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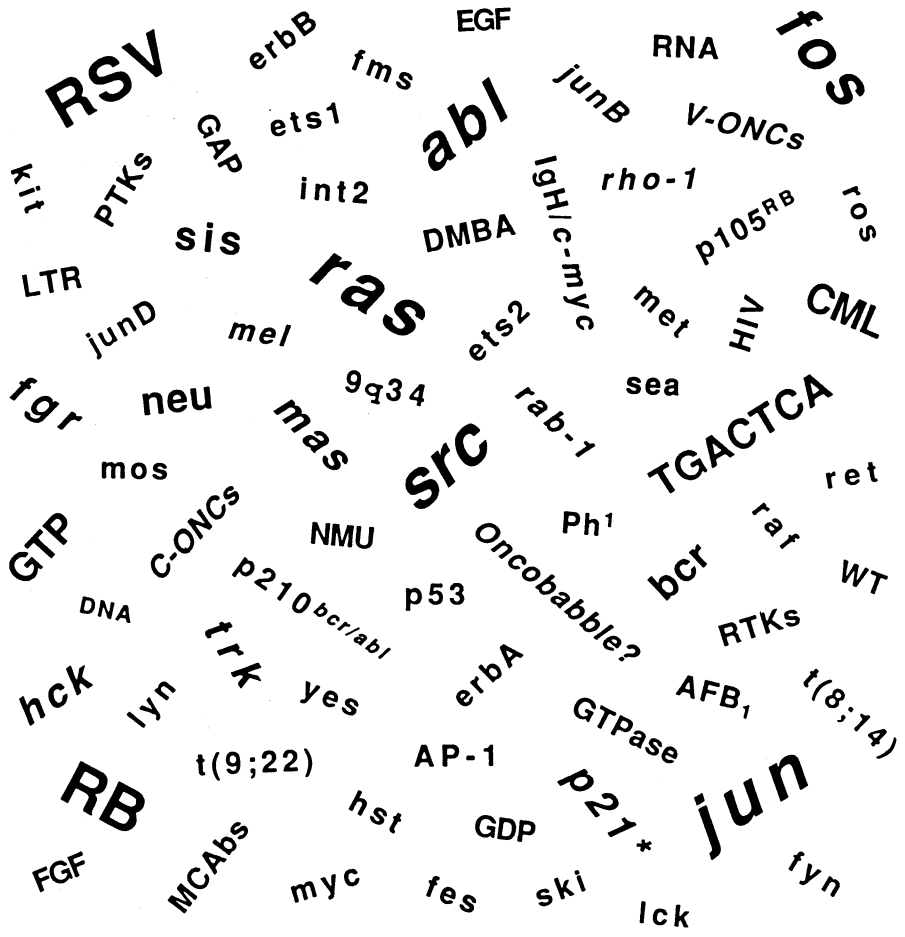
EDITORIAL COMMENTS

This issue marks the end of the sixth volume of the Newsletter. It is also my final issue as editor - after three years of enjoying my role in encouraging, collecting, editing and coordinating the Newsletter. I hope you will be as pleased as I was to learn that a frequent contributor, Doug Hamm, will take up editorial responsibilities. His past articles in these pages have been much requested, and his sense of humor has brightened articles on several topics.

The progress of the Newsletter over the past few years owes much to its regular contributors. Several individuals, whose roles with Workers' Compensation, Alberta Health, private companies or as clinical investigators and academics at our universities have put them in direct contact with occupational medicine, have generously taken the time to contribute their expertise to these pages. Their knowledge and views have benefited many who practice occupational medicine behind the front lines - in their own clinical practices. I know that Doug will benefit from these individuals' continued support. I am also sure he will appreciate reader's comments and enquiries, as I have. We appreciate the continued support of Alberta Health in providing this service to our readership.

Best wishes
Heather Bryant, M.D., FRCPC



The Puzzle of Occupational Cancer - Will this cryptic code reveal the solution? See the following pages.

Prepared in the Department of Community Health Sciences, Faculty of Medicine The University of Calgary, through funding by Alberta Health

OCCUPATIONAL CANCER: Oncogenes and Insights from "Molecular Oncology"

R. Douglas Hamm, M.D., C.C.F.P.

Introduction

"Cancer might be likened to a juvenile delinquent: a derivative of one's own self but without the controls necessary for appropriate socialization".

