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Gene Expression during Post-Embryonic Development: Metamorphosis as a Model

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Dramatic advances have been made in the last decade in advancing our understanding of gene expression during early embryogenesis, made largely possible by the intensive application of new techniques of molecular genetics and the availability of excellent experimental systems. Relatively little effort has similarly gone into seeking the molecular mechanisms underlying post-embryonic development, a period during which the adult phenotype is expressed and established. This review first outlines the major differences between early and late embryonic development, and then describes some of the biochemical information on the products of 'adult' genes expressed during post-embryonic development. Metamorphosis in invertebrates and vertebrates, a process highly conserved through evolution, is an ideal model for studying mechanisms of post-embryonic development. It also shares many similarities with mammalian development in the perinatal period. The precocious induction *in vivo* and in culture of insect and amphibian metamorphosis by exogenous ecdysteroids and thyroid hormones, and its retardation of inhibition by juvenile hormone and prolactin, respectively, have allowed the analysis of such characteristic features of post-embryonic development as morphogenesis, tissue remodelling, gene reprogramming, and programmed cell death. Recent studies on metamorphosis have revealed the important role played by such processes as auto- and cross-regulation of hormone receptor genes and by cell death or apoptosis, as in the maturation of the central nervous system, tissue restructuring and organolysis. Finally, this article considers new questions and future prospects leading to a better understanding of post-embryonic development, the next major frontier in developmental biology.

Key Words: Gene expression, Post-embryonic development, Metamorphosis, Developmental Biology

Introduction

Anyone attending one of the numerous conferences currently being held on molecular biology of development or reading recent reviews, quite commonly gets the impression that the most important period of animal development is around gastrula or neurula stages. The reason for such an emphasis on early embryogenesis is easy to understand in view of the dramatic impact of molecular genetics, recombinant DNA technologies and experimental transgenesis on immediate post-fertilization development. Yet the important questions tackled are almost the same as those posed a hundred years ago (Davidson 1986, Wilkins 1986, Browder et al. 1991, Gilbert 1991). The purpose of this article is not in any way to diminish the impressive progress now being made in defining, in molecular terms, such phenomena of early embryogenesis as maternal influences, growth factors, patterning, axis formation and homeobox genes (Gehring 1987, Gurdon et al 1989, Kessel &

Gruss 1990, Green & Smith 1991, Stern 1992, Krumlauf 1992). It is rather to point out the limitations of this focus on early developmental processes in understanding how the less well studied expression of genes specifying the adult phenotype are regulated during post-embryonic development and to speculate on the possible new areas of knowledge of developmental biology that this will open up.

Research on Early Embryogenesis is Currently Trendy

That the exploitation of the present-day techniques of molecular biology has not had an equal impact on all branches of developmental biology, but has been largely restricted to early embryogenesis, is quite clear. This is partly because early embryos are easier experimental objects to study, but also because molecular biologists (although not exclusively) tend to be attracted by trendiness. One way of illustrating this point is by surveying the literature over the last 15 years, a period of dramatic increase in the number of publications on developmental molecular genetics.

Figure 1A shows how interest in the frog *Xenopus laevis* as a model organism for developmental studies has grown since 1977, when a significant number of publications first appeared on the applications of techniques of molecular genetics to developmental problems (Dawid & Sargent 1988). By 1990 there had taken place an 8-fold increase in published papers and abstracts (mostly conference proceedings), exceeding 10-fold by mid-1992. When these figures are broken down for the three topics, shown in figure 1B, there is clearly a sharp trend away from exploring developmental processes other than early embryogenesis. For example, the number of papers published (excluding abstracts) on early embryonic development had gone up from about 17 in 1977 to nearly 600 in 1990. During the same period, papers on diverse questions relating to gametogenesis, sex determination, post-embryonic development and sexual differentiation increased from about 45 and 30 to only 150 and 60, respectively. The same would be true of developmental studies on *Drosophila* and mouse, two other currently popular organisms of molecular embryologists.

Molecular Genetics of Early Embryogenesis

Not surprisingly, with the above shift in emphasis and the ever-growing involvement of molecular biologists, a vast amount of information has been gathered on questions concerning gene expression during early embryogenesis. It is worth considering briefly some of these recent findings in order not only to better define those processes which characterize later development, but also to critically assess some generalization arising from these investigations.

