4th Altenberg Workshop in Theoretical Biology 1999

ORIGINS OF ORGANISMAL FORM: BEYOND THE GENE PARADIGM

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The topic

The fields of developmental and evolutionary biology are undergoing ferment. Progress in the last few years in characterizing the genetic mechanisms involved in embryonic development has demonstrated unexpected degrees of functional redundancy in these processes, as well as unanticipated discordances between conserved forms and conserved genes. In addition, evolutionary studies have revealed surprising extents of homoplasy and other forms of parallel morphological evolution in disparate lineages, as well as evidence that extensive morphological diversity appeared much earlier in the history of multi-cellular life than previously thought. These phenomena have raised new questions concerning the relationship between gene content and activity and the generation of biological form, and have suggested that the solution to these puzzles will emerge with the development of a new paradigm for understanding the evolution of developmental systems. A recurrent theme in such discussions has been a heightened interest in the evolutionary implications of epigenetic processes of form generation, including generic physical mechanisms of tissue morphogenesis and inductive and mechanochemical tissue interactions.

Program

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Abstracts

Bodyplans in the Cambrian "Explosion"

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That there was an "explosion" across the Precambrian-Cambrian boundary is beyond dispute: but was it an "explosion" of fossils or animals? If the former, then the best explanation is the breaching of a preservational (taphonomic) threshold; if the latter then it might be a genuine evolutionary event. Either hypothesis poses awkward and unresolved questions. Was there a protracted and deep Precambrian history of animals, with the "explosion" a dramatic scaling up of bodyplans? If so, what exactly did these cryptic metazoans look like? The available fossil record suggests, however, that there was a genuine evolutionary event. In many cases we may be on the threshold of documenting major transitions in bodyplan organization from the Cambrian fossil record. Such hypotheses seem to make sense in terms of function and ecology, but the bearing molecular biology has on these ideas is to some extent opaque. But the field is changing quickly, and the flood of molecular data is being matched by some remarkable new discoveries in the fossil record.

Convergent Evolution of Organismal Form

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Convergent evolution is prevalent, in current morphology, in fossils, and (even) in molecules. Similar morphology in unrelated taxa with common adaptive pressures is self-explanatory. But simple physical effects can produce similar morphologies without natural selection, so that conservation of morphology despite different adaptive pressures is also common. Thus organisms and their constituent parts may be highly (deceptively) similar yet taxonomically distant. Various examples may be used as illustrations of this: segmentation, appendages, ophophores, and larvae.
This may appear at odds with "genetic toolbox" ideas, and the evident conservation across phyla or whole kingdoms of developmentally-acting genes, which for a while seemed to subvert older views of homology, convergence, and phyletic archetypes. However, gene-based certainities are unravelling again; developmental genes may be recruited very differently to fashion differing body-plans. This may force a redefinition of convergence, and increased recognition of its effects. A particular gene may often or always be linked with a particular morphological entity (whatever an "entity" is); but we cannot infer that the morphology only evolved once, much less that all such morphologies are homologues. Perhaps only the underlying genetic programmes for pattern are common, and beyond that divergence and convergence have had rather free rein.

Open Questions of Morphological Evolution - and Some Answers

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This talk is meant to provide an overview of the conceptual background of the workshop theme, by identifying the major theoretical problems we will repeatedly return to, and to propose tentative solutions to some of the unsolved questions in morphological evolution. I will concentrate on three central issues: a) the relationship of genes with organismal form, b) the relationship of epigenesis with organismal form, and c) the problem of morphological organization. It will be argued that the major innovations at the phenotypic level of evolution arise from new epigenetic interactions that become secondarily captured and routinized by genetic circuitry. After the resulting morphological character sets stabilize during further evolution, their respective molecular, genetic, and developmental underpinnings are again free to change. In this scenario the generative factors in morphological evolution are distinct from those ensuring developmental control and heritability.

Three Personal Insights into Developmental Models of Form Generation

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Three personal insights come from a model of development from egg to adult including the logically required unknown processes. 1/ Self-assembly of each cell occurs using macromolecules supplied at the right time as set by nuclear control factors and signaling systems, and self-assembly of organs from the resulting cells is controlled by cell adhesion and adhesion rejection controls during cell movement. In a grand feedback the cell adhesion and signaling systems determine the nuclear factor patterns. 2/ At all stages it is only the detailed interactions of binding, adhesion, regulatory and signaling molecules that make life work. There are no overall control systems having information that specifies form. 3/ Specific binding molecule mutations are not an overwhelming part of developmentally important mutations. Thus these molecules probably result from multiple nearly identical genes. The evolution of such shared function genes is a fascinating and general issue.

