

Petri net based model of the body iron homeostasis [☆]

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Abstract

The body iron homeostasis is a not fully understood complex process. Despite the fact that some components of this process have been described in the literature, the complete model of the whole process has not been proposed. In this paper a Petri net based model of the body iron homeostasis is presented. Recently, Petri nets have been used for describing and analyzing various biological processes since they allow modeling the system under consideration very precisely. The main result presented in the paper is twofold, i.e., an informal description of the main part of the whole iron homeostasis process is described, and then it is also formulated in the formal language of Petri net theory. This model allows for a possible simulation of the process, since Petri net theory provides a lot of established analysis techniques.

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1. Introduction

Iron is a key component of many reactions in the body and is required for a variety of normal cellular functions and vital for proper growth and development. However, natural iron is quite insoluble and excess of iron is harmful, since it can catalyze the formation of potentially damaging toxic oxygen radicals. Humans also have very limited capacity to excrete iron. Therefore, cells have developed mechanisms to improve solubility of iron to control intracellular iron concentrations at the point of iron absorption in the small intestine and other tissues. The intestinal iron absorption is modulated in response to the level of iron

stored by the body and by the amount of iron needed for erythropoiesis. This regulation is thought to operate through two regulators, namely, the store regulator and the erythroid regulator. When the concentration of iron in the stores decreases, the store regulator increases the iron uptake until the reserves are replete. When the iron stores are increased, it reduces the intestinal iron absorption, thus preventing iron overloading. Both the store regulator and the erythroid regulator are believed to be components of the plasma being able to communicate between the sites of iron utilization and mobilization, respectively, and the intestinal cells. So far, the signaling pathway and molecular components involved in the regulation of iron absorption through these two regulators have remained elusive. Despite many components of this complex process have been described in the literature, to the best of our knowledge the complete model of the whole process has not been proposed. It should be also noted that these descriptions are usually informal and hence not very precise (they are not expressed in the language of Petri net or other mathematical theory). Formulation of a complete

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model of the main part of the body iron homeostasis process in a language precise enough for a possible analysis and simulation of the process is the main goal of the paper. For this purpose a mathematical structure called Petri net has been chosen. It is a structure for modeling complex systems proposed in the early 1960s by Carl A. Petri [1]. Until recently the most popular area of applications of Petri nets were technical systems. The situation has changed in the last decade with a rapid growth of computational biology. Since then Petri nets are being used for describing and analyzing various biological processes, e.g., metabolic pathways, signal transduction pathways, and gene-regulatory networks (cf. [2–4]). The reason for choosing Petri nets as a language for modeling the iron homeostasis is their ability to model the system under consideration very precisely. Thus, the developed Petri net model is introduced in this paper to illustrate the biological processes described below. An interested reader is invited to concern with a specification of the model development, the detailed analysis of the Petri net and a validation of the model in [5]. Moreover, in [6] some biological results obtained on the basis of our Petri net can be found. These results are verifications of some hypotheses and answers to some open questions concerning the human body iron homeostasis.

The organization of the paper is as follows. In Section 2 a brief introduction to Petri nets will be given. It should be helpful for non-specialists in understanding the presented model. In Section 3 the iron homeostasis process will be described in a less formal way first and then the model based on Petri nets will be presented. The paper ends with *Conclusions* where some remarks on the possible applications of the presented model are given.

2. Materials and methods

As it has been explained in the previous section, the model of the body iron homeostasis presented in this paper is based on Petri net theory. This approach in qualitative modeling of biochemical networks comprises simulation visualization tools [7] as well as several analysis techniques [8]. In the following, we give a short overview of Petri nets. More formal definitions are given e.g., in [9–11].

Petri nets are bipartite graphs (for an introduction to graph theory see [12]). That means there are two kinds of nodes in the net, called places and transitions, and arcs connect only two nodes of different types. While the places typically stand for the passive system elements as biochemical components (e.g., Fe^{2+} or apotransferrin (ApoTf)), which may be interpreted as conditions, the transitions represent the active system elements as events or biochemical reactions (e.g., Fe^{2+} oxidation by hephaestin (Heph) or binding of ApoTf and Fe^{3+}). Thus, every arc in the net connects an active and a passive element and since there are only directed arcs in Petri nets, they are represented by arrows. In graphical representations, places are depicted as circles and transitions as rectangles. According to the arc direction, every place has a set of pretransitions and a set of

posttransitions. Since the presented net is transition-bordered, these sets are non-empty. In turn, transitions without preplaces (postplaces) are called input (output) transitions and are depicted as flat rectangles. The firing of input transitions is not restricted from the net, i.e., the event modeled by an input transition may take place any time. Contrary to that, a transition with preplaces represents an event, which is only enabled to take place when all the preconditions of that event are fulfilled. The fulfillment of a condition is realized via tokens residing in places. If all preplaces of a transition are marked sufficiently with tokens (i.e., if the corresponding biochemical components are available), this transition may fire (i.e., the reaction takes place). If a transition fires, one token is removed from each of its preplaces and added to each of its postplaces. Thus, the tokens are the dynamic elements of the system. Altogether, arcs connect an event with its preconditions which must be fulfilled to trigger this event, and with its postconditions which will be fulfilled when the event takes place. Principally, a place in a discrete net may carry any integer number of tokens, indicating different degrees of fulfillment. Since the number of preplaces of a transition generally has not to be equal to the number of postplaces of this transition, the number of tokens in the whole net is not conserved. If a condition must be fulfilled, but the firing of a connected transition does not remove any tokens from it, these nodes are connected via two converse arcs. In the following, these arcs are represented by bidirectional arrows as a short-hand notation, and are called read arcs.

