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Research paper

A systems biology approach to propose a new mechanism of regulation of repetitive prophylaxis of stable iodide on sodium/iodide symporter (NIS)

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ABSTRACT

Our group showed that repetitive dose of potassium iodide (KI) for eight days offers an efficient protection for exposure to repeated radioactive emissions without adverse effects on adult rats. However, differential expression of genes implicated in Wolff-Chaikoff effect was observed. To understand the Wolff-Chaikoff regulation and its molecular constituents during repetitive administration of KI, a biochemical reaction network was constructed as a "geographical" map of the thyrocyte depicting iodide and thyroid hormone synthesis. Path analysis of the network has been performed to investigate the presence of a regulatory circuit of the node iodide to the node "*nis* transcription". NIS is responsible for the uptake of KI and plays an important role in the Wolff-Chaikoff effect. The map is a source for the most updated information about iodide and thyroid hormone metabolism. Based on this map, we propose a hypothesis that shows a putative mechanism behind NIS regulation and KI uptake.

1. Introduction

Upon a nuclear accident, a high amount of radio-active iodines (^{131}I) is released into the environment where it can contaminate individuals by ingestion and inhalation [1]. The thyroid is an organ that easily absorbs 10–30% ^{131}I and the absorbed ^{131}I can then cause thyroid cancer [2]. To evade this uptake, a single dose of potassium iodide (KI) is given to the to-be-exposed-public, 130 mg of KI in adults, 65 mg in children between 3 and 12 years and 32 mg in children less than three years old and 2.6 mg for neonates less than one month of age [3]. KI saturates the thyroid with non-radioactive iodides and thereby blocks the transport of radio-active iodide into the thyrocytes by the Sodium/Iodide symporter (NIS) and the production of thyroid hormones [4].

During the nuclear accident at the Fukushima Daiichi Nuclear power plant in 2011, radio-active iodine releases occurred during several occasions over a prolonged time period [1]. The World Health Organisation (WHO) 1999 has recommended a single administration of KI for an exposure to radio-active iodines for several hours up to no more than 2 days. Based on the Fukushima and the Chernobyl accidents, a single dose of KI and perhaps a second dose is not sufficient in protecting the thyroid during prolonged exposure and therefore more than one administration of KI might be more effective [1,5].

Until recently, it has been unknown what dose would be effective, which dose regimen to apply and the maximal duration of the treatment without introducing adverse effects in case of repetitive administration of KI. Our group has recently shown that administration of KI at 1 mg/kg/day is an optimal dose for the saturation and the protection of the thyroid in rats [6]. However, for a prolonged time period the pharmacokinetics have still to be determined to model adequate dose regimens for protracted prophylaxis [6]. More recently, Lebsir and colleagues have shown that repetitive KI administration of 1 mg/kg/day show adapted thyroid protection without changing the thyroid hormones' levels in rats [7]. No toxic effects have been observed but a change in gene expression of *nis*, pendrin (*pds*), thyroid peroxidase (*tpo*) and monocarboxylate transporter 8 (*mct8*) has been observed after administration for eight days. According to the authors a two-step Wolff-Chaikoff effect, which consists of a fast down-regulation of *nis*, *tpo* and to a blockage of the thyroid hormone synthesis upon high intra-cellular concentration of iodide [8], has been induced: an early Wolff-

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Chaikoff effect due to down-regulation of *nis* and *mct8* and a late effect marked by a down-regulation of *tpo* and up-regulation of *pds*.

The gene *nis* encodes for a sodium-iodide symporter NIS that transports two NA^+ ions and one I^- ion over the basal membrane due to electrochemical Na^+ gradient [9]. The molecular mechanisms behind NIS down-regulation for a single dose of KI administration are known and have been nicely summarised by Dayem and colleagues [10]. In short, a high intra-cellular concentration of iodide leads to a reduction in iodide in the thyrocyte due to fast down-regulation of *nis* and to a blockage of the thyroid hormone synthesis (Wolff-Chaikoff effect) due to down-regulation of *nis, tpo,* dual oxidase 2/dual oxidase maturation factor 2 (*duox2/a2*), *pds* and *mct8* [8,9]. In contrast, the gene regulatory network during repetitive KI prophylaxis over a longer time period has not been described well or not extensively enough for the combinations of these genes: *nis, tpo, duox2/a2, pds* and *mct8*.

In order to attempt elucidating a possible mechanism behind the gene expression pattern observed [7], a systems biology approach has been applied in this study. Systems biology is the scientific field that focusses on studying complex systems (e.g. living organism, tissue, cell, etc.) using systems-wide scale analysis that includes (high-throughput) datasets as representatives of an entire phenotype [11]. This approach has been readily applied in cancer research [12], drugs research [13], immunology [14] and in toxicology [15].

