DNA Sequencing

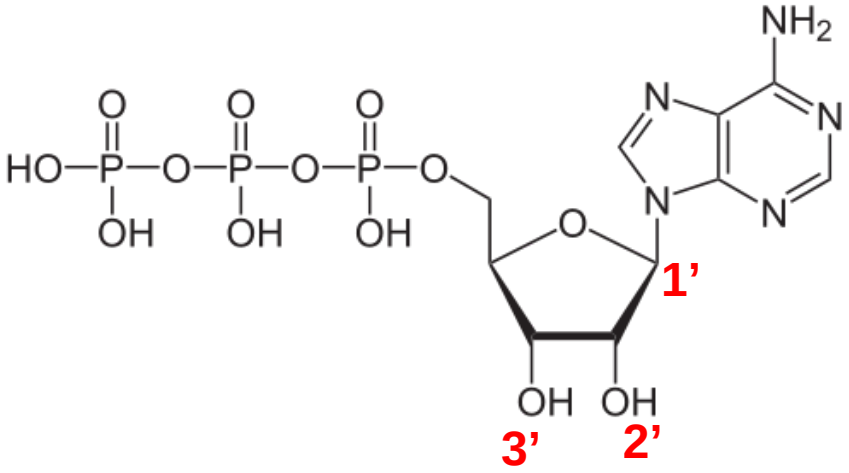
mobility inversely proportional to length in a denaturing gel (e.g. containing urea)

Sanger et al.: DNA polymerase copies a single strand starting with a primer in a soup of the 4 deoxy-nuleotide triphosphates, spiked with 10% of di-deoxy form of one (for example G').

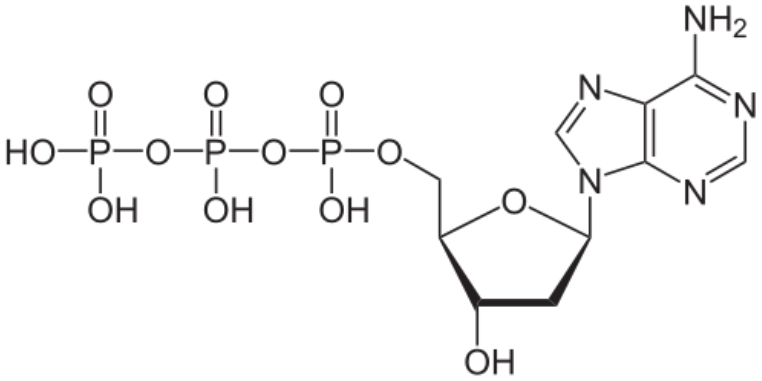
The G' can be incorporated by the enzyme but it cannot extend the chain, thereby creating a ***random*** distribution of chain lengths, ***all ending with G'*** which is now typically labeled with a fluorescent dye.

The soup could also contain 10% of C', A', and T', each with a different colored dye.

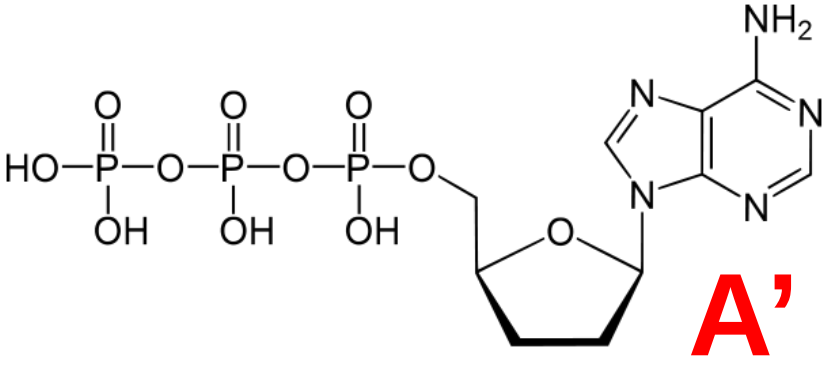
Gel electrophoresis then gives all the possible lengths, each with the color of the base at that position.



