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Regulation of Nuclear Oncogenes Expressed in Lung Cancer Cell Lines

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Abstract Lung cancer is a major cause of mortality in the United States and accounts for the majority of all cancer deaths in both men and women. It is hoped that through broadening our understanding of the mechanisms involved in transformation of bronchial epithelial cells we will be able to improve methods of diagnosis and treatment of this disease, with the ultimate goal of reducing on lung cancer mortality. A knowledge of the molecular mechanisms involved in processes such as cell division and differentiation is paramount to this task, because it is known that aberrant responses to growth factors or cytokines found in the normal cellular milieu can lead to abnormal cell growth and/or transformation. Signals initiated at the cell membrane by tumor promoters, growth factors, or cytokines are transduced from the cell membrane to the nucleus and are, in part, mediated centrally by transcription factors encoded by nuclear protooncogenes. The transcription factor *myc, jun,* and *fos* have been characterized in both normal and transformed lung epithelial cells through detailed studies using cell lines. In this manuscript, we review what is known about the expression and regulation of these nuclear protooncogenes in normal and malignant epithelial cells of the lung, and their role in the development of lung cancer.

Key words: lung cancer, cell lines, nuclear oncogenes, *myc* genes, c-*jun*, c-*fos*, transcription factors

Transcription factors encoded by nuclear proto-oncogenes control important cellular processes such as cellular proliferation, differentiation, and transformation by modulating the expression of certain genes. Transcription factor activity is modulated by tumor promoters and growth factors via signal transduction pathways which transmit signals to the nucleus from the cell membrane. Alteration or disruption of these tightly regulated signal transduction pathways can lead to uncontrolled cellular proliferation or transformation. Several proto-oncogenes with important functions in lung cancer have been described and include *myc, jun,* and *fos*. Understanding the role of transcription factors in cellular proliferation and transformation of the bronchial epithelium has been facilitated by studies using lung cancer cell lines established at the National Cancer Institute (NCI) and other centers. These cell lines have provided a means by which to extensively examine the functions and regulation of transcription factors in lung cancer.

The first transcription factors extensively characterized in these lung cancer cell lines were members of the *myc* gene family. More recently, the expression and regulation of other nuclear oncogenes, such as the early response genes c-*jun* and c-*fos*, have been defined in lung cancer cells. In this manuscript, we review the roles of the nuclear oncogenes c-*myc*, c-*jun*, and c-*fos*, the responses of these transcription factors to extracellular stimuli, and the mechanisms governing regulation of their expression in lung cancer cells.

myc GENE FAMILY

Members of the *myc* gene family, c-*myc*, N-*myc*, and L-*myc*, are the most fully characterized transcription factors in lung cancer cell lines. Data from multiple experimental systems have established that the *myc* genes play central roles in cellular proliferation, transformation, and differentiation pathways and that c-*myc* is involved in the proliferation/differentiation switch [1]. A strong correlation between the up-regulation of c-*myc* expression and increased

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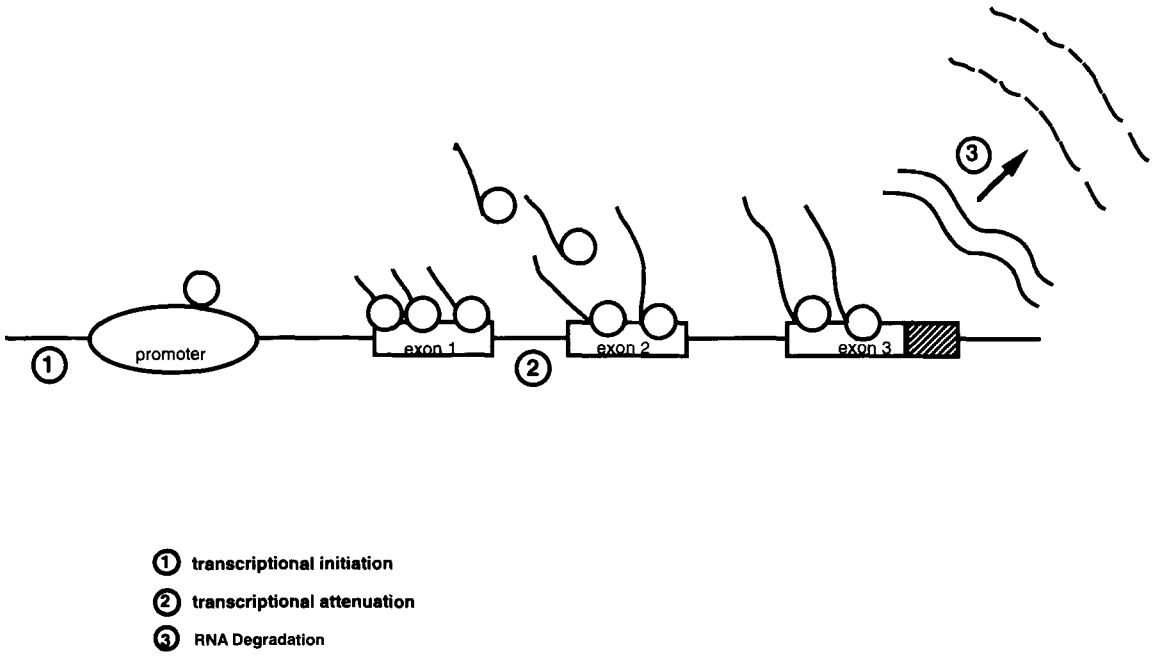

Fig. 1. Mechanisms of transcriptional regulation of the *myc* oncogene. *myc* expression is regulated by several mechanisms which include transcriptional initiation (1), transcriptional attenuation (2), and an altered rate of RNA degradation (3). Expression of the *myc* oncogene is under tight control by these regulatory mechanisms.

rate of cellular proliferation has been well characterized, and expression of c-*myc* has been shown to increase early in the G_0/G_1 phase of the cell cycle as part of the competence process allowing the cell to progress into S phase. In addition, c-*myc* may be necessary for S phase traversal [for review see ref. 2]. Down regulation of c-*myc* expression is associated with cellular differentiation in several different cell types, and constitutive expression leads to a block in differentiation in other cell types [for review see ref. 3].

