Table 2. The seven known genes of phage S13.

Complementation group	Function
I	Coat protein structure
II	Unknown—possibly SS DNA synthesis
IIIa	Unknown—possibly SS DNA synthesis
IIIb	Coat protein structure
IV	RF DNA synthesis
V	Lysis
VI	Unknown—possibly SS DNA synthesis

give equivocal results, *su*HT205 is clearly in a separate functional unit from group V because, unlike group V mutants, it does not produce mature phage intracellularly. Furthermore, cultures of C122 multiply-infected with *su*HT205 clear at the normal time, but when infected with a group V *su* mutant the culture remains turbid.

The test with the group IV mutant *su*HS113 shows the poor-rescue phenomenon discovered by E. S. Tessman (*1*, *9*). Mutant *su*HT205 and *su*HS113 clearly complement each other, but the burst consists almost entirely of the *su*HT205 type, the group IV mutant being very poorly rescued. The fact that *su*HT205 is rescued in all the complementation tests is additional evidence that it is in a functionally different group from IV.

The existence of a new complementation group has been confirmed by another set of tests with a temperature-sensitive false revertant of *su*HT205, labeled *ts*HT205.26.1. This mutant complements *su* mutants in groups I, II, IIIa, IIIb, and IV, but it does not complement nor recombine with *su*HT205.

The functions of some of the complementation groups are known in a general way. Groups I and IIIb determine, at least in part, the phage coat (*1*, *8*). Group IV controls the replication of the replicative form (RF) DNA (*9*). Group V mutants produce intracellular phage normally, but cell lysis is delayed (compare ϕX174, *4*).

The function of VI might involve DNA replication. This possibility was studied by measuring the synthesis of infectious double-stranded RF and single-stranded (SS) phage DNA in the nonpermissive host C122 after infection with *su*HT205 at a multiplicity of infection of 3. The RF DNA was measured by the hydroxylamine inactivation method of Howard, Tessman, and Howard (described in *9*), and SS DNA was assayed by the heat treatment of I. Tessman (described in *10*) which releases DNA from the phage coat so that both naked and encapsulated SS DNA are measured together. In comparison with the parent *su*+, *su*HT205 produces essentially normal amounts of RF DNA, the differences being within the range of variation observed from experiment to experiment for the wild-type phage (Fig. 1). But no SS DNA is made by the group VI mutant. For both RF and SS DNA, the *su*+ results are consistent with previous results (*9*).

With the addition of the new complementation group there are now five functional units of S13 (groups I, II, IIIa, IIIb, VI) of which mutants have no significant effect on RF DNA synthesis but prevent the appearance of SS DNA. Since groups I and IIIb appear to be structural genes for coat proteins (*8*) their roles in the control of SS DNA synthesis must be indirect; perhaps the unprotected SS DNA is degraded or is converted into RF DNA. Thus, if a part of the phage genome has the function of specifying one or more enzymes for synthesizing SS DNA, then groups II, IIIa, and VI are likely candidates for that function (Table 2).

With the addition of the seventh gene, have all the genes in S13 (and also ϕX174) been identified by mutation? The fact that only one mutant site in group VI has been found so far makes it obvious that other groups might not yet be represented at all. And some undiscovered genes could be nonessential and thereby escape detection by conditional-lethal mutants.

However, a rough coding calculation gives an idea of how close we may be to accounting for all the phage genes. Given an RF DNA length of 1.64 ± 0.11 μ (*11*) and assuming a spacing of 3.4 Å of the nucleotides along the chain (*12*) there are 4800 ± 320 nucleotide pairs per RF molecule. If we assume a triplet code, the phage can specify approximately 1600 amino acids. Because 160 amino acids seems to be a low average number of amino acids for a protein subunit (compare *13*), we estimate that ten polypeptide species are a generous maximum limit to what can be coded by the phage; the one phage protein analyzed (in ϕX174, *14*) has 225 amino acids per subunit. Thus, seven complementation groups should account for at least 70 to 100 percent of the phage genes.

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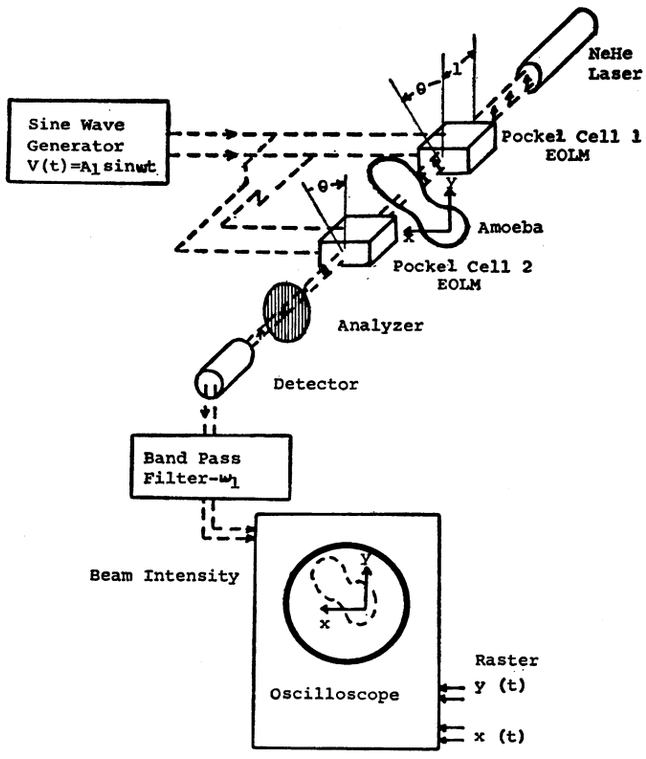
Optical Differentiation of Amoebic Ectoplasm and Endoplasmic Flow

