HHS Public Access

Author manuscript

Mutat Res. Author manuscript; available in PMC 2018 July 01.

Published in final edited form as:

Mutat Res. 2017 July ; 773: 274–281. doi:10.1016/j.mrrev.2016.08.006.

Potentiation of Hydrogen Peroxide Toxicity:

From Catalase Inhibition to Stable DNA-iron Complexes

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Abstract

Hydrogen peroxide (H_2O_2) is unique among general toxins, because it is stable in abiotic environments at ambient temperature and neutral pH, yet rapidly kills any type of cells by producing highly-reactive hydroxyl radicals. This life-specific reactivity follows distribution of soluble iron, Fe(II) (which combines with H_2O_2 to form the famous Fenton's reagent), — Fe(II) is concentrated inside cells, but is virtually absent outside them. Because of the immediate danger of H_2O_2, all cells have powerful H_2O_2 scavengers, the equally famous catalases, which enable cells to survive thousand-fold higher concentrations of H_2O_2 and, in combination with adequate movement of H_2O_2 across membranes, make the killing H_2O_2 concentrations virtually impractical to generate in vivo. And yet, low concentrations of H_2O_2 are somehow used as an efficient biological weapon. Here we review several examples of how cells potentiate H_2O_2 toxicity with other chemicals. At first, these potentiators were thought to simply inhibit catalases, but recent findings with cyanide suggest that potentiators mostly promote the other side of Fenton's reaction, recruiting iron from cell depots into stable DNA-iron complexes that, in the presence of elevated H_2O_2, efficiently break duplex DNA, pulverizing the chromosome. This multifaceted potentiation of H_2O_2 toxicity results in robust and efficient killing.

Keywords

hydrogen peroxide; Fenton's reaction; iron metabolism; nitric oxide; cyanide; catastrophic chromosomal fragmentation

Life-specific reactivity of H_2O_2

Hydrogen peroxide, H_2O_2, is a metastable oxygen species and an important intermediate in the redox pathway linking molecular oxygen, O_2, to the fully reduced oxide in water, H_2O. The complete pathway (1), $O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow$ (2)$OH\cdot \rightarrow$ (2)H_2O, also includes a relatively unstable superoxide (O_2^-), as well as the extremely reactive hydroxyl radical

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

(OH·), which is the species responsible for the "reactivity" of all reactive oxygen species (2). Paradoxically, hydrogen peroxide is generally non-reactive with organic compounds (3–5); in everyday life, 3% (~0.8 M) hydrogen peroxide solution is a common household antiseptic, stored in plastic bottles for years. H_2O_2 does not directly interact with pure biopolymers (nucleic acids, demetallated proteins, polysaccharides, lipids); perhaps, the only exceptions are thiol-based sensor proteins (like OxyR in bacteria), reacting to the presence of micromolar levels of H_2O_2 with formation of disulfide bonds that are used for signaling (6). At the same time, hydrogen peroxide is a surprisingly potent bio-toxin, as the same 3% H_2O_2 solution kills all kinds of cells within several minutes (7, 8), that is as soon as H_2O_2 penetrates the cell wall barrier.

The main reason H_2O_2 is generally stable in abiotic environment, yet becomes so reactive upon contact with life, is the differential availability of soluble iron. Since H_2O_2 by itself does not react with organic compounds (with the above exception of select thiol groups in a few signaling proteins), it would be completely innocuous, if not for the fact that iron is broadly employed by all types of cells in catalysis of many essential metabolic reactions and transitions (9). Iron can be found in two forms: the soluble Fe(II) and the practically insoluble Fe(III) (10). In the current oxidative atmosphere of the Earth, at least in the oxic environments, the trace amounts of soluble Fe(II) iron are rapidly oxidized by usually more abundant H_2O_2 to Fe(III) iron (11), which does not react with the remaining H_2O_2, explaining the general H_2O_2 stability in abiotic environment. But exactly due to this reason, the only soluble iron in the environment outside cells is represented by the (highly variable) trace amounts of Fe(III) complexed with natural organic ligands (12–14). The limited and unpredictable availability of Fe(III) iron forces cells of all types to actively procure and stockpile iron to maintain their metabolism and support multiplication (15), accumulating 0.1–1.0 mM total iron (10, 16–18). But even inside the cell, the availability of free Fe(II) iron is limited and tightly controlled (15), because when free Fe(II) and H_2O_2 meet, Fenton's reaction occurs.

