Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Trends in Cell Biology

CellPress

Opinion

Tracking Mechanisms of Viral Dissemination *In Vivo*

Raphael Gaudin[1,2,6,*,@] and Jacky G. Goetz[3,4,5,7,*,@]

Dissemination and replication of viruses into hosts is a multistep process where viral particles infect, navigate, and indoctrinate various cell types. Viruses can reach tissues that are distant from their infection site by subverting subcellular mechanisms in ways that are, sometimes, disruptive. Modeling these steps, at appropriate resolution and within animal models, is cumbersome. Yet, mimicking these strategies *in vitro* fails to recapitulate the complexity of the cellular ecosystem. Here, we will discuss relevant *in vivo* platforms to dissect the cellular and molecular programs governing viral dissemination and briefly discuss organoid and *ex vivo* alternatives. We will focus on the zebrafish model and will describe how it provides a transparent window to unravel new cellular mechanisms of viral dissemination *in vivo*.

Highlights

The zebrafish model allows *in vivo* investigations of virus-induced molecular processes at subcellular resolution.

Viruses have evolved multiple strategies for disseminating over long distance, including by indoctrinating host cell types with high migration potential.

Organoids derived from stem cells emerge as powerful alternatives to unravel new molecular mechanisms of viral dissemination.

Studying Viral Dissemination *In Vivo*: The Zebrafish Model

To disseminate into hosts, pathogens had to evolve strategies to navigate and indoctrinate complex three-dimensional environments that cannot be fully recapitulated in two-dimensional monolayer cell culture. They deploy molecular stratagems to hijack resident stromal cells of multiple tissues and favor their dissemination and replication. While mimicking these strategies *in vitro* or *ex vivo* can be useful, either through the use of organoids or tissue explants (Box 1), established animal models are powerful systems for unraveling cellular and molecular programs favoring viral dissemination. Cell biologists have adapted relevant *in vivo* platforms, such as zebrafish embryos and mice, to develop exciting approaches allowing dissection of *in vivo* subcellular processes that foster viral spreading. More recently, *ex vivo* tissue culture and organoids derived from human embryonic stem cells or induced pluripotent stem cells have been developed to study viral dissemination strategies in complex multidimensional settings (Box 1). While bacterial dissemination *in vivo* was recently reviewed [1], we aim here at discussing recent work in zebrafish related to the cell biology of virus dissemination *in vivo*, list the current technical limitations, and propose future developments.

Command and Conquer

Viral dissemination into host refers to the ability of viral particles to reach tissular compartments remotely located from their initial infection site. This includes fluid-to-tissue, tissue-to-fluid, and tissue-to-tissue dissemination, in addition to cell-to-cell virus transmission [2,3]. Such large-scale transitional stages require that the virus builds well-orchestrated subversion molecular mechanisms, which can be either disruptive or, *a contrario*, quiet. In any case, it is critical to decipher the subcellular processes involved in viral dissemination at the multiorgan scale and, to this end, relevant *in vivo* models represent instrumental approaches.

Tracking Viral Dissemination from the Bloodstream Using Zebrafish Embryos

A common way for viruses to disseminate is to use afferent fluid canals that naturally irrigate the organism. The bloodstream is the perfect culprit as it provides a fast track for distant viral

[1]Institut de Recherche en Infectiologie de Montpellier (IRIM), CNRS, 34293 Montpellier, France
[2]Université de Montpellier, 34090 Montpellier, France
[3]INSERM UMR_S1109, Tumor Biomechanics, Strasbourg, France
[4]Université de Strasbourg, Strasbourg, France
[5]Fédération de Médecine Translationnelle de Strasbourg (FMTS), Strasbourg, France
[6]www.irim.cnrs.fr/index.php/en/researchh/teams/membrane-dynamics-viruses
[7]www.goetzlab.com

*Correspondence:
raphael.gaudin@irim.cnrs.fr (R. Gaudin) and jacky.goetz@inserm.fr (J.G. Goetz).
@Twitter: @Gaudinlab (R. Gaudin) and @GoetzJacky (J.G. Goetz).

