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CELL SELECTION IN DEVELOPMENT

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CONTENTS

I. INTRODUCTION

How does order emerge during development? How do the individual cells that make up complex organisms come to compose the higher structure that we recognize as tissues, organs, body plan and so on? The fate of a cell, we imagine, is determined by *instruction and selection.* A cell is said to be *instructed* if it responds to a signal by turning on a specific programme of gene expression. It is *selected*, on the other hand, when it is caused either to live or to die (Roux, 1881; Till & McCulloch, 1980; Mintz, 1969). Here I will outline four developmental systems in which cellular selection may play a role: the immune system, the development of the vertebrate limb, the formation of mammalian pigment pattern and the switch from fetal to adult characteristics that occurs in human red blood cells.

II. THE IMMUNE SYSTEM

The immune system is perhaps the best characterized example of a developmental process organized by selection. The production of an antibody with a high degree of specificity for antigen is the result of a process of cellular selection among the lymphocytes which compose the immune system (Jerne, 1967; Burnet, 1959). Long before exposure to antigen, each immature lymphocyte generates a unique cell surface antibody by a process of random modification of the DNA coding for immunoglobulin genes. When challenged by a foreign substance, that very small number of lymphocytes that express, purely by chance, a complementary antibody, bind the antigen and divide. In this way, antibody-producing cells are *selected* by antigen.

The production of a highly specific antibody is made possible because clonal selection draws upon the huge library of randomly generated immunoglobulins. The molecular-biological basis of this antibody diversity has been well characterized in the last few years, and a number of different mechanisms have been found to contribute to the generation of immunoglobulin randomness, including utilization of multiple variable region genomic sequence, variation in the site of DNA rearrangement, and point mutation (Tonegowa, 1983). This illustrates a critical and non-intuitive point: *In a system like the immune system, moulded by cellular selection, precision of the end product is made possible by introducing the highest possible degree of randomness at the earliest stages.*

III. MORPHOGENESIS

(1) *Development of the vertebrate limb*

Limb development begins with a small outgrowth, the limb *bud*, composed of mesodermal cells enclosed by a boundary of ectoderm (Fig. 1). After a period of growth, condensations of chondrogenic and myogenic cells appear roughly in the general pattern of bones and muscles that will constitute the mature limb (Hinchliffe & Johnson, 1980). Although it has been suggested that limb morphogenesis is a process in which undifferentiated mesodermal cells are instructed to become muscle or bone (by the action of diffusible 'morphogens' and other signals), a number of studies suggest that undifferentiated mesodermal cells with potential to become either myogenic or chondrogenic probably do not exist in the limb. In fact, these two cell types appear to be derived from entirely distinct non-interconvertible cell lineages. This has been demonstrated by experiments utilizing grafts between chicken and quail embryos; since cells from these two species can be distinguished microscopically it has been possible to determine that the limb muscle is derived from the somites while the bone originates from the somatopleural mesoderm (Christ, Jacob & Jacob, 1977; Chevallier, Kieny & Mauger, 1977; Lewis, Chevallier, Kieny & Wolpert, 1981; Chevallier, Kieny & Mauger, 1978; Kieny & Chevallier, 1979; Agnish & Kochhar, 1977; Pinot, 1970). These experiments substantiate earlier observations from Holtzer's laboratory that while limb mesoderm can, upon culturing, give rise to clonal colonies of either myogenic or chondrogenic potential, colonies containing both types of cell have never been seen (Abbott *et al.*, 1974; Dienstman *et al.*, 1974).

It seems likely, then, that although the mesoderm *appears* to be a uniform population of cells, it is, in fact, a salt-and-pepper mixture of cells with separate potentiality. What,

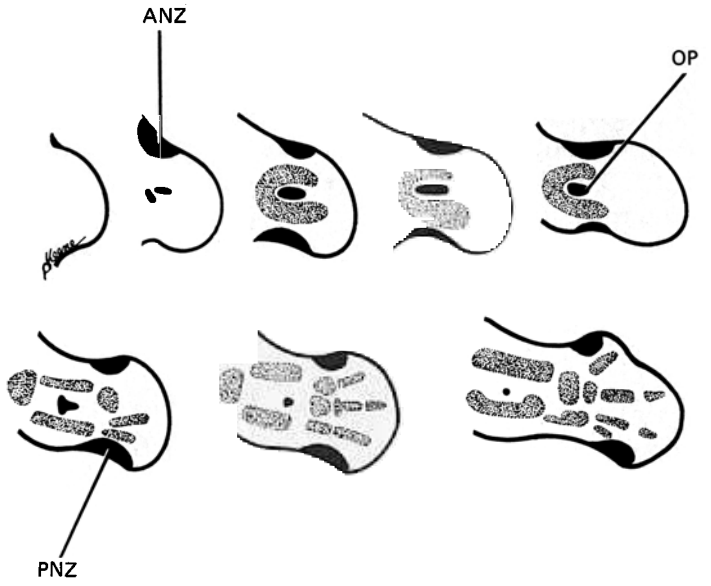

Fig. 1. Development of the chick wing, showing areas of highest cell death (black) and areas of chondrogenic condensation (grey). ANZ, anterior necrotic zone; PNZ, posterior necrotic zone; OP, opaque patch. From Hinchliffe & Johnson (1980), Saunders *et al.* (1962), Hinchliffe (1962), and others.

then, accounts for the appearance of limb structure? It has been suggested that limb morphogenesis proceeds by a process of *selection* through destruction of inappropriate cells in the 'wrong' areas of the limb and maintenance of those cells which are lucky enough to be in the right place at the right time (Abbott *et al.*, 1974; Dienstman *et al.*, 1974; Ahrens *et al.*, 1979). That such a selective process is occurring at the time of condensation is indicated by the appearance of massive amounts of cellular elimination seen as cell death (Saunders, 1966; Zwilling, 1964; Dawd & Hinchliffe, 1971; Saunders & Fallon, 1967; Hinchliffe, 1980; Saunders, Gasseling & Saunders, 1962; Hurle & Hinchliffe, 1978; Ballard & Holt, 1968; Ede & Flint, 1972; Fallon & Cameron, 1977). Large areas of such cell death are seen at the front and back of the limb bud, the *anterior* and *posterior necrotic zones* and in a central area named the *opaque patch* (Fig. 1). Since bone will later appear only in those areas where cell death is not seen, this cell death could account for the selective elimination of chondrogenic cells. Direct experimental support for such a possibility can be seen in studies by Searles (Searles, 1965, 1973). Chondrocytes deposit a matrix rich in chondroitin sulfate, which can be visualized by incorporation of $^{35}SO_2$ and autoradiography. Before the appearance of cellular death, $^{35}SO_2$ can be seen diffusely throughout the limb. After condensation, this diffuse general incorporation is not detected in the non-chondrogenic areas where much cell death is seen, suggesting that the cell death seen in the limb is preferential killing of cells of the chondrogenic lineage (Cooke & Summerbell, 1980; Summerbell, 1981; Searles & Janners 1971; Janners & Searles, 1970; Ede, 1971).

