Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

Dendrite enlightenment

Gaia Tavosanis[1,2]

Abstract

Neuronal dendrites acquire complex morphologies during development. These are not just the product of cell-intrinsic developmental programs; rather they are defined in close interaction with the cellular environment. Thus, to understand the molecular cascades that yield appropriate morphologies, it is essential to investigate them *in vivo*, in the actual complex tissue environment encountered by the differentiating neuron in the developing animal. Particularly, genetic approaches have pointed to factors controlling dendrite differentiation *in vivo*. These suggest that localized and transient molecular cascades might underlie the formation and stabilization of dendrite branches with neuron type–specific characteristics. Here, I highlight the need for studies of neuronal dendrite differentiation in the animal, the challenges provided by such an approach, and the promising pathways that have recently opened.

Addresses

[1]German Center for Neurodegenerative Diseases (DZNE), Venusberg-Campus 1/99, Bonn, 53127, Germany

[2]LIMES Institute, University of Bonn, Carl-Troll-Str. 3, Bonn, 53115, Germany

Corresponding author: Tavosanis, Gaia (Gaia.tavosanis@dzne.de)

Current Opinion in Neurobiology 2021, **69**:222–230

This review comes from a themed issue on **Molecular Neuroscience**

Edited by **Frank Bradke** and **Yukiko Goda**

For a complete overview see the Issue and the Editorial

Available online 13 June 2021

https://doi.org/10.1016/j.conb.2021.05.001

Introduction

Neurons can develop highly complex and neuron type–specific branched dendrites that have fascinated neuroscientists for more than 100 years [1]. The morphology of dendrites has tight functional constraints related to the localization and distribution of inputs received by the neuron and to the way in which information is integrated and processed along dendrites, defining the output of the neuron [2–4]. Not surprisingly, thus, the mechanisms that support the establishment of the neuron type–specific morphology of dendrites during development have been a major focus of research for the past three decades [5–8].

The definition of specific dendrite morphologies as well as of other neuron type–specific properties depends on intrinsic transcriptional codes that are established during development [9]. Cascades of events triggered by positional information relative to the body axes lead to the expression of combinations of transcription factors that define the morphology of the neuron. A well-studied example is afforded by the dendrite arborization (da) neurons of the *Drosophila* larva that can be subdivided into four classes (c1da–c4da) with distinctive functional and morphological properties [10]. Manipulating the relative abundance of key transcription factors shifts the morphology of a particular da neuron from one class to another [11,12]. Only a few of the downstream factors that translate the information contained in those codes into structural traits are known [13–16]. The general outcome of this transcriptional information is likely represented by the expression of several effectors, including regulators of the cytoskeleton and receptors for extrinsic signals. The activation and localization of those effectors represent the next layer of regulation requiring precise spatiotemporal information.

Owing to these intrinsic programs, some aspects of neuron type–specific morphology are preserved also in culture after the neuron has been extracted from its natural environment, its processes severed, and it has been induced to regrow in a petri dish [17]. Multiple aspects of the complex and neuron type–specific morphology of dendrites, though, depend on localized signals and specific interactions with substrates and partners. Pyramidal hippocampal neurons in culture become highly complex and multipolar [18,19]. In contrast to their *in vivo* morphology, though, they do not form a primary dendrite (S. Dupraz and F. Bradke, personal communication). Neurons in the insect central nervous system are mostly unipolar as their cell bodies are positioned outside of the neuropile [20]. Those same neurons, such as the Kenyon cells of the *Drosophila* mushroom body, acquire a multipolar morphology in culture [21]. Clearly, the context plays a central role in the definition of dendrite morphology. This is particularly important for the formation of maps of sensory inputs in the developing nervous system, such as in the barrel cortex of rodents, in which the position of dendrites sustains the formation of discrete maps [22].

Therefore, to understand how dendrite patterns are established, it is mandatory to investigate neuronal differentiation in the animal.

In this review, I will focus on the progress in understanding the cell biology of dendrite differentiation in a developing animal. I will summarize the key tools and techniques that currently advance our experimental access to the subcellular organization of neurons *in vivo*, an essential prerequisite for understanding the cell biology of differentiating neurons during animal development.

The dynamics of dendrite differentiation

Visualizing neuronal differentiation *in vivo* is challenging. This is particularly true in organisms in which the relevant developmental stages happen in the womb, but observing individual neurons over time in the same animal, while development is happening, presents a number of complications in every system. Tissues undergo morphogenic rearrangements, whereas the observed neurons migrate and change in size and shape sometimes quite rapidly, with fine process dynamics happening on the scale of minutes. The choice of the system is therefore critical. Pioneering work took advantage of transparent developmental stages of organisms such as *Xenopus laevis* tadpoles or zebrafish larvae to investigate the pattern of extension and retraction of dendrites of optic tectal neurons or the stepwise process that leads to retinal ganglion cell dendrite laminated organization [23–26]. Via DiI uptake or the expression of genetically encoded fluorescent markers, the dendrites of optic tectal neurons could be imaged by confocal microscopy over multiple days, revealing distinct phases of dendrite growth, including a period of exuberant extension and branching and a late phase of stabilization [24,25,27]. Already in those early studies, the initial observation of dendrite dynamics raised important questions about the factors that decide which dynamic branches become stabilized and which ones retract. The formation of a synaptic contact supports the stabilization of a branch favoring its maintenance [23,28–30].