Box 1. Alternatives to Animal Models

With current ethical concerns regarding animal research, non-animal models mimicking complex 3D environments have been developed. Human explants probably represent the most relevant alternative to measure physiological viral infection *ex vivo* (see, for instance [40–42]), but the administrative and technical difficulties in obtaining human-derived samples, associated with the complicated methods required to image and genetically manipulate them, makes such an approach cumbersome to implement in cell biology studies. In contrast, stem cell-derived organoids represent attractive alternatives to study physiological viral dissemination in-a-dish [43,44]. Nature, size, genetics, imaging, and reproducibility are properties justifying why organoids are acclaimed. Recently, for instance, intestinal organoids were used to study SARS-CoV-2 intracellular replication [45]. They observed viral particles in double membrane vesicles, Golgi apparatus, and inside the endomembrane system, while virus secretion was occurring both from the apical and basolateral side (Figure 1), which is consistent with the observation that virus particles shed in feces from infected patients [46]. Organoids can also be adequately used to study apoptosis or cell division, as exemplified with ZIKV-infected cerebral organoids [47,48], but should prove useful in a variety of other subcellular studies. As opposed to animal models, organoids offer a unique human system, while working in tissue-like conditions. Although it is not fully appropriate to study adaptive immune responses or interorgan physiopathology, they should be considered as relevant animal-free models to address the cell biology of viral dissemination in greater detail.

dissemination, which may occur through various molecular mechanisms [4]. The zebrafish embryo (until 2.5 days postfertilization) and larvae (until 6 weeks postfertilization) (*Danio rerio*) represent a very attractive model to study virus dissemination from the bloodstream toward various organs [5]. Indeed, the genetic manipulation of zebrafish is straightforward and fluorescent fish lines allowing tracking of multiple cell types (endothelial, immune cells, etc.) are readily available [6]. The zebrafish model displays both **innate and adaptive vertebrate-type immunity** (see Glossary). In addition, although it might be less relevant for studying viral dissemination, recent developments now offer genetically immunocompromised strains, useful for xenotransplantation of human patient samples and for unraveling the contribution of key immune elements to pathophysiology [7]. Moreover, the major advantage of zebrafish is in allowing *in vivo* high-throughput screening [8], a feature that is ethically and technical impossible to perform in mice. This strategy is currently considered to identify potent antiviral small molecules, and associated toxicity, active against the **severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)**, the causative agent of coronavirus disease 2019 (COVID-19) (Box 2) [9]. Furthermore, microscopic studies are eased by the transparency of the embryo and its small size, avoiding body hindrance under the microscope. The model has been widely used in developmental and cell biology, as well as in cancer biology [10,11], and recently emerged as a powerful system to track viral dissemination *in vivo* [12,13].

Studies investigating the cell biology of fish viruses have used the zebrafish model extensively [14]. However, in the case of mammalian-tropic viruses, zebrafish embryos are mostly employed to study immune responses as well as pathogenesis, antiviral agent potency, and toxicity [15–22]. This model is also powerful for identification of **viral tropism**, as it has recently been exemplified for the **human norovirus**, which can be detected in cells of the hematopoietic lineage and the intestine [16]. Hence, this powerful *in vivo* model offers exciting opportunities to decipher molecular mechanisms of pathogen dissemination.

Box 2. Zebrafish in the Context of the SARS-Co-2 Pandemic

Numerous labs are rushing toward the development of a zebrafish model to study SARS-CoV-2, but no reports have been formally published to date, suggesting that the virus causing COVID-19 may not vigorously infect zebrafish. The SARS-CoV-2 human receptor angiotensin-converting enzyme 2 (ACE-2) and the entry factor TMPRSS2 protease show 69% and 54% similarities with the zebrafish (*Danio rerio*) proteins (pairwise sequence alignment using EMBOSS Needle [49]). Thus, genetically engineered zebrafish-expressing human receptors could promote zebrafish susceptibility to SARS-CoV-2. One could think that a respiratory disease is difficult to be recapitulated in an organism deprived of lungs. However, the well-known respiratory virus, **influenza A virus**, was shown to infect and cause significant pathological phenotypes in zebrafish [21], thus, the zebrafish model still hold promise in the fight against COVID-19.

