A time-space translation mechanism for patterning the vertebrate anteroposterior axis

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Chapter 5

A tribute to D'Arcy Wentworth Thompson: Elucidation of a developmental principle

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D'Arcy Thompson

Nature is inspiring and beautiful. She has her own elegant logic. Put differently, and applied to embryogenesis: the shapes and forms in which organisms develop are governed by specific physical and mathematical laws. These define 'Developmental Principles', which can perhaps be viewed as the unitary building blocks for development. This was the area of D'Arcy Thompson's pioneering endeavours.

D'Arcy Wentworth Thompson (1860-1948), was a mathematical biologist, educated at Edinburgh and Cambridge, before becoming professor in Dundee and later St. Andrews. He published more than 300 books and articles, culminating in his most influential work: D'Arcy Wentworth Thompson 'On Growth and Form' (Thompson, 1917). This book greatly influenced architects, anthropologists, artists and mathematicians as well as developmental biologists. The theoretician Brian Goodwin and the geneticist John Maynard Smith were strongly influenced by this book. It inspired the mathematician Alan Turing to his seminal insights into how regular body patterns such as those seen in radially symmetric sea anemonies can be organised by a system of simple chemical reactions (Turing, 1952). Turing's work in turn inspired a generation and led on to investigation of dissipative structures in media like Beloussov-Zhabotinsky reagent and Dictyostelium. These ideas were still being debated furiously between Maynard Smith and his associates and Goodwin and his at the University of Sussex when one of us (AD) was there around 1970. D'Arcy Thompson has been criticised for being descriptive rather than analytical but what else can you expect, considering the stage at which he was working? That is also the nature of radical originality. It concerns opening new lines of investigation, not completing them. None of D'Arcy Thompson's discoveries have yet been elucidated at the molecular level.

A free thinking, radically original pioneer, working in a time before there was any substantial experimental embryology or methodological basis for investigation, D'Arcy Thompson explored the areas above. He gained brilliant fundamental insights into how different organisms, including the vertebrate embryo are made. He drew many interesting conclusions including that plant phyllotaxis can conform to a Fibonacci sequence, and that the Nautilus shell is a logarithmic spiral. Most famously, he saw that different fish body shapes are mathematically connected. They conform to different transformations of a

common Cartesian coordinate system (Fig. 1A). He thus laid bare a developmental principle. He challenged the accepted wisdom of the day, that the forms generated in embryogenesis are entirely the result of Darwinian Natural Selection.

That is how some of the most radically original departures in science are made. We need to understand what Nature's logic is. Simple observations are generally the key. Pre-existing accepted scientific wisdom (dogma) and technology are considered essential for progress but are a double-edged sword. They channel ideas and can thus stunt imagination and hinder originality. Radically original major breakthroughs can only be made by going back to basics and breaking out from dogma. Surprisingly, this simple pioneer is still potentially inspirational for any who strive to introduce original concepts into developmental biology, up to the present day.

