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Research Article

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Expression of Two "Immediate Early" Genes, *Egr-1* and c-*fos*, in Response to Renal Ischemia and during Compensatory Renal Hypertrophy in Mice

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Abstract

To identify specific genetic regulatory mechanisms associated with renal ischemia, we measured the accumulation of *Egr-1* and c-*fos* mRNAs in the mouse kidney after occlusion of the renal artery and reperfusion. At 1 h after right nephrectomy and arterial occlusion of the contralateral kidney for 10 or 30 min, *Egr-1* mRNA levels were three to five times greater in these kidneys as compared with those in control animals that had sustained unilateral nephrectomy alone and were much greater than levels in the normal organ. Whether ischemia was imposed for 10 or for 30 min, renal *Egr-1* mRNA contents were equivalent and remained elevated after 24 h of reperfusion subsequent to 30 min of ischemia. Although c-*fos* mRNA also accumulated in response to ischemia and reperfusion, the pattern differed from that of *Egr-1* in that c-*fos* mRNA content varied with the duration of ischemia and was undetectable 24 h after injury. Contralateral nephrectomy was not necessary to see the marked accumulation of *Egr-1* and c-*fos* mRNAs with unilateral ischemia. Reflow was necessary, however, since only minimal sequence accumulation occurred by the end of the ischemic period. After left uninephrectomy alone, *Egr-1* mRNA levels in the remaining kidney were maximal 30 min after surgery, but were not detectable thereafter; c-*fos* mRNA levels did not change after unilateral nephrectomy. Differential expression of early growth-related genes implicated in transcriptional activation may influence tissue recovery after renal ischemia. (*J. Clin. Invest.* 1990. 85:766–771.) acute renal failure • anoxia • messenger RNA • zinc finger

Introduction

Egr-1 is an early growth-response gene that is rapidly and transiently induced by a variety of agents during induction of cell proliferation (1–4) and during neuronal (2, 5) and cardiac (2) differentiation. Like c-*fos*, *Egr-1* mRNA accumulates in the presence of cycloheximide and is a component of the "immediate early response" (6). Since many viral and mammalian "immediate early" genes are involved in transcriptional activation (7–10), a role in the modulation of transcription early in growth has been proposed for the *Egr-1* gene product (2–4). The zinc finger-coding domains of *Egr-1* are a common feature of transcription factors, further implicating *Egr-1* in transcriptional activation of genes in response to mitogens, as well as suggesting a broader role as a nuclear intermediary in other signal transduction processes (2, 5). If the *Egr-1* gene product participates in transcriptional control, the abundance of the mRNA at the peak of induction in growth suggests that it may interact with many other genes, functioning as a general transcription factor in the growth response. In cultured cells stimulated by mitogens, the kinetics of *Egr-1* mRNA accumulation and decay are similar to those of c-*fos* mRNA (1). Since recovery from acute renal ischemia is dependent upon epithelial cell hypertrophy and proliferation (11), we hypothesized that *Egr-1* and c-*fos* would be induced in response to renal ischemia.

We studied regulation of the c-*fos* and *Egr-1* mRNAs in response to renal ischemia because of the potential roles of the gene products during epithelial cell growth after injury. Transient occlusion of the renal artery causes severe morphological abnormalities and deficits in function, including loss of proximal tubule brush border, epithelial cell necrosis, obstruction to tubular flow, and increased passive backflow of glomerular filtrate through the damaged epithelium (11–17). If the ischemic injury is not too severe, the surviving epithelial cells proliferate, restoring the tubular epithelium, and function improves (15, 18). The contributions of growth-promoting and growth-inhibitory genetic responses to tissue recovery after ischemic acute renal failure have not been characterized.

Egr-1 and c-*fos* mRNA expression was investigated after unilateral nephrectomy as well as after renal ischemia. In most mammals, unilateral nephrectomy is followed by compensatory hypertrophy of the proximal tubule cells of the remaining kidney, but with little hyperplasia (19–21). Compensatory renal hypertrophy is characterized by a 20–40% increase in kidney ribosome content after 1 d and by a two- to threefold increase in the overall rate of rRNA gene transcription in the first 6–24 h (22, 23). Induction of protooncogene expression, which correlates with hyperplastic responses in mammalian cells, has not been demonstrated during induced hypertrophy of the kidney (24, 25).

Methods

Animals and surgical procedures. Mice were 35–45-d-old outbred Swiss males ([Crl:CD-1] [1CR]BR) purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA) fed freely, and maintained under alternating 12-h cycles of light and dark (22).

