Computerised Modelling for Developmental Biology

Bertens, L.M.F.

CHAPTER 5. AN ALGORITHMIC PROCESS MODEL: MODELLING GRADIENTS USING PETRI NETS

Omnia mutantur, nihil interit.

Everything changes, nothing perishes.

- Ovid, *Metamorphoses* XV, v.165

The third category of modelling approaches discussed in chapter 1 comprises algorithmic process models, among which Petri nets, and the current chapter presents a case study of the application of Petri nets to the field of developmental biology. We have chosen to model the process of gradient formation, since this process is modular and concurrent in nature and can be placed in a hierarchical structure with other simultaneous and interlinked processes. Petri nets are distinguished by their ability to model these features; the combination of modelling approach and the process to be modelled therefore provides an optimal context to fully explore the possibilities of this approach for developmental biology. Here a qualitative method is applied, not taking into account exact numerical data. In the next chapter a more quantitative approach is applied to the same developmental process.

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5.1 INTRODUCTION

Petri nets (Reisig and Rozenberg, 1998) have been shown to be very promising for molecular and cellular biology, in particular for metabolic, signalling and gene-regulatory networks (see *e.g.* Banks, 2009; Banks *et al.*, 2009; Chaouiya, 2007; Gilbert and Heiner, 2006; Gilbert *et al.*, 2007; Heiner *et al.*, 2008; Koch *et al.*, 2004; Steggles *et al.*, 2006; Talcott and Dill, 2006). We believe Petri nets to be useful for higher level developmental processes as well, *e.g.* on tissue and organ level. Therefore this chapter concerns the use of Petri nets as an abstract modelling tool for higher level processes in the organism, taking cells as central elements. To this end we have selected one developmental process, the formation of a morphogen gradient, as a case study for this approach. This process helps instigate the differentiation of cells along the developing axis in the organism. In early development, gradients are crucial (Wolpert, 2002) and finding a modelling solution for the generic process of gradient formation will not only serve the theoretical goal of investigating the use of Petri nets for developmental biology. It will also, in a more practical sense, be useful for the modelling of other developmental processes in which gradients play a role. By staying very close to the biological sequence of events in gradient formation, rather than focusing on a concrete outcome, the model should be generally applicable and robust. The implications of this approach are further addressed in chapter 6.

Throughout this chapter the emphasis will be on abstraction and modelling decisions, as opposed to implementation of specific biological data (which will be addressed in chapter 6). The main question dealt with in this chapter is: how can Petri nets be used to model higher level developmental processes, which focus on cells as the central units? We present a basic Petri net, modelling gradient formation, which serves as a proof of concept for our approach. In the remainder of this chapter we outline the biological background of gradient formation, we describe our modelling decisions and we present the model. In the last section the possibilities of the model and future work are discussed.

5.2 PT-NETS WITH ACTIVATOR ARCS

For a general introduction to Petri nets we refer to (Reisig and Rozenberg, 1998). Here, we use PT-nets with activator arcs (Kleijn and Koutny, 2007) and a maximally concurrent execution rule (Burkhard, 1983).

Petri nets are defined by an underlying structure consisting of *places* and *transitions*. These basic elements are connected by directed, *weighted arcs*. In the Petri net model considered in this chapter, there are moreover *activator arcs* connecting places to transitions. In modelling, places are usually the passive elements, representing

local states, and transitions the active elements. Here, global states, referred to as *markings*, are defined as mappings assigning to each place a natural number (of *tokens* corresponding to available resources).

A *PTA-net*, is a tuple $N = (P, T, W, Act, m_0)$ such that:
– P and T are finite disjoint sets, of the *places* and *transitions* of N, respectively.
– $W : (T \times P) \cup (P \times T) \to \mathbb{N}$ is the *weight function* of N.
– $Act \subseteq P \times T$ is the set of *activator arcs* of N.
– $m_0 : P \to \mathbb{N}$ is the *initial marking* of N.

In diagrams, places are drawn as circles, and transitions as boxes. Activator arcs are indicated by black-dot arrowheads. If $W(x, y) \geq 1$, then (x, y) is an *arc* leading from x to y; it is annotated with its weight if this is greater than one. A marking m is represented by drawing in each place p exactly $m(p)$ tokens as small black dots. We assume that each transition t has at least one input place (there is at least one place p such that $W(p, t) \geq 1$).

