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Emerging zoonotic viruses: new lessons on receptor and entry mechanisms
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Viruses enter the host cell by binding cellular receptors that allow appropriate delivery of the viral genome. Although the horizontal propagation of viruses feeds the continuous emergence of novel pathogenic viruses, the genetic variation of cellular receptors can represent a challenging barrier. The SARS coronavirus, henipaviruses and filoviruses are zoonotic RNA viruses that use bats as their reservoir. Their lethality for man has fostered extensive research both on the cellular mechanisms underlying both virus entry and pathogenesis. These studies have allowed new insights into the diversity of the molecular mechanisms underlying both virus entry and pathogenesis.

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Mankind is under a permanent threat from novel pathogens qualified as emerging [1,2,3]. Here I review the receptors and mode of entry of three emerging zoonotic viruses, responsible for rare but deadly diseases, whose natural reservoir is the bat: severe acute respiratory syndrome coronavirus (SARS-CoV), Hendra (HeV), Nipah (NiV), Ebola (EboV), and Marburg (MarV) viruses.

SARS-CoV: a dangerous affinity
SARS-CoV has a ~30 kb positive RNA genome and the crown-like shape typical of the *Coronaviridae*. A regular array of viral spike glycoprotein (S) trimers constitutes the viral envelope. S mediates binding to the cellular receptor Angiottensin Converting Enzyme 2 (ACE2) [4,5], and ensures the viral-cell membrane fusion that allows virus entry.

As an ectometalloprotease with monocarboxypeptidase activity, ACE2 cleaves the vasoconstrictor Angiotensin II octapeptide into the vasodilatator Ang1-7 heptapeptide. ACE2 protects the heart, lung and kidney from deleterious vasoconstriction and prevents the onset of an acute respiratory distress syndrome [4,5,8]. The tissue distribution of ACE2 (pneumocytes I and II, lung epithelium progenitor cells, small intestine enterocytes, kidney, heart cardiomyocytes and endothelium) mostly correlates with the known replication sites of SARS-CoV, and could explain the poor lung repair following SARS infection [4,9].

The ectopeptidase Type II transmembrane protease subfamily member 2 (TMPRSS2) was recently identified as a companion molecule of ACE2 [10,11,12]. TMPRSS2 is detected on the epithelium of the small intestine and respiratory tract, that is the major cell targets of SARS-CoV, but not on the endothelium, which is refractory to SARS-CoV infection [13,14]. TMPRSS2 and ACE2 physically interact [10]. Only a few S proteins get cleaved by TMPRSS2 to allow a pH- and cathepsin-independent efficient entry of SARS-CoV [10,12]. TMPRSS2 cleaves S protein at sites distinct from those ascribed to trypsin and cathepsin L [12]. Upon contact of ACE2 with S protein, ACE2 is also cleaved by TMPRSS2 [10]. When expressed on opposing membranes, SARS-CoV S and the ACE2 + TMPRSS2 complex induce intercellular fusion [11]. However, newly expressed S proteins escape cleavage by TMPRSS2 allowing the production of virions decorated with uncleaved S [10,11,12], possibly because the tripartite association is prevented intracellularly.

The present model of virus entry predicts the following (Figure 1a): SARS-CoV S protein binds to the ACE2 receptor via the concave S494 region of the receptor binding site (RBD) that cradles over 17 nm² of the outer surface of the N-terminal lobe of the ACE peptidase domain, that is outside the enzymatic site [15]. This activates TMPRSS2 to cleave a few S proteins into fusion-competent S1-S2 homodimers [10,11,12,16], which immediately undergo typical class I fusion protein structural changes [17] which permit the viral envelope to fuse with the plasma membrane. S1-S2 heterodimers are probably too unstable to be incorporated into infectious virus particles [16,18]. Moreover, activated TMPRSS2, and possibly ADAM17/TACE (TNFα converting enzyme) [4,19], concomitantly cleave ACE2. This results in the massive shedding of ACE2 ectodomains [10], probably due to amplification of the constitutive pathway [5].

