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Intracellular motility and the evolution of the actin cytoskeleton during development of the male gametophyte of wheat (*Triticum aestivum* L.)

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Intracellular motility and the evolution of the actin cytoskeleton during development of the male gametophyte of wheat (*Triticum aestivum* L.)

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SUMMARY

The uniaperturate pollen of wheat is dispersed in a partially hydrated condition. Amyloplasts are concentrated in the apertural hemisphere where they surround the two sperms, while vigorously moving polysaccharide-containing wall precursor bodies (P-particles) together with the vegetative nucleus occupy the other. This disposition is the product of a post-meiotic developmental sequence apparently peculiar to the grasses. During vacuolation of the spore after release from the tetrad, the nucleus is displaced to the pole of the cell opposite the site of the germination aperture, already defined in the tetrad. Following pollen mitosis, the vegetative nucleus migrates along the wall of the vegetative cell towards the aperture, leaving the generative cell at the opposite pole isolated by a callose wall. As the vacuole is resorbed, the generative cell rounds up, loses its wall and follows the vegetative nucleus, passing along the wall of the vegetative cell towards the aperture where it eventually divides to produce the two sperms. Throughout this period of nucleus and cell manoeuvrings, minor inclusions of the vegetative cell cytoplasm—including mitochondria, lipid globuli and developing amyloplasts—move randomly. Coordinated vectorial movement begins after the main period of starch accumulation, when the amyloplasts migrate individually into the apertural hemisphere of the grain, a final redistribution betokening the attainment of germinability. In the present paper we correlate aspects of the evolution of the actin cytoskeleton with these events in the developing grain, and relate the observations to published evidence from another monocotyledonous species concerning the timing of the expression of actin genes during male gametophyte development, as revealed in the synthesis of actin mRNA.

1. INTRODUCTION

The mature pollen of wheat is released at anthesis in a partly hydrated state. There is no imposed interval of dormancy, and the various inclusions of the vegetative cell (VC) cytoplasm are in a state of continuous movement throughout the brief period of viability. At the time of dispersal the cytoplasm of the VC in the vicinity of the single germination aperture (the proximal pole with reference to the anther wall) contains a closely packed population of amyloplasts, which conceal the two sperms and mask other organelles except near the cell surface (Watanabe 1961). At the opposite, distal, pole the cytoplasm contains fewer amyloplasts, and the most conspicuous inclusions are the vegetative nucleus (VN), mitochondria, lipid globuli and numerous polysaccharide-containing wall-precursor bodies (P-particles; Heslop-Harrison & Heslop-Harrison 1982). In the ungerminated mature grain at the time of dispersal the movement of the amyloplasts is slow and restricted, especially in the congested proximal region of the VC, but movement is very vigorous in the distal domain, where the P-particles travel in short linear trajectories, with local groups executing rapid rotational movements (Heslop-Harrison & Heslop-

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Harrison 1992*b*). We have noted many of these characteristics in the pollens of species of six tribes of the Gramineae, namely Maydeae, Phalarideae, Agrostideae, Aveneae, Festuceae and Hordeae, suggesting that the condition is likely to be widespread in the family.

Both amyloplasts and P-particles carry surface myosin (Heslop-Harrison & Heslop-Harrison 1989*a*, 1997), and their movement in the mature grain is associated with actin fibrils. Shorter randomly disposed fibrils are dispersed between the congested amyloplasts at the proximal pole of the VC, and also near the surface in the form of thicker strands focused upon the germination aperture, a disposition that defines in advance of germination the prospective pathways of organelle movement during the later emergence of the pollen tube. Towards the distal pole these strands break up into fine fibrils, merging into a population of still finer fibrils of indeterminate length interpenetrating the mass of vigorously moving P-particles. The actin skeleton of the VC is thus preadapted in the mature pollen grain for the deployment and directional transport of organelles and other inclusions of the cytoplasm during germination and tube emergence, a circumstance not hitherto reported from any other pollen type. Wheat pollen, accordingly, is in a state allowing almost immediate function on a receptive stigma (within as short a period as 60 s; Chandra & Bhatnagar 1974) in contrast with the condition in liliaceous pollens where a protracted period of reorganization of the actin cytoskeleton is required during hydration on the stigma before germination can occur (Heslop-Harrison & Heslop-Harrison 1992*a*).

Although reports such as that of Tanaka & Wakabayashi (1992) have dealt with the organization of the actin cytoskeleton in naked pollen protoplasts extracted before germination, there have been no accounts of the development of the actin cytoskeleton in *intact* pollen grains, where the influence of the wall structure is a paramount factor in determining polarity and in consequence the pregermination architecture of the actin system. The aim of the present study has been to follow the evolution of the actin cytoskeleton of the male gametophyte of wheat in the intact grain from the early division stage onwards, and to relate this both to the structure of the enclosing pollen wall and to the establishment of the characteristic disposition of the VC contents present at the time of pollen dispersal.

