GENETICS AND MOLECULAR BIOLOGY

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Keywords: AP-1 (activator protein-1), *Arabidopsis thaliana*, *Caenorhabditis elegans*, CaM-kinase (calmodulin dependent protein kinase), DNA methylation, *Drosophila melanogaster*, Epigenetics, GTP-binding proteins, Growth factor receptors, H_2O_2 (hydrogen peroxide), *Homo sapiens*, I-κB (inhibitor κB), JAK (Janus kinase), MAP (mitogen activated protein) kinase cascades, *Mus musculus* (mouse), *Neurospora crassa*, NF-κB (nuclear factor-κB), NO (nitric oxide), Nucleoside diphosphate kinase, Oxidative and reductive stress, Polycomb group (PcG), Protein kinase A (PKA), Protein kinase C (PKC), Protein kinase G(PKG), Quelling, Reactive oxygen species (intermediates), Repeat induced point mutation (RIP), RNA interference, STAT (signal transducers and activatorsof transcription), Src tyrosine kinase, $\cdot O_2^-$ (super oxide), SOD (super oxidedismutase), Trithorax group (trxG)

Contents

Summary

Living organisms on the earth survive by responding to ambient environmental factors such as visible light, UV (ultraviolet) light, temperature, reactive oxygen intermediates (ROIs), chemicals, nutritional salts, wounding (including inflammation), hormones, growth factors, and infections by viruses, bacteria, and fungi.

The genes in eukaryotes are regulated by the mechanism revealed by classical genetics, which include the upstream regions, promotors, transcribed regions encoding primary transcripts, and downstream regions. The primary transcripts are processed by removing introns by splicing mechanisms remaining exons forming mature messenger (m)RNAs, which are constituted of untranslated regions (UTRs) and coding regions. The transcription regulator proteins, such as AP-1, activate the upstream region, stimulating the transcription of mRNA. These transcription regulators receive the signals from second messengers from the receptors in the surface of cell, the plasma membrane. However, recent genetic and cell biology analysis has revealed epigenetic processes, which involve the higher order hierarchic regulation of genes without change to the nucleotide sequence. This kind of regulation of genes may be derived from protective processes against attack by genetic materials, for example infection by retroviruses.

Signal transduction from plasma membrane to gene expression: Upon reception of glucagon by the stimulating receptor, and the binding of GTP to stimulatory GTP-binding protein, Gs will enhance the activity of adenylyl cyclase and produce cyclicAMP, which can function as a second messenger. The increase in the concentration of cyclicAMP will results in the activation of cyclicAMP dependent protein kinase (A-kinase) by the binding of cyclicAMP to the regulatory subunits (R2). A-kinase is known to down-regulate Raf-1, possibly by phosphorylation. Thus the activation of A-kinase will stop the proliferation of the cell by down-regulating MAP kinase cascade. A-kinase also phosphorylates CREB and AP-1, increasing the rate of transcription of specific genes.

Upon reception of the factors designated *first messengers*, specific receptors—for example the EGF (epidermal growth factor) receptor on the extracellular side—stimulate the autophosphorylation of tyrosine on the cytoplasmic side. This is recognized by Grb2/Ash (growth factor receptor bound protein 2/abundant SH), followed by binding with Sos (son of sevenless), and stimulates the exchange of GDP for GTP by the GTP-binding protein, Ras. It is suggested that the activated Ras, Ras-GTP, may stimulate the phosphorylation of Raf-1 (MAPKKK), causing phosphorylation of MAPKK and enhancing the phosphorylation of MAPK. Thus the MAP kinase cascade is activated following the activation of Ras. Transcription factor NF-κB (nuclear factor-κB) forms a complex with I-κB (inhibitor of κB), in which NF-κB is

inactive. A MAPKKK (MEKK1) phosphorylates I-κB, leading to its proteolytic degradation. Another MAPKKK phosphorylates the NF-κB precursor, leading to proteolytic processing to a mature NF-κB.

Non-receptor type Src family protein tyrosine kinases (Src tyrosine kinases) sense the oxidative stress caused by H_2O_2 and NO after UV irradiation. Src tyrosine kinases are located on the cytoplasmic side of the plasma membrane, and at the NH_2-terminus have a myristoylated Gly that supports anchoring to the plasma membrane. Src tyrosine kinases contain a Cys-X-X-Cys sequence, with which they form a complex with receptor molecules such as CD4 and CD8: they are activated by oxidative stress, enhancing the autophosphorylation of Tyr394, and are further activated by phosphotyrosine phosphatase via the dephosphorylation of Tyr505. The activated Src tyrosine kinase phosphorylates I-κB, forming a complex with NF-κB; this leads to the proteolytic degradation of I-κB, stimulating the translocation of NF-κB from cytoplasm to nucleus. NF-κB activated by thioredoxin stimulates the transcription of inducible NO (nitric oxide) synthetase, iNOS, co-operatively with AP-1.

Signal transduction of light: Our eyes perceive light signals at the retinal rod cells' outer segments, which contain rhodopsin. Upon reception of light rhodopsin is activated to metarhodopsin II, which forms a complex with heterotrimeric GTP-binding protein, transducin (Gt), and stimulates the cyclicGMP phosphodiesterase (PDE). The activation of PDE results in the decrease in the concentration of cyclicGMP, which functions to open the Na^+ channel on the plasma membrane. The reception of light signals results in the closure of the Na^+ channel, causing electrical stress on the plasma membrane. This causes the start of electrical pulse transmission alone, with neuronal systems such as bipolar cells, horizontal cells, amacrine cells, and glia cells connecting to the brain.

Signal transduction of UVA to blue light (320 to 460 nm) was partly revealed by the use of a filamentous fungus, *Neurospora crassa*. Blue light increased the phosphorylation of nucleoside diphosphate kinase-1 (NDK-1). The phosphorylation of NDK-1 is dependent on WC-1 and WC-2, putative photoreceptor-forming heterocomplexes suggested to be transcription factors. NDK-1 is presumed to have histidine kinase activity and showed:

- γ-phosphate transferring activity for ATP + GDP → ADP + GTP
- autophosphorylation activity
- protein kinase activity, which may include phospho-relay from histidine kinase

NDK-1 is suggested to provide GTP in the vicinity of GTP-binding protein. The mutant protein NDK-1Pro72His showed a deficiency in both autophosphorylation activity and protein kinase activity, which may include phospho-relay from histidine kinase. In stem sections of etiolated seedlings of the pea plant *Pisum sativum*, light signals transmitted via the plant photoreceptor phytochrome stimulated the phosphorylation of Pea NDK1.

