Embryogenèse somatique végétale : mécanismes et perspectives

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Biology of the Cell and Regeneration (BCR)

Plant development biology

In vitro culture engineering

Model species and crops

Theme 1. Organogenesis

Theme 2. Somatic embryogenesis

Theme 3. Methods

Team BCR - the people

Y. & M.-C. Chupeau

C. Pannetier

2009 2011 2013 2015 2017 2019

L. Chelysheva

H. Wehbi

P. Rech

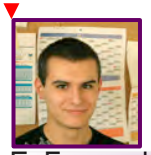
C. Soulhat

F. Aissa Abdi

O. Bouchabké

M. Da Costa

M. Louis


E. Fernandez

P. Hilson

Y. Fierlej

A. Richer

B. Kraiem

N. Wilmot

J. Saffar

R. Boumlik

Permanent

CDD

PhD

M2, Lic. Pro

1. Introduction
2. What, where, when
3. Genetics, molecular events
4. Productive perturbations
5. Perspectives

It is a long story

1902	Haberlandt: concept of in vitro cell culture
1904	Hannig: cultured embryos from cruciferous species
1926	Went: IAA discovery
1939	Gautheret, White and Nobecourt: endless proliferation of callus cultures
1941	Overbeek: addition of coconut milk for cell division in *Datura*
1946	Ball: whole *Lupinus* plants from shoot tip culture
1954	Muir: single cells from callus tissues into single cells
1955	Skoog and Miller: discovery of kinetin as cell division hormone
1957	Skoog and Miller: hormonal control (auxin:cytokinin) of organ formation
1959	Reinert and Steward: embryos from carrot callus clumps and cell suspension
1960	Cocking: protoplast isolation by enzymatic degradation of cell wall
1962	Murashige and Skoog: MS medium with higher salt concentration
1964	Guha and Maheshwari: haploid plants from *Datura* pollen grains (anther culture)
1970	Power et al.: protoplast fusion
1971	Takebe et al.: plant regeneration from protoplasts
1972	Carlson: interspecific hybrid of *Nicotiana tabacum* by protoplast fusion
1977	Integrated of Ti plasmid DNA from *Agrobacterium tumefaciens* into plant cells
1981	Larkin and Scowcroft: introduction of the term “somaclonal variation”
1983	Pelletier et al.: intergeneric cytoplasmic hybridization in radish and grape
1984	Transgenic tobacco plants produced by transformation with Agrobacterium
1987	Klien et al.: biolistic gene transfer method for plant transformation

