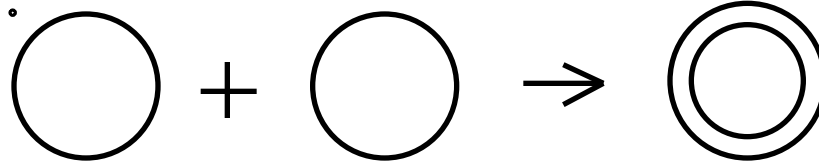


1. The electrophoretic mobility of a naturally occurring negatively supertwisted covalently-closed circular DNA 5040 base pairs long is measured in the presence of varying concentrations of an intercalating agent chloroquine. At a concentration of chloroquine in which 360 molecules bind per circular DNA molecule, the DNA was found to migrate at a speed identical with that of a fully relaxed or nicked circular DNA 5,040 base pairs long. Assuming the each molecule of chloroquine that binds to the DNA unwinds the helical twist by 26° upon binding, how many supertwists (writhe) were present in the original DNA sample. What is the average number of writhe are present after binding 360 molecules of chloroquine?

**Answer: -26 Writhe = $-(26^\circ \times 360) / 360^\circ$ per twist.
relaxed DNA has ZERO writhe on the average (Boltzman distribution about 0).**

2. Which of the following reactions can be mediated by a prokaryotic type I or a prokaryotic type II DNA topoisomerase? For each mechanism, state which class of topoisomerase, or neither or both, can catalyze the reaction shown.

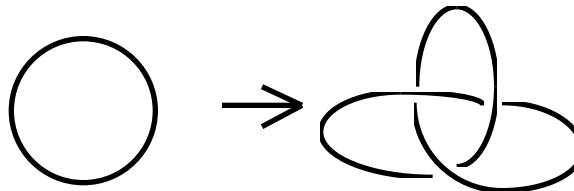
A. Renaturation of complementary single stranded circles.



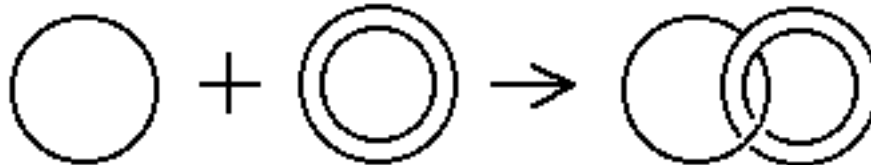
B. Catenation of duplex circles.



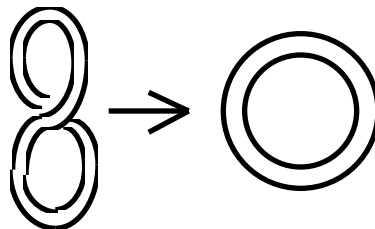
C. Knotting of single-stranded circles.



D. Catenation of a single-stranded circle and an intact duplex circle.



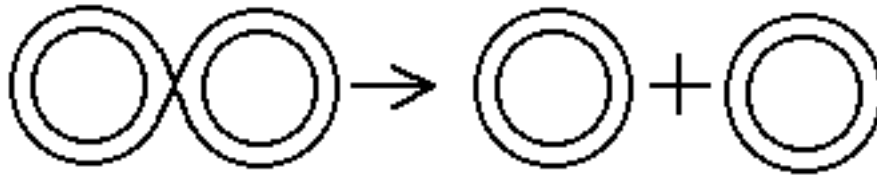
E. Relaxation of a covalently-closed-circular DNA with a single negative writhe.



F. Decatenation of interlocked circular duplexes.



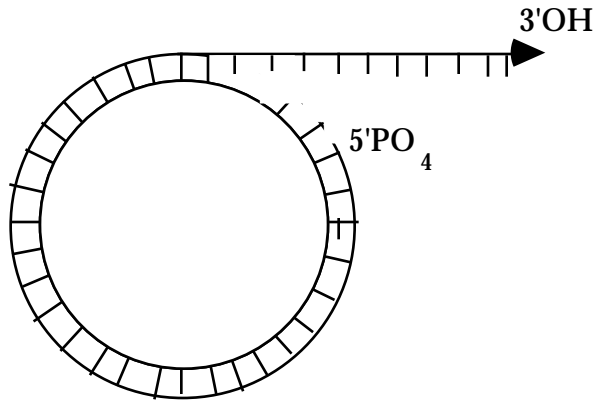
G. Resolution of two duplex circles joined in a Holliday structure.



