instead of flow as a whole plot factor. There was no significant ($P > 0.05$) time effect or time by treatment interaction for total animals or metamorphosed larvae.

22. Larvae exposed to 3% formalin or 90% ethanol in seawater spread their provisional chaetae and were readily discernable (after staining with rose bengal) from juveniles that had lost their chaetae during metamorphosis.
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Participation of the TATA Factor in Transcription of the Yeast U6 Gene by RNA Polymerase C

FLORENCE MARGOTTIN, GENEVIÈVE DUJARDIN,* MATTHIEU GÉRARD, JEAN-MARC EGLY, JANINE HUET, ANDRÉ SENTENAC

Fractionation of transcription extracts has led to the identification of multiple transcription factors specific for each form of nuclear RNA polymerase. Accurate transcription in vitro of the yeast U6 RNA gene by RNA polymerase C requires at least two factors. One of them was physically and functionally indistinguishable from transcription factor IID (TFIID or BTF1), a pivotal component of polymerase B transcription complexes, which binds to the TATA element. Purified yeast TFIID (yIID) or bacterial extracts that contained recombinant yIID were equally competent to direct specific transcription of the U6 gene by RNA polymerase C. The results suggest the formation of a hybrid transcription machinery, which may imply an evolutionary relation between class B and class C transcription factors.

THE MAJOR CLASSES OF EUKARYOTIC genes that are transcribed by RNA polymerase C (pol C), the tRNA and 5*S* RNA genes, have intragenic control regions (*1*). However, some class C genes are controlled by regulatory elements that lie upstream of the structural gene (*2*) and that are similar to those found in class B genes (*3, 4*). These include the 7SK gene and the U6 gene, the latter of which encodes a small nuclear RNA required for splicing of precursor RNA. That transcription of these genes is carried out by pol C is inferred on the basis of a number of convincing, albeit indirect, criteria, including a characteristic α-amanitin sensitivity and the presence of a typical pol C thymidine (T)–rich termination signal (*2*). The upstream elements of the U6 and 7SK genes include a sequence about 30 bp upstream of the start site of transcription that is similar to the pol B TATA box. This TATA sequence is required for efficient transcription by pol C of *Xenopus*, human and plant U6 RNA genes (*5–7*), and the human 7SK gene (*3*).

The U6 gene from *Saccharomyces cerevisiae* is transcribed by pol C both in vitro and in vivo (*8*). The template used in these previous studies [pTaq6 plasmid DNA (*9*)] harbors the whole SNR6 coding region (−120 to +125) with the T-rich termination site and 120 bp of upstream flanking sequence that contains a consensus TATA element at −30. This template contains the minimum sequence information necessary for directing accurate transcription in a reconstituted in vitro system that contains purified pol C and partially purified transcription factor IIIB (TFIIIB) (*8*). The other class C transcription factors, TFIIIA and Tau (TFIIIC), are not required for transcription with this template (*8*). Size fractionation by gel filtration of a TFIIIB preparation in high salt buffer (*10*) resolved two components that, on their own, did not support U6 RNA synthesis by purified pol C (Fig. 1, lanes 1 to 3). However, U6 transcription could be fully restored when both fractions were combined (lanes 4 and 5), as shown by the production of the full-length U6 RNA transcript [114 nucleotides (nt)]. Therefore, at least two distinct factors, eluted in peak 1 (fractions 55 to 59) and peak 2 (fractions 63 to 68), participated in U6 RNA synthesis (Fig. 1B). A nuclease activity that cleaves the 114-nt transcript and generates a 90-nt RNA species was found to partly overlap with peak 2 on the Superose column (Fig. 1B) (*11*). The larger component in peak 1 (~130 kD) (Fig. 1B) had TFIIIB activity, as monitored by transcription of the $tRNA_3^{glu}$ gene in the presence of Tau and purified pol C (90-nt transcript) (Fig. 1A, lanes 6 and 7). The smaller component (~30 kD) (Fig. 1B, peak 2) did not correspond to any known pol C transcription factor.

A TATA box at position −30 is present in all U6 genes in vertebrates and yeast, and has been shown by mutagenesis to be important for transcription of vertebrate genes (*5, 6*). Therefore, we performed a transcription-competition experiment to explore the possibility that the small component was a U6-specific TATA binding factor. Oligonu-