Epigenetic regulation of gene expression: Recent research has uncovered complex gene regulations, or *epigenetics*, which manifest as a heritable gene regulation without change in the DNA sequence.

The epigenetic mechanisms observed in fungi, animals, and plants fall within at least

four major categories:

- RNA interference (RNAi) caused by the introduction of double stranded (ds)RNA, or in some cases by the introduction of DNA. This induces the degradation of messenger RNA, suppressing the gene expression.
- Repeat-induced point mutation (RIP), observed in a haploid filamentous fungus *Neurospora crassa*, which includes the process of methylation in cytosine(C) residues at the duplicated region prior to entering into meiosis. The methyl C is deaminated and converted to T.
- Maintenance of the genetic program by *Polycomb* group (*PcG*) genes, which suppress gene expression, and *trithorax* group (*trxG*) genes, which stimulate gene expression.
- Genetic regulation of development and differentiation through the methylation of DNA, mainly referring to the CpG methylation in mammals.

1. Introduction

Living organisms on earth manifest their capacities to survive by responding to changes in ambient circumstances, such as levels of visible light, UV light, low and high temperatures, draught, nutritional salts, wounding (including inflammation), hormones, growth factors, oxidative stresses, and infections by viruses, bacteria, and fungi. The genetic and biochemical approaches to analyzing these phenomena made by G. W. Beadle and E. L. Tatum (1941) reported biochemical mutants in *Neurospora* requiring vitamin B1 (thiamin), vitamin B6 (pyridoxine), and *p*-amino benzoic acid. This created the basis of molecular biology with reference to the "one gene–one enzyme" theory.

Living organisms evolved on the earth, which orbits the sun with a rotation every 24 hours, typically allowing living organisms to receive periodic sunlight for about 12 hours daily. Sunlight reaching the earth is composed mainly of UV light, along with visible lights ranging from violet to far red and infrared. Such a periodic sunlight regime causes various organisms to generate a circadian (approximately one-day) rhythm. Circadian rhythms are defined by the following three characteristics:

- the period length of the phenomenon is about (circa) one day (dian) under constant conditions
- the period length of the rhythm can be adjusted (entrained) by light irradiation, with a cycle of about 24 hours (light-induced phase response)
- the period length of the rhythm can be maintained constantly, independent of ambient temperature.

In the case of humans, the sleeping-and-waking rhythm is about 25 hours in darkness, and therefore about one hour of adjustment of time can occur every day in sunlight. Not only our eyesight but also our biological clock system, like the avian pineal body, is able to receive light signals. In the case of eyesight, the photoreceptor in the retinal rod cell outer segment is rhodopsin. The reception of light by rhodopsin stimulates the binding of GTP to GTP-binding protein, transducin (Gt). Transducin activated by the binding of GTP will stimulate cyclicGMP phosphodiesterase (PDE) by the removal of the inhibitory γ-subunit of PDE. The activated PDE causes a decrease in the

concentration of cyclicGMP, resulting in the closing of the sodium channel on the plasma membrane stopping the influx of Na^+. The stoppage of influx of Na^+ in response to light signals will reduce the concentration of Na^+ in the inside of the rod cell inner segment, which will cause the onset of electrical stress to be transmitted to the brain. The light signal is the first messenger while the corresponding cyclicGMP is termed the second messenger, as in the case of glucagon as a first messenger and cyclicAMP as the second messenger.

In the case of the circadian rhythm (with a biological clock period of about 24 hours) the photoreceptor is suggested to be a family of rhodopsins in vertebrates, and also cryptochrome in *Drosophila*. However, the molecular structure of the timekeeping mechanism underlying circadian rhythm remains to be clarified.

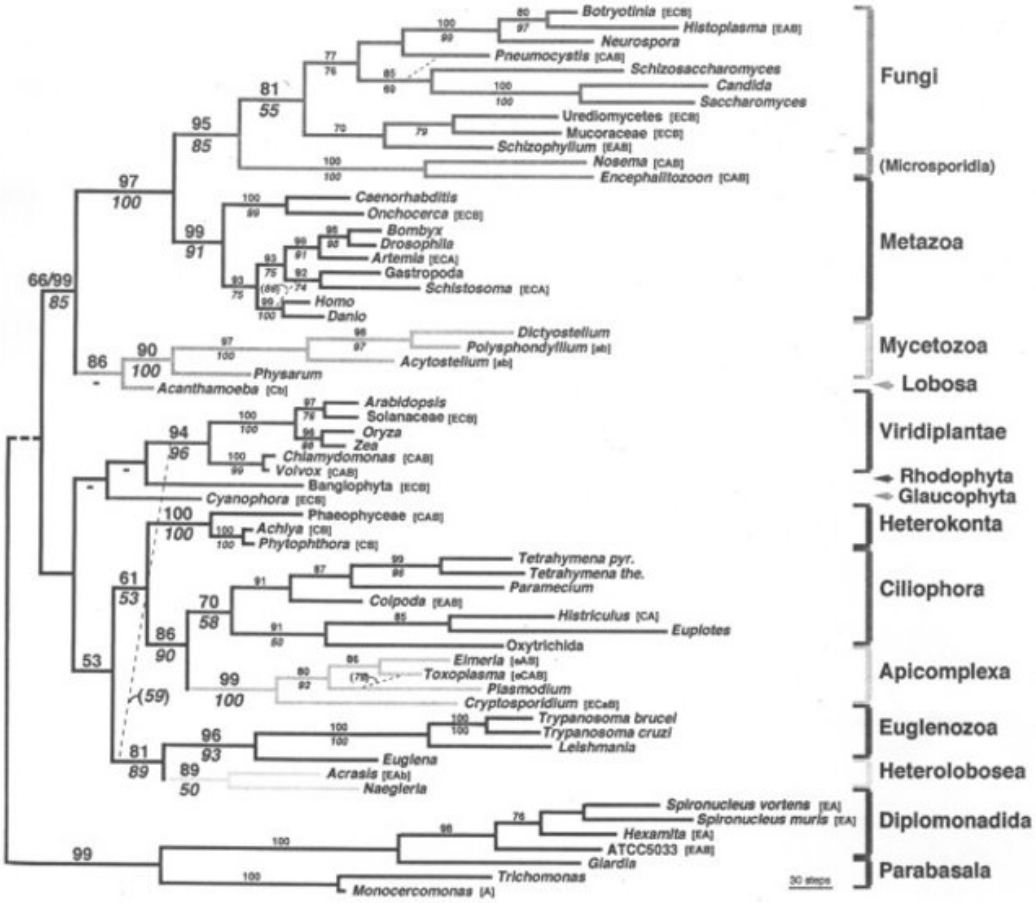

Figure 1. A kingdom-level phylogeny of eukaryotes, based on combined protein sequences.

