DREF, a Transcriptional Regulatory Factor Related to Cell Proliferation

Midori Maruyama[1], Makiko Inoue[1], Akiko Koike[1], Tomoko Shuto[1], Akiko Ikeda[1], Hideki Yoshida[2], Masamitsu Yamaguchi[2], Fumiko Hirose[3] and Akio Matsukage[1]
[1]Department of Chemical and Biological Sciences, Japan Women's University,
[2]Kyoto Institute of Technology and
[3]Aichi Cancer Center Research Institute

Abstract: Coordinate expression of many cell proliferation-related genes is required for the cellular shift from resting state into proliferating state. For a candidate of the regulatory factors involved this process, we found a transcription regulatory factor named DREF (DNA replication-related element-binding factor) in *Drosophila* and then in mammalian systems. *Drosophila* DREF consists of a homodimer of the polypeptide of 709 amino acid residues, and shears about 22% identities of amino acid sequences with the human homolog which consists of 694 amino acid residues. *Drosophila* DREF binds specifically to the DRE sequence (5'-TATCGATA-3') in the promoters of many DNA replication/cell proliferation-related genes to activate their transcription. Ectopic expression of DREF in larval imaginal discs induce abnormal DNA synthesis, apoptosis and failures in differentiation . Both *Drosophila* and mammalian DREF's interact genetically and physically with regulatory factors related to chromatin structures, suggesting that DREF activates the expression of proliferation-related genes through modification of chromatin structures. By searching the *Drosophila* genome, we found about 150 genes which carry DRE sequences in their promoter regions, interestingly many of which are related to reactions required for cell proliferation such as DNA replication, transcription, transcriptional regulation, cell cycle regulation, growth signal transduction, protein metabolism. Thus, DREF is a master key-like factor for cell proliferation.

1. Introduction

Coordinate expression of many genes is required for the cellular shift from resting state into proliferating state. On this important biological subject, we can propose two major questions: the first is how many and what kinds of genes are related cell proliferation. And second question is what is the key regulatory mechanism(s) for the expression of proliferation-related genes. Furthermore, it should be pointed out that expression of many proliferation-related genes such as those related to DNA replication is reduced during cell differentiation processes in various tissues (1). Since cell proliferation is shut off during the differentiation in many organs, the above mentioned regulatory mechanism may be a target of negative regulation by differentiation signals. In order to answer these two major questions, we have made efforts to find out common regulatory mechanisms for transcription of cell proliferation-related genes and found a candidate, a transcription regulatory factor named DREF (DNA replication-related element-binding factor). The present paper describes the finding, characterization and function of *Drosophila* and mammalian DREF.

2. Finding of DRE and DREF

Previously, we observed that the protein factors involved in DNA replication such as DNA polymerase α are clearly correlated to cell proliferation (1). Thus, we started to work on the transcription regulatory mechanism for DNA replication-related genes. We isolated the *Drosophila* gene encoding the proliferation cell nuclear antigen (PCNA), which is an important factor in DNA replication and cell cycle regulation. Then, we have determined the transcriptional promoter of this gene (2). Furthermore, we also found that expression of the PCNA gene is repressed by the product of the *zerknullt* (*zen*) gene (3), a homeobox-containing regulatory protein required for differentiation of the dorsal structure of *Drosophila* embryo (4). Later, the repression by Zen was resulted from reduction of the

DREF, the regulatory factor for many genes including one for PCNA (5).

In addition to the PCNA gene, we cloned the gene for *Drosophila* DNA polymerase α, a central enzyme in DNA replication (6). We found that a common palindromic 8 bp sequence 5'-TATCGATA-3' in the promoter regions of both genes is required for the transcriptional activation, which is termed the DNA replication-related element (DRE). The requirement of DRE for promoter activity was confirmed in both cultured cells (7-11) and transgenic flies (12-14). Then, we identified a specific DRE-binding factor (DREF) consisting of an 80-kDa polypeptide homodimer by a band mobility shift assay and other methods (7).

3. cDNA cloning and characterization

cDNA for DREF has been cloned as followed (15). DREF was purified from the nuclear extracts of *D. melanogaster* Kc cells using latex beads carrying DRE sequences. By using primers synthesized on the basis of partial amino acid sequences, cDNA for DREF was isolated with the reverse-transcriptase polymerase chain reaction method, and followed by screening of cDNA library (15). Deduced from the nucleotide sequences of cDNA, DREF is a novel polypeptide of 709 amino acid residues. Deletion analysis of the bacterially expressed DREF fused with glutathione S-transferase (GST-DREF) indicated that a part of the N-terminal basic amino acid region (16-115 amino acid positions) is responsible for specific binding to DRE and dimer formation.

