Korean J Biol Sci 2: 117-122, 1998

Roles of Transcription Factor Binding Sites in the *D-raf* Promoter Region

Eun Jeong Kwon, Hyeong In Kim, In Ju Kim, and Mi Ae Yoo*

Department of Molecular Biology, Pusan National University, Pusan 609-735, Korea

Key Words:
Drosophila
Raf
Promoter
Transcription factor binding site

D-raf, a *Drosophila* homolog of the human c-raf-1, is known as a signal transducer in cell proliferation and differentiation. A previous study found that the *D-raf* gene expression is regulated by the DNA replication-related element (DRE)/DRE-binding factor (DREF) system. In this study, we found the sequences homologous to transcription factor C/EBP, MyoD, STAT and Myc recognition sites in the *D-raf* promoter. We have generated various base substitutional mutations in these recognition sites and subsequently examined their effects on *D-raf* promoter activity through transient CAT assays in Kc cells with reporter plasmids p5'-878*Draf*CAT carrying the mutations in these binding sites. Through gel mobility shift assay using nuclear extracts of Kc cells, we detected factors binding to these recognition sites. Our results show that transcription factor C/EBP, STAT and Myc binding sites in *D-raf* promoter region play a positive role in transcriptional regulation of the *D-raf* gene and the Myo D binding site plays a negative role.

Raf belongs to a family of serine/threonine protein kinase and acts as an important mediator of signals involved in cell proliferation, differentiation, and development (Rapp, 1991; Crews and Erikson, 1993; Davis, 1993). Mammalian cells contain 3 *Raf* genes encoding Raf-1 (otherwise known as c-Raf), A-raf, and B-raf, whereas *C. elegans* and *D. melanogaster* appear to have only Raf kinase; lin45 and D-raf, respectively (Marais et al., 1997).

D-raf, a *Drosophila* homolog of the human *c-raf-1* gene, has been cloned and mutants defective for this gene have been identified (Nishida et al., 1988; Ambrosio et al., 1989). Through the *D-raf* mutant phenotypes, it was found that D-raf functions for the regulation of cell proliferation as mammalian c-raf and for the determination of cell fates at embryonic termini and is expressed through most of the development stages (Ambrosio et al., 1989; Hata et al., 1994). However, little is known about the regulatory mechanism of *D-raf* gene expression. Previous studies found that the expression of the *D-raf* gene is specifically regulated according to developmental stages and tissues (Ryu et al., 1994, 1996), is regulated by the DNA replication-related element (DRE)/ DRE-binding factor (DREF) system, and is another target of the Zerknüllt (Zen) protein (Ryu et al., 1997).

C/EBP (CCAAT/enhancer binding protein), a DNA-binding protein first isolated from rat liver, is a tissue- and development-specific transcription factor that is expressed predominantly in adult liver, adipose tissue, and kidney and has a critical role in regulating the balance between cell proliferation and differentiation (van Dijk et al., 1992). The *Drosophila* C/EBP (DmC/EBP) is expressed predominantly during late embryogenesis in the nuclei of a restricted set of differentiating cell types, such as the lining of the gut and epidermis, similar to the mammalian tissues that express C/EBP (Rorth and Montell, 1992).

MyoD, one member of a family of muscle-specific transcription factors, triggers a muscle differentiation programme in non-muscle cells and induces a complete block of cell proliferation (Robertson, 1990; Trouche et al., 1993). MyoD also regulates its own transcription (Robertson, 1990; Trouche et al., 1993). The functional sequences of myogenic regulatory genes are highly conserved over vast evolutionary distances (Michelson et al., 1990). The *Drosophila* myogenic regulatory genes, for instance, *nautilus* (*nau*) and *Dmyd*, have sequence similarity to those of mammals and are expressed in temporal and spatial patterns on embryonic and adult *Drosophila* muscle (Michelson et al., 1990; Paterson et al., 1991).

STATs (signal transducer and activator of transcription) are a family of transcription factors rapidly activated by various cytokines, hormones, and growth factors (Ihle, 1995). Upon ligand binding and cytokine receptor dimerization, STAT proteins are phosphorylated in tyrosine residues, then form homo- or heterodimers, translocate to the nucleus, and induce transcription (Levy et al., 1996; Moriggl et al., 1997). The *Drosophila stat* gene (*D-stat*) has been identified and it has been confirmed that the JAK-STAT pathway regulates

* To whom correspondence should be addressed.
Tel: 82-51-510-2278, Fax: 82-51-513-9258

multiple developmental processes in *Drosophila*, particularly the regulation of pair-rule genes and fly hematopoiesis (Yan et al., 1996; Hou and Perrimon, 1997).