In this famous reaction (19–21) (Fig. 1), soluble Fe(II) iron donates one electron to a hydrogen peroxide molecule, causing its decomposition: $Fe(II) + H_2O_2 \rightarrow Fe(III) + OH\cdot + OH^-$ that produces hydroxyl radical capable of reacting with any organic compound at diffusion rates (5, 22). Hydroxyl radicals kill via DNA damage, as indicated by the exquisite H_2O_2 sensitivity of DNA repair mutants (23–25). Fenton's reaction is the reason why otherwise relatively innocuous extracellular H_2O_2 becomes a potent poison once inside the cell. At the same time, due to its small size and lack of charge, H_2O_2 shows substantial permeability through the membrane barrier (26, 27), so exposed cells cannot simply block the entry of this "conditional" poison. This life-specific reactivity in combination with substantial membrane permeability makes hydrogen peroxide a popular weapon in bio-warfare: our immune cells use it to kill invading microbes (28–30), bees use it as a honey preservative (31), lactic acid bacteria generate it to kill off the competition (32) (and are one of the few life forms that learned to do without iron in their metabolism (33)), ants (if given a choice) take it as an anti-fungal medication (34), while plants employ hydrogen peroxide to reduce grazing by herbivores (35).

H_2O_2 is impossible to concentrate in vivo

However, this strategy of production of a diffusible life-specific source of hydroxyl radicals suffers from the problems of targeting and is also undermined by efficient detoxification. Because superoxide and hydrogen peroxide in sub-micromolar concentrations are byproducts of aerobic metabolism (36), and because both H_2O_2 and O_2^- are commonly employed in pathogen-defense mechanisms, the cells are equipped with powerful catalases (37) (Fig. 1) and superoxide dismutases (38). Both are rare examples of diffusion-limited enzymes — the fastest enzymes possible (39) — capable of scavenging up to low millimolar concentrations of hydrogen peroxide during acute exposures without adverse consequences for the cell, once H_2O_2 is removed. Even though 3 mM concentrations of H_2O_2 will eventually kill during prolonged exposures (40), the H_2O_2 concentrations that kill cells within minutes start around 30 mM (24, 41). This creates a classic engineering problem: besides the obvious caveat that such high H_2O_2 concentrations will be dangerous to the producing cell itself, no known cells are actually capable of producing a short burst of 30 mM H_2O_2, or of maintaining a several-hour 3 mM levels of H_2O_2 (perhaps with the exception of lactobacilli (42)).

In fact, for our leucocytes, the real problem appears several orders of magnitude greater. Due to the abovementioned substantial H_2O_2 permeability, it is almost impossible to achieve a significantly higher concentration of H_2O_2 in any cellular compartment relative to the rest of the cytoplasm, especially if its production is slow and indirect, which is thought to be the case in the phagosome (43, 44). That is, if a leucocyte targets superoxide production (that generates H_2O_2 by dismutation) exclusively to the phagosome around a captured bacterium, the continuous escape of H_2O_2 from the phagosome will keep the maximal H_2O_2 concentration ~3 μM (43, 45, 46), about 1,000 times lower than that required for slow killing, not to mention the fast killing. So, how do the cells solve this problem of creating lethal local concentrations of a readily-diffusible toxin?

Potentiated toxicity of H_2O_2

Theoretically, this engineering problem has an elegant chemical solution. Indeed, the cellular systems that negate H_2O_2 effectiveness (for example, H_2O_2 scavengers or DNA repair pathways) could be inactivated with a different agent, to either increase the effective intracellular H_2O_2 concentrations or to make DNA damage irreparable. In particular, catalases have heme in their active centers (37), so any simple chemical that binds heme iron tightly (NO, CN, H_2S (47, 48)) will inhibit catalases and thus will reduce the killing concentrations of H_2O_2. In fact, catalases *could have been* the original target of the evolutionary arms race, as the cells have a second hydrogen peroxide scavenging enzyme, called alkylperoxidase, which is effective against low H_2O_2 concentrations and that has reaction chemistry very different from the chemistry of catalase (49), meaning that the same inhibition principle will not work against both enzymes. Catalase poisoning has been offered to explain potentiation of H_2O_2 toxicity by other chemicals (Fig. 1), however the inefficiency of this poisoning limits the power of this explanation (41, 50–52). Important for our discussion, though, is the concept of catalase inhibition by a separate agent, which

highlights a general strategy for solving the *in vivo* hydrogen peroxide concentration problem, the strategy of **potentiated toxicity**.