Answer:

- A. Type I only.
- B. Type II only.
- C. Type I only.
- D. None.
- E. Type I only.
- F. Type II only.
- G. None.

3. List, in order, the enzymatic steps that *Escherichia coli* DNA polymerase I would catalyze using the following DNA template/primer under physiological conditions of nucleotide precursors (0.1 mM each), salt (0.15 M), temperature (37°), pH (7.5) and divalent ions (5 mM Mg⁺⁺):



ANSWER

- 1) binds to 3'OH terminus
- 2) 3'exonuclease to paired region
- 3) fill gap in circle (gap filling)
- 4) nick translation around circle
- 5) at 37°, eventually strand displacement will occur leading to a rolling circle product with a long displaced 5' end

4. Measurements of helical twist in both crystal structures of B-form DNAs and in solution have indicated that the twist angle varies greatly from one base pair to the next as a function of the actual DNA sequence. The helical twist angle between different base pairs can vary from as little as 25° to as much as 47° compared to the average of 34.6° for B-DNA in solution (10.4 base pairs per turn). Describe the primary force causing of the deviation of the helical twist angle from the average and describe how this force changes the helical twist. Describe two other changes in the structure of DNA observed in crystals from those in the classical Watson-Crick B-DNA caused by these forces.

ANSWER

- 1) Primary force = hydrophobic stacking interaction between hydrophobic base pairs.**
- 2) Hydrophobic interaction is maximized by twisting base pairs from flat plane (propeller twist).**
- 3) Propeller twist of purine-pyrimidine or pyrimidine-purine sequences results in purine purine clash.**
- 4) Purine-purine clash is relieved by either increasing or decreasing helical twist angle.**

Changes in structure relative to Watson-Crick structure can include:

- propeller twist**
- purine-purine-clash**
- bending of helix**
- kinking of helix**
- helical twist**
- sugar pucker (2' endo to 3' endo)**
- base-pair displacement**

6. Unlike prokaryotes, where the superhelix density of DNA is the result of the combined action of two topoisomerases, DNA gyrase and topoisomerase I, the superhelical density of DNA isolated from eukaryotes is the result of the topological folding of eukaryotic DNA in chromatin. Many aspects of chromatin folding contribute to the average superhelical density measured in eukaryotic DNA. Assuming an average of 200 base pairs of DNA per nucleosome (140 base pairs of core DNA and 60 base pairs of linker DNA) estimate the number of writhes each of the following aspects of chromatin folding would contribute to the total writhe of DNA per nucleosome.

DNA folding in chromatin	Number of writhes per nucleosome
Wrapping of nucleosomal DNA in left handed solenoidal path around core histones twice per nucleosome. .	
Reduction in the pitch of DNA from 10.4 bases per turn to 10.0 base pairs per turn in 140 base pair of core DNA.	
Wrapping of the linker DNA in a left handed solenoidal path in the 300 Å diameter chromatin fiber (assume a pitch of 6 nucleosomes per turn of the solenoid).	
Total writhe expected per nucleosome (sum of above)	

6. ANSWER

DNA folding in chromatin	Number of writhes per nucleosome
Wrapping of nucleosomal DNA in left handed helical path around core histones twice per nucleosome. .	-2

<p>Reduction in the pitch of DNA from 10.4 bases per turn to 10.0 base pairs per turn in 140 base pair of core DNA.</p>	$W_r = -T_w = -(140/10.4 - 140/10.0) = +.54 W_r$
<p>Wrapping of the linker DNA in a left handed solenoidal path in the 300 Å diameter chromatin fiber (assume a pitch of 6 nucleosomes per turn of the solenoid).</p>	$W_r = -1 \text{ writhe/solenoid} = -1/6 \text{ per nucleosome} = -0.17$
<p>Total writhe expected per nucleosome (sum of above)</p>	<p>-1.63 writhe / nucleosome</p>

7. List three similarities and three differences between the mechanism of initiation of DNA replication at oriC in bacteria and initiation of SV40 DNA replication by the T-antigen.

Similarity 1

Similarity 2

Similarity 3

Difference 1

Difference 2

Difference 3

ANSWERS

7.

A) **Similarities between SV40 and oriC initiation include:**

Origins contain short repeated sequences for binding

Sequence specific binding involved (dnaA and T-antigen)

Binding proteins melt region at origin

ATP required for initiation

Origin appear to be bent during initiation

Unwinding requires a single-strand DNA binding protein

Extensive unwinding requires topoisomerase.

