科技资料

Chemical Carcinogenesis

2

Modulating Factors

Chemical Carcinogenesis 2

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PRÉFACE

"Chemical Carcinogenesis" is the general title of the series of international meetings which are held, biannually, in Sardinia (Italy) since 1981. Despite the generality of the title, the main effort of the Scientific Committee has been to pursue a coherent line around one of the most distinguishing features of carcinogenesis: the "multifasic" development of the process.

Given that many chemical compounds are known to cause "experimental cancer", many questions still remain unresolved or are given too simplistic answers. The very first question concerning the interaction between the chemical carcinogen and the molecular target in the cell is a debatable one despite the overwhelming literature in this field. It is certain that chemical carcinogens are toxic to cells and cause DNA damage: however it is still an open question as to how to relate these changes to the different stages of carcinogenesis including initiation. We have not to forget that 1/3 to 2/3 of the average life time of a given species elapses between the experimental administration of the carcinogen and the appearance of "cancer". The experimental manipulation of carcinogenesis through the use of "adequate biological models" has not simplified the problems about cancer, but it has led us to face the complexity of otherwise elementary biological properties, cell proliferation and differentiation. The understanding of this biological complexity is not just theoretical, but it may give us those adequate conceptual tools to approach "cancer" also from a medical point of view.

Carcinogenesis can be modulated by altering the physiological properties of the target parenchyma. Toxic cell injury, dietary, metabolic or hormone imbalances may modify the course of chemical carcinogenesis, ultimately by altering the homeostasis of cell proliferation between the parenchymal cells and the preneoplastic lesions. We know that the path to "cancer" is not unidirectional. This very simple concept suggests that the battle against cancer is not just fighting "malignancy", but taking those adequate measures of prevention and/or therapy at each step along the sequential path of carcinogenesis.

The Fifth Sardinian International Meeting on Chemical Carcinogenesis was held in Villasimius (Cagliari, Italy) on September 19th through the 22nd, 1989. The main topic "Modulating Factors in Multistage Carcinogenesis" was divided into four sessions:

1. Oncogenes and Cancer Development

2. Genetic Factors

3. Metabolic Regulation in Cancer Development

4. Cell Death and Multistage Chemical Carcinogenesis.

With the helpful collaboration of the Scientific Committee and suggestions from those chairing the sessions, we hope to have not taken the Warholian road of "repetition and fragmentation", but that towards a unifying vision of the "cancer" problem.

Our efforts were generously supported by public and private institutions, for which we are very thankful. We are very grateful to the "European Association for Cancer Research", the "Società Italiana di Cancerologia", and the "Società Italiana di Patologia" for having sponsored the Fifth Sardinian Internation Meeting on Chemical Carcinogenesis. Finally, we would like to express our special thanks to Miss Laura Giacomini for her secretarial collaboration, the organization of the Meeting, and editing of the Proceedings. As for the preparation of this volume we are also indebted to Miss Franca Fadda.

The Editors

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SECTION I

ONCOGENES AND CANCER DEVELOPMENT

NUCLEAR ONCOGENES AS TRANSCRIPTION FACTORS

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Molecular biology studies have begun to clarify mechanisms responsible for eukaryotic transcriptional regulation by isolating and characterizing factors which have positive or negative (or both) effects on gene expression. The answer to how the integration of positive and negative regulatory factors can influence the pattern of gene expression may provide a clue as to how a reproducible pathway for cell growth and differentiation is achieved. In the long run, it is hoped that by understanding normal patterns of gene expression, aberrant patterns anticipated for cases of neoplasia and genetic-based diseases can be identified and perhaps rectified.

CLASSES OF TRANSCRIPTIONAL REGULATORS

Cellular proteins playing a role in transcriptional regulation have been generally identified as trans-acting factors. Their function, whether inhibitory or inductive of gene expression, is carried out through interactions with specific cis-acting promoter elements[1-3]. The number of trans-acting factors identified increases daily thanks to the development of several methods for detecting DNA sequence-specific binding proteins. Significantly, some factors identified in such assays exhibit the characteristics of oncoproteins, nuclear hormonal receptors or homeotic proteins thus indicating a crucial role in differentiation and development[4].

