Endosomal Cysteine Proteases: Essential Host Factors for Ebola and Marburg Virus Infection

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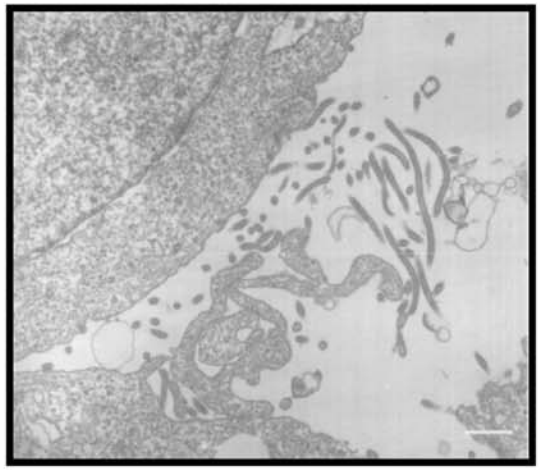
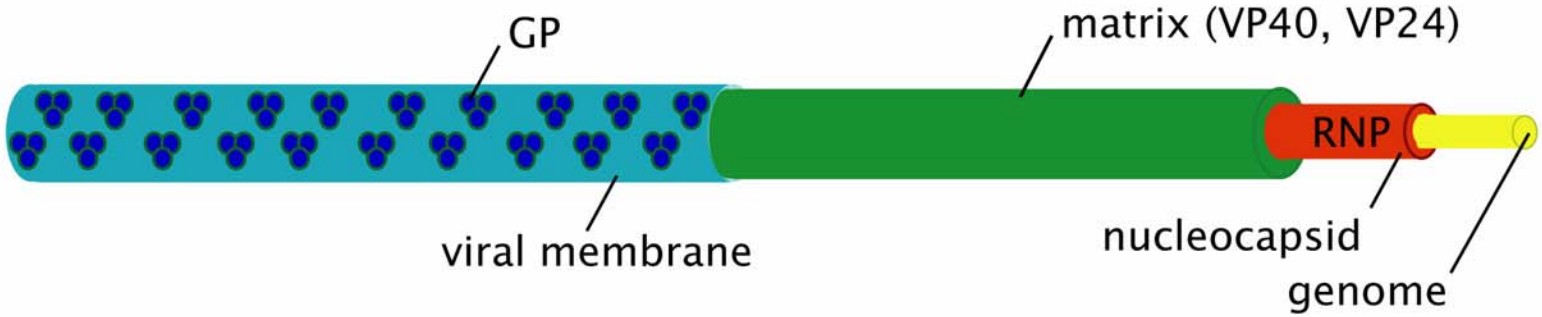
Ebola and Marburg viruses (family *Filoviridae*)

- *Nonsegmented negative strand RNA genomes*
- *Filamentous enveloped virions*
- *Rapidly fatal hemorrhagic fever*
- *> 20 outbreaks in equatorial Africa since 1976*
- *Macrophages and dendritic cells are early and sustained infection targets in vivo*
- *Broad tissue tropism in vitro*
- *Viral glycoprotein GP is proposed to be a major determinant of tissue tropism and cell injury*

