspensions at 63, 72 and 90°C in three different media [Tris-magnesium gelatin (TMG) buffer: 10 mmol l^{-1} Tris-Cl, 10 mmol l^{-1} MgSO(4) and 0.1% w/v gelatin; Man Rogosa Sharpe (MRS) broth and reconstituted nonfat dry skim milk (RSM)]. A marked heat sensitivity was evident in both phages, as 15 min at 72°C was enough to completely inactivate (6 $\log_{10}$ reduction) them. No clear influence was demonstrated by the suspension media. The phages also showed similar resistance to biocides. Peracetic acid and sodium hypochlorite (800 ppm) were the most effective ones, destroying the phages within 5 min. Concentrations of 75 and 100% ethanol were not suitable to inactivate phage particles even after 45 min. Isopropanol did not show an effect on phage viability. CONCLUSIONS: The data obtained in this work are important to design more effective control procedures in order to inactivate phages in dairy plants and laboratories. SIGNIFICANCE AND IMPACT OF THE STUDY: This work will contribute to enhance the background knowledge about phages of probiotic bacteria.

7. **The chromosome of *Shigella flexneri* bacteriophage Sf6: complete nucleotide sequence, genetic mosaicism, and DNA packaging. Casjens, S., Winn-Stapley, D. A., Gilcrease, E. B., Morona, R., Kuhlewein, C., Chua, J. E. H., Manning, P. A., Inwood, W., Clark, A. J. (2004). *Journal of Molecular Biology* 339:379-394.** *Shigella flexneri* temperate bacteriophage Sf6 is of interest in part because its prophage expresses the oac gene that alters the antigenic properties of the surface O-antigen polysaccharide of its host bacterium. We have determined the complete sequence of its 39,044 bp genome. The sequence shows that Sf6 is a member of the canonical lambdoid phage group, and like other phages of this type has a highly mosaic genome. It has chromosomal regions that encode proteins >80% identical with at least 15 different previously characterized lambdoid phages and prophages, but 43% of the genome, including the virion assembly genes, is homologous to the genome of one phage, HK620. An analysis of the nucleotide differences between Sf6 and HK620 indicates that even these similar regions are highly mosaic. This mosaicism suggests ways in which the virion structural proteins might interact with each other. The Sf6 early operons are arranged like a typical lambdoid phage, with "boundary sequences" often found between functional modules in the "metabolic" genome domain. By virtue of high degree of similarity in the encoding genes and their DNA target sites, we predict that the integrase, early transcription anti-terminator, CI and Cro repressors, and CII protein of Sf6 have DNA binding specificities very similar to the homologous proteins encoded by phages HK620, lambda, 434 and P22, respectively. The late operon contains two tRNA genes. The Sf6 terminase genes are unusual. Analysis of in vivo initiation of the DNA packaging series showed that the Sf6 apparatus that recognizes DNA for packaging appears to cleave DNA for initiation of packaging series at many sites within a large region of about 1800 bp that includes a possible pac site. This is unlike previously characterized phage packaging mechanisms.

8. **Cyanophage diversity, inferred from g20 gene analyses, in the largest natural lake in France, Lake Bourget. Dorigo, U., Jacquet, S., Humbert, J. F. (2004). *Applied and Environmental Microbiology* 70:1017-1022.** The genetic diversity of the natural freshwater community of cyanophages and its variations over time have been investigated for the first time in the surface waters of the largest natural lake in France. This was done by random screening of clone libraries for the g20 gene and by denaturing gradient gel electrophoresis (DGGE). Nucleotide sequence analysis revealed 35 distinct cyanomyovirus g20 genotypes among the 47 sequences analyzed. Phylogenetic analyses showed that these sequences fell into seven genetically distinct operational taxonomic units (OTUs). The distances between these OTUs were comparable to those reported between marine clusters. Moreover, some of these freshwater cyanophage sequences were genetically more closely related to marine cyanophage sequences than to other freshwater sequences. Both approaches for the g20 gene (sequencing and DGGE analysis) showed that there was a clear seasonal pattern of variation in the composition of the cyanophage community that could reflect changes in its biological, chemical, and/or physical environment.

9. **Identification of peptide sequences that induce the transport of phage across the gastrointestinal mucosal barrier. Duerr, D. M., White, S. J., Schluesener, H. J. (2004). *Journal of Virological Methods* 116:177-180.** To investigate whether specific peptide sequences could induce virion transport across the intestinal barrier, we used phage display to both identify signalling peptides capable of inducing trans-intestinal transport and also provide a suitable model of virion translocation. We utilised simple, single-round high input in vivo biopanning protocol using a 7-mer random amino acid phage display library. Phage were applied by gavage and translocation across the intestinal barrier assessed by phage recovery from the spleen 2h later. Following isolation, a number of phage were sequenced and several homologies with HIV gp120 were identified. Immunocytochemical analysis of phage translocation across the intestinal barrier by a phage bearing the peptide YPRLLTP demonstrated that phage were actively transported along specific channels. It is concluded that utilisation of in vivo phage display (IVPD) has provided evidence for a specific peptide-guided transport of undegraded cargo across the intestinal barrier, modelled by M13 phage.

10. **Resuscitation of a defective prophage in *Salmonella* cocultures. Figueroa-Bossi, N., Bossi, L. (2004). *Journal of Bacteriology* 186:4038-4041.** Widely studied *Salmonella enterica* serovar *Typhimurium* strains ATCC 14028s and SL1344 harbor a cryptic ST64B prophage unable to produce infectious virions. We found that coculturing either strain with an isogenic sibling lacking the prophage leads to the appearance of active forms of the virus. Active phage originates from reversion of a +1 frameshift mutation at a monotonous G:C run in a presumptive tail assembly pseudogene.

11. **Isolation and characterization of the bacteriophage WO from *Wolbachia*, an arthropod endosymbiont. Fujii, Y., Kubo, T., Ishikawa, H., Sasaki, T. (2004). *Biochemical & Biophysical Research Communications* 317:1183-1188.** *Wolbachia* is a group of obligate symbiotic bacteria found in many insects and other arthropods. The presence of *Wolbachia* alters reproduction in the host, but the mechanisms are unknown. Molecular biological studies of *Wolbachia* have delayed significantly, and one of the reasons is the lack of transformation techniques of this bacterium. In the present study, bacteriophage particles were isolated from *Wolbachia* for the first time. The purified phage had an isometric head that was approximately 40 nm in diameter and contained linear double-stranded DNA of approximately 20 kbp. Partial sequence information (total of 20,484 bp) revealed that there were 24 open reading frames including a structural gene module, and genes for replication and lysogenic conversion. This bacteriophage is the only known mobile genetic element potentially used for transformation of *Wolbachia*.

12. **Diversity, distribution and specificity of WO phage infection in *Wolbachia* of four insect species. Gavotte, L., Vavre, F., Henri, H., Ravallec, M., Stouthamer, R., Bouletreau, M. (2004). *Insect Molecular Biology* 13:147-153.** The bacteriophage WO was recently characterized in *Wolbachia*, a strictly intracellular bacterium that causes several reproductive alterations in its arthropod hosts. To gain insights into the phage-*Wolbachia* relationships, we studied the phage presence among *Wolbachia* infecting four insect species sharing several *Wolbachia* strains, two *Drosophila* and two of their parasitoid wasps. Based on the phage sequence of ORF7, we identified five different phages in six *Wolbachia* strains. Among these five bacteriophages, some are specific for a given bacterial strain whereas others are not, but globally phage infection appears stable on a large geographical scale and across insect generations. Their specificity contrasts with the absence of congruence between *Wolbachia* and phage phylogenies, suggesting phage exchanges between different *Wolbachia* lineages

