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Abortive Assembly of the Lactose Transport System in *Escherichia coli*†

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ABSTRACT: Induction of the lactose operon of *Escherichia coli* has been studied in an unsaturated fatty acid auxotroph grown at different temperatures with a variety of essential fatty acid supplements. Induction of a fully functional transport system, as deduced from the ratio of transport activity to the activities of either β-galactosidase or thiogalactoside transacetylase, proceeds normally with decreasing growth temperature until a critical temperature is reached. At this temperature, induction of the transport activity becomes abortive. The critical temperature at which abortive transport system induction is first observed varies with the essential fatty acid supplement employed, and is identical with the temperature at which transport has previously been demonstrated to undergo a physiological transition. The critical temperature for abortive transport induction is also identical to the temperature at which the membrane lipids undergo a change of state that can be detected by physical probes. The phenomenon of abortive transport induction is not a reflection of any gross defect in complex lipid biosynthesis, nor is it caused by any substantial increase in the lability of the transport system, since preformed transport activity is not markedly labilized below the critical temperature for abortive induction. The most likely explanation for abortive transport system induction is abortive assembly, which occurs when transport is induced at a temperature where both the preexisting and newly synthesized membrane lipids are in a nonfluid, immobile state.

The activities of numerous membrane systems are highly sensitive to the fatty acid composition of the membrane lipids (Fox, 1972; Getz, 1972). Mutants which require unsaturated fatty acids have enabled investigators to alter the fatty acid composition of membrane lipids in microorganisms (Resnick and Mortimer, 1966; Silbert and Vagelos, 1967; Silbert *et al.*, 1968; Keith *et al.*, 1969). Processes such as cellular growth, transport, and respiration have markedly different responses to temperature in mutants grown with different unsaturated fatty acid supplements (Schairer and Overath, 1969; Wilson *et al.*, 1970; Overath *et al.*, 1970; Fox *et al.*, 1970; Wilson and Fox, 1971a; Overath *et al.*, 1971; Raison *et al.*, 1971), and the morphogenesis of transport systems and the cellular respiratory apparatus is highly dependent upon the cellular ability to synthesize complex lipids containing unsaturated fatty acids (Fox, 1969; Proudlock *et al.*, 1969). Using unsaturated fatty acid auxotrophs of *Escherichia coli*, Overath and his colleagues and workers in this laboratory have demonstrated transitions in β-galactoside transport as a function of the temperature for transport assay (Wilson *et al.*, 1970; Overath *et al.*, 1970). Arrhenius plots which describe transport rate as a function of assay temperature are biphasic in slope, intersecting at a point which has been termed the transition temperature. The transition temperatures for the independent β-galactoside and β-glucoside transport systems are identical when assayed in cells grown with the same unsaturated fatty acid supplement, and are modified in parallel when the cells are grown with different unsaturated fatty acid supplements. The identical response of two transport systems which share no common functional protein to an alteration in lipid fatty acid composition was interpreted to be the result of a change of state in the membrane lipids (Wilson *et al.*, 1970). This conclusion is supported by the results of a study which compares the transition temperatures for physiological functions with the onset temperature for a change in physical properties of the extracted membrane lipids (Overath *et al.*, 1970).

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The transition temperature for transport correlates well with the critical temperature for a change of state detected in a phospholipid monolayer at the air–water interface, where lipids extracted from the cells utilized for measuring the physiological transitions were used for the monolayer studies. The critical temperature for a change of state in a phospholipid monolayer also correlated in some instances with the temperature at which transitions were observed in other physiological phenomena, such as respiration and cellular growth.

The influence of membrane lipid fatty acid composition on cellular growth is of particular interest. The correlation between the critical temperature for a change of state detected in extracted phospholipids in monolayers and the temperature below which cellular growth ceases or is markedly impeded indicates that certain crucial events in membrane assembly, or perhaps membrane assembly in general, may be exquisitely dependent upon the physical state of the membrane lipids. In order to further define the influence of lipid physical properties on membrane assembly, we have studied in detail the effects of lipid fatty acid composition and temperature on the morphogenesis of a single, well-described membrane system, that responsible for lactose transport.

Methods

Growth of Bacteria. The properties of strain 30E, an unsaturated fatty acid auxotroph of *Escherichia coli* K12, are described elsewhere (Fox *et al.*, 1970). Growth was in medium A (Davis and Mingioli, 1950) supplemented with 1% Difco casamino acids, 5 μg/ml of thiamine·HCl, 0.5% Triton X-100 (Rohm and Haas), and the essential fatty acid at 0.02%.

