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Summing up: conservation and diversification in metazoan eukaryotic cells

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The pre-meeting announcement raised the question: 'The cell is a marvel of evolution. How has an apparently common set of mechanisms present in the eukaryotic cell evolved to generate the variety of cellular processes found in multicellular organisms?' such as, I might add here, the organism's development, physiology and behaviour, and how have they generated the variety of organisms themselves, particularly metazoa?

The meeting was stimulating and successful. It was inspiring to hear speakers summarize progress in understanding the common (conserved) processes of eukaryotic cells of single-celled and multicellular organisms, namely: the functioning of the cytoskeleton; the basis of motility; the core reactions and feedbacks in the cell cycle; the elements of transcription and transcriptional regulation; the steps of signal transduction pathways and their crosstalk; the means of specificity in vessicle trafficking and compartmentation; the extracellular matrix and cell adhesion molecules; and the various means of generating osmotic balance and membrane potential. Structural conservation of protein components of these processes is apparent from sequence comparisons and crystallography. Functional conservation is apparent from interphylum gene substitution. Prokaryotes do not share these processes. The meeting was unusual in the speakers' balancing of data and speculation. Several said openly and others implied that once you really know the components of the processes and the interactions of components at the biochemical level, it is not hard to imagine how the process arose in the course of eukaryotic cell evolution from processes and components of a prokaryotic ancestor. Sometimes a comparison of *E. coli*, yeast and mouse was used, but sometimes the speculations came without comparisons, just from the layered organisation of the process itself. It is a reward of such studies that plausible integrative schemes can now be devised where nothing could be said a short time ago.

Still, although all these processes are fundamental to eukaryotic cell behaviour in general, and although many of them distinguish eukaryotic cells from prokaryotic cells, there was little speculation on what allowed the transition of eukaryotic cells from single-celled lifestyles to multicellularity, a transition that prokaryotic cells have barely made. What was or were the key innovations that opened up the possibilities of metazoan evolution? (K. Liem has referred to key innovations as those past changes which in hindsight can be seen as pregnant with possibilities because so much evolution followed upon them). The eukaryotic cell is indeed a marvel of evolution, but what distinguishes it in its metazoan manifestation? Was it the ability to shed a hard wall while still maintaining osmotic balance, or to reach large size and to phagocytose prokaryotes (making it the precambrian top carnivore) and to gain complex organellar compartmentation, or to engage in meiotic sexual reproduction by way of cell fusion, or to enlarge the genome while keeping down errors and noise. Or was it no one process in particular that made multicellularity possible; perhaps multicellularity was tried repeatedly at all points in cell evolution, whenever a minor barrier to aggregation was removed. Perhaps the more complex the individual cell and the more complex its interactions with the environment before entering the multicellular path, the more complex the multicellular organisms to evolve from it. It remains a mystery for a future meeting to explore this further.

But once the eukaryotic cell had reached the multicellular state, what in its repertiore of conserved processes was most made use of? It is striking that the great diversity and complexity of metazoa are underlain by a common cell biology. If this is conserved, what is diversified? It seems a paradox or contradiction that diversification is generated from conservation, as implied by the call to the meeting. Of course, conservation is not perfect: there are small changes of DNA and protein sequence, and although components may have a conserved catalytic or binding function, they may differ slightly in their interactions with other cell components. Also, the seeming paradox depends on a preconception that conservation implies constraint (an inability to change) and is antithetical to evolution. However, the reverse may be true: these processes may not only fulfill single cell function but may support and facilitate the evolution of multicellular organisms. M. Kirschner alluded to this theme in his discussion of exploratory mechanisms, ones characterized by a great variety and number of outputs, single ones of which are then selected upon and stabilized by interactions with varying components of the peripheral environment.

What then is the relation of conserved processes to diversified ones? Does the conservation of some allow the diversification of others? Three examples will be taken from the meeting.

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1. TRANSCRIPTION

As M. Ptashne and M. Levine discussed, the basic biochemistry of transcription and the means of regulating transcription have been conserved, but the contingency or conditionality of transcription has varied greatly (a point emphasised elsewhere by M .Kirschner, whose terminology I've adopted here). Whether a particular gene is transcribed or not depends on conditions that can be very complex. The gene's transcription is contingent on certain conditions. Embryonic development is filled with compelling examples of genes with complex *cis*-regulatory DNA sequences 5–50 kb in length containing multiple modular elements that set rules for when and where the gene is expressed. Specific regulatory proteins bind at these elements, interact with each other,and affect the RNA polymerase complex at the promoter. As Ptashne has described, the polymerase seems inherently active but is held in a barely inactive complex by a set of inhibitory proteins, and the regulatory proteins bound at distant regulatory elements contact the complex and relieve the inhibition. The polymerase complex seems built to be easily activated; many kinds of contact suffice. Given all this, the rules of expression of genes can be very complex and of great variety. A large number and variety of regulatory proteins can be bound at a large number and variety of separate *cis*-regulatory elements. The rules can change by way of changes in the DNA sequences of regulatory elements, and these changes seem nearly unconstrained. The regulatory proteins are not overly specific in their sequence requirements for binding; they seem on the edge of binding anyway, and new sequences for their binding seem easy to originate. They also seem also able to interact with a variety of other proteins. Thus they seem well suited, even selected, for establishing contingency of gene expression.

