INDUCED MUTATIONS

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1. Induced mutations

- Mutations are categorized as *induced* or *spontaneous*. **Induced mutations** are defined as those that arise after purposeful treatment with **mutagens**, environmental agents that are known to increase the rate of mutations.

- Spontaneous mutations are those that arise in the absence of known mutagen treatment. They account for the "background rate" of mutation and are the ultimate source of natural genetic variation that is seen in populations. The frequency at which spontaneous mutations occur is low, generally in the range of one cell in $10^5$ to $10^8$.

- Therefore, if a large number of mutants is required for genetic analysis, mutations must be *induced*. The induction of mutations is accomplished by treating cells with mutagens.
2. Spontaneous vs induced mutations

- Recognize that the distinction between induced and spontaneous is purely operational. If we are aware that an organism was **mutagenized**, then we infer that any mutations that arise after this mutagenesis were induced. However, this is not true in an absolute sense. The mechanisms that give rise to spontaneous mutations also are in action in this mutagenized organism. In reality, there will always be a subset of mutations recovered after mutagenesis that are independent of the action of the mutagen. The proportion of mutations that fall into this subset depends on **how potent a mutagen is**. The higher the rate of induced mutations, the lower the proportion of recovered mutations that are actually "spontaneous" in origin.

- Induced and spontaneous mutations **arise by generally different mechanisms**. After considering these mechanisms, we shall explore the subject of biological mutation repair. Without these repair mechanisms, the rate of mutation would be so high that cells would accumulate too many mutations to remain viable and capable of reproduction. Thus, the mutational events that do occur are those rare events that have somehow been overlooked or bypassed by the repair processes.
3. Mutation induction

- The task of finding rare mutations in multicellular organisms is difficult compared with that in microorganisms.

- In 1928, Hermann J. Muller devised a method of searching for any lethal mutation on the X chromosome in Drosophila.
  - He first “constructed” an X chromosome called ClB. The C stands for a chromosomal rearrangement called an inversion; it causes suppression of crossover because, in a female fly carrying this special ClB chromosome and a normal X, the X chromosome chromatids do not recombine.
  - The ClB chromosome also bears “l”, a recessive lethal allele, and the allele B, which determines the dominant bar-eye phenotype. ClB/Y males die because of hemizygosity for the lethal allele, but the chromosome can be maintained in heterozygous ClB/C+l+B+ females.

- This special ClB system allowed Muller to screen for lethal mutations anywhere on the X chromosomes in samples of male gametes.
4. Muller’s protocol

**The ClB test for new X chromosome mutations in Drosophila.** The symbol \( m \) represents a recessive lethal mutation anywhere on the X chromosome. Observing presence versus absence of males in each individual progeny amounts to scanning—by genetic analysis—a sample of gametes from the male parent of the bar-eyed daughter. Bar-eyed daughters from the cross of females heterozygous for the \( ClB \) chromosome and wild-type males are crossed individually with wild-type males. Each bar-eyed daughter lays her eggs in a separate culture vial. When the progeny hatch, the vials are examined for the presence of males. If there was a new lethal recessive mutation on an X chromosome in one of the original male gametes, then the F1 female carrying that chromosome will not produce any viable male progeny.
5. Increase of mutation rates

- Muller found the recessive frequency of such mutations occurring spontaneously to be about 1.5 per 1000 chromosomes, still a relatively low value for an entire chromosome.

- Muller then asked whether there were any agents that would increase the rate of mutation. Using the ClB test, he measured X-linked lethal frequencies after irradiating males with X rays and observed frequencies that were much higher than those in unirradiated controls.

- His results supplied the first experimental evidence of a mutagen—in this case, the X rays.
6. Induced mutation rate

- It is now known that many kinds of radiation increase mutations. Radiation is often categorized as *ionizing* or *nonionizing*, depending on whether ions are produced in the tissue through which it passes. X rays and $\gamma$ (gamma) rays, for example, do produce ions.

- The harnessing of nuclear energy has become a social issue because of the powerful mutagenic effect of nuclear radiation. No containment system is infallible, and recent decades have seen many examples of accidental release of nuclear isotopes. Furthermore, disposal of nuclear wastes is not as easy as had been supposed.

