Programming Cell Death in the 1960s: Developmental Biology beyond Dichotomy

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Summary: Programmed cell death (PCD) has been one of the most significant topics in modern biomedical research. Its broad importance in many biological and pathological phenomena, including morphogenesis, autoimmune disease, and cancer, demonstrates that its origin deserves a historical examination. By analyzing the role of developmental biology of the 1960s in shaping the notion of a program, this paper explains the emergence of a close correlation between not only life and death, but also the normal and the pathological in the postwar study of cell death.

Keywords: programmed cell death, developmental biology, life, death, the normal, the pathological

Research on programmed cell death (PCD) has grown into a large industry in the contemporary biosciences. As of May 2015, a PubMed search with “programmed cell death” as a keyword shows more than 290,000 entries. Indeed, scientists around the world are finding the death of cells in various circumstances, including the formation of digits during embryogenesis, the deletion of self-reacting immune cells, and the destruction of possible aberrant tissues with abnormal DNA. The phenomenon of PCD has come to the fore in the biomedical sciences, because these diverse instances of cell death are known to be relevant to a number of critical illnesses, such as autoimmune disorders, Alzheimer’s disease, AIDS, and cancers. PCD is thus commanding the attention of scientists in contemporary global biomedical enterprises.

This paper discusses what I believe is the most critical decade in the history of PCD, the 1960s. I think that it is important to review scientific research on PCD in these years because the idea of "programming" the death of cells was established at the time. Although cellular death itself was observed as early as the mid-nineteenth century, it was in the 1960s that several scientists, including Richard Lockshin, Carroll Williams, John Saunders, and John Fallon, conceived the idea that somatic cells died through intricate genetic and epigenetic programs.[1] Admittedly, a more formal theory of programming emerged in the 1980s through Robert Horvitz and his colleagues' experiments on *C. elegans*.[2] However, I contend, Lockshin's and others' research in the 1960s was a key predecessor. How did they think that there was a program that controlled cell death? What was the impact of their thoughts on future research on PCD? My answer to these questions addresses the historical origins of the ideas of programming cell death, which have not been a focal point of investigation in the history of bioscience and biomedicine.[3]

This essay illustrates how developmental biology, a new discipline replacing its precursor, embryology, contributed to the construction of the notion of a cell death program, which made possible a close correlation between not only life and death but also the normal and the pathological. As Hannah Landecker has stated, biomedical research on cells after the mid-

[1] Richard A. Lockshin and Carroll M. Williams, "Programmed cell death—II. Endocrine potentiation of the breakdown of the intersegmental muscles of silkmoths," *J Ins Physiol* 10 (1964): 643-649; John W. Saunders, Jr., "Death in embryonic systems," *Science* 154 (1966): 604-612; John W. Saunders, Jr. and John F. Fallon, "Cell death in morphogenesis," in Michael Locke (ed.), *Major Problems in Developmental Biology* (New York: Academic Press, 1966): 289-314.

[2] A key paper is Harry M. Ellis and Robert Horvitz, "Genetic control of programmed cell death in the nematode *C. elegans*," *Cell* 44 (1986): 817-829.

[3] There are many retrospective accounts of research on PCD written by scientists. See, for example, P. G. H. Clarke and S. Clarke, "Nineteenth century research on cell death," *Exp Oncol* 34 (2012): 139-145; D. L. Vaux, "Apoptosis timeline," *Cell Death Differ* 9 (2002): 349-354; N. Maghsoudi, Z. Zakeri, and R. A. Lockshin, "Programmed cell death and apoptosis—where it came from and where it is going: from Elie Metchnikoff to the control of caspases," *Exp Oncol* 34 (2012): 146-152; Richard A. Lockshin and Zahra Zakeri, "Programmed cell death and apoptosis: origins of the theory," *Nat Rev Mol Cell Biol* 2 (2001): 545-550. But there are only a small number of papers written by historians. See Hannah Landecker, "On beginning and ending with apoptosis," in Sarah Franklin and Margaret Lock (eds.), *Remaking Life & Death: Toward an Anthropology of the Biosciences* (Santa Fe: School of American Research Press, 2003): 23-59; Michel Morange, "What history tells us XXI. Apoptosis and programmed cell death: when biological categories are blurred," *J Biosci* 35 (2010): 177-181; Lijing Jiang, "History of apoptosis research," in *eLs* (Chichester: John Wiley & Sons, 2012) 1-7; Andrew S. Reynolds, "The deaths of a cell: how language and metaphor influence the science of cell death," *Stud Hist Philo Biol Biomed Sci* 48 (2014):175-184.

twentieth century highlighted the significance of death "on which life is dependent, or at least….with which life and disease are inextricably bound."[4] This statement overlaps with William Albury's view that "both life and death fall under the aegis of a perpetual maintenance programme" in twentieth century biomedicine.[5] In this new situation, I think, a key contribution was made through the novel discipline of developmental biology, born with a synthesis among embryology, cell biology, genetics, and molecular biology. This new science created a research space where cellular death became closely associated with its life. There the normal also found a novel form of coalition with the pathological, as developmental biology contributed to realigning their relationship.

In disentangling this relationship, my research method will be historical. It means that I use scientific vocabulary in the form that past scientists used within the context of their time. Hence, in the main body of my discussion, I do not use the word, "apoptosis," a morphological term that was often been used alongside "programmed cell death," despite some scholars' continued efforts for distinction between the two.[6] The exception is the final section in which I trace how the earlier study of PCD influenced the scholars who conceived the notion of apoptosis. Likewise, I do not use the term "active cell death," although a number of current biologists use it to avoid confusion and clarify the nature of their study subject.[7] Whereas the major students of PCD in the 1960s stressed that PCD was actively controlled, they did not call it "active cell death."