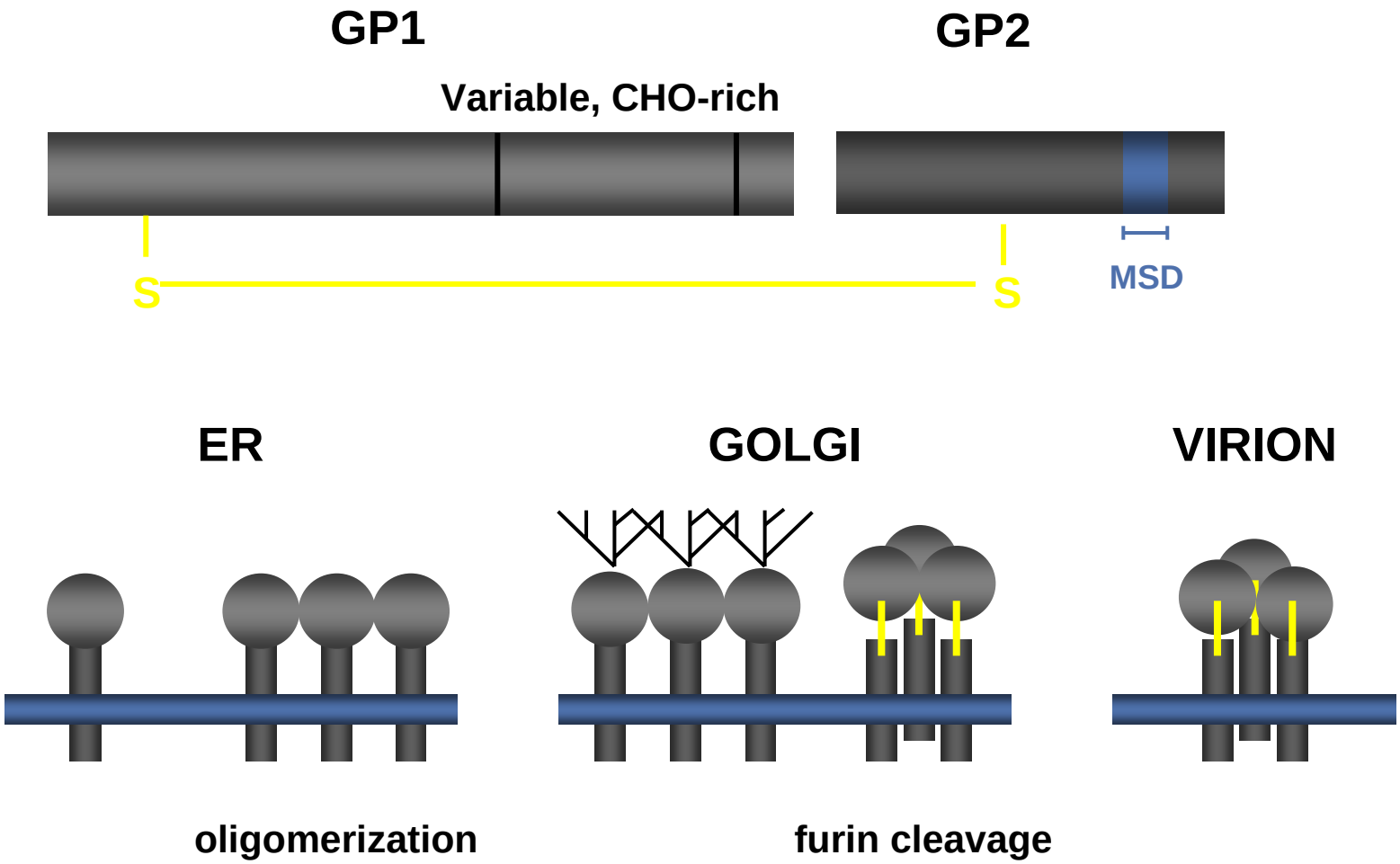


Architecture of Ebola virus virions

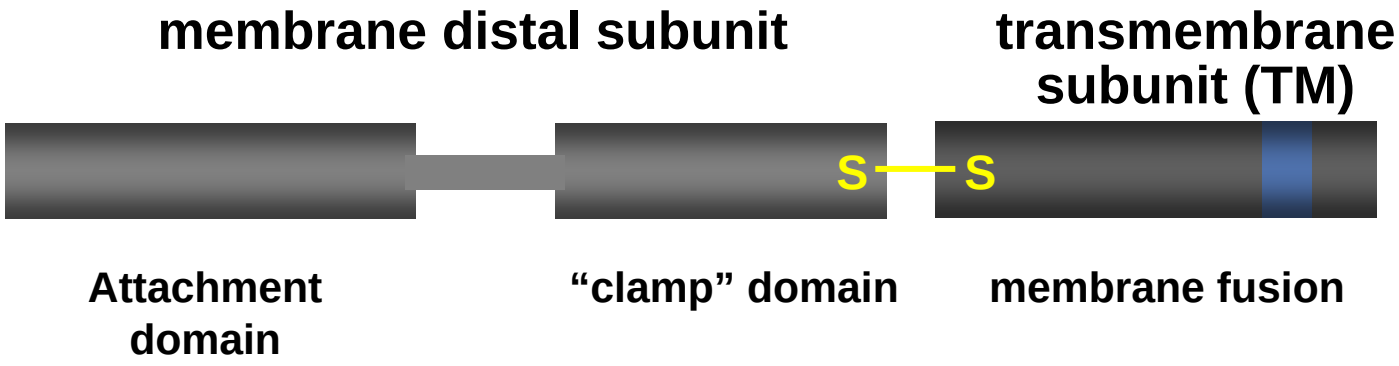


- *Viral envelope glycoprotein GP mediates membrane fusion and cell entry*

Assembly of Ebola Virus Envelope Glycoprotein



“Spring-loaded” model of Type I Env GP function



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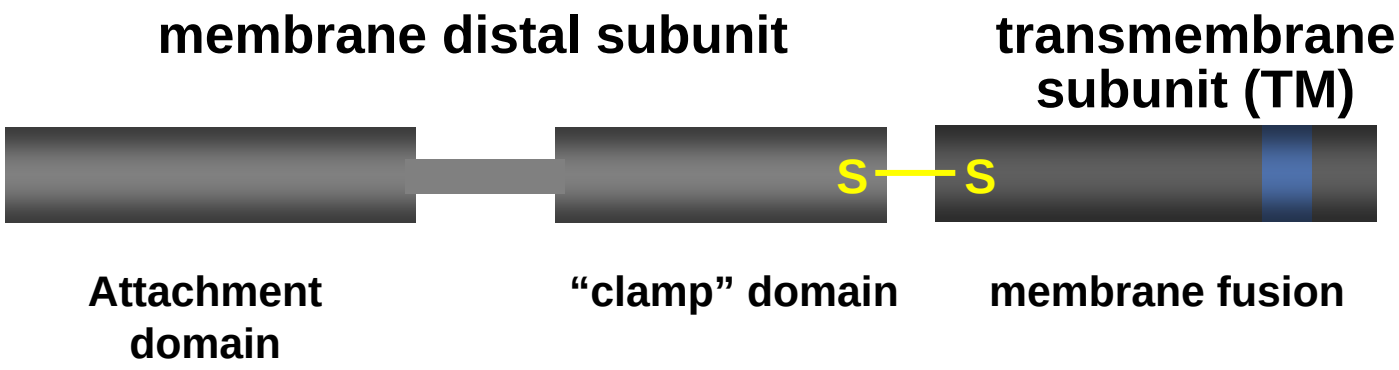
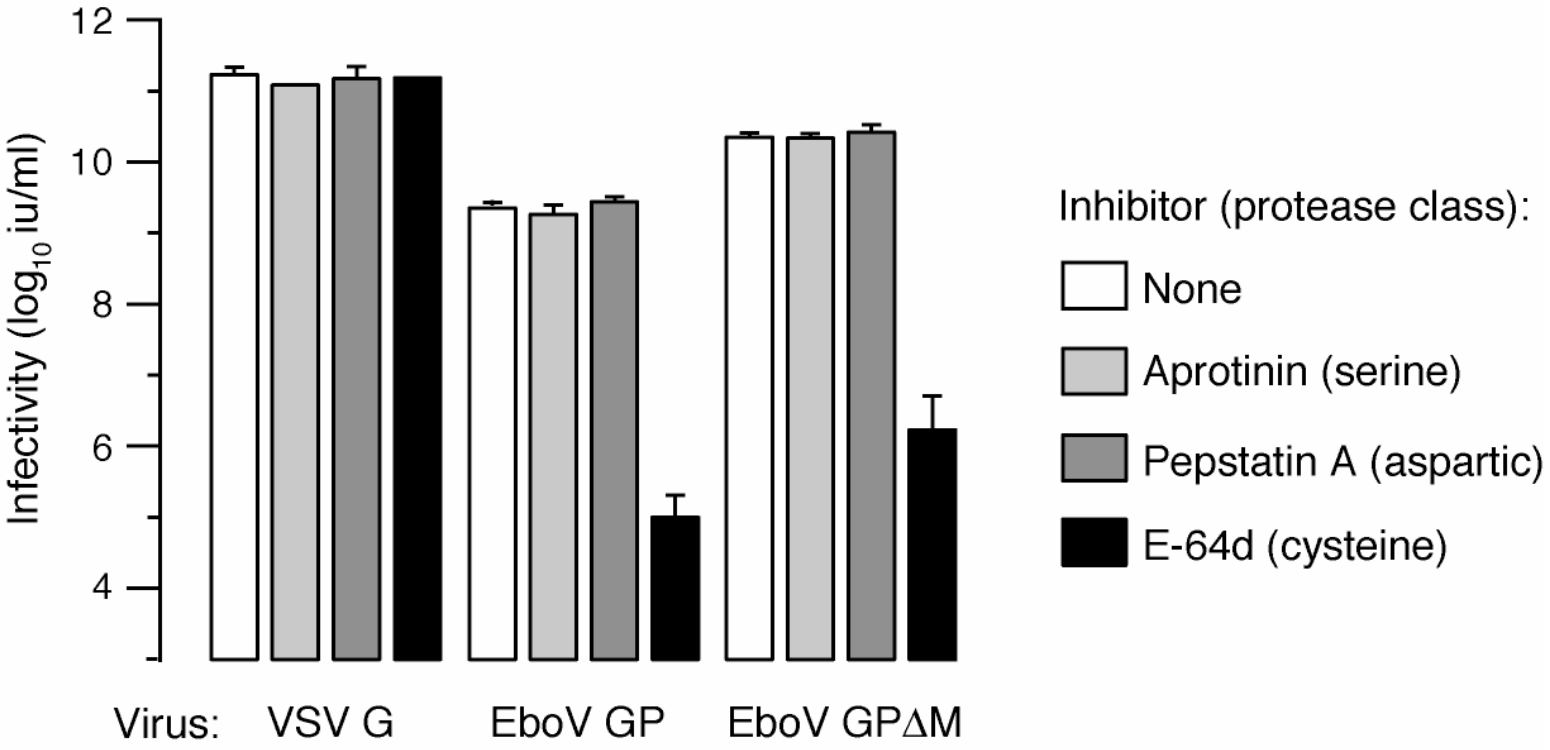


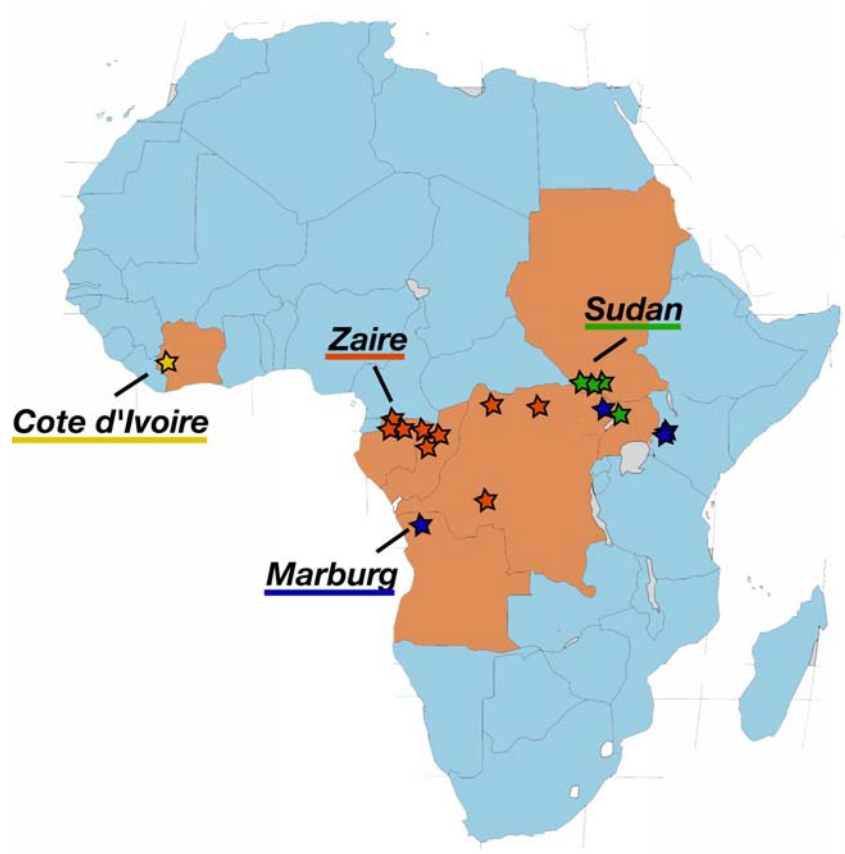
Receptor binding and/or endosomal acid pH are known host factors that release “clamp”:

