The Study of Supernumerary Incisor Formation in *rSey* Rat

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A mutant rat with small eyes, *rSey*

rSey inherits a dominant mutation in *Pax-6* gene and this mutation leads to impaired migration of anterior midbrain neural crest cells resulting in lack of eyes, nose and facial cleft in the homozygote.

Facial morphology of new born rat

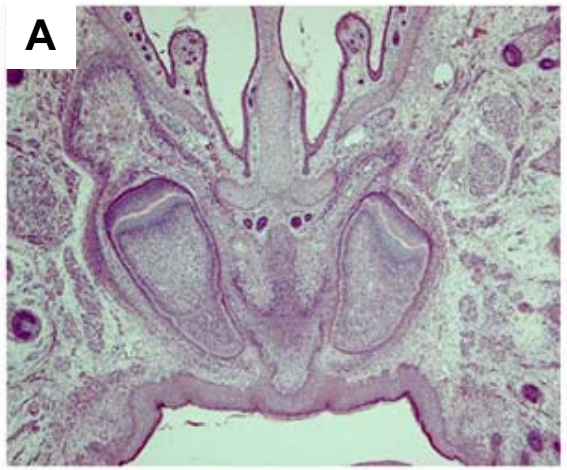
wild type

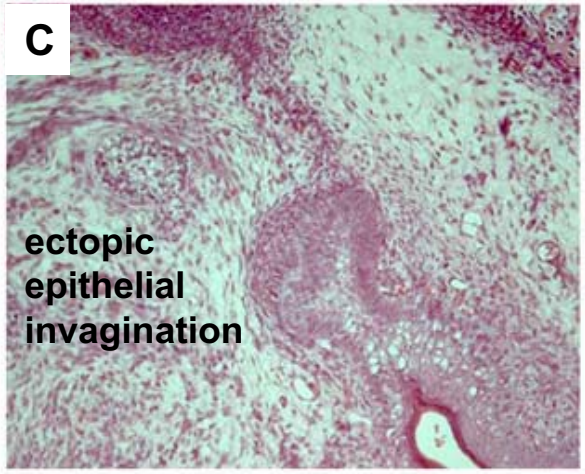
homozygote (*rSey/rSey*)

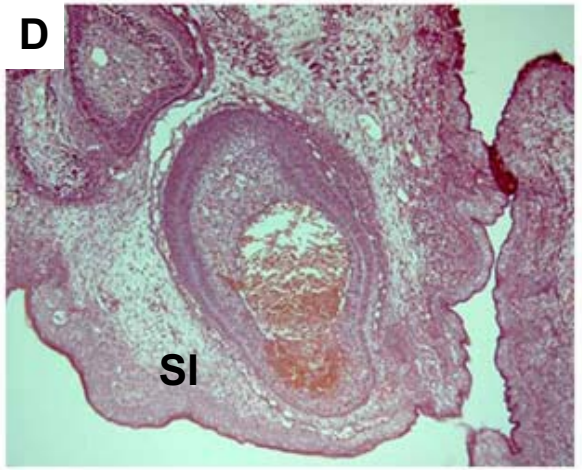
20% of homozygotes develop supernumerary incisor-like structures at E 20

wild type

homozygote (*rSey/rSey*)







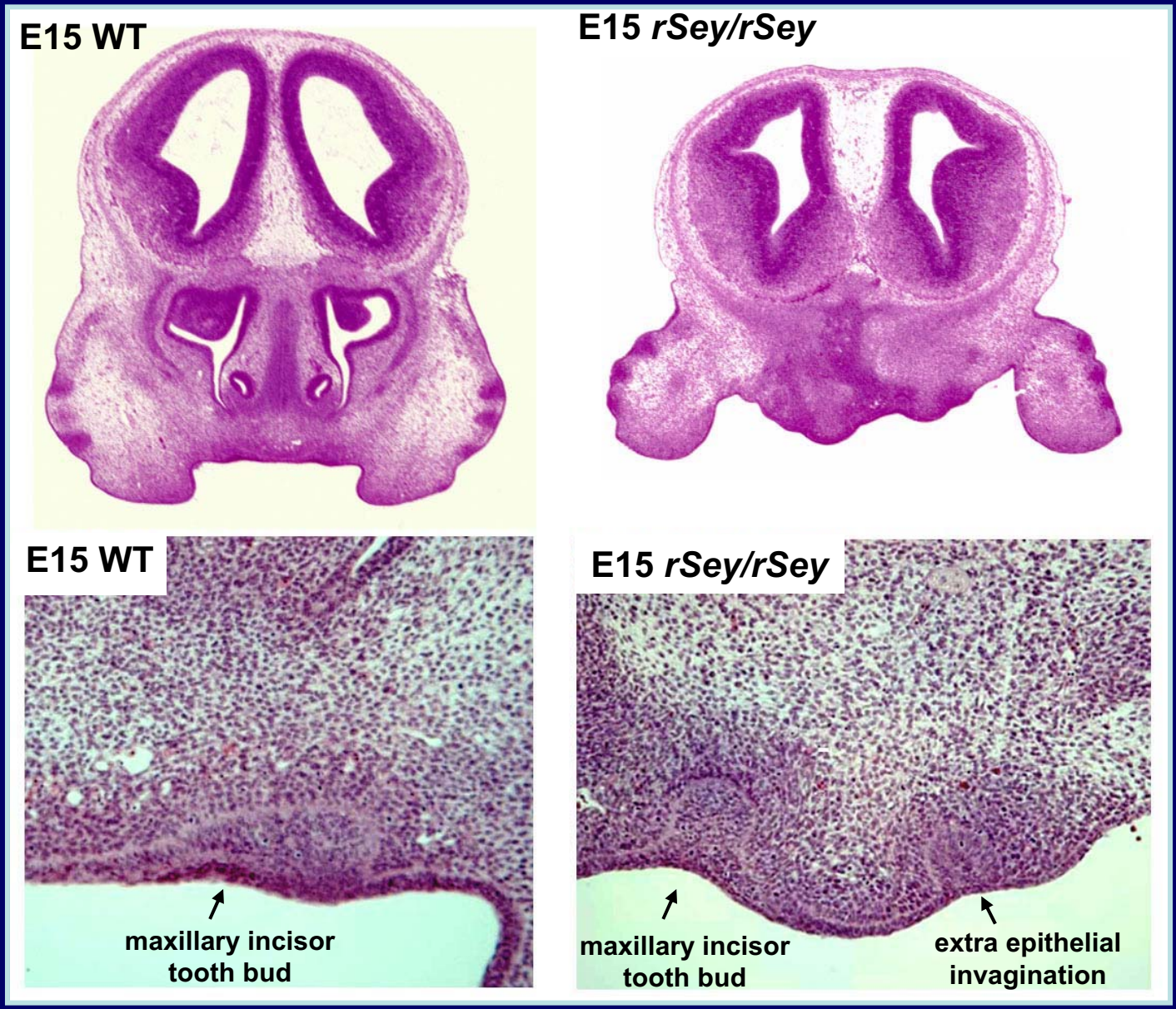
A: wild type (WT)

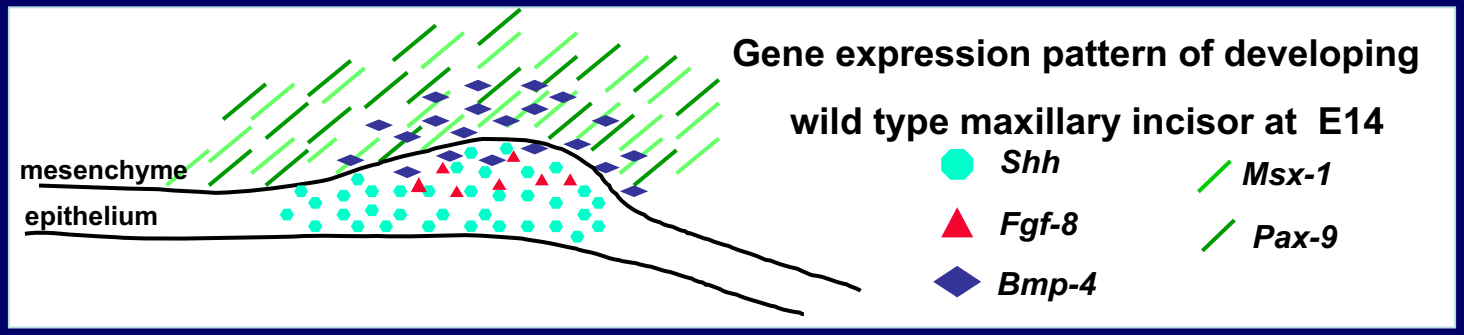
B-D: homozygote (*rSey/rSey*)

