to 70,000 cal yr B.P.). It remained partly emergent during the warm Karginsk/MW Interval (23,000 to 50,000 cal yr B.P.). Paleo valleys of several major rivers (Lena, Yana, and others) can be traced on the Arctic shelf to a depth of 50 m at this time (23). The Yana RHS offers bifacial technology with no sign of blade making. By contrast, the Dyukha culture combines bifaces with a blade industry based on wedge-shaped cores (3). Its earliest appearance in the Bering Land Bridge region dates to about 12,000 to 11,000 cal yr B.P. (24). Yana is 27,000 radiocarbon yr B.P. In theory, the Yana people may have crossed over the land bridge toward the end of Karginsk Interval.

It is difficult to assess similarities between Yana RHS and Clovis faunas. Thousands of kilometers and roughly 16,000 years separate them. Their similarity is intriguing, and they both have bifacial industries. Although a direct connection remains tenuous, the Yana RHS site indicates that humans extended deep into the Arctic during colder Pleistocene times.

References and Notes

13. Apparently, these terraces present Terrace 3 (the higher one, 35 to 40 m a.w.l.) and Terrace 2 (lower terrace, 16 to 18 m a.w.l.). Terrace 1 is not presented within the bluff. Presumably, it is totally eroded here. The floodplain terrace, or Terrace 0, exists here in the form of small sections.
14. First 14C dates for the site obtained by L. D. Sulerzhitsky (Geological Institute, Russian Academy of Sciences, Moscow).
15. Species identification for 14C samples provided by E. A. Vangenheim (Geological Institute, Russian Academy of Sciences, Moscow).
16. The Yana RHS bone collection offers the full range of carnivore species present at the end of the Karginsk Interval. Carnivore bones, especially those of the large species, rarely appear in natural exposures. The appearance of so many bones in one place suggests human activity in the past.
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oplasia of the cerebral hemispheres (Fig. 1A) (23, 24). However, the principal mechanism by which Foxg1 regulates this early step in cortical development has remained elusive.

**Most cortical neurons in Foxg1 null mutants become CR cells.** To address which cortical cell types are generated in the Foxg1−/− mutants, we examined the expression of layer-specific markers at embryonic day 18.5 (E18.5), the latest time point at which these mutants are viable. In the E18.5 wild-type cortex, only the deeper layers (layers 4 to 6) have achieved their mature laminar organization. These layers are characterized by the expression of Otx1, Otx2, and ROBβ (Fig. 1C) (25–28). In situ analysis of the telencephalon revealed that the Foxg1−/− mice failed to express any of these genes, demonstrating that these cortical laminae are absent in these mutants.

In contrast, we observed that the earliest born CR neurons were not only present but supernumerary in these mice (Fig. 1C, bottom right panel). CR neurons can be identified by a number of criteria. Foremost among these is their expression of Reelin. In addition, CR cells express both calretinin (29) and CXCR4 (30). Consistent with the Foxg1 telencephalon’s possessing increased numbers of CR cells, we observed the widespread expression of both of these markers in the cortex of the mutant mice (Fig. S1).

To determine how this overproduction of CR neurons occurs, we examined progressive stages of corticogenesis in these mutants. The cortex of Foxg1−/− mice appeared phenotypically indistinguishable from wild-type animals from E8.5 through E10.5. Furthermore, at E10.5, when the normal production of CR cells is occurring, we observed that the distribution and number of these cells is comparable in Foxg1 null mice compared with wild-type littermates. During normal cortical development, although all postmitotic cells express microtubule-associated protein 2 (MAP2), only those cells in the most superficial layer of the cortex express CR-50. In contrast, in the Foxg1 null mutant cortex, the entire MAP2 population appears to express CR-50 (fig. S2 and Fig. 2).

