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24. We have generated several other plasmid constructions that may be useful to investigators. These include a pBluescript II KS (+) derivative (TU#65) containing a Kpn I–Eco RI fragment encoding GFP with an Age I site 5′ to the translation start and a Bsm I site at the termination codon. Also available are *gfp* versions (TU#60 to TU#63) of the four *C. elegans lacZ* expression vectors (pPD16.43, pPD21.28, pPD22.04, and pPD22.11, respectively) as described (*27*) except that they lack the Kpn I fragment containing the SV40 nuclear localization signal.
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RNA Polymerase II Initiation Factor Interactions and Transcription Start Site Selection

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An RNA polymerase II transcription system was resolved and reconstituted from extracts of *Schizosaccharomyces pombe*. Exchange with components of a *Saccharomyces cerevisiae* system was undertaken to reveal the factor or factors responsible for the difference in location of the transcription start site, about 30 base pairs and 40 to 120 base pairs downstream of the TATA box in *S. pombe* and *S. cerevisiae*, respectively. Two components, counterparts of human transcription factor IIF (TFIIF) and TFIIH, could be exchanged individually between systems without effect on the start site. Three components, counterparts of human TFIIB, TFIIE, and RNA polymerase II, could not be exchanged individually but could be swapped in the pairs TFIIE-TFIIH and TFIIB–RNA polymerase II, which demonstrates that there are functional interactions between these components. Moreover, exchange of the latter pair shifted the starting position, which shows that TFIIB and RNA polymerase II are solely responsible for determining the start site of transcription.

Synthesis of mRNA in eukaryotes requires RNA polymerase II and accessory factors, some which are general and act at most, if not all, promoters, and others of which confer specificity and control. Five general factors—a, b, d, e, and g—have been purified to homogeneity from the budding yeast *Saccharomyces cerevisiae* and have been identified as counterparts of human-rat factors TFIIE-ε, TFIIH-δ, TFIID-τ, TFIIB-α, and TFIIF-βγ, respectively (*1–8*). Because these factors assemble at a promoter in a complex with RNA polymerase II, interactions among them are assumed to be important for the initiation of transcription.

Most studies of general factor interactions have focused on binding (*8*). The results have shown that the order of assembly of the initiation complex on promoter DNA begins with factor d (TFIID), is followed by factor e (TFIIB), and then by polymerase and the remaining factors (6, 9). Factors b (TFIIH), e, and g (TFIIF), however, bind directly to polymerase II, and as many as four of the five factors may assemble with the polymerase in a holoenzyme (*10*) before promoter binding. There are a couple of limitations implicit in these findings: The functional significance of interactions revealed by binding is questionable because only a few percent of initiation complexes give rise to transcripts, and there is little indication of the roles of the various interactions in the initiation process.

We have used a functional approach to analyze general transcription factor interactions on the basis of the ability of factors to be exchanged between transcription systems. Exchange between *S. cerevisiae* and mammalian systems is of interest because of a marked difference in location of the transcription start site, 40 to 120 base pairs downstream of the TATA box in the former versus about 30 base pairs in the latter (*11*). The TATA-binding component (TBP) of factor d (TFIID) is functionally interchangeable between *S. cerevisiae* and humans (*4, 12, 13*), but the transcription start site remains characteristic of the particular transcription system, irrespective of the source of TBP. The factor or factors responsible for start site selection could not be identified by this approach because neither the other factors nor the polymerase proved interchangeable between *S. cerevisiae* and higher eukaryotic systems. We decided to use a *Schizosaccharomyces pombe* system because of its similarity to higher eukaryotes in the location of RNA polymerase II transcription start sites and its closer evolutionary relation to *S. cerevisiae*. Initiation from *S. pombe* promoters occurs about 30 base pairs downstream of the TATA box, and initiation from mammalian promoters introduced in *S. pombe* occurs at the same sites as in mammalian cells (*14*).