Fig. 1 depicts an example of a Petri net, which illustrates the firing process. With the firing of input transition $t1$ one token is put at place $p1$. This token enables the transitions $t2$ and $t3$ to fire, i.e., both of them are able to fire but only one of them can really fire, if there is only one token at their common preplace (i.e., $p1$). Since $t2$ is an output transition, it can only remove this token from $p1$. The firing of $t3$ would remove this token and put one token at each place $p2$ and $p3$. A token from place $p3$ is removed by firing of its posttransition $t4$.

The distribution of the tokens over all places describes a certain system state and is called the marking of the net. According to this, the initial marking of a net describes the system state before any transitions have fired.

To avoid immoderate arc crossings, the construct of logical nodes is used in the graphical representation of the net. A logical node is represented by its name and exists in

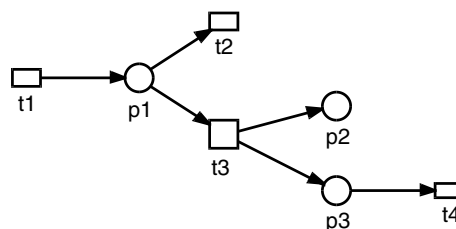


Fig. 1. An example of a simple Petri net.

multiple copies in the net, which are logically identical. Thus, a node defined as a logical one is identically equal to every other logical node with the same name and they are identified as one node which occurs in several graphical copies in the net. To distinguish these special nodes from ordinary ones, they are tinted grey. The model is one connected Petri net, but in the following it is presented and discussed in the form of three subnets which are connected via logical nodes in the original model.

3. Results and discussion

In this section a description of the iron homeostasis is given. It is followed by a description of the Petri net based model of this process.

3.1. Informal description of the body iron homeostasis

The form in which iron is presented to the digestive tract affects the iron absorption, and inorganic iron ions change their oxidation state during the absorption process. There are two major forms of dietary iron: heme iron (ferrous, Fe^{2+}) and non-heme iron (ferric, Fe^{3+}). Heme iron ($\text{Hem}(\text{Fe}^{2+})$), found primarily in red meats, is the most easily absorbed form. Heme iron is ingested as myoglobin and hemoglobin. In the presence of gastric acid, the globin molecule is split off and the ferrous iron is liberated and transported with its phosphorin ring from the stomach into the duodenum and jejunum via a not yet characterized duodenal heme receptor [13,14]. Heme is oxygenated by Heme oxygenase (HO) before it reaches the labile iron pool (LIP) in the cell. Other forms of iron are bound to some other organic constituent of the food. Cooking tends to break these interactions and increase iron availability. Iron ions undergo important changes of oxidation state during digestion and absorption. The change occurs in the stomach. Here, iron Fe^{3+} is reduced to Fe^{2+} . This reduction is favored by the low pH (in the range from 2 to 5). Reducing agents, such as ascorbic acid, assist this process. Reduction is important because the ferrous iron dissociates from ligands more easily than the ferric one. Fe^{3+} when ingested into a stomach unable to produce acid, forms insoluble iron complexes, which are not available for absorption. Then Fe^{2+} is transported into the mucous membrane of the small intestine by divalent metal transporter-1, which is also known as natural resistance-associated macrophage protein-2 or divalent cation transporter-1 (DMT1/Nramp2/DCT1) situated on the apical side of mucous membrane. If the iron is orally supplemented (in the Fe^{2+} form) it is also directly transported by the transporter mentioned above. If the iron (in the Fe^{3+} form) is administered parenterally it needs to be reduced to ferrous ion by ferri-reductase, called duodenal cytochrome *b* (Dcytb) situated like DMT1/Nramp2/DCT1 at the luminal site of the small intestine mucous membrane [15,16].

The prevailing view is that all forms of iron entering cells reach a cytosolic pool of metabolically active or labile

iron. The LIP is the compartment from which most of the metal is either: metabolically drawn into Fe-dependent enzymes, transported into mitochondria for heme synthesis or incorporated into ferritin for secure storage and/or detoxification. LIP is comprised of forms of iron bound to a variety of medium and low affinity binding ligands. Those might vary in composition and quantities in different physiological settings. Operationally, LIP can be regarded as that component of cell iron that is accessible to a particular chelator or a class of chelators. Topologically, it is identified primarily with the cytosol and as such, it is regarded as the crossroad of the cellular iron traffic. Biochemically, LIP has been defined as the ‘regulatory’ or ‘regulated’ pool of cell iron, since depending on the cells system, its level is apparently maintained within a constant range [17]. The limits of the latter are such that, on the one hand, they meet the cellular requirements for iron and, on the other hand, they prevent excesses from developing and triggering reactive oxygen species (ROS) formation. Most cells regulate their LIP by iron responsive proteins (IRPs) that sense its level, and in turn control the translation of transferrin receptor (TfR) and ferritin in a compensatory manner [18,19]. A rise in iron uptake increases the LIP and results in IRPs inactivation. The latter concomitantly evokes ferritin synthesis and blocks TfR synthesis by inducing TfR mRNA degradation. The iron deprivation causes opposite effects. The IRP mode of action on ferritin and TfR synthesis and erythroid heme synthesis is via specific mRNA–protein interactions. Two IRPs have been invoked in those interactions, but only the activity of the IRP1 is apparently directly affected by the cytosolic iron (i.e., LIP) concentrations and modulated by heme, cell redox status, and other factors. Other regulatory modalities found in some cells comprise the increase in LIP by transcriptional activation of TfR expression and the withdrawal of iron from the LIP via a transcriptional induction of ferritin synthesis. So, some fraction of the iron taken up from the LIP is deposited as ferritin within the enterocyte, and the remainder is transferred by ferroportin (Fpn) at the basolateral site of the small intestine mucous membrane to the plasma-bound transferrin (Tf). When the enterocyte defoliates, iron deposited as ferritin is lost into the intestinal lumen. This mechanism of iron loss is probably overwhelmed by the large amounts of iron ingested. The amount of iron entering the body depends largely on two factors: total body iron concentration and the rate of erythropoiesis.