Chemicals. Oleic (*cis*-Δ⁹-octadecenoic) acid was purchased from Hormel Institute, Austin, Minn., and elaidic (*trans*-Δ⁹-octadecenoic) acid from the Aldrich Chemical Co., Milwaukee, Wis. The synthesis of bromostearic acid (a mixture of the 9-bromo and 10-bromo derivatives) has been described (Fox *et al.*, 1970). Isopropyl 1-thio-β-D-galactopyranoside (iPrSGal)[1] was purchased from Sigma Chemical Co., St. Louis, Mo.; NphGal from Pierce Chemical Co., Rockford, Ill.; and $SGal_2$ was synthesized by the method of Cerny *et al.* (1963). Acetate-labeled [^{3}H]acetyl-CoA (980 mCi/mol) and [9,10-^{3}H]oleic acid were purchased from New England Nuclear, Boston, Mass. [^{3}H]Elaidic acid was synthesized using the technique described by Fusari *et al.* (1951). A mixture of 880 mg of oleic acid and 2 mCi of [^{3}H]oleic acid (5 mCi/0.62 mg) was isomerized by heating under nitrogen gas for 10 min at 185–203° in the presence of 8.8 mg of selenium. The reaction mixture was then cooled, dissolved in acetone, and filtered through a fine fritted glass filter to remove selenium. The product was crystallized at −20° from acetone, recrystallized from acetone at −7°, and dried under vacuum. The weight of the product was 236 mg, a 26.8% yield based on the weight of precursor oleic acid. The melting point was 45.2–45.6° (uncor), and the specific radioactivity was 1.44×10^6 cpm/mg. The methyl ester of [^{3}H]elaidic acid was subjected to gas chromatography and had a retention time identical to that of a commercial sample of methyl elaidate (Hormel Institute).

Induction and Assay of Transport and Enzymes. Cultures of strain 30E were grown in exponential phase with aeration by rotary agitation to a culture density of 5×10^8 cells/ml and shifted to growth at the temperature to be used for induction. After an incubation at this temperature for at least 10 min, induction of the proteins of the lactose operon was initiated by the addition of iPrSGal at 0.5 mM final concentration. Cell samples for the assay of transport and enzymes of the lactose operon were prepared as previously described (Wilson *et al.*, 1970). Transport was assayed by measuring the rate of NphGal hydrolysis mediated by intact cells (Wilson *et al.*, 1970). The fatty acid used for growth was included in the transport assay incubation mixture at 0.01% (oleic and elaidic acids) or 0.005% (bromostearic acid). All transport assays were performed at 30°. After cell disruption by sonic irradiation, β-galactosidase was assayed at 30°, except where otherwise indicated in the text. The same reaction mixture utilized for transport assay was employed, except that detergent and fatty acid were omitted. The assay system for thiogalactoside transacetylase contained 20 μmol of pH 7.2 potassium phosphate buffer, 0.3 μCi of [^{3}H]acetyl-CoA, 0.035 mmol of iPrSGal, and cell extract in a total volume of 0.2 ml. Control reactions were incubated in the absence of iPrSGal. The procedure was that described by Fox and Kennedy (1967), except that the acetyl-CoA concentration was 1.4 μM. The concentrations of cell extract (heated for 10 min at 65° before assay to inactivate phosphotransacetylase activity) were such that less than 10% of the radioactive substrate was utilized.

Extraction and Analysis of Lipids. For lipid extraction, cells were suspended in 5 ml of methanol and heated for 10 min at 60°. Ten milliliters of chloroform was added, and the mixture was incubated for 16 hr at room temperature. Nonlipid radioactivity was eliminated by equilibrating the chloroform phase 3 times with 10 ml of a 2 M KCl solution. Aliquots of the chloroform phase were then assayed directly to determine the total incorporation of [^{3}H]elaidic acid into phospholipids. Individual classes of phospholipids were separated on silicic acid thin-layer plates (Analtech, Inc.) which were developed with chloroform–methanol–acetic acid (7:3:1, v/v). The positions of individual phospholipid classes were visualized by exposing the plates to iodine vapor, and radioactivity was quantitated by scraping the appropriate areas of silica gel into vials for the determination of radioactivity. Radioactivity was determined by liquid scintillation counting employing the solution described by Patterson and Green (1965) containing 3:1 (v/v) of toluene and Triton X-100.