That a vast diversity of messenger RNAs are stored in the egg and whose translation is activated upon fertilization has now been firmly established (Jefferey 1983, Pratt et al. 1983, Davidson 1986, Browder et al. 1991). A

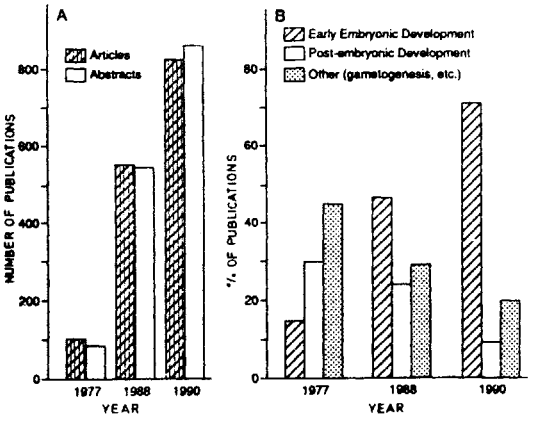

Figure 1A & B A literature search of publications between 1977 and 1992 on topics related to development of the frog *Xenopus laevis*. **A**, The rapid rise in total number of publications; **B**, Breakdown of the increase according to topics: Early embryogenesis; post-embryonic development (including metamorphosis); gametogenesis and other topics

number of proteins encoded by these mRNAs have been identified and shown to play an important role as signalling molecules or their receptors and determining the onset of transcription of zygotic genes (Pratt et al. 1983, Bender 1985, Gall 1986, Green & Smith 1991, Gurdon 1992, Smith 1993). This fact provides a molecular explanation for the phenomenon, known to developmental biologists for many decades, of "maternal effects" in early embryogenesis. Another important advance is the identification of some of the stages when zygotic genes are activated and, in some instances, the processes they control. Among these is the group of genes that control patterns and body plans, such as those regulating muscle-specific genes which are now known to be expressed in somites derived from the mesoderm (Gurdon et al. 1989, Green & Smith 1991). What has emerged as a generalization from findings of this kind is that homeobox genes, which are highly conserved, play the same role in the early development of flies, frog and mice (Stern 1992, Krumlauf 1992). Many of the above genes and the processes they control appear to be autonomously regulated, a major difference underlying early versus late embryonic or foetal development.

Differences between Early- and Post-embryonic Developmental Processes

A large number of maternal mRNAs encode components of machinery for such processes as protein synthesis, cell division, respiration and growth factors for use in the immediate post-fertilization period (Jefferey 1983, Pratt et al. 1983, Kirschner et al. 1985, Gurdon et al. 1989, Green & Smith 1991). Some of the maternally-derived transcripts also code for proteins or transcription factors that "switch on" the zygotic genes which are responsible for the onset of substantial cellular differentiation and initial organogenesis. However, further differentiation leading to the expression of adult structures and functions does not occur for a considerable period of time. This can vary from a few hours for some invertebrates to months, and even years, for some amphibia, reptiles and mammals. This long period or "pause" is characterized by considerable growth of the embryo or foetus with relatively little differentiation by switching on new genes, compared with the considerably more rapid events during early embryogenesis. The developmental "pause" is abruptly terminated by the activation of many genes that encode 'adult' proteins and cellular components. Importantly, these genes are henceforth expressed constitutively and thus serve both to establish and maintain the adult phenotype.

Some of the principal features that distinguish early embryogenesis from post-embryonic or foetal gene expression, are listed in table 1. The signal molecules for early embryonic development seem to be peptides not made in specialized cells whose mRNA is intially maternally derived and which act, as autocrine or paracrine growth factors, such as activin, bFGF and members of the TGF-β-like family (Green & Smith 1991, Stern 1992, Smith 1993). The signals

Table 1 *Some differences between early embryogenesis and post-embryonic development*

Early embryogenesis	Post-embryonic development
Developmental signals are mostly paracrine	Development signals mostly endocrine
Signals control determination and differentiation of undifferentiated cells	Signals control completion of differentiation in partially differentiated cells
Sex determination	Sexual differentiation
Cell death not extensive and occurs locally	Cell death extensive and can occur locally or lead to lysis of entire organs
Cell-cell interactions initiate differentiation	Cell-cell interactions are part of the inductive response

