to 1 × 10^8 spleen cells from Fos mutant mice. Transplanted mice were analyzed radiographically and histologically after 3 weeks, and the presence of donor hematopoietic cells in the irradiated recipients was confirmed by Southern blot analysis of hematopoietic tissues (*4*, *5*). All specimens are 5-μm paraffin sections stained with hematoxylin and eosin.

28. Primary osteoblasts were isolated from calvaria of 3- to 5-day-old mice with a sequential collagenase digestion procedure (*25*). For coculture experiments, primary osteoblasts (10^4 cells per 16-mm well) and spleen cells (5 × 10^5 cells per 16-mm well) from either wild-type or Fos mutant mice were cultured in α–minimum essential medium containing 10% fetal bovine serum and 10^{-8} M 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] (*15*). For virus infection experiments, P1.15 osteoblastic cells (*3*) (10^5 cells per 16-mm well) were cultured with either wild-type or Fos mutant spleen cells (10^6 cells per 16-mm well) that had been infected previously in suspension with either a control virus (pMV7) or a c-*fos*–expressing virus (pMV-c-*fos*) for 6 hours at 37°C. Cells were cultured in the presence of 10^{-8} M 1,25-$(OH)_2D_3$ and 10^{-7} M Dexamethasone. After 8 days in culture the cells were fixed in 3.7% formaldehyde and stained for TRAP with a commercially available kit (Sigma) in the presence of 50 to 100 mM sodium tartrate. TRAP-positive cells containing three or more nuclei were counted as osteoclasts. Osteoclast resorption activity was measured by plating of primary calvarial osteoblasts (5.6 × 10^3 cells per 6-mm well) and spleen cells (7 × 10^4 cells per 6-mm well) from either wild-type or Fos mutant mice, with slices of ivory as a mineral substrate. After 8 days in culture in the presence of 10^{-8} M 1,25-$(OH)_2D_3$, resorption pits were quantified as described previously (*25*).

29. Tissue specimens were prepared as described (*24*). Immunostaining for F4/80 antigen and Mac-2 antigen (clone M3/38, Boehringer Mannheim) was done according to the indirect peroxidase-conjugated streptavidin procedure (*25*). In situ hybridization was done with sense and antisense riboprobes synthesized from a cDNA for murine c-*fms* (*11*). Exposure times were 3 to 4 days, and all hybridizations with sense riboprobes did not show any specific signals.

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Differential Regulation of RNA Polymerases I, II, and III by the TBP-Binding Repressor Dr1

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RNA polymerases I, II, and III each use the TATA-binding protein (TBP). Regulators that target this shared factor may therefore provide a means to coordinate the activities of the three nuclear RNA polymerases. The repressor Dr1 binds to TBP and blocks the interaction of TBP with polymerase II– and polymerase III–specific factors. This enables Dr1 to coordinately regulate transcription by RNA polymerases II and III. Under the same conditions, Dr1 does not inhibit polymerase I transcription. By selectively repressing polymerases II and III, Dr1 may shift the physiological balance of transcriptional output in favor of polymerase I.

Three RNA polymerases (Pols) are responsible for the transcription of nuclear genes. Although much has been learned about how these polymerases are individually controlled, little is known about how they are regulated with respect to each other. Coordination of the three polymerases must be important for cellular metabolism, because unbalanced activity would be wasteful. An obvious way to coordinate the regulation of the polymerases would be to regulate shared components. A strong candidate for such regulation is TBP, which is used by RNA Pols I, II, and III (*1*).

Although TBP is used by all three polymerases, it is assembled into polymerase-specific complexes (*1*). These complexes are called SL1 for the Pol I system (*2*–*4*), TFIID for the Pol II system (*5*), and TFIIIB for the Pol III system (*6*–*11*). TFIID can nucleate transcription complex formation at Pol II promoters (*12*). Recruitment of polymerase to this complex requires TFIIB, which binds directly to both TBP and Pol II (*12*). TFIIIB contains a polypeptide called TFIIB-related factor (BRF; also known as TDS4 or PCF4), which is homologous to TFIIB and binds to TBP and Pol III (*13*). TFIIB and BRF therefore perform analogous functions in directing Pol II or Pol III, respectively, to the appropriate complexes. It is not yet clear whether the Pol I system also contains a TFIIB-like factor.

