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Temperature adaptation in *Dictyostelium*: role of $\Delta 5$ fatty acid desaturase

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Membrane fluidity is critical for proper membrane function and is regulated in part by the proportion of unsaturated fatty acids present in membrane lipids. The proportion of these lipids in turn varies with temperature and may contribute to temperature adaptation in poikilothermic organisms. The fundamental question posed in this study was whether the unsaturation of fatty acids contributes to the ability to adapt to temperature stress in *Dictyostelium*. First, fatty acid composition was analysed and it was observed that the relative proportions of dienoic acids changed with temperature. To investigate the role of dienoic fatty acids in temperature adaptation, null mutants were created in the two known $\Delta 5$ fatty acid desaturases (FadA and FadB) that are responsible for the production of dienoic fatty acids. The *fadB* null mutant showed no significant alteration in fatty acid composition or in phenotype. However, the disruption of *fadA* resulted in a large drop in dienoic fatty acid content from 51.2 to 4.1% and a possibly compensatory increase in monoenoic fatty acids (40.9–92.4%). No difference was detected in temperature adaptation with that of wild-type cells during the growth phase. However, surprisingly, mutant cells developed more efficiently than the wild-type at elevated temperatures. These results show that the fatty acid composition of *Dictyostelium* changes with temperature and suggest that the regulation of dienoic fatty acid synthesis is involved in the development of *Dictyostelium* at elevated temperatures, but not during the growth phase.

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INTRODUCTION

The physical properties of phospholipids mainly depend on the degree of unsaturation of the fatty acids esterified to the glycerol backbone. An increased degree of unsaturation increases the mobility of the acyl chains and consequently increases the fluidity of the membrane formed from them (Murata & Wada, 1995). Membrane fluidity may, in turn, affect the function of various membrane proteins and their appropriate targeting within the cell, as well as membrane trafficking (Gurr *et al.*, 2002).

When food is supplied, *Dictyostelium* amoebae live as a unicellular organism. Upon starvation, a developmental program is initiated that leads to the formation of the multicellular structure. *Dictyostelium* cells grow and develop in leaf litter of temperate forests and so must be able to thrive over a range of temperatures. The growth temperature of *Dictyostelium discoideum* influences its subsequent

development. The effect of growth temperature on *Dictyostelium* development has been analysed by Das *et al.* (1980). They reported that an increase in the growth temperature enhanced development at higher temperatures. However, they also reported that the fatty acid composition of *Dictyostelium* lipids did not change significantly with the growth temperature (Das *et al.*, 1980). This would be unusual, given the adaptation of fatty acid composition to temperature known from other organisms (Los & Murata, 1998), and we therefore re-examined this observation. To determine whether the degree of unsaturation of fatty acids contributes to temperature adaptation, we altered the degree of unsaturation of fatty acids by manipulation of the genes that encode the enzymes responsible for the desaturation of fatty acids.

The major fatty acids present in *Dictyostelium* lipids are C16 and C18, and these are mainly unsaturated at positions $\Delta 5$ and $\Delta 9$ (Weeks & Herring, 1980). These modifications are introduced into the alkyl chains by specific desaturase enzymes. By the use of sequence information from the *Dictyostelium* cDNA project (Morio *et al.*, 1998), we have previously identified two potential $\Delta 5$ fatty acid desaturases, FadA and FadB, encoded by *fadA* and *fadB*, respectively, and

Nomenclature: C_x:y(a,b), a fatty acid containing x carbon atoms and y double bonds – the numbers in parentheses (a,b) indicate the position of the double bonds; *des5-1* and *des5-2* genes have been renamed as *fadA* and *fadB*, respectively, according to the recommended genetic nomenclature for *Dictyostelium*.

confirmed that both enzymes are responsible for the production of dienoic fatty acids in the slime mould (Saito & Ochiai, 1999; Saito *et al.*, 2000).

