The Carnegie Department of Embryology at 100: Looking Forward

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I would like to start this talk by extending my sincere thanks to the Carnegie Institution for supporting my laboratory for the last 33 years. As a budding young biologist, getting a job in the Embryology Department felt like winning the lottery. And that was before I could appreciate the full value of what Carnegie offers, or how much the Institution would shape my career by providing consistent, crucial support at every level. What the Department means to all of us who work here has been beautifully expressed during this anniversary Symposium, and in videotaped recollections from former Carnegie developmental biologists, some of which are available at www.ciwemb.edu.

Carnegie's biggest impact over the years has always been the people who train here and then migrate to locations throughout the research and education enterprise. One could almost think of the Department as a stem cell, its graduates as transit-amplifying cells, and the graduates' own trainees as downstream cells. It is extremely gratifying to see how successful Carnegie's progeny have been, to read their papers, and to communicate, visit, and interact with many on an almost daily basis. Notice that I did not describe Carnegie's offspring as "differentiated cells," because it is now clear that Embryology is not just any stem cell, but a germline stem cell, so there need be no limit to the process we are celebrating. Because of this, and because the last 100 years have been described so eloquently throughout the day, I propose to talk this evening about the future of developmental biology.

Speaking about the future of science is a hazardous business. Whether it has ever been done successfully is doubtful. About the only thing you can be sure of is that the future will scarcely resemble whatever is predicted. In the spirit of Carnegie, however, I will nonetheless proceed. Mostly, I wish to focus on a more limited question: Is the scientific style that has characterized research here still relevant for advancing our field? Can a department with 10 laboratories make a significant impact in an era of worldwide mega-biology? Or have we become an institution that is starting to look back too much on its glorious past? In fact, I believe that Embryology's scientific style is critically needed as biology goes forward, perhaps more than at any time during the last 100 years.

The biggest insight from developmental research so far gets less attention than it deserves. It has come from comparing development across different animal groups, or "evo-devo," a field that Nipam Patel helped get started while he was an Embryology Staff Associate in the

mid-80's. Comparative studies have now revealed a remarkable, and largely unexpected, unity of metazoan life. All major multi-cellular animal groups on Earth today appear to have developed from a single early metazoan, a creature that already displayed most of the organs, tissues, cells, pathways, and genes that constitute the basic ground plan of animal life. This realization represents one of the greatest insights in the history of science, and makes the task facing the field of developmental biology seem, for the first time, truly feasible. It is simply amazing and wonderful to know that humans share a basic body plan, organ systems, cell types, and genes with so many of Earth's other creatures—that we are literally variants of a common design.

When I was growing up, I heard that Albert Schweitzer valued all animal life so greatly that he refused even to slap a mosquito. It seemed impossible to me then that mosquitoes could be comparable to ourselves. Now we know that mammals and insects do share core developmental mechanisms. But I still slap mosquitoes. How can I justify that, you might ask? I do it to help them—as a species—evolve to evade humans even better than they already do.

If we can understand this core developmental program, we can understand the development of any particular animal group and species in terms of how they have diverged from that foundation during evolution. Understanding developmental strategies this way should become a unifying paradigm for thinking about animal groups and their characteristics. It means that the insights gained from studying any organism's development can contribute to our knowledge of both its special features and the core. Thus, evo-devo reveals a sort of natural biological coordinate system for thinking about the great diversity of life around us.

The powerful model genetic organisms that have dominated our attention during recent decades are defining major aspects of the core developmental program. Studying species with unusual properties or phylogenetic positions is becoming increasingly valuable as the core becomes better defined. The Embryology Department has done much to advance technology in the major model systems, and also to pioneer new systems, such as the work by Alejandro Sanchez-Alvarado and Phil Newmark on *Planaria*. Curiously, during my time at Carnegie, most faculty members have worked on different organisms. But we have been studying a common program of metazoan development.

Despite the exciting side benefit of understanding life's evolutionary history, the science of developmental biology today is at a turning point. Some of the most important gaps in our knowledge can now be addressed as a result of recently established experimental methods. These are largely the "high throughput genomic" techniques that have generated so much excitement since the days of the genome projects. Embryology's own technical contributions, such as Andrew Fire's discovery of RNAi, rank along with sequencing among the most valuable such technologies. With the approaches now available, we can realistically contemplate closing the gap between genotype and phenotype, the holy grail of developmental genetics. If basic developmental genetic research continues to be supported and pursued by talented researchers, we will map the core program of development at single-cell resolution in several model organisms and determine the effects of altering every important gene and control element. Carrying out such a program in conjunction with

fundamental biochemistry and cell biology has the potential to reveal the basic physiological processes underlying metazoan life, and to change the way we think about many of them. The undertaking might require 25–50 years, but would ultimately be successful. With such knowledge, DNA sequences would become catalogues of detailed information about phenotype and disease susceptibility, transforming biology and medicine.

