Calcium-independent Phospholipases from Guinea Pig Digestive Tract as Probes to Study the Mechanism of Lipocortin*

Ama Gassama-Diagne†, Josette Fauvel, and Hugues Chap§
From Institut National de la Santé et de la Recherche Médicale Unité 326, Phospholipides Membranaires, Signaulation Cellulaire et Lipoproteines, Hôpital Purpan, 31059 Toulouse, France

Two calcium-independent phospholipases isolated from guinea pig pancreas (lipase Ia, 37 kDa) and from guinea pig intestine (phospholipase B, 97 kDa) have been used to probe the mechanism of phospholipase inhibition by lipocortin. In the presence of calcium, both enzymes were inhibited by lipocortin I in a manner very similar to the inhibition of pig pancreas phospholipase A2. By using phospholipases that lack a requirement for calcium, we have for the first time been able to dissociate enzymatic activity from the role of calcium in the inhibitory process. It was found that lipocortin was without effect against phospholipase A1 and phospholipase B in the absence of calcium, under which conditions the inhibitory protein is unable to interact with anionic phospholipid surfaces. The same behavior toward phospholipase A1 was observed with two other related proteins, endonexin II or lipocortin V, and ß68/67-kDa calelectrin or lipocortin VI. Together with the observation that lipocortins are active only in the presence of limited amounts of substrate, these data give further support to the "surface depletion model" of lipocortin inhibition, rather than to a mechanism involving a direct interaction between enzyme and inhibitor.

Lipocortins have been defined as proteins inducible by glucocorticoids and able to inhibit intracellular phospholipase A2, resulting in a decreased synthesis of eicosanoids such as prostaglandins or leukotrienes, and of platelet-activating factor or platelet-activating factor-acether (1). A great advance about the knowledge of these proteins has been recently achieved by elucidation of the sequence of either purified proteins (2) or of their corresponding cDNA (3). As recently reviewed by Klee (4), these studies revealed that lipocortins actually belong to a protein family which includes calpactins I and II, identical to lipocortins II and I, respectively (5-7), endonexin I, protein II or chromobindin 4 (8-9), 67-kDa calelectrin or ß68 (10-12), anchoring CII (13, 14), lipocortin III (15), and uroteroglobin (16). Also included in this family is endonexin II or lipocortin V, which is recognized as an inhibitor of blood coagulation and has been isolated from human placenta and from human endothelial cells (17-21). All of these proteins display a high degree of homology (30-60%) first described by Geisow et al. (2), who identified a 17-amino acid consensus sequence present in the four repeats (eight in the case of ß68) forming the core of these various proteins, which also have become known by the general term of annexins (22).

However, annexins differ by their N-terminal end, which is the site of specific interactions with other proteins, such as protein I, the heterotetrameric form of calpactin I (23). The N-terminal end also offers specific phosphorylation sites to various protein kinases including protein kinase C (24, 25), cAMP-dependent protein kinase (25), calmodulin-dependent kinase (25), and oncogene or growth factor-related tyrosine kinases (4). In some instances, it has been suggested that these phosphorylations modulate the antiphospholipase A2 activity of these proteins (26, 27).

Besides being responsible for immunological cross-reactivity (28, 29), the homologous core appears to support various common properties of annexins, especially their calcium-dependent binding to negatively charged phospholipids (4). This binding to phospholipids rather than a specific interaction with the enzyme has been recently proposed as the mechanism of phospholipase inhibition (30, 31). In favor of this, phospholipase A2 inhibition by lipocortins can be reversed by increasing the concentration of phospholipid substrate, i.e. under conditions where the total membrane surface is no longer covered by adsorbed proteins. On this basis, Davidson et al. (30) proposed a so-called "surface depletion model" to explain phospholipase inhibition by lipocortins. This is consistent with the fact that all of them have been shown to achieve the same in vitro inhibition of phospholipase A2 under similar conditions (32). Furthermore, in addition to inhibiting the low molecular mass, calcium-dependent phospholipases A2 isolated from pancreas, snake venom (see Ref. 4 for review) or rat liver mitochondria (31), lipocortin was previously shown to also inhibit Clostridium perfringens phospholipase C (26), phosphoinositide-specific phospholipase C (33), as well as phospholipase D from cabbages (26) or from mammalian cells (34). Finally, in addition to the placentald protein isolated for its anticoagulant properties (17-19, 21), four of these annexins were recently found to inhibit the conversion of prothrombin into thrombin by a complex of factors Xa and Va under conditions involving a limited amount of anionic phospholipids (32).