Mammalian systems are in general less amenable to *in vivo* imaging during development. In adult rodents, live longitudinal imaging of individual neurons has been pioneered already in the early 2000s through the injection of cell-permeant indicators or the use of transgenic mice expressing cytoplasmic green fluorescent protein (GFP) in random subsets of defined neuronal populations [31,32]. Implanting permanent cranial windows in these transgenic mice and performing 2-photon imaging revealed dendrite and spine dynamics in superficial layers of the adult cortex and later even in deeper regions such as the hippocampus [33–38]. In spite of this progress, access to the developing nervous system has been particularly challenging, as parts of neuronal differentiation happen while the embryo is in the womb. Nevertheless, in the rat and even in the mouse, cranial windows were used at neonatal stages to investigate the dynamics of dendritic spines in somatosensory cortex neurons after sensory deprivation or the interplay between intrinsic and stimulus-driven activity in shaping the refinement of the circuits in the mouse barrel cortex [39–41]. Cerebellar granule neurons (CGNs), the most abundant cell type in the cerebellum, elaborate their dendrites in postnatal stages and are therefore more approachable for imaging early steps of dendritogenesis [42]. CGNs display on average four dendrites, each terminating with a claw that enwraps a mossy fiber axonal bouton, and respond to the coincident activation of multiple mossy fiber boutons [43–45]. To achieve this very specialized dendrite organization, multiple exuberant processes are initially formed, of which only 3–5 become stabilized and form a claw in the course of about five days [46]. Claw formation is not necessary for the selection of the branches that will be stabilized, but once the claw is formed, the branch will remain stable [46]. In addition, the refinement of barrel organization in the barrel cortex happens in early postnatal stages in the mouse, and it involves the asymmetric distribution of the dendrites of stellate neurons [47]. By imaging the differentiation of individual stellate neurons *in vivo* every few hours using 2P microscopy, it became clear that the asymmetric arborization of those dendrites toward the center of the barrel is obtained by differential stabilization of the branches. In fact, dendrite branches oriented toward the outside of the barrel are formed, but are very likely to retract [48]. Thus, longitudinal imaging of dendrite growth, even at low time resolution, can help to elucidate the logic underlying the establishment of specific dendrite morphologies.

A major boost in the understanding of the molecular genetics of dendrite differentiation followed the establishment of *Drosophila* lines that express cytoplasmic GFP in a subset of neurons of the peripheral nervous system of the larva [49]. The four classes of multidendritic dendrite-arborization neurons or da neurons, mentioned previously, develop their dendrites in a 2-dimensional field underneath the transparent cuticle of the fly larva and are thus ideal for *in vivo* imaging by confocal microscopy. In this system, genetic tools to visualize and manipulate individual neurons were soon combined with large-scale mutagenesis screens to identify core and novel factors involved in regulating neuronal morphology [6]. Findings obtained through those screens over the years have helped to define the molecular players of multiple key phenomena, including 'self-avoidance' and 'tiling', central conserved rules for the establishment of dendrite trees, reviewed elsewhere [7,50]. Thanks to the accessibility of these neurons, it has been possible to image dendrite elaboration in the same neuron every few hours, similarly to what

described previously for the mouse experiments [51]. In addition, the larvae can be immobilized, and da neuron dendrites can be imaged every few seconds, allowing approaching the cell biological mechanisms of branch site selection, extension, retraction, and stabilization [52–57]. The c4da neuron high-order branches extend and retract in an exploratory fashion, changing their direction of growth. In contrast, fascin-enriched high-order branches of c3da neurons tend to extend and retract, at a velocity of approximately 0.4 μm/min, in a linear trajectory [13,56]. Finally, the initial formation of higher-order branches in c1da neurons is stochastic, and their velocity of growth varies with developmental time [52]. The main branches of da neurons extend at early stages of development that have been less accessible to imaging. Only recently, studies have followed the detailed elaboration of dendrites starting from the embryonic stages in which the primary branches become defined [51,52,55]. Importantly, these studies have delivered the primary data for computational models describing neuronal dendrite differentiation. Through the early phases of primary branch elaboration of the complex and space-filling c4da neurons, the c3da neurons just as the sparse and selective c1da neurons follow a highly conserved pattern of branching that is random and simply based on the parsimonious usage of cable length [51,55,56]. The c4da neurons follow these simple conserved rules throughout their differentiation stages to fill in their entire receptive field [51]. In contrast, the proprioceptive c1da neurons transition to a phase of branch loss that is stochastic and molecularly driven by *dscam1* and that results in comb-like–shaped dendrites, in which second-order branches are parallel to the direction of body contraction [52,55]. This is important because, owing to their orientation, these branches experience a large curvature rise during contraction, which is suggested to increase the opening probability of mechanogated ion channels initiating the cell response [55,58,59]. Finally, the differentiation of c3da neuron dendrites includes a second, divergent phase in which local rules dominate over the basic limitation of cable length [56].

Some of the da neurons completely shed off their branches approaching metamorphosis and reform dendrite branches with renewed patterns during the pupal stages [60,61]. Visualization of primary branch growth is more approachable in those stages, which thus represent an interesting option [57]. Furthermore, because da neurons do not receive direct synaptic input, few laboratories turned to investigating dendrite dynamics in the central nervous system of *Drosophila* [62,63].

Similar to the da neurons, dendrites of the somatosensory PVD neuron of *Caenorhabditis elegans* are also ideal for live imaging because they form an orthogonal array of superficial branches with a highly reproducible pattern, right under a transparent cuticle [64–66]. This has allowed a detailed description of the stepwise elaboration of dendrites in this neuron, supported by the dynamic extension and withdrawal of branches organized by contact-dependent self-avoidance [66].

In summary, the chance of gaining a glimpse into the early stages of dendrite differentiation *in vivo* currently strictly depends on the model organism, on the developmental stage, and on the particular neuronal type. Thus, the dynamics of the morphological steps leading to dendrite maturation have been carefully described in a limited selection of neurons. Nevertheless, the neurons of choice have very distinct morphologies, from the space-filling c4da neurons of the *Drosophila* larva to the highly selective CGNs of the mammalian cerebellum, allowing asking general versus specific aspects. Based on these data, dendrite elaboration appears to start with the formation of exuberant processes that are dynamic and extend and retract over the course of minutes. A second phase might follow, in which neuron type-specific branches are formed or in which subsets of branches are stabilized while others are eliminated. The proportion, timing, and properties of these events depend on the particular neuronal type analyzed.

Subcellular localization of molecular effectors *in vivo*

The morphological description of dynamics combined with genetic and molecular analysis has pointed to an increasing number of key factors that control dendrite differentiation *in vivo* [5,8,67]. Nonetheless, the dynamic cellular context in which those factors operate *in vivo* might remain elusive. Indeed, biochemical reactions are controlled in cells to a large extent by the specific subcellular localization of the molecules involved [68]. For cellular events to take place, the local concentration of multiple relevant factors might need to reach a certain threshold related to their specific chemical properties. In broad terms, these concepts have important implications for how we think of dynamic molecular cascades of events happening during cellular differentiation. In fact, if the subcellular localization of molecular players and their local concentration are key elements of the regulation of cellular processes, it is of essential importance to reveal when and where molecular interactors come together to initiate a cellular phenomenon. This is particularly relevant for highly polarized cells, such as neurons, that form dendrites arborizing with specific morphological characteristics and dynamics in the complex context of the developing nervous system.