We have described the derivation of a chromatographic fraction from *S. pombe* that, upon addition of TBP, will support promoter-dependent RNA polymerase II transcription

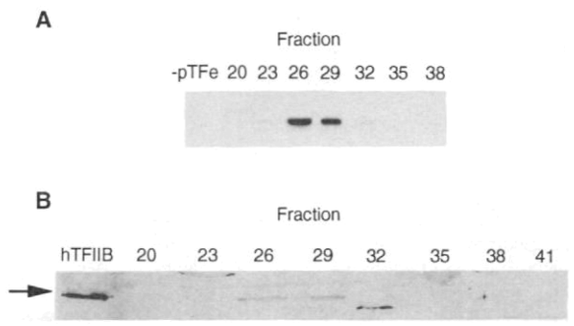

Fig. 1. Factor e of *S. pombe* copurifies with a 35-kD polypeptide cross-reactive with human TFIIB antiserum. (**A**) Assay of fractions (2 μl) from HAP (*16*) for pTFe activity. Assays were performed with the complete *S. pombe* system (*16*), except for the omission (first lane) of pTFe. (**B**) Immunoblot analysis of fractions (40 μl) from HAP. Trichloroacetic acid precipitation, 12% SDS–polyacrylamide gel electrophoresis, and blotting onto nitrocellulose were followed by successive incubations with polyclonal human TFIIB antiserum (1:300) for 18 hours at 4°C and with goat monoclonal antibody to rabbit (1:2000) for 1 hour at 24°C as described (*26*). The 35-kD polypeptide (indicated by arrow) was the only cross-reactive species seen when smaller amounts of protein were loaded. Lane 1 contained 10 ng of human TFIIB.

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(*15*). Here we resolve a set of general transcription factors and RNA polymerase from this *S. pombe* fraction by procedures similar to those used for resolution of the *S. cerevisiae* system. A whole cell extract of *S. pombe* was chromatographed on Bio-Rex 70 and DE52 (*16*). Although the 0.4 M potassium acetate eluate from DE52 required only TBP for transcription (*15*), the majority (about 90%) of another essential factor could be recovered from the DE52 flow-through fraction by further chromatography on hydroxyapatite (HAP). This factor was identified as the *S. pombe* counterpart of factor e (TFIIB) and was designated pTFe on the basis of two criteria: (i) The factor could be substituted for its counterpart in the *S. cerevisiae* system and (ii) activity of the factor in the *S. pombe* system co-chromatographed on HAP with a polypeptide cross-reactive with TFIIB antibodies (Fig. 1). Similar results were obtained with antibodies to *S. cerevisiae* factor e and human TFIIB, although each antibody preparation revealed different contaminants. The apparent mass of the cross-reacting polypeptide in *S. pombe*, 35 kD, was comparable to that of *S. cerevisiae* factor e and TFIIB (38 and 35 kD, respectively) (*2*, *3*, *17*).

The *S. pombe* 0.4 M potassium acetate eluate from DE52 was further chromatographed (*16*) to yield fractions identified as pTFa, pTFb, pTFg, and RNA polymerase II on the basis of substitution in the *S. cerevisiae* system. Transcription could be reconstituted with the complete set of *S. pombe* fractions from both the *S. pombe ADH1* and adenoviral major late promoters. Although the fractions were not homogeneous, transcription was abolished by α-amanitin (10 μg/ml) and was absolutely dependent on TBP, which demonstrated that it was entirely the result of RNA polymerase II activity. The fractions were well resolved from one another: Omission of pTFa, pTFb, pTFe, pTFg, or RNA polymerase II reduced transcription to 10, 5, 0, 20, and 0%, respectively, of the level attained with the complete system.

Three highly purified *S. cerevisiae* factors could be substituted for fractions in the *S. pombe* system (Fig. 2A and Table 1): Factor b (TFIIH) could fully replace pTFb; *S. cerevisiae* TBP was fully active in place of *S. pombe* TBP, as reported (*15*); and factor g (TFIIF) could substitute for pTFg to a limited extent. These results identify pTFb and pTFg with their counterparts in *S. cerevisiae*.

No *S. cerevisiae* components could substitute individually for pTFa, pTFe, or *S. pombe* RNA polymerase II, but combinations of the components were effective (Fig. 2B and Table 1): *S. cerevisiae* factors a (TFIIE) and b could partially replace pTFb and a second component thereby identified as pTFa; and *S. cerevisiae* factors a, b, and g could replace pTFa, pTFb, and pTFg. Whereas the pair of factors a and b was much less effective than factor b alone, the triple combination of factors a, b, and g was similar in activity to the a, b pair or to g alone (Table 1). These results suggest that factors a and b interact, but that factor g functions independently.

