The Biology of Complex Organisms

Creation and Protection of Integrity

Edited by Klaus Eichmann

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Klaus Eichmann
Freiburg, August 8, 2002

Preface

On December 6, 1961, a contract was signed by which the research institute of the Wander AG in Freiburg became the Max-Planck-Institut für Immunbiologie. The transfer of ownership took place during a happy expansion phase of the Max-Planck-Society in which a growing economy in Germany allowed the foundation of many new research institutes by the Max-Planck-Society and other organizations. Nevertheless, it was a remarkable event. The acquisition by an academic organization of an institute formerly operated by an industrial company was rather unusual, not to speak of the fact that not only the facilities but also the entire scientific personnel were taken over. Retrospectively, the 40 years of the institute in the Max-Planck-Society can be divided into 2 very different phases of 20 years each. The first 20 years were characterized by a continuation of the research that had begun in the Wander institute and centered on the structure and function of the bacterial compound endotoxin. During the second 20 years, the institute more than doubled in size and developed into an interdisciplinary research center that focuses on the development and organization of multicellular systems by combining studies in two fields of research: immunology and developmental biology.

The 40th anniversary of the foundation of the Max-Planck-Institute was celebrated by a ceremony including a scientific symposium. The first part of this volume presents the lectures given at the symposium by six leading biologists. They present their views on principles nature uses in the evolution and generation of complex organisms such as mice and man, and how the immune system manages to protect their integrity in a hostile environment. The lectures were initially not meant for written publication. All of the lectures, however, were very well received by the attendees not only because they were given in a style appreciated by a general audience. Although by no means covering the subjects in any comprehensive or complete sort of way, they nevertheless appear to present well focused and thoughtful accounts of some of the issues that are in the center of present day scientific and public interest. In addition, the lectures lend strong support to the philosophy of the institute that understanding complex organisms is one of the continuing challenges of biology and that the general principles that govern such organisms are best unraveled by studying multicellular systems in their various forms. As a result of the very positive feedback, it seemed worthwhile to compile the materials in a book.

This volume is a *Festschrift* celebrating the 40th anniversary of the Max-Planck-Institut für Immunbiology. Accordingly, the scientific lectures are complemented by historical accounts of the early and late phases of the Max-Planck-Institute and its standing within the history of immunological research in the city of Freiburg. In addition, the volume contains a DVD video diskette featuring the speakers in short sections of their talks, for those who are interested in the human individual behind the science.

Klaus Eichmann
Freiburg, August 8, 2002

Part I: Symposium

The Biology of Complex Organisms –
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Evolution of development

Lewis Wolpert

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Thank you very much, I am flattered to be invited to talk at your celebration – congratulations on your successes. The relationship between evolution and development is a very fashionable field at the moment. My approach is rather different as I focus on how the embryo and multicellularity evolved. I do not know the answers so it is in a sense a just-so story. Rudyard Kipling wrote wonderful just-so stories. There is, for example, a tale of how the camel got its hump and how the elephant got its nose – the crocodile pulled it; my story is rather of that category, it is a just-so story. The supporting evidence is not very strong.

The basic organisation and functions shared by all eukaryotic cells but not prokaryotes, must have been present at least 2 billion years ago, before single-celled eukaryotes diverged. This conservation would include their large size – 1,000 x the volume of the prokaryotic cell – their dynamic membranes capable of endocytosis and exocytosis, their membrane-founded organelles like the nucleus, mitosis and meiosis, sexual reproduction by cell fusion, a cdk/cyclin-based cell cycle, actin- and tubulin-based dynamic cytoskeletons, cilia and flagella, and histone/DNA chromatin complexes. These ancient processes which evolved in the single-celled prokaryotes and early eukaryotes long before metazoa, constitute the core biochemical, genetic, and cell biological processes of metazoa.

These eukaryotic cells were doing very well. Why did they bother to get together? And what had to be invented in addition to what the eukaryotic cell already had to make the embryos? Let me make my position clear. The miracle, and I do not mean it in the religion sense, I mean it in the evolutionary sense, the miracle of the evolution, is the cell. While there are theories involving an RNA world and self-organising, it remains a mystery. Once you had the eukaryotic cell from the point of view of evolution and development it was downhill all the way, very very easy.