In studies of renal ischemia, mice were injected intraperitoneally with 3 ml of lactated Ringer's solution and 5 U of heparin (heparin

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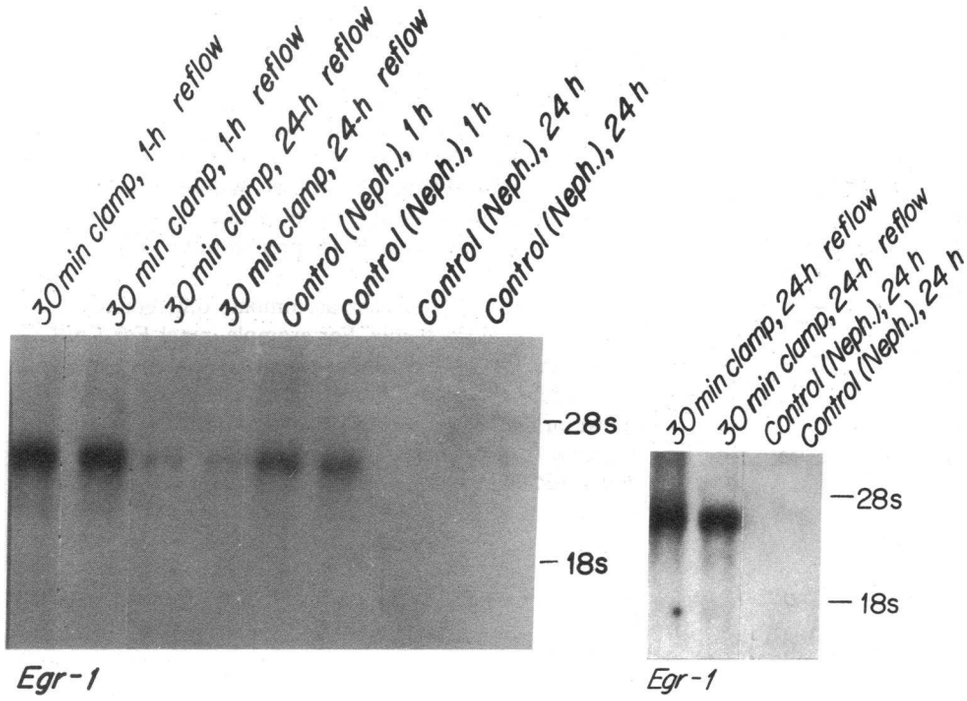

Figure 1. Egr-1 mRNA accumulation in renal ischemia. Mice were subjected to right nephrectomy and occlusion of the contralateral renal artery for 30 min followed by 1 or 24 h of reflow. Controls were subjected to right nephrectomy, left celiotomy, and manipulation of the left kidney with sterile swabs. Lanes contain RNA from individual mice treated as shown. Blots were hybridized with the *Egr-1* cDNA probe, and films were exposed for 2.5 h (*left panel*) or 7 h (*right panel*). Reading left to right, relative laser densitometry values in each lane of the left panel: 100, 70.4, 4.2, 3.6, 34.1, 20.5, 0.8, and 0.2. Bars denote rRNA markers of 4618 (*upper*) and 1864 (*lower*) nucleotides.

sodium injection, U.S. Pharmacopeia) 5 min before ether anesthesia, and right nephrectomy was performed through a flank incision, sparing the adrenal gland (26). The left kidney was exposed via a flank incision and elevated with sterile cotton swabs. Ischemia was induced by occluding the artery using a microaneurysm clamp (Roboz Surgical Instrument Co., Inc., Washington, DC). After closure of the peritoneum with hemostatic forceps, mice were maintained lightly anesthetized on heating pads under lamps for the 10 or 30 min period of renal ischemia. The hemostat was then removed, the incision was closed, and the mice were returned to cages on heating pads. Control animals sustained right nephrectomy, left celiotomy, elevation, and manipulation of the left kidney with sterile swabs; they were maintained anesthetized for 10 or 30 min before the incision was closed.

In studies of compensatory renal hypertrophy, left unilateral nephrectomy was performed under light ether anesthesia through a flank incision, sparing the adrenal gland (22). Right kidneys of sham nephrectomized mice were used as controls. Sham nephrectomy consisted of anesthesia, celiotomy, and massage of the left kidney with blunt forceps.

Controls for ischemia (see Figs. 1–3) and kidneys responding to left unilateral nephrectomy (see Fig. 6) were treated differently. The controls in Figs. 1–3 were left kidneys from mice that had sustained a right nephrectomy, had the left kidney exposed and lifted with swabs, and had been kept under anesthesia for 30 min with the peritoneum sealed by a hemostat before closure of the peritoneum with surgical clips. In contrast, kidneys in Fig. 6 were from mice that sustained only left unilateral nephrectomy: a procedure that requires only 2 min of anesthesia; the experimental right kidney was not manipulated before its removal for isolation of RNA.

Isolation of RNA. RNA was purified from intact whole kidneys by deproteinization with guanidinium isothiocyanate (GIT[1]; Bethesda Research Laboratories, Inc., Gaithersburg, MD) and phenol/chloroform/isoamyl alcohol (23, 27, 28). Individual kidneys were homogenized at room temperature in 10 ml of GIT buffer containing 4 M GIT, 50 mM Tris-Cl (pH 7.6), 100 mM 2-mercaptoethanol, and 2% Sarkosyl. RNA precipitated at −20°C with 0.5 vol ethanol was further deproteinized by two extractions with GIT buffer, two extractions with 6 M guanidine-HCl, 100 mM potassium acetate (pH 5.0), and by exhaustive phenol/chloroform/isoamyl alcohol extraction. RNA was quantitated by absorbance at 260 nm. RNA was dissolved in sterile water and stored frozen.

