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The wild-type circadian period of *Neurospora* is encoded in the residual network of the null *frq* mutants

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Abstract

We model theoretically the response of the widely studied circadian oscillator of *Neurospora crassa* to inactivation of the *frq* gene. The resulting organism has been termed "arrhythmic" under constant conditions. Under entrainment to periodic temperature cycles Roenneberg, Merrow and coworkers have shown that the phase angle at which spore formation occurs depends on the entrainment period, curiously even in the null *frq* mutants (frq^9 and frq^{10}). We show that such a response does not imply the presence of a self-sustained free-running oscillator. We derive a simple candidate model (a damped harmonic oscillator) for the null *frq* mutants that successfully reproduces the observed phase angle response. An endogenous period of 21 h for the damped harmonic oscillator coincides with the endogenous period of wild-type *Neurospora*. This suggests that the (noise driven) "residual system" present in the mutants may have a significant timekeeping role in the wild-type organism. Our model (with no change of parameters) was then used to investigate spore formation patterns under constant conditions and reproduces the corresponding experimental data of Aronson et al. (Proc. Natl. Acad. Sci. USA 91 (1994) 7683.)

Keywords: *Neurospora*; Circadian; Oscillator; Temperature entrainment; *frq* gene

1. Introduction

Circadian rhythms are observed both in unicellular organisms and in higher eukaryotes, but the mechanism behind the rhythmicity of the circadian clock consists of intracellular processes (Aschoff, 1981; Dunlap, 1999; Goldbeter, 1996; Roenneberg and Merrow, 2001). The circadian system of the fungus *Neurospora crassa* is one such genetic model system that has been widely studied (Bell-Pedersen et al., 2001; Crosthwaite et al., 1997; Dharmananda, 1980; Feldman and Hoyle, 1973; Lakin-Thomas, 1998; Liu et al., 1998; Loros and Feldman, 1986; Merrow et al., 1997, 1999, 2001; Roenneberg and Merrow, 2001; Russo, 1988; Sargent et al., 1956; Dunlap, 2003). Many of the circadian components in *Neurospora* are now known although the precise manner in which they interact is still not fully characterized.

In *Neurospora* a negative feedback loop is thought to give rise to regular oscillations with the observed period of 21 h (Dunlap, 1999; Roenneberg and Merrow, 2001) although, we will demonstrate that this period also manifests itself in the null *frq* mutants. The output of this circadian oscillator controls, amongst other things, spore formation in *Neurospora*. The point in the cycle at which this spore development occurs is used as the clock "read-out", since it is a readily observable event that occurs periodically with a period identical to that of the clock components. It is not yet clear from the experimental data that *frq* plays the exclusive time-keeping role that this discussion suggests and indeed other regulatory elements, only some of which are mentioned above, certainly play important roles. Rhythmic spore formation can be observed in *frq* null mutants under various conditions (Aronson et al., 1994; Lakin-Thomas and Brody, 2000; Loros and Feldman, 1986; Merrow et al., 1999).

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As well as exhibiting regular oscillations circadian rhythms must also exhibit sensitivity to external conditions. Detection of light in *Neurospora* causes changes in *frq* RNA levels (Crosthwaite et al., 1997) and acts to reset the circadian clock so that the phase of the clock coincides with the phase of the external world (Dharmananda, 1980; Pittendrigh, 1965). Temperature is another factor that can affect the synchronization of the cell. The *Neurospora* oscillator can be phase reset by temperature changes (Liu et al., 1998; Merrow et al., 1999) but its period is carefully temperature compensated (Gardner and Feldman, 1981; Loros and Feldman, 1986). Entrainment of circadian systems to a periodic external signal (*zeitgeber*) such as a temperature cycle, is mediated by repeated phase resetting.

Entrainment phenomena are believed to occur in a number of different systems. In response to periodic stimulation, endogenous rhythms can become phase locked to the stimulus giving periodic dynamics. The effects of brief periodic stimulation of circadian oscillators was presented by Pittendrigh and others (e.g. Pittendrigh, 1965).

