Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine\textsuperscript{1–4}

Tak Yee Aw

ABSTRACT Recently, it has become increasingly apparent that oxidants, in addition to being agents of cytotoxicity, can play an important role in mediating specific cell responses and expression of genes involved in degenerative pathophysiologic states, such as inflammation and cancer. In particular, nuclear transcription factor κB (NF-κB), a multisubunit transcription factor, has been implicated in the transcriptional up-regulation of inflammatory genes in response to oxidants or changes in cellular oxidation-reduction status. This paper provides an overview of the cellular responses to oxidative stress and oxidation-reduction imbalance and the role of NF-κB in these responses and summarizes the current strategies used to study NF-κB activation and nuclear translocation, particularly in relation to dietary oxidant-mediated pathophysiology of the intestine. Am J Clin Nutr 1999;70:557–65.

KEY WORDS Dietary lipid peroxides, intestine, oxidation-reduction imbalance, oxidative stress, apoptosis, proliferation, nuclear transcription factor κB, NF-κB, cell cycle responses, peroxidized lipids, carcinogenesis

INTRODUCTION

Two major conceptual breakthroughs in recent years have significantly affected our current understanding of oxidant regulation of cellular function. The first is the recognition that oxidants, in addition to their widely accepted roles as agents of cytotoxicity, can serve as important mediators of specific cellular and molecular responses and expression of genes involved in degenerative pathophysiologic conditions, such as inflammation and cancer. The second is the increasing recognition that oxidant effects in cellular and molecular regulation may be mediated by oxidant-induced cellular oxidation-reduction (redox) imbalance. This paper summarizes the current status of our knowledge of this subject. The primary focus will be on the intestine and the effect of dietary lipid peroxides because of the role that the intestine plays in the nutrition of the organism and because substantial luminal accumulation of lipid hydroperoxides occurs with consumption of highly unsaturated fats. However, because our current knowledge of oxidant and redox regulation of molecular responses in vascular inflammatory processes is state of the art, the present discussion will include, where appropriate, salient highlights of what is currently known about oxidant-mediated transcriptional expression of endothelial cell inflammatory genes and the role of nuclear transcription factor κB (NF-κB). This discussion will be pertinent for conceptualizing dietary oxidant and thiol redox regulation of intestinal cellular and molecular responses and the implications of these changes in the development of pathologies of the intestinal epithelium, such as colon cancer.

THE INTESTINE AS AN INTERFACE BETWEEN THE ORGANISM AND ITS LUMINAL ENVIRONMENT

The intestine is unique among all fully differentiated organs in that it is a labile organ wherein enterocytes turn over within 48–72 h (1). Because the intestine sits at the interface between the organism and its luminal environment, it represents a critical defense barrier against luminal toxic agents. Thus, in addition to being exposed to luminal nutrients, the intestinal mucosa is constantly challenged by diet-derived oxidants, mutagens, and carcinogens as well as by endogenously generated reactive oxygen species (2). To preserve cellular integrity and tissue homeostasis, the intestine possesses several defense mechanisms such as the ability to maintain high antioxidant concentrations (glutathione, tocopherol, and ascorbic acid), to up-regulate antioxidant enzyme systems [glutathione peroxidase, glutathione reductase (NADPH), and superoxide dismutase], and to induce cell death by apoptosis to dispose of injured or spent enterocytes.

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PEROXIDIZED LIPIDS AND OXIDATIVE STRESS IN PATHOLOGY

Lipid peroxides and malignant transformation of the intestine

Lipid hydroperoxides represent a class of dietary oxidants of major nutritional and toxicologic importance. Lipid hydroperoxides are potentially toxic products of peroxidized polyunsaturated fatty acids derived from dietary fats (3, 4). They are among the natural mutagens and carcinogens present in the human diet that can initiate degenerative processes through the generation of oxygen radicals, which may ultimately lead to disorders of the digestive system (5, 6), including intestinal inflammation and cancer (2).