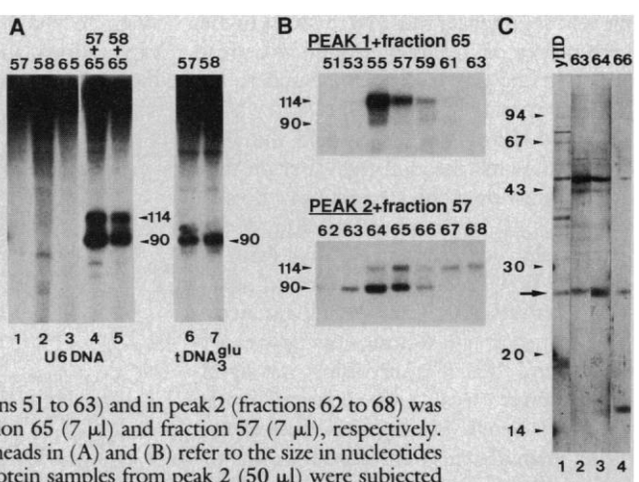

Fig. 1. Two factors required for in vitro transcription of the U6 gene. (**A**) Fractions from the Superose 12 column (*10*) were assayed for transcription activity with the U6 gene (U6 DNA, lanes 1 to 5) or the $tRNA_3^{glu}$ gene (lanes 6 and 7). Transcription mixtures (*10*) received 7-μl aliquots of different column fractions as indicated. Transcripts were analyzed on polyacrylamide-urea gels and revealed by autoradiography. (**B**) Factor activity in peak 1 (fractions 51 to 63) and in peak 2 (fractions 62 to 68) was assayed in the presence of fraction 65 (7 μl) and fraction 57 (7 μl), respectively. The numbers next to the arrowheads in (A) and (B) refer to the size in nucleotides of the RNA transcripts. (**C**) Protein samples from peak 2 (50 μl) were subjected to electrophoresis on a 12.5% SDS-polyacrylamide gel, which was then silver-stained. Lane 1, yIID (G-3000-SW fraction) (*13*); lanes 2 to 4, peak 2 fractions 63, 64, and 66, respectively. The arrow indicates the 27-kD yIID polypeptide. The arrowheads indicate molecular size markers in kilodaltons.

F. Margottin, G. Dujardin, J. Huet, A. Sentenac, Département de Biologie, Service de Biochimie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur-Yvette Cedex, France.
M. Gérard and J.-M. Egly, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'INSERM, 11 rue Humman, 67085 Strasbourg Cedex, France.

*Present address: Centre de Génétique Moléculaire du CNRS, 91191 Gif-sur-Yvette Cedex, France.

cleotides that encompassed the TATA sequence (*9*) (U6-TATA; nucleotide positions −39 to −11) or a proximal sequence that is conserved in mammalian U6 genes (U6-PS; nucleotide positions −68 to −46) were separately incubated with a transcription mixture (legend to Fig. 2) before template addition. Incubation with U6-TATA oligonucleotide inhibited U6 RNA synthesis, whereas the U6-PS oligonucleotide had little effect at the same concentration (*12*). The presence of TATA box binding activity was further evidenced in electrophoretic mobility shift assays with the ^{32}P-labeled U6-TATA oligonucleotide as a probe. A complex of the same electrophoretic mobility was observed when the U6-TATA probe was incubated with peak 2 fractions or purified yeast TFIID factor (*12*). Further experiments indicated that the U6 transcription factor activity in peak 2 copurified with the pol B TATA factor (TFIID or BTF1). When the polypeptide content of peak 2 was analyzed by SDS–polyacrylamide gel electrophoresis (PAGE), a major polypeptide with a mobility of 27 kD was highly enriched in the most active fractions (Fig. 1, B and C; compare fraction 64 to 63 or 66). The two other observed polypeptides (49 and 16 kD) did not coelute with transcription factor activity. The 27-kD polypeptide comigrated on an SDS-polyacrylamide gel with purified yIID (*13*) (Fig. 1C, lane 1). Peak 2 fractions substituted for human TFIID when transcribing from the adenovirus-2 major late promoter (Ad2MLP) in a reconstituted HeLa cell transcription system containing pol B, pol B transcription factors 2 and 3 (BTF2 and BTF3), and stimulating transcription factor (STF) (*14*). A 309-nt run-off transcript was obtained with either peak 2 fractions (Fig. 2A, lanes 3 and 5), the HeLa IID factor (lane 2), or a purified preparation of yIID (lane 4). Addition of α-amanitin at a concentration that completely inhibits mammalian pol B activity suppressed peak 2–dependent transcription (lane 6). Furthermore, the peak of U6 transcription activity (pol C system) and the activity of TFIID, as assayed with the Ad2MLP template (pol B system), coeluted from a Superose 12 column (Fig. 2, B and C).

