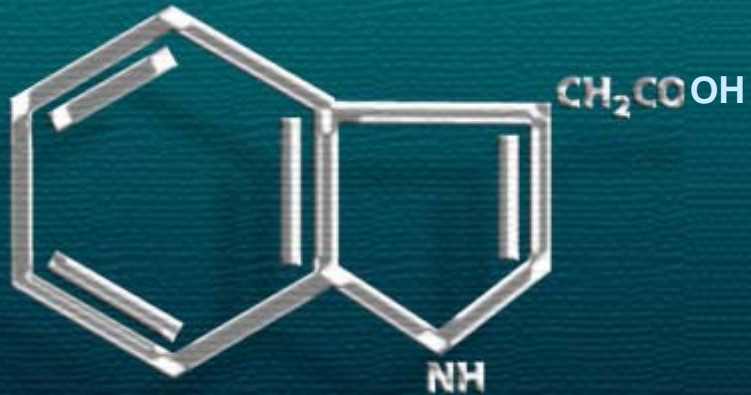


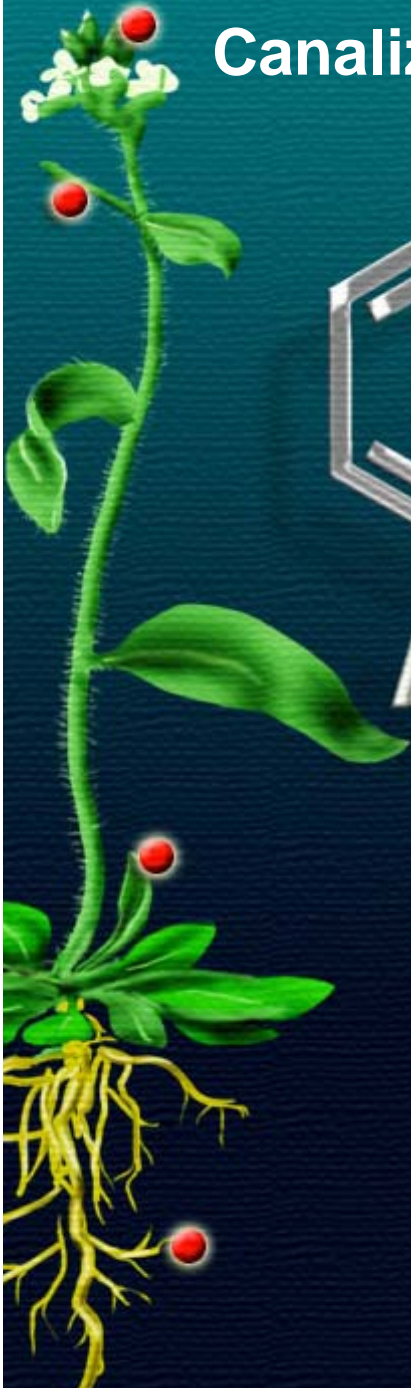
# Canalization of auxin flow by regulation of PIN polarity



Auxin

Ivan Paponov

Department of Botany, University of Freiburg



# Outline

## **Overarching scientific context**

System under study

Key scientific questions addressed

Current methodologies (models) employed

System components

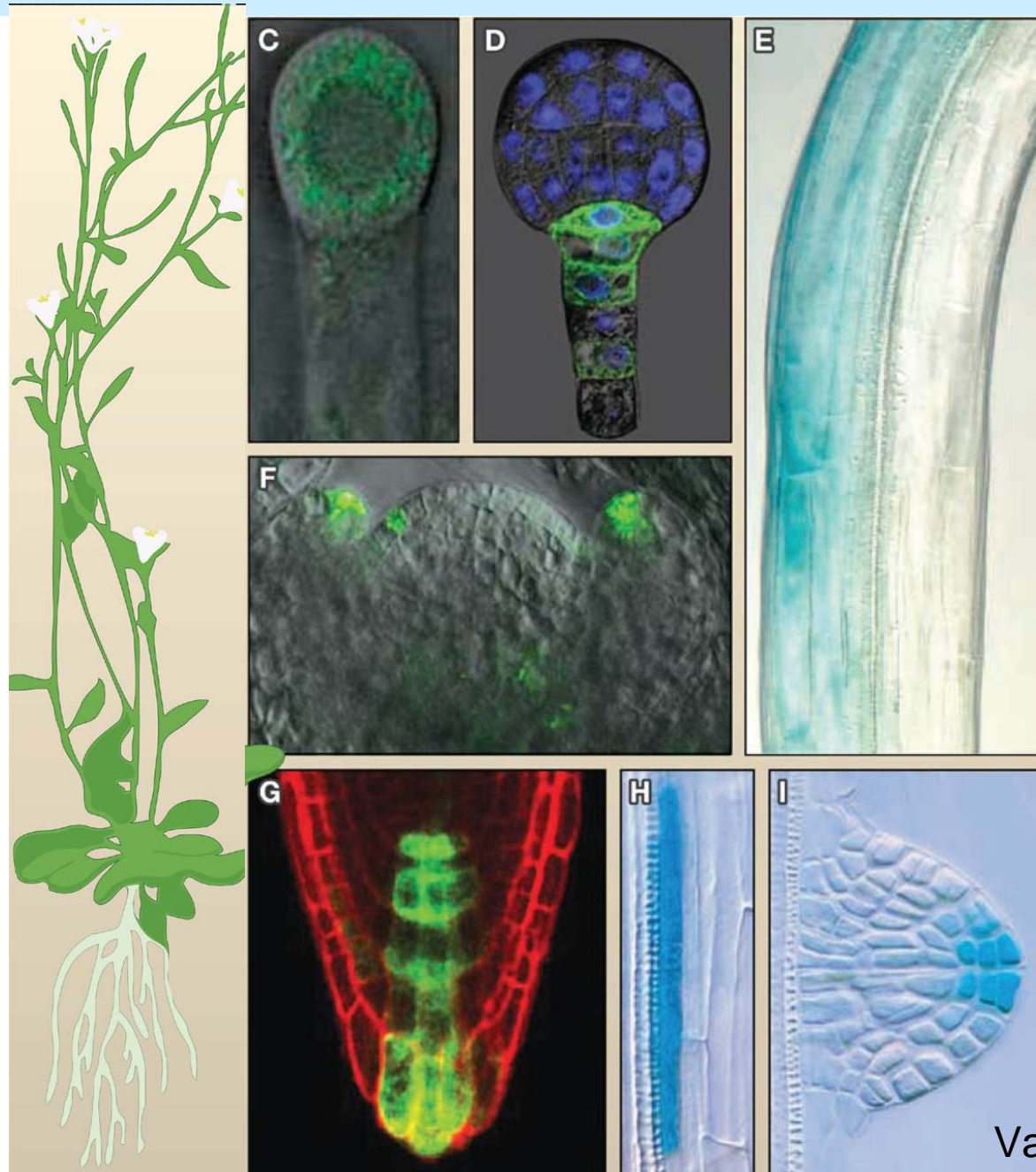
Key component behaviours

Discrepancy of current models

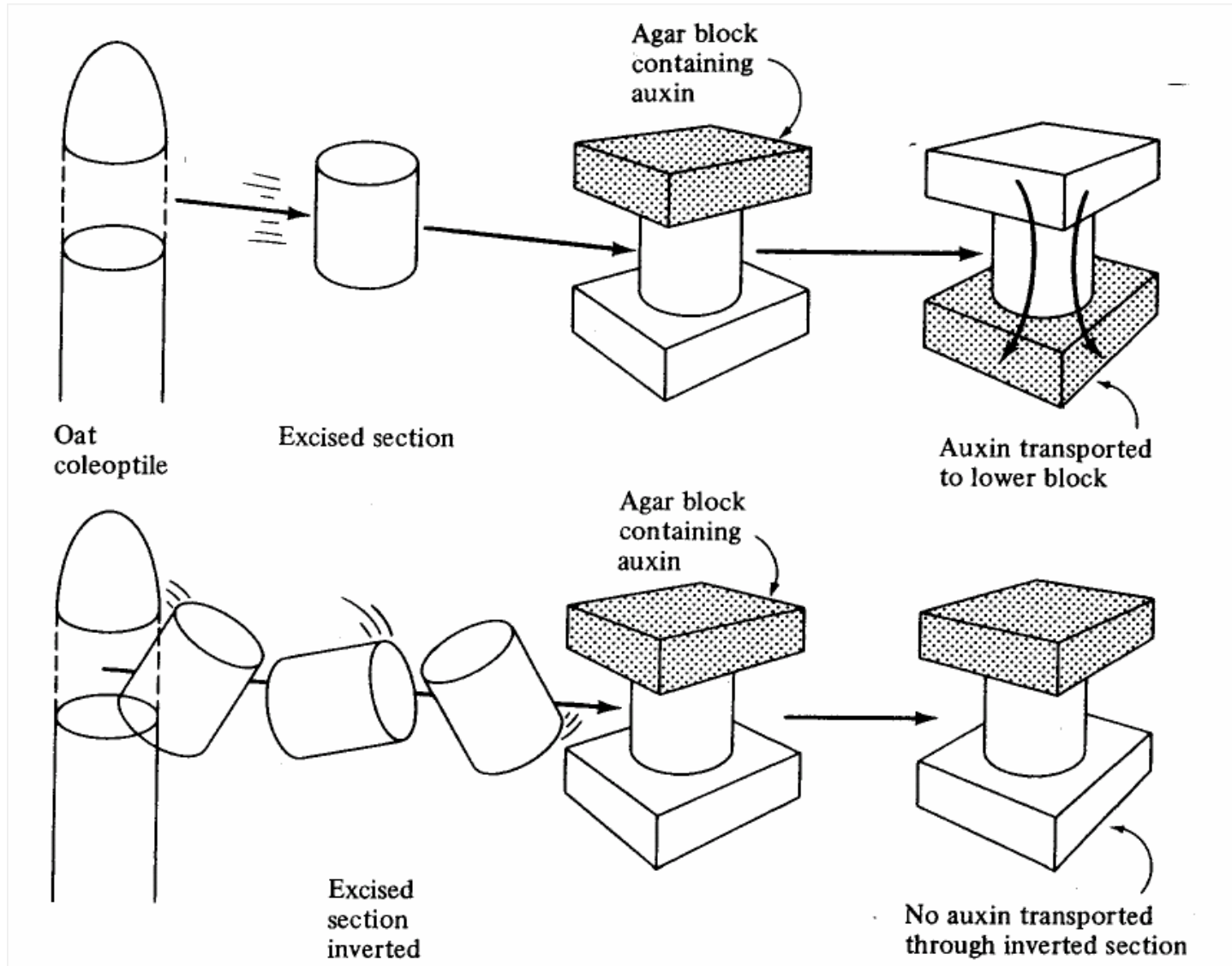
(Experimental) Results

Possible applications

# Auxin as morphogen



# Polar auxin transport



## IAA is a weak acid



$$K_d = \frac{[\text{IAA}^-][\text{H}^+]}{[\text{IAA}]} \quad \text{at pH 4.7, } \frac{[\text{IAA}^-][\text{H}^+]}{[\text{IAA}]} = 1$$