This comment by Robert Oldham (1987) reflects our growing understanding of cancer as a state of disordered "cellular socialization" which seems to be driven by aberrant inter- and intra-cellular communication (signalling). This article will present readers with a simplified overview of the oncogene paradigm that provides the scientific support for Oldham's remark. The story of oncogenes begins with a generally neglected discovery in chickens that proved to be of far-reaching significance in clarifying the "biodelinquency" of cancer.

From RSV to src

In 1910, while working at the Rockefeller Institute, Peyton Rous reported a transmissible sarcoma in Plymouth Rock hens and in 1911 he showed its induction by a cell free tumour extract. The Rous Sarcoma Virus (RSV) thus became one of the first infectious oncogenic agents to be described. It has set the stage for much of our current knowledge of retroviral oncogenes, being the first solid tumor-producing retrovirus to be identified. Fortuitously, RSV was able to replicate without a helper virus and was easily subjected to deletional mutations. These properties enabled it to lead researchers into the mysteries of retroviral gene structure and replication.

Among the RNA containing viruses, retroviruses are distinguished by their "reverse transcriptase" enzyme (RNA-dependent DNA polymerase), discovered in 1970. The retroviridae changed from curiosity status to notoriety in 1983 when a human retrovirus (initially called HTLV-III by Robert Gallo's group at the National Cancer Institute and LAV-1 by Luc Montagnier's group at the Pasteur Institute), was identified as the "AIDS virus", now known as the Human Immunodeficiency Virus or HIV.

You may be excused for wondering what such animal and human retroviral diseases have to do with occupational cancer. In fact, the molecular biology of retroviruses has taken our understanding of carcinogenesis to new levels of resolution and complexity. This quantum leap in our understanding has occurred in only the past few decades as retroviruses have introduced us to the world of "oncogenes", a term proposed by Huebner and Todaro in 1969.

Returning to the Rous Sarcoma Virus (which eventually brought Rous his Nobel Prize), we find that by the early 1970's, its modest 9 kilobase genome was found to carry a single gene responsible for its ability to initiate and maintain neoplastic transformation. This sarcoma producing gene was called "src" (pronounced "sarc" for SaRC-oma) and it has become the prototype oncogene. An oncogene is a genetic element that contributes to the neoplastic transformation of cells and in some cases may be inserted into the cellular genome by a retrovirus using its reverse transcriptase. According to George and Eva Klein (1985), "Thanks to the recombinational accidents of the retroviral lifestyle, we now have access to the dominantly acting oncogenes as an unexpected gift from viral oncology".

Oncogene "Alphabet Soup"

It had initially appeared that cellular oncogenes might be entirely a result of genetic transfer or transfection by retroviruses ("insertional mutagenesis"), but during the 1970's, DNA copies (called complementary DNA or "cDNA") of portions of the RSV ribonucleic acid (RNA) genome were found to hybridize with *normal* cellular DNA from many vertebrates and even unicellular organisms. This surprising finding suggested that src genes are ubiquitous in nature (highly conserved) and are components of the normal cellular genome with important functions in non-neoplastic cells. Further cDNA analysis of other retroviral oncogenes demonstrated that homologous cellular counterparts to such viral oncogenes were present in normal cells. In order to distinguish the normal "socialized" cellular genes from their "delinquent" (oncogenic) variants, the former were called proto-oncogenes. Under certain conditions (see below), proto-oncogenes can be changed into cellular oncogenes and neoplastic transformation can result (the term "c-onc" distinguishes cellular oncogenes from the virally borne "v-onc" genes, e.g., c-src and v-src). Comparisons of v-oncs with their c-onc counterparts has shown a variety of "activating" point mutations, deletions and genetic substitutions in the viral alleles (Bishop, 1987).

We now recognize a whole family of src-type oncogenes with a strange sounding "alphabet soup" nomenclature. The names of oncogenes are usually derived from the retroviruses in which they were first identified (see Table 1). For example, the "fgr" oncogene was first identified in F-eline G-ardner R-asheed sarcoma virus and the "yes" oncogene is a homolog of the viral oncogenes of Y-73 sarcoma virus and E-sh S-arcoma virus. A recently discovered oncogene was called "jun" because it was found in Avian Sarcoma Virus-17 and the Japanese word for seventeen is "ju-nana". One of the most readable current reviews of oncogenes is the book by Burck, Liu and Larrick (*Oncogenes*, Springer-Verlag 1988) which catalogs over fifty oncogenes. Table 1 lists the best known oncogenes by functional classes. Racker (1989) has said that "oncogene products are the fifth column in a war for the control of metabolism and growth". Figure 1 illustrates the cellular locations of these oncogenic Trojan horses.