We previously purified from guinea pig pancreas and intestine, respectively, two cationic lipases with high phospholipase A2 activity (35) and a phospholipase A2 with lysophospholipase activity or phospholipase B (36). These enzymes are structurally unrelated to classical phospholipases A2 (M, are 37,000 and 42,000 for the phospholipases A2 from guinea pig pancreas, and 97,000 for the intestinal phospholipase B, and they are characterized by a lack of calcium requirement. The present investigation took advantage of the latter property to study in more details the mechanism of phospholipase

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
† Recipient of a fellowship from Fondation pour la Recherche Médicale.
§ To whom correspondence and reprint requests should be addressed.
Calcium-independent Phospholipases and Lipocortins

inhibition by lipocortins. Indeed, in contrast to previous investigations dealing with calcium-dependent processes such as blood coagulation and hydrolysis by classical, low molecular mass phospholipases A₂, guinea pig phospholipases should allow the study of the interaction of these enzymes with their phospholipid substrates under conditions where their catalytic activity is unaltered by calcium concentration.

**EXPERIMENTAL PROCEDURES**

**Materials**—[3H]Palmid acid (500 mCi/mmole) was obtained from the Radiological Center (Amersham, United Kingdom), [3H]Oleic acid (500 mCi/mmole) and [3H]palmitoyl-[1-14C]oleoyl-phosphatidylcholine (phosphocholine (5.26 mc/mmole) were from Du Pont-New England Nuclear, Dreieich, Federal Republic of Germany. Phosphatidylerine from pig brain (95% purity), sodium deoxycholate, CHAPS, and pig pancreas phospholipase A₂ (700 units/mg protein) were purchased from Sigma.

**Guinea Pig Phospholipases**—The molecular form designated lipase Ia (M₂ = 37,000), which was shown to display a high phospholipase A₂ activity (37), was purified from guinea pig pancreas until apparent homogeneity, as determined by polycrylamide gel electrophoresis under nondenaturing conditions (35). Specific activity (as measured using rat liver phosphatidyicholine under optimal conditions) was 54 μmol/min/mg. Two different forms of pig intestine phospholipase B were alternatively used. One displayed a specific activity of 22.9 μmol/min/mg and was purified to about 90% purity (as detected by sodium dodecyl sulfate-polycrylamide gel electrophoresis), as previously described (36). Another preparation, although displaying a relatively high specific activity (29.9 μmol/min/mg), still showed several contaminating bands at 110, 64, and 55 kDa, in addition to the 97 kDa protein previously shown to support the phospholipase B activity (36).

**Lipocortin I**—Lipocortin I was purified from pig lung as described (29, 38). Briefly, this included selective extraction with EGTA, chromatography on DEAE TSK 545 (21.5 × 150 mm), gel filtration on ACA 44, affinity chromatography on polycrylamide gel immobilized phosphatidylinositol and chromatography on hydroxyapatite. Whereas lipocortin I was detected in the flow-through fractions of the DEAE TSK 545 at pH 7.0, a 33 kDa calcium- and phospholipid-binding protein was retained on the column and eluted with 0.2 M NaCl. Further purification involved affinity chromatography on polycrylamide-imobilized phosphatidylinerine and chromatography on hydroxyapatite. Whereas lipocortin I was detected in the flow-through fractions of the DEAE TSK 545 at pH 7.0, a 33 kDa calcium- and phospholipid-binding protein was retained on the column and eluted with 0.2 M NaCl. In contrast to its bovine homolog previously mentioned (39), the pig protein displayed a single band at 33 kDa upon sodium dodecyl sulfate-polycrylamide gel electrophoresis and was tentatively identified as lipocortin I or endoxemnin II, on the basis of physicochemical properties, immunoreactivity, and by the fact that it substrates calcium-dependent kinase C. The 67 kDa calceceitin, also referred to as lipocortin VI (15), was purified from pig liver using the same procedure as previously described (38).

