INTRODUCTION

Neurons and glia cells constituting the cerebral cortex are derived from progenitor cells in the ventricular and subventricular zones. Not only are multiple cell types produced from the neural epithelium, indicating a complex series of regulatory steps, but also a balance between proliferation and differentiation must be maintained within the progenitor cell population (McConnell, 1995). These processes ultimately involve precise regulation of transcription.

The early phase of development of the cortex is mostly devoted to expansion of the progenitor cell population with a comparatively small proportion of the cells exiting the cell cycle (Caviness et al., 1995). As cells become postmitotic, they leave the ventricular zone and migrate outwards to form the cortical plate. The first neurons born are destined to form the deep layers. The progenitor population undergoes a process of development such that later born neurons are destined for superficial layers, with layer II neurons the last to be generated (McConnell, 1995). These processes ultimately involve precise regulation of transcription.

Homozygous mice show a lack of large pyramidal cells in layer V of the cortex, which is reflected in a reduction in the number of Otx1-positive neurons in this layer during postnatal development. Homozygous mice also show a reduction in the number of GAD67-positive interneurons throughout the cortex. Our results suggest that Querkopf is an essential component of a genetic cascade regulating cell differentiation in the cortex, probably acting in a multiprotein complex regulating chromatin structure during transcription.

Key words: Histone acetyltransferase, Cerebral cortex, OTX1, Interneuron, Pyramidal neuron, Mouse, querkopf
was cross-extracted to determine if a recessive phenotype resulted from the insertion of the gene trap vector.  

5° RACE was performed as described in Chenchik et al. (1996).

Oligos used in this procedure were the kind gift of Dr V. Tarabykin. Northern analysis, Southern analysis and cDNA library screening were performed using standard molecular biology techniques. Probes were labelled by incorporating 32P-labelled dCTP using the Amersham random priming kit (Amersham life Science).

Skeletal preparations and Cresyl violet staining of sectioned Bouin’s-fixed brains were performed according to standard histological techniques. For in situ hybridisation and immunohistochecmistry, mice older than postnatal day 4 were perfused with 4% paraformaldehyde, and the brain removed and fixed overnight in 4% paraformaldehyde. Brains dissected from younger mice or whole embryos were fixed in 4% paraformaldehyde without perfusion. In situ hybridisation was performed essentially as described previously (Hogan et al., 1986). Briefly, sections were dewaxed, rehydrated through graded concentrations of alcohol, incubated for 10 minutes (or 30 minutes for adult brains) at room temperature in 10 mg/ml proteinase K, fixed in 4% paraformaldehyde 10 minutes, then dehydrated through graded concentrations of alcohol. Sections were air dried, and hybridisation solution containing 5×105 cts/minute/μl in vitro transcribed cRNA probes was placed over the section. Slides were incubated overnight at 56°C and then washed as described (Hogan et al., 1986).

The probes used were: neurofilament light chain (GenEMBL M13016, bases 2613-4258 cloned in Bluescript) and Otx1 (Simeone et al., 1992). The neurofilament light chain probe was cloned in our laboratory and checked by sequencing.

To analyse the numbers of neural precursors at the S phase, heterozygous females were mated to heterozygous males and injected with 100 μg/g body weight bromodeoxyuridine (BrdU) at 12:00 noon. 1 hour later the concepti were recovered. The extraembryonic membranes were used for genotyping by Southern analysis. Embryos (E11.5-E14.5) or brains (E15.5-E17.5) were processed for BrdU immunohistochecmistry. Briefly, they were fixed in 4% paraformaldehyde, infiltrated and embedded in paraffin. 5 μm thick serial sections were cut, dewaxed and rehydrated. Endogenous peroxidase activity was blocked with H2O2 and the sections were treated with intervening washes in PBS with HCl and then Na2B4O7, pepsin, anti-BrdU antibody (Bio-Science Products), biotinylated anti-mouse/rabbit IgG (Vector Laboratories), avidin:biotinylated horseradish peroxidase complex (Vector Laboratories) and diaminobenzidine. Sections were mounted, and viewed and photographed with differential interference contrast optics using a Zeiss Axioshot microscope. Sections of three homozygous and three wild-type controls were analysed at each gestational stage shown in Fig. 7. BrdU-positive nuclear profiles in four sections of the dorsal parietal neocortex were counted. The longest diameters of 50 nuclei selected at random in each section was measured in digital images taken at 630× magnification. The mean values of the longest nuclear diameters were not found to differ significantly between wild-type and homozygous mice at these embryonic stages. From this, we concluded that the accuracy of cell counts was similar in wild-type and homozygous mice and so ratios of BrdU-positive and -negative cell profiles could be compared (Abercombie, 1946).

For the TUNEL assay, embryos and brains of embryos or pups recovered from heterozygous intercrosses between E12.5 and PN13 were processed for paraffin sections and analysed for cells undergoing apoptosis by TdT-mediated dUTP-biotin nick-end labelling using an in situ apoptosis detection kit (Apotag, Oncor) according to the manufacturer’s instructions. Sections were viewed using a fluorescence microscope (Zeiss Axioshot). TUNEL-positive cell profiles were counted on sections in the neocortex.

