6.5 Termination of Transcription

When the polymerase reaches a **terminator** at the end of a gene it falls off the template, releasing the RNA. *E. coli* cells contain about equal numbers of two kinds of terminators. The first kind, known as **intrinsic terminators,** function with the RNA polymerase by itself without help from other proteins. The second kind depend on an auxiliary factor called **rho** (ρ). Naturally, these are called rho-dependent terminators. Let us consider the mechanisms of termination employed by these two systems, beginning with the simpler, intrinsic terminators.

Rho-Independent Termination

Rho-independent, or intrinsic, termination depends on terminators consisting of two elements: an inverted repeat followed immediately by a T-rich region in the nontemplate strand of the gene. The model of termination we will present later in this section depends on a "hairpin" structure in the RNA transcript of the inverted repeat. Before we get to the model, we should understand how an inverted repeat predisposes a transcript to form a hairpin.

Inverted Repeats and Hairpins Consider this inverted repeat:

5′-TACGAAGTTCGTA-3′
•
3′-ATGCTTCAAGCAT-5′

Such a sequence is symmetrical around its center, indicated by the dot; it would read the same if rotated 180 degrees in the plane of the paper, and if we always read the strand that runs 5′→3′ left to right. Now observe that a transcript of this sequence

UACGAAGUUCGUA

is self-complementary around its center (the underlined G). That means that the self-complementary bases can pair to form a hairpin as follows:

U • A
A • U
C • G
G • C
A • U
A U
G

The A and the U at the apex of the hairpin cannot form a base pair because of the physical constraints of the turn in the RNA.

The Structure of an Intrinsic Terminator The *E. coli trp* operon (Chapter 7) contains a DNA sequence called an attenuator that causes premature termination of transcription. The *trp* attenuator contains the two elements (an inverted repeat and a string of T's in the nontemplate DNA strand) suspected to be vital parts of an intrinsic terminator, so Peggy Farnham and Terry Platt used attenuation as an experimental model for normal termination.

The inverted repeat in the *trp* attenuator is not perfect, but 8 bp are still possible, and 7 of these are strong G–C pairs, held together by three hydrogen bonds. The hairpin looks like this:

A • U
G • C
C • G
C • G
C • G
G • C
C • G
C • G A
U U
A A

Notice that a small loop occurs at the end of this hairpin because of the U–U and A–A combinations that cannot base-pair. Furthermore, one A on the right side of the stem has to be "looped out" to allow 8 bp instead of just 7. Still, the hairpin should form and be relatively stable.

Farnham and Platt reasoned as follows: As the T-rich region of the attenuator is transcribed, eight A–U base pairs would form between the A's in the DNA template strand and the U's in the RNA product. They also knew that rU–dA base pairs are exceptionally weak; they have a melting temperature 20°C lower than even rU–rA or dT–rA pairs. This led the investigators to propose that the polymerase paused at the terminator, and then the weakness of the rU–dA base pairs allowed the RNA to dissociate from the template, terminating transcription.

What data support this model? If the hairpin and string of rU–dA base pairs in the *trp* attenuator are really important, we would predict that any alteration in the base sequence that would disrupt either one would be deleterious to attenuation. Farnham and Platt devised the following in vitro assay for attenuation (Figure 6.45): They started with a *Hpa*II restriction fragment containing the *trp* attenuator and transcribed it in vitro. If attenuation works, and transcription terminates at the attenuator, a short (140-nt) transcript should be the result. On the other hand, if transcription fails to terminate at the attenuator, it will continue to the end of the fragment, yielding a run-off transcript 260 nt in length. These two transcripts are easily distinguished by electrophoresis.

When these investigators altered the string of eight T's in the nontemplate strand of the terminator to the sequence TTTTGCAA, creating the mutant they called *trp a*1419,