From the various types of protein factors characterized, several DNA-binding motifs have been modeled. The helix-turn-helix model was originally postulated for some prokaryotic regulatory proteins. With the aid of x-ray crystallographic analysis the critical features were identified as two α-helical regions separated by a ß-turn. These proteins bind as dimers and utilize both subunits to recognize target sequences and stabilize the DNA-protein interactions. Also some mammalian transcription factors have the helix-turn-helix motif, which is contained in a large conserved region constituted by a 60 amino acid homeodomain, a spacer and another 76-78 amino acid domain. This region is unique to

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pituitary-specific factor Pit-1 and the two octamer binding proteins Oct-1 and Oct-2, and is referred to as the POU-specific domain[5]. A second DNA-binding-domain, characterized by a zinc-finger motif was originally described for TFIIIA, a transcription factor required for 5S RNA gene transcription by RNA polymerase III. Zinc-finger motifs have been subsequently identified or proposed in a variety of eukaryotic proteins from steroid receptors to factors associated with sex determination[6]. These structures contain DNA-binding motifs which require coordinate binding of zinc atoms through properly spaced cysteines and/or histidines to impart tetrahedral symmetry to the coordinate complex. The interspaced residues between the coordinated amino acids then loop out in a finger-like projection. Finger-swapping experiments demonstrated an essential role for these structures in DNA binding and specificity[7]. Recently, it has also been demonstrated that a single amino acid change in the glucocorticoid receptor zinc-finger produces a receptor with mutated specificity[8].

A new DNA-binding motif know as the leucine-zipper (LZ) was recently proposed by Landschulz et al.[9] for C/EBP, a dimeric protein which binds to both enhancer-core and CCAAT recognition sites. This model described the protein dimerization domain as a region containing a repeat of five leucines (designated L1 to L5 in Figure 1) and an adjacent DNA-binding domain containing clusters of basic amino acid. Subsequent work by O'Shea et al.[10] indicated that the leucine-zipper of the monomers are associated

