**Abstract.** The N-myc proto-oncogene is expressed during embryogenesis, suggesting that it plays a role in normal development. Since the myc-family oncogenes have been implicated in the control of cell growth, the embryonic expression may reflect rapid proliferation known to occur in development. Alternatively, N-myc expression may be involved in specific differentiation stages. In many embryonic tissues, early and late differentiation events occur in different locations. By in situ hybridization of tissue sections, we now demonstrate a restricted expression of N-myc mRNA to a few tissues and to areas where the first differentiation stages occur. N-myc expression was most strongly expressed in the developing kidney, hair follicles, and in various parts of the central nervous system. In these tissues, expression was restricted to a few cell lineages. In all lineages, expression was confined to early differentiation stages, and, at onset of overt differentiation, the level of expression decreased dramatically. Several rapidly proliferating tissues showed very little, if any, N-myc expression. In the brain, post-mitotic but not yet differentiated cells expressed high levels of N-myc mRNA. Therefore, N-myc expression is not a simple marker for proliferation in the embryo. Rather, N-myc expression seems to be a feature of early differentiation stages of some cell lineages in kidney, brain, and hair follicles, regardless of the proliferative status of the cell. The results raise the possibility that N-myc may participate in the control of these early differentiation events.

**Proto-oncogenes** have been shown to be expressed during embryonic development (Müller et al., 1982) suggesting that a physiological role of oncogenes is to control normal development (for reviews see Bishop, 1983; Weinberg, 1985; Müller, 1986; Adamson, 1987). It is not yet clear whether the expression of oncogenes in embryonic cells is related to proliferation, to differentiation processes, or to both. Although several lines of evidence point to a role of oncogenes in cell cycle control, they may have additional roles, unrelated to proliferation, during development.

The members of the myc-oncogene family encode nuclear proteins believed to be important for regulation of cell growth. Three well-defined members of the myc-family are known to date, c-myc (Sheinnes and Bishop, 1979; Sheinnes et al., 1980; Alt et al., 1986), N-myc (Kohl et al., 1983; Schwab et al., 1983), and L-myc (Nau et al., 1985; Legouy et al., 1987). They are found in many species and the mRNA sequences have been deduced from cDNAs of both human and mouse. The known myc oncogenes are all expressed at high levels in the embryo (Jacobovits et al., 1985; Zimmerman et al., 1986; Sejersen et al., 1987), and it has been clearly documented that the expression of N-myc and L-myc is very restricted with respect to tissue and stage in the newborn mouse, while that of c-myc is more generalized. Northern blotting of total RNA revealed particularly high levels of N-myc expression in developing brain and kidney in newborn mice (Zimmerman et al., 1986).

For the developing brain, the areas of proliferation have been characterized previously (Miale and Sidman, 1961; Hinds, 1968) and there are also data on proliferation of some cell types in the developing kidney (Ekblo et al., 1983). In the developing kidney, proliferation and differentiation occur in the same anatomical compartments, and cells seem to proliferate both during early and late differentiation. In some areas of the developing brain, however, post-mitotic but not yet differentiated cells can be found in defined locations. In such cases, the differentiation process occurs after cessation of cell proliferation and migration to the target locations (Miale and Sidman, 1961; Angervin and Sidman, 1961). Thus, these embryonic tissues can be used to study the relationship between cell proliferation, cell differentiation, and expression of the N-myc oncogene. We have therefore performed in situ hybridization of sections of mouse embryos with an N-myc probe.

Since Northern hybridization of total RNA showed strong signals in developing brain and kidney in newborn mice and
embryos (Zimmerman et al., 1986; this work), we expected to see expression in many cell lineages in these tissues. In situ hybridization revealed, however, that N-myc mRNA was remarkably restricted to a few cell lineages in the developing kidney and brain. Furthermore, expression occurred only during the earliest stages of differentiation of the cells and it was shut off when overt differentiation started. An unexpected strong signal was seen in developing hair, but also here, the expression was confined to the early differentiation stages.

