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Gene Transfer to a Myxobacterium by Escherichia coli Phage P1

Abstract. Myxococcus xanthus *is a bacterium with an interest for studies of development because it has an organized multicellular phase in its life cycle. Bacteriophage P1 can adsorb to* M. xanthus *and inject its DNA into this organism despite the wide taxonomic gap separating myxococcus from* Escherichia coli, *the source of P1. A specialized transducing derivative of P1, called P1CM, can carry a gene for chloramphenicol resistance from* E. coli *into* M. xanthus *and generate unstable drug-resistant strains.*

Fruiting myxobacteria can propagate indefinitely as independent vegetative cells that have typical gram-negative bacterial morphology (*1*). However, when appropriately starved, myxobacterial cells stop dividing and aggregate to form a multicellular fruit of species-specific shape in which individual cells become spores (*2*). The behavior of myxobacteria during aggregation and fruiting shows a high degree of coordination between the actions of separate cells and suggests that they might be appropriate organisms in which to study communication between cells (*3*). To advance a combined biochemical and genetic attack on this problem we have sought a mode of genetic exchange, because none had yet been described for any of the myxobacteria. We now report that phage P1 can transfer genes to *Myxococcus xanthus*. P1 is a general transducing phage isolated from *Escherichia coli* Li that can infect many species of enteric bacteria (*4*). Because *Myxococcus* forms multicellular fruiting bodies, exhibits gliding motility, and has a DNA base composition of 60 to 70 percent guanine • cytosine pairs, it has been placed in a taxonomic group far from the enteric bacteria (*5*). Gene transfer between the two groups is, therefore, surprising.

When a drop of a P1 suspension was placed on the surface of an agar plate seeded with cells of *M. xanthus* strain FB, bacterial growth under the drop was inhibited and a zone of clearing was produced. Five different stocks of P1 were tested and all produced clearing. Material from a clear zone, suspended and placed on a fresh bacterial lawn, did not produce clearing. The titer of P1 in this material (measured on *E. coli* K12) was no greater than that expected for dilution of the drop into the agar, that is, no phage production was detectable. To test whether clearing was due to the phage itself or to some other lytic agent, lysozyme for example, a P1 lysate was sedimented to equilibrium in CsCl and the phage-containing band was isolated. This purified P1 had the same clearing activity as the original lysate.

P1 adsorbed efficiently to *M. xanthus* cells. When P1 was mixed with FB cells at a density of 2×10^8 per milliliter, suspended in a solution of 2 percent Bacto-Casitone containing 2.5 mM $CaCl_2$ and 0.1 mM $MgCl_2$, and incubated at 32°C for 15 minutes, more than 99 percent of the P1 was removed from the supernatant after sedimentation of cells. A cell-free supernatant of FB had some capacity to inactivate P1, but the rate of inactivation was 3.5 times greater in a cell suspension than in a cell-free culture supernatant.

Table 1. Transfer of chloramphenicol resistance to *M. xanthus* by P1CM. The indicated number of phage particles or the number of molecules of DNA extracted from phage with phenol (*13*) were mixed with 1.5×10^8 exponentially growing FB cells in a total volume of 0.3 ml of 2 percent Bacto-Casitone containing 2.5 mM $CaCl_2$, and incubated for 17 hours at 32°C with aeration. Finally the mixture was divided into four portions and plated on Casitone agar containing chloramphenicol (25 μg/ml). Colonies were counted after incubation at 30°C for 4 days. The total number of colonies for the four portions is reported.

Addition	Chloramphenicol-resistant colonies
7×10^9 P1CM particles	83
3.5×10^9 P1CM particles	36
3.5×10^9 P1 particles	<1
None	<1
8×10^9 P1CM DNA molecules	3

To test whether phage DNA entered the cells, phage P1CM was applied to strain FB. Phage P1CM carries a gene specifying chloramphenicol transacetylase from an R factor (*6*). Bacteria and P1CM were mixed, incubated, and plated on Casitone (*7*) agar containing chloramphenicol. As indicated in Table 1, chloramphenicol-resistant colonies of *M. xanthus* arose from the cultures containing P1CM, but not from controls that contained P1 rather than P1CM. The number of drug-resistant colonies obtained was proportional to the number of P1CM particles added, with an efficiency of about 10^{-8} resistant colonies per phage.

To test whether P1 injected its DNA into the cell or released it at the cell surface to be taken up by a transformation-like mechanism, DNA extracted from P1CM was added to FB. Although some chloramphenicol-resistant colonies were observed (last line of Table 1), there were 25 times fewer colonies than from an equivalent amount of phage. The more efficient transfer from phage as compared to transfer from free DNA favors (but does not prove) injection. Despite the taxonomic gap separating *M. xanthus* from *E. coli*, the ability of P1 to adsorb to DNA and its apparent ability to inject DNA into myxobacterial cells suggests the presence of related surface structures. Lipopolysaccharide is the adsorption receptor for P1 in *E. coli* (*8*), and recently *Myxococcus* was shown to possess lipopolysaccharide similar to that from *E. coli* and *Salmonella* (*9*).

Chloramphenicol-resistant bacteria that arose from P1CM infection of FB grew on both solid and liquid media containing 25 μg of chloramphenicol per milliliter. Cultures have been carried through serial single colony isolations, representing more than 60 cell generations, without loss of drug resistance. In spite of that, the chloramphenicol-resistant character of these bacteria is unstable, and colonies that formed on solid medium without chloramphenicol contain drug-sensitive cells. The growth rate of the chloramphenicol-resistant bacteria in chloramphenicol liquid medium was one-third that of parental cells without chloramphenicol. The chloramphenicol-resistant bacteria failed to produce P1 phage, detectable by

ability to form plaques on *E. coli* K12 or on *M. xanthus* FB.