When a single transition t occurs ('fires') at a marking, it takes tokens from its input places and adds tokens to its output places (with the number of tokens consumed/produced given by the weights of the relevant arcs). Moreover, if there is an activator arc $(p, t) \in Act$, then transition t can only be executed at the given marking if p contains at least one token, without the implication of tokens in p being consumed or produced when t occurs. Thus, the difference with a *self-loop*, *i.e.,* an arc from p to t and vice versa, is that the activator arc only tests for the presence of tokens in p.

We define the executions of N in the more general terms of simultaneously occurring transitions. A step is a multiset of transitions $U : T \to \mathbb{N}$. Thus $U(t)$ specifies how many times transition t occurs in U. (Note that if we exclude the empty multiset, single transitions can be considered as minimal steps.) Step U is *enabled* (to occur) at a marking m if m assigns enough tokens to each place for all occurrences of transitions in U and, moreover, all places tested through an activator arc by a transition in U, contain at least one token. Formally, step U is enabled at marking m of N if, for all $p \in P$:

– $m(p) \geq \Sigma_{t \in T} U(t) \cdot W(p, t)$
– $m(p) \geq 1$ whenever there is a transition t such that $U(t) \geq 1$ and $(p, t) \in Act$.

If U is enabled at m, it can be *executed*, leading to the marking m' obtained from m through the accumulated effect of all transition occurrences in U:

– $m'(p) = m(p) + \Sigma_{t \in T} U(t) \cdot (W(t, p) - W(p, t))$ for all $p \in P$.

Finally, a step U is said to be *max-enabled* at m if it is enabled at m and there is no step U' that strictly contains U (meaning that $U' \neq U$ and $U(t) \leq U'(t)$ for all transitions t) and which is also enabled at m. And we write $m[U\rangle m'$ if U is max-enabled at m and execturion of U at m leads to m'. A (max-enabled) *step sequence* is then a sequence $\sigma = U_1 \ldots U_n$ of non-empty steps U_i such that $m_0 [U_1\rangle m_1 \ldots m_{n-1} [U_n\rangle m_n$, for some markings $m_1, \ldots, m_n$ of N. Then m_n is said to be a reachable marking of N (under the maximally concurrent step semantics).

To conclude this preliminary section, we elaborate on the choice of this particular net model. First, it should be observed that it follows from the above definitions that the semantics allow *auto-concurrency*, the phenomenon that a transition may be executed concurrently with itself. This approach makes it possible to use transitions for a faithful modelling of natural events like the independent (non-sequential) occurrence in vast numbers of a biochemical reaction in a living cell. Note that the degree of auto-concurrency of a transition can easily be controlled by a dedicated place with a fixed, say k, number of tokens connected by a self-loop with that transition implying that never more than k copies of that transition can fire simultaneously.

Activator arcs were introduced in (Janicki and Koutny, 1995) as a means of testing for the presence of at least one token in a place, and so they are similar to other kinds of net features designed for the same reason. We mentioned already self-loops by which the presence of a token in a place can be tested only by a single transition (which 'takes and returns' the token) and not simultaneously by an arbitrary number of transition occurrences in a step. Two other mechanisms related to activator arcs, which do allow such multiple testing are *context arcs* (Montanari and Rossi, 1995) and *read* (or *test*) *arcs* (Vogler, 2002). Both, however, display important differences when compared with activator arcs. A context arc testing for the presence of a token in place p by transition t indicates that after a step in which t participates has been executed, p must still contain a token which precludes the occurrence in the same step of transitions that have p as an input place. A read arc is also different, but less demanding in that there must exist a way to execute sequentially (*i.e.*, one-by-one) all transition occurrences in the step, without violating the read arc specification. In both cases, one can easily see that activator arcs are most permissive since they only check for the presence of a token before the step is executed (this is often referred to as *a priori* testing). We feel that *a priori* testing is more appropriate for biological applications as the 'look ahead' implied by the other two kinds of test arcs is hard to imagine in reality.

Finally, we rely in this chapter on *maximal concurrency* in the steps that are executed which reflects the idea that execution of transitions is never delayed. This may also be viewed as a version of time-dependent Petri nets where all transitions have a

firing duration of 1. However, the maximal concurrency we apply here does not derive from Petri nets with time, but rather from Petri nets with *localities* (Kleijn *et al.*, 2006) leading to *locally maximal* semantics. This semantics is what we plan to use to model other aspects of the development as well. Here one may think of *e.g.* the locally synchronous occurrence (in pulses) of reactions in individual compartments of a cell.

