

Genetic approaches identify adult pituitary stem cells

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Adult tissues undergo continuous cell turnover in response to stress, damage, or physiological demand. New differentiated cells are generated from dedicated or facultative stem cells or from self-renewing differentiated cells. Here we describe a different stem cell strategy for tissue maintenance, distinct from that observed for dedicated or facultative stem cells. We report the presence of nestin-expressing adult stem cells in the periluminal region of the mature anterior pituitary and, using genetic inducible fate mapping, demonstrate that they serve to generate subsets of all six terminally differentiated endocrine cell types of the pituitary gland. These stem cells, while not playing a significant role in organogenesis, undergo postnatal expansion and start producing differentiated progeny, which colonize the organ that initially entirely consisted of differentiated cells derived from embryonic precursors. This generates a mosaic organ with two phenotypically similar subsets of endocrine cells that have different origins and different life histories. These parallel but distinct lineages of differentiated cells in the gland may help the maturing organism adapt to changes in the metabolic regulatory landscape.

anterior pituitary | Cre | Lhx3 | nestin | nestin-GFP

Tissue maintenance in the adult organism requires a constant supply of new cells to replace differentiated cells lost to stress and damage or destroyed as part of a normal cell death program. This replacement is made possible through the activity of tissue-specific dedicated stem cells (exemplified by stem cells for blood, hair follicles, and brain), facultative stem cells (e.g., oval cells of the liver producing hepatocytes upon injury), or fully differentiated cells, which can undergo self-duplication when required for tissue maintenance (e.g., hepatocytes and pancreatic β cells) (1, 2). For both the dedicated and facultative stem cells of the adult tissues, the lineage can be traced back to particular embryonic precursors; these same embryonic precursors are also responsible for the generation of differentiated cells of the tissue.

Anterior pituitary gland is a key regulator of the endocrine balance in the adult organism. The gland of newborns already contains a full set of terminally differentiated hormone-producing cells (3, 4). However, the postnatal gland undergoes extensive remodeling during the lifetime of the animal. Soon after birth, the anterior pituitary enters a phase of growth that results in a dramatic increase in the size of the gland (5, 6). Furthermore, composition of the gland undergoes changes in response to hormonal signals (7). These observations are compatible with the existence of stem-like cells in the maturing and adult pituitary; this notion is also supported by observations that a fraction of pituitary tumors of monoclonal origin are plurihormonal and contain several pituitary cell types (8, 9). However, the existence of multipotent stem cells in the anterior pituitary has not yet been demonstrated. Several different cell types, including folliculostellate cells (10–13), rapidly dividing nestin-expressing cells (14), and side population cells of the anterior pituitary (15), have been recently proposed as stem cells (16); however, there is no evidence that any of the proposed candidates are able to generate all differentiated cell types and participate in the process of cell renewal in the anterior pituitary

gland *in vivo*. We here demonstrate the existence of stem cells in the adult pituitary and describe a distinct strategy that these stem cells employ.

Results

Nestin-Expressing Cells of the Adult Anterior Pituitary. To identify stem cells of the anterior pituitary, we used transgenic mice expressing GFP driven by regulatory elements of the *nestin* gene (17). Expression of this transgene has been found in several types of tissue-specific multipotential stem cells, including adult and embryonic neural stem cells (17), stem cells in the bulge of the hair follicles (18, 19), precursors to the Leydig cells of the testis (20), satellite cells of the skeletal muscles (21), and oval cells in the liver (22). We therefore examined whether nestin-GFP expression may also mark stem cells for the pituitary gland. We found GFP-positive cells in both the anterior and intermediate lobes of the pituitary gland of 3- to 4-week-old mice, residing predominantly around the lumen that separates the anterior and intermediate lobes (Fig. 1 *A* and *B*); identical results were obtained with an independently generated mouse line (23) carrying cyan fluorescent protein fused to a nuclear localization signal driven by the same regulatory elements (data not shown). A smaller number of such cells was found at the border between the intermediate and posterior lobes. The GFP-positive cells expressed *Lhx3* (Fig. 1*C*), a key marker of all six pituitary lineages (24, 25), suggesting a histogenetic relation to the endocrine cell types of the pituitary. They also expressed *Sox2*, whose expression is associated with several categories of stem cells; furthermore, they express epithelial markers cytokeratin 8 and EpCAM (Fig. 1 *D* and *E*). The nestin-GFP cells were negative for the markers of terminal differentiation of the pituitary lineages, indicating their undifferentiated phenotype. A smaller number of weakly GFP-positive cells, presumably of mesenchymal origin, did not express *Lhx3*, cytokeratin 8, or EpCAM and were mostly found associated with blood vessels (Fig. 1*F*).