<table>
<thead>
<tr>
<th>Type of radiation</th>
<th>Percentage of male X chromosomes bearing recessive lethal mutations after a dose of 1000 roentgens*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible light (spontaneous)</td>
<td>0.15</td>
</tr>
<tr>
<td>X rays (25 Mev)</td>
<td>1.7</td>
</tr>
<tr>
<td>$\beta$ rays, $\gamma$ rays, hard X rays</td>
<td>2.9</td>
</tr>
<tr>
<td>Soft X rays</td>
<td>2.5</td>
</tr>
<tr>
<td>Neutrons</td>
<td>1.9</td>
</tr>
<tr>
<td>$\alpha$ rays</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* The roentgen (r) is a unit of radiation energy

The effects of several types of radiation on increasing mutation frequencies in *Drosophila*
7. Dose-response curves

It is known that, within a certain range of radiation dosage, induction of point mutations is linear; that is, if we double or halve the radiation level, the number of mutants produced will vary accordingly.

Radiation doses generally are cumulative. If a population of organisms is repeatedly exposed to radiation, the frequency of mutations induced will be in direct proportion to the total amount of radiation absorbed over time.
8. Ionizing radiation

- Ionizing radiation results in the formation of ionized and excited molecules that can cause damage to cellular components and to DNA. Because of the aqueous nature of biological systems, the molecules generated by the effects of ionizing radiation on water produce the most damage. Many different types of reactive oxygen species are produced, including superoxide radicals, such as \( \cdot \text{OH} \). The most biologically relevant reaction products are \( \cdot \text{OH}, \text{O}_2, \text{and H}_2\text{O}_2 \). These species can damage bases and cause different adducts and degradation products. Among the most prevalent, which result in mutations, are thymine glycol and 8-oxodG. Ionizing radiation can cause **breakage of the N-glycosydic bond**, leading to the formation of AP sites, and can cause **strand breaks** that are responsible for most of the lethal effects of such radiation.

DNA damage products formed after attack by oxygen radicals. dR = deoxyribose
9. Ultraviolet light

- Ultraviolet light generates two different lesions that occur at adjacent pyrimidine residues: the cyclobutane pyrimidine photodimer and the 6-4 photoproduct. These lesions interfere with normal base pairing; hence, induction of the SOS system is required for mutagenesis. The insertion of incorrect bases across from UV photoproducts is at the 3’ position of the dimer, and more frequently for 5’-CC-3’ and 5’-TC-3’ dimers. The C→T transition is the most frequent mutation, but other base substitutions (transversions) and frameshifts also are stimulated by UV light, as are duplications and deletions.

(a) Structure of a cyclobutane pyrimidine dimer. Ultraviolet light stimulates the formation of a four-membered cyclobutane ring (green) between two adjacent pyrimidines on the same DNA strand by acting on the 5,6 double bonds.

(b) Structure of the 6-4 photoproduct. The structure forms most prevalently with 5’-CC-3’ and 5’-TC-3’, between the C-6 and the C-4 positions of two adjacent pyrimidines, causing a significant perturbation in local structure of the double helix.
10. Incorporation of base analogs

- Some chemical compounds are sufficiently similar to the normal nitrogen bases of DNA that they occasionally are incorporated into DNA in place of normal bases; such compounds are called **base analogs**. Once in place, these analogs have pairing properties unlike those of the normal bases; thus, they can produce mutations by causing incorrect nucleotides to be inserted opposite them in replication. The original base analog exists in only a single strand, but it can cause a nucleotide-pair substitution that is replicated in all DNA copies descended from the original strand.

- For example, **5-bromouracil (5-BU)** is an analog of thymine that has bromine at the C-5 position in place of the CH$_3$ group found in thymine. This change does not affect the atoms that take part in hydrogen bonding in base pairing, but the presence of the bromine significantly alters the distribution of electrons in the base.

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The normal structure (the keto form) of 5-BU pairs with adenine, as shown here. 5-BU can frequently change to either the enol form or an ionized form; the latter pairs in vivo with guanine.
11. Intercalating agents

- The **intercalating agents** form another important class of DNA modifiers. These agents are planar molecules, which mimic base pairs and are able to slip themselves in (intercalate) between the stacked nitrogen bases at the core of the DNA double helix (see figure). In this intercalated position, the agent can cause single-nucleotide-pair insertions or deletions. Intercalating agents may also stack between bases in single-stranded DNA.
12. Bulky addition products

- Aflatoxin B$_1$ (AFB$_1$) is a powerful carcinogen, that may contaminate human food. It generates **apurinic sites** following the formation of an addition product at the N-7 position of guanine. Studies with apurinic sites generated in vitro demonstrated a requirement for the SOS system and showed that the SOS bypass of these sites leads to the preferential insertion of an adenine across from an apurinic site. Thus agents that cause depurination at guanine residues should preferentially induce GC→TA transversions.

