Synchronized and Differentiated Modes of Cellular Dynamics

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1. Introduction

Intercellular communication serves a variety of purposes in metazoan systems, including, (i) the recruitment of individual cells into multicellular aggregates, as in the transition from vegetative growth to the slug stage in the slime mold *Dictyostelium discoideum* (Dd hereafter), (ii) the initiation and/or synchronization of collective activities such as the repetitive contraction in the myocardium, (iii) the initiation and coordination of spatial differentiation in developing systems, and (iv) the guidance of cell movement during morphogenesis. It is convenient to classify the different modes of intercellular communication that have evolved for these purposes as either long-range or short-range, according as the distances involved are greater or less than about 0.25 mm, this being the distance for which the relaxation time for diffusion is of the order of one minute. Long-range signal transmission can occur via convective transport, as in hormonal interactions via a circulatory system, or it may involve diffusion coupled with a spatially-distributed mechanism for regenerating and relaying the signal, such as occurs in nerve impulse conduction and in the aggregation phase of Dd.

At least three distinct modes of short-range communication between cells have been identified: (i) direct exchange of diffusible substances such as ions, cyclic nucleotides, small metabolites, and neurotransmitters via gap junctions and synapses, (ii) indirect interactions by uptake of nutrients or other essential substances from a common pool or by release of substances that activate or inhibit cellular functions into the pool, and (iii) surface interactions that result from mechanical stresses or that occur via receptor molecules that are embedded in the cell membrane. Direct exchange occurs frequently in developing systems and plays a central role in reaction-diffusion models of pattern formation [1]. For instance, it has been found that the ionic permeability of gap junctions in developing insect epidermis is hormonally controlled and varies with the developmental stage [2]. Furthermore, it has been demonstrated that cell-to-cell communication via gap junctions is responsible for the synchronization of activities in the myocardium and in smooth muscle [3]. Indirect interaction occurs in suspensions of Dd, which releases CAMP into the external medium via vesicles [4], and in suspensions of the yeast cell *S. carlsbergensis* [5]. It is also used as an alternative to the conventional mode of synaptic transmission in certain types of neurons in the marine mollusk *Aplysia* [6].

In many other cases the mode of interaction between cells is not known because several modes may be possible and the molecular species involved has not yet been identified. Since it is to be expected that different modes lead to different dynamical behavior in a cellular aggregate, it may be helpful in identifying the mode used to contrast the dynamical behavior for the various modes, and this is done here for directly- and indirectly-coupled systems. The following section deals with the question of global synchronization and gives conditions under which all cells relax to the same instantaneous state, irrespective of whether that state is time-invariant, time-periodic, or shows some more complicated temporal behavior. These conditions prove to be very stringent and one can ask whether weaker conditions will ensure that a synchronized system remains synchronized in the presence of small

In Dynamics of Synergetic Systems, H. Haken, ed., Springer-Verlag (1980)

disturbances. This question is dealt with in the third section. The fourth section is devoted to a detailed analysis of the simplest example of direct and indirect coupling, namely, a system of two cells with two active species in each cell. The range of dynamical behavior exhibited in this simple system illustrates the difficulty inherent in determining the complete structure of the solution set for more complicated systems.

2. Global Synchronization

For many purposes an aggregate of cells coupled by gap junctions can be regarded as a reacting continuum and this viewpoint is adopted here. Let Ω be a bounded region of R_p ($p = 1,2$ or 3) with outward normal $\underset{\sim}{n}$. We assume that the flux of species i is given by $j_i = -\bar{\mathcal{D}}_i \nabla C_i$ where the $\bar{\mathcal{D}}_i$ are positive constants, and that the boundary is impermeable. The governing equations are

$$\begin{aligned} \frac{\partial C}{\partial t} &= \bar{\mathcal{D}}\bar{\nabla}^2 C + \bar{R}(C) \quad &&\text{in} \quad \Omega \\ \underset{\sim}{n}\cdot\nabla C &= 0 \quad &&\text{on} \quad \partial\Omega \\ C(\underset{\sim}{r},0) &= C_0(\underset{\sim}{r}) \quad &&\text{in} \quad \Omega \end{aligned} \tag{1}$$

where $C = (C_1,\ldots,C_n)^T$, $R(C)$ is the reaction rate vector, r is the space coordinate in Ω, and $\bar{\mathcal{D}}$ is the diagonal diffusion matrix. For the purpose of casting these equations into a dimensionless form, let L be a measure of Ω, let $\bar{C}_i$ be a reference concentration for species i, let κ^{-1} be a time scale characteristic of the kinetics, and let $\delta = \max \bar{\mathcal{D}}_i$. Set

$$\underset{\sim}{\zeta} = \underset{\sim}{r}/L, \quad \tau = \kappa t, \quad c_i = C_i/\bar{C}_i, \quad \mathcal{D}_i = \bar{\mathcal{D}}_i/\delta \quad \text{and} \quad \nabla = L\bar{\nabla}.$$

Then (1) becomes

$$\begin{aligned} \frac{\partial c}{\partial \tau} &= \Delta_1 \mathcal{D}\nabla^2 c + R(c) \quad &&\text{in} \quad \Omega \\ \underset{\sim}{n}\cdot\nabla c &= 0 \quad &&\text{on} \quad \partial\Omega \\ c(\underset{\sim}{\zeta},0) &= c_0(\underset{\sim}{\zeta}) \quad &&\text{in} \quad \Omega \end{aligned} \tag{2}$$

where $\Delta_1 \equiv \delta/\kappa L^2$ and $R_i(c) = \bar{R}_i(\bar{C}_1 c_1,\ldots,\bar{C}_n c_n)/\kappa\bar{C}_i$.

