Memorial/Obituary

Lewis Wolpert (1929 – 2021)

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1.Summary

Lewis Wolpert was a brilliant and inspiring scientist who made hugely significant contributions which underpin and influence our understanding of developmental biology today. He spent his career interested in how the fertilised egg can give rise to the whole embryo (and ultimately the adult) with one head, two arms, two legs, all its organs and importantly how cells become different from each other and how they 'know' what to become. His ideas revolutionised the way developmental biology was perceived and also reinvigorated, in particular, the key question of how pattern formation in embryonic development is achieved. He published over 200 scientific articles and received many accolades over his career for his work and services to science in the UK. These included a CBE (Commander of the Order of the British Empire) from the Queen, being elected a Fellow of the Royal Society and a Fellow of the Royal Society of Literature. He was also a recipient of the Waddington Medal from the British Society for Developmental Biology and was awarded The Royal Society's top honour, the Royal Medal in 2018. Lewis was also a gifted teacher and communicator, including being the author of a textbook on developmental biology used around the world to train the next generation of developmental biologists. This contribution was recognised in 2003, by the award of the Viktor Hamburger Outstanding Educator Award from the Society of Developmental Biology in the USA. Lewis always enjoyed giving talks and lectures, having an infectious and persuasive enthusiasm coupled with a sharp sense of humour (Figure 1). He also published articles in popular science journals (aimed at the public) such as New Scientist, Scientific American and The Scientist. Lewis also wrote several popular science books. He was a passionate advocate for the public understanding of science and was the Chair of The Royal Society/Royal Institution/British Association for the Advancement of Science Committee for Public Understanding of Science (1994-1998). For this contribution he was awarded The Royal Society Michael Faraday Medal for "excellence in communicating science to UK audiences". He presented the prestigious Royal Institution Christmas Lectures in 1986 entitled 'Frankenstein's Quest: development of life'. These lectures, six in total, are presented by leading scientists and aimed at the general public and broadcast on national television. On a personal level, Lewis influenced all who came into contact with him, shaped his students and postdocs careers and instilled in them, and the community as whole, a life-long love of developmental biology.

1.1 His early career

Lewis was born in Johannesburg, South Africa in 1929. As a young man he studied civil engineering as he wanted to do science of some sort, but he was unsure at the time. He also got involved with politics even meeting and helping Nelson Mandela in the early 1950s. He came to the UK in 1954 and studied soil mechanics at Imperial College London before realising his calling was cell and developmental biology. He carried out his PhD at Kings College London with Dr James Danielli, a biophysicist, and studied the mechanics of cell division and measured the mechanical forces used in cell division in sea urchin embryos [1]. To continue his research on sea urchin embryos he would travel to Sweden most Summers in order to have access to the embryos and published widely on their development [2, 3]. In 1966 he took up the position of Chair of Biology as Applied to Medicine at the Middlesex Hospital Medical School (now part of University College London) and initially studied regeneration in the freshwater invertebrate *Hydra* [4]. He was also interested in the basis of polarity of the *Hydra*, that is, how does the *Hydra* know its head from its tail [5] (also see

Section 1.2). He soon moved into the developing chick limb as a model system to study development, because he felt the developing limb was more appropriate at a Medical School [6].

1.2 His scientific contributions and the concept of Positional Information

Wolperts studies on early development of the sea urchin contributed to him coining the famous quote '*it is not birth, marriage or death, but gastrulation which is truly the most important time of your life*' celebrating the essential and wondrous event that occurs in all early vertebrate embryos that converts a mass of cells into germ layers that gives rise to all the organs and tissues of the body. Wolpert is equally famous for his concept of Positional Information also known as the French Flag problem [7, 8].

Wolpert chose *Hydra* to work on originally because it regenerates and regulates following tissue loss, so enabled him to investigate the specification of the spatial organisation of an embryo in a simple organism. Lewis, admittedly, was not very practical in the lab, he was a theoretician, and he worked with his technician Amata Hornbruch for a large part of his career and who was 'his hands' in the lab [9]. Lewis also hired gifted and talented students and postdocs who carried out experiments and creatively discussed/debated his ideas (for a list of Wolpert Lab Staff, see Vargesson [10]). In the *Hydra* he demonstrated that the head was formed by the creation of a diffusible inhibitory gradient, that prevented the head forming in the incorrect place [4, 5, 11]. He also showed that a second gradient was present to determine where the head would form [4]. Wolpert was also influenced by work from Hans Driesch, who separated the two-cell stage sea urchin embryo into single cells and found each made complete embryos but were half the normal size, and which indicated the cells had an idea of position and spatial awareness [12]. Together, his work on *Hydra* and the work by Driesch provided the basis for his concept of Positional Information by devising the "French Flag Problem" [7]. This is where Wolpert realised that the embryo was behaving like a flag, where the pattern remains the same irrespective of the size of the embryo. The "problem" was how does a line of cells, then create three different colours or patterns to produce the French flag? [7]. He proposed that a concentration gradient of a signalling molecule or morphogen, or through cells counting cell divisions, could provide positional information so that cells acquire different positional values depending on their position [8]. Cells then interpret their positional values according to their developmental history and behave appropriately to produce specific cell types and patterns [8]. He proposed this model could account for the patterns being the same for 'flags' (tissues/embryos) of different sizes [8] (for further detail on the origins of the Positional Information concept see Vargesson [10]).