Protein Determinations. Protein was determined by the method described by Lowry *et al.* (1951).

Results

We have utilized the lactose transport system as a probe for studying membrane assembly in a number of previous instances (Fox, 1969; Hsu and Fox, 1970; Wilson and Fox, 1971a,b). The operator proximal and distal genes of the operon code for the synthesis of the soluble enzymes, β-galactosidase and thiogalactoside transacetylase, and the actual amount of synthesis of these proteins can be readily assessed from activity measurements (Zabin, 1963). The gene which codes for the transport protein (M protein) is located between the genes which code for the synthesis of the soluble enzymes of the operon (Fox and Kennedy, 1965; Fox *et al.*, 1967). Thus the efficiency of transport induction can be determined by comparing the extent of transport induction with the extent of induction of β-galactosidase or thiogalactoside transacetylase. A comparison of the ratio of induced β-galactosidase (or thiogalactoside transacetylase) activity to transport activity can therefore yield a valid estimate of the

[1] Abbreviations used are: iPrSGal, isopropyl 1-thio-β-D-galactopyranoside; NphGal, *o*-nitrophenyl β-D-galactopyranoside; $SGal_2$, thiodigalactoside, β-D-galactosyl 1-thio-β-D-galactopyranoside.

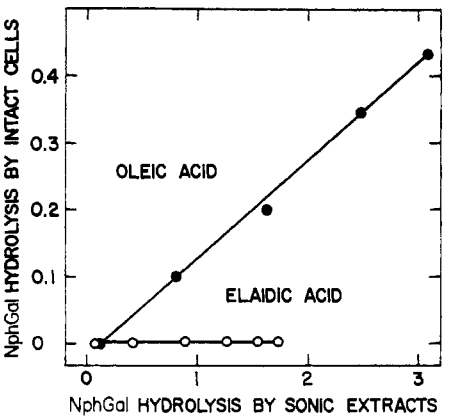

FIGURE 1: Efficiency of lactose transport induction at 25° in media supplemented with elaidic acid or oleic acid. Cultures of strain 30E were grown as described in Methods in media supplemented with elaidic acid (○) or oleic acid (●) at 37°. At a culture density of 5×10^8 cells/ml, the cultures were shifted to 25° and induced for the proteins of the lactose operon. Aliquots of cells were removed at various times after initiating induction, and were processed for the determination of β-galactosidase and transport activities. Assays for transport (NphGal hydrolysis by intact cells) and β-galactosidase (NphGal hydrolysis by extracts) were performed at 30° as described in Methods. Units are μmol of *o*-nitrophenol released from NphGal hr^{-1} mg^{-1} of protein.

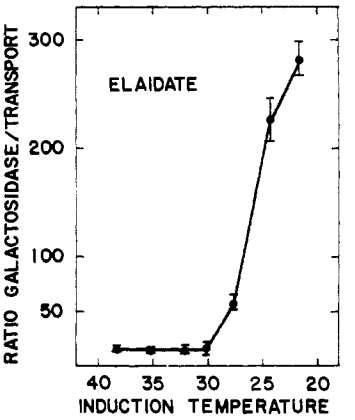

FIGURE 2: Relative efficiency of β-galactosidase induction to transport induction at various temperatures in medium supplemented with elaidic acid. A culture of strain 30E was grown at 37° in medium supplemented with elaidic acid. At a culture density of 5×10^8 cells/ml, the cells were shifted to the indicated temperatures and induction was initiated by the addition of iPrSGal at 0.5 mM. The induction times at 28.3–38.2° ranged from 20 to 35 min, and at 21.6–24.3° from 20 to 50 min. The procedures for β-galactosidase and transport assay are described in Methods. The experimental variation at each point is indicated by the vertical bar.

efficiency of incorporation of newly synthesized M protein into a fully functional transport system.

