Minireview

Transcriptional control of dendritic patterning in *Drosophila* neurons

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Published: 28 July 2006

Genome ***Biology*** 2006, **7:**225 (doi:10.1186/gb-2006-7-7-225)

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2006/7/7/225

Abstract

How the morphology of individual neurons is controlled remains poorly understood. A recent *in vivo* genome-wide screen based on RNA interference identified a large number of transcriptional factors that regulate the stereotyped growth and branching of dendrites on some *Drosophila* sensory neurons.

Many neurons receive most of their input through thin processes, called dendrites, that form synapses with the axons of other neurons. Dendrites can be highly branched and have complex architecture. The shape and size of the dendrites of a single neuron determine the number and specificity of the axonal contacts it receives. Therefore, the development of the correct pattern of dendritic branching, or arborization, on different neurons is essential for the proper functioning of the nervous system. Neuronal subtypes in mammals and insects can have specific patterns of dendrite branching [1,2], and several unbiased genome-wide 'forward' genetic screens in *Drosophila* have identified factors that regulate dendritic morphogenesis in both the peripheral and central nervous systems [3-5]. But although these studies uncovered dozens of genes that might have essential roles in dendritic morphogenesis, most of the genes have yet to be cloned and characterized, and the information on genetic pathways has been sparse. Except for a few identified transcription factors that have been implicated in dendritic morphogenesis in flies and mammals [6-14], little is known about the overall transcriptional programs that specify the characteristic patterns of dendritic arborization in different neurons.

Over the past few years, however, large-scale 'reverse' genetic approaches such as RNA interference (RNAi) have emerged to help characterize molecular pathways. Screens based on RNAi have been used to analyze cellular processes in cultured insect cells [15,16] and in intact *Drosophila* embryos [17,18]. In a recent paper in *Genes and Development*, Parrish *et al*. [19] report the results of an *in vivo* RNAi-based screen set up to examine transcription factor networks that control several aspects of dendritic morphogenesis in *Drosophila*.

Identification of three functional groups of transcription factors

Parrish and colleagues [19] took advantage of the simple and stereotyped dendrite branching patterns of type I dendritic arborization (DA) neurons in the *Drosophila* peripheral nervous system. They prepared double-stranded RNAs (dsRNA) representing 730 known and putative transcription factors, injected them into early embryos, and screened for dendritic defects at the end of embryogenesis. To avoid false positives, only genes showing dendritic phenotypes as assessed in several blind tests were chosen for further analysis. Because the effectiveness of RNAi silencing could not be systematically quantified, RNAi phenotypes were evaluated qualitatively. The authors identified 76 genes for transcriptional regulators that could be divided into three functional groups (Figure 1). Group A comprises genes coding for

