where (SC) is the number of all the undifferentiated cells; and, G_1, S, G_2, and M are the numbers of cells in each of these phases of the cell cycle;

$$dG_1/dt = -[A - a(SC)]G_1 - bG_1 + 2cM$$

where a is a constant that represents the rate of decrease, with increasing $[SC]$, in the probability that a cell can leave G; A is a constant computed from the minimum average time that a cell is assumed to spend in G_1; b is a constant representing fractional loss of undifferentiated cells to the circulation and loss by accidental death in the narrow; c is a constant computed from the average time a cell is assumed to spend in mitosis (M);

$$dS/dt = [A - a(SC)], - eS$$

where e is a constant computed from the average time a cell is assumed to spend in S;

$$dG_2/dt = [eS - (f + g)eS] - hG_2$$

where f and g are the fraction of cells, leaving S, that are triggered into erythroid or nonerythroid differentiation, respectively; h is a constant computed from the average time a cell is assumed to spend in G_2; and

$$dM/dt = hG_2 - cM$$

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Phloem Differentiation: Induced Stimulation by Gibberellic Acid

Abstract. *Gibberellic acid solutions supplied through micropipettes to explants from dormant branches of white pine* (Pinus strobus *L.*) *stimulate changes in the cambial zone. Immature sieve cells expand radially and exhibit cytological changes usually associated with spring maturation. Differentiation of sieve cells continues in response to treatment and is recognized by the birefringence of secondary walls when examined with the polarizing microscope.*

Evidence accumulated in recent years suggests that the induction and differentiation of vascular tissues in plants is regulated by the participation of endogenous growth regulators. Experimental findings from the laboratories of Jacobs (*1*), Torrey (*2*), and Wetmore (*3*) provide strong support for the involvement of auxin, indole-3-acetic acid (IAA), in various stages of xylem regeneration and differentiation. While IAA is generally recognized as a limiting factor for xylem differentiation, other growth substances (*4*), sugars (*5*), and minerals (*6*) also may be involved.

Physiological studies on the role of growth substances in regulating phloem differentiation are limited. LaMotte and Jacobs (*7*) view phloem regeneration in wounded *Coleus* plants as proceeding basipetally, stimulated by the availability of endogenous IAA flowing towards the base from shoot organs. Indole-acetic acid appears, then, to be a limiting factor for phloem regeneration, although other factors could be limiting when IAA is present in adequate amounts. Sucrose, alone or in combination with IAA, was not effective in inducing phloem differentiation in their system (it was probably never limiting in their green *Coleus* plants which were illuminated 16 hours per day) but has been implicated as a major factor in another experimental system (*3*).

Gibberellic acid has been noted to stimulate the differentiation of secondary phloem in woody species (*8*), and the stimulation is enhanced when this acid and IAA are supplied in combination. However, Wareing *et al.* (*9*) have been unable to detect stimulated differentiation of sieve elements with gibberellic acid treatment.

An evaluation of existing data leads to the consideration that phloem differentiation—like xylem differentiation—is controlled by a variety of factors, one or more of which may become limiting at some stage during development from cell initiation to maturation. Evidence presented in this report establishes a functional role for gibberellic acid during sieve cell differentiation in explants of white pine (*Pinus strobus* L.). To evaluate the activity of the acid in phloem differentiation, explants from dormant branches of white pine were utilized. Transections of these branches are characterized by a cambial zone of six to ten rectangular cells with thickened tangential walls, limited internally by latewood tracheids and externally by sieve cells in various stages of maturation (Fig. 1*A*). Except for the cambial initials, certain cells of the cambial zone, even at this stage, are already destined to become phloem cells. Abbe and Crafts (*10*) demonstrated that the initial response to growing conditions in the spring is seen in the swelling of cells in the cambial zone. Associated with the swelling are thinning of the tangential walls, cytoplasmic changes, loss of the nucleus, and deposition of secondary wall—a sequence of changes depicting sieve cell differentiation. These anatomical changes, easily seen by microscopic techniques, provide a convenient assay for detecting substances capable of stimulating phloem differentiation.