Both origins require the action of a DNA helicase.

B) Differences between SV40 and oriC initiation include:

SV40 T-antigen binds and protects origin while origin DNA appears to wrap around the dnaA complex

Denaturation of SV40 origin requires ATP binding but not hydrolysis. melting by dnaA requires ATP hydrolysis.

17bp AT tract at SV40 origin can bend by itself, but oriC requires a protein such as integrative host factor to bend the DNA near the origin.

T-antigen serves as its own helicase once the origin is melted. dnaB serves as the helicase in oriC initiation.

T-antigen moves 3' to 5' on the strand to which it is bound

dnaB moves 5'-3' on the strand to which it is bound (it appears T-antigen moves along the leading strand template while dnaB moves along the lagging strand template).

8. Circle whether each of the following properties is true or false for the base-pairs found in B-DNA crystals:

The base pairs contain complementary rather than identical bases TRUE or FALSE

The base pairs are flat-coplanar structures TRUE or FALSE

Base pairs are connected by non-covalent hydrogen bonds TRUE or FALSE

Purines are in syn conformation about glycosylic bonds TRUE or FALSE

There is a constant twist angle between each base pair in the DNA TRUE or FALSE

An obtuse angle between the two glycosylic bonds on the paired bases gives rise to a major and minor groove TRUE or FALSE

The bases have a propeller twist with respect to each other giving rise to a purine-purine clash TRUE or FALSE

Answers to number 8. (1 point each)

Complementary bases rather than identical bases TRUE

Flat-coplanar base-pairs FALSE

Base-pairs connected by non-covalent hydrogen bonds TRUE

Purines are in syn conformation about glycosylic bonds FALSE

There is a constant twist angle between every base-pair in the DNA **FALSE**

An obtuse angle between the two glycosylic bonds on the paired bases gives rise to a major and minor groove. **TRUE**

The bases have a propeller twist with respect to each other giving rise to a purine-purine clash. **TRUE**

9. Explain the inverse relationship between the Twist and the Writhe in a covalently closed circular DNA molecule. Define both terms and give a formula relating their relative value assuming that the covalent structure of the DNA is not interrupted.

Answer to 9.

The Twist of DNA is the total helical twist along the entire axis of the circular DNA helix.

The Writhe is a measure of the number of supercoils that the helical path of the DNA exhibits.

For a covalently closed circular DNA the sum of the Twist and the Writhe is a constant known as the linking number. Either of the following two formulas for this relationship are valid:

