Promoter strength has been defined as the relative production of transcripts from a promoter. For T7 transcription it has frequently been observed that T7 class III promoters are qualitatively stronger than T7 class II promoters. In previous work it was observed that the maximum rates of initiation of three class III and three class II promoters show no class distinctions (Ikeda, R. A., Lin, A. C., and Clarke, J. (1992) J. Biol. Chem. 267, 2640–2649). This suggests that the efficiency of the conversion of the polymerase initiation complex to a stable transcription complex contributes to the overall strength of T7 promoters.

The class differences in the strengths of T7 class II and class III promoters are confirmed by measuring the relative synthesis of run-off transcripts. These results show that the relative strengths of the class III promoters, φ6.5, φ10, and φ13, are all comparable ranging from 0.61 for φ6.5 to 1.00 for φ10, while the relative strengths of the T7 class II promoters, φ1.1B, φ1.3, and φ3.8, vary widely. One T7 class II promoter, φ1.1B (relative strength = 0.34), approaches the strength of the class III promoters, while the other T7 class II promoters, φ1.3 (relative strength = 0.045) and φ3.8 (relative strength = 0.070) are nearly inactive.

The efficiency of promoter clearance is then determined by measuring the relative production of small transcription products in comparison with the production of run-off transcripts. These measurements clearly distinguish the T7 class III promoters from the T7 class II promoters. It is found that 68–75% of all initiations at the T7 class III promoters φ6.5, φ10, and φ13 produce a run-off transcript, while only 16–36% of the initiations at the T7 class II promoters φ1.1B, φ1.3, and φ3.8 produce a run-off transcript. Clearly, promoter clearance contributes to the difference in promoter strengths of the T7 class II and III promoters.

Transcription of the bacteriophage T7 genome is unidirectional from left to right across the T7 chromosome; however, complete transcription of T7 requires two different RNA polymerases, *Escherichia coli* RNA polymerase and T7 RNA polymerase. The early genes or class I genes are transcribed by *E. coli* RNA polymerase, while the middle and late genes or class II and III genes are transcribed by T7 RNA polymerase. Although promoters for T7 RNA polymerase consist of a highly conserved sequence of 23 continuous bases (1–3), it is known that transcription from T7 promoters both in vivo and in vitro is sequence dependent (4–11). In vitro, the relative strength of T7 class II and class III promoters depends not only upon promoter sequence but also upon reaction conditions, specifically ionic strength, temperature, and the super helicity of the DNA template: factors that are known to affect helix stability (5, 11–21). In vivo, T7 transcription from the class II promoters, which differ in sequence from the consensus T7 promoter, is weaker than transcription from the absolutely conserved class III promoters (6, 7, 22).

The nature of the transcriptional differences between T7 class II and III promoters has been addressed, and different studies have provided different answers. With T7 restriction fragments McAllister and Carter (5) showed that class II promoters are preferentially inhibited by conditions that stabilize the DNA helix. They also noted that the establishment of stable RNA-polymerase complexes required the synthesis of longer RNA transcripts at class II promoters (5, 20). On cloned T7 promoters Smeeckens and Romano (11) found no differences in the binding strength of T7 RNA polymerase to the class II and class III promoters φ1.1B and φ13. Similarly, Burgess and co-workers’ comparison (23) of T7 RNA polymerase footprints on a class III promoter and a class II/III hybrid promoter showed that there was no difference in promoter binding in the absence of GTP, but that in the presence of the initiating nucleotide the binding constant for the class III promoter was almost 3 times that of the hybrid promoter. All of this suggested that the efficiency of initiation is reflected in the stability of the initiated polymerase-protein complex, and that the overall process of initiation might differentiate strong T7 class III promoters from the weaker T7 class II promoters.

