CLASSICAL TO MODERN GENETICS

Kohji Hasunuma and Naoto Yabe
Yokohama City University, Kihara Institute for Biological Research, Graduate School of Integrated Science, Yokohama, Japan

Keywords: *Agrobacterium tumefaciens, Aspergillus nidulans, Bacillus subtilis, dim-2* (defective in methylation), DNA transformation, heterothalism, homothalism, methylation induced premiotically (MIP), *Neurospora crassa*, parasexual (mitotic) genetic cycle, repeat induced point mutation (RIP), restriction fragment length polymorphism (RFLP), sporulation, Ti plasmid, T-DNA

Contents

Summary

The history and the research results obtained by the recent research by use of genetic approach were briefly summarized in the organisms, *Bacillus subtilis, Agrobacterium tumefaciens, Neurospora crassa, Aspergillus nidulans*, and *Lotus corniculatus L. var*. japonicus *Regal*, with special focus on *Neurospora crassa*, since the organism played important roles in the development of genetics and molecular biology. In *Bacillus subtilis* the molecular analysis of the process of sporulation is deseribed. In *Agrobacteium tumefaciens*, which forms crown gall in the plant, the nature of Tumer inducing (Ti) plasmid is reported. In *Neurospra crassa* history and relatively precise

research results of genetics and epigenetics are summarized. Not only classical genetics but the recent development of epigenetics induding repeat induced point mutation (RIP) mediated by the specific methylation of cytosine will provide new fields in the genetic analysis of higher eukaryote. *Aspergillus nidulans* shows specific parasexual (mitotic) genetic cycle, and Lotus corniculatus L. provided excellent systems to analyze the molecular mechanism of *Rhizobium*-Legume symbiosis

1. Introduction

During the development of the research on genetics and molecular biology, *Neurospora crassa* played very important roles. The process of development of early stages of research are well described in the preface of "The Neurospora Compendium, Chromosomal Loci, eds. Perkins, D. D. *et al.*, 2001." In it Dodge, B.O., who analyzed sexual cycle of *Neurospora crassa* in 1927, pioneered to establish the life cycle. He was very much impressed by the advantages for genetic analysis, and he tried to persuade Morgan, T.H. and his *Drosophila* group to adopt the organism. Morgan encouraged a graduate student, Lindegren, C. to examine potential nature of Neurospora. Ten years later the research results issued from Dodge, and Lindegren led Beadle, G.W. and Tatum, E.L. to adopt *Neurospora* in their reseach for nutritional mutants. The results endowed *Neurospora* to gain wide recognition, and to be the fungal counterpart of *Drosophila*. The work "Control of Biochemical Reactions in *Neurospora*" broke down the barriers that had separated biochemistry and genetics. The work of *Neurospora* went on to those in *Drosophila*, *maize*, and other higher eukaryotes. The *Neurospora* work was also expanded to other micro organisms, such as *Escherichia coil, Chlamidomonas, Aspergillus, Sordaria, Ustilago, Saccharomyces, Schizosaccharomyces, Coprinus, Podospora, Ascoboius,* and so on. In the present review the author would like to focus on *Neurospora crassa*.

2. Bacillus subtilis

Bacillus subtilis was characterized in 1876, which is Gram positive and grow in an aerobic condition. Under nutritionally rich condition it grew forming typical rod-shaped vegetative cells. However, under nutritionally deficient condition *B. subtilis* forms endospores.

At the beginning of 1960, it was recognized as a cellular differentiation system from vegetative cells to endospores, and provided excellent system to analyze the development of endospores by genetical methods.

Further it was discovered to be transformed by DNA, which supported the genetic analysis of sporulation.

2.1 Life cycle of *Bacillus subtilis*

As shown in Fig. 1, the life cycles of *Bacillus subtilis* are composed of two alternative growth phases, vegetative cell growth (Stage 0 and I) and entering into the process of sporulation (Stage, II~VII). Limitation for the source of carbon, nitrogen or phosphorous made the vegetative cells to entering into the developmental phase of sporulation.

