Rho-independent termination requires an inverted repeat (palindromic) sequence followed by a U-rich segment in the transcribed RNA. The assay in the diagram above is an example of how termination can be studied. The rate at which the RNA polymerase is able synthesize the transcript determines whether attenuation occurs or not.

RNA polymerase pauses at AU-rich sequences. Formation of a hairpin in the RNA at the location of an inverted repeat further destabilizes the complex, increasing the probability that the nascent RNA chain will be released from the complex.

Rho decreases the net rate of RNA synthesis. Rho reduces the size of the RNA product. Plot shows the distribution of labeled RNA in a sucrose gradient. The larger molecules sediment more rapidly, toward the bottom of the tube at the left.

Rho releases RNA from the DNA template. Upper plot in the absence of Rho. Lower plot in the presence of Rho. Again, these are sucrose gradients with sedimentation right to left. Red represents the $^3$H label in RNA; blue represents DNA, measured by the absorbance.

Rho attaches to the nascent RNA at a “loading site” and migrates 5’ to 3’ towards the RNA polymerase. When it reaches a hairpin structure near the RNA polymerase it promotes dissociation.
Rho-dependent termination and ATPases in transcript termination
John P. Richardson

Diagram of a transcription elongation complex before and after attachment of Rho to the nascent RNA. Rho acts to pull the nascent transcript from the elongation complex, presumably in an ATP-dependent manner.

Lambda is a temperate, not virulent, phage.
Weaver pp 215-230

N and cro are the first two genes transcribed after infection. Cro represses transcription of the cl gene, N is an antiterminator. Q, also an antiterminator, allows the late genes encoding the phage coat proteins and lytic functions to be expressed. cl encodes the λ repressor. O, P, Q favor the lytic option; cII and cIII favor the lysogenic response. S and R control release of the phage from the cell.
When N protein is present in the cell it binds the N-utilization site in the mRNA and interacts with the RNA polymerase and host proteins called *nus*, for N-utilization substances.

The presence of N in the transcribing complex allows transcription to proceed past the termination site, may act to suppress pausing.

NusA tethers N and boxB to the RNA polymerase; additional proteins bind to boxA, strengthening the complex, enhancing its processiveness and making it more resistant to termination.

Q binds directly to the gur (Q utilization) site in the DNA. In the absence of Q, RNA polymerase pauses but then continues to the downstream termination site. When Q is present, it binds to the polymerase, altering it such that hairpin formation and termination at the downstream terminator are suppressed.

Establishing lysogeny requires suppression of the lytic response and integration of the phage DNA into the host chromosome.

cII facilitates binding of RNA polymerase to PRE; cIII protects cII from cellular proteinases.

Dnase footprint analysis of the interaction of cII with PRE and P1 in the -35 box. Stimulates repression and integration.

Methylation protection with DMS. Left: Protection by cII. Right: Protection by RNA polymerase +/- cII.
The cI repressor binds to O\textsubscript{x} and O\textsubscript{y}, blocking leftward and rightward transcription. At the same time it stimulates transcription of the cI gene.

Figure 8.26

DNA fragment used to assay transcription from cI and cro promoters

Figure 8.27

Products of transcription from the 790 bp fragment as a function of cI concentration. Cro transcription is shut down first, followed by cI.

Figure 8.28

Key is to provide plasmids with a collection of random mutations in the rpo gene to the cell.

Only cells receiving a plasmid that encoded a mutant rpoD gene that compensated for the mutation in the cI gene could grow in kanamycin.

Figure 8.30

Principle of intergenic suppression: normal situation

Mutated repressor that cannot bind to RNA polymerase.

Compensating mutation in the RNA polymerase restores the ability to transcribe.

Figure 8.29

Activation by contacting sigma, the lambda repressor in this case.

Figure 8.31
Competition between cro and cI determine the response. cII is critical, aided by the stabilizing action of cIII.

Inactivation of the repressor induces the lysogen.