The gene for DREF was isolated from *Drosophila virilis* (*D. virilis*), and shears 71% identity in amino acid sequences with the *D. melanogaster* homologue (16). Three highly conserved regions were identified at amino acid positions 14 to 182 (CR1), 432 to 568 (CR2) and 636 to 730 (CR3) of the *D. virilis* DREF, with 86.4%, 86.1% and 83.3% identities, respectively. The CR1 contains the domain required for DRE-binding and dimer formation.

We have also isolated cDNA for a human homologue of DREF (hDREF). hDREF consists of the polypeptide of 694 amino acid residues and shares 22% identity and 21% similarity with the *Drosophila* homologue. The CR1 was highly conserved in hDREF. The binding sequence (5'-TGTCGCGAC/TA) for hDREF was identified.

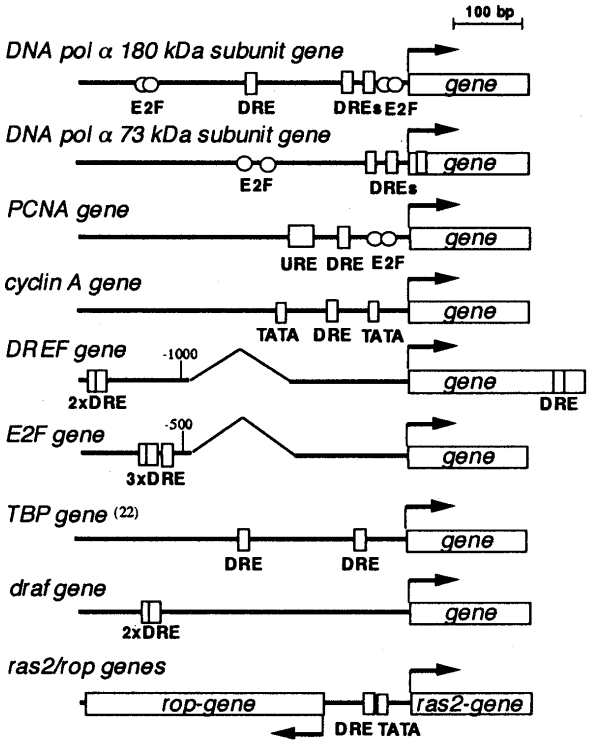

Fig. 1. *Drosophila* genes regulated by the DRE. Except for the *ras2*/*rop* genes, involvement of DREF in transcription regulation has been confirmed.

4. DREF function in cultured cells and individuals

DRE can activate transcriptional promoters through DREF binding, when it is located within the 2 kb upstream region from the transcription initiation site (7). The promoter activating function of the DRE/DREF system was confirmed using the chloramphenicol acetyl transferase (CAT) assay using cultured cells and specific binding assay methods following genes: PCNA (7), DNA polymerase α 180kDa (6) and 73kDa subunits (10), raf (11), ras2/rop (8), E2F (14), TBP (17), and DREF itself, cyclin A (9) (Fig.1). It should be notice that these genes are related with either DNA replication, growth signal transduction, transcription regulation, or cell cycle regulation, all important reactions for cell proliferation.

Functions of DRE and DREF were further examined in individuals by making many transgenic flies. We developed two types of transgenic flies. The first types were made as to carry genes consisting of promoters from above mentioned genes with various mutations and the reporter β-galactosidase gene. The genes were inserted into chromosomal DNA using P element-mediated transduction into early embryos. By detecting β-Gal activities biochemically and histochemically, one can analyse the requirements for regulatory elements in promoters in various tissues through developmental stages.

Second types of transgenic flies are for expressing the gene in desired cells at desired developmental stages, which made possible to know the consequence of gene expression there. For this purpose, two kinds of transgenic flies were required: one is a so called enhancer trap line which expresses the yeast transcription factor GAL4 under either of various promoter/enhancers specific to cell types, and the other is one carrying the transgene made by ligating the GAL4-binding regulatory element (UAS) and the desired cDNA. When two lines of flies are mated to make hybrids, the desired protein such as DREF can be expressed under the control of the specifically supplied GAL4 (18).

