The 1995 Nobel Prize in Medicine: A tribute to the power of formal genetics

One of the most remarkable areas of achievements in biology during the past few decades has been the elucidation of the mechanisms that an apparently unstructured egg cell uses to transform itself into a complex-patterned multicellular organism. The first glimpse of this genetic blue-print was obtained through genetic studies with the fruit fly *Drosophila*. Fortunately, these results were obtained at a time when the recombinant DNA and molecular biological techniques were spreading like wild fire and therefore, even these esoteric genetic studies with *Drosophila* attracted the imaginations of biologists working with all kinds of organisms. Very soon the commonality of organization of all living organisms was reaffirmed and scientists could hope to decipher the genetic programme that controls development of as complex an organism as *Homo sapiens*.

Therefore, the committee for this year's coveted Nobel prize in Medicine has very rightly selected three *Drosophila* geneticists for their pioneering work on the genetic control of embryonic development and differentiation using simple but powerful tools of conventional genetic analysis. They are Edward B. Lewis of The California Institute of Technology, USA, Christianne Nüsslein-Volhard of the Max-Planck Institute for Entwicklungsbiologie, Tubingen, Germany and Eric Wieschaus of Princeton, USA. Lewis has been working with *Drosophila*, mostly by himself, for more than 50 years and published[1] a summary and analysis of the data collected by him over many years in his well-known *Nature* paper in 1978; Nüsslein-Volhard and Wieschaus published[2] their seminal paper, also in *Nature*, in 1980 when both were at the EMBO Laboratory in Heidelberg, Germany. These two papers have changed the course of contemporary biology in more than one way by allowing a new look at the transformation of a seemingly structureless egg into a complex, patterned and organized organism. The concepts generated in these papers have found very wide applications in studies dealing not only with animals but plants as well. It is remarkable indeed, that these two papers were based on simple methodologies of 'pure' or formal genetics with no 'sophisticated' or 'advanced' molecular biological techniques being employed. As is the wont of geneticists, they simply obtained a large number of mutations, selected those that affected early development, mapped the mutations on linkage maps and characterized the consequences of either individual mutations or specific combinations of the different mutations on the developmental phenotype of the individual. The only 'advanced' analytical techniques employed by these scientists were scanning electron and/or dark-field microscopy! It was the systematic analysis and a foresight in rationalization of the phenotypic effects of the various mutant genes that led them to formulate general principles of Nobel prize-winning consequence.

Study of embryonic development has fascinated biologists for a long time. Curiosity to know how a single-celled egg develops into a complex organism led to the growth of the whole field of Embryology to describe the morphological and anatomical changes taking place in a developing embryo. In biology it is often necessary to examine the abnormal so that the normal may be understood. Thus a conventional experimental embryological approach to the study of a complex process has been to disrupt one step in the chain of events and follow its consequence. This path was followed by generations of eminent embryologists during this century and their efforts did help in providing some