Potentiated toxicity is one of the two types of a more general phenomenon of **synergistic toxicity**, when two agents in sublethal concentrations that do not kill individually, efficiently kill when used together (53–55). Even though synergistic toxicity conceptually sounds like the bio-analog of the principle behind binary weapons (projectiles loaded with two relatively non-toxic chemicals, which combine into a potent toxin during their short flight toward the target (56)), the mechanisms behind synergistic toxicity are different. One of the two basic scenarios of how two biotoxins synergize is *redundancy*, when both toxins target the same essential cellular process, and their combined action overwhelms the robustness limit of this process, producing "co-toxicity". According to the co-toxicity rules, either agent alone in high enough concentrations is expected to be toxic. In contrast, the second basic scenario of biotoxin synergy, *potentiated toxicity*, envisions that one of the agents is not capable of killing the cells in any concentration, but inactivates an antidote against the second agent, which is the real toxin (41, 57, 58). A classic example from everyday life is clavulanic acid, an inhibitor of beta-lactamase, that potentiates the action of amoxycillin against resistant bacteria without being toxic itself (59). The helpful aspect of H_2O_2 toxicity potentiation is that if the second agent is a charged molecule, it will not be able to easily cross the membrane barrier and escape from the phagosome, where the cell tries to accumulate H_2O_2. In other words, the concentration of H_2O_2 may be only ten times higher inside the phagosome than outside of it, but it is the concentration of the charged potentiating agent inside the phagosome that will make H_2O_2 toxic only within the compartment. Thus, potentiated toxicity not only dramatically reduces the killing concentrations of the toxin that the cell has to produce, but also provides the means to target specific cellular compartments.

How NO potentiates H_2O_2 toxicity

Of the several examples of H_2O_2 toxicity potentiation by other simple chemicals, the best known case is observed in our immune cells that kill infecting microbes with a mixture of hydrogen peroxide and nitric oxide (NO) (28–30). NO is bacteriostatic for wild type bacteria (28, 60, 61), inhibiting their general metabolism by binding to heme iron and thus blocking respiration (62, 63). For targeted NO production, our leucocytes have a phagosome-associated nitric oxide synthase (64); together with (phox) NADPH oxidase that generates superoxide (65) (which then dismutes to H_2O_2 (44)), this produces the potently-toxic NO + H_2O_2 mix only within the phagosome.

The mechanism of NO potentiation of H_2O_2 toxicity turns out to be complex, as NO affects many enzymes and processes. By readily binding to heme iron, NO inhibits catalases (66, 67), increasing the effective intracellular H_2O_2 concentrations (52, 68) (Fig. 1, scenario #1). In addition, NO binding to cytochrome oxidases inhibits respiration (69–71) decreasing the cellular ATP pools, which should inhibit any repair of complex DNA lesions that requires ATP hydrolysis (72, 73). Finally, NO directly inhibits Fpg DNA glycosylase, that removes the major types of oxidized bases from DNA (74, 75), which should further increase susceptibility of NO-treated cells to oxidative DNA damage. Thus, NO could potentiate

H_2O_2 toxicity by interfering both with H_2O_2 scavenging and with subsequent repair of oxidative DNA lesions (Fig. 1).

Investigation into the metabolic underpinnings of NO + H_2O_2 co-toxicity in *E. coli* has substantially broadened the picture, though. First, iron chelation blocks the NO + H_2O_2 co-toxicity (30, 76), indicating participation of free intracellular iron. It turns out that NO-induced respiration inhibition causes accumulation of NADH and, indirectly, greatly expands the pool of reduced flavin mononucleotides (FMNs) (76), used by iron-siderophore reductases to recycle insoluble Fe(III) back into soluble Fe(II) (77), which promotes Fenton's reaction (Fig. 1, scenario #2). Remarkably, the proposed mechanism (76) expands the emphasis of NO potentiation of the H_2O_2 toxicity from simple interference with H_2O_2 scavenging and/or repair of oxidative DNA damage to the increased availability of the second component of Fenton's reaction, the free reduced iron (Fig. 1). The mechanistic diversification of the potentiation phenomenon makes it robust and efficient.

