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Chemiexcitation and its Implications for Disease

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Abstract

Quantum mechanics rarely extends to molecular medicine. Recently, the pigment melanin was found to be susceptible to chemiexcitation, in which an electron is chemically excited to a high energy molecular orbital. In invertebrates, chemiexcitation causes bioluminescence; in mammals, a higher-energy process involving melanin transfers energy to DNA without photons, creating the lethal and mutagenic cyclobutane pyrimidine dimer that can cause melanoma. This process is initiated by NO[•] and O₂^{•-} radicals, the formation of which can be triggered by ultraviolet light or inflammation. Several chronic diseases share two properties: inflammation generates these radicals across the tissue and the diseased cells lie near melanin. We propose that chemiexcitation may be an upstream event in numerous human diseases.

Keywords

chemiexcitation; triplet state; melanin; macular degeneration; deafness; Parkinson's disease

A Quantum Link to Disease

In a typical biochemical reaction, bonds rearrange when pairs of electrons shared between atoms simply change partners while remaining in their lowest energy state [1]. When the reaction requires energy, molecular collisions excite the molecules' vibrational or rotational states. Light-driven reactions, such as photosynthesis or synthesis of vitamin D, are different [2]. A photon excites a single electron in chlorophyll b or 7-dehydrocholesterol to a higher-energy orbital. When an electron is excited (Fig. 1), the electron's partner is left behind and

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the newly occupied orbital has an energy up to 10-fold higher than that of ATP. These high energies – ~100 kcal/mol or 50,000 K or 4 eV, depending on whether you are a biochemist or a physicist – accompany drastically altered orbital shapes, allowing reactions that cannot happen in the ground state. The separated electron pair also reacts like two **radicals** (see Glossary), turns a double bond into a single bond that allows the molecule to rotate around it, and is a stronger oxidizing or reducing agent. In this world, a key distinction is singlet state *vs* triplet state. A **singlet** state retains the two electrons' opposite spins (Fig. 1), whereas in a **triplet** state the spins are parallel. Triplet states have lower energy than the corresponding singlet and have a long lifetime that provides time to collide with other molecules and initiate unusual physical or chemical reactions.

This exotic world can be entered through a rarer door, **chemiexcitation**, in which it is a chemical reaction that excites the electron, leading to the same downstream processes as described for a photon. For many years it was thought impossible for biochemical reactions to reach these energy levels, but bioluminescence was found to have this origin [3]. Recently, we discovered that chemiexciting the skin pigment **melanin** sends **melanocytes** down the path to melanoma [4, 5], a disease whose risk was thought to accrue only while a person was exposed to the sun [6]. First, a brief exposure to ultraviolet radiation (UV) activates the enzymes nitric oxide synthase (NOS) and NADPH oxidase to generate the radicals nitric oxide ($\text{NO}^\bullet$) and superoxide ($\text{O}_2^{\bullet-}$) for 2–6 hours; these combine instantly to form the powerful oxidant peroxynitrite (ONOO^-) [7]. Peroxynitrite is one of the few biological molecules capable of creating a **dioxetane** moiety, a high energy strained 4-atom ring containing C–O–O–C (Fig. 2). Peroxidase enzymes can also do it [8]. In the second stage, melanin is oxidized to its dioxetane derivative. In the third and crucial stage, the dioxetane's high-energy ring spontaneously splits between the two oxygen atoms and between the two carbon atoms to yield a pair of carbonyls ($\text{C}=\text{O}$), one of which typically receives most of the bond energy. That bond energy excites one of the carbonyl's electrons and causes a spin-flip, creating a long-lived triplet state with as much energy as a UV photon and having the same potential for havoc. The excited melanin-derived carbonyl can transfer its energy to DNA to make the lethal and mutagenic **cyclobutane pyrimidine dimer** (CPD), just as sunlight would (Fig. 2). For clarity, we will refer to the CPDs made by direct photon absorption as "CPDs", and the identical photoproducts made in the dark by chemiexcitation as "dark CPDs" (dCPDs).

Replicating DNA past a CPD leads to C→T mutations in the daughter cells (Fig. 2). In skin, sunlight-induced mutations in genes such as *TP53*, *PTCH*, and *RAC1* lead to the keratinocyte tumors squamous and basal cell carcinoma or the melanocyte tumor melanoma [9–11]. It now appears that half at most of the CPDs responsible came directly from sunlight, with the rest produced later by chemiexcitation in the dark [4]. Melanin may be a beneficial sunscreen but, in light of this evidence, may also act as a pro-mutagen.

The three chemical steps of this process would, logically, also occur in other disorders: $\text{NO}^\bullet$, $\text{O}_2^{\bullet-}$, and ONOO^- are generated by inflammation [7]. Melanosomes are present in the cochlea, retinal pigment epithelium, and heart; **neuromelanin** accumulates without melanosomes in the human brainstem's substantia nigra, locus coeruleus, dorsal nucleus of the vagus, and raphe nuclei (Fig. 3) [12–14]. These melanin-containing epithelia and

neurons are the cells that die in macular degeneration, noise- or drug-induced deafness, Parkinson's disease, and Alzheimer's disease, which are often accompanied by inflammation. We hypothesize that chemiexcitation may therefore be an upstream step in these disorders. For cell death, the CPD's lethality [15, 16] would be more important than its mutagenicity. Pursuing this lead requires us to identify, for each diseased tissue: the sources of excited states; the melanin chemistry that hosts the excited state; the DNA or protein alterations that result; and the way these alterations contribute to disease.