Glossary

Chikungunya virus: enveloped single-stranded positive-sense RNA virus (*Togaviridae* family).
Correlative light and electron microscopy (CLEM): imaging of a specific object (cellular, subcellular) within a sample using both photonic and electron microscopy.
DNA traps: extracellular fibers forming a 'net' primarily composed of DNA from neutrophils.
High-resolution intravital imaging: microscopy approaches that allow visualization of cellular and subcellular phenomenons within living organisms.
Human cytomegalovirus (HCMV): enveloped double-stranded DNA virus (*Herpesviridae* family).
Human norovirus: nonenveloped single-stranded positive-sense RNA virus (*Caliciviridae* family).
Infectious hematopoietic necrosis virus: enveloped single-stranded negative-sense RNA virus (*Rhabdoviridae* family).
Influenza A virus: enveloped single-stranded negative-sense RNA virus (*Orthomyxoviridae* family).
Innate and adaptive vertebrate-type immunity: while innate immunity refers to nonspecific defense mechanisms that are immediate upon infection, adaptive immunity is a long-lasting immune response that is specific to the pathogen. In zebrafish larvae, only innate immunity is active.
Proteins of the complement: part of the innate immune system that promotes inflammation and attacks lipid bilayer membranes.
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): enveloped single-stranded positive-sense RNA virus (*Coronaviridae* family).
Sindbis virus: enveloped single-stranded positive-sense RNA virus (*Togaviridae* family).
Vaccinia virus (VACV): enveloped double-stranded DNA virus (*Poxviridae* family).
Vesicular stomatitis virus: enveloped single-stranded negative-sense RNA virus (*Rhabdoviridae* family).
Viral tropism: ability of a virus to productively infect specific cell types or tissues.
Zika virus (ZIKV): enveloped single-stranded positive-sense RNA virus (*Flaviviridae* family).

The bloodstream carries a number of viruses and bacteria, but this environment is very hostile, as it contains a large number of host defenses, including immune cells, antibodies, **proteins of the complement**, and **DNA traps** [23]. To quickly escape the bloodstream, the easiest strategy is probably to infect endothelial cells lining blood vessels. Although relatively efficient at first, endothelial leakage or dysfunction is permanently under tight surveillance, thus, this strategy does not preserve the virus from the host's immune defenses. The *Tg(fli1:GFP)* zebrafish line exhibiting fluorescent endothelial cells is an ideal model to study such a process, as exemplified with the **infectious hematopoietic necrosis virus**, which strongly disrupts the blood vessels for dissemination [24]. To a lesser extent, **chikungunya virus** was also shown to infect endothelial cells of the brain vasculature of zebrafish larvae, leading to neuroinvasion [12]. In contrast, the authors of this later study found that **Sindbis virus** was infecting the central nervous system (CNS) independently of endothelial infection. This represents one of the first studies to our knowledge investigating various routes of neuroinvasion, which was made possible thanks to advanced zebrafish embryo imaging [12]. In contrast, the invasion of the CNS by **Zika virus (ZIKV)** was recently investigated in interferon-α/β receptor (IFNAR)-deficient mice, but the outcome was less evident [25]. Indeed, the authors nicely showed that the blood–brain barrier (BBB) was not disrupted, but it remains to be determined whether the virus reaches the CNS through basolateral release, transcytosis, or transinfection processes [25]. Such information requires **high-resolution intravital imaging**, which is technically difficult to implement in mice (Box 3).

Disseminating Using a Trojan Horse

Viruses also evolved a subtler method to invade remote organs, which consists of hiding in blood cell components, such as platelets or monocytes [13,26], conferring a protective shell to them. When hiding, the virus may either replicate in the host cell, or just be carried in an intracellular compartment of the circulating host cell. This latter strategy, however, is also hazardous for the virus, as the particles will need to escape the cell later on to disseminate, while avoiding being

