Bacteriophage Ecology Group (BEG) News

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

April 1, 2004 issue (volume 20)

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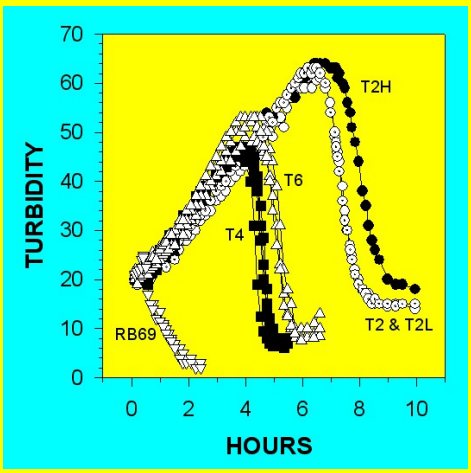
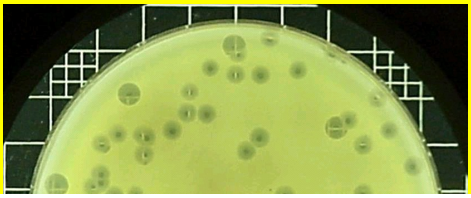
Editorial

A Brief History of Phage as Art

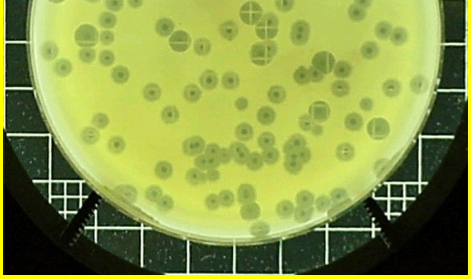
Allow me to go out on a limb and declare that phages are inspiring and beautiful and, therefore, are objects of art. This art can be intentional, or not, and scientifically relevant, or not. And I□m not just talking about virion particles. The earliest of phage "art" likely were the glassy, partially dissolved colonies of Frederick Twort. What follows is a brief tour of the history of phage art.

Twort□s colonies were followed by the performance art of Félix d' Hérelle. d' Hérelle□s whose bottles of rapidly diminishing turbidity are still awe inspiring today. Even graphical representations of this lysis, so-called lysis profiles, can be a wonder to behold. Note: click on the following images to view larger displays or links to larger displays.



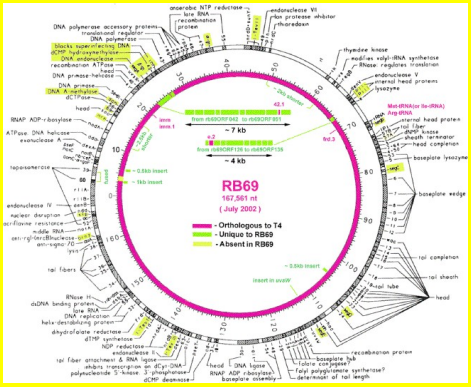


The great artistic contribution of d' Hérelle, however, were his plaques□a quasi-static, reasonably durable, phage macroscopic aesthetic.

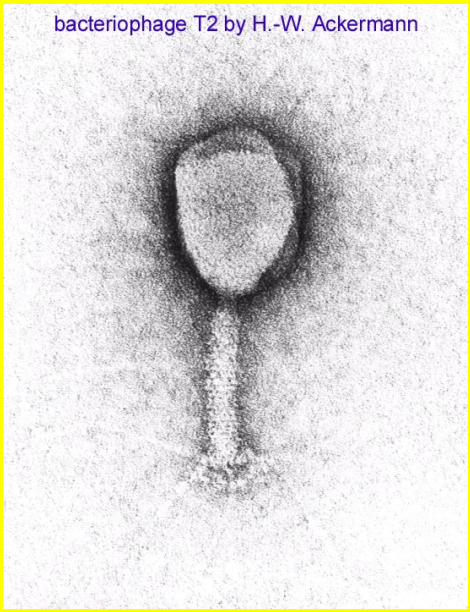


Nobody, of course, ever argued against human intervention into the doings of phage, and plaque-based □art□ is certainly no exception, as the example to the right exemplifies.

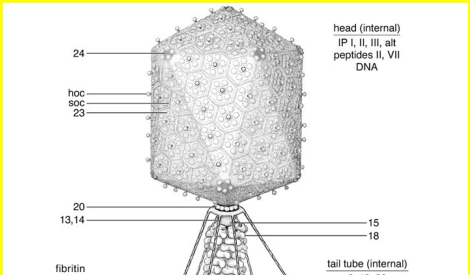
Phage genetics has also made its contribution.



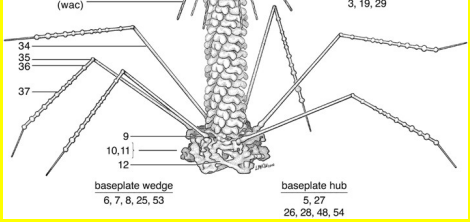
The greatest contributor to phage art, however, has been the electron microscope and its utilization for visualizing phage virions.



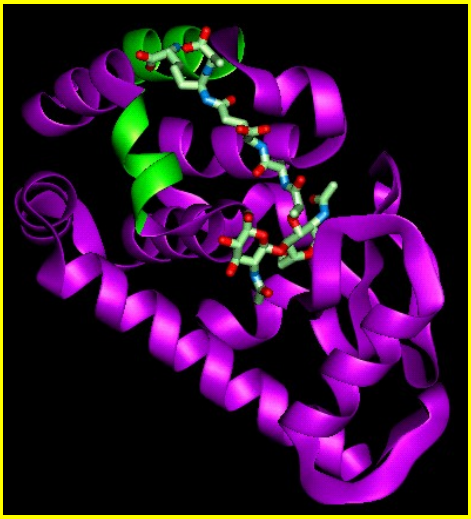
Naturally, people were compelled to make drawings of what they saw (or thought they saw) in electron micrographs, with subsequent drawings informed by much more than just electron micrographs but also the characterization (e.g., by X-ray diffraction) of the three-



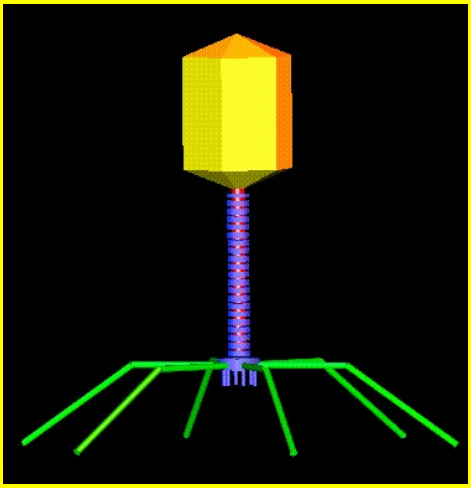
dimensional structure of individual proteins and multi-protein complexes.



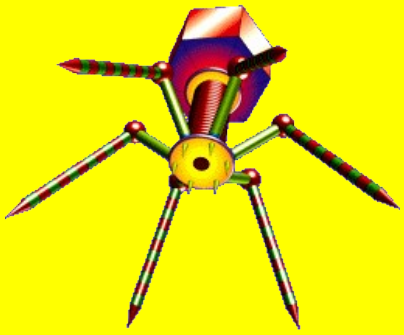
Individual proteins themselves make for an incredible aesthetic.



The next step, naturally, was to make representative cartoons of phage virions.

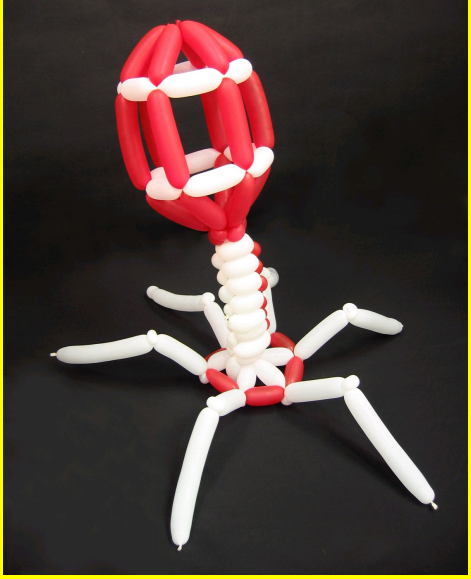


Some of which were highly stylized.



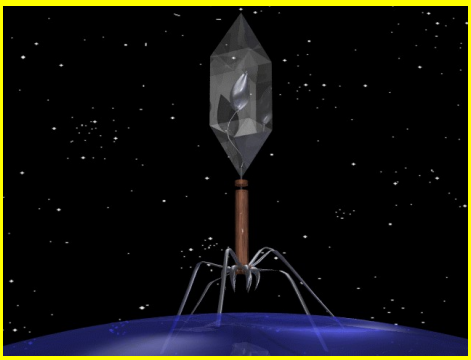
Or which move.

Or are made from balloons.



Or even gingerbread!

Of course, it was once kids with computers discovered how neat phage virions look that phage art really came into its own.



The real trick, however, is to use a computer to generate both accurate and aesthetic representations of a phage particles, especially starting with structural data on individual virion components. With complex virions this takes time, both because the renderings are painstaking to produce and because, being data driven, they can be produced only as fast as structures are elucidated. For a retrospective on the ongoing attempts to computer generate a lifelike phage T4 based on T4 structural data, see this issue□s submission. Or, if that□s not good enough for you, check out this quarter□s phage image, a phage-virion pin!

Editorial Archive

- 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

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.

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New BEG Members

Please welcome our newest members

name (home page links)	status	e-mail	address
Sergio F. Martinez Diaz	PI	sdiaz @ipn.mx	Interdisciplinary Center of Marine Science, Microbiology Lab, Playa el Conchalito sn, La Paz BCS, Mexico CP 23060
	interests:	Relation between phage and pathogenic bacteria. We isolate Vibriophages from samples of the California Gulf. Our interest in phages to *Vibrio* is because the importance of this genus in the marine environment and in marine aquaculture. (contents | BEG members | top of page)	
Lorenzo Drago	---	lorenzo.drago @unimi.it	Microbiologia e Microbiologia Clinica, Dipartimento di Scienze Cliniche "L.Sacco", Universit degli Studi di Milano, Via G.B. Grassi, 74 20157 Milano
	interests:	Phage therapy against multi-drug resistant bacteria. (contents | BEG members | top of page)	
Kwang-Pyo Kim	---	kimk @foodsci.purdue.edu	Purdue University
	interests:	Identification of how immune systems react with phages to improve the efficacy of phage therapy. (contents | BEG members | top of page)	
Eli Magen	PI	elimgen2 @netvision.net.il	Clinical Immunology and Allergy unit, Barzilai Medical center, Medicine B Department, BenGurion University of Negev, Ashkelon, Israel
	interests:	Phage therapy and bacteriophage-host interactions from immunological point of view. (contents | BEG members | top of page)	
Meto Onwuamaegbu	PI	meto.onwuamaegbu @cddah.nhs.uk	The Education Centre, University Hospital of North Durham, North Road, Durham DH1 5TW, United Kingdom
	interests:	Bacteriophage and cellulites; cell-wall deficient bacteria and cardiovascular infections; hypothetical role of *Chlamydia* spp. in acute coronary syndromes and atherosclerosis. (contents | BEG members | top of page)	
Poh-Choo Pang	---	pohchoopang @yahoo.com	Institute of Biological Sciences (Genetics), Faculty of Science, University of Malaya, 50603 KUala Lumpur, Malaysia
	interests:	Isolation and characterization of vibriophages from the environment. (contents | BEG members | top of page)	
Anupama Byrappa Ramalinga	---	anudna @yahoo.com	#30, BHEL (EPD) Township B, 19th Cross, Malleshwaram, Bangalore, Karnataka 560055 INDIA
	interests:	Bacteriophage therapy, especially for vancomycin-resistant enterococci and *Pseudomonas* infections, and biology of phages isolated from natural sources. (contents | BEG members | top of page)	
	---	jumpeiu @hotmail.com	Japan

Jumpei Uchiyama	interests:	Relationship between the human normal microflora and bacteriophage, bacteriophage therapy, and the relation between environmental bacteriophage and humans. (contents | BEG members | top of page)	
Nicola Walker	PI	nicola.walker @agresearch.co.nz	Rumen Microbiology Group, Agresearch Ltd, Grasslands Research Centre, Tennent Drive, Private Bag 11008, Palmerston North, New Zealand
	interests:	Rumen and hindgut microbial ecology; molecular biology and ecology of phage which affect and interact with the microbial ecosystems in the rumen of sheep and cattle, and the hind-gut of the horse. The potential of using phage to manipulate gut fermentation and target specific key bacterial populations; phage host specificity; induction of temperate phage. (contents | BEG members | top of page)	
Ry Young	PI	ryland @tamu.edu	Dept. of Biochemistry and Biophysics,Texas A&M University 2128 TAMU, College Station TX USA 77843-2128
	interests:	Phage biology, especially phage lysis, phage genomics, the adsorption-injection process, and phage-based therapeutics and prophylaxis. (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.

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Meetings

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

ASM Conference on the New Phage Biology

See last quarter's editorial and phage image for links and details. See you all in Florida!

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.

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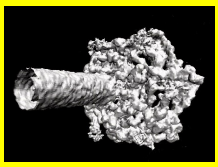
Submissions

Zooming Through the Tail Tube – A Steve McQuinn Perspective on Phage T4

by Steven McQuinn

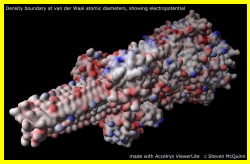
0. Get free Quicktime player (plug in); you will need if (if don't already have it) to be able to view ".mov" files.

1. Turntable view of T4 baseplate. Double click on the image (below) to view the movie.

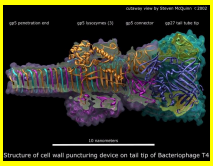


2. Take a trip through the T4 tail tube. Double click on the image (below) to view the movie.

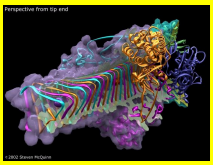
3. Surface scan of the tail-tube tip. Originally presented in BEG News 14.



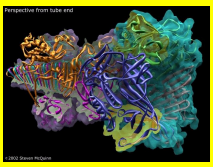
4. Labeled cutaway of tail-tube tip (side view). Originally presented in BEG News 14.



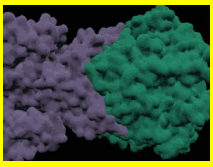
5. Labeled cutaway of tail-tube tip (business-end view). Originally presented in BEG News 14.



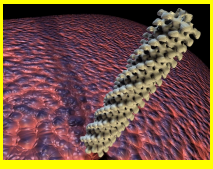
6. Labeled cutaway of tail-tube tip (tube-end view). Originally presented in BEG News 14.



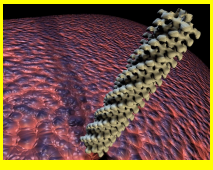
7. Movie of attachment (separation, actually) of tip to tube. Originally presented in BEG News 14.



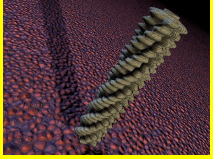
8. Leaning Tower of Phage (revision 1).



9. Leaning Tower of Phage (revision 2).

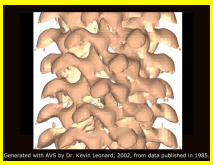


10. Leaning Tower of Phage (original version). Originally presented in BEG News 12.

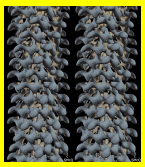


11. Phage T4 contractile sheath (disembodied). Originally presented in BEG News 13.

12. Phage T4 contractile sheath ("raw" data). Originally presented in BEG News 13.

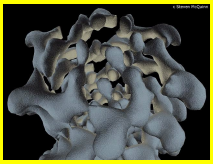


13. Phage T4 contractile sheath (side, stereoscopic view). Originally presented in BEG News 13.

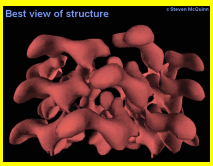


14. Phage T4 contractile sheath (looking up, stereoscopic view). Originally presented in BEG News 13.

15. Phage T4 contractile sheath (contacts between gp18 and gp19). Originally presented in BEG News 13.

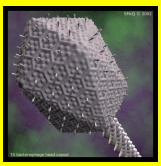


16. Phage T4 contractile sheath (animated comparison of data sets). Originally presented in BEG News 13.

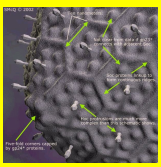


17. Full relief of phage T4 head (on transparent background). Originally presented in BEG News 12.

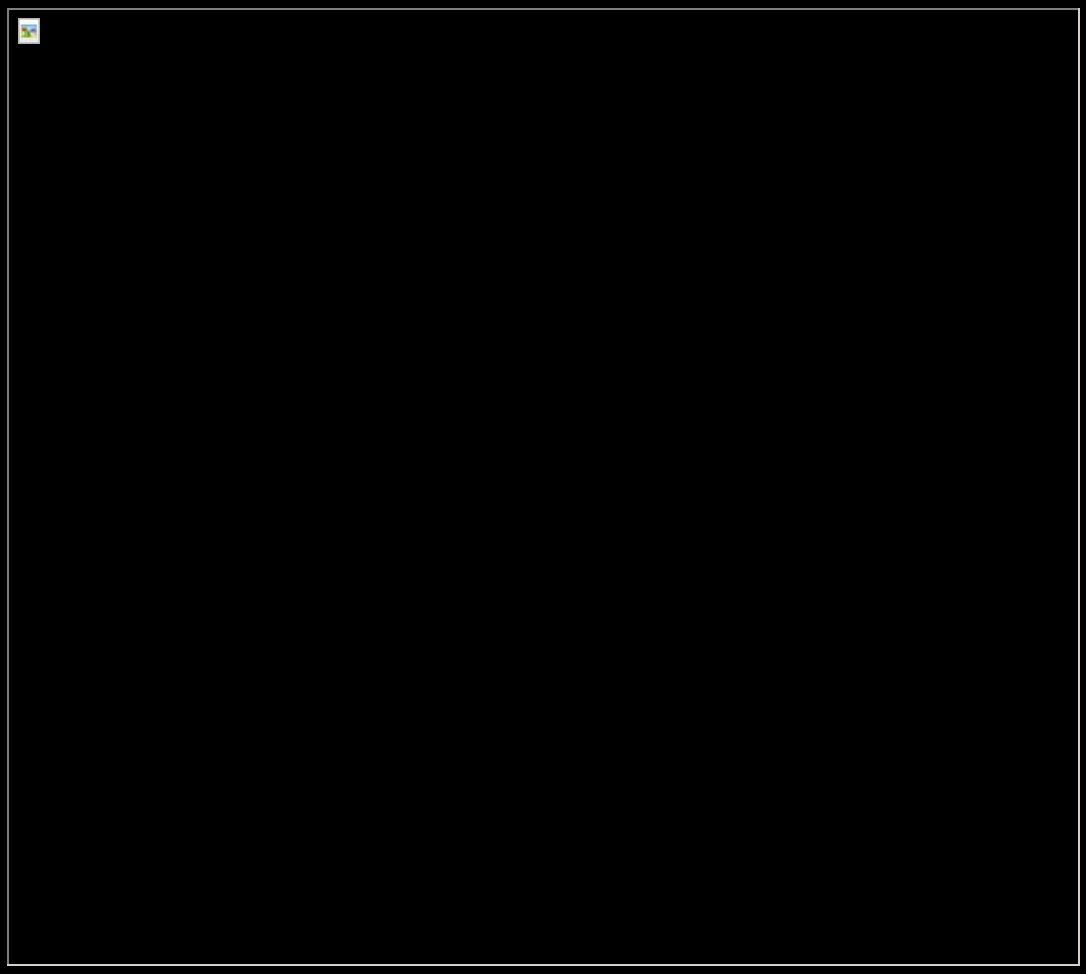
18. Phage T4 head with tail attached. Originally presented in BEG News 12.