Lingering bottlenecks despite wide range of applications

Large-scale clonal propagation of elite cultivars

Regeneration of transgenic or genome-edited plantlets

Yet…

Not possible with all plant species

Genotype dependent

Always requires optimization, still mostly via empirical approaches

Efficiency often limiting

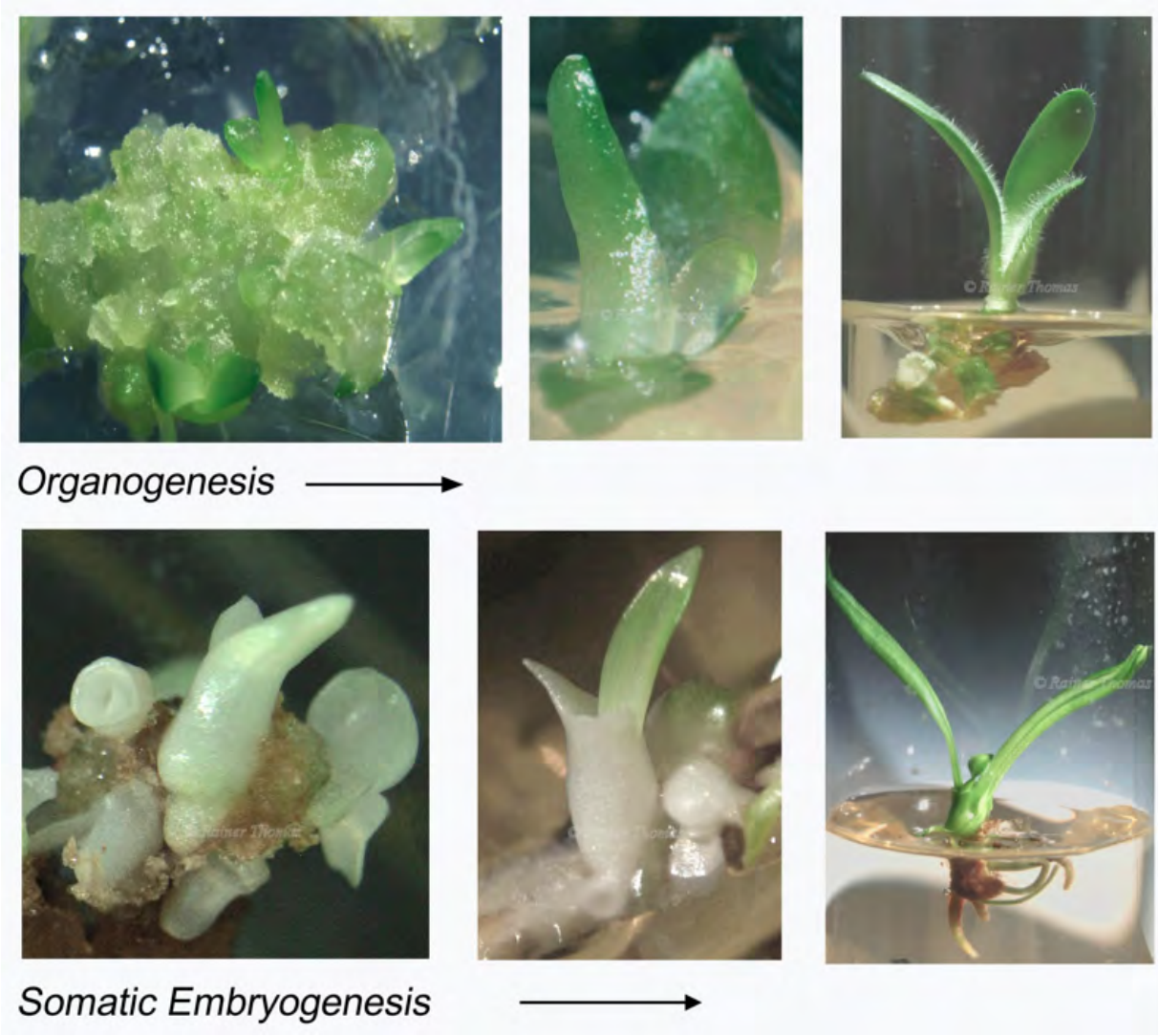
May last months, even years

Laborious and costly

Regenerative ability decreases over time

Development of somatic embryos tends to be non-synchronous

Organogenesis vs. Somatic embryogenesis



UNIPOLAR structure

