JCB Journal of Cell Biology

SPOTLIGHT

A close shave: How SARS-CoV-2 induces the loss of cilia

Barbara F. Fonseca[1,2] and Lisa A. Chakrabarti[1]

Wang et al. report in this issue (2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202108015) that the SARS-CoV-2 protein ORF10 increases the activity of the E3 ligase CUL2^{ZYG11B}, leading to the degradation of multiple ciliary proteins. The resulting loss of cilia may facilitate the spread of SARS-CoV-2 in the respiratory tree.

The first line of defense against respiratory pathogens relies on motile cilia. The epithelium that lines the airways contains cells harboring one to two hundred cilia at their apical surface and cells that secrete a viscoelastic mucus. Inhaled particles, including viruses, stick to the mucus layer, which is moved towards the pharynx and swallowed thanks to the coordinated beating of cilia. It is not surprising, then, that respiratory pathogens have evolved a variety of strategies to counter this clearance mechanism (1). SARS-CoV-2, for instance, preferentially infects ciliated cells (2) and causes the loss of motile cilia at the surface of infected cells, leading to an impairment of mucociliary clearance (3, 4). Wang et al. (5) now provide insights into the mechanism of cilia loss, by showing that the small viral protein ORF10, which is only 38 amino acids long, is sufficient to cause cilia loss by promoting the ubiquitin-dependent degradation of an array of ciliary proteins.

An interaction of the ORF10 protein with the E3 ubiquitin ligase adapter ZYG11B was previously reported in a large analysis of the SARS-CoV-2 interactome by affinity-purification mass spectrometry (6). E3 ubiquitin ligases are enzymes that catalyze the addition of ubiquitin to proteins, which are then targeted for degradation by the proteasome in the majority of cases. E3 ligases work in concert with E1 ubiquitin-activating enzymes and E2 ubiquitin-conjugating enzymes to covalently attach ubiquitin (itself a small 8 kD protein) to target proteins. E3 adapters are the enzymatic subunits that confer substrate specificity. ZYG11B, for instance, is part of the CUL2-ElonginBC ubiquitin ligase complex, and targets proteins with an N-terminal glycine for proteasomal degradation (7). Multiple viruses have evolved strategies to usurp the ubiquitin/proteasome pathway by encoding proteins that mimic E3 adapters and induce the degradation of cellular proteins involved in antiviral defense. For example, the HIV-1 proteins Vif, Vpx, Vpr, and Vpu target the intrinsic defense proteins APOBEC3G/F, SAMHD1, UNG2, and BST2, respectively, through the recruitment of these defense proteins to distinct E3 complexes (8). Inspired by this notion, Wang et al. (5) asked whether the ORF10/ZYG11B interaction had functional consequences that could promote SARS-CoV-2 dissemination.

The authors first confirmed the interaction of ORF10 and ZYG11B by pull-down experiments and found that addition of recombinant ORF10 to CUL2^{ZYG11B} immunocomplexes in the presence of relevant E1 and E2 enzymes increased ubiquitination activity in vitro (Fig. 1, A and B), yet the precise mechanism remains elusive. To evaluate the impact of ORF10 on the cellular proteome, they compared the profile of ORF10-expressing and mock-transfected HEK 293T cells by Tandem Mass Tag (TMT) proteomics analysis. These experiments revealed a marked effect of ORF10 expression, which caused the downregulation of 352 proteins and the upregulation of only two proteins, suggesting that ORF10 interferes with protein stability. Strikingly, the majority of downregulated proteins were annotated as involved in ciliogenesis or cilium structure. Western blot analyses confirmed the downregulation of key proteins involved in cilium biogenesis and/or maintenance, such as TALPID3, TTBK2, BBS4, SEPTIN2, and IFT46. Of note, a previous study had also used TMT proteomics to analyze the effect of ORF10 expression in HEK 293T cell but had reported only limited changes in cellular proteome profiles (9). To explore reasons for these divergent findings, Wang et al. (5) evaluated the dose dependency of the ORF10 effect and found that downregulation of ciliary proteins did require high ORF10 expression. Furthermore, they observed that the pcDNA-ORF10 plasmid vector used in their study resulted in higher ORF10 expression than the pHAGE-ORF10 vector used in the prior work (9), which likely explains some of the differences between the two studies.

Wang et al. (5) moved on to study the effect of ORF10 expression on ciliation, first by using transformed cell line models. Ciliogenesis and cellular division are mutually

[1]Control of Chronic Viral Infections Group, Virus and Immunity Unit, Institut Pasteur, Université Paris Cité, CNRS UMR 3569, Paris, France; [2]Biomaterials and Microfluidics Core Facility, Institut Pasteur, Paris, France.

Correspondence to Lisa A. Chakrabarti: chakra@pasteur.fr.