The idea of modeling general circadian systems using coupled oscillators and feedback loops has been investigated by many authors, including Roenneberg and Merrow (1998,1999). However, these systems can be mathematically quite complex, and it is our intention in the present work to rather focus on the construction of a *minimal* mathematical model for the residual circadian network of a mutant organism that does not exhibit regular oscillations.

The present work is motivated by the experimental studies of Roenneberg and Merrow (Merrow et al., 1999; Roenneberg and Merrow, 2001) who use temperature entrainment to probe the circadian oscillator of *Neurospora*. Several different *frq* mutants were entrained to temperature cycles of varying periods, but we are primarily interested in the behavior of two null mutants. The frq^9 allele is a mutation that is thought to result in a truncated protein (Loros et al., 1986), and it shares the same properties as frq^{10} (which produces no protein at all) (Aronson et al., 1994). These mutants have been described as "lacking circadian rhythms" under conditions of constant temperature in the dark. This is quite a subjective definition, but is generally accepted as meaning that their rhythms are markedly less regular than those of the wild type under standard experimental conditions. We shall use this as a definition of "arrhythmic" throughout. The rhythm of the mutants can be made more regular by entraining them to temperature cycles of varying periods. For periods not too different from 24 h, they entrained stably (period-locked) to the *zeitgeber* cycle such that their spore formation occurred once per cycle.

It has been observed (Merrow et al., 1999; Roenneberg and Merrow, 2001) that the phase angle at which spore development occurs is dependent on the period of the entrainment, even for the null mutants (which remains a controversial observation (Dunlap, 2003)). This might be regarded as somewhat surprising in view of the fact that *frq* had been described as an integral component of the circadian system, and yet the organism still showed a response that suggested some sort of internal timekeeping. An early suggestion that the influences of FRQ and *frq* on each other did not describe the whole system was by Lakin-Thomas et al. (Lakin-Thomas and Brody, 2000). The residual timing system has since been dubbed the *FRQ-less oscillator* (FLO) (McWatters et al., 1999) and its biochemical components are unknown. Our goal is to establish the minimum mathematical requirements for this observed behavior. As we will see, a good place to start is with a linear approximation (an approach generally overlooked by those building mathematically complex nonlinear systems).

In order to proceed, we first make some qualitative observations of the data (Merrow et al., 1999; Roenneberg and Merrow, 2001) which we will require that our model reproduces. The phase angle ϕ at which spore formation occurs in null mutants entrained to temperature cycles of varying periods T_ω is reproduced in Fig. 1. This data represents a few measurements sampled from

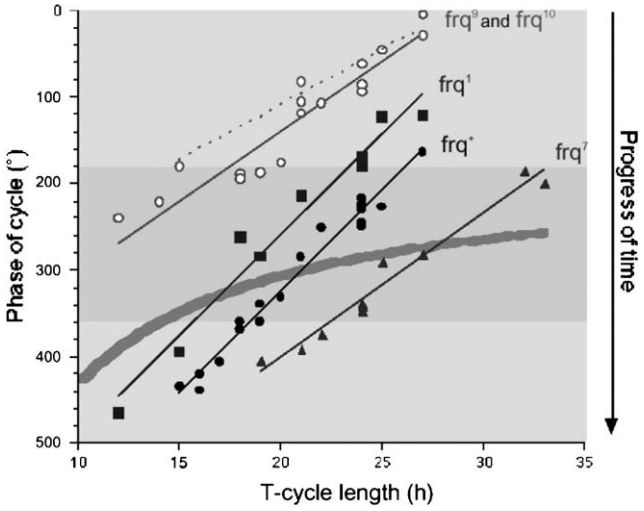

Fig. 1. Reproduction of the experimental results of Roenneberg and Merrow (2001). The graph shows the phase angles (ordinate) at the onset of spore formation under entrainment by temperature cycles of different length (abscissa). Various *frq* mutants of *Neurospora* are displayed: wild-type frq^+, short frq^1 and long frq^7 period mutants and "null" mutants frq^9 and frq^{10}. Light gray areas represent 27°C, darker gray 22°C, a convention that is maintained in later figures. Phase angles are calculated in degrees of the respective temperature cycle (the beginning of the warmer period being 0°). The very dark gray curve was present in the original figure of Roenneberg and Merrow and represents the phase angle of a hypothetical event that occurs a constant 7 h after the warm to cold transition, irrespective of the length of the temperature cycle. This curve has no significance within the context of the present work.

the unknown function $\phi(T_\omega)$ for the wild-type frq^+ and the mutants frq^1, frq^7, frq^9 and frq^{10}. No significant difference was observed between the two arrhythmic mutants frq^9 and frq^{10}. The behavior of these arrhythmic mutants was the focus of our investigation.