In a preliminary study we observed movement of minor inclusions in the thin peripheral layer of cytoplasm in microspores soon after vacuolation, indicating that actin is present during the post-meiotic interphase preceding microspore mitosis. However, we record here that the principal accumulation of actin occurs after the mitosis, over a period when electron microscopy shows that the ribosome population of the cytoplasm increases substantially (El-Ghazaly & Jensen 1986*a*, *b*) presumably signalling an interval of active protein synthesis. Early changes in the nature of the protein content in the developing pollen of wheat were reported by Vergne & Dumas (1989). While the results recorded by these authors did not provide any quantitative measure of protein accumulation during gametophyte development, they did give clear evidence of progressive change in the protein pattern throughout the interval from the uninucleate free spore to the late tricellular stage. Notably, in SDS-PAGE preparations heat-treated extracts, three protein bands with molecular masses corresponding to 86, 29 and 15 kD first appeared from late bicellular pollen, increasing thereafter. Others, exemplified by a band at 32 kD, progressively increased in relative concentration from the earlier uninucleate microspore stage onwards.

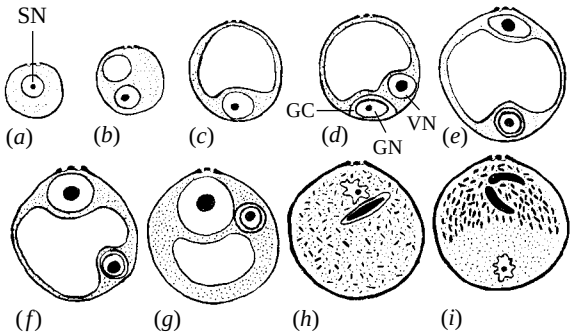

Figure 1. Diagram of the development of the male gametophyte of wheat: SN, spore nucleus and nucleolus; VN vegetative nucleus and nucleolus; GN, generative nucleus and nucleolus; GC, generative cell. In each sketch the germination aperture is uppermost. (*a*) Spore. (*b*) Early vacuolation. (*c*) Vacuolate spore ('signet ring' stage), with the nucleus displaced to a site near the wall at the distal pole. (*d*) VN migrating along the wall towards the aperture after pollen mitosis, leaving the GC with its enclosing callose wall at the distal pole. (*e*) VN and GC at opposite poles, the former in register with the aperture. (*f*) GC migrating along the wall towards the VN at the proximal pole; vacuole partly resorbed. (*g*) Late vacuolate stage, VN and GC together in the vicinity of the aperture; amyloplasts with early starch. (*h*) Vacuole resorbed; amyloplasts with rodlet starch. VN condensed with infolded envelope, and GC elongated preparatory to the division that produces the two male gametes. (*i*) Amyloplasts congregated in the proximal hemisphere and enclosing the two male gametes. Shrunken VN in the distal hemisphere, now heavily populated with P-particles.

Presumably actin was present in the initial extracts of Vergne & Dumas (1989), but their results provided no direct basis for identification of specific proteins, neither for those appearing first at specific periods of the developmental sequence, nor for those increasing in relative concentration throughout. In another monocotyledon with an essentially similar course of male gametophyte development, *Tradescantia paludosa*, Stinson *et al.* (1987; review, Mascarenhas 1992), using an actin cDNA clone as a probe, detected actin mRNA as a minor component during the interphase before pollen mitosis, and showed that it increased dramatically after the division to reach a maximum during late development before decreasing in maturing pollen. If, as seems entirely probable, the gene-expression programme in wheat is comparable with that in *Tradescantia*, this

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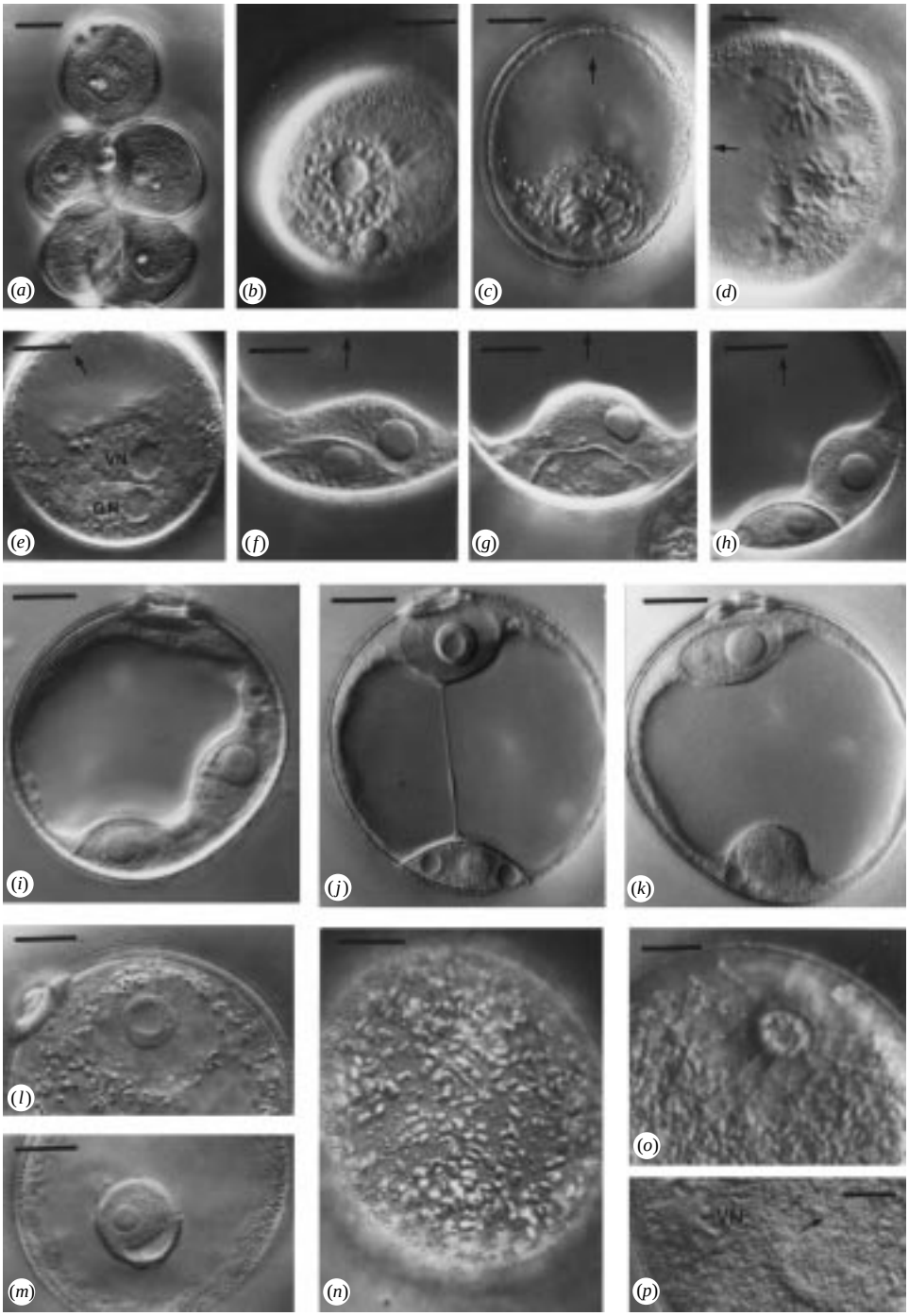

