Using Petri Nets in Higher Level Development Biology: a case study on the AP axis development in Xenopus laevis. Extended abstract

Bertens, L.M.F.; Jansen, H.J.; Kleijn, J.; Koutny, M.; Verbeek, F.J.

Using Petri Nets in Higher Level Developmental Biology: A case study on the AP axis development in *Xenopus laevis* Extended Abstract

Laura M.F. Bertens
LIACS
Leiden University, The Netherlands
lbertens@liacs.nl

Hans J. Jansen
IBL/Moleculaire celbiologie
Leiden University, The Netherlands
h.j.jansen@biology.leidenuniv.nl

Jetty Kleijn
LIACS
Leiden University, The Netherlands
kleijn@liacs.nl

Maciej Koutny
School of Computing Science
Newcastle University, U.K.
maciej.koutny@ncl.ac.uk

Fons J. Verbeek
LIACS
Leiden University, The Netherlands
fverbeek@liacs.nl

1 Introduction

Static models like schematic diagrams are commonly used by biologists to survey and further their understanding of complex biological processes. Recently, with the development of the area of computational systems biology, also dynamic modeling methods have become available and their benefits for gathering more insight into such processes have been recognized (see, e.g., [6]). To understand a living organism however, not only its components and their dynamics should be analyzed, but also their interactions. Consequently, Petri nets as a formal model for concurrent systems, appear to provide a natural and promising modeling technique and may be of help to understand the complex processes behind real-life phenomena. In fact, Petri nets have already been shown to be very promising for molecular and cellular biology, in particular for metabolic, signaling and gene-regulatory networks (see, e.g., [25, 2, 1, 18, 7, 8, 10, 26]). So far the use of Petri nets in biology has mainly focused on the molecular and cellular levels. In this project we aim at extending their use to higher level processes in the organism, e.g., on tissue and organ level. In order to explore new ways in which Petri nets can be applied to developmental biology, we propose a case study. This case study was chosen because it comprises several different aspects, found in many biological processes, that require modeling solutions.

Petri net modeling methods developed for this relatively small case study will, at a later stage, enable us to model larger processes, incorporating multiple levels of both spatial and temporal information. In this extended abstract we present the initial ideas underlying a project based on a close and balanced collaboration between biologists and computer scientists. Such collaboration should ensure that the model we develop, will be as realistic and close to the actual biological processes as possible, while at the same time enabling precise mathematical analysis and predictions. This extended abstract gives an overview of the setup, approached from the biological point of view. It provides the basic layout and points out the modeling challenges, but does not include any formal definitions or results.

2 Petri nets and membrane systems

Petri nets are an abstract model of information flow [23]. They have both a graphical representation and a formal, mathematical, definition, making their use for biologists intuitively attractive while allowing

Submitted to:
compmod09

at the same time a precise analysis (see [22, 24, 4]). A Petri net consists of places (representing passive entities like resources, sometimes called actors) and transitions (representing actions or events), and a flow relation relating them. Thus a Petri net is a bipartite directed graph with places (usually depicted as circles) and transitions (depicted as rectangles) as its nodes, and arcs between them (representing the flow relation). For example, Figure 1(b) shows a Petri net consisting of 6 places representing different resources and 2 transitions describing possible changes of the quantities of these resources (the initial distribution of resources is indicated by the black tokens drawn inside places). In particular, executing the lower transition consumes one unit of resource $(b,2)$ and one unit of resource $(c,2)$, and produces two units of resource $(c,2)$.

