Phosphorylation of Geranyl and Farnesyl Pyrophosphates by Nm23 Proteins/Nucleoside Diphosphate Kinases*

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The biochemical mechanism(s) by which Nm23 proteins/nucleoside diphosphate kinases suppress tumor metastasis, inhibit cell motility, and affect cellular differentiation are not known. Here we report that Nm23 proteins can phosphorylate geranyl and farnesyl pyrophosphates to give triphosphates. Wild type Nm23-H1 had higher geranyl and farnesyl pyrophosphate kinase activities than did mutants of Nm23-H1 that do not inhibit cell motility. The phosphorylation of farnesyl pyrophosphate appears to occur in vivo as cells with an elevated level of Nm23-H1 contained more farnesyl triphosphate than did control cells. To our knowledge, this is the first report that farnesyl triphosphate exists in cells. The phosphorylation of farnesyl pyrophosphate by Nm23 proteins could alter isoprenoid metabolism, and cells with an elevated level of Nm23 proteins were found to contain more farnesylated 46- and 24-kDa proteins than did control cells. The phosphorylation of geranyl and farnesyl pyrophosphates by Nm23 proteins provides a novel mechanism by which these proteins might exert their biological effects.

Nm23 proteins have been implicated in the regulation of tumor metastasis (1–3), cell motility (4), and cellular differentiation (5–7). Significant correlations between low nm23 expression and high metastatic potential have been observed in cohorts of human breast, hepatocellular, gastric, ovarian, and cervical carcinomas and melanomas (2). Overexpression of nm23 genes reduces the metastatic potentials of highly metastatic murine melanoma cell lines (8–11), rat mammary adenocarcinoma cells (12), and human breast carcinoma cells (9, 13, 14). Expression of human nm23-H1 has been correlated with low metastatic potential (2), and transfection of human breast carcinoma cells with nm23-H1 inhibits their motility (4).

Nm23 proteins are nucleoside diphosphate kinases (NDP kinases) (15) and catalyze the phosphorylation of nucleoside diphosphates to triphosphates by a ping-pong mechanism involving a high energy phosphohistidine intermediate. NDP kinases are thought to be responsible for maintaining nucleoside triphosphate pools; however, this activity does not account for all of the biological effects of Nm23 proteins. Transfection experiments have shown that different Nm23 proteins, although they have similar NDP kinase activities, have very different effects on the metastatic potential of rat mammary adenocarcinoma cells (12), on the motility of human breast carcinoma cells (4), and on Drosophila development (16). It has been suggested that Nm23 proteins might phosphorylate other kinds of substrates.

Nm23 proteins have protein kinase or phosphotransferase activities (17–21). However, with the exception of the transfer of phosphate from Escherichia coli NDP kinase to a histidine on a Tar/EnvZ chimera (19), these phosphotransferase reactions have not been shown to occur in vivo, and it has not been shown under which physiological conditions E. coli NDP kinase phosphorylates proteins.

The binding of ADP to Nm23 proteins is primarily through the sugar and pyrophosphate; the base lies in a hydrophobic cleft and forms no specific polar interactions with the enzyme (22). The lack of specific interaction between the base and the enzyme accounts for the ability of NDP kinase to phosphorylate a variety of nucleoside diphosphates and suggests that Nm23 proteins might also phosphorylate other kinds of diphosphates. Here we report that Nm23 proteins phosphorylate geranyl pyrophosphate (GPP) and farnesyl pyrophosphate (FPP) to give the corresponding triphosphates in vitro and that cells with an elevated level of Nm23-H1 contain an elevated level of farnesyl triphosphate (FTP).

Isoprenoid pyrophosphates are important metabolic intermediates. FPP is a branch point in isoprenoid metabolism and is a precursor of geranylgeranyl pyrophosphate (GGPP), cholesterol, heme A, ubiquinone, and dolichol phosphate (23, 24). FPP and GGPP are utilized for the isoprenylation of a number of proteins (25), many of which play critical roles in signal transduction and cell growth regulation. We have found that cells with an elevated level of Nm23-H1 contained an increased level of protein farnesylation.

