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Circadian rhythms in *Neurospora crassa:* a clock mutant, *prd-1,* is altered in membrane fatty acid composition

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The fatty acid compositions of the phospholipids of *Neurospora crassa* mutants with altered periods were determined to test the possibility that some of these mutants might have altered membrane composition. In liquid shaker culture in constant light the *bd* (band) strain, which has a normal period (21.6 h), exhibited a growth-dependent increase in linoleic acid content and a decrease in linolenic acid content during early log phase growth. By late log phase, fatty acid composition was essentially constant. The phospholipid fatty acid compositions of *bd* strains containing mutations at the *frq* (frequency) and *chr* (chrono) loci were indistinguishable from that of the *bd* strain under the conditions used. However, a *bd* strain containing a mutation at the *prd-1* (period) locus, as well as *prd-1* segregants from a cross of this strain to a *bd* strain, had altered patterns of growth-dependent fatty acid composition; linoleic and linolenic acid contents changed more slow than in the *bd* strain and continued to change throughout growth. In addition, the fatty acid composition of a *bd prd-1* strain on solid medium differed from that of the *bd* strain. It is proposed that the *prd-1* mutation leads to altered membrane homeostasis, which in turn affects circadian rhythmicity because some or all components of the rhythm-generating system are membrane-localized.

Introduction

Many, it not all, eukaryotic organisms display circadian rhythms, physiological or biochemical oscillations with a period of about one day which persist under constant conditions. Such rhythms are temperature-compensated, the periods varying little with temperature, and are sensitive to light, which causes a shift to a different phase of the rhythm.

Despite intensive research the biochemical mechanism by which rhythmicity is generated remains unknown. The involvement of cellular membranes has been proposed by several investigators [1–4], and circumstantial evidence has accumulated which suggests such involvement. For example, a rhythm has been described in the sensitivity of the spheroplasts of *Gonyaulax polyedra* to valinomycin and external potassium [5] as well as a rhythm in intracellular potassium, which suggests rhythmic changes in membrane permeability [6]. Rhythms in the fatty acid composition of the phospholipids of *Neurospora* [7] and of *Phaseolus coccineus* pulvini [8] have been reported. In *Neurospora*, the *cel* (chain elongation) mutant [9], which is partially blocked in the synthesis of fatty acids [10], has lost temperature compensation of

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its conidiation rhythm [11] and has a period sensitive to supplemental fatty acids [12,13]. We have previously shown that the *cel* mutant also has an abnormal phospholipid fatty acid composition [14], which might explain the altered properties of its rhythms, if the rhythm-generating system has membrane-localized components. We have therefore analyzed the membrane phospholipid fatty acid compositions of a number of other mutants of *Neurospora* with periods longer or shorter than the wild-type period.

We report here that one period mutant of *Neurospora*, the *prd-1* mutant, has abnormal phospholipid fatty acid composition. This is consistent with the hypothesis that some components of the rhythm-generating system are membrane-localized, and further suggests that the primary defect of the *prd-1* mutation might be in the homeostasis of membrane properties.

Materials and Methods

Strains

All strains used in this study contained the *bd* (band) mutation [15]. This mutation makes conidiation relatively insensitive to carbon dioxide so that the conidiation rhythm can be clearly seen on petri plates. The underlying circadian oscillator does not appear to be affected by this mutation [16]. In addition, strains used for determining fatty acid composition of plate-grown cultures contained the *csp-1* (conidial separation) mutation [17] which prevents re-inoculation of plates during manipulation.

Period mutants at the following loci were used in this study: *chr* (chrono) [18], *prd-1* (period) [19], and *frq* (frequency) [20]. The period of the rhythm, 21.6 h in the *bd* strain [20], is lengthened in both *chr* and *prd-1*, to 23.5 and 25.8 h, respectively. Four alleles of the *frq* locus were studied: *frq-1, -2,* and *-3* [20], and *frq-8* [18], which have periods of 16.5, 19.3, 24.0 and 29.0 h, respectively.