Achieving the temporal and spatial resolution required to reveal the dynamics of subcellular localization of molecules *in vivo* in defined neuronal populations is challenging. For instance, the main approach to visualize

the subcellular localization of a molecule *in vivo* requires generating transgenic animals that express a fluorescently tagged version of this molecule in a defined subset of neurons. The impact of the tag on the function and localization of the molecule, as well as the effect of potential overexpression, needs to be carefully controlled. Regardless, for certain molecular contexts, for instance the molecular control of actin dynamics, tools to achieve this in cultured motile cell types or dissociated neurons undergoing differentiation have been actively developed for years [69]. The complex dynamics of actin can be revealed via fluorescent molecules that reversibly associate with filamentous actin in those contexts or by GFP-tagged actin (e.g. [70,71]). In addition, the localization of GFP-tagged actin regulators helps to infer the local dynamics of actin filament nucleation, elongation, or severing [53,69,72,73]. In the past few years, super-resolution imaging of actin even in the thin dendrites of superficial layers of the cortex in transgenic mice could be achieved using *in vivo* 2-photon stimulated emission depletion (2P-STED) microscopy. This permitted a direct view into actin dynamics within dendrite spines, which is related to the functional potentiation of individual synapses [74,75].

In the invertebrate model systems that I described previously, genetically encoded fluorescently tagged constructs are frequently used to investigate the subcellular spatiotemporal distribution of molecules [76–78]. Transient actin patches were thus correlated in dendrites of c4da neurons of the *Drosophila* larva with the initiation of collateral branch formation [53,54]. Those patches are locally initiated by the activation of Arp2/3 complex-mediated nucleation of branched actin filaments triggered by the WAVE complex (Figure 1). The transient recruitment of Arp2/3 complexes can be detected as a punctum that predicts the site at which a novel branch will form [53] (Figure 1A and B). The site of activation of this cascade in *C. elegans* PVD neurons is positioned by a multiprotein ligand–receptor complex that thus defines the branching site accurately [79]. A combination of genetic analysis and time-lapse imaging can thus help to clarify the role of actin regulatory proteins in dendrite differentiation [56]. The actin-bundling protein fascin is enriched only in the high-order branches of c3da *Drosophila* neurons, but not in the other da neurons, and it accumulates in particular onto elongating branchlets. In the absence of fascin, these branches become bent and branched. This led to the suggestion that fascin bundles and stabilizes actin filaments in the extending branchlets, stiffening them and guaranteeing their typical straight elongation [13].

These types of experiments address the dynamic localization of proteins in the cell. However, many proteins can be rather ubiquitously localized in the cell, whereas their activation is tightly restricted to spatially defined domains. Thus, the specific location of the active protein gives relevant insight. Multiple tools to detect activated signaling cascades in neurons are described in an accompanying review [80], and genetically encoded tools are used routinely for recording neuronal activity, using transient surges in Ca^{2+} concentration [81]. An important set of conserved molecules in regard to the establishment of neuronal dendrite morphology are the small GTPases of the Rho, Rac, and Cdc42 families [82]. An approach to reveal the subcellular domains of Cdc42 activation *in vivo* took advantage of the reversible binding of Cdc42 to a specific peptide (CBD) on activation. Binding of the activated Cdc42 to a co-expressed CBD could be revealed by fluorescence resonance energy transfer (FRET) [83]. These constructs helped to correlate the localized, Pak1-dependent, activation of Cdc42 in *Drosophila* motor neurons during embryogenesis with the site at which dendrite branches form [63,83].

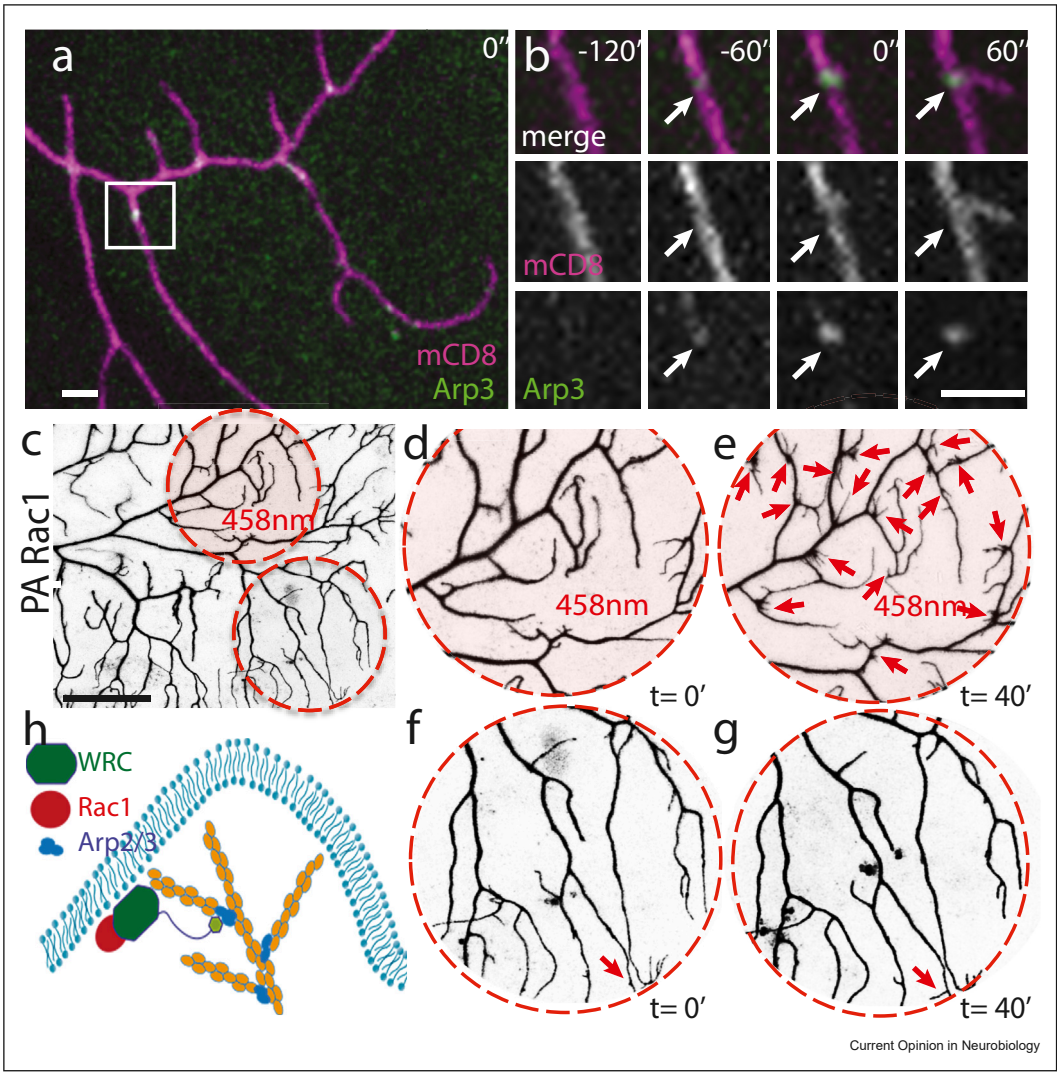
Taken together, via a combination of fluorescent markers and rapidly improving microscopy solutions, the subcellular localization and in specific cases also the localized activation of various types of molecules can be detected. This allows inferring where and when biochemical cascades are in action to induce morphogenic cellular changes.