$$L_k = T_w + W_r$$

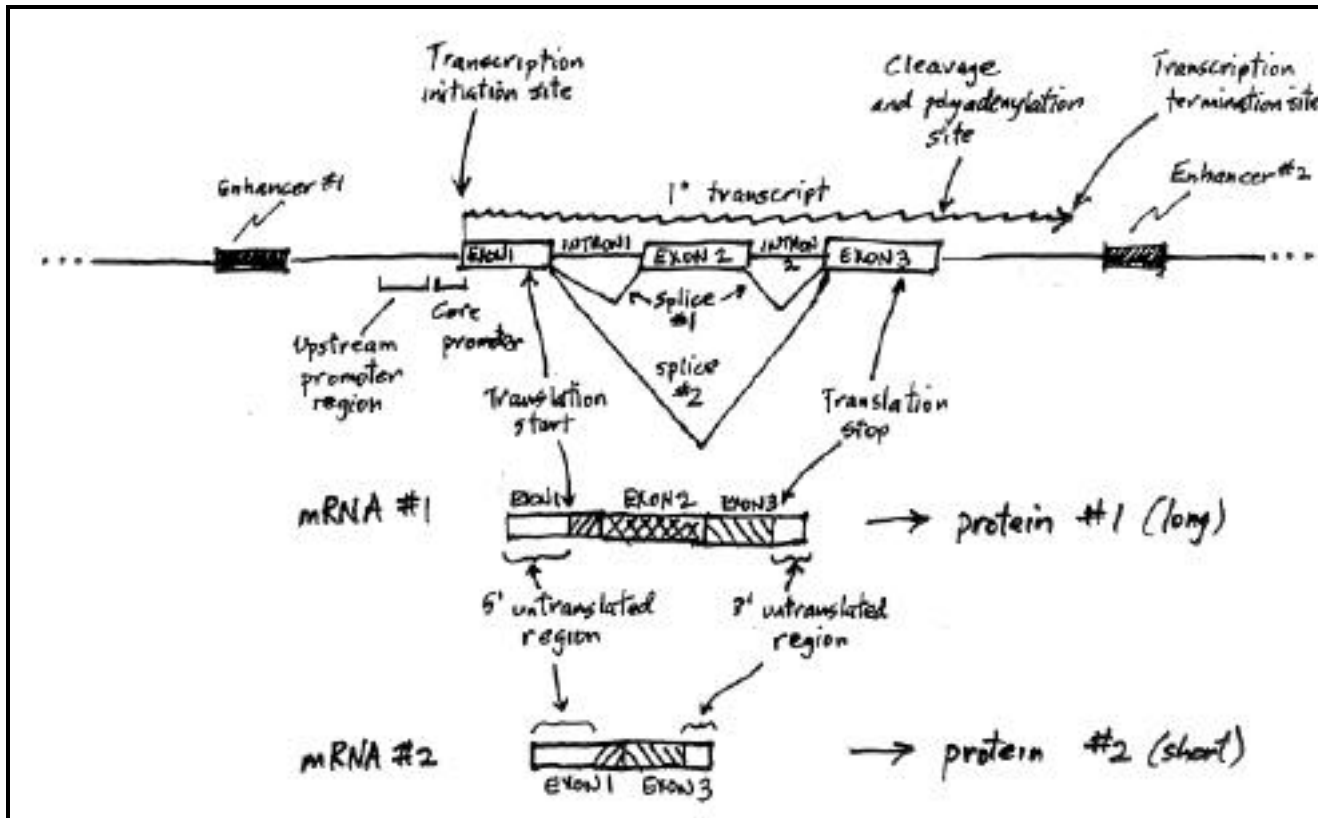
$$0 = T_w + W_r$$

10. Diagram a hypothetical eukaryotic gene with 2 distributed enhancers, 3 exons and 2 introns. Diagram how it is differentially spliced to give two different protein forms.

Label the following in your diagram:

- | | |
|----------------------------------|---|
| 1. enhancers | 7. translational start |
| 2. exons | 8. translational stop |
| 3. introns | 9. 3' untranslated region |
| 4. core promoter | 10. 5' untranslated region |
| 5. cleavage/polyadenylation site | 11. transcriptional initiation site |
| 6. upstream promoter region | 12. transcriptional termination site(s) |

ANSWERS:



11.

A. Where in a typical mammalian cell is most mRNA processing believed to take place? Does it take place throughout the organelle or at specific locations?

B. Very recently (Nature 385, 357 (1997)) it was found that the C-terminal domain of the largest subunit of RNA polymerase II is required for efficient mRNA splicing and cleavage/polyadenylation. Why was this a surprising finding?

B. It was also found that this domain interacts with several splicing and polyadenylation factors. What functional consequences might the association of these factors with RNA polymerase II have for gene expression in the cell?

Answers:

A)

In localized domains (~20-100) within the nucleus.

B)

RNA polymerase II carries out transcription of protein-coding genes. This results suggests that the enzyme is also required for efficient processing of the mRNA.

C)

This results suggests that there is a molecular coupling between the transcriptional machinery and the RNA processing machinery. This would ensure that the RNA processing machinery is efficiently directed to newly synthesized transcripts.

12. Differential gene expression is usually investigated by measuring changes in the levels of mRNA's. Briefly discuss the advantages and disadvantages of choosing to measure this particular parameter among the many that can reflect differential gene expression.

(No answer given)

13. Among the genes controlling yeast mating type, there are two silent copies of the alpha- and a-type mating hormone genes called HML-ALPHA and HMR-a and an active copy of one of the genes at the MAT locus in between the silent copies. In addition, there is a gene called HO that encodes an endonuclease that initiates the switching of the mating type gene.

A. Describe the role of histone and non-histone proteins that are responsible for repressing the silent mating type loci. Name the non-histone proteins and the site on the DNA where they act and describe their interaction with the nucleosomal histones on the silent genes.

B. Describe the gene products involved in the switching of mating types and describe their role in the expression of the HO locus.