One possible interpretation of the experimental data shown in Fig. 1 is that some (hidden) endogenous period of the null mutants (frq^9 and frq^{10}) can be determined by considering the known free-running periods of the other mutants. The endogenous periods at 25°C of the wild type (frq^+), the long period mutant (frq^7) and the short period mutant (frq^1) are 21, 29 and 16 h, respectively. This argument is based on the observation that in each case when the driving period T_ω is the same as the endogenous period T_Ω, spores form in the middle of the colder period. The argument then continues that the endogenous period of the null mutants can be found by extrapolating the straight line through the experimental data to the middle of the cold period and assuming that the driving period that caused the spore formation at that point is the endogenous period of the null mutants. This would suggest that the endogenous period of the null mutants is about 12 h (Merrow et al., 1999; Roenneberg and Merrow, 2001). We do not favor this interpretation and devote much of the rest of this article to the analysis of an alternative.

Our goal is now to investigate what kind of residual circadian system gives a qualitative response such as that observed in the null mutants, and to determine what is the simplest such model that also gives a satisfactory quantitative fit to the data.

2. The circadian oscillator as a system of coupled rate equations

The state of the circadian oscillator in *Neurospora*, and indeed in any organism, at a given time may be described by the concentrations of all the distinguishable clock components. This may separately include the concentrations of any partially or fully phosphorylated proteins, their RNA and, indeed, these concentrations in each different cellular compartment, most obviously the cytoplasm and nucleus. Within this scheme, it seems reasonable to neglect the effects of diffusion not adequately treated by such a compartmentalization approach on the grounds that characteristic diffusion time for a protein to traverse a typical cell is on the scale of seconds. These N concentrations can be represented as an N-dimensional vector $\mathbf{u}(t)$, where every component of the vector $u_i(t)$ is one such concentration that can vary in time.

The advantage of this specification of the state of the system is that it allows a rather compact mathematical description of the circadian oscillator particularly if one invokes a "mean field" approximation which amounts to neglecting random fluctuations in concentrations and assumes that it is only the rate of change (first derivative) of these concentrations which is controlled by the state of the system, rather than the second or higher derivatives (which would correspond to *direct* control of rates of acceleration of concentration).

The resulting mathematical description, which allows for high-order chemical kinetics is

$$\frac{\mathrm{d}u_i}{\mathrm{d}t} = b_{ij}u_j + c_{ijk}u_ju_k + d_{ijkl}u_ju_ku_l + \cdots, \tag{1}$$

where the order in $\mathbf{u}$ at which this expansion is truncated, corresponds to the highest order chemical kinetics present. The matrix elements b_{ij}, c_{ijk}, d_{ijkl}, etc. can be identified with first-, second- and third-order rate constants, respectively. Most oscillators, including the van der Pol oscillator, can be constructed from such a system of equations. Their relevance to us here is that removing components such as *frq* from the circadian system reduces the dimensionality of $\mathbf{u}$—we must now specify fewer component concentrations. The ultimate extrapolation of this is to one (and ultimately zero) dimensions. The one-dimensional version of this equation, truncated at linear order in $\mathbf{u}$ ($c_{ijk} = d_{ijkl} = \cdots = 0$) is the first-order decay model considered later. It is, in this sense, the simplest possible residual circadian system for the null mutants. The two-dimensional version of this equation, also truncated at linear order in $\mathbf{u}$, can be shown to yield the damped harmonic oscillator (also considered later).

The actual biochemical system is likely to be of the form of a weakly attracting nonlinear system, but we have no reason to assume any form for the nonlinearity would be better than another. These linear systems are good approximations on which to base such a weakly attracting nonlinear model.

