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Chemical excitation of electrons: A dark path to melanoma

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ABSTRACT

Sunlight's ultraviolet wavelengths induce cyclobutane pyrimidine dimers (CPDs), which then cause mutations that lead to melanoma or to cancers of skin keratinocytes. In pigmented melanocytes, we found that CPDs arise both instantaneously and for hours after UV exposure ends. Remarkably, the CPDs arising in the dark originate by a novel pathway that resembles bioluminescence but does not end in light: First, UV activates the enzymes nitric oxide synthase (NOS) and NADPH oxidase (NOX), which generate the radicals nitric oxide ($NO^{\bullet}$) and superoxide ($O_2^{\bullet -}$); these combine to form the powerful oxidant peroxynitrite ($ONOO^-$). A fragment of the skin pigment melanin is then oxidized, exciting an electron to an energy level so high that it is rarely seen in biology. This process of chemically exciting electrons, termed "chemiexcitation", is used by fireflies to generate light but it had never been seen in mammalian cells. In melanocytes, the energy transfers radiationlessly to DNA, inducing CPDs. Chemiexcitation is a new source of genome instability, and it calls attention to endogenous mechanisms of genome maintenance that prevent electronic excitation or dissipate the energy of excited states. Chemiexcitation may also trigger pathogenesis in internal tissues because the same chemistry should arise wherever superoxide and nitric oxide arise near cells that contain melanin.

1. Introduction

The origin of skin cancer – melanoma and basal or squamous cell carcinoma – can be traced to the quantum mechanical level. Electron excitation creates non-DNA structures ("damaged" DNA) that, when copied by a polymerase, write altered information into chemically-normal DNA (mutation). At this quantum mechanical level, our laboratory recently encountered a surprise: Chemical excitation of electrons, termed "chemiexcitation", is a new source of genome instability in melanocytes and in the keratinocytes that receive melanin from them. This discovery raises questions about how chemiexcitation operates in mammals and whether it underlies additional diseases. It also draws attention to little-noticed mechanisms of genome maintenance that protect us even prior to DNA repair.

The cyclobutane pyrimidine dimer (CPD) is the classic DNA photoproduct created by ultraviolet radiation present in sunlight [1]. It joins two adjacent pyrimidine bases, thymine or cytosine (T or C), by two single bonds that create a 4-carbon ring between the bases (the "cyclobutane" ring) (Fig. 1). This structure disrupts base pairing and distorts the DNA helix from its normal B form. The CPD is lethal and mutagenic, and the "UV signature" of C→T mutations at sites of adjacent pyrimidines is seen in melanomas, non-melanoma skin cancers, precancerous lesions, and sun-exposed skin [2–11]. Poor CPD repair in xeroderma pigmentosum increases childhood melanoma 10,000-fold whereas removing CPDs prevents UV-induced skin cancer in mice. The CPD has long been known to be generated by UV photons; we now find that it can be generated by chemiexcitation when melanin is present [12].

2. CPDs in the dark

Induction of CPDs in DNA by direct absorption of UVC photons requires <1 psec [13], so the usual result of irradiation experiments is an immediate peak in CPD formation followed by slow excision repair. We exposed murine melanocytes to UVA radiation and measured CPDs over time using an anti-CPD antibody [14] for ELISA. Surprisingly, melanin-containing murine melanocytes continued

Abbreviations: CPD, cyclobutane pyrimidine dimer; chCPD, chemiexcitation induced CPDs; NOS, nitric oxide synthase; NOX, NADPH oxidase; UVA, ultraviolet A radiation (320–400 nm); UVB, (280–320 nm); UVC, (100–280 nm).

