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Neural stem cell pools in the vertebrate adult brain: Homeostasis from cell-autonomous decisions or community rules?

Nicolas Dray[1] | Emmanuel Than-Trong[1,2] | Laure Bally-Cuif[1]

[1] Zebrafish Neurogenetics Unit, CNRS, Team supported by the Ligue Nationale Contre le Cancer, Institut Pasteur, UMR3738, Paris, France

[2] Ecole doctorale Biosigne, Le Kremlin Bicêtre, Université Paris-Saclay, France

Correspondence
Laure Bally-Cuif, Zebrafish Neurogenetics Unit, Institut Pasteur, UMR3738, CNRS, Team supported by the Ligue Nationale Contre le Cancer, Paris 75015, France.
Email: laure.bally-cuif@pasteur.fr

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Abstract

Adult stem cell populations must coordinate their own maintenance with the generation of differentiated cell types to sustain organ physiology, in a spatially controlled manner and over long periods. Quantitative analyses of clonal dynamics have revealed that, in epithelia, homeostasis is achieved at the population rather than at the single stem cell level, suggesting that feedback mechanisms coordinate stem cell maintenance and progeny generation. In the central nervous system, however, little is known of the possible community processes underlying neural stem cell maintenance. Recent work, in part based on intravital imaging made possible in the adult zebrafish, conclusively highlights that homeostasis in neural stem cell pools may rely on population asymmetry and long-term spatiotemporal coordination of neural stem cell states and fates. These results suggest that neural stem cell assemblies in the vertebrate brain behave as self-organized systems, such that the stem cells themselves generate their own intrinsic niche.

KEYWORDS
dynamic homeostasis, intrinsic niche, neural stem cell, population behavior, spatiotemporal coordination, zebrafish

INTRODUCTION – HOMEOSTASIS IN STEM CELL POOLS: FACTS AND MODELS

Adult tissues maintain their structure and function over extended periods of time during the life of an individual. Part of this involves cell replacement or cell addition, and there is now considerable evidence that the de novo generation of new functional cells within adult organs results from the activity of so-called stem cells (SCs). SCs are functionally defined by the long-term capacity to self-renew and to generate differentiated progeny. Cell populations harboring such properties have been identified in many organs, although, as discussed in this review, these may often reflect the transient fluctuating potential of individual cells.

Adult SC systems are frequently organized in pools, suggesting not only that these SCs share a collective niche but also that they have the potential to influence each other. The fate of individual SCs within these pools has been clonally traced, usually by means of genetic Cre-lox labeling. Quantitative biophysical modeling of the resulting clonal size and composition led to strikingly reproducible interpretative models where SC pool maintenance involves a process referred to as "population asymmetry," where individual SCs are endowed with equivalent potential, and choose stochastically between asymmetric

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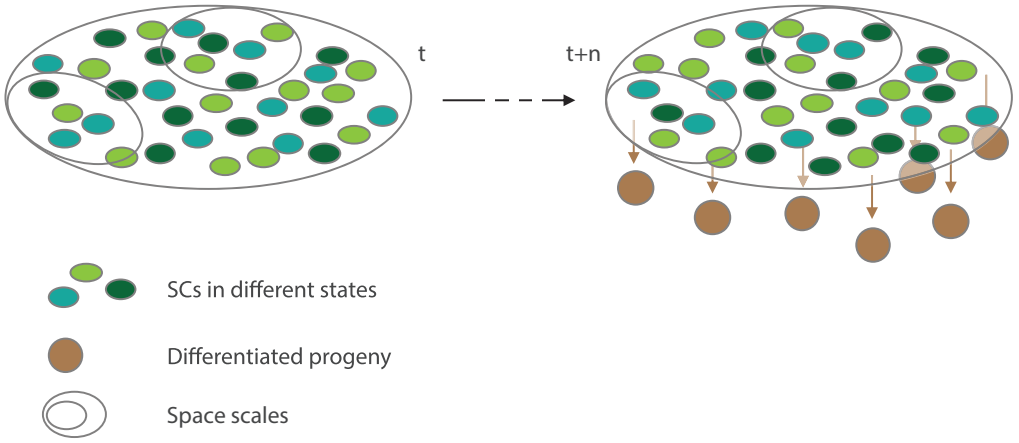


FIGURE 1 Dynamic equilibrium of adult stem cell populations. Adult SC populations consist of a heterogeneous assembly of progenitor cells in different, fluctuating and interconvertible states (green) that, at the population level, show "dynamic stability." This dynamic stability operates across a range of space scales (ovals, within which SC states can be randomly organized or patterned) and time scales (t, t+n). Drifts may appear at minimal space (number of cells) and time intervals (e.g., short temporal fluctuations) that need to be identified for each system. This dynamic stability also accommodates the generation of differentiated progeny cells (red, here shown with a homogeneous distribution)

division, amplification, or differentiation at each fate decision time point. Homeostatic mechanisms balance amplification and differentiation events at the population level (reviewed in[1,2]). Such models predict clonal SC behavior in, for example, the interfollicular epidermis, the male germ line, intestinal crypts, and the esophageal epithelium in mouse, and in the human airway epithelium. It is important to note that the systems studied share a number of attributes that may underlie some of their apparent similarities: their constituent tissues display a relatively high turn-over (i.e., the SC pools generate differentiated progeny at a high frequency) and their associated SC pools do not grow, remaining in a state of overall equilibrium referred to as homeostasis.

Notwithstanding, the concept of population asymmetry bears two important correlates, namely feedback and dynamic homeostasis:

- The occurrence of feedback is first inferred by logic: it is unlikely that tight SC fate equilibrium is maintained over life-long time frames based solely on the balance, by chance, of individual stochastic cell fate decisions.[3] Indeed, negative feedback in studies of lineage dynamics was experimentally demonstrated to take place along a commitment series, that is, from progeny back onto their mother SCs, to adjust SC proliferative activity and produce the amount of progeny cells needed. This is the case in, for example, the mouse olfactory epithelium and hair follicle SCs.[4,5] Feedback may also impact SC fate choices such as division modes. In addition, the feedback-generating cells may not be restricted to direct progeny cells, but could also involve organ function.

- Population asymmetry also implies that the state of an individual SC can vary with time, as illustrated by its decision to divide or not, or to take on a particular fate upon division at time *t*. Thus, not only can a SC population be heterogeneous at any given time, but this heterogeneity is a freeze-frame and would be different, at the individual cell level, at a different time. This dynamic heterogeneity, or flexibility of cellular states, is known at the population level as dynamic homeostasis. The notion of an SC as a cell harboring stable and permanent SC properties therefore appears elusive, and SC properties are rather reflected at the level of the population at any given time.

