Transcriptional Control of the *Agrobacterium* Virulence Genes by Two Proteins VirA and VirG

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The *Agrobacterium* virulence (*vir*) genes that are essential for pathogenicity on plants are induced through the sensor protein VirA and the transcription factor VirG by phenolic compounds released from plant wounded sites. We have delineated the molecular mechanism of this VirA-VirG signal transduction system by elucidating biochemical characteristics of these proteins.

Keywords: Two-component regulatory system/ DNA-binding protein/ Phosphotransfer/ Plant-microbe interaction/ Virulence regulon

The hairy-root-inducing plasmids (pRi) confers tumorigenic symptoms at wound sites on a wide variety of dicotyledonous plants upon infection by its host bacterium, *Agrobacterium rhizogenes*. Tumorigenesis by pRi is caused by the transfer of a defined DNA segment (T-DNA) from the plasmid into the plant nuclear genome and the subsequent constitutive production of plant phytohormones directed by the T-DNA. The 25-base-pair imperfect direct repeats at both extremities of T-DNA are indispensable for T-DNA transfer, but no other portions inside T-DNA are required. Plasmid genes essential for T-DNA transfer are located in the virulence (*vir*) loci outside T-DNA. The plasmid *vir* genes (about 20 genes) constitute six transcriptional units, *virA*, *virB*, *virC*, *virD*, *virE*, and *virG*. Their expression is tightly regulated as a regulon by the *virA* and *virG* gene products, being inducible by plant phenolic compounds such as acetosyringone [1]. Here we describe biochemical characteristics of the VirA (829 amino acid residues) and VirG (241 amino acid residues) proteins, and delineate molecular mechanisms of transcriptional activation of the *vir* genes by plant factors.

For plant factors to signal *vir* expression, extracellular recognition is required. This process appears to be mediated by VirA because its N-terminal half contains the periplasmic portion (220 residues) flanked by the two membrane-spanning regions. The C-terminal domain of VirA is well conserved among the sensor components of every two-component regulatory system. However, unlike other sensor components, VirA has an additional domain at the most C-terminal end (115 residues). This portion is called VirG-like domain (VGL domain) because of its resemblance to the N-terminal half of VirG. Deletion and point mutants within the VGL domain show poorly inducible expression of the *vir* genes by acetosyringone, and generate no or reduced tumorigenic symptoms on plants.

To characterize VirA biochemically, N-truncated versions of VirA (VirA') have been overproduced in

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Scope of research

Attempts have been made to elucidate structure-function relationships of genetic materials and various gene products. The major subjects are mechanisms involved in regulation of gene expression, initiation of DNA replication, signal transduction responsive to environmental stimuli, morphogenesis of plant leaves and flowers, and plant-microbe interaction. As of December 1994, study is being concentrated on signal transduction for plant morphogenesis, and identification of protein kinases and phosphoprotein phosphatases essential for plant signal transduction.

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Escherichia coli. These VirA′ derivatives are capable of phosphorylating their own His-474 residue in the presence of ATP provided their deletions do not extend beyond His-474. Furthermore, VirA′ molecules with larger N-truncations are generally higher autophosphorylating activities [1]. Substitution of Gln for His-474 abolishes autophosphorylation function, the ability to induce *vir* expression, and pathogenicity on plants, indicating autophosphorylation as being essential in signal transduction for transcriptional activation. Based on these facts and other several lines of circumstantial evidence, we believe that the active center of VirA kinase is usually masked by its N-terminal half, and upon recognition of plant factors, becomes unmasked for activation.

When the VirG protein that has been purified from overproducing *E. coli* cells is mixed with VirA′ in the presence of ATP, VirG together with VirA′ is phosphorylated. The phosphate group of phospho-VirG comes from phospho-VirA′ but not ATP because VirG is similarly phosphorylated by the purified phospho-VirA′, concurrently with dephosphorylation of phospho-VirA′. The Asp-52 residue of VirG has been found to be the phosphorylation target [1]. Thus, the N-terminal half of VirG is the signal receiver domain. Site-directed mutagenesis toward Asp-52 leads to an extreme reduction of pathogenicity. These facts support the view that phosphotransfer is an important process involved in signal transduction required for transcriptional activation.

The VGL domain of VirA resembles the VirG signal receiver domain as described above. In particular, three residues (Asp-9, Asp-52, and Lys-102) critical for acquiring the phosphate group are completely conserved. This VGL domain does not contribute to the enzymatic activity of autophosphorylation but considerably enhances phosphotransfer from VirA′ to VirG *in vitro* [2]. Therefore, VirA-VirG interaction appears to occur through two homologous regions, namely the VGL domain of VirA and the N-terminal signal receiver domain of VirG, presumably mimicking the oligomerization process of VirG molecules [3]. Although this modest decrease in phosphotransfer activity *in vitro* does not seem to account for the drastic phenotype alteration *in vivo*, this contradiction is probably derived from the difference of VirA molecules tested *in vivo* and *in vitro*: the former VirA is the native membrane-anchored protein (VirA), while the latter VirA is the N-truncated cytoplasmic proteins (VirA′).

To confirm that VirA and VirG are sufficient for inducible *vir* expression, we have constructed an *in vitro* transcription system, consisting of the *Agrobacterium* RNA polymerase, VirG, and template DNAs containing the *vir* promoter regions. In this system, RNA is synthesized entirely depending on VirG, and its start site is exactly identical to that of *in vivo* mRNA. VirG works as a positive transcription factor but not as an alternative sigma factor because the RNA polymerase core enzyme is unable to replace the holoenzyme in this system [4].

This transcription system has been coupled with the phosphotransfer reaction from VirA′ to VirG. Under neutral conditions for transcription (pH 7.3–7.7), coupling shows no effects or slight reduction of the VirG-dependent transcription activity. Under moderate acidic conditions for transcription (pH 6.5–7.0), VirG-dependent RNA synthesis is significantly enhanced by the coupling. Since phosphotransfer reactions are done in the same conditions, it is obvious that VirG has enough potential for transcriptional activation without being phosphorylated and that phospho-VirG promotes transcriptional activation more competently than nonphospho-VirG in the rather acidic conditions. This peculiar pH-dependency may relate to the fact that *vir* induction by plant factors *in vivo* occurs only under acidic conditions. Since phosphotransfer from VirA to VirG appears critical for *vir* expression *in vivo*, functional nonphospho-VirG *in vitro* seems to be interpreted as that VirG phosphorylation is essential for transcriptional activation when the concentration of VirG is low, and function of phospho-VirG can be compensated by an excess of nonphospho-VirG.

There is a point mutant of VirG (VirG I77V), containing a substitution of Val for Ile-77. Its phenotype is hypersensitive *vir* induction by plant factors. Phosphotransfer from VirA′ to VirG I77V is considerably higher than to the wild-type VirG. Thus, its phenotype has been attributed to an elevated affinity of VirG I77V to VirA [5]. Another mutant (VirG N54D) carrying an amino acid substitution near the phosphorylation target, Asp-52, shows constitutive *vir* expression *in vivo*, independent of both plant factors and VirA. In comparison to the wild-type VirG, VirG N54D has a higher activity for transcriptional activation *in vitro*, but has comparable activity for phosphotransfer from VirA′. In addition, no enhancement in RNA production has occurred by phosphorylation of VirG N54D. Therefore, VirG N54D is likely to have a molecular conformation close to the phosphorylated from of the wild-type VirG [5]. All of these experimental results obtained *in vitro* correctly reflect *in vivo* phenomena of *vir* expression induced by plant factors.

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