Development requires turning genes on and off, cell signalling and transduction and cell motility. The ancestral cells had these. Lower eukaryotes, such as flagellates, slime moulds, ciliates, and yeast cells, have many control mechanisms known from metazoa. Cell differentiation depends on different genes being active in different cells and the cell cycle can be thought of as a developmental programme. There were kinases turning processes on and off, and also genes being turned on-off. There was also signal transduction of stimuli arriving at the cell membrane. Signalling in unicellular eukaryotes was believed to be confined to mating factors in, e.g., ciliates and yeast cells. It is now evident that unicellular eukaryotes depend

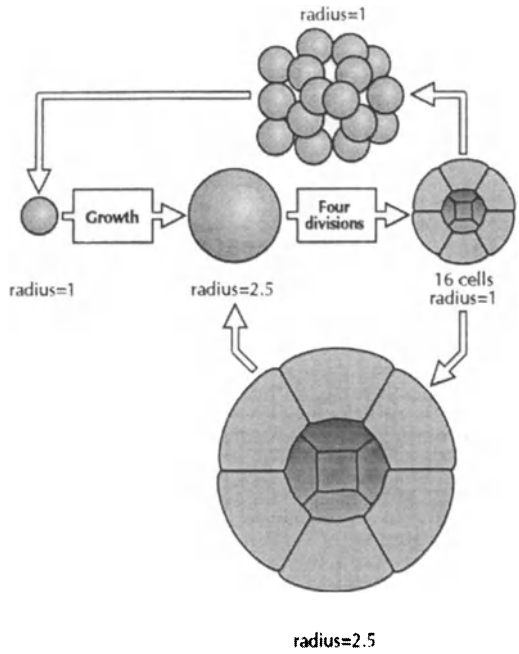

Figure 1. Generation of multicellularity by growth and division.

on extensive signalling systems for their existence. There are also many similarities of the intracellular transduction systems in uni-and multicellular organisms. Eukaryotic cells had motility and chemotaxis. While the slime moulds have nothing to do with the origin of the embryo, they branched from the metazoan line shortly before plants and fungi, they have cell-cell signalling involving several components shared by metazoa, such as cAMP, G-protein linked receptors, a variety of protein kinases, and JAK/STAT transcriptional control. From their unicellular past, early metazoa had a lot to draw upon in the evolution of intercellular signalling.

Single cell organisms have molecular motors and these could provide the forces for morphogenesis. Chemotaxis in the slime mould *Dictyostelium* provides an important model for cytoskeletal organisation and signal transduction and chemotaxis is important in its own right. The chemotactic cell is polarised and polarity is fundamental to many developmental processes. Ligand binding leads to rearrangement of the cytoskeleton-actin polymerisation at the anterior end and results in filopodial extension while myosin at the rear contracts to bring it forward. This illustrates how complex the cytoskeleton already was.

So I want to say my first argument is that eukaryotic cells had everything. We know they did not have collagen or other extracellular matrix molecules , but I am not impressed by that argument as that could come easily. Among the basic components required for development, I can think of virtually nothing that eukaryotic cells did not have which is required for the developmental processes. And so, the real question is why did they bother to get together and what was the adaptive advantage?

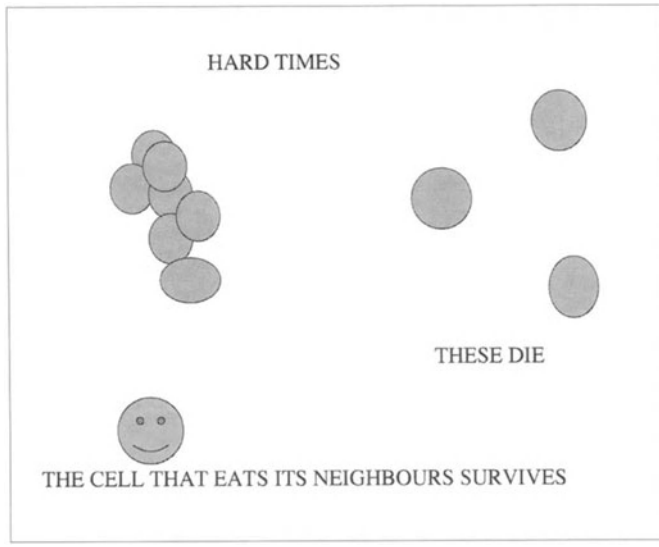

Figure 2. Selection for multicellularity by altruistic cannibalism.