3. Method 1: modeling using a first-order decay equation

The simplest candidate behavior for the residual circadian system in the null mutants is that of a first-order decay process. By this we mean that the rate of production of the component of the circadian system that directly controls spore formation under entrainment is a function of the temperature and that this component undergoes simple decay characterized by a single time constant τ. The concentration of this component is determined by the variable x which may be thought of as the deviation from its average concentration. In what follows, we study only phase *differences* between the response of our model systems and the entrainment cycle. The absolute phase is arbitrary within our model and amounts to the choice of whether spore development occurs when x is at a maximum, a minimum, or at any other fixed point in its

cycle. Mathematically the simple decay process we have described is equivalent to

$$\frac{dx(t)}{dt} + x(t)/\tau = f(t), \tag{2}$$

where the entrainment signal $f(t)$ is proportional to temperature and represents the effect of temperature on the rate of production of the spore formation controlling component x. We consider first the most simple idealization where the entrainment signal is sinusoidal with some amplitude A and some frequency ω (where $2\pi/\omega$ gives the length of the entrainment period, T_ω):

$$f(t) = A\cos\omega t. \tag{3}$$

4. Results and discussion

The solution to Eq. (2) is

$$x(t) = \frac{A\tau}{\sqrt{1+\omega^2\tau^2}}\cos(\omega t + \phi), \tag{4}$$

where the phase angle varies with entrainment period according to

$$\tan\phi = -\frac{2\pi\tau}{T_\omega}. \tag{5}$$

Fig. 2 shows the variation of ϕ with period length T_ω that we can compare with the data of Fig. 1. It can be seen that the response characterized by Eq. (5) fails to be a good fit to the data. Experimentally, the total change in phase angle between entrainment at the shortest and longest periods is approximately 180° and certainly greater than 90°. The line of best fit exhibits a maximum phase angle change of 90° between very short and very long period entrainment and, for the range of periods studied experimentally (Merrow et al., 1999; Roenneberg and Merrow, 2001), only a small fraction of 90°. On these grounds, we eliminate this first-order decay model as a candidate to model the residual circadian system in the null mutants.

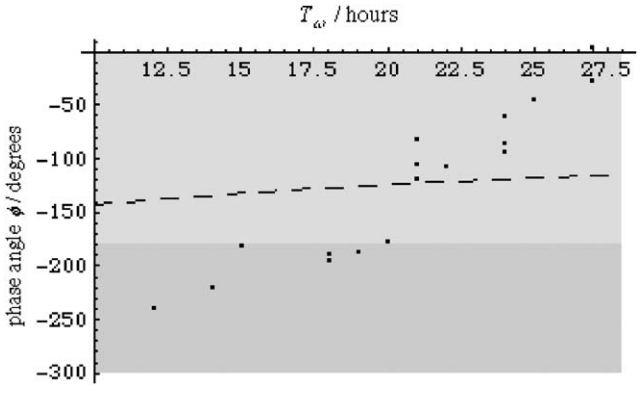

Fig. 2. The experimentally observed phase angle ϕ at which *frq*-inactive *Neurospora* (*frq*^{9} and *frq*^{10}) conidiate as a function of the period of the temperature entrainment cycle T_ω (points). Data courtesy of Roenneberg and Merrow (2001). Also shown is the curve of best fit to a simple first order decay as given by Eq. (5), corresponding to $\tau = 3.07$. It can be seen that this model gives a bad fit to the experimental data.

5. Method 2: modeling using a damped harmonic oscillator

As the driven, first-order decay equation has been found lacking, we investigate the next most simple model for the FLO within a scheme that models the system as a system of differential equations with an increasing number of components and chemical kinetics. This residual circadian system might more successfully reproduce the phase angle behavior observed in the null mutant experiments. With x again the concentration difference, from its average value, of the component that controls spore development we may define the driven, damped harmonic oscillator as

$$\frac{d^2x(t)}{dt^2} + 2v\frac{dx(t)}{dt} + kx(t) = f(t) \tag{6}$$

with $f(t)$ again some driving term proportional to temperature that models the temperature entrainment signal and v and k are as yet unspecified (fit) parameters. It is important to note that while this second-order equation explicitly involves the concentration of only one clock component (x) it can be shown to be equivalent to the special case of Eq. (1) corresponding to a system of two coupled first-order linear differential equations, i.e. *two* relevant clock components controlling each other's rates of production. Thus, it is the natural next choice of candidate to describe the *Neurospora* data in which one could trace the origin of the apparently arbitrary parameters v and k to (combinations of) the underlying rate constants in the corresponding kinetic equations.

