The tyrosinase of *Streptomyces antibioticus* is encoded by the second open reading frame, melC2 of the melanin operon (melC). The upstream open reading frame melC1 specifies a 146-amino acid protein with a typical NH$_2$-terminal signal-peptide characteristic of a secretory protein. The MelC1 protein is involved in the transfer of copper ion to apotyrosinase MelC2 via binary complex formation (Lee, Y.-H., Chen, C.-R., Wu, S.-Y., Leu, W.-M., Lin, J.-J., Chen, C. W., and Lo, S. J. (1988) *Gene* (Amst.) 65, 71–81; Chen, L.-Y., Leu, W.-M., Wang, K.-T., and Lee, Y.-H. W. (1992) *J. Biol. Chem.* 267, 20100–20107). To investigate whether the export of tyrosinase is also dependent on MelC1, a mutational study of its signal-peptide sequence was performed. Four different mutants were obtained. Mutation at the positively charged region (mutant M-6LE, Arg$^2$-Arg$^7$ → Leu$^2$-Glu$^7$) or the hydrophobic region (mutant M-16D, Val$^{16}$ → Asp$^{16}$) led to Mel$^*$ phenotypes. These lesions caused a severe 7–10-fold reduction of the export of both the MelC1 and MelC2 proteins and a concomitant accumulation of the two proteins in the cytosolic fraction. The cell-associated tyrosinase activity in M-6LE but not in the M-16D mutant was dramatically reduced to 4% of the activity found in the wild type strain, suggesting that the basic NH$_2$ terminus of MelC1 is also important for the trans-activation function of this protein. Nevertheless, the defects on the trans-activation and/or secretory functions of MelC1 in mutants M-6LE and M-16D are not due to the impairment of the formation of the MelC1-MelC2 complex. The translation of melanin operon genes in these two mutants also decreased. In contrast, the tyrosinase activity and the secretion of MelC2 were not affected if the mutations occurred at the putative cleavage site of the signal peptidase (e.g. mutant M-29SM, Arg$^{26}$-Ala$^{30}$ → Ser$^{26}$-Met$^{30}$ or mutant 29-SMG, Arg$^{26}$-Ala$^{30}$-Asp$^{31}$ → Ser$^{26}$-Met$^{30}$-Gly$^{31}$). Additionally, tyrosinase activity and its export were abolished in a MelC1-negative mutant, M-850. Taken together, these results demonstrate that a functional MelC1 is essential for tyrosinase secretion and activity. Furthermore, the results suggest that like other secretory proteins, basic and hydrophobic residues in the MelC1 signal sequence are an important feature of the signal-peptide and play a pivotal role in the secretion of both the MelC1 and MelC2 proteins. These results also establish a novel role for MelC1 on tyrosinase transport in addition to a role in copper transfer to tyrosinase.

Tyrosinase (EC 1.14.18.1) is a copper-containing monooxygenase that catalyzes the formation of melanin pigment from tyrosine (Lerch, 1981). In *Streptomyces*, production of melanin is widespread among different species (Kuster, 1976). The synthesis and secretion of tyrosinase in this genus have interesting determinants and regulation (Gregory and Shyu, 1961; Gregory and Huang, 1964; Baumann and Kocher, 1976; Kuster, 1976; Kieser et al., 1981; Crameri et al., 1982; Held and Kutzner, 1990). For instance, while synthesis of tyrosinase is inducible and the enzyme secreted extracellularly in the wild type strain of *Streptomyces glaucescens*, mutant strains have been isolated which are defective in tyrosinase activity, tyrosinase secretion, or inductive regulation (Baumann and Kocher, 1976; Baumann et al., 1976; Kieser et al., 1981; Crameri et al., 1982).

