Week 13

(Preparing for) **Tuesday's lecture:**

Budgeting homework time (45 min): Chapter 1, section 1.5 on Epigenetics is 1840 words in length. At 200 words per minute, reading section 1.5 should just take 10 minutes. But when done properly, when you pause to review figures, read and think about a few of the Integrating & Review Questions, and take careful notes, this homework assignment should take you more like 45 minutes (if you focus).

1. _____ **For Tuesday's lecture,** continue **Chapter 1: Heritable Material** by reading section 1.5: "Is all genetic information encoded linearly in the DNA sequence?" and take careful handwritten notes.

2. _____ **Try to answer some Integrating Questions** and **Review Questions**.

3. _____ **Prepare to explain (aloud) Figures 1.19 (the method), and do a Trifecta for Figures 1.20, and 1.21 in class** (Purpose, Methods, Findings).

1.5 Is all genetic information encoded linearly in the DNA sequence?

Biology Learning Objectives

- Describe the epigenetic code using methylcytosine and its effects on gene activity.
- Evaluate experimental design and analyze data from research on DNA as molecular information.

Normal Bases

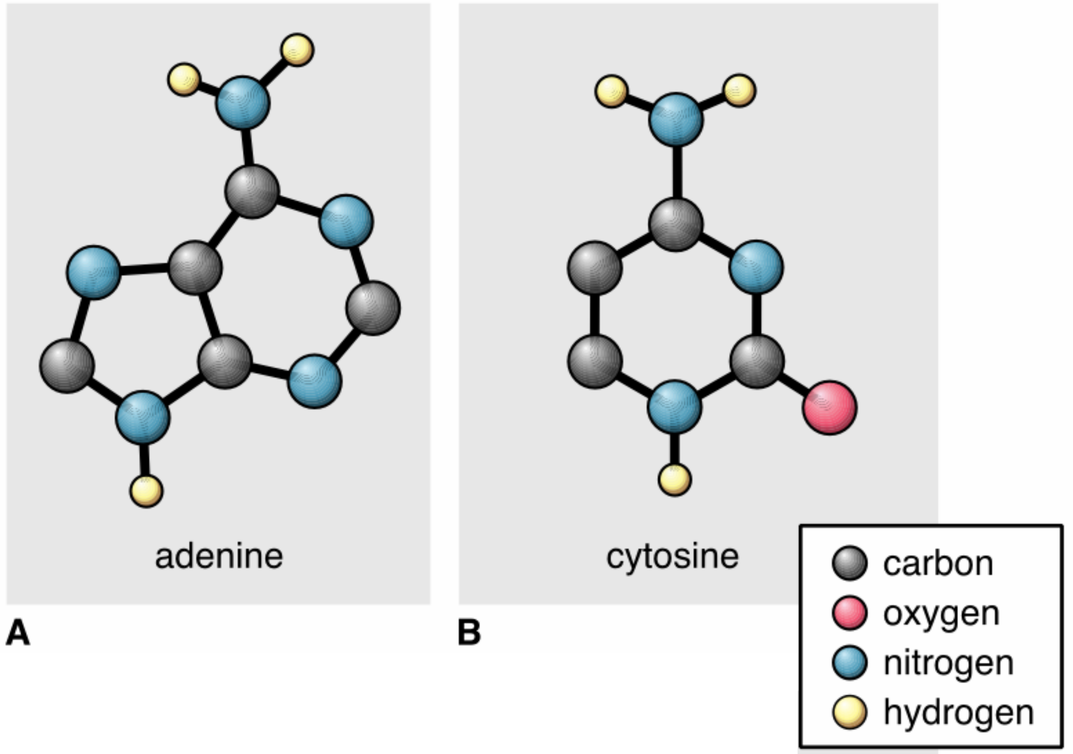

Fig. 1.18

Methylated Bases

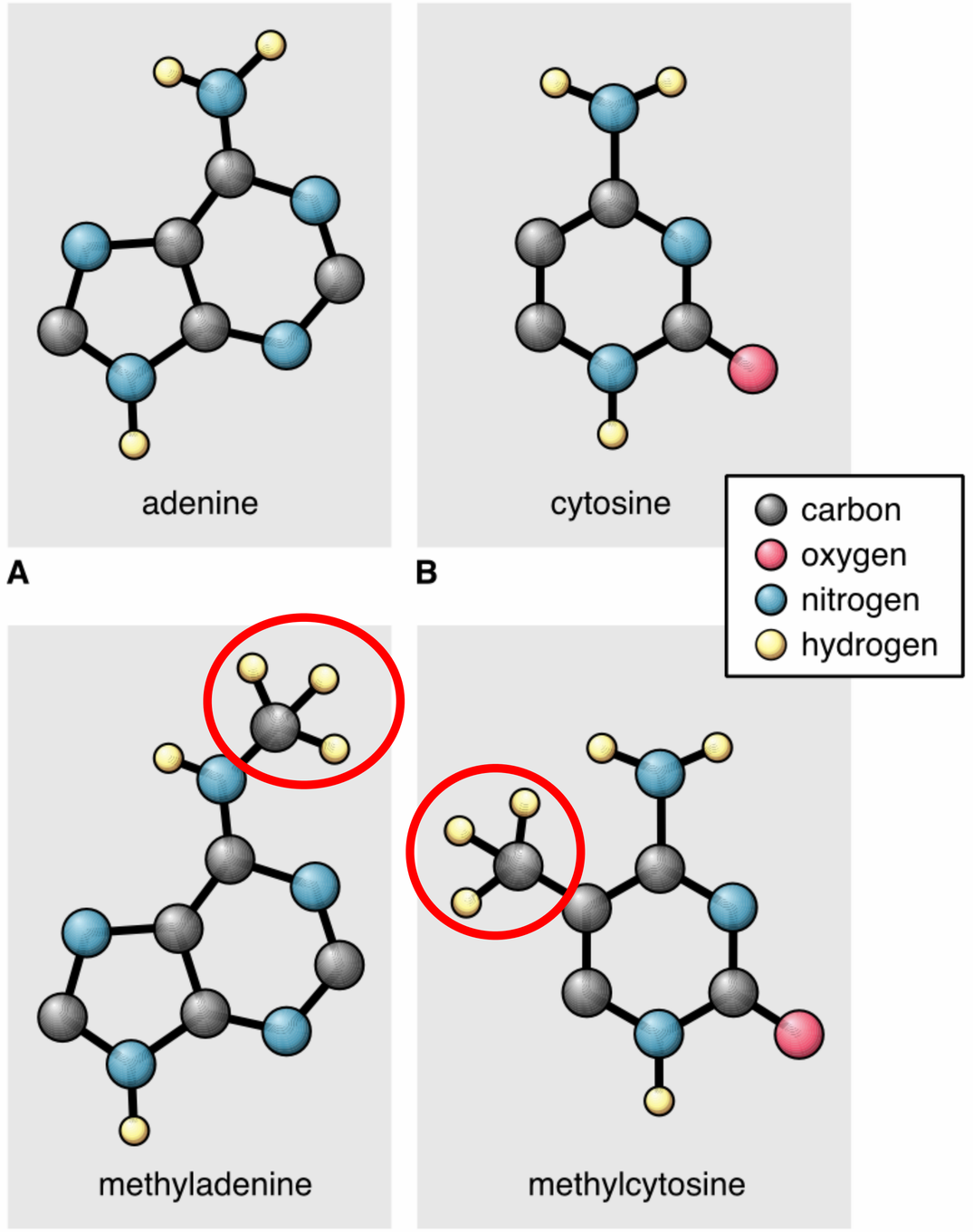

Fig. 1.18

Methylated Bases

different chemical structures

different physical properties

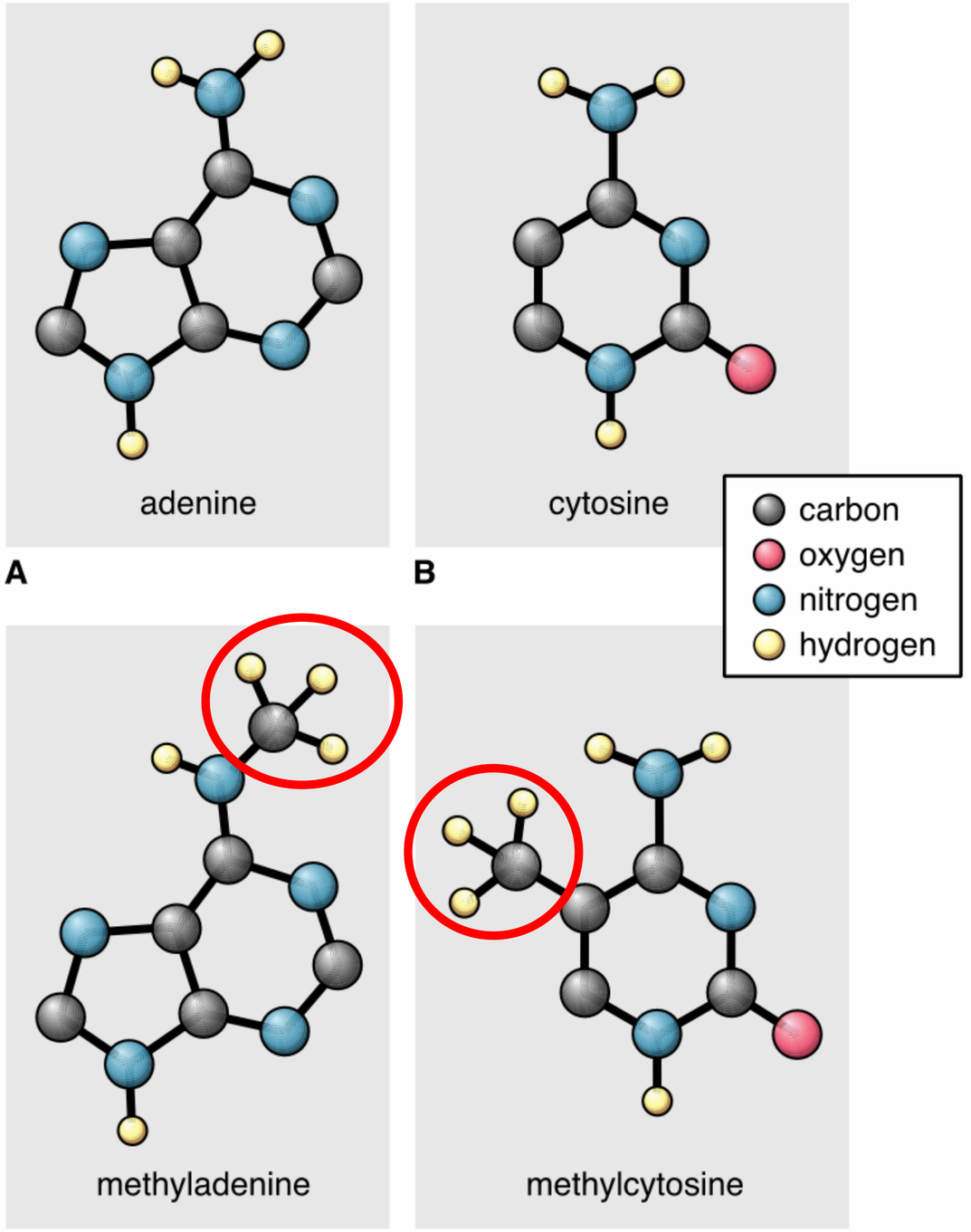

Fig. 1.18

Methylated Bases

these are NOT mutations!