**Determination of Phospholipase A₂ Activity in Guinea Pig Intestine Phospholipase B and Comparison with Pig Pancreas Phospholipase A₂**—In the first experiments, the phospholipid substrate consisted of sonicated phospholipid vesicles containing 1-acyl-2-[14C]oleoyl-phosphatidylcholine and brain phosphatidylinositol in the molar ratio 7:3, previously used to study inhibition of both phospholipase A₂ and prothrombinase (32). However, owing to the poor interaction of phospholipase B with phospholipase vesicles, no hydrolytic activity could be detected under these conditions, in agreement with our previous observation that the enzyme requires mixed micelles of phospholipids with sodium deoxycholate (36, 44).

As shown in Fig. 1, lipocortin I promoted a dose-dependent inhibition of both pig pancreas phospholipase A₂ and guinea pig intestine phospholipase B under identical conditions of assay, i.e. 1.2 mM sodium deoxycholate and 5 mM CaCl₂. Although the specific activities of the two phospholipases measured under these conditions were different, the two inhibition curves were remarkably parallel, suggesting a similar mechanism for the inhibition of the two enzymes. Maximal inhibition (75–80%) was attained for both enzymes at 10 μg/ml of lipocortin I, while 50% inhibition occurred in the presence of 3.75 μg/ml of lipocortin I.

**RESULTS**

**Effects of Lipocortin I on Phospholipase A₂ Activity of Guinea Pig Intestine Phospholipase B and Comparison with Pig Pancreas Phospholipase A₂**—In the first experiments, the phospholipid substrate consisted of sonicated phospholipid vesicles containing 1-acyl-2-[14C]oleoyl-phosphatidylcholine and brain phosphatidylinositol in the molar ratio 7:3, previously used to study inhibition of both phospholipase A₂ and prothrombinase (32). However, owing to the poor interaction of phospholipase B with phospholipase vesicles, no hydrolytic activity could be detected under these conditions, in agreement with our previous observation that the enzyme requires mixed micelles of phospholipids with sodium deoxycholate (36, 44).

As shown in Fig. 1, lipocortin I promoted a dose-dependent inhibition of both pig pancreas phospholipase A₂ and guinea pig intestine phospholipase B under identical conditions of assay, i.e. 1.2 mM sodium deoxycholate and 5 mM CaCl₂. Although the specific activities of the two phospholipases measured under these conditions were different, the two inhibition curves were remarkably parallel, suggesting a similar mechanism for the inhibition of the two enzymes. Maximal inhibition (75–80%) was attained for both enzymes at 10 μg/ml of lipocortin I, while 50% inhibition occurred in the presence of 3.75 μg/ml of lipocortin I.

**Note**: The abbreviations used are: CHAPS, 3-[cholamidopropyl]-dimethy lamonium); I-propanecolonic acid; EGTA, (ethylenecarbamonyl)-oxyethyl) enenitirilo)tetraacetic acid.

---

1. I. Vilgrain, E. Chambaz, J. Pauvel, and H. Chap, unpublished results.
The presence and in the absence of calcium, respectively). As high activities were measured in the presence of calcium or EGTA at detergent concentrations above 4 mM, phospholipase B was no longer inhibited by lipocortin at concentrations up to 20 pg/ml (not shown). Here again, addition of lipocortin I resulted in a dramatic inhibition of phospholipase A1 activity when CaCl2 was present in the assay medium, whereas EGTA abolished the inhibitory effect of lipocortin I against phospholipase A1, whose activity was slightly enhanced (Fig. 5). The same conditions of assay were selected to compare the antiphospholipase A1 activity of three different lipocortins: I, V, and VI, corresponding, respectively, to calpactin II, endonexin II and p88, according to Pepinsky et al. (15). As shown in Table I, the three proteins displayed very similar if not identical inhibition of phospholipase A1 in the presence of calcium, whereas EGTA completely suppressed this effect.

In order to find conditions which would allow us to detect identical phospholipase B activities in the presence or in the absence of calcium, the effect of sodium deoxycholate concentration on phospholipase B activity was examined. From data of Fig. 3, it is clear that sodium deoxycholate concentrations producing the highest phospholipase B activities were shifted to lower values in the presence of calcium. As a result of this, calcium appeared to increase phospholipase B activity at sodium deoxycholate concentrations up to 2.4 mM, but equally high activities were measured in the presence of calcium or EGTA at detergent concentrations above 4 mM, in agreement with our previous data (36). However, at these higher concentrations of detergent, phospholipase B was no longer inhibited by lipocortin at concentrations up to 20 μg/ml (not shown). This was probably due to a large excess of anionic lipid/water interface, as previously described by others (30, 31).

