

## Conrad H. Waddington's contributions to avian and mammalian development, 1930-1940

CLAUDIO D. STERN\*

*Department of Genetics and Development, Columbia University,  
New York, NY, USA*

### Introduction

Few developmental biologists have been as productive, diverse or influential as Conrad Hal Waddington (1905-1975) (Fig. 1). His output includes more than 200 primary papers, 27 books and numerous 'popular' science and other writings in his 48-year-long publishing career (1929-1977) (see Robertson, 1977 for a detailed list of publications, albeit with some errors and omissions). His interests spanned Palaeontology, Evolution, Genetics, Developmental Biology of plants and animals, Growth, Cancer, Biochemistry, Biophysics, Mathematics and Theoretical Biology, Education, Ethics, Politics and Philosophy. Reviewing such a career would be a formidable task. But it is somewhat disconcerting that even his contributions to the embryology of amniote embryos, which are among his most important, are often forgotten. It is for this reason that I have decided to concentrate on his first decade of embryological experiments using amniote embryos and on some of the concepts that he developed based on his experimental results, rather arbitrarily emphasising some of the experiments and concepts for which his contribution should have received more credit.

Waddington was certainly not the first to perform transplants in young avian or mammalian embryos, nor the first to show that the amniote organiser resides in the anterior primitive streak, nor was he responsible for the concepts behind words like 'competence'.

But he had a great capacity for synthesis, and few if any embryologists before him have been able to think at so many different levels simultaneously, trying to understand mechanisms to explain the molecular basis of induction together with the contributions made by cell movements, patterning and the evolutionary consequences of these mechanisms. As Robertson (1977) states, Waddington was "a great coiner of neologisms", and was responsible for finding English words for many embryological concepts that are, or should be, in use today.

Waddington's working life was a quest for a unifying theory of life –one that would bring together his many interests and in this sense he was a true Renaissance man. His first full-fledged attempt at putting forward such a theory was in 1940, in his book *Organisers and genes*, where he proposes the idea of 'epigenetic landscapes'. He proposes that development and evolution can be viewed as a succession of relatively stable states, separated by periods of instability and change. He illustrates this as a series of valleys and ravines that branch as they slope downhill (Fig. 2). Unfortunately this 'theory' only provides a visual representation of some aspects of development and does not provide any specific explanation for developmental or evolutionary processes, as he himself recognised. The quest continued well after 1940 and, needless to say, failed. But travelling along the road was more important than reaching the end, and along this road there are many treasures. In my opinion his most important contribution was the lucidity with which he

\*Address for reprints: Department of Genetics and Development, Columbia University, 701 West 168th Street #1602, New York, NY 10032, USA. FAX: (212) 923 2090. e-mail: [cgs20@columbia.edu](mailto:cds20@columbia.edu)

asked mechanistic questions about development – questions that are still as valid as when he first addressed them and which are still crying out for answers.

### Some experiments and conclusions

Having taken a First in the Cambridge Natural Sciences Tripos Part II in Geology (1926), Waddington obtained a scholarship in Palaeontology and Philosophy but never presented his thesis on the taxonomy of Ammonites because at this time his friendship with Gregory Bateson caused his interest to shift to Genetics and Embryology, and started him reading Spemann's work on the amphibian organiser. After two early papers on other topics (Waddington, 1929a,b), Waddington's excursions into experimental embryology begin when he visits Honor Fell at the Strangeways laboratory (probably in 1929) to look at her organ culture technique using plasma clots on a watch glass, and to assess its suitability for the culture of avian and mammalian embryos. Immediately thereafter he starts an extensive series of manipulations that include extirpations as well as transplants, both within and across species. His 1932 paper is a summary of some 650 operations which he performed with this technique in his first 3 years as an experimental embryologist.

The following sections review some of the major findings on amniote development made by Waddington between 1930 and 1940. The order of these sections is somewhat arbitrary. It was impossible to follow a chronological order according to when the experiments were performed, since Waddington tackled many questions simultaneously. It is also difficult to respect the order in which these events take place during development, because the findings from his work on different problems are often closely interrelated. The result is somewhere between the two, with perhaps some over-emphasis on issues of current wide interest, but I have purposely refrained from referring to recent work on these subjects.

#### *A role for the endoderm in axial development*

Until fairly recently, work on early development has concentrated somewhat disproportionately on the formation and patterning of the mesoderm and ectoderm, but the role of the endoderm has been neglected. In the last 5 years or so, this germ layer (and particularly the components with an extraembryonic fate, such as the visceral endoderm of the mouse and the hypoblast of the chick) has received renewed attention from work in several different vertebrate classes. Surprisingly, Waddington's pioneering work on this is only rarely cited. It was he who discovered that the early endoderm of the avian embryo (a layer now generally called the 'hypoblast', whose fate is to contribute to the yolk sac stalk) plays an important role in directing the cranio-caudal orientation of the embryonic axis. He thought very deeply about whether this was an example of an inductive interaction between the endoderm and the rest of the embryo, and it is a good lesson to follow his arguments closely.

