observations to support the data to be acquired by the Galileo mission at Jupiter by providing extended vertical and temporal coverage of atmospheric conditions across the planet.

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A Class III Transcription Factor Composed of RNA

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It is generally assumed that the machinery that transcribes genes is composed entirely of polypeptides. However, in vitro transcription by silkworm RNA polymerase III requires a transcription factor that is not a polypeptide. This component, TFIIIR, is distinct from the previously identified transcription components: RNA polymerase III, and the accessory factors TFIIIA, TFIIIB, TFIIIC, and TFIIID. The newly discovered TFIIIR is a macromolecule that appears to be composed of RNA. It is resistant to heat, detergent, phenol, protease, and deoxyribonuclease, but it is sensitive to alkali and ribonuclease.

THE PROMOTERS RECOGNIZED BY RNA POLYMERASE III require at least three transcription factors in addition to polymerase itself (*1–3*). These discrete and readily separable components appear to act as a unit during in vitro transcription of silkworm class III genes (*4–6*). Although protein-protein interactions could potentially account for the cooperative action of multiple transcription components, we considered the possibility that a nucleic acid might contribute to the structural integrity of the active RNA polymerase III transcription complex. While such a proposal has no precedent, a variety of other key biological processes such as protein synthesis, protein secretion, RNA processing, and telomere maintenance use machinery that is organized in ribonucleoprotein particles (*7–13*). We therefore set out to determine whether a nucleic acid, apart from the template, plays an essential role in transcription by silkworm RNA polymerase III.

We examined the sensitivity of the transcriptional activity of crude extracts to nuclease treatment in order to determine whether the RNA polymerase III transcription machinery includes an essential nucleic acid component. Micrococcal nuclease was chosen because its action can be stopped by EGTA-chelation of calcium ions—a procedure that does not interfere with subsequent assay of transcription activity in the treated extract. Micrococcal nuclease completely destroyed the ability of a silk gland nuclear extract to transcribe a gene coding for silkworm alanine transfer RNA ($tRNA_C^{Ala}$)(Fig. 1). Control experiments showed that loss of transcriptional activity requires the simultaneous presence of both calcium ions and micro-

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coccal nuclease. Thus, inactivation is not due to a calcium-independent contaminant in the micrococcal nuclease, or to destruction of the transcription machinery by exposure to calcium. To test the possibility that the transcription machinery might have been poisoned by a diffusible product of nucleolytic action (fragments of bulk nucleic acid, for example), we determined the transcriptional activity of mixtures composed of different proportions of treated and untreated extract. In all cases, the activity of the mixture was proportional to the amount of untreated extract. Thus, loss of activity appears to be due to the destruction of a positively acting component, rather than to the creation of a diffusible, negatively acting one.