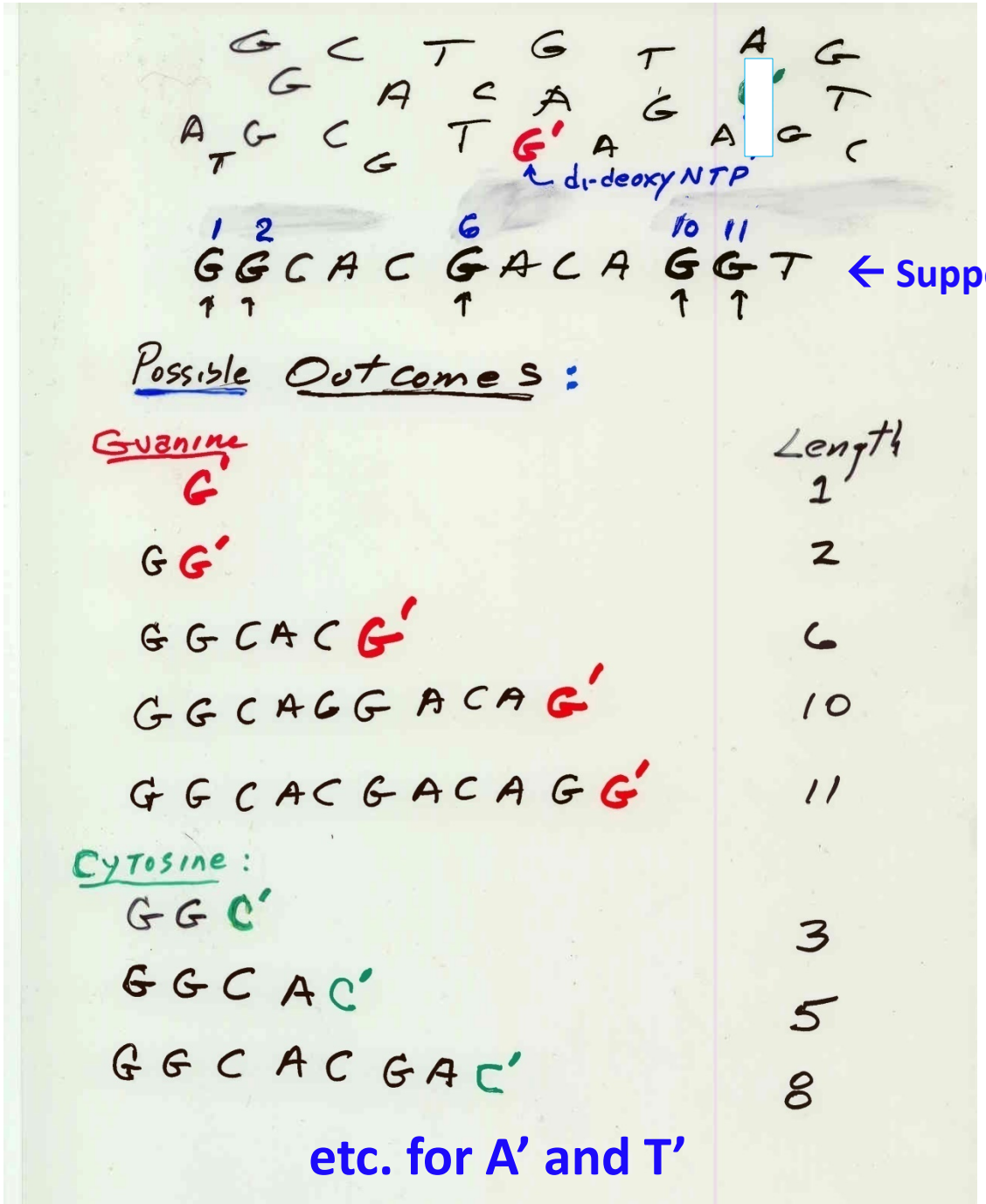
adenosine triphosphate
(in **RNA)**



2’-deoxyadenosine triphosphate
(in **DNA**)



2’,3’-dideoxyadenosine triphosphate
(in **No NA**



Spiked soup
+ template
+ polymerase

← Suppose primer codes for this:

The di-deoxy nucleotides incorporate, but cannot extend the chain.