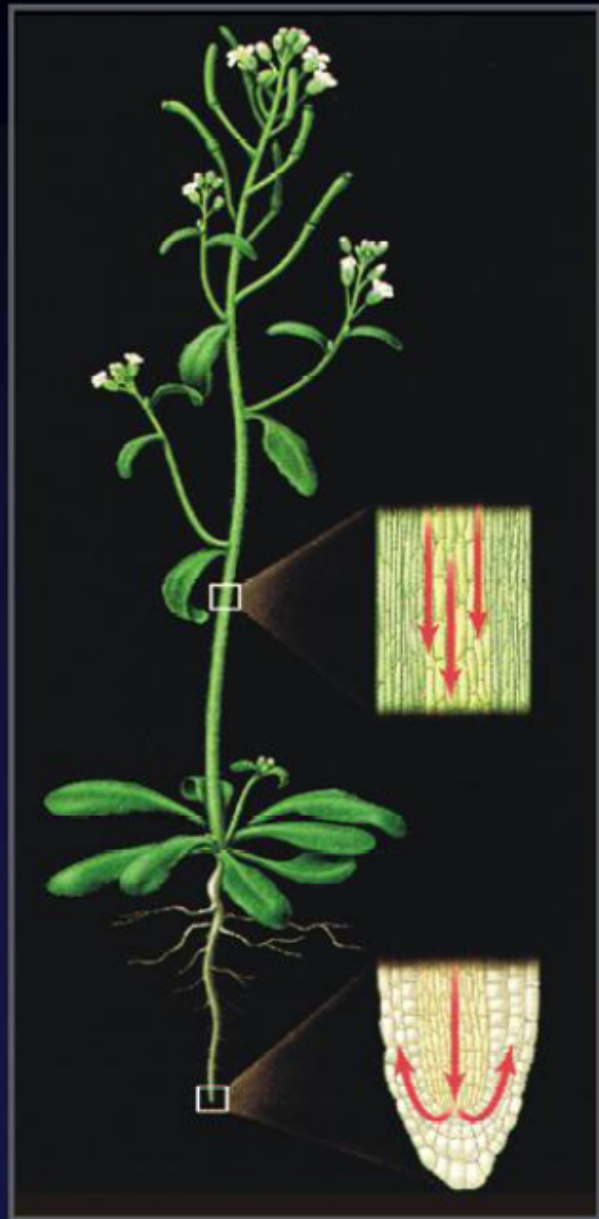
**Apoplast,  
pH = 5.0**

**Cytosol,  
pH = 7.2**

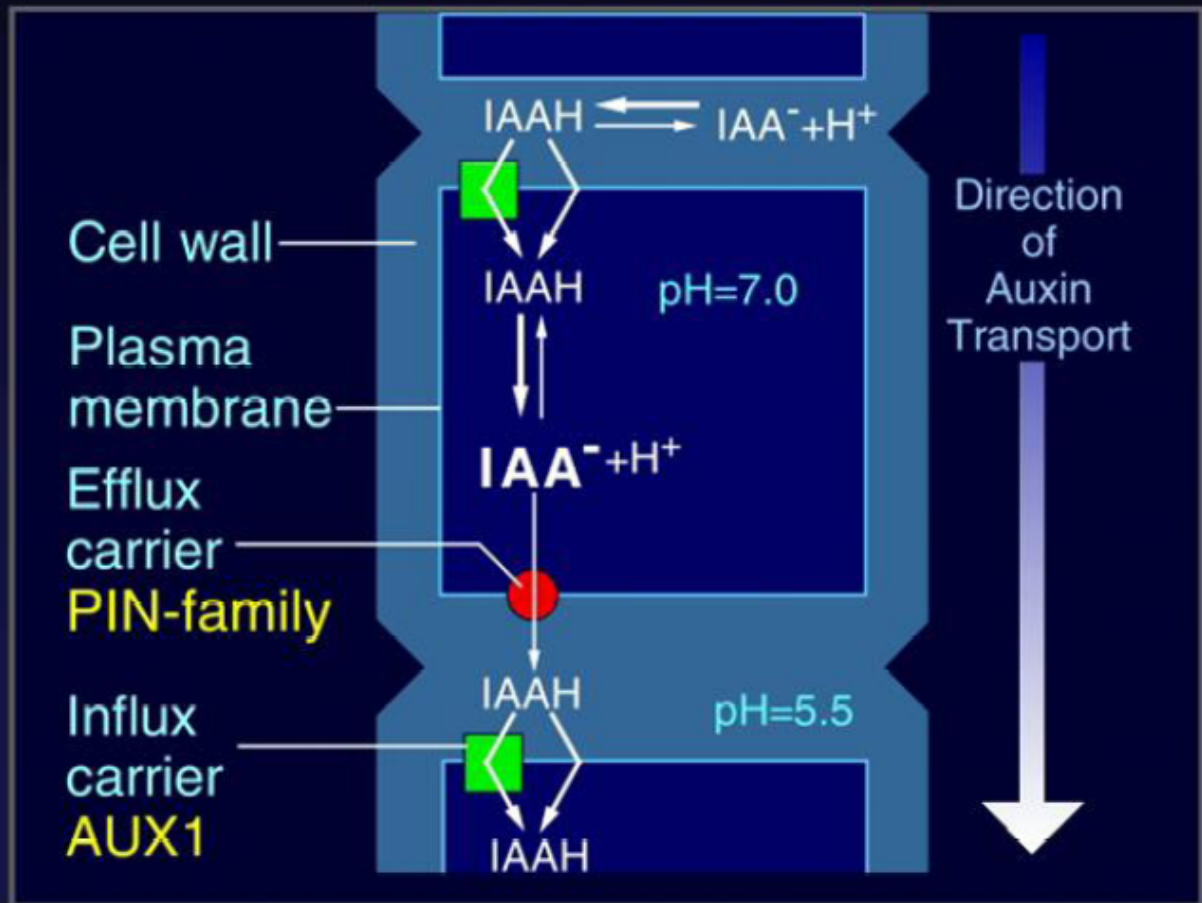
**IAA / IAA<sup>-</sup> >**

**IAA/IAA<sup>-</sup>**

# Auxin Transport

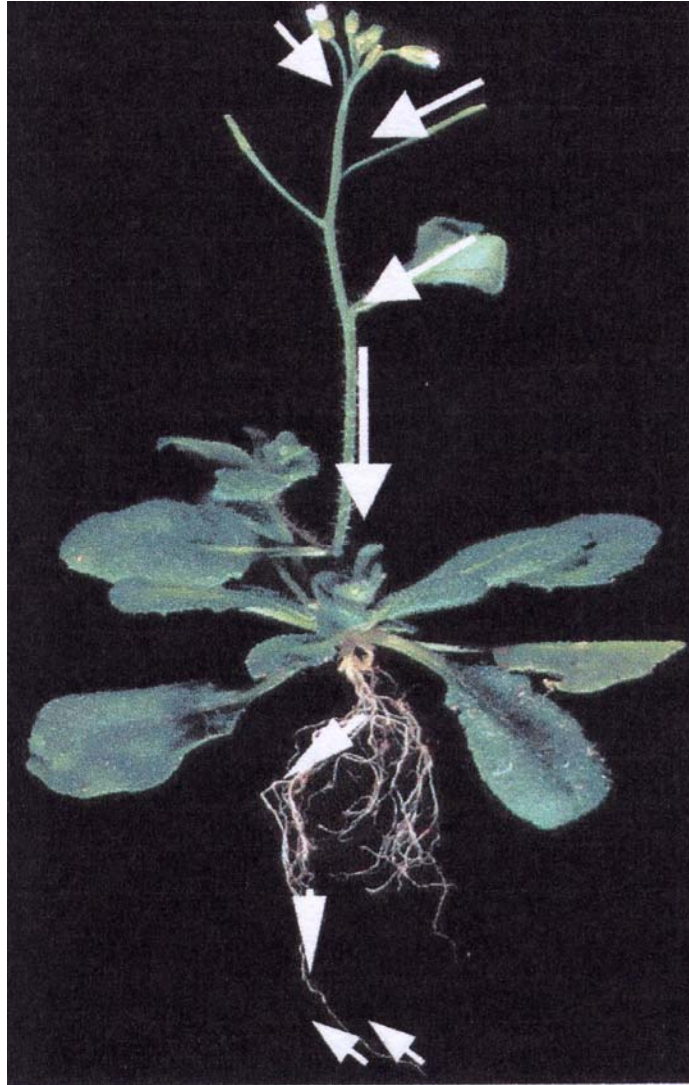


## Chemiosmotic hypothesis

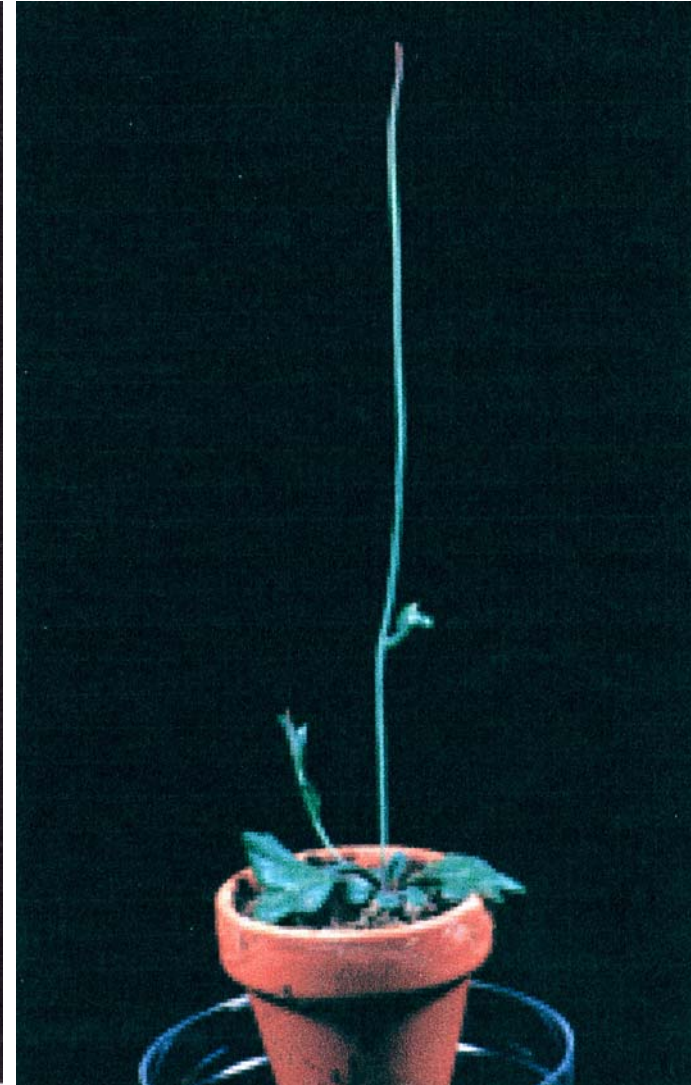


## Phenotype of *pin1*

Wild type