What Biochemical Reactions Excite Electrons?

Two biochemical paths excite electrons, in cells or a test tube [8, 17, 18].

Radical-radical reactions

An encounter between two alkoxyl or two peroxy radicals ($-O^{\bullet}$ or $-O_2^{\bullet}$), typically on carbon chains such as membrane lipids, converts a C–O to an excited triplet-state carbonyl ($C=O^*$, where chemists use * in this context to denote an excited state). The peroxy reaction additionally releases an excited O_2 molecule in its singlet state, **singlet oxygen**, which we will write as $^1O_2^*$.

Dioxetane cleavage

Ambient heat will break the strained O–O bond in the dioxetane ring. The C–C then also breaks, resulting in two carbonyls; one is excited. The energy required to create the strained ring ultimately came from the high energy contained in free radicals, such as the precursors $NO^{\bullet}$ and $O_2^{\bullet-}$ or a pair of lipid radicals.

The dioxetane family are perhaps unique in nature for their ability to release large amounts of energy in a single reaction and direct it toward creating long-lived, high-energy, electronically excited triplet states. Dioxetanes are created in three ways [3, 18–21]:

1. Invertebrate luciferases add ambient O_2 to a luciferin to form $-O-OH$. This hydroperoxide then cyclizes at an electron-deficient bond, creating a ring containing C–O–O–C.
2. A molecule that has been oxidized to a radical – by peroxynitrite, horseradish peroxidase, or other enzymes – is then a target for ambient O_2 , which adds itself at the radical site then cyclizes. The peroxynitrite route is biologically important because $NO^{\bullet}$, $O_2^{\bullet-}$, and $ONOO^-$ are stable enough to diffuse across a cell and $NO^{\bullet}$ can cross membranes [7].
3. Singlet oxygen, $^1O_2^*$, can add to an electron-deficient bond and cyclize.

Dioxetanes then cleave in one of several ways [3, 17, 22, 23]. In bioluminescence, an electron is injected into the O–O bond and leads to short-lived singlet states that release fluorescence. An autonomous path is decomposition by ambient heat, usually leading to triplet states. Underlying that deceptively simple process is a confluence of quantum mechanical events that showcase the dioxetane's special properties (Box 1).

We see that only a small set of rather unusual chemical reactions lead to electronically excited states in biology.

Which Biomolecules Can Host an Electronically Excited State?

A colored object is an excited object. It reflects the wavelength responsible for that color and absorbs the others, with the absorbed photons exciting pigments in that tree or painting as long as daylight remains. But these are short-lived singlet states that dissipate as heat. For triplet states, the ideal host for a dioxetane loses an electron easily, providing a site for O₂ attack, and also has an electron-deficient site to which the –O–OH can cyclize. Molecular rings containing delocalized electrons (**aromatic** molecules) are candidates and several are important in biochemistry, including melanin, indoles, and flavins.

Melanin has many unusual properties. It is an effective protective pigment because of its broad and peakless light absorption spectrum ranging from UV to infrared. But it also absorbs sound, hosts persistent radicals, scavenges free radicals, and binds large amounts of Ca⁺⁺ and metal ions; when exposed to UV, it ionizes easily, generates O₂^{•–} and ¹O₂^{*}, and can rapidly dissipate energy by proton transfer [24–27]. Melanin's structure is uncertain, but it appears to be a stack of planar aromatic oligomers. Its monomers are **quinones**, aromatic rings containing a pair of carbonyls that readily interconvert with an –OH form to 'transduce' between 1-electron and 2-electron transfer reactions, facilitate charge transfer, and form charge-transfer complexes that bind drugs which are facile electron donors [28, 29]. These properties may be the reason melanin is found throughout the body – ion buffering and radical scavenging have been proposed – but they also augment the electron transfer and excitation reactions that underlie chemiexcitation. Yet melanin may be the tip of the iceberg, with other families of molecules also able to host chemiexcited states.

Melanin and its eumelanin monomers are members of the broader class of **indoles**, a merger of two electron-rich rings that lose electrons easily and undergo n→π* transitions. The indole family includes tryptophan, the hormone melatonin, and the neurotransmitter serotonin, each of which can form a dioxetane [21, 30]. A ring of four pyrroles constitutes the porphyrin of heme, the iron-containing compound found in hemoglobin and a cofactor in many enzymes where it transfers electrons. Melanin's catechol ring may also form dioxetanes [31] and it is similar to the catecholamine neurotransmitters such as dopamine and noradrenaline. Polymers of oxidized serotonin, dopamine, or related neurotransmitters, bound to protein and lipid, constitute neuromelanin [32].

A separate class of electron-exchanging molecules are **flavins**. These three-ring compounds contain two nitrogens that can accept hydrogen, allowing it to also conduct 1- or 2-electron reactions. These are also frequently cofactors in enzymes.

Non-aromatic dioxetane hosts also exist, including chains of conjugated double bonds such as polyunsaturated lipids [33]. Carbonyls and imine compounds (C=N) can also do this; an example is a sugar that acquires a =N by reacting with an amino acid (the "Maillard reaction") [34, 35].

We see that cells contain a surprisingly large collection of molecules whose electrons can be excited, even if excitation is not the molecule's normal function.

Which Degenerative Diseases Might Involve these Reactions or Enzymes?