So, Fe^{3+} transported by Fpn must be reduced by a ceruloplasmin (Cp) homologue–Heph [20], mostly expressed through the small intestine and colon, to be prepared for the Tf transport. Normally, all of non-heme iron in the circulation is bound to holotransferrin (HoloTf). Only about 30% of Tf binding sites are occupied, so that most of the protein, which is called apotransferrin is free of iron and is able to bind one or two Fe^{3+} ions in transferrins’ N- and/or T-terminal ends ($\text{Nt}(\text{Fe}^{3+})\text{HoloTf}$ or $\text{HoloTf}(\text{Fe}^{3+})\text{Ct}$ or $\text{HoloTf}(\text{Fe}^{3+})_2$). The ferrous ion in connection

with ApoTf is oxidized to the ferric one. A majority of serum iron is carried by HoloTf, and is taken up by cells as an iron bound HoloTf. On the cell surface iron bound HoloTf binds to transferrin receptor 1 (TfR1) and as the HoloTf(Fe³⁺)-TfR1 complex is internalized by receptor mediated endocytosis (RME) through clathrin coated pits into endosomes. HoloTf binds to the TfR in 2:2 (Tf: TfR subunit) stoichiometry [21,22]. After internalization of this complex, iron Fe³⁺ is released from HoloTf under the endosomal acidic conditions (pH varies with the cell type, ranging from 5.5 to 6.0 [23]) and occurs in the reduced form as Fe²⁺. HoloTf after Fe²⁺ releasing is called ApoTf. Next, ApoTf and TfR1 complex (ApoTf-TfR1) is exocytosed. After the return of the complex to the cell surface, the extracellular pH triggers the release of ApoTf, allowing another round of binding and RME to begin. Then the ferrous ion is transported by DMT1/Nramp2/DCT1 transporter to the cytosol or mitochondria of the all-proliferative cells and reaches the LIP [24]. The expression of these transport genes is strongly related to the body iron homeostasis, since the small intestine expression of DMT1, Dcytb, Fpn and to a lesser extent of Heph is increased in the iron deficiency anemia [25,26].

Expression of TfR1 on cells surface is also regulated at the transcriptional level by the status of cellular proliferation and oxygen saturation. Another well-characterized mechanism of TfR1 expression regulation is performed at the posttranscriptional level. When the iron concentration is insufficient, the iron regulatory proteins (IRPs) bind to iron responsive elements (IREs) of TfR1 mRNA resulting in stabilization of the transcript. In excess of the iron concentration, IRPs are released from IREs and the transcript is degraded. The recently discovered Tf receptor, called transferrin receptor 2 (TfR2), is similar to TfR1 [27,28]. However, if the main function of TfR2 is to transport iron into the cells, the iron overload would be expected to suppress the expression of TfR2 to keep cellular iron homeostasis. This paradoxical expression profile of TfR2 may be suggesting that it has another function besides the cellular iron uptake. Alternatively, TfR2 may simply be another receptor for Tf, and cells may be controlling the iron influx by using two different receptors for Tf; one is the high affinity receptor TfR1, whose expression is regulated by the cellular iron status, and the other is the low affinity receptor TfR2, whose expression depends on the cell cycle rather than the iron status, because its transcript lacks an IRE [29].

It is well known that on the erythroid precursors cells surface is the vest TfR1 concentration. Erythroid precursors need an extraordinary amount of iron to support the hemoglobin synthesis and differentiation into mature red cells. The density of TfR on the cell surface is changing during the erythroid maturation. A strict correlation exists between the iron requirement and the transferrin receptors number, indicating that the abundance of transferrin receptors on the cell surface is a major determinant of the erythroid iron uptake [30]. Because maturing red cells shed

their transferrin receptors, the quantity of the soluble transferrin receptor in plasma reasonably reflects erythropoiesis. It is known, that the mature red cells synthesis is stimulated by erythropoietin (EPO), a hormone produced by the kidney. In the case of a low serum iron concentration, the EPO synthesis is increased to enhance the erythrocytes production.

The red blood cells live in the human body about 120 days and after this period of time one-nuclear cells phagocytose them. These cells play a key role in the iron metabolism, as they are responsible for the recirculation of iron derived from effete red blood cells, so that it may enter the circulation, bind to Tf, and be transported to the bone marrow for red cell production [31]. Before that, heme iron from red blood cells must first be released from hemoglobin by HO and transported by natural resistance-associated macrophage protein-1 (Nramp-1) from a phagolysosome to the one-nuclear cell cytoplasm. Another way of iron delivery to one-nuclear cells cytoplasm is the iron transport via HoloTf(Fe³⁺)-TfR1 complex which is able to take up significant amounts of iron from HoloTf. This process proceeds like the RME route. Next, Fe²⁺ reaches LIP, the compartment from which it is metabolically drawn into enzymes, transported into mitochondria for the heme synthesis or incorporated into ferritin for secure storage and/or detoxification, as shown above. Furthermore, changes in the iron metabolism during immune and inflammatory responses which result in reduced serum iron (hypoferrremia) and anemia are thought to be due, at least in a part, to changes in iron handling by one-nuclear cells. What is going on with LIP depends on the body iron status. If there is a low concentration of iron, iron (Fe²⁺) from one-nuclear's LIP is mostly transported out by Fpn. Ferroportin, situated in the small intestine membrane and in the one-nuclear cells is strongly regulated by hepcidin, a liver expressed antimicrobial peptide, which is upregulated by bacterial lipopolysaccharide. Then, iron is oxidized to ferric ion by ceruloplasmin (Cp), a plasma metaloprotein synthesized and secreted by the liver, and in this form is able to connect with ApoTf and to be transported to the cells with TfR on its surfaces. Ceruloplasmin may play an essential role in determining the rate of iron efflux from cells with mobilizable iron stores. On the other hand, in case of an abnormal higher body iron concentration, iron from the LIP is mostly transferred to the body iron store (ferritin), where iron is oxidized and which synthesis is regulated by iron requirements via IRP system. The mobilization of the iron from the ferritin is much more poorly understood than the deposition in the storage protein [32].