The repressor Dr1 inhibits Pol II transcription by binding directly to TBP and blocking its interaction with TFIIB (*14*). We tested whether Dr1 could also regulate Pol III activity. Transcription of the $tRNA^{Glu6}$ gene was repressed by Dr1 that had been substantially purified from HeLa cells (hDr1) (*15*) (Fig. 1A). Furthermore, ~90% pure recombinant Dr1 (rDr1) (*15*) also inhibited expression of this gene (Fig. 1B) and that of other Pol III templates, including the VA_I (Fig. 1C) and U6 genes (*16*). This was not a nonspecific response to added protein, because the corresponding control fraction from bacteria that lacked the Dr1 expression vector (*15*) had little effect (Fig. 1C). Dr1 can repress transcription of Pol III templates by as much as 70-fold. Indeed, it inhibited $tRNA^{Glu6}$ transcription as much as it inhibited that of the Pol II template G_6TI (Fig. 1B). α-Amanitin treatment confirmed the polymerases responsible for this transcription (*16*). Repression still occurred if the Pol III factors were preassembled on the VA_I gene (*16*). Clearly,

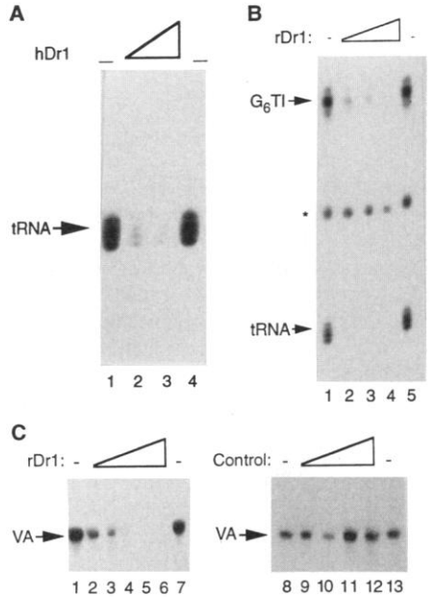

Fig. 1. Dr1 is a potent repressor of Pol III transcription. (**A**) Fractionated factors (*21*) were preincubated without hDr1 (lanes 1 and 4) or with 2 μl (lane 2) or 4 μl (lane 3) of hDr1. pGlu6 (500 ng) (*22*) was added with nucleotides to begin transcription (*23*). (**B**) Nuclear extract (30 μg) (*21*) was preincubated with 100 ng of pG_6TI (*22*) and 100 ng of pGlu6 either without (lanes 1 and 5) or with 1.5 μl (lane 2), 3 μl (lane 3), or 5 μl (lane 4) of rDr1. Nucleotides were added to begin transcription. The asterisk reflects template-independent end-labeling of endogenous small RNAs. (**C**) Fractionated factors (*21*) were preincubated with the following amounts of bacterially expressed protein (*15*): lanes 1, 7, 8, and 13, none; lane 2, 0.5 μl of rDr1; lane 3, 1 μl of rDr1; lane 4, 2 μl of rDr1; lane 5, 4 μl of rDr1; lane 6, 8 μl of rDr1; lane 9, 1 μl of bacterial control; lane 10, 2 μl of control; lane 11, 4 μl of control; and lane 12, 8 μl of control. pVA_I (500 ng) (*22*) was added with nucleotides to begin transcription.

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Dr1 is a potent regulator of both Pol II and Pol III activity.

Add-back experiments were used to identify which Pol III factor was inactivated by Dr1. Repression of VA_1 transcription by rDr1 was efficiently overcome by the addition of partially purified TFIIIB (Fig. 2A). The specificity of this effect is shown by the fact that TFIIIC did not overcome repression (Fig. 2A), even though TFIIIC is the limiting factor in the absence of added Dr1 (*17*). That it is the TBP subunit of TFIIIB that is targeted is suggested by the fact that repression of Pol III transcription involves the TBP binding site of Dr1 (*16*). Furthermore, cloned TBP alone can restore VA_1 transcription in the presence of rDr1 (Fig. 2B). In contrast, TBP does not activate in the absence of rDr1 and instead has an inhibitory effect at high concentrations (Fig. 2B). This is because TBP is normally in relative excess for VA_1 transcription in HeLa systems (*17*). These results show that Dr1 specifically lowers the effective concentration of the TBP subunit of TFIIIB so that it becomes rate-limiting for Pol III transcription.