MI: maxillary incisor

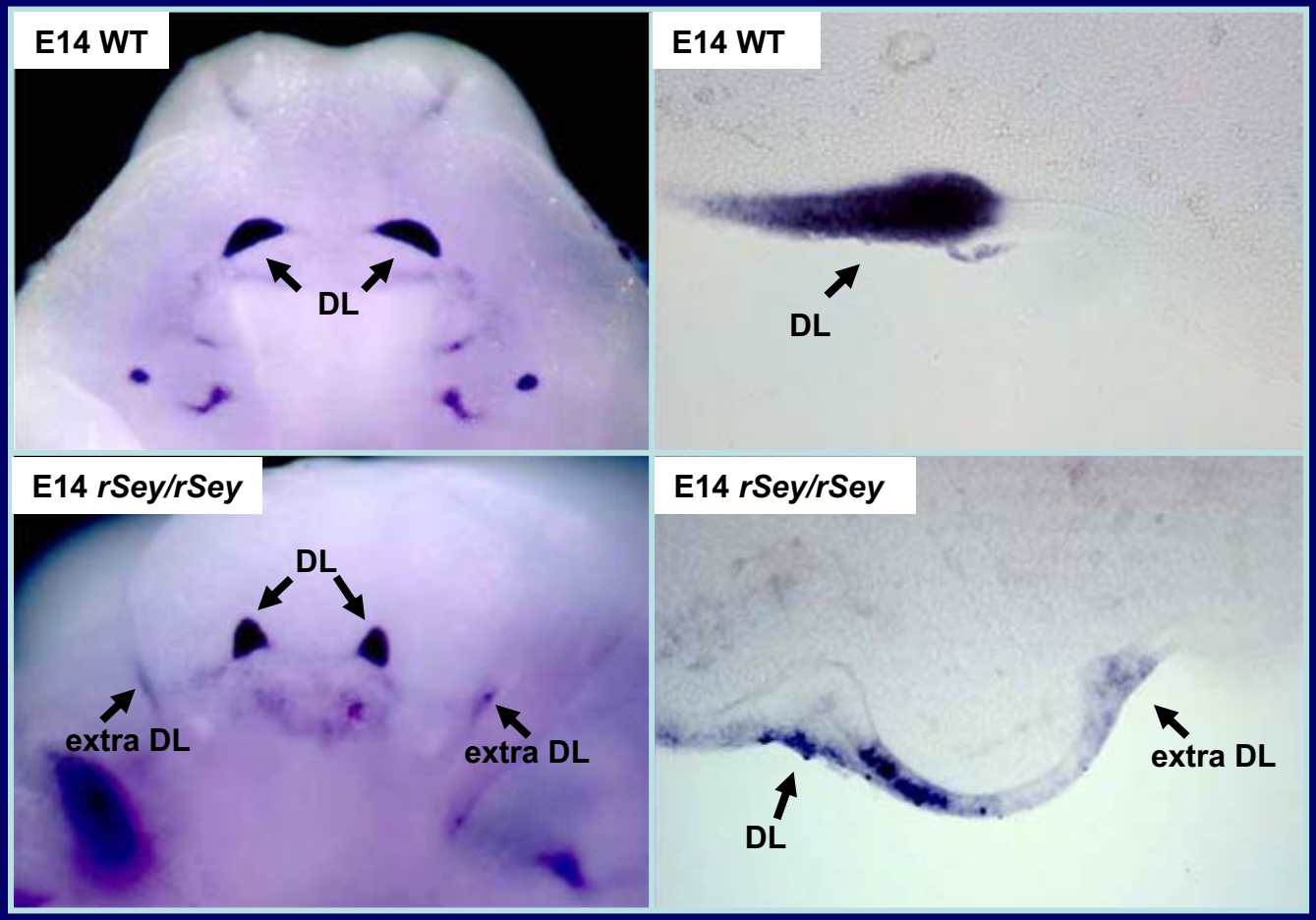
SI: supernumerary incisor-like structure