Explants were excised from surface-sterilized pine branches (Fig. 2*A*), and sterilized micropipettes (*11*) were inserted into the soft tissue of the cambial zone. Explants with pipettes were immediately planted upright in sterile test tubes containing agar-solidified Knudson's medium with 1 percent sucrose (Fig. 2*B*). Sterilized solutions of gibberellic acid to be tested for their ability to induce differentiation of sieve cells were supplied to the cambial zone via the micropipettes. Tubes were sealed with polyethylene and maintained in a temperature-controlled room (25° ± 2°C). They received 12 hours of illumination daily (about 2200 lu/m^2) supplied by a combination of fluorescent and incandescent lamps. Solutions were maintained at a constant level and all experimental treatments were prepared in triplicate on three separate occasions during February. Controls consisted of explants supplied with empty pipettes. Explants were collected at weekly intervals for a 3-week period, processed for microscopic examination, and studied by light and polarized light microscopy.

Control explants viewed with polarized light (Fig. 1*B*) exhibit the usual features associated with the dormant condition. The over-wintering cambial zone and immature sieve cells are nonbirefringent, as are the large, banded parenchyma cells which delimit seasonal increments of phloem differentiation and help form detectable "growth rings." Situated between these two nonbirefringent zones, and in striking con-

trast, are radial rows of four to five mature sieve cells with birefringent secondary walls. These cells represent phloem which matured during the previous growing season (*12*).

When treated with solutions of gibberellic acid at 10 or 100 parts per million, sectioned explants displayed signs of differentiation similar to those observed by Abbe and Crafts (*10*) and analogous to the condition seen in sectioned branches 10 to 12 days after artificially induced bud break (*13*). The first visible sign of activity occurs 1 to 2 weeks after treatment and is seen in the swelling of cells in the cambial zone. Three weeks after treatment with gibberellic acid, most cells of the cambial zone had enlarged two to four times their over-wintering radial diameter, and their thickened tangential walls were considerably thinned and tenuous (Fig. 3*A*). Maximum swelling was observed in cells situated beneath the pipette orifice and directly in the path of the flowing gibberellic acid solutions. Not uncommonly, explants were found with regions largely unaffected by the acid treatment. When present, these regions were on either tangential side of an activated cambial zone, indicating that in this system the acid stimulus was extremely localized in its action. In none of the many explants examined was complete stimulation by the acid observed.

That gibberellic acid treatment stimulates swelling of immature sieve cells and enlargement of phloem components in the dormant cambial zone in pine is without question (Fig. 3*A*). These findings support the observations of Wareing *et al.* (*9*) that new phloem is formed in *Populus* and *Vitis* in response to gibberellic acid treatment. The induced differentiation of sieve cells by the acid, however, is not as obvious, and has not been established previously with certainty. The deposition of secondary walls in sieve cells of pine and their unequivocal recognition in polarized light presents a simple, effective technique for detecting newly differentiated sieve cells and for estimating the amount produced during the experimental period (*14*). Polarized light examination of sections from explants treated with gibberellic acid (Fig. 3*B*) reveals a broad, radial cambial zone of enlarged, swollen, nonbirefringent cells. When compared with Fig. 2*B*, it is readily apparent that many of these cells are immature sieve cells which were derived from the thick-walled, rectangular cells in the dormant cambial zone. Rows of sieve cells, characterized in polarized light by having incomplete or complete deposition of secondary walls, extend from the zone of swollen cells to the nonbirefringent "growth ring" of phloem parenchyma cells. The average number of cells in each row of the birefringent zone was determined by numerous counts to be eight. This number represents more than a 60-percent increase in the amount of mature sieve cells noted in the same region of control sections and provides the most convincing evidence that gibberellic acid is active in stimulating the differentiation of new sieve cells in addition to causing enlargement of immature cells of the cambial zone.

Whether the gibberellic acid effect is direct or indirect is not immediately apparent. Conifers are known to be lacking in hormone production during the dormant period (*15*) and the possibility is remote that the acid exerts its effect by increasing the transport of IAA as suggested by previous workers and recently reviewed by Jacobs (*16*). In addition, explants were removed from the known major sources of hormone supply (shoot and root apex), and it is unlikely that the phloem tissue is influenced by a stimulus from the dormant, nonactive cambium. The use of dormant pine explants further precludes complex interactions between the usual supply of hormones and externally applied growth substances.

