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Phase behaviour of the membrane lipids of the thermophilic blue-green alga *Anacystis nidulans*

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The phase behaviour of total membrane lipid extracts of the blue-green alga *Anacystis nidulans* is compared with that of the individual lipid classes present in such extracts using fluorescence probe, differential scanning calorimetry, wide-angle X-ray diffraction and freeze-fracture techniques. Marked differences are observed in the properties of the isolated lipids as compared to the total lipid extracts. In particular, purified samples of monogalactosyldiacylglycerol and phosphatidylglycerol form complex high melting-point gel phases on storage which are not found in the membrane extracts. Addition of Mg^{2+} ions to the extracts is also shown to lead to an extensive phase separation of monogalactosyldiacylglycerol from the extracts. The enthalpy changes associated with phase separations occurring in the lipid extracts are found to be approx. 30% higher than those for the corresponding membranes, suggesting that the presence of other components, such as membrane proteins, may influence the phase behaviour of the lipids. The significance of these observations is discussed in terms of the factors limiting the stability of membrane systems.

Introduction

The thylakoid membranes of higher plant chloroplasts contain four main classes of polar lipids; monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), sulphoquinovosyldiacylglycerol (SQDG) and phosphatidylglycerol (PG). These same four lipid classes are also the main polar lipid components of the thermophilic blue-green alga *Anacystis nidulans*. The two sets of lipids differ very greatly, however, in their acyl substitution patterns. The membrane lipids found in higher plant chloroplasts contain high proportions of polyunsaturated residues while those of *A. nidulans* contain only saturated or monounsaturated residues (see Ref. 1 for a comparative review).

The presence of high proportions of polyunsaturated residues in the lipids of higher plant thylakoids is usually explained in terms of a requirement for ensuring an adequate membrane fluidity at the growth temperature of the plant. The gel-to-liquid crystal phase transitions of the MGDG and DGDG fractions of higher plant chloroplasts have, however, been estimated to occur at about $-35°C$ and $-55°C$, respectively [2]; far below the temperatures most plants are likely to encounter. Studies performed in this laboratory [3] suggest that the requirements for such residues in these lipids may be associated with the fact that aqueous dispersions of polyunsaturated molecular species of MGDG tend to form inverted hexagonal phases at normal physiological temperatures [2,4]. The ready formation of non-bilayer struc-

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tures, such as spherical and cylindrical inverted lipid micelles, in dispersions of mixtures of MGDG and DGDG isolated from higher plant chloroplasts and total polar lipid extracts of chloroplast membranes is well documented [5–10]. Equivalent structures are not normally found in the native membranes but they can be induced by heat stress [11] and by changes in pH and the action of phospholipases [12]. These observations have led to the suggestion that the ability of MGDG to form non-bilayer structures may be important in the packaging of the photosynthetic light-harvesting apparatus within the thylakoid membrane [13,14].

The more saturated membrane lipids found in *A. nidulans* undergo gel-to-liquid crystal phase transitions at temperatures well above 0°C. The occurrence of such transitions in the thylakoid membranes of intact cells, and in lipid extracts of such cells, has been demonstrated by differential scanning calorimetry [15,16], by wide-angle X-ray diffraction [17] and freeze-fracture electron microscopy [15,18–21]. The possible occurrence of bilayer/non-bilayer transitions in these systems has not, however, been investigated. In this paper, we report a series of studies on the phase behaviour of *A. nidulans* membranes and their lipid extracts and relate them to the phase behaviour of the individual lipids found in such membranes.

Materials and Methods

Cell culture. A. nidulans obtained from the Cambridge Culture Collection (U.K.) was grown in a modified version of medium C of Kratz and Myers [22] containing three times the normal amounts of iron. The cultures, which were maintained at 28 or 38°C, were bubbled with 5% CO_2 in air. They were illuminated by fluorescent lights providing an incident light intensity of approx. 4000 lux.

Lipid extraction and analysis. Cells were harvested in their logarithmic growth phase and their lipids extracted by the method of Kates [23]. The extract was concentrated to dryness, redissolved in a minimum volume of chloroform and passed through a short silicic acid column [24] using 3.5 column volumes of chloroform, chloroform/methanol (2 : 1, v/v) and methanol as eluants. The chloroform fraction, which contained the pigments and neutral lipids, was discarded. The other two fractions, which contained the polar membrane lipids were combined, dried under vacuum and redissolved in a minimum volume of chloroform/methanol (2 : 1, v/v). This crude polar lipid extract was then subjected to TLC on Silica gel G-60 plates with petroleum ether (60–80°C b.p.)/diethyl ether/acetic acid (8 : 2 : 1, v/v) as the developing solvent, to yield a purified total polar lipid extract. Samples of purified MGDG, DGDG, SQDG and PG were obtained from the original crude polar lipid extract eluted from the silicic acid column using a column of acid-washed Florisil [25,26] eluted with 3.5 column volumes of chloroform, chloroform/acetone (1 : 1, v/v) and 2 column volumes of acetone, chloroform/methanol (2 : 1, v/v), methanol. The chloroform fraction was again discarded. The chloroform-acetone fraction was concentrated to dryness and subjected to TLC on Silica gel G-60, using chloroform/methanol/water (65 : 25 : 4, v/v), to yield pure MGDG. The remaining fractions containing DGDG, SQDG and PG were combined and dried under vacuum. PG was separated from DGDG and SQDG on ammonium sulphate impregnated TLC plates developed with acetone/benzene/water (91 : 30 : 8, v/v) [27]. Finally, DGDG and SQDG were separated using the same TLC method employed for MGDG isolation.