To obtain further insights into the genesis of the nestin-GFP cells of the adult gland, we traced the development of these cells during the embryonic and perinatal periods of pituitary histogenesis. The first nestin-GFP-expressing cells were detected in Rathke's pouch, the primordium of the anterior pituitary, at embryonic day 11.5. At this time, single GFP-expressing cells were located in the dorsal part of Rathke's pouch, the region that contributes to the future intermediate and anterior lobes of the

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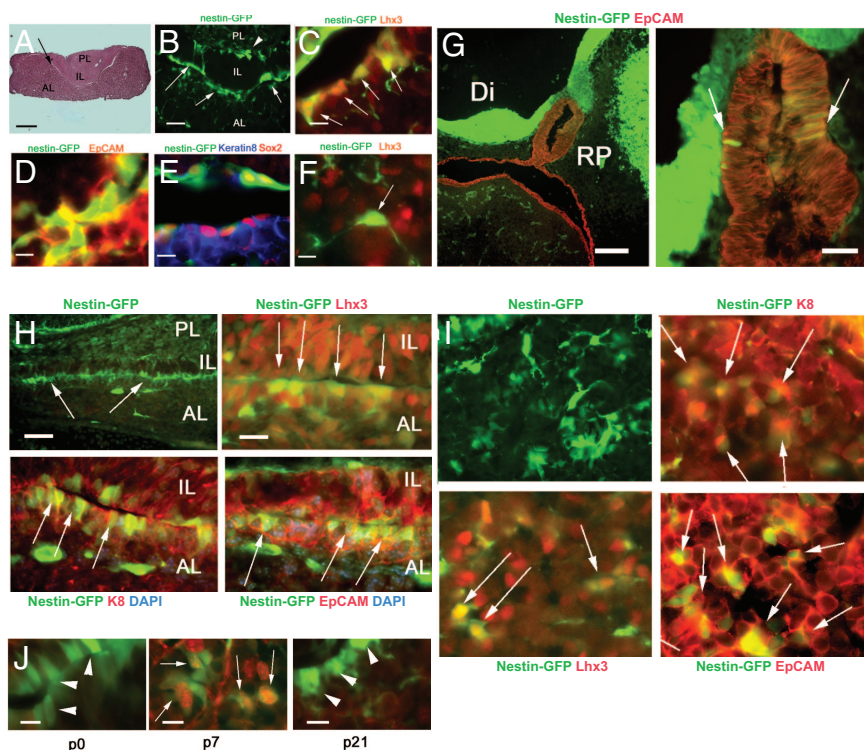


Fig. 1. Nestin-GFP cells in the adult and developing pituitary. (A) General view of adult mouse pituitary. PL, posterior lobe of pituitary; IL, intermediate lobe; AL, anterior lobe. An arrow indicates the lumen that separates intermediate and anterior lobes. The periluminal area is a remnant of embryonic primordium of the anterior pituitary, Rathke's pouch. (B) In the pituitary of nestin-GFP transgenic mice the majority of GFP-positive cells are located in the periluminal area (arrows). Several GFP-positive cells are seen at the border separating intermediate and posterior lobes (arrowhead). (C–F) GFP-positive cells express Lhx3 (C, arrows), EpCAM (D), and Sox2 and cytokeratin 8 (E). GFP expression was also found in the cells with long processes that were dispersed throughout the gland and did not express Lhx3 (F, arrow). [Scale bars: 400 μm (A), 35 μm (B) and 10 μm (C–F).] (G) Nestin-GFP-positive cells in the primordium of the anterior pituitary, Rathke's pouch (RP; Di, ventral diencephalon), are first detected at embryonic day 11.5 (Left). They localized in the upper portion of Rathke's pouch and express epithelial markers, such as EpCAM (Right, arrows). [Scale bars: 50 μm (Left) and 12.5 μm (Right).] (H) At p0, GFP-positive cells are seen almost exclusively in the periluminal area of pituitary (Upper Left) and are Lhx3-positive (Upper Right), cytokeratin 8-positive (Lower Left), and EpCAM-positive (Lower Right). [Scale bars: 75 μm (Upper Left) and 15 μm (Upper Right and Lower).] (I) A significant number of GFP-positive cells is seen in pituitary parenchyma at p7 (Upper Left) at the beginning of the postnatal wave of the pituitary growth. These cells express cytokeratin 8 (Upper Right, arrows), Lhx3 (Lower Left, arrows), and EpCAM (Lower Right, arrows). (J) Proliferation of transgene-positive cells. At p0, transgene-positive cells located in periluminal zone are negative for Ki67, a marker of proliferation (Left, arrowheads). Later, during a postnatal wave of pituitary growth nestin-GFP-positive cells that migrate into glandular zone are often positive for Ki67, a marker of proliferation (Center, arrows, p7). At p21, the majority of transgene-positive cells reside in the periluminal zone. They are negative for Ki67 (Right, arrowhead). (Scale bars: 15 μm .)

anterior pituitary (Fig. 1G). At the end of embryonic development and immediately after birth [embryonic day 18.5 to postnatal day 1 (p1)], the number of GFP-expressing cells gradually increased, and they were still confined mostly to the periluminal areas. As in the adult pituitary, these cells were positive for Lhx3 and for the epithelial markers cytokeratin 8 and EpCAM (Fig. 1H); however, they did not express markers of terminal differentiation.