Box 3. Beyond the Zebrafish: The Mouse Model to Study Viral Dissemination

The mouse represents an attractive model to study virus–host interactions [50]; however, they are more difficult to image than zebrafish embryos and because viruses can target various tissues, intravital microscopy (IVM) requires specific settings according to the organ to be imaged. A detailed protocol for IVM of the lung to study influenza virus infection was recently released [51] and while this protocol could be adapted to study SARS-CoV-2 infection, for instance, it is less relevant to viral infections targeting other organs. Moreover, a large number of mouse models of viral infection require immunocompromised or humanized mice, which makes this approach nontrivial and not fully relevant to human disease. Yet, the mouse model has the advantage of having an immune system more closely related to humans than zebrafish and better recapitulates pathogenesis of viral infections. A pioneering study developed IVM in mouse lymph nodes to monitor the dissemination of the human immunodeficiency virus type 1 (HIV-1) through virus-induced migration of T cells [52], after a CD169-dependent virus transfer from sinus-lining macrophages [53]. Visualization of fluorescently tagged **vesicular stomatitis virus** particles in live mice showed circulating leukocytes transporting the oncolytic virus toward tumors [54]. These studies could track the migratory properties of infected cells, but underlying molecular mechanisms were not proposed. Murooka and colleagues intriguingly showed that the HIV-infected T cells exhibited unusually elongated, thin, and branched trailing edges [52], which must result from dramatic cytoskeletal rearrangements that remain to be fully characterized *in vivo*. Using IVM and electron tomography in the bone marrow of HIV-infected mice, the description of several scenarios leading to HIV cell–cell transfer was further depicted (Figure 1) [55] and, here again, host proteins involved in these complex processes remain to be identified. Recently, the **vaccinia virus (VACV)** was shown to enhance rapid and directed cell motility through the subversion of EGFR signaling [56]. The authors could highlight *in vitro* that inhibitors of the host factors ADAM10, EGFR, MAPK, and FAK reduced the radial velocity and directional migration of infected cells and that depletion of the viral-encoded protein VGF also decreases virus-induced cell migration. Mouse IVM revealed that VGF was responsible for virus spread and lesion formation in mice ear pinnae. Because FAK is involved in focal adhesion formation, one may envision that VACV controls the dynamics of focal adhesion of infected cells to promote cell migration. Finally, *Drosophila melanogaster* has been used to track and understand viral dissemination of the Nora virus, notably through the creation of transgenic reporter lines that emit fluorescence upon infection [57]. However, flies have not been used to study mammalian viruses, as relevance would rightfully be questionable.

directed toward degradative endolysosomes [27]. Nevertheless, an associated benefit from subverting a blood cell is the possibility to hitch a ride *incognito* over long distances, opening the door to long-range dissemination. It also allows the virus to sneakily cross tight endothelial barriers, usually impermeable to microbes, without disrupting them, thus, without inducing a strong immune response. This phenomenon is referred to as the Trojan horse hypothesis