When $R(c) \equiv 0$, the initial distribution relaxes to a spatially uniform one exponentially in time and the Fourier series solution of (2) in that case shows that the rate is controlled by the smallest (in magnitude) nonzero eigenvalue of the Laplacian on Ω. The same should be true when $R(c) \neq 0$, provided that the appropriate relaxation time for diffusion is short compared to that for reaction. To make this precise, we must assume that solutions of (2) are bounded for all τ, pointwise in ζ. Let $\|\cdot\|_E$ and $\|\cdot\|_{L_2}$ be the Euclidean and L_2 norms, respectively, set $\tilde{m}(\Omega) = \int_\Omega d\underset{\sim}{\xi}$, and let

$$\bar{c}(\tau) = \frac{1}{m(\Omega)} \int_\Omega c(\underset{\sim}{\zeta},\tau)\,d\underset{\sim}{\zeta}.$$

Further, let

$$\hat{k} \equiv \sup_c \left\| \frac{\partial R}{\partial c} \right\|_E$$

and let $-\alpha_1^2$ be the smallest nonzero eigenvalue of the problem

$$\begin{aligned} \nabla^2\phi &= -\alpha^2\phi \quad &&\text{in} \quad \Omega \\ \underset{\sim}{n}\cdot\nabla\phi &= 0 \quad &&\text{on} \quad \partial\Omega. \end{aligned} \tag{3}$$

Then it can be shown [1,7] that if

$$\Delta_1\alpha_1^2(\min_i \mathcal{D}_i) > \hat{k} \tag{4}$$

then

$$\| c(\underset{\sim}{\zeta},\tau)-\bar{c}(\tau)\|_{L_2} \to 0$$

exponentially in τ. Thus the appropriate relaxation times for reaction and diffusion are $\tau_R = (k\kappa)^{-1}$ and $\tau_D = L^2/\alpha_1^2(\min \bar{\mathcal{D}}_i)$, respectively, and (4) is equivalent to the condition $\tau_D < \tau_R$. Typically $\tau_R \sim 10$ seconds but for some metabolic processes it may be up to 9 hours [8]. In a one dimensional system of length L, $\alpha_1^2 = \pi^2$ and if $\min \bar{\mathcal{D}}_i \sim 10^{-7}$ cm^2/sec, then the lengths for which (4) is satisfied for the foregoing τ_R are ~0.03 mm and 1.8 mm, respectively. A typical cell diameter is ~10μ and so one can expect synchrony over at least 3ϕ's *if all species in question pass through the gap junctions.*

A different mathematical description is required when diffusible substances can pass between cells only via the extracellular medium. While it is possible to formulate the governing partial differential equations for a random suspension of cells, it is difficult to extract information from them and a different approach is taken here. Imagine that the cells form a monolayer at the bottom of a petri dish and that the cells are covered by a thin layer of culture medium. Suppose that reactions occur only in the cells, that the composition within a cell is uniform, and that there is no direct communication between cells. Further, suppose that the overlying fluid layer is very thin compared to the dish diameter so that vertical nonuniformity decays rapidly compared to horizontal nonuniformity. If the flux between fluid and cells is linear in the concentration difference, then the governing equations, written in dimensionless form, are

$$\begin{aligned} \frac{\partial u}{\partial \tau} &= \Delta_1 \mathcal{D}\nabla^2 u + \varepsilon\Delta_2 H(v-u) \\ \frac{\partial v}{\partial \tau} &= -\Delta_2 H(v-u) + R(v). \end{aligned} \tag{5}$$

Here u is the vertical average concentration in the fluid, v is the composition in the cells, ε is the ratio of total cell volume to fluid volume, and H is a constant diagonal matrix whose entries are $h_i = \bar{h}_i/(\max \bar{h}_i)$. The $\bar{h}_i$ are the dimensional transfer coefficients, $\Delta_2 \equiv (\max \bar{h}_i)a/$, and a is the interfacial area per unit cell volume. These equations provide an exact description in the limit as the cell diameter tends to zero and otherwise are adequate whenever the scale of concentration variation in the fluid is greater than a cell diameter.

Since there is no direct exchange between cells, the conditions under which both phases relax to a uniform state will necessarily involve the h_i's as well as the diffusion coefficients. To formulate these, let V^c and V^f denote the cell and fluid volume, respectively, let $V = V^c + V^f$, set $\underline{h} = \min_i h_i$, and let

$$\bar{u}(\tau) = \frac{1}{V}\int_V u(\underset{\sim}{\xi},\tau)d\underset{\sim}{\xi} \qquad \bar{v}(\tau) = \frac{1}{V}\int_V v(\underset{\sim}{\xi},\tau)d\underset{\sim}{\xi}. \tag{6}$$

Here u and v are defined to be zero in V^c and V^f, respectively. Then it can be shown [9] that if

$$\underline{h} > 2\hat{k}/\Delta_2 \tag{7}$$

and

$$\Delta_1\alpha_1^2(\min \mathcal{D}_i) > \frac{\varepsilon\Delta_2^2(1-\underline{h}^2) + 2\varepsilon\Delta_2\hat{k}}{\Delta_2\underline{h}-2\hat{k}} \tag{8}$$

then

$$\| u(\underset{\sim}{\xi},\tau)-\bar{u}(\tau)\|_{L_2} \quad \text{and} \quad \| v(\underset{\sim}{\xi},\tau)-\bar{v}(\tau)\|_{L_2}$$

tend to zero exponentially in τ.

If we define a relaxation time for interphase transport as $\tau_T = [(\min \bar{h}_i)a]^{-1}$ and use the previous definitions of τ_R and τ_D, then (7) and (8) can be written as

$$\tau_T < \frac{1}{2}\tau_R \tag{9}$$

and

$$\tau_D < \frac{\underline{h}^2\tau_T}{\varepsilon}\left[\frac{\tau_R - 2\tau_T}{\tau_R(1-\underline{h}^2)+2\underline{h}\tau_T}\right]. \tag{10}$$

As $\underline{h} \to 1$, in which case all h_i's are equal, (10) reduces to

$$\tau_D < \frac{\tau_R - 2\tau_T}{2\varepsilon}. \tag{11}$$

Furthermore, if $\underline{h} = 1$ and $\bar{h}_i \to \infty$, $\tau_T \to 0$ and so (9) is always satisfied, while (11) reduces to $\tau_D < \tau_R/2\varepsilon$. This is to be compared with the inequality $\tau_D < \tau_R$ that ensures synchronization for the directly-coupled case. It should be noted that if $\underline{h} = 0$ or if $\underline{h} \in (0,1)$ and $\min \bar{h}_i \to \infty$, the right-hand side of (10) reduces to zero. Thus the synchronization conditions cannot be satisfied unless all $\bar{h}_i > 0$ and if $\min \bar{h}_i \to \infty$, all $\bar{h}_i$ must be equal as well. A later example will show that the uniform state can in fact be unstable when $\underline{h} = 0$.

3. Destabilization of a Synchronized State

The conditions that guarantee global asymptotic stability of the set of all uniform solutions provide estimates of the region in parameter space in which no spatial differentiation between cells can persist in time. Outside that region it may happen that a spatially-uniform solution is unstable to certain small-amplitude nonuniform disturbances and the problem is to determine conditions on the kinetic mechanism and the transport coefficients under which this is possible. We first consider the destabilization of uniform steady states in directly-coupled systems.