More than 40 years ago, Andrews et al (7) and Kameda et al (8) documented a causal relation between the toxicity of dietary polyunsaturated oils and the peroxide content in rats, suggesting a potential cytotoxic effect associated with excessive consumption of hydroperoxides in vivo. In reality, human consumption of cytotoxic concentrations of lipid peroxides is unlikely. However, the concentrations of lipid peroxides found in common foods cooked in oils or fats (2–15 μmol/L; Table 1) can produce mucosal oxidative stress and redox imbalance, which can have far-reaching effects on intestinal metabolic homeostasis. Early epidemiologic studies implicated dietary fat as a major risk factor for malignant transformation of the gut in humans. For example, the formation of adenomatous polyps of the colon was shown to be associated with the ingestion of high-fat diets (11–13). What was unknown then, and is only currently being better appreciated, is that a significant intake of lipid peroxides accompanies the high consumption of dietary polyunsaturated fatty acids. The luminal accumulation of lipid hydroperoxides can promote oxygen radical generation and propagation by increased lipid peroxidation. Consequently, the enhancement of tumorigenesis associated with high fat intakes can be attributed to the oxygenated derivatives of unsaturated fatty acids (14–16). Indeed, Bull et al (14) showed that intrarectal instillation of hydroperoxy and hydroxy fatty acids provokes proliferative responses in rat colonic mucosa that are correlated with the stimulation of DNA synthesis and induction of ornithine decarboxylase. These results are consistent with a substantial perturbation in normal intestinal cell turnover in response to lipid hydroperoxide challenge. Importantly, these studies underscore the fact that oxidative modification of fatty acids increases their tumorigenic potential.

Oxidative stress and inflammation

The role of oxidants in mediating inflammatory reactions in a variety of experimental models is well recognized and has been intensely studied. The most commonly examined inflammatory stimuli include cytokines (tumor necrosis factor and interleukin 1), bacterial toxins (lipopolysaccharide), ischemia-reperfusion, and lipid mediators (platelet activating factor). Central to all inflammatory processes, regardless of stimuli, is the induction of cellular oxidative stress. In the intestine, damage to the epithelium resulting from an inflammatory response is generally viewed as a secondary event. The primary event is the systemic inflammatory cascade of neutrophil adherence to vascular endothelial cells, disruption of the endothelial barrier, and subsequent infiltration of inflammatory cells into the intestinal interstitium, where oxidants and proteases are released and produce mucosal injury. To what extent diet-derived lipid peroxides contribute to systemic inflammation is unknown. A strong correlation was observed between a high fat intake and an increased frequency of atherosclerotic lesions (11). Additionally, oxidized forms of cholesterol and lipid hydroperoxides have been directly implicated in atherogenesis (17), and oxidized LDL was found to be more atherogenic than native LDL (18). Thus, it appears that dietary lipid hydroperoxide can be an important contributor to the genesis of vascular pathologies such as atherosclerosis.

Although diet-derived lipid peroxides are implicated in gut and cardiovascular pathologies, we little of the underlying mechanisms in the development of these diseases. A key issue that is pivotal for understanding the molecular mechanisms of oxidant-induced metabolic aberrations that has recently gained recognition is oxidant-mediated cellular redox imbalance and its role in the regulation of gene expression.

GLUTATHIONE, OXIDATIVE STRESS, AND CELLULAR THIOL REDOX BALANCE

Glutathione is an important naturally occurring antioxidant that functions to detoxify reactive oxygen metabolites of endogenous or exogenous origins. Glutathione is a ubiquitous tripeptide (γ-Glu-Cys-Gly) present in high concentrations in tissues (19, 20), including the intestine (21–23). Normal cellular glutathione homeostasis is maintained through de novo synthesis from sulfur-containing precursor amino acids (cysteine and methionine), through regeneration from glutathione disulfide (19, 20), and through glutathione uptake from exogenous sources via Na+-dependent transport systems. Glutathione transport has been shown in a variety of cell types (24–26), including the intestine (23, 27). Notably, the ability of intestinal cells to transport luminal glutathione has important implications for the control of intestinal thiol redox balance, especially under oxidative conditions, because the human diet varies considerably in glutathione concentrations (28, 29) and lipid peroxide content (30–32; Table 1).

