Extrinsic factors in cellular differentiation

RICHARD L. GARDNER*

Imperial Cancer Research Fund, Developmental Biology Unit, Department of Zoology, Oxford, United Kingdom

ABSTRACT An impressive feature of cellular differentiation in metazoa is its stability. This has led to widespread acceptance of the view that the determined state is a heritable property of individual cells. There are, in fact, rather few types of cell for which this has been demonstrated convincingly. Not only is it clear that gene expression is subject to continuous regulation but there is also evidence for an increasing variety of cells that changes in differentiation can be induced by manipulating their environment. Such findings suggest that extrinsic factors may play a more significant role in maintaining the differentiated state of cells than is generally assumed.

KEY WORDS: *differentiation, determination, transdifferentiation*

While environmental factors are widely acknowledged to play a vital part in directing the differentiation of cells, they tend to be relegated to a purely permissive role once determination has taken place. This is largely because the view has gained wide currency that determination entails the establishment of heritable restrictions in patterns of gene expression. In fact, we remain completely ignorant about the molecular basis of the determined state.

Determination is defined purely operationally by experimental embryologists and is used to denote the restriction of cells to a predictable repertoire of differentiation notwithstanding their isolation or relocation. The notion that it is perpetuated through cell heredity has arisen as a result of various studies testifying to its stability. Among the examples most widely cited in support of this notion is the behavior of serially transplanted imaginal disks in *Drosophila*. There are two points worth noting in this context. The first is that one is not dealing with single cell grafts. In fact, Hadorn (1966) concluded that at least one hundred imaginal disk cells need to be transplanted in order to secure a viable graft. The second point is that from studies of Gehring (1967) on the transplantation of genetically mosaic disks, it is difficult to escape the conclusion that transdetermination occurs in groups of cells rather than single ones. Bearing in mind that compartments are invariably polyclonal, is it conceivable that determination is itself a collective rather than an individual cell property in *Drosophila*?

Two types of experiment are generally cited in support of the notion that determination is a clonally heritable cellular state in *Drosophila*, neither of which specifically addresses the issue in relation to cells of the imaginal disks. The first is the sorting out of cells of different provenance in artificial aggregates, and the second their autonomous differentiation on heterotopic transplantation. While cell sorting clearly demonstrates the existence of distinct surface properties, it is too short-term to establish whether these are heritable. Most of the transplantation experiments providing evidence that heterotopically grafted cells differentiate autonomously entail multiple rather than single cell grafts. In the study of Simcox and Sang (1983), for example, 6 cells were engrafted in each host blastoderm in order to attain an acceptable frequency of chimerism of about 10%. When grafts were reduced to 3 cells, only 3.5% of hosts were chimeric. No chimeras at all were recorded among 127 specimens transplanted with single cells. Greater success with single cell transplants has been achieved more recently by Technau (1987), whose results suggest that, apart from neuroblasts which are precocious in commitment, cells in the early gastrula are only restricted with respect to germ layer of origin in their developmental potential. In another series of experiments in *Drosophila*, it was found that ectoderm cells behaved as determined when transplanted in small groups but not when transplanted singly (Stuttem and Campos-Ortega, 1991). Most interestingly, recent application of a photoactivatable cell marker has shown that expression of engrailed, a gene believed to confer a posterior compartmental fate on cells, is not clonally heritable during early embryogenesis in *Drosophila* (Vincent and O'Farrell, 1992). Thus, mixed clones in which some cells have ceased to express the gene are found at the posterior but not the anterior border of engrailed stripes. This implies that continued expression of engrailed depends, at least initially, on local environmental cues.

The only compelling examples of heritability of the determined state by individual cells come from *in vitro* cultures in which the capacity to engage in a specific pattern of differentiation has been found to survive repeated cloning at limiting dilution. (e.g. Ursprung, 1968; Folkman and Haudenschild, 1980; Barrandon and Green, 1987). These examples are rather few at present, possibly because of the difficulty of characterizing all except the most distinctive types of cells *in vitro*.

That differential gene activity can be propagated clonally as a stable heritable cell trait is very clearly illustrated by the phenom-

***Address for reprints**: Imperial Cancer Research Fund, Developmental Biology Unit, Department of Zoology, South Parks Road, Oxford, OX1 3PS, United Kingdom. FAX: 0865-281.310.

0214-6282/93/$03.00

enon of X-chromosome inactivation in female eutherian mammals (Lyon, 1961). In the course of normal development this inactivation process is only reversed in germ cells on their entry into meiosis (Monk and McLaren, 1981).