The role of cell death in the emergence of form in the limb is also suggested by the many studies that indicate that antimitotic agents cause limb malformation through the induction of an increased amount of cell death (Kochhar, Penner & McDay, 1978;

Kochhar & Agnish, 1977; Scott, Ritter & Wilson, 1977, 1980). Also suggestive are observations that a variety of mutations that cause limb defects have as their earliest detectable effect variation from the usual distribution and amount of cell death (Knudsen & Kochhar, 1981; Hinchliffe & Ede, 1967; Hinchliffe & Thorogood, 1974).

Cellular elimination appears to direct another aspect of limb morphogenesis, the formation of digits. The limb bud is originally paddle-shaped. Subsequently, areas of cell death, the 'interdigital necrotic zones', carve out the separations between the digits.

Cellular selection can also appear without cell death, for example by differential cell growth. In fact, subtle regional differences in mitotic activity in the developing limb have been described by a number of workers (Cooke & Summerbell, 1980; Summerbell, 1981; Searles & Janners, 1971; Janners & Searles, 1970; Ede, 1971).

(2) *Experimental manipulation of limb morphogenesis*

Surgical manipulation of certain areas of the limb may have dramatic effects on its development (Hinchliffe & Johnson, 1980). For example, removal of the narrow ridge at the tip of the bud, the Apical Ectodermal Ridge (AER), results in failure of the limb bud to grow and differentiate. Transplantation of an additional ridge causes development of an additional limb, while rotation of a ridge results in a rotated limb (Zwilling, 1955, 1956*a*, *b*, *c*; Saunders, 1948; MacCabe, 1974; Rubin & Saunders, 1972; Summerbell, 1974; Rowe & Fallon, 1981, 1982; Errick & Saunders, 1976). Another critical area of the limb is the region of mesoderm at the posterior, the Zone of Polarizing Activity (ZPA). When the ZPA is transplanted to the anterior part of the limb it causes the outgrowth of a double limb (Saunders, Gasseling & Errick, 1976; Saunders & Reuss, 1974; MacCabe, Gasseling & Saunders, 1973; MacCabe & Parker, 1976; Tickle, Summerbell & Wolpert, 1975; Summerbell, 1979, 1981; Fallon & Cameron, 1977; Tickle *et al.*, 1976).

The actions of the ZPA and AER are consistent with modification of the pattern of cellular selection in the limb. Removal of the AER causes increased cell death and decreased mitotic activity in the limb bud (Summerbell, 1977). A factor derived from the AER has been shown to prevent cell death of limb mesoderm grown in culture (Cairns, 1975). Removal of the ZPA results in increased cell death in the anterior mesoderm (Hinchliffe & Gumpel-Pinot, 1981), while a graft of an extra ZPA to the anterior wing, a manipulation which will cause a duplicated limb, has as its earliest effect increased mitotic activity (Cooke & Summerbell, 1981, 1980). Furthermore, the ZPA has been shown to yield a growth factor which prevents cell death of the apical ectoderm in vitro (Calandra & MacCabe, 1978; MacCabe & Richardson, 1982).

(3) *Would selection be an accurate generator of morphogenesis?*

The development of the vertebrate limb, both descriptively and experimentally, gives the appearance of a selective process, but can a selective process be capable of the kind of precision which characterizes development (Summerbell & Wolpert, 1973)? Two studies, one theoretical and the other experimental, seem to indicate that selection is indeed capable of morphogenesis. Swindale (1980) has asked whether a striped pattern, such as that seen in a zebra's skin or in the ocular dominance stripes of the visual cortex, can be generated by cellular selection alone. He has shown that with a computer-simulated model of cellular competition, and starting from a random mixture of cells

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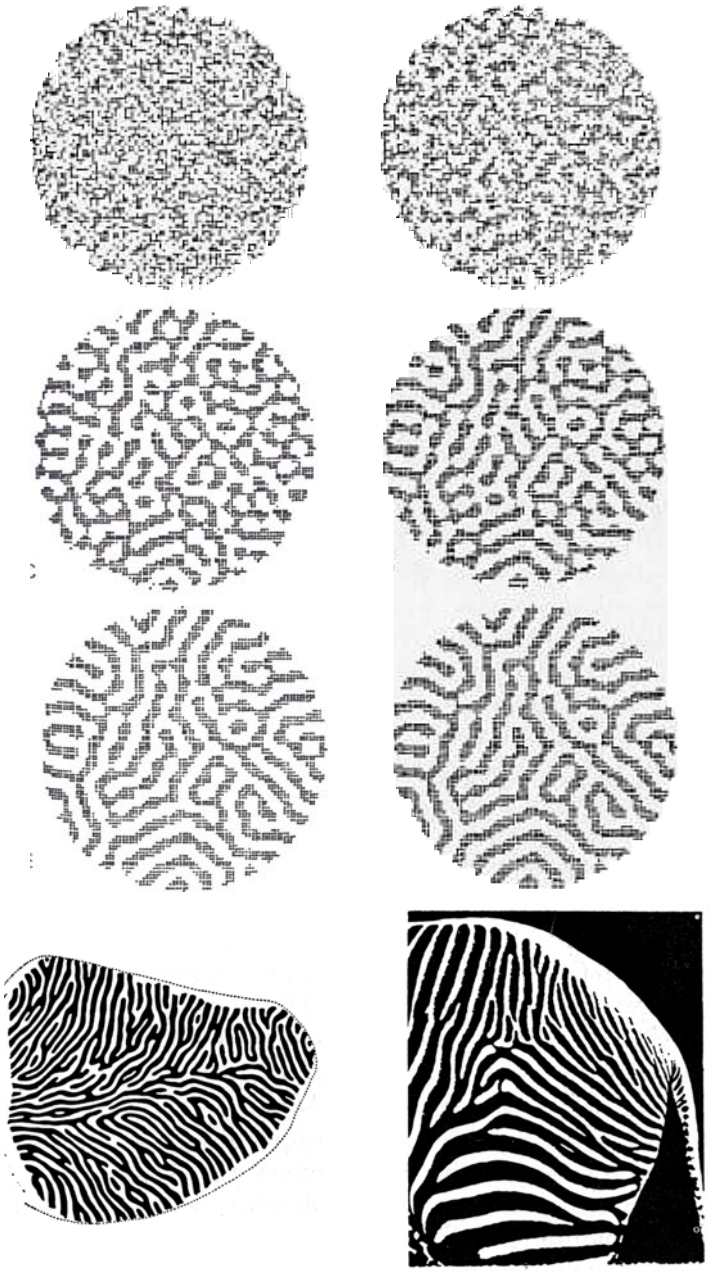