At the end of the first postnatal week, the pituitary gland starts to undergo a second wave of growth accompanied by a dramatic increase in cell proliferation. At this time, we observed a significant migration of GFP-expressing cells that expressed cytokeratin 8, EpCAM, and Lhx3, from the periluminal area into the glandular territory. (Fig. 1I). Among these cells, a small fraction also expressed Pit-1; these cells may correspond to a transitory form between the multipotent stem cells and their more narrowly committed progeny. A large fraction of nestin-GFP-expressing cells both in the glandular zone and in the periluminal area was positive for Ki67, a marker of dividing cells. Importantly, such active migration and proliferation of nestin-GFP cells was observed only for this period of pituitary development. It was not seen immediately after birth (p1) or at the end of the second wave of pituitary growth (p21) (Fig. 1J).

Genetic Lineage Marking of the Nestin-GFP Stem Cells. The observations of the stem-like nature and phenotype of nestin-GFP cells, their dissimilarity from the embryonic pituitary precursors, and their involvement in the postnatal wave of pituitary growth together suggest that nestin-GFP cells correspond to the multipotent stem cells of the adult pituitary. They further suggest that these cells are different from the embryonic precursors that generate the bulk of the cells that make up the gland during embryonic development. We sought to support this hypothesis by using a lineage analysis technique that employs a recombination-based reporter system in which transgenic mice expressing Cre recombinase under the control of nestin regulatory elements, the same elements used to generate the nestin-GFP line (26), are crossed with mice of the ROSA26-loxP-stop-loxP-GFP (ROSA-lsl-GFP) reporter line. In the progeny, the differentiated cells that are derived from nestin-expressing precursors (which have undergone Cre-mediated recombination) will be permanently marked by GFP expression.

In the pituitary of newborn nestin-Cre/ROSA-lsl-GFP mice, we found scattered terminally differentiated GFP-positive cells in the intermediate and anterior lobes. The most significant contribution of GFP-positive cells to the newborn gland was in the rostral tip of the pituitary, a transient structure that disappears soon after birth

