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The nuclear receptor COUP-TFI represses differentiation of Cajal-Retzius cells

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Abstract

The cellular diversity of neurons located in the marginal zone (MZ) of the cortex has a crucial role in cortical development. However, little is known about the molecular mechanisms involved in how these different neuronal cell types are specified. Here, we show that in the MZ, the nuclear receptor COUP-TFI is localized in calbindin-positive cells and not in reelin-positive cells. High expression of *COUP-TFI* has been detected in preplate (PP) and subplate (SP) cells, suggesting that this nuclear receptor is down-regulated during preplate differentiation towards the Cajal-Retzius (CR) cell lineage. By maintaining high ectopic expression of *COUP-TFI* in preplate cells, we show that *COUP-TFI* represses the CR cell markers reelin and calretinin, and with lower efficiency the transcription factor Tbr1. Furthermore, general differentiation is not affected, strongly suggesting that COUP-TFI represses differentiation of CR cells.

Keywords: Cerebral cortex; Electroporation; Development; Reelin; Negative regulation

1. Introduction

During corticogenesis, the cells of the neuroepithelium, the ventricular zone (VZ) lining the caudodorsal parts of the telencephalic ventricles, produce diverse neuronal phenotypes from a morphologically homogeneous pool of precursor cells. After completing their last mitosis, the cells exit the cell cycle, leave the ventricular zone and develop into particular neuronal cell types. Lateral inhibition mediated by the Delta-Notch signalling pathway apparently regulates the numbers of neurons produced [7], while a subset of proneural genes regulates the selection of neuronal versus glial fate [5,22]. However, little is known regarding the determining factors for the different neuronal cell types that are responsible for the laminar organization of the cerebral cortex.

The earliest generated neurons in the developing mammalian cortex migrate towards the pial surface and form the preplate layer (PPL), a transient layer of differentiated neurons that lies superficial to the proliferative cells of the ventricular zone. The PPL neurons are distinct from the cortical plate (CP) neurons, and they represent a primitive cortical organization that is common to amphibians, reptiles and mammals. It is widely accepted that the derivatives of the PPL that settle in the marginal zone (MZ) are Cajal-Retzius (CR) cells, which represent the main neuronal population in the MZ. CR cells express reelin, a secreted glycoprotein that regulates the laminar organization of the cortex and that plays a pivotal role in cortical development. Disruption of any component of the *reelin* signalling pathway [30] produces an inverted laminar order and no splitting of the PPL. In addition, disruption of the reelin product or of any regulatory gene involved in the differentiation of CR cells, results in a neocortical migratory phenotype [11]. For example, *Tbr1*-deficient mice have decreased levels of reelin, which results in defective PPL splitting and an early reeler-like cortical migration disorder [15]. Moreover, in *Emx2* mutant mice, *reelin* signalling is impaired when the cortical plate is generated and the late phases of radial migration are defective in a *reeler*-like way [18]. Thus, accurate differentiation of CR cells at

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the PPL stage is a prerequisite for the correct organization of the cortical laminar structure and its connections. However, little is known about the molecular mechanisms of CR-cell specification.

Previous reports have shown that the orphan members of the COUP-TFI and COUP-TFII nuclear receptor superfamily and their orthologues in *Drosophila* are implicated in neurogenesis, cell differentiation and cell-fate determination [24]. The COUP-TFs were generally considered to be repressors of transcription; however, there is growing evidence that the COUP-TFs can also function as transcriptional activators [23]. In cell lines induced to differentiate with retinoic acid, *COUP-TFI* over-expression results in the blocking of morphological differentiation after induction to differentiate [1,21], suggesting that COUP-TFI is involved in regulating the differentiation of specific subpopulations of neuronal cell types. Indeed, in the absence of *COUP-TFI*, subplate (SP) cells and layer-IV neurons fail to differentiate properly, and instead undergo excessive cell death [32].

