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# Suppression of chaos by periodic oscillations in a model for cyclic AMP signalling in *Dictyostelium* cells

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*Abstract.* We investigate how the introduction of cells oscillating periodically affects the behaviour of a suspension of *Dictyostelium discoideum* amoebae undergoing chaotic oscillations of cyclic AMP. The analysis of a model indicates that a tiny proportion of periodic cells suffices to transform chaos into periodic oscillations in such suspensions. A similar result is obtained by forcing the aperiodic oscillations by a small-amplitude, periodic input of cyclic AMP. The results provide an explanation for the observation of regular oscillations in suspensions of a putatively chaotic mutant of *Dictyostelium discoideum*[12]. More generally, the results show how chaos in biological systems may disappear through the coupling with periodic oscillations.
*Key words. Dictyostelium discoideum*; cAMP oscillations; biological rhythms; coupled oscillators; chaos; nonlinear dynamics.

In contrast to wild type *Dictyostelium discoideum* amoebae which display periodic waves of chemotactic movement in response to pulses of cyclic AMP (cAMP) emitted at regular intervals by aggregation centers after starvation[1–4], the mutant *Fr 17* aggregates in an aperiodic manner[5]. Preliminary studies of suspensions of the related mutant *HH 201* provided evidence for erratic oscillations of cAMP[6]. The occurrence of chaotic oscillations in a model for cAMP signalling in *D. discoideum* suggested that the aperiodic behaviour of the mutants *Fr 17* and *HH 201* might provide an example of autonomous chaos at the cellular level[7,8] (in most examples known in biology[9–11], chaos is nonautonomous as it results from the periodic forcing of an oscillatory system). The possible occurrence of chaos in *Dictyostelium* was tested by studying light scattering changes, which accompany cAMP oscillations, in suspensions of the mutant *HH 201*[12]. Regular oscillations similar to those occurring in the wild type were observed in these experiments instead of chaos. By analyzing the model for cAMP oscillations we show that these observations might be explained by the fact that a tiny proportion of periodic cells suffices to suppress chaos in cell suspensions. This result is of general significance as it shows how chaos in chemical or biological systems can disappear through the coupling with periodic oscillations.

The model for cAMP oscillations in *Dictyostelium*[13] is based on the positive feedback exerted by extracellular cAMP on its intracellular production[14], and on desensitization of the cAMP receptor through reversible phosphorylation[15] (for a scheme of the model, see also fig. 1 in Martiel and Goldbeter[7]). In addition to accounting for periodic oscillations of cAMP and for the relay of suprathreshold cAMP pulses[13,16], this model predicts the occurrence of more complex phenomena such as the coexistence of two stable rhythms[17], and complex oscillations in the form of bursting or chaos[7,8]. The model has also been used to simulate propagating waves of cAMP in the course of slime mould aggregation on agar[18,19].

To examine the effect of the presence of periodic cells in a suspension of chaotic amoebae, we consider the simplest case of mixing two populations, one periodic and the other chaotic (the results generalize to the mixing of a larger number of distinct populations); the situation considered is schematized in figure 1. The two populations differ by the value of a single parameter, which is either the net rate of ATP supply within the cells (v), or

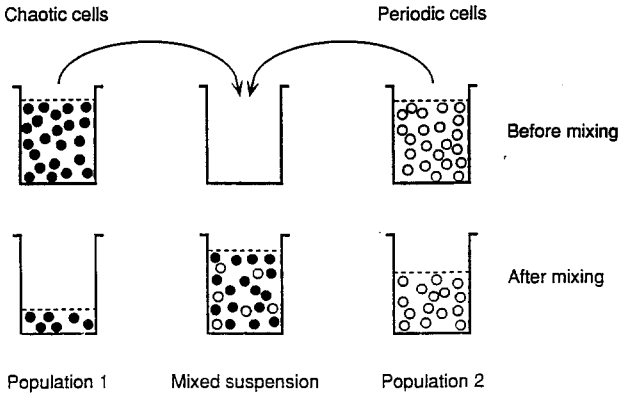


Figure 1. Schematic representation of the mixing of various proportions of *D. discoideum* amoebae behaving (initially) in a chaotic or periodic manner. Cells from population 1 undergo aperiodic oscillations of cAMP, while cells from population 2 oscillate periodically. Cells from population 1 (black circles) are mixed with cells from population 2 (empty circles); the fraction of the two types of cells in the final mixed suspension is denoted by $F_1$ and $F_2$, respectively ($F_1 + F_2 = 1$). The volume of the mixed suspension as well as the number of cells it contains are the same as those of each of the two populations before mixing.