13. **Hot new virus, deep connections. Hendrix, R. W. (2004). *Proceedings of the National Academy of Sciences, USA* 101:7495-7496.** [first two paragraphs] Biologists of the 17th, 18th, and 19th centuries-call them natural historians-somehow got along without PCR, chip technology, mass spectrometry, or highspeed computers. From our vantage point in the 21st century we may wonder how our scientific ancestors, lacking our sophisticated tools, could have accomplished anything that we would recognize as forward progress in understanding how biology works. Yet they were in the enviable position that much of their biological world was completely unexplored; all they had to do to make a name for themselves was step out of their home habitat, usually Europe, and with a little luck they might find a new and wonderful organism, unlike anything previously known to science. Many examples of such discoveries can be cited (cycads, duck-billed platypuses, giant tortoises, Komodo dragons, and animalcules among them) and, in the most successful cases (think of Mr. Darwin), the new creature(s) dramatically enhanced our understanding of the structure and history of the biological world as a whole. Fortunately for the excitement quotient of modern-day natural historians, Mother Nature's reservoir of undiscovered bizarre and wonderful organisms is not yet empty, and a new one makes the transition from unknown to known with the report by Rice *et al.* (1) in this issue of PNAS. ¶ The new entry is a virus plucked from the near-boiling water of a thermal pool in Yellowstone National Park, and it is every bit as interesting to 21st century science as something like the Galapagos marine iguana (Fig. 1*A*) was to European science when it first came on the stage a few centuries ago. The new virus's host is the hyperthermophilic archaeon *Sulfolobus sulfataricus*, which grows happily at temperatures above 80°C and a pH of 2. Very few viruses of Archaea have been described to date [they amount to <1% of the viruses enumerated by the International Committee on the Taxonomy of Viruses (2)] but these early indications suggest that archaeal viruses likely are just as diverse as the more extensively characterized viruses of Bacteria and Eukarya. The viruses that infect the archaeal halophiles are so far confined to ones that have the same virion morphology and even occasional sequence similarity with the familiar tailed bacteriophages, but the viruses of the hyperthermophiles are a strange and diverse group with virion morphologies including filaments as well as shapes resembling food items such as lemons and corndogs. In this context, perhaps it is not surprising that the new virus would not look quite like anything described before. It is a spherical or, more properly, an icosahedrally symmetric virus (Fig. 1*B*), and, like most such viruses, the surface morphological features follow the rules enunciated by Caspar and Klug (3), although it has a previously undescribed triangulation number of 31. The most dramatic morphological feature of the virion is the protruding "turrets" that extend 13 nm above the capsid surface at the 12 fivefold symmetrical positions of the icosahedron. The function of the turrets is not known, but a plausible guess is that they have a role in attaching the virus to the cell and initiating infection. The morphological features are the basis for the authors' name for the virus: STIV, for *Sulfolobus* turreted icosahedral virus (1).

14. **Isolation of phages infecting Actinoplanes SN223 and characterization of two of these viruses. Jarling, M., Bartkowiak, K., Robenek, H., Pape, H., Meinhardt, F. (2004). *Applied Microbiology and Biotechnology* 64:250-254.** Phages infecting the industrially important *Actinoplanes* strain SN223 were isolated from soil samples collected at the shores of inland waters in Germany. The genome sizes range from 53 kb to 58 kb. Preliminary

analyses revealed G+C contents comparable with the G/C bias of the host. Electron microscopy of three selected viruses displayed no obvious morphological differences, the phage heads being icosahedral and their tails non-contractible. Two of the phages (FAsp2, FAsp3.1) characterized in more detail are capable of provoking putative pseudolysogenic growth of the host bacterium. The carrier state for FAsp2, in which cells are tightly packed with viruses, was demonstrated by electron microscopy. The latter phage is apparently widely distributed, as it was isolated from regions which are distantly located, i.e. more than 600 km apart from each other.

15. **The persistence and removal of enteric pathogens in constructed wetlands. Karim, M. R., Manshadi, F. D., Karpiscak, M. M., Gerba, C. P. (2004). *Water Research* 38:1831-1837.** Sedimentation is thought to be one of the mechanisms of microbial reduction from wetlands used for wastewater treatment. This study compared the occurrence and survival of enteric indicator microorganisms and pathogens in the water column and sediments of two constructed surface flow wetlands in Arizona. On a volume/wet weight basis the concentration of fecal coliforms and coliphage in the water column and sediment was similar. However, on a volume/dry weight basis the numbers were one to two orders of magnitude higher in the sediment. Giardia cyst and Cryptosporidium oocyst concentrations were one to three orders of magnitude greater in the sediment compared to the water column. The die-off rates of all the bacteria and coliphage were greater in the water column than the sediment. The die-off rates of fecal coliforms in the water and sediment were 0.256 $\log_{10}day^{-1}$ and 0.151 $\log_{10}day^{-1}$, respectively. The die-off rates of *Salmonella typhimurium* in the water and sediment were 0.345 $\log_{10}day^{-1}$ and 0.312 $\log_{10}day^{-1}$, respectively. The die-off rates of naturally occurring coliphage in water column and sediment were 0.397 $\log_{10}day^{-1}$ and 0.107 $\log_{10}day^{-1}$, respectively, and the die-off rates of and PRD-1 in water and sediment were 0.198 $\log_{10}day^{-1}$ and 0.054 $\log_{10}day^{-1}$, respectively. In contrast Giardia die-off in the sediment was greater compared to the water column. The die-off rates of Giardia in water and sediment were 0.029 $\log_{10}day^{-1}$ and 0.37 $\log_{10}day^{-1}$, respectively. Coliphage survived the longest of any group of organisms in the sediment and the least in the water column. In contrast Giardia survived best in the water column and least in the sediment.

16. **Bacteriophages that infect the cellulolytic ruminal bacterium *Ruminococcus albus* AR67. Klieve, A. V., Bain, P. A., Yokoyama, M. T., Ouwerkerk, D., Forster, R. J., Turner, A. F. (2004). *Letters in Applied Microbiology* 38:333-338.** AIM: To isolate bacterial viruses that infect the ruminal cellulolytic bacterium *Ruminococcus albus*. METHODS: Four phages infecting *R. albus* AR67 were isolated under anaerobic conditions using the soft-agar overlay technique. The phages were characterized on morphology, solvent stability, nucleic acid type and digestion characteristics. Two phages, ϕRa02 and ϕRa04 comprised icosahedral virions with linear double-stranded DNA and appeared to belong to the family Tectiviridae. The other two phages are most likely filamentous phages with circular single-stranded DNA of the family Inoviridae. SIGNIFICANCE OF THE STUDY: Viruses of the families Tectiviridae and Inoviridae have not previously been isolated from rumen bacteria. The phages isolated in this study are the first phages shown to infect the cellulolytic bacteria of the rumen. This suggests that the cellulolytic populations of the rumen are subject to lytic events that may impact on the ability of these bacteria to degrade plant fibre and on the nutrition of the animal.

17. **Group I intron homing in *Bacillus* phages SPO1 and SP82: a gene conversion event initiated by a nicking homing endonuclease. Landthaler, M., Lau, N. C., Shub, D. A. (2004). *Journal of Bacteriology* 186:4307-4314.** Many group I introns encode endonucleases that promote intron homing by initiating a double-stranded break-mediated homologous recombination event. In this work we describe intron homing in *Bacillus subtilis* phages SPO1 and SP82. The introns encode the DNA endonucleases I-HmuI and I-HmuII, respectively, which belong to the H-N-H endonuclease family and possess nicking activity in vitro. Coinfections of *B. subtilis* with intron-minus and intron-plus phages indicate that I-HmuI and I-HmuII are required for homing of the SPO1 and SP82 introns, respectively. The homing process is a gene conversion event that does not require the major *B. subtilis* recombination pathways, suggesting that the necessary functions are provided by phage-encoded factors. Our results provide the first examples of H-N-H endonuclease-mediated intron homing and the first demonstration of intron homing initiated by a nicking endonuclease.

18. **Involvement of colicin in the limited protection of the colicin producing cells against bacteriophage. Lin, Y. H., Liao, C. C., Liang, P. H., Yuan, H. S., Chak, K. F. (2004). *Biochemical & Biophysical Research Communications* 318:81-87.** The restriction/modification system is considered to be the most common machinery of microorganisms for protection against bacteriophage infection. However, we found that mitomycin C induced *Escherichia coli* containing ColE7-K317 can confer limited protection against bacteriophage M13K07 and I infection. Our study showed that degree of protection is correlated with the expression level of the ColE7 operon, indicating that colicin E7 alone or the colicin E7-immunity protein complex is directly involved in this protection mechanism. It was also noted that the degree of protection is greater against the single-strand DNA bacteriophage M13K07 than the double-strand bacteriophagel. Coincidently, the K(A) value of ColE7-Im either interacting with single-strand DNA ($2.94x10^{5}M^{-1}$) or double-strand DNA ($1.75x10^{5}M^{-1}$) reveals that the binding affinity of ColE7-Im with ssDNA is 1.68-fold stronger than that of the protein complex interacting with dsDNA. Interaction between colicin and the DNA may play a central role in this limited protection of the colicin-producing cell against bacteriophages. Based on these observations, we suggest that the colicin exporting pathway may interact to some extent with the bacteriophage infection pathway leading to a limited selective advantage for and limited protection of colicin-producing cells against different bacteriophages.

