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A ρ -dependent termination site in the gene coding for tyrosine tRNA su_3 of *Escherichia coli*

Hans Küpper* and Takao Sekiya†

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Martin Rosenberg

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

James Egan and Arthur Landy

Section of Microbiology and Molecular Biology, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912

Abstract

A set of partially overlapping DNA restriction fragments that support promoter-dependent transcription of the tRNA^{Tyr}₁ gene of *Escherichia coli* has been used to study site-specific termination *in vitro*. Transcription termination occurs at a specific site 224–226 nucleotides beyond the end of the structural gene and is completely dependent on ρ -factor. Certain features of this site suggest differences from other termination sites previously studied. A role for specific sequence recognition is suggested.

Initiation and termination of RNA synthesis are two discrete steps in gene expression. These two events occur at different specific sites along the DNA template and are due to certain sequences or structural features which act as signals for the transcribing RNA polymerase. Both events can be modulated by the encoded sequences themselves or in conjunction with specific transcriptional control factors (see ref. 1 for review). In *Escherichia coli* the correct initiation of RNA synthesis is absolutely dependent on the presence of the σ factor². The ρ factor, on the other hand, causes termination of RNA synthesis *in vitro*³, and there is evidence that this termination factor is essential for *in vivo* function. However, until now, all termination sites studied *in vitro* have been independent of the ρ factor to varying degrees^{5–9}.

We report here an *in vitro* analysis of a completely ρ -dependent site-specific termination in the tyrosine tRNA su_3 gene (tRNA^{Tyr}₁). Our results suggest that in addition to the possible role of structural features in site-specific termination, specific sequences in the DNA template may also play a part in the ρ -polymerase termination reaction.

Extensive use has been made of a family of small DNA restriction fragments that support promoter-dependent transcription of the tRNA^{Tyr}₁ gene and that extend for different distances in the downstream direction^{10,11}. Using this same *in vitro* system, we have previously shown that (1) purified RNA polymerase, in a σ -dependent reaction, initiates RNA synthesis *in vitro* with the same sequence as it does *in vivo*; (2) no termination of RNA synthesis occurs

Correspondence to: Arthur Landy.

*Present address: Institut für Molekulare Genetik, Im Neuenheimer Feld 230, 6900 Heidelberg, Germany.

†Present address: National Cancer Center Institute, 5 Chome, Tsukigi Chuo-ku, Tokyo, Japan.

within the 70 bases following the CCA end of the tRNA sequence in the absence or presence of the ρ factor; and (3) the two RNA species coded by the phage $\Phi 80\text{psu}_{111}^{+,-}$ are transcribed as a common precursor.

Effect of ρ on transcription from various DNA restriction fragments

The DNA fragments used in this study were derived by digestion of $\Phi 80\text{psu}_{111}^{+}$ DNA with various restriction endonucleases. The three major fragments, *Cla*, *HpaII* and *Bla*, extend 73, 372 and approximately 880 base pairs, respectively, beyond the sequences coding for the mature tRNA (see Fig. 1). The transcripts made by purified RNA polymerase from these DNA fragments and the effect of ρ factor on their lengths are shown in Fig. 2.

Transcription of *Cla* yields a main RNA species of 200 nucleotides, and the presence of ρ has no effect on the product size (Fig. 2, lanes 1 and 2). The number of nucleotides beyond those encoding the 3' end of the mature tRNA is known (see also Fig. 4), so this result indicates that transcription from the $\text{tRNA}_{\text{Tyr}}^{\text{I}}$ promoter₁₁ proceeds to the end of this restriction fragment even in the presence of ρ .

Transcription of the *HpaII* fragment yields a major RNA species of 500 nucleotides which indicates that here also transcription proceeds up to (or close to) the end of the fragment. With this template, however, the presence of ρ has a strong effect on product size, and the resulting major RNA species has a length of 350 nucleotides (Fig. 2, lanes 3 and 4).

