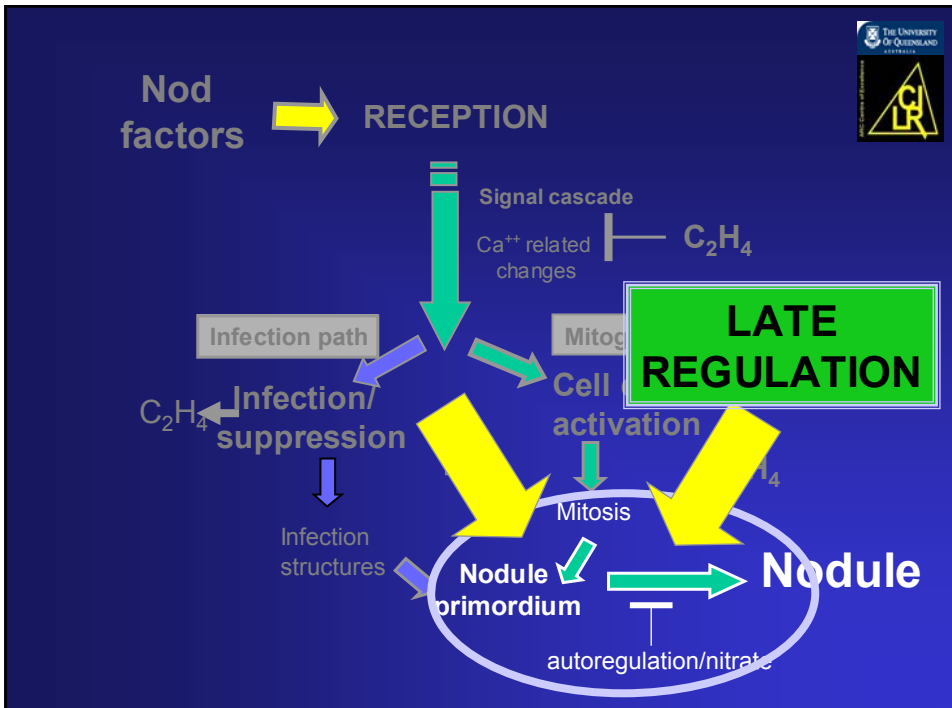
Internal regulation of cell proliferation:

Autoregulation of nodulation

Reading:
Searle et al (2003) Science 299: 109-112



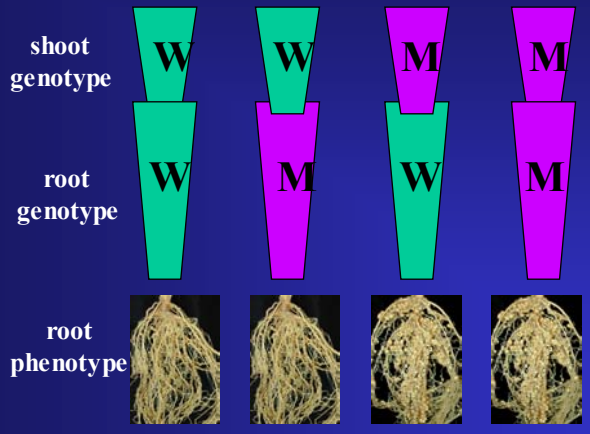
EMS

?

Wild type

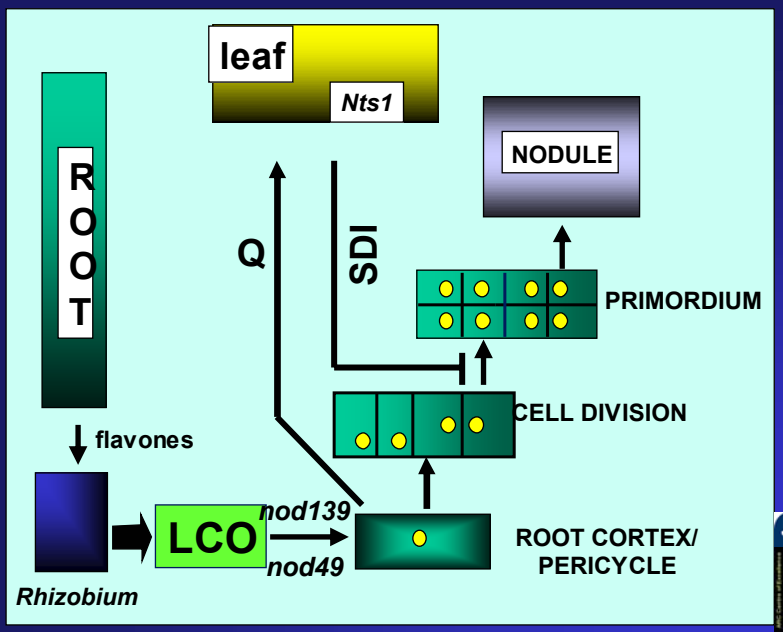
nts1007

Shoot genotype controls nodule numb



:: this demands a SIGNAL

The AON Model:

