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Radicals in Berkeley?

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In a previous autobiographical sketch for *DNA Repair* (Linn, S. (2012) Life in the serendipitous lane: excitement and gratification in studying DNA repair. *DNA Repair* 11, 595–605), I wrote about my involvement in research on mechanisms of DNA repair. In this Reflections, I look back at how I became interested in free radical chemistry and biology and outline some of our bizarre (at the time) observations. Of course, these studies could never have succeeded without the exceptional aid of my mentors: my teachers; the undergraduate and graduate students, postdoctoral fellows, and senior lab visitors in my laboratory; and my faculty and staff colleagues here at Berkeley. I am so indebted to each and every one of these individuals for their efforts to overcome my ignorance and set me on the straight and narrow path to success in research. I regret that I cannot mention and thank each of these mentors individually.

My Scientific Experiences Outside of Berkeley Provided a Plethora of the World's Most Distinguished Mentors

I was educated in public schools of the then-unincorporated West Hollywood district of Los Angeles. In high school, I was fortunate to have been exposed to "new math" and both classical and modern physics. In particular, Dr. Melvin Greenstadt was responsible for defining my enthusiasm for chemistry and science in general. This enthusiasm was not limited to me, as ten members of my 1958 high school graduating class applied to Caltech. Seven of us were accepted and enrolled into the class of 1962 with fewer than 200 members. The others were enticed by "new" physics and "new math" as majors, while I focused on chemistry and biology. How could I not pursue molecular biology/biochemistry, having taken courses and had numerous informal discussions at Caltech with the likes of Linus Pauling, Richard Feynman, George Beadle, Norman Horowitz, Norman Davidson, Renato Dulbecco, and Jerry Vinograd? Also, I did research in the laboratory of Henry Borsook, studying the expression of hemoglobin in his reticulocyte system, which went on to be used worldwide. Each one of these faculty members imparted knowledge of techniques and concepts that I applied in my later research. By chance, I also took an elective course on electroanalytical chemistry, which provided the necessary background for me to be able to study free radicals and reactive oxygen species (ROS) later in my career.

With all of my exposure to biochemistry and molecular biology (and my California upbringing), I had only one obvious choice for graduate school: the newly formed biochemistry department at Stanford Medical School. For my thesis research, I worked with Bob Lehman on two DNases from *Neurospora crassa*, one of which was mitochondrial. In this way, not only did I learn how to manipulate DNA on gels, etc., but I also learned how to study mitochondria, an important aspect later in the study of free radicals. Moreover, I learned bacterial genetics from Dale Kaiser, Charles Yanofsky, and colleagues and aspects of DNA sequencing and oligonucleotide synthesis and manipulation from Gobind Khorana, who was on a sabbatical leave at Stanford while I was there. Finally, Joshua Lederberg, who was in an adjacent laboratory, made me appreciate the future applications of computer technology to biological studies, while Phil Hanawalt taught me about DNA repair. Although I did not work with Arthur Kornberg, we had regular graduate student

seminars, during which he was extremely demanding; we all knew that if you could survive Arthur, you could survive giving a talk at any meeting anywhere. This pressure turned out to have one other important aspect: it prepared me for giving lectures to large undergraduate classes at Berkeley, in which hundreds of students consider it fair game to play "let's bait the prof." Indeed, I am often asked why I seem so relaxed giving talks (or expert witness testimony), and my response is that "if I can survive a large Berkeley undergraduate class, I can be relaxed at any other presentation."

Between Caltech and Stanford, it would appear that I had been playing "let's meet who's who in biochemistry and molecular biology." However, this did not include important personalities in Europe, so I went from Stanford to a postdoctoral position at the University of Geneva with Werner Arber. I studied host-controlled restriction and modification from a biochemical point of view and, in the process, learned more skills in bacterial genetics. Moreover, being in the department with Eduard Kellenberger, I learned techniques of electron microscopy, which were extremely useful in later studies of DNA damage and repair by ROS. I had one fortunate event during this period: I did a short study (supported by the European Molecular Biology Organization) with John Smith at the Medical Research Council in Cambridge, United Kingdom, to study the *Escherichia coli* B modification site. I could not have picked a luckier time for this visit. Of course, I interacted with and profited from discussions with the usual Medical Research Council Cambridge suspects, John Smith, Sydney Brenner, Fred Sanger, Francis Crick, etc., but also with a number of other visitors, including Joan and Tom Steitz (who were running DNA gels), Gobind Khorana (who was visiting), and Harry Noller.

Given my exposure and interactions with so many pillars of modern biochemistry and molecular biology, I cannot believe what good fortune I have had in my life. Indeed, I am sure that in my case, whatever successes I have had in science were 95% due to luck in being at the right place with the right colleagues at the right time and 5% due to talent.