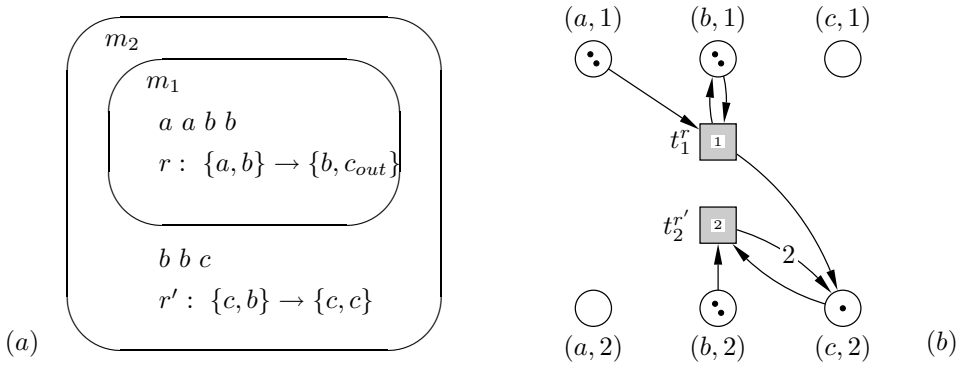

Figure 1: A membrane system (left), and the corresponding Petri net (right).

Places can for instance represent genes, proteins or cells, while transitions can represent chemical reactions or cell-to-cell interactions. The flow may be inscribed, e.g., by weights indicating a multiplicity of resources consumed or produced by a transition when it occurs. The dynamic behaviour of a Petri net is based on a firing rule which describes the conditions (determined by its neighboring places) under which an individual transition can take place and the effect when it does. Hence the model makes it possible to define when and how events may occur concurrently or independently.

Since Petri nets are dynamic and executable, when properly applied as a modeling tool, they can be used to simulate and investigate possible evolutions of a system and hence, e.g, to predict behavior under new circumstances. The project relies on Petri nets as a well-established model of concurrent and distributed systems featuring a wealth of tools for the analysis and verification of their behavioural properties [5]. We start from Petri nets extended with the concept of transition locality, which has recently been introduced ([14]) with the aim of faithfully modeling various classes of membrane systems ([20, 21]).

A membrane system (see, e.g., Figure 1(a)) is a computational representation of a cell divided by membranes into compartments where biochemical reactions take place. When translated into a Petri net, each place corresponds to a certain type of molecule in a specific compartment of the cell and each transition to a reaction rule. However, since the standard Petri net model does not support a notion of an environment to which one or more transitions may belong, the net model is extended. Each transition (representing a reaction rule) is associated with a locality to indicate the compartment to which its rule belongs. Features like inhibitors, catalysts, and promoters, are easily added using the existing corresponding Petri net extensions. As shown in [17], this translation extends smoothly to enhanced concepts as complicated as membranes with a dynamically changing structure due to thickening and dissolving of membranes. In principle, a variety of analytical techniques for Petri nets could be applicable to mem-

brane systems through Petri nets with localities. Already existing techniques ignore however transition localities and the possibility of locally synchronized occurrences of reactions. Therefore, as a first step towards the development of effective analysis techniques, the process semantics of ordinary Petri nets has been extended to nets with localities ([14, 15]). With such a semantics, the causality relations between the occurrences of events during an evolution of the system become explicit and can thus be analysed.

In this project, we aim to investigate the possibilities of applying Petri nets with localities to the modeling, simulation, and analysis of biological processes on higher levels in the organism. If needed we will extend them with additional features, to increase their modeling power. By using hierarchical nets (i.e., creating subnets as done in the Coloured Petri Nets model of [13]), both high level processes, dealing with organs and organism as a whole, and lower level processes, dealing with, for instance, gene expression patterns and cell-to-cell signaling, can be incorporated into the same model. We believe that the already extensive tool set currently available for building and analyzing Petri nets can be augmented with new tools or modifications of existing ones.

3 The Case study

We will be looking at the embryonic development of the anterior-posterior (i.e. head-to-tail) axis in the model organism *Xenopus laevis*, the African clawed frog. The development of this model embryo has been studied thoroughly and a huge amount of literature is available to draw from when building the model ([11, 3]). The formation of the anterior-posterior (AP) body axis is influenced by inhibition of certain processes, movement of cell layers and chemical signaling between cells in two axes. This great variety of processes and acting agents combined with the relatively small scale and the clear boundaries (temporal and spatial) of the process makes the axis development an excellent case study.

