Antiviral defense in plant stem cells

Forum

Antiviral Defense in Plant Stem Cells

Jie Li[1,6], Elizabeth Hong[2,6], Pengcheng Zhang[3,4,6], Mahmut Tor[4], Jianjun Zhao[1], Stephen Jackson[5], Yiguo Hong[1,3,4,5,*]

[1] State Key Laboratory of North China Crop Improvement and Regulation, Hebei International Research Centre of Vegetable Functional Genomics, College of Horticulture, Hebei Agricultural University, Baoding 071000, China

[2] St George's University Hospitals NHS Foundation Trust, London SW17 0QT, UK

[3] Research Centre for Plant RNA Signaling, College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China

[4] School of Science and the Environment, University of Worcester, Worcester WR2 6AJ, UK

[5] School of Life Sciences, University of Warwick, Coventry CV4 7AL, UK

[6] These authors contributed equally to this work.

[*] Correspondence:

Hong, Y. (yg.hong@hebau.edu.cn; y.hong@worc.ac.uk; yiguo.hong@warwick.ac.uk)

ORCID:

Li, J.: 0000-0002-9958-2850

Hong E.M.: 0000-0003-2223-162X

Zhang P.: 0000-0003-2939-6443

Tor, M.: 0000-0002-4416-5048

Zhao, J.: 0000-0001-7364-1974

Jackson S.: 0000-0002-5093-8668

Hong, Y.: 0000-0002-3352-9686

Corresponding author's website:

Hong, Y.: https://yuanyi.hebau.edu.cn/info/1055/2255.htm

https://www.worcester.ac.uk/about/profiles/professor-yiguo-hong

https://warwick.ac.uk/fac/sci/lifesci/people/5/

Abstract

Undifferentiated plant and animal stem cells are essential for cell, tissue and organ differentiation, development, and growth. They possess unusual antiviral immunity which differs from that in specialized cells. Here, in comparison with animal stem cells, we discuss how plant stem cells defend against viral invasion and beyond.

Keywords: Antiviral immunity, Meristem virus exclusion, Plant stem cell, RNAi, Shoot apical meristem

Stem cells have not undergone differentiation but maintained their pluripotency to develop into various specialized cells. In animals, stem cells are of embryonic and somatic origins although some mature body cells can also be stimulated to de-differentiate back into stem cells. In contrast, plant cells are mostly pluripotent and can regenerate into progeny plants. *Bona fide* stem cells exist in plant growth tips such as root and shoot apical meristem (SAM), and vascular meristem. These plant stem cells are destined to develop into various tissues and organs such as roots, leaves, flowers, and seeds. One common feature of plant and animal stem cells is that they have evolved distinct antiviral mechanisms and are often resistant to viral invasion.

Antiviral defense in animal stem cells

Animal stem cells appear more resistant to viral infection compared to specialized/differentiated cells [1]. Apart from the adaptive antibody-mediated immune system, specialized/differentiated cells often use an interferon (IFN)-triggered response to combat viral infection. Upon attack by invasive viruses, these cells respond with rapid secretion of IFNs that bind to cell receptors. This leads to the induced expression of hundreds of antiviral IFN-stimulated genes which form the first line of antiviral defense [2]. However, animal stem cells are IFN refractory and do not produce inducible IFN-stimulated genes. Instead, they only constitutively express a subset of IFN-stimulated genes as intrinsic arsenals that can prime defence to alert and subvert viral invasion [3]. In addition, RNA interference (RNAi) has emerged as an effective means to withhold virus infection in stem cells that lack the canonical IFN-based antiviral defence [4]. However notably, the extent of RNAi-mediated antiviral defence remains open to debate in animals. Nevertheless, a

specific Dicer isoform can cleave viral double-stranded RNA (dsRNA) and thus help protect animal stem cells from viral infection [5].

