HISTORY AND SCOPE OF GENETICS; EPIGENETIC REGULATION OF GENE EXPRESSION

Kohji Hasunuma
Yokohama City University, Kihara Institute for Biological Research, Graduate School of Integrated Science, Yokohama, 244-0813, Japan

Keywords: Arabidopsis thaliana, Caenorhabditis elegans, Centromere, ddm1 (decrease in DNA methylation), dim-2, DNA maintenance methylase, Double stranded RNA (ds RNA), Drosophila melanogaster, Epi-allele, Fungi, Gene quelling, Genome imprinting, Locus control region (LCR), Neurospora crassa, Polycomb Group (PcG), PcG response element (PRE), Position effect variegation (PEV), Post-transcriptional gene silencing (PTGS), qde-1, Repeat induced point mutation (RIP), RNA interference (RNAi), RNA dependent RNA polymerase (RdRP4), Transcriptional gene silencing (TGS), Trithorax Group (TrxG)

Contents

Summary

Modern genetics revealed the molecular mechanism of gene regulation based on the linear DNA base sequences of a gene composed of upstream region, promoter region, transcribed regions encoding primary transcript, and downstream region. The primary transcripts of premature messenger (m)RNAs are processed by the splicing mechanism removing introns. The mature mRNAs are constituted of untranslated regions (UTRs) and coding region. However, recent research results discovered epigenetics, which includes heritable gene regulation without change in DNA base sequence.

Epigenetics in fungi, animals and plants includes at least four major categories. These are as follows, i) RNA interference (RNAi) caused by the introduction of double stranded (ds) RNA, ii) Repeat induced point mutation (RIP) observed in *Neurosora crassa*, which includes methylation of DNA, iii) The maintenance mechanism of genetic program by Polycomb group (PcG) genes and Trithorax Group (TrxG) genes, and iv) Gene regulation through the methylation of DNA in plants and animals. In the last case the epigenetic regulation by CpG methylation in DNA in mammals is described in Patterns of Heredity and Genetic Alteration: Epigenetics of Mammals.

RNAi is found in *Caenorhabtidis elegans*, where the introduction of dsRNA of a gene specifically inhibit the expression of the gene by the effective degradation of the mRNA of the gene, and therefore RNAi could be observed after the transcription of the gene.

Repeat induced point mutation (RIP) observed in *Neurospora crassa* includes following phenomena. A sizeable DNA segment introduced into the genome of *Neurospora crassa* cells by transformation causes point mutations in the region of duplicated DNA segments on the sides of both residential DNA and transforming DNA in one nucleus during the pre-meiotic dikaryon during the process of sexual cycle before entering into meiosis.

The dual epigenetic regulation of genes first analyzed by *Drosophila melanogaster* is the gene regulation caused by PcG genes, which regulate the genes over the cell cycles by the repressive mechanism. Conversely, TrxG genes regulate the genes over the cell cycles by the stimulating mechanism.

Epigenetic regulation observed in *Arabidopsis thaliana* seems to be similar to those observed in mammalian gene regulation via DNA methylation. A mutation caused in the *FWA* gene showing gene inactivation did not show any of the change in the DNA sequence. However, it caused the methylation of upstream region of *FWA* gene. Such a mutation was called as epi-allele. Although the title of this Topic Perspectives is “History and Scope of Genetics, the part of history could be observed in several Topic Perspectives, the author wish to focus on “Scope of Genetics”, that is, “epigenetic regulation of gene expression”.

1. Introduction

Epigenetic regulation of gene expression is classified into two groups, which are the genetic regulation of a gene at the transcriptional level, and that at the post

transcriptional level. RNAi observed in *Caenorhabtidis elegans*, post-transcriptional gene silencing (PTGS) in plants and gene quelling in *Neurospora crassa* are classified into the gene regulation at the post transcriptional level. These phenomena appeared as the suppression of gene expression are caused by the introduction of double stranded RNA (dsRNA) or DNA into the target cells. In this case the genetic expression of a homologous gene in the targetted cells is suppressed by the destruction of the messenger RNA (mRNA). The molecular systems are considered to be genetic defense systems from viruses such as retrovirus and from mobilization of transposable elements.

