Induction of the epibranclial placodes

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SUMMARY

The cranial sensory ganglia, in contrast to those of the trunk, have a dual embryonic origin arising from both neurogenic placodes and neural crest. Neurogenic placodes are focal thickenings of ectoderm, found exclusively in the head of vertebrate embryos. These structures can be split into two groups based on the positions that they occupy within the embryo, dorsolateral and epibranclial. The dorsolateral placodes develop alongside the central nervous system, while the epibranclial placodes are located close to the top of the clefts between the branchial arches. Importantly, previous studies have shown that the neurogenic placodes form under the influence of the surrounding cranial tissues. In this paper, we have analysed the nature of the inductive signal underlying the formation of the epibranclial placodes. We find that epibranclial placodes do not require neural crest for their induction, but rather that it is the pharyngeal endoderm that is the source of the inductive signal. We also find that, while cranial ectoderm is competent to respond to this inductive signal, trunk ectoderm is not. We have further identified the signalling molecule Bmp7 as the mediator of this inductive interaction. This molecule is expressed in a manner consistent with it playing such a role and, when added to ectoderm explants, it will promote the formation of epibranclial neuronal cells. Moreover, the Bmp7 antagonist follistatin will block the ability of pharyngeal endoderm to induce placodal neuronal cells, demonstrating that Bmp7 is required for this inductive interaction. This work answers the long standing question regarding the induction of the epibranclial placodes, and represents the first elucidation of an inductive mechanism, and a molecular effector, underlying the formation of any primary sensory neurons in higher vertebrates.

Key words: Epibranclial, Placode, Induction, Sensory Neurons, Pharyngeal endoderm, Phox2a, BMP7, Chick

INTRODUCTION

While the primary sensory neurons of the trunk are derived exclusively from the neural crest, those in the head have a dual embryonic origin (Ayer Le Lievre and Le Douarin, 1982; D’Amico-Martel and Noden, 1983). A proportion is derived from neural crest, but the majority arises from neurogenic placodes: focal thickenings of the embryonic ectoderm found exclusively in the head of vertebrate embryos (Webb and Noden, 1993). The importance of this dual embryonic origin lies in the role of the neurogenic placodes in the development of the cranial peripheral nervous system as a whole. The placodal neurons differentiate early and establish both peripheral and central projections before the neural-crest-derived neurons initiate axonogenesis (Webb and Noden, 1993). Furthermore, in their absence the neural-crest-derived ganglia fail to establish normal peripheral projections and motor neurons within the brain fail to undergo their normal migratory reorganisation (Moody and Heaton, 1983).

The neurogenic placodes form in stereotypical positions in all vertebrates and they can be split into two groups, dorsolateral and epibranclial (Fig. 1a). The dorsolateral placodes, the vestibular and trigeminal, develop close to the CNS. In contrast, the epibranclial placodes, the geniculate, petrosal and nodose, lie at the dorsoanterior margin of the branchial arches. The fact that the placodes arise in stereotyped positions is presumably a reflection of the localised inductive interaction(s) that underlie their formation and, interestingly, transplantation studies have shown that the neurogenic placodes, both dorsolateral and epibranclial, form under the influences of the surrounding cranial tissues (Vogel and Davies, 1993). However, there is scant information regarding the source, and nature, of such inductive signals, particularly with respect to the epibranclial placodes.

The epibranclial placodes develop at the top of the branchial clefts in close proximity to two embryonic tissues, the cranial neural crest and the pharyngeal endoderm, both of which have been suggested as placodal inducers (Fig. 1b) (Webb and Noden, 1993). However, there has never been compelling experimental evidence demonstrating a direct role for either. Although previous workers noted that epibranclial placodes could form after crest extirpation, suggesting a primary role for
the pharyngeal endoderm, these experiments were fraught with
difficulty, as it was impossible to ensure that there had been no
neural crest regeneration. Consequently, the results from such
experiments were equivocal. A central role for the pharyngeal
endoderm in the induction of these placodes was also
dismissed on the grounds that the trigeminal placode does not
form in close proximity to this tissue (Webb and Noden, 1993).
Implicit in this criticism was the assumption that this placode
would share a common inductive mechanism with the
epibranial placodes.