By using the first types of transgenic flies, we studied on the functions of regulatory elements in the PCNA gene. The PCNA gene has at least three different elements: in order from the transcription initiation site to the distal, two E2F-binding sites, a DRE and a URE (upstream regulatory element). Various deletion and point mutations were introduced to see the functions of these elements in tissues. The results showed that the sequence containing URE enhances the promoter activity in embryos and turned out to be almost absolutely required in the larvae (19). When a part or all of the DRE sequence was further deleted, any β-Gal expression was not seen through developmental stages except in the adult females. This means that DRE is essential for expression in embryos and larvae but not in adult females (13). These observations were also confirmed by histochemical-detection of β-Gal in larval and ovary tissues. Mutations in E2F sites I and II, even if the URE and DRE were left intact, eliminated completely the expression through all stages of development. The region containing the cap site and E2F sites can support maternal expression in the ovary and is essential during the rest of development (13, 20). DRE is essential for expression in embryos and larvae, and URE is necessary for expression in larval tissues (13) (Fig.2). Thus, with development advancing more, the promoter needs more elements to maintain high activity, although the physiological significance of this has to be clarified.

To analyse the function of DREF, we developed the second types of transgenic flies. The transgenic flies which is expressing the full length or N-terminal fragment (1-125 amino acid residues) of DREF under the control of the salivary gland-specific promoter, the eye imaginal disc-specific promoter or the wing disc-specific promoter were developed. In salivary glands of the transgenic larva, the N-terminal fragment forms a homo-dimer and by itself a hetero-dimer with the endogenous DREF, and both capable of DRE-binding. Ectopic expression of the N-terminal fragment in salivary gland cells resulted in reduction of the contents of mRNA's for the 180 kDa subunit of DNA polymerase α and dE2F, and also the extent of DNA endoreplication. Ectopic expression of the N-terminal fragment in the eye imaginal discs also reduced significantly DNA replication in cells at the second mitotic wave (21). These lines of evidence suggest that the N-terminal fragment can impede the endogenous DREF function in a dominant negative manner, indicating that DREF is required for normal DNA replication in both mitotic and endo-cell cycles.

The full length DREF was expressed in the area posterior to the morphogenic furrow of the eye imaginal disc of the larvae using the specific *Glass* gene promoter. This induced ectopic DNA replication and apoptosis, and resulted in rough eye phenotypes in adults (22). Interestingly, this ectopic DREF expression enhanced the E2F gene promoter. Thus, the excess expression of DREF in the cells after differentiation onset causes the ectopic DNA replication and apoptosis, and inhibits normal differentiation.

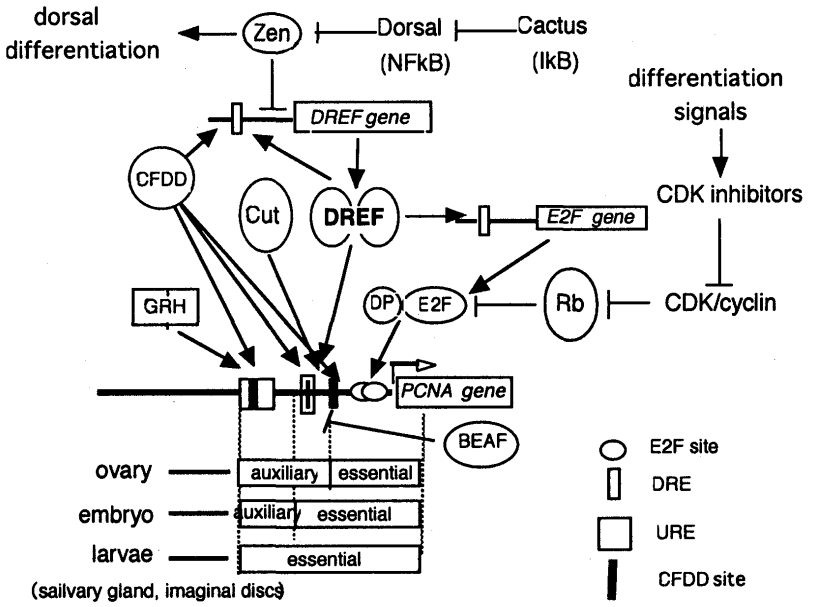

Fig. 2. Regulatory network for transcription of the Drosophila PCNA gene. DREF plays a central role in this network. Requirements of regulatory elements change during development.