Northern blots. Samples of RNA (10 μg) in 50% formamide and 6% formaldehyde (29) were heated at 65°C for 10 min and electrophoresed at 30 V for 18–24 h in 2% agarose gels in buffer containing 40 mM 3-(*N*-morpholino)propanesulfonic acid, pH 7.0, 10 mM sodium acetate, 1 mM EDTA, and 6% formaldehyde. RNA was transferred by capillarity to GeneScreen *Plus* membranes using 10× standard saline citrate (1× SSC = 150 mM NaCl, 10 mM sodium citrate) as the transfer buffer. Membranes were prehybridized at 42°C in a solution of 50% formamide, 1% sodium dodecyl sulfate (SDS), 1 M NaCl, 10% dextran sulfate, and denatured herring sperm DNA (100 μg/ml). Probes were labeled in vitro with Klenow fragment after priming with random hexamers (30, 31) using [^{32}P]dCTP (3,000 Ci/mmol, DuPont New England Nuclear Products, Inc., Boston, MA). After hybridization, membranes were washed twice with 2× SSC at room temperature for 5 min, twice at 65° with 2× SSC, 1% SDS for 60 min, and twice at room temperature with 0.1× SSC for 30 min. After autoradiography, hybridization was quantitated by laser densitometry. The *Egr-1* clone was OC3.1 (2); v-*fos* DNA was purchased from ONCOR, Inc., Gaithersburg, MD.

Results

Egr-1 and c-fos mRNA accumulation with renal ischemia. Kidney levels of *Egr-1* mRNA were three- to fivefold higher than those of controls 1 h after contralateral nephrectomy and arterial occlusion for 30 min (Fig. 1, *left panel;* Fig. 2). Under the conditions of the assay, *Egr-1* mRNA was not detected in Northern blots of total renal RNA from unoperated mice (Fig. 3). As shown in Figs. 1 and 2, *Egr-1* mRNA was abundant 1 h after a 30-min ischemic insult, and it persisted even 24 h after reperfusion (Fig. 1, *right panel*). Furthermore, accumulation of *Egr-1* and c-*fos* mRNAs was observed in the postischemic kidney 1 h after ischemia and reoxygenation regardless of

1. *Abbreviations used in this paper:* GIT, guanidine isothiocyanate; SRE, serum response element.

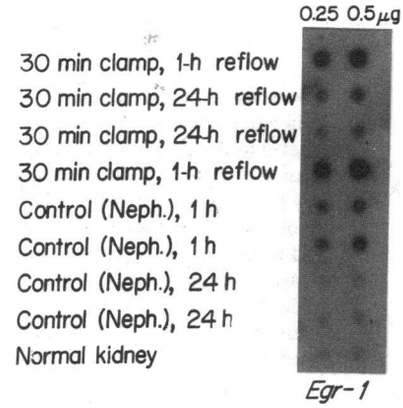

Figure 2. RNA dot blot measurement of *Egr-1* mRNA accumulation in renal ischemia. Samples of total renal RNA from mice treated as in Figure 1 (*left*) were applied directly to GeneScreen *Plus* membranes in quantities as indicated and hybridized to the *Egr-1* cDNA probe. Reading from top to bottom of the 0.25-μg column, relative laser densitometry values were 80.9, 15.3, 9.6, 100, 16.1, 18.3, 1.7, 3.0, and 2.5.

whether contralateral right nephrectomy preceded clamping of the left renal artery (see Figs. 3 and 5). The two mRNAs accumulated to only low levels at the end of the ischemic insult before reflow (Fig. 3). When the kidney was made ischemic with the contralateral kidney in place, the level of sequence induction on the clamped side after 1 h of reflow exceeded that of the contralateral kidney by at least 30-fold. The validity of these findings was confirmed by the invariant quantity of P31 (32) mRNA in all renal RNA preparations (Fig. 3, *lower panel*).

