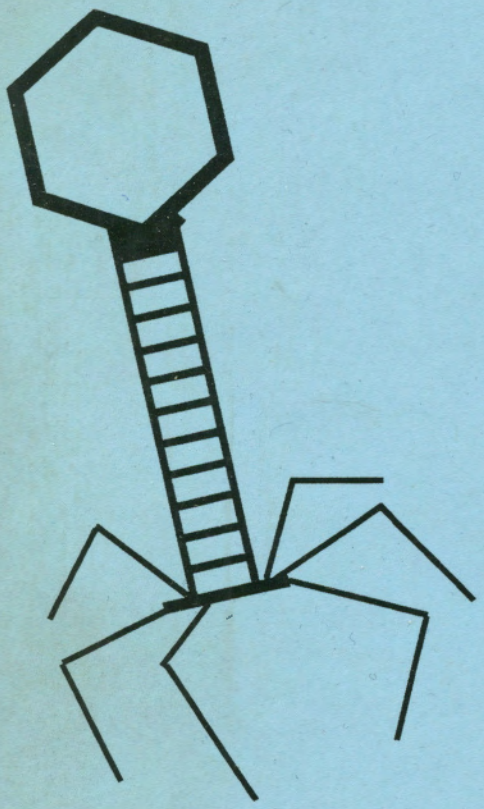
McGill

Dr. Michael S. DuBow
Department of Microbiology and Immunology
McGill University
Montreal, Quebec, Canada



Millennial Phage Biology Meeting

Montreal, 7-11 June 2000

POWERED BY:

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BETTY KUTTER
EVERGREEN STATE COLLEGE

PROGRAM

Wednesday, June 7, 2000:

SESSION I **Chair: Betty Kutter**

7:30 - 7:45 p.m. OPENING REMARKS
Michael DuBow

7:45 - 8:45 p.m. FELIX D'HERELLE AND THE ORIGINS OF MOLECULAR BIOLOGY
William Summers

8:45 - 9:05 p.m. FREQUENCY OF PHAGE DESCRIPTIONS IN THE YEAR 2000
Hans Ackermann

9:05 - 9:30 p.m. FROM PHAGE TO PHILOSOPHY: EXPLORING THE SUPPOSITION THAT LIFE IS ONE BIG CHEMICAL REACTION
David Coombs et al.

Thursday, June 8, 2000

SESSION II **T7 and DNA Ejection**
Chair: Linda Reha-Krantz

9:00 - 9:40 a.m. MODES OF T7 GENOME TRANSLOCATION FROM THE VIRION AND INTO THE BACTERIAL CELL CYTOPLASM
Ian Molineux

9:40 - 10:00 a.m. THE LYTIC TRANSGLYCOSYLASE MOTIF OF THE T7 INTERNAL HEAD PROTEIN gp16 AND ITS ROLE IN DNA EJECTION
Michael Moak

10:00 -10:20 a.m. THE INTERNAL HEAD PROTEIN gp16 CONTROLS DNA EJECTION FROM THE BACTERIOPHAGE T7 VIRION
William Robins

COFFEE BREAK

SESSION III: **Structure**
Chair: Eleanor Spicer

10:40 – 11:00 a.m. THE ROLE OF BASEPLATE PROTEIN gp29 IN BACTERIOPHAGE T4 TAIL ASSEMBLY
Yuefei Lou et. al.

11:00 – 11:20 a.m. RECOGNITION OF RECEPTOR LIPOPOLYSACCHARIDES
Minoru Inagaki et al.

11:20 – 11:40 a.m. THE KINETIC ROLE OF CYSTEINE RESIDUES ON THE IN VIVO FOLDING AND FUNCTION OF *SALMONELLA* PHAGE P22 TAILSPIKE
Cameron Hasse-Pettingell et al.

11:40 – 12:00 noon MOLECULAR ARCHITECTURE OF BACTERIOPHAGE T4 CAPSID AND ITS DEVELOPMENT DURING MATURATION
Kenji Iwasaki et al.

LUNCH

SESSION IV: **Recombination and Phage Tools**
Chair: Ian Molineux

7:30 - 7:50 p.m. A BIPARTITE BACTERIOPHAGE T4 SOC AND HOC RANDOMIZED PEPTIDE DISPLAY LIBRARY: DETECTION AND ANALYSIS OF PHAGE T4 TERMINASE (gp17) AND LATE SIGMA FACTOR (gp 55) INTERACTION
Lindsay Black

7:50 - 8:10 p.m. TRANSGENE INTEGRATION IN *SCHIZOSACCHAROMYCES POMBE* MEDIATED BY THE STREPTOMYCES BACTERIOPHAGE PhiC31 SITE-SPECIFIC RECOMBINATION SYSTEM
Lynn Thomason et al.

8:10 - 8:30 p.m. REQUIREMENT OF PHAGE LAMBDA RECOMBINATION FUNCTIONS FOR PHAGE-PROPHAGE MARKER RESCUE
Sidney Hayes et al.

COFFEE BREAK AND POSTER SESSION

POSTER SESSION: 8:30 P.M. - 10:30 p.m.

IN VITRO SELECTION OF RNA APTAMERS THAT BIND INTACT BACTERIOPHAGE
Timothy Dean et al.

PARTIAL SEQUENCE OF *PSEUDOMONAS putida* PHAGE gh-1 USING SHOTGUN SEQUENCING STRATEGY, EVIDENCE FOR RELATIONSHIP TO THE T7 GROUP
Irina Kovalyova et al.

DEVELOPMENT OF THERAPEUTICS FOR THE TREATMENT OF BACTERIAL INFECTIONS USING LADS
David Schofield

DEVELOPMENT OF THERAPEUTICS FOR THE TREATMENT OF BACTERIAL INFECTIONS USING LADS
Laura Kasman

TREATMENT OF EXPERIMENTAL INFECTIONS IN EMBRYONATED HEN EGGS AND MICE
Laura Kasman et al.

THE COMPLETED SEQUENCE OF THE GENOME OF *SALMONELLA typhimurium* PHAGE P22
Andrew M. Kropinski

BIOLOGICAL CONTROL OF ERWINA *amylovora* WITH BACTERIOPHAGES
J.J. Gill et al.

THE FREQUENCY OF LYSOGENY AND POLYLYSOGENY IN BACTERIA ISOLATED FROM NORTHERN ADRIATIC SEA
David Stopar et al.

ANTIBIOTIC-INDUCED "PSEUDOLYSOGENY": IMPLICATIONS FOR ANTIBIOTIC/ PHAGE COMBINATION THERAPY
Richard Weld

EFFECTS OF HOST PHYSIOLOGY ON THE INTRACELLULAR GROWTH OF PHAGE T7
Lingchong You

TESTING THE MOBILITY OF FIMBRIAL OPERONS BY PHAGE INDUCTIONS EXPERIMENTS
Hojabr Dezfulian et al.

ELABORATION OF THE NEW MEDICINAL-PROPHYLACTIC BACTERIOPHAGES AGAINST PURULENT INFECTIONS
Inga Giorgadze

LYSOGENIC CONVERSION BY P2-RELATED PHAGES: A NOVEL METHYLASE ENCODED BY PHAGE Wphi
Gail Christie et al.

INJECTION OF LAMBDA DNA INTO *E. coli*
Sutton Mooney

Friday, June 9, 2000

SESSION V: **Gene Expression**
Chair: Rich Calendar

9:00 - 9:20 a.m. TRANSLATIONAL REPRESSOR regA PROTEINS FROM PHAGE T4 AND RB69 HAVE CONSERVED RNA BINDING DOMAINS BUT DIFFERENT RNA BINDING SPECIFICITIES
Eleanor Spicer et al.

9:20 - 9:40 a.m. ROLE OF mRNA SECONDARY STRUCTURE IN THE TRANSLATIONAL INITIATION REGION OF GENE 25 OF T4-TYPE BACTERIOPHAGES
Rimas Nivinskas

9:40 - 10:00 a.m. DETERMINANTS FOR DNA BINDING BY A MEMBER OF THE P2 Ogr FAMILY OF ZINC-FINGER TRANSCRIPTION FACTORS
Gail Christie et al.

10:00 – 10:20 a.m. ORGANIZATION OF THE GENOME OF P1 BACTERIOPHAGE: TRACKING THE DEVELOPMENTAL PROGRAM OF EXPRESSION OF P1 PHAGE GENES
Malgorzata Lobocka et al.

10:20 - 10:40 a.m. THE CONSERVED Ner FAMILY OF TRANSPOSABLE PHAGES
Michael S. DuBow et al.
COFFEE BREAK

SESSION VI: **Lysis**
Chair: Donna Duckworth

11:00 – 11:20 a.m. NEW INSIGHTS ON THE FOUNDING PHENOMENON OF MOLECULAR GENETICS: THE MECHANISM OF LYSIS-INHIBITION
Erian Ramanculov et al.

11:20–11:40 a.m. DISSECTION OF MOLECULAR EVENTS LEADING TO FORMATION OF THE LAMBDA S HOLIN-DEPENDENT LESION IN THE CYTOPLASMIC MEMBRANE
Udo Bläsi et al.

11:40 – 12 noon: A PLETHORA OF LYSIS-TIMING VARIATION
Stephen T. Abedon

LUNCH BREAK

Friday, June 9, 2000

SESSION VII: **Phage Therapy**
Chair: Lindsay Black

2:15 - 2:35 p.m. OVERVIEW OF PHAGE THERAPY
Betty Kutter

2:35 - 2:55 p.m. CHARACTERIZATION OF TWO THERAPEUTIC STAPHYLOCOCCAL AND COLI-DYSENTERIAL BACTERIOPHAGES
Revaz Adamia

2:55 - 3:15 p.m. USE OF *VIBRIO vulnificus* BACTERIOPHAGE TO PREVENT DISEASE IN A MOUSE MODEL OF INFECTION
Donna Duckworth et al.

COFFEE BREAK

3:30 - 3:50 p.m. A BACTERIOPHAGE LETHAL AGENT DELIVERY SYSTEM FOR THE TREATMENT OF BACTERIAL INFECTIONS
Caroline Westwater

3:50 - 4:10 p.m. BIOLOGICAL CONTROL OF ERWINA *amylovora* WITH BACTERIOPHAGES
J.J. Gill et al.

4:10 - 4:45 p.m. DEVELOPMENT AND COMMERCIALIZATION OF PHAGE THERAPY IN THE UNITED STATES
Alexander Sulakvelidze et al.

4:45 - 5:30 p.m. DISCUSSION OF PHAGE THERAPY

Saturday, June 10, 2000

SESSION VIII: **Phage Evolution**
Chair: Gisela Mosig

9:00 - 9:20 a.m. STRUCTURAL, GENETIC AND EVOLUTIONARY ANALYSES OF MICROVIRIDAE SCAFFOLDING PROTEINS
Bentley Fane

9:20 - 9:40 a.m. DARWINIAN EVOLUTION OF THE STRUCTURAL GENE CLUSTER IN SIPHOVIRIDAE?
Harald Bruessow et al.

9:40 - 10:00 a.m. T-EVEN PHAGES AND THE REAL WORLD
Betty Kutter

10:00 –10:20 a.m. MOLECULAR ANALYSIS OF LYTIC *YERSINIA enterocolitica* SEROTYPE 0:3-SPECIFIC BACTERIOPHAGE phi-Ye03-12
Maria Pajunen et al.

COFFEE BREAK

10:40 – 11:00 a.m. PARTIAL GENETIC CHARACTERIZATION OF COLIPHAGE LG1
Lawrence Goodridge

11:00 – 11:20 a.m. CHARACTERISATION OF THE LYSOGENIC PHAGE IN SALMONELLA ENTERICA SEROVAR TYPHIMURIUM
Princess Mmolawa et al.

11:20 – 11:40 a.m. BACTERIOPHAGE PHYLOGENETICALLY RELATED TO T4 INFECT A WIDE RANGE OF HOST BACTERIAL SPECIES
Mzia Kutaleladze

LUNCH BREAK

Saturday, June 10, 2000

SESSION IX: **T4 DNA Replication**
Chair: Steve Abedon

2:00 - 2:20 p.m. EVIDENCE THAT DNA POLYMERASE PROOFREADING CREATES AN OPPORTUNITY FOR MISALIGNMENT MUTAGENESIS. STUDIES OF THE BACTERIOPHAGE T4 DNA POLYMERASE PROOFREADING PATHWAY
Linda Reha-Krantz et al

2:20 - 2:40 p.m. THE CHARACTERISATION OF A UNIQUE MUTANT OF T4 TOPOISOMERASE THAT IS HYPERSENSITIVE TO A BROAD RANGE OF CLEAVAGE-INDUCING ANTITUMOR AGENTS
Erin O'Reilly et al.

2:40 – 3:00 p.m. REPAIR OF TOPOISOMERASE-MEDIATED DNA DAMAGE IN BACTERIOPHAGE T4
Brad Stohr

3:00 – 3:20 p.m. LOADING ENZYMES ONTO ssDNA—T4 PHAGE AND THE RMP
Scott Morrical et al.

COFFEE BREAK

3:40 – 4:00 p.m. AN UNCONVENTIONAL USE OF ITERONS AT THE PHAGE T4 ORIGIN E OF DNA REPLICATION
Gisela Mosig et al.

4:00 – 4:20 p.m. PHAGE T4 rI AS A MUTATION-REPORTER GENE
Gerry Carver et al.

4:20 – 4:40 p.m. THE FIDELITIES OF PHAGE-RB69 DNA POLYMERASES COMPARED *IN VIVO* AND *IN VITRO*
Jan Drake et al.

6:30 - ? DINNER AND CRUISE ON THE ST-LAWRENCE RIVER

Sunday, June 11, 2000

SESSION X: **Phage and Pathogenesis**
Chair: David Coombs

9:00 – 9:20 a.m. THE ENDOGENOUS BACTERIOPHAGES OF STREPTO- COCCUS PYOGENES STRAIN SF370 DISCOVERED BY WHOLE GENOME SEQUENCING: IMPLICATIONS FOR PATHOGENESIS

William McShan

9:20 – 9:40 a.m. PHYLOGENETIC RELATIONSHIP BETWEEN COLIPHAGE T4 AND VIBRIOPHAGE KVP40

Shigenobu Matsuzaki

9:40 – 10:00 a.m. DISCOVERY OF PERTUSSIS TOXIN SEQUENCES PRESENT IN BORDETELLA AVIUM BACTERIOPHAGES

Derrick Lenz et al.

COFFEE BREAK - FOLLOWED BY MEETING SUMMARY AND GENERAL DISCUSSION

Title:MD, Professor
First Name:Hans
Last Name:Ackermann
Address:Department of Medical Biology
Faculty of Medicine
Laval University
Quebec, Qc, G1K 7P4
Phone:(418) 656-2131 # 2558
Fax:(418) 656-7666
E-mail:ackermann@mcb.ulaval.ca

Abstract Title:Frequency of Phage Descriptions in the Year 2000

Approximately 5000 bacterial viruses, excluding nonviable and nonpropagated phages or viruses without known hosts, have been examined in the electron microscope since 1959. About 96% are tailed and about 240 (4%) are icosahedral, filamentous, or pleomorphic. Bacteriophages belong to 12 families and occur in over 130 eubacterial and archaebacterial genera. Although phage hosts represent 32 groups of Bergey's Manual, the vast majority are low-GC eubacteria and g3 proteobacteria. The tailed phages belong to the Myoviridae (24%), Siphoviridae (62%), and Podoviridae (14%) families. The other 9 families are mostly small and sometimes include a single member. All basic types of nucleic acid (ssDNA, dsDNA, ssRNA, dsRNA) are repesented. Thermus phages offer an example of interphyletic phage transmission.

Title:Dr.
First Name:David
Last Name:Coombs
Address:Dept. of Biology
University of New Brusnwick
Fredericton NB Canada
E3B 1P6
Phone:506-453-4812
E-mail:dhc@unb.ca

Abstract Title:From Phage to Philosophy: Exploring the supposition that life is one big chemical reaction.

COOMBS, D. & FERGUSON, P. University of New Brunswick, Dept. of Biology, Fredericton, NB, Canada E3B 1P6

I have been trying to come up with an experiment that will take me through my normal career and well into my retirement so that I never run out of things to do. So far this one looks pretty good. It involves peering into a living cell (without invoking the Heisenberg Principle) to see if I can build a mathematical model of a living process, virus assembly. The ultimate goal is to test the supposition that life is nothing special and can be fully understood at the chemical level as the sum of thousands of simultaneous reactions inside the cell. This is only a stone's throw from the realm of philosophy: if all we are is the sum of our reactions then how can we be held responsible for our actions. Our existence was preordained from the moment of our conception and by extension from the moment of the creation of the universe. Pretty heady stuff. On a more immediate and practical level, the hope is that there might be some new principles guiding living processes that we do not observe *in vitro*.

The first attempt we made was to observe T4 tail assembly in vivo using a pulse-chase protocol. The concept is straightforward: if you pulse infected cells, chase them with cold amino acid, and then sample the culture repeatedly for the completed structures, in this case the finished tails, the first protein to show the label and then the chase will be the last one added before the structure entered the "finished pool". Conversely, the last protein to show the pulse-chase will be the one furthest back in the pathway, the initiator. The analogy of the automobile assembly plant is apt.

On the whole, the experiment worked. The first proteins to chase were the tail sheath and core, followed by the late baseplate proteins and then a mix of the hub and wedge species. However, there were several important deviations from the pathway of Kikuchi and King, chief among them the appearance of gp10, a wedge initiator, in the middle of the wedge pathway rather than at the beginning. This led us to do some modeling and we discovered that the quality (rapidity) of the chase can influence the timing of its appearance in structural proteins if the pools of soluble protein subunits were of different sizes. Looking back at our data, we also found that we had chased after much of the pulse had already been taken up by the cells and that this "self-chasing" as we call it could easily account for the gp10 result if gp10 had a small pool while the other wedge co-initiator, gp7, had a large one. The model shows that the gradual chase can cause an initial delay of 0.7 min in the chase time between a large and small pool speices and that this delay is amplified to 4 min by pools of assembly intermediates.

So where do we go from here? We can reduce or eliminate the influence of the size of a protein's pool size on chase time if we can make the chase more effective, so we will be trying various tricks to accomplish that. Then there is the T4 head pathway and the T7 pathway, both of which are to be completed soon. The steepest part of the learning curve is behind us and the subjects of interest are too numerous to contemplate. My retirement is not imminent.

First Name:Ian
Last Name:Molineux
Address:Molecular Genetics and Microbiology
University of Texas
Austin, Texas 78712-1095
Phone:(512) 471-3143
Fax:(512) 471-7088
E-mail:molineux@mail.utexas.edu

Abstract Title: Modes of T7 genome translocation from the virion and into the bacterial cell cytoplasm.

MOLINEUX, IAN J., CHANG, CHUNGYU, and KEMP, PRISCILLA. University of Texas, Austin, Texas, 78712-1095 USA

After binding to the lipopolysaccharide of the *E. coli* outer membrane, a signal is transduced from the cell surface through the tail fibers and at least one tail protein up into the head to initiate infection. Several proteins are then ejected from the phage particle into the cell, in part to make a channel for DNA transport. Three proteins, gp14, gp15, and gp16, that together form an internal head core structure, are localized primarily to membrane-associated fractions of the bacterium. The 18 copies of gp14 is thought to make a channel across the outer membrane, whereas the 12 copies of gp15 and 3 copies of gp16 traverse the peptidoglycan layer, the periplasm and the cytoplasmic membrane. These last two proteins are then imagined to constitute a proton-motive force-powered molecular motor that ratchets the phage genome into the cell at a constant rate of ~75 bp/sec at 30°C. After 850 bp have entered the cell, this motor stops, and the remainder of the genome normally enters the cell as a result of transcription, initially by *E. coli* RNA polymerase and then by the T7 enzyme. This presentation will provide an overview of the overall process of T7 genome entry, certain aspects will be given in more detail by Michael Moak and Bill Robins.

Title:Mr.
First Name:Michael
Last Name:Moak
Address:100 W.24th Street
ESB 502
Dept. of Molecular Genetics and Microbiology
University of Texas at Austin
Austin, Texas 78712
Phone:(512)471-3143
Fax:(512)471-7088
E-mail:moakm.e@mail.utexas.edu

Abstract Title:The lytic transglycosylase motif of the T7 internal head protein gp16 and its role in DNA ejection

Abstract:The predicted catalytic glutamate residue for transglycosylase activity of bacteriophage T7 gp16 is not essential for phage growth but is shown to be beneficial during infection of *Escherichia coli* cells grown to high cell density, conditions where murein is more highly cross-linked. In the absence of the putative transglycosylase, internalization of the phage genome is significantly delayed during infection. The lytic transglycosylase motif of gp16 is essential for phage growth at temperatures below 20°C, indicating that these growth conditions also lead to increased cross-linking of peptidoglycan. Overexpression of *slt*Y, *Escherichia coli* soluble lytic transglycosylase, partially complements the defect in infection of mutant phage particles, allowing them to infect at higher efficiencies. Conversely, an *slt*Y deletion increases the latent period of wild-type phage.