5. Network of regulatory mechanism consisting of DREF and related factors

Factors interacting genetically and (or) physically with DREF were identified by several methods, and biological significance of interaction between DREF and these factors have been analysed. The transgenic flies expressing DREF in eye-imaginal discs mentioned above is useful for screening mutants for genes whose products affect the phenotype induced by DREF expression. Transgenic lines expressing the rough eye phenotype were mated with mutant lines with defective alleles in various genes. If a mutant line carries defect in a gene encoding protein inhibitory to the DREF function, the rough eye phenotype is expected to be enhanced in the hybrid progenies. On the other hand, those suppressive to the rough-eye phenotype carry defects in the genes cooperative with DREF. Using this genetic analysis, we identified a number of genes whose products might genetically interact with DREF. One of such examples showing cooperation with DREF is the E2F gene: we found that reducing the copy number of the active E2F gene from two to one suppressed the DREF-induced rough eye phenotype. One of mechanism of the cooperation was clarified by the finding that DREF bounds directly to DRE sequences in the promoter of the E2F gene to enhance transcription (14) (see also Fig.1). Reaction to supply the E2F by the DRE/DREF regulatory system in combination with physiological regulation of E2F by the Rb protein and G1-cyclin/CDK system may be a key molecular process for regulation of cell proliferation.

In addition to DREF and E2F/DP systems (23), we isolated CFDD (common regulatory factor for DNA replication and DREF genes), a novel protein factor(s), from nuclear extracts of *D. melanogaster* Kc cells. CFDD, which recognize two unique nucleotide sequences (5'-CGATA and 5'-CAATCA), binds to promoter region of PCNA gene, and makes an important contribution to promoter activity in both cultured cells and in living flies. Also multiple CFDD sites were found in promoters of the DNA polymerase α and DREF genes (24). Therefore, CFDD may play

important roles in regulation of *D. melanogaster* DNA replication-related genes. However, the functional relationship between CFDD and other factors remains unclear.

6. Interactions between DREF and chromatin-modifying factors

Another interaction, between DREF and BEAF-32 (boundary element association factor of 32 kDa) was found. BEAF-32 can bind to the boundary element consisting of 5'-CGATA-3', a sequence present in DRE (5'-TATCGATA-3'), to block the effect of upstream regulatory elements such as enhancers. DREF antagonizes BEAF-32 to activate transcription of genes regulated by BEAF-32 (25).

The boundary elements (BEs) in *D. melanogaster* was identified on the the scs' (special chromatin structure) region in the *hsp* 70 gene locus, and is known important role in limiting the spread of open and closed chromatin states by binding BEAF-32 (26). As described above, BEAF-32 and DREF share common binding site, DREF may be a key factor for shift of many DNA replication/cell proliferation-related genes from inactive to active states by impeding BEAF-32 (25).

In addition, by the yeast two-hybrid screening method using hDREF as a bait, several cDNA encoding proteins related to modification of chromatin structures including Polycomb and Zinc finger helicase (ZFH) were isolated (our unpublished data). Polycomb group proteins are involved in the establishment of heterochromatin (26), while ZFH is a member of chromatin-remodeling factors (27).

To understand significance of interactions, we constructed plasmids for expression of hDREF in a form of fusion protein with Flag-tag and of Polycomb or ZFH in a form of fusion protein with hemagglutinin (HA)-tag in mammalian cells (our unpublished data). When Flag- hDREF and HA- Polycomb were co-expressed, these proteins showed transient co-localization, and then, dissociated. Similar results were obtained with the co-expression of Flag-hDREF and HA- Zinc finger helicase (our unpublished data). This change in localization of DREF through interaction with these factors possibly correlates to the shift of gene expression through the chromatin remodeling. Furthermore, immuno-coprecipitaiton experiments confirmed the interaction between Flag- hDREF and HA-Polycomb. Furthermore, we found that the genetic interaction between DREF and Polycomb or trithorax group proteins were confirmed using the transgenic *Drosophila* system (18).From these results, it is suggested that DREF interacts with chromatin-modifying factors to activate genes carrying DREs in the promoters.

Table 1. Genome wide screening of DRE-containing genes in *Drosophila*

	Functions	Numbers of genes
1	DNA metabolism (replication, repair, synthesis of precursors)	12
2	Transcriptional regulation	15
3	Nuclear or chromatin structures	9
4	Protein metabolism	35
5	Signal transduction and the phosphorylation	25
6	Germ line formation	8
7	Cell cycle regulation	7
8	Miscellaneous	14
9	Unknown functions	31

DRE sequences found within 1kb from their transcription initiation sites.

