Incubation of HeLa cells with the IMP dehydrogenase inhibitors: ribavirin (100 μM, 4 h), tiazofurin (100 μM, 4 h), selenazofurin (100 μM, 4 h), or mycophenolic acid (10 μM, 4 h) resulted in ~70% reduction in cellular GTP pools and shifting of nucleophosmin/B23 from nucleoli to nucleoplasm as detected by immunofluorescence (B23-translocation). Enzyme-linked immunosorbent assay and Western blot assay showed there is no loss or degradation of nucleophosmin/B23 protein during drug treatment. This translocation effect could be prevented by co-incubation with guanosine (100 μM) or reversed by addition of guanosine (100 μM) to the culture medium after B23-translocation had been induced by these inhibitors. Under these conditions of guanosine supplementation, cellular GTP pool concentrations were maintained at the control level. These results indicate that localization of nucleophosmin/B23 into the nucleus is dependent on the cellular GTP level.

Nucleoli of rapidly proliferating cancer cells are large, pleomorphic, and hyperactive (1). These highly structured and specialized organelles are the sites of active transcription of rDNA and ribosome assembly. Numerous nucleolar proteins, RNA, and other elements are involved in these intricate processes to produce ribosomes (2, 3).

Nucleophosmin/B23 (protein B23, NO38, numatrin) is a major nucleolar phosphoprotein which is significantly more abundant in tumor and growing cells than in normal resting cells (4-6). Its localization in the granular region of the nucleolus (7) along with its association with preribosomal particles (8, 9) suggest that it may be involved in ribosome assembly or processing. Induction of mitogenesis in B-lymphocytes is characterized by significant increases in nucleolar RNA, and other elements are involved in these intricate processes to produce ribosomes (2, 3).

We have developed a B23-translocation assay which is a simple and rapid functional assay that directly indicates the effect of certain anticancer drugs, particularly the intercalators (9, 11-16). The principle of this assay is based on the redistribution of nucleophosmin/B23 from nucleoli to nucleoplasm when cells are exposed to these anticancer drugs. B23-translocation has been demonstrated in cell cultures to correlate with drug effects, and the assay is useful in detecting drug-resistant cancer cells (13-16). B23-translocation has also been demonstrated to occur in an animal system, P388D1 leukemia implanted in DBA2 mice (17). The mechanism(s) of drug-induced redistribution of nucleophosmin/B23 is currently under investigation. The fact that only certain cytotoxic compounds have the capacity to induce B23-translocation (18) may provide some insight into the translocation mechanism, as well as into the function of nucleophosmin/B23 in the cell. We now report that depletion of GTP pools by IMP dehydrogenase inhibitors (19-27) also causes B23-translocation. This is the first report that adequate GTP levels are necessary for the localization of nucleophosmin/B23 in the nucleolus.

**MATERIALS AND METHODS**

**Drugs and Antibodies**—Ribavirin, tiazofurin, and selenazofurin were synthesized (28-30). Torcanomycin was kindly provided by the Drug Synthesis Branch of the National Cancer Institute. Mycophenolic acid, actinomycin D, guanosine, guanine, hypoxanthine, xanthine, adenosine, inosine, and xanthosine were purchased from Sigma. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG was also obtained from Sigma.

**Cell Culture Studies**—HeLa cells were grown as a monolayer in minimum essential medium supplemented with 10% newborn bovine serum in a 5% CO2 humidified incubator at 37 °C. For immunofluorescence studies, cells were subcultured over glass slides in a Petri dish 2 days before use. Fresh media were added 2 h before the beginning of each experiment. Generally, cells were either incubated with single drugs or co-incubated with drugs and normal nucleosides for 4 h to assess B23-translocation. In reversal studies, cells were first induced to B23-translocation, and then guanosine or other purines were added to the culture media for 2 to 4 additional hours.

**Immunofluorescence Staining**—Immunofluorescence staining was performed as previously described (13). Briefly, cells were fixed in 2% paraformaldehyde and then permeabilized in cold acetone prior to overnight incubation with monoclonal antibody (diluted 1:16 in PBS) that recognizes only nucleophosmin/B23. Fluorescein-conjugated goat anti-mouse IgG served as the second antibody. Slides were examined for the localization of nucleophosmin/B23 using fluorescence microscopy.