I originally thought that a cell by mistake grew large and then subdivided into quite a large number of cells (Fig. 1). But I could not see the adaptive advantage of that. There are arguments, that being multicellular allows it to have division of labour and specialised functions. But that cannot have been the original selective advantage. So, our argument goes like this. There was mutation in a single cell so that when it divided the cells stuck together. Further division resulted in a loose colony of cells. Now this had an advantage or might have been an advantage if there were other sorts of unicellular predators around. They were more difficult to eat. But what was the real advantage? In hard times when there was no food around and single cells could not survive, some cells in the colony could then eat each other and so survive. If some cells died, then cells could eat them and survive, and that is a major advantage (Fig. 2). It is also the origin of cell death.

There is current evidence to support this idea. If you take planaria or hydra and you starve them, they get smaller, keep their normal form and the way they do is by the cells eating each other. The way the sponge egg develops is by phagocytosing neighbouring cells. In certain fish and annelids what happens at the time of reproduction is that the adult eats an enormous amount of food and devotes all of its body almost to feeding the egg. In fact, muscle cells are actually broken down and phagocytosed, the eggs are laid and the animal dies.

At a later stage in the evolution of this simple multicellular organism it was an advantage to identify the cell which was going to be fed by the others and become large. This is the origin of the egg. The cell might have been under different external conditions, for example near the centre of the colony, such that it would enable it not to die, and therefore to feed on the neighbours. The other advantage of course of having the egg was that it avoided general conflict. While a colony of inde-

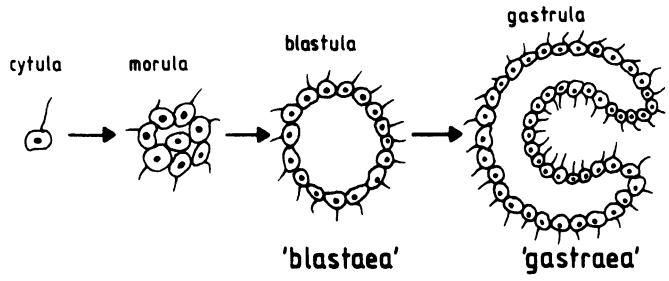

Figure 3. Gastrulation.

pendently reproducing cells could have been successful, mutations in all the individual lineages would have occurred and accumulated. This would have had two severe disadvantages. The first would have been at the level of how cells interacted in the colony. The cells would acquire different genetic constitutions and this would have led to competition rather than cooperation between the lineages. Secondly it would have been difficult for the colonies to lose deleterious mutations or mutations in general, including those reverting to unicellular state. The solution to these problems lay in the evolution of the egg; if the various colonies arose from a few germ-like cells with low mutation rate, then the competition and the mutation problems would both disappear. It is not too difficult to imagine a series of mutations which would have given the inner cells an advantage with respect to eating their neighbours so that in hard times the outer cells died. Our origin, I claim lies in what one might think of altruistic cannibalism.

Haeckel really played a very important role in thinking about evolution and development. He had the idea about ontogeny recapitulating phylogeny which turned out to be incorrect. Ontogeny does not recapitulate phylogeny, the reason why we have something like fish gill slits in our embryonic development is that we partly retain ancestral early embryonic stages. However on one evolutionary change I think he was right, and that is in relation to gastrulation. He has near the bottom of his evolutionary tree what he calls the gastraea which evolves from the simple blastea (Fig. 3). Here there is real evidence that ontogeny really does recapitulate phylogeny. All animals pass through a gastraea – like stage, they gastrulate. Why is gastrulation so similar in all animals? I want to argue that it does actually recapitulate an ancient ancestor.