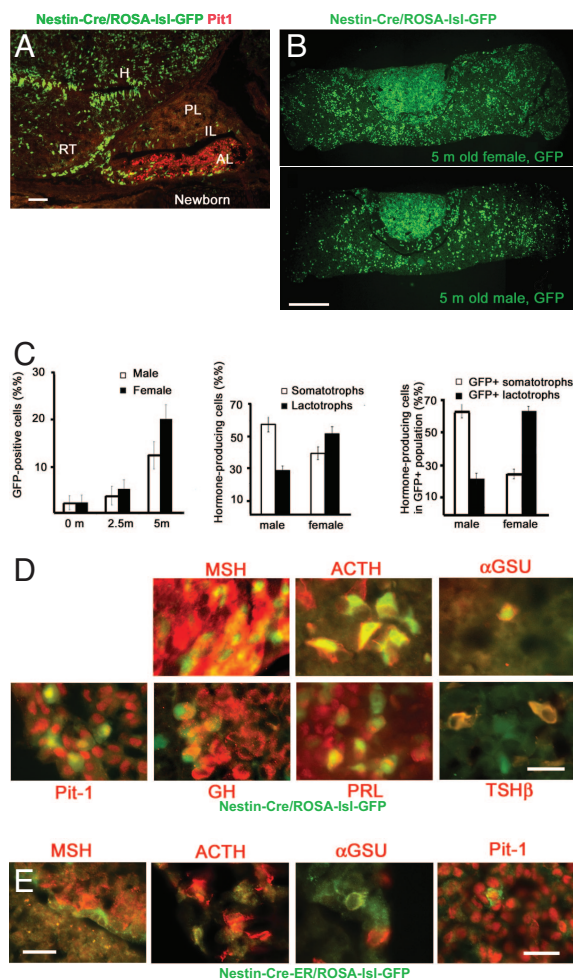


Fig. 2. Nestin-expressing precursors/stem cells contribute to all six terminally differentiated cell types in the anterior pituitary. (A) GFP-positive cells are seen in the brain and pituitary gland of newborn (p0) nestin-cre recombinase transgenic mice crossed with the ROSA-IsI-GFP reporter strain. H, hypothalamus; AP, anterior pituitary; RT, rostral tip of the anterior pituitary. In the anterior pituitary, a few cells express GFP. The majority of rostral tip thyrotrophs are GFP-positive. Note that the majority of the differentiated neurons in the hypothalamus are GFP-positive, which indicates the effectiveness of Cre-induced recombination in neural stem cells. The expression of GFP in this particular mouse reporter line is weak but can be effectively visualized with anti-GFP antibodies. (Scale bar: 75 μm .) (B) Accumulation of GFP-positive cells in the anterior pituitary of nestin-Cre transgenic mice crossed with ROSA-IsI-GFP reporter mice. The number of GFP-positive cells reaches a peak around age 5 months. Female pituitaries (Upper) contain significantly higher numbers of GFP-positive cells than males (Lower). (Scale bar: 400 μm .) (C) Accumulation of nestin-Cre-derived cells in the anterior pituitary is age- and sex-dependent. The percentage of GFP-expressing cells gradually increases between 2.5 and 5 months. Between ages 5 and 8 months, a significantly higher number of stem cell-derived differentiated cells was found in the female anterior pituitary. Male pituitaries contain almost twice the percentage of somatotrophs in comparison with lactotrophs, whereas female pituitaries contain many more lactotrophs than somatotrophs. Correspondingly, the majority of GFP-positive progeny of nestin-expressing stem cells in males differentiated into somatotrophs, whereas in female most of them became lactotrophs. (D) GFP-positive cells contribute to each endocrine lineage: melanotrophs in the intermediate lobe and corticotrophs in the anterior lobe, visualized with anti-MSH and anti-ACTH antibodies correspondingly; gonadotrophs (anti- αGSU antibody); and Pit-1-dependent cell lineages (anti-Pit-1 antibody) that include somatotrophs (anti-GH antibody), lactotrophs (anti-PRL antibody), and thyrotrophs (anti-TSH β antibody). Yellow shows colocalization of transgene and corresponding marker. (Scale bar: 30 μm .) (E) Nine-month-old mice carrying Cre-ER fusion protein driven by the nestin regulatory elements and the ROSA-IsI-GFP transgene were treated with tamoxifen twice with a 24-h interval and killed 1 month later. Expression of GFP reporter was found in

(Fig. 2A). Not counting these cells, the number of GFP-positive cells in the newborns was only $\approx 2\%$ (Fig. 2A and C). This demonstrates that the bulk of the pituitary cells derived from cells that did not express nestin at any stage of their development, supporting our hypothesis that nestin-expressing cells represent a separate lineage of pituitary precursors.

Providing further support for the notion of nestin-positive cells serving as adult stem cells, the number of GFP-positive cells in both the intermediate and anterior lobes of the pituitary gradually increased after birth and reached 20% of all cells of the anterior pituitary by 5 months of age (Fig. 2B and C). GFP expression was found in all six terminally differentiated pituitary endocrine cell types—melanotrophs, corticotrophs, gonadotrophs, somatotrophs, lactotrophs, and thyrotrophs (Fig. 2D). Thus, the input of adult stem cells increases 10-fold during the first months of life, highlighting their contribution to the structure of the adult gland.

Further supporting the notion of a separate adult stem cell lineage, the differentiation choices differed between the embryonic- and adult stem cell-derived progeny; for instance, in females the ratio of lactotrophs to somatotrophs was 1.8 times higher for GFP-expressing cells (i.e., cells derived from the presumptive adult stem cells) than for all of the cells of the gland (a mix of embryonic- and adult-generated cells), whereas in males it was 0.7 times lower for the GFP-expressing cells as compared with all cells of the gland (Fig. 2C). Together, these results indicate that a substantial fraction of the differentiated cells in the adult pituitary derive from adult, but not embryonic, stem cells and demonstrate complex dynamics of the stem cell-derived population in the gland.

It is potentially possible that differentiated cells in the above experiments derive from committed progenitor cells with a more limited differentiation repertoire that persist in the adult gland, rather than from multipotential stem cells. To discriminate between these possibilities, we used another lineage-marking approach, genetic inducible fate mapping (27), where the same ROSA-IsI-GFP reporter mice were crossed with animals expressing Cre recombinase fused to the tamoxifen-responsive estrogen receptor ligand binding domain under the control of the nestin gene regulatory elements that had been used to generate the nestin-Cre and nestin-GFP lines (28). In the progeny, recombination, and thus GFP expression, occurs upon addition of tamoxifen and activation of the recombinase; thus, it is possible to achieve a temporal control over the initiation of the lineage analysis. We found that, even in animals of advanced age (10 months), tamoxifen-induced marking revealed terminally differentiated endocrine cells (Fig. 2E). (Note that tamoxifen-induced recombination may be effective in a fraction of potential target cells; thus, we cannot quantify the overall contribution of adult stem cells using this approach.) These results confirm that new differentiated cells are generated in the adult gland and indicate that these cells are derived from nestin-positive multipotential stem cells.

Nestin-GFP Cells Generate Pituitary Lineages *in Vitro*. To extend this *in vivo* evidence of the presence of multipotent stem cells in the anterior pituitary gland, we developed a protocol for isolation and cultivation of nestin-GFP-expressing cells from the adult pituitary [Fig. 3 and supporting information (SI) Fig. S1]. These cells can give rise to exponentially growing colonies. There are $\approx 1,000$ clonogenic cells in the adult anterior pituitary gland, representing ≈ 0.1 – 0.2% of the cells in the gland. These clono-

periluminal precursors as well as in terminally differentiated cells—ACTH-positive, αGSU -positive, and Pit-1-positive cells (fourth image). [Scale bars: 60 μm (first image) and 30 μm (other images).]