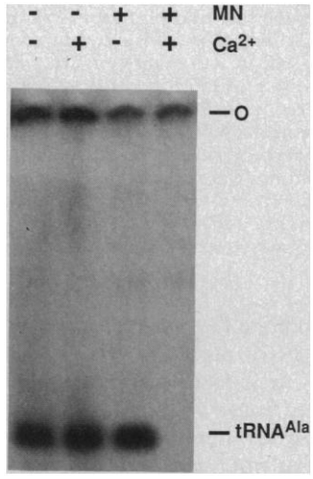

Fig. 1. Sensitivity of unfractionated class III transcription machinery from silkworms to micrococcal nuclease. Silk gland nuclear extract (*4, 14*) was treated with micrococcal nuclease (+ MN) or with an equal amount of bovine serum albumin (BSA) (− MN) in the presence (+) or absence (−) of $CaCl_2$ (Ca^{2+}). Micrococcal nuclease treatment was with 2.5 units (*38*) of Mono S–purified enzyme in 13 μl of 30 mM tris-HCl (pH 7.5), 150 mM KCl, 2 mM $CaCl_2$, 3 mM $MgCl_2$, 0.6 mM dithiothreitol (DTT), and 12 percent glycerol for 30 minutes at 37°C. Digestion was stopped by the addition of EGTA to a final concentration of 4 mM. To make all mixtures equivalent in subsequent transcription assays, $CaCl_2$ was added to the reaction mixtures that lacked it initially. The level of calcium-independent nuclease activity that contaminates most commercial preparations of micrococcal nuclease is too high for experiments that require large amounts of enzyme. Therefore, commercial micrococcal nuclease (Worthington) was further purified by chromatography on Mono S (Pharmacia), with a 0 to 500 mM gradient of KCl in 50 mM Hepes (pH 6.7). The slope of the gradient was 20 mM KCl per milliliter; calcium-dependent nuclease activity eluted between 355 and 375 mM KCl. Fractions with this activity were identified by measuring degradation of purified silkworm ribosomal RNA (rRNA) (*17*) in the presence and absence of calcium ions. After dialysis against distilled water (*39*), these fractions were stored at −70°C. The treated samples were tested for transcriptional activity on a $tRNA_C^{Ala}$ gene under the following (standard) conditions: 50 mM tris-HCl (pH 7.5); 65 mM KCl; 5 mM $MgCl_2$; 1 mM DTT; 10 percent glycerol; ATP, CTP, and GTP at 600 μM each; 25 μM UTP; and 5 μCi of [α-^{32}P]UTP (New England Nuclear). Each reaction mixture (20 μl) contained 100 ng (0.032 pmol) of template [a wild-type $tRNA_C^{Ala}$ gene inserted in pBR322 (*40*)] and 8 μl of extract. Incubation was for 2 hours at 22°C. The products of transcription were fractionated by polyacrylamide gel electrophoresis and detected by autoradiography (*41*). The position of the $tRNA_C^{Ala}$ transcript ($tRNA^{Ala}$) relative to the gel origin (O) is shown.

Discovery of a class III transcription factor composed of RNA. In an independent line of investigation, the silkworm transcription machinery was fractionated into multiple components, each of which is required for transcription of tRNA genes. These components are RNA polymerase III, the transcription factors TFIIIB, TFIIIC, and TFIIID (*2*), and a newly discovered component that we call TFIIIR. We first deduced the existence of TFIIIR from the behavior of different preparations of silkworm TFIIIB. Preparations of TFIIIB with identical activities in a standard TFIIIB complementation assay (*2*) varied in their ability to complement the transcriptional activity of the most highly purified preparations of the other silkworm transcription components. In the most highly purified reconstituted system, some preparations of TFIIIB gave high levels of transcription, and others gave none—an indication that the active TFIIIB fractions might contain an essential component that was distinct from all of the previously identified components. Mixtures of the known components that did not support transcription provided a specific assay for the putative novel component and allowed us to resolve it from the rest of the transcription machinery. The TFIIIB fraction was impractical as starting material for this isolation because it contained relatively little TFIIIR. The low speed (1000*g*) supernatant generated during preparation of silk gland nuclear extracts (*4, 14*) was a rich source of TFIIIR, however. TFIIIR was separated from other class III transcription components present in this fraction by DEAE Sephadex chromatography and gel filtration (see legend to Fig. 2).

TFIIIR appears to be a general class III transcription factor since it is required for transcription of both $tRNA_C^{Ala}$ and 5*S* RNA genes (Fig. 2) and also for silkworm $tRNA_{SG}^{Ala}$ (*15*) and BmX (*6*) genes. Transcription of BmX and tRNA genes also requires each of the previously identified transcription factors (TFIIIB, TFIIIC, and TFIIID) and transcription of 5*S* RNA genes requires TFIIIA as well (Fig. 2) (*16*). We were concerned that the apparent requirement for the TFIIIR fraction might be due to quantitative effects, rather than to true dependence on a qualitatively distinct activity provided by this fraction. Specifically, two fractions might supply the same dilute component in sufficient quantity to overcome a threshold. This possibility was tested by determining the effect of omitting one component while systematically doubling the amounts of each of the