This prompted us to determine phospholipase B activity in the presence of CHAPS, a zwitterionic detergent. Using total phospholipids from E. coli, experimental conditions could be defined where phospholipase B activity remained completely independent on calcium (38 and 44 nmol × min⁻¹ × mg⁻¹ in the presence and in the absence of calcium, respectively). As shown in Fig. 4, the phospholipase B inhibitory effect of lipocortin I absolutely required calcium under these conditions of assay. Half-maximal inhibition occurred at 9 μg/ml lipocortin I and a total inhibition at 40 μg/ml. In contrast, lipocortin remained without effect in the presence of EGTA.

Effect of Lipocortins on Guinea Pig Pancreas Phospholipase A1 Activity—At variance with the previous experiments, assay of phospholipase A1 activity showed no effect of calcium, even when using sodium deoxycholate as a detergent. As shown in Fig. 5, phospholipase A1 activity using [3H]palmitate-labeled phospholipids from E. coli was 12.6 and 10.8 nmol × min⁻¹ × mg⁻¹ in the presence or in the absence of calcium, respectively. Here again, addition of lipocortin I resulted in a dramatic inhibition of phospholipase A1 activity when CaCl2 was present in the assay medium, whereas EGTA abolished the inhibitory effect of lipocortin I against phospholipase A1, whose activity was slightly enhanced (Fig. 5).

FIG. 1. Inhibition of guinea pig intestine phospholipase B (PLB) and pig pancreas phospholipase A2 (PLA2) by lipocortin I. Phospholipase A2 activity was determined under identical conditions for both enzymes as described under “Experimental Procedures,” using 1-palmitoyl-2-[14C]oleoyl-phosphatidylcholine-unlabeled phosphatidylserine (7:3, molar ratio) in the presence of 1.2 mM of sodium deoxycholate. Specific activities of pig pancreas phospholipase A2 and guinea pig intestine phospholipase B were 97.4 and 2.3 nmol × min⁻¹ × mg⁻¹, respectively. Data are means of two determinations.

FIG. 2. Effects of calcium and of lipocortin I on phospholipase A2 activity of guinea pig intestine phospholipase B in the presence of sodium deoxycholate. Phospholipase A2 activity was determined as described under “Experimental Procedures” using 1-palmitoyl-2-[14C]oleoyl-phosphatidylcholine-unlabeled phosphatidylserine (7:3, molar ratio) in the presence of 1.2 mM of sodium deoxycholate, 5 mM of CaCl2, or 1 mM EGTA. Data are means of two determinations.

FIG. 3. Effect of sodium deoxycholate on phospholipase A2 activity of guinea pig intestine phospholipase B in the presence or in the absence of calcium. Enzyme activity was determined as described under “Experimental Procedures” using 1-palmitoyl-2-[14C]oleoyl-phosphatidylcholine-unlabeled phosphatidylserine (7:3, molar ratio) in the presence of various concentrations of sodium deoxycholate, with addition of either CaCl2 (6 mM) or EGTA (1 mM). Data are means of two determinations. DOC, sodium deoxycholate.

FIG. 4. Effect of calcium on the inhibition of phospholipase A2 activity of guinea pig intestine phospholipase B by lipocortin I. Phospholipase A2 activity was determined as described under “Experimental Procedures” using 1-acyl-2-[3H]oleoyl-phospholipids from E. coli as a substrate and CHAPS as a detergent, in the presence of 5 mM CaCl2 or 1 mM EGTA. Data are means of three determinations.

FIG. 5. Phospholipase A1 activity using [3H]palmitate-labeled phospholipids from E. coli was 12.6 and 10.8 nmol × min⁻¹ × mg⁻¹ in the presence or in the absence of calcium, respectively. Here again, addition of lipocortin I resulted in a dramatic inhibition of phospholipase A1 activity when CaCl2 was present in the assay medium, whereas EGTA abolished the inhibitory effect of lipocortin I against phospholipase A1, whose activity was slightly enhanced (Fig. 5).
phospholipase B, respectively, molecular mass (37 and 97 kDa for phospholipase A1 and A2)

Here again, some stimulation of phospholipase A1 was even observed.