How are feedback and dynamic homeostasis controlled? Because most clonal analyses and quantitative biophysical modeling focus on clonal size and composition but ignore cell positioning, both feedback and dynamic homeostasis have largely been considered with respect to time (i.e., the temporal control or variations of SC states and fate decisions). An important remaining issue is that of space. Indeed, SC pool homeostasis must not only account for numbers (of remaining SCs and of progeny cells) at a given time, but also for the fact that the different SC fate choice events occur at the right place within the population to maintain SCs and generate progeny cells homogeneously (or in a spatially controlled manner) across the tissue. The overall challenge of dynamic homeostasis, including its spatiotemporal requirements, is illustrated in Figure 1.

Within this framework, this review will address a major current conundrum in the SC field: our present understanding of and speculations on SC dynamics in the adult vertebrate brain. We will discuss the existence and spatiotemporal parameters of dynamic homeostasis in neural stem cell (NSC) pools, and speculate on relevant mechanisms potentially coordinating NSC states. The recent development in the adult zebrafish of intravital imaging methods, capable of tracking entire populations of NSCs in their niche over long time periods, which reveals for the first time NSC behavior in the context of their neighbors, will be used as a strong support for the validity of these speculations.

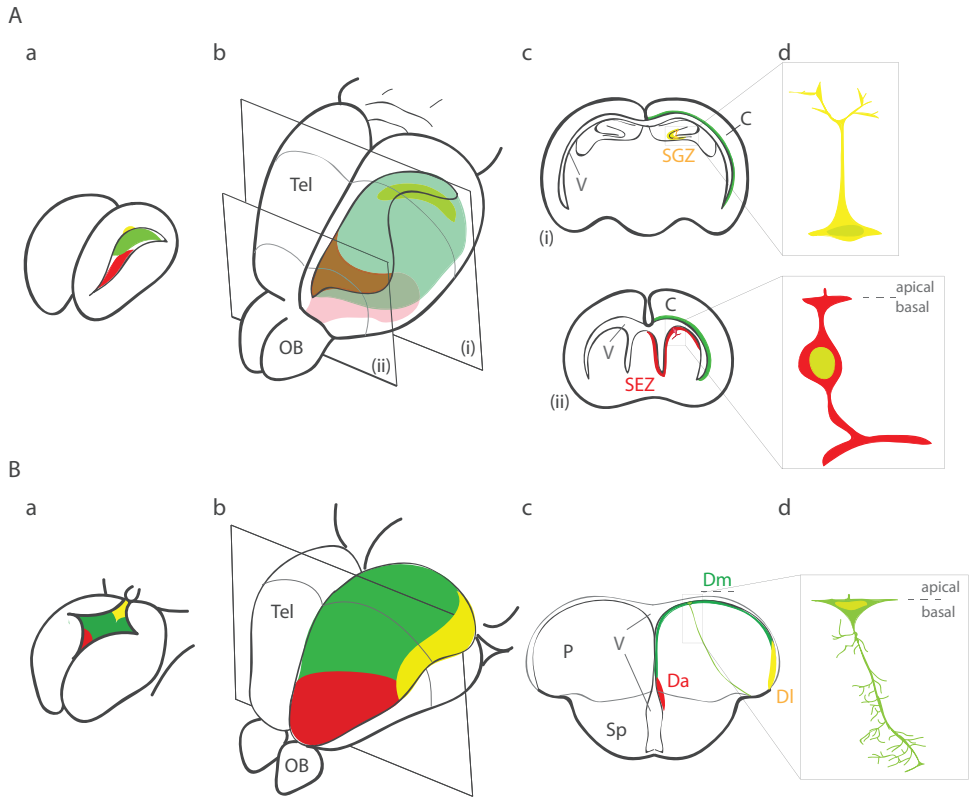


FIGURE 2 Comparison of neurogenic domains and NSCs in the adult mouse (A) and zebrafish (B) telencephalon. Schemes illustrate whole-mount views of the telencephalic hemispheres at embryonic (**a**) and adult (**b**) stages (dorso-anterior views), representative cross-sections (**c**, at the levels indicated in **b**), and the morphology of the astroglial cells identified as NSCs in each location (**d**). Homologous ventricular zones between the two species are color-coded. The Dm is ontogenetically and functionally homologous to the mammalian neocortex. Its ventricular zone remains neurogenic in the adult, while the neocortical ventricular zone does not. Note that a morphogenetic process of eversion exposes the ventricular side of the Dm. In the SEZ and Dm, astroglial NSCs form a tightly arranged assembly in contact with the ventricle and display apico-basal polarity. C: neocortex; Dm: medial domain of the dorsal part of the telencephalon; OB: olfactory bulb; P: pallium; SEZ: sub-ependymal zone; SGZ: sub-granular zone; Sp: sub-pallium; Tel: telencephalon; V: ventricle

QUANTITATIVE DYNAMICS OF ADULT NEURAL STEM CELL POOLS SUGGESTS MAINTENANCE THROUGH FEEDBACK MECHANISMS AND DYNAMIC HOMEOSTASIS

Adult neural stem cells in mouse and zebrafish are maintained through the control of quiescence and stemness-related fate choices

Neural progenitor populations were identified in adult vertebrate brains, within which some astroglial cells, when traced in situ, behaved as NSCs. Rodents (notably mouse) and zebrafish are the most studied model systems, and will be the sole models considered here, with a focus on the dorsal telencephalon (pallium), where most single cell and quantitative analyses were performed. NSCs in the rodent telencephalon are organized into two major pools: the sub-ependymal zone of the lateral ventricle (SEZ) and the subgranular zone of the dentate gyrus of the hippocampus (SGZ) (Figure 2A). These cells express astroglial markers but differ from niche or parenchymal astrocytes in their constitutive neurogenic activity and their transcriptome under physiological conditions; notably, NSCs express markers generally associated with the progenitor state such as Sox2.[6–10] A continuous germinal zone bridges the SEZ and SGZ equivalent domains in the zebrafish adult pallium, as NSCs and neurogenesis remain constitutively active in the area homologous to the neocortex (Dm) (Figure 2B). In contrast with mouse, parenchymal astrocytes have not been described in the adult zebrafish pallium, and NSC and astrocytic functions are believed to be performed by the same cell type (reviewed in).[11] Single cell lineage tracing in both mouse and zebrafish provide evidence that at least some SEZ, SGZ and Dm astroglial cells are capable of long-term self-renewal and neuron generation (SEZ[12]; SGZ[13,14]; Dm[15,16]). While the specific function and properties of adult-born neurons in the Dm remain to be studied, mouse SEZ- and SGZ-derived neurons populate the olfactory bulb and dentate gyrus, respectively, and display critical periods of increased plasticity that permit pattern separation and overall cognitive flexibility during the formation of memory episodes.[17,18]

The physiological function of adult NSCs makes it critical to understand how NSC populations achieve the balance between recruitment and maintenance across a lifetime. At any given time, most NSCs are

found in a state of quiescence, defined by the non-expression of cell cycle markers, but have the capacity to reactivate (i.e. re-enter the cell cycle) upon appropriate stimulation. The frequency of NSC activation impacts NSC maintenance. To date, quiescence control mechanisms were largely studied at the individual NSC level (reviewed in).[19,20] However, one notable exception was recently provided in the adult zebrafish pallium, where quiescence control was considered across space and time in the NSC niche (see the further chapters of this review). A second prime parameter in the homeostasis of individual NSCs and NSC pools are SC-related fate decisions (division mode, differentiation). A number of recent studies, based on quantitative clonal dynamics, analyzed these decisions at the population level, and will be discussed below.