5.3 BIOLOGICAL BACKGROUND AND MODELLING DECISIONS

5.3.1 Mechanisms of biological gradient formation

In biology, the term gradient is used to describe a gradual and directed change in concentration of a morphogen through a group of cells, *e.g.*, a tissue. Morphogens are signalling molecules that cause cells in different places in the body to adopt different fates and thereby help establish embryonic axes. Morphogens are produced in a localized source of a tissue, the source cell(s), and emanate from this region, forming a concentration gradient (Gurdon and Bourillot, 2001; Teleman *et al.*, 2001). A morphogen gradient has an immediate effect on the differentiation of the cells along it; cells are able to 'read' their position along the gradient and determine their developmental fate accordingly. They have a range of possible responses and the morphogen concentration dictates which response will be exhibited (Gurdon and Bourillot, 2001; Teleman *et al.*, 2001).

The mechanisms by which the morphogen travels through a cell layer have been the topic of some debate and are not yet fully understood. Three mechanisms have been described, shown schematically in Figure 5.1: (A) diffusion through the extracellular matrix (Fischer *et al., 2006; Gurdon et al.*, 1994; Lander *et al.*, 2002), either passively, like a drop of ink in water (Gurdon *et al.*, 1994), or facilitated by receptors on the cell surface which guide the morphogens along (Fischer *et al.*, 2006), as shown in the figure; (B) sequential internalization of the morphogen molecules in vesicles in the cells, a process called endocytosis, and subsequent re-emission (Entchev and Gonzalez-Gaitan, 2002; Fischer *et al.*, 2006; Teleman *et al.*, 2001); (C) direct contact between the cells by means of tentacle-like threads of cytoplasm, called cytonemes, connecting the cells (Gurdon and Bourillot, 2001). These mechanisms are not necessarily mutually exclusive and some studies conclude that a combination of mechanisms underlies the formation of a gradient, (*cf.* Kicheva *et al.* 2007, on which the case study in 6.4 is based). It is important to note that both diffusion and endocytosis take place between neighbouring cells, while cytonemes connect all cells directly to the source. This makes it very different from a modelling perspective, as will be discussed below. In this and the next chapter we will solely be concerned with communication between neighbouring cells, *i.e.* through diffusion and endocytosis.

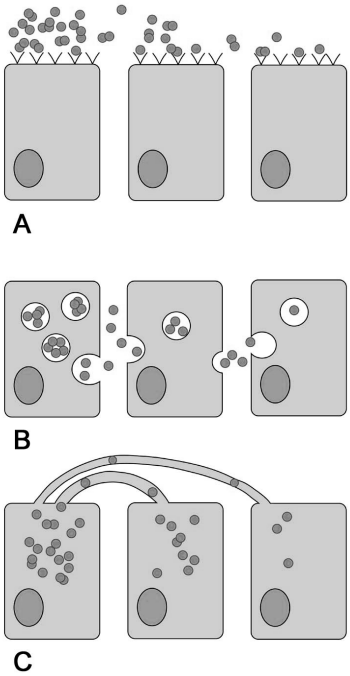

Figure 5.1. Three possible mechanisms for gradient formation: diffusion (A), endocytosis subsequent re-emission (B) and transport through cytonemes (C).

Unfortunately, knowledge of the exact concentrations and shapes of most gradients is often limited. This is mainly due to the transient nature of morphogen gradients and the low concentrations at which they are effective, both of which make it difficult to visualise the morphogens (Gurdon *et al.*, 1994). Many morphogens are rapidly degraded or prevented from binding to receptors by antagonistic proteins (Gurdon *et al.*, 1994). Much of the information on gradients is therefore obtained indirectly, by observing their effect, *i.e.*, the responses of the cells involved (Gurdon *et al.*, 1994). A qualitative approach, such as the one presented in this chapter, circumvents this issue by not relying on exact quantitative data. However, in cases for which quantitative data is available, incorporating this in the model will yield a more detailed and practical model of process under study. Such a quantitative model is presented in chapter 6, along with a case study based on experimental observations.

5.3.2 Modelling decisions

We have chosen **cells as the elementary units** in our model, to be represented by places in the Petri net. Earlier studies (Bonzanni *et al.*, 2009; Krepska *et al.*, 2008; Matsuno *et al.*, 2003) have successfully modelled cell-to-cell signalling, starting from a lower biological level, using places to represent genes and proteins. Although this allows a

high level of detail, it also complicates the net and makes it difficult to identify single cells. In our approach the cellular level represents the intermediate level between the subcellular levels, on which the morphogen signalling between cells takes place, and the tissue/organ level, where whole cell layers may move.