There is a very simple organism, *Trichoplax*, which is made up of just a single layer of cells and a hollow interior. It is rather like Haeckel's blastea.What is remarkable in *Trichoplax* is that it undergoes a change similar to early gastrulation while feeding. Particles of food or microorganisms that it is going to eat are moved into a digestive chamber.

The basic idea is that a two-layered primitive organism fed on the bottom and it formed an infolding to aid feeding. This basic idea comes from Jaegerstern. In a blastea-like organism, a hollow organism made of a single layer of cells, the feeding was encouraged by currents from cilia.

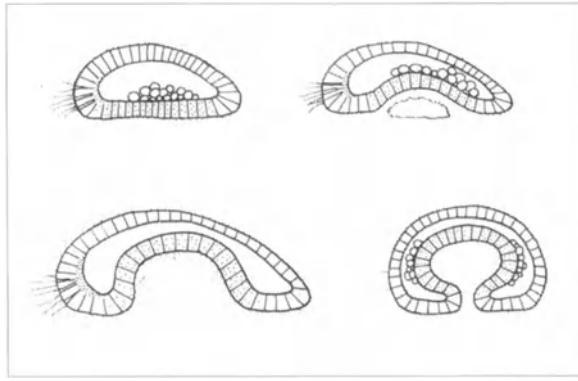

Figure 4. Evolution of the gastrula from feeding.

Living on the bottom it formed an invagination to sweep the food into a primitive gut where the cells are that would engulf the food (Fig. 4). It takes no stretch of the imagination to see that all it had to do is to fuse this infolding with the sheet on the other side and you then have a mouth, a gut, and an anus.

Little had to be invented to reach this stage, everything was there in the cell, and in a way the embryo is really much less complicated than individual cells. The complexity of the developmental biology does not lie in the embryo, but lies in the individual cells. If you look at the signals between cells there are less than 10 grand families of signalling molecules between cells and this is trivial by comparison with what goes on inside cells.

One of the things that need to be thought about in relation to the evolution of the embryo is what of the selection pressures on the embryo itself. Now, I like to liken the embryo to medical students at my university. Medical students can play around, not come to lectures, spend their parents' money, get drunk. Only one thing matters, they have to pass the final exam. It is the same with embryos. They do not have to look for a home, they do not have to mate, and energy expenditure is trivial; the only thing they have to do is to reliably give rise to the adult. About 25% of the cell's ATP goes on keeping sodium out of the cell. So just being alive is expensive. Making one more gene or movement from an evolutionary point is trivial. What matters is reliability. There may be little selection for a more efficient way of, for example, gastrulation. Cnidaria take very different pathways to the planula (Fig. 5). This is a standard way by invagination giving it with two-layers but in yolky egg cell death is the mechanism.

How did cells in the colony evolve different identities and so establish well-defined patterns of cell activities? There are really only two major mechanisms by which cells acquire identity, one is by asymmetric cell division together with cytoplasmic localisation. The other relies on interactions (Fig. 6). An important mechanism in the evolution of the spatial patterning of the embryo could have involved the Baldwin effect which I learnt about from one of my teachers, Conrad Waddington. An environmental stimulus, such as contact with the substratum which

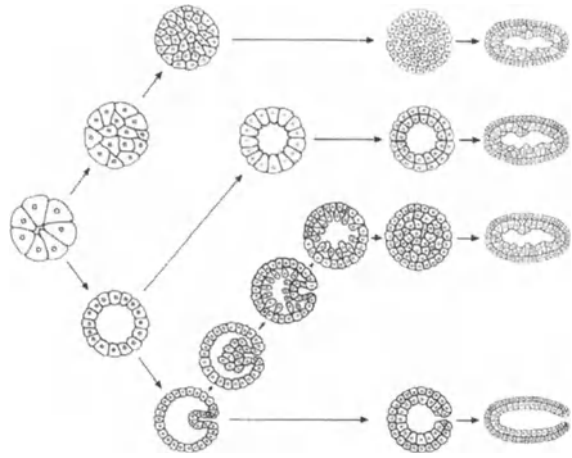

Figure 5. Cnidarian forms of gastrulation.