By analogy to the Pol II system, Dr1 may disrupt TFIIIB by preventing TBP from binding to BRF. To test this idea, we used a pull-down assay to measure directly the TBP-BRF interaction (*18*). BRF was fused to glutathione-S-transferase (GST), expressed in bacteria, and purified on glutathione-Sepharose beads. The beads were then incubated with in vitro–translated radiolabeled human TBP. A substantial amount of labeled TBP remained bound to GST-BRF beads after centrifugation and extensive washing (Fig. 3). This reflects binding to BRF, because beads linked to GST alone bound very little TBP. The interaction between BRF and TBP was abolished when rDr1 was included in the reaction. In contrast, a buffer control or the corresponding protein fraction from bacteria that lack the Dr1 expression vector had no effect on this reaction. These results establish that Dr1 can efficiently disrupt the BRF-TBP interaction, which accounts for its ability to inactivate TFIIIB.

Because Pol I transcription also uses TBP (*2–4*), we tested its response to Dr1. However, α-amanitin–insensitive ribosomal RNA (rRNA) synthesis by Pol I was unaffected by concentrations of either hDr1 (Fig. 4A) or rDr1 (Fig. 4B) that strongly inhibit Pols II and III. A tRNA internal control was substantially repressed in the same experiment (Fig. 4B). This differential effect provides further evidence that Dr1 functions in a specific fashion. Although we cannot exclude that Pol I transcription might respond under other assay conditions, these results suggest that it is much less sensitive to Dr1.

Dr1 appears to repress Pols II and III by very similar mechanisms. In each case it prevents TBP binding to a class-specific factor (TFIIB or BRF) that is required for Pol recruitment. It is unknown why Pol I is unresponsive. Pol I transcription is less susceptible than Pols II or III to inhibition with TATA box oligonucleotides or antibodies to TBP, which implies that SL1 is less readily disrupted than TFIID or TFIIIB (*4*). This may account for its resistance to Dr1. Alternatively, the TFIIB binding site on TBP may not interact with a Pol I factor. Certain mutations in the region of TBP implicated in contacting BRF produce a decrease in Pol III transcription and a concomitant increase in Pol II activity (*19*), which suggests that TFIIB competes with BRF for binding to an overlapping surface on TBP. The same mutations do not affect Pol I transcription (*19*). Therefore, the Pol I system may not have a TFIIB-like factor and may not use the TBP surface that is blocked by Dr1.

We have confirmed recently that Dr1 binds to TBP in living cells (*20*). Furthermore, transfected Dr1 represses transcription in a manner that is dependent on its ability to bind TBP (*20*). These results confirm that the biochemical properties of Dr1 are directly relevant to its behavior in vivo.

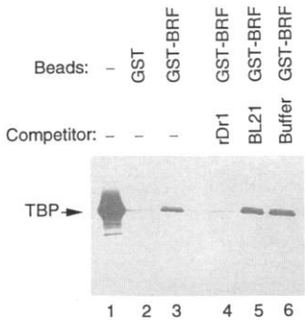

Fig. 3. Binding of TBP to BRF is disrupted by Dr1. Lane 1 shows the input level of radiolabeled TBP. Lanes 2 to 6 show the amount of TBP bound to beads linked to GST alone (lane 2) or GST-BRF (lanes 3 to 6). Four microliters of rDr1, bacterial control protein, or buffer alone was included in the reactions in lanes 4, 5, and 6, respectively.

