A Gene Expressed during Sexual and Asexual Sporulation in Phytophthora infestans Is a Member of the Puf Family of Translational Regulators

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Mating, sexual sporulation, and asexual sporulation are central to the life histories of many lower eukaryotes. Mating is important because this increases genetic fitness and diversity and because sexual spores are often thick walled and thus more durable than vegetative structures. Also, both sexual and asexual spores are the main agents of dispersal for many species. Detailed molecular analyses of these pathways have been executed only for selected groups, particularly the cellular slime molds, true fungi such as ascomycetes and basidiomycetes, and green algae (2, 5, 10, 13, 31). Very limited information is available for most other lower eukaryotes, including oomycetes.

Most oomycetes, which include important parasites of plants and animals, undergo both sexual and asexual sporulation. Despite their generally filamentous, fungus-like pattern of growth, oomycetes are diploid and share a close taxonomic relationship with chrysophytes, diatoms, and brown algae rather than with true fungi (3). One of the best-known oomycetes is Phytophthora infestans, which caused the Irish potato famine in the 1840s. The late blight diseases caused by P. infestans continue to have devastating effects on agricultural production and threaten food security (12).

The physiology of sporulation in oomycetes is well described, but little is known at a molecular level. In heterothallic Phytophthora, sexual development occurs in response to hormonal interactions between A1 and A2 mating types (4, 16, 19). Male and female gametangia termed antheridia and oogonia form, and then oogonia penetrate antheridia and develop into oospores. Meiosis occurs in both gametangia, after which haploid antheridial and oogonial nuclei fuse to form a zygote within the developing oospore. Cross-walls appear, which delimit gametangia from the normally aseptate hyphae, and the oospore becomes dormant.

The induction of mating also arrests asexual sporulation, which occurs in nonmating aerial hyphae upon aging or nutrient limitation (11). This involves the differentiation of hyphal tips into sporangiophores (16, 33). Terminal swellings then form, which become the sporangia, each containing several diploid nuclei. Mitosis stops within sporangia, even though their cytoplasm remains active and undesiccated, unlike the conidia of true fungi (16). Germination usually involves cleavage of each sporangium into 6 to 12 mononucleate, motile zoospores which later encyst and extend germ tubes that transition into hyphae (15).

To enhance our understanding of sexual development, cDNAs identifying eight genes that are up-regulated during mating were isolated and partially characterized (11). Three encoded potential RNA-interacting proteins, suggesting that stabilization or degradation of RNA participates in the transition from hyphae to sexual spores. This paper focuses on one of those genes, M90, which encodes a member of the Puf family of developmental regulators that bind and inhibit translation of specific mRNAs (21, 25, 35, 36, 40). Presented are details of the structure and activity of M90, including its spatial and temporal pattern of expression during development as discerned by using a fusion between its promoter and the β-glucuronidase (GUS) reporter gene. M90 was induced early in sexual development in both male and female gametangia. It was also induced at an early stage of development of asexual spores, which was unexpected since the two spore types are structurally and biochemically distinct (15). Interestingly, during the preparation of this paper it was reported that a Puf gene was also expressed in zoospore cysts of another oomycete,
Saprolegnia parasitica (1). Although the gene was induced in a different developmental stage in S. parasitica than in P. infestans, it appears that this family of RNA-binding proteins may play a universal role in oomycete sporulation.

**MATERIALS AND METHODS**

**Strains and culture conditions.** P. infestans isolates 1306 (A1 mating type, United States), 8811 (A1, United Kingdom), 618 (A2, Mexico), 88069 (A1, The Netherlands), and 6.11 (self-fertile [11]) and their transformants were routinely cultured on rye A agar (6) at 18°C. Some cultures employed rye A broth (rye A clarified by centrifugation at 5,000 × g) or defined medium (38).

**Characterization of gene structure.** M90 was identified as a short cDNA, later found to represent nucleotide (nt) 2495 to 2879 of the primary transcript, from a subtraction library enriched for mating-specific genes (11). Genomic sequences were obtained from a B. lactucae isolate from a polycarbonate membranes (0.4-μm pore size), upon which parallel strips of A1 and A2 inocula were placed separated by 2 cm. Oospores were from 22-day mating cultures, which were homogenized five times for 2 min each in 50 ml of extraction buffer at 4°C in a Brinkman Polytron (speed 7) and passed through a 100-μm nylon mesh. The oospores, which remained intact after this treatment, were concentrated by centrifugation for 10 min at 2,000 × g for three times with extraction buffer, resuspended in 0.3 ml, and ground in liquid nitrogen prior to RNA extraction. RNA from a self-fertile strain was obtained by using the poly-carbonate procedure or rye broth cultures.