Antiviral defense in plant stem cells

Most plant organs, tissues and cells are susceptible to virus/viroid infection. However, plant stem cells in meristems, particularly SAM, are often excluded from virus invasion, a phenomenon known as virus meristem exclusion. However, virus meristem exclusion does not always occur, and many exceptions exist. For instance, seed-transmissible viruses comprise approximately 25% of plant viruses and can enter SAM and infect meristematic stem cells and stem cell-derived reproductive tissues including ovule, megaspore mother cell, egg, pollen mother cell and pollen grains, although virus seed transmission is more a quantitative trait than an all or nothing response [6]. In contrast, non-seed transmissible viruses can only reach the basal part of SAM and fail to overcome a yet unidentified defensive SAM barrier that prevents invasion of meristematic L1, L2 and L3 cells [7] (see Figure 1). This protective barrier appears to have evolved some selective mechanisms that can grant or block the ability of certain macromolecules to enter SAM [7,8]. Indeed, some specific signalling mRNAs such as the mobile florigen *Flowering Locus T* (*FT*) mRNA can move from distal leaf cells into SAM stem cells [8-10]. Intriguingly, acting *in cis*, mobile *FT* mRNA can also enable entry of a non-seed transmissible virus, potato virus X (PVX), into the SAM and subsequent infection of *Nicotiana benthamiana* stem cells [8-10].

Antiviral defense in plant stem cells is complex and may rely on specific host-virus interactions [7,11,12]. For instance, the SAM-specific master regulator WUSCHEL participates in the protection of *Arabidopsis* stem cells from infection by cucumber mosaic virus (CMV) [11]. WUSCHEL is a homeodomain transcription factor and plays a key role in maintaining stem cell identity via a WUSCHEL-CLAVATA3 feedback loop (Figure 1). In *Arabidopsis*, WUSCHEL responds to CMV infection repressing viral protein synthesis via WUSCHEL-mediated transcriptional inhibition of S-adenosyl-L-methionine-dependent methyltransferases (MTases), the latter of which are involved in rRNA methylation to stabilize rRNA and ribosome structure for translation. Consequently, this sabotages CMV replication and its ability to invade stem cells. The WUSCHEL-MTase module is also known to prevent

invasion of stem cells by tobacco rattle virus (TRV), turnip crinkle virus (TCV), and turnip mosaic virus (TuMV) [11].

Unlike animal stem cells, plant stem cells possess fully functional RNAi machinery. Mobile small RNA (sRNA) signals in leaf source cells can spread systemically to induce silencing in distant cells and tissues, but such sRNAs cannot move into and induce silencing in SAM [7,13]. However, if non-seed transmissible viruses are engineered to overcome virus meristem exclusion and are able to enter SAM, these viruses can trigger RNAi in meristematic stem cells. For example, PVX/FT-GFP fused with the mobile *FT* RNA induces RNAi in *GFP*-transgenic *N. benthamiana* SAM whereas PVX/GFP alone cannot [9,10]. Likewise, defective RNAi machinery allows virus entry and infection of meristematic cells. Plants with a dysfunctional RNA-dependent RNA polymerase-6 (RDR6), one of the components in plant RNAi pathway, fail to prevent PVX and potato spindle tuber viroid infection of the SAM [7]. Moreover, virus-encoded RNA silencing suppressors such as TRV 16-kD and CMV 2b proteins can facilitate the bypassing of RNAi-mediated virus meristem exclusion [7].