For preparation of GAD67 immunohistochecmistry, brains were fixed by paraformaldehyde perfusion, embedded in paraffin and processed using standard techniques. In brief, deparaffinised sections were
treated with intervening PBS washes with 
H2O2 to block endogenous peroxidase, with anti-glutamate decarboxylase (GAD, larger form, 67 kDa) polyclonal antibody (Chemicon), biotinylated anti-mouse/rabbit IgG (Vector Laboratories), avidin/biotinylated horseradish peroxidase complex (Vector Laboratories) and diaminobenzidine. Slides were viewed and photographed with differential interference contrast optics using a Zeiss Axioscope microscope. Profiles of positively staining cells were counted on sections of four homozygous and four wild-type littermate control animals. Besides positively staining cytoplasm, stained axon terminals were also visible but not counted.

For histone acetyltransferase activity assays, fragments coding for the MYST domain of Querkopf were cloned into pGEX4t-2. pQkf80 contained bases coding for amino acids 362 to 980, and pQkf82 contained sequences coding for amino acids 429 to 674, cloned in frame to GST. Fusion proteins were purified using glutathione bound to sepharose4B according to the manufacturer’s instructions (Pharmacia). The purity of fusion proteins was assessed by SDS-PAGE. Histone acetyltransferase assays were performed essentially as described (Bannister and Kouzarides, 1996). Briefly, approximately 0.5 pmol of fusion protein were incubated for 30 minutes in a buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 5 mM EDTA, 0.5% NP40, 0.1 mM PMSF) containing 20 μg of calf thymus histones (Sigma) and 0.25 μCi [3H]acetyl-coenzyme A (Amersham life Science, 152 GBq/mmol) and then the reaction was spotted on Whatman P81 filter paper, washed and counted in a liquid scintillation counter. For fluorography, the reactions were performed as above except that [14C]acetyl-coenzyme A (Amersham life Science, 1.85-2.29 GBq/mmol) was used and, in some cases, 1 μg of purified histones (Boehringer Mannheim) were used.

For the statistical analysis of the thickness of the telencephalon, the preplate and the cortical plate, cell counts, the number of BrdU-positive nuclear profiles, unstained cell profiles or GAD67-positive cell profiles were counted in sections of specific areas of the cortex (see results). Data were analysed by one- or two-way analysis of variance, with genotype or genotype and age as the determining parameter (SAS System 6.12, SAS Institute Inc., NC). Results are stated as mean ± standard error of the mean and P value when statistically significant differences were observed. The volumes of cortex and cerebellum were compared by Student’s t-test and results are given as mean ± standard deviation.

RESULTS

Identification of querkopf

We performed a gene trap screen in murine embryonic stem (ES) cells as described previously (Voss et al., 1998b). Using this procedure, we identified an ES cell line in which the insertion event occurred in a gene strongly expressed in the telencephalon. We applied the 5′ RACE technique to RNA purified from the cortex of neonatal mice and obtained a product of 110 base pairs. Clones covering the entire coding sequence were obtained by screening an E14.5 random primed cDNA library from a postnatal day 20 brain. This also produced overlapping clones covering half the coding sequence. The sequence was identical to that obtained from the E14.5 brain library. From this, we conclude that the sequence that we submitted to the GenEMBL database (AC# AF222800) represents the sequence of the predominant querkopf mRNA species found in brain. This completed sequence showed a high level of similarity over the entire coding sequence to a human EST, KIAA0383 (Nagase et al., 1997) and to a recently described human protein MORF (Champagne et al., 1999), suggesting that MORF is the human querkopf homologue. We called the allele in which the gene trap insertion occurred ‘querkopf Kn’ (qkff). Transcription of the querkopf gene produces a mRNA of 6909 bases containing a coding region of 5292 bases.

The insertion of the gene trap vector occurred at base 76 of the complete querkopf cDNA sequence. We cloned and sequenced the insertion site, confirming that this occurred in a 5′ untranslated exon. Southern analysis showed that between this exon and the initiating methionine there is at least 1 intron. The sequence at the insertion site is: 5′ AACG GAT TGG ccc ccc ccc tgc 3′, where the upper case letters indicate the querkopf sequence and the lower case letters indicate the vector sequence. Using a 5′ probe of 125 base pairs, which includes the insertion site, as a Southern probe, we were able to find an RFLP in the homozygous DNA using BglII. Using cDNA probes further 3′ of the insertion site or other probes 5′ of the coding sequence did not produce RFLPs, suggesting that the mutated locus does not contain major rearrangements. As the insertion event occurred in a 5′ untranslated exon, leaving all coding exons intact, there is no possibility that interpretation of the phenotype is complicated by the presence of truncated protein products that may have dominant negative effects.

