Development 103 Supplement, 61–62 (1988)
Printed in Great Britain © The Company of Biologists Limited 1988

Morphogenesis of the head and face: discussion report

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Key words: somitomeres, morphogen, cell lineage, immunolocalization, neural crest, growth factors.

The discussion following the session on evolution and morphogenesis of the head and face concentrated upon two major issues:

(1) How can one test models of development, particularly at biochemical and molecular levels?

(2) Are the cell populations of the early facial primordia heterogeneous and when might this heterogeneity arise?

The Chairman, J. Z. Young (London), had suggested in his introductory remarks that research into craniofacial development was at last becoming more specialized, having previously dealt with principles and model systems rather than with issues of practical importance. The ensuing lectures clearly demonstrated the direction and advances in current research, both in evolutionary aspects and at the level of morphogenesis.

Robert Greene (Philadelphia) opened the general discussion. He emphasized the need to examine biochemical and molecular aspects of craniofacial development. In his view, the conceptual chasm between the gene and metazoan embryogenesis was wide and deep and had remained so in recent years. For example, the factors controlling growth and segmentation patterns at the molecular or cellular level are largely unknown.

The Chairman, although struck by the progress in understanding craniofacial development, was anxious to know what types of molecules are involved in development and how they are distributed. "Are they segregated in any pockets? Are there gradients with peak points at which substances are produced and thus make a positional gradient along which differentiation can take place?" He would liked to have heard more, at some point, about the synthesis of some of these substances.

The Chairman emphasized that we need to link the factors involved in development with the genome. The craniofacial system may be a good system in which to do this since one may have different factors in different parts of the facial rudiments. One might expect there to be differences in the enzymatic activities or in the distribution of RNA molecules in relation to these, perhaps graded, factors. He suggested that the field of developmental biology had as many general theories in the 1920s as we have today. Such theories suggest what to look for, but they do not tell us much about the molecules.

Once the discussion was opened to the floor, Lewis Wolpert (London) responded to the concern that the field still had a long way to go. "We've got to be a little patient," he said. "It's a hard problem." He compared the "man/woman hours" spent on developmental biology research with the much greater time devoted to other biological problems, such as the action of insulin at the cellular level. He estimated that there was more than a million-fold difference. Yet the action of insulin remains obscure.

Wolpert felt that there *had* been progress and that we should be encouraged by the breakthroughs that have come in understanding other areas of development. He pointed out that retinoic acid, a potential morphogen, had been found in a graded distribution in the chick limb bud (Thaller & Eichele, 1987). He next cited the advances in understanding insect development. In insect research, "very specific models of pattern formation and positional information, at both the genetic and physiological levels are being tested all the time".

Brigid Hogan (NIMR, London) referred to preliminary *in situ* hybridisation work and outlined some of the problems in examining the role of growth factors at the molecular and biochemical level. Using probes to some of the growth factors, they have found that the RNA is present in most mouse embryonic cells at low levels. She emphasized that there are also problems in examining the proteins using immunolocalization, since antibodies raised to the same protein can give different patterns of expression. In addition, one does not know whether antibodies are localizing active, stored or bound protein.

Mark Ferguson (Manchester) enlarged upon this issue. He stated that it has recently been published that the expression of insulin-like growth factors is controlled at the translational level (Haselbacher, 1987). Another problem with immunolocalization is that growth factors are soluble molecules and change with tissue fixation. Referring to work in his laboratory, where they have used gold probes to immunolocalize growth factors in the embryonic chick palate, he said that "the probes appear to be everywhere. It's even more complicated because the extracellular matrix molecules bind the growth factors." The matrix may store growth factors and when remodelled, may release them. This means that the appearance of growth factors, as determined by immunolocalization, is not necessarily temporally or spatially related to their synthesis. Ferguson suggested that one can separate the effects of growth factors from the extracellular matrix by developing artificial systems for supporting epithelial cell differentiation *in vitro*.

At a molecular level one can, in theory, study differential gene expression in the three types of palatal epithelium. But this is also not easy. In addition to requiring vast numbers of cells, all at exactly the same stage of development, there would be, as pointed out by Hogan, a strong chance of picking up only the abundant molecules such as cytokeratins.

Having worked through some of the difficulties in analysing craniofacial development at the biochemical and molecular level, the discussion moved onto the second major issue of the session, that of cell heterogeneity and its origin.