The *myc* genes encode nuclear phosphoproteins that bind to DNA in a sequence specific manner [4]. Initially, it was thought that Myc proteins directly affected DNA replication [5]. However, more recent data suggest that these proteins have an indirect role in DNA replication and RNA processing, and function as transcriptional regulators of gene expression [6,7]. Structurally, Myc and its binding partners, Max and Mad, contain leucine zipper and helix-loop-helix motifs which are common to proteins that function as transcription factors [8,9]. The Myc protein binds as heterodimer with Max or Mad to a specific core consensus sequence, CACGTG, and is implicated in the control of proliferation in normal cells as well as in transformation and differentiation [10,11].

Recent work from several laboratories has provided insight into molecular mechanisms involved in the regulation of *myc* gene expression. In most cell systems, c-*myc* expression is highly dependent upon the growth conditions of the cell. For instance, c-*myc* gene expression increases with serum stimulation or exposure to a variety of growth factors or mitogens in cell culture [12,13]. Conversely, in other cell systems, c-*myc* expression levels decrease dramatically with exposure to agents such as retinoic acid which induce cellular differentiation [14]. These changes in c-*myc* gene expression are, in part, dependent upon transcriptional and post-transcriptional regulatory mechanisms including transcriptional initiation, transcriptional attenuation, or mRNA degradation (Fig. 1) [15]. Transcriptional initiation is a process where the core polymerase complex binds (along with various co-factors) to specific target sequences in the gene's promoter and begins RNA transcription. In many cell types, this process is often the key regulatory step for c-*myc* expression.

Transcriptional attenuation is another mechanism of gene regulation which has only recently been characterized [16,17]. The biological importance of transcriptional attenuation as a regulatory mechanism is evident in cell systems where c-*myc* expression is down regulated in

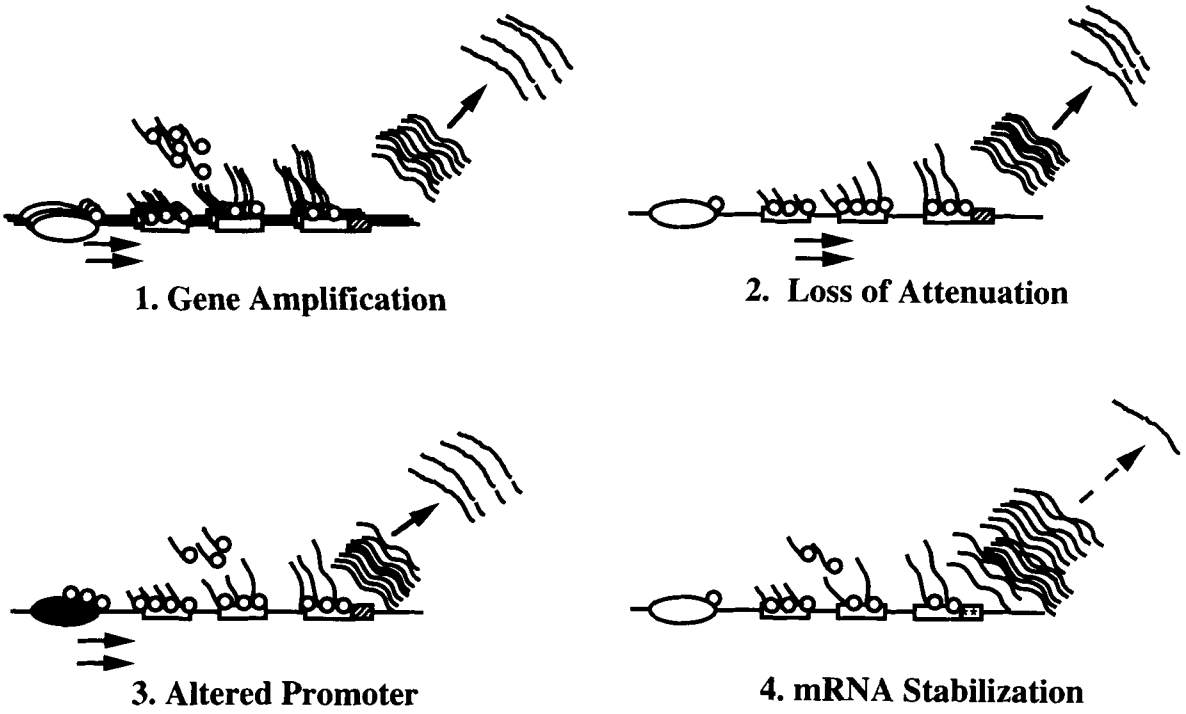

Fig. 2. Possible alterations in *myc* transcriptional regulatory mechanisms. Gene amplification leads to increased transcriptional machinery (**1**); loss of attenuation leads to "transcriptional read-through" (**2**); mutations in the promoter or its flanking sequences can alter levels of transcriptional initiation (**3**); and mutations in the destabilizing sequences found in the 3′ untranslated regions of *myc* genes lead to a decrease in mRNA degradation (**4**). Each of these alterations results in elevated steady state levels of *myc* mRNA and protein.