The AP axis formation takes place during gastrulation, the developmental stage in which the three germ layers, endoderm, mesoderm and ectoderm, are formed and positioned in the embryo. During gastrulation the mesoderm starts off as a ring around the embryo, between ectoderm on the animal pole of the animal and endoderm on the vegetal pole (see Figure 2(A)). Subsequently the mesoderm moves along this ring-shape towards one point, the blastopore (Figure 2(B)). At the blastopore both the endoderm and the mesoderm move inwards (a process called involution), in a direction perpendicular to the original ring-shape of the mesoderm, as can be seen in Figure 2(C,D). In this way the three germ layers come to lie on top of each other, with the mesoderm in between endo- and ectoderm.

The mesoderm layer is of special importance in coordinating the AP axis formation. It contains a dorsal region, the Spemann organizer, which is an important player in the process and behaves in a functionally different way from the rest of the layer, the non-organizer mesoderm; both these mesoderm regions will therefore be modeled as separate units in the Petri net. The organizer mesoderm is illustrated in the schematic drawing of Figure 3(B) (the thick black line). In Figure 3(A), a schematic cutaway side view of the embryo (corresponding to Figure 2), the movement of the mesoderm along the ring (from ventral to dorsal, at the arrow) and the subsequent involution (from posterior to anterior) at different developmental stages can be seen (explanation of the figure and the different colours in the mesoderm is given below).

The process of axis formation can be divided into two stages: the activation and transformation steps, as originally described by [19]. When modeling the entire process, these two stages will be represented in subnets, which are temporally sequential. During activation, ectodermal cells overlying the organizer mesoderm are induced to become neural cells. This happens through what can be described (and modeled) as double inhibition; in a default state ectodermal cells will adopt a neural fate. However, in the

whole embryo this default is inhibited by the protein BMP, inducing the cells to develop into epidermis. In a select group of cells, close to the Spemann organizer, the default is restored by BMP antagonists (like the proteins Noggin and Chordin), secreted by the organizer. Ectoderm in this region will therefore become anterior neurectoderm.

When modeling this first step several actors and events can be distinguished. Neither spatial nor temporal factors are necessary in this subnet, since there is no strict temporal sequence in the events and the spatial heterogeneity of the mesoderm can be accounted for by modeling organizer and non-organizer mesoderm as two separate entities.

In the second step, transformation, an AP axis is established in the anterior neurectoderm through graded posteriorisation, i.e. the (originally anterior) neurectoderm is induced to become more and more posterior in the direction of the future tail. Two mechanisms seem to be responsible for this. First of all, gradients of several proteins, amongst others FGF and Retinoid acid, exist in the neurectoderm along the future AP axis. Individual cells sense their position along these gradients and take on a more posterior identity according to the concentration of the posteriorising protein ([12], p.9), thereby starting the formation of an AP axis. This mechanism of signaling through the gradients in the neurectoderm is called planar signaling. The gradients and different degrees of posteriorisation pose a challenge when modeling this step. In a Petri net, gradients could be modeled by making the execution of the relevant transitions conditional on the relative concentration of certain entities implemented using range arcs [16].

In addition to the planar signaling, a second mechanism plays an important role in the transformation step; the mesoderm gives off position specific signals, called vertical signals, which posteriorise the neurectoderm directly overlying it in differing degrees, a mechanism called vertical signaling (since the signaling between the two layers is perpendicular to the future AP axis, as opposed to planar signaling, which occurs parallel to this axis), schematically illustrated in Figure 3.