Repeat induced point mutation (RIP) was discovered in a filamentous fungus *Neurospora crassa* and thereafter related process, methylation induced premeiotically (MIP) was discovered in a filamentous fungus, *Ascobolus immersus*. RIP and MIP inactivate the duplicated DNA sequences in a pair-wise manner by the methylation of cytosine(C) forming 5mC. Two copies of homologous sequences in one nucleus are affected simultaneously by the RIP or MIP process. RIP induces several point mutations in the duplicated DNA by deamination of 5mC causing C to T mutation. However, MIP induces methylation of cytosine in the duplicated region without causing deamination of 5mC.

Polycomb group (PcG) genes and Trithorax group (TrxG) genes are known to regulate the hometic genes such as *HOM-C* gene by the specific mechanism to maintain the gene regulation for a specific duration at a defined cells during the development of the organism. PcG proteins suppress gene expression, while TrxG proteins antagonistically activate the gene expression. PcG gene is not detected in *Saccharomyces cerevisiae*. In *Caenorhabtidis elegans* the existence of two PcG genes was identified. In *Drosophila malanogaster* about 100 genes with PRE (Polycomb response elemnt) were detected, and 30 genes were defined to be PcG genes. Among them 16 genes were identified to show the function of PcG genes. In *Arabidopsis thaliana* two genes with the function of PcG were identified. To control the highly organized program for the morphogenesis of higher organisms epigenetic mechanisms such as the suppressive function of PcG genes and stimulative function of TrxG may be evolved.

The epigenetic regulation of gene expression in *Arabidopsis. thaliana* has special importance for the genetic analysis of the relationship between genetic regulation by DNA methylation and by antagonistic regulation by PcG genes and TrxG genes, since either *Saccharomyces cerevisiae, Caenorhabtidis elegance* or *Drosophila melanogaster*, which are model systems for genetic analysis, show limited levels of methylation of DNA.

2. RNA interference

Epigenetic gene regulations are largely composed of two major categories, one of which is regulated during the process of transcription of genes (TGS: transcriptional gene silencing) and the other is regulated after transcription. Post-transcriptional gene silencing (PTGS) observed in plants, gene quelling that occurs in *Neurospora crassa*, and RNA interference (RNAi) first reported in *Caenorhabtidis elegans* are included in the latter categories. The latter three phenomena are caused by the DNA or dsRNA introduced exogeneously, and results in the suppression of the genes with homologous

sequences. These phenomena originally found in plants, fungi and *Caenorhabtidis elegans* are mutually related in the sense that these processes are closely related to the molecular defense mechanism from the genetic materials such as retro viruses and also from retro transposons.

2.1. Process of RNA interference (RNAi)

In the process of RNAi the dsRNA introduced have the ability to suppress the expression of homologous genes. The first report was made in *Caenorhabtidis elegans*, and then in *Drosophila melanogaster* and *Arabidopsis thaliana* similar phenomena are reported.

In the cells accepting dsRNA mRNA with the homologous base sequence will be degraded. The amount of dsRNA required for the suppression of homologous gene expression is exceedingly small in the amount suggesting that before the process of mRNA degradation dsRNA is suggested to be propagated or the dsRNA may have catalylic activity. The effect of RNAi can be observed through the germ cell line and to the next generation.

2.2. The genes regulating the process of RNAi

In *Neurospora crassa* mutations related to the genes regulating the occurrence of gene quelling were isolated by use of transgene of *al-1*. The introduction of *al-1* gene to wild type caused *albino* mutation as a result of gene quilling, from which revertants producing orange colored conidia were isolated. One of the quelling defective (*qde*) mutants, *qde-1* was cloned. The putative amino acid sequence of *qde-1* gene product is similar to an RNA dependent RNA polymerase found in the potato. The presence of *qde-1* homologues in *Caenorhabtidis elegans ego-1*, ORF of *Schizosaccharomyces pombe* Z98553 (*pom*), ORF of *Arabidopsis thaliana* AF080120 (*araB*) and tomato (*Lycopersicon esculentum*) Y10403 (RaRP) indicates that a conserved gene–silencing mechanism may exist, which may have evolved to maintain the integrity of the genome and to protect the genome against naturally occurring transposons and viruses.

