Early events in erythroid differentiation: accumulation of the acidic peroxidoxin (PRP/TSA/NKEF-B)

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The acidic peroxidoxin [also named thiol-specific antioxidant protein (TSA) or protector protein (PRP)], which plays a role in the response against oxidative stress, is one of the major proteins of red blood cells. In this work, we show that this protein is induced at early stages of erythroid differentiation prior to haemoglobin accumulation, which suggests that it may play a role at the erythroblast stage, where haemoglobinized,

nucleated and genetically active cells are submitted to a maximally dangerous oxidative stress. The early accumulation of this protein has been demonstrated both on transformed cell systems and on normal differentiating human erythroid cells. This suggests that this protein may play an important role in the differentiation of the erythroid cells.

INTRODUCTION

Organisms living under aerobic conditions require mechanisms preventing, or at least limiting, cellular damage caused by reactive oxygen species ($O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$) arising from the incomplete reduction of oxygen during respiration or exposure to external agents such as light, ionizing radiations or some redox drugs [1,2]. These reactive oxygen species can damage the components of the living cells, leading to lipid peroxidation, protein modification and inactivation, and DNA base modification and strand breaks. To counter these destructive processes, cells have evolved protective systems, which either repair the damage (e.g. DNA repair enzymes) or act to prevent the damage by destroying the reactive oxygen species. Such systems include the superoxide dismutases, which destroy the superoxide anion and produce oxygen and peroxide, and catalases or peroxidases, which destroy the peroxide.

Another protein implicated in the protection against oxygen- or sulphur-derived reactive species was first described in the yeast *Saccharomyces cerevisiae* [3]. This protein [named TSA (thiol-specific antioxidant protein), or PRP for protector protein] is induced when yeast cultures are submitted to oxidative stress. Under aerobic conditions, and especially under oxidative stress conditions, yeast strains lacking PRP show a significantly reduced growth rate compared with a wild-type strain, indicating that PRP has a physiologically important role. In fact, it has been recently shown that PRP is able to destroy both peroxide and $OH^{\cdot}$ radicals [4]. $OH^{\cdot}$ destruction was shown by the ability of PRP to prevent $OH^{\cdot}$ -mediated DNA cleavage, while other proteins, even those destroying peroxide such as catalase, are not able to protect DNA from cleavage [4]. This protection also takes place for proteins and lipids [5]. PRP seems to destroy

reactive oxygen species using a reactive cysteine [4,6], which is regenerated in its active form either with a thiol [4], such as reduced glutathione, or with an enzymic system [7].

PRP is a member of a family of proteins (peroxidoxins) defined by a significant amino acid conservation from prokaryotes to higher eukaryotes [8]. To date, in addition to PRP, this family includes prokaryotic alkylperoxide reductases [9], a mitochondrial protein induced during the differentiation of Friend erythroleukaemia cells [10] also known as SP22 [11], a protein induced by oxidative stress in the macrophage [12], a protein overexpressed in fibroblasts upon transformation by the *ras* oncogene [13], and two proteins showing a natural killer cell-enhancing action *in vitro* and named NKEF-A and -B [14,15]. The *nkef-a* and *pag* genes are identical (M. T. Prosperi and G. Goubin, unpublished work), and NKEF-B and PRP are variants of the same protein.

Among the cell types present in higher organisms, erythroid cells are probably one of the cell types which are most prominently exposed to oxidative stress. This arises from the fact that these cells are the vehicle for oxygen in the body via its binding to haemoglobin, and therefore handle large amounts of oxygen. Moreover, haemoglobin may act as a Fenton reagent, which further increases the oxidative stress by catalysing the production of $OH^{\cdot}$ radicals [16]. This $OH^{\cdot}$ production may be due, in fact, to iron released from haemoglobin [17,18]. Iron release seems to be catalysed both by oxidants such as peroxides and by reducers such as glutathione [19].

It is noteworthy that the main enzymes protecting against oxidative stress, namely Cu,Zn-superoxide dismutase and catalase, are present at high levels in erythrocytes. Recent work has demonstrated that PRP or its analogues are also present at high levels in mature erythrocytes [5,14].

Abbreviations used: BFU-E, erythroid burst-forming units; CFU-E, erythroid colony-forming units; HMBA, hexamethylene bisacetamide; IL, interleukin; IMDM, Iscove modified Dulbecco's medium; IPG, immobilized pH gradient; PAG, proliferation-associated gene; PMA, phorbol myristate acetate; PRP, protector protein; PVDF, polyvinylidene difluoride; SCF, stem cell factor.

