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Integrative feedback and robustness in a lipid biosynthetic network

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The homeostatic control of membrane lipid composition appears to be of central importance for cell functioning and survival. However, while lipid biosynthetic reaction networks have been mapped in detail, the underlying control architecture which underpins these networks remains elusive. A key problem in determining the control architectures of lipid biosynthetic pathways, and the mechanisms through which control is achieved, is that the compositional complexity of lipid membranes makes it difficult to determine which membrane parameter is under homeostatic control. Recently, we reported that membrane stored elastic energy provides a physical feedback signal which modulates the activity *in vitro* of CTP:phosphocholine cytidylyltransferase (CCT), an extrinsic membrane enzyme which catalyses a key step in the synthesis of phosphatidylcholine lipids in the Kennedy pathway (Kennedy 1953 *J. Am. Chem. Soc.* **75**, 249–250). We postulate that stored elastic energy may be the main property of membranes that is under homeostatic control. Here we report the results of simulations based on this postulate, which reveal a possible control architecture for lipid biosynthesis networks *in vivo*.

Keywords: simulation; model; networks; stored elastic energy; membrane torque tension; homeostasis

1. INTRODUCTION

Lipid membranes are highly complex dynamic chemical systems characterized by the rich diversity of the amphiphilic species that constitute them. This compositional diversity arises because in a typical eukaryotic membrane there can be up to 20 or so classes of lipids, excluding sterols, which differ from each other in the chemical nature of the hydrophilic headgroup. Further, each of these classes can be composed of between 20 and 60 homologues (differing in the composition of the hydrophilic moieties) giving a total diversity of between 400 and 1200 chemically distinct species. Additionally, these species occur with widely different number densities and spatial distributions, both of which are subject to temporal changes.

Although in principle each membrane lipid species could be under homeostatic control, this is extremely unlikely: not only is there no evidence in support of this level of control, but also the energy costs to an organism implementing such a control architecture might be expected to be prohibitive. These considerations suggest that a more parsimonious control system may operate *in vivo*, in which only a small number of key species, or perhaps membrane properties, are under homeostatic control. Our recent studies of CTP:phosphocholine cytidylyltransferase (CCT) suggest that the latter could be the situation that pertains in eukaryotic cells.

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CCT, which has been the subject of considerable interest over the past two decades, mediates a reaction regarded as the rate-limiting step in the CDP-choline pathway for the biosynthesis of phosphatidylcholine (PC) lipids, which in many organisms comprise the most abundant class of lipid constituents of cell membranes. CCT catalyses the reaction between CTP and phosphorylcholine to produce inorganic phosphate and CDP-choline. The latter then reacts with a diacylglycerol (DAG) molecule to produce a PC molecule. CCT is an extrinsic membrane protein that is essentially inactive when in the cytosol but becomes active when it partitions onto a membrane.

There have been many attempts to elucidate how the lipid composition of biomembranes modulates the binding of CCT and therefore its activity. Explanations have included hydrophobic and electrostatic interactions with phospholipids in the membrane and the alteration of phospholipid headgroup packing. The alteration of headgroup packing theory (Cornell 1991*b*) relates the enzyme activity to the physical structure of the membrane according to the phospholipid headgroups. Crucially however, this does not explain inhibition by lysoPC or its synthetic analogues (Boggs *et al.* 1995) which share the same headgroup as PC. Electrostatic interactions have also been suggested as driving the binding of CCT to membranes (Cornell 1991*a*; Arnold & Cornell 1996), with anionic phospholipids controlling the partitioning of CCT onto the membrane owing to the attractive interaction with cationic amino acid residues present in the amphipathic helix of CCT.

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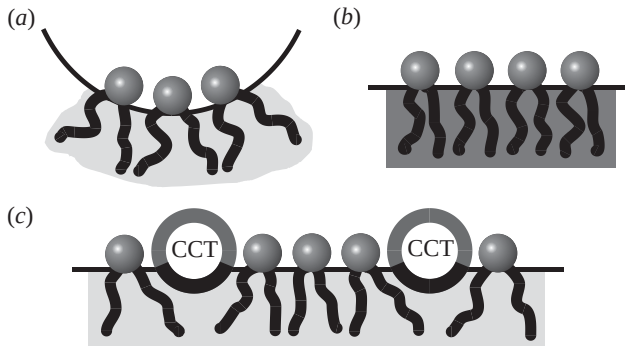

Figure 1. Model for CCT regulation. (*a*) Monolayer with optimum and large spontaneous curvature. (*b*) Monolayer constrained to lie flat in a bilayer; large stored elastic energy indicated by darker shading. (*c*) CCT α-helices partition into the membrane, relieving stored elastic energy.

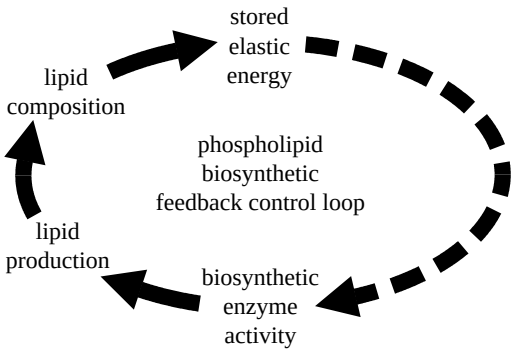

Figure 2. Feedback hypothesis: the relationship between the lipid composition of a membrane, and therefore its stored elastic energy, and the activity of the enzymes of lipid biosynthesis would complete a feedback loop and ensure homeostatic control of the torque tension.

However, this explanation does not account for the modulation of CCT activity by neutral lipids like DAG, which have minimal electrostatic effect on the membrane, or the bimodal activation of CCT by monoolein (MO). By contrast, the effects of DAG, MO and lysoPC on CCT are readily explicable in terms of the effect they have on the stored elastic energy. Our previous studies have shown that the partitioning of CCT onto model membranes is driven by the stored elastic energy (or torque tension) of the host membrane (Attard *et al.* 2000) and that this effect outweighs electrostatic interactions.