shoot OR root primordium

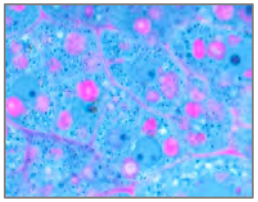
vascular system IS connected to explant

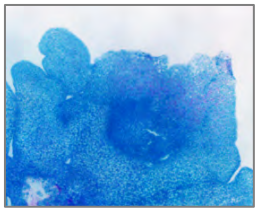
BIPOLAR structure

shoot AND root axes

vascular system IS NOT connected to parent tissue

The successive steps of somatic embryogenesis





embryonic cell fate specification

↓

cell proliferation

↓

morphogenesis meristems

↓

embryo development

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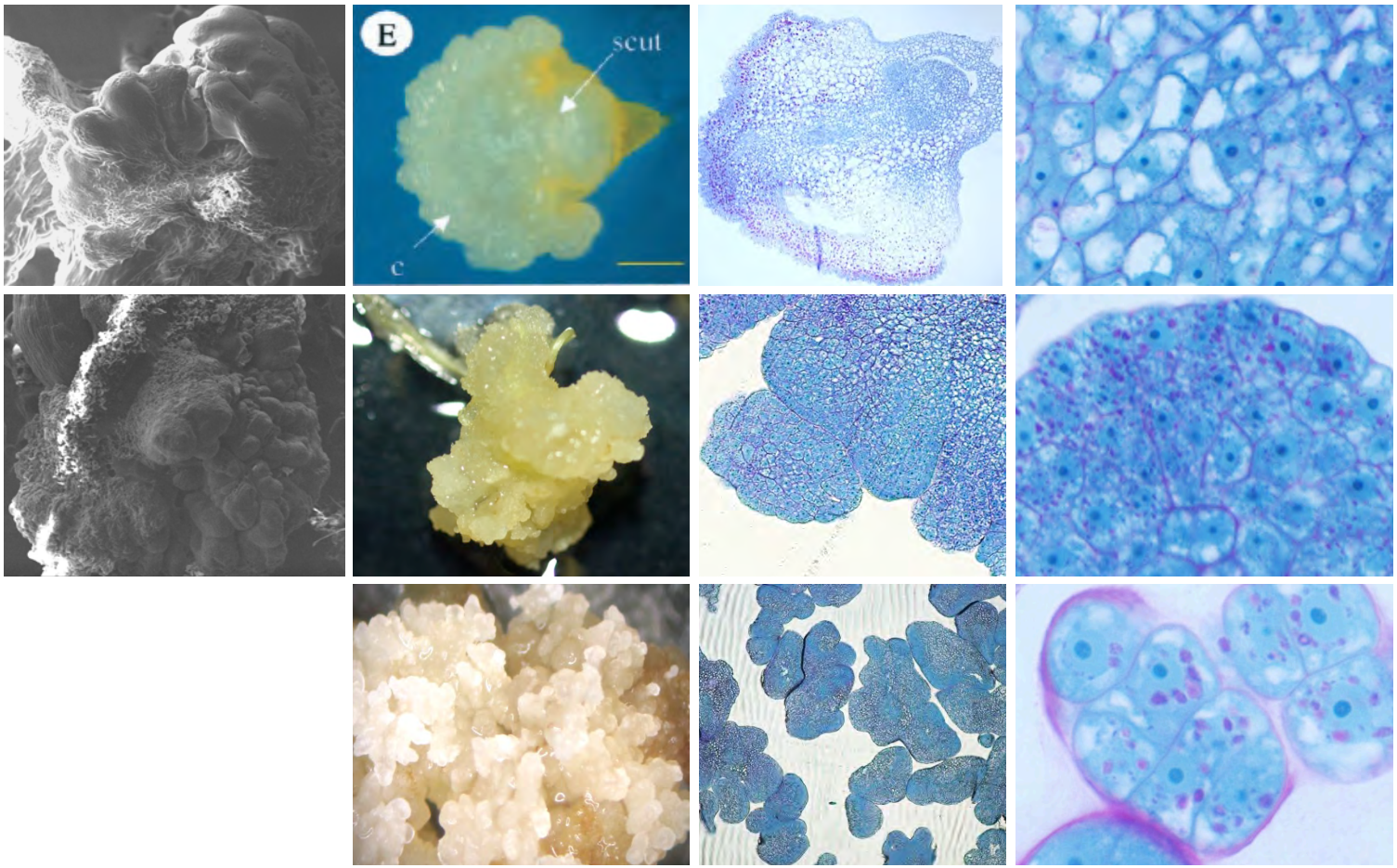
Specification of embryogenic cells occurs early in plant explants