Furthermore, the process lends itself to be modelled using a **modular approach**; for each of the neighbouring biological cell pairs identical modules of places and transitions are used. This makes it easy to change or extend the model or to adjust parameter values according to different experimental data.

We let **tokens represent morphogen levels**, conducted from cells to neighbouring cells by the transitions. By changing the interpretation of the tokens a range of levels is possible, from exact quantitative modelling, in which each token corresponds to a precise morphogen number, to strictly qualitative modelling (Kleijn *et al.*, 2006), in which markings become binary, indicating merely the presence or absence of morphogens in a cell. In an intermediate, semi-qualitative approach, increasing token numbers equal increasing morphogen levels, without exact molecular numbers. Petri nets allow modelling at all these different levels. Biological gradients often work in a rather discrete, semi-qualitative manner; a number of cell responses (such as activation of a particular gene) exists for a given gradient and threshold values in morphogen concentration demarcate the boundaries between these responses, resulting in a stepwise change in cellular behaviour throughout the tissue. Due to this, both semi-qualitative and quantitative ways of modelling can represent biological situations realistically; our Petri net model is applicable to both. Moreover, it is possible to model the formation of a gradient in a quantitative manner, but let other processes which depend on the morphogen levels do so in a semi-qualitative way, by using threshold values. Since we do not use experimental quantitative data in the current chapter, the model can be interpreted as semi-qualitative; in the next chapter an example will be presented of an entirely quantitative approach, in which tokens numbers directly corresponds to numbers of morphogen molecules.

Instead of merely calculating the final distribution of the tokens, we want our net to model the gradual process of morphogen movement through the tissue, *i.e.* to **represent all intermediate steps**. This will allow the user to simulate experiments in which the process is altered while running; *e.g.* grafting experiments, in which parts of the tissue get removed or replaced, can be simulated by taken cells out of the net at a certain moment during the process or depleting them of tokens.

Our model focuses on **local signalling between neighbouring cells**. Therefore we take into account cell-to-cell communication mechanisms, *e.g.* endocytosis and diffusion, but not long distance transport mechanisms, *e.g.* through cytonemes. In the situation of local signalling, the number of morphogens to be transported from one cell

to the next depends solely on the difference in morphogen level between these two neighbouring cells; cells have no 'knowledge' of morphogen transport in other parts of the tissue. In order to accurately reflect this situation we base the computation of transported tokens solely on the difference in token numbers between the neighbouring cells. This makes the model easily scalable, *i.e.* the number of cells in the tissue is irrelevant to the computation and can be adjusted without altering the workings of the model.

5.3.3 Implementation

Often exact quantitative data for the processes of morphogen transport and degradation between neighbouring cells are not known, and these may vary depending on the gradient considered. Therefore we do not discern the molecular mechanisms of diffusion, endocytosis and degradation of morphogens in this model but we introduce a parameter ρ in our model to represent the effective **ratio of concentration levels** between neighbouring cells and to determine the amount of tokens to be transported between places during the simulation of gradient formation. In other words, ρ represents the final ratio of morphogens between neighbouring cells and morphogen degradation is implicit. In the next chapter we present a model in which production, transport and degradation are modelled explicitly.

In the organism, gradient ratios arise passively as a consequence of physical laws. However, to accurately reflect the biological process of gradient formation underlying the spread of morphogens from cell to cell, our formal model has to compute the number of tokens passed on based on the ratio ρ. Hence, the model includes an **explicit separate computational unit** for each pair of neighbouring cells, to perform the necessary calculations. In particular, these parts of the net control the transport of tokens between places. In this way a close relation to the biological process can be maintained in one part of the net, with the underlying computations being performed in the background by another part of the net. At all times, the marking of the places representing biological cells will be consistent with biological observations of (the effect of) the gradient, *i.e.*, the ratio is maintained and places corresponding to cells further away from the source will never have more tokens than places (cells) closer to it.

Another important feature of the model is the **use of concurrent steps** rather than individually occurring transitions. Morphogen transport between cells is not directly influenced by events taking place in non-adjacent cells, which means these processes should be able to take place concurrently and non-adjacent cells can be simultaneously involved in the transport of morphogens. This leads to an execution mode consisting of *concurrent steps*. Moreover, since in the biological situation

morphogens move to the next cell as soon as this is possible, we have chosen to use a **maximally concurrent steps**.