brings about a change in the cell so that it becomes different from its neighbours, then having established that machinery it becomes intrinsic. For example there are mutations which lead to the thickening of the soles of the feet due to pressure, then this thickening can become genetically determined and occurs only in the feet. I think it is a nice idea, because I think to get thickening autonomously localised to the feet from the very beginning would be much more difficult. And in the same way, one could think of the cell like this, for example, just having contact with a substratum and therefore this region could, for example, secrete a protein and then this could have been used by neighbouring cells, and so perhaps morphogen gradients and positional information could have evolved. Then later on could this become genetically determined. In the same way the cell at the centre of that colony that I

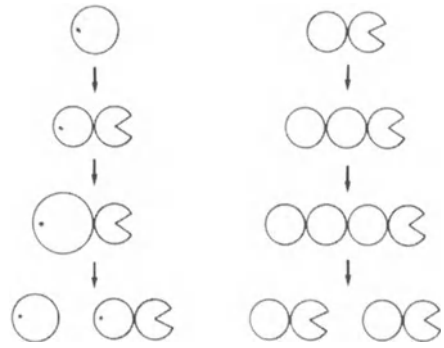

Figure 6. Early development – asymmetry or interaction?

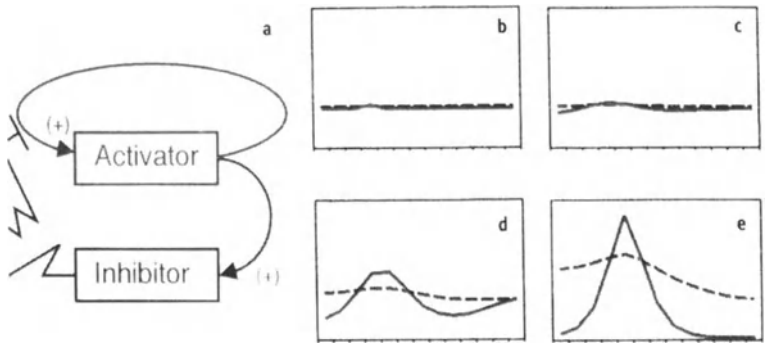

Figure 7. Turing's model of pattern formation.

spoke about at the very beginning, the one that is going to be fed by all the other cells, could make use of the Baldwin effect. It was in the centre of the colony, would have a slightly different environment, and therefore become adaptive and eventually become genetically determined as the egg.

When one is thinking about how patterning evolved it would be very attractive if it used reaction diffusion. The idea of reaction diffusion came from that British genius, Alan Turing who broke your code in the war. He showed that if you had two diffusible molecules, one an activator and one an inhibitor and the activator had a positive feedback, but it also stimulated the inhibitor which inhibited the synthesis of the activator, then with appropriate values of rates this system would self organise (Fig. 7). You could start off with uniform concentrations but it would develop a peak in the activator. And if you increased the length you could get 2 peaks. It is an important possible mechanism of self-organisation. Now, it would be lovely to believe that early patterns in the developing embryo could be based on such a mechanism. Alas, there is not a shred of evidence for it. I do not know of any evidence in the whole of the biological literature which persuades me that the reaction diffusion is actually in operation.

Gene duplication as an important mechanism is so obvious to an audience like you, I am not going to pursue but of course gene duplication was absolutely essential; the virtue of gene duplication is that the cells have now two copies of a gene that is functioning, and so they do not need the other one and it can acquire a new function. And of course, I think that was absolutely fundamental to the evolution of development of the embryos.