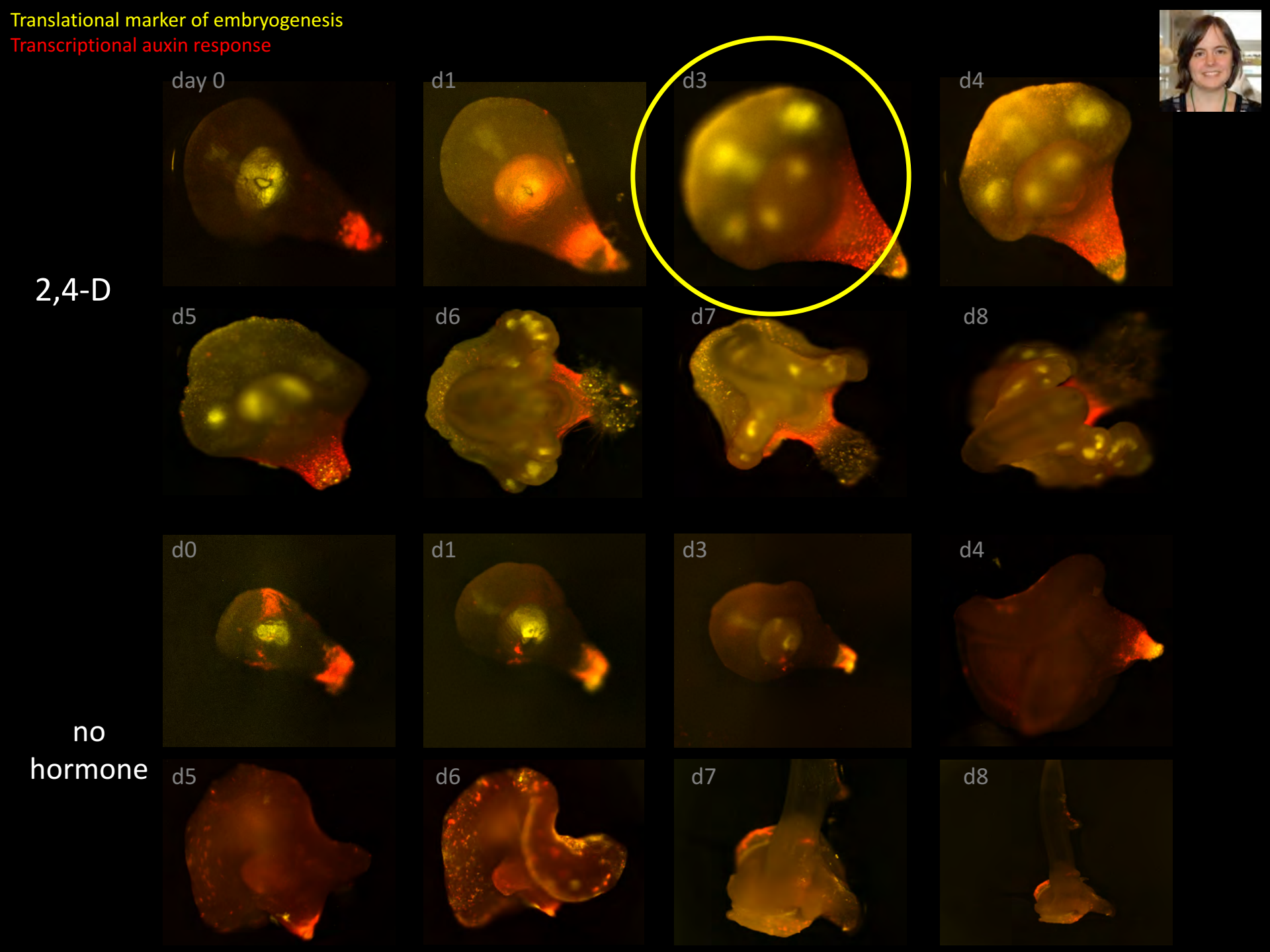
Brachypodium distachyon

week 1

week 2

week 3




Translational marker of embryogenesis
Transcriptional auxin response
day 0
d1
d3
d4
2,4-D
d5
d6
d7
d8
d0
d1
d3
d4
no hormone
d5
d6
d7
d8

Somatic embryogenesis initiation

- Occurs early in plant explants…
  long before it is macroscopically observed

- Is restricted to defined developmental windows…
  possibly explaining discrepancy between labs and part of genotype dependency

- Takes place in various tissues or cell types
  - microspores
  - protoplasts or protoplast-derived calli
  - epidermis (hypocotyl, root, cotyledon, leaf)