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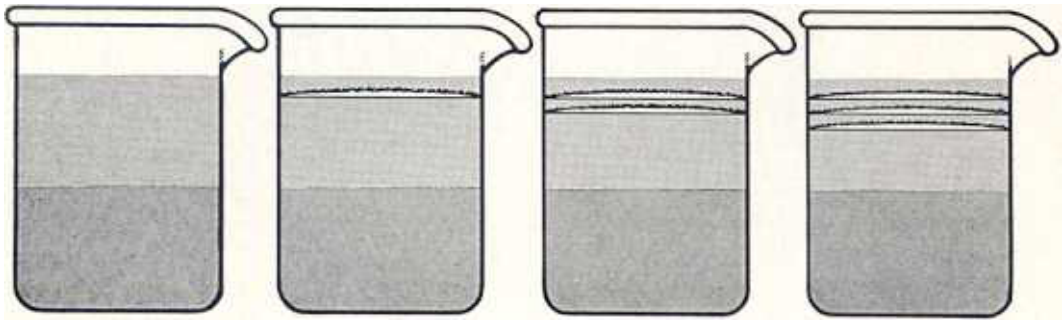

Fig. 3. Emergence of pattern in bacterial cultures by differential cell growth. Initially, bacteria are seeded evenly throughout the upper layer of flask. Bands arising in a flask of Bacillus sp. at 0, 2, 4 and 6 days of incubation. Redrawn from Wimpenny (1982, 1981).

interacting through diffusible substances, a highly characteristic striped two-dimensional pattern can emerge (Fig. 2). A similar conclusion has been reached experimentally; Wimpenny (1982) has demonstrated that microbial growth in gel stabilized media can give rise to a highly ordered pattern. He found that when bacteria are suspended in a gel in a beaker, a very characteristic pattern of bands can be seen to appear as a result of differential growth (Wimpenny *et al.*, 1981) (Fig. 3). These studies demonstrate that selection alone is capable of giving rise to an accurate pattern.

Another system in which development arises through a process of selection is the mammalian ovarian follicle (Lacker, 1981). In a young woman, approximately one thousand immature follicles begin to grow during the course of each menstrual cycle. Of this large number of follicles all but one will die. In non-human mammals that give birth to multiple offspring, the final number of surviving follicles may be larger, but it is still small in comparison to the number of follicles that begin growth during each cycle. Therefore, a small number of surviving ovarian follicles is *selected* from a large pool of candidate follicles. This process of follicle selection is apparently mediated by hormones. The ovarian follicle produces estradiol which in turn induces pituitary release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), leading to the feedback control of the growth of the somatic cells that compose the follicle. A mathematical model of this process has revealed the accuracy of this process of selection. (Lacker, 1981; Lacker & Peskin, 1981; Akin & Lacker, 1984.) Furthermore, this analysis has indicated that the process of selection has important properties that are not obvious but which can be seen by examining the features of the idealized model. For example, computer simulations indicate that if each cycle began with considerably less than the usual 1000 or so immature follicles, the final number of follicles would rise, and the timing of the cycle would become less accurate. This is precisely what is known to occur in women; as age increases the number of incipient follicles gradually decreases, the incidence of twinning increases, and the menstrual cycle becomes less accurate. This result appears to account for why in young women there is such a luxuriant waste of follicles in each cycle; in exchange for the inefficiency of a large initial follicle number, there is a great gain in accuracy.

(4) *Cell death in embryology*

Although cell death is only one form of morphogenetic cellular selection, and the most extreme form at that, it is nonetheless a common observation in embryology. Embryonic cell death has been extensively reviewed by Glucksman (1950), and Saunders (1966) has provided an excellent discussion of the same subject with special reference to the limb (see also Bowen & Lockshin, 1981; Lockshin & Beaulaton, 1974; Wyllie *et al.*, 1980; Menkes, Sandor & Ilies, 1970.) There are numerous examples of embryonic cell death (Glucksman enumerated 74 separate instances) which include such common cases as regression of the Wolfian or Müllerian duct in sexual differentiation, destruction of larval tissues in amphibian metamorphosis, closure of the palate, invagination of the optic-cup, shaping of the nose, and creation of the digestive tract and heart (Hurle, Lafarga & Ojeda, 1977). Cell death is a particularly common feature during the formation of the nervous system and a number of authors have suggested that selective cell death may play a role in the nervous system's developmental specificity (Swindale, 1980; Ashwell & Watson, 1983; Oppenheim & Nuñez, 1982; Pittman & Oppenheim, 1979; Lamb, 1981).

Morphogenetic cell death has a characteristic appearance (Wyllie *et al.*, 1980), and such death can be readily distinguished from necrotic cell death such as that caused by lack of oxygen. Perhaps there is a specific cellular mechanism by which self-destruction occurs upon receipt of an appropriate biological signal, and this process gives rise to the characteristic appearance of cells that die during development.

IV. DEVELOPMENTAL GENETICS

(1) *Skin pigment pattern*

The formation of white and coloured areas in the skin of spotted animals is, like limb development, an example of morphogenesis. As in the case of limb development, the embryology of pigment pattern formation is well described; the melanocytes that produce these pigments are derived from cells which migrate out of the neural crest and settle in the skin (Rawles, 1947, 1948). Pigment-pattern formation, however, is of particular interest because of the large number of mutant genes which modify these spotting patterns (Table 1) (Silvers, 1979; Searle, 1968).

