sive to a low-salt diet. The *p*H profiles for plasma renin activity differ between S and R rats (*11*), suggesting some qualitative strain difference in either renin or renin substrate.

Plasma renin originates largely from the kidneys. Since renin generates angiotensin I, which is converted into the pressor peptide angiotensin II, one expects high plasma renin activity, not low plasma renin activity, to be associated with increased blood pressure. Renin is, however, expressed in many tissues besides the kidney, including the brain (*14*), but how such nonrenal renin functions is obscure. It is also worth noting that about 25% of human essential hypertensives have low plasma renin without any obvious autonomous mineralocorticoid excess to suppress the plasma renin activity. This has led to the concept of low-renin essential hypertension (*15*).

Rats and mice have been selectively bred for high and low renal juxtaglomerular granularity. This resulted in strains with high and low renal and plasma renin levels. Blood pressures of these strains were, however, exactly the opposite of what was expected, that is, the high renin strains had lower blood pressure, and the low renin strains had higher blood pressure (*16*). These results appear compatible with the present experiments showing that the S-rat renin allele, which is presumably responsible for the low tissue and plasma renin of S rats, cosegregates with a positive increment of blood pressure.

In summary, the polymorphic renin alleles in Dahl S and R rats cosegregate with part of the blood pressure difference between these strains. This means that either the renin molecules coded by the S and R renin alleles account for the associated blood pressure differences or that alleles at some unknown locus, genetically linked to the renin locus, cause these blood pressure differences. At the very least, then, the data mean that a gene for blood pressure regulation has been localized to a part of the genome close to, or identical to, the renin locus.

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Repression of the IgH Enhancer in Teratocarcinoma Cells Associated with a Novel Octamer Factor

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Embryonal carcinoma (EC) cell lines are models for early cells in mouse embryogenesis. A 300–base pair fragment of the heavy chain enhancer was inactive in F9 EC cells, unlike in other nonlymphoid cells where it has significant activity. Alterations of the octamer motif increased enhancer activity. Nuclear extracts from F9 cells contained an octamer binding protein (NF-A3) that was unique to EC cells; the amount of NF-A3 decreased upon differentiation. It is proposed that NF-A3 represses specific regulatory sequences that contain the octamer motif. Thus, the same DNA sequence mediates either negative or positive transcriptional effects, depending on the cell type.

EMBRYONAL CARCINOMA (EC) CELL lines are derived from the stem cells of teratocarcinomas and have properties of ectodermal cells of the embryonic inner cell mass (*1*). One EC cell line, F9, when induced with retinoic acid and cyclic AMP, differentiates into extraembryonic parietal endoderm (*2*). Enhancer sequences from Simian virus 40 (SV40), polyoma virus, and Moloney murine leukemia virus (MuLV) do not function efficiently in undifferentiated F9 cells (*3, 4*). Upon differentiation, negative regulation of the viral enhancers is removed and viral expression is augmented (*3, 4*).

The 300-bp Pvu II–Eco RI fragment of the mouse immunoglobulin (Ig) heavy chain enhancer (μ300) (Fig. 1A) is active in nonlymphoid cells (*5, 6*). Located upstream of a truncated c-*fos* promoter–chloramphenicol acetyltransferase (CAT) fusion gene, the μ300 enhancer increases transcription seven times in BALB/c 3T3 cells (Fig. 1C) (*5*). The same construct bearing the wild-type μ300 enhancer in undifferentiated F9 cells was inactive in F9 cells (*7, 8*) (Fig. 1A); the CAT enzyme activity was comparable to that with no enhancer (Fig. 1, B and C).

Because the lack of activity could be due to either the absence of positive regulatory factors or the presence of negative regulatory factors, we analyzed mutations in the enhancer that abrogate binding of known trans-acting factors (*5*) (Fig. 1A). Mutations that eliminate binding of the NF-μE3 factor ($E3^-$) had no significant effect on activity (Fig. 1C). Mutations in the octamer motif ($OCTA^-$), however, increased the CAT activity more than six times (Fig. 1, B and C; O^-). To determine whether elimination of the ability to bind octamer factors was the critical effect of this mutation (as opposed to generating a novel factor-binding site), we created an 8-bp deletion of the octamer site (*9*) (Fig. 1A; DO1). Again, this increased enhancer activity (Fig. 1C).

