be interesting to consider the use of patterned MoO_3 films as masks for high-resolution x-ray lithography. In a more general sense, it should be possible to use these ideas for nanomachining other inorganic thin layers, such as SiO_x on Si, when the substrate (for example, Si) wears at a rate significantly slower than the inorganic coating (for example, SiO_x) (*20*).

We can also go beyond the level of simply patterning a surface and make distinct objects that can be manipulated and in principle incorporated into complex nanostructures. The basis for this new idea is the fact that the MoO_3 crystallites are not strongly bound to the underlying MoS_2 substrate; therefore, it is possible to separate nanomachined MoO_3 objects from the MoS_2 surface. This concept is demonstrated in Fig. 2. In this series of AFM images we first define a triangle at the edge of a MoO_3 crystallite (Fig. 2, A to C). The line pattern defining the triangle was machined with a force of 1×10^{-7} N (*21*). The most remarkable feature of this series of images is that the triangular structure patterned at the edge of the MoO_3 can be separated from the original crystallite by scanning across the entire crystal with a force of $\sim 1 \times 10^{-7}$ N (Fig. 2D). In Fig. 2D the triangular object was moved ~100 nm after a single high-load scan. We can further manipulate the triangular MoO_3 object on the MoS_2 with this procedure and show a second ~100-nm translation step in Fig. 2E. Importantly, these translation steps can be imaged without perturbation by using low loads ($\leq 10^{-8}$ N). Hence, we are not only able to nanomachine free objects, but we can also translate and observe these objects on the MoS_2 surface with an AFM tip. The objects we create and manipulate with the AFM are several orders of magnitude smaller than those currently produced by micromachining techniques (*22*). For future applications it is important to note that the electronic properties of MoO_3 can be readily varied from insulating through metallic by doping, and that MoO_3 and related metal oxides exhibit photochromism. Because it should be possible to lift these small objects electrostatically with the tip (in addition to translating them), it is interesting to speculate whether one can assemble nanostructures with novel electrical and optical properties by using these techniques.

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20. A stable tip is required to obtain reproducible nanomachining. For other oxide systems the Si_3N_4 tips may not be sufficiently robust; however, cantilevers with diamond tips should be applicable to a wide range of materials.
21. The line resolution at the surface of the MoO_3 is lower than in Fig. 1 because this crystallite is three times thicker (42 Å) than the one unit cell–thick MoO_3 layer patterned with HU.
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Recovery from Hemophilia B Leyden: An Androgen-Responsive Element in the Factor IX Promoter

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One form of the inherited, X-linked, bleeding disorder, hemophilia B, resolves after puberty. Mutations at −20 and −26 in the clotting factor IX promoter impair transcription by disrupting the binding site for the liver-enriched transcription factor LF-A1/HNF4. The −26 but not the −20 mutation also disrupts an androgen-responsive element, which overlaps the LF-A1/HNF4 site. This explains the improvement seen in patients with the −20 mutation and the failure of the −26 patient to recover.

Patients with Hemophilia B Leyden present in childhood with severe bleeding symptoms and <1% of the normal amounts of plasma factor IX. After puberty, the clinical symptoms improve gradually and plasma factor IX concentrations rise to 60% of normal. The first known patient had a T to A mutation at −20 in the factor IX promoter (*1*). Other Leyden-like patients, have point mutations at nucleotides −6, +6, +8, or +13 (*2*). Here we study a new patient who failed to improve after puberty and suggest an explanation for the clinical improvement.

The patient, Hemophilia B Brandenburg, had a G to C mutation at −26 in the promoter region (*3*). Like the classical Leyden patients, he had <1% normal amounts of factor IX clotting activity before puberty, but unlike them, his factor IX clotting remained low and there was no clinical recovery (*3*). To test whether this −26 mutation and the −20 mutation (*1*) impaired transcription from the factor IX pro-

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Table 1. CAT activity in HepG2 cells comparing normal, Brandenburg, and Leyden promoters. Each construct contained −189 to +21 of the factor IX promoter fused to the CAT gene in the promoterless vector pCAT00 (*4*). Mean and standard errors of three independent experiments are shown.