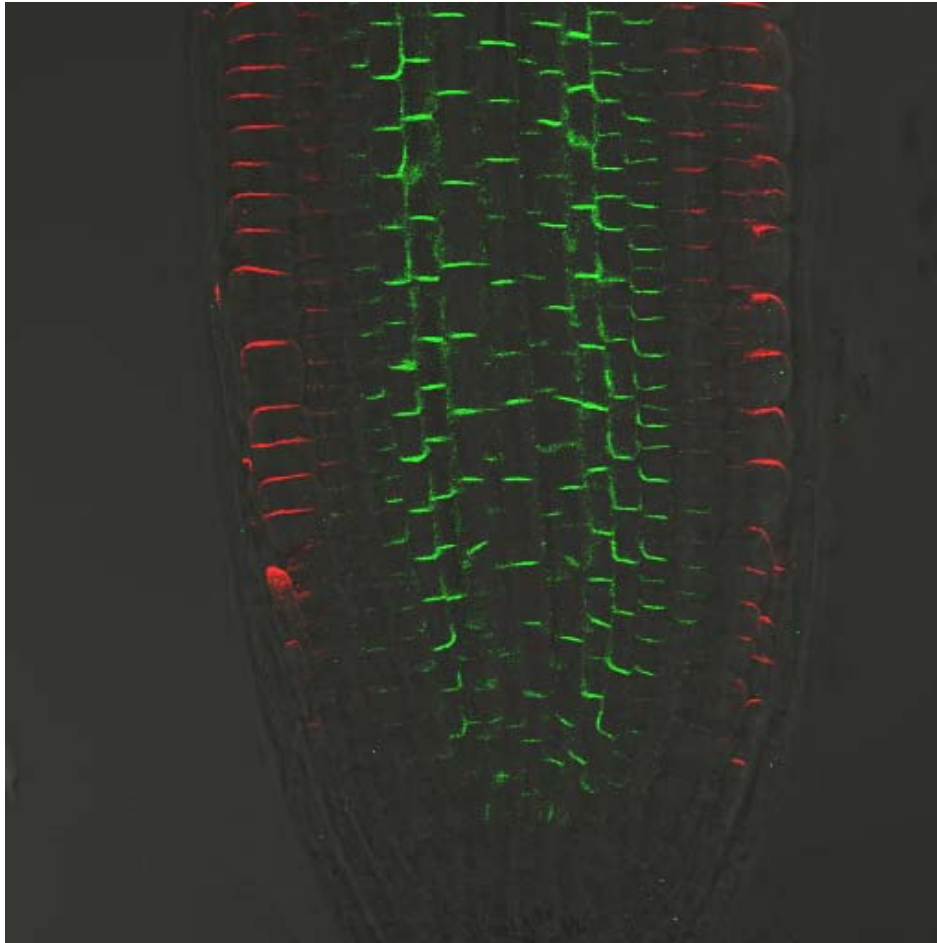


*pin1*

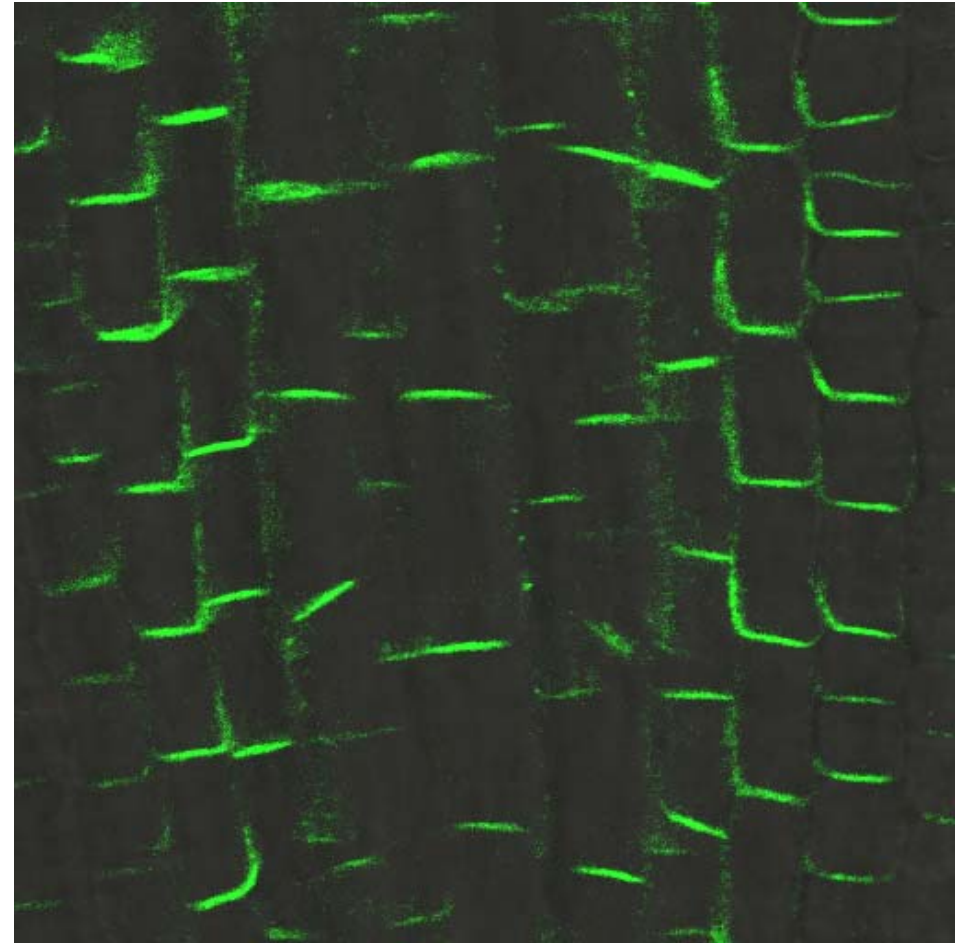


## Polar localization of auxin efflux carriers

**PIN1 PIN2**

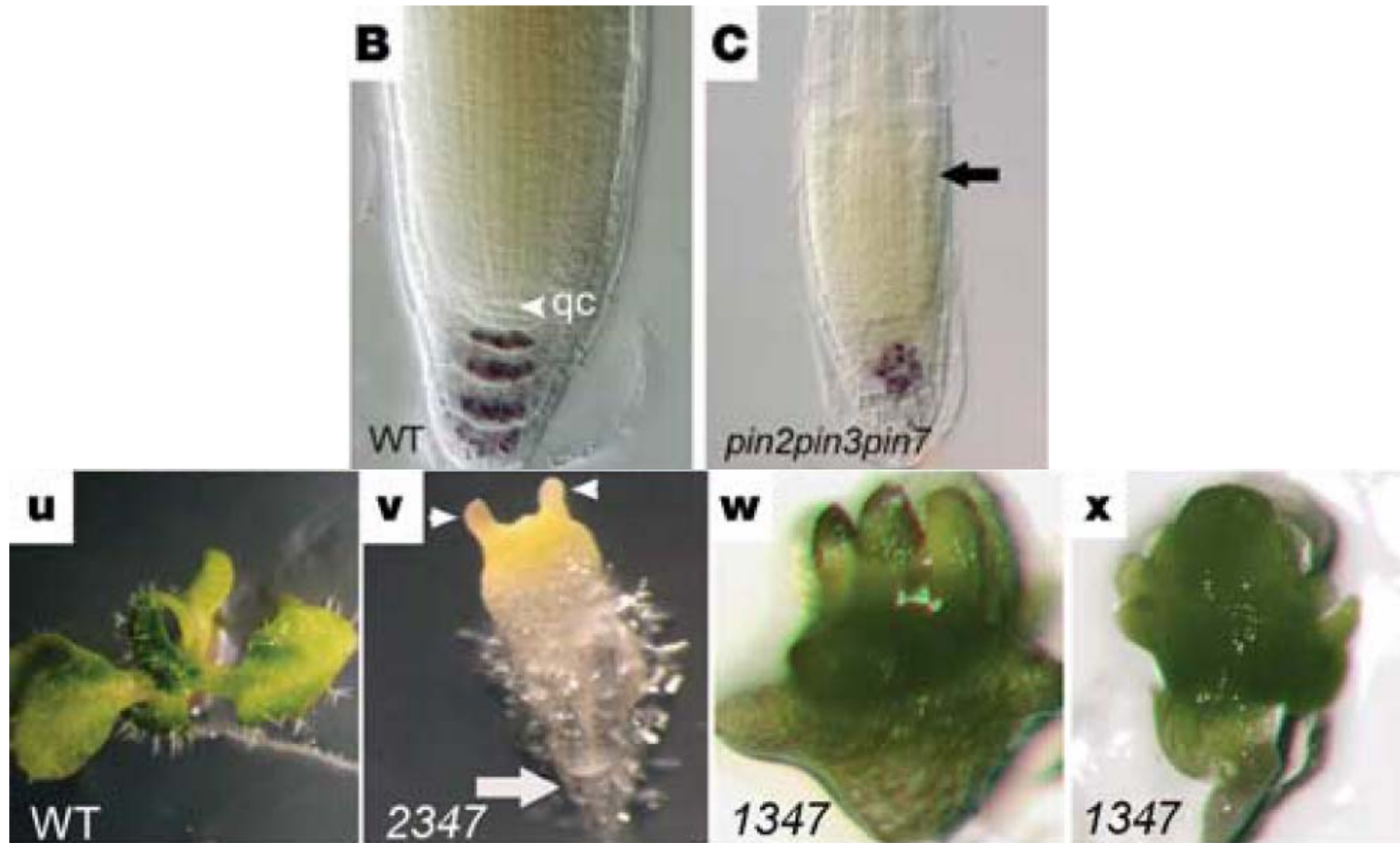


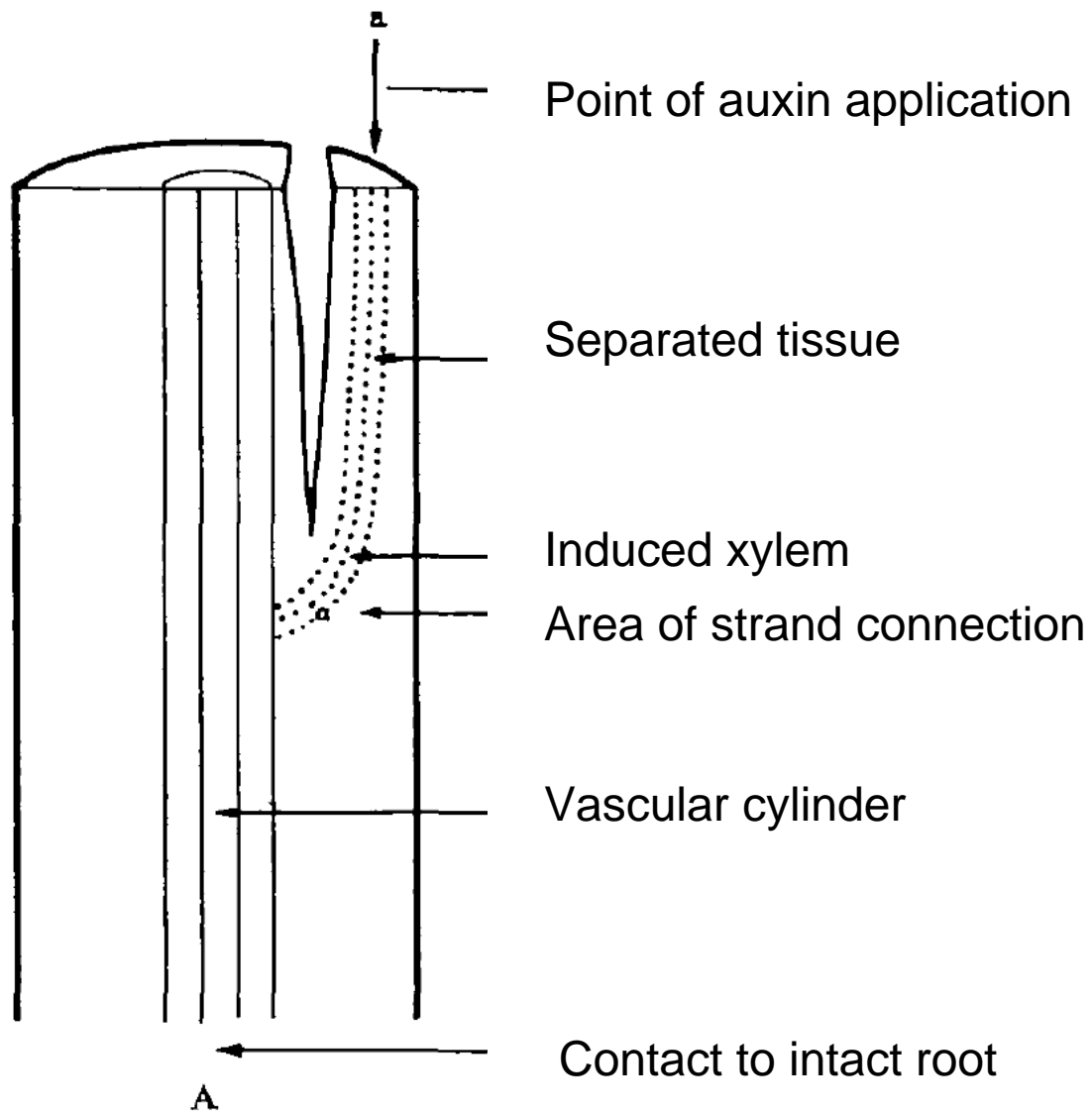
**PIN1**





## Strong defects in triple and quadruple mutants





From Sachs T. 1968 Ann.Bot.