The amount of peroxidized lipids is given as thiobarbituric acid–reactive substances. Rats fed a diet containing 2–10 μmol peroxides/d (20 g diet) for 2 wk exhibited intestinal oxidative stress, redox imbalance, and perturbations of intestinal apoptotic and proliferative activity (9, 10).

### TABLE 1

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<th>Food</th>
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1The lipid peroxide contents of selected foods cooked in fat or oil.

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reactions from normal metabolism or toxicologic perturbations can cause the redistribution of some or all of these forms. Thus, enhanced oxidant challenge, such as by lipid peroxides, would be expected to result in depletion of the cellular glutathione pool and a corresponding increase in glutathione disulfide.

Cells are rendered more oxidized by the excessively high accumulation of intracellular glutathione disulfide during oxidative stress. This altered thiol-disulfide status coupled with the resultant oxidation of protein sulphydryls has profound effects on metabolic processes. These include altered specific cell cycle responses, impaired functions of a variety of enzymes (33) and proteins, and activity of transcription factors, such as NF-κB. It is apparent that a loss of redox balance corresponds directly to the development of cellular oxidative stress and that the resultant redox imbalance could have deleterious consequences for metabolic regulation, cell integrity, and organ homeostasis. These considerations are particularly pertinent to the intestine given that the epithelium is often challenged by dietary oxidants, including peroxidized lipids. The potential effect of oxidative stress and redox imbalance on intestinal cellular and molecular responses is considered in the following sections.

OXIDATIVE STRESS AND REDOX IMBALANCE IN SPECIFIC CELL CYCLE RESPONSES

Fully differentiated tissues such as liver, kidney, brain, and intestine are characterized by an arresting of cells in the quiescent state. Imposition of a severe oxidant stress typically results in cytotoxicity. However, previous studies in immune cells introduced a new perspective on cell responses to oxidative stress. Central to this concept is that subtoxic oxidative stress can induce phase transition of a cell from a quiescent state to a proliferative, apoptotic, or necrotic state. It has long been recognized by scientists studying cell cycle responses that the entry of cells into proliferation or death is governed by regulatory genetic or environmental barriers (34). Regarding oxidant regulation of cellular responses, evidence shows that shifting these control checkpoints in the direction of reductants or oxidants can result in a cell that favors quiescence, proliferation, or death.

In a recent review, Flores and McCord (35) described 3 possible cellular responses to increased oxidative stress. Depending on the differentiation or proliferative state, each cell type exhibits a characteristic response to oxidative challenge as illustrated in Figure 1. Cells that are terminally quiescent or fully differentiated, such as hepatocytes, have a biological constraint to proliferate as a result of a mitotic block (curve A in Figure 1). With elevated concentrations of oxidants, cells die either by apoptosis or necrosis. In some differentiated cells, a genetic program is maintained that allows the cells to proliferate, but only when subjected to a stimulus such as an antigen, a mitogen, or increased oxidants, or a combination of these (curve B). In these cells, the initial stimulus may provide the priming event that lowers the barrier of regulatory checkpoints (eg, G0 to G1 transition), much like an enzyme that lowers the activation energy to catalyze a thermodynamically unfavorable biochemical reaction. Subsequent increases in oxidative stress can then drive the cell to proliferate. Intestinal cells may represent such a cell type. In these cells, subtoxic concentrations of peroxides can cause an oxidative shift in the cellular redox status sufficient for an enhanced mitogenic response (9, 10). At higher oxidative stresses, cells die by apoptosis or necrosis (36). In the case of transformed, tumor, or cancerous cells, there are few barriers to proliferation and a subsequent subtoxic oxidant dose may provide a further stimulus for greater proliferation. Alternatively, if the oxidant dose is sufficiently large, the cells may be pushed beyond the maximal point of cell division into the apoptotic phase (curve C). Indeed, anticancer drugs principally operate by forcing actively proliferating tumor cells into the apoptotic phase (37–39). Whether this phase transition occurs via an oxidative shift in cellular redox status is not known. Collectively, these examples illustrate the fundamental concept that cellular responses to oxidative stress are not linear but rather are bell shaped. Additionally, unless the concentrations of oxidants are cytotoxic, necrotic cell death is not necessarily an obligatory endpoint of oxidative stress.
This concept of differential cell responses to oxidative stress has been tested in lymphocytes incubated with various doses of paraquat (S Flores, personal communication, 1996). In these studies, mild oxidative stress caused the lymphocytes to proliferate. At higher paraquat concentrations, the cells began to undergo apoptosis and at even higher oxidative stress, the lymphocytes died by necrosis. In more recent studies, we found that human intestinal cells respond differentially to the degree of oxidative stress. At mild oxidant concentrations (<10 μmol/L), intestinal proliferative activity increases up to a point; at higher oxidant stress (10–50 μmol/L), cells die by apoptosis. At even higher concentrations of lipid peroxides (>100 μmol/L), there is significant necrotic cell death (36).