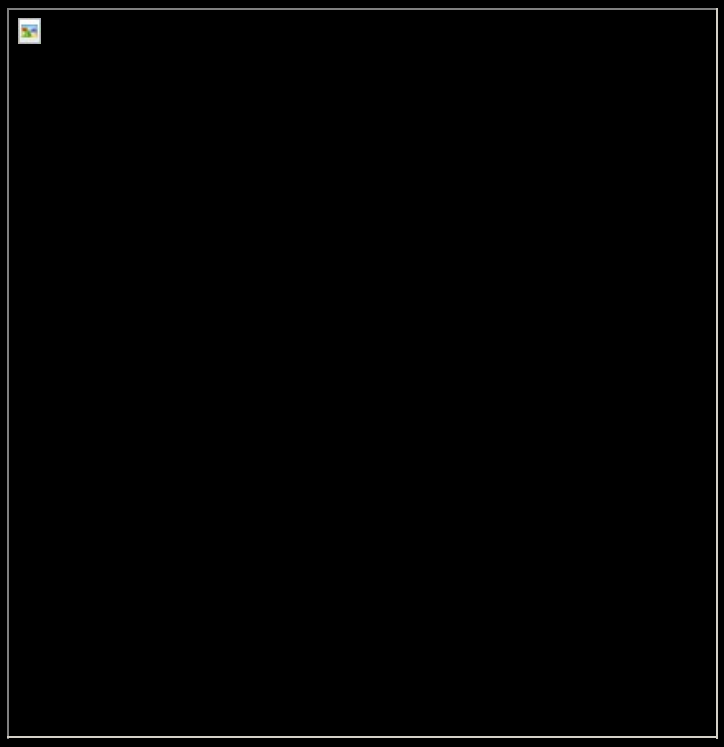
19. Phage T4 head with details labeled. Originally presented in BEG News 12.



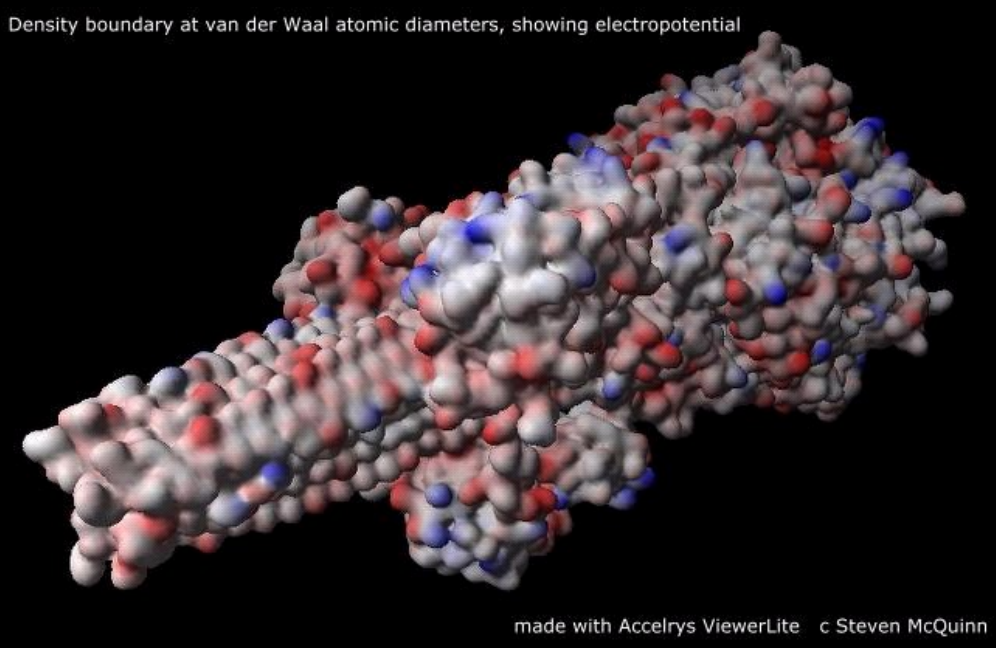
20. Animated view of T4 head as cut-out toy. Originally presented in BEG News 12.



This is a rendering of the phage T4 baseplate as a "turntable" movie. Double click image to start or to pause the movie. Don□t forget that you will need the free Quicktime player (plug in) to view the movie. [return to image summary]

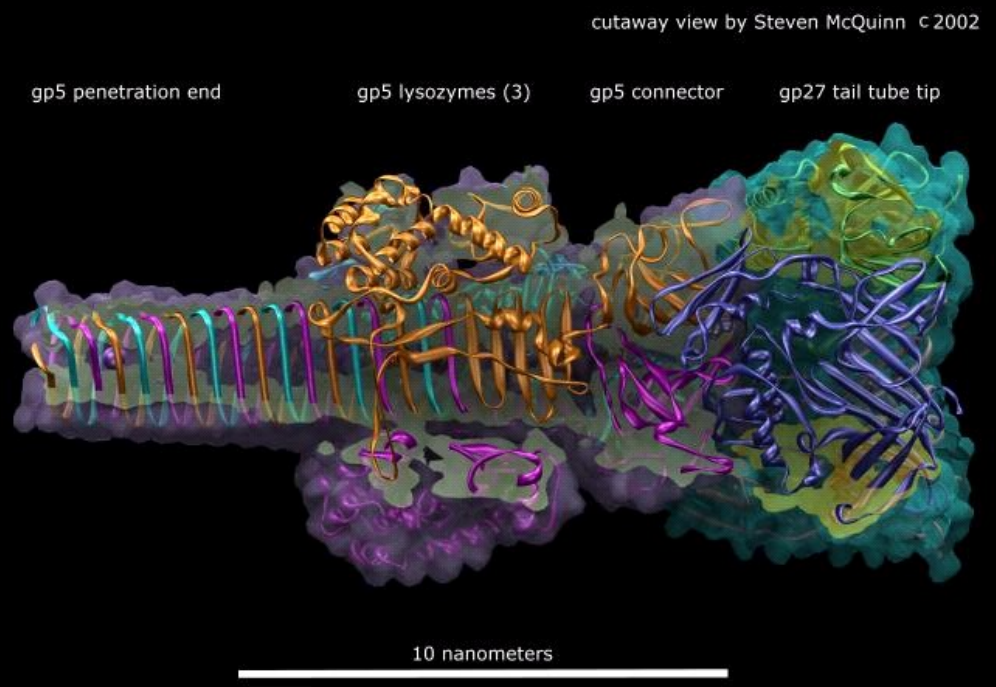


Take a trip down the T4 tail tube, as though you were a piece of DNA. Double click image to start or to pause the movie. Don't forget that you will need the free Quicktime player (plug in) to view the movie. [return to image summary]



The elaborate infection mechanics of Bacteriophage T4 are often illustrated as Nature's nanoscale version of a hypodermic syringe. This misleading analogy has flourished in graphics used by introductory textbooks, biology lectures and the popular media, even though phage researchers have long known that "it ain't necessarily so."

The real story is infinitesimally more complicated and not fully understood. The recent revelation of structural detail in the cell-puncturing tip of T4 may eventually provide answers to the question, "How does T4 thread its long strand of DNA through the host cell wall into the cytoplasm?" [return to image summary]



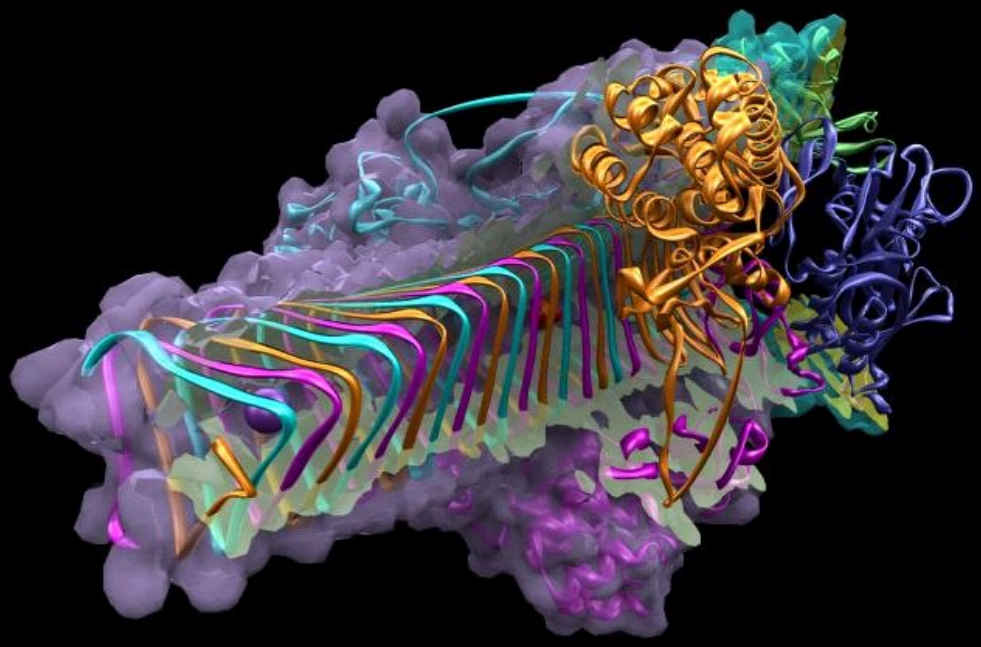
Structure of cell wall puncturing device on tail tip of Bacteriophage T4

The syringe analogy pictures the phage tip plunging completely through the "skin" of the host, forcibly injecting phage DNA through a penetrating pipe that extends well into the cell interior. Evidence contradicts this model, while suggesting an alternative.

The cell envelope of the gram negative bacterium, *Escherichia coli*, is a laminate similar to a Kevlar flack vest, with an inner and outer membrane and a tough layer of fiber between. The T4 tail tip, pressed into the cell wall by tail sheath contraction, seems to be structured for pushing aside the lipid outer membrane, then cutting through the peptidoglycan

fibers using the three lysozyme "scissors" loosely and flexibly deployed around its central barrel. [return to image summary]

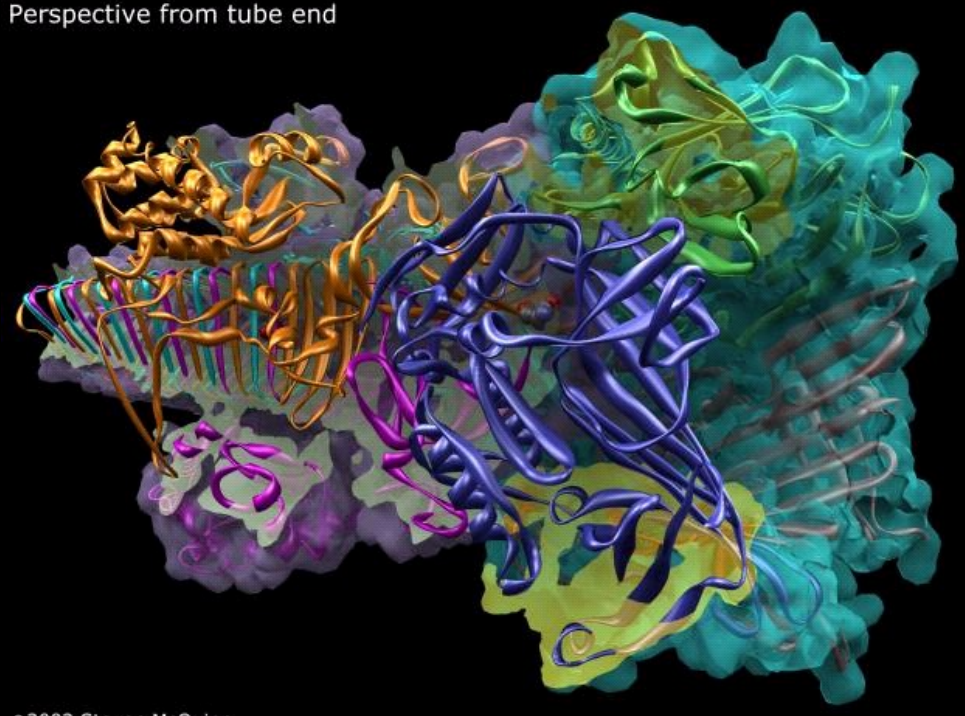
Perspective from tip end



While the literature is not completely clear about the penetration of the inner membrane, the inner and outer membranes apparently fuse across the puncture zone to create a channel through the cell envelope for the hollow tail tube pushing the tail tip. Electron micrographs show that the hollow tail tube usually extends down to, but not into, the cytoplasm.

X-ray crystallography to 2.9 Angstrom resolution reveals that the penetrating point itself provides no hollow passageway for DNA. However, the penetrating tip is connected to the tail tube via a hollow ring, allowing a route for DNA, or a leader for the DNA, to reach the backside of the tail tip via the tail tube. [return to image summary]

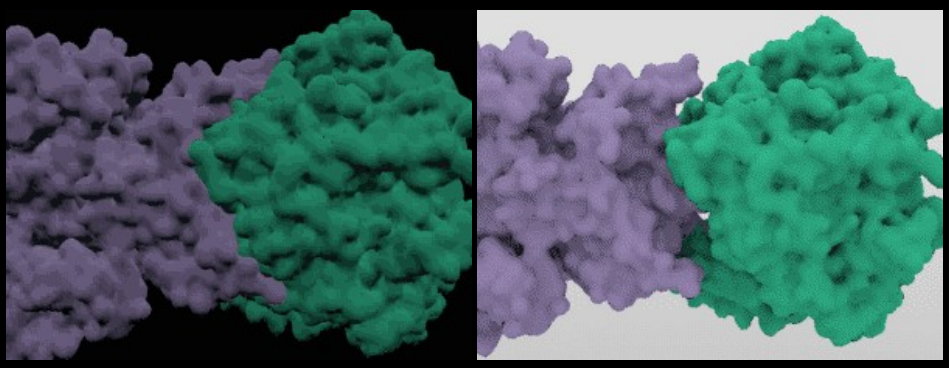
Perspective from tube end



Thus poised at the edge of the cytoplasm, what now draws the tail tip and the DNA into the guts of the cell? Again, the structure of the tail tip may provide clues. Researchers have speculated that a voltage difference across the inner membrane (the Proton Motive Force) may act upon both the tail tip and the DNA strand, moving them into the cytoplasm. The distribution of electro-potential on the surface of the tail tip might support that hypothesis.