Materials and Methods

Embryos and Tissues

Hybrid mouse embryos 129 X NMRI were used. The day of the vaginal plug was designated as day 0. To minimize RNA degradation, tissues collected for Northern blotting of total RNA were immediately frozen on dry ice after microsurgery, and care was taken to perform the microsurgery immediately after decapitation. Frozen sections from embryonic kidneys and newborn mice were prepared as described by Holland et al. (1987) except that embryonic kidneys were fixed in 4% paraformaldehyde for 1 h only.

Isolation of RNA

For preparation of total RNA, 0.5 g of frozen tissue were homogenized in 5 ml of 4 M guanidinium thiocyanate (Fluka AG, Buchs, Switzerland; pu.

Preparation of Labeled Probes

For nick translation, the 2.3 kb Bgl II/Eco RI fragment of the murine genomic clone p2773 carrying parts of the second intron and the whole third exon of the N-myc gene (DePinho et al., 1986) was purified from a 1% agarose gel and labeled with 30-40 μCi [α-32P]dCTP (6000 Ci/mmol; Amersham-Buchler, Buchsweig, FRG) using a nick translation kit (Bethesda Research Laboratories, Bethesda, MD). The reaction was carried out in a final volume of 50 μl according to the supplier's instructions. The reaction was stopped by addition of 40 μl TNE (10 mM Tris-Cl, 10 mM NaCl, 0.15 M NaCl, 10 mM EDTA, pH 8.0). The labeled DNA was collected by isopropanol precipitation for 15 min at room temperature (RT) and then twice in 0.1× SSC/0.1% SDS for 15 min each followed by 0.1 M ethanol. The RNA pellet was dissolved in 10 mM Tris-Cl, 0.25 mM EDTA, pH 8.0, and chloroform were added. After incubation for 15 min at 37°C, the RNA was extracted once with phenol/chloroform (1:1) and once with chloroform. Unincorporated nucleotides were precipitated by the labeled RNA (2 M ammonium acetate, 2 μl ethanol) for 10 min on dry ice. The RNA was then redissolved in 10 μl EDTA. The antisense (−) strand (i.e., complementary to N-myc mRNA) was synthesized using SP6 RNA polymerase after linearizing the template DNA with Hind III. The sense (+) strand was synthesized using T3 RNA polymerase after linearizing with Eco RI, and was used for control hybridization. Probe length was reduced to an average size of 100-200 nucleotides by limited alkaline hydrolysis (Cox et al., 1984) and was checked by formaldehyde agarose gel electrophoresis (Lerch et al., 1977). Probes were stored in 50% formamide and 10 μM EDTA at −20°C.

Northern Blotting

Equal amounts of total RNA subjected to electrophoresis on 1% agarose gels after denaturation with glyoxal (McMaster and Carmichael, 1977). After electrophoresis, the lane with the molecular weight marker (Hind III/Eco RI-cut λ-DNA) was cut from the gel and stained with ethidium bromide (5 μg/ml). Glycerol followed by boiling the filters under vacuum at 80°C for 2 h. Blots were prehybridized in 50% deionized formamide, 5× SET (0.75 M NaCl, 0.15 M Tris, 10 mM EDTA, pH 8.0, 2.5× Denhardt's solution, 0.5% SDS, and 0.1 mg/ml herring sperm DNA for 1-2 h at 42°C. The heat-denatured, nick translated N-myc probe (2.4 × 10^8 cpm/ml) was added to the prehybridization mixture and hybridization was performed at 42°C for 15-20 h in several standard hybridization conditions. Blots were washed twice in 2× SSC/1% SDS at 42°C for 15 min, then twice in 0.1× SSC/0.1% SDS for 15 min at 42°C. Control hybridizations with the same filters were performed under the same conditions using the nick translated 1.2-kb Pst I fragment (2.7 × 10^8 cpm/ml) of the murine β-actin cDNA clone pAL41 (Mimny et al., 1983). For removal of the probe before subsequent rehybridization, blots were washed in boiling water for 1-10 min. Northern blots were exposed to Kodak X-OMAT AR x-ray films for various lengths of time at −70°C using intensifier screens. In the liver, however, the β-actin expression decreased with advancing development, and actin expression could therefore not be used to monitor the amount of RNA in this tissue. For the different stages of liver development, we therefore stained the filter for 28 and 18 S RNA with 0.004% methylene blue (Maniatis et al., 1982).

