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The ectodermal placodes: a dysfunctional family

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The ectodermal placodes are focal thickenings of the cranial embryonic ectoderm that contribute extensively to the cranial sensory systems of the vertebrates. The ectodermal placodes have long been thought of as representing a coherent group, which share a developmental and evolutionary history. However, it is now becoming clear that there are substantial differences between the placodes with respect to their early development, their induction and their evolution. Indeed, it is now hard to consider the ectodermal placodes as a single entity. Rather, they fall into a number of distinct classes and it is within each of these that the members share a common development and evolution.

Keywords: ectodermal placodes; head development; induction; evolution; special sense

1. INTRODUCTION

The term 'ectodermal placode' has been in use for well over a century (Von Kupffer 1891) and its definition can be readily found in textbooks and primary articles. A simple definition of the ectodermal placodes is as focal thickenings of the ectoderm found in the embryonic vertebrate head, which contribute extensively to the special sense organs of the head. The ectodermal placodes also have an added significance in that they have long been thought to be vertebrate specific and to have evolved with this subphylum (Gans & Northcutt 1983). Similarly, the placodes seem to show a number of common developmental features (Jacobson 1966). However, any discussion of the ectodermal placodes invariably breaks down into a categorization dealing with the specifics of each placode: their induction, their development and the derivatives they produce. Here we argue that this tendency to work 'list-fashion' through the ectodermal placodes reflects the fact that this term does not describe a coherent group of structures. Rather, we suggest that the structures lumped together under the umbrella of 'ectodermal placode' are best dealt with in separate groups, within which each member shares a common development, evolution and later function.

2. THE ECTODERMAL PLACODES

There are a number of ectodermal placodes and importantly they form in stereotypical positions in all vertebrates (figure 1). The two most anterior placodes derive from the anterior neural ridge: the adenohypophyseal placode arises from the medial portion and the olfactory from slightly more lateral regions (Couly & Le Douarin 1985; Jacobson 1966; Verwoerd & Van Oostrom 1979). However, the spatial relationship between these two structures alters during embryogenesis as subsequent morphogenetic movements cause the adenohypophyseal placode to lie ventral to the forebrain and the olfactory placode to occupy a more medial frontal position. A further placode associated with the developing forebrain is the lens placode, which forms opposite the optic vesicles (Couly & Le Douarin 1990; Jacobson 1966; Verwoerd & Van Oostrom 1979). Caudal of these placodes are the ophthalmic and maxillomandibular trigeminal placodes, which emerge at the level of the midbrain–hindbrain junction (D'Amico-Martel & Noden 1983; Northcutt & Brandle 1995; Verwoerd & Van Oostrom 1979). Sitting more caudal again, opposite the central hindbrain, is the very prominent otic placode (Jacobson 1966; D'Amico-Martel & Noden 1983; Northcutt & Brandle 1995; Verwoerd & Van Oostrom 1979) and, unique to aquatic anamniotes, the lateral line placodes (Northcutt & Brandle 1995; Northcutt *et al*. 1994). The number of lateral line placodes can vary but it has been suggested that ancestrally there were six: three post-otic and three pre-otic (Northcutt 1997). The final group of ectodermal placodes, the epibranchial placodes, arise close to the tips of the clefts between the pharyngeal arches (D'Amico-Martel & Noden 1983; Northcutt & Brandle 1995; Verwoerd & Van Oostrom 1979).

3. DERIVATIVES OF THE ECTODERMAL PLACODES

When considered *en masse* it is apparent that the ectodermal placodes generate a vast array of derivatives (see table 1). Some, the adenohypophyseal, olfactory and otic placodes, generate complex organs containing several cell types: the adenohypophyseal placode generates the anterior pituitary (Couly & Le Douarin 1985), the olfactory gives rise to the olfactory sensory epithelia, receptor cells, gonadotrophin-releasing hormone (GnRH) neuroendocrine cells and glia (Couly & Le Douarin 1985), and the otic gives rise to the sensory neurons, specialized epithelia and receptors of the inner ear (D'Amico-Martel & Noden 1983; Torres & Giraldez 1998). Similarly, the lateral line