Suppose that $\bar{c}$ is such that $R(\bar{c}) = 0$ and set $x = c - \bar{c}$; then x satisfies

$$\begin{aligned} \frac{\partial x}{\partial \tau} &= \Delta_1 \mathcal{D}\nabla^2 x + Kx + F(x) \quad \text{in } \Omega \\ \frac{\partial x}{\partial n} &= 0 \quad \text{on } \partial\Omega \end{aligned} \tag{12}$$

where K is the Jacobian of R at $\bar{c}$ and $\|F(x)\|_E \sim \mathcal{O}(\|x\|_E)$ as $\|x\|_E \to 0$. If asymptotic stability of $\bar{c}$ is defined in terms of either the L_2 or L_∞ norm, it can be shown that in noncritical cases stability is governed by the linear terms in (12). The linear equation gotten by dropping $F(x)$ has the solution

$$x(\underset{\sim}{\zeta},\tau) = \sum_{n=0}^{\infty} e^{(K-\mu_n\mathcal{D})\tau} y_n \phi_n(\underset{\sim}{\zeta}), \tag{13}$$

where $\mu_n \equiv \alpha_n^2\Delta_1$, and therefore asymptotic stability is governed by the spectrum of the set $\{K-\mu_n\mathcal{D}\}$. For any given smooth domain Ω there are only a countable number of matrices to test, but to arrive at results valid for any domain, we replace μ_n with a continuous parameter $\mu \in [0,\infty)$. Furthermore, we assume that the steady state is asymptotically stable as a solution of the kinetic equations $dc/d\tau = R(c)$, in which case the spectrum of K lies in the left-half plane. A number of special cases that are asymptotically stable at all wavelengths μ are known, including the case of symmetric K and the case where the diffusion coefficients do not differ too much from each other. Since we are interested in determining when instabilities can arise, we are primarily concerned with establishing necessary conditions for asymptotic stability at all wavelengths.

Let $\sigma(K)$ denote the spectrum of K, let LHP ($\overline{\text{LHP}}$) denote the open (closed) left-half complex plane, and let $K[i_1,\ldots,i_p]$ denote a $p \times p$ principal submatrix of K formed from rows and columns $i_1,\ldots,i_p$ for $1 \leq p \leq n-1$.

Theorem 1 Let $\mathcal{D}$ be diagonal with $\mathcal{D}_i \geq 0$. In order that $\sigma(K-\mu\mathcal{D}) \subset$ LHP for all such $\mathcal{D}$ and all $\mu \in [0,\infty)$, it is necessary that

(i) $\sigma(K) \subset$ LHP

(ii) $\sigma(K[i_1,\ldots,i_p]) \subset \overline{\text{LHP}}$ for all p^{th}-order submatrices of K, where $1 \leq p \leq n-1$.

We shall not give the proof here but will merely indicate how an instability arises when one of the conditions is violated. Suppose that there is a $p \times p$ principal submatrix of K whose spectrum intersects the open right-half plane. Without loss of generality we may assume that it lies in the first p rows and columns, and we partition K as follows

$$K = \begin{bmatrix} K_1 & K_2 \\ K_3 & K_4 \end{bmatrix} \tag{14}$$

Here K_1 is $p \times p$, K_4 is $(n-p) \times (n-p)$, etc. Since K_1 has at least one eigenvalue with a positive real part, choose $\mathcal{D}$ so that the first p $\mathcal{D}_i$'s are zero and the remaining n-p $\mathcal{D}_i$'s are one. Then it can be shown that for $\mu \to \infty$ the asymptotic expansions of the eigenvalues have the form

$$\lambda_j = \lambda_j^{K_1} + \mathcal{O}(\mu^{-\ell_j/p}) \qquad j = 1,\ldots,p$$

$$\lambda_j = -\mu + \mathcal{O}(1) \qquad j = p+1,\ldots,n$$

where $\ell_j \leq p$. Thus $K-\mu\mathcal{D}$ will have at least one eigenvalue with a positive real part if μ is large enough. We have not required that K_1 be the smallest submatrix that has an eigenvalue with a positive real part and as a result, it can happen that for $n > 2$ either stationary or oscillatory instabilities arise, depending on the choice of p and $\mathcal{D}$.

The physical interpretation of these instabilities is as follows. The amplitude $y_k = (y_{1k}, y_{2k})^T$ of a small disturbance evolves according to the equation

$$\frac{d}{d\tau}\begin{bmatrix} y_{1k} \\ y_{2k} \end{bmatrix} = \begin{bmatrix} K_1-\mu_k\mathcal{D}_1 & K_2 \\ K_3 & K_4-\mu_k\mathcal{D}_2 \end{bmatrix}\begin{bmatrix} y_{1k} \\ y_{2k} \end{bmatrix},$$

and for $k = 0$, $y_{10}(\tau)$ grows exponentially if $y_{20}(\tau) \equiv 0$. Of course this is not possible in an isolated uniform system and the instability in the kinetic subsystem whose matrix is K_1 is suppressed through stabilizing interactions with the remainder of the network. For the earlier choice of $\mathcal{D}$, namely, $\mathcal{D}_1 = 0$ and $\mathcal{D}_2 = I$, diffusion has no direct effect on the evolution of the amplitudes of the components y_{1k}. However there is an indirect effect, because for large μ_k, an asymptotic analysis shows that y_{2k} decays rapidly and remains small and thus the stabilizing effect of the remainder of the network is lost. This analysis predicts that the largest wave numbers are fastest growing, which is due to the fact that $\mathcal{D}_1 = 0$, but if $\mathcal{D}_1 = \varepsilon I$ where $\varepsilon << 1$, an upper cut-off in μ exists and the preceding argument still goes through. CROSS [10] has given a proof of Theorem 1 based on Rouche's Theorem, but his proof does not show that the unstable modes arise from K_1 for large μ and so the preceding interpretation cannot be made. SEGEL and JACKSON [20] have discussed the origin of such instabilities in two-component systems.