The G' is in ***low*** concentration so incorporation is equally probable at all positions

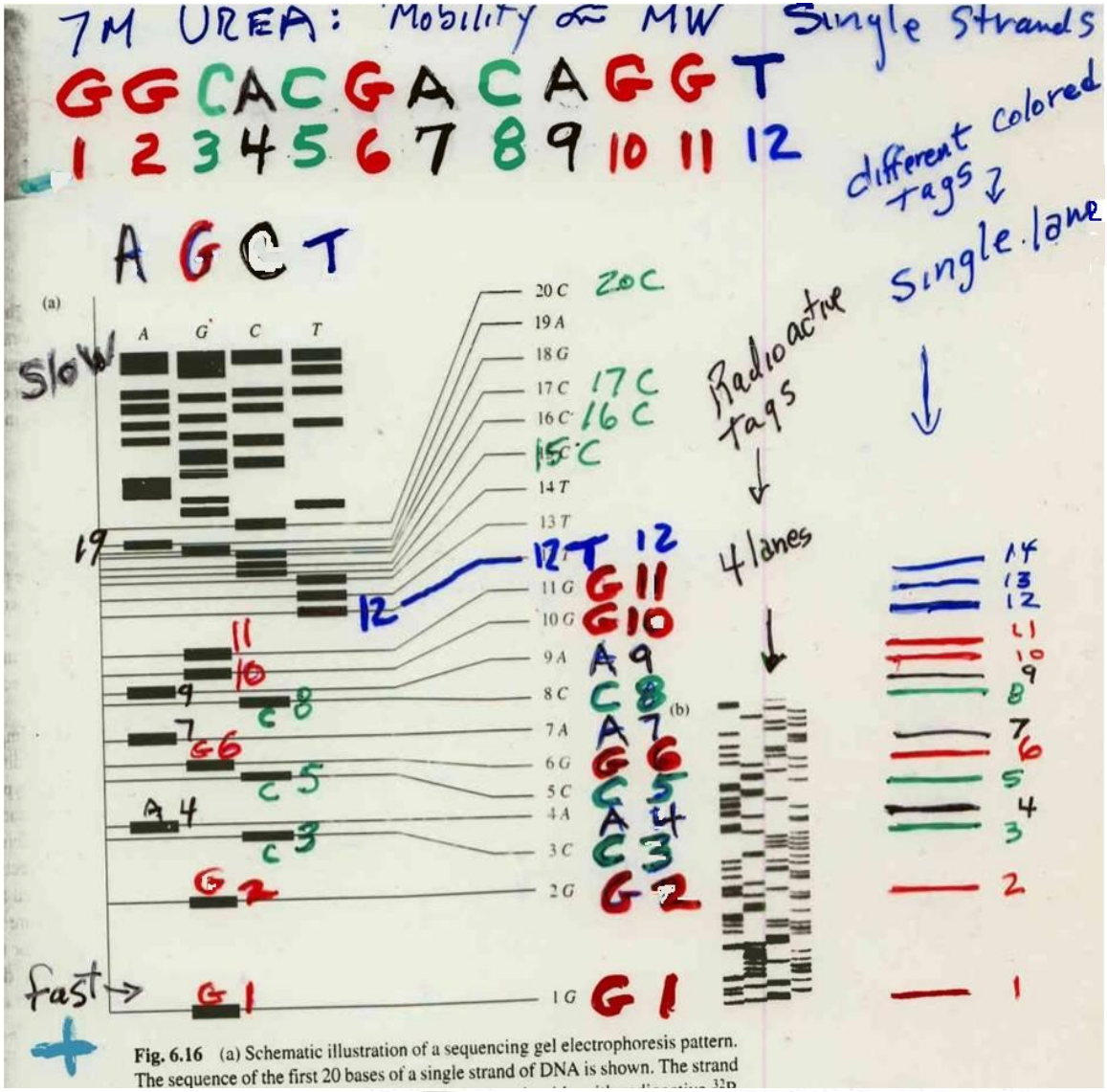


Fig. 6.16 (a) Schematic illustration of a sequencing gel electrophoresis pattern. The sequence of the first 20 bases of a single strand of DNA is shown. The strand