Transcription of *Bla* yields a more heterogeneous collection of RNA species. Although transcripts longer than 500 nucleotides are made, the majority of transcriptions do not proceed to the end of the fragment. The heterogeneity of the RNA pattern is due, in part, to contamination of the *Bla* fragment during isolation with other DNA fragments of similar size (see ref. 10). The ρ factor again reduces the size of the transcripts to yield a major RNA species of 350 nucleotides, the same size as is synthesised from the *HpaII* fragment in the presence of ρ (Fig. 2, lanes 5–8).

To make sure that the observed effect of ρ is not due to a nuclease contaminant in the preparation, transcripts made in the absence of ρ were incubated with the same amounts of ρ , and in the same conditions, as used in the transcription experiments. No change in the size or amount of transcript could be detected (data not shown). When different preparations of ρ were used, the same effect on RNA size was always observed (Fig. 2, lanes 6–8). The transcription products from *Cla*, *HpaII*, and *Bla* made in the absence or presence of ρ indeed contain the tRNA sequence because they can be processed by an *E. coli* S100 extract to yield the $\text{tRNA}_{\text{Tyr}}^{\text{I}}$ (ref. 11, and unpublished results).

The patterns of lower molecular weight RNA species seen on the acrylamide gels are highly reproducible, although varying in intensity (see also Fig. 3). These shorter transcripts may occur *in vitro* because of pausing of the RNA polymerase or because of inefficient termination events¹². The pattern might even be due to an alignment of polymerase molecules, as polymerase in these experiments is in 10- to 15-fold excess over DNA, and the distances between the bands observed are 50–60 nucleotides. The presence of ρ does not change this pattern, although it reproducibly decreases the amount of background synthesis especially of higher molecular weight bands (Fig. 2).

These results indicate that the *E. coli* tRNA₁^{Tyr} gene contains a relatively efficient ρ -dependent termination site *in vitro* approximately 225 nucleotides 'downstream' from the 3' end of the encoded tRNA sequence.

Influence of transcription conditions on ρ activity

The restriction fragment *Cla* provides a very useful means of separating the possible effects of ρ on initiation from those on termination; as *Cla* does not have a ρ -dependent termination region, the amount of tRNA₁^{Tyr} gene product is influenced solely by events at the promoter.

Figure 3b shows that the effect of ρ factor on initiation is not dramatic, even over an approximate 10-fold range of polymerase to DNA (1.8:1 to 15:1). Although we see a reproducible small stimulation on addition of ρ factor (up to twofold, lanes 3 and 4, Fig. 3b legend), we could never achieve the very high stimulation reported for intact $\Phi 80\text{psu}_{111}^+$ DNA_{13,14}. Our results are therefore more in agreement with studies on ρ in other systems (refs. 3,15,16; see also discussion in ref. 17).

The catalytic action of ρ (ref. 15) at the termination site was established using the *HpaII* restriction fragment. Even a polymerase: DNA ratio as high as 24:1 (at a constant ρ :DNA ratio of 2:1) does not allow any additional polymerase molecules to read through the termination signal (data not shown). Addition of the polyanion heparin (20 $\mu\text{g ml}^{-1}$) completely inhibits the action of ρ , either when the two are added simultaneously, or when ρ is preincubated along with the polymerase, DNA, and GTP + CTP (see ref. 11) before addition of the inhibitor (data not shown).

It has been observed that the activity of ρ is relatively insensitive to ionic strengths up to 0.1 M KCl, whereas at high ionic concentration (>0.1 M KCl) ρ activity becomes gradually inhibited (see ref. 18 for review). In contrast, we find that termination at the tRNA ρ -dependent site is somewhat more sensitive to ionic concentration. Termination efficiency of the 350-nucleotide ρ -dependent product is not affected at 0.05 M KCl concentration; however, at 0.1 M KCl an approximate 50% inhibition of termination is observed (Fig. 3a). Note that the RNA chains that are extended beyond this ρ site at the higher salt concentration do not continue to the end of the fragment. Instead, a second major RNA product about 500 nucleotides in chain length is produced, suggesting the occurrence of a less sensitive, perhaps more efficient, termination site several hundred nucleotides downstream. This potential second termination site is discussed below in terms of the repeated DNA sequence that occurs within the tRNA gene.