for post-embryonic development are synthesized in highly specialized endocrine cells and include protein and non-protein hormones such as neuropeptides and pituitary trophic factors, prolactin, juvenile hormone, thyroid hormones, ecdysone and glucocorticoids (Baulieu & Kelly 1990). The central nervous system, in both vertebrates and invertebrates, plays a key role in initiating the cascade of endocrine signals, whose function is essentially to tune in the developing organism with environmental factors. In post-embryonic development, the different target cells of developmental hormones are partially differentiated and the hormone initiates the completion of the process, whereby different target cells respond to the same hormonal signal quite differently according to distinct developmental programmes. Among other features that set apart the early and late developmental processes are cell death, sexual differentiation and cell-cell interactions. Cell death during early embryogenesis is usually local whereas programmed cell death during post-embryonic development is very extensive, often initiated by the same developmental signal that promotes morphogenesis (Snow 1987, Tomei & Cope 1991, Gerschenson & Rotello 1992). The latter may be spatially restricted, as in the maturation of the brain and remodelling of the gut, or may involve elimination of whole organs such as gills and the tail of the metamorphosing frog tadpole (Beckingham et al. 1976, Gilbert & Frieden 1981, Burd 1990). Sex determination usually occurs during post-embryonic development, its timing being controlled by environmental and hormonal signals (Gilbert 1991, Gallien 1953, Witschi 1956, Shapiro 1990, Browder et al. 1991). Cell-cell interactions, with extracellular matrix (ECM) molecules themselves acting as developmental signals, play an important role in early embryogenesis (Dawid & Sargent 1988, Gurdon et al. 1989, Gurdon, 1992), whereas this is not marked or essential during later development.

Expression of 'Adult' Genes during Post-embryonic Development

A large body of biochemical information has been assembled over the last 50 years concerning the appearance of specific gene products and processes that establish the adult phenotype during post-embryonic development, some of which are listed in table 2 (Weber 1967, Grahani & Wareing 1976, Tata 1991, 1993). It should be emphasized that these are not examples of *adaption* to new demands made upon the organisms, but represent activation of genes in *anticipation* of new demands or a change in environment, nutrition, etc. Some of the biochemical studies have been extended to specific genes, such as the switching from foetal to adult haemoglobin and from α-foetoprotein to albumin (Nienhuis &

Table 2 *Example of vetebrate post-embryonic developmental expression of adult genes and processes in partially differentiated tissues*

Process	Genes and tissues
Gene switching	α-Foetoprotein → albumin Foetal (or larval) → adult haemoglobin
Morphogenesis	Formation of limbs, lungs, cuticle; chondrogenesis
Neural differentiation	Extensive neuronal cell turnover, acquisition of new functional and behavioural characteristics
Tissue restructuring	Keratinization of epidermis, remodelling of gut
Hormonogenesis	Activation of hormone producing genes in endocrine tissues
Induction of new functions	Urea excretion; new cell adhesion molecules
Cell death	Removal of tissues or organs by induction of lytic enzymes (patterning of limbs; loss of tail and gillls)
Sexual differentiation	Activation of genes for sex determination; differentiation of accessory-sexual tissues

Stamatoyannopoulos 1978, Nahon 1987). Other examples of functional remodelling and genetic induction during post-embryonic development include switching to adult type of visual pigment, skin keratization, cuticle formation in insects, differentiation and selective loss of neurons and expression of lung surfactants. These few examples suffice to emphasize the importance of the post-embryonic developmental period for the establishment of the adult phenotype, although relatively little is known about the detailed structure and function of the genes involved.

A major reason for the relative paucity of our knowledge about post-embryonic development at the molecular level is the lack of a suitable mammalian model system. Thus the advantages of genetics in *Drosophila*, the micromanipulation of the large amphibian and avian egg and early embryo, and the technique of transgenesis in mice, all of which have contributed enormously to progress in the area of early development, cannot be easily applied to the study of gene expression during post-embryonic development in mammals. The close physical and chemical association between the mother and developing foetus in mammals is another advantage which prevents manipulation of the embryo and not allowing a clear separation of maternal from foetal signals and responses. However, a developmental system of free-living embryos in invertebrates and non-mammalian vertebrates which obviates the above difficulties, namely metamorphosis, offers many advantages for analyzing the regulation and expression of 'adult' genes during post-embryonic development.

Metamorphosis: An Ideal System for Studying Post-embryonic Development

Metamorphosis, which simply means a change in form or structure, is a most dramatic example of post-embryonic development. It occurs widely in all free-living embryos, from simple invertebrates to highly evolved non-mammalian vertebrates. The process is highly conserved through evolution and allows certain generalization to be made about post-embryonic development as a whole.