The reciprocal substitutions of *S. pombe* fractions into the *S. cerevisiae* transcription system were less effective (Table 2): pTFb supported a low level of transcription in place of *S. cerevisiae* factor b (Fig. 2C), and pTFg failed to substitute for factor g. This low level of activity of the *S. pombe* fractions in the *S. cerevisiae* system could be the result of the lesser purity and concentration of the *S. pombe* components (*18*), or it might reflect a lack of reciprocity in protein-protein interactions (*19*).

Factor e and *S. cerevisiae* polymerase II failed to substitute in the *S. pombe* system, nor were pTFe and the *S. pombe* polymerase individually able to replace any component of the *S. cerevisiae* system. Together, pTFe and the *S. pombe* polymerase were active in the *S. cerevisiae* system (Fig. 3), and the transcription start site was switched to that characteristic of *S. pombe*. Transcription was absolutely dependent on the *S. cerevisiae* factors (a, b, and g), which shows that the pTFe and *S. pombe* polymerase fractions used did not contribute *S. pombe* counterparts of any of these factors. A role of the *S. cerevisiae* factors in start site selection was ruled out by the finding that substitution of factors a, b, and g in the *S. pombe* system did not switch the initiation site (Fig. 2). We conclude that the combination of factor e and RNA polymerase II alone determines the location of the transcription start site.

The functional interaction between *S. cerevisiae* factors a and b demonstrated here may underlie the stimulatory effect of TFIIE on the COOH-terminal domain kinase activity of TFIIH recently observed in several laboratories (*20*). Although *S. cerevisiae* factor a requires a species-specific interaction with factor b for function in *S. pombe*, factor b and pTFb exhibit no such requirement (*21*). This lack of reciprocity provides a basis for assessing the significance of the stimulatory activity of TFIIE: A stimulatory effect of pTFa on *S. cerevisiae* factor b kinase, but lack

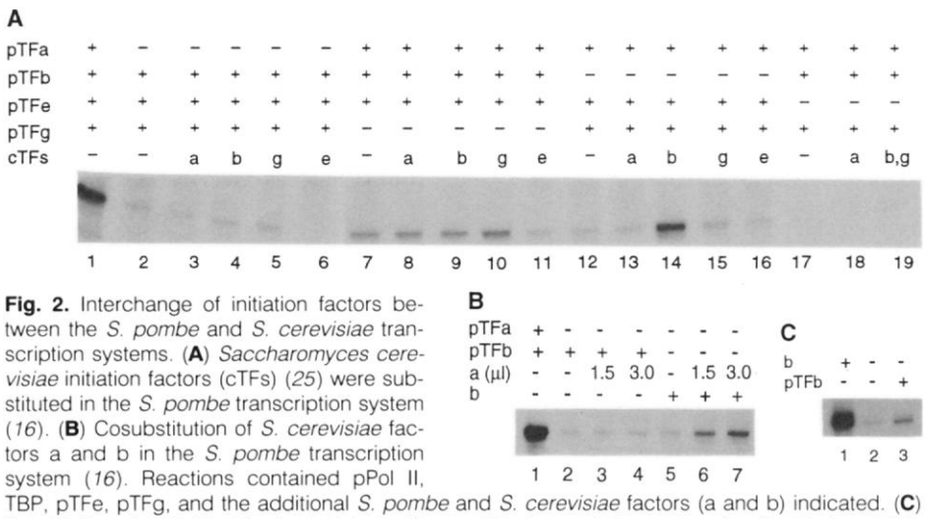

Fig. 2. Interchange of initiation factors between the *S. pombe* and *S. cerevisiae* transcription systems. (**A**) *Saccharomyces cerevisiae* initiation factors (cTFs) (*25*) were substituted in the *S. pombe* transcription system (*16*). (**B**) Cosubstitution of *S. cerevisiae* factors a and b in the *S. pombe* transcription system (*16*). Reactions contained pPol II, TBP, pTFe, pTFg, and the additional *S. pombe* and *S. cerevisiae* factors (a and b) indicated. (**C**) Substitution of pTFb (2 μl) for factor b in the *S. cerevisiae* transcription system [purified factor a (*27*), factor b (*7*), recombinant TBP and factor e, and factor f (*25*)].