The development of early embryos was probably rather messy, they were not very reliable nor canalised to limit the effects of variations in unrelated genes. It did not matter, they had time. We are talking of hundreds of millions of years for things to evolve. They could play around as I told you, so long as some of them passed the exam and gave a good phenotype. Reliability in development has not received the attention that it deserves. Odell's group made a mathematical model to explain an early aspect of patterning in the *Drosophila* embryo in relation to segment polarity genes. What turned out was a surprise, as with their complex network of

gene interactions with detailed parameters and rate constants, they could get the same results with quite large variations in the rate constants. Yet if you take isogenic nematode worms you let them grow up under exactly the same conditions. They all die after about 15 days, but there is considerable variation in the time of death. The idea that clones are identical should be treated with suspicion. Examination of the hypothalamus of human identical twins shows that the number of cells in the hypothalamus can vary by as much as 20%. Development is reliable, yes, it passes the exam but sometimes there are variations in the degree of success.

Division of labour could provide advantage once organisms were multicellular. This has been widely claimed to be a crucial step in the evolution of multicellular organisms, though the benefit has never been established. Bell & Koufopanou, for example, suggest that the unexpectedly high rates of increase shown by colonial algae are made possible by the division of labour between somatic and germ cells. For example, if the somatic cells are a source and the germ cells a sink, then there is the possibility that end product inhibition which may act as a negative feedback mechanism for resources could be reduced.

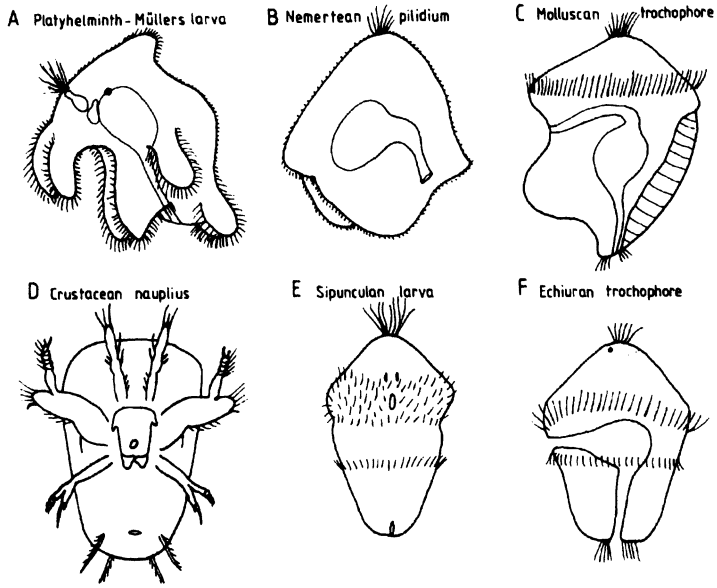

Figure 8. Larvae.

Larval forms are extremely wide spread throughout the animal kingdom and so is metamorphosis, that dramatic change from the larvae into the adult. And the question is how could it have evolved and why do so many larval forms look rather similar (Fig. 8)? It is generally accepted that the larvae of insects were intercalated. The original fly already was a fly-like animal that would develop wings. Only later did they evolve larvae to make use of vegetation and food. However Eric Davidson and his group have argued that in evolution, and this is important from the point of view of evolution of development, the larva is the primitive form and adults came