methylation is epigenetic change

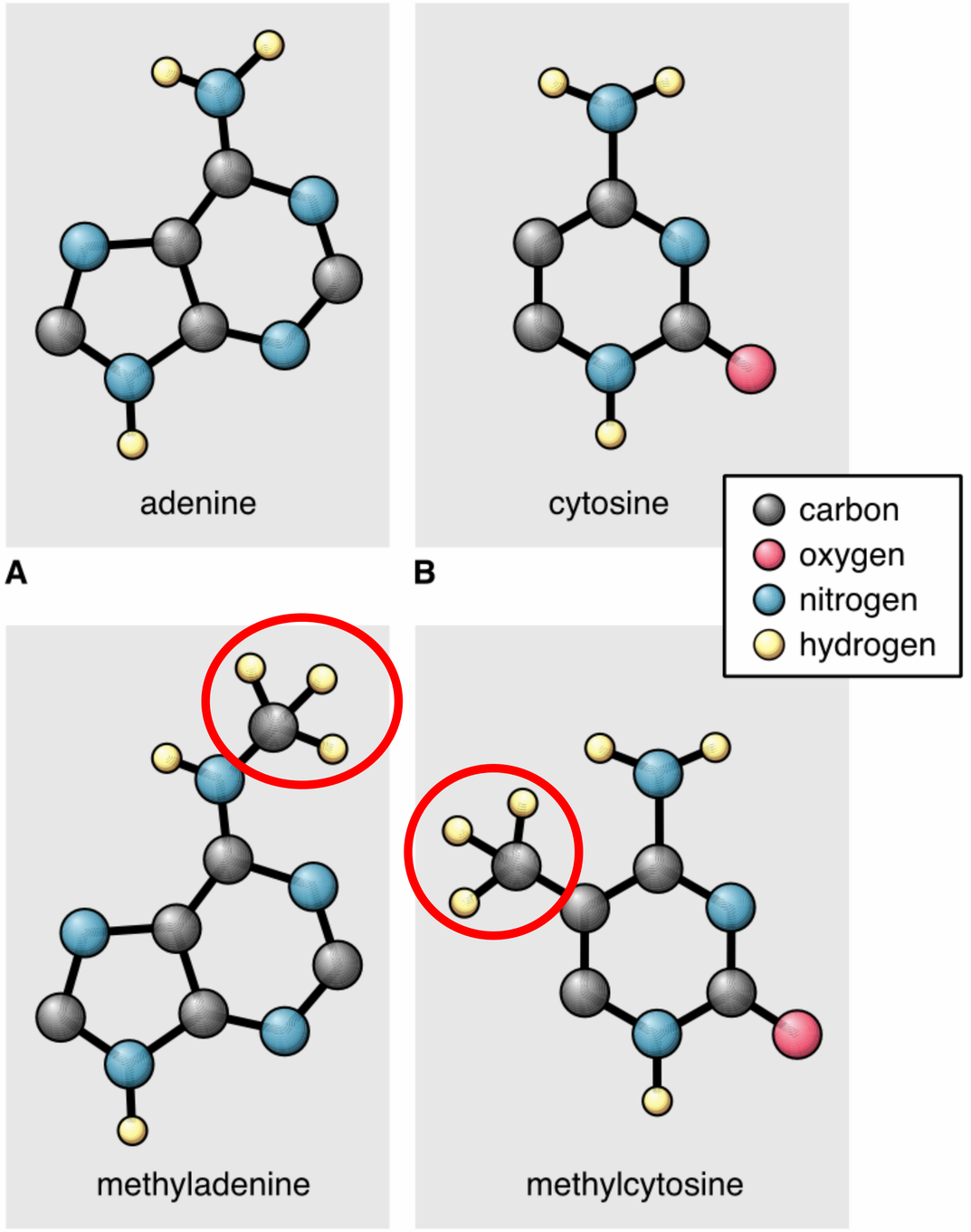

Fig. 1.18

Method?

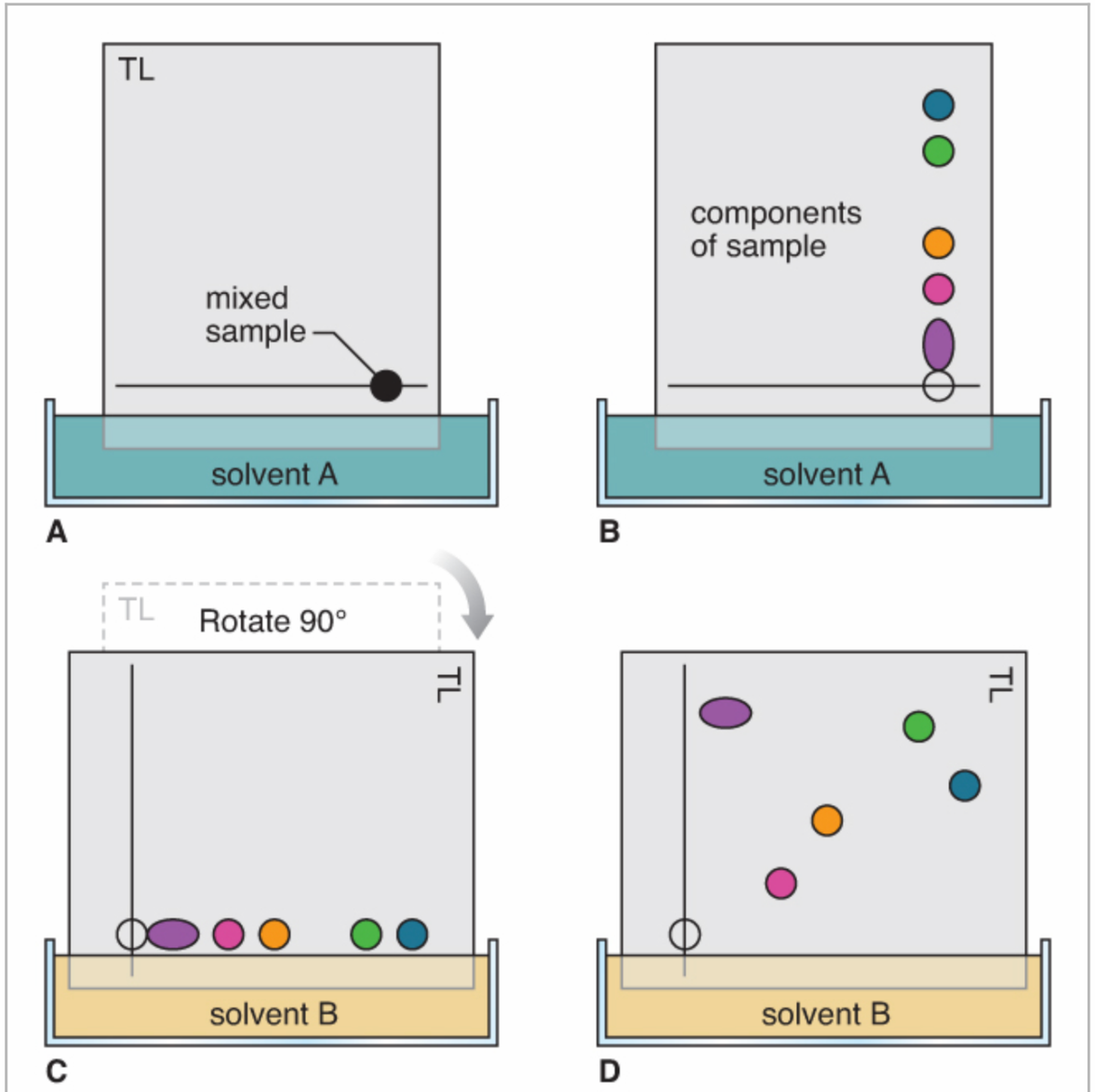

Figure 1.19 Two dimensional thin layer chromatography (TLC) technique. **A,** Complex mixture of a sample is pipetted on the line and then the bottom edge is dipped in solvent A. **B,** Sample components migrate at different rates depending on their chemical structures. **C,** The sheet is rotated and dipped into a second solvent where **(D)** the components again migrate

Thin Layer Chromatography

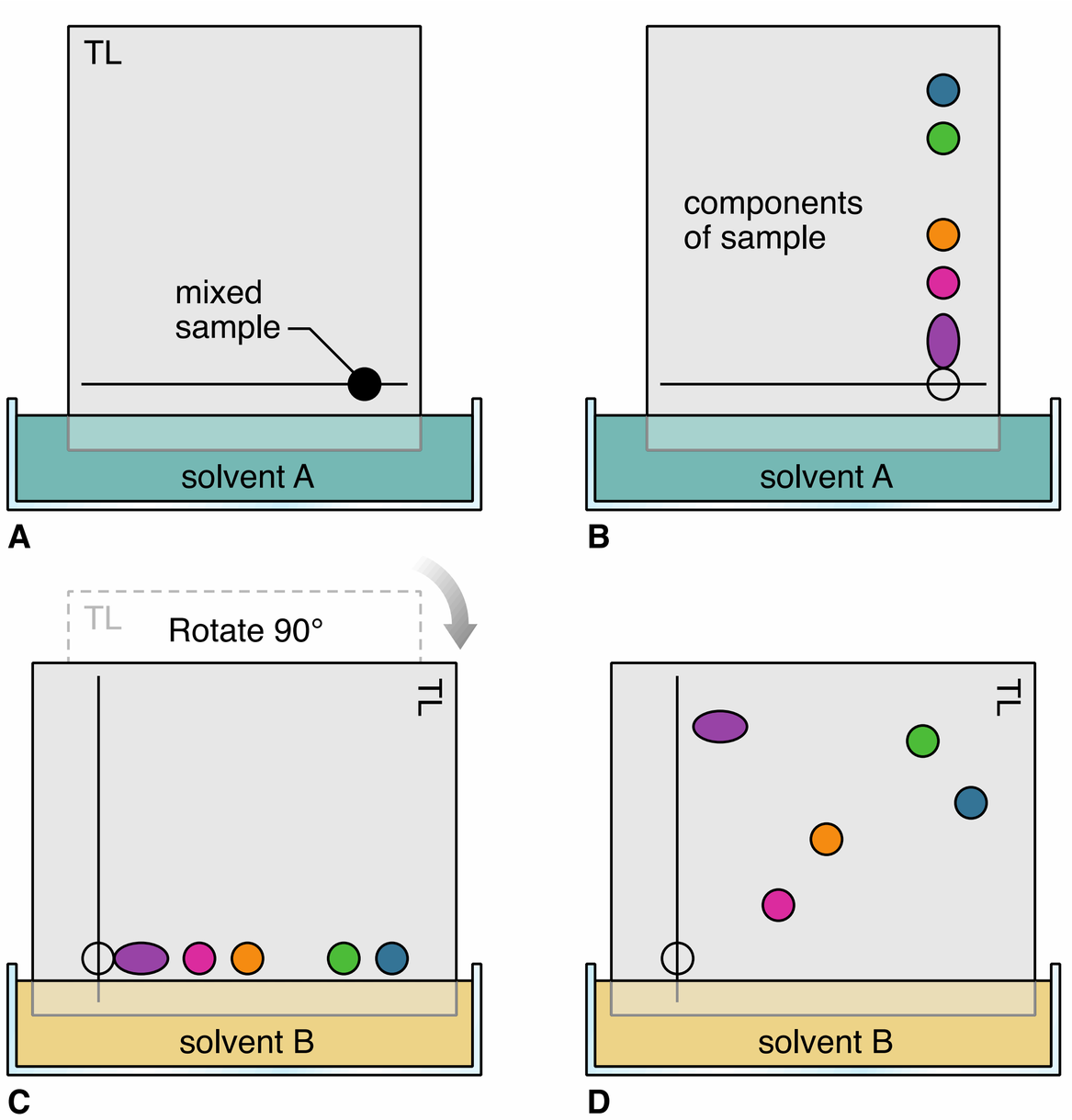

Fig. 1.19

Thin Layer Chromatography

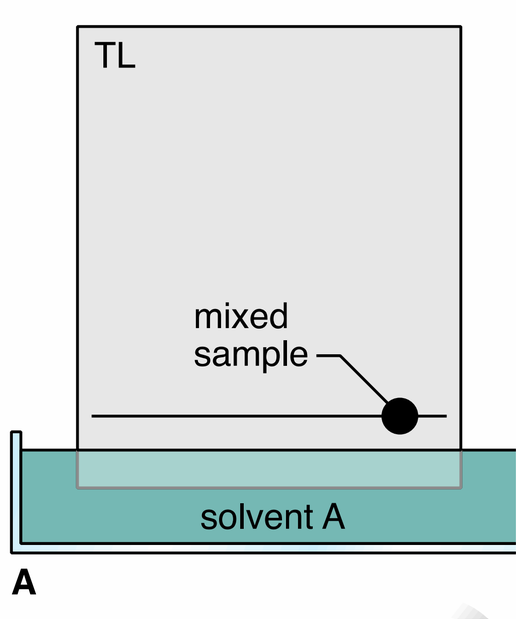

Fig. 1.19

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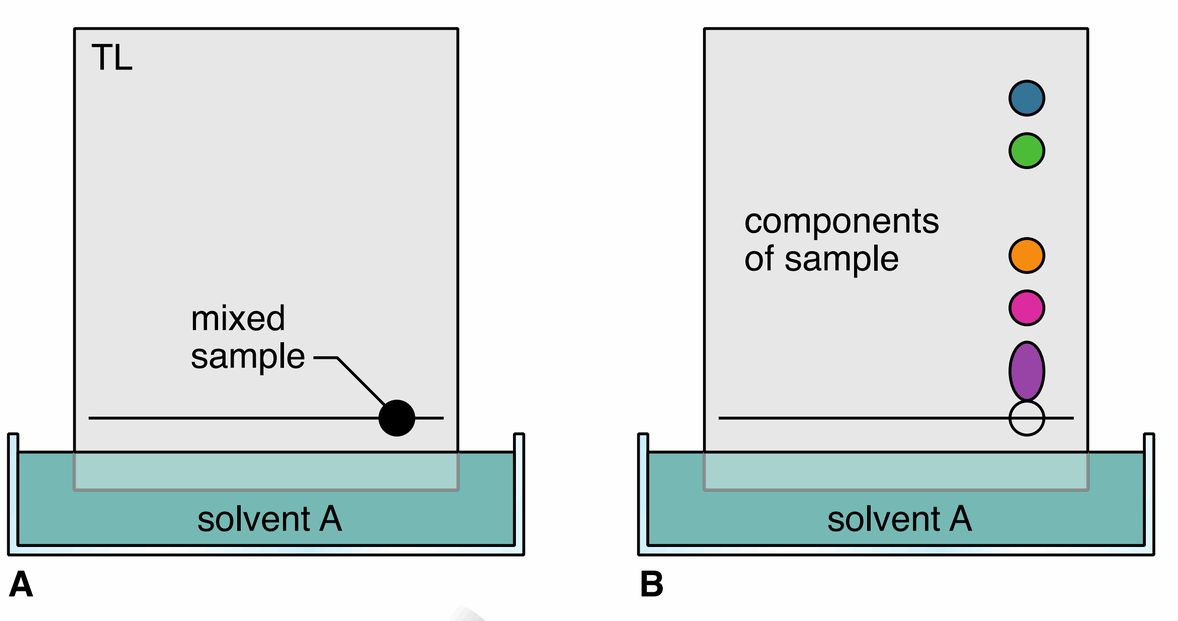

