Cell Cycle Regulation of Membrane Phospholipid Metabolism*

Suzanne Jackowski‡

From the Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38101 and the University of Tennessee, Memphis, Tennessee 38163

This review focuses on the phospholipid metabolism regulated by the cell cycle. Phospholipids are the major cellular constituents required for the assembly of biological membranes, and cells must double their phospholipid mass to form daughter cells. It seems reasonable that this event should coincide with the synthesis of other cellular components such as DNA, stable RNA, etc.; however, the biochemical mechanisms that coordinate macromolecular and bulk membrane phospholipid production are largely unknown. The importance of these regulatory processes to cell physiology is obvious. Discordant regulation of phospholipid accumulation by only a few percent per cell cycle would rapidly result in cells with either a large excess or deficit of membrane surface leading to abnormalities in cell size and/or intracellular lipid accumulation. Thus, stringent control mechanisms must be in place to keep the phospholipid content in tune with the cell cycle. This discussion will explore the state of our knowledge in cultured mammalian cell systems, although cell cycle-regulated phospholipid accumulation occurs in lower eukaryotes such as Saccharomyces cerevisiae (1) and Caulobacter crescentus (2). This review is limited to a discussion of events that are directly tied to the cell cycle. Phospholipid metabolism in response to mitogenic stimulation will not be addressed as these biochemical events are generally associated with the G0 to G1 transition and are ligand-regulated rather than being orchestrated by the cell cycle.

G1 Phase

The G1 phase of the cell cycle is characterized as having a high rate of membrane phospholipid turnover. Increased incorporation of label into phospholipids and elevated levels of intracellular soluble phospholipid precursors were noted after the mitogenic stimulation of cells (3, 4), although these studies did not address whether the increase in labeling represented net phospholipid synthesis or enhanced phospholipid turnover (5–11). Jackowski (12) examined both synthesis and degradation in a macrophage cell line using a double label experiment and attributed the increase in choline incorporation into PtdCho during G1 (4) to rapid PtdCho turnover (12). Importantly, the rate of PtdCho degradation decreased by an order of magnitude in S phase and then accelerated again as the cells entered the next G1 period, thereby establishing that PtdCho turnover in G1 was associated with the cell cycle and not a property of the G0 to G1 transition (12). The cessation of PtdCho degradation in S phase is likely an important contributor to the net accumulation of phospholipid during this time; however, nothing is known about the biochemical processes that govern the periodicity of PtdCho turnover.

PtdCho turnover may be an important aspect of phospholipid metabolism during G1 that is necessary, and in some cases, sufficient for entry into S phase. PtdCho hydrolysis by phospholipase C and/or D pathways is triggered by a wide array of agonists (9), and both exogenous bacterial PtdCho phospholipase C (13) and PtdCho phospholipase D (14) added to the medium mimicked the mitogenic effect of platelet-derived growth factor. The fact that PtdCho phospholipase C, like growth factors, was required throughout G1 for maximal mitogenic effect (15) supports a causal relationship between PtdCho turnover and G1 progression. The precise signal transduction pathways activated by PtdCho phospholipase C remain to be clarified, although activation of the Ras-Raf pathway (16, 17) and/or protein kinase Cζ (18) may be involved.

S Phase

The net accumulation of phospholipid is a periodic event associated with S phase of the cell cycle. The first experiments established that phospholipid synthesis occurred during interphase as opposed to mitosis (19, 20). Subsequent measurements of the amount of phospholipid per cell during the first 12 h after mitogenic stimulation revealed little increase, whereas there was a significant rise in cellular phospholipid content between 12 and 24 h (21, 22). In these studies, the first 12 h corresponded to the exit from G0 together with the G1 phase of the cell cycle, whereas the time between 12 and 24 h corresponded to S, G2, and M phases. A more detailed analysis revealed that phospholipid content doubled specifically during S phase (12, 20). This pattern of net phospholipid accumulation was consistent among a number of mammalian cell types including fibroblasts (21), HeLa cells (19), macrophages (12), mast cells (20), and thymocytes (22). A key experiment was to follow the pattern of phospholipid accumulation through the second cell cycle following synchronization to convincingly distinguish that membrane phospholipid acquisition was a cell cycle-regulated process rather than a growth factor-triggered event that took several hours to initiate (12). The distribution of major phospholipid classes in whole cells was essentially constant throughout the cell cycle in NIH fibroblasts (11). Likewise, the content of PtdIns, PtdIns-P, and PtdIns-P2 was relatively constant during the cell cycle (23), although specific changes have been observed in the nuclear compartment (see below).