19. **Standardised evaluation of the performance of a simple membrane filtration-elution method to concentrate bacteriophages from drinking water. Mendez, J., Audicana, A., Isern, A., Llaneza, J., Moreno, B., Tarancon, M. L., Jofre, J., Lucena, F. (2004). *Journal of Virological Methods* 117:19-25.** The bacteriophage elution procedure described further after adsorption to acetate-nitrate cellulose membrane filters allows better recovery of phages concentrated from 1l of water than elution procedures used previously. The improvement is due to the combined effect of the eluent (3% (w/v) beef extract, 3% (v/v) Tween 80, 0.5M NaCl, pH 9.0) and the application of ultrasound instead of agitation or swirling. Average recovery of somatic coliphages, 82 +/- 7%, was the greatest, and that of phages infecting *Bacteroides fragilis*, 56 +/- 8%, the lowest, with intermediate values for F-specific and F-specific RNA bacteriophages. Thus, the method allowed recovery of over 56% for all the phages

suggested as surrogate indicators. The method was then validated according to an International Standardisation Organisation validation standard procedure and implemented in routine laboratories, which obtained reproducible results.

20. **Factors influencing the replication of somatic coliphages in the water environment. Muniesa, M., Jofre, J. (2004). *Antonie van Leeuwenhoek* 86:65-76.** The potential replication of somatic coliphages in the environment has been considered a drawback for their use as viral indicators, although the extent to which this affects their numbers in environmental samples has not been assessed. In this study, the replication of somatic coliphages in various conditions was assayed using suspensions containing naturally occurring somatic coliphages and *Escherichia coli* WG5, which is a host strain recommended for detecting somatic coliphages. The effects on phage replication of exposing strain WG5 and phages to a range of physiological conditions and the effects of the presence of suspended particles or other bacteria were also assayed. Phage replication was further tested using a strain of *Klebsiella terrigena* and naturally occurring *E. coli* cells as hosts. Our results indicate that threshold densities of both host bacterium and phages should occur simultaneously to ensure appreciable phage replication. Host cells originating from a culture in the exponential growth phase and incubation at 37 °C were the best conditions for phage replication in *E. coli* WG5. In these conditions the threshold densities required to ensure phage replication were about 10^4 host cells/ml and 10^3 phages/ml, or 10^3 host cells/ml and 10^4 phages/ml, or intermediate values of both. The threshold densities needed for phage replication were higher when the cells proceeded from a culture in the stationary growth phase or when suspended particles or other bacteria were present. Furthermore *E. coli* WG5 was more efficient in supporting phage replication than either *K. terrigenae* or *E. coli* cells naturally occurring in sewage. Our results indicate that the phage and bacterium densities and the bacterial physiological conditions needed for phage replication are rarely expected to be found in the natural water environments.

21. **Detection of enteric viruses in shellfish from the Norwegian coast. Myrmel, M., Berg, E. M. M., Rimstad, E., Grinde, B. (2004). *Applied and Environmental Microbiology* 70:2678-2684.** Common blue mussels (*Mytilus edulis*), horse mussels (*Modiolus modiolus*), and flat oysters (*Ostrea edulis*) obtained from various harvesting and commercial production sites along the Norwegian coast were screened for the presence of norovirus by a real-time reverse transcription (RT)-nested PCR assay and for possible indicators of fecal contamination, i.e., for F-specific RNA bacteriophages (F-RNA phages) by plaque assay and for human adenoviruses and human circoviruses by nested PCR assay. The aims were to obtain relevant information for assessing the risk of transmission of enteric viruses by shellfish and to investigate the potential of various indicator viruses in routine screening. Noroviruses were detected in 6.8% of the samples, and the indicators were detected in 23.8% (F-RNA phages), 18.6% (adenoviruses), and 8.0% (circoviruses) of the samples. A seasonal variation was observed, with the exception of circoviruses, with more positive samples in the winter. A positive correlation was found between F-RNA phages and noroviruses. However, F-RNA phages were present in only 43% of the norovirus-positive samples. The results show that mussels from the Norwegian coast can constitute a risk of infection with enteric viruses and that routine testing of samples may be justified. Advantages and disadvantages of various options for screening are discussed.

22. **Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. Noble, R. T., Lee, I. M., Schiff, K. C. (2004). *Journal of Applied Microbiology* 96:464-472.** AIM: The survival of indicator micro-organisms in aquatic systems is affected by both biotic and abiotic factors. Much of the past research on this topic has been conducted using laboratory-generated cultures of indicator bacteria. For this study, we used natural sources of faecal contamination as inoculants into environmental water samples, thereby representing the wide diversity of organisms likely to be found in faecal contamination. METHODS AND RESULTS: Rates of inactivation of water quality indicators, total coliforms (TC), *Escherichia coli*, enterococci (EC) and F+-specific coliphage were studied in three experiments using inoculants of sewage influent, sewage effluent and urban storm drain run-off. Effects of temperature, nutrients, total suspended solids, bacterial load and solar irradiation were studied in fresh and seawater matrices. Results demonstrated that temperature and solar irradiation had significant effects upon rates of inactivation (anova, $P < 0.001$). Inactivation rates were similar, regardless of the inoculant type. EC degraded the slowest in the dark with T90s of 115-121 and 144-177 h at 20 and 14°C, respectively. When incubated in sunlight, EC was inactivated significantly more rapidly than either *E. coli* or F+-specific coliphage ($P < 0.001$). CONCLUSIONS: Inactivation of indicator bacteria is not dependent upon the original source of contamination. Inactivation rates of indicator bacteria were similar in fresh and seawater matrices. However, EC degraded more rapidly in sunlight than E. coli. SIGNIFICANCE AND IMPACT OF THE STUDY: This study suggests that the source of faecal contamination is not an important factor to inactivation rates of indicator bacteria. However, rates of inactivation of indicator bacteria are likely system specific.

23. **Testing for viral penetration of non-latex surgical and examination gloves: a comparison of three methods. O'Connell, K. P., El Masri, M., Broyles, J. B., Korniewicz, D. M. (2004). *Clinical microbiology and infection* 10:322-326.** Currently, there are no international standards based on microbiological methodology for testing the ability of medical examination or surgical gloves to prevent the passage of viruses. Three protocols for the direct examination of the viral barrier properties of non-latex gloves were compared with 1080 gloves (270 gloves from each of two surgical brands and two medical examination brands). In two of the methods, gloves were filled with and suspended in a nutrient broth solution, and bacteriophage ϕX174 was placed either inside or outside the glove, while the entire test vessel was agitated. Gloves tested using the third method were filled with a suspension of bacteriophage and allowed to rest in a vessel containing nutrient broth. Gloves were tested directly from the manufacturer's packaging, or after being punctured intentionally or subjected to a stress protocol. The passage of bacteriophage was detected with plaque assays. Significant differences in failure rates between glove brands were apparent only among gloves that had been subjected to the stress protocol. Overall, the two methods in which bacteriophage were placed inside the gloves provided more sensitivity than the method in which bacteriophage was spiked into broth outside the gloves. Thus the placement of bacteriophage inside test gloves (or the use of pressure across the glove barrier during testing), and the use of a standardised stress protocol, will improve significantly the ability of a glove test protocol to determine the relative quality of the barrier offered by medical examination and surgical gloves. Further research is needed to provide test methods that can incorporate reproducibly both the use of bacteriophage and simulated glove use in an industrial quality control setting.

24. **Characterization of three *Lactobacillus delbrueckii* subsp. *bulgaricus* phages and the physicochemical analysis of phage adsorption. Quiberoni, A., Guglielmotti, D., Binetti, A., Reinheimer, J. (2004). *Journal of***

***Applied Microbiology* 96:340-351.** AIMS: Three indigenous *Lactobacillus delbrueckii* subsp. *bulgaricus* bacteriophages and their adsorption process were characterized. METHODS AND RESULTS: Phages belonged to Bradley's group B or the Siphoviridae family (morphotype B1). They showed low burst size and short latent periods. A remarkably high sensitivity to pH was also demonstrated. Indigenous phage genomes were linear and double-stranded DNA molecules of approx. 31-34 kbp, with distinctive restriction patterns. Only one phage genome appeared to contain cohesive ends. Calcium ions did not influence phage adsorption, but it was necessary to accelerate cell lysis and improve plaque formation. The adsorption kinetics were similar on viable and nonviable cells, and the adsorption rates were high between 0 and 50 degrees C. SDS and proteinase K treatments did not influence the phage adsorption but mutanolysin and TCA reduced it appreciably. No significant inhibitory effect on phage adsorption was observed for the saccharides tested. This study also revealed the irreversibility of phage adsorption to their hosts. CONCLUSIONS, SIGNIFICANCE AND IMPACT OF THE STUDY: The study increases the knowledge on phages of thermophilic lactic acid bacteria.