ACE2 shedding is not required for SARS-COV entry [5,20], but is probably responsible for the associated major lung failure. Indeed, soluble S both induces ACE2 shed-
ding and worsens the clinical signs of SARS [21]. In humans and according to virus strain diversity, S/ACE2 affinity correlates with the efficacy of virus entry, the level of ACE2 cleavage and the intensity of the pathology. Furthermore, the highest affinity is associated with inter-human transmission [20,22] (Figure 1b). Antibodies targeting the S binding site on ACE2 strongly inhibit viral infection [23]. The correlation between S/ACE2 affinity and SARS-CoV pathogenicity extends to the host range for other mammal species (except bats) in determining whether a particular ACE2 protein can act as a receptor for SARS-CoV or not (including among bats) (Figure 2b) [23–27]. Consequently, the accuracy of an animal model of human infection critically depends on the capacity to mimic the affinity between the human ACE2 and viral S protein. In mammals, except bats, SARS-CoV induces a ‘toxic-like’ syndrome by triggering a devastating massive cleavage of the cellular receptor used for entry, the molecular basis of which remains to be documented. Why bats do not exhibit clinical signs of infection by SARS-CoV remains puzzling.

**Henipaviruses: a universal receptor?**

NiV and HeV constitute the *Henipavirus* genus of the *Paramyxoviridae* family and are responsible for fatal respiratory and neurological diseases. Their non-segmented negative strand RNA genomes code for two envelope glycoproteins. The fusion protein F is synthesized as a precursor maturated into a functional F1–F2 heterodimer by cathepsin L via a clathrin-mediated recycling endosomal pathway [28,29]. The attachment protein G is a tetramer consisting of two disulfide bridged dimers. Like the *morbillivirus* H and *parainfluenzae* HN proteins, its C-terminal globular head is folded into a six β-sheet blade propeller surmounting a stalk, transmembrane region and cytosolic tail. The sugar-free β1–β6 dimer interface is
G protein attaches to the cellular receptors ephrinB2 and ephrinB3 [32]. Ephrins are ligands of Eph receptor tyrosine kinases involved in cell homeostasis [33*]. EphB4 and its ligand ephrinB2 are expressed on the endothelial cells of veins and small arteries, respectively [34]. Their reciprocal trans-endocytosis acts as a repulsive signal during vasculogenesis [33*]. EphrinB2 is also expressed in the smooth muscle cells of vessels [34]. The tissue distribution of ephrinB3 is restricted to the spinal cord and the corpus callosum [35]. EphrinB2/B3 expression correlates with the tropism of NiV in infected humans [32].

Ephrin binding sites on NiV and HeV G map to the top of the globular head over the β5 and β6 blades [36*], that is away from the center and side of the propeller, where sialic acid and protein receptors bind parainfluenza HN proteins and measles H, respectively [37]. Astonishingly, both the physiological ligands of ephrinB2, EphB2, EphB4 and EphA4, and the G proteins of NiV and HeV bind the same site (F113–K131) on the G–H loop [30,31,36*,38,39] (Figure 2). However, the henipavirus G proteins exhibit the highest affinity to ephrinB2 [38,40*].

EphrinB2 from mammal species, including human, horse, mouse, pig, cat, dog, and bat, can act as efficient cellular receptor for NiV. Indeed, the G–H loop FTTKQFE(–F,Y)SPN_LWG(L,H)EF sequence is highly conserved between ephrinB2 and ephrinB3 including in those from the distantly related species zebrafish and chicken as supported by successful replication of NiV in chicken embryo [41]. Bronchial epithelium gets infected in the pig and cat [42–44] but not in human [45] suggesting ectopic expression of ephrinB2/3 in the former species. A LW/YM substitution prevents ephrinB1 from acting as a receptor for NiV and HeV and binding to EphB4 and EphB2 receptor [35,46,47]. Correlatively, (i) all ligands compete with each other, (ii) the ephrinB2/3 binding site on NiV-G is exquisitely neutralized by specific antibodies in vitro and in vivo [48,49*] and (iii) there is a cross-protection between NiV and HeV [50]. The affinity of
NiV-G and HeV-G for ephrinB2/B3 correlates with the efficiency of virus entry [48,51].