Attempts to reactivate the inactive X-chromosome in somatic cells provide an ample testimony to its remarkable stability (Chapman, 1986). However, extensive data from studies utilizing nuclear transplantation and cell fusion argue against the notion that X-inactivation provides a general model for silencing the genes that are not expressed in cells of a particular lineage (Harris, 1970; Gurdon, 1986). These data show that exposing nuclei of differentiated cells to a foreign cytoplasmic environment can readily induce quite alien patterns of gene expression such as, for example, muscle proteins in hepatocytes or keratinocytes (Blau, 1989). As discussed recently by Blau and Baltimore (1991), this implies that differentiation requires continuous regulation of gene expression. The question is to what extent this regulation depends on the sustained input of information from outside the individual cells. Put another way, how susceptible are differentiated cells to re-programming as a consequence of alterations in their environment? In considering this issue the environment of cells is taken to include their neighbors, the extracellular matrix, as well as hormones, growth factors and other molecules.

Histopathologists in particular have long accepted the notion that various types of differentiated cells may not be as rigidly fixed in their state of determination as has generally been supposed, as the following quotation from Willis (1962) testifies:

«The student of normal histology is apt to assume that the different kinds of adult tissues and cells are rigidly fixed invariable structures, distinctly immutable species each capable of producing by proliferation cells of the one kind only. But as soon as he pays attention to pathological histology — that is, to what the various cells and tissues can be and do in all manner of abnormal environments — he realizes that great transformations of cellular structure are possible in most tissues. The cells have much wider potencies for differentiation than are ever displayed in health; abnormal conditions are needed to reveal their dormant potencies or plasticity».

A complication in interpreting the histopathological data is the uncertainty in specific cases as to whether one is really dealing with metaplasia rather than the consequences of a developmental anomaly which led to the placement of cells of one tissue type at the locus of another. However, there is a growing list of well-documented examples where re-programming of cells that have already embarked on differentiation has been clearly demonstrated following some form of perturbation of their environment. The terminology used to describe such changes is confusing because it has not been applied consistently, metaplasia, transdetermination or transdifferentiation often being employed more or less interchangeably. This may be because it is often uncertain whether one is dealing with a change in phenotype of overtly differentiated cells or re-direction of the differentiation of more primitive 'stem' cells within a tissue. In the following discussion use of the term transdifferentiation is reserved for cases where the former clearly applies.

One of the earliest and most spectacular examples of transdifferentiation is the regeneration of a lens from dorsal marginal iris epithelium following lentectomy in urodele amphibians. Although often referred to as Wolffian regeneration, it was independently discovered somewhat earlier by Colucci (Okada, 1991). That this entails transdifferentiation is evident from the fact that initially pigmented epithelial cells can be seen to lose their pigmentation and eventually synthesize crystallin proteins as they reorganize into a lens. Studies on the developing eyes of various vertebrates including birds and mammals have shown that interconversion of various cell types can occur, including iris and pigmented retinal epithelium and neural retina into lens as well as retinal pigment epithelium into neural retina and vice versa. The developmental status of the cells in the neural retina that yield lentoid differentiation is not clear except in the studies of Moscona and his colleagues on relatively advanced chick embryos where strong biochemical and other evidence exists that they are Muller glia (reviewed in Moscona, 1986). Unquestionably, the most spectacular transdifferentiation is that exhibited by mononucleated striated muscle cells in the hydrozoan anthomedusa, *Polycoryne carnea* (Schmid and Alder, 1986). These and other well-established cases of re-programming of cells which, by virtue of their seeming irreversibility, cannot be regarded simply as modulations will not be discussed further here since they have been reviewed recently by Okada (1991). Rather, the remainder of the article will be devoted to considering additional examples of changes in cell state that do not feature in this review.