The Radicals at Berkeley

In the fall of 1968, I arrived in Berkeley to take up a position of assistant professor of biochemistry. Having come from the calm, apolitical (at least on the surface) atmosphere of Switzerland, it was quite a shock to arrive in the midst of anti-war demonstrations, police barricades, and tear gas. However, with the realization that I had in my midst yet another collection of "pillars," including Bruce and Giovanna Ames, Horace Barker, Melvin Calvin, John Clark, Harrison (Hatch) Echols, Seymour (Sy) Fogel, Dan Koshland, Bob Mortimer, Joe (Iron Man) Neilands, Howard Schachman, Wendell Stanley, Gunther Stent, Allan Wilson, and so many others, I began to study a number of problems, all related to DNA transactions, damage, and repair.

I began my research with a continuation of studies of the restriction and modification enzymes of the EcoB system (1, 2). Then, when John Clark informed us that phage restriction is defective in *recB* and *recC* mutants of *E. coli*, we characterized the RecBC(D) nuclease (3, 4).

In 1974–1975, I took a sabbatical leave from Berkeley to study aging with Robin Holliday at the Medical Research Council in London. I was immediately impressed with the plethora of interests that Robin was pursuing with the utmost vigor. In addition to aging, he was studying recombination mechanisms in *Ustilago maydis*; DNA excision repair in *Ustilago*; and, what is truly impressive in retrospect, a role for what is now called "epigenetics" in development, differentiation, and stress responses (5). Indeed, coming into the lab every day was like reading an issue of *Cell*, in both the quality and variety of the science discussed.

Although I did not find the fountain of youth in the Holliday lab, I did observe that in extracts from human cell strains, DNA polymerase fidelity dropped with passage number (6). After my return to Berkeley, I continued to study this phenomenon and finally concluded that what we were seeing was the replacement of the accurate, replicative polymerases with the less faithful, DNA repair "sloppier copier" lesion bypass enzymes (7). However, in the process, we also discovered a new replication DNA polymerase, polymerase ϵ, which we went on to characterize (8).

During the winter just prior to my year in the Holliday lab, my family went skiing at Squaw Valley. Unfortunately, it rained continually while we were there, and my two preschool children were suffering extreme bouts of cabin fever. Fortunately for me, however, the cabin that we rented was only a few hundred meters from the site of the ICN-UCLA (now Keystone) meeting on DNA repair. Escaping from preschool chaos to the meeting, I quickly concluded that there was really very little true understanding of the chemistry and enzymology of DNA damage and repair in mammalian cells and vowed to enter that field when I returned from my sabbatical leave. Conversely, I also realized that quite a bit was known about the *E. coli* enzymes that took part in DNA repair, so these could be

relatively easily purified and utilized as reagents for studying the mammalian systems.

We studied activities that recognized and cleaved DNA at baseless (apurinic or apyrimidinic (AP)) sites and found two classes. Class II (now called Apn1, ADE/Ref-1, or HAP in various laboratories) cleaves DNA to leave a 3′-hydroxyl group and a 5′-baseless sugar phosphate. A property of HAP that we discovered is that it is inhibited by NAD^+ and no other pyridine nucleotide, and it is also inhibited by adenine. We proposed that the cell avoids extensive base excision repair when it has reduced energy stores. Perhaps related is the fact that sirtuins are active only in the presence of stores of NAD^+.

The second group, which we called Class I, cleaves damaged DNA such that the final product contains a baseless fragment on the 3′ terminus. This group of enzymes was ultimately found to be a class that also contained DNA glycosylase activity for the damages; it could cleave off the damaged base prior to cleaving the phosphodiester bond. Moreover, we noted that this group of enzymes tended to recognize damages that could be caused by ROS. At least some members of this enzyme class were also present in mitochondria. Reference 9 gives a more detailed summary of this work, along with another major study in our laboratory: the mammalian DNA damage-binding protein (DDB) and its role in p53 responses and preventing various degenerative diseases of aging.

The topic of study in our laboratory that produced the most unanticipated and novel results was the follow-up on our observations that so much of a prokaryotic or eukaryotic cell's capacity for DNA damage recognition and repair was directed toward damages by ROS. Because it would have been difficult to irradiate large volumes of bacterial or mammalian cell cultures with γ-rays or ultraviolet light, we decided to use hydrogen peroxide as the damaging agent. Initial attempts to induce enzymes in *E. coli* that might repair oxidative damage were carried out by Bruce Demple, a graduate student in the laboratory at the time who went on to do postdoctoral work in the laboratory of Tomas Lindahl, and an undergraduate, James Halbrook, an undergraduate researcher who went on to graduate school at UCLA. These experiments initially appeared not to have been successful. However, on reanalyzing the results after they left Berkeley, Demple and Halbrook realized that the treated cells became resistant to subsequent hydrogen peroxide exposure. Lindahl very generously allowed Demple to explore the observation further in his laboratory, and Demple reproduced the results and also showed that the treatment made cells resistant to γ-radiation as well. Demple and Halbrook published their results in *Nature* (10).