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We have undertaken a study aimed at defining early markers of erythroid differentiation, using high-resolution two-dimensional electrophoresis as the screening tool. Markers were defined as proteins whose level increased in the early phases of induced erythroid differentiation in various *in vitro* model systems. During the course of this study, we have shown that levels of acidic peroxidoxin significantly higher than in other blood cell types are reached in early steps of differentiation in cells of the erythroid lineage, prior to haemoglobin accumulation.

MATERIALS AND METHODS

Cell culture

Cell lines

Friend murine erythroleukaemia cells (line F4NW0) were cultured in minimal essential medium supplemented with 10 mM Hepes buffer, non-essential amino acids and 10 % (v/v) fetal-calf serum. Cells were seeded at 10^4 cells/ml and harvested at $(2-5) \times 10^5$ cells/ml.

Cell differentiation was induced with 4 mM hexamethylene bisacetamide (HMBA) for various times up to 5 days, with one medium change after 3 days.

HEL cells were cultured in RPMI 1640 medium supplemented with 10 % (v/v) fetal-calf serum, seeded at $(2-5) \times 10^4$ cells/ml and harvested at $(2-5) \times 10^5$ cells/ml.

The cells were induced to differentiate either with 0.1 mM haemin (predissolved at 4 mM in 1 M NaOH) or with 0.15 μ M phorbol myristate acetate (PMA) in the culture medium.

In both cases, the extent of erythroid differentiation was measured by the haem-induced benzidine reaction. Briefly, the cells were harvested, washed and resuspended in PBS. For each volume of cell suspension, an equal volume of a solution containing 2 g/l benzidine tetrahydrochloride and 1 % (v/v) of 30 % H_2O_2 was added. After 10 min, the cells were counted and the proportion of blue cells (haem-containing) was recorded.

The other blood cell lines (obtained from the American Type Culture Collection, except for the BASC6 line which was a gift from Jacklyn Pierce, NIH, Bethesda, U.S.A.) were cultured in RPMI 1640 medium or Dulbecco's modified Eagle's medium (DMEM) supplemented with 10 % (v/v) fetal-calf serum.

Normal cells

Normal human peripheral blood cells collected from cytophereses were first separated on a Ficoll Hypaque column to eliminate red blood cells and granulocytes. The floating cells were then cultured, as described in [20], to induce erythroid differentiation. Briefly, the cells were first plated overnight in Iscove modified Dulbecco's medium (IMDM) supplemented with 5 % (v/v) fetal-calf serum to remove monocytes. The non-adherent cells were collected and seeded at 5×10^6 cells/ml in IMDM supplemented with 10 % (v/v) fetal-calf serum, 1 μ g/ml cyclosporin A, 2 ng/ml interleukin (IL)-3 and 20 ng/ml stem cell factor (SCF). Cells were cultured for 5 days in this medium. The non-adherent cells were harvested, washed and reseeded in the same volume of IMDM supplemented with 30 % (v/v) fetal-calf serum, 1 μ g/ml cyclosporin A, 1×10^{-6} M dexamethasone and 1 unit/ml erythropoietin. The cells were cultured for 4 days in this medium. The non-adherent cells were collected, centrifuged and the medium was saved. The cell pellet was suspended in a small volume of culture medium and layered on to a cushion of 45 % (w/v) Percoll in PBS ($d = 1.0585$) and centrifuged at 2000 g for 20 min. The upper layer was collected and diluted in PBS to recover the cells by centrifugation. At this stage, examination of the cell

Table 1 Composition of erythropoietin-induced cultures

Results given as percentages. Abbreviation: ND, not determined.

Cell type	Day 4 ('proerythroblasts')	Day 7 ('erythroblasts')
Megakaryocytes	1	ND
Lymphocytes	12.5	6
Monocytes + macrophages	3	3
Proerythroblasts	76	2.5
Total erythroblasts	7	88.5
Basophilic erythroblasts	ND	13
Polychromatophilic erythroblasts	ND	19.5
Orthochromatic erythroblasts	ND	56

population by May-Grunwald-Giemsa staining gave the composition described in Table 1 (column 1). These cells were either collected for analysis or returned to the conditioned erythropoietin-containing medium for culture for an additional 3 days. After this time, the cell population had the composition described in Table 1 (column 2). The cells were then collected for subsequent analysis.

