Although the human CD4–immunoglobulin chimaera reported by Capon *et al.*[10] was secreted, they found that a similar hybrid protein, based on the mouse γ_1 heavy chain, was retained intracellularly. We have also noticed that hybrid molecules containing the C_H1 domain, for example CD4-1gM chimaeras, are not secreted (unpublished observation) and we suspect that in the absence of immunoglobulin light chains, the hydrophobic face of the C_H1 domain interacts strongly with the heavy chain binding protein, thus preventing secretion[30,31].

We believe that hybrid proteins which combine the specificity of CD4 with the multivalency and effector functions of different immunoglobulin subclasses could provide a realistic approach to AIDS therapy. We also think that our approach to designing hybrid immunoglobulin molecules could be applied more generally for building novel immunoglobulin molecules. □

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Activation of HIV gene expression during monocyte differentiation by induction of NF-κB

George E. Griffin*†, Kwanyee Leung*, Thomas M. Folks‡, Steven Kunkel§ & Gary J. Nabel*‖

* Howard Hughes Medical Institute, University of Michigan Medical Center, Departments of Internal Medicine and Biological Chemistry, Ann Arbor, Michigan 48109, USA
‡ Retrovirus Diseases Branch, Centers for Disease Control, Atlanta, Georgia 30333, USA
§ Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA

THE latent period of AIDS is influenced by factors which activate human immunodeficiency virus (HIV) replication in different cell types. Although monocytic cells may provide a reservoir for virus production *in vivo*[1–8], their regulation of HIV transcription has not been defined. We now report that HIV gene expression in the monocyte lineage is regulated by NF-κB, the same transcription factor known to stimulate the HIV enhancer in activated T cells[9]; however, control of NF-κB and HIV in monocytes differs from that observed in T cells. NF-κB-binding activity appears during the transition from promonocyte to monocyte in U937 cells induced to differentiate *in vitro* and is present constitutively in mature monocytes and macrophages. In a chronically infected promonocytic cell, U1, differentiation is associated with HIV-1 replication as well as NF-κB binding activity. These findings suggest that NF-κB binding activity is developmentally regulated in the monocyte lineage, and that it provides one signal for HIV activation in these cells.

We transfected monocytic cell lines from progressive stages of differentiation with a plasmid containing the HIV enhancer linked to the chloroamphenicol acetyltransferase (CAT) gene. Twenty-four hours after transfection, cells were incubated in medium alone or in the presence of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Expression of the HIV enhancer was induced by TPA in two immature monocyte leukaemia lines, a human granulocyte-macrophage leukaemia, HL-60, and the human promonocytic line, U937 (Fig. 1*a*). TPA treatment did not augment CAT expression in the mature macrophage leukaemic cells, THP-1, P388D1, or PU5-1.8, which showed higher basal activity (Fig. 1*b*; note scale changes).

Using a mutant HIV-CAT plasmid containing alterations in both κB sites[9], we showed that induction of HIV-CAT expression in the immature lines, HL-60 and U937 (Fig. 1*a*), and constitutive expression in the mature lines was dependent on the κB sites (Fig. 1*b*). This suggested that NF-κB is present in the induced progenitors and in the mature cells, and we therefore looked for NF-κB binding activity in nuclear extracts from these cell lines. NF-κB binding activity in the immature lines, HL-60 and U937, was induced by TPA, whereas in the mature macrophage lines, THP-1, PU5-1.8, and P388D1, it was constitutively expressed (Fig. 2*a*). We then determined whether NF-κB binding activity is present in normal human monocytes and/or macrophages. Nuclear extracts were prepared from human peripheral blood monocytes or adherent mononuclear cells, and NF-κB binding activity was found in both cell types as well as in mouse peritoneal macrophages (Fig. 2*b*). NF-κB binding is therefore constitutively active in normal and neoplastic mature mononuclear phagocytes, including blood monocytes and adherent macrophages.