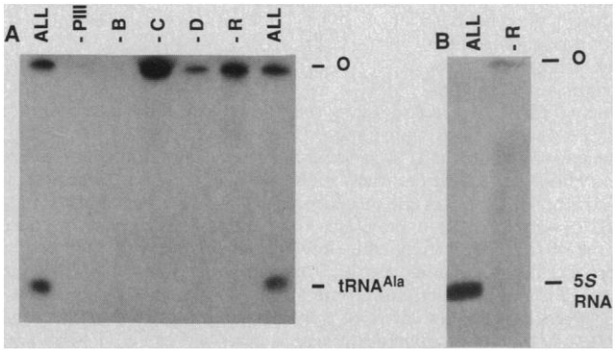

Fig. 2. TFIIIR requirement for transcription of tRNA and 5*S* RNA genes. Transcription of the $tRNA_C^{Ala}$ gene (**A**) or a 5*S* RNA gene (**B**) was carried out in the presence of all components (ALL), or in the absence of each single component (−). Transcription reaction conditions were as for Fig. 1; the total volume was 38 μl. Amounts of fractionated transcription machinery were chosen on the basis of previous titrations against a fixed amount of TFIIID in the presence of excess template (*40, 42*) and the other fractions. The concentration of each fraction that just saturated the transcription rate under these conditions was used. The functional TFIIID concentration (0.05 fmol per reaction mixture) was determined independently in single round transcription assays (*22, 43*). Accumulation of transcript was linear for at least 3 hours in the reconstituted system. For transcription of 5*S* RNA genes, silkworm TFIIIA (0.08 fmol, quantified in single round transcription assays) was added. Fractionation of the silkworm class III transcription machinery to obtain RNA polymerase III, TFIIIA, TFIIIB, TFIIIC, and TFIIID was as described (*2, 44*). TFIIIR was isolated from the low-speed (1000*g*) supernatant generated during preparation of silk gland nuclear extracts (*4, 14*). To resolve TFIIIR from the other transcription components, 30 ml of the fraction (protein, 8 mg/ml) was placed on a DEAE Sephadex A-25 column (3 by 14 cm) equilibrated in buffer D [50 mM tris-HCl (pH 8), 0.1 mM EDTA, 0.5 mM DTT, 20 percent glycerol, and 0.1 μM leupeptin] containing 100 mM KCl. The column was washed with 1.1 column volumes of the same buffer and eluted with 1.1 column volumes of buffer D containing 500 mM KCl. TFIIIR was eluted along with small amounts of TFIIIB and RNA polymerase III. No TFIIIC or TFIIID activity was detected. After dialysis to 50 mM tris-HCl (pH 7.5), 100 mM KCl, 4 mM $MgCl_2$, and 20 percent glycerol, the TFIIIR fraction (0.5 to 0.7 ml) was placed on a 25-ml HR 10/30 Superose 6 column (Pharmacia) (flow rate 0.25 ml/min) equilibrated in buffer S [50 mM tris-HCl (pH 7.5), 125 mM KCl, 5mM $MgCl_2$, and 10 percent glycerol]. Fractions with TFIIIR activity (see legend to Fig. 3) were pooled and stored at −70°C after BSA had been added to 0.5 mg/ml.

others. Since in no case did doubling the amount of any transcription factor eliminate the requirement for any of the other factors, each component, including TFIIIR, is qualitatively distinct from the others.

Because the unfractionated silkworm class III transcription machinery showed sensitivity to nuclease, we tested each of the resolved fractions to identify a component having the properties of a nucleic acid. Preliminary experiments drew our attention to TFIIIR. This factor appeared to be a macromolecule since it did not pass through a dialysis membrane, and its apparent molecular mass by gel filtration was about 45 kilodaltons with respect to protein standards. Nonetheless, TFIIIR displayed unusual thermostability, in that heating for 10 minutes at 65°C did not reduce its activity, and boiling for 10 minutes reduced activity by only 40 percent. Furthermore, TFIIIR was completely resistant to protease treatment under conditions that eliminated the activity of the complementing fractions (Fig. 3) and was resistant to extraction with a mixture of detergent (SDS) and phenol (Fig. 4).

