spleen, and the Thy1.2$^+$, CD4$^+$, and CD8$^+$ subpopulations of splenocytes were not reduced at day 7 by the MAb treatment. However, flow cytometry (indirect immunofluorescence) analysis of LFA-1 and ICAM-1 expression on splenocytes of allografted mice indicated that the MAb treatment led to significant reduction of LFA-1– and ICAM-1–positive cells at day 7 after transplantation (Fig. 3). This down-regulation of the antigens may account for the inability to detect alloreactive CTL activity at this time (Table 2) and could be responsible for the induction of tolerance against alloantigens. However, the expression of LFA-1 and ICAM-1 was normal 40 and 75 days after transplantation, although alloreactive CTL activity was still not detected. Thus, the unresponsiveness is probably maintained by some mechanism other than down-modulation of LFA-1 and ICAM-1 molecules on allo responding cells.

The combination of both anti–ICAM-1 and antibody to CD11a (anti-CD11a) MAbs was required to induce tolerance. Each MAb used in this study alone can inhibit in vitro cell-mediated cytotoxicity (*10, 13*). However, our in vivo results showed that each MAb has only a modest effect on the prolongation of graft survival. Persistent acceptance of the grafts was achieved only by simultaneous administration of these two MAbs. Although further investigations are needed, the redundancy of adhesion pairs may partly account for this synergism. LFA-1 has at least three ligands, ICAM-1, ICAM-2 (*18*), and a third, unknown ligand (*19*). In addition, ICAM-1 has other counter-receptors, Mac-1 (*20*) and CD43 (*21*). Mac-1 and LFA-1 bind to discrete domains on ICAM-1 (*20*). However, the involvement of these adhesion molecules in allograft rejection and tolerance induction remains to be determined. Whatever the mechanism, the ICAM-1 and LFA-1 interaction is important in the pathogenesis of allograft rejection. This mode of immunosuppression could perhaps be applied to individuals undergoing organ transplantation.

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Isolation of Two Genes That Encode Subunits of the Yeast Transcription Factor IIA

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The yeast transcription factor IIA (TFIIA), a component of the basal transcription machinery of RNA polymerase II and implicated in vitro in regulation of basal transcription, is composed of two subunits of 32 and 13.5 kilodaltons. The genes that encode these subunits, termed *TOA1* and *TOA2*, respectively, were cloned. Neither gene shares obvious sequence similarity with the other or with any other previously identified genes. The recombinant factor bound to a TATA binding protein–DNA complex and complemented yeast and mammalian in vitro transcription systems depleted of TFIIA. Both the *TOA1* and *TOA2* genes are essential for growth of yeast.

IN ADDITION TO RNA POLYMERASE II (RNAPII), initiation of mammalian mRNA synthesis requires at least eight general transcription initiation factors, termed TFIIA, B, D, E, F, G, H, and J. These general factors are required for transcription from a core promoter containing a TATA element and a transcription initiation site (*1, 2*). The mechanism by which the general factor TFIIA acts is not well understood. Although some fractionated transcription systems lack a TFIIA requirement (*3*), the most highly purified human systems have a strong TFIIA dependence (*2, 4*). In a fractionated yeast transcription system, yeast TFIIA (yTFIIA) is required for high levels of transcription promoted by an acidic transcriptional activator (*5*). Several results suggest that TFIIA may perform an important regulatory function by blocking the action of inhibitors of transcription (*2, 4, 6*). While not itself a DNA binding protein, TFIIA tightly binds to a TATA binding protein (TBP)–DNA complex (*5, 7, 8*). Below, we refer to the TATA binding protein as TBP and refer to TFIID as the multisubunit transcription factor that contains TBP (*9*).

The reported subunit composition of TFIIA from different sources is variable (*2, 10*). Purified yTFIIA consists of two polypeptides of 32 and 13.5 kD (*5*), which we term TOA1 and TOA2, respectively. Together, these two polypeptides can bind to a TBP-DNA complex and restore transcription to both yeast and mammalian in vitro transcription systems depleted of TFIIA. Neither subunit alone shows any activity in transcription or TBP-DNA binding (*5*). To better understand the function of TFIIA, we have cloned the genes encoding both subunits of the yeast factor.