Interestingly, whereas NiV G and F induce fusion of cells expressing ephrinB2/B3, NiV preferentially enters after internalization via macropinocytosis [52**, though acidic pH is not required [52**,53]. Virus entry, but not membrane fusion, is inoperative when the cytosolic tail of ephrinB2 has its PDZ-binding motif deleted or Tyr304 mutated [52**]. These two motifs recruit Grb4 and the P21-activated kinase 1 (Pak1)/CdC42/Rac1 complex that govern macropinocytosis [33*,54,55,56*,57–59]. The need for macropinocytosis while the fusion machinery is operative at the cell surface is puzzling. Macropinocytosis occurs very rapidly upon contact [60] and could be faster than the fusion step but then macropinocytosis inhibitors would not be expected to prevent virus entry. Several hypotheses can be proposed: (i) fusion requires a specific Ca** and/or Na+ ionic environment as documented for Semliki Forest virus [62]. (ii) NiV replication requires a specific conditioning of the cytoplasm induced by contacting ephrinB2/B3. (iii) The nucleocapsid needs to reach a particular cytoplasmic location deeper in the cells, more favorable for viral polymerase activity. The latter would not be unprecedented since forced rerouting of virus normally entering by fusion at the cell surface into the endocytic pathway results in hampered infectivity as shown for pseudotyped measles virus and lentivectors [63–65].

Filoviruses: an elusive receptor

The Filoviridae EboV and MarV cause severe hemorrhagic fevers and septic-like shock in humans [66]. Their non-segmented negative RNA genomes code for the envelope glycoprotein GP which ensures both attachment to a (still elusive) cellular receptor and membrane fusion. GP is cleaved by a furin-like protease into mature GP1–GP2 heterodimers [67]. Curiously, mutation of the furin-cleavage site does not abolish GP-mediated virus entry due to alternative cleavage [68]. GP is heavily glycosylated with sugar moieties recognized by LESCtin and DC-SIGN/R lectins that can enhance but not mediate infection [69–72]. This high glycan content shields MHC class I and β-integrin from antibody recognition [73,74], a finding that explains the previously reported apparent downregulation of the latter [75].

Figure 3

Model of filovirus entry. Virus binds to the cell surface via recognition of sugar moieties (blue branches) of GP1 by DC-SIGN or LESCtin (light green) (1) to be immediately internalized by macropinocytosis (2) and migrate through the endocytic pathway (3) until the mucin-like domain (mucin) is cleaved off from GP1 by resident cathepsin B (cath B). This results in RBR (yellow star) being accessible for binding to the postulated receptor (dark green question mark) (4). An additional activation event (disulfide bridge reduction?) occurs (5). This triggers the conformational changes of GP2 (yellow) that mediates fusion of viral and endosomal membranes and ensures the delivery of the nucleocapsid (NC) into the cytosol (pale yellow background) where replication occurs (6). Because the postulated receptor (question mark) is predicted to be expressed at the cell surface, it has been included in every step of virus internalization. Although not yet documented, endosome structures 4 to 6 may successively represent early, maturing, late and possibly endolysosomes, because of the ~1 h delay between the cell attachment and membrane fusion steps.
A cellular receptor of glycoprotein nature is predicted on the basis of saturable binding of soluble GP [76] and loss of binding after protease, periodate or tunicamycin treatment [76,77]. In infected animals, the virus disseminates in many tissues [66]. The EboV receptor is stocked in trans-Golgi network membranes in all cell types including the non-permissive T and B lymphocytes. It is exported to the cell surface upon cell adhesion and internalized via a microtubule-dependent and actin-dependent pathway, respectively [78,79]. EboV and MarV GP cross-compete for binding suggesting the use of a common receptor [76,80]. However, 3 out of 4 key lysines (at positions 114,115 and 140) defining the receptor binding region (RBR) of EboV GP1 [76] are not conserved in MarV GP1 [81**]. The structure of a soluble trimeric form of GP1–GP2 reveals a GP1-based chalice form, lined by the RBR. The fusion competent GP2 trimers cradle the chalice stem, with the internal fusion peptide flanked by two β-sheets. The RBR is mostly shielded by a glycan cap and a mucin-like domain [82**], the cleavage of which by cathepsins strongly enhances GP1–GP2 binding to the cell surface [76,83]. However, lowering the pH neither allows EboV entry at the cell surface, nor cell–cell fusion by mucin-deleted GP1–GP2, and the GP/receptor interaction is stable at acidic pH [76,77,84**].

