The Swarm: Causes and consequences of HIV quasispecies diversity

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August 14, 2008
Success of HIV largely due to its ability to “escape” control of immune system/drug treatment via rapid mutation

$10^9 - 10^{10}$ viral particles produced each day, ~ 3 mutations per particle $\Rightarrow$ enormous diversity possible
Mutation, mutation, mutation

- Success of HIV largely due to its ability to “escape” control of immune system/drug treatment via rapid mutation
- $10^9 - 10^{10}$ viral particles produced each day, $\sim 3$ mutations per particle $\Rightarrow$ enormous diversity possible
- Vast majority of mutations cripple virus, but small number confer evolutionary advantage
Mutation, mutation, mutation

- Success of HIV largely due to its ability to “escape” control of immune system/drug treatment via rapid mutation
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**The Swarm**

Within an infected person, HIV exists as a genetically diverse population of competing **quasispecies**
Quasispecies

Questions:

- How do we characterize HIV sequence diversity in an infected patient?
- What is the balance point between ability to resist selective pressure and overall viral function?
- How is quasispecies diversity maintained in the face of this pressure?
- What are the consequences of viral diversity?
1 HIV Infection, briefly
2 Sequencing Techniques
3 Viral Fitness
4 Inducing Diversity
5 Maintaining Diversity
6 Impact of Diversity
7 Summary
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Natural Course of Infection

- **Motivation**
- **Infection**
- **Sequencing**
- **Viral Fitness**
- **Inducing Diversity**
- **Maintaining Diversity**
- **Impact of Diversity**
- **Summary**

**Natural Course of Infection**

- **Primary Infection**
  - Wide dissemination of virus
  - Seeding of lymphoid organs

- **Acute HIV syndrome**
- **Clinical Latency**
- **Opportunistic Diseases**
- **Constitutional Symptoms**
- **Death**

- **CD4+ T Lymphocyte Count (cells/mm^3)**
  - 0 to 1200 cells/mm^3

- **HIV RNA Copies per ml Plasma**
  - 10^2 to 10^7 copies/ml
Stage 1: Acute/Early Infection

- Virus enters body, infects a number of possible target cells (e.g., Langerhans, dendritic, macrophage, CD4)
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- Flu-like symptoms
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- Very high viral loads, CD4+ (T “helper” cell) levels drop dramatically
- Flu-like symptoms
- Vigorous CD8+ (T “killer” cell) response, killing many HIV-infected cells. CD4+ levels rebound, but do not reach original levels.
Stage 2: Immune Control

- Virus remains active in lymphoid tissue, mutations accumulate
- Reservoirs persist in other parts of the body (i.e. gut)
- Gradual decrease in CD4+ levels over a two-week to 20-year period
Stage 3: Immune Failure

- CD4+ count drops to level where cell-mediated immunity is no longer effective
- Viral load rises sharply, progression to AIDS
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7. Summary
Basic Sequencing

Issues

• Viral sequences may differ across tissues
• May miss rare sequences, misrepresent population diversity
• If small number of genomes in original sample (e.g., very low viral load), may amplify same template sequence more than once

Liu 1996
Basic Sequencing

**Issues**

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Motivation | Infection
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Sequencing | Viral Fitness
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Impact of Diversity | Summary
Basic Sequencing

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\(^a\) Liu 1996
Limiting Dilution

- Isolate individual sequences via dilution, then amplify to get enough copies for sequencing
- Limited by number of individual genomes which can be sequenced in reasonable amount of time
Pyrosequencing

- Yields large number of short (200-400 bp) “reads”
- Can be automated \(\Rightarrow\) fast
- Computational techniques for haplotype/frequency estimation
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Limitations

- Works best when known template sequence available
- Subject to quality of algorithms applied
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Genetic variability causes individual viruses to have different abilities to:

1. Infect cells
2. Replicate within cells
3. Cause clinical disease
Viral fitness

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- Genetic variability causes individual viruses to have different abilities to:
  1. Infect cells
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  3. Cause clinical disease
- For our purposes, *Viral fitness = Replication Capacity / Persistence in vivo*
- May differ from fitness *in vitro*
In vitro vs. in vivo