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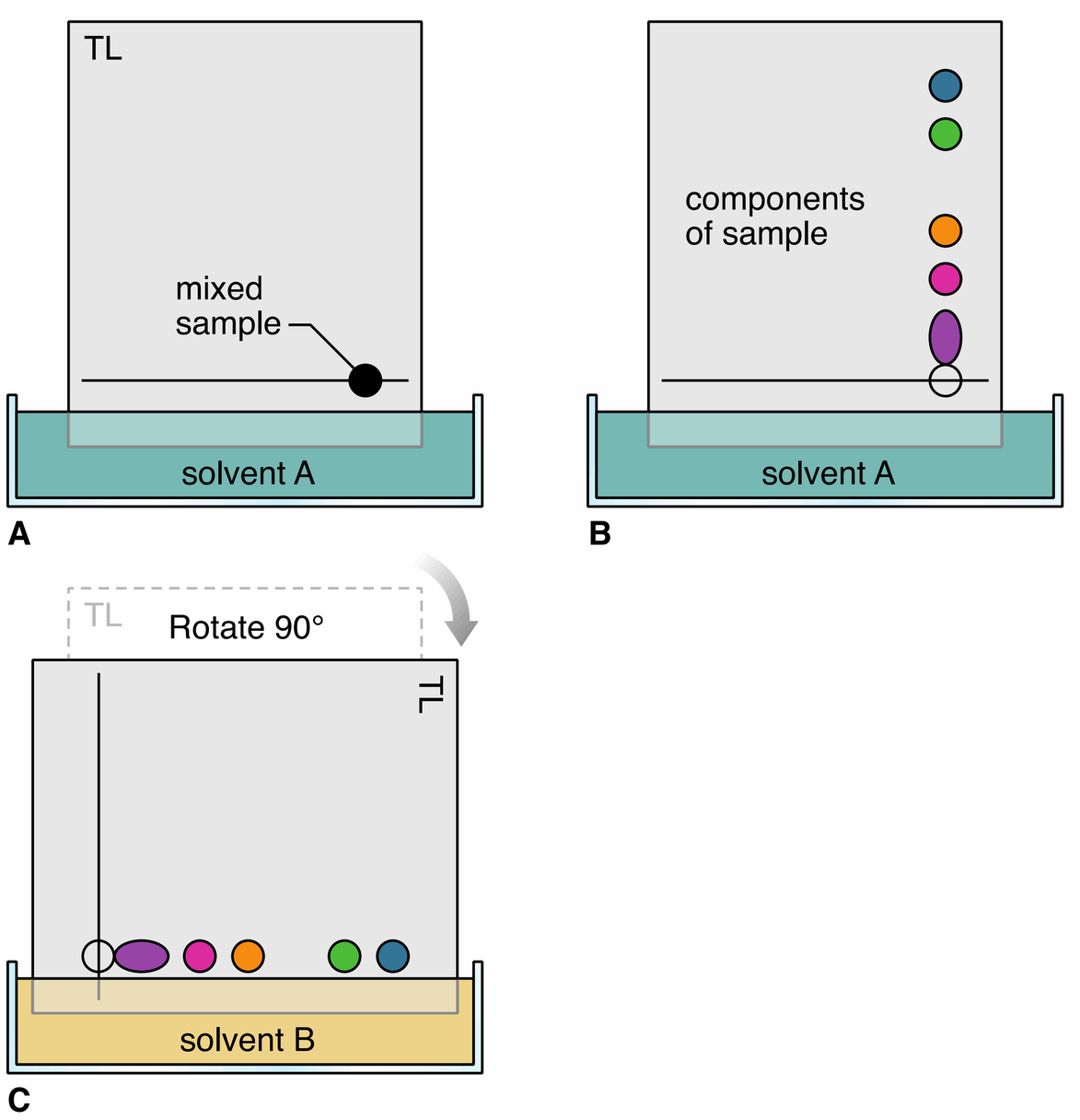

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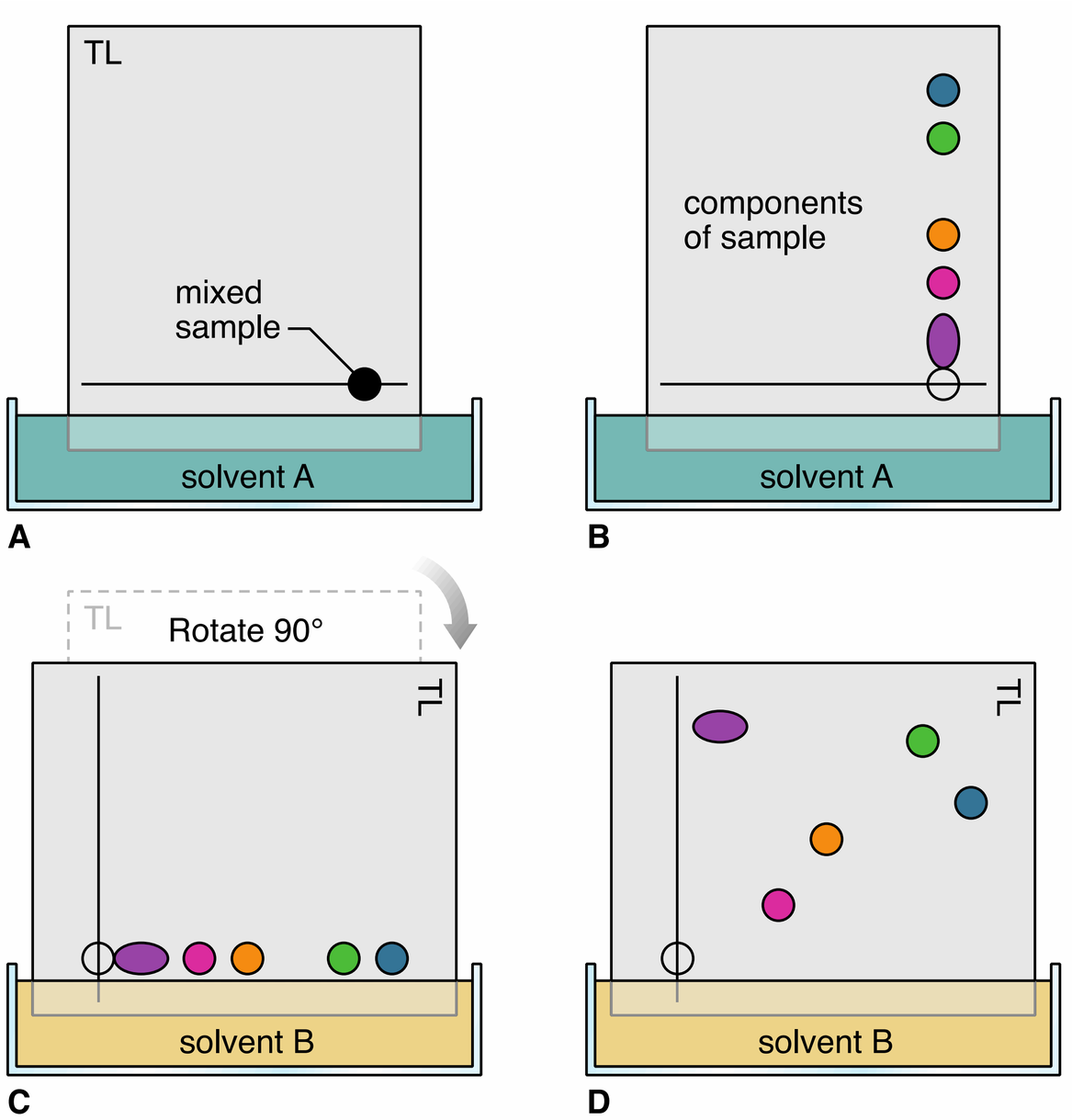


Fig. 1.19

Trifecta?

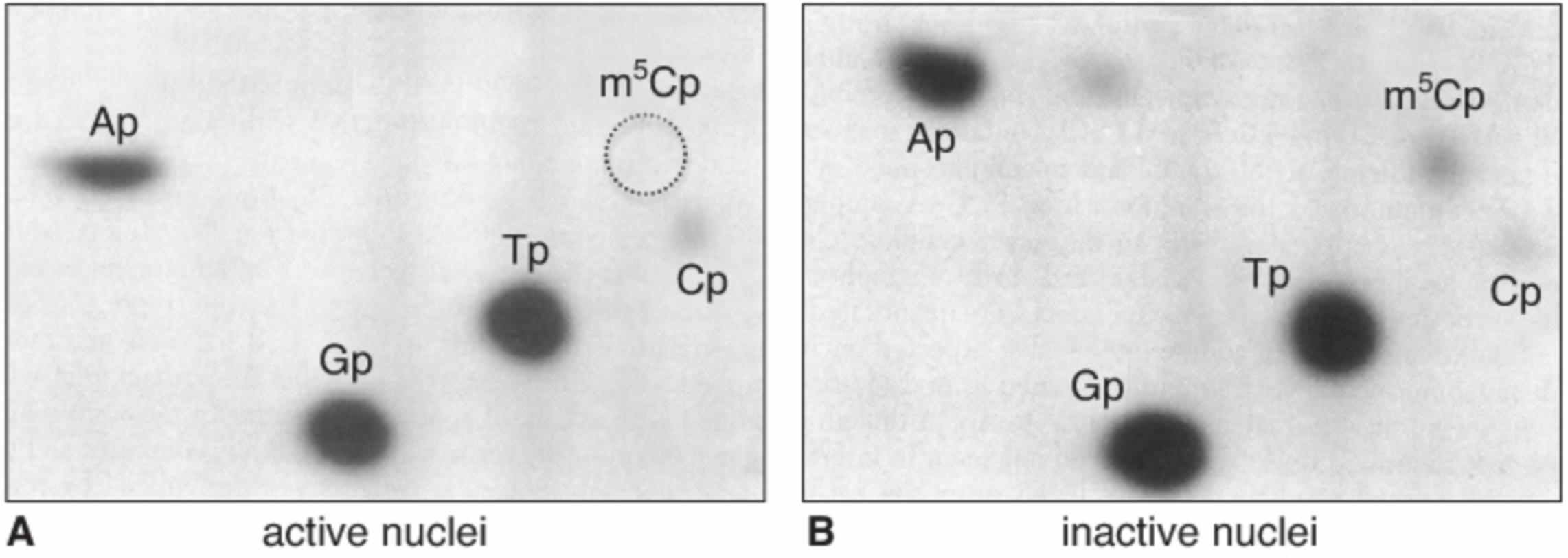


Figure 1.20 Two dimensional thin layer chromatography (TLC) results showing the five bases of DNA. Radioactive phosphorus "p" labels DNA fragments from **(A)** nuclei with known active genes and **(B)** nuclei with genes known to be inactive. The degree of darkness indicates how much of each base was present. The dotted circle shows you where to look for $_{m}^{5}{}_{Cp}$. Naveh-Many, Tally and Howard Cedar.