Source: adopted from Baldauf et al. (2000).

Notes: The tree shown is one of the two shortest trees found by parsimony analysis of concatenated EF-1α, actin, α-tublin, and β-tublin amino acid sequences. The tree is 5,056 steps long with branches drawn to scale. Boots strap values >50% are shown above and below the lines, respectively, for amino acid parsimony (aaBP) and maximum likelihood analysis of second codon-position nucleotides (ntBP). Parentheses indicate the aaBP for the grouping of animals + fungi plus lobosa + mycetozoa in

analyses omitting Bangiophyceae and *Cyanophoa*. Dashes (–) below lines indicate nodes not tested in the ntBP analyses. For taxa with missing data, the sequences used are indicated in brackets to the right of taxon names in uppercase and lowercase letters for complete and partial sequences, respectively (E = EF-1α, C = actin, A = α-tublin, B = β-tublin). The lowest common taxonomic designation is given for sequences combined from different taxa. The shortest trees differ only in their placement of Pneumocystis, as shown by the thin dashed line; all other slanting dashed lines indicate alternative groupings found with ntBP > 50%. The horizontal dashed line (left center) indicates the tentative placement of Diplomonadida and Parabasalia.

Living organisms may be classified as viruses, bacteria (including domain archaea and domain bacteria), prokaryophyta (including cyanobacteria), and eukaryotes (domain eucarya). A kingdom-level phylogeny of eukaryotes, based on combined protein sequences, is presented in Figure 1. Fungi (including *Neurospora*, *Saccharomyces*, and *Schizosaccharomyces*) and Metazoa (animals) including *Caenorhabditis*, *Drosophila*, *Homo* (human) and *Danio* (Zebra fish) are closely related. Mycetozoa including *Dictyosterium* and *Physarum*, and Viridiplantae including *Arabidopsis*, *Oryza*, and *Chlamydomonas*, are distantly related to Metazoa.

Single and double stranded RNAs and single and double stranded DNAs are the genetic components of viruses. In bacteria, double stranded DNAs with a molecular size of around 4.5 Mbp are the genetic material, and the process of sexuality can be detected, which is promoted by a sex factor such as fertility factor in *Escherichia coli*. Eukaryotes have a nucleus containing DNA as the genetic materials, which forms a specific structure called a chromosome. For example the genetic material in *Neurospora crassa* comprises seven chromosomes with a total of 42.9 Mbp: in other words, roughly ten times the DNA in *Escherichia coli*. Chromosomes are composed of a centromere, replication origins, and telomeres controlling the ends of the chromosome.

In the filamentous fungus *Neurospora*, which is haploid except during meiosis, introduction of a gene segment by transformation results in the random integration of the transgene, and in the duplication of the gene segment in the nucleus. The duplicated DNA segments are methylated premeiotically with cytosine, and during the process of meiosis the G:C with methylated cytosine is frequently converted into A:T. During the process, the 5-methyl cytosine is deaminated to produce thymidine. Thus, meiosis stimulates the recombination of genes and also modifies the DNA, resulting in mutations. In *Drosophila melanogaster*, however, very little DNA methylation could be detected. No telomere could be observed in the chromosome structure; furthermore, no telomerase activity could be detected, suggesting that the telomere of a chromosome may not be necessary.

The pattern of gene regulation from virus to eukaryote may be described as follows. In a retrovirus the plus strand RNA is the genome, and the genes for *gag*, *pro*, *pol* and *env* overlap with each other and encode different proteins with different reading frames. In bacteria, characteristic gene expressions result in polycistronic messengers (m)RNA, as observed in the tryptophan operon. The initiation of mRNA synthesis is regulated by transcription factors, including transcription regulators. The release of initiated mRNA from DNA is also controlled by transcription attenuation based on the concentration of

tryptophan. Even in *Escherichia coli* epigenetic regulation of gene expression in the lysogenic phase and lytic phase of lambda phage can occur.

The regulation of homeotic genes is a model system to analyze the change in chromatin structure during development and differentiation in *Drosophila melanogaster*. *Polycomb* group (*PcG*) genes suppress the expression of homeotic genes such as *Ubx*, whereas *trithorax* group (*trxG*) genes stimulate genes such as the heat shock gene, *hsp70*, whose product can supply proteins for thorax differentiation functioning as a molecular chaperone. In humans, the gene homologous to *PcG* was cloned as a tumor suppressor.

In eukaryotes, most of the mRNAs are spliced, splitting off the introns. In lower eukaryotes, such as *Saccharomyces cerevisiae*, the spliced segments are restricted to a relatively small area. In specific cases the spliced area in mitochondria encodes mRNA maturase having a specific function. In higher organisms such as humans a gene can extend over more than 100 kbp, including huge spaces of introns which also contain several elements of retrotransposons, enabling the expression of the gene via several promotors.

The ability of transgenes such as double stranded RNA (dsRNA) to silence the expression of homologous loci—a process referred to as PTGS (post transcriptional gene silencing) and also RNAi (RNA interference)—was first detected in plants and later observed in *Caenorhabditis elegans, Neurospora crassa, Drosophila melanogaster*, and *Trypanosoma brucei*. Homology-dependent trans-silencing effects are divided into two categories:

- the gene expression of the target locus is not affected, but the half-life of the target RNAs is severely decreased
- the homology-triggered processes exert a primary effect on the template DNA of the target loci including methylation, termed TGS (transcriptional gene silencing).

The genome of an adult vertebrate cell comprises 60–90 percent cytosines, in CpG dinucleotide methylated by DNA methyl transferase. DNA methylation affects recognition by the transcriptional regulators and the structural proteins assembling chromatin. DNA methylation can control gene expression, either by changing the activity at a single promotor and enhancer, or by changing the activity through global mechanisms such as X chromosome inactivation by the Xist gene. It is suggested that PTGS and the methylation of DNA evolved as a mechanism of host-defense to protect the genome against genomic parasites, such as retrotransposons, which represent at least 35 percent of the genome in humans.