In this study, we have readdressed the question of the how
the epibranchial placodes are induced. We have reanalysed
the effects of crest ablation on the development of the epibranchial
placodes using new molecular markers and find that they can
form in the absence of neural crest. Furthermore, we show,
using tissue recombination experiments, that pharyngeal
endoderm is the source of the inductive signal. Our tissue
recombination experiments also demonstrate that while cranial
ectoderm is competent to respond to this signal trunk ectoderm
is not. We show that the signalling molecule, Bmp7, is
expressed at precisely those sites where the pharyngeal
endoderm contacts the ectoderm and that the addition of Bmp7
protein to cultures of isolated cranial ectoderm results in the
production of epibranchial neuronal cells. Finally, we show
that the Bmp7 antagonist, follistatin, causes a reduction in the
number of neuronal cells induced in the cranial ectoderm by
the pharyngeal endoderm showing that Bmp7 is required for
this inductive event.

MATERIALS AND METHODS

Embryo manipulations
Fertile Rhode Island Red hens’ eggs (Needle Farm, Enfield, England)
were incubated at 38°C in a humidified atmosphere. Hindbrain
ablations were carried out using a tungsten needle to remove the entire
neural tube from the level of the midbrain to the first somite of chick
embryos at Hamburger-Hamilton (HH) stage 9 (Hamburger and
Hamilton, 1951). Embryos were re-incubated for 24 hours and fixed
in 4% paraformaldehyde for analysis.

In situ hybridisation
Whole-mount in situ hybridisation using digoxigenin probes for Bmp7
(Houston et al., 1994), Phox2a and Dlx2 was carried out as described
by Henrique et al. (1995). The protocol was modified for double in
situ using digoxigenin Phox2a probe and fluorescein Dlx2 probe such
that the digoxigenin probe was developed as described (Henrique et
al., 1995), the embryos refixed and the fluorescein probe subsequently
detected using anti-FITC antibody (1:8000; Boehringer) with fast-red
colour substrate (Sigma).

Immunohistochemistry
Whole-mount immunohistochemistry was carried out as described by
Storey et al. (1992) using the anti-neurofilament medium chain
antibody (1:10000; clone RMO-270, Zymed).

Explant cultures
Cranial ectoderm explants were taken from the region lateral to
rhombomere 3 of embryos at HH stage 9 (C in Fig. 3c), while trunk
ectoderm explants were taken from over somites of the trunk region
(somite 7 onwards) (T in Fig. 3c), or from regions lateral to the
presegmental plate of embryos at HH stage 10. For recombination
experiments, endoderm was taken from the forming branchial arch
region of embryos at around HH stage 11. All tissues were treated
with dispase (1 mg/ml in L-15 media) for 10 to 15 minutes prior to
final dissection to ensure the removal of all underlying tissues. The
explants were isolated using flame-sharpened tungsten needles and
collected into L15 supplemented with 1% calf serum. Explants were
immobilised in collagen (Cellon), one explant per well and cultured
for 24 hours in F12 supplemented with N-2 (Gibco). Where Bmp7
treatment is described, recombinant Bmp7 (Genetics Institute,
Boston) was added to both the collagen and medium at the appropriate
concentration (1 or 10 ng/ml).

For recombination experiments including follistatin beads, Affi-Gel
Blue beads mesh 100-200, 75-150 μm (BioRad) were washed in two
changes of phosphate-buffered saline (PBS) and soaked in follistatin
(1 mg/ml) (kindly donated by Dr Ketan Patel, Reading) for 1 hour at
room temperature, control beads were soaked in 0.1% serum in PBS.
Beads were used to deliver the follistatin as this method allows one
to establish a highly localised dose of this antagonist. The beads were
moved into place, directly abutting the recombinations, with a flame-
sharpened tungsten needle.

Analysis of the cultures by immunohistochemistry was carried out as
described for whole-mount staining. For in situ hybridisation, only
the initial washes of the protocol described above were modified to
include 1% Tween.

Isolation of chick Phox2a clone
The chick Phox2a clone was isolated by RT-PCR using degenerate
primers, corresponding to the amino-acid sequences flanking residues
16 to 142 of the mouse gene (Valarche et al., 1993).

Isolation of chick Dlx2 clone
The chick Dlx2 cDNA clone was identified by low-stringency
hybridisation of an E10.5 chick cDNA library with a probe made from
the mouse Dlx2 cDNA (Bulfone et al., 1993). Sequencing of the clone
revealed that the chick Dlx2 homeodomain was identical to that of
mouse Dlx2.