We previously showed that initiation at T7 promoters can be assayed by following the rate of appearance of small initiation products. These studies showed that the *in vitro* initiation activities of the T7 class II promoters φ1.1B, φ1.3, and φ3.8, and the T7 class III promoters φ6.5, φ10, and φ13 only mimic the *in vivo* observation that T7 transcription from class II promoters is weaker than transcription from class III promoters when the T7 class II and III promoters are assayed on linear templates (24). On these linear templates, the class II promoters generally required a higher promoter concentration to produce half of the maximum rate of initiation ([P]vmax/2 values) than the class III promoters. Although the [P]vmax/2 measurements differentiated the T7 class II and III promoters, curiously there was no class difference observed in the maximum rates of initiation (Vmax values) of the six promoters. Based on Vmax only the T7 φ10 promoter is significantly more active than any of the other five promoters.

This similarity in the maximum rates of initiation at the three T7 class II promoters, φ1.1B, φ1.3, and φ3.8, and the two T7 class III promoters, φ6.5 and φ13, seems to contradict the many observations that T7 transcription from its class III promoters is qualitatively much stronger than transcription...
tation from its class II promoters. What is the basis for this apparent contradiction?

Since promoter strength has been defined as the relative production of transcripts from a promoter (25), promoter strength is the net combination of the rate of promoter binding/opening, the rate of formation of the initial internucleotide bonds, and the rate/efficiency of conversion to a stable transcription complex. \( [P] \text{V}_{\text{max}}/2 \) and \( V_{\text{max}} \) values for the production of small initiation products are measures of the efficiency of the early stages of initiation and reflect both the characteristics of promoter binding/opening and the rate of formation of the initial internucleotide bonds (24). \( [P] \text{V}_{\text{max}}/2 \) and \( V_{\text{max}} \) values for the production of small initiation product do not measure the efficiency of the late stages of initiation and do not reflect the rate/efficiency of conversion to a stable transcription complex (24). The similarity of the \( V_{\text{max}} \) values for the three T7 class II promoters, \( \phi 1.1B, \phi 1.3 \), and \( \phi 3.8 \), and the two T7 class III promoters, \( \phi 6.5 \) and \( \phi 13 \), suggests that the rate/efficiency of conversion to a stable transcription complex is a significant factor in the determination of the overall strength of these promoters.

Relative promoter strengths and efficiencies of conversion to a stable transcription complex are measured for the three T7 class II promoters, \( \phi 1.1B, \phi 1.3 \), and \( \phi 3.8 \), and the three T7 class III promoters, \( \phi 6.5, \phi 10 \), and \( \phi 13 \). These measurements are made on the same templates previously used to measure \( [P] \text{V}_{\text{max}}/2 \) and \( V_{\text{max}} \) values (24) to allow direct comparison of the two studies. It is found that the relative strengths of the class III promoters are generally stronger than the class II promoters and that the class III promoters are much more efficient than the class II promoters at converting from the initiation complex to a stable transcription complex. This suggests that the promoter clearance step is a significant factor in differentiating the T7 class II and III promoters.

### EXPERIMENTAL PROCEDURES

#### Materials

**Enzymes**—Restriction endonucleases and Klenow fragment of DNA polymerase I were purchased from New England Biolabs and Boehringer Mannheim Biochemicals.

**Chemicals**—Buffers, acrylamide, \( N,N',N' \)-methylenebisacrylamide, ammonium persulfate, TEMED, and urea were electrophoresis grade. Media and all other chemicals were reagent grade.

**Nucleotides**—[\( \alpha \text{-}^{32} \text{P} \)]ATP and [\( \beta \text{-}^{32} \text{P} \)]ATP (800 Ci/mmol) were purchased from Du Pont–New England Nuclear. Unlabeled, high-purity dNTPs and NTPs were purchased from Pharmacia LKB Biotechnology Inc., and the purity of the unlabeled NTPs was confirmed by thin layer chromatography on PEI-cellulose F (EM Science) (26, 27). No contaminants were detected in the unlabeled NTPs.

**Plasmids**—The plasmids pSR135, pSR381, pSR652, phH1.0, pH1.1B, and pH1.3 contain the T7 promoters \( \phi 13, \phi 3.8, \phi 6.5, \phi 10, \phi 11.1B \), and \( \phi 13.1 \), respectively. The construction of these plasmids has been previously reported (24, 28). Plasmids pSR135, pSR381, and pSR652 were obtained from Dr. R. Rubin (Sloan–Kettering Institute) (28).