Protein-Tyrosine Kinases

In 1978, Collett and Erikson, at the University of Colorado, discovered that the v-src oncogene product, known as $p60^{v\text{-}src}$ ("p" denotes a protein, "60" refers to its molecular weight in kiloDaltons, and the superscript denotes the encoding gene), catalyzes the addition of a phosphate molecule to other proteins, a process that is termed protein phosphorylation. Enzymes that phosphorylate proteins are called protein kinases and have been well described since the purification of phosphorylase kinase in 1959. Hunter (1987) has compared protein kinases to cellular "transistors" that act as amplifiers and switches in the biochemical circuitry of cells. Phosphorylation of proteins is an important mechanism for regulating enzymatic functions in the complex molecular circuitry of the cell. However, rather than phosphorylating the serine or threonine amino acids of proteins, as was the case for other known protein kinases, $p60^{v\text{-}src}$ was found to add phosphate (from ATP) to tyrosine, the only other amino acid with a hydroxyl group. This surprising tyrosine specificity (normally less that 0.1% of cellular protein phosphate is tyrosine bound) prompted a search for similar activity in other oncoproteins and a diverse group of protein-tyrosine kinases (PTKs) are now considered members of the src oncogene family (see Table 1). It is not yet known whether src-family oncoproteins phosphorylate unusual substrates, or simply phosphorylate their usual substrates unusually well (Perlmutter et al., 1988). In any case, tyrosine kinases have proved to be promiscuous in their choice of intracellular targets so we do not yet know their key oncogenic substrates.

ras Oncogenes

One of the most thoroughly studied models of oncogene activation in carcinogenesis is the ras oncogene system, named for the RA-t S-arcoma virus in which the oncogene was first found. Of special interest in occupational cancer is the fact that ras oncogenes appear to have an important role in carcinogen-induced tumors (see Table 2). The three prototype members of the ras family are H-ras (Har-

TABLE 1

ONCOGENES	RELATED ONCOPROTEIN FUNCTIONS (See Fig. 1 for cellular locations)	CHROMOSOME LOCATION p = short arm q = long arm prefix numeral = chromosome suffix numeral = sub-bands
cytoplasmic src-family		
abl	Protein-tyrosine kinase	9q34
fgr	Protein-tyrosine kinase	1p36
fyn	Protein-tyrosine kinase	6q21
hck	Protein-tyrosine kinase	20q11
lck/tck	Protein-tyrosine kinase	1p32
lyn	Protein-tyrosine kinase	8q13
src	Protein-tyrosine kinase	20q13
yes	Protein-tyrosine kinase	18q21
growth factor receptor-family		
erbB-1/erbB	EGF receptor homology	7p11
fms	GM-CSF receptor homology	5q34
kit	PDGF receptor homology	4q11
mas	angiotensin receptor function	?
met	receptor with unidentified ligand	7q21
neu/erbB-2	receptor with unidentified ligand	17q21
ret	receptor with unidentified ligand	?
ros	Insulin receptor homology	6q22
sea	receptor with unidentified ligand	11q13
trk	tropomycin receptor kinase	?
serine protein kinase-family		
ets-1	?	11q23
mos	cytoplasmic serine-threonine kinase	8q22
raf/mil	cytoplasmic serine-threonine kinase	3p25
extracellular growth factor-family		
int-2	Fibroblast Growth Factor homology	11q13
hst	FGF-related homology	?
sis	B chain of PDGF (PDGF-2) homology	22q12
GTP binding/GTPase-family		
H-ras	membrane based signal transduction	11p15
K-ras	membrane based signal transduction	12p12
N-ras	membrane based signal transduction	1p22
DNA binding-family		
erbA	receptor for thyroid hormones	17p11
ets-2	?	21q23
c-myc	role in DNA and hnRNA processing	8q24
N-myc	role in DNA and hnRNA processing	2p23
L-myc	role in DNA and hnRNA processing	1p32
myb	DNA transcriptional trans-activator	6q22
fos	DNA transcriptional modulation	2q21
ski	?DNA transcription	1q12
jun	DNA transcriptional trans-activator	1p31
Anti-Oncogene family		
RB	suppressor gene in retinoblastoma	13q14
WT	suppressor gene in Wilms' tumour	11p13
p53	suppressor gene in colorectal CA	17p?

