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Intrinsic programs regulating dendrites and synapses in the upper layer neurons of the cortex

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Dendrites and spines are key regulators of neuronal function often affected in cognitive disorders. Neuronal subclasses are characterized by a wide range of dendritic morphologies that aid their specific functions. However, how subclass-specific dendritic trees arise during vertebrate development remains largely unknown. We have recently reported that the restricted expression of *Cux1* and *Cux2* genes in the upper layers of the cerebral cortex determines the specific morphology of dendrites and spines and the function of these neurons. Since *Cux* genes are the vertebrate homologs of Drosophila *Cut*, which specifies the dendritic morphologies of certain sensory neuron populations, our findings suggest that mechanisms of dendrite differentiation are conserved between Drosophila and mammals, which had yet to be demonstrated. Importantly, we found that *Cux* genes not only modulate dendritic branching, but also dendritic spine morphogenesis, the functional synapse and cognition. Dendritic spine stabilization was partly mediated by direct repression of genes of the *Xlr* family, previously implicated in cognitive defects in a model of Turner syndrome. Hence, our work indicates that neuronal subclass specific determinants may intrinsically affect synaptic activity beyond expected. The functions of *Cux1* and *Cux2* were additive and complement each other to establish the final pattern of the dendritic tree and the number and strength of the synapses. This work unravels novel mechanisms of dendritogenesis and synaptogenesis and illustrates how regulating dendritic structures contributes to the specialization of upper layer neurons. It will be interesting to dissect how these mechanisms regulate cortical activity, area specialization and cognitive functions.

Dendritic branches and spines are key regulators of neuronal function. Number, growth and orientation of dendritic branches modify the way information is integrated and select axonal targets.[1,2] Dendritic spines are specialized structures that modulate the activity, strength and stability of the synapse.[3-5] Both structures are often altered in number and morphology in mental disorders.[6-8] From the times of Cajal we learned to identify the myriad of different types of neurons by their stereotyped dendritic morphologies, and we now begin to understand how these dendritic patterns serve their specialized functions.[1] However, how subclass-specific neuronal features are defined during development is largely unknown. Based in studies on Drosophila, dendritic architecture is thought to be instructed by the selective expression of transcription factors (TFs), but few of such TFs have been described in vertebrates.[2,9] Moreover, the possibility that these subclass specific intrinsic factors may affect the formation of dendritic spines and synapses has not been proposed or explored.

Cux1 and *Cux2* are two transcription factors selectively expressed in the pyramidal neurons of the upper layers (II, III and IV) of the mouse cortex.[10,11] These neurons have elaborated dendritic morphologies and profusion of spines[12] that allow them to integrate intracortical circuits involved in the higher cognitive tasks of the brain. They might be considered as a late evolutive addition, since they appear in mammals and are highly represented in the primate cerebral cortex, especially in humans.[13] We have recently found that *Cux* genes exert an intrinsic control of the dendritic structures of the upper layer neurons of the mouse cerebral cortex (**Fig. 1**).[15] Our knockout and knockdown studies demonstrate that the homeobox *Cux1* and *Cux2* are early regulators of dendrite branching in a cell autonomous manner. *Cux* genes are the vertebrate homologs of Drosophila homeobox *Cut*, which specifies the dendritic morphology of certain sensory neurons.[14] Our findings support the existence of conserved mechanisms of dendritic differentiation between flies and mammals. They may also imply that the activity of Drosophila *Cut* in specifying simpler neuronal types might have been co-opted during cortical evolution to generate the more complex neurons of mammals. But perhaps more unexpected, we found that *Cux1* and *Cux2* instruct also genetic programs that control the number and morphology of the dendritic spines. In the absence of *Cux* genes, the dendritic spines adopt a more immature morphology, with longer necks and smaller heads. Correspondingly, electrophysiological recordings show reduced number and strength of the synapses. A few other TFs, such as MEF2, have been previously implicated in activity dependent spine formation and synaptogenesis,[8,16,17] but these mechanisms apply to most neuronal populations. The implication of *Cux1* and *Cux2* on neuronal plasticity and in normal brain function remains to be understood. Why is it important to selectively restrict or promote synapse formation by intrinsic factors? Do presynaptic axons need this constrains to define their connectivity?