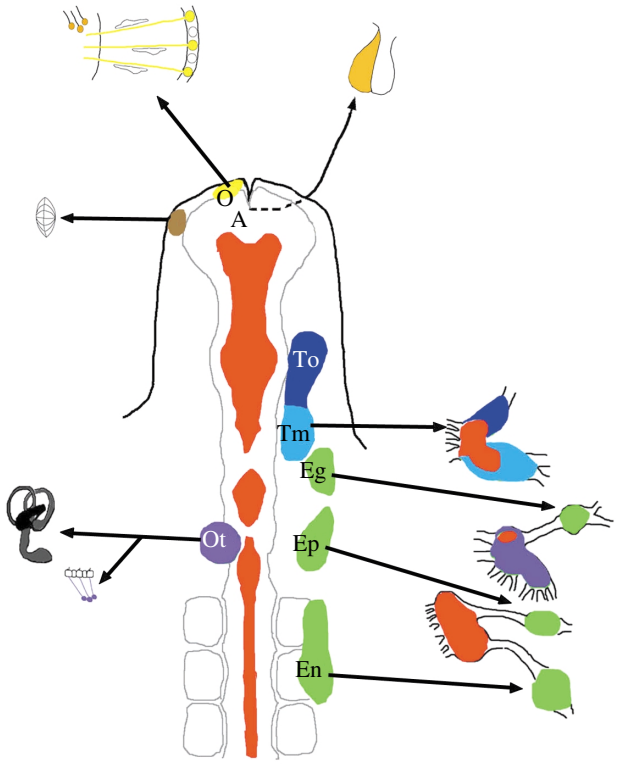

Figure 1. Schematic of fate map and derivative structures of the ectodermal placodes in the chick. The olfactory placode (yellow) gives rise to the olfactory sensory epithelium: receptor cells (yellow), supporting cells, glia and neuroendocrine cells (orange). The adenohypophysis (orange) arises from a placode lying ventral to the forebrain and contains a range of endocrine and supporting cells. The lens placode (brown) produces the lens of the eye. The otic placode (purple) gives rise to the inner ear, associated receptor cells and sensory neurons. The majority of the sensory neurons of the cranial ganglia are placodal: ophthalmic (dark blue) and maxillomandibular (light blue) lobes of the trigeminal ganglia; vestibuloacoustic (purple); epibranchial placodes—geniculate, petrosal and nodose (green). Neural crest and neural-crest-derived sensory neurons are shown in red.

placodes, of fish and amphibia, generate both receptor cells and sensory neurons (Northcutt 1997). Others, however, have somewhat simpler fates. The epibranchial placodes, the geniculate, petrosal and nodose, give rise to primary sensory neurons that convey viscerosensory and gustatory sensation from the oropharyngeal cavity (D'Amico-Martel & Noden 1983), while trigeminal placodes, the ophthalmic and the maxillomandibular, exclusively produce somatosensory neurons (D'Amico-Martel & Noden 1983). Finally, there is the lens placode, which simply produces an epithelial structure, the lens of the eye (Jacobson 1966).

4. INDUCTION OF THE ECTODERMAL PLACODES

Although, it has always been clear that the ectodermal placodes arise at distinct sites in the embryo, and eventually produce distinct derivatives that fulfil quite different functions, it has for a long time been believed that the ectodermal placodes all shared a common early development. Obviously, one manifestation of this is the fact that they are all evident as focal thickenings of the ectoderm. More than that, it has previously been proposed that the inductive events that lead to the formation of the ectodermal placodes share a number of features (Jacobson 1966). The first step is the generation of a common placodal state, with individual placodes then forming through the action of specific multiple serial inducers. For example, the induction of both the lens and olfactory placodes have been shown to involve signals from the mesoderm and the endoderm, as well as from the forebrain. Similarly, the otic placode was found to require signals from the mesoderm and the central nervous system (CNS), although in this instance from the hindbrain.

Many early studies on placode induction focused on the olfactory, lens and otic placodes for the simple reason that these placodes generate readily discernible structures: noses, lenses and ears. As such these experiments did not actually focus on the induction of the placodes themselves but rather on the signals that are necessary to promote the formation of these complex, more developed structures. Obviously, in each case, be it the generation of a nose, lens or ear, the appropriate placode must have been induced, but, in the absence of early placodal markers, these workers were not able to assay for direct specific placodal inducers: ones which did not necessarily promote development beyond the point of placode formation. Consequently, it is not clear whether the scenario, in which a common placodal state is first induced and each placode subsequently elaborated through the action of multiple serial inducers, generally holds true. Furthermore, little consideration has been given in the past to the inductive events that drive the formation of the other placodes. Recent analysis of these other structures, however, has proven to be very informative.