It appears that the white areas in spotted skin are the result of selective elimination of pigment cell precursors, as has been shown by the simple histological observation that melanocytes are absent from these white areas (Silvers, 1958). The mechanisms by which these cells are eliminated have been studied by Mayer and colleagues. These workers utilized a simple procedure in which embryonic neural and epidermal tissues, differing in genotype, were combined and allowed to differentiate (Mayer, 1965, 1967, 1973*a*, *b*, 1975, 1979; Mayer & Green, 1968; Mayer & Maltby, 1964; Mayer & Oddis, 1977; Mayer & Reames, 1962). In one such study, neural tubes from 11-day-old embryos carrying the spotting gene *piebald* were found to be incapable of donating pigment to *wild-type* skin also taken from 11-day embryos. This demonstrated that the *piebald* defect occurs in the melanocyte precursor (Mayer, 1979) rather than in the skin environment. Furthermore, Mayer found that melanocyte precursors taken from *piebald* neural tissue of 11-day-old mice, although incapable of populating 11-day-old skin, were

Table 1. *Spotting genes of the mouse and their pleiotropic effects*

Gene	Gene symbol	Site of action on pigmentation	Effects	Cell affected
Microphthalmia	Mi^{wh}	Melanocyte precursor	Spotting	Melanocyte precursor
Piebald	*s*	Melanocyte precursor	Spotting	Melanocyte precursor
			Megacolin	Ganglion cells
Lethal spotting	*ls*	Melanocyte precursor	Spotting	Melanocyte precursor
			Anaemia	Blood forming cells
			Sterility	Germ cells
Dominant spotting	*W*	Melanocyte precursor	Spotting	Melanocyte precursor
			Anaemia	Blood forming cells
			Sterility	Germ cells
Flexed tail	*f*	Melanocyte precursor	Spotting	Melanocyte precursor
			Anaemia	Blood forming cells
			Bone defects	Bone
Belted	*bt*	Skin environment	Spotting	Melanocyte precursor
Steel	*Sl*	Skin environment	Spotting	Melanocyte precursor
			Anaemia	Blood forming cells
			Sterility	Germ cells

able to grow in the embryologically abnormal environment of 13-day-old skin. This established that melanocyte precursors are apparently given off by the *piebald* neural crest and deposit in the skin, but later die.

Mayer found that other spotting genes act in the skin by producing an inhospitable skin environment. For example, neural tube–skin recombination experiments of the spotting mutant *Steel* gave results opposite to those described above for *piebald*. *Steel* neural tissue was found to contain melanocyte precursors that can occupy *wild-type* embryonic skin, but *Steel* embryonic skin is incapable of supporting pigment cell growth from neural tubes of any genotype.

These experiments demonstrate that melanocyte precursors are deposited throughout the skin in spotted mice, but perish in areas destined to be white by a process of selection. Experiments of three types substantiate this viewpoint by establishing that spotting genes exert their influence on melanocyte precursors by reducing their fitness:

(*a*) *Dermal–epidermal reconstitution*

Embryonic dermal and epidermal tissue can be recombined at a time when both contain melanoblasts. Mayer has shown that when one of the two layers carries a mutant gene for spotting, the mutant melanoblasts are displaced by wild-type melanoblasts from the other layer (Mayer, 1973*a*; Mayer & Oddis, 1977).

(*b*) *Chimeric mice*

Chimeric mice composed of two cell lines of independent origin may be made by fusing together two early mouse embryos, and reimplantation into a pseudo-pregnant female. If the two donor embryos are marked in some way, such as by being of contrasting coat colour genotype, the resulting animal will grow into an adult with many patches of contrasting colour (Mintz, 1969, 1971, 1974, 1976) (Fig. 4).

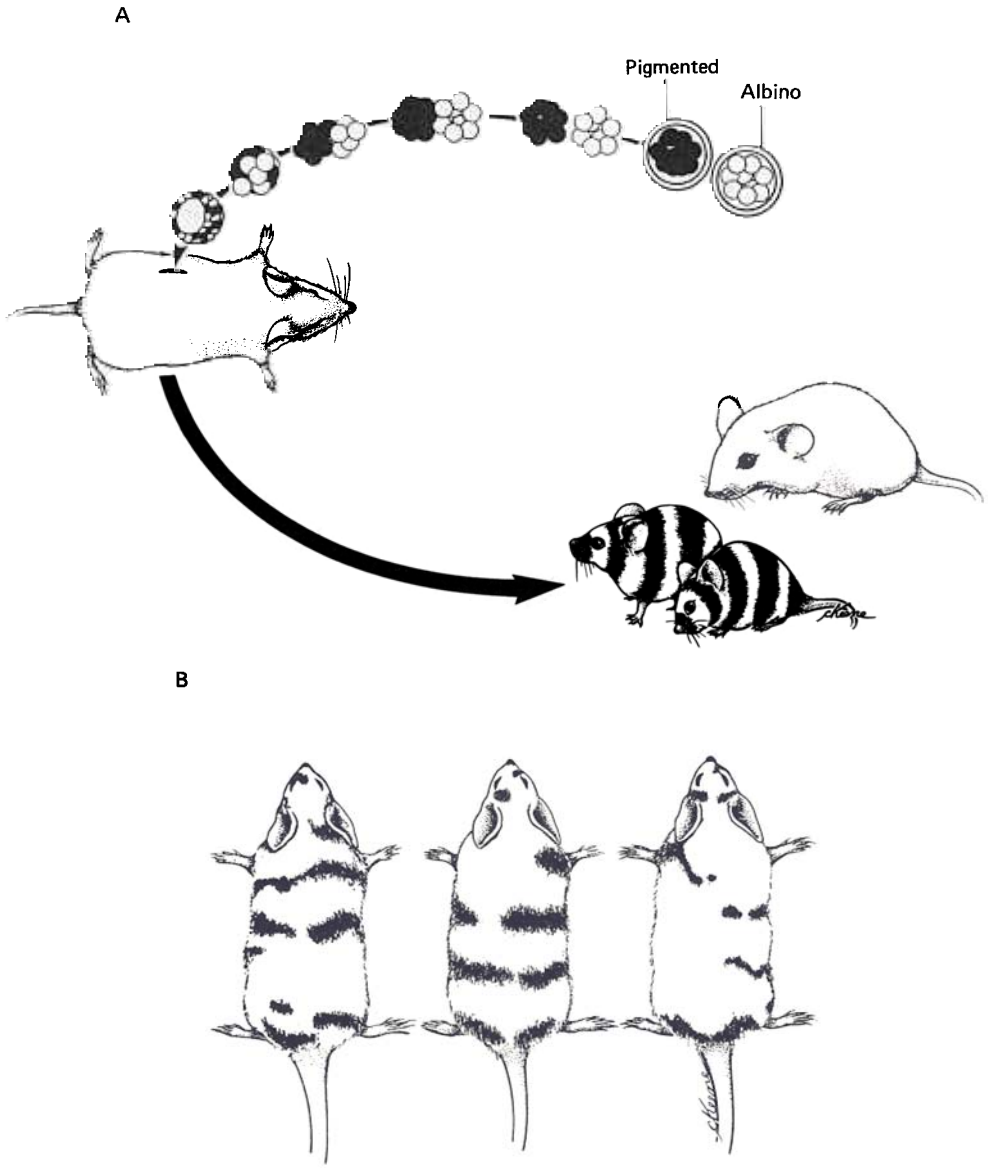

Fig. 4. (A) Production of chimeric mice from the fusion of two embryos, one of which is marked by the selectively-neutral coat colour gene *albino*. (The albino phenotype is not the spotting type but is due to the failure of the pigment-producing enzyme tyrosinase.) (B) Three examples of chimeric mice of this composition. Redrawn from Silvers (1979).

