Dictyostelium AMPKa regulates aggregate-size and cell-type patterning

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Figure S1. Construction of the overexpressing strain (ampkα<sup>OE</sup>). (A) Diagrammatic representation of ampkα overexpression construct. (B) PCR amplification of the ampkα gene using gene-specific primers from the genomic DNA. (C) Confirmation of clone yielded 6.8 Kb vector backbone and a ~2.6 Kb insert. (D) Transformed cells expressing AMPKα–Eyfp fusion protein. [M- DNA Marker].
Figure S2. Creation and confirmation of \textit{ampka} knockout construct and strain. (A) PCR amplification of the 5’ targeting region was approximately 0.7 Kb. (B) PCR amplification of the 3’ targeting region was approximately 0.65 Kb. (C) Confirmation of 5’ and 3’ targeting region and bsr cassette via restriction digestions. (D) Diagrammatic representation of \textit{ampka} knockout construct obtained. The primer positions and expected amplicons sizes are marked. (E) Confirmation of \textit{ampka} knockout strain by PCR from Ax2 and \textit{ampka\textsuperscript{-}} genomic DNA using various primer combinations. (F) Whole-cell extracts were prepared from Ax2 and \textit{ampka\textsuperscript{-}} vegetative cells and AMPK\textalpha protein expression was measured by western blotting (anti-AMPK\textalpha antibody; CST and anti-Actin antibody; CST). [M- DNA Marker; P1, P2, P3, P4, P5 and P6 are different primers used for knockout screening.]
Figure S3. Comparison of aggregates number and size. (A) and (B) Graph shows the number and size of aggregates formed in various chimaeric mixtures (5-90% ampka− cells). ampka− cells cause the Ax2 aggregates to break up into smaller ones. (C) and (D) Graph representing the aggregates number and size formed by Ax2 and ampka− cells in buffer, Ax2 and ampka− conditioned medium. Ax2 cells form smaller aggregates when developed in ampka− CM, whereas ampka− cells were small-sized in both Ax2 and ampka− CM. Values were compared and significance was plotted against control. [The values represent mean ± standard deviation; n=4; ***P < 0.001, **P <0.01, *P < 0.05 (Student’s t-test)].

Figure S4. Comparison of aggregate numbers and sizes formed in the presence of exogenous glucose. (A) and (B) Graph shows the number and size of aggregates formed by Ax2 and ampka− cells in presence of 5 mM glucose. Values were compared and significance was plotted against control. [The values represent mean ± standard deviation; n=4; ***P < 0.001, **P <0.01, *P < 0.05 (Student’s t-test)].
Figure S5. Analyses of cell-type specific marker gene expression during development. Relative abundance of the various transcripts in both, Ax2 and ampkα− cells during development were analysed. (A) ecmA, (B) ecmB and (C) pspA. [V- Vegetative, Str-Streaming, M- Mound, MS- Migratory Slug, EC- Early Culminant, C- Culminant. The values represent mean ± standard deviation; n=4; ***P < 0.001, **P <0.01,*P < 0.05 (Student’s t-test)].

Figure S6. Western blots showing AMPKα activation during starvation. Ax2 cells were starved in 1xKK2 buffer and samples were collected every 2 hours till 8 hours. Cell lysates were prepared and protein expression was measured by western blotting using phosphorylated AMPKα (pThr172) and AMPKα antibody. β- Actin was used as a loading control. [V- Vegetative; all antibodies were purchased from Cell Signalling Technology].
Figure S7. Cloning for *in-situ* hybridisation studies. (A) PCR amplification of ~0.74 Kb genomic region for the preparation of probe. (B) Confirmation of *in-situ* clone yielded 3.0 Kb vector backbone and a ~0.74 Kb insert. (C) The unhydrolysed and hydrolysed product of antisense and sense probes. [M- DNA Marker].
Table S1: List of oligonucleotides used in this study. Sequence underlined marks the restriction enzyme site.

| Gene name | Primer | Primer sequence (5′------- 3′) | Genomic position |
|-----------|--------|--------------------------------|------------------|
| in-situ   | Forward| CATCCTCGAGCCAAGCAATAGCAATAGCATCA| 1514-1536 |
|           | Reverse| GCTCTAGAATTTGTTGTTGTTGTTGTTGTAATCTCTG | 2235-2257 |
| ampka RT  | Forward| GGAAATAGTGAATGGTAGCAACAGC | 1859-1882 |
|           | Reverse| CTTCCATTAACCATTCTACATCTT | 2495-2519 |
| ampka αOE| Forward| CATCGGTACCAGXXCATATCAACAAATCCCATA | 4-27 |
|           | Reverse| CTACGGCATCAGATCCCCCAAATCCATCACAAA | 2617-2642 |
| ampka 5′ targeting region | Forward| CGATGGGCCCCCTTTGGGTGTCTATATGGCAGTGGA | 34-57 |
|           | Reverse| GACTCTAGACAGAGATAACTCAGGTGCAGGTAAT | 731-757 |
| ampka 3′ targeting region | Forward| CTCAGTCGACGTGTAGGCAACAGCAATAANAAA | 1866-1890 |
|           | Reverse| GTGACTGCGAGGGGTTTCCATTAACATTCTACCTC | 2497-2521 |
| rnlA RT   | Forward| TGAATTGAAGTCTGAGTAAACGG | 1795-1817 |
|           | Reverse| TAGATAGGGACAAACTGTCTCAC | 3065-3042 |
| ctnA RT   | Forward| ATTTTACGCTTTATCCTTGTCAAC | 22-45 |
|           | Reverse| GTGTAAGCAATCGAGGAGGTGAAT | 468-491 |
| cadA RT   | Forward| TCTGTGTAGCACAATAAAGTAAA | 4-28 |
|           | Reverse| ATAGTCATATGGTGATAGTGGTGT | 583-559 |
| csA RT    | Forward| GTGAACGACTCTATTTAATCTCTGCT | 406-430 |
|           | Reverse| AGTTGGAGTGTCTGGAATTGATA | 1374-1350 |
| ecmA RT   | Forward| GATGATGGAATAGATGGTCAACA | 220-243 |
|           | Reverse| GTACATTGGTTATTATCATCGACA | 1236-1213 |
| ecmB RT   | Forward| GTGGGTGGTTACTCTACACTCACAATTCGTTT | 2611-2637 |
|           | Reverse| CATGGAAACATGAATACATTTACACCACC | 3366-3340 |
| pspA RT   | Forward| GATAGGATCCCCAGTTGTTGTGCTTACATGATGTC | 94-117 |
|           | Reverse| ACTTCTCGAGGTTGTTGATGTTTGGGATGG | 434-415 |
Table S2: List of oligonucleotides used for *ampka* knockout screening.

| Gene name | Primer P1 | Primer sequence (5′------- 3′) |
|-----------|-----------|-------------------------------|
| Primers for *ampka* knockout screening | TTTGTCCATTGAAACTGCA |
| | TGCAGTTTCGAATGGACAAA |
| | CGATGGGGCCCTTTGTTGTGTCTATATTGGCAGTGGA |
| | GTGACTGAGGGCTTTCATTAACCATTCTACATC |
| | TCCAGCCCTATATATATCCAC |
| | TAAGTATTGATAAAAAAGAGCACTC |