QUERKOPF, A HISTONE ACETYLTRANSFERASE, IS ESSENTIAL FOR EMBRYONIC NEUROGENESIS.

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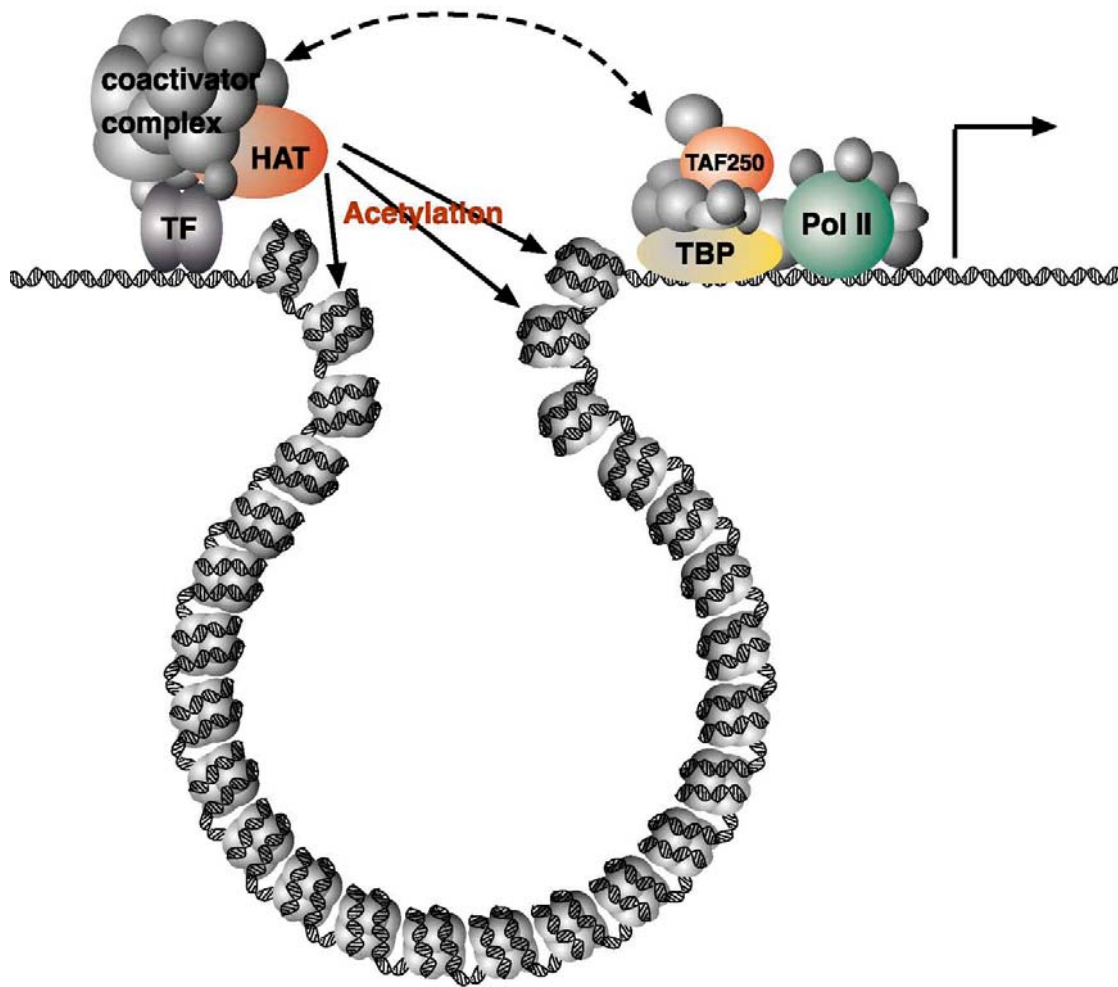
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1. ABSTRACT

DNA binding transcription factors require the presence of co-activators in order to exert their effects on the pattern of gene expression in a cell. An essential element of co-activator complexes is one or more proteins that have histone acetyltransferase activity. In a gene trap screen for mutations affecting brain development, we identified a member of the MYST family histone acetyltransferases, Querkopf. Querkopf is the mouse homologue of the human protein MORF and both these proteins are closely related to MOZ. Querkopf shows a dynamic pattern of expression in the telencephalon. It is initially expressed strongly in the dorsal telencephalon and then in the ventral telencephalon. This suggests that, unusually for a histone acetyltransferase, part of its activity is regulated at the transcriptional level. Mice carrying a mutation in the *querkopf* gene have defects in the development of the cerebral cortex. At all stages of fetal development *querkopf* mutant mice show a reduced number of cells in the cortical plate resulting in a reduction in the size of the adult cortex. The adult cortex in these mice contains less large pyramidal cells and a reduced number of interneurons. In addition Querkopf is also involved in adult neurogenesis. In this short review we examine the role of co-activators of transcription in general and the function of Querkopf in particular.