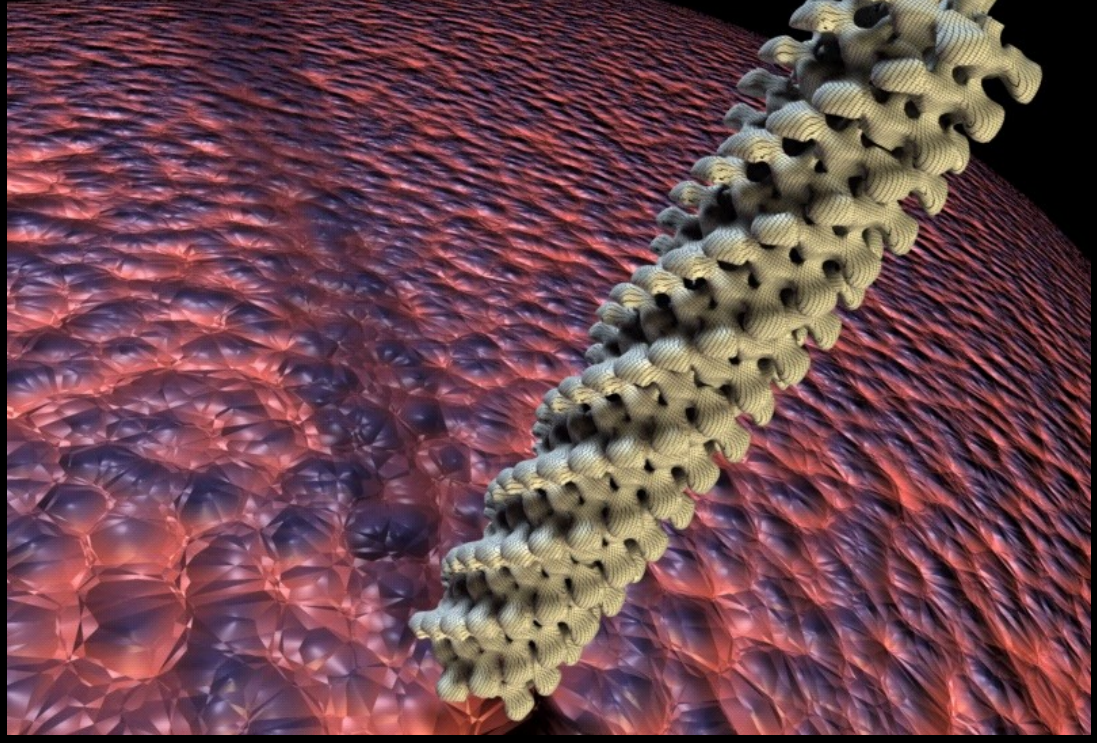
It is clear that the tip, gene product 5 (gp5), is structured to break away from the hollow ring connecting it to the tail tube, gene product 27 (gp27). It is not clear whether the separated tip plays a role in DNA entry or is simply removed to make

way for the DNA. In at least one electron micrograph, a string of expelled phage DNA seems to dangle a little bauble that looks suspiciously like a tail tip. One is tempted to make comparisons with a hooked trout spooling the line off a fishing reel, but as can be seen with hypodermic syringes, reasoning by analogy can be dangerous. [return to image summary]

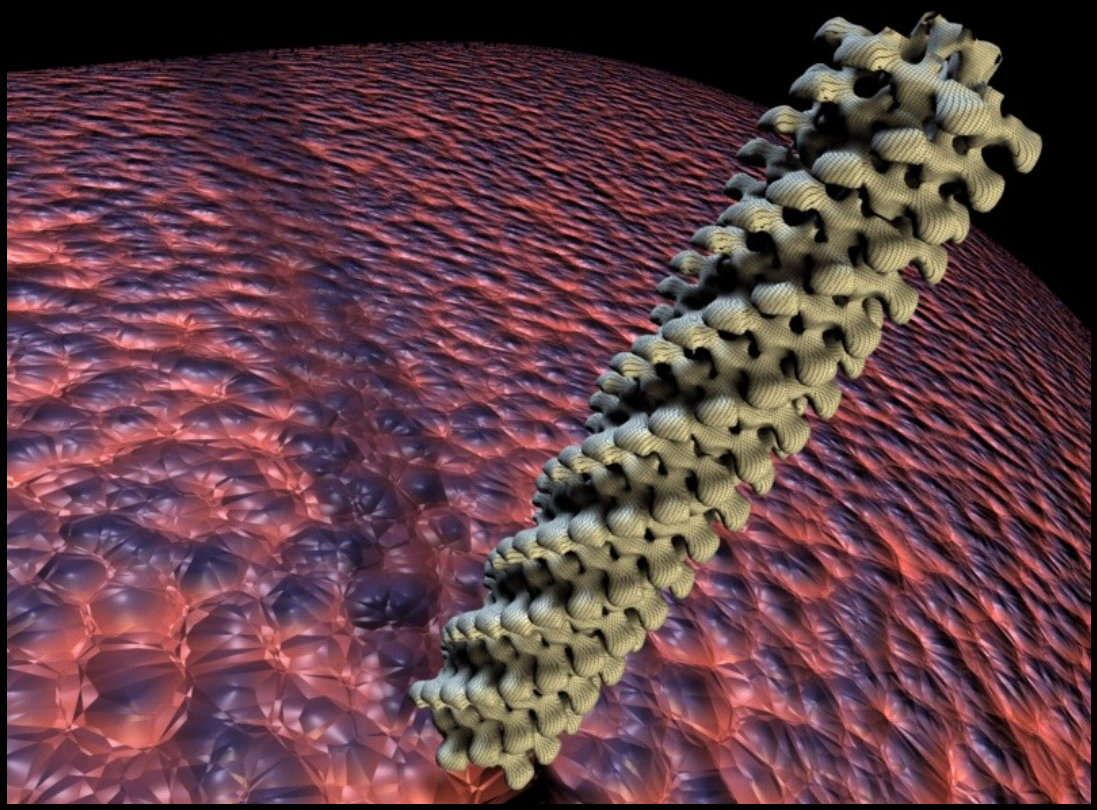


These images were constructed from data obtained from the web-based Atlas of Macromolecules via Protein Explorer, the web-based interface for the molecular visualization engine, Chime. The Atlas version of 1K28 provides a full model of the gp5/gp27 complex, whereas the Protein Data Bank version, 1K28.pdb, provides only one third of the full structure.

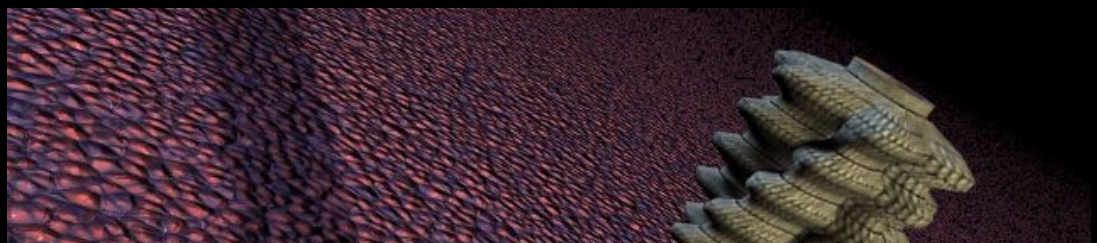
After saving the full structure from Chime as a .pdb file, I opened it in the free molecular visualization program, Accelrys ViewerLite, which I used for the electro-potential surface rendering. Exporting the surface and the ribbon as VRML files, I cut the surface mesh using Amapi 6, then assembled and rendered the cutaway view in Carrara Studio 2. [return to image summary]

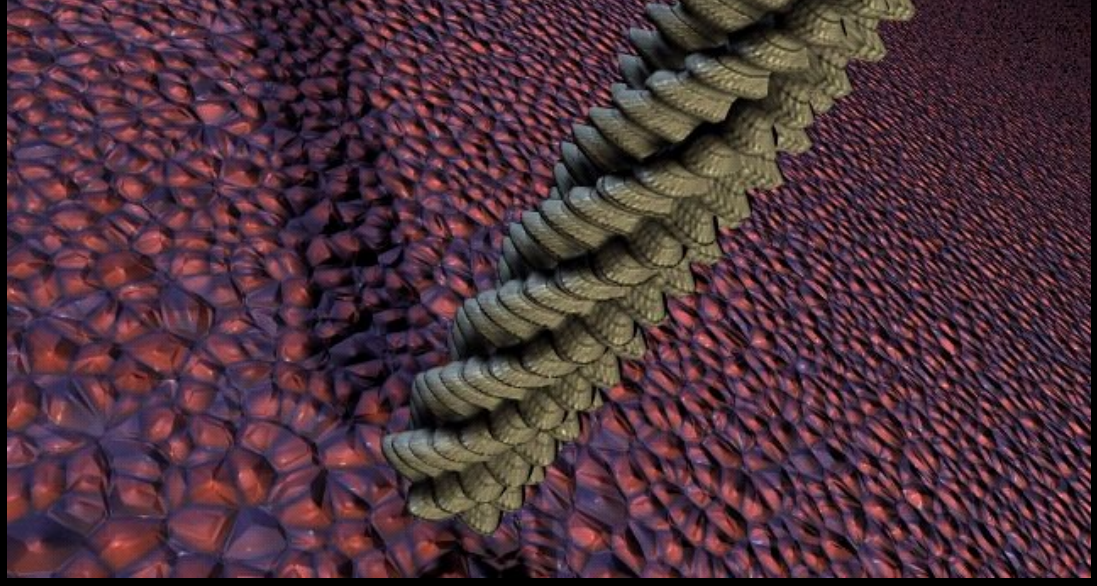


Above is a re-rendering of the "Leaning Tower of Phage" showing **greater detail in deeper grooves**. Image is based on data presented by Lepault, J., and Leonard, K. (1985). Three-dimensional structure of unstained, frozen-hydrated extended tails of bacteriophage T4. Journal of Molecular Biology 182(3):431-441. Click here for bitmapped rendition of figure (1,407 Kbyte). [return to image summary]



Above is a re-rendering of the "Leaning Tower of Phage" showing **greater contrast**. Image is based on data presented by Lepault, J., and Leonard, K. (1985) Three-dimensional structure of unstained, frozen-hydrated extended tails of bacteriophage T4. Journal of Molecular Biology 182(3):431-441. Click here for bitmapped rendition of figure (1,407 Kbyte). [return to image summary]

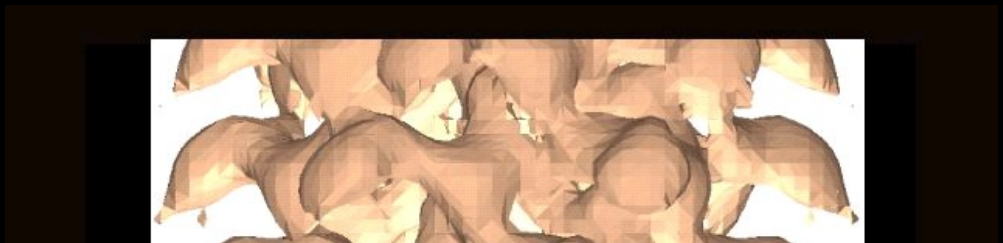


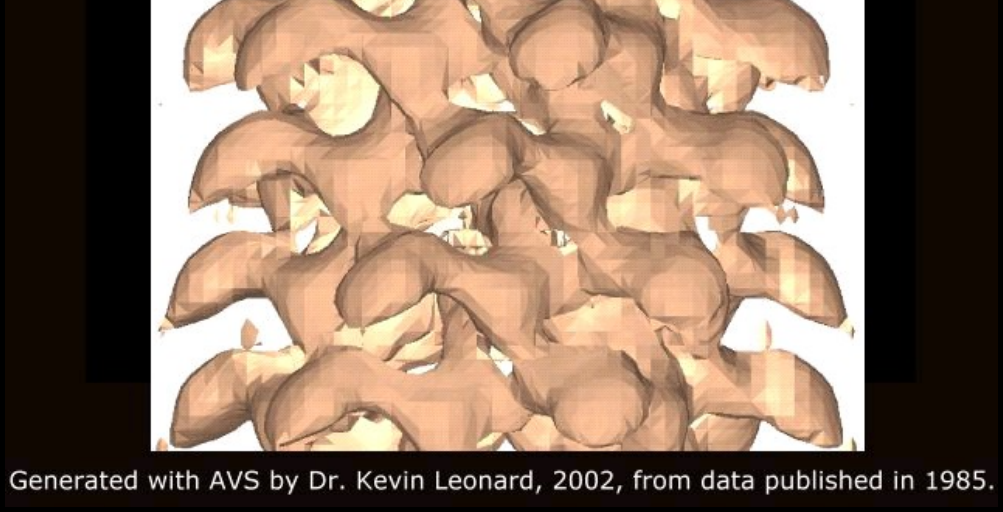


Above is the original ("in-preparation" but awesome) rendering of the "Leaning Tower of Phage." Image is based on data presented by Lepault, J., and Leonard, K. (1985) Three-dimensional structure of unstained, frozen-hydrated extended tails of bacteriophage T4. Journal of Molecular Biology 182(3):431-441. Click here for bitmapped rendition of figure (1,407 Kbyte). [return to image summary]

Above is the phage T4 contractile-tail sheath shown with surface texture and fancy lighting.

What you are seeing. These are synthetic photographs of data. While it is tempting to say, "this is what the extended tail sheath of bacteriophage T4 really looks like," such a statement makes no sense in the nanoscale microcosm where visible light washes over phage the way ocean swells move through plankton. Rather, phage must be probed using the severely short end of the electromagnetic spectrum. Electron microscopists and x-ray crystallographers examining phage details compile data sets of infinitesimal measurements and clever mathematical calculations. Such data sets can be visualized in various ways to illustrate protein morphology, even protein molecular structure. When data is furnished thus to the eye it becomes comprehensible in the most fundamental way, though the caveats of method should never be slighted. We are not looking at a thing, we are looking at the result of a process. [return to image summary]

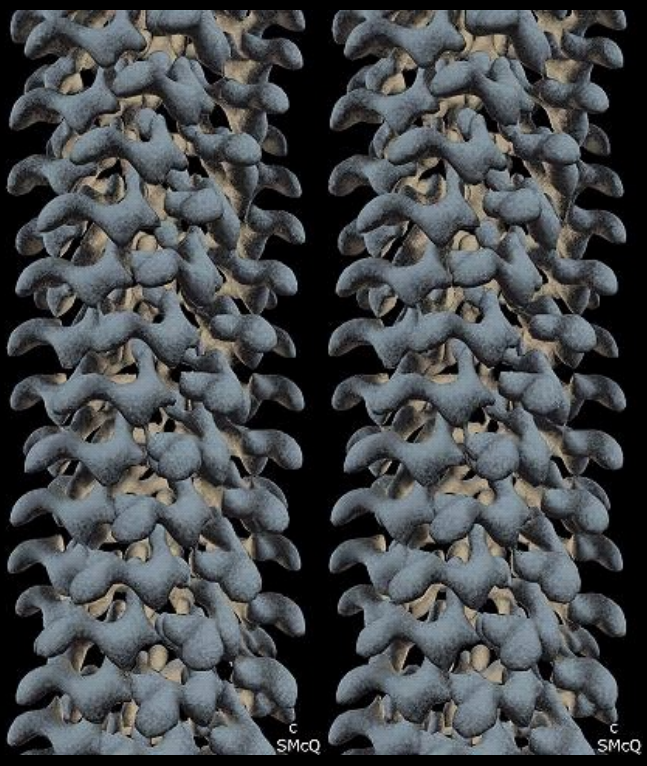




Generated with AVS by Dr. Kevin Leonard, 2002, from data published in 1985.

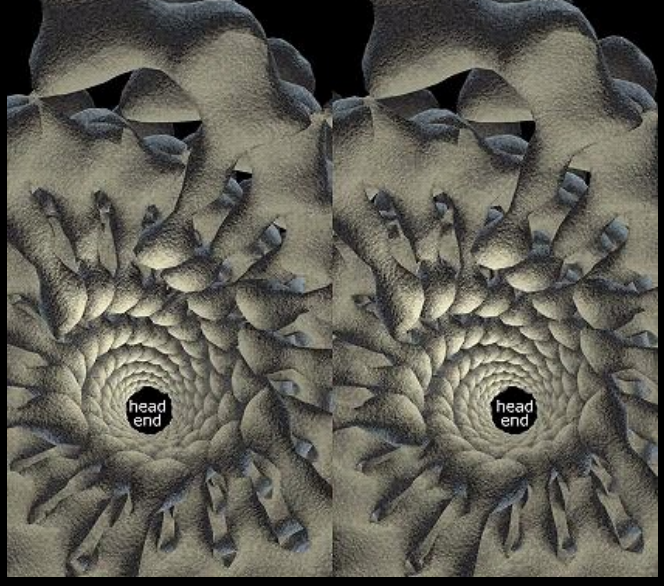
Above is a straight-forward, unadorned image of one density surface data set for the T4 tail sheath, extended configuration. Note the fusion between gp18 proteins.

Where It Came From. Anyone in middle age can appreciate how reassuring it is to see 20-year-old data looking splendid when dressed up in modern fashion. The images here were created from cryo-electron microscopy density surfaces calculated for a paper published in 1985 in the Journal of Molecular Biology. Kevin Leonard kindly dug up the old mag tapes, converted the files for use with contemporary visualization software (AVS), exported them as VRML files, compressed and sent them to me via email and ftp. All this over his weekend and during his busy workweek. [return to image summary]



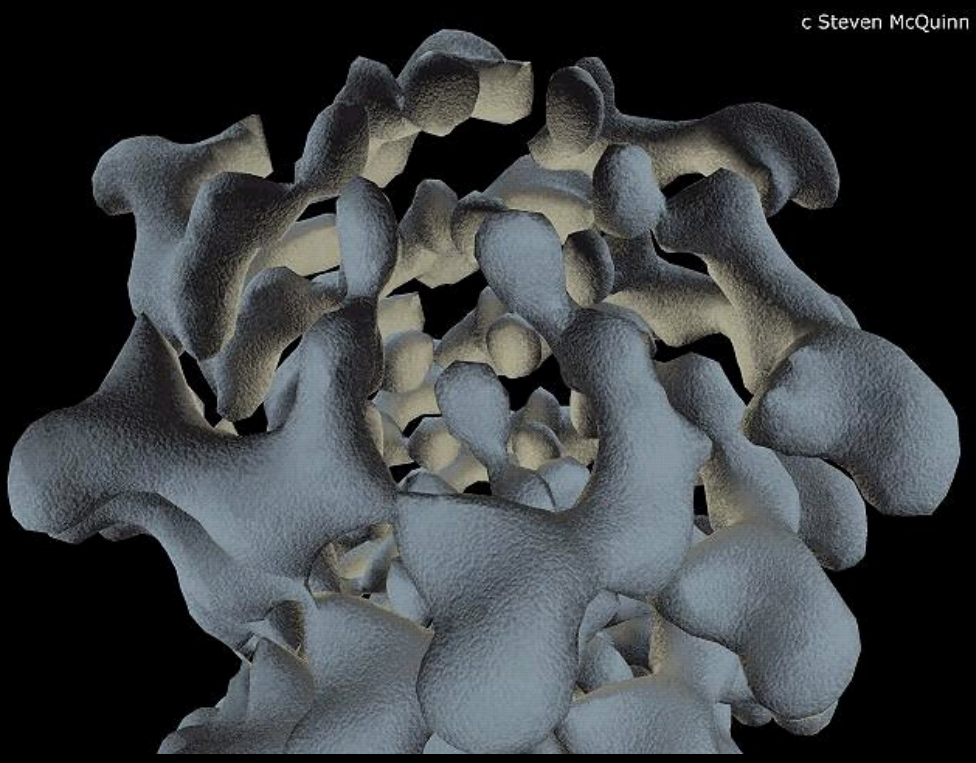
Above is a side view of the sheath, in stereo, with exaggerated depth. See below for how to visualize stereo pairs.

