WORKING GROUP 1. DEVELOPMENT AND EVOLUTION

Chairpersons: R Radlanski and PE Witten

12 May 2005

18h : Poster session. Chair: C Martínez-Álvarez

- WG1: ROLE OF THE MEDIAL EDGE EPITHELIUM EXTRACELLULAR MATRIX IN THE ORIGIN OF THE CLEFT PALATE PRESENTED BY TGF-b3 NULL MUTANT MICE
  E. Martínez-Sanz; E. Resel; E. Trinidad; C. Izquierdo; M. Lopez; B. Garcillán; M. Amorós; A. Del Río; C. Barrio; M. Martinez; J. Murillo; C. Martínez-Álvarez
- WG1: EARLY ODONTOGENESIS IN THE PIG
  Livia Pruskova , Ivan Misek
- WG 1:The developing human deciduous dentition: a reminiscence of reptilian ancestors
  Maria Hovorakova, Herve Lesot, Miroslav Peterka, Renata Peterkova.
- WG1: LAYING DOWN ORDER: GENE EXPRESSION AND INITIATION OF DENTAL PATTERN IN FISH
  Fraser, G. J., Graham, A. and Smith, M. M.

13 May 2005

9h30' **: Keynote Lecture. Invited Speaker Fritson GALIS from Leiden University (Netherlands). " The evolutionary conservation of body plans: Developmental mechanisms, internal selection and medical implications."**

10h30' **Oral Communications** . **Chair: JY Sire**.

10h 30' THE ROLE OF THE ENAMEL ORGAN IN DETERMINING TOOTH SHAPE IN CLOSELY RELATED CICHLIDS (TELEOSTEI; CICHLIDAE; ERETMODINI)
E. Vandervennet, K. Wautier E. Verheyen & A. Huysseune

10h45' THE MORPHOLOGICAL BASIS OF DIVERSITY IN ORAL TOOTH SHAPE IN ERETMODINE CICHLIDS (CICHLIDAE, TELEOSTEI)
Wautier, K, Vandervennet, E., Verheyen, E. & Huysseune, A.

11h00' INVOLVEMENT OF EPIPROFIN IN THE ORGANOGENESIS OF TOOTH AND GENITAL DEVELOPMENT.
S. de Vega, L. Jimenez, T. Nakamura, A. Vilaxa, Y. Yamada, F. J. Unda

11h15' IMMUNOHISTOCHEMICAL EXPRESSION OF P63 IN HUMAN PRENATAL TOOTH PRIMORDIA
Marianne Kock, Dorrit Nolting, Klaus W Kjaer, Birgit Fischer Hansen, Inger Kjær

11h30': MICROSCOPIC STRUCTURE AND MINERAL DISTRIBUTION IN TOOTH AND PERIODONTAL TISSUES IN A ROBUST AUSTRALOPITHECINE FOSSIL HOMINID FROM KOOBI FORA, KENYA. A preliminary report.
Randi Furseth Klinge, M. Christopher Dean, Anette E. Gunnæs, Meave G. Leakey and Alan Walker.

11h 45' Coffee break

12h: **Chair: Ricardo Pérez-Tomàs. Oral Communications**

12h00' BARX1 AND THE EVOLUTION OF FEEDING
Isabelle Miletich and Paul T Sharpe

12h15' THE REASONS WHY CHICKEN CAN NO LONGER HAVE TRUE TEETH
Jean-Yves Sire and Marc Girondot.

12h30' WNT EXPRESSION DURING EPITHELIAL HISTOGENESIS *IN VITRO*
Nadiri A., Hu B., Kuchler-Bopp S. and Lesot H.

12h45' THE INTEGRITY OF THE DENTAL MESENCHYME IS ESSENTIAL TO CONTROL TOOTH MORPHOGENESIS
Bing Hu, Amal Nadiri, Sabine Bopp-Küchler, Songlin Wang and Hervé Lesot

13h00 APOPTOSIS AND CELL PROLIFERATION DURING EARLY UPPER MOLAR MORPHOGENESIS IN THE FIELD VOLE
Setkova J , Lesot H, Matalova E, Matulova P, Misek I

13H15' D1 MEDIATES DEGRADATION OF MYOGENIN BY BMP-2
Francesc Viñals and Francesc Ventura

THE EVOLUTIONARY CONSERVATION OF BODY PLANS. DEVELOPMENTAL MECHANISMS, INTERNAL SELECTION AND MEDICAL IMPLICATIONS

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Body plans are those combinations of morphological characters of a taxon that have been unusually conserved during evolution. Yet, a) even the highly conserved characters of body plans virtually always display minor intraspecific variation and b) new mutants with major effect on those characters are common. This suggests that evolutionary conservation of body plans is usually caused by strong stabilizing selection.