Systems biology approach can lead to a more holistic and dynamic view of complex biological processes. In order to ensure such view, all necessary data about a certain biological process needs to be either sampled by using high-throughput technologies (holistic view) in time series (dynamic interaction) or has to be collected as such information is dispersed among many published articles. Representing this knowledge as a network in the form of a map is easier to understand the relations/interactions between entities (e.g. proteins, DNA, RNA, etc.) than text-based-only database [16]. The map represents biological mechanisms and through analysis of the of map, either as a static network (topology) or dynamic one (simulations) structural details and regulations can be revealed behind certain biological phenomena [17–21].

In order to identify a putative mechanism behind *nis* regulation during repetitive KI prophylaxis, we have constructed manually a process description (PD) diagram that represents the biochemical reaction network that describes the thyroid hormone synthesis and iodide metabolism in the form of a map. Due to the amount of details or granularity of the biochemical reaction network this type of diagram enables mechanistic descriptions [22]. After performing network analysis, a sub-network has been selected with nodes that correspond to major participants of the thyroid hormone synthesis and iodide metabolism. Subsequently this sub-network has been translated into an activity-flow (AF) network. The edges connecting the nodes have either a positive or a negative influence on their neighbours and they can represent a sequence of events, however these AF diagrams are not mechanistic but can be used for the transfer of information [16,22]. The use of this type of diagrams can reduce the complexity of the network and the number of nodes. By sequential reduction of the AF network that includes only the nodes/genes that have been analysed by Lebsir and colleagues, we have obtained a final network that might be able to explain the regulation behind the observed gene expression during repetitive prophylaxis of KI shown by Lebsir and colleagues.

2. Materials and methods

A possible workflow to investigate a putative effect of iodide on the regulation of NIS after multiple administration of KI during prolonged or repetitive exposure to ^{131}I can be found in Fig. 1. The

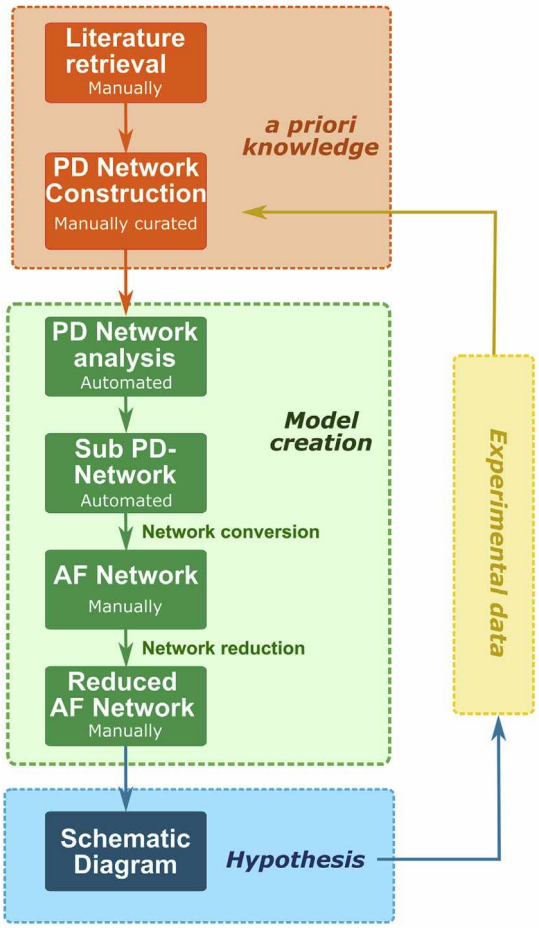

Fig. 1. A schematic view of how systems biology can be of help to formulate a hypothesis that may explain the observed experimental data (yellow box). The observed experimental data can consist of data obtained from transcriptomics, proteomics, metabolomics, whole genome sequencing, mutation data, etc. These data are the bases for constructing a network. This network can be inferred statistically if quantitative data is present or the network can be constructed manually based on information derived from the scientific literature (as in our case). Using data from publications, a process descriptive (PD) network can be constructed using e.g. CellDesigner software [23]. The obtained network is a directed, sequential and mechanistic one [22]. The next step is to analyse the network using Cytoscape software [24] and the BiNoM plugin to retrieve a sub-graph with connected nodes of interest. The CellDesigner software is not suitable to perform such analysis. This sub-graph will be then converted manually in to an activity flow (AF) diagram and subsequently reduced by removing nodes that are of no interest. The most reduced AF diagram can then be used for postulating a hypothesis often visualised in a schematic diagram. For a more detailed explanation see text in materials and methods.