Figure 1 compares β-galactosidase activity and transport activity induced after shifting cells grown at 37° in media supplemented with oleic or elaidic acid to growth at 25°. Though the ratio of activities of induced transport to β-galactosidase is not appreciably different when induction proceeds during growth at 37° (compare Figures 2 and 3), induction at 25° leads to a dramatically decreased efficiency of formation of the fully functional transport system in the cells grown in medium supplemented with elaidic acid. A ratio of β-galactosidase activity to transport activity of approximately 13 was observed throughout the entire course of induction in cells grown with an oleic acid supplement. This ratio increased to approximately 400 in cells grown with an elaidic acid supplement, indicating a decrease in the efficiency of transport induction of over 30-fold. The induction of β-galactosidase and thiogalactoside transacetylase was studied at 25 and 37° in cells grown with an elaidic acid supplement to test the possibility that synthesis of the soluble proteins of the lactose operon might be affected differently by the temperature shift (Table I). No significant difference in the relative extents of synthesis of these two enzymes was observed.

The efficiency of transport induction as a function of induction temperature during growth with three different essential fatty acid supplements is described in Figures 2–4. In these experiments, the cells were first grown at 37° and then shifted to incubation at the indicated temperatures for induction of the lactose operon. The efficiency of transport induction remains constant or varies only slightly until some critical temperature is reached. Below this critical temperature there is a dramatic decrease in the efficiency of transport induction. The critical temperatures at which abortive transport system induction is initially observed are compared in Table II with the transition temperatures in transport rate for cells induced

TABLE I: Induction of β-Galactosidase and Thiogalactoside Transacetylase at 25 and 37° in Medium Supplemented with Elaidic Acid.[a]

	Growth Temperature			
	37°		25°	
	Induction Time		Induction Time	
	30 min	60 min	30 min	60 min
β-Galactosidase	14.8	23.2	3.66	6.97
Thiogalactoside transacetylase	15.6	22.9	3.38	7.31
β-Galactosidase: transacetylase	0.95	1.01	1.08	0.96

[a] Cultures of strain 30E were grown as described in Methods in medium supplemented with elaidic acid at 37°. At a culture density of 5×10^8 cells/ml, the culture was divided into two parts. One was induced for proteins of the lactose operon at 37°, and the other, at 25°. Assays for β-galactosidase and thiogalactoside transacetylase were performed at 37° as described in Methods. Units for β-galactosidase and thiogalactoside transacetylase activities are μmol hr^{-1} mg^{-1} of protein and nmol hr^{-1} mg^{-1} of protein, respectively.

TABLE II: Effect of Temperature and the Essential Fatty Acid for Growth on Transport Rate and Transport System Assembly.[a]

Fatty Acid	Transition Temp for Transport[b] (°C)	Abortive Transport System Induction (°C)
Elaidate	30	28–30
Bromostearate	22	20–22
Oleate	13	13–15

[a] See text for details. [b] Wilson and Fox (1971a).

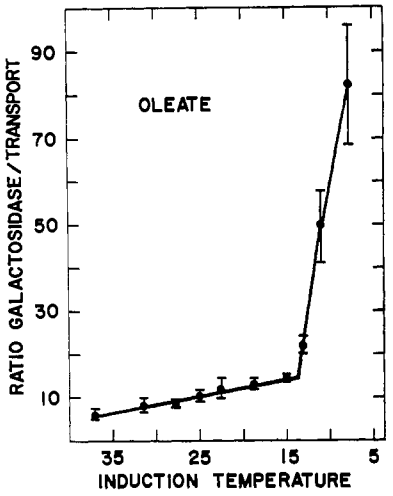

FIGURE 3: Relative efficiency of β-galactosidase induction to transport induction at various temperatures in medium supplemented with oleic acid. The procedures are those described for Figure 2. The induction times at 22.6–37.0° ranged from 20 to 40 min, at 18.6° from 30 to 70 min, at 13.1–15.0° from 60 to 120 min, and at 7.7–10.9° from 70 to 150 min. The variation in the β-galactosidase: transport ratios at single induction temperatures below the critical temperature at which abortive transport induction was initially observed followed a regular pattern, as it did in the experiments described in Figure 4. The ratio of β-galactosidase: transport induction decreased as the time period of induction was increased. This could be a reflection of the cellular ability to adapt to lower growth temperatures by increasing the ratio of unsaturated: saturated fatty acids incorporated into phospholipids (Marr and Ingraham, 1962; Haest *et al.*, 1969; Esfahani *et al.*, 1969).