The structural gene (melC2) for tyrosinase of *Streptomyces antibioticus* (Katz et al., 1983) or *S. glaucescens* (Hintermann et al., 1985) is located in a similar polycistronic operon (melC). Both tyrosinase genes (melC2) are preceded by an upstream gene, melC1, specifying a conserved protein (146 or 134 amino acid residues) with an NH$_2$-terminal 30-amino acid signal sequence that is removed after secretion (Bernan et al., 1985; Huber et al., 1987; Chen et al., 1992). The MelC2 protein does not possess a signal-peptide sequence, although the secretion of this protein is required for the formation of melanin (Crameri et al., 1982). No NH$_2$-terminal peptide sequence of MelC2 is removed during secretion (Crameri et al., 1982; Bernan et al., 1985). The integrity of melC1 is essential for melanin operon expression (Katz et al., 1983; Bernan et al., 1986; Lee et al., 1988). Genetic and biochemical studies showed that the MelC1 protein is involved in the copper-transfer process that activates apotyrosinase (Lee et al., 1988; Chen et al., 1992). In addition, the presence of a 30-amino acid signal-peptide sequence in the MelC1 protein suggests a role for tyrosinase transport (Bernan et al., 1985). In support of this supposition, we have detected complex formed by tyrosinase and MelC1 both inside and outside of the cell (Chen et al., 1992).

In this study, we examined the effect on the secretion of tyrosinase by various mutations within the signal-peptide sequence of MelC1. Our results indicate that both the hydrophobic region and the basic NH$_2$ terminus of the MelC1.*
signal-peptide are crucial for secretion of tyrosinase and MelCl itself.

**EXPERIMENTAL PROCEDURES**

**Materials**—Restriction endonucleases and DNA-modifying enzymes were purchased from New England Biolabs, Bethesda Research Laboratories, or Boehringer Mannheim Biochemicals. 32P-Labeled deoxynucleotides were purchased from Amersham Corp. Bacterial Strains, Plasmids, and Culturing Conditions—Streptomyces lividans TK64 (proc-2, str-6) (Hopwood et al., 1983) was used as the host for recombinant plasmids. Streptomyces plasmid pIJ702 (Katz et al., 1983), containing the thiostrepton-resistance determinant (tsr) and the melanin operon (melC), was kindly provided by Professor E. Katz (Georgetown University, Washington, D.C.). Plasmid pPP90, a melC1 melC2 derivative of pIJ702, and plasmid pSAI, a melC1 melC2 derivative of pIJ702, have been described previously (Lee et al., 1988). Plasmid M13 mp18-800 was generated by inserting a 0.8-kilobase PstI-SstI fragment containing the mutations on the M13 mp18-800 derivative as described earlier (Chater et al., 1982). Oligonucleotide Synthesis and in Vitro Mutagenesis—The oligonucleotide-directed site-specific mutagenesis was performed essentially as described by Kunkel et al. (1987). Uracil-containing single-stranded template was prepared in Escherichia coli strain CJ236 (dut ung) (Bethesda Research Laboratories) as described. Single-stranded DNA of M13 mp18-800 was used as the template for mutagenesis. Oligonucleotides designed for site-directed mutagenesis were synthesized in an Applied Biosystem automatic DNA synthesizer (model 380A) and purified by polyacrylamide gel electrophoresis (Maniatis and Efstratiadis, 1980). The primers used for site-directed mutagenesis is given as shown. Each primer was designated by the position of the mutated amino acid in the MelC1 protein and the nature of the amino acid (one-letter symbol) after the mutation. The asterisks indicate mutated bases; new restriction enzyme sites created by the mutagenesis. Oligonucleotides designed for site-directed mutagenesis are as shown. Each primer was designated by the position of the mutated amino acid in the MelC1 protein and the nature of the amino acid (one-letter symbol) after the mutation. The asterisks indicate mutated bases; new restriction enzyme sites created by the mutagenesis.

