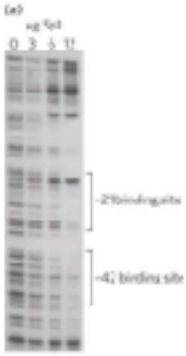
Rationale:
To find where transcription factor Sp1 binds on the DNA (DNA promoter section).
Steps:
Using the 1 radiolabeled end of the gene of interest. Incubate DNA with proteins from the cell so that interacting proteins will bind to that region. Remove the proteins that did not interact/bind and add DNAse I to the DNA. Finally, separate any proteins bound to the DNA and run it on a gel.
They first found that in SV40 vs human adenovirus genes because the RNA poly II attaches to the SV40 but does not to the other one. This shows Sp1 doesn't just attach anywhere and helps RNA pol II attach. When they put the two different sequence templates in a dish and added Sp1, the SV40 started getting transcribed while the adenovirus got inhibited by 40%. Using a deletion mutation in the promoter region of SV40, they found about 70-100 base pairs upstream where transcription starts so it needs tandem repeats of a specific 21 bp sequence.They used DNA footprinting to show that.
DNA footprinting showed that Sp1 would attach to that 21 bp sequence. Then, they added a bunch of SV40 DNA that had the promoter region and also added a bunch of Sp1. So now you have a mixture of Sp1 protein and a bunch of SV40 DNA with promoters for Sp1 to attach to. They added DNAse I to it which basically cuts and degrades DNA but what they discovered is that the promoter section was not getting cut and degraded because Sp1 would attach there and keep it safe. The area that is clear means there isn't any DNA. Why? Because Sp1 kept it safe from being cut up.
Results:
The transcription factor Sp1 bound to both the -21 and -42 binding sites and it also binds to SV40 which is a specific promoter sequence and location specific which then it helps RNA poly II bind to the DNA to replicate it.



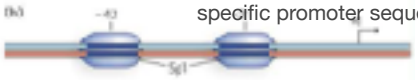


FIGURE 2 Sp1 leaves its footprint on a promoter. (a) The Sp1 footprint, seen as bands in the gel that decrease in intensity as the Sp1 concentration increases, is visible at sites flanking positions −21 and −42 with increasing concentrations of Sp1. The band that increases in intensity indicates a base pair in the DNA that becomes more susceptible to cleavage by DNase I on Sp1 binding—hinting at a change in the DNA structure that is induced by protein binding. (b) Sp1 binds (Wild DNA near positions −21 and −42. (Source: Dynan WS, Tjian R, Cell, 1983, Nov 35 (1), 79–87, with permission from Elsevier.)

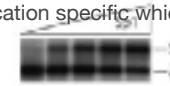
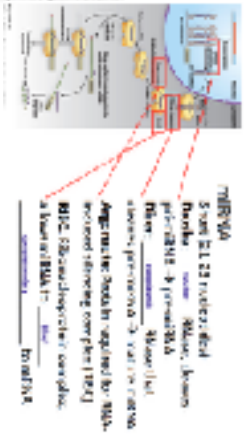
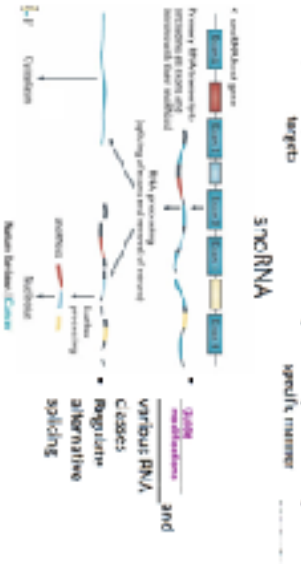


FIGURE 1 Sp1 activates transcription of SV40 DNA, but not human adenovirus DNA. Purified RNA polymerase and increasing amounts of Sp1 were added to a mixture of DNA containing an SV40 promoter and an adenovirus promoter; transcripts initiated from each promoter were separated and analyzed by gel electrophoresis. (Source: Data from W. S. Dynan and R. Tjian, Cell 22:669, 1983. Fig. 5, with permission from Elsevier.)