In addition to the triphosphate concentrations described in the legends to Figs 2 and 5, experiments have been carried out with several different combinations of three triphosphates at high concentration (0.5–1.0 mM each) and one at low concentration (10–15 μM). The ρ -dependent termination of transcription was observed in all conditions (including an ATP concentration curve from 5 μM to 1 mM), but the relative efficiencies in the various conditions were not quantified.

Experiments are in progress to further characterise the tRNA₁^{Tyr} gene termination reaction in a range of different conditions. In particular, this system affords the unique opportunity to investigate the effect of several important parameters (temperature, triphosphate concentration, dielectric constant of the solvent) on the relative termination efficiency at each of three successive potential termination sites (see below). These studies are being carried out in conjunction with an investigation of *in vivo* termination in this stable tRNA gene.

An unusual repeated structure in the distal portion of the tRNA^{Tyr}₁ gene

Sequence analysis of the distal portion of the tRNA^{Tyr}₁ gene and a discussion of the gene structure are presented elsewhere¹⁹. In this report we discuss those features that are specifically related to termination of transcription. The most striking feature of the sequence is a 178-base pair unit that is repeated 3.14 times. The final sequence is presented in Fig. 4 in such a way as to facilitate a comparison of the repeated units. The strand of DNA which has the same sequence as the mature tRNA is shown. The first repeat begins within the sequence corresponding to the mature tyrosine tRNA, and a 19-base pair sequence from the 3' terminus of the mature tRNA occurs without any alterations at the beginning of the four repeat units. There are only 14 sites at which one of the repeats differs from the others.

Also indicated in Fig. 4 are the 'downstream' endpoints of the restriction fragments used in the transcription studies reported here. As the size of the RNA product made from the *Hpa*II or *Bla* fragments is dependent on the presence or absence of ρ factor, and the RNA product made from *Cla* is not, the ρ -dependent termination site must be between positions 199 and 497. The size of the ρ -dependent transcript from either *Bla* or *Hpa*II further narrows the location of the termination point to the region of 320–380.

Precise location and sequence of the ρ -dependent termination site

Two different methods were used to determine precisely what portion of the DNA sequence specifies the ρ -dependent termination site. In the first, T1 oligonucleotides from the *Hpa*II transcript made in the presence or absence of ρ were compared. These results are shown in Table 1 along with the T1 oligonucleotides found in the transcript from *Cla*. Those oligonucleotides most crucial for placing the terminus of the + ρ transcript are marked with an asterisk. The absence, or reduction by one relative mole, of oligonucleotides 3, 10c, 14 and 18 in the + ρ transcript indicates that this RNA terminated before position 357 (the first base in oligonucleotide 14) but after position 341 (the last base in oligonucleotide 10b). This is confirmed by the presence in the + ρ transcript of oligonucleotide 10b as well as all of the oligonucleotides preceding this one in the sequence.

A second method was used to confirm the above results and to define more precisely the 3' terminus of the + ρ transcript. Highly labelled gel-purified transcript made from the *Hpa*II restriction fragment in the presence of ρ was digested with T1 ribonuclease and passed over a column of dihydroxyboryl-substituted cellulose^{16,20}. All internal oligonucleotides, which have a 3'-phosphate as a result of the T1 digestion, pass through the column; only those oligonucleotides derived from the 3' end of the transcript contain a cis-diol and are selectively retained.

The material eluted from the borate column was found to be heterogeneous (Fig. 5). Analysis of these oligonucleotides indicates that this heterogeneity is due to the sequential addition of bases within only one T1 oligonucleotide. The longest sequence obtained is CAAUCAAAU and could only be obtained from T1 oligonucleotide 3, as predicted from the experiments described above.

This result shows that termination occurs within a region of five bases, between positions 348 and 352. Position 351 seems to be the preferred termination point, where one third of all chains are terminated. Position 352 is the second strongest point of termination, and the remaining 30–35% of the molecules have termini that are distributed about equally at positions 348, 349, and 350.

