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Craniofacial development: a summing up

L. WOLPERT

Department of Anatomy & Developmental Biology, University College and Middlesex School of Medicine, London W1P 6DB, UK

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It is convenient to distinguish between three related problems in development: cell differentiation; pattern formation, which is about spatial organization; and morphogenesis in the strict sense, which is about change in form, particularly of cell sheets, but includes cell migration (Wolpert, 1981; Wolpert & Stein, 1984). All these need to be linked to gene action. If one looks forward over the next five to ten years then the future of craniofacial biology lies in molecular cell biology. This is not to say that all the problems at the tissue level have been solved, quite the contrary, but rather that the emphasis must now be at the cell and molecular level.

One can illustrate some of the problems of cell differentiation – and the approaches involved – with the differentiation of the cells of the haemopoietic system. Here we have a stem cell that can give rise to all the different types of blood cell. The extent to which the different pathways are determined by intrinsic factors as distinct from extrinsic factors, i.e. environmental influences, is not yet resolved (Gordon, Riley, Watt & Greaves, 1987; Clark & Kamen, 1987). Nevertheless, it is interesting to use it as a model for considering the differentiation of the neural crest. Weston (see reports by Wedden and by Bee & Newgreen, this volume) has suggested that the neural crest differentiation might start by the intrinsic generation of a number of different cell states or types which then undergo selection during migration. This selection may be either positive, some cell types are preferentially stimulated to divide, or negative, some types being killed. The essence of this mechanism is that the early neural crest population is heterogeneous and selection then operates. This differs from the conventional view in which the initial population is more or less homogeneous, and the cells become committed to the different pathways of differentiation, during migration, depending on their local environment. It may be necessary in order to resolve these problems to proceed along the lines of those who have studied the differentiation of the haemopoietic system *in vitro*. They have developed culture systems in which the various stages can be followed and, most importantly, provide an assay for the differentiation and growth-promoting factors (Clark & Kamen, 1987). In this way, a number of proteins have already been identified. It will thus be necessary to develop similar *in vitro* systems for the differentiation of the different classes of neural crest cells. But this requires that one can identify these different classes at early stages of differentiation and for this the most appropriate techniques may be surface markers using immunological techniques. This has been successful with the haemopoietic system – for example, Greaves' (1986) work with lymphocytes – and with the early development of glial cells of the optic nerve as studied by Temple & Raff (1984). It does seem that unless one has such markers it will not be possible to make much progress. Immunological techniques are not the only way into the problem: the techniques of recombinant DNA can equally provide markers at either the mRNA and protein level (e.g. Weeks & Melton, 1987). Just to get the appropriate markers is going to require a great deal of work.

Even when one can identify the various cell types and have an understanding of their differentiation, it is necessary to understand how they become located in the right place in the head. Somehow the cells cooperate. However, our perception of patterning in the craniofacial system may be very different from that of the cells. If you asked the cells how they make, say, the nose, the reply might be 'Nose! Nose? I don't know what you're talking about. We're just "ZANY-O" neural crest talking to ....' So we need to know the cells' language for organizing themselves into structures. Are the cells organized into compartments as in insects? There may well be a group of cells who would happily admit to being mandibular archists – but at this stage we really don't know. There is no reason to believe that what we perceive as a structure is what the cells, too, perceive as a structure. 'Craniofacial', too, may be meaningless to them.

It would be very nice if I could suggest that in order to understand patterning in the head you could use the development of another system, say the limb (e.g.

Wolpert & Stein, 1984; Tickle, Lee & Eichele, 1985; Wolpert & Hornbruch, 1987). That might be thought of by some as trying to explain the unknown in terms of the unknown. Nevertheless, one can think of some classes of patterning in terms of positional information (Wolpert, 1981; Wolpert & Stein, 1984). There is a soft sense in which the term positional information can be used: i.e. that cells in different positions are different. But it is in the hard sense that I want to use it. By positional information, I mean that there is some cellular parameter that correlates with position, for example the concentration of a morphogen or some combination of molecules. Positional value, then, is some more-permanent cell parameter that actually provides the cells with an address. Positional value implies a cell parameter that varies as if the cells were in a coordinate system. Our most recent model for the limb involves both prepattern and positional information (Wolpert & Hornbruch, 1987). A wave-like prepattern may generate the cartilage elements and positional information will then 'name' them and generate their special character.

As far as I can see there is very little, if any, evidence for gradients or positional information in the hard sense in the head. If one views the head as part of the main body axis then the question is how is position along this main axis specified. It is an uncomfortable truth that, at this stage, one has very little idea as to what specifies the character of the various structures along this main axis: skull, vertebrae and so on. Some evidence that it may involve positional information comes from experiments on the early amphibian embryo in which increasing doses of u.v. light to the vegetal pole of the egg removes increasing sectors of anterior structures (Scharf & Gerhart, 1980; Cooke, 1985). Even in the system about which we know most – the early insect embryo – it is not clear how the pattern along the main axis is specified (Akam, 1987). While gradients may be involved (Mlodzik & Gehring, 1987) there seems to be a complex set of interactions. The mechanism for segmentation seems to be different to the mechanism for naming the segments. For the dorsoventral axis however it does seem as if a positional gradient is operating (Anderson, 1987).