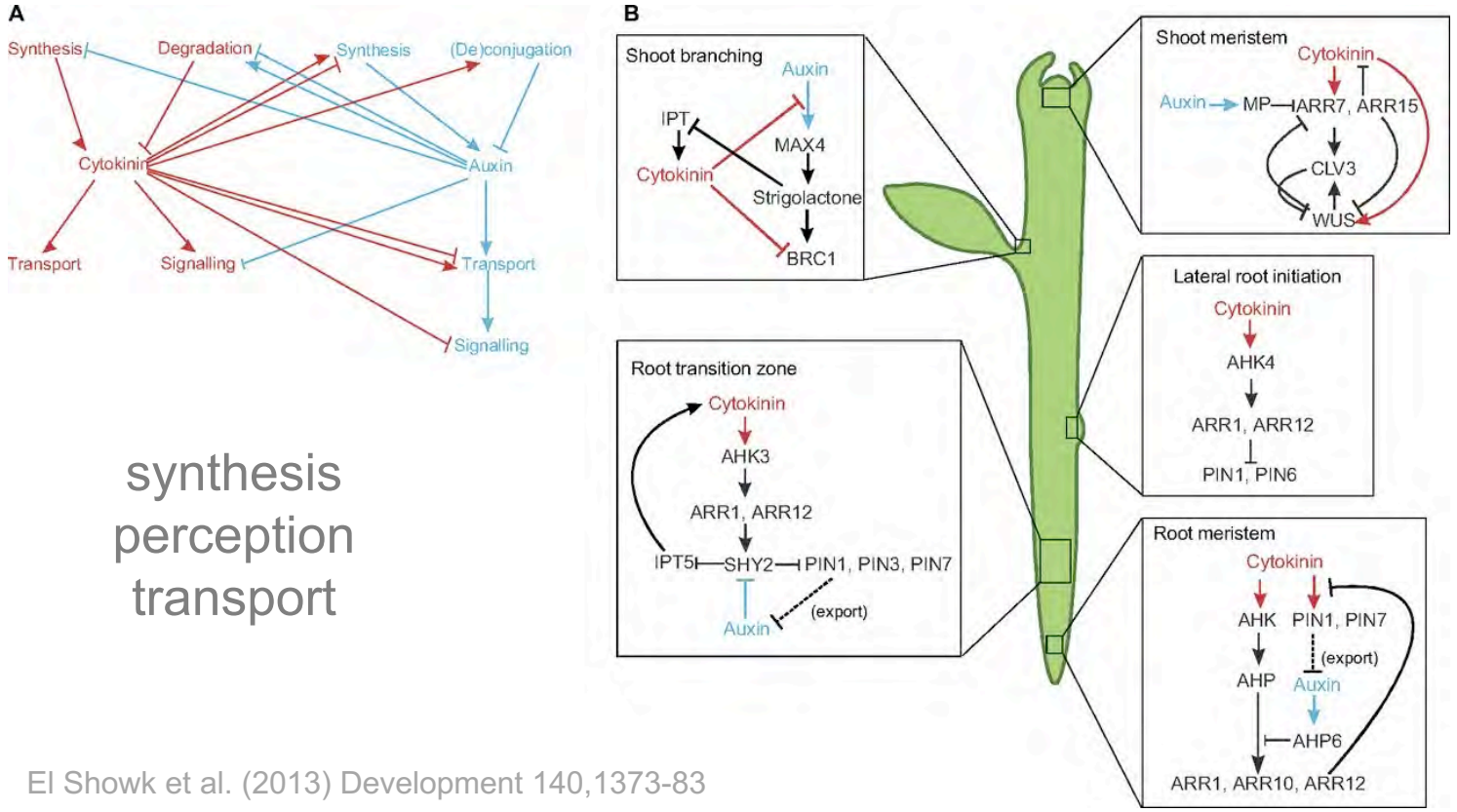
What happens in explants flooded with exogenous hormones ?

auxin > proliferation

cytokinin > differentiation

But…

- Exogenous hormone treatments and in vitro culture transfers induce stress
- Multiple cross-talks are part of the *in vitro* response, depending on context



synthesis
perception
transport

El Showk et al. (2013) Development 140,1373-83

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Genetics pinpoints functional modules involved