In Waddington's first two papers using experimental manipulation of cultured avian embryos (Waddington, 1930, 1932), he reports that primitive streak stage chick embryos deprived of the primitive endodermal layer develop more or less normally. However, an interesting result is obtained when the endodermal layer is rotated by 90° at early to late primitive streak stages. He finds that

when the endodermal layer (hypoblast) is rotated in the duck, the primitive streak "bent round towards the anterior part of the endoderm: in later stages the embryos became straight, lying in a direction midway between those of the ectoderm and endoderm". He concludes that "both the layers have a polarity, and that under the conditions of these experiments, both layers play a part in the determination of the axial direction of the embryo." He asks the question, rather more lucidly than many modern papers studying a similar tissue interaction, of whether this is due to induction or to cell movements: "... although it is clear that the endoderm has an important influence on the differentiation of the ectoderm, it is impossible to decide whether this influence affects only the form-building movements, or whether it affects also the actual qualitative developmental fate of the tissues." (Waddington, 1932, p. 203). An extraordinarily visionary statement, taking into account that, without the benefit of time-lapse analysis or cell marking, he was unable to analyse cell movements directly.

A few years later (Waddington, 1933a), he takes these experiments a step further. He attempts to tackle directly the question of whether the endoderm (hypoblast) can initiate, rather than merely redirect, the movements of cells in the epiblast, albeit not with a particularly elegantly designed experiment. He rotates the endoderm by 180° rather than 90° as in the earlier paper. In the majority of cases he sees, again, bending of the primitive streak. However, in a few cases he finds that a second primitive streak forms. In one, rather convincing case of a duck embryo operated in this way (no. 716, pp. 512-513, Fig. 11b) two almost complete axes develop, with the posterior ends coming from opposite ends but sharing common head structures. He concludes that the endodermal layer has inducing ability, but that this is "probably an induction of form-building movements, rather than of a specific organ or tissue" (p. 503) and that "the thing which is induced by the endoderm is a set of tissue movements" (p. 517).

Despite Waddington's careful initial observations and interpretations, his own conclusions from the same experiments go much further in later years, even to the point of mentioning an 'endoderm organiser' (Waddington, 1940; p. 10). It is this larger claim that has been perpetuated by many recent authors and so found its way into most textbooks, leaving by the way-side Waddington's keen observations and profound thought.

#### *Induction by the primitive streak in birds and mammals*

In amphibians, Spemann and Mangold had shown in the mid-1920's that a special region, which they called the 'organiser', resides at the dorsal lip of the blastopore at the gastrula stage. When this is transplanted elsewhere, an ectopic axis, which is often complete, develops. Spemann emphasised that almost all of the neural tissue in the ectopic axis was derived from host ectoderm whose fate was not to become nervous system but epidermis. The importance of this discovery was two-fold: first, it is the first unambiguous demonstration of a true inductive interaction, where one tissue affects the fate of another. Second, it reveals that the interaction is more than a simple change of fate, because the resulting axis is fully patterned.

Having entered into experimental embryology inspired by the work of Spemann, it is not surprising that Waddington turned his attention early on to addressing the question of whether amniote embryos possess an 'organiser' as well. First (Waddington, 1930, 1932) he applied Honor Fell's culture technique to chick and duck

embryos, and showed that the anterior half or so of the primitive streak has the ability to elicit the formation of an ectopic neural plate upon transplantation. At the same time, he attempted to do the same with rabbit embryos, which failed at first, but eventually succeeded in 1937.

#### *Induction by the avian organiser*

In the 1930 and 1932 papers, Waddington reports that grafts of duck primitive streak can induce neural folds in a chick host, particularly (but not only) when the anterior end (Hensen's node) is included. These findings lead him to the conclusion that in birds, as in amphibians, the primitive streak is a functional organiser, but that certain other regions of the embryo at this and later stages also have some ability to induce. He concludes his 1930 paper in awe of the complexities of early developmental events: "... the developmental mechanics of the chick blastoderm are decidedly complicated. Probably there are several regions capable of acting as organisers in Spemann's sense, and, ... the blastoderm retains for a considerable time enough plasticity to be affected by organising regions".

During the amphibian egg laying season of 1933, Waddington, accompanied by his close colleagues Joseph and Dorothy Needham, visits Berlin to conduct experiments to elucidate the chemical nature of the inducer in amphibians, grafting either coagulated or otherwise denatured organisers, or extracts thereof, as well as certain pure substances. During this period they come into close contact with Otto Mangold and Johannes Holtfreter, with whom they discuss their experiments extensively. The result is a large series of papers (Waddington *et al.*, 1933a,b, 1935, 1936a,b; Needham *et al.*, 1934a,b; Waddington and Needham, 1935) concluding that the inducer is probably a definite chemical substance, "probably of a lipoidal nature", soluble in petrol-ether, and that compounds of a 'steroidal nature' can act as inducers. While the search for a chemical inducer may seem obvious to us today, in the early 1930's it represented a radical departure from the more vitalist ideas of Spemann.

On his return to England, Waddington attempts to repeat these experiments in chick embryos, by implanting organisers that had been killed by boiling for 30 seconds (Waddington, 1933b, 1934b). He noticed that the graft often became enveloped by host cells and that it often induces a notochord as well as neural structures, as well as that the pattern of the induced structures appeared to be determined by the host. Unfortunately, the possible problem that the inducing influences might emanate from some of the invading cells is not directly acknowledged either here (Waddington, 1933b) or in his many subsequent papers on this topic.