There are several repeated patterns in the head that require special attention. These are the pharyngeal arches, the somitomeres and, at a later stage, the neat pattern of bristles. The arches and somitomeres clearly involve a mechanism similar to somite formation and the latter are very like feather patterns. We don't know how any of these repeated patterns are set up but a recent paper by Nagorcka, Manoranjan & Murray (1987) shows how a reaction–diffusion mechanism coupled to cell traction could specify a hexagonal spacing pattern. This may be particularly relevant to the different bristle patterns seen in mice, lions and so on. Like feathers, individual bristles may be different, each with its own positional value. There does seem to be a general principle in development which involves repeated structures and this has a possible evolutionary basis. One can think of the limb, for example, being, in some senses, a repeated structure. If one tries to imagine how it could have evolved it is very hard to believe that positional information could provide the primitive limb, since too many thresholds would be required to be established at once. However, a mechanism involving the generation of a repeated pattern, such as a wave-like prepattern, is much more plausible: this pattern could then become modified during evolution. This principle may well be fundamental to understanding the head.

Even though there is no evidence for positional information in the head most of us believe that there are regional intrinsic differences between cells of the same class. What is known is that at an early stage of development, even before the somites have formed, the character of the different vertebrae is specified. That is, they have become nonequivalent (Lewis & Wolpert, 1976). If presumptive somite tissue from the neck region is grafted to a more-posterior site, then neck vertebrae will develop at this inappropriate location (Kieny, Mauger & Sengel, 1973).

One of the interesting ideas to have come out from this meeting is that two cell classes, embryonic muscle and vascular tissues, are equivalent. They appear to be totally egalitarian without any intrinsic differences between the cells. Their behaviour is determined by the surrounding connective tissue. At least as far as muscle is concerned this is in agreement with limb development where the equivalence of the embryonic muscle cells has been demonstrated (McLachlan & Wolpert, 1980). Muscle cells derived from cervical somites can develop a normal pattern of muscles within the limb.

One of the earliest problems of pattern formation in the head is the specification of the cells that are going to become neural crest. The neural plate is specified by induction by the underlying mesoderm, but what then specifies that a group of cells at the edge will form the neural crest? It is quite a nice simple and unsolved problem in pattern formation. We also don't know how fine grained differences between cells are. It's worth remembering that genes are cheap and that, in principle, with 50 genes one could specify, combinatorially, each cell in the body uniquely. Each cell would have its own zip-code. It may be that we only will resolve the question of fine grainedness when we have the molecular tools to distinguish small differences between cells.

An important question in patterning is the relation between the ectoderm and its underlying mesenchyme. How are the two patterns coordinated? I have always taken the view that the mesenchyme is the 'dominant' tissue and induces the pattern in the overlying ectoderm. On this view, induction can be considered as a coordinating mechanism (Wolpert, 1981). The mesoderm induces the overlying ectoderm to become neural tissue and the spinal cord and vertebral column are thus properly positioned. Wherever there are two tissues which form a combined structure such coordination is necessary. An attractive hypothesis is that many cases of mesenchymal–epithelial induction involve the inprinting of positional values from the mesenchyme to the overlying epithelium (Wolpert, 1981). In this way, the ectoderm would acquire a set of positional values and would interpret them according to its developmental history. There is some experimental evidence for such a mechanism; it has the further attraction that it treats the face, for example, as a positional field. Rather than thinking of the induction of specific structures, the structures develop at specific positions. This would account for the development of bristles in various different locations on the face. It also would imply that the eye does not induce a lens but a set of positional values which is interpreted by the cells becoming lens. In spite of the attraction of such a unifying principle, the model is clearly wrong in a number of instances. Ferguson's (this volume) studies on trans-species induction of palatal structures clearly shows that the mesenchyme induces mesodermal, rather than ectoderm-specific responses: the induction is instructive in the sense that it causes cellular responses specific to the mesoderm, such as keratinization and cell death.

In thinking about induction of structures in the head one must be careful, again, not to assume that our description is similar to that of the cells. We speak about, for example, the induction of the lens, or bristles or the ear. But these subdivisions may not correspond to anything in the logic of the embryo. Just because there are no obvious features in the cheek region does not mean that no induction is occurring. My guess is that one should view the face and head as a unit whose properties are determined by the underlying mesenchyme. This unity is related to the seamless junction between the tissues derived from the mesoderm and the neural crest. There is no structure at the junction, no detectable join, though lineage analysis could reveal boundaries analogous to compartments in insects. It must mean that the behaviour of the cells in the mesenchyme is determined by some field-like property. It is unlikely that the cells of the neural crest are so specified before migration to make such a seamless junction with the mesoderm. Such a field requires signalling and cell-to-cell interactions. As yet we have no evidence for either.