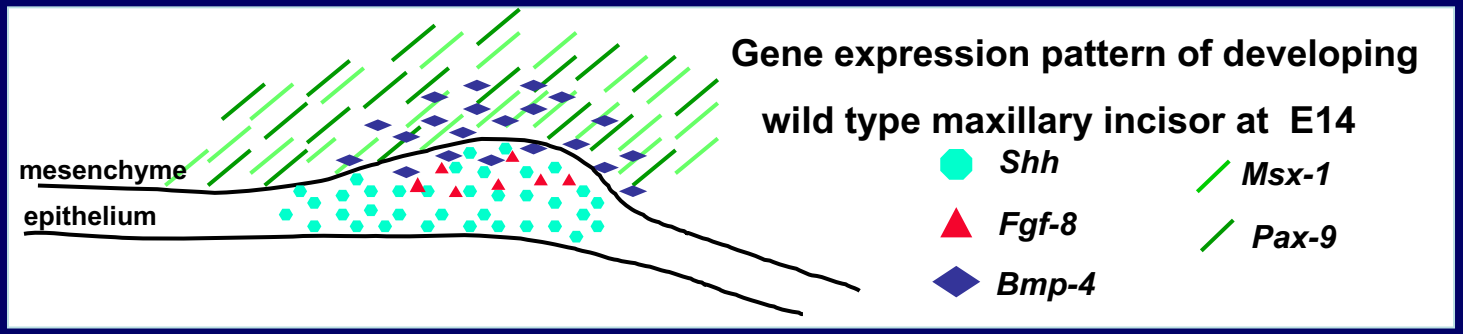
Histology of wild type and homozygote



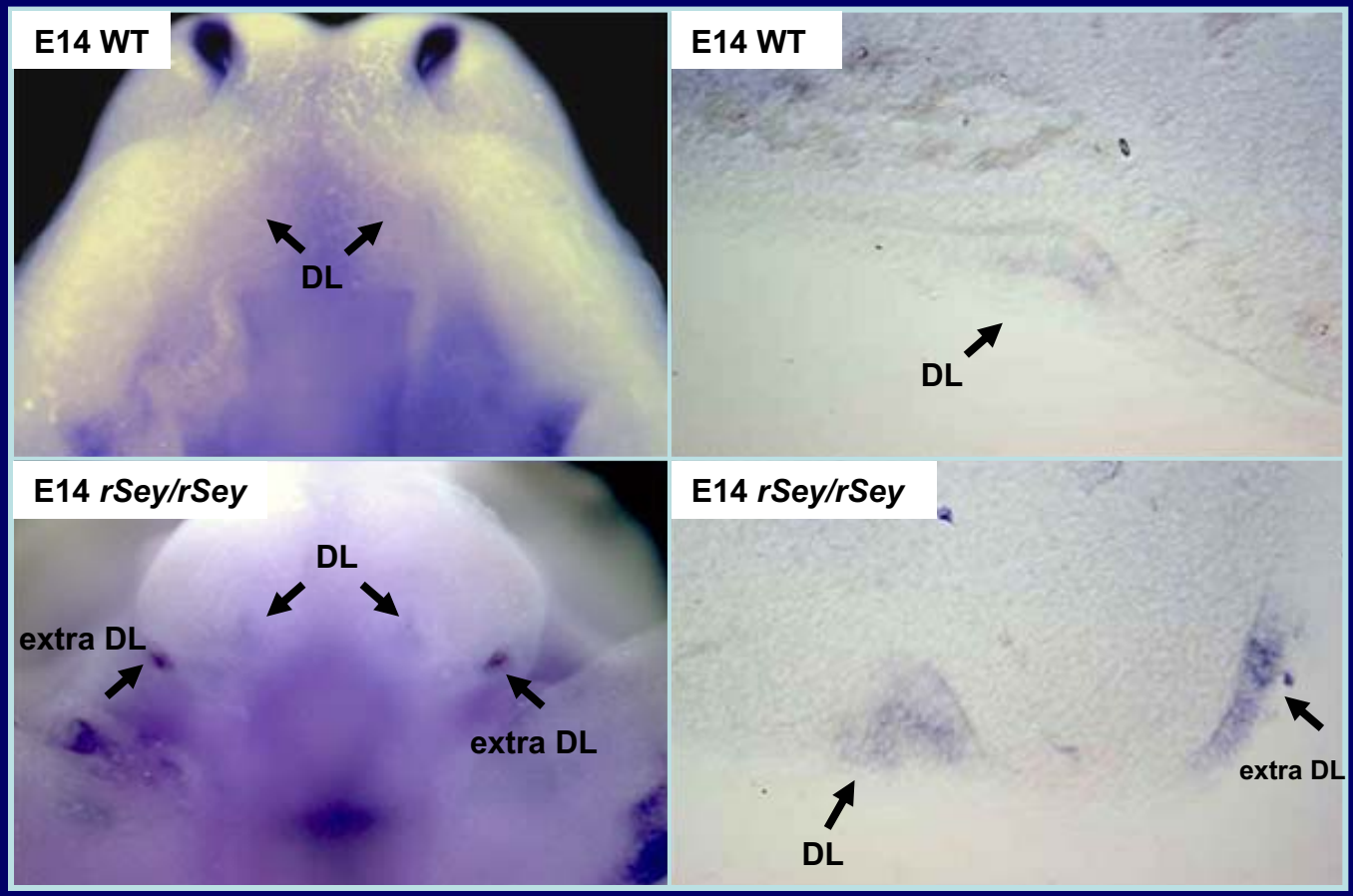


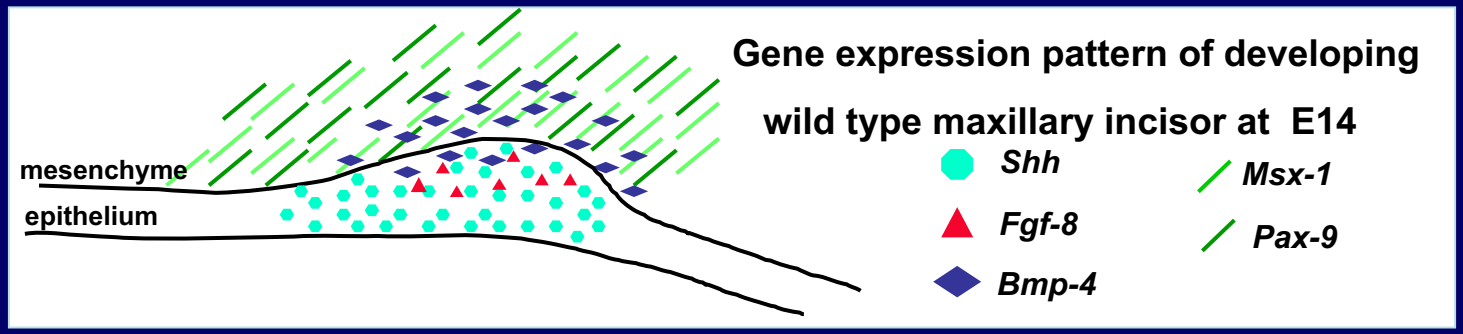
Whole mount in *situ* hybridization of *Shh*



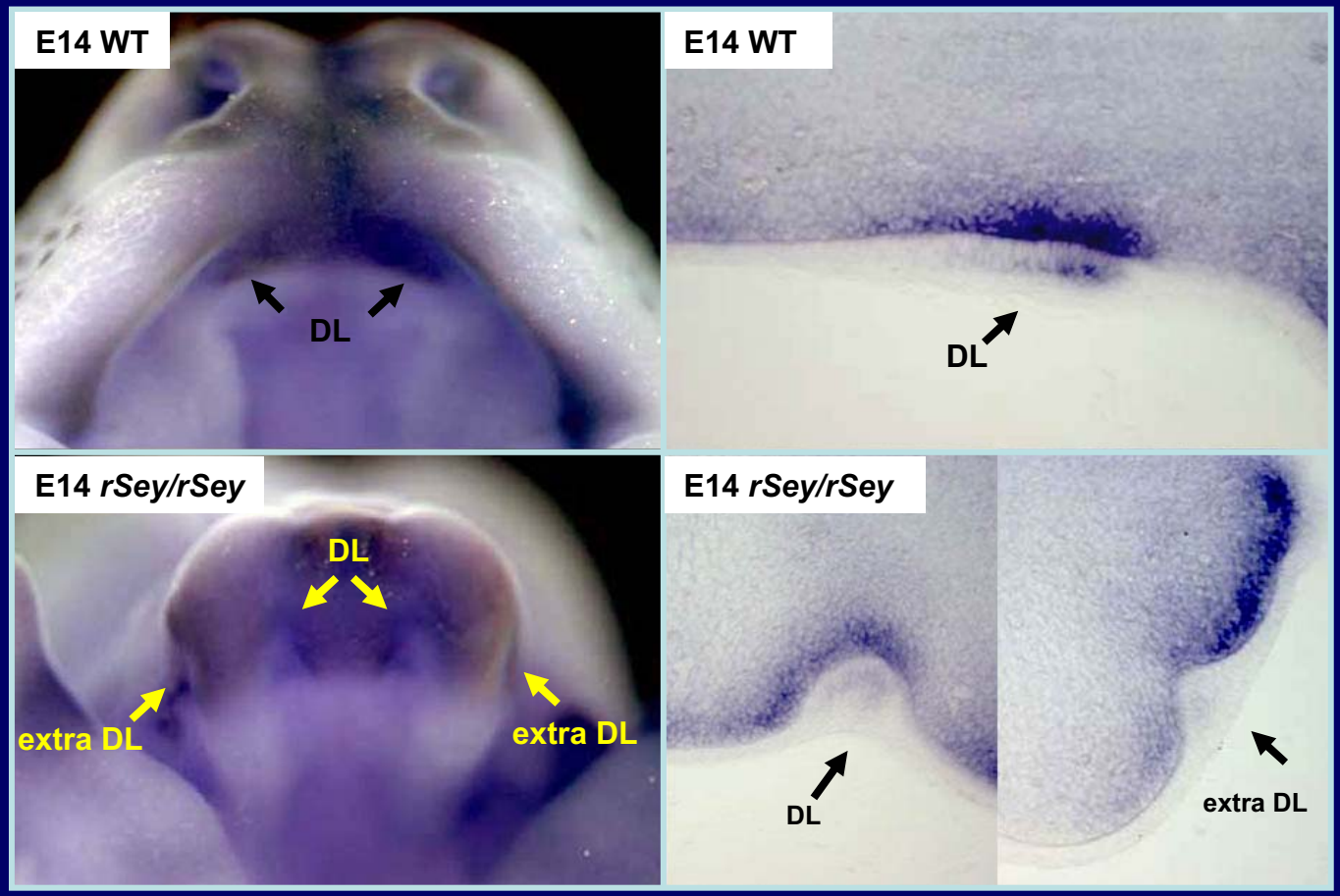


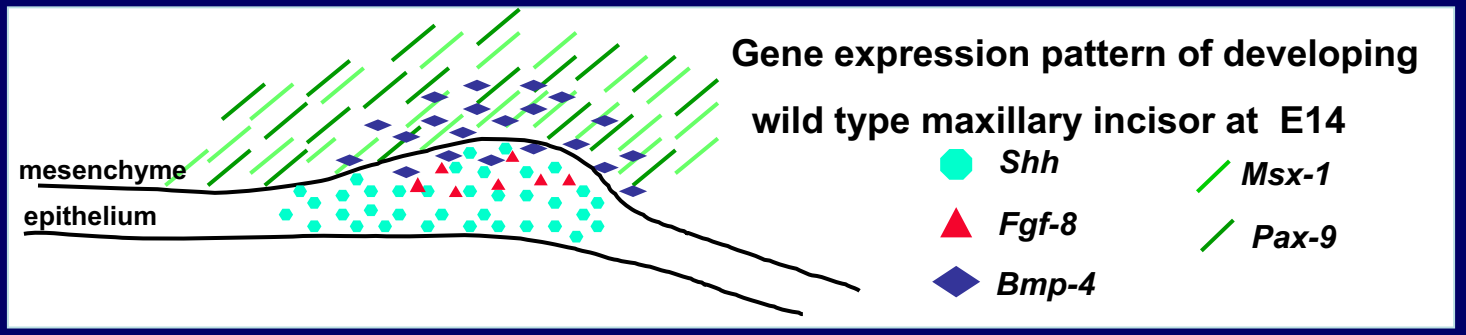
Whole mount in *situ* hybridization of *Fgf-8*