Title:Mr
First Name:William
Last Name:Robins
Address:421 W. San Antonio #J4
San Marcos, TX 78666
Phone:512-392-8117
E-mail:robinsbp@mail.utexas.edu

Abstract Title: The Internal Head Protein Gp16 Controls DNA Ejection from the Bacteriophage T7 Virion

Abstract:Robins, W., Schlinke-Struthers, J., Kemp, P. and Molineux, Ian. University of Texas; Department of Molecular Genetics and Microbiology, and Institute for Cell and Molecular Biology. University of Texas,Austin,Texas 78712-1095

A wild-type T7 virion ejects about 850 bp of the 40 kb genome into the bacterial cell by a transcription-independent process. Internalization of the remainder of the genome normally requires transcription. Inhibition of transcription-independent DNA translocation beyond the leading 850 bp is not absolute but the time taken by a population of phage genomes in overcoming the block averages about 20 min at 30sC. There are additional blocks to transcription-independent translocation and less than 20% of infecting DNA molecules completely penetrate the cell cytoplasm after four hours of infection. Mutant virions containing an altered gene 16 protein either prevent the blocks to transcription-independent DNA translocation or effect rapid release from blocking sites and allow the entire phage DNA molecule into the cell at a constant rate of about 75 bp per sec. This rate is likely the same at which the leading 850 bp is ejected into the cell from a wild-type virion. All mutations fall into two clusters contained within 380 bp of the 4 kb gene 16, suggesting that a 127 residue segment of gp16 controls DNA ejection from the phage particle. We suggest that this segment acts as a clamp to prevent transcription-independent DNA translocation.

First Name: Yuefei
Last Name: Lou
Address:Toronto Western Hospital
399 Bathurst Street
Toronto, ON
M5T 2S8
Phone:416-603-5420
E-mail:ylou@playfair.utoronto.ca

Abstract Title: The role of baseplate protein gp29 in bacteriophage T4 tail assembly

Abstract:Yuefei Lou AND Coombs D. University of New Brunswick; Dept. of Biology, Fredericton, N B, Canada.

Previous studies of bacteriophage T4 tail assembly suggested that the gene product 29 (gp29) could be an initiator of central hub assembly and a template for tail length. However, how gp29 interacts with other tail proteins and plays a role in tail assembly is not clear. Using anti-gp29 serum, its assembly was investigated by isolating various tail substructures and doing Western blots to see if they contained gp29. It was immediately apparent that there were two forms of gp29; full length gp29 and a cleaved form of gp29, about 5 kDa smaller. In complete tails and phage, only the full length form was observed, indicating that proteolysis was occurring during the analysis. Using various lysis methods, it was found that gp29 remained intact until after cell lysis, when it was cleaved to the smaller version by cell proteases unless it was protected by the tail. This artifact was used as a probe of gp29 structure during assembly. Further study showed that gp26 and 28 were required for gp29 folding, and that it remained sensitive to proteolysis until the tail tube, gp19, formed on the baseplate. Anti-gp29 antiserum failed to immunoprecipitate the hub structure, but was able to precipitate complete baseplates, indicating that the hub, as a structural intermediate, may not exist. Expression of recombinant N- and C-terminal His-tagged gp29 in E. coli revealed that the preferential cleavage sites were located at the N-terminus of gp29, suggesting that the N-terminus of gp29 protrudes from the baseplates and acts as the tail length ruler. Expressed N-terminal His-tagged gp29 complemented 29- amber mutants, so the tolerance of the His-tag by gp29 in the tail structure offers a promising avenue for further study of the function of gp29 in tail assembly.

Title:Mr.
First Name:MINORU
Last Name:INAGAKI
Address:Laboratory of Biofunctional Chemistry,
Department of Bioscience, Faculty of Bioresources,
Mie University,
1515 Kamihama, Tsu, Mie 514-8507, Japan
Phone:81-59-231-9618
Fax:81-59-231-9684
E-mail:inagaki@bio.mie-u.ac.jp

Abstract Title: Recognition of Receptor Lipopolysaccharides by Spike H Protein of Bacteriophage øX174

Abstract:°INAGAKI, M., WAKASHIMA, H., TANAKA, A., KAWAURA, T., SUZUKI, R., KARITA, S.,† NISHIKAWA, S., and KASHIMURA, N.

Bacteriophage øX174 is a small icosahedral virus with a singe-stranded closed circular DNA and four structural proteins, F, G, H, and J. At each of twelve vertices, the phage has spikes which consist of five G and one H proteins, and can infect some rough mutants of enterobacteria such as *Escherichia coli* C and *Salmonella typhimurium* Ra. In infection process, the phage recognizes lipopolysaccharides (LPSs) in the outer membrane of the host bacteria as a receptor molecule. Within the spike, the H protein consisting of 328 amino acids has been considered to recognize the receptor LPS from several lines of indirect evidence. However, there has been no report to date about the isolation of H protein and direct evidence for interaction with LPS. Thus, we prepared the spike H protein as a hexa histidine-tagged fusion (HisH) by using genetic engineering techniques, and examined whether HisH could, by itself, recognize the receptor LPS.

The DNA fragment encoding H protein was amplified by polymerase chain reaction (PCR) using Ampli Taq DNA polymerase and øX174 RF DNA as a template. The forward and reverse primers were designed to include *Bam*HI and *Hin*dIII restriction sites, respectively. The amplified fragment was inserted into the *Bam*HI/*Hin*dIII sites of pQE30 (Qiagen) to yield an expression vector, pQE-H. The expressed HisH in the cell lysate of *E. coli* M15 harboring pQE-H was fractionated by Ni-NTA agarose affinity chromatography. Further purification was accomplished by DEAE-Cellulofine anion-exchanged chromatography to obtained the purified HisH as a single band having an expected molecular weight of 37 kDa on sodium polyacryl amide-gel electrophoresis (SDS-PAGE).

The interaction of HisH with the LPSs of øX174-sensitive and -insensitive strains was examined by the enzyme-linked plate assay using streptavidin-peroxidase complex (STP-POD). HisH (0.5 µg/mL) in 100 µL of Tris-HCl buffer saline (pH 7.4) was absorbed on a polystyrene assay plate (Nunc, Immuno Plate II), and treated with various concentrations of the biotin-labeled LPSs. The biotin-label bound was conjugated with STP-POD, and detected as an orange color of the oxidized o-phenylendiamine dihydrochloride (OPD) at 490 nm. The LPSs of øX174-sensitive strains, *E. coli* C and *S. typhimurium* TV119 (Ra mutant), were shown to bind HisH strongly, whereas, the LPSs of insensitive strains, *E. coli* F583 (Rd2) and E. coli O111:B4 (smooth), bound very weakly to HisH. Since only the LPS with biotin-label was detected in the enzyme-linked assay, the LPSs without biotin-label competitively inhibited the binding of the biotin-labeled LPS of E. coli C. By comparing concentration for 50 % inhibition (I50%), the order of affinity of various LPSs for HisH was calculated. When the binding of the LPS of *E. coli* C was referenced as 100 %, the relative affinity of the LPSs were: *S. typhimurium* TV119 (Ra, 92 %), S. typhimurium SL684 (Rc, 5.7 %), *E. coli* J5 (RcP+, 3.3 %), *E. coli* F583 (Rd2, 1.9 %), and *E. coli* O111:B4 (smooth, 0.6%), respectively.

Fluorescence spectrum of HisH was changed dose-dependently in the presence of the LPS of *E. coli* C. The fluorescence intensity around 300-340 nm increased and an emission peak lmax shifted to the shorter wave length side, which was attributable to hydrophobic interaction with the LPS. The binding equilibrium was analyzed by fluorometric titration to determine the dissociation constant Kd, 7.02 ± 0.37 µM, and the Gibbs free energy change ?G0, -29.1 kJ mol-1 (at 22 °C, pH 7.4). Based on the temperature dependence of Kd in a van't Hoff plot, the standard enthalpy change H0 and the entropy change S0 were estimated to be +23.7 kJ mol-1 and 179 J mol-1 K-1 at 22 °C, respectively, and this binding thereby concluded to be an entropy-driving.

The Kinetic Role of Cysteine Residues on the *In Vivo* Folding and Function of Salmonella Phage P22 Tailspike

Cameron Haase-Pettingell, Scott Betts, , Steve Raso,, Anne Robinson* and Jonathan King Dept of Biology, MIT, Cambridge, MA 02139 and *Dept of Chem. Eng., University of Delaware, Newark, DE

Abstract

The predominantly beta sheet tailspike trimer of the salmonella phage P22 contains an interdigitated beta sheet region in which strands from each of the three subunits contribute to a local beta sheet. The 8 cysteines/666 residue chain are buried and unreactive in the native trimer. Though the native tailspike lacks disulfide bonds, transient interchain disulfide bonds are required for formation of the native trimer both in vivo{1} and in vitro{2} These bonds occur in the protrimer intermediate. Since they are absent from the native state, the transient disulfide bonds must play a kinetic role in chain folding and assembly.

To examine the role of the tailspike cysteines in the maturation pathway, each of the 8 cysteines was replaced with serine by site-speicifc mutagenesis. These mutant genes were expressed from pET vectors in E. coli. Seven of the mutant substitutions C267S, C287S, C290S, C458S, C496S, C613S and C635S when expressed at 37°C, were impaired in their folding or assembly. These chains did mature into their native state when expressed at 30°C as measured by the intracellular accumulation of SDS resistant trimers. C496S was particularly impaired, requiring the lower temperature of 17°C to reach its native state. C169S folded and assembled at all temperatures tested.

Native states of all 8 substitutions were purified. All 8 retained their thermostability measured at 65°C. When tested for their biological activity –conversion of phage capsids to infectious virus and absorption to the host cell - they all exhibited wildtype activity. Thus these cysteines thiols make only very limited contribution to the stability and activity of the native state. However at least 3 of them are important for the successful folding and assembly. Apparently through formation of a S-S disulfide bond in the protrimer.

Molecular Architecture of Bacteriophage T4 Capsid and its Development During Maturation

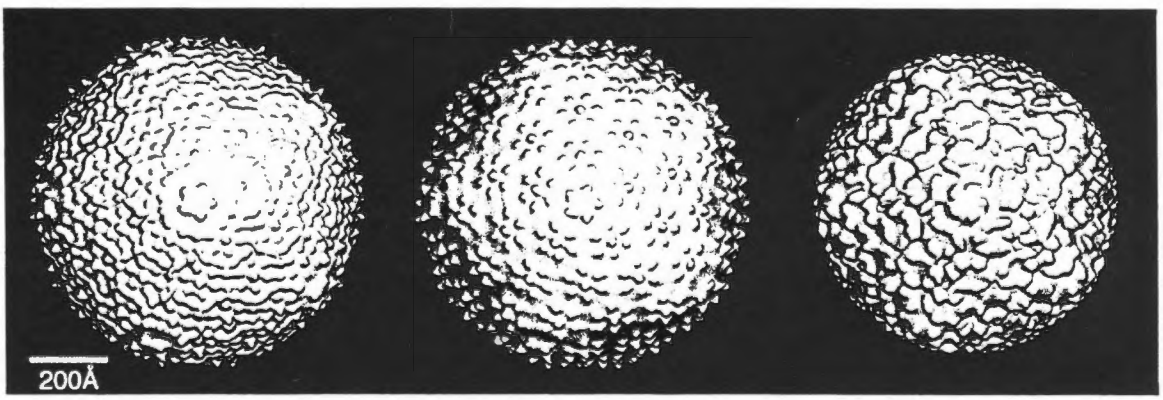
Kenji Iwasaki [1], Benes L. Trus [1,3], Paul T. Wingfield [2], Naiqian Cheng [1], Gregorina Campusano [1,4], Venigalla Rao [4],1 and Alasdair C. Steven [1]*

1Laboratory of Structural Biology and 2 Protein Expression Laboratory, National Institute of Arthritis, Musculoskeletal and Skin Diseases; 3 Computational Bioscience and Engineering Laboratory, Center for Information Technology, National Institutes of Health, Bethesda, MD 20892; 4 Department of Biology, The Catholic University of America, Washington, DC 20064.

T4 encodes two accessory proteins, Soc(9kDa) and Hoc(40kDa) that bind to the outer surface of the mature capsid. We have studied the three-dimensional structure of T4 capsid by reconstructing cryo-electron micrographs of isometric capsids produced by a point mutant in gene 23, for both Hoc+.Soc+ and Hoc-Soc- phages†. To prepare isometric and empty capsids, the mutant *pt*21-34c was crossed in a packaging-defective mutation in gene 17. To suppress accumulation of revertants, *i.e.*, prolate capsids, propagation was restricted to two cycles, starting from single petite plaques and produced ~81% isometric capsids. The isometric capsid showed T=13 *l* lattice. A protrusion at the center of each gp23* hexamer represents an averaged Hoc. Each vertex of icosahedral particle is composed of pentamer of gp24*. Although sequence homology between gp24* and gp23* is not high, the monomer protrusion of gp24* is similar to that of gp23* in size and shape, suggesting they may have the same fold. However, gp24* binds neither Hoc nor Soc. The additional binding of Soc to the capsid stabilizes the capsid against extremes of alkaline pH and temperature. In our three-dimensional reconstruction of Hoc+.Soc+, Soc is visualized as trimers at the trigonal points of the gp23* lattice and as monomers at the sites closest to the vertices. Soc is a folded protein that is monomeric in solution, as determined by analytic ultracentrifugation. Therefore, its trimerization on the capsid surface is imposed by a template of three symmetry-related binding sites. Comparison of the Soc+ and Soc- 3D reconstructions suggests that each Soc subunit interacts with two gp23* capsomers. Soc subunits bridge the intercapsomer interfaces, with possible reinforcement by secondary Soc-Soc intercations at the center of the trimer. We can also show three-dimensional reconstruction of the cleaved and unexpanded capsids (ESP) before maturation. Although unexpanded precursors of previously described phages (λ, P2/P4, HK97, P22, T7) are round, ESP has near-planar facets. ESP's hexons deviate from 6-fold symmetry, however, compared with HK97, this deviation is relatively small. It therefore appears likely that cleavage induces substantial conformational changes in the T4 surface lattice, prior to expansion.

† K. Iwasaki, B. L. Trus, P. T. Wingfield, N. Cheng, G. Campusano, V. Rao and A. C. Steven. (2000). Virology. *in press*.

Hoc+.Soc+ Hoc+.Soc- ESP



First Name:Lindsay
Last Name:Black
Address:Department of Biochemistry and Molecular Biology
University of Maryland School of Medicine
Baltimore, Md 21201-1505 USA
Fax: (410) 706-8297
Phone:410-706-3510
E-mail:lblack@umaryland.edu

Abstract Title:
A bipartite bacteriophage T4 SOC and HOC randomized peptide display library: detection and analysis of phage T4 terminase (gp17) and late sigma factor (gp55) interaction.

Abstract:

A bipartite phage peptide library of ~2 x 107 sequences was created by displaying on tetra-alanine linker peptides five randomized amino acids from the carboxyterminus of T4 phage SOC (~960 copies per capsid) and five randomized amino acids from the aminoterminus of T4 phage HOC (~160 copies per capsid). A single ten residue carboxy-terminal SOC library was also constructed. The bipartite library was tested by biopanning against the phage T4 terminase large subunit protein gp17. Among the selected phage a strong consensus sequence showed homology to gp55, the phage T4 late gene transcription RNAP sigma factor homolog. Immunoprecipitation and affinity column chromatography demonstrated direct binding of gp17 and gp55, suggesting regulatory interaction between late transcription and DNA packaging.

First Name:Lynn
Last Name:Thomason
Address:2168 Shattuck Avenue
Berkeley. CA 94704
Phone:510-647-0690 ext 127
E-mail:lynno@sequence.molsci.org

Abstract Title:Transgene integration in *Schizosaccharomyces pombe* mediated by the *Streptomyces bacteriophage* PhiC31 site-specific recombination system

Abstract:THOMASON, L.[1], CALENDAR, R.,[1], AND OW, D.[2*] [1]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720-3202. [2]Plant Gene Expression Center, U.S. Department of Agriculture, 800 Buchanan St., Albany, CA 94710.

The *Streptomyces* bacteriophage PhiC31 site-specific recombination system has been shown to function in the fission yeast *Schizosaccharomyces pombe*. To engineer a chromosome target, the bacteriophage attachment site attP was placed into chromosome II at the *S. pombe leu*1 locus. This target strain was subsequently transformed with a plasmid that contains the bacterial attachment site attB linked to a ura4+ selectable marker. When co-transformed with a second plasmid harboring the PhiC31 integrase gene, high efficiency transformation to Ura+ was observed under conditions where the integrase gene was expressed. Southern analysis of the integration events shows insertion of the attB-ura4+ plasmid into the attP site of the leu1 locus. Nucleotide sequence of the hybrid junctions revealed that the attB x attP recombination reaction is precise. As the PhiC31 integrase alone does not catalyze a freely reversible reaction, the PhiC31 attB x attP recombination is stable. This property sets it apart from site-specific recombination systems currently in use for eucaryotic cells, such as the Cre-lox or FLP-FRT system, where the recombination reactions can readily reverse. Deployment of the PhiC31 recombination system in eucaryotic cells will provide new opportunities for directing stable transgene and chromosome rearrangements.

Title:Dr.
First Name:Sidney
Last Name:Hayes
Address:Department of Microbiology and Immunology
College of Medicine
University of Saskatchewan
Saskatoon, SK S7N 5E5
Phone:306 9664307
Fax:306 9663411
E-mail:hayess@duke.usask.ca

Abstract Title: Requirement of phage lambda recombination functions for phage-prophage marker rescue.

Abstract:HAYES, S., BOOTH, J. AND ASAI, K. University of Saskatchewan; Dept. of Microbiology and Immunology, College of Medicine, Saskatoon, SK. S7N 5E5 Canada.

We have examined recombination that occurs between a cryptic lambda prophage fragment integrated within the *E. coli* chromosome and infecting heteroimmune lambda phages. Recombinant infecting phage, acquire by marker rescue (i.e., a double recombination event), the nonhomologous immunity region from the prophage. The region of nonhomology to be rescued from the prophage was 2662 base pairs and is straddled by regions of 2200 base pairs (or more) of DNA sharing homology with the infecting phage. Phage lambda encodes recombination functions clustered within dispensable regions of the phage genome. One such region, the int-kil interval, termed here *Rec*L, includes the Red-Gam functions. The other region, called *Nin*R, includes ten open reading frames between genes P-Q on the lambda map, and includes known genes ren, orf (orf:*nin*B) and rap. We found that in a *Rec*A+ host, marker rescue occurred efficiently without *Rec*L or *Nin*R, but not both. We name the phage-encoded ability to capture genes from the chromosome the kleptomania (KM) phenotype.
We found that functions encoded by either the *Rec*L or *Nin*R regions can bring about phage-prophage recombination in the absence of RecA activity. Among the required genes within *Nin*R, the orf:*nin*B function can be substituted by an activity of *Rec*A encoded by the host. However, the rap:*nin*G function is an essential component of the marker rescue activity provided by *Nin*R. The difference in marker rescue for infections with lambda-bio275imm434-deletion:d2 [*Rec*L and orf:*nin*B both deleted] from *Rec*A+ and RecA- hosts was 4750-fold. However, for infections with lambda-bio275imm434 [i.e. *Nin*R+ phage expressing orf:*nin*B] the effect of the *Rec*A- mutation on marker rescue was completely suppressed, suggesting that orf:*nin*B must play a key role in marker rescue. We have also examined the requirement of cryptic prophage induction/replication, and requirements of host recombination functions *Rec*F, *Rec*J and *Rec*Q for phage-prophage marker rescue. Marker rescue was enhanced about 25-fold when the cryptic prophage was induced to initiate replication (assays at 39C), but was also seen for infections conducted at 30C. Our data suggest that NinR may encode a product with an activity like *Rec*J; in the absence of *Nin*R, *Rec*Q is essential for marker rescue at both 30 and 39C; and *rec*F mutations inhibit marker rescue at 30C, but not at 39C where the prophage is induced to replicate.

