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Contents

Sequential Cell Polymorphism: A Fundamental Concept in Developmental Biology

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1 Introduction

Many years ago, Wigglesworth (e.g. 1954) introduced the concept of sequential cell polymorphism. The outstanding example was the production of distinct types of cuticle and of cuticular derivatives by insect epidermal cells undergoing metamorphosis in a succession of moults. For historical reasons, the concept has been primarily associated with developmental changes occurring during discrete moulting cycles, under the impetus of changing hormonal states. I believe that this formulation of the concept is only a special one, and that the concept is fundamentally applicable to a very wide variety of developmental phenomena. I believe that developmental regulation centrally involves the programmed expression of specific gene sets in an orderly and often overlapping sequence. The programming of this sequential expression is what makes cell types fundamentally different from each other, and must ultimately be understood if we are to understand development. The actual sequential expression can be

described as a succession of visibly differentiated states. According to the physiological exigencies of the system, this succession may or may not be entrained by hormonal changes; sequential polymorphism may be relatively gradual, as well as saltatory. In this formulation, sequential polymorphism refers to a succession of distinguishable but possibly overlapping states of determination and differentiation—regardless of the physiological mechanisms which may have evolved to control the cell's progress through the succession. The value of this concept to the developmental biologist is that it gives proper attention to the dynamic, temporal aspects of regulation, instead of placing the commonly excessive emphasis on a static end state of differentiation.

In this paper, I will discuss polymorphism while reviewing the work that my colleagues and I have performed over approximately the last ten years. Many additional examples (including some that are more apt) could be adduced to illustrate the concept of sequential polymorphism in this extended formulation.

2 Cellular metamorphosis in the saturniid labial gland

The paired labial glands of larval silkworms are known as silk glands. In saturniids, as in *Bombyx* (Yamanouchi, 1921), they are long tubes, ending blindly at their distal end and opening together in the spinneret on the labium. Two main parts can be distinguished. The posterior division is secretory, producing and storing the silk proteins, fibroin and sericin. The anterior division (Kafatos, 1971) is a narrower duct, which serves to conduct the liquid silk from the secretory division to the spinneret during spinning. This duct consists of approximately 1000 highly polyploid cells. Their main function appears to be the production and maintenance of a thick (up to 20 μm) cuticle, which forms a rigid tubular channel. Shearing forces developing during the extrusion of silk through this narrow channel may serve to orient the metastable fibroin molecules, facilitating their subsequent crystallization into an insoluble thread (Lucas *et al.*, 1958). It is also possible that the duct serves of modify the silk solution in subtle ways, by addition or removal of components. However, comparison of the abundance (relative to dry mass) of radioisotopes of Ca^{2+}, K^+ and Cl^- in the cocoon and in stored liquid silk gives no evidence that the duct adds ions to silk. If anything, slight reabsorption of K^+ and Cl^- from liquid silk may occur during spinning.

At pupation, the secretory division completely degenerates (as does the entire silk gland in *Bombyx*). The duct, however, is preserved. The cells shed their characteristic larval cuticle and their cytoplasm regresses. The

nuclear branches, highly extended in the larval cell, are pulled together into a compact mass, surrounded by a thin layer of cytoplasm (Fig. 1 in Selman and Kafatos, 1974). Evidently, the pupal cells are at a developmental standstill. However, during the subsequent adult development they grow again and undergo a dramatic metamorphosis. First they secrete a thin (<1 μm), loosely fibrous cuticle, strikingly different from that in the larva. Then their mitochondria proliferate and become intimately associated with deep plasma membrane infolds which develop, first in the basal and then in the apical side of the cells. At or near the time of adult emergence, much of the cell consists of these mitochondria-membrane associations (Fig. 2 in Selman and Kafatos, 1974). This structural specialization corresponds to the new function of the cells: the gland now secretes copious amounts of essentially isotonic $KHCO_3$ solution (Kafatos, 1968). Physiological experiments suggest that active transport of K^+ by the cell, from the blood into the lumen, may drive the production of the secretion as a whole. The rate of secretion is very high: the volume of liquid produced per minute approaches the volume of the secretory cells. The copious secretion promotes the digestion of the cocoon by the enzyme cocoonase, in those species which lack an escape valve (Kafatos and Williams, 1964).

