Background

The proper development of an embryo from a fertilized egg has long posed a fascinating and difficult question. To examine complex processes, biologists often use genetic approaches, which involve collecting mutant organisms that differ from the normal or “wild-type” organism. In studying developmental biology, a geneticist’s approach involves looking for mutant organisms that display an obvious defect in overall formation. Early work uncovered a number of genes involved in the development of the fruit fly Drosophila melanogaster.

In the first genes examined, mutations resulted in the birth of flies with obvious physical defects, such as the presence of an extra set of wings. Because this approach relied on examining viable flies with physical malformations, it missed many developmentally important genes that, when mutated, result in the death of the fly embryo.

In the late 1970s, Nüsslein-Volhard and Wieschaus began their pioneering work on the development of Drosophila embryos. They sought to identify as many genes in the developmental process as possible by looking for genes that resulted in the death of the embryonic fly. Their work unveiled several key genes active in the early development of not only Drosophila, but higher organisms as well.

The Experiment

Geneticists develop systematic methods, known as genetic screens, to search for mutations that affect biological processes. Nüsslein-Volhard and Wieschaus had to consider several previous observations on Drosophila development when they designed their screen. First, they knew that genes expressed in the egg, called maternal-effect genes, as well as genes expressed after fertilization in the developing embryo, called zygotically active genes, controlled the early development of an embryo. They chose to focus on isolating zygotically active mutants. Second, they had to consider that the Drosophila genome is diploid, which means that the progeny receives a copy of each gene from both parents. Scientists had previously demonstrated that Drosophila required only a single wild-type copy of most genes in order to develop into a viable fly. This made it likely that the developmentally active mutants that the screen was looking for would be recessive. Therefore, to see defects resulting from mutations in these genes required breeding the mutant Drosophila such that it was homozygous for the mutations.

The overall mutation rate in a naturally occurring population is quite low. If a geneticist were to search for mutants in a natural population, he or she would have to examine a large number of individuals. To circumvent this difficulty, Nüsslein-Volhard and Wieschaus induced mutations
in a population at the onset of the screen, then created inbred lines to assure that each fly they examined would carry
the induced mutation on each chromosome. They fed a mutagenic chemical to male flies, then mated them to a genetically
defined population of female flies in a process known as a genetic cross. The resulting progeny would be heterozygotes because they would have the mutation only on
the chromosome they received from the father. To assure the homogeneity of the genetic background, the heterozygote males were mated again to females of the same genetic
background, establishing an inbred line. Finally, males and females from the inbred line were mated to each other, and
the progeny were examined for the desired phenotype, embryonic death.

Using this screen, Nüsslein-Volhard and Wieschaus amassed a large collection of mutants. The next step was to assign the mutant Drosophila to specific classes, based
on their phenotype. They focused on the segmentation of the larvae. Whereas all mutants in this screen necessarily
displayed the phenotype of embryonic lethality, they differed greatly in their segmentation defects. To classify these
defects, Nüsslein-Volhard and Wieschaus examined the lar-
vae under the microscope. They compared the body pattern
of a wild-type larva (see Figure 14.1, far left), which is vi-
able, to those of the embryonic lethal mutants. By comp-
paring these patterns, they uncovered three classes of genes
that affect segmentation, which they called segment polarity, pair-rule, and gap.

Gap mutants are missing up to eight segments from the
overall body, without regard to symmetry (see Figure 14.1),
which results in a smaller body type. Three mutants—
knirps, hunchback, and the previously characterized Kru-
pel—fell into this class. The next class of mutants, the segment polarity mutants, has the same overall number of segments as the wild-type larvae. The mutation resulted in a
deletion of the body pattern within a segment. The deleted segment was replaced by a mirror image of the portion that
remained. Nüsslein-Volhard and Wieschaus’s initial screen
uncovered six mutants of this class, three of which, goose-
berry, hedgehog, and patch, had not been previously ob-
served. The final set of mutations, the pair-rule mutation,
resulted in deletion of alternating segments of the body,
which caused a shorter body formation. Five previously un-
characterized mutants, paired, even-skipped, odd-skipped,
barrel, and runt, as well as one known mutant, engrailed, were placed in this class.

Discussion

By the first report of their screen, Nüsslein-Volhard and Wieschaus had identified 15 mutants that affected segmentation. Of these, only five were previously identified genes. When they completed the study—often referred to as the Heidelberg screens—they had identified 139 different genes that, when mutated, resulted in embryonic death. These mutations fell into 17 different classes. These mutants formed the base for the past 20 years of research into the development of Drosophila. As molecular techniques evolved, scientists cloned many of these genes and characterized their gene products.

The majority of proteins encoded by the genes have been shown to be transcription factors, but the screen also uncovered signaling molecules, receptors, enzymes, adhesion molecules, cytoskeleton proteins, and proteins whose functions remain unknown. Scientists interested in mammalian development have studied homologues of the Drosophila genes uncovered by Nüsslein-Volhard and Wieschaus, and have shown them to be important in mammalian development as well. In 1995, the Nobel Foundation awarded its prize for Physiology and Medicine to Nüsslein-Volhard and Wieschaus for their pioneering work.