Net phospholipid accumulation is coordinated with S phase of the cell cycle, but phospholipid synthesis is not dependent on DNA synthesis. Cell cycle arrest of a macrophage cell line in mid-G1 with dibutyryl-cAMP or at the G2/S boundary with aphidicolin prevented S phase DNA synthesis; however, net phospholipid accumulation continued (12). These data illustrated that the decision to double the phospholipid mass was...
made in early G₂, or perhaps M phase of the previous cell cycle, and proceeded concurrently, but independently, of DNA replication. The nature of the signal that licenses the cell to double its membrane phospholipid mass is unknown but is likely to involve the expression or modification of key regulators in early G₁.

The overall phospholipid composition of isolated nuclei is not markedly different from the whole cell; PtdCho, PtdEtn, and sphingomyelin are the predominant phospholipid species, and PtdIns and its phosphorylated derivatives are represented as minor components (see Ref. 24 and references therein). Interestingly, nuclear phospholipids appear to be distributed within the interphase nucleus in addition to their presence in the nuclear membrane. Amorphous lipoprotein complexes were identified morphologically in the interphase cell nucleus, localized mainly in the interchromatin spaces and in the nucleolar domain. Furthermore, a decrease in the overall nuclear phospholipid content was associated with DNA replication (25); however, the morphological analysis did not reveal whether this decrease was due to the disappearance of a specific phospholipid class. The apparent co-localization of phospholipids and ribonucleoproteins suggested a role for phospholipid in the mechanism of transport and release of transcripts. For example, the release of ribonucleoproteins after phospholipase A₂ digestion of the nucleus indicated that phospholipids may mediate the binding between ribonucleoprotein and the nuclear matrix (24). The importance of these morphological observations to the biochemical events taking place in the nucleus is a challenging area for future research.

Nuclear inositol phospholipid metabolism is a significant S phase-specific event. The enzymes of polyphosphoinositide turnover occur in the nucleus (for review see Ref. 26), and there is considerable evidence for PtdIns-P₂ synthesis (27, 28) and degradation in the nuclear matrix (29–31). Nuclear PtdIns, PtdIns-P, and PtdIns-P₂ decreased coincident with S phase in HeLa cells (23), and the levels of all of the inositol phosphates increased at both the G₇/S boundary and in S phase in the nucleus of synchronized neuroblastoma cells (32). These data suggested that the PtdIns cycle was activated in S phase nuclei and implied the presence of a nuclear PtdIns-P₂ phospholipase C that was specifically regulated during S phase. Phospholipase Cδ1 is present in the nuclei of Swiss 3T3 cells and was postulated to be involved in the rapid responses of quiescent (G₀) cells to insulin growth factor-1 (33). More recently, Asano et al. (34) purified a novel phospholipase C isoform that was only detected in the nuclei of regenerating rat liver. Subsequently, a new isoform of PtdIns-specific phospholipase C (Cδ4) was cloned, purified, and characterized by two laboratories (35, 36). Importantly, phospholipase Cδ4 was primarily present in the nucleus, dramatically increased at the G₇/S transition, and virtually disappeared by the time cells entered the next G₁ phase (36). Thus, phospholipase Cδ4 has the biological properties anticipated for the enzyme that regulates nuclear phosphoinositide metabolism during S phase, and it will be important to determine if its activity is regulated by cell cycle-specific expression alone or whether there is an additional level of activity regulation by cyclin-dependent protein kinases.