RDR1, another component of the RNAi machinery, once activated by phytohormone salicylic acid (SA), can upregulate RNAi and inhibit TuMV proliferation in stem cells [12]. TuMV can invade L1, L2 and L3 but is subsequently cleared from L1 and L2 in the later infection stages, although it persists in L3. Loss of *RDR1* and sRNA-processing *DICER-LIKE* genes in *Arabidopsis rdr1* and *dcl234* mutants, however, enables TuMV to persist in L1, L2 and L3. *RDR1* is involved in dsRNA synthesis and sRNA amplification. This leads to produce mobile TuMV-specific sRNAs for anti-TuMV defense in stem cells. Interestingly, TuMV infection increases biosynthesis of SA which upregulates *RDR1* expression, resulting in TuMV clearance and meristem exclusion. In contrast, in plants expressing a bacterial enzyme NahG that degrades SA, TuMV invades and persists in L1, L2 and L3. Thus, an elevated SA/RDR1/sRNA pathway determines TuMV meristem exclusion, linking for the first time a plant defense hormone to RNAi for effectively enacting virus meristem exclusion [12]. Moreover, the collective sRNA products of DCL2, DCL3 and DCL4 are sufficient for stem cell exclusion of turnip yellow mosaic virus (TYMV) or TCV, and the SA/RDR1 pathway is not necessary for TYMV and TCV exclusion since neither *rdr1* nor NahG plants show stem cell invasion by either of these viruses [12]. RNAi is also required for TYMV/TCV-infected plants to produce seeds, but stem cell

exclusion of TuMV through RDR1 or sRNAs alone does not rescue seed production in *rdr1* plants, suggesting that virus meristem exclusion as such is insufficient to safeguard fertile seed development [12].

Concluding remarks and prospective

In this article, we briefly review recent discoveries of antiviral defense in animal and plant stem cells (Figure 1). (1) Animals have evolved distinct antiviral pathways in stem versus specialized/differentiated cells. The IFN-signalling and immune systems act primarily to protect specialized/differentiated cells from virus attack, whilst specific RNAi along with a limited number of constitutive IFN-stimulated genes sustain the primordial antiviral role in animal stem cells [1-5]. Similarly, plants may have evolved a stem cell-specific WUSCHEL-MTase module for virus immunity [7,10]. (2) When animal stem cells start to differentiate into specialised cell types, they gradually lose stem cell-specific defensive mechanism, become responsive to IFNs and eventually acquire the authentic IFN signalling response for antiviral defence [3]. Such a trend also exists in plant stell cells as evidenced by a gradual decrease in antiviral activity from L1/2 to L3 to cells outside SAM [7,10,12]. (3) Unlike in animals, RNAi is a well-documented innate defense mechanism which operates at intracellular, intercellular, and systemic levels against virus infection of plant differentiated and meristematic cells [7,10,12,14].

Significant progress has been made towards dissecting how plant stem cells restrict virus invasion. However, many burning questions and conflicting findings remain to be addressed. Firstly, does a general antiviral mechanism exist in plant stem cells? Both the WUSCHEL-MTase module and RNAi are sufficient for meristem exclusion of some viruses, but certainly not all [7,10,12,15]. Virus meristem exclusion may also not be persistent. This is particularly true for seed-transmissible viruses including well-studied CMV and TuMV. Secondly, antiviral mechanism in stem cells can be virus- or/and host-specific [7]. Moreover, whether other forms of antiviral defenses including physical barriers (specific stem cell wall/membrane structures, and size limitations of plasmodesmata), chemical defense (stem cell-specific secondary metabolites), defense hormones (auxin, SA, jasmonic acid, and ethylene), autophagy, and *R*-gene-mediated immunity exist in plant stem cells is largely overlooked. Each of these defensive mechanisms, individually or collectively, could be involved in stem cells against virus invasion. Finally, the biological relevance of

stem cell-localised antiviral defense shall be investigated to seed development and fertility, to the mechanism behind how some viruses can whilst others cannot be vertically transmitted via seed to progenies, and to the molecular basis of how temporary versus persistent meristem exclusion of (non) seed-transmissible viruses affects plant symptom recovery. Well-designed spatio-temporal experiments coupled with advanced single-cell/nucleus multi-omics technology may now offer promising avenues to resolve these challenging complex questions at single-cell resolution.