Adult neural stem cells dynamics in mouse reveals population asymmetry

NSC maintenance in the mouse SEZ and SGZ has been extensively studied by means of single cell or population tracing with, for example, retroviruses, Cre-lox genetic fate mapping, or, in one recent publication, intravital imaging. Conclusions diverge widely, and NSC pools exhaustion (SEZ[21–24]; SGZ[25,26]), maintenance (SEZ[12]; SGZ[13,14,27,28]), and even amplification (in the SGZ[29]) were reported (summarized in Table 1). A number of experimental issues can explain these variations, among which the most likely are differences in the choice of promoters or in Cre induction efficiencies, which leads to the tracing of a subset of fates. These biases are in themselves interesting, and it is now of great importance to understand which type of heterogeneity these subsets indicate. The heterogeneous nature of adult NSCs, at the ontogenetic, morphological, physiological, cellular and transcriptional levels, is increasingly appreciated (recently reviewed in).[30–33] Among these, genetic lineage tracing has captured molecular heterogeneities, which could notably reflect commitment differences, sub-lineages, or dynamic transient states "frozen" at the time of Cre induction. These will now need to be integrated into a global dynamic model of NSC pool evolution.

Overall, however, we do not currently have comprehensive knowledge of which clonal dynamics and heterogeneities underlie the long-term evolution of adult NSC pools. A quantitative analysis using biophysical modeling based on clonal tracing was conducted in the mouse SEZ.[12] Clones labeled by recombination in Troy+ astroglia (comprising a large fraction of gfap+ ventricular astrocytes, and including both their quiescent and activated pools) were followed over 8 months, and analyzed for their composition and localization, with a focus on NSCs. Troy+ NSCs were capable of symmetric divisions, and both the number of Troy+ NSC-containing clones and their NSC content appeared constant, suggesting that transient increases in the number of NSCs per clone increase the chance that some NSCs will differentiate upon activation. This particular type of population asymmetry contrasts with the adult epithelial tissues mentioned above, as homeostasis in the SEZ is also maintained at the level of individual clones, with respect to both NSC number and state (quiescence or activation). This raises the intriguing possibility that "intra-lineage" regulatory process(es) operate within clones (Figure 3A). In the SGZ, Urban et al. analyzed the function of the ubiquitin ligase Huwe1, a negative regulator of the bHLH transcription factor Ascl1, which drives neurogenesis and NSC activation.[28,34] In the absence of Huwe1, activated NSCs fail to return to quiescence and eventually exhaust, but this phenotype only affects a subset of NSCs, while a major pool of NSCs remains intact. Thus, the authors propose that two NSC sub-pools with different properties coexist in the SGZ: a large long-lasting dormant sub-pool, from which NSCs rarely activate to populate another sub-pool, where NSCs oscillate between quiescence and activation under Ascl1 control, and which is biased towards differentiation (Fig. 3B). This model, based on population assessments in a mutant context, remains to be challenged by a quantitative clonal approach. The live imaging study of Pilz et al. illuminates the behavior of a committed NSC state biased towards neurogenesis, and which may position itself within the latter sub-pool, in a state close to final commitment.[25]

Overall, the results obtained in mouse support the proposal that adult NSC pools are maintained over time, via population asymmetry. They further suggest additional levels of complexity, such as the existence of functionally different NSC sub-pools, and/or some degree of intra-lineage regulation of NSC fate choices, that perhaps differ between the SEZ and SGZ niches.

A unifying model of adult neural stem cell dynamics revealed through genetic tracing and intravital imaging in zebrafish

The distinctive architecture of the zebrafish adult pallium, where the ventricular zone and NSCs lie as a superficial monolayer, permits fully noninvasive intravital imaging methods, where individual fish are imaged every 1-3 days over several weeks.[35,36] The slow dynamics of the NSC population, and the absence of NSC migration, allows reliable tracking of all individual NSCs (typically > 300 cells per hemisphere) in the context of their neighboring NSCs.[36] These studies revealed the occurrence of different NSC fates, including symmetric amplifying, asymmetric and symmetric neurogenic divisions, as well as direct neuronal differentiation events, the frequency of which can be biased upon injury, further supporting the role of non-cell-autonomous components in their regulation.[35,36] Finally, the neurons produced from individual NSCs in the Dm delaminate and stack below their mother NSC, in a continuous process that is paralleled by pallial growth and ventricular zone expansion.[37,38] This absence of cell migration makes it possible to account for the full cell complement of individual clones.

We recently exploited these unique attributes in a comprehensive study combining short-term (1 month) intravital imaging and long-term (18 months) whole mount clonal lineage tracing, integrating a range of dynamic and static measures with statistical analyses based on biophysical modeling.[16] Our analysis of marker genes demonstrated that expression driven by regulatory elements of the bHLH transcription factor gene *her4* is overall identical to that of the glial fibrillary acidic protein Gfap, labeling over 90% of pallial radial glia. Intravital