The products of polyphosphoinositide breakdown are thought to play a role in DNA synthesis, consistent with their formation during S phase. Treatment of the nuclear matrix with phospholipase C released nucleic acid suggesting that polyphosphoinositides mediated the association of DNA with the matrix (37), an interaction that may have to be disrupted for efficient DNA synthesis. Alternatively, the diacylglycerol released by phospholipase C may activate nuclear protein kinase C, which, in turn, has been found to phosphorylate and activate DNA polymerase and topoisomerase (30, 38). Yorf et al. (39) demonstrated the existence of a nuclear inositol polyphosphate-1-phosphatase. The overexpression of this inositol polyphosphate-1-phosphatase inhibited DNA synthesis, thus providing compelling evidence for inositol polyphosphates as determinants in the control of DNA synthesis. One likely candidate for nuclear signaling is inositol 1,4-bisphosphate since this compound could bind to DNA polymerase and enhance the affinity of the enzyme for DNA template/primer (40). Alternatively, the identification of an inositol 1,4,5-trisphosphate receptor in the inner nuclear membrane that mediated calcium release into the cytoplasm (41) indicated that inositol polyphosphates played a role in regulating nuclear calcium concentration, which in turn could influence DNA replication and gene transcription (42). Establishing the critical nuclear target(s) and determining their function in the initiation and control of S phase DNA replication will be an interesting challenge.

### G₂ and M Phases

Much less is known about phospholipid metabolism in G₂ and M phases. There was little overall phospholipid synthesis or degradation occurring in these latter stages based on metabolic labeling of macrophase cells synchronized by growth factor withdrawal (12). The paucity of information is due in part to the difficulty in analyzing pure populations of cells in these phases of the cell cycle. The G₂ and S phases are between 6 and 12 h in length making it relatively easy to isolate adequate numbers of cells. However, the G₂/M phases are considerably shorter (2–4 h), and synchronous cell populations in G₂/M are contaminated with cells in S and G₁ phases. Nocodazole is an effective agent that arrests cells in M phase; however, the use of cell cycle blockers can be problematic without corroboration from experiments with synchronous or elutriated cells.

The most significant event involving phospholipids in M phase is the cessation of membrane trafficking concomitant with the destruction and reassembly of the nuclear membrane (43). While the dynamic aspects of the assembly of nuclear membrane protein components have received considerable experimental attention (44), the phospholipid components have not been studied in detail. Metabolic labeling of Chinese hamster ovary cells indicated that at least 50% of the nuclear envelope phospholipid present in G₂ was used to resynthesize the nuclear envelopes of the daughter cells (45). Studies with Amoeba proteus are worthy of mention because of their unique approach (46). Autoradiographic observations following implantation of [³H]choline-labeled nuclei into unlabeled cells revealed little turnover of nuclear membrane phospholipid during interphase; however, during mitosis the label was dispersed throughout the cytoplasm coincident with the degradation of the nuclear envelope. The cytoplasmic label was subsequently divided equally among the daughter cell nuclei. These data indicated that the nuclear envelope was reconstructed from pre-existing phospholipids as the cells exited M phase and entered early G₁. The role of phospholipid trafficking in the dissolution and reformation of the nuclear envelope promises to be an exciting area for future investigation.

### Biochemical Mechanisms

The search for the biochemical mechanism underlying the periodic accumulation of membrane phospholipid has focused on the control of PtdCho synthesis and degradation. PtdCho is not only the most abundant membrane phospholipid, but it is also serves as the precursor for the other two predominant phospholipid species, PtdEtn (47) and sphingomyelin (48). Most cells are capable of synthesizing PtdEtn via CTP:phos-
The G1 phase is characterized by rapid synthesis and degradation of phospholipids in preparation for cell division. Although DNA replication and expansion of the membrane phospholipid pool are coordinated with the cell cycle, they are not dependent on each other since inhibition of DNA replication did not block phospholipid accumulation and the inhibition of phospholipid synthesis did not have an immediate impact on DNA replication.

Do Lipids Regulate the Cell Cycle?