Comparison of the deduced Querkopf protein sequence with the GenEMBL database show that this protein has similarity in four domains with MOZ (Fig. 1A). At the N-terminal end, Querkopf has a PHD zinc-finger domain. The PHD finger is found in several proteins involved in transcriptional regulation including CBP (Aasland et al., 1995). This domain is highly similar to comparable domains in NeuroD and Requiem (Borrow et al., 1996). Between MOZ and Querkopf, the similarity extends over a region of 153 amino acids and, within this domain, there are 93% similar or identical amino acids. The Querkopf MYST histone acetyltransferase domain is 88% similar or identical to the MYST domain of MOZ. Comparison of the MYST domains from Querkopf, KIA0383 (equivalent to MORF), MOZ, MOF (Hilfiker et al., 1997), Tip60 (Yamamoto and Horikoshi, 1997), ESA1 (Smith et al., 1998), SAS2 and SAS3 (Reifsnnyder et al., 1996) and an EST obtained from the sprouting tip of a carrot are show in Fig. 1B. Querkopf, MOZ and the human EST KIA0383 are more similar to each other than to other proteins containing a MYST domain (Fig. 1B) and so form a subfamily of MYST domain proteins. At the C-terminal end, there are two domains unique to MOZ, Querkopf and the human counterpart MORF. These include a domain rich in serines followed by a domain rich in methionines. In MOZ, these two domains are separated by a stretch of prolines and glutamines (‘PQ’ domain). In Querkopf, the serine-rich and methionine-rich domains are not separated by a ‘PQ’ domain. The similarity or identity between MOZ and Querkopf is 96.5% in the serine-rich domain. The similarity between MOZ and Querkopf in the methionine-rich domain is 68% and 85% of the methionines are in conserved positions. The serine-rich domain and the methionine-rich domain are not found in any other proteins in the Swissprot database. MOZ and Querkopf are not similar in the acidic domain described (Borrow et al., 1996). However, this region of Querkopf is rich in glutamic acid making this part of the protein acidic.

The querkopf gene maps to the A3-B region of chromosome 14 (SeeDNA Biotech Inc., data not shown) and KIA0383 maps to human chromosome 11 (Nagase et al., 1997).
Fig. 1. (A) Structure of the *querkopf* mRNA. The insertion of the gene trap vector occurred in a 5′ untranslated exon at base 76 of the mRNA as indicated by the arrowhead. The region coding for the 1763 amino acid protein is boxed. The initiating methionine is at base 343. The position of sequences coding for the PHD fingers (bases 828-1285), MYST (bases 1600-2416), acidic (bases 2736-3855), serine-rich (bases 4080-4585) and methionine-rich domains (bases 4590-5517) is indicated by shading. The diagram is drawn to scale. Probes 19 (1.2 kb) and 34 (1.2 kb) are indicated.

(B) Amino acid comparison (Macboxshade) of the MYST domains from *Querkopf*, KIAA0383 (AC# AB2381), MOZ (AC# Q92794), Tip (AC# Q92993), AB012703, ESA1 (AC# Q08649), MOF (AC# O02193), Q10325, SAS3 (AC# P34218), SAS2 (AC# P40963). Identical amino acids are indicated by black boxes, similar amino acids are indicated by grey boxes.

(C) Northern analysis of RNA purified from littermate wild-type (wt) or mutant (mt) brains. The left-hand panel shows a northern using RNA from adult brains probed with probe 19. The right-hand panel, northern probed with probe 34, the first three lanes contain RNA from 3-week-old mice (littermates). Lane 1 wild type, lanes 2 and 3 mice with severe symptoms of starvation. The last four lanes contain RNA from adult mice (two pairs of wild-type and homozygous littermates). (D) Southern of a *Bgl*II digest of wild-type (wt), heterozygous (ht) and homozygous (mt) DNA probed with the 5′ RACE product, which contains the first 86 bases of the *querkopf* cDNA. The position of the mutant (*qkf*^-^) and wild-type allele (*Qkf*) is shown.
Transcription of the \textit{querkopf} gene generates a 7.2 kb polyadenylated and processed mRNA in brain (Fig. 1C). In other tissues, a larger mRNA species of approximately 7.7 kb is also present in equal abundance. In all tissues examined, there is also an RNA species of approximately 1 kb. This RNA species is particularly abundant in the brain where the level of this RNA is much higher than the 7.2 kb species. The 1 kb RNA species is not polyadenylated (data not shown). It can be detected with the 5’ 1.2 kb probe (probe 19, Fig. 1A) but not with the more 3’ probe (probe 34, Fig. 1A).

In homozygous \textit{querkopf} mice, there is normal coding mRNA produced at a level of about 10% of the wild type showing that the mutant \textit{querkopf} \textsuperscript{gt} allele is a hypomorphic allele (Fig. 1C). Northern gel analysis showed that all three species of RNA were present and showed similar reduction in intensity. In total we compared the level of \textit{querkopf} RNA present in seven homozygous mouse brains with five wild-type brains. There was some variation in the level of mRNA produced by the mutated allele in the brain (Fig. 1C). However, the levels in 3-week-old homozygous brains of mice that were severely runted did not contain lower levels of \textit{querkopf} RNA in the brain than those that survived to adulthood. In situ hybridisation using probes both from the 5’ end (probe 19, Fig. 1A) and a probe containing the histone acetyltransferase domain (probe 34, Fig. 1A) showed that the mRNA in homozygous mice was uniformly reduced in abundance in all tissues of the embryo and postnatal brain (data not shown). We have identified alternatively spliced variants of the \textit{querkopf} 5’ untranslated sequence. Presumably, normal mRNA is produced in the homozygous mice by splicing out of the exon in which the insertion occurred, in manner similar to that described previously (Voss et al., 1998a).

