Induction by sodium dodecylsulfate of the circadian rhythm of conidiation in *Neurospora crassa*

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Summary. The circadian rhythm of conidiation expressed by the mutant *band* of *Neurospora crassa* can also be induced in the wild strain by Na-dodecylsulfate. This correlates with the induction of growth rhythms in fungi caused by changes in the hyphal membranes. The circadian character suggests an additional independent circadian center acting upon the chemically or mutationally triggered rhythm.
Key words. Circadian conidiation; *Neurospora crassa;* sodium dodecylsulfate.

Rhythmic growth and/or reproduction are well known in fungi, mostly in the form of concentric bands or rings (zonations)[1]. Under natural conditions this behavior is normally due to alternating environmental factors like light or temperature. This type of rhythm is called 'diurnal' according to Bünning[2]. The endogenous circadian rhythms known as the 'biological clock' in other eucaryotic organisms, however, are very rare in fungi and mainly have not been exhaustively tested. The few well-documented cases are hence more interesting.

The best known system is the circadian rhythm of conidiation, found in mutants of *Neurospora crassa,* like *band (bd)* or the *frequency (frq)* mutations; the study of which has revealed that the expression of this circadian rhythm depends on a single gene only while the endogenous period is affected by further mutations[3]. A similar mutant has been isolated from *Chlamydomonas reinhardii*[16]. Attempts to find comparable strains in nature were made with *Sclerotinia fructigena* by Jensen and Lysek[4]. They found a small percentage of strains which exhibited endogenous and even circadian growth rhythms.

The occurrence of circadian rhythms in *Neurospora crassa* suggested the possibility that agents which cause non-circadian rhythmic growth in other fungi could be used to induce circadian conidiation in *Neurospora crassa.* This attempt has been successful with rubidium ions, which induce rhythmic growth in *Podospora anserina* and circadian conidiation in *Neurospora crassa*[5,6]. In continuation of these experiments, surfactants were applicated, which also induce growth and fructification rhythms in various fungi[7-9]. As reported in this paper, sodium dodecylsulfate successfully induced the circadian rhythm of conidiation in *Neurospora crassa,* too.

Materials and methods. The mutant *csp-2* (conidial separation defective), strain No 2525 FGSC was used throughout the experiments. The conidia of this strain are not liberated but adhere together; hence self-infection of the cultures is avoided.

The experimental cultures were grown on a solid minimal medium containing Westergaard mineral solution and D-fructose as nutrient sugar. Sodium dodecylsulfate (sodium laurylsulfate, SLS) was added to the medium before autoclaving. Colonies were grown in Petri dishes (mainly 15 cm diameter) or racing tubes[10].

Temperature for incubation was 27 °C, unless otherwise noted. Light regimes were continuous dark (DD), light-dark periods 10:14 (LD), and continuous light (LL). Light intensities were circa 300 lx from fluorescent tubes.

Each value is the mean of 5 or more replicas; the experiments were repeated at least three times.

Results. It can be seen from the table that sodium laurylsulfate induced rhythmic growth and conidiation in *Neurospora crassa* strain 2525. This effect is more pronounced in the presence of potassium chloride. The table also shows that this induction is associated with a reduced linear growth rate, an effect which is well known from similar experiments in other fungi[9,11]. Thus the growth and conidiation rhythm induced in *Neurospora* is comparable to rhythms in other fungi.

In addition, the results in the table indicate that this induced rhythm also fulfills every demand for circadian rhythms: entrainment by light-dark cycles; free running period in permanent dark and at constant temperature; damping out in permanent light and temperature compensation, i.e. the free running, endogenous period is not or nearly not influenced by the temperature.

Induction of the circadian conidiation in *Neurospora crassa* csp at various experimental conditions by sodium dodecylsulfate + KCl, and the effect on the linear growth rate

Additions to medium	Temperature °C	Light regime	Linear growth rate mm/d	%	Period h
None (control)	27	DD	51.1	100	No rhythm
None (control)	32	DD	73.2	143.2	No rhythm
0.04 g · l^{-1} sodium	27	LD	7.7	15.1	24.1
dodecylsulfate	27	DD	8.5	16.6	23.1
+ 0.3 mol · l^{-1}	27	LL	8.8	17.2	No rhythm
KCl	32	DD	19.9	38.9	22.4

Discussion. The induced rhythm in *Neurospora crassa* reveals, in contrast to other fungi, a circadian component. Since the type and morphogenesis of the bands found resemble those of other fungi, the interpretation developed for those rhythms[1,12] may be applied also to *Neurospora* conidiation. In that model the plasmalemma plays a central role; rhythms are caused by events which enhance the concentration of protons in the hyphal cytoplasm by increased production or influx[12]. Among the immediate reactions to this acidification is an enhanced activity of the plasmalemma-ATP-ase. This accounts for the increase in respiration also found to be characteristic of rhythmically growing strains[13,14]. The primary effect of SLS – like that of other surfactants – is easily integrated into the model; sodium laurylsulfate is probably incorporated into the lipid phase of the membranes, the structure of which is weakened. Increasing concentration would eventually lead to enhanced permeability and hence to an enhanced proton influx.