Table 1. Ectodermal placodes: induction and derivatives.

placode	inducers	derivatives
adenohypophysis	ventral forebrain	endocrine cells of the anterior pituitary
olfactory	forebrain mesoderm endoderm	olfactory receptor cells olfactory epithelium GnRH neuro-endocrine cells glia
lens	forebrain mesoderm endoderm	lens
trigeminal	midbrain–hindbrain	somatosensory neurons of Vth ganglion
otic	hindbrain mesoderm	sensory epithelia sensory neurons sensory receptor cells
lateral line	?	sensory receptor cells sensory neurons
epibranchials	pharyngeal endoderm	gustatory and viscerosensory neurons of the distal VIIth, IXth and Xth ganglia

5. INDUCTION OF THE OPHTHALMIC TRIGEMINAL PLACODE

As described previously, the trigeminal placodes arise alongside the neural tube at the level of the midbrain–hindbrain junction. High level expression of the transcription factor *Pax-3* is a very early marker of the ophthalmic trigeminal placode, and this fact has been exploited in an analysis of the induction of this structure (Baker *et al.* 1999; Stark *et al.* 1997). These studies have shown that this placode forms as the result of an inductive signal, which is probably diffusible, emanating from the midbrain–hindbrain region, exerting its effect on the adjacent ectoderm. This signal seems to be instructive and both general early cranial ectoderm and epiblast ectoderm, from gastrula stage embryos, are competent to respond. Curiously, however, it was found that presumptive ophthalmic placodal ectoderm could be induced to form this placode by neural tissue from all axial levels, suggesting that the localization of the ophthalmic placode could in part be due to a restriction in the competence of ectoderm to respond to the inductive stimuli (Baker *et al.* 1999). Alternatively, this observation could reflect the fact that the ectoderm used in this experiment was already specified and was in fact responding to a general permissive signal that is found along the neural tube rather than to a direct instructive signal. It is interesting that the ophthalmic placode is induced by the midbrain–hindbrain regions as this neural territory contains an important organizer of neural patterning, the isthmus (Wassef & Joyner 1997). Many of the isthmus functions can be mediated by FGF8; therefore it may be that the induction of ophthalmic trigeminal placode is influenced by this structure and through the action of FGF8 (Crossley *et al.* 1996; Irving & Mason 2000; Shamim *et al.* 1999).

6. INDUCTION OF THE EPIBRANCHIAL PLACODES

In contrast to all the other placodes, the epibranchial placodes form some distance from the CNS emerging somewhat ventral, close to the clefts between the pharyngeal arches. The epibranchial placodes, the geniculate, petrosal and nodose, form in the vicinity of two embryonic tissues, the neural crest and the pharyngeal endoderm, both of which have been implicated in their induction (Webb & Noden 1993). However, recently it has been shown that the neural crest is not required for the induction of these structures and that they are induced by the pharyngeal endoderm (Begbie *et al.* 1999). Again this is an instructive interaction, one which can drive non-presumptive epibranchial ectoderm to an epibranchial fate. The mediator of this inductive interaction has also been identified as being the signalling molecule Bmp-7 (Begbie *et al.* 1999). This molecule is expressed prior to the formation of the epibranchial placodes in the pharyngeal endoderm as it contacts the ectoderm. Further, if recombinant Bmp-7 is added to cranial ectoderm in culture it will elicit the formation of epibranchial neuronal cells, marked by their expression of *Phox-2a*, in the explanted tissue. Finally, if Bmp-7 function is blocked, using the antagonist follistatin, then pharyngeal endoderm is unable to induce the formation of the placode.