**Querkopf has histone acetyltransferase activity**

In order to show that the Querkopf MYST domain has acetyltransferase activity, we cloned fragments containing the MYST domain into \textit{pGSX-4T2} to produce GST fusion proteins in bacteria. \textit{pQkf80} coded for amino acids 362 to 980 and \textit{pQkf82} coded for amino acids 429 to 674, both cloned in frame with GST. A histone acetyltransferase assay using tritiated acetyl-CoA as a substrate was performed as described in the experimental procedures section. This assay was repeated three times on two independent protein preparations. The Querkopf/GST fusion protein produced from \textit{pQkf80} was capable of catalysing the transfer of the acetyl group from acetyl-CoA to histones with similar efficiency to that reported for CBP and Tip60 (Fig. 2A). The Querkopf/GST fusion protein produced from \textit{pQkf82} was not sufficient to increase the histone-bound radioactivity above background levels obtained when GST alone or no GST proteins were added. The fusion protein produced from \textit{pQkf82} is lacking the conserved amino acids at the end of the MYST domain. This region contains highly conserved leucine and proline residues. In order to determine the substrate specificity of Querkopf, the same experiment was repeated using \textit{[14C]acetyl-CoA} and the reaction products were separated SDS-PAGE gel electrophoresis. As can be seen in Fig. 2B, Querkopf predominantly acetylates histone H3 and histone H4. Histone H2A was also acetylated to a lesser extent. In the absence of core histones, Querkopf will acetylate histone H1. However, in a natural mixture of histones from calf thymus, histone H1 is not acetylated (Fig. 2B). Note that in Fig. 2B, the lane containing the natural mixture of histones contains approximately twice the amount of histone H1 (Coomassie blue stained gel lanes 1 and 2) than the purified histone H1.

![Fig. 2. Histone acetyltransferase assays.](image-url)

(A) Radioactivity incorporated into calf thymus histones after incubation with Querkopf/GST fusion proteins, Qkf80 and Qkf82, in the presence of tritiated acetyl-CoA. (B) Left-hand panel shows Coomassie-blue-stained gel containing calf thymus histones (20 µg) or purified histone H1. Prior to loading the histones were incubated with Querkopf/GST fusion proteins in the presence of \textit{[14C]acetyl-CoA} as described in the methods section. The right-hand panel shows a fluorograph of the stained gel.
(Coomassie blue stained gel lanes 3 and 4) which was strongly labelled. The in vitro histone acetyltransferase activity of Querkopf is similar to that previously reported for Tip60 and P/CAF (Yamamoto and Horikoshi, 1997; Yang et al., 1996) and is the same as that recently reported for the histone acetyltransferase activity of MORF (Champagne et al., 1999). Although Querkopf and CBP can use histone H1 as a substrate for acetylation in vitro, there is no evidence that histone H1 is acetylated under physiological conditions (Herrera et al., 1997). The yeast MYST family member, ESA1, which also has a preferred substrate specificity of histones H3 and H4 can acetylate chicken histone H2B when this is the only available substrate (Smith et al., 1998).

**querkopf expression pattern**

The *querkopf* gene is strongly expressed in ES cells and in the inner cell mass of blastocysts. At E9.5 expression is low (not shown). During the period of neurogenesis, a dynamic pattern of *querkopf* expression was seen, particularly in the forebrain. Starting at E10.5, a strong domain of expression appears in the dorsal telencephalon. Expression becomes stronger at E11.5 in the telencephalon and new domains of strong expression appear in the frontal nasal process and in the maxillary process (not shown). As shown in Fig. 3A, at E12.5 strong expression of *querkopf* continues in the dorsal telencephalon and in the mesoderm of the frontal nasal process. At this stage, no expression is seen in the anlage of the pallidum. The pattern of expression in the brain at E13.5 and E14.5 (not shown) shows progressive development to the pattern at E15.5. Strong expression is seen at E15.5 in the ventricular and subventricular zone. Expression is also seen in the cortical plate (Fig. 3C). The expression in the ventricular zone of the hippocampus becomes progressively reduced after E15.5, correlating with the progression of neurogenesis. *Querkopf* continues to be expressed at high levels in the ventricular zone of the cortex and the olfactory bulbs until E17.5. At postnatal day 1, cells in deep layers of the cortex express *querkopf* more strongly than other cells in the cortex and in other parts of the central nervous system. At E15.5, strong expression develops in ventral telencephalon, in the striatum and in the septum (Fig. 3C). By E17.5, strong expression is seen in the pallidum as well as the septum, the striatum and the cortex (Fig. 3E). Also, from E15.5
Querkopf is required for cerebral cortex development onwards, somewhat higher levels of expression are found in
the anlage of the inferior colliculi and the cerebellum. Expression in the postnatal period declines (Fig. 3G) to the low
levels found in the adult brain (not shown).