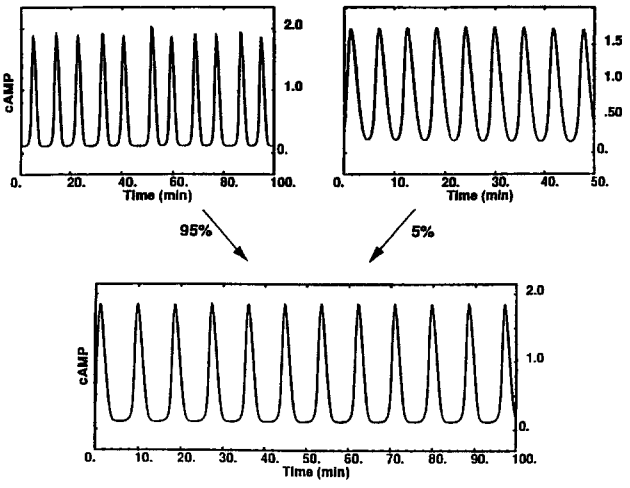


Figure 2. The coupling of 5% periodic cells with 95% chaotic cells results in periodic oscillations of cAMP whose normalized, extracellular concentration ($\gamma$) is shown as a function of time (lower panel). The chaotic behaviour of the homogeneous population 1 (upper panel, left) is obtained for $k_{e1} = 2.6257\ min^{-1}$ while the periodic behaviour of the homogeneous population 2 (upper panel, right) is obtained for $k_{e2} = 2.4257\ min^{-1}$. The dynamics of each homogeneous population is governed by three kinetic equations [17]. The curve in the lower panel was obtained by numerical integration of the five kinetic equations which govern cAMP synthesis in the mixed suspension [20], after elimination of transients. Initial conditions were $\rho_{T1} = 0.124$, $\rho_{T2} = 0.112$, $\alpha_1 = 1.063$, $\alpha_2 = 1.048$ and $\gamma = 0.538$. Parameter values are $k_1 = 1.125\ min^{-1}$, $k_2 = 0.45\ min^{-1}$, $k_i = 4.5\ min^{-1}$, $k_t = 3\ min^{-1}$, $v_1 = v_2 = 4.39807 \times 10^{-3}\ min^{-1}$, $\sigma = 0.75\ min^{-1}$, $L_1 = 316.2277$, $L_2 = 10^{-4} L_1$, $\theta = \lambda = 0.01$, $\varepsilon = 0.2$, $c = 100$, $q = 4000$, $h = 5$.

the total activity of the membrane-bound and extracellular forms of phosphodiesterase ($k_e$) which hydrolyze extracellular cAMP. Continuous variation of either v or $k_e$ elicits the transition from periodic oscillations to chaos within a given population. The values of the two parameters for populations 1 and 2 will be denoted by $v_1$, $k_{e1}$ and $v_2$, $k_{e2}$, respectively.

We wish to determine the behaviour of a suspension in which various proportions of chaotic and periodic cells are mixed (see fig. 1). The dynamics of the mixed suspension is governed by five kinetic equations [20]. Four of these relate to the evolution within each population of the two intracellular variables, namely, ATP ($\alpha$), which serves as substrate for cAMP synthesis, and the fraction of active, dephosphorylated cAMP receptor ($\rho_T$). The fifth equation governs the evolution of extracellular cAMP ($\gamma$) through which coupling occurs between the two populations present within the mixed suspension. Thus, in contrast to the connection of cells through diffusion that is generally considered in studies of coupled oscillators, coupling occurs here through an extracellular signal that is released by all amoebae in the stirred suspension and modulates their behaviour upon binding to specific membrane receptors.

The chaotic behaviour of population 1 and the periodic behaviour of population 2 prior to mixing are represented in figure 2 (upper panels). The dynamic behaviour of the mixed suspension markedly depends on the relative proportions of cells from the two populations. Most striking is the result that a tiny proportion of cells from population 2 sometimes suffices to impose its periodic properties on a suspension containing a large majority of cells from chaotic population 1. Thus, in the specific case considered in figure 2, the mixing of 5% periodic cells with 95% chaotic cells results in periodic oscillations of cAMP (lower panel).