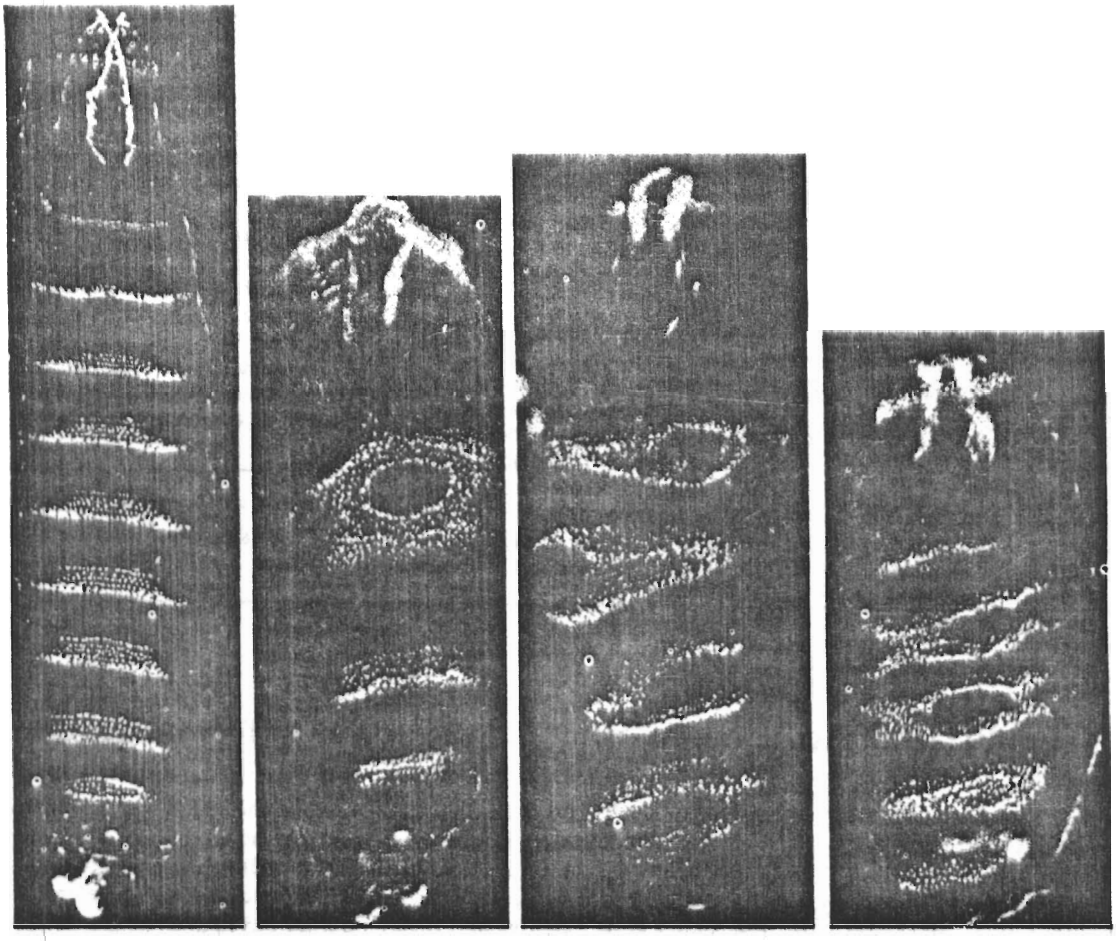

Figure 1. Examples of disruptions in normal segmentation of late *Drosophila melanogaster* embryos due to mutations in some of the segmentation genes identified by Nüsslein-Volhard and Wieschaus: the first panel shows the segmentation pattern in a normal late embryo; the second is an embryo mutant for the *krüppel* gap gene (with the prothoracic to 5th abdominal segments missing), the third one is mutant for the *paired* pair-rule gene (with the posterior compartment of all odd-numbered and the anterior compartment of all even numbered segments missing) while the last one has a mutation at the *engrailed* segment-polarity gene (with posterior compartments being affected). Identification of such defects in segment patterns in mutant embryos led the scientists to interpret the functions of the wild-type genes in normal pattern regulation.

cause–effects relationships in the complex events taking place during embryonic development. However, none of these approaches could address the central question of what molecules actually caused a particular cell in the embryo to decide or to know that it belongs to say head or tail region of the future animal or the leaf or root of the plant. With the growing realization that like all other things in living organisms, the development was also under genetic control, a geneticist would approach this issue by inducing random mutations in genes and identifying, not only the gene that has mutated but also the consequence of that mutation on the phenotype of the individual. It is this approach that led the three Nobel-prize winning scientists to provide the first insights into the complex signalling and regulatory events that 'tell' the undifferentiated cells to be committed to and follow a particular path of differentiation during early embryonic development.

To appreciate and understand the significance of these two papers[1,2], we need to briefly look at the way the *Drosophila* embryo develops (it is interesting to note that the basics of *Drosophila* embryology were properly understood only because of these and other subsequent papers, yet to understand these papers *today* we first need to look at what was historically learnt later: this highlights the farsighted intuition of these scientists as well as the power of genetic analysis). Unlike any other well-studied case of embryonic development, the *Drosophila* embryo follows a very unusual pattern of cleavage divisions during which only the nucleus divides repeatedly without any cell division; this results in a syncitial blastoderm stage which quickly cellularizes. This is followed by the demarcation, along the anteroposterior axis, of the repeating units of a defined number of body segments (parasegments) with the visible segment boundary bisecting each parasegment. As a result, each anatomical segment includes the posterior half and the anterior half of consecutive parasegments. Depending upon the antero-posterior and dorso-ventral coordinates, cells in each half (compartment) of the segment are committed to generate specific adult structures. A variety of genetic studies had established that even at the syncitial blastoderm stage, much before the morpho-

