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Investigating the Turing conditions for diffusion-driven instability in the presence of a binding immobile substrate

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ABSTRACT

Turing's diffusion-driven instability for the standard two species reaction–diffusion system is only achievable under well-known and rather restrictive conditions on both the diffusion rates and the kinetic parameters, which necessitates the pairing of a self-activator with a self-inhibitor. In this study we generalize the standard two-species model by considering the case where the reactants can bind to an immobile substrate, for instance extra-cellular matrix, and investigate the influence of this dynamics on Turing's diffusion-driven instability. Such systems have been previously studied on the grounds that binding of the self-activator to a substrate may effectively reduce its diffusion rate and thus induce a Turing instability for species with equal diffusion coefficients, as originally demonstrated by Lengyel and Epstein (1992) under the assumption that the bound state dynamics occurs on a fast timescale. We, however, analyse the full system without any separation of timescales and demonstrate that the full system also allows a relaxation of the standard constraints on the reaction kinetics for the Turing instability, increasing the type of interactions that could give rise to spatial patterning. In particular, we show that two self-activators can undertake a diffusively driven instability in the presence of a binding immobile substrate, highlighting that the interactions required of a putative biological Turing instability need not be associated with a self-activator–self-inhibitor morphogen pair.

1. Introduction

Alan Turing (1952) wrote his seminal paper on biological pattern formation, in which he showed that a system of chemicals (which he termed morphogens) undergoing reaction and diffusion can lead to the counter-intuitive phenomenon of diffusion-driven spatial heterogeneity. That is, a spatially uniform steady state, stable in the absence of diffusion, could be driven unstable by diffusion, evolving into a spatially heterogeneous state, a pattern. Furthermore, with non-dimensionalisation of the system equations to a fixed size domain, the diffusion coefficients acquire a domain-size dependence and hence one can deduce that Turing's instability will induce symmetry breaking from fluctuations as a domain adiabatically grows beyond a critical size. Consequently, this instability can drive the spontaneous formation of pattern, triggered simply by domain growth rather than any exquisite long-range cellular communication, and Turing proposed that this mechanism could induce a pre-pattern for cell differentiation in early developmental biology. However, this hypothesis laid largely ignored until the seminal paper of Gierer and Meinhardt (1972) 20 years later, which analysed the two chemical cases in detail. This demonstrated two ways in which pattern could arise, one of which for instance is referred to as "short-range-activation, long-range-inhibition". Further, one can readily demonstrate that the Turing instability in general for the two-species system, in the absence of a binding substrate, necessitates a short range morphogen which is a self-activator, i.e. it upregulates its own production,[1] interacting with a long range

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[1] At least in the neighbourhood of the steady state so that the corresponding diagonal entry in the Jacobian matrix evaluated at the steady state is positive. The concept of self-activation in the presence of a binding substrate is also further discussed in Section 2.

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morphogen which is a self-inhibitor and thus analogously down-regulates its own production (Murray, 2002).

Thus, implicit in the latter constraint is one of the key conditions for diffusion-driven-instability (DDI) with two chemical species, namely that their diffusion coefficients have to be different. While in principle they can be arbitrarily close to each other, this requires extensive parameter fine tuning for a Turing instability to still exist (Pearson and Horsthemke, 1989; Baker et al., 2008) and, in practice, interacting chemical molecules will typically have very similar diffusion coefficients. This led to great difficulty in identifying real Turing structures, but in 1991 they were eventually determined in a chemical system by Castets et al. (1990) and Ouyang and Swinney (1991) due to a substrate, introduced as a marker, binding to one of the chemicals and reducing its diffusion coefficient sufficiently. Furthermore, such binding dynamics have been implicated with diffusible gene-products such as fibroblast growth factor (FGF) indicating that this mechanism for inducing differential transport may potentially be active in biological systems exhibiting long range self-organisation (Miura, 2007).

We note that binding with an immobile substrate is not the only mechanism that has been highlighted as providing a means of circumventing the constraint of equal diffusion coefficients in Turing's mechanism. For finite amplitude perturbations, it is also possible for spatial patterns to arise with equal diffusion coefficients (Vastano et al., 1987). However, this is outside the scope of simple linear stability analysis and, more importantly, it is also outside the scope of fluctuation induced instability from an essentially homogeneous steady state and thus it cannot explain a core feature of Turing's instability, namely symmetry breaking from a near perfect spatial homogeneity, and is thus not considered further here. A second manner of evading the constraint of equal diffusion coefficients concerns receptor dynamics. In particular, a focussed model of hair follicle patterning (Klika et al., 2012) has also revealed that patterning can occur with equal diffusion coefficients. Coupling receptor dynamics to Turing's mechanism results in a system of coupled ordinary and partial differential equations, as also studied by Marciniak-Czochra in the context of hydra self-organisation (e.g. Marciniak-Czochra, 2003), which presents a mathematical framework with rich behaviour. Below, we do not consider the complexities associated with genuine receptor dynamics (Klika et al., 2012; Marciniak-Czochra, 2003), but instead focus on the influence of simple reversible binding with an immobile substrate such as extra-cellular matrix, representing a particularly simple class of coupled ordinary and partial differential equations with biological motivation that generalise the standard Turing model.