How are we to make progress? A little luck would help. We need to keep a close watch on work in insects and mice. The homeobox may be a way in. It is necessary to take a chance – a little like a lottery ticket where the probability of winning may be low but at least it is finite. We also need to think about growth factors, such as EGF and FGF, and the more recently fashionable TGF family (Massague, 1987), and other possible molecules that may be involved. There are only about 50 or so known molecules that might act as signals during development. If one guesses how many might actually be involved, and if this is of the order of thousands, then we have a very long way to go. In addition, we must keep a watch on the phosphorylation of proteins (Hunter, 1987) which I guess will turn out to be fundamental to controlling pattern formation. This is a bit pessimistic and things might turn out to be much simpler than at present seems likely.

Turning now to morphogenesis, or change in form in the true sense. It is here that we seem to be making the most progress. The invaginations of the lens and placodes can be understood in terms of the change in form of cell sheets. The models of Odell, Oster, Burnside & Alberch (1985) on how a wave of contractions can bring about a variety of changes in form are very encouraging. One requires patterning in the sheet to specify the origin and boundaries of the contracting cells and it is in this way that patterning and morphogenesis are linked. It would be very nice if these ideas on the folding of sheets could also be applied to the development of teeth, whose form is related to the epithelium. How does one investigate the mechanical basis for such changes? One would like a marker that correlated reliably with localized contraction: perhaps localized accumulation of actin filaments will have to suffice. In more general terms, it seems that it is the epithelium that determines the overall form of the face. It is attractive to think that both teeth shape and palatal shelf elevation, as raised by Linda Brinkley in discussion, are mechanically controlled by the epithelium. The epithelium can be thought of as mechanically constraining the underlying mesoderm, the change in shape of such systems being thought of in terms of directed dilation (Rooney, Archer & Wolpert, 1984). The energy for such changes in form seems to be rather small. The estimates for sea urchin gastrulation and amphibian neurulation suggest that the energy required is about 10^6 times less than that produced by the embryo (Gustafson & Wolpert, 1963). Even if this estimate has to be increased because only a few cells are producing the force, say one cell per thousand, the

energy requirements are still small.

Another issue relates to the role of the extracellular matrix. There is no doubt about its importance in affecting cell behaviour but the key question is whether it signals directly or whether its influence is indirect: that is, it acts as a substratum which alters cell shape. Most of the actions of the matrix suggest that it is the latter mechanism that is almost invariably operating (Watt, 1986). Exceptions are, however, beginning to accumulate. For example, Thorogood (this volume) implicates collagen II in directing where cartilage will form and Menko & Boettiger (1987) have some evidence for the matrix controlling muscle differentiation directly. The mode of action of the matrix is particularly puzzling in relation to epithelial–mesenchymal interactions. Why has it been so difficult to identify the nature of the signal? There does not seem to be any other mode of communication between these two layers other than the matrix, yet the matrix alone does not seem to be the inducer. Perhaps we have to think of the matrix as a channel for an, as yet, undiscovered diffusible signal. We need some novel new approach – we must put away our Nucleopore filters.

Then there is the question of teratology. Is there anything to be learned from teratogenic agents? In general, I would argue, teratology has taught us virtually nothing. In spite of all the numerous studies on the effects of a myriad of agents on a variety of organs in development, hardly a single agent has emerged as being interesting; interesting, that is, in the sense of giving insight into developmental processes or into the nature of the chemicals that are involved in development. Almost all the teratogenic agents seem to simply make cells sick to varying degrees. It was thus of great interest to hear of Skulik's (this volume) results suggesting that teratogens act by increasing areas of programmed cell death. Two exceptions to my lack of enthusiasm for teratogens are retinoic acid (Tickle *et al.* 1985; Thaller & Eichele, 1987), which seems to be able to alter positional values, and lithium, which may act in a similar manner.

Finally, one has to face the lack of genetics in craniofacial studies. There are some encouraging prospects as outlined by Moore *et al.* (this volume) in relation to human abnormalities. There also may be ways round this problem. One is looking to see if there are genes controlling craniofacial development which have some homology to genes controlling pattern and form in other systems. Perhaps homeobox-containing genes will be involved. Another approach comes from insertional mutation using transgenic mice. An inherited limb deformity has been created by this method (Woychick *et al.* 1985). However, one should not underestimate the difficulty in understanding the abnormality even when a protein has been identified. The route from genes to pattern and form, regrettably, may be more tortuous and complicated than I would have hoped.

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