Dictyostelium discoideum (countin3−) Forms Small Fruiting Bodies

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In the cellular slime mold Dictyostelium discoideum, Countin and Countin2 proteins are thought to control the size of the multicellular structure since the countin strain forms a huge fruiting body and countin2 strain forms a small fruiting body. Recently, the countin3 gene encoding a polypeptide homologous to Countin and Countin2 was identified in the D. discoideum genome. The countin3 strain formed a 1.8-fold larger number of aggregates, resulting in smaller fruiting bodies compared to those formed by the wild-type. The extent of cell-cell adhesion was reduced in the mutant, indicating that Countin3 protein regulates size by controlling the amounts of proteins responsible for the adhesion.

Key words: countin3, Dictyostelium discoideum, size

It is important for organisms to form multicellular structures of appropriate sizes. However, little is known about the mechanism by which the size of such structures is determined. The cellular slime mold Dictyostelium discoideum is a suitable model for studying the molecular basis of size control. D. discoideum, a predatory amoeba feeding on bacteria of forest soil, grows as a unicellular free-living microorganism1. When starved, the cells aggregate and form a fruiting body consisting of about 105 cells. In this process, D. discoideum cells differentiate into two basic cell types, stalk and spore cells1. D. discoideum forms a fruiting body to hold the spore mass as high off the ground as possible in order to disperse spores. If the fruiting body is too small, spores will not be dispersed over a large area. On the other hand, if there are too many cells in a fruiting body, the stalk will bend and spores will not be dispersed. Thus, regulation of the size of the fruiting body is important for optimal spore dispersal. D. discoideum cells should produce factors that regulate cell number in a multicellular structure. Countin and Countin2 proteins have been reported as such factors. Countin is thought to limit the maximum size of the multicellular structure since the countin strain forms a huge fruiting body compared to that of the wild-type2. Countin2 protein is thought to limit the minimum size of the multicellular structure since countin2− cells form a smaller fruiting body3.

Recently, we identified the countin3 gene encoding a protein 49% identical to Countin and 39% identical to Countin24. To analyze the function of Countin3, we established a gene disruption mutant. A DNA fragment (1758 bp) containing countin3 was recovered from genomic DNA by PCR. The primers (CNT3-1 and CNT3-4) used in the reaction are shown in Fig. 1A. The reactions were carried out as described in our previous report4. The blasticidin resistance (BSR) gene cassette (1.35 kb) was inserted into the HinClI site in the countin3 coding region. The construct was introduced into D. discoideum Ax2 (wild-type strain) by electroporation, and homologous recombinants were selected in
HL5 medium\(^5\) containing 5 mg/L of blasticidin S (Funakoshi, Tokyo, Japan). Gene disruption was confirmed by PCR and RT-PCR with primers (CNT3-1, -2, -3 and -4) that were designed to amplify a 0.59-kb fragment and a 0.48-kb fragment from the coding region on the chromosome and mRNA, respectively. Genomic DNA samples were prepared from the wild-type and transformant to use as templates in PCR analysis. Comparison of the sizes of the PCR products indicated that a 1.35-kb DNA fragment had been inserted into the countin3 gene in the transformant (Fig. 1B). We next confirmed the gene expression by RT-PCR. Total RNAs extracted from the strains were used as templates in the reactions. The RT-PCR product (0.48 kb) was detected only when RNA extracted from the wild-type was used in the reaction, indicating that the transformant did not express countin3 mRNA (Fig. 1C). We concluded from these results

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**Fig. 1.** Establishment of the gene disruption mutant.

(A) The sequence of the recovered fragment including the countin3 gene. The BSR cassette was inserted into the Hin\(_{cII}\) site in the coding region. The primers (CNT3-1, -2, -3 and -4) are indicated by arrows. (B) PCR analysis. Lane 1, size marker; lane 2, wild-type; lane 3, countin3\(^-\). (C) RT-PCR analysis. RT-PCR product amplified by countin3-specific primers are shown. RT-PCR was performed using a One Step RNA PCR Kit (Takara Shuzo, Kyoto, Japan) according to the manufacturer’s instructions. The EtBr staining pattern of rRNAs is presented in the bottom panel. Lane 1, size marker; lane 2, wild-type; lane 3, countin3\(^-\).
that a countin3 gene disruption mutant had been established. Equal numbers of parental and countin3- cells were separately plated on a non-nutrient agar to compare their multicellular structures. In this condition, *D. discoideum* cells aggregated to form a multicellular mass “mound”, entered the multicellular stage of development, and completed the fruiting body formation. As shown in Fig. 2, countin3- formed small mounds and fruiting bodies compared with those of the wild-type. We observed that the countin3- cells formed a 1.8-fold larger number of aggregates, resulting in the formation of smaller fruiting bodies (data not shown).

One of the size control mechanisms is mediated by cell-cell adhesion. It has been reported that disruption of the countin gene caused an increase in cell-cell adhesion resulting in the formation of a large fruiting body30. In contrast, a decrease in adhesion was observed in countin2- strain which formed a small fruiting body31. We examined whether the cell-cell adhesion would be altered in countin3- cells. Vegetative cells cultured in HL5 medium were harvested, washed, suspended in the phosphate buffer and shaken slowly. Cells were starved, expressed cell-cell adhesion proteins and formed aggregates under the conditions. The aggregates in the buffer were photographed (Fig. 3). The aggregates of countin3- were smaller than those of the wild-type, indicating that the cell-cell adhesion was reduced in the mutant. The countin3- cells would form small fruiting bodies because of the altered adhesion.

Two independent null clones exhibited the same phenotype (our observation). Thus, the disruption of countin3 is responsible for the small fruiting bodies. The expression kinetics of the genes for the adhesion in the null cells is under investigation. Further analysis to determine the functional difference between countin2 and countin3 genes is in progress.
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References