In effect, EboV mostly enters by macropinocytosis with a requirement for lipid rafts, the Na+/H+ exchanger, Pak 1, Rac1, Rab5, Rab7, RhoC GTPase and the vacuole closure protein C-terminal binding protein 1 of E1A, CtBP/BARS [59,85**,86**,87–89]. Constitutive macropinocytosis in dendritic cells and macrophages fits with their permissiveness to EboV infection [90,91]. Activation of Ax1 enhances both macropinocytosis and EboV entry [92] although the latter may be mediated by serum Gas6, which was recently reported to mediate non-specific entry for several enveloped viruses [93].

The EboV (and MarV) entry process lasts for about 1 h [94,95] and can be schematized as follows (Figure 3): Firstly, (i) EboV attaches to the cells via the GP1/GP2 interaction with DC-SIGN/R and/or LECStin and is (ii) immediately internalized by constitutive and/or virus-contact-induced macropinocytosis. (iii) After ~30 min of endocytic trafficking, EboV reaches a late endosomal compartment, where (iv) the resident cathepsin B cleaves off the mucin-like domain [83,84**,96] to (v) expose GP1’s RBR so that the putative receptor can be recruited; then, (vi) a late pH-dependent activation step of the mucin-deleted GP1/GP2 complex triggers the fusion activity of GP2, possibly via the reduction of a disulfide bridge [84**].

In conclusion, several lessons can be taken home. (i) Susceptibility to a disease can be driven by the affinity level between the viral attachment glycoprotein and its cellular receptor. (ii) Evolutionary conserved orthologs of a viral receptor can allow an extended host-range. (iii) A timely proteolytic activation of membrane fusion can occur only upon binding to the receptor. (iv) A viral glycoprotein may follow a complex maturation pathway during endosomal trafficking.

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest  • of outstanding interest

1. Snowden FM: Emerging and reemerging diseases: a historical perspective. Immunnol Rev 2008, 225:9-26.

2. Morens DM, Folkers GK, Fauci AS: Emerging infections: a perpetual challenge. Lancet Infect Dis 2008, 8:710-719.

An historical overview that allows easy understanding of the "emerging infection" concept.

3. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P: Global trends in emerging infectious diseases. Nature 2008, 451:990-993.

4. Kuba K, Imai Y, Ohno-Nakanishi T, Penninger JM: Trilogy of ace2: a peptidase in the renin-angiotensin system, a sars receptor, and a partner for amino acid transporters. Pharmacol Ther 2010, 128:119-128.

A review describing known physiological functions of ACE2 that allow the understanding of its key role in the severe lung injury induced by SARS-CoV.

5. Jia HP, Look DC, Tan P, Shi L, Hickey M, Gakhar L, Chappell MC, Wohlford-Lenane C, McCray PB Jr: Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. Am J Physiol Lung Cell Mol Physiol 2009, 297:L84-96.

6. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA et al.: Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005, 436:112-116.

7. Oudit GY, Herzenberg AM, Kassiri Z, Wong D, Reich H, Khokha R, Crackower MA, Backx PH, Penninger JM, Scholey JW: Loss of angiotensin-converting enzyme-2 leads to the late development of angiotensin ii-dependent glomerulosclerosis. Am J Pathol 2008, 168:1808-1820.

8. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, da Costa J, Zhang L, Pei Y, Scholey J et al.: Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002, 417:822-828.

9. Chen Y, Chan VS, Zheng B, Chan KY, Xu X, To LY, Huang FP, Khoo US, Lin CL: A novel subset of putative stem/progenitor cells is the major target for sars coronavirus in the human lung. J Exp Med 2007, 204:2529-2536.

10. Shulla A, Heald-Sargent T, Subramanya G, Zhao J, Perlman S, Gallagher T: A transmembrane serine protease is linked to the severe acute respiratory syndrome coronavirus receptor and activates virus entry. J Virol 2011, 85:873-882.

These three convergent and complementary papers describe the TMPRRSS2 ectopeptase as a partner of ACE2 receptor responsible for both activation of SARS-CoV protein allowing virus entry and shedding of ACE2 ectodomain.