Influenza:	acid pH
HIV:	CD4+CXCR
Avian retrovirus:	LDL-R+acid pH
Ebola virus:	?+acid pH

Effect of protease inhibitors on VSV-EboV GP (Zaire) infection

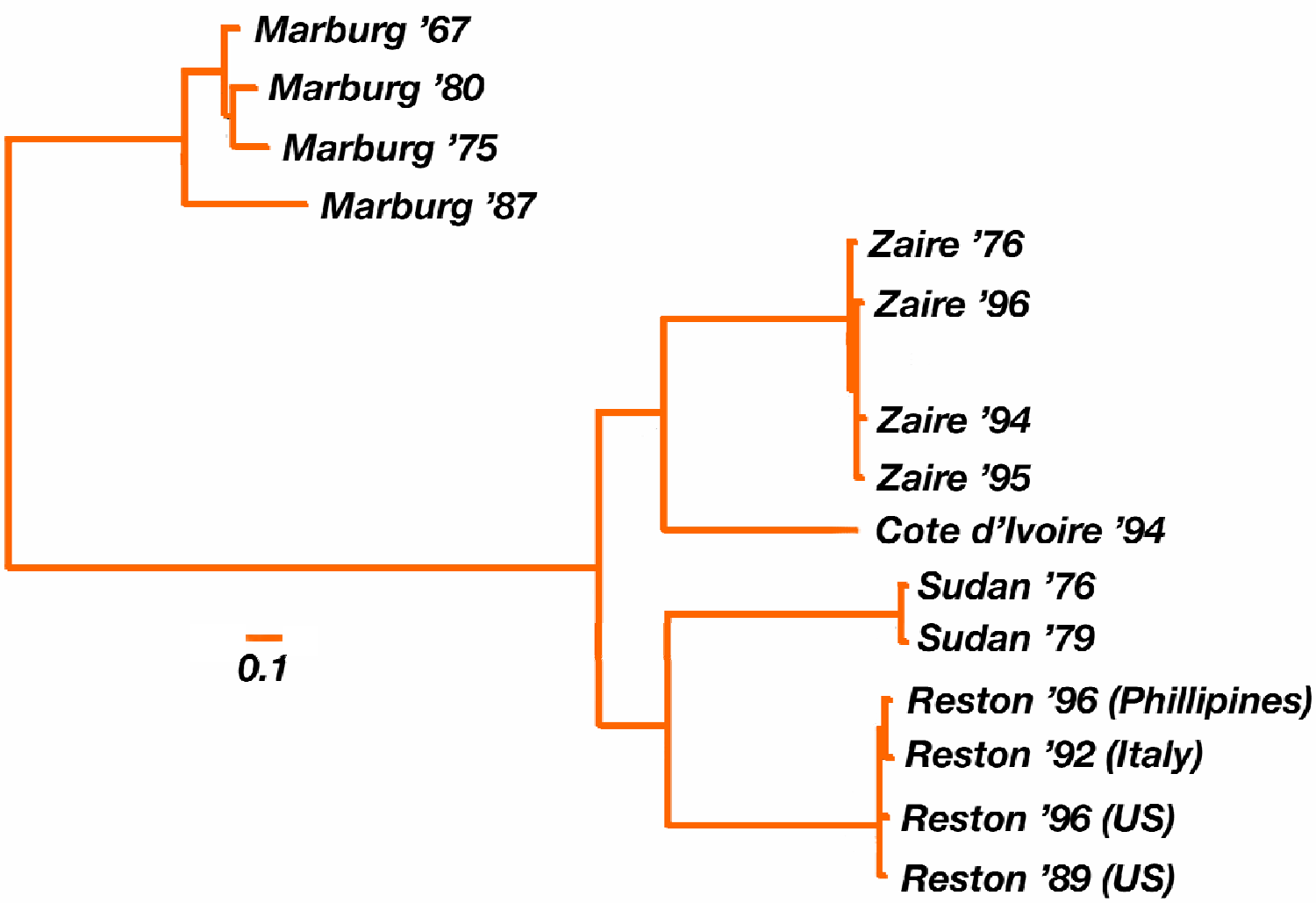


Independent Ebola/Marburg virus outbreaks in central Africa



Reston - Phillipines

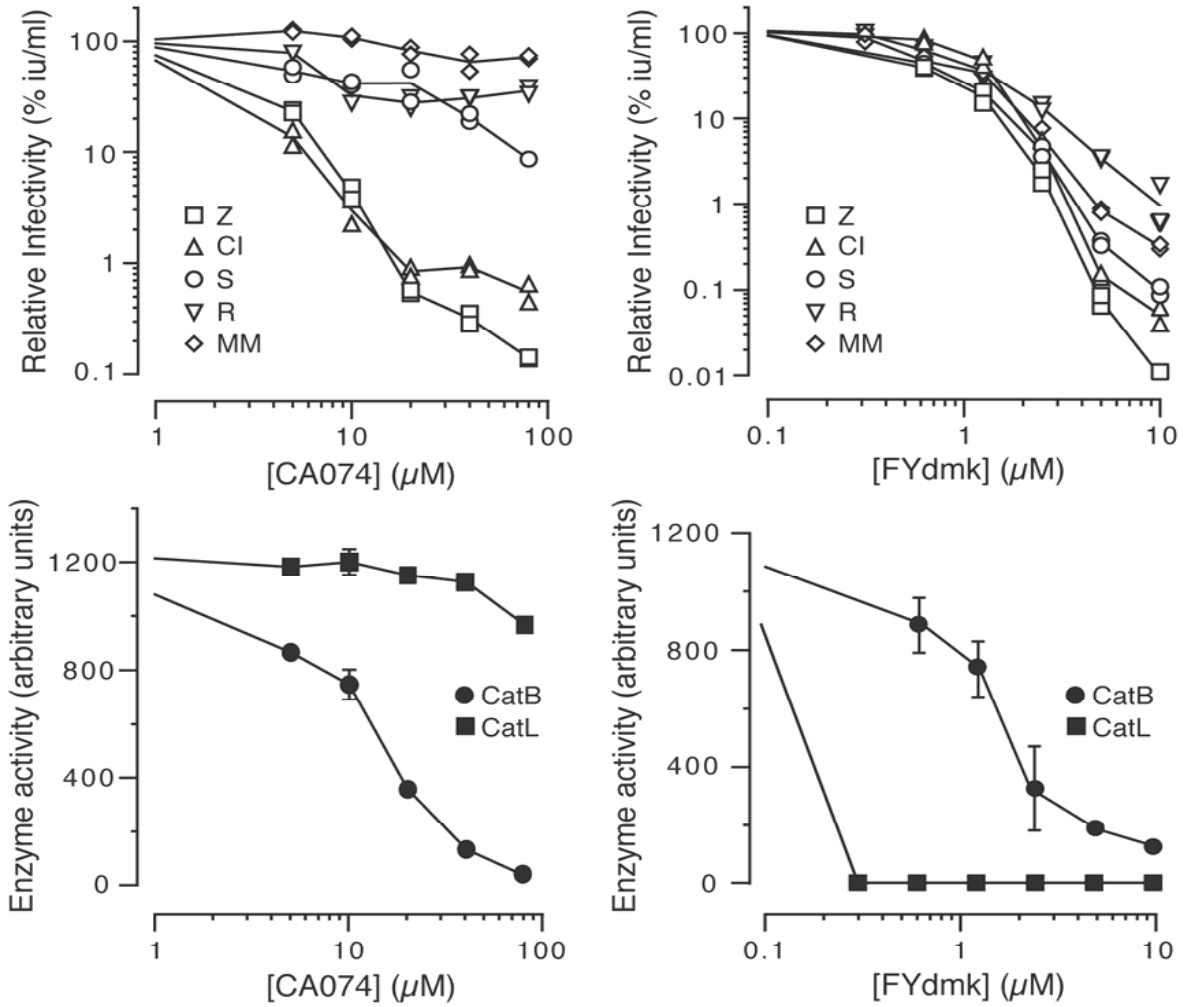
Phylogeny of Ebola and Marburg virus isolates



