Induction and Competence
Precise arrangement of tissues (e.g. in sense eye and sense organs) cannot be disturbed without impairing its function
- coordination in construction of organs accomplished by one group of cells changing the behavior of an adjacent set
- causing them to change their shape, mitotic rate, or fate

- induction (proximate interaction) interaction at close range between two or more cells or tissues with different histories and properties
- induction has two components:
  1. inducer: tissue that produces a signal(s) that changes cellular behavior
  2. responder: tissue being induced
- not all tissues can respond to inducer signal
  - e.g. optic vesicle induces lens tissue in head ectoderm
  - however, if optic vesicle placed under ectoderm of flank - no lens
  = only the head ectoderm is competent to respond

- competence - the ability of a cell or tissue to respond to a specific inductive signal
  - not passive state
  - actively acquired (and can also be transient)
    - e.g. Pax6 proteins’ role in mammalian eye: Pax6 gene expression seen in head ectoderm (responds to optic vesicle by forming lens); Pax6 gene not seen in other regions of surface ectoderm
- Pax6 - competence factor

NOTE - there is no single inducer of lens
- in amphibians the first inducer may be the pharyngeal endoderm and heart-forming mesoderm that underlie lens-forming ectoderm during early and mid-gastrula stages
- the second inducers: anterior neural plate (including signal that promotes Pax6 synthesis in anterior ectoderm)
- therefore, although the optic vesicle appears to be the inducer, anterior ectoderm has already been induced by at least two other factors
- the optic vesicle appears to secrete two induction factors
  - BMP4 (bone morphogenetic protein 4) induces Sox2 and Sox3 transcription factors
  - Fgf8 (fibroblast growth factor 8)

Cascades of induction: Reciprocal and sequential inductive events
Many inductive interactions are reciprocal; e.g.
- once the lens has formed, it can then induce other tissues
- optic vesicle becomes induced
  - optic vesicle becomes optic cup
  - wall of optic cup differentiated into pigmented retina and neural retina
  = **reciprocal inductions**
- lens induces ectoderm to become cornea
- a structure does not need to be fully differentiated in order to have a function
  - optic vesicle induces before it becomes the retina
  - lens placode reciprocates by inducing the optic vesicle before the lens forms

**Instructive and permissive interactions**

**Instructive interaction:** a signal from the inducing cell is necessary for initiating new gene expression in the responding cell
- w/o inducing cell, responding cell is not capable of differentiating (in that particular way)
- e.g. optic vesicle placed under new region of head ectoderm

**Permissive interactions:** responding tissue has already been specified; needs only an environment that allows the expression of these trait
- e.g. many tissues need solid substrate containing fibronectin or laminin in order to develop
- matrix does not alter the type of cell produced, but enables what has already been determined to be expressed

**Epithelial-mesenchymal interactions**

**epithelia** - sheets or tubes of connected cells
- originate from any germ layer

**mesenchyme** - loosely packed, unconnected cells
- derived from mesoderm or neural crest

all organs consist of an epithelium and an associated mesenchyme
- epithelial-mesenchymal interactions among the most important phenomena in nature

**Regional specificity of induction**
- e.g. skin: composed of two main tissues:
  - **outer epidermis** (epithelial tissue derived from ectoderm)
- **dermis** (mesenchymal tissue derived from mesoderm)
- epidermis secretes proteins that signal underlying dermal cells to form condensations
  - condensed dermal mesenchyme responds by secreting factors that cause the epidermis to form regionally specific cutaneous structures; e.g.
    - feathers (broad or narrow)
    - scales
    - claws
- dermal mesenchyme responsible for regional specificity of induction in the competent epidermal epithelium
  - when epithelium and mesenchyme are separated and recombined in alternate ways, same epithelium develops cutaneous structures according to the region from which the mesenchyme was taken
  - i.e. mesenchyme plays an instructive role; initiating different sets of gene activity in responding epithelial cells

**Genetic specificity of induction**
- mesenchyme instructs epithelium as to what sets of genes to activate; however, responding epithelium can only comply within the limits of its genome

**Paracrine Factors: The Inducer Molecules**
- some inductions could take place across filters (i.e. soluble induction factors)
  - soluble factors diffuse between cells = **paracrine interactions**
    - **paracrine factors** (inducing factors)
  - or - **growth and differentiation factors (GDFs)**
  - others needed contact (juxtaposition - **juxtacrine interactions**)
    - cell membrane proteins interact with receptors on receiving (responding) cell

- **endocrines** - factors produced by one set of cells and secreted into the bloodstream

**Paracrine factor families:**
1. Fibroblast growth factor (FGF)
2. Hedgehog family
3. Wingless (Wnt)
4. TGF-β superfamily
   - TGF-β family
   - activin family
   - BMPs
   - Vg1 family
   - etc.
- also **NOTE** - **autocrine regulation**
  - same cell produces factors as uses factors
    - e.g. cytotrophoblast
Signal Transduction Cascades: The Response to Inducers.