But is biological chemiexcitation dangerous? Excitation energy eventually goes somewhere, because an electron in an excited state eventually returns to the ground state. The triplet carbonyl has several options for energy dissipation, some harmless and some detrimental (Box 2) [18]. Then, after the carbonyl has returned to the ground state, it can still be a disruptive aldehyde that makes adducts with other molecules or crosslinks them together. This is a small set of chemical mechanisms, but their cellular targets are widespread: chemiexcitation can affect nucleic acids, proteins, sugars, lipids, and small molecules such as neurotransmitters and hormones [8, 18, 21, 30]. In which diseases might it be important? Several disorders feature the ingredients for the $\text{NO}^\bullet + \text{O}_2^{\bullet-}$ pathway to CPDs found in skin melanocytes, as well as clues that that this pathway is active.

Deafness

Intense sounds and ototoxic drugs such as aminoglycoside antibiotics or the anticancer drug cisplatin lead to deafness due to death of hair cells in the cochlea's organ of Corti. This loss is preceded by cell death in the stria vascularis, an epithelium responsible for the cochlea's endolymphatic voltage potential, and is accompanied by death of neurons in the modiolus, the axis of the cochlea. Both the stria and modiolus contain melanocytes; their absence correlates with pathology [12, 36, 37]. Reduced inner ear melanin correlates with light skin and eye color, and these correlate with greater temporary hearing loss after loud sound and with noise-induced deafness [12, 38, 39]. In Africa, Meniere's disease (hearing loss, vertigo, and tinnitus) is rare in native Africans, who have more melanin in both their skin and cochlea, but is common in the European subpopulation [12]. Conversely, hearing loss due to ototoxic drugs is greatest in individuals with dark eye and skin color [12, 40]. Mice lacking melanocytes in the stria fail to generate the endolymphatic potential, leading to degeneration of the stria, hair cells, and neurons, in that order [41, 42]. Autoradiographic studies show that ototoxic drugs accumulate at melanin sites in proportion to the amount of melanin [12, 43].

In rodents, ototoxic drugs or intense sounds trigger $\text{NO}^\bullet$, $\text{O}_2^{\bullet-}$, and ONOO^- , killing strial melanocytes and hair cells and causing deafness [36, 37, 42, 44, 45]. Chemiexcitation actors are therefore present in the absence of UV.

Strikingly, xeroderma pigmentosum patients – who have frequent skin cancers due to a genetic defect in the enzyme complex that repairs sun-induced CPDs – have profound hearing loss in the absence of intense sound, with atrophy of the stria vascularis, organ of Corti, and modiolus [46, 47]. Cockayne syndrome, another genetic deficiency in the same nucleotide excision repair pathway, also features hearing loss with death of hair cells and the modiolus [48, 49]. The implication is that a DNA lesion repairable by nucleotide excision repair – a dCPD or an unknown bulky lesion [50] – is created in melanin-containing cochlear cells in the dark, kills these cells and thence hair cells, and leads to deafness. The process is exacerbated by loud sounds, drugs, or the absence of nucleotide excision repair.

Because $\text{NO}^\bullet$, $\text{O}_2^{\bullet-}$, and ONOO^- are observed in the case of loud sound or drugs, a prime candidate for the initial lethal lesion in the stria vascularis is the chemiexcitation-induced dCPD. Excited states do make DNA lesions in addition to dCPDs, such as oxidatively generated strand breaks, 8-oxo-7,8-dihydroguanine, and **formamidopyrimidine** [51], but these are repaired by other DNA repair pathways [50].

Age-related macular degeneration

AMD is the loss of central vision with age, resulting from degeneration of the retinal pigment epithelium (RPE) and then the overlying photoreceptors [52]. The RPE is a monolayer of epithelial cells, containing eumelanin melanosomes, that enfold photoreceptor cells. The most phagocytically active cells in the body, each morning they engulf the photooxidized rhodopsin-containing disc at the photoreceptor's tip. As a result, they accumulate **melanolipofuscin** granules that are aggregates of melanosomes, polyunsaturated lipids, and aberrant metabolites of retinal, the light-sensing component of rhodopsin [13, 52–54]. Fluorescent pigment accumulates throughout the retina, but melanolipofuscin and cell death are greatest in the macula [55]. Degenerating RPE cells then create extracellular deposits below the RPE termed drusen, which contains molecules such as amyloid and complement that indicate prior immune responses and which is itself a trigger of inflammation. Drusen alone are not invariably associated with vision loss, so other factors are involved [52].