The value of the minimum fraction, $F_{2min}$, of cells from the periodic population capable of suppressing chaos in the mixed suspension depends on the parameters of the model. Thus $F_{2min}$ decreases from a value above 70% to less than 15% as parameters $k_{e2}$ and $v_2$ of the periodic population move further away from the values $k_{e1}$ and $v_1$ which give rise to chaos in population 1. Numerical simulations show that when population 2 is sufficiently far away from the domain of aperiodic oscillations, a tiny fraction of periodic cells, sometimes as small as a few percent, suffices to suppress chaos in the mixed suspension; such a situation is exemplified in figure 2.

What is the origin of the unequal influence of periodic and chaotic cells on the behaviour of the mixed suspension? The analysis of the model (Li et al., manuscript in preparation) indicates that the stronger effect of periodic cells directly results from the smallness of the domain of chaos in parameter space. Thus, mixing two populations which differ only by the activity of phosphodiesterase ($k_{e1} \neq k_{e2}$) will produce a synchronized population with an effective value for $k_e$ equal to $k_{e1}F_1 + k_{e2}F_2$, where $F_1$ and $F_2$ denote, respectively, the fraction of cells from population 1 and population 2 in the mixed suspension. Given that the range of $k_e$ values corresponding to periodic behaviour is much larger than that corresponding to

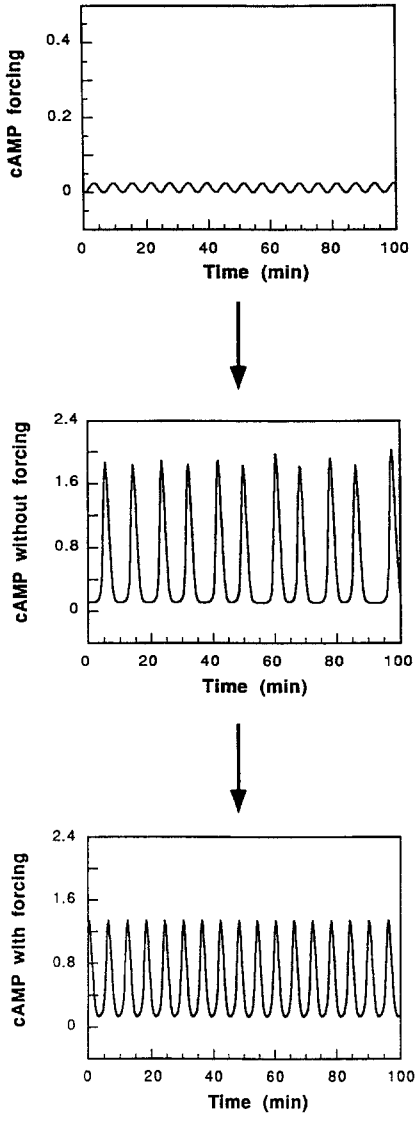


Figure 3. Suppression of chaos by a small-amplitude, periodic input of cAMP. The chaotic oscillations of cAMP (middle panel) are the same as those considered for population 1 in fig. 2. The system is subjected to a sinusoidal input of cAMP (top panel); this input corresponds to a term of the form $A[1 + \sin(2\pi t/T)]/2$ which is added into the right-hand side of the kinetic equation for variable $\gamma$ (see equations [1] in Goldbeter and Martiel[17]). Parameter values are A = 0.025 and T = 6 min; the latter value compares with the period of oscillations in population 2 in fig. 2.

chaos, the chance that the value of $k_e$ resulting from mixing will lie in the periodic domain is higher. Hence, the tilting of the mixed suspension towards periodic rather than chaotic oscillations. Similar results are observed for parameter v, although these cannot be shown analytically as for $k_e$. The above discussion in terms of $k_e$ further explains why the coupling of two distinct chaotic populations, instead of suppressing chaos, generally leads to the synchronization of the two populations to an intermediary regime of aperiodic oscillations.

An alternative way of looking at the results of figure 2 is to interpret the effect of the small proportion of periodic cells as equivalent to the forcing of the chaotic population by a small-amplitude periodic input. To test this hypothesis, we have considered the behaviour of a homogeneous aperiodic cell population (middle panel of fig. 3) and subjected it to a sinusoidal input by adding to the evolution equation for extracellular cAMP ($\gamma$) a term of the form $A[1 + \sin(2\pi t/T)]/2$, where A and T denote, respectively, the amplitude and the period of the periodic input. The value of T was chosen so as to be close to the period of the periodic population in the situation considered in figure 2.