We purified TFIIA from yeast using a TBP-DNA affinity column (*5*), and sequenced tryptic peptides generated from both the TOA1 and TOA2 subunits (*11*) (Fig. 1). The peptide sequences were used to design degenerate polymerase chain reaction (PCR) primers for amplification of small segments of each gene (*12*). These PCR products were then used as probes in screens of yeast genomic libraries to isolate the entire coding sequences for *TOA1* and *TOA2* (*13*). *TOA1* consisted of an open

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reading frame of 286 amino acids encoding a protein with a predicted molecular size of 32.2 kD that contained the sequences of the two tryptic peptides from TOA1 (Fig. 1A). *TOA2* consisted of an open reading frame of 122 amino acids encoding a protein with a predicted molecular size of 13.5 kD that contained the sequences of the two tryptic peptides from TOA2.

The TOA1 protein has a net negative charge with a predicted isoelectric point of 4.1. Plots of charged residues as well as hydropathy analysis revealed that except for the NH_2-terminus, the protein is largely hydrophilic and has two regions of concentrated acidic residues (greater than 57%) between positions 169 to 188 and 221 to 241. TOA2 has a predicted isoelectric point of 4.8. Hydropathy analysis and a plot of charged residues showed several alternating hydrophilic and hydrophobic regions with positive and negative charges distributed throughout the polypeptide. Comparison of the amino acid sequence of TOA1 and TOA2 with the available protein and nucleic acid databases revealed no obvious similarity to other known sequences. Further, TOA1 and TOA2 had no similarity with each other and contained no repeated sequences.

To test whether *TOA1* and *TOA2* together encoded TFIIA activity, each gene was cloned downstream of the T7 RNA polymerase promoter. The two genes were separately transcribed, and the resulting two mRNAs were translated both individually and simultaneously in a rabbit reticulocyte lysate system (*12*). Translation of *TOA1* and *TOA2* generated polypeptides with apparent molecular sizes of 43 and 13 kD as measured by SDS polyacrylamide gel electrophoresis (SDS-PAGE). The aberrant mobility of the TOA1 32-kD polypeptide was also seen with the native subunit purified from yeast (*5*). The translated proteins were tested in an electrophoretic mobility shift assay for the ability to bind to a TBP-DNA complex. When individually translated TOA1 or TOA2 was incubated with purified recombinant TBP and DNA, no alteration in the mobility of the TBP-DNA complex was observed (Fig. 2). The weak background bands were a result of proteins in the reticulocyte lysate (*14*). However, cotranslated TOA1 and TOA2 proteins bound to TBP-DNA, forming a complex with a mobility identical to that obtained when purified yTFIIA is used in the assay. Cotranslated TOA1 and TOA2 did not bind to the TATA-containing DNA fragment in the absence of TBP (*14*).

To test whether recombinant TFIIA could function in transcription, we needed a more concentrated source of protein than could be obtained from in vitro translation (*5*). The TOA1 and TOA2 subunits were expressed separately in *Escherichia coli*, denatured, renatured together, and purified (*15*). The specific activity of recombinant TFIIA, as measured by electrophoretic mobility shift assay was the same as that of native TFIIA. Neither subunit expressed individually in *E. coli* showed any activity in this assay.

Native TFIIA purified from yeast complements both yeast and mammalian in vitro transcription systems depleted of TFIIA (*5, 8*). To test for complementation with the recombinant proteins, we used a fractionated yeast system that had been separated by chromatography into five fractions, all of which were required to restore maximum levels of activated transcription (*16*) (Fig. 3A). We used this system to test whether recombinant TFIIA could promote transcription from a plasmid that contained binding sites for the yeast transcriptional activator protein GAL4 and the yeast *CYC1* TATA and promoter initiation elements (*17*). Basal transcription is nearly undetectable in our system, but is greatly induced when the GAL4-VP16 activator fusion protein is added (*5*). Transcription from the *GAL-CYC1* promoter produces a heterogeneous series of transcription start sites that are identical to those seen in vivo (*18*) (Fig. 3B). Transcription was reduced when the TFIIA-containing fraction (III) was omitted

A 1 MSNAEASRVY EIIVESVVNE VREDFENAGI DEQTLQDLKN
41 IWQKKLTETK VTTFSWDNQF NEGNINGVQN DLNFNLATPG
81 VNSSEFNIKE ENTGNEGLIL PNINSNNNIP HSGETNINTN
121 TVEATNNSGA TLNTNTSGNT NADVTSQPKI EVKPEIELTI
161 NNANITTVEN IDDESEKKDD EEKEEDVEKT RKEKEQIEQV
201 KLQAKKEKRS ALLDTDEVGS ELDDSDDDYL ISEGEEDGPD
241 ENLMLCLYDK VTRTKARWKC SLKDGVVTIN RNDYTFQKAQ
281 VEAEMV