The torque tension of membranes stems from the desire of each leaflet of the lipid bilayer to curve. Optimum curvature cannot be achieved, as the apposed leaflets want to curve in opposite directions, and the flat bilayer stores elastic energy in direct proportion to its preferred curvature, c_o (assuming both leaflets of the bilayer have the same composition) and to the elastic constant for bending deformations, κ_M (Helfrich 1981). CCT can 'sense' stored elastic energy because part of the molecule folds into an α-helix with a hydrophobic strip. When this domain of CCT partitions onto a membrane it releases a proportion of the stored elastic energy, and it is the release of this energy that provides the driving force which favours the association of CCT with the membrane (figure 1). Consequently, the enzyme is strongly activated by lipids, such as DAG and phosphatidylethanolamine (PE), which favour negative mean curvature and therefore induce high stored elastic energy. These types of lipids are referred to as type II lipids to distinguish them from lipids that favour the formation of flat interfaces (type 0 lipids) or those that favour the formation of interfaces with positive mean curvature (type I lipids). Both type I and type 0 lipids have been shown to deactivate CCT. The relationship between modulation of CCT activity and the spontaneous curvature accounts for the observation by Vance that the degree of CCT activation is related to the ratio of bilayer- to non-bilayer-forming lipids (Jamil *et al.* 1993).

The significance of the observation that the stored elastic energy of a membrane can influence the activity of CCT lies in the fact that this establishes a feedback relationship between the lipid composition of a membrane, its stored elastic energy and the activity of one of the key enzymes in lipid biosynthesis (illustrated in figure 2). Additionally, unlike other types of feedback loops reported in biological systems, the control of CCT does not rest with the concentration of a single species. Instead, the activity of CCT is determined by the balance of type II lipids and the sum of type I and type 0 lipids. We term this type of feedback 'integrative' as the net signal to CCT is integrated, or summed, over the contributions of all the lipid species. The non-specific feedback through which CCT appears to be controlled could provide the cell with a method to maintain homeostatic control over its membrane composition. This insight raises the possibility that physical membrane forces may play key roles in the regulation of other enzymes involved in lipid biosynthesis and degradation. With cells using membrane torque tension as a feedback signal to regulate phospholipid synthesis, it is reasonable to suppose that the torque tension may in fact be one of the main membrane properties that are under homeostatic control.

Membrane torque tension is an excellent candidate for homeostatic control because it affords single-parameter regulation of membrane properties, such as membrane permeability and fluidity, which are essential for membranes to function as partitions of intracellular space while also providing a communication mechanism between membrane proteins. The possibility that a membrane physico-chemical property is controlled homeostatically has been discussed for a number of years. In particular, Gruner suggested the possibility that the spontaneous curvature of each leaflet of a bilayer might be a key membrane property under homeostatic control (Gruner 1985; Gruner 1994). Furthermore, a number of experimental observations suggest that the regulation of several extrinsic and intrinsic membrane proteins through stored elastic energy might be a widespread and generic feature of lipid biosynthesis. For example, studies of *Acholeplasma laidlawii* have shown that the ratio of monoglucosyldiacylglycerol to diglucosyldiacylglycerol, the major lipid components in this organism, appears to be maintained within very tight bounds (Lindblom *et al.* 1986).

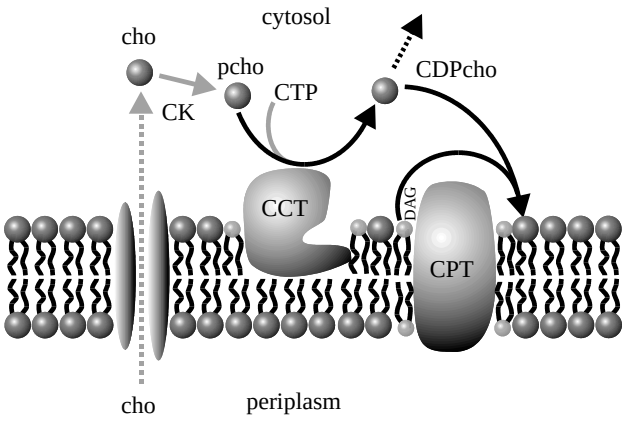

Figure 3. The location of the substrates and products of the CCT and CPT catalysed reactions (Jackowski & Baburina 2002). In our simulations, CDPcho, formed at the membrane, is modelled as a variable species. The concentrations of cho and pcho, which are external to the membrane, are clamped.

Similarly, in the case of *Clostridium butyricum*, the ratio of (PE+plasmenylethanolamine)/glycerolacetal to plasmenylethanolamine is also maintained within narrow limits (Johnston & Goldfine 1992).

We have set up a large-scale *in silico* simulation of a 'typical' eukaryotic lipid biosynthetic network in order to understand how a control architecture based on an integrative feedback that uses stored elastic energy as the feedback signal might work. In particular, we have addressed the question of which reactions in the network are most likely to be affected by integrative feedback if the torque tension of a membrane was to be kept under homeostatic control.

2. METHODS

Our *in silico* studies were based on the core of the phospholipid reaction network of a typical mammalian cell. Here we outline the features of the model and the analyses performed.

2.1. Model topology

The topology of the network, as outlined in table 1 and figure 4, was constructed from the literature reports of lipid biosynthesis and degradation reactions in eukaryotic cells (e.g. as embodied in the Boehringer Mannheim Biochemical Pathways Chart) and applies to a 'generic' mammalian eukaryotic cell (but not a liver cell, since we excluded the conversion of PE to PC by sequential methylation). Our model encompasses the most abundant lipid species, as well as several that are of primary importance in lipid signalling, and provides a test bed for assessing the possible role of integrative feedback and torque tension in the control of lipid biosynthesis. The model was restricted to headgroup classes and the number of hydrocarbon chains only (i.e. two-chain versus lyso lipids) and did not take into account different acyl (or alkenyl) chain patterns. The rationale for this simplification is that to a zeroth-order approximation the headgroup of a lipid is one of the key factors that determine spontaneous curvature. Clearly, by not including the hydrophobic components of the lipids in the model we are not able to capture subtleties that arise from the contribution of acyl or alkenyl chains to c_o and κ_M.

In our model, species that are not normally integral membrane components (or are synthesized at the membrane) are clamped (treated as inexhaustible pools). The localization of the enzymes CCT and cholinephosphotransferase (CPT) and their substrates and products are illustrated in figure 3. Choline (cho) and phosphocholine (pcho) are external to the membrane and are therefore clamped, while CDP-choline (CDPcho) is synthesized at the membrane by CCT and is therefore a variable of the model. This rudimentary level of compartmentalization makes no allowance for further localization within the membrane. The model therefore treats the membrane as a well-mixed solution, ignoring the subcellular distribution and localization of the enzymes. The asymmetry in the lipid composition of the leaflets of the bilayer is also omitted in our approach.