#### T7 RNA Polymerase

T7 RNA polymerase was prepared from E. coli HSM12/pGP1-1/pGP1-5 (28). This strain was a gracious gift from Drs. Stanley Tabor and Charles Richardson (Harvard Medical School). The T7 RNA polymerase was purified as previously described (30), and the activity of the purified enzyme was assayed under standard conditions (31). The T7 RNA polymerase had a specific activity of 485,000 units/mg (1 unit of activity is defined as the amount of enzyme necessary to incorporate 1 nmol of AMP into RNA in 1 h at 37°C).

1. The abbreviations used are: TEMED, \( N,N',N',N' \text{-tetramethyl-} \) ethylenediamine; nt, nucleotide(s); NTP, nucleotide triphosphate.

### Preparation of Template DNA

All plasmids were prepared by standard procedures and were confirmed by restriction mapping (32). The purified, supercoiled plasmids were then cut with an appropriate restriction enzyme to produce linear transcription templates (Table I). For pH1.3 and pSR135, the overhanging ends created by linearization with AflIII were repaired with E. coli large fragment DNA polymerase I and the 4-dNTPs.

The restricted DNA was analyzed on a 1% agarose gel to check for complete cleavage, and afterward, the DNA was purified by extraction with phenol and chloroform and precipitation with ethanol. The repaired pH1.3 and pSR135 templates were purified over a Schleicher & Schuell Etiduct-d column to remove unincorporated dNTPs, and the purified DNA was dissolved in 10 mM Tris-HCl, pH 7.8, 1 mM EDTA, and DNA concentrations were determined by measuring the absorbance of the solutions at 260 nm.

### Measurement of Promoter Strength

Promoter strength was determined by measuring the production of run-off transcripts from two different templates in a single transcription reaction. The 25-μl reactions contained 40 mM Tris-HCl, pH 8.0, 20 mM MgCl₂, 5 mM dithiothreitol, 800 μM GTP, 400 μM ATP, 400 μM UTP, 400 μM CTP, 15–20 μCi of [\( \alpha \text{-}^{32} \text{P} \)]ATP, 200 nM T7 RNA polymerase, 40 μg/ml bovine serum albumin, 20 nM pH110/EcoRI, and 20 nM of a second template. The specific activity of the [\( \alpha \text{-}^{32} \text{P} \)]ATP in the reaction mixture was determined for each individual experiment and ranged from 1800 to 3900 cpm/pmol.

In general, the reactions minus T7 RNA polymerase were preincubated at 37°C for 5 min to warm the mixture. The reactions were then started by the addition of T7 RNA polymerase. At 3, 6, and 9 min, 7-μl samples were withdrawn from the reaction and were stopped by addition of an equal volume of stop buffer (90% formamide, 50 mM EDTA, 0.1% bromphenol blue, 0.1% xylene cyanol).

The samples were heated in a boiling water bath for 30 s and cooled on ice. Samples of 7 μl were loaded onto a 5% polyacrylamide denaturing gel, and the samples were electrophoresed under standard conditions (32). After electrophoresis, the gels were soaked in 10% methanol, 10% acetic acid to remove the urea and were subsequently dried. The products were visualized by autoradiography using Kodak XAR-5 x-ray film. The bands corresponding to the run-off transcriptions were cut out of the gel, and the bands were quantitated by liquid scintillation.

### Measurement of the Efficiency of Run-off Transcription

The efficiency of producing a run-off transcript was determined by measuring the ratio of small transcription products to run-off transcripts produced in a T7 transcription reaction. Reaction conditions were identical to the reactions described in the previous section except for the inclusion of only one template at 20 nm, the use of 20–25 μCi of [\( \alpha \text{-}^{32} \text{P} \)]GTP to label the RNA, and the withdrawal of samples at 3, 9, and 27 min. The specific activity of the [\( \alpha \text{-}^{32} \text{P} \)]GTP in the reaction mixtures was determined for each individual experiment and ranged from 2100 to 2600 cpm/pmol.