There follow several more publications on induction in the chick. In 1933 he reports that the mesodermal parts of the primitive streak, the "head process and sinus rhomboidalis" and the neural plate can all induce an axis in the host, while the notochord cannot (Waddington, 1933c). He comments that the orientation of the induced axis is controlled at least in part by the graft, suggesting that the early primitive streak already has polarity. Waddington and Schmidt (1933) continue these experiments using duck/chick combinations and even attempt to film some of the grafts in time-lapse, in collaboration with Dr. R.G. Canti at St. Bartholomew's Hospital in London (who had previously aided Honor Fell in producing films of the growth of joint cartilages *in vitro*). The experiments, and particularly the visualisation of cell movements



Fig. 1. Conrad H. Waddington. Reproduced from Robertson (1977).

associated with the graft, led them to the conclusion that the orientation of the induced neural tube is dependent on both graft and host. In addition, they argue that there are quantitative differences in inducing power along the axis of the primitive streak: "The node, I would suggest, is to be regarded merely as the most active part of the definitive primitive streak, differing in its developmental functions from the rest of the streak only in a quantitative and not in a qualitative manner" (p. 547). This conclusion angered some of Waddington's colleagues, particularly in America, and led to a long debate about the uniqueness of Hensen's node as the avian organiser, to which we shall return later.

#### *Induction by the mammalian organiser*

Waddington devoted a considerable amount of effort to an experimental demonstration that mammalian embryos contain an organiser, and that this too is located in the primitive streak. He followed a similar course as he had previously done with avian embryos. First, he extended the use of Honor Fell's plasma clot culture technique to rabbit embryos (Waddington and Waterman, 1933), reporting that these can be cultured successfully from the full primitive streak stage for about one day. The following year (Waddington, 1934c), he succeeds in obtaining inductions by the

chick primitive streak grafted into rabbit embryos in culture, and concludes (p. 226) that “the ectoderm of the rabbit at this stage ... is ‘competent’ to form neural plate..., and thus make it probable that the embryonic development of the mammals is influenced by factors similar to those which have become familiar in the Amphibia and birds. They are also a demonstration of the lack of species specificity of the inducing agents involved in embryonic organisers.”

Despite several attempts, however, Waddington did not succeed in obtaining inductions by rabbit tissue until 1936, a finding first published as a brief and rather unconvincing report in *Nature* (Waddington, 1936a). By the following year, he had conducted numerous experiments and felt ready to summarise all of his mammalian experiments in a single, landmark paper (Waddington, 1937a). This contains reports of rabbit-rabbit, rabbit-chick and chick-rabbit organiser grafts made between 1932 and 1935, as well as isolation experiments of portions of the rabbit embryo (discussed in the next section). He finds that grafts of portions of the rabbit primitive streak into chick hosts give an ectopic neural plate. He had less success with rabbit-to-rabbit grafts, mostly because of uncertainties about whether the neural tissue came from graft or host. In addition, “No trace of inducing action has been found when the grafts were made into the extra-embryonic trophoblastic area, and it may be that in the Rabbit this region is not competent to form neural tissue, unlike the extra-embryonic parts of the Chick blastoderm (Waddington, 1934a)” (Waddington, 1937a, p. 285).

#### **Regulation after ablation of Hensen’s node, and a debate about the uniqueness of the node as the organiser**

Waddington’s conclusion that organiser activity was not unique to Hensen’s node but also extended into the anterior half of the primitive streak was further strengthened by his finding that embryos from which this region had been ablated nevertheless continue to develop perfectly if the operation is conducted early enough. Together, these observations led him to assert that organiser activity is not unique to Hensen’s node but is rather more widely distributed in the embryo. This fuelled a heated debate between Waddington and other contemporary avian embryologists (notably Hunt, Rawles and Willier in the USA and Dantschakoff and Wetzel in Germany), who maintained that Hensen’s node is rather special although they also acknowledged that its boundaries are difficult to define.

As early as 1930, Waddington already reported that removal of the entire primitive streak “including the primitive pit and Hensen’s knot” is followed by regeneration (Waddington, 1930, 1932). This finding was confirmed in three subsequent papers (Waddington, 1935; Waddington and Cohen, 1936; Abercrombie and Waddington, 1937). He reports that when Hensen’s node or the anterior primitive streak is ablated, this is followed by regulation provided that the injury is made “only before the onset of differentiation indicated by the appearance of the head process” (Waddington and Cohen, 1936; p. 225). The following year, Waddington found that rabbit embryos are also capable of extensive regulation: the posterior part of an embryonic shield, not including Hensen’s node, can develop into part of an embryo with neural tube and somites (Waddington, 1937a).

Waddington’s conclusion that Hensen’s node is not the only region of the early embryo endowed with organiser properties conflicted with those reached by other contemporary authors (see

above), who were reporting special properties associated with the node. With hindsight, it is relatively easy to find the origin of the confusion. Waddington’s fate maps of the embryo at this stage show a rather smaller prospective neural plate than other contemporary maps (see for example, Waddington, 1952 –Fig. 10 on pp. 30-31). Therefore in many of his experiments, Waddington’s grafts were placed within the territory of the prospective neural plate, and the results he obtained are consistent with a *recruitment* of these prospective neural plate cells into a separate neural tube.