Some other common potentiators of H_2O_2

A few other simple chemicals potentiate H_2O_2 toxicity in both bacterial and mammalian cells. Ascorbic acid (AA) potentiates H_2O_2 toxicity, both *in vivo* (78–81) and *in vitro* (82). The enhancement of the intracellular oxidative potential of H_2O_2 by AA can be blocked by intracellular iron chelators, suggesting that AA increases the pools of intracellular free iron (83). Curiously, many cancer cell lines are sensitive to extracellular AA concentrations innocuous for normal cells, and this sensitivity is relieved by extracellular catalase, indicating the AA + H_2O_2 co-toxicity as the underlying cause of their sensitivity (84).

Amino acid L-histidine potentiates H_2O_2 toxicity (85–88) (reviewed in (89)). The His + H_2O_2 co-toxicity is associated with formation of double-strand DNA breaks and is blocked by iron chelation (86, 87, 90, 91). Histidine also potentiates pure DNA nicking by Fenton's reaction *in vitro* (91, 92).

L-cysteine, the only redox-active amino acid, also potentiates hydrogen peroxide toxicity (85, 93, 94). Cystine, the oxidized dimeric form of cysteine, also potentiates H_2O_2 toxicity, but in a transient way, by temporarily increasing the intracellular cysteine levels (93–95). Cysteine potentiation is blocked by *in vivo* chelation of iron (93, 94). Cysteine also potentiates pure DNA nicking by Fenton's reaction *in vitro* (94).

Potentiation by cysteine may work via its intracellular conversion into hydrogen sulfide (H_2S) (85), which is another known potentiator of cell killing by H_2O_2 (50, 51, 85). Treatment with hydrogen sulfide inhibits cellular catalase, suggesting the obvious mechanism of its potentiation of H_2O_2 toxicity (50, 51) (Fig. 1). As with NO or L-Cys above, intracellular iron chelation abolishes H_2S + H_2O_2 co-toxicity, suggesting that H_2S enhances the availability of free intracellular iron (85).

Recently, the findings that NO (96) and H_2S (97) protect *E. coli* against antibiotics were interpreted in terms of protection against oxidative damage, based on another recent proposal that lethal oxidative damage is the common mechanism behind antibiotic action (98). However, this review presents enough evidence that both NO and H_2S *potentiate* H_2O_2

poisoning, rather than reduce it, in contrast to the prediction from NO or H_2S protection against antibiotics (96, 97). In light of the recent strong evidence of no oxidative damage associated with antibiotic treatment (41, 99), the NO and H_2S protection most likely works by slowing down cellular metabolism, which would make bacterial cells more tolerant to a broad spectrum of cell-damaging treatments.

The paradoxes and complexities of CN potentiation of H_2O_2 toxicity

The emerging pattern of H_2O_2 potentiation, while leaving aside the importance of control over the intracellular H_2O_2 concentration, does emphasize the role of available iron. A recent study of yet another strong H_2O_2 potentiator, cyanide (25, 41), while further solidifying the critical role of iron, brings back into focus stable DNA-iron interactions and highlights yet another important aspect of this multifaceted toxicity — timely DNA repair.

Like NO above, cyanide (CN) is a “classic” strong-field isoelectronic diatomic ligand for heme iron and a strong potentiator of H_2O_2 poisoning (100). Investigation of the mechanisms of CN potentiation of H_2O_2 toxicity (101) was a trailblazer for the subsequent study of NO potentiation (76). By inhibiting heme-containing enzymes (the preferred target of CN binding) and thus blocking respiration, CN was found to increase the intracellular pool of NADH, which would translate into a higher pool of reduced FMNs and would, therefore, elevate the pools of free soluble Fe(II) iron (101). In fact, this general scenario would work for all known H_2O_2 potentiators, since NO, CN, H_2S, as well as the amino acids cysteine and histidine, are all heme ligands (47, 62, 102–104). Ascorbate is the only exception in this list, but it acts as an electron donor for some heme-containing enzymes (105), and so could be considered a “transient ligand”.

However, equating H_2O_2 potentiators with heme ligands creates a paradox, as the best diatomic competitor of O_2 for binding heme iron is the notorious carbon monoxide (CO) (48, 102), which, according to the hypothesis above, should be among the strongest potentiators of H_2O_2 toxicity. To the contrary, CO is known to protect mammalian cells against H_2O_2 toxicity (106–111). In other words, binding to heme iron may be a common property of the H_2O_2 potentiators, but it does not have to be the mechanism of potentiation.