Genes inducing or repressing plant tissue regeneration

Locus	Common Name	Protein Family	Predicted Function	References
AT2G42430[a]	*LBD16*	LOB-domain transcription factor (TF)	Auxin response/lateral root formation	Fan et al. (2012)
AT2G42440[a]	*LBD17*	LOB-domain TF	Auxin response	Fan et al. (2012)
AT2G45420[a]	*LBD18*	LOB-domain TF	Auxin response/lateral root formation	Fan et al. (2012)
AT3G58190[a]	*LBD29*	LOB-domain TF	Auxin response/lateral root formation	Fan et al. (2012)
AT3G16857[a]	*ARR1*	GARP TF	Cytokinin response	Sakai et al. (2001)
AT5G07210[a]	*ARR21*	GARP TF	Cytokinin response	Tajima et al. (2004)
AT1G12980[a]	*ESR1/DRN*	AP2/ERF TF	Cytokinin response/shoot regeneration	Banno et al. (2001)
AT1G24590[a]	*ESR2/DRNL/BOL*	AP2/ERF TF	Cytokinin response/shoot regeneration	Ikeda et al. (2006); Marsch-Martinez et al. (2006)
AT1G78080[a]	*WIND1/RAP2.4b*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT1G22190[a]	*WIND2/RAP2.4d*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT1G36060[a]	*WIND3/RAP2.4a*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT5G65130[a]	*WIND4*	AP2/ERF TF	Wound-induced cell dedifferentiation	Iwase et al. (2011a, 2011b)
AT1G21970[a]	*LEC1*	CCAAT-box binding TF	Embryogenesis	Lotan et al. (1998)
AT1G28300[a]	*LEC2*	B3 domain TF	Embryogenesis	Stone et al. (2001)
AT5G13790[a]	*AGL15*	MADS box TF	Embryogenesis	Harding et al. (2003)
AT5G17430[a]	*BBM*	AP2/ERF TF	Embryogenesis	Boutilier et al. (2002)
AT5G57390[a]	*EMK/AIL5/PLT5*	AP2/ERF TF	Embryogenesis	Tsuwamoto et al. (2010)
AT1G18790[a]	*RKD1*	RWP-RK domain TF	Gametogenesis	Kőszegi et al. (2011)
AT1G74480[a]	*RKD2*	RWP-RK domain TF	Gametogenesis	Kőszegi et al. (2011)
AT5G53040[a]	*RKD4*	RWP-RK domain TF	Embryogenesis	Waki et al. (2011)
AT2G17950[a]	*WUS*	Homeodomain TF	Stem cell maintenance	Zuo et al. (2002)
AT3G50360[b]	*KRP2*	CDK inhibitor	Negative regulation of cell proliferation	Anzola et al. (2010)
AT5G48820[b]	*KRP3*	CDK inhibitor	Negative regulation of cell proliferation	Anzola et al. (2010)
AT1G49620[b]	*KRP7*	CDK inhibitor	Negative regulation of cell proliferation	Anzola et al. (2010)
AT5G49720[b]	*TSD1/KOR1/RSW2*	Endo-1,4-β-D-glucanase	Cellulose biosynthesis	Frank et al. (2002); Krupková and Schmülling (2009)
AT1G78240[b]	*TSD2/QUA2/OSU1*	S-adenosyl-L-Met–dependent methyltransferase	Pectin biosynthesis (?)	Frank et al. (2002); Krupková et al. (2007)
AT2G23380[b]	*CLF*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004)
AT4G02020[b]	*SWN*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004)
AT4G16845[b]	*VRN2*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004); Schubert et al. (2005)
AT5G51230[b]	*EMF2*	PRC2	Histone H3 Lys-27 trimethylation	Chanvivattana et al. (2004); Schubert et al. (2005)
AT3G20740[b]	*FIE*	PRC2	Histone H3 Lys-27 trimethylation	Bouyer et al. (2011)
AT2G30580[b]	At *BMI1A*	PRC1	Histone H2A Lys-119 ubiquitination	Bratzel et al. (2010)
AT1G06770[b]	At *BMI1B*	PRC1	Histone H2A Lys-119 ubiquitination	Bratzel et al. (2010)
AT2G25170[b]	*PKL*	CHD3/4-like chromatin remodeling factor	Histone H3 Lys-27 trimethylation and histone deacetylation (?)	Ogas et al. (1997, 1999)
AT2G30470[b]	*VAL1/HSI2*	B3 domain TF	Termination of embryogenesis	Tsukagoshi et al. (2007)
AT4G32010[b]	*VAL2/HSL1*	B3 domain TF	Termination of embryogenesis	Tsukagoshi et al. (2007)

Ikeushi et al. (2013) Plant Cell 25, 3159–3173.

auxin & cytokinin signaling

TFs / developmental transition

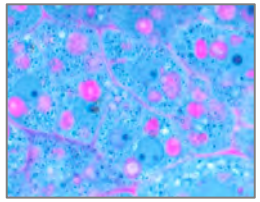
TFs / meristem homeostasis

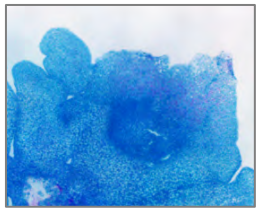
TFs / wound responses

cell wall remodeling

chromatin remodeling

Not trivial to infer sequential gene actions





embryonic cell fate

↓

cell proliferation

↓

morphogenesis
meristems

↓

embryo development

auxin
maximum ← ?