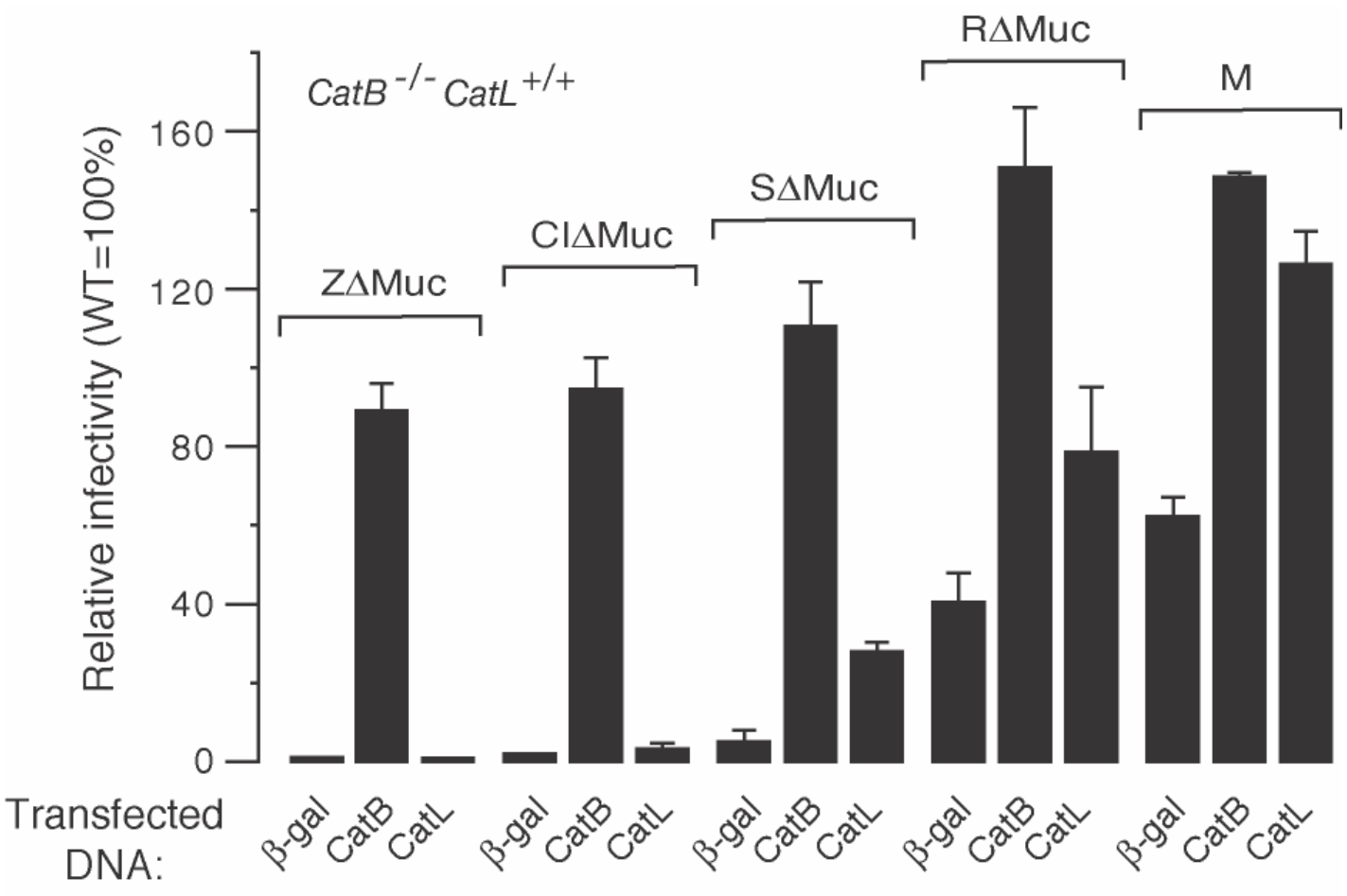
E-64 sensitive cysteine proteases

- *Family of as many as eleven related proteases (cathepsins)*
- *Localized in endosomes/lysosomes, $pK_a = 5.5$*
- *Endo- and exopeptidases, do not recognize specific sequence motifs*
- *Cathepsin L and cathepsin B: broad cell type expression*
- *Function in development, antigen presentation wound healing, metastasis*

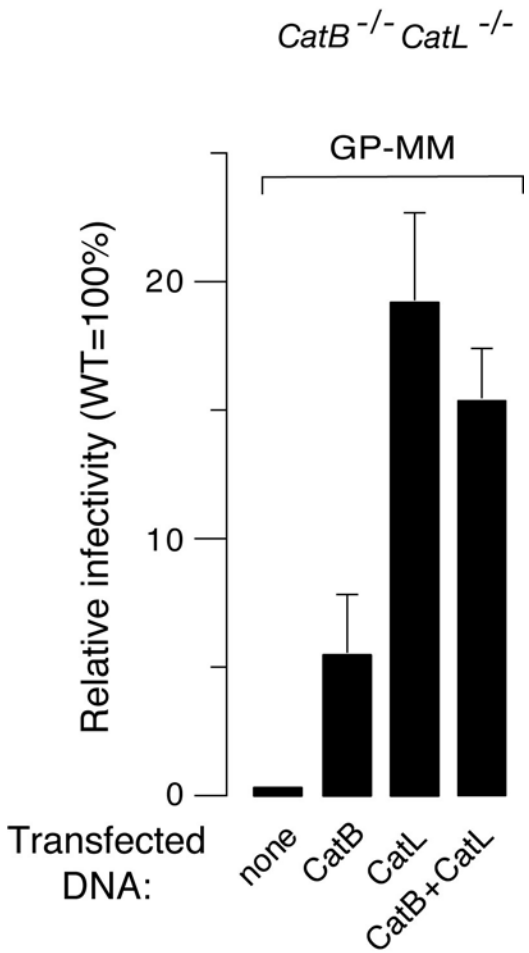
EboV Zaire and Cote d'Ivoire GP infection are strongly dependent on Cat B activity



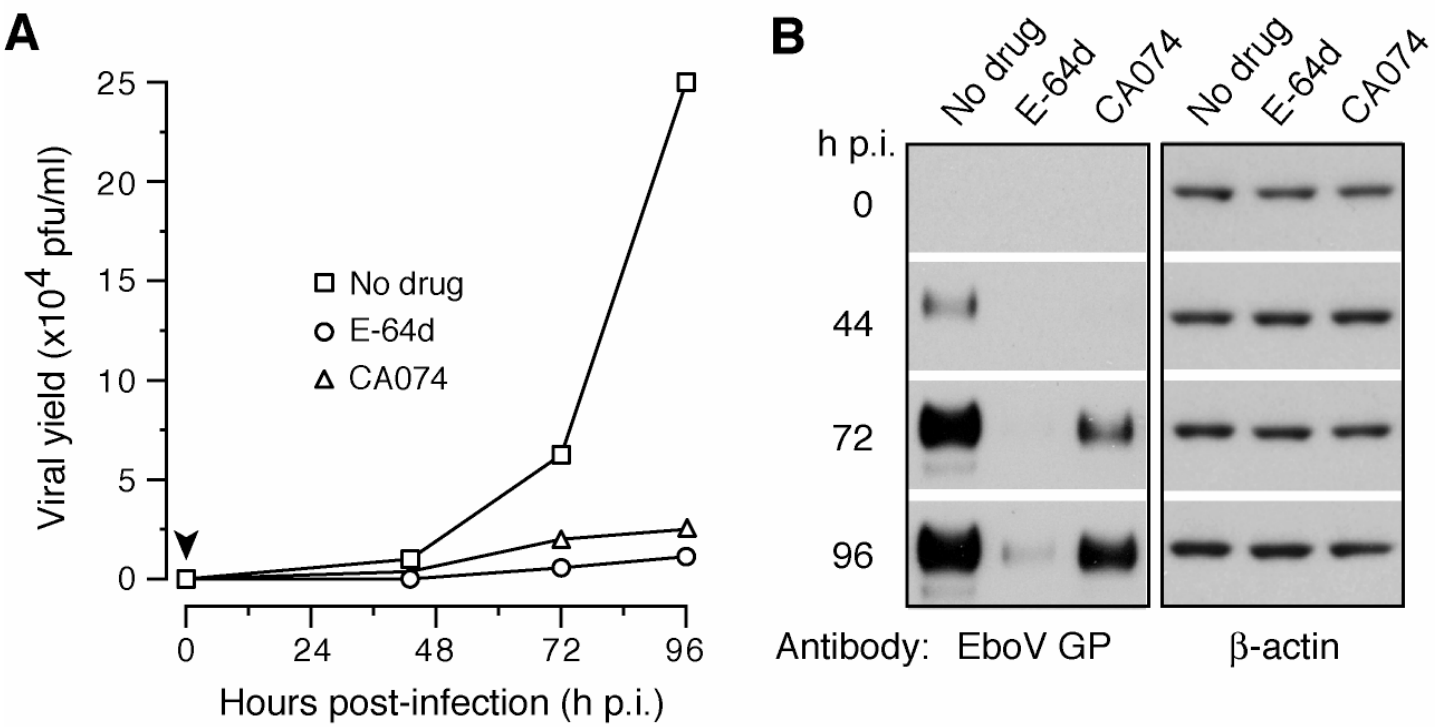
Virus-specific differences in dependence on Cat B and Cat L