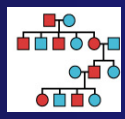


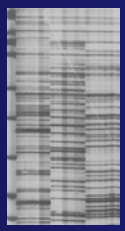
How does one find a gene that affects cell proliferation, when

a) humans, worms and flies do not have nodulation
b) neither do rice and Arabidopsis (model plants)?
c) one is the first to do this?
d) there is no obvious metabolic step to be complemented?

ANSWER? Map-based cloning

Map-based cloning of genes



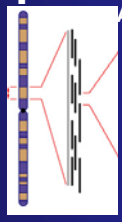


1

- Genetic mapping of DNA markers
  AFLP, DAF, RAF, RFLP
  relative to locus

2

- Use closest DNA markers each side to isolate AC clones



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50
007  MRSCVCYTLL LFIFFIWLRV ATCSSFTDME SLLKLKDSMK GDKAKDDALH
S55  MRSCVCYTLL LFIFFIWLRV ATCSSFTDME SLLKLKDSMK GDKAKDDALH
N4   MRSCVCYTLL LFIFFIWLCV ATCSSFTDME SLLKLKDSMK GDKAKDDALH
WM   MRSCVCYTLL LFIFFIWLRV ATCSSFTDME SLLKLKDSMK GDKAKDDALH

BRG  DWKFFPSLSA HCFFSGVKCD RELRVVAINV SFVPLFGHLP PEIGQLDKLE
100
007  DWKFFPSLSA HCFFSGVKCD RELRVVAINV SFVPLFGHLP PEIGQLDKLE
S55  DWKFFPSLSA HCFFSGVKCD RELRVVAINV SFVPLFGHLP PEIGQLDKLE
N4   DWKFFPSLSA HCFFSGVKCD RELRVVAINV SFVPLFGHLP PEIGQLDKLE
WM   DWKFFPSLSA HCFFSGVKCD RELRVVAINV SFVPLFGHLP PEIGQLDKLE

BRG  NLTVSQNNLT GVLPKELALL
                150
007  NLTVS----- ----------
S55  NLTVS----- ---------
N4   NLTVSQNNLT GVLPKELALL
WM   NLTVSQNNLT GVLPKELALL
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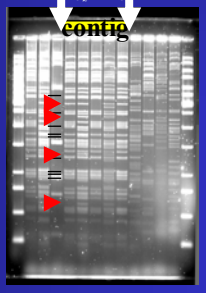
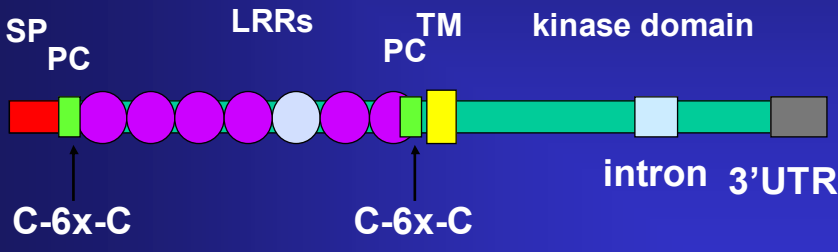
4 Allelic sequencing



3 BAC fingerprinting and contig construction

Characterisation of the *Nts-1 locus*:

GmNARK gene structure = *AtCLAVATA1-like*



GmNARK = Nodule Autoregulation Receptor Kinase

Sciencexpress Report

Long-Distance Signaling in Nodulation Directed by a CLAVATA1-like Receptor Kinase

Iain R. Searle,[1,2*†] Artem E. Men,[3†] Titeki S. Laniya,[3] Diana M. Buzas,[3] Inaki Iturbe-Ormaetxe,[3‡] Bernard J. Carroll,[1,2§‖] Peter M. Gresshoff[3]

