Perspective

Oncogenes and Transcriptional Control

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IN THE CHRONICLE OF ONCOGENETICS, EVERY SEASON HAS ITS rumor. Observers of seasons past will recall the gossip that human transforming genes are mutant *ras* genes (winter of 1982), that the c-*sis* proto-oncogene encodes a chain of platelet-derived growth factor (summer of 1983), that c-*erb*B is the epidermal growth factor receptor gene (spring of 1984), that c-*erb*A is related to steroid receptor genes (fall of 1985) and (1 year later) that it encodes a thyroid hormone receptor, and that the *Drosophila* homolog of *int*-1 is *wingless* (spring of 1987). The latest conversational buzz (summer of 1987) has it that the cellular progenitor of the retroviral oncogene, v-*jun*, is closely related (if not equivalent) to the gene for the transcription factor AP1. As documented in this issue of *Science* (*1*), the latest story, like the others (*2, 3*), appears to be true. And just as the earlier surprises often merged oncogenetics with other disciplines, such as the endocrinology of growth control or developmental genetics, this new advance seems likely to build a stronger alliance between oncogenetics and the study of eukaryotic transcription.

Some curious turns of scientific history have made this alliance particularly interesting. Before the late 1970's, when ideas about oncogenic proteins were still in a primitive stage, the conventional wisdom was that primary events in cellular transformation were most likely to occur in the nucleus where they might directly affect DNA replication or RNA synthesis. In those early days this view was sustained largely by studies of the only oncogenic protein then in hand, the large T antigen of simian virus 40 (SV40), a protein manifest in the nucleus and implicated in replication and transcription of the viral genome (*4*).

By the early 1980's however, a torrent of new information about the protein products of viral and cellular oncogenes moved the principal locus of neoplastic action toward the periphery of the cell—the cytosol, the plasma membrane, and even the extracellular space—where growth factors, growth factor receptors, protein kinases, and GTPases (guanosine triphosphatases) that transduce external signals normally reside (*5*). Even a numerically minor but functionally important species of SV40 T antigen has now been reported to be associated with the plasma membrane (*6*). In the model for carcinogenesis that emerged from these findings, excessive amounts of cytoplasmic or extracellular growth regulating proteins, or mutant versions of them, would perturb the control network by sending persistent signals for unbridled growth to the nucleus, through still unidentified messengers (*7*). Ultimately the transcriptional program would be affected, but as a final common pathway mediated by epigenetic events, not as a primary mechanism for neoplastic change through mutations of transcriptional regulatory genes.

The growing tendency to look to the cell's outer margins for oncogenic activities has been partially balanced by a countercurrent of new evidence for crucial nuclear events in the past few years. The most tantalizing of this evidence suggests that certain oncoproteins located in the nucleus can influence—augment or diminish, directly or indirectly—the activity of various viral and cellular transcriptional promoters (*8*). Putative transforming proteins encoded by the adenovirus E1A gene (*9*), by the *X* (or *tat*) genes of human T cell leukemia viruses (*10*), by papilloma and polyomaviruses (*4, 11*), and by *fos* (*12*) and *myc* (*13*) genes have all been to a greater or lesser degree implicated in transcriptional control. Identification of c-*erb*A as a gene for a thyroid hormone receptor (*3*) offered a second sign that transcriptional control may play a primary role in transformation (in this case by the relatively subtle oncogene of avian erythroblastosis virus, v-*erb*A), since thyroid hormone receptors are believed to act in the manner of steroid receptors, that is, as hormone-dependent, gene-specific stimulators and repressors of transcription (*14*).

Now the most dramatic links between transcriptional events and neoplasia have been forged in the story of *jun*. This is a story, like many others, that charms through coincidence. Isolation of the v-*jun* oncogene required persistent devotion to the idea that chicken retroviruses, which have already delivered a remarkable set of oncogenes (*src, myb, myc, fps, yes, erb*A, *erb*B, and *ros*, among others), remain a rich source of novel and interesting genes, despite the several other means now at hand to isolate active or potential oncogenes (*15*).