The reduced fitness of pigment cell precursors caused by spotting genes is demonstrated in a chimeric mouse in which one of the two embryos carries genes for spotting. In such a mouse, the coat colour phenotype has been found not to be composed of patches, but to be uniform like that of a normal, wild-type mouse. This non-chimeric appearance of the pelt is seen even though internal chimerism is present (Silvers, 1979; Mintz, 1969, 1971, 1974, 1976) (Fig. 5). Apparently, the wild-type cells in the chimeric

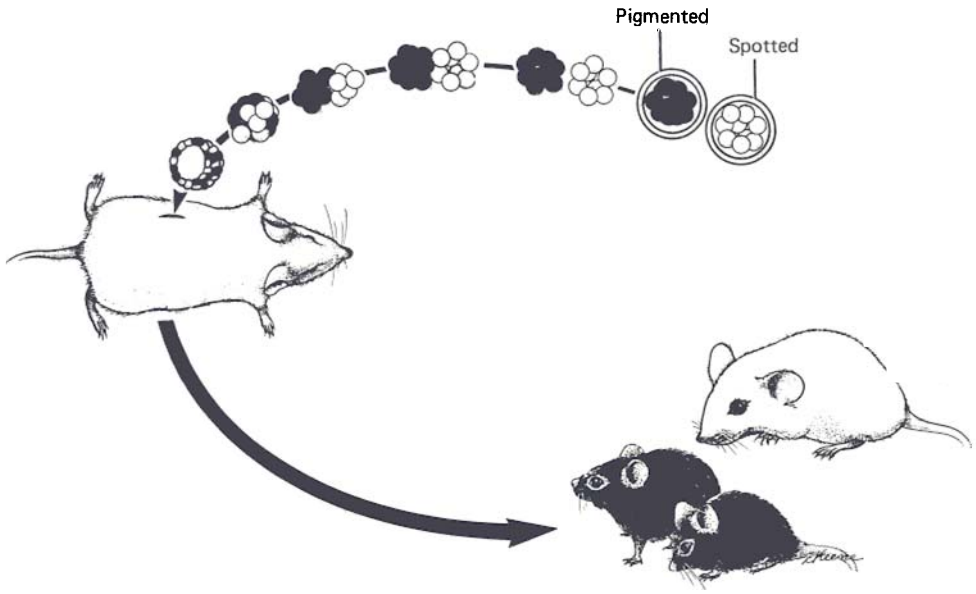

Fig. 5. Chimeric mice in which one of the embryos is of spotted genotype. The uniform pigmentation of these mice demonstrates the selective elimination of spotted pigment cell precursors. (Internal chimerism may, as expected, be found.) Occasionally such mice may show a partial or complete spotted phenotype, but never the striped pattern (Fig. 4), expected in the absence of selection.

embryo are able to 'elbow out' cells of spotted genotype and become the predominant occupiers of the skin.

A particularly impressive example of this phenomenon was provided by Gordon (1977) for the action of the spotting gene W^v. $W^v/+$ heterozygous mice are mostly pigmented; only a small spot is seen on the belly. It is only in W^v/W^v homozygotes that extensive areas of white are seen. Nonetheless, Gordon has found that even a single dose of W^v is enough to decrease the fitness of melanoblasts; the melanocytes of $W^v/+ \leftrightarrow +/+$ chimeric mice are almost entirely of $+/+$ genotype.

(c) *The clonal constitution of pigment cells*

The melanocyte clones which appear as patches in chimeric mice can also be visualized in female mammals which are heterozygous for X-linked coat-colour genes. The basis of this patched phenotype, known as X-linked variegation, is due to the random inactivation of X-linked genes as first described by Lyon (1961). For example, only one X-chromosome in a melanocyte of a female mouse heterozygous for the X-linked gene *Mottled* (*Mo*/+) will be active, expressing either the *Mo* or the + phenotype, but not both.

Since the X-chromosome inactivation event occurs early in development, before the formation of the neural crest, all of the melanocytes of a clonal patch formed by a single melanocyte progenitor will share the same X-chromosome phenotype; in the case of *Mo*/+ female mice, each clonal patch will be of either *Mottled* or *wild-type* phenotype. The effect of spotting genes on the reduction of melanocyte precursor fitness can be seen in such mice. For example, mice which are doubly mutant (*Mo*/+ ; *s*/*s*) for *Mottled*

(*Mo*) and the spotting gene *piebald* (*s*) will have a strikingly smaller number of clonal patches than (Mo/+ ; +/+) mice which do not carry the spotting gene (Silvers, 1979). Apparently, the introduction of *piebald* (*s*/*s*) causes the death of many melanocyte clonal progenitors.

A more familiar case is known to cat lovers. Females heterozygous for the X-linked gene *orange* (*O*/+) display the black–yellow variegated phenotype known as tortoiseshell. With the addition of the spotting gene *tabby*, *O*/+ females display the characteristic tabby–tortoiseshell pattern with a much reduced number of patches of black and orange (Searle, 1968) (Fig. 6).

Female mice which are *Mottled* and *belted* (*Mo*/+ ; *bt*/*bt*) likewise show a decrease in the number of clonal patches. There is some evidence that *belted* acts in the skin environment (Mayer & Maltby, 1964). Therefore, we see in this example a spotting gene acting externally on melanocyte precursors, causing the selective elimination of these cells.