Promoter construct	Relative CAT activity (%)
Normal	100 ± 4.6
Brandenburg (−26 G to C)	2.9 ± 0.8
Leyden (−20 T to A)	3.8 ± 0.5
pCAT00	2.7 ± 1.1

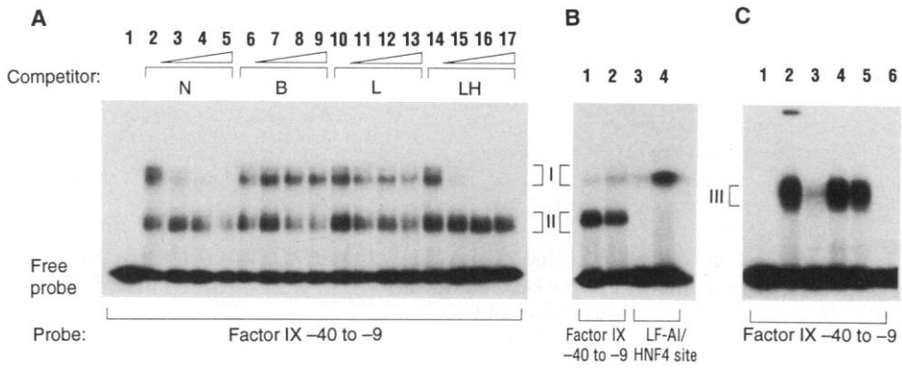

Fig. 1. Gel retardation assays showing LF-A1/HNF4 binding. (**A**) A 5′ ^{32}P-labeled factor IX DNA fragment, −40 to −9, was incubated with rat liver nuclear extract (5 μg except in lane 1) and resolved by 4% polyacrylamide gel electrophoresis (PAGE) (*6, 15*). Competition experiments with homologous, mutant, or −137 to −103 (LF-A1/HNF4 site LH) α1-antitrypsin oligonucleotides had about 10-, 100-, and 200-fold excess of unlabeled competitor DNA. N, normal, f1X −40 to −90; B, Brandenburg, −26 G → C; L, Leyden, −20 T → A. (**B**) Gel retardation comparing labeled promoter regions of factor IX, −40 to −9 (lanes 1 and 2), and α1-antitrypsin, −137 to −103 (lanes 3 and 4). Lanes 1 and 3 contained 2.5 μg of rat liver nuclear extract; lanes 2 and 4 contained 5 μg. (**C**) Gel retardation with LF-A1 (all except lane 1 contained 0.5 μg) and labeled factor IX, −40 to −9. Competition was with a 100-fold excess of unlabeled homologous DNA (lane 3), −26 G to C mutant (lane 4), −20 T to A mutant (lane 5), and α1-antitrypsin −137 to −103 promoter (lane 6).

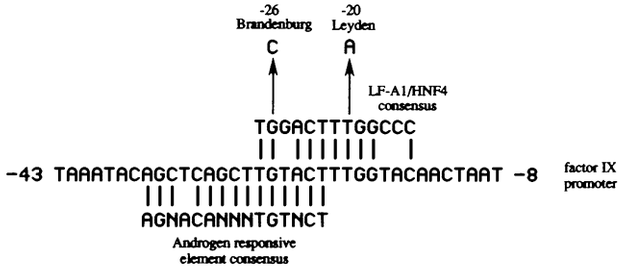

Fig. 2. Sequence of overlapping ARE and LF-A1/HNF4 binding site in the factor IX promoter. Residues matching the LF-A1/HNF4 consensus (*6*) above and the ARE consensus (*16, 17*) below are aligned.