Let us single out a particular parameter in (12) and label it p, and suppose that when p increases through p_0 a real eigenvalue of $K-\mu_k \mathcal{D}$ crosses from the left-half to the right-half plane for some k. Write (12) as

$$\frac{\partial x}{\partial \tau} = L(p)x + F(x,p) \tag{15}$$

and for $|p-p_0| \sim \mathcal{O}(\varepsilon)$, $\varepsilon << 1$, write

$$\sigma(L) = \sigma_1(L) \cup \sigma_2(L)$$

where

$$\sigma_1(L) = \{\lambda \in \sigma(L) |\ |\text{Re}\ \lambda| \leq \gamma\}$$

$$\sigma_2(L) = \{\lambda \in \sigma(L) | \text{Re}\ \lambda < -\gamma\}$$

and γ is a small positive constant. Such a separation is possible because L(p) has a compact inverse on $L_2(\Omega)$. Let P_1 and $P_2 = I-P_1$ be the projections associated with this decomposition of the spectrum and write $u = P_1 u + P_2 u \equiv \varepsilon(v+\bar{w})$; then v and $\bar{w}$ satisfy the equations

$$\begin{aligned} \frac{\partial v}{\partial \tau} &= L_1 v + \varepsilon F_1(v,\bar{w},p,\varepsilon) \\ \frac{\partial \bar{w}}{\partial \tau} &= L_2 \bar{w} + \varepsilon F_2(v,\bar{w},p,\varepsilon), \end{aligned} \tag{16}$$

which are gotten by applying the projections to (15). Under the standing assumption that $\|u\|_{L_2} \sim \mathcal{O}(\varepsilon)$ for all $t \geq 0$, it can be shown that $\|\bar{w}\|_{L_2} \sim \mathcal{O}(\varepsilon)$ for $t > 0$ if $\|\bar{w}(\underline{r},0)\|_{L_2} \sim \mathcal{O}(\varepsilon)$ and γ is large enough. We assume that this is true and write $\bar{w} = \varepsilon w$; then

$$\begin{aligned} \frac{\partial v}{\partial \tau} &= L_1 v + \varepsilon F_1(v,\varepsilon w,p,\varepsilon) \\ \frac{\partial w}{\partial \tau} &= L_2 w + F_2(v,\varepsilon w,p,\varepsilon) \end{aligned} \tag{17}$$

If F(x,p) is C^k, $k \geq 3$, for (x,p) near $(0,p_0)$, F_1 and F_2 have the expansions

$$\varepsilon F_1(v,\varepsilon w,p,\varepsilon) = \varepsilon Q^1(v,v,p) + \varepsilon^2\{2Q^1(v,w,p) + C^1(v,v,v,p)\} + \mathcal{O}(\varepsilon^3)$$

$$F_2(v,\varepsilon w,p,\varepsilon) = Q^2(v,v,p) + \varepsilon\{2Q^2(v,w,p) + C^2(v,v,v,p)\} + \mathcal{O}(\varepsilon^2)$$

where Q^i and C^i are homogeneous of degree two and three respectively. Therefore, to $\mathcal{O}(\varepsilon^2)$ v evolves independently of w while to $\mathcal{O}(\varepsilon)$, w is 'forced' by v through the term $Q^2(v,v,p)$. This partial separation of the unstable or nearly unstable modes from the rapidly decaying forced modes is in effect a first step toward the complete separation that can be obtained formally by invoking the Center Manifold Theorem for flows generated by partial differential equations [11].

Since the eigenvalue that crosses zero at $p = p_0$ is simple, bifurcation is known to occur and the bifurcating solution can be constructed as an expansion in the amplitude parameter $\varepsilon \equiv \langle\psi^*,u\rangle_{L_2}$ [12]. If $p_1(\varepsilon)$ is defined by setting $p-p_0 = \varepsilon p_1(\varepsilon)$, then one finds that

$$p_1(0) = \frac{\langle y^*,Q(y,y,p_0)\rangle}{\langle y^*,\frac{\partial K}{\partial p} y\rangle} \int_\Omega \phi^3 d\Omega. \tag{18}$$

If this is nonvanishing then a nontrivial solution exists on both sides of $p = p_0$ and an exchange of stability occurs at p_0. Evidently $p_1(0)$ will vanish for purely geometric reasons if $\int\phi^3 d\Omega = 0$, which happens in one dimension because $\phi = \cos n\pi x/L$. In such cases higher-order terms must be examined. When the other factor in the numerator vanishes the kinetics have a degeneracy in the quadratic terms in the direction of y^*, and again the properties of the bifurcating solution are governed by higher order terms. AUCHMUTY and NICOLIS [13] and others have constructed the bifurcating solutions for the trimolecular reaction scheme.

Bifurcation is certain to occur at an eigenvalue of odd multiplicity but if the multiplicity is greater than one, the number of bifurcating branches cannot be determined a priori. In this case and the case of even multiplicity one has to examine the bifurcation equations to determine the number of solutions. One source of eigenvalue degeneracy is symmetry of the domain Ω and an example of this is given in [12]. For other results on bifurcation in the presence of symmetry see [14] and the references therein.

Next let us suppose that the kinetic equations have an orbitally asymptotically stable (OAS hereafter) periodic solution $\Phi(\tau)$ of least period T. Under Neumann boundary conditions $\Phi(\tau)$ is also a solution of (2), and we can write

$$c(\underset{\sim}{\zeta},\tau) = \Phi(\tau) + \sum_k y_k(\tau)\phi_k(\underset{\sim}{\zeta}). \tag{19}$$

For small disturbances the amplitudes satisfy the linear equation

$$\frac{dy_k}{d\tau} = (K(\tau)-\mu_k\mathcal{D})y_k \tag{20}$$

where $K(\tau+T) = K(\tau)$. It can be shown that if the $\mathcal{D}_i$'s are not too different or if all of them are large enough, then $\Phi(\tau)$ is an OAS solution of the partial differential equation as well [1], but we are again more interested in necessary conditions for stability, and a result that parallels Theorem 1 can be obtained as follows. Partition $K(\tau)$ as in (14) and choose $\mathcal{D}$ as before, replace μ_k by a continuous variable $\mu \in [0,\infty)$, and let $\varepsilon = \mu^{-1}$. Then (20) becomes

$$\begin{aligned} \frac{dy_1}{d\tau} &= K_1(\tau)y_1 + K_2(\tau)y_2 \\ \varepsilon\frac{dy_2}{d\tau} &= \varepsilon K_3(\tau)y_1 + (\varepsilon K_4(\tau)-I)y_2 \end{aligned} \tag{21}$$

and associated with this is the degenerate system

$$\begin{aligned} \frac{d\bar{y}_1}{d\tau} &= K_1(\tau)\bar{y}_1 \\ \bar{y}_2(\tau) &\equiv 0 \end{aligned} \tag{22}$$

obtained by setting $\varepsilon \equiv 0$. For initial conditions such that $y_1(0) = \bar{y}_1(0)$ and $y_2(0) = \bar{y}_2(0) = 0$, it can be shown, using results in [15], that for any τ in a closed subinterval of $\mathbb{R}^+$,

$$\begin{aligned} \|y_1(\tau)-\bar{y}_1(\tau)\| &\leq \Lambda(\varepsilon) \\ \|y_2(\tau)\| &\leq \Lambda(\varepsilon) \end{aligned} \tag{23}$$

Here $\Lambda(\varepsilon)$ is a continuous nonnegative function that vanishes at zero. It follows that if $K_1(\tau)$ has one or more characteristic exponents with a positive real part, the uniform periodic solution is unstable to disturbances of sufficiently short wavelength. This conclusion leads to the following set of necessary conditions for stability.