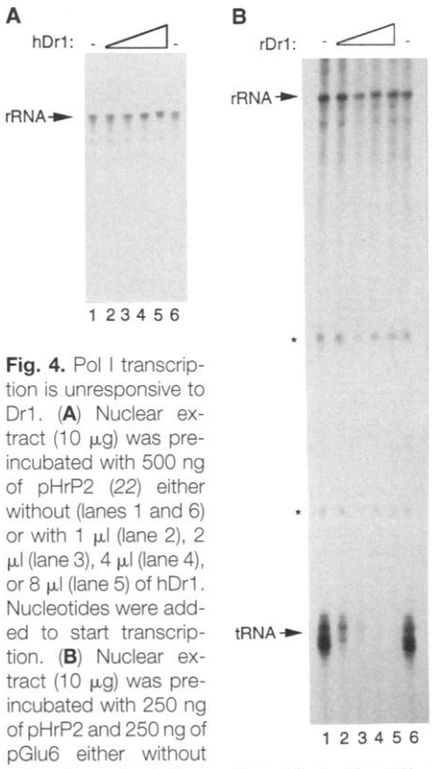

Fig. 4. Pol I transcription is unresponsive to Dr1. (**A**) Nuclear extract (10 μg) was preincubated with 500 ng of pHrP2 (*22*) either without (lanes 1 and 6) or with 1 μl (lane 2), 2 μl (lane 3), 4 μl (lane 4), or 8 μl (lane 5) of hDr1. Nucleotides were added to start transcription. (**B**) Nuclear extract (10 μg) was preincubated with 250 ng of pHrP2 and 250 ng of pGlu6 either without (lanes 1 and 6) or with 1 μl (lane 2), 2 μl (lane 3), 4 μl (lane 4), or 7 μl (lane 5) of rDr1. Nucleotides were added to start transcription. The slightly reduced rRNA signal in lane 3 is the result of incomplete loading, as can be seen by comparison with the asterisked bands, which represent end-labeling of endogenous small RNAs.

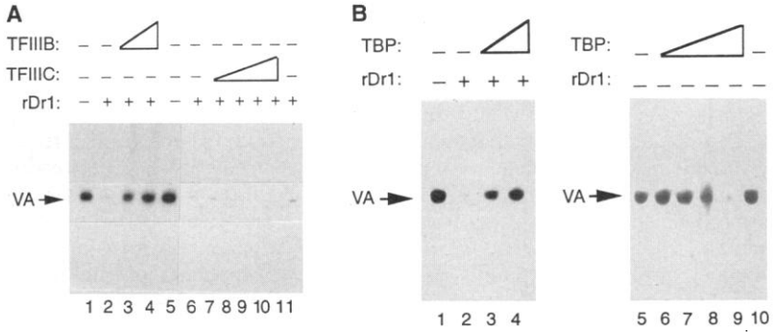

Fig. 2. Dr1 specifically inactivates TFIIIB. (**A**) Fractionated factors were preincubated with either no added Dr1 (lanes 1 and 5) or with 2 μl of rDr1 (other lanes). Mono Q–purified TFIIIB (4 μl or 8 μl) (*24*) was included in reactions 3 and 4, respectively. Reactions 7 to 10 contained 1 μl, 2 μl, 4 μl, or 8 μl, respectively, of affinity-purified TFIIIC (*25*). pVA_1 (500 ng) was added with nucleotides to begin transcription. (**B**) Fractionated factors (*21*) were preincubated with either 2 μl of rDr1 (lanes 2 to 4) or without rDr1 (other lanes). The following amounts of recombinant TBP (*26*) were also included: lanes 1, 2, 5, and 10, none; lanes 3 and 6, 10 ng; lane 7, 20 ng; lanes 4 and 8, 30 ng; lane 9, 40 ng. pVA_1 (500 ng) was added with nucleotides to begin transcription.

We consider that in vitro studies are the most informative for identifying direct effects of Dr1 on individual polymerases. This is because the sustained overexpression of Dr1 in vivo will inevitably perturb all three polymerases, because each depends on the products of the others to maintain production of transcription factors and polymerase subunits. We speculate that the cellular role of Dr1 is in fine-tuning, allowing transitory adjustments in polymerase output in response to environmental changes.

We have established the potential of Dr1 to directly coregulate Pols II and III. In contrast, Pol I transcription appears immune to this factor. Dr1 may therefore serve to shift the overall balance of nuclear metabolism in favor of Pol I. This could be of considerable value when rRNA levels are limiting. Factors such as Dr1 that form a regulatory network to interlink the three transcriptional systems are likely to be of great importance to the overall cellular economy.