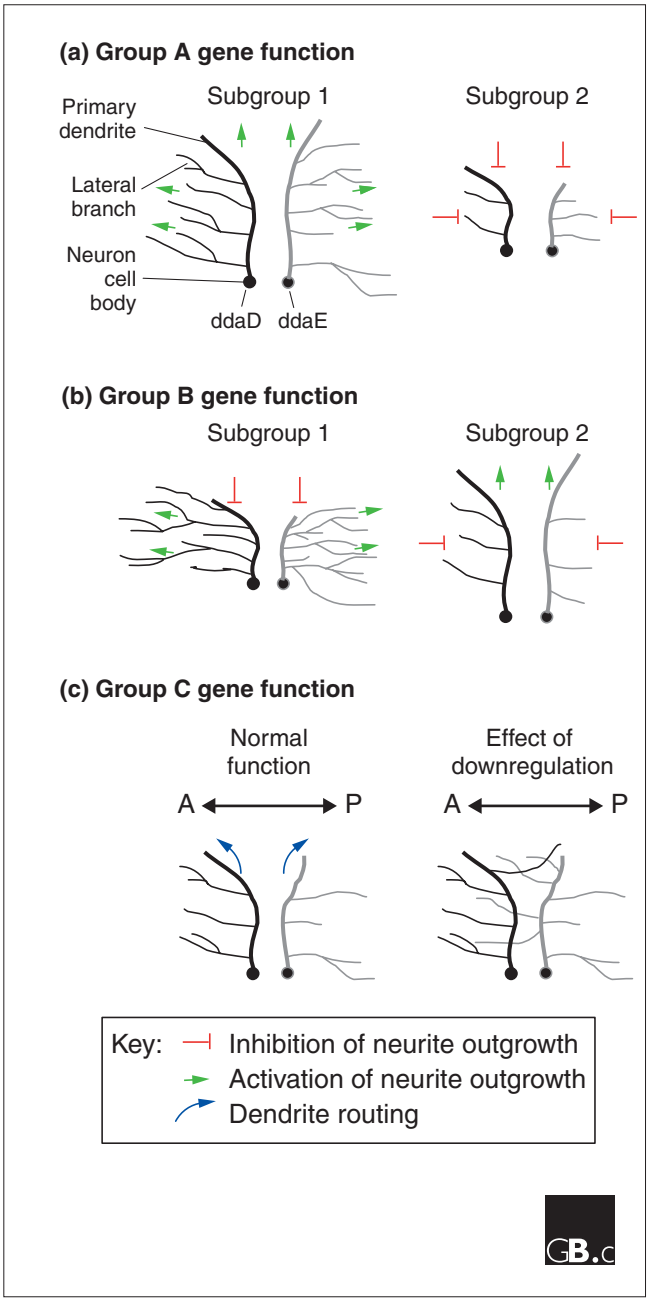

Figure 1
Schematic representation of the functions of each transcription factor group in dendritic patterning of type I DA neurons in the *Drosophila* peripheral nervous system. Subtypes of DA neurons have simple and stereotyped branching patterns (the black neuron represents the ddaD subtype and the gray neuron the ddaE subtype). **(a)** Group A genes fall into two different subgroups. One group (19 genes) promotes overall dendrite outgrowth (left), whereas the other (20 genes) inhibits overall outgrowth (right). **(b)** Group B also includes genes with opposing functions. Most of this group (19 genes) promote dendritic branching and inhibit primary dendrite extension (left). The other two members inhibit dendritic branching and promote primary dendrite extension (right). **(c)** Group C genes (10 genes) influence the routing, or direction of growth, of dendrites. The normal branching pattern is shown on the left. The abnormal routing of dendrite lateral branches observed when a group C gene is downregulated is shown on the right.

transcription factors that regulate dendrite outgrowth and branching, and thus the size and complexity of a neuron's dendritic 'field'. Group B includes genes with opposing effects on dendrite outgrowth and dendrite branching. Group C includes genes that specifically control the orientation of the developing dendrites of embryonic type I DA neurons.

The 39 genes in group A were further divided into two subgroups. In one subgroup were 19 genes whose silencing reduced the size of the dendritic field, indicating that these genes normally promote both dendrite outgrowth and branching. Although silencing of most of these genes affected dendritic branching and outgrowth equally, RNAi of the genes encoding the nuclear ecdysteroid hormone receptors Ultraspiracle (Usp) and EcR (the ecdysone receptor), appeared to affect mostly the extension of primary dendrites without notably altering the number and length of lateral branches. The second subgroup, of 20 genes, had opposing functions to those of the first group, in that their normal function appeared to be to limit both dendrite branching and outgrowth. This group included the gene *abrupt*, which restricts dendritic branching and outgrowth of type I DA neurons [12,13], as well as other transcriptional regulators with unknown function in neurons, such as *Elongin c*. Interestingly, the second subgroup included four genes of the same group of transcriptional regulators, the Polycomb group. The downregulation of the genes - *Su(z)12, E(z), esc* and *Caf1* - resulted in increased dendritic branching and an expanded dendritic field in type I DA neurons, suggesting that the Polycomb group plays an important role in the transcriptional repression of dendrite outgrowth and branching.

Of the 21 genes in group B, the normal function of 19 appeared to be to restrict the extension of the primary dendrite in favor of lateral branch formation and outgrowth (see Figure 1b). In contrast, the normal function of the two remaining genes, *glial cells missing 2* (*gcm2*) and the histone acetyltransferase gene *pcaf*, appeared to be to promote primary dendrite extension and restrict lateral branch formation, as their downregulation caused increased lateral branching and decreased primary dendrite extension. For both these genes, this phenotype was observed only in one of the two type I DA neurons in the dorsal cluster, the ddaE neurons. Whether the loss of function of *gcm2* and *pcaf* leads to the absence of ddaD neurons, the other type I of DA neurons in the dorsal cluster, or to their inability to extend any neurites (the growing ends of dendrites or axons) is not clear. Nevertheless, the existence of these two antagonistic transcriptional pathways suggests that the outgrowth of primary dendrites could inhibit the outgrowth of lateral branches, and *vice versa*, and that transcriptional regulation might be critical to the redistribution of the cellular machinery for neurite extension from the main dendrite to the lateral processes.

Finally, Parish *et al.* [19] identified ten transcription factor genes (group C) that regulate the orientation of the growing

dendrites. The ddaD and ddaE neurons normally extend the lateral branches of their dendrites towards the anterior and posterior boundaries, respectively, of an embryonic body segment. The silencing of the group C transcription factors disrupted this pattern: in ddaE neurons, for example, lateral branches even extended toward the anterior boundary of the segment. Laser ablation of all other DA neurons in the dorsal cluster did not affect the extension of lateral branches of ddaE neurons toward the posterior segment boundary [20], so it is possible that the orientation of these neurites is mainly driven by extracellular attractive cues, and that group C transcription factors regulate the expression of proteins responsible for receiving and integrating these signals.