The focus of this review lies on the molecular mechanisms of signal transduction, with special emphasis on the reactive oxygen intermediates (ROIs), light signal transduction, and epigenetics including *Polycomb* group (*PcG*) genes, RNAi, and complex gene regulation by DNA methylation. These areas of research have seen astonishing advances during the last ten years, and will constitute important fields in “genetics and molecular biology” in the decade to come.

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Jones, P. A. 1999. The DNA Methylation Paradox. *Trends in Genetics*, No. 15, pp. 34–7. [The methylation of CpG islands is often equated with transcriptional inactivity, and there is overwhelming evidence that this is the case for islands located in gene promotors. Such methylation is probably part of a mechanism to silence the activities of genes permanently, including those on the inactive X chromosome. Methylation of CpG islands downstream of transciption initiation does not block elongation of mRNA in mammalian cells.]

Kamijo, R.; Harada, H.; Matsuyama, T.; Bosland, M.; Gerecitano, J.; Shapiro, D.; Le, J.; Koh, S. I.; Kimura, T.; Green, S. J.; Mak, T. W.; Taniguchi, T.; Vilcek, J. 1994. Requirement for Transcription Factor IRF-1 in NO Synthase Induction in Macrophages. *Science*, No. 263, pp. 1612–5. [Production of NO by macrophages is important for killing intracellular infectious agents. Interferon (IFN)-γ and lipopolysaccharide stimulate NO production by transcriptionally up-regulating the inducible NO synthase (iNOS). Macrophages from mice with a targeted disruption of the IFN regulatory factor-1 (IRF-1) gene (IRF-1-/- mice) produced little or no NO, and synthesized barely detectable quantities of iNOS messenger RNA in response to stimulation.]

Kass, S. U.; Pruss, D.; Wolffe, A. P. 1997. How Does DNA Methylation Repress Transcription? *Trends in Genetics*, No. 13, 444-9. [There may be transcriptional repressors specific for methylated DNA and chromatin assembly. The assembly of nucleosomal structures on methylated DNA may provide the DNA segment to silence transcription more efficiently than conventional chromatin.]

Keyse, S. M.; Tyrrell, R. M. 1989. Heme Oxygenase is the Major 32-kDa Stress Protein Induced in Human Skin Fibroblasts by UVA Radiation, Hydrogen Peroxide, and Sodium Arsenite. *Proceedings of the National Academy of Science of the USA*, No. 86., pp. 99–103. [UVA (320-380nm) radiation, hydrogen peroxide, and sodium arsenite induce a stress protein of about 32 kDa in human skin fibroblasts, which is identified as heme oxigenase.]

Kinoshita, T.; Yadegari, R.; Harada, J. J.; Goldberg, R. B.; Fischer, R. L. 1999. Imprinting of the *MEDEA* Polycomb Gene in the Arabidopsis Endosperm. *Plant Cell*, No. 11, pp. 1945–52. [A maternal loss-of-function *mea* allele results in embryo abortion and prolonged endosperm production irrespective of the genotype of the paternal allele.]

Kinoshita, T.; Harada, J. J.; Goldberg, R. B.; Fischer, R. L. 2001. Polycomb Repression of Flowering During Early Plant Development. *Proceedings of the National Academy of Science of the USA*, No. 98, pp. 14,156–61. [PcG proteins play a central role in repressing flowering early in the plant life cycle. Mutations in the *Fertilization Independent Endosperm (FIE)* PcG gene caused the seedling shoot to produce flower-like structures and organs.]

Kiyosue, T.; Ohad, N.; Yadegari, R.; Hannon, M.; Dinneny, J.; Wells, D.; Katz, A.; Margossian, L.; Harada, J. J.; Goldberg, R. B.; Fischer, R. L. 1999. Control of Fertilization-Independent Endosperm Development by the *MEDEA* Polycomb Gene in *Arabidopsis. Proceedings of the National Academy of Science of the USA*, No. 96, pp. 4186–91. [A mutant, F644, was isolated, that allows for replication of a

central cell and subsequent endosperm development without fertilization.]

Lakin-Thomas, P.; Cote, G.; Brody, S. 1990. Circadian Rhythms in *Neurospora crassa*: Biochemistry and Genetics. *Critical Review of Microbiology*, No. 17, pp. 365–416.[The putative molecular mechanisms of circadian rhythm in *Neurospora crassa* are summarized and discussed.]

Lakin-Thomas, P.; Brody, S. 2000. Circadian Rhythms in *Neurospora crassa*: Lipid Deficiencies Restore Robust Rhythmicity to Null *Frequency* and *White-Collar* Mutants. *Proceedings of the National Academy of Science of the USA*, No. 97, pp. 256–61. [Two mutants, *cel* (chain-elongation) and *chol-1* (choline-requirer), are defective in lipid synthesis and affect the period and temperature compensation of the rhythm. The double mutant strains *chol-1 frq9, chol-1 frq10, chol-1 wc-1, chol-1 wc-2, cel frq9, cel frq10,* and *cel wc-2* have been constructed. These strains are robustly rhythmic when assayed under lipid-deficient conditions, indicating that free-running rhythmicity does not require the *frq*, *wc-1*, or *wc-2* gene products.]

Lee, W.; Haslinger, A.; Karin, M.; Tjian, R. 1987. Activation of Transcription by Two Factors that Bind Promotor and Enhancer Sequences of the Human Metallothionein Gene and SV40. *Nature*, No. 325, pp. 368–72. [The authors report the identification of two cellular DNA-binding proteins that interact selectively with sequences governing the basal level expression of hMIIA. One of these factors is a novel activator protein (AP1) that interacts with sequences in the BLE of hMTIIA, and also binds to a site within the 72-base pair (bp) repeats of the simian virus 40 (SV40) enhanced region. The second protein has been purified to homogeneity and shown to be the transcription factor Sp1, which recognizes and binds to a single GC-box element within the hMTIIA promotor.]

Linden, H.; Macino, G. 1997. White Collar 2, a Partner in Blue-Light Signal Transduction, Controlling Expression of Light-Regulated Genes in *Neurospora crassa*. *EMBO Journal*, No. 16, pp. 98–109. [WC-2, the second partner of this light signal transduction system, encodes a functional zinc finger DNA-binding protein with a putative PAS dimerization and transcription activation domain.]