first step is to construct a PD network that summarises all possible information about iodide metabolism and thyroid hormone synthesis from published articles. This formation is dispersed among many (e.g. a PubMed search with the key words: iodide, metabolism, thyroid, and synthesis results in more than 5700 hits) scientific articles. The second step is to run the "path analysis" function in a network visualisation software between the following nodes: NIS, AIT, PDS, TPO, Tg, DUOX2, DUOXa2 and MCT8. This function reveals a subgraph with the nodes and edges that are connected to the above mentioned nodes. This sub-graph is then converted into an AF network and subsequently, this AF network will be reduced in the number of nodes by removing nodes from the AF network in several steps. Based on the reduced network, a hypothesis can be formulated how the observed gene expression of NIS, AIT, PDS, TPO, Tg, DUOX2, DUOXa2 and MCT8 [7] may be regulated. For a more detailed description of the workflow see

Cohen et al. [16].

2.1. PD network construction

To manually construct a biochemical network depicting thyroid hormone biosynthesis and iodide metabolism, all relevant information from scientific articles was retrieved from the PubMed database using the molecule(s) of interest as key word(s), e.g. "nis" was used as a query or the combination of "iodide" and "nis" was used as query. Pendrin was used as separate query either in combination with key words or not. No data-mining tools were used. The CellDesigner software (version 4.4) [23] was used to represent molecular biological mechanisms, derived from the literature, resulting in a structured network representation (process-descriptive diagram) compliant with Systems Biology Markup Language (SBML) level 2 that is suitable for further computational analysis [25]. Each reaction has been annotated in the "reaction note" at least once with a corresponding scientific article. The CellDesigner's graphical notation [26] can be converted into the Systems Biology Graphical Notation (SBGN) standard [27] by using the CellDesigner's internal convertor. The file can be downloaded from the supplementary material section in the xml format.

2.2. Structural analysis of the PD network

The constructed PD network, in the shape of a map, contains many details (e.g. post-translational modifications) and many connections which make it difficult to comprehend intuitively which edges and nodes are implicated in the regulation of *nis* upon repetitive administration of KI. Network reduction using the Cytoscape plug-in BiNoM allows obtaining a simplified influence (Fig. 3) network with nodes of interest (NIS, TPO, Tg, PDS, Duox2/A2 and MCT8). The reduced network allows postulating a putative mechanism without the need to display the full details. Such strategy has been applied successfully before for proposing mechanisms behind observed biological phenomena [18].

The PD network that depicts the thyroid hormone biosynthesis and iodide metabolism was analysed using Cytoscape v2.8.3 [24] and the plugin BiNoM v2.5 [28]. The function "Path analysis" that can be found under "BiNoM analysis" section in the plugin, was carried out to extract paths that might be present between the nodes: iodide, NIS, Thyroglobulin, MCT8, Duox2, DuoxA2, Pendrin and TPO. The parameters selected for the path analysis function were "Finding direct path", "the shortest paths", and "use finite search radius" was deselected. The "path analysis" function selected nodes and edges that are connected to the nodes iodide, NIS, Thyroglobulin, MCT8, Duox2/A2, Pendrin and TPO. These nodes and edges were used to create a sub-network in the cytoscape program. A workflow of the path analysis can be found in the Supplementary Information Fig. 1.

2.3. Network conversion and reduction

The sub-network obtained after path analysis of the initial network was manually converted into an activity-flow diagram in order to understand the influence of iodide on the other nodes [18]. A protein catalysing a reaction will have an activating influence on the formed product; an enzyme that inhibits a reaction will have a negative influence on the formed product. On the other hand, the catalysing protein will have a negative influence while the inhibiting enzyme will have a positive effect on the reactant (see Supplemental Information Fig. 2A). The network will contain only arrows instead of arches that depict biochemical interactions. The complexity of the activity-flow diagram was reduced sequentially by removing constituents that do not affect the regulation by iodide on the nodes implicated in thyroid hormone biosynthesis. The aim of the reduction of the AF network is such that only the nodes iodide, NIS, Tg, Pendrin, Duox2, MCT8, TPO and AKT are kept with their regulation on their surrounding neighbours. The other nodes (see Supplemental Information Fig. 2B) are removed in sequential steps i.e. they were not removed all at once but rather one by one to better understand the underlying regulation. Reductionism allowed us to understand the organisation of the network and to find the core paths that are preserved throughout all the reduction steps.

3. Results

3.1. Biochemical network

We have manually constructed and curated a PD network that depicts the biosynthesis of thyroid hormones and the metabolism of iodide in a single thyrocyte (Fig. 2). The network is displayed as a "geographical" map, a way to easily comprehend the displayed information. The map summarises all known up-to-date information about thyroid hormone synthesis and iodide metabolism. The manually constructed network contains 283 chemical species (including 93 proteins, 34 genes and 34 RNA) that are connected with 201 arches/reactions. In total 217 annotations were included to justify each reaction/edge derived from 111 unique scientific articles. The annotations can be found in the Supplementary Information. The complete network can be downloaded either as the xml-file generated by the CellDesigner software or as a svg-file which can be opened by any internet browser.