The peripheral blood stem cells are composed almost exclusively of erythroid burst-forming units (BFU-E) with very few granulocyte/macrophage colony-forming units (CFU-GM) and virtually no erythroid colony-forming units (CFU-E). Hence, by using appropriate culture conditions [20], the ordered erythropoietic differentiation steps, starting from the BFU-E through the CFU-E, proerythroblasts and ultimate erythroid maturation, may be obtained in 12 days by a two-step culture sequence. In the first culture, the BFU-E are induced to proliferate and mature into CFU-E by IL-3 and SCF, while cyclosporin prevents the expansion of the lymphocytes and induces them to degenerate slowly. In the secondary culture, CFU-E are induced by erythropoietin to differentiate first into large proerythroblasts (4 days); these can be highly enriched by a Percoll separation step that removes most of the remaining lymphocytes (these still represent more than 75 % of the cells prior to the Percoll separation). The additional 3 days of culture of the proerythroblast-enriched fraction in the presence of erythropoietin allows the final erythroid differentiation through basophilic, polychromatophilic and acidophilic stages, the latter being post-mitotic cells.

The non-erythroid cells from the same donors were collected from a small aliquot of the Ficoll Hypaque-separated cells. Monocytes and macrophages were removed by adherence to plastic, and the lymphocytes were recovered in the supernatant. At this stage, the cells were collected for further analysis.

Two-dimensional electrophoresis

The cell pellets, washed in PBS, were lysed in 9 M urea/3 % CHAPS/20 mM spermine base/40 mM dithiothreitol ($\approx 400 \mu$ l for approx. 50 μ l of cell pellet). After lysis for 1 h at room temperature, nucleic acids were eliminated by ultracentrifugation (200000 g for 1 h at 20 °C in a Kontron TFT 80.4 rotor). The concentration of protein was determined by a modified dye-binding assay [21], using BSA dissolved in lysis buffer as a standard.

Two-dimensional electrophoresis was performed as described in [22]. Isoelectric focusing was performed using immobilized pH gradients (IPGs) of pH 4 to pH 8, the protein being absorbed by the IPG strip during the rehydration step [22]. After focusing for approx. 50000 Vh the strips were equilibrated and transferred on to the SDS/polyacrylamide gel. After the second migration, the

Figure 1 Protein expression in Friend cells, BASC6 pro B cells and mouse erythrocytes

Whole-cell extracts obtained from control cells (a) or from Friend cells induced to differentiate for 48 h with HMBA (b) were analysed by two-dimensional electrophoresis. Some of the spots showing a significant variation are shown with arrowheads. The spot identified as acidic peroxidoxin is shown by an arrow. Whole-cell extracts from BASC6 pro B cells (c) and from normal mouse erythrocytes (d) are also shown. Arrows mark acidic peroxidoxin. Arrowheads: molecular-mass markers (from top to bottom: 200, 116, 97, 68, 46, 30, 21 and 14 kDa). First dimension: immobilized pH gradient, linear from pH 4 (left) to pH 8 (right).

gels were stained with silver according to a previously published protocol [22]. The silver-stained gels were scanned with a Molecular Dynamics Personal Densitometer, and the images analysed with ImageQuant and HERMeS software [23]. A dose-response curve was determined for PRP by analysing serial dilutions of the same extract separated by two-dimensional electrophoresis and silver stained in the same experiment. A linear response with a coefficient of 1.0 was found over the range encountered during the analytical experiments with the different cells and conditions tested. These experiments were carried out in triplicate (starting with different cultures) except for the normal cells (in duplicate). The values of the spots were normalized to p.p.m. of the sum of all the spots present on the gels. These values were found to differ by less than 15 % from one experiment to another.