Recent studies have shown that these vertical signals are induced by the expression in the mesoderm of a specific group of genes, called *Hox* genes; the signals might even be expression products of these very genes. *Hox* gene expression in the mesoderm is quite complex and comprises the most interesting modeling dilemma in this case study, including both spatial and temporal aspects.

Hox genes lie on chromosomes in ordered clusters and the onset of expression of each of the genes in the mesoderm is determined by their chromosomal position; genes start to be expressed in the mesoderm sequentially in the order in which they lie on the chromosomes, from the 3' end of the chromosomal cluster towards the 5' end, a peculiar phenomenon known as temporal colinearity. This temporal colinearity in turn leads to spatial colinearity, i.e. the pattern of expression of the different *Hox* genes along the AP axis is also sequential, again according to the position of the genes on the chromosomes. This spatial and temporal collinearity is preserved in evolution and can be found in many different species. Figure 4 illustrates the collinearity in Drosophila melanogaster, the fruit fly.

How temporal and spatial colinearity come to be linked during AP axis formation can be understood when looking at Figure 3. Expression of new *Hox* genes starts in the non-organizer mesodermal ring (NOM) in a temporal sequence, one at a time (each new gene is depicted in a different color in Figure 3). These mesodermal cells then move towards the organizer and start involuting (IM, cf. Figure 2), thereby coming to lie under the neurectoderm (NE). The organizer gives off signals to these mesodermal cells, which stabilize the *Hox* gene expression at that moment in time and thereby create the spatial colinearity; new non-organizer cells expressing new *Hox* genes keep moving towards the organizer, their *Hox* codes get stabilized and they involute in the anterior direction, resulting in a spatial sequence, from anterior to posterior, identical to the temporal sequence. During this process, the Hox gene expression from the mesoderm is copied in the neurectoderm directly overlying it, thereby creating the AP axis in this germ layer.

The two mechanisms of transformation, described above, take place simultaneously, involving different entities and actions, and need to be modeled as such. As a first approximation to model the spatial aspects, we propose to view the system as having locations (similar to the compartments in a membrane system) and thus localised events. The organizer would be a separate part of the system and the moving entities (non-organizer mesoderm), timed by the *Hox* genes, would follow a path towards and subsequently alongside the organizer and on their way influence (via transitions) corresponding areas in the neurectoderm. This would model the vertical signaling. A clock would regulate the onset of the expression of the *Hox* genes. To distinguish between the different *Hox* genes we would use coloured tokens (as in the Coloured Petri Nets model).

4 Future work

To investigate the possibilities of applying Petri nets as a suitable and useful tool for the modeling of dynamic aspects of higher level developmental biological processes, we plan to model in detail the AP axis formation in *Xenopus laevis*. As discussed, we envisage that this will lead to an extended high-level net model supporting concepts like locality, hierarchy, and time. This in its turn will make it necessary to reconsider the existing tools and analysis techniques for Petri nets. Initially, we will focus on the proper rendering of causalities (dependencies) in the biological process. Later we should also investigate the possibilities of a more quantified analysis relating to time and stochastics.

One of the aims of modeling this biological process using a Petri net is to enable simulation of the system under differing circumstances, i.e. predict what would happen if for instance the organizer were to be removed or inactivated. Of particular interest would be predicting outcomes of experiments which are hard to realise in the wet lab. It order to make this possible the model would have to be thoroughly validated first. This can be done using results from previous wet lab experiments. A great number of experiments has already been performed and published ([11, 3, 27, 12]). By simulating these experiments and checking the obtained predictions against the wet lab results we will be able to assess the prediction strength of the model and increase this by adjusting the model. When the model is sufficiently accurate further predictions can be the basis for new wet lab experiments.

On a broader scale this case study will pave the way for higher level modeling of developmental processes using Petri nets. The modeling solutions found for this project apply to many general developmental processes and will serve as tools when modeling larger systems.