How Much Artistic License. The VRML files, imported into my 3D graphics software as a polygon mesh, defined 4 annular rings in a stack, the top and bottom rings clipped somewhat. I trimmed the geometry down to the fully intact middle two rings, assembling duplicates to make a full helical stack, 24 rings high. The bump map supplying texture to the gp18 proteins serves purely for displaying the surface curvature and has no structural significance. Ideally, the surface of the protein would show the lumpiness of constituent atoms with van der Waal radii and be colored to indicate surface charge, but I cannot find any such data; apparently the molecular structure of gp18 has not yet been worked out. The 3D synthetic lighting sources consist of a warm-colored tube light extending up the middle of the tail sheath and a cool-colored ring light encircling the sheath. [return to image summary]



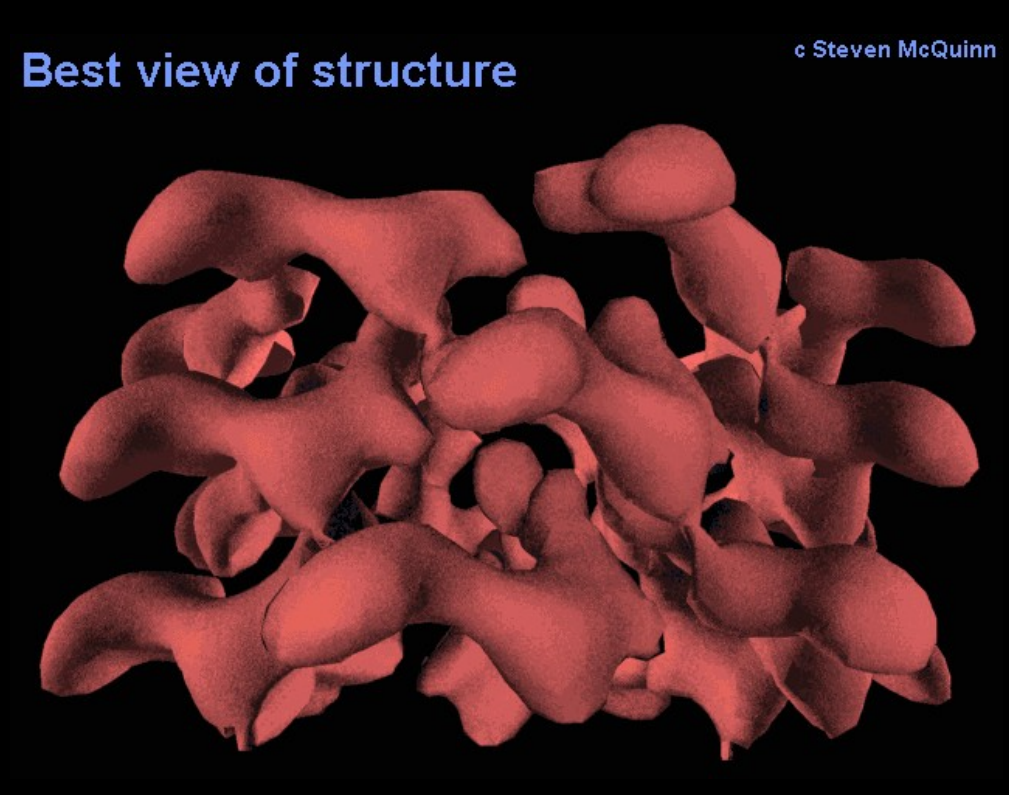
Here (above) you are looking up through the sheath from the baseplate position toward the head. The tail tube fits inside.

How To View The Stereo Pairs. Stare through the stereo pairs as if your thoughts were lost in a distant daydream, gazing off into space; suddenly the right/left images will fuse into one. Stereo fusion requires the eyes to drift apart, exactly the opposite of looking cross-eyed. To help you achieve this fusion, the paired images here are set apart the same distance as the separation of your two eyes--if you view the images on my high resolution monitor. However, it may well be that your monitor displays lower resolution than mine, making the paired images more widely separated and thus harder to fuse. In this case, open the link to the PDF version and use the percentage controls in Acrobat Reader to resize the images for best effect. (It is possible to look distantly while focusing closely if you wear strong reading glasses, which you can borrow from someone nearby who is older than 50.) [return to image summary]



The gp18 proteins in a ring kick up their legs like synchronized swimmers, as seen above. The "feet" (upper, middle of image) connect with the gp19 proteins of the tail tube inside the sheath (gp19 is not shown).

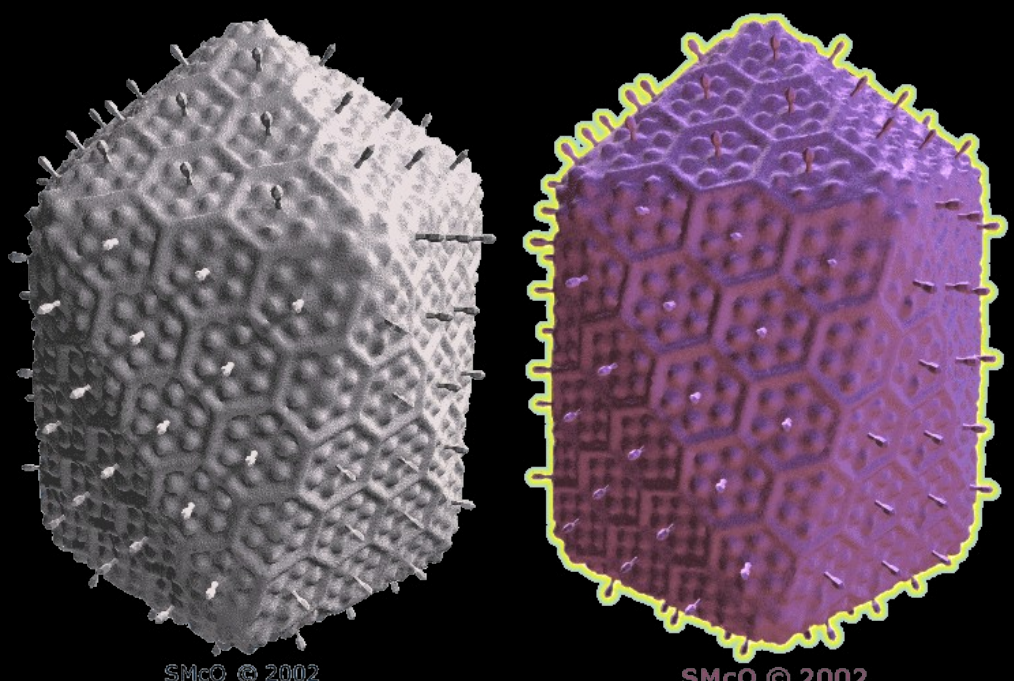
What It All Means. You can see how the gp18 proteins arranged in a helical stack seem to link bulge-to-bulge with their immediate neighbors. Depending on how the data is visualized, these bulges can appear as fusions, and likely represent the bonds between the proteins that hold the extended sheath together. However, the extended sheath would not be stable were it not for the tail tube which extends down through the sheath. The tail tube is not included in these visualizations but you can infer it by the arrangement of the inner ends of gp18 positioned like legs with knees and toes pointed upward. Each end "foot" of gp18 is matched by a corresponding gp19 protein in the tail tube. It is thought that the attraction between the tail tube and the inner structure of the tail sheath holds the sheath in extended position. When bacteriophage T4 infects its *E. coli* host, the baseplate at the bottom of the sheath (not illustrated) springs open, initiating an upward cascade of broken gp18/gp19 connections, allowing the sheath to contract into a different helical arrangement with shorter length and greater radius. It is thought that sheath contraction physically drives the tail tube tip into the host cell wall. [return to image summary]



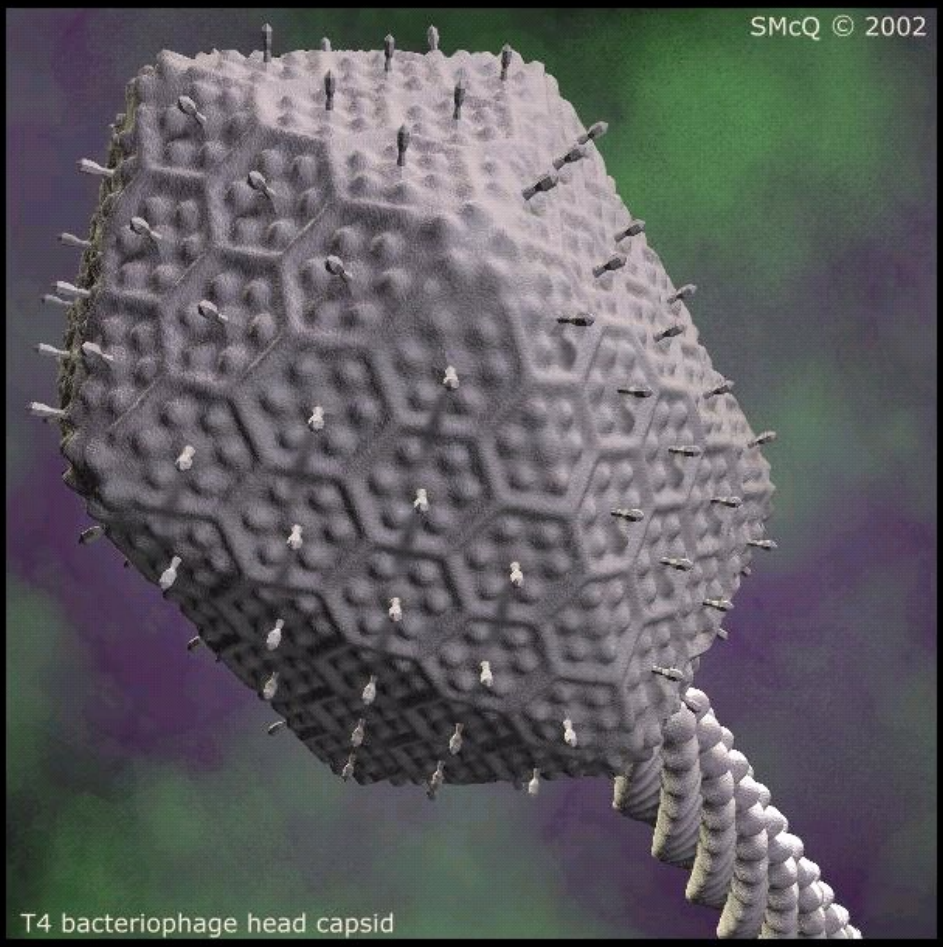
Shown above is an animated comparison of two data sets from Lepault and Leonard. The one without correction (red) shows a slim version of gp18, allowing a view of how distinct units fit together. The version with phase-contrast correction (blue) is thought to be the best representation of shape and size, but the units are hard to distinguish. Shown are three annular rings, essentially, of 6 gp18 subunits each. The red one is a little clipped on top, the blue a little extended on the bottom.

Remember Those Caveats. If only I could simply leap to the conclusion that these crisp, clear images of structure represent Truth, my task in animating tail sheath contraction would be a bit easier. The particular data set I used for the above views shows a slim, distinct gp18; however, the data are uncorrected. Lepault and Leonard determined that a different data set, corrected for the phase-contrast transfer function, represents the best display of gp18 size and shape, though the unit proteins in that view are too fused to be distinct. Superposition of the two data sets shows that they are very similar in general structure, with ambiguity about how the gp18 bond together. My challenge as a 3D animator will be to separate artifact from architecture, basing my interpretations on a synthesis of both data sets. It seems that even with data-derived imagery there is no escaping the need for creativity.

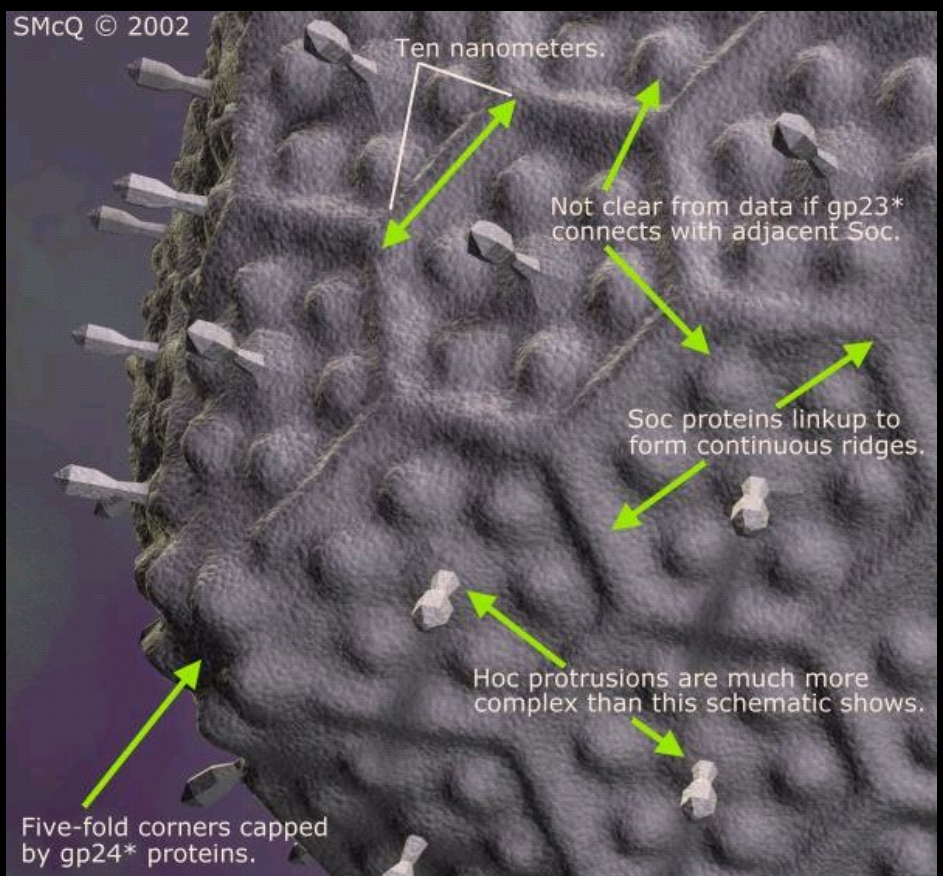
The construction of T4 in 3D is an ongoing project. These first pictures (below), mostly of the phage T4 head, are high-polygon-count meshes modeled to approximate published, data-derived imagery. Researchers who are teasing out the fine details of T4 morphology, from the capsid head to the tail-fiber toes, will eventually publish more accurate meshes generated directly from their analyses. Nevertheless, there is some value in graphical interpretation, especially when showing functionality through animation. I am directing my efforts accordingly, eventually turning toward the creation of simplified low polygon meshes that can be used with interactive web3D formats such as Viewpoint. [return to image summary]



Above are two full-relief gif of T4's head on a transparent background. [return to image summary]



Above is the T4 head, with tail attached. For more on the T4 tail, see this month's phage image. [return to image summary]



Above is a close up of the head structure with various proteins labeled. [return to image summary]

Above is a computer-graphic (movie) rendering of a cut-out model of a T4 head. For a paper-cutout model of the T4 icosahedral head, click here (warning, PDF file is large: 1.6 megabytes). Below is an animated rendering of the paper-cutout model. [return to image summary]

References

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2. Coombs, D. H. & Arisaka, F. in Molecular Biology of Bacteriophage T4, Chapter 21, p. 259-281.
3. Goldberg, E., Grinius, L. & Letellier, L. in Molecular Biology of Bacteriophage T4 (ed. Karam, J. D.) Chapter 34, p. 347-356 (American Society for Microbiology, Washington, DC, 1994).
4. Kanamaru S, Leiman PG, Kostyuchenko VA, Chipman PR, Mesyanzhinov VV, Arisaka F, Rossmann MG. Structure of the cell-puncturing device of bacteriophage T4. Nature. 2002 Jan 31;415(6871):553-7.
5. Kutter, E., Guttmann, B., Carlson, K., in Molecular Biology of Bacteriophage T4, Chapter 33, p. 343-356.
6. Leonard, K., Group Leader (http://mac-leonard4.embl-heidelberg.de/index.html) with the European Molecular Biology Laboratory (http://www.embl-heidelberg.de/)
7. Lepault, J., Group Leader in the Methods and Electron Microscopy division of Le Laboratoire de Virologie Moleculaire & Structurale (formerly Le Laboratoire de Genetique Des Virus, http://www.gv.cnrs-gif.fr/) of CNRS (http://www.cnrs.fr/index.html)
8. Lepault J, Leonard K. Three-dimensional structure of unstained, frozen-hydrated extended tails of bacteriophage T4. J Mol Biol 1985 Apr 5;182(3):431-41.
9. Mosig, G., Eiserling, F. (1988). In *The Bacteriophages* Plenum Press, New York 2:521.
10. Olson, N. *et al.* (2001). The structure of isometric capsids of bacteriophage T4. *Virology* 279:385-391.
11. Qu, C. The Icosahedral Server. mmtsb.scripps.edu/viper/chunxuqu/.