Pathways that might control dendrite arborization

The formation of stereotyped dendritic arborization not only requires overall dendrite outgrowth but also the coordination of the extension of the primary dendrite with the formation of lateral branches and with the direction of growth. On the basis of RNAi phenotypes, Parrish and colleagues [19] classified 76 transcription factors into three functional groups that affect different aspects of dendrite arborization. But is there any unity of mechanism behind the functional unity of each group? The answer is likely to be yes. For instance, several group A genes, such as those for the Polycomb group proteins and the nucleosome-remodeling complex NURF, are associated with the regulation of Hox gene expression [21]. Hox proteins are key developmental transcription factors that control cell proliferation and differentiation. Moreover, RNAi of group A genes resulted in the highest rate of embryonic lethality, suggesting that these transcription factors control basic pathways responsible for general cell growth and survival. The vast majority of group B genes identified in this screen control the balance between primary dendrite outgrowth and lateral branching, and most of them participate in protein complexes that repress transcription. It is worth noting that *runx1*, the mammalian homolog of the group B gene *runt*, regulates the diversification of sensory neurons in mice [22]. Thus, Runx-family transcription factors might be involved in both cellular identity and morphogenesis in vertebrate and invertebrate peripheral nervous system neurons. Last but not least, three of the ten group C genes encode components of the Brahma protein complex, which regulates the expression of the signaling protein Decapentaplegic (Dpp) in wing imaginal discs [23], raising the possibility that some group C genes regulate dendritic routing through a common molecular pathway.

Temporal action and relationships between the different groups of transcription factors

Does this RNAi-based screen allow the characterization of all transcription factors involved in dendritic arborization among the 730 genes tested? The answer is probably no. Indeed, when the authors reproduced their screen with higher dsRNA concentrations, they identified three additional genes (*bonus*, *stat92e*, and *rpd3*) missed in their previous screen. This was presumably due to the difficulty of silencing genes that have a high maternal contribution in the embryo. And since the effectiveness of RNAi silencing could not be quantified, other transcription factors involved in dendritic morphogenesis might have been overlooked. Of the 76 candidate genes that Parrish *et al*. [19] identified in their screen, 32 have available mutant alleles and the authors have analyzed the morphology of type I DA neurons in these mutant flies. In most cases, the mutant alleles produced a phenocopy of the RNAi phenotype, validating the accuracy of the approach. Analysis of the mutant flies revealed that some of the candidate genes are also involved in later stages of dendritic morphogenesis when type I DA neurons do not extend new branches. Thus, some transcription factors seem to be required to continuously maintain the dendritic arborization and allow type I DA neurons to retain their capacity to form new dendrites.

Identification of a large number of transcription factors through reverse genetic screens offers an exciting opportunity to map the transcriptional network that controls dendritic morphogenesis. Parrish *et al*. [19] also explored the effect of simultaneously disrupting two group A genes with opposite effects on dendrite outgrowth. The loss of function of either of two genes normally required for dendrite extension was epistatic to the loss of *abrupt*, which normally antagonizes overall dendrite outgrowth. The different group A transcription factors are thus likely to positively or negatively regulate a common set of target genes responsible for overall dendrite extension. As outlined earlier, group B genes can act as transcriptional switches between primary dendrite outgrowth and lateral branch extension, and this raises the question of the epistatic relationship between these transcription factors and the group A genes that control overall extension of the dendritic tree. In a further experiment, the RNAi phenotype of four group A genes appeared to override the mutant phenotype of the gene *senseless*, which functionally belongs to both group B and group C. The group A genes targeted here either promoted or limited dendrite extension, suggesting that the loss of regulation of overall dendrite outgrowth is epistatic to the loss of the correct balance between primary dendrite extension and the extension of lateral branches.

To sum up, the RNAi-based screen carried out by Parrish *et al*. [19] identified an extensive list of transcription factors that regulate dendrite growth and the pattern of dendrite arborization of type I DA neurons. Overlapping but distinct sets of transcription factors may be required for dendritic morphogenesis in other types of DA neurons in *Drosophila*. As the dsRNAs were injected into early-stage embryos, which are still in the syncytial stage, the cell-autonomous function of these genes in either precursor cells or postmitotic neurons

needs to be further assessed for a better understanding of the mechanism of action of the transcription factors they encode. Nevertheless, this comprehensive study offers a powerful entry point to the task of dissecting the transcriptional networks in postmitotic neurons and precursors that are ultimately responsible for the morphology of each subtype of DA neurons. Finally, most of the genes identified in this screen have homologs in mammals. A recent screen based on *in situ* hybridization characterized 349 transcription factors with expression patterns restricted to different anatomical regions of the mouse brain [24]. It will be interesting to examine more closely all the transcription factors identified in both screens with the aim of understanding the transcriptional programs that regulate the dendrite morphology of specific neuronal subtypes in mammals.

Acknowledgements

We thank S. Ordway and G. Howard for editorial assistance, and lab members for comments. This work is supported by the NIH (F.-B.G.).

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