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1568-7864/

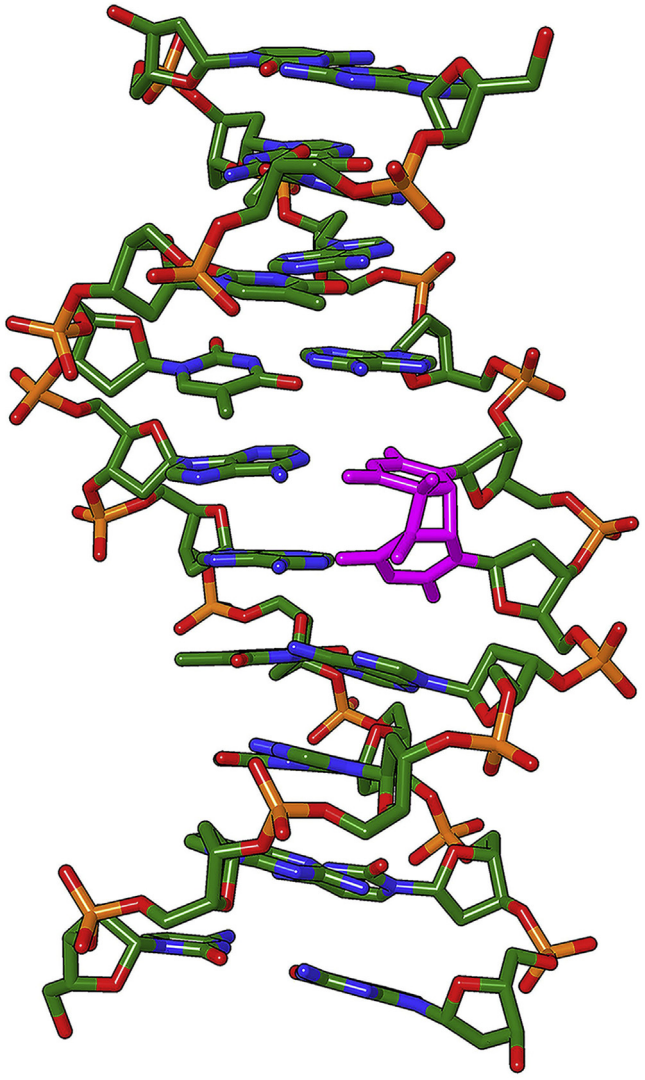

Fig. 1. Thymine-thymine cyclobutane pyrimidine dimer in DNA.
The violet color indicates a four-carbon cyclobutane ring (in the vertical plane) joining two adjacent thymine bases at the site of the former 5–6 double bonds. Orientation is 5′-3′ from bottom to top. The bases are no longer parallel, the 5′ thymine has lost one of its hydrogen bonds with adenine, and the helix is distorted, rendering DNA polymerases error-prone at this site. Modified from [59].

to generate CPDs for at least three hours after UVA exposure, after which generation was offset by DNA repair (Fig. 2) [12]. In contrast, melanocytes derived from albino mice reached the peak of CPD induction immediately upon exposure, as expected; so did murine fibroblasts. The delayed CPDs therefore depended on melanin. Similar results were observed using a comet assay after treating lysed cells with a nucleotide excision repair enzyme to induce DNA breaks at CPD sites, also indicating that the delayed CPDs resided in the nucleus.

These results were intriguing because delayed CPD production had occasionally been reported in other cell types after UVC or UVB exposure [15–19], often with wide variability. It seemed possible that the melanin-dependent mechanism would be more susceptible to experimental study. The important implications for skin cancer prevention and sunbed use made this mechanism worthy of elucidation.

The gold standard assay, mass spectrometry, revealed delayed production of TT, TC, and CT CPDs, only in melanocytes that contained melanin. The cytosine-containing CPDs are the species that generate UV-signature C → T mutations. The effect was larger when

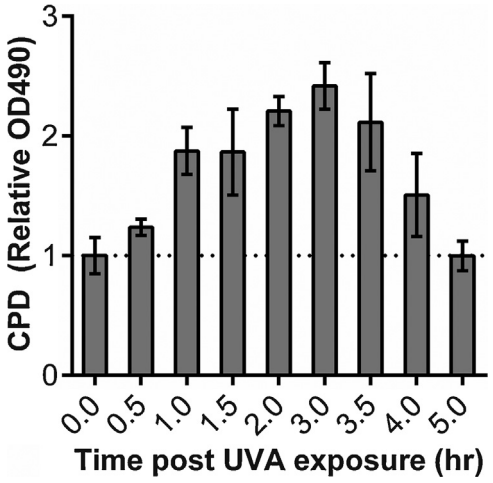

Fig. 2. Cyclobutane pyrimidine dimers (CPDs) generated long after the end of UV exposure.
In melanin-containing melanocytes, CPDs continue to increase for 3 h after UVA exposure ends. In albino murine melanocytes or in fibroblasts, the peak of CPD induction is at time 0, followed by repair. CPDs were assayed by DNA ELISA and similar results were obtained by an endonuclease-sensitive sites assay using comet and by mass spectrometry. Figure from [12].

concurrent repair was knocked down using siRNA against *Xpa* or *Xpc* transcripts. Delayed CPDs constituted half of the total CPDs. This two-fold increase in DNA photoproducts is substantial because xeroderma pigmentosum complementation group D individuals are only 60% defective in CPD repair [20]. With UVB, most CPDs in melanocytes were created in the dark even without repair knockdown.

Human melanocytes also generated delayed CPDs after UVA or UVB, but there was inter-individual variation. For human melanocytes having modest repair of CPD after exposure, but no obvious delayed CPDs, the delayed CPDs were revealed upon siRNA knockdown of *XPA* or *XPC*. Individual variation may stem from variable repair or melanin type. The phenomenon was then studied in vivo using transgenic mice that contained epidermal melanocytes due to Kit ligand expression in keratinocytes, thereby mimicking human skin. Delayed CPDs were seen throughout the epidermis. Most cells were keratinocytes, which receive pigment from the melanocytes, implying that the crucial requirement is melanin content rather than melanin synthesis. Both initial and delayed CPDs were more frequent in mice whose melanocytes synthesized red-yellow pheomelanin than in black mice. This suggests that pheomelanin is both a poorer shield against normal CPD formation and a more potent generator of delayed CPDs. This difference could underlie the increased sunburn and skin cancer sensitivity of individuals who have red or blonde hair.

Yet, creating CPDs requires extraordinarily high energies usually found only in ultraviolet light. Where would this energy come from in a cell? To fully appreciate the dilemma and the solution, it is useful to first highlight the photophysics of CPD production by photons.

3. CPDs from photons

Some reactions cannot proceed when the reactants' electrons are in the ground state, the state in which electrons fill orbitals from the lowest energy upward. The Woodward-Hoffman rules, based on orbital shapes, forbid ground state [2 + 2] cycloadditions – rings created when 2 atoms connected by a double bond undergo a concerted reaction with 2 atoms of another molecule. Cyclobutane pyrimidine dimer formation requires a [2 + 2] cycloaddition, so an electron in a DNA base must first be raised to an excited state.

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