The transformation of the larval, predominantly cuticle-producing cells into the predominantly salt-secreting cells of the adult is under hormonal control (direct or indirect). The transformation occurs during the metamorphic moults, which are triggered by ecdysone acting in the near or total absence of juvenile hormone (JH). If JH is injected into the pupa at the very beginning of adult development (Hakim, 1972), the cells become pupal-adult intermediates, with variable development of the mitochondria-membrane associations. A cuticle is always produced, whereas the pupal cells apparently lack a cuticle (Hakim, 1972). However, the cuticle in juvenile hormone-injected animals is usually considerably thicker than in normal adults. In fact, in some cases (including some glands forced by transplantation and JH injection to develop beyond the pupal state twice in the presence of JH), the ultrastructural appearance of the cuticle is somewhat reminiscent of that in the larval duct (Hakim, 1972). Formally, this cannot be considered a case of reversal of metamorphosis, since the pupa does not produce a cuticle. It can be argued that the information for producing a larval-type cuticle is included in the "pupal gene set", but is normally not implemented because secretion of *any* cuticle is blocked by a mechanism which thereafter becomes insensitive to the presence or absence of the hormone. In any case, the abnormal cuticle and the unequal development of the adult specializations of the labial gland in different JH-injected animals (Hakim, 1972) suggest that the metamorphosis of the labial gland may not be a unitary process under all-or-none control by the

hormone. The period of JH-sensitivity is limited to the beginning of adult development (Hakim, 1972).

The cells of the labial gland are polyploid and thus do not undergo cell division. Later than the period of hormone sensitivity, and at about the time the mitochondria-membrane associations begin to appear, DNA synthesis does occur. This could be associated either with further growth of the cells which is occurring at that time, or with a necessity for new copies of the genome for "reprogramming" into the adult state of differentiation. The results of inhibitor experiments suggest that the former explanation may be correct: the DNA synthesis can apparently be inhibited without interfering with the metamorphosis of the cells (Selman and Kafatos, 1974).

In conclusion, the labial gland cells of saturniids are an example of sequential cell polymorphism under hormonal control. The transformation into the adult state does not appear to require DNA synthesis. The existence of pupal-adult intermediates suggests that several, partially dissociable processes may be involved in this transformation. Salt secretion, the function of the adult state, is certainly not unique to these cells—but it is so extensively developed as to constitute a state of differentiation as distinct as, say, that of the cecropia mid-gut (Anderson and Harvey, 1966), or the Malpighian tubules (Berridge and Oschman, 1969).

3 The sphingid labial gland: a more complex case of cellular metamorphosis

In the sphingid, *Manduca sexta*, the duct of the larval labial gland again undergoes transformation during metamorphosis (Hakim, 1972; Hakim and Kafatos, 1976). In this case, the larval duct has a cuticle thinner than that of saturniids, but similar in construction. The secretory division produces a lubricant, rather than silk fibres, and the duct may play a more active role than in saturniids, since it has moderately prominent mitochondria-membrane associations (Figs 7-9 in Hakim and Kafatos, 1976). The secretory division of the larval gland degenerates during pupation, and the duct transforms into the complex salivary gland of the adult (Hakim and Kafatos, 1974, 1976).

In contrast to the uniform saturniid adult glands, each of the paired sphingid adult glands is divided into four distinct regions (Hakim and Kafatos, 1974). In a posterior to anterior division, these are region I, which consists of cells specialized for the production of invertase; region II, the cells of which produce a K^+-rich salt solution and are as rich in mitochondria-membrane associations as the saturniid adult gland; region III, which contains squamous cells with sclerotized cuticle, apparently functioning in the forward conduction of the dilute invertase solution produced

by regions I and II; and region IV, which appears to function in the reabsorption of salts and the concentration of the saliva. In addition to the paired glands, a short common duct (region V) exists; it appears to be derived from the larval common duct and to be purely conductive in function.

All four cell types of the paired glands are derived from the polyploid cells of the paired larval ducts (Hakim and Kafatos, 1976). During adult development the pupal cells, which were initially uniform in appearance, begin to be distinguishable by their cytoplasmic growth pattern. Then they secrete distinctive cuticle, different for the various gland regions. Finally, they assume the cellular morphology that corresponds to their respective adult functions.