**RESULTS**

Alteration of the MelCl Signal Sequence Blocked the Phenotypic Melanin Formation—The NH2-terminal 30 amino acid residues of MelCl protein exhibit the features of a typical prokaryotic signal-peptide: 3 positively charged amino acids (Arg4, Arg5, Arg6), followed by a 12-amino acid long hydrophobic stretch (Ala4 through Val15) and a small side chain amino acid at the signal-peptide cleavage site (Ala18 and Asp19) (Fig. 1) (Berman et al., 1985; Chen et al., 1992; Perlman and Halvorson, 1983). These three regions were chosen as the targets of site-directed mutagenesis for their effect on activation and secretion of tyrosinase. Four melCl signal-peptide

**FIG. 1. Sequence of the wild type and mutant MelCl signal-peptides.** The amino acid sequence of the wild type MelCl signal-peptide is shown as standard single-letter abbreviations. The mutant signal-peptides are listed below the wild type and only those changed from the wild type are shown. Each mutant as indicated is designated by the position of the mutated amino acid in the signal-peptide and the nature of the amino acid residue after mutation. An arrow marks the cleavage site of signal-peptidase (Chen et al., 1992).
mutants were obtained (see “Experimental Procedures” for mutant constructions). These mutation sites were (i) Arg^G to Leu^G (mutant M-6LE); (ii) Val^G to Asp^G (mutant M-16D); and (iii) Arg^Gto Ala^G, Asp^G to Ser^G (mutant M-29SM) or Arg^G to Ser^G, Met^G to Asp^G (mutant M-29SMG) (Fig. 1). When examining the effects of these mutations on melanin production on R2YE agar plates (containing 0.12 μM copper ion and 0.05% tyrosine), mutants M-29SM and M-29SMG displayed the Mel^ phenotype in the colony itself as well as its surrounding zone, whereas mutants M-6LE and M-16D exhibited the Mel^ phenotype (Fig. 2A). Addition of 0.1 mM copper ion to the agar plate only induced a trace amount of melanin production in the M-6LE and M-16D colonies but not in the surrounding area, indicating that the tyrosinase in these two mutants was either inactive or was not secreted (Fig. 2B). It is to be noted here that in melCl mutant M-950, in which MelCl was not synthesized, the colony phenotype was Mel^- (Fig. 2), which is consistent with our previous observation (Lee et al., 1988).

The MelCl Signal-Peptide Mutants M-6LE and M-16D Are Defective in the Export of Tyrosinase—The intracellular tyrosinase activity in the melCl signal-peptide mutant M-6LE (4% of wild type activity) was drastically decreased, while it was essentially unchanged in mutants M-29SM and M-29SMG (Table I). In contrast, a slight enhancement of intracellular enzymatic activity was found in mutant M-16D. Results also indicated that the tyrosinase found in the medium of wild type, M-29SM, and M-29SMG cells was about 58–65% of total activity, but it decreased to 5–8% in mutants M-6LE and M-16D, and was completely undetectable in mutant M-950 (Table I). Quantitation of the tyrosinase exported by immunoblot suggested that in the wild type, M-29SM, and M-29SMG strains, approximately 50–55% of the total cellular tyrosinase was secreted into the medium, while in mutants M-6LE and M-16D less than 15% (11–14%) of the MelCl protein was secreted (see Fig. 3). The secretion of tyrosinase was also completely blocked in the melCl^- mutant M-950 (Fig. 3). The amount of intracellular tyrosinase retained in this mutant also decreased to 13% of the wild type, which probably resulted from the instability of the tyrosinase in the absence of functional MelCl protein, or from the polarity effect as a consequence of the frameshift mutation in melCl (Lee et al., 1988).

Taken together, we conclude that MelCl is required for the expression and export of tyrosinase. While alterations of the basic NH_2 terminus and the hydrophobic region of the signal-peptide caused a pronounced effect on the secretion of tyrosinase, alteration of the signal-peptide cleavage site was not critical for the functions of MelCl on the tyrosinase export and trans-activation.

**TABLE I**

<table>
<thead>
<tr>
<th>Clone*</th>
<th>Phenotype</th>
<th>Relative tyrosinase activity</th>
<th>Cellular distribution of tyrosinase activity</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>Mel^*</td>
<td>100 ± 19</td>
<td>64.5 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>M-950</td>
<td>Mel^-</td>
<td>0.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>M-6LE</td>
<td>Mel^-</td>
<td>4 ± 1</td>
<td>7.6 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>M-16D</td>
<td>Mel^-</td>
<td>165 ± 28</td>
<td>5.4 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>M-29SM</td>
<td>Mel^*</td>
<td>100 ± 13</td>
<td>58.7 ± 6.7</td>
<td></td>
</tr>
<tr>
<td>M-29SMG</td>
<td>Mel^-</td>
<td>115 ± 19</td>
<td>60.4 ± 7.7</td>
<td></td>
</tr>
</tbody>
</table>

*The clone designation is similar to that described in Fig. 1.