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[4] Landecker, "On beginning and ending," 56.
[5] William R. Albury, "Ideas of life and death," in W. F. Bynum and Roy Porter (eds.), *Companion Encyclopedia of the History of Medicinee*, vol. 1 (London: Routledge, 1993): 273.
[6] J. F. R. Kerr, A. H. Wyllie, and A. R. Currie, "Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics," *Br J Cancer* 26 (1972): 239-257.
[7] For example, see Robert S. Sloviter, "Apoptosis: A Guide for the Perplexed," *Trends in Pharmacological Sciences* 23 (2002): 19-24; Wilfried Bursch, Adolf Ellinger, Herald Kienzl, Ladislaus Török, Siyaram Pandey, Marianna Sikorska, Roy Walker, and Rolf Schulte Hermann, "Active Cell Death Induced by the Anti-estrogens Tamoxifen and ICI 164 384 in Human Mammary Carcinoma Cells (MCF-7) in Culture: The Role of Autophagy," *Carcinogenesis* 17 (1996): 1595-1607.

Early Research on Cell Death: Development, Evolution, and Immortality

Scientists began to observe cell death after Matthias Schleiden (1804-1881) and Theodor Schwann (1810-1882) proposed the cell theory in the 1830s.[8] First of all, the German scientist Carl Vogt (1817-1895) described the degeneration of the cells constituting the notochord during amphibian development.[9] This observation was important for understanding the process of development in general, since it indicated that the creation of an organ should accompany a complete or partial destruction of temporary structures. As Vogt claimed, the formation of vertebrae demanded the dissolution of the notochord during the development of amphibians. This was a crucial process of shaping the normal, healthy body.

However, scientists found that cell death could occur in a diseased body as well. After proposing his theory that cells were the ultimate sites of disease, the German pathologist Rudolf Virchow (1821-1902) made a distinction between "necrosis" and "necrobiosis."[10] Whereas necrobiosis was a naturally occurring death of cells due to a physiological necessity, necrosis was a pathological and violent cell death. According to Virchow, cells died under varying circumstances, which could be both normal and pathological.

Afterwards, other cases of cell death were discovered. Most notably, the German zoologist August Weismann (1834-1914) observed a massive breakdown of larval cells during insect metamorphosis, and Carl Josef Eberth (1835-1926) saw the degeneration of cells of the tadpole's tail during development. The Russian zoologist and Nobel laureate Elie Metchnikoff (1845-1916) also made a crucial contribution to the study by showing that phagocytes were responsible for eliminating unnecessary cells during growth processes. Strikingly, cell death was

[8] Jiang, "History of apoptosis research," 2; Clarke and Clarke, "Nineteenth century research," 139.
[9] Carl Vogt, *Untersuchungen über die Entwicklungsgeschichte der Geburtshelferkröte (Alytes obstetricans)* (Solothurn: Jent und Gassmann, 1842).
[10] Rudolf Virchow, *Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre* (Berlin: Verlag von August Hirschwald, 1859): 292-293.

not limited to the earlier phases of life, because it could occur in a mature healthy body as well. In effect, American embryologist Charles Minot (1852-1914) noted that epithelial cells in an adult body would gradually die while moving toward the surface, as younger cells in the deeper layers of the skin continued to multiply.[11] Likewise, cells lining the digestive track died and were cast off as fresh cells were generated to replace the lost ones.

These findings had evolutionary implications. Above all, the changes that occurred during development due to cell death seemed to support the biogenetic law, which postulated that an animal's embryogenesis "recapitulated" its evolutionary past. For example, the disappearance of a tadpole's tail during its metamorphosis into a frog could be understood as a piece of evidence for the evolutionary transformation from fish to amphibians. Cell death was also relevant to theories that did not refer to the biogenetic law. As early as 1889, Weismann proposed one such theory, based on his distinction between eternal "germ plasm" and temporary "soma." To him, the soma, unlike the germ plasm, had a limited life due to the necessity of maintaining the individual body's form that had been shaped through natural selection. Its somatic cells should stop growing and eventually die, because it must keep a certain form that it had acquired through its long evolutionary past in its natural environment.[12] In a similar vein, the British biologist G. P. Bidder (1863-1954) claimed that all somatic cells of terrestrial animals had to die at a certain point in their life, because oversized bodies were more prone to predators' attack.[13] Under the force of gravity, terrestrial animals could grow up only to a definite size limit so that they would remain swift enough to avoid their predators' aggression in their natural habitat. In contrast, the somatic cells of certain marine animals might indefinitely proliferate because the buoyancy of water could enable them to reduce the effect of gravity and maintain the

[11] Charles S. Minot, *The Problem of Age, Growth, and Death: A Study of Cytomorphosis* (London: John Murray, 1908): 71-72.
[12] August Weismann, *Essays upon Heredity and Kindred Biological Problems*, E. B. Poulton, S. Schönland, and A. E. Shipley (eds.) (Oxford: Clarendon, 1889): 31.
[13] G. P. Bidder, "Senescence," *Brit Med J* 2 (1932): 585.

speed they needed. Hence, they could live forever and die only through accidents, diseases, or predation.