Strains carrying each of these mutations and the *bd* mutation were kindly supplied by Dr. Jerry Feldman of the University of California, Santa Cruz. The *csp-1* mutation was introduced into these strains by standard crossing techniques [21].

Stock cultures on Vogel's minimal medium [22] plus glucose and agar were kept at 4° C. From these stock cultures, fresh cultures on the same medium were prepared and allowed to grow for 7–14 days at room temperature before being used for inoculation in an experiment.

Growth of cultures

Strains were grown in petri plates on solid medium prepared with Difco Bactoagar (Difco Laboratories, Detroit, MI). Linear growth rate on plates and the period of the conidiation rhythm were measured as described [12]. Calculations were performed on an Apple IIe computer with an Apple Graphics Tablet using a program developed by Robin Ralls and one of us (G.G.C.). Strains were grown in liquid culture as previously described [14].

Determination of phospholipid fatty acid composition

Cultures growing on liquid or solid medium were harvested as described [14]. Fatty acids were prepared, as methyl esters, from the phospholipids of the harvested mycelia and were analyzed by gas-liquid chromatography, also as described [14].

Statistical methods

Standard regression techniques were used to fit lines to the data of fatty acid levels as functions of residual dry mass. Extra sum-of-squares analyses were performed as previously described [14] to determine whether the regression patterns of a given fatty acid in two different strains were significantly different.

Results

Growth of the strains in liquid culture

Growth in liquid culture was measured as a function of time. Representative curves for the *bd, bd frq-1, bd chr* and *bd prd-1* strains are shown in Fig. 1. Growth curves for the other *frq* strains were essentially the same as that for *bd frq-1*. Despite very different growth rates the strains all reached roughly the same final dry mass.

As was previously noted for the *cel* strain [14], the growth of a given strain as a function of time varied from experiment to experiment, so that time of growth was not a good predictor of dry mass. This was especially true with the *prd-1*

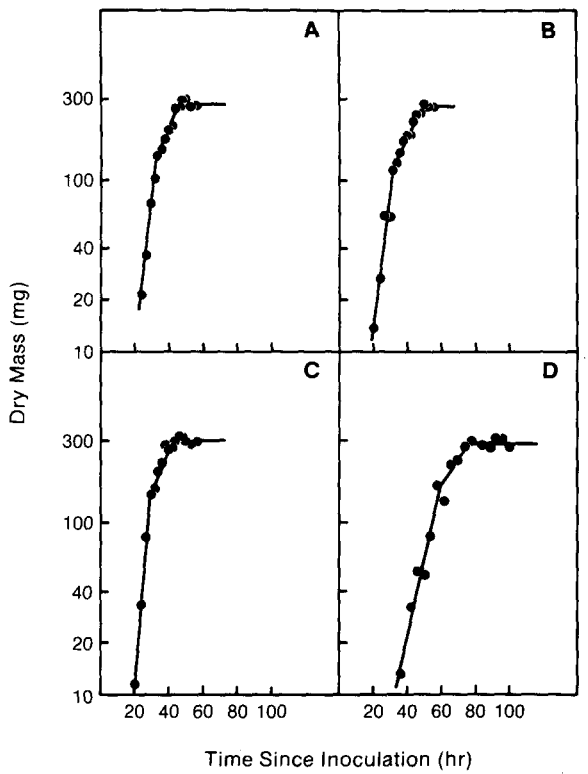

Fig. 1. Growth of rhythm mutants in liquid shaker culture. Several cultures of each strain were grown in liquid shaker culture with 2% glucose at 25° C in constant light. Cultures were harvested at various times, the mycelia were dried overnight at 65° C and weighed, and the logarithm of mass was plotted against time. Representative curves are shown for each strain. The lines drawn through the data were fitted by eye. (A) *bd*; (B) *bd frq-1*; (C) *bd chr*; (D) *bd prd-1*.

mutant, which appeared to have an initial lag period of variable length before significant growth occurred. This variability made it difficult to reproducibly harvest cultures at any specific stage of growth; therefore, fatty acid composition was studied as a dynamic function of mass.

