Looking for the Homunculus in Drosophila

Alan Garen

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511

By 1965 it was evident that the reductionist approach of molecular biology to complex biological phenomena was proving remarkably effective, at least as applied to bacteria and their viruses. The basic mechanisms underlying the genetic control of cellular processes seemed sufficiently well understood for Jacques Monod to proclaim that what is true for Escherichia coli is true for elephants. Such magisterial confidence in a future as yet uncharted was bolstered by the elegant simplicity and generality of the operon model of gene regulation and expression, derived mainly from studies of β-galactosidase synthesis in E. coli. Nevertheless, the obstacles facing anyone hoping to achieve a comparable understanding of developmental processes in higher organisms were daunting. I recall the humbling impact of viewing a time-lapse film on early Drosophila development. The kaleidoscopic cascade of coordinated changes in cell number, movement, morphology and function, compressed into a brief span of 24 hr, seemed beyond the scope of molecular dissection with techniques then available. What was needed as a first step into the field was a conceptual framework for transforming such enormous phenomenological complexity into experimentally manageable problems.

I can trace my beginnings as a developmental biologist back to the summer of 1965 when I had the good fortune of attending a lecture at Yale by Ernst Hadorn, a pioneer in reestablishing the vital link between genetics and development that had been forged earlier by Thomas Hunt Morgan but surprisingly neglected afterward. Focusing on Drosophila, the geneticists’ favorite eucaryotic organism, Hadorn (and also Donald Poulsdon at Yale and Edward Lewis at Cal Tech) used mutants to identify several key genes involved in development and to characterize the mutant phenotypes. These early studies demonstrated the value of mutants as starting material for dissection of complex developmental processes, and provided insights into the developmental roles of individual genes which would serve as valuable guides for subsequent molecular studies.

In Hadorn’s lecture I learned about a basic aspect of Drosophila development, namely that early in embryogenesis two separate developmental pathways are established, one for larval structures and another for imaginal (adult) structures (Hadorn 1965). The imaginal pathway involves an initial stage of cell proliferation during which separate populations of undifferentiated imaginal cells are generated within the imaginal discs; each disc subsequently differentiates during the pupal stage into a particular adult structure. Hadorn and his students at the University of Zurich had succeeded in culturing undifferentiated imaginal cells in vivo, using the abdomens of adult flies as incubators, for extended periods spanning virtually an unlimited number of cell divisions. When the cultured imaginal cells were transplanted back into a larval host, the transplanted cells usually differentiated according to their original fate; for example, cultured wing disc cells differentiated into wing structures, similarly cultured eye disc cells differentiated into eye structures, and so forth. This result was a revelation for me: it provided a powerful simplifying concept of development by demonstrating a clear operational distinction between the programming of cells for specific developmental fates, called determination, and the expression of the program, called differentiation. Furthermore, imaginal cell determination was shown to be a stable heritable state, which was a puzzling finding because determination presumably has an epigenetic rather than a primary genetic basis. Hadorn’s seminal achievement in developing reliable and accessible methods for culturing and assaying specifically determined imaginal cells provided an opening for entering the field, and helped to define as a clear if distant goal the elucidation of the genetic and molecular basis of determination.
Given the sophisticated level of Drosophila genetics, in contrast to the limited options available at that time for molecular studies of complex biological systems, we chose as a first project to search for mutants affecting imaginal determination. One important class of such mutants was already known, namely the homeotic class which exhibits striking imaginal transformations, for example an antenna to leg transformation in antennapedia or a haltere to wing transformation in bithorax. The homeotic mutants, in common with most other Drosophila mutants that had been characterized, develop into viable and fertile adults, because mutants were generally identified by their morphological or behavioral abnormalities as adults. This was a serious limitation to the scope of classical genetic analysis in Drosophila, because many developmentally important genes, including those controlling imaginal determination, are likely to generate mutants which are predominantly if not exclusively lethals. Therefore, a screen for lethal mutants affecting imaginal development was initiated in 1967 with ALLEN SHEARN, who had just completed his doctoral thesis on Neurospora genetics in NORMAN HOROWITZ's laboratory. Designing such a screen required assumptions about the mutant phenotype, which we anticipated would be a late larval lethal. The assumptions were that larval development involves a separate and independent pathway from imaginal development and therefore would not be affected by defects of imaginal development; also, that at least some of the genes controlling the imaginal pathway are specific for that pathway. Focusing first on zygotic-effect mutants which die as late third-instar larvae, we found that in about 50% of the mutants some or all of the imaginal discs were either missing or morphologically and developmentally defective, indicating that the assumptions were valid (SHEARN et al. 1971; SHEARN and GAREN 1974). It could be estimated that as many as 1000 zygotically active genes are specifically involved with the imaginal pathway, possibly including early steps of determination.