First Name:Timothy
Last Name:Dean
Address:NC State University
Dept. of Microbiology
Box 7615
Raleigh NC 27695
Phone:919-515-7230
Fax:919-515-7867
E-mail:trdean@unity.ncsu.edu

Abstract Title:In vitro selection of RNA aptamers that bind intact bacteriophage

Timothy Dean and Eric S. Miller. Department of Microbiology, North Carolina State University, Raleigh, NC USA

SELEX (Systematic Evolution of Ligands by Exponential Enrichment) is a combinatorial chemistry technique used to isolate individual nucleic acid sequences (RNA or DNA) that have novel binding or catalytic properties (Tuerk and Gold, 1990; Ellington and Szostak, 1990; Jayasena, 1999). In one application, RNA aptamers can potentially bind and detect specific viruses and phages present in complex environments, without extensive enrichment or isolation. Starting with approximately 1013 different molecules an RNA pool randomized at 40 positions (N40) was used to select aptamers that bind intact, viable R17 phage. Concurrent with selection for R17-binding aptamers, we sought to obtain RNAs that simultaneously failed to bind the closely related phage Qβ. The coat protein sequences of these phages are 41% similar and the capsid structures are nearly superimposable, providing stringent criteria for selecting RNA aptamers with high binding specificity. Selection cycles incorporating increased stringency for R17 binding, subtraction of RNAs that bind nitrocellulose filters, and removal of Qb-binding species, were used to drive aptamer specificity. Results of SELEX to obtain the phage-specific RNA aptamers will be presented.

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Ellington AD, Szostak JW. 1990. Nature 346(6287):818-22.
Jayasena SD. 1999. Clin Chem. 45(9):1628-50.

Title:Ms
First Name:Irina
Last Name:Kovalyova
Address:Department of Microbiology and Immunology
Queen's University
Kingston, Ontario
K7L 3N6
Canada
Phone:613-533-2450
Fax:613-533-6796
E-mail:8ivk@qlink.queensu.ca

Abstract Title: Partial sequence of *Pseudomonas putida* phage gh-1 using shotgun sequencing strategy, evidence for relationship to the T7 group.

Abstract: Irina V. Kovalyova and Andrew Kropinski, Department of Microbiology and Immunology, Queen's University, Kingston, Ontario K7L 3N6, Canada

Virulent bacteriophage gh-1 for the common soil bacterium *Pseudomonas putida* is presumably related to the well-studied *E. coli* bacteriophage T7 that is of significant biotechnological importance. A shotgun strategy was used to clone random fragments of the 40 kb double stranded DNA of gh-1, sequence, and subsequently identify genes highly homologous to other members of the T7 group (T7, T3, phi-YeO3-12, K11, SP6, Kvp1) using BLASTP at the NCBI site. To date approximately 10 kb of gh-1 DNA has been obtained. Sequencing of random cloned gh-1 segments revealed significant homology with a number of the T7 group proteins (RNA polymerase, ssDNA-binding protein, endonuclease, lysozyme, DNA ligase, tail tubular protein, tail fiber protein, major and minor capsid proteins, and internal virion protein). The gh-1 RNA polymerase gene sequence was completed using primer walking and presents the evidence of a close evolutionary relationship to RNA polymerases of the T7 group. The sequencing and functional analysis of gh-1 could allow for characterization and isolation of novel proteins potentially leading to design of unique vector elements for enhanced genetic manipulation in *Pseudomonas*. Gh-1 complete genome analysis (statistical and dynamic correlations in DNA sequences) could contribute to better understanding of evolution of virulent bacterial viruses and their phylogeny.

Title:Dr.
First Name:David
Last Name:Schofield
Address:Department of Microbiology & Immunology
PO Box 250504
Medical University of South Carolina
171 Ashley Avenue
Charleston, South Carolina 29425
Phone:(843) 792-8499
Fax:(843) 792-2464
E-mail:schofida@musc.edu

Abstract Title:Development of Therapeutics for the Treatment of Bacterial Infections Using LADS

Abstract: To combat the increasing number of antibiotic resistant infections, we are developing a number of gene therapeutics capable of treating bacterial diseases. The genetic information encoding these therapeutics will be delivered via a lethal agent delivery system (LADS, see Westwater *et al.*). The toxic agents will take the form of proteins that when expressed in the bacterial host, will cause the organism to die. We have taken advantage of naturally occurring phage, plasmid and chromosomally encoded toxic proteins and have demonstrated their effectiveness as antimicrobial therapeutic agents in clinically relevant Gram-negative and Gram-positive bacteria. Using LADS the effectiveness of the toxic proteins as antimicrobial agents has been demonstrated in an embryonated hen egg model (see Kasman *et al.*). Expression of the lethal agent is under the control of regulated- or species-specific promoters. Anr and *pro*C promoters, which are expressed preferentially in *P. aeruginosa*, have been isolated and shown to target specificity of the lethal agent to this pathogenic bacterium. The availability of species-specific promoters will help ensure that indigenous commensal bacteria are protected from the toxic actions of the therapeutic.

Funded by Hexal Pharmaceutical Inc.

Title:Dr.
First Name:Laura
Last Name:Kasman
Address:Department of Microbiology & Immunology
PO Box 250504
Medical University of South Carolina
171 Ashley Avenue
Charleston, South Carolina 29425
Phone:(843) 792-8499
Fax:(843) 792-2464
E-mail:kasmanl@musc.edu

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Funded by Hexal Pharmaceutical Inc.

Title:Doctor
First Name:Laura
Last Name:Kasman
Address:Department of Microbiology & Immunology
Medical University of South Carolina
PO Box 250504
171 Ashley Avenue
Charelston, SC 29425
Phone:(843) 792-8499
Fax:(843) 792-2464
E-mail:kasmanl@musc.edu

Abstract Title:
KASMAN, L., WERNER, P. A., WESTWATER, C., SCHOFIELD, D., HOEL, B., DOLAN, J., SCHMIDT, M. and NORRIS, J.S. LADSTM: Treatment of Experimental Infections in Embryonated Hen Eggs and Mice.

Abstract: A P1 phage-derived lethal agent delivery system (LADS) has been developed as a new strategy for the treatment of gram negative antibiotic-resistant bacterial infections (See Schofield *et al.* and Westwater, *et al.*). In order to test the efficacy of LADS in vivo prior to scaling up production of the modified phage, models of infection were needed which required low doses of bacteria to establish disease. Chicken embryos, which have a well developed circulatory system but immature immune system provided an ideal model for such studies. Human clinical isolates of *E. coli* and *P. aeruginosa* are described which produced lethal infection in chicken embryos at doses <100 cfu/ egg when introduced onto the chorioallantoic membrane at 10 days gestation. Treatment with LADS carrying the bacteriocidal gene doc increased survival of *E. coli* infected chicken embryos whereas LADS carrying a control plasmid did not. Results with *P. aeruginosa* infections were similar, but less consistent. Plasmid carrying a marker gene delivered by LADS was shown to be transferred in vivo to both *E. coli* and *P. aeruginosa*, in eggs.
In related studies, we have used mice to study the immune response of healthy animals to injection with phage. Purified P1 phage, introduced into normal mice by intraperitoneal injection, produced a robust antibody response in less than 18 days. Circulating antibodies were capable of neutralizing phage titers in vitro, and resulted in rapid clearance of phage from the circulation *in vivo*. A long-circulating variant of P1, selected by the method of Merrill *et al.*, was similarly affected, even when immunization was with the parent unselected phage. Results in immunocompromised mice, which may better represent phage therapy target populations, are also reported. Supported by a research contract with Hexal Pharmaceuticals, Inc.

Title:Dr.
First Name:Andrew M.
Last Name:Kropinski
Address:Department of Microbiology & Immunology
Queen's University
Kingston
Ontario K7L 3N6
Canada
Phone:(613)533-2459
Fax:(613)533-6796
E-mail:kropinsk@post.queensu.ca

Abstract Title:The completed sequence of the genome of Salmonella typhimurium phage P22.

Salmonella bacteriophage P22 possesses a linear double-stranded DNA genome, which is terminally repetitious and circularly permuted. It has been considered to be phylogenetically related to coliphage lambda and indeed will recombine with the latter yet possesses unique features including a regulatory region (*imm*I) not found in lambda, headfull packaging, LPS receptor specificity and it belongs to the viralfamily Podoviridae. This phage has been used extensively in chromosomal mapping by transduction.

GenBank contained 24 phage sequences, which collapsed into four contigs of 0.6 to 24.6kb with 17 discrepancies. Using primer walking we have corrected the mistakes and completed the genomic sequence. To get around the circular permutation and the terminally redundancy the map, which we will present, has been opened adjacent to a rho-independent terminator downstream of the tail spike protein gene (gp9). The unique genome is 41.7-kb and we have located the positions of over 60 genes. Among the new genes discovered are those involved in serotype conversion. These have been cloned into *S. typhimurium* giving rise to a strain which displays antigen O1.

Title: Dr.
LastName: Gill
FirstName: J.J.
Address: Dept. of Biological Sciences, Brock University
St. Catharines, Ontario

Abstract:GILL, J. J. (1) (Presenting author), SVIRCEV, A. M. (2), SMITH, R. (3), MYERS, A. L. (2), CASTLE, A. J. (1) Biological Control of Erwinia amylovora with Bacteriophages. 1: Department of Biological Sciences, Brock University, St. Catharines, ON; 2: Agriculture and Agri-Food Canada SCPFRC, PO Box 6000, 4902 Victoria Ave. N., Vineland Station, ON, L0R 2E0; Department of Plant Science, University of Western Ontario, London, ON.

Forty-four bacteriophage isolates of *Erwinia amylovora*, the causal agent of fire blight, were collected from sites in and around the Niagara Region of Southern Ontario in the summer of 1998. Phages were isolated only from sites where fire blight was present. Thirty-seven of these phages were isolated from the soil surrounding infected trees, with the remainder isolated from aerial tissue samples. A mixture of six *E. amylovora* host strains was used to enrich field samples in order to avoid the selection bias of a single-host system. Molecular characterization of the phages with a combination of PCR and restriction endonuclease digestions showed that six distinct phage types were isolated. Ten phage isolates related to the previously characterized *E. amylovora* phage PEa1 were isolated, with some divergence of molecular markers between phages isolated from different sites. The host ranges of the phages revealed that certain types were unable to efficiently lyse some *E. amylovora* strains, and that some types were able to lyse the epiphytic bacterium *Pantoea agglomerans*. Biological control of *E. amylovora* by the bacteriophages was assessed in a bioassay using discs of immature pear fruit. Twenty-three phage isolates were able to significantly suppress the incidence of bacterial exudate on the pear disc surface. Quantification of the bacterial population remaining on the disc surface indicated that population reductions of up to 97% were obtainable by phage treatment.

Title:Dr.
First Name:David
Last Name:Stopar
Address:University of Ljubljana
Biotechnical Faculty
Department of Food Technology
Vecna pot 111
1000 Ljubljana
Slovenia
Phone:+386 61 123 33 88
Fax:+386 61 273 390
E-mail:david.stopar@uni-lj.si

Abstract Title:FREQUENCY OF LYSOGENY AND POLYLYSOGENY IN BACTERIA ISOLATED FROM NORTHERN ADRIATIC SEA

Abstract:CERNE, A. AND STOPAR, D. University of Ljubljana, Biotechnical Faculty, Department of Food Technology, Vecna pot 111, 1000 Ljubljana, Slovenia.

It has been suggested in the literature that temperate bacteriophages may be the most numerous phages in aquatic environments and that 21 to 60 % of member species are lysogenic. In this study 28 fast growing bacteria, isolated from different environments of Northern Adriatic Sea (i.e. coastal waters, estuarine waters and saltpan waters) were examined for lysogeny by treatment with Mitomycin-C. Induction was followed by decrease in culture optical density, bacterial viable count, as well as bacterial and viral direct count with SYBR Green epiflourescence microscopy. All lysates were examined for phage particles by electron microscopy. After prophage induction 71 % of all bacterial isolates yielded phage particles of varying morphology. Viruses with capsid diameter of 30-60 nm were the most abundant virus fraction. 51 % of all phages were tailed. Number of lysogenic bacterial isolates was significantly higher in coastal waters as compared with estuarine waters and saltpan waters. A significant proportion of bacterial isolates were polylysogenic (46 %). Among polylysogenic isolates 62 % had two and 38 % had three or more morphologically different phages. Except in one case, the dominant phage in polylysogenic isolates was always the smallest one. Our results suggest that temperate phages are important component of virioplankton in the Northern Adriatic Sea.

Title:MR
First Name:Richard
Last Name:Weld
Address:Canterbury University,
Private Bag 4800,
Christchurch,
New Zealand.
Phone:03 364 2987 ext7645
Fax:03 364 2999
E-mail:r.weld@botn.canterbury.ac.nz

Abstract Title: Antibiotic-Induced "Pseudolysogeny": Implications for Antibiotic/Phage Combination Therapy.

WELD, R.,COOPER, T., ADAMS, B. AND HEINEMANN, J.A.. University of Canterbury; Dept. of Plant and Microbial Sciences, Private Bag 4800, Christchurch, New Zealand.

We have demonstrated that the bacteriophages lambda and T7 can infect bacterial cells treated with bacteriostatic antibiotics that inhibit gene expression. This effect of antibiotics may be similar to the effect of starvation in inducing the pseudolysogenic state, where lytic bacteriophages are de facto "prophages". As has been proposed to occur during pseudolysogeny, the phage DNA that enters antibiotic-inhibited cells appears to remain dormant until the host cell is released from stasis. On dilution of the antibiotic, phage genes are expressed and the phage is fully revived. Pseudolysogeny may be an important factor in bacteriophage ecology, increasing the survivability of bacteriophages especially in harsh environments. We have proposed that antibiotic-induced pseudolysogeny might increase the effective half-life of bacteriophages used in combination with antibiotics to treat infectious diseases. Here we examine further the interaction between phage and bacteria during antibiotic treatment. We compare this interaction with pseudolysogeny and consider its implications for bacteriophage/ antibiotic combination therapy.

Title:Mr.
First Name:Lingchong
Last Name:You
Address:Department of Chemical Engineering
University of Wisconsin-Madison
1415 Engineering Drive,
Madison, WI, 53705
Phone:(608)-262-8450
E-mail:you@cae.wisc.edu

Abstract Title:Effects of host physiology on the intracellular growth of phage T7

YOU, L., SUTHERS, P. and YIN, J.* Department of Chemical Engineering, University of Wisconsin-Madison, 1415 Engineering Drive, Madison, WI 53706-1691, USA

The one-step growth behavior of a bacteriophage depends not only on the mechanisms and rates of its genome-encoded functions, but also on the levels of its intracellular resources. To quantitatively explore this idea, we expanded a mechanistic model of the phage T7 intracellular growth cycle to probe in silico how phage-host interactions affect T7 development. The simulation suggests that the rate of T7 progeny production is limited by the synthesis of phage proteins. This production rate is very sensitive to the physiological state of the *E. coli* host, in particular, to changes in the number and the elongation rate of its ribosomes, which characterize the translation capacity and efficiency of the host cell. In contrast, phage production is insensitive to changes in the number and the elongation rate of the *E. coli* RNA polymerases, which characterize the transcription capacity and efficiency of the host cell. Further, the energetic costs of translation are found to be significantly higher than for transcription. This work shows how a computer simulation can quantitatively account for the interactions defined by a genome-encoded process, while serving as a foundation to probe system-level behavior.

Title:Mr.
First Name:Hojabr
Last Name:Dezfulian
Address:Université de Montréal; Fac. de Médecine Vétérinaire, Dept. Pathologie & Microbiologie (GREMIP), C. P. 5000, Saint-Hyacinthe, Québec J2S 7C6, Canada
Phone:450-773-8521 ext.8377
Fax:450-773-8108
E-mail:dezfulih@magellan.umontreal.ca

Abstract Title:Testing the Mobility of Fimbrial Operons by Phage Induction Experiments

Abstract:DEZFULIAN, H., NASSAR, A., AND HAREL, J. Université de Montréal; Fac. de Médecine Vétérinaire, Dept. Pathologie & Microbiologie (GREMIP), C. P. 5000, Saint-Hyacinthe, Québec J2S 7C6, Canada

The virulence properties of many pathogenic bacteria are due to proteins encoded by large gene clusters called pathogenicity islands, which are found in a variety of human, animal and plant pathogens including *Escherichia coli*, *Salmonella*, *Shigella*, *s*, *Helicobacter pylori*, *Vibrio cholerae*, *Dichelobacter nodosus* and *Pseudomonas syringae*. Although the presence of pathogenicity islands is a prerequisite for many bacterial diseases, little is known about their origins or mechanism of transfer into the bacterium. Vibrio cholerae, contains a bacteriophage known as cholera-toxin phage (CTXphi), which encodes the cholera toxin, and a large pathogenicity island called the VPI (for V. cholerae pathogenicity island) which itself encodes a toxin-coregulated pilus that functions as a colonization factor and as a CTXphi receptor. *Staphylococcus aureus* pathogenicity island (SaPI1) which encodes tst, the gene for toxic shock syndrome toxin-1 (TSST-1) also shown to be mobile by bacteriophage. Moreover phage-like sequences are common in PAIs. The presence of PAI(s) was studied in 18 strains of septicemic *E. coli* which originally isolated from calves or piglets, and express F165 fimbriae. These strains contain fimbrial operons that are virulence factors of *E. coli* strains causing extraintestinal diseases such as urinary tract infections, meningitis, and septicemia. All these strains harbours an operon associated to pap operon (pyelonephritis-associated pili). In particular strain 4787 contains foo, a pap-like operon, and fot, an operon related to septicemia fimbrial adhesin/sfa.
To determine the mobility and/or stability of these two operons and the possible role of bacteriophage(s) in transferring these virulence genes, different concentrations of mitomycin C were used. The lysates were examined by electron microscopy for induction of possible prophage(s) in strain 4787. Very few phages, probably from the Myoviridae family were observed, only after induction with 10ml/ml of mitomycin C. Current experiments will determine if virulence genes are present in these particles.

Title:Mr.
First Name:Lingchong
Last Name:You
Address:Department of Chemical Engineering
University of Wisconsin-Madison
1415 Engineering Drive,
Madison, WI, 53705
Phone:(608)-262-8450
E-mail:you@cae.wisc.edu

Abstract Title:Effects of host physiology on the intracellular growth of phage T7

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The one-step growth behavior of a bacteriophage depends not only on the mechanisms and rates of its genome-encoded functions, but also on the levels of its intracellular resources. To quantitatively explore this idea, we expanded a mechanistic model of the phage T7 intracellular growth cycle to probe in silico how phage-host interactions affect T7 development. The simulation suggests that the rate of T7 progeny production is limited by the synthesis of phage proteins. This production rate is very sensitive to the physiological state of the *E. coli* host, in particular, to changes in the number and the elongation rate of its ribosomes, which characterize the translation capacity and efficiency of the host cell. In contrast, phage production is insensitive to changes in the number and the elongation rate of the *E. coli* RNA polymerases, which characterize the transcription capacity and efficiency of the host cell. Further, the energetic costs of translation are found to be significantly higher than for transcription. This work shows how a computer simulation can quantitatively account for the interactions defined by a genome-encoded process, while serving as a foundation to probe system-level behavior.

Title:Mr.
First Name:Hojabr
Last Name:Dezfulian
Address:Université de Montréal; Fac. de Médecine Vétérinaire, Dept. Pathologie & Microbiologie (GREMIP), C. P. 5000, Saint-Hyacinthe, Québec J2S 7C6, Canada
Phone:450-773-8521 ext.8377
Fax:450-773-8108
E-mail:dezfulih@magellan.umontreal.ca

Abstract Title:Testing the Mobility of Fimbrial Operons by Phage Induction Experiments

Abstract:DEZFULIAN, H., NASSAR, A., AND HAREL, J. Université de Montréal; Fac. de Médecine Vétérinaire, Dept. Pathologie & Microbiologie (GREMIP), C. P. 5000, Saint-Hyacinthe, Québec J2S 7C6, Canada

The virulence properties of many pathogenic bacteria are due to proteins encoded by large gene clusters called pathogenicity islands, which are found in a variety of human, animal and plant pathogens including *Escherichia coli*, *Salmonella*, *Shigella*, *s*, *Helicobacter pylori*, *Vibrio cholerae*, *Dichelobacter nodosus* and *Pseudomonas syringae*. Although the presence of pathogenicity islands is a prerequisite for many bacterial diseases, little is known about their origins or mechanism of transfer into the bacterium. Vibrio cholerae, contains a bacteriophage known as cholera-toxin phage (CTXphi), which encodes the cholera toxin, and a large pathogenicity island called the VPI (for V. cholerae pathogenicity island) which itself encodes a toxin-coregulated pilus that functions as a colonization factor and as a CTXphi receptor. *Staphylococcus aureus* pathogenicity island (SaPI1) which encodes tst, the gene for toxic shock syndrome toxin-1 (TSST-1) also shown to be mobile by bacteriophage. Moreover phage-like sequences are common in PAIs. The presence of PAI(s) was studied in 18 strains of septicemic *E. coli* which originally isolated from calves or piglets, and express F165 fimbriae. These strains contain fimbrial operons that are virulence factors of *E. coli* strains causing extraintestinal diseases such as urinary tract infections, meningitis, and septicemia. All these strains harbours an operon associated to pap operon (pyelonephritis-associated pili). In particular strain 4787 contains foo, a pap-like operon, and fot, an operon related to septicemia fimbrial adhesin/sfa.
To determine the mobility and/or stability of these two operons and the possible role of bacteriophage(s) in transferring these virulence genes, different concentrations of mitomycin C were used. The lysates were examined by electron microscopy for induction of possible prophage(s) in strain 4787. Very few phages, probably from the Myoviridae family were observed, only after induction with 10ml/ml of mitomycin C. Current experiments will determine if virulence genes are present in these particles.