Nominating RNA was extracted from single isolates grown on polycarbonate cultures, which supported the development of asexual spores. Nonsporulating, submerged cultures were from 4-day rye broth cultures inoculated with sporangia. Carbon- and nitrogen-starved cultures were obtained by passing hyphal mats from 3-day rye broth cultures through two water washes, followed by 15 h of incubation in defined medium lacking glucose or (NH₄)₂SO₄. Sporangia were isolated from 6-day polycarbonate cultures by vortexing the hyphal mats in water, purifying the sporangia through a 70-μm nylon mesh, and concentrating the filtrate by centrifugation at 1,000 × g for 5 min. Directly germinated sporangia were prepared after incubation at 18°C for 10 h in half-strength rye broth, followed by centrifugation at 1,000 × g. Zoospores were prepared by incubating sporangia in water at 10°C for 4 h, followed by passage through a 15-μm nylon mesh and concentration of the filtrate by centrifugation at 400 × g at 4°C for 5 min. Germinated zoospore cysts were obtained by vortexing zoospores in 1 mM CaCl₂ for 30 s, followed by incubation for 4 h in half-strength rye broth at 18°C and centrifugation at 1,000 × g.

**Blot analysis.** DNA blotting was performed with ethidium bromide-stained total RNA and 1.2% agarose–6.6% formaldehyde gels (27). The RNA was transferred to nylon membranes by capillary blotting in 10× SSPE (1.8 M NaCl, 0.1 M NaHPO₄, 0.01 M EDTA [pH 7.7]) and fixed by UV cross-linking. DNA blotting was with 0.8% agarose gels in 1× TBE (89 mM Tris, 89 mM H₂BO₃, 2 mM EDTA) and alkaline blotting to nylon membranes.
Hybridizations were performed with 32P-labeled randomly primed probes (18). Probes were made during either a KpnI-XbaI fragment of the M90 gene (nt 208 to 3362) (Fig. 1), which were generated as a genomic actin clone (actA), or cDNA clones for elongation factor-1 (Ef1) or 28S rRNA. Signals were detected by phosphorimager analysis and quantified by using the Quantity One software for Macintosh (Bio-Rad, Richmond, Calif.). High-stringency washes were in 0.2% SSPE–0.2% (wt/vol) sodium dodecyl sulfate–0.1% (wt/vol) sodium pyrophosphate at 65°C. Low-stringency washes were in 1× SSPE–0.2% sodium dodecyl sulfate–0.1% sodium pyrophosphate at 55°C.

RESULTS

M90 is expressed as a spliced transcript. Starting from a short cDNA fragment identified by suppression subtractive hybridization-PCR (11), the structure of M90 was determined by analyzing a genomic clone plus products of RT-PCR, 5′ RACE, and 3′ RACE (Fig. 1). The primary transcript of the gene was 3,069 nt and included introns of 75 and 70 nt at positions 514 and 645, respectively. The two introns had 5′ splice junctions of AG/GTACTT and TG/GTACGT and 3′ junctions of CAG/G and TAG/G, respectively. These resemble the consensus from 55 other P. infestans introns, which were recorded as A2G6/G1T1G100A4G60G10 for the donor sites and C58A100G100T100A66A44G86T60 for the acceptor sites (H. S. Judelson, unpublished results). The sizes of the M90 introns were similar to those of introns previously examined.

A search of the promoter for potential transcription signals identified, between nt −68 and −50, a sequence matching the GCTCATTYYNCAATTT element present in several other oomycete genes (Fig. 1A) (30). No matches to transcription factor sites in the Proscan and TRES databases were detected, although it should be noted that no such sequences have yet been defined in oomycetes. An analysis of the M90 promoter and coding region along with two other mating-induced genes by using the Oligo Analysis, Dyad Detector, and Gibbs Motif Sampler programs did not reveal significantly overrepresented motifs that might represent transcription factor binding sites.

M90 encodes a member of the Puf protein family. An open reading frame corresponding to 875 amino acids, which encoded a 97.5-kDa protein, was detected within the M90 transcript. Two in-frame methionine codons were present at +30 and +48 in the contexts of TCCATGG and TCGATGA, respectively. The former is suggested to be the predominant start site, since it is a better match to the ATG sequence that is optimal for translational initiation in eukaryotes (20).