Ironically, Bidder's view was linked to what appeared to be opposite to the death of the cell—cellular immortality. His argument that marine animals could grow indefinitely unless they were accidentally killed reflected the widespread belief that cells were inherently immortal.[14] Even though somatic cells, as Weismann had claimed, should usually die, their death was due not so much to their inherent limitation as to their external context that might have been formed during evolution. Cells of plants, invertebrates, protozoa might live an indefinite life, and even vertebrate somatic cells could be induced to live forever in a special condition. In effect, German botanists in the 1880s had already observed that some plants had extremely long lives, and their cells might be rendered immortal in some conditions.[15] Herbert Spencer Jennings (1868-1947) and other biologists also found that most protozoa could enjoy an eternal life, and Charles Manning Child (1869-1959) studied how some coelenterates and flatworms could prolong their life by "rejuvenating" their cells.[16] Likewise, Edwin Conklin (1863-1952) suggested that polyzoa and tunicates might become immortal through a regular removal of a part of their cytoplasm with metabolic wastes.[17] Based on these studies, the American biologist Raymond Pearl declared that "life itself is inherently continuous."[18]

The most influential scholar in promoting this idea of immortality was the French surgeon Alexis Carrel (1873-1944). As a major contributor to the technique of tissue culture, he argued that vertebrate somatic cells in culture would indefinitely continue their life, if scientists

[14] Ibid.
[15] Friedrich Hildebrand, "Die Lebensdauer und Vegetationsweise der Pflanzen, ihre Ursache and ihre Entwickelung," *Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie* 2 (1882): 51-134.
[16] Herbert Spencer Jennings, "Senescence and Death in Protozoa and Invertebrates," in Edmund Vincent Cowdry (ed.), *Problems of Ageing: Biological and Medical Aspects* (Baltimore: Williams and Wilkins, 1939): 32-52; Charles Manning Child, *Senescence and Rejuvenescence* (Chicago: University of Chicago Press, 1915): esp. 246, 257, 461.
[17] Edwin G. Conklin, "The size of organisms and of their constituent parts in relation to longevity, senescence and rejuvenescence," in *The Harvey Lectures*, series 8 (Philadelphia: Lippincott, 1913): 252-279.
[18] Raymond Pearl, *The Biology of Death* (Philadelphia: Lippincott, 1922): 48.

provided them with the best possible condition with a timely removal of their wastes and an optimal supply of fresh nutrients.[19] In contrast, the somatic cells *in vivo* would have only a limited lifespan because their natural surroundings did not provide such a condition. Due to lingering wastes or the lack of adequate nutrition, somatic cells in the body would eventually die. Starting from this assumption, Carrel and his colleagues at the Rockefeller Institute for Medical Research tried to find out the growth-accelerating and growth-inhibiting substances that controlled cellular lifespan, and attempted a long-term culture of chicken heart cells by placing them in what was believed to be an ideal culture condition.[20] They argued that a batch of chicken heart cells survived for more than 30 years under their perfect control of nutrients, wastes, temperature, and humidity.

Some historians have attributed the start of serious research on PCD in the 1960s to the demise of these theories of immortality. In particular, Landecker has argued that research on apoptosis—the term that she found is "used interchangeably" with PCD by many people including herself—began only after the repudiation of the immortality theories that fostered "particularly inhospitable conditions" for the study of cell death.[21] To Landecker, the idea of controlled cell death in a healthy body emerged only when the two American scientists, Leonard Hayflick and Paul Moorhead, challenged an early-twentieth century discourse of the inherent immortality of life in their 1961 paper, which claimed that vertebrate somatic cells had a limited

[19] Alexis Carrel, "Rejuvenation of cultures of tissues," *J Am Med Assoc* 57 (1911): 1611. In this paper, Carrel did not propose any evolutionary reason for cell death that he observed.
[20] Alexis Carrel and Albert H. Ebeling, "Age and multiplication of fibroblasts," *J Exp Med* 34 (1921): 599-623.
[21] Landecker, "On beginning and ending," 29-30, 41. Although Landecker does mention the continued "attempts to clarify the terminology," her paper does not make a clear distinction between apoptosis and PCD. Even though Ellis and Horvitz did not use the term "apoptosis" in their 1986 paper, she discusses their work as an extension of early research on apoptosis.

span of life.[22] Melinda Cooper has also asserted that "one of the most successful" consequences of Hayflick's work was the emergence of "the concept of programmed cell death."[23]

However, this assertion is questionable. Most importantly, none of the major scholars who established the notion of PCD cited Hayflick and Moorhead's paper. According to my research, the discovery of the ultimate death of somatic cells in culture did not influence researchers of PCD, because they were more interested in another problem, namely, the way in which cell death was *controlled* in the body. In a sense, Saunders, one of the students of PCD in the 1960s, was closer to Carrel than Hayflick. Like Carrel—who searched for the growth-accelerating and growth-inhibiting substances—Saunders sought factors controlling the fate of cells in culture. He indeed argued that "in the presence of certain externally supplied factors" the cells "whose normal prospective fate is death finally become stabilized in a condition leading to indefinitely prolonged life."[24] To Saunders, cell death was controlled by a set of substances—whose nature was uncertain but appeared similar to hormones—that could make possible cellular immortality.

Other hypotheses on the conception of PCD are also less than persuasive. One of them, proposed by scientists themselves, stresses the importance of new visual technologies, including electron and phase-contrast microscopes and microcinematography, which supposedly made possible the emergence of the notion of PCD in the mid-twentieth century.[25] However, the new visual technologies were a cause, rather than *the* cause, because their use requires the formation of a "visual culture" shared by scientists in a community, in which something novel and

[22] Leonard Hayflick and Paul S. Moorhead, "The serial cultivation of human diploid cell strains," *Exp Cell Res* 25 (1961): 585-621.
[23] Melinda Cooper, "Resuscitations: stem cells and the crisis of old age," *Body Soc* 12 (2006): 2.
[24] Saunders and Fallon, "Cell death in morphogenesis," 302.
[25] Lockshin and Zakeri, "Programmed cell death and apoptosis," 546; Maghsoudi, Zakeri, and Lockshin, "Programmed cell death and apoptosis," 147.

interesting, that is, a cell death managed by a program, could be found and discussed.[26] Even with their crude microscopes, many researchers did see cell death in the late nineteenth century but did not conceive of any idea of programming. Moreover, the electron microscope was already available in the 1930s, but the notion of programming cell death was still not forthcoming.