Another point of focus in future work is the inclusion in the model of visual information in addition to the textual information obtained from the literature. We are currently building a series of 3D reconstructions of the whole embryo at different developmental stages, in which different Hox gene expression patterns are visualized. These models will be included in the Petri net, as visual aids, and will be able to show predicted outcomes of in silico experiments (e.g. differing combinations of *Hox* expression patterns). In this way we hope to optimize the use of this Petri net model and of future models in developmental biology.

References

[1] R.Banks: Qualitatively Modelling Genetic Regulatory Networks: Petri Net Techniques and Tools. Ph.D. Thesis, Newcastle University, 2009

[2] R.Banks, V.Khomenko, L.J.Steggles: A Case for Using Signal Transition Graphs for Analysing and Refining Genetic Networks. ENTCS 227, 2009, 3–19

[3] N.Bardine: Hox in Frogs. PhD Thesis, University of Leiden, 2008
[4] J.Desel, G.Juhas: "What is a Petri Net?" Informal Answers for the Informed Reader. LNCS 2128, 2001, 1-25
[5] J.Desel, W.Reisig, G.Rozenberg (Eds.): Lectures on Concurrency and Petri Nets, LNCS 3098, 2004
[6] S.P.Ellner, J.Guckenheimer: Dynamic Models in Biology. Princeton University Press, 2006
[7] D.Gilbert, M.Heiner: From Petri Nets to Differential Equations - an Integrative Approach for Biochemical Network Analysis. LNCS 4024, 2006, 181–200
[8] D.Gilbert, M.Heiner, S.Lehrack: A Unifying Framework for Modelling and Analysing Biochemical Pathways Using Petri Nets. LNBI 4695, 2007, 200–216
[9] D.Gilbert, H.Fuss, X.Gu et al.: Computational Methodologies for Modelling, Analysis and Simulation of Signalling Networks. Briefings in Bioinformatics 7, 2009, 339–353
[10] M.Heiner, D.Gilbert, R.Donaldson: Petri Nets for Systems and Synthetic Biology. LNCS 5016, 2008, 215–264
[11] H.J.Jansen: Anterior-posterior Axis Formation in *Xenopus laevis*. PhD Thesis, University of Leiden, 2009
[12] H.J.Jansen, S.A.Wacker, N.Bardine et al.: The role of the Spemann organiser in anterior-posterior patterning of the trunk. Mechanisms of Development 124, 2007, 668–681
[13] K.Jensen: Coloured Petri Nets. Basic Concepts, Analysis Methods and Practical Use. Volume 1, Basic Concepts. Monographs in Theoretical Computer Science, Springer-Verlag, 1997
[14] J.Kleijn, M.Koutny, G.Rozenberg: Process Semantics for Membrane Systems. J. Autom, Lang and Comb 11, 2006, 321–340
[15] J.Kleijn, M.Koutny: Processes of Membrane Systems with Promoters and Inhibitors. Theoretical Computer Science 404, 2008, 112–126
[16] J.Kleijn, M.Koutny: Processes of Petri Nets with Range Testing. Fundamenta Informaticae 80, 2007, 199–219
[17] J.Kleijn, M.Koutny: A Petri Net Model for Membrane Systems with Dynamic Structure. Natural Computing, 2009, to appear
[18] I.Koch, B.H.Junker, M.Heiner: Application of Petri Net Theory for Modelling and Validation of the Sucrose Breakdown Pathway in the Potato Tuber. Bioinformatics 21, 2004, 1219–1226
[19] P.D.Nieuwkoop: Activation and Organisation of the Central Nervous System in Amphibians. III. Synthesis of a New Working Hypothesis. Journal of Experimental Zoology 120, 1952, 83–108
[20] Gh.Păun: Membrane Computing. An Introduction, Springer-Verlag, 2002
[21] Gh.Păun, G.Rozenberg, A.Salomaa (Eds.): The Oxford Handbook of Membrane Computing. Oxford University Press, to appear
[22] J.L.Peterson: Petri Nets. Computing Surveys 9, 1977, 223–252
[23] C.A.Petri: Kommunikation mit Automaten. PhD Thesis, Universitaet Bonn, 1962
[24] W.Reisig and G. Rozenberg (Eds.): Lectures on Petri Nets I and II, LNCS 1491 and 1492, 1998
[25] L.J.Steggles, R.Banks, O.Shaw et al.: Qualitatively Modelling and Analysing Genetic Regulatory Networks: a Petri Net Approach. Bioinformatics 23, 2006, 363–343
[26] C.Talcott, D.L.Dill: Multiple Representations of Biological Processes. Transactions on Computational Systems Biology, 2006
[27] S.A.Wacker, H.J.Jansen, C.McNulty et al.: Timed Interactions Between the Hox Expressing Non-organiser Mesoderm and the Spemann Organiser Generate Positional Information During Vertebrate Gastrulation. Developmental Biology 268, 2004, 207–219
[28] L.Wolpert: Principles of Development. Oxford University Press, 2002