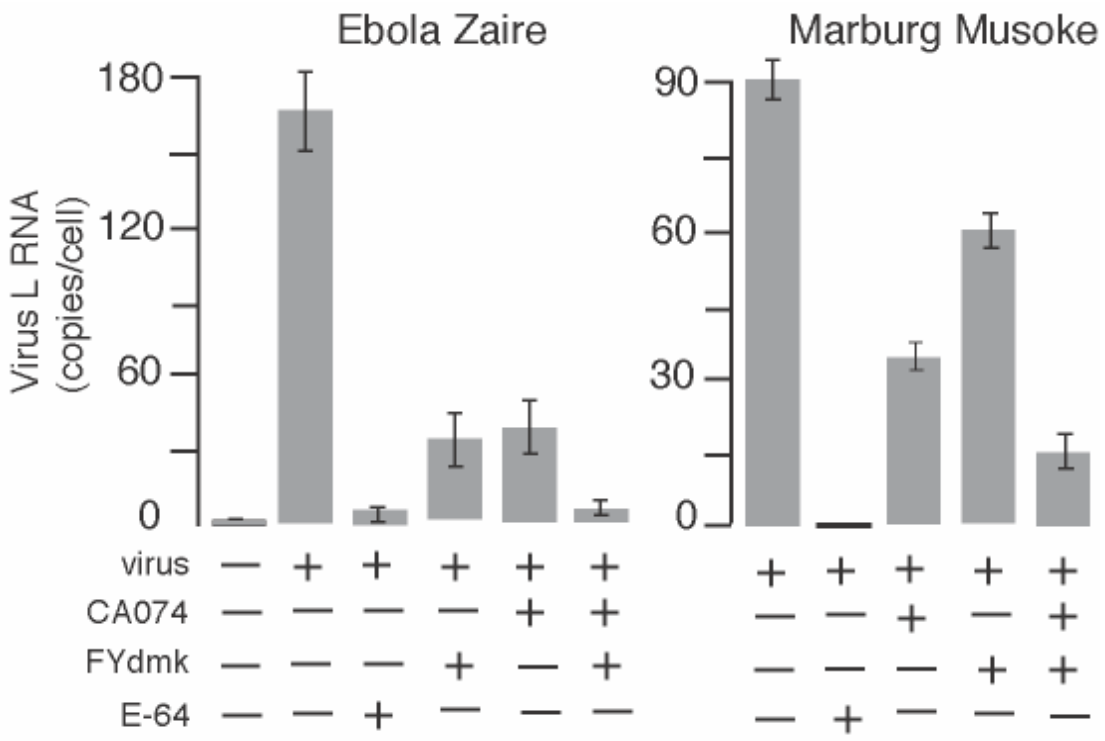
Either Cat L or Cat B is required for Marburg GP-dependent infection of murine embryo fibroblasts



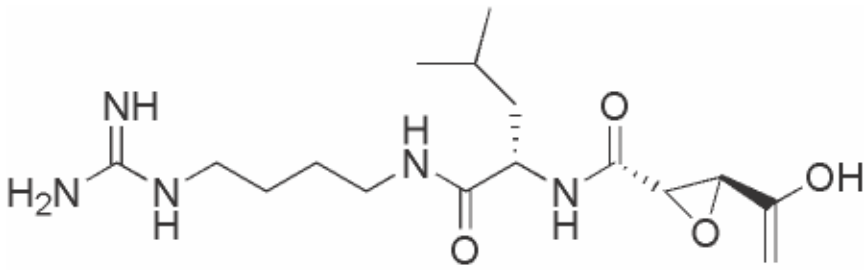
Cysteine protease inhibitors slow growth of *Ebola Zaire* virus



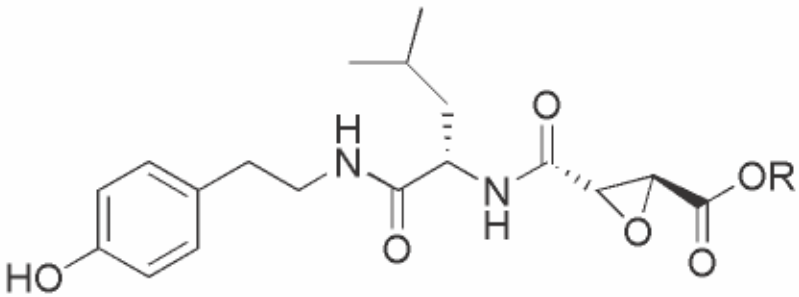
Chandran et al Science, April 2005


Ebola Zaire
Marburg Musoke
Virus L RNA
(copies/cell)
180
120
60
0
90
60
30
0
virus
CA074
FYdmk
E-64