The predicted protein closely matches the Puf family of proteins, which was initially defined by Pumilio of D. melanogaster (25) and FBF of C. elegans (21). These proteins bind the 3′ untranslated regions of target mRNAs, resulting in translational repression (39, 40). Each Puf protein contains a highly conserved RNA-binding region named Pum-HD (Pumilio homology domain) flanked by nonconserved sequences. These features are conserved in the P. infestans protein (Fig. 1B and 2). In P. infestans, Pum-HD resides between amino acids 513 and 868, contains the standard eight RNA-binding motifs, and is flanked by two other sequences (Csp1 and Csp2) found in most Puf proteins. The eight repeated motifs show the consensus II.LLM.D.YGNNRIQKL.EH.A, where upper- and lowercase letters represent amino acids that are identical and conserved in at least half of the repeats, respectively, and periods represent nonconserved residues.

Within Pum-HD, the amino acid identity of the M90 protein versus others ranged from a high of 51% in the case of S. parasitica Puf1 to 28% for C. elegans FBF2. The proteins showed no obvious similarities outside this domain, except for a short region with the consensus (S/T)L/(IV)/E(D/R)IQEDF PRTSPS(V/E)(Y/F)(G/S)(Q/Y) starting at amino acids 261 of M90, 180 of S. parasitica Puf1, and 202 of the P. tremula protein. An alanine- and glutamine-rich region found N terminal to Pum-HD in several Puf proteins (37) was absent in the M90 protein and its relatives from S. parasitica and P. tremula.

Pumilio is represented by a single-copy gene in P. infestans. Most organisms produce multiple Puf-like proteins from either diverged members of small gene families or isoforms from a single gene (28, 29, 37, 40), although there are exceptions (1). To test whether multiple M90-like sequences were in P. infestans, blot analysis was performed at low stringency against genomic DNA digested with SacI, EcoRV, or PstI (Fig. 3). Accounting for the digestion of some of the enzymes within the gene, the data indicated that a single Puf-like gene was present.

M90 RNA is induced during mating and concentrates in oospores. Previously (11), the accumulation of M90 RNA was measured at 3 and 7 days after cultures were inoculated with parallel strips of A1 and A2 strains on rye agar, as diagrammed in the upper left corner of Fig. 4. After 3 to 4 days, when parental hyphae were just starting to meet and form hyphal swellings in the zone between the A1 and A2 inocula, M90 RNA was not detected. A strong signal was detected after 7 days, when young oospores were present (11).

To further explore the expression of M90, a more extensive study was performed, involving RNA from purified oospores, different positions in the mating culture, and older cultures. In time courses, the concentration of oospores peaks at 10 to 13 days, after which their number stabilizes, they continue to mature and become dormant, and the surrounding hyphae vacuolate. In a 4- to 11-day experiment, the M90 signal was barely detectable at 4 days, was moderate at 7 days, and was strong at 11 days (Fig. 4A). In an experiment that extended to 16 days, M90 RNA peaked by 13 days (Fig. 4B). The highest level was detected in purified oospores, based on standardization to Ef1. A slightly lower level of M90 RNA could be calculated for oospores if actin mRNA was used for standardization.

M90 was also expressed in a self-fertile (homothallic) strain of P. infestans grown on agar medium, a condition conducive to...
oo sporogenesis (Fig. 4B, lane SF-Oo+). M90 RNA was less abundant in the self-fertile strain than in normal interisolate matings, which was consistent with observations that the self-fertile strain produced fewer oospores. That transcript accumulation in the self-fertile strain, as well as in normal matings, was due to oospore formation and not to aging was demonstrated by the absence of M90 RNA in a 15-day culture of the self-fertile strain maintained under submerged conditions, which suppress oospore formation (Fig. 4B, lane SF-Oo-H11002).

Experiments were also performed to compare M90 RNA levels in the zone between the A1 and A2 parents with those in the region external to the inoculum strips, where few oospores develop. One initial reason for doing this was to test whether diffusion of mating hormones through the culture affected gene expression. M90 RNA was easily detected in the region external to the mating zone, even though no oospores had formed (Fig. 4B, OM "outside mating" lanes). Although explanations for this included the transduction of a mating signal through the medium or hyphal cytoplasm, subsequent experiments indicated that the gene was induced during asexual sporulation.