Treatment of immature monocytes with TPA, or the water-soluble phorbol-12, 13-dibutyrate[10] (PDB) (which is more easily removed from cells) causes differentiation into mature monocytes and macrophages, as judged by changes in cell growth, morphology, surface glycoproteins, and phagocytic function (Fig. 3, see also refs 11–15). HL-60 cells treated with PDB acquired characteristics of mature macrophages, displaying growth arrest, increased phagocytosis, adherence, FcR and Mo 1 expression. At the same time, these cells began to express NF-κB binding activity which persisted even two days after

† Permanent address: Department of Communicable Diseases, St George's Hospital Medical School, London, UK.
‖ To whom correspondence should be addressed.

removal of PDB (Fig. 3*a*). U937 cells matured similarly and also began to express NF-κB binding constitutively, both in the presence of PDB or tumour necrosis factor-α (TNF-α) (Fig. 3*b*), another known inducer of NF-κB (ref. 16) and monocyte differentiation (Fig. 3*b*, see also refs 14, 15). In contrast to HL-60 cells, U937 cells did not become adherent after incubation with PDB or TNF-α. These findings, together with the detection of NF-κB in circulating monocytes (Fig. 2*b*), suggests that NF-κB induction is associated with the transition from promonocyte to monocyte.

To determine whether monocyte differentiation and NF-κB induction affect production of intact virus, the latently infected U1 promonocytic line[17] was incubated with PDB or TNF-α. U1 cells exposed to these agents demonstrated morphological,

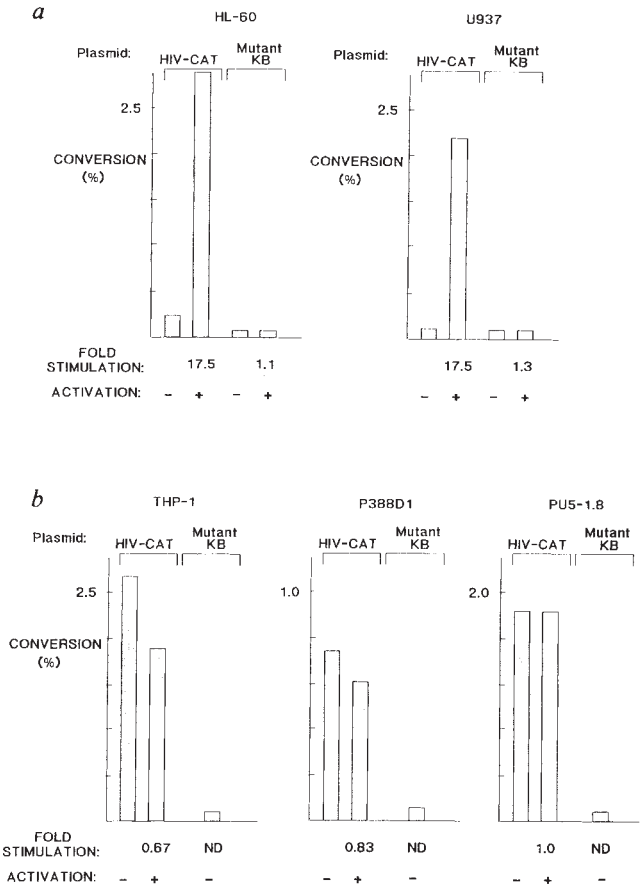


FIG. 1 Activation of HIV-CAT expression in immature cells of the monocytic lineage, constitutive activity in mature cells, and dependence on κB sites. The indicated monocytic leukaemia lines (10^7) were transfected with an HIV-CAT or a mutant plasmid (10 μg) altered in both κB sites using DEAE-dextran[9]. Twenty-four hours after transfection, an aliquot of cells was maintained in medium alone (−) or incubated for a further 20 h in the presence of 20 nM TPA (+) (Sigma). The monocytic leukaemia cells were representative of progressive stages of maturity and included (*a*) immature cells HL-60 (human granulocyte-macrophage progenitor) or U937 (human promonocyte), and (*b*) mature cells THP-1 (human macrophage), P388D1 (mouse macrophage), or PU5-1.8 (mouse macrophage) leukaemia lines.
METHODS. Cells were transfected as previously described[9], except that cells were treated with 10 μM chloroquine for 30 min after transfection. Cell extracts were prepared 44 h after transfection, protein concentrations normalized and conversion of chloramphenicol to its acetylated forms assayed by standard methods[29]. Results are representative of at least two independent transfections and the standard deviations of each determination was <10%. Per cent conversion of [^{14}C]chloramphenicol to its acetylated forms are shown.