It is only in the special case $v = 0$ that sustained, periodic oscillatory variations in x can occur in the absence of any driving (entrainment) term, $f(t) = 0$. In this case, the equation reduces to that of a simple harmonic oscillator with frequency $\sqrt{k}$. For values $v > 0$ of interest to us in what follows there are no oscillatory solutions in the absence of any driving term (see later section on driving with noise). The only steady-state solution in this case is a constant concentration. However, the response of Eq. (6) to entrainment is still rather different, depending on the sign of $k - v^2$. If this is negative then the oscillator is *overdamped* and Eq. (6) actually reduces to that of a first-order decay, Eq. (2). On the other hand if this quantity is positive, as we may therefore assume in what follows, the system exhibits damped oscillatory response. A familiar feature of a damped oscillator would be the appearance of transient

oscillations, with decaying amplitude, when entrainment is switched off.

6. Results and discussion

We first consider the case where the periodic temperature variation used to entrain the null mutants is sinusoidal according to Eq. (3). Eq. (6) can be solved to find $x(t)$ and in so doing a variable Ω (with the dimensions of frequency) naturally appears. It is the characteristic frequency of the damped oscillator and is given by

$$\Omega = \sqrt{k - v^2}. \tag{7}$$

It can be shown that $x(t)$ is given by

$$x(t) = \frac{A\cos(\omega t + \phi)}{\sqrt{v^4 + 2v^2(\omega^2 + \Omega^2) + (\omega^2 - \Omega^2)^2}}, \tag{8}$$

where the phase angle ϕ is now rather different to Eq. (5)

$$\tan\phi = \frac{-2v\omega}{v^2 + \Omega^2 - \omega^2}. \tag{9}$$

It can be seen from Eq. (8) that the response of the driven harmonic oscillator is sinusoidal with the same frequency ω as the sinusoidal driving force, but lags the driving force by a constant phase angle ϕ, given by Eq. (9). Since this phase angle is related to ω, and $2\pi/\omega = T_\omega$ gives the period of the driving force, we can plot the variation of the phase angle with the period of the driving force. This can then achieved using a best fit to the experimental results (Merrow et al., 1999; Roenneberg and Merrow, 2001), where the driving force is the temperature cycle.

If the experimental data points are represented by $\Phi(T_\omega)$, and the fitting function is represented by $\phi(T_\omega)$, then the object is to minimize the least-squares residue, as this will give the best fit. The least-squares residue is defined as

$$E = \sum |\Phi(T_\omega) - \phi(T_\omega)|^2. \tag{10}$$

The best least-squares fit of our curve to the data is shown in Fig. 3 with best fit parameter values of $v = 1/55.6\,\mathrm{h}^{-1}$ and $\Omega = 2\pi/20.7\,\mathrm{h}^{-1}$. Our curve gives a least-squares residue of 9156 (the best straight line gives a least-squares residue of 9162, showing that our curve is the slightly better fit).

Thus, the damped harmonic oscillator, which we here employ as a model for the null mutants residual circadian system, is rather lightly damped but has a characteristic period $T_\Omega = 20.7\,\mathrm{h}$ that is extremely close to the 21 h endogenous period of wild-type *Neurospora* (*frq*$^+$). Therefore, the endogenous period of the wild-type organism may be encoded into what remains of the circadian circuitry of the null mutants. The same