Features of the ρ -dependent termination of transcription

The transcription termination described here for tRNA₁^{Tyr} and for the λ_{R1} site²¹, differs in several striking properties from other termination sites studied thus far. The latter all have transcripts which end in a stretch of four to seven uridine residues, and while they are enhanced by the ρ termination factor, they still show significant amounts of termination in its absence (see ref. 18 for review). In contrast to this, the termination described here is completely dependent on the presence of ρ factor, and the transcripts do not end with a stretch of U residues. An additional important distinguishing feature of these two ρ -dependent termination sites is their similarity in sequence, which is discussed below.

A common structural feature noted at other sites of transcription termination is the occurrence of a potential RNA stem and loop structure just before the end of the transcript (reflecting a region of hyphenated dyad symmetry in the DNA)^{5–9,21–23}. There is some evidence that suggests that this secondary structural feature is important to the termination reaction^{21,22,24}. The absence of such potential structures immediately upstream of the tRNA₁^{Tyr} termination site is therefore quite striking (see also ref. 19). [The two closest loci for potential stem and loop structures are 50 base pairs (positions 287–296 with 195–186) and 100 base pairs (positions 249–255 with 245–239) upstream from the tRNA₁^{Tyr} termination site (see Fig. 4)—considerably greater than the distance of 10–15 base pairs observed for other termination sites.]

A second feature of this site, which again contrasts with other sites of termination, is a relatively stable G-C-rich region (10 of 12 consecutive base pairs, positions 356–367) just beyond the A-T-rich termination site. Beyond other sites of transcription termination an A-T-rich region has been noted^{7,22,25}. In addition, a small cluster of four G-C base pairs occurs 10 base pairs upstream from the tRNA₁^{Tyr} termination site. Maizels and Gilbert^{12,26} have suggested that such a cluster of G-C residues will generate a pause in transcription 7–10 residues downstream. (The configuration of a high A-T region preceded by a G-C cluster of variable length is found in seven of the eight published prokaryotic termination sequences²⁷.)

Thus it is tempting to speculate that the apparent requirement for a ‘structurally induced pause’ in transcription may be satisfied at the tRNA₁^{Tyr} termination site by a stable helical region in the DNA template, both preceding and distal to the point of termination, which is difficult for RNA polymerase to read through. This further suggests that there may be a variety of alternative features encoded in the DNA template, various combinations of which may signal a transcription termination event.

An analogous situation, where a high G-C region affects the interaction of RNA polymerase with DNA, is seen in the promoter of the tRNA₁^{Tyr} gene¹¹. This analogy between the interaction of RNA polymerase at promoter and terminator sites can be extended further. Just as the termination of transcription is not a precise event, the initiation of RNA synthesis can also occur within a region of four or five base pairs if complementary dinucleoside monophosphates are provided^{11,28}, with certain positions within this variable region being preferred¹¹. RNA polymerase prefers purines to a high degree over pyrimidines as the initiating triphosphate. For termination U and A seem to be preferred as the terminal nucleotides. Although the homology among known promoter sequences is not great and is limited to small sections, severe changes in promoter function are brought about by the change of a single base¹. The results reported here indicate that the same situation can be true for the termination reaction as discussed in the following section.

Features of the termination in the tRNA₁^{Tyr} gene

In spite of the fact that the DNA sequence is reproduced in each of the 178-base pair repeat units with very few changes, termination of transcription takes place in the second repeat unit, at positions 350–353, but not in the first repeat unit (at positions 172–175) (see Fig. 4). Within a stretch of 100 nucleotides surrounding the termination region, there are only five positions that are different in the two repeat units (328, 329, 331, 333, 346; Fig. 4). One or more of these five bases may hold a key position in the sequence necessary for ρ -dependent termination.

The base change at position 346 is the closest to the termination region and interrupts a stretch of 11 A·T base pairs. This difference seems to be the most likely candidate responsible for triggering the termination in the second repeat. The importance of this base change is further supported by the fact that it occurs within a seven-base pair sequence, CAATCAA, which is also found at the ρ -dependent λ_{R1} termination site²¹. These two facts taken together strongly suggest that this specific sequence may be recognised by the ρ factor or the ρ -polymerase complex.