Theorem 2. Suppose that $\Phi(\tau)$ is an OAS periodic solution of the kinetic equations and that $\mathcal{D}$ is diagonal with $\mathcal{D}_i \geq 0$. Then $\Phi(\tau)$ is an OAS periodic solution of (2) (in the L_2 norm) only if all the characteristic exponents of every pth-order submatrix $K[i_1,\ldots,i_p](\tau)$ of $K(\tau)$ have nonpositive real parts for $1 \leq p \leq n-1$.

The similarity between the foregoing conditions and those in Theorem 1 is apparent; in each case the presence of a subsystem that would be unstable if it were isolated from the remainder of the kinetic network is sufficient for instability, given the proper choice of diffusion coefficients. Of course the criterion for stability of the subsystems is different in the two cases because the underlying basic solutions are different.

Theorem 2 enables us to delineate a large class of kinetic systems for which the uniform periodic solution can be destabilized by diffusion. Suppose that the OAS periodic solution $\Phi(\tau)$ of the kinetic equations bifurcates from the steady state $c^s(p)$ by a Hopf bifurcation as p increases through p_0, and that there is at least one species for which $k_{jj} > 0$ in a neighborhood of $c^s(p_0)$. Then the uniform periodic solution of (2) can be destabilized by diffusion if $p-p_0$ is sufficiently small and positive. Said otherwise, if there is autocatalysis present in the linearized kinetics at $c^s(p_0)$, then all small amplitude solutions can be destabilized. If there are only two species present, a Hopf bifurcation can *only* occur if there is autocatalysis in the linearized kinetics and d(trace K)/dp > 0, and therefore *a uniform OAS periodic solution of a two-species system can always be destabilized when the kinetic parameters are sufficiently close to the values that yield a Hopf bifurcation.* An example of such an instability is given in the following section.

The type of solution that exists beyond the critical parameter value p_c at which $\Phi(\tau)$ loses stability to nonhomogeneous disturbances depends on how the multipliers π_i cross the unit circle. In two-species systems they can only cross at ±1, and the bifurcating solution is necessarily periodic [1]. In general, if $\pi_i^n(p_c) \neq 1$, n = 1,2,3,4, an invariant torus bifurcates from the periodic solution [16,17], and the bifurcating solution is quasi-periodic. There may still be a periodic solution in this case, or in some cases where the above non-resonance conditions aren't met, but in any event one must generally determine the parametric behavior of the multipliers numerically.

Finally, we consider the stability properties of uniform steady states of (5). We assume that $h_i > 0$ for all i and therefore these steady states are given by $u^s = v^s = R^{-1}(0)$. The differences $x \equiv u-u^s$ and $y \equiv v-v^s$ satisfy

$$\begin{aligned}\frac{\partial x}{\partial \tau} &= \Delta_1 \mathcal{D} \nabla^2 x + \varepsilon \Delta_2 H(y-x) \\ \frac{\partial y}{\partial \tau} &= -\Delta_2 H(y-x) + Ky + F(y),\end{aligned} \tag{24}$$

and the matrix of the linear system is

$$L = \begin{bmatrix} -\mu_n \Delta_1 \mathcal{D} - \varepsilon \Delta_2 H & \varepsilon \Delta_2 H \\ \Delta_2 H & K - \Delta_2 H \end{bmatrix} .$$

Now suppose that $\mathcal{D} = H = I$ and $\sigma(K) \subset \text{LHP}$. By Lyapunov's theorem there exists a positive definite W such that $WK + K^T W$ is negative definite, and if we set $A = \text{diag}\ \{I, \sqrt{\varepsilon} I\}$ and $B = \text{diag}\ \{W, W\}$, then it follows that

$$B(ALA^{-1}) + (ALA^{-1})^T B$$

is negative definite. Thus a kinetically-stable steady state can be destabilized in the present case only if the diffusion coefficients and the mass transfer coeffients are sufficiently different. This is analogous to the requirement for directly-coupled systems and there are other parallels. The characteristic equation of L is

$$\det \{K(\mu_n \Delta_1 \mathcal{D} + \varepsilon \Delta_2 H) - \mu_n \Delta_1 \Delta_2 H\mathcal{D} - \lambda((1+\varepsilon)\Delta_2 H + \mu_n \Delta_1 \mathcal{D} - K) - \lambda^2 I\} = 0 \tag{25}$$

and the potential bifurcation loci for steady states are subsets of the loci along which

$$\det \{K(\mu_n \Delta_1 \mathcal{D} + \varepsilon \Delta_2 H) - \mu_n \Delta_1 \Delta_2 H\mathcal{D}\} = 0. \tag{26}$$

We have assumed that $h_i > 0$ for all i and therefore the multiplier of K is invertible for $\varepsilon > 0$ and $\Delta_2 > 0$. Thus (26) can be written

$$\det \{K - T^{(n)}\} \det \{\mu_n \Delta_1 \mathcal{D} + \varepsilon \Delta_2 H\} = 0 \tag{27}$$

where $T^{(n)}$ is a diagonal matrix whose elements are

$$\frac{1}{T_i} = \frac{1}{\Delta_2 h_i} + \frac{\varepsilon}{\mu_n \Delta_1 \mathcal{D}_i} . \tag{28}$$

This form stems from the fact that the transport steps are in series and either step can control the rate. Indeed, as $\Delta_2 \to \infty$ or $\mu_n \to 0$, $T_i \to \mu_n \Delta_1 \mathcal{D}_i / \varepsilon$ and diffusion controls, while interphase transport controls for large n (short wavelengths) or as $\Delta_1 \to \infty$.

When it is expanded, $\det \{K - T^{(n)}\}$ is a polynomial in the T_i and hence a rational function in the h_i and $\mathcal{D}_i$, and one can prove [9] that it is nonvanishing under the following conditions.