The proportions of the different membrane lipids were determined by two-dimensional TLC of the total membrane lipid extracts followed by gas chromatography. The TLC plates were developed first with chloroform/methanol/water (65 : 25 : 4, v/v) and then with chloroform/methanol/acetic acid/water (85 : 15 : 10 : 3, v/v). The individual lipid spots were detected under ultraviolet illumination after spraying with 0.01% aqueous solution of Rhodamine 6G. Each spot was then extracted into chloroform/methanol (2 : 1, v/v), and analysed by gas chromatography [28] to determine its fatty acid composition.

Fluorescence measurements. The fluorescence probe 1,6-diphenyl-1,3,5-hexatriene (DPH), dissolved in ethanol, was added to the lipid dispersions in the ratio 1 probe molecule: 500 lipid molecules. The samples were incubated for about 30 min at temperatures above that of the gel-to-

liquid crystal phase transition. A Perkin Elmer MPF-44A fluorimeter fitted with polarisers on the excitation and detection sides of the sample was then used to measure the fluorescence polarisation of the samples calculated according to the equation:

$$P = \frac{(I_{\perp\perp}/I_{\parallel\parallel}) - (I_{\perp\parallel}/I_{\parallel\perp})}{(I_{\perp\perp}/I_{\parallel\parallel}) + (I_{\perp\parallel}/I_{\parallel\perp})}$$

where I is the intensity of fluorescence and the subscripts refer to the relative orientations of the two polarisers with respect to the reference frame of the laboratory.

Differential scanning calorimetry (DSC). DSC measurements were made using a Perkin Elmer DSC-2 with liquid nitrogen as the coolant and helium as the purge gas. Cells of *A. nidulans* harvested by centrifugation were resuspended in 1–2 ml of aqueous ethylene glycol (1 : 1, v/v) and recentrifuged. The pelleted cells (70–80 mg) were then sealed in stainless steel pans. Appropriate reference pans were made up using Sephadex G-150 swollen in aqueous ethylene glycol. Lipid samples were prepared and sealed in small aluminium pans as described elsewhere [29]. Calorimeter operating conditions were as specified in the figure legends. Enthalpy values were calculated from the recorder tracings with the aid of an Apple II microcomputer fitted with a graphics tablet. The lipid content of the samples was estimated by gas chromatography.

X-ray diffraction. Freeze-dried lipids dispersed in aqueous ethylene glycol (1 : 1, v/v) saturated with N_2 were sealed in thin-wall (0.01 mm) glass capillaries (1 mm diameter). Wide-angle X-ray diffraction patterns were measured using a Philips PW 1024 Debye-Scherrer powder camera. X-rays were produced using a Philips generator fitted with a copper fine-focus stationary anode tube.

Electron microscopy. Freeze-dried lipids were ultrasonically dispersed in either water or aqueous ethylene glycol (1 : 1, v/v) in the presence, or absence, of 10 mM $MgCl_2$. The dispersions were equilibrated for at least 10 min at the required quench temperature prior to freezing in a slurry of liquid and solid nitrogen. The samples were then fractured at −115°C in a Polaron E7500 unit and immediately shadowed with carbon-platinum. The replicas were washed with chloroform/methanol (2 : 1, v/v) and examined in a Philips EM301 electron microscope.

Results

Whole cells and membrane lipid extracts

Lipid composition. Details of the fatty acid content of the main classes of membrane lipids present in cells of *A. nidulans* grown at 38°C are set out in Table I. In agreement with earlier studies [30], adaptation to lower growth temperatures (results not shown) leads to an increase in palmitoleic acid (16 : 1) at the expense of palmitic acid (16 : 0) and a small increase in the proportion of MGDG to DGDG.

Fluorescence depolarisation. Measurements of the variation with temperature of the fluorescence polarisation, P, of DPH intercalated into aqueous dispersions of membrane lipid extracts of 28°C and 38°C grown cells are presented in Fig. 1. In both cases, there is a continuous decrease in the value of P over a span of about forty degrees suggesting that any phase transitions occurring in these dispersions are likely to be extremely broad. It is noteworthy that the polarisation values at the growth temperatures of the two sets of cells from which the lipids were extracted are almost identical. Similar correlations between growth temperature and membrane fluidity, as reflected by DPH fluorescence polarisation values [31], or spin-label

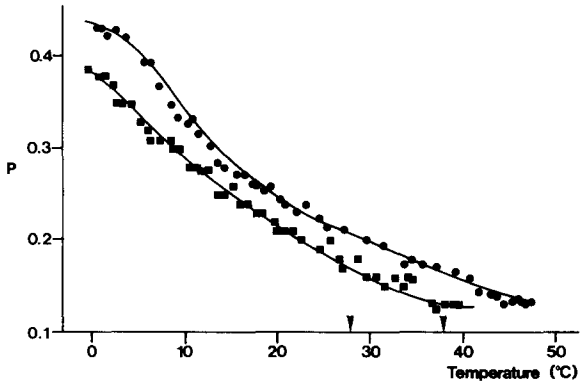

Fig. 1. Plot of fluorescence polarisation, P, of DPH intercalated into aqueous dispersions of membrane lipid extracts prepared from *A. nidulans* grown at 28°C (■) and 38°C (●) as a function of temperature.

TABLE I

FATTY ACID COMPOSITION OF THE LIPID CLASSES ISOLATED FROM *A. NIDULANS* GROWN AT 38°C

Lipid cells	Fatty acid composition (mol%) [a]				Double bond index
	16:0	16:1	18:0	18:1	
MGDG	44.3±0.5	41.3±0.9	2.2±0.5	12.2±0.5	1.07±0.02
DGDG	49.0±0.7	39.6±1.7	1.8±0.8	9.6±1.6	0.98±0.02
SQDG	52.4±1.1	34.4±1.1	2.5±0.5	8.9±1.1	0.87±0.02
PG	47.5±0.8	32.4±3.1	4.0±1.5	16.1±2.4	0.97±0.02

[a] Values are averages of eight independent measurements ± S.E.

mobility [32], have previously been reported for lipids extracted from higher plant chloroplasts.

