The York (UK) meeting held at the York University (July 17 to 22), gathered for the first time the 4 WGs of this Action to the internationally recognized eighth International Conference on “tooth morphogenesis and differentiation”. This meeting created a unique opportunity for COST, North American, Japanese, and Korean participants to formalize scientific interactions. Thus, 129 communications (oral and posters) were presented and 184 researchers attended the meeting. The COST participants contributed 2/3 of all presentations.

COST ACTION B23

“Oral Facial Development and Regeneration”

3rd MANAGEMENT COMMITTEE AND WORKING GROUPS 1, 2, 3 AND 4 MEETINGS

in connection with

Eighth International Conference on Tooth Morphogenesis and Differentiation
17-21 July 2004
University of York, Heslington, York YO10 5DD, UK

Scientific report

WG1 “Development and Evolution” Meeting

Date: Sunday July 18th, 2004.

The meeting of the COST Action B23 WG1 (Evolution and Development) at the Eighth International Conference on Tooth Morphogenesis and Differentiation communicated the most recent advances in the research area of the evolution and development of various animal system. These animal systems are studies to understand functions and dysfunction's of human tooth and craniofacial development. Latest molecular and morphological techniques were applied to established animal models (Mouse, Zebrafish). In addition, "new organisms" with interesting phenomena of tooth loss and continuos tooth renewal (Carp, Salmon) were introduced to the scientific community. The meeting of WG1 was subdivided into four fruitful
sessions (Initiation, Morphogenesis. Evolution and Development x2). Each session elucidated vivid discussions and several new collaborations between the participants resulted from these discussions.

**Isabelle Miletich,** Martyn T. Cobourne and Paul T. Sharpe (Department of Craniofacial Development, Dental Institute, Kings College London) reported about the **Dual role of the Gas1 gene during mouse tooth development.** The studies of the authors regard the localized expression and tight control of Sonic hedgehog (Shh), required for the positioning of future teeth. In a toothless area between mouse molars and incisors Shh protein diffusion is rendered inactive to prevent tooth formation. The diastema epithelium plays an important role in restricting Shh via Gas1 expression. In the absence of the epithelium Shh target genes *Patched1* (*Ptc1*) and GO are ectopically induced. Studies on the Gas1 functions as a Shh antagonist at the bud stage of tooth development suggests that Gas1 may have a dual role in odontogenic and non-odontogenic tissues of the mandibular process.

**Aapo T. Kangas** together with Tuija Mustonen, Irma Thesleff, and Jukka Jernvall (Institute of Biotechnology, University of Helsinki) talked about **Ectodysplasin signalling and formation of dental placodes.** Ectodysplasin (Eda), is a Tumour Necrosis Factor (TNF) family member and one of the earliest markers of the molar placode. A Lack of functional Ectodysplasin leads to ectodermal dysplasia, characterized by missing teeth and simplified molar shapes in mice and men. The authors analysed the initiation of molar development in three groups: 1. Lack of Eda, 2. Normal amount of Eda and 3. Overexpression of Eda. The study revealed significant changes in tooth organogenesis and changes in the final tooth shape. This work, together with research on other ectodermal organs (hairs, skin, glands), contributes to the understanding of the dynamics of placodes and suggests that Edar signalling stimulates the formation and function of ectodermal placodes.

**Heiko Peters** (Institute of Human Genetics, Newcastle UK) presented research about **Genetic and genomic approaches to understand Pax9 function in tooth development.** Pax9 encodes a transcription factor required for tooth formation in both mice and man but the molecular mechanisms that are regulated by Pax9 are largely unknown. To address the function of Pax9, the group started different projects using the mouse as a model system. Experimental approaches include generation of novel hypomorphic and conditional Pax9 mutant alleles,
genome-wide expression profiling of wild-type and Pax9 mutant tooth rudiments, and analyses of genetic interactions involving Pax9, Msx1, and Bmp4.