Figure 2. Development of the male gametophyte, differential interference contrast (DIC). (*a*)–(*o*) Living grains; (*p*) grain fixed with 1.5% glutaraldehyde (GDA) in 0.05 M phosphate buffer at pH 6.8. Arrows indicate direction of the aperture where it is not present in the micrograph. Scale bars, 10 μm. (*a*) Dissociating meiotic tetrads. (*b*) Individual spore with the nucleus at mid prophase. (*c*) Late prophase. (*d*) Anaphase. (*e*) Cell plate forming between the VN (upper) and the GN (lower) nuclei. There is no fixed orientation for the cell plate, which can be initiated even at right angles to the wall. (*f*) Growing GC wall, with overlapping callose plates which eventually link up. (*g*) Continuous GC wall, callosic at this stage and continous with the early intine around its periphery. The VN still in contact with the GC wall. (*h*) VN moving away from the GC and beginning its migration towards the proximal pole of the VC. (*i*) Later in the VN migration; starch synthesis in the amyloplasts begins at this time. (*j*) VN in register with the aperture, in this instance linked by a strand of cytoplasm with the GC, which still retains its callose wall. (*k*) The GC, now without the callose wall, rounding up and pulling away from the inner surface of the intine. (*l*) The much-enlarged VN is seen through the pollen grain wall. The resorption of the vacuole has begun and the amyloplasts contain pin-point starch. (*m*) Almost spherical GC in a grain at the same stage as that of figure 2*l*. (*n*) Surface view of the peripheral cytoplasm overlying the residual vacuole during the main period of starch synthesis. Amyloplasts with rodlet starch; the minor inclusions identifiable as mainly lipid globuli and mitochondria. (*o*) VC surface at the apertural pole of a maturing grain. The aperture is the focus of conspicuous radiating strands which extend over the congested amyloplast mass. The state of the actin cytoskeleton at this stage is seen in figure 7*g*. (*p*) Cytoplasm in the distal hemisphere of a maturing grain. The main inhabitants of this region are the P-particles and the VN, the latter having returned to the distal hemisphere from its site near the aperture.

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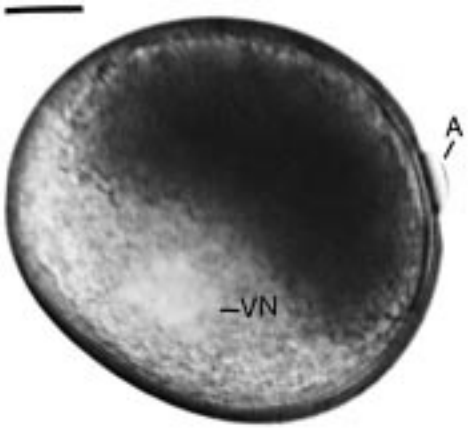

Figure 3. Single mature pollen grain, GDA fixation, I/KI staining for starch. The movement of the amyloplast mass into the vicinity of the aperture (A) described by Watanabe (1961) is complete, and the VN has moved into the dense P-particle population in the distal hemisphere. Scale bar, 10 μm.