Theorem 3. Let ε, Δ_1, and Δ_2 be positive and let μ_n be nonnegative. Then $\det \{K - T^{(n)}\} \neq 0$ for all $\mathcal{D}_i \geq 0$ and $h_i > 0$ if and only if

(i) $(-1)^n \det K > 0$

(ii) $(-1)^p \det K[i_1, \ldots i_p] \geq 0$ for all pth-order submatrices of K, where $1 \leq p \leq n-1$.

Under these conditions there can be no bifurcation of steady states from the uniform steady state (u^s, v^s). An analogous result, in which the conditions on the kinetics are identical, can be stated for directly-coupled systems.

Despite the foregoing similarities, there can be substantial differences between the dynamical behavior of directly- and indirectly-coupled systems. Some of these differences are illustrated in the example that follows.

4. A Comparison for a Model Reaction

The simplest system that will illustrate the differences is one with only two active chemical species and two cells. A number of back-activation mechanisms of the type used to model glycolytic reactions can produce oscillations for suitable parameter values, and the simplest of these gives rise to the following kinetic equations [18, 19]:

$$\begin{aligned} \frac{dx}{d\tau} &= \delta - \kappa x - xy^2 \equiv F(x,y) \\ \frac{dy}{d\tau} &= \kappa x + xy^2 - y \equiv G(x,y). \end{aligned} \tag{29}$$

This system has the unique steady state $(x^s, y^s) = (\delta/\kappa + \delta^2, \delta)$ and when $\kappa < 1/8$ it has a periodic solution for $\delta \in (\delta_-, \delta_+)$, where

$$\delta_\pm = \frac{1-2\kappa \pm \sqrt{1-8\kappa}}{2} \quad . \tag{30}$$

Now suppose that these reactions occur in each of two identical cells that are separated by extracellular medium in which no reaction occurs. Let the volume of the cells and the medium be V_c and V_0, respectively, let $\varepsilon = 2V_c/V_0$, and let '0' denote concentrations in the extracellular medium. The governing equations are

$$\begin{aligned}
\frac{dx_i}{d\tau} &= F(x_i, y_i) + 2\mathcal{D}_x(x^0 - x_i) \\
\frac{dy_i}{d\tau} &= G(x_i, y_i) + 2\mathcal{D}_y(y^0 - y_i) \qquad i = 1,2 \\
\frac{dx^0}{d\tau} &= 2\,\varepsilon\,\mathcal{D}_x\left(\frac{x_1+x_2}{2} - x^0\right) \\
\frac{dy^0}{d\tau} &= 2\,\varepsilon\,\mathcal{D}_y\left(\frac{y_1+y_2}{2} - y^0\right)
\end{aligned} \tag{31}$$

Here $\mathcal{D}_x$ and $\mathcal{D}_y$ represent the diffusion coefficients across a double membrane, which accounts for the factor of two. When $V_0 = 0$ ($\varepsilon = \infty$) the cells are in direct contact and (31) reduces to

$$\begin{aligned}
\frac{dx_i}{d\tau} &= F(x_i, y_i) + \mathcal{D}_x(x_j - x_i) \\
\frac{dy_i}{d\tau} &= G(x_i, y_i) + \mathcal{D}_y(y_j - y_i) \quad , \qquad i \neq j
\end{aligned} \tag{32}$$

If gap junctions or other low-resistance pathways form when the cells come into contact, the diffusion coefficients may increase substantially.

It follows from (31) that the steady state solution $S \equiv (\bar{x}_1, \bar{y}_1, \bar{x}_2, \bar{y}_2, \bar{x}^0, \bar{y}^0)$ is independent of ε and is given by

$$\begin{aligned}
\bar{x}_{1,2} &= \frac{\delta \pm (1+2\mathcal{D}_y)}{\kappa + (\delta \pm \zeta)^2} \\
\bar{y}_{1,2} &= \delta \pm \zeta \\
\bar{x}^0 &= (\bar{x}_1 + \bar{x}_2)/2 \qquad \bar{y}^0 = (\bar{y}_1 + \bar{y}_2)/2,
\end{aligned} \tag{33}$$

where ζ is a solution of

$$\zeta[\zeta^4 + 2(\kappa - \delta^2 + \mathcal{D}_x)\zeta^2 + \frac{\kappa+\delta^2}{1+2\mathcal{D}_y} \det\,(K - 2\mathcal{D})] = \zeta[\zeta^4 + 2b\zeta + c] = 0$$

The first four components of S give the steady state solutions of (32). When $\zeta = 0$ the uniform steady state (USS) is recovered while $\zeta \neq 0$ gives a nonuniform steady state (NUSS). The number of NUSSes is given by the following proposition [19], and the regions in (ii) and (iii) are shown in Fig. 1.

Proposition Let

$$\mathcal{D}_y^* \equiv \frac{1}{2}\,\frac{1-\kappa/\mathcal{D}_x-\sqrt{(\kappa/\mathcal{D}_x)^2+2\kappa/\mathcal{D}_x}}{1+\kappa/\mathcal{D}_x+\sqrt{(\kappa/\mathcal{D}_x)^2+2\kappa/\mathcal{D}_x}} \; .$$

Then

(i) if $\mathcal{D}_y > \max\left(\frac{1}{6}, \mathcal{D}_y^*\right)$ there are no NUSSes for any $\delta > 0$.

(ii) if $\mathcal{D}_y^* > 1/6$ and $\mathcal{D}_y \; \varepsilon \; (1/6, \mathcal{D}_y^*)$ there exists a region in the $\mathcal{D}_x$-δ^2 plane in which there is a pair of NUSSes. There is never more than one pair.

(iii) if $\mathcal{D}_y \; \varepsilon \; [0, 1/6)$ there is one region in which there are two pairs of NUSSes and a contiguous region in which there is one pair.

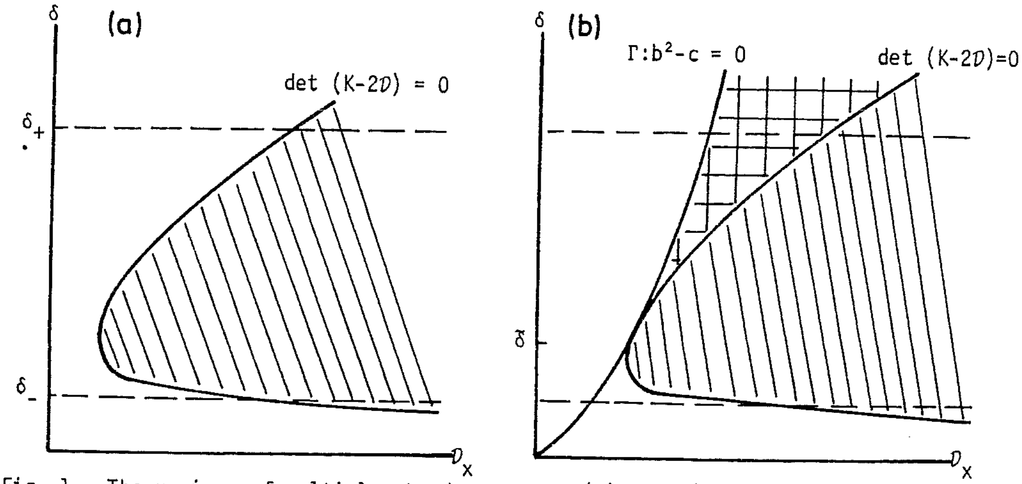

Fig. 1 The regions of multiple steady states: (a) case (ii); (b) case (iii). \\\\\\ one pair of NUSSes, xxxx two pairs of NUSSes, elsewhere; no NUSSes.