During the inflammatory process the body iron metabolism is changing, among others iron is not released from body iron stores (ferritin), to enable the development of microorganisms which need iron for their growth. Infectious and inflammatory disease commonly results hypoferrremia which if prolonged can lead to anemia (the anemia of chronic disease, ACD). It is generally thought that inflammation alters macrophage iron homeostasis, resulting in an

increased iron retention and a reduced iron release, thus giving rise to the hypoferremia and anemia, although in the latter event defects in erythropoiesis may also be involved [33]. One might speculate that the iron sequestration is of benefit during the early acute stages of infectious disease in order to reduce the iron availability to microorganisms, but that the subsequent development of an adaptive immune response requires iron to be ‘unlocked’ in order to allow a proper immune function. A protein whose production is modulated in response to inflammation (positive correlation), anemia and hypoxia (negative correlation) is hepcidin. An elevated expression of HAMP (hepcidin antimicrobial peptide) gene, which is responsible for the high hepcidin production during inflammatory process, is mediated among others by the higher interleukin-6 (IL-6) serum concentration [34]. This situation results in two phenomena: a reduced iron intestinal absorption, and in an iron increase in the one-nuclear cells [35,36]. The first phenomenon follows from the fact that the iron uptake by duodenal enterocyte appears to be largely regulated by hepcidin. In rats, changes in hepcidin expression have shown a close temporal relationship with changes in the duodenal iron transporter expression, like (DMT1/Nramp2/DCT1) and Dcytb [37,38]. Recently, it has been found that hepcidin regulates the cellular iron efflux by

binding to Fpn and through ligand-induced internalization and degradation [39,40]. In the latter phenomenon inflammatory-induced hepcidin expression, causing Fpn degradation might account for the iron sequestration within the one-nuclear cells in the reticuloendothelial system (RES). Moreover, the expression of hepcidin appears to be sensitive to the activity of the erythroid bone marrow. In a recently suggested model for the regulation of hepatic hepcidin expression [40], hepatocyte surface HFE (the hemochromatosis protein) competes with HoloTf(Fe^{3+}) for the binding on surface TfR1 which is TfR2 competitor. The unbound surface HFE and a higher concentration of HoloTf(Fe^{3+})–TfR2 complex were proposed to increase the hepatic hepcidin expression and its release. According to the model, the iron deficiency would lead to a decrease of circulating HoloTf(Fe^{3+}), and the number of free surface TfR1s would increase, resulting in a decreasing fraction of free surface HFE, and a lower concentration of HoloTf(Fe^{3+})–TfR2 complex.

3.2. Petri net based model

As mentioned above, we have developed a Petri net which models the main part of the whole processes of the human iron homeostasis. The stepwise development of