Jonathan Cooke (NIMR, London) commented on an earlier mention of Drew Noden's work. It had been suggested that the mesenchymal contribution of a particular somitomere to a particular region of the head was not sharply defined and was only "statistical". He was surprised at this. If the cells already have their "genetic, real names", and even if they migrate around, their future contributions should be circumscribed, as they are in insect development.

Drew Noden (Cornell) replied that in the transplantation experiments one cannot see boundaries between the somitomeres. It is possible that overlapping sections of the paraxial mesoderm may be grafted. "What

we've generated in terms of muscles is a stacking order – which probably doesn't represent any true boundaries." He added that myogenic cells migrate into regions of neural crest cells and it is this act of moving in that gives spatial information to the myogenic lineage. However, when one transplants connective tissue precursors from different regions of the paraxial mesoderm, the precursors develop largely according to their original position. He emphasized that in terms of the kinds of information the tissue has, the issue is "less whether a tissue is paraxial mesoderm, lateral mesoderm, prechordal plate or neural crest-derived, but whether the precursor is one of connective tissue mesenchyme or nonconnective tissue mesenchyme"

Following Noden's comments, Weston (Eugene) outlined the issue that was to dominate the remainder of the discussion. He suggested that when one tries to construct an experimental paradigm to test the developmental ability of tissues, there is usually the assumption that the embryonic tissues, at early stages, are homogeneous. He emphasized, however, that this assumption is gratuitous since, as early as one can look, the tissues are heterogeneous. He suggested that one of the major questions to be addressed, in terms of how individual cells respond to different factors, is "where, when and under what conditions does this cellular heterogeneity, within an apparently morphologically homogeneous tissue, arise?" In his view, some of this heterogeneity arises autonomously.

Weston remarked that there appears to be an implicit notion that when a cell changes phenotype, it does so in response to some environmental signal. In fact, the cell may do so spontaneously, according to some intrinsic programme. This may be obscured, depending upon the resolution with which one views the system. When asked to give an example, he drew an analogy with the immune system. When viewed from a distance, there appears to be a directed response to a specific stimulus. Yet, when viewed more closely, one has an autonomous generation of diversity within the population; this diverse population then responds to specific cues. In commenting on whether there was a pattern in this, he said that "the pattern is a specific response, the production of antibodies, to a specific stimulus".

Weston emphasized that in neural crest transplantations one has had the assumption that the cell populations are developmentally homogeneous. However, when they have looked, the cells are developmentally and phenotypically heterogeneous. In reply to the Chairman's comments that the cells have histories, Weston suggested that the heterogeneity arises at or before the time the crest cells can be identified as individuals.

The discussion of cellular heterogeneity was continued by Nicole Le Douarin (Nogent). She suggested that the cell populations were heterogeneous when arriving at the site of differentiation. They then receive their cues which select amongst the various potentialities that they have. "It is the cues, which are local, which generate the pattern in the differentiation of the neural crest cells." She suggested that the capacity to respond to such cues is probably acquired autonomously. At the site of gangliogenesis, for example, there may be a growth factor for a particular cell committed to be a certain type of neurone or non-neuronal cell. The responding cells, during determination, will have acquired the receptor and will therefore respond by proliferation or differentiation.

In reply to the Chairman's question of what is making the cells differentiate, Le Douarin suggested that the commitment of cells makes them differentiate in a particular direction. This commitment, she added, is autonomous or intrinsic and lies in the ability of certain genes to be transcribed. She suggested that this makes vertebrate development "a little close to that of *Caenorhabditis*". In *Caenorhabditis*, the largely autonomous development of cells is well documented (Sulston, Schierenberg, White & Thomson, 1983), but there are also cell–cell interactions. In some cases, the cells may be committed but they cannot express the phenotype until they interact with other cells.

Lewis Wolpert put this issue in a slightly different form and questioned whether lineage mechanisms, as in *Caenorhabditis*, could generate diversity in vertebrates or whether it is always cell–cell interactions. He stated that he did not know of a single case in vertebrates, even in the haemopoietic system, where different cell types are generated by anything other than environmental factors or cell–cell interactions. He suggested that such interactions were instructive since there is a choice and the branch is actually determined by the nature of the environment

The discussion closed with the comment from Peter Thorogood that the avian embryo is particularly inappropriate for studying cell commitment. Unlike mammals, the chick embryo is meroblastic. What we call stage 0 consists of some 50 000–60 000 cells, having already had 22–24 h of development *in utero*. As he pointed out, if one wants to examine questions concerning the role of cell–cell interactions, the avian embryo should be taken at fertilization or it shouldn't be taken at all.

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