Sorting of the Specific Granule Protein, NGAL, During Granulocytic Maturation of HL-60 Cells

By Véronique Le Cabec, Jero Calafat, and Niels Borregaard

The different types of human neutrophil granules (azurophil, specific, and gelatinase granules) are formed sequentially during maturation of neutrophils from the promyelocyte stage to the band cell stage. The promyelocytic HL-60 cells can mature to segmented granulocytes but are incapable of activating the transcription of any known intragranular protein, normally located in specific or gelatinase granules. To study the sorting of granule proteins during maturation, we transfected HL-60 cells with the specific granule protein NGAL, inserted under control of a cytomegalovirus promoter. We previously showed that NGAL is sorted to azurophil granules and colocalizes with myeloperoxidase in undifferentiated HL-60 cells. We show here that, when such transfected HL-60 cells differentiate into granulocytes, newly synthesized NGAL is not retained in granules but is constitutively secreted. This indicates that highly specific mechanisms must exist that are responsible for diverting transport vesicles into storage granules, and that HL-60 cells not only lack the ability to activate transcription of specific granule proteins, but also lose the ability to form storage granules during maturation.

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and undifferentiated cells were analyzed in parallel for nuclear morphology
conjugated rabbit antimouse antibody (DAKO F313) were purchased
(DAKO A072), rabbit Ig fraction (DAKO X903) and fluorescent-
(MoAb; DAKO M741), rabbit anti-MPO antibody (DAKO A398), anti-CD11b monoclonal antibody
Becton Dickinson (San Francisco, CA).

as previously described,13 were expanded in culture in parallel with
ten NGAL under control of the constitutively active CMV promoter,
subclones, transfected with the cDNA encoding for the specific pro-
American Type Culture Collection (Rockville, MD). HL-60 cell
was used at a final concentration of 1 mmol/L. RA was prepared as a 10-mmol/L stock solution in DMSO and
PMA was dissolved in DMSO at a concentration of 1 mg/mL and
was from Aldrich Chemical Company Inc. (Steinheim, Germany)
Pharmacia (Uppsala, Sweden). Diisopropyl fluorophosphate (DFP)
from Amersham Corp (Arlington Heights, IL). Protein A-Sepharose
subsequently present in the supernatant was used as a crude preparation.

**Table 1. Maturation of HL-60 Cells**

<table>
<thead>
<tr>
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<th>Undifferentiated HL-60 Cells</th>
<th>Differentiated HL-60 Cells</th>
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<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td>PM, 98%; M + MM, 2%; B + S, 0%</td>
<td>PM, 28%; M + MM, 56%; B + S, 16%</td>
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<tr>
<td><strong>NBT-test</strong></td>
<td>5.7 ± 2.2 (n = 12)</td>
<td>70.5 ± 14.6 (n = 16)</td>
</tr>
<tr>
<td><strong>CD11b (MFI)</strong></td>
<td>3.8 ± 4.5 (n = 12)</td>
<td>251 ± 128 (n = 12)</td>
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Differentiated cells (DMSO [1.3%] and 1 μmol/L RA for 3 to 4 days) and undifferentiated cells were analyzed in parallel for nuclear morphology in cytospin preparations and classified as promyelocytes (PM), myelocytes and metamyelocytes (M + MM), and band and segmented cells (B + S). Results are expressed as the mean percentage of cells showing the corresponding morphological features in four independent experiments. The ability to mount a respiratory burst was assessed by NBT reduction and given as percentage of cells containing blue intracellular formazan deposits after 30 minutes of stimulation by 0.2 μg/mL PMA at 37°C. Cell-surface expression of CD11b was determined by FACS analysis and expressed as MFI. The differentiation of wild-type and transfected HL-60 cells was identical, and results from both cell types were combined in the statistical analysis of the data. Values are mean ± SD of the number of experiments shown in parentheses.