These three properties—thermostability, insensitivity to protease, and resistance to organic solvents—are consistent with the possibility that TFIIIR is composed of nucleic acid rather than protein. To test this idea directly, we treated the native TFIIIR fraction with micrococcal nuclease. This treatment completely destroyed TFIIIR activity in a calcium-dependent manner (Fig. 5), whereas the activity of the complementing components (RNA polymerase III plus TFIIIB, TFIIIC, and TFIIID) was not affected. Additional treatments (Fig. 6) showed that TFIIIR activity is sensitive to alkali and to ribonuclease A (RNase A), but is resistant to deoxyribonuclease (DNase), indicating that the active principle is RNA. At present, the identity of the particular RNA or RNA's with TFIIIR activity is not known since the TFIIIR fraction contains a variety of RNA species. The partial loss of activity that occurs when TFIIIR is boiled could indicate that preservation of secondary structure is important for TFIIIR function.

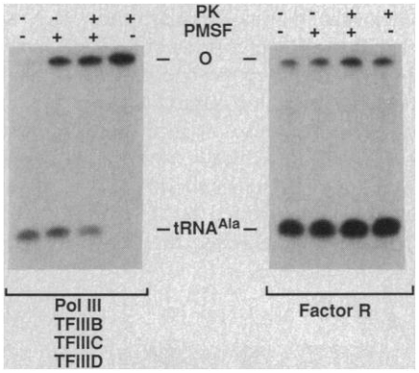

Fig. 3. Resistance of TFIIIR to proteinase K. (Right) Native TFIIIR was incubated with proteinase K (+PK) or with an equal amount of BSA (−PK) in the presence of phenylmethyl sulfonyl fluoride (+PMSF) or an equal amount of the PMSF solvent, 95 percent ethanol (−PMSF). (Left) Incubation of a mixture of the other class III transcription components (supplied by the fractions used to complement TFIIIR in standard assays, see below) under the same conditions. Protease treatment was with 1 mg of Proteinase K (Sigma) per 50 mg of protein for 3 minutes at 22°C in buffer S. Protease digestion was stopped by the addition of PMSF to the "+PK,−PMSF" reaction mixtures. The transcriptional activity of the treated fractions was determined in a standard TFIIIR complementation assay. This assay exploits the fact that the concentration of TFIIIR is frequently low in one or the other of the two phosphocellulose fractions (*2*) that together contain all of the previously identified class III transcription components. Thus, certain combinations of these fractions give low levels of transcription unless supplemented with TFIIIR. Transcription conditions were as described in Fig. 1, with a final volume of 44 μl and saturating template (75 to 100 ng). This complementation system was specific for TFIIIR, since transcription was not stimulated by the addition of concentrated sources of any of the other components (RNA polymerase III, TFIIIB, TFIIIC, or TFIIID), either individually or in combination. Typically, the background of the assay in the absence of added TFIIIR was about 2000 Cerenkov counts per minute (= 14 fmol of transcript per hour) and the activity of added TFIIIR was detected linearly to ~14,000 Cerenkov counts per minute (= 100 fmol of transcript per hour).

