Tyrosinase is a type I membrane protein regulating the pigmentation process in humans. Mutations of the human tyrosinase gene cause the tyrosinase-negative type I oculocutaneous albinism (OCAI). Some OCAI mutations were shown to delete the transmembrane domain or to affect its hydrophobic properties, resulting in soluble tyrosinase mutants that are retained in the endoplasmic reticulum (ER). To understand the specific mechanisms involved in the ER retention of soluble tyrosinase, we have constructed a tyrosinase mutant truncated at its C-terminal end and investigated its maturation process. The mutant is retained in the ER, and it is degraded through the proteasomal pathway. We determined that the mannose trimming is required for an efficient degradation process. Moreover, this soluble ER-associated degradation substrate is stopped at the ER quality control checkpoint with no requirements for an ER-Golgi recycling pathway. Co-immunoprecipitation experiments showed that soluble tyrosinase interacts with calreticulin and BiP/GRP78 (and not calnexin) during its ER transit. Expression of soluble tyrosinase in calreticulin-deficient cells resulted in the export of soluble tyrosinase of the ER, indicating the calreticulin role in ER retention. Taken together, these data show that OCAI soluble tyrosinase is an ER-associated degradation substrate that, unlike other albino tyrosinas, associates with calreticulin and BiP/GRP78. The lack of specificity for calnexin interaction reveals a novel role for calreticulin in OCAI albinism.

Tyrosinase (monophenol, dihydroxy-phenylalanine: oxygen oxidoreductase; EC 1.14.18.1) is the rate-limiting enzyme involved in melanin biosynthesis (1, 2). This protein, consisting of a large catalytic luminal domain that is anchored to the membrane by a C-terminal transmembrane domain and a short cytosolic tail (3), undergoes glycosylation prior to being subjected to the endoplasmic reticulum quality control (4). Although misfolded chains are sorted for the endoplasmic reticulum (ER)\(^1\)-associated degradation (ERAD) pathway, folded tyrosinase is exported out of the ER through the secretory pathway targeting the pigmentation site organelle, the melanosome (5, 6).

Mutations in the tyrosinase gene result in the absence of pigmentation and are responsible for oculocutaneous albinism type I (OCAI) in humans (7). OCAI was proposed to be an ER retention disease in which misfolded tyrosinase mutants are retained in the ER by the quality control (8, 9). Calnexin and calreticulin, as components of the quality control, were shown to interact transiently with the monoglucosylated N-glycans of the misfolded polypeptides (10, 11). These lectin chaperones engage the chains into the de-glucosylation/re-glucosylation cycle catalyzed by glucosidase II and glucosyltransferase, with the latter recognizing only incompletely, folded chains. Although the correctly folded polypeptides leave the cycle and the ER, the incompletely folded ones are re-glucosylated and retrieved by calnexin/calreticulin (12, 13). Both lectins bind to membrane and soluble glycoproteins, but calreticulin was proposed to bind preferentially to soluble substrates. In general, the role of calreticulin has been largely overlooked, because until recently, it was assumed that the two lectins can substitute for each other in the ER quality control (14). This cycle is also believed to be effective in the case of some albino tyrosinases (8, 9, 15). Interestingly, calnexin was shown to associate extensively with these mutants, whereas calreticulin contribution was only rarely reported (8).

Over 100 mutations have been identified in the tyrosinase gene of OCAI patients (16). Although many of them are clustered in the luminal domain, a number of mutations were found to affect, directly or indirectly, the transmembrane domain of tyrosinase. Thus, a base insertion within the TM domain resulting in a dramatic reduction of its hydrophobicity leads to an albino tyrosinase (3). A deletion of 10 bases occurring at the beginning of the tyrosinase TM domain was recently reported in a tyrosinase-negative OCAI patient (19). It has been proposed that these mutations would interfere with the insertion of tyrosinase into the membrane, hence impairing the whole pigmentation process. Breimer et al. (17) reported a mutational hot spot leading to a soluble tyrosinase in two patients with a temperature-sensitive form of albinism. Because the patients also showed a mutation at codon 422, the temperature sensitive phenotype was associated with the point