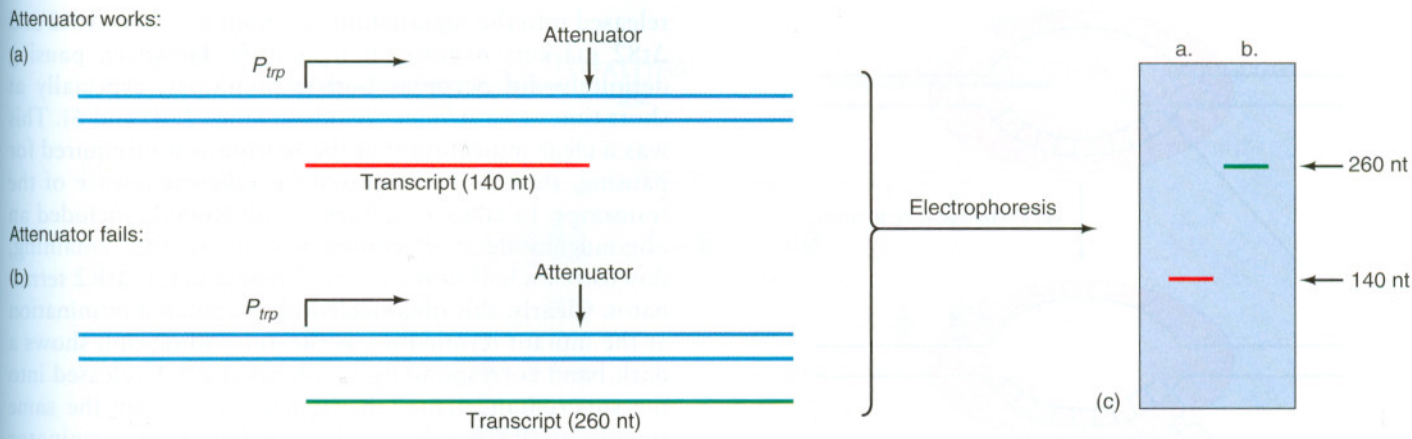

Figure 6.45 An assay for attenuation. (a) When the DNA fragment containing the *trp* promoter and attenuator is transcribed under conditions in which the attenuator works, transcription stops in the attenuator, and a 140-nt transcript (red) results. **(b)** When the same DNA fragment is transcribed under conditions that cause the attenuator to fail, a run-off transcript of 260 nt (green) is the result. **(c)** The transcripts from the two different reactions can be distinguished easily by electrophoresis. Using this assay, one can tell whether the attenuator works under a variety of conditions.

attenuation was weakened. This is consistent with the hypothesis that the weak rU–dA pairs are important in termination, because half of them would be replaced by stronger base pairs in this mutant.

Moreover, this mutation could be overridden by substituting the nucleotide iodo-CTP (I-CTP) for normal CTP in the in vitro reaction. The most likely explanation is that base-pairing between G and iodo-C is stronger than between G and ordinary C. Thus, the GC-rich hairpin should be stabilized by I-CMP, and this effect counteracts the loss of weak base pairs in the region following the hairpin. On the other hand, IMP (inosine monophosphate, a GMP analog) should weaken base-pairing in the hairpin because I–C pairs, with only two hydrogen bonds holding them together, are weaker than G–C pairs with three. Sure enough, substituting ITP for GTP in the transcription reaction weakened termination at the attenuator. Thus, all of these effects are consistent with the hypothesis that the hairpin and string of U's in the transcript are important for termination. However, they do not identify the roles that these RNA elements play in pausing and termination.

SUMMARY Using the *trp* attenuator as a model terminator, Farnham and Platt showed that intrinsic terminators have two important features: (1) an inverted repeat that allows a hairpin to form at the end of the transcript; (2) a string of T's in the nontemplate strand that results in a string of weak rU–dA base pairs holding the transcript to the template strand.

A Model for Termination Several hypotheses have been proposed for the roles of the hairpin and string of rU–dA base pairs in the mechanism of termination. Two important clues help narrow the field of hypotheses. First, hairpins are found to destabilize elongation complexes that are stalled artificially (not at strings of rU–dA pairs). Second, terminators in which half of the inverted repeat is missing still stall at the strings of rU–dA pairs, even though no hairpin can form. This leads to the following general hypothesis: The rU–dA pairs cause the polymerase to pause, allowing the hairpin to form and destabilize the already weak rU–dA pairs that are holding the DNA template and RNA product together. This destabilization results in dissociation of the RNA from its template, terminating transcription.