Before an adequate explanation is advanced to explain the gibberellic acid effect, more must be learned about the specific action of other growth regulators and their interactions in phloem differentiation in pine. The technique

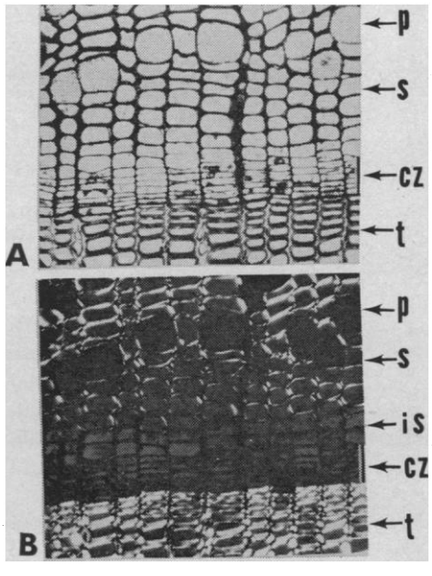

Fig. 1. Transverse section of an explant removed from a dormant branch of *Pinus strobus*. (*A*) Light microscope photograph illustrating anatomical features of the overwintering cambial zone and vascular tissues. (*B*) Polarized light microscope photograph of section in Fig. 1*A* (× 195). Abbreviations: *p*, phloem parenchyma; *s*, sieve cell; *cz*, cambial zone; *t*, tracheid; *is*, immature sieve cell.

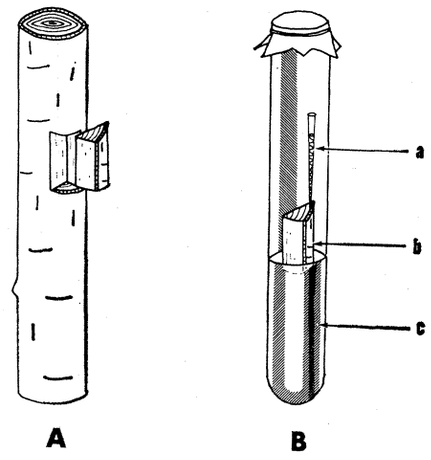

Fig. 2. (*A*) Explant removed from dormant branch of *Pinus strobus*. (*B*) Explant with micropipette (*a, b*) in agar-hardened Knudson's medium (*c*).

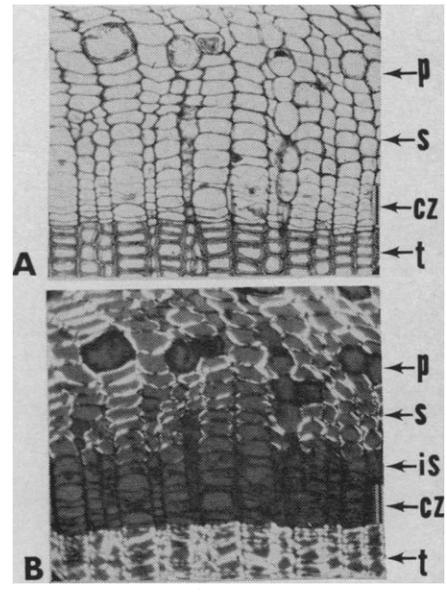

Fig. 3. Transverse section of an explant treated with 100 parts per million of gibberellic acid solution. (*A*) Light microscope photograph illustrating swelling of cells in the cambial zone. (*B*) Polarized light microscope photograph of section in Fig. 3*A* (× 195). See Fig. 1 for meanings of letters.

outlined here serves well for such studies and provides the possibility of distinguishing the actions of various growth regulators on the enlargement and differentiation of immature sieve cells.

