appears to be rather unreactive since it is only attacked by a small number of bacteria.

In a separate study fresh detritus was collected from the mouth of Ward Creek, a tributary presently under intensive investigation (*14*). The detritus consisted mainly of remains of attached algae (periphyton) which had broken loose. The detritus was homogenized and sterilized by autoclaving at 130°C for 15 minutes. It was then divided equally among 150-ml dialysis bags made from tubing tied off and sealed by dipping in hot paraffin at both ends. The bags allowed free diffusion of nutrients and metabolic waste products, but were impermeable to detritus and associated microorganisms. Two sets of dialysis bags were used. One set contained sterile detritus and a 50-ml inoculum of lake water (20-m depth) containing live microorganisms. The other set had similar contents but was sterilized by immersion in 0.001*M* $HgCl_2$ for 12 hours after the bags were sealed. Sterility tests, consisting of plate counts, heterotrophic activity measurements, and microscopic observations, were made on the contents of these bags after a 4-week incubation in Lake Tahoe. Tests showed that microorganisms did not survive sterilization, nor did new cells enter the sealed bags.

Both sets of bags were allowed to incubate at a depth of 20 m in Lake Tahoe (20 m was the depth of maximum heterotrophic activity during the sampling period). Duplicate sterile and nonsterile bags were taken out daily to make observations of the contents by phase light microscopy.

Experiments with in situ dialysis bags gave an indication of the fate of fresh detritus entering from streams. A sequence of light-microscope pictures of the contents of dialysis bags supporting live microorganisms shows aggregation of small detrital particles into larger particles over a period of 3 days (Fig. 3, A and B). After 3 days the particle size in sterilized bags was only 10 percent of the particle size in bags with living microorganisms. Sterile bags showed a slight aggregation of particles. This is possibly due to the attraction of oppositely charged particles or to adsorption of dissolved organic matter on random surfaces (*15*). Aggregation of particles in unsterilized bags may be partly attributable to charge attraction and adsorption, but is largely due to microbial adhesion to particles. Observation of live detritus by SEM shows that particles are not adhering to each other but are trapped by web-like structures associated with bacteria and fungi. Concurrently, particles increase in size and sinking rate.

Structural modification of detritus through aggregation is at least in part attributable to fungal and bacterial growth initiated on small particles. Most of the cementing activity occurs in near-surface waters, where fresh inputs of particulate organic carbon and photosynthetic production of organic carbon are highest. Since the amounts of dissolved organic carbon are relatively low in Lake Tahoe (400 to 500 μg/liter) the pool of particulate organic carbon represents an important source of nutrition for heterotrophic bacteria and fungi. Exploitation of this pool appears to be extensive in the upper 75 m since both heterotrophic activity and attachment are highest in this zone.

Observations show that bacteria and fungi do not necessarily need stalks or elaborate structural modification for attachment. It appears that bacterial secretions and fungal mycelia cause microorganisms to adhere to particulate substrata. Due to their "stickiness," such microorganisms play a significant role in determining the ecology of detritus.

HANS W. PAERL

Institute of Ecology,
University of California, Davis 95616

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Circadian Rhythms in Neurospora: Spatial Differences in Pyridine Nucleotide Levels

Abstract. *A growing colony of a mutant strain of* Neurospora crassa *had two morphologically distinct areas which were formed as a result of a rhythmic spore-forming (conidiation) process. The total pyridine nucleotide content of these two areas was the same, but the levels of NADH, NADPH, and NADP were lower in the conidiating area, while the NAD level was higher. These biochemical differences in the adjacent areas of a single colony were only found in newly formed areas, and were not a permanent record. It is not known whether these pyridine nucleotide changes are a result of the conidiation process, or whether they are tied more directly to some underlying metabolic oscillation. However, it is speculated that the changes in the levels of these key coenzymes could have far-reaching effects on many areas of metabolism.*