ER-associated degradation; WT, wild type tyrosinase; ST, soluble tyrosinase; TM, transmembrane domain; NB-DNJ, N-buty1-deoxinosinijrmycin; LLN, N-acetyl-leucyl-leucyl-norleucinal; PBS, phosphate-buffered saline; CNX, calnexin; CRT, calreticulin; CHAPS, 3-(3-cholamidopropyldimethylammonio)-1-propanesulfonic acid; MeSO, dimethyl sulfoxide; CHO, Chinese hamster ovary; HEK, human embryonic kidney; ERGIC, ER/Golgi intermediate compartment; MEF, mouse embryonic fibroblasts; HCV, hepatitis C virus. 

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§ The abbreviations used are: ER, endoplasmic reticulum; ERAD, ER-associated degradation; WT, wild type tyrosinase; ST, soluble tyrosinase; TM, transmembrane domain; NB-DNJ, N-buty1-deoxinosinijrmycin; LLN, N-acetyl-leucyl-leucyl-norleucinal; PBS, phosphate-buffered saline; CNX, calnexin; CRT, calreticulin; CHAPS, 3-(3-cholamidopropyldimethylammonio)-1-propanesulfonic acid; MeSO, dimethyl sulfoxide; CHO, Chinese hamster ovary; HEK, human embryonic kidney; ERGIC, ER/Golgi intermediate compartment; MEF, mouse embryonic fibroblasts; HCV, hepatitis C virus.
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Mutation previously reported to result in a similar phenotype (20). On the other hand, Berson et al. (21) have shown that the C-terminal truncation of both wild type and a temperature-sensitive mutant tyrosinase determined their ER retention.

All of the above OCAI frameshift mutations introducing termination codons, which either delete or decrease the hydrophobicity of the single TM domain, are associated with a complete absence of tyrosinase activity (3, 16, 17, 19). Although these studies indicate the ER retention of different forms of truncated tyrosinases as the cause of albinism, the molecular basis for this ER retention has not been addressed as yet. Herein, the ER maturation pathway of a human soluble tyrosinase construct was investigated. We found that, unlike wild type, the truncated tyrosinase did not associate significantly with calnexin, being retained in the ER by calreticulin and BiP/GRP78. This tyrosinase mutant is an ERAD substrate that does not require ER-Golgi transport and retrieval for degradation. Our findings qualify calreticulin as a major component in the ER retention of albino C-terminally truncated tyrosinases.

MATERIALS AND METHODS

Reagents, Cell Lines, and Antibodies—CHO, B16F1, and HEK293T cells were obtained from the European Collection of Animal Cell Cultures (Porton Down, UK). MEF (6–5) and wild type MEF cells were a gift from Prof. T. Elliott (University of Southampton, Southampton, UK) and Prof. M. Michalak (University of Alberta, Edmonton, Alberta, Canada). The cells were grown in RPMI 1640 medium (Invitrogen) containing 10% fetal calf serum (Sigma), 50 units/ml penicillin, and 50 mg/ml streptomycin (Invitrogen) and maintained at 37 °C with 5% CO2. Rabbit anti-calnexin antiserum was a kind gift from Dr. J. J. Bergeron (McGill University, Montreal, Quebec, Canada) (10). Mouse monoclonal anti-human ERGIC-53 IgG1 antibody was a gift from Dr. H. P. Hauri (Biozentrum, University of Basel, Basel, Switzerland) (22). T311 (Neo-Markers, Fremont, CA) is a monoclonal antibody IgG2a recognizing human BiP (32). Mouse monoclonal anti-calreticulin C-17 was a gift from Dr. J. Dubuisson (CRNS-Institut Biologie de Lille, Lille, France) (23). The construct pTrEx1.1-E2HCV was a kind gift from Dr. Olivier Argaut (Oxford University, Oxford, UK), and the pTrEx1.1-TYR37 was a gift from Viorica Lastun (Institute of Biochemistry, Bucharest, Romania). Rabbit anti-calreticulin (calregulin C-17) antiserum was purchased from Santa Cruz Biotechnology. Rabbit anti-BiP (anti-GRP78) antiserum was a gift from Dr. J. Dubois (Laboratoire de Biologie Cellulaire et Moléculaire, Université de Nantes, Nantes). The monoclonal anti-human GRP78 antibody (10C) was purchased from DAKO (Glostrup, Denmark). Anti-calreticulin (calregulin C-17) antiserum was purchased from Santa Cruz Biotechnology. Rabbit anti-BiP (anti-GRP78) antiserum was a gift from Dr. J. Dubois (Laboratoire de Biologie Cellulaire et Moléculaire, Université de Nantes, Nantes). The monoclonal anti-human GRP78 antibody (10C) was purchased from DAKO (Glostrup, Denmark).