Submissions Archive

- Rendering Phage Heads
- The Contractile-Tail Sheath, In Three Dimensions
- Eye On The Needle: Phage T4 Puncturing Point May Answer Penetrating Questions
- Pioneering genetic researcher Gisela Mosig dies
- Updated Eiserling T4 Virion
- Some Recent Phage and Phage-Related U.S. Patents (1976-present)
- Some Images of BEG Members
- Early Phage References, pre-1950
- Zooming Through the Tail Tube A Steve McQuinn Perspective on Phage T4

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.

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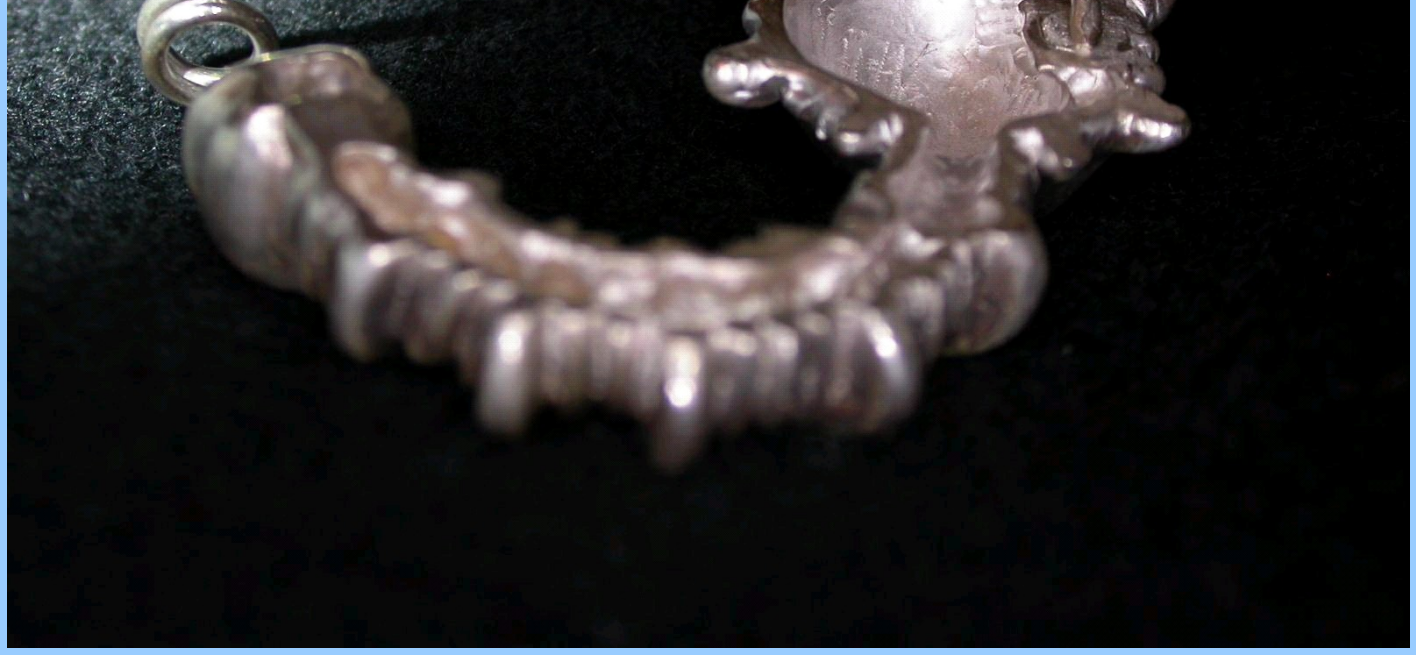
Phage Images

Images are two views of a Siphovirus pin made by Jutta Loeffler, MD (Laboratory of Bacterial Pathogenesis, The Rockefeller University, Box 172, 1230 York Avenue, New York, N.Y. 10021).



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

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!

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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. "My enemy's enemy is my friend." Using phages to fight bacteria. Bradbury, J. (2004). *Lancet* 363:624-625. [PRESS FOR ABSTRACT]

2. Pressure inactivation kinetics of phage λ cI 857. Chen, H., Joerger, R. D., Kingsley, D. H., Hoover, D. G. (2004). *Journal of Food Protection* 67:505-511. [PRESS FOR ABSTRACT]

3. New dawn for phage therapy. Dixon, B. (2004). *The Lancet infectious diseases* 4:186. [PRESS FOR ABSTRACT]

4. Removal of coliphages in secondary effluent by microfiltration-mechanisms of removal and impact of operating parameters. Farahbakhsh, K., Smith, D. W. (2004). *Water Research* 38:585-592. [PRESS FOR ABSTRACT]

5. Big questions, small worlds: microbial model systems in ecology. Jessep, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B., Bohannan, B. J. M. (2004). *Trends in Ecology and Evolution* 19:189-197. [PRESS FOR ABSTRACT]

6. Population and evolutionary dynamics of phage therapy. Levin, B. R., Bull, J. J. (2004). *Nat. Rev. Microbiol.* 2:166-173. [PRESS FOR ABSTRACT]

7. Genomic and genetic analysis of *Bordetella* bacteriophages encoding reverse transcriptase-mediated tropism-switching cassettes. Liu, M., Gingery, M., Doulatov, S. R., Liu, Y., Hodes, A., Baker, S., Davis, P., Simmonds, M., Churcher, C., Mungall, K., Quail, M. A., Preston, A., Harvill, E. T., Maskell, D. J., Eiserling, F. A., Parkhill, J., Miller, J. F. (2004). *Journal of Bacteriology* 186:1503-1517. [PRESS FOR ABSTRACT]

8. Characterizing spontaneous induction of Stx encoding phages using a selectable reporter system. Livny, J., Friedman, D. I. (2004). *Molecular Microbiology* 51:1691-1704. [PRESS FOR ABSTRACT]

9. Bacteriophage contamination: is there a simple method to reduce its deleterious effects in laboratory cultures and biotechnological factories? Los, M., Czyz, A., Sell, E., Wegrzyn, A., Neubauer, P., Wegrzyn, G. (2004). *Journal of applied genetics* 45:111-120. [PRESS FOR ABSTRACT]

10. Evolutionary potential of an RNA virus. Makeyev, E. V., Bamford, D. H. (2004). *Journal of Virology* 78:2114-2120. [PRESS FOR ABSTRACT]

11. Rapid detection of *Escherichia coli* O157:H7 by using green fluorescent protein-labeled PP01 bacteriophage. Oda, M., Morita, M., Unno, H., Tanji, Y. (2004). *Applied and Environmental Microbiology* 70:527-534. [PRESS FOR ABSTRACT]

12. Estimation of septic tank setback distances based on transport of *E. coli* and F-RNA phages. Pang, L., Close, M., Goltz, M., Sinton, L., Davies, H., Hall, C., Stanton, G. (2004). *Environment International* 29:907-921. [PRESS FOR ABSTRACT]

13. The *Pasteurella multocida* toxin is encoded within a lysogenic bacteriophage. Pullinger, G. D., Bevir, T., Lax, A. J. (2004). *Molecular Microbiology* 51:255-269. [PRESS FOR ABSTRACT]

14. The genome and proteome of coliphage T1. Roberts, M. D., Martin, N. L., Kropinski, A. M. (2004). *Virology* 318:245-266. [PRESS FOR ABSTRACT]

15. Immunological factors that affect the *in vivo* fate of T7 phage in the mouse. Srivastava, A. S., Kaido, T., Carrier, E. (2004). *Journal of Virological Methods* 115:99-104. [PRESS FOR ABSTRACT]

16. Are viruses driving microbial diversification and diversity? Weinbauer, M. G., Rassoulzadegan, F. (2004). *Environmental microbiology* 6:1-11. [PRESS FOR ABSTRACT]

17. Ecology of Prokaryotic Viruses. Weinbauer, M. G. (2004). *FEMS Microbiology Reviews* 28:127-181. [PRESS FOR ABSTRACT]

18. Impact of virioplankton on archaeal and bacterial community richness as assessed in seawater batch cultures. Winter, C., Smit, A., Herndl, G. J., Weinbauer, M. G. (2004). *Applied and Environmental Microbiology* 70:804-813. [PRESS FOR ABSTRACT]

19. Genotoxicity of water extracts from the River Yamuna at Mathura, India. Aleem, A., Malik, A. (2003). *Environmental Toxicology* 18:69-77. [PRESS FOR ABSTRACT]

20. AFV1, a novel virus infecting hyperthermophilic archaea of the genus Acidianus. Bettstetter, M., Peng, X., Garrett, R. A., Prangishvili, D. (2003). *Virology* 315:68-79. [PRESS FOR ABSTRACT]

21. Dynamic of isolation of the Phytopathogenic bacteria's phages from a leaf and a root of sugar beet. Andriychuk, O. M., Semchuk, L. I., Romashov, S. A., Ignatenko, T. A., Yatskovska, L. I., Boyko, A. L. (2003). *Bulletin of the University of Kiev, series Biology* . [PRESS FOR ABSTRACT]

22. Detection of the phytopathogenic bacteria' phages in Antarctica. Boyko, A. L., Semchuk, L. I., Voytsitsky, V. M., Andriychuk, O. M., Romashev, S. A., Ignatenko, T. O., Yatskovska, L. I., Vaschenko, V. M., Delimat, A. (2003). *Agroeclogical magazine* 12-15. [PRESS FOR ABSTRACT]

23. Detection of phages of phytopathogenic bacterias *Pseudomonas*, *Xanthomonas*, *Erwinia* and *Bacillus* in agrocenosises. Boyko, A. L., Semchuk, L. I., Romashev, S. A., Andriychuk, O. M., Yatskovska, L. I. (2003). *Agroecological magazine* 24-26. [PRESS FOR ABSTRACT]

24. Evaluation of F+ RNA and DNA coliphages as source-specific indicators of fecal contamination in surface waters. Cole, D., Long, S. C., Sobsey, M. D. (2003). *Applied and Environmental Microbiology* 69:6507-6514. [PRESS FOR ABSTRACT]

25. *Shigella dysenteriae* type 1-specific bacteriophage from environmental waters in Bangladesh. Faruque, S. M.,

Chowdhury, N., Khan, R., Hasan, M. R., Nahar, J., Islam, M. J., Yamasaki, S., Ghosh, A. N., Nair, G. B., Sack, D. A. (2003). *Applied and Environmental Microbiology* 69:7028-7031. [PRESS FOR ABSTRACT]

26. The physical environment affects cyanophage communities in British Columbia inlets. Frederickson, C. M., Short, S. M., Suttle, C. A. (2003). *Microbial Ecology* 46:348-357. [PRESS FOR ABSTRACT]

27. Bacteriophage ecology and plants. Gill, J. J., Abedon, S. T. (2003). *APSnet Feature* http://www.apsnet.org/online/feature/phages/. [PRESS FOR ABSTRACT]

28. Bacteriophage treatment of a severe *Escherichia coli* respiratory infection in broiler chickens. Huff, W. E., Huff, G. R., Rath, N. C., Balog, J. M., Donoghue, A. M. (2003). *Avian Diseases* 47:1399-1405. [PRESS FOR ABSTRACT]

29. Lateral gene transfer: when will adolescence end? Lawrence, J. G., Hendrix, R. W. (2003). *Molecular Microbiology* 50:739-749. [PRESS FOR ABSTRACT]

30. Diagnostic and therapeutic applications of lytic phages. Mandeville, R., Griffiths, M., Goodridge, L., McIntyre, L., Ilenchuk, T. T. (2003). *Analytical Letters* 36:3241-3259. [PRESS FOR ABSTRACT]

31. Use of fluorescently labeled phage in the detection and identification of bacterial species. Mosier-Boss, P. A., Lieberman, S. H., Andrews, J. M., Rohwer, F. L., Wegley, L. E., Breitbart, M. (2003). *Applied spectroscopy* 57:1138-1144. [PRESS FOR ABSTRACT]

32. Detection of the phytopathogenic bacteria phages in the gills of the Black Sea fishes. Semchuk, L. I., Stepanova, O. A., Boyko, A. L., Romashev, S. A., Andriychuk, O. M., Ignatenko, T. O., Yatskovska, L. I. (2003). *Bulletin of the University of Kiev. , series Biology* . [PRESS FOR ABSTRACT]

33. Viral abundance and a high proportion of lysogens suggest that viruses are important members of the microbial community in the Gulf of Trieste. Stopar, D., Cerne, A., Zigman, M., Poljsak-Prijatelj, M., Turk, V. (2003). *Microbial Ecology* 46:249-256. [PRESS FOR ABSTRACT]

34. Transduction of porcine enteropathogenic *Escherichia coli* with a derivative of a shiga toxin 2-encoding bacteriophage in a porcine ligated ileal loop system. Tóth, I., Schmidt, H., Dow, M., Malik, A., Oswald, E., Nagy, B. (2003). *Applied and Environmental Microbiology* 69:7242-7247. [PRESS FOR ABSTRACT]

35. Searching for the advantages of virus sex. Turner, P. E. (2003). *Origins of Life and Evolution of the Biosphere* 33:95-108. [PRESS FOR ABSTRACT]

36. Rapid selection of phage-resistant mutants in Streptococcus thermophilus by immunoselection and cell sorting. Viscardi, M., Capparelli, R., Iannelli, D. (2003). *International Journal of Food Microbiology* 89:223-231. [PRESS FOR ABSTRACT]

37. Bacteriophages as an efficient therapy for antibiotic-resistant septicemia in man. Weber-Dabrowska, B., Mulczyk, M., Gorski, A. (2003). *Transplantation proceedings* 35:1385-1386. [PRESS FOR ABSTRACT]

38. Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic waters. Weinbauer, M., Brettar, I., Höfle, M. (2003). *Limnology and Oceanography* 48:1457-1465. [PRESS FOR ABSTRACT]

39. Comparing the effects of resource enrichment and grazing on viral production in a meso-eutrophic reservoir. Weinbauer, M. G., Christaki, U., Nedoma, J., Simek, K. (2003). *Aquatic Microbial Ecology* 31:137-144. [PRESS FOR ABSTRACT]

40. Sampling natural viral communities from soil for culture-independent analyses. Williamson, K. E., Wommack, K. E., Radosevich, M. (2003). *Applied and Environmental Microbiology* 69:6628-6633. [PRESS FOR ABSTRACT]

41. Imbroglios of viral taxonomy: genetic exchange and failings of phenetic approaches. Lawrence, J. G., Hatfull, G. F., Hendrix, R. W. (2002). *Journal of Bacteriology* 184:4891-4905. [PRESS FOR ABSTRACT]

42. The adaptation and survival of phages in nature. Semchuk, L. I., Ignatenko, T., Romashev, S. A., Andriychuk, O., Yatskovska, L. (2002). *Bulletin of the University of Kiev, series Biology* 38:54-56. [PRESS FOR ABSTRACT]

43. Detection of populations of phages of phytopathogenic bacterias and their biological properties. Boyko, A. L., Semchuk, L. I., Romashev, S. A., Andriychuk, L. N. (2001). *Bulletin of the Agroscience* 51-53. [PRESS FOR ABSTRACT]

44. Ecological and biological aspects of research phages phytopathogenic bacteria. Semchuk, L. I., Andriychuk, E. M., Romashev, S. A., JatskÏvska, L. I. (2001). *Bulletin of the University of Kiev, series Biology* N35:11-13. [PRESS FOR ABSTRACT]

45. Effect of resource supply rate on host-pathogen dynamics. Bohannan, B. J. M. (2000). in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers*. Atlantic Canada Society for Microbial Ecology, Halifax, Canada. [PRESS FOR ABSTRACT]

46. Genetic analysis of a bacterial genetic exchange element: the gene transfer agent of *Rhodobacter capsulatus*. Lang, A. S., Beatty, J. T. (2000). *Proceedings of the National Academy of Sciences, USA* 97:859-864. [PRESS FOR ABSTRACT]

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. **"My enemy's enemy is my friend." Using phages to fight bacteria. Bradbury, J. (2004). *Lancet* 363:624-625.** [first paragraph] Bacteriophages, viruses that prey upon bacteria, typically attack only a single bacterial strain. This specificity, together with the killing capacity of "phages", says phage researcher Martin Loessner, makes them the "natural enemies" of bacteria. "We are now endeavouring to make this enemy our friend", says Loessner, a professor of food microbiology at the Swiss Federal Institute of Technology in Zurich, turning phages into potentially important allies in our battle against bacteria.

