mately 1000-fold. A similar response (600-fold increase) was observed when arabinose was used to induce light in the *ara-lux* fusion mutant. The induction of light production in these fusion mutants was characteristic of *lac* and *ara* gene control in wild-type strains.

Fusion mutants of *Vibrio parahaemolyticus* were also isolated. Transducing phage Pl, specifically Plclr100CM, was used to mobilize mini-Mu*lux*(Tc^R) into *Vibrio* bacteria. Bacteriophage Pl packages mini-Mu*lux*(Tc^R) DNA and infects *V. parahaemolyticus* but does not replicate in this host (*9, 10*). Since this host was resistant to kanamycin, mutagenesis was performed with mini-Mu*lux*(Tc^R). Mutants resistant to tetracycline and defective in lateral flagella function were collected. Mutants in lateral flagella were incapable of swarming over agar surfaces. Transcription of many lateral flagella genes (*laf*) takes place only when bacteria are propagated on the surface of nutrient agar plates and not when grown in liquid media (*9*). A typical *laf-lux* fusion mutant produced light only when the bacteria were grown on the surface of a nutrient agar plate (Fig. 3). As in the previous examples, light production mirrored the regulatory characteristics of the target gene.

Light was measured by means of x-ray or photographic film, a scintillation counter in chemiluminescence mode, or visual observation. It is convenient to identify luminescent colonies or to study the influence of physiological factors on gene expression in fusion strains by making exposures with x-ray film placed in close proximity to culture plates in a darkroom. The colonies were easily visible in a darkened room (Fig. 3). However, fully induced *ara-lac* fusion strains were barely visible. Several factors may influence the intensity of the light produced by *lux* fusions. The host bacterium supplies the energy source, $FMNH_2$, for the light reaction, and the amount of this reductant may vary with bacterial strain or physiological conditions. The stability or activity of luciferase and the associated *lux* enzymes and the capacity to transcribe or translate *lux* genes may also be influenced by the particular host. *Escherichia coli* and *V. parahaemolyticus* were suitable hosts for light production and for mini-Mu*lux* transposition. It is likely that other genera of bacteria can be used as well. Since mini-Mu*lux*, like phage Mu, integrates into the host genome at random sites, it should be possible to use mini-Mu*lux* to study the regulation of a great variety of gene systems. In contrast to fusions with *lacZ* as the indicator gene, use of mini-Mu*lux* fusions does not require that the host bacterium be Lac^-. Light can be measured simply, sensitively, and without perturbing the cell. Strains can also be marked with a traceable phenotype by transposing the *lux* genes into the genome.

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11. Approximately 30 to 40 percent of the Lac^- or Ara^- mutants produced detectable amounts of light. Fifty percent of mini-Mu*lux*(Km^R) insertions would be expected to be Lux^+ if the orientation of insertion were random. Transposon-generated terminal deletions would reduce this frequency as would insertion in control regions or in regulatory genes such as *araC*; expression of *araC* would not be induced by arabinose.
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Interspecific Morphogens Regulating Prey-Predator Relationships in Protozoa

Abstract. *The ciliate* Euplotes octocarinatus *and some close relatives of it are triggered by predator-released substances to undergo morphogenetic changes that inhibit their engulfment. The changes occur within a few hours and do not require cell division. They are perpetuated during reproduction so long as the concentration of the morphogen is maintained. The ability of* Euplotes *to respond to predator-produced signals by a defensive change in cell architecture probably provides an effective mechanism for damping population oscillations of both prey and predators and fosters coexistence. The signal-induced cell transformation merits study for its own sake because of its developmental implications.*

Although prey-predator relationships are often extremely complex, population geneticists elect to study them because they provide valuable insights into subtle processes of evolution. Particularly interesting are mechanisms that damp population oscillations of prey and predator and thus facilitate their coexistence. Heterogeneity of the prey population provides an example of one such mechanism. The ability of predators to switch to alternative food when the prey becomes scarce is another. A third mechanism, not yet well studied, is predator-induced defense. Many plants (*1*) and a few animals (*2–4*) mobilize inducible defenses in response to attack by consumers. Inducible defenses are produced only in response to stimuli from consumers and disappear shortly after the stimuli are withdrawn. The nature of the signals that trigger the defensive responses in the prey organisms is in most cases unknown. We now describe a newly discovered predator-induced defensive response occurring among protozoa. The system we studied provides favorable conditions for identifying the signal released by the predator, in this case most probably a polypeptide. It enables us, in addition, to study the development of the defensive response, a drastic change in cell sculpture that makes engulfment by the predator more difficult or even impossible. The signal-induced cell transformation is not only of ecological importance. It also provides an easy-to-handle model system to study morphological changes in cells. When the morphogen is present, the changes can be induced at will, under strictly controlled conditions, in large numbers of cells, in a synchronized fashion. This property could be useful for studying the process induced by the binding of the morphogen to specific cell receptors and the consequent morphogenetic events.