vey ras), K-ras (Kirsten ras) and N-ras (Neuroblastoma ras). Other recently discovered ras-related oncogenes include rho-1, 2, and 3, ral, R-ras, and rab-1, 2, 3 and 4. The mel oncogene at chromosome 19q13 may also be a member of the ras family which all express a 21 kiloDalton protein ($p21^{ras}$) anchored to the inner side of the cell membrane. $p21^{ras}$ binds GTP and GDP with high affinity and is activated by GTP-binding which enables further interaction with a GTPase activating protein called "GAP". GAP converts p21-GTP (the activated form) to p21-GDP (the inactive form) and simultaneously sends an intracellular "downstream signal" via an undefined effector molecule which may participate in the phosphatidylinositol pathway (See Figure 2).

In most malignancies, all three prototype ras genes can be oncogenically activated but N-ras activation predominates in hematopoietic cancers and the K-ras and H-ras genes predominate in carcinomas. We now know that single nucleotide substitutions in the ras proto-oncogenes at critical codons (nucleotide triplets) such as codons 12 and 61 can specify an altered amino acid in the p21 product (such a mutated form is designated p21*) which modifies its molecular structure so as to reduce its intrinsic GTPase activity and thus produce a constitutive (i.e., unregulated) oncogenic "signal" from the p21*-GAP-effector complex (See Figure 2).

NMU-induced mammary CA in rats produces a consistent G to A transition in the second nucleotide of codon 12 (GGA) in H-ras, whereas DMBA mutates the two adenine residues of the H-ras codon 61 (CAA). Experimental evidence thus suggests that ras oncogenes provide several mutational "hot-spots" for chemical carcinogens.

Receptor Tyrosine Kinases

Following discovery of the tyrosine kinase activity of the src oncoprotein, further investigations revealed a family of oncogene encoded protein products having both cell surface receptors and intrinsic protein tyrosine kinase activity (see Figure 1). These molecules are known as Receptor Tyrosine Kinases (RTKs) and have a common architecture (in three subclasses) consisting of an extracellular ligand-binding domain (receptor) of 500 to 850 amino acids, a membrane spanning (transmembrane) domain of 20 to 30 amino acids and an intracellular (cytoplasmic) domain of 500 to 600 amino acids containing a catalytic (tyrosine kinase) site. RTKs are transmembrane allosteric enzymes, i.e., ligand binding induces a conformational change which activates their kinase function. Loss of such allosteric regulation can confer pathogenicity on proto-oncogene products, e.g., via altered substrate specificity or kinetics.

The integrated receptor-catalytic structure of RTKs suggests a transmembrane signalling function for these molecules. Indeed, the oncogenic potential of these molecules appears due to their ability to generate a mitogenic signal. Such a signal can be produced because of:

a) overexpression of RTKs resulting from oncogene dysregulation or amplification (as has been shown for the neu oncogene in human breast cancer)

b) mutation of the ligand-binding domain so as to produce conformational activation of the catalytic site in the absence of ligand (as shown for the erbB oncoprotein), or

c) mutation of the transmembrane or cytoplasmic domains so as to consti-

tutively activate (i.e., produce unregulated on-signalling) the catalytic site (as shown for the fms oncoprotein).

Conservation of RTKs does not extend to unicellular organisms, suggesting that their function relates uniquely to the physiological requirements of multicellular organisms (Yarden and Ullrich, 1988).

It has been found that some oncogene derived RTKs are homologous to known receptors for growth factors or other biological messengers. For example, the erbB oncogene encodes a truncated receptor (involving deletion of most of the extracellular domain) for epidermal growth factor (EGF) and the mas oncogene product contains a functional angiotensin receptor. The ligands for the met, kit, trk, ret, and ros oncoproteins have not yet been identified but many of these may be growth factor or hormone receptors. It has been found that cells can become "turned on" by so-called autocrine feedback when the cell detects its own growth factors and triggers its own transformation. Oncogenes can set the stage for such a process by short-circuiting mitogenic signal pathways.