In short, the map (Fig. 2) shows the canonical pathway of the uptake of iodide by the NIS symporter at the basal membrane in a single thyrocyte. Within the cell, iodide is transported from the basal to the apical side where it is released into the follicular lumen by the ion-channel Pendrin and/or AIT or Ano1 [29–31]. Within the follicular lumen, iodide is "organified" with a thyroglobulin molecule with the aid of TPO and Duox2/A2 proteins. The organification of iodide can result in the thyroid hormones thyroxine (T3) and 3,5,3′,5′-tetariodothyronine (T4) but still coupled to the thyroglobulin molecule. After coupling, the thyroglobulin with T3 and/or T4 is endocytosed and fused with a lysozyme that digest the thyroglobulin molecule and subsequently T3 and T4 are released into the cytosol. The thyroid hormones are then released into the blood stream by the MCT8 thyroid hormone transporter at the basal membrane [32,33].

3.2. Path analysis and network reduction

Path analysis was performed and resulted in a sub-network with 95 nodes and 147 edges (see Supplemental Information Fig. 3). Subsequently, this sub-network has been manually converted into an AF network and the AF network has been reduced in sequential steps (removing nodes in several steps in contrast to removing nodes all at once) to only keep the nodes that have been analysed by Lebsir et al. and the node AKT (Fig. 3). The green and the red arches in the AF network (Fig. 3) show either an activating or inhibiting effect, respectively, on their connected neighbours or on it-self. Two nodes show a self-inhibiting effect: iodide and high-iodinated thyroglobulin molecule (Tg_high). These two nodes have a negative effect on their own activity that goes through other nodes that have been removed during the network reduction but the mechanism can still be found in Fig. 2: e.g. iodide has a negative influence on both *nis* transcription and mRNA maturation (see Fig. 2) and will negatively affect the presence of the NIS symporter in the basal membrane resulting in a less intake of iodide. Besides inhibiting their own activity, iodide and Tg_high have a negative