2. INTRODUCTION.

In the last decade a large number of DNA binding transcription factors required for normal patterning of the brain during embryonic development have been identified. Despite the progress in identifying the necessary components of the regulatory systems involved in brain patterning the question remains, how does the action of these transcription factors ultimately result in anatomically distinct brain regions?

Results from targeted mutations have shown us two basic principles of transcription factor action, which need to be accommodated in any theory of how patterning occurs during development. Firstly, key DNA binding transcription factors, those regulating the development of whole structures, i.e. master control genes, typically regulate a variety of developmental processes in diverse cell types. An example of this is the paired domain transcription factor, Pax6. Pax6 is critical for eye development in both vertebrates and invertebrates and has been described as the master control gene for eye development (1). In addition Pax6 has essential roles in cranio-facil development, cortex development and the formation of glucagon producing cells in the pancreas (2). Not only does Pax6 regulate different sets of genes but it also acts at different levels in the regulatory hierarchy in different cell types. Secondly, and perhaps more surprisingly, very little of the protein sequence is essential for the action of DNA binding transcription factors. For example Otx2, a homeo domain transcription factor related to the *Drosophila* protein orthodentical, is required for gastrulation and for forebrain development. However, despite the fact that only a small portion of the protein, the DNA binding homeo domain, is homologous to the *Drosophila* protein, the *Drosophila* gene *otd* can rescue the loss of function phenotype in mice provided that all regulatory sequences necessary for its expression in the early embryo are present (3). The apparently paradoxical observation, that key regulators of development have different functions in different cells types while, despite the diversity of their functions, appear to be very simple proteins shows that in general DNA binding transcription factors have a single function namely to provide specificity through their interaction with their binding sites in the DNA. This also indicates that the machinery necessary to generate cell type specific patterns of gene expression requires other proteins and that DNA binding transcription factors alone are not sufficient to explain the development of diverse structures. In order to reconcile the multiplicity of function amongst transcription factors with the highly reproducible nature of differentiation and development it has been suggested that regulation of chromatin structure is an integral part of these processes (4).
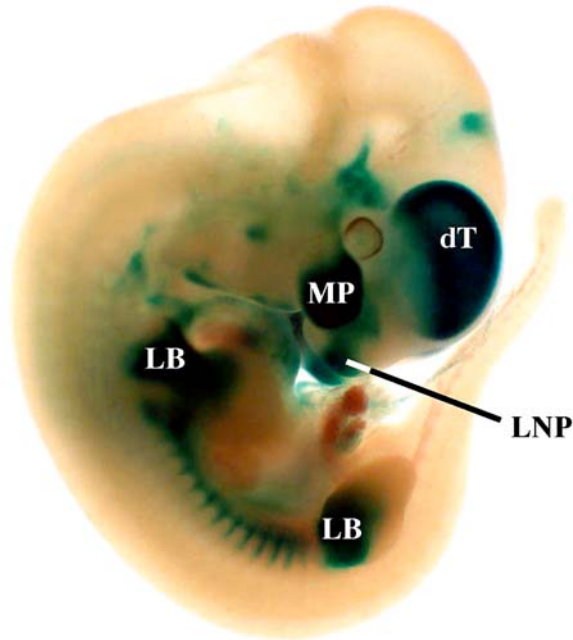
Figure 1. A schematic diagram showing a model of how co-activators complexes may link DNA binding transcription factors at distant binding sites to the basal transcriptional apparatus.

In the last few years it has become apparent that large protein complexes, co-activators, linking DNA binding transcription factors to the basal transcriptional apparatus have an essential role to play in regulating the activity of transcription during development (Figure 1). The recruitment of one or more co-activator complexes appears to be required for gene transcription (5). The use of different co-activator complexes explains how in some circumstances a DNA binding transcription factor can act as an activator but in other circumstances acts as a repressor (6, 7).