Inflammation is widely believed to have a causal role in AMD because variants in a complement factor gene confer AMD susceptibility and symptoms are ameliorated by anti-inflammatories [56]. **Reactive oxygen species** are generated by activated macrophages and microglia; we can infer that they, too, contribute to AMD pathogenesis because symptoms are ameliorated by antioxidants. NOS and ONOO^- are observed in the membrane underlying the RPE in AMD or elderly humans [52, 54]. Only visible light reaches the RPE and, in population studies, AMD was related to the level of a person's exposure to visible light rather than UV [57]. Blue light, the highest-energy visible wavelength, stimulates lipofuscin and retinal metabolites to generate $\text{O}_2^{\bullet-}$, $^1\text{O}_2^*$, and triplet states [13, 58, 59]. It also coincides with a peak in the action spectrum for formamidopyrimidine damage to DNA, which can be generated by dioxetanes [51, 60]. Patients with Cockayne syndrome or another nucleotide excision repair defect, trichothiodystrophy, show accelerated AMD [48, 61], again implicating an excision-repairable lesion such as a dCPD in disease pathogenesis. RPE melanin has not previously been considered as a contributor to AMD because this melanin is equally dark in ethnicities with light and dark skin, whereas AMD is ~40-fold more frequent in individuals with light skin and their lipofuscin concentration is significantly higher [53]. However, dark skin correlates with a dark iris, which is a better filter against blue light reaching the RPE's melanolipofuscin. In summary, these facts can be reconciled if age-related melanolipofuscin accumulation in RPE cells leads to occasional cell death – perhaps due to $\text{NO}^\bullet$ and $\text{O}_2^{\bullet-}$ triggered by blue light or due to the dCPDs these radicals generate. When release of extracellular melanolipofuscin forms immunogenic drusen, and the inflammation and further blue light exposure elevate the level of $\text{NO}^\bullet$ and $\text{O}_2^{\bullet-}$, the resulting chemiexcitation creates an amplification loop of cell death, drusen, inflammation, chemiexcitation, lethal DNA damage, and cell death.

Hypertrophic scar

Thick, elevated scars interfere with re-epithelialization, can lead to contracture, and itch persistently; in burn patients they are a significant source of morbidity. They are built of disorganized collagen containing excessive collagen type I, arranged in whorls instead of parallel bundles. They arise when inflammation, the second stage of wound healing, is prolonged beyond about one week; this leads to excessive proliferation of fibroblasts and myofibroblasts [62–64]. The prevailing view is that excessive scarring is related to abnormally high expression of fibroproliferative or pro-fibrotic cytokines, such as IGF-1 and TGF β 1. Activated mast cells are more abundant in hypertrophic scars than in normal scars; they release cytokines, histamine, and serotonin, and are required for myofibroblast proliferation, wound contracture, and hypertrophic scarring [65–67]. Inflammatory cells, including mast cells, generate both NO $^{\bullet}$ and O $_2^{\bullet-}$. But these observations do not explain the strong dependence that development of hypertrophic scars have with the amount of natural skin pigmentation.

Hypertrophic scars are most common in Black and Native American skin and are a clinical problem for Hispanic and Asian skin. The severity of scarring correlates with an allele of the *MC1R* gene that codes for melanin type and hair color [68]. In animals, the Red Duroc pig develops significant hypertrophic scarring after an excisional wound whereas the Yorkshire White does not. In the pig, scarring is associated with the presence of NO $^{\bullet}$ in biopsies from the healing wound [69]. The pigment-scar correlation could arise in several ways. One potential way is chemiexcitation of melanin by inflammation. The ONOO $^-$ could become a chronic source of dCPDs and persistent DNA damage signaling might induce inflammatory cytokines that abnormally prolong inflammation [70]. Another potential way is chemiexcitation of melanin or mast cell serotonin, causing mast cell degranulation. This is possible because a key receptor for degranulation has ligands resembling the chemiexcitation products of melanin and serotonin [4, 71, 72]. Unlike the situation in ear and eye, the inflammation – chemiexcitation – inflammation cycle proposed here would alter tissue differentiation rather than killing cells.

Parkinson's Disease

The movement disorders observed in Parkinson's disease (PD) are due to loss of neurons in a specific region of the midbrain's substantia nigra: the ventrolateral tier of the dopamine-releasing pars compacta (Fig. 3). There is less neuronal loss in adjacent subregions, no loss of astrocytes, and far less degeneration in nearby brain nuclei [73–75]. An earlier loss of neurons in the locus coeruleus that release epinephrine alters mood, sleep, and posture. Still lower in the brainstem, PD patients lose neurons in the dorsal motor nucleus of the vagus (which are important for the proper functioning of the vocal cords and swallowing) and in the serotonin-releasing raphe nuclei of the reticular formation (loss of which can cause depression). PD patients also lose catecholaminergic neurons of the gut. The initiating cause is unknown, and it may vary between patients because inherited mutations and environmental exposures can contribute [76]. The dominant explanatory paradigm is that PD arises, in an unspecified way, from intraneuronal **Lewy bodies** containing aggregates of α -synuclein [73, 77]; indeed, PD and related diseases are termed "synucleinopathies". Other causes have been hypothesized based on human genetic syndromes predisposing to PD,

findings in post-mortem tissue, the epidemiology of environmental exposures, animal models induced by neurotoxins, and cell culture studies. These causes, which are not mutually exclusive, include oxidative neuronal damage or autoimmune reaction from injured mitochondria, neurotransmitter turnover reactions, and neurotransmitter-derived quinones, perhaps as sequelae of neuronal overstimulation (**excitotoxicity**) or immune attack and perhaps contributing to Lewy body formation [75, 77–79]. Yet, Lewy bodies are often present in neurons that otherwise appear normal [79, 80] and the aforementioned cellular and chemical phenomena occur in people who never get PD. So additional factors for development of PD are required.