Many of the established systems for studying transdifferentiation do not lend themselves readily to detailed analysis of the phenomenon. One complication, exemplified by the neural retina in particular, is initial cellular heterogeneity. Another is the considerable length of time often required for the change in phenotype to occur, and the consequent scope for possible selection of minor subpopulations of cells that may be unrepresentative of the tissue as a whole. Desirable attributes of such a system have been considered recently elsewhere (Gardner and Davies, 1992) in relation to the visceral endoderm of the rodent conceptus. This tissue forms the outer absorptive and secretory epithelium of the yolk sac placenta. It originates from the primitive endoderm which differentiates on the blastocoelic surface of the inner cell mass at the late blastocyst stage. The other, parietal layer, of the extra-embryonic endoderm is also derived from the primitive endoderm and, when cloned by blastocyst injection, individual primitive endoderm cells have been found to yield both visceral and parietal colonization (Gardner, 1984). The visceral and parietal endoderm are among the best characterized tissues of the mouse conceptus, both morphologically and biochemically, and differ profoundly despite their common origin (Jollie, 1968; Enders *et al.*, 1978; Hogan *et al.*, 1982; Poelmann and Mentink, 1982; Gardner, 1983; Hogan and Newman, 1984; Meehan *et al.*, 1984; Shi and Heath, 1984; Cockroft, 1986). Initial indications that visceral endoderm could form parietal endoderm came from examination of ectopic grafts of early postimplantation conceptuses (Solter and Damjanov, 1973; Diwan and Stevens, 1976). More compelling evidence was provided *in vivo* by blastocyst injection experiments (Gardner, 1982) and *in vitro* by culture of partially dissected egg-cylinders (Hogan and Tilly, 1981). However, these earlier studies were limited to relatively early postimplantation conceptuses in which the visceral endoderm was still at an early stage in differentiation. Hence, the possibility that metaplasia rather than transdifferentiation was involved could not be discounted. More recently, it has become clear from the rate of formation of parietal cells, especially by later visceral endoderm, that the phenomenon qualifies as an example of transdifferentiation (Gardner and Davies, 1992). Recent findings do not support an earlier suggestion by Hogan and Newman (1984) that such

transdifferentiation is a facet of normal development (Gardner and Davies, 1992).

In considering transdifferentiation of the visceral endoderm, we are concerned with its change into one other specific type of cell. Recently, however, evidence has emerged which argues that cells of this tissue are susceptible to much more spectacular re-programming even later in development. Payne and Payne (1961) were the first to show that the mature visceral yolk sac placenta could engage in novel patterns of differentiation when transplanted ectopically in rodents. They found in rats that approximately half the transplants of 14-15 day visceral yolk sac to the omentum produced enduring grafts that included various fetal tissues. Most prominent was intestinal epithelium with associated smooth muscle and clusters of neurones that resembled the myenteric plexus. The next most frequent structures were epidermoid cysts, which occasionally included sebaceous glands and hair follicles. Relatively rarely, the grafts contained skeletal elements such as cartilage nodules or stages in ossification which could include bone marrow.

Subsequently, unequivocal teratomas were obtained in both the rat and mouse simply by exteriorizing part of the yolk sac from the uterus following fetectomy early in the second half of gestation (Sobis and Vandeputte, 1974, 1979). These tumors displayed a much wider range of differentiation than the omental grafts and occasionally included more esoteric tissues such as bronchiolar epithelium, salivary and thyroid gland, gastric epithelium, thymus, pancreas and, in a single instance in the mouse, hepatic tissue (Sobis and Vandeputte, 1979). Hence yolk sac tumors rival those derived from embryonal carcinoma (EC) and embryonic stem (ES) cells in the spectrum of tissues they produce although, unlike the latter, they have so far proved refractory to transplantation.

Initially, the favored candidate stem cell of yolk sac teratomas was the primordial germ cell, which is associated with the yolk sac prior to migration to the genital ridge. However, the subsequent failure of chemical and genetic means of deleting such cells to lower the incidence of teratomas (Sobis and Vandeputte, 1976, 1982) made this unlikely. Strong support for the view that the tumors are of visceral endodermal origin has been provided recently by Sobis *et al.* (1991) through use of mutant mice that are deficient for the enzyme glucose-6-phosphate dehydrogenase (G6PD), which is encoded by a gene on the X-chromosome that is subject to inactivation in female conceptuses. The rationale of their study is that while maternal and paternal X-inactivation occurs with essentially equal frequency in precursor cells of the mesodermal component of the yolk sac, the paternal X- is inactivated in most or all cells in the endoderm (West *et al.*, 1977). Hence if visceral yolk sac teratomas are of mesodermal origin, they should be composed of a mosaic of G6PD stained and unstained cells regardless of whether they are from female conceptuses produced by mating hemizygous deficient males with normal females or by mating homozygous deficient females with normal males. In contrast, if they originate from the endoderm, they should be composed wholly of stained cells in the former case and of unstained cells in the latter. The pattern of histochemical staining observed in the teratomas was consistent with their originating from the visceral endoderm rather than the mesoderm. Since the visceral endoderm of the mature yolk sac is a uniformly differentiated tissue that is devoid of immature cells, this finding argues that it retains the capacity for extensive re-programming of gene expression. However, it remains to be established whether the wealth of cell types formed by yolk sac teratomas derive from a single type of «stem» cell, as in the case of EC and ES-derived tumors, or to the cooperative differentiation of several types of cell each of which is relatively restricted in potency.