Waddington had the elements to distinguish between induction and recruitment. He had described a tendency of grafted pieces of primitive streak to generate new cell movements in the host (e.g. in Waddington and Schmidt, 1933), and had already tried to make a distinction between ‘induction’ and ‘organisation’ by his introduction of the concepts of ‘evocation’ and ‘individuation’ (see the section on ‘concepts’ below). His 1937 paper on determination in the rabbit embryo also acknowledged the importance of assessing a change of fate: “Grafts which are made into the embryonic area may very easily lie under the host’s neural plate, since, at least in the anterior part of the embryonic shield, the neural plate is very wide and covers the greater part of the shield.” (Waddington, 1937a). And although in 1934 he describes that even the extraembryonic ectoderm of the chick can form a nervous system in response to grafts, and that only the anterior tip of the streak is effective in this region (Waddington, 1934a), he never adopts this as a test for induction. It is therefore particularly curious that, even armed with this extensive knowledge, Waddington continues to insist that the node is not endowed with unique properties, as late as his 1952 (*‘Epigenetics of Birds’*) and 1956 (*‘Principles of Development’*) books.

#### **Hensen’s node and floor plate development**

Waddington’s node and primitive streak ablation experiments also led him to propose that there is a close lineage relationship between the notochord and the floor plate of the neural tube particularly for caudal regions of the embryonic axis. In embryos deprived of the node after the emergence of the head process, “comparatively small injuries to the primitive pit will cause the floor of the neural groove to be absent over a considerable length of the embryo, extending well into the region of the somites. This is an expression of the fact that the presumptive material for the floor of the tube is concentrated anteriorly in the primitive streak stage and moves back later to its final position. ... In embryos which are split through the lack of the floor material, the sides of the neural tube become united to the endoderm below. The notochord is usually missing in the region of the split, though it may be present in more posterior regions. This would seem to indicate that the presumptive notochord material is located very near the presumptive floor material and tends to be removed with it.” (Waddington, 1936; p. 224). This result is consistent with the observations of Spemann and his colleagues in amphibians a decade earlier, who had noted that organiser grafts are often accompanied by a cellular contribution of the graft not only to the notochord but also to the floor of the nervous system.

#### **Induction of mesoderm from ectoderm**

In 1937, Waddington and Taylor attempted to extend a finding first made by Otto Mangold, that when ectoderm is placed in the proximity of the blastopore, the grafted ectoderm acquires meso-

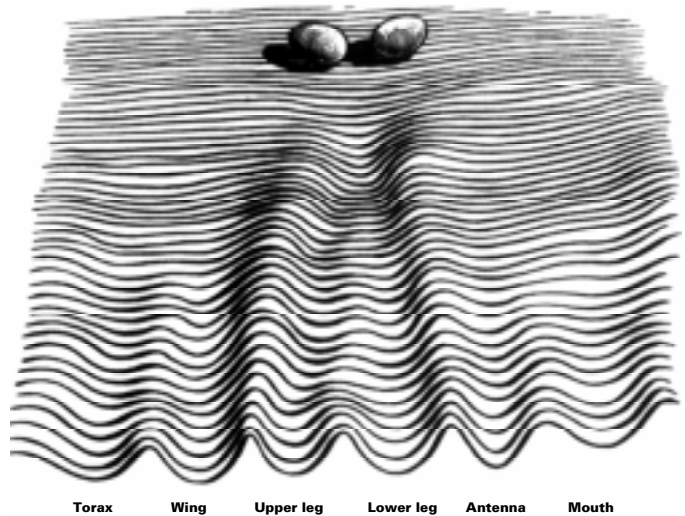
dermal fate. Waddington and Taylor perform the equivalent grafts in the chick and reach the same conclusion. However, they are not able to distinguish graft from host, and more convincing experiments were being done simultaneously by Michael Abercrombie that also supported this view. Even though this topic was among his many interests, Waddington clearly does not deserve credit for having discovered mesoderm induction. A convincing demonstration of this cell interaction had to await the more definitive experiments of Nieuwkoop in the late 1960s.

**Later development: placodes, left-right asymmetry, the pronephros**

Waddington's embryological work was not confined to the earliest stages of development; his interests also extended to many different aspects of organogenesis, although he never studied these problems in depth. In some cases, his contribution was limited to a brief mention of an interesting observation derived from an experiment designed for a different purpose. One of the first papers to be devoted specifically to the study of later events is his 1936 study into head morphogenesis. Here, Waddington and Cohen (1936) report that following damage to the midline, paired head rudiments can develop separately, with two hearts that are mirror images of one another. They also report that the young forebrain has a high capacity to regulate, and that nasal, optic and otic placodes can form from non-presumptive placodal epidermis (Waddington and Cohen, 1936). The following year Waddington (1937c) performs many careful extirpation and recombination experiments to determine the time at which the induction of the otic vesicle occurs, and which tissues emit inducing signals. He concludes that both the neural tube and the surrounding mesenchyme may play a role, and that determination begins quite some time before a visible placodal thickening becomes apparent. But once again these were not entirely new concepts and even Spemann himself had devoted much of his early work at the turn of the century to the problem of placode induction.