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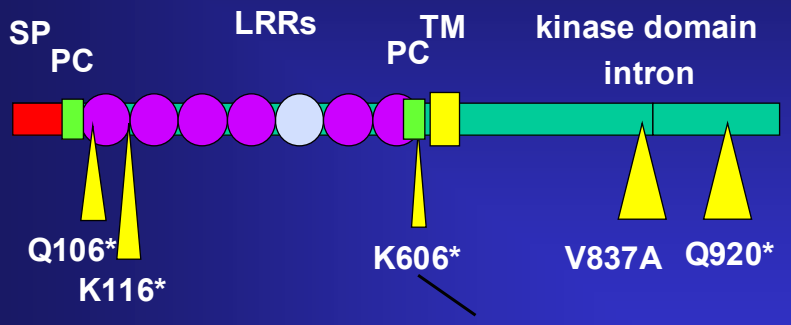
- 31. October 2002 on line - 3. January 2003 hard copy

***First map-based cloning of any soybean gene**

***First (concurrent with *Lotus*) isolation of an autoregulation gene in any legume**

GmNARK gene structure

GmNARK: 465
LjNARK: 1576
AtCLV1: 79
GmCLV1: 76



Nomenclature:

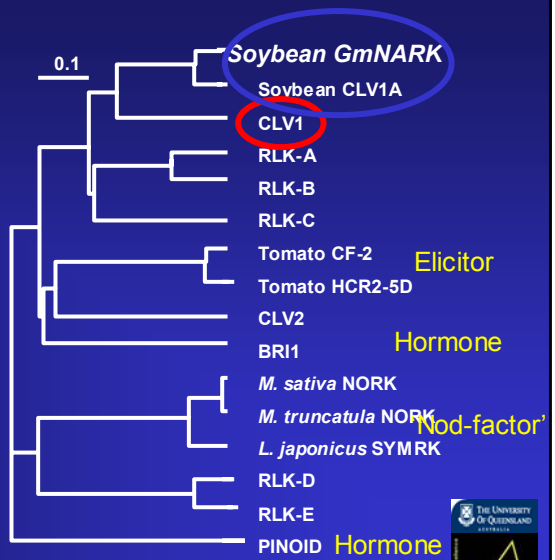
Nts-1 **has similarity to receptor kinase genes, specifically CLAVATA1 from *Arabidopsis***

Since it is crucial for nodule autoregulation,

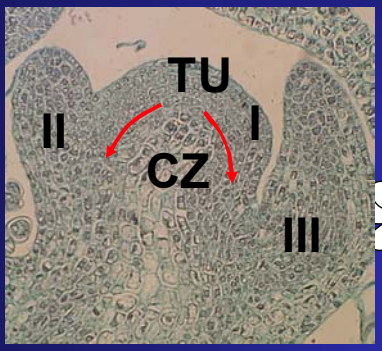
NARK

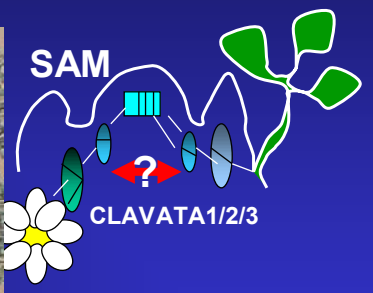
(Nodule Autoregulation Receptor Kinase)

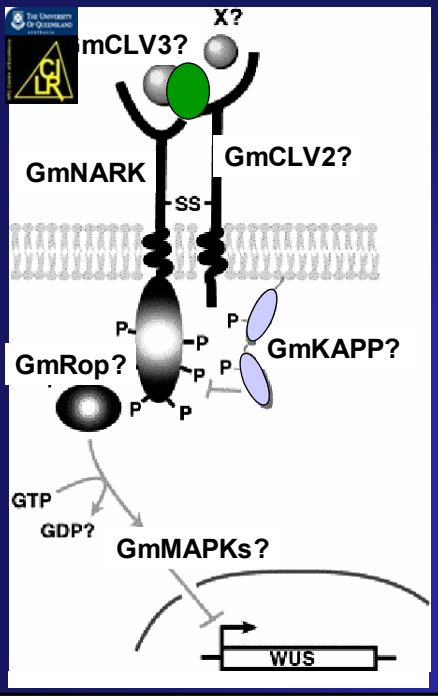
GmNARK **is closely related to other protein kinases involved in the regulation of cell proliferation**



CLAVATA complex: short distance signalling for control of meristem cell proliferation and fate





