Melding the best of two worlds: Cecil Pickett’s work on cellular oxidative stress and in drug discovery and development

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Many chemicals and cellular processes cause oxidative stress that can damage lipids, proteins, or DNA (1). To quickly sense and respond to this ubiquitous threat, organisms have evolved enzymes that neutralize harmful oxidants such as reactive oxygen species and electrophilic compounds (including xenobiotics and their breakdown products) in cells.

These antioxidant enzymes include GSH S-transferase (GST),2 NADPH:quinone oxidoreductase 1, thioredoxin, hemeoxygenase-1, and others (2, 3). Many of these proteins are commonly expressed in cells exposed to oxidative stress.

The antioxidant response element (ARE) is a major regulatory component of this cellular stress response. The ARE is a conserved, 11-nucleotide-long DNA motif present in the 5′-flanking regions of many genes encoding antioxidant proteins. The laboratory of Cecil Pickett (Fig. 1) at the Merck Frosst Centre for Therapeutic Research in Quebec discovered ARE, a finding reported in the early 1990s in two JBC papers recognized as Classics here (4, 5).

ARE’s discovery was spurred in large part by Pickett’s career choice. After completing a PhD in biology and a 2-year postdoc at UCLA in the mid-1970s, he began to work in the pharmaceutical industry. Recruited to Merck in 1978 by its then head of research and development (and later CEO), Roy Vagelos, “I became interested in how drug-metabolizing enzymes were induced by various xenobiotics,” Pickett says.

According to Pickett, Vagelos encouraged researchers at the company “to really start a program where we could build our research careers. But he also said, ‘Keep in mind the long-term mission of Merck—and that is to discover novel medicines that could help people.’”

Pickett remembers joining Merck at a unique time in the pharmaceutical industry. “[Vagelos] really had an incredible vision for research and development.” Inspired by Vagelos’ vision, Pickett launched a vigorous research program that attracted many talented scientists.

To find xenobiotic-metabolizing enzymes, Pickett and his team treated rats with various chemicals, including phenobarbital and 3-methylcholanthrene, to induce genes involved in xenobiotic metabolism and breakdown. To isolate these genes, the team used an in vitro system for translation of the mRNAs isolated from the livers of the animals.

This approach yielded promising results. “I noticed that the profile of the in vitro translated material from induced animals was very different from that of noninduced animals,” says Pickett. “And there tended to be a low-molecular-weight protein that was always induced.”

At first, it was unclear what this small protein might be. But a colleague at Merck, Anthony Lu, had a hunch that it might be GST, Pickett says.

Acting on Lu’s idea, Pickett and his team applied a technique called polysomal immunoprecipitation. The researchers used GST-specific antibodies from Barbara Hales’ laboratory at McGill University in Montreal to detect and isolate mRNA-ribosome complexes that contained GST-encoding mRNA sequences (6).

“That allowed us to synthesize cDNAs and isolate the structural genes for the GSTs,” says Pickett.

Figure 1. Cecil Pickett (pictured) and colleagues first described the ARE motif, present in the 5′ regions of many genes whose expression is up-regulated by oxidative stress and xenobiotics. Photo courtesy of Cecil Pickett.
With the GST gene sequences in hand, the researchers could home in on cis-acting regulatory motifs in the 5′-flanking regions of these genes (reviewed by Pickett and Thomas Rushmore in Ref. 7).

ARE was one of the motifs the researchers discovered as being required for xenobiotics-induced activation of GST gene transcription. Its location in the promoter of the gene encoding the Ya subunit of GST was delineated by extensive deletion and point mutation analyses. These findings were reported in a series of articles (8, 9), including the two Classics papers (4, 5), the second of which reported the ARE consensus sequence.

The discovery of ARE by Pickett and colleagues caused a major shift in thinking about how xenobiotic-metabolizing genes are regulated. Up to that point, it was thought that the aryl hydrocarbon (Ah) receptor, a transcription factor that binds to the xenobiotic-responsive element (XRE) in gene promoters, activated all the genes encoding drug-metabolizing enzymes.

Pickett’s work made it clear that antioxidant genes such as GST have multiple regulatory elements in their promoters that are responsive to specific cellular stressors (Fig. 2). Subsequent work in Pickett’s laboratory and by others helped identify the transcription factors that recognize the ARE and activate the transcription of GST and other oxidative stress response genes (2, 10, 11).

One of these transcriptional regulators is NFE2-related factor 2 (NRF2), identified in 1994 by another research group (11). Because NRF2 binds ARE, suggesting that it helps activate oxidative stress responses, NRF2 soon became a major focus of Pickett’s team, which helped uncover key aspects of how NRF2 itself is regulated by proteasomal degradation and protein kinases (2, 12, 13).

“The discovery of the ARE really laid the groundwork for much of the work that has been done in the field on NRF2,” Pickett notes.

Given the many fundamental discoveries coming out of Pickett’s laboratory, one may be forgiven for thinking that his main focus was on fundamental research. But while running a very prolific research program, Pickett’s talents for designing and overseeing drug discovery programs soon led him to also direct major drug development efforts at Merck.

This meant working with multidisciplinary research teams. “It was a large group of people—chemists, pharmacologists, molecular biologists, and biochemists,” says Pickett. “I oversaw a more integrated approach to how drugs need to be discovered and developed.”