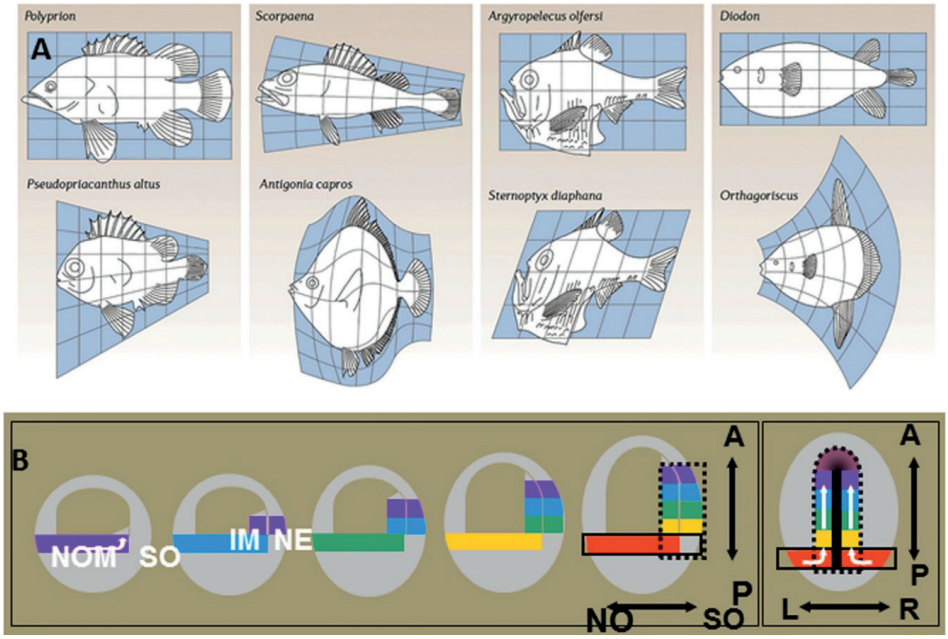

Figure 1. A. Cartesian Fish. Representation of different fish shapes as different transformations of Cartesian coordinates à la D'Arcy Thompson. Reproduced with permission from (Arthur, 2006) © (2006) Nature Publishing Group. **B. The time space translation hypothesis** (Durston and Zhu, 2015). Timed interactions between the *Hox* expressing non-organiser mesoderm and the Spemann organiser generate positional information during vertebrate gastrulation. The drawings show 2-dimensional representations of *Xenopus* gastrulae. The first 5 drawings are parasagittal (ventral to dorsal) representations of gastrula profiles, starting at the beginning of gastrulation and then at sequential stages till the end. The last (6th) drawing shows end of gastrulation, from the dorsal side (profile at the level of the dorsal axial mesoderm). *Hox* expressing tissue: NOM, NO and IM and later NE is represented by different colours, each of which represents a particular *Hox* code. Initially, the sequential spectral shades of the coloured bar represent temporal collinearity in NOM. The later dorsal internal coloured blocks represent historical fixed *Hox* codes in IM, due to SO signals. The adjacent blocks in the wall of the embryo are NE, with identical *Hox* codes. SO is only in the last drawing, as the heavy median black line. The first 5 drawings represent a lateral level where SO is not encountered. The black dotted line in the last drawing depicts the sphere of SO influence. NE: neurectoderm, NO/NOM: non-organiser mesoderm; SO: Spemann organiser; A: Anterior; P: Posterior; L: Left; R: Right. The white arrows reflect directions of cell movement flow. For further explanation see main text and (Durston and Zhu, 2015). Reproduced with permission from Elsevier © 2004.

A Tribute: Attempt at Elucidation of a Developmental Principle

We present the following modest tribute to the memory of D'Arcy Thompson. We attempt molecular elucidation of a Developmental Principle. A body of recent novel evidence (from simple observations of our own and from recent literature) shows that the vertebrate anterior-posterior (A-P) body axis is made by a conserved, Hox collinearity based, BMP- anti-BMP dependent time-space translation (TST) mechanism (the principle) (Durston and Zhu, 2015; Wacker et al., 2004a). This analysis of simple observations challenges current dogma and introduces novel insight and inspiration in a field that had somewhat lost its way. The dogma concentrates on only a fragment of the complex collinearity mechanism and loses sight of the most important point that this mechanism is multiscale (Almirantis et al., 2013). The mechanism connects subcellular behaviour with tissue wide patterning. We remedy this defect by filling in what is missing. Because of his evident appreciation of Nature's beauty, his realisation that her logic reveals developmental principles, and his radical dogma free originality, this is a fitting tribute to D'Arcy Thompson. Please note that, due to space limitation in this 2000-word article, it is impossible to present all of the relevant evidence here. This is easily accessible but needs to be sought in the most relevant source articles especially (Durston and Zhu, 2015).

Timers and Decision Points in Axial Patterning

There is evidence that making the vertebrate anterior-posterior (A-P) body axis depends on developmental timing (Eyal-Giladi, 1954; Gamse and Sive, 2000; Gamse and Sive, 2001; Nieuwkoop, 1952; Wacker et al., 2004a). This involves relatively simple timed 'decision points' separating axial domains (Durston and Zhu, 2015), but also explicit timers that encode extensive stretches of spatial information via a single timing mechanism. In parallel with known timers, the axial decision points are also clearly connected together in a timer (Durston and Zhu, 2015). Axial development seems to involve different timers interacting via facultative or obligatory connections (Durston and Zhu, 2015).