The life cycle of *Bacillus subtilis*, showing the separate phases of vegetative growth and sporulation. The vegetative cycle is proliferative and comprises alternating round of growth by elongation and central division. On starvation, the sporulation cycle is chosen. It begins with a modified cell division, in which an asymmetric septum is produced. A series of intermediate morphological states then ensues, leading to the production of a mature spore. Completion of the septum is defined as *Stage II*. The edges of the septal disc, which comprises a pair of lipid bilayers, migrate towards the pole of the cell, engulfing the smaller cell, known now as the prespore, within the cytoplasm of the larger “mother cell”. During *Stage IV*, a layer of cell-wall-like material, known as the cortex is synthesised in the space between the membranes surrounding the prespore. In *Stage V*, a multilayered proteinaceous coat is deposited on the outside surface of the spore. During the final period of development, known as “maturation”, the extreme resistance and dormancy properties that typify the mature spore develop, with little over change in morphology. Finally, the completed spore is released by lysis of the mother cell (*Stage VII*). The cycle is completed by spore germination and outgrowth of a new vegetative cell, which occurs when conditions suitable for the resumption of growth recur.

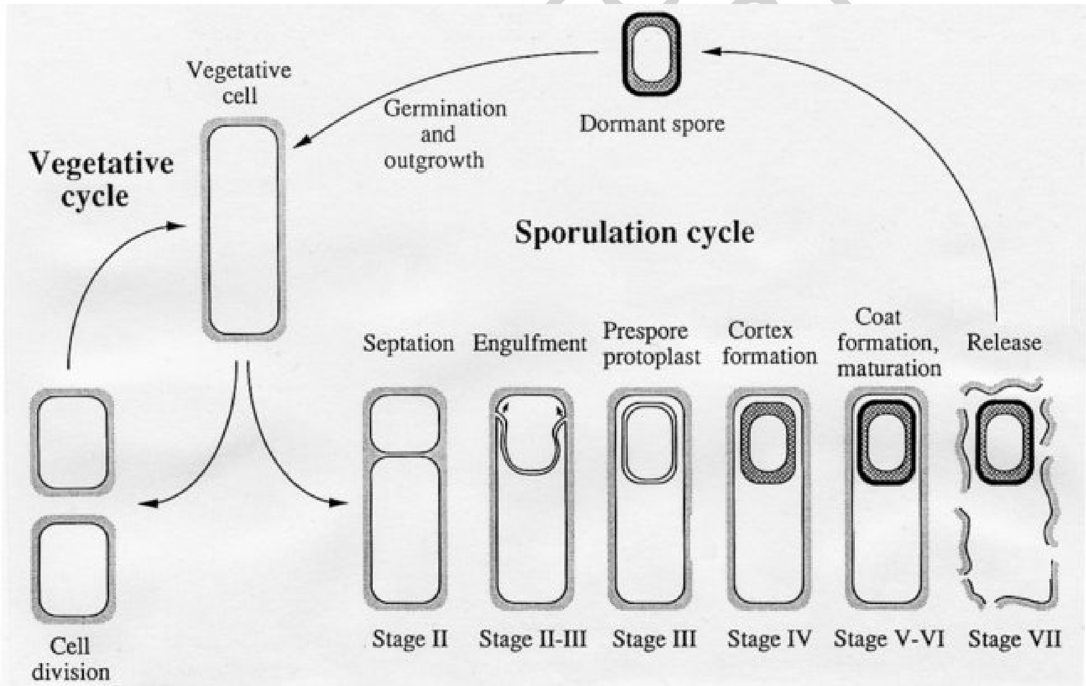

Figure1: The life cycle of *Bacillus subtilis* (Errington, J. 1992)

The processes of sporulation initiated on starvation are comprised of seven distinct morphological stages including 0, II, III, IV, V and VI as shown in Fig. 1. About 50 genes (*Spo*) regulating the sporulation are identified to drive the process.