EXPERIMENTAL PROCEDURES

Materials—[1-3H]GPP, [1-3H]GGPP, [1-3H]FMP, [1-3H]FPP, [1-3H]FTP, and RS-[5-3H]mevalonolactone were purchased from American Radiolabeled Chemicals Corp. Geranyl monophosphate, GPP, GGPP, FMP, FPP, isopentenyl pyrophosphate, and dimethylallyl pyrophosphate were purchased from Sigma. C100 and H1-177 cell lines, a gift from Dr. Patricia Steeg (NCI, National Institutes of Health), were grown as described in Ref. 20. Anti-2',3'-cyclic nucleotide 3'-phosphodiesterase antibodies were a gift from Dr. David M. Jacobowitz (National Institute of Mental Health).

Purification and Phosphorylation of Rat Liver NDP Kinase and Nm23-H1—E. coli strain BL21(DE3) containing expression plasmids for Nm23-H1, Nm23-H1F96S, Nm23-H1S120G, Nm23-H1S120A, and Nm23-H1H118F (18) were gifts from Dr. Patricia Steeg. Recombinant human Nm23-H1 proteins and rat liver NDP kinase were purified as described in Refs. 20 and 21. Coomassie Brilliant Blue-stained SDS-polyacrylamide gels showed that the isolated rat liver NDP kinase and the recombinant human Nm23 proteins were greater than 99% pure.

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**RESULTS**

**Phosphorylation of GPP and FPP by $[^{32}P]$NDP Kinase—**To examine whether phosphorylases, other than nucleoside diphosphates, are substrates for Nm23 proteins, autophosphorylated rat liver NMD kinase, $[^{32}P]$NDP kinase, was incubated with 1 mM sodium pyrophosphate, 1 mM 5′-phosphorylribosyl pyrophosphate, 1 mM thiamine pyrophosphate, or 100 μM GPP for 10 min. Only the incubation with GPP resulted in the removal of $^{32}$P from the NDP kinase (data not shown).

Thin layer chromatography was used to examine the phosphorylation of the isoprenoid phosphorylases by NDP kinases. Incubation of $[^{32}P]$NDP kinase with GPP, FPP, and GGPP resulted in the formation of new $^{32}$P-labeled compounds (Fig. 1). The $^{32}$P-labeled compound formed by the incubation of FPP with $[^{32}P]$NDP kinase had the same mobility as the $[^{3}H]$FPP standard and less than those of $[^{3}H]$PPP and $[^{3}H]$FMP. The $^{32}$P-labeled compounds formed by the incubation of GGPP and GPP with $[^{32}P]$NDP kinase had lower mobilities than the $[^{3}H]$PPP and $[^{3}H]$GPP standards (Fig. 1). $[^{32}P]$NDP kinase, nucleoside diphosphates, nucleoside triphosphates, and inorganic phosphate all remained at the origin. No new $^{32}$P-labeled compounds were formed when GPP, FPP, and GGPP were incubated with $[^{32}P]$NDP kinase in EDTA or with denatured $[^{32}P]$NDP kinase in MgCl₂.

The products from the phosphorylation of GPP, FPP, and GGPP by $[^{32}P]$NDP were also analyzed by reverse phase, ion pair HPLC. The $^{32}$P-labeled compound formed by the incubation of FPP with $[^{32}P]$NDP kinase eluted at the same time as the $[^{3}H]$FPP standard and after the $[^{3}H]$FMP and $[^{3}H]$PPP standards. The $^{32}$P-labeled compounds formed by the incubation of GGPP and GPP with $[^{32}P]$NDP kinase eluted just after the corresponding triphosphate standards (data not shown).