In fact, our recent investigation of CN + H_2O_2 co-toxicity was inconsistent with the respiration block scenario of H_2O_2 toxicity potentiation, although we did confirm the involvement of reduced FMNs in the process (41). Instead, we found evidence for a specific multi-stage scenario (Fig. 2): 1) cyanide recruits (releases) iron from intracellular iron depots, ferritin being one such iron donor; 2) the recruited (released) iron is somehow delivered directly to DNA and forms stable iron-DNA complexes; 3) these DNA-bound iron atoms, if not promptly removed (for example, by the ferritin-like Dps proteins), catalyze ROS-generating Fenton’s reaction right on the most sensitive target of the cell, the chromosomal DNA; 4) cyanide further potentiates this DNA-self-targeted Fenton’s reaction in an unknown way; 5) expeditious repair of the resulting single-strand breaks and lesions (with which CN may also interfere) somehow facilitates iron removal from DNA, giving DNA one more chance to avoid imminent double-strand breaks, — otherwise catastrophic

chromosomal fragmentation ensues (25, 41). Below we discuss some aspects of CN potentiation of H_2O_2 toxicity.

Iron depots and lockups, stable iron-DNA complexes, and ligand-dependent Fenton's variations

Ferritins are 24-subunit thick-walled spheres that manage the levels of cytoplasmic Fe(II) iron using molecular oxygen and depositing excess iron as crystals of Fe(III) oxide (15, 112). At the same time, ferritins not only can accumulate up to 4,500 Fe(III) atoms, but they also release the sequestered iron on specific cues to proteins that are to be iron-metallated (15, 112), functioning as iron-depot proteins. Due to ferritin's general affinity for DNA (113–115), cyanide-compromised ferritins should release iron (Fe(II)) in the vicinity of DNA, as a result, stable DNA-Fe(II) complexes will form (116). In the presence of H_2O_2, stable DNA-iron complexes become the sites of formation of hydroxyl radicals on chromosomal DNA. To emphasize the role of these stable DNA-iron complexes in H_2O_2-promoted DNA demise, we introduced the term "DNA-self-targeted Fenton's reaction", which describes the unique susceptibility of nucleic acids to iron-catalyzed reactive oxygen species.

Ferritin homologs Dps proteins of bacteria are called mini-ferritins, because they assemble into much smaller 12-subunit spheres, packing only 500 Fe(III) atoms (15, 112). Dps mini-ferritins are important for bacterial DNA protection against oxidative damage, especially in stationary cells: in contrast to "maxi-ferritins" that oxidatively deposit iron using O_2, mini-ferritins use H_2O_2 for this purpose. In effect, when H_2O_2 appears in the bacterial cytoplasm, mini-ferritins sequester all free iron, functioning as "iron-lockup" during H_2O_2 stress. Since, in addition, Dps proteins have a high affinity for DNA (117), mini-ferritins represent a perfect "sponge" for mopping up iron from DNA-iron complexes in the presence of H_2O_2. At the same time, Dps does not appear to be the predominant iron storage in stationary cells, as at least half of iron in stationary *E. coli* is still found in ferritin (118). The complete resistance of stationary cells to CN + H_2O_2 treatment could be due to either the efficient lockup of the released iron by Dps, or due to DNA binding by this protein overproduced in stationary phase; the sensitivity of the stationary *dps* mutant cells to CN + H_2O_2 treatment is consistent with either idea (41).

Presumably, a low level of free iron binding to DNA and removal from DNA by specialized proteins happens in the cell continuously (82, 119, 120). Iron has a high affinity to DNA and binds it tightly in solutions. Two binding sites for iron on DNA, one at the backbone, while the other at the bases, have been proposed (121–123). In fact, it was suggested on multiple occasions that when iron has a freedom to bind DNA in the cell, the bulk of lethal oxidative DNA lesions comes from such stable DNA-iron complexes (25, 124–128). Remarkably, the rate of formation of oxidative lesions at such stable DNA-iron complexes in cells, apparently limited by the very low intracellular concentration of H_2O_2, is slow enough to allow DNA repair to expedite iron removal from the damaged DNA, effectively eliminating formation of double-strand breaks. At the same time, when the concentration of H_2O_2 increases during the treatment, it takes only a couple of minutes for such stable DNA-iron complexes to,

figuratively speaking, “burn through” DNA, breaking both strands in the same location (25) (Fig. 2).