For micropreparative purposes, 5 to 10 mg of whole-cell extracts from Friend cells were loaded on to the 6-mm-wide IPG

strip. The two-dimensional electrophoresis was carried out as for the analytical gel, but the gel was fixed for 30 min in 30 % ethanol/0.5 % acetic acid, stained for 30 min by 0.2 % Coomassie Blue R in the same solution, and destained in repeated changes of 30 % ethanol. The spots of interest were excised, further destained with 30 % ethanol for 1 h at room temperature, washed twice with 20 ml of 100 mM ammonium hydrogen carbonate in 50 % acetonitrile for 15 min at 30 °C, washed again twice with 20 ml of the cleavage buffer (25 mM Tris/HCl, 1 mM EDTA, 10 % acetonitrile, pH 8.5) and partially dehydrated in a Speed Vac concentrator. The gel slices were then rehydrated with 10 μ l of the endoproteinase Lys-C solution (0.1 μ g/ml). The digestion was carried out for 36 h at 37 °C. Peptides were extracted from the acrylamide matrix in a two-step procedure: (1) with 200 μ l of a 100 mM ammonium hydrogen carbonate solution containing 10 % acetonitrile and 0.1 % hydrogenated Triton X-100; (2) with 200 μ l of a 100 mM ammonium hydrogen carbonate solution

containing 60 % acetonitrile and 0.1 % hydrogenated Triton X-100 (adapted from [24]). Both extracts were pooled and then partially dehydrated in a Speed Vac concentrator. Peptides were separated by reverse-phase microbore HPLC (column VYDAC C4, 2 mm $\times$ 150 mm). The fractions corresponding to the recorded peaks were manually collected, and the peptides present in these peaks were analysed for amino acid sequence. Edman degradation was performed on an automated Applied Biosystems sequenator (477A) connected with an on-line phenylthiohydantoin-amino acid analyser (Model 120A).

For blotting experiments, 1 mg of protein was loaded on to a 4-mm-wide IPG strip. After two-dimensional electrophoresis, the gel was blotted on to a polyvinylidene difluoride (PVDF) membrane in a semi-dry set-up with the aminohexanoic acid discontinuous buffer system [25]. SDS (0.05 %) was added to the cathodic buffer and 30 % ethanol to the anodic buffer. The PVDF blot was rinsed with water and stained overnight with colloidal gold [26]. After three rinses in PBS (5 min each) the blot was saturated for 1 h with polyvinyl pyrrolidone (PVP) and Tween 20 [27], and the zone of interest was excised and probed with the anti-proliferation-associated gene (PAG) antibody (D. Ferbus and G. Goubin, unpublished work) in an indirect immunoassay. Detection was carried out with an enhanced chemiluminescence assay.

RESULTS

Acidic peroxidoxin expression during Friend cell differentiation

The present work is the result of a research project aimed at the identification of proteins whose levels vary significantly during the early stages of the induced differentiation of Friend murine erythroleukaemia cells.

Two-dimensional electrophoresis was carried out to separate the proteins of whole-cell extracts prepared from Friend cells at various stages of the induced differentiation process. Figure 1 shows two-dimensional gels obtained from untreated cells and from cells induced to differentiate with HMBA for 48 h. The spots showing a strong variation were identified by peptide microsequencing. For the spot shown by an arrow on Figure 1, the following peptidic sequences were obtained: EGIAYRGLFII and EYFS.

Screening of databases clearly identified the spot as a member of the peroxidoxins superfamily. The spot was clearly a member of the acidic peroxidoxin subfamily (PRP/TSA/NKEF-B), since peptides identical with those obtained by microsequencing are present in human PRP [28], NKEF-B [15] and rat PRP [8]. This subfamily is named acidic peroxidoxin because of its acidic pI (around 5.3–5.4). This identification was moreover in good agreement with the experimental pI and molecular mass of the spot. The mature MER5 protein has a theoretical pI of 6.2, but does not share exactly the same peptides. The homologous peptides have the following sequences: AGIALRGLFII and EYFE [10]. The basic peroxidoxins (NKEF-A/PAG, MSP23) have theoretical pIs around 8–8.5, and the peptides homologous to those of the spot have the following sequences: EGISFRGLFII and EYFS [8]. Both results (pI and peptide sequence matching) argue almost unambiguously in favour of the identification of the spot as an acidic peroxidoxin.

