Membranes from Ehrlich ascites tumor cells possess an activity which, in the presence of farnesyl pyrophosphate, incorporates $[^{14}C]$isopentenyl pyrophosphate into a product which is soluble in chloroform/methanol and retained by DEAE-cellulose. The product co-migrated with authentic farnesyl pyrophosphate on thin layer chromatography but was degraded to neutral labeled compounds when subjected to mild acid hydrolysis, suggesting the presence of an unsaturated $\alpha$-isoprene unit. Triton X-100 (2%) liberated the activity from the membranes and the resulting solubilized preparation was further characterized. The enzyme was found to be sensitive to sulfhydryl reagents and stimulated by ionic strength. A strong dependence of activity on the addition of farnesyl pyrophosphate was observed, allowing several compounds to be tested for their ability to serve as potential primers. Geranyl pyrophosphate, neryl pyrophosphate, all-trans farnesyl pyrophosphate and all-trans geranylgeranyl pyrophosphate were found to be effective substrates, although to different extents. Citronellyl pyrophosphate (which has a saturated $\alpha$-isoprene) was inactive as a substrate. The chain length of the products generated was investigated by using a double label isotope procedure and by reverse-phase high performance liquid chromatography. All active primers yielded a product containing 16–19 isoprene units; the distribution of the individual isoprene species was essentially identical regardless of the primer used as substrate. These findings indicate that the specificity lies in the absolute chain length of the product released and not the number of isoprenes added. High performance liquid chromatography of the $[^{14}C]$polyisoprenol resulting from enzymatic dephosphorylation of the reaction product indicated the presence of an unsaturated $\alpha$-isoprene unit in a cis-configuration. It is proposed that the enzyme is the long chain cis-prenyltransferase involved in the biosynthesis of dolichyl phosphate.

cis-Prenyltransferases are ubiquitous enzymes that catalyze the addition of isoprene units from isopentenylpyrophosphate to a preformed pyrophosphate primer (see Ref. 1 for a review). In bacteria, these enzymes are involved in the synthesis of undecaprenyl pyrophosphate, which acts as a glycosyl carrier in lipopolysaccharide and peptidoglycan synthesis. Allen et al. (2) and Baba and Allen (3) have partially purified the undecaprenyl pyrophosphate synthetases from Micrococcus luteus and Lactobacillus plantarum and carried out substrate specificity studies which demonstrated that these enzymes do not exhibit a strict stereochemical requirement for the pyrophosphate primer.

In animals, a major product of the cis-prenyltransferases is dolichyl pyrophosphate, the long chain polyisoprenoid involved in $N$-linked glycoprotein synthesis. The priming substrate for dolichol synthesis is probably trans,trans-farnesyl pyrophosphate since, as in undecaprenol, the dolichol backbone consists of two trans units adjacent to the $\omega$ end of the molecule followed by a variable number of cis units. A basic difference between the eucaryotic dolichol and the procaryotic undecaprenol is the presence of a saturated $\alpha$-isoprene in the former.

Recent studies indicate that the level of dolichyl phosphate may play a role in regulating the rate of $N$-linked glycoprotein synthesis. The demonstration in this (7) and other laboratories (8–10) that the cis-prenyltransferase is rate limiting for de novo dolichyl phosphate synthesis therefore raises the possibility that this enzyme may be subject to regulation. However, before initiating studies on regulation, it is first necessary to carry out a positive identification and preliminary characterization of the enzyme. Although several reports have appeared in recent years describing activities that catalyze the incorporation of radioactivity from $[^{14}C]$isopentenyl pyrophosphate into chloroform/methanol-soluble material with the chromatographic properties of dolichyl phosphate or pyrophosphate (11–14), proof that such activities catalyze cis addition, or that the product has the expected number of isoprenes, has not been provided. Clearly, this type of evidence is crucial toward identifying the enzyme which carries out dolichyl phosphate biosynthesis.

In the present report, we have solubilized and characterized a prenyltransferase from Ehrlich ascites tumor cell membranes. Evidence is presented that this enzyme is the cis-prenyltransferase involved in dolichyl phosphate biosynthesis.

