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Location of Protein Synthesis During Morphogenesis of *Mucor racemosus*

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The intracellular location of active protein synthesis was examined during the emergence of germ tubes from both sporangiospores and yeast-phase cells of *Mucor racemosus*. It was determined that protein synthesis occurs in all regions of the cell and not preferentially at the growing tip.

Mucor racemosus (*M. lusitanicus*) ATCC 1216B is a dimorphic phycomycete that can be stimulated to undergo a rapid and synchronous morphological change by altering the composition of the atmosphere under which the cells are grown (4, 6). The cells grow as budding yeasts under a CO_2 atmosphere in the presence of a hexose sugar. Changing the atmosphere from CO_2 to air results in the emergence of hyphal germ tubes from the yeast cells (4, 6). The organism, when grown on a solid medium under air, produces aerial hyphae that yield many sporangiospores. Upon introduction to liquid medium, the sporangiospores swell and, if under CO_2, develop into budding yeasts or, if under air, form hyphal germ tubes (4, 6). A characteristic acceleration in the specific rate of protein synthesis occurs during the emergence of germ tubes from both yeast (7) and sporangiospores (Orlowski and Sypherd, submitted for publication). As further growth proceeds by hyphal elongation, the rate of protein synthesis decreases. It is possible that the protein made during this period of rapid synthesis is predominantly located in some specific region or structure of the cell. The emergence of germ tubes might be dependent upon the rapid synthesis of such hypothetical structures or areas, which could include mitochondria, "spitzenkörper" (2), or cell-wall proteins. Bartnicki-Garcia has previously demonstrated that the only location where the synthesis and deposition of new cell wall occurs in developing germ tubes of *Mucor rouxii* is at the hyphal tips (2, 3). Making use of whole-cell autoradiographic techniques (1, 5), we examined the possibility that protein synthesis is restricted to or most active in the region of the hyphal tip during germ tube emergence from yeasts and sporangiospores of *M. racemosus*. In addition, we have employed density gradient fractionation techniques to determine whether any subcellular component is enriched in protein newly made during the morphlogical conversion.

Growth media and cultivation conditions that induce germ tube emergence from sporangiospores or yeast have been described (6, 8). Cells at appropriate times of germ tube emergence were pulse-labeled with L-[^{14}C]leucine (2.5

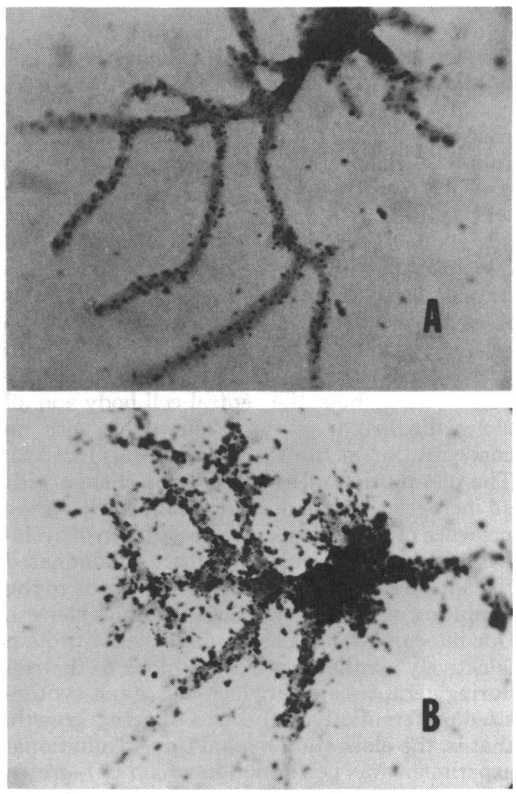

FIG. 1. *Autoradiographs of whole cells pulse-labeled with* L-[^{14}C]*leucine during a yeast-to-hyphae (CO_2-to-air) shift. (A) Pulse duration of 3 min; (B) pulse duration of 5 min.*

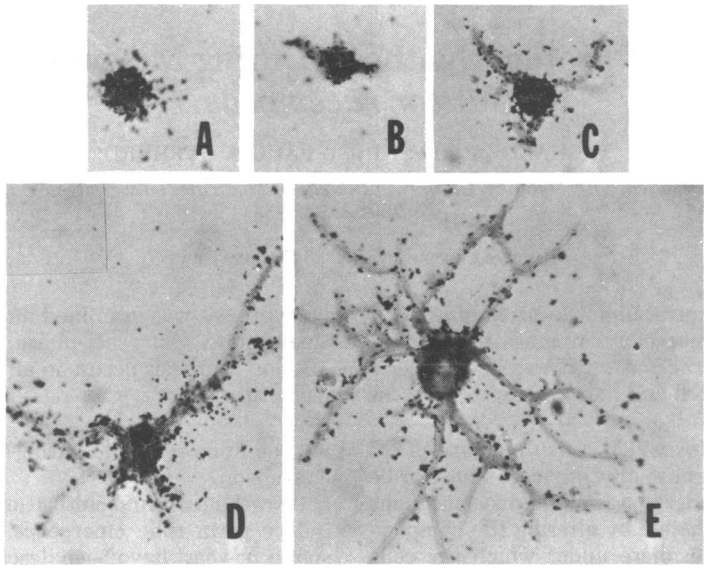

FIG. 2. *Autoradiographs of whole cells pulse-labeled with L-[^{14}C]leucine during sporangiospore germination in air. A 3-min pulse was given. Various stages of germ tube emergence are shown. (A) Swollen sporangiospore; (B and C) newly emerged germ tubes; (D and E) elongating hyphae.*

μCi/ml, 290 Ci/mol) for variable intervals (1, 3, 5, and 15 min). The cells were fixed with 4% osmium tetroxide, extracted with 10% trichloroacetic acid, affixed to glass slides, coated with Ilford G5 photographic emulsion, incubated at 4°C for about 4 days in the dark, developed with Kodak D-19 developer, and viewed microscopically under oil immersion (1, 5). We had demonstrated previously that L-[^{14}C]leucine is incorporated exclusively into protein (7). Grains were observed to be distributed throughout the cells at all stages of germ tube emergence from either sporangiospores or yeasts. Grains appeared throughout the central cell body and all along the length of the germ tubes, with no concentration at the hyphal tips (Fig. 1 and 2). The distribution of grains did not change with an increasing length of incubation of cells in the presence of L-[^{14}C]leucine. The addition of cycloheximide to the incubation mixture eliminated the appearance of grains, presumably due to the inhibition of protein synthesis (data not shown). Our interpretation of these data is that protein is actively synthesized in all regions of the cell during germ tube emergence and is not synthesized preferentially in the area of active growth, that is, the elongating hyphal tip. An additional experiment was performed in which CO_2-grown cells were prelabeled with L-[^{14}C]leucine to a constant specific radioactivity, pulse-labeled with L-[^{3}H]leucine after a shift to fresh medium and an air atmosphere, and fractionated on Renografin density gradients. The distribution of the two radioisotopes on the gradients indicated that there was no large concentration of newly made protein (^{3}H enriched) localized in a discrete structure that could be isolated on the basis of density (data not shown).

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