The spot identified in the Friend cells was also present at high levels in two-dimensional maps of mouse erythrocytes (Figure 1), further confirming its identification as acidic peroxidoxin by analogy with the high amounts of acidic peroxidoxin observed in human erythrocytes [5,14]. Initial evidence for an erythroid specificity was provided by analysis of non-erythroid blood cell

Table 2 Level of acidic peroxidoxin during Friend cell differentiation and in various murine blood cell lines

Cell type	Peroxidoxin expression (in p.p.m. of the sum of the spots detected on the two-dimensional gels)*
Friend cells	
Control	3300
HMBA 16 h	3550
HMBA 48 h	5750
HMBA 120 h	6100
Other murine blood cell lines	
BASC 6 (pro B)	950
MPC 11 (plasmacyte)	480
S49.1 (T lymphocyte)	1500
J 774 (macrophage)	480
Normal erythrocyte	7950

* Variation of the level from one experiment to another is less than 15%. This range of variation was also found in the experiments described in Figures 2 and 3.

lines. An example is given in Figure 1 with the two-dimensional map of the pro-B BASC6 line [29], which was selected because its doubling time is similar to that of Friend cells (11 h), thereby eliminating any proliferation effect such as those observed for other members of the superfamily such as PAG [13]. It can be easily seen that the acidic peroxidoxin spot is much more intense (3-fold), even in undifferentiated Friend cells, than in BASC6 cells. Other cell lines representing either mature B- or T-lymphocytes (MPC 11 and S 49.1 respectively) or myeloid cells such as the J 774 macrophage cell line also showed an acidic peroxidoxin spot significantly less intense than Friend cells, as shown in Table 2.

As for the variation during the induced erythroid differentiation of Friend cells, Table 2 shows that a significant increase of the acidic peroxidoxin spot (approx. 2-fold) occurs during the first 48 h, but is not detected at 16 h of induction. A lesser increase (less than 1.5-fold) takes place during the late phases of the differentiation process (day 5 compared with day 2).

This 48 h period corresponds to the time when cells are committed to differentiate [30], but do not yet express high levels of haemoglobin (less than 5% of the cells are positive to benzidine at this time, while more than 60 % are positive at 5 days of differentiation). This shows that the accumulation of acidic peroxidoxin is an early event preceding haemoglobin accumulation.

Acidic peroxidoxin expression in HEL cells

In order to check whether this high expression of the acidic peroxidoxin during erythroid differentiation was restricted to the Friend cell system or not, experiments were carried out with the human erythroleukaemia HEL cells. These tumour cells can be induced to differentiate *in vitro* in the erythroid pathway with haemin [31] and in the thrombopoietic pathway with PMA [32]. In these cells, the acidic peroxidoxin spot was identified by co-migration with acidic peroxidoxin prepared from human red blood cells and by blotting through a cross-reaction with an anti-PAG polyclonal antibody. The peroxidoxin level was then measured by two-dimensional electrophoresis. The results, presented in Figure 2, show that peroxidoxin expression increased when the cells were directed toward erythroid differentiation with haemin, while peroxidoxin remained at its basal level when

Figure 2 Acidic peroxidoxin expression in HEL cells

Whole-cell extracts from HEL cells either non-induced (**a**) or induced to differentiate with haemin (**b**) or PMA (**c**) were analysed by two-dimensional electrophoresis. The acidic peroxidoxin is shown by an arrowhead, and the intensity of the peroxidoxin spot (in p.p.m. of the total of the spots present on the gels) is indicated below each panel.

the cells were directed toward thrombopoiesis with PMA. This result also shows that there is a correlation between peroxidoxin expression and erythroid differentiation.

Acidic peroxidoxin expression in normal erythroid and non-erythroid cells

To assess the relevance of the correlation between the expression of the acidic peroxidoxin and erythroid differentiation further, a cellular model of *in vitro* erythropoiesis was carried out. For this purpose, BFU-E from peripheral blood were induced to proliferate and differentiate into CFU-E by SCF and IL-3. These CFU-E were then induced to proliferate and differentiate into proerythroblasts first, and then into erythroblasts, by erythropoietin [20]. Whole-cell extracts from proerythroblasts and erythroblasts were analysed by two-dimensional electrophoresis. The acidic peroxidoxin spot was identified by co-migration with acidic peroxidoxin prepared from human red blood cells and by blotting through a cross-reaction with an anti-PAG polyclonal antibody (D. Ferbus and G. Goubin, unpublished work). The level of acidic peroxidoxin was determined in proerythroblasts, erythroblasts and non-erythroid cells (lymphocytes, monocytes and platelets) from the same donor. The results, shown in Figure 3, demonstrate that the acidic peroxidoxin level is much higher in the proerythroblasts and in the erythroblasts than in other cell types. Since high levels are reached at the proerythroblast stage, this suggests that acidic peroxidoxin accumulation also precedes haemoglobin accumulation in normal erythropoiesis.