Conidiation rhythm periods of the mutant strains

Circadian rhythmicity has not been demonstrated in log-phase liquid shaker cultures of *Neurospora*; therefore, rhythmicity was monitored by examining the rhythm of conidiation on solid medium in constant darkness. The linear growth rates and periods observed at 22° C are listed in Table I. Because the temperature in the liquid culture studies was 25° C, growth rates and periods of the *bd* and *bd prd-1* strains were also measured at this temperature. The observed periods at both temperatures were similar to those reported in the literature (see Materials and Methods), while, of all the rhythm mutations, only *prd-1* impaired the linear growth rate.

TABLE I

GROWTH RATES AND PERIODS OF RHYTHM MUTANTS

Strains listed were grown on solid medium in constant darkness at the temperature shown. Supplemented medium contained 18:2 at $4 \cdot 10^{-3}$% (w/v). Linear growth rates and periods were determined as described. Data for *bd csp-1 chr* were provided by Lorraine Chuman of this laboratory. Periods of all rhythm strains were significantly different from the period of *bd csp-1* at 99% confidence. The periods listed in part C for *bd csp-1* and *bd csp-1 prd-1* on 18:2-supplemented medium did not differ significantly from the periods in part B for growth on unsupplemented medium.

Strain	Growth rate (mm/h)	Period (h)	S.E. [a]	N [b]
(A) At 22° C				
bd csp-1	1.3	21.3	0.3	63
bd csp-1 prd-1	0.8	25.2	0.3	14
bd csp-1 chr	1.3	23.4	0.3	6
bd-csp-1 frq-1	1.2	16.3	1.7	16
bd csp-1 frq-2	1.2	19.1	0.9	11
bd csp-1 frq-3	1.2	25.6	0.7	11
bd csp-1 frq-8	1.2	28.4	0.6	8
(B) At 25° C				
bd csp-1	1.7	21.3	0.3	10
bd csp-1 prd-1	0.8	24.2	0.3	36
(C) At 25° C, supplemented with 18:2				
bd csp-1	1.3	20.9	0.6	22
bd csp-1 prd-1	0.7	24.5	0.3	33

[a] Standard error of the mean.
[b] Number of periods used to calculate average.

Fatty acid composition of the bd strain in liquid culture

The fatty acid composition of the *bd* strain grown in liquid culture was not constant, but varied as a function of residual dry mass. This is the mass of the dried residue remaining after extraction of lipids, presumably mostly cell wall material, as well as denatured protein, nucleic acids, etc. The residual dry mass-dependent composition pattern for the *bd* strain was reported earlier [14] and is also shown as the lines superim-

posed over the plotted data in Fig. 2. Levels of the saturated fatty acids, 16 : 0 and 18 : 0, appeared not to vary during growth, while the monounsaturated fatty acid, 18 : 1, increased only about 10 mol% as the mass increased from 20 to 300 mg. The polyunsaturated fatty acids, 18 : 2 and 18 : 3, on the other hand, showed dramatic changes; 18 : 2 rose from 36 mol% (at 20 mg residual dry mass) to a final plateau value of 61 mol%, while 18 : 3 dropped from 39 mol% to a plateau value of 10 mol%. These changes in the levels of the unsaturated fatty acids balanced each other so that the total mol% of all unsaturated fatty acids showed no correlation with residual dry mass, averaging 79.5 mol%. The double bond index, which is defined as mol of double bonds per 100 mol of fatty acid, decreased from 201 at 20 mg residual dry mass to a plateau value of 162, reflecting the replacement of 18 : 3 by 18 : 2.

Once the cultures had reached about 200 mg residual dry mass, the changes in fatty acid composition were essentially complete, although the cultures continued to grow, increasing in residual dry mass by as much as another 100 mg.