The *myc* proto-oncogene family has been implicated in multiple cellular processes, including proliferation, differentiation, and apoptosis (Hann et al., 1994). Myc heterodimerizes with a partner protein termed Max and the heterodimeric complex binds to E-box sequences and activates transcription from these sites (Desbarats et al., 1996). Homologs of the *myc* and *max* genes were cloned from *Drosophila* and their products (dMyc and dMax) were shown to act as their vertebrate homologs (Gallant et al., 1996). The dMyc, encoded by *diminutive* (*dm*), is highly expressed at the embryo stage and is an integral regulator of *Drosophila* growth and development (Gallant et al., 1996).

In this study, we found that the *D-raf* gene promoter contains various transcriptional regulatory elements homologous to C/EBP, MyoD, STAT, and Myc binding sites. We also report roles of the binding sites for the *D-raf* promoter activity through transient expression assays and gel mobility shift assays.

Materials and Methods

Oligonucleotides

For obtaining the fragment containing various transcription factor binding sites and their base-substituted derivatives in the *D-raf* promoter, the following primers were chemically synthesized.

Draf-C/EBP 1 wild type (wt)
5′-GGATCCTAACTTATCGGCCAAGCCATCATCAACAGCAATT-3′
3′-CCTAGGATTGAATAGCCGGTTCGGTAGTAGTTGTCGTTAA-5′
Draf-C/EBP 1 mutant (mut)
5′-GGATCCTAACTTATCGGCCAAGCCATCATCAACAGtcATT-3′
3′-CCTAGGATTGAATAGCCGGTTCGGTAGTAGTTGTCagTAA-5′
Draf-MyoD 1 wt
5′-TCTTCCATCACTACCACCTGCACCTGTATATGGTTAGTTG-3′
3′-AGAAGGTAGTGATGGTGGACGTGGACATATACCAATCAAC-5′
Draf-MyoD 1 mut
5′-TCTTCCATCACTACtcCCTatcCCTaTATATGGTTAGTTG-3′
3′-AGAAGGTAGTGATGagGGAcagGGAtATATACCAATCAAC-5′
Draf-STAT wt
5′-CGAAATGTAGTAAAATTCGCGGAAAGTAAATAAATTGTTA-3′
3′-GCTTTACATCATTTTAAGCGCCTTTCATTTATTTAACAAT-5′
Draf-STAT mut 1
5′-CGAAATGTAGTAAAATgCGCGcAAAGTAAATAAATTGTTA-3′
3′-GCTTTACATCATTTTAcGCGCgTTTCATTTATTTAACAAT-5′
Draf-STAT mut 2
5′-CGAAATGTAGTAAAATTCtCtGAAAGTAAATAAATTGTTA-3′
3′-GCTTTACATCATTTTAAGaGaCTTTCATTTATTTAACAAT-5′
Draf-Myc wt
5′-CAGCTCCTTTGGAAAATCCTCAAGTTCAGCTGCTTCTGCA-3′
3′-GTCGAGGAAACCTTTTAGGAGTTCAAGTCGACGAAGACGT-5′
Draf-Myc mut
5′-CAGCTCCTTTGGAAAATCCTCAAGTTtcGCTaCTTCTGCA-3′
3′-GTCGAGGAAACCTTTTAGGAGTTCAAagCGAtGAAGACGT-5′

Substituted nucleotides are shown by minuscule letters.

Plasmid constructions

Site-directed in vitro mutagenesis was accomplished by the Kunkel method (Sambrook et al., 1989). Uracil-containing single-stranded template DNA generated from the plasmid p5′-878*Draf*BluescriptKS(-) and a synthetic oligonucleotide having base substitutional mutation was hybridized and then the oligonucleotide was extended with T4 DNA polymerase to create a double-stranded structure. The base substitutional mutants were confirmed by sequencing analysis using a sequencing kit (Pharmacia). To determine activity of the *D-raf* promoter carrying various base substitutional mutations, the mutated *D-raf* promoter fragments were isolated by digestion with *Xba*I and *Sac*I, and then inserted between the equal sites of the plasmid pSK(-)CAT.