RESULTS

Immunofluorescence—B16F1 cells were plated on coverslips and transfected with pTrEx1.1-TYR and pTrEx1.1-STYR using the method specified above. After 24 h, the cells were rinsed with PBS and fixed and permeabilized with methanol at −20 °C for 5 min. Cells were incubated with 20 μg/ml mouse anti-mouse IgG1-4 for 2 h at room temperature. Following three washes with PBS, they were mounted in Vectorshcire mounting medium (Vector Laboratories) and viewed with a Nikon Eclipse E 600 fluorescent microscope. Images were processed using Adobe Photoshop 5.0 software.

Soluble Tyrosinase Is Retained in the ER of Melanocytic and Non-melanocytic Cells—Constitutively synthesized only by melanocytes and melanoma cells, wild type tyrosinase is localized in specialized organelles named melanosomes. With melanin being synthesized exclusively in melanosomes, sequestration of tyrosinase in any pre-melanosomal compartment leads to an albino phenotype. To study the intracellular localization of a soluble albino tyrosinase, we have constructed a mutant truncated at the 474 codon and expressed it in the mouse melanoma cell line B16F1. Both wild type and truncated tyrosinase were transiently transfected and analyzed by immunofluorescence microscopy (Fig. 1A). It can be seen that wild type...
Tyrosinase displays mainly a distinct punctate cytoplasmic pattern visible also along the dendrites, consistent with an intracellular vesicular distribution specific to a melanosome. Some of the protein displays a perinuclear staining co-localizing with the ER resident protein calnexin. In contrast, soluble tyrosinase is massively co-localized with calnexin, suggesting an ER localization. This has been previously shown for a soluble tyrosinase truncated at the 444 codon and expressed in HeLa cells (21). Although the two tyrosinases were truncated at different codon positions, they displayed similar ER localizations, indicating that truncations occurring ahead of the TM domain result in ER retention.

Next, to confirm the immunofluorescence localization, transiently transfected B16F1 cells were characterized by Western blotting (Fig. 1B). Cell lysates were divided in two, and half of each sample was digested with EndoH and run next to a non-digested control in reducing SDS-PAGE. Digestion with EndoH, which removed high mannose and hybrid N-glycans, resulted in a soluble tyrosinase form running as a single band at ~54 kDa (Fig. 1B). In contrast, the wild type tyrosinase was expressed as two glycoforms, one EndoH-resistant and one EndoH-sensitive running at ~58–60 kDa, corresponding to the polypeptide (Fig. 1B). Therefore, the ectopic expression of human wild type tyrosinase in B16F1 cells results in a protein able to exit the ER in a proportion of 38% (Fig. 1C) and to be transported through the secretory pathway. In the same system, truncation of the TM domain dramatically decreases the proportion of the ER-exported form, confirming the ER localization observed by immunofluorescence. To address the possibility that this behavior is specific only to melanocytes, we expressed soluble tyrosinase in two non-melanocytic cell lines, CHO and HEK293. As seen in Fig. 1B, we found a similar EndoH pattern as that found in melanocytes. We were not able to detect soluble tyrosinase in the medium of the tested cells referred to above (data not shown). Our results show that the soluble tyrosinase mutant remains in the ER of melanocytic and non-melanocytic cells, consistent with a similar localization of a soluble tyrosinase expressed in HeLa cells (21).