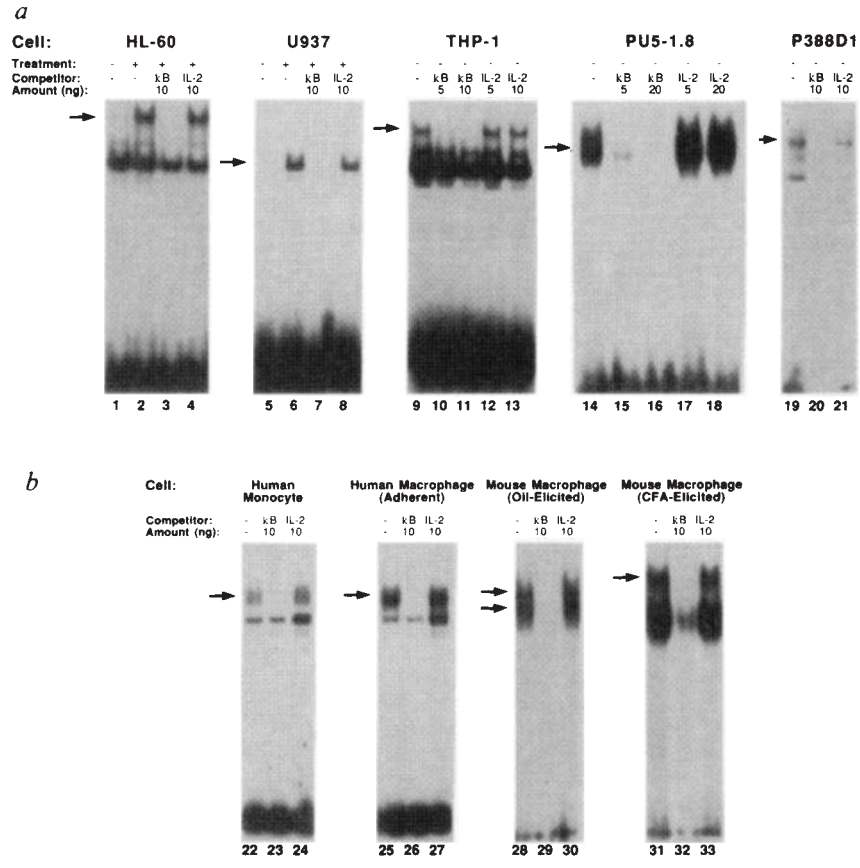


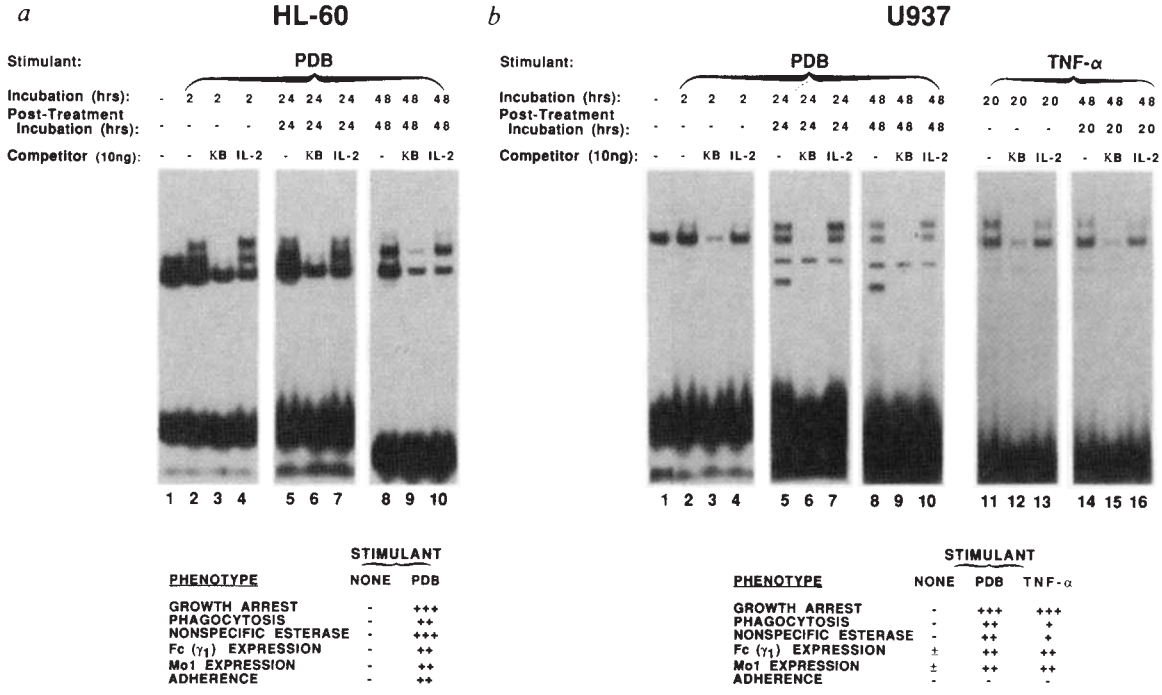
FIG. 2 NF-κB binding is induced by TPA in immature monocytic lines and found constitutively in mature macrophage lines. Nuclear extracts were prepared from cells treated in medium alone (−) or for 2 h in the presence of 40 nM TPA (+) and analysed using the electrophoretic mobility shift assay with a κB probe[30]. *a*, Nuclear extracts from transformed leukaemic lines: HL-60 leukaemia cells (lanes 1–4), U937 promonocytic cells (lanes 5–8), the THP-1 human myelomonocytic leukaemia line (lane 9–13), the PU5-1.8 mouse macrophage leukaemia line (lane 14–18) or the P388D1 mouse macrophage line (lane 19–21). *b*, Nuclear extracts from normal cells: human monocytes (lanes 22–24), human adherent macrophages (lanes 25–27), mouse mineral oil-elicited adherent macrophages (lanes 28–30) or mouse complete Freund's adjuvant-elicited adherent macrophages (lanes 31–33). Adherent cells were ≥95% pure and mononuclear cells ~80% enriched, (as confirmed by phagocytosis, histochemical analysis, and Mo 1 immunofluorescence). THP-1 cells expressed low levels of Fc receptor and low levels of Mo 1 antigen, which increased in response to TPA, together with adherence. TPA also induced rapid secretion of IL-1 and TNF-α, suggesting a mature phenotype. Extracts were incubated with a κB probe alone or in the presence of the indicated amounts of double-stranded oligonucleotide competitor containing the κB site[30] or an unrelated IL-2 promoter fragment[31]. Arrows denote specific inducible complexes competed by double-stranded κB oligonucleotide.
METHODS. The electrophoretic mobility shift assay was performed using a κB probe as previously described[30], with 10 μg nuclear extract in the presence of 1 μg dIdC. Extracts were prepared for analysis from these cells by a rapid method previously described[16].