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22. The pVA_1 [N. Dean and A. J. Berk, *Nucleic Acids Res.* **15**, 9895 (1987)], pGlu6 [J. P. Goddard, M. Squire, M. Bienz, J. D. Smith, *Nucleic Acids Res.* **11**, 2551 (1983)], pG_6TI [B. F. Pugh and R. Tjian, *Cell* **61**, 1187 (1990)], and pHrP2 [A. Lescure *et al.*, *EMBO J.* **13**, 1166 (1994)] plasmids are as described. pG_6TI and pHrP2 were linearized before use.
23. Preincubations were for 15 min at 30°C. Total volume and salt concentrations were kept constant with buffer. Transcription reactions were performed as described [R. J. White, D. Stott, P. W. J. Rigby, *Cell* **59**, 1081 (1989)].
24. TFIIIB was purified from the PC-B fraction (*21*) by Mono Q gradient chromatography as described (*11*).
25. B-block oligonucleotide affinity resin was prepared as described [N. Dean and A. J. Berk, *Nucleic Acids Res.* **15**, 9895 (1987)]. PC-C fraction in BC-70 was incubated on ice with 30 μg of poly dI·dC per milligram of protein for 10 min, then passed 6 times through the affinity resin over 75 min at 4°C. After washing with BC-350, TFIIIC was eluted in BC-2000.
26. Recombinant human TBP was expressed in bacteria and purified to >95% homogeneity as described [M. G. Peterson, N. Tanese, B. F. Pugh, R. Tjian, *Science* **248**, 1625 (1990)].

27. We thank C. Hagemeier and A. Bannister for help with pull-down assays, A. Berk for pVA_1, and I. Grummt for pHrP2. B.C.-E.K. is a member of the University of Cambridge Medical School M.B./Ph.D. Program and is supported by a Wellcome Trust Prize Studentship. This work was funded principally by project grant SP21 43/0101 from the Cancer Research Campaign.

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Rescue of T Cell–Specific V(D)J Recombination in SCID Mice by DNA-Damaging Agents

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Assembly of antigen receptor V (variable), D (diversity), and J (joining) gene segments requires lymphocyte-specific genes and ubiquitous DNA repair activities. Severe combined immunodeficient (SCID) mice are defective in general double-strand (ds) DNA break repair and V(D)J coding joint formation, resulting in arrested lymphocyte development. A single treatment of newborn SCID mice with DNA-damaging agents restored functional, diverse, T cell receptor β chain coding joints, as well as development and expansion of thymocytes expressing both CD4 and CD8 coreceptors, but did not promote B cell development. Thymic lymphoma developed in all mice treated with DNA-damaging agents, suggesting an interrelation between V(D)J recombination, dsDNA break repair, and lymphomagenesis.

Lymphocyte development requires the participation of a site-specific recombinase system to somatically juxtapose widely dispersed V, D, and J gene segments that comprise the variable recognition domains of T and B cell antigen receptors. In V(D)J recombination-deficient mice resulting from natural mutation or created by gene-targeting techniques, precursor T (pre-T) and pre-B cell antigen receptors cannot be expressed by lymphocyte progenitors, which accounts for the absence of mature lymphocytes in these animals (*1*). The most well-defined components of the V(D)J recombinase machinery are developmentally regulated, lymphocyte-specific genes, such as the recombinase-activating genes *RAG-1* and *RAG-2* (*2*) and terminal deoxynucleotidyl transferase, which adds short stretches of nontemplated nucleotides (*3*). The murine *scid* gene defines a dsDNA break repair activity (*4*) that is also required for efficient joining of free V(D)J coding ends (*5–7*). Additional DNA repair activities may also participate in V(D)J recombination (*8*). In contrast to the lymphocyte-specific components of the recombinase machinery, genes encoding many of the general DNA repair activities have not been isolated and little is known about their regulation and precise functions. We now show that treatment of newborn SCID mice with DNA-damaging agents restored normal T cell receptor (TCR) β recombination and T cell maturation. However, all of these mice eventually developed thymic