**DISCUSSION**

A first purpose of this study was to see whether two phospholipases which are different from the calcium-dependent phospholipases A2 can be inhibited by lipocortin through the same mechanism. Indeed, the two phospholipases used in the present study display great differences compared with known pancreas or venom phospholipases A1. In addition to a higher molecular mass (37 and 97 kDa for phospholipase A1 and phospholipase B, respectively, versus 14–15 kDa for phospholipases A2), the two guinea pig phospholipases are not resistant to heat and acid treatment, differ in substrate and positional specificity, do not require calcium for catalytic activity, and are not inhibited by bromophenacyl bromide. The latter reagent is currently used to inhibit calcium-dependent low molecular mass phospholipases A2 by alkylating histidine residue 48 in the active site (45), whereas both guinea pig pancreas phospholipase A1 (38) and guinea pig intestinal phospholipase B are unaffected by the same treatment.4 Thus, the observation that the two phospholipases examined here displayed the same sensitivity to lipocortin as pig pancreas phospholipase A2 strongly argues in favor of an inhibitory action involving an effect on the substrate rather than a direct interaction with the enzymes, whose active site is certainly different. This further extends previous reports concerning inhibition by lipocortins of phospholipases C and D (26, 33, 34). It is also in full agreement with the fact that lipocortin I behaves as a potential anticoagulant agent by suppressing the stimulatory effect of negatively charged phospholipids on prothrombin conversion by a complex of factor Xa and factor Va (32). It thus appears that lipocortin probably inhibits phospholipases upon binding to anionic phospholipid surfaces in the presence of calcium, thus competing for the phospholipid substrate, which is no longer available to the enzyme. However, the role of calcium in this mechanism cannot be explored directly with calcium-dependent target proteins such as pancreatic phospholipase A2 or enzymes from the blood coagulation system.

In this respect, the use of calcium-independent phospholipases provides a unique opportunity to study the effect of calcium on the inhibitory effect of lipocortin. In other words, if lipocortin depresses the activity of phospholipases according to the substrate depletion model (30), this effect should display an absolute requirement for calcium.

Such a goal required that we find conditions where the activity of the enzymes is not influenced by the presence of calcium. As illustrated in this study, this requirement is not always evident, since calcium is able to modify the activity of phospholipases by interacting with the lipid interface, particularly when the substrate or detergent carry negative charges. Such an effect of calcium involving a modification of the lipid/water interface has been previously observed with pancreatic lipases, which do not require calcium in their active site (46–48). Our data fully support the view that this is also the case for guinea pig phospholipase A1 and phospholipase B, in agreement with our previous reports (35–37, 44).

Taking advantage of the observation that a similar phospholipase B identified in rat intestine can be activated by sodium deoxycholate as well as by CHAPS (49), the zwitterionic detergent allowed us to find conditions where calcium effects on the substrate dispersions was minimized, resulting in identical phospholipase B activities in the presence of CaCl2 and of EGTA.

Thus, with both phospholipase A1 and phospholipase B, our results clearly demonstrate that lipocortin inhibition of phospholipases absolutely requires the presence of calcium. Altogether, our data support the view that phospholipase inhibition by lipocortins involves adsorption of these proteins at the lipid/water interface. It could be argued that such an adsorption of the proteins on the phospholipid surface is just a prerequisite, which allows direct interaction of the enzyme with its inhibitor. However, such a view is not supported by the observation that various lipocortins display very similar efficiencies in the inhibition of different phospholipases. Such a conclusion is in line with previous studies (30, 31), but it differs from that of Miele et al. (16), who suggested a more specific interaction between phospholipase A2 and lipocortin, uteroglobin (a closely related protein), or some peptides common to both proteins. The reasons for such a discrepancy are not entirely clear, but it should be recalled that the in vivo anti-inflammatory activity reported for these peptides (16) as well as for recombinant lipocortin (50) could not be observed in a similar model with purified lipocortin (51).