Half of each sample was loaded onto a 19% polyacrylamide (acrylamide to bisacrylamide ratio = 9:1) denaturing gel to analyze the small transcription products, and the remainder of each sample was loaded onto a 5% polyacrylamide denaturing gel (32) to analyze the run-off transcripts. The bands corresponding to the run-off transcripts and the bands corresponding to transcripts that were 3–8 nucleotides long were cut out of the gels. These bands were then quantitated by liquid scintillation analysis.

### RESULTS

#### Relative Strengths of T7 Promoters

It has been previously noted that the experiment-to-experiment variability of T7 RNA polymerase assays makes it difficult to accurately determine the absolute activity of T7 RNA polymerase (24, 33). These same experimental variations also make it difficult to measure the absolute strengths of T7 promoters; consequently, the strengths of the T7 class II promoters \( \phi 1.1B, \phi 1.3, \) and \( \phi 3.8 \) and the T7 class III promoters \( \phi 6.5 \) and \( \phi 13 \) were determined in a comparative assay in relation to the strength of the T7 class III promoter \( \phi 10 \). Measurements were made by comparing the synthesis of run-off transcripts in a
transcription reaction containing an excess of T7 RNA polymerase, a linear transcription template bearing the φ10 promoter, and a second linear template bearing the promoter to be assayed. This comparative, run-off assay minimizes the problems associated in determining absolute promoter strength with an enzyme whose apparent activity can vary from day to day. Furthermore, the assay produces specific RNA transcripts by run-off synthesis. Fortunately, this eliminates the requirement for an efficient transcriptional terminator, since the known T7 terminator, Tφ, is not sufficiently active (34) for this assay. Finally, the assay minimizes the effects of template-dependent pausing and premature termination on the measurement of promoter strength by exploiting the sequence-independent processivity of the stable T7 transcription complex (19).

To prepare the run-off templates for the assay, plasmids containing the T7 promoters φ1.1B, φ1.3, φ3.8, φ6.5, φ13, and φ10 were linearized by cleavage with restriction enzymes (Table I). These plasmids were used to allow direct comparison of the relative promoter strengths obtained in this study to the previous measurements of promoter V₉₀₀ (31) and [P]ₖ₉₀₀ (32) (24). The restriction enzymes for linearizing the plasmid templates were chosen with three criteria in mind. First, the restriction enzyme must have a unique cleavage site on the plasmid to eliminate the generation of promoter-less DNA fragments that might compete for T7 RNA polymerase. Second, restriction of the plasmid must result in flush ends to avoid the possible competitive binding of T7 RNA polymerase to staggered ends (35). Third, where possible, the plasmids should be cut so that the T7 promoter is placed 200–500 bases upstream of the end of the DNA. This was not possible with the plasmids pSR135 and pH11.3; consequently, pH11.3 and pSR135 were cleaved with AflIII, and the overhanging ends created by cleavage with AflIII were repaired with E. coli large fragment DNA polymerase I and the 4 dNTPs. Typically, excess T7 RNA polymerase (200 nM) was added to a prewarmed (37 °C) transcription reaction containing 20 nM pSR10/EcoRV and 20 nM of a second transcription template. Samples taken at 3, 6, and 9 min were electrophoresed on a 5% acrylamide denaturing gel to separate the transcripts. The products were visualized by autoradiography; the bands corresponding to the run-off transcripts were cut out of the gel, and the amount of each transcript was determined from scintillation analysis after correcting for the adenylate content of the transcript. Since it was observed that the accumulation of transcripts was linear with respect to time (data not shown), the rate of synthesis of each transcript was determined from the slope of a least squares analysis of the data. The relative strength of a promoter was then defined to be the ratio of the rate of transcript synthesis from the promoter/template being assayed to the rate of transcript synthesis from the pH110/EcoRV template.