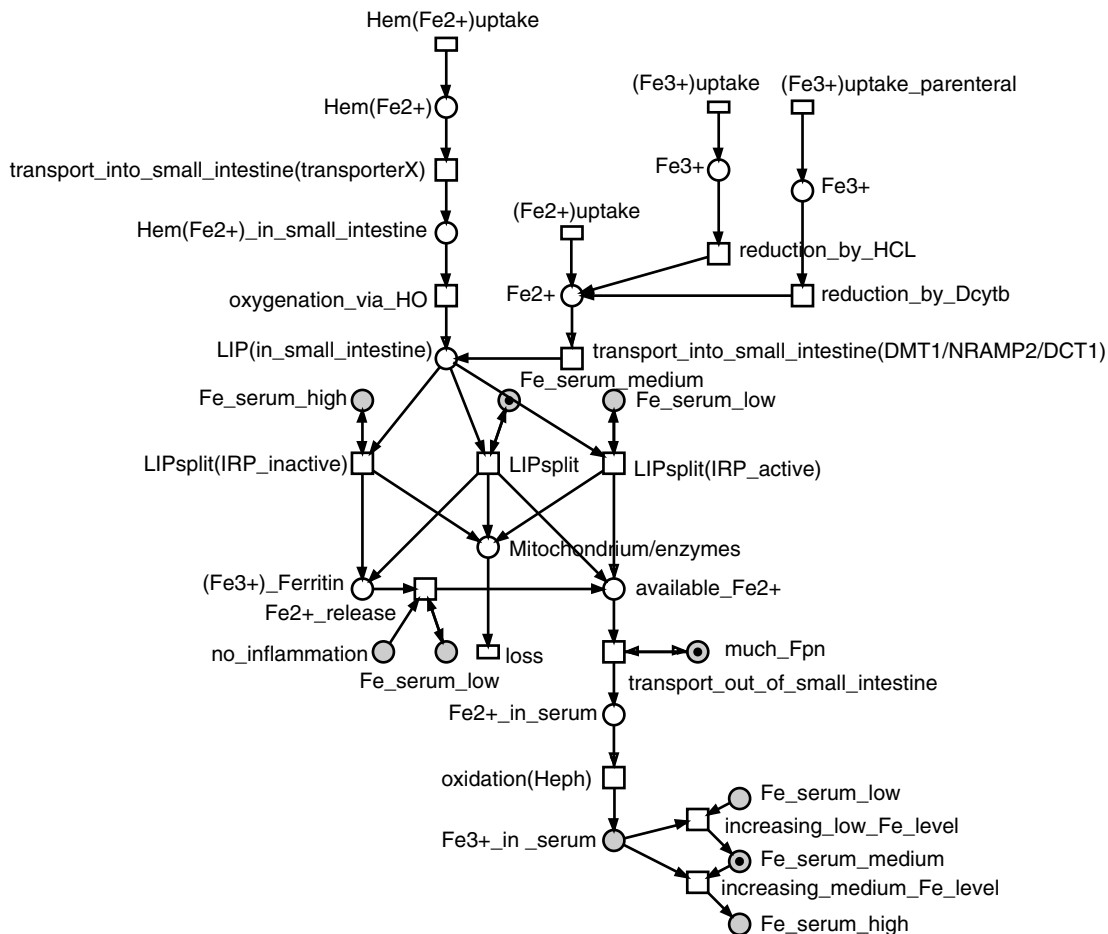


Fig. 2. The part of the Petri net modeling iron uptake and iron transport through the small intestine.

the model as well as the Petri nets analysis and the model validation is described in discussed [5]. The model is a graph, which is presented in the following as three subnets, shown in Figs. 2–4, respectively. These subnets are connected with each other via some logical nodes. Fig. 5 shows in which ways these logical places connect the three subnets with each other. An overview of the whole model is given in Fig. 6.

Considering the presented model, there are several tokens residing in places in the initial marking of the net. These tokens indicate the presence of some components previous to any iron uptake and they give the initial marking representing that system state which is assumed to be the body state on normal physiological conditions.

3.2.1. Subnet 1—iron uptake and transport through the small intestine

The part of the Petri net modeling the iron uptake and the iron transport through the small intestine is shown in Fig. 2. In the digestive tract, there are different ways of iron absorption. Therefore, the model includes four input transitions representing different forms of iron: heme iron through the transition *Hem(Fe²⁺)uptake* and non-heme iron through the transitions *(Fe²⁺)uptake*, *(Fe³⁺)uptake*, and *(Fe³⁺)uptake_parenteral*, respectively. Not depending on the fact which of the input transitions puts tokens in the net, all these tokens arrive at the place *LIP(in_small_intestine)* after passing several places, according to the special iron form during the uptake and the corresponding processing and transport of the iron ions.

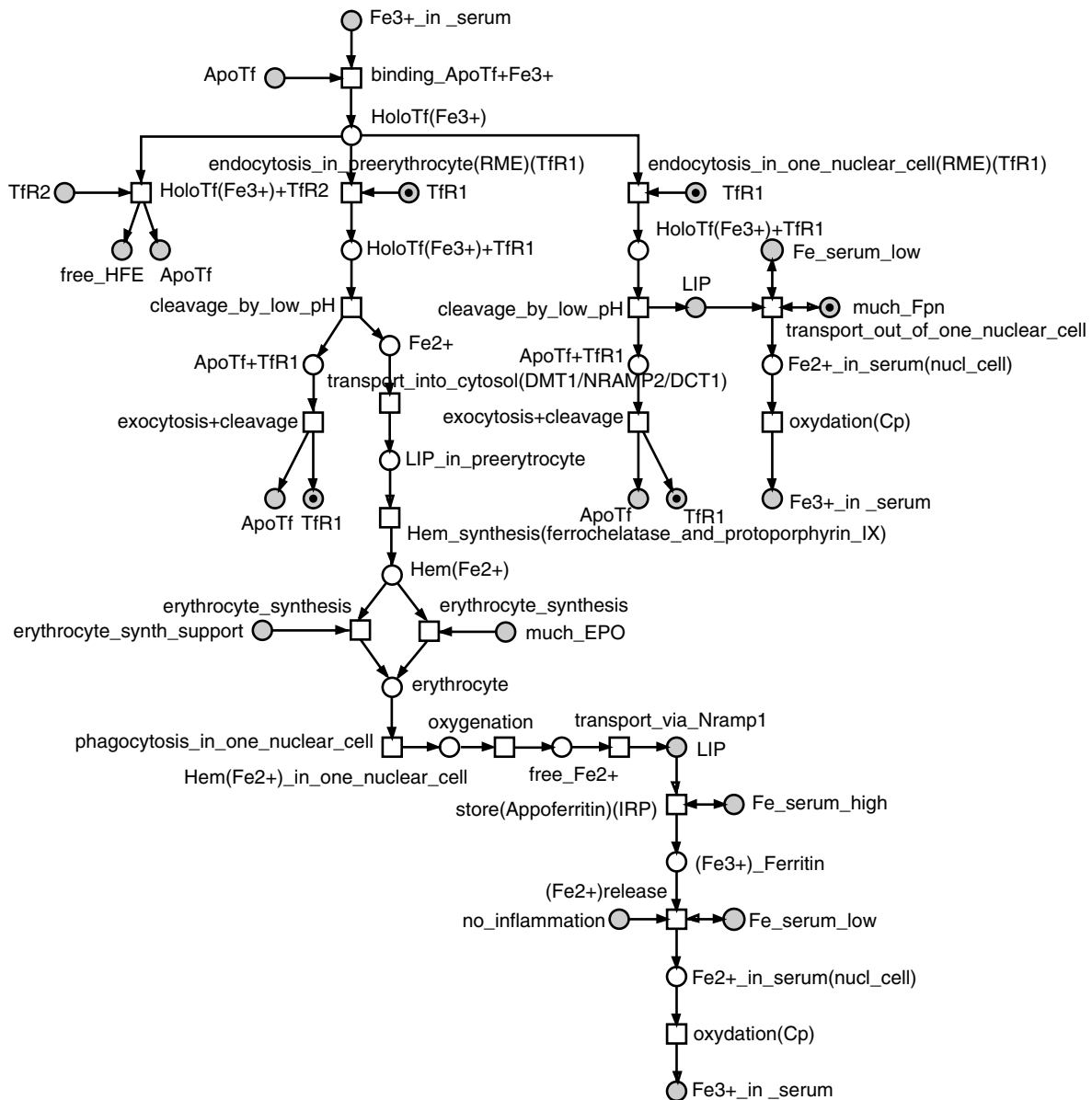


Fig. 3. The part of the Petri net modeling iron pathway through the erythrocyte cell and the one-nuclear cell.