SERKs

RKD2

BBM, PLTs

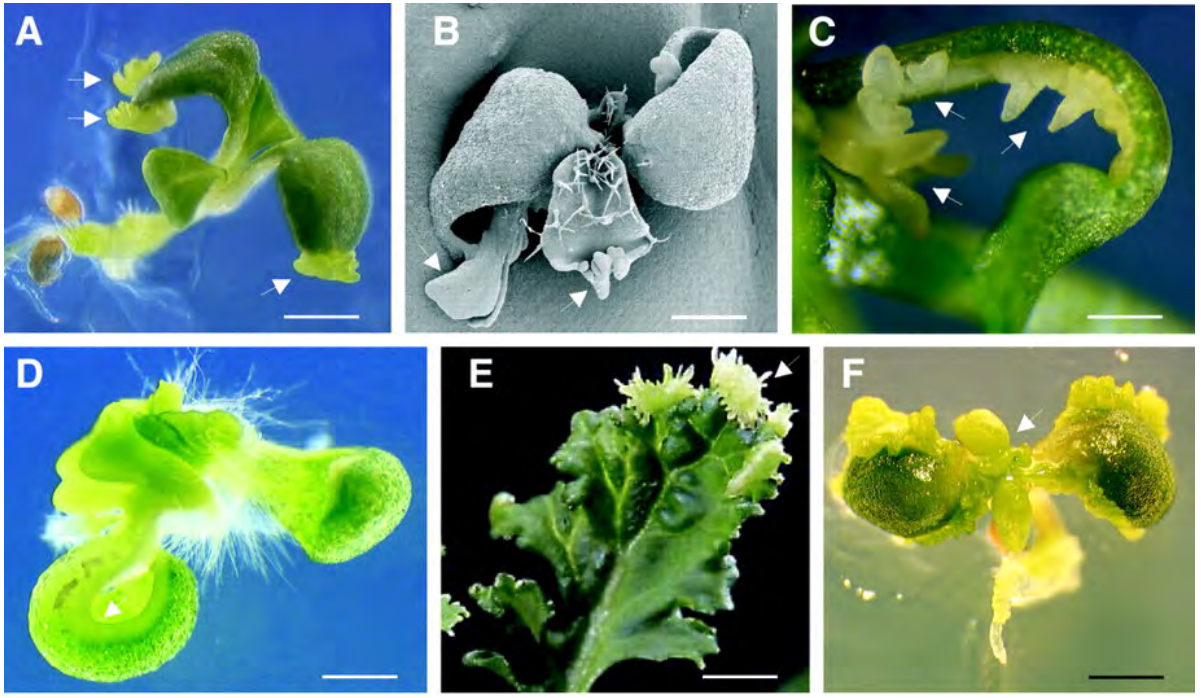
WUS

LEC1
LEC2
AGL15

PKL

signal perception
transcription factors
chromatin remodeling

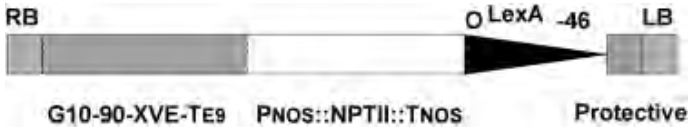
Boutilier et al. (2002) Ectopic expression of *BBM* induces somatic embryo formation in *Arabidopsis* and *Brassica*. Plant Cell 14:1737-1749