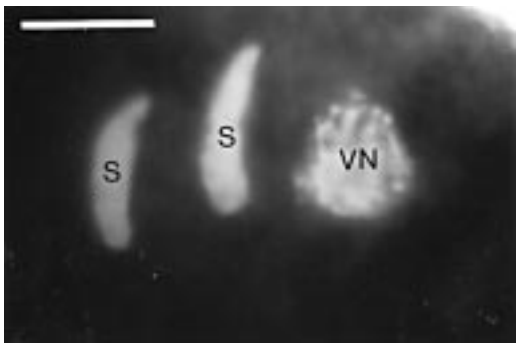

Figure 4. The VN and the nuclei of isolated male gametes (S) in a protoplast released from a pollen grain nearing maturity; DAPI staining for DNA. The VN has contracted from the size illustrated in figure 2*l* and its envelope has collapsed into deep folds. Scale bar, 15 μm.

can be taken to indicate that actin is likely to be present already in the uninucleate spore, if in minor amount, before the subsequent accumulation following pollen mitosis. This would be in accord with the fact that organelles show evidence of movement in living wheat microspores before the division.

2. MATERIALS AND METHODS

The observations were made on field-grown wheat (*Triticum aestivum* L.). Anthers were dissected from developing florets, and the state of the contents established by differential interference (DIC) microscopy, or by staining the nuclei with the DNA-specific fluorochrome, 4,6-diamidino-2-phenylindole (DAPI).

Intracellular motility was followed in samples mounted in Whitmore oil (BDH) in glass cells on microscope slides, or in some instances in a growth medium (GM) containing 1 mM $Ca(NO_3)_2$ and 1 mM H_3BO_3 at pH 6.8, with a sucrose concentration adjusted empirically to produce minimal distortion of the suspended cells. Continuous video records were made from the living preparations, and selected sequences were recorded photographically from the monitor screen with an automatic camera.

F-actin was localized with fluorochrome-labelled phalloidin using a non-fixation direct permeabilization technique. Samples of anther contents at the required stages were dispersed evenly in GM with the appropriate sucrose concentration on poly-L-lysine-coated slides, drained completely and then flooded with 0.05 M phosphate buffer at pH 7.0 containing 10% DMSO and 1 μg ml^{-1} phalloidin labelled with tetramethyl-rhodamine B isothiocyanate (Tr-Ph; Sigma), and exposed immediately to microwave radiation for 8–10 s in a 600 W oven. The staining medium was then withdrawn and the samples washed twice with buffer before being flooded with dilute Citifluor for observation. Where necessary the excitation field in the microscope was restricted to reduce flare.

Starch-containing amyloplasts were identified by polarization microscopy or by I/KI staining (0.2% iodine in 2% potassium iodide) lipid globuli with scarlet R saturated in 50% ethanol, and callose with aniline blue at 0.05% decolorized at pH 11 (DAB) as a fluorochrome.

The actin inhibitor cytochalasin D (CD) was dissolved in dimethyl sulphoxide (DMSO) at a concentration of 1 mg ml^{-1}, and diluted for use with GM containing 20% sucrose to give a concentration of 5 μg ml^{-1}. Pollen samples suspended in GM in glass cells on microscope slides were perfused with this CD-containing medium, and the effects recorded over a period of 4 min. Recovery was then induced by draining off the medium and perfusing twice with fresh GM while the sample was under continuous observation. DMSO in a concentration corresponding to that in the final CD medium had no effect on motility in the VC.

3. RESULTS

(*a*) *The developmental sequence*

Romanov (1966, 1971), who published the first accounts of the post-microspore development of wheat pollen to be based upon direct observation of living material, traced and illustrated the sequence of events from the newly released spore to the period of vacuole resorption and the beginning of starch accumulation in the amyloplasts. His observations revealed a previously unsuspected fact, namely that after the separation of the VN and GC following pollen mitosis these bodies undergo a series of regular migrations within the VC. He reported similar observations from *Secale cereale* (rye) and *Avena sativa* (oats), and a corresponding sequence was described by Oryol (1969) from *Zea mays* (maize). Fragmentary reports from other grass species (for example, *Sorghum bicolor* (sorghum) by Hsu & Peterson (1981)) suggest that the spatial rearrangements may be characteristic for grasses in general. The events following the breakup of the meiotic tetrads up to