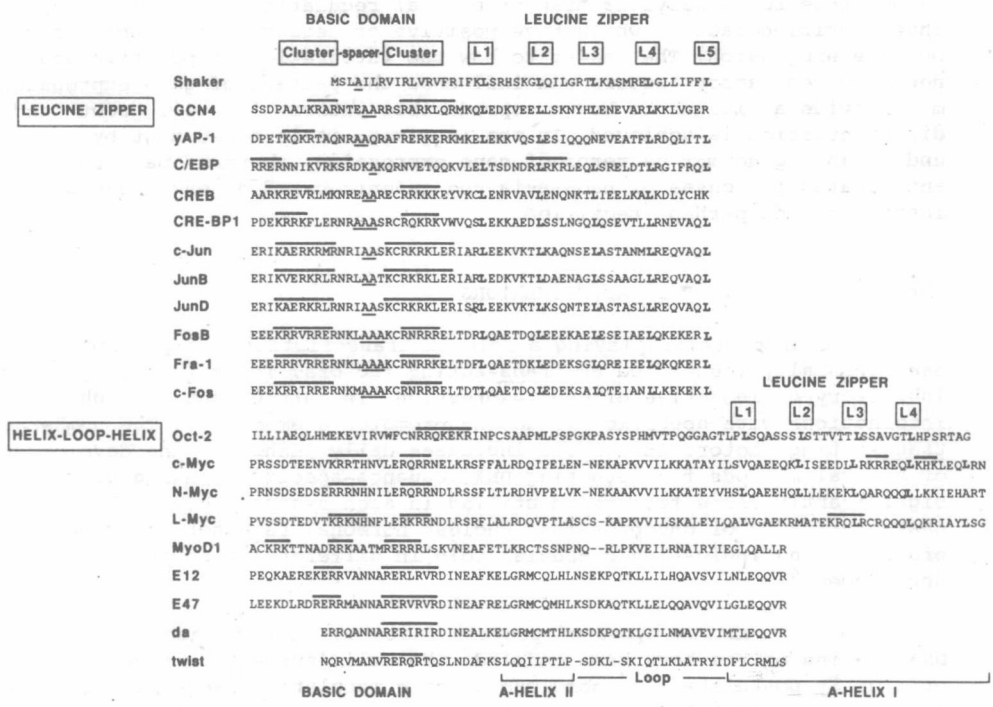

Figure 1. Amino-acid alignement of homologous protein domains for LZ (leucine-zipper) and HLH (Helix-loop-helix) gene regulatory proteins. Positions of the leucines in the LZ motif are indicated from L1 to L5. The adjacent basic domain is represented as organized in a cluster-spacer-cluster structure and the stretches of basic residues are indicated by solid bars over the sequence. Position of the spacer is indicated by the underlined sequence.

as a coiled-coil during dimer formation. The LZ dimerization motif is highly conserved in several other transcription factors and has been identified in many non-nuclear proteins as well (Figure 1). As shown, L_1 - L_5 are also conserved in the Fos and Jun families, whereas in GCN4 and CREB L_5 is substituted by arginine and lysine, respectively. Notable is the yeast transcription factor yAP-1, which has an asparagine substitution at L_3 but still forms functional homodimers. The DNA-binding domains also show strong conservation of structure in most of the included examples. The basic amino acids are found adjacent to the leucine-zipper domain with regular spacing in the Fos and Jun families and with similar distributions in CREB, C/EBP4 and yAP-1. C-myc, which like Fos and Jun is an oncoprotein, is the exception in that it shows a low content of basic amino acids in the region immediately adjacent to the LZ and instead has a basic domain located further away toward the caboxyterminus. C-myc along with other family members including N-myc, L-myc, the octamer binding protein Oct-2, the muscle determination factor MyoD1 and myogenin, the Kappa enhancer-binding proteins E12 and E47, the Drosophila gene products of daughterless, twist and achaetescute all fall into this similar pattern which has been defined as the amphipathic helix-loop-helix motif as summarized in[11]. This may suggest that members of this family have an alternate mechanism for binding to DNA.

FOS AND JUN

Both fos and jun oncogenes belong to the "early response class" which is composed of genes whose main characteristic is that they are rapidly induced upon stimulation of cellular proliferation. These genes are likely to play a pivotal role in normal cell growth, promotion of the transforming phenotype and differentiation[12,13]. Both fos and jun belong to families of genes, several members of which have been characterized and cloned (see Figure 1). Recent studies on jun B, jun D, Fra-1 and fos B indicated that their kinetics of transcriptional activation differs from those of c-jun and c-fos, suggesting differential roles of these proteins at diverse stages of cellular physiology.

An important step in the understanding of jun function has been its identification as one form of the transcriptional factor AP-1[14]. AP-1 was first characterized as a nuclear factor that recognized the enhancer elements of SV40, the metallothionein IIA gene, and the control region of genes containing TPA-responsive promoter elements (TREs). The consensus core DNA sequence TGACTCA, the palindromic binding site of AP-1, has been found within several positive and negative transcriptional regulatory elements. AP-1, biochemically purified via its specific DNA-binding activity, was shown to contain several polypeptides ranging in size from 35 to 50 kDA, possibly either products of different members of the jun gene family or Fos-related proteins. Interestingly, jun/AP-1 contains a regions of homology to the DNA-binding domain of the yeast transcription factor GCN4, which is know to bind as a dimer to a target sequence identical to an AP-1-binding motif. Fos and Jun oncoproteins have been found associated in several transcription complexes, apparently regulating promoter activity in both positive and negative fashion[15]. Whether their cooperativity in transcriptional regulation is reflected in their oncogenic potential is still unknown. Interestingly, Fos protein members heterodimerize with any Jun protein member (R. Bravo, personal communication), dramatically increasing the combinatorial possibilities for gene regulation.