Cell culture, DNA transfection, and CAT assay

Drosophila Kc cells and S2 cells were grown in M3 medium (Sigma) supplemented with 2% and 10% fetal calf serum, 0.5% and 1% penicillin streptomycin, respectively. 5×10^6 cells were plated into 60 mm plastic dishes 16 h before DNA transfection, transfected with 10 μg of each plasmid DNA by the calcium phosphate coprecipitation method (Nocera and Dawid, 1983), and then harvested at 48 h after transfection (Gorman et al., 1982; Yamaguchi et al., 1988). Cell extracts were prepared and CAT activities were normalized to protein amounts, determined using a BioRad protien kit. Radioactivities of spots corresponding to acetylated [^{14}C]chloramphenicol were quantified with an imaging analyzer BAS1500 (Fuji film).

Preparation of nuclear extract and gel mobility shift assay

Kc cell nuclear extracts were prepared essentially as described by Hirose et al. (1993). The cells were harvested and lysed in buffer A containing 10 mM Hepes (pH 7.6), 15 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM dithiothreitol, 0.25 mM phenylmethylsulfonylfluoride, and 10 μg/μl leupeptin. After centrifugation at 1500×g for 10 min, the nuclear extracts were resuspended in buffer B containing 15 mM Hepes (pH 7.6), 110 mM KCl, 3 mM $MgCl_2$, 0.1 mM EDTA, and 1 mM dithiothreitol. Ammonium sulfate (4 M, pH 7.6) was added dropwise to a final concentration of 0.36 M and lysis of nuclei was carried out by gentle mixing for 30 min at 4℃. The extracts were centrifuged at 135,000 ×g for 30 min at 4℃ and redissolved in buffer C containing 25 mM Hepes (pH 7.6), 40 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 10% glycerol, and 10 μg/μl leupeptin. After centrifugation at 10,000×g for 10 min at 4℃, the supernatant was divided into aliquots and stored at -80℃. These were then added to a reaction mixture containing 15 mM Hepes (pH 7.6), 40 mM KCl, 0.1 M EDTA, 1 mM dithiothreitol, 12% glycerol, 0.5 μg

sonicated calf thymus DNA, double stranded ^{32}P-labeled oligonucleotides (20,000 cpm), and unlabeled oligonucleotides as competitor and incubated for 15 minutes on ice. DNA-protein complexes were electrophoretically resolved on 4% polyacrylamide gels in 50 mM Tris-borate (pH 8.3), 1 mM EDTA, and 2.5% glycerol at 25 ℃. Gels were dried and autoradiographed.

Results

In the 5'-flanking region of the *D-raf* gene, we found sequences homologous to various transcription factor recognition sites (Fig. 1). The promoter region of the *D-raf* gene contains sequences homologous to C/EBP binding site located at positions -844 to -840 and -778 to -774, MyoD binding sites at positions -540 to -529 and -384 to -379, STAT binding site located at position -297 to -289, and Myc binding site at position -137 to -132 with respect to the transcription initiation site.

C/EBPs bind to related elements of the TT/GNNG-CAAG/T (Akira et al., 1990). *Drosophila* C/EBP contains a basic region/leucine zipper DNA-binding domain very similar to that of mammalian C/EBP and binds to DNA with the same sequence specificity (Rorth et al., 1992). MyoD recognizes the E-box, CACCTG, as a target promoter sequence conserved in evolution (Michelson et al., 1990; Paterson et al., 1991; Gallant et al., 1996). STATs recognize related elements consisting of the inverted repeat TTCNNNGAA (Ihle, 1995). In *Drosophila*, the phosphorylated protein, D-STAT protein binds specifically to the consensus sequence TTCCCGGAA and the dyad symmetric sequence TTCnnnGAA affords maximal D-STAT binding (Yan et al., 1996). c-Myc binds specifically to sequence with a core of CACGTG which is conserved between vertebrates and invertebrates (Papoulas et al., 1992; Gallant et al., 1996)(Fig. 1B).