With contingency comes linkage (a concept also emphasised by M. Kirschner), in this case the coexpression of different genes in response to a single condition of availability of regulatory proteins because the same ones can bind to regulatory elements of two or more genes. This allows an unlimited variety of combinations of expressed genes with particular conditions related to time and place in the multicellular organism. Changes of contingency and linkage allow the compartmentalization of processes, then the diversification of their use after duplication. Contingency and linkage have evolved greatly in metazoan evolution. The processes for establishing contingency and linkage are part of the cell's evolvability. The conserved components and processes excel at diversification. This would seem a selectable trait.

2. SIGNAL TRANSDUCTION

As H. Bourne discussed, the biochemical steps of signal transduction pathways have been largely conserved among multicellular eukaryotes (and indeed some pathways such as those of tyrosine-kinase receptors seem exclusive to metazoa), but there is near unlimited diversification at the two ends of pathways; namely, with regard to the ligand-binding sites of receptors themselves and to the target proteins. All pathways seem to lead to the activation of one or more of a conserved set of five or six kinases or to Ca^{2+} release. Target proteins are either modulated (activated or inhibited) by phosphorylation by one or more of these kinases or by the binding of Ca^{2+}-calmodulin. Both reactions effectively alter protein activity. Receptor families such as seven-pass serpentine receptors are conserved in the intracellular parts that interact with the G-proteins but their extracellular ligand binding sites vary greatly, there being over 300 of these receptors now identified in different metazoa, not including the myriad odorant receptors. Any cellular protein is a potential target of these kinases or of Ca^{2+}-calmodulin. By variation and selection, target proteins gain sequences that are phosphorylatable or suitable for calmodulin-binding; this is probably easily done because the kinases have low sequence specificity and calmodulin can be bound in several ways. Allostery probably underlies the activation or inhibition of target proteins, and this makes modulation easy. The whole system seems readily diversifiable in terms of which extracellular conditions can be connected to which intracellular responses. By these means, all conserved cell processes including transcription are made contingent on extracellular conditions. Again, the contingencies of basic eukaryotic cell processes have evolved greatly (the when and where of their use) but the processes themselves have not, nor have the basic means to establish contingencies. The metazoan cell's evolvability is also contained in these conserved signal transduction pathways.

Other examples were given by the speakers but these two are especially summarizable and exemplify the highly diversified and highly diversifiable means for contingency and linkage of the eukaryotic cell's conserved molecules and processes. The cell's capacity to discriminate conditions outside and within itself, its capacity to generate conditions to which other cells respond, and its capacity to give individualized responses, all these arise out of contingency and linkage. All are integral to metazoan evolution.

Prokaryotes have contingency and linkage too, though less of it. It would seem they could have diversified their receptors and target proteins (for they have several signal transduction systems), and could have diversified their transcriptional regulatory elements (for they do have such sequences). Why didn't they? Perhaps because the selective context of multicellularity wasn't there, in which the varied conditions set up among cells become so important. There seems to be little constraint on the eukaryotic cell's evolution of new contingencies and linkages. The conserved processes in signal transduction and transcription provide the means to diversify. They exemplify the cell's evolvability, a concept dear to evolutionary biologists, but I think less convincingly apparent in their anatomical organismal examples than in examples to be found at the molecular-cell biological-process level.

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3. HOX GENES AND BODY PLANS

Conservation and diversification also go hand in hand in the complex development of the metazoan body plan, the highest level of conserved multicellular organization. M. Akam discussed the central place of the Hox gene complex and its pattern of gene expression in the body plan of arthropods, with some reference to chordates as well. These genes encode members of the homeodomain family of DNA-binding proteins, which are transcriptional regulatory proteins creating contingency and linkage for a wide variety of target genes. The 8 genes of arthropods, the 10–13 of amphioxus and the 38 of vertebrates, although different in numbers, are conserved with respect to their clustered organisation (one cluster in arthropods and amphioxus, four in vertebrates) and in their colinear order of expression. That is, each gene is expressed in a spatial domain in the anteroposterior dimension of the body, and the order of domains in the body is the same as the order of genes in the gene cluster on the chromosome. There seem to be many cross-activations and cross-inhibitions among the genes and proteins of the complex, which together constitute a complex network. The genes are first expressed at a middle stage of embryonic development, the so-called phylotypic stage, which arises after cellularization, morphogenesis, and an initial minimal establishment of regional differences within the embryo. Other gene products activate the Hox genes, but once activated at the phylotypic stage, the Hox genes persist in expression and activate many other genes contributing to later development. There are two aspects of diversification to consider.