Here, we show that COUP-TFI is expressed in PPL cells, and after the cortical plate cells have begun to be positioned, in SP cells. However, the lack of expression of COUP-TFI in CR cells suggests that it is down-regulated in the MZ during the process of PPL cell differentiation. To determine the functional role of COUP-TFI in the differentiation process of PPL cells, we have ectopically-expressed *COUP-TFI* in the outermost layers of the E12.5 cortex in organotypic cultures, with the aim of maintaining the expression of COUP-TFI during PPL differentiation. After 48 h of incubation, we find that CR markers, such as reelin, calretinin and Tbr1 are down-regulated. Therefore, these data suggest that down-regulation of COUP-TFI is required for the correct specification of CR cells in the MZ layer during the process of PPL differentiation.

2. Materials and methods

2.1. Expression vector, brain slices and electroporation

The complete coding sequence of the mouse *COUP-TFI* gene (a kind gift of M.J. Tsai, Baylor College, USA) was cloned into the *Eco*RI/*Xho*I sites of the plasmid pCAGGS/ES, a chicken β-actin-promoter-driven expression vector [28]. Green fluorescence protein (GFP) was subcloned into the same expression vector and used as a control vector, or it was electroporated together with the *COUP-TFI*-expression vector. Both of the vectors were electroporated at a final concentration of 2 mg/ml into the outermost layers of the E12.5 brain layer, using the same methodology and conditions as given by Stuhmer et al. [28]. When the GFP-expressing vector is co-electroporating alongside, an equivalent vector carrying the DNA of interest, virtually all of the GFP-expressing cells (>90%) co-label with the other vector [28; Studer et al., unpublished data]. Preparation and maintenance of the brain slice tissue cultures were according to Anderson et al. [2]. After electroporation, coronal slices of rostral and caudal cortices were returned to culture for 24–48 h, and only those slices with a strong expression in the most marginal zone of the brain (monitored by GFP fluorescence) were subsequently processed for marker analysis.

2.2. Fixation and re-sectioning of slices

After incubations, the brain slices were fixed in 4% paraformaldehyde in PBS, washed in PBS, and then embedded in Tissue-Tek for further sectioning with a cryostat. The sections were subjected to standard in situ hybridization and/or immunohistochemistry procedures.

2.3. Immunohistochemistry and antibodies

Immunohistochemistry was performed as described previously [2]. The generation of a rabbit COUP-TFI polyclonal antibody has been described elsewhere (Tripodi et al., [31]). The G10 mouse monoclonal anti-reelin antibody was a kind gift of A. Goffinet (University of Louvain Medical School, Brussels, Belgium; 1:1000), the rabbit polyclonal anti-calretinin and anti-calbindin antibodies were from Swant (Bellinzona, Switzerland; 1:2000 and 1:15,000, respectively), the anti-Tbr1 antibody was a kind gift of R. Hevner (University of Washington, Seattle, USA; 1:200), the SDL.3D10 mouse monoclonal anti-βIII-tubulin antibody was from Sigma–Aldrich (St. Louis, MO, USA; 1:400), the AP20 mouse monoclonal anti-MAP2 antibody was from Boehringer (Ingelheim, Germany; 1:500), and the CS-56 mouse monoclonal anti-chondroitin sulphate proteoglycan (CSPG) antibody was from Sigma (1:200). The Alexa series of the fluorescent secondary antibodies were from Molecular Probes (Eugene, OR, USA).

3. Results

3.1. COUP-TFI expression in the marginal zone does not include the Cajal-Retzius cells

To follow the distribution of COUP-TFI during cortical differentiation, we generated a polyclonal antibody against the N-terminal of the protein, and then analysed its cellular localization in the E12.5–E15.5 layers of the mouse cortex. As has already been described, *COUP-TFI* is expressed in the proliferative zone in a rostral-to-caudal and a ventral-to-dorsal gradient, with a higher expression caudo-laterally and a lower expression rostro-medially (data not shown and [10,17,33]). To determine the type of cortical cells that express the COUP-TFI protein during cortical differentiation, we used double immunohistochemistry for COUP-TFI and for markers of specific cell types. Consistent with its role as a transcription factor, COUP-TFI was detected in the cell nuclei (Fig. 1). At E12.5, COUP-TFI was localized in the