To investigate roles of these sites for the activity of the *D-raf* promoter, we introduced base-substitutional mutations in each site by in vitro mutagenesis (Fig. 1B). In the case of the STAT binding sequence in *D-raf* promoter, It includes also the sequence homologous to E2F recognition site known as TTTCGCGC. Therefore we generated two base substitutional mutations STAT mut1 and STAT mut2, respectively (Fig. 1B). The *D-raf* promoter fragment carrying the mutations in each binding site was placed upstream of the CAT gene in a CAT vector. We examined the effects of these mutations on the *D-raf* promoter activity by transient expression assays in cultured Kc cells and S2 cells using plasmid carrying the constructs (Fig. 2A). The plasmid carrying mutations in C/EBP site showed 83% of CAT expression of the wild type construct in Kc cells (Fig. 2B). In S2 cells, 71% of the CAT expression was observed (data not shown). The plasmid carrying STAT mut1 showed 55% of the CAT expression of the wild type construct. STAT mut2

Fig. 1. Nucleotide sequence of 5'-upstream region of the *D-raf* promoter and constructs of *Draf*CAT fusion genes. A, Nucleotide sequence of 5'-upstream region of the *D-raf* promoter is shown. The major transcription initiation site numbered +1 is indicated by an arrow. The sequences homologous to each transcription factor binding site are also shown by the shaded boxes. B, Construct of the wild type 5'-878*Draf*CAT fusion gene is shown. The sequences homologous to transcription factor binding sites are indicated in boxes and their sites are numbered. The nucleotides substituted for wild type sequence are shown by small letters in each mutant in boxes. Consensus sequences of transcription factor binding sites in *Drosophila* are also shown.

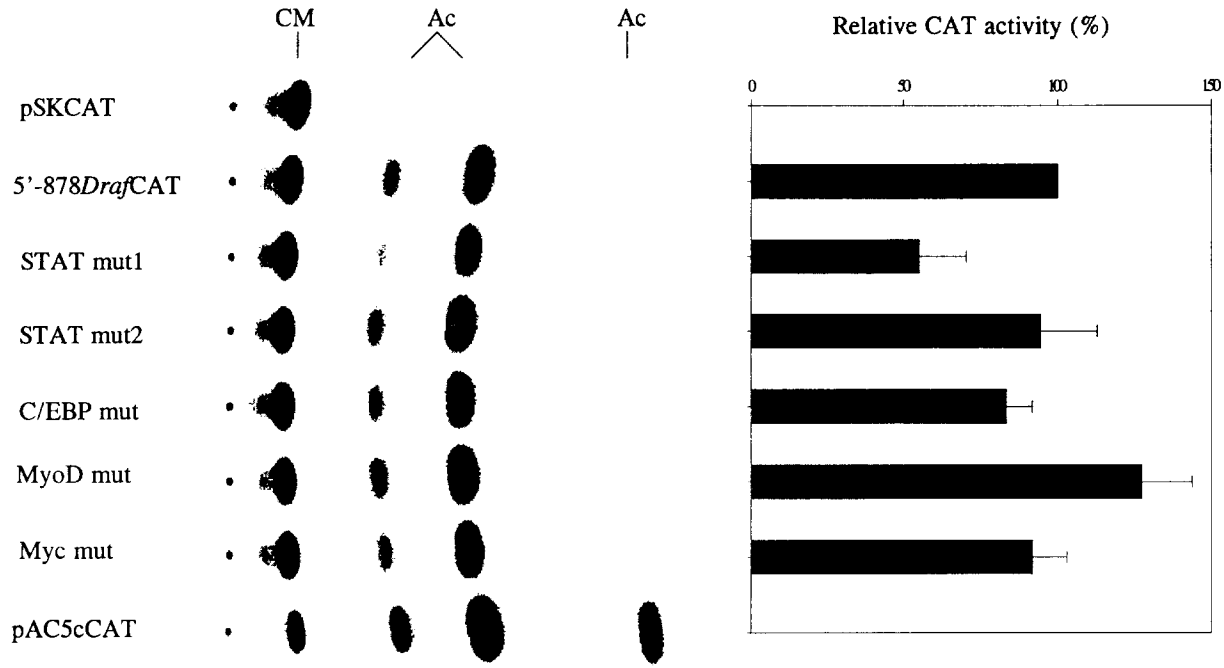

Fig. 2. Effects of mutation in transcription factor binding sites on *D-raf* promoter activity in Kc cells. A, 10 μg of CAT plasmids harboring wild type or mutants *D-raf* promoters were transfected into Kc cells and after 48 h extracts were prepared to determine the CAT expression levels, normalized to protein amounts. The promoterless plasmid pSK-CAT and the plasmid pAct5C-CAT are included as controls. Acetylated and nonacetylated forms of [^{14}C]chloramphenicol are marked by Ac and CM, respectively. B, The CAT sensitivities were quantified and are given as CAT activity relative to that of the wild type plasmid p5'-878*Draf*CAT. The relative values are averages of results from five independent transfections and are shown by filled bars.