Subsequently, back at Berkeley, a graduate student in my laboratory, James Imlay, wanted to study the chemistry/biochemistry of the adaptation. (Simultaneously, Mike Christman, Gisela Storz, Louis Tartaglia, and others in Bruce Ames' laboratory went on to study the molecular biology of the induced adaptation.) Fortunately, Imlay took quite seriously my mantras of "believe your results" and "if you believe you are right, fight for your beliefs regardless of who disagrees."

Imlay began his studies with the obvious necessity of knowing the toxicity of H_2O_2 exposure for *E. coli*. He shortly came to me with a curve for the wild-type strain AB1157 (Fig. 1). He was excited to point out that there was a dip and recovery near 1–2 mM in the shoulder region of the survival curve for AB1157 upon challenge with H_2O_2, after which the shoulder continued until ~15–20 mM. Looking at the curve, I immediately responded that the dip was not going to be reproducible, that it was most likely due to a variety of factors, and that we ought to move on with our studies. Fortunately, Imlay ignored my remarks, went back to the lab, and several weeks later, showed me how wrong I had been by producing an effect with orders of magnitude differences when using strains lacking exonuclease III (defective in excision repair), active RecA protein (defective in recombination), or active DNA polymerase I (defective in both) (Fig. 1). He quickly went on to show that the effect was exaggerated during anoxic growth and that it required active metabolism (*i.e.* a carbon source), but not protein synthesis (*i.e.* it was not affected by chloramphenicol). When we submitted these results to the *Journal of Bacteriology* for publication, a reviewer stated, "This is the most obscure and unintelligible paper which I have ever been asked to review." We took the statement to imply both that the results were novel and exciting and that Imlay's undergraduate double major in English was not quite beneficial for writing a scientific paper. In any event, the paper was eventually accepted (11).

Looking back at the enhanced sensitivity of *E. coli* to H_2O_2 when grown anaerobically, I propose that one ought to study the organism in anaerobic and aerobic environments. For example, many DNA repair mutant combinations are toxic under aerobic (but not anaerobic) conditions, and the DNA polymerase *polA1* mutant is very "sick" in aerobic conditions, but normal when grown anaerobically. Moreover, when studying *E. coli* as a contributor to the intestinal microbiota, *in vitro* experiments should certainly be done anaerobically.

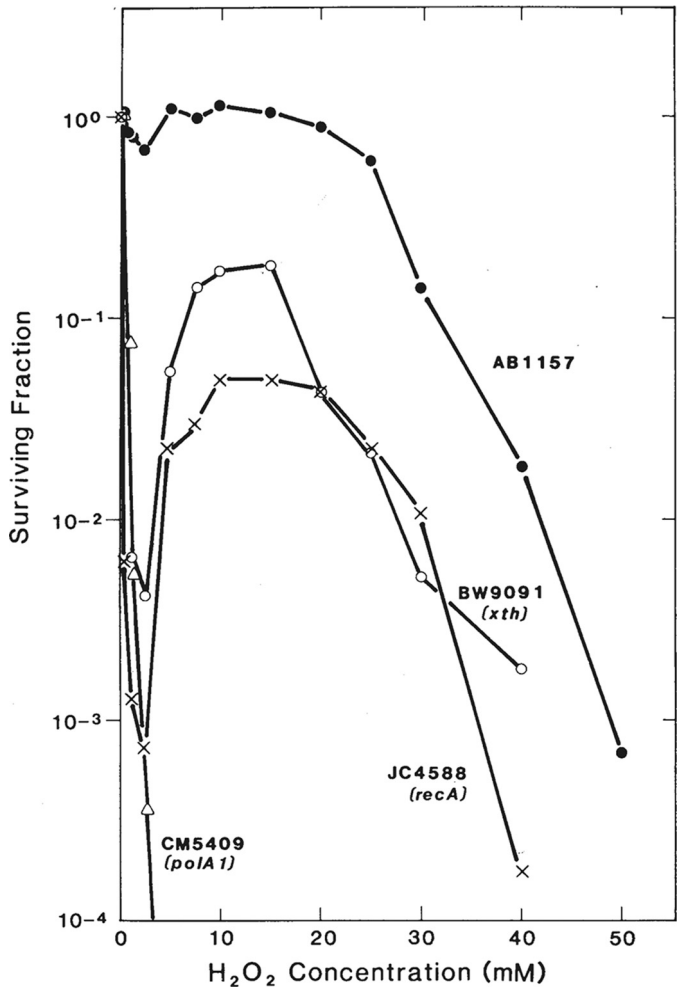

FIGURE 1. **Survival of *E. coli* strains after exposure to H_2O_2.** Liquid cultures were grown to mid-log phase with vigorous shaking in minimal medium, and H_2O_2 was then added to the final concentrations indicated. After 15 min, the cultures were extensively diluted and plated onto L agar plates. Colonies were counted after 24–48 h. Data are from experiments such as those described in Refs. 11, 13, and 15. During such 15-min challenges, other experiments showed that the reductions in survival followed semilogarithmic relationships.