Vogt and his colleagues have recently recovered about 30 avian sarcoma viruses (ASV's) from tumors encountered at commercial poultry houses, and one of these, ASV-17, contained a novel oncogene, v-*jun*, that has been an anomaly from the outset. Even its christening (if it can be called that) was curious; identified by a Japanese postdoctoral fellow, Yoshio Maki, it was named with part of the Japanese word for seventeen (*ju-nana*) and pronounced like the month (June). Like most retroviruses bearing transduced cellular sequences, ASV-17 is replication defective; its genome fuses part of the viral core protein gene (*gag*) to a cell-derived open reading frame of 935 base pairs (v-*jun*), allowing synthesis of a hybrid *gag-jun* protein (*16*). Although all previously isolated ASV's have transduced genes that encode tyrosine protein kinases, the sequence motifs that predict kinase activity were not found in the deduced amino acid sequence of *jun* protein. Instead, a computer search for family members turned up a startling similarity between a portion of *jun* protein and the DNA binding carboxyl terminus of the yeast *GCN4* product (*17*), a protein that positively regulates transcription of several genes required for amino acid synthesis (*18*).

From this point, things happened quickly. To show that the similarity between v-*jun* and *GCN4* proteins was more than a superficial resemblance between two proteins that bind different sequences in DNA, Struhl replaced the 3′ portion of a *lexA-GCN4* fusion gene with the relevant region of v-*jun* and found that the resulting pastiche (*lexA-GCN4-jun*) complements *gcn4*$^-$ mutants (*19*). (The *lex*A portion is probably required for dimer formation, not for correct DNA binding, since the tripartite protein regulates *HIS3* genes that lack a *lexA* operator.) Furthermore, the protein partially encoded by v-*jun* responds to regulatory mutations at the *GCN4* binding site in the *HIS3* promoter, very much like protein encoded entirely by *GCN4*.

Even before Struhl's work appeared, it was evident that yet more potent connections might be made between *jun* and eukaryotic

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transcriptional regulators. Early this year, a novel transcription factor called activator protein-1 (AP-1) in HeLa cell extracts was discovered through its binding both to a component of a human metallothionein promoter essential for constitutive expression and to a nearly identical heptamer within the 72–base pair enhancer of SV40 DNA (*20*). The relevance of binding to transcriptional stimulation was established with the use of mutant promoters and in vitro transcription systems to show a correlation between AP-1 binding and stimulation of transcription. Because binding sites were found in promoter regions of several genes induced by treatment of cells with phorbol esters such as TPA, it was suggested that AP-1 mediates some responses to TPA (with transcriptional activation dependent on phosphorylation of AP-1 by TPA-stimulated protein kinase C). This idea was supported when TPA-inducibility was conferred upon genes by addition of consensus binding sites (*21*); moreover, treatment of cells with TPA augmented AP-1 binding activity without a requirement for new protein synthesis, as also shown for NF-κl, another mediator of TPA-stimulated transcription (*22*). Most pertinent to the story at hand, the DNA sequence protected by and responsive to AP-1 (TGA$^{C}_{G}$TCAG) is compatible with the palindromic binding site previously established for *GCN4* protein (ATGA$^{C}_{G}$TCAT) (*18*), suggesting that if *jun* protein is related to *GCN4* protein, it might also be related to AP-1.

At this juncture, a logical question arose. Does the cellular progenitor of v-*jun* encode AP-1? Several pieces of evidence are now presented by the Tjian and Vogt laboratories in favor of this idea (*1*). (i) Antibodies to v-*jun* peptides (identical to peptides predicted from the newly determined sequence of human c-*jun*) recognize both the *gag-jun* protein and a 47-kilodalton protein in a nearly homogeneous preparation of AP-1. This is so even though the peptides originate from regions both outside and within the putative DNA binding domain of *jun* protein. (ii) A small protein programmed in bacteria with the 3′ coding region of c-*jun* protects the same sequence of SV40 DNA from nuclease digestion as does AP-1 and binds with the same efficiency as AP-1 to mutant binding sites for *GCN4* protein in the *HIS3* promoter. (iii) Sequences of four short peptides from AP-1 are in accord precisely with the protein sequence predicted from the nucleotide sequence of c-*jun*.