DSC studies. Typical thermograms obtained from cells of *A. nidulans* grown at 28°C and 38°C are shown in Fig. 2a. The corresponding thermograms for total membrane lipid extracts of such cells are presented in Fig. 2b. In all cases the transitions, as predicted from the fluorescence studies, are extremely broad. Estimated values of the temperatures corresponding to the maxima and the upper and lower limits of these transitions are listed together with their corresponding en-

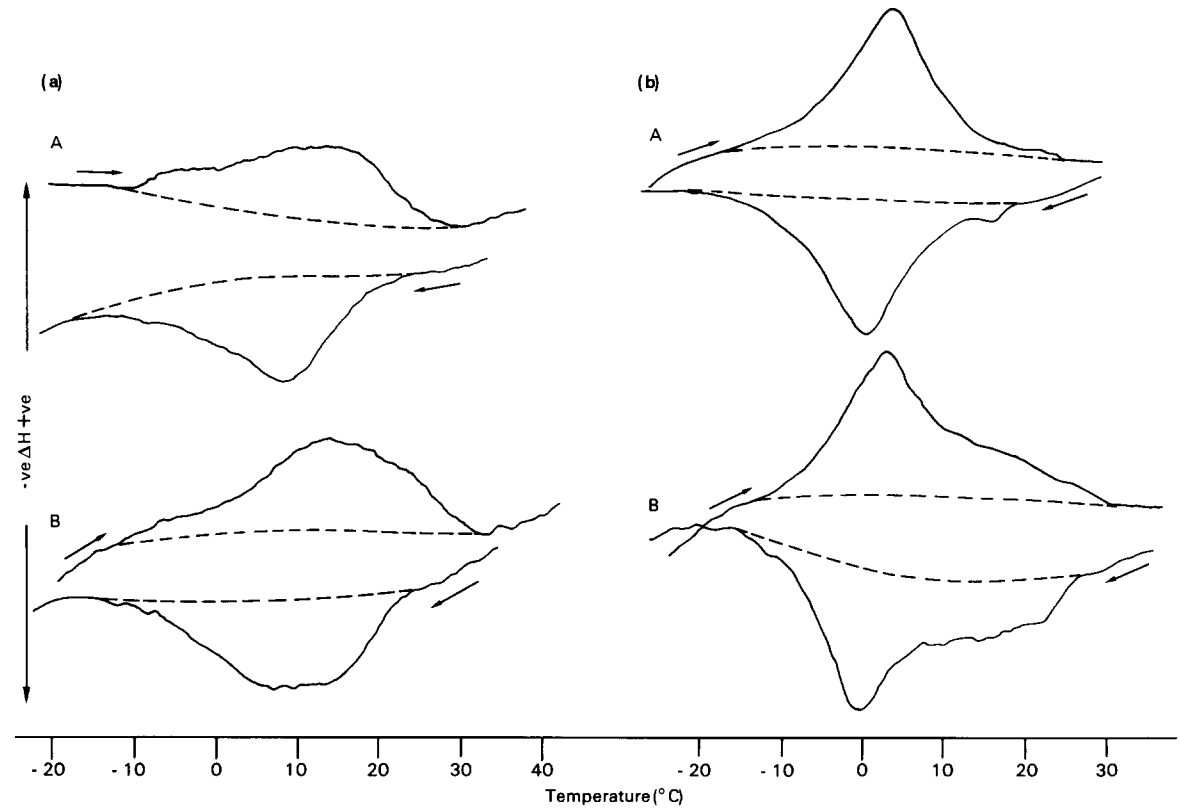

Fig. 2. Thermograms of (a) cells of *A. nidulans* grown at (A) 28°C and (B) 38°C and (b) total lipid extracts of these cells. Samples were prepared as described in methods section. Measurements were performed at a scan rate of 5 Cdeg·min^{-1} on a sensitivity setting of 0.2 mcal·s^{-1}.

TABLE II

TRANSITION TEMPERATURES AND ENTHALPY VALUES FOR CELLS AND TOTAL MEMBRANE LIPID EXTRACTS OF *A. NIDULANS* GROWN AT 28°C AND 38°C

Sample	Transition temperature (°C) [a]		ΔH (kJ·mol^{-1})	
	Endotherm	Exotherm	Endotherm	Exotherm
28°C	−13	−17		
extract	2	0	17.1	−16.1
	24	19		
38°C	−10	−12		
extract	4	1	15.4	−15.2
	10	26		
28°C	−10	−14		
cells	14	10	11.7	−13.3
	28	21		
38°C	−7	−9		
cells	14	14	12.8	−12.9
	31	26		

[a] Temperatures correspond to the on-set, maximum and end-point of the transitions, respectively.

thalpy values in Table II. The transitions show a certain amount of hysteresis and the exotherms are normally displaced 5–10 Cdeg towards lower temperatures with respect to their corresponding endotherms. This displacement is much smaller than that originally reported by Furtado et al. [15] but is in good agreement with the recent findings of Ono et al. [16].

X-ray diffraction studies. Typical examples of wide-angle X-ray diffraction patterns obtained from total membrane lipid extracts of *A. nidulans* grown at 28°C and 38°C are presented in Fig. 3. Depending on the temperature at which the measurement is carried out, the patterns are dominated either by a sharp 0.42 nm diffraction maximum or a diffuse 0.46 nm maximum which reflect the packing of the acyl chains of the lipids in their gel and liquid-crystalline phases, respectively, [33]. As the measurement temperature is increased there is a progressive replacement of the sharp 0.42 nm maximum by the diffuse 0.46 nm maximum reflecting the melting of the gel-phase. Measurements (not shown) of the relative intensity of the 0.42 nm maximum as a function of temperature for the two sets of samples indicate that there is a sharp increase in the proportion of lipids in the