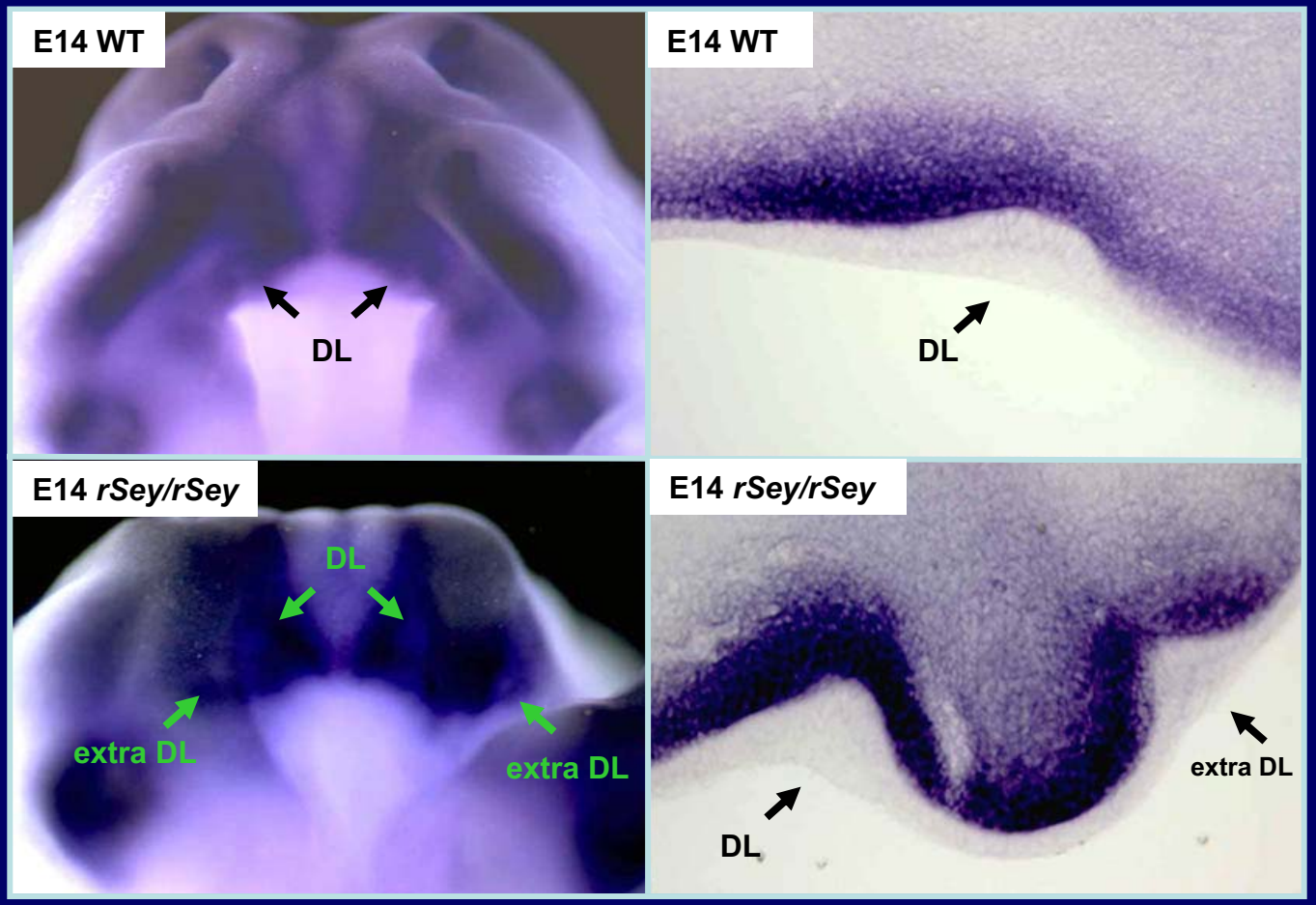


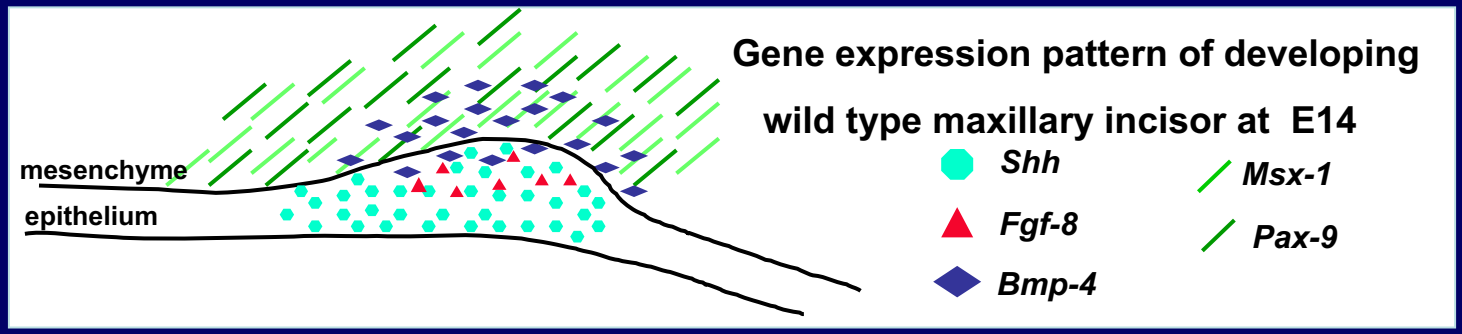
Whole mount in *situ* hybridization of *Bmp-4*