Figure: HIV quasispecies fitness maps for two individuals [Fernandez 2007]
Characteristics which affect a virus’ ability to survive and replicate *in vivo* over a long period of time include:

- Epitope sequences
- Tissue tropism
- Ability of viral proteins (eg. protease, reverse transcriptase) to function efficiently in presence of Abs/ART
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Initial Infection

- Early quasispecies diversity limited by transmission bottleneck\(^1\) (unrelated to replication capacity?)
- Quasispecies which become established are not necessarily fittest/most virulent of infecting population

\(^1\)Karlsson 1998
\(^2\)Bergstrom 1999
Initial Infection

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- Quasispecies which become established are not necessarily fittest/most virulent of infecting population
- May decrease viral fitness over sequential transmissions\(^2\)

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\(^1\) Karlsson 1998

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After infection is established, three major selection forces act on HIV quasispecies:

1. The host’s **natural immune response**, mediated by antibodies and T cells
2. **Competition** between quasispecies for limited resources
3. **Drug therapy**, if applicable
Antibodies attach to outer surface of circulating virus particles, neutralizing them.

In HIV, main target is viral envelope (encoded by \textit{env} gene).

Constant interplay\textsuperscript{3} between

- Quasispecies mutation to avoid detection by antibodies
- Production of antibodies which recognize currently circulating quasispecies

\textsuperscript{3}Richman 2003
Natural Immune Response - T cells

- T cells recognize **epitope**, small piece of viral protein presented on surface of infected cell
- Recognition of viral epitope triggers T-cell-mediated destruction of cell
- Viruses with “unrecognizable” epitopes can remain undetected in a cell
- Epitope presentation/recognition by CD8+ varies by individual’s HLA type\(^a\)

\(^a\)Lichterfeld 2005
Viral Competition

- Fitness cost associated with escape mutations (CTL or HAART) can be high
- Viruses carrying these mutations may be out-competed by more efficient (e.g., wild-type) virus in absence of selective pressure\(^4\)
- Potential loss of resistance mutations forms the basis of “drug holiday” and “intermittent therapy” regimens

\(^4\) Leslie 2004, Friedrich 2004
Quasispecies theory\textsuperscript{5} shows why single-drug regimens (eg. AZT) failed:

- Initial therapy causes reduction in viral load, increases supply of uninfected cells

\textsuperscript{5}Frost 1994
\textsuperscript{6}Petravic 2008
Drug Therapy and Quasispecies

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- Initial therapy causes reduction in viral load, increases supply of uninfected cells
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**Idea**

Raise barriers to escape so that viruses which carry necessary mutations are likely to have low replication capacity

\(^5\)Frost 1994  
\(^6\)Petravic 2008
• Highly Active Anti-Retroviral Therapy now standard treatment in developed countries
• Involves a combination of three or more drugs from the following categories:
  1. Nucleoside Reverse Transcriptase Inhibitors (NRTI)
  2. Non-Nucleoside RTIs (NNRTI)
  3. Protease Inhibitors (PI)
  4. Integrase Inhibitors
  5. Entry Inhibitors
  6. Maturation Inhibitors
• Difficult for HIV to mutate to evade \( \geq 3 \) drugs simultaneously without severe loss of replicative capacity
HAART exerts extreme selective pressure on HIV, yet:

- “Drug holiday” and “intermittent therapy” regimes have had limited success in practice
- Recent studies\textsuperscript{7} have found quasispecies resistant to multiple drugs

\textsuperscript{7}Quan 2008
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**An Implacable Foe?**

Intense selective pressure tends to create evolutionary bottlenecks which decrease quasispecies diversity...