Title:Dr., Prof.
First Name:Inga
Last Name:Giorgadze
Address:3,Gotua st.
Tbilisi, 380060
Georgia.
Phone:+99532 379405
E-mail:liana@select.acnet.ge

Abstract Title:ELABORATION OF THE NEW MEDICINAL-PROPHYLACTIC BACTERIOPHAGES AGAINST PURULENT INFECTIONS

Abstract:Timely diagnostic of infectious disease, determining of pathogenic agent, and assessment of individual sensitivity towards the preparations of different groups, provides physician with the means for efficient and purposeful treatment execution. We have studied and analyzed bacterial flora of the pus from mouth cavity, gums, pharynx, nose, eyes, ears, and of urine, in the patients of various age groups. Out of 1587 patients *Staphylococcus* only was revealed in 19.5% of cases, *Staphylococcus* plus one additional microbe - in 74%, *Staphylococcus* plus two other microbes - in 6%, and, finally, *Staphylococcus* plus three other microbes - in 0.5%. In 11-12% of the cases the fungal associations were found as well. Therefore, it could be noted that association of Staphylococcus with one other microbe (80% - *Streptococcus*, 16% - *Escherichia coli*, 4% - other) significantly exceeds the number of other variants. Considering the results obtained we have elaborated the new bacteriophages - Fersisi, in the following combination (Staphylococcus and Streptococcus), and SES (*Staphylococcus*, *Streptococcus*, and *E. coli*). Fersisi is a stable sterile filtrate of the *Staphylococci* (*S. aureus*, *S. epidermidis*, *S. saprophiticus*) and the *Streptococci* (*S.pyogenes*, *S.sanguis*, *S. salivarius*, *S. agulactiae*) bacteria phagolysate. SES is a sterile filtrate of the *Staphylococcal* (*S. aureus*, *S. epidermidis*, *S. saprophyticum*), the *Streptococcal* (*S. pyogenes, S. sanguis, S. salivarius*), and *E. coli* serovars' (011, 035, 026, 0125, 0113, 0128, 018, 044, 025, 020) phagolysates. Specific activity of the liquid preparation is no less than 10-4-106 (according to Appelman). Insofar the sensitivity to the bacteriophages in the contingent investigated made 50-60%, it seems much advisable to use the phages in those compositions, which have been revealed individually.

First Name:Gail
Last Name:Christie
Address:Department of Microbiology and Immunology
Virginia Commonwealth University
PO Box 980678
Richmond, VA 23298-0678
USA
Phone:804 828-9093
Fax:804 828-9946
E-mail:christie@hsc.vcu.edu

Abstract Title: Lysogenic conversion by P2-related phages: a novel methylase encoded by phage Wphi

Abstract: LOOM, F., ESPOSITO, D., SCHMIDT, B., and CHRISTIE, G. Life Technologies, Molecular Biology Research and Development, 9800 Medical Center Drive, Rockville MD 20849-6482, USA and Virginia Commonwealth University, Department of Microbiology and Immunology, Box 980678, Richmond, VA 23298-0678, USA.

DNA isolated from *Escherichia* coli strain W is resistant to cleavage by several restriction enzymes. A fragment of DNA encoding this resistance function was isolated from an E. coli W cosmid library. Subcloning and sequence analysis identified a gene encoding a polypeptide related to DNA methylases from the restriction-modification systems of a variety of bacterial species. An analysisof the restriction enzymes blocked by this activity defined the recognition specificity of this modification enzyme as (C/T)GGCC(G/A). Further sequence analysis revealed that the methylase gene and several flanking unidentified open reading frames were adjacent to sequence with striking homology to temperate bacteriophage P2. No associated restriction function was found. The A+T content of this block of DNA was markedly higher than that of the DNA homologous to P2, suggesting that this region of DNA was acquired by horizontal transfer.

Spontaneously released phage were isolated from *E. coli* W and used to create lysogens of *E. coli* C and K-12 strains. These lysogens expressed the methylase activity, as judged by resistance of DNA to restriction by NotI, and were immune to infection by the previously identified P2-related phage from *E. coli* W, Wphi. The identity of this phage to Wphi was confirmed by several criteria: (1) the att site of this phage in *E. coli* W was the same as that reported for Wphi; (2) an *E. coli* C strain lysogenic for Wphi also expresses the methylase activity, and (3) sequence from the C-cox region of this phage was identical to that reported for Wphi.

Bacteriophage P2 carries two lysogenic conversion genes, old and tin, which interfere with the growth of lambda and T-even phages, respectively. Early studies of the exclusion of lambda and T-even phages by *E. coli* W led to the conclusion that the Wphi prophage was responsible for this phenotype. We expected, therefore, to find homologues of the *old* and *tin* genes in Wphi. The region of P2 containing these lysogenic conversion genes is replaced in Wphi by the block of DNA carrying the methylase function, and we have been unable to identify homologues of old and tin in this part of Wphi by DNA sequence analysis or in the remainder of the genome by PCR. Furthermore, we have been unable to duplicate the reported exclusion of lambda in Wphi lysogens.

First Name: Sutton
Last Name: Mooney
Address:UNIVERSITY OF TEXAS AT AUSTIN
AUSTIN, TEXAS 78712-1095
Phone:512-471-3143
Fax:512-471-7088
E-mail:smooney@mail.utexas.edu

Abstract Title:INJECTION OF LAMBDA DNA INTO *E. COLI*

Sutton L. Mooney, Lynn O. Thomason,and Ian J. Molineux
Molecular Genetics and Microbiology, University of Texas, Austin, Texas 78712-1095

Despite all of the information that has been collected on bacteriophage lambda, one aspect of its lifecycle that still remains unresolved is how the 48.5 kb genome passes from the phage head into the cell. It is known that lambda gpJ, the tail tip, interacts with *E. coli* LamB, the porin for maltose and maltodextrin uptake. Study of pel (penetration of lambda) mutants also suggest a role for components of the mannose phosphotransferase system; however, more recent work indicates that pel only prevents ejection of lambda deletion mutants, lambda wildtype plates normally on pel mutants. Lambda mutants that grow on *pel* strains are altered either in gpV, the tail tube protein, or gpH, the tail tape-measure. The latter has also been implicated in DNA ejection. Upon injection of DNA into liposomes containing the LamB receptor, gpH becomes insensitive to proteinase and is not associated with the lambda particle. Mutants of lambda, selected for their ability to grow at low temperature, and containing mutation(s) in a tail gene(s), also grow on pel mutants. As a way to analyze lambda DNA entry into the cell, we are using a transvestite phage in which T7 DNA is packaged into the lambda virion. This allows us to use the well developed T7 entry assay to measure methylation of a few DpnI sites and avoid not only complexities associated with 116 DpnI fragments of a lambda genome but also cross-hybridization to the bacterial chromosome. After adsorption at 0°C and a temperature-shift to initiate DNA ejection the lambda genome appears to be internalized extremely rapidly, within one to two minutes. Unlike the rate of T7 genome entry, that of lambda does not seem to be affected by temperature. We are investigating ways to slow this rate of lambda genome entry in order to examine its kinetics and mechanism of injection.

Title:DR.
First Name: Eleanor
Last Name: Spicer
Address:Department of Biochemistry and Molecular Biology
Medical University of South Carolina
173 Ashley Avenue
Charleston, SC 29425
Phone:843-792-1417
Fax:843-792-4850
E-mail:spicerek@musc.edu

Abstract Title: Translational Repressor regA Proteins from Phage T4 and RB69 Have Conserved RNA Binding Domains but Different RNA Binding Specificities.

Abstract:Sengupta, T.K., Gordon, J. and Spicer, E.K. Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, SC 29425.

The regA proteins from the bacteriophage T4 and RB69 are translational repressors that control the expression of multiple phage mRNAs. RegA proteins from the two phages are 78% homologous; however, *in vivo* expression studies have suggested that the RB69 regA protein binds target RNAs with a higher affinity than T4 *reg*A protein (Joswik and Miller, 1992). To compare the RNA-binding properties of T4 and RB69 *reg*A proteins more directly, quantitative RNA gel shift assays were performed with synthetic RNAs corresponding to recognition elements in target mRNAs. Comparative gel shift assays demonstrated that RB69 *reg*A protein has ~ 7 fold higher affinity for T4 gene 44 recognition element (RE) RNA than T4 regA protein. RB69 *reg*A protein also binds RB69 gene 44 RE RNA with a 4 fold higher affinity than T4 *reg*A protein. On the other hand, T4 *reg*A exhibited a higher affinity than RB69 *reg*A protein for RB69 gene 45 RE RNA. With respect to their affinities for cognate RNAs, both regA proteins exhibited the following hierarchy of affinities: gene 44 > gene 45 > *reg*A. Interestingly, T4 *reg*A exhibited the highest affinity towards RB69 gene 45 RE RNA, whereas RB69 *reg*A protein had the highest affinity for T4 gene 44 RE RNA. Homology modeling of the structure of RB69 *reg*A protein reveals that the helix-loop groove RNA binding motif of T4 *reg*A protein (Gordon *et al.*, 1999) is fully conserved in RB69 *reg*A protein. Interestingly, divergent residues are clustered in two areas of the surface, producing two regions of high conservation, which presumably also play a role in RNA binding.

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Jozwik, C.E. and Miller, E.S. (1992) Proc. Natl. Acad Sci. USA 89: 5053-5057.

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Title:Ph.D.
First Name:Rimas
Last Name:Nivinskas
Address:Laboratory of Gene Engineering,
Institute of Biochemistry
Mokslininku 12
Vilnius 2600
Lithuania
Phone:(370-2) 729146
Fax:(370-2) 729196
E-mail:rimasn@bchi.lt

Abstract Title: Role of mRNA secondary structure in the translational initiation region of gene 25 of T4-type bacteriophages

Abstract:MALYS, N., KLAUSA, V. AND NIVINSKAS, R. Institute of Biochemistry, Laboratory of Gene Engineering, Mokslininku 12, Vilnius 2600,
Lithuania mRNA secondary structures that enhance or facilitate translation initiation are rare. In our previous studies, it was shown that such structure may exist in the case of bacteriophage T4 gene 25 translational initiation region, which contains three potential Shine-Dalgarno sequences (Nivinskas *et al.*, 1993,1999). The proposed hairpin structure includes SD1 and SD2 sequences and brings the SD3, the most typical of these Shine-Dalgarno sequences, to a favourable spacing with the initiation codon of gene 25. This stem-loop structure is proposed to consist of a 7-nt duplexed stem and a 4-nt single-stranded loop, AGUU.

To determine directly whether this mRNA secondary structure could arrange in vivo, we used selective dimethylsulphate modification of unpaired bases in RNA, coupled with reverse transcription to detect the modified bases. Our results demonstrate that all 7 nucleotides predicted to be duplexed in the stem were apparently protected from methylation, since reverse transcriptase did not terminate in the stem region. As expected, reverse transcriptase termination was observed at the loop base A. The results of these studies are in good agreement with the existence of the mRNA secondary structure *in vivo*.

Comparison of nucleotide sequence in the translation initiation region of gene 25 of ten T4-related phages revealed absolute conservation in the stem region of the mRNA secondary structure. The absence of potential Shine-Dalgarno sequence SD2 was determined in the case of phages T6, Ox2, and RB70. Nevertheless, the level of synthesis of gp25 was observed the same in the case of all plasmids carrying the cloned genes 26 and 25. Therefore, we conclude that the SD2 sequence is not essential for efficient expression of gene 25.

First Name:Gail
Last Name:Christie
Address:Department of Microbiology and Immunology
Virginia Commonwealth University
PO Box 980678
Richmond, VA 23298-0678
USA
Phone:804 828-9093
Fax:804 828-9946
E-mail:christie@hsc.vcu.edu

Abstract Title: Determinants for DNA binding by a member of the P2 Ogr family of zinc-finger transcription factors

Abstract: McALISTER, V. AND CHRISTIE, Virginia Commonwealth University, Department of Microbiology and Immunology, Box 980678, Richmond, VA 23298-0678, USA.

Positive control of late gene expression in P2- and P4-related phages is dependent upon small, phage-encoded transcriptional activators. These activators, exemplified by P2 Ogr, constitute a highly homologous family of novel prokaryotic zinc-finger transcription factors. All members of this family are functionally interchangeable, at least to some extent, and bind upstream oflate promoters at an unusual site that includes an interrupted element of dyad symmetry and is predicted to span three helical repeats of the DNA major groove.The binding of these activators to DNA has been investigated using NucC, a member of the P2 Ogr family encoded by a cryptic prophage in *Serratia marcescens*. DNA bending by NucC was measured by a gel mobility shift assay, using fragments derived from a circular permutation vector carrying a NucC binding site. DNA determinants for NucC recognition were identified using a variety of base-specific chemical modifications and examining the outcome via protection and interference studies. The results support previous genetic studies that implicated specific nucleotides within the dyad repeat elements, and are consistent with NucC contacts spanning three adjacent major grooves.

Title:Ph.D.
First Name:Malgorzata
Last Name:Lobocka
Address:Department of Microbial Biochemistry,
Institute of Biochemistry and Biophysics
of the Polish Academy of Sciences,
Ul. Pawinskiego 5A,
02-106 Warsaw,
Poland
Phone:01148228237188
Fax:0114839121623
E-mail:lobocka@ibbrain.ibb.waw.pl

Abstract Title:Organization of the Genome of P1 Bacteriophage: Tracking the Developmental Program of Expression of P1 Phage Genes.

Abstract:LOBOCKA1, M., ROSE2, D., RUSIN3, M., SAMOJEDNY3, A., YARMOLINSKY4, M., LEHNHERR5, H. AND BLATTNER2, F. C. Department of Microbial Biochemistry, Institute of Biochemistry and Biophysics, PAS, Ul. Pawinskiego 5A, 02-106 Warsaw, Poland1; Laboratory of Genetics, University of Wisconsin, 445 Henry Mall, Madison, WI 53706, USA2; Department of Molecular Biology, Institute of Oncology, Ul. Wybrzeze Armii Krajowej 15, 44-100 Gliwice, Poland3; Laboratory of Biochemistry, National Cancer Institute, NIH Bethesda, MD, USA4; Institute for Mikrobiology, Ernst-Moritz-Arndt-Universitaet Greifswald, Friedrich-Ludwig-Jahnstrasse 15a, 17487 Greifswald, Germany5

P1 is a temperate bacteriophage of *E. coli* and several other enteric bacteria. After injection into a host cell, viral DNA cyclizes via recombination between redundant sequences to enter a lytic or lysogenic path. As a lysogen, P1 is a stable plasmid maintained at about one copy per bacterial chromosome. The lysis/lysogeny decision is dictated by the interplay of environmental factors with the components of the immunity circuit. Onset of lytic development depends on inactivation of the phage repressor, C1, which plays a central role in a switch between lysis and lysogeny. We present an overview of developmental regulation of P1 gene expression that emerged from analysis of the entire sequence of P1 genome combined with results of previous studies. Of 110 genes of P1 identified, five, organized in three operons, can be associated exclusively with plasmid maintenance functions. The remaining genes are organized in 47 operons. Expression of the majority of them during lytic growth of phage follows a strict temporal pattern that leads to production of mature phage particles within less than one hour. Nineteen operons are under the direct control of C1. In addition to proteins that antagonize C1 repression, they encode functions that include those involved in enhancement of homologous recombination, the modification and replication of phage DNA, and the activation of expression of late genes. Transcription of the C1-controlled operons starts from s70 promoters early during lytic growth. Operons whose expression starts in a late stage of phage development are transcribed from promoters that are recognized by the *E. coli* RNA polymerase only in the presence of the product of the C1-controlled gene, lpa. The late promoters differ from the s70 promoters by the absence of a typical -35 hexamer. Instead they have a conserved 9-bp inverted repeat that is centered at position -22 of transcription start site and binds Lpa. The Lpa controlled genes include those involved in phage tail and head morphogenesis, DNA packaging into phage particles, in cell lysis, and in certain functions that are not essential for phage lytic growth. A subset of four of eleven late operons appears to be under the control of both a C1-regulated s70 promoter and a Lpa-activated late promoter, allowing for differential expression of the respective genes during early and late development. Additional control of timing of phage development is achieved at a level of translation. One of the C1-controlled operons (trn) appears to encode three species of tRNA. Of them, two recognize codons that are rare in *E. coli* genes but overrepresented in certain genes of P1. The pattern of distribution of the rare codons in selected P1 genes indicates that the abundance of P1 encoded tRNAs may be essential for efficient expression of some P1 lytic functions.

Title:Dr.
First Name:Michael
Last Name:DuBow
Address:McGill University, Department of Microbiology and Immunology,
3775 University Street, Montreal, Quebec, Canada H3A 2B4.
Phone:514-398-3926
Fax:514-398-7052
E-mail:msdubow@microimm.mcgill.ca

Abstract Title: The Conserved Ner Family of Transposable Phages

Abstract: Ahmad, F., DuBow, M.S., Rongy-Mimouni, M., Salmon, K. and Thiam, M. The Conserved Ner Family of Transposable Phages. McGill University, Department of Microbiology and Immunology, 3775 University Street, Montreal, Quebec, Canada H3A 2B4.

Our laboratory has been studying the lytic/lysogenic switch in transposable coliphages Mu and D108. A key player in this decision is the Ner protein, expressed from the first gene of the early operon, which acts in an analogous (but not homologous) manner to Lambda Cro. It has subsequently been found to be a member of a family of highly conserved DNA-binding, gene regulatory proteins, including the Ner (negative early repressor) proteins of transposable coliphages Mu and D108, transposable *Pseudomonas aeruginosa* phage D3112, the Nlp (Ner-like protein) proteins of *Escherichia coli*, *Neisseria meningitidis*, *Salmonella typhimurium*, *Photorhabdus luminescens*, a plasmid protein of *Erwinia carotovora*; and finally the TMF/ARA160 (TATA-element modulatory factor, Androgen Receptor co-activator) proteins of humans, mice and rats. The Ner proteins of Mu and D108 negatively regulate transcription from two divergent and overlapping promoters, termed Pe (early) and Pc (Repressor), respectively. The D108 ner operator contains two 11 bp inverted repeats separated by an 8 bp AT-rich region; the regulatory regions are located between the repressor and the early genes. The *ner* gene is the first gene encoded by the early operon during lytic development and its role is to inhibit repressor expression, and ultimately turn down early operon expression late in the lytic cycle. The D108 and Mu Ner proteins are 50% homologous, yet recognize different DNA binding sites. In contrast to many other DNA-binding proteins such as Fis, IHF and CRP, which bind unbent DNA and bend it, D108 Ner binds to its bent operator and unbends it, allowing limited Pe expression while repressing expression from Pc. The E. coli Nlp protein (also called Sfs7) acts as a positive activator of several sugar-metabolizing operons (e.g. *mal*), but only in strains with *cya-crp**1 mutations. Nlp is expressed as a 300 nt mRNA, the gene is located at 69.3 map units between *mur*Z and *isp*B, but is non-essential for cell viability under laboratory conditions. The Nlp protein is nonetheless highly conserved among the Enterobacteriaceae. The Mu and D108 Ner proteins can also activate mal operon expression in *cya-crp**1 mutants. Moreover, the amino acid sequence of Nlp was found to be highly homologous (62 and 63%) to the Mu and D108 Ner proteins, respectively. However, Nlp does not bind to the Mu or D108 *ner* operators *in vitro*, nor confer pseudoimmunity to phage superinfection *in vivo*. We have used gene fusions to identify candidate genes which Nlp may regulate, and thus determine the role of this phage homolgue in bacterial physiology.