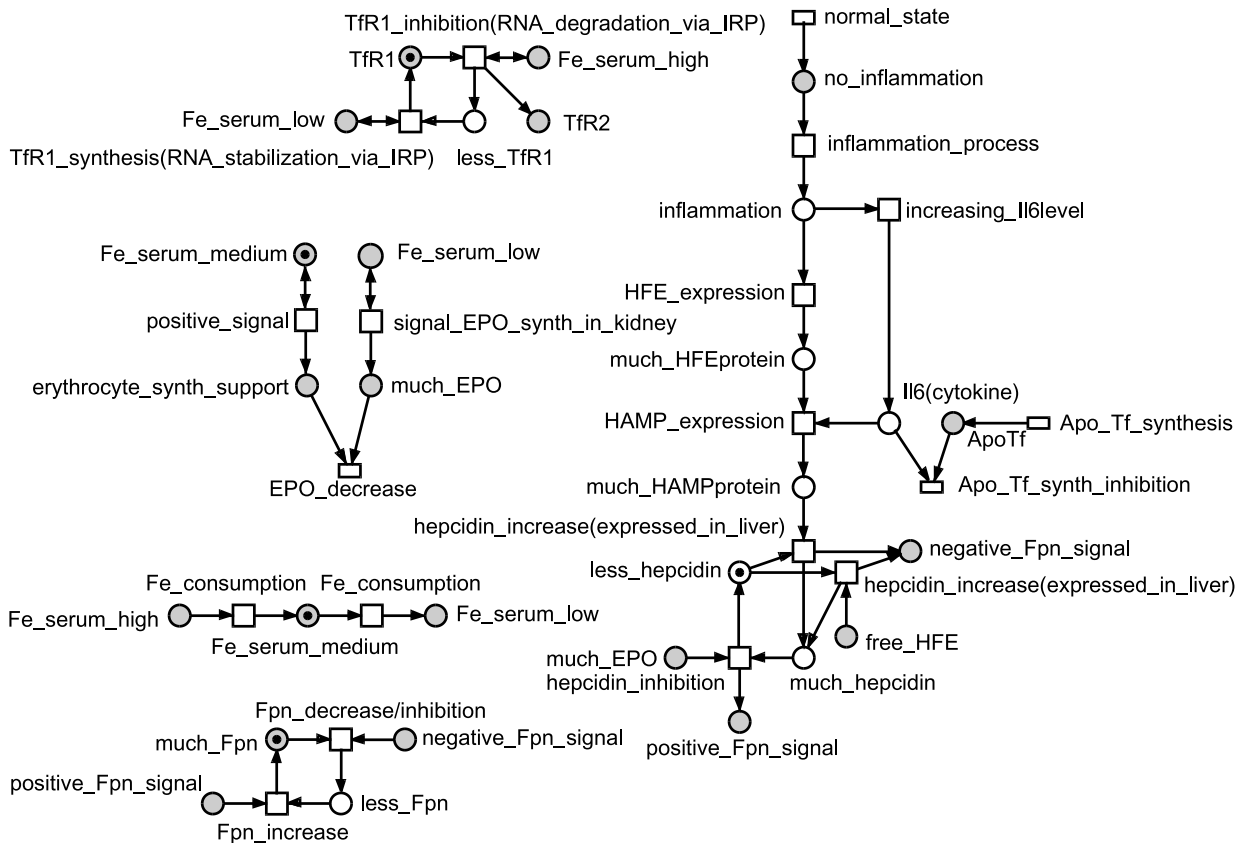


Fig. 4. The part of the Petri net corresponding to some auxiliary conditions necessary for the model.

In the model three levels of iron in serum are distinguished, represented by the places *Fe_serum_high*, *Fe_serum_medium*, and *Fe_serum_low*, respectively. Independent of the firing of any transitions, there is exactly one token, which only circulates between those three places, indicating the actual iron level. In the initial marking, this token is placed at the medium iron level, which stands for a normal state. Depending on that level, the LIP in the small intestine is spitted to its use. In the case of a high iron level (which is measured by inactive IRP), the iron from LIP is on the one hand transported into mitochondria for Fe-dependent enzymes. On the other hand, the iron is incorporated into ferritin for storage. If the iron level decreases to a low level and if there is no inflammation in the system (modeled in subnet 3), the iron from this store is released and is then freely available in the small intestine. In the case of a low iron level (measured by active IRP), the iron from LIP is also transported to mitochondria and is directly available in a free form. In the case of a medium iron level, the LIP is split to all these three usages.

To transport the available iron out of the small intestine, Fpn is needed. Subnet 3 shows how the Fpn level is affected by the level of hepcidin. If there is enough Fpn available, the transition *transport_out_of_small_intestine* is enabled to fire, removes a token from place *available_Fe2+* and puts a token to place *Fe2+_in_serum*. A token at this place is removed by firing of transition *oxidation(Heph)*, which

puts a token to place *Fe3+_in_serum*. Tokens at this place can lead to an increase of the iron level or they are alternatively used in subnet 2, where this place occurs as an input place.