Egr-1 and c-*fos* mRNAs accumulate differentially in response to graded ischemia. For example, renal *Egr-1* mRNA levels were elevated to similar extents whether ischemia was imposed for 10 min or 30 min (Fig. 4). The brief film exposure shown in the lower panel of Fig. 4 demonstrates that the dose response of the film had not been exceeded, confirming that *Egr-1* mRNA levels were the same regardless of the duration of

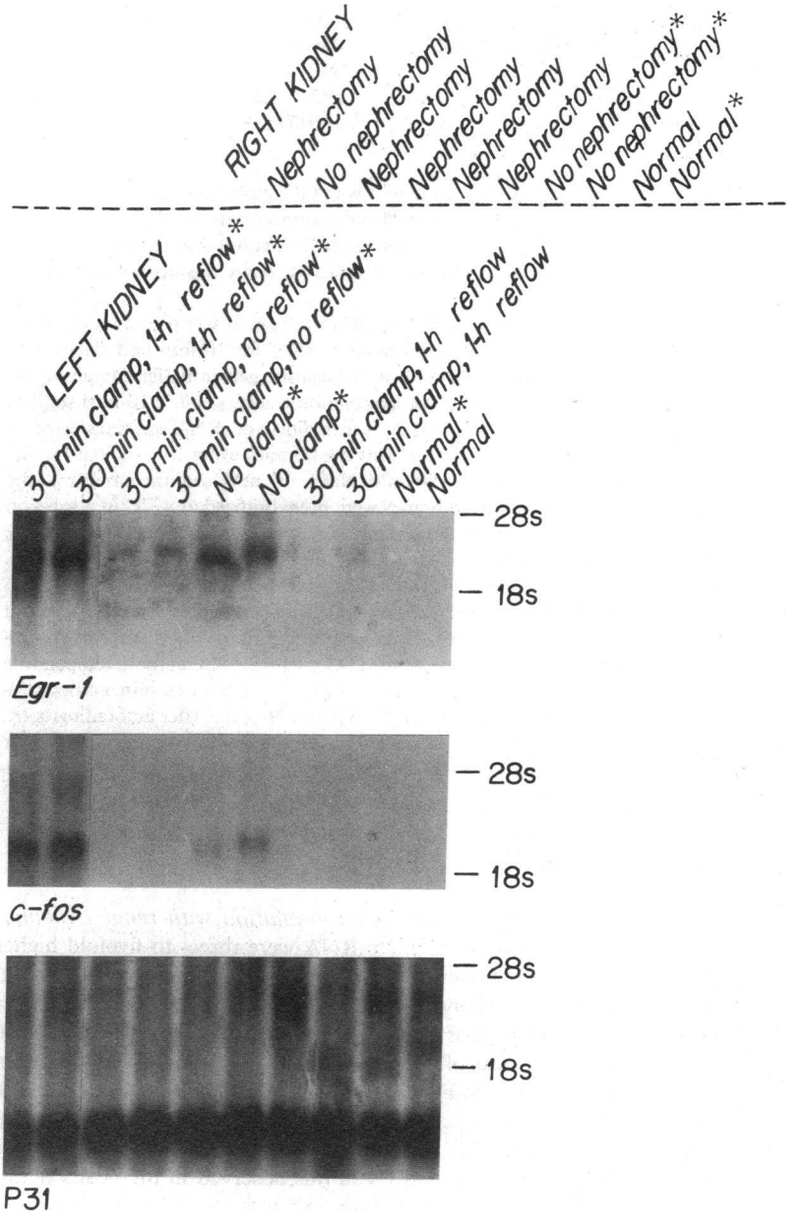

Figure 3. Effects of reoxygenation and preparative contralateral nephrectomy on *Egr-1* and c-*fos* mRNA accumulation in renal ischemia. Replicate Northern blots containing total RNA from kidneys of mice treated as indicated were hybridized to *Egr-1* (*upper*), c-*fos* (*center*), and P31 (*lower*) cDNA probes. Headings over each lane describe treatment of both kidneys, and asterisks in headings above lanes denote the kidney from which RNA was isolated. Reading left to right, relative laser densitometry values in each lane: (*upper panel*) 100, 66.5, 6.8, 5.0, 24.2, 24.3, 1.1, 1.1, 0, and 0; (*middle panel*) 100, 82.2, 1.3, 0.9, 4.8, 11.7, 0, 0, 0, and 0. Bars denote rRNA markers of 4618 (*upper*) and 1864 (*lower*) nucleotides.

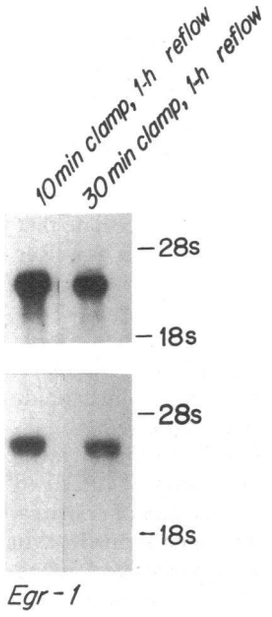

Figure 4. Effect of ischemic duration on *Egr-1* mRNA accumulation. Left renal arteries of mice subjected to right nephrectomy were occluded for 10 or 30 min. Renal RNA purified from these mice after 1 h of reflow was assayed in Northern blots using the *Egr-1* cDNA probe (Methods). Reading left to right, relative laser densitometry values in each lane of the lower panel: 151, 100.

ischemia. c-*fos* mRNA also accumulated rapidly and transiently after ischemia, but, in contrast to *Egr-1* mRNA, accumulation occurred in proportion to the duration of the ischemic injury (Fig. 5). After 1 h of reflow, c-*fos* mRNA levels were approximately three to five times greater in kidneys clamped for 30 min than in those clamped only for 10 min (Fig. 5). Under conditions in which *Egr-1* mRNA persisted for up to 24 h after initiating reflow (Figs. 1 and 2), c-*fos* mRNA content was elevated for less time, declining to below levels of detection by 24 h after reoxygenation (Fig. 5, *right panel*). At 24 h after 30 min of ischemia the histological appearance of kidneys was characteristic of ischemic renal injury, with loss of proximal tubule brush border, dilatation of proximal tubular lumen, and flattening of the epithelium. Histological examination of kidneys at 24 h after 10 min of ischemia, disclosed no evidence of renal abnormality.