**Foxg1 cell-autonomously represses CR cell identity.** To evaluate whether the repression of CR cell production is cell-autonomously regulated by Foxg1, we assessed the normal expression of Foxg1 in this population with a Foxg1loxP/loxP transgenic. Except for the presence of β-galactosidase–positive cells, Foxg1loxP/loxP heterozygous mice are phenotypically indistinguishable from wild-type mice (23). Previous studies have demonstrated that Foxg1 is expressed in progenitor cells (20). We found that expression persisted in postmitotic neurons in the cortical plate (Fig. 3A). However, we observed that LacZ expression was excluded from the early-born CR neurons. At E18.5, individual CR cells were seen within the marginal zone (MZ), a region only sparsely populated with cells (Fig. 3C). Although a few LacZ-positive cells were also detected in the MZ of Foxg1−/−, these likely represent cells that migrated from the ventral telencephalon during later neurogenesis (31). Consistent with this notion, these cells never coexpressed CR-50 (Fig. 3C).

Our results suggest that Foxg1 cell-autonomously represses CR cell identity. Conversely, we found evidence that Foxg1 expression was repressed in CR cells. Specifically, the complementary expression of LacZ and CR-50 cells persisted in Foxg1loxZ/loxZ null cortex. In these mice, LacZ was expressed in neural progenitor cells before CR cell differentiation but was absent in mature CR cells in the cortex (fig. S3). Taken together, these results suggest that early neuronal fate is suppressed by Foxg1 in later progenitors in the wild-type cortex.

The absence of Foxg1 expression in CR cells in the cortex raises the question of whether these two populations are segregated from the onset of neural development. To address this issue, we crossed Foxg1Cre+ mice (32) onto a ROSA26 reporter (R26R) line (33). This allowed us to identify cells
that expressed Foxg1 at any point of their development by evaluating whether β-galactosidase was produced. Examination of the Foxg1tetO-cre; R26R cortex revealed that the majority of the CR cells were LacZ-positive (Fig. 4, A and B). This suggests that most CR cells express Foxg1 at some point during their development. We envisioned two scenarios: (i) The total complement of CR cells may be committed before the onset of Foxg1 expression, making them refractory to the effects of Foxg1; or (ii) continued Foxg1 expression may be required to suppress neurons born later in cortical development from adopting an early neuronal fate. This raises the question of whether the inability of later progenitors to give rise to CR cells reflects a loss of competence or suppression by Foxg1.

To differentiate between these possibilities, we used the tet-transactivator (tTA) system (24) to conditionally remove the Foxg1 gene function in the progenitor cells after the normal birthdate of CR cells had passed. To achieve this, we replaced the endogenous Foxg1 gene with a tTA-regulated Foxg1 gene by generating Foxg1lacZ-tTA-tetOFoxg1-IRESlacZ mice (Foxg1-rescued mice, designated as Foxg1tetO-flox1-lacZ, IRES, internal ribosome entry site). We confirmed that in mice where Foxg1 expression was rescued, cortical lamination was restored (fig. S4) and the CR neurons were confined to the superficial layer as in wild-type animals.

Removal of Foxg1 function at E13 reinitiates CR cell production. We next selectively removed Foxg1 gene function in these mice at E13 by administering doxycycline (2 mg/ml daily in drinking water). We refer to these mice as Foxg1tetO-flox1-E13Doxy. In wild-type mice (and in Foxg1tetO-flox1 mice), this is the time point when layer 5 neurons are being generated. Interestingly, the removal of Foxg1 expression at E13 resulted in the resumption of CR cell production in the cortex (Fig. 5A, compare the left and middle panels), indicating that a significant number of CR cells were born later in cortical development from adopting a CR fate. The CR cells that were born later in normal siblings were excluded from both E14.5 (A) and E18.5 (C) MZ (marginal layer) CR cells. Arrows in (C) and (D) indicate CR cells in the MZ in the E18.5 cortex, all of which are LacZ-negative.

