Tooth formation is highly regulated at the molecular level:
- terminal differentiation of specific cell types
- epithelial-mesenchymal interactions
- secretion of specific extracellular matrices
- controlled processing of those matrices
- regulation of ion deposition
- mineralization of the dental tissues

Tooth Formation is dependent on:
- Genetic Factors
  - Hundreds to several thousand genes likely involved (polygenic)
- Environmental Factors
  - Nutrition
  - Physical phenomenon
  - Infection

Molecular Determinants of Tooth Formation:
- Over 10,000 genes involved in making a tooth
- Most genes involved in odontogenesis are expressed in non-dental tissues
- Some genes are relatively specific for development of the dental tissues (e.g. amelogenin gene)
Environmental Influences of Amelogenesis

- Nutrition
  - Major and minor components
    - Calcium, phosphorus, protein, fluoride etc...
- Hypoxia
- Hyperthermia
- Infection
  - Congenital rubella, syphilis, CMV, etc...
- Physical Determinants
  - Space
  - Trauma

Interactions Leading to Developmental Dental Defects

Host Factors
- Genetic Constitution
- Disease, Age, Sex,

Environmental Stressors
- Nutrition, Disease
- Socio-economic Status

Cell Physiology
- Duration
- Intensity
- Timing

Secretion/Maturation

Dental Defects

Tooth formation occurs over a very long period of time!

Stages Required for Tooth Formation

- Initiation
- Histodifferentiation
- Morphodifferentiation
- Apposition
  - Secretory Phase
  - Transition Phase
  - Maturation Phase

- Stages are not discrete for any given tooth.
- Teeth develop over years beginning with the coronal portion of the crown.
- Can be mineralizing at the cusp tips while cervically cells are differentiating.
Early stages of cell recruitment, signaling, differentiation, and matrix production.

Tooth Bud Initiation

Tooth Development

Dental Developmental Fields

MSX Expression During Odontogenesis

Abnormalities of Tooth Number

- Hypodontia – small or missing teeth
- Anodontia – missing all teeth
- Hyperdontia – increased number of teeth
Supernumerary teeth most often occur in the maxilla

- Mesiodens is most common supernumerary tooth.
- Rare in primary dentition
  - 0.5% of children
- Permanent dentition
  - 1 – 3% of children

Hereditary Conditions with Supernumerary Teeth

- Cleidocranial dysplasia
- Gardner Syndrome
- Cleft lip/cleft palate

Gemination

Congenitally missing maxillary lateral incisors, premolars, 3rd molars

- MSX1 mutation causes autosomal dominant inheritance of congenitally missing teeth.
- Most common form of missing teeth.

Severe Hypodontia PAX9 Gene Deletion

Pax9 Deficient and Wild type Mice

**Ectodermal Dysplasias**

- Diverse group of conditions that can affect normal development of teeth.
- There are over 130 different ectodermal dysplasias that are clinically and genetically heterogeneous.

Freire-Maia & Pinheiro, Ectodermal Dysplasia: A clinical and genetic study 1984

**Ectodermal dysplasias**

- Clinically and genetically diverse group of conditions affecting development of tissues derived from ectoderm. Two affected tissues (e.g. hair, fingernails, teeth, skin).
- Two main types – Hypohidrotic (lack of sweat gland, hair, hypodontia)
- Hidrotic – normal perspiration levels (variable hair, teeth, nail abnormalities)

**Ectodermal Dysplasia Molecular Defects**

- First ED gene defect was reported in 1996
- Molecular defects have now been identified in 10 to 20 ectodermal dysplasias
  - Hypohidrotic X linked ED
  - Reiger Syndrome
  - Tricho Dento Osseous Syndrome
  - Autosomal Dominant/Recessive ED
  - Clouston ED
  - Incontinentia Pigmenti

**Ectodermal Dysplasias: Defining the Molecular Defects**

- X-linked hypohidrotic ED
  - Novel transmembrane protein (ectodysplasin-A)
- Autosomal dominant and recessive hypohidrotic ED
  - Novel tumor necrosis factor receptor (Downless DL)
- Rieger Syndrome
  - Homebox gene (RIEG, PITX2)
- Tricho-dento-osseous syndrome
  - Homebox gene (DLX3)

**Rieger Syndrome**

PITX2 gene defect: involved in anterior posterior patterning.

**Formation of Tooth Mineralized Tissues**

- The formation of dentin, enamel, and cementum required specialized cells that from unique extracellular matrices.
- These tissues perform highly unique functions that are dependent on the tissues composition and structure.
Dentin/Odontoblasts

- Most voluminous mineralized tissue of teeth.
- Largely determines morphology of teeth.
- It is avascular
- Functions to support rigid enamel outer tissue and serves as interface between the dental crown and bone.
- Provides part of mechanism for neurosensory function of teeth.
- Provides mechanism for repair and tissue maintenance.