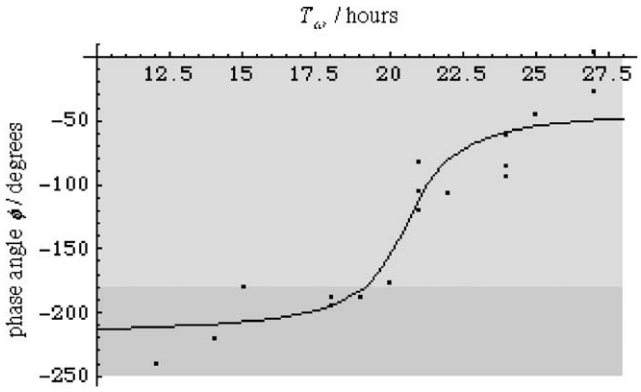

Fig. 3. The experimentally observed phase angle ϕ at which *frq*-inactive *Neurospora* (*frq*^{9} and *frq*^{10}) conidiate as a function of the period of the temperature entrainment cycle T_ω (points). Data courtesy of Roenneberg and Merrow (2001). Also shown is the curve of best fit to a driven, damped harmonic oscillator, corresponding to $v = \frac{1}{55.6}\,\mathrm{h}^{-1}$ and $T_\Omega = 2\pi/\Omega = 20.7\,\mathrm{h}$. It can be seen that this model gives a good fit to the experimental data.

qualitative conclusion might be reached merely by identifying an inflection in the $\phi(T_\omega)$ curve near $T_\omega = 21\,\mathrm{h}$, also indicating that this period is built into the null mutants. We have shown that *frq* is not uniquely required to generate the circadian period in *Neurospora*. One possibility is that *frq* does not play a true time-keeping role, but rather powers the FLO that remains in the null mutants (*frq*^{9} and *frq*^{10}) such that the clock is able to sustain itself more efficiently, an idea proposed by Roenneberg and Merrow (1998). The spring in a mechanical clock plays a similar role by providing energy to a separate time-keeping device, such as an inertial pendulum.

The driving term in the above analysis, $f(t)$, was chosen to be sinusoidal for simplicity. We have also examined numerically the effect of a square wave driving signal (employed experimentally) where the temperature was held constant for half of the period, and then changed to a different temperature for the other half. We conclude that the response observed is rather similar, indeed probably experimentally indistinguishable, from the sinusoidal driving previously considered, as can be seen from Fig. 4.

In view of this quantitative similarity, Eq. (9) can probably be employed as an analytic estimate for the phase angle even under conditions of a square wave entrainment signal.

7. Method 3: driving the model using internal noise

Experimental evidence produced by Aronson et al. (1994) shows that the *average* delay between sporadic spore formation events in the null mutants under "constant" conditions, is 21.4 h. This is similar, both to the endogenous period of wild-type *Neurospora* in

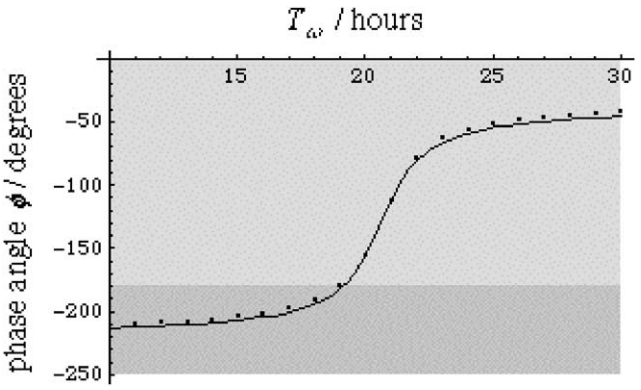

Fig. 4. The phase angle ϕ of the signal from a driven damped harmonic oscillator as a function of the entrainment period for (i) a square wave using the values of $v=\frac{1}{55.6}\,\mathrm{h}^{-1}$ and $\Omega=2\pi/20.7\,\mathrm{h}^{-1}$, as found from the experimental data fit in Fig. 3 (points) (ii) a sinusoidal driving force fitted to these data points (solid line). The parameter values for the curve obtained from the sinusoidal driving force are $v=\frac{1}{56.8}\,\mathrm{h}^{-1}$ and $\Omega=2\pi/20.8\,\mathrm{h}^{-1}$.

constant conditions and to the characteristic frequency of our model of the null mutants under temperature cycles. The remainder of this section will be devoted to demonstrating that our model for the null mutants can also predict the same *average* delay between spore formation events under constant conditions.