**Paracrine factors** bind to membrane receptors, which initiates a series of enzymatic reactions within the cell; these reactions result in the
- regulation of transcription (i.e. differential gene expression)
- or - regulation of the cytoskeleton, such that responding cells alter their shape or are permitted to migrate
- pathways may have several end-points (i.e. different series of genes expressed)
- pathways referred to as signal **transduction cascades**

- typical receptors (e.g. **tyrosine protein kinases**) (Fig 6.10):
  - extracellular region
  - transmembrane region
  - cytoplasmic region

- **ligand** (paracrine factor) binds to its receptor in extracellular region
  - induces a conformational change
  - change transmitted through the membrane into the cytoplasm
  - usually promotes enzymatic activity in cytoplasmic region
    - enzyme is often a kinase (protein phosphorylation)
    - kinases use ATP to phosphorylate specific tyrosine residues on particular proteins
    - kinase activity often triggers phosphorylation cascade
    - eventually activates transcription factor or cytoskeletal protein

- transcription factors divided into three categories
  1. constitutively present in all cells; e.g. Sp1
  2. active whenever a cell acquires them by cytoplasmic localization (e.g. bicoid protein) or by induction (Pax6)
  3. transcription factors whose functions are activated by cell signal transduction cascades (e.g. MITF)

**Paracrine factors:** **Fibroblast growth factors (FGF)** - RTK pathway, JAK-STAT pathway
- FGF gene family consists of about 24 structurally related members
- capable of making hundreds of protein isoforms through
  - splice variants
  - differential initiation
- FGFs can often substitute for each other, but expression patterns of FGFs and their receptors give them separate functions
  - Fgf1 (acidic FGF) - important during regeneration
  - Fgf2 (basic FGF) - important in blood vessel formation
  - Fgf7 (keratinocyte growth factor) - critical in skin formation
  - Fgf8 important in limb development and lens induction
    - Fgf8 usually made by optic vesicle that contact the outer head ectoderm
- after contact between outer ectoderm and optic vesicle, \textit{fgf8} gene expression becomes concentrated in the region of the presumptive neural retina
- Fgf8-containing beads will induce ectoderm to form lens

- FGFs can often substitute for one another, expression patterns of FGFs and their receptors give them separate functions

**Receptor Tyrosine Kinase (RTK) pathway**
- FGFs often work by activating a set of receptor tyrosine kinases (RTKs) called \textit{fibroblast growth factor receptors (FGFRs)}
  - kinase activated - phosphorylates substrate proteins (including other FGF receptors within the responding cell)
  - phosphorylation activates the substrate proteins

- RTK pathway extremely widespread
- ligands that bind to RTKs at the cell surface include
  - fibroblast growth factors (FGF)
  - epidermal growth factors (EGF)
  - platelet-derived growth factors
  - stem cell factors
- each RTK can bind only one or a small set of these ligands

**RTK signal transduction** (Fig. 6.12)
- RTK spans the cell membrane
  - binds ligand; undergoes a conformational change that enables it to \textbf{dimerize} with another RTK
  - the conformational change activates latent kinase activity of each RTK
  - dimerized receptors phosphorylate each other (i.e. ligand binding causes \textbf{autophosphorylation} of cytoplasmic domain of receptor)
- phosphorylated tyrosine on the receptor is recognized by an \textbf{adaptor protein}
- the adaptor protein serves as bridge that links the phosphorylated RTK to an intracellular signaling system
- adaptor protein also activates a \textbf{G protein (e.g. Ras)}
  - G proteins ordinarily inactive, GDP-bound state
  - the activated receptor stimulates the adaptor protein to activate guanine nucleotide releasing factor (GNRP)
  - GNRP exchanges PO$_4$ from GTP to bound GDP to re-form GTP
  - GTP-bound G protein is an active form that transmits the signal
  - after the signal is delivered, GTP on the G protein is hydrolyzed back into GDP
  - catalysis greatly stimulated by the complexing of the Ras protein with the \textbf{GTPase-activating protein (GAP)}
- G protein returned to its inactive state (await further signaling)

NOTE - without the GAP protein, Ras cannot catalyze GTP well, and remains in its active configuration
- mutation in RAS gene account for a large proportion of cancerous human tumors
- **oncogenic mutations of RAS** inhibit the binding of the GAP protein