It is interesting to note that the mutants of Maizels, which affect RNA polymerase pausing, also occur 7–10 nucleotides upstream from the pause point^{12,26}—a spacing which approximates one turn of the DNA helix and which is also found in a critical region of *E. coli* promoters²⁹.

Inspection of the sequence in the third repeat suggests that it should also serve as a functional termination site (that is at positions 528–531), since it has the same sequence as the second repeat in the region of termination (Fig. 4). The experiments described here do not provide a rigorous test of this prediction since addition of ρ factor always results in termination in the preceding repeat unit. However, in those experiments where high salt was used to interfere with termination, we did find some suggestion of termination at the predicted site in the third repeat (Fig. 3a). The few base differences between the third repeat unit and the other two units create the potential for a stable seven-base pair stem and six-base loop structure (positions 499–519) which is quite similar to that observed at other termination sites as discussed above. If it is postulated that this stem and loop would serve to facilitate termination, then the three repeat units constitute a graduated set of termination sites of increasing strength. While there are several roles that might be ascribed to such an arrangement of termination sites, there is at present no *in vivo* evidence that bears on this question. The only *in vivo* tRNA₁^{Tyr} precursor isolated thus far extends just a few bases beyond the CCA terminus of the mature tRNA, and this is most likely the product of some processing enzyme rather than the primary transcript. The repeated structure of the tRNA₁^{Tyr} gene is not peculiar to the tRNA₁^{Tyr} transducing phage, but it does exist in the *E. coli* chromosome in all strains analysed so far (J. Rossi and A. L., in preparation).

It should be noted that, in addition to the sequence differences discussed above, the three potential termination sites are distinguished from each other by the structure of newly synthesised RNA behind the polymerase. For example, in the second and third repeats, the polymerase is linked to RNA that is an almost identical copy of the sequence being transcribed. Although at present there is no evidence, from this gene or others, that bears on this general question, the arrangement of restriction cut sites within the repeated units will facilitate the type of genetic rearrangements which might be useful in pursuing this point.

In addition to the experiments reported here using purified restriction fragments as templates, several other laboratories have been studying transcription of the tRNA₁^{Tyr} gene using intact

$\Phi 80\text{su}_{111}^+$ transducing phage DNA as template. In most cases the sizes observed for the tRNA-containing transcripts were consistent with our results^{13–30,31}, although in one report¹⁴ a considerably smaller transcript would seem to correspond better to a termination in the first repeat unit.

One striking difference from our results is that in most of the experiments using intact $\Phi 80\text{su}_{111}^+$ transducing phage DNA as a template, the size of the tRNA-containing transcript was unaffected by the addition of ρ factor, although the quantity of tRNA sequences assayed was enhanced considerably. We do not have an explanation for this apparent termination in the absence of ρ factor, although it may be related to differences in RNA polymerase preparations. All ρ -independent termination sites analysed so far end with an uninterrupted sequence of four to seven U residues. Nowhere in the distal portion of the tRNA^{Tyr}₁ gene, that is, within 500 nucleotides of the CCA terminus of the mature sequences, is there a sequence coding for an uninterrupted cluster of more than three U residues, or even a singly interrupted cluster of four or more U residues. In fact, the only moderately U-rich cluster within the repeated sequences comes from the termination site described in this report.

While it is possible that intact transducing phage DNA as a template does respond differently to ρ factor in termination of the tRNA^{Tyr}₁ gene, there are some reservations. The problems of working with an extremely large multigenic template are considerably more complex than with a purified restriction fragment in which initiation at the tRNA^{Tyr}₁ promoter is readily monitored and the primary gene transcripts can be isolated with high purity.

Conclusions

In the system described above, the site-specific termination of transcription depends completely on the addition of ρ factor. This termination is sensitive both to salt and to heparin, and the action of ρ factor is catalytic. No striking stimulation of initiation of transcription by ρ factor (twofold or less) was observed.