To establish the degree to which induction of the *Egr-1* and c-*fos* genes is elicited specifically by ischemia and reperfusion as compared with nephrectomy itself, we measured the corresponding mRNAs in mice after left unilateral nephrectomy alone. Relative to controls, *Egr-1* mRNA content of the remaining kidney was elevated 30 min after contralateral nephrectomy (Fig. 6). The extent of *Egr-1* mRNA accumulation after unilateral nephrectomy was lower than that following ischemia and was not detectable after 60 min or longer. Consistent with reported results (24), c-*fos* mRNA was not detected in the contralateral kidney after unilateral nephrectomy alone (data not shown). In comparison with data from unilaterally nephrectomized mice (Fig. 6), controls for ischemic kidneys (Figs. 1, *left panel,* 2, and 3) had a 30-min period of anesthesia instead of 2 min and received two flank incisions instead of one, and the kidney from which RNA was isolated was physically manipulated (See Methods). *Egr-1* and c-*fos* mRNAs were not detected in unmanipulated kidneys after the contralateral kidney had been clamped for 30 min and reoxygenated for 1 h (Fig. 3).

Discussion

One objective of these studies was to characterize genetic responses to ischemia and to relate them to the renal response to unilateral nephrectomy. Activation of early growth response genes may facilitate recovery of the kidney from ischemia by inducing cell growth, and the enhanced expression of *Egr-1* and c-*fos* genes during reperfusion after ischemia implicates these two growth-related genes in particular. The persistence of *Egr-1* mRNA differs from the transient stimulation of c-*fos,* which also increases to high levels, but then disappears rapidly (7, 9). Detection of *Egr-1* mRNA 24 h after initiating reflow

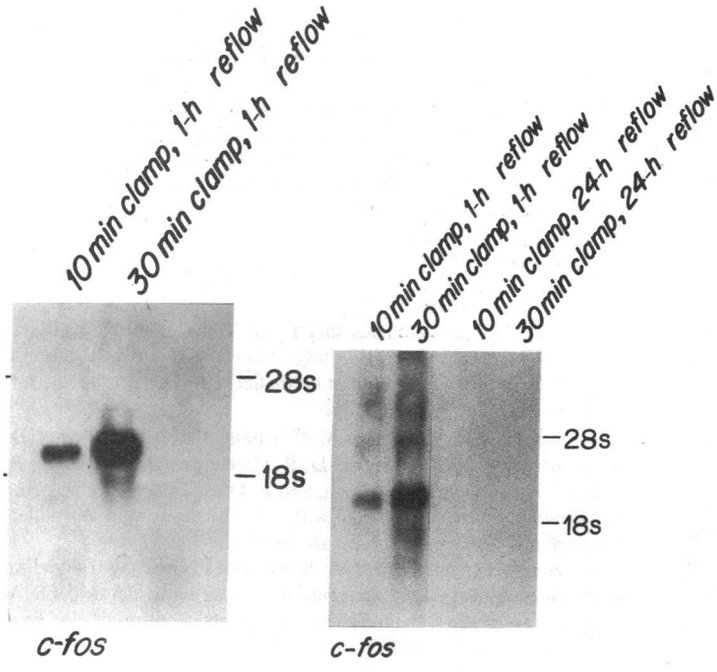

Figure 5. Effect of ischemic duration on c-*fos* mRNA. (*Left panel*) Blots identical to those in Fig. 4 were hybridized with the v-*fos* DNA probe. (*Right panel*) As in left panel but containing different preparations of renal RNAs and including RNA isolated from kidneys 24 h after initiating reflow. Reading left to right, relative laser densitometry values for each lane: (*left*) 21.1, 100; (*right*) 31.6, 100, 0, 0.