The concentrations of nucleoside 5′-triphosphates used for the measurements of promoter strength are the same concentrations that were used for the previously reported initiation studies (24) and are well above the reported K₉₀₀ values for the utilization of GTP, ATP, UTP, and CTP. The K₉₀₀ values measured for GTP, ATP, UTP, and CTP during transcription are 160, 47, 60, and 81 μM (31), respectively, and the K₉₀₀ for the initiating GTP has been calculated to be 600 μM (42). In the presence of the standard concentrations of nucleoside 5′-triphosphate, where the nucleotide concentrations are higher than each of the individual K₉₀₀ values, measurements of the relative strength of the class II promoter φ3.8 yielded reproducible data; conversely, in the presence of 400 nM GTP, a nucleotide concentration lower than the calculated K₉₀₀ for the initiating GTP, measurement of the relative strength of φ3.8 yielded more variable results (Table II).

Additional experiments demonstrated that the relative strength of φ3.8 was insensitive to the concentration of T7 RNA polymerase, to changes in the length of incubation, and to changes in the order of addition of the nucleotides and RNA polymerase (Table II). Conversely, the relative strength of φ3.8 was sensitive to high total template concentration in the assay. If the concentration of φ10/EcoRV plus the concentration of φ3.8/SmaI was comparable with the concentration of T7 RNA polymerase then the apparent strength of φ3.8 decreased; however, if T7 RNA polymerase was present in excess the apparent strength of φ3.8 was constant and insensitive to promoter concentration (Table II). Consequently, to avoid problems promoter strengths were measured with 20 nM of each promoter and excess (200 nM) T7 RNA polymerase.

Measurement of the strengths of promoters φ6.5, φ13, φ1.1B, φ1.3, and φ3.8 relative to φ10 (Fig. 1 and Table III) revealed that the class III promoters φ6.5, φ10, and φ13 are comparable (note: the strength of φ10 relative to itself was defined as 1.00). Their relative strength ranges from 0.61 ± 0.09 for φ6.5 to 1.00 (as defined) for φ10. The class II promoters, however, show much larger variations in strength. The class II promoter φ1.1B with a relative strength of 0.34 ± 0.05 is nearly as strong as the class III promoters, while the class II promoters φ1.3 and φ3.8 are only 0.045 ± 0.009 and 0.070 ± 0.012 as strong as the φ10 promoter, respectively.

Efficiencies of Run-off Transcription—The large differences in relative promoter strength (Table III) contrast with the smaller differences previously observed in the maximum rates of initiation (24). This dichotomy suggests that the efficiency

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**Table I**

| Plasmid | Promoter | Cleavage site* | Run-off transcript* |
|---------|----------|----------------|---------------------|
| pH11.1B | φ1.1B    | EcoRV          | 435                 |
| pH11.3  | φ1.3     | AflIII*        | 481                 |
| pSR385  | φ3.8     | SmaI           | 342                 |
| pSR652  | φ6.5     | SspI           | 443                 |
| pRI10   | φ10      | EcoRV          | 220                 |
| pSR135  | φ13      | AflIII*        | 425                 |

*The unique restriction site used to linearize the plasmid DNA.

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**Table II**

| [P], the concentration of each promoter in the assay; [RNAP], RNA polymerase concentration; [GTP], GTP concentration. | [GTP] | [RNAP] |
|---|---|---|
|   | 400 μM | 800 μM | 800 μM with NTP addition |
| nM | nM |     |         |
| 20 | 20 | 0.032 | 0.086 | 0.061 |
| 20 | 40 | 0.029 | 0.089 | 0.058 |
| 20 | 80 | 0.028 | 0.061 | 0.044 |
| 20 | 200 | 0.022 | 0.075 | 0.073 |
| 20 | 500 | 0.021 | 0.040 | 0.079 |
| 5 | 200 | 0.088 |
| 10 | 200 | 0.085 |
| 20 | 200 | 0.085 |
| 40 | 200 | 0.085 |
| 80 | 200 | 0.085 |
of conversion from an initiation complex to a stable transcription complex is a significant factor in the determination of the overall strength of T7 promoters. The efficiency for producing a run-off transcript (the efficiency of promoter clearance) was determined by measuring the percentage of total initiations that produce a run-off transcript during T7 transcription.