If this ρ -dependent termination site also functions *in vivo*, then these results provide a solution to the long-standing problem of defining an unprocessed primary transcript from a tRNA gene and should greatly facilitate further progress in this area.

The ρ -dependent termination is not precise but takes place over a distance of approximately four or five adjacent base pairs, as has been observed for other termination sites not so tightly coupled to the presence of ρ factor. Whereas the immediate vicinity of the termination site is very high in A·T, the adjacent sequences on both sides are extremely rich in G·C.

In addition to the possible role of structural features in site-specific termination, our results suggest that specific sequences in the DNA template may in fact be recognised in the ρ -polymerase termination reaction.

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Fig 1.

DNA restriction fragments used as templates for promoter-dependent transcription of the tyrosine tRNA su_3 ($tRNA_{I}^{Tyr}$) gene. The isolation of *Cla* and *Bla* from the 'singlet' transducing phage, $\Phi 80psu_3^{+32}$ has been described previously¹⁰. The *HpaII* fragment is obtained by digestion of *Bla* with *HpaII* and purification by preparative polyacrylamide gel electrophoresis^{10,19}. The approximate size of the fragments in base pairs is given in parentheses^{10,19}. The 85 nucleotides coding for the mature tRNA sequence are indicated by the hatched box. These are preceded by 41 nucleotides coding for the 5' end of the primary transcript or precursor molecule³³. The end of the promoter and the startpoint of transcription is marked (P). The ρ -dependent termination site is marked (T), and a potential downstream termination site is marked (T'). The 178-base pair repeat unit (see Fig. 4) is indicated by overhead brackets. Promoter-dependent transcription ($\sim$) in the presence and absence of ρ factor is indicated.

Fig 2.

Effect of ρ factor on transcription from various templates carrying the $tRNA_{I}^{Tyr}$ gene. Standard conditions for RNA synthesis were 40 mM Tris-HCl pH 7.9, 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.1 mM EDTA Na_3 and 15% glycerol in a final volume of 10 μ l. Nucleotides were added to a final concentration of 500 μ M CpC, 500 μ M GTP, 15 μ M each ATP, CTP and UTP. [α - ^{32}P] UTP as the labelled triphosphate had a specific activity of 15,000 c.p.m. $pmol^{-1}$ (New England Nuclear). The dinucleoside monophosphate CpC was added to stimulate initiation of transcription¹¹. *E. coli* RNA polymerase (RNA nucleotidyl-transferase EC 2.7.7.6.) was prepared according to Burgess³⁴ and was present at 1.5 pmol per 10 μ l.

Termination factor ρ (prepared according to Roberts³) was present as indicated, and the DNA fragments were added as follows: lane 1, 0.15 pmol *Cla*; lane 2, 0.15 pmol *Cla* and 0.25 pmol ρ ; lane 3, 0.06 pmol *HpaII*; lane 4, 0.06 pmol *HpaII* and 0.25 pmol ρ ; lane 5, 0.08 pmol *Bla*; lane 6, 0.08 pmol *Bla* and 0.25 pmol ρ ; lane 7, 0.08 pmol *Bla* and 0.2 pmol ρ (a gift from Dennis Kleid); lane 8, 0.08 pmol *Bla* and 0.2 pmol ρ (a gift from Elizabeth Bikoff). Incubation was for 60 min at 37 °C. RNA synthesis was stopped by the addition of 50 μ l electrophoresis loading buffer (7 M urea, 0.1% SDS, 90 mM Tris base, 90 mM boric acid, 4 mM EDTA Na₂, and the dye markers xylene cyanol and bromophenol blue), and heating for 2 min to 100 °C with subsequent quick cooling on ice. The RNA products were separated on a 4.5% polyacrylamide slab gel (20 $\times$ 20 $\times$ 0.2 cm) containing 7 M urea (acrylamide: *N,N'* methylene bis acrylamide, 20:1). Electrophoresis was performed in TBE buffer (90 mM Tris base, 90 mM boric acid, 4 mM EDTA Na₂) at 50–70 mA until the bromophenol blue marker reached the bottom of the gel. M1 and M2 indicate the positions of the dye markers, bromophenol blue and xylene cyanol, respectively. The approximate size of the major transcription products (no. of nucleotides) is indicated in the left margin. The minor band which migrates just ahead of the ρ -dependent product (lanes 3–8) has not been analysed but is visually estimated to contain less than a few per cent of the total counts incorporated.