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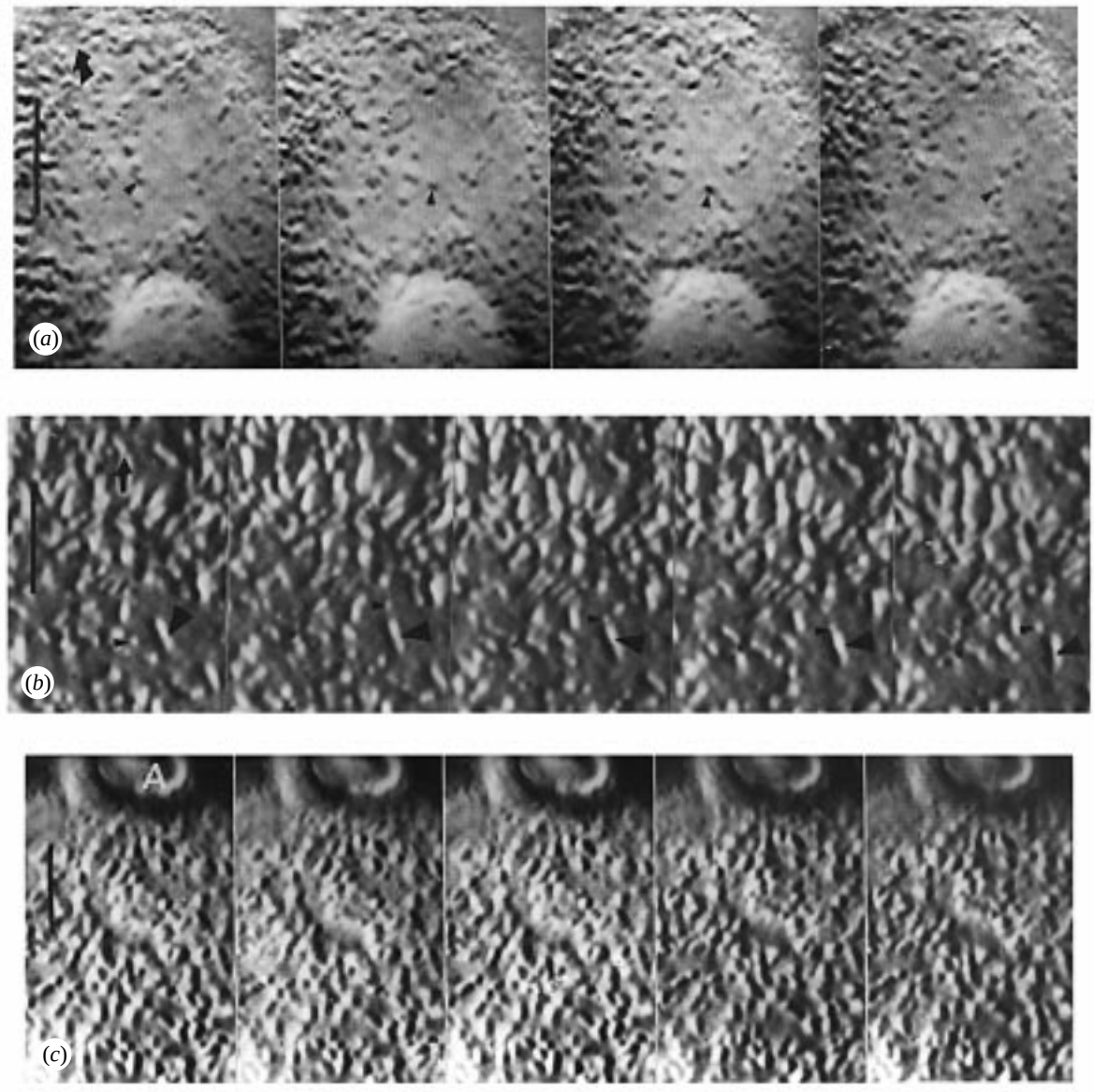

Figure 5. Video sequences of moving organelles in the VC. (*a*) Surface of the peripheral cytoplasmic layer of a vacuolated VC at a stage corresponding to that of figure 2*n*. The large arrow indicates the direction of the aperture. Interval between frames, 0.8 s. The spherical GC is seen out of the focal plane at the bottom of each frame. The body moving to the right in the sequence (small arrow heads) is probably a lipid inclusion; over the distance traversed it achieved a rate of about 2.8 μm s^{-1}. Scale bar, 15 μm. (*b*) Video sequence of orienting amyloplasts after the main period of starch accumulation following the resorption of the VC vacuole. The large arrow indicates direction of the aperture. Interval between frames 3 s. An example of quite rapid reorientation can be seen in the pair of amyloplasts lying end-to-end (large arrow heads). A smaller body (small arrow heads), possibly a lipid globulus, moves erratically, first towards the end of the pair of amyloplasts, and then away again. Scale bar, 15 μm. (*c*) Video sequence of the amyloplast population seen through the pollen grain wall in the vicinity of the aperture (A) in a pollen grain approaching the state seen in figure 3. The amyloplasts are undergoing slow jostling movement, with minor inclusions moving erratically between them. Interval between frames, 3 s. Scale bar, 10 μm.

the mature germinable pollen grain of wheat are summarized diagramatically in figure 1, and illustrated in figure 2 from living material. Pollen mitosis occurs after the migration of the spore nucleus to the distal pole (figures 2*a–e*), and the subsequent events follow the normal pattern for early development of the angiosperm gametophyte, with the formation of a callose wall setting off the generative cell in contact with the inner surface of the intine (figures 2*f*, *g*). Figures 2*h–k* illustrate the migration of the VN through the peripheral layer of cytoplasm as described by Romanov (1971). The electron micrographs of El-Ghazaly & Jensen (1986*a*, *b*) show that the principal particulate inclusions in the early vacuolate period are mitochondria ranging from 0.5 to 1.25 μm in length, and occasional larger lipid globuli. Endoplasmic reticulum and vesiculate bodies, possibly undifferentiated plastids, are also present. By the stage illustrated in figure 2*k*, small starch grains feature abundantly in the thickening cytoplasmic layer. Throughout its passage, the VN remains in close contact with the pollen wall, settling eventually near the

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Table 1. *Response of pollen of wheat at a stage corresponding approximately to that in the video sequence of figure 5b to the actin inhibitor cytochalasin D at* 5 μg ml^{-1} *in GM*

	state of VC cytoplasm and nucleus
time[a]	
1 min 20 s	vectorial movement of organelles arrested; Brownian movement still evident
1 min 30 s	cytoplasm coagulating to form amorphous local masses; organelles randomly dispersed
3 min 45 s	VN contracted into an irregular sphere
recovery[b]	
20 min	minor inclusions exhibiting local movement
40 min	sluggish movement throughout the VC cytoplasm
3 h	local cytoplasm aggregates dispersed; active movement of minor inclusions; amyloplasts moving slowly, but still scattered

[a]After transfer to CD medium.
[b]In GM after transfer at 4 min.

proximal pole, usually within 15° of the shaft of the aperture. The two nuclei differentiate after the isolation of the GC, the GN remaining condensed in the telophase state with the VN progressively enlarging (figure 2*l*).