**Western Blotting**—Control and ribavirin-treated HeLa cells were dissolved in SDS sample buffer (62 mM Tris, pH 6.8, 5% β-mercaptoethanol, 10% glycerol, 2.3% SDS, 0.001% bromphenol blue) at ~2 x 10⁶ cells/ml, and the cell extract was fractionated by 10% SDS-polyacrylamide gel electrophoresis. The separated protein was then electrophoretically transferred to a nitrocellulose sheet. The nitrocellulose sheet was then soaked in a blocking solution (100 mM Tris-HCL, pH 8.0, 3% bovine serum albumin, 10% chicken serum, 150 mM NaCl) overnight in the refrigerator. The nitrocellulose sheet was then incubated for 2 h in monoclonal antibody to nucleophosmin/B23 (diluted 1:10,000 in Nonidet P-40 solution (150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.5, 0.65% Nonidet P-40, 0.25% gelatin)), washed (3 x 10 min) with TBST buffer (60 mM Tris-HCL, pH 8.0, 150 mM NaCl, 0.05% Tween 20), and incubated for 1 h in alkaline phosphatase-conjugated goat anti-mouse antibody (diluted 1:5000 in Nonidet P-40)

**The abbreviations used were:** PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography.
solution). The sheet was then washed (3 × 10 min) in TBST buffer before incubation with Promega substrates for color development.

**ELISA Studies**—Monolayer HeLa cells were rinsed with PBS, then scraped and suspended in RSB buffer (10 mM Tris, pH 7.4, 10 mM NaCl, 1.5 mM MgCl₂, 0.1 mM phenylmethylsulfonyl fluoride, 10 μM leupeptin, 0.1 mM p-chloromercuri phenylsulfonic acid, and sonicated with an Ultrasonic cell disrupter for ~30 s. After centrifugation (30 min, 27,000 × g) to remove debris, the supernatant (whole cell extract) was serially diluted in PBS containing 0.001% bovine serum albumin and loaded into wells of a microtiter plate (ImmunoIn II). The ELISA was performed as previously described by Yung et al. (9). The monoclonal antibody to nucleophosmin/B23 was diluted 1:20,000 in PBS before use.

**Measurement of Cellular GTP Pools**—High-performance liquid chromatography (HPLC) was used to quantitate GTP in HeLa cell extracts after drug treatments. Preparation of cell extracts and chromatography were performed essentially as described by Matsumoto et al. (31). Briefly, following incubation of cells in culture medium containing the compounds, cells were scraped from the flask, counted, and centrifuged to pellet. The macromolecules were precipitated by adding ice-cold 0.4 N perchloric acid to the cell pellet (100 μl per 2 × 10⁶ cells). The precipitates were removed by centrifugation at 3000 g for 5 min, and the acid extract was neutralized with trioctylamine in freon (32). The nucleotides were separated by anion exchange chromatography on an Altex Ultrasil-AX column (4.6 x 250 mm) at a flow rate of 1.5 ml/min with a gradient of solution A (10 mM potassium phosphate, pH 3.83) and solution B (0.35 M potassium phosphate, 0.5 M KCl, pH 3.6) added in the proportions: 0 to 5 min, 0% solution B; 5 to 11 min, 0 to 30% solution B; 11 to 12.5 min, 30 to 60% solution B; 12.5 to 22.5 min, 60 to 100% solution B. HPLC was performed on a Waters 501 and the eluent was monitored at 254 nm. Retention times for ATP and GTP were obtained by using authentic standards.

**RESULTS**

**B23-translocation Induced by Ribavirin**—Fig. 1 shows phase contrast photographs and the immunofluorescence pattern of nucleophosmin/B23 in cultured HeLa cells incubated with 100 μM ribavirin for 4 h compared to that observed in untreated control cells. Control cells have large and pleomorphic nucleoli with bright fluorescence (Fig. 1, A and C), and the nucleoplasmic fluorescence is relatively weak. In contrast, ribavirin-treated cells show a uniform and higher nucleoplasmic fluorescence with decreased nucleolar size and fluorescence (Fig. 1, B and D) which indicates that nucleophosmin/B23 has shifted from nucleoli to nucleoplasm. Lower dosages (25 and 50 μM for 4 h) and shorter incubation times (100 μM for 2 h) resulted in noticeable but lesser effects (data not shown). To determine whether B23-translocation occurred from a reduction in the protein, the content of nucleophosmin/B23 in whole cell extracts before and after drug treatment was determined by ELISA assay. Fig. 2 shows that there is no significant decrease of nucleophosmin/B23 in drug-treated cells. Nucleophosmin/B23 was also analyzed by Western blot assay. A single immunoband at Mr = 37,000 was observed in both control and ribavirin-treated cells (Fig. 3). There is no detectable degradation of nucleophosmin/B23 after the drug treatment. Taken together, these results indicate that ribavirin treatment causes redistribution of nucleophosmin/B23 from nucleoli to nucleoplasm.