(2) *Formation of the clonal constitution of skin pigment in normal mice*

The number of clonal patches in normal (non-spotted) animals, as revealed by chimeric and X-variegated mice, is not large, and Mintz has proposed that pigment cells of the mouse are derived from the clonal progeny of only 34 founder cells (Mintz, 1969, 1971, 1974, 1976) (Fig. 4). It could well be that these 34 cells are themselves selected from a larger pool of cells, the existence of which leaves no trace after the time of skin pigment formation. Perhaps such a process of selection could occur in a manner analogous to the way in which a small number of ovarian follicles are selected from a large precursor pool (Lacker, 1981; Lacker & Peskin, 1981; Akin & Lacker, 1984).

(3) *The genetics of cellular selection*

If cellular selection is an important mechanism in development, we would like to be able to identify the genes which act in controlling selection, and spotting genes provide just such an opportunity (Table 1). Several spotting genes act through the environment of the skin and may identify the types of signals by which selection is carried out. Other spotting genes exert their effect directly in the melanocyte precursor, and thus may code for parts of the cell's own process for determining life or death.

Although these mutant genes have been identified by their influence on pigmentation, they may have other effects also (Table 1). The action of these genes on other developmental processes indicates that their functions are not specific to pigmentation but are used generally in development.

V. SELECTIVE GENE EXPRESSION

(1) *Fetal-adult switch in erythrocyte phenotype*

A gradual change in the nature of the human red blood cell occurs around the time of birth, the features of which include reduction in cell size, change in carbonic anhydrase levels (Alter, 1979), alteration in the antigenic makeup of the membrane and a switch in the composition of the non-α subunit of haemoglobin from fetal (γ) to adult (β) type (Nathan, 1983; Stamatoyannopoulos & Nienhuis, 1979, 1981, 1983; Bard, Makowski, Meschia & Battaglia, 1970; Bard, 1973, 1974, 1975, 1976; Garby, Sjolin &

Fig. 6. Left: variegated pattern of female cat heterozygous for the X-linked gene *orange* ($O/+$). The pelt of this animal is composed of a large number of small patches of black and yellow (indicated in this drawing by black and grey). The common name for such a phenotype is tortoiseshell. Right: female cat of the same X-linked genotype ($O/+$) plus the autosomal spotting gene *tabby*. This animal has areas of black, yellow (shown here by grey) and white. Note that in the non-white areas, the number of black and yellow patches is decreased.

Vuille, 1962; Beaven, Ellis & White, 1960; Saglio *et al.*, 1979; Terrenato, Bertilaccio, Spinelli & Colombo, 1981; Bunn, 1981). This change is in haemoglobin phenotype provides for the different metabolic requirements of the fetus and child (Bunn, 1981).

The genes for γ-globin and β-globin are located in the same region of chromosome 11 (Maniatis *et al.*, 1980); there are actually two forms of γ-globin coded by two closely linked γ-genes named ${}^{G}\gamma$ and ${}^{A}\gamma$ to indicate a single amino acid difference in the gene products. This region of chromosome 11 also encodes genes for an embryonic form of globin named ϵ, as well as δ-globin which is produced in low quantity in adulthood.

The fetus produces predominantly γ-globin (with a small amount of β), the neonate contains a mixture of γ and β, while adults produce mostly β-globin. (Kazazian 1973.) At each of these times, γ-globin is not uniformly distributed in blood, but is confined to a subpopulation of erythrocytes called 'F-cells' (Boyer *et al.*, 1975; Dover, Boyer & Zinkham, 1979; Nute *et al.*, 1980; Popat *et al.*, 1977; Zago *et al.*, 1979). Erythrocytes which do not contain γ-globin are labelled 'A-cells'. *Therefore, γ/β constitution is a*

reflection of the relative mix of different populations of erythrocytes in the blood (Bunch *et al.*, 1979).

(2) *The choice between γ- and β-globin in erythroid precursors appears to be a random process*

We know something about how the mix of 'A-cells' and 'F-cells' is generated through analysis of erythroid progenitors. Tissue culture conditions have been developed which permit the growth of colonies of erythroid cells from primitive progenitors called BFU-E's (Burst Forming Units-Erythroid) (Eaves, Humphries & Eaves, 1979; Johnson, 1981). Colonies arising from BFU-E's display two remarkable qualities with respect to their expression of globin. *First*, they tend not to reflect the peripheral blood globin phenotype of the donors from which they come; BFU-derived colonies from adults express higher than expected amounts of γ-globin (Papayannopoulou, Brice & Stamatoyannopoulos, 1976; Papayannopoulou *et al.*, 1978; Kidoguchi *et al.*, 1978; Kidoguchi, Ogawa & Karam, 1979; Peschle *et al.*, 1980; Darbre *et al.*, 1981) while BFU derived colonies from fetuses express a considerable amount of β-globin (Rowley *et al.*, 1979; Gianni *et al.*, 1980; Hassan *et al.*, 1979). *Second*, the γ/β constitution of colonies arising from BFU-E's is highly variable. Kidoguchi *et al.* (1978, 1979) and Comi *et al.* (1980) have documented this by measuring the amounts of γ and β globin in single BFU-E derived colonies; they found that the γ/β constitution of a series of individual colonies analysed from a single tissue culture plate was not uniform, but displayed a normal variation. Stamatoyannopoulos *et al.* (1981) have also found that single BFU-E's generate progeny that appear to be randomly committed to γ or β globin expression. They were able to demonstrate this by taking advantage of the fact that the BFU-E and its early progeny are mobile; the early daughters in a colony migrate apart and generate visually distinguishable clonal subcolonies (Papayannopoulou & Stamatoyannopoulos, 1980, p. 211). By immunofluorescence sub-clones of both γ^+ and γ^- phenotypes were seen, and this generation of both γ^+ and γ^- progeny from a single parental cell was found to be best explained by a probabilistic process (Stamatoyannopoulos, 1981; Darbre *et al.*, 1981; Papayannopoulou, Kalmantis & Stamatoyannopoulos, 1979; Stamatoyannopoulos *et al.*, 1981; Ogawa *et al.*, 1981; Peschle *et al.*, 1981). *Therefore, the results of the studies of both Kidoguchi and of Stamatoyannopoulos suggest that the choice between γ- and β-gene expression in an erythroid precursor appears to be determined by a random process.*

Perhaps the most dramatic piece of evidence indicating the random nature of γ-globin gene expression comes from studies which demonstrate that each of the allelic $^{G}\gamma$ genes on opposite chromosomes is activated independently and randomly. This can be seen in individuals heterozygous at the $^{G}\gamma$ locus; such persons carry the $^{G}\gamma$-variant haemoglobin-F malta-I (amino-acid change: His→Arg, position 117) on one chromosome, and normal $^{G}\gamma$ gene on the other. The BFU-Es in a tissue culture plate of cells derived from such an individual were found not to show any tendency to express both allelic $^{G}\gamma$ products together; indeed there was such great burst-to-burst variation in the expression of the two allelic $^{G}\gamma$-globin molecules as to indicate that the two allelic $^{G}\gamma$ genes are not co-ordinately controlled but activated randomly.