However, there are many people today, mostly outside developmental biology, who argue against a research program focused on basic studies of embryos and tissues. They think like the old classical geneticists, that it is possible to deduce one's way across the genotype-phenotype divide simply by looking at the nature of mutations and their final outcomes. These people believe that adding a few single-cell assays and building some models using high throughput data will reveal how the genome controls phenotype without ever confronting the complexity of actual developing embryos and tissues. Given the appeal of a short cut to politicians and to many leaders of biomedicine, it is not clear whether our society still possesses the wisdom and long-term vision to complete the genetic analysis of metazoan development and physiology, despite the incalculable benefit that it would provide. Choosing the path of shortcuts and hype would likely surrender our current leadership in biological science and medicine.

Why do we need Embryology and places like it to advance the systematic analysis of metazoan development? Even if our will holds and our technology proves adequate for analyzing development bit by genomic bit, our current intellectual picture of how the genome encodes developmental information may fail us. Biology today resembles the world of classical physics at the end of the 19th century. As the story goes, this was a time when physicists thought all the laws of the universe were known and the only remaining challenge would be to calculate fundamental constants to greater and greater precision. Biological enthusiasts today believe that every important biological law has already been discovered. Information is being collected on how proteins interact and where they bind to chromatin, in databases of greater and greater precision. This information is valuable, especially if one were to compare data from a single cell type (which enthusiasts rarely do). But data analysis cannot begin to address the fact that critical laws of developmental genetics have not yet been discovered. There is a lot we still don't understand, even in principle, about how genomes encode metazoan form and function. We need places like the Department of Embryology in order to develop the biological equivalent of quantum mechanics.

But can't one find missing laws by taking vast biological databases and subjecting them to mathematical analysis? Hasn't biology always been driven more by techniques than ideas? Well, as long as one is guided by a good set of ideas, experiments are often rate limiting. But ideas eventually have their own limits that no amount of data can overcome. To Darwin, doing experiments in geology without reference to ideas was like counting the stones in a gravel pit. In fact, the world is full of data, everywhere we turn. But most data one shouldn't even want to know, and knowing those data is likely only to slow productivity. Data can be accumulated wisely and take on revolutionary meaning only if collected and examined in the context of the right ideas. Our brains are devices that filter out the vast majority of sensory input, and then in fiendishly clever ways construct something sensible from a highly processed fraction. Our brains evolved to protect us from big data. The proponents of data

mining will mostly discover how frequently statistical significance does not correspond to biological or medical significance. Abandoning the search for new ideas represents the perfect corollary to abandoning the study of the actual organisms we seek to understand.

How can new ideas and experimental directions that have the potential to change developmental genetics be encouraged? There is no known way to guarantee such research, but the chance of discovering new principles and mechanisms can be maximized by a department's scientific style and research atmosphere. This atmosphere, like a niche, has a big role in controlling what the departmental stem cells produce. Of primary importance is ensuring that true stem cells are in residence, cells that are flexible and multipotent, rather than committed to a single product. The Embryology Department has never had a specific mission or scientific program. It feels safe to doubt anything here—if you can defend your position. However, stem cells can proliferate rapidly only in a supportive and nutrient-rich microenvironment. The Department is most fortunate to reside in the new Maxine Singer Building, one of the best niches ever for developmental research. We have access to state-of-the-art equipment and are assisted by highly talented specialists. Finally, we recognize the influence of niche signals on stem cell behavior. We place the highest value on discovery, and believe that working in new areas and using new techniques should be encouraged. In 1981, neither Gerry Rubin nor I had ever injected a *Drosophila* embryo. We planned most of our experiments on transformation of *Drosophila* during a Gordon Conference coffee break.

There is nothing so stimulating of stem cell activity as what I think of as “creative disbelief”—doubting an experiment, a paper, a fad, and especially questioning some tenet of our current science. Disbelief suggests many new experiments, whereas belief suggests only experiments that are already being done by others. Overturning an old idea frequently leads to something new; sticking with existing ideas means you will always work in the mainstream. Fields that have accepted a fundamentally wrong idea cannot progress further until they are rescued by creative disbelief. Developmental biology probably has some intellectual roadblocks that need to be cleared away in the coming years at places like Carnegie. In contrast, at many venues, creative skepticism has become less welcome, and it is too often being replaced with hype.

