Synthetic Biology and Vaccines

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CASW NEW HORIZONS IN SCIENCE
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Synthetic Biology and Vaccines

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Lance Eckerle

Coronavirus

Noroviruses

Dengue

Systems Biology

Systems Genetics
Synthetic Biology and Vaccines

**Themes**

- **Introduction:**
- **Synthetic Biology:**
  - Applications
  - Risks/Benefits

---

**Synthetic Virology**

**Coronavirus**

**Systems Biology**

**Noroviruses**

**DENGUE**

**Systems Genetics**

**RP Vectors**
**Introduction:**

**Synthetic Biology:**
- Applications
- Risks/Benefits

**Emerging Viruses:**
- Human Coronavirus: Origins
- In silico resurrection of Viruses
- Perils/Dual Use

**Virus Vaccine Design:**
- Platform Technologies
- Rewiring/Recoding Virus Genomes

**Summary:**

**Goal:** Use of high-throughput technologies to develop rapid response strategies to control future emerging disease outbreaks, using coronaviruses as model platforms
Synthetic Biology

• Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature.

• This engineering perspective may be applied at all levels of the hierarchy of biological structures—from individual molecules to whole cells, tissues and organisms.
  – In essence, synthetic biology will enable the design of ‘biological systems' in a rational and systematic way
  – It joins the knowledge and techniques of biology with the practical principles and techniques of engineering
    • Synthetic Biology: Applying Engineering to Biology: Report of a NEST High Level Expert Group
Synthetic Biology

• Novel Tool/Probe:
  – Biological function of genes
  – Structure and function
  – Genome organization
  – Replication Strategy
  – Mechanism of Pathogenesis
  – Evolution

Microbial Pathogenesis
Synthetic Biology

• Novel Tool/Probe:
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• Outcomes:
  – New Therapeutic Targets
  – New Vaccine Strategies

Microbial Pathogenesis

Improved Public Health
Synthetic Biology

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  – Mechanism of Pathogenesis
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• Outcomes:
  – New Therapeutic Targets
  – New Vaccine Strategies

• Dual Use Concerns
  – Designer Pathogens/Biothreats

Microbial Pathogenesis

Improved Public Health
Many Challenges in Controlling New Emerging Diseases

- High Morbidity, Mortality and Severe Economic Hardships
  - HIV, 1918 flu, H5N1, SARS-CoV, BSE, Chikungunya virus, Dengue, etc.
  - 356 New or Emerging Pathogens Identified since 1940

- Challenges:
  - How do you protect against a future occurrence that is hard to predict?
  - How do you develop drugs/therapeutics against an unknown?
  - Where do you target scarce resources?

Heterogeneous Pool
Many Challenges in Controlling New Emerging Diseases

- **High Morbidity, Mortality and Severe Economic Hardships**
  - HIV, 1918 flu, H5N1, SARS-CoV, BSE, Chikungunya virus, Dengue, etc.
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- **Challenges:**
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  - How do you develop drugs/therapeutics against an unknown?
  - Where do you target scarce resources?

- **Genomics Related Technologies**
  - Synthetic Biology, Phylogenomics, Structure Modeling, High Throughput Sequencing, Functional Genomics, etc.
  - SARS-CoV as a model
    - Emerged 2002-03, reemerged 2004, epidemic strains may be extinct in the wild,
    - Huge reservoir pool (Future source of new outbreaks?)
**Synthetic Genomics**

- **Dual Use Technology**
  - Cost: (~0.30$/base; projected to be pennies/base within about 5 yrs)
  - Size: (520Kb mycobacterium genome synthesized-infectious)
  - Methods Synthesize/Recover Most RNA/DNA Viruses Exist

- **Benefits**
  - Doesn’t require access to biological sample
  - Rapid Response platform: Emerging and Biodefense Pathogens
  - Unprecedented mutagenic control/genome design

- **Risks: Synthetic Pathogen Design**
  - Chimeric pathogen design from component parts
  - Engineering strains that deliberately circumvent existing vaccines/therapeutics
  - Host shifted zoonotic pathogens
Synthetic Genomics