7. A COMMON PLACODAL STATE

As mentioned, it has often been suggested that a first step in the formation of the ectodermal placodes is the induction of a common placodal state (Jacobson 1966; Torres & Giraldez 1998). This idea comes from both fate mapping and experimental studies. Fate maps have shown that the olfactory, lens and otic placodes all derive from a region of ectoderm, shaped like a horseshoe, which flanks the anterior neural plate at early stages of development (Jacobson 1966). Experimental analysis has found that cranial ectoderm is generally competent to respond to a variety of placodal inducing signals (Jacobson 1966). A number of lines of evidence, however, suggest that there is no need to assume the initial induction of a common placodal state, but that this state corresponds to a general competence for naive ectoderm to make placodes. First, fate mapping studies in the chick have shown that the epibranchial placodes arise from ectoderm that lies distal and posterior to the neural primordium and the horseshoe-shaped olfactory, lens and otic placodal territory (D'Amico-Martel & Noden 1983). Studies on the induction of both the otic and trigeminal placodes have also shown that gastrula ectoderm, which has never 'seen' any placodal inducers, can be specifically directed to generate each of these placodes (Baker *et al.* 1999; Gallagher *et al.* 1996). Furthermore, it has been found that the lack of lateral line placodes in embryonic stages of the direct developing frog, *Eleutherodactylus coqui*, is not due to any deficiency in the inductive signals but because the ectoderm has specifically lost the competence to respond (Schlosser *et al.* 1999). Indeed, it seems that young ectoderm in all species is inherently competent to respond, but that this is lost as development proceeds. Thus, one feature involved in the formation of all the placodes seems to be that with age competence becomes restricted to the presumptive placodal territories—and clearly this could have an important role in delineating the ectodermal placodes during development.

8. ECTODERMAL PLACODES HAVE SPECIFIC DIRECT INDUCERS

The studies on the induction of the ophthalmic trigeminal placode and the epibranchials are important as they make the clear point that these ectodermal placodes can be induced by a single specific tissue: the ophthalmic by the midbrain–hindbrain and the epibranchials by the pharyngeal endoderm. This work further showed that both of these interactions are instructive, exerting their effect on cranial ectoderm, which seems to be generally competent to respond to these signals. Furthermore, a recent study has shown that when presumptive ophthalmic ectoderm is grafted to the site of the nodose placode it then goes on to produce *Phox-2a* positive nodose neurons (Baker & Bronner-Fraser 2000), which reinforces the idea of specific instructive placodal induction by specific tissues.

Recent studies have re-addressed the induction of the otic placode using early markers. The transcription factor *Pax-2* is expressed in the otic placode at very early stages and expression of this gene can be induced in competent ectoderm when adjacent to the central hindbrain (Groves

& Bronner-Fraser 2000). However, *Pax-2* expression is not induced by either the midbrain or spinal cord, suggesting that the primary inductive signal for the otic placode is specifically localized to the central hindbrain region. Similarly, experiments using the *Lmx-1* gene, which also labels the otic placode, have shown that the expression of this gene is dependent on signals emanating from the hindbrain (Giraldez 1998).

The ventral forebrain has likewise been identified as the primary inducer of the adenohypophyseal placode (Gleiberman *et al.* 1999). Interestingly, although the initial inducer of the adenohypophysis is the ventral forebrain, the surrounding mesenchyme is also required to support this interaction. By extension it could be the case, in those other instances where multiple inducing tissues have been implicated, that one tissue is the source of the primary inducing signal while the other tissues are required for support. It is also clear, however, that placodes that generate the more complex structures, the adenohypophyseal, olfactory and otic placodes, are likely to require additional signals. These placodes could be further patterned and elaborated through the action of signals that may emanate from adjacent tissues.

9. A LINK BETWEEN INDUCTION AND LATER FUNCTION

There is a further significance to the fact that there are specific inducers for individual ectodermal placodes—that there seems to be a link between the primary inducer and the later derivatives of the placode. The epibranchial placodes are induced to form by the pharyngeal endoderm. An intrinsic feature of the pharyngeal endoderm is its ability to generate taste buds (Northcutt & Barlow 1998) and the gustatory neurons that innervate these structures are derived from the epibranchial placodes (Ariens Kappers *et al.* 1960). Similarly, the adenohypophysis is induced to form by the region of ventral forebrain, which subsequently generates the neurohypophysis (Gleiberman *et al.* 1999). The forebrain is also implicated in the induction of the olfactory placode and it is from this region of the brain that the olfactory nuclei arise (Couly & Le Douarin 1985). Likewise, the lens is induced to form by the presumptive optic region (Henry & Grainger 1990). This situation also seems to apply to the hindbrain. The central hindbrain region is the site of origin of the vestibuloacoustic nuclei as well as being an inducer of the otic placode (Marin & Puelles 1995; Torres & Giraldez 1998). Finally, the midbrain–hindbrain region involved in the induction of the trigeminal is that which gives rise to the other components of the trigeminal complex: the mesencephalic trigeminal nucleus, the trigeminal sensory column and the trigeminal motor nucleus (Lumsden & Keynes 1989; Marin & Puelles 1995; Narayanan & Narayanan 1978).