2. **Pressure inactivation kinetics of phage λ cI 857. Chen, H., Joerger, R. D., Kingsley, D. H., Hoover, D. G. (2004). *Journal of Food Protection* 67:505-511.** Inactivation curves of phage lambda cI 857 inactivated by high hydrostatic pressure were obtained at three pressure levels (300, 350, and 400 MPa) in buffered media and ultrahigh-temperature 2% reduced fat milk. Pressurization of phage lambda in buffered media at 300 MPa for 300 min, 350 MPa for 36 min, and 400 MPa for 8 min reduced the titer of phage lambda by 7.5, 6.7, and 7.7 log, respectively. Pressurization of phage lambda in milk at 300 MPa for 400 min, 350 MPa for 80 min, and 400 MPa for 20 min reduced the titer of phage lambda by 5.4, 6.4, and 7.1 log, respectively. Tailing was observed in all inactivation curves, indicating that the linear model was not adequate for describing these curves. Among the three nonlinear models studied, the Weibull and log-logistic models consistently produced best fits to all inactivation curves, and the modified Gompertz model the poorest. Because there were no significant differences in the values of shape factor (n) for suspension medium buffer, we reduced the number of parameters in the Weibull model from two to one by setting n at the mean value. The simplified Weibull model produced a fit comparable to the full model. Additionally, the simplified Weibull model allowed predictions to be made at pressures different from the experimental pressures. Menstruum was found to significantly affect the pressure resistance of phage lambda. Comparison of pressure inactivation of hepatitis A virus and phage lambda indicated that phage lambda is more sensitive to pressure than hepatitis A virus in Dulbecco's modified Eagle medium with 10% fetal bovine sera

3. **New dawn for phage therapy. Dixon, B. (2004). *The Lancet infectious diseases* 4:186.** [first two paragraphs] Perhaps Antony Twort was 10 years too early in publishing his father Frederick's biography. A marvellous portrait of the eccentric co-discoverer of the bacteriophage, whose work helped to usher in the era of molecular biology, the book appeared only after numerous rejections from publishers (*Lancet Infect Dis* 2003; **3:** 58). It also received little review attention, because literary editors are largely unaware of the role of science and scientists in shaping the modern world. ¶ However, the decade since publication of *In Focus, Out of Step* (Stroud, UK: Alan Sutton) has seen increasing interest in phages, especially in administering them therapeutically. Most recently there have been promising advances towards real applications. Now, thanks to work in Vienna, Austria, the major obstacle to phage therapy seems well on the way to being removed. At a time when antibiotic resistance is provoking real concern even in the most sober quarters, this is excellent news.

4. **Removal of coliphages in secondary effluent by microfiltration-mechanisms of removal and impact of operating parameters. Farahbakhsh, K., Smith, D. W. (2004). *Water Research* 38:585-592.** The efficacy of a microfiltration (MF) pilot plant in removing somatic coliphages (referred hereafter as coliphages) present in the secondary effluent was evaluated during this study. The impact of operating parameters such as feed coliphage concentrations, permeate flux and membrane fouling on the removal of coliphages by the MF plant was investigated. The study showed that membrane fouling was beneficial for removing coliphages by MF. It was also shown that the removal of coliphages by MF was initially governed by adsorption on membrane surface or in membrane pores. As the membrane fouled, however, the removal of coliphages was primarily governed by direct interception on the cake layer formed on the surface of the membrane. Increases in feed coliphage concentrations resulted in the passage of larger numbers of coliphages when the MF was clean but had little impact on the passage of coliphages when the membrane became fouled. Increasing permeate flux lowered log-removal values (LRVs) for the clean membrane but resulted in an initial increase in LRVs for the fouled membrane followed by a drop in LRVs with further increases in permeate flux

5. **Big questions, small worlds: microbial model systems in ecology. Jessep, C. M., Kassen, R., Forde, S. E., Kerr, B., Buckling, A., Rainey, P. B., Bohannan, B. J. M. (2004). *Trends in Ecology and Evolution* 19:189-197.** Although many biologists have embraced microbial model systems as tools to address genetic and physiological questions, the explicit use of microbial communities as model systems in ecology has traditionally been more restricted. Here, we highlight recent studies that use laboratory-based microbial model systems to address ecological questions. Such studies have significantly advanced our understanding of processes that have proven difficult to study in field systems, including the genetic and biochemical underpinnings of traits involved in ecological interactions, and the ecological differences driving evolutionary change. The use of microbial model systems is not without criticism, however. Many ecologists have voiced concern that microbial microcosm experiments are too simplified, contrived, and small in spatial and temporal scale to adequately address ecological questions. We argue that these concerns reflect a misunderstanding of the purpose of microcosm studies. It is the simplicity of microbial model systems that makes them such powerful tools for the study of ecology; such simplicity allows the high degrees of experimental control and replication necessary to address many questions that are inaccessible through field observation or experimentation. Furthermore, the tractability of the microbial model systems also allows ecologists to bridge ecological and evolutionary questions, and to analyze experiments post hoc to better understand the mechanisms underlying particular results.

6. **Population and evolutionary dynamics of phage therapy. Levin, B. R., Bull, J. J. (2004). *Nat. Rev. Microbiol.***

2:166-173. Following a sixty-year hiatus in western medicine, bacteriophages (phages) are again being advocated for treating and preventing bacterial infections. Are attempts to use phages for clinical and environmental applications more likely to succeed now than in the past? Will phage therapy and prophylaxis suffer the same fates as antibiotics--treatment failure due to acquired resistance and ever-increasing frequencies of resistant pathogens? Here, the population and evolutionary dynamics of bacterial-phage interactions that are relevant to phage therapy and prophylaxis are reviewed and illustrated with computer simulations

7. **Genomic and genetic analysis of *Bordetella* bacteriophages encoding reverse transcriptase-mediated tropism-switching cassettes. Liu, M., Gingery, M., Doulatov, S. R., Liu, Y., Hodes, A., Baker, S., Davis, P., Simmonds, M., Churcher, C., Mungall, K., Quail, M. A., Preston, A., Harvill, E. T., Maskell, D. J., Eiserling, F. A., Parkhill, J., Miller, J. F. (2004). *Journal of Bacteriology* 186:1503-1517.** Liu *et al.* recently described a group of related temperate bacteriophages that infect *Bordetella* subspecies and undergo a unique template-dependent, reverse transcriptase-mediated tropism switching phenomenon (Liu *et al.*, Science 295: 2091-2094, 2002). Tropism switching results from the introduction of single nucleotide substitutions at defined locations in the VR1 (variable region 1) segment of the mtd (major tropism determinant) gene, which determines specificity for receptors on host bacteria. In this report, we describe the complete nucleotide sequences of the 42.5- to 42.7-kb double-stranded DNA genomes of three related phage isolates and characterize two additional regions of variability. Forty-nine coding sequences were identified. Of these coding sequences, bbp36 contained VR2 (variable region 2), which is highly dynamic and consists of a variable number of identical 19-bp repeats separated by one of three 5-bp spacers, and bpm encodes a DNA adenine methylase with unusual site specificity and a homopolymer tract that functions as a hotspot for frameshift mutations. Morphological and sequence analysis suggests that these *Bordetella* phage are genetic hybrids of P22 and T7 family genomes, lending further support to the idea that regions encoding protein domains, single genes, or blocks of genes are readily exchanged between bacterial and phage genomes. *Bordetella* bacteriophages are capable of transducing genetic markers in vitro, and by using animal models, we demonstrated that lysogenic conversion can take place in the mouse respiratory tract during infection

8. **Characterizing spontaneous induction of Stx encoding phages using a selectable reporter system. Livny, J., Friedman, D. I. (2004). *Molecular Microbiology* 51:1691-1704.** Shiga toxin (Stx) genes in Stx producing *Escherichia coli* (STEC) are encoded in prophages of the lambda family, such as H-19B. The subpopulation of STEC lysogens with induced prophages has been postulated to contribute significantly to Stx production and release. To study induced STEC, we developed a selectable *in vivo e*xpression technology, SIVET, a reporter system adapted from the RIVET system. The SIVET lysogen has a defective H-19B prophage encoding the TnpR resolvase gene downstream of the phage P_R promoter and a *cat* gene with an inserted *tet* gene flanked by targets for the TnpR resolvase. Expression of resolvase results in excision of *tet* , restoring a functional *cat* gene; induced lysogens survive and are chloramphenicol resistant. Using SIVET we show that: (i) approximately 0.005% of the H-19B lysogens are spontaneously induced per generation during growth in LB. (ii) Variations in cellular physiology (e.g. RecA protein) rather than in levels of expressed repressor explain why members of a lysogen population are spontaneously induced. (iii) A greater fraction of lysogens with *stx* encoding prophages are induced compared to lysogens with non-Stx encoding prophages, suggesting increased sensitivity to inducing signal(s) has been selected in Stx encoding prophages. (iv) Only a small fraction of the lysogens in a culture spontaneously induce and when the lysogen carries two lambdoid prophages with different repressor/ operators, 933W and H-19B, usually both prophages in the same cell are induced.

9. **Bacteriophage contamination: is there a simple method to reduce its deleterious effects in laboratory cultures and biotechnological factories? Los, M., Czyz, A., Sell, E., Wegrzyn, A., Neubauer, P., Wegrzyn, G. (2004). *Journal of applied genetics* 45:111-120.** Infection of bacterial cultures by bacteriophages as well as prophage induction in the host cells are serious problems in both research and biotechnological laboratories. Generally, prevention strategies (like good laboratory/factory hygiene, sterilisation, decontamination and disinfection) are necessary to avoid bacteriophage contamination. However, it is well known that no matter how good the laboratory/factory practice and hygiene are, bacteriophage infections occur from time to time. The use of immunised or resistant bacterial strains against specific phages may be helpful, but properties of the genetically modified strains resistant to phages are often worse (from the point of view of a researcher or a biotechnological company) than those of the parental, phage-sensitive strains. In this article we review recent results that may provide a simple way to minimise deleterious effects of bacteriophage infection and prophage induction. It appears that low bacterial growth rates result in a significant inhibition of lytic development of various bacteriophages. Moreover, spontaneous prophage induction is less frequent in slowly growing bacteria

10. **Evolutionary potential of an RNA virus. Makeyev, E. V., Bamford, D. H. (2004). *Journal of Virology* 78:2114-2120.** RNA viruses are remarkably adaptable to changing environments. This is medically important because it enables pathogenic viruses to escape the immune response and chemotherapy and is of considerable theoretical interest since it allows the investigation of evolutionary processes within convenient time scales. A number of earlier studies have addressed the dynamics of adapting RNA virus populations. However, it has been difficult to monitor the trajectory of molecular changes in RNA genomes in response to selective pressures. To address the problem, we developed a novel *in vitro* evolution system based on a recombinant double-stranded RNA bacteriophage, phi 6, containing a beta-lactamase (bla) gene marker. Carrier-state bacterial cells are resistant to ampicillin, and after several passages, they become resistant to high concentrations of another beta-lactam antibiotic, cefotaxime, due to mutations in the virus-borne bla gene. We monitored the changes in bla cDNAs induced by cefotaxime selection and observed an initial explosion in sequence variants with multiple mutations throughout the gene. After four passages, a stable, homogeneous population of bla sequences containing three specific nonsynonymous mutations was established. Of these, two mutations (E104K and G238S) have been previously reported for beta-lactamases from cefotaxime-resistant bacterial isolates. These results extend our understanding of the molecular mechanisms of viral adaptation and also demonstrate the possibility of using an RNA virus as a vehicle for directed evolution of heterologous proteins.

11. **Rapid detection of *Escherichia coli* O157:H7 by using green fluorescent protein-labeled PP01 bacteriophage. Oda, M., Morita, M., Unno, H., Tanji, Y. (2004). *Applied and Environmental Microbiology* 70:527-534.** A previously isolated T-even-type PP01 bacteriophage was used to detect its host cell, *Escherichia coli* O157:H7. The phage small outer capsid (SOC) protein was used as a platform to present a marker protein,

green fluorescent protein (GFP), on the phage capsid. The DNA fragment around soc was amplified by PCR and sequenced. The gene alignment of soc and its upstream region was g56-soc.2-soc.1-soc, which is the same as that for T2 phage. GFP was introduced into the C- and N-terminal regions of SOC to produce recombinant phages PP01-GFP/SOC and PP01-SOC/GFP, respectively. Fusion of GFP to SOC did not change the host range of PP01. On the contrary, the binding affinity of the recombinant phages to the host cell increased. However, the stability of the recombinant phages in alkaline solution decreased. Adsorption of the GFP-labeled PP01 phages to the *E. coli* cell surface enabled visualization of cells under a fluorescence microscope. GFP-labeled PP01 phage was not only adsorbed on culturable *E. coli* cells but also on viable but nonculturable or pasteurized cells. The coexistence of insensitive *E. coli* K-12 (W3110) cells did not influence the specificity and affinity of GFP-labeled PP01 adsorption on *E. coli* O157:H7. After a 10-min incubation with GFP-labeled PP01 phage at a multiplicity of infection of 1,000 at 4ºC, *E. coli* O157:H7 cells could be visualized by fluorescence microscopy. The GFP-labeled PP01 phage could be a rapid and sensitive tool for *E. coli* O157:H7 detection

12. **Estimation of septic tank setback distances based on transport of *E. coli* and F-RNA phages. Pang, L., Close, M., Goltz, M., Sinton, L., Davies, H., Hall, C., Stanton, G. (2004). *Environment International* 29:907-921.** Setback distances between septic tank systems and the shorelines of Lake Okareka, New Zealand were determined from model simulations for a worst-case scenario, using the highest hydraulic conductivity and gradient measured in the field, removal rates of the microbial indicators (*Escherichia coli* and F-RNA phages) determined from a column experiment, and maximum values of the design criteria for the disposal system, and assuming an absence of an unsaturated zone, a continuous discharge of the raw effluent from a failed or non-complying treatment system (both indicators at concentrations of $1x10^7$ counts/100 ml) into the groundwater and no sorption of pathogens in the aquifer. Modelling results suggest that the minimal setback distances were 16 m to satisfy the New Zealand Recreational Water Quality Guidelines for *E. coli* <126 per 100 ml (Ministry for the Environment, 1999) and 48 m to meet the Drinking-Water Standards for New Zealand 2000 for enteric virus <1 per 100 l (Ministry of Health, 2000). These distances may be applicable for other lakeshores in pumice sand aquifers with groundwater velocities <7 m/day. Findings of laboratory column and batch experiments provided an insight into the microbial attenuation and transport processes in pumice sand aquifers. Bacterial removal was predominately through filtration (87-88%) and partially by die-off (12-13%), while viral removal was by both die-off (45%) and filtration (55%). In addition, microbial die-off in groundwater without aquifer material (i.e., free microbes) was much lower than die-off in groundwater with aquifer material (i.e., sorbed microbes) and contributed only 2-6% to the total removal. This implies that the setback distances estimated from die-off rates for the free microbes, determined in the laboratory without considering aquifer media and other removal processes, which are often reported in the literature, could be larger than necessary

13. **The *Pasteurella multocida* toxin is encoded within a lysogenic bacteriophage. Pullinger, G. D., Bevir, T., Lax, A. J. (2004). *Molecular Microbiology* 51:255-269.** Toxigenic strains of *Pasteurella multocida* produce a 146 kDa toxin (PMT) that acts as a potent mitogen. Sequence analysis of the structural gene for PMT, toxA, previously suggested it was horizontally acquired, because it had a low G + C content relative to the *P. multocida* genome. To address this, the sequence of DNA flanking toxA was determined. The sequence analysis showed the presence of homologues to bacteriophage tail protein genes and a bacteriophage antirepressor, suggesting that the toxin gene resides within a prophage. In addition to phage genes, the toxA flanking DNA contained a homologue of a restriction/modification system that was shown to be functional. The presence of a bacteriophage was demonstrated in spent medium from toxigenic *P. multocida* isolates. Its production was increased by mitomycin C addition, a treatment that is known to induce the lytic cycle of many temperate bacteriophages. The genomes of bacteriophages from three different toxigenic *P. multocida* strains had similar but not identical restriction profiles, and were approximately 45-50 kb in length. The prophages from two of these had integrated at the same site in the chromosome, in a tRNA gene. Southern blot analysis confirmed that these bacteriophages contained the toxA gene.