TABLE 1 Analyses of NSC self-renewal in the mouse and zebrafish adult pallium

Reference	Species	Niche	Methods	Analysis	Maintenance	Self-renewal mode
[97]	mouse	SEZ	Long-term genetic lineage tracing of *Gli1*-expressing NSCs	bulk	yes	not determined, expansion
[98]	mouse	SEZ	Quantification of ventricle-contacting type B1 cells (GFAP+ and harbouring a single primary cilium)	population	no	not determined
[23]	mouse	SEZ	Multicolor (Confetti) genetic lineage tracing of *Glast*-expressing type B cells	clonal	no	repetitve asymmetric divisions followed by exhaustion
[99]	mouse	SEZ	Retrovirus-mediated barcoding / genetic lineage tracing of *Nestin*-expressing cells	clonal	no	not determined
[12]	mouse	SEZ	Long-term lineage tracing and clonal analysis of *Troy*-expressing NSCs / mathematical modelling	clonal	yes	population asymmetry, mostly involving symmetrical NSC divisions
[21]	mouse	SEZ	Short-term retrovirus-based (RCAS-TVA system) and clonal analysis of *Gfap*-expressing NSC fates / quantification of NSCs in young and old individuals	clonal, population	no	population asymmetry, mostly involving symmetrical NSC divisions
[7]	mouse	SEZ	FACS of GLAST$^+$ PROM$^+$ cells and quantification of SOX2+ embryonic LRCs within the TLX lineage / mathematical modelling	population	no	symmetrical NSC divisions (supposed based on Basak et al. and Obernier et al.)
[97]	mouse	DG	Long-term genetic lineage tracing of *Gli1*-expressing NSCs	bulk	yes	not determined, expansion
[100]	mouse	DG	Lentivirus- and retrovirus-based lineage tracing of Sox2-expressing progenitors	bulk and clonal	to some extent	symmetric and asymmetric division
[101]	mouse	DG	Quantification of *Hes5*-expressing NSCs in young and old individuals	population	to some extent	not determined
[26]	mouse	DG	Short-term lineage tracing of sparsely labelled *Gli1*-expressing NSCs / BrdU pulse-chase experiment / double thymidine analog protocol	semi-clonal / population	no	three asymmetric divisions followed by differentiation into astrocyte
[14]	mouse	DG	Long-term clonal analysis of the *Nestin*-expressing NSC fates	clonal	at least to some extent	population asymmetry with a sizeable contribution of asymmetric divisions
[30]	mouse	DG	Long-term genetic lineage tracing of *Nestin*-expressing NSC fates	bulk	yes	not determined, expansion
[101]	mouse	DG	Mid-term genetic lineage tracing of *Hes5*-expressing NSC fates	bulk	yes	not determined
[25]	mouse	DG	Time-lapse live imaging of clones derived from *Ascl1*-expressing NSCs / mathematical modelling	clonal	no	developmental-like program: NSCs transition from an amplification phase to a self-renewing phase before getting exhausted
[102]	mouse	DG	Long-term genetic lineage tracing of *Hes5*-expressing NSC fates	bulk	to some extent	not determined
[15]	zebrafish	pallium	Lentivirus-mediated lineage tracing and clonal analysis	clonal	yes	expansion
[35]	zebrafish	pallium	Time-lapse live imaging of the fate of *gfap*-expressing NSCs following their labelling by plasmid electroporation	clonal	no	Imbalance between the direct neuronal differentiation of NSCs and their symmetric and asymmetric self-renewing divisions
[104]	zebrafish	pallium	Quantification of *gfap*-expressing NSCs	population	yes	not determined

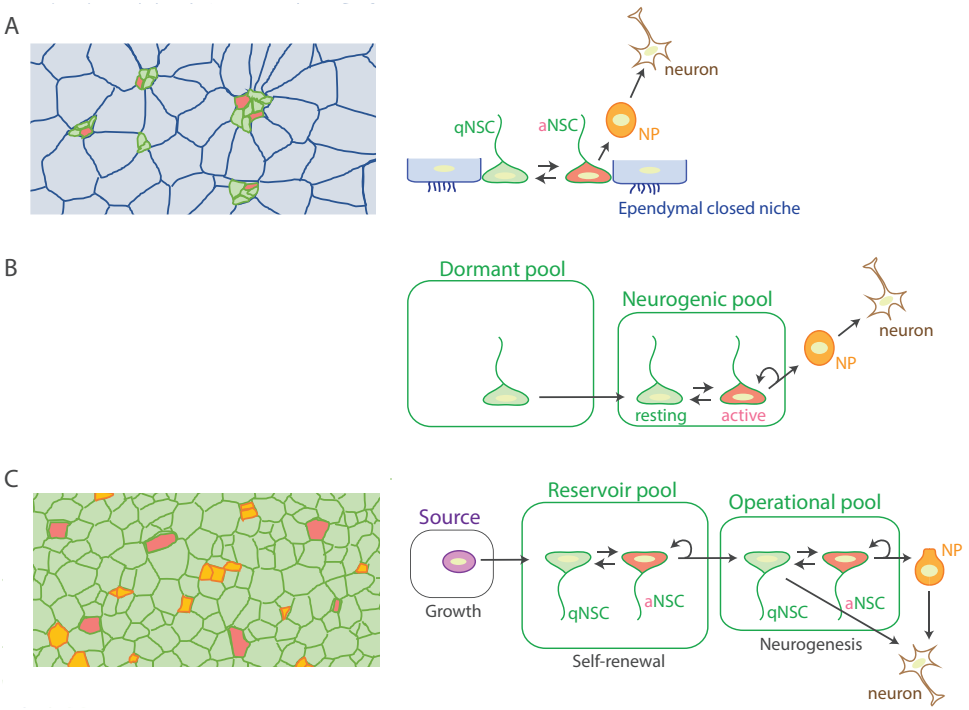


FIGURE 3 Current understanding of the lineage dynamics underlying the maintenance of adult NSC pools in the mouse SEZ (A), mouse SGZ (B), and zebrafish Dm (C). When known, the spatial arrangement of NSCs at the population level is schematized in the left panels (SEZ, Dm, ventricular views). **(a)** The apical domains of small NSC clusters (green surrounding) are tightly juxtaposed within the center of ependymal rosettes (blue). NSCs can be found that are quiescent (qNSCs, green cytoplasm) or dividing (activated NSCs, aNSCs, pink cytoplasm), generating committed neural progenitors (NPs, orange) and neurons (brown). The equilibrium between quiescence and activation operates at a local scale, possibly defined by the closed niche formed by ependymal rosettes.[12] **(b)** In the adult mouse SGZ, population analyses suggest that NSC maintenance may involve subdivision into a dormant and a neurogenic pool. In the neurogenic pool, NSCs can switch back and forth between a resting (quiescent) and an active state, generating NPs and neurons (same color code as in A).[28] **(c)** In the adult zebrafish Dm, the ventricular zone consists of a pseudo-stratified monolayer of progenitors including qNSCs, aNSCs and NPs. Quantitative modeling of clonal and population dynamics suggest that NSC pool maintenance involves a hierarchical organization into three functionally specialized sub-pools: one responsible for the amplification of the NSC population ("source" pool), a second for its self-renewal (reservoir pool, dividing asymmetrically) and a third for its neurogenic activity (operational pool, where all division modes as well as direct differentiation are observed). The reservoir and operational pools contain NSCs in a balance between quiescence and activation. To date the exact location and nature of source cells remains unknown. Same color code as in a. In both b and c, the size of the pools is represented to scale based on experimental/modeling data

