Tip Growth in Micrasterias

Abstract. *Methylation of polysaccharides in the growing primary wall of* Micrasterias *is localized at specific points on the wall which, by analogy with hyphal growth in fungi, are sites of maximum wall extension. Rather than being a direct cause of extension at these points, methylation may indicate an incorporation of hemicellulosic matrix substance that accompanies extension.*

In fungal hyphae, root hairs, pollen tubes and some algal cells, elongation is largely the result of growth at the tip of the elongating structure (*1*). In these cases, marking experiments and autoradiography indicate that extension of existing wall and incorporation of new wall are confined to a very restricted region of the wall. The growth region is characteristically a hemisphere and growth is such that a cylinder of nongrowing wall is produced at its base.

Given that an area of wall participates in tip growth, some of the changes that take place can be described in physical terms. Besides increase in size, there may be changes in the thickness or shape of the area or in the rate of its passage through the growth region (*2*). However, cell walls are structurally too complex for their growth to be reduced to simple physics and geometry. Lytic and synthetic events both participate in tip growth (*3*), and so some consideration of wall fine structure and biochemistry is unavoidable. Incorporation of new wall is a necessary feature of steady state wall growth since wall thickness would otherwise diminish to zero. Studies of radiolabel incorporation and of fine structure in growing hyphae and root hairs confirm that wall substance is continually added at their tips (*3, 4*). Wall synthesis and deposition is far from being sufficient for growth, since walls may thicken without expanding in area.

From Robertson's observation that normally plastic hyphal tips harden over or "set" when growth is arrested (*5*), he proposed a model of hyphal growth that depends on two competing processes, wall extension and wall hardening. Wall extension occurs continuously and is maximal at the hemisphere apex. Hardening acts throughout the growth hemisphere so as to render the new and plastic wall of the apex progressively more rigid. The area characterized by rapid extension is therefore small compared to that being hardened. Our work with tip growth in lobes of *Micrasterias rotata* semicells reveals two distinct patterns of autoradiographic labeling that one can compare with Robertson's two hypothetical processes. Methyl groups from methionine are incorporated into very restricted regions of the wall only, and for this reason could be associated with the process of extension. Glucose incorporation into the microfibrillar fraction of the wall is spatially less restricted as would be expected of a process associated with hardening. If these comparisons should prove to be meaningful, Robertson's model would acquire an additional degree of generality sufficient to include the growth of *Micrasterias* lobes, a new and interesting example of the phenomenon of tip growth.

After mitosis in vegetative cells of *Micrasterias*, a septum of primary wall forms between the semicells and then grows as these separate so as to develop within several hours an elaborate shape similar to that of the parent semicells (Fig. 1a). Individual lobes of the developing semicell appear to grow by tip growth alone. If irradiated by a laser microbeam, lobe growth is arrested only when the very tip of a lobe is damaged. Damage to other regions of the wall may leave a mark, but fails to arrest lobe growth (*6*). Autoradiography of primary cell wall ghosts, with the use of tritiated glucose and methionine (*7*), further implicates tip growth in semicell development (Fig. 1b). With brief pulses, label appears only at the tips of growing lobes. With progressively longer pulses, the labeled area enlarges at a rate corresponding to that at which wall area is itself increased by lobe growth. Primary wall ghosts show different patterns for incorporation of the two labels, with the methionine label being far more restricted (Fig. 2). This difference cannot be due to a relatively slower uptake of methionine since the area labeled by glucose at a hypothetical zero time is still substantially larger than that labeled by brief exposures to methionine. In fact, saturation of precursor pools is equally rapid for both compounds, if we judge by how sharply bounded the labeled regions appear (*8*). Glucose label appears to be incorporated into microfibrils since it is not removed by the chromic acid treatment shown to remove all but the microfibrillar fraction in primary walls of closely related desmids (*9*). Methionine label is removed by all treatments with alkali and by pectin methyl esterase, but not by trypsin or Pronase (*10*). This is consistent with its presence in methyl groups esterified to an acidic

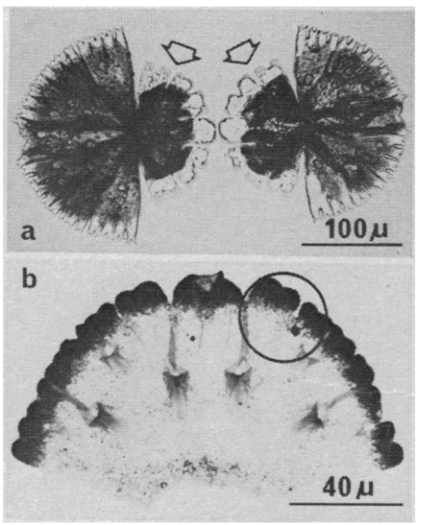

Fig. 1. (a) A developing *Micrasterias* cell 2½ hours after mitosis. The new semicells (arrows) are each contained within a primary wall. (b) Autoradiogram of a primary wall ghost from a developing cell allowed to grow for 30 minutes in medium containing [methyl-^{3}H]methionine (200 μc/ml) and then lysed to remove the protoplast. Label is confined to the tips of semicell lobes, and labeled regions are sharply bounded. A circle encloses the upper wing lobe traced in Fig. 2.