In Situ Hybridization of Tissue Sections with Single-stranded RNA Probes

In situ hybridization was performed according to P. Holland's protocol as described in Hogan et al. (1986). Sections (8-10 μm) were cut on a cryostat (Reichert and Jung, Nussloch, FRG) at −15°C, collected on polylysine-coated slides, masked and dried on a hot plate at 30°C for 3 min, and air-dried for 1-2 h at RT. Sections were postfixed in 4% paraformaldehyde in PBS (20 min, RT), rinsed in PBS (three times for 5 min at RT), and dehydrated with increasing ethanol series. After air-drying, slides were stored desiccated at −20°C. Pretreatment of sections was performed as described by Hogan et al. (1986) except that the incubation step in 2× SSC at 70°C was omitted. After incubation in 0.2 M HCl (20 min, RT) to remove basic proteins, sections were treated with self-digested pronase (0.125 mg/ml) for 10 min at RT. The pronase reaction was subsequently blocked with 0.2% glycine in PBS (30 s, RT). The slides were then rinsed in PBS, fixed in 4% paraformaldehyde, acetylated, dehydrated, and air-dried. Hybridization conditions were as described by Ingham et al. (1985). Probes were used at a final concentration of ~10 μg/ml. Posthybridization washes including RNase A digestion were performed as described (Hogan et al., 1986). For autoradiography, the slides were dipped in emulsion (K2, Ilford Ltd., Basildon, Essex, UK), diluted 1:1 with 1% glycerol in water. After air-drying, slides were exposed at 4°C for 7-11 d, developed in Kodak D19 developer (2 min at 20°C), stopped in 1% acetic acid (1 min at 20°C), fixed in 30% sodium thiosulphate (5 min at 20°C), and rinsed in distilled water. Sections were stained in toluidine blue (0.02% for 30 s), dehydrated, and mounted. Photographs were taken under bright-field and dark-field illumination.

Results

Previously performed hybridization of total RNA had shown that N-myc mRNA was present in the mouse embryo during...
preimplantation development, in certain teratocarcinoma cell lines, and during late organogenesis of brain and kidney (Jacobovits et al., 1985; Zimmerman et al., 1986; Sejersen et al., 1986). Only a limited amount of information was, however, available for early and late prenatal organogenesis. Differentiation is asynchronous in organs, and many rather undifferentiated cells, intermixed with terminally differentiated cells, are present during late organogenesis. It was therefore unclear whether N-myc expression during late organ development in newborn mice reflected N-myc expression by cells in early or late differentiation stages. To study this issue in more detail for the solid organs, we performed Northern blotting experiments of earlier developmental stages, and in situ hybridization of tissue sections. Embryonic day 12 was selected as a starting point since one primary interest was to study kidney development. Kidneys from 12-d embryos are composed predominantly of cells in their earliest differentiation steps. Heart, lung, and liver organogenesis, however, already starts somewhat earlier in embryonic development. Brain development also starts earlier but continues postnatally (for details of mouse development, see Theiler, 1972; Jacobson, 1978; Hogan et al., 1986).

**RNA Blot Analysis of N-myc Expression**

We assayed total RNA of several major organs (brain, kidney, heart, lung, liver) using electrophoresis and subsequent hybridization to the N-myc probe. In tissues from the youngest embryos studied (12-d old), abundant expression of N-myc mRNA was seen in the brain and kidney. A somewhat weaker expression was seen in the lung and heart (Fig. 1), and a very weak signal could be detected in the liver from the youngest embryos (Fig. 2). In the kidney, lung, heart, and liver, expression gradually decreased with advancing embryonic development, and adult tissues showed no expression. Expression seemed to remain more constant during embryonic brain development (Fig. 1), but as also shown previously (Zimmerman et al., 1986), we found that it declined during postnatal life. In adult brain, no N-myc mRNA was detected with the methods used. These data suggested that N-myc expression indeed could be characteristic for the early stages of organogenesis, but with this method it was not possible to judge whether all cells or only a few cell types in the organs expressed N-myc mRNA.