Nuclear Oncogenes

An "average" human chromosome contains about 2500 genes. Oncogenes are literally "buried" throughout the cellular genome (see Table 1) which is thought to consist of about 100,000 genes. Whereas oncogenes are located in the cell nucleus, their translation products (oncoproteins) are located throughout the cell and may even be secreted as growth factors (Figure 1). Within the past few years, much attention has focused on the role of oncogenes and their oncoproteins in cellular gene regulation. In particular, research on the control of gene transcription and cell growth has converged on the jun oncogene and its role in nuclear signalling.

Jun, Fos and Myc

Work on jun is so recent that *The Oncogene Handbook* (Elsevier, 1988) only makes passing mention of it. It appears that the discovery of jun, a "nuclear" oncogene, has many parallels to that of src, a "cytoplasmic" oncogene. Like src, jun was discovered in an avian retrovirus (Maki, 1987). It was found to be the 0.9 kb transforming sequence in the genome of Avian Sarcoma Virus 17 (ASV 17) and, like src, jun produced sarcomas in chickens.

Just as src proved to be the prototype oncogene, jun is the first definitive example of a transcription factor gene inducing cancer (Vogt and Bos, 1989). Gene regulation is accomplished by cis-acting ("upstream") sequences called promoters. It has been found that a nucleotide sequence (5' TGACTCA 3') in gene promoters recognizes the human transcription factor-family known as AP-1. AP-1 binding