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<table>
<thead>
<tr>
<th>Genome</th>
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<td>Human Genome</td>
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<td>Mycobacterium genitalium*</td>
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<td>Poliovirus*</td>
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Synthetic Genomes/Reverse Genetics
Background

- N Protein
- M Glycoprotein
- E Protein
- S glycoprotein

Receptor Binding/entry
Tissue Tropism
Species Specificity
Neutralizing epitopes
Major component protective immunity

Perlman et. al. Nature Reviews Immunology, 2005
Coronavirus Phylogeny

Origins of Human Coronaviruses

Five Human Coronaviruses
- HCoV-OC43
- HCoV-HKU1
- SARS-CoV
- HCoV-229E
- HCoV-NL63

Sixth Human Coronavirus
- HCoV-SA1 (HCoV-2c EMC/2012)
- New betacoronavirus (2 patients/50% mortality)

No Cross Protection: Strains
- Rapid response platforms using synthetic genome design
- Possible to create subgroup specific antigen vaccines?
Origins of Human Coronaviruses

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 No Cross Protection: Strains

- Rapid response platforms using synthetic genome design
- Huge animal reservoir is antigenically distinct (source of future epidemics)
Part 1: Origins of HCoV

A. Sampled N. American Bats

[Map of Maryland and West Virginia]

Hyuhn et al., 2012 Sep 19. [Epub ahead of print]
Part 1: Origins of HCoV

A. Sampled N. American Bats

B. Identified New Bat Coronaviruses

Hyuhn et al., 2012 Sep 19. [Epub ahead of print]
Part 1: Origins of HCoV

A. Sampled N. American Bats

- Bat cell lines support HCoV NL63 Growth

B. Identified New Bat Coronaviruses

- Tricolored Bat Lung Cells

C. Bat cell lines support HCoV NL63 Growth

Hyuhn et al., 2012 Sep 19. [Epub ahead of print]
Part 1: Origins of HCoV

A. Sampled N. American Bats

B. Identified New Bat Coronaviruses

C. Bat cell lines support HCoV NL63 Growth

D. Bat Cells Support SARS-CoV Growth

- Zoonosis and Reverse Zoonosis are Common

Hyuhn et al., 2012 Sep 19. [Epub ahead of print]
Conclusions

- HCoV 229E and HCoV NL63 likely emerged from bats about 200 and 400 yrs ago, respectively.
  - HCoV OC43 (group 2a)-100 yrs ago from cattle
  - SARS-CoV and HCoV 2c EMC/2012 last 10 yrs
  - Several Full length N. American Bt CoV sequenced
  - Unique cell lines available

- Coronaviruses undergo host shifts frequently
  - S glycoprotein is very plastic
  - Tolerates mutations/recombination events (viable virus)
  - Multiple pathways to host shift/virulence (receptor interactions key)

Synthetically reconstructed BtCoV in different phylogenetic subclusters
- Most Relevant HKU5 (closest relative to HCoV 2c EMC/2012)
- Therapeutic testing (proteases very closely related)

Broad Vaccine Design (3 platforms)
- VRP multivalent, VRP chimeric S or Live Attenuated Chimeric S
- Will chimeric S vaccines protect against group 2b challenge?
Receptor Driven Cross Species Transmission
Part 2. Synthetic Reconstruction of Early SARS-CoV Isolates

Paradigm: SARS Evolved from Viruses Circulating in Zoonotic Pools

Palm Civets
(Raccoon Dogs)
(critical intermediate host)

Evolution towards efficient infection of human cells (ACE2)

2002-2003 Epidemic: ~8,000 cases/800 deaths

Guan et al., 2003; Li et al., 2005, Song et al., 2005
SARS Coronavirus Molecular Epidemiology

SARS-CoV Isolates

Urbani—Late phase isolate

Deming et al., 2006
SARS-CoV Isolates

Urbani - Late
CUHK-W1 - Middle
GZ02 - Early
GD03 - Sporadic Human Case; Mild disease.

HC/SZ/61/03 - Late civet
SZ16: Early civet.