The specific activity of tyrosinase present in the cell pellets was determined and expressed as percent of the wild type. The specific activity of wild type tyrosinase was 4.6 units/mg protein. The data were obtained from 3 transformants in three to four experiments.

The percentage of total tyrosinase activities that was secreted to the medium.

**FIG. 3. Immunoblot analysis of the secretion of tyrosinase protein in melCl mutants.** S. lividans TK64 harboring plJ702 or its mutant derivatives were cultured in TSB medium for 30 h at 30°C. The intracellular extracts from the cell pellets were prepared by sonicnation (Lee et al., 1988). The protein in culture medium was precipitated by 2 volumes of cold acetone and the recovered pellets were dissolved in the sample buffer (Laemmli, 1970). Both fractions were analyzed by SDS-PAGE (0.1% SDS-15.5% gel) (Laemmli, 1970) and immunoblotted with anti-MelC2 antiserum (1:1000 dilution). The proteins from 100 μl of cultures were applied to each lane. The band intensity was quantitated by the soft laser densitometer (Biomed). The clones designation were as described in the legend to Fig. 1. M-SA1, cell harboring melC2^- plasmid pSA1 (see “Experimental Procedures”).

**FIG. 2. Phenotypes of melCl mutants.** S. lividans TK64 harboring either wild type or the melCl defective plasmid plJ702 were cultured on an R2YE plate containing 0.05% tyrosine and 0.01% thiostrepton (A) or on the same plate as described but with an additional 100 μM of copper ion (B) for 2 days. WT, host carrying wild-type melCl gene in plJ702; M-950, host carrying plasmid pPF950; M-6LE, M-16D, M-29SM, M-29SMG, host carrying mutant plJ702 with melCl signal-peptide mutation as described in the legend to Fig. 1.
plIJ702S-16D (M-16D) displayed a 25–60-fold decrease in secreted tyrosinase as compared with the wild type strain (Fig. 4B). These signal-peptide mutants also showed 300–600-fold reduction in the secreted MelC1 protein (Fig. 4A). However, a less than 2-fold difference for the amounts of MelC1 and MelC2 retained in the cytosolic and membrane fractions was observed (Fig. 4, A and B).

Quantitation of the subcellular distribution of both MelC1 and MelC2 proteins in these two signal-peptide mutants and the wild type strain shown in Fig. 4 is summarized in Table II. Both proteins accumulated in the cytosolic fraction of the mutant strains and accounted for 84–88% of the total MelC1 or MelC2 synthesized. While in the wild type strain, the majority (86–98%) of these two proteins was in the medium fractions. Only a slight increase of both proteins in the membrane fractions of the mutant strains was found. The finding that the melC1 signal-peptide mutations elicited the same level of interfering effect on the export of MelC1 and MelC2 (7–10-fold reduction) strongly suggests that the secretion of these two proteins might be coupled (see “Discussion”). It is also noted that both mutants, M-6LE and M-16D, exhibited a severe reduction in the total amount of the MelC1 protein to a level of 2–3% of the wild type strain, and a moderate reduction on the MelC2 protein to 20–30% of the wild type (see Table II). A similar phenomenon has been reported for other export proteins defective in the signal-peptide region (Oliver, 1985; Gennity et al., 1990). This may reflect the coupling of translation to secretion (Oliver, 1985; Gennity et al., 1990). Nevertheless, it is also likely that the mutations in M-6LE and M-16D cause the instability of both MelC1 and MelC2.

