

Theses

of the PhD dissertation

Regulation of nopaline uptake in
Agrobacterium tumefaciens

Dr Ferenc Marincs

Agricultural Biotechnology Center

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Introduction

Cellular processes are regulated by different mechanisms at specific levels. The first of these levels is the regulation of transcription, which ensures the appropriate temporal and spatial presence of gene products for cellular processes. Regulation of transcription is mediated by DNA binding proteins, which can be either repressors or activators, depending on their effect on gene expression. The interaction of regulatory proteins with special DNA sequences called operators results in repression or activation of the regulated promoters and their driven genes.

The position of the operator sequence relative to the regulated promoter is a very important factor in terms of the mechanism by which a regulatory protein mediates gene expression. Repressor proteins usually bind to the regulated promoter, thus physically preventing initiation of transcription by the RNA polymerase. In contrast, activator proteins usually bind outside of the regulated promoter, thus they can interact with RNA polymerase to promote transcription. There are regulatory proteins

which have a dual function, and regulate gene expression both as a repressor and an activator.

The interaction of regulatory proteins with operator sequences may be influenced by smaller signal molecules (also called inducers, co-repressors or co-activators) which by interacting with the protein modulate the protein-DNA interaction, thus affecting gene expression. The effects of these molecules on the regulatory protein-DNA interaction can be various. They either affect the affinity of the regulatory protein for its target DNA sequence, i.e. the regulatory protein does or does not bind to its target sequence in either the presence or absence of the appropriate molecules. This type of modulation is common amongst repressor and activator proteins. In the case of regulatory proteins with dual repressor-activator function, the proteins usually bind to the operator both in the presence and absence of the small signal molecule, which however either influences the strength of binding or causes a conformational change in the protein-DNA complex. Either effect can act as a switch between the repressed and activated states.

In this thesis I describe the investigation of a dual function repressor-activator protein and its role in the regulation of gene expression in the nopaline catabolism operon of *Agrobacterium tumefaciens*.

A. tumefaciens is a soil-born plant pathogen bacterium. Virulent strains of the bacterium harbour a large, so called tumour-inducible (Ti) plasmid, and cause tumour formation on diverse plants. After infecting a wounded site in response to plant exudates, which also induce the virulence operon genes of the Ti plasmid, a particular portion of the Ti plasmid, called the T-DNA, is transferred into the nucleus of the plant cells by the products of the virulence genes and integrated into the plants genome. Following this natural transformation of the plant cells, expression of several genes of the integrated T-DNA results in overproduction of plant growth hormones, which cause the uncontrolled proliferation of the transformed cells leading to tumour formation.

Naturally, the formation of the tumour is not the aim, but only a tool for the bacterium in this process. By inducing

tumour formation in the plant, the bacteria create a niche for their survival and reproduction. Namely, the integrated T-DNA contains genes which are expressed inside the plant genome and direct the synthesis of unique chemicals. These molecules, collectively called opines, are organic acid and amino acid, or sugar and amino acid conjugates and can serve as sole nitrogen and carbon source for the tumour-inducing bacteria. In that relatively nutrient-rich environment, bacteria can divide and conjugate their Ti plasmids into other bacterial cells. The opine catabolism genes are also part of the Ti plasmids, but they are located outside the T-DNA, and therefore are not transferred into the plant genome. Their expression is induced by that particular opine which is produced by the tumour induced by the inciting bacteria. Nopaline is such an opine synthesised in tumours induced by *Agrobacterium* strains harbouring a nopaline-type Ti plasmid, for example pTiT37. My aim during this work was to investigate the regulation of gene expression in the nopaline catabolism operon of the Ti plasmid pTiT37 of *A. tumefaciens*.

The work was done at the research institute DSIR/AgResearch, Palmerston North, New Zealand in the period of 1989 to 1995, and was published in four first authorship publications as listed at the end of the theses.

Materials and methods

During my work, I used various molecular biology approaches and techniques.

Construction of plasmids involved isolation of nucleic acid, cloning, site-directed mutagenesis, sequencing, PCR and building reporter-gene fusions, which were either performed using standard methodologies or are described in the published papers.

Spectrophotometry, agarose gel electrophoresis, topoisomer agarose gel electrophoresis, denaturing acrylamide protein electrophoresis and enzyme assays have been employed for nucleic acid and protein analysis as described in the published papers.

Protein-DNA interactions were studied by gel retardation assay and DNaseI footprinting.