**In Situ Localization of N-myc mRNA in Sections**

In tissues which by Northern blotting showed strong N-myc expression, the developing brain and kidney, it was possible by in situ hybridization of sections to identify the cells expressing N-myc. A remarkable, stage- and cell-lineage specific expression was noted. In addition, we found a strong expression in developing hair follicles of newborn mice. In the other tissues from newborn mice, the hybridization signal was considerably weaker, and in most tissues from newborn mice, no clear signal was obtained. The expression of N-myc in the mouse embryo was found to be very different from that of c-myc in the human embryo (see Pfeifer-Ohlsson et al., 1985).

**In Situ Hybridization of N-myc in Embryonic Kidneys**

In the developing kidney, at least four different cell lineages are present: the ureter epithelium, the endothelium, and two different mesenchymes. The differentiation of the ureter epithelium and the endothelium is largely an ingrowth and branching process, whereas the two mesenchymal cell popu-

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*Figure 1. N-myc RNA expression in different tissues during murine development. Total cellular RNA (20 μg per lane) from various tissue samples isolated from days 12–18 p.c. (12–18 d), newborn (nb), and adult (a) mice was hybridized to the N-myc probe. To show the quality of RNA, the same filter was also hybridized to a β-actin probe. Autoradiographic exposure time was 22 h for N-myc (top) and 1.75 h for β-actin (bottom). Note that hybridization for actin mRNA reveals the expected shift from β-actin to α-actin during embryonic heart development.*
Lineages occur in the cortical parts of the embryonic kidney, part of the mesenchyme will become stroma (Aufderheide et al., 1987), and another part will convert into a new epithelium (Figs. 3 and 5). The differentia-
tion process: condensation, comma-shape, S-shape, tubule elongation, and folding of the podocytes of the glomeruli (Fig. 4). Larger magnification of the cortical areas showed that N-myc mRNA was expressed exclusively during early tubule differentiation, namely in the condensates (Fig. 5, a and b) the comma- and S-shape stages (Fig. 5, c and d), but not in the subsequent stages (Fig. 5, c-f). Due to the continuous branching of the ureter and a proliferation of mesenchymal cells in the periphery, previously induced mesenchymal cells will be gradually displaced towards the inner parts of the kidney as development proceeds (Osathanondh and Potter, 1963). Because of this, the later differentiation events (tubule elongation and podocyte folding in the glomerulus) occur in the inner part of the developing kid-
neys. As can be seen both in low magnification (Fig. 3) and high magnification (Fig. 5) of 16-d-old kidneys, the inner parts, which contain the still proliferating elongating tubules and maturing glomeruli, were not expressing N-myc (Fig. 3, c and d; Fig. 5, c-d), whereas the areas which contain mesenchymal cells in their early differentiation stages around the ureter tips in the cortex were strongly positive for N-myc mRNA (Fig. 5, a-d). Hence, N-myc expression seems to be restricted to the precursors of one cell lineage, the mesenchymal cells which will differentiate into kidney tubules.

In Situ Hybridization of N-myc in Developing Skin

In the skin of newborn mice, N-myc expression could be detected in the hair follicles but it was restricted to the hair bulbs. The epidermis and the dermis were both negative for N-myc mRNA (Fig. 6, a-d). The hair bulb, the germinative zone of the hair follicle, consists of a bell-shaped mass of proliferating epithelial cells enclosing the mesenchymal dermal papilla. As soon as the cells leave this compartment, they undergo terminal differentiation. Concomitantly, the proliferation rate drastically decreases (Epstein and Mai-
bach, 1969; Sengel, 1976). As it is known that the great majority of the hair follicles are built up during embryonic and early postnatal development (Sengel, 1976), it is not surpris-
ing that, in newborn mice, N-myc mRNA was present in all hair follicles. The bell-shaped mass of N-myc mRNA-positive cells is readily distinguishable from both the N-myc-negative dermal papilla and the hair shaft in sagittal sections of the developing hair (Fig. 5, c and d). Thus, in the developing skin, N-myc expression seems to be confined to one cell lineage, and to its earliest developmental stages.