Bases of Active vs Inactive DNA

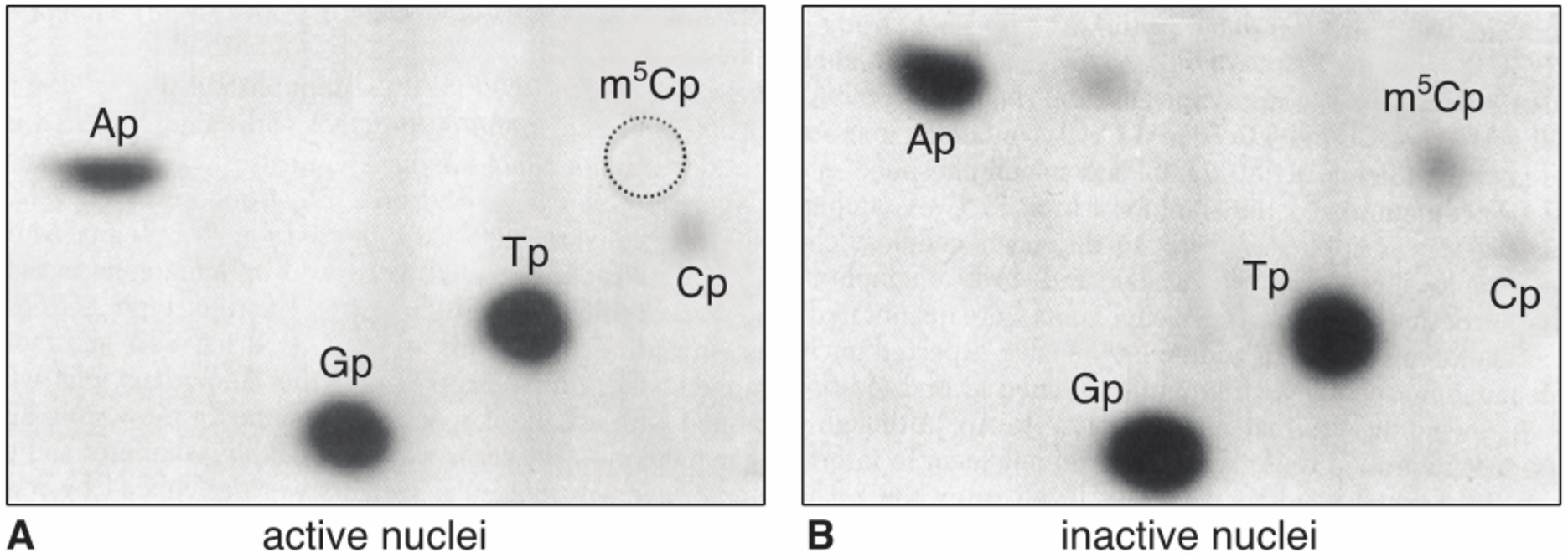
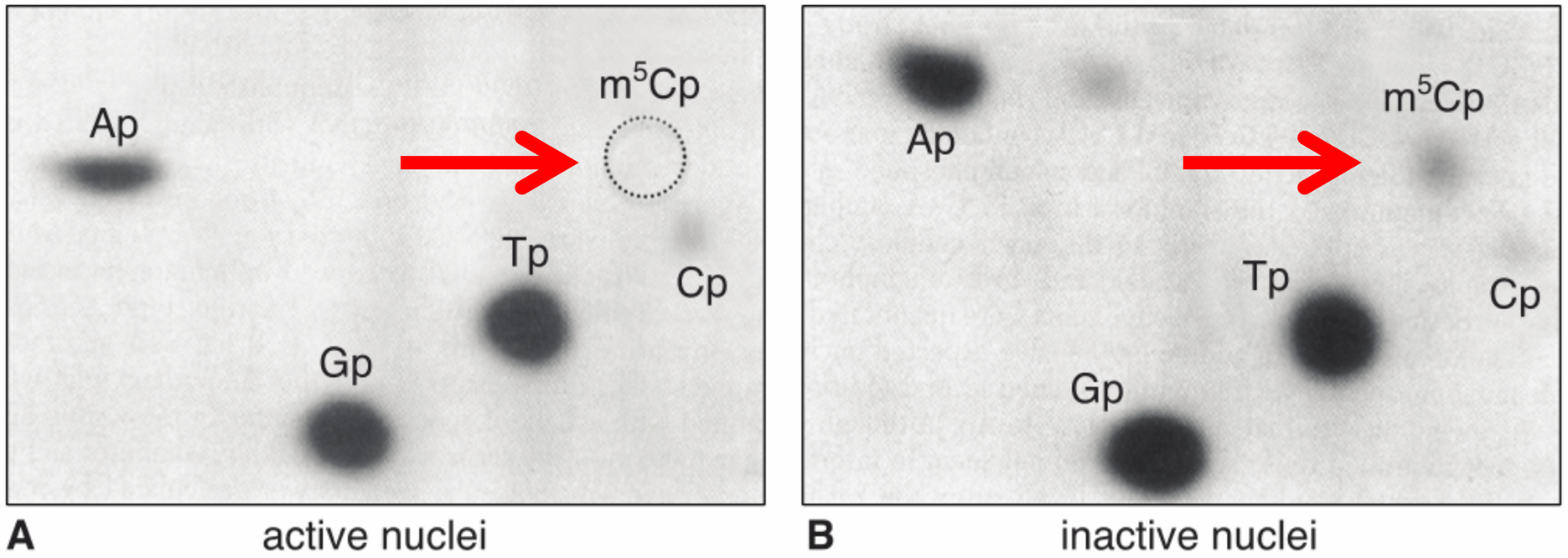


Fig. 1.20

from Naveh-Many and Cedar. 1981.

Bases of Active vs Inactive DNA



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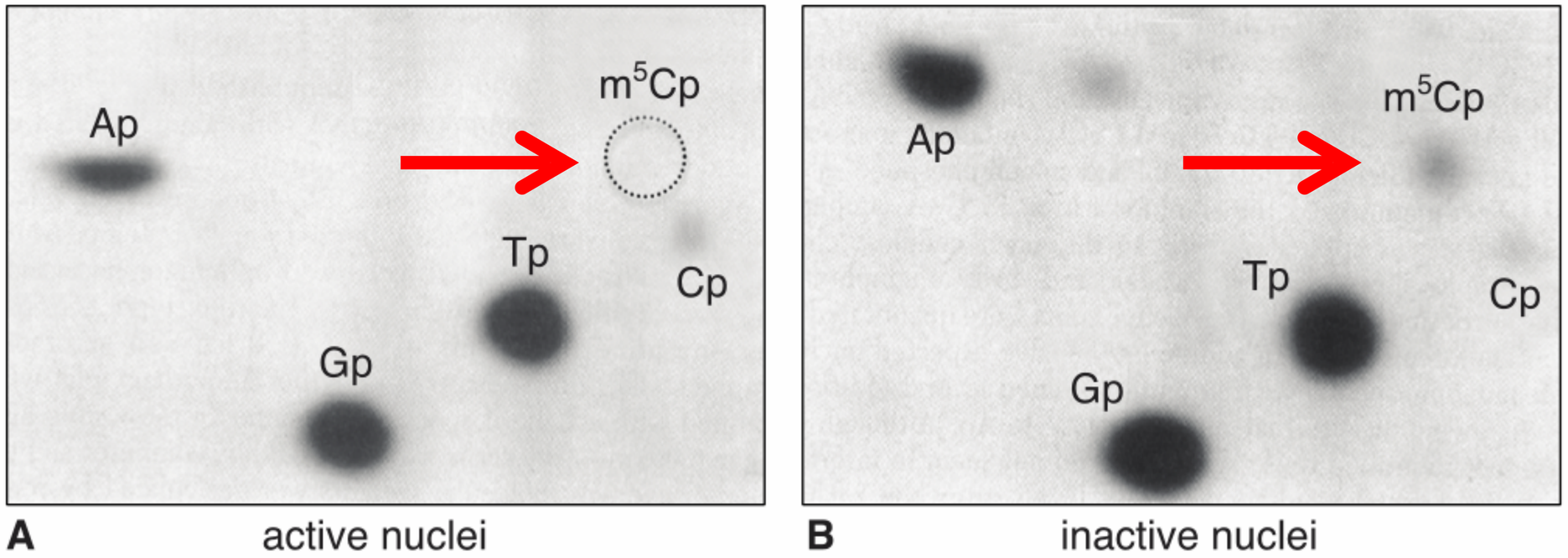
from Naveh-Many and Cedar. 1981.

Bases of Active vs Inactive DNA

What is the general rule about gene activity and methylation?



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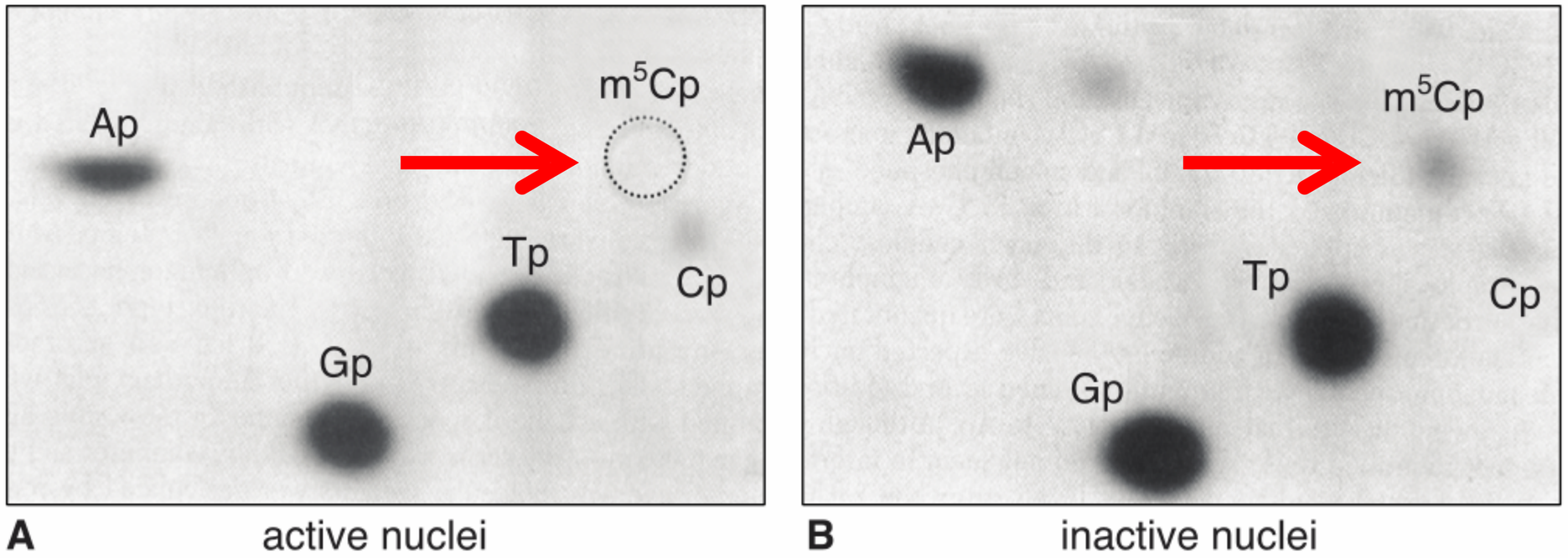


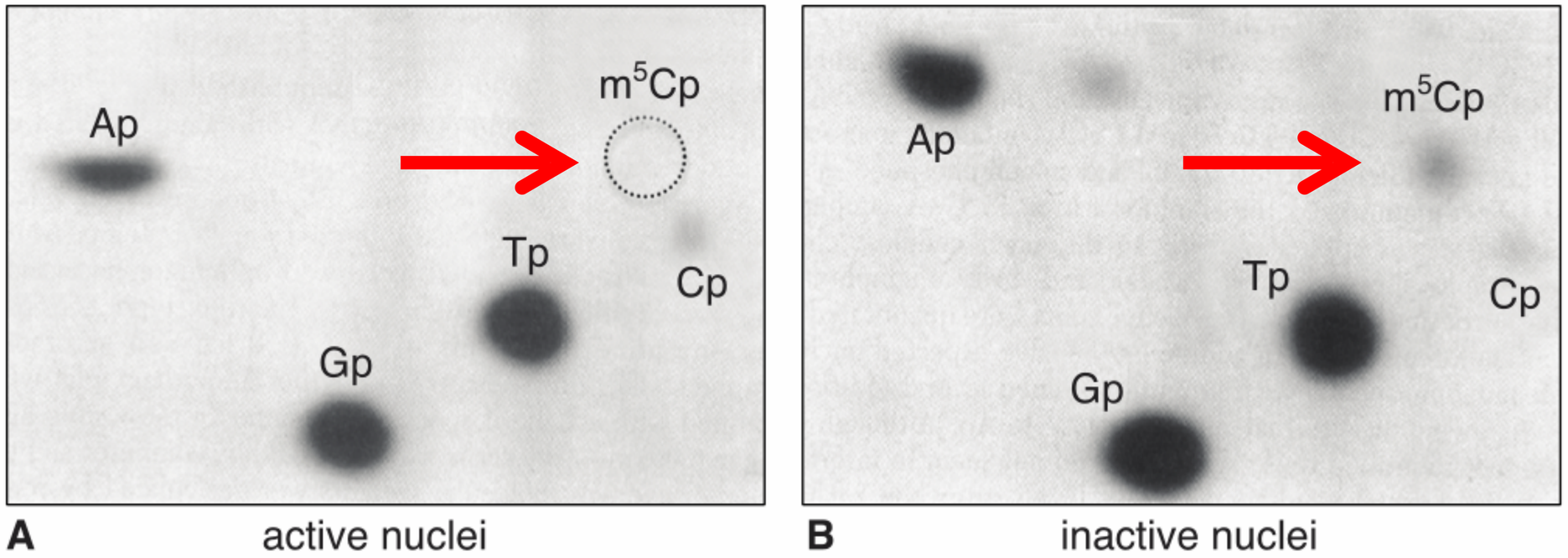
active genes are *hypo*methylated

inactive genes are *hyper*methylated

Fig. 1.20

from Naveh-Many and Cedar. 1981.

