Virus-Based Technologies

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Viruses are by far the most abundant biological entities on earth. In the oceans they comprising approximately 94% of the nucleic-acid-containing particles.

However, because of their small size they comprise only about 5% of the biomass.
Phage viruses influence planetary geochemical processes such as the production of oxygen with up to 50 million particles per ml of surface ocean water.

The viral shunt moves material from heterotrophs and photoautotrophs (represented by red and green arrows, respectively) into particulate organic matter (POM) and dissolved organic matter (DOM).
We are continually exposed to viruses via infection, air, water and food. Fortunately, viruses are highly host specific. Thus, the vast majority of viruses that we come in contact with do not infect humans.

Examples of Plant Viruses in our environment:

1. Tomato mosaic virus has been found in fog events along the east coast. (C. Bachand, J. Castello, SUNY ESF)
2. Tobacco mosaic virus is a common component of cigarette smoke.
3. Tobasco sauce contains $10^7$ pepper viruses per ml. (Colson et al., 2010, PLOS One)

HONR269V: Virus Hunting

Fall Semesters

Plant viruses make up the bulk of RNA viruses present in the human gut (Zhang et al., 2006, PLOS Biol).
Symbiotic Viruses: three way interaction between a virus, a fungus and a plant

A mutualistic association between a fungal endophyte and a tropical panic grass allows both organisms to grow at high soil temperatures. A mycovirus infecting the fungal endophyte is required for the enhanced temperature tolerance.

Marilyn J. Roossinck Lab
Science 26 January 2007: _ol._ 315, no. 5811, pp. 513-515
Symbiotic Viruses:


_Campoletis sonorensis Ichnovirus_
- DS DNA virus
- Integrated in wasp genome as provirus
- Transmitted in wasp via germline
- Virion form only in reproductive tissues
- Wasp inoculate lepidopteran hosts as they deposit eggs
- Virus infects lepidopteran host immune cells
- Expresses a cys-motif protein that disrupts lepidopteran immune system
~8% of the Human Genome is Composed of Retroviral genomes.

Viral Elements Control Host Gene Expression
e.x. Human Amylase Expression in Syliva

Viral proteins have been purloined by the host for specific functions
e.x. viral glycoproteins are key to placental formation
Therapeutics, Tools and Vectors
Bio-control of Bacterial Pathogens Using Lytic Phage

- E. coli 0157
- Salmonella
- Clostridium
- Pseudomonas aeruginosa
- Staphylococcus aureus
- Listeria monocytogenes

Food Safety
Spray on meat

Environmental Sanitation
Spray in feed lots

Veterinary Applications
Animal feed

Human Therapeutics
Place on wounds

PhagoDerm
Viruses as bio-control agents for invasive species

TMGMV - *SolviNix™*
- A bio-control agent for soda apple
  induces systemic necrosis
- Infected plants are also more susceptible to herbicide treatments

*Tropical soda apple*
TMGMV: Some Field Application Methods

- Mow & spray
- Chain-link fence application
- Carpet application
- High-pressure foliar spray

Using Virus Sequences To Engineer Plants for Disease Resistance

RNAi Silencing Overview

Most plant viruses are RNA viruses that replicate through a double-stranded RNA intermediate.

Viral ssRNA

Virus-encoded RNA-dependent RNA polymerase

Viral dsRNA

Double-stranded RNA is cleaved by DCL to produce siRNA which associates with AGO to silence virus replication and expression.

siRNAs target viral RNA for degradation or inactivation via AGO / RISC complex
The first commercialized resistance transgene

Tricoli et al. (1995)
Bio/Technology
13:1458-1465

Fuchs & Gonsalves (1995)
Bio/Technology
13:1466-1473
28 months after transplanting, at which time the severely *Papaya ringspot virus* (PRSV)-infected matrix plants (Sunrise) are clearly distinguishable from the resistant transgenic Rainbow plants by PRSV infection.

2011 - Danforth Center (Claude Fauquet) has grants totaling $11.9 million to support its efforts to develop and distribute a virus-resistant strain of cassava to farmers in sub-Saharan Africa. Targeting Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD).

1996 - Virus-resistant transgenic squash released (cucumber mosaic virus, zucchini yellow mosaic virus, and watermelon mosaic virus)
As of 2008 – 2012 represents ~12% of the U.S. crop.

1998 - Virus-resistant transgenic papaya cultivars released (papaya ringspot virus)
As of 2008 – 2012 represents ~60% of production in Hawaii.

1999 – Potato resistant to PVY and Potato leaf role virus.
As of 2000 – withdrawn from market due to McDonalds ban on GM products.