In Situ Hybridization of N-myc in Developing Brain

Within the developing brain of newborn mice, several distinct areas with high levels of N-myc expression were discernible. In the cerebellum, the external granular layer showed strong N-myc expression (Fig. 7, a and b). This layer, the second
germinal layer in the cerebellum, is known to be an extremely mitotically active zone during later fetal life and the first 2 wk of postnatal murine development (Miale and Sidman, 1961). Undifferentiated neuroblasts originating from this germinal layer migrate deeper into the cerebellar cortex to their respective target layers where they terminally differentiate. One of these cortical layers, the inner granular layer, was also positive for N-myc although the signal intensity was much weaker. In contrast to the growth in the cerebellum, the increase in size of the neocortex and the bulbus olfactorius is due to accretion of post-mitotic neuroblasts that are generated in the respective germinal zones around the ventricles. After migration to their target layers, the neuroblasts finally differentiate into mature neurons (Miale and Sidman, 1961; Hinds, 1968; Jacobson, 1978). We found that the post-mitotic but not yet differentiated cells in the neocortex (Fig. 7, c and d) and the bulbus olfactorius (Fig. 7, e and f) were expressing N-myc. In the bulbus olfactorius, N-myc expression was restricted to two of the six layers, namely to the mitral cell and the internal granular layer, which are composed of postmitotic but not terminally differentiated cells. In contrast, the three more externally located layers with differentiated cells were negative for N-myc (Fig. 7, e and f).

**Discussion**

In the embryo, overt cell differentiation is in most organs preceded by one or more distinct cell determination stages. During these stages, stem cells become progressively more determined towards certain differentiation pathways. The early determination stages can also be viewed as the first differentiation stages as opposed to terminal differentiation. In general, the determined state of a cell would not be apparent by direct inspection of any means now available but is
The hydration of the kidney, the ingrowth of the vessels, and the conver-

Figure 4. Scheme of kidney differentiation in vivo showing the branching of the ureter, the ingrowth of the vessels, and the conversion of the induced mesenchyme to epithelium, redrawn according to Kazimierzak (1971) and Ekblom (1984). The four different cell lineages, the ureter (1), the blood vessels (2), the uninduced mesenchyme (3), and the induced mesenchyme (4) are first seen clearly in the condensation stage. After this stage, the induced mesenchyme first condenses, develops into a comma-shaped and S-shaped structure, the tubules elongate, and simultaneously the podocytes in the glomeruli fold.

only deduced from its later behavior (Dawid and Wahli, 1979). The location of the cells which are still in these early differentiation stages has been well-defined morphologically for many organs, although very few molecular markers are available. In the present study, we show by in situ hybridization that N-myc expression is a feature of the earliest differentiation stages of some cell lineages in the developing brain, kidney, and hair follicles. It remains to be seen whether c-myc and L-myc expression shows a similar pattern, but Northern blotting (Zimmerman et al., 1986) suggest that each member of the myc-oncogene family could have a unique tissue distribution.

When sufficiently young embryonic tissues were analyzed, some N-myc mRNA could be detected in several other tissues as well (lung, heart, liver) and expression was, in all cases studied, strongest in the tissues from the youngest embryos. Thus, it is possible that some expression of N-myc could be a more general feature of early differentiation stages in the embryo. However, the cells expressing it could be assessed with certainty only in the tissues which showed exceptionally strong expression, namely in the brain, kidney, and hair follicles.