Patient-specificity may reflect the step in a single pathway that was that patient's starting point. The potential pathway to developing PD may be clearest if we first describe it for environmental toxins that lead to Parkinson's [76]: Occupational exposure to the insecticide Rotenone or recreational exposure to the neurotoxin **MPTP** inhibits the mitochondrial electron transport chain. The resulting ATP depletion depolarizes neurons and thus irreversibly opens an ion channel termed the NMDA receptor, leading to excitotoxicity and allowing an influx of Ca^{++} that activates NOS to synthesize $\text{NO}^{\bullet}$, resulting in ONOO^{-} . The Ca^{++} also depolarizes the mitochondria, exacerbating electron leakage, $\text{O}_2^{\bullet-}$ production, and neuron death [78]. The firing pattern of substantia nigra neurons may make them particularly sensitive to this phenomenon; nigra neurons surviving in PD patients have few NMDA receptors, as if this was protective [75, 78]. Initial toxic reactions are amplified by immune responses: Microglia – the resident innate immune cells of the brain – are activated by toxins, air pollution, viral infection, or head trauma; they are elevated in post-mortem tissue from exposed humans or animal models, including PD substantia nigra [81–83]. When activated, microglia generate bursts of $\text{O}_2^{\bullet-}$, $\text{NO}^{\bullet}$, ONOO^{-} , and pro-inflammatory cytokines that kill additional neurons; signs of these events are found in post-mortem substantia nigra of PD patients [81, 83, 84]. Intraneuronal α -synuclein modified by ONOO^{-} or oxidized dopamine forms aggregates and Lewy bodies; not only do these α -synuclein aggregates further activate microglia but microglial presentation of α -synuclein fragments activates T cells and the acquired immune system [82, 83, 85]. Once triggered, the cycle of neuron death and neuroinflammation can therefore self-escalate and the neuroinflammatory state can persist for years [82, 83]. Other PD etiologies may enter this same pathway at either the toxicity or inflammation points. For example, the endogenous excitotoxin glutamate also induces $\text{NO}^{\bullet}$ in neurons [86] and is released during ischemia-reperfusion such as in stroke or sleep apnea, which are accompanied by ONOO^{-} [7]. Still, these events could occur in any neuron and would not be limited the specific brain regions that die in PD.

Cell specificity can be provided by chemiexcitation's requirement for $\text{NO}^{\bullet}$ and $\text{O}_2^{\bullet-}$. A causal role for neuromelanin has long been suggested: The substantia nigra neurons lost in human postmortem samples are nearly exclusively those containing neuromelanin [87, 88]. The nigra's neuromelanin derives from dopamine; locus coeruleus neurons contain noradrenaline-derived neuromelanin; the dorsal motor nucleus of vagus contains neuromelanin that may be dopaminergic; and the raphe nuclei contain neuromelanin derived from serotonin [14]. Brain nuclei containing neuromelanin form a nearly contiguous chain (Fig. 3). Neuromelanin is sparse or absent in non-human primates and rodents, which do not

contract sporadic PD [89, 90]; correspondingly, artificial induction of PD in mice requires 100-fold the mg/kg dose of MPTP required in rhesus monkeys [91, 92]. Dying neurons release neuromelanin and these extracellular granules activate local microglia [93]. NADPH oxidase and ONOO⁻ are elevated in the substantia nigra of PD patients but not in healthy controls [94, 95]. Interestingly, children with xeroderma pigmentosum have disturbed REM sleep patterns resembling those of PD patients [96]. Therefore, chemiexcitation as a final common pathway in people experiencing chronic inflammation or excitotoxicity could explain PD's predilection for neuromelanin-containing neurons in the relatively few people who contract the disease, whereas proposals that involve solely α -synuclein, mitochondrial reactive oxygen, excitotoxicity, or oxidized neurotransmitters do not.

Similarly, the earliest stages of Alzheimer's disease are neuron loss in the substantia nigra pars compacta, locus coeruleus, and raphe nucleus [89, 97]. Before cell death, neurons contain intracellular inclusions of tau protein, but these are also present in normal brain [98]. Activated microglia, NADPH oxidase, and signs of peroxynitrite are prominent [99]. We see that each of these unsolved disease states contains the reactants needed for chemiexcitation and contains them in the appropriate location.

Preventing or Quenching Chemiexcitation Therapeutically

The hours-long production of CPDs in the dark after a brief UV radiation exposure provides several opportunities for triplet-state preventers or triplet-state quenchers. **Antioxidants** that scavenge NO^{*}, O₂^{*}, their product ONOO⁻, or singlet oxygen ¹O₂^{*} would be expected to prevent the triplet state from being created [4]. A caveat is that chemoprevention trials of antioxidants have shown increased incidence of heart disease, diabetes, or cancer. Chemical cleavage of dioxetanes can also prevent chemiexcitation. Electron donors such as transition metal ions, amines, glutathione, vitamin C, vitamin E, NADH, and FADH₂ split the O–O bond of a dioxetane in a way that is not followed by the C–C cleavage and yields harmless diols [100–103].

Alternatively, a molecule having a triplet energy that is lower than DNA's will siphon the chemiexcited molecule's energy before it can damage DNA. This is the role of *cis*- β -carotene in a chloroplast [104]. Other triplet quenchers include sorbate, vitamin D3, coenzyme Q10, Natamycin, curcumin, lycopene, and indigoids. Triplet quenchers for therapeutic purposes must be chosen carefully because some, such as riboflavin, then donate their new energy to ambient oxygen, creating singlet oxygen. Singlet oxygen not only makes dioxetanes but is much more reactive than ordinary oxygen. This is because O₂ is one of the few molecules in nature whose ground state is a triplet state, with parallel-spin electrons; molecules with the usual opposing-spin electron pairs cannot form a bond with it. Singlet oxygen is no longer bound by this restriction.