EXPERIMENTAL PROCEDURES

Isotopes—$[1,^{14}C]$Isopentenyl pyrophosphate (64 Ci/mmol) was obtained from Amersham-Searle.

Dolichyl $[^{32}P]$phosphate was prepared using the method of Cramer et al. (15). This method produces the monophosphate ester as the primary product. Briefly, bistrichylammonium $[^{32}P]O_3^- (1.0 \text{ mmol}, 1.0 \text{ Ci})$ was incubated with dolichol (0.7 mmol) in 0.1 ml of acetonitrile/dichloroethane (1:1) at 50 °C in a screw-capped Reactivil (Pierce). After 60 min, the reaction was diluted into 2 ml of chloroform/methanol (2:1) and an equal volume of water was added. After centrifuging to separate the phases, the organic phase was retained and washed with 50% aqueous methanol. Residual inorganic phosphate was removed by chromatography on DEAE-cellulose (acetate form) equilibrated with chloroform/methanol (1:1). Elution of the product was carried out with 0.3 M ammonium acetate in chloroform/methanol (1:1). Water was added to effect phase separation and, after
washing with 50% aqueous methanol, the lower phase was concentrated to a small volume. The yield was about 30 μCi dolichyl [32P] phosphate which gave a single radioactive band on silica TLC in solvent D (see below for composition of solvent D).

Isopentenyl (α,β-8-P)phosphopyrophosphate (2 Ci/mmol) was synthesized from α,β-8-P-pyrophosphate (0.5 Ci/mmol) by the procedure of Cramer and Böhm (18). The product was dissolved in 0.15% alcohol by thin layer chromatography using solvent A. The radioactive band migrating with authentic isopentenyl pyrophosphate was scraped from the plate and the [32P]-labeled product was eluted with 0.1 M NH4OH overnight at room temperature. Yield: 7.5 μCi. Rechromatography showed a single peak of radioactivity.

Preparation of Enzyme—Ehrlich ascites tumor cells were obtained from Dr. Joseph G. Cryer of this department and were used to inoculate 25-g female ICR mice. After 6 days, the mice were killed by cervical dislocation and the tumor cells removed from the peritoneal cavity with a syringe. After washing the cells three times with 8 volumes of a solution containing 0.15 M NaCl, 5 mM Tris-Cl, pH 7.5, 1 mM 2-mercaptoethanol, the cells were suspended by sonication in 8 volumes of hypertonic buffer consisting of 5 mM Tris-Cl, pH 7.5, 2 mM CaCl2, 1 mM EDTA, 1 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride. After 10 min on ice, the cells were disrupted using a Dounce homogenizer (3-5 strokes, tight pestle). Nuclear and cell debris were removed by centrifugation at 250 g for 5 min. The supernatant was removed and centrifuged at 100,000 × g for 45 min to sediment the remainder of the cellular membranes. The membrane pellet was suspended in an equal volume of 0.25 M sucrose and stored at −70 °C.

Preparative reverse-phase HPLC of polyprenols was carried out on a HPLC Magnum-9 ODs-3 column (0.9×25 cm) at a flow rate of 0.5 ml/min and a temperature of 37 °C. The chromatography was carried out at room temperature. Preparative reverse-phase HPLC runs (Partisil-5, 0.1% alcohol in hexane). Purity of the trans-polyprenol-18 product was >95%.

Stability of Long Chain Prenyltransferase—The assay mixture contained, in a total volume of 0.5 ml: Tris-Cl, pH 7.0, (50 μmol); MgCl2 (0.25 μmol); [32P]isopentenyl pyrophosphate (4 nmol, 500,000 dpm); dihydrotestosterone (0.25 μmol), farnesyl pyrophosphate (70 nmol); NaCl (250 μmol); and enzyme. After incubation for 1 h at 37 °C, the reaction was terminated by the addition of 2 ml of chloroform/methanol (2:1). Samples were then treated with 0.4 ml of 0.86% KCl, vortexed, and centrifuged. After washing the aqueous phase with 1 ml of chloroform, the resulting upper phase, containing the released inorganic phosphate, was assayed by liquid scintillation counting.