To compare the time course maturation of wild type and
soluble tyrosinases, we performed a pulse-chase experiment in B16F1-transfected cells. The cells were pulse-labeled with [35S]methionine/cysteine for 20 min followed by chase for time periods up to 2 h. Immunoprecipitation of the labeled cell lysates with T311 anti-tyrosinase antibodies was followed by SDS-PAGE analysis (Fig. 1C). The mock immunoprecipitation in non-transfected B16F1 cells show the absence of nonspecific bands (Fig. 1C). After a 30-min lag period, a gradual reduction in the amount of the labeled immunoprecipitated soluble and wild type protein was observed during the chase. In cells transfected with ST, digestion with EndoH reduced the pool to a single band running at the polypeptide mass (Fig. 1C). As shown by EndoH digestion experiments, WT is synthesized as a high mannose precursor that acquires complex-type glycans in the Golgi in ~30 min of chase and is degraded rapidly during 2 h of chase (Fig. 1C). As reported previously (8), a significant population (62%) of the labeled WT remains EndoH-sensitive, indicating a reduced level of productive folding of this protein (Fig. 1D). Although membrane tyrosinase has a half-life of 1.8 h, soluble tyrosinase degrades with a half-life of 1.5 h. Because the half-life of the ER-retained WT (WT-sensitive, >2 h) is at least twice as high as the one of the secreted population (WT-resistant, 1 h), we analyzed the two populations separately. As seen in Fig. 1D, the WT-sensitive population degrades slower than the soluble tyrosinase population.

Although it has been proposed that soluble tyrosinase behaves like the WT-sensitive form of the membrane tyrosinase (21), our degradation kinetics studies indicate an accelerated degradation of the soluble tyrosinase mutant versus the ER-retained form of membrane tyrosinase. This prompted us to investigate further the degradation pathways of soluble tyrosinase.

Soluble Tyrosinase Is an ERAD Substrate—Proteins that are not able to fold correctly are stopped by the ER quality control checkpoint in the ER and targeted to degradation. These ERAD substrates are retrotranslocated in the cytoplasm and degraded in proteasomes. Sometimes the misfolded proteins require transport to the early Golgi and retrieval to the ER prior to degradation (25).

To determine whether soluble tyrosinase is an ERAD substrate, we first asked the question as to whether its ER retention was a true ER retention or was the result of exit and retrieval back into the ER. To answer this question, we analyzed the localization of the soluble mutant in the presence and absence of nocodazole, which inhibits the retrograde transport Golgi-ER (26). Immunofluorescence experiments of the transfected cells show only partial co-localization of ST with the ERGIC-53 protein known to recycle between ER and Golgi and mostly localization in the ER/Golgi intermediate compartment (ERGIC) (22, 27) (Fig. 2). The distinct localization of the two proteins is even more obvious after the treatment of cells with nocodazole, which induces the distribution of ERGIC in the early Golgi (28). In these conditions, soluble tyrosinase is localized in a different compartment than ERGIC, as seen by the lack of merge between the two proteins. Although ERGIC-53 has a typical peripheral early Golgi pattern, soluble tyrosinase displays an ER distribution, which indicates that ST is not transported beyond the ER, being retained in this compartment without any retrieval steps.