RESULTS

Molecular markers used in this study
One of the most versatile markers of the neurogenic placodes
is neurofilament and these structures can be readily visualised
through staining with anti-neurofilament antibodies. Fig. 1c
shows a side view of a stage 16 chick embryo whole mount
stained with an anti-neurofilament antibody. The
branchial clefts (Fig. 1a,c), while the epibranchial placodes can be located at, and slightly caudal of, the
branchial clefts (Fig. 1a,c). In section (Fig. 1d), the NFM
staining can been seen to reveal the presence of neuronal cells
within the embryonic epidermal ectoderm. Fig. 1d, which
details the most rostral epibranchial placode, the geniculate,
also shows neuronal cells migrating away from the placode
towards the site of ganglion formation. Another marker that
we have exploited is the homeobox-containing gene Phox2a
(Tiveron et al., 1996). The expression of this gene is of
particular interest as it does not label the dorsolateral placodes,
but specifically labels the epibranchial placodes and is
necessary for their proper development (Morin et al., 1997).
Fig. 1e shows the localised expression of Phox2a in the
forming geniculate, petrosal and nodose ganglia, which are
located at the dorsal anterior margin of their respective arches.
In section (Fig. 1f), Phox2a-positive cells can be found within
the placodal ectoderm, as well as migrating away towards the
site of the forming ganglion. The last marker that we have
employed in this analysis is another homeobox gene, \textit{Dlx2}, which labels the cranial neural crest. This gene is expressed strongly in the developing branchial arches and specifically in their neural crest component (Fig. 1g,h). This is apparent in section (Fig. 1h), where the \textit{Dlx2}-expressing cells are found lining the surface of the arch, where the neural crest cells are located, and not in the core of the arches, which is mesodermal.

**Epibranchial placodes develop in the absence of neural crest**

It has been suggested previously that the epibranchial placodes could be induced by the neural crest, which comes to underlie these structures (Fig. 1b). To analyse the role of this embryonic tissue in the development of the epibranchial placodes, we have assessed the impact of ablating the crest upon the formation of these structures. To remove the neural crest from the pharyngeal region, the neural tube from the level of the midbrain to the first somite was extirpated at stage 9, which is prior to the emigration of crest from this level of the neuraxis (Tosney, 1982; Lumsden et al., 1991). The embryos were then re-incubated for 24 hours, and in the first instance analysed through NF-M staining. Fig. 2a shows the result of such a manipulation \((n=10)\). It is clear from the neurofilament staining that much of the hindbrain has been removed. However, it is also apparent that this drastic surgery did not affect the development of the epibranchial placodes. In Fig. 2a, the geniculate placode can be seen to be forming in its usual position: ventral to the otocyst and at the dorsal margin of the second branchial arch. Fig. 2b shows a section through such an embryo, and the epibranchial neurons are evident, even though the neural tube at this axial level has been ablated. One problem with this type of analysis, and one that dogged similar experiments previously, is that one cannot be sure that the neural crest has not regenerated. This is an important issue, as it is possible that the placodes are still in contact with crest even after such an extensive ablation. To address this point, we analysed the ablated embryos for the presence of both epibranchial placodes, through \textit{Phox2a} expression, and neural crest cells, with \textit{Dlx2}, using double in situ hybridisation. \textit{Dlx2} is a particularly useful crest marker as, in contrast to genes such as \textit{slug}, it still labels the crest once it has migrated into the branchial arches (Nieto et al., 1994). The results are shown in Fig. 2c and d and, importantly, we find that epibranchial placodes do develop free of neural crest \((n=12)\). Focussing on the geniculate placode, which is marked by an asterisk in Fig. 2c, it is apparent that, while \textit{Phox2a} expression highlights the presence of this ganglion, the lack of \textit{Dlx2} staining in the associated branchial arch demonstrates that this placode has developed free from the influence of neural crest. This is also shown in section in Fig. 2d, where again the \textit{Phox2a}-positive cells of the epibranchial placode can be seen to have developed without associated neural crest.
Pharyngeal endoderm can induce neurogenic placodes