# Outline

Overarching scientific context

## **System under study**

Key scientific questions addressed

Current methodologies (models) employed

System components

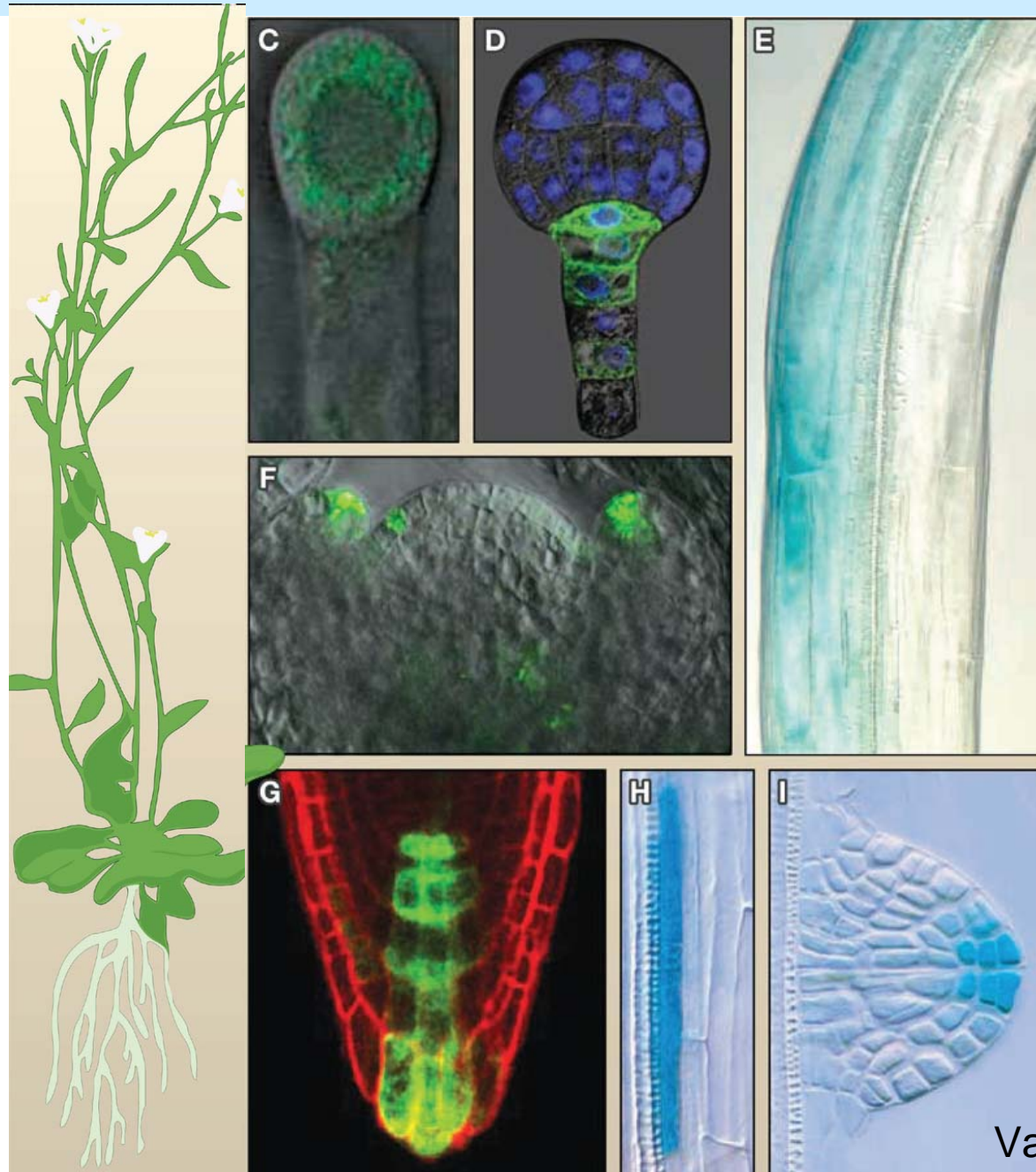
Key component behaviours

Discrepancy of current models

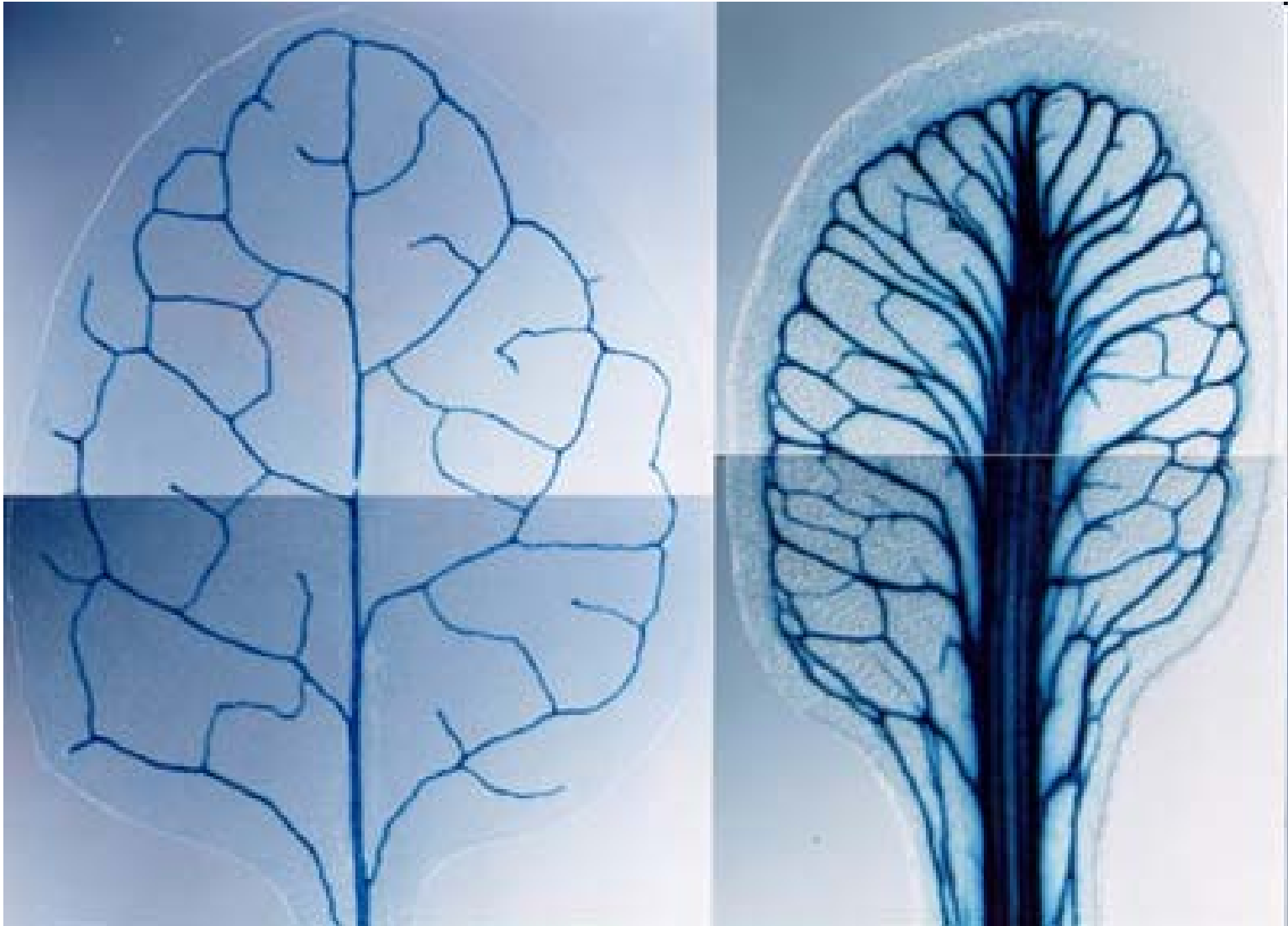
(Experimental) Results

Possible applications

# Auxin as morphogen



## Vein formation



## DR5 signal in roots



# Outline

Overarching scientific context

System under study

**Key scientific questions addressed**

Current methodologies (models) employed

System components

Key component behaviours

Discrepancy of current models

(Experimental) Results

Possible applications

## **Key scientific questions addressed**

**How does auxin regulate the plant development?**

**How does auxin regulate the vascular development, phylotaxis,...?**

**How does auxin regulate PIN localization?**



# Outline

Overarching scientific context

System under study

Key scientific questions addressed

**Current methodologies (models) employed**

System components

Key component behaviours

Discrepancy of current models

(Experimental) Results

Possible applications

## Models of auxin-mediated cell polarity

### Flux-based

Cells of the provascular strand have a high auxin flux but a low auxin concentration

No auxin flux sensor has been found

### Concentration-based

Rely on a feedback between PIN polarity and differences in mean auxin concentration between adjacent cells.

Rely on unknown and unspecified signal to communicate information about auxin concentration between adjacent cells

# Outline

Overarching scientific context

System under study

Key scientific questions addressed

Current methodologies (models) employed

## **System components**

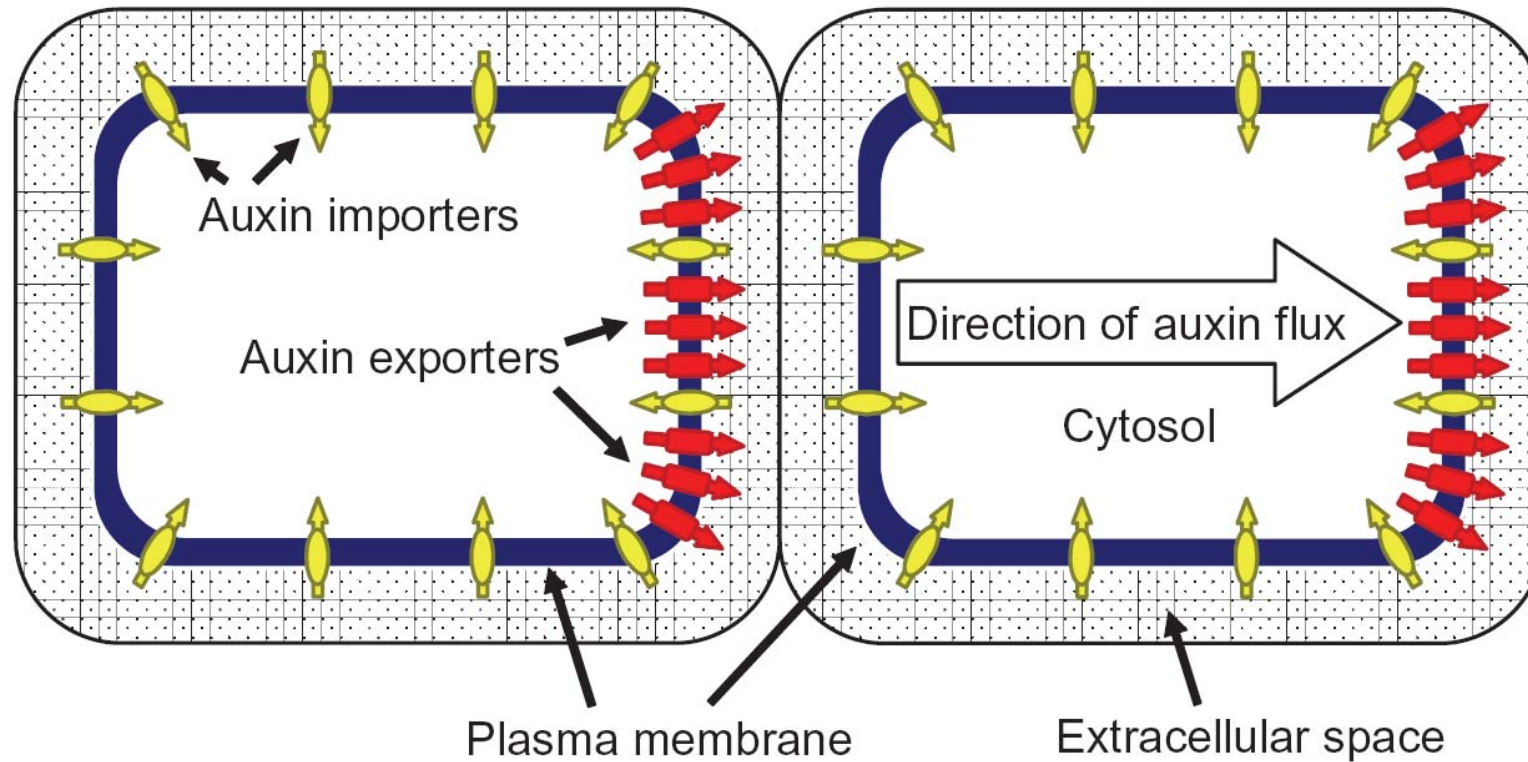
Key component behaviours

Discrepancy of current models

(Experimental) Results

Possible applications

## System components



From Smith and Bayer (2009)