So what might be developmental biology's roadblocks? For starters, the field should beware of relying too heavily on gain-of-function experiments to define fundamental aspects of biology. What cells can do in a situation for which evolution never prepared them is a very shaky foundation on which to build biological understanding. Another looming danger is trying to use simple cell cultures to address developmental rather than cell biological problems. The study of stem cells reveals just how profoundly cells depend on their microenvironment to maintain both their immediate activities, such as cell cycling, and their state of differentiation. It would be fabulous to create an artificial niche that could cause a *Drosophila* stem cell to respond normally to a wide range of challenges. However, today the order in which such experiments can be done successfully is usually reversed. One must thoroughly understand the biology *in vivo* first, in order to guide *in vitro* system development, rather than the reverse.

However, the biggest roadblock may be the “standard model” of developmental genetics itself. This model is based on the notion that cells of various “cell types” have well-defined programs of differential gene expression. Understanding development would mean understanding how cytoplasmic and environmental asymmetries, along with the starting pattern of gene expression, induce an altered program of expression in daughter cells. If a closed cycle of such changes can be understood throughout the life cycle, and if the changes at each step are predicted by the functions of a cell’s expressed genes, then the entire program of gene expression would be explained.

Hopes have declined for predicting gene activity from lists of expressed regulators now that the dominant role of chromatin in controlling gene expression has become clear. Every cell’s chromatin state, by determining how gene regulatory sequences are organized and how they interact with binding factors, specifies both its constitutive and inducible expression program. Acquiring a stable chromatin state must largely correspond to the process of cell differentiation, even though some changes in chromatin state can still take place throughout life. Unfortunately, how chromatin states are specified and how they regulate gene expression remain very poorly understood. It was much easier to imagine predicting gene activity from local combinatorial transcription factor binding, than from chromatin-modifying factors. The idea of enhancer looping remains too simplistic to bridge this gap. How chromatin states are duplicated during S phase and accurately inherited by daughter cells remains equally mystifying.

Our limited understanding of chromatin is not the only problem with the standard model. In a strange parallel to classical physics, the model has been highly successful in explaining cell behavior at a “high” level, where the properties of many similar cells are averaged together. But gene expression in individual tissue cells can be harder to reconcile with the concept of a few uniform cell types. Some discrepancies are probably caused by undescribed, minor cell types with their own expression patterns, something that was found in the *Drosophila* ovary. However, there is increasing evidence of epigenetic and genetic differences among tissue cells of the same type.

Cellular individuality implies that individual chromatin states are not exactly the same, even among cells of the same type. Differences may arise during differentiation because of imperfections in chromatin inheritance or local external influences experienced by only some of the cells. Regulation does not only go in one direction, and differences in metabolism, mechanical forces and microenvironment may feed back and alter chromatin. As a result, how individual tissue cells will behave is subject to a small amount of uncertainty. Developmental programs seem to finesse cellular differences by using patterning mechanisms that are non-autonomous. The exact number of cells and their lineage relationships in a developing tissue rarely matter within broad limits. When cells synthesize a product, signals and feedback systems ensure that the overall product distribution is more accurate than the autonomous responses of the component cells. The weaker or more distracted cells either synthesize longer or their portion of the structure is supplemented by material from neighbors.

The deterministic standard model encounters further problems with progenitor cells, i.e., cells that proliferate prior to the onset of differentiation. Gene expression in progenitor cells varies much more than in differentiated cells, and these changes are stochastic but transiently heritable. The chromatin states of progenitor cells must be subject to organizational shifts that influence expression levels. Progenitor epigenetic instability may be related to their ability to differentiate into multiple cell states. But the molecular nature of these instabilities and how they are resolved during differentiation remain virtually unknown. Current ideas include changes in histone modification or in the levels of higher-order compaction.

Another instability likely derives from the genome itself. On average, it is clear that most cells retain most of the germline genome. But that is very different from assuming, as we do today, that the entire genome is precisely replicated in all somatic cells except for immune cells. Additional diversity outside that coded in the genome appears to be valuable in multiple situations. Site-specific recombination and somatic mutation diversify immunoglobulin proteins, RNA editing alters the structure of membrane-spanning regions of channel proteins, and somatic transposition is proposed to diversify neurons in developing brains. Replication errors provide a significant source of genome variation, especially if a region fails to complete duplication prior to the next cell cycle. The technical means to accurately detect sequence differences between individual tissue cells has been lacking, so we know very little about their nature and frequency. It is not necessary to have an army of clones to run a tissue any more than it is to run a research lab. Tissues cells may resemble cooperating individuals with different strengths and weaknesses. How cell-to-cell genetic variation is produced and integrated with epigenetic differences represents another challenge in moving beyond the standard model of development.