While it is apparent that the cell cycle controls bulk phospholipid and membrane biogenesis, it is not clear whether the cell cycle is in turn influenced by lipid content. Several studies reported that the inhibition of fatty acid and/or phospholipid synthesis by nutritional deprivation (i.e. biotin or choline starvation) led to the accumulation of several cell types in G2 (58–61). When C3H/10T1/2 fibroblasts were deprived of choline and synchronized in G1 by incubation in low serum, the cells did not efficiently enter S phase following serum restimulation suggesting that PtdCho synthesis was a requirement for S phase entry (61). However, cells deprived of choline continued to divide for several days (61), and Chinese hamster ovary cells with a temperature-sensitive defect in CT activity continued to grow for several doublings in the absence of PtdCho synthesis via the de novo pathway (62). These latter data suggest that proliferating cells do not detect phospholipid content as a determinant of cell cycle progression. However, examining the state of cyclins and their associated kinases in cells arrested by choline starvation or in the temperature-sensitive CT mutants may reveal a relationship between phospholipid metabolism and cell cycle control that is not apparent from these experiments. Alternatively, the accumulation of cells deficient in phospholipid in G1 may reflect a requirement for the high degradation or turnover of PtdCho that occurs during this phase of the cell cycle, which in turn demands an accelerated rate of lipid synthesis.

Concluding Remarks

The experiments to date lead to a conceptual model for the modulation of phospholipid metabolism during the cell cycle (Fig. 1). The G1 phase is characterized by rapid synthesis and degradation of PtdCho that continues up to the G1/S boundary. PtdCho metabolism is so rapid that some cells turn over about 75% of their total PtdCho during G1. PtdCho turnover ceases, and the cells double their membrane phospholipid content in preparation for cell division. Although DNA replication and expansion of the membrane phospholipid pool are coordinated with the cell cycle, they are not dependent on each other since inhibition of DNA replication did not block phospholipid synthesis. CT is a key regulatory enzyme in PtdCho biosynthesis and hence phospholipid formation (50). CT is extensively phosphorylated on its carboxyl-terminal domain in vivo (51), and CT phosphorylation is associated with the inhibition of enzyme activity (12, 52). Importantly, the extent of CT phosphorylation correlates with the cell cycle, and maximum CT phosphorylation occurs in the G2/M phase and correlates with the cessation of phospholipid synthesis (12). Nuclear CT (53–56, 68) is in the correct subcellular compartment for regulation by cyclin-dependent kinases, and the observation that CT is phosphorylated to some extent in vitro by Cyclin B/Cdc2 kinase (57, 69) suggests that the regulators of the cell cycle control the pace of phospholipid synthesis through the direct phosphorylation of CT. However, CT activity regulation by cyclin-dependent protein kinase phosphorylation has not been directly demonstrated, and there are likely to be additional enzymes (i.e. those responsible for PtdCho degradation) that contribute to the observed periodicity in membrane formation. Identifying these enzymes and their modes of regulation is obviously important to completing the understanding of cell cycle regulation of phospholipid metabolism.

FIG. 1. Relationship between phospholipid metabolism and the cell cycle. G1 phase is characterized by a high rate of PtdCho degradation and resynthesis that is dependent on growth factor and terminates at the G1/S boundary. Doubling of the phospholipid mass occurs in S phase due to continued phospholipid synthesis but with drastically reduced phospholipid turnover. The turnover of nuclear polyphosphoinositides is an additional S phase event that may be a component of a regulatory network that governs DNA replication. The G2 and M phases are characterized by the cessation of phospholipid metabolism.
phospholipid synthesis reaches its nadir in the G2 and M phases, a stage characterized by the activation of Cyclin B/Cdk2 kinase. CT is currently the only candidate for a key regulator of cell cycle-dependent membrane phospholipid biosynthesis; however, other candidate regulators are likely to arise from the ongoing investigation of the biochemical mechanisms responsible for coordinating membrane formation with cell division. The role of phospholipid trafficking in the dissolution and reformation of the nuclear envelope is also a fertile area for investigation. The solutions to these questions present major experimental challenges, but the answers promise exciting new insights into the biochemical processes that govern membrane formation and cell division.