Co- activator complexes typically contain proteins, which have chromatin modifying activity. Chromatin is, in general, repressive to transcription (8, 9). However, the N-terminal tails of histones are subject to covalent modifications. These covalent modifications, which include acetylation, methylation and phosphorylation (10), greatly influence transcriptional activity at a given locus. Since differentiation and development are processes requiring the establishment of cell and tissue specific patterns of gene expression it is to be expected that regulation of chromatin structure is an integral part of this process (11). It has been suggested some time ago that

establishing an appropriate chromatin structure is important in preparing or "poising" undifferentiated cells for differentiation along a given lineage (12). Direct evidence that the regulation of chromatin structure is intimately involved in developmental processes is suggested by recent studies of the myeloid cell differentiation. In the lysozyme locus the chromatin structure in the undifferentiated precursor cells resembles that of the mature myeloid cells suggesting that the chromatin may be patterned prior to the transcription of the locus in the fully differentiated cell (13).

Different types of post-translational changes contribute to the overall pattern of histone modifications leading to a co-ordinate effect on gene activity in a given locus. Methylation of lysine in histone H3 tails is associated with either inactivation or activation of transcription depending on which lysine is modified. Methylation of lysine 9 is associated with developmental inactivation of globin genes and low levels of acetylation, whereas methylation on lysine 4 is correlated with active regions of the locus and interestingly, elevated acetylation of histone H3 (14). There is evidence that deacetylation of histones is connected to DNA methylation since the methyl-CpG-

Figure 2. A *querkopf*^{GT} heterozygous embryo (E11.5) stained for ß galactosidase activity. The pattern of *lacZ* expression shows the strong expression domains of *querkopf* in the dorsal telencephalon (dT), the lateral nasal process (LNP), maxillary process (MP) and anterior aspects of the fore and hind limb buds (LB).

binding protein is found in a complex that includes histone deacetylases (15). These observations have lead to the hypothesis that the pattern of covalent modifications on the N-terminal tails of histones represents a code and this histone code dictates the transcriptional activity of a given locus proposed (16). This theory proposes that each modified residue in the histone tails can interact with specific binding proteins. This in turn directs large protein complexes to a particular region of chromatin and so leads to specific effects on gene regulation. Several proteins with chromatin modifying activity, such CBP, contain bromodomains. This domain has been reported to bind acetylated histones (17). On the other hand the silencing protein HP1 contains chromodomains, which are reported to bind to methylated lysine 9 associated with transcriptional inactivation (18, 19).

Although relatively few examples have been studied in depth, it is clear that co-regulators of transcription with chromatin modifying activity play a critical role in development (20). For example the terminal differentiation of muscle cells requires the histone acetyltransferase activity of P/CAF (21). Co-activator complexes may be limiting in a cell so that different differentiation pathways may compete for co-activator complexes. The proneural gene neurogenin may simultaneously promote neurogenesis and inhibit gliogenesis by appropriating the available co-activator (22). The maintenance of silencing is also important in linage determination. The presence of the nuclear receptor co-repressor, NCoR, is required to suppress gliogenesis (23).
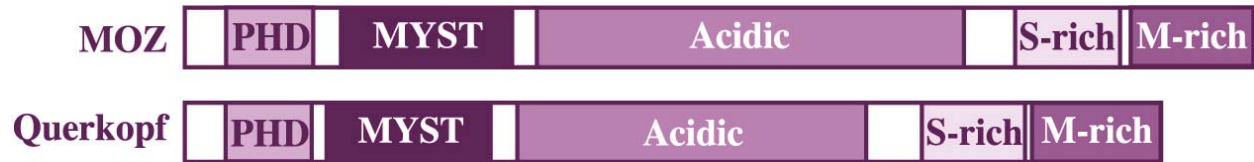
A large number of DNA binding transcription factors with essential roles during development have been identified. However, in the majority of cases the co-activator complexes necessary for their activity are unknown. Since there are only a small number of histone acetyltransferases presumably individual histone acetyltransferases can act in co-activator complexes for a number of different DNA binding transcription factors. It has been shown that different classes of DNA binding transcription factors use the general co-activators CREB binding protein (CBP) and the p300/CBP associated factor (PCAF). In this short review we present work which suggests that a histone acetyltransferase, Querkopf, may have very specific functions in the regulation of development in the forebrain.