Another historical hypothesis found the cause in the demise of the predominant philosophy that life was "defined as the absence or resistance of death."[27] Only after this philosophy was abandoned in the 1960s, could scientists say that cell death was controlled under a program for modulating life. However, Albury has shown that this philosophy in medicine and biology was already overthrown by the new idea of "life and death as correlatives" in the nineteenth century.[28] Life and death were then thought to be close associates in several new discoveries and inventions in biology and medicine, including the life-saving surgical operation made possible through patients' apparent death with anesthesia, the death of humans caused by the proliferation of bacterial life, and the new life forms created through natural selection that killed unfit individuals. Although Albury has not mentioned anything about PCD, its emergence in the 1960s further promoted, rather than initially introduced, the idea of "correlation" of life and death into the biological and medical sciences.

How, then, was this idea further advanced in the 1960s? How did life and death find their correlation at the cellular level? The key, I think, was the rise of developmental biology in which Lockshin, Williams, and Saunders pursued their novel research.

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[26] Visual technology in science has been studied by many historians and sociologists. For a recent work, see Nancy Anderson and Michael R. Dietrich (eds.), *The Educated Eye: Visual Culture and Pedagogy in the Life Sciences* (Hanover: Dartmouth College Press, 2012).

[27] Jiang, "History of apoptosis research," 2.

[28] Albury has argued that twentieth century medicine replaced this idea with the newer emphasis on controlling both life and death, which were now defined as "contraries." But I think that the idea of correlation between life and death did not disappear in the twentieth century. Albury's examples of correlation in the nineteenth century continued to make sense in the twentieth century. See Albury, "Ideas of life and death," 254-272.

Conceiving the Program: Developmental Biology and Cell Death

In the late 1950s, embryologists were marginalized and sidelined. Their science was cut off from the major changes in the life sciences of their time.[29] Most of all, embryology was barely relevant to the Modern Evolutionary Synthesis, which created a new hybrid field reflecting the latest developments in population genetics, paleontology, and evolutionary biology. Embryology also had little relation to the rise of molecular biology. The novel approaches to life at the level of DNA and proteins brought forth a fundamental shift in scientists' methodological outlook, but embryology was rather isolated from the new direction of research. Nor could embryologists contribute to the postwar emergence of biomedicine, which, with the assistance of molecular biology and new financial and research institutions, rearticulated the relationship between "the normal and the pathological in such a way that research in one is immediately available to research in the other."[30]

It was in this context that embryology was recast as developmental biology. Facing the charge that their science was outmoded, some embryologists, along with molecular biologists, geneticists, and cell biologists, established a new field with a new name.[31] With this renamed discipline, students of embryogenesis would attract young researchers as well as financial and social support from potential patrons and the public. The Society for the Study of Growth and Development was thus renamed the Society for Developmental Biology in 1964 after launching a new journal, *Developmental Biology*, in 1959. In 1968, the International Institute of Embryology also became the International Society of Developmental Biologists.

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[29] Nick Hopwood, "Embryology," in Peter J. Bowler and John Pickstone (eds.), *The Cambridge History of Science*, vol. 6 (Cambridge: Cambridge University Press, 2009): 308-312.

[30] Peter Keating and Alberto Cambrosio, *Biomedical Platforms: Realigning the Normal and the Pathological in Late-Twentieth-Century Medicine* (Cambridge, Mass.: MIT Press, 2003): 72. For a brief review of the historiography of biomedicine, see Ilana Löwy, "Historiography of biomedicine: 'bio,' 'medicine,' and in between," *Isis* 102 (2011): 116-122.

[31] Evelyn Fox Keller, *Refiguring Life: Metaphors of Twentieth-Century Biology* (New York: Columbia University Press, 1993): 25-26.

This reorganization is reflected in *Major Problems in Developmental Biology* (1966), which included the presented papers of the annual symposium of the Society for Developmental Biology shortly after proposing its new name. In the introductory chapter, Jane Oppenheimer (1911-1996) at Bryn Mawr College stated,

Since genetics and the study of development have converged during the intervening quarter-century, together with the study of molecules and macromolecules; of proteins, enzymes, nucleo-proteins, and others; of cells and organelles; of metabolic pathways and immune reactions; of microbes and protozoans and fungi; and since their convergence has transformed biology and has carried it to depths hardly dreamed of when this Society first met as a Society in 1940, it may be appropriate to inquire to what degree the Society….may have reflected, or possibly have contributed to the development of the new biology.[32]

Her subsequent discussion of the body's immune response to pathogens as well as the significance of genetics and molecular biology bespoke developmental biologists' novel agenda and scope. The new science should place the phenomena of development amid the contemporary biological and biomedical study of life and death as well as the normal and the pathological. In effect, cancer was related to development, because its growth could be understood as a modification of the normal path of cellular differentiation. Immunology was also linked to development, since Frank Macfarlane Burnet (1899-1985) and Peter Brian Medawar (1915-1987) had found the creation of immune cells during embryogenesis.[33]

[32] Jane Oppenheimer, "The growth and development of developmental biology," in *Major Problems in Developmental Biology*: 1.
[33] Frank Macfarlane Burnet, *The Clonal Selection Theory of Acquired Immunity* (Cambridge: Cambridge University Press, 1959); H. W. Park, "'The shape of the human being as a function of time': time, transplantation, and tolerance in Peter Brian Medawar's research, 1937-1956," *Endeavour* 34 (2010): 112-121.