The human TMF protein is a 1093 amino acid protein that binds to the TATA sequence of the HIV-1 LTR (which consists of two imperfect direct repeats flanking an AT-rich region) and represses transcription from this promoter, as well as from the adenovirus major late promoter. The TMF protein contains a DNA-binding domain (~85 amino acids) highly homologous to Ner and Nlp. The *Ner*-homologous region of TMF has been PCR amplified and shown to activate mal operon expression in *cya-crp**1 mutants, as does its bacterial homologue, Nlp. Studies of this family of prokaryotic and eukaryotic DNA-binding proteins will lead to a better understanding of their role in the regulation of gene expression and their enormous conservation.

First Name:Erlan
Last Name:Ramanculov
Address:Department of Biochemistry and Biophysics, r.301, Texas A&M University, College Station, TX, 77843
Phone:(979)845-2853
Fax:(979)862-4718
E-mail:erlan@tamu.edu

Abstract Title:New insights on the founding phenomenon of molecular genetics: the mechanism of lysis-inhibition

Abstract: Erlan Ramanculov* and Ryland Young. Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, 77843

In the 1940s, the Doermann showed that T-even phages have the ability to delay the onset of lysis for hours in response to the presence of free T-even phage particles in the culture medium. This phenomenon can be considered as the phage version of quorum sensing, which interprets the presence of free T4 particles in the medium as an indication of the shortage of the available host cells. Significant lysis delay prevents waste of new progeny by not releasing phage particles when available hosts are already infected. Besides it allows more exhaustive use of host resources leading to significantly increased bacteriophage burst size. This ability of T-even phages to inhibit host cell lysis in response to an environmental signal is called lysis inhibition (LIN). Although LIN was described more than 50 years ago, and LIN mutants of bacteriophage T4 were widely used as a genetic tool, the mechanism of lysis inhibition remains unknown. Previous genetic data showed that at least two T4 genes are involved in LIN - rI and t (rV). We have approached LIN problem through step-by-step reconstitution of LIN system in the 8 phage, where phage specific lysis was thoroughly characterized. We have cloned T4 t into 8 to replace the canonical holin gene S. T exhibits typical holin behavior, causing saltatory lysis after lysogenic induction at a strictly defined time, programmed into the T holin itself. Coexpression with a functional allele of T4 rI, but not rIII, leads to the inhibition of holin action, suggesting that rI is the primary effector of the LIN signal transduction. Monitoring the intracellular accumulation of T revealed that during LIN, T is present at high concentration in a reversibly inactive form. Moreover, *in vivo* cross-linking experiments show that T is present in high molecular weight complexes with RI.

RI is predicted to be a periplasmic protein. Previous data suggest that the LIN signal is also periplasmic. Therefore it is of interest to find out which part of the T holin is exposed to periplasm and can be responsible for sensing the LIN signal. T is significantly larger than average holin and has a unique hydrophobic profile among more than 100 other holins currently identified. Vast majority of holin genes fall into two groups, according to their primary structure. Class I holins, represented by the 8 S holin, has three transmembrane domains, and class II, represented by the phage 21 S holin, is thought to have only two transmembrane domains. Strikingly, only one clearly defined transmembrane domain can be discerned in the predicted amino acid sequence of the T4 holin. Unique regulatory capacity of T may provide a rationale for the unusual structure of this holin. We have constructed series of phoA-lacZ sandwich fusion constructs with t and have found that the C-terminal half of T is exposed to the periplasm and is most likely to be responsible for reception of the LIN signal.

These and other results allow us to propose a comprehensive model for the mechanism of LIN.

Title:Prof
First Name:Udo
Last Name:Bläsi
Address:Vienna Biocenter
Dr. Bohrgasse 9
1030 Vienna
Austria
Phone:++43-1-4277-54609
Fax:++43-1-4277-9546
E-mail:udo@gem.univie.ac.at

Abstract Title:Dissection of molecular events leading to formation of the lambda S holin-dependent lesion in the cytoplasmic membrane

Abstract:GRÜNDLING, A.[1/2], GRASCHOPF, A.[1], YOUNG, R.[2] and BLÄSI,U.[1]. [1]Institute of Microbiology & Genetics, Vienna Biocenter, Dr. Bohrgasse 9, 1030 Vienna, Austria. [2]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Tx 77843-2128, USA.

Bacteriophage lambda uses a holin-endolysin system for host cell lysis. R, the endolysin, has muralytic activity and accumulates in the cytosol. S, the holin, is a small membrane protein which permeabilizes the inner membrane at a precisely scheduled time after infection. The S-mediated lesion allows the endolysin access to the murein which results in immediate host cell lysis. The lambda S gene represents the prototype of holin genes with a dual-start motif, which leads to the synthesis of two polypeptides S105 and S107. Both products differ only by residues Met1 and Lys2 at the beginning of S107. Despite the minor difference, the two proteins have opposing functions in lysis with S107 being an inhibitor and S105 being an effector of `hole formation´ in the inner membrane. Functional assembly of the S-dependent hole in the membrane requires translocation of the N-terminus of both S105 and S107 to the periplasm. Our data revealed that S107 with a reversed topology of its N-terminus interacts with S105 and poisons `hole formation´. Upon depolarization of the membrane, translocation of the N-terminus of S107 to the periplasm results in functional assembly of S proteins, i.e. `hole formation´. There are several steps required for `hole formation´: dimerization, oligomerization and a profound structural rearrangement of the S assemblies in the membrane. Based on genetic and biochemical studies of different S-mutant proteins a three step model for S-dependent hole formation will be presented.

Title:Dr.
First Name:Stephen T.
Last Name:Abedon
Address:Department of Microbiology
The Ohio State University
1680 University Dr.
Mansfield, OH 44903
Phone:(419) 755-4343
Fax:(419) 755-4327
E-mail:microdude+@osu.edu

Abstract Title:A Plethora of Lysis-Timing

Evolution assures that bacteriophages are optimized for producing bacteriophage and babies; at least in some systems, at least to some extent. Thus we should expect phages to be pretty good at adsorbing hosts and making baby phages. Still, any organism must face a tradeoff in the complexity of progeny versus their cheapness; with more expensive/more complex phages better at doing what they do though not necessarily produced as quickly or in enormous quantities. T-even phages, for example, presumarly are well biased towards complexity over cheapness. In this talk I will discuss yet another variable available for phage optimization, the phage burst size versus latent period tradeoff. For any given obligately lytic and otherwise optimized phage, any increase in phage burst size must come at a lengthened-latent-period cost. It is suspected that this burst size-latent period tradeoff is equivalent to the virulence-avirulence dynamic presumably present in the evolution of all pathogen-host symbioses. I have been looking at phages from this perspective of latent-period evolution for over a decade (e.g., Abedon, S. T., 1989, Selection for bacteriophage latent period length by bacterial density: A theoretical examination, Microbial Ecology 18:79-88) and have been slowly identifying factors that produce T-even bacteriophage lysis-timing variation and acquiring a small collection of T-even bacteriophage lysis-timing variants. These I will review for you.

For additional information, see:
http://www.phage.org/homepage_publications.htm
http://www.phage.org/homepage_research_interests.htm

Title:Dr.
First Name:Revaz
Last Name:Adamia
Address:laboratory of Genetic Engineering and Biotechnology
G.Eliava Institute of Bacteriophages, Microbiology and Virology
3, Gotua st., Tbilisi, 380060, Georgia
Phone:+995 32 37 12 18
Fax:+995 32 93 14 76
E-mail:defence@parl.gov.ge

Abstract Title:Characterization of two Therapeutic Staphylococcal and Coli-Dysenterial Bacteriophages

Abstract:Molecular biological investigation and detailed characterization of two therapeutic bacteriophages - Sb-1 active to *Staphylococcus aureus* and Coli-Dysenterial Phl-1 were carried out. Sb-1 displays wide spectrum (97%) of lytic activity and high specificity to *S. aureus* species. Phl-1 phage lytic activity (62%) is checked on different coli-dysenteric bacterial strains. According to the electron microscopy investigation of the viral particles, these phages are representatives of the Myoviridae family.

Detailed structure of Sb-1 and Phl-1 phage tails has been studied. Three-dimensional structure of phage tails was reconstructed by the method of synthesis of modified projected functions. Crystallographic parameters were determined for the virions with intact and contracted tails. Intact tails of the phages are characterized by identical principles of structure and have a symmetry axis of sixth degree. Models of Phl-1 phage tails were assembled as a result of reconstruction of three-dimensional space structure. It is determined that Phl-1 phage virion has identical structure and sheath contraction mechanisms as T4.

Sb-1 and Phl-1 phage genomes belong to A-T type, do not contain unusual bases or extra-saccharide components. The conformation of Phl-1 and Sb-1 DNAs was studied in situ and in vitro. Protein free DNAs of the phages are characterized by a double chain spiral structure and reveal typical B configuration in standard conditions. 1/3 of in situ DNA of Phl-1 phage is in an irregular conformation. Sb-1 phage, on the contrary, is characterized by the same regular structure of DNA in nucleoprotein and solution.

Restriction analysis of the phage genomes showed that Phl-1 phage is rather resistant to endonucleases. Sb-1 phage DNA is fragmented by all endonucleases except the enzymes that contain GATC nucleotide sequence in the center of their recognition site.

It is determined that Phl-1 and Sb-1 bacteriophages perform protection from cellular restriction enzymes by elimination, i.e. counterselection of specific recognition sites for some endonucleases.

Title:Dr.
First Name:Donna
Last Name:Duckworth
Address:Department of Molecular Genetics and Microbiology
Box 100266, Health Science Center
University of Florida
Gainesville, Fl 32610
Phone:352-392-0681
Fax:352-392-3133
E-mail:duckwort@mgm.ufl.edu

Abstract Title: Use of *Vibrio vulnificus* Bacteriophage to Prevent Disease in a Mouse Model of Infection

CERVENY, K.,[1] DOYLE, T.,[1] DePAOLA, A.,[2] GULIG, P.,[1] AND DUCKWORTH, D.[1]
[1]University of Florida; Department of Molecular Genetics and Microbiology, Box 100266, Health Science Center, Gainesville, Florida 32610, and [2]Gulf Coast Seafood Laboratory, U.S. Food and Drug Administration, Dauphin Island, Alabama 36528

With the specter of bacteria resistant to all known antibiotics looming very large, there is renewed interest in research on alternative antibacterial therapies. We are studying the use of bacteriophage as therapy for *Vibrio vulnificus* infection in a mouse model of disease caused by this organism. *V. vulnificus* is the leading cause of death associated with consumption of seafood in the USA. During the summer, virtually every oyster harvested from the Gulf of Mexico is contaminated with *V. vulnificus*. People with liver damage (cirrhosis) or a genetic mutation that leads to excess uptake of iron (hemochromatosis) can die from systemic infection within 24 hours of consuming contaminated oysters. The bacterium can also cause serious wound infection that requires extensive surgical intervention and can lead to septicemia and death. A number of phage active against *V. vulnificus* have been isolated from oyster-bed sediment in the Gulf of Mexico or from oysters themselves. To study the therapeutic use of these phage, mice were injected intraperitoneally with iron dextran and were then infected subcutaneously with various strains of the bacteria. Phage were administered intravenously at the time of bacterial inoculation or at various times thereafter. Control mice not receiving the phage became moribund and died or had to be euthanized within 18 hours. These mice had large edematous lesions at the site of injection that contained up to 108 CFU/gram of tissue. Their livers contained up to 106 CFU/gram. Most (80-90%) of the mice that received phage at the time of bacterial injection remained healthy and exhibited only minor subcutaneous lesions, many of which did not contain detectable levels of bacteria. The livers of all animals treated with phage had no detectable bacteria. These data demonstrate that phage have a definite therapeutic potential in this model. We are planning to use this model system to study the pharmacokinetics of phage and are also planning on developing strategies using phage for the "depuration" of contaminated oysters.

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Title:Dr.
First Name:Caroline
Last Name:Westwater
Address:Department of Microbiology & Immunology
PO Box 250504
Medical University of South Carolina
171 Ashley Avenue
Charleston, South Carolina 29425
Phone:(843) 792-8499
Fax:(843) 792-2464
E-mail:westwatc@musc.edu

Abstract Title:A Bacteriophage Lethal Agent Delivery System for the Treatment of Bacterial Infections

Abstract:We are developing a non-replicating phage-based gene therapy for the treatment of multidrug-resistant bacteria. Genetic information encoding antibacterial therapeutics will be delivered by the bacteriophage P1. The delivery vehicle contains all the essential signals for packaging, a selectable marker for transfer detection, the lethal genetic information and a plasmid origin of replication for *Escherichia coli* or a broad host range origin capable of replicating in Gram-negative bacteria. The vector is maintained in a P1 lysogen which provides all the replication factors needed to activate the lytic cycle and all the structural components to form mature viral particles but will be unable to package its own DNA. Phage particles will inject vector DNA, but not viral DNA, into pathogenic bacteria and expression of the lethal agents under the control of environmentally-regulated or species-specific promoters will result in cell death. Lethality has been demonstrated with plasmid and bacteriophage associated toxic proteins and the conditionally lethal genes located on the *E. coli* chromosome (see Schofield et al.). Using this system we have demonstrated transfer of genetic information via the bacteriophage P1 delivery system in vitro and in a mouse peritonitis model of infection. The efficacy of LADS therapy is being tested in the embryonated hen egg model (see Kasman *et al.*).

Funded by Hexal Pharmaceutical Inc.

doc protein kills { Staph. Ps. Coli

Title: Dr.
LastName: Gill
FirstName: J.J.
Address: Dept. of Biological Sciences, Brock University
St. Catharines, Ontario

Abstract:GILL, J. J. (1) (Presenting author), SVIRCEV, A. M. (2), SMITH, R. (3), MYERS, A. L. (2), CASTLE, A. J. (1) Biological Control of Erwinia amylovora with Bacteriophages. 1: Department of Biological Sciences, Brock University, St. Catharines, ON; 2: Agriculture and Agri-Food Canada SCPFRC, PO Box 6000, 4902 Victoria Ave. N., Vineland Station, ON, L0R 2E0; Department of Plant Science, University of Western Ontario, London, ON.

Forty-four bacteriophage isolates of *Erwinia amylovora*, the causal agent of fire blight, were collected from sites in and around the Niagara Region of Southern Ontario in the summer of 1998. Phages were isolated only from sites where fire blight was present. Thirty-seven of these phages were isolated from the soil surrounding infected trees, with the remainder isolated from aerial tissue samples. A mixture of six *E. amylovora* host strains was used to enrich field samples in order to avoid the selection bias of a single-host system. Molecular characterization of the phages with a combination of PCR and restriction endonuclease digestions showed that six distinct phage types were isolated. Ten phage isolates related to the previously characterized *E. amylovora* phage PEa1 were isolated, with some divergence of molecular markers between phages isolated from different sites. The host ranges of the phages revealed that certain types were unable to efficiently lyse some *E. amylovora* strains, and that some types were able to lyse the epiphytic bacterium *Pantoea agglomerans*. Biological control of *E. amylovora* by the bacteriophages was assessed in a bioassay using discs of immature pear fruit. Twenty-three phage isolates were able to significantly suppress the incidence of bacterial exudate on the pear disc surface. Quantification of the bacterial population remaining on the disc surface indicated that population reductions of up to 97% were obtainable by phage treatment.

First Name:Alexander
Last Name:Sulakvelidze
Address:University of Maryland School of Medicine
Department of Epidemiology and Preventive Medicine
MSTF Bldg., Room 9-34
10 S. Pine Street
Baltimore, MD 21201
USA
Phone:410-706-4587
Fax:410-706-4581
E-mail:asulakve@medicine.umaryland.edu

Abstract Title:Development and commercialization of phage therapy in the United States.

A. SULAKVELIDZE, J. G. MORRIS, Z. ALAVIDZE, and T. BROWN. University of Maryland School of Medicine, and Intralytix, Inc., Baltimore, Maryland 21201, U.S.A.

The past decade has seen the increasing emergence of pathogenic bacteria resistant to most, if not all, currently available antibiotics. This phenomenon has become a critical problem in modern medicine, particularly because of the concomitant increase in immunosuppressed patients (including patients undergoing organ transplantation or cancer chemotherapy, or persons infected with the HIV). The concern that humankind is re-entering the "pre-antibiotics" era has become very real, and the development of alternative anti-infection modalities has become one of the highest priorities of modern medicine/biotechnology.

Prior to the discovery and widespread use of antibiotics, it was suggested that bacterial infections could be prevented and/or treated by the administration of bacteriophages (viruses which kill bacteria). Although the early clinical studies with bacteriophages were not extensively pursued in the United States and Western Europe, phages continued to be utilized in the former Soviet Union and Eastern Europe, with general acceptance of their safety and efficacy. However, the recent emergence of bacterial pathogens highly resistant to antibiotics has rekindled the interest in phage therapy in the West, and investigators and start-up biotech companies in the US, Canada, and Western Europe are beginning to explore the utility of bacteriophages for prophylaxis or treatment of bacterial infections. These investigations include efforts to eradicate carriage of antibiotic-resistant organisms by patients, prophylaxis to prevent invasive disease in colonized patients undergoing organ transplantation or cancer chemotherapy, and treatment of persons having invasive disease due to these bacteria. Since many of the Soviet and Eastern European studies do not meet the current standards for clinical trials, biotech companies face challenges in their efforts to bring phage therapy to the cutting-edge biotech level and to the point where therapeutic or prophylactic phage products are ready for human clinical trials in the West. For example, several rigorous studies must be performed, and proper regulatory approvals must be obtained, before Phase I safety trials of therapeutic phages can be initiated in the United States. However, if these preliminary studies result in the identification of phage preparations having appropriate activity against and specificity for the pathogens of interest, and if these phages can be shown to be safe and efficacious in animal models, there would be a strong basis for rapidly moving forward with phase I and phase II trials of bacteriophage therapy in humans. Such trials, in turn, could lead to licensure of specific phage products for use in the United States, Canada, Japan, and Western Europe. Therapeutic phages are unlikely to supplant the use of antibiotics or vaccines in managing bacterial infections, but they can potentially be an important part of an overall strategy to combat infectious diseases of bacterial origin. As noted above, there is a very real and urgent market for such products, and this need should serve as a strong stimulus for further product development.

Title:Dr.
First Name:Bentley
Last Name:Fane
Address:Department of Veterinary Sciences and Microbiology
University of Arizona
Tucson AZ, 85721
Phone:520-626-6634
E-mail:bfane@u.arizona.edu

Abstract Title:Structural, genetic and evolutionary analyses of Microviridae scaffolding proteins.

Microviridae morphogenesis is dependent on two scaffolding proteins; an internal and external species. Structural and genetic analyses suggest that the COOH terminus of the internal protein is critical for coat protein recognition. Chimeric internal scaffolding genes between coliphages øX174, G4 and a3 were constructed and expressed in vivo. The chimeric proteins are functional in complementation assays. However, efficient complementation is only observed when the viral coat and the COOH-terminus of the internal scaffolding proteins are of the same origin. The external scaffolding protein is the most conserved protein within the coliphage Microviridae. Despite 70% homology on the amino acid level, over-expression of a foreign Microviridae external scaffolding protein is a potent cross-species inhibitor of morphogenesis. To define the requirements for, and constraints on scaffolding protein interactions, chimeric external scaffolding proteins have been constructed *in vivo* and analyzed for effects on assembly. The results of these experiments suggest that at least two cross-species inhibitory domains exist within these proteins. One domain inhibits the formation of the DNA packaging complex, while the other domain affects an earlier stage in procapsid morphogenesis.

A newly isolated phage of Chlamydia, Chp2, has been characterized. Though a member of the Microviridae, it appears to have no scaffolding proteins. The primary functions of the coliphage external scaffolding proteins are the mediation of two-fold interactions: the placement of spike protein pentamers on the coat protein, and the organization of the coat protein at the three-fold axes of symmetry. None of these functions may be required in Chp2. The phage appears to be spike-less. A large insertion loop in the coat protein most likely organizes three-fold axes. Finally, a structural protein, VP3, may mediate two-fold interactions. VP3 shares homology to the coliphage internal scaffolding proteins, which also make two-fold interactions. Amino acid residues in the øX174 procapsid known to mediate interactions between the viral coat protein and internal scaffolding proteins are conserved in the Chp2 coat and VP3 proteins. We suggest that VP3 performs scaffolding - like function but has evolved into a structural protein.