It can be shown that the solution to Eq. (6) in the absence of any driving term $f(t)=0$ always approaches $x=0$, regardless of initial conditions. However, there will always be some variation in the internal conditions of the cell in the input pathway, due to stochastic fluctuations in molecular concentrations, metabolic levels, etc. (Thattai and van Oudenaarden, 2001; Vilar et al., 2002). We will denote what drives this variation as the "internal noise" of the system. In what follows, we examine the response of the damped harmonic oscillator model of the residual circadian system driven by a generic form of noise. We are motivated by the belief that this is more representative of the behavior of the residual circadian circuit under what might be experimentally termed "constant" conditions and aim to compare our results with the experimental data (Aronson et al., 1994).

We assume a canonical form for the noise $f(t)=\Psi(t)$ in Eq. (6) with $\Psi(t)$ a Gaussian random variable with zero mean $\langle \Psi(t)\rangle = 0$ (fluctuations are no more likely to drive concentrations up than down) and second moment given by

$$\langle \Psi(t)\Psi(t')\rangle = \varepsilon\delta(t-t') \tag{11}$$

which specifies that the fluctuations are uncorrelated in time (a fluctuation that drives the concentration up now makes it no more or less likely that it is driven up again at any later time).

8. Results and discussion

The use of Eq. (11) suggests that Eq. (6) now has only stochastic solutions. Some of the statistical properties of the response can be understood from the resulting correlation function describing the average value of x at two different times

$$\langle x(t)x(t+\tau)\rangle = -\frac{\varepsilon\exp(-v\tau)}{4vk\Omega}(v\sin\Omega\tau+\Omega\cos\Omega\tau). \tag{12}$$

The correlation function is oscillatory with the same period as the natural frequency of the harmonic oscillator, T_Ω. The fact that the noise is now the only driving signal means that the correlations decay with characteristic time $1/v$ (mathematically this is due to the exponential term in Eq. (12)), see Fig. 5. Thus, roughly speaking, knowledge of the state of the system at an earlier time only gives significant information on its state for times up to $1/v$ later. This result implies that each subsequent spore formation event is *likely* to occur approximately T_Ω after the previous one. Thus, the response $x(t)$ resembles a clock that remains roughly in phase for $1/v$ hours. Indeed, a computer simulation of Eq. (6) with such a Gaussian random driving term gives precisely this behavior. The result of one typical simulation run is shown in Fig. 5. It can be seen that most reasonable choices of a criterion for spore development result in such events occurring roughly every 21 h, as might have been expected from our knowledge of the correlation function. This behavior is not sensitive to the amplitude of the noise (the system still functions in the same way even if the noise is very small). Thus, this is a reasonable simulation of "constant" experimental conditions and our earlier (implied)

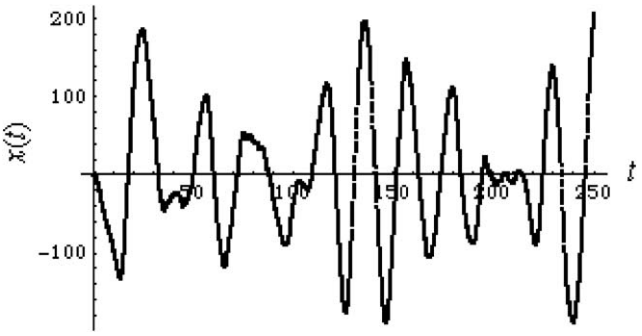

Fig. 5. Our model for the behavior of the residual circadian circuit under what might be experimentally termed "constant conditions". A single realization of the concentration of the molecular component controlling spore formation $x(t)$ (arbitrary units) is plotted against time t (hours). This is obtained from a numerical simulation of the damped harmonic oscillator model for the residual circuit (Eq. (6)) driven by generic Gaussian noise, as defined in the text. The model parameters are those obtained previously $v=\frac{1}{55.6}\,\mathrm{h}^{-1}$ and $\Omega=2\pi/20.7\,\mathrm{h}^{-1}$.