Eventually the GC withdraws from intimate contact with the intine and loses its callose wall (figure 2*m*) before itself migrating along the wall to the proximal pole of the grain to join the VN. The subsequent progressive resorption of the central vacuole of the VC is accompanied by an increase in the amyloplast population with enlargement of each as starch grains accumulate (figure 2*n*). As the grain matures, the amyloplasts begin to migrate towards the apertural pole. The GC elongates as it moves into the amyloplast mass (figure 1*h*), where it assumes a spindle shape before dividing (Korobova 1974). Later, prominent strands develop at the surface of the VC cytoplasm, focused upon the aperture site (figure 2*o*). Figure 2*p* shows that the VN, now much contracted with its bounding envelope collapsed into folds, has moved back into the distal domain of the grain, where it lies among the abundant population of P-particles undergoing constant amoeboid shape changes (Heslop-Harrison & Heslop-Harrison 1992*b*). I/KI staining illustrates more clearly the polarized disposition of the VC contents in a germinable grain (figure 3). The condensed VN and the nuclei of the two male gametes are illustrated in figure 4, from a mechanically isolated wheat VC protoplast after DAPI staining.

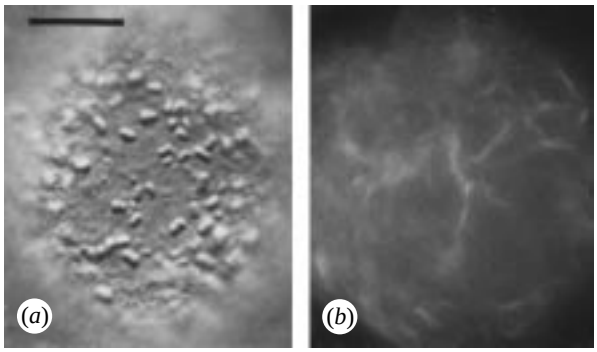

Figure 6. Surface views of the peripheral cytoplasmic layer of vacuolated VCs at the stage comparable with that of the video sequence of figure 5*a*. (*a*) DIC micrograph of a living grain showing amyloplasts and minor inclusions in active movement at the time of the exposure. (*b*) The sparse population of actin fibrils in the corresponding layer of a grain. Tr-Ph staining; preparation as in the text. Scale bar, 10 μm.

(*b*) *Intracellular movement*

The video sequence of figure 5*a* illustrates the movement of organelles in the peripheral layer of cytoplasm in a vacuolated grain comparable with that of figure 2*n*. Minor inclusions identifiable as mitochondria and lipid globuli move relatively rapidly—sometimes erratically, but occasionally more consistently—along pathways of several microns. The amyloplasts with developing starch show little evidence of sustained movement at this time, nor of any preferred orientation. The first evidence of directional movement appears within the amyloplast mass following the main period of starch accumulation (figure 5*b*). In this sequence the long axes of some 50% of the amyloplasts are oriented towards the aperture within 20°, and a further 40% within 40°. The directional movement ceases as the grain matures, and movement within the amyloplast mass of the grain in the germinable state is restricted and random (figure 5*c*).

(*c*) *The actin cytoskeleton*

Because of the osmotic sensitivity of the highly vacuolate VC, satisfactory images of the distribution of actin could not be obtained from earlier stages than that illustrated in figure 2*n*. The cytoplasmic layer at the surface of a VC at this time is shown in figure 6*a*, and the disposition of actin fibrils in a similar VC in figure 6*b*. The fibrils are tenuous and sparse, but several in the thin surface layer of cytoplasm are continuous over many microns in the paramural plane, suggesting that they could provide pathways for consistent organelle movement over distances at least as great as that defined by the arrowheads in figure 5*a*.

During the increase of starch in the amyloplasts after the resorption of the vacuole, actin appears not only in occasional fibrils but in dense local accumulations, up to five in number, near the surface of the VC (figure 7*a*). Figure 7*b* shows three such