Table 1. Substitution of *S. cerevisiae* initiation factors (cTFs) for *S. pombe* factors (pTFs) in the *S. pombe* transcription system. Transcription reactions (*16*) were performed with the set of *S. pombe* components, with substitutions by *S. cerevisiae* components (*25*) (+) or with no substitution (−).

cTFs	*S. pombe* factor					Activity (%)
	e	b	g	a	pPol II	
None	−	−	−	−	−	100
e	+	−	−	−	−	0
a	+	−	−	−	−	0
b, g	+	−	−	−	−	0
a	−	+	−	−	−	0
b	−	+	−	−	−	90
e	−	+	−	−	−	0
g	−	+	−	−	−	0
a	−	−	+	−	−	0
b	−	−	+	−	−	0
e	−	−	+	−	−	0
g	−	−	+	−	−	15
a	−	−	−	+	−	0
b	−	−	−	+	−	0
e	−	−	−	+	−	0
g	−	−	−	+	−	0
Pol II	−	−	−	−	+	0
b	−	+	−	+	−	0
b, a	−	+	−	+	−	14
b, g	−	+	−	+	−	0
b	−	+	+	−	−	0
b, a	−	+	+	−	−	0
b, g	−	+	+	−	−	0
a, g	−	−	+	+	−	0
a, b, g	−	+	+	+	−	20

of effect of *S. cerevisiae* factor a on pTFb kinase, would indicate a correlation between the stimulatory activity and the essential factor a–factor b interaction, suggesting the former is also essential for transcription.

The capacity of pTFe (TFIIB) and *S. pombe* RNA polymerase II to support transcription in the *S. cerevisiae* system only in concert with one another is indicative of a functional interaction between these two components as well. The interaction may be involved in transcription start site selection because the components involved determine the location of the site. The role of factor e (pTFe/TFIIB) in start site selection might be limited to this interaction: The factor could serve merely to recruit RNA polymerase to a TBP-promoter complex, as previously suggested (*6*), leaving the polymerase responsible for all details of the initiation process. Alternatively, factor e might play a more active role in initiation: For example, the factor could facilitate the translocation of DNA duplex melting, nucleated by TBP at the TATA element (*22*), to RNA polymerase at the start site of transcription. A direct role of factor e in start site selection seems most likely because mutations in the factor e gene, termed *SUA7* (*2*), have been shown to perturb the locations of transcription start sites in *S. cerevisiae* (*23*). Mutations in several *SPT* genes have similar effects (*23, 24*), and whereas the products of these genes are absent from a purified *S. cerevisiae* RNA polymerase II transcription system [except for SPT 15 (*25*)], they may influence initiation in vivo.

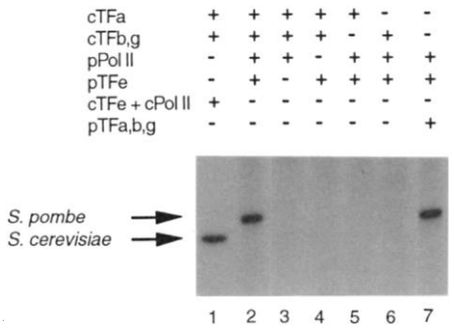

Fig. 3. Factor e (TFIIB) and RNA polymerase II are solely responsible for determining the location of the transcription start site. Reactions contained *S. cerevisiae* initiation factors a (*27*), b, g (DEAE-5-PW fraction) (*25*), TBP, e (*3*), and RNA polymerase II (cPol II) (*28*) and *S. pombe* initiation factors a, b, and g (pTFa, b, g), e (pTFe), and RNA polymerase II (pPol II), as indicated. Reactions were otherwise as described (*16*). Part (5%) of a complete *S. cerevisiae* reaction was analyzed in lane 1; part (20%) of a complete *S. pombe* reaction was analyzed in lane 7. The locations of bands attributable to transcripts from *S. cerevisiae* and *S. pombe* start sites are indicated.