**Inhibition of IMP Dehydrogenase Causes B23-translocation**—Two ribavirin analogs, tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide) and selenazofurin (2-β-D-ribofuranosylselenazole-4-carboxamide), were also tested for the ability to induce B23-translocation. Both analogs were found to be effective (Table I). Ribavirin and its analogs were previously reported to inhibit IMP dehydrogenase (19–26). To investigate whether B23-translocation is related to inhibition of this enzyme’s activity and is not related to the structural similarity of these nucleoside analogs, a non-nucleoside inhibitor of IMP dehydrogenase, mycophenolic acid, 6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalany1)-4-methyl-4-hexenoic acid (27), was tested. It was found that mycophenolic acid also effectively induced B23-translocation (Table I). These results indicate that inhibition of IMP dehydrogenase causes B23-translocation.

**Depletion of GTP by IMP Dehydrogenase Inhibitors and B23-translocation**—IMP dehydrogenase catalyzes the conversion of inosine monophosphate (IMP) to xanthosine monophosphate (XMP), which is a precursor of guanosine monophosphate (GMP). Inhibition of this enzyme causes a reduction in de novo GMP synthesis resulting in depletion of cellular guanine nucleotide pools (33). To investigate the relationship between B23-translocation and GTP pools, the GTP content of drug-treated cells was determined. Results are shown in Table II. We found that the GTP pool concentration in control HeLa cells is 0.81 ± 0.14 nmol/10⁶ cells while cells treated with ribavirin (100 μM, 4 h) is 0.24 ± 0.01 nmol/10⁶ cells, a reduction of ~70%. The reduction in GTP concentration persists after 6 and 8 h of drug treatment (Table II) as does the B23-translocation.

If depletion of GTP pools induced by ribavirin is the cause of B23-translocation, then replenishment of GTP should reverse the translocation. As shown in Fig. 4D, addition of guanosine together with ribavirin effectively prevented B23-translocation. At the same time, we observed that the GTP content of these cells was slightly higher than in untreated controls (Table II). B23-translocation induced by tiazofurin, selenazofurin, or mycophenolic acid could be similarly blocked by guanosine (Table I). Of the other purines tested (adenosine, inosine, hypoxanthine, xanthine, xanthosine, and guanine), only co-incubation with guanine effectively prevented ribavirin-induced B23-translocation.

In other experiments, guanosine was capable of reversing
Nucleolar Localization of Nucleophosmin/B23 Requires GTP

FIG. 2. ELISA analysis of nucleophosmin/B23 in HeLa cells after ribavirin treatment. Total cell extracts of control or ribavirin-treated cells were serially diluted and loaded into microtiter plates for ELISA assay (see "Materials and Methods"). There is no significant difference in the quantity of nucleophosmin/B23 in control versus drug-treated cells.

FIG. 3. Whole HeLa cell extracts before and after drug treatment were analyzed by 10% SDS-gel electrophoresis and subsequent Western blot staining (see "Materials and Methods"). Lanes: A and a, undiluted extracts; B and b, C and c, D and d, 1/2 serial dilutions made in SDS sample buffer. The arrow indicates the nucleophosmin/B23 immunoband.