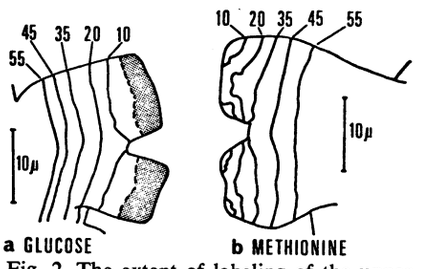

Fig. 2. The extent of labeling of the upper wing lobe is shown for a series of unextracted primary wall ghosts from cells exposed to label for varying lengths of time, all of which had reached the same stage of development at the time of lysis. Each diagram combines tracings by camera lucida from 20 cells. Numbers indicate the duration of label pulse in minutes and include in every case 5 minutes for a standardized wash and lysis procedure. (a) Walls labeled with [1-^{3}H]glucose. The shaded area indicates the minimum area which, by extrapolation, must have begun incorporating at zero time in order to produce the pattern observed with 10 minutes' labeling. (b) Walls labeled with [methyl-^{3}H]methionine. A similar extrapolation to zero time here would indicate initial incorporation at four individual points.

polysaccharide, but not with its presence in a cell wall protein or cytoplasmic residue. Methyl groups may be esterified to the hemicellulosic matrix of the primary wall which, in the case of *Micrasterias*, is rich in uronic acids (*11*).

Autoradiography therefore reveals two patterns of incorporation of material into cell wall which are spatially and biochemically distinct. Comparing these with Robertson's two hypothetical processes, extension and hardening, we could conclude that methionine incorporation is associated with wall extension and glucose incorporation (microfibril synthesis) with hardening. The participation of microfibril synthesis in hardening of plastic wall is not surprising since Robertson has argued that this is the case in hyphal growth (*5*). Nor is it difficult to envisage a role for microfibrils in a process that probably involves stiffening and reinforcement. The relation between methylation and Robertson's extension process is not so easily dealt with. Extension probably depends on wall loosening and some wall synthesis, although a precise understanding of the extension process in tip growth and in other types of cell wall growth has been elusive (*12, 13*). There is no simple causal relation between methylation of existing wall and extension, although inhibition of methylation may reduce cell elongation in some cases (*14*). It may be, in *Micrasterias*, that methylation accompanies the incorporation of new matrix into wall, and that matrix incorporation is linked to wall extension. Physical properties of the wall do depend upon the amount and kind of matrix present (*15*), and during growth matrix incorporation could act to force microfibrils apart or serve as a lubricant to facilitate microfibril slippage (*13, 16*). If extension were more directly the result of enzymatic hydrolysis or the breaking of acid-labile bonds, matrix incorporation must in any case accompany these changes in order that the original wall thickness and composition be maintained in regions of extension. Methylation, matrix incorporation, and wall extension are probably interrelated in some fashion. Tip growth in *Micrasterias* may provide an additional opportunity for study of this interrelation.

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7. Cells normally grown in Waris medium were allowed to develop for from 5 to 90 minutes in Waris medium containing [1-^{3}H]glucose or [methyl-^{3}H]methionine (200 μc/ml). Cells were washed twice with fresh medium and lysed with an ultrasonic cleaner (20 khz), with the time through washings to lysis standardized at 5 minutes. Primary walls were picked out by hand and mounted on slides for standard autoradiographic processing with the use of Ilford L-4 emulsion.
8. Resolution of the method is not a problem. Wall preparations are sufficiently thin that resolution is close to the theoretical limit for tritium autoradiography.
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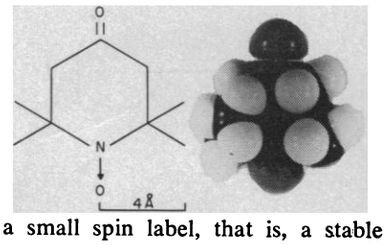
Viscosity of Cellular Protoplasm

Abstract. *The protoplasmic viscosity was studied by using a small spin label having high permeability and broad solubility properties and nickel chloride as an extracellular spin-subtracting agent to localize signal inside cells. The viscosity is variable and in some cells is many times that of water or phospholipids, suggesting that lateral diffusion in biological membranes is important to cell function.*

The internal viscosity of cells is undoubtedly important to many cellular processes and cells might be expected to have considerable diversity in internal viscosity based on size differences and amount of internal membrane structure. Several approaches have been used in attempts to determine intracellular viscosity. In one study, centrifugation coupled with microscopy was used to observe the rate at which intracellular organelles diffuse through the cytoplasm. For eggs of the sea clam *Spisula*, the protoplasmic viscosity was estimated to be 4.3 centipoise (cp) (*1*). Another method involved the incorporation of magnetic particles into cells and subsequent observation of the viscous drag they experience in response to an external magnetic field. The internal viscosity of the giant amoeba *Chaos chaos* was estimated to be 3 to 11 cp by this method (*2*). In a detailed study of the interior of cultured chicken cells, Crick and Hughes (*3*) found a somewhat more rigid protoplasm with evidence of thixotropy, and suggested a value for the modulus of rigidity of about 10^2 dyne/cm^2. The magnetic particle method was also used in these experiments. More recently, nuclear magnetic resonance experiments employing the spin-echo technique were carried out to make approximations of the self-diffusion coefficient of water protons. In yeast, the diffusion coefficient was about 3.4 times less than in water (*4*). With the exception of this last study, all reports have dealt with a bulk or macroviscosity. It is not obvious nor perhaps expected that the microviscosity relevant to the diffusion and rotation of small molecules would mimic the bulk viscosity of a solution or of protoplasm.

Tempone (2,2,6,6-tetramethylpiperidone-*N*-oxyl radical), whose structure is shown below,



is a small spin label, that is, a stable organic radical with an unpaired electron. We have measured the molecular motion of tempone in several different types of cells (*5*). The spin label was allowed to passively diffuse into cells, and spin label signal originating from outside the cell boundaries was eliminated by having 0.5*M* $NiCl_2$ in the medium (*6*). Nickel is paramagnetic yet gives no visible electron spin resonance (ESR) signal in liquid medium