functional and cell-surface characteristics of differentiation similar to those seen in U937 cells[17,18], and NF-κB binding was induced (Fig. 4*a*, lanes 2 and 3). At the same time, reverse transcriptase and p24 antigen levels increased dramatically (Fig. 4*b*, *c*). Both NF-κB and HIV production persisted even 24–48 h after the stimulus was removed (Fig. 4).

Transient exposure of immature lines to PDB (<2 h) did not lead to constitutive expression of NF-κB binding, and had no effect on cell morphology (data not shown). Once monocyte differentiation occurred, however, NF-κB binding activity remained, as it does in normal monocytes (Fig. 2*b*). Constitutive NF-κB binding activity, found previously only in mature B-cell leukaemia lines[19], is therefore associated with mature cells of the monocyte lineage. NF-κB binding is also inducible in different cells by several agents, including TPA, TNF-α, and IL-1 (refs 9, 16, 20).

In these experiments, NF-κB binding was induced when growth was inhibited, raising the possibility that it is associated with growth arrest. TNF-α, which also induces macrophage differentiation and growth arrest, (Fig. 3*b*; refs 14, 15) stimulates NF-κB binding activity[16]. TNF-α also activates HIV expression in T cells[16,21]. This cytokine is elevated in the blood of AIDS patients[22], and may be important in the activation of HIV *in vivo*.

The HIV enhancer can be activated through κB-independent mechanisms[23] and contains additional transcription factor binding sites[24,25]. Our data show that NF-κB contributes to the activation of HIV in monocytes, but it is not the sole activator of virus in these cells. Granulocyte-macrophage colony-stimulating factor, GM-CSF, which does not induce NF-κB binding[16], activates HIV expression in mature macrophages[26] which already contain NF-κB (Fig. 2*b*), and, in combination with TNF-α, in the U1 cell line (data not shown). These findings imply that at least two signals modulate HIV production in



PHENOTYPE	STIMULANT NONE	PDB
GROWTH ARREST	-	+++
PHAGOCYTOSIS	-	++
NONSPECIFIC ESTERASE	-	+++
Fc (γ_1) EXPRESSION	-	++
Mo1 EXPRESSION	-	++
ADHERENCE	-	++

PHENOTYPE	STIMULANT NONE	PDB	TNF-α
GROWTH ARREST	-	+++	+++
PHAGOCYTOSIS	-	++	+
NONSPECIFIC ESTERASE	-	++	+
Fc (γ_1) EXPRESSION	±	++	++
Mo1 EXPRESSION	±	++	++
ADHERENCE	-	-	-