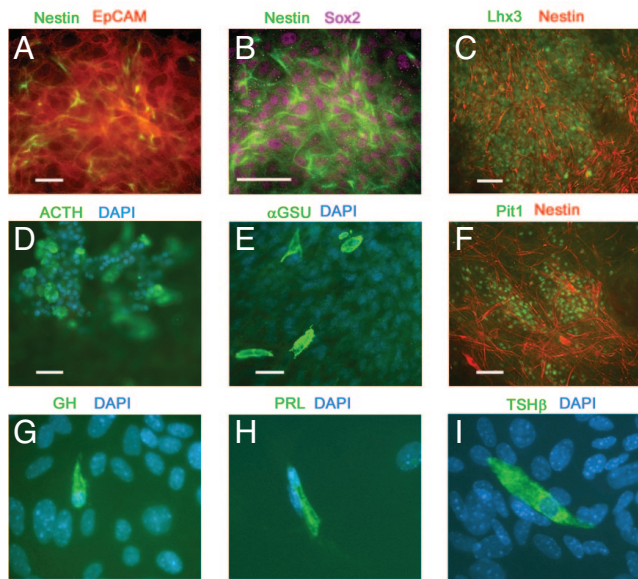


Fig. 3. Nestin-GFP-positive cells can self-renew and differentiate along all pituitary lineages *in vitro*. (A) Epithelial colonies grown from nestin-GFP transgenic mice undergo spontaneous differentiation after exiting from exponential growth. At day 8, the majority of the cells still express EpCAM (A) and Sox2 (B) as Sox2 and Lhx3/nestin double positive cells (A–C). Cell aggregates often contain ACTH-positive cells (D), dispersed α GSU-positive cells (E), and rare Pit-1-positive cells (F) increasing at day 12. (G) GH-positive somatotrophs. (H) PRL-positive lactotrophs. (I) TSH β -positive thyrotrophs. (Scale bars: A, 70 μ m; B, 35 μ m; C and F, 100 μ m; D and E, 10 μ m; the bar in E corresponds to 5 μ m in G–I.)

genic cells can undergo at least 25 divisions, suggesting that they may have stem cell-like properties.

During exponential growth, cells of the colony express the same markers as the nestin-GFP cells of the pituitary gland (GFP, nestin, Sox2, cytokeratin 8, cytokeratin 18, Lhx3, and EpCAM), but they do not express markers of pituitary differentiation (see also *SI Text*). When the cells of the colony exit the exponential growth phase, they start to differentiate and can produce cells of all six anterior pituitary lineages: scattered cells in the colony express adrenocorticotrophic hormone (ACTH), α -subunit (α GSU) of the glycoproteins follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH), and Pit-1, a POU-homeodomain transcription factor that determines the differentiation of somatotrophs, lactotrophs, and thyrotrophs; furthermore, we found cells expressing growth hormone (GH), prolactin (PRL), and TSH β , which mark terminal differentiation of the somatotrophs, lactotrophs, and thyrotrophs, respectively (Fig. 3). These results were confirmed by RNA expression analysis (see *SI Text*). Thus, nestin-GFP cells isolated from the anterior pituitary are undifferentiated and capable of self-renewal and, upon prolonged cultivation, can produce differentiated cells of all anterior pituitary lineages. Together, these results provide additional evidence that nestin-GFP cells of the anterior pituitary correspond to stem cells of the adult gland.

Nestin-GFP Stem-Like Cells in Pituitary Tumors. An emerging concept in the biology of stem cells is their potential connection to carcinogenesis (1, 29, 30). Because nestin-expressing stem cells in the anterior pituitary are the only cell type in the organ that possesses a self-renewal potential, we speculated that these cells may be targeted for the initiation of pituitary tumorigenesis. An attractive genetic model of neuroendocrine tumorigenesis are mice that carry one functional allele of retinoblastoma Rb-1 gene