Fatty acid compositions of bd chr and the bd frq strains in liquid culture

Several cultures each of the *bd chr* strain and of each of the *bd frq* strains were analyzed for fatty acid composition. The results are compared to the fatty acid composition of *bd* in Fig. 2. The lines drawn in this figure represent the fatty acid composition of *bd* as described above, while the plotted points represent actual data for the *bd chr* and *bd frq* strains. These strains did not differ in fatty acid composition from the *bd* strain at the residual dry masses tested, as the observed data for these strains were well within the 95% confidence intervals of the regression model. As these strains appeared to be identical to *bd* in fatty acid composition they were not investigated further.

The fatty acid composition of bd prd-1 differed from that of bd in liquid culture

A number of cultures of the *bd prd-1* strain, covering a range of residual dry masses, was analyzed. The results are plotted against residual dry mass in Fig. 3, along with a calculated regression model for these data. This strain, in contrast

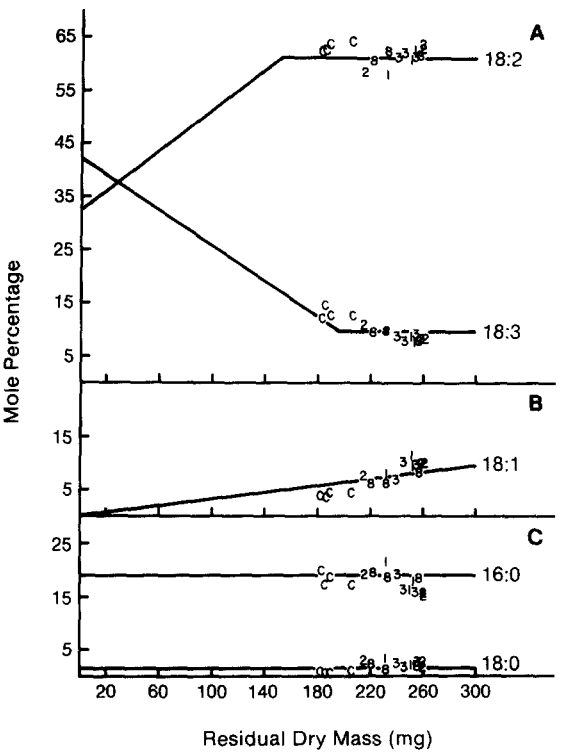

Fig. 2. Phospholipid fatty acid composition of rhythm mutants in liquid shaker culture. Cultures of the *bd chr* strain and each of the *bd frq* strains were grown in liquid shaker culture with 2% glucose at 25 ° C in constant light. Cultures were harvested and analyzed for phospholipid fatty acid composition as described in the text. The data for these strains were plotted against residual dry mass, but the lines drawn through the data represent the control (*bd* strain) fatty acid composition pattern (see text). (A) Polyunsaturated fatty acids. (B) Monounsaturated fatty acid, (C) Saturated fatty acids. Symbols: 1, *bd frq-1*; 2, *bd frq-2*; 3, *bd frq-3*; 8, *bd frq-8*; C, *bd chr*.

to the other rhythm mutants, had a fatty acid composition quite different from that of the *bd* strain.

In *bd prd-1*, as in *bd*, the levels of 16 : 0 and 18 : 0 did not vary with residual dry mass, although these levels were somewhat higher than in *bd*, 21 mol% 16 : 0, and 3.5 mol% 18 : 0, as compared to 19 mol% and 1.5 mol%, respectively, in *bd*. These differences were statistically significant at the 99% confidence level (*t*-test for the difference between means).