cells which have been induced to differentiate. Transcriptional attenuation results from blockage of elongation of nascent mRNA molecules at the exon 1/intron 1 border of the gene. For example, in undifferentiated HL60 cells, the ratio of exon 1 to exon 2 transcripts is 3:1 reflecting a decrease in the density of transcription complexes as one proceeds 5′ → 3′ down the gene. In cases where these cells are induced to differentiate with retinoic acid, c-*myc* gene transcriptional attenuation is augmented, and the exon 1/exon 2 ratio increases to 15:1, resulting in a marked decrease in steady state mRNA levels [16]. These results suggest that, at least in this cell system, the major component of c-*myc* transcriptional regulation occurs at the level of elongation rather than at the level of initiation [16]. Another commonly observed mechanism of c-*myc* regulation is at the level of mRNA degradation with modulation in the half-life of c-*myc* mRNA. The regulation of mRNA degradation of c-*myc* and other short-lived mRNAs involves elements in both the 3′ untranslated and 5′ regions. Thus, the transcriptional regulation of *myc* is indeed complex and clearly involves multiple cis- and trans-acting transcriptional regulatory elements [15].

Deregulated c-*myc* expression can result from alterations in any one of the above-mentioned regulatory mechanisms (Fig. 2). First, an increase in gene copy number resulting from gene amplification can provide for increased transcriptional initiation and ultimately lead to elevated steady state levels of mRNA. Second, a decrease in transcriptional attenuation has been shown to produce transcriptional "read-through," resulting in elevated steady state levels of mRNA and protein. Third, mutations in the promoter region or its flanking sequences can have a profound effect on the expression levels of these genes by altering transcriptional initiation. Finally, mutation or deletion in the destabilizing sequences found in the 3′ untranslated regions of this gene can produce prolongation of mRNA half-life and therefore enhance steady state levels of mRNA [for review see ref. 15].

Lung cancer cell lines have proven to be an excellent resource to delineate specific mechanisms of overexpression and deregulation of the

TABLE I. Characterization of the *myc* Gene in NCI Lung Cancer Cell Lines*

Cell line	Relative copy number of oncogene			Relative mRNA level of oncogene [half-life (min)]			Reference
	c-*myc*	N-*myc*	L-*myc*	c-*myc*	N-*myc*	L-*myc*	
SCLC							
H82	25	1	1	23 (50)	—	1 (153)	[25]
H146	1	1	1	8 (36)	—	—	[25]
H187	1	1	1	—	1 (ND)	—	[25]
H249	1	150	1	—	10 (72)	—	[25]
H209	1	1	1	—	—	17 (90)	[25]
H510	1	1	15	1 (ND)	—	110 (130)	[25]
N390	3.8	1	1	0.9 (ND)	—	—	[20]
H60	38	1	1	35 (ND)	—	—	[20]
N417	47	1	1	25	—	—	[20]
H446	20	1	1	15	—	—	[20]
NSCLC							
H23	20	1	1	ND	ND	ND	[20]
A549	1.4	1	1	ND	ND	ND	[20]
H157	0.8	1	1	ND	ND	ND	[20]
H125	1.4	1	1	ND	ND	ND	[20]

*ND, not determined.

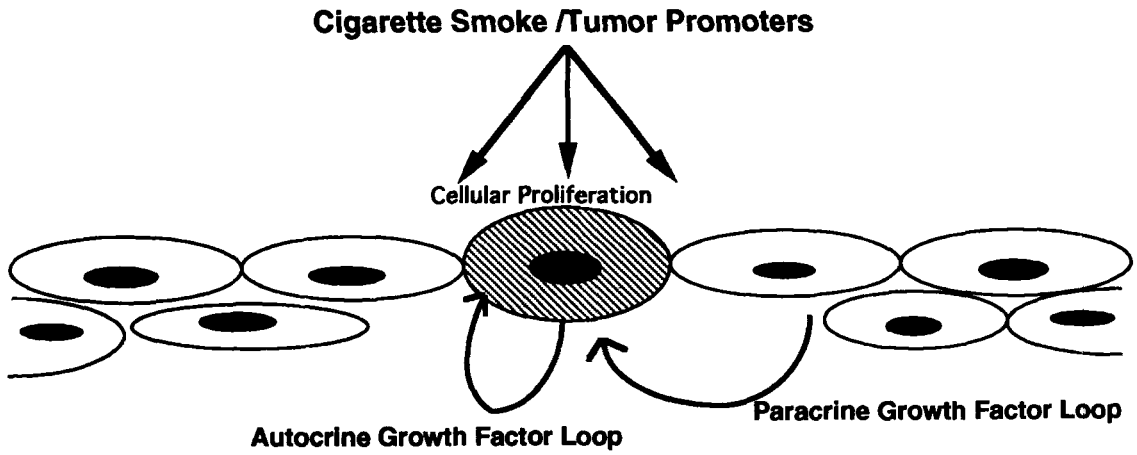

Fig. 3. Mechanisms involved in tumor promotion of the tracheobronchial epithelium. Malignant transformation of the respiratory epithelium is a multistage process which involves the steps of initiation (mutations in regulatory oncogenes), promotion (clonal expansion of the initiated cells), and progression (additional mutational changes in oncogenes by carcinogenic insults). Tobacco smoke contains factors which act as promotional agents on the respiratory epithelium. These substances promote growth of initiated bronchial epithelial cells through a network of cytokines and growth factor pathways which can work via autocrine or paracrine mechanisms. Examples of growth factors which work through these mechanisms include epidermal growth factor (EGF), transferrin, insulin-like growth factor (IGF-1), and gastrin-releasing peptide (GRP).