The origin of the adult regions from specific areas of the larval duct has been investigated by extirpation, transection and transplantation experiments (Hakim and Kafatos, 1976). While the cells are expressing their larval differentiation, they are already programmed for specific adult states. At least in the fifth larval instar, particular areas of the duct are already determined to form specific adult regions. Preliminary experiments with earlier larval stages suggest that at least some determination may exist before the fifth instar.

In the sphingid gland, sequential polymorphism is again punctuated by the moults of metamorphosis. The polymorphism is more dramatic than in saturniids, in that it results in the formation of several distinct cell types from the duct cells of the caterpillar. Moreover, one of these cell types secretes a digestive enzyme, invertase, which is undetectable in the larval gland. In this system, it is clear that a number of different (adult) determination states can coexist with the same (larval) state of overt differentiation. Since determination is the first step of differentiation, the results emphasize the temporal overlap between the states of sequential polymorphism.

4 The cocoonase organules of the silkmoth galea: multistage sequential polymorphism in epidermal derivatives

Cocoonase is a trypsin-like proteolytic enzyme which digests sericin, loosening the silk fibres and permitting the moth to escape from the cocoon (Kafatos and Williams, 1964; Kafatos *et al.*, 1967a, b; Hruska and Law, 1970; Hixson and Laskowski, 1970; Hruska *et al.*, 1973; Felsted *et al.*, 1973; Kramer *et al.*, 1973). It is produced in the form of a zymogen, which is activated as it appears on the surface of the animal, one to two days before adult emergence (Berger *et al.*, 1971; Berger and Kafatos, 1971a; Felsted *et al.*, 1973). The cells which produce the zymogen are

found in epidermal organules (Lawrence, 1966), in the lateral aspects of two modified mouthparts, the maxillary galeae (Kafatos and Williams, 1964; Kafatos and Feder, 1968; Kafatos, 1970; Kafatos, 1972a; Selman and Kafatos, 1975).

The galeae first appear as two conical protuberances of the epidermis during the metamorphosis of larva into pupa. At that time they consist of a seemingly uniform population of epidermal cells, indistinguishable from those found in other parts of the integument. The cells produce the thick pupal cuticle and remain otherwise inactive throughout the pupal life. This epidermal cell population gives rise to the zymogen-producing organules during adult development (the pharate adult stage). Formation of the organules presupposes action of ecdysone in the absence of JH: when sufficient JH is injected at the beginning of the moult cycle, so as to cause the development of a "second pupa" instead of an adult, formation of the zymogen organules is blocked and the cells remain in the state characteristic of the pupa (unpublished observations). The period of JH sensitivity does not extend beyond the first two days of adult development. In gross terms, the sequential polymorphism is punctuated by the action of developmental hormones, and may seem to be saltatory (from a cuticle-producing to a zymogen-producing state).

Closer attention to the process of zymogen organule formation reveals that this description is oversimplified (Kafatos, 1970; Kafatos, 1972a; Selman and Kafatos, 1975). The hormonal state prevalent at the beginning of adult development does not bring about in one step the characteristic adult function (zymogen production), but instead initiates an entire sequence of "adult" developmental states, one of which corresponds to production of zymogen during a limited time period. This is particularly evident from the ultrastructural studies (Selman and Kafatos, 1975). Sequential polymorphism is saltatory in an overall sense, but gradual or multistage in detail.

Early in adult development, the epidermis retracts from the pupal cuticle, leaving behind the moulting gel. Some cellular growth occurs, and then mitotic figures appear in the lateral epidermis of the galea. These are presumed to be differential divisions, because of the vertical orientation of the spindle, which contrasts with the horizontal orientation in proliferative divisions of the insect epidermis (Lawrence, 1966). By day 5 of the three-week period of adult development, the epithelium is pseudostratified and several cell types begin to be distinguishable. The organule forms from the specific association of three cells, which are probably derived from the differential divisions. Two of these cells become polyploid and are ultimately destined to synthesize zymogen (cells Z_1 and Z_2), and the third (cell D) forms a unicellular cuticular "main duct" which permits extrusion

of the zymogen from the organule to the surface of the galea. These cells surround each other in their apical region, forming three coaxial cytoplasmic layers, with the cytoplasm of cell D outermost and that of Z_1 innermost; more basally, the coaxial arrangement is disrupted and the cells are separate.