**MATERIALS AND METHODS**

**Materials.** RPMI-1640 culture medium, methionine-deficient medium, fetal calf serum (FCS), L-glutamine, streptomycin, and penicillin were purchased from Gibco-BRL (Gaithersburg, MD). RA (all-trans), NBT, and phorbol myristate acetate (PMA) were from Sigma (St Louis, MO). DMSO was purchased from Merck (Darmstadt, Germany). L-[^{35}S]-methionine (1,175 Ci/mmol) was from New England Nuclear-DuPont (Boston, MA), and Amplify was from Amersham Corp (Arlington Heights, IL). Protein A-Sepharose CL-4B and CNBr-activated Sepharose 4B were purchased from Pharmacia (Uppsala, Sweden). Disopropyl fluorophosphate (DFP) was from Aldrich Chemical Company Inc. (Steinheim, Germany) PMA was dissolved in DMSO at a concentration of 1 mg/mL, and was used to stimulate the cells at a final concentration of 200 ng/mL. RA was prepared as a 10-mmol/L stock solution in DMSO and was used at a final concentration of 1 μmol/L in culture. Rabbit anti-MPO antibody (DAKO A398), anti-CD11b monoclonal antibody (MoAb: DAKO M741), rabbit anti-β2-microglobulin antibody (DAKO A072), rabbit Ig fraction (DAKO X903) and fluorescent-conjugated rabbit antihuman antibody (DAKO F313) were purchased from DAKO (Glostrup, Denmark). Purified mouse IgG, was from Becton Dickinson (San Francisco, CA).

**Cells and culture conditions.** HL-60 cells were purchased from American Type Culture Collection (Rockville, MD). HL-60 cell subclones, transfected with the cDNA encoding for the specific protein NGAL under control of the constitutively active CMV promoter, as previously described,17 were expanded in culture in parallel with wild-type HL-60 cells. Three different clones, referred to as clones A, B, and C, respectively, as well as wild-type HL-60 cells were used in the experiments. The cells were maintained in RPMI-1640 medium supplemented with 10% (vol/vol) heat-inactivated FCS, 2 mmol/L L-glutamine, 100 U/mL penicillin/streptomycin (complete medium), and 1 mg/mL Geneticin (Gibco-BRL), except for wild-type HL-60 cells, in a humidified atmosphere of air:CO₂ (19:1). Cell cultures were passaged 3 times weekly to maintain a cell density between 2 × 10⁶ and 6 × 10⁶ cells/mL. 

**Induction of differentiation.** Exponentially growing HL-60 cells were harvested by centrifugation and resuspended at a density of 5 × 10⁶ cells/mL in complete RPMI culture medium supplemented with 1.3% (vol/vol) DMSO and 1 μmol/L RA for 3 to 4 days. The medium was renewed after 2 days of differentiation. The combined treatment of HL-60 cells with RA and another polar solvent, N,N-dimethylformamide, has previously been shown to result in maximal granulocytic differentiation.18 Viability was assessed by the ability of the cells to exclude 0.1% trypan-blue dye.

**Assessment of cell differentiation.** The extent of differentiation/maturation was assessed by monitoring growth arrest, morphological changes, and capacity for superoxide generation as assessed by the NBT test and by quantitation of a differentiation marker (CD11b antigen) by FACScan (Becton Dickinson, Mountain View, CA) analysis.

Growth of HL-60 cells was assessed by counting the number of trypan-blue–negative cells by light microscopy. The differentiation response was also apparent on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein profiles (Coomassie blue) of undifferentiated versus the differentiated HL-60 cells as previously reported (Meyer and Howard11 and data not shown). For the morphological assessment of the cells, 10⁵ cells were harvested, centrifuged, and resuspended in 0.1 mL of phosphate-buffered saline (PBS). Slides were prepared by centrifugation of the cells in a cyto-centrifuge, stained with May-Grünwald-Giemsa stain, and ex-
Fig 2. Biosynthesis and sorting of NGAL in undifferentiated and differentiated transfected HL-60 cells. Undifferentiated (A) and differentiated (B) HL-60 cells transfected with NGAL cDNA under control of a CMV promoter were pulse-labeled with [35S]methionine for 3 hours followed by chase for up to 8 hours. At indicated points, 2 × 10⁶ cells were withdrawn, pelleted by centrifugation, and solubilized. Immunoprecipitation with anti-NGAL antibody was performed on the cell lysates (Cells) and the corresponding culture supernatant (Medium). In addition, NGAL was immunoprecipitated from the supernatant obtained after the 3-hour pulse (S1). The immunoprecipitates (10⁶ cell equivalents) were subjected to a 10% SDS-PAGE and visualized by fluorography. The fluorograms were exposed for 24 hours (A) and 7 days (B). The position of NGAL is indicated with arrows on the right. Molecular weight markers are shown on the left. The results of one experiment, representative of four, are shown. The lower panel of the figure shows the quantification of the radioactivity in NGAL extracted from the gels. Lines are results from cells; hatched bars are from the corresponding culture media; and filled bars are S1, medium from pulse. Results are expressed as the percentage of total labeled NGAL present at the start of chase. Error bars represent the SD of four independent experiments.