The finding that mirror-image hearts can occur following damage to the midline of the embryo (Waddington and Cohen, 1936) led Waddington further to address questions about how left-right asymmetry may be established. He had observed that the heart and the head of the embryo always turn in a reproducible direction, which begs the question of whether there is a causal connection between these events. To address this, he removes the heart (the first of the two structures to develop morphological asymmetry), and finds that head turning is abolished. As a result he proposes a connection between the two. However, he also noticed that the normal flexure arising between forebrain and midbrain still appeared in these operated embryos, and therefore proposed that this flexure is independent of the heart (Waddington, 1937b).

As a final example of Waddington's interest in organogenesis, he became curious about the possible function of the pronephros, an organ he considered 'vestigial' because of its apparent lack of function in higher vertebrates. Extirpation, injury and transplant experiments lead him to conclude (Waddington, 1938) that the pronephros is determined just before the early neural plate stage at the level opposite any given somite and thereafter develops independently of the somite or lateral plate mesoderm, that the Wolffian duct grows backwards by multiplication of its own cells, and that when cut, it ceases to elongate posterior to the cut. In the absence of the Wolffian duct, the mesonephros fails to develop properly, and he therefore proposes an inductive role for the duct:



**Fig. 2. Two visual representations of 'epigenetic landscapes'.** (A) "A symbolic representation of the developmental potentialities of a genotype in terms of a surface, sloping towards the observer, down which there run balls each of which has a bias corresponding to the particular initial conditions in some part of the newly fertilised egg. The sloping surface is grooved, and the balls will run into one or other of these channels, finishing at a point corresponding to some typical organ". Figure and legend from Waddington, 1956; p. 351. (B) Epigenetic landscapes depicted as branching valleys (from Waddington, 1940, reproduced with kind permission of Cambridge University Press).

"The Wolffian duct may therefore be said to induce the mesonephros, which, however, has a feeble power of self-differentiation as a double assurance" ... "This provides confirmation of previous

suggestions that an organ which is vestigial in the sense that it has lost its physiological function may be retained in ontogeny because it fulfils the morphogenetic function of providing a stimulus essential for the development of other, physiologically more important, structures.”

It is probably fair to say that although Waddington did show an interest in these later morphogenetic events, this was mostly in the context of a search for further interactions between tissues at different stages of development - an attempt to extend the organiser concept. This is intimately connected with his quest for a universal model mentioned in the Introduction; in this model, at least some of the branch points in the epigenetic landscape correspond to inductive interactions that help cells decide between one path and another (see Fig. 2).

## Some concepts and their origin

### Competence

The introduction of the English word into Embryology is due to Waddington, and arises in his 1932 paper: “In this middle period it [the ectoderm] is *competent* to form neural plate ... The idea of competence covers two concepts already in use, *Reaktionsfähig* and *labil determiniert*. *Reaktionsfähig* means that the tissue to which the adjective is applied is competent to differentiate to a certain other tissue but requires a definite stimulus, while *labil determiniert* means that it is competent so to differentiate without a definite stimulus.” It is not totally clear why Waddington chooses to introduce a new term to cover two different concepts. A more accurate translation of the German word (‘able to react’) is closer to what we now interpret as ‘competence’. By contrast, *labil determiniert* (‘determined but labile’) is a concept that is still used in its original sense and as interpreted by Waddington – tissue that does not require an inductive stimulus to continue to develop in its determined direction, but can still be redirected to a new fate with an appropriate stimulus.

By 1940, idea of competence changes to become equal to the German term and this time he separates the two concepts. In *Organisers and genes* he writes: “The notion of competence implies an ability to react; it is similar to ... *Reaktionsfähigkeit*. ... It is important to compare the idea of competence with that of potency. A potency implies an ability to perform an action. ... we can say that a piece of tissue has a potency to develop into something if, in the course of time, it does become that thing. ... On the other hand, ... when a tissue is competent, it can carry out certain reactions which it cannot perform at any other time, namely reactions with the appropriate organiser”. The difference is subtle, but it is made. Therefore in this later definition he uses ‘potency’ as an alternative to ‘fate’, and lability is not an essential part of this concept. But the source of the problem appears to reside in Waddington’s use of the word ‘appropriate’ adjacent to organiser. He views competence, or the ability to react to a signal from the organiser, only in the context of what the tissue will normally do when exposed to such a signal. He does not apply it to a different tissue whose fate is not to see this signal but which can nevertheless respond to it by altering its developmental course and follow a new direction. Despite this, his paper on competence for amphibian lens formation (Waddington, 1936b) contains this: “a competent tissue should be thought of as an unstable system which has *two or more ways* of change open to it, the decision as to which way

it actually follows being taken by the relevant organiser” (p. 86). It appears, therefore, that although Waddington attempted to define ‘competence’ fairly precisely, his use of the word tended to break from the confines of his own definition.

### Evocation and individuation

Waddington was probably the first to attempt to separate explicitly the two major properties of the organiser: its inducing ability (‘change of fate of the responding tissue’) and its capacity to generate a coherent, patterned axis that includes its own as well as surrounding cells. To distinguish these two aspects of organiser activity, he coined the words ‘evocation’ for the former and ‘individuation’ for the latter. However, as we shall see below, the precise meaning of these words kept changing among several of his papers.