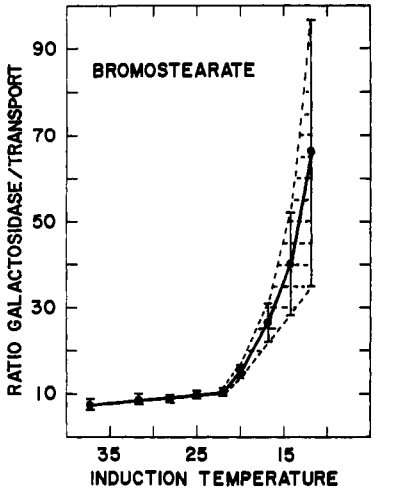

FIGURE 4: Relative efficiency of β-galactosidase induction to transport induction at various temperatures in medium supplemented with bromostearic acid. The procedures are those described for Figure 2. The induction times at 24.9–37.3° ranged from 30 to 60 min, at 16.7–21.9° from 30 to 90 min, at 14.3° from 90 to 150 min, and at 11.7° from 90 to 300 min. The area indicated by the dashed lines is the range over which the β-galactosidase: transport ratios varied. This variation followed the same pattern described in the legend of Figure 3.

at 37°. The transition temperatures for transport are in excellent agreement with the critical temperatures for abortive transport induction.

Previous reports from this laboratory have described decreases in transport induction efficiency under conditions of starvation for an essential fatty acid or complete interruption of phospholipid biosynthesis (Fox, 1969; Hsu and Fox, 1970). The extent of incorporation of [^{3}H]elaidic acid at 25 and 37° was therefore compared with the extent of induction of β-galactosidase and transport. Figure 5 describes the incorporation of [^{3}H]elaidic acid into total *E. coli* lipids. Though incorporation of [^{3}H]elaidic acid into total lipids is reduced by approximately 50% at 25°, this reduction is not more than that expected for a temperature decrease of over 10°. An examination of the two major classes of *E. coli* phospholipids shows that label incorporation into phosphatidylglycerol relative to incorporation into phosphatidylethanolamine is increased approximately 2-fold at 25° (Figure 6). Table III compares the ratios of label incorporation into lipid to the extent of β-galactosidase and transport induction at 25 and 37°. Inspection of these data shows that the formation of labeled lipid relative to β-galactosidase synthesis is actually 3- to 4-fold higher at 25° than at 37°, indicating that abortive transport induction is unlikely to be the result of an altered cellular ability for complex lipid biosynthesis.

The stability of lactose transport induced in cells grown at 37° in medium supplemented with elaidic acid was studied at 25° to determine if the apparent decrease in transport induction efficiency could be accounted for by an increased lability of the preformed system (Figure 7). Though the lability of preformed transport activity is enhanced by incubation of the cells with aeration below the critical temperature for abortive transport induction, an inactivation of only 25% was observed under conditions where the efficiency of transport induction is reduced by 15- to 30-fold. A shift to incubation with aeration in medium supplemented with oleic acid protects the transport system from inactivation during incubation with aeration at 25°. Under transport assay conditions (no aeration), no significant inactivation of transport activity was observed during the incubation periods used for transport assay.

Discussion

Our studies reported here indicate that assembly of a fully

TABLE III: Ratios of β-Galactosidase and Transport Induction to Incorporation of [^{3}H]Elaidic Acid into Membrane Lipids During Growth at 37 and 25°.[a]

	Temperature			
	37°		25°	
	Induction Time		Induction Time	
Ratios	30 min	60 min	30 min	60 min
β-Galactosidase: lipid[b]	1140	2010	444	492
Transport: lipid[b]	115	172	2.86	2.84
β-Galactosidase: transport[c]	12.6	11.7	155	173

[a] Aliquots of cells utilized for β-galactosidase and transport assay were taken from the 30- and 60-min samples used for lipid analysis in the experiment described in Figure 5. The assays were performed at 30° as described in Methods. [b] Units are nmoles of *o*-nitrophenol liberated hr^{-1} mg^{-1} of protein per 1000 cpm of [^{3}H]elaidic acid incorporated into lipid mg^{-1} of protein. [c] Units of β-galactosidase and transport used for computing this ratio are nmoles of *o*-nitrophenol liberated hr^{-1} mg^{-1} of protein.