Preliminary studies from our laboratory showed further that the differential induction of cell proliferation or apoptosis tracks well with the progressive induction of cellular redox imbalance with increasing doses of lipid peroxides (T-G Wang, Y Goto, and TY Aw, unpublished observations, 1998). Taken together, these results show that lipid peroxide–induced intestinal cell proliferation or death exhibits a clear bell-shaped function that is directly dependent on cellular redox status.

**OXIDATIVE STRESS AND REDOX CONTROL OF NF-κB ACTIVATION AND GENE EXPRESSION**

Our current knowledge of the oxidant and redox control of gene expression involving NF-κB is derived largely from studies using in vitro and in vivo models of inflammation. To fully appreciate the role of NF-κB in the redox regulation of gene expression in other experimental models and pathophysiological processes, such as the expression of apoptotic and proliferative genes in intestinal pathologies like colon cancer, a synaptic presentation of the relation between NF-κB activation and vascular inflammatory reactions is warranted.

**Redox control of NF-κB in inflammation**

Recently, the concept that reactive oxygen intermediates may be important mediators in the expression of genes involved in inflammatory processes has gained momentum and is now the subject of intense investigation. In particular, NF-κB has been specifically implicated in the activation of expression of a variety of genes during inflammatory responses, including genes encoding endothelial cell adhesion molecules (reviewed in reference 40). Studies with tumor necrosis factor (TNF)-mediated inflammation have linked TNF-induced mitochondrial oxidant production with the signal transduction of TNF-induced NF-κB activation (41–43). In other studies, an endotoxin (lipopolysaccharide) was similarly found to activate NF-κB. This transcription factor activation can be blocked by the antioxidant pyrrolidine dithiocarbamate (44), a finding consistent with a role of oxygen radicals in intracellular signaling of lipopolysaccharide. Furthermore, activation of NF-κB is specific for hydrogen peroxide (45). Recently, we found that anoxia-reoxygeneration of endothelial cells similarly elicits an inflammatory response. The enhanced adherence of neutrophils to endothelial cells is mediated by endothelial-derived oxidants and involves activation of NF-κB and transcriptional up-regulation of specific adhesion molecules (46). Collectively, these studies linking the action of cytokines, endotoxin, or anoxia-reoxygeneration with endothelial-derived hydrogen peroxide support the role of oxidants as mediators of gene regulatory signaling pathways that involve the activation of NF-κB during an inflammatory episode.

In parallel with our recognition of the involvement of oxidants in NF-κB activity, there is an increasing awareness of a role for cellular thiol redox status in NF-κB activation and gene expression. Studies of cytokine-mediated activation of NF-κB and transcription of human immunodeficiency virus 1 revealed that gene activation and expression are responsive to cellular redox status (47, 48). Staal et al (47) showed that low thiol concentrations promote NF-κB activation, suggesting that intracellular thiol status has a key role in regulating gene activation. Consistent with this finding, Mihm et al (48) showed that exogenous supplementation with cysteine and N-acetylcysteine inhibited viral NF-κB activity. In addition, TNF-induced activation of NF-κB, gene expression of intercellular adhesion molecule 1, and leukocyte adhesion in endothelial cells is inhibited by N-acetylcysteine (49, 50).