Title:Dr.
First Name:Harald
Last Name:Bruessow
Address:Nestlé Research Centre
Bioscience Department / Microbiology
CH-1000 Lausanne 26 Vers-chez-les-Blanc Switzerland
Tel. 0041 21 785 8676
Fax. 0041 21 785 8925
e-mail: harald.bruessow@rdls.nestle.com

Abstract Title:Darwinian Evolution of the Structural Gene Cluster in Siphoviridae?

Abstract:F. DESIERE, C. MAHANIVONG, A. HILLIER, B. DAVIDSON, H. BRUESSOW (presenting author). Nestlé Research Centre CH-1000 26 Lausanne, Switzerland.

Bacteriophages from lactic acid bacteria are not genetic mosaics as predicted by the classical modular theory of phage evolution. Horizontal gene transfer was limited to a few phage types infecting the same bacterial species. Comparative genomics of the phage structural gene clusters defined several lineages of Siphoviridae in low GC content Gram-positive bacteria. Genome analysis of one of these lines (the proposed Sfi21-like phage genus of Siphoviridae) revealed a hierarchy of relationships that covered high and low DNA sequence similarity, high and low protein sequence similarity and similarity in genome organization in absence of sequence similarity. The observed gradient of similarity between the investigated phages correlated with the evolutionary distance separating their bacterial hosts. The data support gradual evolution of the morphogenesis gene cluster from an ancestor phage leading to several extant lines of Siphoviridae in Gram-positive and Gram-negative Eubacteria and Archaea.

Title:Dr.
First Name:Elizabeth
Last Name:Kutter
Address:Lab I, Evergreen State College
Olympia, WA 98505
Phone:360 866 6000 X6099
Fax:360 867 5430
E-mail:t4phage@elwha.evergreen.edu

Abstract Title:T-Even Phages and the Real World

We are trying to develop a better understanding of the ecology and evolution of the T-even phages, in light of both their important role in phage therapy and their inherent complexity and interest in the general field of viral origins and evolution, as well as in molecular biology.

Despite the completion of the T4 genomic sequence, it is clear that there is much that we do not understand about T4. Only 69 of its 283 probable protein-encoding genes are essential under standard lab conditions, Only 34 show clearcut homologies with non-phage proteins in the genomic databases and only a few others have been found to be closely related to proteins from non-T-even phages; this remains true despite the many genome sequences completed since our initial analysis. The two most like E. coli proteins - the anaerobic ribonucleotide reductase and its cofactor - clearly diverged well before the separation of H. influenzae and E. coli, and there is no evidence of sequence similarities that could support recombination with its host. The eucaryotic homologues include relatives of each of the 3 known families of mobile endonucleases first identified in the mitochondrial introns of filamentous fungi, but these 13 genes are all widely diverged from each other and from any known outside genes. We still have little sense of the specific functions of almost half of T4's, except to know that most of the uncharacterized genes are expressed immediately after infection and are likely to be involved in some way in the transition from host to viral metabolism. Despite managing to clone and purify some of those proteins, we have not yet made much progress in determining their function or their targets in the host.

While T4 itself was only isolated once from nature, T-even phages attacking gram-negative bacteria are very common, isolated from patients recovering from dysentery, from zoo animals, from sewage. These all have substantial homologies with T4. We have been comparing the patterns of infectivity of nearly 100 of these phages from around the world on various lab and clinical strains, including the 72 members of the classic ECOR collection and pathogens infecting pigs and humans. Some T-even phages infect 60% of the hosts, while others infect only 2 or 3; all are hit by at least one of the phages, and one can put together a cocktail of several that attack almost all of them. There is no simple obvious relationship between geographic origin, evolutionary relatedness of the bacteria, infectivity by other phages or pathogenicity and the patterns of sensitivity. There are also significant differences in the efficiency of plating on various strains that we do not understand, despite the HMC in the DNA of most of them.

One assumes that the major site of T-even phage replication is likely to be in the mammalian gut, where it would be expected to encounter stationary-phase cells. The "common wisdom" is that T4 cannot infect stationary phase cells. We find that while it indeed does not generally make a burst in stationary phase, it can enter into a sort of "hibernation mode" even after many days in stationary phase, In this mode, it can still make infective centers. This ability is reflected in the fact that a very similar pattern of synthesis of a limited number of proteins is seen just after nutrients are re-added in infected and uninfected cells, but not long after the patterns diverge drastically and the infected cells make phage. The relative ability of T-even phages to adapt to different conditions and eventually reproduce may well be one aspect of their natural roles in maintaining microbial balance and be relevant to their therapeutic potential.

Web site, with further information about T4, phage therapy history and issues and
the nonprofit PhageBiotics Foundation: http://www.evergreen.edu/user/T4/home.html

First Name:Maria
Last Name:Pajunen
Address:University of Turku
Department of Medical Biochemistry
Kiinamyllynkatu 10
20520 Turku
FINLAND (Europe)
Phone:+358 2 333 7444
Fax:+358 2 333 7229
E-mail:maria.pajunen@utu.fi

Abstract Title:Molecular Analysis of Lytic *Yersinia enterocolitica* serotype O:3 -specific Bacteriophage phi-YeO3-12

Abstract:PAJUNEN, M.I., KILJUNEN, S.J., SÖDERHOLM, M.E.-L. AND SKURNIK, M. University of Turku; Dept. of Medical Biochemistry, Kiinamyllynkatu 10, 20520 Turku, FINLAND

phi-YeO3-12 is a lytic double-stranded DNA phage whose receptor is a homopolymer of 6-deoxy-L-altropyranose that forms the LPS O-chain. Phage phi-YeO3-12 can also infect recombinant *E. coli* expressing the *Y. enterocolitica* O:3 O-antigen. Morphologically phi-YeO3-12 is a typical member of the T7 group with approximate dimensions (PT) of 57 nm for the head and 15 x 8 nm for the tail. Using *Y. enterocolitica* 6471/76-c as a host, phage phi-YeO3-12 had a burst size of 120 ± 20 and a latent period of 25 min.

The complete nucleotide sequence of the 39 600 bp genome was determined (Acc. No. AJ251805) including redundant direct terminal repeats of 232 bp. Three gene products of the phage particle were analyzed by protein microsequencing. Altogether 56 putative open reading frames (orfs), all of them organized in the same direction of transcription, were identified. On the basis of comparison of the deduced amino acid sequences to known proteins in databases and by searching for conserved motifs, putative functions could be assigned to the products of 29 orfs. For 18 orfs homologies were detected in databases, but no function could be inferred for them. The remaining 9 orfs showed no homologies to any known proteins.

The most striking feature of the phi-YeO3-12 genome was an extensive homology with the coliphages T3 and T7; more than half of the orfs had marked homology to T3 genes with > 70% identity, the promoter regions were identical for the two phages, and the gene arrangements of the genomes of phi-YeO3-12 and T3 were almost identical. In addition, phi-YeO3-12 was found to share several common features with T3, including the morphology and non-subjectibility to F exclusion. These findings indicate that phi-YeO3-12 is a T3-like phage well adapted to *Y. enterocolitica* O:3, and provide clear evidence of the intergeneric spread and evolution of bacteriophages. This is the first report to determine the whole genome sequence of dsDNA phage in Yersinia species.

Title:Mr
First Name:Lawrence
Last Name:Goodridge
Address:Department of Food Science
University of Guelph
Guelph, Ontario, Canada
N1G 2W1
Phone:1-519-824-4120 ext 3911
Fax:1-519-824-6631
E-mail:lgoodrid@uoguelph.ca

Abstract Title: Partial Genetic Characterization Of Coliphage LG1

Goodridge, L., Gallaccio, A., and Griffiths, M. University of Guelph, Canadian Institute for Food Safety, Department of Food Science, Guelph, Ontario, Canada, N1G 2W1

Reporter bacteriophage assays represent a sensitive and elegant method for the detection of bacterial pathogens. We describe here the partial genetic characterization of coliphage LG1. This phage belongs to the family Myoviridae, possesses the ability to infect a wide range of *E. coli* serogroups and serotypes, and is therefore potentially useful in a reporter bacteriophage assay for detection of *E. coli* in food and clinical samples. A partial genetic characterization of phage LG1 was undertaken in order to determine its tractability in the construction of such an assay. Pulsed Field Gel Electrophoresis (PFGE) analysis showed phage LG1 to have a chromosome 48.5 kb in size. Restriction digestion studies showed that the nucleic acid was DNA, and the restriction profiles identified several fragments that were identical in size to fragments of coliphage T4, generated by the same restriction enzyme. Southern blot analysis using Dig labeled genomic T4 DNA as a probe showed that one LG1 EcoR V fragment, 9.3 kb in size, hybridized with the T4 probe. Primers were designed against the corresponding fragment in T4, and were employed in a Polymerase Chain Reaction (PCR), to try to identify genes in LG1 that were homologous to their T4 counterparts. One PCR yielded a fragment that was 98% homologous to the T4 gene uvsW. The implications of this discovery are discussed, as well as the sequence results obtained from the production of a LG1 random genomic library. The possible sequence identification of a capsid gene in LG1 is also discussed.

Title:Ms
First Name:Princess
Last Name:Mmolawa
Address:IMVS, Infectious Diseases Laboratories
Box 14
P.O. Rundle Mall
Adelaide
South Australia
5035
Phone:+618 82223274
Fax:+618 82223543
E-mail:princess.mmolawa@imvs.sa.gov.au

Abstract Title:Characterisation of the Lysogenic Phage in *Salmonella enterica* serovar Typhimurium.

Abstract: [1,2]MMOLAWA, P.T., [1]THOMAS C. AND [2]HEUZENROEDER, M.W.
[1]University of Adelaide, Department of Microbiology and Immunology, [2]Institute of Medical and Veterinary Sciences, Infectious Diseases Laboratories, Adelaide, 5000, South Australia.

Salmonella enterica serovar Typhimurium is the most common serovar causing outbreaks in Australia. The most common phage types isolated are 9, 64 and 135. Bacteriophage conversion studies using a lysogenic phage lysate from S. Typhimurium phage type 64 was able to convert *S. typhimurium* phage type 9 to phage type 64 and phage type 135 to phage type 16; this has serious implications for organism tracing during an outbreak of salmonellosis as phage typing is used to differentiate isolates of the same serovar. Induction of phages with mitomycin C from phage type 64, followed by purification of phage particles on caesium chloride (CsCl) gradient resulted in two phage bands, namely the top and the bottom. However, only the bottom phage band was seen in phage types 9 and 135. Restriction analysis with SmaI shows that DNA from the crude phage lysate (not CsCl purified) is a combination of DNA from the top and the bottom phage bands. Transmission Electron Microscopy showed both bands to have icosahedral phage heads with no visible tails. DNA extracted from these phage particles and restricted with SmaI showed different patterns of banding. *S. typhimurium* phage type 64 bottom band phage genome was cloned into pGEM-7zf(-) vector and sequenced. Sequence analysis of this phage genome will be discussed.

Title:Dr.
First Name:Mzia
Last Name:Kutateladze
Address:Laboratory of Genetic Engineering and Biotechnology,
G.Eliava Institute of Bacteriophages, Microbiology and Virology
3, Gotua str., Tbilisi, 380060, Georgia
Phone:+ 995 32 37 12 18
Fax:+ 995 32 93 14 76
E-mail:defence@parl.gov.ge

Abstract Title:Bacteriophage phylogenetically related to T4 infect a wide range of host bacterial species

Abstract: We have examined a number of bacteriophage with T4-like morphology that propagate in different bacterial species (*E. coli, Shigella, Salmonella, Yersinia, Vibrios, Aeromonas, Pseudomonas* etc.). Most of these phage had morphology and genome size similar to T4. However, a few of them had more elongated heads and larger genomes. Nevertheless, all these phage are phylogenetically related, since they each had sequences homologous to the capsid gene (gp23) and contractile tail genes (gp18 and gp19) of T4. A sequence comparison indicates that the T4-type phage can be classified into four subgroups that are increasingly divergent from T4: the T-evens, the mezzoT-evens, the pseudoT-evens and the schizoT-evens. In general, phage that infect closely related host species are phylogenetically closer to each other than phage that infect distant hosts. Some of the phage appear to be chimeras, indicating that some genome shuffling must occur between the different T4-type subgroups. The compilation of a number of gene 23 sequences reveals a pattern of conserved motifs that are separated by sequences that differ in the T4-type subgroups. These variable patches in the gene 23 sequence may determine, among other things, the size of the virion head and consequently the viral genome length.
The sequence analysis of capsid gene and tail genes of Coli-Dysenterial Phl-1 therapeutic bacteriophage showed that it belongs to pseudoT-even subgroup.

Title:Professor
First Name:Linda
Last Name:Reha-Krantz
Address:
Department of Biological Sciences
CW405 BioSciences Building
University of Alberta
Edmonton, Alberta T6G 2E9
Canada
Phone:780-492-5383
Fax:780-492-9234
E-mail:LREHA@gpu.srv.ualberta.ca

Abstract Title:Evidence that DNA Polymerase Proofreading Creates an Opportunity for Misalignment Mutagenesis. Studies of the Bacteriophage T4 DNA Polymerase Proofreading Pathway.

Abstract:REHA-KRANTZ, L. J.*, HADJIMARCOU, M. I.*, KOKOSKA, R.**, AND PETES, T. D.** *University of Alberta, Dept. of Biological Sciences, Edmonton, Alberta T6G 2E9 and the **University of North Carolina, Chapel Hill, NC 27599-3280.

We propose that DNA polymerases occasionally misalign the primer and template strands during exonucleolytic proofreading. During proofreading, the 3'-end of the primer strand is separated from the template and transferred to the exonuclease active center where the terminal nucleotide is excised. The primer strand is then returned to the polymerase active center and DNA replication resumes. If the primer-end reanneals to the template strand in a misaligned configuration, however, primer extension will result in addition or loss of one or more nucleotides. This hypothesis was tested by measuring misalignment mutagenesis *in vivo* by bacteriophage T4 DNA polymerase mutants that are defective in different steps of the proofreading pathway (1,2). The studies were extended to the protein-sequence related yeast DNA pol delta. The results indicate that misalignment mutations are produced during DNA polymerase exonucleolytic proofreading. Our results provide a mechanism for "DNA polymerase slippage" that was originally proposed by George Streisinger for generating frameshift mutations and an explanation for the instability of simple repeat sequences in phage T4, yeast, and in humans.

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Title:Dr.
First Name:Erin
Last Name:O'Reilly
Address:Duke University Medical Center
Department of Microbiology
Box 3020
Duham, NC 27710
Phone:(919) 684-2566
Fax:(919) 681-8911
E-mail:oreilly@abacus.mc.duke.edu

Abstract Title:THE CHARACTERIZATION OF A UNIQUE MUTANT OF T4 TOPOISOMERASE THAT IS HYPERSENSITIVE TO A BROAD RANGE OF CLEAVAGE-INDUCING ANTITUMOR AGENTS.

Abstract:O'REILLY*, E AND KREUZER, K. Duke University Medical Center, Dept. of Microbiology, Durham, NC 27710 USA.

Bacteriophage T4 provides a useful model system for the dissection of the mechanism of action of antitumor agents that target type II DNA topoisomerases. Many of these inhibitors act by trapping the enzyme in the cleavage intermediate. Previous analysis of drug resistant mutations in the T4 topoisomerase has fortuitously led to the discovery of a hypersensitive mutant (Cancer Res. 58:1260-1267). A single amino acid substitution (G269V) in topoisomerase structural gene 52 was shown to confer dramatic hypersensitivity in vivo to the cleavage-inducing inhibitors, oxolinic acid and 4'-(9-acridinylamino)methanesulfon-m-anisidide (m-AMSA). To determine if the G269V mutant was indeed hypersensitive to a broad range of cleavage-inducing inhibitors, we purified the mutant enzyme and analyzed its drug sensitivity with DNA cleavage assays. As expected from the in vivo data, the purified mutant enzyme was hypersensitive to m-AMSA and oxolinic acid. The mutant enzyme was also hypersensitive to a number of other cleavage-inducing inhibitors that cannot be tested in vivo such as VP-16, ellipticine, and mitoxantrone. Surprisingly, in the absence of any inhibitors, the hypersensitive enzyme appears to remain in the cleavage complex for a longer period of time compared to the wild type enzyme. Thus, this mutant seems to cause hypersensitivity in a unique manner by increasing the frequency of cleavage complexes available to inhibitors. We believe the study of hypersensitive mutants provides a unique window into the mechanism of drug action.

First Name:Brad
Last Name:Stohr
Address:Box 3020
Duke University Medical Center
Durham, NC 27710
Phone:919-684-2566
Fax:919-681-8911
E-mail:stohr@abacus.mc.duke.edu

Abstract Title:Repair of Topoisomerase-Mediated DNA Damage in Bacteriophage T4

Type II topoisomerase inhibitors are important clinical agents used in the treatment of both tumors and bacterial infections. These drugs act by stabilizing DNA-enzyme cleavage complexes that consist of the topoisomerase protein covalently linked to the 5' phosphates of a double-strand break.
It is believed that these reversible cleavage complexes are converted to lethal double-strand DNA breaks, perhaps due to collision of a replication fork. Genetic studies using cell sensitivity as an endpoint have demonstrated that recombinational repair can eliminate this topoisomerase-mediated DNA damage. To explore this repair pathway more directly, we have developed an in vivo plasmid-based assay that allows physical analysis of the repair products from one particular topoisomerase cleavage site. This assay utilizes a bacteriophage T4 model system. We show that in the presence of the antitumor agent m-AMSA (4'-(9-acridinylamino)methanesulphon-m-anisidide), the T4 type II topoisomerase is stabilized at a strong topoisomerase cleavage site on the plasmid, thereby stimulating recombinational repair of the lesion. The resulting repair products are detectable by Southern blot analysis of plasmid DNA. These drug-dependent repair products do not form in the absence of functional topoisomerase and appear at lower drug concentrations with a drug-hypersensitive topoisomerase mutant. The appearance of repair products is replication origin-dependent, consistent with the idea that replication fork collision may play a role in generating the lesions. Finally, genetic analysis demonstrates that repair product formation is absolutely dependent on genes 32 and 46, largely dependent on genes *uvs*X and *uvs*Y, and only partly dependent on gene 49. Very similar genetic requirements are observed for repair of endonuclease-generated double-strand breaks, suggesting mechanistic similarity between the two repair pathways. A clear understanding of how topoisomerase-mediated DNA damage is repaired may ultimately allow design of drugs that block this repair, thereby increasing the clinical efficacy of the type II topoisomerase inhibitors.

Title:Dr.
First Name:Scott
Last Name:Morrical
Address:Department of Biochemistry
University of Vermont College of Medicine
B411A Given Bldg.
Burlington, VT 05405
Phone:802-656-8260
Fax:802-862-8229
E-mail:smorrica@zoo.uvm.edu

Abstract Title:Loading Enzymes onto ssDNA-- T4 Phage and the RMP

MORRICAL, S., XU, H., BLEUIT, J., MA, Y., AND WANG, Y. University of Vermont College of Medicine; Dept. of Biochemistry, B411A Given Bldg., Burlington, VT 05405 U.S.A.

Studies of recombination-dependent replication (RDR) in the T4 system have revealed a class of factors necessary for the timely and productive loading of specific enzymes onto ssDNA during DNA recombination, replication, and coupled RDR processes. These factors, which we term "Recombination/Replication Mediator Proteins" or "RMPs" (cf. Beernink & Morrical, 1999, Trends Biochem. Sci. 24, 385-389), include the T4 uvsY and gp59 proteins. *Uvs*Y is necessary for the proper assembly of the T4 presynaptic filament (uvsX recombinase cooperatively bound to ssDNA) during homologous recombination. Gp59 is required for the assembly of gp41, the DNA helicase component of the T4 primosome, onto the lagging template strand during RDR transactions.

*Uvs*Y protein exists as a hexamer of identical 15.8 kDa subunits in solution and interacts with ssDNA as a hexamer. The interaction of ssDNA with multiple subunits within a uvsY hexamer is essential for high-affinity *uvs*Y-ssDNA binding. High-affinity uvsY-ssDNA binding is responsible in turn for the observed destabilization of gp32-ssDNA complexes, which is a prerequisite for the uvsY-dependent nucleation of *uvs*X-ssDNA presynaptic filament assembly. Studies of mutant uvsY and gp32 protein species have helped to delineate the roles of *uvs*Y-ssDNA, uvsY-gp32, and *uvs*Y-*uvs*X interactions in presynaptic filament assembly. Results are consistent with a model in which wrapping of ssDNA around a *uvs*Y hexamer destabilizes filaments of cooperatively bound gp32, making way for its displacement by incoming *uvs*X molecules. These ideas, along with results of recent efforts to determine the X-ray crystal structure of *uvs*Y-ssDNA complexes, will be presented and discussed.

