Review

Offerings from an Urchin

Susan G. Ernst *

Department of Biology, Tufts University, Medford, MA 02155, USA

A B S T R A C T

There is a natural curiosity about how organisms give rise to offspring like themselves through a series of reproducible developmental events and how, once mature, these offspring mate and continue the process giving rise the next generation. In the mid-1800 s investigators started using gametes and embryos to explore this process. Although the observations and experimental approaches changed over time, embryologists and developmental biologists after them, sought understanding of development and inheritance through the study of gametes and embryos. It is argued here that in their quests to understand these processes embryologists made major conceptual advances that were seminal to the origins of genetics and to the origins of molecular biology. Furthermore these advances derived from the distinct perspective of those investigators with focused interest on the development of the organism. In this essay fundamental discoveries that originated with the sea urchin embryo as an experimental system are used to illustrate this position. The sea urchin has a long and uninterrupted history as a model organism that helped prepare the ground for the emergence of genetics and contributed important aspects to understanding of the central dogma of molecular biology. As molecular biology came of age new concepts and technology of the discipline were transformative for developmental biology and to this day the reciprocal inductive interactions between molecular biology and developmental biology continue to revitalize each other.

© 2011 Elsevier Inc. All rights reserved.

Contents

Introduction

The 70th anniversary of the Society for Developmental Biology is an ideal opportunity to celebrate the study of the embryo and those individuals whose fascination with embryos leads them to search for understanding of perhaps the most amazing of all biological phenomena. Insight gained from investigations of organisms on their journey from the fertilized egg to the adult have been used to answer questions contributing to our basic concepts and understanding of not only development, differentiation, and inheritance but also to the origins of genetics and molecular biology. This essay will build the case that questions central to the study of development, the experiments carried out to answer these questions, and the subsequent results were foundational to the origins of genetics and

to the origins of molecular biology. Experiments with sea urchin embryos will be used to illustrate this thesis. The undeniable central role sea urchins have played in our understanding of embryogenesis amply justifies using an echinoderm as an example of the importance of the study of the embryo to the emergence of these fields. These new fields, genetics and molecular biology, spawned in part by developmental biology, have by reciprocal interactions expanded and invigorated the study of development and differentiation.

Origins of disciplines can be murky and are often better understood when viewed with the benefits of hindsight. There is no doubt that individual events, experiments or insights such as those of Mendel, Morgan or Watson and Crick can announce unequivocally that a new era had arrived in experimental biology. More frequently increased understanding of biological phenomena builds a body of knowledge within a particular discipline, which over time can emerge as an entirely independent area of inquiry. This essay chronicles the history and some of the major scientific advances of investigative embryology using sea urchins that have significantly contributed to the origins of genetics and of molecular biology. In relating this history it is important to keep in mind that our knowledge and perceptions of a particular field change over time and that this can result in proper credit to individual investigators becoming under or over estimated or completely forgotten with the passage of time. An example particularly relevant to this essay is the excellent work of several people who have carefully documented that gene theory has its roots deep in embryology (Churchill, 1987; Coleman, 1965; Gilbert, 1978; Gilbert, 1987; Maienschein, 1989) and yet, this connection is still frequently under recognized except by developmental biologists.

Developmental biology, genetics and molecular biology operate in the realm of a shared knowledge base. The pioneering contributions to this knowledge base from embryologists stemmed from a need to know how it is that once fertilized, the egg begins to develop and differentiate. Throughout the decades and up until the present, this remains the central intellectual problem that developmental biologists have pursued albeit within new conceptual frameworks. As elaborated in this essay, this context for framing questions is responsible, at least in part, for the defining nature of the contributions made by embryologists and developmental biologists after them.

Table 1 contains a list of discoveries made first by researchers using gametes or embryos of sea urchins or other echinoderms or where the results of these investigations have greatly contributed to a particular discovery. This is not an exhaustive list, but rather a selective one drawing attention to some of those investigations that have shed light directly on our understanding of what would become genetics and the central dogma of molecular biology. There are many excellent reviews where one can form a more comprehensive view of the many landmark contributions made by sea urchin embryologists (Briggs and Wessel, 2006; Czihak, 1975; Davidson, 1976, 1986, 1989, 2001, 2006a,b,c; Ettensohn and Ingersoll, 1992; Ernst, 1996; Ernst, 1997; Fantini, 2000; Giudice, 1973; Gilbert and Greenberg, 1984; Giudice,

1986; Horstadius, 1973; Monroy, 1986; Pederson, 2006; Stearns, 1974).

The information in Table 1, serves to illustrate that the value of an organism as an experimental system may wax and wane over the years as new investigators with new ideas and technologies conduct experiments, however, sea urchins have been and continue to be instrumental in breaking new scientific ground. Maderspacher in a recent essay points out how in the 1870 s there was a growing interest in using marine invertebrates for many experiments and wrote that "in a sense, sea urchins were on their way to becoming one of the first real model organisms" (Maderspacher, 2008).

Favorable qualities of the sea urchin contributed to understanding the role of the nucleus in the cell and pronuclear fusion during fertilization

So why and how did the sea urchin emerge and continue to this day as a "model system"? Embryology was primarily an observational science until about the last quarter of the 19th century and the breathtaking optical clarity of sea urchin eggs and embryos drew many excellent cytologists to the urchin. As anyone who has ever had the pleasure of observing fertilization and development of a sea urchin egg can attest, the opportunity to witness the developmental processes firsthand invites questions that beg to be answered. As early as the 1840s Derbes, Dufosse and von Baer had each taken advantage of the clarity of these eggs to study fertilization and embryogenesis (Briggs and Wessel, 2006). In their paper Briggs and Wessel bring back to life the work of these three investigators and present an interesting analysis of the times suggesting reasons why their work on fertilization was "lost" (Briggs and Wessel, 2006). Further advantages of using sea urchins are that the adults are easy to obtain and maintain and can readily be induced to spawn copious quantities of eggs that upon fertilization divide synchronously. As Chabry found in the late 1880 s when he observed normal development after he pierced an egg with a fine glass needle, sea urchin eggs are also sturdy (Sander and Fisher, 1996).

The importance of scientific developments is often best appreciated in the context of the day. The basic tenets of Robert Remak's cell theory were in place by the late 1850 s and had been succinctly summarized by Virchow in 1855, who wrote, "*All cells come from preexisting cells.*" (Coleman, 1965; Wolpert, 1995). Simple as it now seems this concept was an essential insight for developmental biologists to formulate their basic syllogism: "*if all cells arise from other cells, and eggs also give rise to cells, it must follow that all cells of the body arise from the fertilized egg*" (Wilson, 1925). By the 1850 s the nucleus had been observed in cells from several plants and animals and thought to be important by some investigators, however, the nucleus was seen to disappear and re-appear throughout the life of the cell. Not surprisingly, this apparent lack of permanency of the nucleus resulted in differences of opinion about the function and the

Table 1
Selected Firsts or Major Contributions From Sea Urchins in Embryology and Developmental Biology to Genetics and Molecular Biology ~1875–1975.

Fusion of sperm and egg nuclei at fertilization	Hertwig, 1877
Fusion of a *single* sperm with the egg at fertilization	Fol, 1877
First account of maturation of germinal vesicle	H. Fol 1879[*]
Reduction divisions of chromosomes by half in oogenesis and spermatogenesis; understanding of the polar bodies	T. Boveri and O. Hertwig 1887-1890[*]
Chromosomes in nucleus are determinants for development	Boveri, 1902
Individual chromosomes possess different qualities	Boveri, 1902
DNA is found in chromosomes of all cells	Brachet 1942[**]
RNA is found in the cytoplasm of all cells	Brachet 1942[**]
First correlation of RNA and protein synthesis	Brachet 1941[**]
Small amount of new protein synthesis is required for each cell division : predicted cyclin	Hultin 1961
Discovery of long-lived maternal mRNAs	Gross and Cousineau, 1963
Cytoplasmic polyadenylation	Wilt, 1973; Slater et al., 1973
Cloning first eukaryotic gene	Kedes et al., 1975

[*]Farley, 1982 [**]Brachet, 1945.

importance of nuclei and of the chromosomes within them. This question of the relative importance of the nucleus in development and hereditary was to remain unresolved for another 50 years or more (reviewed: Allen, 1978; Gilbert, 1978; 1987).

