CnrN regulates Dictyostelium group size using a counting factor-independent mechanism

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One of the simplest examples of a complex behavior is the aggregation of solitary Dictyostelium discoideum amoebae to form a 20,000-cell fruiting body. A field of starving amoebae first breaks up into territories. In each territory, the cells form a spider-like pattern of streams of cells. As part of a negative feedback loop, counting factor (CF), a secreted protein complex whose concentration increases with the size of the stream, prevents over-sized fruiting bodies from being formed by increasing cell motility and decreasing cell-cell adhesion, which causes the breakup of excessively large streams. Cells lacking the phosphatase CnrN (cnrN cells) form small aggregation territories and few streams. In this report, we present computer simulations that suggest that in the absence of stream formation, CF should be unable to affect group size. As predicted, cnrN group size is insensitive to the addition or depletion of CF. Together, the data indicate that CnrN regulates group size by regulating both the break-up of a field of cells into aggregation territories and stream formation during development, and that CnrN-mediated and CF-mediated group size regulation use different mechanisms.

Much remains to be understood about how eukaryotic organisms precisely regulate the size of multicellular structures. A number of previous observations suggested that secreted factors are involved in size regulation. For example, myostatin secreted by myoblasts inhibits myoblast proliferation, and this negative feedback loop maintains the muscle size. During Dictyostelium development, starving cells secrete counting factor (CF), a protein complex, as a diffusible signal to regulate group size. When there are too many cells in an aggregation stream, the local concentration of CF is high, causing increased cell motility and decreased cell-cell adhesion, which in turn results in the breakup of the stream into groups. Each group contains approximately 20,000 cells and develops into a fruiting body. A lack of CF thus results in an inability to break large streams, which results in the formation of huge groups.

To elucidate the mechanisms of CF-mediated group size regulation, we conducted a second-site suppressor screen by random insertional mutagenesis on smlA cells which over-accumulate CF during development and form very small fruiting bodies. In this screen, we identified cnrN, which encodes a PTEN-like protein. Although an insertion at the cnrN locus causes smlA cells to form large fruiting bodies, an essentially complete deletion of cnrN causes smlA cells to form small fruiting bodies. Similarly, deleting cnrN in wild-type cells also results in a small fruiting body phenotype, which can be rescued by expressing exogenous CnrN.

We first compared the development processes of different cell lines. Unlike smlA cells, which form normal aggregation streams that are then broken up into very small groups because of over-accumulated CF, cells lacking CnrN (cnrN cells) form very small aggregation territories and few streams, which barely undergo stream breakup and directly coalesce into groups. Previously, computer simulations predicted, and experiments showed, that CF decreases cell-cell adhesion and increases random cell motility to cause streams to break. To examine if CF might be able to theoretically cause groups such as those formed by cnrN cells to break into smaller groups, we did computer simulations of a stream of cells as well as a group of cells, varying the cell-cell adhesion and motility, the varying the cell-cell adhesion and motility. Under conditions where a stream of cells broke into groups, a disk of cells did not break (Fig. 1). Varying the cell-cell adhesion and/or varying the motility in either the simulations or experimentally changes the number of groups that streams break into, while in the computer simulations changing the same parameters for the disk of cells never caused the disk to break into groups (Fig. 1). The computer simulations thus predicted that even if CF alters the adhesion and/or motility of cnrN cells, with fewer streams, there will be correspondingly less stream breakup, and with the cnrN groups tending to have a larger diameter than the wild-type stream thickness, CF should be unable to alter the cnrN group size.

The prediction by computer simulations was confirmed by several experimental observations. First, after 6 hours starvation, compared to wild-type cells, cnrN cells secrete and accumulate higher level of countin, which is a key component of CF and which alone
has partial CF activity. Second, although CF did not appear to significantly affect cell-cell adhesion ofcnrN cells, high levels of CF did cause an increasedcnrN cell motility. Third, neither thecnrN aggregate size nor the number of aggregates was altered when the effective concentration of CF was increased or decreased. Together with the previous finding that CF does not affect aggregation territory, the computer simulations presented here and our previous experimental findings suggest that CnrN regulates group size by using a CF-independent mechanism.

Our knowledge of how a field of cells breaks up into a number of territories or functional groups is still very limited. In Dictyostelium, the cAMP pulse size during development and the chemotactic response to cAMP are considered to be two key factors that affect territory formation. Excessively high levels of cAMP were previously reported to cause the formation of small territories. In agreement with the previous findings, we foundcnrN cells, compared to wild-type cells, accumulate much higher levels of cAMP. TreatingcnrN cells with exogenous cAMP phosphodiesterase rescues the small territory phenotype, which indicated that a large cAMP pulse size causes the small territories.

Cells with chemotaxis defects such astorA or pten cells form small aggregation territories or do not aggregate at all. To determine whethercnrN cells have chemotaxis defects which then contribute to the formation of small territories, we conducted small droplet chemotaxis assays. As shown in Figure 2, the chemotactic responses ofcnrN cells toward 10 nM to 1 μM cAMP were similar to those of wild-type cells. In contrast, overexpression of CnrN incnrN cells caused slightly weaker chemotactic responses toward low concentration of cAMP. This result was supported by Boyden Chamber assays, in whichcnrN cells showed similar chemotaxis behaviors to wild-type cells (data not shown). Overall, these results suggest that over-accumulated cAMP during development, rather than chemotaxis defects, causescnrN cells to form small aggregation territories and eventually small fruiting bodies.

Our work indicates that CnrN, a novel group size regulator, regulatesDictyostelium group size by regulating territory formation, a process prior to CF-regulated stream breakup during development, via a CF-independent mechanism. Although CnrN shares some sequence and function similarities to PTEN, whereas PTEN clearly affects chemotaxis, and the loss of PTEN causes no aggregation at all, CnrN regulates territory formation by regulating cAMP pulse size rather than chemotaxis.

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