In the present report, we describe the changes in the relative proportions of fatty acids in *Dictyostelium* according to the temperature and the disruption of *fadA* and *fadB*. Using these mutants, we address the question of whether dienoic fatty acids are involved in temperature adaptation.

METHODS

Cell culture and development. *Dictyostelium discoideum* axenic strain Ax2 was used as the parental strain and was grown in HL-5 medium at 22 °C (Watts & Ashworth, 1970). To examine the effects of growth temperature on fatty acid composition, cells grown at 22 °C were inoculated at a density of 5×10^4 cells ml⁻¹ and incubated in a shaking suspension (160 r.p.m.) at 15, 22 and 25 °C, respectively, until they reached a density of 1×10^6 – 2×10^6 cells ml⁻¹. The effect of successive and accurate changes of growth temperature was examined with wild-type cells incubated in a temperature gradient incubator (Advantec Toyo, TVS126MA).

Morphogenesis was observed on nitrocellulose filters (Millipore) on top of filter pads soaked in 17 mM phosphate buffer (pH 6.2), or under submerged conditions. Wild-type and *fadA* null mutant cells were incubated at 22 °C, harvested and washed with phosphate buffer. Then the cells were placed in a 12-well titre plate at a density of 5×10^5 cells cm⁻² or on a nitrocellulose filter at a density of 2×10^6 cells cm⁻² and incubated at 22 and 27 °C. Photographs were taken with a digital camera (Olympus HC-300z/OL) attached to a stereomicroscope (Olympus SZX12). To examine the change in fatty acid composition with respect to development, cells were incubated with axenic medium and washed with phosphate buffer (pH 6.2), and then spread on phosphate buffer containing 1.5% agar at a density of 1×10^6 – 2×10^6 cm⁻².

To examine the effect of thermal stress on growth, the number of surviving cells was counted after 24 h incubation at 30 °C with shaking.

Spore formation was examined by counting the number of Triton X-100-resistant cells after 40 h incubation on a nitrocellulose filter at 22 and 27 °C (Kay, 1989).

Fatty acid analysis. Total lipids were extracted (Bligh & Dyer, 1959) from wet cells of *Dictyostelium* and subjected to methanolysis with 10% acetyl chloride in methanol for 3 h at 100 °C. Cells grown in a temperature gradient incubator were subjected to methanolysis directly with 10% acetyl chloride in methanol. Analysis of the methyl esters by GC was performed as described by Matsuoka *et al.* (2003).

Plasmid construction and transformation. *fadA* and *fadB* disruption cassettes were constructed by PCR. The genomic fragment of each gene was amplified and ligated into pT7Blue T-vector (Novagen) as described previously (Saito & Ochiai, 1999; Saito *et al.*, 2000). The *fadA* and *fadB* genomic fragments were digested with *Hind*III and *Bgl*II, respectively. Then the blasticidin S resistance gene cassette was inserted into the *Hind*III site of *fadA* and the *Bgl*II site of *fadB*, respectively. These plasmids were purified with the Plasmid Maxi Kit (Qiagen). Fifteen micrograms of each plasmid was introduced into Ax2 cells via electroporation, using the same conditions described by Adachi *et al.* (1994).

Selection at 5 µg blasticidin S ml⁻¹ (Funakoshi) was started on the day after transformation, and after 7–10 days, resistant cells were plated in

association with *Klebsiella aerogenes* on SM agar plates. Clones were screened by genomic PCR and RT-PCR to confirm disruption of each gene. All the transformants were grown under selective conditions.

To confirm the disruption of each gene, RT-PCR was performed using the following primers: *fadA*, 5'-AGATGGGTACCATTACATCCAG-GTGG-3' and 5'-GGTTCATCTGGTCTTTCAGGGGTAGC-3'; *fadB*, 5'-CGATGCATGACGATTGCCACACAGC-3' and 5'-GCCCAATCT-TGATTGAAGTTGTTGG-3'. The reactions were performed according to the manufacturer's instructions (One Step RNA PCR kit; Takara).