0.67% methylene bisacrylamide, 7 *M* urea. After electrophoresis (the positive electrode is at the bottom of the gel), autoradiography for 8 hr produced the pattern shown. The sequence is now simply read off. [A. M. Maxam and W. Gilbert,

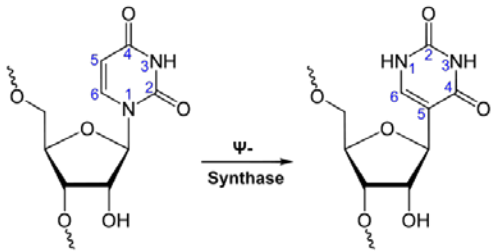
Finger Printing

Only ~2% of human DNA codes for proteins (genes). The rest is simple repeating sequences unique to each individual (except identical twins).

These regions are now known to code for small regulatory RNAs, a rapidly exploding area of knowledge

Wikipedia RNAi

Many RNAs are involved in modifying other RNAs.
Introns are spliced out of pre-mRNA by spliceosomes, which contain several small nuclear RNAs (snRNA),[1] or the introns can be ribozymes that are spliced by themselves.[46]
RNA can also be altered by having its nucleotides modified to other nucleotides than A, C, G and U. In eukaryotes, modifications of RNA nucleotides are in general directed by small nucleolar RNAs (snoRNA; 60-300 nt),[27] found in the nucleolus and cajal bodies. snoRNAs associate with enzymes and guide them to a spot on an RNA by basepairing to that RNA. These enzymes then perform the nucleotide modification. rRNAs and tRNAs are extensively modified, but snRNAs and mRNAs can also be the target of base modification.[47][48]
RNA can also be methylated.