11. Matsuyama S, Nagata N, Shirato K, Kawase M, Takeda M, Taguchi F: Efficient activation of the severe acute respiratory syndrome coronavirus spike protein by the transmembrane protease TMPRSS2. J Virol 2010, 84:12656-12664.

see ref. [10]
12. Glöckler A, Bertram S, Muller MA, Allen P, Soilleux E, Pfefferle S, Steffen I, Tsegaye TS, He Y, Gnirss K, Niemeyer D, Schneider H, Drosten C, Pohlmann S: Evidence that TMPRSS2 activates the severe acute respiratory syndrome coronavirus spike protein for membrane fusion and reduces viral control by the humoral immune response. J Virol 2011, 85:4122-4134.

13. Vaarala MH, Porvari KS, Kellokumpu S, Kylötone AP, Vihko PT: Expression of transmembrane serine protease TMPRSS2 in mouse and human tissues. J Pathol 2001, 193:134-140.

14. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GJ, van Goor H: Tissue distribution of ace2 protein, the functional receptor for sars coronavirus. A first step in understanding sars pathogenesis. J Virol 2004, 80:631-637.

15. Li F, Li W, Farzan M, Harrison SC: Structure of sars coronavirus spike receptor-binding domain complexed with receptor. Science 2005, 309:1864-1868.

16. Belouzard S, Chu WC, Whittaker GR: Activation of the sars coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. Proc Natl Acad Sci USA 2009, 106:5871-5876.

17. Roche S, Albertini AA, Lepault J, Bressanelli S, Gaudin Y: Structures of vesicular stomatitis virus glycoprotein: membrane fusion revisited. Cell Mol Life Sci 2008, 65:1716-1728.

18. Kam YW, Okumura Y, Kido H, Ng LF, Bruzzone R, Altmeyer R: Cleavage of the sars coronavirus spike glycoprotein by airway proteases enhances virus entry into human bronchial epithelial cells in vitro. PLoS ONE 2009, 4:e7870.

19. Haga S, Yamamoto N, Nakai-Murakami C, Osaka Y, Tokunaga K, Sata T, Sasazuki T, Ishizaka Y: Modulation of mfn-alpha-converting enzyme by the spike protein of sars-cov and ace2 induces mfn-alpha production and facilitates viral entry. Proc Natl Acad Sci USA 2008, 105:7809-7814.

20. Glöckler A, Bertram S, Herzog P, Pfefferle S, Steffen I, Muench MO, Simmons G, Hofmann H, Kuri T, Weber F, Eichler J et al.: Differential downregulation of ace2 by the spike protein of severe acute respiratory syndrome coronavirus and human coronavirus niv3. J Virol 2010, 84:1198-1205.

21. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L et al.: A crucial role of angiotensin converting enzyme 2 (ace2) in sars coronavirus-induced lung injury. Nat Med 2005, 11:875-879.

22. Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, Song SK, Huang IC, Xu K, Vasilieva N, Murakami A et al.: Receptor and viral determinants of sars-coronavirus adaptation to human ace2. EMBO J 2005, 24:1634-1643.

23. The solved structure illuminates the viral spike binding site on ACE2 receptor.

24. Li W, Wong SK, Li F, Kuhn JH, Huang IC, Choe H, Farzan M: Animal origins of the severe acute respiratory syndrome coronavirus: insight from ace2-s-protein interactions. J Virol 2008, 82:4211-4219.

25. Li F: Structural analysis of major species barriers between humans and palm civets for severe acute respiratory syndrome coronavirus infections. J Virol 2008, 82:6984-6991.

26. Graham RL, Baric RS: Recombination, reservoirs, and the modular spike: Mechanisms of coronavirus cross-species transmission. J Virol 2010, 84:3134-3146.

27. Hou Y, Peng G, Yu M, Li Y, Han Z, Li F, Wang LF, Shi Z: Angiotensin-converting enzyme 2 (ace2) proteins of different bat species confer variable susceptibility to sars-cov entry. Arch Virol 2010, 155:1563-1569.

28. Yu M, Tachedjian M, Crameri G, Shi Z, Wang LF: Identification of key amino acid residues required for horsehose bat angiotensin-i converting enzyme 2 to function as a receptor for severe acute respiratory syndrome coronavirus. J Gen Virol 2010, 91:1708-1712.