Preparation of Prenels and Prenyl Phosphates—Geraniol and nerol were obtained from commercial sources. Allylic pyrophosphates were prepared and purified by the method of Cornforth and Popjak (18). Citronellyl pyrophosphate, all-trans farnesyl pyrophosphate and all-trans geranylgeranyl pyrophosphate were generous gifts from Dr. Charles Allen, University of Florida. Purity of all substrates was assessed by thin layer chromatography on silica gel G plates (solvent A); detection was with phosphate or anisaldehyde spray reagents. Concentrations of allylic pyrophosphates were determined by phosphatase analysis following acid hydrolysis (19). Polyprenols were prepared from the needles of Juniperus communis, Pinus elliottii, or Juniperus virginiana obtained locally, according to the method of Stone et al. (20). Individual isoprenologues were obtained either by preparative HPLC or from Calbiochem.

α-trans-Polyenol-18 was prepared from α-cis-polyenol-18 (Calbiochem) as follows: 20 mg of polyprenol in 1.0 ml of hexane was stirred with 50 mg of MnO2 for 1 h at room temperature, after which time thin layer chromatography (solvent B) showed nearly complete conversion to a new anisaldehyde-positive band (RF = 0.7) which was also UV positive and well separated from the starting material (RF = 0.3). The reaction was diluted with hexane and the MnO2 removed by centrifugation. After reducing the volume to 0.7 ml, cis-trans isomerization of the α-cis-aldehyde was effected by incubating the hexane solution in a quartz cuvette mounted in front of a UVS-54 watt UV lamp (UV Products) with the filters removed. Irradiation was for 30 min, after which time thin layer chromatography showed a new UV-positive band migrating slightly slower than the α-cis-aldehyde and of about the same intensity. Further irradiation gave no increase. The mixture of cis- and trans-polyprenols was dissolved in 0.2 ml of dry tetrahydrofuran and reduced for 16 h at room temperature with excess LiAlH4. The use of NaBH4 as the reductant was avoided in order to minimize 2,3-dihydropolyprenol (dolichol) formation resulting from a side reaction which accompanies borohydride reduction of allylic aldehydes and ketones (21). The reaction was terminated by the addition of 0.2 ml of aqueous 0.1 M NaOH and the mixture was diluted into 1 ml of chloroform/methanol (2:1). Water was then added to form two phases. An aliquot of the organic phase, when analyzed by TLC (solvent C) showed two closely spaced UV-negative anisaldehyde-positive bands, the faster band (RF = 0.25) co-migrating with α-cis-polyenol-18. The two stereoisomers were separated by repetitive HPLC runs (Partisil-5, 0.1% alcohol in hexane). Purity of the α-trans-polyprenol-18 product was >95%.

Isolation of Dolichol and Dolichol Phosphate—Ehrlich ascites tumor cell membranes, g, wet weight, were homogenized in 5 ml of 0.5 M sucrose containing 8 ng/ml Triton X-100. The reaction was then carried out in a final volume of 0.1 ml containing 10 μCi dolichyl [32P] phosphate (0.25 μmol), 3NHCl (250 μmol); and enzyme. After incubation for 1 h at 37 °C, the reaction was terminated by the addition of 2 ml of chloroform/methanol (2:1). After vigorous mixing, the reaction tubes were centrifuged at 100,000 × g for 45 min. The supernatant, which contained, in a total volume of 0.5 ml: Tris-Cl, pH 7.0, (50 μmol); MgCl2 (0.25 μmol); [32P]isopentenyl pyrophosphate (4 nmol, 500,000 dpm); dihydrotestosterone (0.25 μmol), farnesyl pyrophosphate (70 nmol); NaCl (250 μmol); and enzyme. After incubation for 1 h at 37 °C, the reaction was terminated by the addition of 2 ml of chloroform/methanol (2:1). Water was then added to form two phases. An aliquot of the organic phase, when analyzed by TLC (solvent C) showed two closely spaced UV-negative anisaldehyde-positive bands, the faster band (RF = 0.25) co-migrating with α-cis-polyenol-18. The two stereoisomers were separated by repetitive HPLC runs (Partisil-5, 0.1% alcohol in hexane). The product of the reaction was isolated by ion-exchange chromatography (see “Experimental Procedures”) and quantitated by HPLC on silica using 0.3% alcohol in hexane. The product was characterized as dolichyl phosphate by comparison with the original samples to monitor purification and correct for yield.

