Mutations that cause autosomal recessive primary microcephaly (MCPH), including MCPH1 through MCPH6, have provided insight into the normal programming that directs brain growth and defines ultimate brain size. The common denominator in these mutations is that they all manifest within neural stem and progenitor cells, decreasing their numbers at various stages of neurogenesis. Microcephalin (MCPH1) and abnormal spindlelike, microcephaly associated (MCPH5) have been the focus of most of the research. However, the recent discovery of microcephaly caused by mutation of the N-myc (also MYCN) proto-oncogene both in mice, where it was directed specifically to neural stem cells, and in the germ line in humans in Feingold syndrome has shed new light on the role of neural stem cells in brain growth. N-myc controls brain growth not only by regulating neural stem cell proliferation, but also through maintaining a neural stem cell identity at least in part via a mechanism involving global chromatin. Interestingly, along with microcephaly, mutation of N-myc also causes chromatin condensation in neural stem cells, while premature chromosome condensation (PCC) is observed with mutation of MCPH1. The fact that 2 genes required for brain growth are also essential for normal chromatin structure suggests that the global chromatin activity state of neural stem cells is a key factor in regulation of brain mass. In this review, we will focus on the links between neural stem cell chromatin and brain growth.

One avenue that has proved fruitful in gaining insight into the regulation of brain size is the study of mutations that impair brain growth in mice and humans. Because brain and head growth are intimately connected, these mutations usually result in both a small head and brain, manifesting as microcephaly, frequently but not always correlating with reduced intelligence. Six loci have been associated with MCPH, with MCPH1 and MCPH5 being the most thoroughly studied. Other mutations cause microcephaly as well, although not MCPH, as these changes are associated with other developmental defects. The notion that neural stem cells play a key role in brain growth is supported by the fact that studies of genes mutated in microcephaly indicate that a common element is their influence on maintenance of appropriate stage-specific neural stem cell populations.

How are neural stem cell pools controlled? In theory, any biological process that changes the number of neural stem cells or the ultimate cell size of their differentiated progeny during neurogenesis could impact the eventual size of the brain since brain mass is largely a product of the number of the cells and their mean cellular mass. Mutation of genes that regulate these processes would in turn impinge on brain growth and size. Besides proliferation there are a host of other cellular processes that, invoked through neural stem cells, could potentially impact brain growth and size: asymmetric vs symmetric division, differentiation, migration, cell survival, and morphogen activity. The diversity of these processes illustrates the complexity of the programs that direct...
neural stem cell biology and in turn drive appropriate brain growth. Although each of these processes has been implicated in murine brain growth, their relative importance for human brain growth remains largely unclear. An emerging but largely unexplored concept is the notion that the global chromatin or epigenetic state of neural stem cells may also be essential for their normal function and, in turn, brain growth.

**CHROMATIN ON THE BRAIN: MCHP1**

Chromatin activity may be of particular importance for neural stem cell biology because it influences numerous aspects of cell function, including pluripotency and self-renewal; however, it is also possible that the importance of neural stem cell chromatin for brain growth is strictly a function of its key role in the regulation of neural stem cell mitosis. The first indication that chromatin plays a key role in brain growth came from studies of cells from patients with MCPH and with PCC syndrome, which both turn out to be caused by mutations in MCHP1. The fact that mutation of MCHP1 causes both PCC and microcephaly suggests the overall chromatin state of neural stem cells is a key determinant of brain growth. However, to my knowledge, the chromatin state specifically of neural stem cells in patients with MCPH or PCC has not been analyzed. Indeed, the link between MCHP1 and chromatin has only been reported in lymphocytes from patients with MCPH and in immortalized HeLa cells after short interfering RNA knockdown of MCHP1. Thus, a key open question is whether MCHP1 regulates chromatin specifically in neural stem cells; however, given the expression of MCHP1 in the neuroepithelium, a function in neural stem cells seems likely.

How does MCHP1 influence chromatin? An obstacle to answering this question is the fact that more generally the function of the MCHP1 protein remains largely unknown. MCHP1 contains 3 BRCT domains with homology to the C terminus of BRCA1. These domains are conserved in proteins that regulate the response to DNA damage and cell cycle checkpoints. The function of the BRCT domains of MCHP1 has not been investigated, but as the only known domains in MCPH1, it is tempting to speculate that they play at least some role in its influence on chromatin. However, MCHP1 could influence chromatin indirectly via changes in the cell cycle; recently, MCHP1 has been shown to regulate entry into mitosis at least in part in conjunction with ATR, another damage-response protein containing BRCT domains. A function for ATR itself in brain growth is indicated by its mutation in Seckel syndrome, which is also characterized by severe microcephaly. An ATR-independent role for MCPH1 in regulating CDK1 phosphorylation is hypothesized to be involved in PCC in MCPH1 mutant cells. At this point, the specific mechanism by which MCPH1 regulates chromatin and the important question of whether it is a direct or indirect mechanism await further study.