Fig 3.

a, Effect of salt concentration on ρ -dependent termination. RNA polymerase (3.2 pmol) was preincubated with *Bla* (0.45 pmol) in the presence of CpC and GTP (500 mM each) in standard conditions (see Fig. 2) in a total volume of 35 μ l for 10 min at 37 $^{\circ}$ C. Aliquots of 7 μ l were withdrawn, and the remaining triphosphates were added to a final concentration of 15 μ M along with ρ (0.25 pmol per assay) and KCl as indicated. The mixtures (final volume 10 μ l) were further incubated for 50 min at 37 $^{\circ}$ C. This preincubation and the prior formation of initiation complexes are necessary because of the salt-sensitivity of the tRNA^{tyrT} promoter (see ref. 11). Transcription was stopped, and RNA products were analysed on a 4% polyacrylamide gel as described in Fig. 2. Lane 1, $-\rho$, $-\text{KCl}$; lane 2, $+\rho$, $-\text{KCl}$; lane 3, $+\rho$, 50mM KCl; lane 4, $+\rho$, 100mM KCl; lane 5, $+\rho$, 150mM KCl. *b*, effect of ρ factor on promoter-dependent initiation of transcription at different polymerase: DNA ratios. Varying amounts of RNA polymerase were incubated in standard conditions with the *Cla* fragment (0.1 pmol per 10 μ l reaction) in the presence or absence of ρ factor (0.2 pmol per 10 μ l). Incubation was 45 min at 37 $^{\circ}$ C, and the RNA products were analysed on a 5% polyacrylamide gel (see Fig. 2). Incorporation of radioactivity into the main product was measured by Cerenkov counting of the excised gel band. The RNA polymerase: DNA ratios for each reaction and the amount of radioactivity in the excised band were as follows: lane 1, 1.8:1, $+\rho$ (1,388 c.p.m.); lane 2, 1.8:1, $-\rho$ (1,400 c.p.m.); lane 3, 3.7:1, $+\rho$ (3,841 c.p.m.); lane 4, 3.7:1, $-\rho$ (2,159 c.p.m.); lane 5, 7.5:1, $+\rho$ (7,611 c.p.m.); lane 6, 7.5:1, $-\rho$ (7,548 c.p.m.); lane 7, 15:1, $+\rho$ (5,692 c.p.m.); lane 8, 15:1, $-\rho$ (7,969 c.p.m.).

Fig 4.

Sequence of the distal region of the tRNA^{Tyr} gene19. The DNA strand having the same sequence as the tRNA is arranged to facilitate comparison of the 178-base pair repeating units. The first base shown (42) corresponds to the first base in the mature tRNA (the 41 nucleotides corresponding to the 5' portion of the primary transcript, or precursor11,33, are not shown). The sequences corresponding to the mature tRNA are indicated by an overhead bracket, and that portion of this sequence which appears at the beginning of each repeat unit by a dashed bracket (see text and ref. 19). Positions in one repeat which differ from the others are marked by an asterisk. The location of the p-dependent termination of transcription is marked (T) (see text and Fig. 5). The downstream termini of the three restriction fragments used as templates for transcription in this study are also indicated (see Fig. 1).

Fig 5.