Table 2. Substitution of *S. pombe* initiation factors (pTFs) for *S. cerevisiae* factors (cTFs) in the *S. cerevisiae* transcription system. Transcription reactions were performed with the set of *S. cerevisiae* components (*25*), with substitutions by *S. pombe* components (*16*) where indicated (+) or with no substitution (−).

pTFs	*S. cerevisiae* factor					Activity (%)
	a	b	e	g	cPol II	
None	−	−	−	−	−	100
pTFa	+	−	−	−	−	0
pTFb	+	−	−	−	−	0
pTFe	+	−	−	−	−	0
pTFg	+	−	−	−	−	0
pTFa	−	+	−	−	−	0
pTFb	−	+	−	−	−	5
pTFe	−	+	−	−	−	0
pTFg	−	+	−	−	−	0
pTFe	−	−	+	−	−	0
pTFa	−	−	−	+	−	0
pTFb	−	−	−	+	−	0
pTFe	−	−	−	+	−	0
pTFg	−	−	−	+	−	0
pPol II	−	−	−	−	+	0
pTFa, b	+	+	−	−	−	0
pTFe + pPol II	−	−	+	−	+	5
pTFg + pPol II	−	−	−	+	+	0
pTFa, b, g	−	+	+	+	−	0

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16. Whole cell extract from *S. pombe* strain YNN36 *leu1-32 ura4-294 ade6-216* was fractionated as described for *S. cerevisiae* (*25*), except that DE52 (Whatman) was used in place of DEAE-Sephacel. The DE52 0.1 M potassium acetate (flow-through) fraction was resolved on HAP as described (*25*) (except without 0.1 M potassium acetate in the buffer) to yield pTFe. The DE52 0.4 M potassium acetate eluate was fractionated on HAP as described (*25*), yielding *S. pombe* RNA polymerase II (pPol II) (peak at 40 mM potassium phosphate) and a pool of initiation factors (eluting between 0.08 and 0.14 M potassium phosphate) that were sufficient when combined with pPol II and TBP for promoter-dependent transcription. The pool of initiation factors from HAP was absorbed to Q Sepharose Fast Flow (Pharmacia), eluted with buffer B (*25*) containing 1.5 M potassium acetate, and further fractionated on a DEAE-5-PW high-performance liquid chromatography column developed with a linear gradient of 0.3 to 1 M potassium acetate in buffer B, yielding pTFb, pTFg, and pTFa (peaks at 0.36, 0.5, and 0.676 M potassium acetate, respectively). Transcription assays were performed with a plasmid template (pMLCG-; 200 ng), containing the adenovirus major late promoter, in 37 mM Hepes (pH 7.5), 110 mM potassium acetate, 0.7 mM adenosine triphosphate, 0.7 mM cytidine triphosphate, 5 μM uridine triphosphate (UTP), 0.5 mCi/ml of [α-^{32}P]UTP (400 Ci/mmol), 2.5 mM dithiothreitol, 5 mM magnesium acetate, 2.5 mM magnesium sulfate, 3.7 mM EGTA, and 100 ng of recombinant *S. cerevisiae* TBP (*12*). The *S. pombe* system used in these assays was composed of pPol II (2 μl), pTFa (1.5 μl), pTFb (1.5 μl), pTFe (2 μl), and pTFg (1.5 μl). Similar results were obtained with the *S. pombe ADH1* promoter (*25*).
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18. Transcription proteins from *S. pombe* were from a smaller culture (15 to 20 liters) and so were less concentrated than those from *S. cerevisiae* (60-liter culture). The complete *S. pombe* system gave 20% the level of transcription obtained with the complete *S. cerevisiae* system.
19. Lack of reciprocity in cross-species protein-protein interactions is not unexpected. For example, if species A protein a (Aa) has a surface bulge that fits into a surface depression on Ab, but Ba does not, then the Ba-Ab interaction may be allowed, whereas the Aa-Bb interaction is not.
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29. We thank M. Carey for human TFIIB antiserum and our colleagues for provision of *S. cerevisiae* transcription proteins. H.T. was a recipient of a Deutsche Forschungsgemeinschaft fellowship. This research was supported by NIH grant GM36659 to R.K.K.

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