- active Ras G protein associates with a kinase called **Raf**
- G protein recruits inactive Raf to the cell membrane; Raf activates
- Raf phosphorylates **MEK**
- MEK phosphorylates **ERK**
- ERK enters nucleus and phosphorylates transcription factors

- RTK critical in activating numerous developmental processes: e.g.
  - the **microphthalmia transcription factor** (Mitf) (Fig. 6.13)
  - produces pigment cells (migrating neural crest) cells of the eye and skin
  - MITF mutation (humans) = not pigmented, microphthalmic, deaf, multicolored irises, white forelock (see Ch. 5)
    - enhancer sequences found in three pigment-cell specific enzymes of the tyrosinase family
  - without MITF, proteins not synthesized properly; melanin not made
- Mitf - basic helix-loop-helix
- Mitf transcribed in pigment-forming melanoblast cells that migrate from the neural crest into the skin and in melanin-forming cells of pigmented retina
  [NOTE - in homozygous **White** and **Steel** mutants, pigment cells fail to migrate]
- **Steel** encodes paracrine protein called **stem cell factor**
- stem cell factor binds to and activates the **Kit** receptor tyrosine kinase encoded by the **White** gene
  - binding of stem cell factor to Kit RTK dimerizes Kit; autophosphorylates
  - phosphorylated Kit activates pathway whereby phosphorylated ERK is able to phosphorylate the Mitf transcription factor
  - phosphorylated Mitf can bind to cofactor p300/CTBP
  - p300/CTBP acetylates nucleosome histones, initiates transcription

**JAK-STAT pathway** (Fig. 6.14)
- Fgfs also activate JAK-STAT pathway
  - e.g. differentiation of blood cells, limb growth, activation of casein gene during milk production
  - ligand bound to FGF receptors (or other receptors) that are linked to JAK (**Janus kinase**) proteins
    - ligand binding phosphorylates STAT (signal transducers and activator of transcription) family of TFs
  - phosphorylated STATs form homodimers, act as transcription factors
**Paracrine factors: the Hedgehog family** (hedgehog pathway)

- used to induce particular cell types and to create boundaries between tissues
  
  NOTE - cholesterol necessary for hedgehog signal to function and also for hedgehog to bind to patched receptor

- vertebrates have 3 homologues of the *Drosophila hedgehog* gene
  
  - *sonic hedgehog* (*shh*)
    - motor neuron formation, somite specification, feather formation, digit positioning
    - often works in conjunction with other paracrine factors, e.g. Wnt, FGF proteins
  
  - *desert hedgehog* (*dhh*)
    - Sertoli cells; mutants = defective spermatogenesis
  
  - *indian hedgehog* (*ihh*)
    - gut and cartilage; postnatal bone growth

**Hedgehog pathway** (example in *Drosophila*)

- Hedgehog binds Patched receptor (not a signal transducer)
- Patched binds to Smoothened - signal transducer
  - unbound Patched inhibits Smoothened

- Cubitus interruptus (Ci) protein is tethered to microtubules in the responding cell
  - cleaved so that a portion enters the nucleus and acts as a transcriptional repressor

- hedgehog binds to Patch:
  - inhibition of Smoothened is relieved
  - Smoothened acts (phosphorylation?) to release the Ci protein from the microtubules and to prevent Ci cleavage
  - intact Ci enters nucleus; acts a transcriptional activator of the same genes it used to repress

- Hedgehog pathway extremely important in vertebrate limb and neural differentiation
- vertebrate homologues of Ci protein are the Gli proteins
  - several syndromes result from problems in *GLI3* gene
    - Pallister-Hall syndrome (lethal after birth); extra digits, poor development of pituitary gland, hypothalamus, anus, kidneys

- mutations that inactivate hedgehog pathway can cause malformations
- mutations that activate pathway ectopically can cause cancers

- cholesterol: needed to cleave hedgehog protein
- Patched needs cholesterol to function
  - some human cyclopia syndromes caused by mutations that encode Shh or enzymes that synthesize cholesterol
**Paracrine factors:** The **Wnt family** (canonical, noncanonical Wnt pathways)
- Wnts are cysteine-rich glycoproteins
- at least 15 members
- active in patterning dorsal cells of the somite to become muscle; involved in the specification of the midbrain; polarity of insect and vertebrate limbs, promoting the proliferation of stem cells, urogenital system development