M90 is also transcribed during asexual sporulation. While asexual sporulation is prevented within regions of cultures showing a mating response (11), asexual spores will form in the outside-mating region after 4 to 5 days, as is also the case in single-isolate cultures. That the M90 signal in the nonmating region was due to asexual sporulation was suggested by the detection of transcripts in 15-day cultures of the A1 and A2 parents grown separately, in which asexual sporulation had occurred (Fig. 4B, lanes A1Sp and A2Sp).

**FIG. 2.** Relationship of the predicted M90 protein to other members of the Puf family. (A) Comparison of 12 Puf proteins and similarity within the RNA-binding domain. The full-length sequence is represented by the open box, black boxes represent the Pum-HD motifs, and grey boxes represent the Csp1 and Csp2 regions. The percent identities to M90 within the numbered region are shown above the respective sequences. For example, amino acids 425 to 766 of *S. parasitica* Puf1 show 51% identity to the amino acids 515 to 868 of M90. Accession numbers are listed in Materials and Methods. (B) Amino acid alignment of the eight Pum-HDs (R1 to R8) and two Csp domains (Csp1 and Csp2) in Puf proteins. Amino acids that are identical or conserved in ≥50% of the sequences are shown by black or grey shading, respectively.
**M90 is expressed in male and female structures during mating.** A fusion between the M90 promoter and the GUS reporter gene was expressed in transformants to address the spatial and temporal patterns of M90 expression. This was expected to be informative, since sexual development occurs asynchronously and thus extrapolation of the developmental stage(s) of expression from RNA blot data can be challenging. For example, after 8 days a mating culture contains a mixture of structures, including A1 hyphae not yet responding to the A2 and vice versa, hyphae swelling in response to mating hormones, young and old antheridia and oogonia (including those in pre- and postmeiotic stages), young oospores containing zygotic nuclei, nearly mature oospores, and hyphae connected to mating structures. Also, GUS fusions would provide data on M90 expression independent of the RNA blot analysis, in which some ambiguity resulted from the observation that normalization standards such as actin and Ef1 varied during different stages of growth and development.

To learn where M90 transcripts accumulate, DNA from nt −1345 to +31 relative to the transcription start site was fused to GUS in a G418 resistance-conferring vector and transformed into A1 and A2 isolates. Transformants and nontransgenic partners were then mated in combinations in which GUS was predicted to be expressed only in male or female structures, due to the system of sexual preference (relative sexual-activity) in *Phytophthora* (17). For example, A2 isolate 618 forms only male structures when coupled with A1 strain 1306 but acts female when crossed with 8811. Preliminary analyses of transformants in single cultures and mating reactions indicated that the 1.38-kb promoter fragment conferred the expected pattern of expression: GUS was present during mating but not in vegetative hyphae. As described below, GUS was also expressed during asexual sporulation.

The pattern of GUS staining during mating indicated that M90 accumulation began early during both male and female development (Fig. 6). In crosses of GUS-positive 8811 (male) with GUS-negative 618 (female), strong staining was observed in hyphal swellings representing antheridial primordia prior being penetrated by oogonia (Fig. 6A). It was not always obvious that such swellings were going to develop into antheridia, but this was predicted since GUS was being expressed only by the male strain. Occasionally some blue coloration was noted in adjacent hyphae, but this was probably due to leakage of the cleaved GUS substrate. At a more advanced phase of sexual development, staining was observed in antheridia attached to oogonia (Fig. 6B and C). In some instances a short hyphal swelling attached to antheridia also stained (Fig. 6D). This likely represents an intermediate stage in development preceding the completion of cytoplasmic migration into the antheridium, rather than leakage of GUS, since stained hyphae were never observed adjacent to more mature oogonia or oospores.

From pairings between GUS-positive 1306 [female] and GUS-negative 618 [male], it was evident that M90 also accumulates early in female development. Figure 6E illustrates a stained young oogonium that just recently penetrated an antheridium. It was more challenging to detect young female structures than young antheridia, suggesting that oogonia quickly penetrate nascent antheridia and then swell. At later stages (Fig. 6F to H), the majority of GUS activity was detected...
in fully expanded oogonia, i.e., those containing the developing oospore. Some activity was frequently detected in the hyphal donors of oogonia (Fig. 6G), which resembled observations described above for antheridia. This was not observed in the more mature mating structures (Fig. 6H), which suggested that such GUS-staining hyphal cytoplasm normally moves into oogonia during maturation.

