Analysis of gamone 1 receptor in the ciliate *Blepharisma japonicum* using a gamone 1 antibody

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SUMMARY

Most ciliated protozoa undergo conjugation under food-deprived conditions. In some species, conjugation-inducing substances (mating substances, mating pheromones or gamones) have been identified. However, receptors for these substances have not been well studied. Conjugation of *Blepharisma japonicum* is induced by specific cell-cell interaction between cells of complementary mating-types I and II, and involves conjugation-inducing substances called gamones. The glycoprotein gamone1, is secreted by type I cells, and is a trigger molecule for conjugation that is specifically recognized by mating type II cells. Previously, we purified gamone1 protein and isolated the *gamone1* gene. In this study, an antibody was raised against a peptide of 14 amino acids in the C-terminal region of gamone1 (named G1_P6 antibody). We showed by western blotting that G1_P6 antibody could detect a small amount of gamone1 in a cell-free fluid, and that the antibody-bound gamone1 retained its conjugation-inducing activity. Using G1_P6 antibody, we localized presumptive gamone1-receptors in gamone1-stimulated type II cells with a confocal laser scanning microscope. After incubation with gamone1 for 1.5 hrs, strong fluorescence signals were detected in the adoral zone of membranelles (AZM), cilia and ciliary rows.