2.2. Model methodology

In broad terms, our strategy for elucidating the control structure in the model network is as follows. Initially we specified a target steady-state (TSS) concentration for each of the lipid species, and used this (together with the assumption that the rate laws of each enzyme followed the stoichiometry of the catalysed reaction) to work out a set of rate constants for each of the reactions in the network. The assumptions made in deriving the initial set of rate constants were tested systematically in order to determine their effect on the properties of the network. We then carried out a sensitivity analysis of the extent to which changes in the rate constant of each reaction affected the magnitude of the torque tension. This enabled us to identify the reactions most likely to be controlled by integrative feedback. In our final series of studies, we implemented integrative feedback at these reactions and investigated how integrative feedback affects the robustness of the network (Barkai & Leibler 1997) by examining the changes in the sensitivity of the torque tension and the lipid species composition.

2.3. Model parameters

The TSS concentration for each lipid component of the network was defined to conform to that reported for the rough endoplasmic reticulum in several cell types. The composition of this 'virtual membrane' broadly reflects the lipid compositions of many eukaryotic membranes. The relative concentrations used are shown in table 3.

In branched networks, the solution of the stoichiometric matrix to yield an effective series of rate constants requires the input of relative magnitudes of the flux through each of the branches in the network (Hofmeyr 1986). For the purpose of this work, we assumed that all fluxes originating at any given species are equal. This is termed the equal branch flux assumption and represents the least biased assumption we could make in relation to the various fluxes. These conditions are added to the matrix which is then solved

Table 1. Reaction list. (Italics denote clamped metabolites; bold type indicates lipid species. The variable species are PC, PE, phosphatidylserine (PS), phosphatidic acid (PA), 1,2-diacyl-sn-glycerol (DAG), lysoPC (LPC), lysophosphatidylethanolamine (LPE), lysoPS (LPS), fatty acid (FA), acyl coenzyme A (AcCoA), CDP-choline (CDPcho), CDP-ethanolamine (CDPeth), CDP-diacylglycerol (CDP-DAG), phosphatidylinositol (PI), phosphatidylinositol 4-phosphate (PIP), phosphatidylinositol 4,5-biphosphate (PIP_2), sphingomyelin (SM), ceramide (Cer), N-acetylsphinganine (NacSa), sphinganine (Sa), sphinganine 1-phosphate (Psa), sphingosine 1-phosphate (Pso), sphingosine (So).)

rxn	stoichiometry	rxn	stoichiometry
R1	$cho + ATP = pcho + ADP$	R28	$\mathbf{LPA} + \mathbf{AcCoA} = CoA + \mathbf{PA}$
R2	$eth + ATP = peth + ADP$	R29	$\mathbf{LPC} + \mathbf{AcCoA} = CoA + \mathbf{PC}$
R3	$pcho + H_2O = cho + Pi$	R30	$\mathbf{LPS} + \mathbf{AcCoA} = CoA + \mathbf{PS}$
R4	$peth + H_2O = eth + Pi$	R31	$\mathbf{LPE} + \mathbf{AcCoA} = CoA + \mathbf{PE}$
R5	$pcho + CTP = Ppi + \mathbf{CDPcho}$	R32	$H_2O + \mathbf{LPC} = cho + \mathbf{LPA}$
R6	$peth + CTP = Ppi + \mathbf{CDPeth}$	R33	$H_2O + \mathbf{LPS} = serine + \mathbf{LPA}$
R7	$gly3pho + \mathbf{AcCoA} = CoA + \mathbf{LPA}$	R34	$H_2O + \mathbf{LPE} = eth + \mathbf{LPA}$
R8	$FApre = \mathbf{FA}$	R35	$CoA + \mathbf{FA} = H_2O + \mathbf{AcCoA}$
R9	$\mathrm{CDPcho} + \mathbf{DAG} = CMP + \mathbf{PC}$	R36	$CTP + \mathbf{PA} = Ppi + \mathbf{CDPDAG}$
R10	$\mathrm{CDPeth} + \mathbf{DAG} = CMP + \mathbf{PE}$	R37	$inos + \mathbf{CDPDAG} = CMP + \mathbf{PI}$
R11	$\mathbf{PC} + \mathbf{cer} = \mathbf{DAG} + \mathbf{SM}$	R38	$ATP + \mathbf{PI} = ADP + \mathbf{PIP}$
R12	$H_2O + \mathbf{PC} = pcho + \mathbf{DAG}$	R39	$ATP + \mathbf{PIP} = ADP + \mathbf{PIP_2}$
R13	$H_2O + \mathbf{PA} = Pi + \mathbf{DAG}$	R40	$H_2O + \mathbf{PIP_2} = inosP3 + \mathbf{DAG}$
R14	$H_2O + \mathbf{PS} = pcho + \mathbf{DAG}$	R41	$\mathbf{SM} = pcho + \mathbf{cer}$
R15	$H_2O + \mathbf{PE} = peth + \mathbf{DAG}$	R42	$serine + \mathbf{AcCoA} = CoA + \mathbf{Sa}$
R16	$ATP + \mathbf{DAG} = ADP + \mathbf{PA}$	R43	$\mathbf{Sa} = \mathbf{Psa}$
R17	$H_2O + \mathbf{PC} = cho + \mathbf{PA}$	R44	$\mathbf{AcCoA} + \mathbf{Sa} = CoA + \mathbf{NAcSa}$
R18	$H_2O + \mathbf{PS} = cho + \mathbf{PA}$	R45	$\mathbf{NAcSa} = \mathbf{cer}$
R19	$H_2O + \mathbf{PE} = eth + \mathbf{PA}$	R46	$\mathbf{cer} = \mathbf{FA} + \mathbf{So}$
R20	$\mathrm{PS} = CO_2 + \mathrm{PE}$	R47	$\mathbf{So} = \mathbf{Pso}$
R21	$eth + \mathbf{PS} = serine + \mathbf{PE}$	R48	$\mathbf{Pso} = peth + \mathbf{FA}$
R22	$serine + \mathbf{PC} = cho + \mathbf{PS}$	R49	$\mathbf{Psa} = peth + \mathbf{FA}$
R23	$serine + \mathbf{PE} = eth + \mathbf{PS}$	R50	$\mathbf{CDPcho} \rightarrow SINK$
R24	$H_2O + \mathbf{PA} = \mathbf{LPA} + \mathbf{FA}$	R51	$\mathbf{CDPeth} \rightarrow SINK$
R25	$H_2O + \mathbf{PC} = \mathbf{LPC} + \mathbf{FA}$	R52	$\mathbf{PC} \rightarrow SINK$
R26	$H_2O + \mathbf{PS} = \mathbf{LPS} + \mathbf{FA}$	R53-	$\mathbf{X} \rightarrow SINK$
R27	$H_2O + \mathbf{PE} = \mathbf{LPE} + \mathbf{FA}$	−73	

to determine a set of reaction fluxes (and a set of rate constants according to the rate laws used, as described in §2.5) that are consistent with the TSS.