The ability of *Euplotes* to develop extended wings and ridges in response to factors released by predatory ciliates was discovered when we fed the hymenostome *Lembadion lucens* with the freshwater hypotrich *Euplotes octocarinatus*. Figure 1 shows *E. octocarinatus* cells before and after exposure to *Lembadion*-conditioned medium. Exposed cells develop prominent lateral wings, which give them an almost circular appearance, an extended ventral projection, and a particularly protuberant dor-

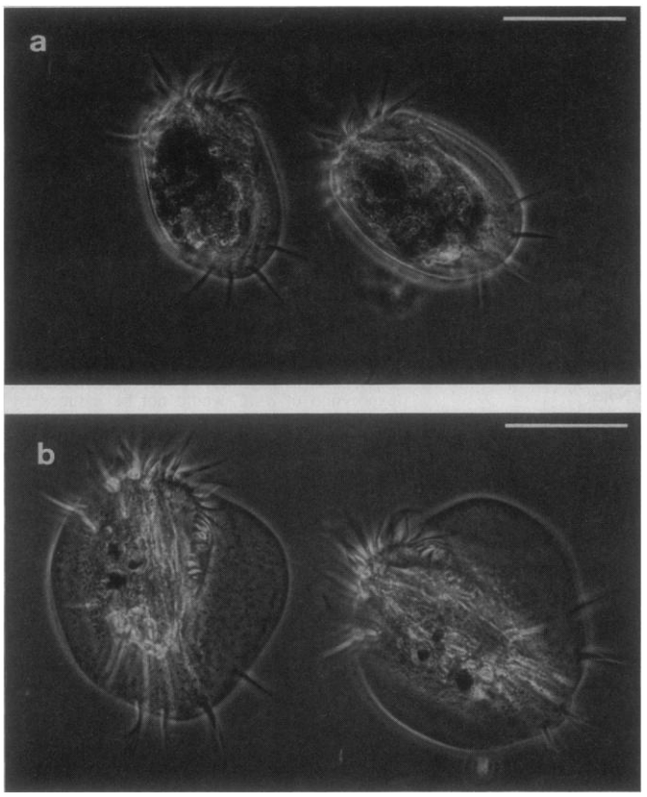

Fig. 1. *Euplotes octocarinatus* before (a) and after (b) exposure to the *Lembadion*-factor. Photomicrographs of living cells, microflash, phase-contrast illumination (scale bar, 50 μm).

sal ridge (Fig. 2). The transformation of the cells from their typical ovoid form (Fig. 1a) into the enlarged circular form (Fig. 1b) takes place within a few hours. The first changes in cell shape can be recognized by light microscopy after 4 to 5 hours of exposure to the *Lembadion*-factor and are clearly visible after 12 hours of exposure. The outgrowth of the cell protuberances may continue for another 24 hours; its speed and extent depend on the concentration of the morphogen.

The increase in size reached by *Euplotes* in response to the morphogen was measured on samples of 36 cells. The mean height of control cells was 22.1 ± 0.9 μm (standard error of the mean) in comparison with transformed cells with a mean height of 41.5 ± 1.0 μm, corresponding to almost a doubling of the height. Similarly, the widths differed, with a mean of 61.4 ± 1.3 μm in the controls compared with a width of 100.5 ± 1.2 μm in treated cells. The mean lengths differed by 85.7 ± 1.1 μm and 107.1 ± 1.3 μm, respectively (*5*). The hymenostome *L. lucens*, which can be grown on *Euplotes*, is not able to swallow the enlarged forms. If *Lembadion* and *Euplotes* are grown in a Boveri dish side by side under conditions that allow *Euplotes* enough time to respond to the *Lembadion*-factor, then *Lembadion* encounters increasing difficulties in swallowing *Euplotes* and eventually dies out. These circumstances are different from those in nature, where the predator can switch to other prey organisms. A concentration of the morphogen high enough to induce maximum protection of *Euplotes* may seldom be reached outside the laboratory. However, we did observe the circular *Euplotes* cells among our field collections, and it was these observations that led us to the discovery of the induced morphogenetic changes.

