宿主－病原菌関係の進化Ⅰ
Evolution of Host-Pathogen Relationships I
Evolution Prior to Development of Basic Pathogenicity

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Synopsis

Evolution of host-pathogen relationships prior to development of basic pathogenicity was discussed. Toxin production and development of cell wall degrading enzymes in potential pathogens are considered closely related to origin of pathogens. At first pathogens invaded in the hosts by intracellular hyphae. An increase in resistance in the hosts and decreases in pathogenicity in the pathogens brought a temporary equilibrium to some extent in the host-pathogen relationships. Later, an increase in resistance and increase in pathogenicity were alternatively repeated, leading to the formation of haustorium by inhibition of the elongation of intracellular hypha. Non-specific resistance in the host developed at least during the following periods: the formation of cell walls, landing of the plant, and standing-up of the plant by lignification and the formation of vascular bundles.

Key words: evolution, origin, host-pathogen relationship, pathogenicity, resistance, toxin

Introduction

Recent rapid advances of molecular genetics are making clear evolutionary change of host-pathogen relationships in a level of DNA’s and proteins. Here, we will propose some new hypotheses on evolution of host-pathogen relation-

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ships with brief general review on this problem in the light of advances of molecular genetics.

**Origin of pathogens**

At present, the Endosymbiotic theory of origin and evolution of eukaryotic cells is generally believed. According to this theory, eukaryotic cells have nuclei containing most of the cells' DNA, packaged as chromosomes. They also generally contain many, even hundreds, of mitochondria. Plant and algal cells contain not only the same organelles that fungus and animal cells contain, but also one, several or even thousands of plastids. Eukaryotic cells are considered to have originated as communities of cooperating entities that had joined together in a definite order; with time, the members of the cooperative, already skilled in their specialities, became organelles. This particular story of the separate origin and development of the parts of eukaryotic cells and their coming together to form the whole has been dubbed by F.J.R. Taylor the Serial Endosymbiotic Theory (Margulis 1981).

The acquirement of photosynthesis by eukaryotes, occurred during and after the establishment of the fundamental eukaryotic organization by eukaryote+photosynthetic prokaryotes. The acquirement of plastids occurred at least several times and involved several distinct species of partners. Hence, the functions of photosynthesis now performed by cell organelles are thought to have evolved long before the eukaryotic cell itself existed (Margulis 1981).

The least accepted—most original and most questionable aspect of the Serial Endosymbiotic Theory is its hypothesis of the origin of undulipodia from spirochete bacteria. Spirochetes are thought to have attached to the nucleocytoplasm-mitochondria consortium for feeding purposes. Natural selection transformed spirochete-host symbioses into highly motile complexes—ancestors of today's mastigotes. This transformation was followed by morphogenesis, the evocation of form, in the development of impressive cellular asymmetries (Margulis 1981).

It has been considered that symbiosis is a state of the most evolved parasites. From these points of view, it is thought that origin of pathogen or parasitism must go back before origin of eukaryotes. However, we will discuss only on the origin of plant pathogen and so on origin of host-pathogen relationship in higher plant in the present paper.

As Heath (1987b) mentioned, one of the problems of discussing the evolution of resistance or susceptibility of plants towards fungal plant parasites is deciding whether to begin with a basically resistant or a basically susceptible plant. Many investigators including Heath (1987a, b) and Verderovsky (1959) considered that basic resistance is original one.

Original basic resistance is, however, considered to occur in prokaryotes,
long before origin of eukaryotes. Then the basic resistance may be broken-down at the origin of pathogens. Later, basic resistance described in recent papers may occur in host plants. At least some changes from susceptibility to resistance may occur at the origin of cell wall in an ancestral plant, and landing and standing-up over ground of higher plants. Formation of cell wall should play an important role to protect ancestral plants from diseases. For landing, the dry conditions on the land should require drought resistance in plants and especially in pathogens. Furthermore, standing-up of plants over ground by production of lignin and development of vascular bundle system should require strong drought resistance especially to pathogens. Dryness over land at least induced a change from soil-borne or water-borne type to air-borne type spores (Kiyosawa and Nomura 1988, 1989); in other words, induced evolution of Ascomycotina and then Basidiomycotina. These mean that general and high resistance (basic resistance) may occur at least three, probably over three times during evolution of higher plants. Therefore, it is reasonable to begin discussion on the evolution of host-pathogen relationship from the general existence of basic resistance.