imaging of > 300 *gfap:dTomato* NSCs allowed us to quantify cell fates and show that asymmetric NSC divisions accounted for only 42% of events, while NSC gains (symmetric amplifying divisions) equaled NSC loss events (symmetric neurogenic divisions or direct differentiation). These results conclusively demonstrate that homeostasis of a traced *her4*-derived population of the NSC pallial pool is homeostatic and that its dynamics rely, at least in part, on population asymmetry. Interestingly, however, long-term quantitative biophysical modeling of *her4:CreERT2*-derived clones indicates that their dynamics is better accounted for by a functional subdivision of the NSC population into two hierarchically connected sub-populations, a "reservoir" population responsible for self-renewal and acquiring asymmetric fates, and an "operational" population involved in neurogenesis and acquiring stochastic fates biased toward differentiation (Fig. 3C). Quantitative predictions further indicate that both sub-populations are strongly quiescent -although to a different extent- and that the reservoir pool is large, accounting for > 60% of NSCs. This organization is qualitatively and quantitatively highly reminiscent of that proposed by Urban et al. in the mouse SGZ. Importantly, however, the homeostasis of the *her4:Cre*-driven lineage does not account for the behavior of the global NSC population, which we found to grow extensively during the tracing period as the result of an additional "source" population responsible for the sustained production of *her4*+ NSCs (Fig. 3C).

These results illustrate how the use of broadly-expressed promoters, combined with analyses of short and long term quantitative clonal dynamics and overall population assessments, are key to attaining a comprehensive perspective on population dynamics. Conceptually, they also highlight that, in NSC pools like those in adult epithelia, stochastic fate choices are an integral part of the homeostatic process. However, they further suggest that these stochastic choices are embedded in a hierarchy of sub-functionalized NSCs, which itself accounts for the key joint characteristics of NSC populations: growth, self-renewal and neurogenic activity. Finally, they provide a comprehensive framework in which to position the data obtained in mouse, and develop hypotheses on the specific significance of the NSC subsets that have been traced.

These results together illustrate the complexity of cellular behaviors that underlie population homeostasis in vertebrate adult NSC pools. Understanding the molecular mechanisms underlying these heterogeneities and their coordination remains a fundamental issue. Single cell transcriptome profiling provides a first step toward this goal, and was conducted in a number of studies in the SEZ,[7,8,12,39–41] SGZ[6,42,43] and zebrafish Dm,[44,45] from micro-dissected tissue or FACS-sorted cells, including NSCs, committed progenitors, neurons, and niche cells. While NSCs could be clustered into distinct transcriptome-based subgroups, pseudo-time analyses highlight that these subgroups hierarchically progress from quiescent NSCs to neurons, and are therefore dominated by broad changes in activation or commitment. These studies, however, provide the material for future, finer analyses that will reveal, between and possibly within clusters, the underlying cell transitions and hierarchies, cell states and/or division fate choices that account for population homeostasis.

A VARIETY OF COORDINATION MECHANISMS UNDERLIES NSC POPULATION HOMEOSTASIS

The quantitative results above suggest the existence of a coordination operating within NSC pools that controls NSC state and fate. Parallel studies have provided evidence for three broad classes of mechanisms that can sustain, in part, this coordination.

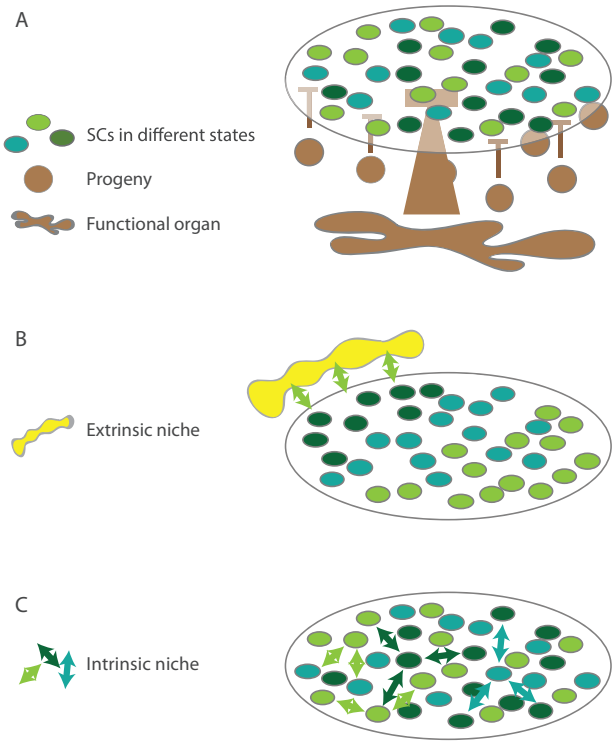


FIGURE 4 Broad mechanism types ensuring the coordination of SC states within adult NSC pools. **(A)** Feedback mechanisms (generally inhibitory) from progeny cells or organ function. **(B)** Interactions with an extrinsic niche biasing the distribution of SC states. **(C)** SC-SC interactions (arrows), at short and long range, and dynamic fluctuations of SC states, generating an "intrinsic niche"

Feedback from progeny

Retroactive control, exerted along a lineage by committed or differentiated progeny to limit the recruitment of mother cells, was modeled by A. Lander as a driver of population asymmetry. It is akin in essence and biological significance to embryonic processes such as lateral inhibition, exerted within developing structures by committed progenitors on their neighbors, to prevent them from committing as well. Such processes convey an equilibrium between output (differentiated progeny) and available input (remaining progenitors) (Figure 4A).

A major molecular mediator of lateral inhibition in embryos is Notch signaling. Within the developing mouse cortex for example, intermediate progenitors activate Notch signaling in mother radial glia to maintain their long-lasting state.[46] Notch signaling also maintains adult NSC quiescence in zebrafish[47,48] and mouse,[49–51] as well as NSC stemness.[52] Interestingly, the selective deletion of the Notch ligand DLL1 from active NSCs and their transit amplifying progenitor (TAP) progeny leads to the reactivation of quiescent NSCs.[53,54] These results highlight a negative feedback mechanism that is exerted on NSCs by their immediate progeny, via Notch signaling.

In rodent adult neurogenic niches, newly post-mitotic neuroblasts, and neurons, also exert a negative feedback on NSCs. The mediators in that case are neurotransmitters (recently reviewed in),[55,56] which can be directly sensed by NSCs via their expression of neurotransmitter receptors, or cell-cell signaling molecules, such as Eph/Ephrins.[57] In the SGZ, a major neurotransmitter of this type is gamma-aminobutyric acid (GABA), released from local interneurons, which inhibits NSC activation.[58,59] In the SEZ, freshly post-mitotic newborn neurons release GABA, and local cholinergic neurons also control the NSC quiescence-activation balance.[60,61] Overall, acetylcholine exerts an effect opposite to GABA, promoting NSC proliferation. These mechanisms may provide NSCs with input regarding progeny cell number and/or global network activity.