The hindbrain ablations demonstrated that the neural crest was not necessary for placodal induction, but also suggested the pharyngeal endoderm as the likely source of the inducer (arrows in Fig. 2b,d). An indication of this came from the fact that, in the ablated embryos, the neuronal cells of the epibranchial placode could be seen to be developing immediately adjacent to the pharyngeal endoderm (see Fig. 2b,d). To test this idea more directly, we used in vitro tissue recombination experiments in collagen gels to assess the ability of the pharyngeal endoderm to induce neurons in cranial epidermal ectoderm. Cranial ectoderm was isolated from the region lateral to the forming rhombomere 3 in stage 9 embryos (Fig. 3), prior to the formation of any neurogenic placodes and also prior to neural crest emigration. We also chose to use ectoderm from this region, as it is not fated to be placodal (D’Amico-Martel and Noden, 1983). We found that this ectoderm when cultured alone for 24 hours produces very few neuronal cells (Fig. 3a). By contrast, if this ectoderm is co-cultured with pharyngeal endoderm a large number of neuronal cells form (Fig. 3b). Counts of NF-M-positive cells within the ectoderm show that this recombination resulted in a 4-fold increase in neuronal number (Fig. 3d). We also used the neural crest marker, HNK-1, to analyse the endodermal/ectodermal recombinations for the presence of these cells, and in all cases analysed we did not detect neural crest in these co-cultures. Thus, these in vitro experiments demonstrate that pharyngeal endoderm alone is able to induce the formation of placodal neuronal cells.

Trunk ectoderm is unresponsive to pharyngeal endoderm

To address whether the pharyngeal endoderm was capable of inducing neurons in ectoderm from other regions, we repeated the recombination experiments using ectoderm from the trunk, which is that region caudal of the fifth somite. We tested the ability of ectoderm isolated from over somites, caudal to somite 7, from stage 10+ embryos, to respond to pharyngeal endoderm. In all of the cases that we examined, NF-M-positive cells were never seen in this ectoderm whether cultured alone (n=8) or co-cultured with pharyngeal endoderm (n=14; Fig. 3e,f). We further analysed the ability of ectoderm from other regions of the trunk to respond to the pharyngeal endoderm. Specifically, we tested whether ectoderm from the region lateral to the segmental plate could be induced by the pharyngeal endoderm. The reason for also probing the competence of this ectoderm was that grafting experiments had suggested that it could form placodal neurons (Vogel and Davies, 1993). However, we found no evidence of NF-M-positive cells in this ectoderm either when cultured alone or in combination with the pharyngeal endoderm.

Bmp 7 mediates the induction of epibranchial neurons by the pharyngeal endoderm

It has been shown for the induction of the sympathetic ganglia that members of the Bone Morphogenetic Protein (Bmp) family of signalling molecules can stimulate Phox2a expression (Reissmann et al., 1996). We therefore analysed the expression patterns of a number of Bmps in the developing pharyngeal endoderm to determine if these molecules could be playing a similar role in the developing epibranchial placodes. While Bmp2 and Bmp4 were not expressed in a spatial and temporal manner consistent with them playing a role in placodal induction, we found that one member of this family, Bmp7, is expressed in a manner consistent with its involvement in the induction of the epibranchial placodes. Interestingly, Bmp7 is expressed at elevated levels in the pharyngeal pouches and not throughout the pharyngeal endoderm (Fig. 4a,b). These structures are the sites where the endoderm and ectoderm intimately contact each other, prior to the formation of the branchial arches. The expression of this gene in the first and second pharyngeal pouches, lying
between arches 1 and 2, and 2 and 3 respectively is shown in Fig. 4a. In section, Bmp7 expression can be seen to be restricted to the site of endodermal/ectodermal contact (arrowhead in Fig. 4b).

To assess the role of Bmp7 in placodal induction, we have used explant cultures. As we have already shown, non-neural cranial ectoderm cultured alone produces very few neuronal cells (Fig. 4c). However, the addition of Bmp7 to the cultures, at concentrations of 1 or 10 ng/ml, greatly increases the number of neuronal cells found in the explants (Fig. 4d). Counts of NF-M-positive cells within the ectoderm show that treatment with either 1 or 10 ng/ml resulted in a similar 2.5-fold increase in neuronal number (Fig. 4e). That Bmp7 can elicit such a response at concentrations as low as 1 ng/ml demonstrates the efficacy of this molecule and that it works at physiological concentrations. In contrast, other members of the TGF-β superfamily, such as activin and TGF-β2, were unable to induce neurogenesis in the explant at any of the concentrations tested (data not shown). While these culture experiments show us that Bmp7 can induce the formation of neuronal cells in epidermal ectoderm, the neurofilament staining does not inform us as to the type of cell induced. To address this question, we tested whether Bmp7 can induce Phox2a expression, and consequently epibranchial neurons. Consistent with the other results presented in this study, cranial ectoderm cultured in isolation does not form Phox2a-positive cells (Fig. 4f). However, when 10 ng/ml Bmp7 is added to the cultures, numerous Phox2a-positive neuronal cells are observed in the ectodermal explants (Fig. 4g). This demonstrates that Bmp7 not only induces the formation of neuronal cells in epidermal ectoderm but specifically of epibranchial neuronal cells. Taken with its expression pattern, these data strongly suggest that Bmp7 is the signalling molecule, secreted by the pharyngeal endoderm, that mediates the induction of the epibranchial neurogenic placodes.