Newborn E13 neurons become CR cells in the absence of Foxg1 gene function. To determine whether CR cells in Foxg1tetO-flox1-E13Doxy mice arose from newborn neurons, we used 5-bromo-2'-deoxyuridine (BrDU) to pulse-label dividing cells in these mice subsequent to the administration of doxycycline. The majority of BrDU-labeled cells in these animals expressed reelin (Fig. 5D, bottom right panel), indicating that these CR neurons likely represent cells that exited the cell cycle subsequent to removal of Foxg1 gene function. These results suggest that in Foxg1tetO-flox1-E13Doxy mutants, neural progenitors that are normally committed to generating deeper layer neurons revert to pro-
As we learn more about the Milky Way Galaxy, extrasolar planets, and the evolution of life on Earth, qualitative discussions of the prerequisites for life in a Galactic context can become more quantitative (3–5). The Galactic habitable zone (GHZ) (4), analogous to the concept of the circumstellar habitable zone (5), is an annular region lying in the plane of the Galactic disk possessing the heavy elements necessary to form terrestrial planets and a sufficiently element environment over several billion years to allow the biological evolution of complex multicellular life. In order to more quantitatively estimate the position, size, and time evolution of the prerequisites for life in a Galactic context can become more quantitative (3–5). The Galactic habitable zone (GHZ) (4), analogous to the concept of the circumstellar habitable zone (5), is an annular region lying in the plane of the Galactic disk possessing the heavy elements necessary to form terrestrial planets and a sufficiently element environment over several billion years to allow the biological evolution of complex multicellular life. In order to more quantitatively estimate the position, size, and time evolution of the

GHZ, we combined an updated model of the evolution of the Galaxy (6) with metallicity constraints derived from extrasolar planet data (7).

Of the factors that determine the location of the GHZ, the abundance of elements heavier than hydrogen and helium (metallicity) is particularly crucial because these elements are what terrestrial planets are composed of. The current metallicity of the Galaxy can be directly measured. However, modeling is needed to identify the metallicity distribution throughout the history of the Milky Way.

We simulated the formation of the Galaxy with the use of two overlapping episodes of accretion that correspond to the buildup of the halo and disk. The gas accretion rate falls off exponentially on a small (~1 Gyea (Gy)) time scale for the first phase and a longer time scale (~7 Gy) for the second phase. Although there is a 1-Gy delay between the onset of halo formation and the onset of thin disk formation, the formation of these two components overlaps in time. In our model, we monitor the creation of heavy elements and the exchange of matter between stars and gas. Model parameters have been chosen to reproduce the key observational constraints, namely, the radial distribution of stars, gases, and metals; the metallicity constraints derived from extrasolar planet data (7).

The Galactic Habitable Zone and the Age Distribution of Complex Life in the Milky Way

Charles H. Lineweaver,1,2* Yeshe Fenner,3* Brad K. Gibson**

We modeled the evolution of the Milky Way Galaxy to trace the distribution in space and time of four prerequisites for complex life: the presence of a host star, enough heavy elements to form terrestrial planets, sufficient time for biological evolution, and an environment free of life-extinguishing supernovae. We identified the Galactic habitable zone (GHZ) as an annular region between 7 and 9 kiloparsecs from the Galactic center that widens with time and is composed of stars that formed between 8 and 4 billion years ago. This GHZ yields an age distribution for the complex life that may inhabit our Galaxy. We found that 75% of the stars in the GHZ are older than the Sun.

References and Notes
34. We thank S. Mungakar, A. Schier, and A. Jaye for critical reading of the manuscript; S. McConnell for Foxg1Cre mice; M. Ogawa for CR-50 antibody; T. Jessell, T. Curran, E. Morrisey, R. Slack, and A. Simeone for BRIT, Reelin, Foxp1, Foxp2, ROR, and Ob1 in situ probe templates; A. Barzadeh, K. Gurijala, and D. Lomonte for technical assistance; and members of the Fishell laboratory for helpful discussions. Work in G.F.’s laboratory was supported by NIH grants (NS59007 and NS6493) and a March of Dimes basic research grant; work in E.L.’s laboratory was supported by NIH grants (HD29584 and EY11124).