7. Genome-wide screening of *Drosophila* genes regulated by the DRE/ DREF system

As described above, DRE/DREF is one of the common regulatory mechanisms for the cell proliferation-related genes. Previously, by screening about 3.5 % of the *Drosophila* genome sequence, we found that 61 genes carry DRE

sequences (28). These genes carry the DRE within 600 base pairs (bp) upstream regions of their transcription initiation sites, and interestingly many of them were related with reactions required for cell proliferation such as DNA replication, transcription, translation, growth signal transduction and other regulatory functions. By simple extrapolation, it was estimated that more than one thousand *Drosophila* genes are regulated by DRE.

We are interested in searching again DRE-containing genes in the whole *Drosophila* genome sequences to find out how many and what kinds of genes are regulated by the DRE/ DREF system. We found 456 of DRE sequences, and 156 genes carry the DRE sequences within 1kb upstreams from their transcription initiation sites (Table 1). Many of them are related to reactions required for cell proliferation such as DNA metabolism, transcriptional regulation, nuclear or chromatin structures, protein metabolism, signal transduction, cell cycle regulation and germ line formation, suggesting that the DRE/DREF system is a common regulator of many cell proliferation-related genes. We have tried to determine the sites for DREF–binding on the larval salivary grands polytene chromosomes using anti-DREF antibody to compare the sites and the maps of the genes found in the genome screening. Several hundreds of antibody-stained bands were detected through the chromosomes, suggesting many genes are under the regulation of DREF in the salivary grands. For example, the *skp* A gene encoding the factor involved in cell cycle regulation and mapped in the 1B region, carries two DREs. The band stained by the anti-DREF antibody was detected in the 1B region of the polytene chromosome.

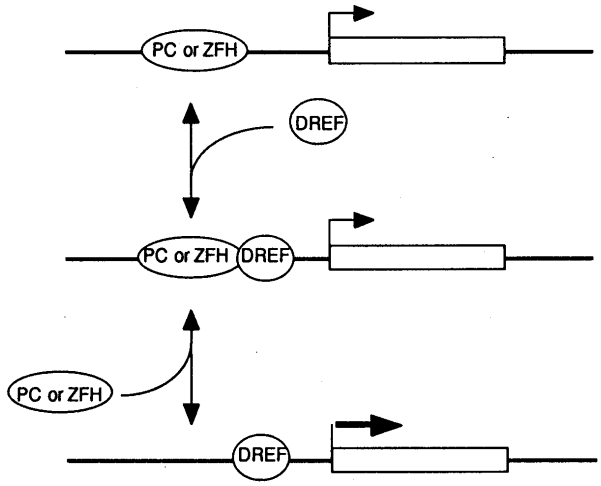

Fig. 3. A model of interaction between DREF and chromatin-modifying factors in regulation of proliferation-related genes.

8. Conclusion and perspectives

From the obtained results, we can conclude as following: 1) *Drosophila* DREF binds specifically to the DRE sequence (5'-TATCGATA-3') in the promoters of many cell proliferation-related genes to activate their transcription. 2) Some differentiation-related signals down-regulates the expression of DREF genes. 3) Ectopic expression of DREF in larval imaginal discs induce abnormal DNA synthesis, apoptosis and differentiation failures. 4) Both *Drosophila* and mammalian DREF's interact genetically and/or physically with regulatory factors related to chromatin structures, suggesting that DREF activates proliferation-related genes through modifying chromatin structures. 5) The DRE/DREF system regulates possibly several hundreds of genes related cell proliferation. From these results, we can suggest the DRE/DREF system may be one of the master key-like regulatory mechanisms for coordinated expression of many cell proliferation-related genes.

Although we can draw a rough model of the DRE/DREF system in regulation of cell proliferation, many questions are still left to be clarified: The first is related the mechanism of activation of the genes by DREF through

interaction with chromatin-modifying factors. One possible mechanism is that DREF may replaced the inhibitory chromatin factors at the promoters containing DREF sequences (Fig. 3). Second question is how DREF is involved in repression of cell proliferation during differentiation. We observed the expression of hDREF was gradually decreased during differentiation of the rat pheochromocytoma cell line, PC12 (our unpublished data), which are known to differentiate into nerve cells by adding nerve cell growth factor (29). This shutting off of DREF may possibly be related to the interaction with inhibitory factors such as Polycomb.