showed 94% of the CAT expression in Kc cells. In S2 cells, they showed 29% and 88% of the CAT expression of the wild type construct, respectively (data not shown). 91.8% of the CAT expression was determined in Myc site mutant. In S2 cells, CAT expression of the Myc mut construct was reduced to 72.8% (data not shown). Mutution in MyoD site1 increased CAT expression to 127%, and a similar result was observed in S2 cells (data not shown).

To elucidate the factors binding to these recognition sites, gel mobility shift assays were carried out using chemically synthesized oligonucleotide containing each site as a probe. As shown in Fig. 3A, a DNA-protein complex could be detected with the C/EBP wild type oligonucleotide. The DNA-protein complex was diminished by adding unlabeled C/EBP as a competitor. But the oligonucleotide C/EBP mut containing base substitutional mutation didn't compete for binding when added to the reaction in excess. When we used the ^{32}P-labeled MyoD wild type oligonucleotide, the DNA-protein complex also was detected and diminished by adding unlabeled MyoD wild type oligonucleotide. The oligonucleotide containing MyoD site mutation didn't compete for binding when added in excess (Fig. 3B). We could detect a factor binding to STAT site using ^{32}P-labeled STAT wild type oligonucleotide. The oligonucleotides containing STAT mut1 and STAT mut2 didn't compete under these conditions (Fig. 3C). The specific DNA-protein complex was also detected using the ^{32}P-labeled Myc wild type oligonucleotide. The oligonucleotide containing Myc site mutation didn't compete for binding when added in excess (Fig. 3D).

Discussion

Multiple regulatory elements should participate in the expression of *D-raf*, and in previous study it was revealed that the DREF system is one of them (Ryu et al., 1997). We have found other regulatory elements, the sequences homologous to C/EBP, MyoD, STAT, and Myc recognition sites, in the *D-raf* promoter region.

In mammals, C/EBP, a tissue-specific transcription factor, is expressed predominantly in liver and is bound to the promoter of many genes (Rorth and Montell, 1992). *Draf-lacZ* transgenic flies showed that *D-raf* is highly expressed in the fat body (Ryu et al., 1996). In *Drosophila*, the fat body is analogous to the vertebrate liver and serves as a storage point for food reserves (Ransom, 1982). Base-substitutional mutation in the sequences homologous C/EBP recognition site in *D-raf* promoter showed reduction of CAT expression (Fig. 2). The DNA binding complex was confirmed through gel mobility shift assay (Fig. 3). These observations suggest that *D-raf* gene promoter activity might be under the regulation of C/EBP.

Terminal differentiation and cell proliferation are in many cases mutually exclusive processes (Trouche et al., 1993). While differentiating myoblasts are withdrawn