Manipulating key molecular players to understand dendrite differentiation

Over the years, the knockout of individual genes has led to the discovery of important pathways controlling dendrite branching *in vivo* [5,7,67]. Nevertheless, the analysis of full knockdown of genes that might have multiple functions in different tissues during development can be complicated by functional compensation or by pleiotropic phenotypes confounding or even making the analysis of dendrite differentiation impossible. Conditional knockouts in defined neuronal populations or even generating individual mutant neurons help overcome multiple of these issues [47,84]. Regardless, the elaboration of dendrites is a rather late step during neuronal differentiation, after migration, polarization, neurite formation, axon extension. Important cellular regulators are likely to have functions early during cellular differentiation that hamper the interpretation of dendrite phenotypes. As an example, although cofilin is required for dendrite establishment, hippocampal neurons isolated from mice mutant for actin depolymerizing factor (ADF)/cofilin do not even form neurites [54,85,86].

One possible solution to this issue is to develop tools for acute activation or inactivation of proteins *in vivo*. The technical aspects behind these approaches in developmental biology have been extensively reviewed recently [87,88]. For *in vivo* applications in the nervous system, the constructs need to be expressed in transgenic animals in subsets of neurons. Furthermore, activation or inactivation should be ideally obtained by noninvasive

Figure 1



Initiation of dendrite branchlet formation in c4da neurons of *Drosophila* larvae. **(a)** Time-lapse imaging of differentiating c4da neurons of second instar *Drosophila* larvae expressing Arp3-GFP (green) and mCD8cherry (magenta) imaged every 60 s. **(b)** Three-minute time lapse of the region boxed in A. Arrows indicate the sites where a new branchlet will form. Scale bars: 10 μm. **(c–g)** Images from time-lapse recordings of a c4da neuron expressing membrane-tagged red fluorescent mCherry to visualize their membrane and photoactivatable constitutively active Rac1 (PA Rac1). The red circles represent a region of interest (ROI) illuminated with 458 nm light to activate PA Rac1 (orange background) and a ROI that was not illuminated. **(d–g)** Images of those two ROIs before (D, F; t = 0′) and after 40 min of photoactivation (E; t = 40′) or 40 min of no illumination (G; t = 40′). The arrows indicate some of the newly formed branchlets or groups of branchlets. **(h)** Schematic representation of the cascade of events leading to the formation of a dendrite branchlet. This starts with local activation of the WRC complex by Rac1, which in turn yields a local recruitment of the active Arp2/3 complex and thus the formation of a patch of branched actin filaments (Adapted from Stürner et al., 2019).

means. In non–warm-blooded animals, temperature-sensitive mutations represent a classical answer to these needs. The *Drosophila* gene *shibire* encodes for the conserved protein dynamin that forms multimers catalyzing the pinching off of endocytic vesicles [89]. Constructs carrying a temperature-sensitive *shibire* mutant can be expressed in specific tissues or neuronal subsets in *Drosophila* [90]. A shift in temperature yields a reversible block of endocytosis in these flies that can also quickly arrest neurotransmitter release. The systematic design of potential temperature-sensitive mutations could be noticeably accelerated by improvements in protein structure prediction [91]. A more common approach takes advantage of light (optogenetics) to modulate cellular functions and was pioneered to control activity in neurons [92]. For more broad cell biological approaches, domains derived from light-sensitive plant proteins were co-opted to modulate the regulation, clustering, or localization of proteins [87,88,93]. Such domains, as the light–oxygen–voltage

(LOV) domain, undergo conformational changes on blue light illumination with low intensity [94,95]. By positioning the LOV domain in a critical position within the structure of the target protein of interest, it is possible for instance to occlude an active domain in a light-dependent, reversible manner. Such an approach has been used to generate an optically switchable constitutive active form of the small GTPase Rac1 [96]. Localized activation of Rac1 obtained with this tool in c4da neurons of the *Drosophila* larva inducted a molecular cascade leading to activation of WAVE and recruitment of the Arp2/3 complex, thus locally initiating the formation of dendrite branchlets [53] (Figure 1C–H). LOV domains, as well as cryptochrome 2 (CRY2)/N-terminal domain of cryptochrome-interacting basic-helix-loop-helix 1 (CIB1), are used to sequester proteins to defined subcellular locations or to induce protein oligomerization [97–100]. In *C. elegans*, constructs including an LOV domain were used to display *in vivo* the role of different motor proteins in polarized trafficking in neurons [101]. Such acute manipulations of protein activity or localization are very exciting as they allow direct testing of causal relationships between certain molecular functions and cellular processes leading to dendrite differentiation. Nonetheless, these methods have many technical limitations. In the case of the LOV domain, the range of activating light is broad, although recently improved, making it difficult to restrict temporally its activation in the living animal. Depending on the wavelength of activation, penetration in deeper tissues might be problematic. Achieving a tightly localized activation, in certain cells or cell domains, such as few dendrites, requires careful titration [102]. In fact, there is no toolbox that works for all proteins. Instead, for each protein of interest, a potential solution needs to be identified and the obtained constructs then carefully tested in cells and finally in the animal. Despite these complications, the advantages of acute protein manipulation to dissect cellular processes during development are so evident that we can expect a large expansion of the toolbox in the coming years.