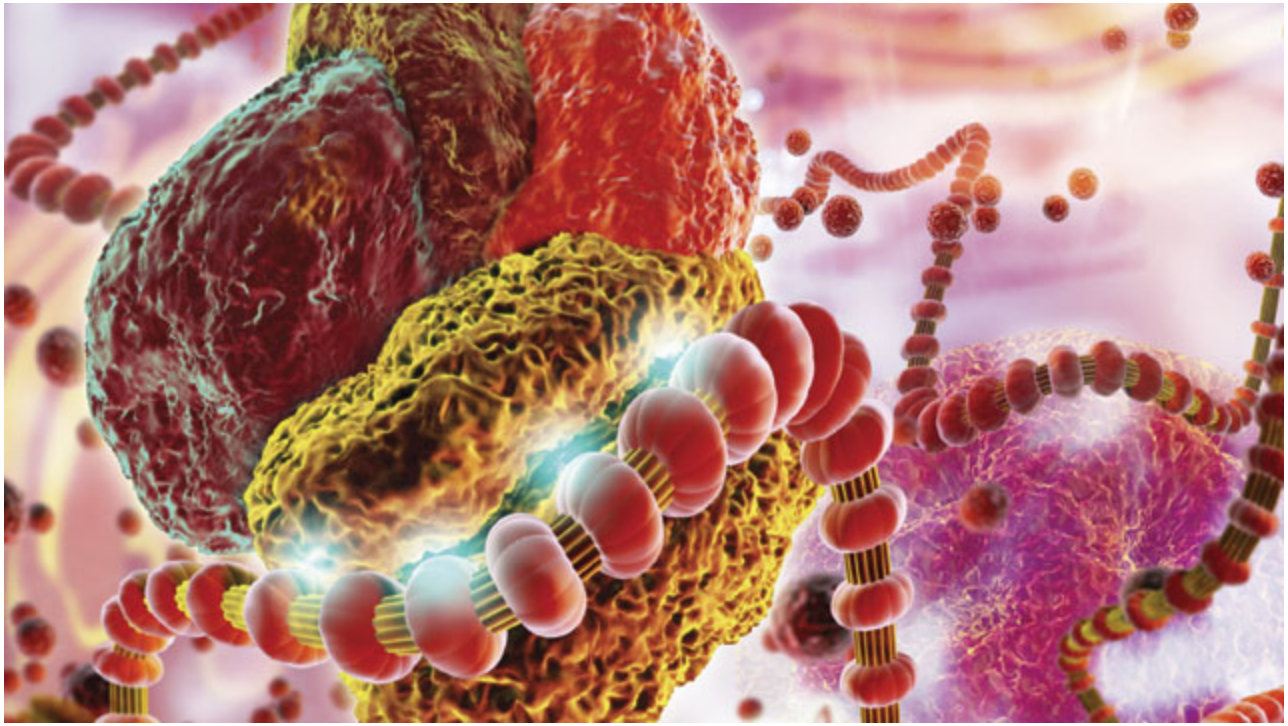


Uridine to pseudouridine is a common RNA modification.

The Second Coming of RNAi

Now showing clinical progress against liver diseases, the gene-silencing technique begins to fulfill some of its promises.

By Eric Bender | September 1, 2014



THE ART OF SILENCING: Small interfering RNA molecules are incorporated into an RNA-induced silencing complex where they bind and degrade target messenger RNAs (yellow with red rings). Taking advantage of this natural RNA interference (RNAi) pathway, researchers are developing therapeutics for liver-based diseases, viral infections, cancer, and more.

When these ***hypervariable fingerprint regions*** are cut up at a specific sequence (such as CAATTG) by a restriction enzyme, the resulting gel is unique to each individual. This is called RFLP analysis, standing for ***restriction fragment length polymorphism analysis.***

Large ***double stranded*** DNA "crawls" through the gel.
This is called "reptation", i.e., snake-like motion.