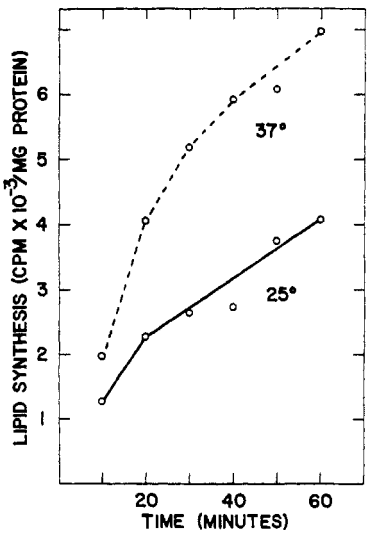

FIGURE 5: Incorporation of radioactivity from [^{3}H]elaidic acid into lipids of cultures grown in medium supplemented with elaidic acid at 37 and 25°. Two 500-ml cultures of strain 30E were grown in parallel at 37° to a culture density of 5×10^8 cells/ml in medium supplemented with elaidic acid. At this point, the temperature of one of the cultures was rapidly lowered to 25° by swirling in an ice water slurry, and growth was resumed at 25°. After 10 min of further growth at the indicated temperatures, [^{3}H]elaidic acid was added to each culture as 1 ml of a 20-mg/ml solution in ethanol; and iPrSGal was added at a final concentration of 0.5 mM. At the indicated times, 70-ml aliquots were removed from the cultures and chilled to ice bath temperature, and the cells were harvested by a 10-min centrifugation at 10,000*g*. The cells were suspended in 10 ml of ice-cold elaidic acid supplemented medium with the radioactive isotope omitted and chloramphenicol added at 50 μg/ml. A 2-ml sample was removed for the determination of transport, β-galactosidase activity, and protein (Table III). Cells from the remaining 8 ml were harvested by centrifugation, and lipids were extracted for the determination of radioactivity as described in Methods.

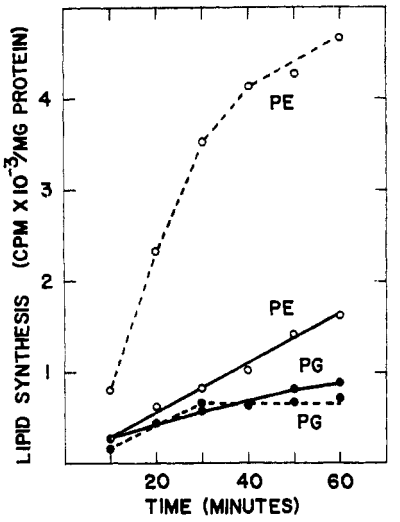

FIGURE 6: Incorporation of radioactivity from [^{3}H]elaidic acid into the major phospholipid classes at 37 and 25°. Lipids from the experiment described in Figure 5 were resolved into individual classes on silicic acid thin-layer plates as described in Methods: PE, phosphatidylethanolamine; PG, phosphatidylglycerol. The 37° experiment is indicated by the broken lines, and the 25° experiment by the solid lines.

functional β-galactoside transport system is defective below a critical temperature which defines a change of state in the membrane lipids. The phenomenon of abortive assembly could be caused by defective incorporation of a portion of the newly synthesized β-galactoside transport proteins (M protein) into the membrane, or by incorporation of all the newly synthesized M protein into membrane in a fashion which leads to a reduced efficiency in transport function for all the transport sites. Though our studies do not identify the explicit mechanism of abortive assembly, they provide a clear indication that the change of state in membrane phospholipids which affects the kinetic properties of the preformed transport system is also a determining feature in the efficacy of its assembly.

Recent spin labeling studies by Shimshick and McConnell (1973) show that mixtures of phospholipids do not undergo a sharp phase transition, but rather, a gradual course of phase separations. Above some critical temperature (t_h), virtually all the fatty acid side chains of membrane lipids are in a fluid, mobile state. Below t_h, the lipids separate into fluid and relatively nonfluid domains. The fraction of the lipids in the nonfluid regions increases as the temperature is decreased until a second critical temperature (t_l) is reached. At this point, virtually all the lipids are in a nonfluid state. The critical temperatures which define the beginning (t_h) and the end (t_l) of the lateral phase separations of lipids in membranes of the bacterial strain used in our study (strain 30E) have been determined for cells grown with a number of unsaturated fatty acid supplements (C. D. Linden, H. M. McConnell, and C. F. Fox, to be published). In all cases, the onset temperature for abortive transport system assembly is identical ($\pm 1°$) with t_l, the critical temperature which defines the end of lateral phase separations in these membranes. The onset of abortive assembly thus occurs at the temperature where virtually all the membrane lipids have lost their fluid character. Or, stated differently, some membrane fluidity is required for effective membrane assembly, at least in the case of the β-galactoside transport system.