Acknowledgments

This work was funded by grants from the National Natural Science Foundation of China (31872636 to Y.H.), Hebei Provincial S&T Bureau (2023HBQZYCSB009 to Y.H.), and Ministry of Education of China (MoE 902/1123401 to Y.H.), and the UK Biotechnology and Biological Sciences Research Council (UK BBSRC–China Partnering Award BB/T018259/1 to S.J. and Y.H.). Y.H. holds a MoE Chair Professorship. All authors were involved in discussing, drafting, writing, revising, and finalizing this Forum article.

Declaration of interests

No interests are declared.

References

1. Belzile JP, Stark TJ, Yeo GW, Spector DH. Human cytomegalovirus infection of human embryonic stem cell-derived primitive neural stem cells is restricted at several steps but leads to the persistence of viral DNA. J Virol. 88: 4021-4039 (2014).
2. Katze MG, He Y, Gale M Jr. Viruses and interferon: a fight for supremacy. Nat Rev Immunol. 2: 675-687 (2002).
3. Wu X, Dao Thi VL, Huang Y, Billerbeck E, Saha D, Hoffmann HH, Wang Y, Silva LAV, Sarbanes S, Sun T, Andrus L, Yu Y, Quirk C, Li M, MacDonald MR, Schneider WM, An X, Rosenberg BR, Rice CM. Intrinsic immunity shapes viral resistance of stem cells. Cell. 172: 423-438.e25 (2018).
4. Anobile DP, Poirier EZ. RNA interference, an emerging component of antiviral immunity in mammals. Biochem Soc Trans. 51: 137-146 (2023).
5. Poirier EZ, Buck MD, Chakravarty P, Carvalho J, Frederico B, Cardoso A, Healy L, Ulferts R, Beale R, Reis e Sousa C. An isoform of Dicer protects mammalian stem cells against multiple RNA viruses. Science. 373: 231-236 (2021).
6. Matsushita Y, Yanagisawa H, Sano T. Vertical and Horizontal Transmission of Pospiviroids. Viruses. 10: 706 (2018).
7. Bradamante G, Scheid OM, Incarbone M. Under siege: virus control in plant meristems and progeny. Plant Cell 33: 2523-2537 (2021).
8. Yu Z, Chen W, Wang Y, Zhang P, Shi N, Hong Y. Mobile Flowering Locus T RNA - Biological Relevance and Biotechnological Potential. Front Plant Sci. 12: 792192 (2022).

9. Li C, Gu M, Shi N, Zhang H, Yang X, Osman T, Liu Y, Wang H, Vatish M, Jackson S, Hong Y. Mobile FT mRNA contributes to the systemic florigen signalling in floral induction. Sci Rep. 1:73 (2011).

10. Li C, Zhang K, Zeng X, Jackson S, Zhou Y, Hong Y. A cis-element within *FLOWERING LOCUS T* mRNA determines its mobility and facilitates trafficking of heterologous viral RNA. J Virol. 83: 3540-3548 (2009).

11. Wu H, Qu X, Dong Z, Luo L, Shao C, Forner J, Lohmann JU, Su M, Xu M, Liu X, Zhu L, Zeng J, Liu S, Tian Z, Zhao Z. WUSCHEL triggers innate antiviral immunity in plant stem cells. Science. 370: 227-231 (2020).

12. Incarbone M, Bradamante G, Pruckner F, Wegscheider T, Rozhon W, Nguyen V, Gutzat R, Mérai Z, Lendl T, MacFarlane S, Nodine M, Scheid OM. Salicylic acid and RNA interference mediate antiviral immunity of plant stem cells. Proc Natl Acad Sci USA. 120: e2302069120 (2023).

13. Chen W, Zhang X, Fan Y, Li B, Ryabov E, Shi N, Zhao M, Yu Z, Qin C, Zheng Q, Zhang P, Wang H, Jackson S, Cheng Q, Liu Y, Gallusci P, Hong Y. A genetic network for systemic RNA silencing in plants. Plant Physiol. 176: 2700-2719 (2018).