Sachiko Iseki together with Rungrun Kriangkrai, Suconta Chareonvit, Michio Fujiwara, Koichi Yahagi and Kazuhiro Eto (Tokyo Medical and Dental University Japan, Chulalongkorn University; Thailand, Naresuan University; Yamanouchi Pharmaceutical Co. Ltd, Japan) presented studies regards the **Involvement of maxillary process epithelium in rat maxillary incisor formation.** To investigate the contribution of the maxillary process (MP) to maxillary incisors the authors labelled the epithelium with a fluorescent dye based on a whole embryo culture, followed by organ culture. The result indicate that MP epithelium forms the most lateral part of the primary dental laminae. Results further suggest that fusion of the facial processes contributes to maxillary incisor formation, to supply all components and possibly to assemble the components at one site. Defect in the fusion in mutants keeps MP epithelium from contributing to the lateral primary dental laminae (L-PDL) and maintains medial-PDL and incomplete L-PDL.

Hervé Lesot, Bing Hu, Amal Nadiri, and Sabine Kuchler Bopp (Faculté de Médecine, Strasbourg, France, Université Louis Pasteur, Strasbourg, France) presented their studies about **Dental epithelial histogenesis in vitro: Positional information does not require the memorization of cell history.** Epithelial histogenesis starts during the bud to cap transition. The authors used the first lower molar from ICR mouse to evaluate the importance of positional information in the epithelial cells, to test the role of the mesenchyme in specifying information. For this purpose, dissociation/reassociation experiments were performed. The observation of the group showed a high plasticity of epithelial cells. Positional information, which is necessary to co-ordinate the histo-morphogenesis of the developing tooth, is a very dynamic process and does not require the memorization of the cell history.

Irma Thesleff (Institute of Biotechnology, Helsinki, Finland) talked about **Epithelial signalling centres and tooth morphogenesis.** Epithelial signalling centres are important regulators of tooth morphogenesis. They appear reiteratively in the dental epithelium and regulate key steps in tooth development. Recent work from the speakers laboratory indicates that the transcription factor p63 is required in the early oral ectoderm for the initiation of the signalling centres in dental placodes. Latest results indicate that epithelial signalling centres are
key regulators of initiation and morphogenesis of teeth, and that modulation of their signalling functions can affect the number, size, and shape of teeth.

**Jo-Maree Courtney**, James Blackburn and Paul Sharpe (Department of Craniofacial Development, Kings College London, UK) presented **NEMO and Friends: The IKK Complex in Tooth Development**. It has become obvious that the transcription factor NFκB plays an important role in the development of ectodermal organs such as teeth, sweat glands and hair. In non-stimulated cells NFκB is sequestered in the cytoplasm by IκB. Activation in response to an external stimulus involves the IκB kinase (IKK) complex which phosphorylates IκB causing the release of NFκB into the nucleus. The IKK complex comprises the catalytic subunits IKKα and IKKβ and the regulatory subunit IKKγ (known as NEMO). The authors have recently shown that mutations in IKKα result in molar teeth with abnormal cusps, further implicating NFκB in cusp morphogenesis, and also an early incisor phenotype which appears to be NFκB independent.

**Xiu-Ping Wang**, Marika Suomalainen, Carolina J. Jorgez, Martin M. Matzuk, Miriam Wankell, Sabine Werner and Irma Thesleff (University of Helsinki, Baylor College of Medicine, Houston, USA, Institute of Cell Biology, Zurich, Switzerland) presented most recent results regards **Modulation of activin/BMP signaling by follistatin is required for the morphogenesis of mouse molar teeth**. Tooth morphogenesis is regulated by sequential and reciprocal interactions between epithelium and mesenchyme. Signaling molecules of several conserved families, such as transforming growth factors, mediate cell communication during tooth development. Follistatin inhibits extracellularly several members of the TGF superfamily and regulates morphogenesis and shaping of the tooth crown. The antagonistic effects between follistatin and TGFβ superfamily signals are critical for the formation and function of the enamel knots and for patterning of the tooth cusps.

**Jean-Yves Sire** together with Sidney Delgado, Tiphaine Davit-Beal, Marie-Lise Couble, Françoise Allizard (Université Paris 6, France & Faculté d’odontologie, Lyon, France) studied the **Dentition pattern, tooth development and amelogenin expression in the lizard, Chalcides viridanus (Scincidae, Squamata)** by in situ hybridization, immunogold labelling, cloning of the amelogenin gene and comparative analysis of available sequences in tetrapods. A sequence analysis confirmed that hydrophilic regions are well conserved during evolution.
In contrast, the hydrophobic region is more variable appears to blur the phylogenetic information. In situ hybridization revealed that amelogenin expression during amelogenesis was similar mammals. However, different from mammals, amelogenin transcripts were never found in the odontoblasts at any stage of their development.