logical segments become apparent, each nucleus 'knows' its location in the embryo and also what its 'fate' is going to be when the cells begin to differentiate. The time gap between this 'commitment' and 'differentiation' can in fact be several days long. The basic question of how the nuclei in the syncitial blastoderm of *Drosophila* 'know' their location and their fate was addressed through genetic studies.

Although Lewis worked much earlier than Nüsslein-Volhard and Wieschaus, the genes that Lewis studied are called upon their duties later in embryonic development than the sets of genes described by the other two. Nüsslein-Volhard and Wieschaus argued that genes that control early embryonic development (and thus affect the critical determination events), if mutated, should cause malformed embryos and therefore be embryonic lethal. Therefore, they generated, through conventional mutagenesis procedures, a large number of random mutations in *Drosophila* genome and selected those that led to death of embryos. A simple microscopic examination of these dead embryos helped them determine the defect in the normal developmental pattern and thereby decipher the role of the mutated gene in the process (see Figure 1). In summary, Nüsslein-Volhard and Wieschaus showed that three sets of genes are sequentially activated after fertilization: i) the 'gap' genes define by their expression, three major domains (anterior, middle and posterior) along the length of the embryo so that a mutation in any of these genes resulted in the loss of a contiguous series of structures along the antero-posterior axis; ii) the 'pair-rule' genes, working after the 'gap' genes, commit the nuclei to one of the 14 repeating segments along the length of the syncitial blastoderm: these were called 'pair-rule' genes since they seemed to work in alternate segments and a mutation in any of these genes caused alternate, odd or even-numbered, segments to be defective, and iii) the 'segment-polarity' genes which finally commit a blastoderm nucleus to the anterior or to the posterior compartment of a given body segment so that a mutation in any of these genes disrupts the polarity in each segment. These three sets of genes thus were shown to 'instruct' the blastoderm nuclei their polar co-ordinates and thereby define segmentation of the body, much before we can actually see it.

A parallel development around this time was the identification, again through mutational analysis, of a number of genes that were active during the period of oogenesis in the mother and whose products were later shown to be very orderly localized in the egg cell; the products of these maternally acting genes behaved like the classical morphogens to directly activate the different gap genes in different domains of the fertilized egg and through them the later acting pair-rule and segment polarity gene cascades.

The genetic system elucidated by Lewis comes into play after the above three sets of genes have carried out their work. Lewis worked with a very interesting family of mutations, designated by *Drosophila* geneticists as the 'Bithorax-complex' (BX-C): this name was given since the flies that carried such mutations typically showed duplication of the thoracic region (see Figure 2). Such mutations belong to an interesting class, the homeotic mutations, a term coined by W. Bateson[3] in 1894 for malformations that substituted the pattern of one body region for another. Very early in the growth of *Drosophila* genetics, a number of homeotic mutations (e. g. the *bithorax, aristapedia, proboscipedia*) were identified. The existence of homeotic mutations signified that certain genes 'tell' the cells at some stage of development as to what they are destined to become and if, as a result of mutation, these genes gave a wrong instruction to a cell, the cell develops a different structure at a wrong place (the homeotic transformation). Thus such mutations provide an unique opportunity to ask what these genes 'tell' the cell and at what time. Lewis approached this goal by analysing the large number of mutations that mapped to this locus and which transformed a thoracic or an abdominal segment of the fly into another in a complex but predictable fashion. He had earlier shown that this locus is a complex locus being composed of several discrete genetic units (hence these individual units were initially termed 'pseudoalleles') which were very closely linked but each had a distinctive effect on the phenotype of thoracic and the abdominal segments. He summarized an enormous wealth of his own experimental data in the 1978