W. S. Yarnell and Jeffrey Roberts proposed a variation on this hypothesis in 1999, as illustrated in Figure 6.46. This model calls for the withdrawal of the RNA from the active site of the polymerase that has stalled at a terminator—either because the newly formed hairpin helps pull it out or because the polymerase moves downstream without elongating the RNA, thus leaving the RNA behind. To test their hypothesis, Yarnell and Roberts used a DNA template that contained two mutant terminators (ΔtR2 and Δt82) downstream of a strong promoter. These terminators had a T-rich region in the nontemplate strand, but only half of an inverted repeat, so hairpins could not form. To compensate for the hairpin, these workers added an oligonucleotide that was complementary to the remaining half of the inverted repeat. They reasoned that the oligonucleotide would base-pair to the transcript and restore the function of the hairpin.

To test this concept, they attached magnetic beads to the template, so it could be easily removed from the mixture magnetically. Then they used *E. coli* RNA polymerase to synthesize labeled RNAs in vitro in the presence and absence of the appropriate oligonucleotides. Finally, they removed the template magnetically to form a pellet and electrophoresed the material in the pellet and

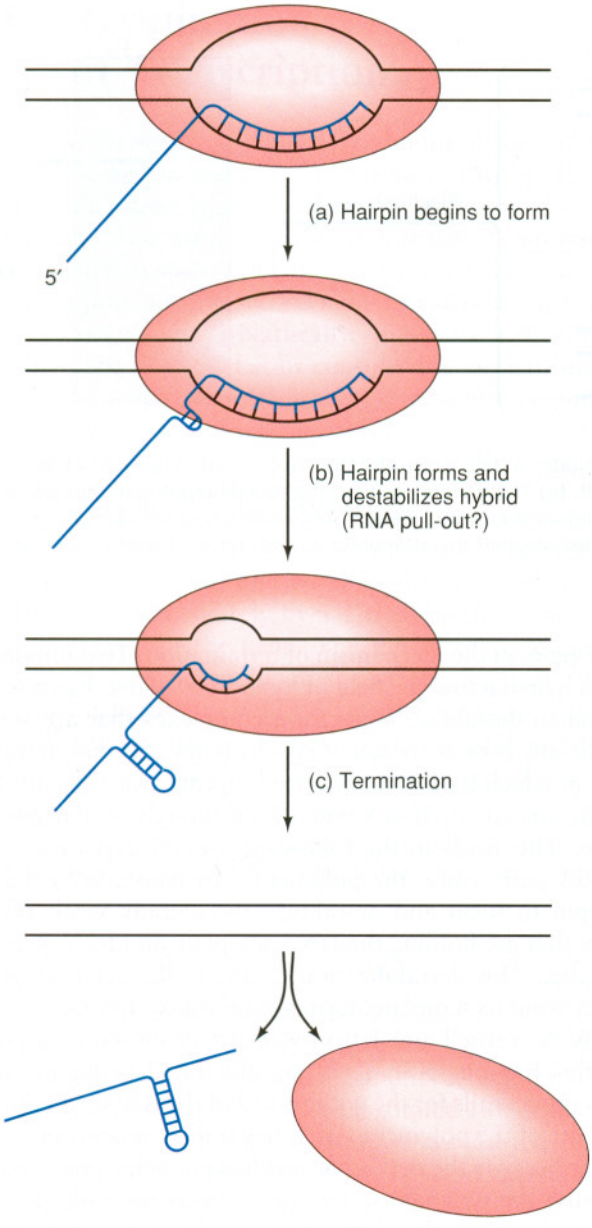

Figure 6.46 A model for intrinsic termination. (a) The polymerase has paused at a string of weak rU–dA base pairs, and a hairpin has started to form just upstream of these base pairs. **(b)** As the hairpin forms, it further destabilizes the RNA–DNA hybrid. This destabilization could take several forms: The formation of the hairpin could physically pull the RNA out of the polymerase, allowing the transcription bubble to collapse; conversely, it could cause the transcription bubble to collapse, expelling the RNA from the hybrid. **(c)** The RNA product and polymerase dissociate completely from the DNA template, terminating transcription.

the supernatant and detected the RNA species by autoradiography.

Figure 6.47 shows the results. In lanes 1–6, no oligonucleotides were used, so little incomplete RNA was released into the supernatant (see faint bands at ΔtR2 and Δt82 markers in lanes 1, 3, and 5). However, pausing definitely did occur at both terminators, especially at short times (see stronger bands in lanes 2, 4, and 6). This was a clear indication that the hairpin is not required for pausing, though it is required for efficient release of the transcript. In lanes 7–9, Yarnell and Roberts included an oligonucleotide (t19) complementary to the remaining, downstream half of the inverted repeat in the ΔtR2 terminator. Clearly, this oligonucleotide stimulated termination at the mutant terminator, as the autoradiograph shows a dark band corresponding to a labeled RNA released into the supernatant. This labeled RNA is exactly the same size as an RNA released by the wild-type terminator would be. Similar results, though less dramatic, were obtained with an oligonucleotide (t18) that is complementary to the downstream half of the inverted repeat in the Δt82 terminator.