While continuing his studies in our laboratory (12–15), Imlay went on to show that similarly complex response curves to peroxide exposure are obtained when monitoring mutagenesis, induction of phage λ, or cell division delay. He defined the two sections of the curve as Mode I (that seen at lower peroxide concentrations, with the dip and rise) and Mode II (that seen at higher millimolar peroxide concentrations, which was independent of peroxide concentration). Realizing that NADH accumulates in anaerobic cells and in those with blocked metabolism, he tested the effect of exposing *ndh* (NADH dehydrogenase) mutants to H_2O_2 and found that they also gave complex curves analogous to those obtained with anaerobic cells or cells treated with KCN.

Imlay proposed an explanation for the Mode I response (Fig. 2): glucose provides electrons for NADH production. NADH normally provides four electrons for the reduction of oxygen to water or two electrons for the reduction of peroxides to water. However, on occasion, NADH can provide a single electron for the reduction of H_2O_2 and form the extremely potent hydroxyl radical. However, H_2O_2 can also oxidize the hydroxyl radical to the much less potent superoxide radical via the Haber-Weiss reaction.

$$H_2O_2 + HO^{\bullet} \rightarrow O_2^{\bullet-} + H_2O + H^+$$

The latter would normally be relatively easily removed by superoxide dismutase. The Haber-Weiss reaction would explain the rise and subsequent drop in H_2O_2 toxicity, as schematized in Fig. 2.

What could be the mediator of the one-electron transfer from NADH to hydrogen peroxide? Imlay proposed that it was iron (14, 15). NADH would reduce ferric ion to ferrous ion, which could then generate a hydroxyl radical via the Fenton reaction.

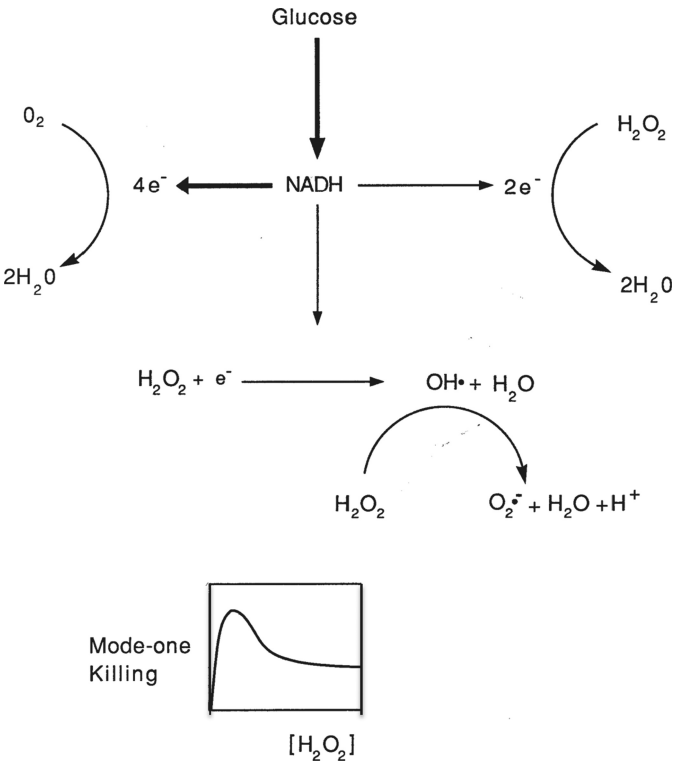

FIGURE 2. **Proposed scheme that gives rise to the complex responses of *E. coli* to H_2O_2 challenges.**

$$Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + HO^{\bullet} + H_2O$$

Imlay then went on to extend these studies as a postdoctoral fellow in Irwin Fridovich's laboratory and afterward as an independent investigator in his own laboratory. Our laboratory went on to study the chemistry of DNA damage by the Fenton reaction with ferrous ion or by the related reaction with NADH and ferric ion. In the former case (16–18), we were literally thrilled to generate a DNA-nicking response *in vitro* that was analogous to the Mode I/Mode II-shaped curve that we had observed *in vivo* in *E. coli* (Fig. 3). In the presence of ferrous iron, DNA nicking was maximal in the micromolar range of hydrogen peroxide; became quenched in the low millimolar range; and was then relatively independent of peroxide concentration up to ~10 mM, after which it increased somewhat. When ethanol, a hydroxyl radical scavenger, was added at various concentrations, it eliminated the Mode I (but not Mode II) portion of the curve. Above 10 mM, ethanol eliminated the slight rise in nicking seen at high concentrations of H_2O_2.