Extra-embryonic tissues are, of course, relatively short-lived and they typically show signs of ageing even before they are discarded at birth. To support the development of the fetus the visceral endoderm has to differentiate precociously and to combine functional activity with rapid growth. It achieves this without the assignment of growth and differentiation to distinct cell populations that is typically seen in epithelia of embryonic origin. Consequently, it is conceivable that regulation of gene expression in extra-embryonic tissues may differ from that in embryonic tissues in ways that render them more susceptible to re-programming. It is interesting to note in this context that DNA has been found to be less methylated in extra-embryonic tissues than in those of the fetus, both globally and with regard to specific gene loci as well as repetitive sequences (Chapman *et al.*, 1984; Razin *et al.*, 1984; Sanford *et al.*, 1985; Monk *et al.*, 1987). Furthermore, genes on the inactive X-chromosome can be re-activated much more readily in extra-embryonic cells than those of the embryo or adult (Chapman, 1986).

While the visceral endoderm may be exceptional among differentiated types of cell in the extent to which it can be re-programmed, there is, nevertheless, growing evidence that various adult cells may not be as rigidly fixed in their state of determination as has generally been supposed. In certain cases it may be difficult to exclude the possibility that one is dealing with modulation of gene expression rather than a genuine change in cell state. There is no such uncertainty in recent experimental studies by Cunha and his colleagues in which epithelia isolated from one type of adult organ were combined with neonatal mesenchyme from another (Cunha *et al.*, 1983, 1991). These studies provide compelling morphological and biochemical evidence that certain epithelia in adult rodents remain susceptible to a change in differentiation appropriate to that of the mesenchyme with which they are combined. First it was shown that the urothelium of the bladder of adult rats could be converted to prostatic epithelium when combined with prostatic mesenchyme (Cunha *et al.*, 1983). More recently, compelling evidence has been provided for the conversion of the epithelia of both adult rodent ureter and vas deferens to seminal vesicle epithelium in combination with neonatal seminal vesicle mesenchyme (Cunha *et al.*, 1991). However, since the results were assessed in grafts of recombined tissues that had resided in the kidney for one month, it has yet to be established how quickly the change occurs and whether it involves transdifferentiation or re-direction of the differentiation of stem cells in these epithelia. Nevertheless, it is important to emphasize that, particularly in the conversions to seminal vesicle epithelium, the change is complete in terms of both pattern of morphogenesis and histogenesis as well as in cytodifferentiation. Coordinated change in all 3 facets is by no means inevitable following epithelial-mesenchyme recombination, even where prenatal tissues have been used. Indeed, striking examples of uncoupling of morphogenesis and cytodifferentiation have been recorded in several such studies (Mizuno and Yasugi, 1990).

There is a strong tendency to accept fixity of programmes of differentiation of cells as the norm and to regard departures from it as aberrant curiosities. This viewpoint is challenged not only by the growing list of cases of transdifferentiation of metaplasia that have been authenticated experimentally but also by the experience of the pathologist, encapsulated in the earlier quotation from Willis, which cannot be ignored. Just as developmental biology can inform

pathology, so is the reverse unquestionably true. And nowhere are the benefits to be gained from dismantling the traditional boundaries between these disciplines illustrated more clearly than in the work of Barry Pierce.

Acknowledgments

I wish to thank Dr. Phil. Ingham for valuable discussion and comments on the manuscript, Mrs. Jo Williamson for help in preparing it, and the Royal Society and Imperial Cancer Research Fund for support.

References

BARRANDON, Y. and GREEN, H. (1987). Three clonal types of keratinocyte with different capacities for multiplication. *Proc. Natl. Acad. Sci. USA 84:* 2302-2306.

BLAU, H.M. (1989). How fixed is the differentiated state? *Trends Genet. 5:* 268-272.

BLAU, H.M. and BALTIMORE, D. (1991). Differentiation requires continuous regulation. *J. Cell Biol. 112:* 781-783.