RESULTS

The fatty acid composition of *Dictyostelium* lipids adapts to their growth temperature

Previous work had suggested that the fatty acid composition of *Dictyostelium* lipids does not change significantly with growth temperature (Das *et al.*, 1980). This would be unusual, since the adaptation of fatty acid composition to temperature is well known from other organisms (Los & Murata, 1998), and we therefore re-examined the fatty acid composition of *Dictyostelium* lipids at different temperatures. Our results show that the proportions of the different unsaturated fatty acids change significantly as the growth temperature is increased from 15 to 25 °C, but with subtle differences between different fatty acids. Table 1 shows the fatty acid composition at various temperatures. The amounts of C16:2(5,9) and C18:2(5,11) decreased according to the increase in temperature (5.2–2.3% and 39.8–28.7%, respectively). On the other hand, the amount of C18:2(5,9) increased (5.6–14.6%). The total amount of Δ5 desaturated products also decreased with the increase in

Table 1. Effect of growth temperature on major fatty acid compositions of total lipid from wild-type cells

Others, minor unidentified acids; Δ5 desaturation, total ratio of Δ5 desaturated fatty acids.

Fatty acid	Percentage (±SD) at:		
	15 °C	22 °C	25 °C
16:0	7.1 (0.7)	6.3 (0.3)	6.6 (0.5)
16:1(9)	4.9 (0.1)	6.2 (0.7)	5.7 (0.4)
16:2(5,9)	5.2 (0.5)	3.0 (0.5)	2.3 (0.2)
17:0	0.4 (0)	0.6 (0.2)	0.7 (0.2)
17:1(9)	1.5 (0.3)	1.5 (0.2)	1.5 (0.2)
17:2(5,9)	1.4 (0.1)	1.5 (0.2)	2.0 (0.2)
18:0	0.5 (0.2)	1.2 (0.4)	1.2 (0.3)
18:1(9)	1.6 (0.2)	2.9 (0.2)	3.8 (0.3)
18:1(11)	29.4 (2.5)	30.3 (1.5)	31.4 (0.8)
18:2(5,9)	5.6 (0.6)	13.1 (0.7)	14.6 (0.9)
18:2(5,11)	39.8 (1.3)	31.4 (0.7)	28.7 (0.8)
Others	2.6 (0.1)	2.2 (0.4)	1.6 (0.2)
Δ5 desaturation	52.0	49.0	47.6

temperature. For further investigation of the effect of successive changes in growth temperature, wild-type cells were incubated in a temperature gradient incubator. Fig. 1 shows the changes in the proportions of C18:2 fatty acids and $\Delta 5$ desaturated acids according to temperature. Since the major change in fatty acid composition was found in the dienoic acids, we suggest that the production of dienoic acids is important for temperature adaptation.

$\Delta 5$ desaturase gene null mutants

We have previously cloned two putative $\Delta 5$ desaturase genes and expressed them in yeast, and have thereby been able to confirm their biochemical activity. Since the proportion of $\Delta 5$ unsaturated fatty acids is strongly regulated by temperature during the growth phase, we considered it likely that FadA and FadB are involved in temperature adaptation. As an initial test of this idea, we asked whether expression of these genes is regulated by growth temperature. However, we could find no effect: levels of both mRNAs, as determined by RT-PCR, were essentially unchanged in cells grown at a range of temperatures (data not shown). It remains possible that enzyme activity is regulated at a post-transcriptional level, or that fatty acid turnover is differentially regulated by growth temperature. We therefore used a genetic test for the role of $\Delta 5$ desaturation in temperature adaptation by making null mutants of the $\Delta 5$ desaturase genes *fadA* and *fadB*. Elimination of *fadB* by homologous recombination resulted in mutant cells which lack any obvious phenotype and whose fatty acid composition is essentially identical to the wild-type (Table 2). In contrast, the *fadA* null mutant showed a significant decrease in the amount of dienoic acids with a concomitant increase in their monoenoic acid precursors. This was most marked for C16 and C17 dienoic