The reaction conditions used for determination of the efficiencies of promoter clearance were identical to the assay conditions used for determination of relative promoter strengths except for the inclusion of only one transcription template (20 nM) in the efficiency assays. This allows direct comparison of the relative promoter strengths and the efficiencies of run-off transcription.

Generally, excess T7 RNA polymerase (200 nM) was added to a prewarmed (37 °C) transcription reaction containing 800 μM GTP, 400 μM ATP, 400 μM UTP, 400 μM CTP, 20–25 μCi of [α-32P]GTP, and 20 nM of a transcription template. [α-32P]GTP was used in this assay so that trinucleotides produced by promoter dependent initiation of T7 RNA polymerase would be labeled. The RNA was analyzed by gel electrophoresis (Fig. 2), and the bands corresponding to run-off transcripts or to small initiation products 3-8 nucleotides in length were cut out of the gel. The quantity of each product was then determined by scintillation analysis after correcting for the guanylate content of the individual products.

Although small initiation products longer than 8 nucleotides are visible on the autoradiographs (Fig. 2), these products are significantly less abundant (data not shown) than the products 3–8 nucleotides long. The exclusion of these longer initiation products from the calculation of the efficiency of run-off transcription does not appreciably affect the results of the assay.

The proportions of small transcription products and run-off products were measured for transcription from T7 promoters on the templates φ0/SpI, φ6.5/SpI, φ13/AIII, φ1.1/EcoRV, φ1.3/AIII, and φ3.8/Smal. These results are listed in Table IV as the percentage of initiation events that result in a run-off transcript and as the number of small initiation products per transcript. The data clearly distinguish the T7 class III promoters φ6.5, φ10, and φ13, from the T7 class II promoters, φ1.1B, φ1.3, and φ3.8, showing that the
class III promoters produce 0.36–0.47 small initiation products per run-off transcript, while the class II promoters produce 1.75–5.14 small initiation products per run-off transcript.

In addition, it was observed that the percentage of initiation events that result in a run-off transcript is constant over time (data not shown) and that the distribution of small initiation events 3–8 nucleotides long differs from promoter to promoter (Fig. 2 and Table IV). For φ1.1B, the amounts of small initiation products synthesized generally decrease as the size of the products increase; however, the pentamer, GGAGA, and the heptamer predominates, while trimer, tetramer, pentamer, and heptamer are the major initiation products observed for φ13.

### DISCUSSION

Transcription by T7 RNA polymerase has been studied extensively, but although much is known about the enzyme, the mechanistic details of T7 transcription have not been fully characterized. The studies of transcription by T7 RNA polymerase suggest that the mechanism for the initiation of transcription by T7 RNA polymerase (Fig. 3) might be similar to the mechanism proposed for *E. coli* RNA polymerase (36–38). Coleman and co-workers (39, 40) have studied the association of T7 RNA polymerase with its promoter in the absence of ribonucleoside triphosphates. They found no evidence of a closed complex (RPi), but did identify an open complex (RPo) on native polyacrylamide gels. This suggests that either the closed complex is difficult to observe or the recognition and binding of a promoter by T7 RNA polymerase is involved in the DNA double helix. Addition of ribonucleoside triphosphates to the binary polymerase-promoter complex allows for the initiation of RNA synthesis. Footprinting indicates that the polymerization of the first few ribonu-
cleotides stabilizes the polymerase promoter complex and suggests that initiation produces a complex (RP) that is different from the open complex (8, 23, 41). Finally, both McAllister et al. (5, 20) and Coleman et al. (19) have shown that T7 RNA polymerase forms a stable transcription complex (RP) after synthesis of an oligoribonucleotide of approximately 8–12 nucleotides.