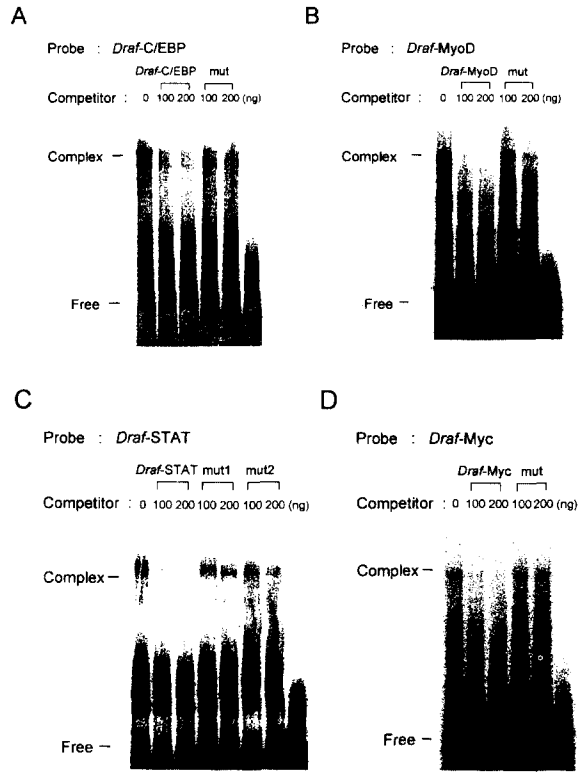

Fig. 3. Competition for complex formation between *Draf*-C/EBP, *Draf*-MyoD, *Draf*-STAT, *Draf*-Myc oligonucleotides and Kc cell nuclear extracts. ^{32}P-labeled double-stranded oligonucleotides were incubated with Kc cell nuclear extracts in the presence or absence (0) of the indicated competitor oligonucleotides. A, *Draf*-C/EBP, oligonucleotide containing wild type *Draf*-C/EBP 1 sequence; mut, oligonucleotide containing base substitutions in *Draf*-C/EBP 1 sequence. B, *Draf*-MyoD, oligonucleotide containing wild type *Draf*-MyoD 1 sequence; mut, oligonucleotide containing base substitutions in *Draf*-MyoD 1 sequence. C, *Draf*-STAT, oligonucleotide containing wild type *Draf*-STAT sequence; mut1, oligonucleotide containing base substitutions in *Draf*-STAT sequence; mut2, oligonucleotide containing base substitutions in other sites of *Draf*-STAT sequence.

from the cell cycle, myogenesis is inhibited by some mitogens and overexpression of some oncogenes (Grossi et al., 1991; Trouche et al., 1993; Ramocki et al., 1997). In our results, when the MyoD binding site was destroyed, CAT expression increased (Fig. 2). Also, in gel mobility shift assays, a factor binding to MyoD recognition site in *D-raf* promoter was detected (Fig. 3B). These observations show that *D-raf* gene promoter activity is regulated negatively by the MyoD binding site.

Recent studies show that the mammalian JAK-STAT pathway transduces signals by various cytokines and growth factors related with MAP kinase cascade (Winston and Hunter, 1995; Stancato et al., 1997). The activated MAPK affects the STAT dimer which binds to the target gene (Winston and Hunter, 1995; Stancato et al., 1997). In *Drosophila*, janus kinase, Hopscotch (Hop), and D-STAT, were identified (Perrimon et al., 1986). It has also been confirmed that the Hop-D-STAT pathway was activated throughout the developing embryo (Perrimon et al., 1986). D-STAT functions in expression of pair rule gene such as even skipped at the cellular blastoderm stage (Yan et al., 1996). In our data, the STAT mut1 construct having base substitutional mutations in STAT site of *D-raf* promoter resulted in extensive reduction of CAT expression, while little reduction was observed in mut2 having base substitutional mutations in E2F site. However, in gel mobility shift assays both the oligonucleotide containing STAT mut1 and the oligonucleotide containing STAT mut2 didn't compete for binding when added in excess (Figs. 2 and 3C). These results indicate that *D-raf* gene promoter activity is regulated positively by STAT binding site.

Transcription factor Myc, which belongs to the helix-loop-helix/leucine zipper (HLH/LZ) family, heterodimerizes with Max and the heterodimer complex functions as a transcription activator of genes (Hann et al.,1994; Vastrik et al., 1994). Usually c-Myc acts as a transgenic activator of genes (Hann et al., 1994; Vastrik et al., 1994), but it can also function as a transcriptional repressor (Kakkis et al., 1989). In other case, overexpressed Max elicits transcriptional repression and blocks transcriptional activation by Myc through the same DNA-binding sites (Amin et al., 1993). Although Myc protein has been demonstrated to function as a transcription factor recognizing as E-box (CACGTG) element, few myc-related genes have been identified and the specific role of myc is still unclear (Tsuneoka et al., 1997). Base substitutional mutation in Myc site of *D-raf* promoter region reduced CAT expression (Fig. 2), and in gel mobility shift assays a factor binding to Myc recognition site was detected (Fig. 3D). These results indicate the possibility that Myc might activate expression of the *D-raf* gene through the E-box element.

Our results show that the *D-raf* gene promoter contains transcriptional regulatory elements homologous to C/EBP, MyoD, STAT, and Myc binding sites and its activity is regulated positively by C/EBP, STAT, and Myc binding sites and negatively by the MyoD binding site. Further in vivo precise analyses about these transcriptional regulatory elements and analyses about the relation of these regulatory elements including DRE element are necessary to confirm the regulation mechanism of *D-raf* gene expression.

Acknowledgements

This study was supported by the Academic Research Fund (GE97) of the Ministry of Education, Korea.