In *bd prd-1*, as in *bd*, the unsaturated fatty acids exhibited major changes as mass increased. The 18 : 1 level showed weak but significant correlation with residual dry mass ($r = 0.70$), rising at

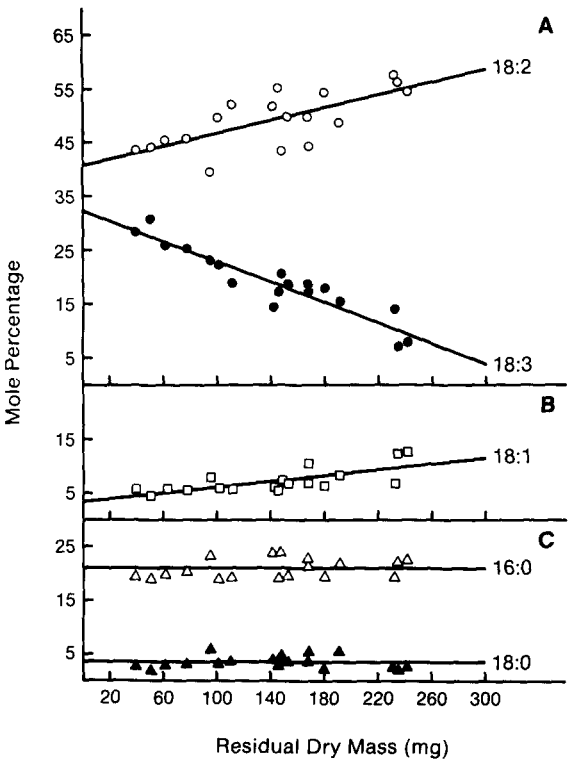

Fig. 3. Phospholipid fatty acid composition of the *bd prd-1* strain in liquid shaker culture. Cultures of *bd prd-1* were grown in liquid shaker culture on 2% glucose at 25° C in constant light. Cultures were harvested and analyzed for phospholipid fatty acid composition as described in the text. The data were plotted against residual dry mass and fitted regression lines were drawn through the data. Lines are horizontal where regression analysis indicated no correlation (i.e., the 95% confidence limits for the correlation coefficient included zero). (A) Polyunsaturated fatty acids: ○, 18:2; ●, 18:3. (B) Monounsaturated fatty acid: □, 18:1. (C) Saturated fatty acids: △, 16:0; ▲, 18:0.

nearly the same rate as in *bd*, although at all masses the level of 18:1 in *bd prd-1* was about 2.5 mol% higher than the level in *bd*. The two strains were very different, however, in the behavior of the polyunsaturated fatty acids. The level of 18:2 rose more slowly in *bd prd-1* than in *bd* and did not appear to reach a final plateau during the growth period. Similarly, the level of 18:3 decreased somewhat less rapidly in *bd prd-1* than in *bd* and also failed to reach a final plateau; however, 18:3 levels in *bd prd-1* were, at all masses, fairly close to the levels in *bd*. The growth-dependent pattern of both the 18:2 level and the 18:3 level in *bd prd-1* were significantly different from the equivalent patterns in *bd* at 99.9% confidence, as demonstrated by extra sum-of-squares analysis (see Materials and Methods).

In *bd prd-1*, as in *bd*, the total mol% of unsaturated fatty acids showed no correlation with residual dry mass, averaging 75 mol%. This was significantly lower than in *bd* at 99.9% confidence (*t*-test for difference between means).

The double bond index dropped from 186 at 20 mg residual dry mass to a plateau value of 157 in *bd prd-1*. Although this pattern was qualitatively similar to that in *bd*, quantitatively it was significantly different at 99.9% confidence (extra sum-of-squares analysis).

Altered fatty acid composition co-segregated with mutant period

A *bd csp*-1 *pan*-2 × *bd prd*-1 cross was kindly provided by Lorraine Chuman. A number of *bd csp*$^+$ *pan*$^+$ progeny were isolated and analyzed for growth rate, period, and fatty acid composition. Seven progeny were judged to be *bd prd*$^+$, based on their growth rates and conidiation rhythm periods in constant darkness at 22° C; they all had fatty acid compositions consistent with the regression curves of the *bd* strain. Another seven progeny were judged to be *bd prd-1*, and had fatty acid compositions consistent with the regression curves of the *bd prd-1* strain (data not shown).