10. THE EVOLUTION OF THE ECTODERMAL PLACODES

It has been suggested that the ectodermal placodes evolved together with the emergence of the vertebrates (Gans & Northcutt 1983), and this has been taken as another reason for thinking of the ectodermal placodes as a single grouping. Yet, recent studies on protochordates suggest that the ectodermal placodes may not have evolved together, but that some of them arose before the vertebrates. Studies in amphioxus, both molecular and ultrastructural, have suggested that there may be a homologue of the olfactory placode in this protochordate. The amphioxus *msx* gene is expressed at early stages in two patches of rostral ectoderm, mirroring the situation in vertebrates in which the olfactory placode is marked by the related gene, *msx-1* (Sharman *et al.* 1999). Correspondingly, groups of rostrally located epithelial sensory cells that show some similarities to the vertebrate olfactory sensory cells have also been described at later stages (Lacalli & Hou 1999). A possible homologue of the olfactory placode has also been suggested to exist in the colonial ascidian, *Botryllus schlosseri*. In this animal the neurohypophyseal duct has features that are reminiscent of the olfactory and adenohypophyseal placodes; this structure forms at the anterior of the animal and cells delaminate and migrate from it (Manni *et al.* 1999). The ascidian, *Halocynthia roretzi*, possesses bilateral atrial primordia, which may further be a homologue of the otic placode. The atrial primordia is an ectodermal thickening that generates ciliated sensory cells, as does the otic placode; this structure is also marked by the expression of *HrPax-258* and the otic placode by *Pax-2* (Krauss *et al.* 1991; Nornes *et al.* 1990; Wada *et al.* 1998). Some placodes, however, do seem to be vertebrate specific. Although amphioxus has pharyngeal pouches there are no associated epibranchial placodes. Similarly, there is no indication of the existence of lateral line placodes outside the vertebrates. Collectively, these studies suggest that the placodes did not evolve together with the emergence of the vertebrates. Rather it seems that some have a more ancient history, the otic and olfactory, while others, the epibranchials and lateral line, are vertebrate specific.

11. THE SIGNIFICANCE OF ECTODERMAL PLACODES

It should be apparent from the issues discussed above that there are substantial differences between the ectodermal placodes. Indeed, in reality, the only feature that is shared by all of them is that they are focal thickenings of the cranial ectoderm—and in fact one has to question how significant this is. All of the placodes emerge from the early cranial ectoderm, which at least in mammals is generally thickened but which then thins out in the non-placodal regions (Verwoerd & Van Oostrom 1979). Thus, in one sense placodes represent focal 'unthinnings' of the ectoderm. It is also worth noting that there are no general placodal markers. A number of genes have been described as labelling placodal ectoderm but invariably they do not label all of the placodes. One gene, *cSix4*, has been reported as labelling all the placodes but it certainly does not label the early trigeminal placodes nor does it specifically label the epibranchial placodes, but labels the whole branchial region (Esteve & Bovolenta 1999). We would argue that the ectodermal placodes are merely a reflection of the action of localized inducers on the cranial ectoderm. Those regions that are subject to inductive cues maintain their thickened morphology,

while the other regions thin and become more squamosal. Furthermore, beyond this initial superficial similarity, the morphogenesis of the placodal structures diverges. While the adenohypophyseal, olfactory, lens and otic placodes all invaginate, the first two form an expanded cavity whereas the latter two form closed vesicles (Webb & Noden 1993). The other placodes, the trigeminals, the epibranchials and the lateral line, do not invaginate but thicken further and are generally evident as dense regions of columnar epithelia from which the neuronal derivatives delaminate.

12. CONCLUSIONS

The ectodermal placodes have for a long time been dealt with as a single entity, sharing a common development and evolution. However, this appears increasingly to be an oversimplification. We would argue that what was previously described as a common placodal state is in fact merely a reflection of the general competence of naive ectoderm to produce placodal structures and, furthermore, that the naive ectoderm is able to produce specific placodes in response to specific inducing signals. Thus it would appear that the early embryonic ectoderm is a very plastic tissue capable of producing all of the placodes and their diverse range of differentiated cell types. The ectodermal placodes should in fact be segregated, according to their inductive mechanisms, subsequent development, evolution and later function. Along these lines, we would separate the placodes as shown in table 1. Thus we would class the epibranchial placodes as one group, the trigeminal placodes as another, the lateral line placodes as a further group and then deal with the remaining placodes as separate distinct entities.

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