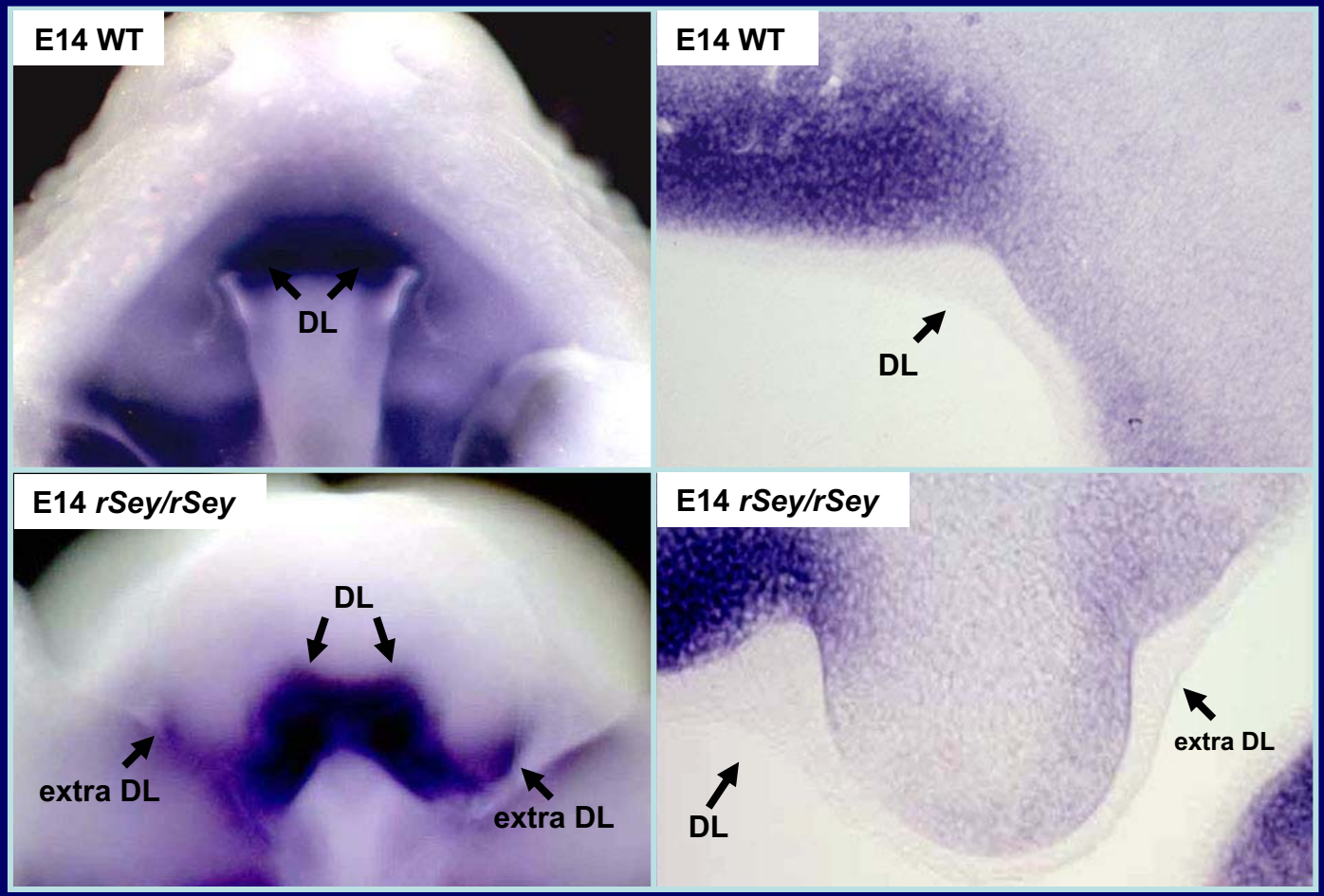


Whole mount in situ hybridization of *Msx-1*

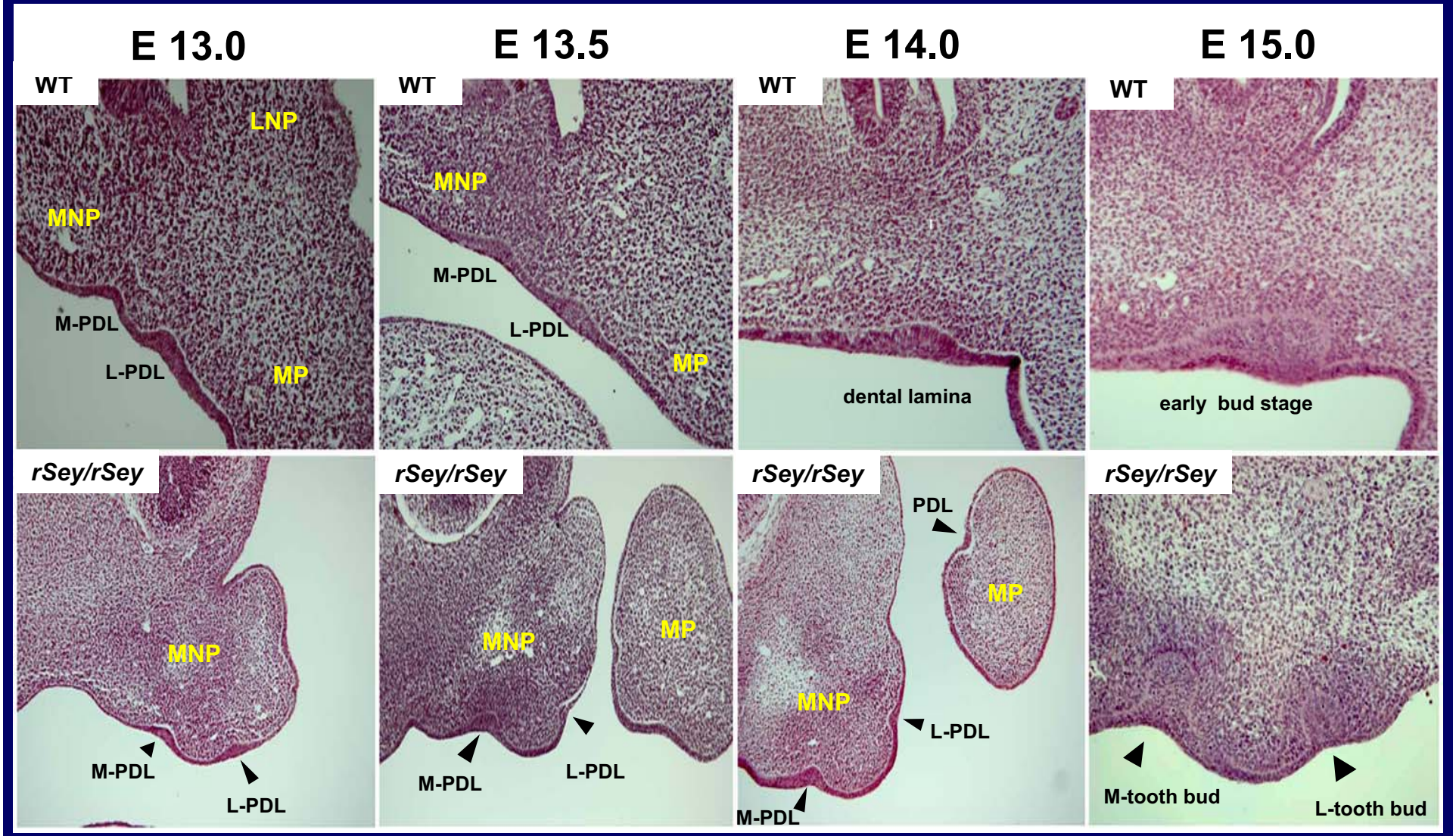




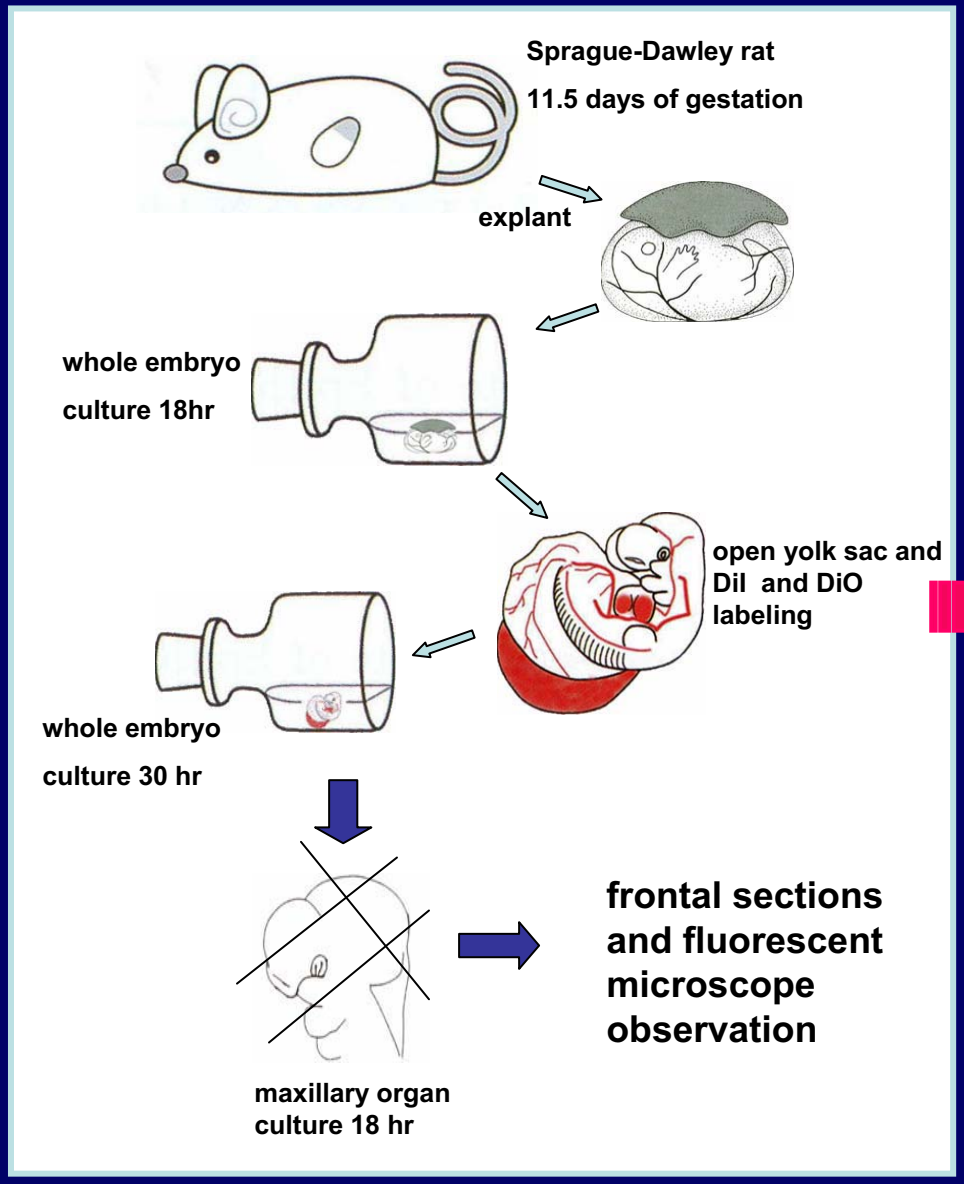
Whole mount in situ hybridization of *Pax-9*