This incredibly simple concept explained how a group of homogenous cells in a tissue can all become different from another and produce different patterns. When Wolpert first proposed the concept of Positional Information to explain pattern formation, it was controversial and was disliked by many of his peers. However, support from Sydney Brenner and Francis Crick encouraged him to publish and it changed and inspired the field [6, 9]. In 2019, his concept celebrated its 50^{th} anniversary since publication and continues to be highly cited, remains a central concept in all the major developmental biology textbooks, created a framework for understanding embryonic development and has influenced multiple generations of students and scientists, which include me, to become developmental biologists [10].

1.2.1 The concept of Positional Information and chick limb development

Wolpert also proposed that Positional Information acts ‘universally’, that is it acts in multiple developmental systems including early embryonic development of sea urchins, amphibians, Hydra, insects, regenerating salamander limbs and the early chick limb [8]. Around this time, he was influenced by the experimental embryologist, John Saunders jr, who had discovered the Zone of Polarising Activity in the chick limb and its ability to duplicate digits, as well as demonstrated the role of the Apical Ectodermal Ridge was to control limb outgrowth in a proximal to distal manner (ie: the humerus forms first, then the radius/ulna and finally the digits) [13, 14, 15]. Wolpert realised from Saunders work that he could study his concept of Positional Information in the chick limb and he and his talented students and postdocs then set about using the developing chick limb as a primary model. The list of people who went through his lab is an amazing legacy and many of whom are leaders in their fields today, underlining the influence he still has on the field, for example some of the students he supervised included Jim Smith, Dennis Summerbell, Nigel Holder, Michael K. Richardson and some of his postdocs included Cheryll Tickle, Jonathan Slack, Julian Lewis, Philippa Frances-West. For a detailed overview of the Wolpert Lab Family Tree, please see Vargesson [10].

Wolpert proposed that in the developing limb Positional Information was specified with respect to a three dimensional coordinate system. Cells needed to be informed of their position in relation to the three main axes of the limb. This was a radically different way of thinking about limb development. He proposed two models. One, the morphogen gradient model, where positional values across the antero-posterior axis (thumb to little finger) were specified by a gradient of a long-range signalling molecule produced by the zone of polarising activity in the posterior-distal margin of the limb bud [16]. Today we now know that molecule is Sonic Hedgehog [17]. In contrast, he proposed another model, the Progress Zone model to explain how positional values along the proximo-distal axis of the limb are specified by a timing mechanism that operates in a Progress Zone model. The Progress Zone is a region of undifferentiated mesoderm cells beneath the apical ectodermal ridge, the thickened rim of ectoderm required for limb bud outgrowth. Depending on how long cells remain in the Progress Zone determines their positional value. Cells that fall out early, become proximal limb elements, whereas cells that remain in the Progress Zone the longest ended up as the digits [18]. His group also showed that when early limbs were X-irradiated this resulted in thalidomide-like phocomelia – the loss of proximal long bones [19]. This could be interpreted in terms of the Progress Zone model as the irradiation kills cells and because the remaining cells stayed in the Progress Zone for longer in order to repopulate the Progress Zone, distal structures develop, at the expense of proximal ones.

Both these models stimulated research in the field as well as many challenges and Lewis always enjoyed debates and controversies with other scientists, but his ideas moved the field forward.

Wolpert was also interested in understanding the basis of ‘handedness’ or left-right asymmetry and establishment of symmetry. Using the chicken embryo he proposed that a molecule displaying asymmetric expression could explain left-right asymmetry differences in embryos, a question still at the forefront of developmental biology today [20].

In the 1990s, the Wolpert Lab had several students and postdocs looking for molecular cues that underpin Positional Information (Figure 2). It was an exciting time as molecular biology and genetic misexpression strategies were taking over science, although still primitive compared to today. In addition, the Wolpert lab adjoined the group of Cheryll Tickle, which altogether made for a stimulating, supportive and productive environment. (for a detailed overview of the Wolpert Lab Family Tree, see Vargesson, [10]).

