its metanephrogenic mesenchyme (*4*), obstruction of urine drainage (*1*), or interference with the development of the ampullae of the ureteral bud branches (*5*). These possibilities require evaluation. Experimental ligation of the fetal or embryonic ureter obstructs urine drainage but is not always associated with the later development of dysplasia (*1, 6–8*). These discrepant results suggest that factors other than simple obstruction must be considered in the genesis of dysplasia.

Renal blastemas were microdissected from chick embryos in days 7 to 8 of incubation. At this stage, the renal blastema is an elongate, branched ureteral bud apposed by condensed metanephrogenic mesenchyme (Fig. 1A); nephrons are not yet present. Seventeen blastemas were cultured on the chorioallantoic membrane as an in ovo graft for up to 10 days. These normal blastemas developed a normal renal architecture (13 specimens) or mild hydronephrosis (four specimens). Twenty-six other blastemas were placed in tissue culture (*9*) for up to 7 days. These explants displayed branching of the ureteral bud, but the metanephrogenic mesenchyme did not remain condensed (Fig. 1B). Finally, 33 renal blastemas deprived of condensed metanephrogenic mesenchyme by preliminary tissue culture were further cultured as chorioallantoic grafts. This procedure resulted in kidneys that displayed normal architecture, normal architecture with a few primitive ducts, many primitive ducts with few renal tubules, or primitive ducts only (no renal tubules) (Table 1). Primitive ducts were especially frequent when blastemas were cultured in vitro for at least 4 days ($P < .005$, chi-square test) (Fig. 1C). Both the primitive ducts induced in the chick embryos and those present in 11 human dysplastic kidneys (Fig. 1D) demonstrated tall epithelium surrounded by whorled mesenchymal cells.

Our results suggest that primitive ducts originate from branches of the ureteral bud that develop without condensed metanephrogenic mesenchyme. Cartilage, another feature of human renal dysplasia, was not seen in this chick model. It may be that the chick metanephrogenic mesenchyme is not capable of metaplastic differentiation or that the brief period of grafting does not permit mesodermal expression into cartilage. That chick renal blastemas explanted in tissue culture did not further differentiate may be accounted for by the inability of new tubules to be fostered in vitro.

There has been little investigative effort to clarify the morphogenesis of genitourinary malformations. Attempts to induce renal dysplasia experimentally have been directed at obstructing the urine drainage of fetal (*6, 7*) or neonatal (*1*) kidneys. These attempts have not been uniformly successful, perhaps because of the timing of the experimental manipulations (*10*). The most severe cases of dysplasia are believed to develop from insults that affect the kidney before nephrogenesis (*11*). The model described here provides a unique means of interfering with the development of the embryonic kidney before the appearance of renal tubules. Obstructing the urine drainage of the embryonic kidney in this model does not cause dysplasia. (*8*).

In conclusion, it appears that perturbing the condensed metanephrogenic mesenchyme of the embryonic kidney leads to changes consistent with renal dysplasia. These experiments support the notion that congenital renal malformations arise from faulty nephrogenesis. They also provide a basis for further manipulation of embryonic tissue to simulate congenital urologic malformations. Such simulations could provide a better understanding of the morphogenesis of congenital malformations.

Table 1. Histological development of renal blastemas cultured in vitro and in ovo. Values are numbers of grafts showing the indicated architectures.

Days in tissue culture	Architecture of graft*			
	Normal	Some primitive ducts	Many primitive ducts	Primitive ducts only
0	17	0	0	0
1 to 3	7	5	1	1
4 to 7	2	4	8	5

*Includes specimens that exhibited dilation of the ureter or tubules; these changes may be interpreted as hydronephrosis.

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Expression of a Cellular Oncogene During Liver Regeneration

Abstract. *The number of transcripts of the cellular oncogene* ras, *which is homologous to the transforming gene of Harvey sarcoma virus, increases during liver regeneration in rats. The increase in these transcripts in liver polysomal polyadenylated RNA occurs at the time of activation of DNA synthesis during the regenerative process induced by partial hepatectomy or carbon tetrachloride injury. The number of* ras *transcripts returns to basal levels within 72 hours. These observations show that transcription of a cellular oncogene increases in a regulated way in a nonneoplastic growth process.*

Transforming retroviruses originate by recombination of type C RNA viruses with cellular sequences of vertebrate hosts (*1, 2*). Studies with temperature-sensitive and deletion mutants indicate that the cellular sequences incorporated into these viruses are necessary for virus-induced cell transformation (*3*) and for rapid production of tumors in vivo. So far, more than a dozen different viral transforming sequences (v-*onc*) have been identified, each with a cellular counterpart (c-*onc*) (*1, 2, 4, 5*). The oncogenicity of several of these cellular sequences has been demonstrated by their capacity to transform cells efficiently when linked to viral control elements (long terminal repeats) (*6, 7*).