Next, we investigated the effect of inhibitors of the ERAD components on the degradation of the truncated mutant. An ERAD substrate is targeted to proteasomal degradation, and therefore, proteasome inhibitors reduce the degradation rate of these substrates. Associations with lectins recognizing monoglycosylated (CNX, CRT) and Man8 (EDEM) glycans have been reported to be required for the dislocation of the ERAD substrate into the cytoplasm (29). To identify soluble tyrosinase as an ERAD substrate, we have used inhibitors of the proteasomal degradation and of the lectin associations and analyzed the degradation rate. Transfected cells were pulse-chased for 0 and 3 h in the presence of various inhibitors, and the cell lysates were immunoprecipitated with T311 antibody and analyzed by SDS-PAGE. As seen in Fig. 3, the percentage of non-degraded labeled protein increased from 30 to 78% in the presence of the proteasome inhibitor lactacystin and to 95% in the presence of LLN, which is another proteasome inhibitor. Kifunensin, which inhibits the N-glycan trimming by ER mannosidase I (29), showed a substantial inhibitory effect on the degradation of soluble tyrosinase. This inhibitor of the ER N-glycan processing pathway increases the accumulation of soluble tyrosinase at 3 h of chase to 94%. Bafilomycin and nocodazole have very little effect on the degradation rate of soluble tyrosinase, confirming that the mutant is targeted to degradation without any recycling through the ERGIC or Golgi (Fig. 3). Taken together, the data show that soluble tyrosinase is an ERAD substrate degraded mainly through the proteasomal pathway.

Association of Soluble Tyrosinase with the ER Chaperones—The reduced degradation rate observed during the inhibition of
the N-glycan processing pathway indicates the contribution of the ER quality control in soluble tyrosinase retention. To address the role of the ER quality control chaperones CNX and CRT in the maturation of ST, we have sequentially immunoprecipitated the metabolically labeled transfected B16F1 cell lysates with antibodies against CNX, CRT, and BiP/GRP78. In these experiments, tyrosinase complexes with chaperones were first immunoprecipitated with chaperone antibodies. Subsequently, the soluble and wild type tyrosinases were recovered from their complexes by immunoprecipitation with anti-tyrosinase antibody. Finally, the unbound protein was precipitated from their complexes by immunoprecipitation with anti-tyrosinase antibody. The samples were run in reducing 8% SDS-PAGE and visualized by autoradiography. B, the autoradiographs in panel A were quantified by scanning densitometry. The level of non-degraded protein after 3 h of chase is expressed as the percentage of the amount present at 0 h of chase for every experiment. Values indicate the mean of three independent experiments ± S.D.

FIG. 3. Soluble tyrosinase is an ERAD substrate. A, cells transfected with pTriEx-1.1-STYR were pulse-chased for 0 and 3 h in the absence (Control) and presence of lactacystin (Lac), N-acetyl-leucyl-leucyl-norleucinal (LLN), kifunensin (Kif), bafilomycin (Baf) and nocodazole (Noc), lysed, and immunoprecipitated with anti-tyrosinase antibody. The samples were run in reducing 8% SDS-PAGE and visualized by autoradiography. B, the autoradiographs in panel A were quantified by scanning densitometry. The level of non-degraded protein after 3 h of chase is expressed as the percentage of the amount present at 0 h of chase for every experiment. Values indicate the mean of three independent experiments ± S.D.

and in the control MEF crt<sup>−/−</sup> cells. Western blot analysis of the transfected MEF crt<sup>−/−</sup> cell lysates revealed that a significant population of the soluble tyrosinase pool was secreted from the ER, as shown by its resistance to EndoH treatment (Fig. 5A, res). In contrast, secreted soluble tyrosinase is almost absent in the calreticulin-positive cells (Fig. 5A), as seen in the other cell lines tested by us (Fig. 1B). These data confirm that CRT is one of the chaperones that retain soluble tyrosinase in the ER and also suggest that, in the absence of CRT, soluble tyrosinase association with CNX is still reduced.

To find out whether a similar ER export is shown by membrane tyrosinase, we have expressed it in both MEF cells. As seen in Fig. 5A, more complex forms than those found in the parental cells were seen in MEF crt<sup>−/−</sup>, indicating that membrane tyrosinase is secreted better in the absence of CRT. We should note, however, that the two tyrosinases have similar molecular masses in the calreticulin-deficient cells, which may be explained by an abnormal glycosylation of the membrane tyrosinase in these cells. Although the mechanisms are not completely understood, we propose that CRT retains soluble tyrosinase and, to some extent, wild type tyrosinase in the ER and that, in its absence, the protein is partially secreted.