CHAPMAN, V.M. (1986). X-chromosome regulation in oogenesis and early mammalian development. In *Experimental Approaches to Mammalian Embryonic Development*, (Eds. J. Rossant and R.A. Pedersen). Cambridge University Press, Cambridge, pp. 365-398.

CHAPMAN, V.M., FORRESTER, L., SANFORD, J., HASTIE, N. and ROSSANT, J. (1984). Cell lineage-specific undermethylation of mouse repetitive DNA. *Nature 307:* 284-286.

COCKROFT, D.L. (1986). Regional and temporal differences in the parietal endoderm of the midgestation mouse embryo. *J. Anat. 145:* 35-47.

CUNHA, G.R., FUJII, H., NEUBAUER, B.L., SHANNON, J.M., SAWYER, L. and REESE, B.A. (1983). Epithelial-mesenchymal interactions in prostatic development: I. Morphological observations of prostatic induction by urogenital sinus mesenchyme in epithelium of the adult rodent urinary bladder. *J. Cell Biol. 96:* 1662-1670.

CUNHA, G.R., YOUNG, P., HIGGINS, S.J. and COOKE, P.S. (1991). Neonatal seminal vesicle mesenchyme induces a new morphological and functional phenotype in the epithelia of adult ureter and ductus deferens. *Development 111:* 145-158.

DIWAN, S.B. and STEVENS, L.C. (1976). Development of teratomas from the ectoderm of mouse egg cylinders. *J. Natl. Cancer Inst. 57:* 937-942.

ENDERS, A.C., GIVEN, R.L. and SCHLAFKE, S. (1978). Differentiation and migration of endoderm in the rat and mouse at implantation. *Anat. Rec. 190:* 65-78.

FOLKMAN, J. and HAUDENSCHILD, C. (1980). Angiogenesis *in vitro*. *Nature 288:* 551-556.

GARDNER, R.L. (1982). Investigation of cell lineage and differentiation in the extraembryonic endoderm of the mouse embryo. *J. Embryol. Exp. Morphol. 68:* 175-198.

GARDNER, R.L. (1983). Origin and differentiation of extraembryonic tissues in the mouse. *Int. Rev. Exp. Pathol. 24:* 63-133.

GARDNER, R.L. (1984). An *in situ* cell marker for clonal analysis of development of the extraembryonic endoderm in the mouse. *J. Embryol. Exp. Morphol. 80:* 251-288.

GARDNER, R.L. and DAVIES, T.J. (1992). Environmental factors and the stability of differentiation in mammalian development. *C.R. Hebd. Acad. Sci. Paris, t. 314 Serie III, Suppl. 9:* 67-69.

GEHRING, W. (1967). Clonal analysis of determination dynamics in cultures of imaginal disks in *Drosophila melanogaster*. *Dev. Biol. 16:* 438-456.

GURDON, J.B. (1986). Nuclear transplantation in eggs and oocytes. *J. Cell Sci. (Suppl.) 4:* 287-318.

HADORN, E. (1966). Dynamics of determination. In *Major Problems in Developmental Biology* (Ed. M. Locke). Academic Press, New York, pp. 85-104.

HARRIS, H. (1970). *Cell Fusion*. Clarendon Press, Oxford.

HOGAN, B.L.M. and NEWMAN, R. (1984). A scanning electron microscope study of the extraembryonic endoderm of the 8th-day mouse embryo. *Differentiation 26:* 138-143.

HOGAN, B.L.M. and TILLY, R. (1981). Cell interactions and endoderm differentiation in cultured mouse embryos. *J. Embryol. Exp. Morphol. 62:* 379-394.

HOGAN, B.L.M., TAYLOR, A. and COOPER, A.R. (1982). Murine parietal endoderm cells synthesize heparan sulphate and 170K and 145K sulphated glycoproteins as components of Reichert's membrane. *Dev. Biol. 90:* 210-214.

JOLLIE, W.P. (1968). Changes in the fine structure of the parietal yolk sac of the rat placenta with increasing gestational age. *Am. J. Anat. 122:* 513-532.

LYON, M.F. (1961). Gene action in the X-chromosome of the mouse. *Nature 190:* 372-373.

MEEHAN, R.R., BARLOW, D.P., HILL, R.E., HOGAN, B.L.M. and HASTIE, N.D. (1984). Pattern of serum protein gene expression in mouse visceral yolk sac and foetal liver. *EMBO J. 3:* 1881-1885.