29. Diederich S, Mall M, Klenk HD, Maisner A: The nipah virus fusion protein is cleaved within the endosomal compartment. J Biol Chem 2005, 280:29899-29903.

30. Pager CT, Craft WW Jr, Patch J, Dutch RE: A mature and fusogenic form of the nipah virus fusion protein requires proteolytic processing by cathepsin L. Virology 2006, 346:251-257.

31. Bowden TA, Crispin M, Harvey DJ, Jones EY, Stuart DI: Dimeric architecture of the hendra virus attachment glycoprotein: evidence for a conserved mode of assembly. J Virol 2010, 84:6208-6217.

32. Bowden TA, Crispin M, Harvey DJ, Aricescu AR, Grimes JM, Jones EY, Stuart DI: Crystal structure and carbohydrate analysis of nipah virus attachment glycoprotein: a template for antiviral and vaccine design. J Virol 2008, 82:11628-11636.

33. Lee B: Envelope-receptor interactions in nipah virus pathobiology. Ann N Y Acad Sci 2007, 1102:51-65.

34. Ptitsyn OS: Nipah virus: a recently emergent deadly paramyxovirus. Arch Virol 2000, 145:2153-2163.

35. Negrete OA, Chu D, Aguilar HC, Lee B: Single amino acid changes in the nipah and hendra virus attachment glycoproteins distinguish ephrinb2 from ephrinb3 usage. J Virol 2007, 81:10804-10814.

36. Bowden TA, Aricescu AR, Gilbert RJ, Grimes JM, Jones EY, Stuart DI: Structural basis of nipah and hendra virus attachment to their cell-surface receptor ephrin-b2. Nat Struct Mol Biol 2008, 15:567-572.

37. Shin D, Garcia-Cardenas G, Hayashi S, Gerety S, Asahara T, Stavrakis G, Isner J, Folkman J, Gimbrone MA Jr, Anderson DJ: Expression of ephrinb2 identifies a stable genetic difference between arterial and venous vascular smooth muscle as well as endothelial cells, and marks subsets of microvessels at sites of adult neovascularization. Dev Biol 2001, 230:139-150.

38. Hashiguchi T, Ose T, Kubota M, Matsu N, Kamishikyori Y, Maenaka K, Yanagi Y: Structure of the measles virus hemagglutinin bound to its cellular receptor clam. Nat Struct Mol Biol 2011, 18:135-141.

39. Qin H, Noberini R, Huan X, Shi J, Pasquale EB, Song J: Structural characterization of the epha4-ephrin-b2 complex reveals new features enabling eph-ephrin binding promiscuity. J Biol Chem 2010, 285:644-654.

40. Himanen JP, Saha N, Nikolov DB: Cell-cell signaling via eph receptors and ephrins. Curr Opin Cell Biol 2007, 19:534-542.

41. Negrete OA, Wolf MC, Aguilar HC, Enterlein S, Wang W, Muhtberger E, Su SV, Bertolotti-Ciarlet A, Flick R, Lee B: Two key residues in ephrinb3 are critical for its use as an alternative receptor for nipah virus. PLoS Pathog 2006, 2:e7.

42. By mutagenesis of two amino acid that differ between ephrinb1 and ephrinb3 within the G-H loop and using binding and virus entry assays, the molecular basis of ephrinb2/b3 exclusive usage as henipavirus receptor was established.

43. Tanimura N, Imada T, Kashiwazaki Y, Sharifah SH: Distribution of viral antigens and development of lesions in chicken embryos inoculated with nipah virus. J Comp Pathol 2006, 135:74-82.

44. Chua KB, Bellini WJ, Rota PA, Harcourt BH, Tamin A, Lam SK, Ksiazek TG, Rollin PE, Zaki SR, Shieh W, Goldsmith CS et al.: Nipah virus: a recently emergent deadly paramyxovirus. Science 2000, 288:1432-1435.

45. Middleton DJ, Westbury HA, Morrissy CJ, van der Heide BM, Eaton BT, Broder CC: Vertical transmission and fetal replication.
of nipah virus in an experimentally infected cat. J Infect Dis 2007, 196:812-816.