***BMP*-anti *BMP* and A-P patterning**

There is evidence that the vertebrate gastrula organiser somehow regulates A-P as well as dorsal–ventral (D-V) patterning. The early D-V, organiser/non organiser *BMP*/anti *BMP* pathway is somehow required for A-P as well as D-V development (Durston and Zhu, 2015). The question is: how does this single pathway simultaneously participate in making two different axes? We present evidence below that this happens because the organiser is part of a timing mechanism.

Trunk (*Hox*) Time-Space Translation

In the posterior part of the vertebrate embryo, *Hox* genes are the major determinants of A-P positional information and *Hox* collinearity is evidently involved in timing and axial patterning. *Hox* genes are in fact central in specifying A-P positional information in all bilaterian metazoan embryos. Gain or loss of function (GOF or LOF) for a *Hox* gene determines and changes the identity of part of the body axis. Two examples: *Xenopus* Hoxc6 LOF turns the whole axis into head-neck, truncating it at the neck/thorax boundary (which is also the *hoxc6* anterior expression boundary) (Zhu et al., 2017b). Ectopic expression (GOF) for *hoxd1*, *hoxb4*, *hoxa7*, *hoxb9* in dorsalised (*Hox*less) *Xenopus* embryos generates in each case an axis segment starting with the expression zone of the ectopically expressed gene and proceeding back to the tail (Zhu et al., 2017a).

Hox genes show collinearity (Duboule and Dolle, 1989; Graham et al., 1989; Lewis, 1978). In most bilaterian metazoans, they are contained in chromosomal clusters where the most 3' gene in each cluster is expressed most anteriorly and successively more 5' *Hox* genes are expressed successively more posteriorly. This is called spatial collinearity (Duboule and

Dolle, 1989; Graham et al., 1989; Lewis, 1978). In *Drosophila,* the collinear Hox sequence extends essentially the whole length of the body axis. In vertebrates, it extends from within the neck to the tail and excludes the head. In some organisms including vertebrates, *Hox* genes also show temporal collinearity (Dolle et al., 1989; Izpisua-Belmonte et al., 1991). The most 3' anterior *Hox* gene is expressed first. Successively more 5' *Hox* genes are expressed successively later.

Here, we review the evidence for a *Xenopus Hox* collinearity time-space translation mechanism (Fig. 1B (Durston and Zhu, 2015)). The importance of *BMP*-anti-*BMP* is that it regulates progress and arrest of a developmental timer. This is a *BMP* dependent ventral non organiser mesodermal timer (details as in (Durston and Zhu, 2015; Wacker et al., 2004b)) whose output is *Hox* temporal collinearity and that is arrested at different developmental times during gastrulation by dorsal anti-*BMP* signals from the organiser. For background on gastrulation and the organiser see (Durston and Zhu, 2015). When stopped, the timer generates a positional value (*Hox* code) identical to the temporally collinear *Hox* code at which it was stopped. The sequential stopping of an A early to P late sequence of mesodermal zones as they converge sequentially towards the dorsal organiser during gastrulation generates the familiar spatially collinear A-P *Hox* pattern, initially in dorsal mesoderm derived from the original temporally collinear gastrula ventral non organiser mesoderm, and then copied zone by zone via vertical signalling to neurectoderm during neural transformation (Bardine et al., 2014; Durston and Zhu, 2015). An important feature of this mechanism is that it utilises positive and negative (collinear) *Hox*-*Hox* interactions (Hooiveld et al., 1999; Zhu et al., 2017a). A positive interaction: posterior induction, whereby more anterior *Hox* genes induce posterior neighbours, underlies temporal collinearity (Faiella et al., 1994; Hooiveld et al., 1999; Zhu et al., 2017a). A negative collinear interaction, whereby posterior *Hox* genes repress more anterior ones (Hafen et al., 1984; Miller et al., 2001; Plaza et al., 2008; Struhl and White, 1985; Yekta et al., 2004), partially mediates time space translation. Non cell autonomous *Hox* autoregulation mediates neural transformation. This time-space translation mechanism is important for A-P patterning in the trunk (neck-thorax-abdomen-tail) part of the axis (Durston and Zhu, 2015; Wacker et al., 2004a). The *Hox* interactions mediate multiscalarity and drive the whole mechanism.