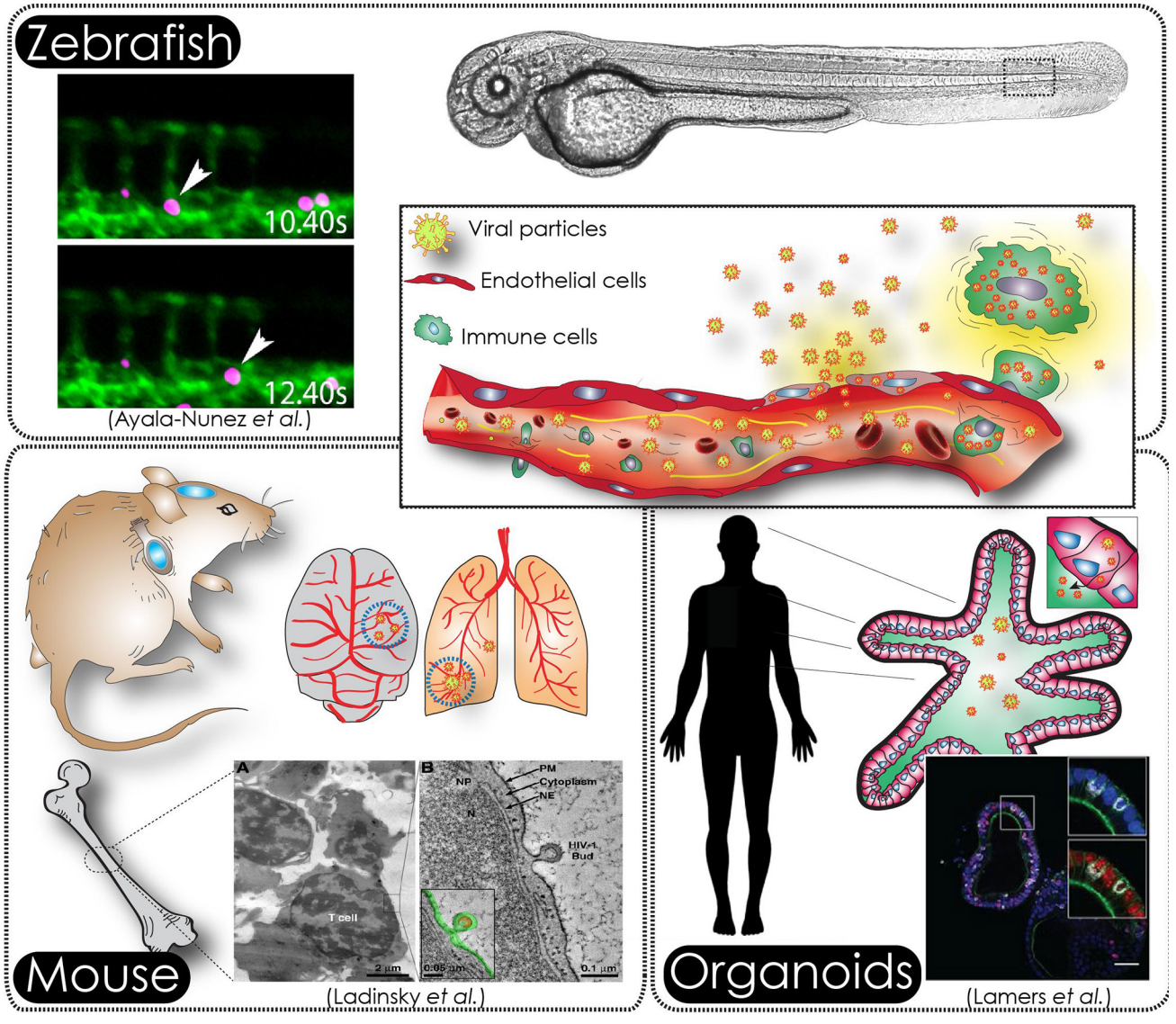

Figure 1. Strategies to Track Viral Dissemination *In Vivo*. Viruses employ several strategies to disseminate and reach adjacent tissues (central scheme). They can freely diffuse through the vascular wall when its integrity is altered; they may infect endothelial cells and are released in the extravascular space; and they can be transported across the vascular wall without infection or they can be transported upon infection of circulating immune cells that can stop and cross the endothelium layer. Animal models such as the zebrafish embryo, the mouse model, and the versatile organoids provide very useful imaging and analysis platforms for tracking, at high spatio-temporal resolution, the dynamics and cellular strategies of viral dissemination. For example, intravascular behavior of Zika virus-infected monocytes can be tracked in real time in zebrafish embryos [13]. Budding of HIV-1 from a T cell can be tracked with electron tomography in mice [55]. The inset shows a profile emanating from the surface of a little cytoplasm between the nuclear envelope (NE) and the budding plasma membrane (PM). Dissemination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be tracked in enterocytes of intestinal organoids [45]. Abbreviation: NP, nuclear pore.

[4,28], described more than 35 years ago [29]. Recent work highlighted that ZIKV could be carried by human circulating monocytes. Tracking the behavior of these monocytes in the bloodstream of zebrafish embryos revealed key cellular strategies used by ZIKV to favor distant dissemination [13] (Figure 1). Indeed, molecular mechanistic insights revealed that intravascular arrest of ZIKV-exposed monocytes was increased when compared with their nonexposed counterparts. These findings correlated with the increased expression of adhesion molecules at the surface of ZIKV-exposed monocytes, suggesting that the virus promotes cell attachment to the endothelial cells. Interestingly, ZIKV not only favors the intravascular arrest of infected monocytes, it further stimulates their extravasation, which is a step that is instrumental for viral dissemination. This *in vivo* experimental approach was originally developed for tumor metastasis [30], highlighting the successful adaptation of powerful tools designed by cell biologists to unravel key strategies of viral dissemination.

