“The only way to get from genotype to phenotype is through developmental processes.”
- Remember the analogy that the zygote contains information almost entirely in the coded form (the genotype), while the adult contains information almost entirely in the expressed form (the phenotype). Development is the program that turns coded information into expressed information.

Notable events and trends in modern experimental embryology/developmental biology:
- early 1920s - split between embryology and genetics; transmission and expression, respectively of inherited traits
- which controlled inheritance - nucleus or cytoplasm?
  - cytoplasm (Thomas Hunt Morgan)
  - nucleus (Edmund Beecher Wilson; Theodor Boveri)
- there was experimental support for both groups
- eventual preponderance of evidence supported nuclear theory and provided the material basis for genes and the gene theory of inheritance
- early splits between genetics and embryology was the result of inadequate methods for genetic analysis, as well as conceptual difficulties combining the presumption of genomic equivalence (all cells contain the same genes and therefore the same potential for gene expression) and the production of differentiated cells

- synthesis of genetics and embryology = developmental genetics
  - mutations in mouse *Brachyury* gene cause aberrant development (Glueckhohn-Schoenheimer); developmental effects of *Brachyury* traced to notochord
  - genes connected to wing mutations in *Drosophila* (Waddington)

**Genomic Equivalence** - with the same genes in every cell, how could development be directed, i.e. how do cells become differentiated?
- amphibian cloning (somatic nuclear transfer) - success of this technique established that nuclei do become irreversibly functionally restricted through development
- cloning requires:
  1. A method for **enucleating** host eggs without destroying them.
  2. A method for isolating intact donor nuclei.
  3. A method for transferring isolated nuclei to enucleated host egg.

NOTE - in frogs and mammals, enucleation techniques also activates the egg; activation can be stimulated by pricking the membrane with a needle; the nucleus need not be removed
- egg activation stimulates the cytological and biochemical changes in the egg that are associated with fertilization (apart from pronuclear fusion)
  - e.g. sperm binding/entry stimulates egg cytoplasmic
rearrangement; resumption of meiosis, protein synthesis, etc.
- glass micropipette used to obtain donor nucleus and transfer it to enucleated host oocyte
- experimental results with *Rana pipiens* (northern leopard frog) showed that blastula cell nuclei could direct complete development
- use of nuclei from later-stage embryos was notably less successful
  - somatic cells appeared to lose their ability to direct development as they became determined and differentiated

- subsequent experiments using *Xenopus laevis* (African clawed frog) showed that nuclei from fully differentiated cells (e.g. from foot webbing) supported development only through early stages of embryogenesis (John Gurdon)
  - serial transplantation experiments [technique: transplant differentiated nucleus to oocyte or early blastomere; allow development through blastocyst stage, then remove the nuclei, transplant them again into an enucleated oocyte, allow development to blastocyst; repeat] showed that the differentiated nuclei would support progressively longer periods of development; established that there was at least some reversibility in differentiated nuclei (see “Dolly” story next)

- cloning mammals (e.g. Dolly, sheep that was the first cloned mammal) - differentiated cells (udder cells, in Dolly’s case) were put into culture (grown outside the body); fused with enucleated oocytes using an electrical pulse (electroporation; see below); transferred into uterus of pregnant sheep
  - this process is very inefficient; e.g. Dolly was the only success in 434 oocyte tries
  - mammalian cloning has been accomplished with multiple species, but efficiency problems remain; also,
  - offspring generally healthy, but they often have multiple problems; e.g. large size at birth, “old” telomeres; premature aging, disease susceptibility, etc.

**Potency** - potential of cell to differentiate into various types of cells
  - **totipotent** - can produce all cell types of the embryo and adult
  - **pluripotent** - can produce many different types
  - **multipotent** - can produce more than one type of differentiated cell

- amphibian cloning experiments established that early blastula cells were totipotent, but potency was lost in later embryonic stages
  - serial transplantation experiments established that degrees of potency could be re-established by manipulating nuclear conditions

- mammalian cloning experiments established that near-totipotency could be obtained by manipulation of conditions in culture (near-totipotency because mammalian embryos originate as two parts, the trophoblast, which establishes the connection
between the embryo and the uterus, and the inner cell mass, which forms the embryo proper, and consequently all differentiated cells in the adult. The differentiated cells used to produce cloned mammals would not be able to make trophoblast cells. More on this later.