14. **The genome and proteome of coliphage T1. Roberts, M. D., Martin, N. L., Kropinski, A. M. (2004). *Virology* 318:245-266.** The genome of enterobacterial phage T1 has been sequenced, revealing that its 50.7-kb terminally redundant, circularly permuted sequence contains 48,836 bp of nonredundant nucleotides. Seventy-seven open reading frames (ORFs) were identified, with a high percentage of small genes located at the termini of the genomes displaying no homology to existing phage or prophage proteins. Of the genes showing homologs (47%), we identified those involved in host DNA degradation (three endonucleases) and T1 replication (DNA helicase, primase, and single-stranded DNA-binding proteins) and recombination (RecE and Erf homologs). While the tail genes showed homology to those from temperate coliphage N15, the capsid biosynthetic genes were unique. Phage proteins were resolved by 2D gel electrophoresis, and mass spectrometry was used to identify several of the spots including the major head, portal, and tail proteins, thus verifying the annotation

15. **Immunological factors that affect the *in vivo* fate of T7 phage in the mouse. Srivastava, A. S., Kaido, T., Carrier, E. (2004). *Journal of Virological Methods* 115:99-104.** Phage display is a powerful method to study organ and tissue specific addresses. As part of our studies on the in vivo panning of tissue-homing peptide libraries, we examined the survival of T7 phage in the blood of C57BL/6J mice to estimate the half-life of T7 phage and the factors responsible for its inactivation. Amplified and purified T7 phage particles with or without random peptide library inserts were injected intravenously into the tail vein of wild-type (C57BL/6J) and immunocompromized (C57BL/6J) female mice. In wild-type mice, both the parent phage as well as phage carrying a peptide library were eliminated quickly from the blood, with only approximately 1% survival of detectable infectious phage after 60 min of injection. In SCID (C57BL/6J-Prkdc^{scid}) mice, phage titers were stable over the same period of time with or without peptide library, suggesting a role for either B- or T cells or both in phage inactivation. The presumed role of B cell was indicated by demonstration of stable phage in the B-cell deficient mouse (C57BL/10-Igh-6^{tm1Cgn}). In other immunocompromized mice, the phage titers were unstable, similar to that found in wild-type mice. In no case, was there a difference between phage with or without random peptide library. These data indicate that the presence of random C-X7-C peptides on the T7 phage coat protein does not affect the clearance of the phage in murine blood. Most likely, host immune factors play a role in the neutralization of T7 phage in blood by reacting with B-cell dependent immunoglobin

16. **Are viruses driving microbial diversification and diversity? Weinbauer, M. G., Rassoulzadegan, F. (2004). *Environmental microbiology* 6:1-11.** Viruses can influence the genetic diversity of prokaryotes in various ways. They can affect the community composition of prokaryotes by 'killing the winner' and keeping in check competitive

dominants. This may sustain species richness and the amount of information encoded in genomes. Viruses can also transfer (viral and host) genes between species. Such mechanisms have probably influenced the speciation of prokaryotes. Whole-genome sequencing has clearly revealed the importance of (virus-mediated) gene transfer. However, its significance for the ecological performance of aquatic microbial communities is only poorly studied, although the few available reports indicate a large potential. Here, we present data supporting the hypothesis that viral genes and viral activity generate genetic variability of prokaryotes and are a driving force for ecological functioning and evolutionary change

17. **Ecology of Prokaryotic Viruses. Weinbauer, M. G. (2004). *FEMS Microbiology Reviews* 28:127-181.** The finding that total viral abundance is higher than total prokaryotic abundance and that a significant fraction of the prokaryotic community is infected with phages in aquatic systems has stimulated research on the ecology of prokaryotic viruses and their role in ecosystems. This review treats the ecology of prokaryotic viruses ("phages") in marine, freshwater and soil systems from a "virus point of view". The abundance of viruses varies strongly in different environments and is related to bacterial abundance or activity suggesting that the majority of the viruses found in the environment are typically phages. Data on phage diversity are sparse but indicate that phages are extremely diverse in natural systems. Lytic phages are predators of prokaryotes, whereas lysogenic and chronic infections represent a parasitic interaction. Some forms of lysogeny might be described best as mutualism. The little existing ecological data on phage populations indicate a large variety of environmental niches and survival strategies. The host cell is the main resource for phages and the resource quality, i.e., the metabolic state of the host cell, is a critical factor in all steps of the phage life cycle. Virus-induced mortality of prokaryotes varies strongly on a temporal and spatial scale and shows that phages can be important predators of bacterioplankton. This mortality and the release of cell lysis products into the environment can strongly influence microbial food web processes and biogeochemical cycles. Phages can also affect host diversity, e.g., by "killing the winner" and keeping in check competitively dominant species or populations. Moreover, they mediate gene transfer between prokaryotes, but this remains largely unknown in the environment. Genomics or proteomics are providing us now with powerful tools in phage ecology, but final testing will have to be performed in the environment.

18. **Impact of virioplankton on archaeal and bacterial community richness as assessed in seawater batch cultures. Winter, C., Smit, A., Herndl, G. J., Weinbauer, M. G. (2004). *Applied and Environmental Microbiology* 70:804-813.** During cruises in the tropical Atlantic Ocean (January to February 2000) and the southern North Sea (December 2000), experiments were conducted to monitor the impact of virioplankton on archaeal and bacterial community richness. Prokaryotic cells equivalent to 10 to 100% of the in situ abundance were inoculated into virus-free seawater, and viruses equivalent to 35 to 360% of the in situ abundance were added. Batch cultures with microwave-inactivated viruses and without viruses served as controls. The apparent richness of archaeal and bacterial communities was determined by terminal restriction fragment length polymorphism (T-RFLP) analysis of PCR-amplified 16S rRNA gene fragments. Although the estimated richness of the prokaryotic communities generally was greatly reduced within the first 24 h of incubation due to confinement, the effects of virus amendment were detected at the level of individual operational taxonomic units (OTUs) in the T-RFLP patterns of both groups, *Archaea* and *Bacteria*. One group of OTUs was detected in the control samples but was absent from the virus-treated samples. This negative response of OTUs to virus amendment probably was caused by viral lysis. Additionally, we found OTUs not responding to the amendments, and several OTUs exhibited variable responses to the addition of inactive or active viruses. Therefore, we conclude that individual members of pelagic archaeal and bacterial communities can be differently affected by the presence of virioplankton.

19. **Genotoxicity of water extracts from the River Yamuna at Mathura, India. Aleem, A., Malik, A. (2003). *Environmental Toxicology* 18:69-77.** Water samples were collected from the River Yamuna at Mathura, India, and concentrated by using XAD resins (Amberlite XAD-4 and XAD-8) and liquid-liquid extraction procedures. The genotoxicities of the extracted water samples were evaluated by the Ames *Salmonella*/mammalian microsome test, DNA repair of defective mutants, and bacteriophage lambda systems. The results of the *Salmonella* test demonstrated that the XAD-concentrated water samples had maximum mutagenicity with the TA98 strain, both with and without metabolic activation. The XAD-concentrated water samples collected in the summer showed maximum mutagenic responses compared with those collected in other seasons, whereas the liquid-liquid extracted samples exhibited maximum mutagenic activity during the postmonsoon season. The damage brought about during DNA repair of defective mutants in the presence of XAD-concentrated water samples was found to be remarkably high compared with the liquid-liquid extracted water samples at a dose level of 20 microL/mL of culture. All the mutants invariably exhibited significant decline in their colony-forming units compared with their isogenic wild-type counterparts. Survival was decreased by 86.7% and 65.1% in the polA(-) strain after 6 h of treatment with XAD-concentrated and liquid-liquid extracted water samples, respectively. A significant decrease in the survival of bacteriophage lambda was also observed when treated with test samples. The damage was more pronounced in lexA mutants when the phage was treated with XAD-concentrated samples. The recA, lexA, and polA mutants of *E. coli* K-12 were found to be sensitive to the test samples, suggesting damage to the DNA of exposed cells as well as to the role of $recA^+$, $lexA^+$, and $polA^+$ genes in coping with the hazardous effect of the pollutants. The results demonstrated substantial genotoxicity and mutagenicity in the water samples tested

20. **AFV1, a novel virus infecting hyperthermophilic archaea of the genus Acidianus. Bettstetter, M., Peng, X., Garrett, R. A., Prangishvili, D. (2003). *Virology* 315:68-79** We describe a novel virus, AFV1, of the hyperthermophilic archaeal genus *Acidianus*. Filamentous virions are covered with a lipid envelope and contain at least five different proteins with molecular masses in the range of 23-130 kDa and a 20.8-kb-long linear double-stranded DNA. The virus has been assigned to the family *Lipothrixviridae* on the basis of morphotypic characteristics. Host range is confined to several strains of *Acidianus* and the virus persists in its hosts in a stable carrier state. The latent period of virus infection is about 4 h. Viral DNA was sequenced and sequence similarities were found to the lipothrixvirus SIFV, the rudiviruses SIRV1 and SIRV2, as well as to conjugative plasmids and chromosomes of the genus *Sulfolobus*. Exceptionally for the linear genomes of archaeal viruses, many short direct repeats, with the sequence TTGTT or close variants thereof, are closely clustered over 300 bp at each end of the genome. They are reminiscent of the telomeric ends of linear eukaryal chromosomes.

21. **Dynamic of isolation of the Phytopathogenic bacteria's phages from a leaf and a root of sugar beet. Andriychuk, O. M., Semchuk, L. I., Romashov, S. A., Ignatenko, T. A., Yatskovska, L. I., Boyko, A. L. (2003).**

Bulletin of the University of Kiev, series Biology **.** Research on studying the dynamics of phage's isolation to indicatory cultures *Pseudomonas, Xanthomonas, Erwinia*, which formed groups on certain taxonomicaly attributes is carried out: 1 - some strains which represents the pathovars of the certain species (8 strain); 2 - strains which represent species of the certain genus (13 strain); 3 - certain genus of phytopathogenic bacteria (*Pseudomonas, Xanthomonas, Erwinia).* Such bacteria can represents the taxonomic hierarchy and represents the model of microflora field agrocenoses. Research of phage's isolation from plants and roots of sugar beet has shown the presence of various phage's isolates by a character of the lytic abilities to the used indicatory bacteria. The direct isolation of phages to 13 indicators strains and a ultra-violet induction of lysogenic microflorae were carried out. Researches carried out in dynamics during 14 months continuously. During the warm period for the analysis selected leaves of sugar beet, roots selected during all year. The reveals wide spectrum of phages was characteristic both for free, and for ultra-violet induction phages.

22. **Detection of the phytopathogenic bacteria' phages in Antarctica. Boyko, A. L., Semchuk, L. I., Voytsitsky, V. M., Andriychuk, O. M., Romashev, S. A., Ignatenko, T. O., Yatskovska, L. I., Vaschenko, V. M., Delimat, A. (2003). *Agroeclogical magazine* 12-15.** During expedition to the Ukrainian Antarctic station " Academician Vernadsky " in 2003 testes of a thawed snow and a ground from islands of the Argentina archipelago have been taken. The analysis on presence of phytopathogenic bacteria phages *Xanthomonas, Burkholderia, Erwinia* and *Pseudomonas.* has shown lytic activity practically in all tests. For the most of them the titres of phages was 1-10 PFU/ml, and 6 gave high spontaneous production of the investigated phages - 10^6 PFU/ml. Processing of the tests by UV-radiation with the purpose of an lysogenic microflorae induction caused the inactivation of phages up to individual negative colonies. Discussion of the received results from the point of view of the investigated region's features is offered.

23. **Detection of phages of phytopathogenic bacterias *Pseudomonas*, *Xanthomonas*, *Erwinia* and *Bacillus* in agrocenosises. Boyko, A. L., Semchuk, L. I., Romashev, S. A., Andriychuk, O. M., Yatskovska, L. I. (2003). *Agroecological magazine* 24-26.** Studied the diffusion of phytopathogenic bacteria' bacteriophages of in [*sic*] Kiev area. The samplings conducted on plantations of sugar-beet. Among discharged bacteriophages the main specific weight was made by phages [of] *Xanthomonas beticola*. In samples [of] seeds of sugar-beet the phages are not detected. Their influencing on number of a pathogenic microflora in biocenoses is discussed.

24. **Evaluation of F+ RNA and DNA coliphages as source-specific indicators of fecal contamination in surface waters. Cole, D., Long, S. C., Sobsey, M. D. (2003). *Applied and Environmental Microbiology* 69:6507-6514.** Male-specific (F+) coliphages have been investigated as viral indicators of fecal contamination that may provide source-specific information for impacted environmental waters. This study examined the presence and proportions of the different subgroups of F+ coliphages in a variety of fecal wastes and surface waters with well-defined potential waste impacts. Municipal wastewater samples had high proportions of F+ DNA and group II and III F+ RNA coliphages. Bovine wastewaters also contained a high proportion of F+ DNA coliphages, but group I and IV F+ RNA coliphages predominated. Swine wastewaters contained approximately equal proportions of F+ DNA and RNA coliphages, and group I and III F+ RNA coliphages were most common. Waterfowl (gull and goose) feces contained almost exclusively F+ RNA coliphages of groups I and IV. No F+ coliphages were isolated from the feces of the other species examined. F+ coliphage recovery from surface waters was influenced by precipitation events and animal or human land use. There were no significant differences in coliphage density among land use categories. Significant seasonal variation was observed in the proportions of F+ DNA and RNA coliphages. Group I F+ RNA coliphages were the vast majority (90%) of those recovered from surface waters. The percentage of group I F+ RNA coliphages detected was greatest at background sites, and the percentage of group II F+ RNA coliphages was highest at human-impacted sites. Monitoring of F+ coliphage groups can indicate the presence and major sources of microbial inputs to surface waters, but environmental effects on the relative occurrence of different groups need to be considered

25. ***Shigella dysenteriae* type 1-specific bacteriophage from environmental waters in Bangladesh. Faruque, S. M., Chowdhury, N., Khan, R., Hasan, M. R., Nahar, J., Islam, M. J., Yamasaki, S., Ghosh, A. N., Nair, G. B., Sack, D. A. (2003). *Applied and Environmental Microbiology* 69:7028-7031.** *Shigella dysenteriae* type 1 is the causative agent of the most severe form of bacillary dysentery, which occurs as epidemics in many developing countries. We isolated a bacteriophage from surface water samples from Bangladesh that specifically lyses strains of *S. dysenteriae* type 1. This phage, designated SF-9, belongs to the Podoviridae family and has a 41-kb double-stranded DNA genome. Further screening of water samples for the prevalence of the phage revealed 9 of 71 (12.6%) water samples which were positive for the phage. These water samples were also positive in PCR assays for one or more *S. dysenteriae* type 1-specific genes, including ipaBCD and stx1, and live *S. dysenteriae* type 1 was isolated from three phage-positive samples. The results of this study suggest that phage SF-9 may have epidemiological applications in tracing the presence of *S. dysenteriae* type 1 in environmental waters

26. **The physical environment affects cyanophage communities in British Columbia inlets. Frederickson, C. M., Short, S. M., Suttle, C. A. (2003). *Microbial Ecology* 46:348-357.** Little is known about the natural distribution of viruses that infect the photosynthetically important group of marine prokaryotes, the cyanobacteria. The current investigation reveals that the structure of cyanophage communities is dependent on water column structure. PCR was used to amplify a fragment of the cyanomyovirus gene (g) 20, which codes for the portal vertex protein. Denaturing gradient gel electrophoresis (DGGE) of PCR amplified g20 gene fragments was used to examine variations in cyanophage community structure in three inlets in British Columbia, Canada. Qualitative examination of denaturing gradient gels revealed cyanophage community patterns that reflected the physical structure of the water column as indicated by temperature and salinity. Based on mobility of PCR fragments in the DGGE gels, some cyanophages appeared to be widespread, while others were observed only at specific depths. Cyanophage communities within Salmon Inlet were more related to one another than to communities from either Malaspina Inlet or Pendrell Sound. As well, surface communities in Malaspina Inlet and Pendrell Sound were different when compared to communities at depth. In the same two locations, distinct differences in community composition were observed in communities that coincided with depths of high chlorophyll fluorescence. The observed community shifts over small distances (only a few meters in depth or inlets separated by less than 100 km) support the idea that cyanophage communities separated by small spatial scales develop independently of each other as a result isolation by water column stratification or land mass separation, which may ultimately lead to changes in the distribution or composition

of the host community

27. **Bacteriophage ecology and plants. Gill, J. J., Abedon, S. T. (2003). *APSnet Feature* http://www.apsnet.org/online/feature/phages/.** Plant biology cannot be fully appreciated absent microbial flora, and plant-associated bacteria are incompletely understood without an awareness of phage -- the viruses of prokaryotes. Phage have been found in association with "buds, leaves, root nodules (leguminous plants), roots, rotting fruit, seeds, stems and straw; crown gall tumors… healthy or diseased alfalfa, barley, beans, broccoli, Brussels sprouts, buckwheat, clover, cotton, cucumber, lucerne, mulberry, oats peas, peach trees, radish, rutabaga, ryegrass, rye, timothy, tobacco, tomatoes, [and] wheat" (4). In this overview we consider the myriad ways that phage can impact ecologically on plant-associated bacteria.

28. **Bacteriophage treatment of a severe *Escherichia coli* respiratory infection in broiler chickens. Huff, W. E., Huff, G. R., Rath, N. C., Balog, J. M., Donoghue, A. M. (2003). *Avian Diseases* 47:1399-1405.** A bacteriophage to a serotype 02, nonmotile *Escherichia coli* was isolated from municipal waste treatment facilities and poultry processing plants. A study was conducted to determine the efficacy of multiple vs. single intramuscular (i.m.) injections of bacteriophage to treat a severe *E. coli* respiratory infection. The birds were challenged at 7 days of age by injection of 6 x 10^4 colony-forming units (cfu) of *E. coli* into the thoracic air sac followed by an i.m. injection into the thigh with either heat-killed or active bacteriophage. There were 16 treatments with three replicate pens of 10 birds. There were four control treatments, which included untreated birds, birds injected with either heat-killed or active bacteriophage, and birds challenged only with *E. coli*. In the remaining treatments, birds were injected with heat-killed or active bacteriophage either once immediately after E. coli challenge or immediately after challenge and at 8 and 9 days of age, once at 8 days of age or at 8, 9, and 10 days of age, and once at 9 days of age or at 9, 10, and 11 days of age. Mortality was significantly decreased from 57% to 13% in the birds given a single i.m. injection of bacteriophage immediately after *E. coli* challenge, and there was complete recovery in birds treated immediately after challenge and at 8 and 9 days of age, which was a significant improvement from the single injection treatment. There was a significant reduction in mortality from 57% to 10% in the birds treated with bacteriophage once at 8 days of age and those birds treated at 8, 9, and 10 days of age, with no difference between single or multiple treatments. The mortality in the single or multiple phage treated birds that started at 9 days of age was reduced from 57% to 28% and 27%, respectively, but was not statistically different from the control. These data suggest that bacteriophage can be an effective treatment when administered early in this experimental *E. coli* respiratory disease and that early multiple treatments are better than a single treatment. The efficacy of bacteriophage treatment diminishes as it is delayed, with no difference between single or multiple treatments. Bacteriophage may provide an effective alternative to antibiotics, but like antibiotic therapy, the effectiveness of phage to rescue animals decreases the longer treatment is delayed in the disease process

29. **Lateral gene transfer: when will adolescence end? Lawrence, J. G., Hendrix, R. W. (2003). *Molecular Microbiology* 50:739-749.** The scope and impact of horizontal gene transfer (HGT) in Bacteria and Archaea has grown from a topic largely ignored by the microbiological community to a hot-button issue gaining staunch supporters (on particular points of view) at a seemingly ever-increasing rate. Opinions range from HGT being a phenomenon with minor impact on overall microbial evolution and diversification to HGT being so rampant as to obfuscate any opportunities for elucidating microbial evolution - especially organismal phylogeny - from sequence comparisons. This contentious issue has been fuelled by the influx of complete genome sequences, which has allowed for a more detailed examination of this question than previously afforded. We propose that the lack of common ground upon which to formulate consensus viewpoints probably stems from the absence of answers to four critical questions. If addressed, they could clarify concepts, reject tenuous speculation and solidify a robust foundation for the integration of HGT into a framework for long-term microbial evolution, regardless of the intellectual camp in which you reside. Here, we examine these issues, why their answers shape the outcome of this debate and the progress being made to address them.