Suppose that δ is fixed and $\mathcal{D}_x$ is regarded as the bifurcation parameter. In (a) a pair of NUSSes bifurcates supercritically as $\mathcal{D}_x$ increases across $\det(K-2\mathcal{D}) = 0$, and whether they or not they are stable depends on δ: if $\delta \; \varepsilon \; [\delta_-, \delta_+]$ they are unstable near the bifurcation point. In (b) the bifurcation along $\det(K-2\mathcal{D}) = 0$ is subcritical and unstable for $\delta > \tilde{\delta}$ and these branches connect to a pair of stable branches along Γ. At the intersection of $\delta = \delta_+$ and $\det(K-2\mathcal{D}) = 0$, the linear system derived from (32) has a zero real eigenvalue and a pair of complex conjugate eigenvalues with zero real part, and such systems exist only on a submanifold of codimension 2 in the space of all 4×4 real matrices. By perturbing such a system one may find solutions other than those that bifurcate along $\delta = \delta_+$ or along $\det(K-2\mathcal{D}) = 0$, and an analysis of the case $\varepsilon = \infty$ has been done [19]. One finds, using a multiple-time-scale analysis, that there are two other bifurcation curves that emanate from the crossover point and on which a nonuniform periodic solution (NUPS) bifurcates. These curves, whose behavior near the crossover point is found analytically, can be continued numerically and the results are shown in Fig. 2(a). The curve $H_3 = 0$ corresponds to bifurcation of a NUPS from the NUSS. On the broken curve, whose location is schematic because only the solid points were computed, a NUPS bifurcates from the UPS. At the computed points the bifurcation is supercritical and the NUPS is stable, and we conjecture that this is true all along the broken curve. The results illustrate the earlier assertion that a UPS of a two-species system can be destabilized by diffusion sufficiently near the bifurcation point of such solutions. Fig. 2(b) shows the global structure of the solution set for a fixed value of δ. The amplitudes shown are only schematic. For further details and other bifurcation diagrams see [19].

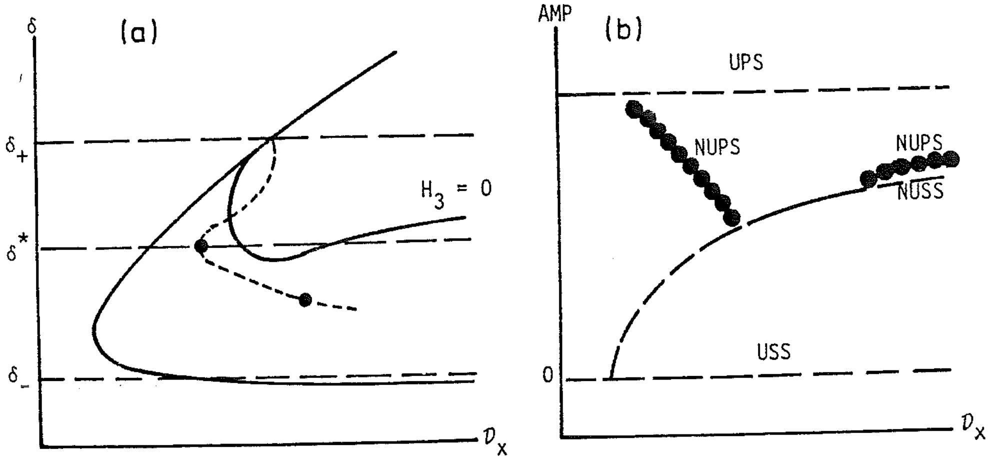

Fig. 2 (a) Fig. 1(a) with the addition of the curves of secondary bifurcation. (b) Amplitude (shown schematically) of the various solutions for $\delta = \delta^*$ in (a).

When ε is large but finite, (32) can be regarded as a singular perturbation of (31) and the structure of the solution set for (31) is similar to that already given. However, differences may arise as ε decreases, and since the steady states are independent of ε, these differences must pertain to the stability of the steady states and the existence of periodic solutions. It is easiest to describe the results for $\mathcal{D}_y = 0$ and we restrict ourselves to that case. In the base case $\varepsilon = \infty$ the bifurcation loci are as shown in Fig. 3(a) and a selected bifurcation diagram is shown in Fig. 3(b).

As before, the linearization of the four-dimensional system around a NUSS has a pair of complex conjugate eigenvalues with zero real part on the curve labelled $H_3 = 0$. On the broken portion of the curve the NUPS bifurcates from the intermediate

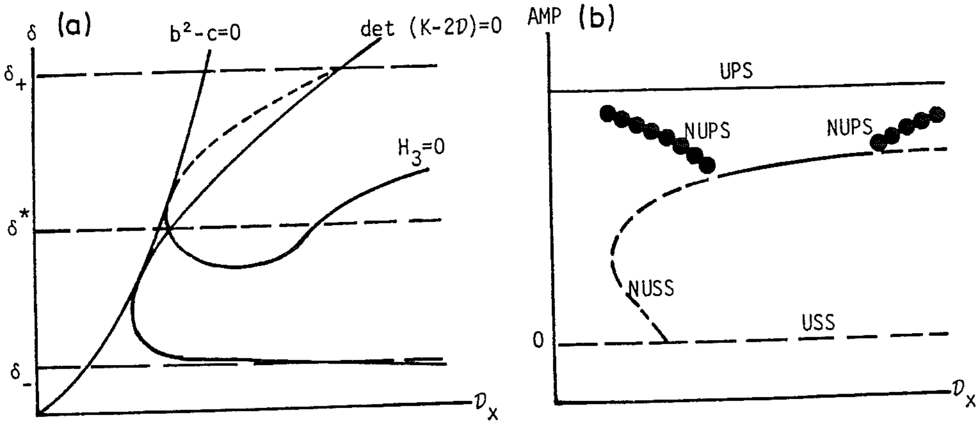

Fig. 3 (a) The bifurcation loci for $\mathcal{D}_y = 0$, $\varepsilon = \infty$. (b) A schematic of the amplitude of the various solutions at $\delta = \delta^*$.