Perhaps the most conserved feature of metamorphosis is that it is tightly controlled by hormones (Beckingham Smith & Tata 1976, Gilbert & Frieden 1981, Tata 1993). The striking similarity of hormonal control of the initiation and completion of metamorphosis in insects and amphibia is illustrated in figure 2. There is, however, a distinction between invertebrates and vertebrates in the manner in which metamorphosis proceeds. As shown in figure 3, the process in amphibia is a continuous larvae → adult transition with a single surge of T_3 and T_4, whereas in insects (as illustrated for *Manduca*) it proceeds through multiple larval and pupal moults, each corresponding to distinct pulses of ecdysone. Whether the process is continuous or in stages, two groups of simple hormonal signals, released from specialized endocrine cells following environmental cues transmitted by the brain, determine the onset, rate and completion of post-embryonic development. One group of hormones, as exemplified by the terpenoid group of substances called juvenile hormone (JH) in invertebrates (Granger & Bollenbacher 1981) and the peptide hormone prolactin (PRL) in vertebrates (Nicoll 1974, White & Nicoll 1981) prevents metamorphosis. This action may be essential in determining the timing of initiation of the programme for further development of the growing but not as yet fully differentiated tissues by the steroid hormone ecdysone in invertebrates (Karlson & Sekeris 1966, Tata 1986, Granger & Bollenbacher 1981, Ohnishi & Ishizaki 1990) and the iodoamino acids thyroid hormones, L-thyroxine (T_4) and triiodo-L-thyronine (T_3) in verte-

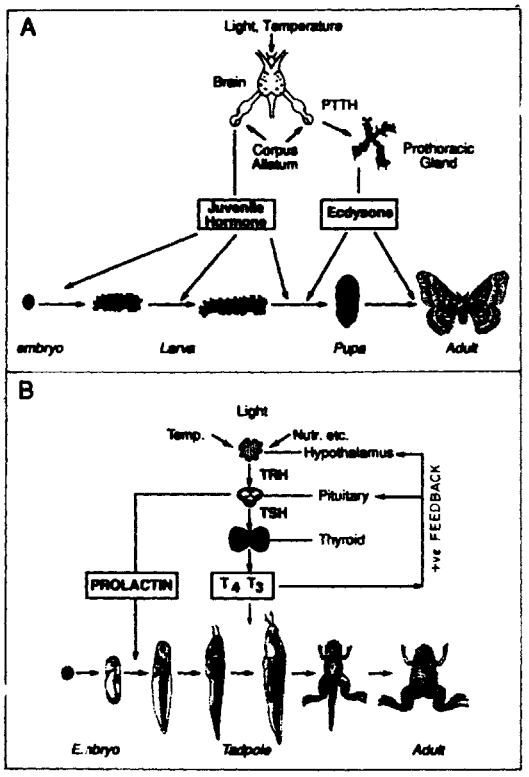

Figure 2A & B Schematic illustration of similarities in hormonal control of insect (**A**) and amphibian (**B**) metamorphosis. In each instance the first hormone (juvenile hormone, prolactin) exerts a juvenilizing action by delaying or preventing the induction of metamorphosis by the second [ecdysone, thyroid hormone (T_4, T_3)]. The timing and onset of post-embryonic development is determined by environmental cues transmitted via the central nervous system by a cascade of signals impinging on specialized endocrine tissues which synthesize these hormones (*NC, neurosecretory cells; TRH*; thyrotrophin releasing hormone; *TSH*, thyroid stimulating hormone)

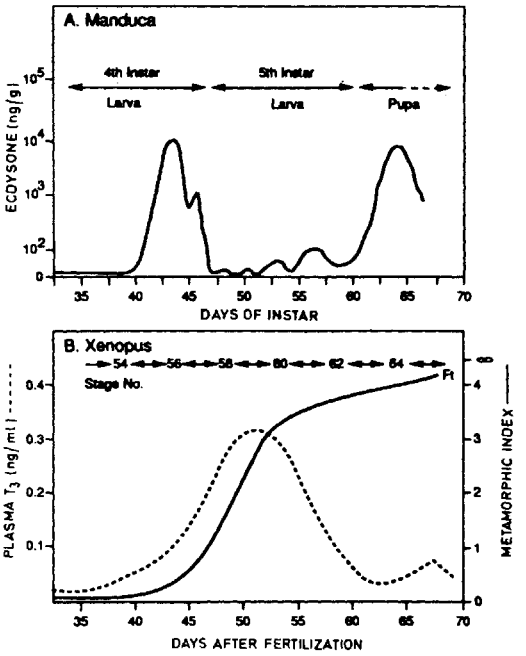

Figure 3A & B Distinction between the serial larval and pupal moults in insects and a single transition from larval to adult forms in amphibian metamorphosis. The idealized curves depict the pulses of ecdysone released (ng/g of whole body) just preceding each of the multiple larval and pupal moults in *Manduca sexta* (**A**) and the single burst of T_3 (ng/ml plasma) just before a continuous transition from the larval to the adult state in *Xenopus laevis* (**B**). Metamorphic Index is ratio of hind limb: tail length. (*Data adapted from Riddiford* 1976)

brates (White & Nicoll 1981, Tata 1993). The action of these hormones is obligatory for the onset and completion of metamorphosis up to the adult stage.