A major conceptual breakthrough towards understanding the importance of the nucleus was made by Oscar Hertwig and Hermann Fol working independently and yet producing observations complementary to each other's. Their collective discovery that a single sperm enters the oocyte and the male and female pronuclei fuse at fertilization brought further attention to the nucleus making this work not only important to embryology but also seminal to what would become the field of genetics. Oscar Hertwig trained with Haeckel. Hertwig was an excellent cytologist and in 1877 he took full advantage of the clarity of sea urchin eggs enabling him to observe, "*the cleavage nucleus [i.e. the zygote] arises from the conjugation of two different sexual nuclei, a female nucleus which is derived from the germinal vesicle and a male nucleus which is derived from the body of the entering spermatozoon*" (Hertwig, 1877). Although he saw pronuclear fusion for the first time in any animal system, Hertwig did not see the penetration of the egg by a single sperm. Almost simultaneously, yet independent of Hertwig's observation, Hermann Fol who was also a student of Haeckel was able to confirm and extend this observation using starfish (Fol, 1877). Fol was the first to observe a single sperm penetrate an egg membrane and the nucleus of that sperm progress toward the egg nucleus for fusion (Farley, 1982). In 1879 Fol also reported for the first time the appearance and disappearance of the fertilization cone and grasped its importance in facilitating sperm entry into the egg, supplying an early dramatic example of the interaction of two living cells (Farley, 1982; Wilson, 1925). He observed the formation of the fertilization membrane as did Derbes (Briggs and Wessel, 2006) and was the first to recognize its role in preventing polyspermy (Farley, 1982). The first correct account of the maturation of the germinal vesicle and the origins of the egg nucleus also came from Fol's investigations (Farley, 1982). Advantaged by the clarity of echinoderm eggs, Hertwig and Fol were able to make these and several other pioneering discoveries. E. B. Wilson reflecting on the far reaching implications of their findings wrote "*…the cleavage- or segmentation- nucleus, gives rise by division to all the nuclei of the body; hence every nucleus of the child may contain nuclear substance derived from both parents; and this gave the first basis for the conclusion, independently announced in 1884–1885 by Hertwig and Strasburger, that it is the cell-nucleus which carries the physical basic of heredity*" (Wilson, 1925).

Although the role of the nucleus in the life of the cell and the behavior and importance of the chromosomes within the nucleus were gaining recognition, the question of whether it was the cytoplasm or the nucleus that was primarily responsible for development, differentiation and inheritance remained (reviewed: Allen, 1978; Gilbert, 1978, 1987). Through a series of meticulous observations carried out primarily by Walter Flemming the process of mitosis was described and separated into the stages that we know today. Using fixed and live material from salamanders Flemming observed that, in contrast to the reports of others, chromatin threads split longitudinally and partition equally into the two nuclei of the daughter cells (Paweletz, 2001). The observation that chromosomes undergo invariant stages of division and that these processes lead to an equal division of the chromosomes represented extraordinary progress. Flemming published his findings in 1882 in *Zellsubstanz, Kern und Zelltheilung* (Cell Substance, Nucleus and Cell Division) (Paweletz, 2001), about two decades before the rediscovery of Mendel's work and did not associate the events of mitosis as playing a central role in heredity.

Boveri and the Chromosome Theory

It took an additional 20 years to supply definitive experimental evidence proving that the nucleus and the chromosomes within the

nucleus were the factors of differentiation and inheritance. A significant portion of the cytoplasm vs. nuclear debate was played out using results from experiments with sea urchins. One of the leading figures was Theodor Boveri, a superb cytologist and a gifted experimentalist (Baltzer, 1962; Laubichler and Davidson, 2008; Maderspacher, 2008; Oppenheimer, 1963). In 1885 after finishing his medical degree at the University of Munich, Boveri joined the lab of Oscar Hertwig's brother Richard. In 1887 while studying with Richard Hertwig he made his first trip to the Stazione at Naples where he was introduced to sea urchin eggs and embryos (Baltzer, 1964). Boveri recognized that although he and others believed that "*the substances giving the definite and hereditary characters of the cell are entirely contained in the nucleus*" there was no experimental evidence to support this belief (Boveri, 1889). In 1887 Oscar and Richard Hertwig had produced egg fragments by shaking sea urchin eggs. Some of these fragments appeared to lack nuclei and when these merogones were fertilized they occasionally showed signs of cleavage (Laubichler and Davidson, 2008). Boveri like many researchers after him, made use of these merogones. In 1889 he "fertilized" egg fragments lacking a nucleus and carried out his hybrid merogones experiments in an attempt to prove that *definite and hereditary characters of the cell are entirely contained in the* nucleus. The results of his studies lead him to conclude that "*herein is demonstrated the law that the nucleus alone is the bearer of hereditary qualities*" (Boveri, 1889). Laubichler and Davidson (2008) recently published a fascinating analysis of Boveri's sea urchin merogones experiments in establishing the role of the nuclear chromosomes in development and the broader lessons to be learned from the way Boveri initially approached this question and later redesigned his experimental approach. Even at the time this work was published, there were a number of biologists who felt that Boveri's experimental procedure left room for error and could not support this conclusion. T.H. Morgan, who translated Boveri's paper in 1893 for *The American Naturalist* commented in the preface to his translation "*results of this importance must be verified over and over again, until all chances of error (by no means small) are eliminated*" (Morgan, 1893).

Morgan's comments are of particular historical interest. At this time he was committed to the idea that the cytoplasm and not the nucleus was responsible for heredity, and development and differentiation (reviewed: Gilbert, 1978; Gilbert, 1987). During the 1894–1895 academic year Morgan took a leave from his associate professor position at Bryn Mawr and went to work with Driesch in Naples (Allen, 1978). In 1887 Oscar and Richard Hertwig had discovered that dispermic eggs do not develop normally and Driesch and Morgan continued these experiments (Boveri, 1902). As discussed by Gilbert (1978) Morgan's experiments in 1895–1896 showed that the abnormal development was the result of unequal distribution of the chromosomes, however, he interpreted these results to support his belief that it was the cytoplasm rather than the nucleus that contains factors responsible for directing inheritance.

In the late 1890 s the answer to the question of whether the nucleus or the cytoplasm was responsible for development and differentiation still remained unresolved although there were many proponents on the side of nuclear control. E.B. Wilson in his extremely interesting and prescient 1895 book *Atlas of the Fertilization and Karyokinesis of the Ovum* makes clear that he considered the chromosomes within the nucleus responsible for development and differentiation of an organism, and for passing traits from one generation to the next. After discussing the importance of an equal number of chromosomes contributed by the sperm and the egg nuclei at fertilization in several species Wilson using sea urchins as a prime example wrote:

" *These facts justify the conclusion that the nuclei of the two germ-cells are in a morphological sense precisely equivalent, and they lend strong support to Hertwig's identification of the nucleus as the bearer*

of hereditary qualities. The precise equivalence of the chromosomes contributed by the two sexes is a physical correlative of the fact that the two sexes play, on the whole, equal parts in hereditary transmission, and it seems to show that the chromosomal substance, the chromatin, is to be regarded as the physical basis of inheritance. Now, chromatin is known to be closely similar to, if not identical with, a substance known as nuclein ($C_{29}H_{49}N_9P_3O_{22}$, according to Miescher), which analysis shows to be a tolerably definite chemical composed of nucleic acid (a complex organic acid rich in phosphorus) and albumin. And thus we reach the remarkable conclusion that inheritance may, perhaps, be effected by the physical transmission of a particular chemical compound from parent to offspring "(Wilson, 1895).

This passage demonstrates that before rediscovery of Mendel's work and formulation of the gene theory some embryologists were hypothesizing that a nucleic acid within the chromosomes constituted the physical basis of inheritance.