B 1 MAVPGYYELY RRSTIGNSLV DALDTLISDG RIEASLAMRV
41 LETFDKVVAE TLKDNTQSKL TVKGNLDTYG FCDDVWTFIV
81 KNCQVTVEDS HRDASQNGSG DSQSVISVDK LRIVACNSKK
121 SE

Fig. 1. Sequence of TOA1 and TOA2. (**A**) Predicted amino acid sequence of the *TOA1* gene. (**B**) Predicted amino acid sequence of the *TOA2* gene. Underlined sequences represent the four sequenced tryptic peptides. The DNA sequence of both genes was determined on both strands. GenBank accession numbers are M85248 for TOA1 and M85249 for TOA2. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

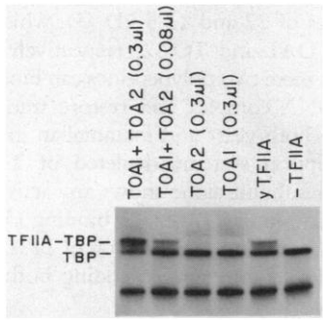

Fig. 2. Binding of TOA1 and TOA2 to a TBP-DNA complex in an electrophoretic mobility shift assay. *TOA1* and *TOA2* were translated with unlabeled methionine in reticulocyte lysates and portions of the lysate were added to binding reactions that contained TBP (yeast TFIID) and a ^{32}P-labeled 112-bp fragment from the adenovirus major late promoter that contained the TATA element (*5*). Reactions contained either no TFIIA, TFIIA purified from yeast (yTFIIA), or the products of in vitro translation reactions (volume added indicated in microliters). The positions of the TBP-DNA and TFIIA-TBP-DNA complexes are indicated.

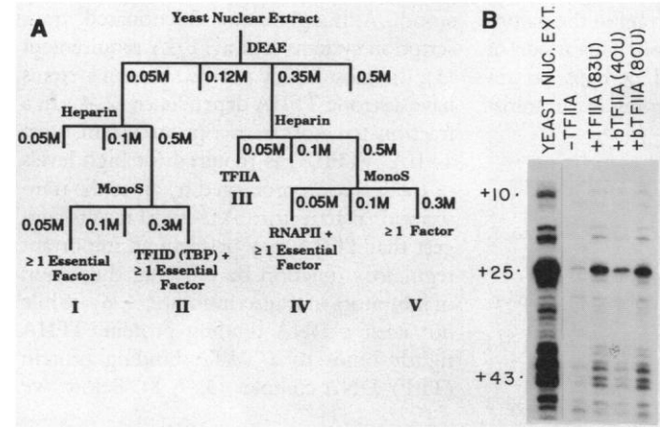

Fig. 3. Complementation of a fractionated yeast in vitro transcription system by recombinant TFIIA. (**A**) Fractionation scheme for yeast nuclear extract. The ammonium sulfate concentrations used in elution of columns are indicated (*16*). Initial steps in the fractionation were as described (*5*). (**B**) Primer extension analysis of RNA produced in a yeast in vitro transcription system. All reactions except the lane labeled yeast nuclear extract contained fractions I, II, IV, and V along with the indicated additions. Lane labeled TFIIA has fraction III and lane labeled bTFIIA has recombinant yTFIIA expressed in bacteria. Units of TFIIA normalized by electrophoretic mobility shift assay (*5, 27*) are indicated. In vitro transcription reactions were performed as described (*5*) except that reactions contained polyvinyl alcohol at 2.4%.

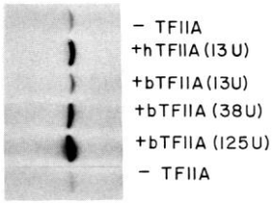

Fig. 4. Complementation of a mammalian transcription system depleted of TFIIA by recombinant TFIIA. All reactions contained recombinant human TBP (*20*), fraction CB (*21*), and calf thymus RNAPII, plus addition of TFIIA as indicated. hTFIIA is fraction AB (*21*) and bTFIIA is recombinant yTFIIA. Units of TFIIA normalized by electrophoretic mobility shift assay are indicated. In vitro transcription was performed as described (*5*).

and was restored by adding an equivalent amount of recombinant TFIIA as normalized by electrophoretic mobility shift assay (Fig. 3B) (*19*).

We next tested whether recombinant TFIIA could function in a mammalian transcription system. To test for transcription from the minimal adenovirus major late promoter, we used a TFIIA-depleted system (*5*) that contained calf thymus RNAPII, bacterially expressed recombinant human TBP (*20*), and fraction CB (*21*), which contains all the remaining general transcription factors. Basal transcription was stimulated five- to tenfold by human TFIIA (Fig. 4). Addition of recombinant yTFIIA to this system stimulated transcription to the same extent as did human TFIIA, although about three times as much yeast TFIIA was required as compared to the human factor. The reason for this discrepancy is not yet clear, but it is also observed with the native yeast factor. The above results indicate that the recombinant TOA1 and TOA2 gene products can substitute for native yeast and human TFIIA.