The interaction of gp59 with the acidic, C-terminal "A-domain" of gp32 is essential for the assembly of gp41 helicase onto ssDNA previously coated with cooperatively bound gp32 molecules. We have employed fluorescence and sedimentation methods to determine the stoichiometry and hydrodynamic shape parameters of gp59 complexes formed with gp32 derivatives containing the A-domain. Our results place significant constraints on the location of the gp32 binding site within gp59, and on the probable orientation of gp59 molecule(s) at the DNA replication fork suggested by the recently published X-ray crystal structure of gp59 (Mueser *et al.*, J. Mol. Biol. 296, 597-612).

Together, *uvs*Y and gp59 play a critical role in the productive coupling of homologous recombination events to the initiation of T4 RDR. *Uvs*Y promotes presynaptic filament formation on 3' ssDNA-tailed chromosomes, the products of origin-specified replication, leading to strand invasion of a homologous chromosomal region and to the initiation of RDR. At the same time, *uvs*Y stabilizes *uvs*X bound to the invading strand, effectively preventing the assembly of gp41 helicase on the branch point. Gp41 helicase assembly is instead directed, via gp59-gp32 interactions and the adapter function of gp59, to the displaced strand of the D-loop/replication fork. This helicase partitioning mechanism enforced by the T4 RMPs guards against anti-recombination activity of the helicase and ensures that recombination intermediates formed by *uvs*X/*uvs*Y will efficiently be converted into semi-conservative DNA replication forks.

Title:Prof.
First Name:Gisela
Last Name:Mosig
Address:Vanderbilt University
Dept. of Molecular Biology
Box 1820 Sta. B
Nashville TN 37235 USA
Phone:615-322-3442
Fax:615-343-6707
E-mail:mosigg@ctrvax.vanderbilt.edu

Abstract Title:AN UNCONVENTIONAL USE OF ITERONS AT THE PHAGE T4 ORIGIN E OF DNA REPLICATION.

Abstract:MOSIG,G., HARVEY, A. AND VAISKUNAITE, R.
An Unconventional Use of Iterons at the Phage T4 Origin E of DNA Replication.
Vanderbilt University, Dept. of Molecular Biology, Nashville, TN 37235 USA

DNA replication of phage T4 is first initiated at one of several potential origins, using transcripts synthesized by host RNA polymerase as primers for leading strand DNA synthesis. Most subsequent replication is initiated from intermediates of recombination. Different origins are preferred under different physiological conditions. Specifically, origin E (oriE) is functional, when global torsional stress in the intracellular T4 DNA is reduced, or when the T4 transcriptional activator MotA is inactive. We have shown that different sequence elements and protein requirements allow functioning of oriE in a motA mutant (Vaiskunaite *et al.* 1999, J. Bacteriol. 181: 7115-25.)

Our recent results indicate that a small T4 protein (RepEB) encoded in the oriE region, and required for oriE function, binds to repeated sequences (iterons) upstream of the primer promoter, if, and only if these sequences are distorted., e.g., by heat denaturation or by RNA polymerase binding to the primer promoter. Transcription from the primer promoter does not depend on RepEB protein. Instead, RepEB affects priming by a primer precursor transcript approximately 2 kb downstream of the promoter and of the iterons. We propose that RepEB binding to distorted iterons can trap localized torsional stress, and facilitate origin function when global torsional stress is reduced. We suggest that RepEB facilitates priming by the 3'ends of the transcript by helping to load the replicative gene 41 helicase at the iteron DNA and that subsequent unwinding of downstream DNA by the helicase promotes formation of an R-loop. This hypothesis is supported, among others, by co-immuno precipitations of the gene 41 helicase with RepEB.

Title:Ms.
First Name:Gerry
Last Name:Carver
Address:Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709-2233
Phone:(919) 541-3458
Fax:(919) 541-7613
E-mail:carver@niehs.nih.gov

Abstract Title: Phage T4 rI as a Mutation-Reporter Gene.

CARVER, GERALDINE, BEBENEK, ANNA, DRESSMAN, HOLLY and DRAKE, JOHN W. Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709-2233

Both classically and today, most fundamental understanding of the mutation process derives from studies using either phage T4 or the bacterium *E. coli*. However, it has gradually become clear that many of the genetic systems used to monitor mutation in T4 have drawbacks. Reversion tests are limited in scope. The rII gene is too large for convenient sequencing, is insensitive to base-pair substitutions (BPSs), and is itself involved in DNA metabolism. The ac gene is also involved in DNA metabolism, and ac mutants are difficult to quantitate. We have recently turned to the rI gene, which is not involved in DNA metabolism, is easy to sequence, and was anticipated to be sensitive to BPSs. Mutations are first detected as traditional r plaques on *E. coli* BB cells, thus excluding rII and probably rIII mutations; about 60% of such mutations map to the classical rI gene. The frequency of spontaneous rI mutations is low, roughly 2 per 100,000, which makes collection tedious; however, this problem quickly disappears with, for instance, mutators. The rI spontaneous mutational spectrum comprises a mixture of BPSs, frameshift mutations, and mutations of large extent. Both warm and hot spots occur, but unfortunately lacks extreme (A:T)6 frameshift hot spots. Two kinds of remarkable complex mutations arise.

Title:Dr.
First Name:Jan
Last Name:Drake
Address:Laboratory of Molecular Genetics
National Institute of Environmental Health Sciences
Research Triangle Park, NC 27709-2233
Phone:(919) 541-3361
Fax:(919) 541-7613
E-mail:drake@niehs.nih.gov

Abstract Title: The fidelities of phage-RB69 DNA polymerases compared *in vivo* and *in vitro*

Abstract:BEBENEK, ANNA,* DRESSMAN, HOLLY,* KARAM, J. D.,Ý KONIGSBERG, W. H. and DRAKE, JOHN W.* Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709-2233, Department of Biochemistry, Tulane University Medical Center, New Orleans, LA, and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT

Phage RB69 DNA polymerase drives T4 DNA replication with the same high fidelity as is achieved with the T4 enzyme, but does not recombine with the diverged T4 gene. Thus, the fidelities of mutated gp43s can be explored both *in vivo* and *in vitro*. We have compared the fidelities of all four combinations of RB69 gp43s that vary in two ways: by a knockout of the proofreading exonuclease activity, and/or by a change in a key tyrosine residue near the polymerase active site. Both of these changes produce strong mutator activities. Neither the Exo defect nor the Pol substitution strongly affect gross Pol activity. Mutations are scored *in vivo* using a newly developed rI system, mutations *in vitro* using a lacZa system. Mutation rates measured *in vitro* are larger than those measured *in vivo* and include types not observed *in vivo*, including a bizarre and inexplicable five-base insertion. *In vivo*, the Exo defect produces similar numbers of transitions, transversions, and frameshift mutations, while this Pol mutator produces mostly transitions. The two mutators in combination are much less strong than the multiplicative product of the strengths of the individual mutators and display a spectrum of mutations intermediate between those characteristic of the two component mutators.

Title:Dr.
First Name:William
Last Name:McShan
Address:BMSB 1053
Dept. of Microbiology and Immunology
Univ. of Oklahoma Health Sciences Center
940 S.L. Young Blvd.
Oklahoma City, OK 73190
USA
Phone:405-271-1202
Fax:405-271-3117
E-mail:William-McShan@ouhsc.edu

Abstract Title:The Endogenous Bacteriophages of *Streptococcus pyogenes* strain SF370 discovered by Whole Genome Sequencing: Implications for Pathogenesis.

Abstract:The association of Streptococcus pyogenes and temperate bacteriophages has long been known, and so it was anticipated that complete or partial phage genomes would be discovered in the genome of SF370, the strain that was the focus of genomic DNA sequencing at the University of Oklahoma. One phage, phage 370.1, containing the gene for erythrogenic toxin C (pyrogenic exotoxin C; speC) and inducible by mitomycin C, was known to be part of the SF370 genome at the onset of sequencing. Whole genome DNA sequencing has revealed the presence of several additional endogenous bacteriophage genomes: two more apparently complete genomes (phages 370.2 and 370.3) and one partial genome (phage 370.4). Phylogenetic analysis shows that phages 370.1 and 370.3 are closely related, with phages 370.2 and 370.4 less related to the other two phages as well as each other. These phage genomes are not clustered in the host genome, but are evenly distributed on the streptococcal chromosome. Three of the phages, 370.1, 370.3, and 370.4, are oriented in the same direction with only phage 370.2 oriented antiparallel to the others. A number of common features are observed by the three complete genomes, including the presence of a hyaluronidase, probable lysin genes, and an integrase. The chromosomal targets for integration of these temperate streptococcal bacteriophages are all contained within host genes, and the DNA duplication is such that the particular host gene remains uninterrupted after integration. Comparative analysis of the phage genomes shows that unique regions as well as regions with sequences common to all of the complete phages are present suggesting that horizontal transfer and recombination have played important roles in phage diversification. The most striking feature shared by these three phages is the presence of one or more genes that are either known to be associated with streptococcal virulence or are highly homologous to such genes. Only one of these phage-associated genes (*spe*C) has been previously described. One new putative virulence factor gene (mf2) was identified in phage 370.1, two genes identified in phage 370.2 (*spe*H and *spe*I), and at least one gene identified in phage 370.3 (mf3). The expression patterns of these genes are not yet known. The phages harbored in the streptococcal genome provide a rich source of mobile genetic material for the transfer of virulence genes, and the information gathered from the sequencing of *S. pyogenes* SF370 should provide a springboard for future studies of the relation between bacteriophages and GAS virulence.

First Name:Shigenobu
Last Name:Matsuzaki
Address:Department of Microbiology
Kochi Medical School
Oko, Nankoku, Kochi 783-8505
Japan
Phone:81-88-880-2323
Fax:81-88-880-2324
E-mail:matuzaki@kochi-ms.ac.jp

Abstract Title: Phylogenetic Relationship between Coliphage T4 and Vibriophage KVP40

Abstract:MATSUZAKI, S., INOUE, T., TANAKA, S., KURODA, M., KIMURA, S., AND IMAI, S. Kochi Medical School; Dept. of Microbiology, Oko, Nankoku, Kochi 783-8505, Japan

We have described a lytic vibriophage KVP40, isolated from seawater with a *Vibrio parahaemolyticus* strain as the host. KVP40 is morphologically similar to T-even coliphages, but has a longer head (140 nm long and 70 nm wide) with a much larger DNA (250 kbp). The present study is a further investigation of the phylogenetic relationship between T4 and KVP40.

(1) A 10.8 kbp region of KVP40 genome including the major capsid protein (Mcp) gene was sequenced. The mcp gene and the region immediately upstream of it (9 kbp) were fairly homologous to the corresponding region of T4 genome, and contained 9 ORFs homologous to T4 genes 17 (DNA packaging protein), 18 (tail sheath protein), 19 (tail tube protein), 20 (head portal protein), 67 (prohead core protein), 68 (prohead core protein), 21 (prohead protease), 22 (prohead core protein), and 23 (major head protein) in the same order as in T4. However, the 1.8 kbp region immediately downstream of the mcp gene was not homologous to the corresponding region of T4 genome. This suggests that the gene group specifying major head and tail proteins in T4 and KVP40 has been evolved as a unit.

(2) We examined the ability of KVP40 Mcp to replace T4 gp23* (major head protein) during T4 morphogenesis, by immunogold electron microscopy using antibodies specific for KVP40 Mcp. The results showed that KVP40 Mcp can partly substitute for T4 gp23* without affecting infectivity or head size of the virion.

These facts, together with the morphological similarity, strongly suggest that T4 and KVP40 are phylogenetically related, and mechanism of phage head assembly has been conserved during evolution of them.

First Name:Derrick
Last Name:Lenz
Address:15 Woodlands Place
Pompton Plains, NJ 07444
Phone:973-835-7552
Fax:973-408-3824
E-mail:dlenz@drew.edu

Abstract Title:Discovery of pertussis toxin sequences present in *Bordetella avium* bacteriophages.

Abstract: LENZ, D. AND TEMPLE, L. Drew University Dept. of Biology, 36 Madison Avenue, Madison, NJ 07444 USA.

Genetic information that is passed directly from one bacterium to the next could cause major changes in the virulence of a bacterial pathogen. The discovery of genes in a bacteriophage that code for pertussis toxin suggests that horizontal transmission of factors causing disease could be occurring. Pertussis toxin is the major virulence factor of *Bordetella pertussis*, the causative agent of whooping cough. Preliminary data indicates that the genes for this toxin are present in a bacteriophage that infects *Bordetella avium*, a bacterium that causes, in birds, a respiratory tract disease similar to whooping cough. Pertussis toxin and the genes encoding it have been reported to be absent in *B. aviumi* and are considered not to be a factor in the avian form of the disease. Why it is present in a bacteriophage, which is a vehicle for the horizontal transmission of genetic information, is a fascinating question. The first step to answering this question is to determine how much of the pertussis toxin genes *B. aviumi* bacteriophages possess to see if these genes could produce active toxin.

In order to determine how much of the pertussis toxin operon is present in the bacteriophage genome, the polymerase chain reaction (PCR) was used to amplify portions of the pertussis toxin operon from the bacteriophage genome. An 800 basepair portion, highly homologous to the pertussis toxin sequence, was amplified, cloned, and sequenced from two phage strains. A second, different PCR product of 1500 basepairs was also amplified, cloned and sequenced from two *B. aviumi* phage strains. Southern blotting has also been performed to identify the location of the toxin sequences on specific restriction fragments. Since these phages possess most of the sequence coding for the pertussis toxin subunits in a highly conserved form, the next step will be to investigate the role these bacteriophages play in the pathogenesis of their natural host, *B. avium*.

REGISTRANTS	
Title:Dr. First Name:Stephen T. Last Name:Abedon E-mail:microdude+@osu.edu Postal Address:The Ohio State Univ. Department of Microbiology 1680 University Drive Mansfield, OH 44906 Telephone (work):419-755-4343 Occupation:Professor Fax:419-755-4327	Title:Dr. First Name:Hans-W. Last Name:Ackermann E-mail:ackermann@mcb.ulaval.ca Postal Address:Dept. of Medical Biology Faculty of Medicine, Laval University Quebec, QC, G1K 7P4 Telephone (work):418-656-2131 ext. 2558 Occupation:Professor Fax:418-656-7666
Title:Dr. First Name:Revaz Last Name:Adamia E-mail:torrey@erols.com Postal Address:Eliava Institute Of Bacteriophage, Microbiology and Epidemiology Georgian Academy of Sciences 3 Gotua St. Tbilisi (Georgia) Occupation:Researcher/Scientist Fax:410-625-2244	Title:Mr. First Name:Faiz Last Name:Ahmad E-mail:fahmad8@po-box.mcgill.ca Dept. of Microbiology & Immunology McGill University 3775 University St. Montreal, QC H3A 2B4 Occupation:Honours Student
Title:Dr. First Name:Zemphira Last Name:Alavidze E-mail:zalavidz@medicine.umaryland.edu Postal Address:University of Maryland, Shchool of Medicine, Department of Hospital Epidemiology. 10 S. Pine St. - MSTF Bld., Rm 9-17. Baltimore, MD USA 21201 Telephone (work):410-706-4586 Occupation:Postdoctoral Fellow	Title:Mr. First Name:David C. Last Name:Alexander e-mail: dalex@microimm.mcgill.ca Dept. of Microbiology & Immunology McGill University 3775 University St. Montreal, QC (Canada) H3A 2B4 Tel:514-398-4434 Occupation: Graduate Student
Title:M. First Name:Costanzo Last Name:Allione E-mail:costanzoa@hotmail.com Postal Address:1507 Eastbay Dr. NE Olympia,WA. 98506 Telephone (work):360-866-6000 ext.6523 Occupation:Undergraduate Student	Title:Dr. First Name:Francis F Last Name:Arhin E-mail:farhin@phagetech.com Postal Address:PhageTech Inc. C.P. 387, Place du Parc Montreal, Quebec (Canada) H2W 2N9 Telephone (work):514-282-0990 Occupation:Researcher/Scientist Fax:514-282-9889

Title:Dr. First Name:Pascale Last Name:Bauda E-mail:bauda@phagetech.com Postal Address:C.P. 387, Place du Parc Montreal, Qc H2W 2N9 Telephone (work):514-282-0990 Occupation:Researcher/Scientist Fax:514-282-9889	Title:Dr. First Name:Dominique Last Name:Bergeron E-mail:bergerdo@phagetech.com Postal Address:PhageTech Inc C.P. 387, Place du Parc Montreal (Quebec) H2W 2N9 Telephone (work):514-282-0990 Occupation:Researcher/Scientist Fax:514-282-9889
Title:Dr. First Name:Lindsay Last Name:Black E-mail:lblack@umaryland.edu Postal Address:Dept. of Biochemistry and Molecular Biology University of Maryland Medical School University of Maryland, Baltimore 108 N. Greene Street, Baltimore, Md. 21201-1503 USA Occupation:Professor Fax:410-706-8297	Title:Professor First Name:Udo Last Name:Bläsi E-mail:Udo@gem.univie.ac.at Postal Address:Vienna Biocenter Dr. Bohrgasse 9 1030 Vienna, Austria Telephone (work):+43-1-4277-54609 Occupation:Professor Fax:+43-1-4277-9546
Title:Ms. First Name:Jill Last Name:Bleuit E-mail:jbleuit@zoo.uvm.edu Postal Address:Room B411 Given Building Dept of Biochemistry University of Vermont Burlington, VT 05405 Telephone (work):(802) 656-8244 Occupation:Graduate Student	Title:Dr. First Name:Torrey Last Name:Brown E-mail:Torrey@erols.com Postal Address:Intralytix, Inc. 351 W. Camden St. Suite 100 Baltimore, Maryland 21201 Telephone (work):410-659-7330 Occupation:Other Fax:410-625-2244
Title:Dr. First Name:Harald Last Name:Bruessow E-mail:harald.bruessow@rdls.nestle.com Postal Address:Nestlé Research Center Vers-chez-les-Blanc P.O. Box 44 CH-1000 Lausanne 26 Switzerland Telephone (work):+41/21-785 86 76 Occupation:Researcher/Scientist Fax:+41/21/785 89 25	Title:Dr. Last Name:Burbutashvili First Name: T. Institute of Bacteriophage Dept. of Microbiology & Virology Gotua St. 3, Tbilisi 380060 (Georgia) e-mail: c/o tevdorav@hotmail.com

Title:Professor First Name:Richard Last Name:Calendar E-mail:rishard@socrates.berkeley.edu Postal Address:MCB-401 Barker Hall University of California, Berkeley, CA Telephone (work):510-642-5951 Occupation:Professor Fax:510-643-6791	Title:Dr. First Name:Richard Last Name:Carlton E-mail:carltonebi@erols.com Postal Address:Exponential Biotherapies, Inc., 150 Main Street Port Washington, NY 11050 Telephone (work):516-883-6883 Occupation:Other Fax:516-883-6903
Title:Ms. First Name:Geraldine Last Name:Carver E-mail:carver@niehs.nih.gov Postal Address:Geraldine Carver N.I.E.H.S. P.O. Box 12233 MD E-01 R.T.P., N.C. 27709 Telephone (work):919-541-3458 Occupation:Technician Fax:919 541 7613	Title:Ms. First Name:Nicha Last Name:Charoensri E-mail:nicha@microimm.mcgill.ca Postal Address:3775 University St, Department of Microbiology and Immunology Montreal, Quebec, H3A 2B4 Telephone (work):514-398-4434 Occupation:Graduate Student
Title:Dr. First Name:Gail Last Name:Christie E-mail:christie@hsc.vcu.edu Postal Address:Department of Microbiology & Immunology Virginia Commonwealth University PO Box 980678, Richmond, VA 23298-0678 USA Telephone (work):804-828-9093 Occupation:Professor Fax:804-828-9946	Title:Dr. First Name:David Last Name:Coombs E-mail:dhc@unb.ca Postal Address:Biology Dept. University of New Brunswick Fredericton, NB Canada E3B 6E1 Telephone (work): 506-453-4812 Occupation:Professor Fax:506-453-3583
Title:Mr. First Name:Timothy Last Name:Dean E-mail:trdean@unity.ncsu.edu Postal Address:NC State University Dept. of Microbiology Box 7615 Raleigh, NC 27695 Telephone (work): 919-515-7230 Occupation:Graduate Student Fax:919-515-7867	Title:Dr. First Name:Mohammed Last Name:Dehbi E-mail:mdehbi@phagetech.com Postal Address:Phagetech Inc PO. Box 387, Place du Parc Montreal, Qc, H2W 2N9 Telephone (work):514-282-0990 Occupation:Researcher/Scientist Fax:514-282-9889