To test further the importance of base-pairing between the oligonucleotide and the half-inverted repeat, these workers mutated one base in the t19 oligonucleotide to yield an oligonucleotide called t19H1. Lane 13 shows that this change caused a dramatic reduction in termination at ΔtR2. Then they made a compensating mutation in ΔtR2 and tested t19H1 again. Lane 14 shows that this restored strong termination at ΔtR2. This template also contained the wild-type t82 terminator, so abundant termination also occurred there. Lanes 15 and 16 are negative controls in which no t19H1 oligonucleotide was present, and, as expected, very little termination occurred at the ΔtR2 terminator.

Together, these results show that the hairpin itself is not required for termination. All that is needed is something to base-pair with the downstream half of the inverted repeat to destabilize the RNA–DNA hybrid. Furthermore, the T-rich region is not required if transcription can be slowed to a crawl artificially. Yarnell and Roberts advanced the polymerase to a site that had neither an inverted repeat nor a T-rich region and made sure it paused there by washing away the nucleotides. Then they added an oligonucleotide that hybridized upstream of the artificial pause site. Under these conditions, they observed release of the nascent RNA.

Termination is also stimulated by a protein called **NusA**, which appears to promote hairpin formation in the terminator. The essence of this model, presented in 2001 by Ivan Gusarov and Evgeny Nudler, is that the upstream half of the hairpin binds to part of the core polymerase called the **upstream binding site (UBS)**. This protein–RNA binding slows down hairpin formation and so makes termination less likely. But NusA loosens the association between the RNA and the UBS, thereby stimulating hairpin formation. This makes termination more likely. In Chapter 8, we will discuss NusA and its mode of action in more detail and see evidence for the model mentioned here.