Supporting Online Material
www.sciencemag.org/cgi/content/full/303/5654/56/
DC1: Materials and Methods
Figs. S1 to S6
References
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Supporting Online Material

Includes: Materials and Methods

Animals

Foxg1⁻/⁻ null mice were obtained by intercrossing Foxg1⁺/⁺ mice as described previously (S1). Mice in which Foxg1 expression was rescued were generated by cloning the Foxg1 sequence into pUHD10.3 plasmid containing multiple tet-Operator sites and the IRESlacZ reporter cassette. The eight transgenic lines generated with this transgene were crossed with Foxg1TA/⁺ mice (S2) to examine these animals for their expression of Foxg1 and the extent to which they compensated for the loss of this gene. Of these, 4 lines were found to have moderate expression levels of Foxg1. These lines were then mated with Foxg1lacZ/⁺ to obtain the Foxg1lacZ/⁺;tetOFoxg1-IRESlacZ mice. These in turn were crossed with the Foxg1TA/⁺ mice to generate Foxg1lacZ/TA;tetOFoxg1-IRESlacZ embryos, resulting in mice whose cerebral cortex resembled wild type animals (S2). We refer to these embryos as Foxg1-rescued animals (for simplicity these are designated as Foxg1tetO-foxg1). These embryos lack the endogenous Foxg1 gene but express Foxg1 under the control of tTA activation. Foxg1 expression was abolished in these embryos when pregnant mothers were fed 0.2µg/ml or higher doxycycline concentrations in the drinking water (which we designate as Foxg1tetO-foxg1-EXDox, where X refers to the date of doxycycline administration). Embryos of this genotype were obtained from timed pregnancies where
noon of the plug date was designated as E0.5. These embryos were fixed in 4%
paraformaldehyde and embedded in OCT. β-gal staining on 10µm frozen sections was
performed as described (S2).

**In situ** hybridization and Immunohistochemistry

**In situ** hybridization using digoxigenin-labeled probes was performed as previously
described (S2). **In situ** cDNA probe templates were kindly provided by T. Curran (reelin),
T. Jessell (ER81), E. Morrisey (Foxp1, Foxp2), A. Simeone (Otx1), R. Slack (RORb).

Double-labeling of CR-50 and MAP2 or CR-50 and LacZ immunoreactivity was
performed using the mouse MOM kit (Vector Labs). Sections were incubated overnight
with mouse monoclonal CR-50 antibody (1:50) (S3). CR-50 immunoreactivity was
visualized using biotinylated α-mouse antibody (1:500) followed by fluorescein-
conjugated streptavidin or Texas Red-conjugated streptavidin (Vector). Sections were then
incubated with α-MAP2 antibody (1:250 Sigma) or α-β-gal antibody (1:100 Biogenesis)
for 1 hr followed by the biotinylated α-mouse antibody or Cy3-conjugated donkey α-goat
antibody.

Double-labeling of LacZ and CR-50 was performed by first staining the sections with
X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) for 3 hours (S1) and subsequently
processing for CR-50 immunohistochemistry as described above. CR-50 immunoreactivity
was visualized using the Vector ABC system (Vector).
BrdU labeling

For BrdU labeling, pregnant dams were given a single injection of BrdU (30mg/kg body weight, i.p.). Embryos were harvested at a given date and fixed and processed for BrdU immunohistochemistry as described previously (S2). Double-labeling of reelin and BrdU or ER81 and BrdU was performed by first amplifying Reelin and ER81 signals with biotinylated-tyramide followed by fluorochrome-conjugated streptavidin, treated with 1N HCl for 10min at 55ºC, then processed for BrdU immunohistochemistry as above.