Reciprocal Interactions between Genes and Tissue Architecture

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Given that every cell within the body has the same genetic information, a significant problem in biology is to understand how cells within a tissue express genes selectively. A sophisticated network of

physical and biochemical signals converges in a highly orchestrated manner to bring about the exquisite regulation that governs gene expression in diverse tissues. Thus, the ultimate decision of a cell to proliferate, express tissue-specific genes or apoptose must be a coordinated response to its adhesive, growth factor and hormonal milieu. The unifying hypothesis examined in this overview is that the unit of function in higher organisms is neither the genome nor the cell alone, but the complex, 3-dimensional (3D) tissue. This is because there are bi-directional connections between the components of the cellular of the cellular microenvironment (growth factors, hormones, and extracellular matrix; ECM) and chromatin structure and lead to selective gene expression. Thus, cells need to be studied "in context", i.e., whithin a proper tissue structure, if one is to understand the bi-directional pathways that connect the cellular microenvironment and the genome.
In the last decades we have used well-characterized human and mouse mammary cell lines in "designer microenvironment" to create an appropriate context to study tissue-specific gene expression. The use of 3D culture assay (cell grown in collagen, basement membrane, polyhema, etc.) has allowed us to develop assays for mammary gland morphogenesis and to distinguish normal and malignant breast cells easily and rapidly. We have shown that opimorphin (syntaxin 2), a molecule synthesized by stromal and myoepithelial cells is a critical morphogen for the mammary gland. However whether it signals for branching or lumen formation is determined by the level of its expression at the cell surface, by the temporal regulation of its cleavage and thus secretion, and by its tissue localization. We have shown further that whereas normal cells become growth arrested and form organized "acini" in 3-D ECM, tumor cells is cultured in, or on, abasement membrane. We have shown that, whereas b 1 integrin and epidermal growth factor receptor (EGFR) signal transduction pathways are integrated reciprocally in 3D, on tissue culture plastic (2-D) these pathways are not coordinated. Finally, we have demonstrated that, rather than passively reflecting changes in gene expression, nuclear organization itself can modulate cellular and tissue phenotype. We conclude that the structure of the tissue is dominant over the genome, and that we may need a new paradigm for how epithelial-specific genes are regulated in vivo. We also argue that unless the structure of the tissue is critically altered, even in the presence of multiple chromosomal mutations malignancy will not progress.

Genes, Form, and the Tempo of Evolution

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Evolution as "descent with modification" stresses the primacy of heredity in evolution and implies, as a corollary, that hereditary changes altering development are responsible for the evolution of form. This "genocentric" point of view requires reconsideration in view of recent findings that different body plans utilise the same developmental genes. I will provide a framework for relating genes, form, and relative rates of evolution in defending the following propositions:
1. All biodiversity is generated from no more than six cell behaviours which are influenced by genes with respect to when and where they occur.
2. There exist different strategies for morphogenesis, two prominent ones are: form generation from epithelial sheets and form generation from tissue interaction. Genes play different roles in these strategies and this has implications for the tempo of morphological evolution.
3. A rationale for rapid changes in morphological evolution is provided by the partial coupling of modules in biological hierarchies from molecules to multicellular structures.

Cell adhesive interactions and tissue self-organization

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An animal zygote progresses through a choreographed series of "stages" to the pre-ordained configuration of cells, tissues and organs that is the larva or the adult. One might well assume that this progression is specified by genetic programs evolved through mutation and natural selection to produce functional structures. But regulative animal embryos can also to some extent restore these normal cell and tissue arrangements after disarrangement ñ a form of self-organizing behavior that is one of their most remarkable attributes. This means that an approximation of the "correct" structure can be arrived at not only by the usual pathway but by any number of abnormal pathways as well. This leads to the remarkable conclusion that the underlying genetic programs must therefore direct the assembly of specific structures without specifying the pathways to be followed! Here we explore evidence that this is accomplished by regulating the adhesive interactions of intrinsically motile cells.