In *Caenorhabtidis elegans* RNA interference-deficient (*rde*) mutants, *rde-1~rde-4* were identified. The gene *rde-1* encodes a protein (RDE-1) with 1,020 amino acids, which form gene family of 22 genes coding homologues of RDE-1. In *Arabidopsis thaliana*, *zwille* (=*pinhead*) and *argonaute1*, and in *Drosopila melanogaster sting* and *piwi* are the homologuous genes of *rde-1*.

Two mutator mutants in Saccharomyces cerevisiae with a high capacity to mobilize transposon, *mut-2* and *mut-7* show exceedingly low activity of RNAi. The putative amino acid sequence of *mut-7* includes a part of bacterial RNase D, DNA Q helicase (protein for Wernar syndrome), 3', 5' exonuclease such as PM-Scl100 and also 3', 5' exonuclease domain in DNA polymerase.

2.3. Putative function of small dsRNA in the process of RNA interference

By use of tomato and tobacco (*Nicotiana tobaccum*) the cell with transgenes with ACO

and also GFP (gelfish fluorescent protein) genes, or by infection with virus, the induction of PTGS resulted in the accumulation of 25 bp double stranded RNA. This short RNA is constituted of sense and antisense strands of mRNA, which included the nucleotide sequence of transgene. For the process of this short dsRNA accumulation the sense strand of transgene should be synthesized and also the multiplication of RNA virus is also required, suggesting that antisense RNA could be produced through the sense RNA as a template, and that the 25 bp dsRNA denatured may hybridize to the mRNA, which facilitates the degradation of mRNA at a specific site.

During the process of RNAi, the accumulation of small dsRNA could be detected. The cultured cell of *Drosophila melanogaster* transformed with dsRNA have the endonuclease activities with high specificity to the small dsRNA accumulated in the cell. By use of this extract from embryo *in vitro* system to analyze the molecular basis of RNAi was developed, and following results were obtained, 1) *in vitro* activity to degrade target mRNA requires ATP, 2) transcription is not required, 3) added dsRNA is split into 21-23 bp, and this process is promoted in the presence of ATP without target mRNA, 4) target RNA can be split at every 21-23 nt at precisely the region covered by dsRNA, and 5) mRNA can be hydrolyzed at uracil. These results indicate that the process of RNAi can be catalized by the two steps including the splitting of the dsRNA to small dsRNA and then the hydrolysis of target mRNA at uracil.

2.4. RNase activities from dsRNA to siRNA, Dicer

One of the nuclease included in the process to split dsRNA was a member of an RNase III family, and it was designated as Dicer, which had two RNase III motifs and at the C-terminus DNA helicase domain. The hydrolysis of dsRNA by Dicer required ATP, and it is assumed that the small dsRNA may be unwound by the Dicer and then recognize the target mRNA by the hybridization to the target mRNA. The biochemical process of to split dsRNA to 22 bp dsRNA by Dicer was separated from the other biochemical process to split target mRNA by a nuclease complex designated as RNA-induced silencing complex (RISC).

-
-
-

Bibliography

Agarwal, S. and Rao, A. (1998). Modulation of chromatin structure regulates cytokine gene expression during T cell differentiation. *Immunity* **9**, 765-775.

Akasaka. T., Tsuji, K., Kawahira, H., Kanno, M., Harigaya, K., Hu, L., Ebihara, Y., Nakahata, T., Tetsu, O., Taniguchi, M. and Koseki, H. (1997). The role of mel-18, a mammalian *Polycomb* group gene, during IL-7-dependent proliferation of lymphocyte precursors. Immunity **7**, 135-146.

Allmang, C., Petfalski, E., Podtelejnikov, A., Mann, M., Tollervey, D. and Mitchell, P. (1999). The yeast exosome and human PM-Scl are related complexes of 3'Å®5' exonucleases. *Genes & Develop*. **13**, 2148-2158.

Alvarado, A.S. and Newmark, P.A. (1999). Double-stranded RNA specifically disrupts gene expression during planarian regeneration. *Proc. Natl. Acad. Sci. USA.* **96**, 5019-5054.

Anandalakshmi, R., Pruss, G.J., Ge, X., Marathe, R., Mallory, A.C., Smith, T.H. and Vance, V.B. (1998). A viral suppressor of gene silencing in plants. *Proc. Natl. Acad. Sci. USA.* **95**, 13079-84.