A031G - Raccoon dog (most divergent)

Deming et al., 2006
RNA Virus Reverse Genetics

Cell Replicate

More Progeny Viruses
SARS Reverse Genetics

1. SARS RNA Genome
2. Transfection
3. Infection
4. Replicate
5. N
6. More Progeny Viruses
SARS Reverse Genetics

Cell

Replicate

SARS RNA Genome

Reverse Transcribe

SARS ds DNA Genome

Transcribe viral RNA

More Progeny Viruses

SARS RNA Genome

Transfect

Infection

SARS ds DNA Genome

Reverse Transcribe
SARS Reverse Genetics

DNA copy of genome allows for genetic manipulation of the genome and recovery of virus.
Approach


Lethal Infection in Aged Animals (ARDS)
SARS-CoV infection of HAE

### SARS-CoV Human Neutralizing Antibodies

**Panel A**

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<td></td>
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<tr>
<td>I. 132</td>
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<td>217.4</td>
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<td>222.1</td>
<td>200</td>
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<td>800</td>
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<td>IV. 110.4</td>
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<td>800</td>
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<td>232.17</td>
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<td>V. 124.5</td>
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### Panel B

[Diagram of SARS-CoV proteins (RBD, RBM, FP, HR2)]

**Group 1**

- Group 3
- Group 4

**Group 2**

- Group 1
- Group 3

**Therapeutic human mABs**

- Neutralize all strains (RBD=\sim16%)
- Protect Young and Aged Animals from Lethal Epidemic and Zoonotic SARS-CoV Infection

- hmAb 227 and 230
- Most robust
Vaccines
(Aged 1 yr old Animals; Lethal Challenge Models-ARDS)

- **Alphavirus Vaccines**: complete protection in young animals; SARS-age related mortality
- **Fail: Aged animals** *(Combination immune senescence/heterologous challenge-225 fold reduction neut titers)*
  - Killed Vaccines; adjuvanted recombinant protein vaccines also fail
  - SARS-CoV mortality >50% in aged populations
  - Live attenuated viruses protect young and aged animals from lethal challenge (later)
Coronavirus Synthetic Genome Design

Focus next few slides

Red = Clones successfully produced viruses; Blue = not viable or untested
CoV Type I Glycoprotein is Modular in Design?

- SARS-CoV and NL63 RBD domains may have been derived by recombination driven processes.
- If so, RBD and fusion core may be interchangeable within members of a virus family.
- Test the hypothesis:
  - Non-cultivatable coronavirus (Bat)
  - SARS S-RBD component exchange to drive cross species transmission.
  - Fusion core mutations-drive host range shifts (MHV)
    - Recombination processes (Natural)
    - Dual use: Deliberate host shift design.

Designing, synthesizing and recovering a SARS-like Bat Coronavirus

Becker et al., 2009; synthesis by Blue Heron
SEQUENCE VERIFIED, SRBD construct viable
Recombinant Bat CoV Growth (HKU3 SRBD)
(Full length Bat-CoV with Urbani RBD)

Not pathogenic in young or aged mice
HKU3-SRBD
Avirulent Strain

Inoculate, Harvest lungs d2, passage serially in mice

C57Bl6  C57Bl6  C57Bl6  C57Bl6  C57Bl6

2day  2day  2day  2day  

N=15 or 20X

20 wk aged mice (BALB/c)

Lethal (Virulent)
Dual Use Research of Concern?

- “United States Government Policy for Oversight of Life Sciences Dual Use Research of Concern”
  - SARS just added to the select agent list (Oct 2012)
  - UNC required DURC response to this program/approved locally
    - Risk assessment plan has been prepared and filed on campus

- Intentionally Host Shifted Zoonotic Viruses, replicate human cells (Fink Report)
  - Bat viruses which grow in human/mouse cells
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  - Bat viruses which grow in human/mouse cells

- Intentionally Increased Virulence in Mice (Fink Report)
  - RBD mutations likely enhance mouse ACE2 receptor recognition

- Intentionally Changed Antigenic Structure: viruses may be resistant to SARS vaccines? (Fink Report)
  - Viruses still susceptible to SARS vaccines
Resistant from Vaccines?
(Does 210 AA SARS RBD provide Cross Protection)