Our studies on fetal and infant deaths in humans indeed show extremely strong ongoing internal selection against variations of body plan characters. The selection against variation appears to be caused by highly deleterious pleiotropic effects. Examples are the conservation of the number of cervical vertebrae and digits.

Many conserved characters of the vertebrate body plan are determined during the early organogenesis stage. This stage is the one of maximum similarity in vertebrates, and in particular in amniotes. The cause for the relatively high conservation appears to be that mutations with an effect during this stage almost invariably lead to deleterious pleiotropic effects in other parts of the body.

Because many adult traits are determined during early organogenesis, early developmental events have a persisting influence. My talk focuses on how such projected effects constrain the power of natural selection in shaping adaptive evolution. Our working hypothesis is that the strong interactivity during the patterning of the embryonic axes is the root cause of the conservation of body plans. Due to this integration, positive mutational changes of some character cause that many negative pleiotropic effects (congenital abnormalities) elsewhere that they are nearly excluded (so-called internal selection).

In a meta-analysis of the literature we have identified specific couplings between the A-P patterning of the mesoderm determining the number of cervical vertebrae and the A-P patterning of other germ layers and/or patterning along other embryonal axes. The multiple, correlated abnormalities that we found in human fetal and infant deaths can be understood as resulting from such couplings.

The data shows that applications of the concepts of evolutionary constraints and pleiotropy provide a novel and unexpected insight into medical risks associated with seemingly harmless anatomical variations, such as cervical ribs and supernumerary digits.

THE ROLE OF THE ENAMEL ORGAN IN DETERMINING TOOTH SHAPE IN CLOSELY RELATED CICHLIDS (TELEOSTEI; CICHLIDAE; ERETMODINI)

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The Eretmodini are closely related cichlids endemic to Lake Tanganyika with very divergent oral tooth shapes, ranging from spatulate in *Eretmodus*, over cylindrical in *Spathodus*, to conical in *Tanganicodus*. It is striking that such closely related cichlids can generate such divergent tooth shapes. To study this we investigated how the enamel organ directs the development of spatulate teeth in *E. cf. cyanostictus* (lineage A), both in ontogeny and in adults, and of conical teeth in adult *T. cf. irsacae*, using 3D reconstructions from serially sectioned tooth germs.

The spatulate oral tooth shape that characterizes adult *E. cf. cyanostictus* (lineage A) is preceded early in ontogeny by a conical tooth shape. We propose two possible hypotheses to account for changes in the folding of the enamel organ (in particular its epithelio-mesenchymal boundary) capable of generating such distinct tooth shapes. Different arguments lead us to favour the hypothesis on an asymmetric growth and differentiation of the enamel organ, such that the tip of a conical tooth corresponds to one "corner" of a spatulate tooth. Applying current models of tooth shape variation would imply the existence of asymmetric fields of inhibition.

Further studies, especially on tooth shapes that are transiently expressed during ontogeny in the different eretmodine taxa could reveal whether eretmodines re-utilise a simple mechanism operating in ontogeny (such as asymmetric progression of the enamel organ) to generate interspecific differences in tooth shape.

THE MORPHOLOGICAL BASIS OF DIVERSITY IN ORAL TOOTH SHAPE IN ERETMODINE CICHLIDS (CICHLIDAE, TELEOSTEI)

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The cichlid tribe Eretmodini, endemic to Lake Tanganyika, comprises four closely related nominal species assigned to three genera with distinct tooth shapes, *Eretmodus*, *Spathodus* and *Tanganicodus*. Yet, this morphology-based taxonomy has proven incongruent with the genetic diversity within this tribe, suggesting a multiple and independent origin of similar tooth shapes. Since variation provides the substrate for natural selection, and hence, the origin of diversity, we engaged in a detailed analysis of eretmodine tooth shape variation at different biological levels (from the individual to the interspecific level). The present overview summarizes the findings of approximately five years of study on this question.

First, our results on tooth shape support earlier statements on the genetic diversity within this tribe, on the existence of cryptic species and on the multiple and independent origin of similar tooth shapes.