Status of Virus Resistance in the Field:

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Viral Vector Systems:  
Made possibly via the construction of infectious viral clones

Vector systems

Viral genome cloned into a bacterial plasmid and used to modify the genome.

In vitro transcription from plasmid template produces genomic viral RNA.

Infect host cell.
Strategy for foreign gene expression from Tobacco Mosaic Virus Vector

Promoter Duplication

CAP

126 K 183 K

SGP V

17 K

30 K

tRNA_{his}

Subgenomic RNA

CAP

17 K

Subgenomic RNA

Polylinker

TMV U1

TMV U2

CAP

Your GENE

Subgenomic RNA
ZMappTM – Anti-Ebola virus treatment composed of three monoclonal antibodies that bind the Ebola glycoprotein
Transforming Animals with virus vectors.

Turkish Angoras - were created by taking skin cells from donor female cats and using a harmless virus to insert the gene for red Fluorescent Protein (RFP) into the nucleus of each cell.

Kong Il-keun, a cloning expert at Gyeongsang National University
Current Vaccine Technology

-Grow in eggs (influenza, vaccinia) then inactivate

-100 million eggs used for influenza in the USA every year

-It takes about one egg to make one dose of vaccine.

Plant Produced Vaccines Using a Virus Based Expression System: Influenza, Rabies, Rotavirus – Like Particle Production

Benefits
- Rapid Scalability
- Less Expensive
- No Animal Contaminants

DARPA Blue Angel's 'live-fire' test, produced 10 million doses of an H1N1 influenza vaccine candidate in one month using this plant based technology.

https://www.youtube.com/watch?v=lAk_HkFi9-s
Characteristics of the Plant-Made VLP Vaccine

- Influenza virus-like particles in plants using only one viral gene (Hemagglutinin)

- No possibility of viral replication

- The VLP vaccine presents wild-type HA in an immunologically-relevant array as a membrane-bound protein
Gene Therapy
GENE THERAPY USING VIRAL VECTORS
SEVERE COMBINED IMMUNODEFICIENCY (SCID)
Correcting Genetic Defects

• Human SCID was first reported by Drs. Glanzmann and Riniker in 1950 (Buckley, 2004).
• In 1972, the first discovered molecular cause of SCID was adenosine deaminase deficiency (Buckley, 2004).
• SCID gained publicity in the 1980s with media coverage of an XSCID patient named David Vetter (1971-1984). He lived in a bubble and died at age 13 because of an unsuccessful bone marrow transplant.
• In the 1990s, SCID became the first human illness treated by human gene therapy.
2010 Update in the SCID Gene Therapy Clinical Trials

- Five of the 20 patients that received ex vivo–transduced hematopoietic progenitor cells for the treatment of SCID developed a T cell leukemic disease. The disease was fatal for one patient and was cured in the four others.
- The immunodeficiency has been corrected in 17 of the patients with SCID (85%).
- Similarly, 27 of 27 treated patients with ADA deficiency are alive and 19 (70%) have a corrected immune system.
- Combined 36 patients (76%) are living a normal life.

Approach:
Retroviral based vectors used to insert a functional copy of the defective gene
In the current study, nine boys with SCID-X1 underwent gene therapy using a vector engineered by the study researchers. Seven boys developed functional T cells at levels comparable to those seen in previous studies and have remained healthy for one to three years after treatment. Analyses of the children’s T cells suggest that the new vector causes fewer genomic changes that could be linked to leukemia.
Researchers have successfully tested a powerful gene therapy, delivered directly into the heart, to reverse heart failure in large animal models.

The new research study findings, published in November 13 2013 issue of *Science Translational Medicine*, is the final study phase before human clinical trials can begin testing SUMO-1 gene therapy. SUMO-1 is a gene that is "missing in action" in heart failure patients.

SUMO-1 gene therapy may be one of the first treatments that can actually shrink enlarged hearts and significantly improve a damaged heart's life-sustaining function," says the study's senior investigator Roger J. Hajjar, MD, Director of the Cardiovascular Research Center at Icahn School of Medicine at Mount Sinai and the Arthur & Janet C. Ross Professor of Medicine at Mount Sinai."

https://www.youtube.com/watch?v=yk78AROydNA
The median survival was 15.2 months, and the 12-month survival rate was 70%; 18- and 24-month survival rates were 43.8% and 29.2%, respectively.

ClinicalTrials.gov

A service of the U.S. National Institutes of Health
Web site to search for government funding clinical trials

2940 studies found for: gene therapy 2013
3723 studies found for: gene therapy 2014
4202 studies found for: gene therapy 2015
4801 studies found for: gene therapy 2016

Review article
Gene therapy as a new treatment option for inherited monogenic diseases

Table 1  Pol F. Boudes  2013
Recent important clinical progresses with gene therapy for monogenic disorders.