David W. Stock, William R. Jackman, and Josh Trapani (Department of Ecology and Evolutionary Biology University of Colorado, USA) presented results about Genetic changes associated with reduction of the dentition in cypriniform fishes. The authors have used the loss of oral teeth in cypriniform fishes as a model to investigate evolutionary mechanisms of tooth loss and tooth gain. The presented data identify loss of FGF signaling to the dental epithelium as a candidate cause of absence of oral teeth in cypriniform fishes. Gain-of-function studies should provide a test of this hypothesis and may yield insight into the potential for re-acquisition of oral teeth in cypriniforms.

Gareth J. Fraser, Imelda McGonnell, Anthony Graham and Moya M. Smith (King's College London, UK) talked about Conservation of process for vertebrate dentitions but each with their own design. Since it has been proposed that basal taxa of stem group gnathostomes each have a unique pattern for tooth addition the authors decided to investigate the genetic mechanisms that pattern and regulate odontogenesis in a crown group gnathostomes, the rainbow trout (Oncorhynchus mykiss). A number of genes were identified as homologous to the murine genetic cascade confirming the conservation of developmental controls at one stage, between trout and mouse.

P. Eckhard Witten, Brian K. Hall, and Ann Huysseune (University of Hamburg, Germany; Ghent University. Belgium; Dalhousie University, Halifax, Canada) raised the question: Are breeding teeth in Atlantic salmon a component of the drastic alterations of the orofacial skeleton? The upriver spawning migration of Atlantic salmon (Salmo salar) involves drastic skeletal alterations, a fast, "tumour-like" formation of chondroid bone in males and a toothless stage, followed by the appearance of a new set of teeth (breeding teeth). The authors studies provide no indication for a complete change of dentition prior to spawning since the pattern of tooth replacement observed in juveniles continues in pre-spawning individuals. Earlier reports regarding complete tooth loss/replacement relate to the proliferation of the oral mucosa that covers and so disguises the teeth and to maceration techniques that have removed all teeth with a base that is not fully mineralised.
Ann Huysseune (Ghent University, Belgium) raises the question: Are epithelial stem cells involved in continuous tooth replacement in non-mammalian vertebrates? Lessons from the zebrafish. Recently, the author hypothesized that epithelial stem cells are required for continuous tooth replacement in non-mammalians. This hypothesis relies on several arguments, largely based on observations on the development of teeth in the zebrafish (Danio rerio): morphological resemblance between the epithelial crypt structures, in vitro and in vivo studies, the fact that initiation of replacement teeth appears to be under a different genetic control than that of firstgeneration teeth, and proliferation data in the enamel organ. The zebrafish will prove its value in future studies aiming to test the hypothesis of epithelial stem cell involvement and Wnt signaling in the process of continuous tooth replacement in non-mammalian vertebrates.

Renata Peterková, H Lesot, L Viriot and M Peterka (Institute of Experimental Medicine, Prague, Czech Republic; Faculté de Médecine, Strasbourg, France; LGBPH - CNRS UMR 6046, Faculté SFA, Poitiers, France).presented the results of the study The "supernumerary" cheek tooth in the tabbyEDA mice - a reminiscence of the premolar in mouse ancestors. Primitive rodents, as well as some of their actual descendants, displayed two maxillary and one mandibular premolars in the dentition. In mice, these premolars disappeared during the evolution. The fossil record documents coinciding process during the disappearance of the mandibular premolar. The authors ontogenetic and phylogenetic data support the hypothesis that this "supernumerary molar" might be related to the premolar loss during mouse evolution.