1. Target genes in late development: the Hox genes are selector genes in the sense that their products activate or repress the expression of a select set of other genes, the target genes. But more than that, the Hox genes are region-specific selector genes. Each is expressed in a unique domain of the body and then confers region-specific expression on its set of target genes. Targets can change easily in evolution, for example, with changing tagmatization in arthropods as M. Akam described. Presumably the change just involves the coming and going of regulatory sequence elements near these target genes. Hox gene products are a marvel of evolution in their capacity to establish contingency and linkage of target genes, and hence their capacity to couple gene expression to position in a multicellular population, one of the big challenges for the evolution of development. Even though homeodomain proteins are ancient, their use in development was new in the new spatial contexts of metazoa.

2. Hox gene activation in early development: these genes are first activated and confined to domains about the time the body plan is first established at the phylotypic stage. Are 38 region-specific conditions needed in the early embryo to activate the 38 Hox genes in the right places? Does the prephylotypic stage have to be as complex as the phylotypic stage? Probably not because the Hox complex seems to have network properties, that is, to behave as an integral unit rather than a collection of individual members. Perhaps only a few region-specific conditions are needed in the early embryo to get the entire network to turn on in its stereotypic ordered way. Perhaps if a few Hox genes are activated, they activate the rest of the members due to network interactions. Thus even though the network may be constrained to change because of the interconnectedness of members, there would be a valuable trade-off. It would be semi-autonomous and partly self-patterning, and would therefore minimize the requirements it places on other processes before and parallel to it as to what it needs to be activated, oriented and scaled. Although internally constrained, it would place only minimal constraints on other processes. Such concentration of constraint in one system might even be selected for if other processes were in turn deconstrained for diversification. Thus the Hox gene complex seems to have two assets: it easily brings any target gene into region-specific expression for use at stages of development after the phylotypic stage, and it places few demands on prephylotypic stages for its initial activation.

The phylotypic body plan, which includes the Hox network in its makeup, shows the same two faces of conservation and diversification. Hox expression domains are a prominent aspect of the anteroposterior organization of the body plan, but there are probably also many selector genes of the dorsoventral dimension and of the germ layers as well (and of segmental units in arthropods and annelids), and all of these come together in the body plan. Many arguments made for the Hox network may apply to the conserved phylotypic body plan. This plan is the set of traits common to all members of a metazoan phylum. Some traits are distinct to the phylum, and some are shared with other phyla. These phylotypic traits show up for the first time at the phylotypic stage in the course of development, before the development of the diversifed traits specific to the classes and orders. In chordates, the pharyngula is the phylotypic stage; it is formed shortly after gastrulation and neurulation, still long before organogenesis, differentiation of cell types, hatching, or birth. The segmented germ band is the arthropod phylotypic stage. Why is this stage conserved whereas stages before and after it diverge widely? It is not enough to say the phylotypic stage is constrained, for although this may be true, many constrained processes have probably disappeared in the 500 Ma that modern body plans have persisted.

Presumably the body plan has selective advantages, and these may be similar to those cited for the Hox complex. The phylotypic body plan is greatly added to and modified at stages after the phylotypic stage, to give the diverse class and order differences of the larva, juvenile and adult. These modifications are adaptations of feeding, movement, defense and reproduction of the hatched animal. As a speculation, the phylotypic body plan may be a multicellular spatial organization to which a particularly large number of additions and modifications can be made, and made with selectable effect in the macroscopic world inhabited by metazoa. That is an advantage worth conserving. It is presumably related to the ease of diversification of targets of the many region-specific selector genes and to

the overall spatial organisation of the selector genes' domains.

What about diversification of development before the phylotypic stage? Early development of different classes of the same phylum differs greatly, but why should this be, when all paths lead to the same phylotypic stage? The differences may not concern the development of the body plan itself, but serve as reproductive adaptations of the class or order for the survival of pre-feeding stages of the life cycle, both as specializations of the egg and of extraembryonic tissues. Just as for the conserved Hox gene network, the conserved bodyplan may have the advantage that its development makes rather few demands on the processes of prephylotypic stages, thereby leaving the stages free for diversification in the realm of reproductive adaptations, which are very important for survival of offspring and entry into new habitats (e.g. the amniote egg). Thus the conserved body plan may be advantageous for different reasons for the diversification at stages before and after it. Its properties may have opened up the life cycle. Although internally constrainted, it only minimally constrains other processes, yet it is facile at creating contingency and linkage of other processes. Its internal constraint may even be selected as the means of its semi-autonomy. Thus constraint does not imply non-evolvability of the cell or organism with regard to its other aspects. One needs to look at the whole cell, whole organism, and whole life cycle. Conservation and constraint may be a strategy for diversification and evolvability. The study of conserved processes may lead to an appreciation of the cell's means for evolvability, and a more complete picture of what has suited eukaryotic cells for multicellular evolution, and of what aspects of various conserved processes support and facilitate evolutionary change.

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