Large regions of the *TOA1* and *TOA2* coding sequence were separately deleted and replaced by the yeast *HIS4* gene (*22*). These two constructs were used to disrupt one copy of *TOA1* or *TOA2* in diploid yeast. Upon sporulation of the *TOA1*- or *TOA2*-disrupted strains, no HIS^+ spores were recovered (0 out of 15 for *TOA1::HIS4* and 0 out of 14 for *TOA2::HIS4*), suggesting that the genes are essential for growth. Second, a plasmid-loss assay was used to prove that *TOA1* and *TOA2* were necessary for growth of yeast and not merely for spore germination. Haploid strains were constructed that contained a disruption of either *TOA1* or *TOA2* on the chromosome and a wild-type copy of the disrupted gene on a *URA3*-marked plasmid. If the *TOA* genes are essential, then no survivors should be recovered when the cells are grown on 5-fluoroorotic acid plates, which selects for cells that have lost the *URA3*-marked plasmid. Yeast cells could not tolerate the loss of either *TOA1* or *TOA2* unless the strain also contained a second copy of the gene on a separate *LEU2*-marked plasmid. This proves that the *TOA1* and *TOA2* genes are necessary for growth of yeast.

The question of whether TFIIA is an essential transcription factor has been controversial, as several fractionated systems apparently lack a TFIIA requirement. One possibility is that a major function of TFIIA is to interfere with the function of an inhibitor and that in such systems this negative factor has been depleted. Such a mechanism has been proposed to explain dependence on TFIIA for in vitro transcription (*2, 6*). In any case, the fact that the *TOA* genes are essential for viability of yeast suggests that TFIIA is likely to be required for transcription in vivo of at least some if not all genes transcribed by RNAPII. Further genetic and biochemical experiments with the cloned *TOA* genes will provide a better understanding of the function of TFIIA in transcription.

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14. J. A. Ranish and S. Hahn, unpublished data.
15. TOA1 and TOA2 expressed separately in *E. coli* under control of the T7 promoter were insoluble and were purified from an insoluble fraction of sonicated bacteria by solubilization in urea, followed by renaturation of the TOA1 and TOA2 subunits together (*25*). The renatured TFIIA was purified to >95% purity by Q Sepharose and gel filtration chromatography. The recombinant protein had a native molecular size of ~100 kD as measured by gel filtration.
16. Fractions I and V contain at least one factor each with as yet unknown functions. Fraction I is essential whereas fraction V stimulates transcription in our assay up to about fivefold. Fraction II contains TFIID (yeast TBP) plus at least one other essential factor, as purified yeast TBP could not substitute for this fraction and further chromatography of fraction II showed it to be composed of at least one component besides TBP (*14*). Fraction III contains TFIIA, and fraction IV contains RNAPII and probably at least one other factor, as the crude 0.05 M DEAE flow-through fraction, which also contained RNAPII, could not eliminate the requirement for fraction IV when substituted for fractions I and II. Fractions were assayed for TBP, RNAPII, and TFIIA by immunoblot analysis. It is not yet clear how some of the components in our fractionated yeast transcription system relate to the mammalian general factors. As described (*5*), our fractionation scheme does not appear consistent with another published yeast in vitro system measuring basal transcription (*26*). In addition, fractions I to V were all heat labile.
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19. Recombinant TFIIA was also tested in the less purified yeast system described by Ranish and Hahn (*5*). This system used the 0.05 M DEAE flow-through fraction (Fig. 3A) and the 0.1 to 0.5 M heparin fraction derived from the DEAE 0.12 to 0.35 M fraction. In this system, the recombinant factor substituted for fraction III (TFIIA), but did so less well than fraction III when normalized by activity in an electrophoretic mobility shift assay. The reason for the reduced activity is under investigation, but one possibility is that the activity of TFIIA may be altered by modifications in yeast or *E. coli*. Another possibility is that fraction III contains an inhibitor of a negative component that has been removed by fractionation in the more purified system. In contrast, in the more purified system, the recombinant factor works as well in transcription as does TFIIA purified from yeast.
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27. One unit of TFIIA activity was defined as the amount necessary to mobility shift one-half of the TBP-DNA complex to the TFIIA-TBP-DNA complex under the assay conditions described (*5*).

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