30. **Diagnostic and therapeutic applications of lytic phages. Mandeville, R., Griffiths, M., Goodridge, L., McIntyre, L., Ilenchuk, T. T. (2003). *Analytical Letters* 36:3241-3259.** The ability of lytic phages to rapidly kill and lyse infected bacteria, the specificity of phages for particular bacteria, and the ability of phages to increase in number during the infection process make phages excellent potential diagnostic and therapeutic agents for fighting bacterial disease. However, temperate phages are of little use in phage diagnostics and therapy.

31. **Use of fluorescently labeled phage in the detection and identification of bacterial species. Mosier-Boss, P. A., Lieberman, S. H., Andrews, J. M., Rohwer, F. L., Wegley, L. E., Breitbart, M. (2003). *Applied spectroscopy* 57:1138-1144.** Phages are viruses whose hosts are bacterial cells. They identify their hosts by specific receptor molecules on the outside of the host cell. Once the phages find their specific receptors, they bind to the bacterial cell and inject their nucleic acid inside the cell. The binding between phage and host can be so specific that only certain strains of a single species can be infected. In this communication, the specificity of phage P22 for *Salmonella typhimurium* LT2 is exploited to allow the detection of *Salmonella* in the presence of other bacterial species. In particular, the dsDNA of P22 is bound to SYBR gold, a highly sensitive, fluorescent nucleic acid stain. When multiple phages infect the same cell, the fluorescence emissions of the phage DNA inside the cell allow it to be imaged using an epifluorescence microscope. The advantages of using phages as the bacterial recognition element in a sensor over antibodies are discussed

32. **Detection of the phytopathogenic bacteria phages in the gills of the Black Sea fishes. Semchuk, L. I., Stepanova, O. A., Boyko, A. L., Romashev, S. A., Andriychuk, O. M., Ignatenko, T. O., Yatskovska, L. I. (2003). *Bulletin of the University of Kiev. , series Biology* .** A biological activity of phages, isolated from the gills of Black Sea fishes, to phytopathogenic bacteria was studied. It was shown the capacity of the testing cultures to support the phages reproduction during the definite time.

33. **Viral abundance and a high proportion of lysogens suggest that viruses are important members of the microbial community in the Gulf of Trieste. Stopar, D., Cerne, A., Zigman, M., Poljsak-Prijatelj, M., Turk, V. (2003). *Microbial Ecology* 46:249-256.** Epifluorescence microscopy and transmission electron microscopy were applied to study virioplankton community in the Gulf of Trieste (northern Adriatic Sea). The total viral abundance was in a range between 2.5 x 10^9/L and 2.9 x 10^{10}/L and was positively correlated with trophic status of the environment.

Viruslike particles were significantly correlated with bacterial abundance in all samples studied. Correlations with other physicochemical or biological parameters were not significant. The data suggest that, because of the substantial fraction of tailed viruses present (26%), bacteriophages are an important component of the virioplankton community in the Gulf of Trieste. The abundance of viruslike particles in the seawater changed at hour intervals in a range from 1.3 x 10^9/L to 5.1 x 10^9/L. A significant fraction (71%) of the bacterial isolates was inducible in vitro by mitomycin C, and a high occurrence (51%) of lysogenic isolates with more than one phage morphotype present in the lysate was detected. The presence of lysogenic bacteria in the seawater was confirmed in situ with a mitomycin C induction experiment on the natural bacterial population. Results suggest that virioplankton is an abundant component of the microbial community in the Gulf of Trieste

34. **Transduction of porcine enteropathogenic *Escherichia coli* with a derivative of a shiga toxin 2-encoding bacteriophage in a porcine ligated ileal loop system. Tóth, I., Schmidt, H., Dow, M., Malik, A., Oswald, E., Nagy, B. (2003). *Applied and Environmental Microbiology* 69:7242-7247.** In this study, we have investigated the ability of detoxified Shiga toxin (Stx)-converting bacteriophages Φ3538 (Δstx(2)::cat) (H. Schmidt et al., Appl. Environ. Microbiol. 65:3855-3861, 1999) and H-19B::Tn10d-bla (D. W. Acheson et al., Infect. Immun. 66:4496-4498, 1998) to lysogenize enteropathogenic *Escherichia coli* (EPEC) strains in vivo. We were able to transduce the porcine EPEC strain 1390 (O45) with Φ3538 (Δstx(2)::cat) in porcine ligated ileal loops but not the human EPEC prototype strain E2348/69 (O127). Neither strain 1390 nor strain E2348/69 was lysogenized under these in vivo conditions when *E. coli* K-12 containing H-19B::Tn10d-bla was used as the stx1 phage donor. The repeated success in the in vivo transduction of an Stx2-encoding phage to a porcine EPEC strain in pig loops was in contrast to failures in the in vitro trials with these and other EPEC strains. These results indicate that in vivo conditions are more effective for transduction of Stx2-encoding phages than in vitro conditions

35. **Searching for the advantages of virus sex. Turner, P. E. (2003). *Origins of Life and Evolution of the Biosphere* 33:95-108.** Sex (genetic exchange) is a nearly universal phenomenon in biological populations. But this is surprising given the costs associated with sex. For example, sex tends to break apart co-adapted genes, and sex causes a female to inefficiently contribute only half the genes to her offspring. Why then did sex evolve? One famous model poses that sex evolved to combat Muller's ratchet, the mutational load that accrues when harmful mutations drift to high frequencies in populations of small size. In contrast, the Fisher-Muller Hypothesis predicts that sex evolved to promote genetic variation that speeds adaptation in novel environments. Sexual mechanisms occur in viruses, which feature high rates of deleterious mutation and frequent exposure to novel or changing environments. Thus, confirmation of one or both hypotheses would shed light on the selective advantages of virus sex. Experimental evolution has been used to test these classic models in the RNA bacteriophage ϕ6, a virus that experiences sex via reassortment of its chromosomal segments. Empirical data suggest that sex might have originated in ϕ6 to assist in purging deleterious mutations from the genome. However, results do not support the idea that sex evolved because it provides beneficial variation in novel environments. Rather, experiments show that too much sex can be bad for ϕ6; promiscuity allows selfish viruses to evolve and spread their inferior genes to subsequent generations. Here I discuss various explanations for the evolution of segmentation in RNA viruses, and the added cost of sex when large numbers of viruses co-infect the same cell

36. **Rapid selection of phage-resistant mutants in Streptococcus thermophilus by immunoselection and cell sorting. Viscardi, M., Capparelli, R., Iannelli, D. (2003). *International Journal of Food Microbiology* 89:223-231.** Immunoselection and flow cytometry allowed the isolation from *Streptococcus thermophilus* strain Str31 of double mutants displaying resistance to the phage ϕ31 and good acid production. Strain Str31 is very sensitive to phage ϕ31. This phage-host system seemed therefore particularly suitable to test the validity of the selection method adopted in this study. Mutants were stable with respect to both characters. The isolation of the double mutants required 4 to 5 days. The approach does not involve genetic manipulations and can therefore be an alternative to genetic engineering when this technology cannot be applied

37. **Bacteriophages as an efficient therapy for antibiotic-resistant septicemia in man. Weber-Dabrowska, B., Mulczyk, M., Gorski, A. (2003). *Transplantation proceedings* 35:1385-1386.** [first paragraph] Acute bacterial infection-induced sepsis, with shock, metabolic acidosis, oliguria, or hypoxemia, remains a major medical challenge, especially at a time when experts believe that we may be returning to the pre-antibiotic era, arising from increasing antibiotic resistance In the USA alone there are at least 500,000 cases of sepsis annually, with mortality rates ranging from 30% to 50% (ie, 150,000 to 250,000 deaths). Assuming a U.S. population of approximately 270 million and a total world population of >6 billion, this would mean at least 11 million cases of sepsis worldwide with at least 3 to 5 million deaths annually (or probably more, as American health-care standards are generally much higher than in many other countries). Several treatments designed to reduce sepsis-associated mortality have been unsuccessful; therefore, finding an effective new therapy for sepsis is urgently needed.

38. **Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic waters. Weinbauer, M., Brettar, I., Höfle, M. (2003). *Limnology and Oceanography* 48:1457-1465.** Lysogeny (bacteria containing inducible prophages) and lytic viral infection (bacteria in a lytic stage of infection) were investigated at the community level in contrasting marine environments such as estuarine versus offshore waters, surface versus deep waters, and oxic versus anoxic waters in the Mediterranean and Baltic Seas. The frequency of lysogenic cells (FLC) in bacterioplankton communities ranged from not detectable to 84% as estimated by prophage induction due to mitomycin C, and highest values were typically found in deep waters (800–2,000 m). Transmission electron microscopy based estimates of virus-induced mortality of bacterioplankton (VMB) ranged from a few percent to 71%, and highest values were found in anoxic waters of the Baltic Sea. FLC and the frequency of infected cells (FIC) were related in form of a negative power function indicating that environments exist where one of the two viral life strategies prevails. Across all investigated environments, FLC was negatively related to bacterial abundance and production, whereas FIC showed a positive relationship with viral and bacterial parameters. FIC was higher and FLC was lower in moderately productive estuarine and offshore surface waters than in less productive mesopelagic and deep waters. Thus, lysogeny seems to be a survival strategy at low host abundance and activity, whereas high host abundance and activity seems to favor the lytic life cycle. The key process for the prevalence of lytic infection compared to prophage replication at high host abundance could be competition due to outnumbering. Between 11% and 88% (average, 35%) of the bacteria contained a functional (lytic or lysogenic) viral genome.

39. **Comparing the effects of resource enrichment and grazing on viral production in a meso-eutrophic reservoir. Weinbauer, M. G., Christaki, U., Nedoma, J., Simek, K. (2003). *Aquatic Microbial Ecology* 31:137-144.** As viral production depends on bacteria, factors which influence bacterial production should also impact viral production. Likewise, viruses and heterotrophic nanoflagellates (HNF) both exploit bacterial prey, so HNF grazing could influence interactions between viruses and bacteria. To examine these relationships, we examined samples from experiments in which natural bacterial populations were subjected to relaxation of nutrient limitation and different levels of grazing pressure from HNF. We observed that stimulation of bacterial production and abundance with the relaxation of nutrient limitation resulted in a higher standing stock of viruses, higher viral production and also a higher virus-induced lysis rate of bacterioplankton. These relationships suggest that the relative effect of virus-induced mortality is higher in more productive environments. We found that viral abundance, viral production and virus-induced mortality of bacteria was highest in the treatments in which grazing rates on bacteria by HNF were highest, and lowest in the treatments where no eukaryotic predators were present. Thus, high grazing rates were associated with high virus production rates. The resource enrichment had a stronger effect on viral production and infection of bacteria than grazing. Averaged over time for single treatments, viruses lysed a significant portion (range, 18 to 66%) of the bacterial production per day.

40. **Sampling natural viral communities from soil for culture-independent analyses. Williamson, K. E., Wommack, K. E., Radosevich, M. (2003). *Applied and Environmental Microbiology* 69:6628-6633.** An essential first step in investigations of viruses in soil is the evaluation of viral recovery methods suitable for subsequent culture-independent analyses. In this study, four elution buffers (10% beef extract, 250 mM glycine buffer, 10 mM sodium pyrophosphate, and 1% potassium citrate) and three enumeration techniques (plaque assay, epifluorescence microscopy [EFM], and transmission electron microscopy [TEM]) were compared to determine the best method of extracting autochthonous bacteriophages from two Delaware agricultural soils. Beef extract and glycine buffer were the most effective in eluting viable phages inoculated into soils (up to 29% recovery); however, extraction efficiency varied significantly with phage strain. Potassium citrate eluted the highest numbers of virus-like particles from both soils based on enumerations by EFM (mean, 5.3×10^8 g of dry soil^{-1}), but specific soil-eluant combinations posed significant problems to enumeration by EFM. Observations of virus-like particles under TEM gave confidence that the particles were, in fact, phages, but TEM enumerations yielded measurements of phage abundance (mean, 1.5×10^8 g of dry soil^{-1}) that were about five times lower. Clearly, the measurement of phage abundance in soils varies with both the extraction and enumeration methodology; thus, it is important to assess multiple extraction and enumeration approaches prior to undertaking ecological studies of phages in a particular soil

41. **Imbroglios of viral taxonomy: genetic exchange and failings of phenetic approaches. Lawrence, J. G., Hatfull, G. F., Hendrix, R. W. (2002). *Journal of Bacteriology* 184:4891-4905.** The practice of classifying organisms into hierarchical groups originated with Aristotle and was codified into nearly immutable biological law by Linnaeus. The heart of taxonomy is the biological species, which forms the foundation for higher levels of classification. Whereas species have long been established among sexual eukaryotes, achieving a meaningful species concept for prokaryotes has been an onerous task and has proven exceedingly difficult for describing viruses and bacteriophages. Moreover, the assembly of viral "species" into higher-order taxonomic groupings has been even more tenuous, since these groupings were based initially on limited numbers of morphological features and more recently on overall genomic similarities. The wealth of nucleotide sequence information that catalyzed a revolution in the taxonomy of free-living organisms necessitates a reevaluation of the concept of viral species, genera, families, and higher levels of classification. Just as microbiologists discarded dubious morphological traits in favor of more accurate molecular yardsticks of evolutionary change, virologists can gain new insight into viral evolution through the rigorous analyses afforded by the molecular phylogenetics of viral genes. For bacteriophages, such dissections of genomic sequences reveal fundamental flaws in the Linnaean paradigm that necessitate a new view of viral evolution, classification, and taxonomy.

42. **The adaptation and survival of phages in nature. Semchuk, L. I., Ignatenko, T., Romashev, S. A., Andriychuk, O., Yatskovska, L. (2002). *Bulletin of the University of Kiev, series Biology* 38:54-56.** The short analyse of the phage's properties changing, their evolution and survival adaptation in the environment is given.

43. **Detection of populations of phages of phytopathogenic bacterias and their biological properties. Boyko, A. L., Semchuk, L. I., Romashev, S. A., Andriychuk, L. N. (2001). *Bulletin of the Agroscience* 51-53.** Excreted bacteriophages to *X. axonopodis* pv. *beticola*, *E. carЇtovora*, *P. syringae* pv. *atrofaciens* in the Kiev area. The bacteriophages showed specific specificity, were look-alike on morphology, sizes, are stable at long-term storage and in range pH 4-10. The role of populations of phages in biocenoses and capability of indication of pathogens with their help is discussed.

44. **Ecological and biological aspects of research phages phytopathogenic bacteria. Semchuk, L. I., Andriychuk, E. M., Romashev, S. A., JatskЇvska, L. I. (2001). *Bulletin of the University of Kiev, series Biology* N35:11-13.** Investigated features of biology and ecology of phages of phytopathogenic bacteria *Xanthomonas* and *Pseudomonas* on sowings of sugar beet.

45. **Effect of resource supply rate on host-pathogen dynamics. Bohannan, B. J. M. (2000). in Bell, C. R., Brylinsky, M., Johnson-Green, P. (eds.) *Microbial Biosystems: New Frontiers*. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.** The dynamics of model host cell (*E. coli*) and model pathogen (bacteriophage) populations were studied in chemostats with different resource supply rates. Resource supply rate was manipulated by altering the concentration of the limiting resource (glucose) in the incoming media. Population responses to increased resource supply rate were influenced strongly by the vulnerability of the host cells to infection. When the host cell population consisted entirely of cells equally vulnerable to infection, both pathogen and host cells responded to increased resource supply rate with an increase in their average densities. In contrast, when the host cell contained some cells that were less vulnerable to infection (i.e., partially phage-resistant *E. coli*), only the pathogen population responded to increased supply rate with a significant increase in average density. Furthermore, when the host cell population contained some cells completely invulnerable to infection (i.e., phage-resistant *E. coli*) only the host cell population responded to increased supply rate with an increase in average density. These responses were in general agreement with the predictions of mechanistic models of resource-consumer interactions.

46. **Genetic analysis of a bacterial genetic exchange element: the gene transfer agent of *Rhodobacter capsulatus*. Lang, A. S., Beatty, J. T. (2000). *Proceedings of the National Academy of Sciences, USA* 97:859-864.** An unusual system of genetic exchange exists in the purple nonsulfur bacterium *Rhodobacter capsulatus*. DNA transmission is mediated by a small bacteriophage-like particle called the gene transfer agent (GTA) that transfers random 4.5-kb segments of the producing cell's genome to recipient cells, where allelic replacement occurs. This paper presents the results of gene cloning, analysis, and mutagenesis experiments that show that GTA resembles a defective prophage related to bacteriophages from diverse genera of bacteria, which has been adopted by *R. capsulatus* for genetic exchange. A pair of cellular proteins, CckA and CtrA, appear to constitute part of a sensor kinase/response regulator signaling pathway that is required for expression of GTA structural genes. This signaling pathway controls growth-phase-dependent regulation of GTA gene messages, yielding maximal gene expression in the stationary phase. We suggest that GTA is an ancient prophage remnant that has evolved in concert with the bacterial genome, resulting in a genetic exchange process controlled by the bacterial cell.

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