From these results, we hypothesized that there were three types of Fenton reaction radicals that can cause DNA nicking. Type I radicals, which cause Mode I damages, are formed by ferrous ions that are loosely coordinated with DNA and thus moderately resistant to ethanol and sensitive to H_2O_2 oxidation. Type II radicals, which cause Mode II damages, are tightly base-coordinated and thus very resistant to ethanol and H_2O_2 oxidation. Finally, Type III radicals are formed by ferrous ions that are free in solution and thus very sensitive to ethanol and sensitive to H_2O_2 oxidation. As was appreciated later, radicals formed by peroxide reacting with ferrous ions sequestered by molecules such as DNA are quite reactive, but they are not chemically identical to those formed with free ferrous ions in solution. This type of radical is now denoted as a ferryl radical.

Although the superoxide anion is not particularly reactive with purified DNA *in vitro*, it is highly mutagenic and DNA damaging *in vivo* (17). It undergoes dismutation either spontaneously or catalyzed by superoxide dismutase to form hydrogen peroxide.

$$2O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2$$

Moreover, it can reduce and liberate ferric ion from ferritin or ferrous ion from iron-sulfur clusters, thus allowing the generation of very reactive oxygen species, such as hydroxyl radical, via Fenton or related reactions.

In a subsequent study in our laboratory at Berkeley, Ernst Henle, Priyamvada Rai, Yongzhang Luo, Zhengxu Han, and Ning Tang explored which DNA base sequences are cleaved by ferrous ion and H_2O_2 *in vitro* (18). We were astounded to find that the specificities were virtually as

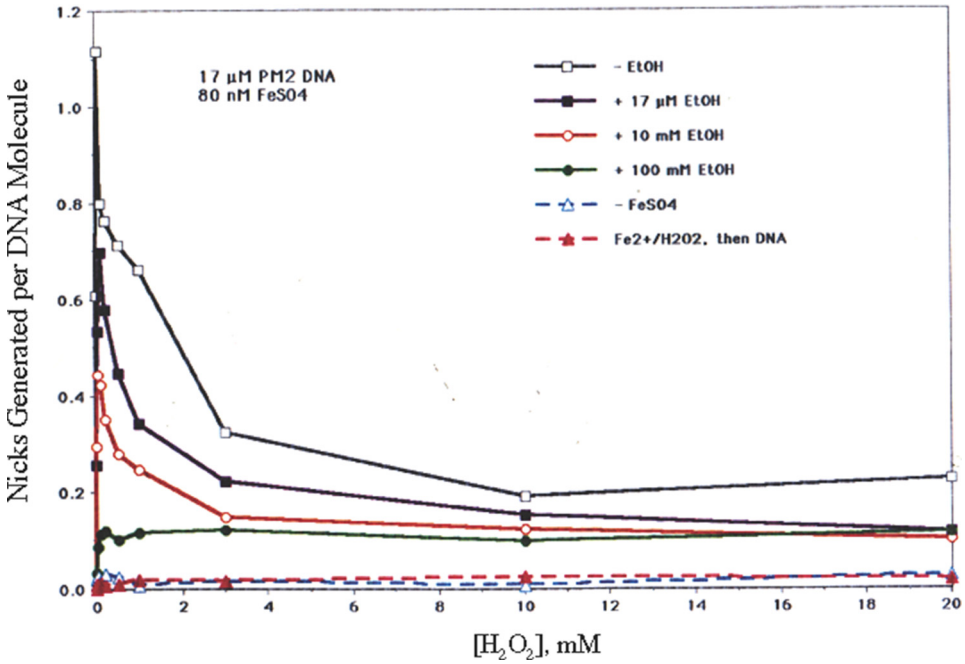

FIGURE 3. **Nicking of PM2 phage DNA by H_2O_2 and Fe^{2+}.** Ethanol was added as indicated to remove any free HO$^{\bullet}$ that formed on Fe^{2+} that was not bound to DNA. Similar experiments are described in Ref. 16.

great as those seen with restriction enzymes. Type I oxidants (formed in 0.5 mM H_2O_2) cleaved DNA at the sequence RT↓GR (where R = A or G, and ↓ indicates the location of the cleavage.) On the other hand, Type II oxidants (formed in 50 mM H_2O_2) cleaved DNA at R↓G↓G↓G or weakly at T↓G↓G↓G. Cleavages occurred 5′ to each of the dG residues, but with decreasing frequency going from 5′ to 3′.

