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Lineage progression from stem cells to new neurons in the adult brain ventricular-subventricular zone

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It has been estimated that ~10,000 new neurons are generated daily in the mouse ventricular-subventricular zone (V-SVZ),¹ an extensive germinal niche present in the walls of the lateral brain ventricles in many adult mammals. Neural stem cells, a subpopulation of GFAP-expressing astrocytes (B1 cells),² contact the ventricle with a small apical process surrounded by ependymal cells forming pinwheel-like structures.¹ B1 cells generate intermediate progenitors (C cells), which differentiate into neuroblasts (A cells).² A cells also divide and migrate out of the V-SVZ along the rostral migratory stream into the olfactory bulb, where they differentiate into local interneurons.^{3,4} A full understanding of this process requires precise information of the dynamics of proliferation of V-SVZ progenitor populations and how many times each divides. This information is now available for the adult mouse brain.⁵

The reporter protein in hGFAP::GFP mice was used to identify B1 cells and immunolabeling for Ascl1, and DCX was used to identify C and A cells, respectively. Of the Ascl1 + cells, only 3.1+/-0.6% were also GFP+, while 15.9+/-1.6% were DCX+, indicating a small overlap between populations, possibly associated to transitional stages between cell types. We used whole-mount preparations, which provide an en face view of the lateral ventricular surface, to analyze the entire neurogenic niche, minimizing the effect of rostral migration of proliferating progenitors on our quantifications. Using a combination of thymidine analogs (CldU and EdU) and

three protocols (CL, cumulative labeling; DA, double analogs and PLM, percentage of labeled mitosis methods), we calculated the growth fraction (GF) and the shortest and longest cell cycle (T_C) for each cell population. We also estimated the length of the different phase of the cell cycle: S (T_S), mitosis (T_M), G_2 (T_{G2}), and G_1 (T_{G1}) for B1, C and A cells. These three methods were used to minimize effects of caveats associated to each one; e.g., CL and PLM estimates may be influenced by cell migration and differentiation.

PLM relies on mitotic cells which are a small subset of the proliferating population and may be too small for reliable quantifications of populations with a low GF such as B1 cells. Importantly, we also found that EdU was toxic as labeled cells attempted a second division. EdU was therefore only used for experiments shorter than T_C .

Interestingly, we found that as many as 8.6% of B1 cells proliferate with a relatively short T_C (<< 17 h) and a short T_S (<< 4.5 h) compared to intermediate progenitors (see below). Given the average number of B1 cells in one mouse (6,200+/-200), we can extrapolate that = 700 B1 cells proliferate daily. This suggests that a sizable fraction of the primary progenitors or stem cells are dividing at any one time, and they do so quite rapidly.

We observed that the majority of the intermediate precursors or transit-amplifying C cells are actively cycling (87%). They have a T_C that varies between 18 and 25 h with a surprisingly long T_S of 12–17 h and a short T_{G1} (2 h). From the

quantification of Ascl1+ CldU+ cells at different survival times and their ratio of mitotic cells, we estimated that C cells divide on average three times before differentiating. The population of Ascl1+ cells that we observed (~8,000 cells) is composed of C cells generated by the division of a B1 cell and the ones generated by the three subsequent divisions of these intermediate progenitors.

Neuroblasts account for almost half of all actively dividing cells in the V-SVZ. About 55% of A cells divide at least once in the V-SVZ ($T_C = 18$ h, $T_S = 9$ h, $T_{G1} = 4$ h) resulting in the << 10,000 new neuroblasts generated daily.

It took 3–4 d for the generation of A cells from B1 cells. Interestingly, B1 cells had a relatively short S phase and long G_1 phase, similar to the cell cycle dynamics of radial glial during embryonic development.⁷ Another important finding is the observation of heterogeneity in cell cycle dynamics of C cells. This heterogeneity was not related to changes with the circadian rhythm or the localization of C cells within different subregions of the V-SVZ. It may be due to differences in cell cycle dynamics depending on whether they are generated from a B1 cell or from other C cells. Consistently, an elongation of the cell cycle as intermediate progenitors progress through the lineage in vitro has been reported.⁸

Our study provides the first overall estimation of cell cycle times of different neural progenitors and the dynamics of progression from stem cells to neuroblasts. This is fundamental new information to understand the mechanisms by which the

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V-SVZ continues to supply the olfactory bulb with thousands of new neurons every day. This knowledge, in turn, would be important to the overall understanding of how new neurons are produced in an adult brain.

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