RESULTS

Characterization of the Enzymatic Reaction—Crude preparations of Ehrlich tumor cell membranes were found to incorporate [1-14C]isopentenyl pyrophosphate into organic solvent soluble material which was retained on columns of DEAE-cellulose. The product of the reaction was isolated by ion-exchange chromatography (see “Experimental Procedures”) and migrated as a single species on thin layer chromatography in solvent D (Fig. 1A). Its mobility was identical with that of dolichyl monophosphate. However, when the 14C-labeled

1 The abbreviations used are: HPLC, high performance liquid chromatography; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate.

2 Alcohol is a specially formulated solvent from Fisher. The composition is isopropanol/ethanol/methanol (5:9:5:5).

3 The nomenclature of polyprenols and dolichols follows that proposed by Cornforth and Popjak (20). Individual isoprenologues were obtained either by preparative HPLC or from Calbiochem.

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6 The nomenclature of polyprenols and dolichols follows that proposed by Cornforth and Popjak (20). Individual isoprenologues were obtained either by preparative HPLC or from Calbiochem.
product was subjected to acid hydrolysis, the radioactivity was found to migrate near the solvent front in solvent D and as several species (presumably tertiary alcohols) moving faster than authentic polyenol-18 in solvent C (Fig. 1B).

**Solubilization of Long Chain Prenyltransferase Activity**—A variety of detergents were found to successfully solubilize the tumor cell enzyme, including Triton X-100, CHAPS, Lubrol WX, and octylglucoside (Table I). Sodium deoxycholate (1%) inactivated the enzyme (data not shown). Removal of detergent either by treatment with Amberlite XAD-2 or dialysis led to an 80% loss of enzymatic activity, which could be fully restored by the addition of Triton X-100 to a final concentration of 0.2%. All subsequent studies were performed with enzyme preparations which were solubilized in 2% Triton X-100. Reactions were linear with time up to 1 h and with protein concentration up to 2 mg/assay (Fig. 2). The pH optimum was 7.5 and, in the presence of 10 μM farnesyl pyrophosphate, half-maximal activity was obtained with 5 μM [1-14C]-isopentenyl pyrophosphate (data not shown). Enzymatic activity was sensitive to sulfhydryl reagents with 0.1 mM p-chloromercuribenzenesulfonic inhibiting 81% and 1 mM iodoacetate giving 80% inhibition. N-ethyl maleimide was less effective: at 1 mM only 20% inhibition was observed. The addition of 1 mM EDTA to the assay abolished enzyme activity, and gel filtration on Sephadex G-25 yielded a preparation which was completely dependent on the addition of 0.5 mM MgCl₂ in the assay for full activity. Because of the apparent requirements for a free SH group and divalent cation, dithiothreitol and MgCl₂ (both at 0.5 mM) were included in all assays. High salt concentrations were found to activate the enzyme about 40% with maximal activation at 0.4 M NaCl (data not shown). This effect was independent of the monovalent cation, since identical results were obtained using KCl.

**Attempts at Purification of the Long Chain Prenyltransferase**—Efforts to purify the prenyltransferase were frustrated by extensive aggregation in all detergents tested. Gel filtration on columns of Sepharose-4B showed that all enzymatic activity eluted in the void volume of the column in a turbid solution (data not shown). Ion exchange, adsorption, and hydrophobic affinity chromatography resulted in either minimal increases in specific activity or unacceptable losses.