RGGG is contained in the majority of telomere repeats. In the same study, when we exposed a related plasmid containing a human telomere to Type II oxidants, the telomere was cleaved with the same frequency at each of the repeats as that of individual RGGG sequences elsewhere in the plasmid. Evidently, telomeres can "soak up" free iron, perhaps linking iron load, telomere damage, and shortening to aging phenomena.

In a subsequent collaboration with David Wemmer in the chemistry department (19), Rai used ^{1}H NMR to characterize the binding of Fe^{2+} to the duplex oligonucleotide CGAGTTAGGGTAGC/GCTAACCCTAACTCG and 7-deazaguanine variants of it. She showed that Fe^{2+} binds preferentially to the GGG sequence, most strongly toward its 5′-end. Moreover, she showed that binding involves two adjacent guanine N7 moieties and that it is accompanied by large changes in specific imino, aromatic, and methyl proton chemical shifts such that a distorted structure forms at the binding site and also 2 bp 3′ to the GGG sequence. Thus, the zero-order dose response seen under Mode II conditions is apparently due to the rate-limiting step of reorganizing the distorted structure to expose the bound Fe^{2+} to H_2O_2 to enable formation of a DNA-damaging ferryl radical.

Looking at the RTGR sequence, Henle *et al.* (18) noted that it is biologically important. It is contained in the (**ATGG**A)$_n$ centromeric repeats and frequently found to be required in promoters for normal responses of many prokaryotic and eukaryotic genes to iron or oxygen stress. Perhaps most dramatic is the case of the human AP endonuclease promoter, which contains three required sites in palindromic variants of RTGR for binding of the human upstream factor for its regulation.

5'GTCACGTGGT
CAGTGCACCA 5'

5'ATCACGTGAC
TAGTGCACTG 5'

5'CCCACGTGGG
GGGTGCACCC 5'

Henle *et al.* (18) then modeled the interaction of Fe^{2+} with RTGR in B DNA. The modeling predicted that the thymine methyl group provides steric hindrance to binding such that the thymine must flip out of the helix to allow coordination of the Fe^{2+} with the three purine N7 residues. Preliminary NMR studies were consistent with such a model. Rai *et al.* (20) then collaborated once again with

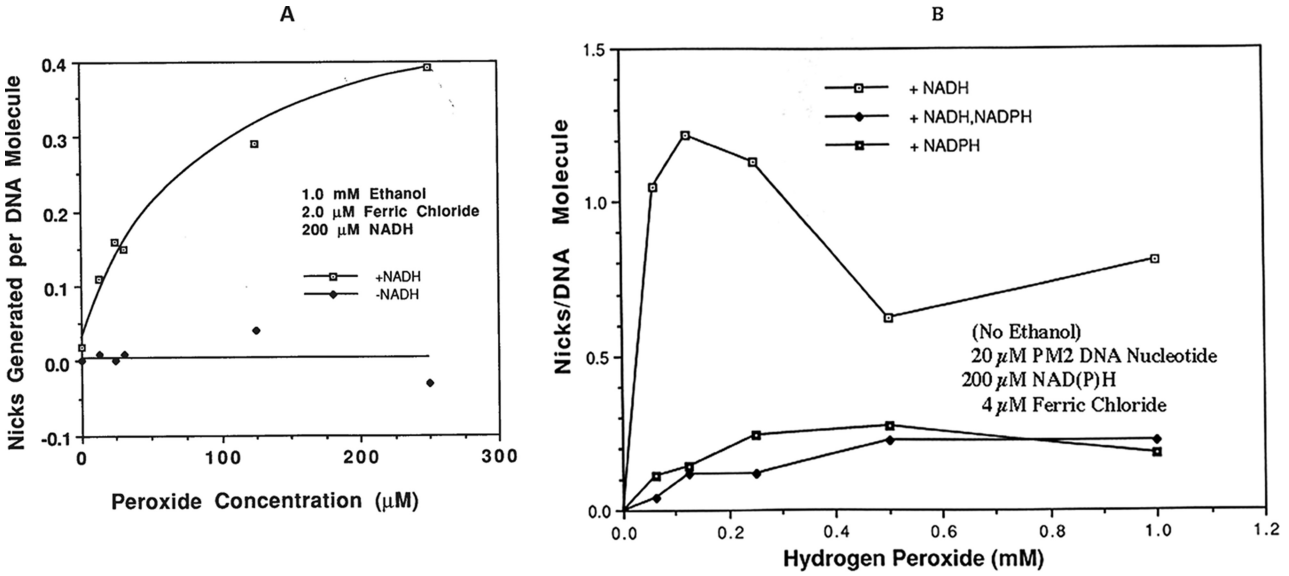

FIGURE 4. **Nicking of PM2 phage DNA by H_2O_2, Fe^{3+}, and NAD(P)H.** *A*, requirement for NADH. Ethanol was added to avoid effects of any contaminating unbound Fe^{2+}. *B*, NADPH does not drive the nicking reaction and inhibits that driven by NADH. Ethanol was not present, and some $HO^{\bullet}$ apparently formed in solution, possibly due to a variety of factors, such as the presence of contaminating Fe^{2+}.