What happens in the head?

The vertebrate A-P axis consists of a *Hox*-dependent posterior part (trunk) and a *Hox* independent anterior part (Head and extreme anterior domain (EAD)) We review the evidence that the same BMP-anti BMP, D-V, time-space translation mechanism that makes the trunk also makes the head part of the axis, via very early timed decisions (Durston and Zhu, 2015; Tuazon and Mullins, 2015). The EAD domain, which becomes face, is the most anterior part of the axis (Dickinson and Sive, 2007). This seems to be specified even earlier than the head (Durston and Zhu, 2015). The anterior determinants may interact among themselves and with the more posterior *Hox* genes via similar ordered interactions as regulate the *Hox* genes (Durston and Zhu, 2015; Zhu et al., 2017a).

***Hox* genes and intercellular signalling**

Hox collinearity is a multiscale process. It requires coordinating and synchronising the collinear behaviour of individual cells into a global multicellular mechanism along the developing A-P axis. This mechanism is crucially dependent on *Hox* interaction driven and *Hox* independent intercellular signalling. The following forms are important.

A. *Hox* proteins as signalling molecules

Hox induced signalling is required, The *Hox* proteins themselves are known to be direct signalling molecules. They travel through membranes in inverted micelles and mediate important actions in neurobiology and cell biology. In axial patterning, *Hox* protein transfer mediates at least one relevant intercellular *Hox-Hox* interaction: vertical signalling from mesoderm to neurectoderm during neural transformation (Bardine et al., 2014).

B. The relation between the axial timer and the somitogenesis clock.

The axial TST timer and the somitogenesis clock run concurrently in a common mesodermal tissue (early gastrula non-organiser-later paraxial mesoderm). These two timers are facultatively connected (Cordes et al., 2004; Peres et al., 2006).

C. Conventional signalling pathways (*retinoids, Wnt, FGF*) directly regulate expression of key *Hox* genes at decision points (Durston and Zhu, 2015), see below.

Decision points and the axis

The vertebrate A-P axis is made in sequential blocks or domains. These are: the EAD, head, neck, thorax, abdomen, tail. This organisation enables construction of a functional animal. Different domains are separated by boundaries characterised by time-space localised 'decision points', involving interactions between the axial timer and external signalling pathways (Durston and Zhu, 2015). Three well characterised cases are explained in (Durston and Zhu, 2015).

How do these ideas relate to accepted wisdom?

The accepted wisdom is that *Hox* collinearity originates from evolutionary tandem duplication of an *ur-Hox* gene (Gehring et al., 2009), and is based on sequential opening of chromatin from 3' to 5' in the *Hox* clusters (Fabre et al., 2015). These ideas are presumably correct. Chromatin opening is backed up by the progressive 3'to 5' deformation of *Hox* cluster chromatin during collinearity, the action of repressive and activating global enhancers (Durston and Zhu, 2015), and 3'to 5'sequential movement of Hox genes from a repressive (*Polycomb* related) to an activating (*Trithorax* related) compartment (Durston and Zhu, 2015; Fabre et al., 2015). However this wisdom deals with only a part of the complex *Hox* axial patterning mechanism. *Hox* collinearity is multiscale (Almirantis et al., 2013). It operates over 100 nm in each *Hox* cluster and also over 1mm in the spatially collinear axial *Hox* pattern and over a similar dimension in the same cells when they are temporally synchronised earlier during temporal collinearity. The key to this multiscalarity is *Hox* driven cell interactions. We presented evidence above that *Hox* driven interactions are the trans acting signals that synchronise the collinear *Hox* clusters in cells, that they mediate or control the intercellular signals that integrate *Hox* collinear cells into collinear tissues, and that they integrate *Hox* collinearity with head and EAD patterning during the complete axial patterning process (Durston and Zhu, 2015).

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