**The “canonical” Wnt pathway** (canonical because it was the 1st one discovered)
- Wnts bind to Frizzled receptor
- Frizzled activates Disheveled
- activated Disheveled inhibits activity of glycogen synthase kinase 3 (GSK3) enzyme
  - active GSK3 prevents dissociation of $\beta$-catenin from the APC protein (which targets $\beta$-catenin for degradation)
  - with Wnt signal, GSK3 inhibited, $\beta$-catenin can dissociate from APC protein and enter nucleus
    - in nucleus, forms a heterodimer with LEF or TCF DNA-binding protein, becomes a transcription factor
    - binds to and activates Wnt-responsive genes

NOTE - Wnt pathway is undoubtedly more complicated; e.g. each of the components are known to have other functions in the cell

NOTE - overriding principle evident in both Wnt and Hedgehog pathways: activation is accomplished by inhibiting an inhibitor

**The “noncanonical: Wnt pathways**
1. Wnt affects actin and microtubular cytoskeleton
- Wnt binds to Frizzled, activates Disheveled
- Disheveled protein interacts with Rho GTPase
  - Rho GTPase activates kinases that phosphorylate cytoskeletal proteins
    - alter cell shape, polarity, motility

2. Wnt signal increases intracellular Ca$^{2+}$
- Wnt binds to Frizzled, activates a phospholipase (PLC)
- PLC synthesizes a compound that releases Ca$^{2+}$ from endoplasmic reticulum
  - Ca$^{2+}$ activates enzymes, TFs, translation factors

**Paracrine factors:** The **TGF-$\beta$ superfamily** (SMAD pathway)
- transforming growth factor-$\beta$ (TGF-$\beta$)
  - over 30 structurally related members
  - carboxy-terminal regions contains the mature peptide
  - peptides dimerize into homodimers or heterodimers (with other TGF-$\beta$ peptides) and are secreted from the cell
- **TGF-β** family
- **activin** family,
- **bone morphogenic proteins (BMPs)**
- **Vg1** family
- etc.

- **TGF-β** important in regulation of formation of extracellular matrix between cells, regulating cell division (+/-)
- also control where and when epithelia branch to form ducts of kidneys, lungs, salivary glands

**NOTE** - **TGF-β** members can substitute for each other; could compensate for loss of other members when expressed as a group

- **BMPs** - many functions: cell division, apoptosis, cell migration, differentiation

**The SMAD pathway**
- **TGF-β** ligand binds to a type II **TGF-β** receptor
- this allows the type II **TGF-β** receptor to bind to a type I **TGF-β** receptor
- when in close contact, the type II receptor phosphorylate a serine or threonine on the type I receptor, thereby activating it
  - activated type I receptor phosphorylates Smad proteins
    - **Activin** or **TGF-β** ligands result in phosphorylated Smads 2 or 3
    - **BMP** ligands result in phosphorylated Smads 1 or 5
  - phosphorylated Smads bind to other Smads (Smad 4) and move into nucleus to act as transcription factors

**Cell Death Pathways**

**apoptosis** - programmed cell death
- takes place both in embryonic development and continuously in the adult
  - e.g. many neurons start development, but die before birth
    - ~3X final number of neurons generated in brain
  - e.g. developing middle ear space, vaginal opening, spaces between fingers & toes
  - frog tails (through metamorphosis), male mammary tissue

- different tissues use different signals for apoptosis
  - e.g. **BMP4** used to differentiate connective tissue into bone, frog ectoderm into skin;
    neural crest and tooth primordia respond to **BMP4** by degrading DNA and dying
  - other cells “programmed” to die
    - need to be rescued by growth factor
      - e.g. mammalian RBCs; need erythropoietin to survive
        - erythropoietin receptor works through JAK-STAT pathway

- mammalian apoptosis pathway involves **caspases (proteases)**
  - activation of caspases causes autodigestion of the cell
  - cleave cellular proteins and fragment the DNA
- mice with loss-of-function mutations for either caspase-3 or caspase-9 dies around birth from massive cell overgrowth in the nervous system
- deletion of Apaf1 (apoptotic protease activating factor 1; activates caspase 3 & 9) have severe craniofacial abnormalities, brain overgrowth, webbing between their toes

- some instances where cell death is the normal state unless some ligand rescues the cell
  - e.g. chick neural tube; Patched (hedgehog receptor) will activate caspases
  - binding of Sonic hedgehog suppresses Patched; caspases not activated