Competition for the niche

At any time, the propensity of a given SC for a particular state or fate may be biased by its specific position relative to external elements. As a whole these elements form a defined environment known as a niche and rely on distinctive tissue architectures that generate spatially limited sub-environments favorable for stemness, for which SCs compete (Figure 4B).[62] Clear examples of this are seen in adult epithelia, such as the mouse intestinal epithelium, where SCs occupy specific positions relative to the base of crypts.[63–65] Likewise, in the hair follicle, SC fate

correlates with cell position relative to the mesenchymal dermal papilla (DP).[66]

In the brain, vasculature and the collection of parenchymal cells underlying the ventricular NSC pool (amplifying progenitors and neurons, but also oligodendrocytes, microglia etc.) are major extrinsic niche components.[67] In addition, the mouse SEZ and zebrafish Dm are bathed by cerebrospinal fluid, and the mouse SEZ is lined with ependymal cells (for reviews[67,68]). These components secrete or transport factors modulating NSC proliferation, division modes and/or stemness. In the case of the vascular niche, direct contact is established between NSC astroglial processes and the extracellular matrix and endothelial cells of blood vessels at locations devoid of pericytes, suggesting that individual NSCs could differ in their access to information.[69–71] So far, however, these processes have been proposed to exert general positive or negative forces primarily on NSC pools, rather than to introduce single cell biases in state and fate that could account for the dynamic heterogeneity that maintains population homeostasis.

The recent report by Basak et al. shows however that the clonal dynamics of NSCs of the mouse SEZ can be best described by a model involving a restricted niche (Figure 3A).[12] Because at any given time *Troy:CreERT*-derived clones contain, on average, the same and constant number of NSCs (generally 1.5), it follows that NSC fate decisions must be influenced by the number of NSCs in the clone: an increasing number of NSCs will favor their differentiation, and indirectly promote activated NSCs to return to quiescence. One of the remarkable strengths of this interpretation is that it is supported by the architectural organization of the SEZ NSC pool. Ventricular ependymal cells arrange in rosettes ("pinwheels") that physically isolate the apical domains of a few NSCs in their center.[72] Thus, the homeostasis observed at the clonal level may be secondary to the existence of the pinwheel, the organization of which could provide a physical substrate for the generation of restricted niches. There are several hypotheses for this niche effect, such as space competition for access to the ventricle, or for access to fractone bulbs, which are extensions of the ependymal extracellular matrix that extend into the center of pinwheels.[73] Ependymal cells and NSCs within pinwheels also establish unique asymmetrical junctions at their apicobasal boundaries, features that may introduce differences between NSCs located at the edge versus the center of the pinwheel hole. Alternatively, pinwheels may act in a non-instructive manner to isolate sub-groups of NSCs among which interactions will control homeostasis (see below). Distinguishing between these hypotheses will require the direct experimental perturbation of pinwheel organization. The absence of ependymal cells in the mouse SGZ and zebrafish Dm (at least prior to old age),[74] as well as our interpretation of lineage dynamics in the Dm, do not support, neither structurally nor by quantitative dynamics, the existence of such niche mechanism in these territories.[16] The important differences in the location and organization of extrinsic neurogenic niches between brain territories and vertebrate species suggest that distinct niche-specific solutions may have been developed.

NSC-NSC interactions: the notion of an intrinsic niche

While the extrinsic niche permits the expression of SC potential, individual SC state and fate decisions may be the result of community interactions rather than the direct readout of instructive or biasing niche cues. In this context, SC pools capitalize on individual SC plasticity to achieve dynamic and collective cell decision-making, readjusted at any time to account for neighboring SC fate decisions or simply for neighboring SC state fluctuations. We refer to these interactions as the "intrinsic niche," where the state of each individual SC at any time integrates that of its neighbors (space) and of the system at preceding time points (time) (Figure 4C).

In embryonic systems, self-organization processes have evolved to generate differences between cells, which are then fixed. In contrast, adult SC pools would aim to generate coordinated systems that ensure (i) spatial homogeneity in potential and (ii) long-term maintenance in a plastic state. Dynamic homeostasis predicts that these coordination mechanisms likely control both division frequency and fate choices (division mode, and, if they exist, direct differentiation events). Although most of the time inferred from population dynamics, these mechanisms are likely local. They may spread across the tissue from cell to cell, in a domino-like process (as exemplified during developmental processes, see[75,76] for reviews). Finally, they must also integrate functionally different SC sub-pools, if these exist. Much about these long-lasting and dynamic systems adjustments remains to be understood.

Resolving the spatiotemporal parameters of dynamic systems necessitates the real-time imaging of large cell populations. The recent development of intravital imaging methods has provided a means for addressing these issues in epithelial SCs in mouse, revealing that, in the skin epithelium, the differentiation and division decisions of neighboring SCs are coordinated in time and causally related.[77–79] The division of an individual SC is in part triggered by the occurrence of SC delamination (following differentiation) in its vicinity (Figure 5A), within a one-cell diameter range and a 1 to 2-day temporal delay. A related example was provided in epithelial SCs of the Drosophila gut, where SC loss or apoptosis trigger neighboring SC division.[80,81]

Whether intrinsic niches operate in adult NSC pools in the vertebrate brain has remained largely unexplored, due to the lack of tools for tracking, on site, the behavior of individual NSCs in the context of their proximate and more remote neighbors. In addition, the dynamics of adult NSC systems is very slow with, on average, one NSC division occurring per cell per month, therefore requiring live analyses over very long time periods. The recent development of a long-term intravital imaging technology in the zebrafish adult brain has opened up the possibility of studying individual NSC behavior, probing the existence of spatiotemporal coordination, and determining its quantitative parameters.