HADORN's studies with cultured imaginal cells had demonstrated that the cells were fully determined by the third-instar larval stage but did not provide information about earlier stages. To address that important point, HADORN and his students G. SCHUBIGER and M. SCHUBIGER-STAUD designed an elegant experiment which involved dissecting genetically marked 10-hr embryos to produce anterior and posterior halves, and then dissociating the embryonic cells to form cell suspensions. The dissociated cells from differently marked anterior and posterior halves were mixed together, cultured in adult hosts to allow the imaginal cells to reach maturity, and then tested for their developmental fates by transplantation into larval hosts. The results were clear and dramatic: anterior head structures were formed only from cells of the anterior half and posterior genital structures were formed only from cells of the posterior half, showing that the imaginal cells are already autonomously determined as early as 10 hr after fertilization, although further processing is needed before the cells acquire the capacity to differentiate (SCHUBIGER, SCHUBIGER-STAUD and HADORN 1969). When HADORN's student WALTER GEHRING joined the laboratory as a postdoctoral fellow, we decided, together with graduate student LILLIAN LING-ChAN, to repeat HADORN's embryo culture experiment using younger embryos. The cellular blastoderm stage was chosen because it marks a major transition point in early development when the syncytial embryo becomes transformed into a multicellular embryo containing several thousand newly formed somatic cells arranged at fixed positions along the inner surface of the egg. During the syncytial blastoderm stage the nuclei are totipotent, because shifting their positions in the embryo results in a corresponding shift in their developmental fates. We expected the embryo culture experiment to provide equivalent information about the developmental potential of the blastoderm cells. The results obtained with cultured cellular blastoderm embryos were the same as those previously obtained with 10-hr embryos, showing that autonomous imaginal cell determination occurs concomitantly with blastoderm cell formation (CHAN and GEHRING 1971). Therefore, earlier stages of embryogenesis and oogenesis must have a key role in establishing the necessary positional information for determination.

Accordingly, we extended the genetic analysis of imaginal development to oogenesis, which traditionally had been neglected mainly for technical reasons because it would have involved an additional genetic cross using homozygous mutant females; the additional cross is not only labor-intensive but also requires homozygous mutant adults and therefore can only be performed with mutants that are not zygotic lethals. Nevertheless, encouraged by the possibility of identifying maternal-effect genes involved in providing positional information specifically for imaginal cell determination, THOMAS RICE, a graduate student, decided to focus his doctoral thesis on a large-scale screen for maternal-effect mutants involving such genes (RICE 1968). The screen was designed to detect a particular maternal-effect mutant phenotype which would exhibit apparently normal development until the cellular blastoderm stage, when imaginal determination occurs; defects of imaginal determination should result in late larval lethality or adult abnormalities as previously shown for zygotic-effect mutants. No mutant with that phenotype appeared in RICE's screen, nor in similar screens for maternal-effect mutants conducted by several other laborato-
ries. It appears that the larval and imaginal pathways are initially interdependent, sharing maternally derived positional information, and that imaginal-specific functions are controlled entirely by zygotically active genes.

Rice's screen yielded 10 rare maternal-effect lethal mutants, representing eight complementation groups, which formed a morphologically normal syncytial blastoderm. In three of the groups blastoderm cell formation was either partially or totally blocked, probably because of a defect in cell membrane synthesis (Rice and Garen 1975); the other five groups formed a morphologically normal cellular blastoderm but shortly afterward showed major abnormalities of gastrulation. Gastrulation-defective mutants have since been shown to involve genes controlling dorsal/ventral polarity in the embryo (Anderson and Nüsslein-Volhard 1986). With the remarkable advances in elucidating the complex genetic control of early Drosophila development, it is becoming evident that maternal-effect genes have a key but limited role in providing positional information for imaginal development. Although the process of imaginal determination begins during oogenesis, it continues into the zygotic stage as zygotically active genes refine the rough maternal sketch for the imaginal homunculus. By the cellular blastoderm stage the individual imaginal cells have become autonomously determined entities, no longer dependent on positional cues in the embryo to pursue their developmental fates.

The phenotypes of Rice's mutants were sufficiently intriguing to attempt an identification of the products encoded by the maternal-effect genes. The strategy was to repair the mutant defect by injecting cytoplasm from normal eggs into the eggs produced by the homozygous mutant females, and then to apply this bioassay to fractions prepared from the normal cytoplasm in order to purify the active component. Briggs and Cassens (1966) at the University of Indiana had already shown that the defect in a maternal-effect Axolotl mutant could be repaired in this way, although no purification experiments had been attempted. Since there was no precedent for an analogous experiment with Drosophila, I spent several months developing an injection technique suitable for the much smaller Drosophila egg. Gehring and I tested the effectiveness of this technique with the maternal-effect lethal mutant deep orange (dor) which was chosen because, unlike Rice's mutants, the dor defect could be repaired by the zygotic activity of a paternal dor* gene and therefore might respond similarly to injection of normal egg cytoplasm. The success of the deep orange tests (Garen and Gehring 1972) opened the way to similar tests with other mutants which were more relevant than deep orange to the analysis of positional information in early development (Anderson and Nüsslein-Volhard 1984).

The entire field of development would soon shift into high gear with the introduction of techniques for gene cloning and expression. Although we reported the cloning of the first Drosophila gene (Lepesant, Kejzlarova-Lepesant and Garen 1978), our focus had changed by then and so the clone contained an ecdysone-inducible gene rather than a maternal-effect gene.

Early Drosophila development is now known to proceed via a highly complex network of coordinated interactions involving many maternally and zygotically expressed genes and their encoded products. A sophisticated understanding of the molecular bases of those interactions is rapidly emerging as a result of the brilliant contributions from several laboratories, notably those of Nüsslein-Volhard, Gehring, Hogness and their colleagues. The elusive homunculus is finally shaping up not as an object but rather as a dynamic process.

LITERATURE CITED