Liu, Y.; Loros, J.; Dunlap, J. C. 2000. Phosphorylation of the *Neurospora* Clock Protein FREQUENCY Determines its Degradation Rate and Strongly Influences the Period Length of the Circadian Clock. *Proceedings of the National Academy of Science of the USA*, No. 97, pp. 234–9. [The mutation of one phosphorylation site at Ser513 leads to a dramatic reduction of the rate of FREQUENCY (FRQ) degradation and a very long period (>30 hour) for the clock's cycle. The data suggest strongly that FRQ phosphorylation triggers its degradation, and that the degradation rate of FRQ is a major determining factor for the period length of the circadian clock under bd genetic background.]

Los, M.; Schenk, H.; Hexel, K.; Baeuerle, P. A.; Droge, W.; Schulze-Osthoff, K. 1995. IL-2 Gene Expression and NF-κB Activation Through CD28 Requires Reactive Oxygen Production by 5-Lipoxygenase. *EMBO Journal*, No. 14, pp. 3731–40. [Lipoxygenase metabolites activate ROI formation, which then induces IL-2 expression via NF-κB activation.]

Malagnac, F.; Wendel, B.; Goyon, C.; Faugeron, G.; Zickler, D.; Rossignol, J.-L.; Noyer-Weidner, M.; Vollmayr, P.; Trautner, T. A.; Walter, J. 1997. A Gene Essential for De Novo Methylation and Development in Ascobolus Reveals a Novel Type of Eukaryotic DNA Methyltransferase Structure. *Cell*, No. 91, pp. 281–90. [A gene for *de novo* methylation of DNA, masc1 in Ascobolus, is characterized. This encodes a protein that contains all motifs of the catalytic domain of eukaryotic 5mC-DNA-methyltransferases, but lacks the NH_2-terminal domain. Crosses between parents harboring the masc1 disruption are arrested at an early stage of sexual reproduction.]

Masters, B. A.; Kelly, E. J.; Quaife, C. J.; Brinster, R. L.; Palmiter, R. D. 1994. Targeted Disruption of Metallothionein I and II Genes Increases Sensitivity to Cadmium. *Proceedings of the National Academy of Science of the USA*, No. 91, pp. 584–8. [The authors inactivated the mouse metallothionein (MT)-I and MT-II genes in embryonic stem cells and generated mice homozygous for these mutant alleles. These mice were viable, and reproduced normally when grown under normal laboratory conditions. They were, however, more susceptible to hepatic poisoning by cadmium.]

May, M. J.; D'Acquisto, F.; Madge, L. A.; Glockner, J.; Pober, J. S.; Ghosh, S. 2000. Selective Inhibition of NF-κB Activation by a Peptide that Blocks the Interaction of NEMO with the IkB Kinase Complex. *Science*, No. 289, pp. 1550–4. [Activation of NF-κB requires the activity of I-kb kinase (IKK) complex, containing two kinases (IKKa and IKKb) and the regulatory protein of NEMO (NF-κB essential modifier). A cell-permeable NEMO-binding domain peptide blocked association of NEMO with the IKK

complex.]

Meister, A. 1994. Glutathione-Ascorbic Acid Antioxidant System in Animals. *Journal of Biological Chemistry*, No. 269, pp. 9397–400. [When glutathione (GSH) deficiency is produced in newborn rats or guinea pigs the animals develop multiorgan illness, and die within a few days. This result is directly related to loss of an essential antioxidant system.]

Moncada, S.; Nistico, G.; Bagetta, G.; Higgs, E. A. (eds.) 1998. *Nitric Oxide and the Cell: Proliferation, Differentiation and Death*. London, Portland. [Based on results up until 1996, the functions of NO in the process of signal transduction are summarized from various view points.]

Myrset, A. H.; Bostad, A.; Jamin, N.; Lirsac, P.-N.; Toma, F.; Gabrielsen, O. S. 1993. DNA and Redox State Induced Conformational Changes in the DNA-binding Domain of the Myb Oncoprotein. *EMBO Journal*, No. 12, pp. 4625–33. [The DNA-binding domain of the oncoprotein Myb comprises three imperfect repeats, R1, R2, and R3. R2 and R3 are required for sequence-specific DNA-binding. Both are assumed to contain helix-turn helix (HTH)-related motifs, but multidimensional heteronuclear NMR spectroscopy revealed a disordered structure in R2 where the second HTH helix was predicted. The disordered region folds into a recognition helix and generates a full HTH-related motif upon binding to DNA. This would move Cys43 into the hydrophobic core of R2.]

Naumann, M.; Scheidereit, C. 1994. Activation of NF-κB *In Vivo* is Regulated by Multiple Phosphorylations. *EMBO Journal*, No. 13, pp. 4597–607. [The authors investigated the modifications imposed on NF-κB/IkB components following stimulation, and showed that the final step of NF-κB induction *in vivo* involves phosphorylation of several members of the NF-κB/IkB protein families. The authors found that the induction by hydrogen peroxide of NF-κB translocation to the nucleus, which is assumed to be triggered by reactive oxygen intermediates, also coincided with incorporation of phosphate into the same subunits that were modified after stimulation by TNF-α.]

Ninnemann, H.; Maier, J. 1996. Indications for the Occurrence of Nitric Oxide Synthetases in Fungi and Plants and the Involvement in Photoconidiation of *Neurospora crassa*. *Photochemistry and Photobiology*, No. 64, pp. 393–8. [Indications of the occurrence of nitric oxide synthetases in Dictyostelium, Neurospora, Phycomyces and leguminous plant *Mucuna hassjoo*, as well as a physiological role for nitric oxide in *Neurospora crassa*, are demonstrated.]

Nishizuka, Y. 1984. The Role of Protein Kinase C in Cell Surface Signal Transduction and Tumor Promotion. *Nature*, No. 308, pp. 693–8. [Protein kinase C, activated by the breaking down of inositol phospholipid producing diacylglycerol (DG) in response to external signals, can be antagonistically controlled by cyclicAMP. Protein kinase C activated by DG can promote cell proliferation.]

Nishizuka, Y. 1995. Protein Kinase C and Lipid Signaling for Sustained Cellular Responses. *FASEB Journal*, No. 9, pp. 484–96. [The author discusses some dynamic aspects of membrane lipid metabolism in the control of intracellular events, and summarizes current knowledge and perspectives of the PKC family's pivotal roles in the intracellular signaling network. Stimulation of a cell surface receptor initiates a degradation cascade of various membrane lipid constituents. Many of their metabolites have potentials to induce, intensify, and prolong the activation of protein kinase C that is needed for sustained cellular responses.]