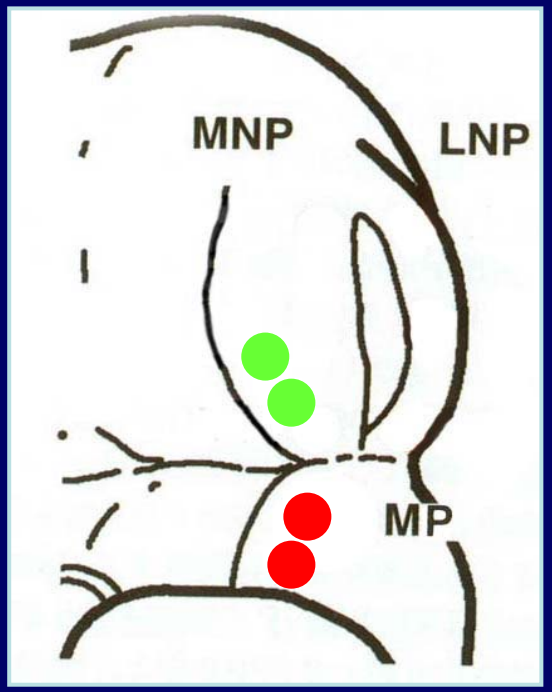


Histological observation of maxillary incisor development



Labeling of MNP and MP epithelia to investigate their contribution to incisor formation