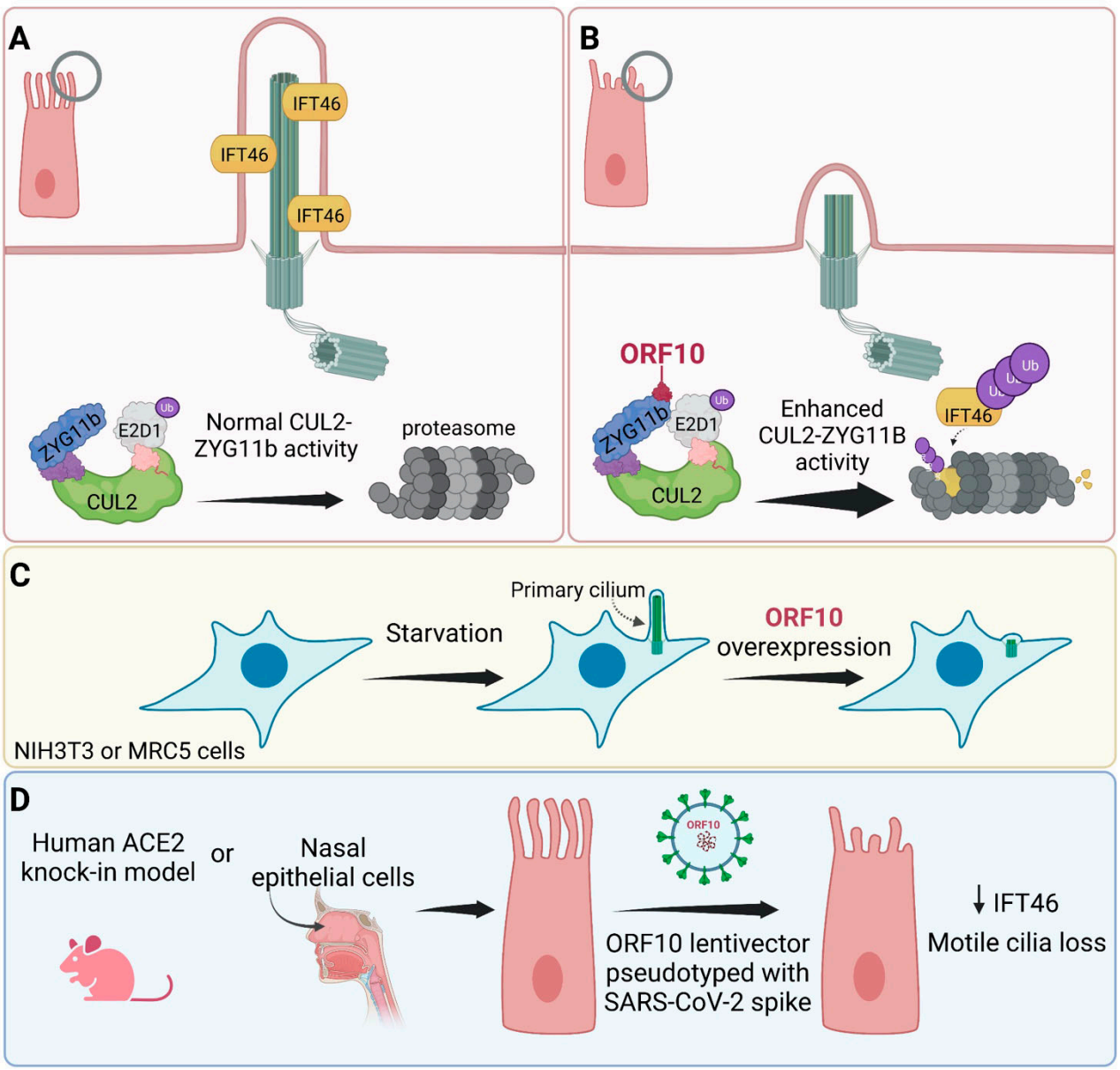

Figure 1. **SARS-CoV-2 ORF10 impairs ciliogenesis by enhancing the activity of the E3 ligase CUL2^{ZYG11B}. (A)** The CUL2^{ZYB11B} RING E3 ligase complex contributes to cellular protein degradation via ubiquitination. **(B)** Upon SARS-CoV-2 infection, the viral protein ORF10 binds the E3 adapter ZYG11B, increasing the ubiquitination activity of the complex, and inducing the proteasomal degradation of ciliary proteins, including IFT46. **(C)** ORF10 overexpression in serum-starved NIH3T3 and MRC-5 cells blocks primary cilium biogenesis and maintenance. **(D)** The lentiviral transfer of ORF10 is sufficient to induce cilia loss in human ACE2 knock-in mice and in primary human nasal epithelial cells, highlighting the role of this viral protein in SARS-CoV-2-mediated cilia disruption.

exclusive, as centrosomes cannot engage in both processes simultaneously. Therefore, the transformed cell lines MRC-5 and NIH3T3 were first serum starved to inhibit cellular division and enable the formation of a primary cilium (Fig. 1 C). This organelle could be detected as a single protrusion positive for a stable microtubule marker, acetylated α-tubulin. Transfection of ORF10 either before or after starvation decreased the percentage of cells carrying a primary cilium, confirming that this viral protein antagonizes both ciliogenesis and cilium maintenance. Overexpression of ZYG11B mimicked the effect of ORF10, while knock-down of ZYG11B attenuated ORF10 effect, consistent with a ZYG11B-dependent inhibition of ciliogenesis by ORF10.