We acknowledge that the zebrafish model has limitations to study pathogenesis and long-term dissemination in the case where the zebrafish cells are not permissive to the infection. Although there is no evidence to our knowledge that ZIKV can efficiently infect zebrafish, a study has taken advantage of the relative permissiveness of this *in vivo* model for xenografts to investigate virus-induced cell extravasation across the vascular wall [13]. Similarly, the **human cytomegalovirus (HCMV)** was shown to induce monocyte adhesion to endothelial cells and transmigration [31], and while the zebrafish is not permissive to HCMV infection [32], one could, for instance, propose injecting HCMV-infected human cells into zebrafish to monitor virus-induced cell migration in an *in vivo* context. Transplanting cells into adult zebrafish has recently been made possible, thanks to the development of immunocompromised models (i.e., rag2 mutant zebrafish [33]), where subcellular imaging can be envisioned upon further generation of optically cleared zebrafish that lack T, B, and natural killer cells (i.e., the prkdc$^{-/-}$, il2rga$^{-/-}$ zebrafish [34]). Nevertheless, such an advantage comes with the drawback that it does not allow understanding of the immune response to viral infection, which shapes viral illness, allowing the development of antiviral medicine [35].

Concluding Remarks

The benefits of detailed *in vivo* cell biology analyses that the transparent zebrafish embryo has to offer should not be overlooked. Indeed, zebrafish allows *in vivo* investigations of virus-induced molecular processes at subcellular resolution, including studies addressing the mechanism of attachment, rolling, and extravasation through the endothelial cell wall, the adhesion molecules involved and drastic actin rearrangements, and the influence of a natural sheer stress in these processes [4,5,12,13]. Yet, this model has limitations and future developments are still needed (see Outstanding Questions). For example, the zebrafish embryo and larvae provide a clear advantage toward imaging approaches compared with adults. In particular, the zebrafish embryo represents a powerful platform to develop correlative imaging approaches designed to track viral dissemination at nanoscale and thereby identify mechanisms of viral subversion leading to dramatic intracellular membranous rearrangement [36]. **Correlative light and electron microscopy (CLEM)** has recently been used in this system for tracking the dissemination and uptake of extracellular vesicles [37] that are similar in many aspects (size, morphology, content, etc.) to viral particles. Alternatively, it would be exciting to apply such CLEM strategies to mouse models of viral infection [38]. The zebrafish embryo not only allows capture of the cellular behavior of viruses, it also allows discovery of new viruses. A recent study reported the creation of a strain that expresses GFP under an interferon-stimulated gene promoter. Here, GFP expression can be used to track immune antiviral response in larvae and thereby provides an ideal platform to develop new strategies for discovering viruses while testing their impact on vertebrate models [39].

Outstanding Questions

Can zebrafish become a key *in vivo* model for the study of the molecular mechanisms involved in viral dissemination?

Can correlative or super-resolution imaging be applied to mouse (or zebrafish) models and allow the study of viral dissemination at high resolution?

Will complex organoid and assembloid models replace animal-based *in vivo* cell biology in the future?

Can we monitor and track single viral particle dynamics *in vivo* with appropriate spatio-temporal resolution?

Acknowledgments

We thank all members of the Gaudin and Goetz Labs for helpful discussions. This work has been funded by institutional funds from CNRS to R.G and by INSERM and University of Strasbourg to J.G.