Cause *vs* Correlation



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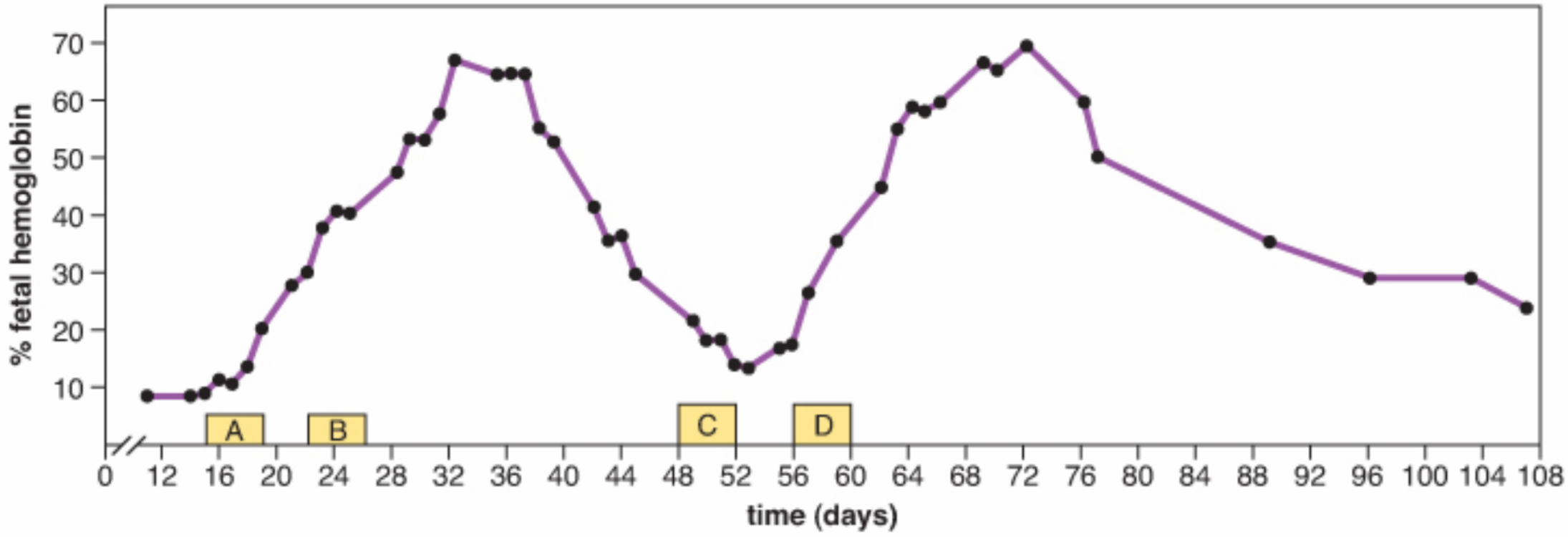

Figure 1.21 Monkey response to 5-azaC injections. Fetal hemoglobin levels were measured after monkeys were injected with 5-azaC at four times (A-D). More 5-azaC was injected at C and D than at A and B. From DeSimone, *et al.*, 1982. Figure 1. Copyright Joseph De

Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4428–4431, July 1982
Medical Sciences

5-Azacytidine stimulates fetal hemoglobin synthesis in anemic baboons

(globin genes/hypomethylation/cytidine analogue/gene expression)

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Veterans Administration West Side Medical Center and Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois

Communicated by Leon D. Jacobson, April 21, 1982

ABSTRACT **In an attempt to stimulate Hb F synthesis in baboons by means other than erythropoietic stress, we considered the possibility that an agent that inhibits methylation of CpG sequences in DNA may be effective. 5-Azacytidine, a cytosine analogue that cannot be methylated, is such an agent. Animals whose packed red cell volume was maintained at approximately 20% by bleeding were given 10 daily intravenous injections of the drug (6 mg/kg) in 12 days. Hb F levels in these animals started to increase on day 5 of this regimen and peak levels, which were 6–30 times higher than those produced by bleeding alone, occurred 5–7 days after the last dose of the drug. In animals previously identified as genetically "high" or "low" Hb F responders, the maximal Hb F levels were 70–85% and 35–40% respectively. In dose–response studies 5-azacytidine given daily at 3–4 mg/kg produced maximal Hb F increases. The drug did not increase the percentage (number) of Hb F-containing cells (F cells) beyond the maximal**

in Hb F synthesis (7–9). The magnitude of this response (high or low) has been shown to be genetically determined (10, 11) and it appeared to be of interest to determine whether these genetic differences could be influenced by 5-azaC. Other myelosuppressive agents [hydroxyurea and 1-β-D-arabinofuranosylcytosine (araC, cytosine arabinoside)] were used in four baboons to test their effect on Hb F synthesis.

METHODS

Initially, four baboons (2, 3, 3, and 5 years old; weight 4–12 kg) were bled to reduce the packed erythrocyte volume (PCV) to 20% within 5 days. Two of the baboons had been found to be high Hb F responders and two were low responders (10, 11). The PCV of 20% was maintained for another 10 days by bleeding; during this time, Hb F levels were measured every day by alkali denaturation (12) to determine the extent of Hb F increase

ABSTRACT In an attempt to stimulate Hb F synthesis in baboons by means other than erythropoietic stress, we considered the possibility that an agent that inhibits methylation of CpG sequences in DNA may be effective. 5-Azacytidine, a cytosine analogue that cannot be methylated, is such an agent. Animals whose packed red cell volume was maintained at approximately 20% by bleeding were given 10 daily intravenous injections of the drug (6 mg/kg) in 12 days. Hb F levels in these animals started to increase on day 5 of this regimen and peak levels, which were 6–30 times higher than those produced by bleeding alone, occurred 5–7 days after the last dose of the drug. In animals previously identified as genetically "high" or "low" Hb F responders, the maximal Hb F levels were 70–85% and 35–40% respectively. In dose–response studies 5-azacytidine given daily at 3–4 mg/kg produced maximal Hb F increases. The drug did not increase the percentage (number) of Hb F-containing cells (F cells) beyond the maximal number achieved by bleeding alone and thus its main effect was to increase Hb F per F cell. The finding that Hb F synthesis can be modulated to such a high degree by a drug may have therapeutic implications—e.g., in sickle cell anemia, in which stimulation of Hb F synthesis may prevent sickling.

The degree of methylation of CpG dinucleotide sequences of

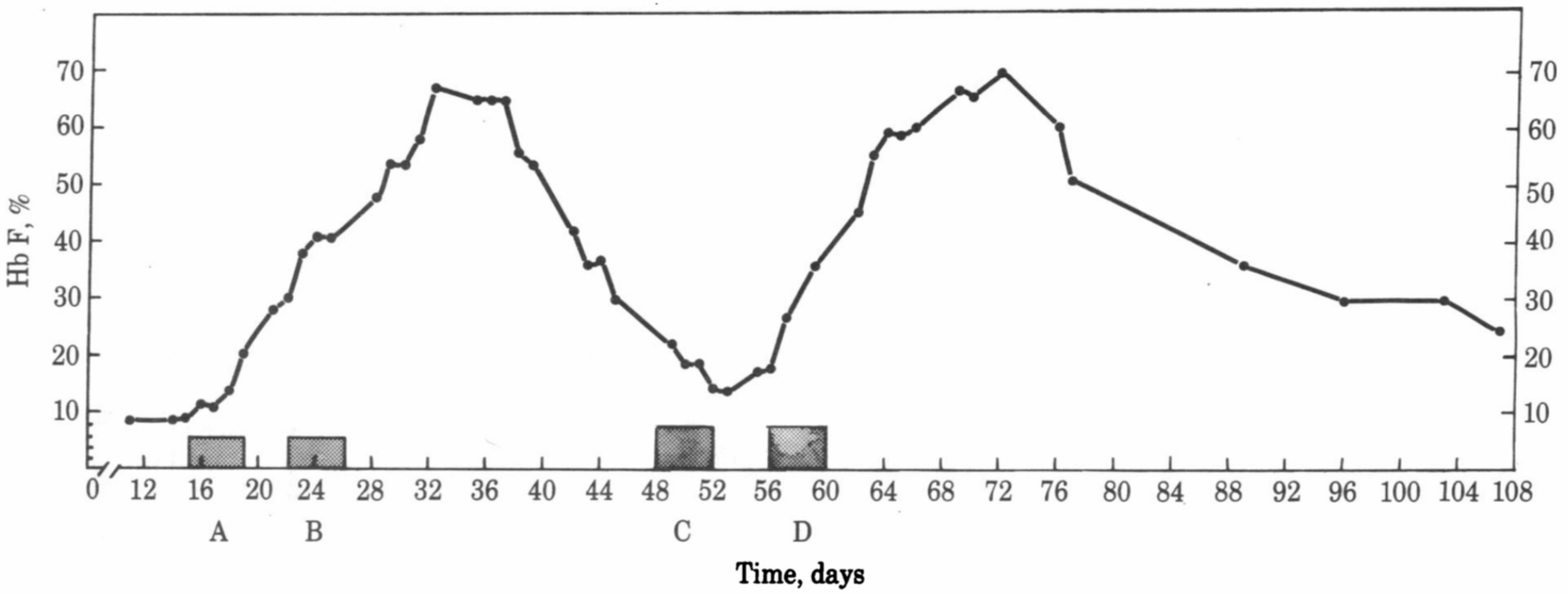


FIG. 1. Changes of Hb F levels in baboons rendered anemic by bleeding and then injected with 5-azaC: A, 6 mg/kg per day on days 15–19; B, 6 mg/kg per day on days 22–26; C, 8 mg/kg per day on days 49–53; D, 8 mg/kg per day on days 56–60.

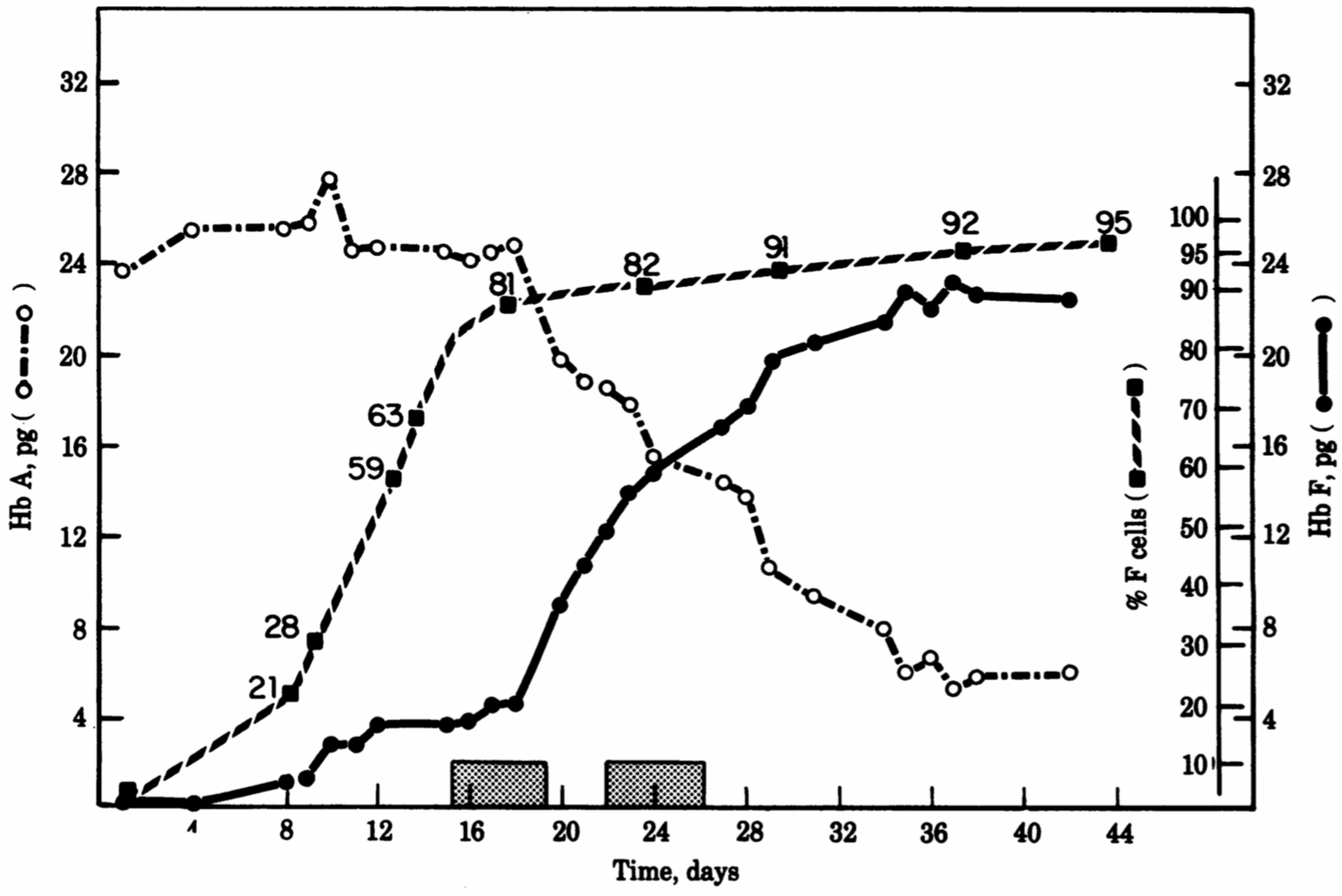
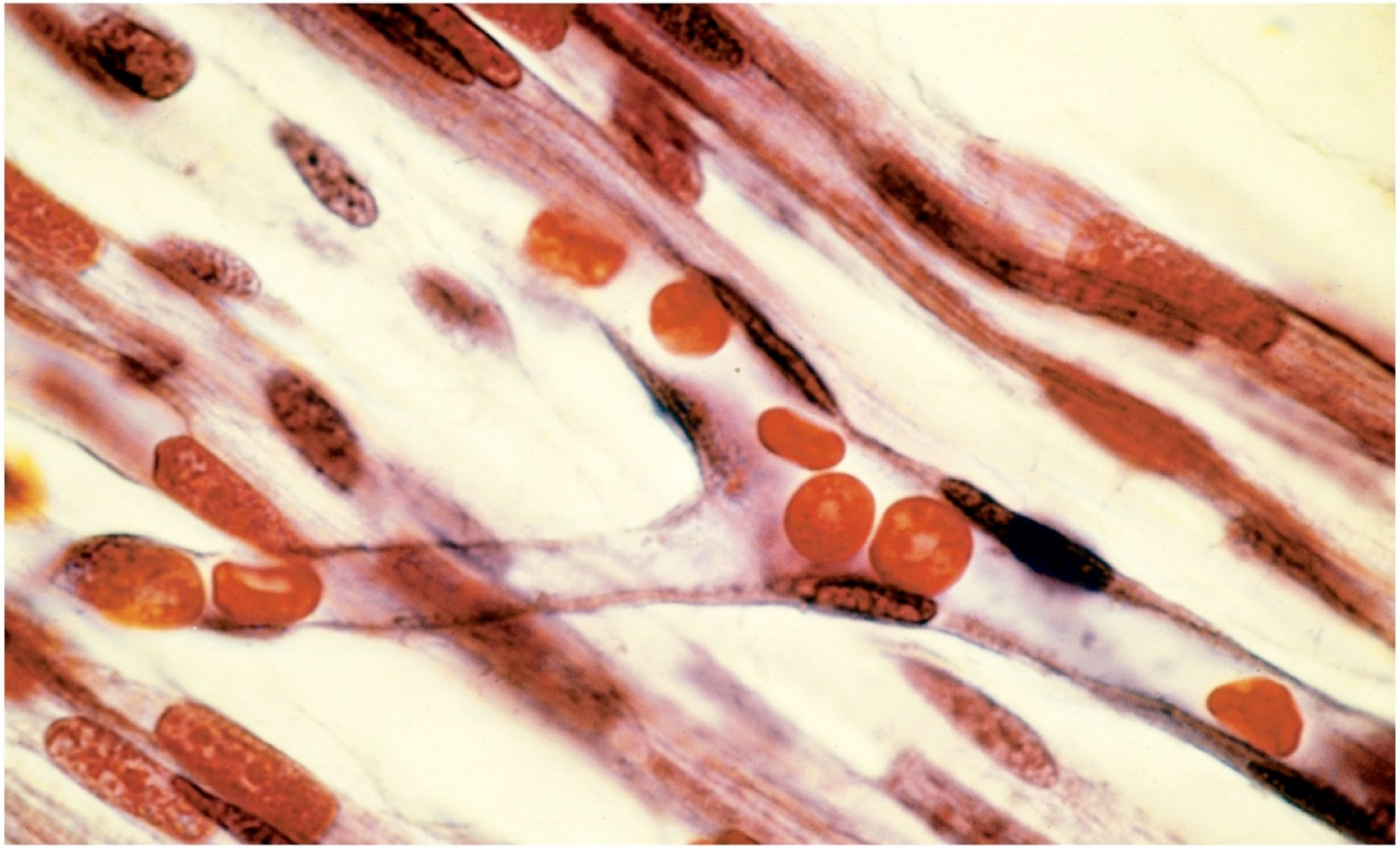