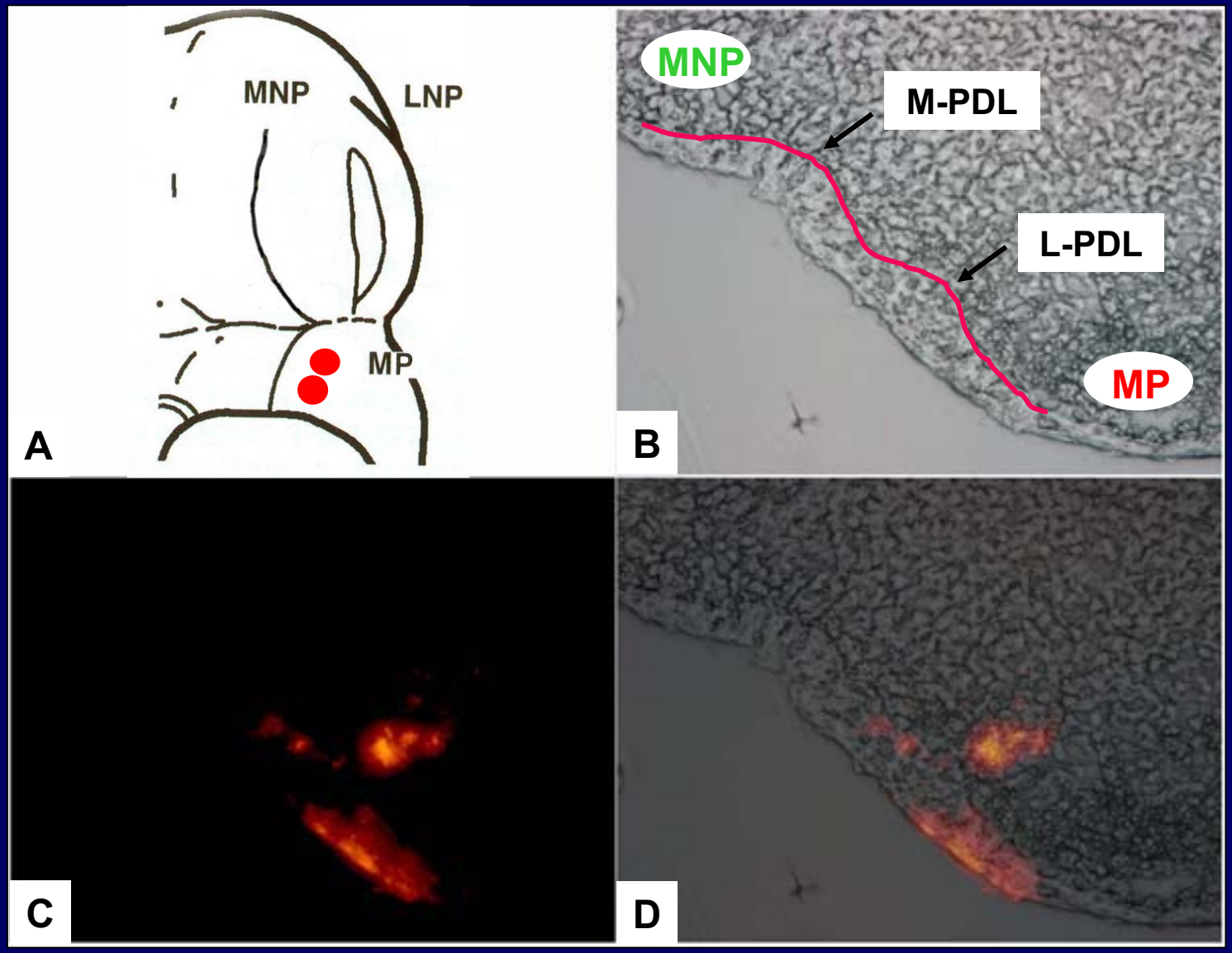




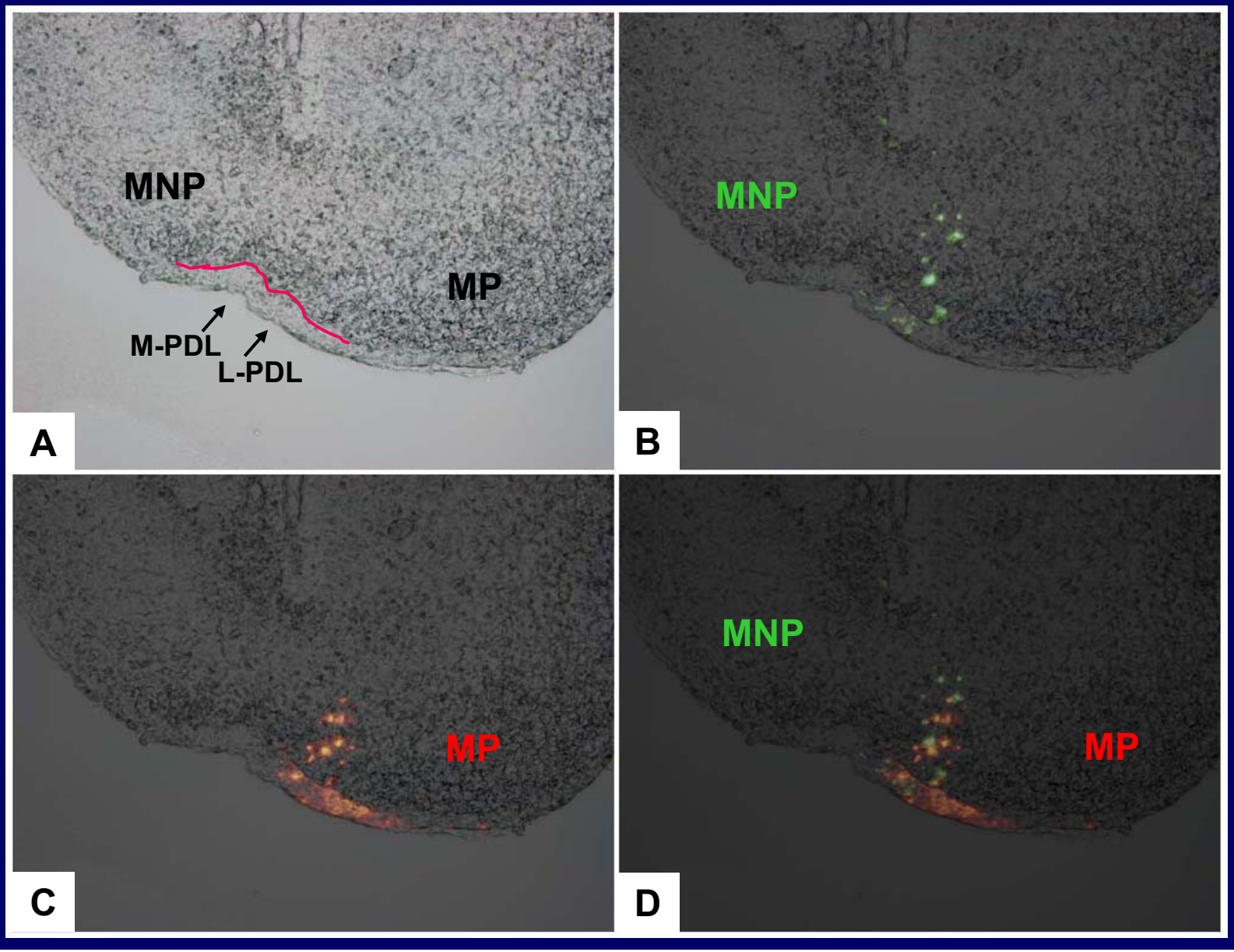
MNP epithelium
DiO labeling

MP epithelium
DiI labeling

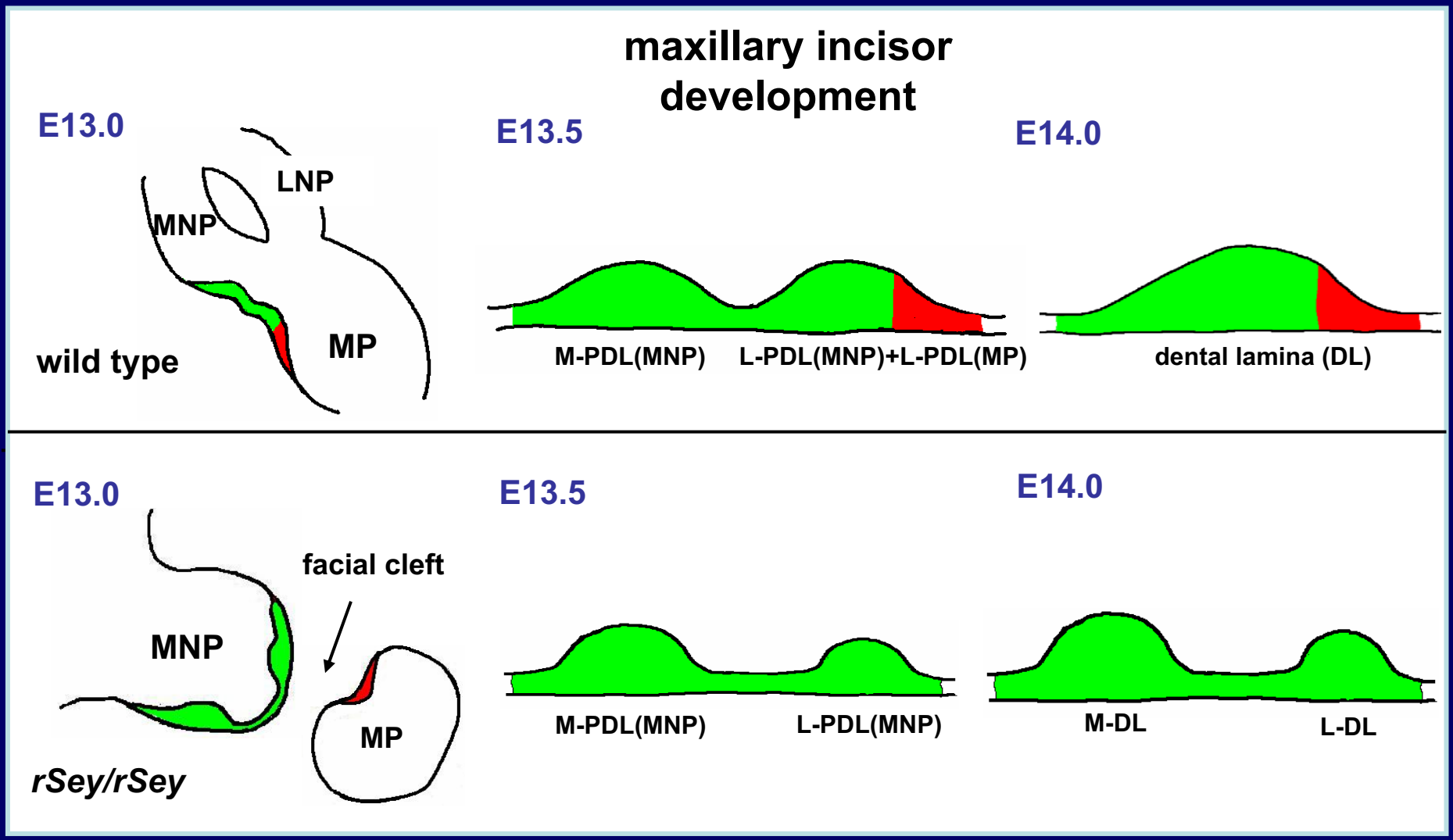
DiI labeling of maxillary process epithelium



DiO and DiI labeling of medial nasal process and maxillary process



Conclusions



This research is supported by the Thailand Research Fund and Grants-in-Aid for Scientific Research from MEXT Japan.

Contribution of maxillary process epithelium in rat maxillary incisor formation

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INTRODUCTION

Mammalian dental formula includes three incisors, one canine, four premolars, and three molars in each dental quadrant. Murid evolution leads to reduction in the number of teeth. It comprises only one incisor separated from three molars by a toothless gap diastema. Classical data suggests that the maxillary incisor corresponds to the middle incisor, while the other two have been lost during murid evolution.

During the early mouse maxillary incisor formation, the several embryonic maxillary incisor placode (epithelial thickening) are identified. Strassburg *et al.* interpreted that there are three maxillary incisor placodes, the middle one develops into functional maxillary incisor and the other two become regressed during early stage of development. However, Peterkova *et al.* described that maxillary incisor dental placode is formed by an assembly of five maxillary incisor dental placodes. Around this period, facial processes, maxillary process (MP), medial nasal process (MNP) and lateral nasal process (LNP) fuse to form the midface. Since maxillary incisor placodes develop in the area of facial process fusion it is suggested that the maxillary process epithelium might participate in the maxillary incisor formation.