Biological rhythms in fungi are expressed in a variety of ways. In *Neurospora crassa,* the periodic formation of conidia (vegetative spores) occurs in certain mutant strains such as patch (*1*), timex (*2*, *3*), or band (*3*), whereas in other mutant strains (clock) the periodicity can only be seen as changes in the branching frequency of the filamentous hyphae (*4*). In the band (*bd*) strain, the conidiation rhythm has the characteristics of a circadian one, in that the rhythm is not appreciably affected by temperature, can be altered by light, and has a periodicity of approximately 1 day (*3*). In the *bd* strain, both the conidiating phase and the nonconidiating phase can be approxi-

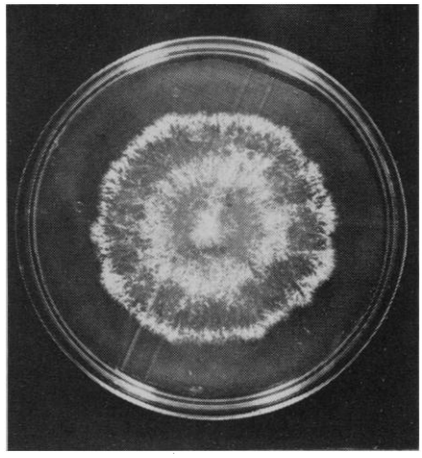

Fig. 1. Concentric areas of spore formation due to a circadian rhythm in a growing colony of *Neurospora*.

mately 12 hours long. The basic periodicity is repeated for more than one cycle, and under certain conditions can proceed undamped for 8 to 10 days (*5*). Many wild-type strains of *Neurospora* will also show the conidiation rhythm, but only if the cultures are vigorously aerated (*5*). Therefore, it appears that there is a basic biological clock in *Neurospora*, and certain mutations, such as those at the *bd* locus, allow this clock to be expressed phenotypically.

The precise biochemical changes that lead to the manifestations of the rhythm in *Neurospora* are not known. By analogy to studies of other fungi (*6*), it appears that some aspects of carbohydrate metabolism and respiration are involved. Previous studies on the biochemical basis of morphogenesis in fungi also indicated the importance of carbohydrate metabolism (*7*). These latter studies showed that morphological mutants containing altered pentose-phosphate shunt enzymes (*8*) had lower levels of pyridine nucleotides, and that the pyridine nucleotides appeared to play a critical role in the morphogenesis of *Neurospora* (*9*). Therefore, we attempted to determine what role the pyridine nucleotides played in the rhythmic morphological changes observed in the *bd* mutant strain.

The *bd* mutant was obtained from Malcolm Sargent, Botany Department, University of Illinois. It was grown at 23°C on top of dialysis tubing that was overlaid on Vogel's minimal agar (*10*) containing maltose (0.5 percent) and arginine (100 μg/ml). Cultures were grown in this way so that they could be quickly and clearly separated from the agar. Circles 15 cm in diameter were cut from dialysis tubing (7.6-cm flat width; Will Scientific), sterilized in ethanol for 20 to 30 minutes, quickly dipped in sterile distilled water, and then placed on the surface of the agar. The next day, any excess water was drained from the top of the tubing, and cultures were then carefully inoculated on top of the tubing in the center of a 15-cm-diameter plate. After inoculation, cultures were exposed to overhead fluorescent light overnight (16 to 18 hours) and were then incubated in constant darkness for the remainder of the experiment. They were examined in red light only, but extracted in fluorescent light. Extraction in red light did not change the values obtained. The appropriate section of the mycelial mat was severed from other areas with a spatula and quickly removed from the dialysis tubing sheet by gently scraping with a flat-ended polypropylene spatula. The mycelial sections from many plates were quickly combined and extracted together. For the analysis of reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH), the mycelia were extracted by plunging into KOH-alcohol at 70°C for 1 minute and then assayed by enzymatic means (*9*). For the analysis of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), mycelia were extracted in 12 percent cold trichloroacetic acid (*9*). Two samples of each extract were assayed simultaneously, and the average value taken as the content of that extract.