	Base length	Function
miRNA (microRNA)	21–23	Translational inhibition or destabilization of the target mRNA (polyA shortening), leading cleavage of the target mRNA in plants
siRNA (small interfering RNA)	21–23	Transcriptional inhibition through cleavage of the target mRNA
piRNA (piwi-interacting RNA)	24–32	Functions in germ cells
rasiRNA (repeat-associated siRNA)	24–33	Functions in germ cells
ta-siRNA (trans-acting siRNA)	20–22	Transcriptional inhibition through cleavage of target mRNA (plants)
nat-siRNA (natural antisense transcripts siRNA)	21,24	Transcriptional inhibition through cleavage of the target mRNA (plants)
hc-siRNA (heterochromatic siRNA)	21–24	Methylation of DNA and chromatin modification

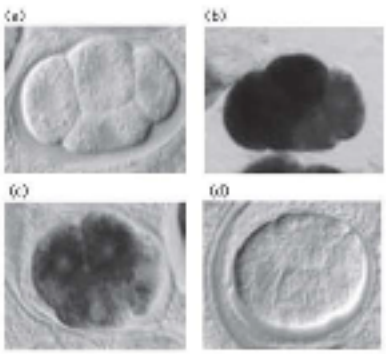
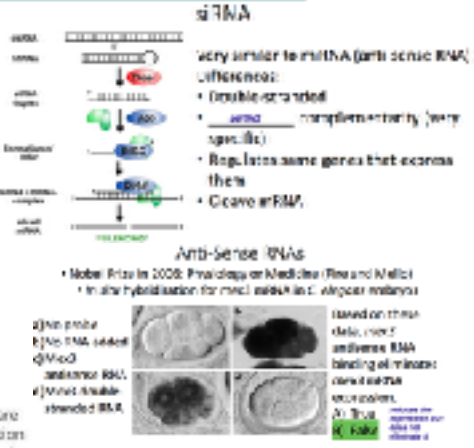
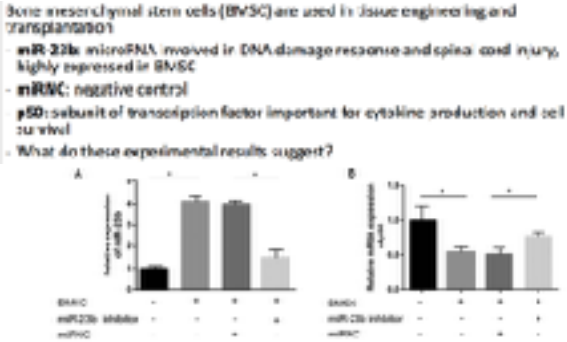
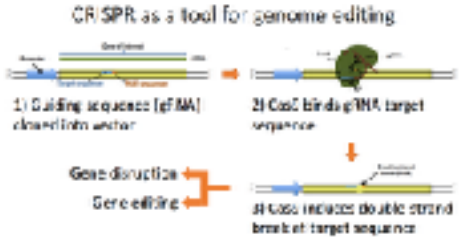


FIGURE 22-15 Silencing of gene expression by RNAi in a nematode. Mex-3 protein is a regulator that is expressed and functions early in nematode development. Four early embryos are shown at identical stages of development, with in situ hybridization used to detect the presence of mex-3 transcripts. (a) No staining is seen in a control, with no hybridization probe added. (b) Staining reveals the normal pattern of mex-3 expression in the embryo. (c) Injecting an embryo with an RNA complementary (antisense) to mex-3 mRNA reduces gene expression somewhat. (d) Injecting an embryo with double-stranded RNA corresponding to mex-3 reduces gene expression dramatically. (Source: Reproduced by





CRISPR:
a. Generate a guiding sequence (gRNA) against alu
b. Clone it into a vector
c. If specifically targeting something (ex. Neurons) then vector must reflect that. In this case, use neuron specific promoters
d. inject/transfer construct into organisms cells
e. CRISPR would disrupt by repressing alu or inducing DSB at targeted region