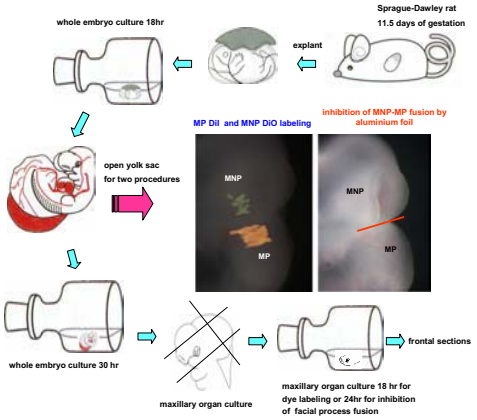
In order to get some insight into maxillary incisor formation and its epithelial origin, we carried out studies on fetuses of wild type SD rat and rat small eye mutant (*rSey*).

MATERIALS AND METHODS

Animals: Fetuses of Sprague-Dawley (SD) rat and *rSey* which was found in the course of breeding SD rats were used. The day on which the vaginal plug was found was designated as embryonic day 0 (=E 0).

Histological analysis: The samples were fixed in Bouin's fixative and embedded in paraffin for hematoxylin and eosin staining.

Epithelial labeling of MNP and MP and inhibition of MNP-MP fusion (see figure below): Whole rat fetuses of E11.5 were cultured followed by maxillary organ culture. During whole embryo culture, the fetus was taken out of the embryonic membrane and subjected to the operation, epithelial labeling with fluorescent dyes or inhibition of MNP-MP fusion aluminium foil insertion.



Epithelial labeling of MNP and MP and inhibition of MNP-MP fusion were carried out with whole embryo culture followed by maxillary organ culture.

RESULTS

MAXILLARY INCISOR DENTAL PLACODE IS COMPOSED OF PRIMARY DENTAL PLACODE (PDP) FUSION DURING THE PROCESS OF FACIAL PROCESS FUSION

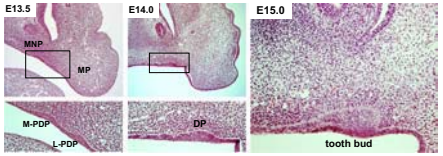
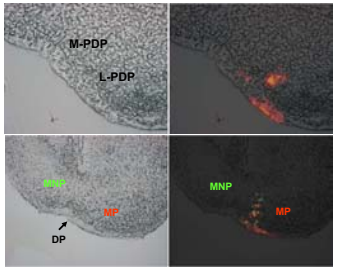


Fig. 1 Histological analysis of wild type maxillary incisor formation shows that at E13.5 MNP and MP have started fusion to make the midface. The thickened primary dental placodes appear aligned around the boundary of MNP and MP, medial and lateral primary dental placodes (M-PDP and L-PDP). They eventually approach each other and fuse to make a single dental placode at E14.0. The dental placode starts invagination at E15.0 indicating early bud stage.

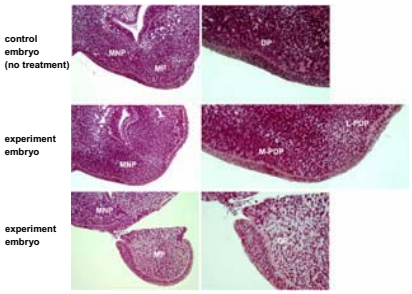
MAXILLARY PROCESS EPITHELIAL CONTRIBUTES TO LATERAL PART OF MAXILLARY INCISOR DENTAL PLACODE



We performed the labeling experiment to investigate the contribution of MP epithelium to maxillary incisor dental placode formation (see Materials and Methods).

Fig. 2 At the end of maxillary culture following whole embryo culture from E11.5, the formation of M-PDP and L-PDP is observed. Fluorescent dye labeling of MP (DiI, red) shows that MP epithelium contributes to the lateral part of the L-PDP. Sometimes we find the formation of a fused single dental placode at the end of culture and double labeling of MNP (DiO, green) and MP (DiI, red) clearly shows that MNP and MP epithelia comprise the dental placode (DP) together.

INHIBITION OF MNP-MP FUSION RESULTS IN SEPARATION OF PDP FORMATION

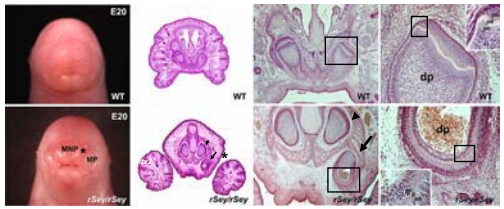


We inhibited the fusion of MNP and MP of the wild type fetus by aluminium foil insertion under the same culture system as Fig. 2. to evaluate the role of MP epithelium in maxillary dental placode formation.

Fig. 3 As shown above, a single dental placode is formed in the explant at the end of culture.

When we disturb the MNP-MP fusion by aluminium foil insertion we observe separated epithelial thickening in a distance in the MNP. We also find the appearance of the epithelial thickening in MP. We show that MP epithelium contributes to the lateral part of maxillary dental placode. Moreover, the defect in facial process fusion leads to develop separated PDP in MNP and MP. These observations suggest that the fusion of facial prominences is required to achieve a single maxillary incisor dental placode.

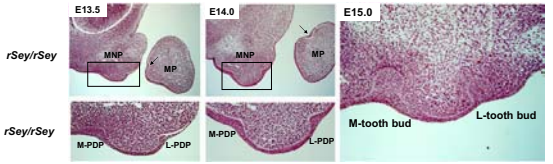
ECTOPIC MAXILLARY INCISOR-LIKE STRUCTURES DEVELOP IN HOMOZYGOUS *rSEY*



We carried out histological observation on maxillary incisor formation in a mutant rat which shows impaired facial process fusion.

Fig.4 Homozygous *rSey* shows facial cleft between MNP and MP (asterisk) due to the lack of LNP. Histological observation on the E20 fetus demonstrates that the maxillary incisor of both wild type and *rSey* homozygotes develops to late bell stage and exhibits amelogenesis and odontogenesis. 25% of homozygous *rSey* shows incisor-like structures (arrow) in addition to its original incisors (arrowhead).

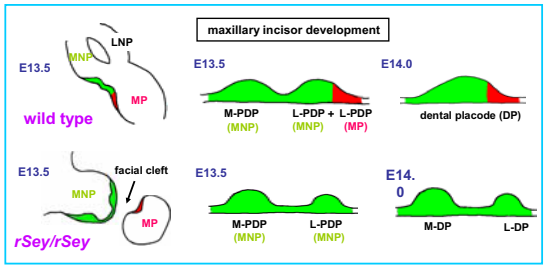
PDP ARE KEPT SEPARATED IN HOMOZYGOUS *rSEY*



We investigated the maxillary incisor development in the *rSey* homozygotes.

Fig.5 Histological study shows that at E13.5 M-PDP and L-PDP develop in MNP. At E14.0 M-PDP and L-PDP are separated in a distance in MNP (compare to wild type in Fig. 1) and the epithelial thickening in MP is still present (arrow). At E15.0 dental placodes in MNP of homozygous *rSey* start invagination separately. The epithelial thickening in MP does not invaginate.

CONCLUSION



Rat maxillary incisor dental lamina contains the epithelial components, in MNP and MP, and they assemble to develop into one functional incisor. The dental placode assembly begins with the incorporation of MP epithelium to L-PDP in MNP at the beginning of MNP-MP fusion. Subsequently M-PDP and L-PDP approach to develop a single maxillary incisor during the process of MNP-MP fusion. Inhibition of facial process fusion resulting in separated PDP, which demonstrates the role of the fusion process in a single maxillary incisor tooth bud formation. These are supported by the study of the homozygous *rSey* which has a facial cleft between MNP and MP. In the mutant separated PDP are developed in each component, M-PDP is adequate to develop an incisor, where as improper L-PDP lacking MP component shows incomplete incisor development.