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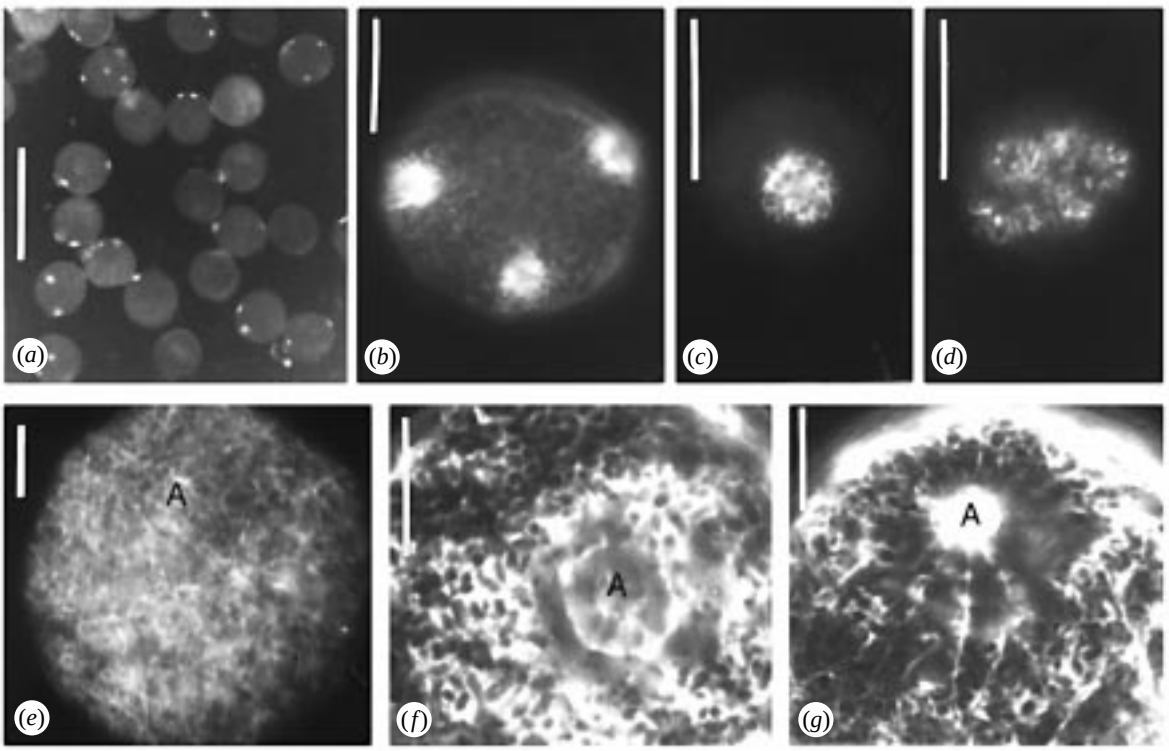

Figure 7. Development af the actin cytoskeleton in the VC; Tr-Ph staining. Preparation as in the text. (*a*) Population of grains released from the anther during the early period of starch accumulation in the amyloplasts. At this stage of development dense local actin aggregates, 1–4 in number, are present in virtually every grain. Scale bar, 100 μm. (*b*) Single grain with three aggregates. There is little indication of actin fibrils in the main amyloplast mass at this time. Scale bar, 20 μm. (*c*) Detail of a single aggregate, with evidence of short radiating fibrils at the periphery. Restricted field excitation. Scale bar, 20 μm. (*d*) Actin aggregate during dispersal. Restricted field excitation. Scale bar, 20 μm. (*e*) Later stage; actin fibrils distributed throughout the VC; (A), aperture site. Scale bar, 10 μm. (*f*) Surface of the cytoplasm at the proximal pole of a maturing grain. Dense actin accumulations encase the amyloplasts and surround the aperture site (A). Scale bar, 15 μm. (*g*) Grain approaching maturity. In the vicinity of the aperture (A) actin is now distributed in radiating fibrils in the cytoplasm over the surface of the amyloplast mass (cf. figure 2*o*). Scale bar, 15 μm.

centres in a single grain, and a detail of one appears in figure 7*c*. This state is transient, the condensed local concentrations passing first into a dispersal phase (figure 7*d*), and then into a period when actin fibrils appear throughout the amyloplast mass, at first in a loose entanglement with no special orientation towards the aperture (figure 7*e*). Later, actin in a seemingly condensed state interpenetrates the whole mass, concentrated especially at the proximal pole (figure 7*f*). As the grain matures, radiating actin fibrils focused upon the aperture develop, with the aperture itself defined by a local concentration in which no detail can be distinguished with the present technique (figure 7*g*). A corresponding system of radiating strands focused upon the aperture is visible with DIC microscopy in the living VC (figure 2*o*). This is the situation in the germinable grain at the time of dispersal (Heslop-Harrison & Heslop-Harrison 1992*b*).

During the translation of the actin cytoskeleton into the state seen in figure 7*g*, the first indications appear of the orientation and directional movement in the amyloplast population illustrated in the video sequence of figure 5*b*. Such an apparently coordinated migration throughout the amyloplast mass could result from interaction between individual myosin-coated amyloplasts and oriented elements of the actin system, and this interpretation receives support from the response to CD (table 1). All movement in the VC was arrested in the CD medium, with the cytoplasm coagulating to form amorphous masses and the organelles, including the amyloplasts, dispersing randomly (figure 8*a*). Movement was resumed after the leaching out of the CD medium, indicating a restoration of an actin system; but significantly the coordinated migration was not resumed (figure 8*b*).

