TIME SPACE TRANSLATION: A CONFIRMED PRIMARY AXIAL PATTERNING MECHANISM IN VERTEBRATES

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Abstract

The vertebrate anterior-posterior (A-P) body axis arises due to time space translation (TST). *BMP* dependent *Hox* temporal collinearity in early embryonic mesoderm generates the initial vertebrate axial pattern because the Hox codes associated with sequential times are frozen sequentially by *BMP* inhibiting signals from the embryonic organiser or node. There are three reasons why it is now opportune to review TST. 1/ It has become clear that this mechanism is highly relevant for current and emergent directions in medicine. Making a particular tailored stem cell or culturing a specific organoid in vitro both depend on it. 2/ This unexpected and perhaps unlikely sounding mechanism has recently been thoroughly validated. 8 recent primary publications from 6 major groups confirm that TST is the mechanism for primary axial patterning in the 4 best investigated vertebrate embryos. 3/ Its mechanism is now becoming clear. Previous publications propose it involves *Hox* regulation of cell movement during gastrulation or sequential stabilisation of *Hox* codes by anti *BMP* as above. Neither of these processes works alone but together they amount to a very convincing mechanism.

Introduction: The early vertebrate anterior-posterior (A-P) axis is made by time space translation (TST)

There is evidence that the vertebrate anterior-posterior (A-P) axis is made in a timed manner from head to tail. This was first shown by classical embryologists in Amphibia [1,2]. The early studies focussed on the anterior-posterior (A-P) patterning of the central nervous system and showed that axial neural tissue is first specified as anterior (presumptive forebrain- telencephlon/diencephalon) and then sequentially posteriorised. This transformation involved first conversion to presumptive mesencephalon, and subsequently to presumptive rhombencephalon, and then to presumptive spinal cord. These findings were confirmed and expanded by more recent studies in various vertebrates [3-7]. Recent work also shows that the head/brain is not the most anterior/early domain in the axis. There is actually a further rostral axial domain: the extreme anterior domain (EAD) , newly discovered by Hazel Sive and colleagues), anterior to the brain [8]. The very recently characterised EAD, which becomes the ventral face and part of the

pituitary gland (and the cement gland, in *Xenopus*), forms via ventralward bending of the anterior end of the dorsal A-P axis so this end faces backward ventrally like the handle of a walking stick [8,9]. ***Fig 1 here***

There is evidence that the timing aspect of vertebrate axial patterning involves collinearity of the Hox genes: the main determinants for different levels in the trunk-tail part of the axis. They complement equivalent determinants in the head-EAD part of the axis [10]. The vertebrate *Hox* genes are contained in 4 genomic clusters, each of which contains an incomplete variant of the vertebrate sequence of 13 *Hox* paralogues. These clusters and the whole *Hox* complement all show both; spatial collinearity (the spatial sequence in which they are first expressed along the embryo's main (A-P) body axis matches their 3' to 5' genomic sequence in the *Hox* clusters); and temporal collinearity (the temporal sequence in which they are first expressed in the embryo also matches their genomic sequence [11-13]). Recent evidence shows clearly that the earlier temporal, collinearity leads to the later spatial collinearity. We have called this aspect 'time space translation (TST)'.

What is the mechanism of TST- Is this cell movement control?

i/ It is has been proposed that *Hox* control of the movement of gastrulating mesoderm cells may be involved in enabling Hox temporal collinearity to generate the spatial pattern. Pourquie and colleagues [14,15] (Iimura and Pourquie, 2006, Denans et al., 2014) showed that the time when chicken epiblast cells ingress (ie begin their gastrulation movements by internalising into the embryo and entering the primitive streak) can be altered by ectopic expression of a *Hox* gene. The more 5' late posterior the ectopically expressed Hox gene, the later ingression begins and if two *Hox* genes are ectopically expressed simultaneously, the more 5', later, more posterior of the two determines the ingression time- a phenomenon that presumably relates to posterior dominance or posterior prevalence. This finding possibly relates to a mechanism that could order cells expressing *Hox* genes with temporal collinearity into a temporally collinear sequence of arrival at the organiser/node and thus in entering the dorsal axis of the embryo. Could this mechanism alone generate the spatial pattern? No, it could not. The problem is that *Hox* temporal collinearity does not stop of its own volition. In an environment that can support temporal collinearity, it runs through to completion in each temporally collinear cell [5] (see below). So each and every gastrula mesoderm cell, regardless of where it is in the *Hox* sequence at