To further confirm this finding, we have abolished the CRT and CNX interactions in the transfected calreticulin-positive MEF crt<sup>−/−</sup> cell line and analyzed tyrosinase secretion. Following treatment of these cells transfected with ST and WT with NB-DNJ (an inhibitor of the ER glucosidases) (6), we have obtained heterogeneously glycosylated WT and ST populations. After the EndoH digestion, it was concluded that the tyrosinase populations are comprised of a mixture of high mannose and complex glycoforms (Fig. 5B). This variety of glycoforms could be due to the increased activity of the Golgi endomannosidase from MEF parental cells that process high mannose glucosylated structures to complex glycans. As a control, calreticulin-deficient cells transfected with soluble and membrane tyrosinase were run next to the NB-DNJ-treated samples (Fig. 5B). The Golgi tyrosinases (Fig. 5B, res) migrate with similar velocities in NB-DNJ-treated parental MEF cells and in calreticulin-deficient MEF cells, confirming the secretion of tyrosinases in the absence of CNX/CRT interactions.

Because both ST and WT were exported out of the ER, we examined the ER retention capabilities of MEF crt<sup>−/−</sup> cells by analyzing the secretion of two control proteins known to be retained in the ER by different mechanisms. Thus, a tyrosinase mutant lacking the last N-glycan (TYR37) retained by CNX (8) and the envelope protein E2 of HCV retained by its TM domain (22) have been transfected in MEF crt<sup>−/−</sup> cells. Both control proteins were EndoH-sensitive at steady state and therefore retained in the ER, as shown by Western blotting analysis (Fig. 5C). This proves that not all ER retention mechanisms are damaged in MEF crt<sup>−/−</sup> cells, supporting the role of CRT in the ER retention of soluble tyrosinase. Taken together, these results show that, in the absence of CRT, soluble tyrosinase escapes from the ER.

DISCUSSION

The major finding of this study is that the truncated tyrosinase is retained in the ER by a different set of chaperones than the previously reported OCAI mutants. We show that the mutant associates with calreticulin and BiP rather than calnexin. This is in startling contrast with the other albino tyrosinases in which ER export is prevented primarily by their prolonged association with calnexin during ER maturation (8, 9, 15).

That tyrosinase association with calnexin is crucial for its folding and maturation is known from previous reports investigating mouse and human tyrosinase (30, 4). We have shown...
that calnexin interacts with specific glycans, and this interaction is required to prevent the accelerated folding of some regions of the lumenal domain (4). However, a calreticulin role in wild type or albino tyrosinase maturation has been only occasionally investigated (6, 8). Thus, the albino tyrosinases T373K and H402A were reported to be retained in the ER by their prolonged binding to both lectin chaperones (8). In our hands, the membrane-anchored chain was assisted by CNX and CRT starting from the early stages until completion of the folding process. In contrast to wild type, soluble tyrosinase showed a weak interaction with calnexin and a stronger affinity for CRT and BiP/GRP78. The post-translational associations with CRT and BiP/GRP78 are unable to rescue the chain from misfolding but appear to be responsible for its ER retention.

To confirm the role of CRT in ER retention, we have expressed the truncated tyrosinase in cells deficient in CRT. Indeed, we found that, in these cells as well as in NB-DNJ-treated cells, a significant population of soluble tyrosinase was secreted. Membrane tyrosinase was also massively exported in ctn−/− cells as compared with ctn+/+ cells, indicating that CRT retains a population of the membrane-anchored chain in the ER. On the other hand, vesicular stomatitis virus G protein, which does not normally bind CRT, was shown to traffic normally in the CRT-deficient cells (14). Therefore, these experiments indicate that, in the absence of calreticulin, the soluble tyrosinase chain escapes the ER quality control and leaves the ER. These cells have been recently characterized, and apart from a slight elevation in the BiP/GRP78 level, the other ER folding factors are expressed at normal levels (14); hence, these effects can be attributed to the CRT knock-out.