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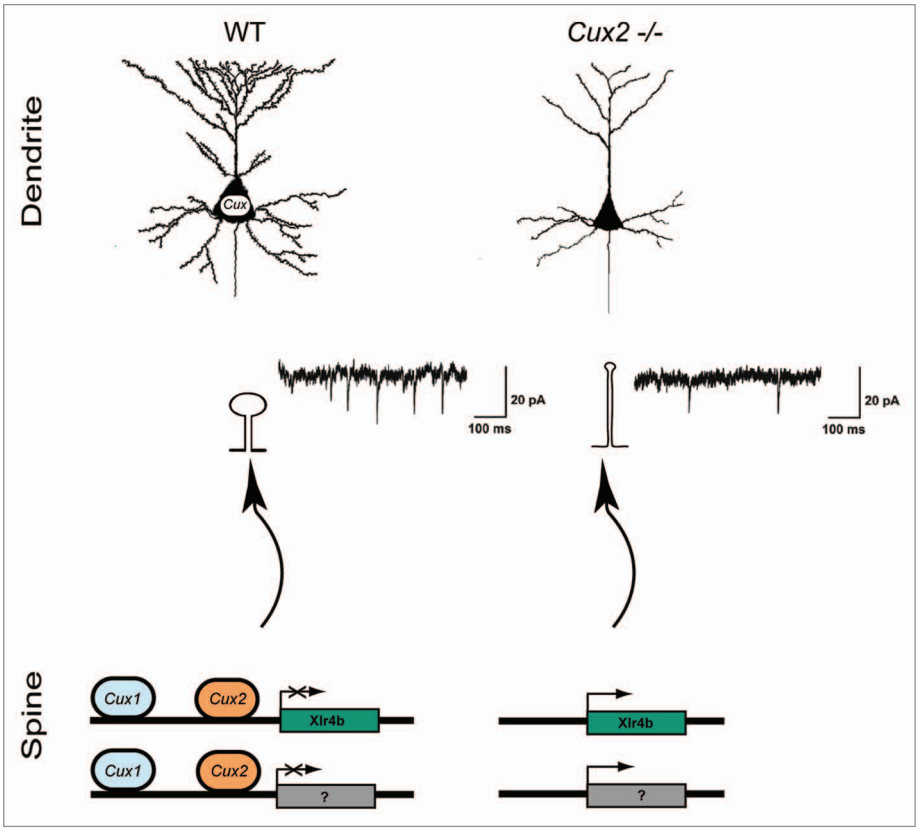

Figure 1. *Cux* genes control dendrite branching and synaptogenesis. *Cux1* and *Cux2* regulate neuronal differentiation and control intrinsic mechanisms of dendrite development, spine formation and synaptic function in upper layers in the cortex. Upper part: Dendritic parterns in WT and *Cux2*$^{-/-}$ pyramidal neurons of the upper layers. Lower parts: Downregulation of *Xlr3b* and *Xlr4b* gene expression by Cux proteins contributes to dendritic spine differentiation. Cux1 and Cux2 bind and regulate different regions in the *Xlr4b* locus. Miniature excitatory postsynaptic current (mEPSCs) from layer II and III pyramidal cells of *Cux2*$^{-/-}$ mice were reduced in amplitude and frequency.

Another interesting point is the additive and complementary functions of *Cux1* and *Cux2*. *Cux1* and *Cux2* label most neurons of the superficial layers and display overlapping patterns of expression in several areas of the cortex. This indicates that they are likely co-expressed in many upper layer neurons, as we formally corroborated for the neurons of the somatosensory cortex. Initially, this suggested us that *Cux1* and *Cux2* might have redundant functions.[10,18] However, we found that *Cux1* and *Cux2* are complementary but not redundant. Upper layer neurons of the somatosensory cortex of both *Cux1* and *Cux2* single mutants show similar reduction in dendritic complexity and comparable defects in dendritic spine numbers and morphologies. Double loss of *Cux1* and *Cux2* expression induced more dramatic defects in dendrites and spines. Ectopic expression of *Cux1* in cingulated neurons, that normally express *Cux2* but lower levels of *Cux1*, increased branching and reproduced the more complex dendritic morphologies of the somatosesory areas. Therefore, we concluded that the functions of *Cux* genes add to each other to stimulate branching and that it is the combinatorial expression of *Cux1* and *Cux2* that defines the final dendritic pattern.

Out of these experiments, it should be highlighted that overexpression of *Cux1* also incremented dendritic spine density and induced more mature dendritic spine phenotypes (**Fig. 2**). Thus, the additive functions were revealed also for the intrinsic control of the spine. Altogether, these suggest that discrete differences in the levels of expression of *Cux1* and *Cux2* may modulate dendritic and spine morphogenesis in a dose-dependent manner in subsets of superficial neurons or regionally, in cortical areas. This would refine their functions and establish a fine tuning of their connectivity.

In search for the downstream elements by which *Cux* genes exert their functions we found mechanisms of synaptogenesis key to cognition, including the regulation of NMDAR2B and PSD95.[8,19-21] Directly downstream of Cux, we found the chromatin remodeling genes of the *Xlr* family (**Fig. 2**). These genes were initially identified as upregulated in the *Cux2*$^{-/-}$ cortex in a screen of genes differentially expressed. Previous report showed that increased level of *Xlr3b* and *Xlr4b* expression correlated with more acute behavioral inflexibility in a mouse model of Turner syndrome.[22,23] Nothing was known about the functions of *Xlr* genes, but dendrite and spine defects associate to mental retardation and therefore, it seemed possible that these genes were involved in the changes in dendritic structures in the absence of *Cux*. Further research identified *Cux* binding sites in the *Xlr3b* and *Xlr4b* locus and proved that Cux proteins bind to these sites in vivo and repress *Xlr* expression. The functional demonstration of the direct implication of *Xlr* genes in the control of the synapse was provided by experiments in which RNAs of interference targeting *Xlr* rescued normal spine density and reduced the proportion of long spines upon *Cux* loss of function. Dendritic tree was not affected, proving to be independently regulated. Interestingly, Cux1 and Cux2 proteins selectively bound and repressed distinct regulatory regions on *Xlr3b* and *Xlr4b* loci, illustrating the mechanisms conveying the additive functions (**Fig. 1**, lower parts).