Title: Mlle. First Name:Carine Last Name:Denoux E-mail:carined@microimm.mcgill.ca Postal Address:Department of Microbiology and Immunology McGill Universirty 3775 University Street Montreal, Quebec, Canada H3A 2B4 Telephone (work):514-398-4434 Occupation:Technician Fax:514-398-7052	Title:Dr. First Name:Katherine Last Name:DeYoung E-mail: Katherine.deyoung@biology.gatech.edu Postal Address:Katherine DeYoung School of Biology - Georgia Tech Atlanta, Georgia 30332 Telephone (work):404-894-8998 Occupation:Professor Fax:404-894-0519
Title:Mr. First Name:Hojabr Last Name:Dezfulian E-mail:dezfulih@magellan.umontreal.ca Postal Address:Universite de Montreal, Faculte de Medicine Veterinaire, Dept. Pathologie Telephone (work):450-773-8521 X8377 Occupation:Graduate Student Fax:450-778-8108	Title:Dr. First Name:John Last Name:Drake E-mail:drake@niehs.nih.gov Postal Address:Laboratory of Molecular Genetics, E3-01, National Institute of Environmental Health Sciences P.O. Box 12233, Research Triangle Park, NC 27709 Telephone (work): 919-541-3361 Occupation:Researcher/Scientist Fax:919-541-7613
Title: Dr. First Name:Michael Last Name:DuBow E-mail:msdubow@microimm.mcgill.ca Postal Address:Department of Microbiology and Immunology McGill University 3775 University Street Montreal, Quebec, Canada H3A 2B4 Telephone (work):514-398-3926 Occupation:Professor Fax:514-398-7052	Title:Dr. First Name:Donna Last Name:Duckworth E-mail:duckwort@mgm.ufl.edu Postal Address:Department of Molecular Genetics and Microbiology Box 100266, Health Science Center University of Florida Gainesville, Fl 32610 Telephone (work):352-392-0681 Occupation:Professor Fax:352-392-3133

Title:Dr. First Name:M. Last Name:Dzuliashvili Eliava Inst. of Bacteriophage Microbiology & Virology Of Georgian Academy Thd@caucasus.net	Title:Dr. First Name:Bentley Last Name:Fane E-mail:bfane@u.arizona.edu Postal Address:Department of Vet. Sci and Microbiology Building #90, U. of Arizona Tucson, AZ, 85721 Telephone (work):520-626-6634 Occupation:Professor Fax:520-621-6366
Title:Dr. First Name:Vincent Last Name:Ferretti E-mail:ferretti@phagetech.com Postal Address: Phage Tech Inc. C.P. 387, Place du Parc Montreal, QC H2W 2N9 Telephone: (514)282-0990 Occupation: Researcher/Scientist Fax: (514) 282-9889	Title:Dr. First Name:K. Last Name:Gachechiladze Eliava Inst. Of Bacteriophage Microbiology & Virology Of Georgian Academy Thd@caucasus.net
Title:Mr. First Name:Alfred Last Name:Gertler E-mail:73130.2055@compuserve.com Postal Address:239 Manning Avenue Toronto, Ontario M6J 2K8 Canada Telephone (work):(416) 603-9063 Occupation:Other Fax:(514) 933-1702 (family fax in Mtl.)	Title:Mr. First Name:Jason Last Name:Gill Address:Department of Food Science University of Guelph Guelph, ON N1G 2W1 Phone:(519) 823-5519 E-mail:jasongil@uoguelph.ca
Title:Mr. First Name:Lawrence Last Name:Goodridge E-mail:lgoodrid@uoguelph.ca Postal Address:Dept. of Food Science, University of Guelph, Guelph, ON N1G 2W1 Telephone (work):519-824-4120 X 3911 Occupation:Graduate Student Fax:519-824-6631	Title:Dr. First Name: Nuban Last Name: Ha E-mail:hun@phagetech.com Postal Address:PhageTech Inc C.P. 387, Place du Parc Montreal (Quebec) H2W 2N9 Telephone (work):514-282-0990 Occupation:Researcher/Scientist Fax:514-282-9889

Title:Dr. First Name:Cameron Last Name:Haase-Pettingell E-mail:chaase@mit.edu Postal Address:68-330 Biology Dept. MIT 77 Mass. Ave Cambridge, MA 02139 Telephone (work):617-253-3545 Occupation:Researcher/Scientist Fax:617-251843	Title:Dr. First Name:Dwight Last Name: Hall E-mail:dwight.hall@biology.gatech.edu SCHOOL OF BIOLOGY GEORGIA TECH 310 FERST DRIVE ATLANTA, GA 30332-0230 (USA) Telephone (work): 404-894-3702 Occupation:Professor Fax:404-894-0519
Title:Dr. First Name:Sidney Last Name:Hayes E-mail:hayes@duke.usask.ca Dept. of Microbiology and Immunology College of Medicine University of Saskatchewan Saskatoon, SK S7N 5E5 Telephone (work):306-966-4307 Occupation:Professor Fax:306-966-4311	Title:Mr. First Name:Michel Last Name:Houde E-mail:Michel.Houde@nrc.ca Postal Address:Biophage Inc. 6100 Royalmount Montréal, Québec H4P 2R2 Telephone (work):514-496-1488 Occupation:Researcher/Scientist Fax:514-496-1521
Title:Ms. First Name:Lisa Last Name:Huang E-mail:lhuang@vet.purdue.edu Postal Address:Purdue Cancer Research Center-Hansen B-036 Purdue University-West Lafayette IN 47907 Telephone (work):765-494-0506 Occupation:Graduate Student Fax:765-496-1795	Title:Dr. First Name:Kenji Last Name:Iwasaki E-mail:Kenji_Iwasaki@nih.gov Postal Address:LSB, NIAMS, NIH, Bldg. 6, Rm. B2-27, MSC-2717, Bethesda, MD20892 Telephone (work):301-435-2587 Occupation:Postdoctoral Fellow Fax:301-480-2717
Title:Dr. First Name:Ru-zhang Last Name:Jiang E-mail:ruzhanhjiang@hotmail.com Dept. of Microbiology & Immunology McGill University 3775 University St. Montreal, QC H3A 2B4 (Canada) Telephone (work):514-398-4434 Occupation:Researcher/Scientist	Title:Dr. First Name:Laura Last Name:Kasman E-mail:kasmanl@musc.edu Postal Address:Department of Microbiology & Immunology PO Box 250504 Medical University of South Carolina 171 Ashley Avenue Charleston, South Carolina 29425 Telephone (work):843-792-8499 Occupation:Postdoctoral Fellow Fax:843-792-2464

Title: Ms. First Name: Irinia Last Name: Kovalyova e-mail: 8ivk@qlink.queensu.ca Dept. of Microbiology & Immunology Queen's University Kingston, ON K7L 3N6 (Canada) Tel.:613-533-2450 Occupation: Graduate Student Fax:613-533-6796	Title:Dr. First Name: Andrew M. Last Name: Kropinski e-mail: kropinsk@post.queensu.ca Dept. of Microbiology & Immunology Queen's University Kingston, ON K7L 3N6 (Canada) Tel.:613-533-2459 Fax:613-533-6796
Title:Dr. First Name:Mzia Last Name:Kutateladze E-mail:torrey@erols.com Postal Address:Eliava Institute of Bacteriophage, Microbiology and Epiemiology Georgian Academy of Sciences 3 Gotua St., Tbilisi - Georgia Occupation:Researcher/Scientist Fax:410-625-2244	Title:Dr. First Name:Elizabeth Last Name:Kutter E-mail:t4phage@evergreen.edu or kutterb@evergreen.edu or phagebiotics@home.com Postal Address:The Evergreen State College - Lab I - Olympia, WA 98505 Telephone (work):360-866-6000 X 6099 Occupation:Professor Fax:360-867-5430
Title:Dr. First Name:T. Last Name:Kvelashvili Eliava Inst. Of Bacteriophage Microbiology & Virology Of Georgian Academy Thd@caucasus.net	Title:Ms. First Name:Marie-Hélène Last Name:Laprise E-mail:htetmhl@interlinx.qc.ca Postal Address:1453, rue McManamy Sherbrooke, Québec J1H 2P3 Telephone (work):819-820-6868 ext. 15846 Occupation:Graduate Student
Title:Mr. First Name:Derrick Last Name:Lenz E-mail:dlenz@drew.edu Postal Address:15 Woodlands Place Pompton Plains, NJ 07444 Telephone (work):973-408-3119 Occupation:Graduate Student Fax:973-408-3824	Title:Dr. First Name:Jing Last Name:Liu E-mail:Jliu@phagetech.com Postal Address:C.P.387, Place du Parc, Montreal, Quebec, Canada H2W 2N9 Telephone (work):514-282-0990 Occupation:Researcher/Scientist Fax:514-282-9889

Title:Dr. First Name: Malgorzata Last Name: Lobocka e-mail: lobocka@ibb.waw.pl Dept. of Microbial Biochemistry Institute of Biochemistry & Biophysics PAS, (Ul. Pawinskiego 5A, 02-106 Warsaw, POLAND) Tel.: 01148228237188 Occupation: Researcher/Scientist FAX: 0114839121623	Title:Mr. First Name:Yuefei Last Name:Lou E-mail:ylou@playfair.utoronto.ca Toronto, ON Telephone (work):416-603-5420 Occupation:Graduate Student
Title:Dr. First Name:Jack Last Name:Maniloff E-mail:jkmf@mail.rochester.edu Postal Address:Department of Microbiology and Immunology University of Rochester Medical Center Box 672 Rochester, NY 14642-0001 USA Telephone (work):716-275-3413 Occupation:Professor Fax:716-473-9573	Title:Dr. First Name:Shigenobu Last Name:Matsuzaki E-mail:matuzaki@kochi-ms.ac.jp Postal Address:Department of Microbiology Kochi Medical School Oko, Nankoku, Kochi 783-8505 Japan Occupation:Researcher/Scientist Fax:81-88-880-2324
Title:Dr. First Name:William Last Name:McShan E-mail:William-McShan@ouhsc.edu Postal Address:BMSB 1053 Dept. of Microbiology & Immunology U. Oklahoma Health Sciences Center 940 S.L. Young Blvd. Oklahoma City, OK 73190 USA Telephone (work):405-271-1202 Occupation:Researcher/Scientist Fax:405-271-3117	Title:Dr. First Name:Eric Last Name:Miller E-mail:esm@mbio.ncsu.edu Postal Address:Department of Microbiology Box 7615 North Carolina State University Raleigh, NC 27695 USA Telephone (work):919-515-7922 Occupation:Professor Fax:919-515-7867

Title:Ms. First Name:Princess Last Name:Mmolawa E-mail:princess.mmolawa@imvs.sa.gov.au Postal Address:IMVS, Infectious Diseases Laboratories – Box 14 P.O. Rundle Mall Adelaide - South Australia 5000 Telephone (work):+618 82223274 Occupation:Graduate Student Fax:+618 82223543	Title:Mr. First Name:Michael Last Name:Moak E-mail:moakm.e@mail.utexas.edu Postal Address:700 E.24th St (Esb 502) Dept. of Molecular Genetics and Microbiology - University of Texas at Austin - Austin, Texas 78712 Telephone (work):512-471-3143 Occupation:Graduate Student Fax: 512-471-7088
Title:Dr. First Name:Greg Last Name:Moeck E-mail:greg.moeck@phagetech.com Postal Address:PhageTech, Inc. C.P. 387, Place du Parc Montreal, QC H2W 2N9 Telephone (work):514-282-0990 Occupation:Researcher/Scientist Fax:514-282-9889	Title:Professor First Name:Ian Last Name:Molineux E-mail:molineux@mail.utexas.edu Postal Address:Molecular Genetics & Microbiology, University of Texas, Austin, TX 78712-1095 USA Telephone (work):512-471-3143 Occupation:Professor Fax:512-471-7088
Title:Ms. First Name: Sutton Last Name:Mooney E-mail:smooney@mail.utexas.edu Postal Address:5504 Joe Sayers Ave. Austin, Texas 78756 Telephone (work):512-471-3143 Occupation:Graduate Student Fax:512-471-7088	Title:Dr. First Name:Scott Last Name:Morrical E-mail:smorrica@zoo.uvm.edu Postal Address:Dept. of Biochemistry Univ. of Vermont College of Medicine B411A Given Bldg. Burlington, VT 05404 Telephone (work):802-656-8260 Occupation:Professor Fax:802-862-8229
Title:Ms. First Name:Ariana Last Name:Murata E-mail:amurat1@po-box.mcgill.ca Dept. of Microbiology & Immunology McGill University 3775 University St. Montreal, QC H3A 2B4 Telephone (work):514-398-4434 Occupation:Honours Student	Title:M. First Name:Kishore Last Name:Murthy E-mail:Kishore.Murthy@nrc.ca Postal Address:Biophage Inc. 6100 Royalmount Montreal, Quebec H4P 2R2 Telephone (work):514-496-1488 Occupation:Researcher/Scientist Fax:514-496-1521

Title:Dr. First Name:Rimas Last Name:Nivinskas E-mail:rimasn@bchi.lt Institute of Biochemistry Mokslininku 12 Vilnius 2600 Lithuania Telephone (work): 370-272-9146 Occupation:Researcher/Scientist Fax: 370-272-9196	Title:Mr. First Name:Patrick Last Name:Noack E-mail:micptn@ttuhsc.edu Postal Address:Dept. of Microbiology, TTUHSC Rm. 5B191 3601 4th Street Lubbock, TX 79430
Title:Dr. First Name:James Last Name:Norris E-mail:norrisjs@musc.edu Postal Address:Department of Microbiology & Immunology PO Box 250504 Medical University of South Carolina 171 Ashley Avenue Charleston, South Carolina 29425 Telephone (work):843-792-7915 Occupation:Professor Fax:843-792-2464	Title:Dr. First Name:Erin Last Name:O'Reilly E-mail:oreilly@abacus.mc.duke.edu Postal Address:Duke University Medical Center Department of Microbiology Box 3020 Jones Building Durham, NC 27710 Telephone (work):919-684-2566 Occupation:Postdoctoral Fellow Fax:919-681-8911
Title:Ms. First Name:Maria Last Name:Pajunen E-mail:maria.pajunen@utu.fi Postal Address:University of Turku Department of Medical Biochemistry Kiinamyllynkatu 10, 20520 Turku FINLAND (Europe) Telephone (work):+358 2 333 7444 Occupation:Graduate Student Fax:+358 2 333 7229	Title:M. First Name:Erlan Last Name:Ramanculov E-mail:erlan@tamu.edu Postal Address:Department of Biochemistry and Biophysics, Rm. 301, Texas A&M University, College Station, TX, 77843 Telephone (work):979-845-2853 Occupation:Graduate Student Fax:979-862-4718
Title:Professor First Name:Linda Last Name:Reha-Krantz E-mail:LREHA@gpu.srv.ualberta.ca Department of Biological Sciences CW405 BioSciences Building University of Alberta Edmonton, Alberta T6G 2E9 (Canada) Telephone (work):780-492-5383 Occupation:Professor Fax:780-492-9234	Title:Mr. First Name:William Last Name:Robins E-mail:robinsbp@mail.utexas.edu Postal Address:University of Texas MGM Department ESB#502 Austin,TX 78712 Telephone (work):512-471-3143 Occupation:Graduate Student Home Address:421 W. San Antonio #J San Marcos, TX 78666

Title:Ms. First Name:Cheryl Last Name:Runke E-mail:cheryl.runke@utoronto.ca Toronto, Ontario Canada Occupation:Undergraduate Student	Title:Dr. First Name:Parviz Last Name:Sabour E-mail:sabourp@em.agr.ca Agriculture & Agri-Food Canada Food Research Program 93 Stone Road, West Guelph, Ontario N1G 5C9 Tel.:519-829-2400, Ext. 3101 Occupation:Researcher/Scientist Fax:519-829-2600
Title:Ms. First Name:Diane Last Name:Schaak E-mail:schaak@rowland.org Rowland Institute for Science 100 Edwin Land Blvd Cambridge Mass 02142 Telephone (work):617-497-4679 Occupation:Researcher/Scientist Fax:617-497-4627	Title:Dr. First Name:David Last Name:Schofield E-mail:schofida@musc.edu Dept. of Microbiology & Immunology PO Box 250504 Medical University of South Carolina 171 Ashley Avenue Charleston, South Carolina 29425 Telephone (work):843-792-8499 Occupation:Postdoctoral Fellow Fax:(843) 792-2464
Title: Dr. First Name:Tapas Last Name:Sengupta E-mail:senguptk@musc.edu Postal Address:Department of Biochemistry & Molecular Biology. Medical University of South Carolina BSB # 746 - 171 Ashley Avenue Charleston, SC 29425 (USA) Telephone (work):843-792-9932 Occupation:Postdoctoral Fellow Fax:843-792-4850	Title:Professor First Name:Richard Last Name:Sharp E-mail:richard.sharp@camr.org.uk The Centre for Applied Microbiology, Porton Down, Salisbury, SP4 OJG (United Kingdom) Telephone (work):00 44 1980 612443 Occupation:Professor Fax:00 44 1980 612731
Title:Mr. First Name: Anthony LastName: Smithyman E-mail: smithyman@cellabs.com.au P.O. Box 421 Brookvale, Sydney NSW 2100 Australia Telephone:61-2-9905-0133 Fax 61-2-9905-6426	Title:Mr. First Name:Brad Last Name:Stohr E-mail:stohr@abacus.mc.duke.edu Postal Address:Box 3020 Duke University Medical Center Durham, NC 27710 Telephone (work):919-684-2566 Occupation:Graduate Student Fax:919-681-8911

Title:Dr. First Name:David Last Name:Stopar E-mail:david.stopar@uni-lj.si Postal Address:Univeristy of Ljubljana Biotechnical Faculty, Dept. of Food Technology, Vecna pot 111 1000 Ljubljana, Slovenia Telephone (work):+386-61-123-33-88 Occupation:Researcher/Scientist Fax:+386-61-273-390	Title:Dr. First Name:Alexander Last Name:Sulakvelidze E-mail:asulakve@medicine.umaryland.edu Univ. of Maryland School of Medicine Department of Epidemiology and Preventive Medicine MSTF Bldg., Room 9-34 10 S. Pine Street Baltimore, MD 21201 USA Telephone (work):410-706-4587 Occupation:Professor Fax:410-706-4581
Title:Dr. First Name:William Last Name:Summers E-mail:william.summers@yale.edu Postal Address:Radiobiology Labs Yale University 333 Cedar Street New Haven, CT 06520-8040 Telephone (work):203-785-2986 Occupation:Professor Fax:203-785-6309	Title:Mr. First Name:Patrick Last Name:Suthers E-mail:suthers@cae.wisc.edu University of Wisconsin-Madison Department of Chemical Engineering 1415 Engineering Drive Madison, WI 53706 Telephone (work):608-262-8450 Occupation:Graduate Student Fax:608-262-5434
Title:Dr. First Name:Louise Last Name:Temple E-mail:ltemple@drew.edu Postal Address:Department of Biology Drew University 36 Madison Avenue Madison, NJ 07940 Telephone (work):973-408-3119 Occupation:Professor Fax:973-408-3824	Title:Dr. First Name:Gabriel (Gabi) Last Name:Teper E-mail:spring@gezernet.co.il Spring Diagnostics LTD - Building 13A Kiryat Weizman Science Park Ness-Ziona 70400 (Israel) Telephone (work):+972-8-9380363 Occupation:Postdoctoral Fellow Fax:+972-8-9408085
Title:Mr. First Name:Madani Last Name:Thiam E-mail:mbthiam@microimm.mcgill.ca Dept. of Microbiology & Immunology McGill University 3775 University Street, Room D-21 Montreal, Quebec, Canada H3A 2B4 Telephone (work):514-398-4434 Occupation:Graduate Student Fax:514-398-7052	Title:Ms. First Name:Chelsea Last Name:Thomas E-mail:chelseatho@hotmail.com Postal Address:Evergreen State College 2700 Evergreen Pkwy NW Olympia, WA 98505 Telephone (work):(360)866-6000 x6523 Occupation:Undergraduate Student

Title:Dr. First Name:Eugene Last Name:Rooney E-mail:eurol@prodigy.net Postal Address:5628 Ottawa Road, Centreville, VA 20120 Telephone (work):703-830-5247 Occupation:Scientist Fax:703-533-5539	Title:Mr. First Name:William B. Last Name:McCall Postal Address:4711 40th Street North, Arlington, VA 22207 USA Telephone (work):703-241-2111 Occupation:Business/Lawyer Fax:703-533-5539