The fatty acid composition of bd csp-1 prd-1 is altered during growth on petri plates

The phospholipid fatty acid compositions were determined for cultures of *bd csp-1* and *bd csp-1 prd-1* grown on solid medium in constant darkness at 22° C. Under these conditions the conidiation rhythm was clearly expressed. Freshly grown mycelia were collected and analyzed for fatty acid composition as described in Materials and Methods. The results, in Table II, show that the *prd-1* strain had a fatty acid composition different from that of the control strain; in particular, the level of 18:2 was lower. This is similar to the situation in liquid culture where 18:2 was lower in *bd prd-1* than in *bd* over much of the growth period. The data reported for the *bd csp-1* strain are similar to results reported for the same strain under similar conditions by Roeder et al. [7], although 18:3 was

TABLE II

FATTY ACID COMPOSITION OF A *bd prd-1* STRAIN GROWN ON PETRI PLATES

Strains listed were grown on solid medium in constant darkness at 22° C, and mycelia were harvested and analyzed for phospholipid fatty acid composition as described in the text. The reported values shown for *bd csp-1* are the averages from eleven samples, and for *bd csp-1 prd-1* the values are the averages from five samples. Each *bd csp-1* sample contained mycelia from three to eight plates and each *bd csp-1 prd-1* sample contained mycelia from nine plates.

		16:0 (mol %)	18:0	18:1	18:2	18:3	Total UFA [a]	DBI [b]
bsd csp-1	mean	20.6	1.3	5.7	40.4	32.0	78.1	183
	S.E. [c]	1.1	0.3	0.7	0.5	1.0	1.4	2
bd csp-1 prd-1	mean	18.4	1.4	4.9	34.3 *	41.1 *	80.3	197 *
	S.E. [c]	0.1	0.1	0.1	0.5	0.5	0.8	1

[a] Total mol% of unsaturated fatty acids.
[b] Double bond index (mol double bonds per 100 mol fatty acid).
[c] Standard error of the mean.
* Significantly different from the *bd csp-1* value at 99% confidence (*t*-test).

somewhat lower and 16:0 somewhat higher than in their report.

Supplementing prd-1 strains with 18:2 restored neither normal period on petri plates nor normal fatty acid composition in liquid culture

Table I also shows periods and linear growth rates of *bd csp-1* and *bd csp-1 prd-1* grown at 25° C with supplemental 18:2 at $4 \cdot 10^{-3}$% (w/v). The 18:2 did not alter the period of either strain as compared to the period of that strain at 25° C without supplementation (Table IB). Similarly, when grown in liquid shaker culture with 18:2 at the same concentration as above, *bd* and *bd prd-1* had fatty acid compositions consistent with the regression models calculated for the same strains without supplement (data not shown).

Discussion

The data reported here demonstrate that the *prd-1* mutant of *Neurospora* has an abnormal growth-dependent pattern of phospholipid fatty acid composition in liquid shaker culture. The data also demonstrate that the *prd-1* mutant has an abnormal phospholipid fatty acid composition on solid medium in constant darkness. The fatty acid composition under these conditions was investigated to demonstrate that the altered fatty acid composition of the mutant occurred under conditions where the rhythm was expressed and thus was not a peculiarity of growth in liquid shaker culture. Rhythmicity has not been reported in log-phase liquid cultures of *Neurospora*; furthermore, constant light, as used in the experiments described here, is thought to prevent rhythmicity [16].

It is unlikely that the differences between the fatty acid composition of the *prd-1* strain and that of the control strain on solid medium could result from sampling at somewhat different phases of the circadian cycle. Fatty acid composition is known to exhibit a circadian rhythm in *Neurospora* [7], and the mycelia harvested for the *prd-1* strain included mycelia covering more of the cycle than the mycelia harvested for the control strain. However, the variation reported in fatty acid composition over the course of the cycle [7] is such that the *prd-1* and control samples would have to be an entire half cycle out of phase for the differences between them to be explained by differences in the phase of sampling.