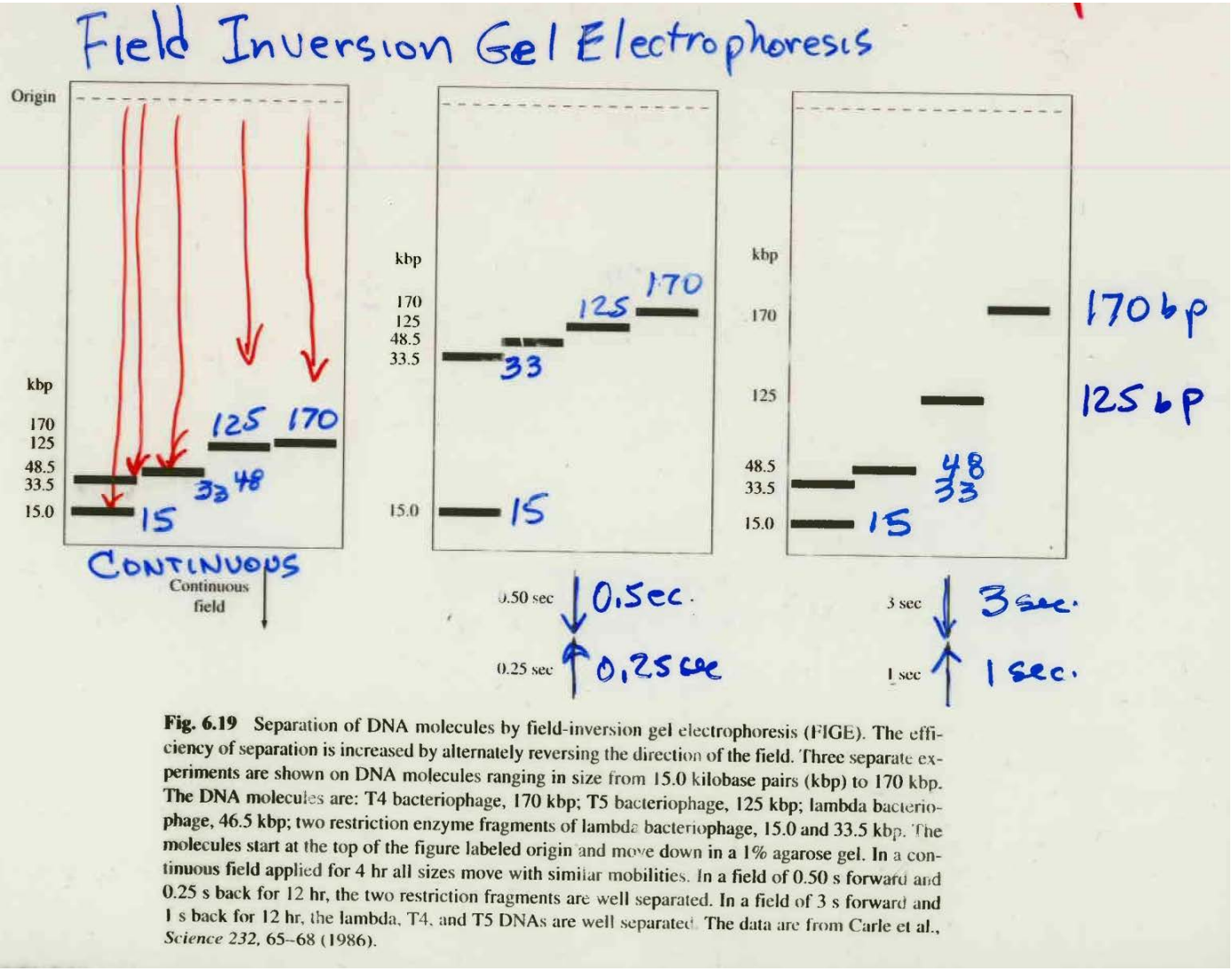


Fig. 6.19 Separation of DNA molecules by field-inversion gel electrophoresis (FIGE). The efficiency of separation is increased by alternately reversing the direction of the field. Three separate experiments are shown on DNA molecules ranging in size from 15.0 kilobase pairs (kbp) to 170 kbp. The DNA molecules are: T4 bacteriophage, 170 kbp; T5 bacteriophage, 125 kbp; lambda bacteriophage, 46.5 kbp; two restriction enzyme fragments of lambda bacteriophage, 15.0 and 33.5 kbp. The molecules start at the top of the figure labeled origin and move down in a 1% agarose gel. In a continuous field applied for 4 hr all sizes move with similar mobilities. In a field of 0.50 s forward and 0.25 s back for 12 hr, the two restriction fragments are well separated. In a field of 3 s forward and 1 s back for 12 hr, the lambda, T4, and T5 DNAs are well separated. The data are from Carle et al., *Science 232*, 65–68 (1986).

CHAPTER 9: CHEMICAL KINETICS

CONCEPTS:

- Require collisions (often)
- Require energy (usually) — temperature dependence
- Concentration dependence (usually)
- Catalysts

TWO DISTINCT PARTS

1. EXPERIMENTAL → RATE LAW

   Measure: Concentration dependence, Temperature dependence, Solvent effects, Catalysts, etc.

   i.e. what actually happens MACROSCOPICALLY

2. MECHANISM: A detailed "movie" of how we think the reaction happens at the molecular level.

   - From the imagination of humans
   - Can be proven wrong, but CANNOT BE PROVEN CORRECT (with certainty)
   - USES ELEMENTARY REACTIONS

Chapter 9: Kinetics: Rates of *Chemical* Reactions

Example:

