2007 UNAIDS

25 million AIDS deaths since 1981
39.5 million people currently living HIV infection
   (17.7 million women and 2.3 million children)
2007 UNAIDS

25 million AIDS deaths since 1981
39.5 million people currently living HIV infection
(17.7 million women and 2.3 million children)

In 2006 Alone:
2.9 million AIDS deaths (380,000 children)
4.3 million new infections (530,000 children)
14,000 new infections per day
25 million AIDS deaths since 1981
39.5 million people currently living HIV infection
   (17.7 million women and 2.3 million children)

In 2006 Alone:
  2.9 million AIDS deaths (380,000 children)
  4.3 million new infections (530,000 children)
  14,000 new infections per day

In USA:
   Minorities disproportionately affected
   African Americans  -  12% of population, but 50% of HIV cases
2007 UNAIDS

25 million AIDS deaths since 1981
39.5 million people currently living HIV infection
   (17.7 million women and 2.3 million children)

In 2006 Alone:
   2.9 million AIDS deaths (380,000 children)
   4.3 million new infections (530,000 children)
   14,000 new infections per day

In USA:
   Minorities disproportionately affected
   African Americans - 12% of population, but 50% of HIV cases

In Washington DC: One out of 20 residents are HIV positive
HIV-1 Life Cycle
HIV-1 Life Cycle
HIV-1 Life Cycle
HIV-1 Life Cycle
Who solved the structures of the HIV-1 proteins?
Who solved the structures of the HIV-1 proteins?

Young people like you!
Structure of the HIV-1 MA protein

Mike Massiah (Oklahoma State)
Mary Starich (NIH)
Chiana Paschall (PhD, UPenn)
HIV-1 NC-RNA complex

Roberto DeGuzman (Kansas)
Chelsea Stalling (MD-PhD UPenn)
Justin Wu (Ohio State)
Second HIV-1 NC-RNA complex

Justin Wu (Ohio State)
Ryan Turner (MD, Harvard)
Gaya Amarasinghe (Iowa State)
Structure of the HIV-1 CA protein

Yasmine Ndassa, MD-PhD, Harvard
Discovery of antiviral CA inhibitors

Chun Tang: Missouri
Isaac Kinde: MD-PhD, JHU
Erin Loeliger: MD-PhD, Harvard
Sampson Kyere: MD-PhD, Maryland
Structure of CA inhibitor

Sampson Kyere: MD-PhD, Maryland
We now know what most of the parts of HIV look like...
We now know what most of the parts of HIV look like...

HOW DO THEY FUNCTION?
Virus assembly occurs at punctate sites on the plasma membrane

Assembly occurs at PM
Paul Bieniasz & co-workers (2006)
Cohen & co-workers (2007)
Krausslich & co-workers (2007)
How is Gag trafficked to these specific assembly sites?

Assembly occurs at PM
Paul Bieniasz & co-workers (2006)
Cohen & co-workers (2007)
Krausslich & co-workers (2007)

Immunofluorescence confocal microscopy

Electron microscopy
Trafficking is directed by the MA Domain
 Trafficking is directed by the MA Domain

- Targeting depends on myristoylation
 Trafficking is directed by the MA Domain

- Targeting depends on myristoylation

- Conservative mutations (e.g. L8I) can retarget Gag to cytoplasm
What triggers myristate exposure?

Recoverin

Calcium-free

Calcium-bound
Phosphatidylinositol phosphates are cellular membrane markers

Behnia & Munro, Nature (2005)
Phosphatidylinositol phosphates are cellular membrane markers

PIP2: Major Landmark for the PM

Behnia & Munro, Nature (2005)
Trafficcing of HIV-1 to the Plasma Membrane is mediated by PI(4,5)P2

5ptaseIV depletes PI(4,5)P2 from the PM

Freed & co-workers, PNAS (2004)
Trafficking of HIV-1 to the Plasma Membrane is mediated by PI(4,5)P2

Green: HIV-1 Gag

Freed & co-workers, PNAS (2004)
HeLa cells transfected with HIV-1

Gag buds from the PM

Freed & co-workers, PNAS (2004)
Gag is re-targeted to endosomes.

Upregulation of ARF6/Q67L induces PI(4,5)P2 enriched endosomes.