We represented about 150 *Drosophila* genes carrying DRE in the upstream regions of transcription initiation sites, most of these genes seem to be related to cell proliferation. Are more genes regulated by the DRE/DREF system? We found that 8 bp sequences with 1-2 bp differences from TATCGATA can also function as DRE to stimulate transcription as seen with the case of dE2F1 gene (14). Thus, additional genes may be found by further screening, which is suggested by results of staining of the salivary grand polytene chromosome by anti-DREF antibody. It is also important question whether human DREF has a similar function on many genes as the *Drosophila* homologue or not.

References

1. Matsukage, A., Kitani, H., Yamaguchi, M., Kusakabe, M., Morita, T. and Koshida,Y.: Differentiation of lens and neural cells in chicken embryo is accompanied by simultaneous decay of DNA replication machinery. *Dev. Biol.* 117: 226-232, (1986).
2. Yamaguchi, M., Nishida, Y., Moriuchi, T., Hirose, F., Hui, C.c., Suzuki, Y. and Matsukage, A.: the *Drosophila* PCNA/cyclin gene: structure and expression during development and specific binding of homeodomain proteins to it's 5'-flanking region. *Mol.Cell. Biol.* 10: 872-879, (1990).
3. Yamaguchi,M., Hirose, F., Nishida, Y. and Matsukage, A.: Repression of *Drosophila* proliferating cell nuclear antigen promoter by zerknullt protein. *Mol.Cell. Biol.* 11: 4909-4917, (1991).
4. Doyle,H.J., Kraut,R. and Levine, M.: Spatial regulation of Zerknullt: a dorsal-ventral patterning gene in *Drosophila*. Genes & Dev. 3: 1518-1533, (1989).
5. Hirose, F., Yamaguchi, M. and Matsukage, A.: Repression of regulatory factor for *Drosophila* DNA replication related gene promoters by zerknullt homeodomain protein. *J.Biol.Chem.* 269: 2937-2942, (1994).
6. Hirose, F., Yamaguchi, M., Nishida, Y., Masamitsu, M., Miyazawa, H., Hanaoka, F. and Matsukage, A.: Structure and expression during development of Drosophila melanogaster gene for DNA polymerase alpha. *Nucleic Acids Res.* 19: 4991-4998, (1991).
7. Hirose, F., Yamaguchi, M., Handa, H., Inomata,Y. and Matsukage, A.: 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, (1993).
8. Lighfoot, K., Maltby, L., Duarte, R., Veale, R. and Segev, O.: Conserved cis-element bind a protein complex that regulates *Drosophila ras2/rop* bidirectional expression. *Br.J.Cancer* 69: 264-273, (1994).
9. Ohno, K., Hirose, F., Sakaguchi, K., Nishida, Y. and Matsukage, A.: Transcriptional regulation of the *Drosophila cycA* gene by the DNA replication–related element (DRE) and DRE binding factor (DREF). *Nucleic Acids Res.* 24: 3942-3946, (1996).
10. Takahashi, Y., Yamaguchi, M., Hirose, F., Cotterill, S., Kobayashi,J., Miyajima, S. and Matsukage, A.: DNA replication–related element cooperate to enhance promoter activity of the *Drosophila* DNA polymerase α 73-kDa subunit gene. *J.Biol.Chem.* 271: 14541-14547, (1996).
11. Ryu, J.R., Choi, T.Y., Kwon, E.J., Lee, W.,H., Nishida, Y., Hayashi, Y., Matsukage, A., Yamaguchi, M. and Yoo, M.A.: Transcriptional regulation of the *Drosophila raf* protooncogene by the DNA replication–related element (DRE)/ DRE-binding factor (DREF) system. *Nucleic Acids Res.* 24: 794-799, (1997).
12. Yamaguchi, M., Nishida, Y. and Matsukage, A.: Role of homeodomain protein binding region in expression of *Drosophila* proliferating cell nuclear antigen gene: Analysis with transgenic flies. *Gene Expression* 4: 183-193,

(1995).