When grown on the surface of the dialysis tubing overlaid on the agar, the *bd* strain exhibited a morphological rhythmicity (Figs. 1 and 2) and grew approximately two-thirds as fast as the wild-type strain (RL3-8A). The wild-type strain showed an increase in the colony radius of 30 to 35 mm/day, whereas the *bd* strain had an average growth rate of 20 to 25 mm/day. The dialysis tubing overlay had no noticeable effect on the growth rate of either strain or on the periodicity of growth of the *bd* strain.

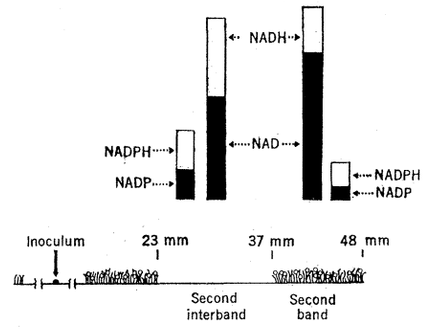

Fig. 2. Schematic illustration of rhythmic spore formation in *Neurospora* and regional pyridine nucleotide composition 53 hours after inoculation.

The two morphologically distinct sections of the agar-grown *bd* culture were assayed separately for pyridine nucleotide composition at specific times after inoculation (Table 1 and Fig. 2). At 53 hours, the second band area as compared to the second interband area had 40 percent less NADH and NADPH, 60 percent less NADP, but 40 percent more NAD. At 77 hours, the newly formed third band contained 40 percent less NADH and NADPH than the corresponding third interband area. Therefore, the basic difference in the spatial pattern apparently holds true at this time as well. We also assayed both the second band and interband (Fig. 2) about 24 hours later; that is, after growth of an additional interband and band area. Under these conditions of delayed assay, the interband content of both NADH and NADPH had decreased, whereas the levels in the band region remained unchanged (Table 1). Similarly, band areas assayed 24 hours after formation had decreased in NAD content (Table 1). Therefore, it would appear that these spatial biochemical differences are not a permanent "fossil record," inasmuch as changes did oc-

Table 1. Pyridine nucleotides in different regions of a *Neurospora* colony. Values are given as micromoles per gram (residual dry weight). Standard errors are given if appropriate, *N*, number of extracts analyzed.

Region	NAD		NADH		NADP		NADPH	
	μmole/g	*N*	μmole/g	*N*	μmole/g	*N*	μmole/g	*N*
			Assayed at 53 hours					
Interband 2	1.70 ± 0.10	5	1.28 ± 0.10	4	0.44	3	0.60 ± 0.06	4
Band 2	2.40 ± .15	5	0.75 ± .02	9	.18	3	.42 ± .02	9
			Assayed at 77 hours					
Interband 2			0.80	2			.44	2
Band 2	1.60	2	.77	2			.38	2
Interband 3			1.23 ± .12	5			.67 ± .10	5
Band 3			0.73 ± .06	5			.41 ± .02	5

cur a day later. Even so, it is curious that these differences in adjacent areas of a colony exist for a period despite considerable cytoplasmic streaming throughout the *Neurospora* colony.

The total pyridine nucleotide content of the two areas was approximately the same (3.9 ± 0.2 μmole/g), but the composition of these two areas differed. The ratio of NAD plus NADH to NADP plus NADPH changed from approximately 3 : 1 in the interband area to approximately 5 : 1 in the band area. More important, the ratio of oxidized to reduced nucleotides differed from one area to another. In the interband area, the ratio of NAD to NADH was approximately 4 : 3; whereas in the band area it was approximately 9 : 3. On the other hand, the ratio of NADP to NADPH was approximately 2 : 3 in the interband region and approximately 1 : 3 in the band region. It is not clear why the two redox couples changed in opposite directions (the NAD ratio toward the oxidized, and the NADP ratio toward the reduced). In contrast, previous studies indicated that neither of these ratios changed in mutants with an altered glucose-6-phosphate dehydrogenase or in strains starved of or supplemented with nicotinic acid, a precursor of the pyridine nucleotides (*11*). The redox changes reported here differ from those in yeast cells in that the latter had a periodicity in minutes and only involved approximately 10 percent of the total pyridine nucleotide content (*12*).