Mammalian cells may contain endogenous triplet preventers or quenchers. Cells contain triplet preventers such as metals, amines, glutathione, and vitamins C and E. Some of these are present in plasma at 0.1–300 μ M [105], well above the nM range of pharmaceuticals that inhibit enzymes but what would be needed to collide with the short-lived precursors of excited states. Triplet-state quenchers present in cells include vitamin D3, coenzyme Q10,

and the water-soluble carotenoids lutein and zeaxanthin [106]. The latter two, originally considered to filter out blue light in the eye, are present at up to 1 mM in the macula, 1000-fold higher than in liver or serum. The fact that many of these compounds are not synthesized by mammals we would suggest indicates that nature is relying on diet to acquire them.

Concluding Remarks

We see that the prevailing explanations for the disorders discussed here are insufficient to account for their patient- and cell-specificity, and so require an additional factor (see Clinician's corner, box 3). The conjunction of chemiexcitation initiators such as $\text{NO}^\bullet$ and $\text{O}_2^{\bullet-}$ with chemiexcitation hosts such as melanin or neurotransmitters we propose fulfills this specificity, and their co-localization sites match the disease targets. Causal agents previously proposed, such as inflammation or excitotoxicity, may in fact supply the chemiexcitation initiators. The combination would create a bootstrapped, self-amplifying chain of pathogenesis that intensifies with time. Biocompatible compounds that interfere with chemiexcitation are known, so these disorders may be preventable; indeed, many beneficial effects attributed to antioxidants may be due to their concomitant activity preventing or quenching electronically excited states.

The chemiexcitation hypothesis is not guaranteed, however. Although the ingredients are surely present, these reactions may not be the rate-limiting step in a particular disease or endogenous triplet-state defenses may provide adequate protection, with the evident exception of skin. Ascertaining the details will be a thorny task even if the hypothesis is correct: excited states are ephemeral, they can induce non-CPD DNA lesions that can also originate from oxidation, they induce non-DNA changes such as isomerization that in a regulatory molecule could be biologically significant, and most of the pertinent tissues can only be obtained post-mortem.

Despite these challenges, key experiments (see Outstanding Questions) have the prospect of connecting the dots reviewed here into a biomedical discipline of mammalian excited-state biology and would take molecular medicine one step deeper, toward quantum-level medicine.

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Glossary

antioxidant

molecule that prevents an oxidant from removing an electron or hydrogen atom from a target molecule, usually because the antioxidant is more easily oxidized; can refer to a molecule that siphons energy from excited singlet oxygen

aromatic molecule

a molecule containing a ring with delocalized electron clouds instead of discrete single and double bonds

chemiexcitation

chemical excitation of an electron to a higher-energy molecular orbital

conical intersection

a molecular geometry at which the ground state and excited state energies become equal

cyclobutane pyrimidine dimer

DNA photoproduct in which adjacent thymine or cytosine bases are joined by two single bonds

Dexter energy transfer

photon-less energy transfer between molecules in which the donor transfers a high-energy excited electron to a nearby acceptor and receives a low-energy electron in exchange

dioxetane

a strained 4-atom ring containing --C--O--O--C--

diradical

a molecule in which two unpaired electrons are on different atoms

excitotoxicity

neuron death due to chronic excitatory activity

flavins

three-ring compounds with two nitrogens that can accept and then donate hydrogen, mediating transfer of one or two electrons cofactors in many enzymes

formamidopyrimidine

oxidized purine base with only one ring remaining

HOMO

highest occupied molecular orbital, after filling with electrons from the lowest energy orbital up

indole

merger of an aromatic 6-carbon ring (catechol) and an aromatic 5-member ring containing nitrogen (pyrrole)

loses electrons easily

especially at the nitrogen

keratinocyte

skin cell that synthesizes keratin and constitutes most of the epidermis

Lewy bodies

intraneuronal aggregates of α -synuclein and other proteins

LUMO

lowest unoccupied molecular orbital

melanin

polymeric pigment that gives skin and hair their color, ranging from black to red

melanocyte

skin cell that synthesizes melanin and donates it to keratinocytes

melanolipofuscin

aggregate of melanin and oxidized lipids

MPTP

(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine): Parkinson's-inducing byproduct of synthesis of an illicit opioid, now used for animal models of PD

 $n \rightarrow \pi^*$ transition

excitation of an electron from a non-bonding n orbital (Fig. 1), where it does not participate in a bond, to a higher-energy π^* anti-bonding orbital, where it weakens existing bonds

neuromelanin

melanin-like pigment formed by polymerization of a neurotransmitter

radical

molecule containing an unpaired electron, making it highly reactive

reactive oxygen species

reactive oxygen-containing compounds such as $O_2^{\bullet-}$, H_2O_2 , $\bullet OH$, $^1O_2^*$, $C=O^*$, and $ONOO^-$

singlet oxygen

excited and highly reactive state of O_2

singlet state

state in which the two electrons of a pair have opposite spins, even if one has been excited into a new orbital

triplet state

state in which the two electrons of a pair have parallel spins, possible only if the electrons are in different orbitals

Box 1 Why a Dioxetane is Exceptional

The dioxetane has many properties that make it unusually good at releasing large amounts of energy to create excited triplet states.