There is some evidence which indicates that these observations from tissue culture accurately reflect the biological reality in vivo. This additional evidence for the

randomness of fetal gene expression can be found in experiments in which the presence of multiple fetal properties have been measured on individual erythrocytes. For example, the distribution of fetal 'i' antigen and γ-globin appears to be random on erythrocytes (Papayannopoulou *et al.*, 1980; Maniatis, Frieman & Bertles, 1977); erythrocytes with both fetal markers are seen at a frequency no higher than that expected from chance alone. A similar lack of association between γ-globin expression and fetal levels of carbonic anhydrase has also been observed (Boyer & Dover, 1979). These observations indicate that the various fetal properties appear not to be co-ordinately controlled, but to be expressed in an independent and random fashion (Stamatoyannopoulos *et al.*, 1983).

(3) *Does the value of the γ/β probability vary?*

The choice of γ or β globin expression in an erythroid precursor appears to be a random process. However, it is not apparent whether the value of this probability changes or is fixed. We might expect that if the value of this probability were determined by environment, then primitive cells placed in an apparently uniform and homogeneous environment, such as a tissue culture dish, might be expected to display a characteristic γ/β ratio. This is not, in fact, observed; the γ/β ratio seen in BFU-E cultures is higher when the cultures are made from fetal cells than adult cells. These BFU-E culture results seem to indicate that the ensemble of precursors from fetus versus adult display different population structures, i.e. differently selected mixtures of precursors from a larger pool of randomly committed cells (Darbre *et al.*, 1981; Papayannopoulou, Kalmantis & Stamatoyannopoulos, 1979; Stamatoyannopoulos *et al.*, 1981; Ogawa *et al.*, 1981; Peschle *et al.*, 1981).

Stamatoyannopoulos *et al.* have demonstrated a factor that affects γ/β ratio in vitro. Whether this factor acts by varying globin gene expression or by cell selection remains to be seen (Stamatoyannopoulos *et al.*, 1983; Johnson, in Stamatoyannopoulos & Nienhuis, p. 360, 1983).

(4) *Might the γ/β switch be a selective process?*

γ-globin expression is known to be associated with high levels of haematopoietic expansion. Fetal haematopoiesis proceeds at a high rate until birth, at which time haematopoiesis all but stops. The concentration of blood cells drops early in life (Finne & Halvorsen, 1972; Terrenato *et al.*, 1981). When blood formation starts up again after several months, it does so at a much lower rate and with a switch-over to β-chain production. The reappearance of γ-globin expression in adult life is almost invariably associated with accelerated levels of haematopoiesis, particularly in response to certain types of anaemia (Beaven, Ellis & White, 1960; Dover, Boyer & Zinkham, 1979; Schiliro, Musemeci & Russo, 1980; Dover *et al.*, 1978; Papayannopoulou, Vichinsky & Stamatoyannopoulos, 1980; Nute *et al.*, 1980; Popat *et al.*, 1977; Boyer & Dover, 1979; Wood & Jones, 1981). The association of γ-globin production with cellular growth has also been observed in vitro, by Porter & Ogawa (1981) who have shown that the BFU-E growth factor, Burst-Promoting Factor (BPA), increases γ expression in culture (Terasawa *et al.*, 1980). *Perhaps the haematopoietic expansion which accompanies γ-globin expression is analogous to the lymphoid expansion which accompanies immunization, in that both are manifestations of selective expansion of primitive proliferative cells randomly committed to the production of a specific protein.*

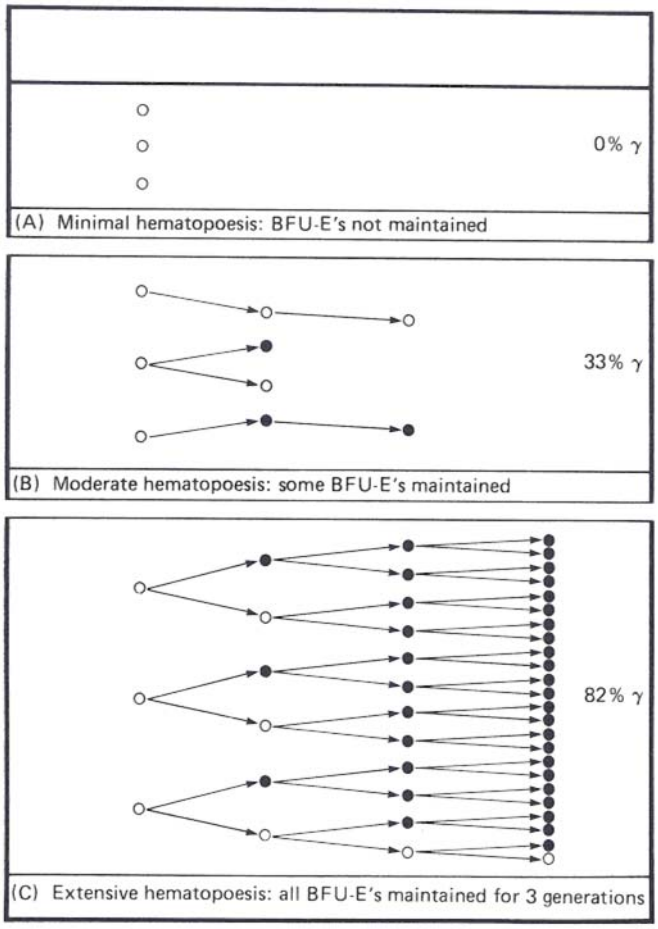

Fig. 7. A selective model of haemoglobin switching. A constant number of BFU-Es, here represented by 3 cells, is shown entering the system. At times of haemotopoietic expansion, cellular signals such as BPA are thought to maintain these BFU-Es, stimulating them to divide and grow. Otherwise these BFU-Es will die before they can yield additional stem cells. In the model, incipient BFU-Es are assumed to be committed to γ-globin expression. In each cell division, each BFU-E entertains a random choice of becoming changed to β-globin expression. In this example, this choice was simulated by flipping a coin.