For several cell lineages, the first differentiation stages are coupled with cell proliferation, and it has been argued that the mitotic process itself is a crucial part of the differentiation of stem cells (Holzer et al., 1975; Lathja, 1979). Proliferation is a feature of early differentiation of those cells in the developing kidney, hair follicles, and cerebellum which showed a strong expression of N-myc. It could therefore not be distinguished whether N-myc expression was due to the rapid proliferation or whether it marked the earliest differentiation stages. In certain regions of the developing brain, however, cells stop proliferating before differentiation begins. Because of subsequent migration of the post-mitotic cells, differentiation and proliferation will occur in different locations. We could show that post-mitotic cells which were still in the early differentiation stages expressed N-myc. Similar findings were recently reported for human embryonic brain (Grady et al., 1987). The N-myc transcripts have a half-life of about 130 min (Sejersen et al., 1987), and the cells which expressed N-myc in the brain had been post-mitotic for more than 24 h (Hinds, 1968). Therefore, it is extremely unlikely that the N-myc mRNA in post-mitotic but not yet differentiated cells represents mRNA synthesized during the earlier, mitotic stages.

Many of the tissues known to continue to proliferate throughout organogenesis showed no expression of N-myc during late organogenesis (liver, lung). Furthermore, within those tissues showing strong expression, some rapidly proliferating cells expressed very little, if any, N-myc. In the developing kidney, for example, the ureter cells are known to grow rapidly, but expression was seen only in the nearby mesenchymal cells. Thus, neither N-myc as shown here, nor c-myc as previously shown (Pfeifer-Olsson et al., 1985; Godeau et al., 1986; Taylor et al., 1986), can be considered as a simple marker for proliferative activity during embryogenesis. The most compelling evidence that N-myc is associated with early cell differentiation steps rather than with cell proliferation is the fact that the post-mitotic, not yet differentiated brain cells are strongly N-myc-positive. In vitro cell culture experiments have also demonstrated that undifferentiated neuroblasts from neuroblastomas and pre-B cells express N-myc, and that decreased expression correlates with initiation of differentiation (Thiele et al., 1985; Zimmerman et al., 1986).

Taken together, current evidence suggest that N-myc expression in the embryo is a feature of certain stem cells which are still in the earliest differentiation stages regardless of the proliferative state of the cells. Therefore, the proto-oncogene N-myc can be considered as a marker for such early differentiation stages. Northern blotting experiments show that N-myc expression occurs also in late organogenesis (Zimmerman et al., 1986) but the in situ hybridization results establish that this is due to not yet differentiated cells which remain present in the tissues also at late organogenesis.

The identification of the cell types that express oncogenes during normal development should help us to clarify the physiological role of oncogenes. It has recently been shown for some other proto-oncogenes that they, like N-myc, show a remarkable tissue-specific and stage-specific expression during embryogenesis (Wilkinson et al., 1987; Shakleford and Varmus, 1987; Dony and Gruss, 1987). The expression of N-myc mRNA seems to be particularly interesting since it occurs during early differentiation stages and is turned off at onset of terminal differentiation. In the developing tissues which express c-fos mRNA, another nuclear oncogene in-
plicated in growth control, the oncogene products were expressed rather late in development, and only during development of a few bones (Dony and Gruss, 1987). Myc and fos gene products have been suggested to have a direct or indirect role in regulating the expression of other genes, a proposal consistent with the fact that these oncogenes encode nuclear proteins (Slamon et al., 1984; Renz et al., 1985; Sambucetti and Curran, 1986). If the proteins were to serve as control genes in the embryo, they should be expressed during early differentiation steps rather than during late differentiation. We have here shown such a remarkable pattern for N-myc. The data raise the possibility that N-myc functions as a control gene during several early differentiation events in the developing embryo. Such functions have been directly demonstrated previously for homeobox and segmentation genes and other developmentally important control genes, which code