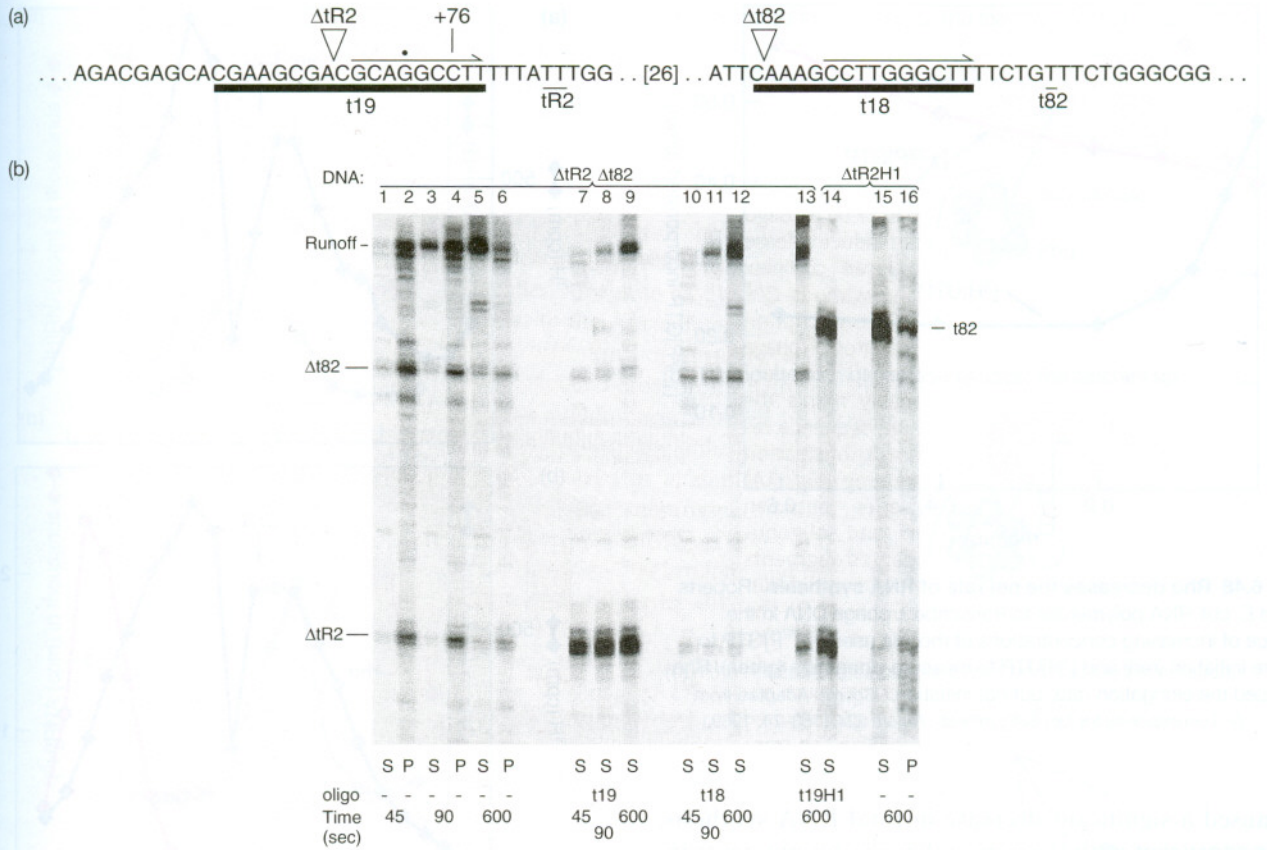

Figure 6.47 Release of transcripts from elongation complexes by oligonucleotides complementary to mutant terminators. **(a)** Scheme of the template used in these experiments. The template contained two mutant terminators, ΔtR2, and Δt82, situated as shown, downstream of a strong promoter. The normal termination sites for these two terminators are labeled with thin underlines. The black bars denote regions complementary to the oligonucleotides used (t19 and t18). The rightward arrows denote the half inverted repeats remaining in the mutant terminators. The dot indicates the site of a base altered in the t19HI oligonucleotide and of a compensating mutation in the DNA template in certain of the experiments. The template was attached to a magnetic bead so it could be removed from solution easily by centrifugation. **(b)** Experimental results. Yarnell and Roberts synthesized labeled RNA in the presence of the template in panel (a) and; no oligonucleotide (lanes 1–6 and 15–16), the t19 oligonucleotide (lanes 7–9), the t18 oligonucleotide (lanes 10–12); and the t19HI oligonucleotide (lanes 13–14). They allowed transcription for the times given at bottom, then removed the template and any RNA attached to it by centrifugation. They electrophoresed the labeled RNA in the pellet (P) or supernatant (S), as indicated at bottom, and autoradiographed the gel. The positions of run-off transcripts, and of transcripts that terminated at the ΔtR2 and Δt82 terminators, are indicated at left. *(Source: (a–b)* Yarnell, W.S. and Roberts, J.W. Mechanism of intrinsic transcription termination and antitermination. *Science* 284 (23 April 1999) 611–12. © AAAS.)

SUMMARY The essence of a bacterial terminator is twofold: (1) base-pairing of something to the transcript to destabilize the RNA–DNA hybrid; and (2) something that causes transcription to pause. A normal intrinsic terminator satisfies the first condition by causing a hairpin to form in the transcript, and the second by causing a string of U's to be incorporated just downstream of the hairpin.

Rho-Dependent Termination

Jeffrey Roberts discovered rho as a protein that caused an apparent depression of the ability of RNA polymerase to transcribe certain phage DNAs in vitro. This depression is simply the result of termination. Whenever rho causes a termination event, the polymerase has to reinitiate to begin transcribing again. And, because initiation is a time-consuming event, less net transcription can occur. To establish that rho is really a termination factor, Roberts performed the following experiments.