Although the 30 to 50 percent change in content of some of the pyridine nucleotides may seem too small to be of general significance, even smaller changes in pyridine nucleotide levels can lead to a dramatic and gross change in the growth rate and morphology of a *Neurospora* colony (*9*). It may be hard to detect these spatial differences in organisms with a different life-style. In *Neurospora*, the spatial localization of the band areas from the interband areas permits one to physically separate and assay these two areas. If the entire colony rather than the separate areas were assayed at 53 or 77 hours, one might expect an NADH value intermediate between the values for the two areas. Furthermore, the NADH level in the total colony might change only slightly as the proportion of bands to interbands changed; that is, as the colony expanded. Therefore, measurements of total NADH content as a function of time might not indicate changes as large as those observed here. It would be interesting to test these predictions, as well as to see if there are any finer spatial differences or gradients in pyridine nucleotide composition within a given band or interband area.

The finding of these spatial differences raises the questions of whether these changes (i) are caused by the rhythmic conidiation; (ii) trigger the conidiation process; or (iii) are just correlated with this process. None of these three questions can be definitively answered now. In reference to the first question, the enzyme NAD glycohydrolase, which is found in large quantities in conidia and is associated with the conidiation process (*13*), does not appear to play a significant role in causing these spatial differences. This conclusion is based on the observation that the NAD level is higher in the conidiating areas than in the interband region (Table 1). To determine if other factors, triggered by the conidiation process, bring about the changes in the pyridine nucleotide ratios will require other experimental approaches. The opposite approach—to determine whether there are spatial differences in a growing colony of the wild-type strain with no obvious pattern of conidiation—might also be fruitful.

Regardless of whether the pyridine nucleotide levels are responding to the conidiation rhythm or to some other metabolic oscillation, one can speculate about the types of metabolic changes produced by the changed pyridine nucleotide ratios. In the band region as compared to the interband region, one might expect an increase in the rate of many NAD-requiring reactions, since there would be more substrate (NAD) and less product (NADH). Reactions of enzymes such as NAD-dependent isocitritic dehydrogenase might then produce more CO_2 under these conditions, provided their substrates were not limiting. Another possible effect of the changed ratios would be on the configuration of an enzyme, since pyridine nucleotides can affect the aggregation state of certain enzymes (*14*). Many other possible effects could be listed, such as the role of pyridine nucleotides as allosteric feedback effectors (*15*), but further speculation is probably unwarranted, since it is uncertain whether the changes in the pyridine nucleotide levels or ratios have any of these effects in vivo.

STUART BRODY
STEPHANIE HARRIS

Biology Department,
University of California, San Diego,
La Jolla 92037

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Leukemia Virus Activation during Homograft Rejection

Abstract. *Activation of murine leukemia viruses, as detected by the mixed culture cytopathogenicity (XC) assay, followed the transplantation of A/J skin onto immunosuppressed BALB/c mice. Virus was found in most of the mice receiving both skin grafts and antilymphocyte serum, but not in animals receiving either the serum alone, skin graft alone, or no treatment.*

Among patients receiving organ transplants, there is an increased incidence of malignant tumors arising de novo months to years after transplantation (*1*). The most common tumors in this population are malignant lymphomas, particularly reticulum cell sarcomas, estimated to occur approximately 4000 times in excess of what would be expected in the general population (*1, 2*). This extraordinary incidence of reticulum cell sarcomas is thought by some investigators to be a result of therapeutic immunosuppression, reducing immune surveillance of malignant cell mutants that are normal-