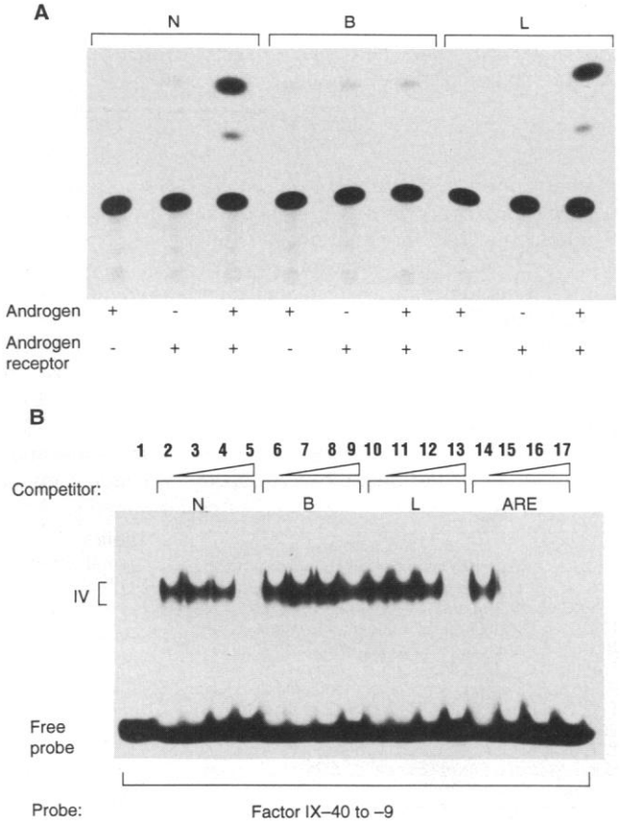

Fig. 3. Evidence for an ARE. (**A**) CAT assays. Four copies of the factor IX promoter, −42 to −17 (wild type or mutant), were cloned into pBLCAT2 (*18*). After transfection of the resultant plasmids (10 μg), with the AR expression plasmid (10 μg) (*11*) or sonicated herring testes DNA (10 μg) into HeLa cells (10^6) maintained on hormone-depleted media (*19*), 5α-dihydrotestosterone (2.5×10^{-8} M, where indicated) was added at 16 hours. CAT activities were determined at 40 hours (*4*). (**B**) Gel retardation with AR–protein A fusion protein and −40 to −9 factor IX promoter. AR–protein A fusion protein was incubated with 5′ ^{32}P-labeled factor IX promoter, −40 to −9, and resolved by poly 4% PAGE (*12*). All lanes except one, which had 1 μg of protein A, contained 0.8 μg of AR fusion protein. Competition experiments [N, −40 to −9 factor IX, wild type (lanes 3 to 5); B, Brandenburg, −26 mutation (lanes 7 to 9); L, Leyden −20 mutation (lanes 11 to 13); and the rat prostatic binding protein ARE (lanes 15 to 17)] were with 50-, 100-, and 500-fold excess of unlabeled DNA.

moter, we assayed promoter function with chloramphenicol acetyltransferase (CAT) constructs and human hepatoma HepG2 cells (*4*). Both mutations reduced transcription markedly (Table 1).

To determine how these mutations interfered with transcription, we tested whether they affected the binding of transcription factors to the promoter. A −40 to −9 fragment of the factor IX promoter when incubated with rat liver nuclear extract formed two retarded complexes, I and II (Fig. 1). Homologous DNA efficiently competed for complex I, but not complex II, suggesting that only complex I was formed by the binding of a sequence-specific DNA binding protein. Neither the −26 nor the −20 mutant oligonucleotides could compete for specific binding. Homology of this region of the factor IX promoter to the liver-enriched transcription factor LF-A1/HNF4 (*5, 6*) binding site (Fig. 2) implicated LF-A1/HNF4 as a possible candidate protein in complex I. Known LF-A1/HNF4 binding sites, including α1-antitrypsin (*7*), apolipoprotein A1 (*6*), and transthyretin (*5*) promoter regions, competed for complex I (Fig. 1A) (*8*). When the LF-A1/HNF4 binding site from the α1-antitrypsin promoter was used in gel retardation assays, the resultant complex comigrated with complex I (Fig. 1B). Finally, purified LF-A1, prepared by affinity chromatography with the use of its binding site in the apolipoprotein B gene promoter, formed a complex III (Fig. 1C) that was specifically competed by both normal factor IX sequences and the α1-antitrypsin site but not by either mutant promoter. Complex III had a slightly faster mobility than complex I, perhaps because the LF-A1 preparation (*6*) apparently contained a 40-kD fragment of the normal 50-kD protein (*5*).

These results suggested that the −26 and −20 mutations disrupt an LF-A1/HNF4 binding site, thereby impairing fac-

tor IX promoter activity, reducing factor IX expression and resulting in hemophilia B. It has been postulated that the factor IX promoter responded to testosterone (*9*), which can increase factor IX concentrations in Leyden patients (*10*). These results suggest that the factor IX promoter contains a functional androgen-responsive element (ARE). The promoter contains a sequence resembling a consensus ARE (nucleotides −36 to −22) (Fig. 2). The −26 G to C mutation but not the −20 T to A mutation disrupts this ARE.

