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
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
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
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

Immunofluorescence confocal 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
Saturated myr group required for targeting rafts

Normal targeting by myrGag
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

Lindwasser & Resh, PNAS (2002)
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)
Trafficking 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)
Gag is targeted to endosomes

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

HeLa cells transfected with HIV-1 Gag buds from the PM

Gag buds from the PM

Freed & co-workers, PNAS (2004)
Upregulation of ARF6/Q67L induces PI(4,5)P2 enriched endosomes.

Gag is re-targeted to 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
- di-C4-Pl(3)P, di-C4-Pl(4)P,
- di-C4-Pl(5)P, di-C4-Pl(3,5)P2
- 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’-PIP2 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

Extracellular

liquid-disordered

GPI-anchored protein

liquid-ordered (raft)

Non-raft-associated:

PIP2

phospholipids with unsaturated acyl chains

Raft-associated:

sphingolipids

phospholipids with saturated acyl chains

cholesterol

dually-acylated protein
Rafts contain lipids with saturated acyl chains

All Enriched in the HIV-1 envelope
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. native PIP2 binds tightly in reverse micelles

Josh Wand & coworkers
3. native PIP2 binds tightly in reverse micelles

HIV-1 MA Myr(+) protein plus PIP2 15N-HSQC overlay
20mM Sodium Phosphate, 40mM NaCl, 10mM DTT, pH 6.53
Referenced to DSS

Native PIP2 binds tightly in reverse micelles

Peaks appear
Peaks disappear

Josh Wand & coworkers
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 *in vivo* is PIP2-dependent (collaboration with E. Freed)
What about HIV-2?

HIV-2 membrane targeting \textit{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 \textit{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

- 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)

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

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

Support: HHMI, NIAID, NIGMS