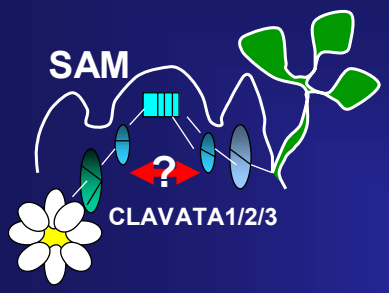


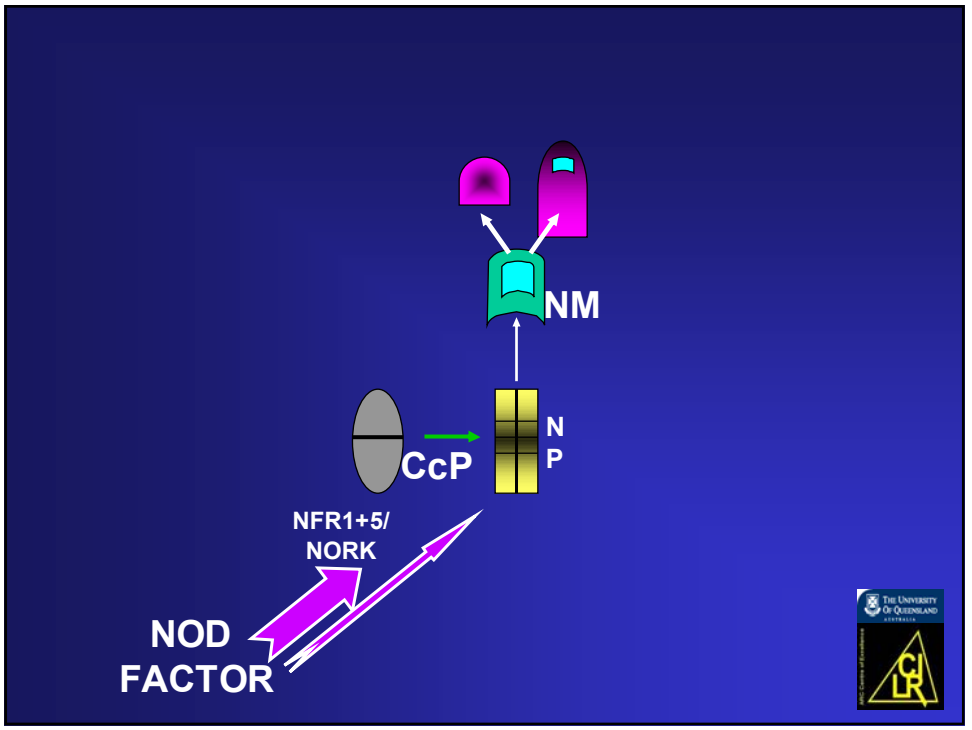
1) Shoot-specific ligands??

The CLV signaling complex (from **J.C. Fletcher**. 2002. Annu. Rev. Plant Biol. 53:45-66)

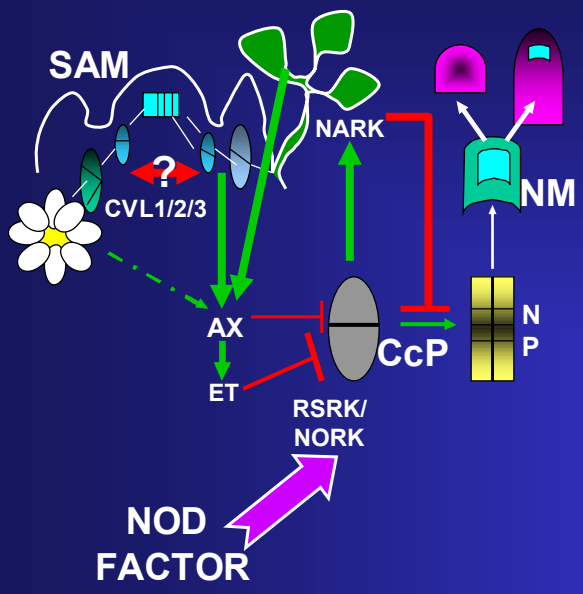
The CLV1 LRR receptor kinase forms a heteromeric complex with the CLV2 LRR receptor-like protein at the plasma membrane of interior SAM cells. Binding of the CLV3 ligand, possibly in association with another protein (X), stimulates assembly of an active signaling complex that also contains a phosphatase (KAPP) and a Rho-like GTPase (Rop). The signal is relayed from the cytosol to the nucleus, potentially via a MAP kinase cascade, to limit *WUS* expression.