Figure 5. Analysis of N-myc expression with an antisense RNA-probe during the condensation of the induced mesenchyme, formation of S-shaped bodies, tubule elongation, and glomerular development in cryostat sections from mouse kidneys. Compare with scheme in Fig. 4. (a and b) Autoradiograph of the nephrogenic zone of the 13-d mouse kidney revealing the label surrounding the ureter tips. Note label only in the induced mesenchyme whereas neither the surrounding loose mesenchyme nor the ureter buds (u) show N-myc expression. Antisense (−) probe, bright field (a) and dark field (b) illumination. (c and d) Autoradiograph of a section from the cortical area reveals high density of silver grain in two S-shaped bodies found around the ureter (u) epithelium which shows very few grains. Note that both the renal capsule above the S-shaped tubules, and the glomerulus (arrows) found in the lower part of the figure show very few grains. Bright field (c) and dark field (d) illumination. (e and f) Autoradiograph of a section from the area with elongating and proliferating tubules. The density of the silver grains is low and does not differ from background levels found outside the sections. Bright field (e) and dark field (f) illumination. Autoradiography was for 11 d. For stages of nephron development, see Fig. 4. Bars, 50 μm.
for nuclear proteins with DNA-binding capacity (White and Wilcox, 1984; Miller et al., 1985; Rosenberg et al., 1986). Another equally intriguing possibility is that expression of myc oncogenes is required to retain the cells in an undifferentiated state. It has been shown for several cell types that experimentally induced constitutive expression of c-myc can inhibit differentiation (Coppola and Cole, 1986; Denis et al., 1987; Lomø et al., 1987). Such an effect could be physiologically important in the embryo because it could ensure the existence of a sufficient amount of stem cells.

Because some pediatric tumors derived from nerve cells and kidney cells express high amounts of N-myc (Schwab et al., 1983, 1984; Nisen et al., 1986), it was important to identify the cell types that express N-myc during normal development. Our data may shed some light to the histogenesis of these tumors. In the Wilms' tumor, the childhood kidney cancer derived from developing kidney cells, high levels of N-myc expression have been reported. It is interesting that there is no amplification of the gene in Wilms' tumor as in other tumors with an elevated N-myc expression (Nisen et al., 1986). This could be due to a large number of determined but yet undifferentiated mesenchymal cells in Wilms' tumor. Differences in the extent of N-myc expression between different Wilms' tumor samples (Nisen et al., 1986) may simply be due to the varying amounts of the mesenchymal cells which fail to differentiate further. In situ hybridization studies on the expression of the N-myc oncogene in these tumors, brain, and skin tumors, should help to clarify these issues. We anticipate that analysis of N-myc mRNA in tissue sections with single-stranded antisense probes in combination with Northern blotting experiments will be useful in diagnostic histopathology.

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Figure 7. In situ hybridization with antisense N-myc RNA to sagittal sections of different areas of the brain of a newborn mouse. (a and b) N-myc mRNA distribution in the cerebellum of a newborn mouse. High density of silver grains are seen in the external granular layer (EG). The internal granular layer (IG) also exhibits N-myc expression although to a lesser degree, whereas the molecular layer (Mol) is devoid of silver grains. Photographed under bright field (a) and dark field (b) illumination. (c and d) Autoradiograph of a section of the neocortex of the newborn mouse. Label is evenly distributed throughout the cortical plate. The patchy label in the upper left corner, outside the brain, represents the strong signals in the hair bulbs. Photographs were taken under bright field (c) and dark field (d) illumination. Layers: (CP) cortical plate; (IZ) intermediate zone; (SZ) subventricular zone; (VZ) ventricular zone. (e and f) Localization of the N-myc mRNA in a section of the bulbus olfactorius. High grain density is present in the mitral cell layer and throughout the internal granular layer. Bright field (e) and dark field (f) illumination. Layers: (IG) internal granular layer; (MC) mitral cell layer; (EP) external plexiform layer; (G) glomerular layer. The innermost subventricular layer and the outermost layer of olfactory nerve fibers are not present in this section. (See Hinds, 1968; nomenclature of the different cells in the respective layers). Autoradiography was for 9 d. Bars, 200 μm.
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