In the mesodermal domains, expression of querkopf is
downregulated as cells differentiate. No expression can be
detected in cartilage condensations, such as bones forming
the base of the skull at E15.5 (Fig. 3C) and the cartilage
condensations in the limbs at E12.5. However, the gene is
expressed strongly in the mesoderm surrounding the cartilage.
These cells are thought to form the future periost and therefore
have the potential to differentiate into osteoblasts. In adult
animals, low-level expression can be detected by northern gel
analysis in all tissues examined: brain, liver, kidney, heart, gut
and muscle. The expression in the adult gut was investigated
by in situ hybridisation. No expression was detected in the
epithelium and only very low level expression could be
detected in the stroma of the villi.

Phenotype of mice homozygous for the querkopf gt allele
Homozygous mice from a querkopf gt+/+ heterozygous
intercrosses characteristically have low body weights and
craniofacial abnormalities. The homozygous mice surviving to
adulthood have short square heads, the ears appear to be
located in a more ventral position and the eyes are small (Fig.
4). Frequently the nasolacrimal duct is
occluded. The majority (65%) of homozygous mice on an inbred 129Sv
background die around the time of
weaning (Fig. 4; Table 1) despite being supplied with wet feed; no body
fat is visible and muscle mass is
reduced. At birth these abnormalities are not apparent but develop in the
postnatal period. The homozygous
mice can first be unambiguously identified at postnatal day 3 because
of their failure to thrive. During the
weaning period, the weight gap
between homozygous and the controls
increases progressively and the more
seriously affected animals become
apathetic before finally dying. The
mice feed normally and at the time of
weaning have continuous access to wet
feed placed in the cage. The cause of their
failure to thrive is not known, histological
examination of the digestive tract did not
reveal any cellular abnormalities.

In order to investigate the nature of the craniofacial abnormalities, we made
preparations of the skeleton. The mice have defects in the calvarial bones (Fig. 5).
The parietal bones are shorter in relation to the frontal bones. The sutures between the
parietal and frontal bones are frequently
irregular. The occipital bones are abnormal
being concave rather than convex. Since
these mice show defects in brain
development, we wanted to eliminate the
possibility that the brain defects were a secondary result of the
defects in development of the skull. Therefore, we made
skeletal preparations at birth (2 homozygous; 3 wild type) and
at 3 weeks of age (4 homozygous; 3 wild type). At birth
the sutures were open as is the case for wild-type mice. In the 3-
week-old homozygous mice, we found that the suture closure
was retarded. In no case was craniosynostosis observed. This
shows that, whilst the skull defects may lead to some distortion
of the brain in those mice surviving to adulthood, it is not the
primary cause of the defects in brain development described
below.

querkopf gt homozygous mice have defects in brain
development
Mutant brains are shorter than wild-type brains. Although, no
nuclei in the brain are missing, the cortex is significantly
smaller than in wild-type mice (Fig. 6A,B). The cerebellum is
essentially normal, although there is a foliation defect. In normal brains, the inferior colliculi meet in the dorsal midline
(Fig. 6A,B). In mutant brains, the inferior colliculi are reduced
in size and so, in the majority of cases (75%), fail to meet in
the midline. The olfactory bulbs are greatly reduced in size.
We have cut and examined serial sections from 13 pairs of
homozygous and littermate control brains from E18.5, PN2, 3-
week-old and adult brains and stained them with cresyl violet.
The reduction in the size of the adult cerebral cortex is shown

![Fig. 4. External appearance of wild-type Qkf+/- and homozygous qkf gt/gt. Left panel shows littermates at 3 weeks of age, the homozygous died shortly after this picture was taken. Right panel surviving homozygous qkf gt/gt and wild type at 3 months of age.](image)

![Fig. 5. Skull preparations of adult wild-type and querkopf mutant mice. (A) Wild type; (B-D) homozygous. Note that, in the mutant skulls, the occipital bones appear concave (arrows in B-D). The skull C is the most severely affected in this respect. Note that, in skulls B and D, the sutures between the parietal and frontal bones are irregular (arrowheads).](image)
in sagittal and coronal sections in Fig. 6. Serial sections were used to measure the volume of the adult neocortex and cingulate cortex in three pairs of homozygous and wild-type controls using the method of Cavalieri as described previously (Coggeshall, 1992). The volume of the homozygous cortex was 77% of the volume of the control mice (mutant 18.0±0.3 mm³, control 23.3±0.4 mm³). As a control, the volume of the cerebellum was also measured. Although the cerebellum showed a minor foliation defect, the volume of the homozygous cerebellum was not significantly different (Student’s t-test) from the volume of the wild type (mutant 13.5±1.0 mm³, control 15.4±1.6 mm³) showing that the cortex is disporportionally reduced in size. The cell density in layers II/III, IV, V and VI was determined in the dorsal frontal, dorsal parietal and dorsal occipital cortex using the optical dissector method (West et al., 1991). No significant difference between control and mutant brains was found. In Fig. 6, the extents of the hippocampus and the piriform cortex have been used to orient the sections for comparison. Since the cortex is shorter in the homozygous animals, the mammillary nucleus is more extensive on the sections in Fig. 6D than the control Fig. 6C. Comparison of coronal sections showed that the posterior cortex is more affected than the frontal cortex.

