I. Model systems

1. Caenorhabditis elegans

*C. elegans* is a microscopic (~1 mm) nematode (roundworm) that normally lives in soil. It has become one of the "model" organisms in biology because:

1. It is a true animal with at least rudiments of the physiological systems – feeding, nervous, muscle, reproductive – found in "higher" animals like mice and humans, but so small that large numbers can be raised in petri dishes (where it is fed *E. coli* – another model organism).
2. It reproduces rapidly.
3. It is transparent so that every cell in the living animal can be seen under the microscope from the fertilized egg to the 556 cells of the newly-hatched worm and, later, the 959 somatic cells, and a variable number of germ cells, of the adult worm.
4. It can be easily transformed with transgenes as well as treated with antisense RNA.
5. Some other features: Its cells contain 5 pairs of autosomes and, usually 2 X chromosomes. These animals are hermaphrodites, producing both sperm and eggs. Most of the time they fertilize themselves, so that any recessive alleles quickly become homozygous and affect the phenotype. On rare occasions, nondisjunction occurs during meiosis with the loss of one X chromosome. Animals with a single X are males and are able to fertilize the eggs of the hermaphrodites (with more success than they have themselves).

*C. elegans* was the first multicellular eukaryote to have its entire genome sequenced. It contains some 19 – 20,000 protein-encoding genes incorporated in 100,258,171 base pairs of DNA. In contrast to other eukaryotes, some 13 – 15% of its genes are grouped in operons containing 2 – 8 genes each.
2. *Drosophila melanogaster*

Within a few years of the rediscovery of Mendel's rules in 1900, *Drosophila melanogaster* (the so-called fruit fly) became a favorite "model" organism for genetics research:

1. The flies are small and easily reared in the laboratory.
2. They have a short life cycle and a new generation of adult flies can be produced every two weeks.
3. They are fecund; a female may lay hundreds of fertilized eggs during her brief life span. The resulting large populations make statistical analysis easy and reliable.
4. The giant ("polytene") chromosomes in the salivary (and other) glands of the mature larvae: these chromosomes show far more structural detail than do normal chromosomes, and they are present during interphase when chromosomes are normally invisible.
5. Its embryo grows outside the body and can easily be studied at every stage of development.
6. The blastoderm stage of the embryo is a syncytium (thousands of nuclei unconfined by cells) so that, for example, macromolecules like DNA injected into the embryo have easy access to all the nuclei.
7. The genome is relatively small for an animal (less than a tenth that of humans and mice).
8. Mutations can be targeted to specific genes.

Examples of things that Drosophila has taught us:

1. In embryonic development:
   How a single fertilized egg can give rise to different kinds of cells.
How transcription factors guide the organization of the body plan of the embryo.
Signals needed to guide the development of the central nervous system.
Genetic controls over the building of wings, legs, eyes.
How the giant chromosomes reveal changing patterns of gene expression.
Forces at work in evolution: sexual selection, the benefits of sexual reproduction.
Genetic control of circadian rhythms, foraging behavior.

3. Zebrafish
The zebrafish, *Danio rerio*, has become another popular "model" organism with which to study fundamental biological questions. It is a small (1–1.5 inches) freshwater fish that grows easily in aquaria (it is available at many pet stores). Some of its advantages for biologists:
(1) It breeds quickly and often (daily).
(2) It is a vertebrate.
(3) Its embryos, like those of most fishes, develop outside the body where they can be easily observed (unlike mice).
(4) Its embryos are transparent so defects in development can be seen easily.
(5) Embryonic development is quick (they hatch in two days).
(6) Individual cells — or clusters of cells — can be transplanted to other locations in the embryo.
(7) They can be forced to develop by parthenogenesis to produce at will homozygous animals with either: a male-derived or female-derived genome.
(8) They can be cloned from somatic cells.
(9) They can be made transgenic (like mice and Drosophila).

4. Making transgenic mice
Two methods of producing transgenic mice are widely used:
* transforming embryonic stem cells (ES cells) growing in tissue culture with the desired DNA;
* injecting the desired gene into the pronucleus of a fertilized mouse egg.

1. The Embryonic Stem Cell Method
Embryonic stem cells (ES cells) are harvested from the inner cell mass (ICM) of mouse blastocysts. They can be grown in culture and retain their full potential to produce all the cells of the mature animal, including its gametes.

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Random vs. Targeted Gene Insertion

The early vectors used for gene insertion could, and did, place the gene (from one to 200 copies of it) anywhere in the genome. However, if you know some of the DNA sequence flanking a particular gene, it is possible to design vectors that replace that gene. The replacement gene can be one that
* restores function in a mutant animal or
* knocks out the function of a particular locus.

In either case, targeted gene insertion requires
* the desired gene
* neor, a gene that encodes an enzyme that inactivates the antibiotic neomycin and its relatives, like the drug G418, which is lethal to mammalian cells;
* tk, a gene that encodes thymidine kinase, an enzyme that phosphorylates the nucleoside analog gancyclovir. DNA polymerase fails to discriminate against the resulting nucleotide and inserts this nonfunctional nucleotide
into freshly-replicating DNA, so ganciclovir kills cells that contain the tk gene.
* Most cells fail to take up the vector; these cells will be killed if exposed to G418.
* In a few cells: the vector is inserted randomly in the genome. In random insertion, the entire vector, including the tk gene, is inserted into host DNA. These cells are resistant to G418 but killed by gancyclovir.
* In still fewer cells: homologous recombination occurs. Stretches of DNA sequence in the vector find the homologous sequences in the host genome and the region between these homologous sequences replaces the equivalent region in the host DNA.
The Cre/loxP System
One of the bacteriophages that infects *E. coli*, called P1, produces an enzyme — designated Cre — that cuts its DNA into lengths suitable for packaging into fresh virus particles. Cre cuts the viral DNA wherever it encounters a pair of sequences designated loxP. All the DNA between the two loxP sites is removed and the remaining DNA ligated together again (so the enzyme is a recombinase).

Using "Method 1" (above), mice can be made transgenic for

* the gene encoding Cre attached to a promoter that will be activated only when it is bound by the same transcription factors that turn on the other genes required for the unique function(s) of that type of cell;
* a "target" gene, the one whose function is to be studied, flanked by loxP sequences.

In the adult animal,
* those cells that
  o receive signals (e.g., the arrival of a hormone or cytokine)
  o to turn on production of the transcription factors needed
  o to activate the promoters of the genes whose products are needed by that particular kind of cell will also turn on transcription of the Cre gene. Its protein will then remove the "target" gene under study.
* All other cells will lack the transcription factors needed to bind to the Cre promoter (and/or any enhancers) so the target gene remains intact.

The result: a mouse with a particular gene knocked out in only certain cells. The Cre/loxP system can also be used to remove DNA sequences that block gene transcription. In such a "knockin" mouse, the "target" gene is turned on in only certain cells.
**Transcription factors**

Promoter

**Cre**

**Target gene**

**loxP**

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**Cre transcribed and translated**

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**Cre recombinase**

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**Target gene destroyed**