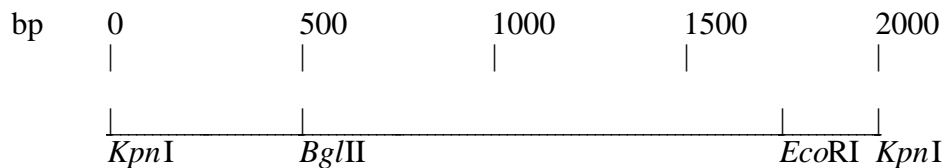


Questions, Chapter 11. Transcription: Promoters and Terminators

11.1 Determining the sequences that encode the ends of mRNAs.

A gene that determines eye color in salamanders, called *almond*, is contained within a 2000 bp *KpnI* fragment. After cloning the *KpnI* fragment in a plasmid, it was discovered that it has a *BglII* site 500 bp from the left *KpnI* site and an *EcoRI* site 300 bp from the right *KpnI* site, as shown in the map below.



In order to determine the positions that correspond to the 5' and 3' ends of the *almond* RNA, the *EcoRI* and *BglII* sites were labeled at the 5' or 3' end. The *KpnI* to *BglII* fragments (500 and 1500 bp) and the *KpnI* to *EcoRI* fragments (1700 and 300 bp) were isolated, hybridized to *almond* RNA and treated with the single-strand specific nuclease S1. The sizes of the probe fragments protected from digestion in the RNA-DNA duplex are shown below (in nucleotides); a 0 means that the probe was not protected by RNA.

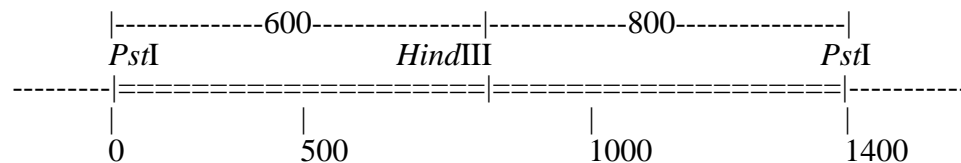
<u>5' end-labeled probe</u>		<u>3' end-labeled probe</u>	
probe	protected fragment	probe	protected fragment
<i>KpnI</i> - <i>BglII</i> *	500 0	<i>KpnI</i> - <i>BglII</i> *	500 100
* <i>BglII</i> - <i>KpnI</i>	1500 1300	* <i>BglII</i> - <i>KpnI</i>	1500 0
<i>KpnI</i> - <i>EcoRI</i> *	1700 0	<i>KpnI</i> - <i>EcoRI</i> *	1700 1300
* <i>EcoRI</i> - <i>KpnI</i>	300 100	* <i>EcoRI</i> - <i>KpnI</i>	300 0

The asterisk denotes the end that was labeled.

- What is the direction of transcription of the *almond* gene, relative to the map above?
- What position on the map corresponds to the 5' end of the mRNA?
- What position on the map corresponds to the 3' end of the mRNA?

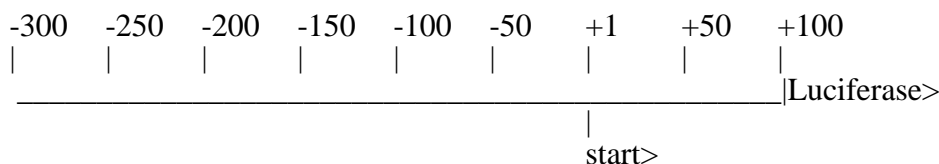
11.2 Determining the sequences that encode the ends of mRNAs.

The gene for histone H2A from armadillo can be isolated as a 1400 bp *PstI* fragment. The map is shown below; the armadillo *PstI* fragment is shown by the double dashed line, and the vector DNA is denoted by the single dashed lines. Sizes are in base pairs. The H2A gene clone was cleaved with *HindIII*, treated with alkaline phosphatase, and incubated with polynucleotide kinase and [³²P] ATP in an appropriate buffer to introduce a radiolabel at the 5' ends of the DNA fragments. The DNA was then extracted with phenol to remove the kinase, and then cut again with *PstI*. The labeled 600 bp and 800 bp *PstI*-*HindIII* fragments were separated by gel electrophoresis and isolated. The isolated fragments were denatured, hybridized to histone mRNA, and treated with nuclease S1. The S1-resistant labeled DNA fragments were identified by gel electrophoresis followed by radioautography. A 200 nucleotide protected fragment was observed when the 600 bp fragment was used in the S1 protection assay, but no protected fragment was observed when the 800 bp fragment was used.

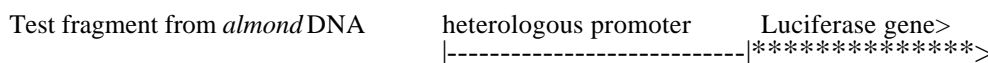


- What is the direction of transcription of the histone H2A gene (relative to the restriction map above)?
- With reference to the numbers below the restriction map, what is the position of the 5' end of the histone H2A mRNA?
- What is the position of the 3' end of the mRNA?

11.3 A 400 bp DNA fragment containing the start site for transcription of the *almond* gene was investigated to find transcriptional control signals. The start site (+1 in the coordinate system) is 100 bp from the right end. The 400 bp fragment is sufficient to drive transcription of a reporter gene (for luciferase) in an appropriate cell line. Two series of 5' and 3' deletions were made in the 400 bp fragment and tested for their ability to drive transcription of the luciferase reporter gene. Each fragment in the 5' deletion series has a different 5' end, but all are fused to the luciferase gene at +100 (see diagram below). Each fragment in the 3' deletion series has a common 5' end at -300, but each is fused to the luciferase gene at the designated 3' position. The amount of luciferase (a measure of the level of transcription) for each construct is shown in the first two pairs of columns in the table. The intact reporter construct, with *almond* DNA (the horizontal line) fused to the luciferase gene, is diagrammed immediately below.