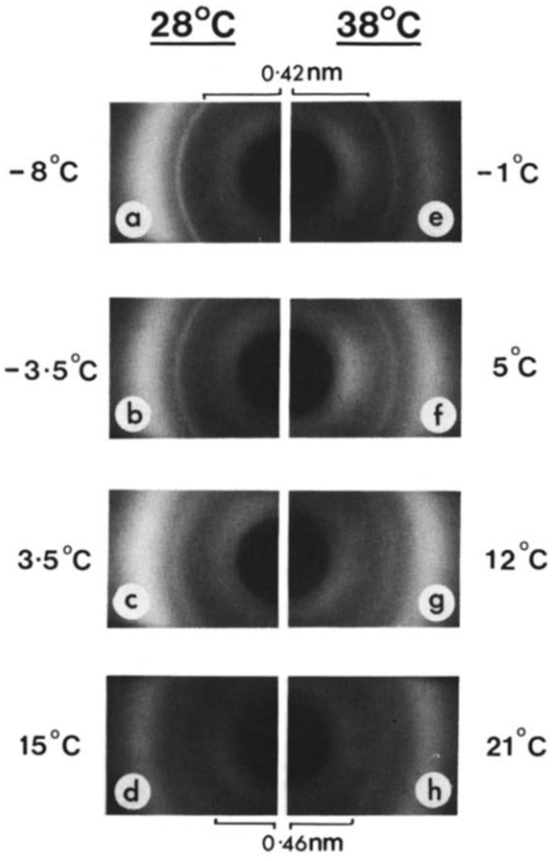

Fig. 3. Wide-angle X-ray diffraction patterns of total membrane lipid extracts of *A. nidulans* grown at 28°C (a–d) and 38°C (e–h). Measurement temperatures are indicated alongside the individual patterns.

liquid-crystalline phase at temperatures above 0°C in the case of lipids extracted from cells grown at 28°C. This increase was found to be much more gradual in the case of 38°C grown cells.

Freeze-fracture studies. Freeze-fracture replicas from total membrane lipid extracts of *A. nidulans* dispersed in distilled water were characterised by the presence of large numbers of liposomes of differing diameter (Fig. 4). No obvious differences in morphology were observed between samples prepared from lipids isolated from cells grown at 28°C or 38°C thermally quenched from temperatures in the range of 2 to 45°C. Sen et al. [5,7] have shown that high concentrations of cryoprotectants leads to extensive phase-separation, and hence the formation of large numbers of spherical inverted lipid micelles, in dispersions of mixtures of MGDG

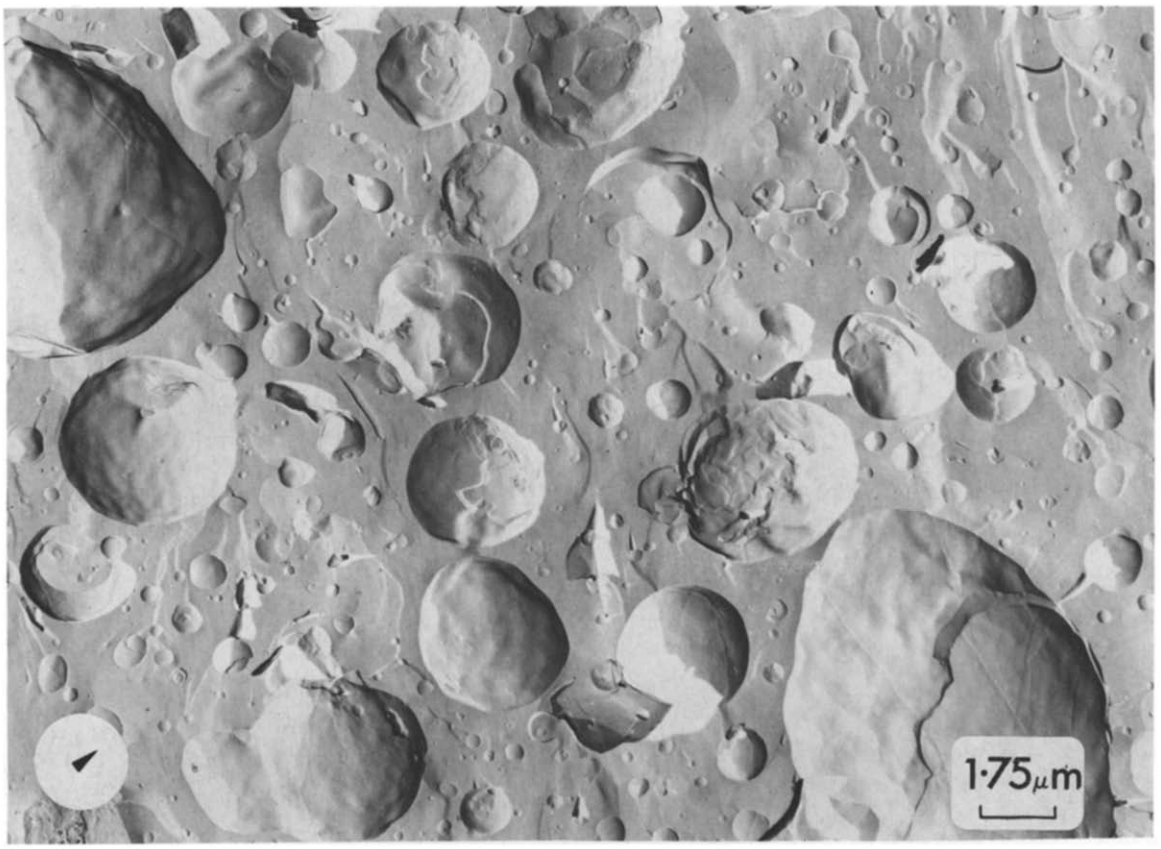

Fig. 4. Freeze-fracture electron micrographs of polar lipid extracts of *A. nidulans* grown at 38°C, dispersed in distilled water and thermally quenched from 45°C.

and DGDG isolated from higher plant chloroplasts. Dispersions of lipid extracts of *A. nidulans* made up in ethylene glycol/water (1 : 1, v/v), however, showed little evidence of phase separation.