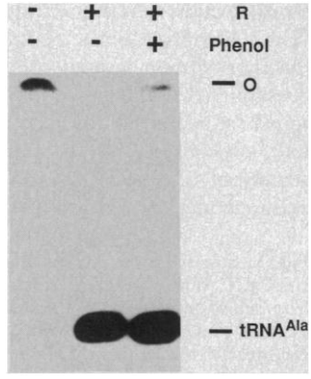

Fig. 4. Resistance of TFIIIR to extraction with SDS and phenol. The transcriptional activity of the native TFIIIR fraction (middle lane), or of the same fraction after extraction with SDS and phenol (right lane) was measured in the standard TFIIIR complementation assay (see legend to Fig. 3.) The two forms of TFIIIR were added in amounts that supplied equal concentrations of nucleic acids in the two reaction mixtures (2 ng/μl). Extraction with an equal volume of phenol and chloroform (1:1) was performed twice after the sample had been made 0.5 percent in SDS and heated at 65°C for 5 minutes. Material precipitable by 0.3 M sodium acetate and three volumes of 95 percent ethanol was collected and examined spectrophotometrically. Its maximum absorbance was at 255 to 260 nm and the ratio of A_{260} to A_{280} was 1.9. The nucleic acid content of the native TFIIIR fraction was determined after correction for the absorbance attributable to added BSA.

At present, experimental work with class III transcription machinery from various organisms is done with components that are only partially purified. It is essential, therefore, to consider the possibility that what appear to be components of the transcription machinery might actually be unrelated to transcription itself. Since the active principle of TFIIIR is a nucleic acid, we were concerned that TFIIIR might be required simply to titrate a nuclease that would otherwise destroy the product of transcription or the template. We therefore tested the stability of preformed transcripts in reaction mixtures that lack TFIIIR, but include the remainder of the transcription machinery. Isolated $tRNA_C^{Ala}$ transcripts were stable both in the presence and in the absence of TFIIIR (Fig. 7).

To test the possibility that TFIIIR inhibits a degradative activity that acts on the template, we determined whether template molecules are functionally impaired by incubation in the absence of TFIIIR. Template was incubated under the conditions of a standard transcription reaction, but TFIIIR was omitted. TFIIIR was then added, and the ability of the template DNA to direct transcription was assayed. Under these conditions, template activity was not affected by incubation in the absence of TFIIIR (Fig. 8, left). Similar experiments with no template present (Fig. 8, right) ruled out the possibility that RNA polymerase III or any transcription factor was

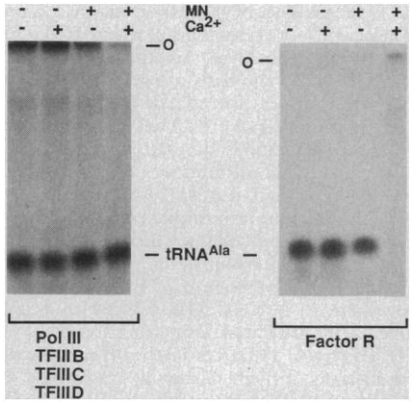

Fig. 5. Sensitivity of TFIIIR to micrococcal nuclease. (Right) Incubation of native TFIIIR with micrococcal nuclease (+MN) or with an equal amount of BSA (−MN) in the presence (+) or absence (−) of $CaCl_2$ (Ca^{2+}). (Left) Incubation of a mixture of the other class III transcription components (supplied by the fractions used to complement TFIIIR in standard assays) under the same conditions. The digestion conditions were as in Fig. 1, except that the incubation was at 22°C for 15 minutes. The ability of the treated fractions to transcribe a $tRNA_C^{Ala}$ gene was determined as in Fig. 3. The position of the resulting transcripts ($tRNA^{Ala}$) after resolution on a polyacrylamide gel (O, origin) is shown.

permanently damaged in the absence of both template and TFIIIR.

Although the incubation experiments provide evidence that TFIIIR does not simply protect the template or the transcription machinery from irreversible damage, they do not rule out the possibility that TFIIIR blocks reversible inhibition. To test for inhibitors that may act reversibly, but are stoichiometrically titrated by TFIIIR, we titrated each of the transcription components, including TFIIIR, against a fixed amount of one factor (typically TFIIID) to find the point at which no component was in excess (see legend to Fig. 2). At that point, the effect of doubling the amount of each single component was determined. If one or more of the fractions contained an inhibitor that could be quantitatively titrated by TFIIIR, this procedure should introduce excess inhibitor that would reduce transcription. In these experiments, we find no inhibitory effect of doubling the amount of any fraction when the amount of TFIIIR is held constant. Thus, by all the tests we have applied, TFIIIR is a bona fide part of the transcription machinery.

