apoptosis, chemotaxis, and motility (Sumida et al., 1993). Several studies have shown that both production and function of cytokines can be regulated by PUFAs and their metabolites, and vice versa (Peplow, 1996). Like other laboratories we have brought evidence that modulation of AA metabolism by specific inhibitors of its enzymatic pathways a) affects murine and human myelopoiesis (Kozubík et al., 1994) and proliferation of various cancer cell lines (Hofmanová et al., 1996) and b) potentiates antiproliferative and differentiating effects of some cytokines, such as TGF-ß1 (Kozubík et al., 1997), and inducers of differentiation, such as all-*trans* retinoic acid (Hofmanová et al., 1998; Hofmanová et al., 2000). It is evident that the regulation of these processes depends on both the cell type and differentiation pathway.

Oxidative stress

Oxidative stress has been defined as "a disturbance in the prooxidant/antioxidant balance in favor of the former, leading to potential damage" (Klaunig et al., 1998). Nowadays it is clear that oxidative stress is capable of inducing direct damage to both DNA and epigenetic modes of action. It means that, besides oxidative damage to cellular DNA, lipids, and proteins, reactive oxygen species (ROS) or nitrogen oxides can alter signal pathways, including the activation of transcriptional factors (p53, AP-1, NFκB, Myc, Erg-1, etc.) or transcription factor inhibitors (such as IκB) (Dalton et al., 1999), and modulate the cell-cell communication without apparent genotoxicity (Trosko, 1998). Thus, various oxidative stress-induced signal transduction mechanisms trigger apoptosis or necrosis and modulate cell proliferation or differentiation (Aw, 1999).

Cytosolic and nuclear receptors and transcription factors

Besides the above receptor-mediated induction of CYP enzymes, specific interactions of xenobiotics with cytosolic receptors or the nuclear receptor superfamily, involving estrogen receptors, PPARs, and receptors for retinoic acids (RAR, RXR), can induce gene expression producing a set of pleiotropic responses or specific adverse effects (Green, 1992). The activation of the AhR is followed by species- and tissue-specific effects including alterations in endocrine homeostasis, immunotoxicity, modulation of cell proliferation and differentiation, and tumor promotion (Denison and Heath-Pagliuso, 1998). Therefore, the AhR-mediated gene expression corresponds to the "dioxin-like" toxic potency of xenobiotics. Similarly, many endocrine-disrupting xenobiotics affect part of nuclear hormone receptor-dependent gene expression and, binding as ligands, are subsequently capable of either activation of the receptor or competition with natural hormones. The major target transcriptional factors are xenobiotic-modulated estrogen, androgen, and thyroid hormone receptors. Imbalances in hormone receptor-controlled processes induce many adverse effects including carcinogenesis; an example thereof is a close association of xenobiotic-enhanced estrogen receptor activation with human breast cancer (Gillesby and Zacharewski, 1998).

Since 1990, the PPARs have been identified in various tissues. These receptors, controlling a variety of genes in lipid metabolism pathways, rank with the steroid/thyroid/retinoid receptor superfamily (Desvergne and Wahli, 1999). Like other steroid hormone receptors, PPARs are ligand-activated transcriptional factors with distinct and even conflicting pleiotropic responses, including increased peroxisomal proliferation, induction of specific enzymes like the peroxisomal acyl-CoA oxidase and CYP4A, oxidative stress, disturbance of fatty acid metabolism, hypertrophy, and hyperplasia. These effects were demonstrated in liver tissue of several mammalian species treated with specific drugs or exposed to environmental contaminants. Both natural ligands (PUFAs and their derivatives) and drugs, such as hypolipidemic compounds (fibrates) and cyclooxygenase inhibitors, have been identified as inducers of one or more PPARs (Clarke et al., 1999). Recently, it has been shown that the differentiation of many cell types (hepatocytes, adipocytes, keratinocytes, monocytes/macrophages, colonocytes etc.), which protect against some malignancies, involves activation of PPARs (Vanden Heuvel, 1999). Distinct biological effects of PPAR ligands and their specificity to the individual PPAR isotypes have been demonstrated in *in vitro* assays using tumor or transfected cells.

The formation of heterodimers with the 9-*cis*-retinoic acid receptor (RXR) is a common mechanism of activation of many nuclear receptors (Machala and Vondráček, 1998). A competition for their common partner can develop between these receptors; moreover, cross-talks of signal pathways of these receptors should be considered (Desvergne and Wahli, 1999). Therefore, a multibiomarker approach using a set of *in vitro* bioassays is necessary to assess the actual significance of xenobiotic-modulated signal pathways and toxic potencies of xenobiotics.

DNA methylation and histone acetylation

DNA methylation (5-methylcytosine content) plays a pivotal role in the development and differentiation, and there is evidence supporting its role in carcinogenesis. Both hypo- and hypermethylation of DNA are considered as epigenetic mechanisms, which involve mainly alterations in the normal gene expression (Klein and Costa, 1997). It has been shown that hypomethylation is associated with increased gene expression and may facilitate clonal expansion of preneoplastic cells. On the other hand, regional hypermethylation in a part of the genome can inactivate tumor suppressor genes (Counts and Goodman, 1995).