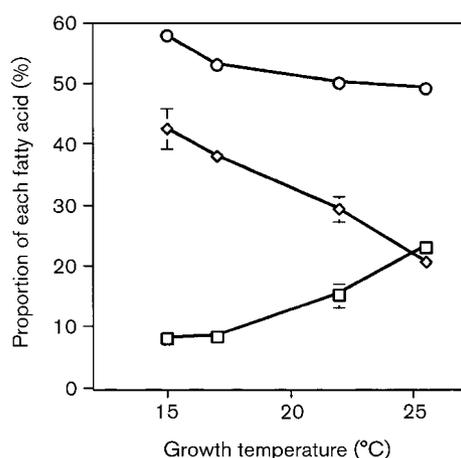


Fig. 1. Temperature-dependent changes in dienoic fatty acids. The effects of successive and accurate changes in growth temperature were examined in wild-type cells. Circles, ratio of total dienoic acids; diamonds, ratio of C18:2(5,11); squares, ratio of C18:2(5,9). The data plotted are the means of three independent experiments \pm SEM.

Table 2. Major fatty acid composition of total lipid from wild-type cells and $\Delta 5$ fatty acid desaturase null mutants

Cells were incubated in HL-5 medium at 22 °C. ND, Not detected; TR, trace (less than 0.2%); others, minor unidentified acids.

Fatty acid	Percentage (\pm SD) in:		
	Wild-type	<i>fadA</i> null	<i>fadB</i> null
16:0	6.3 (0.3)	1.6 (0.4)	6.1 (0.5)
16:1(9)	6.2 (0.7)	19.2 (2.3)	6.1 (0.4)
16:2(5,9)	3.0 (0.5)	ND	3.1 (0.4)
17:0	0.6 (0.2)	0.2 (0.2)	0.5 (0.3)
17:1(9)	1.5 (0.2)	4.2 (0.1)	1.5 (0.2)
17:2(5,9)	1.5 (0.2)	ND	1.6 (0.3)
18:0	1.2 (0.4)	1.6 (0.8)	1.0 (0.2)
18:1(9)	2.9 (0.2)	16.2 (1.7)	2.9 (0.4)
18:1(11)	30.3 (1.5)	52.8 (0.5)	31.2 (1.3)
18:2(5,9)	13.1 (0.7)	TR	13.6 (0.6)
18:2(5,11)	31.4 (0.7)	4.1 (1.3)	30.3 (1.1)
Others	2.2 (0.4)	TR	2.1 (0.3)
Saturated fatty acids	8.1	3.4	7.6
Monoenoic fatty acids	40.9	92.4	41.7
Dienoic fatty acids	51.2	4.1	50.7

acids, which were undetectable in the mutant. The presence of a residual amount of C18 dienoic acid in the *fadA* null mutant is presumably due to FadB, which acts mainly on C18 fatty acids, whereas FadA acts on both C16 and C18 fatty acids (Saito & Ochiai, 1999; Saito *et al.*, 2000). These results indicate that FadA is mainly responsible for the production of dienoic fatty acids, whereas FadB seems to have a supplementary function. We isolated several independent clones which shared the same phenotype and fatty acid composition and used two clones for further experiments. Table 3 shows the effect of temperature on the fatty acid composition of the *fadA* mutant. This shows that the higher temperature does not affect the fatty acid composition of the *fadA* mutant, but the lower temperature does. From this result, we speculate that the fatty acid composition of the *fadA* mutant is suitable for higher temperatures.

$\Delta 5$ fatty acid desaturation and temperature adaptation of growth

To see the relationship between $\Delta 5$ fatty acid desaturation and temperature adaptation of growth, the growth rate of the *fadA* null mutant cells was compared to their parent at different temperatures. We measured the growth rates of mutant cells in suspension and found that they grow at essentially the same rate as the wild-type at 22 °C. Unexpectedly, the growth rates of the mutant were also the same with wild-type cells, even at elevated temperatures. Fig. 2 shows typical examples of the growth curves at 22 and 27 °C. At 30 °C, both wild-type cells and the *fadA* null mutant could not grow and the number of cells was reduced.