David Wemmer to study the binding by ^1H NMR. They showed that Fe^{2+}, but not Fe^{3+}, preferentially binds to the RTGR sequence because of its unique structure, but the binding is relatively weak and reversible and does not strongly perturb the structure in duplex DNA. Because the binding is weak, the Fe^{2+} is subject to oxidation in the unbound state by H_2O_2. The resulting $HO^{\bullet}$ is quenched in a first-order Haber-Weiss reaction. These results would explain the peculiar dose response for Mode I phenomena and led our laboratory to propose that the presence of RTGR in promoters of genes regulating responses to stress by ROS allows the promoters to detect the oxidation state of free iron cations in the cell and respond by positively or negatively regulating these genes. For example, the strong binding of Fe^{2+} by the human AP endonuclease promoter would act to regulate AP endonuclease production in anticipation of DNA damage by Fenton-type reactions due to the presence of free Fe^{2+}.

A final subject of our ROS studies had to do with the role of nicotinamide nucleotides in the phenomena (21), a forerunner to conclusions in our laboratory and many others on the importance of this group of molecules in regulating cell metabolism and responses to stress. Following his initial observation that *ndh* mutants of *E. coli* were especially sensitive to killing by H_2O_2, Imlay had shown that NADH could be oxidized by peroxide in the presence of catalytic amounts of Fe^{3+}, and, more to the point, DNA could be damaged by peroxide and NADH in the presence of catalytic amounts of Fe^{3+} (15). These observations were then reproduced (Fig. 4*A*) and extended (21) in our laboratory by Julia Brumaghim, Ying Li, and Henle, who observed that although NADH could drive DNA nicking in the system, NADPH could not. Moreover, NADPH inhibited the nicking driven by NADH (Fig. 4*B*). The interactions of NADH and NADPH with Fe^{3+} and Ga^{3+} were also studied by ^1H, ^{13}C, and ^{31}P NMR spectroscopy (21). We found that association with NADH occurred primarily with the adenine N7 and amino group, which put it adjacent to the nicotinamide ring, but for NADPH, a large fraction of the cations were sequestered by the 2′-phosphate group, removing them from the vicinity of the redox-active nicotinamide.

We were excited to find that NADPH could protect DNA from iron-mediated damage, but alas, *E. coli* had scooped us. When we analyzed the bacterium's pools after exposure to H_2O_2 (21), we found that total NAD(H) remained near 850 μM, but the ratio of NADH to NAD^+ changed from 3 to <0.0001 as the H_2O_2 challenge increased from 0 to 10 mM. Meanwhile, total NADP(H) remained near 250 μM, but the ratio of NADPH to $NADP^+$ changed from 0.1 to 0.04 as the challenge increased. Hence, the ratio of NADPH to NADH increased from 0.03 to >80 with the increasing H_2O_2 challenge. These changes were shown to be accompanied by 2.5–3-fold inductions of NADPH-dependent peroxidase, NADH/$NADP^+$ transhydrogenase, and glucose-6-phosphate dehydrogenase.

These responses are reflected in mammalian cells, in which NAD^+ is converted to poly(ADP-ribose) and nicotinamide in response to DNA damages, thus reducing both NADH and NAD^+ levels. One final caveat: Imlay surprisingly observed that mutants of *E. coli* that lack both

catalases (KatE and KatG) are not abnormally sensitive to peroxide challenge. It is also noteworthy that humans who lack catalase have only a relatively mild phenotype of proneness to infections by peroxide-resistant bacteria that cause gingivitis and periodontal diseases. So, what is now being added to toothpaste to brighten our teeth? Hydrogen peroxide. I imagine that the idea is that although our teeth will be caused to fall out, they will be bright and beautiful in the process.

In a related marketing fad, we are bombarded with all sorts of antioxidants that are supposed to keep us young and beautiful, yet even the most understood antioxidants (vitamins A, C, and E and β-carotene) can cause disease when taken at high doses. So, what is the secret to a long, healthy life? Perhaps it is having a satisfying career such as observing radicals at Berkeley.