Though we find a clear and consistent correlation between the low-temperature boundary of a change of state in the membrane lipids detected by electron spin resonance and physiological transitions in transport activity and transport system morphogenesis, the overall picture regarding physiological transitions in membranes is not so clear. Esfahani *et al.* (1971) have compared the transition temperatures for proline transport and succinate dehydrogenase activity with a change of state in the membrane lipids that can be detected by X-ray diffraction (Engelman, 1970). Using an unsaturated fatty acid auxotroph grown with a variety of essential fatty acid supplements, they observed no clear correlations either between the two physiological transitions or between either physiological transition and the change of state in membrane lipids detected by X-ray diffraction. The disparities between the temperatures where changes of state in membrane lipids are revealed by X-ray diffraction and physiological transitions led these authors to the interpretation that membranes are organized in local domains which differ considerably in lipid composition, or that some physiological transitions reflect unique lipid–protein interactions between individual functional protein species and an essentially random lipid phase. Mavis and Vagelos (1972) have likewise interpreted the results of a similar study to indicate a heterogeneity between membrane-associated enzymes and phospholipids. Overath *et al.* (1970) showed that physiological transitions in membrane-associated processes other than transport (*e.g.*, respiration) do in some instances occur at temperatures other than the transport transition temperature. In addition, respiration was observed to undergo more than one transition in cells

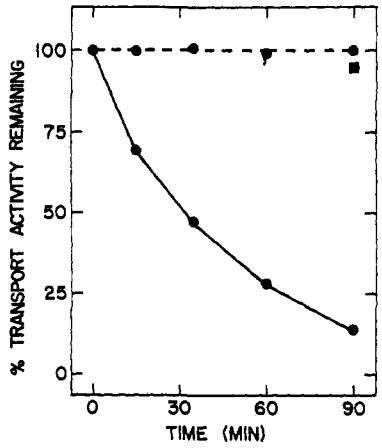

FIGURE 7: Stability of lactose transport induced in medium supplemented with elaidic acid. A culture of strain 30E was grown in medium supplemented with elaidic acid at 37° to a culture density of 5×10^8 cells/ml, and iPrSGal was added at 0.5 mM for a 30-min period of induction. The culture was then divided into three portions, and the cells of each were harvested at 37° by a 10-min centrifugation at 10,000*g*. Maintaining the temperature at 37°, two portions of the cells were suspended in medium supplemented with elaidic acid and the third in medium supplemented with oleic acid. The centrifugation was repeated, and the cells of each portion were suspended in the same medium as before, adjusting the cell density in the three portions to 5×10^8 cells/ml. One portion of cells suspended in medium supplemented with elaidic acid was incubated with shaking at 37°, and the other portion at 25°. The portion suspended in medium supplemented with oleic acid was incubated with shaking at 25°. An aliquot was removed from each portion when shaking was initiated (100% transport activity remaining), and subsequently at the indicated times. No increase in β-galactosidase activity per unit volume of culture was observed indicating that further induction of proteins of the lactose operon did not occur. Previously induced transport activity remaining after incubation in medium supplemented with elaidic acid at 25° is indicated by ●—●, at 37° by ■, and after incubation in medium supplemented with oleic acid at 25° by ●--●.

grown with oleic or elaidic acid supplements. This too indicates that whereas certain processes such as transport do reflect a well-defined change of state in the membrane lipids which can be detected by electron spin resonance and other physical probes, this change of state may not be the sole temperature-dependent parameter by which physical alterations in membrane lipids can influence the kinetic properties of biological processes.

Though we do not disagree with the contention of Esfahani *et al.* (1971) or Mavis and Vagelos (1972) that certain physiological transitions in membrane functions could arise from unique lipid–protein interactions, there are other explanations for the discrepancies noted by these authors, and the multiple transitions observed by Overath *et al.* (1970). The course of phase separations in *E. coli* membranes has a distinct beginning and end, and in some instances transport transitions have been observed at both t_h and t_l (the beginning and end of the course of phase separations) in cells grown with a single essential fatty acid (Linden *et al.*, 1973). It is conceivable that some physiological processes might be affected by the change of state in the membrane lipids occurring at t_h, others at t_l and still others at both t_h and t_l. Proteins might also place constraints upon the continuity of lipids in the plane of the membrane. This could depress t_h, t_l, or both where such constraints exist. The Kelvin equation for curved surfaces indicates that depressions of the temperatures defining changes of state in the membrane lipids can be caused by a breakdown in the continuity of a lipid monolayer. This relationship predicts that melting point is a function of the size of a domain and can also be influenced by its shape. [For a more thorough treatment of this relationship and its applicability to biological membranes, we refer the reader to Kezdy (1972)].