STRUCTURAL FEATURES

The leucine-zipper appears to be a protein domain which promotes

dimer formation. As discussed above, most proteins bearing this structural feature (GCN4, C/EBP, CREB, Jun, yAP-1, Myc) exist as homodimers in solution and dimer formation has been shown, at least in some cases, to be required for efficient DNA-binding. The unique know exception to this rule is Fos. Although its leucine-zipper apparently contains all the structural features to induce formation of homodimers (see Figure 1), Fos protein does not homodimerize, but forms heterodimers with Jun oncoproteins. Remarkably the Fos/Jun heterodimer appears to be 500-1000 fold more stable than the Jun homodimer[16-22]. Corresponding transfection analysis using a TRE reporter gene construct indicated that cotransfections of fos and jun results in a higher transcriptional activation level than with jun alone[17].

a) Dimerization

The consensus amino acid sequences required for DNA-binding function include the leucine-zipper as the protein dimerization domain and a DNA-binding domain which is enriched in basic amino acids (see Figure 1 and Figure 2 for the Fos and Jun helical wheel analysis). The leucines in the LZ are spaced every seventh residue in the primary sequence such that they align on a common face every second turn of a 4/3 α-helix[10]. Site-directed mutagenesis studies of Jun and Fos suggest that single mutations at L_1 may not[21,22] affect the dimerization of Fos and Jun as characterized by their ability to co-immunoprecipitate with Fos directed antibodies. They are, however, sufficient to abolish TRE-specific binding[21] and trans-activation through a TRE[20]. Single conservative mutations of leucines L_{2-5} of either Fos or Jun did not, however, alter the proteins ability to heterodimerize with its wild-type counterpart[21]. In general, combinations of leucine mutations in Fos seemed to be more effective in altering dimerization than those in Jun. Most of the reported

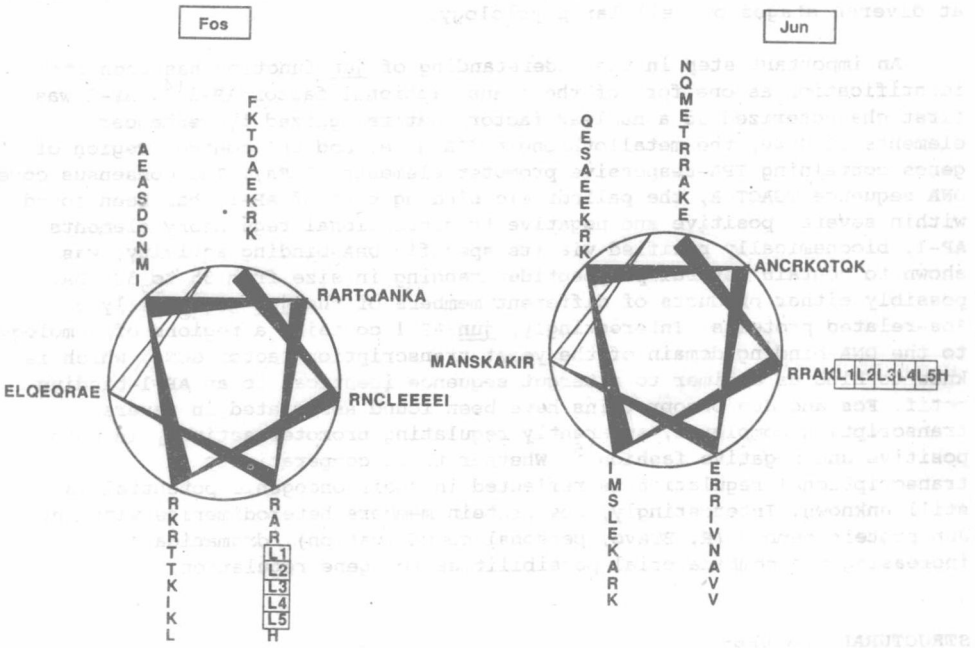

Figure 2. α-helical wheel depiction of Fos and Jun protein domains containing both the leucine-zipper and the basic region.