A molecular clock linked to vertebrate segmentation

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We have identified and characterised a novel type of molecular clock involved in segmentation of the vertebrate embryo by demonstrating the periodic expression of the mRNA of avian homologues of the Drosophila segmentation gene, hairy. These genes called c-hairy1 and c-hairy2 are expressed in the presomitic mesoderm (PSM) where their mRNA exhibits a cyclic posterior-to-anterior wave of expression whose periodicity corresponds to the formation time of one somite. The Notch signaling pathway has been recently implicated in somitogenesis control. We have shown that a component of this pathway, Lunatic Fringe is expressed in a rhythmic fashion similarly to c-hairy1. Lunatic Fringe and c-hairy1 expression domains in the PSM are similar, indicating that they are regulated by the same clock. Thus, clock control on the local modulation of the Notch signaling pathway could confer the periodic arrangement of the boundaries that underly the segmental body plan in vertebrates.

Reaction-Diffusion and other Mechanisms of Pattern Formation in Development

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Our understanding of the mechanisms responsible for pattern formation during the development of organisms has been greatly enhanced by recent findings in developmental genetics. In the course of the past decade, the study of pattern formation has been transformed from a purely theoretical into an

almost entirely empirical science. Many of the mechanisms predicted by theory have indeed been found to operate, and new mechanisms have been uncovered for which there exists as yet no adequate theoretical treatment. It is therefore instructive to compare the current findings from experimental developmental biology with the predictions of earlier theoretical modeling efforts. The work of Turing stimulated an early emphasis on reaction-diffusion as the principal mechanism for pattern formation, and extensive mathematical work in subsequent decades elucidated the general principles of self-organization in chemical and biological systems. In general, short-range positive feedback, coupled with a long-range negative feedback mechanism appears to provide both necessary and sufficient conditions for the generation of self-organizing patterns, starting from initially homogeneous conditions. Real biological systems are, however, never initially homogeneous. In many systems, pattern formation appears to rely on coupled diffusions-threshold mechanisms, or on short-range nearest-neighbor signal exchanges between cells that have already acquired different properties through an earlier patterning event. Although these mechanisms appear at first sight to be substantially simpler than reaction-diffusion systems, the multiplicity of interactions makes their overall behavior quite complex. The reactants in biological reaction-diffusion systems do not all diffuse, nor do they always interact via an activation-inhibition mechanism, like those of classical theory.

From Physics to Development: The Evolution of Developmental Mechanisms

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Organismal forms have not always been generated by the highly integrated genetic programs characteristic of modern multicellular species. It is proposed that physical forces and other conditional processes played a more prominent role at earlier stages of evolution, establishing morphological templates that were consolidated by later genetic change. In particular, the most ancient multicellular creatures must have been simple cell aggregates that arose by adhesion of originally free-living cells, or by the failure of the same to separate after mitosis. Once this occurred, living organisms became susceptible to a set of physical determinants that were not relevant during the unicellular phase of evolution: diffusion, reaction-diffusion coupling, differential adhesion, and decoupling between the cell cycle and cyclical regulation of intercellular adhesion. These mechanisms, in turn, made inevitable developmental gradients, tissue multilayering, lumen formation, and segmentation. The idea that with regard to the generation of three-dimensional form the earliest multicellular organisms were more like certain materials of the non-living world than are their modern, highly evolved counterparts helps explain findings that are difficult to reconcile with the standard neo-Darwinian model, such as the burst of body plans in the early Cambrian and the punctuated character of morphological innovation.

Genetic and Epigenetic Factors in the Origin of the Tetrapod Limb

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The origin of the tetrapod limb is discussed as an example of a morphological innovation. The ultimate challenge in establishing causality of evolutionary innovation requires the study of both genetic and epigenetic factors. Genetic factors are the most likely avenue through which novel phenotypes can be

transmitted from generation to generation. Hence, identification of the genes which have been changed in the origin of the character is essential. Epigenetic factors are also important, as genes do not contain explicit information about the developmental process but must function within a given epigenetic context. In the case of the tetrapod limb, the morphological innovation is the origin of a developmentally individualized autopodium together with its morphological elements, the digits including the metapodium and the mesopodium. We propose, in agreement with the models by Newman, Müller, and Seilacher, that the origin of the autopodium consists of at least two stages: the origin of the structures contained in the autopodium and the developmental individualization of these elements. Recent paleontological data suggest that digit-like elements preceded the origin of the autopodium proper. From a review of the genetic factors implicated in fin and limb development, we hypothesize that evolution of Hoxa-11 and Hoxa-13 gene regulation may have played a key role in the developmental individualization of the autopodium. Here, we discuss our strategy to test this hypothesis and present preliminary data based on sequence comparisons, functional tests and transgenic technology in support of this hypothesis. These results will also contribute to investigations into the epigenetic context in which these genes act.