The proposed new model of H_2O_2 toxicity potentiation needs rigorous experimental testing, as cyanide by itself is a strong iron ligand (129), and the resulting ferrocyanide complexes, especially ferric ferrocyanide (“Prussian blue”) (130), are notoriously inert compared to the moderately reactive ferrous iron. Because of this, for example, cyanide completely blocks a classic *in vitro* Fenton’s reaction that measures decomposition of an organic dye by hydroxyl radicals (41). At the same time, similar to cysteine (94) or histidine (91, 92), cyanide further accelerates Fenton’s reaction with DNA strand scission as a read-out (41), demonstrating that, in complexes with DNA, iron reacts to cyanide differently from iron in solution lacking DNA. It could be that the stable DNA-iron complexes (121, 122, 131) have a configuration resistant to CN chelation, but capable of accepting CN potentiation of the unique DNA self-targeted Fenton’s reaction.

Conclusion

The truly multifaceted mechanism of CN potentiation of H_2O_2 toxicity (comprising all the processes listed in Figures 1 and 2) makes the resulting cell killing robust and unavoidable, but is it applicable to other potentiators of H_2O_2? Remarkably, the CN route of H_2O_2 potentiation via iron Fe(II) recruitment from ferritins (41) is likely shared with other H_2O_2 potentiators, which are also known to release iron from ferritin, including NO (132), H_2S (133), ascorbate (134, 135), cysteine (135, 136) and flavin mononucleotides (135, 137, 138). In other words, iron recruitment from ferritins may represent a general strategy, rather than a peculiarity of CN potentiation of H_2O_2 toxicity. Moreover, two or more different potentiators could work together, for example, in the same cellular compartment (phagosome), enhancing each other’s action. Such a co-potentiation between NO and CN has been already reported before in *E. coli* cultures and was proposed to occur in our immune cells (60). By pumping several different membrane-impermeable potentiators into the same compartment (phagosome), the cell would be able to significantly reduce the killing concentrations of the poison, H_2O_2, in this compartment.

Potentiated toxicity is a unique phenomenon of one agent inducing a non-lethal change in the cellular metabolism that renders cells vulnerable to lethal poisoning by a different agent and can be used to design two-component biocides to fight infections and cancer. It is a part of the more general phenomenon of synergistic toxicity, when two agents, both in safely sublethal concentrations when used alone, cooperate to cause massive lethality when applied together. The phenomenon of synergistic toxicity has a genetic counterpart in the phenomenon of synthetic lethality, in which a combination of two mutations with small individual effects proves nonviable, revealing a gap in our understanding of cell’s metabolism (139–141).

Synthetic lethality is a highly sought-after phenomenon, because isolation of its suppressors (a third mutation that makes the combination viable again) frequently provides a mechanistic understanding of the lethality (142, 143). Similar to synergistic toxicity, there are two general explanations for synthetic lethality (143). The redundancy explanation posits that the

products of the two inactivated genes perform essentially the same enzymatic function, removing a poisonous substrate or synthesizing an essential product. In this sense, the two missing functions are “metabolically proximal”, and the suppressors of synthetic lethality all act to restore the missing function, typically by activating a cryptic activity that functionally overlaps the two mutated activities. In contrast, the potentiation explanation (also known as “defect-damage-repair cycle” (143) or “avoidance-repair couple”) posits that one mutation inactivates a damage-*avoidance* function, while the other mutation inactivates the damage-*repair* function. The two inactivated functions in this case perform completely unrelated, “metabolically distal” enzymatic reactions, and the suppressors of synthetic lethality in this case inactivate the gene(s) responsible for poisoning.

Synthetic lethality is considered to add a second dimension to an otherwise linear mutation analysis, while isolation of suppressors of synthetic lethals makes genetic analysis three-dimensional. Similarly, the phenomenon of synergistic toxicity adds another dimension to the mechanisms of cell poisoning. In the future, it should be instructive to apply a genetic dimension to the analysis of co-toxicity, by isolating resistant mutants.

Acknowledgments

We would like to thank Glen Cronan for enthusiastic discussion of our results and for copyediting. Experimental research in this laboratory was supported by grant # GM 073115 from the National Institutes of Health.