Our data indicate that morphogenesis of the β-galactoside transport system requires some fluidity in the cellular phospholipids. In the accompanying manuscript (Tsukazoshi and Fox, 1973), we extend these studies to show that *stable* membranous domains of different lipid compositions do not normally exist, but can be generated during morphogenesis under rigorously controlled conditions.

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Transport System Assembly and the Mobility of Membrane Lipids in *Escherichia coli*†

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ABSTRACT: Arrhenius plots describing the logarithm of the rate of β-galactoside transport *vs.* the reciprocal of the absolute temperature of transport assay are biphasic in slope with intersects (transport transition temperatures) at 30 and 13° for cells of an *Escherichia coli* unsaturated fatty acid auxotroph grown at 37° in medium supplemented with elaidic acid and oleic acid, respectively. When transport is induced at 37° for a short time period (less than one-eighth of a generation of growth) after shifting from growth with an oleic acid supplement to growth with an elaidic acid supplement or *vice versa*, a single transition temperature for β-galactoside transport is detected in each case, and the temperatures at which the transitions are observed indicate that transport is primarily influenced by the average fatty acid composition of the membrane lipids. If transport induction proceeds at 25° after the fatty acid shift, however, the Arrhenius plots are triphasic. The two observed transport transition temperatures reflect the properties of cells with membranes composed primarily of oleic acid or elaidic acid derived lipids. When the cells induced at 25° are subsequently incubated at 37° before commencing transport assay, only a single, new transport transition is detected and the transition temperature is similar to that observed with cells induced at 37° after the fatty acid shift. We interpret these results to indicate that under the conditions where two transport transition temperatures are observed, a portion of the newly formed transport protein is incorporated into membrane with, and influenced by, the lipids synthesized concomitantly. Subsequent incubation of these cells at elevated temperature leads to a mixing of the preformed and newly synthesized lipids within the membrane bilayer matrix. Thus the mobility of lipids in membranes may, at least in certain instances, be grossly restricted below a defined critical temperature.

Several independent studies point to an interrelationship between protein and lipid biosynthesis during the morphogenesis of certain microbial transport systems. Following a period of starvation for an essential fatty acid, induction of a fully functional lactose transport system in unsaturated fatty acid auxotrophs of *Escherichia coli* is blocked, even though the induction of β-galactosidase and thiogalactoside transacetylase proceeds normally (Fox, 1969; Overath *et al.*, 1971a). Addition of an essential fatty acid subsequent to the starvation period does not result in a recovery of transport activity (Fox, 1969). Induction of functional lactose transport system activity in *E. coli* and *Staphalococcus aureus* is blocked when glycerol auxotrophs of these organisms are starved for a source of glycerol or glycerol phosphate for complex lipid biosynthesis. The transport proteins, however, are incorporated into membrane and exhibit normal binding activity in *E. coli*, and phosphotransferase activity in *S. aureus* (Hsu and Fox, 1970; Mindich, 1971). Transport can be activated by subsequent growth in the presence of glycerol in *S. aureus*, but not in *E. coli*. Uptake and accumulation of amino acids are defective in pantothenic acid deficient *Lactobacillus plantarum* (Holden and Bunch, 1972). Transport activity is restored to normal when the pantothenate-starved cells are incubated with long-chain fatty acids.

Properties of transport systems induced in unsaturated fatty acid auxotrophs of *E. coli* have been exploited in the study of membrane structure–function relationships (Schairer and Overath *et al.*, 1970; Wilson and Fox, 1971; Esfahani *et al.*, 1971) and membrane assembly mechanisms (Fox, 1969; Wilson and Fox, 1971; Tsukagoshi *et al.*, 1971; Overath *et al.*, 1971a; Robbins and Rotman, 1972; Tsukagoshi and Fox, 1973). A report from this laboratory provided evidence supporting the view that assembly of the lactose-transport system proceeds by a coordinated incorporation of newly syn-

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