Specificity of nucleic acids with TFIIIR activity. Additional evidence that TFIIIR is an essential component of the silkworm transcription machinery comes from the specificity of the nucleic acids that have TFIIIR activity. The nucleic acids extracted from the TFIIIR fraction were active at low concentrations and were equivalent in specific activity to the nucleic acids supplied by the native TFIIIR fraction (Fig. 9) (the half-saturating concentration for each is ~0.5 ng/μl). In contrast, bulk RNA from yeast is completely inactive even at ten times higher concentration. Two different commercial preparations of yeast RNA gave identical results. Excess

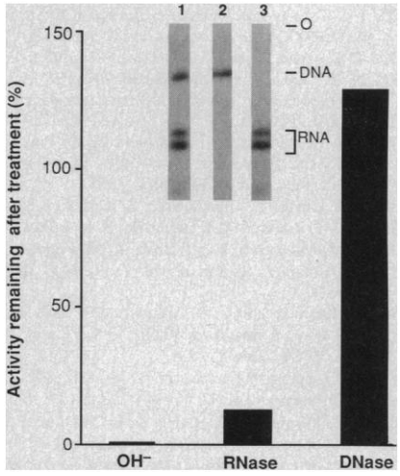

Fig. 6. Sensitivity of TFIIIR to RNase A, alkali, and DNase I. TFIIIR that had been extracted with a mixture of SDS and phenol was subjected to the indicated treatments. Treated samples were assayed for transcriptional activity in the standard TFIIIR complementation assay (see Fig. 3, legend). The results are presented as a percentage of the activity of mock-treated samples. Alkaline hydrolysis was carried out by incubating 500 ng of TFIIIR nucleic acid in 250 μl of 0.2 N KOH, 22 mM tris-HCl (pH 7.5) at 37°C for 16 hours. The solution was then neutralized by the addition of HCl to ~0.2 N. For the mock-treated control, the same 0.2 N KOH, 22 mM tris-HCl solution was neutralized with HCl before incubation with TFIIIR nucleic acids. The effectiveness and specificity of hydrolysis were checked by parallel incubations with ^{32}P-labeled $tRNA_C^{Ala}$ transcripts and gel-purified DNA fragments (*43*). The conditions for pancreatic RNase digestion were: 500 ng of nucleic acid, 1.2 μg of RNase A (Sigma), 40 mM tris-HCl (pH 7.5), 4 mM $MgCl_2$, 16 percent glycerol, 120 mM KCl, in a total volume of 100 μl, incubated at 37°C for 30 minutes. The conditions for DNase digestion were: 500 ng of nucleic acid, 1 μg of DNase I (Worthington), 8 mM tris-HCl (pH 7.5), 10 mM $MgCl_2$, and 1 mM DTT, in a total volume of 100 μl, incubated at 22°C for 30 minutes. For both treatments, in the mock reactions BSA (Boehringer) replaced the nuclease. Digestion was stopped, and nucleic acids were recovered from the reaction mixtures by extraction with 0.1 percent SDS and a mixture of phenol and chloroform (1:1); the extract was then precipitated with ethanol in the presence of glycogen carrier. Inset: DNase and RNase treatments are specific. A mixture of ^{32}P-labeled, gel-purified DNA fragments (DNA) and tRNA transcripts (RNA) were incubated with either BSA (lane 1), a sample of the RNase digestion mixture (lane 2), or a sample of the DNase digestion mixture (lane 3). The species remaining after digestion with nucleases were identified by polyacrylamide gel electrophoresis and autoradiography (O = origin).