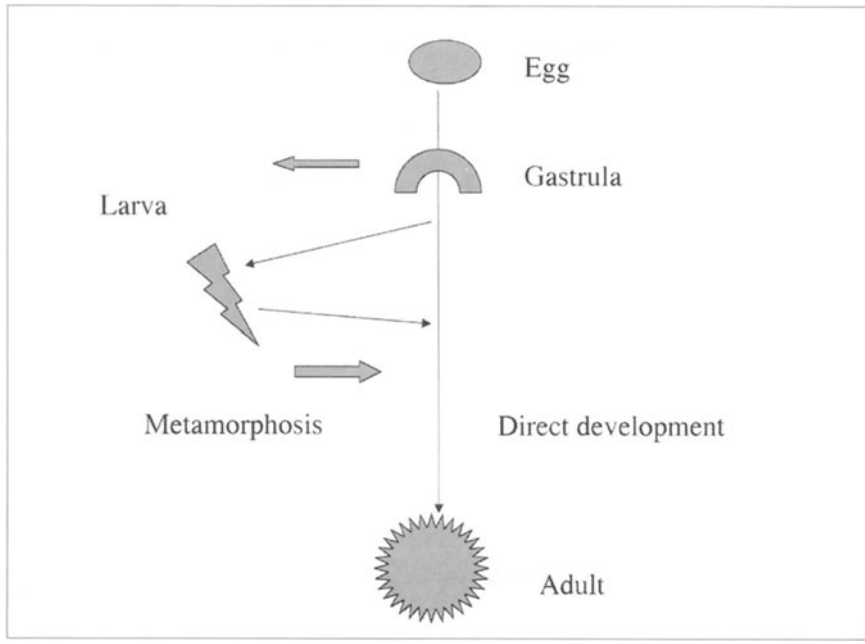

Figure 9. Intercalation of larvae.

later. The argument they make is the following: that the larvae were primitive and then for reasons we do not understand a group of cells called set-aside cells developed. And they are those that in later evolution gave rise to the adult which involved metamorphosis. Therefore, the larval form was the original organism and metamorphosis evolves later.

I wish to argue this is totally and utterly impossible. Just think of the frog. When a frog begins to develop a larvae is made, the tadpole, and then you get metamorphosis. I cannot imagine any of you wishing to argue that the tadpole was what evolved into the frog. What clearly happened with the frog was that an early talebud stage in normal development became a bit motile, it swam away a little bit. What is the function of a larva? Larvae only have two functions, one is dispersal. The last thing you want to be if you want to succeed is to be with all your siblings. When the eggs are laid, you want to get away. The other evolved later was the ability to feed and therefore to get further away and to be able to get bigger. And do not think any rational person really argues about the tadpole being primitive and evolving into the frog.

For these reasons the evolution of larva and metamorphosis must be due to intercalation of the larval stage into a directly developing animal (Fig. 9). A stage of embryonic development or early growth becomes modified to form the larva and that metamorphosis is essentially a return to the original direct developmental program. It is not possible to image a scenario in which set-aside cells in a larval-like form could evolve to give an adult whose form is different to that of the larva.

It is only by modification of the direct development that metamorphosis becomes possible.

One of the points that Davidson rightly makes is that the larvae of very different groups are extremely similar. This is a quip from one of their papers, in which it says 'It seems to us the epitome of hand waving to accept that the larvae of distinct sets of phyla could be due to parallel evolution'. I intend to wave my hands because that is precisely what it is. And let me now persuade you that that is the case.

In the metamorphosis of the sea urchin larva into the adult quite a lot (by no means all) of the adult comes from a small group of cells on the left hand side of the larva, and these are what Davidson calls set-aside cells. I will offer a bottle of champagne to anybody who can give a plausible scenario whereby in evolution first of all you could have selection for set-aside cells; but more important, given a small group of cells like this, that could evolve in the future metamorphosis to give rise to a sea urchin. I claim it is totally and utterly impossible. I wish to argue the complete contrary, namely that all larvae are intercalated into direct development just as in the case of the insect and the frog. What metamorphosis is about is that during direct development an early form shortly after gastrulation becomes the larva, able to disperse. Metamorphosis is to bring it back onto the direct developing programme and to develop into the adult. So these are two very strong counter views.

The reason why larvae look alike is because of gastrulation. They all developed from a gastrula and all invertebrate gastrulae are rather similar. Here is a recent paper on the protostome and the deuterostoma and the gastrulae in both; the way the mouth forms is fundamentally different. In the sea urchin for example you get the invagination, this structure will become the anus, this structure will become the mouth. In the proteostomes there is a complicated process where this structure becomes divided and the mouth and the anus form from the same region, but ultimately end up very similar as larvae and closely related to the gastrula. And so, the hand waving is not that it is surprising that all larvae look alike, it is inevitable. Because that is how they evolve from the gastrulae which are so similar in very, very many forms. Incidentally, once larvae had evolved, the evolution of metamorphosis to give the adult form is quite complicated, and it is hormonally based. All the current evidence is that the hormones that are used were those that were related to those already there, because they were for the control of growth in the adult.