In the course of this study, we observed one heterozygous and one wild-type mouse with severe runting. In the case of the wild-type mouse, this was due to cystic kidney disease. The gross anatomy of the brains of these mice were examined and the brains were serially sectioned. The brains of these mice were smaller than those of healthy mice. However, the reduction in size was proportional and they did not exhibit the pattern of defects seen in the querkopf mice. This analysis shows that the querkopf mutation produces specific defects in cortex development that can be distinguished from the effects of runting.

### Analysis of cell numbers in the cortex during embryogenesis

The thickness of the cerebral cortex was reduced in homozygous querkopf mutant mice and the cortical plate of mutant animals contained fewer cells than wild-type animals. There was a significant difference in the thickness of the telencephalon at E11.5 (mutant, 44.7±2.2 μm, control 65.0±5.1 μm; P<0.01) resulting from a reduction in cell number (Fig. 7E). At E15.5, the cortical plate of the homozygous brains was significantly thinner than that of the control embryos (mutant, 72.1±4.3 μm, control 88.4±4.7 μm; P<0.05). From E13.5 to E17.5, significantly fewer cells were present in the preplate or the cortical plate of querkopf homozygous mice (Fig. 7). The largest difference in cortical plate cell numbers was seen at E15.5 (Fig. 7C,D,F).

We labelled proliferating cells in querkopf mutant and wild-type brains with BrdU. BrdU incorporation into cells of the telencephalon of homozygous embryos was compared to that in wild-type embryos at the following time points: E11.5 (n=3 homozygous and wild-type pairs), E12.5 (n=1 pair), E13.5
Querkopf is required for cerebral cortex development

2545

Fig. 7. Analysis of cell numbers in the cortex during embryogenesis. (A-D) Sections of the parietal cortex at E13.5 (A,B) and E15.5 (C,D). Note the reduction in the number of cells in the preplate (Ppl) and cortical plate (CP) of homozygous mice (E13.5 compare A and B; E15.5 compare C and D). Brown-stained nuclei indicate cells incorporating BrdU. Scale bar: 25 μm. (E,F) Quantitation of cell profiles in the ventricular zone (E), and in the cortical plate (F), at the embryonic stages indicated. Cell profiles were counted in segments (base of segment equals 72 μm along the lumen of the ventricle; height equals full thickness) of wild-type and homozygous neuroepithelium (E11.5 in E), ventricular zone (E13.5 and E15.5 in E), preplate (E13.5 in F) and cortical plate (E15.5 and E17.5 in F). The height was the whole thickness of the neuroepithelium, the ventricular zone, the preplate or the cortical plate, respectively. White bars, homozygous mice; black bars, wild-type mice. At E11.5 the number of cell profiles in the neuroectoderm of homozygous animals is significantly different to wild type (a:b: P<0.05). At all stages examined the number of cell profiles counted in the cortical plate is significantly lower in the homozygous brains (a:b: P<0.01), with the largest difference at E15.5 (F).

(n=3 pairs), E14.5 (n=1 pair), E15.5 (n=3 pairs), E16.5 (n=1 pair) and E17.5 (n=3 pairs). BrdU-labelled and -unlabelled cell profiles were counted in segments of wild-type and homozygous neuroepithelium (E11.5) or ventricular zone (E13.5 and E15.5). The segments' base were 72 μm along the lumen, the height was the whole thickness of the neuroepithelium (E11.5) or ventricular zone, the preplate or the cortical plate, respectively. White bars, homozygous mice; black bars, wild-type mice. At E11.5 the number of cell profiles in the neuroectoderm of homozygous animals is significantly different to wild type (a:b: P<0.05). At all stages examined the number of cell profiles counted in the cortical plate is significantly lower in the homozygous brains (a:b: P<0.01), with the largest difference at E15.5 (F).

We compared the rates of cell death in the brains of homozygous mice with wild-type mice at E12.5, E13.5, E14.5, E16.5, E17.5, PN3, PN7 and PN13. No significant difference in number of TUNEL-positive cell profiles was observed and no increase in cell death was visible histologically.

Markers of the large pyramidal cell population are reduced in querkopf homozygous mice

Detailed examination of Cresyl-violet-stained sections of adult mice showed that layer V did not exhibit normal morphology. In all adult and 3-week-old homozygous brains examined (6 pairs), with one exception, there was a large reduction in the number of large pyramidal cells. In the most severely affected homozygous brains, large pyramidal cells were absent from the parietal cortex (Fig. 8A,B).