Reference

Akira S, Isshiki H, Sugita T, Tanabe O, Kinoshita S, Nishio Y, Nakajima T, Hirano T, and Kishimoto T (1990) A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. *EMBO J* 9: 1897-1906.

Ambrosio L, Mahowald AP, and Perrimon N (1989) Reqirement of the *Drosophila raf* homologue for torso function. *Nature* 342: 288-291.

Amin C, Wagner AJ, and Hay N (1993) Sequence-specific transcriptional activation by Myc and repression by Max. *Mol Cell Biol* 13: 383-390.

Crew CM and Erikson RL (1993) Extracellular signals and reversible protein phosphorylation: what to MEK of it all. *Cell* 74: 215-217.

Davis RJ (1993) The mitogen-activated protein kinase signal transduction pathway. *J Biol Chem* 268: 14553-14556.

Desbarats L, Gaubatz S, and Eilers M (1996) Discrimination between different E-box-binding proteins at an endogenous target gene of *c-myc*. *Genes & Dev* 10: 447-460.

Di Norcera PP and Dawid IB (1983) Transient expression of genes introduced into cultured cells of *Drosophila*. *Proc Natl Sci USA* 80: 7095-7098.

Gallant P, Shiio Y, Cheng PF, Parkhurst SM, and Eisenman RN (1996) Myc and Max Homologs in *Drosophila*. *Science* 274: 1523-1527.

Gorman CM, Moffat LF, and Howard BH (1982) Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol Cell Biol* 2: 1044-1051.

Grossi M, Calconi A, and Tato F (1991) *v-jun* oncogene prevents terminal differentiation and suppress muscle-specific gene expression in ASV-17-infected muscle cells. *Oncogene* 6: 1767-1773.

Hann SR, Dixit M, Sears RC, and Sealy L (1994) The alternatively initiated c-Myc protein differentially regulate transcription through a noncanonical DNA-binding site. *Genes & Dev* 8: 2441-2452.

Hata M, Inoue YH, Yoo MA, and Nishida Y (1994) Multiple functions of *raf* proto-oncogene during development from analysis of a temperature-sensitive mutation of *Drosophila*. *Int J Dev Biol* 38: 329-335.

Hirose F, Yamaguchi M, Handa H, Inomata Y, and Matsukage A (1993) Novel 8-base pair sequence (*Drosophila* DNA replication-related element) and specific binding factor involved in the expression of *Drosophila* genes for DNA polymerase α and proliferating cell nuclear antigen. *J Biol Chem* 268: 2092-2099.

Hou XS and Perrimon N (1997) The Jak-STAT pathway in *Drosophila*. *Trends Genet* 13 105-110.

Ihle JN (1995) Cytokine receptor signalling. *Nature* 377: 591-594.

Kakkis E, Riggs KJ, Gillespie W, and Calame K (1989) A transcriptional repressor of *c-myc*. *Nature* 339: 718-721.

Levy JB, Schindler C, Raz R, Levy DE, Baron R, and Horowitz MC (1996) Activation of the JAK-STAT signal transduction pathway by oncostatin-M cultured human and mouse osteoblastic cells. *Endocrinology* 137: 1159-1165.

Marais R, Light Y, Paterson HF, Mason CS, and Marshall CJ (1997) Differential regulation of *Raf-1, A-Raf,* and *B-Raf* by oncogenic Ras and tyrosine kinases. *J Biol Chem* 272: 4378-4383.

Michelson AM, Abmayr SM, Bate M, Arias AM, and Maniatis T (1990) Expression of a *MyoD* family member prefigures muscle pattern in *Drosophila* embryos. *Genes & Dev* 4: 2086-2097.

Moriggl RM, Berchtold S, Friedrich K, Standke GJ, Kammer W, Heim M, Wissler M, Stocklin E, Gouilleux F, and Groner B (1997) Comparison of the transactivation domains of Stat5 and Stat6 in lymphoid cells and mammary epithelial cells. *Mol Cell Biol* 17: 3663-3678.

Nishida Y, Hata M, Ayaki K, Ryo H, Yamagata M, Shimizu K, and Nishizuka Y (1988) Proliferation of both somatic and germ cells in affected in the *Drosophila* mutants of *raf* proto-oncogene. *EMBO J* 7: 775-781.

Papoulas O, Williams NG, and Kingston RE (1992) DNA binding activities of c-Myc purified from eukaryotic cells. *J Biol Chem* 267: 10470-10480.