Bird, A.P. and Wolffe, A.P. (1999). Methylation-induced repression--belts, braces, and chromatin. *Cell.*, **99**, 451-454.

Brink, R.A., Styles, E.D. and Axtell, J.D. (1968). Paramutation: Directed genetic change. *Science* **159**, 161-170.

Cairns, B.R., Kim Y-J., Sayre, M.H., Laurent, B.C. and Kornberg, R.D. (1994). A multisubunit complex containing the *SWI1/ADR6*, *SWI2/SNF2*, *SWI3*, *SNF5*, and *SNF6* gene products isolated from yeast. *Proc. Natl. Acad. Sci. USA.* **91**, 1950-1954.

Cambareri, E.B., Jensen, B.C., Schabtach, E. and Selker E.U. (1989). Repeat-induced G-C to A-T mutations in Neurospora. *Science* **244**, 1571-1575.

Cambareri, E.B., Singer, M.J. and Selker, E.U. (1991). Recurrence of repeat-induced point mutation (RIP) in *Neurospora crassa*. *Genetics*. **127**, 699-710.

Cao, X., Springer N.M., Muszynski, M.G., Phillips, R.L., Kaeppler, S. and Jacobsen, S.E. (2000). Conserved plant genes with similarity to mammalian *de novo* DNA methyltransferases. *Proc. Natl. Acad. Sci. USA.* **97**, 4979-4984. [The genes for *de novo* DNA methyltransferase from *Arabidopsis* and maize were cloned, that show sequence similarity to mammalian *de novo* methyltransferase, *Dnmt3*.].

Chuang, C-F. and Meyerowitz, E.M. (2000). Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA. S* **97**, 4985-4990.

Chan, C-S., Rastelli, L. and Pirrotta, V. (1994). A *polycomb* response element in the *Ubx* gene that determines an epigenetically inherited state of repression. *EMBO J*. **13**, 2553-2564.

Chuang, C-F. and Meyerowitz, E.M. (2000). Specific and heritable genetic interference by double-stranded RNA in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci*. **97**, 4985-4990.

Cogoni. C. and Macino, G. (1999). Gene silencing in *Neurospora crassa* requires a protein homologous to RNA-dependent RNA polymerase. *Nature*, **399**, 166-169. [Quelling-defective (*qde*) mutants of *N. crassa*, in which transgene-induced gene silencing is impaired, have been isolated. The *qde-1* gene product is very similar to RNA-dependent RNA polymerase.]

Elbashir, S.M., Lendeckel, W. and Tuschl, T. (2001). RNA interference is mediated by 21-and 22-nucleotide RNAs. *Genes & Develop*. **15**, 188-200.

Festenstein, R., Tolaini, M., Corbella, P., Mamalaki, C., Parrington, J., Fox, M., Miliou, A., Jones, M. and Kioussis, D. (1996). Locus control region function and heterochromatin-induced position effect variegation. *Science* **271**, 1123-1125.

Festenstein, R., Sharghi-Namini, S., Fox, M., Roderick, K., Tolaini, M., Norton, T., Saveliev, A., Kioussis, D. and Singh, P. (1999). Heterochromatin protein 1 modifies mammalian PEV in a dose-and chromosomal-context-dependent manner. *Nature Genetics*, **23**, 457-461.

Finnegan, E.J., Peacock, W.J. and Dennis, E.S. (1996). Reduced DNA methylation *Arabidopsis thaliana* results in abnormal plant development. *Proc. Natl. Acad. Sci. USA.* **93**, 8449-8454. [By the transformation with an antisense construct of methyltransferase cDNA (*MET1*), the plants showed reduced cytosine methylation. The transformed plants showed developmental abnormality such as altered flowering time.]

Franke, A., DeCamillis, M., Zink, D., Cheng, N., Brock, H.W. and Paro, R. (1992). *Polycomb* and *polyhomeotic* are constituents of a multimeric protein complex in chromatin of *Drosophila melanogaster*. *EMBO J*. **11**, 2941-2950.

Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E. M. and Goupland, G. (1997). A polycomb-group gene regulates homeotic gene expression in *Arabidopsis. Nature* **386**, 44-51. [The *CURLY LEAF* gene of *Arabidopsis* is necessary for stable repression of floral homeotic gene and encodes a protein with homology to the product of the Polycomb-group gene *Enhancer of zeste*.]