Answer:

A) The silent mating type loci are flanked by SILENCER regions. A group of non-histone proteins referred to as SIR proteins bind at the SILENCER region and one

in particular, the SIR3 protein interacts with the N-terminal tails of histones H4 and H3 to help maintain the adjacent genes in the repressed state.

B) There are at least three genes (SW1, SW2 and SW3) and probably two additional ones (SNF1 and SNF6, since SNF2 = SW2) that are involved in the remodeling of chromatin near sets of genes beginning to undergo transcription. These proteins form a remodeling complex that converts an inactive HO gene to an active one (100 fold stimulation). A complex is believed to interact with the acidic domains of gene specific transcription factors binding at enhancer sites near the beginning of inducible genes. The SW/SNF complex is believed to convert the chromatin from a repressed state to an active state and permit histone acetylation although the complex itself is not a histone acetylase.

14. Obtaining Gene & Genome Function from DNA Sequence (Davis)

Two different approaches have recently been developed to determine the phenotype of a disruption in virtually every region of the yeast genome. The first approach uses a transposon to generate, in a parallel fashion, a large number of random insertions in the genome, followed by serial analysis of each 500 base region by gel electrophoresis. The second approach uses a molecularly tagged deletion to generate, in a serial fashion, a precise deletion of any region by homologous recombination, followed by parallel analysis of the deleted regions by hybridization of the tag sequence to a DNA chip.

Suppose a new organism was being analyzed. Given the listed constraints, which of the methods - transposition or deletion - would be the preferred form of whole genome analysis? Circle the correct answer.

A. A very large number of growth conditions are to be analyzed	transposition or deletion
B. Only the essential genes are to be determined	transposition or deletion
C. Non-homologous recombination occurs at a much higher level than homologous recombination	transposition or deletion
D. A large number of small (10 bp) DNA binding sites are to be analyzed	transposition or deletion
E. There are a large number of tandem copies of genes to be analyzed	transposition or deletion

ANSWERS: (2 points each)

A.	deletion
B.	transposition
C.	transposition
D.	deletion
E.	deletion

15. Provide a rationale for the complexity of pre-mRNA splicing, especially in contrast to the relative simplicity of self-splicing introns.

(NO ANSWER PROVIDED)

16. Semi-conservative DNA replication in both prokaryotes and eukaryotes is a complex process requiring many discrete enzymes, each catalyzing a specific kind of reaction. Discuss succinctly the following features of DNA replication. Please indicate the important similarities and differences that have been found between prokaryotes and eukaryotes.

- A. Initiation of replication.
- B. Formation of primers.
- C. Semidiscontinuous DNA synthesis, i.e. leading and lagging strand synthesis.
- D. Joining of discontinuously synthesized "Okazaki fragments."

(NO ANSWER PROVIDED)

17. State which of the following statements about type I and type II DNA topoisomerases are true or false:

Statement

True or False

Type I topoisomerases always require ATP in their reactions	
Type I topoisomerases always require a divalent metal ion	
Type I topoisomerases always cleave only one DNA strand during their reaction	
Inhibitors of type I topoisomerases are useful anti-cancer drugs	
Type II topoisomerases always change the linking number of DNA in steps of two	
Type II topoisomerases always relax supercoiled DNA	
Type II topoisomerases always cleave both strands of DNA during their reaction	
Inhibitors of type II topoisomerases are useful anti-cancer drugs	

17. Answer

Statement	True or False
Type I topoisomerases always require ATP in their reactions	False
Type I topoisomerases always require a divalent metal ion	False
Type I topoisomerases always cleave only one DNA strand during their reaction	True
Inhibitors of type I topoisomerases are useful anti-cancer drugs	True
Type II topoisomerases always change the linking number of DNA in steps of two	True

Type II topoisomerases always relax supercoiled DNA	False
Type II topoisomerases always cleave both strands of DNA during their reaction	True
Inhibitors of type II topoisomerases are useful anti-cancer drugs	True

18. State which of the following statements about the replication of the SV40 minichromosome in vitro is true or false:

True or False

(a) Replication is initiated at a specific nucleotide sequence and is bidirectional. _____

(b) The primase that catalyzes the synthesis of the initiating oligoribonucleotide resembles the E. coli primase in that it is associated with a DNA helicase as part of a primosome. _____

(c) A heterotrimeric single strand DNA binding protein is required. _____

(d) It requires two distinct DNA polymerases, Pol _ and Pol __. _____

(e) An RNA copy of the SV40 chromosome serves transiently as a template. _____

(f) It proceeds by a semidiscontinuous mechanism. _____

(g) A type II topoisomerase is required to resolve the two circular products of the reaction. _____