Ralf Kist, Xiaomeng Wang, Colin Miles and Heiko Peters (Institute of Human Genetics, University of Newcastle, UK) talked about Generation of an allelic series of Pax9 mutant mice: a model for oligodontia caused by mutations in the human PAX9 gene. Pax9 encodes a transcription factor that is essential for tooth formation in both mice and man. To better understand the aetiology of oligodontia, we have generated a novel, mutant Pax9 allele (Pax9-neoflox) in mice resulting in reduced expression of the wildtype Pax9 mRNA. Preliminary analysis shows that homozygous Pax9-neoflox and Pax9-neoflox/Pax9lacZ compound mutant mice exhibit tooth agenesis with variable severity and thus mimic the predominant phenotype observed in PAX9 oligodontia patients. The data show that the allelic series of Pax9 mutant mice is a suitable model to investigate gene dosage-dependent functions of Pax9.
I. Stem cells

Reports on stem cells dealt with their roles in normal tooth development and replacement, as well as their potential use for tissue regeneration. Huysseune presented a hypothesis proposing that Wnt-regulated epithelial stem cells are required for continuous tooth replacement in non-mammalian species. Sharpe showed that stem cells of different origins, including ES cells, embryonic neural stem cells and adult mesenchymal bone marrow cells can be utilized to create a “dental” mesenchyme which will respond to signals from oral epithelium. Furthermore, dental mesenchyme formed from bone marrow mesenchymal stem cells developed into tooth crown when transplanted to the renal capsule. In other experiments aiming at tooth engineering, dissociated dental epithelial and mesenchymal cells from rats were re-aggregated and co-cultured. The fact that tooth germ regeneration was initiated under these conditions may indicate that cell-based bioengineering of teeth may not require artificial scaffolds (Hu et al.). Nonetheless, scaffolds were used in other efforts to create teeth from pig dental stem cells (Yelick). Stem cell-based attempts to create replacement tissue for articular condyle or fibro-osseous tissue of e.g. cranial suture or periodontal ligament type were demonstrated by Mao. Here, stem cells were seeded or encapsulated in biocompatible and biodegradable polymer scaffolds which were moulded into the shapes of the organ that was to be replaced.

IIa. Hard tissue formation

- odontoblasts and pulp cells

Stem cell-based both tooth engineering and tissue regeneration therapy will require a full understanding of the odontoblast cell phenotype. Using several both RNA and protein
analysis techniques, *Priam et al.* have characterized a series of immortalized clonal tooth germ cell lines with the aim of obtaining an odontoblast “fingerprint” expression pattern. In addition to the combination of various known genes obtained in that study, *Carrouel* reported on the presence of a new gene, which is overexpressed in odontoblasts as compared to other pulp cells. *Magloire et al.* showed that the human odontoblast expresses two specific types of neural voltage-gated sodium channels, and proposed that these channels may have roles in the sensory transduction from the pulp-dentin border region to the pulpal nerve fibers. Other “neural” molecules detected in odontoblasts, such as reelin and semaphorin 7A, were suggested to influence the structural relationship between odontoblasts and adjacent dental pulp nerves (*Maurin et al.*). Additional reports on the characterization of odontoblasts included descriptions of $\alpha$ 3 integrin localization in these cells (*Farges et al.*), and DNA microarray analyses of genes related to the TGF-1 pathway, a signalling system which previously has been identified as crucial in odontoblast function (*Durand et al.*). To increase the understanding of how genetic mutations in tooth pulp cells may produce dental disorders, *Chen et al.* analyzed pulpal cells from patients with cleidocranial dysplasia (CCD). Here, it was concluded that changes in Runx2 gene expression associated with CCD in tooth pulp cells affects the expression of a number of downstream genes, which may cause the dental phenotype seen in CCD. Finally, *Tecles et al.* demonstrated that the proliferating dental stem cell-like cells that are activated after tooth pulp injury probably have a perivascular pulpal origination.

**IIb Hard tissue formation**

- ameloblasts and enamel

Enamel synthesis is dependent on epithelial-mesenchymal interactions between mesenchymal odontoblasts and epithelial ameloblasts. *In vitro* growth of dental epithelial cells is notoriously difficult, which has hampered the analysis of the ameloblast phenotype. However, here *DenBesten et al.* showed that ameloblast-like cells can be grown selectively under standardized conditions and further analyzed for gene expression patterns. Amelogenin is a major component in enamel which is secreted from ameloblasts. In addition, amelogenin appears to facilitate periodontal regeneration. To further the understanding of amelogenin in these processes, *Deutsch et al.* provided molecular data on amelogenin structure and expression patterns. A specific system for the analysis of amelogenin gene induction in dental
epithelial cells was presented by Ruspita et al., while the results of Catón et al. indicate that the expression of amelogenin, as well as of enamelin and ameloblastin, can be enhanced by Insulin-like growth factors (IGFs). Furthermore, Brookes et al. provided evidence that amelogenin proteins form “nanospheres” prior to secretion from ameloblasts, which eventually influence crystal formation.