Curiously, the two words are first used in different papers. ‘Evocation’ is first defined by Needham *et al.* (1934a), who use it as a synonym of ‘induction’ – contact with an inducing stimulus has caused a new structure to be formed, ideally involving a change of fate. ‘Individuation’ is first used by Waddington and Schmidt (1933), to indicate the formation of a morphologically organised structure accompanying the induction: “an organiser tends to rearrange the regional structure both of itself and of any tissue lying near it in such a way as to make that tissue part of a complete embryo. This is then a true unitbuilding activity of the organiser, which works, not by controlling the process of induction, but by modifying the products of that process” (Waddington and Schmidt, 1933; p. 550). But the difference is not indicated with sufficient precision: “the production of an embryonic axis as such, which is called Evocation; and the determination of the regional, e.g., antero-posterior, character of that axis, which is called Individuation.” The use of ‘embryonic axis’ implies some degree of organisation beyond a mere change of fate.

A subtle change of meaning becomes apparent in his later publications. For example in *Epigenetics of Birds* (1952; p. 113) he states: “evocation” ... is the part of the induction process that can be brought about by a stimulus which has itself no organisation to impart, but simply pulls a trigger and leaves the rest to the reacting partner”. Therefore, the distinction that Waddington wishes to make here is between which tissue is the source of the regionalising information: if it is the inducing tissue, then this is an individuation, while if the responding tissue is entirely responsible for its own organisation once its fate has been redirected, then it is an evocation. Where this distinction is particularly useful is in a molecular dissection of the regionalisation process, but unfortunately Waddington never turned his attention to this problem which was one of the foci of interest of his German colleagues in the 1930s.

In fact, Waddington muddied the waters even further by stating that ‘individuation’ is a synonym of morphogenesis (Waddington, 1956, p. 12). But he recognised some of the weaknesses of his own definition: “crudely speaking, in fact, individuation is the process of formation of an organ, and it necessarily suffers from such degree of vagueness as attaches to the concept of an organ: to pursue the definition further carries us into more or less philosophical realms.” (Waddington, 1952, p. 113). And here again, Waddington fails to recognise the importance of a change of fate as part of the inductive process, or to differentiate sufficiently clearly between his own two concepts: “Individuation obviously involves something else be-

sides physical effects on the anatomy of the tissues, since we have seen that changes in presumptive fate may occur, and tissues be produced which would not have occurred in a straightforward self-differentiation" (Waddington, 1952, p. 126). And it is this that earned him the criticism of his contemporaries such as Holtfreter, who pronounced that "these terms confuse rather than clarify the issue" (Holtfreter, 1951).

### 'Epigenetics'

The word 'epigenetics' was coined by Waddington as an English equivalent of *Entwicklungsmechanik* –the branch of science that undertakes a causal analysis of embryonic development by experimental means. The reasons for this appear in his textbook *Principles of Embryology* (1956; p. 10): "epigenetics" ... is derived from the Greek word epigenesis, which Aristotle used for the theory that development is brought about through a series of causal interactions between the various parts; it also reminds us that genetic factors are among the most important determinants of development." The Greek prefix means 'above', and therefore epigenetics might be translated as 'above genetics', or 'above the genes'. Were it not in current use in other contexts, it might now be interpreted as 'mechanisms that control gene expression', and could therefore be appropriate as a description of some developmental mechanisms. But its parent term in Waddington's etymology, epigenesis, has other connotations –reminiscent of the heated debate between two schools of thought on models for development ('preformation' and 'epigenesis') throughout the 17<sup>th</sup> and 18<sup>th</sup> centuries. The original German term, *Entwicklungsmechanik*, is now usually translated as 'experimental embryology', which, despite Waddington's accusation of this as an awkward term, is certainly less ambiguous than 'epigenetics'. Waddington probably remains as the only user of 'epigenetics' in the sense in which he described it, and used it even as a title of one of his books (*Epigenetics of birds*, 1952), whose frontispiece contains the definition: "'Epigenetics'. The science concerned with the causal analysis of development".

### Canalisation and the 'epigenetic landscape'

Finally we should consider the terms that were at the centre of Waddington's first attempt at a comprehensive model of development: epigenetic landscapes, and their visual representations as a series of branching valleys as depicted in Waddington (1940) (Fig. 2B), or as a sloping surface whose undulations dictate the path taken by a ball (Waddington, 1956, p. 351) (Fig. 2A). Waddington first came up with the idea explicitly as a way to explain development as a succession of 'evocations' and 'competencies': "The whole progress of development may therefore be considered as resulting from an unstable configuration of substances which leads the embryonic tissue to change towards a more stable state; and the periods of competence are secondary instabilities when there are two or more alternative modes of progression towards stability, the choice between them depending on outside conditions (the presence or absence of the appropriate organiser)." (Waddington, 1940, p. 45). This concept easily accommodates the idea of 'negative' inductions which have received much attention in amphibian development recently. In Waddington's own words: "... in anurans the ectoderm has an inherent tendency to form suckers, which has to be restrained in the non-sucker region by an inductive influence of the mesoderm. This might be called a negative induction."