These results indicate that GPP and FPP, and to a lesser extent GGPP, were phosphorylated by $[^{32}P]$NDP kinase to give the corresponding triphosphates. These kinase reactions showed specificity as incubation of $[^{32}P]$NDP kinase with isopentenyl pyrophosphate, dimethylallyl pyrophosphate, geranyl monophosphate, or FMP did not result in the formation of any new $^{32}$P-labeled compounds.

The time courses of $^{32}$P transfer from $[^{32}P]$NDP kinase to GPP and FPP are shown in Fig. 2, A and B. The half-lives of transfer of $^{32}$P to GPP and FPP were 9 and 28 min, respectively (Fig. 2C). After 60 min, almost all of the $^{32}$P was transferred from $[^{32}P]$NDP kinase to GPP. Half the maximum rate of transfer occurred in about 125 μM GPP and in about 60 μM FPP.
GPP and FPP were also phosphorylated when incubated with NDP kinase and [γ-32P]ATP. The rates of phosphorylation of GPP and FPP by rat liver NDP kinase at room temperature were 1.2 ± 0.2 and 0.72 ± 0.07 nmol/min/mg, respectively (Table I), and at 37 °C the rates of phosphorylation of GPP and FPP were 4.5 ± 0.3 and 2.1 ± 0.3 nmol/min/mg, respectively. No phosphorylation was observed when GPP and FPP were incubated with [γ-32P]ATP and no added NDP kinase. The phosphorylation of farnesyl and geranyl pyrophosphates by NDP kinase was catalytic; one molecule of NDP kinase transferred phosphates to many molecules of geranyl or farnesyl pyrophosphate.

**Wild Type Nm23-H1 Has Higher GPP and FPP Kinase Activities than Nm23-H1 Mutants That Do Not Inhibit Cell Motility**—Transfection of human breast carcinoma cells with wild type nm23-H1 suppresses the motility of these cells, but transfections with nm23-H1^{P96S} or nm23-H1^{S120G} do not suppress motility (4). The serine 120 to glycine mutation of Nm23-H1S120G was found in 6 of 28 aggressive childhood neuroblastomas (32). The Nm23-H1^{P96S} contains a proline to serine substitution at residue 96 and is homologous to the Drosophila and w^{P96S} mutation (4, 15). Transfection with nm23-H1^{P96S} gave mixed results, inhibiting migration toward fetal calf serum but not toward autotaxin. Extracts from these different cells all contain similar levels of NDP kinase activity (4).

The phosphorylation of GPP by Nm23-H1 and by mutants of Nm23-H1 in the presence [γ-32P]ATP is shown in Fig. 3A. Nm23-H1 and Nm23-H1^{S120A} Nm23 proteins that suppress cell motility, had 2–3-fold higher GPP and FPP kinase activities than did Nm23-H1^{S120G} and Nm23-H1^{P96S}. Nm23 proteins that do not suppress cell motility (Table I). Neither GPP nor FPP were phosphorylated when incubated with [γ-32P]ATP and

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**TABLE I**

| Nm23 protein     | GPP  | FPP  |
|------------------|------|------|
| Nm23-H1          | 2.2  | 0.78 ± 0.08 |
| Nm23-H1^{P96S}   | 0.51 ± 0.14 | 0.41 ± 0.03 |
| Nm23-H1^{S120G}  | 0.79 ± 0.03 | 0.38 ± 0.05 |
| Nm23-H1^{S120A}  | 2.7 ± 0.3 | 1.0 ± 0.1 |

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**Fig. 2.** Time courses of 32P transfer from rat NDP kinase to GPP and FPP. 0.5 μM [32P]NDP kinase was incubated with either 270 μM GPP (A) or 160 μM FPP (B). The products were separated by TLC and visualized by autoradiography. A, PEI sheet developed with 0.5 M NaCl and 0.75 M Tris, pH 7.5. B, PEI sheet developed with 0.2 M ammonium bicarbonate/isopropyl alcohol/acetonitrile (1:1:1). C, transfer of 32P from 0.5 μM [32P]NDP kinase to 270 μM GPP (○) and 160 μM FPP (■).