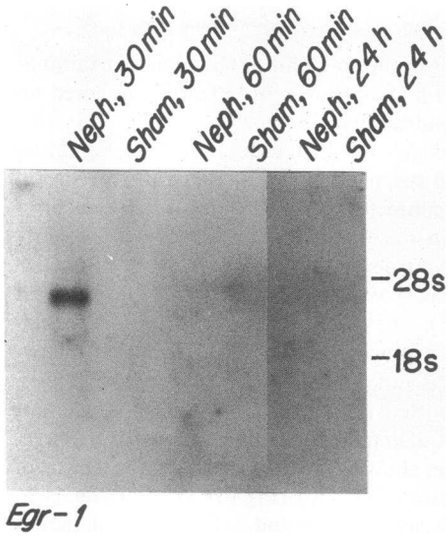

Figure 6. Egr-1 mRNA accumulation early in compensatory renal hypertrophy. Left unilateral nephrectomy was performed; controls sustained left celiotomy and massage of the left kidney with blunt forceps. At the times shown after surgery, RNA was purified from right kidneys of individual mice, and analyzed in Northern blots. Blots were analyzed as in Fig. 1.

may reflect the continued stimulus for cells to hypertrophy or divide well after the ischemic insult itself.

Our results support the conclusion that the *Egr-1* gene is more sensitive than the c-*fos* gene to induction by ischemia or contralateral nephrectomy. First, *Egr-1* is induced maximally by 10 min of ischemia (Fig. 4), but c-*fos* accumulation varies with the duration of the insult (Fig. 5). Secondly, *Egr-1* mRNA, but not c-*fos* mRNA accumulates in response to unilateral nephrectomy (Fig. 6). A structural explanation for these differences can be inferred from a comparison of the upstream sequences of these two genes. For example, the *Egr-1* promoter contains six copies of the $CC(A/T)_6GG$ sequence that constitutes the inner core of the serum response element (SRE [33]); in contrast, the upstream region of the c-*fos* gene contains only one such element (34). Furthermore, deletion analyses show that SREs are responsible for the dramatic induction of *Egr-1* by mitogens, which is fivefold greater than that of c-*fos* (X. Cao and V. P. Sukhatme, unpublished). Because the single SRE in the c-*fos* promoter mediates response of the gene to ultraviolet light (35) as well as to serum (34) and phorbol esters (36, 37), SREs also may regulate the *Egr-1* and c-*fos* genes after ischemia, providing the basis for their differential activation (Figs. 4 and 5). Appearance of *Egr-1* mRNA early in compensatory renal hypertrophy without c-*fos* gene induction implies that the *Egr-1* gene is independent of c-*fos*/c-*jun* transcriptional activation (38, 39). Independence of *Egr-1* activation from fos/jun is consistent with *Egr-1* gene induction by mitogens in the presence of cycloheximide (1, 3, 4).

Egr-1 is an early growth-response gene induced in compensatory renal hypertrophy. Many genes activated in mammalian cells by proliferative stimuli (40–42) are not induced early in compensatory renal growth as judged by the lack of accumulation of corresponding mRNAs (24, 25). Characterization of *Egr-1* as an "immediate early gene" and its three potential zinc finger domains suggests a role in transcriptional activation (43, 44). *Egr-1* may participate as a transcription factor in the activation of sets of genes, perhaps in stimulating the overall rate of rRNA gene transcription (23). The studies described here do not permit the localization of *Egr-1* and c-*fos* gene induction within the cell populations of the nephron or the kidney. Until *in situ* hybridization and immunohistochemical experiments are completed, the direct relation of these gene induction events to the response of the tubular epithelium to ischemia will remain unresolved.

Because mechanisms associated with induction of growth and response to stress appear to be related, we speculate that *Egr-1* mRNA accumulates in response to renal stress as well as having a role in kidney growth. The HSP70 heat shock gene is activated in cells stimulated to proliferate with serum (45) or mitogens and lymphokines (46), and it is related to a gene family essential for growth in yeast (47). Since *Egr-1* is superinduced in serum-stimulated cells exposed to cycloheximide (1) and since ischemia impairs protein synthesis (16, 48), diminished renal protein synthesis after ischemia may contribute to the enhanced and persistent expression of the *Egr-1* gene. The level of renal *Egr-1* mRNA at the end of ischemia, but before reoxygenation, is modest compared to the level in reoxygenated kidneys perhaps because nucleoside triphosphates, depleted after 30 min of ischemia, are partially restored after resumption of oxidative metabolism. In addition, the higher *Egr-1* and c-*fos* mRNA levels after 1 h of reflow may represent a stress response to reperfusion injury potentially resulting from reactive oxygen species, or increases in intracellular $[Ca^{2+}]$ that may occur upon reoxygenation (49).

In conclusion, we have identified an increase in mRNA of an early growth-response gene, *Egr-1*, during compensatory renal hypertrophy and activation of both *Egr-1* and c-*fos* genes after ischemic injury. Although the specific roles of Fos and *Egr-1* proteins in growth and tissue recovery are not known, characterization of the *Egr-1* and c-*fos* gene responses constitutes an important first step in understanding tissue regeneration after ischemic injury. With this enhanced understanding, it may be possible to potentiate recovery from acute renal failure.

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References

1. Sukhatme, V. P., S. Kartha, F. G. Toback, R. Taub, R. G. Hoover, and C. H. Tsai-Morris. 1987. A novel early growth response gene rapidly induced by fibroblast epithelial cell, and lymphocyte mitogens. *Oncogene Res.* 1:343–355.