Rho Affects Chain Elongation, But Not Initiation Just as Travers and Burgess used [γ-^{32}P]ATP and [^{14}C]ATP to measure transcription initiation and total RNA synthesis, respectively, Roberts used [γ-^{32}P]GTP and [^{3}H]UTP for the same purposes. He carried out in vitro transcription reactions with these two labeled nucleotides in the presence of increasing concentrations of rho. Figure 6.48 shows the results. We see that rho had little effect on initiation; if anything, the rate of initiation went up. But

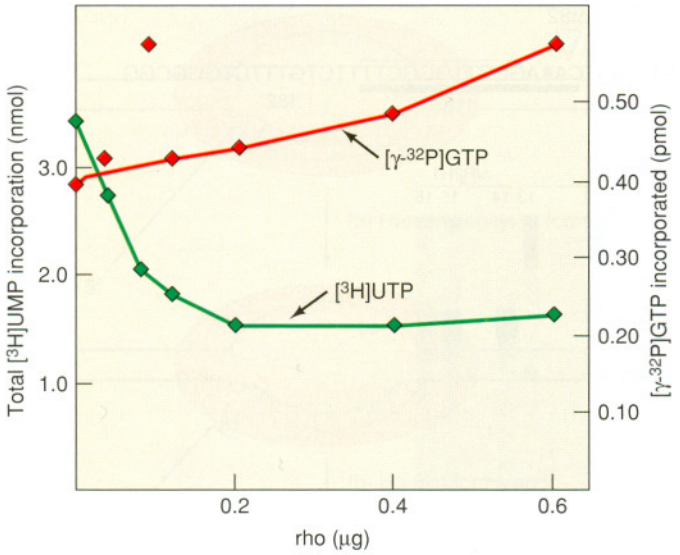

Figure 6.48 Rho decreases the net rate of RNA synthesis. Roberts allowed *E. coli* RNA polymerase to transcribe λ phage DNA in the presence of increasing concentrations of rho. He used [γ-^{32}P]GTP to measure initiation (red) and [^{3}H]UTP to measure elongation (green). Rho depressed the elongation rate, but not initiation. (*Source:* Adapted from Roberts, J.W. Termination factor for RNA synthesis, *Nature* 224:1168–74, 1969.)

rho caused a significant decrease in total RNA synthesis. This is consistent with the notion that rho terminates transcription, thus forcing time-consuming reinitiation. This hypothesis predicts that rho would cause shorter transcripts to be made.

Rho Causes Production of Shorter Transcripts It is relatively easy to measure the size of RNA transcripts by gel electrophoresis or, in 1969, when Roberts performed his experiments, by ultracentrifugation. But just finding short transcripts would not have been enough to conclude that rho was causing termination. It could just as easily have been an RNase that chopped up longer transcripts into small pieces.

To exclude the possibility that rho was simply acting as a nuclease, Roberts first made ^{3}H-labeled λ RNA in the absence of rho, then added these relatively large pieces of RNA to new reactions carried out in the presence of rho, in which [^{14}C]UTP was the labeled RNA precursor. Finally, he measured the sizes of the ^{14}C- and ^{3}H-labeled λ RNAs by ultracentrifugation. Figure 6.49 presents the results. The solid curves show no difference in the size of the preformed ^{3}H-labeled RNA even when it had been incubated with rho in the second reaction. Rho therefore shows no RNase activity. However, the ^{14}C-labeled RNA made in the presence of rho (red line in Figure 6.49b) is obviously much smaller than the preformed RNA made without rho. Thus, rho is causing the synthesis of much smaller RNAs. Again, this is consistent with the role of rho in terminating transcription. Without rho, the transcripts grew to abnormally large size.