Table 3. Effect of growth temperature on major fatty acid composition in the *fadA* mutant

Cells were grown in axenic medium at various temperatures. ND, Not detected; TR, trace (less than 0.2%); others, minor unidentified acids; $\Delta 5$ desaturation, total ratio of $\Delta 5$ desaturated fatty acids.

Fatty acid	Percentage (\pm SD) at:		
	15 °C	22 °C	25 °C
16:0	1.5 (0.1)	1.6 (0.4)	1.7 (0.2)
16:1(9)	26.3 (3.2)	19.2 (2.3)	17.3 (1.6)
16:2(5,9)	ND	ND	ND
17:0	TR	0.2 (0.2)	0.4 (0.3)
17:1(9)	4.6 (0.1)	4.2 (0.1)	4.2 (0.2)
17:2(5,9)	ND	ND	ND
18:0	0.6 (0.1)	1.6 (0.8)	1.3 (0.2)
18:1(9)	8.0 (0.3)	16.2 (1.7)	19.0 (0.7)
18:1(11)	46.6 (0.8)	52.8 (0.5)	51.5 (0.7)
18:2(5,9)	TR	TR	TR
18:2(5,11)	12.5 (2.1)	4.1 (1.3)	4.8 (0.9)
Others	TR	TR	TR
$\Delta 5$ desaturation	12.5	4.1	4.8

To examine the effect of this high-temperature stress, the number of the cells was counted after incubation at 30 °C for 24 h. Of the wild-type cells, 65.1% could be counted after 24 h heat shock compared to 66.0% for the *fadA* mutant.

Thus we conclude that fatty acid composition is not involved in the growth rate at elevated temperatures.

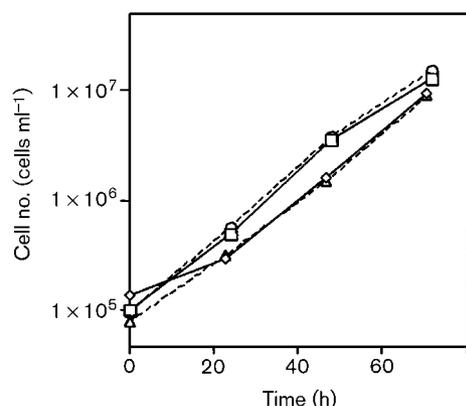


Fig. 2. Growth curves of *fadA* mutant and wild-type cells. Cells were transferred to fresh HL-5 medium at a density of 0.7×10^5 – 1×10^5 cells ml^{-1} . Growth was monitored by haemocytometer counts. The figure shows typical examples of four independent experiments. Squares, wild-type at 22 °C; circles, *fadA* mutant at 22 °C; diamonds, wild-type at 27 °C; triangles, *fadA* mutant at 27 °C.

$\Delta 5$ fatty acid desaturation and temperature adaptation of development

Development is triggered by starvation, but can also be regarded as a response to adverse conditions, possibly including extremes of temperature (Maeda, 1984). It may therefore be advantageous to *Dictyostelium* in the wild to be able to develop at temperatures considerably removed from their normal growth temperature. We therefore tested the ability of the *fadA* mutant and wild-type cells to develop at elevated temperatures, following growth at 22 °C. As a first step, we analysed how the fatty acid composition of mutant and wild-type cells changes during development. As can be seen in Table 4 and Fig. 3, the dienoic fatty acid composition of the *fadA* mutant showed a change at the slug stage even at 22 °C. The amount of dienoic acids increased from 4.1 to 17.6% at the slug stage; in contrast, the wild-type showed only a slight change at the slug stage (Fig. 3). To our surprise, we found that *fadA* mutant cells developed more efficiently than wild-type cells at 22 °C and this efficient development became clearer at elevated temperatures. At 27 °C, *fadA* mutant cells aggregated faster than wild-type cells. After 10 h incubation at 27 °C, mutant cells started to aggregate even under submerged conditions, but the wild-type cells remained as single cells (Fig. 4a, b). On the nitrocellulose filter, the development of the *fadA* mutant was also faster than the wild-type cells at 27 °C (Fig. 4c, d).