# Outline

Overarching scientific context

System under study

Key scientific questions addressed

Current methodologies (models) employed

System components

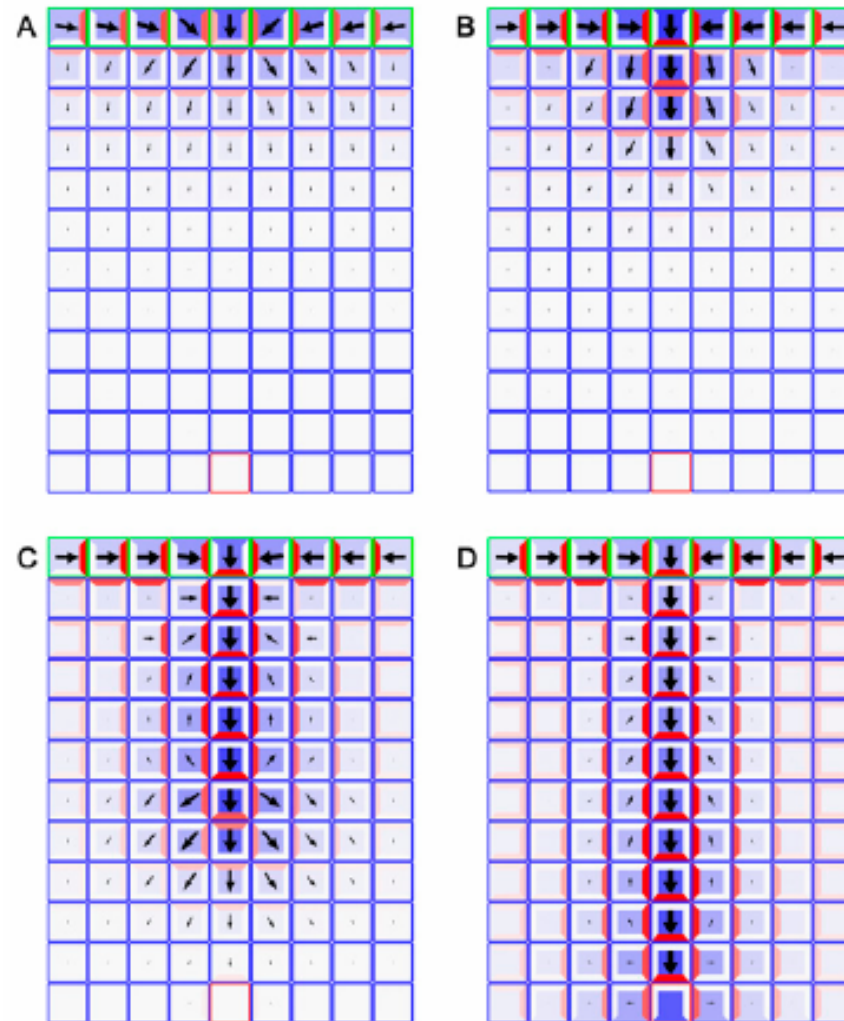
**Key component behaviours**

Discrepancy of current models

(Experimental) Results

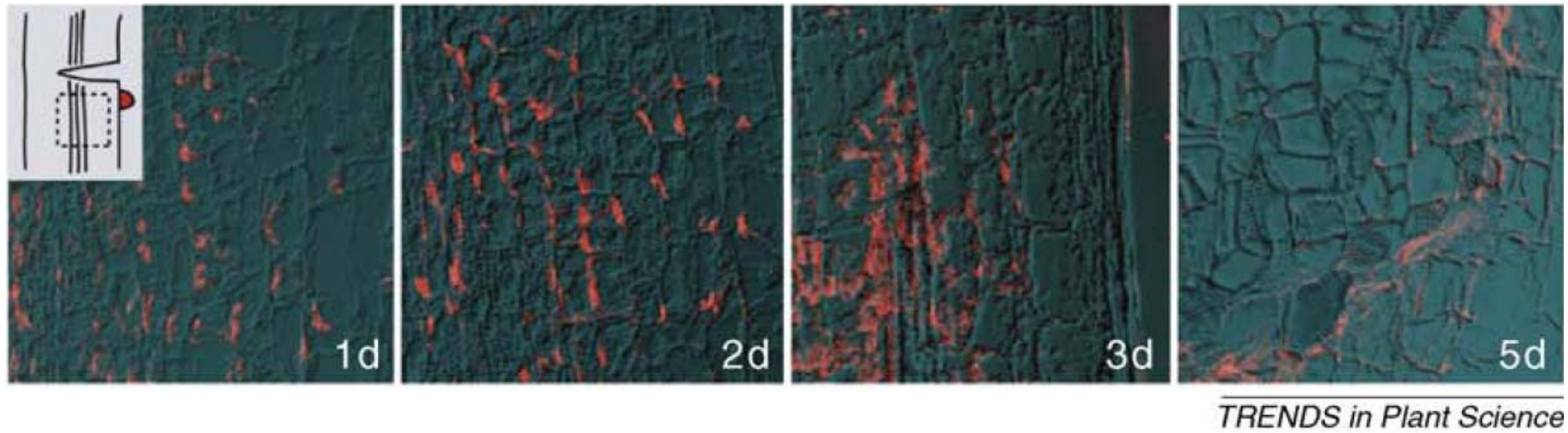
Possible applications

# Concentration-based model



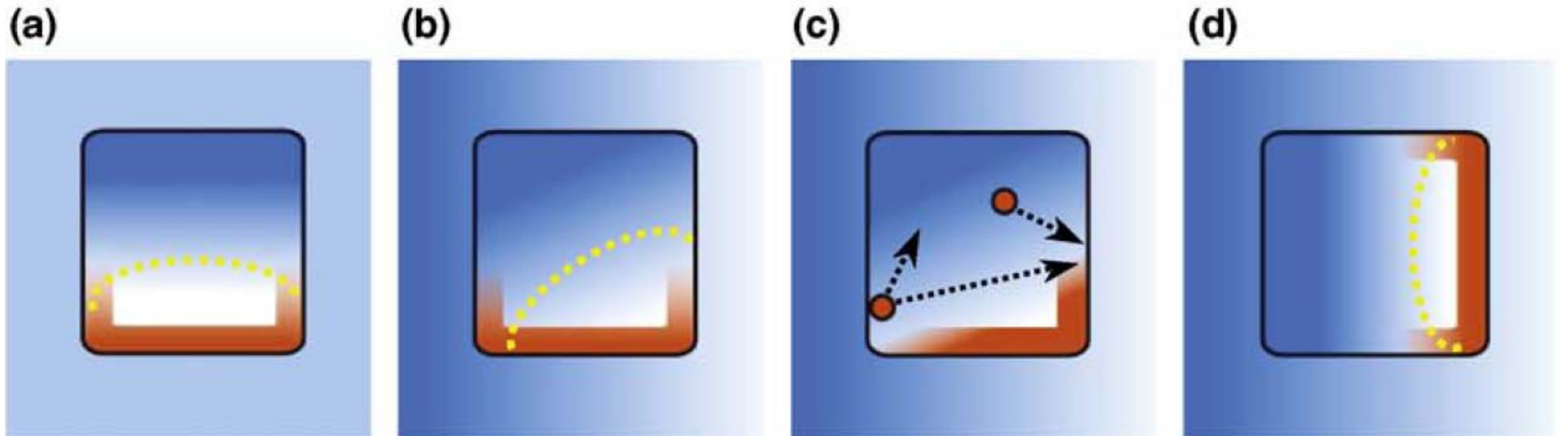
Bayer et al. (2009) Genes Dev

## PIN1 polarity during vascular development



Sauer et al. (2006) *Genes Dev.*

Sketch of the proposed interaction between the cytoplasmic auxin gradient and PIN efflux carriers (red).