To directly test the possible involvement of TFIID in U6 gene transcription, we assayed various preparations of TFIID in our reconstituted U6 transcription system. Both a purified yIID (Fig. 3A, lane 5) and an *Escherichia coli* extract from a strain that expressed yIID (*13*) (Fig. 3A, lanes 2 to 4) allowed U6 RNA synthesis. Transcription directed by the *E. coli* extract was α-amanitin– and rifampicin-resistant as expected for yeast pol C (Fig. 3B, lanes 3 to 5), and required the addition of peak 1 fraction (lane 2) and the pTaq6 DNA template (lane 8). No U6 transcript was produced with a control *E. coli* extract supplemented or not with peak 1 fraction (Fig. 3B, lanes 6 to 7). The accuracy of transcription directed by the different factor preparations was investigated by S1 nuclease mapping with a 191-bp Taq I–Eco NI fragment as the hybridization probe (Fig. 3C) (*15*). The U6 RNA transcripts obtained with partially purified TFIIB (lane 1), the Superose peak 2 fraction (lane 2), or with the bacterial extract from cells that expressed cloned yIID (lane 3) generated the same size protected DNA fragments as partially purified yeast U6 RNA (lanes 4 and 5). Therefore, yIID allowed correct initiation of transcription of the U6 gene.

Several conclusions can be derived from the present work. First, at least two factors are required for in vitro U6 RNA synthesis by pol C. The largest component (130 kD) probably corresponds to TFIIIB, as it is required along with factor Tau (TFIIIC) for in vitro transcription of tRNA genes (*16*). Yeast TFIIIB activity has been ascribed to a 60-kD polypeptide (*17*), yet the size and polypeptide composition of this factor is still uncertain (*18*). The present results also provide evidence, based on chromatographic, functional, and structural criteria, that the second required factor is TFIID. The function of TFIID still remains to be demonstrated in vivo. The U6 template used in our experiments does not contain a B-block element (*1*) that lies downstream of the T-rich termination signal (*19*). The presence of this element, essential for in vivo transcription of the U6 gene, strongly suggests an additional requirement for factor Tau (*19, 20*).

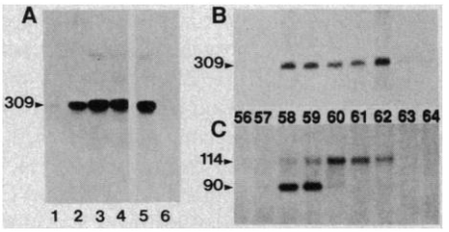

Fig. 2. Substitution of the U6 transcription activity (peak 2) for the human TATA factor in a reconstituted HeLa cell pol B system. (**A**) Transcription reactions that contained HeLa cell RNA pol B, STF, BTF2, and BTF3, the Ad2MLP pM34–Sal I template (run-off transcript, 309 nt) (*14*) were supplemented with different protein fractions as a source of the TATA factor: lane 1, none; lane 2, HeLa TFIID factor preparation (1 μg, SP-5PW step) (*14*); lanes 3, 5, and 6, peak 2 fraction (4 μl, 20 ng of protein); lane 4, yIID (1 μg, Heparin-Ultrogel step) (*13*). Transcription mixtures in lanes 5 and 6 received 0 and 2 μg of α-amanitin per milliliter, respectively. After a short incubation period, RNA synthesis was allowed to proceed for 45 min, and the transcripts were analyzed by gel electrophoresis. (**B** and **C**) Peak 2 fractions from a Superose 12 column similar to that shown in Fig. 1 were assayed for TATA factor activity with (B) the Ad2MLP template in the human pol B system (*14*) and (C) for U6 factor activity (*10*), respectively. Fraction numbers are indicated. The 309-nt Ad2MLP run-off transcript and the 114-nt and 90-nt U6 transcripts are indicated with arrowheads.