One of the problems in evolution of development is how the complexity of signal transduction can be understood. This can be illustrated by a Rube Goldberg cartoon and it is my model for signal transduction whose design I do not understand. The signal is rain, and the end result is to ensure that a person can smoke his cigar, and so an umbrella goes up. So the rain falls on a prune, which moves a lever, which lights a lighter, which lights a candle, which boils this kettle, whose steam blows a whistle which frightens a monkey. This monkey jumps onto a swing which cuts a cord holding a balloon which goes up, and releases the door of a cage so birds fly out and pull the umbrella up. I think that is a simplified version of signal transduction as there are many monkeys.

I think it is wonderful, but the question I am interested in is how could this have evolved? How could all that signal, and the only clue that I can offer to you is from hearing a talk by Adrian Thompson, who worked at the University of Sussex. There is an interesting group at the University of Sussex, where biologists and engineers have got together and the engineers are making use of biology to apply to engineering, one of the things which they have. Adrian uses genetic algorithms to design a circuit, he is an electronic engineer and he wants to design a circuit in which he says, Go, light goes on, and he says Stop, light goes off.

He sets up on his computer little circuits almost randomly connected to the unit. Then he looks, he tests them to see if any have some of the properties that he is looking for. And then he mutates it and repeats the process. He goes through around 600-800 iterations. In each case he is simply selecting the circuit that is getting closer and closer to what he wants. At the end, he has a circuit that works. He then looks at the circuit and has not the foggiest notion how it works, it takes him 3 to 4 months to find out how that circuit actually works. In some cases it has actually invented a clock. And the point that I am making is that when we come to look at signal transduction and many of the other pathways we are looking at them as we would have designed them. Evolution is not like that. All that matters is that it works. I think the experience of these genetic algorithms here is a very important way to begin to try and understand and get some insights into the evolution of development.

Thank you for listening.

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The cloning of mammals: what are the problems?

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I want to talk about a topic which is of public interest, the cloning of mammals. When you look at the media, for example the New York Times Magazin´s cover a couple of months ago, what emerges is the issue of the mad scientist, of out of space sects which believe that life came to earth by cloning, and so on. I think this is a ludicrous debate and I am not going to go further into that. There are rather serious and interesting issues behind this and these issues have been raised already half a century ago, in the seminal experiments with frogs done by Briggs and King, Gurdon, DiBernardino and by others. The principal questions which were posed then were: Does differentiation involve loss of nuclear potency, and is there nuclear differentiation? Can a nucleus of a terminally differentiated cell be reprogrammed to participate in development of an animal and the differentiation of all lineages. These are essentially the same questions we ask now, in one way or the other, and I will discuss these questions. In the second part of my talk I will actually bridge the topics of this institute. I will talk about immunology, including B and T cells.

Let me come to history. In the 50ies and 60ies the people I mentioned above took donor nuclei from either early frog embryos or from later embryos, tadpoles, from gut cells for example, implanted them into enucleated oocytes and asked the question, can they direct development to animals like tadpoles or adult frogs. And very rarely, Gurdon indeed got frogs. However, success was so rare that the origin of the donor cell was never really certain. I will come back to this problem. I think this is a major problem also in present day cloning experiments. The interpretation of the results was yes, the nucleus can retain totipotency but this appears to diminish with development. This was not a really clear conclusion. In the early 80ies, a very important experiment was done by Davor Solter by transferring nuclei from very early cleavage stage embryos into enucleated zygotes. He observed that all clones died within a few divisions. I think that these were very carefully controled experiments. It was a very important issue at this time and it suggested in a way that mammals could not be cloned. By another approach, using the oocyte as a recipient, it was clearly shown later that mammals could be cloned, for example sheep (Dolly) and then a number of other species that have been cloned in succession. What are the questions here? I think nuclear cloning is a really important tool for science. A number of different questions concern the epigenetic state in particular, in which I have been interested for a long time. We have a number of cell types in the body, we have embryonic stem cells, we have adult somatic stem cells, most of which are elusive, and we have terminally differentiated cells. One of the issues