We tested the effects of the wild-type and mutant octamer enhancers in a stable transfection assay with the use of a fusion gene, which consisted of an enhancerless MuLV promoter and the neomycin resistance (neo^r) gene (Fig. 2A) (*10, 11*). In NIH 3T3 cells, the wild-type enhancer increased the number of neo^r colonies two and one-half times in the A orientation and four times in

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the B orientation (Fig. 2B; 3T3). In F9 cells, both orientations of the wild-type enhancer increased colony formation two to three and one-half times (Fig. 2B; F9). However, in F9 cells, but not 3T3 cells, the octamer mutation caused an additional increase in the number of neo^r colonies (Fig. 2B). These results further indicate that alterations of the octamer relieve repression of the enhancer in undifferentiated F9 cells.

We also investigated combinations of mutations in the binding sequences within the μ300 enhancer. A combination of alterations in the E4 and octamer sequences yielded an increase in enhancer activity similar to that of the octamer mutation alone (Fig. 1C). Conversely, simultaneous alterations of the E3 (E3$^-$), E4 (E4$^-$), and OCTA (O$^-$) sequences produced activity comparable to that of the wild-type enhancer (Fig. 1C). No decrease in the basal activity of the c-*fos* promoter resulted from the E3$^-$ mutation alone (Fig. 1C). This suggests that the wild-type octamer may interfere with positive effects at the E3 motif but does not reduce basal c-*fos* promoter activity.

The transcriptional effects of the octamer sequence in both lymphoid and nonlymphoid cells can be attributed to specific DNA-binding proteins (*12–15*). We examined nuclear extracts from F9 cells for proteins that bind to the octamer sequence. As in all other lymphoid and nonlymphoid cells, we detected the NF-A1 factor (Fig. 3, lane 1) (*12, 13*). As expected, the lymphoid-specific factor, NF-A2, was not present (Fig. 3, compare lanes 1 and 2). In addition, a nucleoprotein complex with a faster mobility than either NF-A1 or NF-A2 was detected (Fig. 3, lane 1). A variety of lymphoid and nonlymphoid cell lines of both human and murine origin lacked this complex, but several independent F9 extracts (for example, lane 3) and those from a different EC cell line, PCC4, had equivalent levels of the complex (*12, 16*). Thus, the faster mobility complex appeared specific to EC cell lines.

We examined the DNA recognition sequence of the novel complex. Specific point mutations in the octamer sequence that disrupt binding of the NF-A1 and NF-A2 complexes also eliminated the EC cell–specific complex (Fig. 3, lanes 5 and 6). To determine the DNA contact residues for this nucleoprotein complex, we used a methylation interference assay (*12*). Methylation within the octamer interfered with the binding of NF-A3 in a pattern that was identical to that seen with NF-A1 (Fig. 4A). The pattern is summarized in Fig. 4B and is the same as that previously determined for NF-A2 (*12*). Thus, three distinct complexes, NF-A1, NF-A2, and the EC cell complex make the same contacts with the octamer motif. We proposed to call the EC cell complex NF-A3.

Given the absence of NF-A3 and the lack of repression of the μ300 enhancer in differentiated cell types, we predicted that differentiation of F9 cells by retinoic acid and cyclic AMP would be accompanied by the