The transformation of the *Euplotes* cell from the noninduced ovoid (o) form into the greatly enlarged circular (c) form is not an all-or-none effect. The degree of enlargement of the cell extensions seems to depend on the concentration of the *Lembadion*-factor. In an experiment in which two chambers were allowed to exchange fluid through a polyester net with an average mesh distance of 10 μm, the factor that causes the changes was shown to be separable from the cell surface of the predator and capable of being exchanged between the chambers. When *Lembadion* and *Euplotes* were united in one chamber and *Euplotes* was left alone in the other chamber, the effect was always stronger in the chamber in which *Lembadion* and *Euplotes* had direct physical contact [with sample size $n = 20$, average width 97.2 ± 1.1 μm (standard error of the mean), compared with 89.7 ± 1.1 μm in the latter case]. Thus, the morphogen might be primarily bound to the cell surface of *Lembadion*, perhaps preferentially to the peristome or buccal region. Nevertheless, cell-free fluid conditioned by *Lembadion* does have an effect on *Euplotes*.

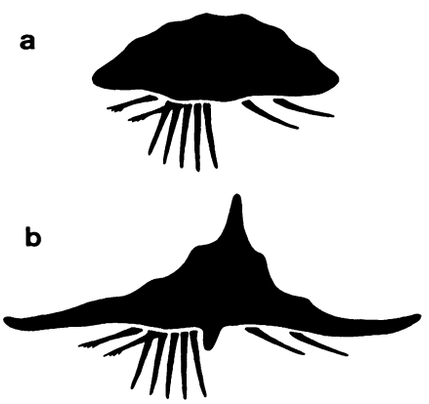

Fig. 2. Optical transverse sections through *E. octocarinatus* viewed toward the posterior end (a) before and (b) after exposure to the *Lembadion*-factor. The five longer ventral projections on the animal's right side (viewer's left side) are the five transverse cirri. The four other cirri (two projecting toward the right side and two toward the left side) are the caudal cirri.

We have used such conditioned fluid to obtain preliminary data on the properties of the *Lembadion*-factor. (i) It is relatively heat-stable (most of the activity is retained after heating the fluid for 5 minutes to 100°C, and some activity is still detectable after heating to 100°C for 30 minutes). (ii) It loses activity over a period of days when it is stored at 20°C. (iii) It passes through a 0.45-μm Millipore filter. (iv) It is held back by Amicon filter PM 10, which excludes material larger than 10,000 in molecular weight. (v) It is not inactivated by ribonuclease from bovine pancreas (25 μg/ml, incubated in culture medium for 60 minutes at 37°C). (vi) It is not inactivated by deoxyribonuclease I (25 μg/ml, incubated in culture medium for 60 minutes at 37°C). (vii) It is not readily inactivated by Proteinase K (250 μg/ml, incubated in culture medium for 60 minutes at 37°C), but is inactivated by Pronase P (25 μg/ml, incubated in culture medium for 60 minutes at 37°C).

So far we have investigated the effects caused by the factor released by *L. lucens*. Similar responses are, however, induced in *Euplotes* by factors released from *Urostyla grandis*, *Stylonychia mytilus*, and *Dileptus anser*, all of which prey on *Euplotes*. Whether the various morphogenetically active substances are similar or perhaps even identical has not yet been determined.

Euplotes octocarinatus is not the only species capable of responding to these morphogens; similar responses are ob-

served also in *E. patella, E. daidaleos, E. plumipes, E. aediculatus*, and to a lesser extent in *E. eurystomus*. No such effect is found in either *E. woodruffi*, which is closely related to the above mentioned species [it has the same pattern of nine type 1 fronto-ventral cirri (*6*)], or in *E. crenosus* (only one stock tested so far) which belongs to the freshwater *Euplotes* species with ten fronto-ventral cirri.

Predator induced defenses have been reported for rotifers (*7, 8*), cladocerans (*3, 9*), and bryozoans (*4, 10*). Our example demonstrates the existence of this ecologically interesting feedback mechanism for protozoa. A chemical characterization of the signal substance that triggers the defensive response in the prey organism has so far been attempted only for the factor released by the carnivorous rotifer *Asplanchna brightwelli* (*8*). On the basis of his finding (*8*) that the activity of *Asplanchna*-conditioned medium was destroyed by treatment with Pronase, Gilbert suggested that the *Asplanchna*-factor is a protein (*8*). We assume that the *Lembadion*-factor which causes *Euplotes* to change its cell shape is also a polypeptide. That it was not found to be readily inactivated by Proteinase K may indicate an unusual amino acid composition. Further studies are, however, required before final conclusions can be drawn.