Inhibitor development for *in vivo* studies

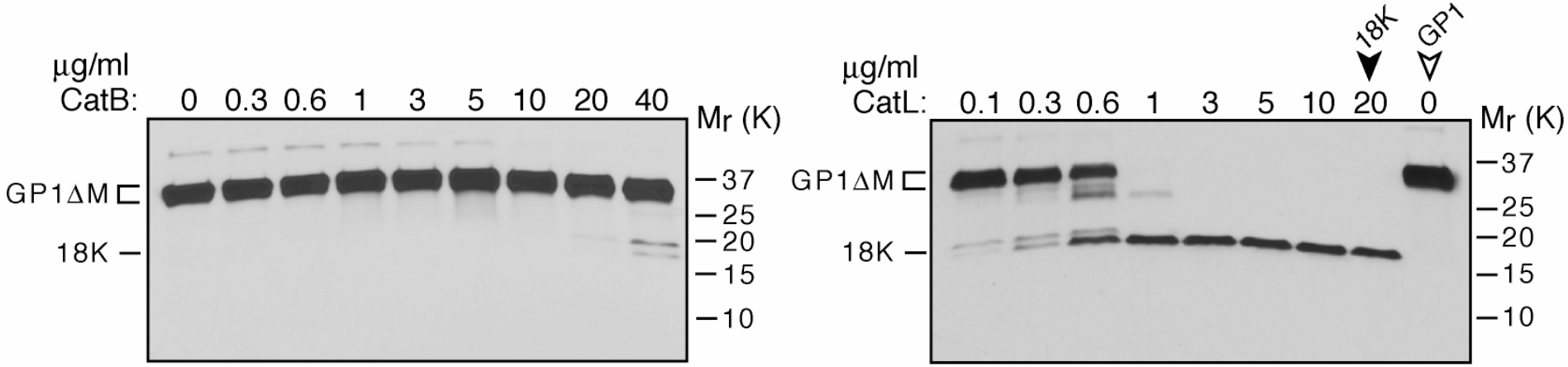


E-64

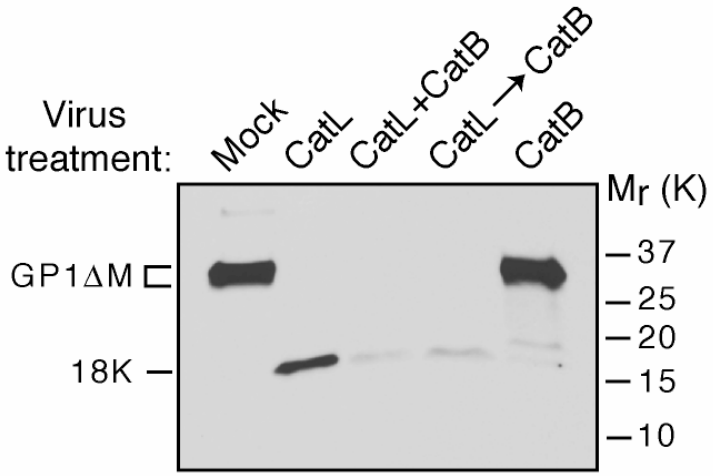


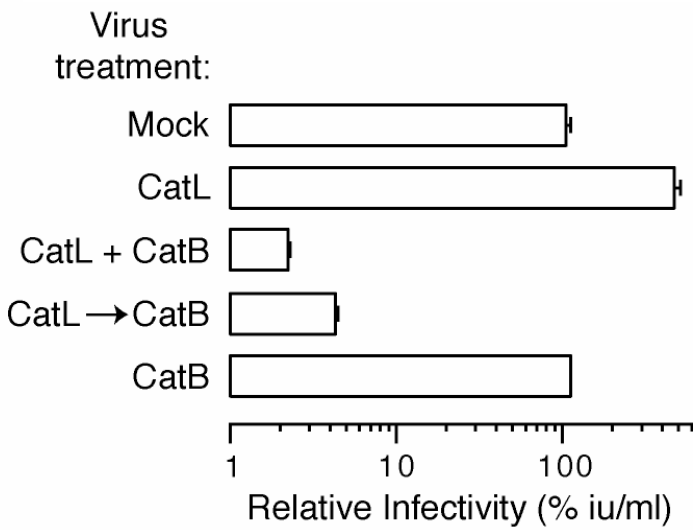
JPM-OEt

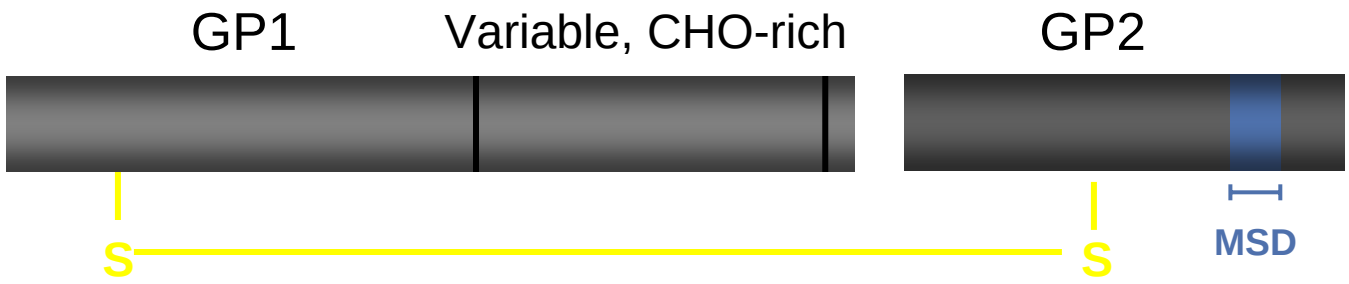
Purified Cat L efficiently cleaves GP1 to an ~18kDa fragment

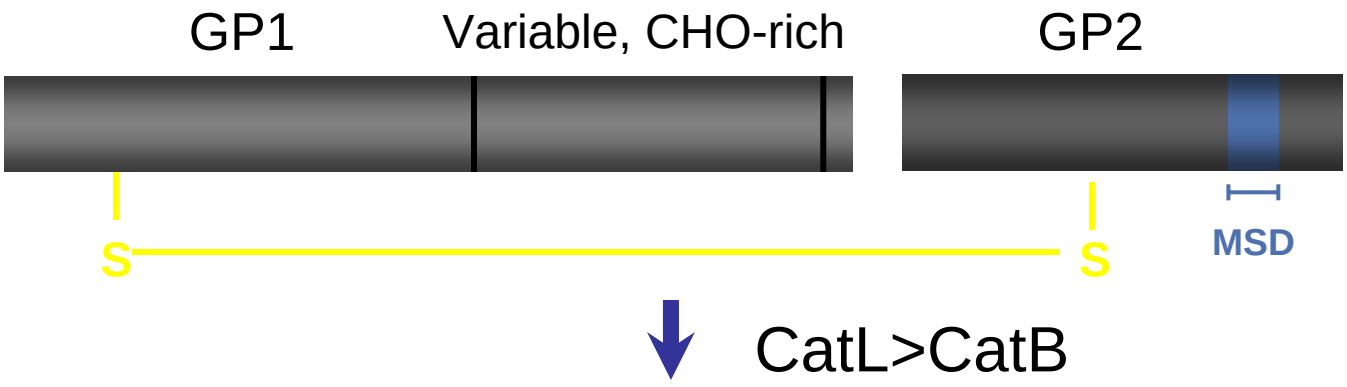


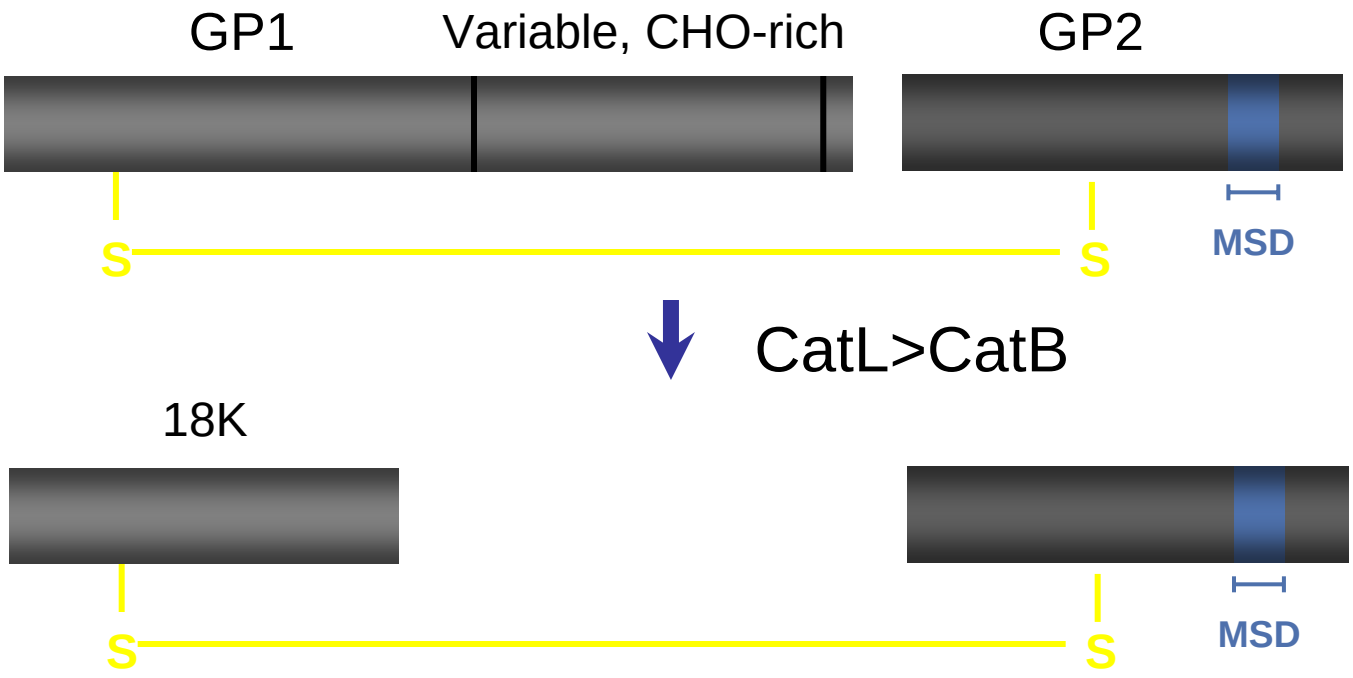
Cat B sensitizes EboV GP1 18K to further cleavage and inactivates infection