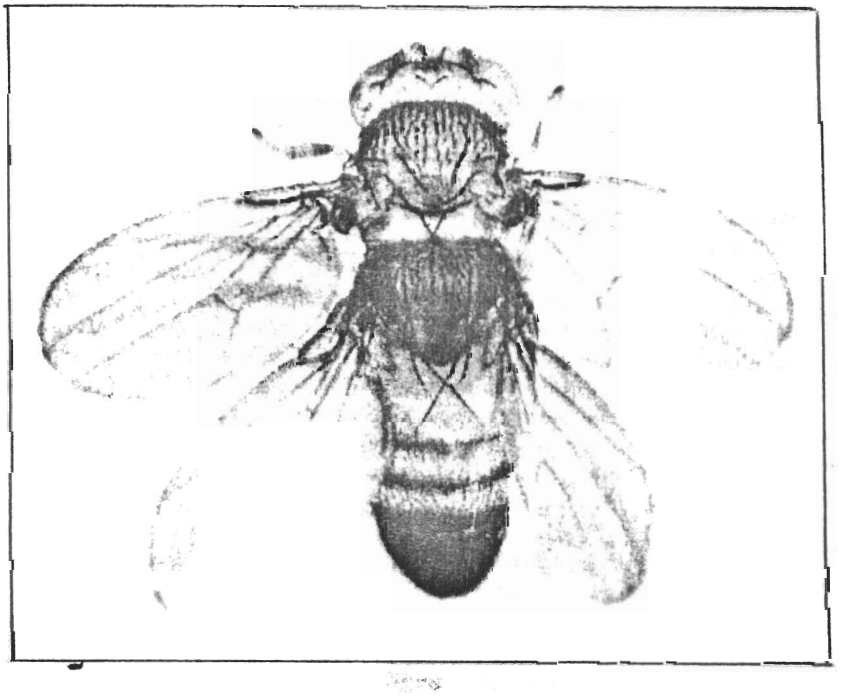

Figure 2. A typical four-winged fly generated in Lewis experiments when certain *bithorax* mutant alleles were brought together in heterozygous combinations: such a combination of the mutant alleles lead to a homeotic transformation of the meta-thorax into meso-thorax so that the normally reduced second pair of wings (the halters) in the meta-thoracic segment now develop into full wings like those in the meso-thoracic segment.

review published in *Nature*[1] to show that the different genetic elements comprising the BX-C control most of the body plan of the fly. He showed that a chromosome that had lost almost whole of the BX-C resulted in an embryo with all its body segments being similar (no abdominal segments are formed) and that addition of the different parts of the BX-C one by one can restore each segment in a defined order. Lewis argued that his results demand that the BX-C contains a minimum of eight genes that 'code for substances controlling levels of thoracic and abdominal development'. A most remarkable point noted by Lewis was that the order of these genes in the BX-C was collinear with the structures along the antero-posterior axis of the fly controlled by them. Lewis also argued that each subsequent posterior segment of the body recruits the services of an additional genetic element in the BX-C linear array. Subsequent studies (see ref. 4) at molecular and genetic levels, however, revealed that Lewis misinterpreted the *cis*-acting regulatory elements in the BX-C for structural protein-coding genes: the BX-C actually has only three structural genes but multiple *cis*-acting regulatory elements which are recruited in a serial fashion to activate one or more of the structural genes of the BX-C, each of which can produce more than one kind of message due to alternative processings of the primary transcripts. However this misinterpretation does not in any way undermine the far-reaching implications of his conclusions that the BX-C genes specified the developmental pathways to build the characteristic pattern of the different body parts and that these genes worked locally. The BX-C and other homeotic genes (like the Antennapaedia-complex or ANTP-C) are also called as the selector genes which take over from the segment-polarity genes and select the specific structures that each compartment has to elaborate as the embryo continues its development[4].