Grayburn, W.S. and Selker, E.U. (1989.) A natural case of RIP: Degeneration of the DNA sequence in an ancestral tandem duplication. *Mole. Cell Biol.* **9**, 4416-4421.

Grishok, A., Tabara, H. and Mello, C.C. (2000). Genetic requirements for inheritance of RNAi in *C. elegans*. *Science* **287**, 2494-2497.

Guo, D., Merits, A. and Saarma, M. (1999). Self-association and mapping of interaction domains of helper component-proteinase of potato A potyvirus. *J. Gen. Virol*. **80**, 1127-31.

Hagemann, A.T. and Selker, E.U. (1996). Control and function of DNA methylation in Neurospora crassa. *Epigenetic Mechanisms of Gene Regulation*, Cold Spring Harbor Lab. Press. pp. 335-343. New York.

Hammond, S.M., Bernstein, E., Beach, D. and Hannon, G.J. (2000). An RNA-directed nuclease mediates post-transcriptional gene silencing in *Drosophila* cells. *Nature* **404**, 293-296.

Irelan, J.T. and Selker, E.U. (1997). Cytosine methylation associated with repeat-induced point mutation causes epigenetic gene silencing in *Neurospora crassa*. *Genetics* **146**, 509-523.

Jacobsen, S.E. and Meyerowitz, E.M. (1997). Hypermethylated *SUPERMAN* epigenetic alleles in *Arabidopsis*. *Science* **277**, 1100-1102. [Heritable but unstable *sup* epi-alleles (the *clark kent* alleles) are associated with excess cytosine methylation within the *SUP* gene and decreased level of *SUP*mRNA. Transformants of antisense DNA methyltransferase showed also a hypermethylated *sup* allele.]

Kakutani, T., Jeddeloh, J.A., Flowers, S.K., Munakata, K. and Richards, E.J. (1996). Developmental abnormalities and epipmutations associated with DNA hypomethylation mutations. *Proc. Natl. Acad. Sci. USA.* **93**,12406-12411. [The decreased DNA methylation (*ddm1*) mutant showed a variety of morphological abnormalities such as defects in leaf structure, flowering time, and flower structure.].

Kennerdell, J.R. and Carthew, R.W. (1998). Use of dsRNA-mediated genetic interference to demonstrate that frizzled and frizzled 2 act in the wingless pathway. *Cell* **95**, 1017-1026.

Ketting, R.F., Haverkamp, T.H.A., van Luenen, H.G.A.M. and Plasterk, R.H.A. (1999). *mut-7* of *C. elegans*, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and Rnase D. *Cell*, **99**, 133-141.

Kinsey, J.A., Garrett-Engele, P.W., Cambareri E.B. and Selker E.U. (1994). The Neurospora transposon *Tad* is sensitive to repeat-induced point mutation (RIP). *Genetics* **138**, 657-664.

Lessard, J., Schumacher, A., Thorsteinsdottir, U. van Lohuizen, M., Magnuson, T. and Sauvageau, G. (1999). Functional antagonism of the *Polycomb*-Group genes *eed* and *Bmi1* in hemopoietic cell proliferation.

Genes Dev. **13**, 2691-2703.

Llave, C., Kasschau, K.D. and Carrington, J.C. (2000). Virus-encoded suppressor of posttranscriptional gene silencing targets a maintenance step in the silencing pathway.

Proc. Natl. Acad. Sci. USA. **97**, 13401-13406.

Mallory, A.C., Ely, L., Smith, T.H., Marathe, R., Anandalakshmi, R., Fagard, M., Vaucheret, H., Pruss, G., Bowman, L. and Vance, V.B. (2001). HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. *Plant Cell* **13**, 571-83.

Margolin, B.S., Garrett-Engele, P.W., Stevens, J.N., Fritz, D.Y., Garrett-Engele, C., Metzenberg, R.L. and Selker, E.U. (1998). A methylated Neurospora 5S rRNA pseudogene contains a transposable element inactivated by repeat-induced point mutation. *Genetics* **149**, 1787-1797.

Misquitta, L. and Paterson, B.M. (1999). Targeted disruption of gene function in *Drosophila* by RNA interference (RNA-I): A role for *nautilus* in embryonic somatic muscle formation. *Proc. Natl. Acad. Sci*.