The concept of epigenetic landscapes is closely allied to another that had been proposed by Waddington some years earlier –'canalisation': "The main thesis is that developmental reactions, *as they occur in organisms submitted to natural selection*, are in general canalised. That is to say, they are adjusted so as to bring about one definite end-result regardless of minor variations in conditions during the course of the reaction". (Waddington, 1942; p. 563. His italics). But the concept stems from his earlier work on competence, and its germ can be found as early as 1934: "Translating this into material terms of a material model, it might be supposed that during the period of competence to form X, there is present in the competent tissue a certain constellation of chemical substances or processes which are in unstable equilibrium, so that the addition of small quantities of other substances (the organiser substances) can cause the whole equilibrium to break down, and the system to alter progressively in a certain direction towards some new equilibrium" (Waddington, 1934a).

### Epilogue

It should be clear from this brief summary that Waddington's contributions to the embryology of avian and mammalian embryos are formidable, particularly for such a short period of his working life. And even during these 10 years his work was not confined to avian and mammalian embryos. It includes numerous papers on various amphibian species, and by 1939-1940 he was starting to turn his attention to *Drosophila* in earnest –a system he found appealing because of its genetics, as another way to get to a unifying theory of development. Just like his 1-page 1930 paper represents a preview of what he would publish over the following 10 years, his 1940 book 'Organisers and genes' tries to present a unifying hypothesis (the 'epigenetic landscapes') by using examples from both genetics and experimental embryology and it is this that will guide his work and thought over the following decade. That his second working decade was considerably less productive was largely due to the onset of the War, from which he emerged with greatly expanded interests, venturing deeply into Evolution, Theoretical Biology, Philosophy and Ethics among many other topics.

Reading through Waddington's scientific output in chronological order reveals many aspects of his style of experimentation, thinking and writing that are not immediately apparent from isolated papers. Most of his primary papers retain the identifying number of each embryo from which it is possible almost to reconstruct his lab notebooks and to unwrap Waddington's active mind. For example, volume 14 of the *Journal of Experimental Biology* (1937) contains four of his papers on very diverse topics, reporting on the results of various manipulations performed in 1935-36. The order in which he performed these is particularly revealing; he might alternate repeatedly between one or two grafts of primitive streak for his collaboration with Abercrombie, an experiment on heart removal to examine its effects on head turning, another to study induction of the ear placode, and yet another to investigate whether the primitive streak can induce mesoderm from ectoderm.

Waddington is often sole author of his papers, and in collaborative studies he is often the dominating force. He appears to have allowed only a few of his collaborators (most notably Joseph Needham and Michael Abercrombie) to contribute intellectually (for example, see Waddington and Schmidt, 1933, where although

Schmidt performed several of the experiments, Waddington claims sole responsibility for the writing, which is also perfectly obvious from the style). In his collaborations with others it is possible not only to discern which of the authors is responsible for which embryo (for examples, see Waddington and Schmidt 1933 and Abercrombie and Waddington, 1937), but also to identify who designed the experiments and who wrote different parts of the paper. The Abercrombie and Waddington (1937) paper provides an excellent example of Abercrombie's experimental and analytical exactitude but is thin on generalisations, while Waddington and Schmidt (1933) is clearly part of Waddington's personal quest for a grand scheme to understand induction and development as a whole.

Robertson (1977) criticises Waddington because "some of his concepts turn out under use to be rather fuzzy at the edges" (p. 613) and our summary contains several examples that justify the accusation. Some of the concepts are indeed woolly even under Waddington's own use. But there is no denying that he was a remarkable man. Not only was he prolific, broad and scholarly, but he was a great instigator of thought and criticism in others. He influenced everyone with whom he came into contact as well as many, including myself, who never had the opportunity to meet him. Most of his lucid questions still remain unanswered more than half a century later and it will continue to be profitable to revisit his writings for a long time to come. Waddington ends his *Principles of Embryology* (1956), with a passage from T.S. Eliot's *Little Gidding*:

"And to make an end is to make a beginning.  
The end is where we start from.  
\* \* \* \* \*

We shall not cease from exploration  
And the end of all our exploring  
Will be to arrive where we started  
And know the place for the first time."

#### Acknowledgements

I am particularly indebted to Jim Smith for sending me a copy of Alan Robertson's (1977) superb *Biographical Memoir of Waddington* which greatly helped to find references to Waddington's more obscure publications as well as an excellent source of biographical information. I am also grateful to Andrea Streit for constructive comments on the manuscript and to Tokindo Okada and Lewis Wolpert for interesting stories on Wad the man, told among heterodox surroundings at University College London.