DISCUSSION

The level of oxidative stress to which a peculiar cell type is submitted depends on many factors, including the oxidative metabolism of the cells and the presence of certain proteins decreasing or enhancing the oxidative stress, the latter acting by

generating the noxious reactive oxygen species. Owing to the high quantities of oxygen handled by red blood cells, these cells are likely to be prone to a fairly high oxidative stress. This may explain why enzymes able to destroy the different products of incomplete reduction of oxygen are present in high levels in the mature erythrocytes: Cu,Zn-superoxide dismutase destroys superoxide but produces peroxide, which is in turn destroyed by catalase. However, haemoglobin or its byproducts (released iron) can function as catalysts in the Fenton reaction and produce hydroxyl radicals [16–18], with thiol compounds, such as glutathione, being used in this reaction. There is therefore competition for the use of peroxide between catalase and reactive iron, the product of the latter being the very noxious hydroxyl radical. It has been shown recently that acidic peroxidoxin can destroy hydroxyl radical [4], thereby eliminating this reactive oxygen species which is not destroyed by the other oxidative stress response proteins. This process requires, however, recycling of the oxidized acidic peroxidoxin either with a thiol [4], such as reduced glutathione, or with an enzymic system [7]. As thio-redoxin is present in mature red blood cells [33], together with high levels of glutathione, both systems can be used in the erythrocyte.

From this point of oxidative stress, it should be noted that the mature red blood cells are generally genetically inactive, either because of the expulsion of the nucleus or by the inactivation of the nucleus through hypercondensation of the chromatin by histone of the H5 type (e.g. avian erythrocytes). This inactivation may prevent the expression of phenotypes linked to DNA damage by reactive oxygen species, which might result in severe genome alterations. However, other types of oxidative damage (e.g. lipid peroxidation and protein carbonylation) can take place in the mature red blood cells, and reduce their functions, i.e. haemoglobin efficiency. The high levels of anti-oxidative proteins present in mature red blood cells may be linked to a need for a permanent protection against reactive oxygen species.

There is, however, another critical stage in the life of erythroid

Figure 3 Protein expression during normal erythroid differentiation and in other normal blood cell types

Whole-cell extracts from proerythroblasts (a), erythroblasts (b), erythrocytes (c), lymphocytes (d), monocytes (e) and platelets (f) were analysed by two-dimensional electrophoresis. The acidic peroxidoxin is shown by an arrowhead, and the intensity of the peroxidoxin spot (in p.p.m. of the total of the spots present on the gels) is indicated below each panel.

cells at the erythroblast stage. At this stage, the cells are highly active at the gene expression level but already contain high levels of functional haemoglobin. Moreover, this phase of haem synthesis requires the handling of large amounts of iron, and produces large amounts of haem. As iron is well known to give rise to Fenton reactions and produce $\text{OH}^{\bullet}$ radicals, extensive damage, and *inter alia* DNA damage, may occur in erythroblasts. The high expression of proteins of the PRP superfamily in the immature erythroid cells may play a major role in the prevention of the oxidative damage which may derive from the peculiar situation of the haemoglobinizing cells.

It should be noted from this work that the PRP is already induced at high levels by the proerythroblast stage, i.e. before the massive haemoglobin production. We tried to determine whether the accumulation of acidic peroxidoxin occurred even earlier, at the CFU-E stage, by preparing CFU-E from cultures induced by SCF and IL-3 but not by erythropoietin. CFU-E were separated on the basis of the presence of the CD 36 antigen. However, the purity of the enriched fraction (< 20%) did not allow us to draw positive conclusions, since the acidic peroxidoxin level was not very different from that of lymphocytes. However, a low level is

present in undifferentiated HEL cells, suggesting that accumulation of the acidic peroxidoxin is not a very early event. Such a conclusion is based on the fact that HEL cells may represent a model of early precursors, since they are able to differentiate either into the erythocytic or thrombocytic pathway.

Together with this early accumulation, it must be noted that the level of the protein in the erythroid cells is very high, of the order of magnitude of actin (approx. half the level of actin in erythroblasts). Linked with the known functions of the protein, such a massive and early expression suggests that this protein is at least an important part of the differentiation programme of erythroid cells, directed towards the detoxification of the reactive oxygen species. It remains, however, to be demonstrated whether this high expression is required for erythroid differentiation to occur.

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