myc gene family members in lung cancer. Extensive work has demonstrated that the majority of lung cancer cell lines express elevated levels of *myc* [18,19]. In general, only one member of the *myc* gene family is overexpressed in any given tumor specimen or cell line, and the mechanism of dysregulation varies among cell lines. The involvement of *myc* in the pathogenesis of lung cancer was initially described in small cell lung cancer (SCLC) cell lines and gene amplification was the first and the most common mechanism of *myc* overexpression to be described [20]. Studies using lung cancer cell lines have demonstrated that both SCLC cell lines and non small cell lung cancer (NSCLC) cell lines display amplification of c-, L-, or N-*myc*. Amplification of these genes ranges from 20 to 115 copies per cell, and results in a proportional increase in steady state mRNA and protein levels [21]. The fraction of NCI lung cancer cell lines amplified

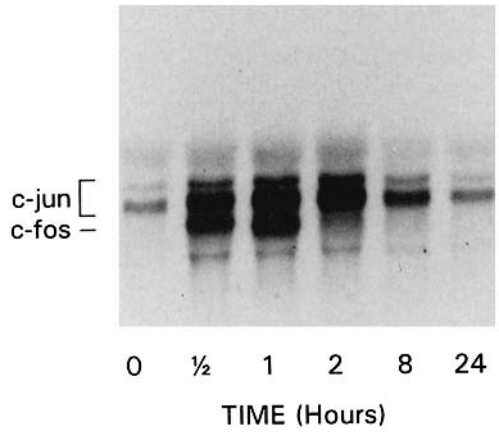

Fig. 4. Serum stimulation of c-*jun* and c-*fos* expression in the NSCLC cell line H125. Cells lines were grown to subconfluence in RPMI-1640 supplemented with 2% fetal bovine serum (FBS). They were starved in serum-free media for 18–20 h and then stimulated by the addition of 2% FBS. Total RNA was harvested at the listed time points and processed as previously described [29]. All lanes contained equal amounts of RNA as determined by ethidium bromide staining (data not shown). Northern blots were probed with ^{32}P-labeled c-*jun* and c-*fos* probes prepared by random priming technique [30]. Baseline levels of c-*jun* mRNA in these cells is greater than c-*fos* mRNA. Upregulation of the expression of both oncogenes is rapid and apparent within 15 to 30 min after serum stimulation. Expression is transient, however, and mRNA levels return to baseline levels within 2 h for c-*fos* and within 24 h for c-*jun*.

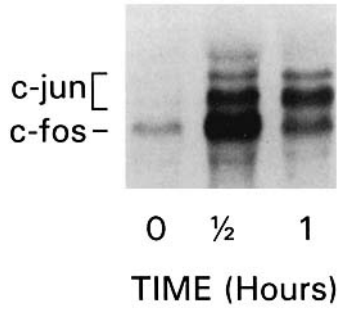

Fig. 5. Serum stimulation of c-*jun* and c-*fos* expression in NSCLC cell line A549. A549 cells were grown and RNA harvested and processed as described in Figure 4. Similar to NSCLC cell line H125, the NSCLC cell line A549 constitutively expresses c-*jun* and c-*fos* mRNA (*fos* > *jun* mRNA levels). These transcription factors are rapidly inducible to 11- and 9-fold baseline levels within 30 min of stimulation with serum.

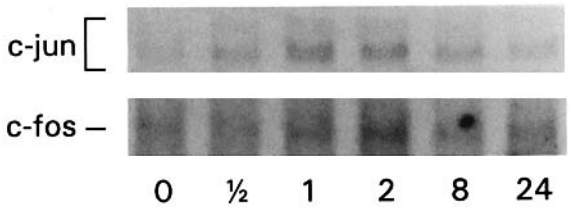

Fig. 6. Serum stimulation of c-*jun* and c-*fos* expression in the SCLC cell line H209. H209 cells were grown and RNA harvested and processed as described in Figure 4. Expression of the c-*jun* and c-*fos* mRNA levels are rapidly upregulated in this SCLC cell line to 3.5-fold baseline within 1 h, and to approximately 2-fold baseline levels within 2 h, respectively, after serum stimulation.

for an *myc* gene family member is 31% of SCLCs, and 20% of NSCLCs [22]. Of interest, amplification of *myc* gene family members has also been studied in primary lung cancer specimens and has been identified in approximately 18% of primary SCLC tumor samples and in 8% of NSCLC tumor samples [22]. The reasons for this discrepancy in *myc* gene amplification between primary tumors and tumor cell lines remain unknown. Potential explanations are that gene amplification is an artifact of cell culture in that the amplification occurs in cell culture during the establishment of the cell line, or cell culture conditions select for a sub-population of cells (already present in the primary tumor) with *myc* gene amplification. More work will be required to distinguish between these different possibilities.

Regardless of the mechanism of *myc* amplification, these data support the concept that it is most likely a late event in the development of SCLC. Biological and clinical data support this notion. Cell lines classified in the "variant" phenotypic subset of SCLC cell lines which overexpresses *myc* have more aggressive growth characteristics than those cell lines in the "classic" group of SCLC which do not overexpress *myc* [20]. The variant subtype is further characterized by a larger cell size and less neuroendocrine differentiation compared to the classic small cell lines which do not overexpress *myc* [23]. In addition, *myc* family DNA amplification occurs more frequently in tumor specimens from patients who have been treated with chemotherapy than in specimens from untreated patients, and DNA amplification of c-*myc* in tumor cell lines established from patients after treatment with chemotherapy is associated with a shortened survival [24].