Several additional points should be noted here. First, multiple MelC1 protein species (two to three forms) with different electrophoretic mobilities in the 15–14-kDa range were detected in all subcellular fractions of the wild type and signal-peptide mutants (Fig. 4A). Second, the sizes of these multiple forms of MelC1 estimated from the electrophoretic mobilities were somewhat larger than the expected size of the processed MelC1 (12 kDa) after removal of the signal-peptide (Chen et al., 1992). The sizes of MelC1 present in the wild type and the signal-peptide variants were also different (Fig. 4A). This might reflect the alteration of electrophoretic mobility or the stability of the MelC1 protein caused by the mutation. Alteration of mobility in gel is a fairly common phenomenon for several signal-peptide mutant proteins (Leinhardt et al., 1987; Freudl et al., 1988; Goldstein et al., 1991).

\[
\begin{align*}
\text{Table II} \\
\text{Subcellular distribution of the MelC1 and MelC2 proteins in melC overproducing strains harboring the wild type or melC1 signal-peptide defective plasmids.} \\
\text{The data displayed in this table were based on the experimental results of Fig. 4, but essentially the same conclusion was obtained from the other sets of experiment.} \\
\text{Clone} & \text{Wild type} & \text{M-6LE} & \text{M-16D} \\
\text{MelC1 protein} & \text{Total amount}^a & \text{Subcellular distribution}^b \\
\text{Cytosol} & 2 & <0.1 & 5 \\
\text{Membrane} & 5 & 10 & 7 \\
\text{Medium} & 98 & 84 & 111 \\
\text{MelC2 protein} & \text{Total amount}^a & \text{Subcellular distribution}^b \\
\text{Cytosol} & 13 & 120 & 23 \\
\text{Membrane} & 4 & 84 & 84 \\
\text{Medium} & 82 & 84 & 84 \\
\text{WT} & 100 & 100 & 100 \\
\text{M-6LE} & 10 & 8 & 8 \\
\text{M-16D} & 10 & 8 & 8 \\
\text{M-29SMG} & 10 & 8 & 8 \\
\text{M-29SM} & 115 & 92 & 92 \\
\text{a All clones carried the derivatives of plIJ702 with melC promoter mutation in addition to the signal-peptide mutation if applicable.} \\
\text{b The amount of MelC1 and MelC2 proteins present in various strains were quantitated and expressed as percent of wild type strain.} \\
\text{c The relative amounts of MelC1 and MelC2 distributed in different subcellular fractions are given.} \\
\end{align*}
\]

\[
\begin{align*}
\text{Table III} \\
\text{Reconstitution of tyrosinase activity in wild type and melC1 mutant strains by copper ion} \\
\text{The intracellular fractions (including the membrane fractions) were prepared from cell pellets and the tyrosinase activity was determined before or after incubation with 0.5 mM copper sulfate at 4 °C.} \\
\text{Clone} & \text{Activity after preincubation with copper ion} & \text{Activity enhanced} \\
\text{0} & \% & 16 h & -folds \\
\text{Wild type} & 100 & 83 & 0.8 \\
\text{M-6LE} & 10 & 8 & 0.8 \\
\text{M-16D} & 107 & 94 & 1.0 \\
\text{M-29SMG} & 100 & 92 & 0.8 \\
\text{M-29SM} & 115 & 92 & 0.8 \\
\end{align*}
\]

In Vitro Reconstitution of Tyrosinase Activity of the melC1 Signal-Peptide Mutants—Our previous studies showed that MelC1 was involved in transferring copper to apotyrosinase (Lee et al., 1988; Chen et al., 1992). It is conceivable that mutations in the signal-peptide of MelC1 may also affect the copper-transfer process. To assess this possibility, the tyrosinase activity in the cellular extracts of melC1 signal-peptide mutants were reconstituted with 0.5 mM of copper ion for 16 h at 4 °C. The results (Table III) demonstrated that no distinct enhancement of the tyrosinase activity was observed by the added copper ion. In fact, a 20% decrease of enzymatic activity was observed for tyrosinase activities in most of the strains examined, except M-16D (Table III). Since the amount of the MelC1 and MelC2 proteins retained in the cell pellet...
of M-6LE was nearly identical to that in the wild type (Fig. 3), the observed low level of tyrosinase activity in the cell pellet of mutant M-6LE (4% of wild type strain) (Table I) is not attributed to the low amount of MelC2 retained in the cell pellet, but rather to defective trans-activation of the MelC1 protein in this particular mutant.