**TABLE I**

| Detergent      | Final concentration | Solubilized activity | Total recovered activity |
|----------------|---------------------|----------------------|-------------------------|
|                | %                   | %                    | cpm x 10⁻²               |
| Triton X-100  | 2                   | 73                   | 73                      |
| Lubrol WX      | 1                   | 57                   | 57                      |
| Octylglucoside| 30 mM               | 64                   | 64                      |
| CHAPS          | 10 mM               | 58                   | 58                      |
| None           |                     |                      | 7                       |

*Represents activity in supernatant compared to total recovered activity.

**Fig. 1.** Thin layer chromatography of the product of the long chain prenyltransferase reaction. A, the 14C-labeled material eluted from DEAE-cellulose was subjected to thin layer chromatography in Solvent D and radio scanned. The arrow marks the position of migration of authentic dolichyl phosphate, B, the post-DEAE-cellulose material was subjected to acid hydrolysis (chloroform, methanol, 1 N HCl, 10/10/3, 95 °C, 1 h) and subjected to thin layer chromatography in Solvent C. The arrow marks the position of authentic polyenol-18. O, origin; SF, solvent front.

**Fig. 2.** Assay of solubilized long chain prenyltransferase activity as a function of time (A) and protein concentration (B). Assay conditions are as described under "Experimental Procedures."
was found to be inactive, providing strong evidence that the allylic pyrophosphates were serving as true substrates and not just stimulating the reaction rate in a nonspecific fashion.

The chain length of the product was determined by two methods. The first method employed [1-14C]isopentenyl [α,β-32P]pyrophosphate as a substrate. Since 2 mol of phosphate are lost for every isoprene incorporated, the change in the 14C/32P ratio is a measure of the number of isoprenes added. Also taken into consideration is the fact that the product contains a single phosphate group. The 14C/32P ratio of the reaction product of the tumor cell enzyme indicated that an average of 14–15 isoprene units had been added to the allylic pyrophosphate substrate (Table III). Considering the chain length of the unlabeled allylic pyrophosphate primer, the total chain length of the product was estimated at 18 isoprene units. This number is probably a slight overestimation since the calculation assumes absolute radiochemical purity of both radioisotopes. For example, a 5% impurity of isopentenyl [32P]monophosphate in the starting mixture would lower the calculated chain length to 17.1.

A more direct assessment of the chain length was made by HPLC analysis. By using the reverse-phase mode, it is possible to resolve the various isoprene species and thus determine the length of the products generated. All allylic pyrophosphates were found to yield products with essentially identical chain length distributions. As shown for farnesyl pyrophosphate in Fig. 4 (open bars) the major product observed was the monophosphate ester of polyprenol-17.

The effect of detergent on product chain length was investigated. With farnesyl pyrophosphate (10 μM) as primer, there was no detectable difference in the chain length distribution using Triton X-100 concentrations in the range of 0.2–1.0% (data not shown).

To approach the stereochemistry of the prenyltransferase reaction, the geometry of the α-isoprene unit was determined by comparing the chromatographic mobilities of standard α-cis- and α-trans-polyprenol-18 with that of the 14C-labeled product after dephosphorylation. The [14C]polyprenyl phosphate reaction product was dephosphorylated with potato acid phosphatase by the method of Fujii et al. (24) and fractionated by reverse-phase HPLC. (The α-cis and α-trans isomers were not resolved by reverse-phase HPLC). The radiolabeled peak of activity on the addition of farnesyl pyrophosphate; it was therefore possible to test several other pyrophosphates as potential substrates. As shown in Fig. 3, the four allylic pyrophosphates examined were found to be active, with all-trans farnesyl pyrophosphate giving the highest maximum velocity. The nonallylic compound citronellyl pyrophosphate

![FIG. 3. Ability of various polyprenyl pyrophosphates to act as primers in the long chain prenyltransferase assay. Assay conditions are as described under "Experimental Procedures." Open bars, farnesyl pyrophosphate; □, neryl pyrophosphate; ▄, geranyl pyrophosphate; ▼, all-trans geranylgeranyl pyrophosphate; □, citronellyl pyrophosphate.](image)