Although Boveri concluded from his 1889 hybrid merogones experiments that the nucleus was responsible for development, differentiation and inheritance he was conscious of potential problems in his experimental design and continued to refine this method (Laubichler and Davidson, 2008). He also took a different approach to solve this problem. Since the experiments reported in his 1902 paper have been discussed by others (Baltzer, 1962; Davidson, 1989; Ernst, 1997; Maderspacher, 2008) they will only be briefly summarized here. Using two sperm to double-fertilize eggs Boveri created triaster and tetraster eggs and observed the effect on development when the first division produced 3 or when it produced 4 cells each with a nucleus. The nuclei within the 3 or 4 cells of the embryo had a variable number of chromosomes. As was already known from the work of Oscar and Richard Hertwig and further analyzed by Morgan and Driesch very few of these double fertilized eggs underwent normal development, however, Boveri improved and extended these experiments in several ways (Boveri, 1902). With the aid of Wein, a physicist friend, Boveri was able to calculated the likelihood that blastomeres from triaster and tetraster double fertilized eggs would contain a full set of chromosomes and realized that he would need large numbers of dispermic eggs to obtain significant results (Baltzer, 1962). In essence he used mathematical modeling to design an experimental protocol that used large sample sizes for separating the three or four original blastomeres that were subsequently cultured separately. Comparisons of the "embryos" derived from cells of the same egg revealed that even when development was abnormal in all of them, they were most frequently "*abnormal in different ways*" (Boveri, 1902). From the analysis of the results of this major experiment, Boveri concluded that

"Thus, only one possibility remains, namely that not a definite number, but a definite combination of chromosomes is essential for normal development, and this means nothing else than that the individual chromosomes must possess different qualities." and that *"the experiments offer us the first exact indications about the role of the nucleus in ontogenesis by the certainty with which they permit us to ascribe the disturbances of development exclusively to the chromosomes."* He went on to say that *"From all these facts, it will have to be concluded that the role of the chromosomes in ontogenesis corresponds rather exactly to the views which have found a brief though not very fitting expression in the designation of these structures as "carriers" of heredity"* (Boveri, 1902).

In his 1902 paper Boveri repeatedly used the term *chromatin* when referring to a component of the chromosomes. He did not address Mendel's newly re-discovered work directly but it was clear that Boveri was aware of his work and had thought about the relationship of Mendel's traits and the chromosomes writing… "*the relevance of this to the results of botanists in studies of hybrids and their descendants, will be discussed separately.*"

Boveri's experiments together with observations of Walter Sutton formed the basis of the Chromosome Theory of Heredity that is also known as the Boveri-Sutton Chromosome Theory or the Sutton-Boveri Chromosome Theory. Sutton was a graduate student with E.B. Wilson at Columbia when he published his 1902 paper. The observations and ideas presented in the paper derived in large part from Sutton's time as a masters student in Kansas in Clarence Erwin McClung's lab (Gilbert, 1978). Interestingly as a graduate student, McClung had spent a semester in Columbia working with Wilson in the late 1890 s (Wenrich, 1946). Though Sutton came to much the same conclusions as Boveri at essentially the same time, he was aware of and appreciated Boveri's work when he published his 1902 paper writing " *The appearance of Boveri's recent remarkable paper on analysis of the nucleus by means of observation on double-fertilized eggs has prompted me to make a preliminary communication of certain results obtained in a general study of the great 'lubber grasshopper', Brachystola magna.*" Again referring to the importance of Boveri's experimental studies he concluded his paper with the statement that " *The evidence advanced in the case of the ordinary chromosomes is obviously more in the nature of a suggestion than that of proof, but it is offered in this connection as a morphological complement to the beautiful experimental researches of Boveri already referred to*" (Sutton, 1902). Although Sutton like Boveri did not discuss his observations in any detail in relation to the recently rediscovered work of Mendel, he went further than Boveri and wrote that " *I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division as indicated above may constitute the physical basis of the Mendelian law of heredity. To this subject I hope soon to return in another place.* "

Only two years after his 1902 paper, Boveri gave a more detailed explanation of his thoughts about chromosomes and Mendel's studies "of hybrids and their descendents". In his 1904 book *Ergebnisse uber die Konstitution der Chromatischen Substanz des Zellkerns* Boveri discussed the chromosomal basis of the Mendelian laws and predicted genetic linkage and crossing over, two prescient insights into the behavior of genes during meiosis (Stern, 1950). In this profound passage translated by Curt Stern (1950) Boveri writes if

"in successive breeding two traits should always appear together or disappear together, then it would be permitted to draw the conclusion, with the highest probably, that the anlagen for these two traits are localized in the same chromosome. And furthermore: if a hybridization experiment included numerous traits and if it should be found in successive breeding that the number of combinations in which the separate traits can occur, is greater than it would correspond to the possibilities of recombination of the chromosomes present then it would have to be concluded that the traits localized in the chromosomes can go independently of each other into one of the other daughter cells which would point to an exchange of parts between homologous chromosomes".

Thus Boveri predicted and laid out the necessary experiments to test for results that would demonstrate genetic linkage and crossing over. It was the following year that Bateson et al. (1905) observed genetic linkage but not until the work of T. H. Morgan (1911) was it explained.

T.H. Morgan, the sea urchin embryologist

These early studies on echinoderm gametes and embryos and other developmental systems yielded information essential for what soon would become the field of genetics. As discussed above much of nascent genetics derived from studies using the sea urchin embryo and so it should come as no surprise that Thomas Hunt Morgan had deep conceptual roots in embryology typified by his work with sea urchins (Morgan, 1894). For two decades before he started working

with *Drosophila* in 1908, Morgan's research and publications were focused on questions of development and differentiation and he never lost interest in experimental embryology (Gilbert, 1978; Maienschein, 1989). His ongoing fascination with development and differentiation and his recognition that the "differentiation problem" was not yet solved were evident in Morgan's, 1934 Nobel Lecture when he said

"*If as is generally implied in genetic work (although not often explicitly stated), all of the genes are active all the time and if the characters of the individual are determined by the genes, why are not all the cells of the body exactly alike? The same paradox appears when we turn to the development of the egg into an embryo. The egg appears to be an unspecialized cell, destined to undergo a prescribed and known series of changes leading to the differentiation of organs and tissues. At every division of the egg, the chromosomes split lengthwise into exactly equivalent halves. Every cell comes to contain the same kinds of genes. Why then is it that some cells become muscle cells, some nerve cells, and others remain reproductive cells?*" (Morgan, 1934).

In this passage we see Morgan, the embryologist, asking the question of how cells differentiate when they are all derived from an apparently unspecialized egg and Morgan, the geneticist, relating this to the then current paradox that all the genes apparently are active all of the time. He then presents three views of how this might happen. Morgan favors one of these. He describes it as

"*an alternative view that can not be ignored. It is conceivable that different batteries of genes come into action one after the other, as the embryo passes through its stages of development ……… it might be possible that in different regions of the egg there is a reaction between the kind of protoplasm present in those regions and specific genes in the nuclei; certain genes being more affected in one region of the egg, other genes in other regions. Such a view might give also a purely formal hypothesis to account for differentiation of the cells of the embryo.*" (Morgan, 1934).

In 1928 Morgan left Columbia to go to Caltech to establish the Division of Biology. In retirement Morgan spent much of his time in Corona del Mar at Caltech's Kerckhoff Marine Laboratory. There he worked with his last PhD student Albert Tyler (Horowitz, 1969). Tyler had come with Morgan from Columbia. Tyler became the first PhD student to graduate from the newly created Division of Biology at Caltech (Pauling, 1970). An embryologists working with sea urchins, Tyler, later carried out pioneering studies on protein synthesis that contributed to the understanding of the information flow from DNA to protein (Monroy and Tyler, 1963; Tyler, 1963a, 1963b).

Embryology and the origins of molecular biology

In hindsight even as genetics and embryology were falling out of step with each other by about 1920 (Fantini, 1985; Gilbert, 1978, 1987), within a decade one can see the origins of what would later emerge as the new field of molecular biology. Not surprisingly, given that there are many schools of thought as to what constitutes the field of molecular biology today (Burian, 2001; Judson, 1979; Olby, 1974; Rheinberger, 1996) there are equally varied views regarding its origins. Whatever one's view about what molecular biology is and how it came about, there were important contributions by numerous investigators from different fields that gave us the understanding that DNA is the genetic material passed from one cell to its daughters and from one generation to the next and that DNA codes for RNA which in turn codes for protein. It is argued here that even with the multiple origins of molecular biology, embryologists approached the understanding of the flow of information from the genetic material to cell specific proteins from the distinct and characteristic perspective of the development and differentiation of the embryo. As with the origins of

genetics, embryologists using sea urchins made important contributions to understanding the fundamentals of the newly emerging molecular biology. Here, however, consideration of the sea urchin's significant contributions to the origins of molecular biology is explored within the boundaries of the central dogma.

In his Third Edition of *The Cell in Development and Heredity* E.B. Wilson presents the prevailing view of RNA and DNA in 1925 with a description of thymonucleic acid (DNA) being present in animal cells and zymonucleic acid (RNA) in the cells of plants (Wilson, 1925). The pace of progress over the next 40–50 years in the understanding of the structure, functions and relationships of these molecules is truly remarkable. Though many experiments were messy, results were frequently full of contradictions, and conclusions were often leaps of faith, the literature is filled with palpable excitement of researchers who were proposing theories, right or wrong, about processes that seemed to hold the secrets of life.