Freed & co-workers, PNAS (2004)
Could membrane targeting be mediated by interactions between MA and PI(4,5)P2?
1H-15N NMR of myrMA

2-component expression system (Yeast NMT & HIV-1(NL4-3) MA)
1H-15N NMR of myrMA

2-component expression system (Yeast NMT & HIV-1(NL4-3) MA)
PIP2 binds HIV-1 myr-MA

$K_d = 150 \pm 30 \mu M$
Electrostatic and hydrophobic interactions contribute to binding.
Electrostatic and hydrophobic interactions contribute to binding

No detectable binding:

di-C4-PI

\[\text{di-C4-PI(3)P, di-C4-PI(4)P, di-C4-PI(5)P, di-C4-PI(3,5)P}_2\]

di-C4-phosphatidylcholine
Allosteric switch

PIP2 binding triggers conformational changes that expose the myr group.
Allosteric switch

PIP2 binding triggers conformational changes that expose the myr group.
PIP2 binding induces a conformational change that triggers Myr exposure.
Allosteric switch mechanism

myr(s)MA
Allosteric switch mechanism

diC4-PI(4,5)P2:myr(e)MA
Myr and 1’-PI(P)2 acyl chains bracket basic residues required for membrane binding
Myr and 1’-PIP2 acyl chains bracket basic residues required for membrane binding

“Extended lipid” similar to models for cytochrome c
Myr and 1’-PIP2 acyl chains bracket basic residues required for membrane binding.

“Extended lipid” similar to models for cytochrome c.

Extended mode preferred for membranes with negative curvature.
Implications for virus assembly at lipid rafts!

Myr and 1’-PIP2 acyl chains bracket basic residues required for membrane binding

“Extended lipid” similar to models for cytochrome c

Extended mode preferred for membranes with negative curvature

Implications for virus assembly at lipid rafts!
Gag co-localizes with raft markers

**GM1:** Raft marker (cholera toxin B subunit)

**TfR:** Non-raft marker (antitransferrin receptor)

Rafts contain lipids with saturated acyl chains
Rafts contain lipids with saturated acyl chains
Model for PIP2-dependent targeting of HIV-1 Gag to lipid rafts
Model for PIP2-dependent targeting of HIV-1 Gag to lipid rafts
Model for PIP2-dependent targeting of HIV-1 Gag to lipid rafts
Additional evidence...
Additional evidence...

1. PIP2 is enriched in the HIV-1 envelope
Additional evidence...

1. PIP2 is enriched in the HIV-1 envelope

2. Point mutations that retarget Gag to cytoplasm (e.g. L8I) turn off myr switch

(Saad et al., 2007)
3. Saturated myr group required for targeting rafts

Normal targeting by myrGag

Lindwasser & Resh, PNAS (2002)
3. Saturated myr group required for targeting rafts

Normal targeting by myrGag

Aberrant targeting by unsaturated acyl chains:
14:1n-9-Gag
14:2n-6-Gag
The PI(4,5)P2 binding site is highly conserved

Substitutions/454 Strains

S77, N80, K/R22: 0
L21, W36, T97: 1
K27: 2
K/R32: 40% substituted
The PI(4,5)P2 binding site is highly conserved

Substitutions/454 Strains

S77, N80, K/R22: 0
L21, W36, T97: 1
K27: 2
K/R32: 40% substituted

Therapeutic target?
What about HIV-2?
What about HIV-2?

HIV-2 membrane targeting \textit{in vivo} is PIP2-dependent (collaboration with E. Freed)
What about HIV-2?

HIV-2 membrane targeting in vivo is PIP2-dependent (collaboration with E. Freed)

The myristyl switch of HIV-2 myrMA is significantly less sensitive than that of HIV-1
What about HIV-2?

HIV-2 membrane targeting *in vivo* is PIP2-dependent (collaboration with E. Freed)

The myristyl switch of HIV-2 myrMA is significantly less sensitive than that of HIV-1

- myr group is more tightly sequestered
What about HIV-2?

HIV-2 membrane targeting *in vivo* is PIP2-dependent (collaboration with E. Freed)

The myristyl switch of HIV-2 myrMA is significantly less sensitive than that of HIV-1

- myr group is more tightly sequestered

~ 5% exposure upon saturation with di-C4-PIP2
Apparently due to different salt bridges
Biological Consequences?