3.1. Identification of a MYST family histone acetyltransferase in brain development.

In order to identify novel genes important in controlling differentiation during embryonic development, particularly brain development, we performed a gene trap screen in mice (24). As a result of this screen we created a mutation in a member of the MYST family of histone acetyltransferases (Figure 2). We called this mutation Querkopf (square head). Sequence analysis showed that of all the members of the MYST family, Querkopf is most similar to MOZ. We, and others, have shown that the MYST histone acetyltransferase domain of Querkopf and its human homologue MORF, can function *in vitro* as a histone acetyltransferase (25, 26).

An important class of covalent modifications to histones is acetylation of lysines in the N-terminal tails. It has been known from the 1960's that actively transcribed regions of the genome have higher levels of acetylated histones than silenced regions of the genome (27). Currently there are six known families of histone acetyltransferases (28). These are defined by there homology in the histone acetyltransferase domain. These include well known transcriptional co-factors CBP and closely related protein p300 as well as the GCN5 family members, GCN5 and PCAF. A member of the basal transcriptional apparatus TAFII250 has histone acetyl transferase activity. Proteins with histone acetyltransferase activity may also acetylate other nuclear proteins leading to the suggestion that acetylation in the nucleus may be a regulatory mechanism analogous to phosphorylation in the cytoplasm (29).

The MYST family is by far the largest of histone acetyltransferases having 5 known members in humans. MOZ (monocytic leukemia zinc finger protein), the founding member of the MYST family was originally identified in a recurrent translocation t(8;16)(p11;p13) associated with acute myeloid leukemia (30). This translocation is present in approximately 0.5% of cases of acute myeloid leukemia and is characteristic of a distinct sub type of acute myeloid leukemia (31). The MYST family of proteins is defined by a highly conserved histone acetyltransferase domain. This family of proteins is present in yeast, plants, insects and mammals.

Figure 3. A schematic diagram showing the domain structure of Querkopf compared to MOZ.

In yeast acetylation of histones H3 and H4 by members of MYST family is essential for cell cycle progression (32). In *Drosophila* a MYST family member, MOF (males absent on the first), is involved in X chromosome dosage compensation. In male flies MOF is responsible for the two fold increase in transcription from the single male X chromosome by hyper-acetylation (33). This is particularly interesting because, unlike other histone acetyltransferases, it does not appear to act at specific loci but apparently has a global effect on the level of histone acetylation on the X chromosome of flies.

3.2. Protein structure of MOZ and Querkopf

In mammals Querkopf and MOZ form one pair of closely related proteins. Both MOZ and Querkopf contain an N-terminal PHD zinc finger domain, a MYST histone acetyltransferase domain, an acidic domain and unique C-terminal serine and methionine rich domains (Figure 3). The MYST domains of Querkopf and MOZ are 88% similar or identical. The PHD finger domains in Querkopf are 93% similar or identical to those found in MOZ. PHD fingers are found in a variety of proteins associated with transcription, such as CBP, and NeuroD, a transcription factor important in neural differentiation (34). At the C-terminus both Querkopf and MOZ contain very similar serine-rich and methionine-rich domains. The serine rich domains are 97% similar or identical and the methionine rich domains are 68% similar or identical with 85% of the methionines in conserved positions. The serine rich and methionine rich domains were not found in any other proteins in the SwissProt database suggesting that Querkopf and MOZ form a unique subgroup of MYST family proteins.

It has been shown *in vitro* that both Querkopf and MOZ interact with Runx proteins (35, 36). Runx proteins DNA binding transcription factors with homology to the *Drosophila* Runt protein. MOZ interacts with Runx1 (AML1). Runx1 is necessary for definitive hematopoiesis (37,38). Runx1 can bind to two regions of MOZ *in vitro*. The strongest interaction takes place through the C-terminal serine-rich and methionine-rich domains. Querkopf has been shown to interact with Runx2 (CBF - 1). Runx2 has been shown to be essential for bone development (39,40)