Investigators using sea urchin eggs and embryos to study nucleic acids and their relation to protein synthesis in cells made major contributions to the fledgling field of molecular biology primarily in two major areas. The first is exemplified by the lifetime work of Jean Brachet and his prophetic understanding of the flow of information in cells from DNA to RNA to protein. Progress in the second area came from the work of several developmental biologists using the sea urchin to study changes that occur in DNA, RNA and protein synthesis following fertilization and the role these changes play in development and differentiation.

Jean Brachet's work with sea urchins giving early insights into the flow of information from DNA to protein

Although his fertile mind lead him into many areas of biology in his incredibly productive scientific career Jean Brachet was, like his father before him, an embryologist. It is his work in chemical embryology where he most frequently used sea urchins and where he and his students and collaborators made significant advances in our early understanding of what would become the central dogma. Brachet was only 18 years old in 1927 when he went to work towards his PhD in Dalcq's laboratory at the Free University of Brussels where he studied the localization of "thymonucleic acid" during oogenesis (Brachet, 1975). In 1933 only eight years following Wilson's description of the localization of nucleic acids in plant and animal cells, Brachet published a major paper showing that RNA was in the cytoplasm and DNA in the nucleus of both plants and animals. In this 1933 paper Brachet concluded "*the synthesis of nucleic acids in the sea urchin is not intelligible unless we admit the presence of a pentose-nucleic acid in the cytoplasm*" (Brachet, 1945). Because this observation flew in the face of the accepted view and because the existing dyes for detecting nucleic acids were somewhat unreliable Dalcq remained skeptical. Brachet searched for methods to improve the cytochemistry procedures for detecting RNA. In the late 1930s he turned to the method of Unna that employed a methyl-green pyronine staining mixture to detect RNA and then he treated every other serial section of cells with ribonuclease (Burian, 1997). Using this procedure, Brachet was able to confirm the presence of RNA in the cytoplasm and also in the nucleolus while DNA was found in the nucleus of sea urchins and many other organisms (Alexandre, 1992; Brachet, 1975). This is likely the first observation of what would become understood as the role of the nucleolus in ribosomal RNA synthesis. Brachet continued work on DNA, RNA and proteins and years later in summarizing a series of his papers from the late 30s early 40s, he wrote

"*I found a completely unexpected correlation between the quantity of RNA in a cell and its capacity to synthesize proteins. This led me to another iconoclastic proposition: proteins are not synthesized by proteolytic enzymes operating backwards, as was generally thought, but by an unknown mechanism implicating RNA. The same*

conclusion was arrived at simultaneously (1941) by T. Caspersson in Stockholm using a completely different technique for the cytochemical detection of nucleic acid" (reviewed: Brachet, 1975; Thomas, 1992).

It is not surprising that given the level of understanding of information flow in 1941 Brachet's and Caspersson's proposals that RNA is involved in protein synthesis did not attract a lot of attention. From the late 1920s on there were many pioneering experiments carried out by excellent scientists employing innovative technologies with a diverse group of organisms giving evidence for what would become the central dogma, proposed by Crick in 1958 (Judson, 1979). The reports of the work of many of these remarkable scientists were somewhat like the discoveries of the six blind men exploring the elephant; they were in large part right, but they were working blindly in different areas and at the time it was not possible to visualize the entire animal. In 1945 almost 10 years before Watson and Crick proposed the structure of DNA, Brachet postulated that it " *is probable that the participation of nucleic acids in the reproduction of genes and viruses as well as the synthesis of proteins will not become clear until such time as we have at our disposal exact determination of the structure of these molecules"* (Brachet, 1945). Indeed elucidation of the structure of DNA a decade later was the "Ah-Ha moment" for many. Watson and Crick published the structure of DNA in 1953, and in conscious understatement, they concluded, *"it has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copy mechanism for the genetic material"* (Watson and Crick, 1953a).

Brachet published *Chemical Embryology* in French in 1944 with a second edition appearing one year later that was translated into English in 1950. The war seriously interrupted the work of Brachet and others at the Free University of Brussels. The University was shut down by the Germans in 1941 and was not reopened until Liberation (Alexandre, 1992; Brachet, 1975; Pirie, 1990). Brachet and eight others were imprisoned for three months and when he was released jobless it was only through the support of some "friends of the University" that Brachet was able to write *Chemical Embryology* (Pirie, 1990). Nevertheless, even though it was written during trying circumstances, Brachet's book was an extraordinary synthesis of his own work and that of others, influencing biologists for years to come (Alexandre, 1992).

In the preface of the first edition Brachet wrote that the field of *"chemical embryology should fill the gaps and provide an exact material basis for those "entities" to which experimental embryologists have had recourse in explaining developmental biology".* Evaluating what was known at the time (1944–1945) he also wrote "thymonucleic acid is a *constant constituent of the chromosomes* (his emphasis), which correspond probably to the genes" (Brachet, 1945). Brachet brought attention to what he described as compelling results from Avery et al. (1944) showing that DNA was the substance inducing specific transformation of pneumococcal types. Furthermore he made explicit his belief of the connection of the genes to development contending that at "*the present time there is no question that genes play a role in the regulation of embryonic development*" (Brachet, 1945). In summarizing the various roles that nucleic acids played in the cell and in the synthesis of proteins and relying heavily on his work in sea urchins and work of Caspersson and Schultz, Brachet wrote, "*the content of thymonucleic acid in the nucleus regulates the amount of ribonucleoproteins in the cytoplasm……….. The ribonucleoproteins of the ergastoplasm are bound to granules……… these various substances probably collaborate to synthesize proteins: the amino acids might be arranged on the surface of the granule in a precise pattern*" (Brachet, 1945).

Following World War II the ways of doing science were changing rapidly. Improvements in fixation of biological materials using osmium tetroxide along with new embedding materials, sectioning techniques and staining methods, for electron microscopy of biological material made it possible to view the fine structure of cells. In 1945 Porter et al. published the first electron micrograph of

intact cell magnified 1,600 times, making visible previously unseen sub-cellular structures (Masters, 2009). Preparative ultracentrifugation and cell fractionation techniques were used to isolate cellular components while advances in biochemical cytology, spectrophotometry and biochemistry facilitated the characterization and quantitation of these newly identified sub-cellular components. Greater availability of radioactive materials for biological research in the late 1940s and the 1950s enabled biologists to measure the synthesis of several molecules within cells and newly discovered antibiotics could be used to inhibit these processes and evaluate the downstream effects. Antibiotics of particular interest to early molecular biologists were actinomycin for the inhibition of DNA synthesis and puromycin that was shown by Darken in 1961 to block protein synthesis both *in vitro* and *in vivo* (Darken, 1964). In addition to the technological advances accelerating the pace of science the size of research laboratories was increasing.

However, even with the transformative changes in the toolboxes available to researchers, sea urchins continued to be an ideal organism for studying development and differentiation using the rapidly emergent technologies. Synchrony of development makes embryos in general and urchin embryos in particular an ideal stopped- start system. An entire culture of millions of embryos from a single female urchin develops synchronously. Therefore, investigators can obtain sufficient quantities of embryos or cells at a desired stage to analyze proteins and nucleic acids. This was particularly useful before modern molecular technology when relatively large amounts of material were necessary for analysis. Furthermore, with radioactive protein and nucleic acid precursors the localization of these processes and the rate of synthesis of these substances could be monitored over time. Addition of DNA, RNA or protein synthesis inhibitors to sea urchin eggs immediately prior to fertilization or at selected stages, gave investigators the means to ask questions about molecular requirements for the synthesis of DNA, RNA or proteins following fertilization and subsequent developmental events.

Structure of DNA and the Central Dogma

The impact of Watson and Crick's two Nature papers in 1953 is well known. In the first they reported the discovery of the structure of DNA (Watson and Crick, 1953a) and the second proposed a model only hinted at in the first paper in which the two strands of the double helix of DNA could serve as a *pair of templates* for the exact duplication of the genetic material (Watson and Crick, 1953b). As reflected five years later in Crick's presentation of his Central Dogma, there was not strong consensus about the information flow from DNA to protein. In his words, the Central Dogma " *states that once 'information' has passed into protein it cannot get out again. In more detail, the transfer of information from nucleic acid to nucleic acid, or from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible. Information means here the precise determination of sequence, either of bases in the nucleic acid or of amino acid resides in the protein. This is by no means universally held"* (Crick, 1958).