Its energy-release properties:

- An O–O bond is weak, especially when in the strained peroxide ring. Heat-induced vibration rotates the two Os apart along the C–C axis until, at an angle of $\sim 70\text{--}180^\circ$, they form a reaction intermediate resembling two $\text{O}^\bullet$ radicals (a diradical). The O–O bond breaks, with the released strain energy facilitating breakage of the C–C bond. Then forming the two very stable C=O bonds frees ~ 90 kcal/mol.
- Nearly all the energy released goes to the carbonyl with the slightly lower triplet energy, determined by the surrounding molecule, allowing that carbonyl to reach the triplet state. Depending on the rest of the molecule, singlet states and heat are competing options.

Its electron-excitation properties:

- Diradicals like the two $\text{O}^\bullet$ in the reaction intermediate have a particularly small energy gap between singlet and triplet states, making it easier to cross into the triplet state.
- If a molecule has a non-bonding n orbital - and the dioxetane's carbonyl end-products do - this is a favored site for excitation because its high energy is closest to the lowest unoccupied orbital (π^* in Fig. 1).
- An $n \rightarrow \pi^*$ transition is geometrically well-suited to flipping an electron's spin to convert a singlet to a triplet, due to a quantum mechanical process termed "spin-orbit coupling", which is beyond our scope here. The $\text{O}^\bullet$ diradical intermediate is particularly efficient in this process.
- Because a molecule vibrates and twists, its energy depends on its exact shape at the moment. A 3D plot of energy vs geometrical coordinates - such as bond distance and the angle between two bonds - forms an uneven 2D surface, with the ground state surface below surfaces for triplet and singlet states. For some molecules, at a specific geometry, these quantum states are no longer neatly distinguishable. The ground state energy rises like a stalagmite and the triplet state energy drops like a stalactite, sometimes touching. At this site, termed a **conical intersection**, it is easy for the molecule to switch between the ground and excited state because no energy need be acquired or lost. A simple dioxetane has a range of geometries like this, forming a long ridge (termed a "seam") between the S_0 and T_1 surfaces. This seam exists whenever the two C–O bonds have rotated between 70° and 180° apart.

Box 2 Where Triplet-State Energy Goes

The energy of a triplet carbonyl is dissipated along both harmless and harmful pathways.

Harmless pathways

- Heat. Occasionally the excited electron flips its spin and the molecule crosses to an excited vibrational level of a singlet state of the same energy. If this state is the ground state, the energy is quickly transferred to solvent molecules as heat.
- Photons. The energy difference can decay as phosphorescence.

Harmful pathways

- Direct energy transfer. Triplet energy can transfer to another molecule within ~ 5 Å by swapping electrons: the donor's high energy electron moves from its **HOMO** (Fig. 1) to the acceptor's **LUMO** and one of the acceptor's low-energy HOMO electrons moves to the donor's half-empty HOMO. This process, **Dexter energy transfer**, is the only electronic energy exchange mechanism available to triplet states and we assume must be the pathway by which melanin fragments transfer triplet state energy to DNA.
- Singlet oxygen. The most common Dexter energy transfer is to ambient O_2 because O_2 is a triplet in the ground state; therefore, no overall spin flip is required. $^1O_2^*$ is much more reactive than ordinary O_2 because it can now react with the plethora of ground state compounds that are singlets.

The remaining options for dissipating triplet energy are easily understood if we recall that the excited state resembles a diradical.

- Isomerization. After energy transfer, the acceptor molecule is now excited. For a molecule having conjugated double bonds, such as sorbate or β -carotene, the diradical character of the newly excited π, π^* double bond allows it to isomerize around the remaining single bond, dissipating the excess energy as heat.
- Cycloaddition. An excited carbonyl's diradical character can also initiate [2+2] cycloaddition reactions, in which two atoms of one molecule react with two atoms of another to form a ring. The carbonyl can add its C and O to another molecule or it can excite another molecule such as a DNA base into undergoing a [2+2] reaction with another base. When two adjacent pyrimidines are excited, their 5–6 double bonds undergo a [2+2] cycloaddition that is not possible in the ground state, making a CPD in the dark.
- Electron abstraction. The excited molecule's unpaired electron can abstract an electron from a nearby molecule, often as an H atom, thereby prompting unwanted chemical reactions.

- C–C bond homolysis. The excited carbonyl diradical is prone to cleaving adjacent to the C, leaving two radicals that can undergo further, unwanted reactions.

The Clinician's Corner (Box 3)

- Melanin – the pigment that gives skin and hair their color – is usually considered beneficial. In skin, it is a sunscreen against UV radiation. In the retina and cochlea, it has been proposed to scavenge free radicals, sequester metal ions, and buffer calcium.
- In the last decade melanin has also been found to be detrimental. Red or yellow pheomelanin in particular predisposes to spontaneous melanoma in mice and enhances apoptosis after UV exposure.
- A recent discovery is that UV induces two reactive oxygen and nitrogen species that combine to excite electrons in melanin, by a physico-chemical reaction termed "chemiexcitation" that is similar to bioluminescence. However instead of generating light, the reaction's high energy transfers directly to DNA to create a cyclobutane pyrimidine dimer, the DNA photoproduct causing the mutations underlying melanoma and other skin cancers. Once triggered, the process continues even in the dark.
- Inflammation and ischemia-reperfusion injury induce the same two reactive oxygen and nitrogen species. When these events occur near melanin or neuromelanin, cell death due to chemiexcitation may underlie degenerative diseases such as macular degeneration, deafness from loud sounds or ototoxic drugs, Parkinson's and Alzheimer's diseases, and Down syndrome.
- This process can be intercepted at several steps, such as diverting the energy from the excited molecule. These interventions offer the prospect of preventing or ameliorating several common degenerative diseases.