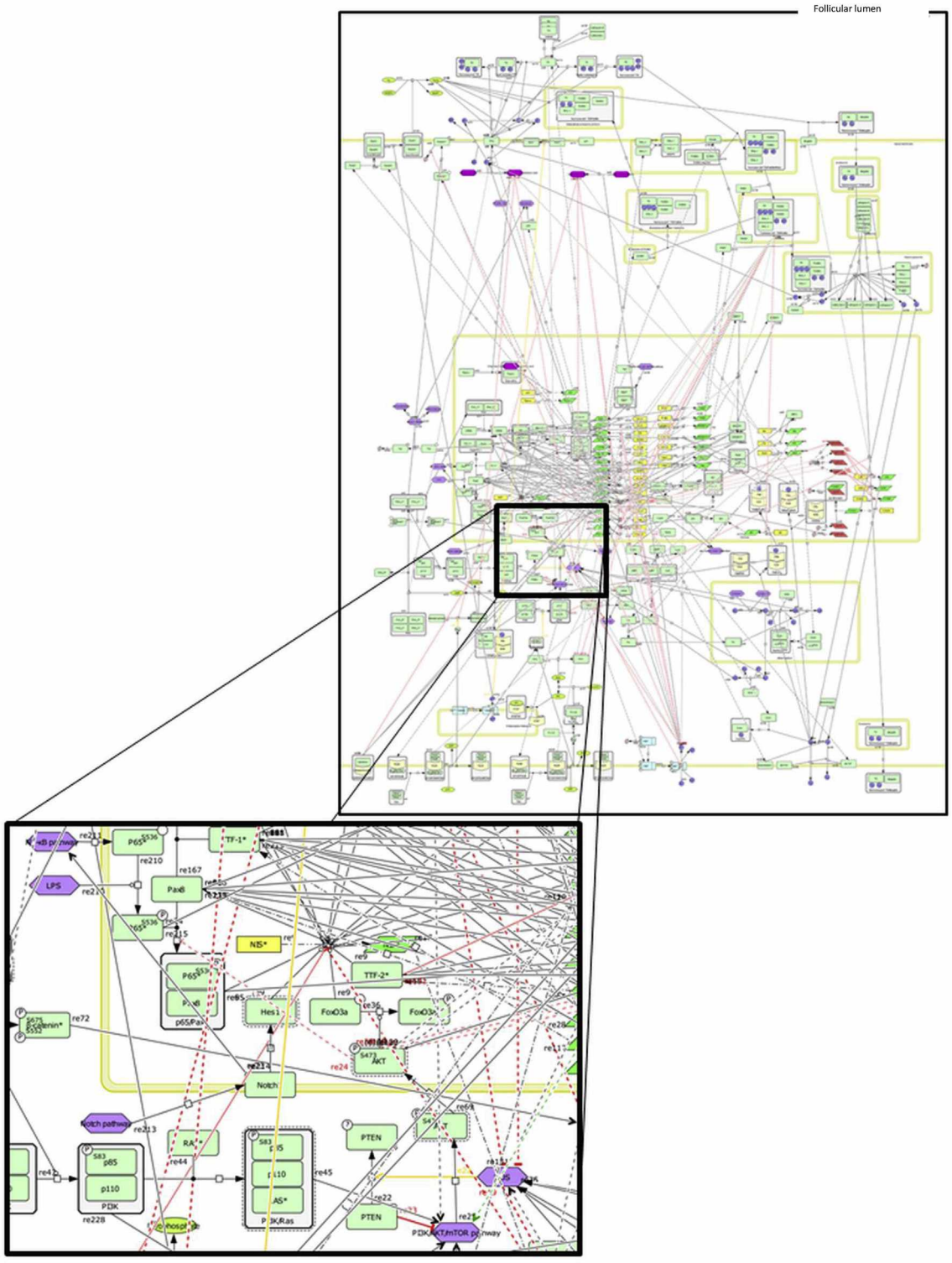

Fig. 2. A "geographical" map of a single thyrocyte. The top shows the apical side of the thyrocyte and the bottom shows the basal membrane of the thyrocyte. The top horizontal line from left to right is the apical membrane. The space above this membrane is the follicular lumen. Within this space there is endosome (rectangle) that contains bound thyroid hormones (T3 and T4). The space under the apical membrane is the cytosol and the large rectangle depicts the nucleus of the thyrocyte containing genes and proteins involved in the biosynthesis of thyroid hormones and the metabolism of iodide. Beneath the nucleus there is a mitochondrion and on the left of the mitochondrion is rectangle depicting the endoplasmic reticulum. The bottom line from left to right is the basal membrane with receptors and other membrane proteins. A process-descriptive (PD) network shows the biosynthetic pathway of thyroid hormones and iodide metabolism. A starting point for the PD network could be the uptake of iodide by the NIS transporter depicted in the middle of the basal membrane. In PD networks, the arches are directed and the networks are sequential [22].

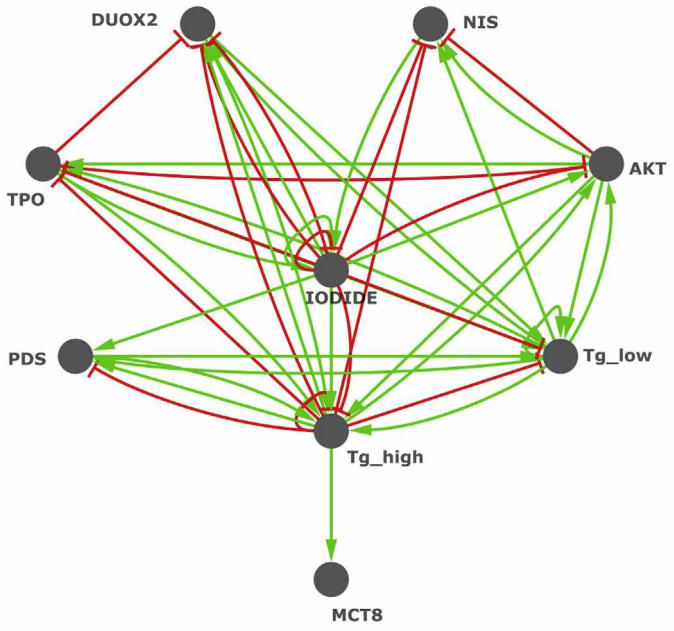

Fig. 3. An activity-flow diagram shows the effect of the connected surrounding neighbours on a node. A red or green arch has a negative or positive effect on a node, respectively. Iodide has clearly a negative influence on many nodes involved in the biosynthesis of the thyroid hormones. The activity of the arches has been deducted using Fig. 1 and the plugin BiNoM in the Cytoscape environment. More details about creating the AF network can be found in the Materials and Method section 2.3.

effect on the other nodes indicating that iodide and Tg_high are inhibiting the thyroid biosynthesis. Furthermore, AKT plays an activating role in thyroid hormone biosynthesis by activating NIS and low-iodinated thyroglobulin (Tg_low).