The authors then focused on the role of the ciliary protein Intraflagellar Transport 46 (IFT46), which was profoundly downregulated upon ORF10 expression. IFT46 interacted with ZYG11B but not with ORF10, supporting a model where ORF10 increased the activity of the CUL2^{ZYG11B} complex towards its substrates. IFT46 overexpression partially rescued ciliogenesis in ORF10 expressing cells, suggesting that IFT46 degradation plays a role in the ORF10-induced ciliogenesis defect. The IFT46 motif recognized by ZYG11B remains to be fully characterized, as the IFT46-ZYG11B interaction did not rely on a canonical mechanism of Gly/N-degron recognition (where ZYG11B binds to an N-terminal glycine).

Rather, Wang et al. found that interaction with ZYG11B required the internal C2 domain of the IFT46 protein. Intriguingly, overexpression of an IFT46 protein lacking the C2 domain still partially rescued ciliogenesis in ORF10 expressing cells, suggesting that cilium recovery did not require a fully functional IFT46 protein nor titration of ZYG11B by excess IFT46. More broadly, whether a specific motif distinct from the Gly/N-degron can target ciliary proteins for ZYG11B-dependent degradation remains to be established. ZYG11B may directly interact with only a subset of the hundreds of ciliary proteins downregulated in the presence of ORF10. Cilia maintenance is highly dynamic, and targeting a master regulator of ciliogenesis can be sufficient to drastically downregulate the expression of multiple ciliary components. SARS-CoV-2 infection of primary ciliated cells was for instance shown to induce an early downregulation of the transcription factor FOXJ1, which is required for cilia formation and maintenance (4). Wang et al. (5) did not observe an effect of ORF10 on FOXJ1 expression, but an effect on another master regulator of ciliogenesis is not ruled out. Interestingly, a role for ORF10 in inducing the autophagic degradation of mitochondria was recently reported (10). The cross-talks between autophagy and ciliogenesis are many (11), raising the possibility that ORF10 may also trigger the autophagic degradation of primary and motile cilia.

A strong point of the study is the demonstration that ORF10 transfer is sufficient to induce cilia loss in vivo (Fig. 1 D). Wang et al. (5) used the intranasal inoculation of a lentiviral vector to transfer ORF10 to human ACE2 knock-in mice. Astutely, the authors pseudotyped the ORF10 and control lentivectors with the SARS-CoV-2 spike, ensuring that ORF10 would be transferred to relevant ciliated target cells. Inoculation of the ORF10 vector was sufficient to induce cilia loss in epithelial cells lining the mice trachea. The spike-pseudotyped control vector had no such effect, ensuring that binding of the spike alone was not sufficient to perturb cilia. The authors then verified that infection of the hACE2 mice with authentic SARS-CoV-2 did also induce cilia loss and IFT46 downregulation. Importantly, the authors also found that ORF10 lentiviral transfer could induce cilia loss in a reconstructed human nasal epithelium in vitro, demonstrating the effect of ORF10 in bona fide human multiciliated cells.

This study illustrates yet another way for viruses to highjack the ubiquitin/proteasome pathway. In most instances reported so far, a viral protein targets a specific protein involved in intrinsic/innate defense for E3 recognition and proteasomal degradation. Here, Wang et al. (5) demonstrate that the viral protein ORF10 induces the degradation of an array of ciliary proteins, leading to the disappearance of a whole organelle involved in viral particle clearance.

Acknowledgments

This work was supported by the Urgence COVID-19 Fundraising Campaign of Institut Pasteur (COROCHIP and PFR4 grants to L.A. Chakrabarti).

The authors declare no competing financial interests.

References

1. Tilley, A.E., et al. 2015. *Annu. Rev. Physiol.* https://doi.org/10.1146/annurev-physiol-021014-071931
2. Hou, Y.J., et al. 2020. *Cell.* https://doi.org/10.1016/j.cell.2020.05.042
3. Zhu, N., et al. 2020. *Nat. Commun.* https://doi.org/10.1038/s41467-020-17796-z
4. Robinot, R., et al. 2021. *Nat. Commun.* https://doi.org/10.1038/s41467-021-24521-x
5. Wang, L., et al. 2022. *J. Cell Biol.* https://doi.org/10.1083/jcb.202108015
6. Gordon, D.E., et al. 2020. *Nature.* https://doi.org/10.1038/s41586-020-2286-9
7. Timms, R.T., et al. 2019. *Science.* https://doi.org/10.1126/science.aaw4912
8. Sauter, D., and F. Kirchhoff. 2018. *Cytokine Growth Factor Rev.* https://doi.org/10.1016/j.cytogfr.2018.02.005
9. Mena, E.L., et al. 2021. *Proc. Natl. Acad. Sci. USA.* https://doi.org/10.1073/pnas.2023157118
10. Li, X., et al. 2022. *Cell. Mol. Immunol.* https://doi.org/10.1038/s41423-021-00807-4
11. Orhon, I., et al. 2015. *Cell Death Differ.* https://doi.org/10.1038/cdd.2014.171