Outstanding questions

Does an inflammatory response in disease generate CPDs in the dark?

Do early stages of an internal degenerative disease, before the target cells have vanished, exhibit dark CPDs, UV signature mutations, or isomerized molecules?

What mammalian enzymes besides nitric oxide synthase and NADPH oxidase can generate dark CPDs *in vivo*?

What biomolecules besides melanin can host dioxetanes *in vivo*? How does UV activate the enzymes that produce reactive oxygen and nitrogen species? Can melanin or its degradation product be observed entering the nucleus?

Can triplet energy or triplet-induced oxidation drive α -synuclein, tau, or amyloid- β to seed an aggregate in their associated neurodegenerative disorders?

HIGHLIGHTS

- Excited electrons underlie photosynthesis and bioluminescence. Oxidizing agents and enzymes can chemically excite electrons in the test tube, but a functional role for this chemiexcitation process in mammals has not previously been found.
- Recently, it was discovered that two UV-induced reactive oxygen and nitrogen species combine to excite electrons in the pigment melanin, with the high energy then transferring to DNA to create the lethal, mutagenic, and carcinogenic cyclobutane dimer.
- Inflammation induces the same reactive oxygen and nitrogen species. Several degenerative diseases feature both inflammation and melanin at the target site, which prompted us to hypothesize that chemiexcitation is a key upstream step in these disorders.

Figure 1.

Exciting Electrons in a Simple Molecule. Electron distribution in the outer molecular orbitals of the C=O of the simplest carbonyl compound, formaldehyde ($\text{H}_2\text{C}=\text{O}$). In the ground state, S_0 , paired electrons in an orbital have opposing spins per the Pauli Exclusion Principle (a **singlet state**, see Glossary). Exciting one electron to a higher orbital can retain the opposing spins, giving singlet excited states S_1 or S_2 . This is a "spin allowed" process. If the excited electron's spin flips, giving the former partners parallel spins, the excited state is a triplet state T_1 and it has lower energy than S_1 . Returning to the ground state S_0 requires the spin to spontaneously flip back and is rare or "spin forbidden"; since triplet states cannot readily return to S_0 , they have long lifetimes that allow a variety of harmless and harmful modes of dissipating the energy. σ and π are bonding orbitals, n is nonbonding, and σ^* and π^* are antibonding. **HOMO** is the highest occupied molecular orbital, after filling with electrons from the lowest energy orbital up according to the Aufbau principle. **LUMO** is the lowest unoccupied molecular orbital, available for entry of an electron or electron pair. Modified from [5, 107, 108].

Figure 2.

The Chemiexcitation Path to Skin Cancer. It has long been known that photons of ultraviolet radiation from sunlight are directly absorbed by DNA, where they excite DNA bases. If two excited pyrimidines (thymine or cytosine) are adjacent, a double bond in each opens to form two single bonds between the bases and makes a cyclobutane pyrimidine dimer (CPD); this [2+2] cycloaddition can only happen if the bases are in an excited state. The CPD disrupts base pairing and distorts the DNA helix, leading to cell death or – when DNA replicates – a C→T mutation. Mutations in oncogenes and tumor suppressor genes are required for cancer. Chemiexcitation instead excites the DNA bases long after sunlight exposure has ended. In this situation, ultraviolet radiation served to activate enzymes that synthesize the radicals NO[•] and O₂^{•-} for hours afterward. These radicals form peroxynitrite, which oxidizes melanin or its monomer DHICA (5,6-dihydroxyindole-2-carboxylic acid) and allows ambient O₂ to create a dioxetane on the melanin. The dioxetane moiety, a strained 4-atom ring containing – C–O–O–C–, is unusual in being able to release large amounts of energy in a single reaction, creating long-lived, high-energy, electronically excited triplet states (denoted by *). This triplet energy can transfer to DNA, resulting in a CPD without the involvement of photons.

Thereafter, an unrepaired CPD would again result in mutations or cell death. The same radicals are formed during inflammation, and the same end product can be created by enzymes like horseradish peroxidase (HRP), prompting our proposal that chemiexcitation can occur in internal organs.

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Figure 3.

The Dioxetane and its Potential Melanin Targets in the Human Body. In mammals, dioxetanes (inset) were recently found to be created on the skin pigment melanin, which gives skin and hair their color. Melanin is found in many locations in addition to melanosomes in the melanocytes that synthesize them or the keratinocytes that receive them. Melanosomes are synthesized in the retinal pigment epithelium and cochlea. Melanocyte-like cells are present in the heart atria and pulmonary veins. Neuromelanin is formed by polymerization of neurotransmitters in the substantia nigra (gray), locus coeruleus (blue), raphe nuclei (green), and dorsal nucleus of the vagus, but without melanosomes. Pathologies associated with sunlight- or inflammation-triggered peroxynitrite, combined with melanin-containing cells, include melanoma, hypertrophic scar, macular degeneration, deafness due to drugs or loud noise, Parkinson's disease, Alzheimer's disease, and late-stage Down syndrome. We propose that chemiexcitation may have a causal role in these disorders.