4. DISCUSSION

As in other monocotyledons (Heslop-Harrison 1971), the site of the aperture of the wheat pollen grain is determined initially by the spindle dispositions and cleavage planes at the two successive meiotic divisions which produce the tetrad of spores (Dover 1972). On release, from the tetrad the spores separate and rotate so as to place the apertures of each against the anther tapetum. Here they form a single rank surrounding a central space in each theca (Romanov 1966), an orientation presumably facilitating the transfer of water, nutrients and possibly

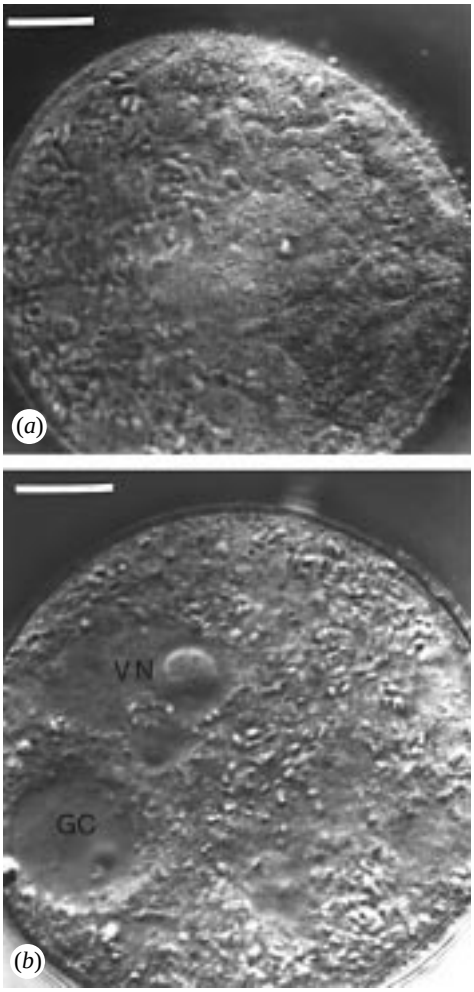

Figure 8. Effect of CD on motility in the VC in maturing pollen grains. (*a*) Movement arrested after 4 min in GM with 5 μg ml^{-1} CD; cytoplasm coagulated locally in amorphous masses. (*b*) Recovery state, 3 h after return to GM. Movement of the amyloplasts and minor inclusions has been restored, but the VC content remains disorganized. Detailed timing in table 1. Scale bar, 10 μm.

more specific determinants from the tapetum into the developing grains. Vacuolation follows rapidly upon the release of the spores, and it is likely that the passage of water into each spore, if it were principally through the aperture, could be a factor in the displacement of the spore nucleus towards the distal pole. This event is critical in that it establishes the polarization later evinced in the nuclear and cell migrations within the VC.

This polarization is first expressed after the division of the spore nucleus, and is maintained during the subsequent movements illustrated in figures 1 and 2. The consistency of these migrations among grass species suggests that they have some adaptive significance, but what this might be remains obscure. Equally enigmatic as yet is the mechanism whereby the movements of the VN and GC are driven. After pollen germination, the placing of VN and GC in the growing tube involves interaction of surface myosin with a linearly disposed system of actin fibrils (Heslop-Harrison & Heslop-Harrison 1989*b*), and an interaction of the same kind may well drive the movements during early gametophyte development. If so, actin must be present in the VC in the form of oriented fibrils demarcating the migration pathways of the VN and GC. The involvement of microtubules seems excluded, since these have never been reported from the VC at this stage.

Watanabe (1961) showed that the redistribution of the VC contents during final maturation of the grain ending in the accumulation of the amyloplasts at the apertural pole (figure 3) is a prerequisite for germination of wheat pollen. As already noted, such a coordinated movement also predicates the presence of an ubiquitous system of polarized actin fibrils in the cytoplasm of the VC, and the fact that the migration is halted and the amyloplast mass randomized by CD treatment (figure 8*a*) supports this interpretation. Again, a technique with greater resolution than that used here would be needed to reveal such a system.

At the beginning of the final period of actin accumulation illustrated in the sequence of figure 7, the VN reaches its maximum size (figure 2*l*), with a massive nucleolus and diffuse DNA (not illustrated). The analogy of *Tradescantia* (Stinson *et al.* 1987) indicates that this would be the interval during which actin mRNA would reach a peak in the VC. Figures 7*a–c* show that the new actin synthesis is initially concentrated in a small number of sites scattered around the periphery of the VC. Thereafter actin is dispersed throughout the cytoplasm before the final establishment of the radiating system of fibrils focused on the aperture which marks the attainment of germinability. The cessation of actin accumulation is accompanied by a shrinkage of the VN, the envelope of which falls into deep folds, a state maintained until reactivation at the onset of pollen tube growth. Notably, the shrinkage of the VN coincides with the period when, from analogy with the findings of Stinson *et al.* (1987), transcription would decline.

While the adaptations described here are undoubtedly shared with other grasses, many would seem to have special significance for the general biology of short-lived grasses like wheat, where there is a premium on rapid and precisely timed reproduction (Heslop-Harrison 1979). To this the structural and functional characteristics seen in male gametophyte development, including the early formation of sperms, undoubtedly contribute. The initial interchanges between pollen and stigma—including water transfer—are extremely rapid; and the preadaptation of the pollen means that at the critical level of hydration germination can take place immediately without extensive reorganization of the VC. This is followed by an exceptionally rapid rate of pollen tube growth and early fertilization. Synchroneity in a whole population with timing linked to environmental conditions would be essential for the success of opportunistic, invasive grass species of emphemeral habitats from which many modern cereals have been derived; and it may be supposed that human selection will have refined and stabilized each of the developmental processes concerned.

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