Based on our ontogenetic study of tooth shape in *Eretmodus cf. cyanostictus* (lineage A), we demonstrate that the Eretmodini possess the necessary developmental tools to generate different tooth shapes, succeeding each other during ontogeny. Remarkably, in juvenile stages, *Eretmodus* transiently expresses a *Spathodus*-like tooth shape, suggesting that heterochrony might have contributed to the evolutionary diversification of tooth shapes within these fish.

In adults, slight but significant differences in tooth shape between the different jaw quadrants can be demonstrated. These are accompanied by significant body shape differences between left and right body side, possibly indicating directional asymmetry. Potentially these differences reflect a case of phenotypic plasticity, related to the use of a preferential body side during feeding.

Finally, high levels of geographic variation in tooth shape and body shape exist in the taxon intensively studied for this purpose, *Eretmodus cyanostictus* (lineage C).

Taken together, the ontogenetic plasticity, together with the hypothesized heterochrony and phenotypic plasticity, may have provided important factors in the generation of tooth shape diversity, and possibly also body shape variation, within the Eretmodini. The geographical differences in tooth shape suggest that the Bauplan in these fishes is capable of a continuous fine-tuning to a spatially and temporally changing habitat such as found in Lake Tanganyika.

Key words: Eretmodini / Cichlidae / Tooth shape / Evolution / Diversity

INVOLVEMENT OF EPIPROFIN IN THE ORGANOGENESIS OF TOOTH AND GENITAL DEVELOPMENT.

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We have previously identified a mouse cDNA encoding a Sp transcription factor, which we have named Epiprofin (Nakamura et al., 2004). We demonstrated that Epiprofin gene is intensely expressed in several developing ectodermal organs, including teeth, limb buds and hair follicle. Now, we show Epiprofin in the distal urethral epithelium (DUE) of genital tubercle from 10.5 old-day mouse embryo. The expression profiles of Epiprofin suggest a role of this transcription factor in the morphogenesis and differentiation processes of such ectodermal organs. In order to study the relationship of Epiprofin with growth factors and signaling molecules during odontogenesis and genitalia development, we cultured first branchial arches, molars and genital tubercles and analyzed the expression of Epiprofin in different experimental conditions. In this work, we showed that Epiprofin is downregulated by FGF-8 in molars and incisors at initiation of odontogenesis. As Fgf-8 expression decreases in prospective molars, Epiprofin is concentrated in dental lamina cells. This suggests that Epiprofin is not important for tooth initiation, but for tooth morphogenesis. At morphogenesis stage, Shh upregulates Epiprofin, which indicates that Epfn could cooperate with Shh in tooth growth and dental cusp formation. Due to the expression pattern, Epiprofin seems to be important both for odontoblast and ameloblast differentiation. Genital tubercle cultures indicated that FGF-8 induced Epiprofin, and SHH could upregulate Epiprofin. These results suggest that Epiprofin is important for external genitalia development. In conclusion, the transcription factor Epiprofin is one of the important molecules involved in organogenesis of several embryo ectodermal tissues, which develop according to epithelial-mesenchymal interactions.

Keywords: Epiprofin (Epfn), FGF, Shh, genital and tooth development.

Acknowledgements: This work has been supported by 1/UPV 00077.327-E-15379/2003 grant and COST ACTION B23. Susana de Vega and Lucia Jimenez have been awarded by Fundacion Gangoiti and UPV/EHU fellowships, respectively.

IMMUNOHISTOCHEMICAL EXPRESSION OF P63 IN HUMAN PRENATAL TOOTH PRIMORDIA

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The aim of this study was to investigate the expression of the p63 gene in normal human tooth buds from different gestational stages. This is the first detailed study of p63 expression in normal human prenatal tooth primordia.

The material consisted of sections of the midaxial tissue block from the cranial base from three human fetuses of gestational ages 11, 15 and 21 weeks. The sections included tooth primordia representing cap stages and bell stages of human tooth morphogenesis.

In the present study immunostaining was carried out by using the primary antibody, Monoclonal Mouse Anti Human p63 Protein, Clone 4A4. The sections were counterstained with hematoxylin Mayer. p63-immunoreactivity was identified by microscopy.

The study showed a positive reaction of p63 in the cap stage and in the bells stage. In both stages positivity was observed in the cells of the oral mucosa, the inner and outer enamel epithelium and in the primary and the secondary dental lamina. In the early cap stage there is a strong positive reaction to p63 in the enamel knot. This positivity is not present in the late cap stage.