The other chapters in *Major Problems* likewise illustrated the expanded scope of the new science. James Ebert and Edward Kaighn's chapter dealt with the relationship between cellular differentiation and DNA replication, and H. Rubin's chapter discussed the development of cancer cells with regard to their surface structure. On the other hand, Conrad Hal Waddington summarized a rather classical subject in embryology, the problem of fields and gradients in development, whereas D. E. Koshland and M. E. Kirtley investigated a newer issue, the structure and function of enzymes in cellular dynamics and differentiation. Anton Lang's chapter on plants' intercellular regulation also indicated that developmental biology had begun to tackle the growth of plants as well as animals as their legitimate study topics.

In many of these inquiries, developmental biologists, unlike embryologists, actively pursued genetic and molecular approaches. Development began to be explained in terms of genes' differential expressions over time, and a number of model organisms—including *Xenopus*, *Drosophila*, and *Arabidopsis*—became the materials for investigating the function of nucleic acids and enzymes engaged in the process of development.[34] Admittedly, the idea that chemicals were responsible for key changes in development had already existed in experimental embryology, a subfield within the traditional discipline of embryology. For instance, Johannes Holtfreter's (1901-1992) research in the 1930s suggested that chemical factors in what Hans Spemann (1869-1941) had called the "organizer" induced the emergence of a secondary axis during amphibian development.[35] Several biologists, including Waddington and Salome Gluecksohn-Schoenheimer, also tried to synthesize genetics and embryology in the 1930s and the 1940s by examining the effects of mutation on embryonic induction, morphogenesis, and

[34] Of course, the use of these organisms as models needed extended discussions and negotiations within scientific communities. See Sabina Leonelli, "Growing weed, producing knowledge: an epistemic history of *Arabidopsis thaliana*," *Hist Phil Life Sci* 29 (2007): 193-223; Evelyn Fox Keller, "*Drosophila* embryos as transitional objects: the work of Donald Poulson and Christiane Nüsslein-Volhard," *Hist Stud Phys Biol Sci* 26 (1996): 313-346.
[35] Johannes Holtfreter, "Reminiscences of the life and work of Johannes Holtfreter," in Scott F. Gilbert (ed.), *A Conceptual History of Modern Embryology* (Baltimore: Johns Hopkins University Press, 1991): 109-127.

other processes.[36] However, developmental biology after 1960 pursued a far more systematic investigation into the macromolecular and cellular mechanisms in development. Developmental biologists had both conceptual and material means to explore the roles of nucleic acids, proteins, and other subcellular components in development.

The notion of a program was introduced into developmental biology at this time. It was a metaphor borrowed from computer science and cybernetics, the novel fields formed during and after World War II.[37] The sciences, reflecting the wartime research on computers and self-regulating weapons, impressed a number of biological researchers in the postwar period. Initially, François Jacob and Jacques Monod used the term in their 1961 paper in accounting for their discovery of the regulation of bacterial gene expression.[38] Similar to computers, Jacob later remarked, bacterial genes had a "programme," which managed the enzyme production by regulating their own expression.[39] Because this "programme" must be a central controlling agent in all forms of life, cells of multicellular organisms should have a similar "genetic programme" regulating their differentiation and function. In the 1960s, cybernetics and computer science shaped another line of thoughts on a program, which was quite different from Jacob and Monod's version. For instance, Michael Apter and Lewis Wolpert argued that the interaction among parts in the entire fertilized egg had an instruction for development, which was likened to a program. The genes in the nucleus were merely a kind of a "sub-routine" in a program that was "called on" when it was needed.[40] In this sense, development was guided by an epigenetic

[36] Scott F. Gilbert, "Induction and the origins of developmental genetics," in *A Conceptual History of Modern Embryology*: 181-206. Also see Kelleer, "Drosophila embryos," 319-323

[37] Evelyn Fox Keller, "Decoding the genetic program: or, some circular logic in the logic of circularity," in Peter J. Beurton, Raphael Falk, and Hans-Jörg Rheinberger (eds.), *The Concept of the Gene in Development and Evolution: Historical and Epistemological Perspectives* (Cambridge: Cambridge University Press, 2000): 162-165.

[38] François Jacob and Jacques Monod, "Genetic regulatory mechanisms in the synthesis of proteins," *J Mol Biol* 3 (1961): 318-356.

[39] François Jacob, *The Logic of Life: A History of Heredity*, tr. Betty E. Spillmann (New York: Pantheon Books, 1973): 9, 226-246.

[40] Michael J. Apter and Lewis Wolpert, "Cybernetics and development. I. Information theory," *J Theor Biol* 8 (1965): 257.

program that did not "exist at particular localized sites"—including nuclear genes—but acted rather "as a dynamic whole" during the process of development.

The subsequent growth of the idea of a program in developmental biology reflected Apter and Wolpert's as well as Jacob and Monod's standpoints. Few denied that the genes in the nucleus were important. Yet development also depended on other organelles, biomolecules, and their interactions that would form new sources of information in the entire body. Indeed, even the idea of the genetic program in Jacob and Monod's work included the notion of an environmental and nutritional feedback upon the gene expression. Since the genes, due to their reliance on various intracellular and extracellular entities, could not wield an overwhelming power on all other portions of the cell, the program in development had to be understood as a complex system with diverse factors interacting with one another. This understanding led scientists to challenge the distinction between the "genetic" and the "epigenetic."[41]

The first paper introducing the term, "programmed cell death," reflected these emerging discourses on programs. In their 1964 paper, Richard Lockshin and Carroll Williams argued that there was a "lethal programme" destroying the cells of the abdominal intersegmental muscles of saturniid moths as they underwent their metamorphosis.[42] According to Lockshin's later recollection, this term was suggested by Carroll Williams, who, as a professor of developmental biology, mentored Lockshin, a graduate student at Harvard. Because "computers were just beginning to be talked about at the time, programmed cell death seemed to be a particularly modern and colorful way of describing" the phenomenon that they studied.[43] To them, a central mechanism in this "programme" was found in the moths' "endocrine conditions"—which may be deemed "epigenetic"—although it also appeared to represent the "genetic information" whose

[41] Keller, "Decoding the genetic program," 159.
[42] Lockshin and Williams, "Programmed cell death—II," 643.
[43] Maghsoudi, Zakeri, and Lockshin, "Programmed cell death and apoptosis," 148.

details were never elucidated.[44] They found that a timely inoculation of juvenile hormones effectively blocked the muscle cells' death. Obviously, this "programme" was "carefully timed" and "highly localized," because the breakdown of the muscles did not occur in any other periods in the moths' life or in other portions of their body.