2.4. The torque parameter

Since there is currently no way to directly determine the stored elastic energy from the lipid composition, we have used, as a proxy, an empirical function which we term the torque parameter, λ

$$\lambda \propto \frac{[\text{type II lipids}]}{[\text{type 0 \& type I lipids}]}. \tag{2.1}$$

In crude terms, the torque parameter reflects the ratio of bilayer- to non-bilayer-forming lipids. This is expressed mathematically by summing, within each lipid class, the product of each lipid's concentration with a coefficient (a_i or b_j) which represents its type II or type 0/I character:

$$\lambda = \frac{\sum_{i=1}^{i=N_{\mathrm{II}}} a_i[\mathrm{S}_i]}{\sum_{j=1}^{j=N_{\mathrm{I}}+N_0} b_j[\mathrm{S}_j]}. \tag{2.2}$$

For example, for a ternary system consisting of PC and phosphatidylserine (PS), both type 0/I lipids, and the type II lipid PE, the value of λ would be given by

$$\lambda = \frac{a_1[\mathrm{PE}]}{b_1[\mathrm{PC}] + b_2[\mathrm{PS}]}. \tag{2.3}$$

The relative magnitudes of the coefficients (a_i and b_j) are chosen on the basis of the phase behaviour of representative lipids for a particular headgroup class (tables 2 and 3) and represent the 'strength' of the type II or type 0/I character of the lipid. This functional form is a simple extension of the parameter suggested by Vance (Jamil *et al.* 1993) and is qualitatively consistent with observations reported for a wide variety of systems.

2.5. Rate laws

With little mechanistic information available for the relevant reactions *in vivo*, the model necessitates the use of arbitrary rate laws for each reaction in the network. Since the aim of our study was to investigate the overall behaviour and control architecture of the lipid biosynthetic network, we adopted the approach used for mass action kinetics where the order of reaction is inferred directly from the stoichiometry of the reaction. While this is an arbitrary decision, it has the benefit of providing a consistent framework for all the reactions in the network and its effect on the

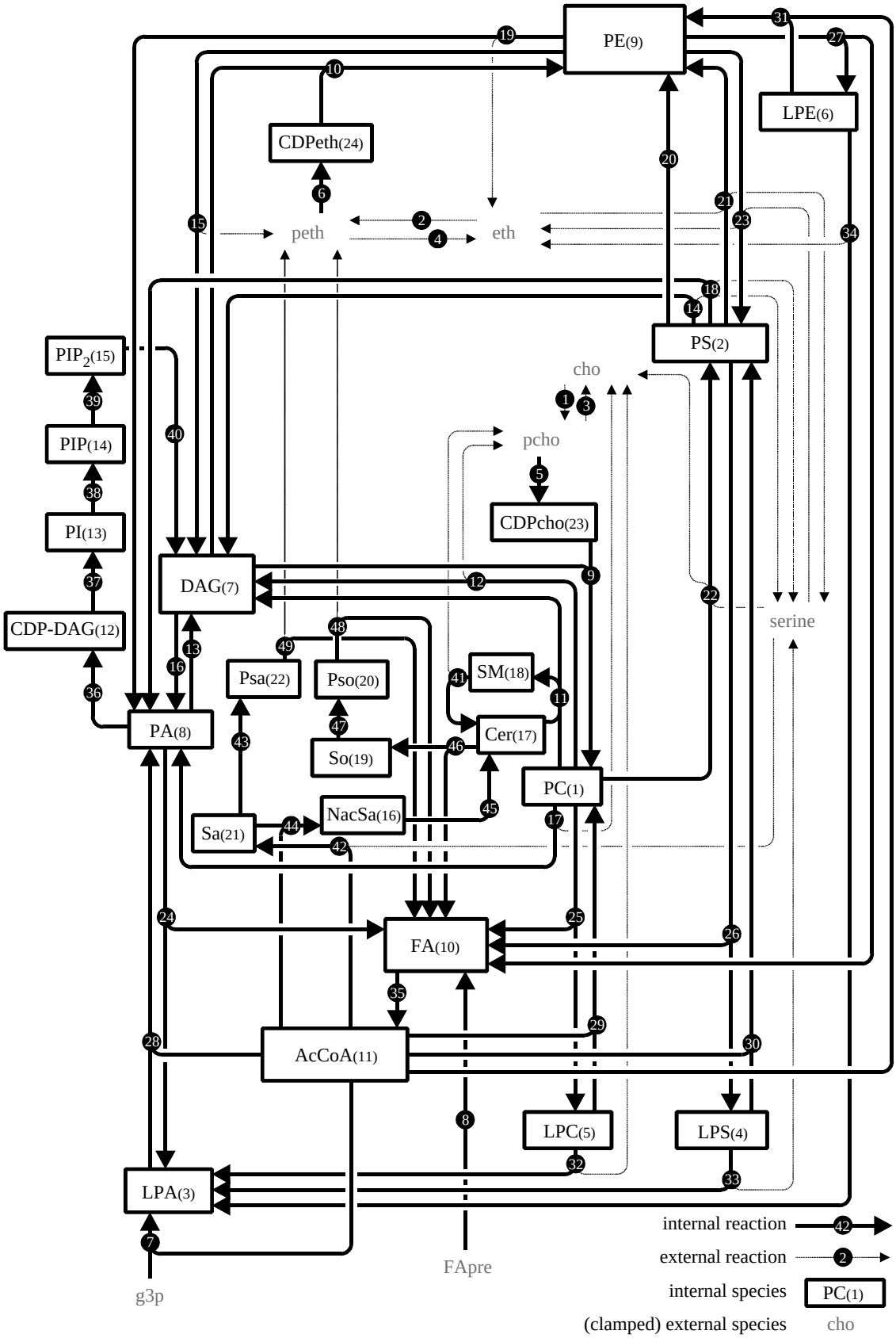

Figure 4. Model network diagram. Note that the drain reactions present on each species have been omitted for clarity.

properties of the system is amenable to modification. Furthermore, our expectation was that feedback control will have a substantial impact on the network such that the system properties would be relatively insensitive to the detailed kinetics of the individual reactions.