3. Agrobacterium tumefaciens

As plant pathogens *Agrobacterium* was detected and isolated from soil. *Agrobacterium* infected to wounded plant deliver the plasmid DNA into host plants, where a part of plasmid T-DNA is integrated to the DNA of host plants. *Agrobacterium tumefaciens* causes crown gall on the infected stem of the plants. *Agrobacterium rhizogenes* infects to plants, and proliferates in the root inducing the roots. The former harbor large (~200 kb) plasmids called Ti for tumor-inducing and the latter harbor Ri for root-inducing. A part of Ti plasmid transferred into plants is integrated in plant chromosome, which was designated as T-DNA (transferred DNA). The T-DNA expressed in the plant causes over-production of or enhanced-sensitivity of plant hormones resulting in the formation of crown gall tumors or in the induction of roots, and causes the production of opines, which is catabolized by the inciting bacteria.

The processes of infection of *Agrobacterium* are as follows:

Wounded plants produce acetosylingone, which chemotactically attract bacteria. The bacteria attach to the plant cell, the process of which is controlled by the chromosomal genes, *att chvA*, *B* and *exoC* controlling virulence in bacteria. The acetosylingone stimulate sensor kinase, VirA, which phosphorylates response regulator, VirG constituting a two-component system. VirG transcriptionally activate *virB*, *virC*, *virD*, *virE* and *virG* on Ti plasmid by attaching to vir-boxes of hexanucleotide motives in the promoter regions of *vir* genes. The concentrations of acetosylingone to attract bacteria to plant is two orders of magnitude lower than those to induce *vir* genes.

The proteins VirD1 and VirD2 control the processing of the T-DNA. VirC1 protein controls the efficiency of T-DNA processing. VirE2 protein is a single stranded DNA binding protein, and VirEl protein was suggested to support the stability of Vir E2 protein. The *virB* operon is composed of 11 genes, the proteins corded by the operon are essential for virulence.

4. Neurospora crassa

A filamentous fungus *Neurospora crassa* is an ascomycete, and heterothalic microorganism. The organism was first described by Payen, A. (1843) in France. “One gene-one enzyme” hypothesis was provided after genetic and biochemical analysis of mutants in *Neurospora crassa* by Beadle, G. W. and Tatum, E. L. (1941). The process of gene conversion was also successfully analyzed in *Neurospora crassa* by Mitchel, M. B. (1955). The history of pioneering genetics to modern biology has been reviewed by Perkins, D. D. (1992).

-
-
-

Bibliography

Ainsworth, G.C.; Sussman, A.S. (eds.) 1965. *The fungi; An advanced treatise*, Academic press, Inc., New York.

Ashworth, J.M.; Dee, J. 1975. *The biology of slime moulds, The institute of biology's study in biology*, no. 56. Edward Arnold, London.

Beadle, G.W.; Tatum, E.L. 1941. Genetic control of chemical reactions in *Neurospora. Proc Natl. Acad. Sci. USA*. No. 27, pp. 499-506.

Beadle, G.W.; Tatum, E.L. 1945. Neurospora. 2. Methods of producing and detecting mutations concerned with nutritional requirements. *Am. J. Bot*. No. 32, pp. 678-86.

Brody, S. 1992. Circadian rhythms of *Neurospora*. In: Russo, V.E.A.; Brody, S.; Cove, D.; Ottolenghi, S. (eds.) *Development; The Molecular Genetic Approach*. pp. 165-176. Berlin, Springer-Verlag.

Catcheside, D.E.A. 1986. A restriction and modification model for the initiation and control of recombination in Neurospora. Genet. Res., No. 47, pp. 157-65. []

Clutterbuck, A.J.; Timberlake, W.E. 1992. Genetic regulation of sporulation in the fungus *Aspergillus nidulans*. In; Russo, V.E.A.; Brody, S.; Cove, D.; Ottolenghi, S. (eds.) *Development; The Molecular Genetic Approach*. pp.103-118. Berlin, Springer-Verlag.

Davis, R.H.; de Serres, F.J. 1970. Genetic and microbiological research techniques for *Neurospora crassa. Meth Enzymol.* No. 17A, pp. 79-143.