NBT dye reduction. Differentiation was confirmed by counting cells capable of reducing NBT in response to PMA stimulation. A total of 1 × 10⁶ cells were harvested by centrifugation and resuspended in 0.1 mL serum-free RPMI-1640 without phenol red, containing 0.1% NBT and 200 ng/mL PMA in a 96-well U-bottomed microtiter plate. The reaction mixture was incubated for 30 minutes at 37°C in an humidified atmosphere containing 5% CO₂. The reaction was stopped by placing the plate on ice. After centrifugation, the cells were resuspended in 0.1 mL of PBS, transferred onto slides by cytopsin, fixed in methanol, and stained with safranine as previously described. Two hundred cells were examined by microscopy for the presence of blue formazan deposits, and the number of positive cells was expressed as percentage of total number of cells.

Flow cytometry. Quantitation of CD11b antigen expression was performed using an FACScan flow cytometer (Becton Dickinson) as previously described. Briefly, cells at 10⁷/mL (50 μL) in PBS were mixed with 50 μL primary MoAb at saturating concentration (5 μg/mL anti-CD11b or 1 μg/mL control mouse IgG1) in microtiter plates and were incubated for 30 minutes at 4°C. The cells were then washed twice, suspended in 50 μL buffer containing 30 μg/mL fluorescein-conjugated rabbit antimouse antibody. After incubation for 30 minutes at 4°C, the cells were washed 3 times, fixed in PBS supplemented with 1% formaldehyde, and diluted 5 times in PBS with 1% formaldehyde just before immunofluorescence flow cytometry analysis. At least 5,000 cells were analyzed for each conditions. All washes were performed in PBS supplemented with 0.5% bovine serum albumin at 4°C by centrifugation of microtiter plates at 200g for 5 minutes. Negative controls were obtained using antibodies without relevant specificity as primary antibody. Data are expressed as mean fluorescence intensity (MFI). Specific fluorescence was obtained by subtracting the background MFI obtained by labeling with irrelevant antibody.

Biosynthetic labeling, chase experiments, and immunoprecipitation. Biosynthetic labeling of NGAL-transfected HL-60 cells was performed as previously described. Cells suspended at 2.5 × 10⁶ cells/mL in methionine-free medium containing 10% dialyzed, heat-
inactivated FCS were incubated for 60 minutes at 37°C, in a humidified atmosphere of air:CO₂ (19:1). The cells were then pulsed for 3 hours by the addition of 50 μCi/mL of [³⁵S]-methionine. In some experiments, cells were incubated with 25 mmol/L DFP during the last 30 minutes of the pulse-labeling. A total of 100 μL of cells was withdrawn and applied to a Whatman filter disk (Whatman, Maidstone, UK) for the determination of the total amount of radioactivity incorporated in Trichloroacetic acid (TCA)-precipitable proteins. The pulse was stopped by pelleting the cells. The supernatant was kept on ice until immunoprecipitation was performed. The cell pellet was washed once, and biosynthetically labeled proteins were chased by resuspending the cells at 10⁶ cells/mL in complete medium and withdrawing 2 mL for immunoprecipitation at timed intervals. Cells in the samples were pelleted and solubilized in radioimmunoprecipitation assay buffer as described. Immunoprecipitations were performed in parallel on the cell lysates and on the corresponding volumes of culture medium, sequentially with affinity-purified rabbit anti-NGAL antibodies (2.5 μg/mL) followed by protein A-Sepharose (4 mg/mL), affinity-purified rabbit anti-MPO, and anti-α₂-microglobulin antibodies coupled to CNBr-activated Sepharose 4B (25 μg/mL). The immunoprecipitates were washed, resuspended in 4 μL of Laemmli SDS-sample buffer, boiled, and centrifuged. Twenty microliters of the supernatant was analyzed by electrophoresis.