We modelled each reaction as a reversible reaction using a reversible Michaelis–Menten equation based on the reaction's stoichiometry. Where there is more than one substrate (or product) we assumed an equal probability of either intermediate. For example, for a

Table 2. Classification scheme for lipid types.

type I	type 0	type II
PIP_2 > LPC > PIP > LPE > LPS > Psa > Pso > PI > AcCoA > SM	PS ≈ PC	Sa < So < NAcSa < CDPDAG < PE < LPA < cer < PA < FA < DAG
positive curvature	← zero curvature →	negative curvature

Table 3. TSS concentrations and torque parameter coefficients (a_i and b_j in equation (2.2)) used in the simulations.

type I/0 species	relative concentration	coefficient b_j	type II species	relative concentration	coefficient a_i
PC	50	1	PE	30	20
PS	12	1	Cer	5	30
SM	7	1	DAG	1	300
PI	1	5	NAcSa	1	20
Pso	1	5	PA	1	50
Psa	1	5	So	1	5
AcCoA	1	5	Sa	1	5
PIP	1	80	FA	0.5	100
LPE	0.5	60	LPA	0.5	20
LPC	0.5	100	CDPDAG	0.01	20
LPS	0.5	10			
PIP_2	0.5	120			

pseudo random bi–uni process ($A + B \rightleftharpoons P$) the rate of reaction is obtained as

$$v = \frac{V_f([A][B] - [P]/K_{eq})}{K_A K_B\left(1 + \frac{[A]}{K_A} + \frac{[B]}{K_B} + \frac{[A][B]}{K_A K_B} + \frac{[P]}{K_P}\right)}. \qquad (2.4)$$

The kinetic constants (V_f, K_{eq}, K_A, K_B and K_P) are then found such that they satisfy the flux solution. These parameters can be systematically varied to investigate their influence on the properties of the network.

2.6. Feedback regulation by torque tension

We implemented explicit feedback by using a simple saturating relationship between torque and the level of modulation of enzyme activity. The rates of the selected reactions were scaled by a factor, σ, which depends on the value of the torque parameter, λ, as defined in equation (2.5)

$$\sigma_{norm} = c_1 \exp(-1/(c_2\lambda)). \qquad (2.5)$$

The use of this type of function means that the reaction reaches a maximum at high values of λ. In this way, σ acts as a tap on the rate of the reaction. It is noted that one of the limitations of the simulation is that it does not take into account the direct modulation of the stored elastic energy by the proteins, as is seen for CCT in figure 1. This approach would require the enzymes to be explicitly included in the calculation of the torque parameter. This is a key reason why our model is best suited to describe static properties rather than dynamic behaviour.

2.7. Implementation

The simulations were implemented using a solution of the ordinary differential equations derived for the model. The model was evaluated using PySCeS 0.1.7 (Olivier *et al.* 2005; for Python 2.4) and Gepasi 3.2.1 (Mendes 1993, 1997) and a custom algorithm for verification.

3. RESULTS

The model we developed was designed to allow the simulation of the effect of feedback, through stored elastic energy, on the robustness of lipid biosynthesis. We used a traditional sensitivity analysis approach to quantify the effects that changing the kinetic constants of individual reactions have on the torque parameter and on the composition of the system. This enabled us to identify key reactions where feedback would be expected to lead to a stabilization of the stored elastic energy. We also investigated the extent to which the results were affected by the key assumptions made in the development of the model.

3.1. Torque parameter sensitivity analysis

The rationale behind using a sensitivity analysis of the torque parameter is that if the torque tension is indeed under homeostatic control, then those reactions that have the biggest effect on the torque tension are the points in the network where integrative feedback will have the most stabilizing effect. Furthermore, we would expect these key reactions to be catalysed by proteins that have clear lipid requirements for activity.

A typical sensitivity analysis plot obtained for the uncontrolled network (without integrative feedback) is shown in figure 5*a*(i). This analysis provides a way of ranking the reactions in the order of their decreasing effect on the torque tension and this is summarized in table 4. From these data, it is clear that the reactions which have the greatest impact on relieving the stored elastic energy are CTP:phosphatidate cytidylyltransferase (PACT),