Table 4. Major fatty acid composition of *fadA* mutant in development

Cells grown at 22 °C in axenic medium were washed with buffer and spread on a phosphate-buffered agar plate (1.5%) at a density of 1×10^6 – 2×10^6 cells cm^{-2} . Slug stage, cells were harvested after 14–15 h incubation; culmination stage, cells were harvested after 17–21 h incubation. In each case, development was observed under a microscope. ND, Not detected; TR, trace (less than 0.2%); others, minor unidentified acids; $\Delta 5$ desaturation, total ratio of $\Delta 5$ desaturated fatty acids.

Fatty acid	Percentage (\pm SD) at:		
	Vegetative stage	Slug stage	Culmination stage
16:0	1.6 (0.4)	2.0 (0.6)	2.4 (0.7)
16:1(9)	19.2 (2.3)	16.6 (2.9)	13.9 (1.8)
16:2(5,9)	ND	ND	ND
17:0	0.2 (0.2)	1.9 (0.5)	2.4 (0.1)
17:1(9)	4.2 (0.1)	4.6 (0.6)	4.4 (0.3)
17:2(5,9)	ND	ND	ND
18:0	1.6 (0.8)	2.3 (1.0)	2.1 (1.2)
18:1(9)	16.2 (1.7)	14.7 (0.6)	14.8 (0.7)
18:1(11)	52.8 (0.5)	39.5 (2.0)	39.1 (0.3)
18:2(5,9)	TR	0.5 (0.1)	0.6 (0.1)
18:2(5,11)	4.1 (1.3)	17.1 (2.5)	19.5 (0.4)
Others	TR	0.6 (0.2)	0.8 (0.2)
$\Delta 5$ desaturation	4.1	17.6	20.1

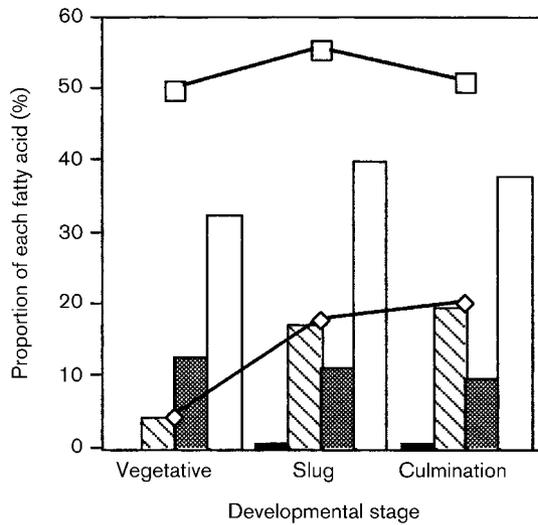


Fig. 3. Change in dienoic fatty acids during development. The ratio of dienoic fatty acids of the *fadA* mutant showed a change at the slug stage, but not so much in the wild-type cells. The total amount of $\Delta 5$ desaturated dienoic fatty acids increased from 4.2 to 17.6% in the *fadA* mutant (diamonds). On the other hand, it remained almost the same in the wild-type cells: 49.6% in the vegetative stage, 55.5% in the slug stage and 51.0% in the culmination stage (squares). Black bars, *fadA* mutant 18:2(5,9); hatched bars, *fadA* mutant 18:2(5,11); stippled bars, Ax2 18:2(5,9); white bars, Ax2 18:2(5,11).