MIZUNO, T. and YASUGI, S. (1990). Susceptibility of epithelia to directive influences of mesenchymes during organogenesis: uncoupling of morphogenesis and cytodifferentiation. *Cell Differ. Dev. 31:* 151-159.

MONK, M. and McLAREN, A. (1981). X-chromosome activity in foetal germ cells of the mouse. *J. Embryol. Exp. Morphol. 63:* 75-84.

MONK, M., BOUBELIK, M. and LEHNART, S. (1987). Temporal and regional changes in DNA methylation in embryonic, extraembryonic and germ cell lineages during mouse embryo development. *Development 99:* 371-382.

MOSCONA, A.A. (1986). Conversion of retina glia cells into lenslike phenotype following disruption of normal cell contacts. *Curr. Top. Dev. Biol. 20:* 1-19.

OKADA, T.S. (1991). *Transdifferentiation*. Clarendon Press, Oxford.

PAYNE, J.M. and Payne, S. (1961). Placental grafts in rats. *J. Embryol. Exp. Morphol. 9:* 106-116.

POELMANN, R.E. and MENTINK, M.M.T. (1982). Parietal yolk sac in early gestation mouse embryos: structure and function. *Bibl. Anat. 22:* 123-127.

RAZIN, A., WEBB, C., SZYF, M., YISREALI, J., ROSENTHAL, A., NAVEH-MANY, T., SCIAKY-GALLILi, N. and CEDFAR, H. (1984). Variation in DNA methylation during mouse cell differentiation *in vivo* and *in vitro*. *Proc. Natl. Acad. Sci. USA 81:* 2275-2279.

SANFORD, J.P., CHAPMAN, V.M. and ROSSANT, J. (1985). DNA methylation in extraembryonic lineages of mammals. *Trends Genet. 1:* 87-93.

SCHMID, V. and ALDER, H. (1986). The potential for transdifferentiation of differentiated medusa tissue *in vitro*. *Curr. Top. Dev. Biol. 20:* 117-135.

SHI, W-K. and HEATH, J.K. (1984). Apolipoprotein expression by murine visceral yolk sac endoderm. *J. Embryol. Exp. Morphol. 81:* 143-152.

SIMCOX, A.A. and SANG, J.H. (1983). When does determination occur in *Drosophila* embryos? *Dev. Biol. 97:* 212-221.

SOBIS, H. and VANDEPUTTE, M. (1974). Development of teratomas from displaced visceral yolk sac. *Int. J. Cancer 13:* 444-453.

SOBIS, H. and VANDEPUTTE, M. (1976). Yolk sac-derived teratomas are not of germ cell origin. *Dev. Biol. 51:* 320-323.

SOBIS, H. and VANDEPUTTE, M. (1979). Teratoma induction in mice and rats in relation to the age of the visceral yolk sac. *Eur. J. Cancer 15:* 143-151.

SOBIS, H. and VANDEPUTTE, M. (1982). Development of teratomas from yolk sac of genetically sterile embryos. *Dev. Biol. 92:* 553-556.

SOBIS, H., VERSTUYF, A. and VANDEPUTTE, M. (1991). Endodermal origin of yolk sac derived teratomas. *Development 111:* 75-78.

SOLTER, D. and DAMJANOV, I. (1973). Explantation of extraembryonic parts of 7 day-old mouse egg cylinders. *Experientia 29:* 701-702.

STUTTEM, I. and CAMPOS-ORTEGA, J.A. (1991). Cell commitment and cell interactions in the ectoderm of *Drosophila melanogaster*. *Development (Suppl.) 2:* 39-46.

TECHNAU, G.M. (1987). A single cell approach to problems of cell lineage and commitment during embryogenesis of *Drosophila melanogaster*. *Development 100:* 1-12.

URSPRUNG, H. (Ed.) (1968). *The Stability of the Differentiated State: Results and Problems in Cell Differentiation* Vol. 1. Springer-Verlag, Berlin.

VINCENT, J-P. and O'FARRELL, P.H. (1992). The state of engrailed expression is not clonally transmitted during early *Drosophila* development. *Cell 68:* 923-931.

WEST, J.D., FRELS, W.I., CHAPMAN, V.M. and PAPAIOANNOU, V.E. (1977). Preferential expression of the maternally derived X-chromosome in the mouse yolk sac. *Cell 12:* 873-882.

WILLIS, R. (1962). *The Borderland of Embryology and Pathology* 2nd ed. Butterworths, London, p. 519.