While this standard Turing model has many applications to pattern formation in biology (see, for example, the books by Meinhardt, 1982; Meinhardt et al., 2003; Murray, 2002) and is highly suggestive due to numerous cases of qualitative agreement with observation (e.g. Nakamasu et al., 2009; Yamaguchi et al., 2007), there is still a lot of scepticism in the biological community because the identification of Turing morphogens remains elusive. Nonetheless, there are a number of recent studies that have begun to move towards identifying possible biological components (Sick et al., 2006; Garfinkel et al., 2004; Solnica-Krezel, 2003; Chen and Schier, 2002; Hamadai, 2012; Muller et al., 2012) and even suggesting that the self-activator–self-inhibitor pair may actually be cells themselves (Yamaguchi et al., 2007; Nakamasu et al., 2009).

In this paper we first briefly revisit the original ideas of the CIMA chemical reaction used to experimentally investigate Turing's instability and, in particular, the theoretical study by Lengyel and Epstein, (1991, 1992), which was motivated by the immobile substrate in the CIMA experiments of Castets et al. (1990) and Ouyang and Swinney (1991). Lengyel and Epstein considered the equations for a Turing pair, in which one of the chemicals (the self-activator) reversibly binds to an immobile substrate and demonstrated this can be reduced using a quasi-steady approximation to a two species system with an altered effective diffusion coefficient ratio that facilities the induction of a DDI even if the two morphogens have an equal diffusion coefficient in the absence of reversibly binding to the immobile substrate. Miura presented an analogous approximation, though with piecewise continuous levels of extra-cellular matrix (ECM), for the interpretation of his experimental results (Miura, 2007), whilst Pearson (Pearson, 1992) extended Lengyel and Epstein's analysis to conditions outside the regime of the quasi-steady state approximation. All the theoretical aspects of these studies were focussed on the constraints for the morphogen diffusion coefficients associated with a DDI, though Pearson additionally assessed the relevance of such models for continuously fed reactors in observations of CIMA Turing instabilities.

However, despite the prevalence of the quasi-steady state approximation in these previous studies, there is no a priori reason to expect this approximation to be universal. For example, fluorescence recovery after photobleaching (FRAP) highlights that VEGF-ECM binding and unbinding occurs on the order of magnitude of 1000 s (Köhn-Luque et al., 2013). In contrast fast developmental events can occur on the timescale of only a few hours as illustrated by Zebrafish gene expression and fate maps for Nodal, a common putative morphogen, which demonstrate that Nodal specifies position-dependent cell fates in Zebrafish before gastrulation, i.e. under 5.25 h from fertilisation at standard conditions (Schier, 2003; Kimmel et al., 1995). Hence the timescale of fast developmental patterning, which must be significantly longer than the kinetic timescales, still need not be multiple orders higher than the timescale of ECM interaction between a diffusible signal and the extra-cellular matrix. In turn, this means that regions of parameter space where the binding-unbinding reaction rates are the same order as other kinetic interactions should not be excluded from studies. Furthermore, there is also no a priori reason to expect that any putative pair of Turing morphogens which interact with the ECM are restricted such that only one of the pair interacts with the ECM.

Hence, we revisit the full system for a Turing pair in the presence of reversible morphogen binding to an immobile substrate, without any quasi-steady approximations, and also briefly consider the system where both diffusing morphogens reversibly bind to the immobile substrate. This modelling framework will reduce to the standard model in the limit of negligible interactions with the immobile substrate so, in particular, our objective is to assess whether the introduction of a mobile substrate allows a *relaxation* of the conditions for a Turing instability. However, our main focus is fundamentally different from the previous work that clearly demonstrated that the 2-species requirement of equal diffusion coefficients needs no longer apply in the presence of reversible binding. In particular, there has been no study of whether it is still necessary to enforce other characteristics of the 2-species DDI, for instance the need to pair a self-activator with a self-inhibitor. Thus, rather than considering diffusive aspects of the diffusion-driven instability, we explore how the presence of a DDI constrains the *kinetics* of interacting morphogens given the presence of reversible binding to an immobile substrate. Furthermore, the diffusible gene-products Nodal and Lefty are the subject of intensive investigation concerning whether they fulfil the criteria of a Turing morphogen pair (e.g. Solnica-Krezel, 2003; Chen and Schier, 2002; Hamadai, 2012; Muller et al., 2012). Thus, more generally we are investigating whether one should refine or generalise the interactions that Nodal and Lefty, or indeed any prospective Turing pair, undertake in order to verify, at the molecular level, that the conditions for Turing's mechanism are satisfied given at least one of the morphogens undergoes reversible binding with an immobile substrate.