Of additional interest is the observation that a substantial number of SCLC cell lines overexpress *myc* mRNA while possessing only a single copy of the gene. This suggests an alternative molecular mechanism of *myc* overexpression and offers a unique opportunity for further study of the transcriptional regulation of *myc* as a transcription factor. Closer examination of these cell lines by Krystal et al. revealed that *myc* mRNA half-life in various SCLC cell lines was essentially unchanged from that found in other cell

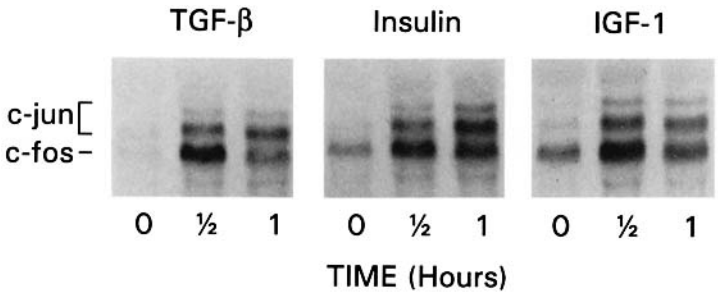

Fig. 7. Peptide growth factor stimulation of c-*jun* and c-*fos* expression in A549 bronchioalveolar cells. Subconfluent A549 cells grown in RPMI were stimulated with the indicated growth factors after a period of 18 h of starvation. The results show that low baseline levels of c-*jun* and c-*fos* mRNA are expressed in the bronchioalveolar cell line A549, and the expression of both of these nuclear oncogenes is rapidly upregulated in the presence of TGF-β (100 pM), insulin (5 μg/ml), and IGF-1 (100 ng/ml).

lines and, thus, the elevated mRNA levels were not due to a prolonged mRNA half-life (Table I) [25]. This finding suggests that the elevated *myc* mRNA levels in these lung cancer cells is most likely the result of alteration in one of two known regulatory mechanisms; that is, increased transcriptional initiation or decreased attenuation.

The nuclear runoff assay allows assessment of nascent mRNA molecule density along a gene of interest by measuring both the process of transcriptional initiation and attenuation. Screening of several lung cancer cell lines has revealed that the *myc* overexpression in these cell lines results from abnormalities in both of these regulatory mechanisms. For instance, it has been demonstrated that small cell lung cancer cell line H146 contains a single copy of the c-*myc* gene but expresses an 8-fold elevation of the level of steady state mRNA (Table I) [25]. In these experiments, the authors used a nuclear runoff assay to demonstrate a 1:1 transcript ratio of exon 1/exon 2 compared to other c-*myc* expressing SCLC cell lines such as H82, which has an amplified number of c-*myc* genes and exhibits an exon 1/exon 2 transcript ratio of 5:1. These results suggest that the block to elongation is not present in the H146 cell line, and that loss of attenuation produces "read-through" by DNA dependent RNA polymerase resulting in elevated steady-state levels of c-*myc* in the absence of gene amplification or prolonged mRNA half-life [25].

Alteration of transcriptional attenuation as a mechanism of deregulation has also been identified for the L-*myc* gene and was first described in the NCI lung cancer cell lines. Small cell lung cancer cell line H209, while possessing a single copy of the L-*myc* gene, expresses high levels of the gene (17-fold) relative to cell line H82 which has a single copy of the gene but does not overexpress L-*myc* [25]. Nuclear runoff assays demonstrated that the ratio of exon 1/exon 2 transcripts in H209 is 1.6:1 while the normal ratio is 4.5:1 [25]. This demonstrates an alteration in L-*myc* transcriptional attenuation leading to overexpression. Transcriptional attenuation has not been identified for the N-*myc* gene. Nuclear runoff assays of single copy N-*myc* overexpression in lung cancer cell lines have revealed an exon 1/exon 2 ratio of 1:1.1 [25]. This suggests that increased N-*myc* gene expression occurs primarily from an alteration in transcriptional initiation [25].

JUN AND FOS GENE FAMILY MEMBERS

The members of the *jun* and *fos* gene families are "early response" genes whose protein products function as transcription factors and provide an immediate link between growth signals originating from the cell membrane and gene expression. These transcription factors contain a leucine zipper motif which allows them to dimerize to each other, forming the AP-1 transcriptional complex. The AP-1 complex binds DNA in a sequence-specific manner, and has been shown to regulate the expression of a wide number of genes. Genes which contain the AP-1 consensus sequence TGAG/CTCA in their promoters are responsive to, and can be upregulated by, the phorbol ester class of tumor promoters.