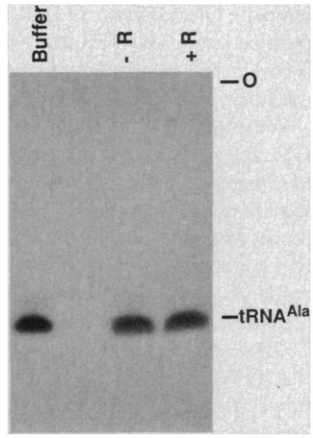

Fig. 7. Stability of $tRNA_C^{Ala}$ transcripts in the absence of TFIIIR. Isolated ^{32}P-labeled $tRNA_C^{Ala}$ transcripts were incubated for 2 hours at 22°C under standard transcription conditions (see legend to Fig. 1) either without other macromolecules (buffer), with the full set of transcription components (+R), or with the subset (see TFIIIR complementation assay in legend to Fig. 3) that lacks TFIIIR (−R). Previously synthesized $tRNA_C^{Ala}$ transcripts were isolated from 8 percent polyacrylamide gels by electroelution, with glycogen as carrier. Independent assays established that neither of two separate transcript preparations contained TFIIIR activity. The properties of the two preparations in stability tests were indistinguishable. The position of the incubated transcripts (tRNA^{Ala}) after resolution on a polyacrylamide gel (O, origin) is shown. The amounts of radioactivity (Cerenkov counts per minute) in $tRNA_C^{Ala}$ transcripts were (left to right): 1498, 1562, and 1350.

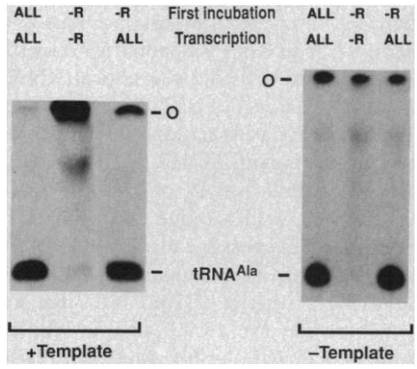

Fig. 8. Prior incubation in the absence of TFIIIR does not permanently damage the template or the transcription machinery. Either all components (ALL) were present or TFIIIR was omitted (−R) during the period of preliminary incubation (first incubation) or transcription. Preliminary incubation was under standard TFIIIR complementation assay conditions (see legend to Fig. 3) for 40 minutes in the absence of radioactively labeled nucleotides only (left panel) or in the absence of both template and labeled nucleotides (right panel). After the preliminary incubation, template was added to all reactions (right panel), TFIIIR was added to each test reaction mixture (right lane, each panel), and transcription was allowed to proceed for 40 minutes in the presence of [α^{32}P]UTP. The position of transcripts (tRNA^{Ala}) after resolution on a polyacrylamide gel (O, origin) is shown. The amounts of radioactivity (Cerenkov counts per minute) in $tRNA_C^{Ala}$ transcripts in the left panel were (left to right): 4505, 229, and 4640; and in the right panel were (left to right): 3146, 459, and 2994.

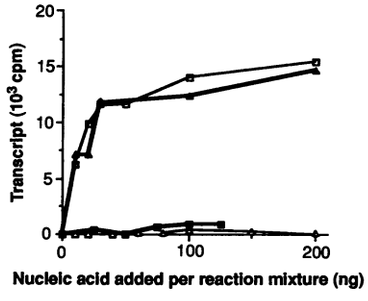

Fig. 9. Specificity of nucleic acids with TFIIIR activity. The transcriptional activity of the native TFIIIR fraction (▲), nucleic acid extracted from the TFIIIR fraction (□), bulk yeast RNA obtained from Sigma (△), or bulk DNA (■, the $tRNA_C^{Ala}$ gene inserted into pBR322) was determined in a standard TFIIIR complementation assay (see legend to Fig. 3). The amount of radioactivity incorporated into transcripts was plotted after subtraction of the negative (−TFIIIR) control value. The native TFIIIR fraction and the TFIIIR fraction that had been extracted with SDS and phenol were added in amounts that supplied equivalent concentrations of nucleic acid. The amounts of DNA plotted are in addition to the 75 ng of plasmid DNA that was provided as template in all reactions. The concentration range tested for DNA was smaller than the range tested for other nucleic acids because transcription was inhibited by more than 200 ng of DNA per reaction mixture.