**TABLE II**

| Primer        | Product 14C/32P | Isoprenes added | Total isoprenes |
|---------------|-----------------|-----------------|-----------------|
| Geranyl-PP    | 25.9            | 16.8            | 18.1            |
| Neryl-PP      | 24.9            | 16.1            | 18.1            |
| All-trans farnesyl-PP | 23.8         | 15.5            | 18.5            |

*a* Isoprenes added = \( \frac{\text{product } ^{14}\text{C}/^{32}\text{P}}{\text{substrate } ^{14}\text{C}/^{32}\text{P}} \times \frac{1}{2} \). The factor of \( \frac{1}{2} \) arises from the fact that the \( ^{32}\text{P} \) specific activity of the monophosphate product is one-half the pyrophosphate substrate.

*a* Found by adding the number of isoprenes added to the chain length of the unlabeled primer.

![FIG. 4. Determination of chain length distribution by reverse-phase HPLC analysis. Open bars, the product of the long chain prenyltransferase reaction using farnesyl pyrophosphate as primer. Solid bars, endogenous dolichyl phosphate isolated from Ehrlich tumor cells.](image)

![FIG. 5. Silica HPLC of the enzymatically dephosphorylated product of the long chain prenyltransferase reaction using as substrates farnesyl pyrophosphate and [14C]isopentenyl pyrophosphate (histogram). The dotted line represents internal standards of α-cis (C) and α-trans (T) polyprenol-18.](image)
co-migrating with authentic polyprenol-18 was mixed with authentic α-cis and α-trans-polyisopren-18 and applied to a silica (Partial-5) column. As shown in Fig. 5, the bulk of the radioactivity was found to migrate with the α-cis-stereoisomer.

**Analysis of Endogenous Dolichol and Dolichyl Phosphate Pools**—The levels of dolichol (representing the free alcohol and any dolichyl fatty acyl esters) and dolichyl phosphate (representing free dolichyl phosphate, dolichyl pyrophosphate, and their glycosylated derivatives) were determined to be, per ml of packed cells, 0.59 ± 0.15 μg (n = 3) and 3.3 ± 0.25 μg (n = 3), respectively. It is noteworthy that the ratio of dolichyl phosphate to total dolichol compounds is substantially higher in tumor cells than in other sources examined (25, 26). This may reflect the fact that these cells are in a state of division and hence must utilize their newly synthesized dolichyl phosphate for glycoprotein synthesis rather than for storage as dolichol.

The chain length of the tumor cell dolichyl phosphate was determined by reverse-phase HPLC (Fig. 4, *solid bars*). The major isoprene species was the monophosphate ester of dolichol-19. The chain length distribution of the endogenous dolichol was found to be nearly identical to dolichyl phosphate (data not shown).

### DISCUSSION

Polyprenols involved in glycan biosynthesis are characterized by an ω-terminus of 2 or 3 trans isoprene units and an extension of 7-20 cis isoprene units (27). In bacteria, the formation of undecaprenyl pyrophosphate proceeds by the addition of 6-8 cis isoprene units to farnesyl pyrophosphate in a reaction catalyzed by a single cis-prenyltransferase (28). The enzymes from *L. plantarum* and *M. luteus* were shown by Allen *et al.* (2) and Baba and Allen (3) to lack strict stereospecificity for the allylic pyrophosphate substrate. Neryl, geranyl, farnesyl, and geranylgeranyl pyrophosphates were all substrates, although they exhibited different kinetic constants. The chain length of the in *vitro* synthesized polyprenyl pyrophosphate product was found to depend on the allylic pyrophosphate primer used and the presence of detergent or phospholipid in the assay mixture.

The prenyltransferase from Ehrlich ascites tumor cells which we have characterized in the present report is similar to the bacterial enzymes in that it lacks strict stereospecificity for the allylic pyrophosphate primer and requires Triton X-100 for activity. However, unlike the bacterial enzyme, the chain length of the product of the tumor cell enzyme is not detectably affected by increasing concentrations of Triton X-100. A second difference is that the chain length distribution of the products of the eucaryotic enzyme was independent of the structure of the allylic pyrophosphate primers, suggesting that the specificity for product release lies in the overall length of the product and not the number of trans isoprenes on the ω end.