Novogrodsky, A.; Vanichkin, A.; Patya, M.; Gazit, A.; Osherov, N.; Levitzki, A. 1994. Prevention of Lipopolysaccharide-Induced Lethal Toxicity by Tyrosine Kinase Inhibitors. *Science*, No. 264, pp. 1319–22. [Protein tyrosine kinase inhibitors of the tyrphostin AG 126 family protect mice against lypopolysaccharide (LPS)-induced lethal toxicity. The protection correlates with the ability of these agents to block LPS-induced production of tumor necrosis factor a (TNF-α) and nitric oxide in macrophages, as well as LPS-induced production of TNF-α *in vivo*. This inhibitory effect correlated with the potency of AG126 to block LPS-induced tyrosine phosphorylation of a p42MAPK protein substrate in the murine macrophage.]

Oda, K.; Hasunuma, K. 1994. Light Signals are Transduced to the Phosphorylation of 15 kDa Protein in *Neurospora crassa*. *FEBS Letters*, No. 345, pp. 162–6. [The first report of blue light irradiation of the crude membrane fraction from WT mycelia stimulating an increase in the phosphorylation of a 15 kDa protein, which is dependent on putative photoreceptors, WC-1 and WC-2 proteins.]

Oda, K.; Hasunuma, K. 1997. Genetic Analysis of Signal Transduction through Light Induced Protein

Phosphorylation in *Neurospora crassa* Perithecia. *Molecular and General Genetics*, No. 256, pp. 593–601. [A mutant form in the phosphorylation of the 15 kDa protein (*psp*) lacked light induced polarity of perithecia. The *psp* (ndk-$1^{Pro72His}$) produced a perithecial beak at random, even under directional irradiation.]

Ogura, T.; Tanaka, N.; Yabe, N.; Komatsu, S.; Hasunuma, K. 1999. Characterization of Protein Complexes Containing Nucleoside Diphosphate Kinase with Characteristics of Light Signal Transduction through Phytochrome in Etiolated Pea Seedlings. *Photochemistry and Photobiology*, No. 69, pp. 397–403. [Red light irradiation of intact etiolated pea seedlings, followed by the preparation of a crude membrane fraction, stimulated phosphorylation of pea NDK-1.]

Ogura, Y.; Yoshida, Y.; Ichimura, K.; Aoyagi, C.; Yabe, N.; Hasunuma, K. 1999. Isolation and Characterization of *Neurospora crassa* Nucleoside Diphosphate Kinase NDK-1. *European Journal of Biochemistry*, No. 266, pp. 709–14. [Purification of NDK-1 and partial determination of its amino acid sequence identified the 15 kDa protein as NDK-1. This showed not only γ -phosphate transfer activity but also autophosphorylation and protein kinase activities.]

Ogura, Y.; Yoshida, Y.; Yabe, N.; Hasunuma, K. 2001. A Point Mutation in Nucleoside Diphosphate Kinase Results in a Deficient Light Response for Perithecial Polarity in *Neurospora crassa*. *Journal of Biological Chemistry*, 276, pp. 21,228–34. [A mutant for the phosphorylation of the 15 kDa protein psp was identified to be ndk-$1^{Pro72His}$, which lacks light induced polarity of perithecia. NDK-$1^{Pro72His}$ protein showed normal γ -phosphate transferring activity. However, it lacked autophosphorylation and protein kinase activity.]

Ohad, N.; Yadegari, R.; Margossian, L.; Hannon, M.; Michaeli, D.; Harada, J. J.; Goldberg, R. B.; Fischer, R. L. 1999. Mutations in FIE, a WD Polycomb Group Gene, Allow Endosperm Development Without Fertilization. *Plant Cell*, No. 11, pp. 407–15. [A female gametophyte with a loss-of-function allele of fie undergoes replication of the central cell nucleus and initiates endosperm development without fertilization.]

Perkins, D. D.; Radford, A.; Sachs, M. S. 2001. *The Neurospora Compendium, Chromosomal Loci*. San Diego, Calif., and London, Academic Press. [This book describes the function and physical location of genes analyzed over the last six decades; with five appendixes including genetic maps.]

Preuss, D. 1999. Chromatin Silencing and Arabidopsis Development: A Role for Polycomb Proteins. *Plant Cell*, No. 11, pp. 765–8. [The function of PcG, genes including FIE, FIS, and MEA, during seed development after double fertilization is discussed. However, there is no data to suggest a contribution by these PcG genes to gene silencing.]

Richard, E. J. 1997. DNA Methylation and Plant Development. *Trends in Genetics*, No. 13, 319–23. [Up to 20–30 percent of cytosines are methylated in the nuclear genome of many flowering plants (angiosperms). Much of this modification is found at the short symmetrical sites, CpG and CpNpG, but noncanonical methylation outside of these sites is also found in angiosperm genomes.]

Robison, G. A.; Butcher, R. W.; Sutherland, E. W. 1968. Cyclic AMP. *Annual Review of Biochemistry*, No. 37, pp. 149–74.

Rodbell, M.; Krans, H. M. J.; Pohl, S. L.; Birnbaumer, L. 1971. The Glucagon-Sensitive Adenyl Cyclase System in Plasma Membranes of Rat Liver. *Journal of Biological Chemistry*, No. 246, pp. 1872–6.

Rudd, C. E.; Trevillyan, J. M.; Dasgupta, J. D.; Wong, L. L.; Schlossman, S. F. 1988. The CD4 Receptor is Complexed in Detergent Lysates to a Protein-Tyrosine Kinase (pp58) from Human T Lymphocytes. *Proceedings of the National Academy of Science of the USA*, No. 85, pp. 5190–4. [The PTK is the human analogue of the murine pp56LSTRA (pp55lck) and has significant homology with c-src, c-yes, and other members of the src family. The identification of the PTK associated with CD4 receptor was made using an antiserum to a synthetic peptide.]

Russo, V. E. A.; Martienssen, R. A.; Riggs, A. D. (eds.) 1996. *Epigenetic Mechanisms of Gene Regulation*. New York, Cold Spring Harbor Laboratory Press. [Epigenetic phenomena including gene silencing, paramutation, imprinted genes, X-inactivation, *Polycomb* group (*PcG*) genes, and DNA methylation were reviewed.]

Sargent, M. L.; Briggs, W. R.; Woodward, D. O. 1966. Circadian Nature of a Rhythm Expressed by an Invertaseless Strain of *Neurospora crassa*. *Plant Physiology*, No. 41, pp. 1343–9.