Notably, this concern was absent in earlier scientific literature on cell death, such as Alfred Glücksmann's 1951 paper, which has often been cited as a classic in PCD history. Glücksmann, a German scientist who moved to the Strangeways Laboratory at Cambridge with the rise of the Third Reich, published a comprehensive survey of all known instances of cell death, classifying them into three types, including "morphogenetic," "histogenetic," and "phylogenetic" degenerations.[45] This survey included Viktor Hamburger and Rita Levi-Montalcini's influential study in 1949 demonstrating the massive cell death in the chick embryo's neuronal development.[46] But Glücksmann did not propose any idea of a program, although he attempted to suggest several possible causes for individual instances of cell death. These causes were also quite distinct from what Lockshin and Williams discussed. Glücksmann's causes were negative in nature, such as "the fading out of stimuli for [cells'] proliferation or for the completion of their differentiation." To Glücksmann, "the fading out of stimuli," rather than their active control through a program, brought about cell death.

John Saunders's 1966 paper was very different, reflecting the changed context of the 1960s. Saunders, a member of the board of directors of the Society for Developmental Biology, situated his research within the ideas and problems of the new field. He was well aware of Lockshin and Williams's work alongside other literature on cell death during development. As a contributor to *Major Problems in Developmental Biology*, he also conducted his own research on

[44] Richard A. Lockshin and Carroll M. Williams, "Programmed cell death—I. Cytology of degeneration in the intersegmental muscles of the pernyi silkmoth," *J Inst Physiol* 11 (1965): 124.
[45] Alfred Glücksmann, "Cell deaths in normal vertebrate ontogeny," *Biol Rev* 26 (1951): 59.
[46] Viktor Hamburger and Rita Levi-Montalcini, "Proliferation, differentiation and degeneration in the spinal ganglia of the chick embryo under normal and experimental conditions," *J Exp Zool* 111 (1949): 457-501.

the chick embryo, which illuminated how the "posterior necrotic zone" (PNZ) of its wing bud was "programmed" to die (Fig. 1).[47]

[Place Figure 1]

By stage 17, PNZ cells seem to undergo a crucial change that would make them die at stage 24, even if they were grafted to the somite region of a different embryo or cultured in a petri dish. However, this death could be prevented if the dorsal tissues of the wing bud were transferred to a site near PNZ cells before stage 22 (Fig. 2).

[Place Figure 2]

The same phenomenon was also observed when PNZ cells were cultured alongside the wing bud's dorsal cells. Although PNZ cells had to die "on schedule" by stage 17, this "death sentence" could become "revocable" by a specific group of cells from the wing bud's dorsal region, which seemed to secrete "diffusible materials" controlling cell death. This was a case of the "epigenetic control."

Saunders also reviewed several instances of the "genetic control" of cell death, which he came to know primarily through his "stimulating discussions" with geneticist Edgar Zwilling.[48] Zwilling's research indeed demonstrated abnormal cell loss during the caudal vertebra development of the chicken with a mutant gene, *rumplessness*, which was a dominant hereditary factor.[49] Likewise, some mice with *Danforth's short tail* underwent extensive cell death during their tail development. Interestingly, the effect of this gene appeared "dosage-dependent."

[47] Saunders, "Death in embryonic systems," 606.
[48] Ibid., 612.
[49] Edgar Zwilling, "The development of dominant rumplessness in chick embryos," *Genetics* 27 (1942): 641-656.

Whereas the homozygote showed the complete loss of its tail, the heterozygotes came to develop tails of varying lengths. These instances made clear that there was a "genetic programming," although the epigenetic control appeared equally important.[50]

Ambiguity and Universality in Cell Death

Despite, or because of, all of these findings, PCD became an ambiguous issue, much like the genetic program that it was partially modeled on. As Evelyn Fox Keller has claimed, there was an "essential ambiguity" in the idea of a genetic program, not only because its definition continued to be controversial, but also because the gene could be both the object and subject of the program's control.[51] Likewise, the role of genes in the program of cell death remained unclear. Saunders and his colleague John Fallon, in their contribution to *Major Problems in Developmental Biology*, stated that "the pattern of genetic readout may set up within a prospectively dying cell a death clock."[52] Lockshin and Williams also argued that individual cells' "deaths represent the decoding and acting-out of a fresh, albeit final, bit of genetic information."[53] Yet they also thought that cell death was under epigenetic control. The juvenile hormones' impact on moths' muscle degeneration and the influence of the "diffusible materials" on PNZ cells' fate were examples of the "tissue-environmental" and "hormonal" control, which, due to their independence from genes, was "epigenetic" in nature.[54] What, then, was the relationship between the epigenetic and genetic controls? To what extent did the genetic and epigenetic factors respectively contribute to the death program? Did the epigenetic factors affect the activation of the genetic factors, or vice versa? There was no clear answer.

[50] Saunders and Fallon, "Cell death in morphogenesis," 312.
[51] Evelyn Fox Keller, *Making Sense of Life: Explaining Biological Development with Models, Metaphors, and Machines* (Cambridge, Mass.: Harvard University Press, 2002): 136.
[52] Saunders and Fallon, "Cell death in morphogenesis," 311.
[53] Lockshin and Williams, "Programmed cell death—I," 124.
[54] Saunders, "Death in embryonic systems," 606, 608.