To further investigate the function of different regions, sub-fragments of the *almond* DNA fragment were added to a construct in which the reporter gene was driven by a different promoter, as diagrammed below. The effects of the almond DNA fragments on this heterologous promoter are shown in the third pair of columns in the table.

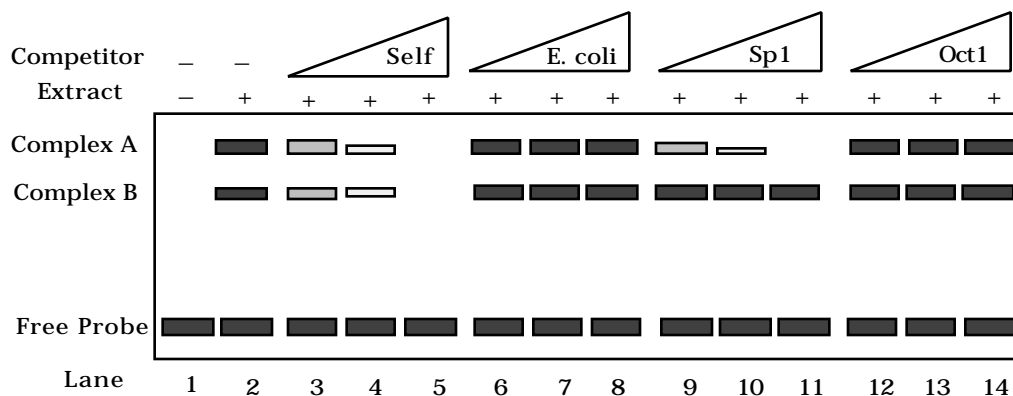


5' deletion endpoints	Amount of expression	3' deletion endpoints	Amount of expression	Test fragment of <i>almond</i>	Amount of expression
-300	100	-200	0	-300 to -250	100
-250	100	-150	0	-250 to -200	500
-200	50	-100	0	-200 to -150	100
-150	50	-50	0	-150 to -100	300
-100	25	+1	100	-100 to -50	300
-50	10	+50	100	-50 to -1	100
-1	0	+100	100	none	100

- What do you conclude is the role of the -250 to -200 fragment?

- b) What do you conclude is the role of the -200 to -150 fragment?
 c) What do you conclude is the role of the -150 to -100 fragment?
 d) What is the role of the -50 to -1 fragment of the *almond* gene?

11.4 An electrophoretic mobility shift assay was used to test for the ability of a short restriction fragment to bind to proteins from the nuclei of kidney cells. The restriction fragment was labeled at one end, mixed with an extract containing the nuclear proteins, and run on a non-denaturing polyacrylamide gel. Lane 1 (below) shows the free probe and lane 2 shows the the probe plus extract; electrophoresis is from the top to the bottom. Complexes between proteins and the labeled DNA probe move more slowly on the gel than does the free probe. Further tests of specificity are shown in the competition lanes, in which the labeled probe was mixed with an increasing excess of other DNA before mixing with the nuclear proteins to test for binding. Competitor DNAs included the unlabeled probe (self competition, lanes 3-5; the triangle above the lanes indicates that an increasing amount of competitor is used in successive lanes), a completely different DNA (sheared *E. coli* DNA) as a nonspecific competitor (lanes 6-8), and two different duplex oligonucleotides, one containing the binding site for Sp1 (lanes 9-11) and the other containing the binding site for Oct1 (lanes 12-14). Thinner, less densely filled boxes denote bands of less intensity than the darker, thicker bands.



- a) How many protein-DNA complexes are formed between the labeled DNA probe and the nuclear extract?
- b) What do lanes 3-8 tell you about the protein-DNA complexes?
- c) What do lanes 9-14 tell you about the protein-DNA complexes?
- 11.5 In order to determine the contact points between a regulatory protein and its binding site on the DNA, a small fragment of duplex DNA was end-labeled (at the 5' terminus of the left end as written below) and treated with dimethyl sulfate so that each molecule on average has one G nucleotide methylated. The regulatory protein was mixed with the preparation of partially methylated DNA, and protein-bound DNA was separated from unbound DNA. After cleaving the DNA at the methylated sites, the resultant fragments were resolved on a "sequencing gel". An autoradiogram of the results showed bands corresponding to all the G's in the labeled fragment for the unbound DNA, but the protein-bound DNA did not have bands corresponding to the G's at positions 14 and 16 below. When the left end of the fragment was labeled at the 3' terminus, no band corresponding to the G (bottom

strand) at position 18 (same numbering system as for top strand) was seen in the preparation of protein-bound DNA.

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          5   10   15   20   25   30
          |   |   |   |   |   |
5'  GATCCGCATGGATGAGTCACGTAACGTGTA
3'          GCGTACCTACTCAGTGCATTGCACAT

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What is the binding site for the regulatory protein?

11.6 Are the following statements about λ and polar effects of some mutations in operons in *E. coli* true or false?

- Nonsense mutations (terminating translation) in the first gene of an operon can have no effect on the transcription of subsequent gene in the operon.
- Mutations in the gene for (*rho* gene) can suppress polarity.
- The hexameric protein λ binds to protein-free RNA and moves along the RNA; when it encounters a stalled RNA polymerase it promotes termination of transcription.
- The protein λ is an RNA-dependent ATPase.