Conclusions and future perspectives

The systematic analysis of the cellular regulation of dendrite morphology establishment *in vivo* is coming of age, thanks to developments in protein visualization and manipulation combined with rapid advancement of microscopy solutions. These will allow asking more systematically important questions such as how are the sites of de novo dendrite branch formation defined, the sources of guidance signals for directed growth, or the cellular mechanisms that lead to selective branch stabilization. When observing the cellular phenomena that control the establishment of the variety of dendrite trees *in vivo*, a major question will be the extent to which common pathways and mechanisms are used. Is there a basic modular set of molecular pathways that can be used in various combinations to achieve neuron type–specific dendrite morphologies, or are *ad hoc* solutions used to solve specific tasks? Such fundamental questions for our understating of the elaboration of those varied morphologies need to be addressed in the appropriate context after all, the developing animal.

Conflict of interest statement

Nothing declared.

Acknowledgements

The author apologizes to the many colleagues whose work could not be represented in this review because of space limitations. She is grateful to present and past members of the Tavosanis laboratory and to Frank Bradke, Martin Furhmann, and Tomke Stürner for critical reading of this manuscript. Work in her laboratory is supported by the DZNE and by the DFG (SPP1464, Project number 170387504; FOR2705, project number 365082554).

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Ramón y Cajal S: *Histología del sistema nervioso del hombre y de los vertebrados. Obra completa (Spanish Edition)*. 1st ed. Agencia Estatal Boletín Oficial del Estado; 2012.
2. Payeur A, Béïque J-C, Naud R: **Classes of dendritic information processing**. *Curr Opin Neurobiol* 2019, **58**:78–85.
3. Poirazi P, Papoutsi A: **Illuminating dendritic function with computational models**. *Nat Rev Neurosci* 2020, **21**:303–321.
4. London M, Häusser M: **Dendritic computation**. *Annu Rev Neurosci* 2005, **28**:503–532.
5. Lefebvre JL: **Molecular mechanisms that mediate dendrite morphogenesis**. *Curr Top Dev Biol* 2021, **142**:233–282.
6. Jan Y-N, Jan LY: **Branching out: mechanisms of dendritic arborization**. *Nat Rev Neurosci* 2010, **11**:316–328.
7. Dong X, Shen K, Bülow HE: **Intrinsic and extrinsic mechanisms of dendritic morphogenesis**. *Annu Rev Physiol* 2015, **77**:271–300.
8. Lin T-Y, Chen P-J, Yu H-H, Hsu C-P, Lee C-H: **Extrinsic factors regulating dendritic patterning**. *Front Cell Neurosci* 2020, **14**: 622808.
9. Puram SV, Bonni A: **Cell-intrinsic drivers of dendrite morphogenesis**. *Development* 2013, **140**:4657–4671.
10. Grueber WB, Jan LY, Jan YN: **Tiling of the Drosophila epidermis by multidendritic sensory neurons**. *Development* 2002, **129**:2867–2878.
11. Corty MM, Tam J, Grueber WB: **Dendritic diversification through transcription factor-mediated suppression of alternative morphologies**. *Development* 2016, **143**:1351–1362.
12. Parrish JZ, Emoto K, Kim MD, Jan YN: **Mechanisms that regulate establishment, maintenance, and remodeling of dendritic fields**. *Annu Rev Neurosci* 2007, **30**:399–423.
13. Nagel J, Delandre C, Zhang Y, Förstner F, Moore AW, Tavosanis G: **Fascin controls neuronal class-specific dendrite arbor morphology**. *Development* 2012, **139**:2999–3009.
14. Iyer SC, Ramachandran Iyer EP, Meduri R, Rubaharan M, Kuntimaddi A, Karamsetty M, Cox DN: **Cut, via CrebA, transcriptionally regulates the COPII secretory pathway to direct dendrite development in Drosophila**. *J Cell Sci* 2013, **126**:4732–4745.
15. Das R, Bhattacharjee S, Patel AA, Harris JM, Bhattacharya S, Letcher JM, Clark SG, Nanda S, Iyer EPR, Ascoli GA, *et al.*:

Dendritic cytoskeletal architecture is modulated by combinatorial transcriptional regulation in Drosophila melanogaster. *Genetics* 2017, **207**:1401–1421.

16. Yalgin C, Ebrahimi S, Delandre C, Yoong LF, Akimoto S, Tran H, Amikura R, Spokony R, Torben-Nielsen B, White KP, *et al.*: **Centrosomin represses dendrite branching by orienting microtubule nucleation**. *Nat Neurosci* 2015, **18**:1437–1445.

17. Fujishima K, Horie R, Mochizuki A, Kengaku M: **Principles of branch dynamics governing shape characteristics of cerebellar Purkinje cell dendrites**. *Development* 2012, **139**: 3442–3455.

18. Dotti CG, Sullivan CA, Banker GA: **The establishment of polarity by hippocampal neurons in culture**. *J Neurosci* 1988, **8**: 1454–1468.

19. Tahirovic S, Bradke F: **Neuronal polarity**. *Cold Spring Harb Perspect Biol* 2009, **1**, a001644.

20. Sánchez-Soriano N, Bottenberg W, Fiala A, Haessler U, Kerassoviti A, Knust E, Löhr R, Prokop A: **Are dendrites in Drosophila homologous to vertebrate dendrites?** *Dev Biol* 2005, **288**:126–138.

21. Kraft R, Levine RB, Restifo LL: **The steroid hormone 20-hydroxyecdysone enhances neurite growth of Drosophila mushroom body neurons isolated during metamorphosis**. *J Neurosci* 1998, **18**:8886–8899.

22. Iwasato T: **In vivo imaging of neural circuit formation in the neonatal mouse barrel cortex**. *Dev Growth Differ* 2020, **62**: 476–486.

23. Niell CM, Meyer MP, Smith SJ: **In vivo imaging of synapse formation on a growing dendritic arbor**. *Nat Neurosci* 2004, **7**: 254–260.

24. Wu GY, Zou DJ, Rajan I, Cline H: **Dendritic dynamics in vivo change during neuronal maturation**. *J Neurosci* 1999, **19**: 4472–4483.

25. Kaethner RJ, Stuermer CA: **Dynamics of process formation during differentiation of tectal neurons in embryonic zebrafish**. *J Neurobiol* 1997, **32**:627–639.

26. Mumm JS, Williams PR, Godinho L, Koerber A, Pittman AJ, Roeser T, Chien C-B, Baier H, Wong ROL: **In vivo imaging reveals dendritic targeting of laminated afferents by zebrafish retinal ganglion cells**. *Neuron* 2006, **52**:609–621.