GUS staining was not detected in mating cultures in hyphae that were unattached to antheridia or gametangia, indicating that M90 expression is narrowly localized to regions undergoing sexual differentiation (Fig. 6G), which resembled observations described above for antheridia. This was not observed in the more mature mating structures (Fig. 6H), which suggested that such GUS-staining hyphal cytoplasm normally moves into oogonia during maturation.

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The M90 promoter is active during asexual sporulation. The GUS-expressing strains were also used to study M90 expression during asexual sporulation (Fig. 6I to M). This revealed that the gene was induced early in the pathway. As described previously (24), sporulation begins when a fraction of hyphal tips evolve into sporangiophores, which are often branched. Apical swellings then develop, cytoplasm and nuclei flow into the swellings, and basal septa form between the sporangiophore and each sporangium. These stages are evident in Fig. 6I, which portrays a hyphal tip presumably developing into a sporangiophore; in Fig. 6J, which shows GUS-staining cytoplasm in both the sporangiophore and young sporangia; in Fig. 6K, where GUS activity is restricted to the sporangia after septation; and in Fig. 6L, which shows mature sporangia detached from the sporangiophore. It was interesting that there was an unstained region near the base of a nearly mature sporangium (Fig. 6J, left). This represents an incipient region of vacuolization, which, along with septation, occurs at the time sporangia become fully developed. Staining was also observed in zoospores released from sporangia (not shown).

M90 (GUS) accumulation appears to be specific to hyphae undergoing sporulation, as regions of aerial cultures not yet

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**FIG. 4.** RNA blot analysis of M90 during mating. The diagram in the upper left corner illustrates the setup of mating reactions between isolates 8811 and 618, labeled with the positions of A1 and A2 inocula, the mating zone (M), and the region outside the mating zone (OM). Six micrograms of total RNA was electrophoresed, blotted, hybridized, and washed at high stringency as described in Materials and Methods. A photograph of the ethidium bromide (EtBr)-stained RNA and hybridization signals against probes for M90, Ef1, actin, and 28S rRNA are shown. (A) RNA from the mating zones of cultures after 4, 7, and 11 days (lanes M4, M7, and M11, respectively). Shown at the bottom are the ratios of M90 signal intensity to Ef1, actin, or rRNA; values are standardized to M11, which was set at 100. (B) RNAs from a separate experiment from the mating zone after 5, 8, 13, and 16 days (lanes M5, M8, M13, and M16, respectively) and outside the mating zone after 5, 8, 13, and 16 days (lanes OM5, OM8, OM13, and OM16, respectively). Also tested were RNAs from purified 8811 × 618 oospores (lane Oo), self-fertile strain 6.11 forming oospores on a polycarbonate membrane culture (lane SF-Oo+), 6.11 from submerged cultures where oospores were not formed (lane SF-Oo−), and individual 15-day cultures of 8811 and 618 producing asexual sporangia (lanes A1Sp and A2Sp, respectively). M90/Ef1, M90/28S, and M90/actin ratios are shown at the bottom, standardized to the maximum ratio, which was set at 100.
plants, animals, and true fungi, including species in which Puf omies (3) indicate that oomycetes are not closely related to Dm), de (lane NonSp), and nonsporulating mycelia from de GermZoo), nonsporulating mycelia from a 4-day rye broth culture broth (lane GermSp), zoospores released from sporangia after 3 h cultures of isolate 1306 (lane Sp), sporangia germinated for 10 h in rye were prepared by using RNA from sporangia puri

gene displayed staining in hyphae as well as spores (Fig. 6 M).

A recurring theme in development is that related proteins can participate in radically different pathways. This study showed that a member of the Puf family of proteins, developmental regulators that are widely distributed in unicellular and multicellular eukaryotes, participates in sexual and asexual sporulation in the oomycete P. infestans. Contemporary taxonomies (3) indicate that oomycetes are not closely related to plants, animals, and true fungi, including species in which Puf function is well understood. Within the framework of the Darwinian evolutionary paradigm, the Puf proteins share a common ancestor and likely function through similar biochemical mechanisms. However, whether the P. infestans protein is a homologue or an orthologue of known Puf proteins is not obvious.