The possibility of inducing *Euplotes* to change its cell shape drastically by exposing it to a defined chemical signal may be useful for studies in developmental biology. Since the cells respond directly to the morphogen, the changes can be induced synchronously under controlled conditions in large numbers of cells. This should make it possible to discern the chain of events occurring from the binding of the morphogen to specific cell receptors and leading to the reconstruction of the cytoskeleton as a function of altered gene activity.

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Diagnostic Ultrasound and Sister Chromatid Exchanges: Failure to Reproduce Positive Findings

Abstract. *Human lymphocytes were exposed in vitro to ultrasound from two clinical devices, one of which was previously reported to have increased the frequency of sister chromatid exchanges. The ultrasonic exposures had no significant effect on the frequency of sister chromatid exchanges from three blood donors. Exposure to ultrasound also had no effect on cell cycle progression. A concomitant positive control (mitomycin C) resulted in a significant increase in sister chromatid exchanges.*

Whether the frequency of sister chromatid exchanges (SCE's) is increased as a result of exposure to diagnostic ultrasound has become an important issue since the initial positive report by Liebeskind *et al.* (*1*). In that study the frequencies of SCE's from lymphocytes in vitro exposed to ultrasound were higher than those from controls. This effect was not observed with comparably exposed HeLa cells (*2*). Subsequently, there have been three additional reports (*3, 4*) of increased frequencies of SCE's with exposure to ultrasound; two of the studies used human lymphocytes (*4*), the third used Chinese hamster ovary cells (*3*).

Many reports, however, have not indicated an increase in SCE's after exposure to ultrasound (*5–8*). Brulfert *et al.* (*5*) and Miller *et al.* (*7*) were unable to verify the positive results of Liebeskind *et al.* (*1*). Although the conditions for the three studies were generally similar, different ultrasound equipment was used. The next logical step, therefore, was to obtain the ultrasound exposures of lymphocyte cultures in vitro with the same clinical devices used by Liebeskind *et al.* (*1, 2*)—a Reflectoscope UM727 (Sperry Rand) and Ekoline-20 (Smith-Kline). We focused on the Liebeskind *et al.* study (*1*) because it was the first to report an increase in SCE frequency with diagnostic ultrasound exposures. We sought to verify their results with their equipment and cell culture facilities to elucidate the physical mechanism of action.

Two sets of experiments were conducted. The first used two ultrasound devices for exposures, and the cells were cultured in Ham's F10 medium with bromodeoxyuridine (BrdU) available continuously; in the second, we used McCoy's 5A medium, added BrdU 22 hours after the culture was initiated and exposed to ultrasound with the Reflectoscope unit.

Blood from three donors was drawn into syringes containing heparin and allowed to sediment for 2 to 3 hours at room temperature so that the leucocytes were separated by gravity from the erythrocytes; one of the donors had also participated in earlier experiments (*1*). For each donor, two 250-ml flasks (Falcon 3024) containing 28 ml Ham's F10 medium (or McCoy's 5A medium), 15 percent fetal bovine serum, phytohemagglutinin (Wellcome) (1 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), 2 m*M* L-glutamine, heparin (4 U/ml), and 9 μg/ml BrdU were seeded with 2 ml of plasma containing leucocytes. In the second set of experiments only 0.5 ml of plasma was added so that the cell concentration was 2×10^5 cell/ml (*1*). For one donor an extra flask was used, with mitomycin C (Sigma) added at a final concentration of 0.033 μg/ml [0.1 μg/ml (*1*)], to provide a positive control with frequent SCE's. Cells were grown in the dark at 37°C in a CO_2 incubator.

At 47 to 48 hours the cultures were exposed to ultrasound. The contents of the two flasks from each donor were mixed and the cultures transferred into three 15-ml plastic tubes. Each tube was completely filled with culture medium and then capped with a sterile sheet of Parafilm. Care was taken to exclude any bubbles under the Parafilm. The tubes were maintained in a water bath at 37°C in the dark; one tube was a control, the others were exposed with one of the ultrasound devices for 30 minutes. The mitomycin C control was also transferred to a 15-ml tube and maintained in