3.3. Hypothesis

Our hypothesis (Fig. 4A) is based on Fig. 3 that shows the effect of nodes on their connected neighbours. In this schematic view, only the node iodide and the nodes referring to genes that have been analysed before by our group [7] are included to simplify the scheme. In short, Lebsir and colleagues have shown that repeated administration of KI (1 mg/kg/day) for eight days doesn't cause modification of thyroid hormones level, but leads to a reversible modification of the expression of genes involved in the synthesis and secretion of thyroid hormones (*nis, pds, ait, tpo, duox2, duoxa2, tg* and *mct8*). According to the authors a two-step Wolff-Chaikoff effect has been induced: an early Wolff-Chaikoff effect due to down-regulation of *nis* and *mct8* and a late effect marked by a down-regulation of *tpo* and up-regulation of *pds*.

The aim of this study was to find an alternative hypothesis how the involved genes are regulated upon repetitive administration of KI (1 mg/kg/day) for eight days in rats. According to our hypothesis, the before mentioned genes are regulated by iodide and thyroglobulin: Before the administration of KI, we assume there is no need for thyroid hormones (Fig. 4B). Therefore, the intracellular level of iodide is low (or at normal homeostatic level) which leads to the formation of low-organified Thyroglobulin (Tg_low), a thyroglobulin to which a low amount of iodide is bound. Tg_low is able to activate six genes indirectly through PKA activation: *pds*, *nis*, *tpo*, *duox2*, *duoxa2* and *tg* [34]. These genes are required for the synthesis of thyroid hormones and their activation prepares the cell to produce thyroid hormones once iodide is available. Upon KI administration, the intracellular iodide concentration will increase due to its uptake by the symporter NIS (Fig. 4C). In the cell, iodide will stimulate the transcription of *pds* that codes for the anion carrier Pendrin [35]. This allows the translocation of iodide from the cytosol into the follicular lumen. At the same time, iodide down-regulates *nis* expression and decreases NIS mRNA stability [36,37]. In addition, iodide blocks TPO activity as it has been observed before and is known as the Wolff-Chaikoff effect [8,38,39].

However, besides down-regulating NIS and TPO, iodide is also capable of activating both TPO and Tg indirectly through activation of AKT [40–43]. AKT in turn can either inhibit or activate *nis* expression (Fig. 3) by phosphorylating the FOX3A transcription factor that induces the transcription of *nis* [44] or by activating the sterol regulatory element binding transcription factor 2 that induces transcription of *nis* [42], respectively. However, it has been shown that iodide inhibits nis mRNA post-transcriptional; iodide shortens the poly-A tail of the nis mRNA and thus increases degradation of the mRNA [37] even after stimulation by AKT. Therefore we conclude that AKT has no effect in the presence of iodide.

The activation of Pendrin, TPO, and Tg allows the cell to organify iodide into the Tg molecule as a high-organified thyroglobulin (Tg_high) due to high concentration of intracellular iodide (Fig. 4C). An efflux of iodide from the thyrocyte into the follicular lumen by Pendrin and the inhibition of influx of iodide due to down-regulation of NIS by both intracellular iodide and Tg_high results in a lower amount of iodide in the thyrocyte. Subsequently, the transcription of the *pds* gene is not stimulated anymore and the repressive action of iodide on the *mct8* gene is relieved. In contrast, Tg_high will continue down-regulating *nis* expression beside the down-regulation of *tpo*, *duox2*, *duoxA2* and *tg* (Fig. 4D). This can be seen as a negative feed-back loop in response to thyroid hormone production: high-iodinated thyroglobulin is a precursor for the thyroid hormones T3 and T4 [45]. Furthermore, Tg_high increases *mct8* expression allowing the release of the T3 and T4 hormones into the blood stream. To release the thyroid hormones, the thyroglobulin protein into which the hormones are incorporated upon the organification of iodide has to be digested (Fig. 4D). As a result the amount of Tg_high lessens, thereby returning to the initial conditions before KI administration (Fig. 4B).

4. Discussion

The mechanisms behind the high-dosage effect of iodide have been investigated thoroughly; the thyroid hormones synthesis is reduced [8] upon high intra-cellular concentration of iodide [9] and this is known as the Wolff-Chaikoff effect. This effect is transient and its escape involves the sodium-iodide transporter (NIS) and the TPO enzyme [9,46–48]. The mechanism that drives the escape is the decrease in *nis* expression which is reflected in a reduced NIS protein abundance [47]. And more recently, a post-transcriptional regulation of *nis* at the level of mRNA stability has been shown [37]. In addition, the activation of the PI3K/AKT pathway by iodide-induced ROS production inhibits *nis* expression [41]. These mechanisms result in a decrease of intracellular iodide concentration thereby relieving the inhibition on thyroid hormones synthesis.