References

1. Torraca, V. and Mostowy, S. (2018) Zebrafish infection: from pathogenesis to cell biology. *Trends Cell Biol.* 28, 143–156
2. Cifuentes-Munoz, N. *et al.* (2018) Direct cell-to-cell transmission of respiratory viruses: the fast lanes. *PLoS Pathog.* 14, e1007015
3. Graw, F. and Perelson, A.S. (2016) Modeling viral spread. *Annu. Rev. Virol.* 3, 555–572
4. Ayala-Nunez, N.V. and Gaudin, R. (2020) A viral journey to the brain: current considerations and future developments. *PLoS Pathog.* 16, e1008434
5. Levraud, J.P. *et al.* (2014) Through the looking glass: witnessing host-virus interplay in zebrafish. *Trends Microbiol.* 22, 490–497
6. Ruzicka, L. *et al.* (2019) The Zebrafish Information Network: new support for non-coding genes, richer gene ontology annotations and the Alliance of Genome Resources. *Nucleic Acids Res.* 47, D867–D873
7. Fazio, M. *et al.* (2020) Zebrafish patient avatars in cancer biology and precision cancer therapy. *Nat. Rev. Cancer* 20, 263–273
8. Varela, M. *et al.* (2017) Modelling viral infections using zebrafish: innate immune response and antiviral research. *Antivir. Res.* 139, 59–68
9. Galindo-Villegas, J. (2020) The zebrafish disease and drug screening model: a strong ally against Covid-19. *Front. Pharmacol.* 11, 680
10. Follain, G. *et al.* (2018) Hemodynamic forces tune the arrest, adhesion, and extravasation of circulating tumor cells. *Dev. Cell* 45, 33–52
11. Osmani, N. and Goetz, J.G. (2019) Multiscale imaging of metastasis in zebrafish. *Trends Cancer* 5, 766–778
12. Passoni, G. *et al.* (2017) Imaging of viral neuroinvasion in the zebrafish reveals that Sindbis and chikungunya viruses favour different entry routes. *Dis. Model. Mech.* 10, 847–857
13. Ayala-Nunez, N.V. *et al.* (2019) Zika virus enhances monocyte adhesion and transmigration favoring viral dissemination to neural cells. *Nat. Commun.* 10, 4430
14. Zou, P.F. and Nie, P. (2017) Zebrafish as a model for the study of host-virus interactions. *Methods Mol. Biol.* 1656, 57–78
15. Langevin, C. *et al.* (2019) IFN signaling in inflammation and viral infections: new insights from fish models. *Viruses* 11, 302
16. Van Dycke, J. *et al.* (2019) A robust human norovirus replication model in zebrafish larvae. *PLoS Pathog.* 15, e1008009
17. Haddad, J.G. *et al.* (2020) The geraniin-rich extract from Reunion Island endemic medicinal plant *Phyllanthus phillyreifolius* inhibits Zika and dengue virus infection at non-toxic effect doses in zebrafish. *Molecules* 25, 2316
18. Burgos, J.S. *et al.* (2008) Zebrafish as a new model for herpes simplex virus type 1 infection. *Zebrafish* 5, 323–333
19. Selakovic, Z. *et al.* (2019) Second generation of diazachrysenes: protection of Ebola virus infected mice and mechanism of action. *Eur. J. Med. Chem.* 162, 32–50
20. Palha, N. *et al.* (2013) Real-time whole-body visualization of chikungunya virus infection and host interferon response in zebrafish. *PLoS Pathog.* 9, e1003619
21. Gabor, K.A. *et al.* (2014) Influenza A virus infection in zebrafish recapitulates mammalian infection and sensitivity to anti-influenza drug treatment. *Dis. Model. Mech.* 7, 1227–1237
22. Vila, I.K. *et al.* (2020) Animal models for the study of nucleic acid immunity: novel tools and new perspectives. *J. Mol. Biol.* Published online August 26, 2020. https://doi.org/10.1016/j.jmb.2020.08.016
23. Hickey, M.J. and Kubes, P. (2009) Intravascular immunity: the host-pathogen encounter in blood vessels. *Nat. Rev. Immunol.* 9, 364–375
24. Ludwig, M. *et al.* (2011) Whole-body analysis of a viral infection: vascular endothelium is a primary target of infectious hematopoietic necrosis virus in zebrafish larvae. *PLoS Pathog.* 7, e1001269
25. Papa, M.P. *et al.* (2017) Zika virus infects, activates, and crosses brain microvascular endothelial cells, without barrier disruption. *Front. Microbiol.* 8, 2557
26. Simon, A.Y. *et al.* (2015) Dengue virus binding and replication by platelets. *Blood* 126, 378–385
27. Robinson, M. *et al.* (2018) Viral journeys on the intracellular highways. *Cell. Mol. Life Sci.* 75, 3693–3714
28. Santiago-Tirado, F.H. and Doering, T.L. (2017) False friends: phagocytes as Trojan horses in microbial brain infections. *PLoS Pathog.* 13, e1006680
29. Peluso, R. *et al.* (1985) A Trojan horse mechanism for the spread of visna virus in monocytes. *Virology* 147, 231–236
30. Follain, G. *et al.* (2020) Fluids and their mechanics in tumour transit: shaping metastasis. *Nat. Rev. Cancer* 20, 107–124
31. Smith, M.S. *et al.* (2004) Human cytomegalovirus induces monocyte differentiation and migration as a strategy for dissemination and persistence. *J. Virol.* 78, 4444–4453
32. Cazorla-Vazquez, S. *et al.* (2019) Human cytomegaloviral multifunctional protein kinase pUL97 impairs zebrafish embryonic development and increases mortality. *Sci. Rep.* 9, 7219
33. Tang, Q. *et al.* (2014) Optimized cell transplantation using adult rag2 mutant zebrafish. *Nat. Methods* 11, 821–824
34. Yan, C. *et al.* (2019) Visualizing engrafted human cancer and therapy responses in immunodeficient zebrafish. *Cell* 177, 1903–1914
35. Goody, M.F. *et al.* (2014) Studying the immune response to human viral infections using zebrafish. *Dev. Comp. Immunol.* 46, 84–95
36. Wolff, G. *et al.* (2020) Double-membrane vesicles as platforms for viral replication. *Trends Microbiol.* Published online June 11, 2020. https://doi.org/10.1016/j.tim.2020.05.009
37. Hyenne, V. *et al.* (2019) Studying the fate of tumor extracellular vesicles at high spatiotemporal resolution using the zebrafish embryo. *Dev. Cell* 48, 554–572
38. Karreman, M.A. *et al.* (2016) Intravital correlative microscopy: imaging life at the nanoscale. *Trends Cell Biol.* 26, 848–863
39. Balla, K.M. *et al.* (2020) Linking virus discovery to immune responses visualized during zebrafish infections. *Curr. Biol.* 30, 2092–2103
40. Tabata, T. *et al.* (2018) Zika virus replicates in proliferating cells in explants from first-trimester human placentas, potential sites for dissemination of infection. *J. Infect. Dis.* 217, 1202–1213
41. Ganor, Y. *et al.* (2019) HIV-1 reservoirs in urethral macrophages of patients under suppressive antiretroviral therapy. *Nat. Microbiol.* 4, 633–644
42. Tajpara, P. *et al.* (2019) A preclinical model for studying herpes simplex virus infection. *J. Invest. Dermatol.* 139, 673–682
43. Lancaster, M.A. and Knoblich, J.A. (2014) Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345, 1247125
44. Marton, R.M. and Pasca, S.P. (2020) Organoid and assembloid technologies for investigating cellular crosstalk in human brain development and disease. *Trends Cell Biol.* 30, 133–143
45. Lamers, M.M. *et al.* (2020) SARS-CoV-2 productively infects human gut enterocytes. *Science* 369, 50–54
46. Wu, Y. *et al.* (2020) Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *Lancet Gastroenterol. Hepatol.* 5, 434–435
47. Watanabe, M. *et al.* (2017) Self-organized cerebral organoids with human-specific features predict effective drugs to combat Zika virus infection. *Cell Rep.* 21, 517–532
48. Tang, H. *et al.* (2016) Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell* 18, 587–590
49. Madeira, F. *et al.* (2019) The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Res.* 47, W636–W641