The Central Dogma supplied a model for information flow from the genes to the proteins. It was only a model and information about the processes responsible for transferring this information was rudimentary at best. Crick frequently corrected people's misconceptions. In 1970 in response to an "unsigned article" in Nature, he published a paper making " *four points about the formulation of the central dogma which have occasionally produced misunderstandings".* The first two of Crick's points, remind us how much was still unknown in 1958. In reference to the central dogma he points out that *"(1) It says nothing about what the machinery of transfer is made of, and in particular nothing about errors. (it was assumed that, in general, the accuracy of transfer was high.) (2) It says nothing about control mechanisms — that is about the rate* at *which the processes work"* (Crick, 1970). Many groups from different areas worked on aspects of replication, transcription and

translation, "the machinery of transfer" and what controls the rate of transfer. Embryologists were no exception and once again the context from which they asked questions was different from that of most other groups and remained centered on the fertilized egg and embryo as it undergoes development and differentiation. The fact that upon fertilization the egg changes from a relative quiescent state to a remarkably dynamic one explains, in part, why developmental biologists employing sea urchins made significant progress investigating the *controls* of the machinery for the transfer of information.

Understanding protein synthesis and translational regulation

In the 1950s protein synthesis was at best poorly understood and even with the work of Brachet and others, it was not yet generally accepted what role, if any, RNA had in the process. Nevertheless, many labs using different systems were actively studying protein synthesis. Notably, Paul Zamecnik and his collaborators were using rat liver to study cancer and Ernest Gale was investigating the action of antibiotics in bacteria (Rheinberger, 1996). Several investigators working with sea urchins continued to focus on the question of what is responsible for the dramatic changes in development and differentiation following fertilization. These researchers were familiar with the many advantages of the sea urchin as a developmental system and readily introduced and adopted new methodologies. An additional advantage, and one that should not be overlooked, was that at this time there were a number of sea urchin labs studying the role of protein, RNA and DNA synthesis following fertilization. The concerted efforts of multiple investigators interested in the same problems and using the same organism made rapid progress possible.

Using various radioactive precursors Tore Hultin measured the changes in protein synthesis following fertilization of the sea urchin egg. Although he varied the precursors and the isotopes the results of all of these experiments were similar in that there was a several fold increase in protein synthesis following fertilization (Hultin, 1950, 1952; Hultin and Wessel, 1952). A few years later, equipped with improved experimental design and using radioactive methionine, Nakano and Monroy (1957, 1958) also saw a major increase in protein synthesis. These investigators and others turned to newly developed cell-free protein synthesis systems to learn more about the components necessary for protein synthesis. As described by Hultin and Bergstrand (1960) "*subcellular fractions of sea urchin embryos at different stages of development were examined in amino acid incorporation systems of varied composition. A rapid increase of the incorporation capacity was consistently observed in the early developmental stages. This was also the case in systems containing partially purified RNP-particles from sea urchin embryos as the only variable constituent. The experiments, therefore, are consistent with the view that populations of protein-synthesizing RNP-particles develop in sea urchin embryos in connection with the process of primary determination.*" Once again these researchers were analyzing the events of proteins synthesis with the ultimate goal of identifying the factors responsible for early development and differentiation.

These experiments established that protein synthesis increased following fertilization, but left open the question of when protein synthesis became essential for development. When Hultin incubated sea urchin eggs in seawater with puromycin, fertilized them and added ^{14}C –L-valine, he found that protein synthesis was inhibited and that this inhibition had a direct effect on the first cell division. Development appeared normal until the clear streak stage and then it stopped (Hultin, 1961). It is during the clear streak stage that nuclear membranes breakdown, chromosome condensation occurs and the mitotic spindles are formed. Surprisingly, those few embryos that escaped arrest at the first cell division exhibited these same characteristics and timing of inhibition of nuclear division at the second division and remained arrested at this stage. From this Hultin concluded that the "*mitotic block-induced by puromycin is probably a*

direct effect of the impaired protein metabolism. Special kinds of proteins of importance for the initiation of mitosis may not become produced in sufficient amounts under these conditions. Which functions these proteins have can only be a matter of conjecture. The present data are consistent, however, with the idea that some of them may be related to the formation of the mitotic spindle" (Hultin, 1961b). Many years later while at Woods Hole, Tim Hunt took advantage of the marvelous synchrony of the early cleavages of sea urchin embryos. Following fertilization he labeled newly synthesized proteins with ^{35}S-metionine and separated the proteins by polyacrylamide gel electrophoresis. He discovered an unusual protein that was synthesized continuously and destroyed at each cell division (Evans et al., 1983). In 2001 Hunt shared the Nobel Prize in Physiology or Medicine for the discovery of cyclin, a protein predicted by Hultin 22 years earlier (Hunt, 2002).

Considering the number of labs that were interested in understanding the major steps of the central dogma and the importance of mRNA and its role as the link between gene and protein, it is not surprising that there are many views of the discovery of mRNA in bacteria (Brenner et al., 1961; Jacob and Monod, 1961; Nirenberg and Matthaei, 1961; reviewed: Volkin, 2001) and the naming of mRNA (Jacob and Monod, 1961). Martin Nemer using a cell-free synthesis system in sea urchins reported the first evidence that ribosomes from eukaryotes were dependent upon messenger polyribonucleotides for protein synthesis (Nemer, 1962). There had been other reports consistent with the necessity of messenger RNA for protein synthesis in mammalian reticulocytes but those fell short of a confirmation (Lamfrom, 1961). Soon after the requirement for protein synthesis following fertilization was discovered in sea urchins, several other urchin labs undertook experiments to determine if RNA synthesis increased following fertilization and, if so, was protein synthesis dependent on this RNA synthesis. Taking advantage of the stopped-start nature of eggs pre and post fertilization, they parthenogenetically activated merogones and found that protein synthesis increased even in the absence of a nucleus, suggesting that RNA synthesis was not necessary for protein synthesis following fertilization (Brachet et al., 1963; Denny and Tyler, 1964; Tyler, 1963b). In a classic paper Gross and Cousineau (1963) used actinomycin, a newly characterized drug, to inhibit RNA synthesis following fertilization. After this "chemical enucleation" they measured the effect of the inhibition of RNA synthesis on protein synthesis and found that protein synthesis continued at approximately the same rate as in the controls. The results of these studies lead to the startling conclusion that maternal messenger RNAs were stored in sea urchin eggs during oogenesis and translated into proteins following fertilization. This first evidence of the existence of long-lived mRNAs was a dramatic example of translational regulation and made stark the differences between eukaryotic and prokaryotic protein synthesis where the measured half-life of mRNAs was about 3 minutes (Jacob and Monod, 1961).

Following the discovery of stored maternal mRNA Raff and colleagues measured the amount of newly synthesized tubulin protein in cleavage embryos using sea urchin eggs as a control, actinomycin D treated eggs and activated merogones. It is interesting to note that in experiments important to the central dogma, activated merogones were still useful for distinguishing between nuclear and cytoplasmic processes and for studying these processes. Methods for producing merogones had progressed since the 1880 s when the Hertwig brothers shook eggs in seawater and were now based on a centrifugation technique developed by Ethel Brown Harvey in the 1930s (Harvey, 1940). The results of Raff's experiment showed that maternal tubulin mRNA serves as a template for the synthesis of tubulin protein following fertilization (Raff et al., 1971, 1972). This was the first demonstration of a specific stored maternal mRNA used to synthesize a protein essential for embryogenesis. The discovery of stored mRNA led to a number of experiments to distinguish between the possibilities that prior to fertilization protein synthesis was inhibited due to "masking" the messenger RNA or it was caused by

defects in the protein synthesis machinery. From these studies came some of the first examples of translational regulation at the level of the ribosomal components and at the level of mobilization of mRNAs (reviewed: Winkler et al., 1985). In 1962–1963 several labs observed polysomes for the first time and proposed that mRNA may hold these ribosomal aggregates together. At that time, Monroy and Tyler (1963) demonstrated that the initiation of protein synthesis following fertilization of sea urchin eggs was the result of the formation of active ribosomal aggregates (polysomes). Experiments with sea urchin embryos helped uncover another novel regulatory mechanism for translational control of mRNA. Slater et al. (1972, 1973) and Wilt (1973) discovered that between fertilization and the 2-cell stage there was greater than a two fold increase in the amount of polyA and that this newly synthesized polyA was covalently linked to maternal mRNA. Furthermore this newly polyadenylated mRNA was rapidly shifted from the sub-ribosomal fraction to the polyribosome fraction of the cell. The messenger RNA population that was polyadenylated contained sequences that were not polyadenylated prior to fertilization and others that were partially adenylated. Wilt (1973) demonstrated that this increase of polyadenylation occurs in activated merogones, confirming that it is a cytoplasmic event. In their 1972 paper Slater et al. noted that it " *is interesting that this first demonstration of net synthesis of RNA following fertilization should implicate a species of nucleic acid believed to be regulatory rather than codogenetic.* "

By exploiting the advantages of the sea urchin system, specifically the fertilized egg to embryo transition these investigators made significant progress in the study of the requirements for protein synthesis and mRNA synthesis. With the continued focus on the development and differentiation of the embryo, their work lead to several "firsts" and early discoveries about translation, one of Crick's "machineries for transfer of information" from gene to protein in higher organisms and about the regulation of translation. In the early days of molecular biology, understanding information flow from gene to protein was driven primarily by experiments in phage and bacteria. However, it was often only a few months before similar aspects of replication, transcription, or translation were understood in eukaryotes. As demonstrated here when it came to protein synthesis, much of this information derived from experiments using sea urchins. In addition, there were several discoveries of translational regulation in sea urchins that are specific to eukaryotic systems including the physical and functional separation of transcription and translation, the presence of long-lived mRNAs and cytoplasmic polyadenylation of mRNAs.