Live Attenuated Virus
MA15
C57BL/6
Challenge
MA15
MA15
BAT-SRBD-MAv
BAT-SRBD-Mav
MA15
10^4
10^5

Mouse adapted virus lethal: BALB/c but not C57Bl/6 mice
Not Engineered Viruses which Escape Existing Vaccines/human mAB therapeutics
Summary

- **Synthetic Genes coupled with Reverse Genetics**
  - Isolation of strains that were never successfully cultured; not available
  - Modular design of CoV S glycoprotein (phylogenetic constraints?)

- **Vaccine Panel: Vulnerable Aged Populations?**
  - Some vaccines work well in young animals
  - Some vaccines induce immune pathology
  - Existing vaccines don’t provide complete protection in aged models
Summary

**Synthetic Genes coupled with Reverse Genetics**
- Isolation of strains that were never successfully cultured; not available
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**Vaccine Panel: Vulnerable Aged Populations?**
- Some vaccines work well in young animals
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**Bat SARS-CoV Progenitor Viruses---More Challenging**
- More Heterogeneous; Can’t be cultured---usually
- Structural Motifs can be exchanged between strains (↑ vaccine breadth)
- Mechanisms of cross species transmission
- Platform to test if Vaccine/therapeutics work against new emergent strains?
Universal Vaccine Strategies

Live virus vaccine problem:
- Reversion to virulence
- Recombination repair
- Rationale design in an outbreak setting

Universal Strategies for Developing Reversion and Recombination Proof Live Attenuated Coronavirus Vaccines

Universal Strategies for Live Virus Vaccine Design
Rationale Design
Coronaviruses encode 3’-5’ exoribonuclease in nsp14

- DEDD Family of 3-5’ exonucleases (bacteria, yeast, mammals)
- SARS-nsp14 ExoN mutants (S-ExoN): are viable, but ……

Eckerle et al., Plos Pathogens 2010
S-ExoN has a 20-fold increased mutation frequency and 13-fold increased mutation rate compared to WT SARS-CoV.

**Mutation Frequency**

**Mutation Rate**

Eckerle et al., Plos Pathogens 2010
Genome Mutation Spectrum
(complete Sequences)

SARS-WT clones (n=10)

MA clones (n=5)

S-ExoN1 clones (n=10)

MA-ExoN1 clones (n=5)
Genome Mutation Spectrum
(complete Sequences)

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S-ExoN1 clones (n=10)

MA-ExoN1 clones (n=5)

MUTATOR PHENOTYPE
RNA dependent RNA Polymerases are error prone and misincorporate nucleotides every $5 \times 10^{-3}$ to $3 \times 10^{-6}$. Coronavirus error rates $\sim 3 \times 10^{-6}$, highest fidelity among positive strand RNA viruses (ExoN-S=mutator phenotype).
Quasispecies Variation is a Virulence Determinant

- Increase PV Fidelity 2-3 Fold = Attenuates pathogenesis
- Decrease SARS-CoV Fidelity ~15-20 fold = ? pathogenesis

RNA dependent RNA polymerase: error prone, no proof-reading activity
Error rates range: $10^{-3}$ to $10^{-6}$; somewhat inversely proportional to genome size
Rapid evolution:
Quasispecies Variation is a Virulence Determinant

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RNA dependent RNA polymerase: error prone, no proof-reading activity
Error rates range: $10^{-3}$ to $10^{-6}$; somewhat inversely proportional to genome size
Rapid evolution:
Dose-dependent mortality in ExoN- and MAwt-infected aged mice

Low Mortality and rapid clearance; passage goes to extinction and does not result in more virulent viruses
Immunodeficient Mice

Weight Loss STAT$^{-/-}$ and RAG$^{-/-}$ Mice

Virus Titer STAT$^{-/-}$ Mice
Immunodeficient Mice

Weight Loss STAT\(^{-/-}\) and RAG\(^{-/-}\) Mice

Virus Titer RAG\(^{-/-}\) Mice
MA-ExoN\(^-\) following 30 day persistent infection in SCID (BALB/c) mice

- Virus from mouse lungs retains DE-to-AA substitutions.
- Virus is attenuated on re-inoculation of aged BALB/c mice
- Virus retains fidelity defect (18X increased mutations)

Graham et al. in press
Percent Initial Mass after lethal MA-SARS challenge

Lung virus titers, day 2 post-challenge.