27. Jontes JD, Buchanan J, Smith SJ: **Growth cone and dendrite dynamics in zebrafish embryos: early events in synaptogenesis imaged in vivo**. *Nat Neurosci* 2000, **3**: 231–237.

28. Haas K, Li J, Cline HT: **AMPA receptors regulate experience-dependent dendritic arbor growth in vivo**. *Proc Natl Acad Sci USA* 2006, **103**:12127–12131.

29. Cline H, Haas K: **The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the synaptotrophic hypothesis**. *J Physiol (Lond)* 2008, **586**: 1509–1517.

30. Sin WC, Haas K, Ruthazer ES, Cline HT: **Dendrite growth increased by visual activity requires NMDA receptor and Rho GTPases**. *Nature* 2002, **419**:475–480.

31. Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, Sanes JR: **Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP**. *Neuron* 2000, **28**:41–51.

32. Stosiek C, Garaschuk O, Holthoff K, Konnerth A: **In vivo two-photon calcium imaging of neuronal networks**. *Proc Natl Acad Sci USA* 2003, **100**:7319–7324.

33. Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K: **Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex**. *Nature* 2002, **420**:788–794.

34. Grutzendler J, Kasthuri N, Gan W-B: **Long-term dendritic spine stability in the adult cortex**. *Nature* 2002, **420**:812–816.

35. Attardo A, Fitzgerald JE, Schnitzer MJ: **Impermanence of dendritic spines in live adult CA1 hippocampus**. *Nature* 2015, **523**:592–596.

36. Dombeck DA, Khabbaz AN, Collman F, Adelman TL, Tank DW: **Imaging large-scale neural activity with cellular resolution in awake, mobile mice**. *Neuron* 2007, **56**:43–57.

37. Gu L, Kleiber S, Schmid L, Nebeling F, Chamoun M, Steffen J, Wagner J, Fuhrmann M: **Long-term in vivo imaging of dendritic spines in the hippocampus reveals structural plasticity**. *J Neurosci* 2014, **34**:13948–13953.

38. ** Pfeiffer T, Poll S, Bancelin S, Angibaud J, Inavalli VK, Keppler K, Mittag M, Fuhrmann M, Nägerl UV: **Chronic 2P-STED imaging reveals high turnover of dendritic spines in the hippocampus in vivo**. *Elife* 2018, **7**.
Impressive combination of 2P-STED microscopy in mice implanted with a hippocampal window that allows high-resolution imaging of spine density and turnover in a deep center, with a key function in memory.

39. * Che A, Babij R, Iannone AF, Fetcho RN, Ferrer M, Liston C, Fishell G, De Marco García NV: **Layer I interneurons sharpen sensory maps during neonatal development**. *Neuron* 2018, **99**:98–116.e7.
The role of intrinsic neuronal activity in circuit refinement is addressed with *in vivo* functional imaging of the barrel cortex using a cranial window in mouse pups starting at postnatal day 6.

40. Lendvai B, Stern EA, Chen B, Svoboda K: **Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo**. *Nature* 2000, **404**:876–881.

41. ** Duan ZRS, Che A, Chu P, Modol L, Bollmann Y, Babij R, Fetcho RN, Otsuka T, Fuccillo MV, Liston C, *et al.*: **Gabaergic restriction of network dynamics regulates interneuron survival in the developing cortex**. *Neuron* 2020, **105**:75–92.e5.
The role of GABAergic signaling in setting up functional assemblies of interneurons and pyramidal cells in the somatosensory cortex during development, investigated using *in vivo* imaging in the first postnatal week in mouse.

42. Altman J: **Postnatal development of the cerebellar cortex in the rat. 3. Maturation of the components of the granular layer**. *J Comp Neurol* 1972, **145**:465–513.

43. Palay SL, Chan-Palay V: **Granule cells**. In *Cerebellar cortex*. Berlin, Heidelberg: Springer Berlin Heidelberg; 1974:63–99.

44. Houston CM, Diamanti E, Diamantaki M, Kutsarova E, Cook A, Sultan F, Brickley SG: **Exploring the significance of morphological diversity for cerebellar granule cell excitability**. *Sci Rep* 2017, **7**:46147.

45. Litwin-Kumar A, Harris KD, Axel R, Sompolinsky H, Abbott LF: **Optimal degrees of synaptic connectivity**. *Neuron* 2017, **93**: 1153–1164.e7.

46. ** Dhar M, Hantman AW, Nishiyama H: **Developmental pattern and structural factors of dendritic survival in cerebellar granule cells in vivo**. *Sci Rep* 2018, **8**:17561.
Detailed description of the maturation of dendrites of cerebellar granule neurons in mouse, imaged *in vivo* by 2P microscopy. Individual cells are imaged longitudinally over several days, revealing the steps of maturation and the relationship between claw formation and dendrite stability.

47. Espinosa JS, Wheeler DG, Tsien RW, Luo L: **Uncoupling dendrite growth and patterning: single-cell knockout analysis of NMDA receptor 2B**. *Neuron* 2009, **62**:205–217.

48. ** Nakazawa S, Mizuno H, Iwasato T: **Differential dynamics of cortical neuron dendritic trees revealed by long-term in vivo imaging in neonates**. *Nat Commun* 2018, **9**:3106.
Individual spiny stellate neurons in the barrel cortex of neonatal mice are imaged *in vivo* over several days. These data elucidate the input-dependent mechanisms that support the orientation of the dendrite of spiny stellate neurons toward the center of the barrel.

49. Gao FB, Brenman JE, Jan LY, Jan YN: **Genes regulating dendritic outgrowth, branching, and routing in Drosophila**. *Genes Dev* 1999, **13**:2549–2561.

50. Zipursky SL, Grueber WB: **The molecular basis of self-avoidance**. *Annu Rev Neurosci* 2013, **36**:547–568.

51. Baltruschat L, Tavosanis G, Cuntz H: **A developmental stretch-and-fill process that optimises dendritic wiring**. *BioRxiv* 2020.

52. ** Palavalli A, Tizón-Escamilla N, Rupprecht J-F, Lecuit T: **Deterministic and stochastic rules of branching govern dendrite morphogenesis of sensory neurons**. *Curr Biol* 2021, **31**: 459–472. e4.

In vivo imaging of the differentiation of c1da neuron dendrites in the *Drosophila* larva. Quantitative description of branchlet dynamics combined with computational models indicate the sequence of steps that define the comb-like structure of this arbor. See also 55.