In addition the rhythm induced by this means in *Neurospora crassa* is circadian; this corresponds to the mutational induction of rhythm, which mainly yields circadian conidiation in *Neurospora crassa*[3]. In the usual terminology of circadian rhythms, the studied oscillating feature is termed the 'hand' and the central circadian oscillator the 'clock'. This would mean that in *Neurospora crassa* the internal 'clock' is composed of two different parts, the biochemical mechanism which generates the oscillations (located in the plasma membrane[15] and triggered by the surfactant), and the regulatory center determining the circadian character of the created oscillations. The 'hand', i.e. the measured rhythmic activity, is the formation of bands and of conidia. This system would allow a mutational alteration of the endogenous, i.e. the free running period without affecting the rhythms otherwise, as is the case with the *frequency*-mutants, studied by Feldman and his group[3]. This view is hence compatible with the existence of strains of *Neurospora crassa* with genetically different endogenous periods, but with otherwise unchanged circadian conidiation. The occurrence of similar mutants in *Chlamydomonas reinhardii*[16] shows that similar conditions may be found in other eucaryotic organisms.

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Effect of pulsing electromagnetic fields on DNA synthesis in mammalian cells in culture[1]

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Summary. DNA synthesis in Chinese hamster V79 cells was significantly enhanced when they were exposed to weak, pulsing electromagnetic fields generated by specific combinations of the pulse width (25 μs), frequency (10, 100 Hz) and magnetic intensity (2×10^{-5}, 8×10^{-5} T). Conversely the DNA synthesis of cells in the fields at 4×10^{-4} T was repressed to 80% of that in controls not exposed to the fields.
Key words. Chinese hamster V79 cells; electromagnetic fields; DNA synthesis.

Weak, pulsing electromagnetic fields were shown to have the ability to stimulate bone healing[3–5] as well as other biological processes including DNA synthesis[6], cell proliferation[7] and cellular transcription[8]. However, the mechanism underlying the modifications of a variety of biological processes by magnetic fields is still largely unexplained. In the present study an attempt has been made to determine the optimal conditions of weak, pulsing electromagnetic fields for the enhancement of DNA synthesis in cell cultures, particularly the pulse width, frequency and magnetic intensity, if any.

Materials and methods. Chinese hamster V79 cells (approximately 2×10^4 cells) were inoculated into 35-mm plastic dishes containing 2 ml Eagle's minimum essential medium supplemented with 10% fetal bovine serum (Gibco) and 0.3 g/l L-glutamine, and were cultured at 37 °C in a humidified atmosphere of 5% CO_2 in air. After 24-h incubation, (methyl-^{3}H)thymidine (78.2 Ci/mmole) (New England Nuclear) was added to the cultures to give a final concentration of 0.05 μCi/ml. Then the cultures were divided into control and experimental groups. The experimental group was placed between a pair of 10 by 10 cm Helmholtz coils placed horizontally in a CO_2 incubator, and received various conditions of pulsing electromagnetic fields[9]. The controls were in a separate incubator and were not perturbed by the magnetic fields. Both of the groups were incubated for two days in the presence of (^{3}H)thymidine and the labeled cells were harvested by trypsinization followed by a brief centrifugation. After addition of cold 10% trichloroacetic acid (TCA), the cells were allowed to stand on ice for 15 min. The lysate was centrifuged to separate the TCA-insoluble materials from the TCA-soluble ones, and radioactivity incorporated into the TCA-insoluble fraction was measured in ACS II scintillation cocktails (Amersham). Statistical analysis was performed using the t-test.

Results. Three independent series of experiments were carried out to determine the effects of pulse width, magnitude and frequency on the DNA synthesis in cell cultures. In the first series, one frequency (100 Hz) and one intensity (2×10^{-5} T) were used for all experiments while varying the pulse width. Of the pulse widths tested so far, only a 25-μs pulse was found significantly to enhance the DNA synthesis; the amount of (^{3}H)thymidine incorporated into the DNA increased by up to 30% ($p < 0.001$, t-test) as compared to the control cultures not exposed to the fields. No significant difference in the DNA synthesis was observed between the experimental cultures exposed to 6-, 10-, 50-, 75-, and 125-μs pulses and their respective controls not in the magnetic fields (fig. 1).

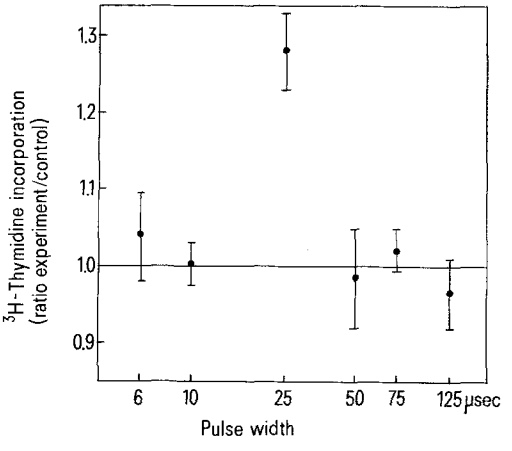

Figure 1. Effect of pulse width on DNA synthesis in Chinese hamster V79 cells exposed to the magnetic fields that are generated with 2×10^{-5} T pulse repeating at 100 Hz. Incorporation of (^{3}H)thymidine into the TCA-insoluble fraction was measured and the ratio of the mean value of experimental cells to that of controls was expressed. Mean values for the experimental cells are given ±SD.