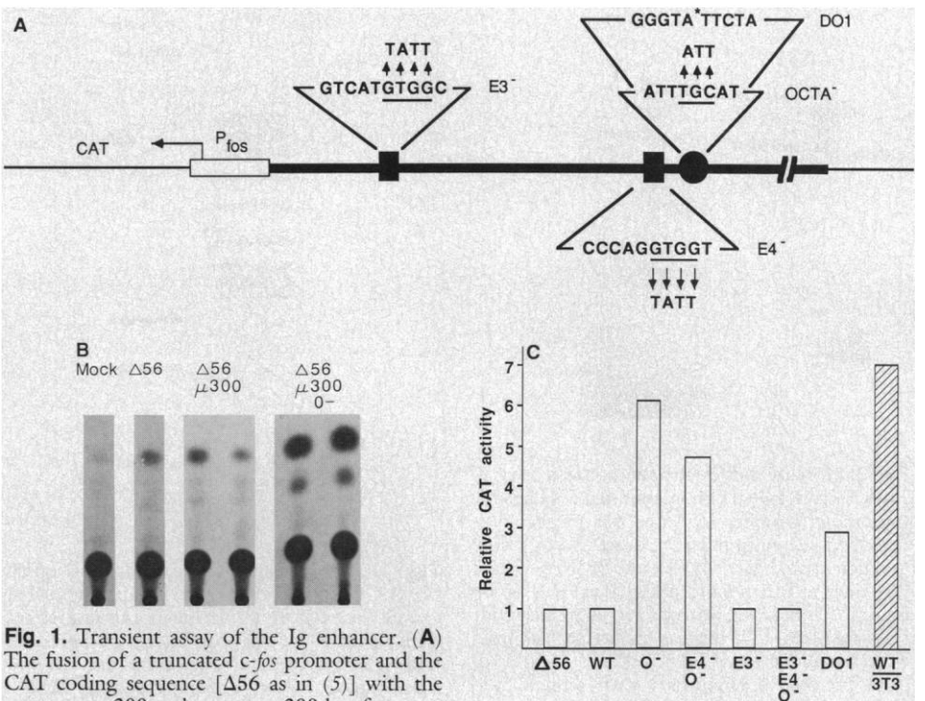

Fig. 1. Transient assay of the Ig enhancer. (**A**) The fusion of a truncated c-*fos* promoter and the CAT coding sequence [Δ56 as in (*5*)] with the upstream μ300 enhancer, a 300-bp fragment extending between Pvu II and Eco RI sites in the Ig heavy chain gene (bold line). Solid symbols are protein binding sites; boxes indicate enhancer (E) motifs and the circle indicates the conserved octamer. The expansion of each protein binding site gives the wild-type sequence and the nucleotides introduced by mutations. The star indicates an 8-bp deletion of the octamer. (**B**) Thin-layer chromatographic separation of chloramphenicol from its acetylated derivatives. F9 cells were transfected with no DNA (mock), with the enhancerless vector (Δ56), with duplicate transfections of the wild-type enhancer (Δ56, μ300), or with duplicate transfections of the enhancer bearing the OCTA$^-$ mutation (Δ56, μ300, O$^-$). (**C**) CAT enzyme activity in F9 cells (open bars) or BALB/c 3T3 fibroblast cells (hatched bar). Mutations are shown in (A). The O$^-$ symbol indicates the OCTA$^-$ mutation. Values are averages of five to eight independent transfections except for E4$^-$/OCTA$^-$ and E3$^-$, which are from two transfections; standard deviations were <25% of the values given. For the wild-type enhancer, absolute conversion to acetylated chloramphenicol was 0.5%. The scale for results of the 3T3 transfection has been normalized to that of the results from the F9 transfection.

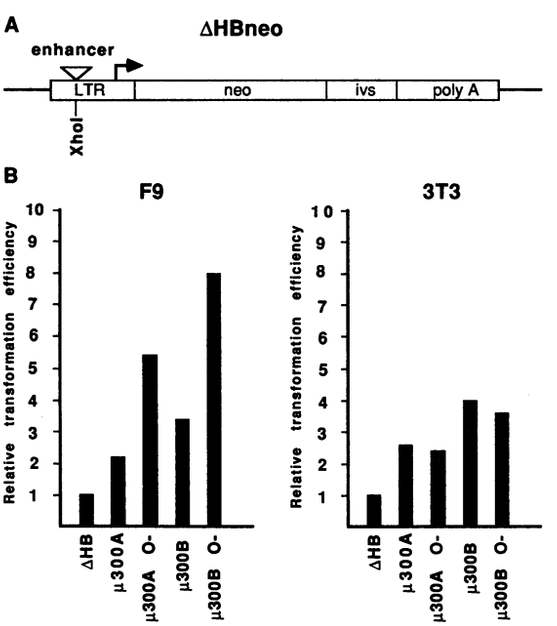

Fig. 2. Enhancement of neomycin resistance in 3T3 and F9 cells. (**A**) Map of ΔHBneo (*11*) showing the Xho I insertion site for the μ300 enhancer. Also shown are the bacterial neomycin resistance gene (neo), the SV40 intervening sequence (ivs), and the SV40 polyadenylation signal (poly A). (**B**) Stable neomycin resistance in 3T3 and F9 cells. The number of colonies for ΔHB control (*1*) was 40 for 3T3 cells and 26 for F9 cells. Transfection of 3T3 and F9 cells was with 100 ng and 2 μg of vector DNA, respectively (*10*). Each bar is the average of nine separate experiments. The standard deviations were <22% of the values given.