Because cellular oncogenes are highly conserved and transcribed in uninfected normal cells, they might play a role in nonneoplastic growth processes and in nonviral neoplastic growth (*1, 2, 4, 5, 8*). We now present data on the expression of the Harvey sarcoma virus *ras* gene during liver regeneration induced by par-

tial hepatectomy or chemical injury. Transforming genes of human bladder and of lung or colon carcinoma cell lines that are homologous to the *ras* genes of Harvey (Ha-*ras*) and Kirsten (Ki-*ras*) sarcoma viruses, respectively, have been reported (*4, 5*). Moreover, Chang *et al.* (*9*), showed that the normal human gene homologous to Ha-*ras*, when linked to control elements, induces transformation in mouse fibroblasts.

In the rat, *ras* is a gene family consisting of at least two genes coding for 21,000-dalton proteins (p21) (*6*). These genes have been acquired by Harvey (HaMuSV) and Kirsten (KiMuSV) sarcoma viruses by passage of murine leukemia viruses through rat cells (*10*). The p21 *ras* protein has been observed in normal tissues and is present at high levels in a hemopoietic stem cell line (*11*). Transcripts of *ras* genes have been detected in various tissues of 10-day-old mice. Transcription of c-*ras* measured in whole mouse embryos varies little throughout prenatal development (*12*).

We used the BS-9 clone derived from the v-*ras* oncogenic sequence of Ha-MuSV (*13*) as a probe to detect cellular *ras* transcripts in normal and regenerating rat liver. Liver regeneration is one of the most striking examples of compensatory growth processes in mammals (*14*). Hepatocytes, which rarely divide in livers of adult animals, undergo DNA synthesis and replication in a partially synchronized manner during liver regeneration induced by partial hepatectomy or chemical injury. We used the "dot-blot" procedure (*15*) to hybridize polyadenylated [poly(A)] polysomal RNA from normal and regenerating livers with ^{32}P-labeled v-*ras* DNA. Figure 1a shows the amount of hybridized ^{32}P-labeled DNA in each spot, determined by punching out the appropriate spots and measuring their radioactivity in a scintillation counter. The data indicate that *ras* transcripts increase during liver regeneration induced by partial hepatectomy or CCl_4 administration. Changes of *ras* gene expression in regenerating liver coincide with the onset of DNA synthesis during both regenerative processes. The peak of DNA synthesis occurs 24 hours after partial hepatectomy and 48 hours after CCl_4 administration (Fig. 1a). In liver regeneration caused by partial hepatectomy, the number of *ras* transcripts, which does not change for about 12 hours, increases between 18 and 36 hours after the operation and returns to normal by 72 hours. After CCl_4 injury, the number of *ras* transcripts increases sharply between 36 and 48 hours and declines by 72 hours. Thus, maximum expression of the *ras* gene takes place 12 to 24 hours later, during regeneration after chemical injury. In a series of three other experiments, we have found that the numbers of *ras* transcripts increase 2.5- to 3-fold after partial hepatectomy and 2.5- to 4-fold in liver regeneration induced by CCl_4 injury. Since the kinetics of hybridization reactions with the dot-blot procedure have not been studied, we also hybridized the labeled BS-9 DNA probe to cellular poly(A)$^+$ RNA in solution, using a large RNA excess. The data confirm the results of the dot-blot procedures by showing a clear acceleration of the hybridization of the labeled probe with total cellular poly(A)$^+$ RNA from 16- and 40-hour regenerating liver. These reactions did not reach saturation and, at high R_0t values (R_0, initial RNA concentration; t, time) there was self-annealing of the probe (*16*). It is therefore difficult to calculate the number of copies of *ras* transcripts per cell. It is clear, however, that there is a two- to fourfold increase in *ras* transcripts after partial hepatectomy.