Current and future value of the sea urchin to developmental biology and molecular biology

By the late 1960s and the early 1970s molecular biology was flourishing and information and technology were exploding. For investigators continuing their quest to understand development and differentiation of the embryo, the questions were now asked and the experiments carried out within the conceptual environment of the central dogma. The reciprocal inductive interactions between developmental biology and molecular biology made this a particularly exciting and productive time for both disciplines. Even as developmental biology contributed to the origins of molecular biology, it was being transformed by it. A comprehensive discussion of these interactions is well beyond the scope of this essay, however, a very few of the many possible examples of studies with sea urchin embryos will suffice to illustrate how at this time advances in developmental biology and molecular biology were and remain intimately related. Examples presented here are restricted to investigations of gene expression during development and differentiation, however, almost all areas of developmental biology have benefited from the knowledge base as well as the technology of molecular biology.

An early instance of the impact that molecular biology was having on developmental biology was reported in 1975 when Larry Kedes working with Stanley Cohen utilized rapidly developing molecular technology and cloned the sea urchin histone genes in *E. coli*, thereby making the sea urchin histone genes the first protein coding eukaryotic genes cloned (Kedes et al., 1975). This achievement enabled Kedes and colleagues to explore the genomic organization of the histone gene families and the regulation of the expression of these genes during development (Maxson et al., 1983). It was also an early and dramatic example of the tremendous potential of molecular technology that would forever alter the study of developmental biology.

Much of the molecular technology developed to study DNA replication, transcription, translation, genome organization and evolution capitalized on the property of DNA that when the two strands are separated, they will find their complementary strand and reassociate (Marmur et al., 1963). Using this property Britten and Kohne disassociated DNA from single nucleotide polymers, *E. coli* and several eukaryotic organisms. Analysis of the rates of reassociation of the DNA from the various sources produced several unexpected results (Britten and Kohne, 1968). In addition to DNA with predicted reassociation kinetics for single copy sequences, it was found that a large fraction of the DNA from higher organisms reassociated more rapidly than would be expected from the DNA content in the cell and that the faster reassociating fractions represented sequences present from 100–100,000 times. They also concluded that during evolution the repeated DNA sequences slowly diverge from each other. Sea urchins have been one of the principal organisms contributing to our understanding of many of the implications arising from these observations, including characterization of the types of repeats, genome organization and repetition frequencies of the repeat families, the expression of repetitive DNA sequences, and repetitive DNA in genome evolution.

The repetitive DNA families prevalent in genomes of higher organisms are an important component of the ingenious model proposed by Roy Britten and Eric Davidson in their 1969 Science paper (Britten and Davidson, 1969). Recognizing the benefits and rich history of the sea urchin as experimental system, they selected it for large-scale analysis of gene expression and regulation during development and differentiation. In describing the major accomplishments and conceptual advances derived from this effort, Thoru Pederson wrote that "*while Drosophila and C. elegans came to the fore for appropriate reasons, no other embryo in the 1970s and early 1980s was subjected to an analysis of gene expression carried out at such a quantitative scale as the Caltech sea urchin gene program*" (Pederson, 2006). Highlights of this work include the first measurement of the number of diverse mRNA sequences in the total polysomes in a eukaryotic system (Galau et al., 1974). In what might be considered the conceptual equivalent of transcriptome analysis on a population level, numerous similar experiments were carried out to determine the sequence complexity of the nuclear, cytoplasmic and polysomal RNA throughout embryonic development and differentiation. From this a comprehensive picture of differential gene expression during embryogenesis of the sea urchin embryo began to emerge and served as a model for embryogenesis in other organisms (reviewed: Davidson, 1986).

By the 1980s there were a number of cloned sea urchin genes making it possible to characterize the expression and regulation of specific genes. Technology advanced to a point where the expression and regulation of lineage specific gene regulation could be analyzed. The endomesoderm specific and progressively restrictive expression pattern of the *Endo16* gene during differentiation (Nocente-McGrath et al., 1989) suggested that it would be an ideal gene for *cis*- regulatory analysis. Extensive examination of the *cis*-regulatory region of the *Endo16* gene identified those elements that are responsible for the temporal and spatial expression of this gene (Yuh et al., 1994; Yuh and Davidson, 1996). Six regulatory modules with over 30 high specificity binding sites and 20 or more sites for factors common to other genes were identified, revealing the amazing complexity of the information

hard-wired in the modular *cis*-regulatory region of an early cell lineage specific gene (Yuh et al., 1994: Yuh and Davidson, 1996).

In 2002 Davidson et al. (2002a, 2002b) produced a "*provisional regulatory gene network for the specification of the endomesoderm of the sea urchin embryo.*" This *tour de force* in sea urchins was the first and most extensive analysis of the specification of a germ layer lineage in any organism. As indicated in the title of paper, the network was provisional in anticipation that more genes and cell signaling events would be added. Investigators from other labs and the Davidson group continue to submit data to expand this GRN. A link to the endomesoderm network and the ectoderm network can be found at http://sugp.caltech.edu/endomes/ . Readers are referred to Davidson, 2006a for a comprehensive discussion of gene regulatory networks in development and evolution.

Molecular biology has forever altered the study of developmental biology. However given selected recent work described above it could be argued that the effective use and further development of these technologies for the study of development and differentiation have contributed greatly to the realm of molecular biology. The same characteristics that made the sea urchin an important experimental system over the last 150 years are still relevant today. A special advantage of the sea urchin that should be mentioned even though it is beyond the scope of this work is the phylogenetic position of the sea urchin as a non-chordate deuterostome. This position has and will continue to result in advances in evolution and in developmental evolution. Confidence in the future of the sea urchin embryo as a model experimental organism that will contribute to new areas of research was reinforced dramatically with the release of the sea urchin genome.

The sequencing, assembly and annotation of the Sea Urchin Genome resulted from a highly successful partnership between the Baylor College of Medicine Human Genome Sequencing Center and the sea urchin community (Davidson, 2006b). The genome was released with close to 9,000 genes annotated by members of the community. This number has increased significantly since then (Cameron et al., 2009). Accompanying the announcement of the sea urchin genome in the Special Issue of Developmental Biology (2006b) and the release in Science (2006) there were 40 papers on different aspects of sea urchins. Many of them are on the forefront of new areas of research. The breath of these studies makes it clear that the sea urchin is still offering up surprising secrets and undoubtedly there are more to come.

Acknowledgments

References

Alexandre, H., 1992. Jean Brachet and his school. Int. J. Dev. Biol. 36, 29–41.

Allen, G., 1978. Thomas Hunt Morgan. Princeton University Press, Princeton.

Avery, O.T., MacLeod, C.M., McCarty, M., 1944. Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a deoxyribonucleic acid fraction isolated from Pneumoccocus type III. JEM 79, 2137–2158.

Baltzer, F., 1962. Theodor Boveri, Leben und Werk eines Grossen Biologen. English translation: Rudnick, D. University of California Press, Berkeley. 1967.

Baltzer, F., 1964. Theodor Boveri. Sci. New Ser. 144, 809–815.

Bateson, W., Saunders, E.R., Punnett, R.C., 1905. Reports to the evolution committee of the Royal Society. Report 111.

Boveri, T., 1889. An organism produced sexually without characteristics of the mother. English translation: Morgan, T.H., 1893. Am. Nat. 27, 222–232.