**Fig. 3.** Phosphorylation of GPP by human nm23 proteins. A, 270 μM GPP was incubated with 10 μM [γ-32P]ATP and 1 μM wild type Nm23-H1, Nm23-H1^{P96S}, Nm23-H1^{S120G}, or Nm23-H1^{S120A} for the times indicated, and the products were analyzed by TLC and autoradiography. B, 270 μM GPP or 230 μM FPP was incubated with 10 μM [γ-32P]ATP and 1 μM wild type Nm23-H1 (Wt) or Nm23-H1^{H118F} (His) for the times indicated, and the products were analyzed by TLC and autoradiography. PEI sheets were developed with 0.2 M ammonium bicarbonate/isopropyl alcohol/acetonitrile (1:1:1).

Nm23-H1^{H118F}, a mutant in which the catalytic histidine has been replaced with a phenylalanine (Fig. 3B).

The nucleoside diphosphate kinase activities of Nm23-H1, Nm23-H1^{P96S}, and Nm23-H1^{S120A}, assayed by measuring the transfer of phosphate from ATP to TDP, were found to be within 5% of each other, and the nucleoside diphosphate activity of Nm23-H1^{P96S} was 80–90% that of Nm23-H1 (data not shown). Others (18, 33) have also reported little differences in the nucleoside diphosphate kinase activities of these different forms of Nm23-H1.

The half-life for the transfer of 32P from [32P]Nm23-H1 (autophosphorylated Nm23-H1) to GPP was 3 min, and the half-
phorylation by the depleted extracts was 54% that of the control extracts, and the rate of GPP phos-
tative in the extracts. After 15 min, most of the 
and H1-177 cell extracts were incubated with 40 
triphosphates. During these incubations, some 32P was also 
the half-life for the transfer of 32P from [32P]Nm23-H1S120G and 
[32P]Nm23-H1S120G to FPP were 50 and 80 min, respectively.

Phosphorylation of GPP and FPP by Cell Extracts—C100
and H1-177 cell lines are stable transfectants of the highly
metastatic human MDA-MB-435 breast carcinoma cell line. The H1-177 line was transfected with 
nm23-H1 as do C100 cells and have a lower metastatic poten-
tial. Extracts from these two cell lines were incubated with 
0.2M ammonium bicarbonate/isopropyl alcohol/acetonitrile (1:1:1).

Kinase Activities of Nm23 Proteins
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A

C100 Cell Extract H-177 Cell Extract

[32P]GerTP [32P]ATP

5 10 15 20 25

51 96 211 250

100 185 329 445

B

C100 Cell Extract H-177 Cell Extract

[32P]FTP [32P]ATP

5 10 15 20 25

46 112 98

138 220 99

C

nm23-H1 nm23-H2

cytosol con dep

Fig. 4. Phosphorylation of GPP and FPP by cell extracts. C100
and H1-177 cell extracts were incubated with 40 μM [γ-32P]ATP and 270
μM GPP (A) or 230 μM FPP (B) for the times indicated, and the products
were analyzed by TLC and autoradiography. PSL units are the relative amounts of [32P]GerTP and [32P]FTP formed as
determined with a Fuji Bio-Image Analyzer.

lives for the transfer of 32P from [32P]Nm23-H1S120G and 
[32P]Nm23-H1P96S GPP were about 10 min. The half-life for the
transfer of 32P from [32P]Nm23-H1 to FPP was 22 min, and
half-lives for the transfer of 32P from [32P]Nm23-H1S120G and 
[32P]Nm23-H1P96S to FPP were 50 and 80 min, respectively.