The stereochemistry of addition of isoprene units is likely to be cis for the following reasons. First, the isoprene at the hydroxyl end of eucaryotic dolichols have been shown to be biogenetically cis (29), and the α-isoprene unit of the product of the Ehrlich tumor cell prenyltransferase reaction is in the cis configuration. Second, all known prenyltransferases maintain specificity with regard to the stereochemistry of addition (30). We therefore suggest that the tumor cell activity which we have characterized in a cis-prenyltransferase, although conclusive proof awaits either the preparation and testing of stereospecifically labeled isopentenyl pyrophosphate substrates (31) or the isolation of sufficient product for NMR analysis.

Based on the products arising from the bacterial cis-prenyltransferases, it is likely that the primary in *vitro* product of the tumor cell enzyme is an allylic pyrophosphate, in spite of the fact that the only product obtained from the in *vitro* assays is a monophosphate ester. The solubilized enzyme from Ehrlich tumor cells therefore must contain a highly active polyprenyl pyrophosphate phosphatase which immediately degrades all pyrophosphate product to the monophosphate form. All efforts to inhibit or separate out this activity have been thus far unsuccessful. Consistent with the concept of a pyrophosphate primary product is the identification by Wellner and Lucas (13) of soluble activity from chicken liver which apparently produces a polyprenyl pyrophosphate from [14C]isopentenyl pyrophosphate and farnesyl pyrophosphate.

The completion of the dolichol moiety requires saturation of the α-isoprene unit, which generates a chiral center at carbon-3. Work from this laboratory has shown that the α-isoprene residues in dolichols from chicken, pig, and human sources are in the S configuration (32). It is presently unknown at what stage during dolichyl phosphate biosynthesis reduction occurs, although work in our laboratory using labeled substrates injected into the portal veins of rats suggests that a phosphorylated form of polyisopren (rather than the free alcohol) is the substrate for the α-isoprene reductase (33). The surprising finding that the average length of the in *vitro* synthesized product is about 1-2 isoprenes less than that of endogenous dolichyl phosphate raises the possibility that the final saturated isoprene unit may be added separately in an as yet uncharacterized reaction. An alternative, and perhaps more plausible, mechanism is that tight coupling exists between the prenyltransferase and the α-isoprene reductase in such a way that the latter enzyme has a higher affinity for the polyprenyl-18 and -19 phosphates, thus shifting the product distribution towards the species observed in *vivo*. In any case, it is clear that in order to elucidate the nature of the saturation step, it will be necessary to develop an in *vitro* assay for reduction. To date, however, all attempts to develop a sensitive, reproducible assay have failed.

The specific activity of the cis-prenyltransferase measured in Ehrlich tumor cell membranes is considerably greater than that found in membranes from rat liver or hen oviduct. This probably reflects the fact that the rapidly dividing tumor cells must synthesize new dolichyl phosphate molecules to maintain a given level of the phospholipid. In liver and oviduct under steady state conditions, new dolichyl phosphate molecules are only required to replenish that which is turned over or secreted. However, conditions could arise (e.g. inflammation followed by synthesis and secretion of acute phase glycoproteins by the liver) in which an increased demand for glycoprotein synthesis might lead to a concomitant increase in dolichyl phosphate levels. Such a situation could be dealt with either by increasing the activity of the de novo pathway (i.e. through elevating cis-prenyltransferase activity) or by phosphorylating pre-existing dolichol molecules via the CTP-dependent dolichol kinase. Both of these enzymes have been localized in the microsomal fraction of rat liver (26, 34, 35) (presumably the endoplasmic reticulum) and have been shown to face the cytosol (36). Thus, both enzymes are candidates for participating in the elevation of dolichyl phosphate levels. The work documented in the present report establishes the validity of an assay for the activity responsible for dolichyl phosphate synthesis and thus enables us to determine what
Long Chain Prenyltransferase

role the de novo pathway plays in elevating the steady state levels of dolichyl phosphate.

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