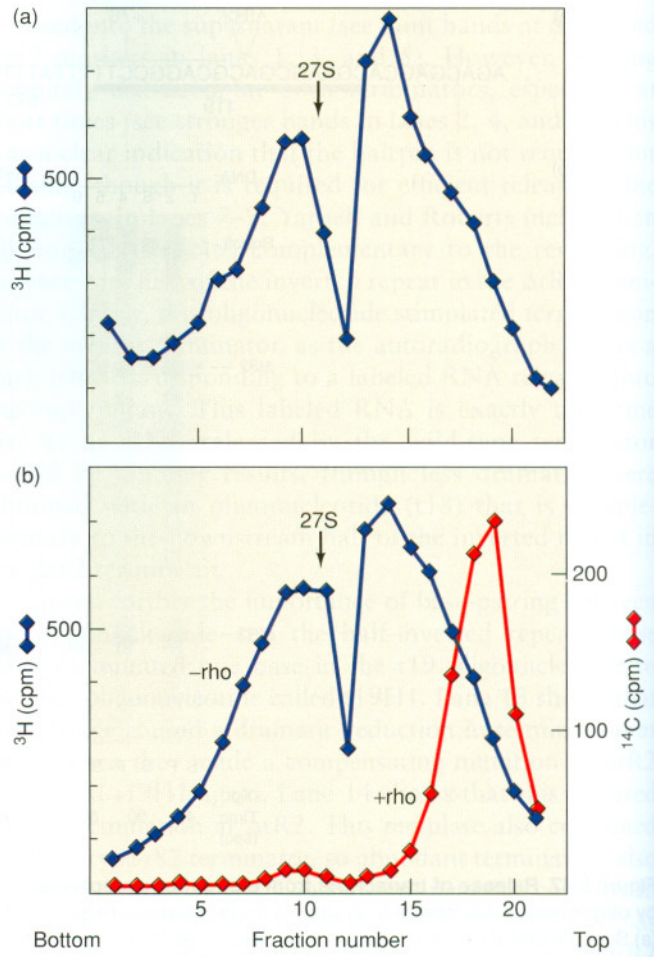

Figure 6.49 Rho reduces the size of the RNA product. (a) Roberts allowed *E. coli* RNA polymerase to transcribe λ DNA in the absence of rho. He included [^{3}H]UTP in the reaction to label the RNA. Finally, he used ultracentrifugation to separate the transcripts by size. He collected fractions from the bottom of the centrifuge tube, so low-numbered fractions, at left, contained the largest RNAs. **(b)** Roberts used *E. coli* RNA polymerase to transcribe λ DNA in the presence of rho. He also included [^{14}C]ATP to label the transcripts, plus the ^{3}H-labeled RNA from panel (a). Again, he ultracentrifuged the transcripts to separate them by size. The ^{14}C-labeled transcripts (red) made in the presence of rho were found near the top of the gradient (at right), indicating that they were relatively small. On the other hand, the ^{3}H-labeled transcripts (blue) from the reaction lacking rho were relatively large and the same size as they were originally. Thus, rho has no effect on the size of previously made transcripts, but it reduces the size of the transcripts made in its presence. (*Source:* Adapted from Roberts, J.W. Termination factor for RNA synthesis, *Nature* 224:1168–74, 1969.)

Rho Releases Transcripts from the DNA Template Finally, Roberts used ultracentrifugation to analyze the sedimentation properties of the RNA products made in the presence and absence of rho. The transcripts made without rho (Figure 6.50a) cosedimented with the DNA template, indicating that they had not been released from their association with the DNA. By contrast, the transcripts made in the presence of rho (Figure 6.50b) sedimented at a much lower

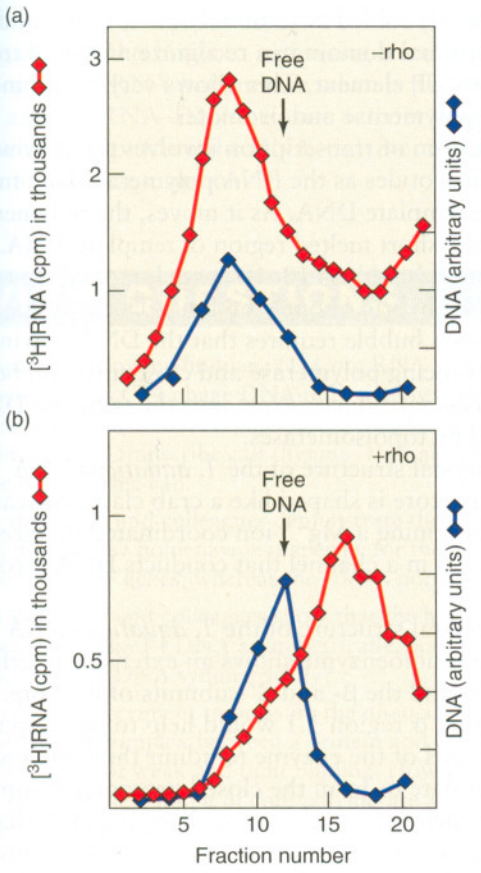

Figure 6.50 Rho releases the RNA product from the DNA template. Roberts transcribed λ DNA under the same conditions as in Figure 6.49, in the **(a)** absence or **(b)** presence of rho. Then he subjected the ^{3}H-labeled product (red) to ultracentrifugation to see whether the product was associated with the DNA template (blue). (a) The RNA made in the absence of rho sedimented together with the template in a complex that was larger than free DNA. (b) The RNA made in the presence of rho sedimented independently of DNA at a position corresponding to relatively small molecules. Thus, transcription with rho releases transcripts from the DNA template. (*Source:* Adapted from Roberts, J.W. Termination factor for RNA synthesis, *Nature* 224:1168–74, 1969.)