To test whether the ARE was functional, we linked it upstream of the herpes simplex thymidine kinase (TK) promoter and the CAT reporter gene and assayed transactivation in HeLa cells by an androgen receptor (AR)–encoding plasmid (*11*) in the presence of testosterone. When the ARE was present in one copy, the response was not significantly above background; with two copies a threefold effect was observed, and with four copies (Fig. 3A) the ARE allowed the TK promoter to be significantly (>50-fold relative to a control in the absence of AR) transactivated when both testosterone and the AR expression plasmid were present. The promoter with the −26 mutation did not support transactivation, whereas that with the −20 mutation (which lies outside the ARE) did.

When the candidate factor IX ARE was bound to a recombinant AR–protein A fusion protein (*12*), a gel-shifted complex IV was formed (Fig. 3B), which was effectively competed by both normal factor IX DNA and by DNA with the −20 mutation but not by DNA containing the −26 mutation. A potent ARE from a rat prostate binding protein gene (*12*) competed even more effectively for this complex (Fig. 3B).

These results show that the ARE in the factor IX promoter can bind AR in vitro. AR mRNA has been identified in human liver and AR protein in rat liver (*13*). The fact that the patient with a mutation at −26 in the ARE did not improve after puberty, whereas patients with mutations at −20, outside the ARE, do improve, shows that this element is important in vivo. Nevertheless, it bound AR less effectively than the well-characterized ARE from the prostatic binding protein gene and only functioned well in CAT assays when multimerized (Fig. 3). In transient transfection systems in HepG2 cells, the factor IX promoter itself supports only a small activation (less than threefold) dependent on androgen and AR (*14*). Additional upstream elements or factors may be required for the full response or the transient assay system may not faithfully reflect the in vivo situation. Although the ARE identified here is necessary, it may not be sufficient for the full response seen in Leyden patients (*10*).

In summary, the mutation in Hemophilia B Brandenburg simultaneously disrupts an LF-A1/HNF4 site and an overlapping ARE, which explains the persistent hemophilia in this patient and also why patients with nearby promoter mutations, which do not disrupt the ARE, recover after puberty. Mutations identified in hemophilia B patients in the site bound by C/EBP (or related proteins) (*4*) and now in the LF-A1/HNF4 binding site of the hepatocyte-specific factor IX promoter emphasize the function of these factors in the regulation of liver-specific genes in vivo.

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Cloning of the γ Chain of the Human IL-2 Receptor

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A third subunit, the γ chain, of the human interleukin-2 receptor (IL-2R) was identified, and a complementary DNA clone encoding this member of the cytokine receptor family was isolated. The γ chain is necessary for the formation of the high- and intermediate-affinity receptors, which consist of αβγ heterotrimers and βγ heterodimers, respectively. The IL-2R on murine fibroblastoid cells can be internalized after binding IL-2 only if the γ chain is present; α and β are insufficient for internalization. Thus, the γ chain is an indispensable component of the functional IL-2R.

The lymphokine IL-2 affects the growth and differentiation of T cells, B cells, natural killer cells, glioma cells, and cells of the monocyte lineage after specifically interacting with IL-2Rs (*1*, *2*). The IL-2R contains at least two distinct subunits, called α and β chains, and exists in three different isoforms: high affinity for IL-2 (the dissociation constant K_d is $\sim 10^{-11}$ M), intermediate affinity ($K_d = \sim 10^{-9}$ M), and low affinity ($K_d = \sim 10^{-8}$ M) (*1*). The high- and intermediate-affinity receptors, which are thought to consist of the α and β heterodimers and the β chain alone, respectively, are effective in IL-2–mediated signal transduction, but the low-affinity receptor, which consists of the α chain alone, is ineffective; therefore, the β chain but not the α chain is indispensable (*3–5*). Several lines of evidence implicate a third lymphoid-specific component that is required for the formation of the high- and intermediate-affinity receptors. (i) The β chain, when expressed on lymphoid

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