So far, this technique has been applied to studying the frequency and location of NSC activation events (Dray et al., in peer review, preprint at biorxiv.org/doi/10.1101/2020.07.15.205021). At any time

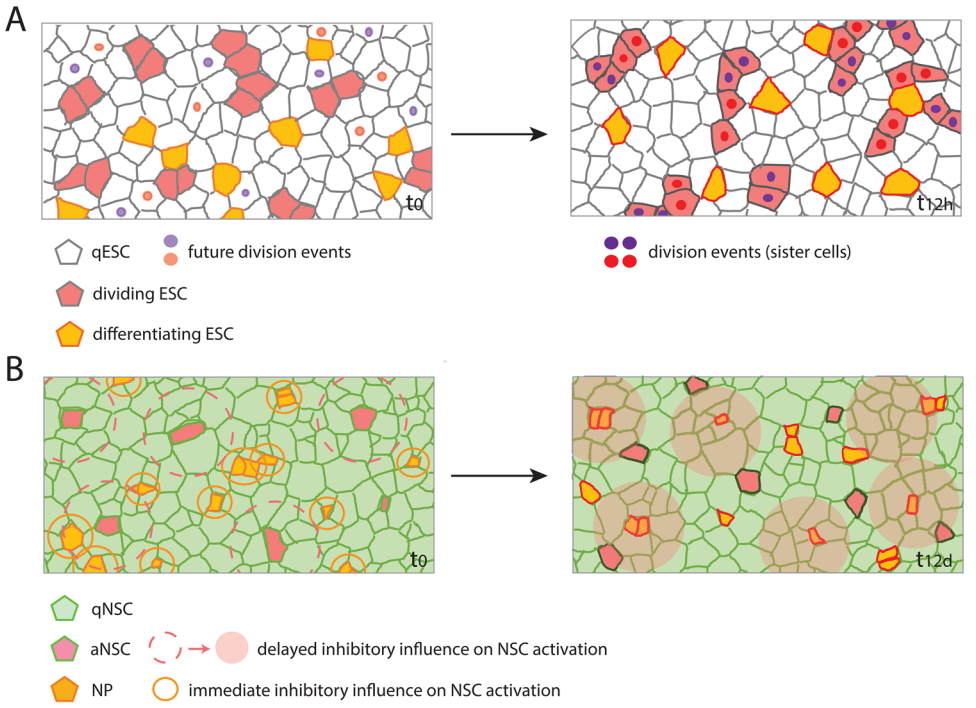


FIGURE 5 Spatiotemporal coordination of SC fates by internal niche mechanisms in the adult mouse skin epithelium and adult zebrafish brain. **(A)** In the adult mouse skin, differentiating epithelial stem cells favor the later occurrence of division events in neighboring epithelial stem cells, with a delay of 12 h. The scheme is re-drawn from,[79] but the positions of dividing cells at t0 and differentiating cells at t+12h are arbitrary, meant to illustrate the temporal propagation of the pattern. **(B)** In the adult zebrafish telencephalon (Dm), the position of NSC activation/division events at any given time is negatively influenced by neighboring neural progenitors and by the position of previously dividing NSCs, with a delay of 12 days (Dray et al., preprint at biorxiv.org/doi/10.1101/2020.07.15.205021). The spatial range of these inhibitory effects is indicated by circles. The panel at t0 is the same as in Figure 3C. In a and b, cell states are color-coded. ESC: epithelial stem cell, qESC: quiescent epithelial stem cells, aNSC: activated neural stem cell, qNSC: quiescent neural stem cell

point within the Dm and in a 3-month-old adult, 5% of NSCs are found in the activated state (defined by the expression of proliferation markers such as Mini-Chromosome Maintenance protein 5 -Mcm5- or Proliferating Cell Nuclear Antigen -PCNA-). This state lasts for a few days and is generally accompanied by cell division, after which most NSCs return to quiescence while other NSCs become activated (Figure 5B). Point pattern analyses indicate that the positions of activation events within the NSC population at any given time are random relative to each other. However, longitudinal intravital imaging reveals that, with a delay of 12 days, division events occur further away from each other than predicted by chance. Thus, coordination events between dividing and activated NSCs in their neighborhood tend to increase, in a time-propagating process, the spatial distance between successive divisions. Because transiently inhibited NSCs will then in turn divide, the inhibitory information may gradually spread across the germinal zone.

These observations highlight that the quiescence/activation balance of individual NSCs within a pool is subject to dynamic spatiotemporal coordination, which originates locally, then presumably propagates to equilibrate population behavior (Figure 5B). When challenged by mathematical modeling, this mechanism appears necessary to maintain a spatially homogeneous distribution of output neurons over the long term (Dray et al., preprint at biorxiv.org/doi/10.1101/2020.07.15.205021).

Activation is the most upstream NSC decision, fundamental to lineage progression and to NSC maintenance. It does not however directly impact clonal composition, which is reliant on the control of NSC fate choices. Whether the latter are also subject to large-scale spatiotemporal coordination remains to be tested.

MECHANISMS UNDERLYING THE DYNAMICS AND PROPERTIES OF INTRINSIC NICHES INTEGRATE SPATIAL INTERACTIONS AND TEMPORAL DELAYS

The mechanisms and molecular components controlling the long-term dynamic equilibrium of intrinsic SC niches remain to be identified. As illustrated by the currently unique examples of the mouse skin and zebrafish brain, these mechanisms ensure a dynamic stability and spatial homogeneity of SC states and fates by integrating spatial and temporal components.

Signaling or readout delays propagate information over time

A delay of 12 h was reported between SC delamination and the division of a neighboring SC in the adult mouse interfollicular epidermis,[79] and division events of NSCs are prevented from occurring in neighboring locations with a delay of 12 days (Dray et al., preprint at biorxiv.org/doi/10.1101/2020.07.15.205021). Such a delayed effect defines the longitudinal component of coordination mechanisms and may be explained in several ways. First, this delay may be "technical," that is, due to the biological time needed to reach our experimental read-out from the starting state. In both examples, the read-out of "SC division" (M phase) is the output of a multistep process (quiescence exit, and progression through the G1-S-G2 phases). Hence, the recipient SC may read the signal at a much earlier time point than the division event itself. In the case of neurogenic divisions, biological time may also correspond to the delay needed to generate more committed neural progenitors that may act by negative feedback. Along these lines, in the zebrafish Dm, a point pattern analysis also showed that activated neural progenitors issued from NSCs (aNPs, equivalent to the transit amplifying progenitors of rodents) do locally inhibit NSC activation events in their vicinity via Notch signaling (Dray et al., preprint at biorxiv.org/doi/10.1101/2020.07.15.205021). These progenitors arise from dividing NSCs after several days, and remain where they were born within the germinal sheet until they delaminate to generate neurons. Their transient nature could account for a local burst of inhibition. Alternatively, or in addition, the delay may reflect the time needed for signal spreading and transduction.

Cell-cell interactions between SCs propagate information in space

The mechanisms sustaining intrinsic niches ensure communication between SCs. At any time, each SC reads the state of its local environment and integrates past information to adjust its own state, and modulates or induces delayed changes in the state of its neighbors.

Rather than direct SC-SC instructive interactions, the adult mouse skin study proposes an indirect mechanical effect, whereby the delamination of the differentiating SC allows for the neighboring cell to expand, a process itself stimulating proliferation.[79] The tight spatial arrangement of NSCs in the Dm (or in the mouse SEZ within pinwheels) makes it possible that mechanical cues are also at play to control quiescence. For example, a division event may create a local compression in neighboring cells that is read as a signal for delayed activation by neighbors. This remains to be tested.

