categories of **conditional mutations**:

1. auxotrophic  (new nutritional requirement for growth relative to wildtype [*prototroph*])
2. host range  (infectivity/virulence) parasite “nutrition”
3. sex-limited/specific (including dominant lethals & steriles)
4. genetically suppressable

5. **temperature-sensitive** (ts)
   
   condition that generates *mutant* phenotype
   
   $$\text{heat-sensitive} \quad (\text{hi temp non-permissive}) \quad (\text{vs. permissive})$$
   $$\text{cold-sensitive} \quad (\text{lo temp. n.-p.}= \text{restrictive})$$

in microbes: generally caused by mutations destabilizing proteins and protein-protein interactions.

in macrobes: causes not so obvious (interfere with temperature compensation?)
Glorious history of ts mutations:

(1) validate 1gene:1enzyme (1 polypeptide) concept concept based on nutritionally supplementable mutants

Delbrück wet blanket (1946) mutants identified only because they were supplementable …hence not valid to conclude they are representative

Horowitz & Leopold dried the blanket:
isolated 161 ts (40C vs. 25C) E.coli mutants on minimal medium
124 (77%) grew at 40C on complete medium

ALSO: ts muts. Identify vital genes (the 23%) in haploid orgnsms.
Glorious history of ts mutations:

(2) genetically dissect bacteriophage T4 (parasitizes E. coli) …especially its assembly (“morphogenesis”) pathway at the restrictive temperature: different t.s. T4 mutants blocked at different steps in assembly (E.M. of phage lysates)

-- *in vitro complementation* of mutant extracts of phage grown at the nonpermissive temp. can one mutant extract provide the material/activity missing from the other mutant extract? …an assay for *purification* of specific gene products

65 T4 genes by ts mutants

genome sequencing: **55 more !!**

Add to reading: pp240-241 of text
ts mutations in “time-of-function” analysis of development

to answer: When is a gene needed?

determination of the “temperature-sensitive period” (TSP)

(Fig. 20.6, p723) zyg-9: a C. elegans ts lethal

permissive temp. throughout: normal development
restrictive temp. throughout: abnormal development (aborts)

subject zyg-9 mutant embryos to a 15 minutes pulse of high temperature

start of pulse

End of the first division

Temperature-sensitive period

Develop
Abort
If a gene functions at different times to do different things, temperature shifts of ts mutants can reveal those various functions.
determination of the “temperature-sensitive period” (TSP) by single temperature shifts up or down.

**Permissive** temp. throughout: *normal* development

**Restrictive** temp. throughout: *abnormal* development (*aborts*)

Point (time) in development when temp. shifted.

- **All OK**
- **All mut**
- **Shift rstrctv to prmsv**
- **Shift prmsv to restrv**
- **Start: when begins drop**
- **End: when wildtype**

**TSP**
determination of the “temperature-sensitive period” (TSP)

Temperature **pulses** (double-shifts) are more definitive:

gene function needed:

all **OK**

all **mut**

point (time) in development when exposed to restrictive temp for |--|
Temperature-sensitive mutant alleles help us recognize (“dissect”) a gene’s pleiotropy (multiple, “unrelated” functions).

WHAT IF:

1. Essential for development
2. Needed for an adult behavior

two TSPs:

all OK

all mut

point (time) in development when exposed to restrictive temp for |--|

终极表型

胚胎 拟雌 成虫 蛹

permissive unshifted

restrictive unshifted
(1) What kinds can we make? (categories)

(2) How do we make them? (mutagenesis)

(3) How do we find them? (mutant screens & selections)

(4) Why bother?
Factors affecting **Mutation Rates**

**MEASURE:** changes per gene per:
- gamete
- cell division
- generation

**Spontaneous** mutations:

DNA mistakes happen (nothing is perfect)

Why do they occur at the **rate** they do?

Consider:

1. It costs **energy** and **time** to avoid making mistakes… **proofreading** of DNA replication

   DNA **synthesis** and **repair** genes determine the spontaneous mutation rate

   "**mutator**" mutations: **increase** spontaneous rate

2. It doesn’t pay to be perfect

   **Natural selection** optimizes spontaneous mutation rate

   redwood trees vs. *E.coli*
Mutagens: agents that increase mutation rate

**Chemicals:** (Fig. 7-10, learn for MCATs)

(1) **base analogs**
- incorporated in place of normal, then misbehave
  - 5-BrU (5-bromouracil) incorp like T, generally pairs w/ A, but tautomerizes to pair with G

(2) **base modifying agents**
- modifications change pairing rules
  - EMS (ethymethane sulfonate) induces replication error
    - Ethylated G pairs with T
    - Ethylated T pairs with G
    - GC > GC > G* > GT > *T > AT  
    - TA > CG

(3) **intercalating agents**
- slip in between bases noncovalently
  - cause **additions** and **deletions**
- acridine orange
Mutagens: agents that increase mutation rate

Chemicals:

(1) base analogs
(2) base modifying agents
(3) intercalating agents

(4) original root beer (sasparilla root)
   organic all-natural carcinogen

(5) urine from smokers

**Ames test** for mutagens  
[p224. Fig 7.16]  
*Salmonella typhimurium*

\[ \text{histidine}^- \rightarrow \text{histidine}^+ \text{ reversion rate} \]

- specific
- molecularly defined mutants

- requires
- does not require

auxotroph prototroph
With chemical mutagens:

(1) …generally easier to induce germline mutations in males than in females.

in sperm: little to “distract” the mutagen from DNA

(2) …often progeny from mutated sperm are **genetically mosaic** for new mutations

only one of the two complementary bases in sperm are modified: \( \text{GC} \rightarrow \text{GC} \rightarrow \text{GT} & \text{GC} \)

in sperm after 1st zygotic division

subsequent rounds of replication do not invariably cause errors: \( \text{GT} \rightarrow \text{GC} & \text{AT} \)
Radiation (the first experimental mutagen discovered)

**Non-ionizing** (lower energy)  UV light

- Photochemical reaction glues adjacent thymines together in cis:

  \[
  \begin{align*}
  \text{AGGCCCTC} & \text{TTCA} \\
  \text{TCCGGAGAAGT} & \rightarrow \\
  \text{AGGCCCTCT} & \text{T=TCA} \\
  \text{TCCGGAGAAGT} & 
  \end{align*}
  \]

  DNA repair machinery called out:
  - light-dependent repair (accurate)
  - excision repair (error prone)

(pity the shoe salesman)

**Ionizing** (higher energy)  X-rays, γ-rays, cosmic rays

generates **free radicals**: chemical bull in the china shop

...can induce double-strand DNA breaks

repairs at any cost!!

...but what repair template available?