template DNA had no TFIIIR activity (Fig. 9). Finally, a mixture of purified large (18*S* plus 28*S*) ribosomal RNA from silkworms (*17*) is completely inactive, even when 100 times more concentrated than the nucleic acids supplied by the TFIIIR fraction (50 ng/μl) (*18*).

We do not yet know whether TFIIIR is a component of the class III transcription machinery in organisms other than silkworms, but its chromatographic behavior indicates that it could be. Although a requirement for TFIIIR has not been revealed during extensive purification of TFIIIB and TFIIIC from other organisms (*19–25*), it is possible that in these cases, TFIIIR activity was in the relatively crude fractions that supplied complementing transcription factor activities. Our observation that TFIIIR can cofractionate with both TFIIIB and TFIIIC at an early stage in factor separation suggests that TFIIIR could be present in various complementing fractions in other systems. Alternatively, a TFIIIR analog could contaminate even the most highly purified preparations of TFIIIB or TFIIIC from other organisms. Since transcription has not been reconstituted in any system with homogeneous preparations of these factors, the possibility that one or both of them contributes TFIIIR activity remains open.

Possible roles of TFIIIR. The idea that RNA polymerase III transcription complexes might resemble ribonucleoprotein (RNP) particles motivated our original investigation. Although we have shown that an RNA is essential for class III transcription in vitro, we do not know whether the critical RNA is part of a stable RNP particle. If native TFIIIR consists of such particles, they are very small. The apparent molecular size of TFIIIR activity in the native, protein-containing TFIIIR fraction is only about 45 kD. Moreover, the high specific activity of isolated TFIIIR-containing RNA seems inconsistent with a requirement for RNP particle reconstitution. Therefore, it is possible that TFIIIR might act as part of a transient complex with other transcription factors, or even as a free RNA molecule. Examples of free RNA's that act with proteins in unusual ways include the tRNA that is required for ubiquitin-directed protein degradation (*26*), the tRNA that participates in chlorophyll biosynthesis (*27, 28*), and RNA's that regulate DNA replication (*29*). In addition, certain RNA molecules catalyze cleavage, ligation, and even polynucleotide polymerization reactions in the absence of any other macromolecules (*12, 30, 31*). It is possible that TFIIIR assists in the assembly of stable pre-initiation transcription complexes. The potential for polypeptide transcription factors to form complexes that include RNA is suggested by the observation that TFIIIA binds specifically to 5*S* RNA, as well as to a site on the 5*S* RNA gene (*32, 33*). Alternatively, TFIIIR might provide a catalytic function during a particular phase of the transcription cycle. It might act to initiate transcription—perhaps as the functional analog of an acidic protein domain in class II transcription activators (*34*)—or it might act during the elongation or termination phases of the transcription cycle. RNA-protein complexes that include particular segments of the nascent transcript are known to influence transcription termination in prokaryotes (*35, 36*) and may do so in eukaryotes (*37*). A detailed analysis of structure and function should give insight into the actual role of TFIIIR.

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44. We obtained silkworm RNA polymerase III, TFIIIB, TFIIIC, and TFIIID as described previously (*2*). Gel filtration of DEII fractions with high TFIIIB to TFIIIR ratios was the most effective procedure for TFIIIB. A fraction containing silkworm TFIIIA was prepared by gel filtration and ion exchange chromatography (T. Smith, unpublished observations) of an extract from pupal oocytes (*14*). This fraction is required for transcription of silkworm 5*S* RNA genes but not tRNA genes, and is functionally interchangeable with *Xenopus* TFIIIA (supplied by R. Roeder).

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