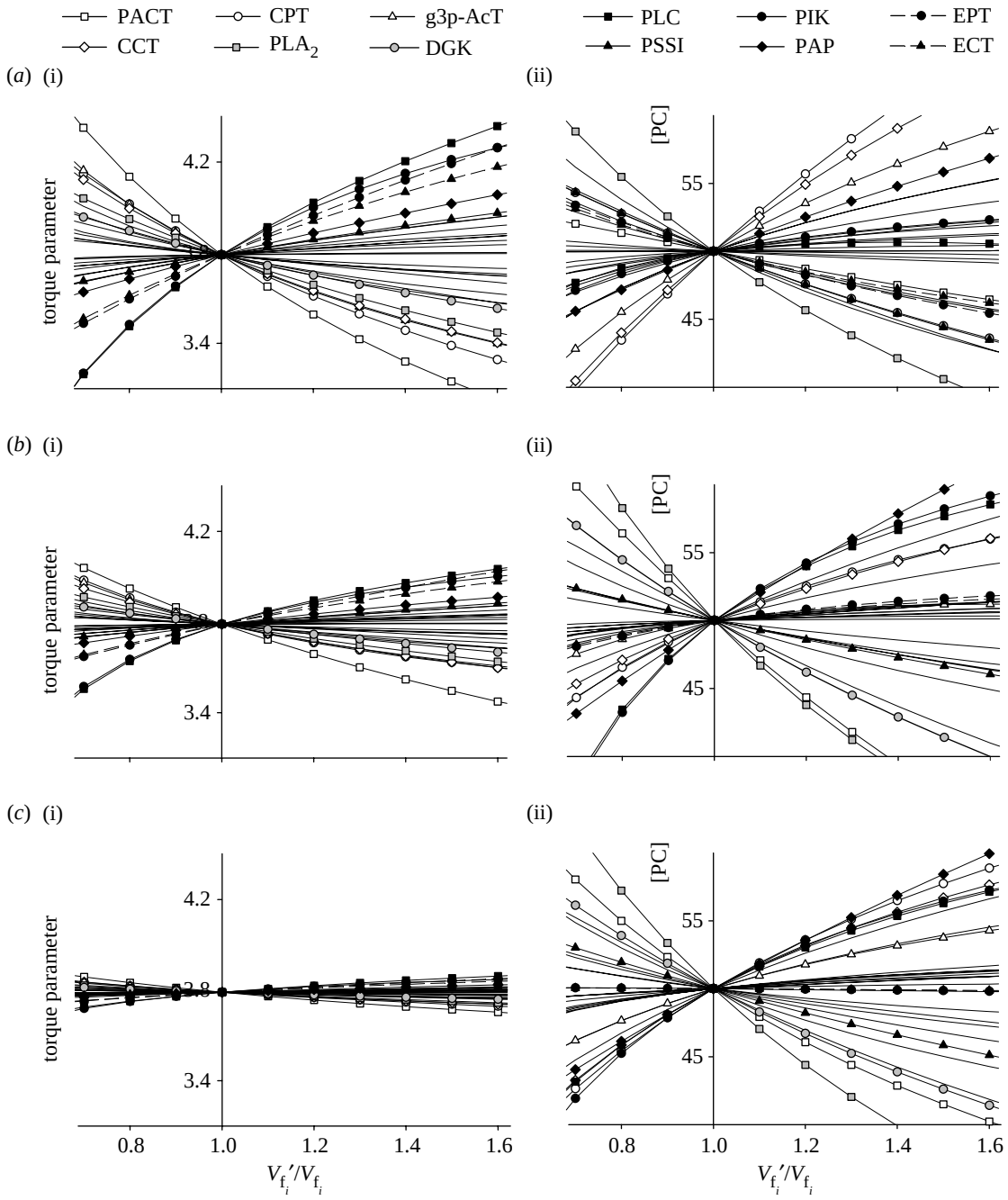

Figure 5. Sensitivity analysis for (i) the torque parameter and (ii) PC concentration for three feedback configurations. (*a*) No feedback. (*b*) Normal feedback (equation (2.5)) at CCT. (*c*) Feedback at multiple (eight) reactions: normal feedback (equation (2.5)) applied to CCT (R5), g3p-AcT (R7), CPT (R9), PACT (R36) and inverse feedback (equation (3.1)) applied to the four PLC mediated reactions (R12, R14, R15 and R40). The plots show how the quantities change as each reaction rate is altered (from the values found for the TSS). The torque parameter is stabilized by feedback; however, the concentrations are not constrained by the feedback, rather they change to maintain the torque parameter. All reaction numbers refer to the reaction list provided in table 1; the enzyme labels used in the key are detailed in tables 4 and 5.

Table 4. Sensitivity analysis results: reactions which decrease the torque parameter.

rank	reaction		enzyme	evidence
1	PA→CDPDAG	PACT	CTP:phosphatidate cytidylyltransferase	
2	DAG+CDPcho→PC	CPT	diacylglycerol cholinephosphotransferase	
3	g3p+AcCoA→LPA	g3p-AcT	glycerol-3-phosphate O-acyltransferase	Green & Bell (1984)
4	Pcho→CDPcho	CCT	CTP:phosphocholine cytidylyltransferase	Attard *et al.* (2000)
5	PC/PE/PS/PA→LPx+FA	PLA_2	phospholipase A_2	Sen *et al.* (1991) and Zidovetski *et al.* (1992)
6	DAG→PA	DGK	diacylglycerol kinase	Thomas & Glomset (1999)

Table 5. Sensitivity analysis results: reactions which increase the torque parameter.

rank	reaction		enzyme	evidence
1	$PC/PS/PE/PIP_2 \rightarrow DAG$	PLC	phospholipase C	Rao & Sundaram (1993)
2	$PIP \rightarrow PIP_2$	PIK	phosphatidylinositol kinase	
3	DAG + CDPeth→PE	EPT	diacylglycerol ethanolaminephosphotransferase	
4	peth→CDPeth	ECT	CTP:phosphoethanolamine cytidylyltransferase	Vermuelen *et al.* (1993); Bladergroen & Van Golde (1997) *contradictory*
5	PA→DAG	PAP	phosphatidate phosphatase	
6	PC→PS	PSS1	phosphatidylserine synthase I	

diacylglycerol CPT, glycerol-3-phosphate O-acyltransferase (g3p-AcT) and CCT. It is noted that we have found the rank order to be relatively insensitive to the TSS compositions that are used in the model.

A fundamental feature to emerge from the sensitivity analysis is that the gradients can be positive or negative. Table 5 shows the ranking of the reactions which increase λ. There is evidence for this type of influence on the membrane (for intrinsic membrane proteins) from studies of gramicidin (Killian & Dekruijff 1986). This implies that, in the network as a whole, simultaneous activation and deactivation of selected enzymes may be exploited to achieve robust control of torque tension. In cases of enzyme deactivation by increased torque tension, inverse feedback according to equation (3.1) provides stabilization

$$\sigma_{\mathrm{inv}} = c_1 \exp(-\lambda/c_2). \tag{3.1}$$

3.2. Model validation

If our postulate that the torque tension is under homeostatic control is correct, then as previously noted, we would expect the reactions identified by sensitivity analysis to be catalysed by enzymes that have distinctive and clear lipid requirements for activity. In principle, this provides a straightforward method to test the predictions of the model and the validity of our postulate using the literature data. However, we note that caution must be exercised in reviewing prior work in this area. For example, assays based on micellar systems do not yield unambiguous conclusions because the stored elastic energy is not representative of that found in bilayer membranes. Furthermore, assays based on small unilamellar vesicles cannot be used reliably due to the substantial contribution from geometric stress to the total torque tension. Unfortunately, these lipid systems are used extensively and consequently a significant proportion of the published data cannot be used to validate our model. However, once data from these systems are discarded, the remaining results are consistent with the putative feedback points identified by sensitivity analysis and the results are detailed in table 4. Notably, g3p-AcT, PLA_2 and DGK have been reported to be activated by type II lipids, consistent with the predictions of our model. Similarly, PLC has been reported to be inactivated by type II lipids (inverse feedback effect) and this is also shown in our sensitivity analysis and summarized in table 5.