TABLE I

B23-translocation induced in HeLa cells by selected cytotoxic agents

| Drug            | Dosage | B23-translocation* |
|-----------------|--------|--------------------|
|                 |        | Drug alone | Drug + guanosine |
| Ribavirin       | 100 µM | +          | -                |
| Tiazofurin      | 100 µM | +          | -                |
| 5-Fluorouracil  | 10 µM  | +          | -                |
| Actinomycin D   | 0.2 µg/ml | +      | +                |

*HeLa cells grown on glass slides were incubated with a cytotoxic drug alone or co-incubated with the drug and guanosine (100 µM) for 4 h before immunostaining (see "Materials and Methods"). + indicates B23-translocation; - indicates no B23-translocation.

translocation previously induced by ribavirin. Fig. 4A shows HeLa cells incubated with ribavirin (100 µM) for 4 h then "rescued" by addition of 100 µM guanosine (100 µM) for 4 h before immunostaining (see "Materials and Methods"). + indicates B23-translocation; - indicates no B23-translocation.

depletion of GTP pools by IMP dehydrogenase inhibitors and B23-translocation which indicates that nucleolar localization of nucleophosmin/B23 depends on cellular GTP levels.

Table I also summarizes the ATP levels in HeLa cells following drug treatment. While the GTP content decreases during ribavirin treatment, the ATP content is not affected (2.88 ± 0.37 nmol/10^6 treated cells versus 2.81 ± 0.58 nmol/10^6 control cells). The ratio of ATP/GTP in ribavirin-treated cells is higher than in control cells (~11 versus 3.5). Although there are variations in the cellular ATP and GTP content in the guanosine supplementation experiments, the ATP/GTP ratios are low (mean = 1.9). The significance of the ATP/GTP ratio for B23-translocation remains to be investigated.

DISCUSSION

IMP dehydrogenase catalyzes the conversion of IMP to XMP. Inhibition of this enzyme results in depletion of cellular guanine nucleotide pools. The current studies show that depletion of cellular GTP and/or alteration in the ATP/GTP ratio by IMP dehydrogenase inhibitors causes the redistribution of nucleophosmin/B23 from nucleoli to nucleoplasm (B23-translocation). This translocation process is reversible because it can be prevented or reversed by addition of guanine or guanosine to the culture medium, which allows the cell to utilize salvage pathways to replenish the depleted GTP pools. The mechanism relating GTP depletion to B23-translocation is not presently clear.

Since GTP is one of the precursors of the nucleic acids, and its depletion can cause reduction of DNA and RNA synthesis, an obvious question is whether nucleic acid synthesis inhibition per se causes the B23-translocation. We found that certain cytotoxic agents (including tubercidin, cytosine β-D-arabinofuranoside, and 5-fluorouracil) which effectively inhibit RNA and/or DNA synthesis (34) do not induce B23-translocation (18). These results suggest that nucleic acid synthesis inhibition and B23-translocation do not constitute a cause/effect relationship.

R. A. Finch, G. R. Revankar, and P. K. Chan, unpublished results.
GTP and GDP are modulators of G-proteins which are involved in the signal transduction mechanisms of hormones, growth factors, and cytokines. The quantity of nucleophosmin/B23 in nucleoli increases with increased cell growth as observed in hypertrophic rat liver (35) and tumor cells (36). Also, a 5- to 20-fold increase in nucleophosmin/B23 is observed when B-lymphocytes are activated by mitogens (6). The increased nucleophosmin/B23 concentration in proliferating cells is associated with higher nucleolar activity. In general, GTP binding activates G-proteins. It is possible that depletion of GTP indirectly inhibits or interrupts the continuity of a G-protein-coupled "nucleolar activation pathway." One of the end results of this interference is the failure of nucleophosmin/B23 to localize in the nucleolus. There are at least two signal transduction pathways affected by G-proteins: 1) activation of adenylate cyclase which raises intracellular cAMP levels and 2) stimulation of phospholipid breakdown and the subsequent activation of protein kinase C and calcium channels. Depletion of GTP could negatively affect these pathways. Preliminary results indicate that the cAMP level is unaffected by ribavirin treatment.