Phosphorylation of GPP and FPP kinase activities of H1-177 cell extracts. H1-177 cell extracts were incubated with either prebleed IgG (Con) or anti-NM23 kinase-antibodies (Dep). After removal of the antibodies, the extracts were incubated with 40 μM [γ-32P]ATP and 270 μM GPP (A) or 230 μM FPP (B) for the times indi-
cated, and the products were analyzed by TLC and autoradiogra-
phy. PSL units developed with 0.2 M ammonium bicarbonate/isopropyl
alcohol/acetonitrile (1:1:1). PSL units are the relative amounts of
[32P]GerTP and [32P]FTP formed as determined with a Fuji Bio-Image
Analyzer, C, relative amounts of Nm23-H1 and Nm23-H2 in control
(Con) and depleted (Dep) cell extracts determined by phosphorylation with [γ-32P]ATP in the presence of EDTA.

control extracts. In three of the four experiments, the depleted
extracts contained about half as much Nm23 proteins as did
the control extracts (Fig. 5C). In one trial, the depleted extract
contained only 8% as many Nm23 proteins as did the control
extract, but it still had about half the GPP and FPP kinase
activities of the control extract.

H1-177 Cells Contain More FPP than Do C100 Cells—C100
and H1-177 cells were labeled with [3H]mevalonolactone in the
presence of lovastatin to inhibit the synthesis of endogenous
mevalonate. C100 and H1-177 cells took up the same amounts
of [3H]mevalonolactone. Isoprenoid phosphates were extracted
from these cells and analyzed by reverse phase, ion-pair HPLC
(Fig. 6A). The gradient used for this column (decreasing tet-
rabutylammonium phosphate and increasing acetonitrile) was
optimized to separate FTP from FPP. Mevalonate phosphate,
mevalonate pyrophosphate, isopentenyl pyrophosphate, and
dimethylallyl pyrophosphate were not retained on the column.
The [3H]FTP standard eluted just after the [3H]FPP standard
(Fig. 6A), and 32P-labeled GerTP, FTP, and geranylgeranyl
triphosphates, made by the phosphorylation of the pyrophos-
phates with [32P]NDP kinase, eluted just after the pyrophos-
phate standards. The [3H]FMP standard eluted just before the
[3H]FPP standard (Fig. 6A).

Four major peaks of 3H were obtained for both C100 and
De phosphorylation was used to show that the $^3$H-labeled compound that eluted from the HPLC column at the same time as the FTP standard contained farnesyl. Extracts from $^3$H]mevalonolactone-labeled H1-177 cells were separated on this reverse phase, ion pair HPLC column, and fractions that eluted at the same times as the FPP and FTP standards were treated with alkaline phosphatase. After dephosphorylation, both fractions contained $^3$H]farnesol (Fig. 6C, lanes PP and TP), the expected product for the dephosphorylation of both $^3$H]FPP and $^3$H]FTP.

These TLCs indicate that the $^3$H-labeled compounds in these cell extracts that eluted from the HPLC column at the same time as the FPP and FTP standards were FPP and FTP, respectively. As shown in Fig. 6A, the C100 cells contained more $^3$H]FPP than did H1-177 cells, and conversely, H1-177 cells contained more $^3$H]FTP than did the C100 cells. The ratio of $^3$H]FPP to $^3$H]FTP in C100 cells was about 2:1, and the ratio in H1-177 cells was about 1:1. Similar differences were observed with two other sets of cells. The unidentified $^3$H peak and that for GGPP were about the same for C100 and H1-177 cells.