Dentin

- Calcified tissue similar to bone but does not remodel.
- Collagen-rich organic matrix
- Carbonate substituted hydroxyapatite crystals
- Number packing and density of crystals largely determine stiffness of tissue.
- Mechanical properties vary site to site.

Types of Dentin

- Primary dentin – rapidly produced dentin formed up to completion of root formation and beginning of tooth function
- Secondary dentin – normal physiological dentin production that proceeds slowly throughout the life of the tooth
- Tertiary dentin – dentin produced in response to external stimulus (e.g. caries, restoration etc.)

Normal Circumpulpal Dentin

Odontoblast Life Cycle

- Differentiated from mesenchymal cells condensing adjacent to the inner enamel epithelium
- Are tall columnar cells during active formation of primary dentin
- Height decreases and less organelles are present as cells become less active

Dentinogenesis

- Complex series of events including cell interactions, differentiation, elaboration of a unique extracellular matrix and mineralization are required to produce dentin
- Numerous environmental and hereditary conditions can influence normal dentinogenesis
Dentinogenesis

- Odontoblasts produce and secrete a collagen-rich extracellular matrix (predentin).
- Odontoblasts then control the deposition of inorganic calcium phosphate into this matrix to produce the final mineralized dentin tissue.

Dentin ECM Components

- Type I collagen – most abundant
- Type III & type V – predentin, not normally in mature dentin
- Dentin sialophosphoprotein
  - Dentin phosphophoryn
  - Dentin sialoprotein
- Dentin Matrix Protein 1
- Proteoglycans – numerous species
- Gla proteins – e.g. osteocalcin

Dentin Collagen

- Type I collagen accounts for 90% of dentin organic matrix
- Made of two α1(I) and one α2(I) to give heterotrimer with triple helical structure
- Rich in glycine (required for helix formation) and proline and hydroxyproline
- Collagen molecules are arranged longitudinally creating alternating overlap zones and gap zones

Dentin Phosphoproteins

- Historically the major dentin phosphoprotein has been called phosphophoryn
- Phosphoproteins make up half of the non-collagenous dentin organic matrix
- There are multiple species of dentin phosphoproteins and the degree of phosphorylation varies.

Dentin Phosphoprotein

- Serine and phosphoserine account for 50% of the amino acid residues
- Very acidic protein
- Secreted at mineralization front and not in predentin
- Associated with insoluble collagen
- Binds Ca\(^{++}\) with strong affinity

Predentin

- Unmineralized dentin matrix
- Always present in normal healthy teeth
- Usually 15 – 20 µm thick and is bounded by odontoblasts on pulp side and dentin on outside
- Predentin exists in a closed compartment with components being determined and regulated by the odontoblasts
Mechanisms of Mineralization in Dentin

- Matrix Vesicles – initiates mineralization in mantle dentin
- Collagen/phosphoprotein complex – required to maintain and continue normal dentin mineralization

Matrix vesicle mediated mineralization in mantle dentin layer.


Dentin Mineralization Front Morphology

- Begins near the DEJ
- Predentin – dentin mineralization front progress pulpally by mineralization and coalescence of the mineralizing calcospherites

Normal Dentin Tertiary Dentin

Collagen – Dentin Phosphoprotein

- Dentin phosphoprotein is bound electrostatically to positively charged areas of type I collagen.
- Conventional dogma - Multiple phosphate esters located on the phosphoprotein are required for mineralization to occur.

Mouse Dentin Sialophosphoprotien (Dspp) Gene Structure

Exons 1-5

Feng et al., J Biol Chem 273:9457-9464, 1998
**DSPP Knock-Out Mouse**

- Mantle dentin and initial circumpulpal dentin mineralization proceeds normally.
- Predentin zone increases in width and irregular dentin mineralization occurs.

**Dentinogenesis Imperfecta (Shields Type II)**

DSPP Mutation in Dentogenesis Imperfecta Shields Type II
Non-sense mutation (Bln45stop) in exon 3 of DSPP gene
Zhang et al., Nature Genetics 27:151-152, 2001

**Dentinogenesis Imperfecta**

- Decreased dentin mineralization
- Enamel fracturing
- Blue-gray to yellow brown opalescent discoloration
- Altered crown and root morphology (increased cervical constriction of crown)
- Decreased number of highly branched dentinal tubules
- Pulp chamber obliteration

