Genetics, genomics, and epigenetics

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Genomics

- Not a method or technique in itself, rather an approach- functional relationsips between genes, gene products and the environment
- Genetics – narrow focus (effects of single genes in isolation), genomics – large scale (interactions between all genes in the genom and with environmental factors)
- Big data, computational power, bioinformatics
- Genome, exome, proteome, transcriptome, metabolome
- Systems biology

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Human Genome Project

- Spawned technologies that can elucidate and analyze information of genome and its expressed products
- Understanding functional relationships between genes, gene product and environment
- Human diseases are influenced by the variation and function of genes
- Expectation to utilize genetic and genomic information in the diagnosis of and treatment for diseases
- The first step towards personalized medicine

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Personalized medicine

By the examination of the patient genome and the properties of the different parts of the genome doctors can decide how the patients with the same diagnosis will react to the same drugs used in the treatment:

- Toxic but beneficial
- Toxic but NOT beneficial
- NOT toxic and NOT beneficial
- Not toxic and beneficial

By the molecular testing of the diseases (eg. several forms of cancer) doctors can decide which treatment (medicine) can be the most effective against the disease. For example sticking with cancer, they can decide whether several rounds of chemotherapy by infusion needed or the drug can be applied by mouth.

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Possible applications of personalized medicine in dentistry

- More effective dental disease prevention
- Reduce the uncertainty of diagnosis and prognosis
- Guide the selection of drugs or treatment protocols (minimize harmful side effects)

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Interindividual variability in pain sensitivity

- Mu opioid receptor variants – 100 Single Nucleotide Polymorphism (SNP) in OPRM1 gene
- Most common: A118G, 2-48% allele frequency
- AA in position 40: from asparagine to aspartate
- Decreased response to opioid painkillers (dosage differencies)

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Oral Diseases

- Dental caries susceptibility: 40-60 % genetically determined, can be heritable. Three genes involved in enamel formation linked to caries development: AMELX, ENAM, KLK4 (SNPs)

- Periodontitis: multiple SNPs in genes involved in NFKB complex mediated inflammation. Cronic and aggressive form can be distinguished by gene expression profiles. Analyzing microbial community composition to assess risks of disease development and response to teraphy

- Head and neck cancer: mutations in ADH+, CYP1A1, GSTM1, GSTT1, and UGT1A7 genes (alcohol dehydrogenase and xenobiotic metabolizing enzimes) → increased risk of tobacco-related HNSCCs

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Epigenetic alterations I. Histone modification

- Post transcriptional modification (Histone methyltransferases, acetyltransferases, and deacetylases)
- Independent of alterations to the DNA sequence
- Acetylation, methylation, phosphorylation, sumoylation, ubiquitination or ADP-ribosylation at the amino-terminal end
- Chromatine architecture remodelling
- Expression through uncoiling or silencing via compacting DNA
- Histone acetylation results in chromatine decondensation, promotion of transcription, inhibition of DNA methylation
- Head and neck sqamous cell carcinoma (HNSCC)

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Chromatin-immunoprecipitation

- A method for the analysis of histone modified DNA fragments
- After DNA-protein cross-linking the target cells lysed and followed by enzymatic digestion or sonication to degrade DNA which is not connected to protein
- Immunoprecipitation of the fragmented cromatin parts with specific antibodies against different histone proteins
- Purification of bound DNA
- DNA analysis by PCR, qPCR, microarray or sequencing

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DNA methylation

- Cytosine–guanine (CpG) dinucleotides are distributed unequally throughout chromosomal DNA
- CpG may be concentrated in regions called CpG islands that typically overlap with gene promoters.
- Methylation of cytosine in CpG dinucleotides is associated with inactive, condensed states of the chromosome.
- DNA methylation involves attachment of a methyl group to the C5 position of cytosine residues in CpG dinucleotide sequences and typically results in silencing of gene transcription.

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Bisulfite sequencing

- A method for the determination of methylation pattern
- Bisulfite treatment converts cytosine residues to uracil, but 5-methylcytosine residues remains. → only methylated cytosines remains in the sequence .
- The specific changes in the DNA sequence depend on the methylation status of individual cytosine residues → single-nucleotide resolution information about the methylation status of the DNA sequence.
- The method employing polimerase chain reaction (PCR) under non-methylation-specific and methylation-specific conditions. → differentiation of bisulfite-generated polimorphisms.