We suggest that p63 may have an important regulatory function in the enamel knot.

Keywords: Tooth, human, p63 gene, development, malformation, histochemical.

Acknowledgements: COST Action B23

MICROSCOPIC STRUCTURE AND MINERAL DISTRIBUTION IN TOOTH AND PERIODONTAL TISSUES IN A ROBUST AUSTRALOPITHECINE FOSSIL HOMINID FROM KOOBI FORA, KENYA. A preliminary report.

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The aim of this study was to determine how well dental tissues preserve at a microscopic level when fossilised and to say something about the process of fossilisation at the site of Koobi Fora in Northern Kenya. We also wanted to study the microscopic structure of mesodermally derived dental tissues and periodontal tissues in fossil hominids, since to our knowledge, no reports on this subject exists.

Ground sections were made and small blocks of tissue were prepared from a poorly preserved early fossil hominid mandible (KNM-ER 1817) containing the root apices of six permanent teeth. Potassium/argon dating techniques of the volcanic tuff layers close by indicates that the mandible was about 1.5 -1.8 million years old. Despite the poor macroscopic preservation of the specimen, a great deal of microscopic anatomy was well preserved.

With routine polarised transmitted light microscopy, fine details of dentine tubules, osteocyte and cemetocyte lacunae and canaliculi, Sharpey's fibres in cementum and incremental markings in dentine and cementum were easily identified. Laser confocal microscopy allowed us to reconstruct small regions of cementum in 3D. Energy dispersive X-ray (EDX) analysis performed in the scanning electron microscope (SEM) revealed that calcium and phosphorus were the main minerals observed in the mineralized tissues, but small amounts of fluoride, magnesium and sodium were also observed. The calcium and phosphorus levels were compatible with biological apatite. The very high levels of calcium as compared to phosphorus found within the periodontal ligament (PDL) space and within the marrow spaces of the trabecular bone, indicate that these spaces had been filled with deposits of calcium carbonate. The PDL and marrow spaces also contained small amounts of manganese. Microradiography revealed that dentine had the highest mineral content followed by cementum and bone (ordered in radiodensity as in living hard tissues). The PDL space had a markedly lower radiodensity as compared to the dentine and cementum. The width of the PDL space however, was still preserved to within normal human physiological limits. Transmission electron microscopy (TEM) of the dental tissues showed densely packed crystallites in the size range 10-50 nm, and this is within the range of crystallite size of mesodermally derived mineralized tissues in living humans. Selected area diffraction and EDX performed in the TEM showed that the crystallites were consistent with hydroxylapatite as found in human teeth.

Overall, we conclude that microscopic preservation of detail in this fossil is exquisite, even after 1.5 million years.

Key words: Fossil hominid, periodontal tissues, microscopic structure.

Acknowledgements: Working group 1, COST ActionB23.

BARX1 AND THE EVOLUTION OF FEEDING

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Evolution of dentitions cannot have occurred in isolation for evolution of other organs required for feeding. The ability to acquire a new feeding niche as a result of new tooth shapes is of little advantage to an organism if the new food cannot be processed and digested. Evolutionary changes in dentitions must therefore have been accompanied by parallel changes in organs involved in food processing and digestion. The *Barx1* homeobox gene is strongly expressed during the development of three organs, molar teeth, salivary glands and the stomach. Using gain-of-function approaches we have shown that *Barx1* misexpression during incisor development results in a transformation of incisors into molars (1). Misexpression of *Barx1* in the developing digestive system results in a transformation of intestine into stomach (2). Using a loss-of-function approach via generation of *Barx1* knockout mice using gene targeting we show that *Barx1* is required for molar tooth development. Moreover in the absence of *Barx1*, the stomach is transformed into intestine.

In the developing stomach we establish that the molecular function of *Barx1* is to regulate the expression of the secreted Wnt inhibitors *Sfrp1* and *Sfrp2* in the mesenchyme that regulate Wnt function in epithelial differentiation. Since *Sfrp1* and *Sfrp2* are locally expressed in presumptive molar mesenchyme and during salivary gland development we believe that *Barx1* function in the development of these different organs involves the same genetic pathway.