3.3. Expression of Querkopf in development.

The *querkopf* gene is ubiquitously expressed at low levels throughout development. However, in addition to low level ubiquitous expression there are also domains of high level expression. *Querkopf* is strongly expressed in the inner cell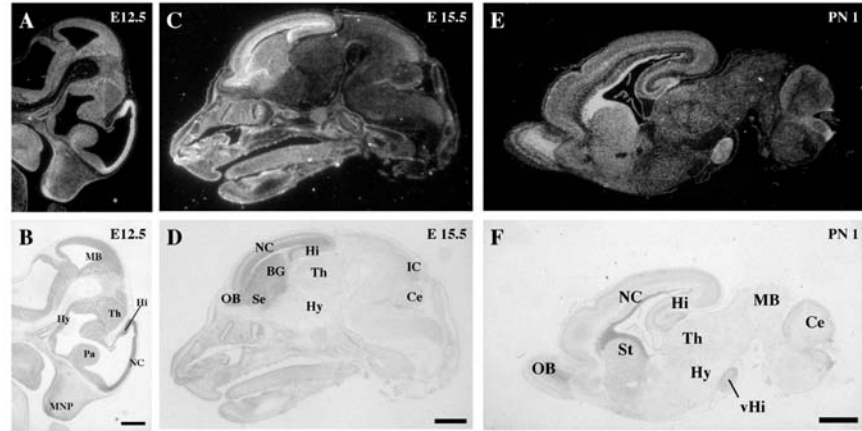 mass of the blastocyst. Subsequently, the expression levels are low until E10.5 when a strong domain of expression appears in the dorsal telencephalon (Figure 4). There is a dynamic pattern of expression in the forebrain during fetal development. Initially *querkopf* is expressed in the dorsal telencephalon, but as development proceeds it becomes expressed in the ventral telencephalon including the basal ganglia and the septum (Figure 4). In the developing cortex *querkopf* is expressed strongly in the ventricular zone and at slightly lower levels in the cortical plate. This suggests that Querkopf has multiple functions and that at least some of these functions are regulated at the level gene expression. This dynamic expression pattern is very unusual for a co-activator of transcription which are generally expressed ubiquitously at uniform levels. Regulation of Querkopf function at the transcriptional level suggests that this histone acetyltransferases may have very specific functions at different stages brain development.

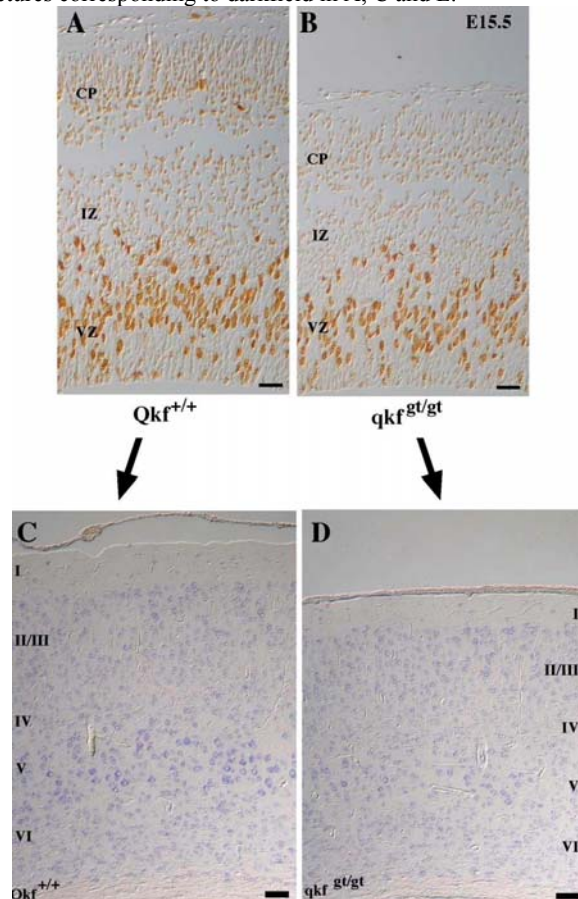**3.4. Phenotype of the Querkopf gene trap mutation**

The mutation in *querkopf* results in a fusion mRNA between the 5 prime UTR of the *querkopf* mRNA and the *lacZ-neo* mRNA encoded by the gene trap vector. Since there is alternative splicing in the 5 prime UTR of the *querkopf* gene, the expression of the *lacZ* reporter gene reflects the promoter activity of the strong expression domains of *querkopf*, but not the low level ubiquitous expression (compare Figures 2 and 4). The gene trap allele does not result in a null mutation. Approximately 10% of the level of normal mRNA are produced in the homozygous mice (26). However, despite the presence of normal mRNA, the *querkopf* homozygous mice display a characteristic phenotype.