**Fig 3.** Biosynthesis and sorting of NGAL in undifferentiated and differentiated transfected HL-60 cells after DFP treatment. Undifferentiated (A) and differentiated (B) transfected HL-60 cells were pulse-labeled with [³⁵S]-methionine as described in the legend of Fig 2, and DFP (final concentration, 25 mmol/L) was added 30 minutes before the end of the pulse. At indicated points, 2 × 10⁶ cells were withdrawn and NGAL was immunoprecipitated from the cell lysates (see legend of Fig 2). The fluorograms were exposed for 48 (A) and 96 hours (B). The results of one experiment representative of two are shown.

**Fig 4.** Quantification of NGAL and MPO in cells and culture medium by ELISA. NGAL and MPO in undifferentiated (ND) and differentiated (D + RA) cells and their corresponding media were assayed by ELISA. Release was calculated as the amount of the proteins in medium, given as the percentage of the total amount in medium plus cells. Mean ± SD is given. The secretion of MPO from undifferentiated cells was not significantly different from the secretion of MPO from differentiated cells (difference, 0.9% ± 8.8% [n = 18]), whereas secretion of NGAL from differentiated cells was significantly higher than that from undifferentiated cells (difference, 41% ± 8% [n = 13]).
RESULTS

Differentiation/maturation of transfected HL-60 cells. Differentiation into the granulocyte lineage and maturation of HL-60 was achieved by combined DMSO (1.3% vol/vol) and RA (1 μmol/L) treatment for 3 to 4 days and was assessed by changes in nuclear morphology, ability to mount a respiratory burst, acquisition of CD11b, and downregulation of MPO synthesis.

After induction, most cells matured to myelocytes or beyond (metamyelocytes, band and segmented), as observed by morphological inspection of May-Grünwald-Giemsa-stained cytospin preparations, and acquired the ability to mount a respiratory burst, as evidenced by PMA-induced reduction of NBT (Table 1). As shown in Table 1, the MFI of cells labeled with an MoAb against CD11b increased from 3.8 to 251 as further proof of maturation of the HL-60 cells. The biosynthesis of an endogenous azurophil granule protein, MPO, and of the plasma membrane HLA-complex was investigated in undifferentiated and differentiated cells. As shown in Fig 1, in agreement with earlier observations, differentiation results in complete shutdown of MPO synthesis (synthesis in differentiated cells 3% ± 1.1% of level in undifferentiated cells; mean of 7 experiments), whereas only a partial reduction was observed in the synthesis of HLA (synthesis in differentiated cells 68% ± 23% of level in undifferentiated cells; mean of 7 experiments).

Sorting of NGAL in undifferentiated HL-60 cells. We have shown before that NGAL localizes to azurophil granules, when synthesized in undifferentiated HL-60 cells, but will be proteolytically degraded. This can be observed in the upper panel of Fig 2A where newly synthesized, cell-associated, NGAL is broken down and eventually disappears. The decrease in amount of NGAL in undifferentiated cells cannot be caused by secretion, because NGAL recovered from the medium was intact throughout the chase period (Fig 2A, middle panel). Furthermore, when cells were treated with 25 mmol/L of the serine protease inhibitor DFP before the chase period, no degradation occurred, and the signal stayed constant during the chase (Fig 3A).

When the fraction of NGAL secreted into medium is measured by ELISA and expressed as the percentage of total NGAL in medium and cells, the degradation of NGAL in cells leads to an overestimation of the release as the percentage of the total. The figure for NGAL secretion to medium
in undifferentiated cells as measured by ELISA is 41% (Fig 4). When the sorting of newly synthesized NGAL is evaluated, the effect of degradation is partly corrected by expressing the amount of biosynthetically labeled NGAL present in medium by the end of the chase as the percentage of total labeled NGAL present at the start of chase (Fig 2A lower panel). This figure amounts to 35% ± 5% (mean ± SD; n = 4) of NGAL being secreted, thus arguing for an effective sorting of NGAL to azurophil granules in undifferentiated cells that is almost as effective as the sorting of MPO, of which 12% was found in the medium (Fig 4).