Barx1 is thus a gene that links the development of teeth, salivary glands and the digestive system. Changes in the morphogenesis of these three organs must have occurred together during mammalian evolution to enable animals to exploit new feeding niches. New feeding must have been accompanied by new methods of food processing (salivary glands) and digestion (complex stomachs). *Barx1* has thus played a key role in the evolution of animal feeding.

1. Tucker A.S., Matthews K and Sharpe P.T. (1998). *Science* 282, 1136-1138. Transformation of tooth type by inhibition of BMP signalling.
2. Kim B-M, Buchner G, Miletich I, Sharpe P.T and Shivdasani R.A. (2005).
Developmental Cell in press. The mesenchymal homeodomain transcription factor Barx1 regulates developing stomach epithelial identity through local inhibition of Wnt signalling.

THE REASONS WHY CHICKEN CAN NO LONGER HAVE TRUE TEETH

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It is well established that the ability to form teeth was lost in a common ancestor of the modern avian lineages, approximately 70 million years ago. The question whether birds could be able to rebuild teeth under appropriate conditions has been experimentally tested several times in the past. Recently, neural crest cell transplantations from mice to chick embryos have shown that it is still possible, in chick, to reactivate the developmental genetic cascade leading to tooth formation (Mitsiadis et al., 2003). However, although these elegant experiments demonstrated that the avian oral epithelium is able to induce a nonavian developmental program in mouse neural crest, resulting in tooth germ formation, true teeth, i.e. composed of dentin covered with enamel, were not obtained. Reactivation of the developmental pathway is necessary, but not sufficient: at the end of the program, structural genes must be activated. In amniotes, four proteins are known to be specific to dental tissues: three enamel matrix proteins, amelogenin (AMEL), ameloblastin (AMBN) and enamelin (ENAM), and one dentin matrix protein, dentin sialophosphoprotein (DSPP). The possible lack of pleiotropy of the genes coding for these proteins, rises the question of their fate during the last 70 million years of avian evolution. Indeed, given the current knowledge of molecular evolution, we suspect these genes to have rapidly disappeared from the bird genomes. However, were these genes really dental specific? Previous molecular attempts to localise one of the major enamel genes, amelogenin were unsuccessful (Girondot and Sire, 1998). Fortunately, the chick genome is now sequenced. In the present study, we have tried to answer the question of the fate of these genes using bioinformatic analysis. In a first step, we blast searched the chick genome with mammalian and/or crocodilian AMEL, AMBN, ENAM and DSPP sequences. No hits were obtained. Next, we compiled all sequences available in databases (more than 50 amniote sequences were used), calculated the ancestral sequences and blast searched the chick genome once more. The results were, again, negative. In a last attempt to find these genes we looked for synteny, a useful method to localise genes if they have diverged much from the ancestral genes. In mammals, ENAM, AMBN and DSPP are located on the same chromosome, along with other genes coding for secretory calcium-binding phosphoproteins (SCPPs): DMP1, IBSB, SPP1. In the chick, we found the SCPP gene cluster (DMP1, SPP1,...) on a single chromosome, as expected for a conserved synteny: neither ENAM, nor AMBN, nor DSPP were found. In mammals, AMEL is located on the X chromosome, close to a large gene, ARHGAP6. The latter gene was found in the chick, but AMEL was not detected in the expected area of this chromosome.

We, therefore, conclude that the genes coding for AMEL, AMBN, ENAM and DSPP have disappeared from the chick genome. We cann't say whether the disappearance of these genes provoked the loss of teeth in a bird ancestor or whether these genes disappeared because teeth were lost. However, our study strongly suggests that these four genes were specific to dental tissues, at least in the last common ancestor of avian lineages.

- Girondot M. & Sire JY. (1998) Evolution of the amelogenin gene in toothed and tooth-less vertebrates. *Eur. J. Oral Sci*., 106 (suppl): 501-508.
- Mitsiadis T.A., Chéraud Y., Sharpe P. & Fontaine-Pérus J. (2003) Development of teeth in chick embryos after mouse neural crest transplantations. *Proc. Nat. Acad. Sci. USA*, 100: 6541-6545.