1. Role of toxin

Verderevsky published many valuable opinions on the evolution of host-pathogen relationship in 1959, although his book has hardly been cited by investigators in foreign countries. Among them, the following three are especially noted and easily acceptable: 1) the first plant was resistant, 2) the first pathogenicity was induced by a toxin which was produced in a potential pathogen, and 3) general resistance occurred earlier than specific resistance.

Toxin may play an important role for acquirement of pathogenicity in a potential pathogen. In this case, the host-pathogen relationship (RR/RS) shown in Table 1 differs from the type of gene-for-gene relationship (RS/SS) in Table 2. When microbe touching to a plant acquired a genetic ability to produce toxin to plant, the toxin killed the plant cells at touching site, and this permitted invasion of the microbe into the plant. As Gabriel (1989) pointed out, epiphytic microbes might have chance more than non-epiphytic microbes to attack plants by production of the toxin.

Pathogens that once acquired pathogenicity strengthened further pathogenicity by increase of production of the same toxin or by qualitative change of toxin for a while (occurrence of necrotrophs). Such strengthening of pathogenicity, however, led to loss of the host and then often led to loss of pathogens themselves. In some cases, pathogens required some mechanism to protect themselves from high toxicity, as some present pathogens have ability of detoxification (Kumada et al. 1988).

The phytotoxin produced by Pseudomonas syringae, coronatine, and growth
Table 1. Host-pathogen relationship of *Helminthosporium* type.

<table>
<thead>
<tr>
<th>Host/Pathogen</th>
<th>Avirulent</th>
<th>Virulent</th>
</tr>
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<tbody>
<tr>
<td>Resistant</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Susceptible</td>
<td>R</td>
<td>S</td>
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Table 2. Host-pathogen relationship of gene-for-gene type.

<table>
<thead>
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<td>S</td>
<td>S</td>
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![Chemical structures]

Coronatine: $R_1 = -\text{NH}$
Jasmonic acid: $R_2 = \text{OH}$
Coronafacic acid: $R_1 = \text{OH}$
Methyljasmonate: $R_2 = \text{OCH}_3$

Fig. 1. Structure of coronatine, coronafacic acid, jasmonic acid and methyl jasmonate (MeJA).
(Feys et al. 1994)

regulator methyl jasmonate (MeJA) caused similar growth-inhibiting effect on *Arabidopsis* seedlings. Fourteen independent mutants were insensitive to coronatine and also MeJA, and were male sterile. This insensitivity is controlled by a single recessive gene, *coil* (coronatine-insensitive). Coronatine and MeJA have similar structure (Fig. 1) (Feys et al. 1994). This suggests that sensitivity of *Arabidopsis* to coronatine is controlled by a dominant gene which is very important gene conferring fertility of the plant. Similar phenomenon has been reported already in maize. Four of male sterile designations of maize were susceptible to *Helminthosporium maydis* (Smith et al. 1971) and some single
cross was susceptible to *Phyllosticta* spp. (yellow leaf blight) (Ayers et al. 1970).

2. Role of enzymes

Following toxin production, several enzymes came to play an important rôle in penetration to hosts in some pathogens. Pectic enzymes and cutinase are the most studied enzymes.

Aspects of pectic enzyme production have been discussed by Collmer et al. (1991) for three dissimilar plant pathogens. *Agrobacterium tumefaciens* biovar 3 produces polygalacturonase and causes both crown gall and root decay of grape. *Pseudomonas syringae* pv. *lachrymans* causes angular leaf spot and fruit rot of cucumber. A mutant derivative of the soft-rot pathogen *Erwinia chrysanthemi*, containing site-directed mutations in genes encoding all of the known extracellular pectic enzymes but still able to macerate plant tissues, was found to produce several novel pectate lyases in planta.

On pectic enzymes, in the past decade it was demonstrated that highly purified pectic enzymes could macerate and kill plant tissues in a manner similar to that occurring in soft-rot diseases; that the same enzymes could be used to disassemble the primary cell wall of dicots, thus revealing the structural importance of pectic polymers in cell-wall architecture; and that a relationship exists between the enzymatic vulnerability of pectic polymers in the cell wall and the regulation of pectic enzyme synthesis in pathogenic fungi (Collmer and Keen 1986).