In addition to the early domains of expression, Otx1 is also expressed in a subset of cells in layers V and in layer VI of the postnatal cortex (Frantz et al., 1994). We found, by in situ hybridisation, a large reduction in the number of cells expressing Otx1 in layer V of the homozygous cortex. This was particularly apparent in the retrosplenial area where no cells expressing Otx1 were seen in any of the sections analysed (Fig. 8 compare D and C). We analysed the expression of Otx1 in the cortex after birth, at postnatal day 2 (one homozygous and one wild-type littermate control) at postnatal day 3, (one homozygous and one wild-type littermate brains), at postnatal day 7 (two homozygous and one wild-type littermate control) and in adult cortex (one homozygous and one wild-type littermate control). Multiple sections were hybridised at regular intervals throughout the cortex. The expression of Otx1 in

[Fig. 7: Analysis of cell numbers in the cortex during embryogenesis. (A-D) Sections of the parietal cortex at E13.5 (A,B) and E15.5 (C,D). Note the reduction in the number of cells in the preplate (Ppl) and cortical plate (CP) of homozygous mice (E13.5 compare A and B; E15.5 compare C and D). Brown-stained nuclei indicate cells incorporating BrdU. Scale bar: 25 μm. (E,F) Quantitation of cell profiles in the ventricular zone (E), and in the cortical plate (F), at the embryonic stages indicated. Cell profiles were counted in segments (base of segment equals 72 μm along the lumen of the ventricle; height equals full thickness) of wild-type and homozygous neuroepithelium (E11.5 in E), ventricular zone (E13.5 and E15.5 in E), preplate (E13.5 in F) and cortical plate (E15.5 and E17.5 in F). The height was the whole thickness of the neuroepithelium, the ventricular zone, the preplate or the cortical plate, respectively. White bars, homozygous mice; black bars, wild-type mice. At E11.5 the number of cell profiles in the neuroectoderm of homozygous animals is significantly different to wild type (a:b: P<0.05). At all stages examined the number of cell profiles counted in the cortical plate is significantly lower in the homozygous brains (a:b: P<0.01), with the largest difference at E15.5 (F).]
homozygous brains was unaltered in layer VI, the habenular nucleus and the dorsal thalamus expression domains.

The large pyramidal cells in layer V express the neurofilament light chain gene at very high levels. Therefore, the large pyramidal cell population can be identified by intense labelling after in situ hybridisation with a neurofilament light chain probe. We found that, in homozygous brains, the number of cells that express neurofilament light chain at very high levels was also reduced in layer V (Fig. 8 compare E and F) confirming the reduction in this cell population in the mutant brains.

**GAD67-positive interneurons are lacking in querkopf homozygous mice**

We compared the occurrence of glutamate decarboxylase immunoreactive neurons in querkopf mutant brains with wild-type brains using an antibody against the 67 kDa form of glutamate decarboxylase (GAD67). This is an enzyme necessary for the synthesis of GABA and a marker for GABA-positive interneurons (Kaufman et al., 1986). We compared the number of GAD67-positive neuron profiles in 4 pairs of homozygous adult mice with the number in wild-type littermate controls. Immunocytochemistry was performed on six serial sections at three levels, the frontal, parietal and occipital dorsal cortex. We found that, in all parts of the cortex, there was a reduction in the number of GAD67-positive neuron profiles (Fig. 8, compare E and F). On average, homozygous brains contained 39% fewer GAD67-positive cells (homozygous brains, 43.5±5.3 GAD67-positive cell profiles; wild-type brains 71.6±6.1 GAD67-positive cell profiles in sections of cortex with 1 mm of ependyma at the base).

**DISCUSSION**

In this paper, we have described a mutation, querkopf<sup>gt</sup>, in a member of the MYST family of histone acetyltransferases. This gene, in contrast to other histone acetyltransferases, which are expressed uniformly, is expressed strongly in discrete cell populations, in particular the ventricular zone of the cortex.

querkopf mutant mice show defects in skeletal development, brain development and gastrointestinal function. In this paper, we have concentrated on cerebral cortex development in these mice. Our analysis of the querkopf mutant mice shows that there are defects in three specific areas of cerebral cortex development. (1) Mice homozygous for the querkopf mutation show a reduction in the size of the dorsal telencephalon at the time when the telencephalon anlage undergoes an expansion. During neurogenesis, a large decrease in cell number in the cortical plate was seen. (2) querkopf mutant mice showed defects in the development of the large pyramidal cell population in layer V, specifically the 

<sup>1</sup>Otx1-expressing cells, of the cortex. (3) querkopf mutant mice showed a large reduction in the number of GAD67-positive interneurons in the cortex.