We have also used nucleic acid hybridization techniques to investigate the pattern of gene expression in normal and regenerating rat liver after partial hepatectomy. We analyzed the extent of genomic expression, levels of messenger RNA (mRNA), and RNA sequence complexity and homology. We concluded that there are quantitative differences, but no obvious qualitative differences, between mRNA populations of normal and regenerating liver (*17, 18*). The pattern of expression of the *ras* gene conforms to these conclusions. The magnitude of the increase in *ras* transcripts detected in regenerating liver is similar to the extent of change of p21 protein expression in human carcinoma cell lines and in NIH 3T3 cells transformed by DNA from bladder carcinomas (*19*). In the context of liver regeneration, it was interesting to compare the changes in *ras* transcripts with variations in the amount of polysomal poly(A)$^+$ RNA and in the level of α-fetoprotein (AFP) mRNA, a messenger that increases in regenerating liver (*20*). Figure 1b shows that the increase in *ras* transcripts starts after the increase in polysomal poly(A)$^+$ RNA has taken place (*21*) but precedes the major changes in AFP mRNA. The extent of the increase of *ras* transcripts is greater than that of AFP mRNA, but the

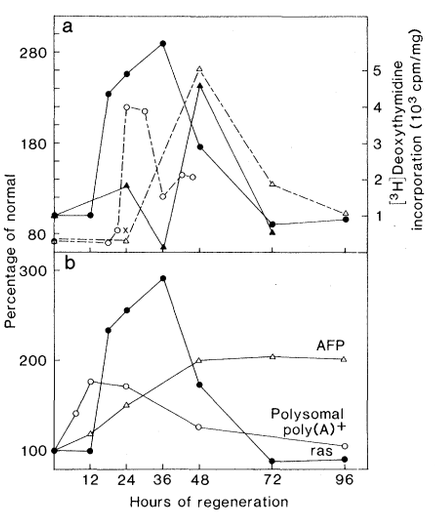

Fig. 1. Expression of the c-*ras* gene during regenerative liver growth. The livers of five to ten rats (male, Sprague-Dawley, Holtzman strain; 140 to 160 g) were used for each RNA preparation. Animals were maintained and partial hepatectomies were performed as described (*17, 18, 22*). CCl_4 was administered as a single intragastric dose (0.5 ml of 50 percent CCl_4 in olive oil per 100 g of body weight). Livers were homogenized, and polysomes were isolated by the magnesium precipitation method (*23*) with several minor modifications. Polysomal RNA was extracted with a mixture of phenol and chloroform, and polyadenylated molecules were isolated by polyuridylate-Sepharose affinity chromatography (*17*). A ^{32}P-labeled Ha-*ras* DNA probe was prepared by nick translation (*24*) of the purified restriction endonuclease fragment BS-9 (*13*). Dot-blot hybridizations were performed with the methods of Thomas (*15*). Polysomal polyadenylated RNA (1 and 5 μg) extracted from normal liver and from regenerating liver (12, 18, 24, 36, 72, and 96 hours) in 5 μl of water were spotted onto nitrocellulose filters. Filters were incubated for 72 hours at 42°C with ^{32}P-labeled BS-9 DNA, 1×10^6 count/min per milliliter of hybridization buffer [quadruple-strength standard saline citrate; 40 percent formamide; 0.016 percent bovine serum albumin, Ficoll, and polyvinylpyrrolidone; ϕ80 DNA (25 μg/ml); 10 percent dextran sulfate (weight to volume). After the hybridization, the filters were washed, placed in contact with Kodak XAR-5 film, and kept at −70°C. A set of standards ranging from 0.002 to 200 pg of unlabeled BS-9 DNA was heat-denatured and spotted on filters to determine the sensitivity of the procedure. *Escherichia coli* transfer RNA spots were included as negative controls. The spots were punched out of the filter and placed in 5 ml of Omnifluor-toluene and their radioactivity was counted. Background levels were determined by counting the radioactivity of random portions of each filter; the background values were subtracted from each experimental value. (a) Hybridization of ^{32}P-labeled Ha-*ras* DNA (BS-9 clone) with regenerating rat liver polysomal poly(A)$^+$ RNA after (●) partial hepatectomy, (▲) CCl_4 injury, or (x) sham operation. Incorporation of [^{3}H]deoxythymidine into regenerating liver DNA (*22*) after (○) partial hepatectomy and (△) CCl_4 injury. (b) Relative levels of (●) c-*ras* transcripts, (△) AFP mRNA (*20*), and (○) polysomal poly(A)$^+$ RNA molecules per milligram of DNA (*21*) in regenerating rat liver after partial hepatectomy. Values expressed as "percentage of normal" were based on values obtained from normal adult rat liver (*25*).

high levels reached at 36 hours after hepatectomy do not persist. In contrast, AFP mRNA remains high until at least 96 hours after the operation. These differences between AFP and *ras* transcripts might be a reflection of the half-lives of these messengers. In contrast to the pattern of change presented in Fig. 1b, albumin mRNA does not increase after partial hepatectomy (*20*).