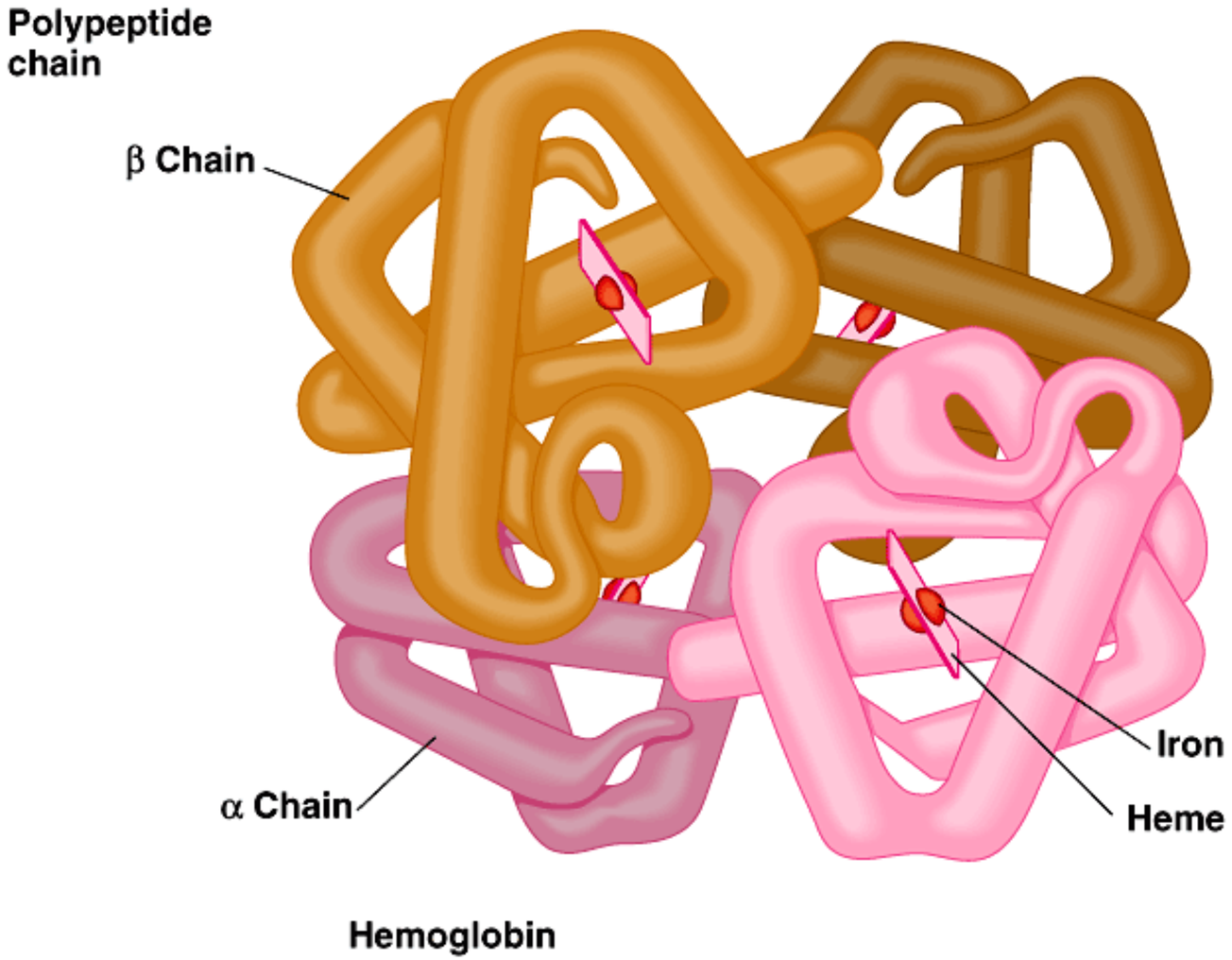
FIG. 2. Calculated mean corpuscular Hb values for Hb A and Hb F during treatment with 5-azaC (6 mg/kg per day) (rectangles) of a high responder adult baboon. Percentages of F cells are also shown. Note the rapid increase in the percentage of F cells with bleeding alone, followed by the increase in the amount of Hb F per erythrocyte.

Capillaries are small and extremely thin walled.



Hemoglobin

The Body's Oxygen Shuttle (and more)



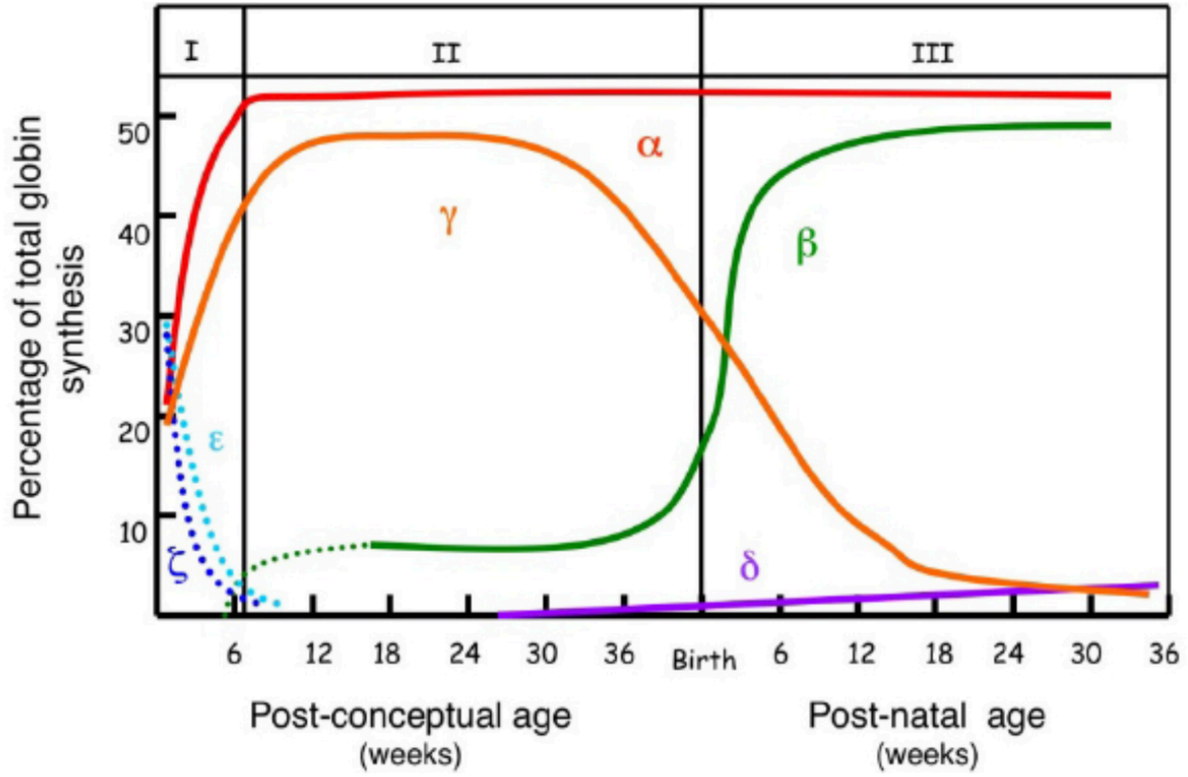
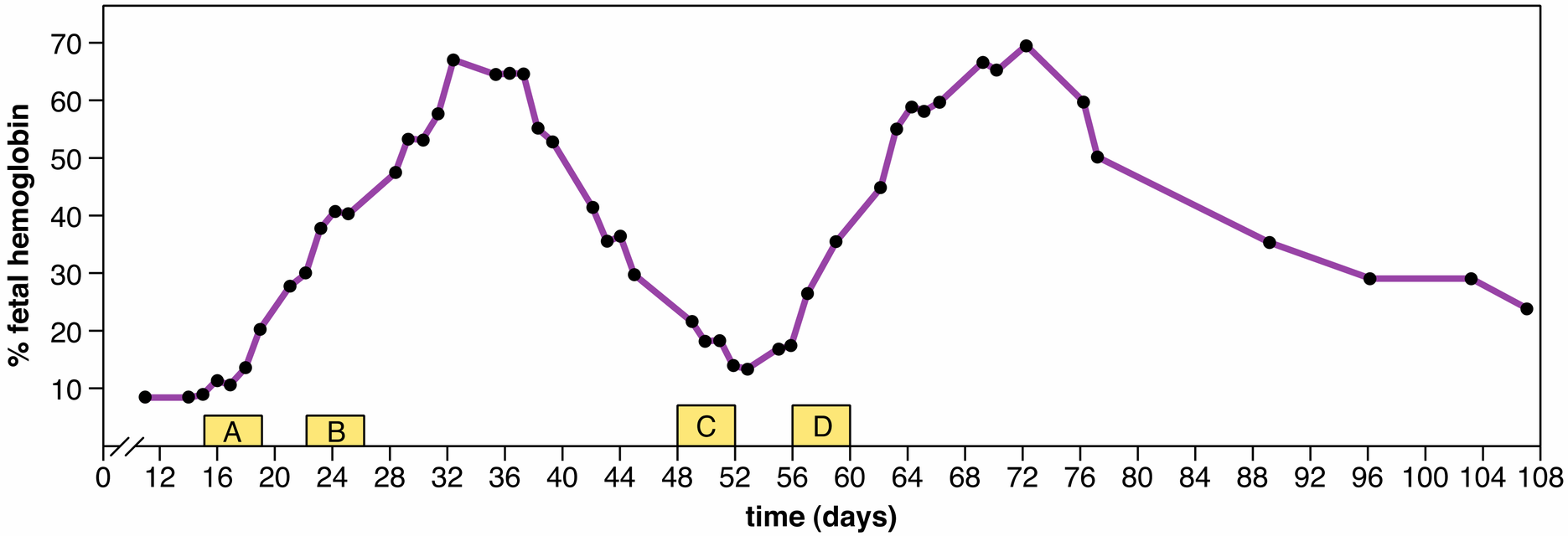


Fig. 3. **Changes in globin gene expression profile during ontogeny.** The x-axis represents the age of the fetus in weeks. The y-axis corresponds to the expression of each globin gene as a percentage of total globin gene expression. Time of birth is denoted with a vertical line. The embryonic genes are expressed during the first six weeks of gestation. The first switch from ε- to γ-globin occurs within 6 weeks after conception, and the second switch from γ- to β-globin occurs shortly after birth.