2. Sukhatme, V. P., X. Cao, L. C. Chang, C. H. Tsai-Morris, D. Stamenkovich, P. C. P. Ferreira, D. R. Cohen, S. A. Edwards, T. B. Shows, T. Curran, M. M. Le Beau, and E. D. Adamson. 1988. A zinc finger-encoding gene coregulated with c-*fos* during growth and differentiation, and after cellular depolarization. *Cell.* 53:37–43.

3. Lemaire, P., O. Relevant, R. Bravo, and P. Charnay. 1989. Two genes encoding potential transcription factors with identical DNA-binding domains are activated by growth factors in cultured cells. *Proc. Natl. Acad. Sci. USA.* 85:4691–4695.

4. Christy, B. A., L. Lau, and D. Nathans. 1988. A gene activated in mouse 3T3 cells by serum growth factors encodes a protein with "zinc finger" sequences. *Proc. Natl. Acad. Sci. USA.* 85:7857–7861.

5. Milbrandt, J. 1987. A nerve growth factor-induced gene encodes a possible transcriptional regulatory factor. *Science (Wash. DC).* 238:797–799.

6. Greenberg, M. G., and E. B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. *Nature (Lond.).* 311:433–438.

7. Lau, L. F., and D. Nathans. 1987. Expression of a set of growth-related immediate early genes in BALB/c 3T3 cells: coordinate regulation with c-*fos* and c-*myc*. *Proc. Natl. Acad. Sci. USA.* 84:1182–1186.

8. Ryder, K., L. Lau, and D. Nathans. 1988. A gene activated by growth factors is related to the oncogene v-*jun*. *Proc. Natl. Acad. Sci. USA.* 85:1487–1491.

9. Rauscher, F. J., III, D. R. Cohen, T. Curran, T. J. Bos, P. K. Vogt, D. Bohmann, R. Tjian, and B. R. Franza, Jr. 1988. Fos-associated protein p39 is the product of the *jun* proto-oncogene. *Science (Wash. DC).* 240:1010–1016.

10. Cohen, D. R., and T. Curran. 1988. *fra*-1: a serum-inducible, cellular immediate-early gene that encodes a Fos-related antigen. *Mol. Cell. Biol.* 8:2063–2069.

11. Toback, F. G. 1984. Phosphatidylcholine metabolism during renal growth and regeneration. *Am. J. Physiol.* 246:F249–F259.

12. Kreisberg, J. I., and M. A. Venkatachalam. 1988. Morphologic factors in acute renal failure. *In* Acute Renal Failure. B. M. Brenner and J. M. Lazarus, editors. Churchill-Livingstone, Inc., New York. 45–65.

13. Reimer, K. A., C. E. Ganote, and R. B. Jennings. 1972. Alterations in renal cortex following ischemic injury. III. Ultrastructure of proximal tubules after ischemic or autolysis. *Lab. Invest.* 26:347–363.

14. Arendhorst, W. J., W. F. Finn, and C. W. Gottschalk. 1975. Pathogenesis of acute renal failure following temporary renal ischemia in the rat. *Circ. Res.* 37:558–568.

15. Finn, W. F., and R. L. Chevalier. 1979. Recovery from post ischemic acute renal failure in the rat. *Kidney Int.* 16:113–123.

16. Kao, R., D. E. Rannels, and H. E. Morgan. 1976. Effects of anoxia and ischemia on protein synthesis in perfused rat hearts. *Circ. Res.* 38(Suppl. I):124–130.

17. Venkatachalam, M. A., D. B. Bernard, J. F. Donahoe, and N. G. Levinsky. 1978. Ischemic damage and repair in the rat proximal tubule: differences among the S1, S2, and S3 segments. *Kidney Int.* 14:31–49.

18. Levinsky, N. G. 1977. Pathophysiology of acute renal failure. *N. Engl. J. Med.* 296:1453–1458.

19. Fine, L. G. 1986. The biology of renal hypertrophy. *Kidney Int.* 29:619–634.

20. Halliburton, I. W., and R. Y. Thomson. 1965. Chemical aspects of compensatory renal hypertrophy. *Cancer Res.* 25:1882–1887.

21. Bucher, N. L. R., and R. A. Malt. 1971. Regeneration of Liver and Kidney. Little, Brown & Co., Boston.

22. Malt, R. A., and D. A. LeMaitre. 1968. Accretion and turnover of RNA in the renoprival kidney. *Am. J. Physiol.* 214:1041–1047.

23. Ouellette, A. J., R. Moonka, A. D. Zelenetz, and R. A. Malt. 1987. Regulation of ribosome synthesis during compensatory renal hypertrophy. *Am. J. Physiol.* 253:C506–C513.

24. Beer, D. G., K. A. Zweifel, D. P. Simpson, and H. C. Pitot. 1987. Specific gene expression during compensatory renal hypertrophy in the rat. *J. Cell. Physiol.* 131:29–35.