Schreck, R.; Rieber, P.; Baeuerle, P. A. 1991. Reactive Oxygen Intermediates as Apparently Widely Used Messengers in the Activation of the NF-κB Transcription Factor and HIV-1. *EMBO Journal*, No. 10, pp. 2247–58. [Micromoler concentrations of H_2O_2 can induce the expression and replication of HIV-1 in a human T-cell line. The effect is mediated by the NF-κB transcription factor. This is potently and rapidly activated by an H_2O_2 treatment of cells from its inactive cytoplasmic form. *N*-acetyl-L-cysteine (NAC) and other thiol compounds also blocked the activation of NF-κB by cycloheximide, double-stranded RNA, calcium ionophore, TNF-α, active phorbol ester, interleukin-1, lipopolysaccharide and lectin. ROIs appear to serve as messengers mediating directly or indirectly the release of the inhibitory subunit IκB from NF-κB.]

Schulze-Osthoff, K.; Beyaert, R.; Vandevoorde, V.; Haegeman, G.; Fiers, W. 1993. Depletion of the Mitochondrial Electron Transport Abrogates the Cytotoxic and Gene-Inductive Effects of TNF. *EMBO Journal*, No. 12, pp. 3095–104. [Studies of L929 fibrosarcoma cells have revealed that the mitochondrial electron transport system plays a key role in inducing TNF cytotoxicity, presumably by the formation of reactive oxygen intermediates (ROIs). Antimycin A, a mitochondrial inhibitor, promotes the generation of ROI, and potentiates TNF-triggered NF-kB activation.]

Selker, E. U. 1997. Epigenetic Phenomena in Filamentous Fungi: Useful Paradigms or Repeat-Induced Confusion? *Trends in Genetics*, No. 13, pp. 296–301. [Epigenetic mechanisms can serve as genome defense systems. In haploid nuclei of special sexual cells of fungi, such as *Neurospora crassa* and *Ascobolus immersus*, duplicated genes are silenced by hypermutation, DNA methylation, or both. DNA introduced into the genome of *Neurospora* cells by transformation can also inhibit homologous genes by a silencing mechanism that does not involve DNA pairing or methylation, and appears to be post-transcriptional.]

Selker, E. U. 1999. Gene Silencing: Repeats That Count. *Cell*, No. 97, pp. 157–60. [Repeat induced methylation of DNA and repeat induced gene silencing observed in *Neurospora crassa*, *Ascobolus immersus* and *Arabidopsis thaliana* are described.]

Sharp, P. A. 1999. RNAi and Double-Strand RNA. *Genes & Development*, No. 13, pp. 139–41. [The post-transcriptional effects of RNAi were directly observed using *in situ* hybridization to follow transcripts of genes suppressed by injection of dsRNA. A total absence of specific mRNA in the cytoplasm could be detected, suggesting that dsRNA establishes an intracellular state that destroys RNA transcribed and spliced from a specific gene.]

Stamler, J. S.; Singel, D. J.; Loscalzo, J. 1992. Biochemistry of Nitric Oxide and its Redox-Activated Forms. *Science*, No. 258, pp. 1898–902. [Nitric oxide (NO•) has been implicated in a wide range of biological functions. The broader chemistry of nitrogen monoxide (NO) involves a redox array of species with distinctive properties and reactivities: NO^+ (nitrosonium), $NO^•$, and NO^+ (nitroxyl anion).]

Storz, G.; Tartaglia, L. A.; Ames, B. N. 1990. Transcriptional Regulator of Oxidative Stress-Inducible Genes: Direct Activation by Oxidation. *Science*, No. 248, pp. 189–94. [The *oxyR* gene positively regulates the expression induced by oxidative stress in *Salmonella typhimurium* and *Escherichia coli*. Purification of the OxyR protein showed that oxidized, but not reduced, OxyR activates transcription of oxidative stress-inducible genes *in vitro*.]

Talora, C.; Franchi, L.; Linden, H.; Ballario, P.; Macino, G. 1999. Role of a White Collar-1–White Collar-2 Complex in Blue-Light Signal Transduction. *EMBO Journal*, No. 18, pp. 4961–8. [The WC proteins from homo- and heterodimers *in vitro*; this interaction could represent a fundamental step in the control activity. The WC proteins are assembled in a white collar complex (WCC), and WC-1 undergoes a change in mobility due to light-induced phosphorylation events.]

Tanaka, N.; Ogura, T.; Noguchi, T.; Hirano, H.; Yabe, N.; Hasunuma, K. 1998. Phytochrome-Mediated Light Signals are Transduced to Nucleoside Diphosphate Kinase in *Pisum Sativum* L. cv. Alaska. *Journal of Photochemistry and Photobiology, B: Biology*, No. 45, pp. 113–21. [Third internodes of etiolated seedlings of pea were irradiated with red light, and crude membrane and soluble fractions then prepared. The phosphorylation of pea NDK-1 is increased by red light, and the purified pea NDK-1 showed autophosphorylation and protein kinase activities.]

Tanaka, T.; Akira, S.; Yoshida, K.; Umemoto, M.; Yoneda, Y.; Shirafuji, N.; Fujiwara, H.; Suematsu, S.; Yoshida, N.; Kishimoto, T. 1995. Targeted Disruption of the NF-IL6 Gene Discloses its Essential Role in Bacteria Killing and Tumor Cytotoxicity by Macrophages. *Cell*, No. 80, pp. 353–61. [To investigate the

role of NF-IL6 *in vivo*, the authors have generated NF-IL6 (-/-) mice by gene targeting. NF-IL6 (-/-) mice were highly susceptible to infection by Listeria monocytogenes.]

Tan, P. B. O.; Kim, S. K. 1999. Signaling Specificity: The RTK/RAS/MAP Kinase Pathway in Metazoans. *Trends in Genetics*, No. 15, pp. 145–9. [This review reports four possible mechanisms that might provide signaling specificity to the RTK/RAS, MAP kinase signaling pathway: i) discrete receptor-activated pathways; ii) differential signaling kinetics; iii) interaction of multiple signaling pathways; and iv) tissue-specific downstream effector.]