Paterson BM, Walldorf U, Eldridge J, Dübendorfer A, Frash M, and Gehring WJ (1991) The *Drosophila* homologue of vertebrate myogenic-determination genes encodes a transiently expressed nuclear protein marking primary myogenic cells. *Proc Natl Acad Sci USA* 88: 3782-3786.

Perrimon N (1993) The torso receptor protein tyrosine kinase signalling pathway: an endless story. *Cell* 74: 219-222.

Ramocki MB, Johnson SE, White MA, Ashendel CL, Konieczny SF, and Taparowsky EJ (1997) Signaling through mitogen-activated protein kinase and Rac/Rho does not duplicate the effects of activated *ras* on skeletal myogenesis. *Mol Cell Biol* 17: 3547-3555.

Ransom R (1982) A Handbook of *Drosophila* Development. Elsevier Biomedical Press, Amsterdam, pp 273-275.

Rapp UR (1991) Role of Raf-1 serine/threonine protein kinase in growth factor signal transdution. *Oncogene* 6: 495-500.

Robertson M (1990) More to muscle than Myo D. *Nature* 344: 378-388.

Rorth P and Montell DJ (1992) *Drosophila* C/EBP: a tissue-specific DNA-binding protein required for embryonic development. *Genes & Dev* 6: 2292-2311.

Ryu JR, Choi TY, Kwon EJ, Lee WH, Nishida Y, Hayashi Y, Matsukage A, Yamaguchi M, and Yoo MA (1997) Transcriptional regulation of the *Drosophila raf* proto-oncogene by the DNA replication-related element (DRE)/DRE-binding factor (DREF) system. *Nucleic Acids Res* 25: 794-799.

Ryu JR, Kim YS, Kim HI, Lee HJ, Lee WH, and Yoo MA (1996) Expression of *raf* proto-oncogene in larval fat body and testis of *Draf-lacZ* transgenic flies. *Korean J Genet* 18: 73- 81.

Ryu JR, Lee WH, and Yoo MA (1994) Expression of *raf* proto-oncogene during *Drosophila* oncogenesis and development. *Korean J Genet* 16: 209-216.

Sambrook J, Fritsch EF, and Maniatis T (1989) Molecular Cloning. Cold Spring Harvor Laboratory Press, New York.

Stancato LF, Sakatsume M, David M, Dent P, Dong F, Petricoin EF, Krolewski JJ, Silvennoinen O, Saharinen P, Pierce J, Marshall CJ, Sturgill T, Finbloom DS, and Larner AC (1997) Beta interferon and oncostatin M activate Raf-1 and mitogen-activated protein kinase through a JAK1-dependent pathway. *Mol Cell Biol* 17: 3833-3840.

Trouche D, Grigoriev M, Lenormand JL, Robin P, Leibovitch SA, Sassone-Corsi P, and Harel-Bellan A (1993) Repression of *c-fos* promoter by MyoD on muscle cell differentiation. *Nature* 363: 79-82.

Tsuneoka M, Nakano F, Ohgusu H, and Mekada E (1997) *c-myc* activates RCC1 gene expression through E-box elements. *Oncogene* 14: 2301-2311.

van Dijk MA, Rodenburg RJ, Holthuizen P, and Sussenbach JS (1992) The liver-specific promoter of the human insulin-like growth factor II gene is activated by CCAAT/enhancer binding protein (C/EBP). *Nucleic Acids Res* 20: 3099-3104.

Vastrik I, Makela TP, Koskinen PJ, and Alitalo K (1995) Determination of sequences responsible for the differential regulation of Myc function by delta Max and Max. *Oncogene* 11: 553-560.

Winston LA and Hunter T (1995) JAK2, Ras, and Raf are required for activation of extracellular signal-regulated kinase/mitogen-activated protein kinase by growth hormone. *J Biol Chem* 270: 30837-30840.

Yamaguchi M, Hayashi Y, and Matsukage A (1988) Mouse DNA polymerase β gene promoter: fine mapping and involvement of Sp1-like mouse transcription factor in its function. *Nucleic Acids Res* 16: 8773-8787.

Yan R, Small S, Desplan C, Dearolf CR, and Darnell JE (1996) Identification of a *Stat* gene that functions in *Drosophila* development. *Cell* 84: 421-430.

[Received November 9, 1997; accepted December 20, 1997]