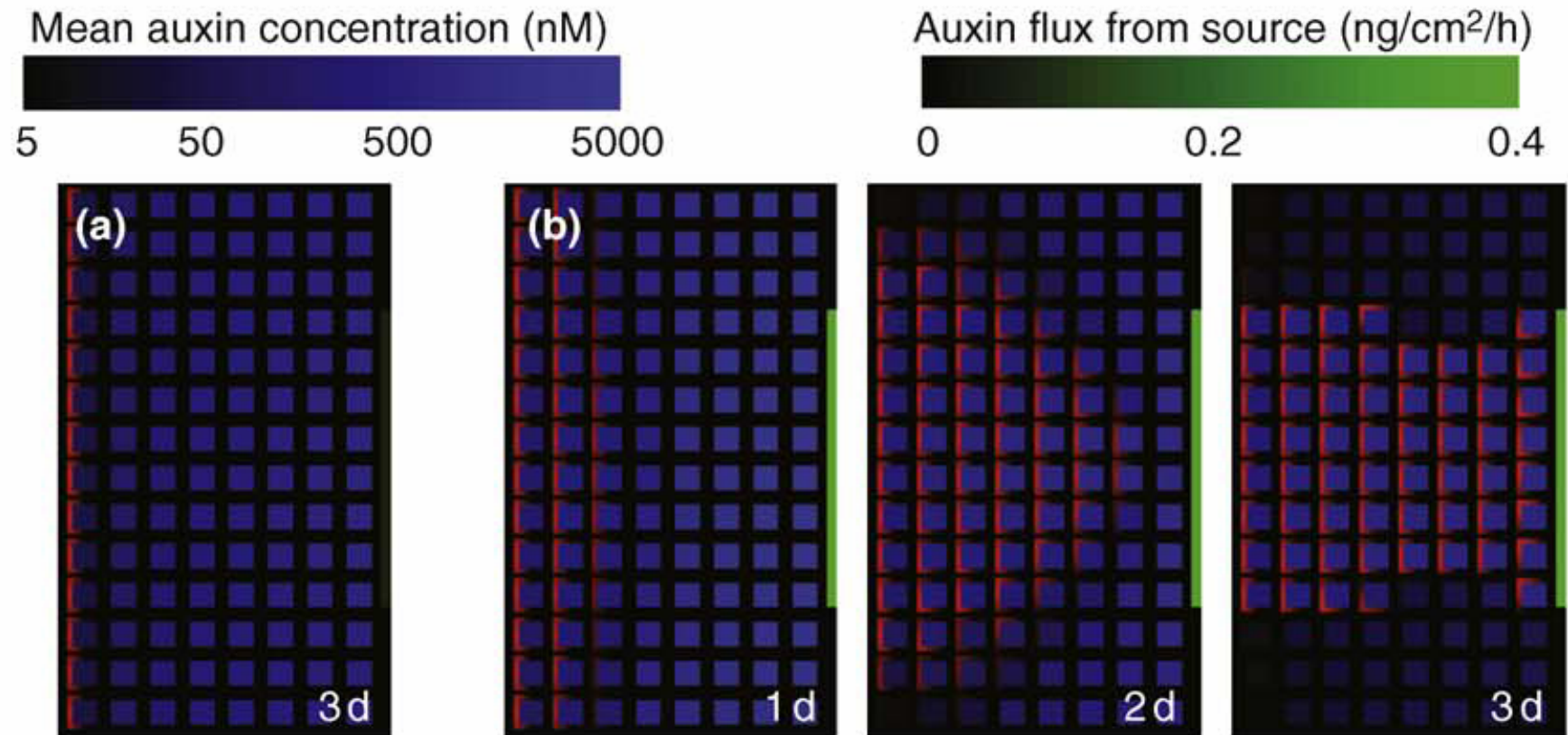


*TRENDS in Plant Science*

Feedback between auxin and PIN

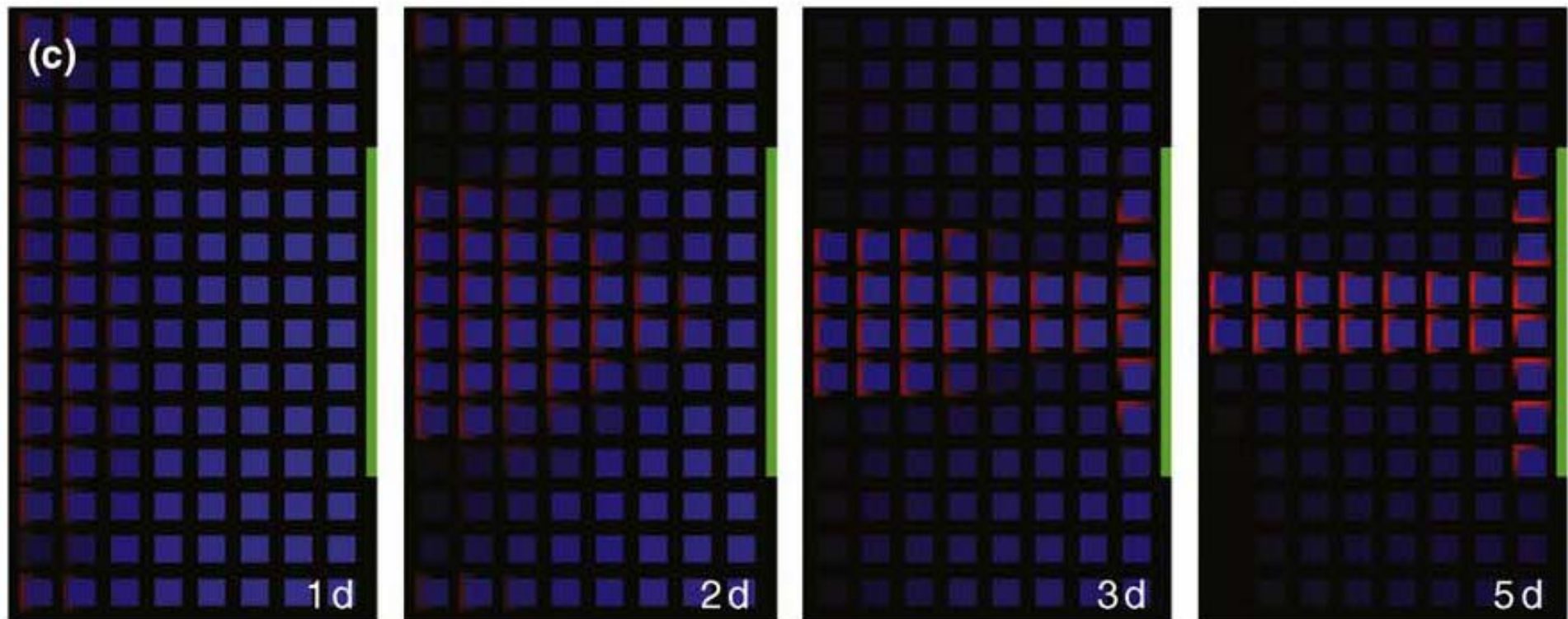


# Model of vascular development

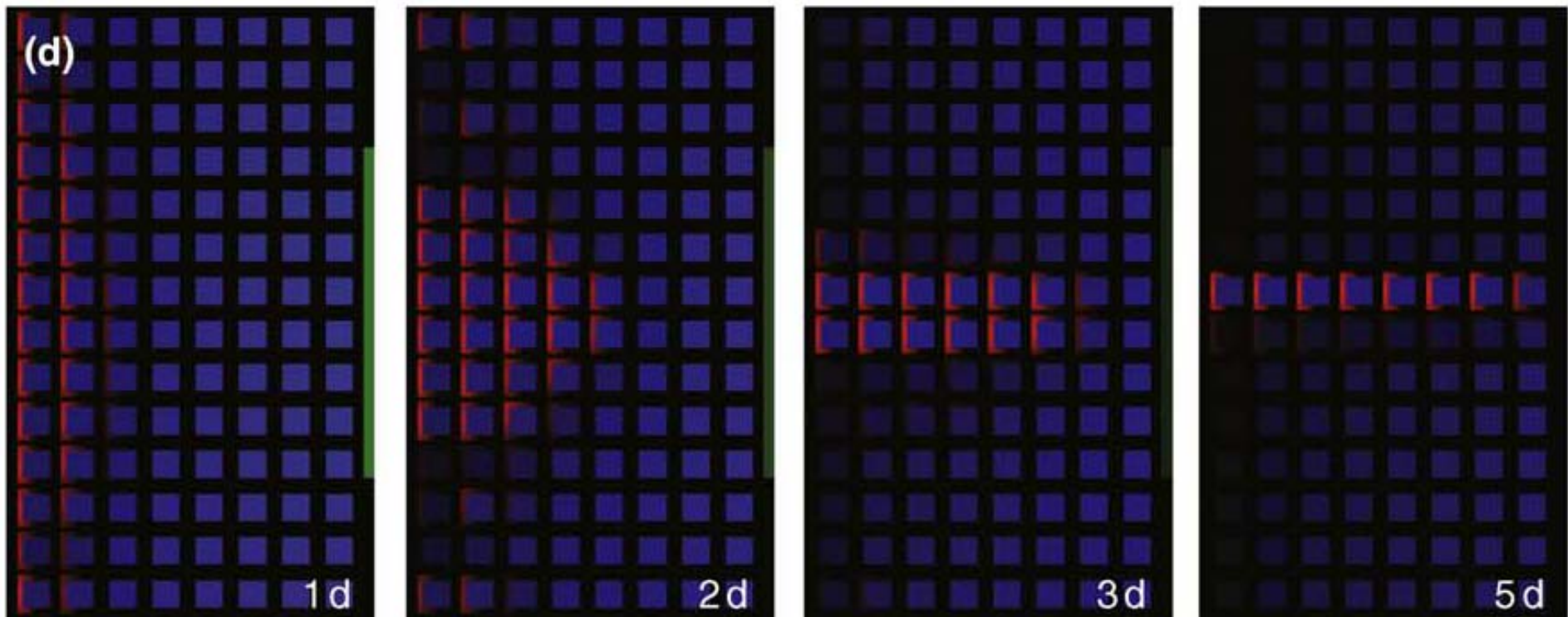


Kramer (2009) TIPS

If the number of auxin carriers per cell is allowed to gradually increased from day 1 to day 5, then the canal narrows to two cell files.