Pickett also served on an internal committee that directed all of Merck’s business in Canada; this unique vantage point further shaped his approach to drug discovery. Pickett’s polymath talents and scientific background in the molecular bases of inflammatory diseases were key for the discovery and development of the anti-inflammatory medicine montelukast (Singular), used chiefly to prevent asthma attacks and other inflammatory lung conditions.

In 1993, Pickett joined Schering–Plough in New Jersey as executive vice president of discovery research, eventually becoming president of the institute. “I had a very good balance between the more fundamental work and also the drug discovery work,” says Pickett of his time at Schering–Plough.

Pickett drove the development of several drugs for managing metabolic disorders and diseases, including cancer, fungal and viral infections, and hypercholesterolemia. He also continued basic research that further deciphered the role of the ARE-binding transcriptional regulator NRF2 in oxidative stress responses. This effort later helped advance drug development at Biogen Idec, a company Pickett joined as head of R&D in 2006.

“Work on NRF2 at Biogen began with some ideas that I had,” says Pickett. “Biogen’s current small-molecule compound Tecfidera [dimethyl fumarate], which is used for the treatment of multiple sclerosis, activates the NRF2 pathway.”

Pickett is now formally retired, but he remains involved in R&D as a member on several advisory boards and committees.
of such organizations and companies as the American Association for Cancer Research and Zimmer Biomet.

Reflecting on the many challenges of directing both fundamental research and drug discovery efforts, Pickett emphasizes the synergy these diverse pursuits offered. “I think having my own lab, being engaged in that research, and reading the scientific literature helped me in making sure that the scientific thoughts in the [drug discovery] programs were solid.”

References
1. Hawkins, C. L., and Davies, M. J. (2019) Detection, identification, and quantification of oxidative protein modifications. J. Biol. Chem. 294, 19683–19708 CrossRef Medline
2. Nguyen, T., Niso, P., and Pickett, C. B. (2009) The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J. Biol. Chem. 284, 13291–13295 CrossRef Medline
3. Raghunath, A., Sundarraj, K., Nagarajan, R., Arfuso, F., Bial, J., Kumar, A. P., Sethi, G., and Perumal, E. (2018) Antioxidant response elements: discovery, classes, regulation and potential applications. Redox. Biol. 17, 297–314 CrossRef Medline
4. Rushmore, T. H., and Pickett, C. B. (1990) Transcriptional regulation of the rat glutathione S-transferase Ya subunit gene: characterization of a xenobiotic-responsive element controlling inducible expression by phenolic antioxidants. J. Biol. Chem. 265, 14648–14653 Medline
5. Rushmore, T. H., Morton, M. R., and Pickett, C. B. (1991) The antioxidant responsive element: activation by oxidative stress and identification of the DNA consensus sequence required for functional activity. J. Biol. Chem. 266, 11632–11639 Medline
6. Pickett, C. B., Telakowski-Hopkins, C. A., Ding, G. J., Argenbright, L., and Lu, A. Y. (1984) Rat liver glutathione S-transferases: complete nucleotide sequence of a glutathione S-transferase mRNA and the regulation of the Ya, Yb, and Yc mRNAs by 3-methylcholanthrene and phenobarbital. J. Biol. Chem. 259, 5182–5188 Medline
7. Rushmore, T. H., and Pickett, C. B. (1993) Glutathione S-transferases, structure, regulation, and therapeutic implication. J. Biol. Chem. 268, 11475–11478 Medline
8. Rushmore, T. H., King, R. G., Paulson, K. E., and Pickett, C. B. (1990) Regulation of glutathione S-transferase Ya subunit gene expression: identification of a unique xenobiotic-responsive element controlling inducible expression by planar aromatic compounds. Proc. Natl. Acad. Sci. U.S.A. 87, 3826–3830 CrossRef Medline
9. Paulson, K. E., Darnell, J. E., Jr., Rushmore, T., and Pickett, C. B. (1990) Analysis of the upstream elements of the xenobiotic compound-inducible and positionally regulated glutathione S-transferase Ya gene. Mol. Cell Biol. 10, 1841–1852 CrossRef Medline
10. Itoh, K., Chiba, T., Takahashi, S., Ishii, T., Igarashi, K., Katoh, Y., Oyake, T., Hayashi, N., Satoh, K., Hatayama, I., Yamamoto, M., and Nabeshima, Y. (1997) An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem. Biophys. Res. Commun. 236, 313–322 CrossRef Medline
11. Moi, P., Chan, K., Asunis, I., Cao, A., and Kan, Y. W. (1994) Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the β-globin locus control region. Proc. Natl. Acad. Sci. U.S.A. 91, 9926–9930 CrossRef Medline
12. Huang, H.-C., Nguyen, T., and Pickett, C. B. (2002) Phosphorylation of Nrf2 at Ser-40 by protein kinase C regulates antioxidant response element-mediated transcription. J. Biol. Chem. 277, 42769–42774 CrossRef Medline
13. Nguyen, T., Sherratt, P. J., Huang, H.-C., Yang, C. S., and Pickett, C. B. (2003) Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element: degradation of Nrf2 by the 26 S proteasome. J. Biol. Chem. 278, 4536–4541 CrossRef Medline