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An example

- In actively progressing periodontal lesions the level of prostaglandin E and prostaglandin-endoperoxide synthase 2 (PTGS2), while it is decreasing in chronic disease
- Hypothesis: altered methylation levels within the PTGS2 promoter
- Site 8 located 12 bp upstream of an NF-κB binding site- impaired activation
- Sustained change in gene expression and phenotype, reduction in the inflammatory infiltrate, tissue tolerance to chronic stress imposed by the biofilm → long-lived alterations in the metastable state of local periodontal tissues.

Zhang et al. 2010. *J Dent Res*

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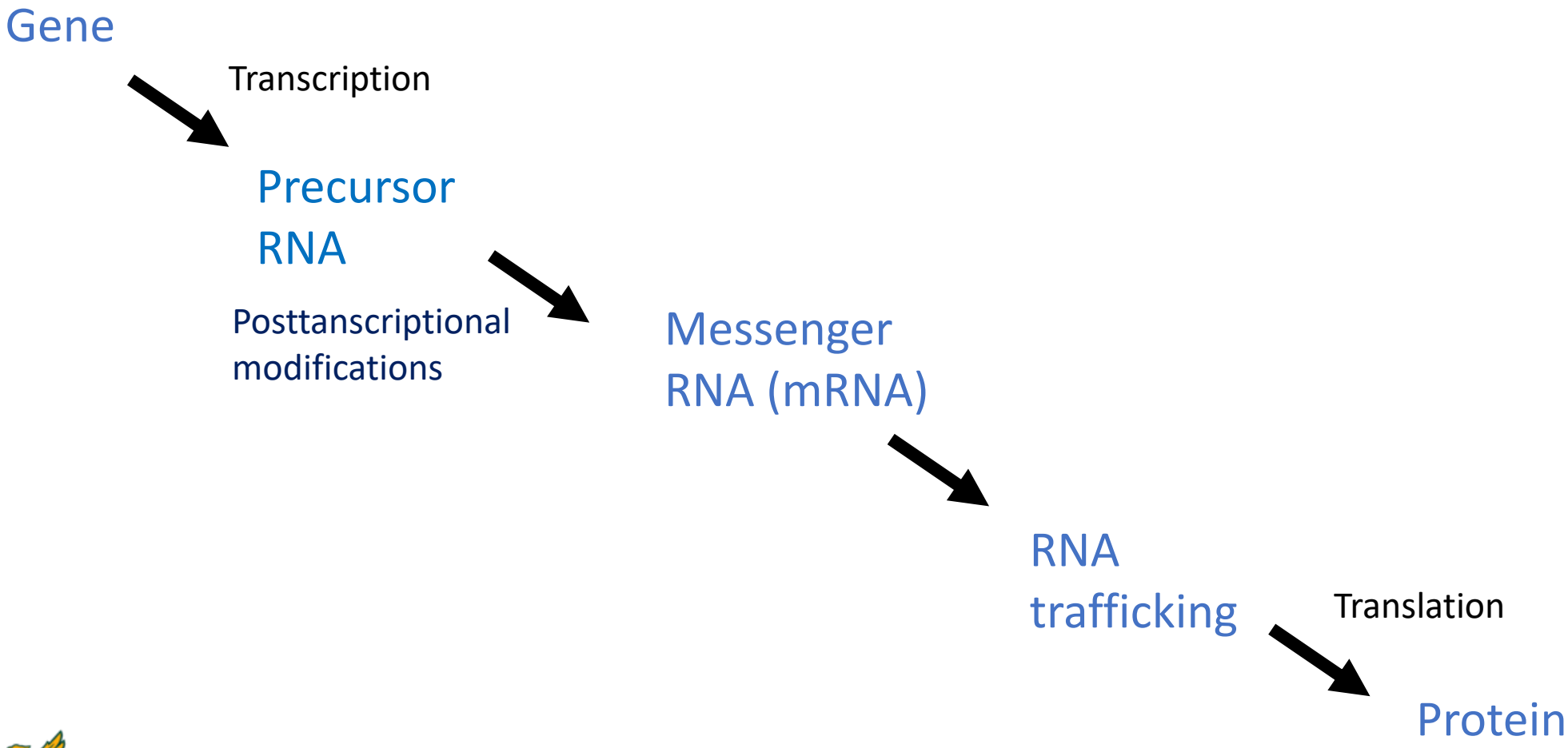
The eukariotic gene expression