5.4 GRADIENTS AND PETRI NETS

5.4.1 Modelling solution

Following the ideas outlined in the previous section, we will propose a formal model for the formation of a gradient. Our assumptions regarding the biological process of gradient formation are as follows. Given is a segment of k adjacent cells with the i-th cell immediate neighbour of the (i+1)-th cell. Morphogens can be transported only between immediate neighbours. Morphogens move from cells with higher concentration to neighbours with lower concentration, as long as the concentration ratio between these cells does not exceed a given gradient ratio $0 < \rho < 1$. We assume that ρ is a rational number, *i.e.*, $\rho = N/M$, where $M > N \geq 1$. Initially, the first cell x_1 (the source) contains a quantity (*i.e.* has a concentration level of) K of a morphogen. These assumptions lead to the following modelling problem.

Given are $k \geq 1$ places $x_1, \ldots, x_k$, representing a segment of k cells with place x_i corresponding to the i-th cell. In the initial marking m_0, the first place x_1 contains K tokens and there are no tokens in the other places. In the net modelling the mechanism of gradient formation, we need to shift tokens from x_1 in the direction of the last place x_k. Places and/or transitions may be added, but in such a way that for any reachable marking m the following hold.

1. The number of tokens in the x_i's remains constant, *i.e.*,
$$m(x_1) + \ldots + m(x_k) = K \qquad \textbf{token preservation}$$
2. The tokens are distributed monotonically along the sequence of k places, *i.e.*,
$$m(x_1) \geq \ldots \geq m(x_k) \qquad \textbf{monotonicity}$$
3. The ratio of the numbers of tokens in two neighbouring places does not exceed ρ, *i.e.*, for every $1 \leq i < k$ with $m(x_i) \geq 1$:
$$(m(x_{i+1})/m(x_i)) \leq \rho \qquad \textbf{ratio}$$
4. Shifting continues until moving even one token would violate the above, *i.e.*, if no tokens are shifted after marking m was reached, then for every $1 \leq i < k$ with $m(x_i) > 1$:
$$(m(x_{i+1})+1)/(m(x_i)-1) > \rho \qquad \textbf{termination}$$

Moreover, the relative position of a place within the sequence plays no role. In particular, the mechanism should be easily scalable and insensitive to the specific values of k and K.

If we look at the above formulation of properties (2) and (3) — monotonicity and preservation of the gradient ratio — and recall that $\rho = N/M$ and $M > N$, it is easy to observe that these two properties are together equivalent to stating that, for every $1 \le i < k$, $N \cdot m(x_i) - M \cdot m(x_{i+1}) \ge 0$. We will call a marking m satisfying this inequality consistent and denote $\alpha_i = N \cdot m(x_i) - M \cdot m(x_{i+1})$, for every $1 \le i < k$. Note that the initial marking is consistent.

Similarly, if we look at the above formulation of properties (2) and (4) — monotonicity and termination — it is easy to observe that together they are equivalent to the statement that, for every $1 \le i < k$, $N \cdot m(x_i) - M \cdot m(x_{i+1}) < M + N$. We will call a consistent marking m satisfying this inequality stable. Note that for a given ρ, k and K, there may be more than one stable marking. For example, if $\rho = 1/2$, $k = 5$ and $K = 111$, then the following are two different stable markings:

x_1	x_2	x_3	x_4	x_5	x_1	x_2	x_3	x_4	x_5
59	29	14	6	3	58	29	14	7	3

We are now ready to propose a generic solution for the above problem. For a given consistent marking m and each $1 \le i < k$, move β_i tokens from x_i to x_{i+1} where $\beta_i \le \left\lfloor \frac{\alpha_i}{M+N} \right\rfloor$, and at least one β_i must be non-zero if at least one of the values $\left\lfloor \frac{\alpha_i}{M+N} \right\rfloor$ is non-zero. We denote the resulting marking by $m_{\beta_1 \dots \beta_{k-1}}$.

An intuitive reason for proposing such a mechanism for shifting tokens is that the number of tokens in x_i that are 'balanced' by tokens in x_{i+1} is $(M/N) \cdot m(x_{i+1})$, because each token in x_{i+1} is equivalent to M/N tokens in x_i. Hence there are $m(x_i) - (M/N) \cdot m(x_{i+1})$ unbalanced tokens in x_i. The 'portion' of each unbalanced token that could be safely transferred to x_{i+1} is $N/(M+N)$. Hence in total we may safely transfer $\left\lfloor \frac{N}{M+N} \cdot \left(m(x_i) - \frac{M}{N} \cdot m(x_{i+1})\right) \right\rfloor$ tokens, which is precisely $\left\lfloor \frac{\alpha_i}{M+N} \right\rfloor$ tokens. Clearly, some of the numbers $\beta_1, \dots, \beta_{k-1}$ can be zero, and by the condition above, all β_i's are zeros if and only if the marking is stable:

Proposition 1. $\beta_1 = \cdots = \beta_{k-1} = 0$ *if and only if m is stable*.