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene/protein</th>
<th>Vector</th>
<th>Delivery</th>
<th>Indication</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glybera®</td>
<td>Lipoprotein lipase</td>
<td>AAV1</td>
<td>Intra-muscular</td>
<td>LPD, approved EU</td>
<td>Decreased pancreatitis. No major safety issue.</td>
</tr>
<tr>
<td>NA</td>
<td>RPE65</td>
<td>AAV2</td>
<td>Subretinal injection</td>
<td>Leber’s amaurosis</td>
<td>No major safety issue. Anemia corrected. No major safety issue.</td>
</tr>
<tr>
<td>Lentiglobin®</td>
<td>β-Globin</td>
<td>Lentivirus</td>
<td>HSC infusion</td>
<td>β-Thalasemia</td>
<td></td>
</tr>
</tbody>
</table>

AAV: adeno-associated virus; LPD: lipoprotein-lipase deficiency; NA: not applicable; HSC: hematopoietic stem cells; ADA-SCID: Severe combined immunodeficiency due to adenosine deaminase deficiency; SCID-X1: X-linked severe combined immunodeficiency; X-ALD: X-linked adrenoleukodystrophy.  
http://dx.doi.org/10.1016/j.ejim.2013.09.009
First-FDA approved oncolytic injected immunotherapy for melanoma 2015

The drug, to be sold under the brand name Imlygic, is an engineered version of a herpes virus that kills cancer cells when injected directly into cancerous lesions on or under the skin, destroying them from the inside, while also priming the immune system to attack the disease.
Templates, Materials and Devices
Phage display

• Phage display is a term describing display of foreign (poly)peptides on the surface of phage particle. This is achieved by splicing a gene encoding such a peptide into a gene encoding a capsid structural protein.

• Phage Display was originally invented by George P. Smith in 1985
Using recombinant DNA technology, collections of billions of peptides, protein variants, gene fragment- or cDNA-encoded proteins presented on phage (so-called phage-displayed libraries) can be constructed and surveyed for specific affinity or activity.
Phage display peptides that bind specific antigens on the surface of colon tumor cells.
Nanofabrication Approaches

Can biology be integrated into the fabrication of functional materials and devices?

Top Down: Torsional Ratcheting Actuator

Bottom Up: Bacterial Flagellum

Surface Assembly of TMV Templates:

1. Attach TMV1cys to Au surface
2. Attach Pd catalyst to TMV1cys surface
3. Ni or Co deposition on Pd\textsuperscript{0} catalyst
TMV Assembled Silicon Anode for Lithium-ion Batteries
Chen, Gerasopoulos, Guo, Brown, Wang Ghodssi, Culver

Step 1
Steel substrate

Step 2
Tobacco mosaic virus

Step 3
Pd catalyst

Step 4
Nickel
golden

Nickel coated virus surface
After silicon physical vapor deposition

(a) (b)
Cell assembly and charge-discharge analysis

Cell assembly

The remaining capacity is ~1100 mAh/g after 120 cycles

Other TMV1cys based applications:

**Antireflective Current Collectors for Photoelectrochemical Cells for Hydrogen Generation**

**Viral templated palladium nanocatalysts for dichromate reduction**

**Scalable Nanomanufacturing of Virus-templated Coatings for Enhanced Boiling**

**The Role of Wickability on the Critical Heat Flux of Structured Superhydrophilic Surfaces**

**An Integrated Approach for Enhanced Protein Conjugation and Capture with Viral Nanotemplates and Hydrogel Microparticle Platforms via Rapid Bioorthogonal Reactions.**
Virus-based piezoelectric energy generation


Genetically modified M13, adding more negative surface charges

Drop cast the modified virus to form a film between electrodes

The device is capable of running a liquid crystal display (6 nA of current and 400 mV)
TMV-VLP electrochemical sensor for selective TNT sensing

Faheng et al., *Chem. Commun.*, 2014, **50**, 12977-12980
Self-assembling biomolecular catalysts for hydrogen production

Paul C. Jordan\textsuperscript{1,2}, Dustin P. Patterson\textsuperscript{3}, Kendall N. Saboda\textsuperscript{3}, Ethan J. Edwards\textsuperscript{1,2}, Heini M. Miettinen\textsuperscript{4}, Gautam Basu\textsuperscript{5}, Megan C. Thielges\textsuperscript{1} and Trevor Douglas\textsuperscript{1,*}
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