Addition of divalent metal cations also facilitates phase separation in dispersions of lipids isolated from higher plant chloroplasts [3,8]. In the case of lipids isolated from *A. nidulans*, addition of 10 mM $MgCl_2$ led to extensive phase separation. The phase-separated lipids, however, were normally in the form of slightly curved lamellar sheets (Fig. 5a) rather than in the inverted micellar form typical of the more unsaturated lipids found in higher plants. Particulate structures, similar in diameter to the inverted spherical micelles seen by Sen and his co-workers, were sometimes observed, especially in the boundary regions between liposomes and the phase-separated sheets (Fig. 5b), but they were comparatively rare features. Little difference was observed on thermally quenching such dispersions from temperatures between 2 and 45°C. However, raising the quenching temperature to 60°C (Fig. 5c) led to a complete replacement of the phase-separated lamellar sheets by regions of inverted hexagonal (Hex_{II}) lipid.

Individual lipid classes

DSC studies. Typical thermograms obtained from samples of purified PG, DGDG and SQDG are presented in Fig. 6. In each case, the thermograms are characterised by a large peak together with an accompanying smaller high-temperature peak or shoulder. The main peaks probably correspond to molecular species containing di-C_{16} fatty acids, which predominate in *A. nidulans* [30], and the high-temperature features to species containing C_{18} substituents. Corresponding thermograms for

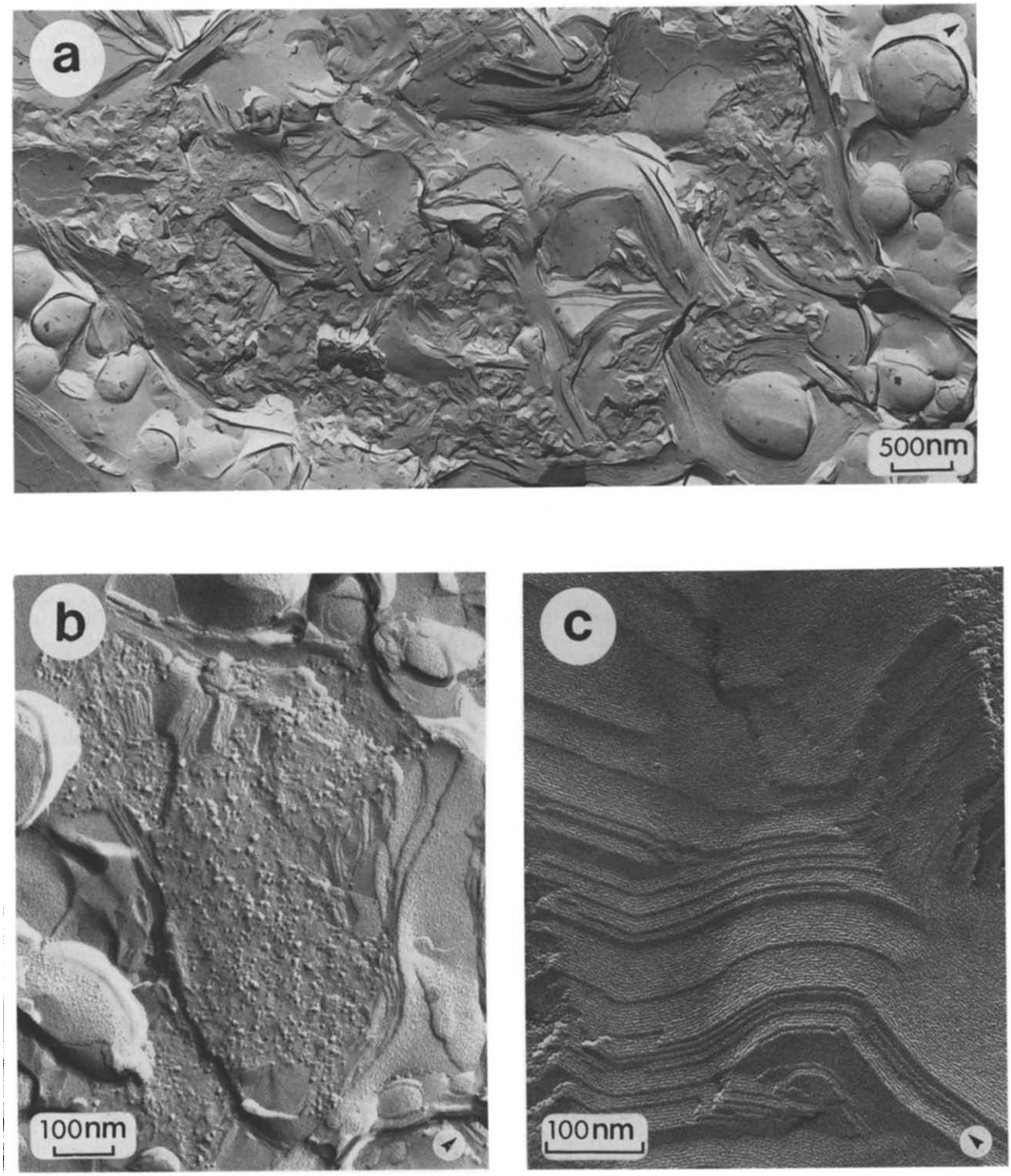

Fig. 5. Freeze-fracture electron micrographs of polar lipid extracts of *A. nidulans* grown at 28°C dispersed in aqueous ethylene glycol (1 : 1, v/v) containing 10 mM $MgCl_2$ thermally quenched from (a) 4°C, (b) 20°C and (c) 60°C. See text for explanations.