Figure 1

LOCATION OF ONCOGENE PRODUCTS

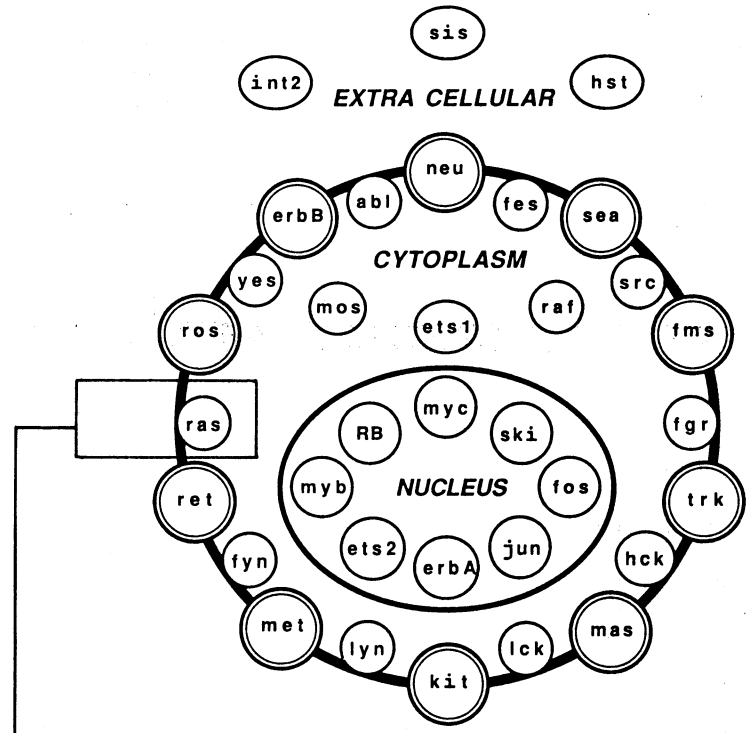


Figure 2

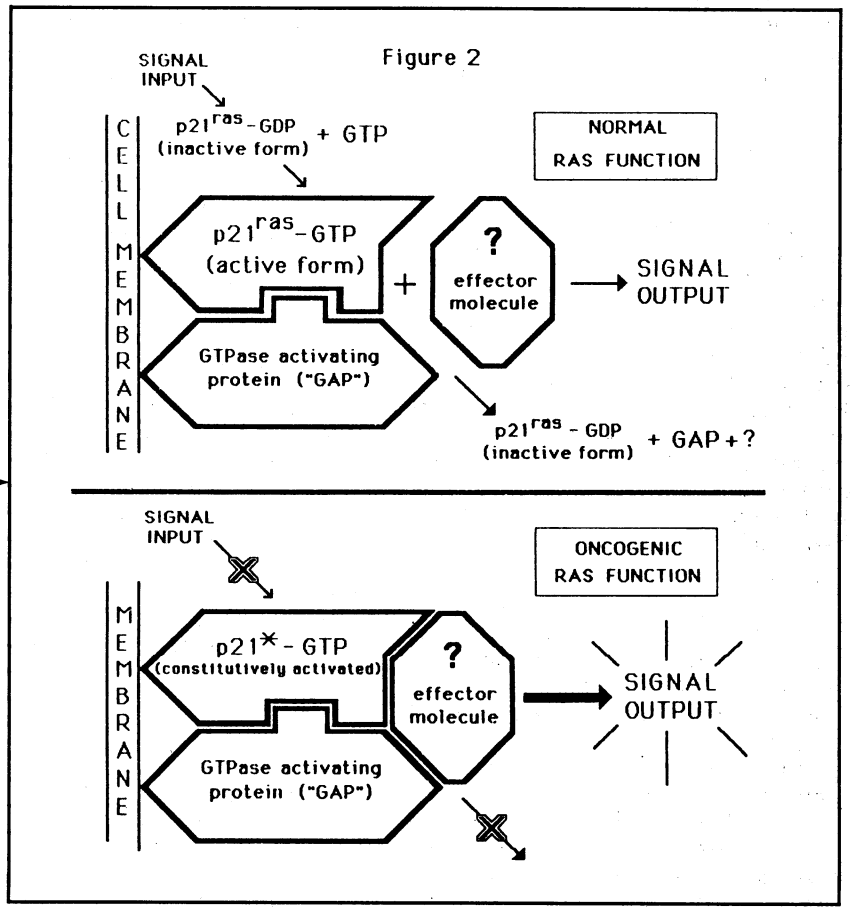


TABLE 2

Activation of ras Oncogenes in Carcinogen-Induced Tumors (from Barbacid, in *Cellular Oncogene Activation* ed. G. Klein, 1988)

Carcinogens	ras Oncogenes	% Activation	Tumour Models Tested
AFB_1	K-ras	25	hepatocellular CA (rat)
B(a)P	K-ras, H-ras	80	hepatocellular CA (mouse)
DBACR	H-ras	80	skin CA (mouse)
DMBA	H-ras	23	mammary CA (rat)
DMBA	H-ras	90	skin CA (mouse)
DMBA	H-ras	75	mammary CA (mouse)
DMBA	H-ras	60	keratoacanthoma (rabbit)
DMN	K-ras	40	kidney mesenchymal (rat)
MCA	K-ras	50	fibrosarcoma (mouse)
MCA	K-ras	83	lymphoma (mouse)
NMU	H-ras	86	mammary CA (rat)
NMU	K-ras	60	kidney mesenchymal (rat)
NMU	K-ras, N-ras	85	lymphoma (mouse)
TNM	K-ras	74	lung CA (rat)
TNM	K-ras	100	lung CA (mouse)
VC	H-ras	100	hepatocellular CA (mouse)

AFB_1 = aflatoxin B_1
B(a)P = benz(a)pyrene
DBACR = dibenz(c,h)acridine
DMBA = dimethylbenz(a)anthracene
DMN = methyl(methoxymethyl)nitrosamine
MCA = 3-methylcholanthrene
NMU = nitrosomethylurea
TMN = tetranitromethane
VC = vinyl carbamates

to DNA is essential for promoter activity and rather unexpectedly, one of the components of AP-1 turned out to be encoded by jun. Two other jun related genes have been termed jun B and jun D.

It has recently been determined that the jun oncoprotein can form a homodimer (jun-jun) but preferentially forms a fos-jun heterodimer with increased binding affinity for the AP-1 DNA consensus sequence (i.e., TGACTCA). Fos alone does not show specific DNA binding and fos homodimers do not form. Fos and jun oncoproteins dimerize because both carry "leucine zipper" amino acid structures (leucines at seven-residue intervals) in alpha helical regions. The contact surface for DNA binding is adjacent to these zipper domains and dimerization constrains the protein structure for proper DNA attachment (See Figure 3).

The myc family (c-, L-, and N-myc) also have leucine zipper domains and appear to be nuclear transcription factors. Of particular interest is the activation of the proto-oncogene myc by chromosomal translocations, usually via a reciprocal t(8;14)(q24;q32). This has been found to occur in about 80% of cases of Burkitt's Lymphoma where during B-cell differentiation, a t(8;14) translocation juxtaposes myc from 8q24 to the immunoglobulin heavy chain (IgH) locus at 14q32 resulting in myc activation by a "head to head" IgH/c-myc gene fusion.