We note that there is one apparently anomalous observation. From the results of sensitivity analysis, CTP:phosphoethanolamine cytidylyltransferase (ECT) emerges as potentially important in controlling the torque tension. ECT is catalytically similar to CCT in that it mediates the reaction between phosphoethanolamine and CTP. Our analysis suggests that this reaction is a probable target for feedback control. However, in mammals there is no evidence that ECT is controlled by membrane lipid composition. A possible explanation for this discrepancy is discussed in §3.5. Overall, however, there is a strong agreement about the nature of the feedback (e.g. normal or inverse) between the results of the sensitivity analysis and the literature data. Further validation of the model could be possible using mass spectrometry data to track *in vivo* changes in lipid concentrations following type I lipid challenge of cells. These experiments are currently underway.

3.3. Explicit feedback

Having identified the potential control points, feedback was implemented explicitly in the simulations by using the torque parameter to modulate (according to equation (2.5)) the rate of one reaction, the reaction catalysed by CCT. As expected, we found that as a consequence of feedback the torque tension was less sensitive to changes in the rate constants of the reactions in the network and this is highlighted in figure 5*b*(i). In other words, the homeostatic robustness of the system was increased considerably. Applying feedback at more than one reaction increases the robustness further, as shown in figure 5*c*(i). One consequence of multiple point feedback is that the system acquires significantly increased effective connectivity, or 'elusive connectivity' (Morowitz *et al.* 1964), and hence enhanced redundancy. This observation is potentially of considerable importance because it indicates how the species complexity and chemical limitations of a cell membrane can be transcended to provide a physico-chemical environment that can be controlled within tight limits and which can allow membrane-associated proteins to function optimally, independent of the detailed lipid composition of the membrane.

3.4. PC concentration sensitivity analysis

Here we note that the sensitivity of the lipid concentrations to the enzyme activities is not reduced by torque tension feedback. This can be seen in figure 5*a*(ii),*b*(ii),*c*(ii), which show sensitivity analysis plots of PC concentration. Owing to its integrative nature, the feedback regime allows considerable variations in both the relative and the absolute concentrations of individual lipid species with minimal change to the torque parameter. The adjustment of reactions subject to feedback acts against the change in the torque parameter by adjusting the concentration of other lipid species. This enables the system to control the balance of type II and type I/0 lipids while increasing the overall concentration of lipid, which *in vivo* is a prerequisite for cell division.

The process in which accumulation of lipid occurs, while the torque parameter remains stable, can be seen clearly by affecting changes to the rates of the system's source reactions. The time-course data for this experiment are shown in figure 6. The plots demonstrate that increasing the influx into the feedback-regulated network results in lipid accumulation and that, in contrast to the uncontrolled network, the feedback control maintains the torque parameter very close to its original value while this occurs. It is noted that this is achieved without the need of the complex mechanisms that would be required to realize this effect by independently balancing the rates of many reactions.

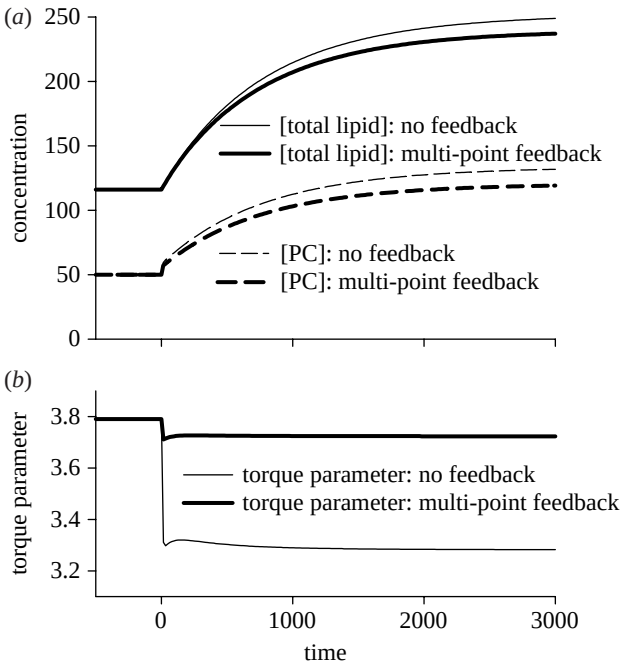

Figure 6. Time-course data for a twofold rate increase (at time point zero) in the source reactions R5 (CCT), R7 (g3p-AcT) and R8 (FA source reaction). The plots contrast the changes in the lipid concentrations and the torque parameter for the network without integrative feedback with a network with the same multi-point feedback configuration outlined in figure 5.

3.5. Contrasting CCT and ECT

As mentioned previously, ECT is cognate in its chemical action to CCT, but there is no evidence to show that it is affected by lipids in the same way as CCT (Vermuelen *et al.* 1993; Bladergroen & Van Golde 1997). In view of this, it might have been expected that the sensitivity analysis would have revealed an insensitivity of the torque parameter to ECT activity. As noted in §3.2, our studies have shown the opposite to be the case. We have also found that when ECT was subjected to the same type of feedback as CCT, there was a significant reduction in the control of the torque parameter and therefore the robustness of the system, as shown by comparison of figure 7*a*,*b*. The only way in which feedback at ECT could contribute to the robustness of the system is if it were to operate in a manner inverse to that at CCT (i.e. according to equation (3.1)). As shown in figure 7*c*, inverse feedback does improve the robustness but this is a relatively modest effect. In view of these observations, we conclude that there is not sufficient benefit to the system for the ECT reaction to be under control through integrative feedback.