Our results show that transcripts of a cellular oncogene increase concomitantly with the burst of DNA synthesis in regenerating rat liver and rapidly return to basal levels. While we have no data demonstrating that the expression of c-*ras* controls compensatory growth, this observation indicates that there is regulated transcription of the cellular *ras* oncogene during a physiological growth process.

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Development of the Place Principle: Acoustic Trauma

Abstract. *Developmental changes in the site of receptor damage following pure-tone acoustic overstimulation were examined in the basilar papillae of embryonic and hatchling chickens. During development, a systematic shift in the position of damage toward the apex of the cochlea was produced by each of three frequencies, suggesting that the transduction properties of the sensory epithelium systematically shift with age. These results imply that neurons in the central nervous system may be maximally stimulated by different sounds during development.*

The basal region of the adult cochlea is maximally responsive to high frequencies, while apical positions are maximally responsive to lower frequencies. This spatial representation of frequency, known as the place principle in the cochlea, is preserved throughout the central auditory pathways. It is thought that this tonotopic organization helps to preserve spectral relations in the pattern of neural activity (*1*). As demonstrated in the classic experiments of von Békésy (*2*), place principle is determined by the position of the maximum amplitude of the traveling wave as a function of frequency. An indirect method used to confirm the "map" of frequency representation in the cochlea is to determine the position of hair cell damage produced by exposure to high-intensity pure-tone or narrow-band noise (*3–5*).

During ontogeny all birds and mammals initially respond to low or relatively low frequency sounds, and mature functional properties usually appear first for relatively low frequencies, whereas high-frequency responsivity is delayed (*6–9*). This sequence suggests that the apical (low-frequency) regions of the cochlea are the first to be responsive to sound and that only later are the basal (high-frequency) regions involved. Paradoxically, it has been repeatedly demonstrated that differentiation of the organ of Corti in mammals and the basilar papilla in birds occurs in the opposite direction (*9–10*). In addition, differentiation of brainstem auditory regions in avian embryos occurs first in the basal projection area and only later in the apical, or low frequency, area (*11, 12*).

This paradoxical relation between the ontogeny of responses to sound and development of the auditory system cannot be accounted for by changes in middle ear transmission (*13*), suggesting that the part of the sensory epithelium that is maximally responsive to low or middle frequencies shifts during development. Specifically, it has been hypothesized that early in development low frequencies are transduced by the basal region of the cochlea; with maturation these same frequencies cause maximum responses at successively more apical positions, while the basal regions respond maximally to progressively higher frequencies (*9, 12*). The hypothesis predicts that the position of hair cell damage produced by exposure to an intense pure tone will shift apically during the late stages of hearing maturation and that the best frequency (*14*) of neurons at any given location in primary central auditory regions will shift toward higher frequencies during maturation. We tested these predictions by examining developmental changes in the functional organization of the cochlea (basilar papilla) and brainstem auditory nuclei of chicks (*4, 15*). In this report we discuss ontogenetic changes in the position of hair cell damage produced by acoustic overstimulation with pure tones; changes in frequency representation in the central nervous system are considered in the following report (*16*).

Domestic chickens (Hubbard × Hubbard) of three ages were used: embryonic day 20 (E20), postnatal day 10 (P10), and postnatal day 30 (P30). At each of these ages at least three animals were exposed to a continuous pure tone of 500, 1500, or 3000 Hz at 125 ± 3 dB (sound-pressure level) for 12 hours (*4, 5, 17*). Animals exposed to the same handling and operative conditions as the experimental chicks (sham exposure) as well as normal animals of the same age served as controls.

After their exposure to sound, the animals were returned to the brooder (the E20 animals invariably hatched during the exposure period) and were allowed to survive for 10 days under normal laboratory conditions. The cochleas (basilar papillae) were then fixed, dissected free, osmicated, embedded in