Davis, R.H. 2000 *Neurospora, Contributions of a Model Organism*, Oxford University Press, Inc, New York.

Downie, A.; Brewin, N. 1992. *The Rhizobium-Legume Symbiosis*. pp. 257-27. In; Russo, V.E.A.; Brody, S.; Cove, V.; Ottolenghi, S. (eds.) *Development, The molecular genetic approach*. Springer-Verlag. Berlin and Heidelberg.

Downie, A.; Brewin, N. 1992. The *Rizobium*-Legume symbiosis. pp.257-269.

Errington, J. 1993. *Bacillus subtilis* Sporulation: Regulation of gene expression and control of morphogenesis. *Microbiol. Rev*. 57, 1-33.

Errington, J. 1992. *Becillus subtilis* sporulation: a paradigm for the spatial and temporal control of gene expansion. pp. 28–43. In: Russo, V.E.A.; Brody, S.; Cove, D.; Ottolenghi, S. (eds.) *Development; The Molecular Genetic Approach*. pp. 165-176. Berlin, Springer-Verlag.

Hayes, W. (ed.) 1962. *The genetics of bacteria and their viruses; Studies in basic genetics and molecular biology* , ** pp. (Publisher's name, City) [This book aimed to introduce a comprehensively principles of genetics from higher organism to bacteria and their viruses to undergraduate and graduate student learning biology]

Hohn, B. 1992. Exploration of *Agrobacterium tumefaciens*. In: Russo, V.E.A.; Brody, S.; Cove, D.; Ottolenghi, S. (eds.) *Development; The Molecular Genetic Approach*. pp. 206-216. Berlin, Springer-Verlag.

Horowitz, N.H. 1985 The origins of molecular genetics: One gene, one enzyme. *BioEssays*, No.3, pp. 37-9.

Jacob, F.; Wollman, E.L. (ed.) 1961. *Sexuality and the genetics of bacteria*, Academic press, Inc., New York [The book aimed at the process of conjugation associated with the transfer of genetic materials]

Mylyk, O.M. 1976. Heteromorphism for heterokaryon incompatibioity genes in natural populations of *Neurospora crassa. Genetics*, No. 83, pp. 275-84.

Perkins, D.D.; Radford, A.; Sachs, M.S. (eds.) 2001. The Neurospora Compendium, Chromosomal Loci. San Francisco, Academic Press. [The historical accumulation of the work of *Neurospora crassa* is described focusing to the genetic analysis of various phenomena issued by *Neurospora crassa* and the closely related ecotypes.]

Pontecorvo G.; Roper J.A.; Hemmons L.M.; MacDonald K.D.; Bufton A.W.J. 1953. The genetics of

Aspergillus nidulans. Adv. Genet. 5, 141-238.

Russo, V.E.A.; Brody, S.; Cove, D.; Ottolenghi, S. 1992. In: *Development; The molecular genetic approach*, ** pp. Springer-Verlag, Berlin and Heidelberg. []

Russo, V.E.A; Pandit, N.N. 1992. Development in *Neurospora crassa*. In: Russo, V.E.A.; Brody, S.; Cove, D.; Ottolenghi, S. (eds.) *Development: The Molecular Genetic Approach*. pp. 88-101. Berlin, Springer-Verlag.

Russo, V.E.A.; Brody, S.; Cove, D.; Ottolenghi, S. (eds.) 1992. *Development; The Molecular Genetic Approach*. Berlin, Springer-Verlag. [From prokaryote to eukaryote the control of development of the organisms is described on the basis of genetic analysis.]

Sargent, M. L.; Briggs, W.R.; Woodward, D.O. 1966. Circadian nature of a rhythm expressed by an invertaseless strain of *Neurospora crassa. Plant Physiol.*, No. 41, pp.1343-9.

Biographical Sketches

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*.

Naoto Yabe, graduated from the Faculty of Science, Department of Biology (Plant Science), Tokyo University, in 1989, and from the Graduate School of Biology (Plant Science) in 1995. Since 1995 he has been research associate of the Kihara Institute for Biological Research, Yokohama City University.