USA. **96**, 1451-1456.

Ngo, H., Tschudi, C., Gull, K. and Ullu, E. (1998). Double-stranded RNA induces mRNA degradation in *Trypanosoma brucei*. *Proc. Natl. Acad. Sci. USA.* **95**, 14687-14692.

Orlando, V. and Paro, R. (1993). Mapping polycomb-repressed domains in the bithorax complex using *in vivo* formaldehyde cross-linked chromatin. *Cell* **75**, 1187-1198.

Paro, R. and Harte, P.J. (1996). The role of polycomb group and trithorax group chromatin complexes in the maintenance of detemined cell states. *Epigenetic Mechanisms of Gene Regulation*, Cold Spring Harbor Lab. Press.

Pirrotta, V. (1996). Stable chromatin states regulating homeotic genes in *Drosophila*. *Epigenetic Mechanisms of Gene Regulation*, Cold Spring Harbor Lab. Press. pp. 489-505. New York.

Pirrotta, V. (1998). Polycombing the genome: PcG, trxG, and chromatin silencing. *Cell* **93**, 333-336. [Silencing by the PcG mechanism is mediated by polycomb response elements (PREs), regulatory regions of several hundred nucleotides that are *in vivo* binding sites for PcG proteins and also for trxG proteins.]

Reuter, G., Giarre, M., Farah, J., Gausz, J., Spierer, A and Spierer, P. (1990). Dependence of position-effect variegation in *Drosophila* on dose of a gene encoding an unusual zinc-finger protein. *Nature* **344**, 219-223.

Riggs, A.D. (1975). X inactivation, differentiation, and DNA methylation. *Cytogenet. Cell Genet.* **14**, 9-25. [Considering bacterial DNA methylase, the author proposed maintenance methylase in mammals and attempted to explain X-inactivation leading to the differentiation of the cells.]

Ronemus, M.J., Galbiati, M., Ticknor, C., Chen, J. and Dellaporta, S.L. (1996). Demethylation-induced developmental pleiotropy in *Arabidopsis*. *Science* **273**, 654-657. [The transformants of cDNA for a cytosine methyltransferase in *Arabidopsis thaliana* were analyzed. The decrease in genomic cytosine methylation showed developmental effects such as changes in meristem identity and organ number.]

Rountree, M.R. and Selker, E.U. (1997). DNA methylation inhibits elongation but not initiation of transcription in *Neurospora crassa*.

Genes Dev. **11**, 2383-2395.

Scheid, O.M., Afsar, K. and Paszkowski, J. (1998). Release of epigenetic gene silencing by trans-acting mutations in *Arabidopsis*. *Proc. Ntl. Acad. Sci. USA.* **95**, 632-637.

Selker, E.U., Rountree, M.R. and Metzenberg, R.L. (1985). Heterogeneity of 5S RNA in fungal ribosomes. Science. **227**, 1340-1343.

Selker, E.U. and Stevens, J.N. (1987). Signal for DNA methylation associated with tandem duplication in *Neurospora crassa*. *Moll. Cell. Biol.* **7**, 1032-1038.

Selker, E.U. (1990). Premeiotic instability of repeated sequences in *Neurospora crassa*. *Annu. Rev. Genet.* **24**, 579-613.

Selker, E.U., Fritz, D.Y. and Singer, M.J. (1993). Dense nonsymmetrical DNA methylation resulting from repeat-induced point mutation in *Neurospora*. Science. **262**, 1724-1728.

Selker, E.U., Richardson, G.A., Garrett-Engele, P.W., Singer, M.J. and Miao, V. (1993). Dissection of the signal for DNA methylation in the ¯ region of Neurospora. Cold Spring Harbor Lab. Press, pp. 323-329. New York.

Selker, E.U. (1997). Epigenetic phenomena in filamentous fungi: useful paradigms or repeat-induced confusion? *TIG* **13**, 296-301.

Selker, E.U. (1998). Trichostatin A causes selective loss of DNA methylation in *Neurospora*. *Proc. Natl. Acad. Sci. USA.* **95**, 9430-9435.