In the assays with phospholipase A1, not only lipocortin

---

**TABLE I**

Effect of three different lipocortins on guinea pig pancreas phospholipase A1: activity in the presence and in the absence of calcium.

| Conditions | 1 mm CaCl2 | 1 mm EGTA |
|------------|------------|-----------|
| Specific activity | Relative activity | Specific activity | Relative activity |
| Control | 12.6 | 100 | 10.8 | 100 |
| Lipocortin I | 3.5 | 28 | 13.3 | 123 |
| Control | 11.1 | 100 | 8.2 | 100 |
| Lipocortin V | 2.5 | 22 | 11.7 | 143 |
| Lipocortin VI | 3.9 | 35 | 12.9 | 157 |

---

*4 A. Gassama-Diagne, J. Fauvel, and H. Chap, unpublished results.*
Calciuin-independent Phospholipases and Lipocortins

inhibition disappeared in the presence of EGTA, but some phospholipase activation could even be detected. At the present time, this increased phospholipase activity in the absence of calcium cannot be correlated to any of the known properties of lipocortin or other calcium- and phospholipid-binding proteins. This is certainly not due to fatty acid removal by the protein, as previously observed with serum albumin (46), since the latter protein was already present in the incubation medium. We cannot exclude the possibility that some lipids bound to the lipocortins might have some activating effect.

In conclusion, the present study gives further support to the view that lipocortins inhibit phospholipase A₂ by a non-specific effect involving interaction with anionic phospholipids present in cell membranes. This is expected to occur preferentially in the inner leaflet of the plasma membrane, which was shown to contain the majority of negatively charged phospholipids such as phosphatidylserine (53–55). Lipocortins have been found to account for up to 1–2% of total proteins from some cells, such as human endothelial cells (29).

However, only rough estimates can be drawn on this basis to conclude whether this is sufficient to cover all of the membrane phospholipids facing the cytoplasm of a cell. Furthermore, some recent studies failed to detect any increase of lipocortin or its mRNA in cells treated by anti-inflammatory steroids (29, 51, 56, 57). Together with other observations that inhibition of prostaglandin synthesis by glucocorticoids does not necessarily involve a reduction of arachidonic acid mobilization from phospholipids (29, 58), these facts give some doubt about the mechanism of the anti-inflammatory action of corticosteroids, which might also modulate the expression of cyclooxygenase (59). They also leave open the question about the real physiological significance of calcium- and phospholipid-binding proteins, which still remains rather obscure (4).
49. Pind, S., and Kuksis, A. (1989) *Lipids* **24**, 357-362.

50. Cirino, G., Flower, R. J., Browning, J. L., Sinclair, I. K., and Pepinsky, R. B. (1987) *Nature* **328**, 270-272.

51. Northup, J. K., Valentine-Braun, K. A., Johnson, L. K., Severson, D. L., and Hollenberg, M. D. (1988) *J. Clin. Invest.* **82**, 1337-1342.

52. van Binsbergen, J., Slotboom, A. J., Aarsman, A. J., and de Haas, G. H. (1989) *FEBS Lett.* **247**, 283-287.

53. Verkleij, A. J., Zwaal, R. F. A., Roelofsen, B., Comfurius, P., Kastelijn, D., and van Deenen, L. L. M. (1973) *Biochim. Biophys. Acta* **323**, 178-193.

54. Chap, H., Zwaal, R. F. A., and van Deenen, L. L. M. (1977) *Biochim. Biophys. Acta* **467**, 146-164.

55. Perret, B. P., Chap, H., and Douste-Blazy, L. (1979) *Biochim. Biophys. Acta* **556**, 434-446.

56. Bronnegard, M., Anderson, O., Edwall, D., Lund, J., Norstedt, G., and Carlstedt-Duke, J. (1988) *Mol. Endocrinol.* **2**, 732-739.

57. Bienkowski, M. J., Petro, M. A., and Robinson, L. J. (1989) *J. Biol. Chem.* **264**, 6536-6544.

58. Wood, J. N., Coote, P. R., and Rhodes, J. (1984) *FEBS Lett.* **174**, 143-146.

59. Bailey, J. M., Makheja, A. N., Pash, J., and Verma, M. (1988) *Biochem. Biophys. Res. Commun.* **157**, 1159-1163.
Calcium-independent phospholipases from guinea pig digestive tract as probes to study the mechanism of lipocortin.
A Gassama-Diagne, J Fauvel and H Chap

*J. Biol. Chem.* 1990, 265:4309-4314.