13. Yamaguchi, M., Hirose, F. and Matsukage, A.: Role of multiple promoter elements of the proliferating cell nuclear antigen gene during *Drosophila* development. *Genes to Cells* 1: 47-58, (1996).
14. Sawado, T., Hirose, F., Takahashi, Y., Sasaki, T., Shinomiya, T., Sakaguchi, K., Matsukage, A. and Yamaguchi, M.: The DNA replication–related element (DRE)/ DRE-binding factor system is a transcriptional regulator of the *Drosophila* E2F gene. *J.Biol.Chem.* 273: 26042-26051, (1998).
15. Hirose, F., Yamaguchi, M., Kuroda, K., Omori, A., Hachiya, T., Ikeda, M., Nishimoto, Y. and Matsukage, A.: Isolation and characterization of cDNA for DREF, a promoter-activating factor for *Drosophila* DNA replication-related genes. *J.Biol.Chem.* 271: 3930-3937, (1996).
16. Takahashi,Y., Hirose, F., Matsukage, A. and Yamaguchi, M.: Identification of three conserved regions in the DREF transcription factors from *Drosophila melanogaster* and *Drosophila virilis* . *Nucleic Acids Res.* 27: 510-516, (1999).
17. Choi, T., Oh, Y., Yoo, M., Matsukage, A., Ryu, Y., Han, K., Yoo, J. and Beak, K.: The DNA replication-related element (DRE)-DRE-binding factor (DREF) system may be involved in the expression of *Drosophila melanogaster* TBP gene. *FEBS Lett.* 483: 71-77, (2000).
18. Brand, A.H. and Perrimon, N.: Targeted gene expression as means of altering cell fates and generating dominant phenotypes. *Development* 118: 401-415, (1993).
19. Yamaguchi, M., Hayashi, Y. and Matsukage, A.: Essential role of E2F recognition sites in regulation of the proliferating cell nuclear antigen gene promoter during *Drosophila* development. *J.Biol.Chem.*. 270: 25159-25165, (1995).
20. Yamaguchi, M., Tanabe, K., Taguchi, Y., Nishizawa, M., Takahashi, T. and Matsukage, A.: Chick embryo DNA polymerase α: Purified enzyme consists of a single Mr=40,000 polypeptide. *J.Biol.Chem.* 255: 7202-7209, (1980).
21. Hirose, F., Yamaguchi, M. and Matsukage, A.: Targeted expression of the DNA binding domain of *Drosophila* transcription factor DREF attenuate DNA replication of salivary gland and eye imaginal disc. *Mol. Cell. Biol.* 18: 6020-6028, (1999).
22. Yoshida, H., Inoue, Y.H., Hirose, F., Sakaguchi, K., Matsukage, A. and Yamaguchi, M.: Over-expression of DREF in the *Drosophila* wing imaginal disk induces apoptosis and a notching wing phenotype. *Genes to Cells* 6: 877-886,(2001)
23. Dynlachet, B.D., Brook, A., Dembski, M., Yenush, L. and Dyson, N.: DNA binding and trans-activation properties of *Drosophila* E2F and DP proteins. *Proc.Natl.Acad.Sci. U.S.A.*91: 6359-6363, (1994).
24. Hayashi, Y., Hirose, F., Nishimoto, Y., Shiraki, M., Yamagishi, M., Matsukage, A. and Yamaguchi, M.: Identification of CFDD (Common regulatory factor for DNA replication and DREF genes) and role of its binding site in regulation of the proliferating cell nuclear antigen gene promoter. *J.Biol.Chem.* 272: 22848-22858, (1997).
25. Hart, C.M., Cuvier, O. and Laemmli, U.K.: Evidence for an antagonistic relationship between the boundary element-associated factor BEAF and the transcription factor DREF. *Chromosoma* 108: 375-383, (1999).
26. Zhao, K., Hart, C.M. and Laemmli, U.K.: Visualization of chromosomal domains with boundary element-associated factor BEAF-32. *Cell* 81: 879-889 (1995)
27. Tyler and Kadonaga: The "dark side" of chromatin remodeling: repressive effects on transcription. *Cell* 99: 443-446,(1999).
28. Matsukage, A., Hirose, F., Hayashi, Y., Hamada, K. and Yamaguchi, M.: The DRE sequence TATCGATA, a putative promoter-activating element for *Drosophila melanogaster* cell proliferation-related genes.: *Gene* 166: 233-236, (1995).
29. Greene, L.A. and Tischler, A.s.: Establishment of noraderenerigec clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc.Natl.Acad.Sci. U.S.A.*73: 2424-2428, (1976)