(h) Proliferating Cell Nuclear Antigen (PCNA) serves _____

to enhance the processivity of deoxynucleotidepolymerization by Pol ____

(i) A DNA ligase is required. _____

Answers:

- (a) T
- (b) F
- (c) T
- (d) T
- (e) F
- (f) T
- (g) T
- (h) T
- (i) F

19. There are certain sites within the E. coli chromosome known as "hot spots" that have unusually high rates of point mutations. Many of these sites contain 5-methylcytosine. How do you explain the existence of such hot spots?

5-methylcytosine, like cytosine, undergoes a facile deamination to produce 5 methyuracil, i.e. thymine. This results in the conversion of a methyl C-G pair to a T-A base pair.

20. E. coli with a mutation in the rec A gene is unable to promote homologous (general) recombination ($<10^{-3}$ of wild type). Such mutants are also defective in the repair of DNA damage resulting from U.V. irradiation. How do you explain this finding?

The presence of pyrimidine dimers resulting from U.V. irradiation at or near a replication fork blocks the DNA polymerase which can, however, reinitiate downstream from the lesion, leaving a gap. The gap is repaired by homologous recombination which depends on the rec A protein.

(I didn't tell them that rec A is also needed for the SOS response which depresses synthesis of the UVrABC complex needed for nucleotide excision repair, but this would also be a correct answer.)

21. The *E coli* Replisome

Compare DNA polymerases (DNAP) with RNA polymerases (RNAP).

A. Cite 3 fundamental similarities

B. Complete table below with "yes" or "no" to identify differences

DNAP RNAP

Can start chains

Uses ribonucleotides

Uses deoxyribonucleotides

Copies very long (multigenic) stretches of DNA

Edits the copies made

Processivity, inherent in the polymerase

(i.e. not dependent on auxiliary proteins)

Uses T to match A

Uses U to match A

Answers

- a. (1) Copy a DNA template.
(2) Use nucleoside triphosphates as building blocks.
(3) Synthesize in 5' → 3' direction.
(4) Use Watson-Crick base pairing.
- b. See above. (Yes and No answers under DNAP and RNAP headings are the answers so should not be typed in exam.)

22. Surveying Physiological Differences in Gene Expression

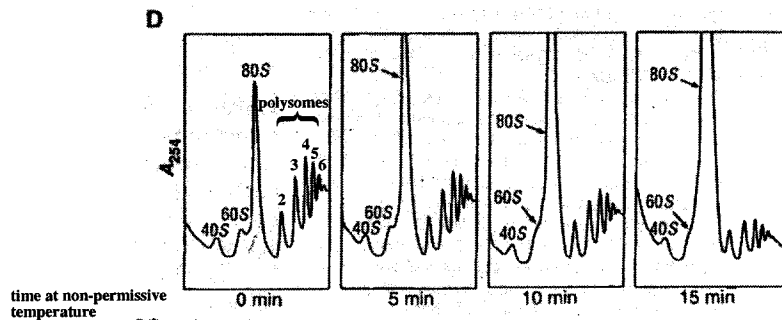
DNA microarrays can be used to monitor thousands of genes in parallel by hybridization of complex nucleic acid mixtures to an ordered array of individual gene sequences.

- a) Describe two properties of genes that can be measured using microarrays.

b) Describe how these properties are measured using these arrays.

23. Translational Control of Protein Synthesis

Chuang, et al., *Science* (1997), **275**, 1468-1471. Ded1p is a newly discovered DEAD box protein in *S. cerevisiae*. Through several lines of evidence the authors have demonstrated that it functions during translation. Shown below are four polysome profiles from a yeast strain bearing a temperature-sensitive allele of the *ded1* gene at different times after a shift to the non-permissive temperature. What do these profiles indicate is the step of translation at which Ded1p operates? Briefly explain your reasoning.



N.B. All profiles are on the same scale/

Answer: initiation is the step at which ded1p appears to operate. the amount of polysomes decreases and the amount of monosomes increases as the protein is inactivated, indicating that initiation is being slowed. If elongation or termination became the rate-limiting phase of translation the amount of high molecular weight polysomes would increase relative to monosomes.

(Also true, but not sufficient: cannot rule out the possibility that ded1p has a small effect on elongation and/or termination).