53. ** Stürner T, Tatarnikova A, Mueller J, Schaffran B, Cuntz H, Zhang Y, Nemethova M, Bogdan S, Small V, Tavosanis G: **Transient localization of the Arp2/3 complex initiates neuronal dendrite branching in vivo**. *Development* 2019, **146**.

This study investigates the initial formation of a dendrite branchlet. It utilizes a powerful combination of genetics, time lapse imaging, *in vivo* detection of dynamic changes of protein localization and electron microscopy to propose that a cascade of localized reactions, starting with activation of the small GTPase Rac1 leads to formation of branched actin filaments and thus an actin patch that predicts the site at which a branchlet is just about to form. Light-induced activation of a photo-activatable Rac1 construct leads to the formation of supernumerary branchlets.

54. Nithianandam V, Chien C-T: **Actin blobs prefigure dendrite branching sites**. *J Cell Biol* 2018, **217**:3731–3746.

55. ** Ferreira Castro A, Baltruschat L, Stürner T, Bahrami A, Jedlicka P, Tavosanis G, Cuntz H: **Achieving functional neuronal dendrite structure through sequential stochastic growth and retraction**. *Elife* 2020, **9**.

Quantitative analysis of the elaboration of c1da neuron dendrite branching *in vivo*. The longitudinal imaging of neurons over development led to the formulation of a model in which dendrite branches are formed and removed stochastically in sequential steps, leading to a morphology that is functionally optimal.

56. * Stürner T, Castro AF, Philipps M, Cuntz H, Tavosanis G: **The branching code: a model of actin-driven dendrite arborisation**. *BioRxiv* 2020.

While overall dendrites of c3da neurons arborize following optimal wiring rules, their high-order branches behave differently. Computational analysis thus led to a two-step model of dendrite elaboration for these neurons. The dynamic properties of these special high-order branches, driven by actin, were defined by genetic analysis combined with time-lapse imaging.

57. * Yoong L-F, Lim H-K, Tran H, Lackner S, Zheng Z, Hong P, Moore AW: **Atypical myosin tunes dendrite arbor subdivision**. *Neuron* 2020, **106**:452–467.e8.

Investigates the role and local regulation of microtubule organization in defining the pattern of primary dendrite branching of sensory neurons of *Drosophila*. Concentrating on the regrowth of dendrites during pupal stages, this work displays impressive time-lapse *in vivo* imaging of dendrite elaboration.

58. Vaadia RD, Li W, Voleti V, Singhania A, Hillman EMC, Grueber WB: **Characterization of proprioceptive system dynamics in behaving Drosophila larvae using high-speed volumetric microscopy**. *Curr Biol* 2019, **29**:935–944.e4.

59. He L, Gulyanon S, Mihovilovic Skanata M, Karagyozov D, Heckscher ES, Krieg M, Tsechpenakis G, Gershow M, Tracey WD: **Direction selectivity in drosophila proprioceptors requires the mechanosensory channel tmc**. *Curr Biol* 2019, **29**:945–956.e3.

60. Satoh D, Suyama R, Kimura K, Uemura T: **High-resolution in vivo imaging of regenerating dendrites of Drosophila sensory neurons during metamorphosis: local filopodial degeneration and heterotypic dendrite-dendrite contacts**. *Gene Cell* 2012, **17**:939–951.

61. Williams DW, Truman JW: **Mechanisms of dendritic elaboration of sensory neurons in Drosophila: insights from in vivo time lapse**. *J Neurosci* 2004, **24**:1541–1550.

62. Sheng C, Javed U, Gibbs M, Long C, Yin J, Qin B, Yuan Q: **Experience-dependent structural plasticity targets dynamic filopodia in regulating dendrite maturation and synaptogenesis**. *Nat Commun* 2018, **9**:3362.

63. Kamiyama D, McGorty R, Kamiyama R, Kim MD, Chiba A, Huang B: **Specification of dendritogenesis site in Drosophila aCC motoneuron by membrane enrichment of Pak1 through Dscam1**. *Dev Cell* 2015, **35**:93–106.

64. Sundararajan L, Stern J, Miller DM: **Mechanisms that regulate morphogenesis of a highly branched neuron in C. elegans**. *Dev Biol* 2019, **451**:53–67.

65. Oren-Suissa M, Hall DH, Treinin M, Shemer G, Podbilewicz B: **The fusogen EFF-1 controls sculpting of mechanosensory dendrites**. *Science* 2010, **328**:1285–1288.

66. Smith CJ, Watson JD, Spencer WC, O'Brien T, Cha B, Albeg A, Treinin M, Miller DM: **Time-lapse imaging and cell-specific expression profiling reveal dynamic branching and molecular determinants of a multi-dendritic nociceptor in C. elegans**. *Dev Biol* 2010, **345**:18–33.

67. Corty MM, Matthews BJ, Grueber WB: **Molecules and mechanisms of dendrite development in Drosophila**. *Development* 2009, **136**:1049–1061.

68. Banani SF, Lee HO, Hyman AA, Rosen MK: **Biomolecular condensates: organizers of cellular biochemistry**. *Nat Rev Mol Cell Biol* 2017, **18**:285–298.

69. Schaks M, Giannone G, Rottner K: **Actin dynamics in cell migration**. *Essays Biochem* 2019, **63**:483–495.

70. Riedl J, Crevenna AH, Kessenbrock K, Yu JH, Neukirchen D, Bista M, Bradke F, Jenne D, Holak TA, Werb Z, *et al.*: **Lifeact: a versatile marker to visualize F-actin**. *Nat Methods* 2008, **5**:605–607.

71. Riedl J, Flynn KC, Raducanu A, Gärtner F, Beck G, Bösl M, Bradke F, Massberg S, Aszodi A, Sixt M, *et al.*: **Lifeact mice for studying F-actin dynamics**. *Nat Methods* 2010, **7**:168–169.

72. Dupraz S, Hilton BJ, Husch A, Santos TE, Coles CH, Stern S, Brakebusch C, Bradke F: **Rhoa controls axon extension independent of specification in the developing brain**. *Curr Biol* 2019, **29**:3874–3886.e9.

73. Krueger D, Quinkler T, Mortensen SA, Sachse C, De Renzis S: **Cross-linker-mediated regulation of actin network organization controls tissue morphogenesis**. *J Cell Biol* 2019, **218**:2743–2761.