In addition, the meaning of the "program" was vague. Above all, the scientists thought, cells would perish under the control of a program, and that cell death itself could also be a part of the program for an embryo's development. Lockshin and Williams wrote that "the death of specific cells and tissues is a part of the 'construction manual'" whereas the cells "that will die have been programmed to do so."[55] What, then, was the meaning of the program they discussed? Did it indicate cell death per se, or something that controlled it?

Furthermore, the metaphors representing PCD were ambiguous. Significantly, it was uncertain whether PCD was a type of "suicide" or "assassination."[56] Cells appeared to kill themselves by releasing their autolytic enzymes, but this occurred only after they were rendered morbid through certain mechanisms outside of the cell. As Andrew Reynolds has noted, this ambiguity was akin to the problems concerning human suicide, because it can occur due to social problems, such as unemployment during an economic recession or increasing cyber-bullying in the age of the Internet.[57] To what degree do such social problems cause people to kill themselves? To what extent did the extracellular mechanism cause a cell's release of its own autolytic enzymes? At another level, it was possible that PCD was neither suicide nor assassination, but rather a mechanical phenomenon. In addition to the aforementioned terms like "construction manual" and "death clock," other mechanical metaphors, such as "booby trap," were often used by PCD researchers. If metaphors play cognitive and heuristic roles in science, how did these divergent metaphorical representations affect PCD researchers' thinking?[58] Were they confusing?

Another dimension of ambiguity is found in the scientists' conflation between the normal and the pathological. Although PCD was considered a normal phenomenon, it could be pathological as well. For instance, Saunders asserted that the "death of cells is a normal

[55] Lockshin and Williams, "Programmed cell death—I," 124.
[56] Saunders, "Death in embryonic systems," 608-609.
[57] Reynolds, "The deaths of a cell," 177, 181.
[58] George Lakoff and Mark Johnson, *Metaphors We Live By* (Chicago: University of Chicago Press, 2003).

component" in embryogenesis, although he also found that PCD could cause pathological changes in the mouse retina development.[59] He noted, "The basic adult pattern of the retina is differentiated by 12 days after birth, but, in [mice] homozygous for *retina dystrophy*, pathological changes set in at this time, and the rods degenerate almost completely in 2 weeks."[60] "A number of congenital anomalies in the human being" could also be attributed to PCD under the control of mutant genes. Unless properly managed, PCD could always bring forth unwanted consequences. In such a situation, can we say that cell death occurred through a program? If the program itself had a defect, to what extent was PCD a normal phenomenon? This problem was exacerbated by the fact that Saunders and Fallon used the term "necrosis" in describing both normal and pathological cell degenerations. "Necrosis" could be seen in the death of PNZ as well as in the abnormal tissue death due to a genetic disease. If this was the case, was Virchow's distinction between "necrosis" and "necrobiosis" still valid?

The problem had an institutional aspect: Lockshin, Williams, and Saunders were all funded by the National Institutes of Health along with the National Science Foundation, and Saunders alone was also supported by the American Cancer Society.[61] Undoubtedly, PCD researchers had a medical implication in mind when they designed their research and applied for a grant. Interestingly, Saunders, with the money earmarked for medical research, conducted his experiment at the Marine Biological Laboratory at Woods Hole, an American bastion of traditional experimental biology, including embryology.[62] In studying PCD, the normal was interlaced with the pathological in terms of institutions as well as scientific concepts.

However, these features of the research on PCD were not necessarily its weakness. Just as the ambiguity in theories of genetic programs—according to Keller—made possible a

[59] Saunders and Fallon, "Cell death in morphogenesis," 289.
[60] Saunders, "Death in embryonic systems," 608.
[61] Lockshin and Williams, "Programmed cell death—II," 643; Saunders, "Death in embryonic systems," 612.
[62] Philip J. Pauly, *Biologists and the Promise of American Life: From Meriwether Lewis to Alfred Kinsey* (Princeton: Princeton University Press, 2000): 145-164.

successful explanation of both heredity and development, the aforementioned ambiguities in PCD at least in part contributed to promoting research on it. In particular, the flexibility of its meaning benefited various scholars pursuing different projects. The program could be either cell death or any agents in charge of it, and death itself could also be understood as suicide, assassination, or the explosion of a booby trap. As Reynolds and Michel Morange have claimed, these diverse metaphors encouraged new insights and approaches, although it might also confuse scientists.[63] The suicide-assassination problem in particular spurred an extensive investigation of the biological processes regulating death because it was necessary to know the conditioning processes prior to starting a cell's self-destruction. The simultaneous use of mechanical and organic metaphors likewise made PCD researchers receptive to new lines of investigations in which biologists and engineers found mutual inspiration from the similarities between living organisms and machines.[64] Just as a fail-safe system was found in both computer engineering and the mammalian developmental processes, a living organism's systems coordinating cellular "suicide" could be a sort of ingeniously designed machinery.

The questions on the genetic and epigenetic control of cell death were interwoven with these new lines of work entangled with the metaphors. Saunders, Lockshin, and Williams's ambiguous standpoint on the genetic and epigenetic control opened up a space where later molecular and biomedical investigators, armed with diverse metaphors, started scrutinizing factors involved in cell death. After the 1980s, scientists actually found that PCD is managed by a number of proteins and their corresponding genes in the intercellular and intracellular networks of signal transduction, composed of caspases, p53, Bcl-2, cytochrome c, and others.