**NGAL and MPO in undifferentiated and differentiated HL-60 cells.** When the HL-60 cells differentiate, they reduce their content of MPO from 1.24 μg/10^6 cells to 0.57 μg/10^6 cells, as observed from Fig 4. This can be explained by one round of cell division after the stop of biosynthesis. In contrast, the cellular content of NGAL is reduced from 33.6 ng/10^6 cells in undifferentiated cells to 4.1 ng/10^6 cells in differentiated cells (of which some are still undifferentiated; see Table 1). We have shown that NGAL and MPO localize in the same granules in undifferentiated cells. Consequently, the loss of previously synthesized NGAL that occurs during differentiation cannot be caused by exocytosis of azurophil granules, because this would affect MPO to the same extent, but must be a result of degradation. This is clearly shown in Fig 5, which shows that undifferentiated cells stain well for both MPO and NGAL, whereas differentiated cells have mainly lost their staining for NGAL but retain the staining for MPO.

**Sorting of NGAL in differentiated HL-60 cells.** The biosynthetic pulse-chase experiment on NGAL in differentiated cells (Fig 2B) shows that newly synthesized NGAL is not retained in the differentiated cells. Furthermore, in contrast to undifferentiated cells, in which NGAL was broken down in the cells, there is no sign of degradation of newly synthesized NGAL in differentiated cells. When the fraction of newly synthesized NGAL, accumulating in medium during the 8 hours of chase is expressed as the percentage of total synthesized NGAL, the figure amounts to 77% ± 8% (mean, n = 4; Fig 2B). When quantitated as the total amount of NGAL by ELISA, 82% of NGAL is secreted in medium (Fig 4).

We addressed the possibility that the decrease of intracellular NGAL in differentiated cells could be caused by an increased intragranular degradation potency activated by differentiation and, in contrast to undifferentiated cells, leaving no detectable proteolysis intermediates on gels. Therefore, pulse-chase experiments were performed on differentiated cells treated with DFP under the same conditions as were shown to inhibit the degradation of NGAL in undifferentiated HL-60 (Fig 3A). In contrast to undifferentiated HL-60 cells, 25 mmol/L DFP had no effect on the decrease in intracellular NGAL in differentiated HL-60 cells (Fig 3B), thus strongly indicating that disappearance of newly synthesized NGAL from differentiated cells is due to secretion. This finding is also confirmed by the paucity of NGAL in differentiated cells as visualized by immunostaining (Fig 5) and by electron microscopy, where NGAL staining was observed only by 4 to 5 gold particles per cell section, localized to endoplasmic reticulum and to the Golgi apparatus (Fig 6A). Exceptionally, one NGAL gold particle was observed in a granule.

Therefore, it is concluded that, when a specific granule protein, in casu NGAL, is artificially transcribed in HL-60 cells that have been differentiated along the granulocytic lineage and are unable to transcribe endogenous specific granule proteins, the transfected specific granule protein cannot be retained in granules but is secreted into the medium. This is in contrast to undifferentiated cells, which effectively retain the specific granule protein. This indicates that these cells, in addition to being unable to transcribe endogenous specific granule proteins, also lose the ability to form storage granules as they differentiate.

Finally, to further address this conclusion, we examined differentiated HL-60 cells for the localization of a membrane protein that, in normal neutrophils, is associated with the membrane of peroxidase-negative granules. This was accomplished by immunoelectron microscopy using an antibody that recognizes the flavocytochrome b_558_. As expected, no labeling was observed in undifferentiated HL-60 cells, but abundant labeling was present on the cell surface of differentiated (NGAL-transfected) HL-60 cells (Fig 6B). In addition to the major labeling of the cell surface, some labeling was observed in vesicles localized underneath the cell membrane. Labeling was also always observed over Golgi stacks but was never observed over granules.

**DISCUSSION**

Introducing the cDNA of NGAL, a specific granule protein, into HL-60 cells has allowed us to study the sorting of NGAL in relation to the maturation of the promyelocytic HL-60 cells. In their undifferentiated form, HL-60 cells are structurally similar to their normal counterpart, the myeloblast/early promyelocyte; however, the maturation of HL-60 cells into granulocytes is characterized by a significant departure from the route taken by normal granulocyte precursors with regards to formation of peroxidase-negative granules. Transcription of genes for proteins located inside peroxidase-negative granules, which normally takes place at the myelocyte, metamyelocyte, and band cell stages, does not occur in HL-60 cells.

A central question is whether these cells, in addition to lacking the ability to activate the synthesis of peroxidase-negative granule proteins, also lose the ability to form storage granules or whether storage granules can be detected if synthesis of a protein, normally located inside these granules is induced artificially by transfecting the corresponding cDNA, which is transcriptionally controlled by a constitutively active promoter. It is known that transfection of cells with von Willebrand factor can induce formation of caveolae. The structure that holds this protein in endothelial cells, and that transfection of lymphocytes with caveolin induces formation of caveolae. The results presented here show that induction of NGAL synthesis in HL-60 cells does not lead to formation of peroxidase-negative granules.