**Juxtactine Signaling**

proteins from inducing cell interact with receptor proteins of adjacent responding cells without diffusing from the cell producing it
  - e.g. **eph receptors** and their **ephrin** ligands
    - ephrin on one cell binds to eph receptors on an adjacent cell, signals are sent to each of the two cells
    - often either attraction or repulsion
      - ephrins often seen where cells are being told where to migrate or where boundaries are forming
  - e.g. **Notch** proteins (bind to a family of ligands exemplified by the **Delta** protein)
    - cells expressing **Delta**, **Jagged**, or **Serrate** proteins activate neighboring cells that contain the Notch protein in their cell membranes
    - Notch extends through cell membrane
    - external surface contacts Delta, Jagged, or Serrate proteins
      - undergoes conformational change that enables a apart of its cytoplasmic domain to be cut off by Presenilin-1
      - cleaved portion enters the nucleus and binds to dormant TF of CSL family
    - activates transcription through requirement of histone acetyltransferases

  - in vertebrate and *Drosophila* nervous system, binding of Delta to Notch cells the receiving cell not to become neural
  - in vertebrate eye, interactions between Notch and its ligand regulate which cells become optic neurons and which become glial (supporting) cells

**The extracellular matrix as a source of developmental signals**

- ECM consist of macromolecules secreted by cells into their immediate environment
- macromolecules form region of noncellular material in the interstices between the cells
  - cell adhesion, migration, of formation of epithelial sheets and tubes depend on ability of cells to form attachments to extracellular matrices
  - some attachments need to be strong
  - some need to be transient (e.g. migrating cells)
  - also, in some cases, ECM merely serves as a permissive substrate to which cells can adhere or migrate
    - provides directions for cell movement
- ECM made up of collagen, proteoglycans, variety of specialized glycoprotein molecules
  - e.g. fibronectin, laminin
  - responsible for organizing ECM and cells into an ordered structure
  - proteoglycans play critically important roles in delivery of paracrine factors
    - core protein (e.g. syndecan)
    - covalently attached glycosaminoglycan polysaccharide side chains
      - e.g. heparan sulfate, chondroitin sulfate
        - heparan sulfate binds many members of TGFβ, Wnt, Fgf families
      - appear to be essential for presenting paracrine factors in high concentration so their receptors
  - fibronectin is a very large glycoprotein dimer (460 kDa) synthesized by numerous cell types
    - general adhesive; links cells to one another and to other substrates; e.g. collagen and proteoglycans
    - several distinct binding sites; important for proper alignment of cells with their ECMs
    - also, forms “roads” for cell migration
  - laminin & type IV collagen: major components of type of ECM called basal lamina
    - characterized by closely knit sheets that surround epithelial tissue

**Integrins, the receptors for extracellular matrix molecules**
- fibronectin receptors; anchorage sites for actin microfilaments
- also bind vitronectin (basal lamina of eye), laminin
- on cytoplasmic side, bind talin and α-actinin; proteins that connect to actin microfilaments

**Direct transmission of signals through gap junctions**
- gap junction channels formed by connexin proteins
- six identical connexins group together in the membrane to form a transmembrane channel with central pore
- channel complex of one cell connects to the channel complex of another cell; joins cytoplasms
- gap junction pass material MW < 1500

**Cross-talk between pathways**
- two signaling pathways reinforce each other through one of the many steps in the pathways
  - e.g. in systems that need two or more TFs for activation (combinatorial systems)
  - e.g. signal from one receptor can block signal from a second

**Maintenance of the Differentiated State**
Four major mechanisms

1) Transcription factor whose gene is activated by a signal transduction cascade can bind to the enhancer of its own gene
   - e.g. MyoD transcription factor in muscle cells

2) A cell can stabilize its differentiation by synthesizing proteins that act on chromatin to keep the gene accessible
   - e.g. proteins in the Trithorax family.

3) Maintain differentiation in an autocrine fashion; i.e. cell can make both the signaling molecule and that molecules’s receptor
   - e.g. Sertoli cells of mammalian testis may maintain their differentiation through such a self-stimulatory autocrine loop

4) Interaction with neighboring cells such that each one stimulates differentiation of the other; part of each neighbors’s differentiated phenotype in the production of a paracrine factor that stimulates the others phenotype
   - e.g. developing vertebrate limb and insect segments

**Community Effect: Autocrine stimulation to maintain development**

- (mechanism 3 above) leads to “mass effect, homotypic induction”; a.k.a. “community effect”
- capacity to express developmental potential only when a critical cell density of induced cells in present
  - once a group of cells has been induced, autocrine factors can sustain that induction and complete their differentiation
  - e.g. Xenopus and mice, once members of TGF-β family have instructed certain mesodermal cells to become muscles, these cells will continue to differentiate only if they are bound together in a group
  - mediated by FGF signaling