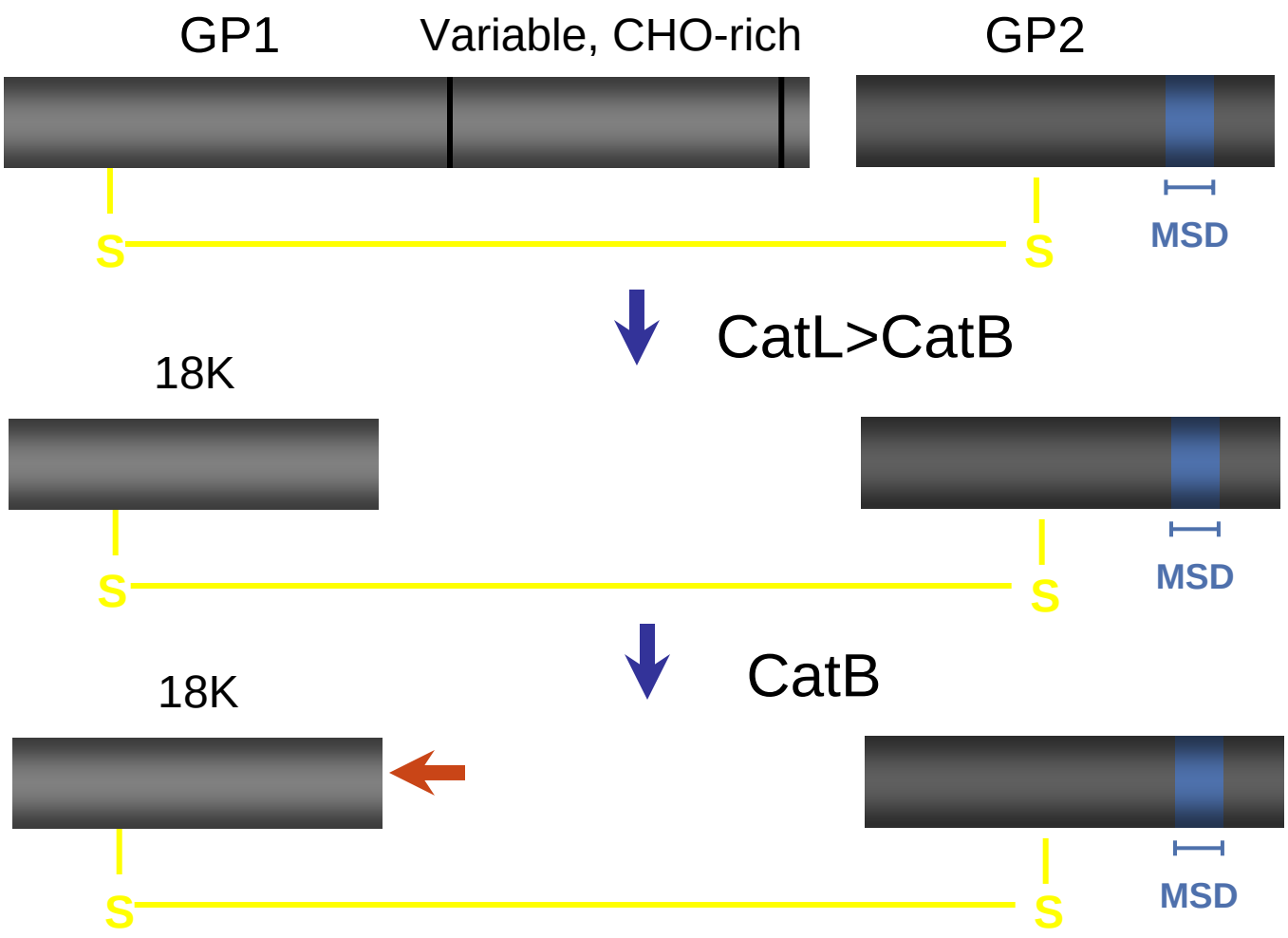


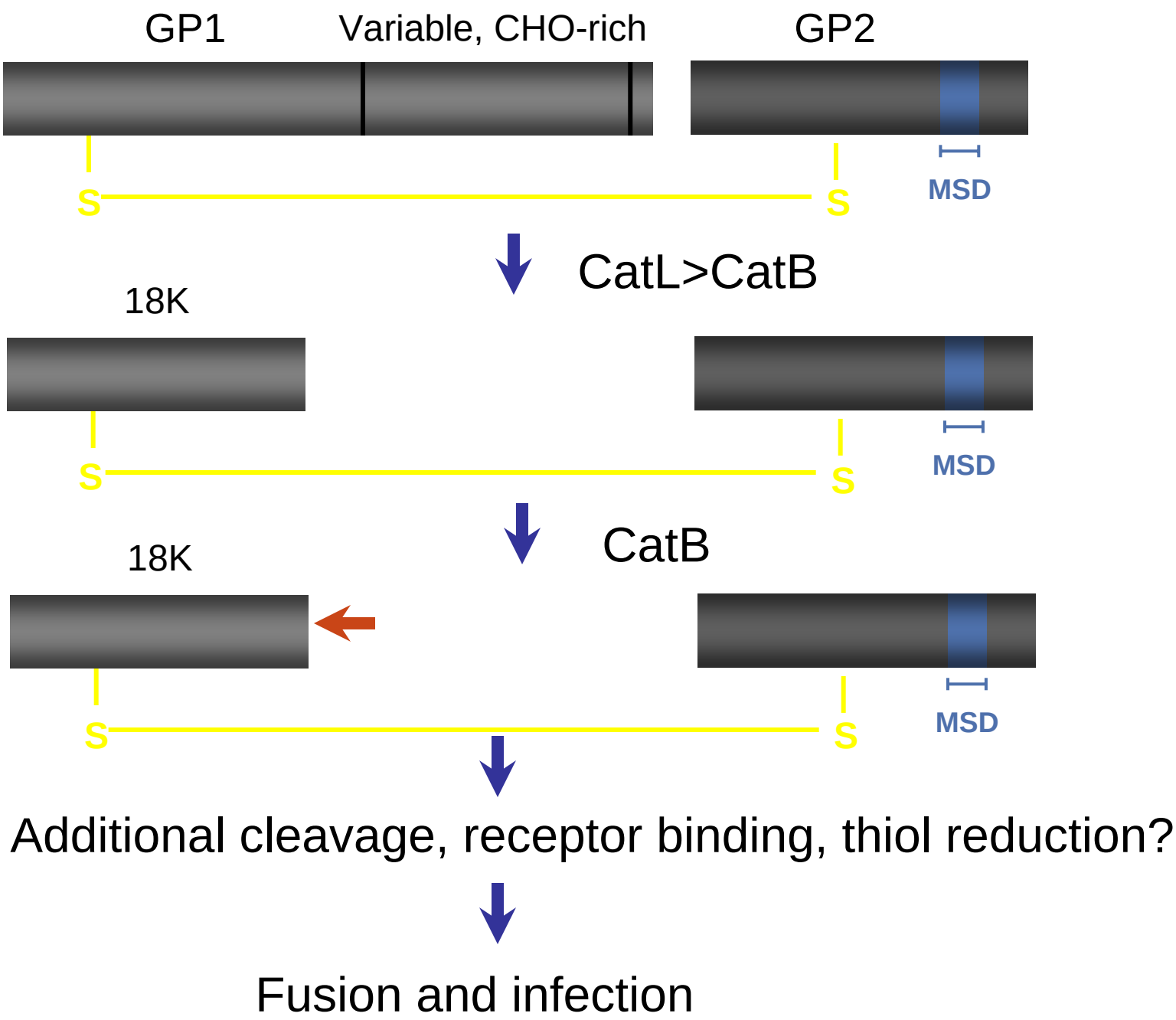



GP1
Variable, CHO-rich
GP2
S
S
MSD


GP1
Variable, CHO-rich
GP2
S
S
MSD
CatL>CatB


GP1
Variable, CHO-rich
GP2
S
S
MSD
CatL>CatB
18K
S
S
MSD


GP1
Variable, CHO-rich
GP2
S
S
MSD
CatL>CatB
18K
S
S
MSD
CatB
18K
S
S
MSD


GP1
Variable, CHO-rich
GP2
S
S
MSD
CatL>CatB
18K
S
S
MSD
CatB
18K
S
S
MSD
Additional cleavage, receptor binding, thiol reduction?
Fusion and infection