During chronic administration of KI, *nis* expression is also down-regulated [49,50] and it has been shown that in addition to differential expression of *nis*, the genes *mct8*, *tpo* and *pds* are differentially expressed as well although other groups have not found any difference [7,50–52]. The mechanism behind the regulation of these genes during repetitive administration of KI is currently not known. However, it has been suggested by Lebsir and colleagues that the Wolff-Chaikoff effect during chronic KI administration

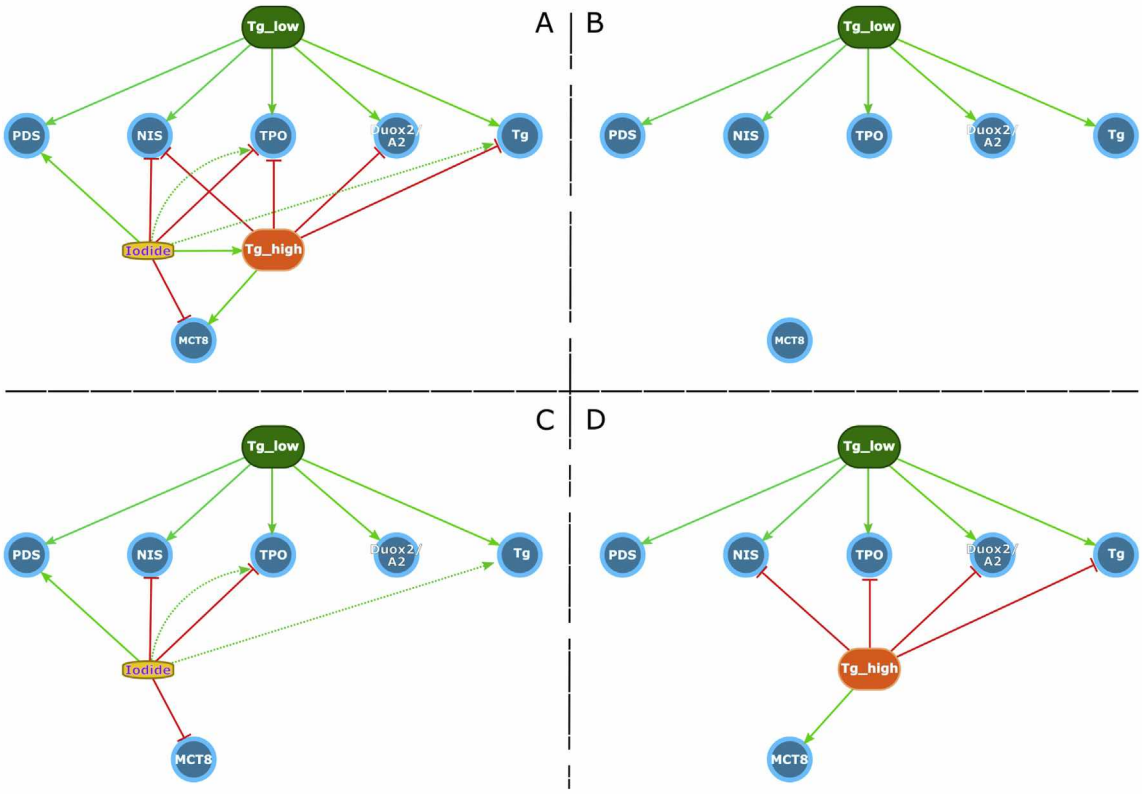

Fig. 4. A) A hypothesis that could explain the gene regulation (blue circles) during repetitive potassium iodide (rectangular box) administration. There are two pools of thyroglobulin: a pool of low-iodinated (Tg_low; green oval) and a pool of high-iodinated (Tg_high; orange oval) thyroglobulin. B) Tg_low is present in the cell when a low concentration of iodide is present in the cell. Tg_low can induce transcription of genes that are involved in the biosynthesis of the thyroid hormones. C) Upon the presence of intracellular iodide, gene expression of *nis* and *tpo* will be inhibited, and transcription of pds and tpo (indirectly) will be activated. This will result in a decrease of intracellular iodide concentration and increase in *de novo* synthesis of high-iodinated thyroglobulin. D) Due to pendrin, there is an absence of iodide which has been incorporated into Tg_high. In the absence of iodide, *nis* is now down-regulated by Tg_high and in addition *tpo, duox2/a2* and *tg* are inhibited to shut down the biosynthesis of the thyroid hormone. In contrast, MCT8 is up-regulated in order to release the unbound thyroid hormones, by digesting Tg_high, in the blood. This will result in the starting point see Fig. 4A.

consists of a two-step mechanism: iodide inhibits *nis* and *mct8* expression promptly upon iodide intake and the second step is a late Wolff-Chaikoff effect that includes inhibition of *tpo* gene expression and increased expression of *pds* both by excessive iodide [7].