So what is the new quantum mechanics of development going to be? It is too early to know. But this brief summary makes clear how much more we need to learn about chromatin states, how they arise, how they regulate gene expression and how they are inherited and stabilized. The most exciting and important discoveries in developmental biology during the coming years will reveal the molecular events underlying these processes. Many of these solutions will be unlike what we are currently thinking about, and will not come from churning more data according current thinking. These solutions will come from creative individual scientists following their own ideas in the spirit of creative disbelief.

The field of developmental biology needs to put more emphasis on testing new ideas and new alternatives to the standard model. What if the core events of development are controlled by conserved intercellular signals and regulatory proteins, but the fine tuning that distinguishes phylogenetic groups and individual species utilizes species-specific genes and chromatin feedback mechanisms from developing tissues? It might mean that you could differentiate generic cell types in vitro from a pluripotent stem cell, but they would lack detailed features requiring signals that cannot be recapitulated outside of an organized tissue. What if known nuclear RNAs that currently lack functions, including lncRNAs, pseudogene transcripts, transposon transcripts, and read through transcripts, can contribute to chromatin states? A subset of chromosome-bound RNAs might undergo duplication during S phase and associate with both sister chromatids to mediate epigenetic inheritance.

One thing seems certain. How long it takes to decipher the laws of chromatin action and epigenetic inheritance will ultimately determine the rate at which we learn to understand our genomes. We should be celebrating the great challenge of solving how metazoans develop and how genomes really work, not data collection and hype. We should be training individuals who are ready to develop the quantum mechanisms of development. The mechanisms will be simple, beautiful, robust and very likely unexpected. They will be discovered by scientists with the freedom and resources to pursue their own ideas.

Thus, the future of developmental biology is promising but not assured. If conditions for progress remain favorable, within 50 years, and perhaps sooner, we will know in detail how genomes control development, allowing genotype and phenotype to be connected at the levels of the cell and the organism. However, this will happen only if society continues to support basic developmental biology research and if the molecular genetic analysis of embryos and tissues remains central to these studies. Career opportunities in developmental biology must continue to attract their share of the brightest young scientists. Finally, the right ideas must be found to explain how genomes control development. Consequently, places like the Carnegie Department of Embryology, where new ideas can be proposed, developed, and criticized, remain critically important for developmental biology to prosper.

It's a privilege to work in developmental biology—so much fun, so fascinating, so rich, so much to discover. But analyzing development also carries a responsibility to society. Nearly all of us have been aided in our careers by public support through taxes, or from charitable foundations. A scientist's first responsibility to society is to do their utmost to produce research that is true. It is very difficult to know in basic research what is important and what is not. But work that is untrue is not going to repay society for its trust and investment.

Working on development also carries a particular responsibility because society is rightly concerned about understanding the connection between genotype and phenotype. There is still widespread fear of genetic manipulation, especially human genetic manipulation. Having engineered thousands of *Drosophila* genes, I am probably the last person to worry that genetic manipulation will lead to superhumans and super diseases. This is not going to happen, because so far only the evolutionary process has shown the capacity to improve fitness in wild populations. But gene therapy will eventually work efficiently in humans, just as it does in other diverse organisms. Developmental geneticists are in a unique position to provide insight and guidance to the public on the ethical issues surrounding advances in gene therapy and genome editing. We should be prepared to provide sensible advice and to oppose those who would play upon fears of the unknown for their own aggrandizement and to advance their personal agendas.

Having the opportunity to do science for a living must already be considered great good fortune. To search for truth, to immerse oneself in the beauty of biological forms, to look for new knowledge that is potentially of great benefit to mankind, all while working with and mentoring exceptionally smart, creative, and fascinating people, is to live without truly working at all. Indeed, it is profoundly satisfying to join the line of humans throughout the ages who searched to understand the universe by applying skepticism and reason rather than remaining satisfied with myth. Working at Carnegie has provided these satisfactions in a

highly enriched form. I would like to close this talk in the same way that I began: by expressing my profoundest gratitude to Carnegie and wishing the Department of Embryology another prosperous century of science.