Long- and short distance signalling for control of meristem proliferation

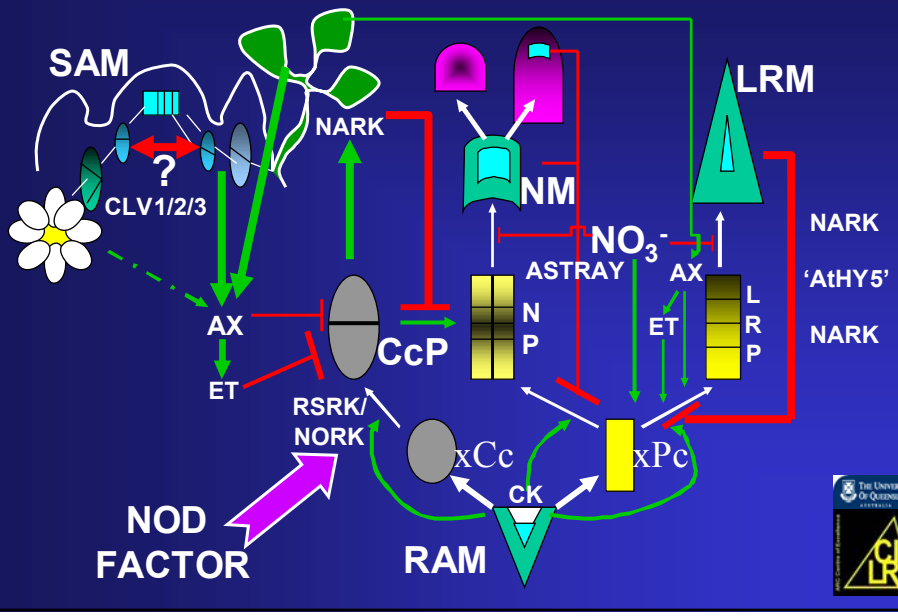




Long- and short distance signalling for control of meristem proliferation



Long- and short distance signalling for control of meristem proliferation



Take-home messages:

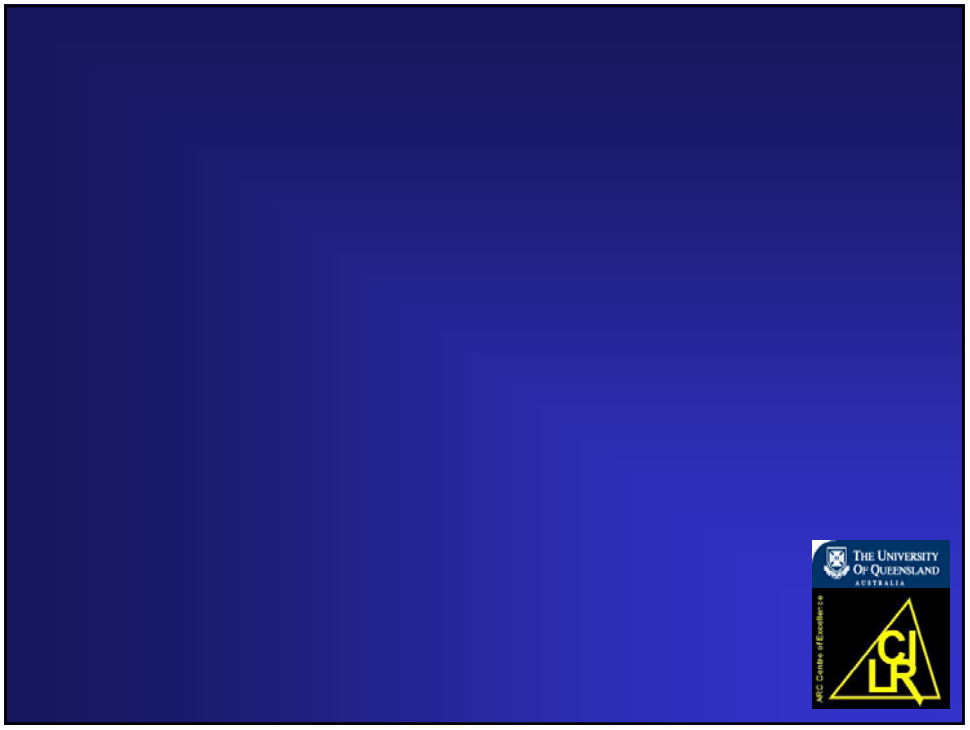
1. Ethylene and autoregulation control nodule development separately
2. Altered receptors influence development
3. Transcriptional regulation can work in reverse
   that is: when the hormone is there and received, the system is not active and transcriptional factors no longer suppress ethylene response genes
4. Mutations can be isolated in the reception pathway
5. Transgenic plants can be used to test the function of genes and illuminate complex developmental pathways
6. Transgenic plants are more accurate than inhibitor studies which tend to be non-specific
7. Autoregulation works by long distance signal
8. Kinase activity can be monitored
9. Developmental processes occur in parallel and are interconnected.

Conclusions

NARKs may be part of a receptor family that negatively controls cell proliferation and thus controls cell fate

Receptors may interact and compensate

These processes may involve new types of long-distance regulatory factors
- such as peptides and RNA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