**Bmp7 is required for placodal induction by pharyngeal endoderm**

To explore whether Bmp7 is actually required for the induction of epibranchial placodes by the pharyngeal endoderm, we analysed the effect of applying an antagonist of this molecule, follistatin, to ectoderm/endoderm recombinations. Follistatin was first identified as an activin inhibitor, but it has also recently been shown to inhibit Bmp7 but not other members of the Bmp family (Liem et al., 1997). Co-culture of cranial ectoderm with pharyngeal endoderm and control beads did not affect the ability of the endoderm to induce the formation of many NF-M cells in the ectoderm (Fig. 5a). In the presence of follistatin-coated beads, however, the number of neuronal cells in the ectoderm was greatly reduced (Fig. 5b). We found that effects were greatest when the beads were located in close proximity to the interface between the tissues; in these cases, counts of NF-M-positive cells showed a 2-fold decrease in the number of neurons in the ectoderm explant (Fig. 5c). We believe this effect of follistatin is a result of it acting to inhibit Bmp7 and not due to the inhibition of activins, as they are not expressed in the pharyngeal endoderm at this time in development (data not shown; Jonathan Cooke, personal communication). This result demonstrates that Bmp7 is required for the induction of the epibranchial neurogenic placodes.
DISCUSSION

In this study, we have identified both the source, and an effector, of the inductive signal underlying the development of the epibranchial placodes. Our evidence that the pharyngeal endoderm induces the epibranchial placodes comes from both in vivo and in vitro experiments. In vivo, we have demonstrated that the epibranchial placodes will form free of the influence of neural crest and, when they do form, it is always in close proximity to the endoderm. We have also obtained direct evidence that pharyngeal endoderm can induce the formation of epibranchial placodes through tissue recombination experiments. If cranial ectoderm is cultured in isolation, few neuronal cells form. However, if this tissue is recombined in vitro with pharyngeal endoderm, then neurogenesis is induced in the ectoderm. By contrast, trunk ectoderm will not elicit this response when cultured with pharyngeal endoderm. We further present strong evidence that the inductive signal is mediated by Bmp7. This gene is expressed in a spatiotemporal manner consistent with such a role, and when added to cultures of cranial ectoderm it induces the formation of epibranchial neuronal cells in these explants. Furthermore, inhibition of Bmp7 function by the inclusion of follistatin beads in the recombination experiments reduces the number of neuronal cells induced within the ectoderm.

The results presented here provide the first direct evidence that the pharyngeal endoderm is the source of an inductive signal mediating the formation of the epibranchial placodes. As already mentioned, this role for the pharyngeal endoderm has been previously dismissed, on the grounds that not all of the neurogenic placodes, particularly the trigeminal, form in close proximity to this tissue (Webb and Noden, 1993). Implicit in this criticism was the belief that all neurogenic placodes would share a common developmental pathway. However, these results, and those emerging from a number of other sources, strengthen the idea that the dorsolateral and epibranchial placodes use different modes of development. While we have shown that the epibranchial placodes are induced by the pharyngeal endoderm; more recently, it has been suggested that the opthalmic lobe of the trigeminal is induced by a signal emanating from the CNS, alongside which it develops (Stark et al., 1997). The fact that the epibranchial and dorsolateral placodes use distinct developmental pathways is also emphasised by the results from the mutational analysis of the mammalian neurogenins. These studies have shown that the dorsolateral placodes depend upon neurogenin1 function for their development, while the epibranchial placodes utilise the related gene neurogenin2 (Fode et al., 1998; Ma et al., 1998).