HIV-2 is Less Pathogenic than HIV-1

- Disease confined mainly to western Africa

- Most live normal lifespans

- Low viral loads, similar to those of HIV-1 long-term non-progressors
Biological Consequences?

HIV-2 is Less Pathogenic than HIV-1

- Disease confined mainly to western Africa
- Most live normal lifespans
- Low viral loads, similar to those of HIV-1 long-term non-progressors

- Osterhaus 2008: Low viral loads NOT due to enhanced immune response
Poor HIV-2 replication might be due to the weaker myr switch.
Poor HIV-2 replication might be due to the weaker myr switch

- HIV-2 Gag does not remain stably associated with membrane assembly sites in some cell lines (Matano JVI 2007)
Poor HIV-2 replication might be due to the weaker myr switch

- HIV-2 Gag does not remain stably associated with membrane assembly sites in some cell lines (Matano JVI 2007)

- N-terminal mutations in HIV-1 Gag that inhibit membrane binding and virus assembly also inhibit myr exposure (Saad JMB 2007)
Conclusions

PI(4,5)P2 binds MA and triggers myristate exposure
Conclusions

PI(4,5)P2 binds MA and triggers myristate exposure
- mechanism for targeting PI(4,5)P2 enriched membranes
Conclusions

PI(4,5)P2 binds MA and triggers myristate exposure
- mechanism for targeting PI(4,5)P2 enriched membranes

The 2’ (unsaturated) acyl chain is sequestered
Conclusions

PI(4,5)P2 binds MA and triggers myristate exposure
- mechanism for targeting PI(4,5)P2 enriched membranes

The 2’ (unsaturated) acyl chain is sequestered
- potential mechanism for targeting Gag to lipid rafts
Conclusions

PI(4,5)P2 binds MA and triggers myristate exposure
- mechanism for targeting PI(4,5)P2 enriched membranes

The 2’ (unsaturated ) acyl chain is sequestered
- potential mechanism for targeting Gag to lipid rafts
- may serve as a general mechanism for lateral organization of membrane proteins
Conclusions

PI(4,5)P2 binds MA and triggers myristate exposure
- mechanism for targeting PI(4,5)P2 enriched membranes

The 2’ (unsaturated) acyl chain is sequestered
- potential mechanism for targeting Gag to lipid rafts
- may serve as a general mechanism for lateral organization of membrane proteins

Potential therapeutic target
Conclusions

**PI(4,5)P2** binds MA and triggers myristate exposure
- mechanism for targeting PI(4,5)P2 enriched membranes

The 2’ (unsaturated) acyl chain is sequestered
- potential mechanism for targeting Gag to lipid rafts
- may serve as a general mechanism for lateral organization of membrane proteins

**Potential therapeutic target**
- PIP2 binding site
Conclusions

Pl(4,5)P2 binds MA and triggers myristate exposure
- mechanism for targeting Pl(4,5)P2 enriched membranes

The 2’ (unsaturated) acyl chain is sequestered
- potential mechanism for targeting Gag to lipid rafts
- may serve as a general mechanism for lateral organization of membrane proteins

Potential therapeutic target
- PIP2 binding site
- helix 1 rearrangement/salt bridge
Conclusions

PI(4,5)P2 binds MA and triggers myristate exposure
- mechanism for targeting PI(4,5)P2 enriched membranes

The 2’ (unsaturated) acyl chain is sequestered
- potential mechanism for targeting Gag to lipid rafts
- may serve as a general mechanism for lateral organization of membrane proteins

Potential therapeutic target
- PIP2 binding site
- helix 1 rearrangement/salt bridge
- Use HIV-2 as a guide
HIV-1 myr-MA:
Chun Tang (Missouri)
Isaac Kinde (MD-PhD, JHU)
Erin Loeliger (MD-PhD, Harvard)

PIP Interactions
Jamil Saad (Alabama)
Janet Tai (PhD, JHU)
Jaime Miller (MD-PhD, UVA)
Andrew Kim, Ruba Ghanam
Kalola Andrews

HIV-1 myr-MA mutants:
Erin Loeliger, Paz Luncsford (MD-PhD, Maryland)
Melissa Liriano (MD-PhD, Maryland) Jamil Saad

Support: HHMI, NIAID, NIGMS
Summer 2009