Some of the work that was ongoing on in the Wolpert Lab when I joined his lab as a PhD student in 1994 included using a reaggregated limb mesenchyme model (where limb mesenchyme is dissociated into single cells and placed in an ectodermal jacket) and found different parts of the limb mesenchyme can reaggregate to make different digits, using a different combination of signalling molecules [21]; Investigating the role of Bmp2 and Bmp4 in skeletal development [22]; Studying feather patterning, specifically as they are formed in periodic patterns, and how they do this was unknown. Work in the lab proposed early globally distributed signals specifying the field (including Shh, Fgf-4) and then localised inhibitors, Bmp2 and Bmp4, triggering feather bud position [23]. My PhD studies focused on cell fate and their relationship to gene expression patterns. I produced detailed (hand-drawn) fatemaps after labelling limb mesenchyme cells with the fluorescent dye, DiI, and in collaboration with Cheryll Tickle and Jonathan Clarke, showed how cell behaviour and movement follows the changes in expression patterns of genes during limb development [24]. Taken altogether, these studies helped begin, along with other labs work, to shed light on the molecular signalling pathways underlying limb and embryonic development. While the molecular basis of Positional Information and determination of positional value in the developing chick limb is still not clear, there is some evidence for such a signal in the regenerating salamander limb, where a gradient of a signal called Prod1 provides positional information [6, 9, 25].

Today, the focus is on interactions between the antero-posterior and proximo-distal axes of the limb rather than a co-ordinate system. Several other models have been proposed to explain antero-posterior and proximo-distal patterning including the Turing reaction-diffusion model which has been shown to play a role in digit specification and which could interact with a graded signal to determine digit specific identities [25, 26, 27]. Yet a timing mechanism and Positional Information remains involved in the process and it is now likely that reaction-diffusion, timing and graded signalling are all involved in limb patterning and outgrowth [17, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36].

1.2.2 Retirement and Awards

Even after retirement and into his late 70s Lewis was still thinking, writing and publishing about positional information [37]. Indeed, in an interview in 2015 for the journal ‘Development’, he also stated ‘if I still had an active lab, finding the molecular basis for positional information would be my objective’ [38], underlining his continued search for answers. He also joined in other scientists lab meetings, specifically the lab of Claudio Stern at University College London, and discussed science with the same twinkle in his eye and excitement for finding out new information, including, publishing a paper on a topic close to his heart (and which started off his science career), gastrulation [39].

For his life long service and impact on developmental biology Lewis Wolpert won the British Society for Developmental Biology Waddington Medal in 2015 [40]. He mentioned how proud he was to have been awarded the medal, not least because he knew Conrad Waddington, the great developmental biologist after whom the medal is named after. Something he was more proud of however, and which underlines the huge legacy he leaves behind and his generosity of spirit, was that several of his former students and postdocs had won the Waddington Medal before he did. He was very pleased about this and mentioned the wonderful and stimulating environment and rewarding discussions he always had with his students and postdocs. His achievements are underlined with the award of The Royal Society Royal Medal in 2018, the highest honour of The Royal Society, for his research on morphogenesis and pattern formation.

1.3 His books and other contributions

Lewis was also a talented writer and communicator and had the remarkable ability to explain complex concepts in simple, logical and clear ways. His textbook 'Principles of Development' was first published in 1998 and is now into its 6th edition [41]. He persuaded many talented developmental biologists to help him write this text book, which has become one of the premier books for undergraduate students. He wrote several popular science books for the public. Perhaps the best known are 'The Triumph of the Embryo', describing in laymans terms how a fertilised cell becomes a fully formed organism; his book 'The unnatural nature of science' where he reviews the history of science and elegantly explains why science is counter-intuitive and hard work and 'Malignant Sadness' where from his personal experience of depression, he writes lucidly and clearly about his own battle with depression. He also wrote popular science books about belief and religion and publically debated his views on science and religion. He was an atheist and was for a long time a vice-president of The British Humanist Association. I recall when he gave an invited lecture in Aberdeen in 2008, he was asked why he was an atheist, he said '*when I was a youth growing up in South Africa I played a lot of cricket. One day while playing in a match I couldn't find the ball, I asked God for help to find the ball. I never found the ball and this led me to atheism*'. However, he also stated that religion does benefit some people.