We have also found that, while cranial ectoderm is competent to respond to the inducing signal emanating from the pharyngeal endoderm in vitro, trunk ectoderm is not. This is in contrast to the results of Vogel and Davies who found that when trunk ectoderm was grafted into the position of the presumptive nodose placode then it could contribute neurons to the distal Xth ganglion. Yet, while both sets of experiments tested ectoderm from the same embryonic region, lateral to the segmental plate, they differed in that we analysed the competence of this tissue to respond in culture while Vogel and Davies tested its ability in vivo. Interestingly, this may reflect the fact that there are additional factors in the cranial environment that can render trunk ectoderm competent to

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**Fig. 4.** The role of Bmp7 in epibranchial placode induction. (a) In situ hybridisation with Bmp7 at stage 13 shows that expression is not present throughout the endoderm but is elevated within the pharyngeal pouches, labelled 1 and 2. Transverse section at the level of the prospective geniculate placode (b) shows Bmp7 expression restricted to the pharyngeal endoderm at the point where it contacts the ectoderm (arrowhead). Cranial ectoderm cultured alone shows very little staining with either NF-M or Phox-2a (c,f). Addition of rBmp7 to the cultures at 10 ng/ml promotes the formation of NF-M-positive cells (d). (e) Graph of numbers of NF-M-positive cells following Bmp7 treatment of cranial ectoderm (untreated: 10±1.7 cells per explant n=16; treated: 1 ng, 27±3.3 cells per explant n=17; 10 ng, 25±2.8 neurons per explant n=19). Treatment with Bmp7 also increases the expression of Phox-2a within the ectoderm explant (g).
stimulating the differentiation of neural crest cells into
(Liem et al., 1995). These same molecules are also secreted by
the pharyngeal endoderm. However, this is currently untestable
as there are no markers of epibranchial placodes which are not
also neuronal markers.

Our results also have an importance outside an
understanding of how epibranchial placodes are formed, as
they represent the first definition of an inductive interaction
underlying the formation of any primary sensory neurons in
higher vertebrates. For example, while we know how dorsal
root ganglia become segmentally organised (Keynes and Stern,
1984), we do not know how these cell types are induced or the
molecules that mediate this event. Similarly, little is known
about the formation of other either crest or placodally derived
sensory ganglia, although in the case of the dorsolateral
placodes there is at least some definition of the tissues that are
likely to be involved.

In this study, we have also identified a role for the molecule,
Bmp7, in the induction of epibranchial placodes. Bmp7 is
expressed at elevated levels in the pharyngeal pouches, and not
in other endodermal regions, and when recombinant Bmp7 protein is added to cultures of cranial ectoderm it will
specifically induce the formation of epibranchial neurons in
ectodermal explants in culture. This role is further reinforced
by the reduction in the number of neurons seen in cranial
ectoderm/pharyngeal endoderm recombinations carried out in
the presence of the Bmp7 antagonist, follistatin. It is intriguing
that Bmp7 is also expressed in the ectoderm overlying the
pharyngeal pouches, and this may indicate that the expression
of this gene is in fact induced in this outer layer through contact
with the pharyngeal endoderm.

It is also noteworthy that Bmps have also been implicated in
the development of other components of the peripheral nervous
system. The neural crest itself is induced by an interaction
between the neural plate and the epidermal ectoderm, and this
event has been shown to be mediated by Bmp4 and Bmp7
(Liem et al., 1995). These same molecules are also secreted by
the dorsal aorta and have been found to be involved in
stimulating the differentiation of neural crest cells into
sympathetic ganglion neurons which form alongside this
structure (Reissmann et al., 1996). This particular case is also
of further interest because, in this instance, as in epibranchial
placode formation, Bmps are acting to stimulate the production
of Phox2a-expressing cells. Lastly, these two molecules, along with other TGF-βs, also act to induce the formation of the
secondary sensory neurons of the dorsal spinal cord (Liem et
al., 1997). However, although these results demonstrate that
Bmp7 plays a role in the development of the nervous system,
no such defects were found in the Bmp7 mutant animals. The
phenotypes that were found to be associated with these animals
related to eye, skeletal and kidney defects (Dudley et al., 1995;
Luo et al., 1995). The lack of neural defects in these animals
may have been due to the fact that the nervous tissues were not
closely analysed, or also because many of these neural cell
types would seem to form under the action of more than one
Bmp.

Finally, it is intriguing that it is the pharyngeal endoderm
that will induce the epibranchial placodes. An intrinsic feature
of the pharyngeal endoderm is its ability to generate taste buds
(Northcutt and Barlow, 1998) and, interestingly, one functional
difference between the epibranchial ganglia and the
dorsolateral ganglia is that gustatory sensory neurons are found
exclusively in the epibranchials (Ariens Kappers et al., 1960).
The induction of the epibranchial ganglia may therefore
represent a situation wherein an embryonic tissue is inducing
its own afferent innervation.

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of cranial sensory ganglia and the potentialities of their component cells