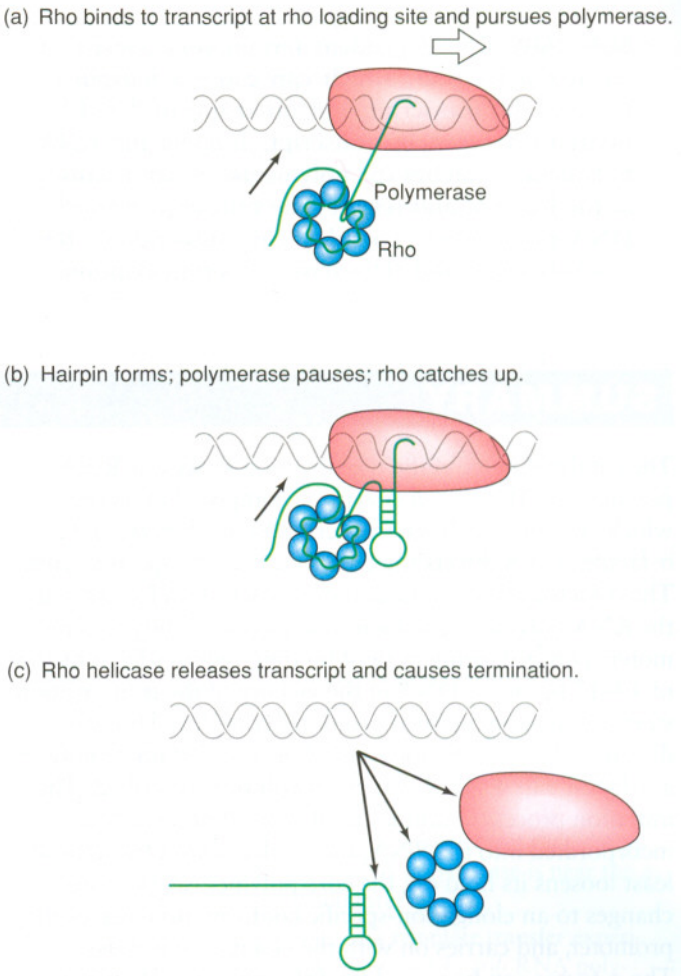

Figure 6.51 A model of rho-dependent termination. (a) As polymerase (red) makes RNA, rho (blue) binds to the transcript (green) at a rho loading site and pursues the polymerase. **(b)** When the hairpin forms in the transcript, the polymerase pauses, giving rho a chance to catch up. **(c)** Rho helicase unwinds the RNA–DNA hybrid and releases the transcript.

rate, independent of the DNA. Thus, rho seems to release RNA transcripts from the DNA template. In fact, rho (the Greek letter ρ) was chosen to stand for "release."

The Mechanism of Rho How does rho do its job? Because we have seen that σ operates through RNA polymerase by binding tightly to the core enzyme, we might envision a similar role for rho. But rho seems not to have affinity for RNA polymerase. Whereas σ is a critical part of the polymerase holoenzyme, rho is not.

Instead (Figure 6.51), rho binds to the transcript upstream of the termination site at a **rho loading site,** a sequence of 60−100 nt that is relatively free of secondary structure and is rich in cytosine residues, Rho consists of a hexamer of identical subunits, each of which has ATPase activity. Binding of rho to the RNA activates the ATPase, which supplies the energy to propel the rho hexamer along the RNA in the 5′→3′ direction, following the RNA polymerase. This chase continues until the polymerase stalls in the terminator region just after making the RNA hairpin. Then rho can catch up and release the transcript. In support of this hypothesis, Terry Platt and colleagues showed in 1987 that rho has RNA–DNA helicase activity that can unwind an RNA–DNA hybrid. Thus, when rho encounters the polymerase stalled at the terminator, it can unwind the RNA–DNA hybrid within the transcription bubble, releasing the RNA and terminating transcription.

The rho hexamer has the shape of a lock washer, an open circle with slightly offset ends. This presumably allows the growing RNA to enter the hexamer through the opening. Each of the six subunits of rho has an RNA binding site, and they all seem to bind the RNA such that it zigzags from one subunit to the next.