Uckun, F. M.; Tuel-Ahlgren, L.; Song, C. W.; Waddick, K.; Myers, D. E.; Kirihara, J.; Ledbetter, J. A.; Schieven, G. L. 1992. Ionizing Radiation Stimulates Unidentified Tyrosine-Specific Protein Kinases in Human B-Lymphocyte Precursors, Triggering Apoptosis and Clonogenic Cell Death. *Proceedings of the National Academy of Science of the USA*, No. 89, pp. 9005–9. [Immune-complex kinase assays on irradiated and unirradiated B-lymphocyte precursors, using antibodies prepared against unique amino acid sequences of p59fyn, p56/p53lyn, p55blk, and p56lck, demonstrated that these Src-family tyrosine kinases were not the primary PTKs responsible for enhanced tyrosine phosphorylation of multiple substrates.]

Veillette, A.; Bookman, M. A.; Horak, E. M.; Bolen, J. B. 1988. The CD4 and CD8 T Cell Surface Antigens are Associated with the Internal Membrane Tyrosine-Protein Kinase p56lck. *Cell*, No. 55, pp. 301–8. [p56lck is associated functionally and physically with CD4/CD8 in normal nurine T lymphocytes; an independent signal is transduced by the interaction of these surface molecules with major histocompatibility complex determinants.]

Verma, I. M.; Stevenson, J. K.; Schwarz, E. M.; Van Antwerp, D.; Miyamoto, S. 1995. Rel/NF-κB/I-κB Family: Intimate Tales of Association and Dissociation. *Genes and Development*, No. 9, pp. 2723–35. [From the finding of NF-κB to its regulation from cytoplasm to nucleus, the history and results of research is described, including that concerning the tight regulation of NF-κB by the formation of a complex with I-κB through phosphorylation followed by proteolysis.]

Vielle-Calzada, J-P.; Thomas, J.; Spillane, C.; Coluccio, A.; Hoeppner, M. A.; Grossniklaus, U. 1999. Maintenance of Genomic Imprinting at the *Arabidopsis Medea* Locus Requires Zygotic DDM1 Activity. *Genes and Development*, No. 13, pp. 2971–82. [*MEA* encodes the PcG protein that regulates cell proliferation by exerting gametophytic maternal control during seed development. Paternally inherited *MEA* alleles are transcriptionally silent in both young embryos and endosperm. Mutations at the decrease in DNA methylation (ddm1) locus are able to rescue *MEA* seeds by functionally reactivating paternally inherited *MEA* alleles during seed development. *DDM1* encodes a putative chromatin-remodeling factor. Chromatin structure is likely to be interrelated with genomic imprinting in *Arabidopsis*.]

Watson, S.; Arkinstall, S. (eds.) 1994. *The G-protein Linked Receptor; Facts Book*. London, San Diego, New York, Boston, Sydney, Tokyo and Toronto, Academic Press. [This book describes materials in three sections: i) superfamily of transmembrane receptor proteins; ii) superfamily of heterotrimeric G-proteins; and iii) G-protein linked effector and second messenger systems.]

Wei, N.; Deng, X.-W. 1999. Making Sense of the COP9 Signalosome. A Regulatory Protein Complex Conserved From *Arabidopsis* to Human. *Trends in Genetics*, No. 15, pp. 98–103. [The COP9 signalosome probably shares a common evolutionary ancestor. A multifaceted role for the COP9 signalosome in cell-signaling processes is implied by its associated novel kinase activity, as well as the involvement of its subunits in regulating multiple cell-signaling pathways and cell-cycle progression.]

Yang, J.-P.; Merin, J. P.; Nakano, T.; Kato, T.; Kitade, Y.; Okamoto, T. 1995. Inhibition of the DNA-Binding Activity of NF-κB by Gold Compounds *In Vitro*. *FEBS Letters*, No. 361, pp. 89–96. [The authors report that gold compounds, especially aurothioglucose (AuTG), have a strong inhibitory effect on NF-κB-DNA binding. Zn^{2+} is a necessary component of NF-κB for its DNA binding and that gold ion can block NF-κB-DNA binding efficiently, presumably through oxidation of the cysteins associated with zinc.]

Yoder, J. A.; Walsh, C. P.; Bestor, T. H. 1997. Cytosine Methylation and the Ecology of Intragenomic Parasites. *Trends in Genetics*, No. 13, pp. 335–40. [Most of the 5-methylcytosine in mammalian DNA resides in transposons, which are specialized intragenomic parasites that represent at least 35 percent of the genome. Suppression of parasitic sequence elements appears to be the primary function of cytosine methylation, with crucial secondary roles in allele-specific gene expression as seen in X-inactivation and

genomic imprinting.]

Yoshida, N.; Yanai, Y.; Chen, L.; Kato, Y.; Hiratsuka, J.; Miwa, T.; Sung, Z. R.; Takahashi, S. 2001. EMBRYONIC FLOWER2, a Novel Polycomb Group Protein Homolog, Mediates Shoot Development and Flowering in *Arabidopsis*. *Plant Cell*, No. 13, pp. 2471–81. [Mutations in *EMF1* and *EMF2* cause *Arabidopsis* to flower directly, bypassing vegetative shoot growth. *EMF2* encodes a zinc-finger protein similar to FERTILIZATION-INDEPENDENT SEED2 and VRN2.]

Ziemiecki, A.; Harpur, A. G.; Wilks, A. F. 1994. JAK Protein Tyrosine Kinases: Their Role in Cytokine Signalling. *Trends in Cell Biology*, No. 4, pp. 207–12. [Members of the JAK (Janus kinase) family of intracellular PTKs (Protein tyrosin kinases; JAK1, JAK2 and TYK2) are characterized by possession of a PTK-related domain and five additional homology domains, in addition to a classical PTK domain. Many cytokine receptors activate the PTK activity of JAK, and transduce the signal to STAT (signal transducers and activators of Janus kinase transcription).]

Biographical Sketch

Kohji Hasunuma graduated from the Faculty of Science, Department of Biology (Plant Science), Tokyo University, in 1966, and from the Graduate School of Biology (Plant Science) in 1971. He was research associate of the Faculty of Arts and Culture, Tokyo University, 1971–; Associate Professor at the National Institute for Basic Biology, 1979–90; and visiting researcher at the Carnegie Institution of Washington, Stanford, in 1990. Since 1990 he has been Professor at the Kihara Institute for Biological Research, Yokohama City University. The Hirase Prize was awarded to Prof. Hasunuma by the Japanese Society of Plant Morphology in 2000 for successfully demonstrating the molecular mechanism of light signal transduction in *Neurospora crassa* and *Pisum sativum*.