25. **[Combined use of active chlorine and coagulants for drinking water purification and disinfection]. Rakhmanin, I. A., Zholdakova, Z. I., Poliakova, E. E., Kir'ianova, L. F., Miasnikov, I. N., Tul'skaia, E. A., Artemova, T. Z., Ivanova, L. V., Dmitrieva, R. A., Doskina, T. V. (2004). *Gigiena i Sanitariia* 6-9.** The authors made an experimental study of the efficiency of water purification procedures based on the combined use of active chlorine and coagulants and hygienically evaluated the procedures. The study included the evaluation of water disinfection with various coagulants and active chlorine; the investigation of the processes of production of deleterious organic chlorine compounds; the assessment of the quality of water after its treatment. The coagulants representing aluminum polyoxychloride: RAX-10 (AQUA-AURATE 10) and RAX-18 (AQUA-AURATE 18), and aluminum sulfate, technically pure grade were tested. The treatment of river water with the coagulants RAX-10 and RAX-18, followed by precipitation, filtration, and chlorination under laboratory conditions, was shown to result in water disinfection to the levels complying with the requirements described in SanPiN 2.1.4.1074-01. RAX-18 showed the best disinfecting activity against total and heat-tolerant coliform bacteria, but also to the highly chlorine-resistant microrganisms--the spores of sulfite-reducing Clostridia, phages, and viruses. Since the coagulants have an increased sorptive capacity relative to humus and other organic substances, substitution of primary chlorination for coagulant treatment may induce a reduction in the risk of formation of oncogenically and mutagenically hazardous chlorinated hydrocarbons.

26. **The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. Rice, G., Tang, L., Stedman, K., Roberto, F., Spuhler, J., Gillitzer, E., Johnson, J. E., Douglas, T., Young, M. (2004). *Proceedings of the National Academy of Sciences, USA* 101:7716-7720.** Of the three domains of life (Eukarya, Bacteria, and Archaea), the least understood is Archaea and its associated viruses. Many Archaea are extremophiles, with species that are capable of growth at some of the highest temperatures and extremes of pH of all known organisms. Phylogenetic rRNA-encoding DNA analysis places many of the hyperthermophilic Archaea (species with an optimum growth 80°C) at the base of the universal tree of life, suggesting that thermophiles were among the first forms of life on earth. Very few viruses have been identified from Archaea as compared to Bacteria and Eukarya. We report here the structure of a hyperthermophilic virus isolated from an archaeal host found in hot springs in Yellowstone National Park. The sequence of the circular double-stranded DNA viral genome shows that it shares little similarity to other known genes in viruses or other organisms. By comparing the tertiary and quaternary structures of the coat protein of this virus with those of a bacterial and an animal virus, we find conformational relationships among all three, suggesting that some viruses may have a common ancestor that precedes the division into three domains of life >3 billion years ago.

27. **Distribution, sequence homology, and homing of group I introns among T-even-like bacteriophages: evidence for recent transfer of old introns. Sandegren, L., Sjoberg, B. M. (2004). *The Journal of biological chemistry* 279:22218-22227.** Self-splicing group I introns are being found in an increasing number of bacteriophages. Most introns contain an open reading frame coding for a homing endo-nuclease that confers mobility to both the intron and the homing endonuclease gene (HEG). The frequent occurrence of intron/HEG has raised questions whether group I introns are spread via horizontal transfer between phage populations. We have determined complete sequences for the known group I introns among T-even-like bacteriophages together with sequences of the intron-containing genes td, nrdB, and nrdD from phages with and without introns. A previously uncharacterized phage isolate, U5, is shown to contain all three introns, the only phage besides T4 found with a "full set" of these introns. Sequence analysis of td and nrdB genes from intron-containing and intronless phages provides evidence that recent horizontal transmission of introns has occurred among the phages. The fact that several of the HEGs have suffered deletions rendering them non-functional implies that the homing endonucleases are of no selective advantage to the phage and are rapidly degenerating and probably dependent upon frequent horizontal transmissions for maintenance within the phage populations. Several of the introns can home to closely related intronless phages during mixed infections. However, the efficiency of homing varies and is dependent on homology in regions flanking the intron insertion site. The occurrence of optional genes flanking the respective intron-containing gene can strongly affect the efficiency of homing. These findings give further insight into the mechanisms of propagation and evolution of group I introns among the T-even-like bacteriophages.

28. **Newly isolated *Vibrio cholerae* non-O1, non-O139 phages. Sarkar, B. L., Ghosh, A. N., Sen, A., Rodrigues, D. P. (2004). *Emerging Infectious Diseases* 10:754-756.** To the Editor: The epidemic cholera caused by *Vibrio cholerae* O1 appeared in Latin America in 1991 after a 100-year absence. Following its explosive appearance in Peru, travelers on the Amazon River brought cholera to Brazil by April 1991. It spread southward along the Atlantic Coast of Brazil, reaching Rio de Janeiro in February 1993. ¶ Phage typing is a useful tool for studying the source or origin of *V. cholerae* for epidemiologic importance. Because of limitations of the Basu and Mukerjee scheme, a new phage-typing scheme for *V. cholerae* O1 was developed at the National Institute of Cholera and Enteric Diseases, India (1-3). During the course of a comprehensive study on the phage typing of *V. cholerae* O1, most strains isolated in Brazil were found to be sensitive with a set of 10 El Tor phages (ATCC 51352-B1-B10) (4). This finding prompted us to explore or ascertain the natural habitat of *V. cholerae* and cholera phages, if any, in an environmental reservoir in Brazil, particularly in Rio de Janeiro.

29. **Phage offer a real alternative. Schoolnik, G. K., Summers, W. C., Watson, J. D. (2004). *Nature biotechnology* 22:505-506.** A News and Views piece by Steven Projan in your February issue offers a gratuitous, pessimistic assessment for the prospects of phage therapy *per se* (*Nat. Biotechnol.* 22, 167-168, 2004). We believe Projan's

criticisms are overly broad and fail to consider the published literature and the impact that contemporary phage biology is having on the development of phage therapeutics. We would not have been moved to respond to his comments were it not for our view that the pharmaceutical industry's capacity to develop truly novel chemical antibiotics or antibacterials is being outstripped through the evolution of antimicrobial resistance by a broad array of infectious agents. Thus, in the spirit of a constructive dialogue, we—participants at a Cold Spring Harbor Banbury Conference—offer the following rejoinder.

30. **[Bacteriophages as antibacterial agents]. Shasha, S. M., Sharon, N., Inbar, M. (2004). *Harefuah* 143:121-5, 166.** Bacteriophages are viruses that only infect bacteria. They have played an important role in the development of molecular biology and have been used as anti-bacterial agents. Since their independent discovery by Twort and d'Herelle, they have been extensively used to prevent and treat bacterial infections, mainly in Eastern Europe and the former Soviet Union. In western countries this method has been sporadically employed on humans and domesticated animals. However, the discovery and widespread use of antibiotics, coupled with doubts about the efficacy of phage therapy, led to an eclipse in the use of phage in medicine. The emergence of antibiotic resistant bacteria, especially strains that are multiply resistant, has resulted in a renewed interest in alternatives to conventional drugs. One of the possible replacements for antibiotics is the use of bacteriophages as antimicrobial agents. This brief review aims to describe the history of bacteriophage and early clinical studies on their use in bacterial disease prophylaxis and therapy, and discuss the advantages and disadvantages of bacteriophage in this regard.

31. ***Burkholderia cenocepacia* phage BcepMu and a family of Mu-like phages encoding potential pathogenesis factors. Summer, E. J., Gonzalez, C. F., Carlisle, T., Mebane, L. M., Cass, A. M., Savva, C. G., LiPuma, J. J., Young, R. (2004). *Journal of Molecular Biology* 340:49-65.** We have isolated BcepMu, a Mu-like bacteriophage whose host range includes human pathogenic *Burkholderia cenocepacia* (formally *B. cepacia* genomovar III) isolates, and determined its complete 36,748 bp genomic sequence. Like enteric bacteriophage Mu, the BcepMu genomic DNA is flanked by variable host sequences, a result of transposon-mediated replication. The BcepMu genome encodes 53 proteins, including capsid assembly components related to those of Mu, and tail sheath and tube proteins related to those of bacteriophage P2. Seventeen of the BcepMu genes were demonstrated to encode homotypic interacting domains by using a cI fusion system. Most BcepMu genes have close homologs to prophage elements present in the two published *Salmonella typhi* genomes, and in the database sequences of *Photorhabdus luminescens*, and *Chromobacterium violaceum*. These prophage elements, designated SalMu, PhotoMu and ChromoMu, respectively, are collinear with BcepMu through nearly their entire lengths and show only limited mosaicism, despite the divergent characters of their hosts. The BcepMu family of Mu-like phages has a number of notable differences from Mu. Most significantly, the critical left end region of BcepMu is inverted with respect to Mu, and the BcepMu family of transposases is clearly of a distinct lineage with different molecular requirements at the transposon ends. Interestingly, a survey of 33 *B.cepacia* complex strains indicated that the BcepMu prophage is widespread in human pathogenic *B.cenocepacia* ET12 lineage isolates, but not in isolates from the PHDC or Midwest lineages. Identified members of the BcepMu family all contain a gene possibly involved in bacterial pathogenicity, a homolog of the type-two-secretion component exeA, but only BcepMu also carries a lipopolysaccharide modification acyltransferase which may also contribute a pathogenicity factor.