That many of these proteins and genes are connected to cancer and immunity also illustrates an outcome of the ambivalent position of PCD between the normal and the

[63] Reynolds, "The deaths of a cell," 180-183; Morange, "What history tells us," 180.
[64] Evelyn Fox Keller, *The Century of the Gene* (Cambridge, Mass.: Harvard University Press, 2000): 103-130.

pathological in contemporary biomedicine.[65] Although Saunders discussed only congenital abnormalities, PCD is also intertwined with the occurrence and regulation of carcinogenesis, since its inhibition permits an uncontrolled proliferation of cells with defective genes, which may engender tumors. Furthermore, cell death has been understood as a critical phenomenon in creating and maintaining the normal immune system, whose breakdown initiates a number of pathological conditions. Certain forms of autoimmune diseases can be tracked down to the defects in factors regulating cell death.

Developmental biology not only made a central contribution to this expansion of research on PCD, but also fostered the notion of universality in studying cell death. Developmental biologists, with their agenda of universality, tried to cover all forms of life in their field, highlighting development as a key problem in the modern life sciences and biomedicine dealing with nucleic acids, proteins, cells, and organs.[66] Likewise, students of PCD in the 1960s, as developmental biologists, projected their new field's vision of universality into the study of cell death.[67] In fact, developmental biologists' work highlighted the complex relationships among a number of apparently opposing categories, such as genes-development, organisms-machines, animals-plants, morphogenesis-carcinogenesis, and entire bodies-macromolecules. PCD researchers appropriated this standpoint into their research, in their effort to understand cell death across these categories. Through this effort, the death of a cell became a phenomenon regulated by a complex network of genes, proteins, and others functioning during development and adult life. With diverse metaphors inspiring varied lines of investigation, cell death was thus represented as an essential portion of the systems managing health and life, but it was also

[65] For a comprehensive review, see James E. Vince and John Silke, "Apoptosis: regulatory genes and disease," in Gary Melino and David Vaux (eds.), *Cell Death* (Chichester: Wiley-Blackwell, 2010): 197-211.
[66] Keller, *Refiguring Life*, 25-42.
[67] According to Landecker, "apoptosis" was considered a "universal feature of cells." See Landecker, "On beginning and ending," 28.

known to contribute to disease and death. Research on cell death became a part of the pursuit of universality beyond dichotomies in the modern bioscientific enterprises.

Cell Death after the 1960s: Apoptosis and a Universal Program

The establishment of PCD as a significant scientific problem in the 1960s heralded the subsequent expansion of research on it. The work of Saunders, Fallon, Lockshin, and Williams became a precedent for later endeavors by an increasing number of scientists interested in cell death. Admittedly, not all of these scientists were developmental biologists, and they had no reason to cling to earlier scholars' standpoints and approaches. Rather, they diversified research on cell death starting from the basic ideas that earlier students of PCD expounded, that is, the notions of universality, the control by a program, and the correlation between life and death as well as health and illness.

In the early 1970s, John Kerr, Andrew Wyllie, and Alstair Currie conducted a particularly important work in this regard. Apparently, they were different from students of PCD in many respects. As pathologists without training in developmental biology or embryology, they used their new morphological term "apoptosis" rather than PCD, and described the processes of cell death with their advanced electron microscopy, which showed details of cellular death hitherto unknown.[68] Nevertheless, their underlying standpoint was not very different from that of advocates of PCD. Most significantly, Kerr and his colleagues cited Saunders and Fallon's paper in 1966 and declared that apoptosis, like PCD, was "an active, inherently programmed phenomenon."[69] Apoptosis was closely associated with life, because it was "implicated in the

[68] The term "apoptosis" came from Greek, meaning the "'falling off' of petals from flowers, or leaves from trees." See Kerr, Wyllie, and Currie, "Apoptosis," 241.
[69] Ibid., 239.

fashioning of developing organs and digits, and in the involution of phylogenetic vestiges in the embryo."

Furthermore, apoptosis, like PCD, occurred in both normal and pathological situations. Admittedly, there was a distinct category of pathological cell death, "coagulative necrosis." It was "invariably caused by noxious stimuli," accompanying extensive inflammation and tissue disruption.[70] Apoptosis, in contrast, was a more controlled phenomenon with its typical morphological features, including "nuclear and cytoplasmic condensation" that led to the creation of fragmented "apoptotic bodies" that would eventually be removed after phagocytosis by other cells (Fig. 3).

[Place Figure 3]

To Kerr and his associates, however, apoptosis was still integrated with pathogenesis at several levels, because it could occur in tumor as well as healthy cells and could be induced by both pathological and physiological stimuli. Apoptosis could take place alongside coagulative necrosis in certain types of tissue injuries caused by hepatotoxins, electromagnetic radiation, and others. Apoptosis was a means to eliminate damaged and misbehaving cells that would possibly cause problems in the body.

This view of Kerr's was in line with Peter Keating and Alberto Cambrosio's depiction of postwar pathologists. According to Keating and Cambrosio, pathologists after World War II viewed their research objects as neither a quantitative deviation from the normal ones nor an ontologically independent entity.[71] In postwar biomedicine, the normal and the pathological were

[70] Ibid., 239, 250.
[71] Keating and Cambrosio, *Biomedical Platforms*, 69-82. This view of Keating and Cambrosio's is based on their interpretation of the contemporary relationship between the normal and the pathological, a longstanding subject discussed in the French philosopher Georges Canguilhem's classic, *Essai sur quelques problèmes concernant le normal et le pathologique* (1943).

conceptually, discursively, and practically interwoven. In this context, cell death became a "universal" phenomenon as a key contributor to the processes of life, in its pathological as well as normal conditions.[72] The new science, developmental biology, played a crucial role in this reassessment of cell death. With the establishment of the novel field, death and illness merged with life and health.

[72] Landecker, "On beginning and ending," 28.