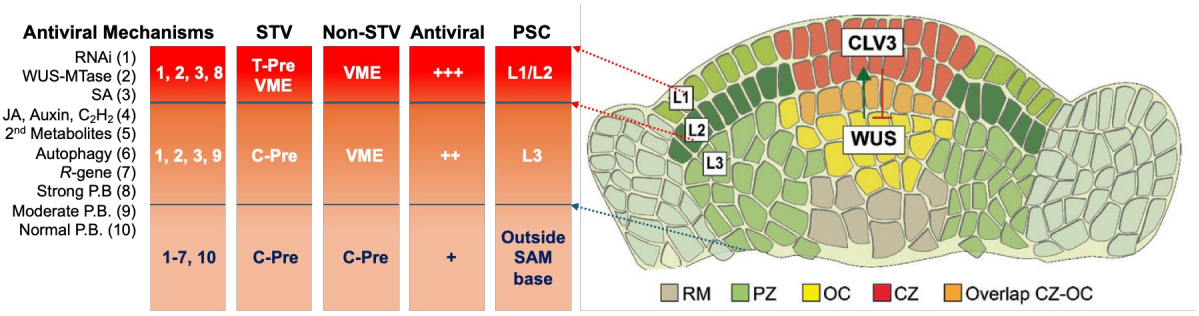
14. Zhang X, Lai T, Zhang P, Zhang X, Yuan C, Jin Z, Li H, Yu Z, Qin C, Tor M, Ma P, Cheng Q, Hong Y. Revisiting mobile RNA silencing in plants. Plant Sci. 278: 113-117 (2019).

15. Medzihradszky A, Gyula P, SOS-Hegedus A, Szittya G, Burgyan J. Transcriptional reprogramming in the shoot apical meristem of CymRSV-infected *Nicotiana benthamiana* plants associated with viral exclusion and the lack of recovery. Mol Plant Pathol. 20: 1748-1758 (2019).

Figure Legend

Figure 1 Antiviral defence in plant stem cells. Typical organization of shoot apical meristem (SAM) is shown on the right. This image is a copy of Fig. 1 in "Lara Lopes F, Galvan-Ampudia C, Landrein B. WUSCHEL in the shoot apical meristem: old player, new tricks. J Exp Bot. 72: 1527–1535 (2021)" with permission. SAM consists of L1, L2 and L3 layers of plant stem cells. Based on strong (+++), moderate (++) and normal (+) strength of antiviral activities, these stem cells can be divided into two types of antiviral zones L1/L2 versus L3 along with plant cells, tissues and organs outside the base of SAM. Non-seed transmissible viruses are often excluded from L1-3 stem cells, indicated by virus meristem exclusion. However, seed-transmissible viruses initially can temporarily invade, but are cleared from L1/L2 at the later stages of virus infection. Many seed-transmissible viruses invade and are constantly present in L3. Various antiviral mechanisms [7,11,12,14,15] found to function in plant stem versus specialized/differentiated cells are shown by number codes in different antiviral niches. Thick and thin blue lines represent physical antiviral-barriers between L1/L2 and L3, and between L3 and cells outside of the SAM base, respectively. The strong, moderate, and normal potency of such physical antiviral-barriers is defined by the capability of cell wall/membrane of L1/L2, L3 and non-stem

cells to block virus cellular entry. The WUSCHEL-CLAVATA3 (WUS-CLV3) feedback loop in maintaining stem cell identity and populations are shown.



Abbreviations: CZ, Central zone; C-Pre, Constant presence of viruses; L1, L2 and L3, Three layers of plant stem cells; Non-STV, Non-seed transmissible viruses; OC, Organizing centre; P.B., Physical antiviral-barriers; PSC, Plant stem cell; PZ, Peripheral zone; RM, Rib meristem; SAM, Shoot apical meristem; STV, Seed-transmissible viruses; T-Pre, Temporary presence of viruses; VME, Virus meristem exclusion.