Mice homozygous for the *querkopf* gene trap allele fail to thrive in the postnatal period and approximately 2/3 die before weaning. The mutant mice have defects in bone growth resulting in characteristic craino-facial abnormalities. They have severe defects in cerebral cortex development (26). During development of the cortex, proliferation takes place in the ventricular zone. As cells leave the cell cycle and begin the process of differentiating into neurons, they migrate away from the ventricular zone and form the cortical plate. The cortical plate subsequently matures to form the adult 6 layered cortex. At all stages studied, the cortical plate of homozygous *querkopf* mutant mice contained less cells than in wild type animals resulting in the adult cortex being smaller (Figure 5). Not only was the cortex smaller, but in adult mice there was a disproportionate reduction in the number of large pyramidal cells in layer 5 (Figure 5) and a large reduction in the number of GABAergic interneurons (Figure 6).
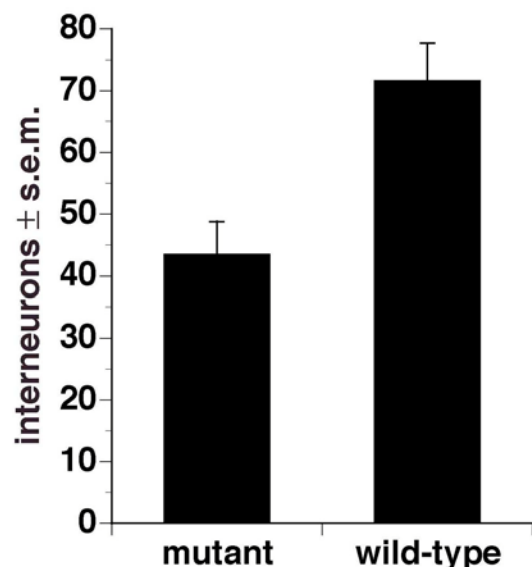
Figure 4. *In situ* hybridisation using a probe complementary to the Querkopf mRNA. Note the strong expression at E12.5 in the dorsal telencephalon (A), by E15.5 (C) strong expression is also seen in the ventral telencephalon. At postnatal day 1(E) expression in the ventricular zone is much reduced, particularly in the posterior aspects of the cortex. BG, basal ganglia; Ce, cerebellum; Hi, hippocampus; Hy, hypothalamus; IC, inferior colliculi; MNP, medial nasal process; MB, midbrain; OB, olfactory blubs; Pa, Pallidum; NC, neocortex; Se, septum; Th, thalamus; vHi, ventral hippocampus. Scale bar are 450μm in B, 850μm in D and F. B, D and F lightfield pictures corresponding to darkfield in A, C and E.

Figure 5. Coronal sections through the cortex at E15.5 (top panels) and adult (lower panels) showing the development of the cortical phenotype in *querkopf* mutant mice (right panels) as compared to littermate controls (left panels). Proliferating cells in the E15.5 animals were labelled with BrdU. There is no statistically significant difference in the proportion of cells in S phase (BrdU positive nuclei vs unlabelled nuclei) between the homozygous and wild type embryos. The upper panels were photographed with Nomaski optics. The adult brain sections were stained with cresyl violet to show different neuronal morphology.

Figure 6. Graph showing the reduction in GAD67 positive interneurons in the cortex in the adult *querkopf* mutant animals compared to wild type littermate controls.

Neurogenesis continues in some regions of the adult brain. Neurons are produced in the sub-ventricular zone of the forebrain lateral ventricles and migrate into the olfactory bulbs where there is a continuous turnover of interneurons throughout life. We noted that the olfactory bulbs in querkopf mutant mice are reduced in size suggesting that the querkopf mutant mice may be lacking adult neural stem cells. Recently a method has been developed to isolate highly purified populations of adult neural stem cells from the lateral ventricles by FACS sorting (41). We applied this method to the querkopf mutant mice and found that this population of cells was substantially reduced in number (41). These results show that querkopf is part of a system involved in the establishment of the adult neural stem cell population as well as being important in embryonic brain development.

4. PERSPECTIVES

The last decades of the 20th century saw tremendous progress in identifying transcription factors important in brain development. It is apparent that the overall pattern of gene expression in a cell during development is the result of multiple transcription factors acting in concert at the same locus and at different loci. How DNA binding transcription factors interact and what the results of these interactions are, is dictated by co-activator and co-repressor complexes. These interactions take place in the context of chromatin and chromatin directly influences the nature of these interactions. Since the pattern of histone modifications in the cell's chromatin is the result of the past actions of co-activators and co-repressors this suggest that the function of these complexes are an integral part of the development of complex structures such as the brain.

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