Crucially, by the mechanism proposed consistent markings are always transformed into consistent markings.

Proposition 2. *If m is a consistent marking then* $m_{\beta_1 \dots \beta_{k-1}}$ *is also consistent*.

According to the above, any number of tokens not exceeding $\left\lfloor \frac{\alpha_i}{M+N} \right\rfloor$ can be moved *simultaneously* from x_i to x_{i+1} (for every $i < k$), and consistency will be

preserved. Clearly, the new consistent marking is different from the previous one if and only if, for at least one i, we have $\beta_i \geq 1$. The idea now is to keep changing the marking on $x_1, \ldots, x_k$ until a marking m has been reached such that $\left\lfloor \frac{\alpha_i}{M+N} \right\rfloor = 0$, for all $1 \leq i < k$, which is equivalent to $\alpha_i < M + N$, for all $1 \leq i < k$. In other words, this m is a stable marking. Since tokens cannot be shifted forever, this procedure will always terminate in a stable marking (formally, we can show this by considering a weighted distance to the end of the chain of the K tokens; it never increases and always decreases in a non-stable state).

Looking now from the point of view of a Petri net implementation of the proposed mechanism, what we are after is a net N_{shift} comprising the places $x_1, \ldots, x_k$ and such that if m is a marking of N_{shift} whose projection on these k places is consistent, then a step U can occur at m if

– it moves at most $\left\lfloor \frac{\alpha_i}{M+N} \right\rfloor$ tokens from x_i to x_{i+1}, for all $1 \leq i < k$;

– at least one token is moved from x_i to x_{i+1} for at least one $1 \leq i < k$, unless the projection of m onto $x_1, \ldots, x_k$ is stable.

In fact, in the proposed implementation, we will be preceding the 'token-shifting' with a 'pre-processing' stage which seems to be unavoidable unless one uses some kind of arcs with complex weights depending on the current net marking.

5.4.2 Implementation

In the implementation of the proposed shifting mechanism, as many tokens as possible should be shifted from one neighbour to the next. That means that, at each stage we have $\beta_i = \left\lfloor \frac{\alpha_i}{M+N} \right\rfloor$ for every $1 \leq i < k$. Moreover, tokens are shifted from a place without any assumptions whether new tokens will come to that place from its other neighbour. Thus we need to provide a Petri net structure capable of 'calculating' the value of expressions like $\left\lfloor \frac{N \cdot m(x_i) - M \cdot m(x_{i+1})}{M+N} \right\rfloor$.

Our proposed gradient forming mechanism distinguishes three phases: I, II and III. An auxiliary net N_{3phase}, shown in Figure 5.2B, is used to schedule the transitions implementing the calculations. It controls these transitions via the places w^I and w^{II} and activator arcs. For the full picture of the system one should combine the figures for all

pairs (x_i, x_{i+1}) with a single copy of the net in Figure 5.2B. Note that all places with identical label (in particular w', w'', and w''') should be identified. That other parts of the encompassing net model do not interfere with the calculations carried out during phases I and II can be ensured by connecting the relevant transitions with the place w''' using activator arcs.

For every $1 \le i < k$, transition t_i is intended to shift tokens from x_i to x_{i+1} (phase III). To achieve this, we use two disjoint sets of new, auxiliary places, $x'_1, \ldots, x'_k$ and $x''_1, \ldots, x''_k$. These places are initially empty. The idea is to fill x'_i with $N \cdot m(x_i)$ tokens and x''_{i+1} with $M \cdot m(x_{i+1})$ tokens (phase I). The latter are used for the removal of $M \cdot m(x_{i+1})$ tokens from x'_i (phase II). After this, there are α_i tokens remaining in x'_i. Finally, for each group of $N + M$ tokens in x'_i, one token is shifted from x_i to x_{i+1}. The construction (for x_i and x_{i+1}) is shown in Figure 5.2A.

The overall mechanism operates in cycles of three consecutive, maximally concurrent steps such that for every $1 \le i < k$:

I. Transition c_i, inserts (in $m(x_i)$ auto-concurrent occurrences) $N \cdot m(x_i)$ tokens into x'_i. In the same step, transition c_{i+1}, inserts (in $m(x_i)$ auto-concurrent occurrences) $M \cdot m(x_{i+1})$ tokens into x''_{i+1}. Simultaneously, transitions e'_i and e''_{i+1} empty x'_i and x''_{i+1} of any residual tokens left from the previous cycle.