**Possible role of NF-κB in intestinal apoptotic and proliferative activity**

Although mounting evidence supports a role for redox modulation of NF-κB activity and transcriptional regulation of gene expression during the inflammatory process, we have little information on the role of NF-κB in mediating intestinal cell proliferative or apoptotic events. Indeed, despite extensive research, a common pathway in cellular apoptosis remains to be elucidated. Early studies in thymocytes showed that hormonal stimuli, such as glucocorticoid, can mediate thymic apoptosis that is preceded by an elevation of cytosolic Ca²⁺, enhanced protein and RNA synthesis, and activation of Ca²⁺-sensitive endogenous endonuclease activity (51–53). In other studies, induction of apoptosis was also linked to inhibition of nuclear topoisomerase activity (39). Although all these mechanisms have been associated with apoptotic cell death in specific tissues, there is no evidence for universal involvement of all mechanisms in any one cell type. Therefore, it is not surprising that we know little of the biochemical mechanisms and molecular regulation of intestinal apoptotic and proliferative events, and in particular, of the role of redox status and the involvement of NF-κB in these processes.

It has been long recognized that toxic or oxidizing agents (eg, tributyltin compounds and 2,3,7,8-tetrachlorodibenzo-P-dioxin) can induce apoptosis in thymocytes (54–57). In the intestine, much of our understanding of oxidant-mediated apoptosis stems from studies in which intestinal cell death was initiated by short-term exposure of the organ to radiation or chemicals (58–60). In recent studies, we found that short-term treatment (2 d) of cultured epithelial cells with subtoxic concentrations of lipid peroxides induces apoptosis that is independent of lytic injury (R Ikawiri and TY Aw, unpublished observations, 1996). Interestingly, in contrast with the acute effects of peroxide exposure, sustained exposure to luminal lipid hydroperoxides appears to inhibit normal intestinal apoptosis and proliferation (9), indicating a fundamental difference in control of intestinal programmed cell death in response to acute or persistent peroxide challenge. Peroxide-induced chronic disruption of control of turnover processes in intestinal cells is significant in that normal induction of apoptosis or proliferation preserves a steady state enterocyte homeostatic balance (1). Consequently, oxidant-mediated perturbation of physiologic apoptotic cell death or proliferative activity could accelerate the loss of organ homeostasis with important implications for the development of intestinal disorders and malignant transformation.

The possibility of lipid peroxide–induced activation of NF-κB in the molecular signaling of intestinal apoptosis or proliferation
has hitherto not been addressed. Because exposure of intestinal
cells to lipid peroxide promotes the formation of reactive oxygen
metabolites and the ensuing induction of thiol redox imbalance,
and because NF-κB is oxidant- and redox-sensitive, it is reason-
able to speculate that NF-κB plays an integral role in the molec-
ular responses of enterocytes to either proliferate or die during
oxidative stress. Indeed, on the basis of the above reasoning, one
could hypothesize that NF-κB may in fact be inextricably linked
to the signaling pathways governing the expression and suppres-
sion of candidate proliferative genes—such as c-myc, cyclins,
cyclin-dependent kinases, and retinoblastoma protein—or of
candidate apoptotic genes—such as p53, p21, bax, and bcl-2
(Figure 2). Although this hypothesis is highly speculative and
remains to be experimentally tested, studies documenting a link
between oxidative stress and oxidative DNA damage with up-
regulation of p53, a gene associated with apoptotic death, and of
p21, a cyclin-dependent kinase inhibitor, appear to support such
a contention (reviewed in reference 61).

FIGURE 2. Working hypothesis of lipid peroxide–induced cell proliferation or apoptosis. NF-κB, nuclear transcription factor κB.