Generation of chloramphenicol-resistant bacteria by infection with P1CM phage shows that DNA carrying the chloramphenicol transacetylase gene can enter myxobacteria and can be replicated through many cell divisions. Loss of drug resistance from bacteria propagated in the absence of chloramphenicol suggests that the CM gene has not stably integrated into the myxobacterial chromosome and that it may be replicating as a plasmid more slowly than the cells are replicating. The clearing effect of P1 on FB growing on solid medium may be due to P1 genes whose expression is toxic to myxobacterial cells. However, there is no evidence for replication of these P1 genes, and no infective phage results from the infection. P1 might be used to transfer other genes from *E. coli* into myxobacteria for which specialized transducing particles exist, such as the genes for lactose metabolism (*10*) and for proline synthesis (*11*). It may be possible to select mutants of either P1 or *M. xanthus* (or both) that would permit P1 to mediate gene transfer between two myxobacterial cells (*12*).

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Concanavalin A: Its Action in Inducing Oocyte Maturation-Inducing Substance in Starfish Follicle Cells

Abstract. *Maturation of starfish oocytes is induced by 1-methyladenine produced in follicle cells under the influence of a gonad-stimulating hormonal peptide released from the nervous system. Concanavalin A stimulates isolated follicle cells to produce 1-methyladenine, which suggests that it has the same capacity as the peptide hormone to indirectly induce oocyte maturation.*

Oocyte maturation and spawning in starfish are caused by 1-methyladenine (*1*), which is secreted by the follicle cells (*2*). The production and secretion of 1-methyladenine are induced by gonad-stimulating substance (GSS) (*3*), which is released from the nervous tissue (*4*). Recently it has been reported that the lectin phytohemagglutinin M (Difco) induces maturation of starfish oocytes (*5*), although this activity was subsequently shown to be due to contaminating 1-methyladenine in the lectin preparation (*6*). We show here that another lectin, concanavalin A (Con A), induces oocyte maturation by stimulating production and release of 1-methyladenine.

When isolated starfish (*Asterina pectinifera*) oocytes with their follicular envelopes were kept in artificial seawater (ASW) (*7*) containing Con A (*8*) at various concentrations (0.04 to 2 mg/ml) for 1 hour, oocyte maturation revealed by the breakdown of germinal vesicles was found to occur at Con A concentrations above 0.12 mg/ml. Experiments to determine if the maturation-inducing activity of Con A was due to contamination with 1-methyladenine showed that this compound was not present in our Con A preparations (*9*).

The maturation-inducing action of Con A was found to be an indirect effect mediated by lectin interaction with follicle cells. When isolated starfish oocytes with intact follicular envelopes were incubated in ASW containing Con A at 25°C for 1 hour, they underwent oocyte maturation. When oocytes whose follicle cells had been removed by treatment with calcium-free ASW were placed in ASW containing Con A, they failed to mature at lectin concentrations up to 2 mg/ml. Treatment with calcium-free ASW does not reduce the capacity of oocytes to mature (*10*). These results indicate that the follicle cells are involved in oocyte maturation induced by Con A.

We next sought to determine whether Con A binding to follicle cells induces the production of a maturation-inducing substance (MIS). Incubation mixtures containing follicle cells (*11*) isolated from approximately 10,000 eggs in ASW, Con A (5 mg/ml) in ASW, and follicle cells plus Con A together in ASW were centrifuged after 60 minutes at 25°C. The supernatants were assayed for MIS with isolated oocytes with their follicular envelopes removed. Maturation-inducing substance was found only in the supernatant of the incubation media containing both follicular cells and Con A, and this activity was equivalent to that of $1.23 \times 10^{-7}M$ 1-methyladenine. These results suggest that Con A can stimulate follicular cells to produce MIS.

Subsequent experiments showed that the MIS induced by the action of Con A on follicle cells was 1-methyladenine. Follicle cells from 4.5×10^6 eggs were incubated at a concentration corresponding to 30,000 eggs per milliliter in ASW with Con A (5 mg/ml) for 2 hours at 25°C. After centrifugation

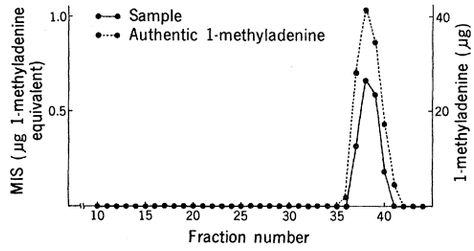

Fig. 1. (Solid line) Fractionation of the pooled active fractions of follicle cells and Con A on a Sephadex G-15 column equilibrated with 0.5*M* pyridine acetate buffer (*p*H 8.5), which is also used as eluant; fraction size, 3 ml; flow rate, 20.6 ml/hour. A part of each fraction (0.1 ml) was dried and the residue was redissolved in ASW and assayed with isolated oocytes without follicles, using authentic 1-methyladenine as the standard reference. (Dotted line) For comparison, the dried residue of 18 ml of 0.03*M* borate buffer (*p*H 8.5) was dissolved in 1 ml of $10^{-3}M$ 1-methyladenine and 1 ml of 0.5*M* pyridine acetate buffer and fractionated on the same column under the same conditions.