The reported $K_d$ values for proteins that bind GTP range from $3 \times 10^{-4}$ to $10^{-7}$ M (37). For example, Ras and EF-Tu have $K_d$ values in the $10^{-6}$ to $10^{-7}$ M range, while phosphoenolpyruvate carboxykinase has a $K_d$ of 30 $\mu$M (38). The intracellular concentration of GTP in HeLa cells as determined in this study (0.81 nmol/10^6 cells, Table II) is about 128 $\mu$M (based on 160 $\times$ 10^6 cells/ml). While a 3-4-fold reduction of this concentration would not affect the GTP binding of Ras or EF-Tu, it might affect the GTP binding of

### Table II

ATP and GTP levels in drug-treated HeLa cells

| Treatment                                      | ATP      | GTP      | % control | ATP/GTP ratio |
|------------------------------------------------|----------|----------|-----------|---------------|
| Control                                        | 2.81 ± 0.58 | 0.81 ± 0.14 | 100       | 100           |
| Ribavirin (4 h)                                | 2.53 ± 0.36 | 0.24 ± 0.01 | 90        | 30            |
| Ribavirin (6 h)                                | 3.14 ± 0.07 | 0.26 ± 0.01 | 112       | 32            |
| Ribavirin (8 h)                                | 3.21 ± 0.25 | 0.24 ± 0.02 | 107       | 30            |
| Ribavirin (4 h) + guanosine (2 h)              | 2.44 ± 0.22 | 1.48 ± 0.04 | 87        | 183           |
| Ribavirin (4 h) + guanosine (co-incubated)     | 2.73 ± 0.19 | 1.08 ± 0.05 | 97        | 133           |
| Ribavirin + guanosine (4 h)                    | 1.72 ± 0.10 | 1.02 ± 0.02 | 61        | 126           |
| Actinomycin D (4 h)                            | 2.64 ± 0.24 | 0.91 ± 0.06 | 94        | 112           |

Note: ribavirin (100 $\mu$M), toyocamycin (10 $\mu$M), actinomycin D (0.2 $\mu$g/ml), guanosine (100 $\mu$M). Values are means ± S.D. of three experiments.
those proteins with high $K_d$ values. The fact that a 3-4 fold reduction of GTP causes B23 translocation suggests that if a G-protein is indeed involved in the pathway for nucleolar activation, it would be one with a relatively high $K_d$ and therefore sensitive to the prevailing GTP concentration.

Regardless of which signal transduction pathway is involved in activation of the nucleolus, the end result is increased production of ribosomes. Intricate mechanisms that mobilize hundreds of proteins, RNAs, and other elements in the nucleolus are involved in ribosome production (3). The mechanism of ribosome production is dependent on a balanced supply of rRNA and ribosomal proteins (39) and a proper conformation of rRNA (40). Multiple steps are involved, such as transport of ribosomal proteins to the nucleolus and binding to nascent pre-rRNA, splicing of the 45 S RNA, packaging of ribosomal proteins into ribosomal subunits, and delivery of the pre-ribosomal ribonucleoprotein to the cytoplasm. Interference with any one of these processes could result in abortion of ribosome synthesis and B23 translocation. Actinomycin D and toyocamycin induce B23 translocation (Refs. 9 and 11, Table I, and Fig. 4, B and F). However, these drugs do not affect cellular GTP or ATP levels in a similar way as ribavirin (Table II). In addition, translocation induced by these drugs is not reversed or prevented by guanosine (Fig. 4, E and F, and Table I). Since B23 translocation induced by toyocamycin and actinomycin D is not associated with reduced GTP pools, we are led to conclude that there is more than one mechanism of B23 translocation.

Recent studies show that the nucleolus may also play an important role in virus replication (41) and transcription. Two HIV proteins, Tat and Rev, which are essential for the early regulation of viral gene expression (42), are found localized in nucleoli (43-45). Fankhauser et al. (46) reported that nucleophosmin/B23 is specifically bound by the HIV Rev protein which is responsible for the transport of unspliced mRNA to the cytoplasm (47, 48). The virus may utilize the nucleolar machinery during the early stages of replication, and nucleophosmin/B23 could serve as a shuttle protein (49) for the transport of viral as well as ribosomal proteins. This process may require GTP. Ribavirin is a clinically useful, broad spectrum antiviral agent (19, 50-52). The fact that ribavirin induces B23 translocation suggests that one of the ribosomal proteins is not reversed or prevented by guanosine (Fig. 4, B and F).