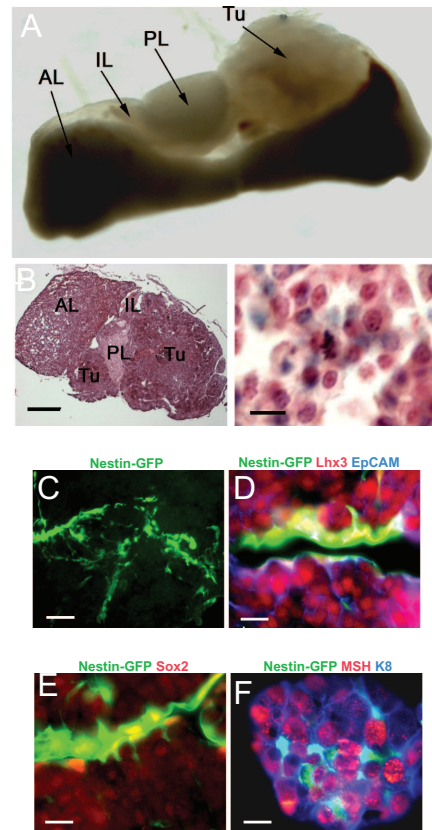


Fig. 4. Nestin-GFP-positive cells in the pituitary tumor. (A and B) Pituitary tumor in a 12-month-old mouse that was *Rb*^{+/-} and carried the nestin-GFP transgene. A macro view (A) and section of the organ (B, H&E) show that the tumor consisted of multiple foci of endocrine proliferating cells arisen in the intermediate lobe that compressed both posterior and intermediate lobes. Divided cells are frequently seen in these nodules (B, H&E). Tu, tumor. [Scale bars: 400 μ m (Left) and 20 μ m (Right).] (C–F) Distribution and phenotype of nestin-GFP-positive cells inside pituitary tumor. Nestin-GFP-positive cells are located mostly on the periphery of tumor nodules (C). They are positive for Lhx3, EpCAM (D), Sox2 (E), and keratin 8 (F), but, in contrast to the majority of tumor cells, they are negative for MSH (F). [Scale bars: 80 μ m (C) and 20 μ m (D–F).]

(*Rb*^{+/-} mice). These animals develop tumors in the intermediate lobe of the pituitary during the second year of life in almost 100% of cases (31, 32). We, therefore, crossed nestin-GFP mice with *Rb*^{+/-} mice to investigate whether cells with a phenotype similar to adult pituitary stem cells could be found in the pituitary tumors. We found well developed tumors growing from the intermediate lobes of 12-month-old *Rb*^{+/-} mice, and consisting of multiple nodules or foci of proliferating endocrine cells (Fig. 4 A and B), predominantly producing melanocyte-stimulating hormone (MSH). Tumor nodules were surrounded by nestin-GFP-positive cells that expressed Lhx3, Sox2, EpCAM, and cytokeratin 8; in contrast to the majority of tumor cells these cells were MSH-negative (Fig. 4 C–F). The similarity of the phenotype of nestin-GFP-positive cells in the pituitary tumors and the adult anterior pituitary stem cells we described above, as well as their spatial distribution inside tumors, is consistent with their representing an undifferentiated cell compartment connected to the initiation and growth of pituitary tumors and with the concept of cancer stem cells.

Discussion

We here describe a population of cells in the adult anterior pituitary that satisfy the criteria for adult organ-specific

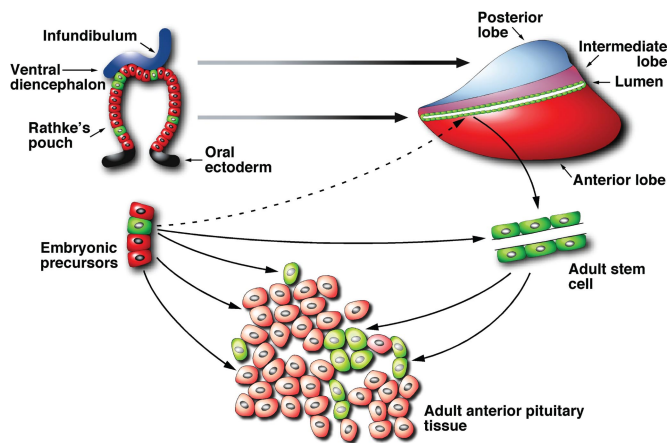


Fig. 5. Schematic representation of the anterior pituitary histogenesis. Anterior pituitary develops from the epithelial primordium (Rathke's pouch), influenced by the contact with ventral diencephalon and regulated by a complex morphogenetic field of growth factors/morphogens. Around embryonic day 11, presumptive adult stem cells arise in the primordium of the anterior pituitary. They reside mostly in the periluminal area and, although normally quiescent, possess high proliferative potential and the ability to generate all terminally differentiated cell types in response to specific physiological stimuli. During postnatal development, they contribute to all of the terminally differentiated endocrine cell types of the anterior pituitary. As a result, the adult anterior pituitary gland became a mosaic of terminally differentiated cells of different origin and with a different life history.

multipotent stem cells: (i) these cells can effectively self-renew in culture; (ii) they can generate cells bearing the markers of all terminally differentiated anterior pituitary lineages *in vivo* and *in vitro*; (iii) they reside in a niche, the periluminal region of the gland; (iv) they contribute significantly to the expansion of all terminally differentiated endocrine pituitary cell types during the postnatal period of pituitary growth; (v) they express key transcription factors that determine pituitary development; (vi) they express nestin, which is a hallmark of an increasing number of adult tissue-specific stem cells; (vii) they represent only a small fraction of cells in the developing pituitary gland, appear later than the embryonic precursors, do not express several important markers of the embryonic pituitary precursors, and contribute minimally to the pituitary of the newborn, all features consistent with their role as stem cells for the adult but not embryonic tissue; and (viii) a small number of cells with the same phenotype reside inside pituitary tumors, compatible with the possibility that they represent tumor stem cells.