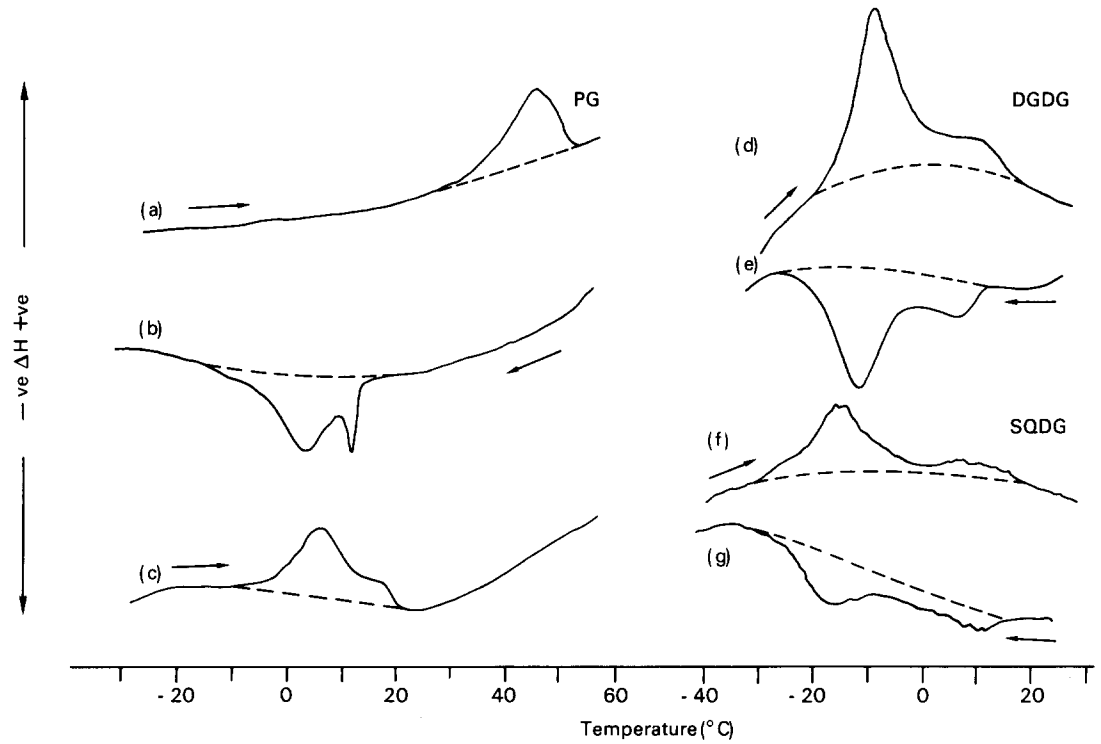

Fig. 6. Thermograms of PG, DGDG and SQDG isolated from *A. nidulans* grown at 38°C. (a) initial heating of PG sample stored at 4°C for 7 days (b) and (c) subsequent cooling and heating thermograms. Traces (d)–(g) heating and cooling thermograms for DGDG and SQDG. The scan rates were 10 Cdeg·min^{-1}. Curve (a) was measured on a 1 mcal·s^{-1} sensitivity scale and curves (b) to (g) on a 0.5 mcal·s^{-1} scale.

MGDG can be found in Ref. 34. The transition temperatures and enthalpy values for all four lipids are listed in Table III.

The phase behaviour of PG is strongly influenced by the thermal history of the samples. Cooling from the liquid-crystalline phase leads initially to the formation of an intermediate lower melting-point gel phase which reverts to a higher melting-point form on storage. This is reflected in the very different endotherms seen in the initial and second heating runs shown in Fig. 6a. Similar changes are seen for MGDG [29,34] but not for DGDG and SQDG. The higher and lower melting-point forms of PG will be referred to as PG_I and PG_{II}, respectively, in agreement with the convention of Sen et al. [29] for MGDG.

X-ray diffraction. Examples of wide-angle X-ray diffraction patterns for the purified lipids are presented in Fig. 7. The patterns obtained from MGDG and PG again depend on the history of the samples. Samples of MGDG that have been stored at low temperatures are characterised by two sharp maxima at about 0.41 and 0.45 nm, of the type reported by Sen et al. [29] for the $MGDG_I$ form of the distearoyl derivative, whilst samples measured shortly after crystallisation from the liquid-crystal state yielded the usual 0.42 nm maximum typical of the $MGDG_{II}$ form. Corresponding patterns were observed for the PG_I and PG_{II} forms. DGDG and SQDG were characterised by a single sharp maximum in the gel state of the type seen for $MGDG_{II}$ and PG_{II}. All four lipids showed the usual diffuse maximum at about 0.46 nm in the liquid-crystalline state.

Freeze-fracture studies. Replicas prepared from aqueous dispersions of DGDG, SQDG and PG were characterised by the presence of conventional liposome structures of the type normally seen for bilayer-forming lipids. Replicas prepared from MGDG, in contrast, showed slightly curved lamellar sheets closely resembling those seen for the total lipid extracts when the lipid samples were

quenched from temperatures below about 45°C or Hex_{II} structures when quenched from higher temperatures (Figs. 8a and 8b). An intermediate structure consisting of spherical inverted lipid micelles sandwiched within lipid bilayers was occasionally seen in samples that had been first preheated, to form the Hex_{II} phase, and then rapidly cooled to about −20°C just prior to thermal quenching (Fig. 8c). Such structures, however, are almost certainly intermediate states of very limited stability trapped in the reversion of the liquid-crystalline state to the gel phase.

TABLE III

TRANSITION TEMPERATURES AND ENTHALPIES OF PURIFIED POLAR LIPID CLASSES DISPERSED IN AQUEOUS ETHYLENE GLYCOL (1 : 1, v/v)

Temperature values are averages of ten estimates and enthalpy values an average of five estimates using at least two independent samples.