To complete and provide new insights into the mechanisms of action involved in the regulation of iodide metabolism in the thyroid, a more holistic approach has been applied to the field of systems biology to study complex interactions within a biological system, i.e. thyrocyte. The aim of the present study is to elaborate on the observed results by Lebsir and colleagues and to propose a putative regulatory circuit that enables the explanation of the observed gene expression during chronic iodide uptake, using a systems biology approach. Such an approach has been used before to hypothesise a possible mechanism that induces the epithelial to mesenchymal transition in colorectal cancer [18]. Furthermore, the application of molecular network as maps in systems biology is becoming more common; there is a scientific community that uses maps of networks as basis for mathematical models, prediction analysis and for hypothesis generation [53,54].

To our knowledge this is currently the most up-dated map created (Fig. 2) with CellDesigner software [23] and it contains more details than similar maps that can be found in the KEGG and Reactome database [55,56]. The thyroid hormone signalling pathway from the KEGG database has 123 entities and 71 reactions, the map of reactome database: regulation of thyroid hormone activity contains 56 entities and 20 arches, respectively. Our map (283 entities, 201 reactions) is manually curated meaning that arches (connections) have been described in published articles and each arch has been annotated at least once (217 annotations from 111 unique articles). As each research group uses his preferred model (e.g. rat, human, mouse) the map contains information from different organisms but the concerned proteins or genes have been conserved among the organisms.

Due to the systems biology approach we have been able to postulate a putative mechanism (Fig. 4) that consists of two main steps: 1) firstly NIS, TPO and MCT8 are inhibited by excessive intracellular iodide which has been proposed as well by Lebsir and colleagues. 2) Secondly, upon depletion of excessive intracellular iodide, high-iodinated Thyroglobulin continues repressing NIS and TPO but activates MCT8 thereby stimulating the release of thyroid hormones into the blood stream.

Systems biology can be used to study a complex biological system by looking at dynamic interactions at different scales or granularity. A fine-grained biological network that displays many details like phosphorylation of proteins or other post-translational modification, like the map in the current study, is called a Process Description diagram. Such graphs summarise the relations between different molecules in a biological system. In addition, looking at the dynamics of the system over time might be necessary to understand (better) a biological system. Mathematical models based on ordinary differential equations (ODE) or partial differential equations (PDE) can model temporal (continuous time treatment) and spatial dynamics in great detail but are limited to the available computational power with increasing model complexity [57]. With logic-based Boolean modelling it is possible to model

large numbers of molecules using time as discrete steps but the results of the modelling are not quantitative but qualitative [16,57]. These type of maps and dynamic models are the bases for Adverse Outcome Pathway or dynamic Adverse Outcome Pathway (AOP) models, respectively [58–60]. The AOP discretizes a process into several events; the molecular initiation event, e.g. binding of compound to receptor, which results in a cellular response, also called a key event(s) [60]. The cellular response can be measured by looking at gene expression, protein abundance, concentration of metabolites, etc. The causal relations between genes, proteins and metabolites can be depicted by using a network. The depending on the granularity of the network (i.e. AF-network) a qualitative model can be build and when more details become available a PD-network can be constructed and quantitative modelling can be applied [60].

In the present study we propose a putative mechanism behind NIS regulation during repetitive administration of KI. Not only iodide is responsible for the regulation of *nis* and *tpo* but sequential regulation by the highly-organified thyroglobulin protein affects *nis* and *tpo*. In addition, the highly-organified thyroglobulin regulates *duox2*, *mct8* and itself (*tg*). Although we have shown a possible path from repetitive excessive iodide to several genes that are involved in the biosynthesis of thyroid hormones and iodide metabolism, a formal mathematical model taking the many regulations on those genes into account has not been performed. Our hypothesis may serve as the basis for constructing a dynamic model in order to study the systems' dynamics, control/regulation mechanism, and design properties [61]. With Boolean modelling for example it is possible to test qualitatively *in silico* simulations [19,20,62]. Of course the ultimate validation would be an experimental one to test if our hypothesis is correct. For example, the effect of KO of the highly-organified thyroglobulin on the gene expression during 8 eight days for every hour could might validate our hypothesis of the sequential regulation by Tg_high on *nis* and *tpo*. Or the effect of AKT KO on the transcription of *Tg* gene.

With this study, we have demonstrated a putative mechanism behind *nis* regulation (this present study) and non-toxic adverse effects upon KI repetitive administration [7] which may help to evaluate the current guidelines for avoiding contamination of the thyroid upon nuclear accidents.

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Declaration of interest

We have no conflict of interest to declare.

Author contribution

Conceived and designed the experiments: DC, MS; Performed the experiments: DC; Analysed and interpreted the data: DC; Contributed reagents, materials, analysis tools or data: DL; Wrote the manuscript: DC; DL; MB; MS. All authors have approved the final article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biochi.2019.04.024.

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