Key words: Enamel matrix proteins, amelogenin, enamelin, ameloblastin, tooth loss, chicken

WNT EXPRESSION DURING EPITHELIAL HISTOGENESIS *IN VITRO*

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During molar development, the cap-stage mesenchyme controls tooth morphogenesis. When reassociated to dental epithelial single cells and cultured *in vitro*, the cap-stage mesenchyme can also induce disorganized epithelial cells to restore a complete histogenesis of the enamel organ. In this system, positional information in the epithelium apparently does not require memorization of cell history (Hu et al., 2005). The epithelial histogenesis *in vivo* progresses together with specific patterns of expression of genes coding for signaling molecules, which stimulate apoptosis as well as cell proliferation and differentiation. Similar observations were made for the receptors of the corresponding proteins. For this reason, we compared the immunolocalization of Wnt signaling molecules in the developing tooth germ *in vitro* with their pattern in cultured reassociations of dissociated epithelial cells with either intact mesenchyme or dissociated dental mesenchymal cells. We investigated the expression of Wnt5a, Wnt10b and their receptor Frizzled (Fz). Indeed, these molecules participate in the control of the cell proliferation, which involves the complex of LEF-1/ß-catenin. Since, the Fgf4 gene is a direct target in the dental germ of the complex LEF-1/ß-catenin, we also compared the localization of ß-catenin and FGF-4 proteins in the three models. Wnt5a and Fz showed similar spatial-temporal distribution in both types of reassociation and in cultured molars. Particularly, the two antigens were detected in the PEK in all cases, illustrating a restoration of signaling in the PEK of reassociations. No apparent difference was observed for the localization of ß-catenin. However, differences were also observed between cultured reassociations and tooth germs. In cultured tooth germs, WNT10b and FGF4 were detected in the epithelial and mesenchymal compartments. However, in the reassociations, the two antigens were detected in the mesenchyme only. To better understand what may cause these different patterns of distribution in the reassociations, possible changes in HSPGs will have to be investigated since they interact with both WNT10b and FGF4. The changes in the expression of WNT10b and FGF4 do not seem to have any major consequence on epithelial histogenesis, cusps formation and cell differentiation, which all take place in the reassociations.

Key words: WNT, Tooth morphogenesis, FGF-4.
Acknowledgements: The authors thank COST Action B23 for the support to this work.

THE INTEGRITY OF THE DENTAL MESENCHYME IS ESSENTIAL TO CONTROL TOOTH MORPHOGENESIS

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For tooth tissue engineering, the achievement of correct crown shape requires the use of specific cell populations, of defined culture conditions and requires the scaffolding of the developing organ. In vitro cultures or grafts of tissue reassociations have shown that the dental mesenchyme controls crown morphogenesis as well as the epithelial histogenesis. However, it was suggested that the integrity of the mesenchyme might be critical. To better analyze this aspect, the fate of reassociations of dental epithelia with dental mesenchymal cells was compared to that of reassociations of either dental epithelial cells with dental mesenchyme or dental epithelial cells with dental mesenchymal cells. In all cases, cells and tissues were prepared from embryonic mouse first lower molars at Embryonic Day (ED) 14. The reassociations were first cultured for 6 or 8 days in the absence of scaffolds *in vitro*, and were then implanted under the skin behind the ears of adult mice. Each type of reassociation was evaluated for its ability to lead to a characteristic molar crown development.

In all three types of reassociation, teeth developed showing odontoblast and ameloblast differentiation. The integrity or dissociation of the epithelial compartment did not seem to interfere with the outcome of the reassociation. However, the crown shape, number and pattern of cusps, were much better achieved when the mesenchymal compartment remained as a tissue.

In conclusion, the integrity of the dental mesenchyme is pre-required to get correct crown shape. The molecular changes after dissociation of the mesenchyme to get single cells will have to be investigated to overcome this point, essential for tooth tissue engineering.

Key words: Tooth morphogenesis, tissue engineering

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APOPTOSIS AND CELL PROLIFERATION DURING EARLY UPPER MOLAR MORPHOGENESIS IN THE FIELD VOLE