Serum dilutions-50% plaque reduction post vaccination

Graham et al. in press
Universal Platform: All Coronaviridae use TRS to express subgenomic mRNA
All Coronaviridae encode ExoN (nsp14)
State of the Ideas

- RNA viruses do not proofread
- Increased mutation rate increases risk / speed of virulence
- Increased mutation rate enhances competitive fitness
- Mutator phenotype decreases safety of working with pathogen
State of the Ideas

• RNA viruses do not proofread
• Increased mutation rate increases risk / speed of virulence
• Increased mutation rate enhances competitive fitness
• Mutator phenotype decreases safety of working with pathogen
New State of the Ideas

• Proofreading occurs in large nidoviruses
• Decreased fidelity trumps adaptation for virulence
• Potential for universal attenuation of any known or emerging coronavirus
• Increased safety of ExoN⁻ attenuated vaccines: sensitivity to RNA mutagens
• Screening for inhibitors of ExoN or other components of proofreading complex
Universal Vaccine Strategies

Universal Strategies for Developing Reversion and Recombination Proof Live Attenuated Coronavirus Vaccines

Universal Strategies for Live Virus Vaccine Design
Recoding Viruses
The Triplet Code/Recoding Viruses

- **Triplet Code**: Each amino acid can be coded for by several three nucleotide combinations (except Met)
  - Degenerate Triplet Code

- **Protein of 881 amino acids (poliovirus ORF)** can be coded in $10^{442}$ different ways
  - Why this particular sequence?
The Triplet Code/Recoding Viruses

- **Triplet Code**: Each amino acid can be coded for by several three nucleotide combinations (except Met)
  - Degenerate Triplet Code

- Protein of 881 amino acids (poliovirus ORF) can be coded in $10^{442}$ different ways
  - Why this particular sequence?

- **Evolutionary Advantage (Sequence Restricted)**
  - Codon Bias (Alanine codon: GCC used 4x more than GCG)
    - Species specific phenomena
  - Codon Pair Bias (CPB) (Poliovirus)
    - Certain codon pairs are unfavorable (GCC-GAA less GCA-GAG)
Follows pioneering work of Olin Kew and Eckhard Wimmer—our strategy is different.
Replicate like wildtype virus
Deoptimization reduces Luciferase expression

1. Mock
2. SARS WT
3. SARS Luciferase
4. SARS Luciferase Ser
5. SARS Luciferase SLR
6. SARS Luciferase SLRVA
Human Codon Deoptimized SARS-CoV Variants

- **Deoptimization**
  - Use rare codons
  - Reduce Protein Expression
  - Translation Block
  - Rheostat

- **Mechanism (?)**
  - Rare tRNAs
  - Codon Pair Bias
  - Other
    - Transcription
    - Disrupt RNA 2° Structure

---

**SARS-M aa 88-103 (Urbani nt 26659-26706)**

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5 Set/SLRVA

1 Set/SLR

3 Set/SLR

1 Set/Ser

WT

Also built 3 and 5 set randomized virus controls
## Deoptimized SARS-CoV Variant Statistics

### E and M Structural Genes

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<th># of Changed Codons (Nucleotides)</th>
<th>E+M # of Deopt Codons</th>
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<th>M- % Deopt Codons</th>
<th>*E- CPB Value</th>
<th>*M- CPB Value</th>
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<tr>
<td>3-Ser/SLR</td>
<td>64 (104)</td>
<td>94</td>
<td>37.7</td>
<td>29.3</td>
<td>-0.011</td>
<td>-0.045</td>
</tr>
<tr>
<td>5-Set/SLRVA</td>
<td>103 (143)</td>
<td>134</td>
<td>53.2</td>
<td>41.9</td>
<td>-0.065</td>
<td>-0.067</td>
</tr>
</tbody>
</table>