Conclusions

1. *Endosomal cysteine proteases are essential host factors for Ebola and Marburg virus infection of Vero cells, murine fibroblasts and primary human macrophages.*

2. *There are virus-specific differences in use of Cat L and Cat B as host factors.*

3. *Ebola virus GP is a substrate for cleavage by Cat L and Cat B.*

4. *Cleavage of Ebola virus GP by endosomal cysteine proteases is necessary for infection.*

5. *The role of cysteine proteases in Ebola virus infection may be analogous to CD4/CCR receptors in HIV infection or acid pH in influenza infection.*

6. *Cysteine protease inhibitors have potential as anti-viral drugs.*

Acknowledgements

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Knockout mice

Tony Sanchez — *CDC*

GP clones, antibodies

Unanswered questions

1. Is proteolysis of EboV GP by cysteine proteases sufficient for infection? EboV receptors?

2. What is location and topology of EboV fusion/entry?

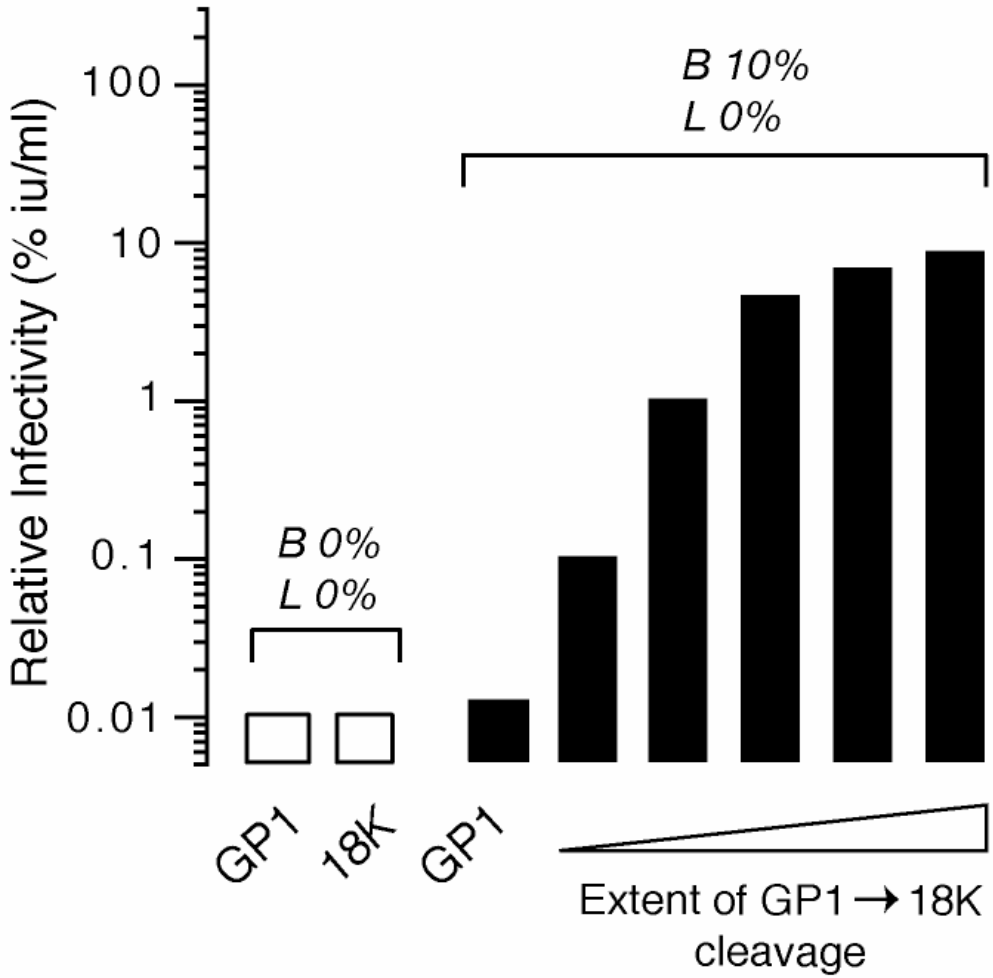
3. Does cell-type specific protease activity underlie EboV tropism?

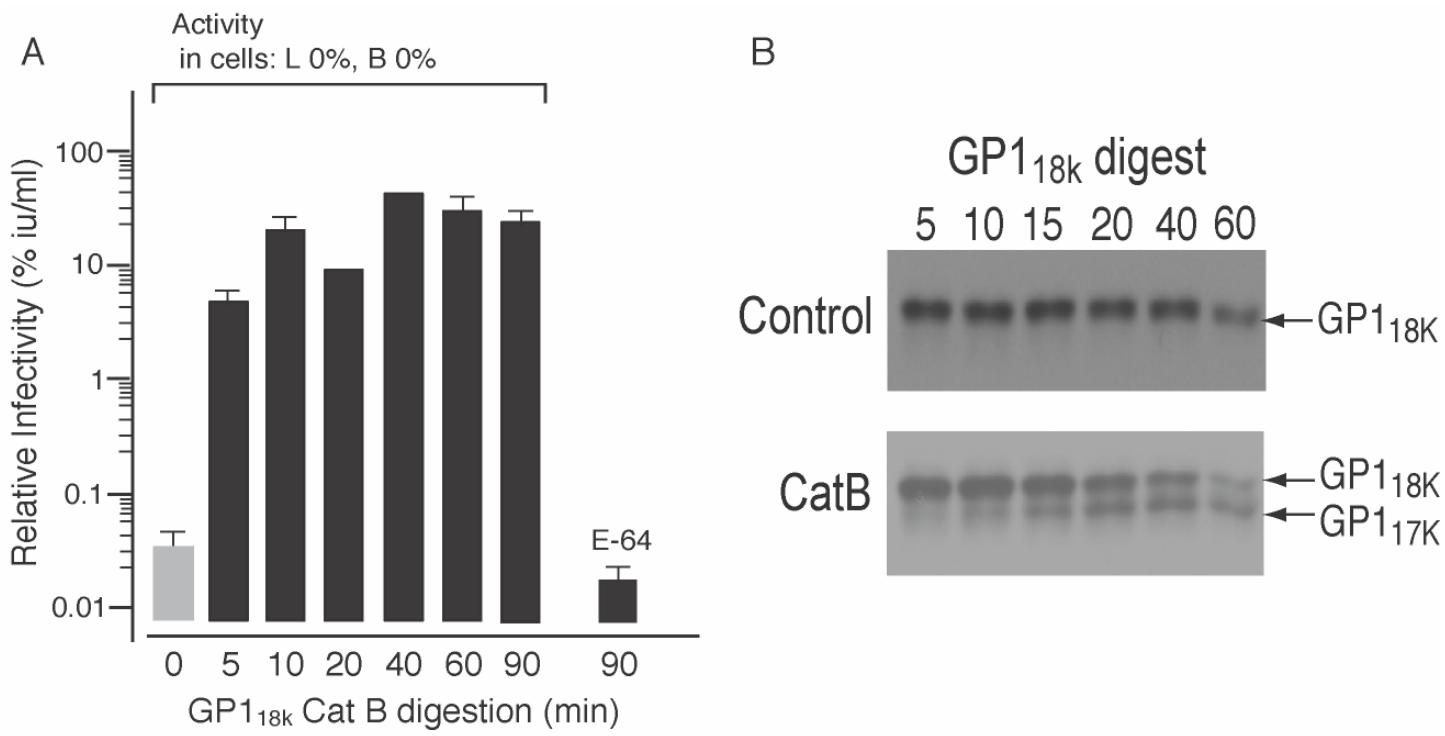
4. Does GP proteolysis influence virus-induced cytokine expression?

5. Is down regulation of cell protease activity necessary for efficient EboV assembly and release?

6. Why is polybasic cleavage site in GP conserved?

Evidence that GP1 is a target for cathepsin cleavage during infection




A
Activity
in cells: L 0%, B 0%
Relative Infectivity (% iu/ml)
100
10
1
0.1
0.01
E-64
0 5 10 20 40 60 90 90
GP1 18k Cat B digestion (min)
B
GP1 18k digest
5 10 15 20 40 60
Control
GP1 18K
CatB
GP1 18K
GP1 17K