Lipid	Sample thermal history	Transition temperature (°C)	ΔH ($kJ \cdot mol^{-1}$)
MGDG	Stored at 4°C initial heating endotherm	37.6 ± 0.5	51.4 ± 2.7
	Cooling exotherms	16.7 ± 0.4 37.7 ± 0.3	−13.2 ± 0.4 −2.3 ± 0.1
	Second heating endotherms	5.7 ± 0.5 39.8 ± 0.4	11.5 ± 0.2 2.3 ± 0.1
DGDG	Heating endotherm	−15.8 ± 0.1	24.4 ± 1.0
	Cooling exotherm	−2.0 ± 0.1 11.1 ± 0.1	−22.1 ± 0.7
SQDG	Heating endotherm	−24.3 ± 0.2	18.4 ± 1.0
	Cooling exotherm	−5.0 ± 0.3 14.7 ± 0.3	−17.5 ± 1.4
PG	Stored at 4°C initial heating endotherm	36.5 ± 0.4	46.8 ± 1.8
	Cooling exotherm	12.9 ± 0.1	−20.8 ± 0.6
	Second heating endotherm	−3.2 ± 0.2	24.1 ± 1.1

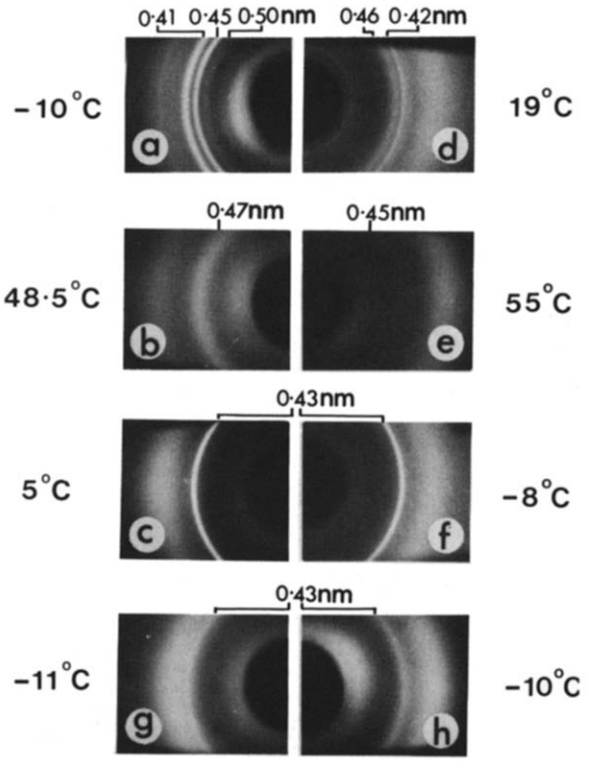

Fig. 7. Wide-angle X-ray diffraction patterns of samples of MGDG, DGDG, isolated from 38°C grown *A. nidulans*. The MGDG and PG samples were initially stored for 7 days at 4°C and −25°C, respectively, in order to allow them to revert to the $MGDG_I$ and PG_I forms (patterns (a) and (d)). They were then heated to yield the corresponding liquid-crystal forms (patterns (b) and (c)) and finally re-cooled to yield the $MGDG_{II}$ and PG_{II} forms (patterns (e) and (f)). The gel-phase patterns of DGDG and SQDG (g) and (h)) were independent of the thermal history of the samples.

Discussion

DSC, fluorescence polarisation and wide-angle X-ray diffraction measurements (Figs. 1–3) all indicate that the gel-to-liquid crystal phase transition occurring in the membranes of *A. nidulans*, or in total membrane lipid extracts of such cells, takes place over a broad temperature range spanning about forty degrees. The temperature ranges for the lipid extracts (Table II) are in good agreement with those recently reported for similar extracts by Ono et al. [16]. Any minor differences between the two sets of results are well within the limitations imposed by difficulties in determining