The simple exposure to T_3 or ecdysone can precociously induce metamorphosis in amphibian or insect larvae, or pupae, as well as in tissues isolated from the late embryos, and induce the same adult gene products or processes as during normal development, as listed in table 3. In every instance, the inducing hormone activates genes specifying adult structures and functions. The same hormone initiates a different developmental programme depending on the tissue such as, for example, extensive morphogenesis in amphibian or insect limbs and wings, the induction of hydrolases and other cell death determinants leading to the loss of whole organs such as the tail, gills, and saliva y gland, functional reorganization of the brain and gut, while producing more subtle changes as in the epidermis and eyes. All the above developmental inductions can be prevented or arrested by raising the concentration of

Table 3 *Some morphogenetic and biochemical changes, characteristic of metamorphosis, induced by ecdysteroids in insect larvae and pupae and by thyroid hormone in amphibia*

Target tissue	Change induced
Ecdysteroids	
Fat body, gut	Lysis, restructuring; induction of digestive enzymes
Salivary gland	Regression; induction of heat shock proteins, hydrolases; chromosomal puffing
Epithelium	Sclerotization, pigmentation, cuticle formation; induction of DOPA decarboxylase, polyphenol oxidase, cocoonase
Wing, limb buds	Morphogenesis; cell division; scale, bristle and pigment formation
Brain	Cell death and restructuring functional reorganization
Eye	*De novo* morphogenesis or restructuring
Thyroid hormones	
Liver	Restructuring; induction of albumin, urea cycle
Gut, pancreas, tail, gills	Resorption and regeneration; induction of hydrolases; cell death
Skin	Keratinization; deposition of collagen; induction of Na-K ATPase
Limb buds, lungs	Morphogenesis; cell division; chondrogenesis; ossification
Brain	Cell death, replacement and functional reorganization
Eye	Induction of adult visual pigments and restructuring

prolactin or juvenile hormone, thus extending the simple experimental manipulation to operationally "freezing" the process of post-embryonic development at any desired stage. That further development can be so easily arrested is a procedure that is not available for manipulating early embryogenesis or mammalian development.

Hormonal induction of metamorphic processes, and the counteracting effects of juvenilizing hormones are direct and not systemic, since they can be reproduced in organ or primary cell cultures. The ease with which many of the hormonal effects can be reproduced in tissue culture has considerably facilitated the study of developmentally programmed morphogenesis, cell death and specific gene expression, and thus allowing a more detailed analysis of the mechanisms underlying late embryonic development (Tata 1966, Smith & Tata 1976, Yoshizato 1989, Beckingham Ishizuyaoka & Shimozawa 1991, Karim & Thummel 1991, Tata et al. 1991, Andres & Thummel 1992, Shimizu-Nishikawa & Miller 1992, Nishikawa et al. 1992). For example, it could be shown in organ cultures of *Drosophila* salivary glands and *Xenopus* tails that the addition of ecdysone and T_3, respectively, induces chromosomal puffs, cell death and complete tissue regression. Recently, the author's laboratory has succeeded in producing limb development by exposing organ cultures of *Xenopus* tadpole hind limb buds to low concentrations of T_3 in the culture medium (Tata et al. 1991). In these experiments, prolactin prevented regression of the tails removed from the same pre-metamorphic tadpoles as the limbs, i.e. the action of prolactin is to prevent both morphogenesis and cell death induced by thyroid hormones. Organ culture experiments have also made it possible to demonstrate that protein synthesis is required for programmed cell death (Tata 1966, Beckingham Smith & Tata 1976, Yoshizato 1989). Thus, in metamorphosis, we have an ideal system for studying both the "stationery" and the "forward" phases before and during post-embryonic development.

Is Metamorphosis Relevant to Mammalian Post-embryonic Development?

An important question arises as to whether or not, or to what extent, metamorphosis bears any resemblance to mammalian post-embryonic development. A rigorous comparison cannot be made since it is

impossible to study the late mammalian embryo or foetus independent of its mother. Also, presumably because of the maternal barrier intervening between environmental factors and the foetus, post-embryonic development in mammals proceeds more as a slowly progressing continuum and not always sharply separated from the embryonic growth phase as in free-living embryos of insects and amphibia (see figure 3).