Additional evidence that these cells contain FTP was obtained using a different HPLC system and a different TLC system. When the reverse phase HPLC column was eluted with a gradient of acetonitrile in NH$_4$CHO$_3$ (35), the elution of the $^3$H]FTP and $^3$H]FPP standards usually overlapped, but $^3$H]FTP started to elute slightly before $^3$H]FPP. The $^3$H-labeled compounds in extracts of H1-177 cells that eluted from this HPLC column at the same times as the FPP and FTP standards were examined by TLC (silica gel G developed with 1-propanol/NH$_4$OH/H$_2$O). Fraction 31 contained a $^3$H-labeled compound with the same mobility as the FTP standard (Fig. 6D). Fraction 33 contained a $^3$H-labeled compound with the same mobility as the FTP standard. The advantage of this TLC system is that mobility of FTP is much less than those of FPP and GGPP. Farnesol and FMP moved with the solvent front. This TLC system could not be used when the HPLC column (Fig. 6A) was eluted with buffers containing tetrabutylammonium phosphate. Under basic conditions, tetrabutylammonium binds to isoprenoid pyrophosphates and triphosphates, causing them to remain at the origin.

**Protein Prenylation**—Prenyl transferases use FPP and GGPP to covalently attach farnesyl and geranylgeranyl groups to cysteines near the C termini of certain intracellular proteins (25). To assess the effect of Nm23 proteins on protein prenylation, C100 and H1-177 cells were labeled with $^3$H]mevalonolactone in the presence of lovastatin (Fig. 7). Although the overall pattern of protein prenylation was the same in these two cell lines, the relative levels of prenylation differed. C100 and H1-177 cells contained comparable amounts of prenylated proteins of about 27 and 25 kDa, but H1-177 contained 80–100% more prenylated 46-kDa proteins and 40–50% more prenylated 24-kDa proteins than did the C100 cells.

Farnesylated or geranylgeranylated proteins can be specifically labeled by incubating cells with either $^3$H]FPP or $^3$H]GGPP (31). When H1-177 and C100 cells were incubated with $^3$H]GGPP, both the pattern and extent of protein geranylgeranylation were comparable (Fig. 7). However, when incubated with $^3$H]FPP, more $^3$H]farnesyl was incorporated into proteins in H1-177 cells than into proteins in C100 cells. The greatest differences (50–100%) were in the amounts of $^3$H]farnesyl incorporated into proteins of 46 and 24 kDa, but there were also smaller differences in several other proteins. Western blots and enzymatic assays showed that C100 and H1-177 cells contain comparable levels of farnesyl transferase (data not shown).
Kinase Activities of Nm23 Proteins

The phosphorylation of GPP and FPP by Nm23 proteins provides a novel mechanism by which these proteins might exert their effects on metastasis, motility, or differentiation. In this paper, we report 1) the phosphorylation of GPP and FPP to the corresponding triphosphate by purified Nm23 proteins and by Nm23 proteins in crude cell extracts, 2) an increase in the concentration of FTP in cells that have an elevated level of Nm23-H1, and 3) an altered pattern of protein prenylation in cells that have an elevated level of Nm23-H1. We also show that Nm23-H1S120G, a mutant found in some aggressive neuroblastomas (32), has reduced GPP and FPP kinase activities and that Nm23 proteins that inhibit cell motility have higher GPP and FPP kinase activities than do Nm23 proteins that do not inhibit cell motility. In contrast, NDP kinase activities and histidine to histidine protein phosphotransferase activities of Nm23 proteins (18, 21) and binding of Nm23 proteins to the c-myc promoter (36) do not correlate with inhibition of cell motility. A histidine to aspartate protein phosphotransferase activity of Nm23 proteins does appear to correlate with inhibition of motility (21); however, the stoichiometry of this phosphotransferase reaction is very low. Only 2–4% of the phosphate bound to NDP kinase is transferred (37). All of the phosphate bound to NDP kinase can be transferred to FTP or FPP.

The rates of phosphorylation of GPP and FPP by Nm23 proteins (1–4 nmol/min/mg) in vitro are much less than rates of phosphorylation of nucleoside diphosphates (500 μmol/min/mg). However, the Km values for the phosphorylation of nucleoside diphosphates (0.2–1 mM), and GPP and FPP effectively competed with nucleoside diphosphates for phosphorylation by Nm23 proteins in crude cell extracts. More importantly, cells with an elevated level of Nm23-H1 contained more FTP than did control cells.