II. Next, transition d_i (in $M \cdot m(x_{i+1})$ auto-concurrent occurrences) empties x''_{i+1} and leaves in x'_i the difference $\alpha_i = N \cdot m(x_i) - M \cdot m(x_{i+1})$.

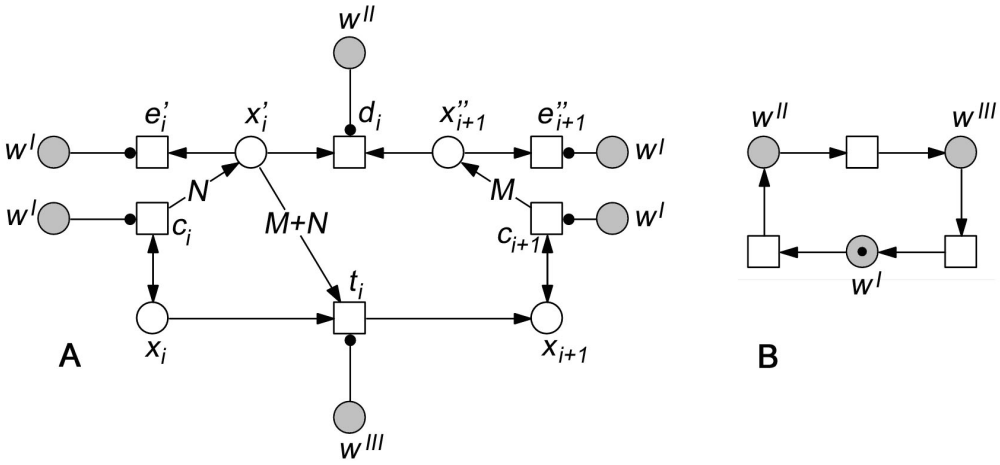

Figure 5.2. (A) The main part of the construction for the solution (note that e''_{i+1} is introduced for later use when one might want to remove or add tokens to the x_i's from 'outside'; in the standard (consistent) situation it is never activated as after phase II, place x''_{i+1} is empty); and (B) the subnet N_{3phase} enforcing the three phases.

III. In the third step, the occurrences of transition t_i transfer $\beta_i = \left\lfloor \frac{\alpha_i}{M+N} \right\rfloor$ tokens from x_i to x_{i+1}.

Proposition 3. Each cycle results in transferring β_i tokens from x_i to x_{i+1}.

Note that in this implementation with the control net N_{3phase}, neighbouring pairs are either all involved in calculations (step I and II of the cycle) or tokens are transferred between neighbours (step III). During the whole operation of the adjustment process (except for the transfer phase), the token numbers in the places x_i, representing the cells, are unchanged and they can be accessed for reading by other transitions (and thus influence neighbouring cells). In other words, calculations are *orthogonal* to the basic operation of the net (the gradient formation). As an example, let us consider the case when ρ = 1/2 , k = 4 and K = 100. Then executing the constructed net in a maximally concurrent manner leads to the following sequence of markings on the x_i after each cycle and eventually to a stable marking:

x_1	100	67	67	60	60	57	57	56	56	55	55	54
x_2	0	33	22	29	25	28	26	27	26	27	26	27
x_3	0	0	11	8	12	10	12	12	13	12	13	13
x_4	0	0	0	3	3	5	5	5	5	6	6	6

The next example shows what happens if we start from a (non-initial) consistent marking (again ρ = 1/2):

x_1	200	167	156	...
x_2	50	67	67	...
x_3	0	16	22	...
x_4	0	0	5	...

The construction works without any problems, if we start with a consistent marking. In case $0 > \alpha_i$ for some i, then transition t_i is not executed, but the transitions t_{i-1} and t_{i+1} may still be executed and lead to an adjustment of the marking causing t_i to become active in the next cycle. A further observation is that adding (or removing) tokens at some point, will trigger a re-adjustment process which tries to re-establish the correct ratios between the markings of adjacent places x_i. This process is unpredictable, but to deal with that case we have included transition e''_{i+1} which in the standard (consistent) situation is never activated since then, after phase 2, place x''_{i+1} is empty.