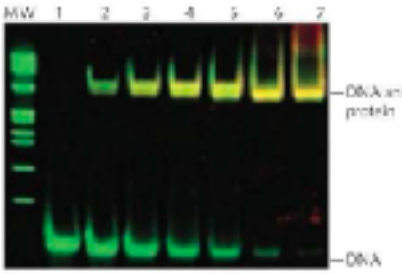


FIGURE 1 Results of an electrophoretic mobility shift assay demonstrate the shift in migration of Lac operator DNA (lane 1) with increasing concentrations of Lac repressor protein (lanes 2 through 7). The nondenaturing polyacrylamide gel preserves the Lac repressor in its folded state so that it can interact with the operator. The gel was stained with fluorophores that bind DNA (green) and protein (red). Yellow bands indicate protein-DNA complexes. (MW indicates the lane with molecular weight markers.) (Source: Photograph used courtesy of Thermo Fisher Scientific, copyright prohibited.)

Genomic Library = entire genome
cDNA = only coding RNA (nRNA)

Gen. Experiment:
a. Harvest tissue and extract DNA or total RNA
b. If total RNA, reverse transcribe to be DNA
c. Fragment DNA using restriction endonuclease
d. Insert fragments into cloning vector

——To distinguish b/t transformed cells and untransformed: Can use specific marker for macular degeneration and run Northern blot— transformed cells will express more of marker

A sample of *E. coli* is incubated in glucose-free and cAMP-free growth media with lactose present. After 24hr, you add cAMP to the media. How will this affect transcription of the *lac* operon?

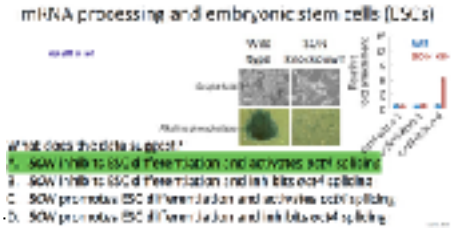
————————————-

Initial: - cAMP; - Glucose; + lactose

cAMP affects activity of CAP.

If you have lactose present, but no cAMP, you will have an inactive CAP, thus, you will have transcription to a very small extent, or none at all.

If you add cAMP-cAMP activates CAP- combined with the lack of glucose, RNA polymerase will be able to bind to the promoter better, and upregulate transcription. Therefore, adding cAMP to the media will increase transcription of the lac operon



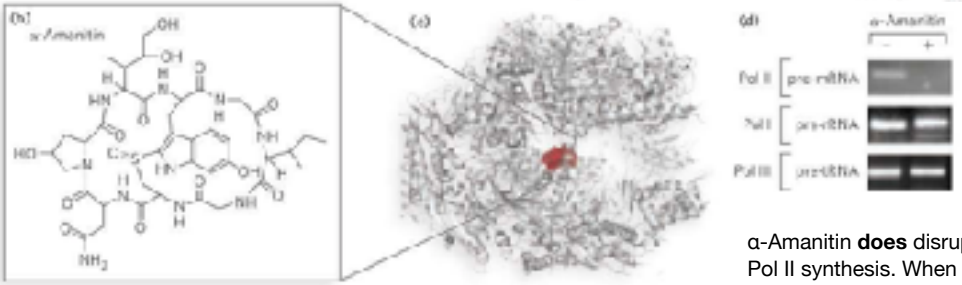
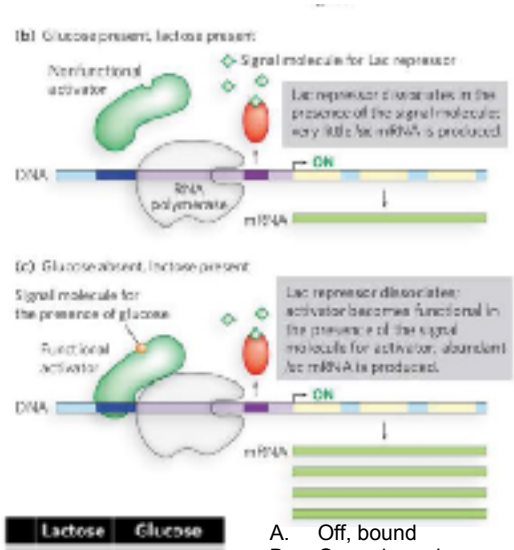