the time of gastrulation should run through to *Hox13* in the spatial pattern.

Clearly, something more is needed to enable an axial pattern to be generated. What could be relevant is if mesoderm cells are transported by *Hox* regulated gastrulation movements from a part of the embryo where *Hox* temporal collinearity is supported to a part where *Hox* temporal collinearity is not supported but is converted to spatial collinearity

It should be added that although *Hox* control of cell migration is an attractive idea, it may not be relevant in every vertebrate embryo. Whereas chicken embryos show ingression of individual mesoderm cells during gastrulation, some embryos (eg *Xenopus*) do not . Mesoderm cells gastrulate (involute) as a largely intact cell sheet with nearest neighbour cell relationships being largely maintained. Initially more dorsal cells gastrulate first and reach the dorsal axis first and the A–P sequence of cells in the dorsal axis is thus already ordered on a first to last arrival basis due to convergence extension movements [16] . In fact, all vertebrate embryos show this aspect [17].

What are the temporal collinearity supporting and temporal collinearity blocking parts of the embryo mentioned above? Temporally collinear *Hox* expression occurs in more ventral mesoderm in the *Xenopus* gastrula embryo [5] or in the posterior primitive streak[18] in the chicken embryo. Mesodermal cell migration during gastrulation brings these ventral cells to the dorsal side of the embryo to within close proximity of the Spemann organiser (or anteriorly to Hensen's node in the chicken). Does the conversion from temporal to spatial collinearity have to do with these cells approaching the organiser?

Ventralised *Xenopus* embryos show only *Hox* temporal collinearity [5]. They show a temporally collinear sequence of *Hox* expression that runs through from 3' early (anterior) to 5'late (posterior) values. Timed grafting of an intact organiser into ventralised embryos at sequential stages blocked the temporal collinearity timer at sequential early to late *Hox* values and therefore fixed timed cell states sequentially. This treatment generated anteriorly truncated spatially collinear embryonic axes with the anterior truncation being at sequentially more 5' posterior positions for sequentially later treatments (implanted organiser: continuous signal source) [5]. I conclude that sequentially repeated interactions between two embryonic parts (ventral mesoderm and

organiser) lead to successive small populations of temporally collinear ventral mesoderm cells being fixed at sequential 3' to 5' time/space points. Presumably,the morphogenetic cell movements in the embryo (possibly *Hox* timed mesodermal cell ingression movements) [14], cause these sequentially timed populations to be arranged in and specify a patterned 3'anterior to 5' posterior spatial *Hox* sequence that becomes the initial A-P pattern.

What is the relevant organiser signal? It is known that the *Hox* expression domains in temporally collinear NOM mesoderm are enlarged (expanded in the animal direction) by ectopic expression of *brachyury* but also expanded in the dorsal direction by ectopic expression of *BMP*[19]. Presumably, *brachyury* expands the mesodermal domain and *BMP* (known as a ventral determinant), ventralises the embryo. This leads to the suspicion that the relevant dorsal organiser signals for TST are *BMP* antagonists like *chordin* and *noggin*. This was tested by timed injection of *noggin* protein into ventralised temporally collinear *Xenopus* gastrulae. Here, it was found that early injection of *noggin* protein (High amplitude anti *BMP* pulse) generated anterior zones in the axial pattern. Later injection gave more posterior zones and sequentially later injections gave sequentially more posterior zones [5].