We have previously shown that NGAL becomes targeted to azurophil granules and colocalizes with MPO in undifferentiated HL-60 cells, thus supporting our hypothesis that all granule proteins synthesized at the same time will localize...
NGAL IN DIFFERENTIATED HL-60 CELLS

Fig 6. Immunoelectron microscopy of differentiated transfected HL-60 cells. (A) Labeling of NGAL with rabbit antibody followed by 10 nm gold attached to goat antirabbit IgG. Staining is observed on Golgi stacks (G) and trans-Golgi network (TGN). (B) Labeling of cytochrome b₅₅₈ with MoAb followed by rabbit antimouse IgG and 10 nm gold attached to goat antirabbit IgG. Extensive labeling can be observed on the plasma membrane (large arrows) and on some vesicles underneath the cell surface (small arrows). No label is observed in the granules (g); nucleus (n). Bars, 200 nm.

to the same type of granules. It is apparent that the ability to divert NGAL from the "default" route of constitutive secretion into storage granules is lost when HL-60 cells mature into myelocytes and metamyelocytes. Thus, the noted lack of peroxidase-negative granules in HL-60 cells cannot be explained solely by the lack of content but is also caused by the loss of the ability to form granules. This loss of ability to form granules clearly does not affect the ability to form transport vesicles capable of transporting newly synthesized protein out of the cell.

It was shown that cytochrome b₅₅₈, which in the normal neutrophil is confined largely to the membrane of peroxidase-negative granules, was localized almost exclusively to the plasma membrane of the differentiated HL-60 cells, in addition to being localized to structures involved in synthesis and routing of proteins. This further testifies to the inability of differentiated HL-60 cells to form granules and argues that, when the route to storage granules is blocked, the default route goes to the surface of the cell, resulting in secretion of soluble proteins (NGAL) and incorporation of membrane proteins into the plasma membrane (cytochrome b₅₅₈).

It is generally assumed that storage granules form by aggregation of smaller immature granules that bud off from the trans-Golgi network. It can be deduced that such immature granules are fundamentally different from the transport vesicles that mediate constitutive secretion of protein, because the immature granules have a tendency for spontaneous homotypic fusion but need a signal for fusion with the plasma membrane (a feature that characterizes them as regulated...
storage granules), whereas transport vesicles lack the ability for homotypic fusion but fuse spontaneously with the plasma membrane. The biochemical basis for this fundamentally different behavior of vesicles budding off the Golgi is unknown, but our results show that the biochemical basis for forming storage granules is lost in HL-60 cells, as these mature beyond the promyelocyte stage.

Another important observation that can be deduced from our results is that degradation of proteins occurs in azurophil granules or their precursors. It has been known for a long time that many of the proteins located in azurophil granules are processed from proforms to their mature form after sorting to storage granules in the trans-Golgi network. This has been shown for MPO,17-38 defensins,40 and cathepsin G.41 This processing is important for functional maturation of the proteins. When NGAL becomes localized to azurophil granules, it is clearly broken down, as previously shown by pulse-chase labeling42 and as evidenced by the disappearance of NGAL staining from granules during maturation of HL-60 cells. This degradation is most likely the result of a serine protease, because that degradation can be blocked by DFP. In contrast, DFP has no effect on the disappearance of newly synthesized NGAL that is formed in more mature HL-60 cells that have stopped forming azurophil granules (as evidenced by the lack of MPO biosynthesis). NGAL in these cells is also recovered intact in the medium, because NGAL is clearly broken down, as previously shown by pulse-chase labeling42 and as evidenced by the disappearance of NGAL staining from granules during maturation of HL-60 cells. This degradation is most likely the result of a serine protease, because that degradation can be blocked by DFP.

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A key question exposed by our results but left to be answered is why do HL-60 cells lose the ability to make storage granules as these cells mature? Because formation of storage granules is dependent on protein synthesis as opposed to formation of transport vesicles mediating constitutive secretion,43 it is possible that HL-60 cells lose the ability to form storage granules because formation of proteins essential for formation of storage granules is downregulated along with the downregulation of azurophil granule protein synthesis.

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