The results of the numerical simulations indicate that a periodic forcing of reduced amplitude is capable of transforming chaos into periodic oscillations (see bottom panel of fig. 3). The amplitude of the sinusoidal input considered in figure 3 is smaller than the amplitude of chaotic oscillations by nearly two orders of magnitude (compare top and middle panels in fig. 3); it is of the order of the periodic contribution of population 2 in figure 2 when cells from the latter population are only a small percentage of the cells in the mixed suspension. These results support the view that the suppression of chaos by a small proportion of cells from the periodic population is equivalent to the transformation of chaos into periodic behavior by the small-amplitude, periodic forcing of a strange attractor. Such a quenching of aperiodic by periodic oscillations can be regarded as one possible method for controlling chaos[21].

The present results suggest an explanation for the occurrence of rather regular oscillations of light scattering in suspensions of the putatively chaotic mutant *HH201*[12]. In a suspension containing a majority of chaotic cells, the presence of a small proportion of periodic cells could arise from a natural heterogeneity in biochemical parameters such as receptor or enzyme concentrations. If the suspension contained a tiny proportion of periodic cells and a majority of cells which would, on their own, oscillate aperiodically, then the strong coupling between the cells in the stirred suspension could well suppress chaotic behaviour. Cell suspension studies would therefore not be appropriate for demonstrating chaos in *Dictyostelium*, in contrast to studies of aperiodic aggregation on agar[5], in which centers would presumably be capable of expressing their chaotic properties in the absence of the strong coupling that exists in stirred suspensions.

An alternative explanation for the absence of chaos in suspensions of *HH201* is of course that mutant cells tested in the light scattering experiments[12] could have developed so as to reach the domain of periodic behaviour in parameter space, which, as mentioned above, is much more extended than the domain of chaos.

The question arises as to the physiological relevance of the present results to other biological systems. Although this study provides a plausible explanation for the suppression of chaos by periodic oscillations in continuously stirred cells suspensions, such a strong coupling may seldom be realized in vivo. It can be expected, however, that the cell suspension situation provides a first approxima-

tion of the behaviour of cells which communicate by means of chemical or electrical signals acting as synchronizing factors. In this light, the present results bear on the occurrence of autonomous chaos in other biological systems such as neuronal or cardiac tissues in which there is a distribution of cellular properties as well as intercellular communication. Given that it is often a rare event in parameter space as compared to periodic behaviour[16,22], chaos could be affected and even suppressed at the cell population level in such heterogeneous systems by the coupling between aperiodic and periodic oscillations.

Note added in proof: The possibility of eliminating chaos by applying a weak periodic forcing has recently been considered in a theoretical study of the periodically driven pendulum (Braiman, Y., and Goldhirsch, I., Phys. Rev. Lett. *66* (1991) 2545).

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## Juvenile hormone I is the principal juvenile hormone in a hemipteran insect, *Riptortus clavatus*

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*Abstract.* Juvenile hormone I (JH I) was identified by combined gas chromatography/mass spectrometry as the predominant JH in the hemolymph of female adults of the bean bug, *Riptortus clavatus* (Thunberg) (Hemiptera: Alydidae). Among JH I, II, and III, JH I was the most effective hormone for inducing the synthesis of yolk proteins in diapause adults.
*Key words.* Juvenile hormone I; *Riptortus clavatus*; cyanoprotein; vitellogenin; adult diapause.

Five different juvenile hormones (JHs) have been identified in insects, i.e. JH I, II, III, 0, and 4-methyl JH I[1–5]. In Hemiptera, however, little has been reported about the precise identification of the JH molecules present, although there have been many endocrinologial studies in this order, such as the pioneer work on the role of the corpus allatum by Wigglesworth[6]. In the Hemiptera, JH III was identified in the developing embryo of *Oncopeltus fasciatus*[5], but more recently the same research group reported the absence of significant levels of the known JHs in eggs and adults of this species[7]. The corpora allata of *Dysdercus fasciatus* were shown to synthesize only JH III in vitro[8]. Furthermore, only JH III was identified from the whole body of *Megoura viciae* and *Aphis fabae*[9]. Thus the few existing reports all showed JH III as the only natural JH in Hemiptera. Nevertheless, Schooley et al.[10] pointed out the possibility of the existence of unknown JH molecules in this order, because of its endocrinological peculiarity among insects. Therefore, it is worthwhile to make further attempts to identify JHs in Hemiptera.