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学位論文題目	Molecular mechanism of ectodermal patterning in *Xenopus laveis*
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論文内容の要旨

During the process of neurulation in vertebrates, the ectoderm is differentiated into several distinct tissue types including neural plate, epidermis, and neural crest. Recent works, mainly in *Xenopus*, have focused upon molecular mechanisms that can direct ectoderm to neural fates. In the models, neural induction is initially caused by inhibition of BMP activities in ectoderm by secreted BMP antagonists *Noggin*, *Chordin* and *Follisitatin* that are induced in Spemann's organizer. Dorsal ectoderm that has low BMP signaling differentiated into neural plate and ventral ectoderm that has high BMP signaling differentiated into epidermis. However, neural crest induction is more complicated. Neural crest is induced at the border between prospective neural plate and prospective epidermis. One model suggests that an intermediate level of BMP signalling that is generated by the balance between the BMPs and BMP antagonists plays a role in establishing the neural crest fate in *Xenopus*. It was also reported that canonical Wnt signalling and fibroblast growth factor (FGF) signalling enhances neural crest induction, cooperating with BMP antagonists. Several transcriptional factors that able to induced neural crest makers such as *Zic*-related genes were reported. But many of them can also induce neural plate makers and they are expressed in not only neural crest region but also neural plate, so it is complicated to understand how ectoderm diverge to neural plate and neural crest fates. In addition, positioning mechanism of neural-epidermal border and mechanism of neural crest induction are poorly understood.

In order to understand of molecular mechanisms of ectodermal patterning, she first focused on the *in vivo* BMP activity that is a basis of the neural and epidermal induction. She performed visualization of endogenous BMP signaling using an antibody that preferentially recognizes BMP-stimulated form of Smads. BMP signaling system composed of several BMP ligands and receptors, and there are several negative regulators. Regulation of downstream target genes and their roles are complex. Therefore it is quite difficult to evaluate BMP signals by analyzing BMP target genes. A preferred method to evaluate BMP signaling *in situ* is to detect activated forms of intracellular signaling molecules specific for BMP. Smads 1, 5, and 8 are best characterized signaling components of BMP signals and believed to be mediating a major part of BMP activity.

BMP signaling was observed uniformly in early blastula, but was restricted to the ventral side of the embryo from the late blastula stage. At gastrula in ventral ectoderm (prospective epidermis) and ventral mesoderm were stained intensively, and dorsal ectoderm (prospective neural plate) and dorsal mesoderm were less stained. These results support the proposed roles of BMPs as ventralizing factors and anti-neurulizing factors in *Xenopus* embryos. From late gastrula, a gradient of staining becomes evident in the dorsal ectoderm, along the anterior-posterior axis. In early neurula staining was gradually reduced along the DV axis in all three germ layers again indicating the presence of a BMP signaling gradient. During the neural tube forming stages, staining was observed at the dorsal part of neural tube. The location of

staining in dorsal neural tube is also consistent with previous findings showing that BMP family members act as dorsalizing factors of the neural tube. The distinct gradient of staining was not observed in neural tube. So it seems that BMP activity is a short-range signal at least in the dorsal neural tube.

Next she performed the functional analysis of a newly isolated homeobox gene that expressed neural-epidermal border. She identified a novel NK-1 class homeobox gene named *Nbx*. Expression of *Nbx* was detected at neural-epidermal border at neural crest forming stages and partially overlapped with neural crest makers. Nbx has an Eh1 repressor motif and act as a transcriptional suppressor. The gain-of-function analysis showed that *Nbx* suppressed neural plate makers. The inhibition of neural induction by *Nbx* overexpression caused expansion of epidermal maker into the neural plate, and suppressed neural crest induction at early neurula. In later stages, however, enhanced expression of neural crest maker was observed at the injected region. *Nbx* is not likely to be a direct neural crest inducer because *Nbx* could not induce neural crest maker alone in the animal caps. Interestingly, co-injection of dominant negative form of BMP receptor and *Nbx* caused melanophore induction efficiently in animal caps. Overexpression of a dominant-negative form of *Nbx* (VP-*Nbx*-GR) expanded the neural plate markers such as *Sox2* and *Otx2*, and suppressed neural crest marker *Slug*. Therefore, she speculated that *Nbx* may be an essential transcription factor to regulate neural-epidermal border by inhibiting the neural plate fate and direct to neural crest induction.

The pattern of BMP signaling visualized in this work supports the model of neural and epidermal induction by BMP activity. Furthermore, she had demonstrated that a novel homeobox gene *Nbx* may be essential for rigorous regional specification on neural-epidermal border and neural crest induction in the downstream process of pattern formation by BMP activity.

論文の審査結果の要旨

動物の三胚葉のうち、外胚葉はその後の背腹軸に沿ったパターン形成によって将来神経あるいは表皮への分化を運命付けられている。申請者は外胚葉パターン形成における細胞増殖因子BMPの細胞分化運命決定への寄与を探るためにアフリカツメガエル初期胚におけるBMP活性の可視化を試みた。そして、BMP刺激によって核内に移行するリン酸化Smadに対する特異抗体を用いた免疫染色により、発生過程における内在BMP活性を可視化することに成功した。その結果、予定表皮領域に強いBMP活性が認められ、神経化が始まる初期原腸胚においては予定神経領域と予定非神経領域の境界付近に核内Smad量の緩やかな勾配が存在することを明らかにし、BMP活性化領域は予定表皮領域と一致すること、BMP活性が形態形成中心であるオーガナイザーから分泌されるBMPアンタゴニストによって負の制御を受けていることを支持する結果を得た。BMP活性の負の制御によって神経領域が決定され、神経板が形成された後、神経板と表皮との境界領域には神経提(neural crest)細胞が分化する。申請者はこのneural crest細胞の誘導機構を明らかにするためにXenopus EST libraryから神経提細胞に限局して発現する、NK1ファミリーに属する新規homeobox geneを単離した。Nbxと名付けられた同遺伝子は原腸胚後期に発現を開始し、神経胚期には表皮-神経板境界と予定運動神経領域後方とに計４本のストライプ状に発現が観察される。申請者は表皮-神経板境界における発現が予定neural crest領域と一致することから、Nbxはneural crest分化に重要な遺伝子と考え、さらに機能解析を行った。そして、Nbxは転写抑制因子としての機能を持つこと、Nbxを胚で過剰発現させるとneural crest領域の拡大が見られること、Nbx活性を阻害する優性阻害型Nbxを胚に過剰発現させるとSox2やOtx2といった神経板特異的なマーカー遺伝子の発現が誘導され、slugなどneural crest特異的遺伝子が抑制されることを見いだした。さらにBMP活性阻害によって神経化させた細胞にNbxを発現させると神経板マーカー遺伝子の誘導を抑制しneural crestを誘導することを確認した。以上の結果は、Nbxが表皮-神経板領域において神経板、すなわち将来の中枢神経系への運命決定を抑制し、neural crestへの分化を促進する機能を持つこと示すものであり、学術的な価値も高く、学位論文として相応しい研究内容であると判断された。

学位論文として提出された研究結果についての口頭発表を行った後、審査委員が論文内容について試問した。さらに、申請者の関連研究分野の一般知識およびその背景となる基礎知識についても口頭試問により審査した。これらの試問に対する申請者の応答はいずれも的確であったことから、関連領域に十分な知識を有しているものと思われる。また、提出された論文は英語で書かれており、二本の論文がすでに国際誌に発表されていることから、英語の能力についても適正であると考える。審査委員会はこれらの結果を総合的に判断し、申請者の学力は学位取得に十分値するものと判断した。