Pharmacological Gene Regulation

Can genes be regulated by epigenetic changes?



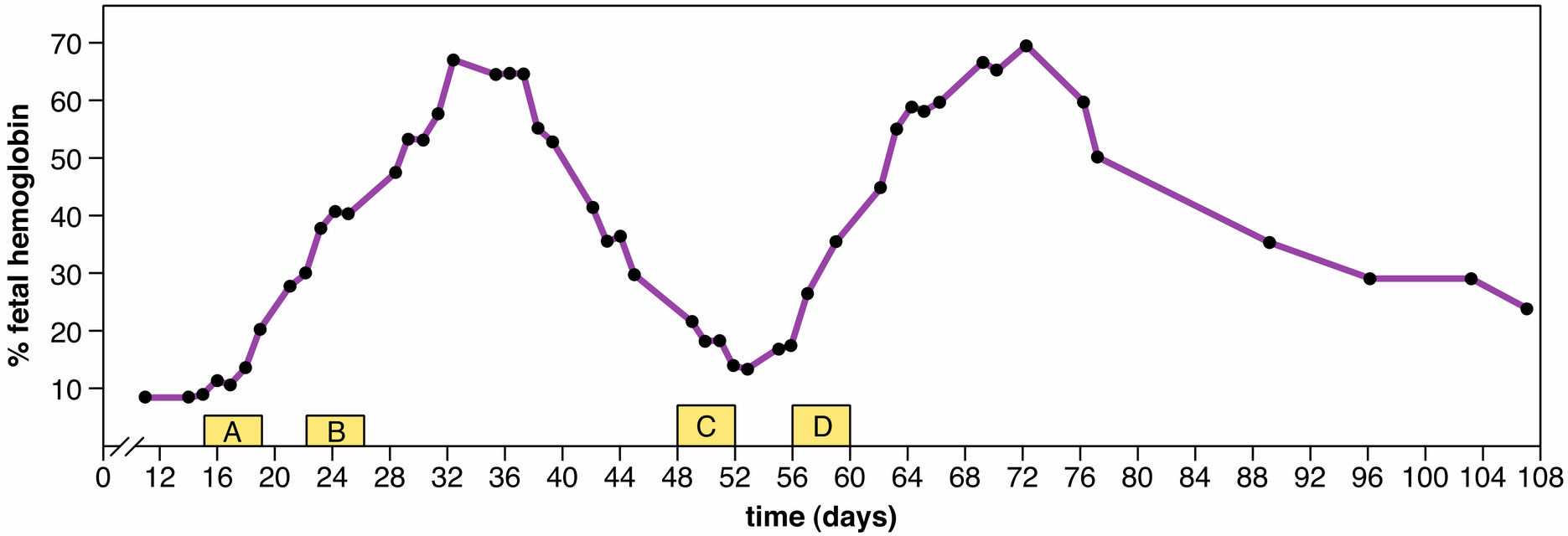
adult monkey response to methylase inhibitor

Fig. 1.21

modified from DeSimone, *et al.*, 1982

Pharmacological Gene Regulation

fetal hemoglobin levels over time



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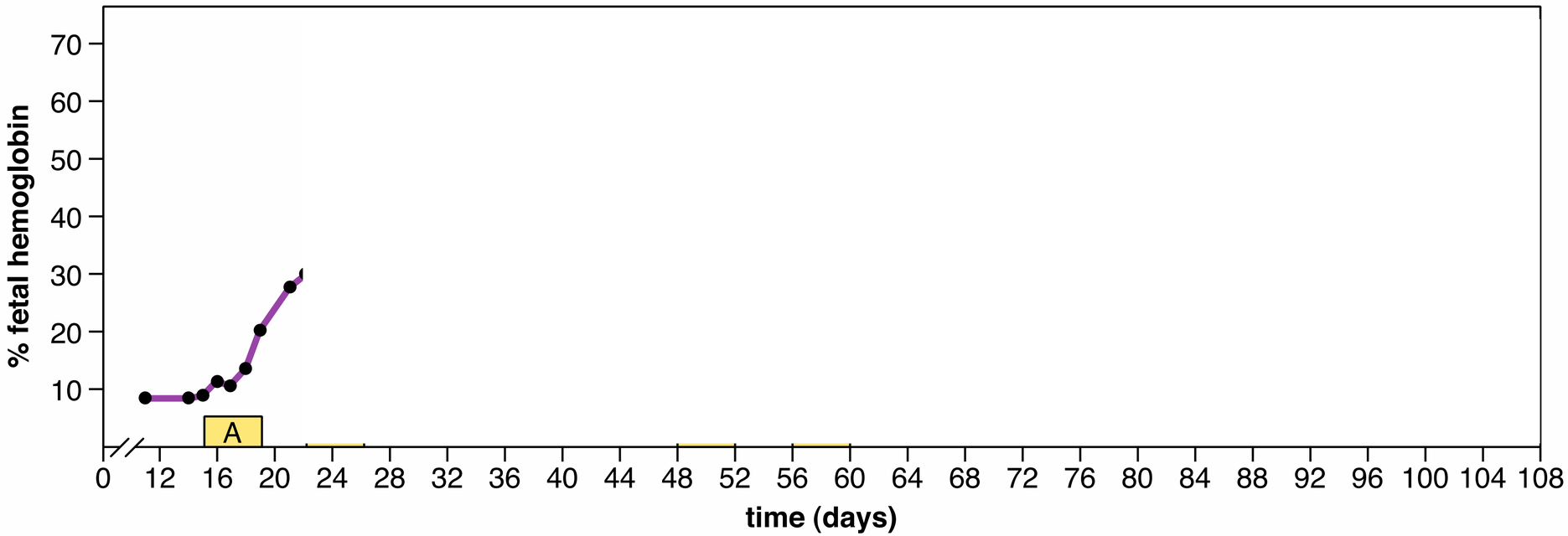

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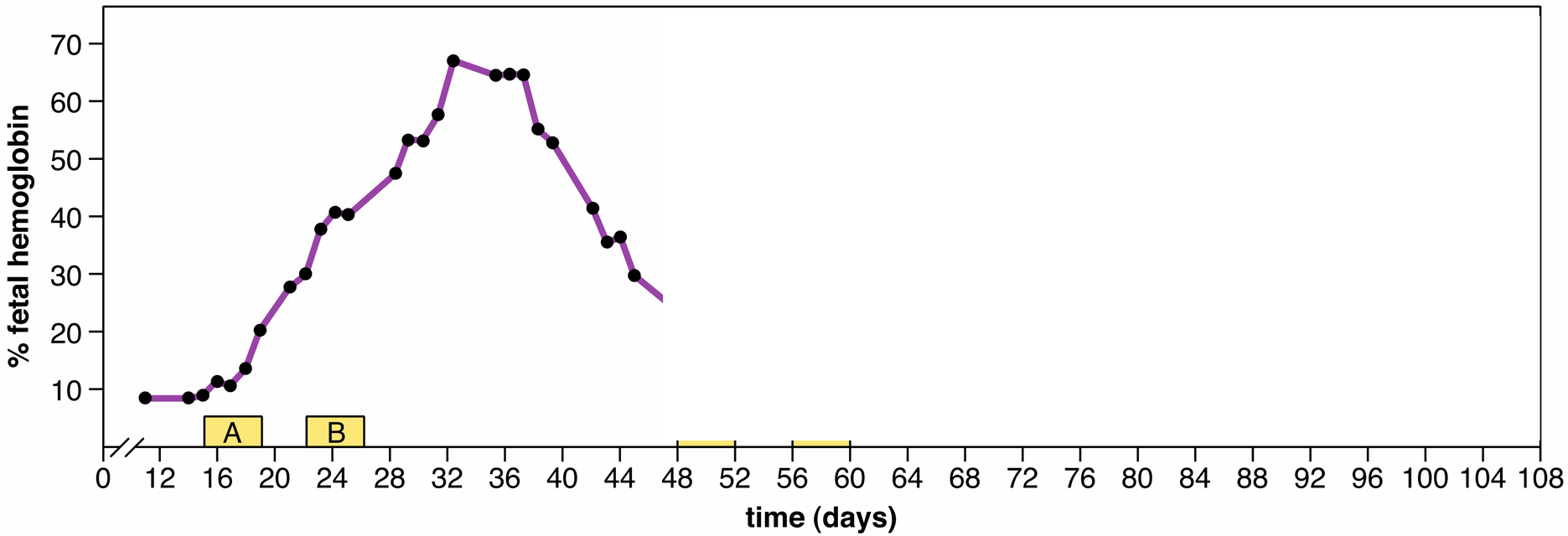

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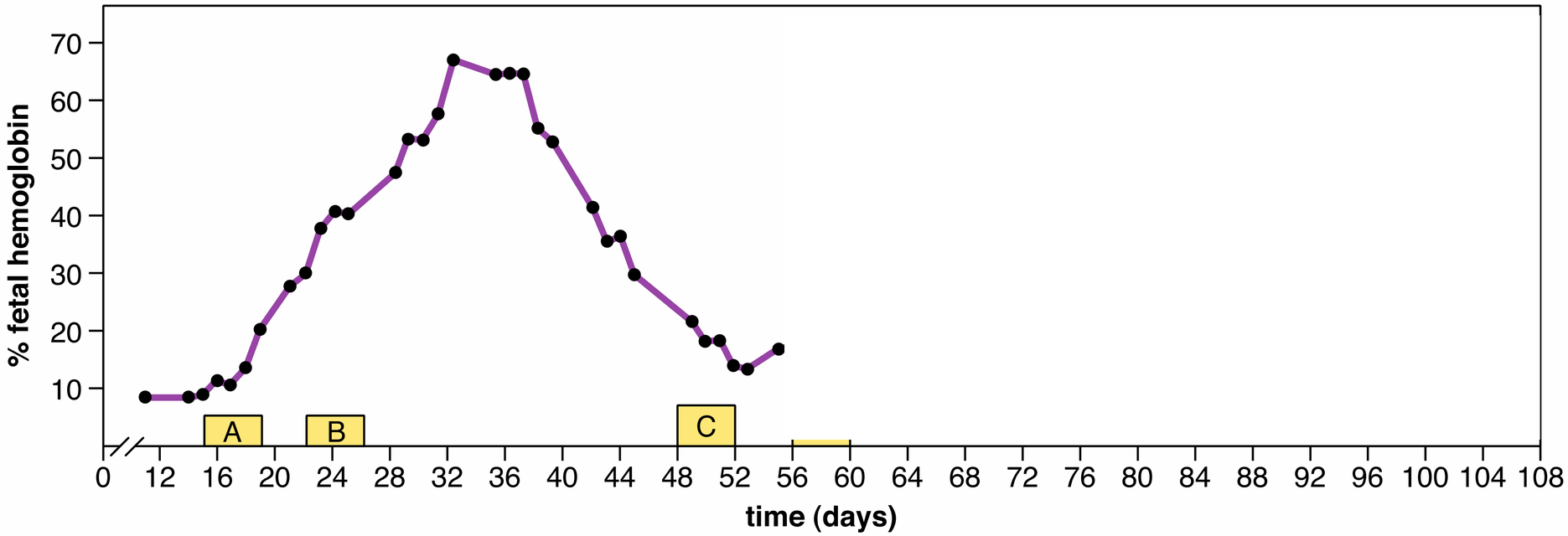
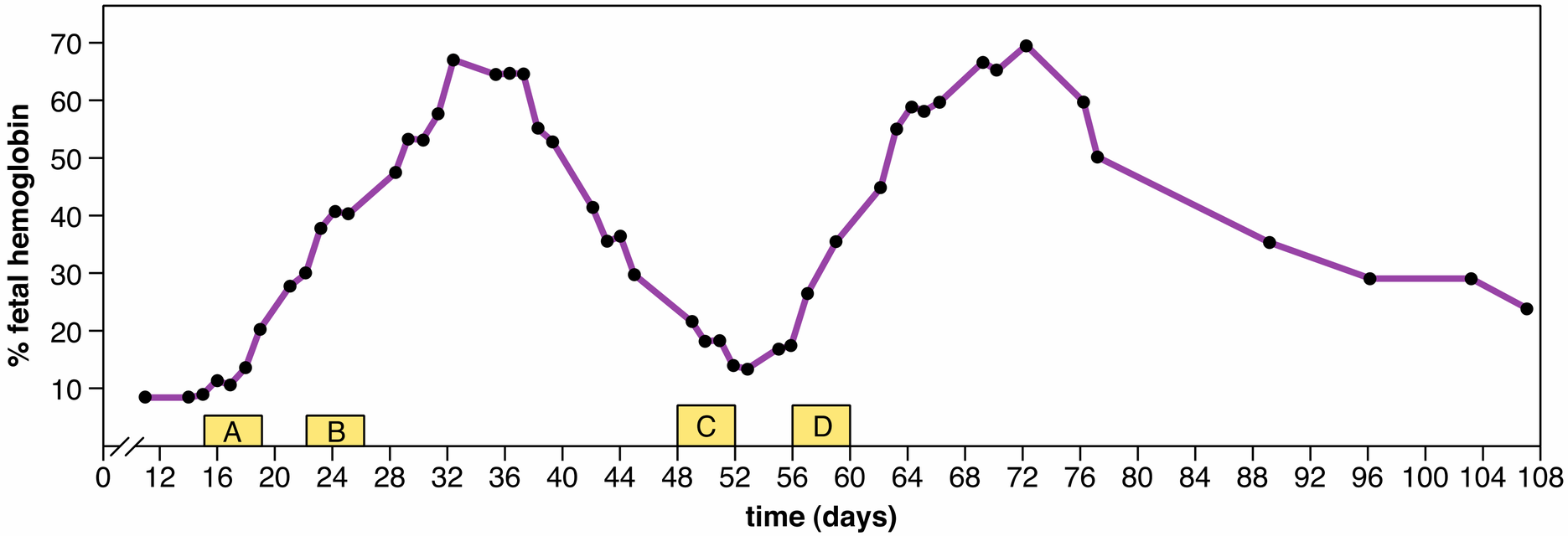


third (higher) dose of methylation inhibitor

Fig. 1.21

modified from DeSimone, *et al.*, 1982

Pharmacological Gene Regulation



What is the consequence of higher inhibitor dose?