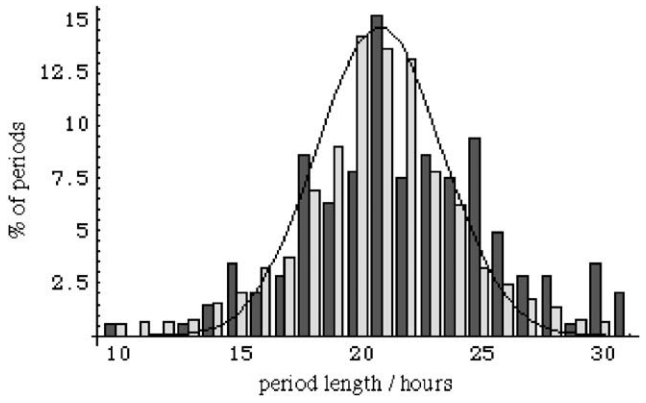

Fig. 6. In dark gray: The results of the experiments of Aronson et al. (1994) for the distribution of period lengths in the null mutants (frq^9 and frq^{10}) at 25°C. The mean period between spore formations is 21.4 h and the standard deviation is 3.98 h. In light gray: A histogram of the periods between spore formations obtained using our model for the residual circadian circuit under what might be experimentally termed "constant conditions". As before this is obtained from a numerical simulation of Eq. (6) driven by generic Gaussian noise with $\nu = \frac{1}{55.6}\,\mathrm{h}^{-1}$ and $\Omega = 2\pi/20.7\,\mathrm{h}^{-1}$. The mean period is 20.7 h, and the standard deviation is 3.36 h. The curve is a Gaussian fit to our data.

assumption that entrainment is likely to suppress this molecular noise is also justifiable.

The results from many simulations similar to that shown in Fig. 5 are shown in Fig. 6, the periods being determined by the time interval between one (upward) crossing of the x-axis and the next. Also shown is the experimental data of Aronson et al. (1994) and a Gaussian curve fitted to the simulation data. It can be seen that our model for the null mutants under temperature cycles also gives an excellent fit to the experimental data under "constant" conditions without modification of the parameters.

9. General conclusions and overview

The phase angle at which spore formation occurs in the null *frq* mutants of *Neurospora* is observed to depend on the period of the entrainment signal (Merrow et al., 1999; Roenneberg and Merrow, 2001). We show that this behavior does not necessarily imply the presence of a fully functional self-sustaining circadian oscillator. A minimum mathematical model for the residual circadian system in the null mutants that approximates the main qualitative features of the experimental data is that of a damped harmonic oscillator entrained (driven) by temperature variation. Biochemically this corresponds to at least two molecular components that linearly control each others rate of production. Roenneberg and colleagues have reached a similar conclusion using a more complex damped oscillator model (Roenneberg and Merrow, 1998). Our simpler model, with a characteristic period of 20.7 h, correctly reproduces the rapid change in the phase angle at which spore formation occurs for entrainment periods around 21 h and is a good fit beyond this. This period is remarkably close to that of the endogenous period of the *Neurospora* oscillator. We therefore claim to have found evidence that this period may be encoded in the null mutants of *Neurospora*. This may signify that the timekeeping part of the oscillator circuitry is intact in the arrhythmic *frq* mutants and thus that the primary timekeeping role may not be exclusively due to *frq* regulation in the wild type (in agreement with Merrow et al. (1999). If this is the case then the FLO may be an essential part of the wild-type oscillator (as opposed to a separate less dominant oscillator unmasked by the removal of *frq*). This supports speculation that the FLO could have evolved to become the circadian clock in present day *Neurospora*.

We also model the free-running null mutants by driving the residual damped harmonic oscillator with noise chosen so as to mimic natural internal variation in molecular composition. We observe that the distribution of period lengths in the response agrees well with the experimental data obtained by Aronson et al. (1994). This further supports our identification of the (noise driven) residual circadian circuitry as resembling that of a (noise driven) damped harmonic oscillator.

This damped harmonic oscillator model for the FLO has been extended to linear models for the wild type and long- and short-period mutants. The results of these will be discussed in forthcoming work (to be submitted).

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