Nevertheless, taking the above factors into consideration and restricting ourselves to vertebrates, it is remarkable as to how many of the thyroid hormone-inducible changes seen in amphibian metamorphosis also occur in late foetal development or during the perinatal period in mammals (compare tables 2 & 3). For example, the activation during metamorphosis of adult haemoglobin and albumin genes in frog tadpole blood cells and liver has a parallel in the foetal → adult haemoglobin and α-foetoprotein → albumin switching in the mammalian foetus. Similarly, skin keratization and urea cycle enzyme induction bear resemblance. Perhaps the most striking parallelism is seen when comparing post-embryonic developmental progression of structures and functions in the central and peripheral nervous systems. Many of the structural and functional changes taking place in the frog brain can also be seen in perinatal mammalian development, and thyroid hormones have been known to exert a strong influence on brain and bone development before birth (Kandel & Schwartz 1985, Duellman & Trueb 1986, Steward 1989, Tata 1991). As shown by the highly idealized representation in figure 4, the period a few weeks before birth is marked by an abrupt appearance of T_4 and T_3 in human foetus, which is soon followed by an increased proliferation of glial and neuronal cells during the perinatal period. This, in turn, is accompanied by the acquisition of several brain functions seen in the newborn mammal.

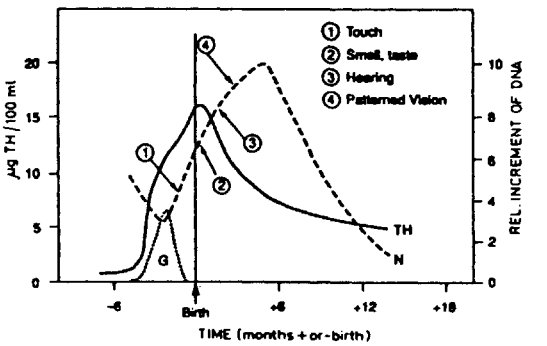

Figure 4 Idealized curves showing the close association between the appearance of thyroid hormones (*TH*) in human foetal plasma, the proliferation of neuronal (*N*) and glial (*G*) cells in the brain, and the onset of acquisition of sensory perceptions (arrows) during the perinatal period and after birth. The proliferation of neural cells is represented as relative increments of DNA at different times during development.

Some newly acquired functions, such as olfactory and optical patterning, are known to be induced by thyroid hormones in metamorphosing tadpoles and during the late stages of chick embryo development (Burd 1990, Hoskins 1990, McEwen et al. 1991, Sjoberg et al. 1992). Ecdysone and juvenile hormone are also known to play a major regulatory role in the structural and functional modifications of the insect CNS during metamorphosis (Weeks & Levine 1990, Restifo & White 1991, Fahrbach 1992). The deleterious effects of thyroid hormone deficiency on human foetal brain development giving rise to a cretinous phenotype, which can be reversed by hormone replacement soon after birth, are well-documented (Dussault & Walker 1983, DeLong et al. 1989, Tata 1991).

Virtually nothing is known about the possible juvenilizing action of prolactin in mammalian development But it is well to consider that the mammalian foetus is exposed, especially at the earlier stages of pregnancy, to high concentrations of placental lactogen. This hormone with similar and multiple biological activities is encoded by the same gene as prolactin which is expressed in the pituitary (Baulieu & Kelly

1990, Petraglia 1991, Southard & Talamantes 1991). It would be most fruitful, therefore, to resolve such unanswered questions as the kinetics of placental lactogen secretion during pregnancy, the possible effects of this hormone on late foetal development and whether it can delay or counteract the action of thyroid hormones in mammalian post-embryonic development.

Expression of Hormone Receptor Genes is Central to Control of Metamorphosis

The frog or insect larva is known to acquire response to thyroid hormones or ecdysteroids very early in development. For example, the 3- to 5-day old *Xenopus* tadpole shows a response to exogenous T_3, which is nearly 2 months before the secretion of endogenous hormone (Tata 1968). Thus the receptor for thyroid hormones (TR) must be expressed early in development, in anticipation of the reception of hormonal signal. Recently, with the cloning of *Xenopus* TR genes, it has been possible to show the presence of small amounts of TR transcripts in early *Xenopus* tadpole (Baker & Tata 1990, Yaoita et al. 1990, Kawahara et al. 1991), chick embryo (Forrest et al. 1990, 1991 Sjoberg et al. 1992), and foetal rat (Mellstrom et al. 1991) tissues. Similarly, the ecdysone receptor (EcR) gene has been cloned, its transcripts detected in early larval tissues and cell lines, and is developmentally regulated (Fahrfach 1992, Segraves 1991). Both receptors are closely related and belong to the steroid/thyroid hormone/retinoic acid family of nuclear receptor gene family (Evans 1988, Andres & Thummel 1992, Chatterjee & Tata 1992).