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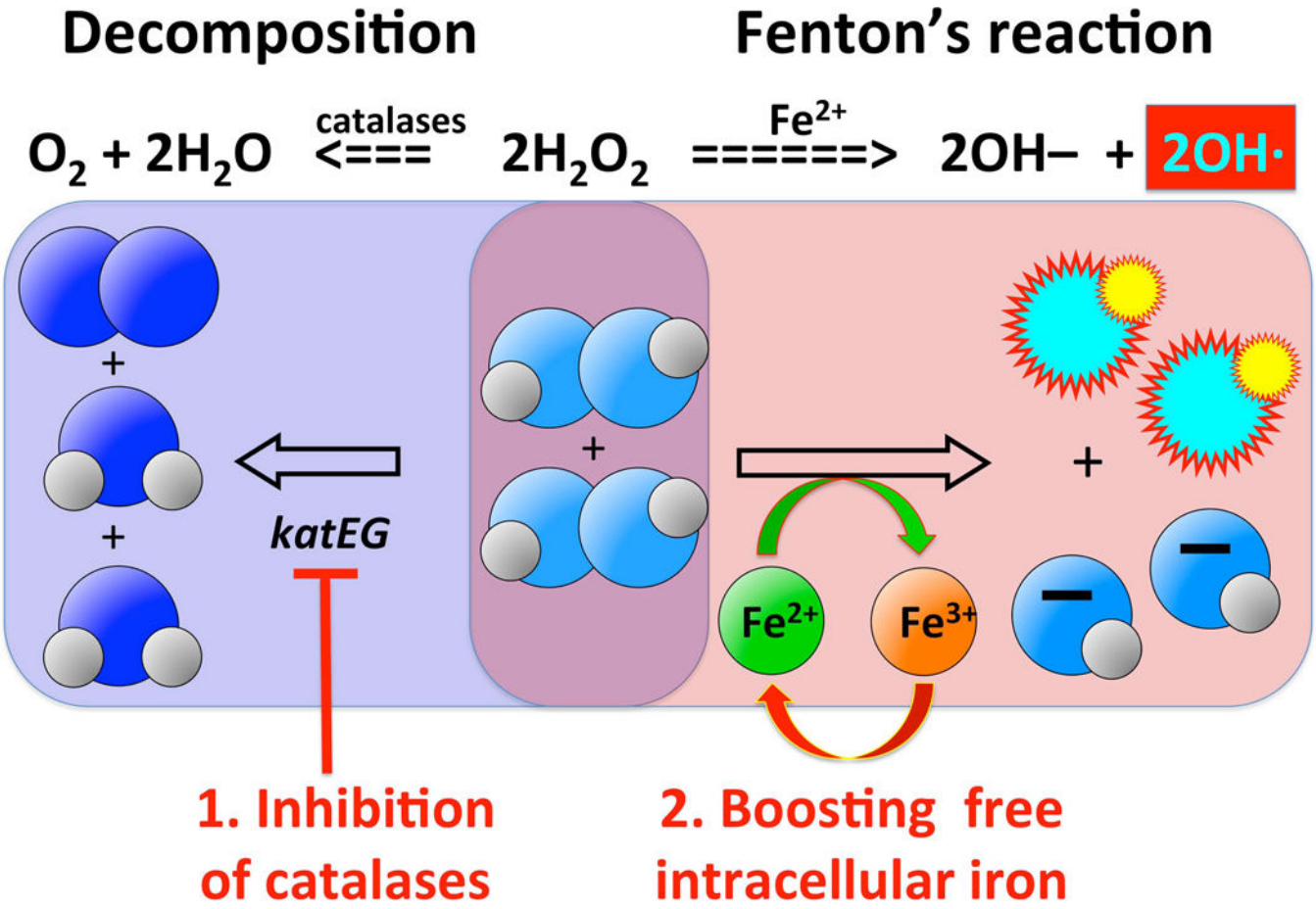

Fig. 1. Hydrogen peroxide scavenging by catalases versus "radicalization" by soluble iron (Fe(II)), and the two obvious strategies to potentiate H_2O_2 toxicity

Hydrogen atoms are small gray spheres. Oxygen atoms are spheres of various shades of blue: the darker the blue, the more stable this oxygen atom is. At the top, in black font, the two opposite in vivo fates of hydrogen peroxide are shown as formulas. At the bottom, in red font and symbols, the corresponding potentiation strategies are indicated: 1) inhibition of H_2O_2 decomposition (or DNA repair); 2) increasing the concentration of soluble intracellular iron.