Abstract. *Optical activity differentiating the flowing and nonflowing amoebic cytoplasm was detected. This evidence indicates molecular alignment in the flow stream and can be used to provide data on the direction of alignment. The results were obtained by utilizing a dynamic polarized-light detection system which is sensitive only to specimens which possess a preferred axis.*

The application of engineering-systems techniques has led to the development of a system capable of detecting low-level optical activity in living specimens which has heretofore escaped detection (*1*, *2*). Optical activity is defined here as any polarized light phenomenon. The system was developed to study the mechanisms of amoeboid motility and is now capable of differentiating cytoplasm optical activity found in the flowing endoplasm from



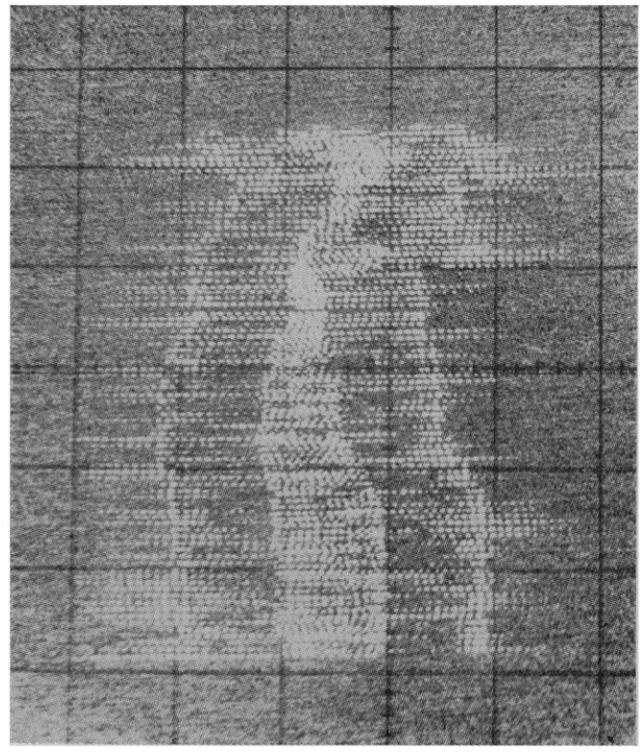


Fig. 1. Schematic diagram of experimental system for detecting optical activity in a streaming amoeba. Fig. 2. Photograph of a portion of the storage tube for a scan of a *Chaos chaos* pseudopodium. Beam diameter, 20 μ; light, λ, 632.8 mμ; raster size, 610 μ by 800 μ. Ringing of the beam modulating information at membrane and flow channel results from low damping in the tuned amplifier.

that of the stationary ectoplasm. However, the present method of storing this information (storage cathode ray tube) limits analysis and quantification of the optical activity data. Extension of the capability of the system is required to substantiate the type of optical activity and to quantify it.

This work was directed toward the development of a system with which the material structure of an amoeba or any macromolecular substance in solution could be characterized by the orientation of its chemical bonds. The structure characterization is achieved by utilizing the changes in the polarized light absorption properties of the substance (*3*). The system makes use of a monochromatic, collimated, and polarized light source (a gas laser) and two electrooptic light modulators (EOLM's) to achieve a dynamic system with a rapidly oscillating polarized beam.

Placing the second EOLM between the specimen and a fixed analyzer enables the system to filter out all optical activities that are independent of their orientation with respect to the polarized light source. The system output information is therefore characteristic of the type of optical activity the specimen possesses and is a function of the EOLM modulation frequency and the orientation of the specimen. The system output is filtered and applied to the z-axis input of a storage tube oscilloscope. The specimen is moved under the polarized light beam by a dual servo system connected to the position control of the microscope stage. A position pick-off from the servo system is applied to the horizontal and vertical axes of the storage tube. The intensity of the storage tube beam can be adjusted so that it stores only optical activity information, that is, the background noise of the system is clipped. The experimental apparatus is shown in Fig. 1.

Figure 2 is a photographic record (photograph of a portion of the storage tube) of differential optical activity of the flowing and nonflowing cytoplasm. At present this data permits comparisons of the regions of molecular alignment in the flow stream (bright regions on the photograph) and those of random alignment (dark regions on the photograph).

Further work is needed to determine the direction of molecular alignment in the flow stream to substantiate the type of activity data and compare the point of maximum intensity of the polarized beam with the angle the beam makes with the flow channel. The analysis of the optical activity data can be achieved by considering the Jones matrices (*4*) for the optical elements to determine the Jones matrix for the specimen optical activity. The direction of molecular alignment in the flow stream should cast some light upon the reasons for the ordering of molecules in the flow stream. From the work of Allen *et al.* (*1*) it is seen that the existence of a preferred axis may arise as a result of flow or stress applied to the substance.

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