The *Erwinia chrysanthemi* has five *pel* genes, arranged in two clusters. One cluster contains the *pelB* and *pelC* genes, which include the neutral isozymes; the other contains the *pelA*, *pelD*, and *pelE* genes, which encode the acidic and alkaline isozymes. Homologies were found between *pelB* and *pelE* and between *pelB* and *pelC*, but homology between *pelC* and other *pel* genes has less than 75%. These observations suggest that the *pel* genes encoding the two neutral isozymes arose by duplication of an ancestral *pel* gene. Recently, one of the *pel* genes from *Erwinia carotovora* strain EC has considerable homology with the *pelB* gene from *E. chrysanthemi* EC16. It was suggested that the *E. carotovora* pel gene and the *E. chrysanthemi* *pelB* and *pelC* genes are derived from a common ancestral gene. The gene order of *pel* genes in *E. chrysanthemi* was determined as follows: met-ile-cya-(*pelB*, *pelC*)-(*pelA*, *pelD*, *pelE*)-purE. The *pel* genes in *Erwinia carotovora* appear to be arranged differently than those in *E. chrysanthemi* (Collmer and Keen 1986). On the other hand, the importance of cutinase in pathogenicity was demonstrated by the following investigations. The following organisms have been found to produce extracellular cutinase activity: *Botrytis squamosa*, *Cladosporium cucumerinum*, *Colletotrichum graminicola*, *Gloeocercospora sorghi*, *Helminthosporium carbonum*, *H. maydis* (race T), *Pythium aphanidermatum*, *P. arrhenomanes*, *P. ultimum*,
Rhizobium solani, Stemphylium loti and Sclerotium rolfsii (Baker and Bateman 1978).

Mycosphaerella sp., which can infect papaya fruits only when the cuticular barrier is mechanically breached, infected fruits with intact cuticle when the surface was first treated with purified cutinase from C. gloeosporioides (Dickman et al. 1982). On intact pea stems Fusarium solani f. sp. pisi isolate T-30 became infective only when the spore suspension placed on the surface was supplemented with exogenous cutinase together with cellulase, pectinase, and pectin methyl esterase, whereas supplementation by the individual enzymes was not effective (Köller et al. 1982).

3. Saprophyte, necrophyte and biophyte

Filamentous fungi involving many pathogens, Zygomycotina and Ascomycotina include many saprotrophic and necrotrophic species. In these families, it is naturally considered that saprotrophy and necrotrophy preceded biotrophy. Even in Basidiomycotina and Ectomycorrhiza in which there are only a small number of saprotrophic or necrotrophic species in plant pathogens, it has been considered that biotrophy was derived from the saprotrophic/necrotrophic direction (Lewis 1987). Generally, biotrophy has been seen as a more advanced trait than necrotrophy (Lewis 1974). This suggests that biotrophic or necrotrophic pathogens are independently derived from saprotrophic bacteria and fungi in these sub-phyla.

Time of evolution of host-pathogen relationship

On the time when new type evolved, Savile (1968) mentioned as follows: new groups do not spring from climax groups, but always from less specialized, genetically plastic groups, and generally diverge early in the principal lineage. This fundamental principle is repeatedly ignored by mycologists.

Such an explanation assumes that new pathogenic relationships are primarily established when the host is undergoing rapid evolution (Savile 1955, 1971). Although there is no inherent reason why such plants should have less effective defence mechanisms than members of climax groups, it is conceivable that during a time of great genetic diversity, slight variations in these defence mechanisms may favour the invasion by certain fungi that fortuitously have the ability to overcome variant forms of resistance (Savile 1971). Although Heath (1987a) mentioned no inherent reason why rapidly evolving plants had less effective defence mechanisms, it is considered that rapid specialization of plant to several new types accompanied the cost. For example, it is thought that Agrobacterium genus which attacks only dicots and almost all genera of dicotyledons occurs at initial stage of the occurrence of dicots.