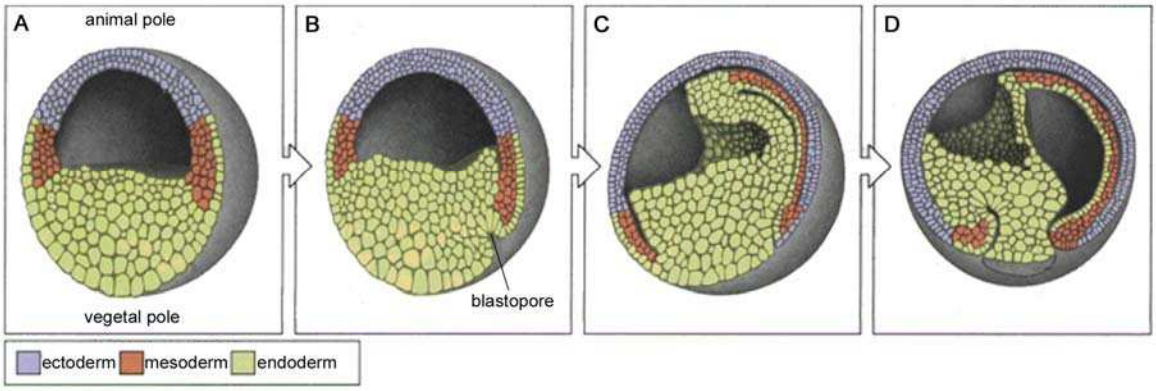

Figure 2: Gastrulation in *Xenopus laevis*; cutaway sagittal view of embryo. Mesoderm starts off as a ring-shape around the embryo (A). Subsequently it moves towards the blastopore (B) and starts to move inwards and upwards, together with the endoderm, through involution (C). In this way the three germ layers come to lie on top of each other, as can be seen in (D). Figure based on [28].