If the auxin source gradually decreases, then the canal also narrows



Kramer (2009) TIPS

# Outline

Overarching scientific context

System under study

Key scientific questions addressed

Current methodologies (models) employed

System components

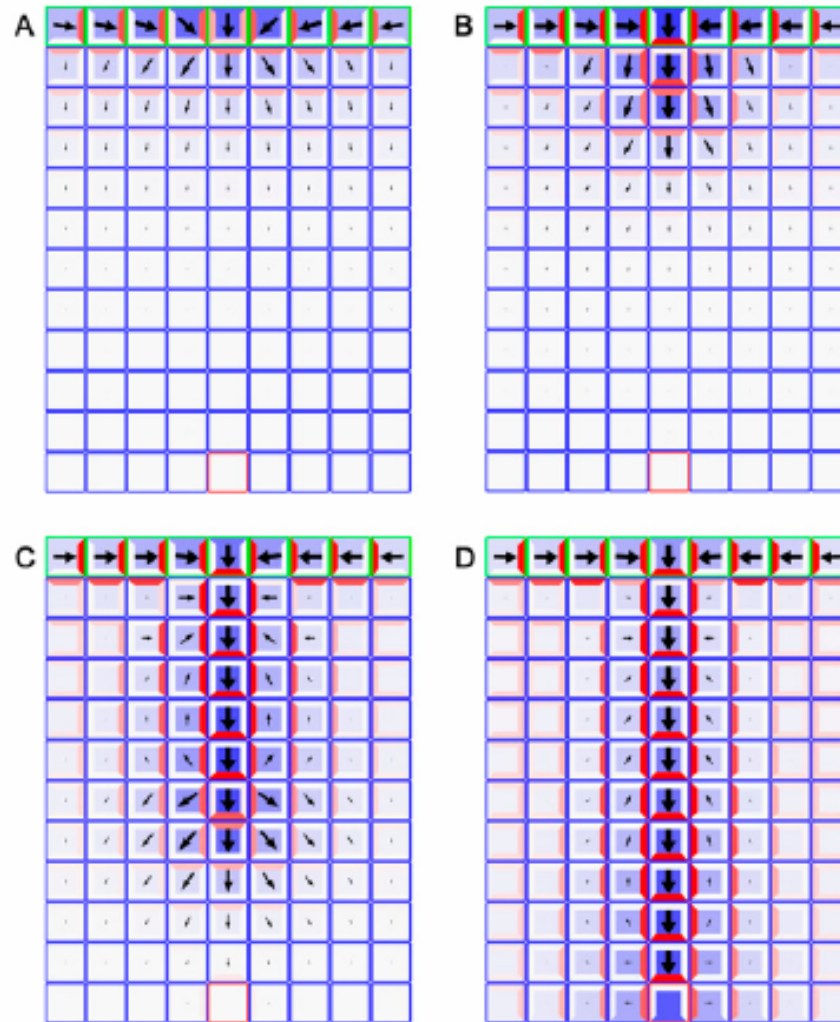
Key component behaviours

**Discrepancy of current model**

(Experimental) Results

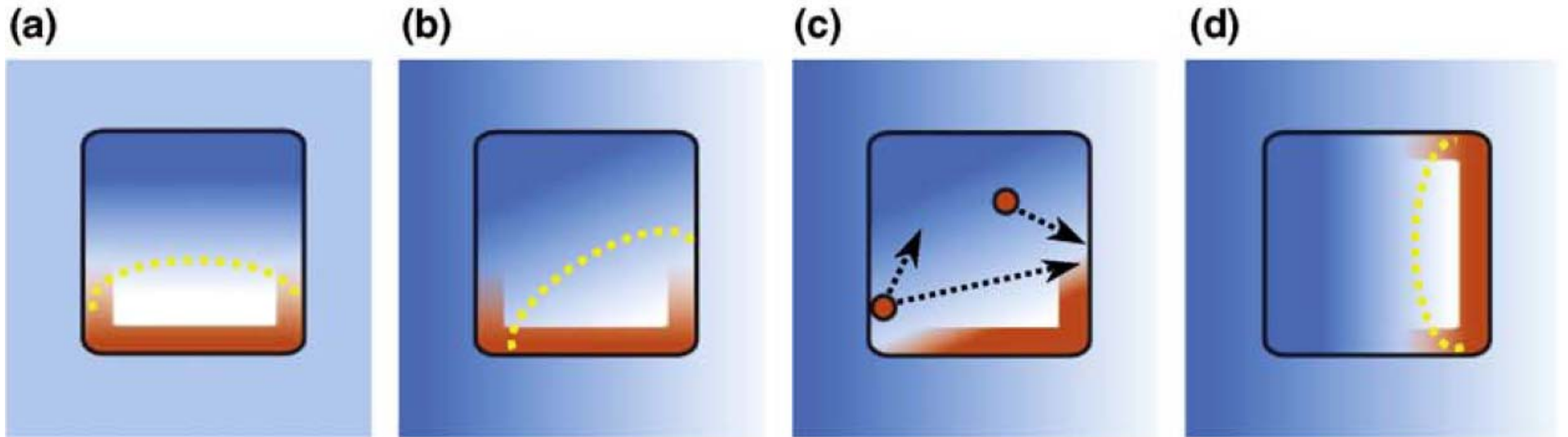
Possible applications

# Concentration-based model



Bayer et al. (2009) Genes Dev

## Modified flux-based model



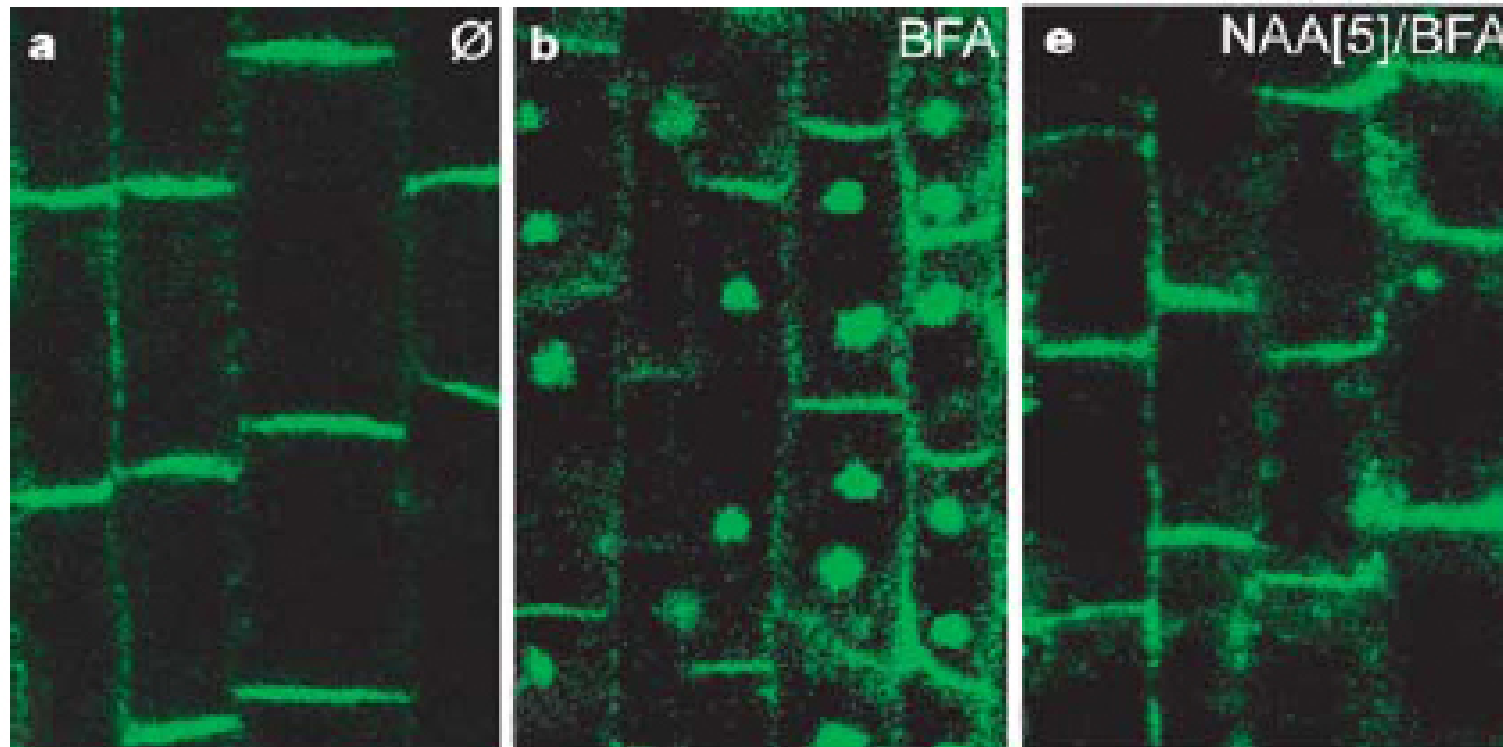
*TRENDS in Plant Science*

**Vascular development is driven by a feedback between cytoplasmic auxin gradient and PIN polarity**

# LETTERS

## Auxin inhibits endocytosis and promotes its own efflux from cells

Tomasz Paciorek<sup>1</sup>, Eva Zažímalová<sup>2</sup>, Nadia Ruthardt<sup>3</sup>, Jan Petrášek<sup>2</sup>, York-Dieter Stierhof<sup>1</sup>, Jürgen Kleine-Vehn<sup>1</sup>, David A. Morris<sup>2,4</sup>, Neil Emans<sup>3</sup>, Gerd Jürgens<sup>1</sup>, Niko Geldner<sup>1</sup> & Jiří Friml<sup>1</sup>