Our data suggest that adult stem cells of the anterior pituitary do not play a significant role in the embryonic development of the anterior pituitary, but start functioning and contributing prominently to the gland soon after birth. This is compatible with the emerging evidence that a population of adult stem cells can be maintained and function only after a specific niche is established in the appropriate tissue (33–35). Once entrenched in the niche (in this case, the periluminal region), these stem cells start to produce progeny that can differentiate along the same lineages as embryonically derived cells and colonize the organ. Thus, soon after birth and throughout adulthood the gland represents a mosaic of differentiated cells with similar phenotypes but of different origin, i.e., those that were generated by the nestin⁻/Lhx3⁺/Lhx4⁺/Hesx1⁺ embryonic precursors and those that were produced by nestin⁺/Lhx3⁺/Lhx4⁻/Hesx1⁻ multipotent adult stem cells (Fig. 5).

What could be the physiological implications of generating parallel sets of terminally differentiated hormone-producing

cells from two different sources hosted by the same organ? It is conceivable that differentiated endocrine cells derived postnatally from multipotential stem cells may be better suited to function in a dynamic fashion in response to the constantly changing hormonal and metabolic landscape of an adult organism. This hypothesis would predict that there are subtle but crucial differences between the two (embryonically derived and adult stem cell-derived) populations of differentiated cells in the anterior pituitary; indeed, there is accumulating evidence in support of this idea. For instance, a physiological heterogeneity has been found in the adult pituitary gland where a spatially distinct subset of somatotrophs expresses the receptor for GHRH. Single-point mutation in the gene coding for this receptor resulted in growth retardation accompanied by loss of this subpopulation of somatotrophs (36). Another example is described for rats where a distinct subset of lactotrophs increases with age in females, reaching a peak at the time of sexual maturity (7). Likewise, the mosaic organization of the pituitary gland may explain recent observations that conditional ablation of the cAMP response element binding protein (CREB-1) using nestin-Cre-mediated recombination (the same nestin-Cre line as used in our experiments) results in adult onset of pituitary hypoplasia accompanied by dwarfism (37). Importantly, the embryonic development of the pituitary was not affected, compatible with the notion of two similar but distinct populations of somatotrophs in the adult gland.

Our results argue that the anterior pituitary exemplifies an unusual strategy for using stem cells to maintain the tissue and respond to incoming signals. This strategy differs from that of tissues with dedicated stem cells (e.g., hematopoietic stem cells), facultative stem cells (e.g., oval cells of the liver), or self-renewal of fully differentiated cells (e.g., hepatocytes). The parallel activity of adult stem cells and embryonic precursors causes the adult anterior pituitary to become a mosaic of terminally differentiated cells of different origin with different life histories. This may make the gland better adapted for dynamic responses to physiological and pathological stimuli.

Materials and Methods

Animals. All experiments were performed by using C57B6 mice and nestin-GFP transgenic mice (17) crossed to C57B6 for at least 10 generations. Nestin-Cre transgenic mice [B6.Cg-Tg(Nes-cre)1Kln/J], ROSA-lsl-GFP [Gt(ROSA)26Sortm2Sho] reporter mice, and *Rb*^{+/-} (B6.129S2-Rb1^{tm1Tyj}/J) mice were obtained from The Jackson Laboratory. Generation of Nestin-CreER mice is described in ref. 28. Use of animals was reviewed and approved by the Cold Spring Harbor Laboratory Animal Use and Care Committee.

Immunocytochemistry. Immunolabeling was performed by following standard protocols for tissue fixation and processing (see *SI Text* for details).

Tissue Culture. Pituitaries from wild-type or transgenic mice were dissociated, and cells were plated at low density and grown in the presence of 20 ng/ml bFGF and 50 ng/ml cholera toxin (see *SI Text* for details).

Real-Time Quantitative PCR. Experiments and statistical analysis of the results were performed as described previously (38) (see *SI Text* and Tables S1 and S2 for the procedure details and the list of primers).

Additional Details. The remaining experimental details can be found in *SI Text*.