3.2.2. Subnet 2—iron pathway through the erythrocyte cell and the one-nuclear cell

In Fig. 3 there is shown the part of the net modeling iron pathway through the erythrocyte cell and the one-nuclear cell, respectively. The input place *Fe3+_in_serum* of this subnet gets tokens from subnet 1. If there is an additional token at place *ApoTf* (what is affected by the presence of cytokine IL-6 in subnet 3), transition *binding_ApoTf+Fe3+* may fire, and it removes those tokens and puts one at place *HoloTf(Fe3+)*. If Tfr1 is present, the *HoloTf(Fe3+)-Tfr1* complex is internalized by the receptor mediated endocytosis either into pre-erythrocyte cells or into one-nuclear cells (shown in the net in form of the middle and the right pathway, respectively). However, the amount of available Tfr1 depends on the iron level (shown in subnet 3) and if this amount decreases, there is relatively more Tfr2, which then triggers the transition *HoloTf(Fe3+)+Tfr2*. Thereby, place *free_HFE* gets a token, which influences the hepcidin level in subnet 3.

Both in pre-erythrocytes and in one-nuclear cells iron is released from the *HoloTf(Fe³⁺)-Tfr1* complex by low pH. Thereafter, the remaining complex is exocytosed and

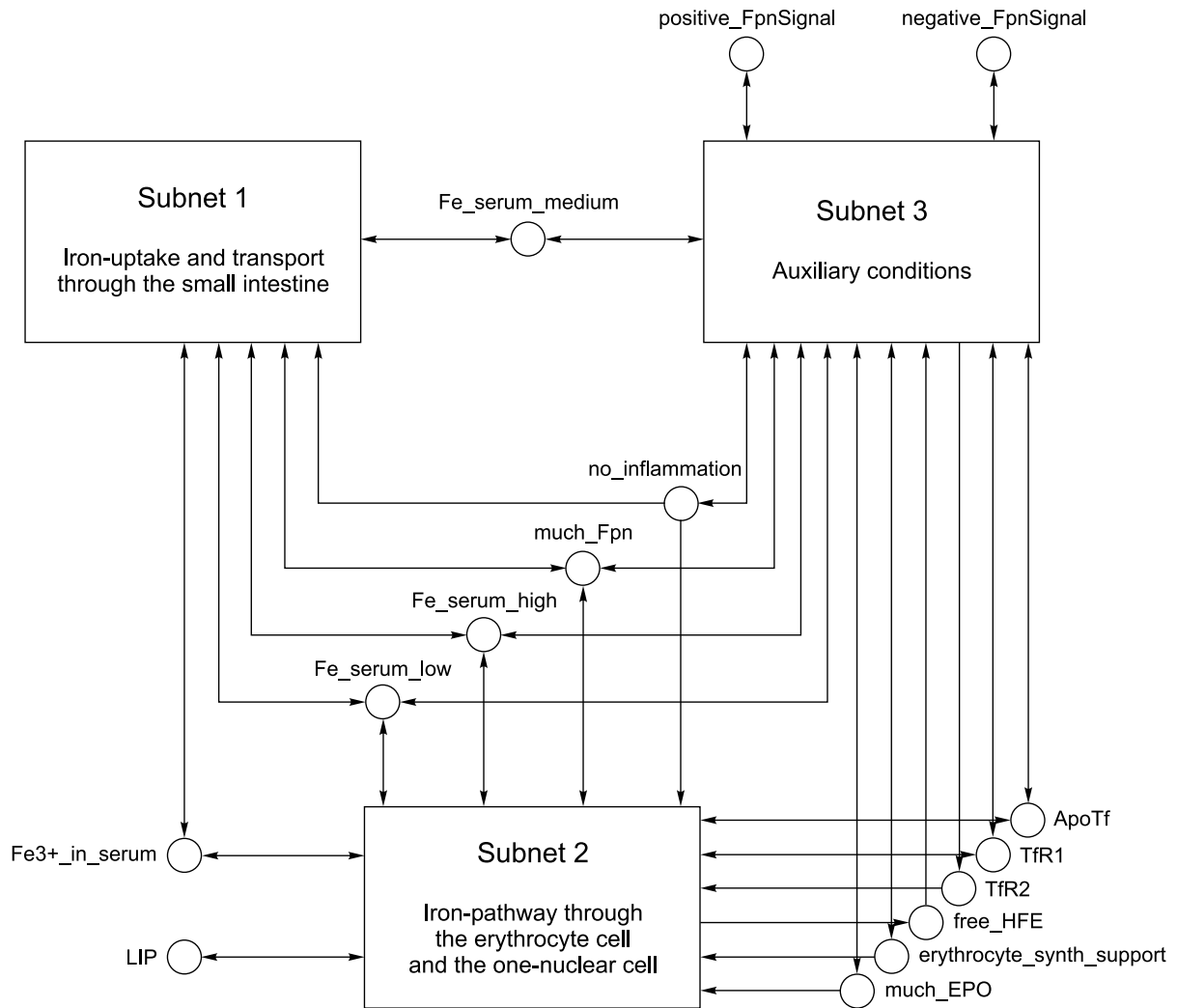


Fig. 5. The general structure of the model—the connections between the three subnets via logical places. The arrows show in which way each place is connected with transitions in the corresponding subnet.

cleaved. In one-nuclear cells the iron is directly admitted to LIP. Under a low level of iron in serum and enough available Fpn, the iron is transported out of the cell. Contrary to that, the iron in the pre-erythrocyte cell is needed to support hemoglobin synthesis and differentiation into mature red cells. The transition *erythrocyte_synth* depends also on tokens at the places *erythrocyte_synth_support* and *much_EPO* which are contained in subnet 3. Since there is no possibility to include any information concerning time in an ordinary, discrete Petri net, the place *erythrocyte* has the posttransition *phagocytosis_in_one_nuclear_cell* (although only the about 120 days old erythrocyte cells are phagocytosed). Via that way the iron in the erythrocyte cell is also admitted to LIP in the one-nuclear cell. Therefore, the place *LIP* is identified with the above mentioned place *LIP* (i.e., a logical place is used here). If the iron level in serum is high, the iron from LIP is incorporated into ferritin for storage. By analogy with the above mentioned processes in the small intestine, the iron is released out of storage if the amount of iron in serum is decreasing to a

low level and if there is no inflammation in the system (see place *no_inflammation* in subnet 3). The iron which is released out of the one-nuclear cell is represented by a token at the logical place *Fe3+_in_serum*. This place also serves as input place for this subnet.