STRATEGIES FOR STUDYING NF-κB ACTIVATION

The general approach to studying NF-κB activation capital-
izes on our current knowledge of the molecular biology of this
transcription factor and the mechanism of its intracellular activa-
tion. Because this section does not cover these topics compre-
hensively, the reader is referred to previous excellent reviews of
the subject (40, 62). A brief introduction to the mechanism of
NF-κB activation is warranted, however, and is relevant to the
discussion of experimental approaches to and strategies for
examining the role of NF-κB in mediating gene expression.

Our present understanding of NF-κB activation and nuclear
translocation is illustrated in Figure 3. NF-κB is composed of
2 functional subunits, p65 and p50, that are associated with an
inhibitory subunit, IκB, in the inactive state. In response to
exogenous stimuli such as oxidants (hydrogen peroxide),
cytokines (TNF and interleukin 1), ischemia and reperfusion,
or endotoxins (lipopolysaccharide), cytoplasmic activation of
NF-κB occurs by a sequence of events. These events include
the phosphorylation and polyubiquination of IκB and the subsequent
degradation of the phosphorylated and polyubiquinated IκB by
a specific intracellular protease complex, the 26S proteasome.
The activated heterodimer p65-p50 is then translocated to the
nucleus where the p65 subunit binds to promoter regions of DNA
to initiate the transcription of genes, most notably genes associ-
ated with mediating the inflammatory processes. In this regard,
the proteasome pathway has been shown to be required for
leukocyte-endothelial adhesion molecule expression mediated
by cytokines (63) and by anoxia-reoxygenation (46).

Use of proteasome inhibitors and κB oligonucleotides

A commonly used strategy is the use of proteasome inhibitors
to prevent NF-κB activation by blocking IκB degradation.
To date, there are 3 major classes of proteasome inhibitors, all of
which are highly selective and effective but with different appli-
cations. These classes are the aldehyde peptides (MG132; Pro
Script Inc, Cambridge, MA), the boronates (MG341; Pro Script
Inc), and lactacystin (64; Figure 4). MG132 is the most widely
used commercially available proteasome inhibitor and is espe-
cially useful in in vitro models of inflammation. Unfortunately, it
is of limited use in vivo because of its cytotoxicity. MG341 has
the advantage of being highly selective; its inhibition constant
($K_i$) for the 26S proteasome is several orders of magnitude lower
(in the subnanomolar range) than that for other known cellular
proteases, eg, chymotrypsin, elastase, cathespin G and B, and
pepsin. Moreover, MG341 has been successfully used in animal
models of inflammation with no detectable side effects. Unfortu-
nately, boronate compounds are not commercially available and
therefore are of limited accessibility. Lactacystin is a class of rel-
atively new proteasome inhibitors that appears to be both highly
selective and applicable to cell and animal models. However, it is
unknown whether deleterious effects are associated with lacta-
cystin use. Cobb et al (65) described an interesting proteasome
inhibitor, nor-LEU (n-acetyl-leucinyl-leucinyl-norleucinal). This
class of inhibitor appears to specifically inhibit NF-κB activation.
and to block interleukin 1β–induced intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 gene expression in endothelial cells without preventing nuclear translocation of NF-κB. Although studies to date generally indicate the successful use of these different classes of synthetic compounds in various models of inflammation, these proteasome inhibitors have yet to be tested in other pathophysiologic models.

A second and more specific approach to defining the role of NF-κB is the use of double-stranded phosphorothioate oligonucleotides as decoys for the transcription factor. An experimental approach used by our laboratory (46) and others (66) is to design a double-stranded 25-mer (25-base-pair oligonucleotide) containing 2 NF-κB binding sites (5′-AGGGACTTTGCTGGGGACTTTCC-3′). The control oligonucleotide is the non-protein-binding sequence 5′-AAAAGTCCCTTGCTGAAAGTCCCTT-3′. The fundamental principle is to provide an alternate “template” for binding of the activated p65-p50 NF-κB dimer, thereby preventing its nuclear translocation and gene expression.