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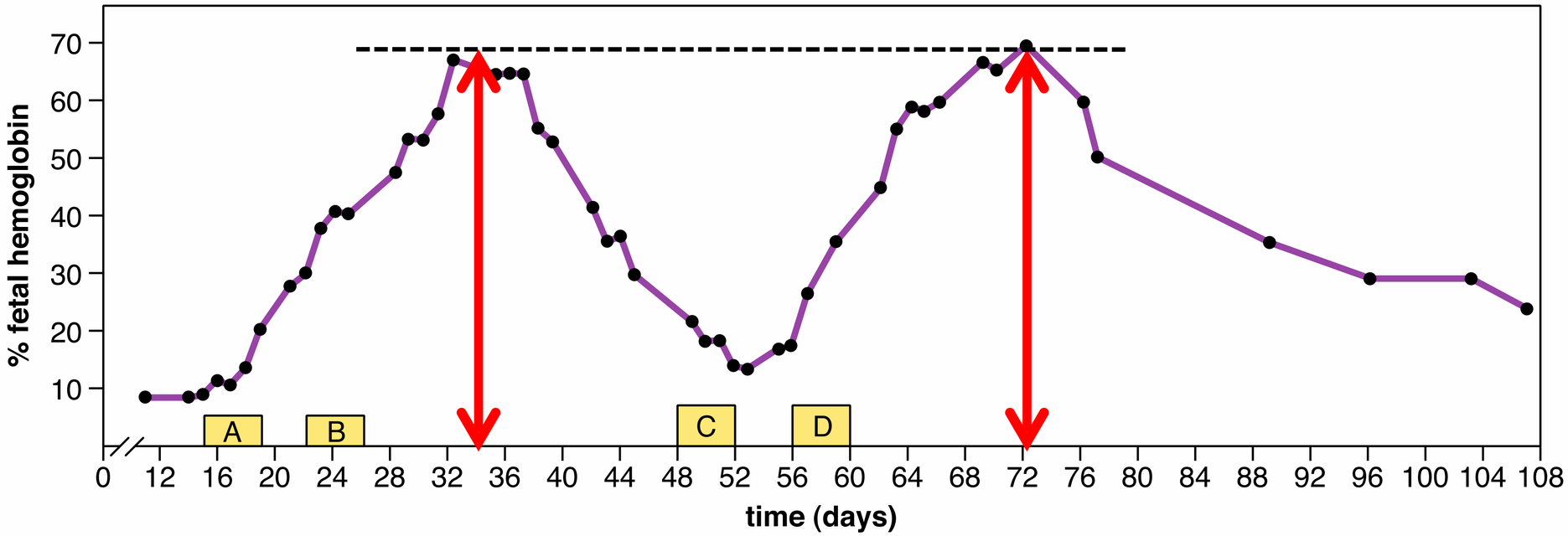
modified from DeSimone, *et al.*, 1982

Pharmacological Gene Regulation



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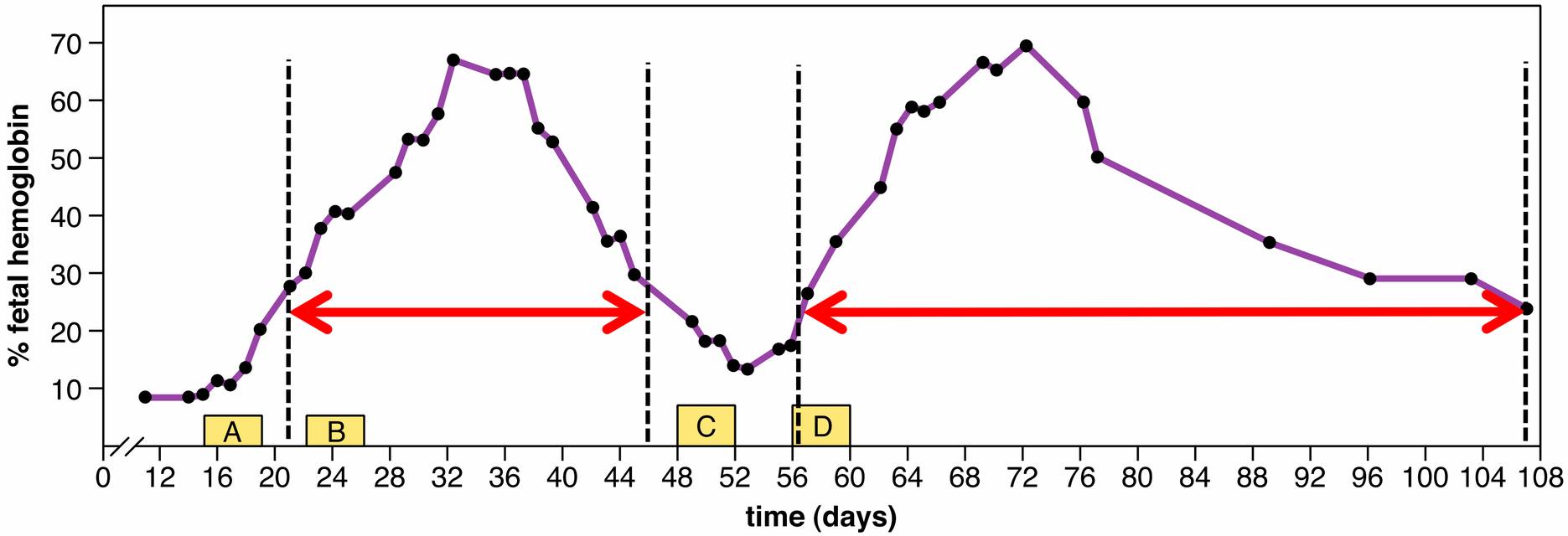

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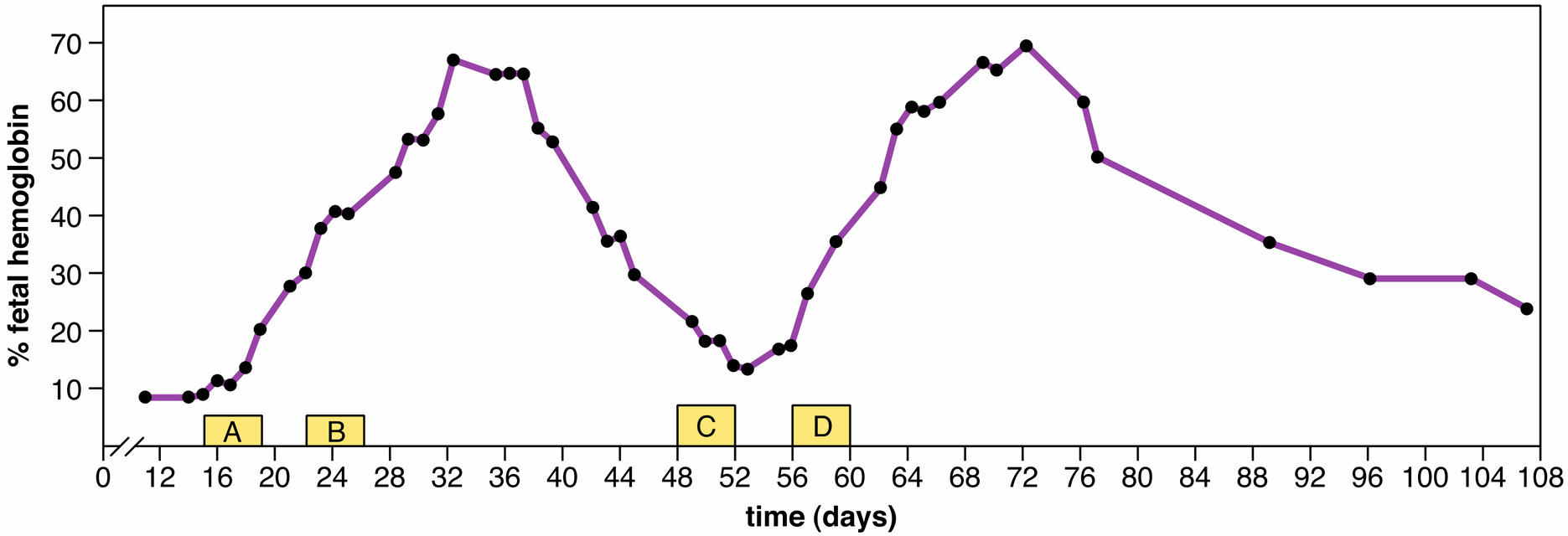
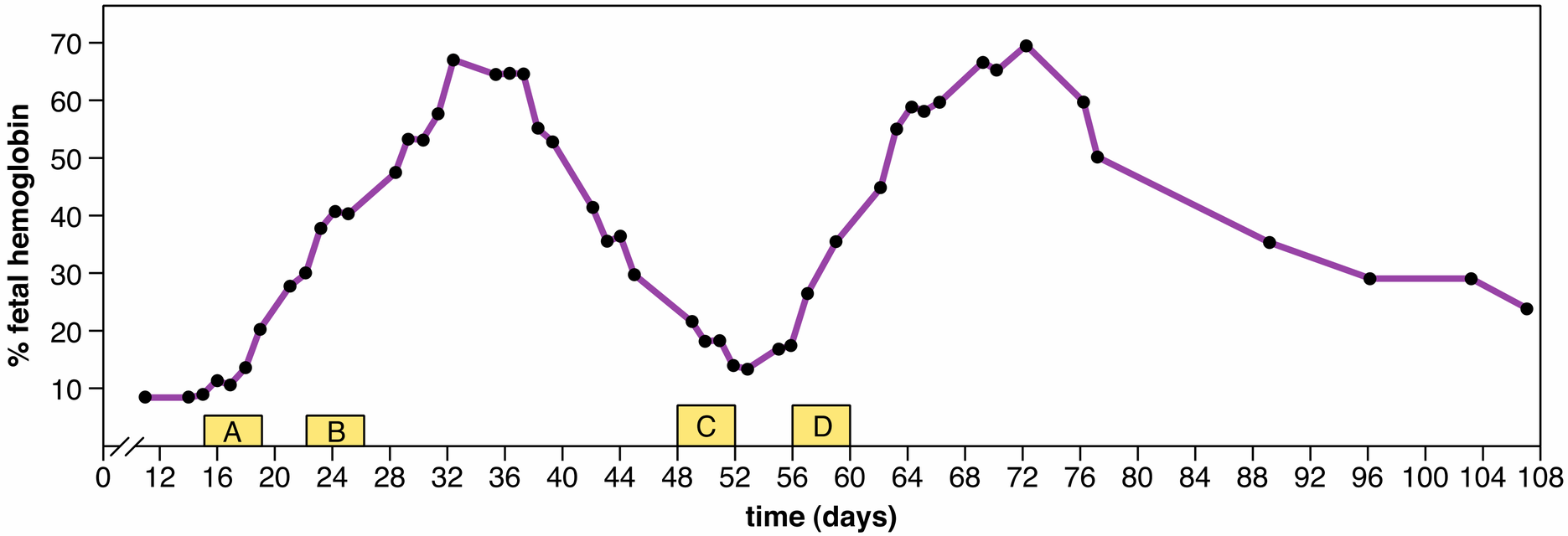


Why was this a bad idea for clinical use?

Fig. 1.21

modified from DeSimone, *et al.*, 1982

Cause *vs* Correlation



Does this experiment show causation?

Fig. 1.21

modified from DeSimone, *et al.*, 1982