Structure/function studies of the Jun and Fos proteins have demonstrated that their transcriptional activity is highly dependent upon expression levels and post-translational modifications. The expression of these proteins is dramatically increased after serum stimulation of cells and

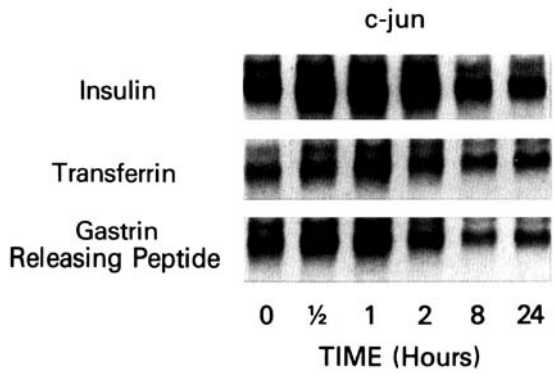

Fig. 8. Peptide growth factor stimulation of c-*jun* expression in SCLC cell line H345. Subconfluent serum deprived cells (as described above) were analyzed for c-*jun* mRNA responses after stimulation with insulin (5 μg/ml), transferrin (10 μg/ml), or gastrin releasing peptide (GRP) (100 nM) in the SCLC cell line H345. c-*jun* mRNA is rapidly upregulated after stimulation with each of these peptide growth factors.

the transition from G_0 to G_1. In addition, phosphorylation of the transactivation domains of these proteins increases their activity while phosphorylation of the DNA binding domain decreases activity [26]. Thus, minor changes in post-translational state of these proteins can result in dramatic changes in protein activity.

Studies of the expression of *jun* and *fos* gene family members in lung cancer have only recently been undertaken. The NCI lung cancer cell lines have been instrumental in this task, and have allowed analysis of c-*jun* and c-*fos* under various in vitro conditions. c-*jun* and c-*fos* are expressed in normal bronchial epithelial cells [27], and their expression is critically dependent upon the cellular growth conditions. Under stimulation by serum and/or various peptide growth factors, the expression of these "early response" genes increases dramatically [27]. The rapid inducibility of these genes by serum suggests that in normal bronchial epithelial cells these genes serve as a critical conduit for many signals originating at the cell membrane. Thus, they may play a crucial role in mediating the biologic effects of factors that function as tumor promoters by stimulating cellular proliferation during the development of lung cancer. Although the nature and identity of tumor promoting substances remain largely unknown for the respiratory epithelium, it has been suggested that these substances may function either directly by action at the cell membrane, or indirectly via the production of growth factors in order to induce cellular proliferation (Fig. 3).

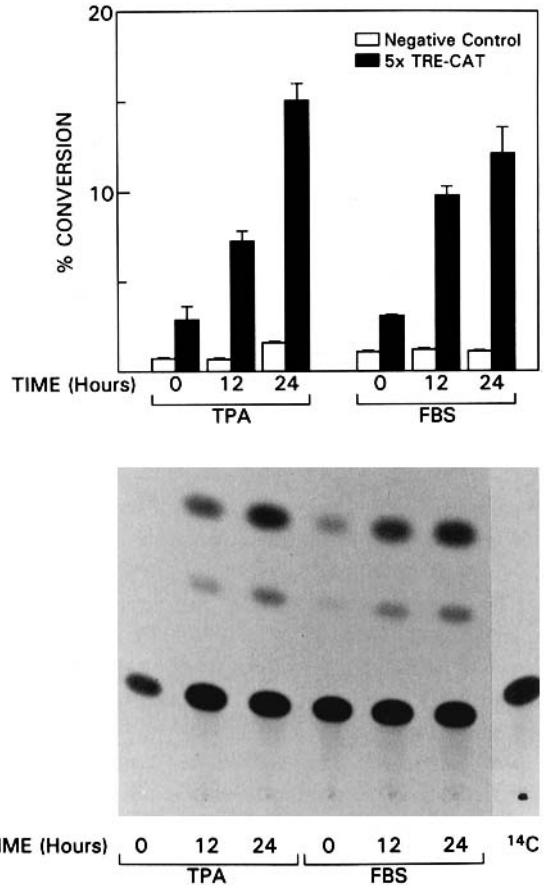

Fig. 9. TPA or serum induced AP-1 Transcriptional activity in A549 cells. DNA was transfected into A549 cells using calcium phosphate precipitation techniques previously described [31], starved and then stimulated with TPA (160 nM) or serum (10%). Cells were harvested, and protein extracts analyzed using CAT assay as previously described [31]. Stimulation of A549 cells with TPA or serum results in a 3- and 2.5-fold increase, respectively, in AP-1 transcriptional activity as measured by the 5XTRE driven CAT reporter construct. No increase in AP-1 activity is seen using the negative control plasmid.

Expression of the proto-oncogenes c-*jun* and c-*fos* has been described in the majority of lung cancer specimens tested and can be rapidly induced by serum stimulation (authors' unpublished observations). For instance, the NSCLC cell line H125, which is an adeno-squamous carcinoma, expresses low but detectable baseline levels of c-*jun* and c-*fos* mRNA (c-*jun* > c-*fos*) and both are rapidly induced by serum over 15–30 min (Fig. 4). Their expression is transient in that c-*fos* is down regulated in 60–120 min while c-*jun* remains elevated beyond 120 min. Another NSCLC cell line A549 (adenocarcinoma) expresses baseline c-*jun* and c-*fos* mRNA (c-*fos* > c-*jun*), which are induced by serum to approximately 11- and 9-fold baseline levels,