**DI Radiographic Features**

- Type I - Associated with osteogenesis imperfecta
  - COL1A1 and COL1A2 mutations
- Type II – Autosomal dominant condition
  - DSPP mutations
- Type III – Autosomal dominant variant with large pulp chambers
  - Allelic to type II (DSPP mutations)

**Osteogenesis Imperfecta**

- Genetically and clinically heterogeneous group of hereditary disorders characterized by
  - Increased bone fragility
  - Blue sclera of eye
  - Hearing loss
  - Joint laxity
  - Dentinogenesis imperfecta (some families)
Osteogenesis Imperfecta

Enamel Formation and Structure

Ameloblast Cell Lineage

Secretory ameloblast
Tall columnar cell with nucleus polarized away from the secretory end (Tomes process).
**Amelogenin and Enamel Formation**

- Amelogenin is thought to control the direction and morphology of crystallite growth.
- Loss or abnormal enamel protein causes marked defects in enamel formation.

**Amelogenesis Imperfecta**

- Group of hereditary conditions caused by mutations in genes important in enamel formation.
- Phenotypes are highly variable depending on the mutation involved.
- Prevalence varies from 1:700 to 1:15,000 depending on population.

**Amelogenin Mutations**

- There are now 15 different mutations in the amelogenin gene (AMELX).
- The phenotypes vary from hypomaturation to hypoplastic defects depending on the type of mutation.

**X-linked Hypomaturation AI**

- Pro70Thr Amelogenin Mutation

**X-linked Amelogenesis Imperfecta**

- Amelogenin gene mutation with C deletion at nucleotides g4114delC.
- Frameshift introduces premature stop codon (L167fsX173).
- Truncates amelogenin protein deleting 18 C terminal amino acids.
The enamel extracellular matrix is a complex mix of multiple proteins, some of which are derived from ameloblasts (enamelin, ameloblastin) and others that are not (albumin).

- Enamelin
- Ameloblastin
- Amelotin
- Amelin
- Tuft protein
- Keratin
- Albumin

Enamelin

- Low abundance glycoprotein immunolocalized to the secretory face of the ameloblast Tome’s process.
- Parent protein is a 186 kD glycoprotein
- Cleaved into multiple smaller polypeptides
- May interact with amelogenin

Hu et al., J Dent Res 76: 1720-1729, 1997

AD Smooth Hypoplastic AI Enamelin Mutation

- Exon 4 base substitution introducing stop codon
- Predicted protein is 52 amino acids vs 1142 in wildtype

Mardh et al., Human Molecular Genetics 11:1069-1074, 2002
Picture from Sundell 1985

New Enamelin Mutation

- Single base deletion causes enamelin protein to be 270 AA vs wildtype 1142 AA in length.

Enamel Matrix Processing

- Enamel matrix must be removed for crystallite growth to occur.
- Controlled matrix processing allows crystallites to grow in a highly ordered orientation.
- Multiple proteases are likely involved in enamel matrix processing.
**Enamelysin (MMP20)**

- Novel matrix metalloproteinases - 483 amino acids (54kD).
- Similar to stromelysins or collagenases.
- Expressed by ameloblasts and odontoblasts.
- Degrades amelogenin

Bartlett et al., Gene 183: 123-128, 1996
AR Hypomaturation
AI Treatment

AI Orthognathic Surgery
- Maxillary LeForti
- 11mm impaction
- Mandible autorotate
- Genioplasty

Hypomaturation AI Treatment

Critical developmental period for fluorosis
- 22 – 27 months of age for permanent central and lateral incisors.
- Corresponds to maturation stage of development
- Excess fluoride interferes with proteinase systems the process enamel matrix.
Fluorotic Enamel

- Appears opaque.
- Has a decreased mineral content compared with normal enamel.
- As severity of fluorosis increases the enamel fluoride content increases and the mineral content decreases.

Enamel Crystallite Matrix Model

- Enamel matrix degradation:
  - MMP20
  - KLK4

Bulk of enamel matrix:
- Amelogenin
- Lesser components
  - Ameloblastin
  - Enamelin

Enamel Composition

By weight:
- Water: 4%
- Protein: 1%
- Mineral: 95%

By volume:
- Water: 13%
- Protein: 2%
- Mineral: 85%

Carbonate Substituted Hydroxyapatite

Hydroxyapatite crystallite

Enamel Crystallite Lattice Structure

Crystallites begin as wide thin needle-like structures and grow in thickness during development.
Human Enamel Prism Structure

Etched Enamel Keyhole Pattern

Enamel Architecture

Crystallites oriented into prisms

Head - Tail Interlocking Prisms

Enamel Bonding via Resin Penetration into Etched Enamel

Diagnosis and Management

Appropriate and optimal management of patients requires making an accurate diagnosis and understanding how the dental tissues can be affected by developmental defects.