During the 120 million years of the Devonian and Carboniferous, vascular
plants evolved in several directions by increase of size, to about 40 m, and by becoming woody (Raven 1986). White rot basidiomycetes degrade lignin most rapidly. As Dennis (1970) demonstrated clamp connections on hyphae in wood of a Carboniferous fern, these basidiomycetes were thought to have originated in the Carboniferous itself (Pirozynski 1976). However, Stubblefield et al. (1985) have now provided convincing evidence for the existence of wood-inhabiting fungi, likely to be basidiomycetes, in the late Devonian, so appropriate organisms for rapid decay of wood existed at the start of the Carboniferous. Considering also on the availability of oxygen, it is clear that basidiomycetes capable of destroying wood either as saprotrophs or necrotrophs were present at least by the start of the Carboniferous (Lewis 1987). From data on host evolution it has been estimated that the Agrobacterium-Rhizobium divergence occurred some 250 Mio years ago (Ochman and Wilson 1987).

An original type (monokaryon) of Basidiomycotina would occur at the stage of occurrence of the most primitive host of Basidiomycotina, and then a dikaryon type would originated at the occurrence of dicots. Thus, mycelium in its monokaryon stage elongates intracellularly and its dikaryon phase attacks the host intercellularly, as mentioned above.

It is often suggested that the antiquity of the host reflects that of the pathogens (Savile 1955), in which case the lack of pathogens on cycads and Ginkgo may merely reflect the paucity of potential pathogen available when these groups evolved.

As Denison and Carroll (1966) pointed out, walled heterotrophs with an invasive mycelium, such as the fungi, would have an advantage over bacteria in their ability to gain quick access to nutrients buried within the dead plant body. In such an environment, a diverse array of saprotrophic mycelial organisms could have arisen (possibly from free-living autotrophic progenitors) to provide the ancestors of the modern terrestrial fungi (Heath 1987a).

Foliar pathogens were apparently not abundant until the Cretaceous period (Pirozynski 1976). One explanation for this observation could be that, at least for the Ascomycotina (Pirozynski 1976), air-borne spores were not initially abundant and most species lacked a reliable means, other than systemic infection, by which the mycelium could easily reach the foliage. Alternatively, the leaves of the vascular plants evolving at the beginning of the Cretaceous period may have had less effective defence mechanisms than previously existing relatives (Heath 1987a).

**Development of resistance**

Plant naturally opposed increase of pathogenicity in the pathogen during the development of basic pathogenicity by various methods including production of enzymes detoxifying the toxin. At least three ways were taken to resist
the pathogenicity. One was to degrade the toxin itself. The second was to protect the plant from cell death by toxin. The third was to inhibit elongation of intracellular hypha of the pathogen.

In the first case, detoxification or degradation of fusaric acid was found in tomato, *Gossypium herbacearum*, and *Pisum sativum* (Patil 1980). Also in *Pseudomonas phaseolicola*, results suggesting contribution of detoxification of a toxin, phaseolotoxin, to host resistance were obtained (Patil 1980). The second case resulted in the resistance in the type of rice-*Pyricularia oryzae* (rice blast) which shows intracellular growth of the hypha without cell death at least immediately after penetration. When the latter was combined with the former, intracellular hypha shortened in living host cell and changed to various types of haustoria, with intercellular growth of hypha in some pathogens (*Puccinia* spp.) and with growth of the hypha on surface of leaves in other pathogens (*Erysiphe* spp.). Thus, biotrophs occurred (Kiyosawa and Nomura 1988, 1989). Intermediate state of haustorium development is found in *Phytophthora magasperma* and *Puccinia* spp. In the former, germ tubes of zoospores of this fungus penetrate soybean root tissues and form haustorium-like bodies. Its hyphae intercellularly and intracellularly elongated in the host tissue (Ho 1969, Klarman and Corbett 1974, Slusher et al. 1974). In the latter, haustorium-like body in monokaryotic stage must be rather called intracellular hyphae (Rijkenberg and Truter 1973, Al-Khesraji and Lösel 1980). Furthermore, it has been found that dikaryon of smut fungus cannot survive on nutrient media, and dies or continues growth only after dissociation to monokaryotic haploid component (Nielson 1968). Such evolutions from saprotrophs through necrotrophs to biotrophs are generally recognized (Lewis 1974, Cooke 1977, Cooke and Whipps 1987, Heath 1987a).

When such a change occurred at the time of differentiation of sub-phyla, genera and species, sub-phylum-, genus- and species-specific resistance were evolved, respectively. Generally these types of resistance belong to general resistance in a present sense. Pathogen itself might weaken its pathogenicity: many negatively acting genes are known (Kondorosi et al. 1989).