It is also reported here that altered fatty acid composition co-segregates with the *prd-1* phenotype. Altered fatty acid composition thus appears to be a property of the mutation at the *prd-1* locus and not a property of other unlinked mutations coincidentally present in the strain used. The altered fatty acid composition might be a property of other mutations closely linked to the *prd-1* locus. However, the *bd prd-1* strain that was used in these analyses had already been backcrossed to

a *bd* strain four successive times in this laboratory to reduce the number of such secondary mutations. We would have liked to examine this question further by analyzing different alleles of the *prd-1* locus but as only one allele has been isolated this was not possible.

Taken together, the results reported here suggest that the *prd-1* mutant has abnormal homeostasis of membrane composition. To our knowledge, this is the first report of any biochemical defect in this mutant.

An alternative explanation which we have considered is that the different intracellular membrane fractions, microsomes, mitochondria, etc., have different fatty acid compositions in *Neurospora* and that the *prd-1* mutant differs in the relative proportions of these subcellular membranes. There is some evidence that the mitochondria of the *prd-1* mutant differ in composition from other cellular membranes [23]; however several laboratories, including our own [14,24,25] found no evidence that the fatty acid compositions of different membrane fractions differed significantly from each other in the wild-type or in other mutant strains. Therefore, this alternate explanation was discounted.

The other rhythm mutations studied, the *chr* and *frq* mutations, appear to have no effect on the composition of the major fatty acids in the membrane. This is consistent with the possibility that these mutations might affect rhythmicity by some process other than by altering membrane composition. However, it is possible that these mutants could differ from the wild-type in the levels of a fatty acid which is a minor membrane constituent. Furthermore, it is also possible that overall membrane fatty acid composition could differ at residual dry masses less than or greater than those tested, or under growth conditions different from those used, such as at other temperatures or on solid medium. We have made preliminary measurements of the fatty acid compositions of the *frq* mutants on solid medium. No significant differences were seen in the mol% of the major fatty acids and no further studies were undertaken.

The apparently normal fatty acid compositions of the *frq* strains would appear to rule out the possibility that altered periodicity per se affects fatty acid composition. In particular, *frq-3*, with a period nearly the same as that of *prd-1*, does not have a grossly abnormal fatty acid composition.

Thus, either altered membrane composition affects rhythmicity, presumably by altering membrane properties, or alterations in both rhythmicity and fatty acid composition are independent results of some other primary defect. The first of these possibilities could be ruled out if normal fatty acid composition or normal period could be restored without restoring the other. An attempt to restore normal fatty acid composition by supplementing with exogenous 18 : 2 was unsuccessful; neither fatty acid composition nor period was affected. It is, of course, possible that the 18 : 2 was simply not taken up by *Neurospora*. This, however, seems unlikely since, in other experiments [23], both strains have been shown to take up at least some fatty acids from the medium.

If *prd-1* is altered in membrane homeostasis it should be defective in other processes which have membrane-localized components. Growth itself is one such process since the electron transport chain and the mitochondrial ATPase are membrane-bound enzyme complexes. The reduced growth rate of *prd-1* might be explained by defective membrane homeostasis. The fact that *frq* and *chr*, which appear to have normal fatty acid compositions, also have normal growth rates is consistent with this interpretation.

An alternative possibility is that altered fatty acid composition and reduced growth rate are independent results of some other primary defect. However, the possibility that reduced growth rate per se affects either rhythmicity or fatty acid composition can be discounted since neither property is affected when the growth rate of the *bd* strain is reduced by supplementation with the fatty acid 18 : 2 [14].

Defective membrane homeostasis should also make *prd-1* sensitive to temperature. In fact, the response of the rhythm to light perturbations is unusually temperature-sensitive in *prd-1*. The amplitude of the light response is much reduced at 25 °C but normal at 20 °C compared to wild type [26].