2A + 3B → C (which is equivalent to: **A + (3/2)B → (1/2)C**)

$$\text{Rate of reaction} = v = \frac{d[C]}{dt}$$ ***always*** has units of **conc. time^{-1}**

$$\text{Note that : } v = \frac{d[C]}{dt} = -\frac{1}{2}\frac{d[A]}{dt} = -\frac{1}{3}\frac{d[B]}{dt}$$

Equally acceptable is ***disappearance of reactants*** :

$$v = \frac{-d[A]}{dt} = -\frac{2}{3}\frac{d[B]}{dt} = 2\frac{d[C]}{dt}$$

$v = \text{function of (concentrations of reactants)} = \text{the } \boldsymbol{RATE\ LAW}$

$\text{often: rate} = k[A]^l[B]^m[C]^n$, (but not always)

l = order with respect to A

m = order with respect to B

n = order with respect to C

$l + m + n =$ **overall order** of the reaction

rate is ***ALWAYS POSITIVE***,

with **units** = **conc. time^{-1}**

And, the **rate constant, k,** is ***ALWAYS POSITIVE***,

with **units** = **conc.$^{-(l+m+n)+1}$ time^{-1}**

(if $v=k[A]^l[B]^m[C]^n$)

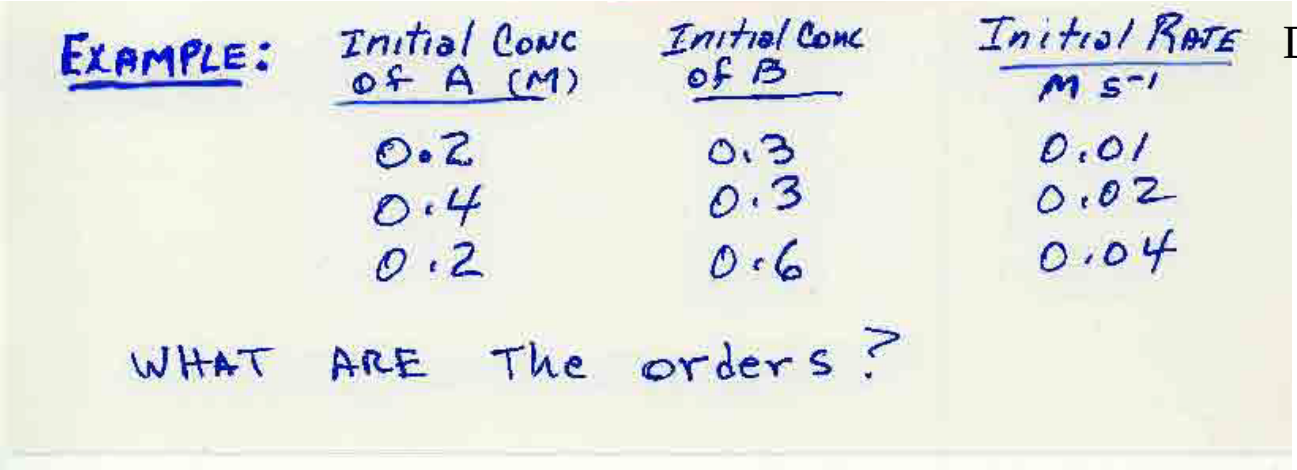
A mechanism ***predicts*** a rate law, **but**

A rate law CANNOT be deduced from a STOICHIOMETRIC Equation. This is *abundantly* clear from the table below.