IIc. Hard tissue formation

- osteoblasts, osteoclasts and bone

When teeth develop, associated bony tissue has to be formed in an orderly fashion. Osteoclasts are important cellular elements in this context, with a capacity to digest calcified bone and dentin matrix. In a broad attempt to further the understanding of the osteoclast phenotype, Helfrich related abnormalities in bone and dentin with molecular alterations observed in osteoclasts. Furthermore, the findings of Lézot et al. and of Aïob et al. show that Dlx2- and Msx2 homeobox genes seem to control the resorptive osteoclast activities involved in this process. Gene expression in osteoblasts was highlighted in a study which has identified and characterized the human Osterix (Osx) gene. The Osx gene is a putative selective marker of bone cell differentiation, and the findings of this study may facilitate subsequent studies of human bone biology (Nourkeyhani et al.). Another important gene/protein in bone formation, Osteopontin (OPN), was analyzed by Sodek et al. This work demonstrated that organ culture with tissue from mice with an OPN gene deletion may provide a powerful tool to study how OPN regulates bone remodelling.

Kaj Fried, WG2 chairperson

WG3 “Genetic anomalies” Meeting

Date: Monday, July 19th, 2004.

This international meeting centred on tooth Morphogenesis and Differentiation organised by Pr Paul Sharpe, Kings College London, devoted half a day (Monday the 19th July, 9:00-13:00) to WG3 Genetic Anomalies.
The keynote speaker Pr Andrew Wilkie presented his work in the field of craniosynostosis, the premature fusion of the cranial sutures occurring in 1-2500 children. This work has led to the discovery of new genes involved in the pathogenesis of these defects like FGFR2, EFNB1 and has given insight into the relationship between developmental malformations and cancer. World known scientists exposed their work and late results in the field of dental genetics. The following diseases were detailed

- Dr Nalin Thakker (familial adenomatous polyposis (FAP), oculofaciocardiodental syndrome (OFCD), Papillon Lefèvre syndrome (PLS), hypophosphatasia, Ehlers Danlos syndrome)
- Dr M.J. Dixon (amelogenesis imperfecta smooth hypoplastic form, AD ; dentinogenesis imperfecta type II, dentin dysplasia type II, oculodentodigital (ODD) syndrome
- Dr Pekka Nieminen (tooth agenesis associated with colorectal cancer)
- Dr Rena N. D’Souza (tooth agenesis, cleidocranial dysplasia)

However many other working groups discussed dental anomalies and genetic issues:

WG1 Development and Evolution

- **Initiation** focused on placode development and missing teeth, hypodontia in a context of ectodermal dysplasia (Drs Aapo T Kangas, Tuija Mustonen, Pr Irma Thesleff, Jukka Jernvall) or as an isolated trait with the involvement of Pax9 (Dr Heiko Peters)
- **Morphogenesis**
  
  Pr Irma Thesleff explained how epithelial signalling centres were keys regulators of initiation and morphogenesis of teeth and how modulation of their signalling functions could affect the number, size and shape of teeth both in the context of transgenic animal models and Human diseases (ectodermal dysplasia, EEC…).
  
  Dr JM Courtney : NEMO and Incontinentia Pigmenti
- **Evo-Devo** Dr Jean Yves Sire : how evolutionary data could assist diagnosis of mutations in the human amelogenin gene involved in X-linked amelogenesis imperfecta disease.

WG2 Stem cells/hard tissue formation

- **Eruption and bone**
  
  Miep Helfich exposed how osteoclast genes were identified by the study of osteopetrotic mutant mice and how important these results were in the understanding of various Human osteopetrotic conditions resulting in defects in bone and tooth eruption (osteopetrosis,
pycnodysostosis, Paget’s disease, familial expansile osteolysis, expansile skeletal hyperphosphatasia…).