FIG. 3 Induction of NF-κB binding activity during macrophage differentiation. Nuclear extracts were prepared from HL-60 (*a*) or U937 (*b*) leukaemia lines after the indicated treatments and analysed using the electrophoretic mobility shift assay. HL-60 cells were maintained in medium alone (−) (*a*, lane 1) or incubated with 20 nM PDB (+) for the indicated time, followed by three washes, and incubated for the additional time indicated in the absence of PDB (*a*, lanes 2–10). U937 cells were similarly incubated alone (*b*, lane 1), with 20 nM PDB (*b*, lanes 2–10), or with 50 ng ml^{-1} TNF-α (*b*, lanes 11–16). Specificity of binding was determined by competition with the indicated double-stranded oligonucleotide competitors. Arrows denote specific inducible complexes competed by double-stranded κB oligonucleotide. Cell lines were analysed for the presence of the Mo1 antigen or FcR using a fluorescence-activated cell sorter (refs 32, 33), and analysed by standard methods[34] for non-specific esterase, phagocytosis, and growth arrest.

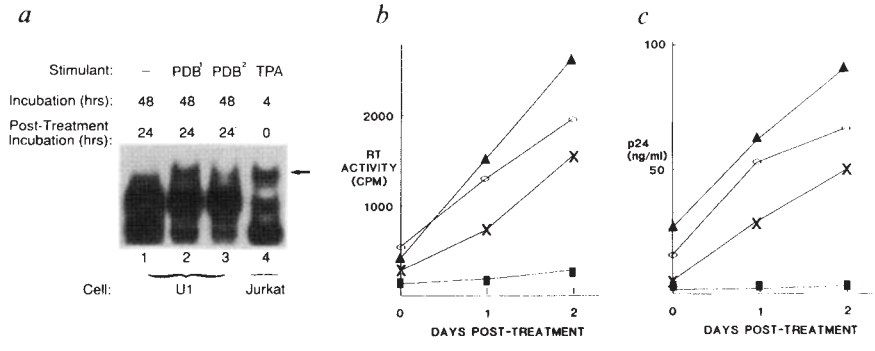


FIG. 4 Increased constitutive expression of NF-κB and HIV in a chronically-infected promonocytic line following cellular differentiation. *a*, NF-κB binding activity was analysed as before. Cellular extracts were prepared from U1 cells incubated in medium alone (lane 1), in the presence of 2 nM PDB (ref. 1) (lane 2) or 20 nM PDB (ref. 2) (lane 3) for the indicated time, followed by washing and further incubation in medium alone for an additional 24 h. A positive control using extract from TPA stimulated Jurkat cells is included (lane 4). Arrows denote specific inducible complex competed by double-stranded κB oligonucleotide. *b* and *c*, Reverse transcriptase (RT) activity (*b*) was determined[17], and p24 antigen levels (*c*) quantitated (Coulter Immunology) in supernatants from U1 cells incubated in medium alone (□) or in the presence of 2 nM PDB (▲), 20 nM PDB (○) or 1,000 U ml^{-1} TNF-α (×) for 48 h, followed by washing, and incubation for an additional 24 or 48 h. Reverse transcriptase values for these groups before the 48 h wash were 101, 379, 526, and 250 c.p.m. respectively.

mononuclear cells. Because hematopoietic stem cells can be infected by HIV[27,28], we suggest that HIV might be induced in latently infected promonocytic stem cells by NF-κB activated during monocyte differentiation, and additionally by signals such as GM-CSF, which may act through an independent pathway. □

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Transfer of a β-turn structure to a new protein context

Thomas R. Hynes*‡, Roger A. Kautz*§, Margaret A. Goodman*‡, John F. Gill*†, & Robert O. Fox*†‖

* Department of Molecular Biophysics and Biochemistry, and † The Howard Hughes Medical Institute, Yale University, 260 Whitney Ave., New Haven, Connecticut 06511, USA
‡ Department of Cell Biology and § The Biophysics Program, Stanford University Medical School, Stanford, California 94305, USA
‖ To whom correspondence should be addressed