While working with BX-C, Lewis did face intellectual opposition from contemporaries since the genetic organization of BX-C defied the then held beliefs about a 'gene': some mutations mapping to this locus suggested that the whole system of the *bithorax* genes was integrated while others pointed to existence of separate and separable elements of function. Unmindful of criticisms and scorn of some contemporary molecular biologists, Lewis pursued the task of understanding the system of bithorax genes which resulted in his great 1978 paper. An interesting point mentioned by Peter Lawrence in his introduction to the historic 1978 *Nature* paper by Lewis in a collection of 'Outstanding Papers in Biology'[5], regarding the publication of this paper is worth noting. To quote Lawrence[5], 'A publication from Ed Lewis is an unusual and special event. In the past 40 years his papers have been few, and their originality and idiosyncrasy has found them natural homes in unrefereed conference reports. In 1978, I had a "phone call from him to ask for help in submitting a new paper to *Nature* – an article which would consist of a 'review' of a vast amount of Lewis's own unpublished work". Peter urged the *Nature* to 'snaffle' without question and to publish unrevised, lest the quibbles over details may cause the paper to be sent elsewhere or even delay it for a decade! As Lawrence further notes 'it is just the kind of contribution that the modern system of reviewing, with its rigid conventions and predilections against imaginative speculation, would censor. We should all be glad that *Nature* decided to publish it.'

How did these papers dealing with 'pure' genetics stimulate such a global interest? In 1978, when Lewis had published his paper, few could appreciate the import of his speculative conclusions (e. g. William McGinnis, one of the discoverers of the 'homeobox' (see later), notes[6] that the Lewis paper 'is oft cited but rarely read in its complex entirety'). Fortunately for Nüsslein-Volhard and Wieschaus, the scientific community in the early eighties had become receptive to prospects of genes controlling development and also this community was now ready to look at the genes from the molecular point of view. Combining the powers of 'formal' genetics with recombinant DNA technology, it was soon possible to clone some of these 'developmental' or 'segmentation' genes and to ask more directly how did they function. Consequently, it was possible to understand the biochemical bases for the observed effects of the various segmentation gene mutations characterized by Nüsslein-Volhard and Wieschaus and to verify the inferences drawn by them from their mutational analysis.

The most impact-making discovery that followed from these papers in the early eighties was the finding of a short stretch of highly conserved DNA sequence in the coding regions of a number of homeotic and segmentation genes. This sequence, aptly termed the 'homeobox' (denoting its association with the homeotic genes), provided a plausible biochemical function for the action of the homeotic genes whose products now appeared to be DNA-binding transcriptional regulators. Discovery of the 'homeobox' was a prelude to a flood concerning homeobox genes and homeodomain proteins, a flood that has channelled into a steady river of homeo-publications, fed by many tributaries[6]. Quickly, the homeobox was making its appearance in genes from as diverse organisms as man, mouse, chick, frog, sea urchin, nematodes and even plants (see ref. 6). Its high degree of conservation provided a simple handle to clone the developmental genes from a large variety of organisms and for many a biologist (as well as the common man since this topic had become the subject of newspaper and magazines in the eighties), the homeobox was (somewhat mistakenly!) equivalent of the Rosetta stone, the universal genetic key to body plan. The most remarkable fact that emerged from these studies was that the organization of the homeotic genes like the BX-C and ANTP-C in *Drosophila* and their counterparts in other organisms (including man) was identical.

These studies thus led to the emergence of the fundamental concept that in all organisms, the events of differentiation and pattern formation result from cascades of activity of specific groups of homologous genes which sequentially 'tell' the cells of their spatial and temporal co-ordinates and accordingly each cell gets committed to a particular path of development. Most of these genes produce transcription factors that regulate the expression of other genes. These studies showed that, as in any language a limited set of alphabets are used to create an endless variety of words and expressions by varying their combinations, in the biological system also, a limited number of regulatory genes can be used to generate a large array of patterns by expressing in different combinations in dif-

ferent cells along the antero-posterior and dorso-ventral axes.

Ever since Thomas Hunt Morgan introduced the fruit fly or *Drosophila* to genetics early in this century, this small insect has been central to the growth of the subject of genetics and thereby to many fundamental concepts in biology. It is interesting to note that Morgan himself was an embryologist who turned to genetics with a view to understanding the central issues of development and differentiation. Although by 1913 Morgan gave up 'efforts to deal simultaneously with genetics as transmission and genetics as development'[7], it is interesting to note that Morgan's objective of understanding development in terms of genetics has finally been achieved and the power of this approach been duly recognized by this year's Nobel prize in Medicine.

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