**FIGURE 3.** The proteasome pathway of nuclear transcript factor κB activation. IkB, inhibitory subunit.

**FIGURE 4.** Classes of proteasome inhibitors. $K_i$, inhibition constant; $K_{\text{inact}}$, inactivation constant. MG132 and MG341; ProScript Inc, Cambridge, MA.
activation. Other than the customized oligonucleotide containing 2 binding sites, a 21-mer oligonucleotide containing a single NF-κB consensus binding site (5′-AGTTGAGGGGACT TTCCCAAGG-3′) from Promega (Madison, WI) is equally effective as a decoy. Experimentally, pretreatment of cells with the NF-κB oligonucleotide sequences is highly effective for delineating the role of NF-κB in the transcriptional up-regulation of endothelial cell adhesion molecules in response to anoxia-reoxygenation (46). Although it is undeniable that the specificity of these oligonucleotides renders this approach superior to the use of chemical proteasome inhibitors, the size of the oligonucleotides poses some limitations for uptake into cells. Consequently, it may be difficult to assess the accumulation of these decoys to effective concentrations within cells. Thus, some caution is indicated in data interpretation when using this approach. Furthermore, because of expense, the general use of oligonucleotides for studying NF-κB activity in vivo is limited.

**Electrophoretic mobility gel shift assays**

Transcription-dependent gene expression associated with NF-κB activation is often confirmed by performing electrophoretic mobility gel shift assays on nuclear extracts prepared from cells or tissues exposed to various stimuli. The nuclear proteins are then probed with double-stranded DNA radiolabeled with 32P for NF-κB under controlled binding conditions and the DNA–NF-κB protein complexes resolved on 4% nondenaturing polyacrylamide gels (46). The specificity of binding is often tested by including in the binding reaction a 50-fold excess of the unlabelled NF-κB oligonucleotide as a positive control or an unlabelled non-NF-κB-binding oligonucleotide, such as activator protein 1, as a negative control. Typically, more than one specific nucleoprotein adduct is evident on the gel shift [eg, in response to anoxia and reoxygenation (46)]. The additional adducts may represent the presence of both the heterodimer (p65-p50) and the homodimer (p50-p50). In such cases, supershift assays can be performed by using antibodies against p50 or p65 to identify the nature of these adducts. The nucleoprotein adducts can be readily quantitated by computer-assisted analysis of scanned autoradiograms with use of an image software program. A popular choice is the NIH Image 1.57 software program (National Institutes of Health, Bethesda, MD).

The above strategies have been used extensively with considerable success to study NF-κB activation and gene expression in various in vitro and in vivo animal models of inflammation. Although these methods have yet to be widely adopted in the study of other normal physiologic (eg, apoptosis, proliferation, and cell cycle responses) or pathologic (eg, carcinogenesis) processes, it is anticipated that as the role of NF-κB in these processes is better recognized, these molecular approaches will become indispensable tools.

**SUMMARY AND PERSPECTIVE**

The growing body of evidence in the literature indicates that cellular oxidative stress and redox status are central to the signaling of expression of inflammatory genes involving activation of NF-κB. The area of oxidant and redox control of pathophysiologic processes in the gastrointestinal tract is relatively unexplored. Recognition of this fact prompted a 1996 Federation of American Societies for Experimental Biology symposium on this topic (67). Our current understanding of oxidative stress and thiol redox imbalance, of redox effects on specific cell cycle responses, and of redox regulation of NF-κB activation and gene expression provides an important conceptual underpinning for future research. On the basis of the existing evidence, it is reasonable to propose that oxidant (eg, lipid peroxide) challenge initiates intestinal oxidative stress and that depending on the degree of thiol redox imbalance, enterocytes will either proliferate or die by apoptosis via NF-κB–mediated expression or suppression of respective proliferative or apoptotic genes (Figure 2). Currently, our challenge lies not in the conceptual acceptance of this hypothesis, but rather in the experimental documentation of the proposed associations. Clearly, the burden of proof rests with future research endeavors.

**REFERENCES**