**MCPH2 THROUGH MCPH6 AND CHROMOSOME DYNAMICS**

Five other autosomal recessive microcephaly genes have also been identified (MCPH2-MCPH6). One common functional link between MCPH1, MCPH5, MCPH3 (CDK5RAP2), and MCPH6 (CENPJ) is that they impinge on mitosis through influence on cell cycle and/or the chromosome dynamics. MCPH3 (CDK5RAP2) and MCPH6 (CENPJ) are both localized and function at centrosomes. MCPH genes likely not only influence the rate of successful neural stem cell mitosis, but also the type of cellular division (asymmetric vs symmetric). Indeed, their influence on chromatin, centrosomes, and the spindle, MCPH genes likely direct the balance between symmetric and asymmetric division. The specific genes at the MCPH2 and MCPH4 loci remain unknown. Their identification is likely to provide further insight into the importance of neural stem cell function in brain growth.

**Myc ON THE BRAIN**

It is clear that many other genes beyond those linked specifically to primary microcephaly play key roles in neural stem cell function and brain growth in addition to their influence on the development of other organs. The Myc family of proto-oncogenes fits into this category. Myc is most famous as one of the first oncogenes identified and arguably the most common oncogene misregulated in human cancer. Shortly after the identification of Myc, a second Myc family member, N-Myc (also MYCN) was identified as an Myc-related sequence amplified in neuroblastoma as well as other tumors, particularly those of the nervous system, including other primitive neuroectodermal tumors such as retinoblastoma and medulloblastoma. N-Myc–related tumors are characterized by
“blast”-like, primitive cells. N-Myc is expressed widely in the murine embryo, with particularly high expression in the ventricular zone of the embryonic neuroepithelium.\textsuperscript{13} Constitutive knockout of N-Myc results in embryonic lethality around midgestation characterized by a broad failure of embryogenesis, consistent with the expansive expression of N-Myc, but yielding little insight into its function in any specific organ system, including the nervous system.\textsuperscript{14} More recent studies using conditional knockout of N-Myc specifically in neural stem cells allowed for a thorough analysis of its role in neurogenesis and neural stem cell function.\textsuperscript{13} Mice with N-Myc–deficient neural stem cells have pronounced microcephaly characterized by a nearly 2-fold reduction in brain mass and a particularly severe disruption of growth of the cerebellum, 3- to 5-fold reduced in growth with dramatic reductions in granule neurons. Neural stem cell cultures, also called neurospheres, represent a powerful yet untapped tool for exploring the mechanisms by which MCPH genes function (Figure 1). For example, neural stem cell cultures derived from the N-Myc–null mice exhibit enhanced neuronal differentiation and slowed proliferation.\textsuperscript{13}

One of the most striking cellular phenotypes of N-Myc knockout neural stem cells is nuclear condensation, present apparently irrespective of their location in the ventricular zone and of their phase of the cell cycle.\textsuperscript{13} Neural stem cells lacking N-Myc also have strongly reduced bromodeoxyuridine incorporation as well as enhanced neuronal differentiation, suggesting a loss of “stemness” and conversely that a normal function of N-Myc is maintenance of a functional stem cell identity. Subsequently, it was shown that N-Myc and c-Myc are global chromatin regulators in neural stem and progenitor cells, required for maintenance of broad domains of active euchromatin.\textsuperscript{15} While the specific mechanism by which Myc regulates global chromatin remains to be defined, evidence suggests it is mediated at least in part directly by Myc on chromatin.