WG4 Tissue regeneration/applications
  -  **Tissue engineering**
    Dr Bruce Rutherford explained the role of the periodontal ligament and his loss as a consequence of periodontal disease. Data from mouse models and Human diseases (craniometaphysial dysplasia, chondrocalcinosis, hypophosphatasia) were analysed to determined the required factors and cells necessary for periodontal regeneration.
  -  **Dentino-and amelo- genesis**
    Pr M Goldberg presented his recent results on the *fro/fro* mice displaying severe forms of osteogenesis imperfecta (Type II and III) and especially the impaired dentin formation (dentinogenesis imperfecta).
    Pr Mary MacDougall offered a synthetic panorama of the SIBLING proteins (among them DSPP, DMP-1, MEPE) and their implications in Human diseases (dentinogenesis imperfecta type II and III, dentin dysplasia type II).

A total of 14 oral communications (on 50) and 15 posters (on 78) were directly devoted to this genetic anomalies field.

**Perspectives**

At York, it was discussed and decided to organise a specific WG3 genetic anomalies meeting in Strasbourg in January 2005.

The following issues will be examined at this meeting:

  - **Diagnosis of cranio-facial and especially dental anomalies:**
    Working towards a consensus on a common protocol to assess dental anomalies, sharing resources and participation to the collaborative database (Diagnosing Dental Defects Database, [Diagnosing Dental Defects Database](#)/ Phenodent.org) project.
- Interdisciplinary management of patients with dental anomalies and partnership with other health professionals (Geneticists…)

- Molecular diagnosis – Genetic testing: what is available in Europe for the diagnosis of these disorders or...where could I send DNA?

- Management of patients with dental anomalies: how is it organised in your area, region, country? What are the links between clinical facilities and research laboratories?

- Census of existing research projects in Europe on rare disorders with oral health involvement

- WG3 relationships with patients family groups and scientific societies: the importance of networking.

- Research on genetic diseases in Europe: what are the legal, ethical issues?

Agnès Bloch-Zupan, WG3 chairperson
Date: Tuesday July 20th, 2004.

The sessions organized under the flag of the WG4 during the conference were excellent. Among the major communications, it should be noted the followings, where new and innovating data were provided.

Paul T Sharpe: Tissue engineering of whole teeth using stem cells. ES cells, neural-crest derived or adult bone marrow stromal cells responded to signals from the epithelium. They expressed gene characteristics from the initiation of tooth development. From these primordia, teeth were formed in renal capsules and recapitulate bone and tooth formation. Transplantating the teeth in the soft tissue of maxillary diastema, embryonic tooth were able to develop in the adult jaw. This was a very stimulating and exciting report.

Yelick’s report was more confusing but evidences that post-natal dental stem cells may be used for tissue engineering applications. At least it provided an overview on the state of the art of our American colleagues.

Attempts to regenerate the primary enamel knot are important with respect to tooth morphogenesis (Cho et al.). Hu et al., showing that an artificial tooth–shaped polymer scaffold is not necessary to obtain a tooth by tissue engineering, evidence regeneration in vitro of dissociated single cells without scaffold. Jeremy Mao was able to reconstruct a condyle and the associated tissue of the musculo tendon complex of the temporo-mandibular apparatus.

Anne Poliard gave a summary of the work of her group tracking the odontoblast phenotype, a prerequisite for tissue engineering dental tissues. Bruce Rutherford studied the regeneration of the periodontium, and so did Rena D’Souza, Francis Hughes, whereas D. Deutsch did the same by using amelogenin.

Altogether, including the posters session, many important informations were brought that will be useful in the next months or years to go further and deeper in the fascinating adventure of dental and peridental tissues generation, or healing and regeneration. It seems that inside the European community, there is a lot of potential that has to be stimulated, and this is the case within the COST action. In brief, it was a great success.

Michel Goldberg, WG4 chairperson
Conclusion: outstanding results

- Increasing contribution of non mammalian vertebrates (zebrafish model) in the understanding of tooth replacement
- Identification of new signalling molecules and new genes during tooth development
- New role of epithelial cells (plasticity) during tooth morphogenesis and replacement
- Use of stem cells of different origins to create a « dental » mesenchyme
- Tooth engineering from stem cells (with or without scaffolds)
- Gene / protein analysis in bone formation surrounding tooth
- Identification of new mutation in gene causing X-linked craniofacial syndrome
- Tooth agenesis associated with colorectal cancer (Wnt gene signalling)

Henry Magloire, COST chair