Microscopically, pathogen that acquired pathogenicity by toxin production elongate intracellularly its hypha in killed cells of the host plant, as present necrotrophic fungi, for example, *Helminthosporium maydis* (Kono et al. 1980). In *Alternaria alternata*, three pathotypes, pear, apple and strawberry, produce different toxins (AK-toxin, AM-toxin and Al-toxin), respectively (Nishimura and Koh moto 1983).

In present bacteria, more complicated systems on toxin production develop: for example, in *Pseudomonas* toxins, phaseolotoxin, tabtoxin or syringomycins, are produced by one or more genes, which is located in plasmid in some strains or in chromosome in other strains. The frequent integration and imprecise
excision of plasmids into the chromosome of \textit{P. syringae} pv. phaseolicola, due to recombination at a homologous repetitive sequences (Szabo and Mills 1984), suggests that plasmids confer plasticity to strains (Gross 1991). Genes required for the synthesis of syringomycin (syringotoxin), coronatine, tabtoxin and phaseolotoxin form clusters (Gross 1991).

\textit{Puccinia} spp. shows intracellular elongation of the hypha in monokaryotic stage and intercellular elongation in dikaryotic stage (Al-Khesraji and Lösel 1980, Rijkenberg and Truter 1973). It seems to support the concept “Ontogeny generally recapitulates phylogeny” (Savile 1968).

**Discussion**

For a plant, the number of nonpathogens is in an overwhelming majority as compared with the number of pathogens, and for a pathogen the number of nonhosts is in an overwhelming majority as compared with that of hosts. This indicates that nonhosts (resistance) and nonpathogens (non-pathogenicity) are general, and hosts (susceptibility) and pathogens (pathogenicity) are exceptions. These factors are more easily explained by considering that a small number of nonpathogens became pathogens or a small number of nonhosts became hosts than a large number of hosts became nonhosts. This is also a reason why it has generally been considered that resistance or non-pathogenicity preceeded susceptibility or pathogenicity.

Toxin production in potential pathogens is considered closely related to origin of pathogens. After the beginning of the host-pathogen relationship, an increase in the amount of toxin production or improvement in quality of the toxins increased the pathogenicity of the pathogens during an evolutionarily short period. However, the increase of pathogenicity in primary pathogens lead to a rapid decrease in the hosts and therefore to the death of the pathogens. Later, increase in resistance in the hosts and probably decreases in pathogenicity in the pathogens brought a temporary equilibrium to some extent in the host-pathogen relationship. Later, an increase in resistance and increase in pathogenicity repeatedly alternated, leading to the formation of haustorium by inhibition of the elongation of intracellular hypha. Growth of hypha in the host changed from intracellular growth to intercellular growth with formation of haustorium.

**Summary**

It is considered that the evolution of the host-pathogen relationship came through as following process. 1) Invasion of a microbe into the plant through a wound or a weaken plant. 2) Development of intracellular growth of the microbe by acquirement of pathogenicity through production of a toxin. 3) Increase of pathogenicity by an increase of toxin production or an improvement
in quality of toxins and/or development of degrading enzymes in the pathogen and a decrease in resistance in the host by it. 4) Formation of a haustorium by an increase in resistance in the host and/or a decrease in pathogenicity in a pathogen.

Non-specific resistance in the host developed at least during the following periods: the formation of cell walls, landing of the plant, and standing-up of the plant by lignification and the formation of vascular bundles.

References


寄生性の進化は原核生物から始まった。初めての真核生物は微生物に対して抵抗性であり、その後植物の進化にともなって病原菌の進化、植物側の新しい抵抗性の獲得を繰り返しながら共進化を続けたものと考えられる。少なくとも細胞壁の形成、リグニンの生成、地上での直立が可能になった時期に非特異的真性抵抗性が生じたものと考えられる。

最初の寄生性は潜在の病原菌が毒素生成能力を獲得し、宿主細胞を殺すことにより生じた。この毒素生成能力の向上とともに宿主の過剰な死により病原菌はかえってその生存を脅かされることになったが、宿主の抵抗性の増加ときには病原菌の中での抑制作用の発達により侵入菌系の生長は抑制され、初めの死細胞への細胞内伸長は抑制され最後には細胞内に吸器として留まり、菌糸は細胞間あるいは表皮上を走るようになった。

付着器は最初は根の上で形成され、感染根の形をしていたがやがて一つの菌糸で付着する能力を獲得し、単一の菌糸で付着するようになった。

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