Involvement of Sib proteins in the regulation of cellular adhesion in Dictyostelium discoideum

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Abstract

Molecular mechanisms ensuring cellular adhesion have been studied in detail in Dictyostelium amoebae, but little is known about the regulation of cellular adhesion in these cells. Here, we show that cellular adhesion is regulated in Dictyostelium, notably by the concentration of a cellular secreted factor accumulating in the medium. This constitutes a quorum-sensing mechanism allowing coordinated regulation of cellular adhesion in a Dictyostelium population. In order to understand the mechanism underlying this regulation, we analyzed the expression of recently identified Dictyostelium adhesion molecules (Sib proteins) that present features also found in mammalian integrins. sibA and sibC are both expressed in vegetative Dictyostelium cells, but the expression of sibC is repressed strongly in conditions where cellular adhesion decreases. Analysis of sibA and sibC mutant cells further suggests that variations in the expression levels of sibC account largely for changes in cellular adhesion in response to environmental cues.

Reference


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Figure A1. Screening of sibC mutant cells.

(A) A sibC knockout vector was derived from a SpeI rescue plasmid as described in the Materials and Methods. The position of the oligonucleotides used for PCR analysis is indicated. (B) The insertion of the plasmid at the sibC locus allows the amplification of a 1.3kb PCR fragment from genomic DNA. Two independent mutant clones are shown. No PCR amplification was seen when genomic DNA from wild-type cells was used. The two sibC mutant clones identified were used with very similar results in all experiments.

Figure A2. A secreted cellular factor inhibits cellular adhesion.

To assess the effect of the medium on phagocytosis, adherent sibA mutant cells were incubated for 4 h in the indicated media. They were then resuspended and their ability to phagocytose latex beads assessed. As detailed in this work, sibA mutant cells are particularly responsive to environmental cues and thus represent the most sensitive system to investigate the nature of these cues. The aim of these experiments was to determine if medium conditioned by growth of Dictyostelium cells lacked a component essential for cellular adhesion, or contained a factor inhibiting adhesion.

A. Conditioned medium (CM) was diluted with four volumes (CM/5) or 24 volumes (CM/25) of fresh medium (FM). Its effect on sibA cells was then assessed. The average and SEM of three experiments are indicated. These results indicate that even diluted conditioned medium has an inhibitory effect on cellular adhesion, suggesting the presence of an inhibitory factor.

B. A dialysis bag (cut-off 5000-8000Da) containing 3ml of conditioned medium was dialyzed first for 3h in 6ml of fresh medium. The six ml of dialysis medium were then recovered and the dialysis bag dialyzed further in 60ml of PBS, then in 60ml of fresh
HL5. The effect of the dialyzed medium (1) and of the first dialysis medium (2) on sibA cells was then tested as described above. The dialyzed medium retained its inhibitory activity, while no inhibitory activity was detected outside of the dialysis bag. As a control, 3ml of fresh HL5 were treated in a similar manner. As expected, dialyzed HL5 (3) and dialysis medium (4) did not exhibit inhibitory activity. These results suggest that conditioned medium contains a large molecule(s), presumably a protein, capable of inhibiting cellular adhesion in Dictyostelium. The inhibitory activity was lost in various purification procedures, preventing further biochemical characterization (data not shown).