32. **The interaction of phage and biofilms. Sutherelandl, I. W., Hughes, K. A., Skillman, L. C., Tait, K. (2004). *FEMS Microbiology Letters* 232:1-6.** Biofilms present complex assemblies of micro-organisms attached to surfaces. they are dynamic structures in which various metabolic activities and interactions between the component cells occur. When phage come in contact with biofilms, further interactions occur dependent on the susceptibility of the biofilm bacteria to phage and to the availability of receptor sites. If the phage also possess polysaccharide-degrading enzymes, or if considerable cell lysis is effected by the phage, the integrity of the biofilm may rapidly be destroyed. Alternatively, coexistence between phage and host bacteria within the biofilm may develop. Although phage have been proposed as a means of destroying or controlling biofilms, the technology for this has not yet been successfully developed.

33. **Toward rational control of *Escherichia coli* O157:H7 by a phage cocktail. Tanji, Y., Shimada, T., Yoichi, M., Miyanaga, K., Hori, K., Unno, H. (2004). *Applied Microbiology and Biotechnology* 64:270-274.** Twenty six phages infected with *Escherichia coli* O157:H7 were screened from various sources. Among them, nine caused visible lysis of *E. coli* O157:H7 cells in LB liquid medium. However, prolonged incubation of *E. coli* cells and phage allowed the emergence of phage-resistant cells. The susceptibility of the phage-resistant cells to the nine phages was diverse. A rational procedure for selecting an effective cocktail of phage for controlling bacteria was investigated based on the mechanism of phage-resistant cell conversion. Deletion of OmpC from the *E. coli* cells facilitated the emergence of cells resistant to SP21 phage. After 8 h of incubation, SP21-resistant cells appeared. By contrast, alteration of the lipopolysaccharide (LPS) profile facilitated cell resistance to SP22 phage, which was observed following a 6-h incubation. When a cocktail of phages SP21 and SP22 was used to infect *E. coli* O157:H7 cells, 30 h was required for the emergence of cells (R-C) resistant to both phages. The R-C cells carried almost the same outer membrane and LPS components as the wild-type cells. However, the reduced binding ability of both phages to R-C cells suggested disturbance of phage adsorption to the R-C surface. Even though R-C cells resistant to both phages appeared, this work shows that rational selection of phages has the potential to at least delay the emergence of phage resistance.

34. **Old dogma, new tricks--21st Century phage therapy. Thiel, K. (2004). *Nature biotechnology* 22:31-36.** As antibiotic resistant bacteria threaten a public health crisis, biotechnology is turning to bacteriophages, nature's tiniest viruses. But can phage therapy overcome its historical baggage?

35. **Bead-based electrochemical immunoassay for bacteriophage MS2. Thomas, J. H., Kim, S. K., Hesketh, P. J., Halsall, H. B., Heineman, W. R. (2004). *Analytical Chemistry* 76:2700-2707.** Viruses are one of four classes of biothreat agents, and bacteriophage MS2 has been used as a simulant for biothreat viruses, such as smallpox. A paramagnetic bead-based electrochemical immunoassay has been developed for detecting bacteriophage MS2. The immunoassay sandwich was made by attaching a biotinylated rabbit anti-MS2 IgG to a streptavidin-coated bead, capturing the virus, and then attaching a rabbit anti-MS2 IgG-β-galactosidase conjugate to another site on the virus. β-Galactosidase converts p-aminophenyl galactopyranoside (PAPG) to p-aminophenol (PAP). PAPG is electroinactive at the potential at which PAP is oxidized to p-quinone imine (PQI), so the current resulting from the

oxidation of PAP to PQI is directly proportional to the concentration of antigen in the sample. The immunoassay was detected with rotating disk electrode (RDE) amperometry and an interdigitated array (IDA) electrode. With an applied potential of +290 mV vs Ag/AgCl and a rotation rate of 3000 rpm, the detection limit was 200 ng/mL MS2 or 3.2 x 10^{10} viral particles/mL with RDE amperometry. A trench IDA electrode was incorporated into a poly(dimethyl siloxane) channel, within which beads were collected, incubated with PAPG, and PAP generation was detected. The two working electrodes were held at +290 and -300 mV vs Ag/AgCl, and electrochemical recycling of the PAP/PQI couple by the IDA electrode lowered the limit of detection to 90 ng/mL MS2, or 1.5 x 10^{10} MS2 particles/mL.

36. **The role of prophage-like elements in the diversity of *Salmonella enterica* serovars. Thomson, N., Baker, S., Pickard, D., Fookes, M., Anjum, M., Hamlin, N., Wain, J., House, D., Bhutta, Z., Chan, K., Falkow, S., Parkhill, J., Woodward, M., Ivens, A., Dougan, G. (2004). *Journal of Molecular Biology* 339:279-300.** The *Salmonella enterica* serovar Typhi CT18 (*S*.Typhi) chromosome harbours seven distinct prophage-like elements, some of which may encode functional bacteriophages. *In silico* analyses were used to investigate these regions in *S*.Typhi CT18, and ultimately compare these integrated bacteriophages against 40 other Salmonella isolates using DNA microarray technology. *S*.Typhi CT18 contains prophages that show similarity to the lambda, Mu, P2 and P4 bacteriophage families. When compared to other *S*.Typhi isolates, these elements were generally conserved, supporting a clonal origin of this serovar. However, distinct variation was detected within a broad range of *Salmonella* serovars; many of the prophage regions are predicted to be specific to *S*.Typhi. Some of the P2 family prophage analysed have the potential to carry non-essential "cargo" genes within the hyper-variable tail region, an observation that suggests that these bacteriophage may confer a level of specialisation on their host. Lysogenic bacteriophages therefore play a crucial role in the generation of genetic diversity within *S.enterica*.

37. **Evaluating microbial purification during soil treatment of wastewater with multicomponent tracer and surrogate tests. Van Cuyk, S., Siegrist, R. L., Lowe, K., Harvey, R. W. (2004). *Journal of Environmental Quality* 33:316-329.** Soil treatment of wastewater has the potential to achieve high purification efficiency, yet the understanding and predictability of purification with respect to removal of viruses and other pathogens is limited. Research has been completed to quantify the removal of virus and bacteria through the use of microbial surrogates and conservative tracers during controlled experiments with three-dimensional pilot-scale soil treatment systems in the laboratory and during the testing of full-scale systems under field conditions. The surrogates and tracers employed included two viruses (MS-2 and PRD-1 bacteriophages), one bacterium (ice-nucleating active *Pseudomonas*), and one conservative tracer (bromide ion). Efforts have also been made to determine the relationship between viruses and fecal coliform bacteria in soil samples below the wastewater infiltrative surface, and the correlation between *Escherichia coli* concentrations measured in percolating soil solution as compared with those estimated from analyses of soil solids. The results suggest episodic breakthrough of virus and bacteria during soil treatment of wastewater and a 2 to 3 log (99-99.9%) removal of virus and near complete removal of fecal coliform bacteria during unsaturated flow through 60 to 90 cm of sandy medium. Results also suggest that the fate of fecal coliform bacteria may be indicative of that of viruses in soil media near the infiltrative surface receiving wastewater effluent. Concentrations of fecal coliform in percolating soil solution may be conservatively estimated from analysis of extracted soil solids.