Significantly, CNX is not able to carry out CRT function and retain the chain in the ER. This may be a consequence of an accelerated folding of the soluble tyrosinase resulting in a rapid export out of the ER. Accelerated folding of two viral proteins has been reported in these calreticulin-deficient cells by Molinari et al. (14), confirming a distinct role for CRT versus CNX. Alternatively, CRT may be required in a distal phase during the multiple release-binding steps of the ER cycle, and in its absence, the chain is released from the cycle and secreted.

It is generally accepted that ERAD substrates may select different pathways in eukaryotic cells. These pathways may vary according to the location of the misfolded domain within the lumen, TM, or cytosolic region of the protein (33). Our results show that the truncated tyrosinase mutant is targeted to degradation with a more accelerated rate than the ER-retained form of wild type. To analyze the ERAD pathway of soluble tyrosinase, we have investigated its localization in the presence of inhibitors of the retrograde Golgi-ER trafficking. By immunofluorescence mi-
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with CRT has been observed, further investigations are required to understand this pathway in more detail.

Interestingly, tyrosinase displays a secreted form in OCAI albinism, where a mutation in the P gene induces the truncation and secretion of tyrosinase (18, 36). Based on the ER location of the P gene, Orlow and collaborators (36) have proposed that tyrosinase folding is impaired by the mutant P protein. Accordingly, this partially misfolded protein would be susceptible to protease cleavage in the ER, or as has been proposed, in a post-ER compartment. As shown above, truncation in the gene results in a soluble tyrosinase retained in the ER and degraded without further transport and recycling from the Golgi. The vast majority of this truncated protein could not be secreted into the medium. Therefore, our data confirm Orlow’s hypothesis (36) that, to be secreted, OCAI tyrosinase undergoes cleavage in a post-ER compartment. This also explains why bafilomycin could reverse the P gene defect in OCAI albinism (18). However, bafilomycin had no effect on the soluble tyrosinase mutant. Therefore, although truncated tyrosinas appear to be the cause for some OCAI albino patients and also for most OCAI patients, a potential treatment has to take into account the different intracellular trafficking pathways of the two albino tyrosinases. Thus, bafilomycin or any other inhibitors that dissipate the pH gradient within the secretory pathway cannot be used as drugs in the case of OCAI albino-truncated tyrosinase. In this form of albinism, what is needed are drugs that induce protein folding at the ER level.

Finally, the ER retention of the soluble form of tyrosinase raises interesting questions related to the role of the TM domain in the folding and maturation of this glycoprotein. Clearly, soluble and wild type tyrosinases have different chaperone interactions, with the soluble form associating with calreticulin rather than calnexin. Whether the TM domain may play an active role in the selection of the appropriate chaperone during the early events of the tyrosinase folding is currently under investigation.

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Fig. 5. Secretion of soluble tyrosinase in calreticulin-deficient cells. A, lysates of crt<sup>+/+</sup> and crt<sup>−/−</sup> cells transduced with pTriEx-1.1-Tyr and pTriEx-1.1-STYR were EndoH-digested and immunoblotted with anti-tyrosinase antibody. Shown is one of at least three representative experiments. res, the EndoH-resistant secreted ST; sens, the high mannose ER-retained ST. B, same as described for A, except that crt<sup>−/−</sup> cells were grown in the presence of 5 mM NB-DNJ. C, as controls, the pTriEx1.1-HCVE2 (coding for the E2 protein of HCV) and the pTriEx1.1-TYR<sup>−/−</sup> (human tyrosinase mutant lacking the last N-glycosylation sequon) constructs were transfected in crt<sup>−/−</sup> cells and analyzed as in A. TYR<sup>−/−</sup> was detected with anti-tyrosinase antibody and E2 with A-11 antibodies.
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