The coaxial arrangement of the apical cytoplasmic regions is related to the morphogenesis of the main duct and of an attached cuticular valve, the end apparatus. The latter regulates extrusion of the zymogen into the main duct, from a storage cavity surrounded by cells Z_1 and Z_2. Beginning with day 6, cells Z_1 and Z_2 retract from the surface, but during this process the intercellular junctions between Z_1 and Z_2, Z_2 and D, and D and normal epidermal cells, respectively, remain intact. As a result, the formerly apical surface of cell D is drawn inwards and comes to line a narrow tubular extracellular channel; this surface then secretes a thin cuticular layer, forming the hollow tubular main duct. The formerly apical surfaces of Z_1 and Z_2 secrete cuticular material, which makes up the end apparatus; cell Z_2 also contributes to the most basal part of the main duct, and to the joint between main duct and end apparatus.

When completed, the main duct and the joint have a continuous epicuticular lining (cuticulin), and are thus presumably impermeable. By contrast, the end apparatus lacks such a lining and consists of fibrous cuticular walls, which are presumably permeable. The end apparatus includes a round ampulla and two long collecting ductules. The lumen of the ductules is free, whereas the ampulla is occluded by two dense fibrous cones, presumably preventing premature passage of the zymogen from the ductules into the main duct and thence to the surface of the animal.

The respective cuticular structures are formed by the three cells of the organule on days 7 to 9. The cuticulin layer, which is initially secreted in contact with numerous microvilli of cell D (Locke, 1969), constitutes a continuous barrier preventing occlusion of the duct by subsequently secreted cuticular material. By contrast, the material that will form the end apparatus penetrates freely into the long invaginated space created by the retraction of cells Z_1 and Z_2 away from the surface. Two long, microtubule-filled processes of cell Z_1 (pseudoflagella) traverse this space and prevent its total occlusion by the cuticular material. The material is first deposited as a solid tubular mass, between the formerly apical surface of cells Z_1 and Z_2 and the axial pseudoflagella. On day 9 this mass is suddenly reorganized into the loose fibrous wall of the end apparatus. The dense cones capping the ampulla are formed somewhat earlier, at the site occupied by the pseudoflagella, as the latter begin to degenerate. Further degeneration of the pseudoflagella creates the lumen of the collecting ductules.

As the cuticular mass secreted by cells Z_1 and Z_2 transforms into the end apparatus, these cells retract away, leaving an extracellular cavity between their apical surface and the apparatus. This cavity is now filled by cocoonase zymogen, which is synthesized and secreted by cells Z_1 and Z_2. As zymogen accumulates the cavity expands tremendously, reaching a length of 200 μm by day 15. It appears that the collecting ductules may elongate in parallel during this time, suggesting continued cuticle production, probably by cell Z_1.

Biochemical studies have revealed more definitively the time course of zymogen synthesis (Berger and Kafatos, 1971b; Kafatos, 1972a). The rate of synthesis is very low on days 7 and 8. Once the end apparatus and the storage cavity have been formed, the rate begins to increase substantially, changing from less than 1 per cent of total protein synthesis in the galea at the end of day 8 to approximately 60 per cent of the total at the end of day 14. The absolute as well as the relative rate of zymogen synthesis increases by nearly two orders of magnitude during this 6-day period. Then, the rate of synthesis decreases precipitously. The stored zymogen (nearly 50 μg per galea, or 2 ng per zymogen cell) is extruded through the end apparatus and the main duct, and appears at the surface of the galea as active cocoonase. The enzyme is used shortly thereafter, in conjunction with the $KHCO_3$ solution of the labial gland, to open a hole in the cocoon at the time of adult eclosion. The organules regress and remain functionless throughout the brief life of the adult moth.

With respect to zymogen production, the main period of differentiation for cells Z_1 and Z_2 is day 9 to day 14. We have called this period of rapid synthesis "full differentiation" or phase II (Kafatos, 1972a). The period of finite but low zymogen synthesis preceding it is phase I and the period of shut-off is phase III (protodifferentiated and modulated states, respectively, according to Rutter *et al.*, 1968). The mechanisms regulating the transitions between the states are unknown. However, the kinetics of change in the rate of zymogen synthesis during phase II are generally consistent with a simple transcriptional control model (Kafatos, 1972a, b; Kafatos and Gelinas, 1974). According to this model, at the beginning of phase II transcription of the zymogen gene is set at a high, nearly maximal rate, and remains so throughout phase II. The zymogen mRNA produced accumulates continuously because of its high stability and is translated at a constant efficiency, thus supporting an ever-increasing rate of protein synthesis. Although the information necessary for testing this model in detail is not available, the key postulates of the model appear to be generally valid for highly specialized cells having a prolonged phase II (long time constant systems: Kafatos and Gelinas, 1974). These postulates are: a single copy of the specific gene per genome, high and essentially constant

transcription of this gene during phase II, high stability of the mRNA, and constant efficiency of translation (polypeptides produced per mRNA molecule per minute).