Wild-type cells form fruiting bodies at 27 °C; however, they are less efficient at forming terminal fruiting bodies. We calculated spore formation activity at 27 °C after 40 h incubation. The mutant showed 77% spore formation

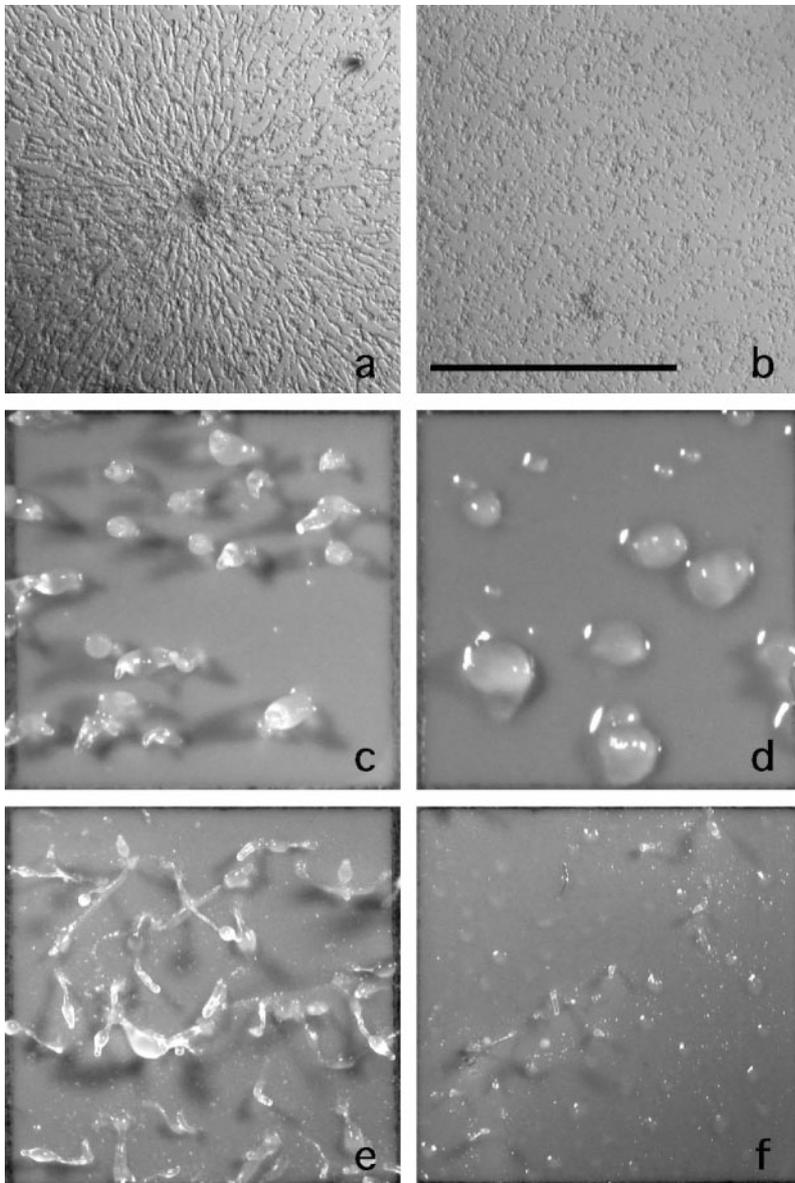


Fig. 4. Visible damage of thermostress. Cells of wild-type and the *fadA* null mutant were incubated at 22 °C, harvested and washed with phosphate buffer. Then cells were placed in a 12-well titre plate at a density of 5×10^5 cells cm^{-2} or on a nitrocellulose filter at a density of 2×10^6 cells cm^{-2} and incubated at 27 °C. Cells of the *fadA* null mutant formed a stream after 10 h under submerged conditions (a) and a tipped aggregate appeared after 10 h on the nitrocellulose filter (c). In contrast, wild-type cells remained as single cells after 10 h under submerged conditions (b) but started to form an aggregate after 10 h on the nitrocellulose filter (d). Bar, 1 mm. When incubated at 30 °C, the *fadA* mutant cells started to form fruiting bodies after 24 h (e) and completed their development by 30–40 h. Wild-type cells made small aberrant structures after 24 h (f) and then their development stopped.