Analysis of the 3'-terminal oligonucleotides of the promoter-dependent, ρ -dependent, transcript. The 360-nucleotide transcript from the *HpaII* reaction (see Fig. 2) was prepared and isolated as described in legend Fig. 2 with the following preparative modifications: total reaction volume, 30 μ l; *HpaII* restriction fragment, 0.3 pmol; RNA polymerase, 5.0 pmol; ρ factor, 0.6 pmol; [α - 32 P]UTP at 15 μ M with a specific activity of $1-1.5 \times 10^5$ c.p.m. μ mol $^{-1}$, GTP, CTP, and ATP each at 500 μ M. The radioactive band was excised from the gel and disrupted in a 5 ml syringe. The RNA was eluted with 1 M NaCl and recovered by ethanol precipitation as described previously¹¹. The RNA was digested with T1 ribonuclease and passed over a column of DEAE-cellulose²⁰ as described previously¹⁶. Oligonucleotides containing a 3'-terminal phosphate were eluted with solvent A (0.05 M morpholinium chloride, 0.1 M MgCl₂, 1.0 M NaCl, 20% dimethylsulphoxide, pH 8.7). Oligonucleotides which contain a nucleoside at their 3' end, and thus should be derived from the 3' terminus of the transcript, were subsequently eluted from the column by simple displacement with solvent A containing 0.1 M sorbitol. The 3'-oligonucleotides were desalted by dialysis against distilled water, concentrated under reduced pressure at 35 °C, and fractionated in the first dimension by electrophoresis on cellogel in 8 M urea at pH 3.5 and in the second dimension by ascending thin layer chromatography on plates of DEAE-cellulose (9:1, cellulose :DEAE-cellulose, 40 $\times$ 20 cm) using homochromatography solvent B35-37. The radioactive spots were eluted, quantified by Cerenkov counting, and subjected to further analysis by pancreatic ribonuclease digestion using standard techniques³⁵⁻³⁷. The schematic diagram next to the autoradiogram shows the sequence deduced for each spot, the single base differences deduced on the basis of mobility shifts between the spots, and the per cent of the total radioactivity found in each spot.

Table 1

Quantitation of T1 oligonucleotides

Oligo no.	Sequence	Position in sequence	<i>Cla</i> - ρ	Mole ratio <i>HpaII</i>	
				-ρ	+ρ
2	CAAUU...G	164–178	0.94	0.94	0.96
3	CAAUC...G	342–356*	–	0.98	0.08
4	ACUCU...G	73–84	0.98	0.90	0.92
5	AACUU...G	146–157	0.95	0.95	0.94
6	AACUC...G	324–333	–	1.01	0.90
7	CAUUACCCG	32–40	1.00	0.97	0.92
10a	CUUCCCG	2–8	1.03	1.03	1.01
b	AAUCCG	158–163	1.03	2.05	2.03
c	ACUAAG	336–341	–	1.03	–
11a	ACUUCG	385–390	–	1.03	–
b	UCAUCG	94–99	1.37	0.97	1.37
c	AUAAG	88–93	1.37	0.97	1.37
13a	UUCCCG	9–13	1.37	0.97	1.37
b	UCCUG	49–54	0.71	1.02	1.02
		140–145	1.43	2.03	2.05
14	CCCAUG	318–323	–	–	–
		179–184	1.12	2.14	1.09
16	ACUCG	357–362	–	1.15	1.14
18	CUUCG	218–222	–	2.25	1.10
		202–206	–	–	–
		380–384	–	–	–

The promoter-dependent tRNA-containing transcript made from the *HpaII* fragment, either in the presence or absence of ρ, or from the *Cla* fragment in the absence of ρ, was isolated from a 5% urea gel as described in Fig. 5 legend. The ³²P label was either in GTP or UTP (in which case all ratios are corrected for the number of U residues in the oligonucleotide). The eluted RNA was digested with T1 ribonuclease and fractionated by electrophoresis at pH 3.5 in the first dimension and homochromatography in the second dimension^{35–37}. Each spot of radioactivity was quantified and then analysed by pancreatic RNase digestion. The pancreatic analysis, in conjunction with the mobility of the spot, permitted an unambiguous correlation of each T1 spot with those predicted from the DNA sequence (see Fig. 4). In the case of unresolved oligonucleotides in a spot (marked by brackets above), this analysis yielded the relative amount of each oligonucleotide in the spot. Those oligonucleotides which are different in the +ρ and -ρ reactions, or which are crucial in positioning the termination site are marked by an asterisk. Oligonucleotides not present in a particular transcript are marked by a dash.