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Development of all multicellular organisms involves a tightly controlled balance between cell elimination and proliferation. During tooth development, as in other organs, controlled proliferation, differentiation and elimination of particular cell populations are considered to determine the final tooth shape, size and position in the jaw.
Despite similar general development, the final tooth shape differs among species. We investigated the 3D pattern of cell proliferation and apoptosis during early tooth development in the field vole - as a suitable model for comparative studies with the mouse - to reveal possible differences in the first upper molar tooth germ.
Embryonic stages E 12.5 to E 15.5 (from bud to cap stage) were used to follow the tooth germ development. Evaluation of apoptotic bodies and mitoses, confirmed by TUNEL test and immunohistochemical localization of PCNA, respectively, were applied as criteria to connect apoptosis and proliferation in a 3D model.
In both species, the mouse and the vole, proliferating cells accumulated in the epithelium surrounding the primary enamel knot (PEK) and later also in developing cervical loop. No major differences were found in mitoses distribution in the field vole compared to the mouse (ED 12.5 – 15.5). Apoptotic bodies were located in non-dividing cell populations. Apoptosis was involved in the elimination of internal cells of the PEK and delimitation of toothless diastema in both species. Nevertheless, the fate of vestigial tooth rudiments in both species seems to differ. In the mouse upper molar, both vestigial rudiments undergo apoptosis, whereas, incorporation of the second rudiment into molar primordium is suggested in the field vole.
In the field vole, apoptotic bodies accumulated also in the longitudinal axis including the area of enamel septum (ED 15, 15.5), which has not been observed in the mouse. Massive apoptotis found along the whole longitudinal axis of the tooth germ may be connected with different positioning of the secondary enamel knots in the vole and the mouse leading to final tooth crown differences. However, later stages and other aspects must be investigated to support these findings.
In all mammalian species studied so far, apoptosis in early odontogenesis showed a highly specific temporo-spatial patterning, particularly in the dental epithelium. Further experiments should confirm, whether apoptosis and proliferation play active morphogenetic roles and if their interplay is necessary to achieve the proper tooth shape formation.

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Keywords: field vole, apoptosis, proliferation

D1 MEDIATES DEGRADATION OF MYOGENIN BY BMP-2

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In this work we describe a new mechanism that participates in the block of the myogenic program by BMP-2, induction of myogenin degradation by the proteasome. In this mechanism Id proteins play an essential role. We show that BMP-2 inhibits the myogenic program stimulated by IGF-1 in C2C12 cells. Induction of myogenin, a specific skeletal muscle transcription factor, is inhibited by BMP-2 blocking not only its transcription but also its protein stability. Thus, inhibition of myogenin protein expression is yet observed at short times after exposition to BMP-2, in absence of any change on myogenin mRNA. Moreover, the decrease of the myogenin protein levels caused by BMP-2 is also observed after the forced expression of myogenin mRNA under the control of an independent promotor. BMP-2 decreases myogenin protein half-life from 60 min in the control cells to 30 min in the presence of BMP-2. Proteasome inhibitors block the BMP-2-induced myogenin protein degradation. Id1 overexpression by transient transfection or under the control of an inducible Tet-off system is sufficient to cause the degradation of myogenin in the absence of BMP-2. Finally, the effect of BMP-2 is blocked by E47 overexpression.

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ROLE OF THE MEDIAL EDGE EPITHELIUM EXTRACELLULAR MATRIX IN THE ORIGIN OF THE CLEFT PALATE PRESENTED BY TGF-b3 NULL MUTANT MICE (POSTER)

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TGF-b3 null mutant mice show all the possible kind of cleft palate, from complete to submucosa, depending on the strain of mice where the gene is targeted, thus implying different degrees of adhesion/fusion of palatal shelves. Our aim has been to analyse the presence/distribution of several extracellular matrix and adhesion molecules in two strains of TGF-b3 null mutant mice to investigate their importance in the process of palatal shelf adhesion and fusion. We have performed immunohistochemistry with antibodies against fibronectin, laminin, collagens IV and IX, a5 and b1 integrins and ICAM-1 on heads of embryonic day (E) 14.5 Albino Swiss and C57 wild type and TGF-b3 null mice. In addition, we have performed E13.5 palate cultures, treated or not with TGF-b3 or treated with blocking antibodies against fibronectin or the integrins a5 and b1. Our results show differences in the presence of the molecules analysed between the wild type and null mutant palates, more severe in the C57 (the one having complete cleft palate) than in the Albino-Swiss strain (where the cleft palate is incomplete). The addition of TGF-b3 rescues the normal presence of these molecules. The use of blocking antibodies against fibronectin and the a5 integrin results in a statistically significant decrease of the adhesion and fusion of the palatal shelves. We therefore conclude that the presence of some extreacellular matrix and adhesion molecules is very important to palatal shelf adhesion/fusion and that is part of the pathogenesis of the cleft palate presented by the TGF-b3 null mice.