REFERENCES

1. Busch, H., and Smetana, K. (1970) The Nucleolus, Academic Press, New York.
2. Hadjioannou, A. A. (1985) The Nucleolus and Ribosome Biogenesis, Springer-Verlag Wien, Vienna.
3. Warner, J. R. (1980) Microbiol. Rev. 45, 256-271.
4. Chan, W. Y., Liu, Q., Roscito, J., Busch, H., Rennert, O. M., Tease, L. A., and Chan, P. K. (1989) Biochemistry 28, 1033-1039.
5. Schmidt-Zachmann, M. S., and Franke, W. W. (1988) Chromosoma (Berl.) 96, 417-426.
6. Feuerstein, N., Chan, P. K., and Mond, J. J. (1988) J. Biol. Chem. 263, 10609-10612.
7. Specter, D. L., Ochs, R. L., and Busch, H. (1984) Chromosoma (Berl.) 90, 139-148.
8. Fresta, C. A., Klomp, G. R., Schneid, D. J., and Busch, H. (1974) Biochemistry 13, 1945-1951.
9. Yung, B. Y.-M., Busch, H., and Chan, P. K. (1985) Biochim. Biophys. Acta 258, 167-173.
10. Feuerstein, N., and Mond, J. J. (1987) J. Biol. Chem. 262, 11398-11397.
11. Yung, B. Y.-M., Busch, R. K., Busch, H., Mauger, A. B., and Chan, P. K. (1985) Biochim. Biophys. Acta 1069-10612.
12. Yung, B. Y.-M., Busch, H., and Chan, P. K. (1986) Cancer Res. 46, 922-927.
13. Chan, P. K., Aldrich, M. B., and Yung, B. Y.-M. (1987) Cancer Res. 47, 3768-3771.
14. Chan, P. K., Aldrich, M. B., and Chakrabarty, S. (1988) Cancer Lett. 40, 143-149.
15. Yung, B. Y.-M., Bor, A. M.-S., and Chan, P. K. (1990) Cancer Res. 50, 5987-5991.
16. Sweet, P., Chan, P. K., and Slater, L. M. (1986) Cancer Res. 46, 677-680.
17. Finch, J. A., and Chan, P. K. (1986) Oncology 46, 225-230.
18. Chan, P. K. (1992) Exp. Cell Res. 203, 174-181.
19. Streeter, D. G., Wiltok, J. T., Khan, G. P., Sidwell, R. W., Buser, R. J., Kolok, W. R., and Simon, L. N. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1174-1178.
20. Szum, C. M., Fontenelle, L. J., Musik, H., Paterson, A. R., Unger, H., Brown, L. W., and Henderson, J. F. (1974) Biochim. Biophys. Acta 2727-2755.
21. Keen, R., Robbins, R. K., and Saunders, P. D. (1982) Biochim. Biophys. Acta 70, 115-1154.
22. Cooney, D. A., Jayaram, H. N., Ghezeyehu, G., Betts, C. K., Kelley, J. A., Nature 14, 835-840.
23. Jayaram, H. N., Dion, R. L., Ghezeyehu, G., Johns, D. G., Robbins, R. K., and Smetana, K. (1986) Biochim. Biophys. Acta 1069-10612.
24. Fankhauser, P. P., Kattan, R., Lai, M. M., and Robbins, R. K. (1983) Mol. Pharmacol. 23, 534-539.
25. Ghezeyehu, G., Marquez, Y. P., Cott, A. V., Cooney, D. A., Kelley, J. A., Jayaram, H. N., Ahluwalia, G. S., Dion, R. L., Wilson, A. J., and Johns, D. G. (1985) J. Med. Chem. 28, 99-105.
26. Jayaram, H. N., Ahluwalia, G. S., Dion, R. L., Ghezeyehu, G., Marquez, V. E., Kelley, J. A., Robbins, R. K., Cooney, D. A., and Johns, D. G. (1983) Biochim. Biophys. Acta 777, 1177-1185.
27. Franklin, T. J., and Cook, J. M. (1990) Biochim. J. 112, 515-524.
28. Wiltok, J. T., Robbins, R. K., Sidwell, R. W., and Simon, L. N. (1972) J. Med. Chem. 14, 1150-1154.
29. Smith, C. M., Fontenelle, L. J., Musik, H., Paterson, A. R., Unger, H., Brown, L. W., and Henderson, J. F. (1974) Biochim. Biophys. Acta 2727-2755.
30. Keen, R., Robbins, R. K., and Saunders, P. D. (1982) Biochim. Biophys. Acta 70, 115-1154.