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Replication of the RNA of Bacteriophage f2

Abstract. *Events occurring after infection of bacteria with wild-type and a temperature-sensitive mutant phage indicate that there are two enzymatic activities necessary to replicate phage RNA. One converts single strands into double strands, while the other uses double strands to synthesize viral RNA. The mutant is deficient in the first activity, probably because the mutation is in the gene specifying the requisite enzyme. On the basis of these and other results, a model is presented for the replication of phage RNA.*

A central problem presented by the growth of a bacteriophage with an RNA genome (*1*) is the mechanism of replication of the viral nucleic acid. There are numerous reports of double-stranded RNA in cells infected by RNA phages (*2–5*). Although the evidence is incomplete, this material may play a critical role in RNA replication. One of the most cogent points for the involvement of this RNA is that after infection some of the parental phage RNA is converted to a double-stranded form (*2, 3, 6*). To carry out such a conversion, an enzyme capable of using single-stranded RNA as template for the synthesis of the complementary RNA strand is required. If double-stranded RNA is an intermediate in RNA replication, a second enzymatic step is required to synthesize the viral RNA.

We now report that these two enzymatic steps are probably catalyzed by different enzymes, and that enzyme I, catalyzing the first step—the synthesis of double-stranded RNA—is synthesized under the direction of the viral genome. We consider in detail a class of temperature-sensitive phage mutants that are blocked primarily in the conversion of single-stranded RNA to double strands (function of enzyme I) and only secondarily in the synthesis of single-stranded viral RNA. This phage function is required throughout infection for the production of phage particles (*7*). Since the mutation is in the phage genome, presumably in the gene specifying enzyme I, these effects indicate that double-stranded RNA is an intermediate in virus replication.

Our experiments are based on the following model for the replication of an RNA bacteriophage. The parental phage RNA molecule, immediately upon entering the cell, becomes attached to a ribosome (*6*) and directs the synthesis of all the necessary enzyme I. Enzyme I now uses the parental RNA as template for the synthesis of the complementary RNA strand, forming a double-helical RNA structure. This double-stranded RNA then becomes a template for the synthesis of single-stranded viral RNA molecules, which is perhaps accomplished by a second enzyme. A small fraction of the newly made single-stranded RNA is converted by enzyme I into double strands that form new templates for the second step. This cyclical process continues throughout infection, and the number of double-stranded RNA molecules increases in parallel with the number of viral single-strands.

Double-stranded RNA was measured in infected cells as follows: 2.0 ml of the labeled cells were added to 0.5 ml of a 5 percent sodium dodecyl sulfate solution and immediately frozen in a mixture of dry ice and acetone. The samples were frozen and thawed twice, mixed with 0.2 mg of yeast RNA (Calbiochem) as carrier, and extracted with an equal volume of water-saturated phenol. The aqueous layer was made 0.3*M* in sodium acetate, and three volumes of ethanol were added. After at least 2 hours at −20°C, the nucleic acids were collected by centrifugation and resuspended in 2.0 ml of buffer (0.10*M* NaCl, 0.001*M* $MgCl_2$, 0.05*M* tris, *p*H 7.4). Deoxyribonuclease (*8*) was added (50 μg/ml) for 30 minutes at 25°C. A portion was added to a solution of 5 percent trichloroacetic acid (TCA) containing 0.1 mg of serum albumin as carrier. The precipitate was collected on fiberglass filters, and the radioactivity was determined (*9*). Another portion was added to 2 ml of buffer, and ribonuclease (50 μg/ml) was added for 30 minutes (*8*). Trichloroacetic acid (to 5 percent) was added, and the precipitate was collected as above. In most experiments portions were also treated with ribonuclease in water (*10*), conditions which degrade double-stranded RNA, to insure that there was no contaminating label not

Table 1. Viral RNA polymerase induced by temperature-sensitive mutants. *Escherichia coli* strain K37 was grown in broth (*24*) at 37°C to a density of 2×10^8 cells/ml. The cells were incubated at the appropriate temperature (second column) for 5 minutes before infection with phage. The cells were harvested for polymerase assay (*12*) at 40 minutes after infection. One unit of enzymatic activity is defined as the incorporation of 100 $\mu\mu$mole of UTP-C^{14} per milligram of protein per 15 minutes at 25°C.

Phage	Temp. of infection (°C)	Polymerase (units)
None	34	0.10
f2	34	2.80
	43	3.20
ts-4	34	2.20
	43	0.10
ts-5	34	.96
	43	.11
ts-6	34	1.70
	43	0.10