Boveri, T., 1902. On multipolar mitosis as a means of analysis of the cell nucleus. Neue Folge 35, 67–90. English translation: Salome Glueckson-Waelsch. In: Willier, B.H., Oppenheimer, J.M. (Eds.), Foundations of Experimental Embryology. Hafner Press, New York, pp. 74–97. 1964.

Brachet, J., 1945. Chemical Embryology. English translation: Barth, L.G., 1950 Interscience Publishers, Inc, New York.

Brachet, J., 1975. From chemical to molecular sea urchin embryology. Am. Zool. 15, 485–491.

Brachet, J., Ficq, A., Tencer, R., 1963. Amino acid incorporation into proteins of nucleate and anucleate fragments of sea urchin eggs: Effect of parthenogenetic activation. Exp. Cell Res. 32, 168–170.

Brenner, S., Jacob, F., Messelson, M., 1961. An unstable intermediate carrying information from genes to ribosomes for protein synthesis. Nature 190, 576–581.

Briggs, E., Wessel, G.M., 2006. In the beginning....animal fertilization and sea urchin development. Dev. Biol. 300, 15–26.

Britten, R.J., Kohne, D.E., 1968. Repeated sequences in DNA. Science 161, 529–540.

Britten, R.J., Davidson, E.H., 1969. Gene regulation for higher cells: A theory. Science 165, 349–357.

Burian, R.M., 1997. Exploratory experimentation and the role of histochemical techniques in the work of Jean Brachet, 1938–1952. Hist. Philos. Life Sci. 19, 27–45.

Burian, R.M., 2001. The dilemma of case studies resolved: the virtues of using case studies in the history and philosophy of science. Presp. Sci. 9, 383–404.

Cameron, R.A., Samanta, M., Yuan, A., He, D., Davidson, E.H., 2009. SpBase: the sea urchin genome database and web site. Nucleic Acids Res. D750–D754, http://www.spbase.org/SpBase/.

Churchill, F.B., 1987. From heredity theory to vererbung: the transmission problem, 1850–1915. Hist. Sci. Soc. 78, 337–364.

Coleman, W., 1965. Cell, nucleus and inheritance: an historical study. Proc. Am. Philos. Soc. 109, 124–158.

Crick, F., 1958. On protein synthesis. Symp. Soc. Exp. Biol. 12, 138–163.

Crick, F., 1970. Central Dogma of Molecular Biology. Nature 227, 561–563.

Czihak, G. (Ed.), 1975. The Sea Urchin Embryo: Biochemistry and morphogenesis. Springer-Verlag, Berlin.

Darken, M.A., 1964. Puromycin inhibition of protein synthesis. Pharm. Rev. 16, 223–243.

Davidson, E.H., 1976. Gene Activity in Early Development, Second Edition. Academic Press, New York.

Davidson, E.H., 1986. Gene Activity in Early Development, Third Edition. Academic Press, Orlando, Florida.

Davidson, E.H., 1989. Genome function in sea urchin embryos: Fundamental insights of Th. Boveri reflected in recent molecular discoveries. In: Horder, T.H., Witkowski, J.A., Wylie, C.C. (Eds.), 8th Symposium of the British Soc. of Developmental Biology. Cambridge University Press, Cambridge, pp. 397–406.

Davidson, E.H., 2001. Genomic Regulatory Systems. Academic Press, San Diego.

Davidson, E.H., Past, J.P., Oliveri, P., Ransick, A., Calestani, C., Yuh, C.-H., Minokawa, T., 2002a. A provisional regulatory gene network for specification of endomesoderm in the sea urchin embryo. Dev. Biol. 246, 162–190.

Davidson, E.H., Rast, J.P., Oliveri, P., Ransick, A., Calestani, C., Yuh, C.-H., Minokawa, T., 2002b. A genomic regulatory network for development. Science 295, 1669–1678.

Davidson, E.H., 2006a. The Regulatory Genome. Academic Press, San Diego.

Davidson, E.H., 2006b. Special issue: The sea urchin genome. Dev. Biol. 300, 1.

Davidson, E.H., 2006c. The sea urchin genome: where will it lead? Science 314, 939–940.

Denny, C., Tyler, A., 1964. Activation of protein biosynthesis in non-nucleate fragments of sea urchin eggs. Biochem. Biophys. Res. Commun. 14, 245–249.

Developmental Biology, 2006. 300, 1–496.

Ettensohn, C.A., Ingersoll, E., 1992. Morphogenesis of the sea urchin embryo. In: Rossomando, E.E., Alexander, S.A. (Eds.), Analysis of the Development of Biological Form. Marcel Dekker, Inc, New York, pp. 189–262.

Ernst, S.G., 1996. Recent advances in early development of the sea urchin embryo. In: Adiyodi, K.G., Adiyodi, R.G., Collier, J.R. (Eds.), Reproductive Biology of the Invertebrates. Progress in Developmental Biology, Vol. 7. Oxford & IBH Publishing Co, New Delhi, pp. 199–224.

Ernst, S.G., 1997. A century of sea urchin development. Am. Zool. 37, 250–259.

Evans, T., Rosenthal, E.T., Youngblom, J., Distel, D., Hunt, T., 1983. Cyclin: A protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. Cell 33, 389–396.

Fantini, B., 1985. The sea urchin and the fruit fly: cell biology and heredity, 1900–1910. Biol. Bull. 168 (suppl), 99–106.

Fantini, B., 2000. Molecularizing embryology: Alberto Monroy and the origins of developmental biology in Italy. Int. J. Dev. Biol. 44, 537–553.

Farley, J., 1982. Gametes and Spores: Ideas about sexual reproduction 1750–1914. Johns Hopkins University Press, Baltimore, pp. 163–165. 1982.

Fol, H., 1877. Sur le commencement de l'henogenie divers animaux. Arch. Zool. Exp. Gen. 6, 145–169.

Galau, G.A., Britten, R.J., Davidson, E.H., 1974. A measurement of the sequence complexity of polysomal messenger RNA in sea urchin embryos. Cell 2, 9–20.

Gilbert, S., 1978. The embryological origins of the gene theory. J. Hist. Biol. 11, 307–351.

Gilbert, S., 1987. In friendly disagreement: Wilson, Morgan, and the embryological origins of the gene theory. Am. Zool. 27, 798–806.

Gilbert, S., Greenberg, J., 1984. Intellectual traditions in the life sciences. II. Stereospecificity. Perspect. Biol. Med. 28, 18–34.

Giudice, G., 1973. Developmental Biology of the Sea urchin Embryo. Academic Press, New York.

Giudice, G., 1986. The Sea Urchin Embryo. Springer-Verlag, Berlin.

Gross, P.R., Cousineau, G.H., 1963. Effects of actinomycin D on macromolecular synthesis and early development in sea urchin eggs. Biochem. Biophy. Res. Commun. 10, 321–326.

Harvey, E.B., 1940. Development of half-eggs of *Arbacia punctulata* obtained by centrifuging after fertilization, with special reference to parthenogenetic merogony. Blot. Bull. 78, 412–427.

Hertwig, O., 1877. Beitrage zur kenntnis der bildung, befruchtung und theilung des thierischen eies. Morphol. Jahr. 3, 1–86. In: Farley, J. (Ed.), Gametes and Spores: Ideas about sexual reproduction 1750–1914. Johns Hopkins University Press, Baltimore, p. 161. 1982.

Horstadius, S., 1973. Experimental Embryology of Echinoderms. Clarendon Press, Oxford.

Horowitz, N.H., 1969. Dedication to Life of Albert Tyler. In: Hanly, E.W. (Ed.), Problems in Biology: RNA in development. The Park City International Symposium on Problems in Biology. University of Utah Press, Salt Lake City.

Hultin, T., 1950. The protein metabolism of sea urchin eggs during early development studied by means of [15] N-labelled ammonia. Exp. Cell Res. 1, 599–602.

Hultin, T., 1952. Incorporation of N[15]-labeled glycine, and alanine into the proteins of developing sea urchin eggs. Exp. Cell Res. 3, 494–496.

Hultin, T., Wessel, G., 1952. Incorporation of C[14]-labeled carbon dioxide into the proteins of developing sea urchin eggs. Exp. Cell Res. 3, 613–616.

Hultin, T., 1961. The effect of puromycin on protein metabolism and cell division in fertilized sea urchin eggs. Experientia 17, 410–411.