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The way of information

The genes consists of:

- Exons (Cap, PolyA, coding sequens)
- Introns (not necessary for translation)

- All translated to RNA

- The mRNA contains only the exon sequences (posttranslational modification → splicing of RNA)

- At translation only the coding sequence turne to amino acids (w/o Cap and polyA)

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Methods for the examination of gene expression

What to examine?

1. Binding of transcription factors to the DNA (to responsive element)
   - Electromobility shift assay (EMSA)
   - Chromatin immunoprecipitation (ChIP)
2. Examination of promoter activity: use of reporter genes
3. Detection of the mRNA of specific genes
   - Northern blot
   - RT-PCR
4. Global examination of all the mRNA in a cell or tissue: microarray

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Electromobility Shift Assay

- The electroforetic mobility of the unbound and protein-bound DNA are different.
- The protein-bound DNA migrates slower.
- Double-stranded DNA and purified (in vitro translated protein extract) protein is needed
- On a non-denaturing agarose gel the bands of free or protein-bound DNA are separated from each other
- Usually the DNA is labelled (radioactive or not radioactive labelling)

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Reporter genes

Reporter gene is a gene which fro the translated protein can be easily determined (eg. by enzyme activity measeurement) and that activity is proportional to the activity of the promoter before the gene.

- The promoter which to be examine is cloned in a vector expressing a reporter gene.
- Transformation into a cell which capable of the synthesis of the reporter protein.
- Change of promoter activity according to our goal → measurement of activity.
- We can determine the effects of certain stimuli on the gene expression through the examined promoter.

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Quantitative Real-Time PCR

- The conversion of RNA into complementer DNA (cDNA) by reverse transcription.
- The cDNA serves as template for PCR reactions and can be multiplied.
- We can determine whether the examined gene(s) is expressed or not, moreover, splice variants can be identifed by using different primers.
- With the application of fluorescent labelling and the detection of the fluorescent products we are able to determine differences in the gene expression level

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Quantitative Real-Time PCR parameters

Baseline: PCR cycles in which a reporter fluorescent signal is accumulating but is beneath the limits of detection of the instrument.

ΔRn: is an increment of fluorescent signal at each time point. The values are plotted versus the cycle number.

Threshold: is an arbitrary level of fluorescence chosen on the basis of the baseline variability. A signal that is detected above the threshold is considered a real signal that can be used to define the threshold cycle (Ct) for a sample.

Ct: the fractional PCR cycle number at which the reporter fluorescence is greater than the threshold. The Ct is a basic principle of real time PCR and is an essential component in producing accurate and reproducible data.

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Microarray

- Global examination of all the mRNA in a cell or tissue
- Glass or nylon membrane platforms
- Microscopic grid of molecular probes (different genes)
- Hundreds of thousands target from 1 sample (DNA, RNA, or protein)
- Differently labeled (fluorescent probes) samples from experiment (eg. treated and untreated, cancer and normal) hibridized to the grid → differentiation of gene expression levels in the different samples

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Deciduous vs. Permanent teeth

- Permanent pulp tissue has higher expressions of genes related to calcium binding and neurotransmission than deciduous pulp tissue.
- Genes related to dentin mineralization were more strongly expressed in permanent dental pulp tissue.
- Gene related neurotransmission (GABA, GRIK1, ST8SIA1, and KCNK10) are strongly expressed in permanent dental pulp tissue → explains the decreased sensitivity of deciduous dental pulp tissue to stimuli compared to permanent tooth clinically.
- Most of the genes that were more abundantly expressed in deciduous dental pulp tissue have barely been discussed with respect to tooth and dental pulp tissues, although most of them have been found in association with the development of other organs or cancer, such as IGF2BP1.
- Genes associated with the immune system, such as HLA-DQA1, were more strongly expressed in deciduous dental pulp tissue. This may be attributable to the characteristic of deciduous teeth relating to root resorption.

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