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, which specifies the dendritic morphologies of certain sensory neurons. 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, 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 constraints to define their connectivity?
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. 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 somatosensory 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 over-expression of Cux1 also incremented dendritic spine density and repressed 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. We identified EAM9A, B and C as the closest orthologs of Xlr genes in human and found sequences containing Cux binding sites in EAM9A, 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-/- mice. Work lies ahead to further investigate these intrinsic mechanisms 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. 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. 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
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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 μm. 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
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