45. Wong KT, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ et al.: Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. Am J Pathol 2002, 161:2153-2167.

46. Chenicke JE, Brooun A, Kraus ML, Kolatkar AR, Han GW, Seifert JM, Widerm H, Auer M, Kuhn P: Structural and biophysical characterization of the ephb4*ephrinb2 protein interaction and receptor specificity. J Biochem 2006, 281:2818-28192.

47. Himanen JP, Rajashankar KR, Lackmann M, Cowan CA, Henkemeyer M, Nikolov DB: Crystal structure of an eph receptor-ephrin complex. Nature 2001, 414:933-938.

48. Bossart KN, Tachdjian M, McEachern JA, Crameri G, Zhu Z, Dube D, Brecher MB, Delos SE, Rose SC, Park EW, et al.: Feline model of acute nipah virus infection and protection with a soluble glycoprotein-based subunit vaccine. J Virol 2006, 80:12293-12302.

49. Aguilar HC, Aspericueta V, Robinson LR, Aanensen KE, Lee B et al.: Different potential of c-type lectin-mediated entry between marburg virus strains. J Virol 2010, 84:5148-5157.

50. Mungall BA, Middleton D, Crameri G, Bingham J, Halpin K, Russell G, Green D, McEachern J, Pritchard LJ, Eaton BT, Wang LF et al.: Dendritic cell function at low physiological temperature. J Virol 2009, 83:357-365.

51. Powlesland AS, Fisch T, Taylor ME, Smith DF, Tissot B, Dell A, Pohlmann S, Drickamer K: Stable transduction of quiescent t cells without ctn in vivo. Proc Natl Acad Sci USA 1999, 96:7691-7695.

52. Wolfsleod RJ, Bates P: Endocytoblastic processing of the ebola virus envelope glycoprotein: cleavage is not required for function. J Virol 1999, 73:1419-1426.

53. Francica JR, Varela-Rohena A, Medvec A, Plesa G, Riley JL, Mateo M, Takada A: Different potential of c-type lectin-mediated entry between ebola virus surface glycoprotein through truncated glycans. J Virol 2008, 82:393-402.

54. Reynard O, Borowiak M, Volchkova VA, Delpeut S, Mateo M, Takada A: A novel method for lentinial vector pseudotyped with measles virus glycoproteins improves titer and selectivity. Gene Ther 2009, 16:700-705.

55. Funke S, Schneider IC, Glaser S, Muhlebach MD, Moritz T, Cattaneo R, Cichutek K, Buchholz CJ: Pseudotyping lentiviral vectors with the wild-type measles virus glycoproteins improves titer and selectivity. Gene Ther 2009, 16:700-705.

56. Amendola CA, Benetti F, Delpeut S, Mateo M, Takada A: A new method for lentiviral vector pseudotyped with measles virus glycoproteins improves titer and selectivity. Gene Ther 2009, 16:700-705.
These two papers demonstrate the intracellular pool of RBR binding protein of EboV that can be exported to the cell surface upon cell adhesion including in lymphocyte, which are refractory to EboV infection probably because they poorly able to prime GP.

79. Dube D, Schornberg KL, Stantchev TS, Bonaparte MI, Delos SE, • Bouton AH, Broder CC, White JM: Cell adhesion promotes ebola virus envelope glycoprotein-mediated binding and infection. J Virol 2006, 80:7238-7242. see ref. [78]

80. Kuhn JH, Radoshitzky SR, Guth AC, Warfield KL, Li W, Vincent MJ, • Towner JS, Nichol ST, Bavari S, Choe H, Aman MJ et al.: Conserved receptor-binding domains of lake victoria marburgvirus and zaire ebolavirus bind a common receptor. J Biol Chem 2006, 281:15951-15958.

81. Brindley MA, Hughes L, Ruiz A, McCray PB Jr, Sanchez A, • Sanders DA, Maury W: Ebola virus glycoprotein 1: identification of residues important for binding and postbinding events. J Virol 2007, 81:7702-7709. A systematic mutagenesis study that has allowed the delineation of key residues of Ebolavirus RBR within GP1.