# Outline

Overarching scientific context

System under study

Key scientific questions addressed

Current methodologies (models) employed

System components

Key component behaviours

Discrepancy of current models

**(Experimental) Results**

Possible applications

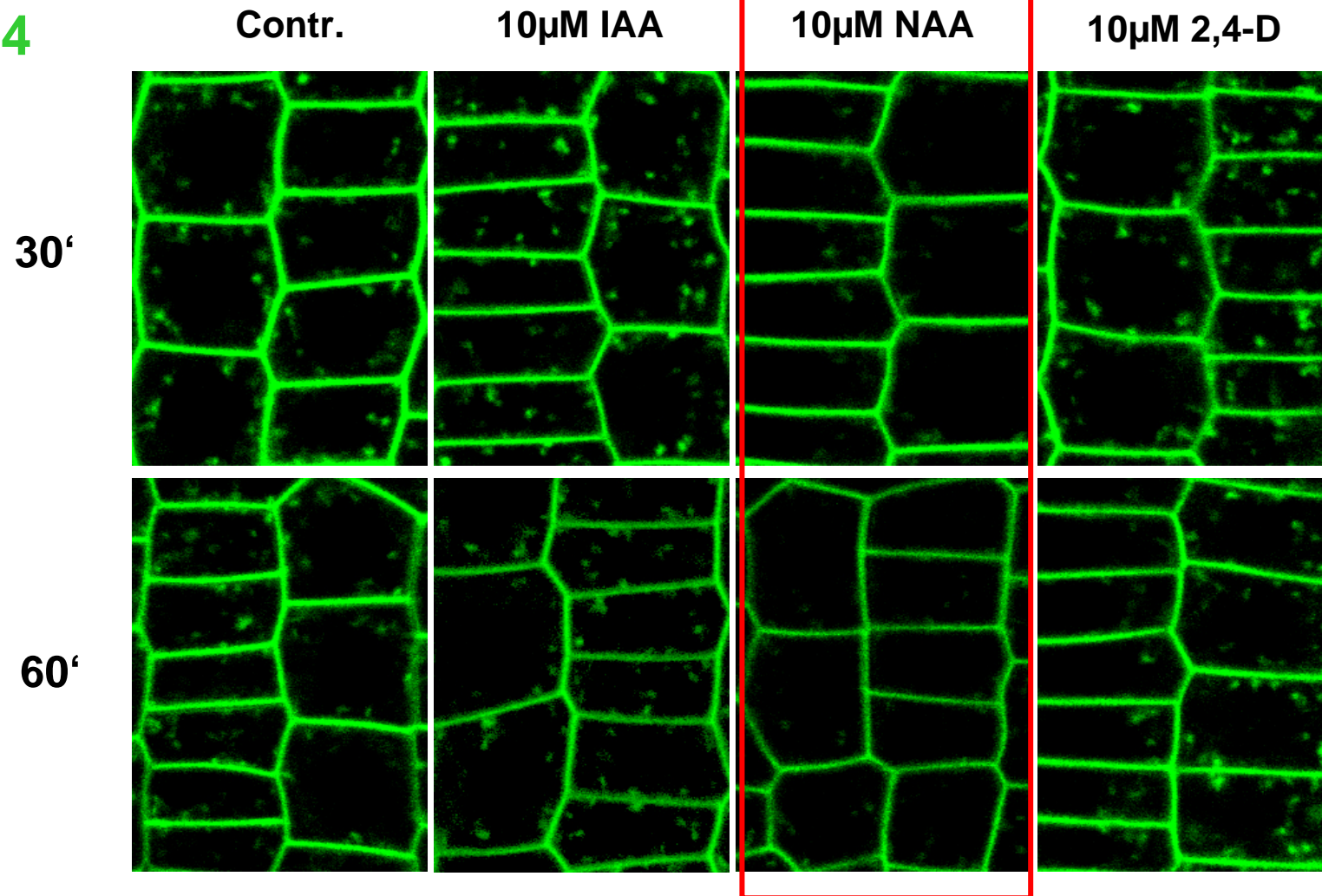


## **Methods to measure endocytosis**

- 1. Using exocytosis inhibitor (BFA)**
- 2. Using endocytosis marker (FM4-64)**

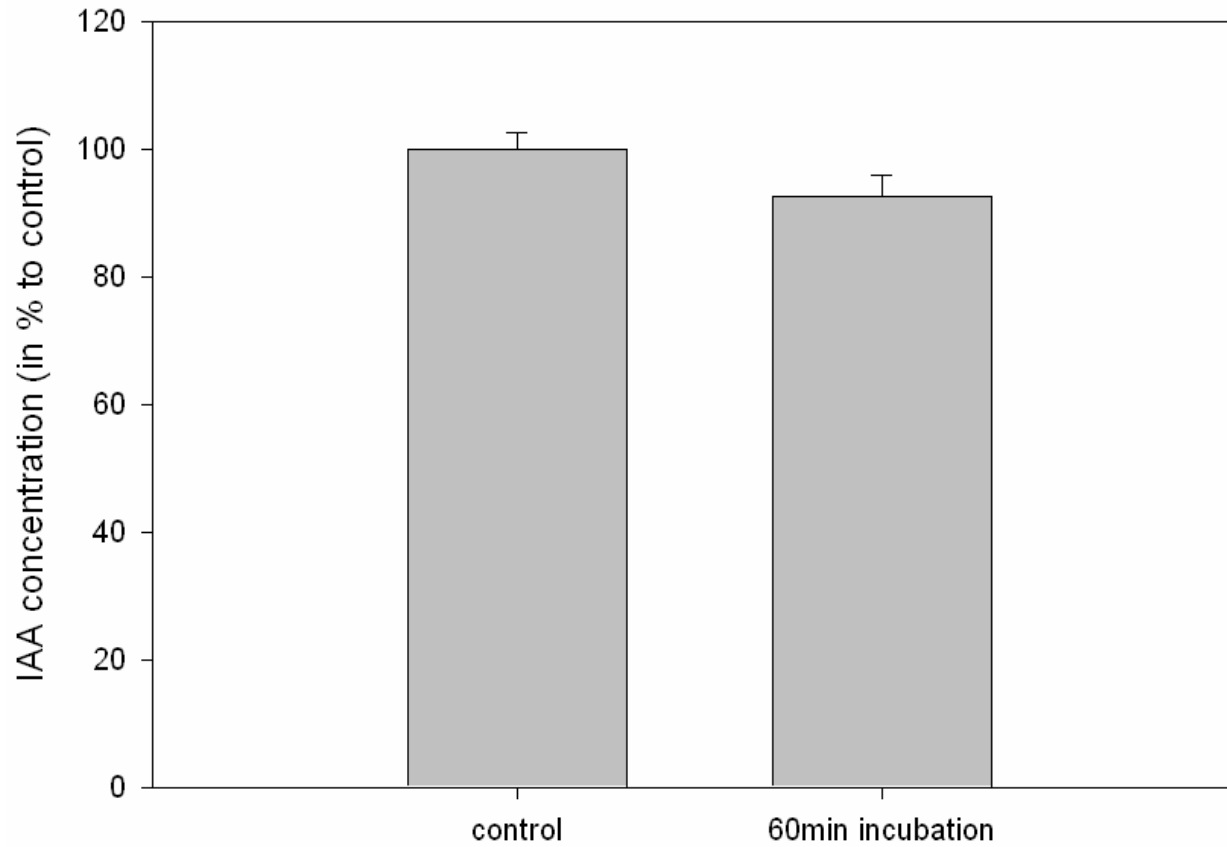
# Only 1-NAA inhibits endocytosis

FM4-64

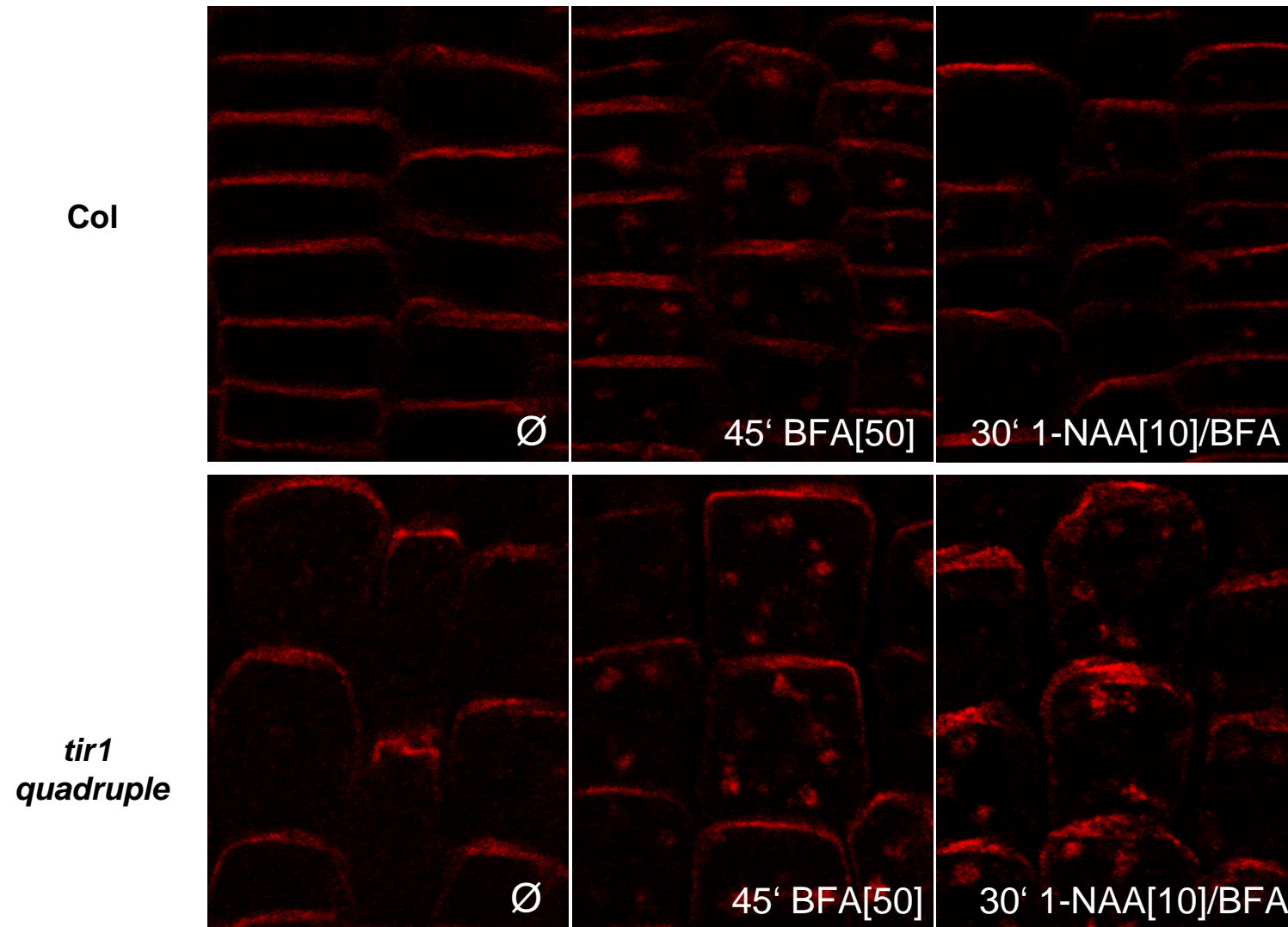


# IAA is not significantly degraded during pre-incubation period

## Concentration of IAA in the incubation solution (by HPLC)

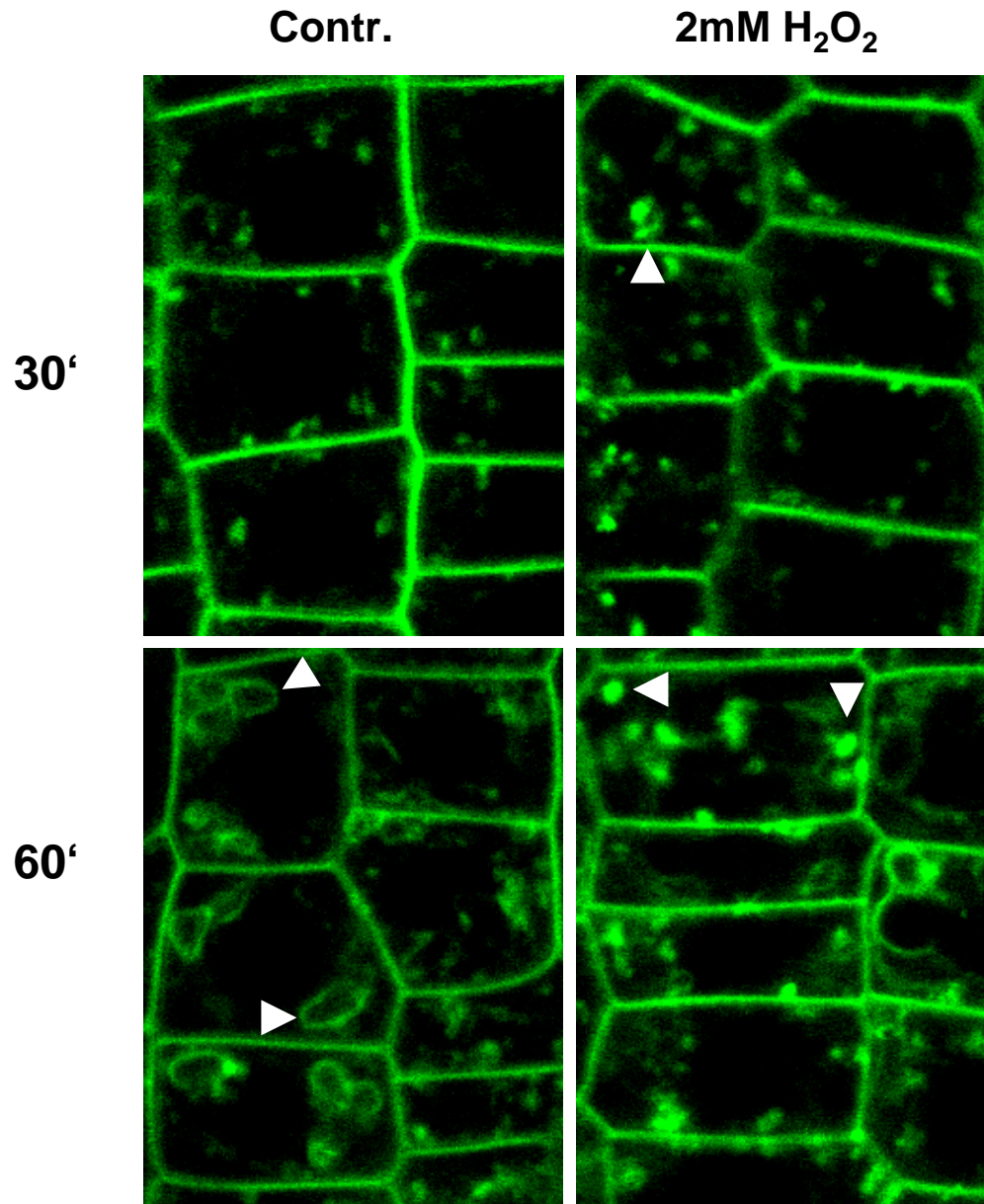


# 1-NAA does not inhibit PIN2-internalization in *tir1 quadruple* mutant



# Effect of Reactive Oxygen Species (ROS) on Endocytosis

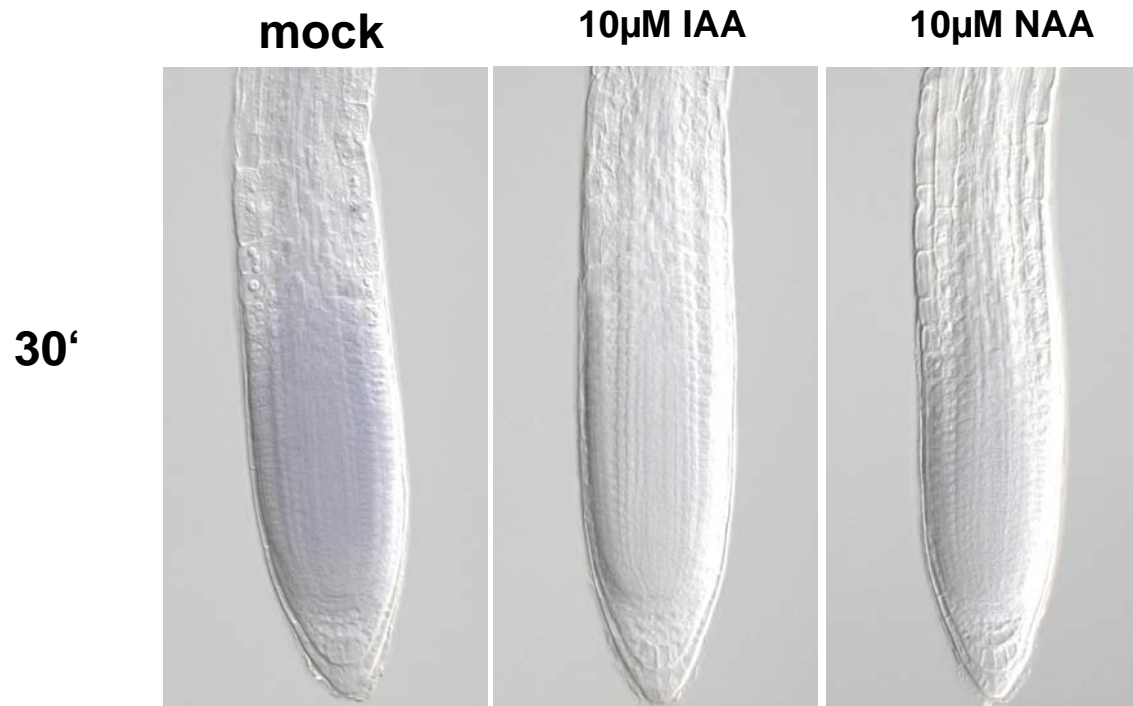
FM4-64



## Auxin reduces ROS level in the roots

Histochemical analysis ( $O_2^-$ )

NBT



**Effect of Auxin on  $O_2^-$ -concentration**

## Outline

Overarching scientific context

System under study

Key scientific questions addressed

Current methodologies (models) employed

System components

Key component behaviours

Discrepancy of current models

(Experimental) Results

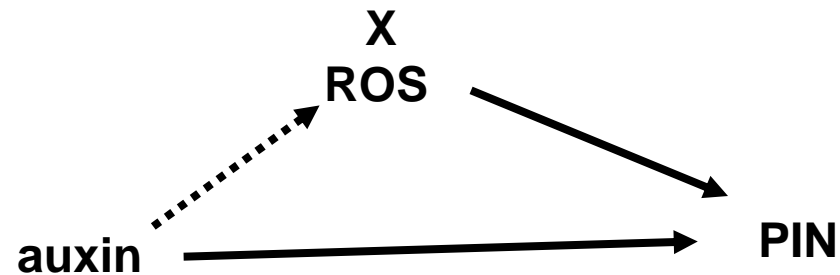
**Possible applications**

## Conclusions and possible applications

Natural auxin, IAA, does not reduce endocytosis.

ABP1 is not a sensor of auxin in regulation of endocytosis

Auxin regulates ROS, which themselves can regulate endocytosis





## Acknowledgments

**Tatyana Khodus (NAA – endocytosis)**

**Martina Paponov (chemical genetics)**

**Perrot-Rechenmann C. (abp1) CNRS, Gif sur Yvette**

**Klaus Palme**