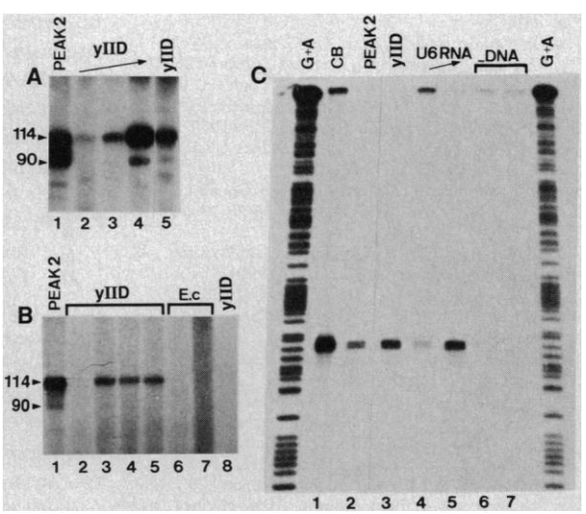

Fig. 3. TFIID directs specific transcription of the U6 gene by pol C. (**A**) U6 transcription mixtures contained either the peak 2 fraction (7 μl, 35 ng) (lane 1), increasing amounts (1, 3, and 9 μl) of an extract from *E. coli* cells that expressed cloned yIID gene (lanes 2 to 4), or purified yIID (200 ng) (*13*) (lane 5). Specific transcripts of 114 and 90 nt are indicated with arrowheads. (**B**) U6 transcription with cloned yIID requires peak 1 factor. Lane 1, control with peak 1 + peak 2 fractions and α-amanitin (100 μg/ml); lane 2, extract of *E. coli* cells (1 μl, 0,33 ng) that expressed the cloned yIID (E.c yIID), and no peak 1; lane 3, E.c yIID + peak 1; lanes 4 and 5, as in lane 3 with α-amanitin (100 μg/ml) and rifampicin (6.5 μg/ml), respectively; lane 6, control *E. coli* extract (1 μl, 0.33 ng) (E.c) and no peak 1; lane 7, E.c + peak 1; lane 8, as in lane 3, without pTaq6 DNA template. (**C**) S1 mapping of in vitro U6 RNA transcripts. In vitro–derived transcripts or yeast RNA were hybridized with a ^{32}P-labeled Taq I–Eco NI DNA probe and treated with S1 nuclease (*15*). Protected DNA fragments were analyzed on a polyacrylamide sequencing gel next to guanine + adenine (G + A) cleavage products of the same probe. Factor preparations used were as follows: lane 1, Cibacron blue fraction (*8*); lanes 2 and 6, peak 1 + peak 2; lanes 3 and 7, peak 1 + E.c yIID; lanes 4 and 5, size-fractionated yeast U6 RNA, 1 and 3 μg, respectively; lanes 6 and 7, pTaq6 DNA was omitted.

Our results suggest that TFIID may have a broader function in gene expression than previously appreciated (*21, 22*). The possibility that a transcription factor is shared by pol B and pol C correlates well with previous indications that upstream activating factors stimulate transcription by the B and C enzymes (*2, 23*). These upstream activator proteins may act via TFIID (*22, 24*). Participation of the TATA factor in the transcription of pol C genes may not be restricted to the U6 (and 7SK) genes, as upstream TATA-like elements have been found in a number of other genes transcribed by pol C (*1*).

In pol B genes, the interaction of TFIID with the TATA element is thought to promote preinitiation complex formation by favoring subsequent binding of TFIIB (or a preformed pol B–TFIIB complex) (*22, 25*). In the case of the U6 gene, TFIID may favor the assembly of TFIIIB that, by itself, does not bind DNA (*16, 18*). A functional and evolutionary relationship may therefore exist between TFIIIB and TFIIB or some other general pol B factor. The relatedness of class C and class B transcription factors would thus parallel the close evolutionary relationship of pol B and pol C, as these two enzymes were found more closely related to each other than to enzyme A (*26*).

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A Genetic Model for Interaction of the Homeodomain Recognition Helix with DNA

STEVEN D. HANES AND ROGER BRENT

The Bicoid homeodomain protein controls anterior development in the *Drosophila* embryo by binding to DNA and regulating gene expression. With the use of genetic assays in yeast, the interaction between the Bicoid homeodomain and a series of mutated DNA sites was studied. These experiments defined important features of homeodomain binding sites, identified specific amino acid–base pair contacts, and suggested a model for interaction of the recognition α-helices of Bicoid and Antennapedia-class homeodomain proteins with DNA. The model is in general agreement with results of crystallographic and magnetic resonance studies, but differs in important details. It is likely that genetic studies of protein-DNA interaction will continue to complement conventional structural approaches.

THE GENE *bicoid* ENCODES A PROTEIN morphogen (Bicoid) that is required for anterior development (*1*). Like many regulatory proteins important for development, Bicoid contains a 60–amino acid sequence known as the homeodomain (*2, 3*). Bicoid exerts its effects, in part, by activating expression of zygotic genes such as *hunchback* and *orthodenticle* (*4*). Bicoid binds to the sequence TCTAATCCC and close variants repeated in the 5′ regulatory region of *hunchback* (*5*). The Antennapedia (Antp) class of homeodomain proteins, such as those encoded by *Antennapedia*, *fushi tarazu*, and *Ultrabithorax*, bind the sequence TCAATTAAAT, which was first identified upstream of *engrailed* (*6–8*), a gene involved in segmentation.

The homeodomain contains a structure similar to the helix-turn-helix motif of prokaryotic transcriptional repressors (*9, 10*). Recognition of specific DNA sites by homeodomain proteins depends on the second α-helix (recognition helix) of this motif (*11, 12*). Bicoid and Antp-class proteins use Lys or Gln, respectively, at position 9 of the recognition helix (Fig. 1A) to distinguish between related binding sites (*11, 12*). When Lys^9 in the Bicoid recognition helix is replaced by Gln, the mutant protein (Bicoid-Q_9) no longer recognizes Bicoid sites, but instead recognizes Antp-class sites (*11*).

Bicoid and Antp-class proteins expressed

Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, and Department of Genetics, Harvard Medical School, Boston, MA 02115.