**The MelC1 and MelC2 Proteins Still Form a Complex in Mutants M-6LE and M-16D**—We have recently found that MelC1 and MelC2 formed a complex both inside and outside of the cell (Chen et al., 1992). Since M-6LE and M-16D were defective in the enzymatic activity and/or the secretion of tyrosinase, it is possible that these mutations may result in a failure in the complex formation. However, immunoaffinity chromatographic studies indicated that MelC1 and MelC2 from both mutants formed a complex (Fig. 5, B and C). Thus, the observed defect in either export or trans-activation functions of the mutated MelC1 in M-6LE and M-16D is not due to the failure to form a MelC1-MelC2 complex. However, it is noted that in mutants the MelC1:MelC2 ratio, as well as the relative elution profiles for the two proteins from the anti-MelC1 antibody column, were different from those for the wild type strain (compare Fig. 5A with B and C). This might reflect the effect of mutational alteration of MelC1.

**DISCUSSION**

Most secretory proteins have an NH2-terminal extension of 20–30 residues known as the signal sequence for translocation across the membrane. Although the primary sequences of the signal-peptide are seldom conserved, they all contain three contiguous parts, a basic NH2 terminus (one or more positive charges), a central apolar region (8–12 hydrophobic residues), and a non-polar carboxyl terminal. Mutagenesis studies have been extensively carried out in several laboratories to analyze the structure-function relationship of these three regions of the signal sequences (for a review, see Gennity et al. (1990)). It has clearly been established that the basic character of the NH2 terminus and the apolar composition of the central domain are essential for protein translocation. Mutational alterations within the basic hydrophilic segment of the signal sequence or the central hydrophobic core can result in defective exportation (Benson et al., 1985; Pollitt et al., 1985; Puziss et al., 1989). In addition, these changes may also cause decreased synthesis. The carboxyl terminus of the signal sequence contains the processing site with consensus Ala-X-Ala sequence (von Heijne, 1983). Mutations in this region usually have little effect on protein exportation even if they block processing (Fikes and Bassford, 1987). Several roles of the signal-peptide in facilitating protein export have been proposed, (i) targeting the protein to the membrane for transport; (ii) maintaining the protein in a transport-competent conformation; (iii) providing a recognition site for components of the cellular export machinery (for reviews, see Giersch (1989) and Randall and Hardy (1989)).

The function of the signal-peptide within MelC1 has not been explored. In this study, we introduced mutations into these three major regions of signal sequence within MelC1. These alterations disrupted one or more features of the signal-peptide that are presumably required for MelC1 secretion. For instance, in M-6LE, a neutral and a negative charged residue replaced the tandem positively charged residues to result in a mutant with no charged NH2 terminus; in mutant M-16D, a negatively charged residue (Asp) was incorporated into the center of the hydrophobic core region to generate a mutant with a shorter hydrophobic core (see Fig. 1). In these two mutants both the secreted MelC1 and tyrosinase were severely reduced as was the total amount of the two proteins present (see Figs. 3 and 4 and also, Table II). This confirms the importance of the basic NH2 terminus and the middle hydrophobic center of the MelC1 signal-peptide. In contrast, the alteration of the processing site in M-29SM and M-29SMG resulted in minimal inhibitory effects on the secretion of MelC2 (see Fig. 3). Although the predicted conformation of these mutant signal-peptides was still helical (data not shown), at least in some cases the conformation of MelC1 and MelC2 had changed. This is based on (i) the defect in tyrosinase activity in M-6LE (Table I); (ii) different migration patterns of mutant MelC1 in SDS-PAGE (Fig. 4A); and (iii) a different immunoaffinity chromatographic profile of mutants MelC1 and MelC2 (Fig. 5). This implies that the NH2-terminally positively charged region is important for the folding of the mature MelC1 and for trans-activation and secretion of tyrosinase. In contrast, the interruption of the hydrophobic core of the MelC1 signal sequence (in M-16D) had no effect on the trans-activation function although it blocked the export of tyrosinase and itself. All these defects on the export of both proteins can be attributed to a conformational incompetence for transport across the membrane. Alternatively, the mutant MelC1 and tyrosinase or their complex are folded in such a way that their tertiary structure is different from that of the native form. As a result, other cellular factors may not be able to recognize it as a secretory protein.