What makes the early appearance of TR and EcR so functionally important is the rather unique phenomenon of autoinduction of receptor genes by the hormone itself (Tata et al. 1993). During normal *Xenopus* tadpole development there is a progressive elevation in the accumulation of both the α and β isoform of TR mRNA until the premetamorphic stage, and then rises abruptly at premetamorphosis. However, the amount of TR α and β mRNAs can be increased rapidly 3-5- and 20-50-fold, respectively, within 24 hours after exposing early tadpoles to T_3 (Yaoita et al. 1990, Kawahara et al. 1991, Baker & Tata 1992, Tata et al. 1993). The phenomenon of receptor auto-induction is not restricted to thyroid hormones, since a similar process may be involved with ecdysone induction of EcR (Koelle et al. 1992). The phenomenon of autoinduction of receptor genes has also been observed for other members of the steroid/thyroid hormone receptor family (see table 4). For example, retinoic acid can upregulate retinoic acid receptor β gene in mice (deThe et al. 1990) and oestrogen receptor is induced by oestradiol during induction of vitellogenesis in *Xenopus* (Shapiro et al. 1989). These findings may reflect a general principle of amplification of response to developmental signals.

Returning specifically to the rapid autoinduction of TR, the process seems to be

Table 4 *Example of auto-induction of nuclear receptors by developmental signals*

Developmental signal	*Receptor*	*Species*	*Function*
Thyroid hormone	TRα & β	*Senopus*	Metamorphosis
Ecdysone	EcR (E75)	*Drosophila*	-do-
Retinoic acid	RARβ	Mouse	Morphogenesis
Estrogen	ER	*Xenopus*	Vitellogenesis

important for the activation of T_3 target genes, such as albumin and keratin. This conclusion was reinforced by results of experiments with prolactin in which the T_3-induced upregulation was abolished by this hormone and which coincided with the abolition of the activation of the two target genes in liver and skin in early *Xenopus* tadpoles. How prolactin acts remains a mystery, but it would be important to know first the mechanism underlying auto-induction of receptor. The upregulation of TR can be reproduced in tissue culture of *Xenopus* cell lines, in which inhibitors of protein synthesis have provided evidence that, even though the process of auto-induction is rapid, it requires the ongoing synthesis of some proteins (Kanamori & Brown 1992, Machuca & Tata 1992). This in turn suggests the possibility of accessory factor(s) exerting a cooperative action with TR which is now thought to be true for all nuclear receptors and transcription factors in general (Diamond et al. 1990, Frankel & Kim 1991, Kliewer et al. 1992). The requirement of upregulation of TR mRNA by T_3 to activate its target genes also suggests differential thresholds of receptor concentration for the induction of TR or target (downstream) genes. A similar model had been presented for the induction of early and late gene puffs in *Drosophila* by ecdysone, but which does not incorporate the involvement of juvenile hormone (Ashburner 1990, Thummel 1990, Segraves 1991, Andres & Thummel 1992). Whether or not such models turn out to be correct, there is no doubt that the receptors for hormones and other developmental signals play a central role in regulating post-embryonic development.

Programmed Cell Death: An Integral Part of Post-embryonic Development

A salient feature of post-embryonic development in all organisms is extensive and selective cell death or apoptosis (Bowen & Bowen 1990, Tomei & Cope 1991, Gerschenson & Rotello 1992). It serves an essential function of remodelling tissues for new functions in the adult organism or the elimination of entire organs not needed for life in a new environment. Examples of the former are neuronal cell death accompanying the developmental organization of brain and peripheral nervous system in all vertebrates and invertebrates, and the loss of amphibian tail and gills for the latter. A general feature of developmental cell death is fragmentation of the nuclear chromatin by endonucleases during the process (Tomei & Cope 1991, Gerschenson & Rotello 1992), while other digestive or hydrolytic enzymes, such as proteases and glycohydrolases are also known to be involved in cell death. The dependence of cell death on newly synthesized proteins, first shown in amphibian and then insect metamorphosis (Tata 1966, Yoshizato 1989, Weeks & Levine 1990), has now been repeatedly verified in numerous examples of programmed cell death, such as neuronal cell death in the developing central nervous system, muscle degeneration and in the immune system (Wadewitz & Lockshin 1988, Schwartz et al. 1990, Tomei & Cope 1991, Rheuben 1992).