25. Norman, J. T., R. E. Bohman, G. Fischman, J. W. Bowen, A. McDonough, D. Slamon, and L. G. Fine. 1988. Patterns of mRNA expression during early cell growth differ in kidney epithelial cells destined to undergo compensatory hypertrophy versus regenerative hyperplasia. *Proc. Natl. Acad. Sci. USA.* 85:6768–6772.

26. Malt, R. A., and D. A. LeMaitre. 1969. Nucleic acids in fetal kidney after maternal nephrectomy. *Proc. Soc. Exp. Biol. Med.* 130:539–542.

27. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* 18:5294–5299.

28. Perry, R. P., J. LaTorre, D. E. Kelley, and J. R. Greenberg. 1972. On the lability of poly(A) sequences during extraction of messenger RNA from polyribosomes. *Biochim. Biophys. Acta.* 262:220–226.

29. Rave, N., R. Crkvenjakov, and H. Boedtker. 1979. Identification of procollagen mRNAs transferred to diaminobezyloxymethyl paper from formaldehyde gels. *Nucleic Acids Res.* 6:3559–3567.

30. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6–13.

31. Feinberg, A. P., and B. Vogelstein. 1985. Addendum: a technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137:266–267.

32. Theodor, L., D. Peleg, and O. Meyuhas. 1985. P31, a mammalian housekeeping protein encoded by a multigene family containing a high proportion of pseudogenes. *Biochim. Biophys. Acta.* 826:137–145.

33. Tsai-Morris, C.-H., Cao, X., and V. P. Sukhatme. 1988. 5′ flanking sequence and genomic structure of *Egr-1*, a murine mitogen-inducible zinc finger encoding gene. *Nucleic Acids Res.* 16:8835–8846.

34. Treisman, R. 1986. Identification of a protein-binding site that mediates transcriptional response of the c-*fos* gene to serum factors. *Cell.* 46:567–574.

35. Buscher, M., H. J. Rahmsdorf, M. Litfin, M. Karin, and P. Herrlich. 1988. Activation of the c-*fos* gene by UV and phorbol ester: different signal transduction pathways converge to the same enhancer element. *Oncogene.* 3:301–311.

36. Gilman, M. Z. 1988. The c-*fos* serum response element responds to protein kinase C-dependent and independent signals but not to cyclic AMP. *Genes Dev.* 2:394–402.

37. Sheng, M., S. T. Dougan, G. McFadden, and M. E. Greenberg. 1988. Calcium and growth factor pathways of c-*fos* transcriptional activation require distinct upstream regulatory sequences. *Mol. Cell. Biol.* 8:2787–2796.

38. Curran, T., and B. R. Franza. 1988. Fos and Jun: the AP-1 connection. *Cell.* 55:395–397.

39. Kouzarides, T., and E. Ziff. 1988. The role of the leucine zipper in the *fos-jun* interaction. *Nature (Lond.).* 336:646–651.

40. Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder. 1983. Cell-specific regulation of c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. *Cell.* 35:603–610.

41. Miller, R., D. J. Slamon, J. M. Tremblay, M. J. Cline, and I. M. Verma. 1982. Differential expression of cellular oncogenes during pre- and post-natal development of the mouse. *Nature (Lond.).* 299:640–644.

42. Goyette, M., C. J. Petropoulos, P. R. Shank, and N. Fausto. 1984. Regulated transcription of c-Ki-*ras* and c-*myc* during compensatory growth of rat liver. *Mol. Cell. Biol.* 4:1493–1498.

43. Klug, A., and D. Rhoads. 1987. 'Zinc fingers': a novel protein motif in nucleic acid recognition. *Trends Biochem. Sci.* 12:464–469.

44. Evans, R. M., and S. M. Hollenberg. 1988. Zinc fingers: gilt by association. *Cell.* 52:1–3.

45. Wu, B. J., and R. I. Morimoto. 1985. Transcription of the human *hsp70* gene is induced by serum stimulation. *Proc. Natl. Acad. Sci. USA.* 82:6070–6074.

46. Ferris, D. K., A. Harel-Bellan, R. I. Morimoto, W. J. Welch, and W. L. Farrar. 1988. Mitogen and lymphokine stimulation of heat shock proteins in T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 85:3850–3854.

47. Craig, E. A., J. Kramer, and J. Kosic-Smithers. 1987. *SSC1*, a member of the 70-kDa heat shock protein multigene family of *Saccharomyces cerevisiae,* is essential for growth. *Proc. Natl. Acad. Sci. USA.* 84:4156–4160.

48. Ouellette, A. J., R. K. Watson, K. Billmire, M. K. Dygert, and J. S. Ingwall. 1983. Protein synthesis in the cultured fetal mouse heart: effects of deprivation of oxygen and oxidizable substrate. *Biochemistry.* 22:1201–1207.

49. Bonventre, J. V. 1988. Mediators of ischemic renal injury. *Annu. Rev. Med.* 39:531–544.