News Release

## Title

Cell cycle–independent transitions in temporal identity of mammalian neural progenitor cells

## Key Points

- ✓ Single cell transcriptome analysis identifies the 'temporal-axis genes', whose expression changes over time but is independent of differentiation status of the progenitor cells in mammalian cerebral development.
- ✓ Cell cycle progression is not necessary for transitions in temporal gene expression and the laminar fate of neural progenitor cells (apical progenitor cells, APs).
- ✓ The results suggest the existence of a cell-autonomous 'timer' in the APs, which is independent of the cell cycle and responsive to environmental cues.

## Summary

Associate Professor Ayano Kawaguchi, Professor Takaki Miyata in Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine (Dean: Masahide Takahashi, M.D., Ph.D.), Dr. Fumio Matsuzaki in RIKEN CDB and their collaborators experimentally demonstrated that progenitor temporal identity arises independent of cell-cycle progression and Notch activation in mammalian cerebral development.

During cerebral development, many types of neurons are sequentially generated by self-renewing neural progenitor cells called apical progenitors (APs). Temporal changes in AP identity are thought to be responsible for neuronal diversity; however, the mechanisms underlying such changes remain largely unknown. The authors performed single cell transcriptome analysis of individual progenitors at different developmental stages, and identified a subset of genes whose expression changes over time but is independent of differentiation status. Surprisingly, the pattern of changes in the expression of such temporal-axis genes in APs was unaffected by cell-cycle arrest. Consistent with this, transient cell-cycle arrest of APs *in vivo* did not prevent descendant neurons from acquiring their correct laminar fates. Analysis of cultured APs revealed that transitions in AP gene expression are driven by both cell-intrinsic and -extrinsic mechanisms. These results suggest that the timing mechanisms controlling AP temporal identity function independently of cell-cycle progression and Notch activation mode.

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## Research Background

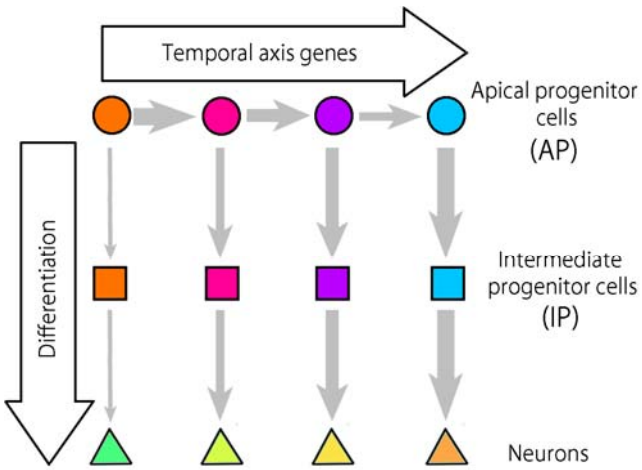
The functional organization of the brain requires the ordered generation of large numbers of diverse neurons and glia during development. The size and diversity of neural cell populations rely on the spatial and temporal diversity of progenitor cells. In mammalian cerebral cortex, self-renewing progenitor cells are formed by elongation of neuroepithelial cells, and repeated

divisions at the apical surface of the ventricular zone (VZ) generate a stratified neuronal organization (these cells are thus termed apical progenitors [APs] or radial glial cells). Over time, these neural progenitor cells undergo temporal progression with respect to two properties. The first is the decision whether divisions are purely proliferative (expansive) or not. APs initially undergo proliferative divisions that generate two APs, and subsequently shift into a differentiating mode in which divisions give rise to non-AP cells, such as neurons or lineage-restricted intermediate progenitors (IPs). In the second, APs progressively change the fates of their differentiating progeny; deep-layer neurons → upper-layer neurons → glia. The mechanisms underlying temporal patterns in neural progenitors are less well understood than those involved in the spatial patterning of these cells.

## Research Results

### (1) Single cell gene-expression profiles of APs and identification of 'temporal-axis' genes

The authors compared global transcriptome profiles of single cells collected from murine dorsal cortices at three different developmental stages by microarrays analysis of cDNAs from those single cells. Based on these profiles, the cells were retrospectively categorized into neurons or progenitor populations, AP or IPs. Using statistical analysis of the APs' and IPs' profiles, the authors resolved the gene expression changes into two components, the temporal and differentiation axes. Of note, these two axes are orthogonal to each other, implying the underlying biological phenomena are separable. They identified genes that contributed highly to the temporal axis, either negatively or positively, as the 'temporal-axis genes' (Figure).




### (2) Cell-cycle arrest does not prevent AP transitions

They next simultaneously overexpressed the the Cdk inhibitor p18 and the intracellular domain of Notch1 (NICD) into embryonic cerebral wall to arrest the cell cycle of APs, and investigated the effects on temporal gene expression of APs by focusing on the 18 temporal-axis genes. The results indicated that cell-cycle progression is not necessary for the transition of temporal identity genes in APs between embryonic day (E) 10/11 and E14.

**(3) Effect of transient cell-cycle arrest on laminar fate**

The authors further investigated whether cell-cycle arrest would affect the temporal laminar fate transition in APs in our experimental system. For temporary expression of both NICD and p18 in APs, the activation and termination of the two genes were controlled by a double *in vivo* electroporation method based on the Cre-recombinase-loxP system. They revealed that the transiently cell-cycle arrested cells become the cells positioned in the upper layers and positive for upper-layer marker. These results suggest that transient cell-cycle arrest *in vivo* does not interfere with the laminar fate transition of APs from deep layers to upper layers during the neurogenic phase.

**(4) Transition of temporal-axis genes in clonally maintained APs**

Finally, the authors investigated to what extent the temporal change in AP gene expression was controlled in a cell-autonomous manner. NICD/p18 co-expressing APs can maintain the one-cell state *in vitro*, thus enabling a stringent examination of cell-autonomous effects.

The results indicated that the changes in gene expression in NICD/p18 co-expressing AP clones were more limited than those observed in neurosphere-derived APs. Surprisingly, even in NICD/p18 co-expressing clonal APs, several genes exhibited temporal expression patterns similar to those seen *in vivo*. These findings lead them to propose a model in which the temporal change in APs is partly mediated by a completely cell-intrinsic mechanism, and that extrinsic cues tune the cell-autonomous change in the APs.

## Research Summary and Future Perspective

Cell cycle progression is not necessary for transitions in temporal gene expression and the laminar fate of APs. These findings, together with the results from *in vitro* culture of single APs, suggest the existence of a cell-autonomous ‘timer’ in the APs, which is independent of the cell cycle and responsive to environmental cues.

The microarray data have been deposited in the GEO database under accession codes: GSE10881 and GSE55981, which provide a solid basis for further analyses to understand the mechanism in which specific types of cells are generated from the neural progenitor cells.

## Publication

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## Japanese ver.

http://www.med.nagoya-u.ac.jp/medical/dbps_data/_material_/nu_medical/_res/topix/2016/cycle_20160421jp.pdf