- AFB$_1$ is a member of a class of chemical carcinogens known as **bulky addition products** when they bind covalently to DNA. Other examples include the diol epoxides of *benzo(a)pyrene*, a compound produced by internal combustion engines. For many different compounds, it is not yet clear which DNA addition products play the principal role in mutagenesis. In some cases, the mutagenic specificity suggests that depurination may be an intermediate step in mutagenesis; in others, the question of which mechanism is operating is completely open.
13. Specific mispairing

- Some mutagens are not incorporated into the DNA but instead alter a base, causing specific mispairing. Certain alkylating agents, such as ethylmethanesulfonate (EMS) and the widely used nitrosoguanidine (NG), operate by this pathway:

![Chemical structures of EMS and NG]

Although such agents add alkyl groups (an ethyl group in EMS and a methyl group in NG) to many positions on all four bases, mutagenicity is best correlated with an addition to the oxygen at the 6 position of guanine to create an O-6-alkylguanine. This addition leads to direct mispairing with thymine, and would result in GC→AT transitions at the next round of replication. As expected, determinations of mutagenic specificity for EMS and NG show a strong preference for GC→AT transitions.
14. Forward mutation frequencies obtained with various mutagens in Neurospora

- The assay measures the frequency of *ad-3* mutants. It so happens that such mutants are red, so they can be detected against a background of white *ad-3* colonies.

<table>
<thead>
<tr>
<th>Mutagenic treatment</th>
<th>Exposure time (minutes)</th>
<th>Survival (%)</th>
<th>Number of <em>ad-3</em> mutants per 10^6 survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment (spontaneous rate)</td>
<td></td>
<td>100</td>
<td>~0.4</td>
</tr>
<tr>
<td>Amino purine (15 mg/ml)</td>
<td>During growth</td>
<td>100</td>
<td>3</td>
</tr>
<tr>
<td>Ethyl methane sulfonate (1%)</td>
<td>90</td>
<td>56</td>
<td>25</td>
</tr>
<tr>
<td>Nitrous acid (0.05 m)</td>
<td>160</td>
<td>23</td>
<td>128</td>
</tr>
<tr>
<td>X rays (2000 r/min)</td>
<td>18</td>
<td>16</td>
<td>259</td>
</tr>
<tr>
<td>Methyl methane sulfonate (20 mM)</td>
<td>300</td>
<td>26</td>
<td>350</td>
</tr>
<tr>
<td>UV rays (600 erg/mm^2 per min)</td>
<td>6</td>
<td>18</td>
<td>375</td>
</tr>
<tr>
<td>Nitrosoguanidine (25 µm)</td>
<td>240</td>
<td>65</td>
<td>1500</td>
</tr>
<tr>
<td>ICR-170 acridine mustard (5 µg/ml)</td>
<td>480</td>
<td>28</td>
<td>2287</td>
</tr>
</tbody>
</table>
15. Specificity of mutagens

The distribution of mutations among 36 sites in the *lacI* gene is shown for three mutagens: **EMS**, **UV light**, and **aflatoxin B₁**. The height of each bar represents the number of occurrences of mutations at the respective site. Some hot spots are shown off-scale, with the number of occurrences indicated directly above the respective peak.

For instance, in the UV-generated collection, one site resulting from a GC→AT transition is represented by 80 occurrences. Each mutational site represented in the figure generates an amber (UAG) codon in the corresponding mRNA. The mutations are arranged according to the type of base substitution.
The previous graphs show the distribution of base-substitution mutations that create chain-terminating UAG codons. The specific sequence changes are known for each lacI site, allowing the graphs to be broken down into each category of substitution.

The graphs reveal the two components of mutational specificity. First, each mutagen shown favors a specific category of substitution.

- For example, EMS and UV favor GC→AT transitions, whereas AFB₁ favors GC→TA transversions. These preferences are related to the different mechanisms of mutagenesis.

Second, even within the same category, there are large differences in mutation rate.

- These differences can be seen best with UV light for the GC→AT changes. Some aspect of the surrounding DNA sequence must cause these differences. In some cases, the cause of mutational hot spots can be determined by DNA sequence studies, as previously described for certain frameshift sites. In many examples of mutagen-induced hot spots, the precise reason for the high mutability of specific sites is still unknown.
17. Summary of mutagenetic mechanisms

- Mutagens induce mutations by at least three different mechanisms.
  - They can replace a base in the DNA
  - They can alter a base so that it specifically mispairs with another base,
  - They can damage a base so that it can no longer pair with any base under normal conditions.