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EARLY ODONTOGENESIS IN THE PIG (POSTER)

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The knowledge of a generally valid pattern of odontogenesis is a prerequisite for detection of developmental changes in the oral cavity in domestic animals as well as man. The pig is a suitable model species for investigation of insectivor-type dentition development. It is known the domestic pig has normodont (44 permanent teeth) and heterodont dentition what means that all tooth types representing incisors (3), canines (1), premolars (4) and molars (3) in each jaw quadrant are present. The pig expresses diphyodont dentition which includes two tooth generations. The main aim of our study was to find fundamental knowledge about early tooth development in the pig.
We have studied pig odontogenesis in collected embryos and foetuses aged from ED 20 to 45 using serial histological sections (duration period in the pig is 115 days). Morphometry and three-dimensional computer-aided reconstructions of the dental epithelium have been used. At ED 20, thickenings of the oral epithelium have been apparent in incisor region of the dental lamina. On histological sections, swellings of incisor primordia were considered to be a morphological sign of odontogenesis initiation. The distribution of tooth primordia expressed an antero-posterior orientation. The dental lamina showed a horseshoe shape and developed from connected thickenings in the incisor region and cheek tooth region. We have focused on tooth development in incisor region to determine swelling, bud, cap and bell stages and to compare acquired data with other mammalian species.
In our study, early stages of tooth primordia have been reconstructed using routine histological sections. However, later stages have been too large for histological processing. Therefore, it will be necessary to use other supplied methods for successful tooth germ reconstruction such as CT and MRI in the near future. This approach to the issue could help to acquire new original data about evolutionary changes in developmental processes during odontogenesis. However, later stages and other aspects must be investigated to support our findings.

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Keywords: tooth development, 3D computer-aided reconstructions, domestic pig

THE DEVELOPING HUMAN DECIDUOUS DENTITION: A REMINISCENCE OF REPTILIAN ANCESTORS (POSTER)

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The medical textbooks on human embryology present classical concept of the early dental development in humans including the origin of two horseshoe-shaped epithelial structures: the dental lamina (giving rise to deciduous tooth primordia), and the externally adjacent vestibular lamina (giving rise to the oral vestibule separating the teeth from the lips and cheeks). However, there is no consensus about the developmental relationship between these laminae. Therefore, we investigated morphogenesis and the developmental relationship of the dental and vestibular epithelium in the human upper jaw from embryonic week 6 to 9. Using computer-aided 3D reconstructions, we showed that the situation is completely different from that, which is generally presented in embryological textbooks. There was no continuous vestibular lamina, but several distinct epithelial structures (bulges and ridges) occur transiently at the place of the developing oral vestibule. The series of vestibular bulges was located anteriorly, in front to the incisor teeth. The different vestibular ridges fused with the dental lamina distally to the deciduous canine, first molar and second molar. These interactions between the developing teeth and vestibular structures could be reminiscent of the situation in some reptiles, where single teeth are paired one-to-one with single tooth glands.

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LAYING DOWN ORDER: GENE EXPRESSION AND INITIATION OF DENTAL PATTERN IN FISH (POSTER)

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It has recently been proposed that stem-group gnathostomes and basal taxa of crown-group gnathostomes each have a unique pattern for establishment of tooth initiation sites. Few studies have generated results focussed on the initial tooth sites and the mechanisms that prime these sites for subsequent odontogenesis. It is proposed that pattern for the sequential order of initiation of tooth sites varies significantly across the jawed vertebrate clade and that primary tooth site positions control the production of subsequent replacement dentition. Not all replace their teeth but primitively a statodont mode is normal, in contrast to the familiar lyodont, or lingual replacement mode. Data from fossil stem gnathostomes (placoderms and acanthodians) has questioned assumptions regarding the homology of vertebrate dentitions as those formed within a dental lamina.

Comparative molecular and morphological data are presented that substantiate the taxon specific pattern in mode of dental initiation and replacement. In the rainbow trout (*Oncorhynchus mykiss*) it is apparent that specific regulatory genes pattern the establishment of primary tooth sites, only set up following a regionally restricted pattern of *Shh* and *Pitx-2* expression, the odontogenic band, clearly related to establishing dental competence for the epithelium. Within the competent region, gene expression (*Shh* and *Pitx-2*) emphasises that the basal epithelial cells at focal loci coincide with the primary tooth sites. Data will also be presented to show that within a species (*O. mykiss*) the different dentate bones, each have unique patterns of sequential tooth initiation. This suggests that they represent separate odontogenic modules but express identical genes with spatio-temporal differences resulting in a variety of patterns. This questions the controls that order and pattern primary tooth sites?