1.4 His personality and my personal experience as a PhD student of Lewis

I joined Lewis Wolpert's lab in October 1994, and left the lab in April 1998, not long before he retired and took up emeritus status (though he never did confirm if it was my performance in the lab that contributed to his decision to retire). Everyone who spoke with or worked with Lewis has their own stories and memories of him. Amongst my favourite memories are when I first met him at my PhD interview. I was an undergraduate student and had been influenced by his 1978 Scientific American article on positional information and his 'The Triumph of the Embryo' book that I had read during my studies at Kings College London. My PhD interview was in his huge office in the Windeyer Building, part of the Middlesex Hospital Medical School. He arrived late, and brought his bike into his office, much to the upset of his secretary (Maureen Maloney). He smiled at me, removed his bicycle helmet and took a seat at his beautiful desk, surrounded by an amazing library of books and pictures and he asked me '*how do you make an elbow?*'. I came up with an answer, which I thought was good (I had done my homework or so I thought) and he simply said '*no that's wrong, my dear boy*' and we then had a wonderful discussion on how he thought the elbow formed. He then called the MRC and asked them to give me a PhD studentship. Years later I looked back on this conversation as one of the reasons for my long-standing interest in understanding thalidomide embryopathy, as an elbow joint forms in many survivors at the expense of many of the other bones. I shall always fondly remember that twinkle in his eye and his questioning excitement when discussing data, limb development, positional information, and our latest research findings.

Lewis was an inspirational supervisor. Always full of advice. One of the lessons Lewis taught me (and others) was to never be afraid of asking questions. He often said '*always ask questions, there is no such thing as a silly question, because if you have a question someone else will as well*'. This was demonstrated sometimes at lab meetings and seminars where he might nod off but he would almost always ask an amazing question at the end. Or, if he didn't understand or thought the speaker was being highly detailed, would look around and find a PhD student and ask '*do you understand what is being said?*'. Of course the PhD student would think *'..i thought I did, but if Lewis doesn't, perhaps I don't*'… Lewis would then raise his hand and politely mention '*this dear child behind me doesn't understand*'. Lewis always encouraged people to think and to question and to not be afraid of saying 'I don't understand'.

Lewis while focused on the principles and the 'big picture' of the questions being addressed [38] would remind his staff and students to always consider this in their experiments or to re-inspire them, if their experiments were not going well. For example, I recall one time when he was giving a Talk at a Scientific Meeting, he asked some starry-eyed students who were all gripped by his ideas and advice *'…to close your eyes, stretch your arms out in front of you, close your hands together and then bring your hands up to your face*'. He asked them 'to open their eyes' and asked '*what do you see?*' Of course, he remarked, '*you see your arms are precisely the same length'*. '*Isn't that amazing. Explain it to me*'.

Lewis was also a very supportive and generous supervisor allowing staff freedom to develop, to think independently and follow their own ideas and took no credit on some publications that came from his lab that were devised and carried out by his students and postdocs (for example, Summerbell and Lewis [42], Smith [43], Tickle [44], Akita [45], Akita et al [46]). Equally, Lewis remained supportive of his students and staff throughout their careers and was always available to glean advice.

Lewis also cared about staff. He always took time to enquire if staff and students were happy, and if they weren't he spent time with them, offered them parental-like advice and/or told them an anecdote to make them smile, or tried to take their mind off things by talking, for example, about his interest in bicycles and playing tennis. One such occasion I shall never forget was when there was an incredibly sad occasion in 1995 when a PhD student from a different lab died suddenly. It affected many of the students and postdocs greatly. On the day we heard the news, Lewis came into the office, pulled up a chair and after sitting down asked how we were and spent a long time with us, checking we were okay, asking how we felt and discussing why we felt the way we do, and genuinely giving us a shoulder to cry on. This underpins what an extremely kind and caring person he was.

1.5 Concluding remarks

Lewis Wolperts remarkable and long career encompassed soil engineering, cell biology and gastrulation in sea urchins, regeneration in *Hydra* and chick limb development. His main passion was understanding how pattern is generated. His concept of Positional Information provided a new way of understanding how cells become different from one another, how they make the right tissues and in the right places, ideas that are still influencing the field today. He trained some of the leaders of the developmental biology field and inspired many others leaving a remarkable, important and sparkling scientific legacy which will continue to influence many more generations. He was also a kind and generous human being who is already deeply missed.

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Figure Legends

Figure 1

Lewis Wolpert enjoying a discussion with colleagues at the 2008 International Limb Development and Regeneration Conference in Madrid, Spain.

Figure 2

Lewis at a party celebrating the achievements of his and Cheryll Tickle's labs at the Windeyer Building, Middlesex Hospital Medical School (UCL) before moving labs to the Medawar Building on Gower Street (UCL) in June 1996.

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