Concluding Remarks

Clearly, I was incredibly fortunate to have been part of the genesis of the biomedical revolution. At this point, I am concerned that with the necessary emphasis on interdisciplinary research brought about largely by advances in technology and data assimilation, we must become "jacks of all trades, but masters of none." Moreover, will it still be possible for an investigator to get funding for truly novel, but small research projects that ultimately could be so important, such as the discovery of host-controlled restriction enzymes or bacterial mating? What about the rapid geneses of online teaching and online interactions with colleagues? Will students still be exposed to hands-on research and its data analysis? Are we going to lose the important and productive informal discussions that take place at meetings while at dinner, at the bar, walking through the woods, etc.? Finally, with published papers having such copious amounts of supplementary information that reading a paper in detail becomes a major, time-consuming chore that may be done only by its reviewers, I wonder how one will be able to keep up with the "literature."

In reading articles in the Reflections series, I noted that many of my contemporaries felt it necessary to transition to administrative duties or to change their scientific home during their independent career. I did my share of administration as the head of the biochemistry department as it transitioned to the biochemistry and molecular biology division of the mega-Molecular and Cell Biology Department. I served on numerous campus committees, including one that reviews promotions of academic employees ("The Budget Committee") and the University of California system-wide Library Committee, which was devising plans for the future of hardcopy libraries in an era of online publications. I also served on the Publication Committee and the Council of the American Society for Biochemistry and Molecular Biology, as well as on study sections for government and other funding agencies. I looked at such service as a duty, not as tools for moving my career into new directions.

Despite numerous offers, I never seriously considered leaving Berkeley. The campus not only provided wonderful facilities and fellow faculty, but also wonderful undergraduate and graduate students. Fig. 5 pictures some of the Berkeley Radicals who so skillfully and brilliantly carried out much of the ROS work that I discussed above.

On a related note, I was so fortunate that the administrative staffers on the Berkeley campus at all levels had a certain sense of pride and devotion that made even the most bureaucratic responsibilities seem almost pleasant. Many of these staffers worked for low pay and for many extra hours to be part of the campus community. Unfortunately, just after my transition to emeritus, the campus, like most others in the western world, has transitioned to a mentality of corporate organization and an emphasis on funding over academic quality. The Berkeley staff members were then subject to reassignments without regard to workload or expertise. These emphases resulted in either early retirements or transitions to industry, where the pay is higher, with a consequent disappearance of institutional memory from the campus. With this in mind, I should like finally to point out that my successes were due not only to my students, postdoctoral associates, and colleagues spending their sabbatical leaves in my group, but also to the Berkeley staffers at all levels. What more could anybody have asked for?

By the above, I do not mean that I am against the corporate American economic system. Indeed, corporate America extends basic academic research discoveries into the large-scale production of agents for treating and preventing diseases worldwide. When I was an undergraduate at Caltech, I spent a summer doing research for a company, Don Baxter, Inc., on the adaptation of laboratory Tris buffers into intravenous agents for controlling blood acidosis, and I had two publications from that work (22, 23). I actually considered accompanying several of my fellow Caltech graduates to business school. Alas, although my draft board in West Los Angeles would give me a deferment from the Vietnam War for a biochemistry graduate program, they would not for a business master's program. Clearly, continuing to pursue a scientific career was preferable to being sent to the Vietnam War.

FIGURE 5. **Some of the Berkeley Radicals who designed and carried out the ROS research described herein.** *A*, the author demonstrating the lost art of mouth pipetting in the lab in 1988. *B*, James Imlay as a graduate student. (Was he caught trying to put his lab notebook into the microwave oven?) *C*, Ernst Henle, Yongzhang Luo, and Rajagopal (Raja) Chattopadhyaya imbibing with a distinguished visitor, Mr. M. Mouse. *D*, Priyamvada (Priya) Rai. *E*, our first attempt at cloning. Although the group was not successful in cloning the author (*front row center*) by this technique, the National Institutes of Health and other regulatory agencies at all levels found the technique not to be at odds with their health and safety guidelines and requirements.

Finally, what would life be if it were only work? I have been quite fortunate to have had the opportunity to also interject many adventurous travel interludes into my life. I have had wonderful adventures on each of the continents, visiting many of the most aspired destinations on the earth's high mountain ranges, forests, plains, and beautiful ocean fronts. Of the many adventures, perhaps the most fulfilling were encounters with relatively uneducated guides whose dialogs and descriptions would put my lectures at Berkeley to shame. The most recent example was on a trip to visit the mass migration of 2 million large mammals in the mountains of the Serengeti in Tanzania in February 2014. In asking the guide about dung beetles, I was immediately given a 45-min lecture on ecological cycles in general and, in this case, on the 2 million mammals that produce their worth of excrement, which the beetles roll into little balls that contain their eggs and that are buried in the grass plain after the animals leave. This action assures not only a perfect environment for the birth of their offspring, but also a source of fertilizer so that the depleted grasses of the plain can regenerate ahead of the next passage of the migration. When I asked the guide where he learned all of this information, he told me that although he had only a nominal early education, he faithfully perused the Internet in the evenings. Such encounters give me a truly positive vision for the future.

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