FIGURE 15-9 Inhibition of transcription by α-amanitin. (a) A mushroom to avoid! *Amanita phalloides* is poisonous to eukaryotes because it produces α-amanitin. (b) The chemical structure of α-amanitin. (c) Binding of α-amanitin (red) to yeast Pol II. (d) α-Amanitin inhibits synthesis of mRNA by Pol II, but not synthesis of rRNA by Pol I or tRNA by Pol III. The image shows a gel matrix in which RNA of a particular size is detected; in the presence of α-amanitin, RNA is made by Pol I and Pol III, but not by Pol II. (Note that pre-RNAs (precursor RNAs), as shown here, are the first products of transcription and are processed to form the mature RNAs.) (Sources: (a) © Webster/Home Images/Rainbow; (c) PDB ID 1T63, pdf Lee Y, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. EMBO Journal. Vol. 23, p. 4051, Fig. 3. © 2004 European Molecular Biology Organization.)

α-Amanitin **does** disrupt Pol II synthesis. When α-Amanitin is added, we can see a decrease in pre-mRNA in the northern blot. This suggests that the synthesis of pre-mRNA is affected.

The picture of the data is a northern blot which can measure the gene expression of RNA. In this case, we have various types of RNA that we want to detect: pre-mRNA, pre-rRNA, and pre-tRNA.



	Lactose	Glucose
A.	-	-
B.	+	-
C.	-	+
D.	+	+

A. Off, bound
B. On, unbound
C. Off, bound
D. Off, unbound

Predict the level of genetc actvity (on/off) of the lac operon as well as the status of the lac operator (bound/unbound), keeping in mind the functon of the CAP
protein (from your reading), under each of the cellular conditons to the right.

The steps in **northern blotting**:

1. **Lyse** the cell to obtain the RNAs; you want to be able to isolate the RNAs that you are interested in.

2. **Dentaure** so that strands are single-stranded and unfolded

3. **Separate** by size using **gel electrophoresis**

4. **Transfer** the RNAs to a **blotting membrane** so that hybridization of probes will be able to bind to the RNA

5. Utilize the **probes** you have created with a **fluorescent or radioactive label** that specifically bind/ **hybridizes** to the pre-mRNA only, pre-tRNA only, or pre-rRNA only

6. **Image** the blots
- Usually with northern blots, you would want to have a housekeeping gene so that you can measure the expression of the RNAs you are interested in observing. The housekeeping gene will also display if you have loaded everything equally.

Complementary RNA against miRNA-122 was added to a sample of liver cells to examine its affect on Hep C virus (HCV).
-Purpose of Actin?
Serves as loading control since actin is a housekeeping gene meaning to should not be affected by experimental treatment; its band density can be used to normalize the amount of HCV detected as shown in figure B
-Based on results, is miRNA required for RNA replication during HCV injection?
Yes, bc adding complementary RNA against miRNA122 which binds to and sequesters miRNA122, significantly decreases expression of HCV—> required
-What would data look like if it were done as a qRT-PCR experiment
Lane 1 to right of control since it has flightless less RNA expression than actin or would overlap with control since expression levels look similar AND Lane 2 would be to right of both control and lane 1 since it was lowest RNA expression

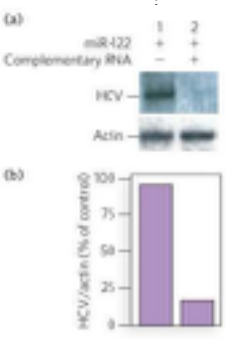


FIGURE 1 Hepatitis C virus replication is reduced in the absence of miR-122 RNA in the host cell. (a) When miR-122 was expressed in a cell harboring HCV, Northern blots showed production of HCV RNAs (lane 1). When an RNA complementary to miR-122 was also present, to sequester miR-122, HCV RNA production declined markedly (lane 2). Northern blots for a housekeeping gene that encodes actin, used as a control, are also shown. (b) Quantification of the results of the same experiments. (Source: (a) From Jopling et al., Modulation of Hepatitis C Virus RNA Abundance by a Liver-Specific MicroRNA, Science 2 September 2005: Vol. 309. no. 5740, pp. 1577–1581, 3c. Reprinted with permission from AAAS.)