50. Sewald, X. (2018) Visualizing viral infection in vivo by multi-photon intravital microscopy. *Viruses* 10, 337
51. Ueki, H. *et al.* (2020) Multicolor two-photon imaging of in vivo cellular pathophysiology upon influenza virus infection using the two-photon IMPRESS. *Nat. Protoc.* 15, 1041–1065
52. Murooka, T.T. *et al.* (2012) HIV-infected T cells are migratory vehicles for viral dissemination. *Nature* 490, 283–287
53. Sewald, X. *et al.* (2015) Retroviruses use CD169-mediated trans-infection of permissive lymphocytes to establish infection. *Science* 350, 563–567
54. Naumenko, V. *et al.* (2018) Visualizing oncolytic virus-host interactions in live mice using intravital microscopy. *Mol. Ther. Oncolytics* 10, 14–27
55. Ladinsky, M.S. *et al.* (2019) Mechanisms of virus dissemination in bone marrow of HIV-1-infected humanized BLT mice. *Elife* 8, e46916
56. Beerli, C. *et al.* (2018) Vaccinia virus hijacks EGFR signalling to enhance virus spread through rapid and directed infected cell motility. *Nat. Microbiol.* 4, 216–225
57. Ekstrom, J.O. and Hultmark, D. (2016) A novel strategy for live detection of viral infection in *Drosophila melanogaster*. *Sci. Rep.* 6, 26250