Acetylation and deacetylation of amino-terminal lysine residues of nucleosomal core histones provide a molecular communication link between chromatin and

signal transduction pathways. Steady-state levels of histone acetylation vary throughout the genome and are maintained by a dynamic balance between histone acetyltransferases and histone deacetylases. Acetylation is believed to facilitate transcription, presumably by modulating the access of transcription factors to nucleosomal DNA or by displacing histones for the passage of the transcription complex. One of the key regulatory molecules whose activity is regulated by the acetylation status of histones is the cyclin-dependent kinase inhibitor $p21^{WAF1,Cip1}$ involved in cell-cycle modulation (Rennie and Nelson, 1999).

Cell proliferation, differentiation, apoptosis, and tissue homeostasis

A large number of excellent reviews on the role of cell proliferation in carcinogenesis have been published (Butterworth and Goldsworthy, 1991; Cohen, 1995; Jones et al., 1996; Foster, 1997). Therefore, only the major points are to be mentioned here. Changes in cell replication are a consequence of alteration of cell cycle regulators, particularly those associated with the G1-phase progression. This regulation is a complex process involving many molecules. Expression and interaction of cyclins, cyclin-dependent kinases (Cdks), Cdk inhibitors and pRB and p53 proteins are largely responsible for this regulation. Alteration of any of these regulators will result in disruption of the G1-phase regulating machinery (Afshari and Barrett, 1993; Kamb, 1995).

Unlimited cell proliferation is also associated with mechanisms responsible for cell immortalization. Activation of telomerase, which adds telomeric repeats to the ends of chromosomes providing immortality to cells, is found in most of tumor cells. A relation between this mechanism and changes of adhesive molecule complexes, activation of specific transcription factors (Tcf, c-myc), and cell-cycle regulators has been demonstrated (Michalides, 1999).

The promotional stages of carcinogenesis can be regarded as cell proliferation that leads to selective clonal expansion of a mutated cell. Sustained increase in cell proliferation will accelerate the accumulation of mutations in tumor cells and the development from the benign to the malignant phenotype. The growth rate of cell clones is determined not only by cell proliferation, but also by the cell death rate. Both processes occur under the control of a growth-regulatory network and can be induced by growth-regulating factors via specific receptors (Roberts et al., 1997). Apoptotic cell death helps to maintain homeostasis of the cell number in tissues and can eliminate damaged cells. An alteration of the regulation of apoptotic cell death has been implicated in both human and rodent carcinogenesis (Goldsworthy et al., 1996). Tumor-promoting epigenetic agents act as survival factors for preneoplastic cells and inhibit apoptosis. They can induce preferential growth of preneoplastic foci by altering the ratio of the rates of cell rise and death. Prolonged inhibition of apoptosis could result in further accumulation of genetic alterations and progression of neoplasia to malignancy (Schulte-Hermann et al., 1994).

During embryonic development and in adult self-renewing systems (like hemopoietic or colonic epithelial tissues), the control of differentiation becomes the third important point in the maintenance of homeostasis. Dysbalance of growth stimulatory and inhibitory signals modulates the processes of proliferation, differentiation, and apoptosis. The cells loose their ability to terminally differentiate and begin to proliferate abnormally in the stage of stem or progenitor cells (Moqattash and Lutton, 1998). During chemically induced hepatocarcinogenesis, tumors may also arise by dedifferentiation of adult hepatocytes (Sell, 1993). Thus, both maturation arrest of determined stem cells and dedifferentiation of mature cells may be the cellular pathway of cancer.

Gap junctional intercellular communication (GJIC)

GJIC plays an important and integrating role in the maintenance of homeostatic control. Gap junctions have been associated with growth control, development, differentiation, apoptosis, and adaptive responses of differentiated cells and are known to be sensitive to environmentally triggered signals which can obstruct intracellular communication. The suppression of GJIC sets in a few minutes after exposure to xenobiotics and is a reversible, restorable event (Ruch, 1994). Aberrant homologous and/or heterologous GJIC was observed in most rodent and human cancer cell lines and in many experimental and human tumors. Based on numerous *in vitro* and *in vivo* studies, it is believed that GJIC disorders play an important role in tumorigenesis. Intracellular signal pathways involved in reversible alterations of gene expression are under the homeostatic control of neighboring cells. An inhibition of GJIC would allow the initiated stem cell to escape the suppressing effects of the surrounding normal cells (Yamasaki, 1990; Yamasaki, 1995; Trosko and Ruch, 1998). Ample evidence that tumor-promoting chemicals downregulate GJIC is available. Therefore, if identified, inhibitors of GJIC are to be regarded as promoters of carcinogenesis.

In vitro detection of modes of action

In addition to *in vitro* genotoxicity assays, investigations into the complex process of carcinogenesis require other systems for exploring epigenetic mechanisms of action of carcinogens at the molecular, biochemical, and cellular levels. Permanent effort to create and validate such *in vitro* systems has been apparent in the recent years. The assays are supposed to include primarily mechanism-based endpoints (Yamasaki et al., 1996). Current methods of molecular biology and biochemistry allow the detection of specific molecules and enzyme