38. **Models of phage growth and their applicability to phage therapy. Weld, R. J., Butts, C., Heinemann, J. A. (2004). *Journal of Theoretical Biology* 227:1-11.** Phage therapy is complicated by the self-replicating nature of phage. It is difficult to extrapolate from *in vitro* phage growth data to *in vivo* expectations, difficult to interpret *in vivo* data and difficult to generalize from one *in vivo* situation to another. Various generic models of phage growth have been used as the theoretical basis for understanding the kinetics of phage therapy. Here, we have experimentally tested the efficacy of such simple models to predict, qualitatively and quantitatively, the growth of phage and the phage proliferation threshold *in vitro*. Naturally occurring, antibiotic-resistant bacteria were used to measure the growth of phage *in vivo*. In homogenous, *in vitro* environments, the models were predictive of T4 phage growth on *Escherichia coli* RR1. However, the models were not able to predict growth of T4 phage or K1-5 phage in the more complex environment of the rat's digestive tract. To explore fully the kinetics of phage therapy, more complex models need to be devised. We suggest that it may be necessary to consider and model the interactions between phage growth parameters and bacterial growth parameters.

39. **Genome function--a virus-world view. Yin, J. (2004). *Advances in experimental medicine and biology* 547:31-46.** By studying viruses one may begin to understand how static genomes can define dynamic processes of development. This talk will describe some of the approaches we are taking, using computer simulations and laboratory experiments, to account for the many molecular-level processes and interactions that occur when a common bacterium, *E. coli*, is infected by one of its viruses, phage T7. We accounted for processes of phage genome entry, transcription, translation, and DNA replication, including protein-DNA and protein-protein regulatory interactions, and we predicted the dynamics of phage progeny formation. The simulations have enabled us to identify limiting host-cell resources in phage growth, discover novel anti-viral strategies, and suggest frameworks for mining data from global mRNA and protein studies.

40. **Bacteriophage observations and evolution. Ackermann, H.-W. (2003). *Research in Microbiology* 154:245-251.** Bacteriophages are classified into one order and 13 families. Over 5100 phages have been examined in the electron microscope since 1959. At least 4950 phages (96%) are tailed. They constitute the order *Caudovirales* and three families. *Siphoviridae* or phages with long, noncontractile tails predominate (61% of tailed phages). Polyhedral, filamentous, and pleomorphic phages comprise less than 4% of bacterial viruses. Bacteriophages occur in over 140 bacterial or archaeal genera. Their distribution reflects their origin and bacterial phylogeny. Bacteriophages are polyphyletic, arose repeatedly in different hosts, and constitute 11 lines of descent. Tailed phages appear as monophyletic and as the oldest known virus group.

41. **Evolution of phage with chemically ambiguous proteomes. Bacher, J. M., Bull, J. J., Ellington, A. D. (2003). *BMC evolutionary biology [electronic resource]* 3:24.** BACKGROUND: The widespread introduction of amino acid substitutions into organismal proteomes has occurred during natural evolution, but has been difficult to achieve by directed evolution. The adaptation of the translation apparatus represents one barrier, but the multiple mutations that may be required throughout a proteome in order to accommodate an alternative amino acid or analogue is an even more daunting problem. The evolution of a small bacteriophage proteome to accommodate an unnatural amino

acid analogue can provide insights into the number and type of substitutions that individual proteins will require to retain functionality. RESULTS: The bacteriophage Qβ initially grows poorly in the presence of the amino acid analogue 6-fluorotryptophan. After 25 serial passages, the fitness of the phage on the analogue was substantially increased; there was no loss of fitness when the evolved phage were passaged in the presence of tryptophan. Seven mutations were fixed throughout the phage in two independent lines of descent. None of the mutations changed a tryptophan residue. CONCLUSIONS: A relatively small number of mutations allowed an unnatural amino acid to be functionally incorporated into a highly interdependent set of proteins. These results support the 'ambiguous intermediate' hypothesis for the emergence of divergent genetic codes, in which the adoption of a new genetic code is preceded by the evolution of proteins that can simultaneously accommodate more than one amino acid at a given codon. It may now be possible to direct the evolution of organisms with novel genetic codes using methods that promote ambiguous intermediates.

42. **Do viruses form lineages across different domains of life? Bamford, D. H. (2003). *Research in Microbiology* 154:231-236.** The scarce characterisation of the viral world has hampered our efforts to appreciate the magnitude and diversity of the viral domain. It appears that almost every species can be infected by a number of viruses. As our knowledge of viruses increases, it appears that this myriad of viruses may be organised into a reasonably low number of viral lineages including members infecting hosts belonging to different domains of life. Viruses belonging to a lineage share a common innate "self" that refers to structural and assembly principles of the virion. This hypothesis has a few consequences. All viruses are old, maybe preceding cellular life, and virus origins are polyphyletic, as opposed to the idea of a monophyletic origin of cellular life.

43. **Isolation and characterization of marine psychrophilic phage-host systems from Arctic sea ice. Borriss, M., Helmke, E., Hanschke, R., Schweder, T. (2003). *Extremophiles : life under extreme conditions* 7:377-384.** Phage-host systems from extreme cold environments have rarely been surveyed. This study is concerned with the isolation and characterization of three different phage-host systems from Arctic sea ice and melt pond samples collected north-west of Svalbard (Arctic). On the basis of 16S rDNA sequences, the three bacterial phage hosts exhibited the greatest similarity to the species *Shewanella frigidimarina* (96.0%), *Flavobacterium hibernum* (94.0%), and *Colwellia psychrerythraea* (98.4%), respectively. The host bacteria are psychrophilic with good growth at 0°C, resulting in a rapid formation of visible colonies at this temperature. The phages showed an even more pronounced adaptation to cold temperatures than the bacteria, with growth maxima below 14°C and good plaque formation at 0°C. Transmission electron microscopy (TEM) examinations revealed that the bacteriophages belonged to the tailed, double-stranded DNA phage families Siphoviridae and Myoviridae. All three phages were host-specific.

44. **Prophage insertion sites. Campbell, A. (2003). *Research in Microbiology* 154:277-282.** Insertion of viral DNA into host chromosomes is an ancient process essential for propagation in the proviral form. Many present-day bacteriophages insert at specific sites on the host chromosome. Insertion by two coliphage families (lambdoid and P4-like) is compared. For both families, insertion sites frequently lie within tRNA genes. The lambdoid phages insert at anticodon loops, whereas the p4-like phages insert in the TψC loops downstream from them. The association of both groups with tRNA genes suggests that the primordial insertion site of both groups may have been within a tRNA gene. The integrase proteins used in phage insertion may have originated at that stage, with subsequent diversification of specificity.

45. **The diversity and evolution of the T4-type bacteriophages. Desplats, C., Krisch, H. M. (2003). *Research in Microbiology* 154:259-267.** Recent studies suggest that viruses are the most numerous entities in the biosphere; bacteriophages, the viruses that infect Eubacteria and Archaea, constitute a substantial fraction of this population. In spite of their ubiquity, the vast majority of phages in the environment have never been studied and nothing is known about them. For the last 10 years our research has focused on an extremely widespread group of phages, the T4-type. It has now become evident that phage T4 has a myriad of relatives in nature that differ significantly in their host range. The genomes of all these phages have homology to the T4 genes that determine virion morphology. Although phylogenetically related, these T4-type phages can be subdivided into four groups that are increasingly distant from T4: the T-evens, the pseudo T-evens, the schizo T-evens and the exo T-evens. Genomic comparisons between the various T4-type phages and T4 indicate that these genomes share homology not only for virion structural components but also for most of the essential genes involved in the T4 life cycle. This suggests that horizontal transmission of the genetic information may have played a less general role in the evolution of these phages than has been supposed. Nevertheless, we have identified several regions of the T4-type genome, such as the segment containing the tail fiber genes that exhibit evidence of extensive modular shuffling during evolution. The T4-type genomes appear to be a mosaic containing a large and fixed group of essential genes as well as highly variable set of non-essential genes. These non-essential genes are probably important for the adaptation of these phages to their particular life-style. Furthermore, swapping autonomous domains within the essential proteins may slightly modify their function(s) and contribute to the adaptive ability of the T4-type phage family. Regulatory sequences also display considerable evolutionary plasticity and this too may facilitate the adaptation of phage gene expression to new environments and stresses.

46. **Haloarchaeal viruses: how diverse are they? Dyall-Smith, M., Tang, S.-L., Bath, C. (2003). *Research in Microbiology* 154:309-313.** Hypersaline lakes are highly productive microbial environments that provide many advantages for microbial ecologists, including stable communities of relatively low diversity (mainly haloarchaea). An important component of these communities is comprised of their noncellular parasites, i.e., their viruses. Few viruses of halobacteria (haloviruses) have been isolated and studied even though a wide selection of host species have been formally described (and easily cultured) for ten years. Hypersaline waters have been shown to contain very high concentrations of virus-like particles (at least 10^7 particles/ml), particularly fusiform particles, but laboratory isolations of new haloviruses have been very slow and the detailed study of selected examples even slower. Here we provide an outline of the reported haloviruses, including fusiform and unpublished isolates from this laboratory, and we discuss their diversity and the future directions for this research.