Shao, Z., Raible, F., Mollaaghababa, R., Guyon J.R., Wu, C., Bender, W. and Kingston, R.E. (1999). Stabilization of chromatin structure by PRC1, a polycomb complex. *Cell* **98**, 37-46.

Sharp, P.A. (2001). RNA interference-2001. *Genes & Develop.* **15**, 485-490.

Simon, J., Chiang, A., Bender, W., Shimell, M.J. and O'Connor, M. (1993). Elements of the *Drosophila* bithorax complex that mediate repression by *Polycomb* group products. *Develpmental Bil*. **158**, 131-144.

Tabara, H., Sarkissian, M., Kelly, W.G., Fleenor, J., Grishok, A., Timmons, L., Fire, A. and Mello, C.C. (1999). The *rde-1* gene, RNA interference, and transposon silencing in *C. elegans*. *Cell* **99**, 123-132.

Tamkun, J.W., Deuring, R., Scott, M.P., Kissinger, M., Pattatucci, A.M., Kaufman, T.C. and Kennison, J.A. (1992). brahma: A regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. *Cell* **68**, 561-572.

Timmons, L. and Fire, A. (1998). Specific interference by ingested dsRNA. *Nature* **395**, 854.

Tuschl, T., Zamore, P.D., Lehmann, R., Bartel, D.P. and Sharp, P.A. (1999). Targeted mRNA degradation by double-stranded Rna *in vitro*. *Genes & Develop.* **13**, 3191-3197.

Van Lohuizen, M., Verbeek, S., Scheijen, B., Wientjens, E., vander Gulden, H. and Berns, A. (1991). Identification of cooperating oncogenes in E_-*myc* transgenic mice by provirus tagging. *Cell* **65**, 737-752.

Vielle-Calzada, J-P., Thomas, J., Spillane, C., Coluccio, A., Hoeppner, M.A. and Grossniklaus, U. (1999). Maintenance of genomic imprinting at the *Arabidopsis medea* locus requires zygotic *DDM1* activity. *Gene & Develop.* **13**, 2971-2982. [*MEDEA* (*MEA*) encodes a SET-domain protein of the *Polycomb* group that regulates cell proliferation by exerting a gametophytic maternal control during seed development.]

Voinett, O. and Baulcombe, D.C. (1997). Systemic signalling in gene silencing. *Nature* **389**, 553.

Walters, M.C., Magis, W., Fiering, S., Eidemiller, J., Scalzo, D., Groudine, M. and Martin, D.I.K. (1996). Transcriptional enhancers act in *cis* to suppress position-effect variegation. *Genes & Dev*. **10**, 185-195.

Watters, M.K., Randall, T.A., Margolin, B.S., Selker, E.U. and Stadler, D.R. (1999). Action of repeat-induced point mutation on both strands of a duplex and on tandem duplications of various sizes in *Neurospora*. *Genetics* **153**, 705-714.

Wolffe, A.P. (1994). Insulating chromatin. *Current Biol*. **4**, 85-87.

Wolffe, A.P. and Matzke, M.A. (1999). Epigenetics: Regulation through repression. *Science*. **286**, 481-486.

Zamore, P.D., Tuschl, T., Sharp, P.A. and Bartel, D.P. (2000). RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. *Cell* **101**, 25-33.

Zink, B. and Paro, R. (1989). *In vivo* binding pattern of a transregulator of homoeotic genes in *Drosophila melanogaster*. *Nature* **337**, 468-471.

Zink, D. and Paro, R. (1995). *Drosophila* polycomb-group regulated chromatin inhibits the accessibility of a *trans*-activator to its target DNA. *EMBO J*. **14**, 5660-567l.

Biographical Sketch

Kohji Hasunuma Graduated from Tokyo University, Faculty of Science, Department of Biology (Plant Science) at 1966, and Graduate School of Biology (Plant Science) at 1971. Research associate of Tokyo University, Faculty of Arts and Culture 1971~1979. Associate Professor of National Institute for Basic Biology, 1979-1990. Visiting researcher at Carnegie Institution of Washington at Stanford at 1990. From 1990 professor of Yokohama City University, Kihara Institute for Biological Research. The Hirase Prize was awarded to Prof. K. Hasunuma by Japanese Society of Plant Morphology in 2000 in the success to prove molecular mechanism of light signal transduction in *Neurospora crassa* and *Pisum sativum*.