82. Lee JE, Fusco ML, Hessler AJ, Oswald WB, Burton DR, • Saphire EO: Structure of the ebola virus glycoprotein bound to an antibody from a human survivor. Nature 2008, 454:177-182. A first structure of prefusion EboV GP1-GP2 heterodimer showing the 82 residues of EboV RBR within GP1.

83. Kaletsky RL, Simmons G, Bates P: Proteolysis of the ebola virus glycoproteins enhances virus binding and infectivity. J Virol 2007, 81:13378-13384.

84. Schornberg K, Matsuyama S, Kabsch K, Delos S, Bouton A, • White J: Role of endosomal cathepsins in entry mediated by the ebola virus glycoprotein. J Virol 2006, 80:4174-4178. First demonstration for a two-step activation of EboV GP occurring in the endosomal pathway using chemical inhibitors, RNA silencing, and biochemical cleavage by cathepsins.

85. Nanbo A, Imai M, Watanabe S, Noda T, Takahashi K, Neumann G, • Halfmann P, Kawao Y: Ebola virus is internalized into host cells via macropinocytosis in a viral glycoprotein-dependent manner. PLoS Pathog 2010, 6:e1001121. Using Ebolavirus-like particles made of GP and VP40, a close mimic of the filamentous Ebolavirus, infectious EboV and dedicated molecular tools and chemical inhibitors, entry by macropinocytosis was demonstrated by these two papers.

86. Saeed MF, Kolokoltsov AA, Albrecht T, Davey RA: Cellular entry of ebola virus involves uptake by a macropinocytosis-like mechanism and subsequent trafficking through early and late endosomes. PLoS Pathog 2010:8. see ref. [85]

87. Quinn K, Brindley MA, Weller ML, Kaludov N, Kondratowicz A, Hunt CL, Sinn PL, McCray PB Jr, Stein CS, Davidson BL, Flick R et al.: Rho gtpases modulate entry of ebola virus and vesicular stomatitis virus pseudotyped vectors. J Virol 2009, 83:10176-10186.

88. Liberati P, Kakkonen E, Turacchio G, Valente C, Spaar A, Perinetti G, Bockmann RA, Corda D, Colanzi A, Marjomaki V, Luini A: The closure of pakt-dependent macropinosomes requires the phosphorylation of ctbp1/bars. EMBO J 2008, 27:970-981.

89. Kalin S, Amstutz B, Gastaoldelli M, Wolfrum N, Boucke K, Havenga M, DiGennaro F, Liska N, Hemmi S, Greber UF: Macropinocytotic uptake and infection of human epithelial cells with species b2 adenovirus type 35. J Virol 2010, 84:5336-5350.

90. Norbury CC, Chambers BJ, Prescott AR, Ljunggren HG, Watts C: Constitutive macropinocytosis allows tap-dependent major histocompatibility complex class i presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. Eur J Immunol 1997, 27:289-298.

91. Norbury CC, Hewlett LJ, Prescott AR, Shastri N, Watts C: Class i mhc presentation of exogenous soluble antigen via macropinocytosis in bone marrow macrophages. Immunity 1995, 3:783-791.

92. Hunt CL, Kolokoltsov AA, Davey RA, Maury W: The tyro3 receptor kinase axl enhances macropinocytosis of zaire ebolavirus. J Virol 2011, 85:334-347.

93. Morizono K, Xie Y, Olafsen T, Lee B, Dasgupta A, Wu AM, Chen IS: The soluble serum protein gas6 bridges virion envelope phosphatidyserine to the tam receptor tyrosine kinase axl to mediate viral entry. Cell Host Microbe 2011, 9:286-296.

94. Saeed MF, Kolokoltsov AA, Freiberg AN, Holbrook MR, Davey RA: Phosphoinositide-3 kinase-akt pathway controls cellular entry of ebola virus. PLoS Pathog 2008, 4:e1000141.

95. Schelhaas M: Come in and take your coat off—how host cells provide endocytosis for virus entry. Cell Microbiol 2010, 12:1378-1388.

96. Chandran K, Sullivan NJ, Felbor U, Whelan SP, Cunningham JM: Endosomal proteolysis of the ebola virus glycoprotein is necessary for infection. Science 2005, 308:1643-1645.