Our previous (Lee et al., 1988; Chen et al., 1992) and present studies show that MelC1 plays a dual role for the expression of tyrosinase, a *trans*-activator and a secretion accessory. A question arises regarding how and where (intracellular or extracellular) MelC1 accomplish these functions. Based on the consideration that both active tyrosinase and the MelC1-MelC2 binary complex were detected intra- and extracellularly (Chen et al., 1992), a model depicting these processes (activation, copper-transfer, and secretion) is proposed as follows (Fig. 6; also see, Chen et al. (1992)). Newly synthesized MelC1 and MelC2 form a transient association. The interaction results in proper folding of apotyrosinase and the secretion of the complex. The copper ion in the medium is transferred to apotyrosinase held in a receptive configuration. Subsequently, the mature tyrosinase dissociates from MelC1 (pathway 1). It is possible that part of the copper transfer and dissociation of the complex takes place intracellularly. It is not clear whether these components may be secreted (pathways 2–4). An *in vitro* translocation assay might help answer this question.

The two roles played by MelC1 could be decoupled as shown by mutant M-16D, which activates but does not secrete tyrosinase. It would be interesting to know whether a *melC1* mutant exists that affects the *trans*-activation but not the
tions involved in tyrosinase secretion. The secretion of tyrosinase has been shown by Crameri genes (the secretion of proteases B and C occurs without a NH2-terminal signal-peptide and is dependent on adjacent genes encoding specific secretion functions (Wandersman, 1989). In E. coli, the secretion of cell-surface anchored lipoprotein pullulanase protein conformations governed by these two processes. The different symbols for MelCl (C1) and MelC2 (C2) designate the possible conformational transition during the MelC2 activation process. The copper forms of tyrosinase and binary complex are indicated by the presence of Cu in the protein. This illustration is not intended to depict the stoichiometry of the copper ion in the complex and/or its components (C1 or C2). C1s represents the putative oligomeric nature of MelC1 protein (Chen et al., 1992). For details see text.

secretory role of MelC1. Further studies on the interactive domains of MelC1 and tyrosinase will be informative for understanding the multiple facets of the MelC1 and MelC2 protein conformations governed by these two processes.

The dependence of other proteins upon one another for export is not uncommon. In Erwinia chrysanthemi, the secretion of proteases B and C occurs without a NH2-terminal signal-peptide and is dependent on adjacent genes encoding specific secretion functions (Wandersman, 1989). In E. coli, the secretion of cell-surface anchored lipoprotein pullulanase from Klebsiella oxytoca requires the presence of 14 other pel genes (the pulC-O operon and pulS) in addition to the six sec genes of the general export pathway (Puglsey et al., 1990). Apart from MelC1, the involvement of other genes in the secretion of tyrosinase has been shown by Crameri et al. (1982) who genetically mapped several chromosomal mutations involved in tyrosinase secretion. The melC1 signal-peptide mutants might be suitable for cloning genes involved in tyrosinase export through isolation of suppressor genes which restore secretion. This approach has been successfully used in the studies of the secretory system of E. coli (for reviews, see Benson et al. (1985) and Bieler et al. (1990)).

Acknowledgments—We thank Drs. C. W. Chen, S. J. Lo, and K. B. Choo for critical reading of the manuscript.

REFERENCES


FIG. 6. Outlines of the proposed activation and secretion pathways for tyrosinase. This scheme illustrates the possible intracellular or extracellular pathways for activation and secretion of tyrosinase (C2) govern by MelC1 (C1). The different symbols for MelC1 (C1) and MelC2 (C2) designate the possible conformational transition during the MelC2 activation process. The copper forms of tyrosinase and binary complex are indicated by the presence of Cu in the protein. This illustration is not intended to exclude the possible involvement of other export components in these processes nor is it intended to depict the stoichiometry of the copper ion in the complex and/or its components (C1 or C2). C1s represents the putative oligomeric nature of MelC1 protein (Chen et al., 1992). For details see text.