47. **Effects of pH and temperature on the survival of coliphages MS2 and Qβ. Feng, Y. Y., Ong, S. L., Hu, J. Y., Tan, X. L., Ng, W. J. (2003). *Journal of Industrial Microbiology & Biotechnology* 30:549-552.** The RNA F-specific coliphages, MS2 and Qβ, have been used as virus indicators in water and wastewater studies. It is therefore useful to have a good understanding concerning the effects of environmental factors on their survival in order to

choose an appropriate candidate for assessing microbial safety in relation to water quality management. The effects of pH and temperature on the survival of these two coliphages were investigated. MS2 survived better in acidic conditions than in an alkaline environment. In contrast, Qβ had a better survival rate in alkaline conditions than in an acidic environment. The inactivation rates of both coliphages were lowest within the pH range 6-8 and the temperature range 5-35°C. The inactivation rates of both coliphages increased when the pH was decreased to below 6 or increased to above 8. The inactivation rates of both coliphages increased with increasing temperature. Qβ behaved peculiarly in extreme pH buffers, i.e. it was inactivated very rapidly initially when subjected to an extreme pH environment, although the inactivation rate subsequently decreased. In general, MS2 was a better indicator than Qβ. However, within the pH range 6-9 and at temperatures not above 25°C, either MS2 or Qβ could be used as a viral indicator.

48. **The role played by viruses in the evolution of their hosts: a view based on informational protein phylogenies. Filée, J., Forterre, P., Laurent, J. (2003). *Research in Microbiology* 154:237-243.** Viruses are often considered as fragments of cellular RNA or DNA that escaped a long time ago from cellular chromosomes and that evolved later on by capturing additional genes from the genomes of their hosts. However, this view has now been challenged by the discovery of surprising homology between viruses with very distantly related hosts, and by phylogenetic analyses suggesting that genes might also have flown from viruses to cells. We present here phylogenetic analyses of four proteins involved in DNA replication and synthesis of DNA precursors (DNA polymerases delta, ribonucleotide reductases, thymidylate synthases and replicative helicases) and we discuss the reciprocal roles of cells and viruses during the evolutionary history of these enzymes. These analyses revealed numerous lateral gene transfer events between cells and viruses, in both directions. We suggest that lateral gene transfers from viruses to cells and nonorthologous gene replacements of cellular genes by viral ones are an important source of "genetic novelties" in the evolution of cellular lineages. Thus, viruses have definitively to be considered as major players in the evolution of cellular genomes.

49. **The great virus comeback—from an evolutionary perspective. Forterre, P. (2003). *Research in Microbiology* 154:223-225.** [first paragraph] Viruses have played a critical role in the development of the field we now know as molecular biology [15]. The study of bacteriophages and animal viruses became fashionable when molecular biologists realized that it was too difficult to directly attack their favorite biological problem in cellular organisms. However, the interest in viruses as model systems has declined with the ensuing years, as powerful experimental tools (often provided by viruses themselves) made it much easier to work directly on "serious" things (the cellular ones). Since we are cellular organisms, this egocentric trend is understandable. The viruses truly belong to another living world, one whose only "*raison d'être*" seems to be to eat us. Most research on viruses now focuses on this conflict for resources between viruses and cells. For many, the only viruses worth studying are "pathogens" that can harm or kill us, in order to understand their vicious tricks and to effectively protect ourselves from them. For a long time, only a small handful of biologists had the scientific curiosity to be interested in viruses for their own sake. This was reinforced by the prejudice, shared by many, that viruses are not really alive, since they are not autonomous. Problems such as the origin of viruses were never considered as serious topics of scientific research. The extent of viral ubiquity and diversity was largely unknown and ignored. The studies on the mechanisms of viral evolution were mainly focused on a small number of "emerging pathogens". Most evolutionists ignored viruses because of the "Woesian revolution" that resulted in the first large-scale experimental studies on the evolution of microorganisms. Indeed, since viruses contain no ribosomal RNA, they had no obvious place in the "universal" tree of life. The development of genomic techniques also had a role in casting viruses aside from evolutionary models. Although viral genomes were the first to be completely sequenced, the nearly exclusive focus of subsequent analysis on cellular genomes shifted the attention of evolutionists toward the cellular world.

50. **Observation of virus-like particles in high temperature enrichment cultures from deep-sea hydrothermal vents. Geslin, C., Le Romancer, M., Gaillard, M., Erauso, G., Prieur, D. (2003). *Research in Microbiology* 154:303-307.** A systematic search was carried out on samples collected in various geographically distant hydrothermal sites located on the East Pacific Rise (EPR 9ºN and 13ºN) and Mid-Atlantic Ridge (MAR 36ºN and 37ºN) to investigate the diversity of virus-like particles (VLPs) from deep-sea vents. Eighty-nine positive enrichment cultures were obtained from one hundred and one crude samples at 85ºC. VLPs were detected by electron microscopy in fifteen different enrichments. Among the different morphotypes observed, the lemon-shaped type prevailed but rods and novel pleomorphic morphologies were also observed. Several observations strongly suggested that host strains of the novel VLPs belong to the hyperthermophilic euryarchaeal order Thermococcales.

51. **New insights into the possible role of bacteriophages in transplantation. Gorski, A., Nowaczyk, M., Weber-Dabrowska, B., Kniotek, M., Boratynski, J., Ahmed, A., Dabrowska, K., Wierzbicki, P., Switala-Jelen, K., Opolski, A. (2003). *Transplantation Proceedings* 35:2372-2373.** Due to the increasing prevalence of drug-resistant bacterial infections in the "post-antibiotic era," bacteriophages (bacterial viruses, BP) may be useful to administer to transplant recipients without exposing them to an increased risk of rejection, which occurs consequent to some viral infections. Herein we present evidence that at least some coliphages (T4) do not pose such risk. Interestingly, they may produce immunosuppressive effects extending transplant survival. Our data suggest that BP may be used in clinical transplantation to treat drug-resistant bacterial infections and perhaps as an adjunct to immunosuppressive therapy.

52. **Bacteriophages with tails: chasing their origins and evolution. Hendrix, R. W., Hatfull, G. F., Smith, M. C. M. (2003). *Research in Microbiology* 154:253-257.** Comparative genomic analysis of the tailed bacteriophages shows that they are genetically mosaic with respect to each other, implying that horizontal exchange of sequences is an important component of their evolution. Horizontal exchange occurs intensively among closely related phages but also at reduced frequency across the entire population of tailed phages. It results in exchange of homologous functions, exchange of analogous but non-homologous functions as with the prophage integrases, and introduction of novel functions into the genome as with the morons. Extrapolation of these processes back in evolutionary time leads to a speculative model for the origins and early evolution of phages.

53. **[Bacteriophage therapy: Stalin's forgotten medicine]. Kaulen, H. (2003). *Deutsche medizinische Wochenschrift* 128:307.**

54. **The view from Les Treilles on the origins, evolution and diversity of viruses. Krisch, H. M. (2003). *Research in Microbiology* 154:227-229.** [first two paragraphs] The traces of the Les Treilles meeting on Origins, Diversity and Evolution of Viruses go back to early 1998. At that time I raised the idea of a meeting on this topic with two of my phage genomics colleagues, Roger Hendrix of Pittsburgh and Harald Brüssow of Lausanne. Although there was enthusiasm for such an endeavour, our efforts in France, Switzerland and the United States to raise the necessary funds were not successful. The idea languished until 1999 when Patrick Forterre of Paris came to Toulouse to give a seminar. During dinner together that evening we decided, based on Patrick's recent successful experience with them, to apply for funding from the Fondation des Treilles in southern France. Less than a year later the meeting became a reality and it was a wonderful experience for all concerned. ¶ The chapters that follow capture the flavour of the results reported during the formal sessions. But for me, and many other participants, the most important aspect of the Les Treilles experience was the ad-libbed discussions among the participants on themes that were too "difficult" to be the subject of formal talks. At one point during the meeting Michael Balter (from the journal *Science*) accused me of being among the rare participants at Les Treilles not liking to speculate. Not true, but unlike some others, I am somewhat shy about doing it in public. Regardless of personal timidity, the ambiance at the Les Treilles meeting made us all think about hard questions that are too easy to dismiss by simply saying: "We will never know". My mentality rapidly became: "Who cares if we never really know, can we at least come up with a plausible scenario?" I now think we can, and that is a significant step forward. What follows is my scenario of the origins, evolution and diversity of viruses. It is my view from Les Treilles: a synthesis of what I heard, said, thought and maybe even dreamed during those too few days and nights near Tourtour. Needless to say it took some additional time and reflection to come up with even this only semi-complete story line.