Another mutant of *Neurospora*, the *cel* (chain elongation) mutant [9], which is deficient in fatty acid synthesis [10], has lost temperature com-

pensation of its rhythm, and also has a growth rate abnormally sensitive to high temperature [11]. Both these properties may be modified by supplemental fatty acids in the growth medium [11]. The phospholipid fatty acid composition of this mutant also differs from that of the control strain [14], which suggests that both growth and rhythmicity are affected by defective membrane homeostasis in this mutant as well.

All of the above evidence is consistent with the possibility that some or all components of the biological system generating rhythmicity are membrane-localized. These membrane-localized components would, of course, be sensitive to changes in membrane properties, but under normal circumstances membrane homeostasis would buffer the system against changes in environmental factors such as temperature. Mutations which alter periodicity would then be of two kinds, direct mutation of soluble or membrane-localized protein components of the system, and mutations which affect membrane homeostasis. The *prd-1* and *cel* mutations appear to alter membrane homeostasis, while the *frq* and *chr* mutations could be candidates for mutations in components of the rhythm-generating system itself.

The evidence is also consistent with the possibility that membranes or membrane properties are themselves components of the rhythm-generating system. An oscillation in membrane phospholipid fatty acid composition has been reported in *Neurospora* [7], but it is not clear whether this oscillation is part of the rhythm-generating system, or merely follows the generated rhythm.

The evidence we have presented does not imply that every change in fatty acid composition must alter the circadian rhythm. Under some conditions, for example, the *cel* mutant has normal period, but greatly altered fatty acid composition [14]. Furthermore, alterations in temperature affect the fatty acid composition, but not the period of the wild-type strain. One would not expect every change in fatty acid composition to affect rhythmicity since membrane properties can also be influenced by adjustments in phospholipid head group composition, in sterol composition, etc. Many different phospholipid fatty acid compositions may thus be compatible with normal membrane properties. This implies that there can be no correlation between period and absolute fatty acid composition. Thus, it is not possible either to predict the period of a strain from its overall fatty acid composition or to predict the pattern of its overall fatty acid composition from its period.

Other work [27] has been interpreted as not supporting the involvement of membranes in rhythmicity. It was found that phenylmethanol and 3-phenylpropanol alter phospholipid fatty acid composition in *Neurospora* but do not affect the period, temperature compensation, or light response of the rhythm. However, the effects of the alcohols on fatty acid composition were demonstrated under very different conditions than were used to test for effects on rhythmicity. The fatty acid composition might have been normal under the conditions used for assaying the period, while the period might have been altered under conditions used for assaying fatty acid composition. In addition, cultures were harvested for lipid determination at only a single time point. Since the pattern of fatty acid composition as a function of residual dry mass in the standing liquid culture system used is not known, it is possible that the drug-inhibited cultures and the control cultures had fatty acid compositions quite different at the time of harvest and yet had the same growth-dependent pattern of fatty acid composition. Furthermore, it is possible that the changes seen in fatty acid composition were homeostatic changes preserving normal membrane properties in the presence of the alcohols.

Scholübbers and co-workers [28] also suggested that membranes might not be involved in rhythmicity since age- and temperature-dependent changes in the fluorescent polarization values of *Gonyaulax* cells labeled with a fluorescent dye did not correlate with changes in the period or phase of the glow rhythm. However, as the authors themselves point out, it is not clear what membrane properties are reflected by the fluorescent polarization values of intact *Gonyaulax* cells.

In conclusions, the strategy of examining the phospholipid fatty acid composition of rhythm mutants has demonstrated that one such mutant, the *prd-1* mutant, may be defective in normal membrane homeostasis. We thus suggest that normal rhythmicity depends not on the overall fatty acid composition, which of course would be differ-

ent at different temperatures, but on the ability of the organism to maintain optimal membrane properties through normal adjustment of membrane composition.

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