Fate of the 63-kDa periplasmic protein of an infectious form of the endonuclear symbiotic bacterium *Holospora obtusa* during the infection process

Fema ABAMO[1], Hideo DOHRA[2] and Masahiro FUJISHIMA[1] ([1]Dept. of Nat. Sci. and Symbiosis, Grad. Sch. of Sci. and Engineering, Yamaguchi Univ., [2]Inst. of Genet. Res. and Biotech., Shizuoka Univ.)

SUMMARY

An infectious form of *Holospora obtusa*, a macronucleus-specific endosymbiotic bacterium of the ciliate *Paramecium caudatum*, was found to secrete a 63-kDa periplasmic protein into its host macronucleus. Soon after the macronuclear infection, the protein appeared in the infected macronucleus and increased remarkably within one day after infection. Using host and bacterial protein synthesis inhibitors, we illustrate that both the pre-existing and newly synthesized 63-kDa proteins were secreted into the host's macronucleus early in the infection. The gene encoding the 63-kDa protein was cloned and sequenced. The deduced amino acid sequence shows that this protein is a novel protein.

INTRODUCTION

The gram-negative bacterium *Holospora obtusa* is a macronucleus-specific symbiont of ciliate *Paramecium caudatum*. It exists as reproductive short form (RF) (1.5-2 μm in length) and grows by binary fission when the host cell is vegetatively growing. When the host starves, the RF stops dividing and differentiates into infectious long form (IF) (13-15 μm in length) forming a cytoplasmic region comprising half the cell length and a large periplasmic region with small invasion tip. At this morphologic change, the bacterium acquires its infectivity[1-3]. The accompanying increase of the periplasmic region suggests that the periplasm may contain some important molecules necessary for its infectivity. Previously, we reported the secretion of a 63-kDa periplasmic protein of the IF into the macronucleus of its host. Our recent study aimed further to elucidate the function of this novel protein.

MATERIALS AND METHODS

P. caudatum strains RB-1(syngen 4, mating type E) free and infected with *H. obtusa* strain F1 and other *H. obtusa*-bearing strains no. 18 and 24 of *P. caudatum* were used. Paramecia were cultivated in 1.25% (w/v) fresh lettuce juice in modified Dryl's solution[4] and inoculated with a non-pathogenic strain of *Klebsiella pneumoniae* one day before use at 25 ± 1°C. IF were isolated from homogenates of *H. obtusa*-bearing paramecia by 70% Percoll density gradient centrifugation[5]. For infection experiments isolated IF ($1x10^6$ ml^{-1}) were mixed with *H. obtusa*-free paramecia ($5x10^3$ ml^{-1}) and the fates of the 63-kDa protein at various time intervals after mixing were traced by indirect immunofluorescence microscopy (IIFM), suspension technique with or without treatment with 1N NaOH[6]. Effects of host protein synthesis inhibitor, emetine, bacterial protein synthesis inhibitors, chloramphenicol and bacterial RNA synthesis inhibitor, rifampicin, on the secretion of 63-kDa protein were evaluated during infection. The concentrations of the antibiotics were described in previous studies[7]. A gene encoding a 63-kDa was cloned and sequenced following the procedures described in previous studies[6].

RESULTS AND DISCUSSION

As reported earlier, the IF of *H. obtusa* secreted a 63-kDa protein into the target host macronucleus soon after invading the nucleus as evidenced by macronuclear fluorescence using IIFM with monoclonal antibodies raised against the protein. Ratio of the host cells with such fluorescence and the fluorescence intensity increased remarkably within one day after infection. Recent results revealed that the fluorescence gradually weakened as the bacteria differentiated into the reproductive form. No significant effects were observed with emetine treatments but chloramphenicol and rifampicin treatments significantly affect the ratio of macronuclear fluorescence as well as the intensity of the fluorescence, evidently one day after mixing the host and the bacteria. These results suggest that, the macronuclear fluorescence was bacterial

in origin.

Almost half of the symbiotic cells showed macronuclear fluorescence at different intensities when examined by IIFM, suggesting that the IF cells leak the antigens continually into the macronucleus. Moreover, immunoblot analysis of the symbiotic host cells at stationary phase of growth also detected a single 63-kDa band, implying that the leaked 63-kDa protein was not degraded in the host macronucleus.

BLAST searches of the cloned gene and its deduced amino acid sequences detected no conserved domains in the deduced amino acid sequence, but Motif Finder (GenomeNet) predicted two DNA-binding motifs. It also found significant match with rhoptry protein of *Plasmodium yoelii*, with 20% identity. An 89-kDa protein of *H. obtusa* also showed similarities with rhoptry proteins[6]. The fact that both 63-kDa and the 89-kDa *Holospora* proteins have similarities with *Plasmodium* proteins suggests that the genes of these proteins might have identical evolutionary origin.

Considering the amounts of the protein secreted into the macronucleus, one can speculate that this protein might inhibit some nuclear receptors or signals from interacting, thereby changing the host gene expression to the advantage of the bacteria. Because the 63-kDa amino acid sequence has DNA-binding motifs, the interactions of the exported proteins with the DNA molecules of the host might block the DNases from digesting the host DNA molecules.

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