Isoprenoid pyrophosphates were detected by labeling cells with [3H]mevalonolactone in the presence of lovastatin, an inhibitor of endogenous mevalonate synthesis. Under these conditions, the concentrations of GPP and FPP are lower than in the untreated cells. Our inability to detect GPP in these cells probably results from it being rapidly metabolized. NIH3T3 cells labeled using a similar protocol contained one-tenth as much GPP as FPP (29).

To our knowledge, neither FTP nor GerTP has been previously reported to be present in any type of cell. A possible explanation for this is that these triphosphates have not been directly looked for and that the published procedures used to fractionate cellular isoprenoid pyrophosphates do not separate triphosphates from pyrophosphates. When phosphorylated isoprenoids extracted from [14C]mevalonolactone-labeled NIH3T3 cells were fractionated by HPLC, a peak of 14C-labeled material eluted immediately before the FPP (29). This peak was not identified, but perillyl alcohol, a monoterpane, caused an increase in both FPP and this unidentified peak. Depending on the solvent system used, FTP elutes immediately before or after FPP.

Two observations suggest that mammalian cells may contain kinases other than Nm23 proteins that can phosphorylate GPP and FTP. 1) H1-177 cell extracts contain 5 times as many Nm23 proteins as do C100 cell extracts but only twice as much GPP and FTP kinase activity. 2) Immunodepleted H1-177 cell extracts contained as little as 8% as much Nm23 protein as did the control extract, but they retained half the FTP and FPP kinase activities. Extracts of the fungus Gibberella fujikuroi contain a kinase that phosphorylates FTP to give FPP (38). Unlike NDP kinases, this kinase is relatively specific for ATP, and it does not phosphorylate FTP.

When labeled with [3H]mevalonolactone, H1-177 cells contained about twice as much FTP as did the C100 cells, and several prenylated proteins were almost twice as abundant in H1-177 cells as in C100 cells. These proteins appear to be farnesylated, since a similar result was obtained when the cells were labeled with [3H]FPP. Thus, at least under this condition, cells with an elevated level of Nm23 proteins have an increased level of protein farnesylation. These results predict that FTP is a substrate for farnesyl transferase, and we have found that farnesy transferase isolated from bovine brain will use FTP to farnesylate recombinant Ras.

The labeling of prenylated proteins was performed in cells treated with lovastatin to inhibit the synthesis of endogenous mevalonate. In cells not treated with lovastatin, the amount of FTP may be sufficient for complete protein farnesylation. When cells not treated with lovastatin were examined, the fractions of Ras and lamin B farnesylated in H1-177 cells appeared to be the same as in C100 cells (data not shown). However, there did appear to be differences in the extent of prenylation of several low molecular weight GTP-binding proteins.

FPP is a branch point in isoprenoid metabolism and is a precursor of GGPP, cholesterol, heme A, ubiquinone, and dolichol phosphate (23, 24). FPP and GGPP are used by prenyl transferases to prenylate proteins, many of which play critical roles in signal transduction, growth regulation, and cell motility. Farnesylation of oncogenic forms of Ras is essential for their transforming activity, and several small GTP-binding proteins involved in regulation of the cytoskeleton are preny-

2 N.-D. Vu and P. D. Wagner, unpublished results.
lated. Cholesterol is involved in membrane structure and affects endocytosis and cell signaling. Dolichol phosphate functions as a saccharide carrier for protein glycosylation and for synthesis of glycosylphosphatidylinositol protein anchors. The conversion of GPP or FPP to triphosphates could alter their synthesis of glycosylphosphatidylinositol protein anchors. The observation that cells with an elevated level of FTP had an increased level of protein farnesylation suggests that cellular FTP is a substrate for geranylgeranyl transferase but not for one or more of the other enzymes that use FPP as a substrate. GerTP and FTP may also have metabolic roles distinct from those of the pyrophosphates.

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