(unstable) branch of NUSSes, while the solid portion corresponds to bifurcation from the upper branch of NUSSes.

Now let ε be finite and consider what happens to the synchronized periodic solution. This solution persists for large ε (apply a perturbation argument) but if ε is sufficiently small the steady state can be stabilized for a range of $\mathcal{D}_x$ and the periodic solution appears to be quenched. To be precise, the uniform steady state is asymptotically stable for $(\mathcal{D}_x,\varepsilon) \in (\frac{1}{2}T^K,\bar{\mathcal{D}}_x) \times (0,\bar{\varepsilon})$ where $\bar{\mathcal{D}}_x$ is the positive solution of det $(K-2\mathcal{D}) = 0$ and $\bar{\varepsilon}$ is the positive solution of

$$-4\varepsilon^2\mathcal{D}_x^2T^K-2\,\varepsilon\,\mathcal{D}_x[(2\mathcal{D}_x-T^K)T^K + 2\mathcal{D}_xk_{22}]-\det\,(K-2\mathcal{D})(T^K-2\mathcal{D}_x) = 0. \tag{34}$$

Here $T^K \equiv$ trace K and δ is fixed in the interval (δ_-,δ_+). Numerical computations show that the trajectories beginning in a finite neighborhood of the USS converge to this steady state, but we have not proven that the periodic solution disappears. Nonetheless, it is certain that there are no small amplitude oscillations, and a degree of quenching exists.

Another effect of finite ε arises when $\delta > \delta_+$, in which case the kinetics have no periodic solution and the directly-coupled system has no uniform periodic solution. Equation (34) gives the locus on which the linearization of (31) has a pair of complex conjugate eigenvalues with zero real part and an analysis of this equation shows that when

$$\delta_+^2 < \delta^2 < 1-\kappa + \sqrt{1-4\kappa} \tag{35}$$

the bifurcation locus is as shown in Fig. 4. Numerical computations show that the periodic solution exists between the two branches of this locus and is stable when $\mathcal{D}_x < \bar{\mathcal{D}}_x$. Thus the cells can oscillate in synchrony if the volume of the intervening dead space is adjusted properly, and these oscillations are stable to both uniform and nonuniform disturbances. It has been suggested [18] that this model can account for the observed effect of cell density in yeast cell suspensions [5] and a somewhat more complicated model can be used for describing Dd suspensions.

In addition to the synchronized oscillations, there are several branches of asynchronous periodic solutions shown in Fig. 4. The bifurcating solutions are

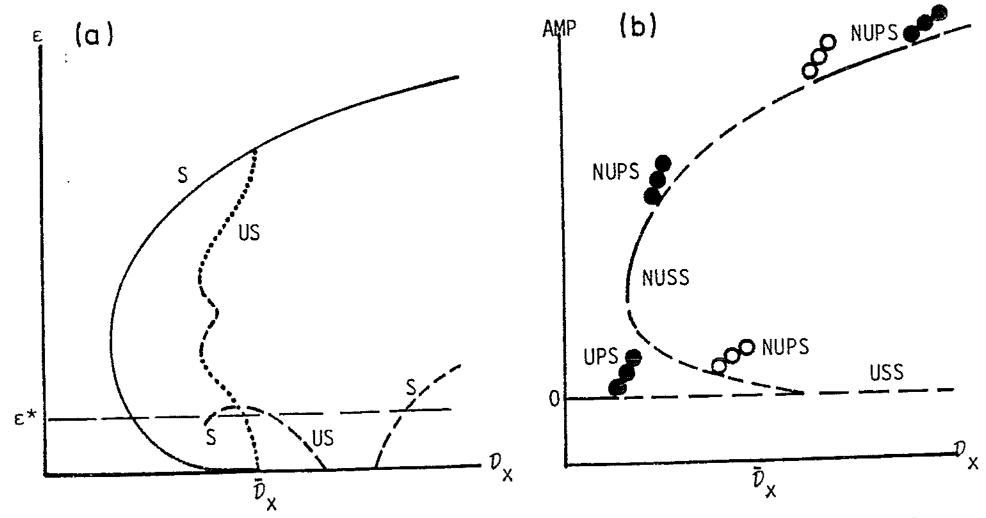

Fig. 4 (a) Bifurcation loci for fixed δ satisfying (35): —: bifurcation locus for UPS; ----(.....) locus for bifurcation of NUPS from upper (intermediate) NUSS. (b) A schematic of the amplitudes for $\varepsilon = \varepsilon^*$.

unstable along curves marked 'US' and stable on those marked 'S'. When its amplitude is small, the solution that bifurcates from the intermediate steady state is stable in a four-dimensional submanifold of the five-dimensional space, but it is not known whether it becomes stable at larger amplitudes. Similarly, it is not known whether any of the other unstable solutions become stable by a tertiary bifurcation. While some of the local features of the bifurcation diagrams are known (cf. Fig. 4(b)), the global structure has not been determined on any cross-section with $\delta > \delta_+$, and there are several singularities of codimension greater than one that must be analyzed. Moreover, it remains to patch together the bifurcation loci in the three-dimensional parameter space. This is easily done for those that correspond to the synchronous oscillations, using results in [18] and those given here.

5. Conclusions

The results given here illustrate how the dynamical behavior of subsystems at one level of organization can influence the dynamical behavior at a higher level of organization. Given a network of intracellular reactions that contains an unstable subnetwork, the diffusive coupling between cells can be chosen so as to destabilize the synchronized steady or time-periodic state of an aggregate of cells. Thus a variety of spatial and spatio-temporal patterns can be generated simply by varying the degree of communication between cells, and TURING first suggested that such patterns could serve as prepatterns for controlling cellular differentiation. The variety of behavior possible with only two cells and two active chemical species points up the fact that relatively tight control of the trajectory in *parameter space* will be needed to achieve a desired sequence of patterns in more complicated systems, but such close control can probably be achieved by turning communication on and off at the appropriate developmental stages. By readjusting internal kinetic parameters during periods in which the cells are uncoupled, quantum steps in the dynamical behavior can be achieved without passing through undesirable types of behavior, and the final destination in parameter space can be reached by a series of zig-zag steps.

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