Results

- The *nocR* gene of the pTit37 plasmid was cloned, sequenced and expressed in *E. coli*.
- Sequence analysis revealed that the NocR protein is a helix-turn-helix DNA-binding protein and belongs to the LysR family of transcriptional activators.
- Using gel-retardation and DNaseI footprinting the binding site of the NocR protein was localised to the *nocR* promoter in the divergently transcribed *nocR-nocB* promoter region, in a remote position relative to the *nocB* promoter.
- Sequence analysis of the binding site revealed a CATGN₄CATG tandem palindrome sequence as the operator for NocR and that the operator overlaps an 18 bp alternating purine-pyrimidine sequence and a putative binding-site for the gyrase protein.
- The *nocR* promoter was mutagenised by site-directed mutagenesis to remove the operator sequence.
- Using gel-retardation it was demonstrated that the operator is necessary for binding of the NocR protein.

- Using *gusA* and *luc* reporter gene fusions it was shown that the NocR protein autoregulates its own synthesis and regulates expression of the divergently transcribed *nocB* gene.
- Using the same gene fusions it was shown that the absence of the operator and transcription of the *nocR* gene itself influence expression of the *nocB* gene, even in the absence of the NocR protein.
- Topoisomer assay revealed that absence of the operator sequence influences plasmid supercoiling.
- A novel model for remote transcriptional control, based on B- to Z-DNA transition and changes in local supercoiling, was developed.

**This PhD dissertation is based on the following
refereed publications:**

Marincs, F. and White, D.W.R. (1993): Nopaline causes a conformational change in the *NocR* regulatory protein-*nocR* promoter complex of *Agrobacterium tumefaciens* Ti plasmid pTiT37. *Mol. Gen. Genet.* 241:65-72.

Marincs, F.* and White, D.W.R. (1994): The *NocR* repressor-activator protein regulates expression of the *nocB* and *nocR* genes of *Agrobacterium tumefaciens*. *Mol. Gen. Genet.* 244:367-373.

Marincs, F.* and White, D.W.R. (1995): Divergent transcription and a remote operator play role in control of expression of a nopaline catabolism promoter in *Agrobacterium tumefaciens*. *J. Biol. Chem.* 270: 12339-12342.

Marincs, F.* and White, D.W.R. (1996): Regulation of gene expression at a distance: the hypothetical role of regulatory protein-mediated topological changes of DNA. *FEBS Letters* 382: 1-5.

* denotes publications with corresponding authorship

Conference publications of the topic:

Talks (as invited speaker):

Marincs, F. and White, D.W.R. (1991): Regulation of *Agrobacterium* opine catabolism gene expression. 1st Annual Queenstown Molecular Biology Meeting, Queenstown, New Zealand.

Marincs, F. and White, D.W.R. (1992): DNA topology controls expression of the *nocB* gene in *Agrobacterium tumefaciens*. Meeting on Molecular Genetics of Bacteria and Phages, Cold Spring Harbor, NY, USA.

Marincs, F. and White, D.W.R. (1993): Regulation of nopaline uptake in *Agrobacterium tumefaciens*: in vivo and in vitro studies. Joint Annual Conference of the New Zealand Microbiological Society and the New Zealand Society for Biochemistry and Molecular Biology. Palmerston North, New Zealand.

Marincs, F. and White, D.W.R. (1994): Effect of upstream sequences on expression of a bacterial promoter. 4th Queenstown Molecular Biology Meeting, Queenstown, New Zealand.

Talks (as co-author):

White, D.W.R. and **Marincs, F.** (1991): Molecular interactions in the activation of *Agrobacterium* opine catabolism gene expression. Australasian Gene Mapping and Molecular Genetics Symposium, Dunedin, New Zealand.

Marincs, F. and White, D.W.R. (1992): DNA topology controls expression of the *nocB* gene in *Agrobacterium tumefaciens*. 2nd Annual Queenstown Molecular Biology Meeting, Queenstown, New Zealand.

Posters:

White, D.W.R., Pritchard, M. and **Marincs, F.** (1990): Genetic and sequence analysis of *Agrobacterium* opine catabolism genes. Molecular Genetics Symposia, Palmerston North, New Zealand.

Marincs, F. and White, D.W.R. (1994): Regulation of the nopaline catabolism genes of pTiT37 in *Agrobacterium tumefaciens*. 7th International Symposia on Molecular Plant-Microbe Interactions, Edinburgh, Scotland.

Marincs, F., Dudas, B. and White, D.W.R. (1994):
Activation of an *Agrobacterium* nopaline catabolism
promoter *in planta*. 4th Queenstown Molecular Biology
Meeting, Queenstown, New Zealand.