Whereas jun and fos are primary nuclear targets of cell signal transduction and act as transcriptional activators via the formation of transcription complexes such as AP-1, jun-fos, etc., other nuclear oncogenes have different mechanisms of gene interaction. The erbA oncoprotein positively or negatively modulates (i.e., up- or down-regulates) a variety of genes by interacting with their promoter regions upon thyroid hormone binding. The myb oncogene has recently (Nishina et al., 1989) been shown to function as a transcriptional trans-activator.

Anti-Oncogenes

One of the most exciting developments in oncogene research has been the discovery of so-called anti-oncogenes or recessive oncogenes. The prototype is the retinoblastoma (RB) gene at 13q14 which encodes a nuclear localized DNA binding protein ($p105^{RB}$) which may regulate transcription of certain genes involved in growth control. Patients with heritable retinoblastoma carry one germ line mutant RB allele and develop a retinal tumor when the corresponding normal allele is deleted or mutated (i.e., reduction to homozygosity for RB^-).

Several DNA tumour viruses have been found to produce nuclear oncoproteins that can bind $p105^{RB}$ leading to neoplastic transformation. The search is now on for other anti-oncogenes (also called tumour or onco-suppressor genes) that by deletion or inactivation can produce malignant transformation. Chromosomal aberrations are prime suspects in tumour induction by anti-oncogene deletion. Genes encoding DNA repair enzymes can also confer a predisposition to cancer when mutated or deleted. However, consideration of their role in carcinogenesis is beyond the scope of this article.

Proto-Oncogene Activation

The ubiquitous proto-oncogenes of normal cells are highly regulated in their expression whereas oncogene expression in neoplastic cells is usually constitutive and amplified. It is thought that each type of tumour may express a unique repertoire of "activated" proto-oncogenes (i.e., oncogenes). The mechanism(s) of proto-oncogene activation by carcinogens continues to be a research frontier in occupational carcinogenesis.

Studies by Hayward et al. (1981) showed that c-myc can be activated by insertion of a retroviral promoter (e.g., the so-called "long terminal repeat" or LTR) resulting in enhanced gene expression and neoplastic transformation (c-erbB, c-mos, c-myb and c-H-ras can be activated by a similar insertional mutagenesis). In 1983, Bartram et al. determined that Chronic Myelogenous Leukemia (CML) is associated with a translocation of c-abl from chromosome 9 to 22, usually by reciprocal t(9;22)(q34;q11). This produces the characteristic shortened chromosome 22 or Philadelphia chromosome (Ph^1), the first consistent chromosomal abnormality noted in human cancer (See Figure 4). The t(9;22) rearrangement places abl under the control of a gene formerly called bcr (for B-reakpoint C-luster R-egion) and now known as phl. This results in a fusion oncoprotein called $p210^{bcr/abl}$.

Point mutations producing oncogene activation have been well documented as noted above for ras and recently have been shown in src, lck, fyn, hck, and neu. Yet to be defined are the mechanisms of proto-oncogene activation related to spontaneous genomic instability since Cairns (1981) argues that chromosomal abnormalities may contribute more to the genetic instability of neoplasms than do point mutations.

Looking Ahead

In occupational settings, chemical genotoxicity presents a likely route of proto-oncogene activation. An earlier article in this *Newsletter* (Volume VI, No.3) discussed carcinogen-DNA interaction. As noted above, ras oncogenes are frequently activated in experimental carcinogen induced tumours and the altered p21 oncoprotein (i.e., p21*) is found in transformed cells. Monoclonal antibodies (MCAbs) have been prepared against a variety of oncoproteins including p21* and it is tempting to consider the use of MCAbs

as probes for oncoprotein markers. Brandt-Rauf and Pincus (1987) have suggested that "Studies using monoclonal antibodies to oncogene proteins offer a new and potentially useful avenue of exploration for molecular epidemiology in the area of markers for occult tumors or premalignant lesions".

The oncogene paradigm or hypothesis proposes that we carry all the genetic elements necessary for malignant transformation. It suggests that neoplastic processes may differ from normal ones only in degree, e.g., gene expression and cellular signalling. This review has focused on gene regulation in carcinogenesis but it should be noted that other models have also been proposed, e.g., intrinsic somatic mutation (Morris, 1988) or chromosome error propagation (Holliday, 1989).

Some researchers (e.g. Burck et al.) have enthusiastically suggested that the oncogene paradigm represents a contribution to scientific thought which is equal in magnitude to earlier cell and germ theories. Will (onco)gene regulation eventually prove as foundational to occupational cancer as microbiology is to communicable disease? In any case, the oncogene paradigm has already provided a tantalizing glimpse into the cellular dynamics of chemical carcinogenesis and promises further revelations.