Human Developmental Plasticity

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This presentation will look at the evidence concerning the genetic determination of physical attributes and behavior. On the biochemical level, humans are characterized by the 100% prevalence of a loss-of-function allele of gulonolactone oxidase. This means that, unlike most mammels, we each require environmental replacement therapy (of ascorbic acid) to survive. This lethal genotype also makes discussions parsing determination into separate genetic and environmental components meaningless. On the physiological level, humans are characterized by an extensive developmental plasticity in their immune responsiveness. Our immune system is a highly refinded version of capacity for environment-induced polyphenisms. On the behavioral level, the retention of our fetal neuron growth rate and the ability to reorganize synapses "allows us to escape the tyranny of our genes, or at least to temper their rule." In addition to reviewing scientific evidence for this plasticity, I will make some hypotheses concerning the popularity of neurogenetic determinism.

Epigenesis and Evolution of Brains

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Brains do not evolve in a piecemeal fashion, adding new divisions one by one, but as developmentally and functionally integrated systems. During early phases of neural development, the neurepithelium is divided, as a result of relatively local cellular and molecular interactions, into numerous distinct units (e.g. neuromeres) that are highly conserved between species. During the second phase of neural development the formation of axonal connections enables a host of long range, yet highly specific, developmental interactions that contribute to the resculpting of previously established brain divisions, the induction of late-appearing divisions, and the establishment of functionally integrated neuronal circuits. Phylogenetic changes affecting this second phase of neural development are likely to generate cascade effects in distant brain regions and therefore to account for many of the more dramatic species

differences in brain organization. Relatively subtle changes in early brain development (e.g. in the relative proportions of neuromeres) can also, however, yield large-scale divergences in subsequent brain development.

Morphological Plasticity in Development and Evolution: Simulations of the Waddington Experiment

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In laboratory experiments on the fruit fly Drosophila, ÝWaddington found that after carrying out indirect selection for an environmentally-induced trait for about fifteen generations, a new, true-breeding phenotype resulted that was absent in the starting population. The phenomenon, originally termed 'genetic assimilation', has been both confirmed and re-discovered by others since then. It continues to attract interest because of the rapidity of the effect and because of its seemingly Lamarckian implications: a major morphological change, involving what Ýappears to be the inheritance of an acquired character, occurs within a small number of generations. Waddington argued that the findings could be accounted by Ýthreshold effects combined with conventional Darwinian selection acting on regulatory genes. A genetic algorithm-based approach developed Ýby us provides an explicit demonstration of his conjecture.
1. C.H. Waddington, ÝAdv. Genetics Ý10: 257-293 (1961)
2. N. Behera and V. Nanjundiah, J.theor.Biol. 188: 153-162 (1997)

Boundary Constraints for the Emergence of Form

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Morphological organization in living organisms arises as the product of interactions among spatial entities during ontogeny. Proportions, orientations, connections, and articulations are the four levels of morphological organization that can efficiently be recognized, separated, and subsequently used for comparative studies. The analysis of boundary patterns between adjacent elements has never been considered as a proxy to study the appearance of novel structures in vertebrate skeletal macroevolution. However, these patterns not only determine the structural scaffold of an organism but are also involved in the generation of morphological constraints in embryonic development. I will show a theoretical analysis on the conditions to generate boundary patterns found in the skeletons of tetrapods. The analysis includes a graph-theoretical approach that provides several estimates about the organization and complexity of boundaries; and a model based on cellular automata that simulates the emergence of patterns given local boundary constraints. Sets of boundary patterns can be generated that preserve the general connectivity relations of real skeletons. These sets form a "morphospace of connections" for various structures and phylogenetic groups that can be used to test the disparity of real skeletal patterns. The analysis of an "evolutionary run" of a computer simulation demonstrates the kinds of constraints that are involved in the emergence of this morphological level of organization.