Most proteins in the family are unrelated outside Pum-HD and participate in distinct developmental processes, usually by repressing the translation of specific mRNAs (14). For example, Pumilio together with Nanos and Brat repress translation of hunchback mRNA to determine cell types in the Drosophila embryo (14, 35). Similarly, Pumilio and Nanos-like proteins repress the translation of an mRNA involved in the sperm-oocyte transition in C. elegans (21). However, rather than target- ing a specific mRNAs the P. infestans protein may exhibit a general RNA-binding activity that protects transcripts in oospores or sporangia until germination. In true fungi and plants, spores and seeds contain preformed mRNAs that are stabilized largely by desiccation (23, 32). However, in oomycetes where spores remain hydrated, other mechanisms such as RNA-binding proteins may be required for RNA stabilization.

Interestingly, one function of Puf that may be conserved between species involves regulation of mitosis. In Drosophila and Xenopus, Puf proteins down-regulate the translation of cyclin B, which normally allows mitotic entry (26, 34, 35). This may explain why M90 is expressed during both sexual and asexual sporulation in P. infestans. Although these processes are dissimilar biochemically and structurally, both involve coordinated nuclear divisions followed by mitotic dormancy (4, 24).

A connection with nuclear dormancy can also be made from a report that a Puf gene is transcribed during certain spore stages of the oomycete S. parasitica (1). There were significant differences in the expression of the Puf-like gene compared to that in P. infestans, however. Transcripts appeared in S. para

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Our interest in M90 has two dimensions: understanding its protein product and identifying factors that govern its activity. In most other species, Puf proteins are expressed in all cell types and are regulated primarily by tissue-specific combinatorial interactions with proteins such as Nanos (14). In contrast, in P. infestans the spatial and temporal patterns of M90 expression are regulated at the level of transcription. The M90 promoter, which is the first developmentally regulated promoter isolated from an oomycete, provides a means for identifying transcription factors and signal transduction pathways regulating sexual and asexual sporulation. Being able to understand and manipulate these pathways has implications related to protecting crops and animals from disease.

DISCUSSION

A recurring theme in development is that related proteins can participate in radically different pathways. This study showed that a member of the Puf family of proteins, developmental regulators that are widely distributed in unicellular and multicellular eukaryotes, participates in sexual and asexual sporulation in the oomycete P. infestans. Contemporary taxonomies (3) indicate that oomycetes are not closely related to plants, animals, and true fungi, including species in which Puf

FIG. 5. Measurement of M90 RNA in nonmating samples. Blots were prepared by using RNA from sporangia purified from 6-day cultures of isolate 1306 (lane Sp), sporangia germinated for 10 h in rye broth (lane GermSp), zoospores released from sporangia after 3 h (lane Zoo); encysted zoospores germinated for 4 h in rye broth (lane GermZoo), nonsporulating mycelia from a 4-day rye broth culture (lane NonSp), and nonsporulating mycelia from defined medium (lane Dm), defined medium lacking the normal (NH₄)₂SO₄ nitrogen source (lane Dm-N), and defined media lacking glucose (lane Dm-C). The blots were prepared and analyzed as described in the legend to Fig. 4, including calculation of the relative levels of the M90 signal versus Ef1, actin, and 28S rRNA controls. EtBr, ethidium bromide.
FIG. 6. Histochemical staining of GUS activity in transformants of *P. infestans* expressing a fusion between the M90 promoter and the GUS gene. (A to D) Matings between a transgenic derivative of 8811 (A1) and nontransgenic 618 (A2), in which 8811 acted male. The interpretative diagram for panel A shows the outline of antheridal initials (ai); the leftmost structure is intertwined with a hypha of uncertain origin. The diagram for panel D shows the antheridial hyphae (ah), antheridia (a) wrapped around the oogonial stalk, and the fully expanded oogonium (o). (E to H) Matings between a transgenic derivative of 1306 (A1) and nontransgenic 618 (A2), in which 1306 acted female. The diagram for panel E shows a young interaction between an oogonium (o) and antheridium (a), and that for panel G shows the oogonial hyphal donor (oh). (I to L) Cultures of transgenic derivatives of 88069 (I) and 618 (J to L) undergoing asexual sporulation. The diagram for panel J shows developing sporangia (s) upon a branched sporangiophore (sp) and a vacuolated (v) boundary forming between a sporangium and a sporangiophore. Panel L shows GUS-stained sporangia detached from sporangiophores and a lysed or germinated spore case devoid of cytoplasm. (M) Transformant expressing GUS behind the *ham34* constitutive promoter, showing staining in spores and hyphae as a control. (N) Mating between the same transformant as in panel M and nontransgenic 618, showing staining in spores and hyphae as a control. (O) Nontransformed strain.
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