The differing regulatory roles of ECT and CCT can be seen more clearly by focusing on the behaviour of the subset of reactions shown in figure 8. This subsystem model includes both the CCT- and the ECT-mediated reactions and the follow-on reactions catalysed by CPT and EPT. In the case of CCT, its activation causes an increase in the concentration of PC, a type 0 lipid, together with an associated decrease in DAG and PE,

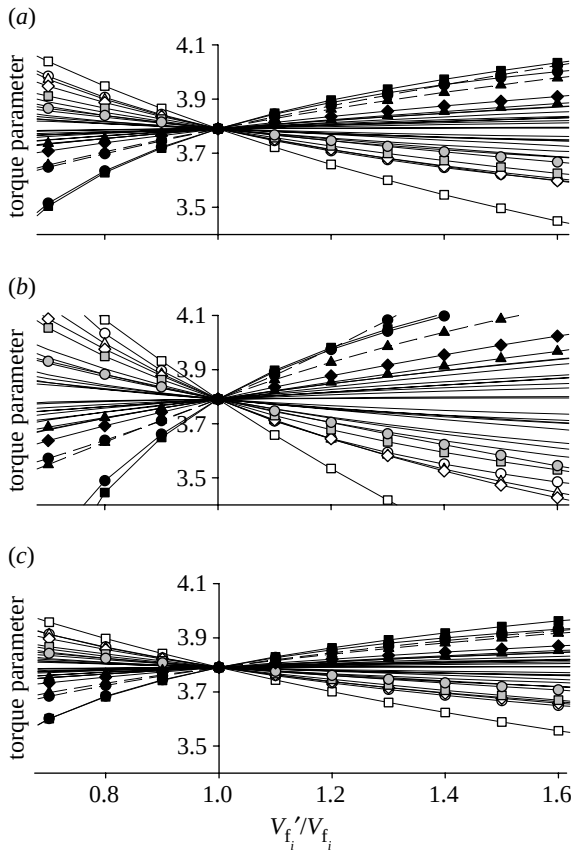

Figure 7. Effect of feedback control at ECT. (*a*) Normal feedback at CCT. (*b*) Normal feedback at CCT and ECT. (*c*) Normal feedback at CCT and inverse feedback at ECT. Keys are the same as given in figure 5.

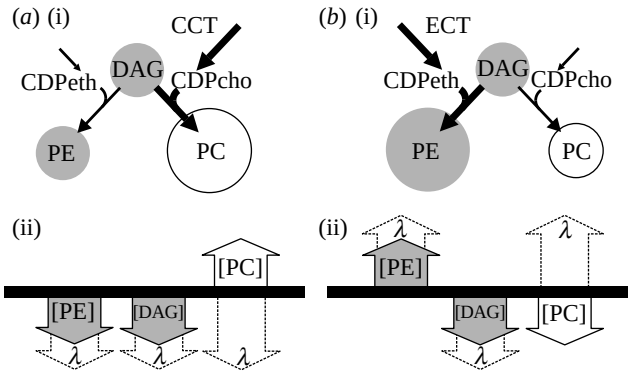

Figure 8. Subsystem model for comparison of changes when the rate of the reaction mediated by (*a*) CCT or (*b*) ECT is increased: (i) the effect on the subsystem components and (ii) the concentration changes for the subsystem's lipids (solid arrows) and the effect of each change on the torque parameter (dotted arrows) (the arrows indicate the increases or decreases in the quantities but do not reflect the magnitude of the changes, the shading indicates type II character with type I/0 lipids shown unshaded).

both of which are type II lipids. It is noted that other lipid concentrations will be affected; however, the changes in PC, DAG and PE are the most significant. These changes act upon the torque parameter in concert and each contribute to a reduction in the torque parameter, as illustrated in figure 8*a*(ii). This provides the signal to attenuate the activity of CCT, stabilizing the stored elastic energy, and leads to an increase in the system robustness. However, in the case of ECT, activation causes an increase in the concentration of PE but a decrease in that of DAG and an associated decrease in the concentration of PC. These changes, shown in figure 8*b*(ii), do not have a consistent effect upon the torque parameter. The direction of the change in the torque parameter will be critically dependent on the balance of the changes in the three lipid concentrations. A consistent effect on the torque parameter upon a change in rate is a crucial requirement for a feedback signal acting on an enzyme to lead to stabilization of the membrane torque tension. Hence, our studies strongly suggest that ECT cannot provide a stabilizing feedback point and we propose that this is the mechanism which accounts for the lack of lipid regulation of ECT.

4. CONCLUSION

The extensive literature on the properties of membrane enzymes that are involved in the synthesis of phospholipids shows that they are affected by the physicochemical properties of the membranes with which they are associated. Our previous *in vitro* work on CCT suggested that the key physical parameter that could affect regulation might be the stored curvature elastic energy. We speculated that this physical quantity could be under homeostatic control *in vivo* through integrative feedback that is dependent on the lipid composition of membranes. To test the consequences of such a control mechanism on the properties of a 'typical' lipid biosynthetic network we set up an *in silico* model.

Our sensitivity analysis of the model highlighted a series of reactions that would be expected to be the targets of integrative feedback that operates though the torque tension. We have found that this series of reactions is consistent with the literature data on enzymes involved in lipid synthesis and degradation that have a lipid requirement for activity. Furthermore, we have found that the lipid requirement is consistent with that predicted by the model, i.e. some reactions are activated by type II lipids (normal feedback), while others are inhibited by this class of lipids (inverse feedback). The only exception to this is ECT, which our sensitivity analysis predicts could have a significant effect on the torque parameter and so would be expected to be a target for feedback. The extensive literature on ECT shows convincingly that, although catalytically it is very similar to CCT, it is not affected by lipids. A detailed analysis of the effect that feedback at ECT has on the properties of the network suggests that ECT cannot provide a stabilizing feedback point and this provides a functional rationale for ECT not being affected by membrane lipid composition.

The results of our studies strongly suggest that membrane curvature elastic energy may play a key role in the *in vivo* control architecture of lipid biosynthesis. Furthermore, any protein that possesses an α-helical membrane-binding motif like that of CCT could, in principle, partition onto a membrane in proportion to the stored curvature elastic energy, and this partitioning might also involve unfolding/refolding of the protein if the relevant energy barriers are sufficiently low. Thus, the effect of varying membrane lipid composition could directly affect fluxes through reaction pathways that do not involve lipid species. Additionally, the partitioning of different proteins onto membranes would be a competitive process and would result in inhibitory interactions between proteins that do not interact with each other except through their effect on the stored elastic energy. As a consequence, the results we have obtained raise new questions about our understanding of the way in which biochemical reaction networks might be regulated. However, the precise *in vivo* role of membrane stored elastic energy, where lipid biosynthesis and degradation pathways intersect with signalling and immune response pathways that also affect lipid composition, remains to be clarified.

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