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\(^7\)Quan 2008
Breaking bottlenecks

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*How is quasispecies diversity maintained?*

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\(^7\)Quan 2008
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Recombination

- A major force for introducing new (possibly resistant) quasispecies into viral population

Charpentier 2006
Recombination

- A major force for introducing new (possibly resistant) quasispecies into viral population
- Possibly also a mechanism for avoiding evolutionary bottlenecks\(^8\):
  - Fitness “peak jumping”
  - Limit size of regions which are homogeneous across quasispecies

\(^8\)Charpentier 2006
Compartmentalization

• Distinct quasispecies populations may become established in different parts of the body, eg. gut, blood, CSF\textsuperscript{9}, breast milk\textsuperscript{10}, genital tract\textsuperscript{11}, and CD4 cells\textsuperscript{12}

\textsuperscript{9}Harrington 2007, Abbate 2005, Caragounis 2008
\textsuperscript{10}Becquart 2002
\textsuperscript{11}Diem 2008
\textsuperscript{12}Fulcher 2004
\textsuperscript{13}van Marle 2007
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- May arise as response to different biological conditions within compartments

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- Some suggestion that reservoirs may exchange genetic information (Diem, Harrington)

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Archiving

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15 Ruff 2002
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- ⇒ Allows for archiving of quasispecies over *entire infection history*\(^{15}\)

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• Macrophages, other cell types may also serve similar archiving role

\textsuperscript{15} Ruff 2002
Archiving

- Resting T cells can harbour replication-competent virus over a long period of time
- ⇒ Allows for archiving of quasispecies over *entire infection history*\(^1^5\)
- Macrophages, other cell types may also serve similar archiving role
- “Rewind” ability allows HIV to adapt quickly to change in biological conditions

\(^1^5\) Ruff 2002
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• Quasispecies diversity plays an important role in ensuring HIV’s survival by
  1. Ensuring the **stability** of the viral population
  2. Improving adaptability to different biological conditions through **cooperative interactions**
  3. Helping to thwart **drug therapy** regimes
Population Stability: Survival of the flattest?

- Theory based on ideas of Eigen and Schuster \(^{16}\), “verified" \(\textit{in silico}^{17}\) and \(\textit{in vivo}^{18}\)
- **Basic idea:** Collection of quasispecies with higher \textit{average} fitness will outcompete one with lower average fitness, even if latter contains individuals with very high fitness

\(^{16}\)Schuster 1988, Eigen 1996

\(^{17}\)Wilke 2001

\(^{18}\)Elena 2008
Motivation

Infection

Sequencing

Viral Fitness

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Maintaining Diversity

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Summary
Cooperative interactions

Quasispecies theory predicts that viral populations, not individual variants, are the target of evolutionary selection.

**Neat experiment [Vignuzzi 2006]**

Compare growth of wild-type (WT) poliovirus to one with high-fidelity (HF) polymerase *in vitro* and *in vivo*
Cooperative interactions, cont’d.

Results - in vitro

• WT outcompetes HF under adverse conditions
  ⇒ Limiting quasispecies diversity lowers replication capacity

• HF highly attenuated as compared to WT, unable to survive in neurological tissue
  • Artificially increasing quasispecies diversity in HF population restored pathogenicity/neurotropism to WT levels
  • Artificially diversified HF and WT brain isolates genetically indistinguishable, but only WT isolate was neurotropic when injected into a new host!

Conclusion

Sequence diversity itself, not particular set of mutations, determines pathogenesis
Cooperative interactions, cont’d.

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Drug resistance

- **Compartmentalization** can limit effectiveness of drug treatment in places where ARs may not penetrate (e.g. brain)
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- **Recombination** restricts evolutionary bottleneck effect to very specific genomic regions, maintains diversity at adjacent regions
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- **Compartmentalization** can limit effectiveness of drug treatment in places where ARs may not penetrate (eg. brain)
- **Recombination** restricts evolutionary bottleneck effect to very specific genomic regions, maintains diversity at adjacent regions
- **Archiving** maintains a catalogue of viruses resistant to previous treatments, making previously-tried drugs ineffective
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Quasispecies diversity is crucial to HIV’s survival in face of these selection forces.

Future vaccine and therapy approaches will need to account for and/or exploit HIV intrahost population dynamics.
Acknowledgements

- Prof. Peter Gilbert
- Prof. Julie Overbaugh
- Anne Piantadosi

Thanks!

Questions?