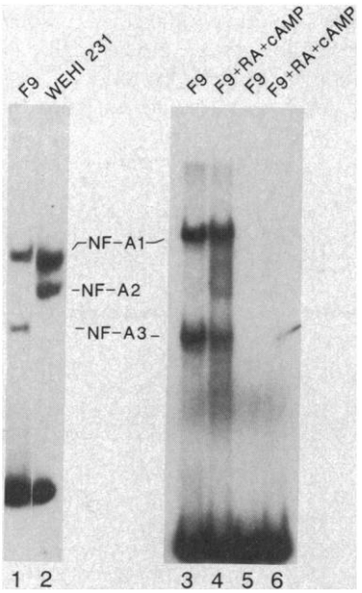

Fig. 3. Mobility shift electrophoresis assay. A ^{32}P-labeled Eco RI–Pvu II fragment that contains the κ-promoter octamer sequence was incubated as described (*12*) with nuclear extract (10 μg) from undifferentiated F9 cells (lane 1) or from WEHI 231 murine B lymphoid cells (lane 2). A 50-bp fragment containing an octamer oligonucleotide (*12*) was used with nuclear extract (8 μg) from either undifferentiated F9 cells (lanes 3 and 5) or from F9 cells differentiated with retinoic acid (RA) and dibutyryl cyclic AMP (cAMP) (lanes 4 and 6) (*2, 3, 12*). Nuclear extracts were prepared in the presence of 0.3 μg per milliliter of leupeptin and antipain, 5 m*M* phenylmethysulfonlyl-fluoride, and 1 m*M* EDTA. The octamer was either wild type (lanes 1 to 4) or contained a single transversion from T to G in the fourth position of the octamer sequence that prevents binding (lanes 5 and 6) (*12*).

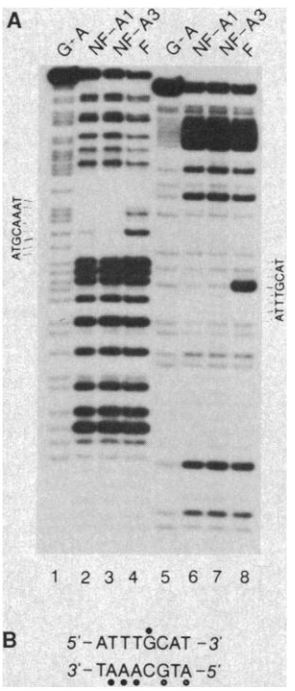

Fig. 4. Methylation interference analysis of the NF-A3 binding factor. (**A**) Either the coding strand (lanes 1 to 4) or the noncoding strand (lanes 5 to 8) of the κ-promoter fragment described in the legend to Fig. 3 was used. Represented are G + A sequencing cleavages (lanes 1 and 5, G + A), the DNA bound into the NF-A1 complex (lanes 2 and 6, NF-A1), the DNA bound into the NF-A3 complex (lanes 3 and 7, NF-A3), and the uncomplexed DNA (lanes 4 and 8, F) retrieved from a mobility shift electrophoresis gel similar to that shown in Fig. 3. (**B**) The pattern of interference on the octamer sequence. Full interference is shown as a closed circle and partial interference by a hatched circle.

loss of NF-A3. Therefore, F9 cells were treated with 1.0 μ*M* retinoic acid and 0.1 μ*M* dibutyryl cyclic AMP for 5 days, which resulted in both morphological changes and increased collagen mRNA production consistent with differentiation (*2*). A comparison of equivalent amounts of nuclear proteins (Fig. 3) showed no change in the amounts of NF-A1, but a decrease of NF-A3 (one-seventh to one-fifth the amount found in undifferentiated F9 cells by densitometric scanning). A similar decrease in NF-A3 was also seen in differentiated PCC4 cells (*16*). Therefore, the NF-A3 response to differentiation is distinct from that of NF-A1 and is consistent with a potential function as a represssor in undifferentiated EC cells.

Our results indicate that a portion of the Ig heavy chain enhancer that has activity in nonlymphoid cells is repressed in undifferentiated F9 cells. Like the polyoma virus enhancer, certain mutations can increase its activity (*4*). The effect of a single point mutation in the polyoma enhancer may both remove repression and allow a positive activator to bind (*4*). By contrast, octamer mutations in the Ig enhancer may only remove a negative influence. The activity of the μ300 in EC cells required NF-μE3 binding. However, the effect of the E3$^-$ mutation depended on whether the octamer was mutant or wild type, suggesting a functional interaction between the sites.

The EC cell–specific factor NF-A3 is a candidate for the transacting protein that mediates the negative regulatory effects of the octamer motif because it was present in undifferentiated EC cells and decreased upon differentiation. The mechanism for the decrease in NF-A3 could be either a decrease in NF-A3 synthesis or a modification of the protein itself. The transcriptional effects of the octamer sequence appear to be governed by distinct types of octamer-binding proteins in different tissues (*12–15*). Recently it was shown that NF-A1 and NF-A2 are the products of distinct genes despite the fact that they make identical contacts with the octamer sequence (*14*). Thus, there may be a family of genes that share highly related DNA binding characteristics but that are independently regulated in different tissues and exert different transcriptional regulatory effects.

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