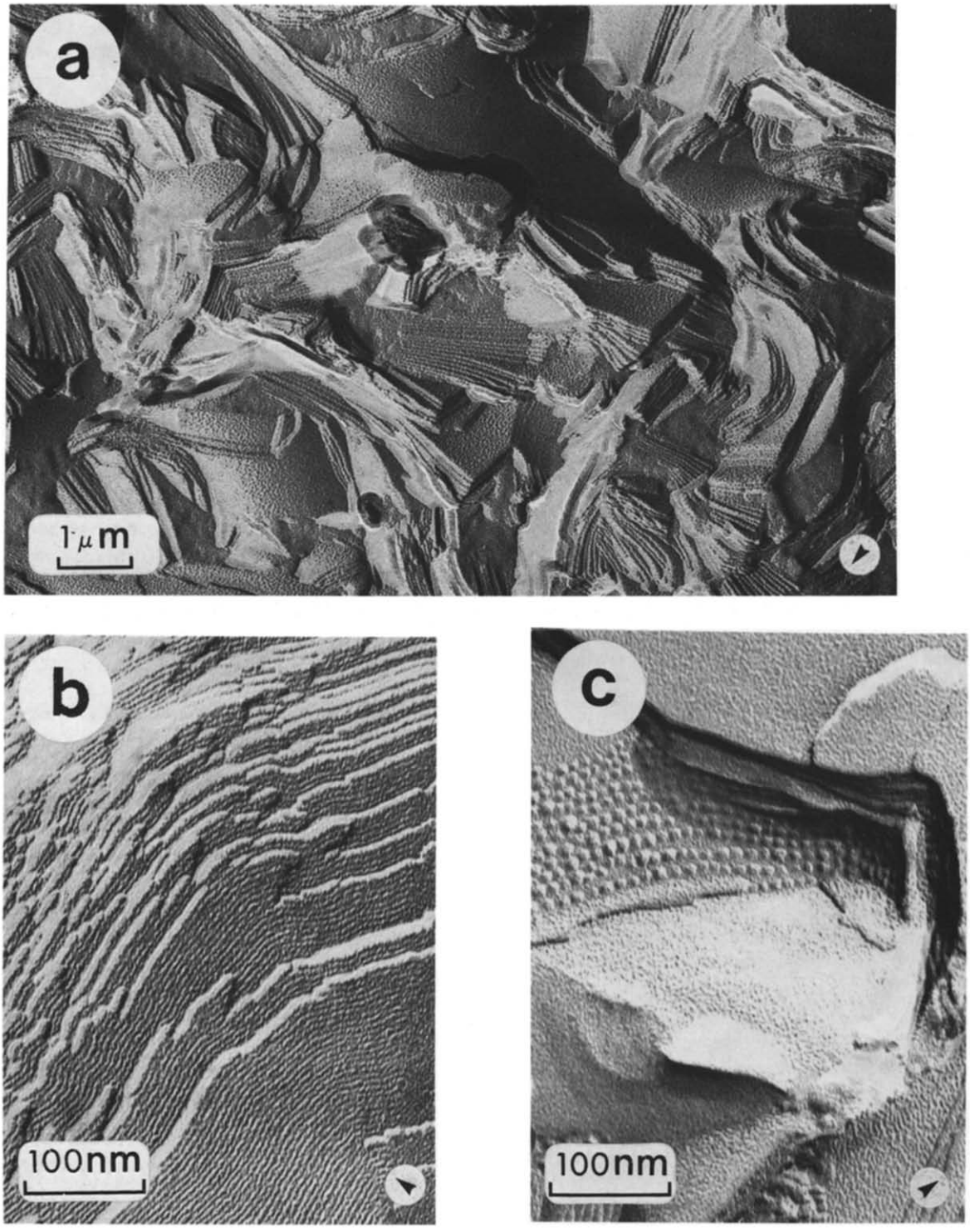

Fig. 8. Freeze-fracture electron micrographs of samples of MGDG isolated from *A. nidulans* grown at 38°C dispersed in distilled water and thermally quenched (a) from 28°C, shortly after pre-heating to 55°C (b) direct from 55°C and (c) from −20°C following rapid cooling from 55°C. See text for explanations.

the precise position of the base-line in such measurements and variations in lipid composition associated with minor differences in growth conditions.

Comparison of thermograms obtained from whole cell preparations with those obtained from the corresponding lipid extracts (Fig. 2) reveals major differences. In particular, while the overall temperature ranges for the different transitions are very similar, the temperatures of their maxima are appreciably different. The maxima seen for the whole cells are typically shifted to temperatures about 10 Cdeg higher than those for the corresponding extracts. These differences are unlikely to be associated with hysteresis effects as they occur for both the endothermic and exothermic transitions. The enthalpy values for the whole-cell transitions were also consistently about 30% lower than those for the extracts. Some caution must, however, be applied in interpreting the significance of these values as they are strongly influenced by choice of the appropriate base-line. Nevertheless, they suggest that other factors, such as lipid-protein interactions, may be playing a part in determining the phase behaviour of the intact membranes.

The thermograms obtained for the membrane extracts are not simply weighted sums of the contributions of the individual lipids. In agreement with earlier studies [29,35,36], the DSC and X-ray diffraction data reported here indicate that MGDG and PG can exist in their purified state in either of two polymorphic forms depending on the thermal history of the samples. However, as in the case of the membrane lipids extracted from higher plant chloroplasts, [3], only the lower melting-point polymorphs, $MGDG_{II}$ and PG_{II} are found in the total lipid extracts of *A. nidulans.* The formation of the higher melting-point $MGDG_{I}$ and PG_{I} forms appears to be supressed both in the extracts and the native membranes. The transitions associated with these two lipids, which account for about 50% and 25% of the total membrane lipids, respectively, are also shifted to lower temperatures in the extracts than in the purified lipid samples. The maximum of the endothermic transition for the extract is at about 4°C whilst the corresponding maxima for MGDG and PG are at about 15°C and 6°C. At the same time, the maxima for the lower melting-point lipids DGDG and SQDG, which are at −14°C and −6°C for the purified lipids, appear to be displaced to higher temperatures. Shifts of this type are often seen for mixtures of related membrane lipids and are believed to reflect the formation of monotectic solutions [37].

Studies performed on membrane lipid extracts of higher plant chloroplasts [3,8] indicate that the addition of metal cations, particularly divalent cations such as Mg^{2+}, leads to an extensive phase separation of the non-bilayer forming lipid MGDG. This is thought to reflect an initial phase-separation of the acidic lipids which leaves a bulk lipid phase consisting mainly of MGDG and DGDG which is then itself capable of undergoing phase separation [8]. In the case of the lipids isolated from higher plant chloroplasts, the phase-separated MGDG is in the liquid-crystalline state and hence tends to form spherical or cylindrical inverted lipid micelles. The more-saturated forms of MGDG present in *A. nidulans* normally form lamellar sheets typical of gel-phase lipid (Figs. 5a and 8b). Similar structures are also seen in hydrogenated extractes of high plant chloroplast lipids [3]. Occasional areas of nonbilayer structures are seen in extracts prepared from *A. nidulans* but it is only at high temperatures (> 50–60°C) that such structures become common (Fig. 5c).

A number of groups have pointed out that lowering the growth temperature leads to changes in the lipid composition [38,39], or lipid/protein ratio [40,41], of the thylakoids of higher plant chloroplasts. These changes are usually interpreted as being part of a homeostatic mechanism for the maintenance of an effectively constant membrane fluidity. It is important to note, however, that the phase-separation of non-bilayer forming lipids is also influenced by lipid saturation and chain length. The changes in lipid composition of *A. nidulans* with growth temperature thus may also play a role in preventing the phase-separation of the non-bilayer forming lipid MGDG in such membranes. It remains to be determined which of these factors is of the greater physiological significance.

Acknowledgements

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