Hultin, T., Bergstrand, 1960. Incorporation of C[14] L-leucine into protein by cell-free systems from sea urchin embryos at different stages of development. Dev. Biol. 2, 61–75.

Hunt, T., 2001. Protein synthesis, proteolysis, and cell cycle transitions. In: Tore, Frängsmyr (Ed.), Nobel Lecture, From *Les Prix Nobel*. : The Nobel Prizes 2001. Nobel Foundation, Stockholm, 2002.

Jacob, F., Monod, J., 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. 3, 318–356.

Judson, H.F., 1979. The Eight Day of Creation. Simon and Schuster, New York.

Kedes, L., Chang, A., Houseman, D., Cohen, S., 1975. Isolation of histone genes from unfractioned sea urchin DNA by subculture cloning in *E. coli*. Nature 225, 533–538.

Lamfrom, H., 1961. Factors determining the specificity of hemoglobin synthesized in a cell-free system. J. Mol. Biol. 3, 241–252.

Laubichler, M.D., Davidson, E.H., 2008. Boveri's long experiment: Sea urchin merogones and the establishment of the role of nuclear chromosomes in development. Dev. Biol. 314, 1–11.

Maderspacher, F., 2008. Theodor Boveri and the natural experiment. Curr. Biol. 18, R279–R286.

Maienschein, J., 1989. T.H. Morgan as invertebrate embryologist. Inverse. Reprod. Dev. 15, 1–6.

Marmur, J., Rownd, R., Schildkraut, C.L., 1963. Denaturation and renaturation of deoxyribonucleic acid. Prog. Nucleic Acid Res. 1, 231.

Masters, B.R., 2009. History of the electron microscope in cell biology. Encyclopedia of Life Sciences John Wiley & Sons, Ltd, pp. 1–9.

Maxson, R., Cohn, R., Kedes, L., 1983. Expression and organization of histone genes. Ann. Rev. Genet. 17, 239–277.

Monroy, A., 1986. A centennial debt of developmental biology to the sea urchin. Biol. Bull. 171, 509–519.

Monroy, A., Tyler, A., 1963. Formation of active ribosomal aggregates (polyribosomes) upon fertilization and development of sea urchin eggs. Arch. Biochem. Biophys. 103, 431–435.

Morgan, T.H., 1894. Experimental studies on echinoderm eggs. Anat. Anz. 9, 141–152.

Morgan, T.H., 1893. Am. Natl. 27, 222–232 Preface to Translation Boveri, T., 1889. An organism produced sexually without characteristics of the mother.

Morgan, T.H., 1911. An attempt to analyze the constitution of the chromosomes on the basis of sex-linked inheritance in Drosophila. J. Exp. Zool. 11, 365–413.

Morgan, T.H., 1934. The relation of genetics to physiology and medicine. Nobel Lecture in Nobel lectures, Physiology or Medicine 1922–1941. Elsevier Publishing company, pp. 313–328. 1965.

Nakano, E., Monroy, A., 1957. A method for the incorporation of radioactive isotopes in the sea urchin eggs. Experientia 13, 416–417.

Nakano, E., Monroy, A., 1958. Incorporation of S[35] methionine in the cell fractions of sea urchin eggs and embryos. Exp. Cell Res. 14, 236–244.

Nemer, M., 1962. Interrelation of messenger polyribonucleotides and ribosomes in the sea urchin egg during embryonic development. Biochem. Biophys. Res. Commun. 8, 511–515.

Nocente-McGrath, C., Brenner, C., Ernst, S.G., 1989. Endo16, a lineage-specific protein of the sea urchin embryo, is first expressed just prior to gastrulation. Dev. Biol. 136, 264–272.

Nirenberg, M.W., Matthaei, J.H., 1961. The dependence of cell-free synthesis in E. coli upon naturally occurring or synthetic polyribonucleotides. Proc. Natl. Acad. Sci. 47, 1588–1602.

Olby, R., 1974. The origins of molecular genetics. J. Hist. Biol. 7, 93–100.

Oppenheimer, J., 1963. Theodor Boveri: The Cell Biologists' Embryologist. Quat. Rev. Biol. 38, 245–249.

Pauling, L., 1970. Dedication to Albert Tyler. Dev. Biol. 21, iii–v.

Paweletz, N., 2001. Walter Flemming: pioneer of mitosis research. Nat. Rev. Mol. Cell Biol. 2, 72–75.

Pederson, T., 2006. The sea urchin's siren. Dev. Biol. 300, 9–14.

Pirie, N.W., 1990. Jean Brachet. Biograph. Mem. Fellows R. Soc. 36, 85–95.

Porter, K.R., Claude, A., Fullam, E., 1945. A study of tissue culture cells by electron microscopy. J. Exp. Med. 81 (3), 233–246.

Raff, R.A., Greenhouse, G., Gross, K.W., Gross, P.R., 1971. Synthesis and storage of microtubule proteins by sea urchin embryos. J. Cell Biol. 50, 516–527.

Raff, R.A., Colot, H.V., Selvig, S.E., Gross, P.R., 1972. Oogenetic origin of messenger RNA for embryonic synthesis of microtubular proteins. Nature 235, 211–214.

Rheinberger, H.-J., 1996. Comparing experimental systems: protein synthesis in microbes and in animal tissue at Cambridge (Ernest F. Gale) and at the Massachusetts General Hospital (Paul C. Zamecnik), 1945–1960. J. Hist. Biol. 29, 387–417.

Sander, K., Fisher, J.-L., 1996. How to dart ascidian blastomeres: The embryological micro-tools of Laurent Chabry (1855–1893). Landmarks in Developmental Biology 1883–1924, pp. 26–28. Sander, K.

Slater, D.W., Slater, I., Gillespie, D., 1972. Post-fertilization synthesis of polyadenylic acid in sea urchin embryos. Nature 240, 333–337.

Slater, I., Gillespie, D., Slater, D.W., 1973. Cytoplasmic adenylation and processing of maternal RNA. Proc. Natl. Acad. Sci. U.S.A. 70, 406–411.

Science, 2006. The sea urchin genome, 314, p. 5801.

Stearns, L.W., 1974. Sea Urchin Development: Cellular and Molecular Aspects Dowden. Hutchinson and Ross, Inc, Stroudsburg, PA.

Stern, C., 1950. Boveri and the early days of genetics. Nature 166, 446.

Sutton, W.S., 1902. On the morphology of the chromosome group in Brachystola magna. Biol. Bull. 4, 24–39.

Thomas, R., 1992. Molecular genetics under the embryologist's microscope: Jean Brachet, 1909–1988. Genetics 131, 515–518.

Tyler, A., 1963a. Action of polyuridylic acid on incorporation of C[14] l-amino acids by sea urchin eggs before and after fertilization. Am. Zool 505.

Volkin, E., 2001. The discovery of mRNA. Mutagen. Res. 488, 87–91.

Tyler, A., 1963b. The manipulations of macromolecular substances during fertilization and early development of animal eggs. Am. Zool. 3, 109–126.

Watson, J.D., Crick, F.H.C., 1953a. Molecular structure of nucleic acids. Nature 171, 737–738.

Watson, J.D., Crick, F.H.C., 1953b. Genetic implications of the structure of deoxyribonucleic acid. Nature 171, 964–967.

Wenrich, D.H., 1946. Clarence Erwin McClung 1870–1946. Science 103, 551–552.

Wilson, E.B., 1895. An Atlas: Fertilization and Karyokinesis of the Ovum. Macmillan and Co., New York.

Wilson, E.B., 1925. The Cell in Development and Heredity, 3rd ed. MacMillan and Company, New York.

Wilt, F.H., 1973. Polyadenylation of maternal RNA of sea urchin eggs after fertilization. Proc. Natl. Acad. Sci. U.S.A. 70, 2345–2349.

Winkler, M.M., Nelson, E.M., Lashbrook, C., Hershey, J.W.B., 1985. Multiple levels of regulation of protein synthesis at fertilization in sea urchin eggs. Dev. Biol. 107, 290–300.

Wolpert, L., 1995. The evolution of the cell theory. Phil. Trans: Biol. Sci. 349, 227–233.

Yuh, C.-H., Ransick, A., Martinez, P., Britten, R.J., Davidson, E.H., 1994. Complexity and organization of DNA-protein interactions in the 5'- regulatory region of an endoderm-specific marker gene in the sea urchin embryo. Mech. Dev. 47, 165–186.

Yuh, C.-H., Davidson, E.H., 1996. Modular *cis*-regulatory organization of Endo-16, a gut specific gene of the sea urchin embryo. Dev 122, 1069–1082.