An important question is: how do *Hox* collinearities and TST work. This is not known. One important clue is that collinearites and TST evidently involve and depend on sequential collinear and autoregulatory non cell autonomous *Hox/Hox* interactions, some of which are evidently *BMP* dependent while others are *BMP* inhibited [20-24]. The sequentiality of these interactions is revealed by 'cascade' phenotypes for *Hox* gain and loss of function (GOF and LOF) (Fig. 3). A second clue is that stabilisation of *Hox* information involves its transfer from dorsalised NOM mesoderm (which has now become paraxial mesoderm:PM) to the overlying dorsal ectoderm which has become neurectoderm: Niewkoop's transformation mechanism . This process seems to involve copying of patterning information [25] (and at the molecular ;level copying of *Hox* codes) between the two tissue layers by (presumably *BMP* inhibited) non cell autonomous *Hox* autoregulation [26].

Is this a generally important mechanism?

TST was discovered in *Xenopus* [5] but also operates during gastrulation in other vertebrates. There is anterior early to posterior late timed stabilisation of axial zones ie generation of a stable spatially collinear axial *Hox* and head pattern by timed anti *BMP*) in early chicken and zebrafish embryos [18, 27, 28]-. *Hox* temporal collinearity in the chicken gastrula also apparently determines the order in which chicken primitive streak cells migrate to the node,[14, 15]. A population of dynamically changing primitive streak cells interacts with a stable organiser derived population to generate the early axial pattern in mouse embryos [29] and *Hox* temporal collinearity during chicken gastrulation generates positional information (forelimb position) in later development [30]. These parallel and complementary findings in other vertebrate embryos establish this timing mechanism as correct and generally relevant. Interestingly, the discoveries above define a believable role for the Spemann organiser, which is well known to be important in A-P patterning. I note that organiserless (ventralised) embryos show transient temporal but not spatial *Hox* collinearity and that reimplantation of an organiser reintroduces and fixes the spatial pattern exactly as predicted above here [5]. It has also become clear that the mouse *Hox* establishment are now convinced that *Hox* temporal collinearity leads to spatial collinearity [31]. This confirms D.Duboule;s original insight that temporal collinearity is significant for making the axial pattern[32] and is despite earlier arguments to the contrary [33]

What is the medical significance of TST?

It has become clear that this mechanism is highly relevant for current and emergent directions in medicine. Making particular tailored stem cells or culturing specific organoids in vitro [26, 27] may both depend on it. Understanding the underlying A-P mechanism enables culture in isolation of different body regions. This is particularly clear from GOF and LOF for *Hox* genes and other axial regulators. See above. Eg. LOF for *Xenopus Hoxc6* cuts off the embryonic axis at the neck-thorax boundary: the whole embryo becomes EAD-head-neck. Suppression of *Wnt 8* also cuts off the posterior axis at the Anterior Head/Posterior Head boundary. GOF for *Hoxb9* in *Hox*less embryos initiates a partial posterior axis, running posteriorly from the thorax-abdomen boundary to the tipip of the tail. These insights will be key to future stem cell and organoid culture applications because different organs form at specific A-P levels in the e body. For example, the forebrain is anterior to all *Hox* genes, the pectoral girdle and heart are in the thorax, the sacrum is at

posterior abdomen level. The findings above enable initiating development of differerent body regions by applying the correct instructions.

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Figures

Fig 1. 1: Timing, Axial patterning, and Time Space Translation

Above: The domain structure of the vertebrate A-P axis Domains with significant Hox genes and other markers. An unexpected element is introduced by the newly characterised EAD: extreme anterior domain,

which makes the face. This is shown as the most anterior part of the straight axis. Actually. the anterior end of the dorsal A-P axis bends backward around to the ventral side of the embryo to face posteriorly-like the handle of a walking stick (not shown). A Head: anterior head (corresponding to telencephalon,diencephalon, mesencephalon). P Head: posterior head (corresponding to anterior rhombencephalon, occipital somites). Neck: cervical somites, posterior rhombencephalon, Thorax: thoracic vertebre, anterior spinal cord. Abdomen: Lumbar and sacral regions, spinal cord. Tail: vertebrae, spinal cord.