### Random Controls

<table>
<thead>
<tr>
<th></th>
<th># of Changed Codons (Nucleotides)</th>
<th>E+M # of Deopt Codons</th>
<th>E- % Deopt Codons</th>
<th>M- % Deopt Codons</th>
<th>*E- CPB Value</th>
<th>*M- CPB Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Set/SLR-R</td>
<td>75 (143)</td>
<td>19</td>
<td>11.7</td>
<td>4.5</td>
<td>0.001</td>
<td>-0.019</td>
</tr>
<tr>
<td>5-Set/SLRVA-R</td>
<td>127 (194)</td>
<td>7</td>
<td>5.2</td>
<td>1.4</td>
<td>-0.042</td>
<td>-0.014</td>
</tr>
</tbody>
</table>

* CPB Value = Codon Pair Bias Values. Calculated from values in Coleman et al. (2008) Science 320(5884) 1784-1787
Target E/M for deoptimization:

Blocks maturation and release

S glycoprotein normally expressed
Recombinant Virus Growth

(Built in mouse adapted rMA15 Backbone)

* dSLR E/M passaged 6X in Vero Cells- grows to near wildtype titer; E protein expression greatly reduced as a function of increasing numbers of deoptimized residues
Recombinant Virus Growth
(Built in mouse adapted rMA15 Backbone)

Blocks Translation/Reduces Growth

Argued the recoding viruses attenuates by “1,000 cuts
Can’t revert to virulence?

Is the deoptimized growth phenotype stable in vitro?

*dSLR E/M passaged 6X in Vero Cells- grows to near wildtype titer; E protein expression greatly reduced as a function of increasing numbers of deoptimized residues
Codon Deoptimization of the E and M genes Abrogates ORF6-subgenomic RNA synthesis
Pathogenesis
(rMA15 Backbone)

SLR Deoptimized Viruses is attenuated; SLR passaged viruses are attenuated as well Protect against lethal challenge (young and aged animal models-ARDS)
Pathogenesis
(rMA15 Backbone)

In Vitro Passage
Does not select for reversion
To Virulence
(need to check in vivo passage)

SLR Deoptimized Viruses is attenuated; SLR passaged viruses are attenuated as well
Protect against lethal challenge (young and aged animal models-ARDS)
Vaccine Efficacy

- Deoptimized viruses induce robust adaptive immune responses in young and aged animals
  - Protect young and aged animals from lethal homologous and heterologous infection
  - Safe and efficacious
  - Similar results reported for poliovirus and influenza viruses
  - Universal Platform for Attenuation/any virus
  - Rapid response platform in an outbreak setting
Dual Use Concerns
Synthetic Genome Design

● Synthetic Gene Companies (Prolific)
  ■ Global distribution-low cost (genomes by computer design)
  ■ Buy DNA synthesizers on E-bay (<$1,000)
  ■ Make one from an ink jet printer

● Synthetic Viruses (Skill required to isolate)
  ■ Poliovirus, H1N1 1918 Influenza, SARS and related viruses
  ■ Most viruses (including extinct ones if sequence is available)
  ■ Chimeric Viruses (HKU3-SRBD)

● Gain of Function Experiments
  ■ Increase Virulence, Alter host range, Alter Tropism
  ■ Alter Immunogenicity, Increase resistance to known anti-virals/drugs
  ■ Increase transmissibility, Unanticipated Consequences
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Classic argument:
altering virus genome attenuates pathogenesis
Examples
(Gain of Function)

- Th2 cytokine expression in mousepox and an assortment of other viruses
  - Increased virulence, escape from vaccines
- PA-X (Flu)-blocks host translation
  - removal enhances flu pathogenesis by inducing cytokine storm
- Transmissible H5N1 Influenza
  - Potential engineering of pandemic strains/high mortality

Problem:
- **Bad actors**: take advantage of findings to harm global health
- **Solutions**: Complex at best (Strong limits can increase vulnerability)
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(Gain of Function)

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Oversight is necessary and appropriate.