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Corrections

DEVELOPMENTAL BIOLOGY. For the article “Genetic approaches identify adult pituitary stem cells,” by Anatoli S. Gleiberman, Tatyana Michurina, Juan M. Encinas, Jose L. Roig, Peter Krasnov, Francesca Balordi, Gord Fishell, Michael G. Rosenfeld, and Grigori Enikolopov, which appeared in issue 17, April 29, 2008, of *Proc Natl Acad Sci USA* (105:6332–6337; first published April 24, 2008; 10.1073/pnas.0801644105), the authors note the following: “We regret that we did not insert a reference to the important manuscript by Fauquier *et al.* [Fauquier T, Rizzoti K, Dattani M, Lovell-Badge R, Robinson ICAF (2008) *Proc Natl Acad Sci USA* 105:2907–2912], which published while our manuscript was under review, into the final galley revision of our manuscript. Fauquier *et al.* demonstrate that adult pituitary contains Sox2-positive cells that, upon cultivation *in vitro*, can generate differentiated pituitary lineages.”

www.pnas.org/cgi/doi/10.1073/pnas.0805200105

MEDICAL SCIENCES. For the article “Next generation of adeno-associated virus 2 vectors: Point mutations in tyrosines lead to high-efficiency transduction at lower doses,” by Li Zhong, Baozheng Li, Cathryn S. Mah, Lakshmanan Govindasamy, Mavis Agbandje-McKenna, Mario Cooper, Roland W. Herzog, Irene Zolotukhin, Kenneth H. Warrington, Jr., Kirsten A. Weigel-Van Aken, Jacqueline A. Hobbs, Sergei Zolotukhin, Nicholas Muzyczka, and Arun Srivastava, which appeared in issue 22, June 3, 2008, of *Proc Natl Acad Sci USA* (105:7827–7832; first published May 29, 2008; 10.1073/pnas.0802866105), the authors note that on page 7831, right column, in *Isolation of Nuclear and Cytoplasmic Fractions from HeLa Cells*, line 3, **V444F** should appear as **Y444F**. This error does not affect the conclusions of the article.

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MEDICAL SCIENCES. For the article “The lipofuscin fluorophore A2E perturbs cholesterol metabolism in retinal pigment epithelial cells,” by Aparna Lakkaraju, Silvia C. Finemann, and Enrique Rodriguez-Boulan, which appeared in issue 26, June 26, 2007, of *Proc Natl Acad Sci USA* (104:11026–11031; first published June 19, 2007; 10.1073/pnas.0702504104), the authors note that the following grant was inadvertently omitted from the Acknowledgments: American Health Assistance Foundation Macular Degeneration Research Grant M2006-081 (to E.R.-B.).

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NEUROSCIENCE. For the article “Activity of human hippocampal and amygdala neurons during retrieval of declarative memories,” by Ueli Rutishauser, Erin M. Schuman, and Adam N. Mamelak, which appeared in issue 1, January 8, 2008, of *Proc Natl Acad Sci USA* (105:329–334; first published December 27, 2007; 10.1073/pnas.0706015105), the authors note that in Fig. 2, panels *E* and *F* were transposed. The corrected figure and its legend appear below.

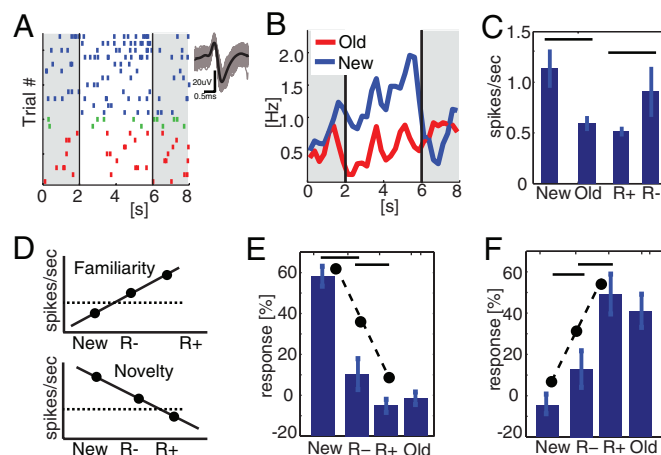


Fig. 2. Single-cell response. (A–C) Firing of a unit in the right hippocampus that increases its firing in response to new stimuli that were correctly recognized (novelty detector). (A) Raster of all trials during retrieval and the waveforms associated with every spike. Trials: new (blue), old and recollected (red, R⁺), and old and not recollected (green, R⁻). (B) Poststimulus time histogram. (C) Mean number of spikes after stimulus onset. Firing was significantly larger in response to new stimuli, and the neuron fired more spikes in response to stimuli that were later not recollected compared with stimuli that were recollected. (D) The hypothesis: The less that novelty neurons fire, the more likely it is that a stimulus will be recollected. The more that familiarity-detecting neurons fire, the more likely it is that a stimulus will be recollected. The dashed line indicates the baseline. (E and F) Normalized firing rate (baseline = 0) of all novelty (E) and familiarity-detecting (F) neurons during above-chance sessions (30-min R⁺). Novelty neurons fired more in response to not recollected items (R⁻), whereas familiarity neurons fired more in response to recollected items (R⁺). Errors are \pm SEM. Number of trials, from left to right: 388, 79, 259, and 338 (E) and 132, 31, 96, and 127 (F).

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