#### References

- ABERCROMBIE, M. and WADDINGTON, C.H. (1937). The behaviour of grafts of the primitive streak beneath the primitive streak of the chick. *J. Exp. Biol.* 14: 319-334.
- HOLTFRETER, J. (1951). Some aspects of embryonic induction. *Growth* 15 (Suppl.): 117-148.
- NEEDHAM, J., WADDINGTON, C.H. and NEEDHAM, D.M. (1934a). Physico-chemical experiments on the amphibian organizer. *Proc. R. Soc. Lond. (Biol.)* 114: 393-422.
- NEEDHAM, J., WADDINGTON, C.H. and NEEDHAM, D.M. (1934b). Physico-chemical experiments on the amphibian organizer. *Arch. Exp. Zellforsch.* 15: 307-309.
- ROBERTSON, A. (1977). Conrad Hal Waddington (1905-1977). *Biogr. Mem. Fell. Roy. Soc.* 23: 575-622.
- WADDINGTON, C.H. (1929a). Notes on graphical methods of recording the dimensions of ammonites. *Geol. Mag.* 66: 180-186.
- WADDINGTON, C.H. (1929b). Pollen germination in stocks and the possibility of applying lethal factor hypothesis to the interpretation of their breeding. *J. Genet.* 21: 193-206.
- WADDINGTON, C.H. (1930). Developmental mechanics of chick and duck embryos. *Nature* 125: 924-925.
- WADDINGTON, C.H. (1932). Experiments on the development of chick and duck embryos cultivated *in vitro*. *Philos. Trans. R. Soc. Lond. (Biol.)* 221: 179-230.
- WADDINGTON, C.H. (1933a). Induction by the endoderm in birds. *W. Roux Arch. EntwMech. Org.* 128: 502-521.
- WADDINGTON, C.H. (1933b). Induction by coagulated organisers in the chick embryo. *Nature* 131: 275-276.
- WADDINGTON, C.H. (1933c). Induction by the primitive streak and its derivatives in the chick. *J. Exp. Biol.* 10: 38-46.
- WADDINGTON, C.H. (1934a). Experiments on embryonic induction. I. The competence of the extra-embryonic ectoderm in the chick. *J. Exp. Biol.* 11: 211-217.
- WADDINGTON, C.H. (1934b). Experiments on embryonic induction. II. Experiments on coagulated organisers in the chick. *J. Exp. Biol.* 11: 218-223.
- WADDINGTON, C.H. (1934c). Experiments on embryonic induction III. A note on inductions by the chick primitive streak transplanted to the rabbit embryo. *J. Exp. Biol.* 11: 224-226.
- WADDINGTON, C.H. (1935). The development of isolated parts of the chick blastoderm. *J. Exp. Zool.* 71: 273-292.
- WADDINGTON, C.H. (1936a). Organisers in mammalian development. *Nature* 138: 125.
- WADDINGTON, C.H. (1936b). The origin of competence for lens formation in the amphibia. *J. Exp. Biol.* 13: 86-91.
- WADDINGTON, C.H. (1937a). Experiments on determination in the rabbit embryo. *Arch. Biol.* 48: 273-290.
- WADDINGTON, C.H. (1937b). The dependence of head curvature on the development of the heart in the chick embryo. *J. Exp. Biol.* 14: 229-231.
- WADDINGTON, C.H. (1937c). The determination of the auditory placode in the chick. *J. Exp. Biol.* 14: 232-239.
- WADDINGTON, C.H. (1938). The morphogenetic function of a vestigial organ in the chick. *J. Exp. Biol.* 15: 371-376.
- WADDINGTON, C.H. (1940). *Organisers and genes*. Cambridge University Press.
- WADDINGTON, C.H. (1942). Canalization of development and the inheritance of acquired characters. *Nature* 150: 563-565.
- WADDINGTON, C.H. (1952). *The epigenetics of birds*. Cambridge University Press.
- WADDINGTON, C.H. (1956). *Principles of development*. London: Allen and Unwin.
- WADDINGTON, C.H. and COHEN, A. (1936). Experiments on the development of the head of the chick embryo. *J. Exp. Biol.* 13: 219-236.
- WADDINGTON, C.H. and NEEDHAM, D.M. (1935). Studies on the nature of the amphibian organisation centre. II. Induction by synthetic polycyclic hydrocarbons. *Proc. R. Soc. Lond. (Biol.)* 117: 310-317.
- WADDINGTON, C.H. and SCHMIDT, G.A. (1933). Induction by heteroplastic grafts of the primitive streak in birds. *W. Roux Arch. Entwicklungsmech. Org.* 128: 522-563.
- WADDINGTON, C.H. and TAYLOR, J. (1937). Conversion of presumptive ectoderm to mesoderm in the chick. *J. Exp. Biol.* 14: 335-339.
- WADDINGTON, C.H. and WATERMAN, A.J. (1933). The development *in vitro* of young rabbit embryos. *J. Anat.* 67: 355-370.
- WADDINGTON, C.H., NEEDHAM, J. and BRACHET, J. (1936a). Studies on the nature of the amphibian organisation centre. III. The activation of the evocator. *Proc. R. Soc. Lond. (Biol.)* 120: 173-198.
- WADDINGTON, C.H., NEEDHAM, J. and NEEDHAM, D.M. (1933a). Physico-chemical experiments on the amphibian organizer. *Nature* 132: 239.
- WADDINGTON, C.H., NEEDHAM, J. and NEEDHAM, D.M. (1933b). Beobachtungen über die physikalisch-chemische Natur des Organisers. *Naturwissenschaften* 21: 771-772.
- WADDINGTON, C.H., NEEDHAM, J., NOWINSKI, W.W. and LEMBERG, R. (1935). Studies on the nature of the amphibian organisation centre. I. Chemical properties of the evocator. *Proc. R. Soc. Lond. (Biol.)* 117: 289-309.
- WADDINGTON, C.H., NEEDHAM, J., NOWINSKI, W.W., LEMBERG, R. and COHEN, A. (1936b). Studies on the nature of the amphibian organisation centre. IV. Further experiments on the chemistry of the evocator. *Proc. R. Soc. Lond. (Biol.)* 120, 198-207.