The cell populations affected most severely are those that express the gene most strongly. Presumably these cell populations have a higher requirement for Querkopf function and the amount of Querkopf produced by the hypomorphic allele is inadequate. Despite the presence normal mRNA produced from the querkopf<sup>gt</sup> allele, 65% of the homozygous die before the age of one month. A null allele would be expected to have a more severe phenotype. The function of the transcriptional co-activator, CBP, is also highly concentration dependent. CBP is also a histone acetyltransferase (Bannister...
Querkopf is required for cerebral cortex development

In conclusion, we have shown that a histone acetyltransferase of the MYST family, Querkopf, is strongly expressed there. It is interesting that the Drosophila MYST family member, MOF, appears to function differently to other histone acetyltransferases described so far. MOF is involved in X chromosome dosage compensation in Drosophila. In male flies, MOF is responsible for a twofold increase in transcription from the single male X chromosome by hyperacetylation (Hilfiker et al., 1997). Rather than being recruited to specific promoters scattered throughout the genome by interaction with DNA-binding proteins, MOF action leads to global acetylation of histones over the whole male X chromosome. A possible mode of action for a protein complex including Querkopf, analogous to the action of MOF, may be maintaining a generally high level of histone acetylation in stem cell populations, such as in the ventricular zone, where it is expressed strongly. In this way, Querkopf may be responsible for maintaining an open chromatin structure and so providing ready access for transcription factors. As other co-activators, such as CBP, have multiple functions, this mode of action does not preclude additional functions at specific promoters.

In conclusion, we have shown that a histone acetyltransferase of the MYST family, Querkopf, is strongly expressed in the cerebral cortex at a time when this cell population undergoes a rapid expansion and during neurogenesis. Analysis of the protein structure suggests that Querkopf acts as a co-activator of transcription. Expression of this gene is strong in many undifferentiated cell populations, particularly the ventricular zone of the cortex. Analysis of the loss-of-function phenotype shows that Querkopf function is required during development of the cerebral cortex. The results presented in this paper support the notion that co-activators of transcription, having chromatin-modifying activity, are another level where regulation of developmental processes takes place.

We gratefully appreciate the excellent technical assistance of

and Kouzarides, 1996). Inactivation of one CBP allele leads to Rubinstein-Taybi disease, characterised by mental retardation and craniofacial abnormalities (Petrij et al., 1995).

We found that, in the querkopf mutant mice, there was a large decrease in the number of cells in the preplate and cortical plate during the period of maximum neurogenesis from E13.5 to E15.5. This means that fewer cells were exiting the cell cycle during this period. In querkopf mutant mice, we observe a lack of large pyramidal cells in layer V of the cortex. It is possible that these cells are present but do not show strong Nissl staining or neurofilament light chain expression. This possibility is unlikely, because, firstly, we do find some large pyramidal cells that show strong Nissl staining and strongly express neurofilament light chain and, secondly, we found a large reduction of Otx1-positive cells. The remaining Otx1-positive cells expressed Otx1 at normal levels. These markers show that there is a specific loss of the large pyramidal cell population. The available evidence suggests that the patterning events that determine the cortical layer identity of neurons in the progenitor population are directly linked to the cell cycle (McConnell and Kaznowski, 1991). The observation that fewer cells are leaving the cell cycle at the time at which deep-layer neurons are produced may indicate that coordination of events regulating the differentiation of neurons is defective in querkopf mutant mice.

In the querkopf mutant cortex, we found a large reduction in the number of GAD67-immunoreactive neurons suggesting that there is a reduction in GABAergic interneurons in these mice. It is possible that interneurons exist in the cortex, but do not contain GAD67 in sufficient levels to be detected above background, possibly producing more GAD65. This latter possibility seems unlikely as some cells are present containing normal levels of GAD67. However, we cannot rule out the possibility that Querkopf is required for the transcription of the glutamate decarboxylase GAD67 gene. Many, if not most, GABAergic interneurons are produced in the lateral ganglionic eminence and migrate into the cortex (Anderson et al., 1997).

No defects were seen in the adult striatum suggesting that there is no generalised defect in the proliferative zone of the basal ganglia, although querkopf is strongly expressed there. An interesting possibility is that querkopf expression in the basal ganglia is important only for those cells that will ultimately reside in the neocortex.

Several lines of evidence suggest that regulation of chromatin structure is intimately involved in the process of development (Gross and Garrard, 1987; Patterton and Wolffe, 1996). Presumably, stem cells have a specific pattern of histone acetylation that allows DNA-binding transcription factors access to binding sites. Since acetylation of histones has been shown to increase accessibility of transcription factors to their binding sites (Lee et al., 1993; Vettese-Dadey et al., 1996), it is possible that a histone acetyltransferase exists that maintains a high level of histone acetylation in the stem cell genome. In this context, it is interesting that the Drosophila MYST family member, MOF, appears to function differently to other histone acetyltransferases described so far. MOF is involved in X chromosome dosage compensation in Drosophila. In male flies, MOF is responsible for a twofold increase in transcription from the single male X chromosome by hyperacetylation (Hilfiker et al., 1997). Rather than being recruited to specific promoters scattered throughout the genome by interaction with DNA-binding proteins, MOF action leads to global acetylation of histones over the whole male X chromosome. A possible mode of action for a protein complex including Querkopf, analogous to the action of MOF, may be maintaining a generally high level of histone acetylation in stem cell populations, such as in the ventricular zone, where it is expressed strongly. In this way, Querkopf may be responsible for maintaining an open chromatin structure and so providing ready access for transcription factors. As other co-activators, such as CBP, have multiple functions, this mode of action does not preclude additional functions at specific promoters.
REFERENCES