Above and below: Time space translation. A biological timer, represented by the clockface below, proceeds from 1-12 (red numbers). The tittimer starts with information needed for making the EAD, proceeds to the anterior head, then to posterior head, then to neck, then to thororax , then abdomen, then tail. The timer needs *BMP* to function so is in tissues like NOM with high *BMP* (yellow/orange). Anti –*BMP* (blue)) (produced by the organiser) interacts with the timer sequentially to freeze the identities of an A-P sequence of axial zones. In the axial sequence, the *Hox* genes are each both a component of the timer at their appropriate times and are sequentially involved in setting up the A–P sequence of axial zones. The genes involved in time space translation in the EAD-head zones are unknown. The heavy red dashed arrows represent transport of cells from the high BMP environment (yellow) to the BMP inhibited environment (blue). The continuous dark red arrows connecting yellow to blue for the head and EAD I indicate that the detaipils here are unknown. The head and tail of the A-P timer are close together because of their representation on a clockface. No statement about molecular identities is intended.

Fig. 2 **Analysis of axial patterning following timed implantation of an organiser into a ventralised embryo or following timed injection of noggin protein into it's blastocoel**. A. Timed implantation of a St. 10 organiser (T=0) into a ventralised gastrula T=0 (St. 10) and 2, 4, and 6 hrs later B. Control. Timed implantation of an aged organiser (0,2,4,6 h) into a T=0 ventralised gastrula. C. Timed injection of noggin protein into the blastocoel of a ventralised gastrula at St,9 ,St. 10, St. 10.5, St. 11.5. The experiments were analysed using a sequence of axial markers: *En* (midbrain-hindbrain boundary), *Krox20* (anterior hindbrain), *Hoxb4* (from posterior hindbrain backward), *Hoxc6* (from anterior thorax), *Hoxa7* (from mid thorax), *Brachyury* (from abdomen), *Hoxd13* (from tail) A combined in situ hybridisation of *Krox20* (arrowed) nd *Hoxd13* is shown in the photos as an example of the analysis and the total analyses are diagrammed in the pictograms by the spectral

sequences of colours (*En* grey then dark blue -anterior (*Krox20*) to red most posterior (*Hoxd13*).

Fig. 3

Fig 3 *Hox* sequences for axial cascade phenotypes Above: Wild type *Ho*x sequence. second and third down: blue diagrams: Loss of function affecting temporal collinearity in EC cells. *Hox 1* and *Hox3* LOF each cut off the *Hox* temporal collinearity sequence from their paralogue position. Fourth and fifth down: Xenopus spatial collinearity, loss of function. Fourth: *Hox1* loss of function (LOF) (all 3 *Hox1* genes knocked down by morpholinos (MOs)). The axis from *Hox1* backward is compromised. The dotted line indicates there is still reduced residual expression for some posterior Hox genes. Fifth down:*Hoxc6* LOF (MO) The axis from *Hoxc6* backward is compromised/deleted. Sixth down: *Hoxb4* gain of function (GOF): ectopic expression of *Hoxb4* in *Hox* free dorsalised embryos. A partial posterior axis is generated, starting with *Hoxb4.*Seventh down: *Hoxb9* gain of function. Details similarly as above for *Hoxb4* gain of function. Please note that: In *Hox1* loss of function, the immediately more anterior marker (*Gbx2)* has enhanced expression, presumably reflecting absence of posterior dominance exerted by *Hox1* genes. Similarly, in *Hoxc6* loss of function, *Hox4* and *Hox5* are hyperinduced, presumably reflecting absence of *Hoxc6* posterior dominance.