The expression of *Cux2* selectively defines the upper layer of the human cortex.[24] We identified *FAM9A, B* and *C*[25] as the closest orthologos of *Xlr* genes in human and found sequences containing *Cux* binding sites in *FAM9A, B* and *C* loci that are conserved between primates and humans. In vitro ChIP experiments in human cell lines demonstrated binding of Cux1 and Cux2 proteins to these regions, indicating that it is possible that similar *Cux* mediated synaptic mechanisms act in humans.

The functions of *Cux* in spine morphogenesis highlight the existence of neuronal subclass specific mechanisms of synaptogenesis that contribute to the establishment of cognitive circuits. Accordingly, we found defects in working memory in *Cux2*$^{-/-}$. Work lies ahead to further investigate these intrinsic mechanisms

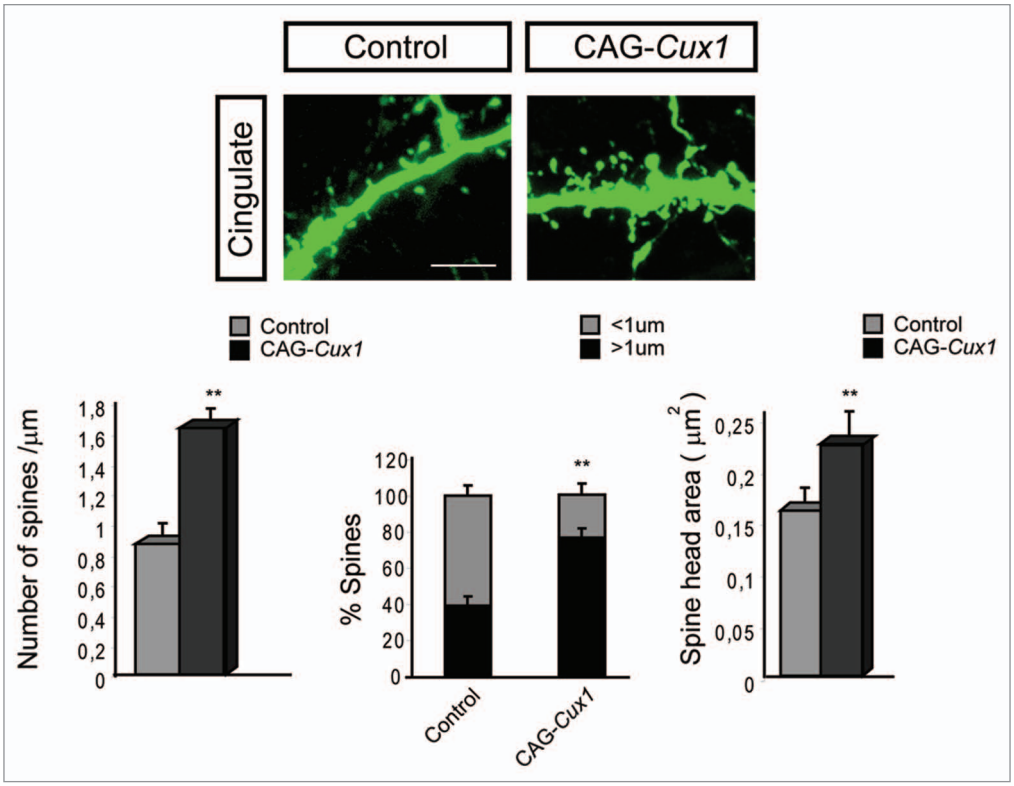

Figure 2. Dendritic spine formation in neurons of the cingulate cortex is stimulated upon *Cux1* overexpression. Upper parts show representative confocal image of GFP positive spines in the cingulate cortex. These neurons had been electroporated with control or *CAG-Cux1* plasmid. Scale bar represents 5 um. Lower parts show quantification of dendritic spine number, spine morphology and spine head area. Data in bar graphs depict mean ± SD. *p < 0.005, **p < 0.001, compared with control. This figure is a modification of Cubelos et al.[15]

of synapse regulation. It will likely reveal genes and proteins affected in cognitive disorders and neurodegeneration. In addition, it will be a challenge to investigate how the selective control of dendritic structures and spines by *Cux1* and *Cux2* contribute to laminar, columnar and area connectivity, and ultimately to the establishment of the intellectual capabilities that rest in the cortex.

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