3.2.3. Subnet 3—auxiliary conditions

The third part of the Petri net is shown in Fig. 4. This subnet is composed of five subnets, which stand for some auxiliary conditions of the model, mainly some regulations. The left depicted nets concern the RNA based regulation of TfR1 via IRP depending on the iron level and the support of erythroid maturation which depends on the iron level in the serum and the regulation of the EPO concentration. The nets below represent the natural iron consumption in the serum and the regulation of Fpn via some signals. These signals result from the firing of the transitions *hepcidin_increase(expressed_in_liver)* (negative Fpn signal) and *hepcidin_inhibition* (positive Fpn signal), respectively (see the net on the right-hand side). The last mentioned net

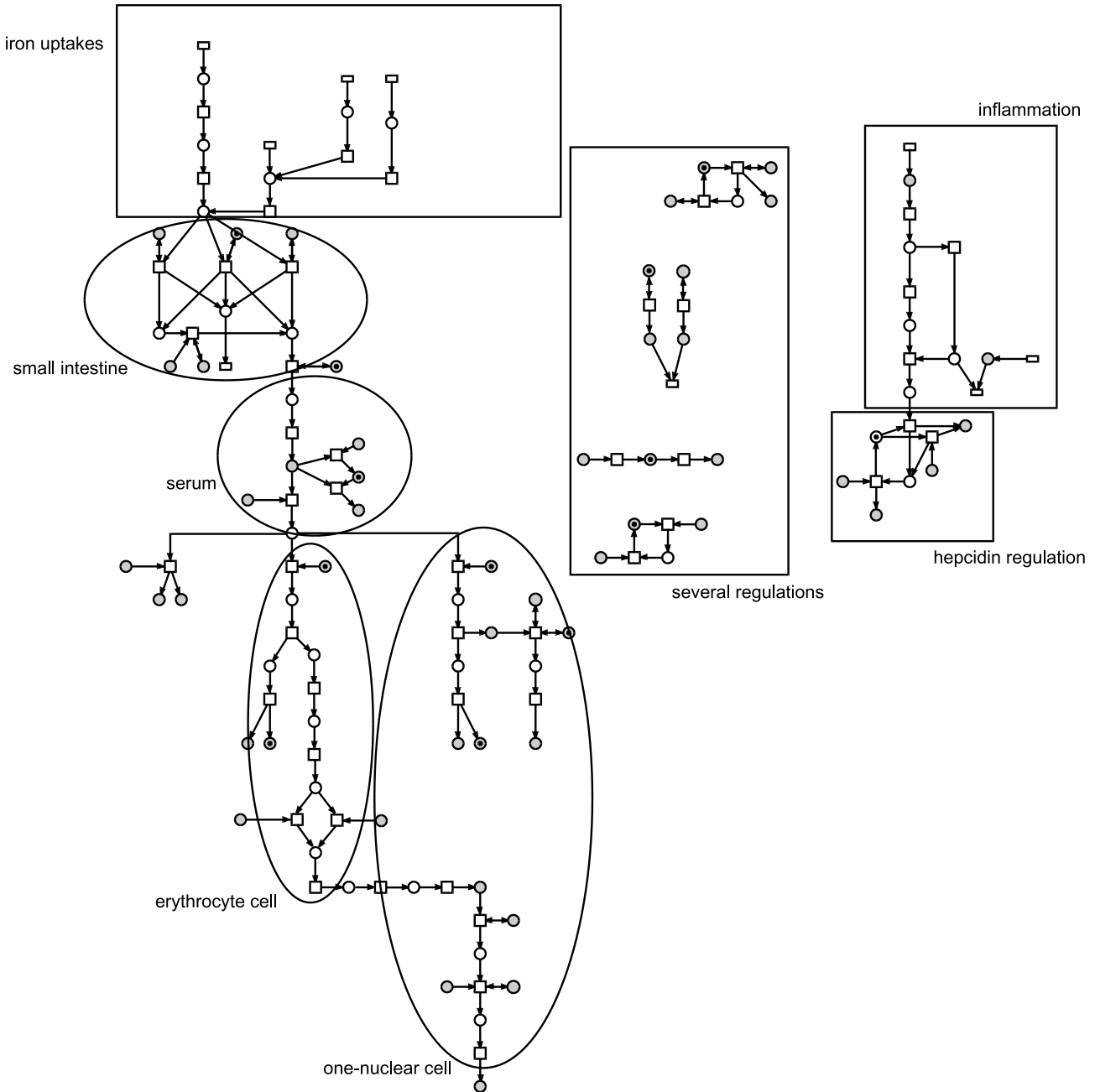


Fig. 6. The whole Petri net model. Functional units are marked with rectangles and locally bounded units with ellipses, each inscribed.

represents the processes during inflammation, including the regulation of ApoTf and hepcidin.

4. Conclusions

In the paper, the main part of the whole process of iron homeostasis has been presented. The process has been described in the language of Petri net theory, which makes the description very precise on one hand, and on the other hand, it allows for a possible simulation of the process. Furthermore, Petri net theory provides a lot of established analysis techniques. Such a net analysis, being a goal of our future research, should make possible a more detailed

investigation of the process. In general, it may lead to a formulation of some hypotheses, which can be then verified in the laboratory or clinical research (cf. [6]).

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