Comparison of the +1 to +8 sequences of the T7 promoters (Table V) (1) with the distributions of initiation products (Table IV) shows that addition of a pyrimidine to an oligoribonucleotide less than 8 nucleotides in length frequently increases the relative frequency of abortive initiation either before the incorporation of the pyrimidine (φ6.5 and φ13) or after the incorporation of the pyrimidine (φ1.1B and φ1.3). Increased abortive initiation has been previously observed when UMP is incorporated into a transcript before the 8th and 12th nucleotides (19, 20), but the observations that the preponderance of abortive initiation products produced by a T7 promoter are 8 nucleotides or less in length and that only 4 of the 17 natural T7 promoters incorporate UMP in the first 8 nucleotides of their transcripts suggest that early CMP incorporation is more important in determining T7 promoter efficiency than UMP incorporation.

The distributions of initiation products not only show that pyrimidinincorporation increases the relative frequency of abortive initiation, but they also show a tendency for abortive initiation to occur more frequently after the incorporation of AMP (φ1.1B and φ3.8) than after incorporation of GMP. This would suggest that even transition mutations from +1 to +6, mutations changing the conserved G nucleotides to A, may increase abortive initiation from a T7 promoter.

Further examination of the distributions of initiation products reveals that the identical T7 class III promoters do not produce identical distributions of initiation products. The relative amounts of pentamer and hexamer produced by the class III promoters φ6.5, φ10, and φ13 differ considerably even though all of the pentamers are GGGAG and all of the hexamers are GGGAGA. This suggests that sequences outside the conserved T7 promoter can influence processes that occur within the conserved sequences of a T7 promoter.

Finally, for all of the promoters assayed, the abundance of short initiation products decreases dramatically after the incorporation of the 8th nucleotide, but in all cases initiation products longer than 8 nucleotides are present (partially visible in Fig. 2). The abundance of these longer initiation products appears to decrease regularly as the length of products increase (data not shown), but at a product length of approximately 13–16 nucleotides, the abundance of the longer initiation products again decreases dramatically, and the even longer initiation products fade into the background. The dramatic decrease in the production of abortive products after the incorporation of the 8th nucleotide has been observed previously, and it has been suggested that a highly processive ternary complex is formed by the T7 RNA polymerase, the DNA template, and the nascent RNA after incorporation of the 8th nucleotide (19). The distributions of initiation products seen here seems to indicate that a processive ternary complex starts to form after the incorporation of the 8th base in a transcript, but it appears that the complex is not fully formed until a transcript 13–16 long has been synthesized.

Components of Promoter Strength—Since promoter strength is the net combination of the rate of promoter binding/opening, the rate of initiation, and the efficiency of the conversion of the initiation complex to a stable transcription complex (25) then relative promoter strength divided by the percentage of initiations that produce full length transcripts should equal the relative maximum velocity for the production of small initiation products. Division of the relative promoter strengths measured here by their respective efficiencies of run-off transcription yields a relative ranking of maximum velocities of initiation that are consistent with the maximum velocities of initiation (Vmax) that were measured previously (24). This confirms that all three measurements (relative promoter strength, the percentage of initiations that produce full length transcripts, and Vmax) are self-consistent and suggests that the three measurements are a reliable reflection of the factors that influence the strengths of T7 promoters.

In conclusion, measurements of the relative strengths of three T7 class II promoters and three T7 class III promoters confirm that the class II promoters are generally weaker than the conserved class III promoters; however, the data also show that the relative strengths of class II promoters vary widely and can range from activities approaching a class III promoter to activities near zero. Interestingly, this distinctive class difference in the strengths of the T7 promoters φ1.1B, φ1.3, φ3.8, φ6.5, φ10, and φ13 is not fully reflected in their the maximum rates of initiation. This suggested that promoter clearance might be a contributing factor to the class differences in the strengths of T7 promoters. It is apparent from the measurements of the efficiencies of run-off transcription that promoter clearance is a major factor in determining the class differences in the strengths of T7 promoters, and that T7 promoter strength is determined by both the maximum rate of initiation of a promoter and its efficiency of promoter clearance.

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