TABLE 9.1 Rate Laws and Kinetic Order for Some Reactions

Stoichiometric reaction	Rate law	Kinetic order
$\text{sucrose} + H_2O \longrightarrow \text{fructose} + \text{glucose}$	$v = k[\text{sucrose}]$	1
$\text{L-isoleucine} \longrightarrow \text{D-isoleucine}$	$v = k[\text{L-isoleucine}]$	1
$^{14}C \longrightarrow {}^{14}N + \beta^-$	$v = k[^{14}C]$	1
$2\ \text{proflavin} \longrightarrow \text{proflavin dimer}$	$v = k[\text{proflavin}]^2$	2
$p\text{-nitrophenylacetate} + 2\ OH^- \longrightarrow p\text{-nitrophenolate} + \text{acetate} + H_2O\ (\text{pH } 9)$	$v = k[p\text{-nitrophenylacetate}][OH^-]$	2 (overall)
$\text{hemoglobin}\cdot 3O_2 + O_2 \longrightarrow \text{hemoglobin}\cdot 4O_2$	$v = k[\text{Hb}\cdot 3O_2][O_2]$	2 (overall)
$H_2 + I_2 \longrightarrow 2\ HI$	$v = k[H_2][I_2]$	2 (overall)
$H_2 + Br_2 \longrightarrow 2\ HBr$	$v = \dfrac{k[H_2][Br_2]^{1/2}}{k' + [HBr]/[Br_2]}$	Complex
$CH_3CHO \longrightarrow CH_4 + CO$	$v \cong [CH_3CHO]^{3/2}$	3/2 (approx.)
$C_2H_5OH \longrightarrow CH_3CHO\ (\text{liver enzymes})$	v constant	0

Orders and rate constant MUST be determined by EXPERIMENT
Below is experimental data for 2A + 3B → C



Defined as rate $= -\frac{d[A]}{dt}$

A is 1st order B is 2nd order

and what is the rate constant?

$$-\frac{d[A]}{dt} = k \times 0.2 \times 0.3^2 = 0.01$$

$$k = \frac{0.01 M s^{-1}}{0.2 \times 0.3^2 M^3} = 0.555 M^{-2} s^{-1}$$

First Order Processes

These ***INCREDIBLY IMPORTANT*** types of processes include
growth of living objects, e.g., humans,
decline of populations,
growth and decay of bank accounts and investments, etc.
or in a word: ?
EXPONENTIAL **decay or growth** (which have same math except for sign)

$$\text{rate} = \frac{dN}{dt} = -kN \quad \text{where N can be in any units}$$ **WHY?**

in words: rate of **loss** of A is directly proportional to A
or rate of **growth** of A is directly proportional to A

$$\text{if rate} = \frac{dN}{dt} = +kN \quad \text{where N can be in any units}$$

or, if we divide both sides by N and multiply by dt (which changes nothing)

$$\text{rate} = \frac{dN}{N} = -kdt$$

in words this says: fractional change is directly proportional to time

$$\text{rate} = \frac{dN}{N} = -kdt$$

IN WORDS this says: **fractional change is directly proportional to time**

How much do we have after time passes?
What is the SUM of FRACTIONAL changes???

$$\int \frac{\mathrm{dN}}{\mathrm{N}} = \pm k \int dt$$

$$\ln\left(\frac{\mathrm{N}_2}{\mathrm{N}_1}\right) = \pm k(t_2 - t_1)$$

or, what is the same thing : $$\frac{\mathrm{N}(\mathrm{t}_2)}{\mathrm{N}(\mathrm{t}_1)} = \mathrm{f} = \mathrm{e}^{\pm \mathrm{k}(\mathrm{t}_2 - \mathrm{t}_1)}$$

The first order mantra : $$\frac{\mathrm{N}(\mathrm{t}_2)}{\mathrm{N}(\mathrm{t}_1)} = \mathrm{f} = \mathrm{e}^{\pm \mathrm{k}(\mathrm{t}_2 - \mathrm{t}_1)}$$

Given any 2 of k, t, or f, find the 3rd

months	cases
1	4000
2	8000
3	16000
4	32000
5	64000
6	128000
7	256000
8	512000
9	1024000
10	2048000
11	4096000
12	8192000
13	16384000
14	32768000
15	65536000
16	1.31E+08
17	2.62E+08
18	5.24E+08
19	1.05E+09
20	2.1E+09
21	4.19E+09
22	8.39E+09

About this time in 2014, **ebola cases doubled in *one month***. Why did this cause concern?

Suppose the doubling rate persisted for 2 years

In less than 2 years:
EVERYBODY would be infected