With respect to sequential polymorphism, it is noteworthy that zymogen production is not the only differentiated function of cells Z_1 and Z_2. At the beginning of adult development, the cells of the galea behave like ordinary epidermal cells, undergoing apolysis and secreting moulting gel. Following the mitotic divisions, cells Z_1 and Z_2 become distinguishable and their apical cytoplasmic regions undergo an intricate morphogenesis. Then these cells secrete specialized cuticular products. At the same time the cells begin production of zymogen at a low rate (phase I). When the cuticular valve is in place, zymogen production shifts to the fully differentiated state (phase II); a low level of cuticle production may persist, at least for cell Z_1. The end of zymogen production coincides with unknown cellular activities, leading to zymogen extrusion and activation and to the regression of the cells.

Except for the initial hormonal requirements, nothing is known about the mechanisms controlling the progression through this sequence of distinct, temporally overlapping functions. One suspects that the timing controls are internal to the organule, rather than external hormonal changes. Only development of the gland in organ culture could answer this question.

5 The follicular epithelial cells of silkmoths; biochemically defined sequential polymorphism

The follicular epithelium in silkmoths consists of a single layer of cells surrounding an oocyte and the seven associated nurse cells. The entire complex is a follicle. A linear array of attached progressively more mature follicles constitutes each of the eight ovarioles in the ovaries of a developing adult. The formation and early functions of the follicle have been reviewed recently (Telfer, 1975).

In response to the hormonal conditions which trigger adult development, the follicles begin their growth, emerge in sequence from the sheath of the germarium, and enter vitellogenesis. During vitellogenesis, intercellular channels open up among the follicular epithelial cells, permitting access of the blood proteins to the oocyte surface. There, specific uptake of vitellogenin takes place, leading to formation of yolk spheres within the oocyte. During this period, the follicular epithelial cells appear to help vitellogenesis not only by the formation of channels, but also by secretion of a histidine-rich product, probably a glycoprotein, which finds its way through the channels into the oocyte; this product may aid the specific

uptake of vitellogenin (Anderson and Telfer, 1969). The epithelium also appears to produce a thin vitelline membrane between itself and the oocyte.

At the end of vitellogenesis secretion of the histidine-rich product stops, the intercellular channels close up, and a substantially thicker vitelline membrane is deposited by the follicular epithelial cells. During this time, the oocyte swells considerably by uptake of water (terminal growth phase; Anderson and Telfer, 1969). It is not known what, if any, contribution the epithelium makes to the uptake of water. Following terminal growth, the epithelium secretes the largely proteinaceous eggshell or chorion. This last developmental stage, choriogenesis, has been studied extensively in recent years (reviewed in Kafatos *et al.*, 1976; and Kafatos, 1975). Upon its conclusion, the follicular epithelium is shed (ovulation), and the oocyte, surrounded by the fully formed shell, is ready for passage into the oviduct, fertilization, and oviposition.

The chorion consists of four size classes of proteins (A, B, C and D, respectively, in order of increasing molecular weight; Paul *et al.*, 1972). Overall, the proteins are unusually small and very distinctive in amino acid composition (e.g. 35 per cent glycine, but essentially histidine-free; compare the follicular cell product synthesized during vitellogenesis). At least 15 to 20 chorion polypeptide species can be distinguished by electrophoresis on SDS-polyacrylamide gels, and more than double that number on isoelectric focusing gels (Regier, 1975; Kafatos *et al.*, 1976). Within each size class the individual components are closely similar to each other; sequencing studies on A proteins suggest that the similarity is due to homologous amino acid sequences (Regier, 1975).