An important characteristics of the proposed solution is that it is purely local and does not assume anything about the number of tokens which may appear in the x_i's nor the length of the chain. In other words, it is truly generic. What's more it also works

if M and N are different for different pairs of neighbouring places, *i.e.*, if rather than a uniform gradient ratio ρ there is a ratio ρ_i for each pair of neighbours x_i and x_{i+1}.

Another feature of our solution is the maximal concurrency semantics intended to reflect the idea of morphogens (simultaneously) moving from cell to neighbouring cell whenever that is possible. The preliminary sequential semantics model we developed (but not reproduced here) is more complicated as it also needs *inhibitor arcs* which test for absence of tokens (to decide whether or not tokens should still be shifted). Moreover, one needs to decide that x_i either receives or sends tokens at each stage. In a step model it can both receive and send. Also, with the maximal concurrency semantics, the number of states of the model is dramatically reduced. The auxiliary net N_{3phase} is used to partly sequentialise the behaviour in order to separate the pre-processing phases from the actual shifting phase. This net could also have been made local to the main construction of the net in Figure 5.2A, with different copies of it assigned to different localities. This would have given the additional possibility of controlling the degree of synchronisation between different parts of the gradient model by using a locally maximal step semantics.

Finally, we would like to point out that the activator arcs in our implementation are used only to control the calculation and can actually be avoided in case there would be a limit on the number of tokens in each place x_i at any time. (Then the activator arcs can be eliminated basically by having separate copies of N_{3phase} for each $1 \leq i < k$, transfer around sufficiently many tokens in a bundle, and replace activator arcs by self-loops). This assumption corresponds to having (or knowing) some capacity bound on the concentration levels of morphogens in a cell and so may be biologically sound.

5.5 CONCLUSION

Starting from gradient formation in the AP axis development in the model organism *Xenopus laevis*, we have presented a novel approach to using Petri nets in developmental biology by focusing on the cellular rather than subcellular levels and abstracting from concrete proteins and genes. This has led to a parameterised Petri net model for the general process of gradient formation through diffusion and endocytosis.

Assumptions regarding gradient formation have been formulated based on essential features of this process as reported in the literature. These assumptions underlie the precise requirements given that should be satisfied by an abstract Petri net model of gradient formation. A crucial point here is the consistency that is maintained during the execution of the model. Hence the realization of the gradient is faithfully reflected. Moreover the close relationship between biological process and evolution of the formal model makes it possible to apply existing Petri net techniques to analyse

what happens during gradient formation. In particular cause-effect relations should be properly reflected in the process semantics of the modelling Petri net (Kleijn and Koutny, 2007; Kleijn and Koutny, 2008).

Another main contribution of this approach is its generic nature, leading to a model that is scalable and applicable to a plethora of specific gradients. Also scalability is a consequence of the faithful reflection of the biological process. Since the final token (morphogen) distribution is not directly computed from the initial amount of morphogen and the length of the chain of cells, but rather simulates the communication between neighbouring cells, the length plays no role in the occurrence of the steps. The model as presented here represents a one-morphogen system without relying on quantitative data, but exact values could be assigned to ratio and individual tokens. Moreover, it provides a basis for simulation of simultaneous gradient formation (different morphogens with different experimental initial markings) and for inhibiting/activating interactions between them. Simulation with actual biological data to validate the model should be a next step; the elaborated model presented in chapter 6 illustrates this. In addition, we will focus our attention on the extension of this still rather basic model to more dimensions, *e.g.*, rather than having just a single line of cells, we consider the spread of morphogens from a source throughout a tissue plane or volume.

In (Bonzanni *et al.*, 2009; Krepska *et al.*, 2008), Petri nets are used to model developmental processes in a way similar to our approach when it comes to the semi-qualitative use of tokens and the use of maximal concurrency. In these papers however, the focus is on subcellular levels. Petri net places are used to represent genes and gene products, where in our approach cells, as basic units in a tissue, are modelled by places. Having cells as basic units should prove to be a useful intermediate position convenient for 'zooming in and out' between subcellular and tissue level. It is our aim to model more subprocesses of the AP axis formation. For instance the different molecular processes underlying diffusion and endocytosis could be modelled in subnets, allowing the user to compare the different effects of these mechanisms. Also the degradation of morphogens could be modelled by a subnet, making the entire process more explicit. In the next chapter a step in that direction is taken, by modelling production, transport and degradation explicitly in the net, by means of parameters derived from an equation based model; a further future development would be to faithfully model the events underlying these subprocesses and thereby have the parameter values arise out of the workings of the subnets, as opposed to providing them to the main as as external information.