Although the evidence falls short of formal proof of identity between c-*jun* and the AP-1 gene, leaving open the possibility that c-*jun* encodes another DNA binding protein with similar specificity to AP-1, it does provide the strongest basis to date for the belief that transcription factors can function directly as oncoproteins. To work with an apparently new type of transforming gene gives new life to questions conventionally asked about any oncogene. How is its oncogenic potential activated? [By gene fusion with *gag*? By amino acid differences between v-*jun* and chicken c-*jun* proteins? (At least two are said to exist.) Upon elevated concentrations of *jun* protein?] What is the potency of the *jun* oncogene? (Does it transform cell types other than fibroblasts? Collaborate with other oncogenes? Participate in nonviral or human tumorigenesis?) But these issues, however interesting, are subservient to another line of questioning that runs to the heart of the mechanism of transformation: What are the functionally significant targets for transcriptional control by the *jun* oncogene and how are they affected? Are the genetic targets different from those normally regulated by AP-1? Have they already been implicated in growth control or cancer? Are the relevant transcriptional effects positive or negative?

The hopes that such questions can now be approached must be balanced against pessimism borne of two long-standing frustrations in oncogenetics: (i) the failure to identify the relevant targets for the neoplastic actions of oncogenes, even when the oncoproteins have provocative biochemical properties, such as protein kinase or guanine nucleotide binding activity; and (ii) the failure to define aspects of the transcriptional program essential for the transition from normal to neoplastic growth. Both of these difficulties can be blamed, in part, on the resistance of animal cells to genetic analysis. Attribution of functional significance to a protein newly phosphorylated or to a messenger RNA of altered abundance is hard to sustain unless mutations tell us that the gene encoding the RNA or protein is essential for oncogene action.

The problems of determining the role of the transcriptional program in neoplastic transformation are compounded by a poor sense of the magnitude of the accompanying changes. For example, infection of chicken fibroblasts by Rous sarcoma virus (RSV) enhances expression of several identified genes, including some, such as embryonic globin genes (*23*), that seem very unlikely to have a role in transformation by v-*src*. An early estimate of the number of genes up-regulated by v-*src* is in the range of 1000 (*24*)—and does not address genes that might be down-regulated. [Despite this large number, two laboratories have recently isolated clones for the same messenger RNA when they used differential complementary DNA cloning to characterize genes more abundantly expressed in RSV-transformed than in normal chicken fibroblasts (*25*).] The definition of important transcriptional changes may be further complicated by regulatory cascades; for instance, stimulation of cell proliferation by polypeptide growth factors or phorbol esters is accompanied by the sequential activation of expression of c-*fos* and c-*myc* (*7*), both of which show signs of being transcriptional regulators themselves (*12, 13*).

Such ruminations are intended as forewarnings that the isolation of *jun* and establishment of its relation to AP-1 are not likely to solve the dilemmas of defining either the relevant targets for oncoproteins or the transcriptional changes essential for transformation. AP-1 is already known to affect promoters in wide use for the dissection of the eukaryotic transcriptional apparatus, such as the SV40 and metallothionein IIA promoters, as well as promoters for some TPA-responsive genes, such as genes for the proteases, collagenase and stromolysin (*21*). Thus the number of genes implicated as potential targets of the *jun* oncogene may prove to be very large, and it is not clear how to sift through them to find those of central importance. Furthermore, attempts to use the *jun* oncogene to probe the long obscure mechanism of tumor promotion by phorbol esters may be stymied by the multiplicity of factors in addition to AP-1 that mediate a transcriptional response to TPA (*22, 26*).

Regardless of the difficulty of identifying essential targets for the *jun* oncoprotein, the strong evidence that *jun* functions as a transcriptional regulator should rekindle efforts to ascribe similar functions to other oncogenes and proto-oncogenes, especially those for which some evidence of transcriptional function already exists. Conversely, there should be renewed interest in the possibility that other genes encoding transcriptional factors are proto-oncogenes. The odds can be better gauged as the impending parade of newly cloned genes of this type marches by.

The newly resuscitated proposal that perturbed transcription is sometimes the primary step in oncogenesis has an additional appeal—multiple options. Mutant regulators could have stimulatory or inhibitory effects upon the transcription of genes that themselves are stimulators or inhibitors of growth. In this way, recessive as well as dominant mutations could be oncogenic. Thus the product of the retinoblastoma locus, which has been characterized as a nuclear phosphoprotein able to bind DNA (*27*), might normally repress genes that foster growth—conceivably even some of the same genes positively affected by AP-1 or *jun* protein. It will be a while before such ideas can be properly evaluated, but events described here leave little doubt that we are now securely tethered to the idea that altered transcriptional control elements can directly mediate oncogenic change.

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