Numerous observations on the requirement for new protein synthesis have intensified a search for a primary gene product initiating a chain of events leading to programmed cell death. As yet such a gene (or genes) has not been definitively identified in vertebrates. At the same time, it is now beginning to appear that cell death may be an active or ongoing process which is suppressed, or kept in check, during the growth phase of a cell. We have already seen how prolactin and juvenile hormone prevent thyroid hormone- and ecdysone-induced cell death as part of a general antimetamorphic juvenilizing action in amphibian and insect metamorphosis, respectively (Nicoll 1974, Riddiford 1976, Granger &

Bollenbacher 1981, Tata et al. 1991). Is programmed cell death during post-embryonic development controlled exclusively by specialized gene products? The first glimpse of an answer has come from studying the nematode *Caenorhabditis elegans* in which programmed cell death has been subjected to genetic analysis and specific genes have been identified as cell death genes (Ellis & Horvitz 1986). The recent identification in Horvitz's laboratory of a gene (*ced*-9) that is responsible for preventing developmental cell death in *C. elegans* is therefore of great significance in this context (Hengartner et al. 1992). Most importantly, recent studies in a variety of vertebrates have revealed that the function of the highly conserved oncogene *bcl*-2 and related members of its family is to prevent the onset or slow down the progression of cell death (Korsmeyer 1992, Veis et al. 1993). If only a few such genes turn out to be specifically responsible for the process of controlling programmed cell death, it would be of considerable importance in not only understanding an important facet of post-embryonic development, but would represent an important advance in elucidating such problems as progression of carcinogenesis and the modulation of immune response.

Future Prospects

Much of the impressive recent progress in elucidating the molecular basis of early embryogenesis has been as much due to the availability of appropriate developmental systems as to the applications of technology of molecular genetics. No single developmental model can serve as an ideal model to answer the different questions, so that it is the combination of such advantages as the genetics of *Drosophila*, mechanical manipulation of *Xenopus* and chick embryos, and transgenic mice which has collectively contributed by offering the most suitable experimental material to answer each of the questions. A similar array of models is not available for investigations on post-embryonic development. In this review, I have attempted to make a strong case for metamorphosis as an ideal model for analyzing the mechanisms underlying various manifestations of late stages of development. The enormous advantages offered by simple hormonal manipulation to induce or prevent further development in free-living embryos is one of several attractive features of this system. Among others are the high degree of tissue-specificity of normal or hormone-induced changes, the analogy with mammalian post-embryonic development, and the possibility of analyzing the regulation of expression of adult genes in organ culture. As the late embryo comprises complex tissues, it will become increasingly necessary to extend the present studies with organ culture to cell lines retaining responses to the same developmental signals and into which specific genes could be introduced by transfection.

The phenomenon of programmed cell death is attracting increasing interest among cell and developmental biologists, immunologists, neurobiologists and cancerologists. Of particular importance is programmed cell death in the developing central nervous system, especially as many sensory and higher functions of the adult organism are established during the post-embryonic or perinatal period of development. This is an area of research which is now poised to provide new insights in developmental neurobiology. Metamorphosis also offers potentially valuable applications in this area as well, since a single, well-characterized hormone can initiate programmed cell death precociously in cells which normally would have survived up to their normal span of life (often for several months), both in culture and *in vivo*. Whether or not the activity of genes that specify programmed cell death is under the control of a unique "master" gene,

irrespective of whether cell death involves structural or functional remodelling of tissues or total organolysis, is a key question. Or, does each situation of cell death represent the consequence of distinct interactions among different sets of gene products? An important corollary to this question is how other genes and gene products prevent cell death from occurring precociously. Whatever the answers, there is no doubt that investigations of programmed cell death promise exciting prospects for the continuing blossoming of developmental biology.

We are clearly aware of how dramatically and rapidly the recent applications of molecular biology have pushed the frontier of our comprehension of early embryogenesis. If details of the diverse mechanisms involved are still incomplete, we are at least now beginning to visualize in molecular terms such phenomena as maternal influences, genes that control the body plan and cell-cell interactions at the onset of embryogenesis. These questions have intrigued biologists for the last century, but only relatively recently have we acquired the tools to unravel them. Compared with the enormous current activity in the area of early embryogenesis, only a fraction of the effort is devoted to investigating post-embryonic development. Important as it is, solving the mysteries of early embryonic development will not suffice to explain how the adult organism is formed which, after all, is the ultimate goal of studying development. If the same effort that has gone into the applications of molecular biology to early embryogenesis were to be devoted to post-embryonic development, for which the foundations have already been laid, it can be safely predicted that it will soon be possible to cross the next exciting frontier of developmental biology.

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