At the outset of choriogenesis, the synthesis of proteins other than chorion declines precipitously. The various chorion proteins then begin to be synthesized in sequence. There are gross changes in terms of synthesis of particular size classes; thus, C proteins are produced early and their synthesis is soon overtaken by A and B synthesis. In addition, there are numerous changes in the synthesis of various subclasses and of individual polypeptides. The absolute or relative rates of synthesis of a large number of chorion components wax and wane individually during choriogenesis. These temporal changes could be described by a series of overlapping curves (synthetic rate versus time), differing in shape, height, width, and time of attainment of the maximum value. Based on the pattern of chorion proteins synthesized at any one time, eighteen distinct stages of choriogenesis can be identified in *Antheraea polyphemus* (Paul and Kafatos, 1975). Each lasts approximately three hours, and thus choriogenesis requires approximately two days.

An important observation is that progression through this sequence of developmental stages is autonomous. When follicles are placed individually

in organ culture, in a completely defined medium, they proceed through the normal sequence of stages at the same rate as they would *in vivo* (Paul and Kafatos, 1975). Moreover, follicles in terminal growth can enter choriogenesis in culture. Thus, the sequential polymorphism of choriogenesis is not under the immediate control of external hormones. An initial hormonal stimulus is necessary to initiate the process of follicular maturation during adult development, but the programme of developmental changes which is thus set in motion becomes independent of external cues at some point. Our long-term goal is to understand the mechanisms internally regulating the temporal sequence of stages in choriogenesis.

The mRNAs for chorion proteins have been identified (Gelinas and Kafatos, 1973), and resolved electrophoretically into a number of bands, after removal of their poly(A) (Vournakis *et al.*, 1975); the poly(A) causes smearing of the intact messages, presumably because it is variable in length (Vournakis *et al.*, 1974). mRNA labelling and cell-free translation studies (Kafatos *et al.*, 1976) suggest that the messages are synthesized throughout choriogenesis, and accumulate in a state suitable for cell-free translation only during this time. Higher molecular weight mRNAs appear to be produced in the early chorionating stages, in parallel with production of the higher molecular weight C proteins. It remains to be determined whether the detailed programme of sequential polymorphism evident at the level of chorion protein synthesis is paralleled in equal detail and is driven by a corresponding transcriptional programme.

6 General discussion

In the four systems discussed in this paper, sequential polymorphism is evident at a morphological, functional, or biochemical level. Since we are dealing with insects, in all cases the hormonal conditions of metamorphosis play a role in permitting the unfolding of this polymorphism. However, the polymorphism is not a simple binary alternative under the immediate control of the hormones. Detailed studies indicate that even in the simplest case, the saturniid labial gland, the "adult state" as it develops can be distinguished into a series of phases (e.g. growth, cuticle formation, and elaboration of mitochondria-membrane associations). It is a matter of perspective whether these phases will be considered distinct stages of differentiation, or will be subsumed under a single temporal programme.

The multistage or progressive nature of polymorphism is most clearly evident when this process can be defined biochemically, as in the follicular epithelium. In that case, we can ultimately look forward to understanding polymorphism not only in terms of what triggers it but also in terms of how it proceeds in detail: we can hope to understand the continuing temporal controls on the expression of identifiable genes. The

controls need not be hormonal, and in fact in the follicular system they appear to be internal to the system itself.

As we learn more about a system, our perspective shifts from the overall gated process to the continuous changes which make it up. For convenience, we may still describe the continuous changes in terms of a succession of stages. This perspective of development as sequential polymorphism, as a temporal progression through distinct states of gene expression, a progression which has strong elements of self-regulation, is widely applicable. One only needs to reflect on the sequential production and disappearance of specific enzymes during slime mould development, or on the sequence of rapid changes occurring during early embryogenesis, even in the absence of a functioning nucleus. Having used the term sequential polymorphism to make the connection with classical studies on insect development, I should now say that it is important to view insect development with the modern perspective of cell differentiation in all organisms. Once used, sequential polymorphism might best be replaced by the term temporal programming.

Insect development offers a number of opportunities for studying temporal programming at this deeper level—and in fact offers the possibility of studying this process directly at the level of gene activity. An example are the elegant studies on the puffing programme initiated by ecdysone action in culture (Ashburner *et al.*, 1974). The involvement of hormones in the control of development in insects is a valuable tool—but it should not obscure the underlying essence of the phenomenon: the stably programmed temporal control of gene activity, which must be maintained through operation of intracellular regulatory circuits. To understand these circuits is to understand much about eukaryotic differentiation.

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