FOUR-RESIDUE β-turns and larger loop structures represent a significant fraction of globular protein surfaces and play an important role in determining the conformation and specificity of enzyme active sites and antibody-combining sites[1,2]. Turns are an attractive starting point to develop protein design methods, as they involve a small number of consecutive residues, adopt a limited number of defined conformations and are minimally constrained by packing interactions with the remainder of the protein. The ability to substitute one β-turn geometry for another will extend protein engineering beyond the redecoration of fixed backbone conformations to include local restructuring and the repositioning of surface side chains. To determine the feasibility and to examine the effect of such a structural modification on the fold and thermodynamic stability of a globular protein, we have substituted a five-residue turn sequence from concanavalin A for a type I′ β-turn in staphylococcal nuclease. The resulting hybrid protein is folded and has full nuclease enzymatic activity but reduced thermodynamic stability. The crystal structure of the hybrid protein reveals that the guest turn sequence retains the conformation of the parent concanavalin A structure when substituted in the nuclease host.

An examination of turn sites in structurally related proteins illustrates that considerable variation is possible in sequence length and backbone conformation[3]. An analysis of protein structures has also identified correlations between β-turn sequence and conformation[4,5], although the local sequence alone does not uniquely determine the fold of short protein

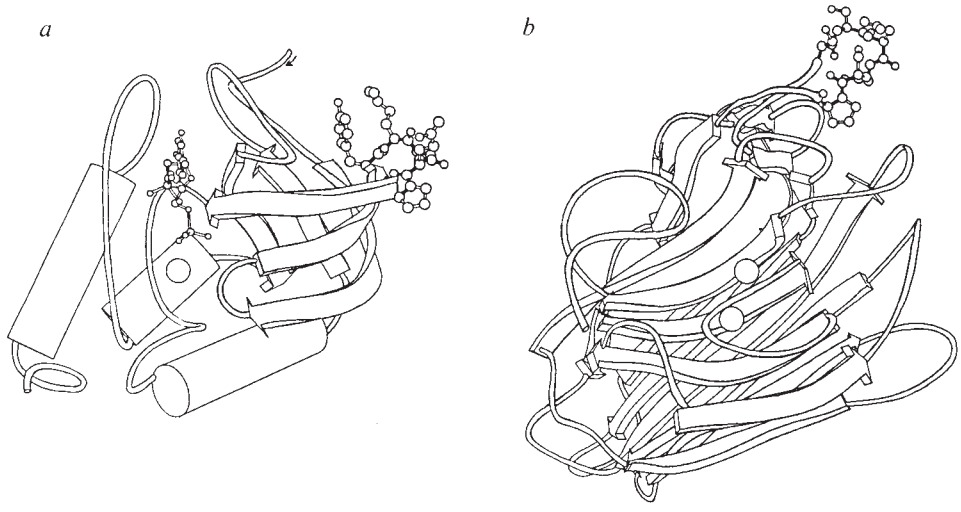

FIG. 1 *a*, The overall fold of nuclease represented as cylinders (α-helices) and arrows (β-strands). The β-turn region, residues 27–31 (Tyr-Lys-Gly-Gln-Pro), is shown in atomic detail in the upper right. A nuclease structure refined in our laboratory as well as a refinement of the Ca^{2+} pdTp complex of nuclease[22] show turn residues 27–30 in a type I′ β-turn conformation differing from the 2SNS nuclease structure[23,24] in the Brookhaven Protein Data Bank[25]. The active site is indicated by bound pdTp nucleotide (small atoms) and Ca^{2+} (large circle). *b*, The overall fold of a concanavalin A monomer unit with arrows representing the β-strands. Turn residues 160–165 (Ser-Ser-Asn-Gly-Ser-Pro) are shown in atomic detail. Residues 160–163 form a type I β-turn. Two structures of concanavalin A have been determined at high resolution[11,12] (2CNA,3CNA). In both cases weak electron density was observed over turn residues 160–165, leading to slightly different models. The primary discrepancy between the two is the orientation of the peptide unit adjacent to the β-turn joining Gly 163 and Ser 164. The bound Ca^{2+} and Mn^{2+} ions are represented as large circles. Both *a* and *b* were drawn with the aid of the program ARPLOT[26].

METHODS. The nuclease gene was subcloned into M13mp18. Two rounds of primer-directed mutagenesis introduced *Hin*dIII and *Bgl*I sites flanking the β-turn sequence at codons 27–31 and removing a *Hin*dIII site within the gene (J.F.G. unpublished data). An oligonucleotide encoding the concanavalin A β-turn sequence was synthesized and subcloned into the vector described above. The hybrid gene was subcloned into the pAS1 expression construct and protein prepared as described[27].