activity compared to that at 22 °C, but the wild-type showed only 27% spore formation activity under the same conditions. To examine this temperature adaptation further the cells were incubated at 30 °C. The *fadA* mutant still managed to form fruiting bodies at this temperature, although they took longer than 24 h and the appearance was untidy. On the other hand, wild-type cells failed to form fruiting bodies in most cases under these conditions (Fig. 4e, f). These results suggest that the lower dienoic acid content of the *fadA* mutant results in a tolerance of elevated temperatures during development, but not during the growth phase.

DISCUSSION

In this report, we found a correlation between growth temperature and fatty acid composition in *Dictyostelium* cells, as shown in other organisms (Los & Murata, 1998). Our results show that in *Dictyostelium* the ratio of two C18 dienoic acids changes according to the temperature. This may explain the relationship between membrane fluidity and growth temperature, because the melting point of fatty acids changes according to the number and position of the double bonds. The change in the ratio of C18 dienoic acids does not seem to agree with the former report by Das *et al.* (1980) who found that there was no systematic temperature-dependent variation in fatty acid composition. This inconsistency was mainly due to the difference in the GC conditions used, since they could not separate C16:2(5,9), C17:0 and C18:2(5,9), C18:2(5,11). We have also found that FadA is mainly responsible for the production of the dienoic fatty acids of *Dictyostelium* and that mutant cells lacking this enzyme have a greatly reduced proportion of these fatty acids in their membranes.

The effect of growth temperature on *Dictyostelium* development was also analysed by Das *et al.* (1980). They reported that an increase in growth temperature enhanced development at higher temperatures. In this report, we found that wild-type cells grown initially at 22 °C were barely able to form fruiting bodies at 27–30 °C. In contrast, the *fadA* null mutant, with its lower ratio of dienoic to monoenoic fatty acids, could form fruiting bodies and spore quite efficiently at higher temperatures.

These results suggest that the regulation of the ratio of dienoic fatty acids is important in the development of *Dictyostelium* at elevated temperatures.

Many organisms are believed to adapt to environmental temperatures by altering the lipid and fatty acid composition of their membranes (Los & Murata, 1998). At high temperatures the degree of unsaturation is expected to decrease and at low temperatures it is expected to increase. Targeted mutagenesis of the gene for $\Delta 12$ desaturase results in drastic changes in cyanobacteria with a considerable decrease in polyunsaturated fatty acids. These strains show severe retardation of growth at lower temperatures (Tasaka *et al.*, 1996; Sakamoto *et al.*, 1997). In higher plants, the

ability to survive at low or high temperatures also correlates with the presence of polyunsaturated fatty acids. Mutants of *Arabidopsis thaliana* that have defects in *sn-2*-palmitoyl desaturase and $\Delta 12$ desaturase are characterized by leaf chlorolysis and growth retardation at low temperatures (Hugly & Somerville, 1992). Transgenic tobacco plants in which the ω -3 fatty acid desaturase gene has been silenced contain a lower level of trienoic fatty acids than wild-type plants and can better acclimatize to higher temperatures (Murakami *et al.*, 2000). These observations explain well the correlation between temperature adaptation and fatty acid composition. In general, the changes are such as to maintain membrane fluidity at a given temperature. This can be achieved by altering the proportion of sterols or saturated fatty acids in the membrane, both of which tend to decrease membrane fluidity (Gurr *et al.*, 2002).

Since fatty acids are the major components of the cell membrane, a decrease in dienoic acids in the *fadA* mutant might affect membrane fluidity and/or membrane microdomain structures, which might be important for membrane physiology and membrane protein functions.

Overall, this study demonstrates that optimization of cellular fatty acid composition is involved in temperature adaptation in *Dictyostelium* and that FadA plays an important role in this process.

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