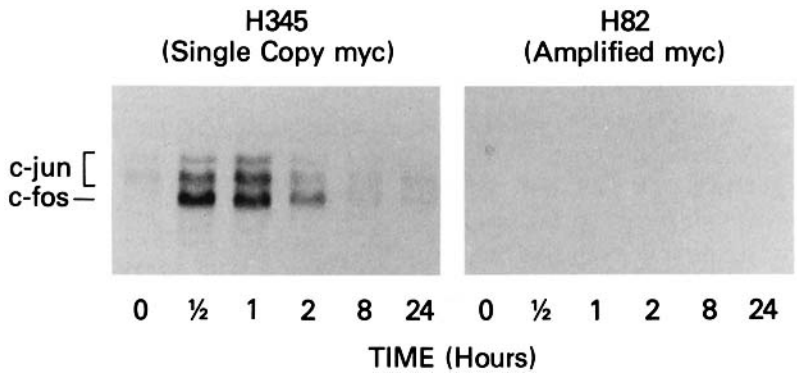

Fig. 10. Serum stimulation of c-*jun* and c-*fos* expression is blunted in SCLC cell lines containing c-*myc* gene amplification. In the cell line H345 which contains a single copy of c-*myc*, c-*jun*, and c-*fos* mRNA levels are rapidly inducible by addition of serum. However, in SCLC cell line H82 which contains 25 copies of the c-*myc* gene, the serum inducibility of these transcription factors is lost. Cells were grown and RNA isolated and processed as described above in Figure 4.

respectively, over 30 min (Fig. 5). Expression patterns of c-*jun* and c-*fos* in SCLC cell lines have also been examined [28]. For instance, cell line H209 expresses baseline c-*jun* and c-*fos*, which transiently increase (3.5-fold within 1 h for c-*jun* and 2-fold within 2 h for c-*fos*) with serum stimulation (Fig. 6). The other members of the *jun* and *fos* groups of nuclear transcription factors include *jun* B, and *jun* D in the *jun* family and *fra*-1, *fra*-2, and *fos*-B in the *fos* family. These protooncogenes have not been well studied in lung cancer cell lines, and their role, if any, in the pathogenesis of lung cancer remains to be elucidated.

The individual components in serum which induce the expression of these genes remain largely unknown, but they most likely include peptide hormones. Treatment of lung cancer cell lines with a variety of peptides including TGFβ, insulin, or IGF-I increases the expression of both *jun* and *fos*. For example, treatment of the NSCLC cell line A549 with TGFβ results in an induction of both c-*jun* and c-*fos* mRNA of 7- and 11-fold, respectively, at 30 min. Likewise, increases in expression of these genes comparable to that seen with serum stimulation (increases of 11-fold for *jun* and 7.5-fold for *fos*), result from stimulation of this cell line by either insulin (10 ng/ml) or IGF-I (10 ng/ml) (Fig. 7). In SCLC cell lines, expression of *jun* and *fos* is also up-regulated when cells are exposed to a variety of peptides. For example insulin, transferrin, or gastrin releasing peptide applied to the SCLC cell line H345 induces similar expression levels of c-*jun* suggesting a convergence of independent stimulatory pathways (Fig. 8). A more thorough examination of the inducibility of these genes has demonstrated that nanomolar concentrations of GRP can induce c-*fos* expression within 30 min [28]. In addition, treatment of the small cell lung cancer cell line H345 with PMA results in dramatic up regulation of c-*fos* after 1 h [28].

An increase in c-*jun* and c-*fos* expression in lung cancer cell lines results in transcriptionally active AP-1 complexes. Stimulation of quiescent A549 cells with TPA (160 nM) or serum (10%) results in an increase in transcriptional activity of 3- and 2.5-fold, respectively, over 24 h. This transcriptional activity is AP-1 specific, as shown by CAT activity from a 5XTRE driven CAT reporter construct but not the control plasmid (Fig. 9).

The inducibility of c-*jun* and c-*fos* mRNA has been shown to be related to the overall sensitivity of the cell to growth factors. For example, cell lines such as the c-*myc* amplified SCLC cell line (H82) are rapidly growing and relatively serum independent. Treatment of H82 with serum results in a blunted or absent stimulation of c-*jun* and c-*fos* expression. Conversely, in cell line H345, which has a low level of *myc* expression and is highly serum dependent, treatment with serum results in high levels of c-*jun* and c-*fos* expression (Fig. 10). These differences may reflect direct effects of *myc* expression on c-*jun* and c-*fos* gene regulation or a loss of key growth factor signaling pathways in cells which are driven to grow rapidly by c-*myc* overexpression.

CONCLUSION

Tremendous progress in our understanding of the molecular biology of neoplastic transforma-

tion has emerged over the last several years. In particular, the identification and characterization of dominant and recessive oncogenes has provided the beginning of a solid foundation for understanding the transformed cell. Understanding of the regulation of nuclear oncogenes and their function as transcription factors has depended, in part, upon the extensive use of transformed human cell lines. For lung cancer, the NCI cell lines have served well in this regard. They have provided a convenient medium by which to test the activity of transcription factors and perhaps, most importantly, an opportunity to characterize their regulation.