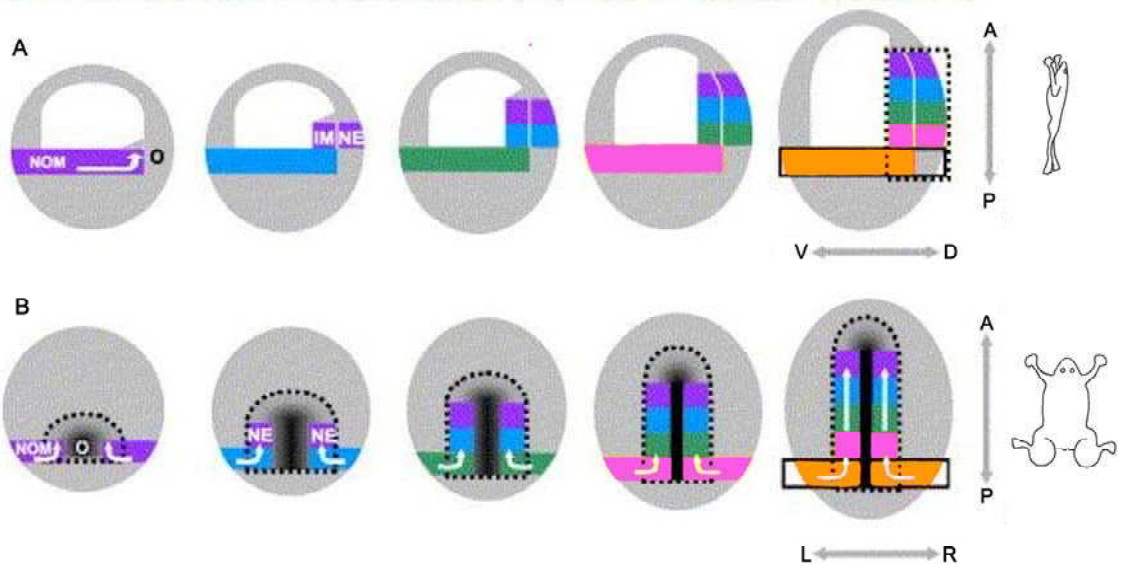

Figure 3: Schematic overview of Hox gene expression and vertical signalling in developmental stages during gastrulation in *Xenopus laevis*; sagittal cutaway view (A) and dorsal view (B); A=anterior, P=posterior, L=left, R=right, NOM=non-organiser mesoderm, O=organiser mesoderm, IM=involuting mesoderm, NE=neurectoderm. The thick black line in the lower series indicates the organiser, the area within the dashed lines indicates the non-organiser mesoderm directly surrounding and receiving signals from the organiser.Within this dashed line the vertical signalling takes place and the neurectoderm, the outer layer on the right in (A) copies the Hox code from the mesoderm underlying it. The white arrows indicate the movement of the mesoderm, first along the ring-shape from ventral to dorsal, and subsequently through involution, from posterior to anterior. The different colours indicate the different Hox genes expressed in the mesoderm and neurectoderm over time. NB: the colours used in this figure, indicating different Hox genes, do not correspond to the colours in Figure 2, indicating the germ layers. Figure based on [11].

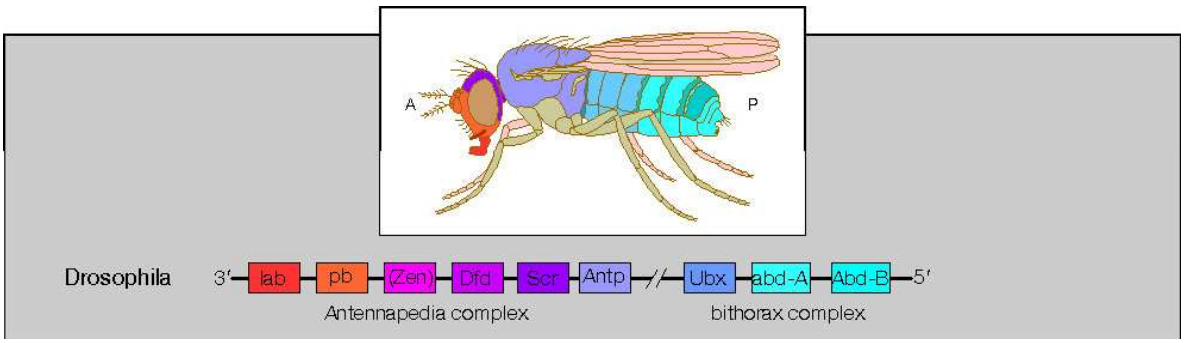

Figure 4: Hox gene collinearity in *Drosophila melanogaster*, the fruit fly. The order in which the Hox genes lie on the chromosome (shown in the coloured boxes) corresponds to the temporal order in which they start being expressed, which results in the same spatial order along the AP axis, as can be seen in the illustration of the fly. Figure from [28].