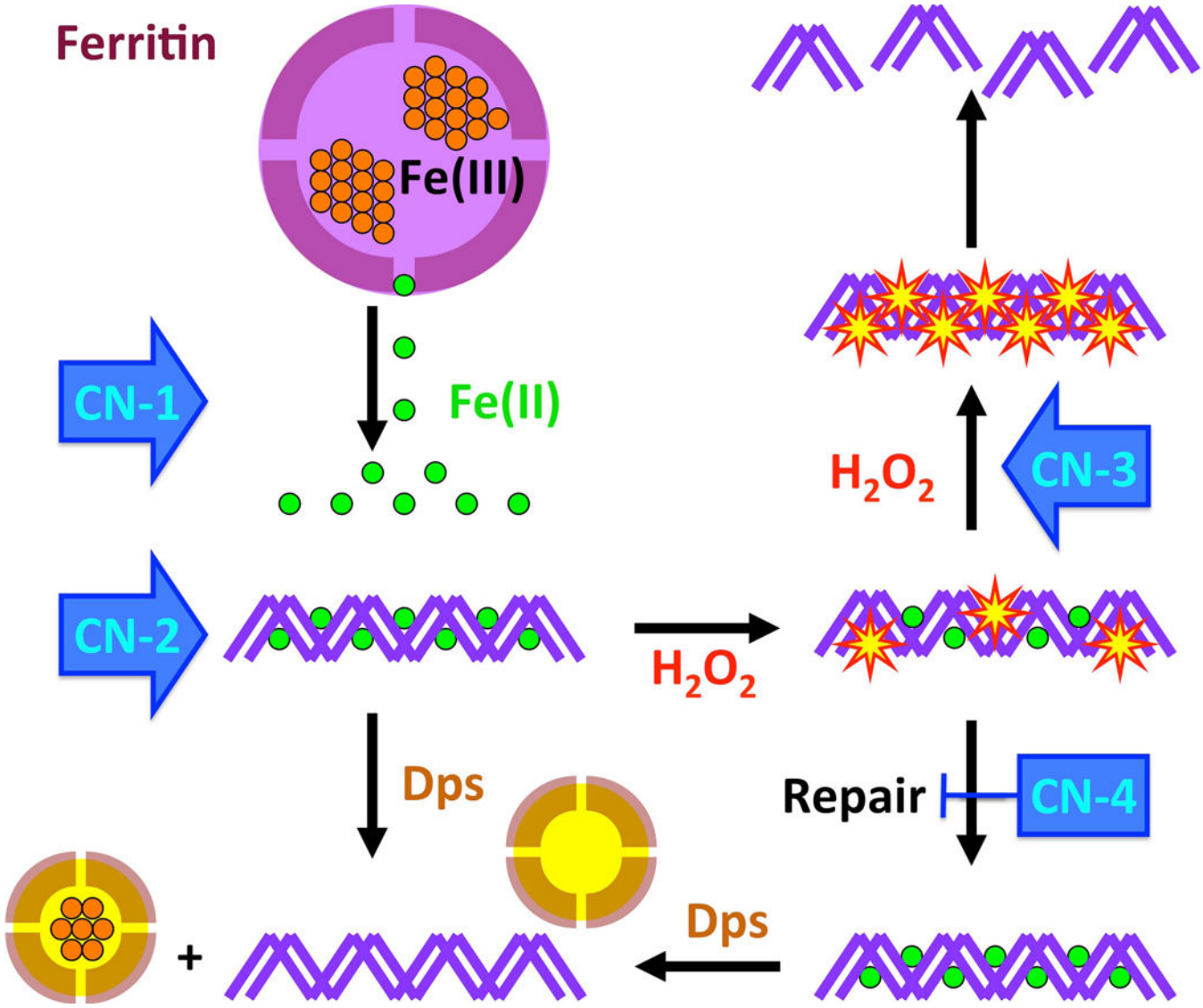

Fig. 2. Cyanide potentiation of hydrogen peroxide toxicity is proposed to happen at least at four separate stages

Small green circles, Fe(II) atoms; small orange circles inside ferritin spheres, Fe(III) atoms. Purple double zig-zag, duplex DNA. The big ferritin depots (purple spheres) release their iron in response to specific signals. Dps lockups (brown-yellow thick-walled spheres) remove iron from the DNA-iron complexes. The distinct opportunities for potentiation, marked by “CN-1, 2, 3, 4” arrows, are: 1) Fe(II) recruitment (“iron release”) from the intracellular iron depots; 2) direct formation of stable DNA-iron complexes; 3) promotion of DNA self-targeted Fenton; 4) Inhibition of one-strand DNA repair.