74. Wegner W, Ilgen P, Gregor C, van Dort J, Mott AC, Steffens H, Willig KI: **In vivo mouse and live cell STED microscopy of neuronal actin plasticity using far-red emitting fluorescent proteins**. *Sci Rep* 2017, **7**:11781.

75. Willig KI, Steffens H, Gregor C, Herholt A, Rossner MJ, Hell SW: **Nanoscopy of filamentous actin in cortical dendrites of a living mouse**. *Biophys J* 2014, **106**:L01–L03.

76. Brüser L, Bogdan S: **Molecular control of actin dynamics in vivo: insights from Drosophila**. *Handb Exp Pharmacol* 2017, **235**:285–310.

77. Hudson AM, Cooley L: **Understanding the function of actin-binding proteins through genetic analysis of Drosophila oogenesis**. *Annu Rev Genet* 2002, **36**:455–488.

78. Lv Z, de-Carvalho J, Telley IA, Großhans J: **Cytoskeletal mechanics and dynamics in the Drosophila syncytial embryo**. *J Cell Sci* 2021, **134**.

79. Zou W, Dong X, Broederdorf TR, Shen A, Kramer DA, Shi R, Liang X, Miller DM, Xiang YK, Yasuda R, *et al.*: **A dendritic guidance receptor complex brings together distinct actin regulators to drive efficient F-actin assembly and branching**. *Dev Cell* 2018, **45**:362–375.e3.

80. Laviv T, Yasuda R: **Imaging neuronal protein signaling dynamics in vivo**. *Curr Opin Neurobiol* 2021, **69**:68–75.

81. Lin MZ, Schnitzer MJ: **Genetically encoded indicators of neuronal activity**. *Nat Neurosci* 2016, **19**:1142–1153.

82. Tapon N, Hall A: **Rho, Rac and Cdc42 GTPases regulate the organization of the actin cytoskeleton**. *Curr Opin Cell Biol* 1997, **9**:86–92.

83. Kamiyama D, Chiba A: **Endogenous activation patterns of Cdc42 GTPase within Drosophila embryos**. *Science* 2009, **324**:1338–1340.

84. Lee T, Winter C, Marticke SS, Lee A, Luo L: **Essential roles of Drosophila RhoA in the regulation of neuroblast proliferation**

and dendritic but not axonal morphogenesis. *Neuron* 2000, **25**:307–316.

85. Flynn KC, Hellal F, Neukirchen D, Jacob S, Tahirovic S, Dupraz S, Stern S, Garvalov BK, Gurniak C, Shaw AE, *et al.*: **ADF/cofilin-mediated actin retrograde flow directs neurite formation in the developing brain**. *Neuron* 2012, **76**: 1091–1107.

86. Rosário M, Schuster S, Jüttner R, Parthasarathy S, Tarabykin V, Birchmeier W: **Neocortical dendritic complexity is controlled during development by NOMA-GAP-dependent inhibition of Cdc42 and activation of cofilin**. *Genes Dev* 2012, **26**:1743–1757.

87. Krueger D, Izquierdo E, Viswanathan R, Hartmann J, Pallares Cartes C, De Renzis S: **Principles and applications of optogenetics in developmental biology**. *Development* 2019, **146**.

88. Johnson HE, Toettcher JE: **Illuminating developmental biology with cellular optogenetics**. *Curr Opin Biotechnol* 2018, **52**:42–48.

89. Ferguson SM, De Camilli P: **Dynamin, a membrane-remodelling GTPase**. *Nat Rev Mol Cell Biol* 2012, **13**:75–88.

90. Kitamoto T: **Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons**. *J Neurobiol* 2001, **47**:81–92.

91. Senior AW, Evans R, Jumper J, Kirkpatrick J, Sifre L, Green T, Qin C, Žídek A, Nelson AWR, Bridgland A, *et al.*: **Improved protein structure prediction using potentials from deep learning**. *Nature* 2020, **577**:706–710.

92. Zemelman BV, Lee GA, Ng M, Miesenböck G: **Selective photostimulation of genetically chARGed neurons**. *Neuron* 2002, **33**:15–22.

93. Wittmann T, Dema A, van Haren J: **Lights, cytoskeleton, action: optogenetic control of cell dynamics**. *Curr Opin Cell Biol* 2020, **66**:1–10.

94. Crosson S, Moffat K: **Structure of a flavin-binding plant photoreceptor domain: insights into light-mediated signal transduction**. *Proc Natl Acad Sci USA* 2001, **98**:2995–3000.

95. Zayner JP, Antoniou C, Sosnick TR: **The amino-terminal helix modulates light-activated conformational changes in AsLOV2**. *J Mol Biol* 2012, **419**:61–74.

96. Wu YI, Frey D, Lungu OI, Jaehrig A, Schlichting I, Kuhlman B, Hahn KM: **A genetically encoded photoactivatable Rac controls the motility of living cells**. *Nature* 2009, **461**:104–108.

97. Wang H, Vilela M, Winkler A, Tarnawski M, Schlichting I, Yumerefendi H, Kuhlman B, Liu R, Danuser G, Hahn KM: **LOVTRAP: an optogenetic system for photoinduced protein dissociation**. *Nat Methods* 2016, **13**:755–758.

98. Niopek D, Benzinger D, Roensch J, Draebing T, Wehler P, Eils R, Di Ventura B: **Engineering light-inducible nuclear localization signals for precise spatiotemporal control of protein dynamics in living cells**. *Nat Commun* 2014, **5**:4404.

99. Kennedy MJ, Hughes RM, Peteya LA, Schwartz JW, Ehlers MD, Tucker CL: **Rapid blue-light-mediated induction of protein interactions in living cells**. *Nat Methods* 2010, **7**:973–975.

100. Taslimi A, Vrana JD, Chen D, Borinskaya S, Mayer BJ, Kennedy MJ, Tucker CL: **An optimized optogenetic clustering tool for probing protein interaction and function**. *Nat Commun* 2014, **5**:4925.

101. Harterink M, van Bergeijk P, Allier C, de Haan B, van den Heuvel S, Hoogenraad CC, Kapitein LC: **Light-controlled intracellular transport in Caenorhabditis elegans**. *Curr Biol* 2016, **26**:R153–R154.

102. Benedetti L, Barentine AES, Messa M, Wheeler H, Bewersdorf J, De Camilli P: **Light-activated protein interaction with high spatial subcellular confinement**. *Proc Natl Acad Sci USA* 2018, **115**:E2238–E2245.