**Differential Gene Expression**

Three postulates of differential gene expression:

1. DNAs of all differentiated cells are identical. *(Genomic Equivalence)*
2. All genes retain the ability to be expressed.
3. Only a small percentage of the genome is expressed in each differentiated cell; i.e. differential gene expression
   - early evidence of differential gene expression see in *Drosophila polytene* chromosomes; these undergo DNA replication in the absence of cell or nuclear division; contain multiple parallel DNA strands; cells expanded to ~150x original volume
   - patterns of chromosomes are identical throughout larvae; i.e. no loss or addition of any region
   - however, different chromosomal regions make different tissue-specific mRNAs, based on cell type
   - chromosome “puffs” transcribe mRNA
   - technique - DNA-RNA hybridization - radioactive labeling of DNA single-stranded RNA binds to complementary region of chromosomal DNA
   - differences in binding showed differences in gene expression in different cell types

**Techniques for establishing mechanisms of differential gene expression**

RNA localization techniques:

- **RT-PCR** *(reverse transcriptase polymerase chain reaction)*
  - converts very small amounts of mRNA into DNA and copies sequences to allow identification and multiplication
- **DNA microarrays**
  - uses RT-PCR to make copies of many or all mRNA from particular cell type or developmental stage; subsequent cDNAs can be hybridized to cDNAs from target cells or developmental stages
- **DNA macroarrays**
  - larger version of microarray technology; allows non-microscopic examination; e.g. with autoradiography
- **in situ hybridization**
  - anti-sense mRNA probe (RNA or DNA can be made with two complimentary strands: sense and anti-sense) is constructed using labeled nucleotides; label is seen only in tissues which are expressing the complementary message
- **whole-mount in situ hybridization**
  - entire embryo, organ system, or structure is stained for particular mRNA
  - 2nd antibody techniques - **digoxigenin** labeled mRNA probe; **alkaline phosphatase** detection system
Determining the function of genes

Transgenic cells and organisms
- insertion of DNA into cells
  - microinjection
  - transfection
  - electroporation
  - transposable elements (retroviral vectors)
  - P elements (*Drosophila*)

- chimeras
  - form transgenic **embryonic stem cells** (ES cells)
    - culture **inner cell mass** (ICM) blastomeres
    - insert DNA sequence
  - add transgenic ES cells to native ICM

- gene targeting (knockouts)
  - similar to chimeric techniques, but replace wild-type genes with mutated genes
    - create ES cells heterozygous for mutant gene
      - add **antibiotic resistance** gene in gene of interest
    - **homologous recombination** will produce some chromosomes with the new gene inserted
    - use modified ES cells to form chimeras in wild-type ICM
    - culture knock-out chimera in presence of antibiotic
      - the only surviving embryos will be ones expressing antibiotic resistance gene (i.e. those heterozygous for knockout gene)

- antisense RNA
  - insert “antisense” RNA into a blastomere
  - forms double-stranded RNA (**dsRNA**)
    - dsRNA is degraded by nucleases in the cell
    = no message; no protein

- morpholino antisense RNAs
  - normal antisense RNA degraded after one round of cell division
  - morpholino nucleotides (morpholine substituted for ribose) do not break down
    - effects of “deleted” message can be followed through multiple developmental stages

- RNA interference (RNAi)
  - dsRNA with same sequence as target mRNA stimulates enzymatic degradation of message
    - Dicer enzyme breaks dsRNA into small pieces - silencing RNA (**siRNA**)
  - siRNA binds to RNA-induced silencing complex (**RISC**) - destroys any
RNAs bound by that small fragment
- effects last through several cell generations
- invertebrates - dsRNA causes degradation of the natural mRNA
- vertebrates - blocks mRNA translation