REFERENCES
1. Cattrell, S. F., Getz, G. S., and Rabiniowitz, M. (1981) J. Biol. Chem. 256, 10973–10978
2. O’Neil, E. A., and Bender, R. A. (1987) J. Bacteriol. 169, 2618–2623
3. Warden, C. H., Friedkin, M., and Geier. P. J. (1980) Biochem. Biophys. Res. Commun. 94, 690–697
4. Tessier, T. G., Rock, C. O., Kalmar, G. B., Cornell, R. B., and Jackowski, S. (1991) J. Biol. Chem. 266, 16261–16264
5. Cunningham, D. D. (1972) J. Biol. Chem. 247, 2464–2470
6. Pasternak, C. A., and Bergeron, J. M. (1970) Biochem. J. 119, 473–480
7. Ristaw, H. J., Frank, W., and Fronlick, H. (1973) Naturforsch. 28C, 188–197
8. Pasternak, C. A. (1972) J. Cell Biol. 53, 231–234
9. Exton, J. H. (1994) Biochim. Biophys. Acta 1212, 26–42
10. Lario, R., Cornet, M. E., Diaz-Meco, M. T., Lopez-Barahona, M., Diaz-Laviada, I., Gudal, P. H., Jhansien, T., and Moscat, J. (1990) Cell 61, 1113–1120
11. Dubos, C., and Rampini, C. (1978) Biochimie 60, 1307–1313
12. Jackowski, S. (1994) J. Biol. Chem. 269, 3858–3867
13. Lario, R., Cornet, M. E., Diaz-Meco, M. T., Lopez-Barahona, M., Diaz-Laviada, I., Gudal, P. H., Jhansien, T., and Moscat, J. (1990) Cell 61, 1113–1120
14. Kondo, T., Inui, H., Konishi, F., and Inagami, T. (1992) J. Biol. Chem. 267, 23609–23616
15. Xu, X., Tessier, T. G., Rock, C. O., and Ackowski, S. (1993) Mol. Cell. Biol. 13, 1522–1533
16. Cai, H., Erhardt, P., Troupmair, J., Diaz-Meco, M. T., Sitanandam, G., Rapp, U. R., Moscat, J., and Cooper, G. M. (1993) Mol. Cell. Biol. 13, 7645–7651
17. Bjorkoy, G., Overvatn, A., Diaz-Meco, M. T., Moscat, J., and Jhansien, T. (1995) J. Biol. Chem. 270, 21299–21306
18. Berra, E., Diaz-Meco, M. T., Dominguez, I., Munizco, M., Sanz, L., Lozano, J., Chapkin, R. S., and Moscat, J. (1993) Cell 74, 553–565
19. Robbins, E., and Scharff, M. (1986) in Cell Synthesis (Cameron, I. L., and Padilla, G. M., eds) pp. 353–360, Academic Press, New York
20. Bergeron, J. J., Warnsly, A. M. H., and Pasternak, C. A. (1970) Biochem. J. 119, 492–499
21. Habenicht, A. J. R., Glomsset, N. J., and Eisele, M. (1981) J. Biol. Chem. 256, 1370–1373
22. Groen, G. J., van der Bliek, A., and Brand, K. (1988) Biochim. Biophys. Acta 962, 220–226
23. York, D. J., and Mayerus, P. W. (1994) J. Biol. Chem. 269, 7847–7850
24. Maraldi, N. M., Mazzotti, G., Capitanio, S., Rizzi, R., Zini, N., Squarzoni, S., and Manzoli, F. A. (1992) Adv. Enzyme Regul. 32, 73–90
25. Maraldi, N. M., Santi, S., Zini, N., Ognibene, A., Rizzi, R., Mazzotti, G., Di Primino, R., Bareggi, R., Bertagalo, V., Pagliarini, C., and Capitanio, S. (1993) J. Cell Sci. 104, 853–859
26. Dicueva, N., Banfic, H., and Irvine, R. F. (1993) Cell 74, 405–407
27. Martelli, A. M., Bareggi, R., Coco, L., and Manzoli, F. A. (1996) Biochem. Biophys. Res. Commun. 218, 182–186