REFERENCES

1. Freytag SO (1988): Enforced expression of the c-myc oncogene inhibits cell differentiation by precluding entry into a distinct predifferentiation state in G0/G1. Mol Cell Biol 8:1614–1624.
2. Marcu KB, Bossone SA, Patel AJ (1992): *myc* function and regulation. Annu Rev Biochem 61:809–860.
3. Prins J, DeVries EGE, Mulder NH (1993): The *myc* family of oncogenes and their presence and importance in small-cell lung carcinoma and other tumor types. Anticancer Res 13:1373–1386.
4. Blackwell TK, Kretzner L, Blackwood EM, Eisenman RN, Weintraub H (1990): Sequence-specific DNA binding by the c-Myc Protein. Science, 250:1149–1152.
5. Studzinski GP, Brelvi ZS, Feldman SC, Watt RA (1986): Participation of c-myc protein in DNA synthesis of Human Cells. Science 234:467–470.
6. Birrer MJ, Minna JD (1989): Gentic changes in the pathogenesis of lung cancer. Ann Rev Med 40:305–317.
7. Blackwood EM, Kretzner L, Blackwell TK, Weintraub H, Eisenman RN (1991): Defining specific DNA and protein interactions of the Myc protein: A key to Myc function. In Brugge J, Crran T, Harlow E, McCormick F (eds): "Defining Specific DNA and Protein Interactions of the Myc Protein: A Key to Myc Function." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 365–375.
8. Blackwood E, Eisenman R (1991): Max: A helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. Science 251:1211–1217.
9. Amati B, Land H (1994): Myc-Max-Mad: A transcription factor network controlling cell cycle progression, differentiation and death. Curr Opin Genet Dev 4:102–108.
10. Kretzner L, Blackwood E, Eisenman RN (1992): Myc and Max possess distinct transcriptional activities. Nature 359:426–429.
11. Amati B, Dalton S, Brooks MW, Littlewood TD, Evan GI, Land H (1992): Transcriptional activation by the human c-Myc oncoprotein in yeast requires interaction with Max. Nature 359:423–425.
12. Armelin HA, Armelin MCS, Kelly K, Stewart T, Leder P, Cochran BH, Stiles CD (1984): Functional role for c-*myc* in mitogenic response to platelet-derived growth factor. Nature 310:655–660.
13. Greenberg ME, Ziff EB (1984): Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene Nature 311:433–438.
14. Kalemkerian GP, Jasti RK, Celano P, Nelkin BD, Mabry M (1994): All-*trans*-Retinoic acid alters *myc* gene expression and inhibits in vitro progression in small cell lung cancer. Cell Growth Differ 5:55–60.
15. Spencer CA, Groudine M (1991): Control of c-myc regulation in normal and neoplastic cells. Adv Cancer Res 56:1–48.
16. Bentley DL, Groudine M (1986): A block to elongation is largely responsible for decreased transcription of c-myc in differentiated HL60 cells. Nature 321:702–706.
17. Nepveu A, Marcu KB (1986): Intragenic pausing and anti-sense transcription within the murine c-*myc* locus. EMBO J 5:2859–2965.
18. Takahashi T, Obata Y, Sekido Y, Hida T, Uedo R, Watanabe H, Ariyoshi Y, Sugiura T, Takahashi T (1989): Expression and amplification of *myc* gene family in small cell lung cancer and its relation to biological characteristics. Cancer Res 49:2683–2688.
19. Kashii T, Mizushima Y, Nakagawa K, Monno S, Yano S (1992): Amplification of the N-*myc* oncogene in an adenocarcinoma cell line of the lung. Anticancer Res 12:621–624.
20. Little CD, Nau MM, Carney DN, Gazdar AF, Minna JD (1983): Amplification and expression of the c-*myc* oncogene in human lung cancer cell lines. Nature 306:194–196.
21. Johnson B, Makuch R, Simmons A, Gazdar A, Burch D, Cashell A (1988): *myc* family DNA amplification in small cell lung cancer patients' tumors and corresponding cell lines. Cancer Res 48:5163–5166.
22. Richardson GE, Johnson BE (1993): The biology of lung cancer. Semin Oncol 20:105–127.
23. Gazdar A, Carney D, Nau M, Minna J (1985): Characterization of variant subclasses of cell lines derived from small cell lung cancer having distinctive biochemical, morphological and growth properties. Cancer Res 45: 2924–2930.
24. Brennan J, O'Connor T, Makuch RW, Simmons AM, Russell E, Linnoila RI, Phelps RM, Gazdar AF, Ihde DC, Johnson BE (1991): *myc* family DNA amplification in 107 tumors and tumor cell lines from patients with small cell lung cancer treated with different combination chemotherapy regimens. Cancer Res 51:1708–1712.
25. Krystal G, Birrer MJ, Way J, Nau M, Sausville E, Thompson C, Minna J, Battey J (1988): Multiple mechanisms for transcriptional regulation of the *myc* gene family in small-cell lung cancer. Mol Cell Biol 8:3373–3381.
26. Boyle WJ, Smeal T, Defize LHK, Angel P, Woodgett JR, Karin M, Hunter T (1991): Activation of protein kinase C decreases phosphorylation of c-Jun at sites that negatively regulate its DNA-binding activity. Cell 64:573–584.

27. Siegfried JM, Birrer MJ, Cuttitta FC (1992): Detection of growth factor effects and expression in normal and neoplastic human bronchial epithilial cells. In Milo GE, Casto BC, Shuler CF (eds). "Detection of Growth Factor Effects and Expression in Normal and Neoplastic Human Bronchial Epithilial Cells." Boca Raton: CRC Press, pp 13–29.
28. Draoui M, Chung P, Park M, Birrer M, Jakowlew S, Moody T (1995): Bombesin stimulates c-Fos and c-Jun mRNAs in small cell lung cancer cells. Peptides 16:289–292.
29. Birrer MJ, Segal S, DeGreve JS, Kaye F, Sausville EA, Minna JD (1988): L-*myc* cooperates with *ras* to transform primary rat embryo fibroblasts. Mol Cell Biol 8:2668–2673.
30. Feinberg A, Vogelstein B (1983): A technique for radiolabeling DNA restriction endonuclease fragments to a high specific activity. Anal Biochem 132:6–13.
31. Alani R, Brown P, Binetruy B, Dosaka H, Rosenberg RK, Angel P, Karin M, Birrer MJ (1991): The transactivating domain of the c-Jun proto-oncoprotein is required for cotransformation of rat embryo cells. Mol Cell Biol 11:6286–6295.