Conclusion

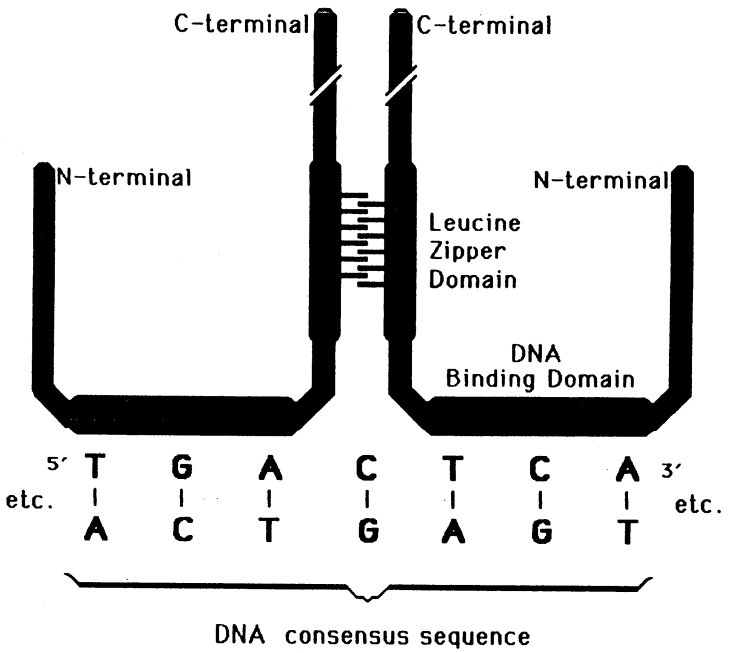
It may be argued that we have far to go before we realize actual worksite applications of oncogene theory. Although it is true that we cannot yet adequately explain or predict the "bio-delinquency" of cancer, concepts such as the oncogene paradigm may provide us with powerful tools for occupational cancer risk assessment in the not too distant future. This brief review has attempted to offer occupational health professionals a perspective on some recent (and explosive) advances in molecular oncology that are fraught with implications for our future practice of occupational medicine.

(References available on request)

Dr. Hamm is a resident in Community Medicine in the Department of Community Health Sciences, Faculty of Medicine, The University of Calgary. This article concludes his two-part series on occupational cancer (the first part is found in Volume VI, Number 3 of the *Newsletter*).

Figure 3

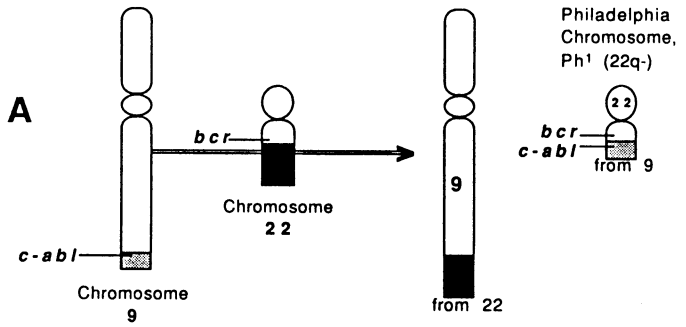
Fos - Jun Dimerization and DNA binding



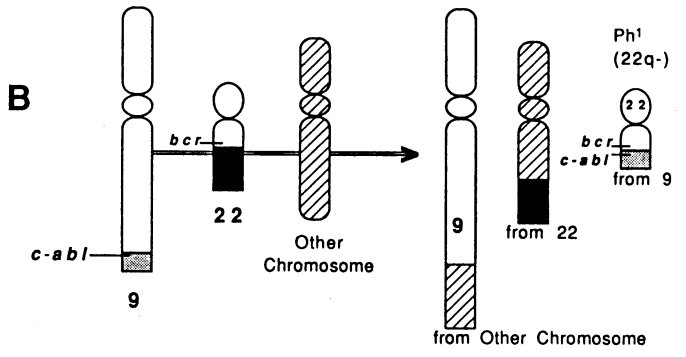
DNA consensus sequence

Figure 4

c-abl ACTIVATION by CHROMOSOMAL TRANSLOCATIONS



Reciprocal Translocation (in >90% of cases of CML)



Complex Translocation (in <10% of cases of CML)