(5) *Selective models of haemoglobin switching*

It is possible to imagine several ways in which the change in γ/β ratio and haematopoiesis could be linked (Fig. 7). For example, perhaps most newly formed BFU-Es arise committed to β production, but in each cell generation engage in a purely probabilistic, Russian-roulette, choice to be irrevocably committed to γ-production. Since during haematopoietic expansion stem cells are maintained and stimulated to divide, this would provide increased opportunities for BFU-Es to change from β to γ type; the *population structure* would tend towards a predominance of γ-producers. Such an example demonstrates one way in which it may be possible for the kinetics of haematopoiesis to determine the γ/β phenotype.

(6) *Is gene activation random?*

Does the random aspect of haemoglobin expression, indicated in the case of the γ/β switch, occur in other systems? There are two cases for which random-gene activation has been unambiguously demonstrated: immunoglobulin gene rearrangement, and X-chromosome inactivation. X-chromosome inactivation of X-linked coat colour genes can be readily seen in the pelt of heterozygous females; the multi-coloured appearance of these individuals is known as *X-linked variegation* (Fig. 6). Of great interest is that coat colour genes on other chromosomes may also show the same phenotype, a phenomenon known as *autosomal variegation.* In the mouse, no less than 5 separate non-X-linked loci have been identified that show variegated phenotypes indistinguishable from X-linked coat colour genes: *pink eye dilution* (chromosome 7: examples p^{un}/p^{un}; $p^{m1}/+$; $p^{m2}/+$), *albino* (chromosome 7: c^m/c^m), *agouti* (chromosome 2: A^{vy}/a; a^m/a^m), *silver* (chromosome 10: *si/si*), *Varitint-waddler* (chromosome 3: $Va/+$) [see Silvers (1979) for discussion and references]. Searle (1968) has pointed out that alleles at the *extension* locus in a number of species also show a variegated phenotype: *tortoiseshell* (e^p) in the guinea-pig, *Japanese* (e^j) in the rabbit, *brindle* (e^{br}) in dogs and cattle and *red-and-black* (e^j) in the pig.

Certain types of the genetic condition Hereditary Persistence of Fetal Haemoglobin may be analogous to autosomal variegated genes, in that γ-globin is found in approximately half of erythrocytes (Boyer *et al.*, 1977). Similarly, several studies indicate that DNA methylation, itself a differentiation-specific cell marker and a possible correlate of gene expression, is also not a stable property of cells, but displays random variation (Reis & Goldstein, 1982; Wolf & Migeon, 1982).

Perhaps the best documented case of randomness in a developmental choice comes from the work of Till, McCulloch & Siminovitch (1964). When bone marrow cells are injected into an irradiated mouse, progenitor cells, operationally called CFU-s, form large clonal colonies in the spleen. Each of these clones contains additional CFU-s, which can be identified and counted by dissecting the colony, dispersing the cells, and injecting them into another irradiated mouse. Till *et al.* reasoned that if the CFU-s in the secondary passage were formed in a deterministic way, then the number of stem cells per clone would be fairly constant and form a Poisson distribution. Results proved otherwise and indeed the distribution found was far more variable than expected, and was in fact consistent with a random process (see also Humphries, Eaves & Eaves, 1981).

Experiments in other systems such as haemoglobin expression in erythroleukemia cells (Orkin, Harosi & Leder, 1975; Gusella *et al.*, 1976), melanin synthesis in melanoma cells (Bennett, 1983) and terminal differentiation in fibroblasts (Smith & Whitney, 1980) indicate that many differentiation choices may have the same random feature seen in haemopoiesis by Till *et al.* (1964) (see also Levenson & Housman, 1981).

VI. SELECTIVE AGENTS

What are the substances that act on cells during developmental selection? For the immune system, antigens are the selective agent, while for haemoglobin switching such substances as Burst Promoting Factor may fill the same role (Gasson *et al.*, 1985). Factors extracted from the ZPA and AER of the developing limb (Cooke & Summerbell,

1981, 1980; Calandra & MacCabe, 1978; MacCabe & Richardson, 1982) are putative 'selective morphogens' in limb development. Hormones are also mediators of growth and death, often with a highly targeted cellular specificity (Underwood & Van Wyk, 1981; Loeb, 1976). Hormone-like growth factors stimulate cell growth (Marquardt *et al.*, 1981), as do neuropeptides (Nilsson *et al.*, 1985, reviewed in Hanley, 1985). Perhaps the most impressive array of agents of cellular selection are cellular growth factors, many of which have been purified and their genes cloned: erythropoietin (Goldwasser, 1975; Lee-Huang, 1984; Jacobs, 1984), nerve growth factor (Green & Shooter, 1980), angiogenin (Fett *et al.*, 1985; Strydom *et al.*, 1985; Kurachi *et al.*, 1985), haemopoetin-1 (Jubinsky & Stanley, 1985; Stanley *et al.*, 1986), epidermal growth factor (Carpenter & Cohen, 1979; Gray *et al.*, 1983; Scott *et al.*, 1983), platelet-derived growth factor (Ross *et al.*, 1986), granulocyte–macrophage colony stimulating factors, of which several have been isolated (reviewed in Metcalf, 1986), γ-interferon (Gray *et al.*, 1982), interleukin-1 (Auron *et al.*, 1984; Lomedico *et al.*, 1984; Mach *et al.*, 1985), interleukin-2 (Kashima *et al.*, 1985; Taniguchi *et al.*, 1983), interleukin-3 (Ihle *et al.*, 1982; Hapel *et al.*, 1985; Fung *et al.*, 1984; Yokata *et al.*, 1984) and interleukin-4 (Noma *et al.*, 1986). Each of these factors has the two essential properties of specificity for a narrow range of cell types and activity for induction of cell growth. Two factors, tumour necrosis factor and lymphotoxin, which induce specific cell death rather than growth, have also been purified and cloned (Gray *et al.*, 1984; Pennica *et al.*, 1984).

VII. OVERVIEW

The signs of selection – an overabundance of cell death and growth, the emergence of order from randomness – can be seen widely in development. For the cases outlined above, the immune system, the limb, skin pigment and the erythron, it is possible to propose selective mechanisms that would account for their development. It might prove useful, then, to consider the possibility that we are, at least in part, the natural consequence of that struggle for existence which goes on within the society of cells of which we are composed.

VIII. SUMMARY

Cell selection is recognized to be the principal mechanism in the generation of the immune response. The role of cell selection in other developmental systems is considered here, with emphasis on the morphogenesis of the vertebrate limb, the formation of pigment pattern in the pelt of mammals and the changes that occur in the human erythrocyte at the time of birth.

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