The pronounced microcephaly in mice with N-Myc–deficient neural stem cells together with N-myc’s role as a potent human neural oncogene suggests that N-myc may be an important regulator of normal human brain growth as well. This hypothesis was confirmed when N-myc was mapped as the mutated gene in Feingold syn-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Model of mechanisms in neural stem cells by which mutations cause microcephaly. Shown are genes whose mutation is linked to microcephaly and the known or proposed mechanisms by which they impinge on neural stem cell (NSC) function to influence brain growth. Most genes influence the nature (asymmetric vs symmetric) or rate of cell division through regulating chromatin.}
\end{figure}
Feingold syndrome is characterized by microcephaly and small stature, interestingly both also characteristics of patients with MCPH and PCC, as well as other defects including esophageal atresia. Remarkably, constitutively null N-Myc mice exhibit many of the same traits as patients with Feingold syndrome, MCPH, and PCC. It will be of great interest to determine if patients with Feingold syndrome also exhibit PCC. N-Myc is clearly distinct from the MCPH genes in a number of ways. First, it is expressed and functions rather ubiquitously during embryogenesis. Second, in addition to its broad chromatin activity, Myc also functions as a transcription factor and indeed may be the first transcription factor implicated in human microcephaly. Finally, unlike the recessive MCPH mutations, mutation of N-myc in Feingold syndrome is present on a single allele and is dominant in humans. It remains unclear how mutation of 1 copy of N-myc causes Feingold syndrome and microcephaly, especially since N-Myc heterozygosity in mice, constitutive or neural stem cell specific, causes little if any phenotype. It is possible that human neural stem cells are more sensitive to N-myc dosage or that compensation by other Myc proteins is more robust in the mouse. Finally, it is also possible that specific mutant N-Myc proteins in Feingold syndrome act as dominant negatives.

A CHROMATIN CONNECTION?

Is there a connection between the chromatin phenotypes observed with loss of MCHP1 and loss of N-myc, and what does this tell us about neural stem cell biology and brain growth? It remains to be seen if there are commonalities between the influence of MCHP1 and N-Myc on chromatin. The influence of MCHP1 on chromatin may be an indirect effect resulting from alterations in the cell cycle, such as premature entry into mitosis. Indeed, there is evidence that MCHP1-deficient cells exhibit impaired chromatin decondensation after exit from mitosis, supporting the notion that MCHP1 is in fact more of a cell cycle gene than a chromatin regulator. Myc, in contrast, appears to influence cell cycle and chromatin through mechanisms that are largely independent of each other. A dual model for Myc regulation of chromatin invokes both indirect, through target gene regulation, and direct mechanisms, via Myc recruitment of histone modifiers including histone acetyltransferases to chromatin. While Myc’s influence on chromatin is most likely not a secondary cause of changes in the cell cycle, changes in the cell cycle are likely to result from Myc’s effects on chromatin as well. While chromatin plays a key role in the action of both proteins and in the microcephaly that results from their mutation, the evidence to date would suggest their regulation of chromatin is through distinct mechanisms. However, importantly, the fact that both MCHP1 and N-myc are required for normal overall chromatin structure and normal brain growth makes a compelling argument that the global chromatin state of neural stem cells is an essential and novel determinant of brain growth.

What is the functional link between stem cell chromatin structure and brain growth? At this point, the best model invokes a combination of several mechanisms (Figure 2). At one level, loss of MCHP1 and N-myc both clearly disrupt normal cell cycling and affect brain growth by reducing neural stem cell populations through impaired proliferation. Another mechanism appears to involve cell survival because the abnormal chromatin state of MCHP1-null cells likely also triggers apoptosis, further reducing neural stem cell populations. However, at this point there is no evidence that N-Myc–deficient neural stem cells have altered survival. A final hypothesis is that the chromatin changes due to loss of MCHP1 and N-myc have a direct effect on the balance between symmetric and asymmetric division. Abnormally condensed chromatin may lead to elevated levels of asymmetric division and differentiation, depleting neural stem cell pools.

FUTURE DIRECTIONS

Additional studies on MCHP1, MCHP3, MCHP5, and MCHP6 as well as the identification and analysis of MCPH2 and MCPH4 should provide further insight into the molecular mechanisms influencing neural stem cell biology and brain growth. However, the power of conditional murine knockouts and the discovery of additional nonprimary microcephaly genes, specifically in neural stem cells, will expand the horizons of the causes of microcephaly as well as their links to neural stem cells and chromatin. By producing phenotypes of primary microcephaly in mice, future neural stem cell–specific conditional murine knockouts will almost certainly add many additional genes to the microcephaly-related category that would otherwise be missed. These genes are likely to shed light on additional novel neural stem cell functions implicated in both murine and human brain growth but also further our understanding of how MCHP1 through MCHP6 function. A greater understanding of the determinants of brain size that manifest through neural stem cell chromatin may aid treatment of brain tumors and promote the development of future neural stem cell–based regenerative medicine therapies that are both safe, namely free from inducing tumors, and effective.

Accepted for Publication: September 11, 2007.
Correspondence: Paul S. Knoepfler, PhD, Department of Cell Biology and Human Anatomy, Institute of Pediatric Regenerative Medicine, Shriners Hospital for Children Northern California, University of California Davis School of Medicine, Sacramento, CA 95817 (knoepfler@ucdavis.edu).

Financial Disclosure: None reported.

REFERENCES