The number of alternative nonmechanical signaling processes and factors that can potentially be exchanged between SCs as a substrate for intrinsic niches is large, exemplified by the processes at play in embryonic systems (reviewed in[82]). In that case, diffusible signals -acting as gradients or cell-cell signals- extracellular vesicles, membrane-bound molecules, or direct cytoplasmic exchanges have all been described. In the context of dynamic plasticity, mechanisms that permit rapid, dose-dependent and reversible information transfer are likely especially relevant. These include bidirectional signaling by membrane ligand-receptor pairs such as Notch-Delta/Jagged, the directionality of which can be rapidly inverted by small changes, for example in cell size.[83] Direct and time-resolved readouts of these different kinds of signaling will be fundamental to quantitating their fluctuations in real-time across germinal tissues. Contributing to information spreading, direct signaling via membrane-bound factors at a distance has been described, via cell protrusions, including for Notch in Drosophila and vertebrates.[84–87] In adult NSC pools such as the Dm, horizontal extensions emitted by activated NSCs can extend several cell diameters.[88] We further observed that the majority of activated NSCs express the Notch ligand DeltaA.[48] Signals exchanged between basal processes, which potentially connect NSCs whose cell bodies are somewhat distant from each other, could also operate.

MODELING APPROACHES PROVIDE INSIGHT INTO THE ESTABLISHMENT AND PERTURBATIONS OF LONG-TERM DYNAMIC HOMEOSTASIS IN ADULT SC SYSTEMS

The systems properties of adult SC ensembles, in particular governed by the intrinsic niche, provide a long-term and dynamic balance of states and fates. Their mathematical modeling provides a unique opportunity to infer the key parameters imposing long-term dynamic stability, such as the cell interaction range, strength, direction, and information content that control the key characteristics of dynamic homeostasis.

Theoretical analyses using artificial lattices of cells with the capacity to divide or differentiate at chosen rates, and involving feedback interactions, were used to model tissue development.[89] The properties of adult cell ensembles in vitro were also modeled to account for the dynamic stability and spatial distribution of cell phenotypic variations, stressing the importance of context-dependent noise in the establishment of cell states in space.[90,91] Very interestingly, the "context" generating or biasing noise can be produced by the cells of interest themselves, for example, it is influenced by cell density (which can modify mechanical and/or chemical cues), which is akin to the notion of an intrinsic niche.[90]

Spatial organization was rarely considered for adult SC populations, however. As one of a few pioneering studies, the closed niche of adult mouse intestinal crypts was modeled in 3D assuming that SC identity at any time is driven by cell position, highlighting the role of crypt geometry, initial cell position, turn-over and signaling levels of niche cells, and cell state plasticity.[92–94] Another example is the adult fly gut epithelium, where a 2D model incorporating contact-mediated feedback on fate and stochastic mobility of each SC across its available space could recapitulate, when SC density reaches a minimal threshold, the proper ratio and distribution of an SC and its differentiated progeny.[95]

Our recent intravital imaging study of adult NSC population behavior in the Dm made it possible to use quantitative experimental parameters to develop a stochastic NSC lattice model that

captures the morphology and spatiotemporal dynamics of individual NSCs in the context of the population (Dray et al., preprint at biorxiv.org/doi/10.1101/2020.07.15.205021). Simulations using this model reveal several important features of NSC population homeostasis. First, they show that the transient nature of aNPs, their downstream position within the NSC lineage, and their intermingling with NSCs within the germinal layer, are sufficient to generate a dynamic lateral inhibition process controlling the spatiotemporal dynamics of NSC activation over the long-term. Second, they predict that this local process is sufficient to spatiotemporally homogenize neuronal production over a lifetime. Third, they suggest that the inhibitory interactions coordinating NSC division may emerge in part from NSC lineage dynamics: encoding the inhibitory activity of aNPs within the model generates a spatiotemporal dispersion of NSC division events with an approximately 12-day delay, as measured in vivo. Together, this illustrates how modeling can help infer causal relationships between actively encoded and emergent interaction rules operating within SC ensembles, and infer their long-term effects across timeframes inaccessible experimentally.

A further important aspect will be to determine how these balanced adult SC systems are generated. Biologically, they are the product of developmental and post-developmental processes, thus of generally progressive changes, for example, in cell states (such as the acquisition of quiescence), cell types (such as the differentiation of ependymal cells in the SEZ) or tissue architecture, that can take place over weeks to months. But it remains largely unknown which cellular dynamics underlie these transformations that generate a steady-state. Proper spatiotemporal models will be instrumental in this endeavor as well. While they currently start from a "mature" situation that mimics the arrangement and states of adult SC populations, they can also be tested for parameters that can generate this initial pattern from various quantitative and spatial compositions of cells. An important question is, for example, whether they can be attained by simple self-organization processes, as a systems stable state. In the mouse SEZ, ependymal cells and NSCs originate from a common precursor.[96] Choices of fates are progressive, concomitant in the two cell types, and involve molecularly ambivalent states expressing both ependymal and NSC genes. Furthermore, in some SEZ domains, they are resolved within the same time frame as the generation of pinwheel patterns, suggesting interactions between these processes in a manner akin to the self-organization of cell fates and patterns.[96]

CONCLUSIONS AND OUTLOOK

The combination of intravital imaging and quantitative genetic tracing in mouse and zebrafish have converged on a model whereby cross-talk between NSCs of a niche plays a major role in the adjustment of their individual states. In particular, analyses of the zebrafish pallium illustrate how lineage progression and cell-to-cell feedback integrate space and time to permit long-term dynamic homeostasis. Such emergence of spatiotemporal NSC coordination is leading us to put forward the concept of an "internal niche," whereby NSC assemblies themselves tailor their own dynamic maintenance. Understanding how SC heterogeneities and state dynamics are coordinated in time and space to ensure the harmonious maintenance of the whole SC complement is one of the most fundamental and substantial challenges of today's SC research, and its relevance is manifold. Functionally, these investigations will provide insight into the mechanisms controlling the maintenance of key cells in adult organ physiology. Conceptually, they will reveal and model how dynamic population-level steady-states can be propagated and maintained spatiotemporally within cell ensembles. These models can further help make predictions regarding how equilibrium shifts, whether in space (generation of inhomogeneities) or in time (generation of imbalances in cell states), will lead to the loss of homeostasis.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study

ORCID

Nicolas Dray https://orcid.org/0000-0002-2632-6004
Emmanuel Than-Trong https://orcid.org/0000-0001-6867-8844
Laure Bally-Cuif https://orcid.org/0000-0001-6611-6274

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