Doxycycline administration

To conditionally knockout the Foxg1 gene function, pregnant females from Foxg1<sup>lacZ</sup>+/tetOFoxg1-IRES lacZ and Foxg1<sup>TA+</sup> matings (i.e. Foxg1<sup>teto-foxg1</sup>) were fed daily from E13 onward 2mg/ml doxycycline in their drinking water with 5% sucrose (which we designate as Foxg1<sup>teto-foxg1-E13Dox</sup>). BrdU (30mg/kg body weight) was injected 6 hrs later to label newly born cells subsequent to doxycycline administration. Embryos were harvested at E16.5 and processed as above.
FIGURE LEGENDS

Fig. S1: Increased CXCR4 and calretinin expressing cells in the Foxg1<sup>−/−</sup> null cortex.

CXCR4 expression is confined to the superficial layer of the cortex, whereas calretinin is expressed in both the MZ and subplate cells in Foxg1<sup>+/−</sup> heterozygotes. In Foxg1<sup>−/−</sup> mutants, both markers are expressed throughout the cortex.

Fig. S2: Supernumerary production of CR neurons in the Foxg1<sup>−/−</sup> cortex. Boxed areas in the cresyl violet stained coronal sections panels on the left represent the regions enlarged in A-C and D-F respectively. (B) The CR cell population in the Foxg1<sup>+/−</sup> telencephalon at E11.5, visualized by CR-50 immunoreactivity (green), represents a subpopulation of the MAP2 neuronal population (A). In Foxg1<sup>−/−</sup> cortex the entire MAP2 population (D) co-expresses CR-50 (E and F).

Fig. S3: Foxg1 expression is repressed in CR cells. Double-labeling of CR-50 (green) and lacZ (red) immunoreactivity in Foxg1<sup>lacZ/lacZ</sup> null mutants have a complementary expression pattern. LacZ is expressed in the VZ progenitor cells in the Foxg1<sup>lacZ/lacZ</sup> telencephalon but is excluded from mature CR cells expressing CR-50. The boxed region in the top right panel indicates an enlarged view, demonstrating that LacZ and CR-50 populations are mixed but distinct.
Fig. S4: Cortical lamination is normal in Foxg1\textsuperscript{tetO-foxg1} (i.e. Foxg1-rescued animals).
Expression of deep-layer cortical markers (Otx1, Foxp2, ER81, Foxp1) is restored in the E18.5 Foxg1\textsuperscript{tetO-foxg1} telencephalon. Reelin-expressing CR neurons are restricted to the marginal zone in Foxg1\textsuperscript{tetO-foxg1} animals and resemble that seen in heterozygote littermates.

Fig. S5: Increased numbers of CR cells are observed in the MZ of Foxg1\textsuperscript{tetO-foxg1}-E13Doxy mice. The number of Reelin and CXCR4 expressing cells is increased in the MZ of Foxg1\textsuperscript{tetO-foxg1}-E13Doxy mutant mice (right panel) compared to the doxycycline fed heterozygote littermates (left panel). Quantitation of number of cells in the MZ of these mice showed an increase of ~50% in mutant versus heterozygote littermates. Single (P<0.01) and double (P<0.001) asterisks indicate statistical significance.

Fig. S6: Newly born cells in the Foxg1\textsuperscript{tetO-foxg1}-E13Doxy mutant cortex do not adopt a deep-layer fate. ER81 (green) and BrdU (red) double-label shows that newborn cells are ER81-negative (middle row). In the heterozygote controls, these two populations partially overlap (top row). The newly born reelin-positive cells (red) do not co-express ER81 (bottom row) suggesting that the neurons rising from progenitor cells in which Foxg1 gene function is removed do not adopt a hybrid deep-layer/CR cell fate.

Supporting References

S1. S. Xuan et al., Neuron 14, 1141-52 (Jun, 1995).