55. ***Myoviridae* bacteriophages of *Pseudomonas aeruginosa*: a long and complex evolutionary pathway. Krylov, V., Pleteneva, E., Bourkaltseva, M., Shaburova, O., Volckaert, G., Sykilinda, N., Kurochkina, L., Mesyanzhinov, V. (2003). *Research in Microbiology* 154:269-275.** Recently we have accomplished the entire DNA sequence of bacteriophage ϕKZ, a giant virus infecting *Pseudomonas aeruginosa*. The 280 334-bp of ϕKZ genome is a linear, circularly permutated and terminally redundant, AT-rich dsDNA molecule that contains no sites for *Not*I, *Pst*I, *Sac*I, *Sma*I, *Xho*I and *Xma*III endonucleases. Limited homology to other bacteriophages on the DNA and protein levels indicated that ϕKZ represents a distinct branch of the *Myoviridae* family. In this work, we analyzed a group of six *P. aeruginosa* phages (Lin68, Lin21, PTB80, NN, EL, and RU), which are morphologically similar to ϕKZ, have similar genome size and low G + C content. All phages have a broad host range among *P. aeruginosa* strains, and they are resistant to the inhibitory action of many *P. aeruginosa* plasmids. The analysis of the genomic DNA by restriction enzymes and DNA-DNA hybridization shows that phages are representative of three ϕKZ-like species: ϕKZ-type (öKZ, Lin21, NN and PTB80), EL-type (EL and RU) and Lin68 which has a shorter tail than other phages. Except for related phages EL and RU, all ϕKZ-like phages have identical N-terminal amino acid sequences of the major capsid protein. Random genome sequencing shows that the EL and RU phages have no homology to the ϕKZ-like phages on DNA level. We propose that the ϕKZ, Lin21, NN, PTB80 and Lin68 phages can be included in a new ϕKZ genus, and that the EL and RU phages belong to a separate genus within the *Myoviridae* family. Based on the resistance to many restriction enzymes and the transduction ability, there are indications that over the long pathway of evolution, the ϕKZ-like phages probably inherited the capacity to infect different bacterial species.

56. **Bacteriophage therapy: an alternative to conventional antibiotics. Mathur, M. D., Vidhani, S., Mehndiratta, P. L. (2003). *The Journal of the Association of Physicians of India* 51:593-596.** Bacteriophage therapy is an important alternative to antibiotics in the current era of multidrug resistant pathogens. We reviewed the studies that dealt with the therapeutic use of phages from 1966-1996 and few latest ongoing phage therapy projects via internet. Phages were used topically, orally or systemically in Polish and Soviet studies. The success rate found in these studies was 80-95% with few gastrointeslinal or allergic side effects. British studies also demonstrated significant efficacy of phages against *Escherichia coli*, *Acinetobacter* spp., *Pseudomonas* spp and *Staphylococcus aureus*. US studies dealt with improving the bioavailability of phage. Problems faced in these studies have also been discussed. In conclusion, phage therapy may prove as an important alternative to antibiotics for treating multidrug resistant pathogens.

57. **[Evaluation of relations between plasmids and phage host range among clinical isolates of *Enterobacter cloacae*]. Nieradko, J., Kurlenda, J. (2003). *Medycyna Doswiadczalna i Mikrobiologia* 55:343-349.** The aim of this study was evaluation the plasmid influence on phage host range of clinical strains of *Enterobacter cloacae*. We found that strains included in restrictive pattern A, displayed reduced host range. Such reduced sensitivity make these strains excellent candidates for search restrictive-modification systems. High discriminative efficacy of isolated phages (specific for strains *Enterobacter cloacae*) make them useful tool for phage typing in epidemiological investigations.

58. **Evolutionary insights from studies on viruses from hot habitats. Prangishvili, D. (2003). *Research in Microbiology* 154:289-294.** The morphological diversity of viruses which parasitize hyperthermophilic archaea thriving at temperatures >=80 °C appears to exceed that of viruses of prokaryotes living at lower temperatures. Based on assumptions of the existence of viruses in the prebiotic phase of evolution and hot origins of cellular life, we suggest that this remarkable diversity could have its source in ancestral diversity of viral morphotypes in hot environments. Attempts are made to trace evolutionary relationships of viruses of hyperthermophilic archaea with other viruses.

59. **Ecological aspects of circulation of the phytopathogevic bacteria' phages in biocenoses. Semchuk, L. I., Andriychuk, O. M., Romashev, S. A., Ignatenko, T. O., Yatskovska, L. I. (2003). *Ecological Bulletin* Special release:498-501.**

60. **Relationships between fuselloviruses infecting the extremely thermophilic archaeon *Sulfolobus*: SSV1 and SSV2. Stedman, K. M., She, Q., Phan, H., Arnold, H. P., Hoz, I., Garrett, R. A., Zillig, W. (2003). *Research in Microbiology* 154:295-302.** The fusellovirus SSV2 from an Icelandic *Sulfolobus* strain was isolated, characterized and its complete genomic sequence determined. SSV2 is very similar in morphology, replication, genome size and number of open reading frames (ORFs) to the type virus of the family, SSV1 from Japan, except in its high level of uninduced virus production. The nucleotide sequences are, however, only 55% identical to each other,

much less than related bacteriophage, related animal viruses and the rudiviruses of *Sulfolobus*, SIRV1 and SIRV2. Nevertheless the genome architecture is very similar between the two viruses, indicating that despite this genomic dissimilarity the virus genomes are mostly homologous. Unlike SSV1, the sequence of SSV2 indicates integration into a glycyl tRNA gene and is completely missing a DNA packaging gene. There is a unique, perfectly tandemly directly repeated sequence of 62 nucleotides in SSV2 that has no similarity to known sequences or structures. By comparison to the SSV2 genome, an integrated partial fusellovirus genome was found in the *Sulfolobus solfataricus* P2 genome further confirming the dynamism of the *Sulfolobus* genome. Clustering of cysteine codon containing ORFs both in SSV1 and SSV2 indicates that these *Fuselloviridae* arose from a genome fusion event.

61. **Sequences and replication of genomes of the archaeal rudiviruses SIRV1 and SIRV2: Relationships to the archaeal lipothrixvirus SIFV and some eukaryal viruses. Peng, X., Blum, H., She, Q., Mallok, S., Brügger, K., Garrett, R. A., Prangishvili, D. (2001). *Virology* 291:226-234.** The double-stranded DNA genomes of the viruses SIRV1 and SIRV2, which infect the extremely thermophilic archaeon *Sulfolobus* and belong to the family *Rudiviridae,* were sequenced. They are linear, covalently closed at the ends, and 32,312 and 35,502 bp long, respectively, with an A1T content of 75%. The genomes of SIRV1 and SIRV2 carry inverted terminal repeats of 2029 and 1628 bp, respectively, which contain multiple direct repeats. SIRV1 and SIRV2 genomes contain 45 and 54 ORFs, respectively, of which 44 are homologous to one another. Their predicted functions include a DNA polymerase, a Holliday junction resolvase, and a dUTPase. The genomes consist of blocks with well-conserved sequences separated by nonconserved sequences. Recombination, gene duplication, horizontal gene transfer, and substitution of viral genes by homologous host genes have contributed to their evolution. The finding of head-to-head and tail-to-tail linked replicative intermediates suggests that the linear genomes replicate by the same mechanism as the similarly organized linear genomes of the eukaryal poxviruses, African swine fever virus and *Chlorella* viruses. SIRV1 and SIRV2 both contain motifs that resemble the binding sites for Holliday junction resolvases of eukaryal viruses and may use common mechanisms for resolution of replicative intermediates. The results suggest a common origin of the replication machineries of the archaeal rudiviruses and the above-mentioned eukaryal viruses. About 1/3 of the ORFs of each rudivirus have homologs in the *Sulfolobus* virus SIFV of the family *Lipothrixviridae,* indicating that the two viral families form a superfamily. The finding of inverted repeats of at least 0.8 kb at the termini of the linear genome of SIFV supports this inference.

contents | BEG News (021) | top of page

Acknowledgements

Big thanks go to Steve McQuinn, Hans Ackermann, and Gary Kaiser for their contributions to this issue of BEG News.

contents | BEG members | top of page

Contact Steve Abedon (microdude+@osu.edu) with suggestions, criticisms, comments, or anything else that might help make this a better site.