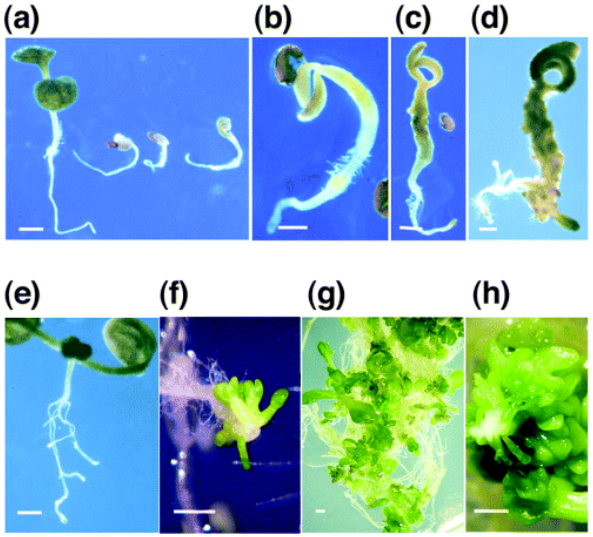


Horstman et al. (2017) Plant Physiology, Epub ahead of print

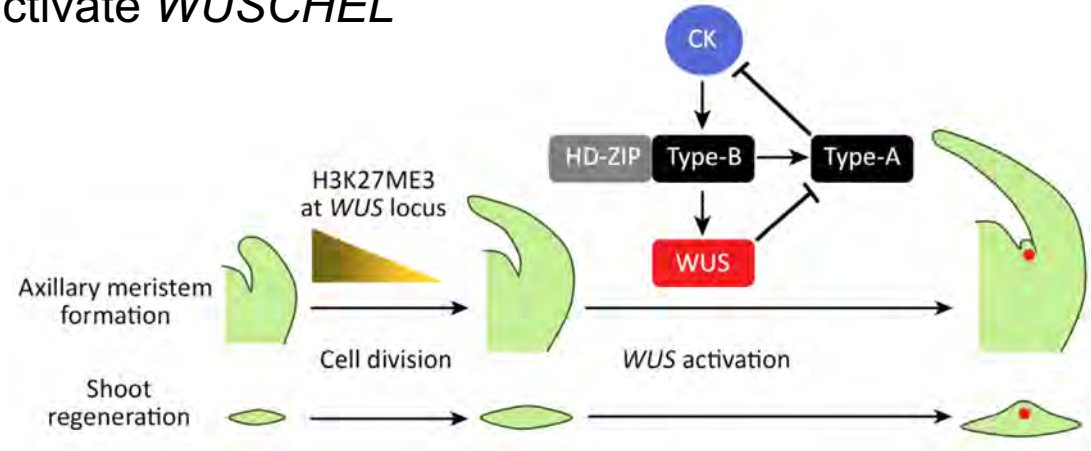
- BBM-mediated SE is dose- and context-dependent
- BBM action is relayed by LAFL genes (LEC1, LEC2, FUS3, ABI3)

Zuo et al. (2002) The *WUSCHEL* gene promotes vegetative-to-embryonic transition in Arabidopsis. Plant J. 30, 349-359



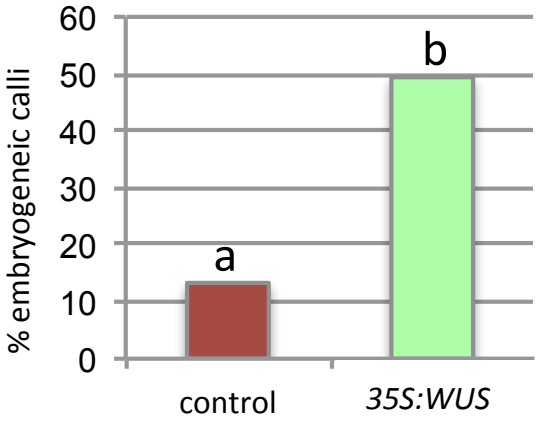


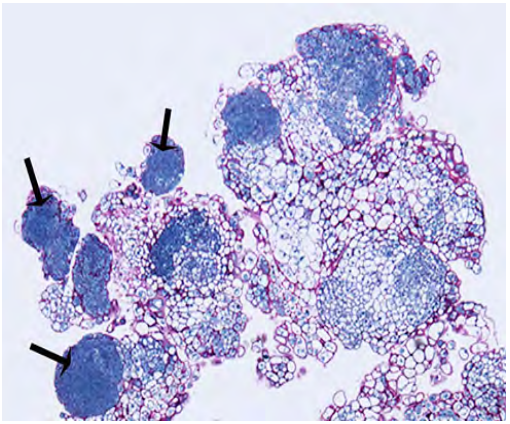
Type-B ARABIDOPSIS RESPONSE REGULATORs (ARRs) relaying the cytokinin signal directly activate *WUSCHEL*



Zhang et al. (2017) Trends Plant Sci, Epub ahead of print

*WUS c*onstitutive expression prevents true-to-type regeneration





AtWuschel promotes somatic embryogenesis and induces organogenesis in cotton

… but regenerants are rare and misshaped

Bouchabké et al. (2013) Plant Cell Rep

Morphogenic Regulators Baby boom and Wuschel Improve Monocot Transformation

by Keith Lowe, Emily Wu, Ning Wang, George Hoerster, Craig Hastings, Myeong-Je Cho, Chris Scelonge, Brian Lenderts, Mark Chamberlin, Josh Cushatt, Lijuan Wang, Larisa Ryan, Tanveer Khan, Julia Chow-Yiu, Wei Hua, Maryanne Yu, Jenny Banh, Zhongmeng Bao, Kent Brink, Elizabeth Igo, Bhojaraja Rudrappa, PM Shamseer, Wes Bruce, Lisa Newman, Bo Shen, Peizhong Zheng, Dennis Bidney, Carl Falco, Jim Register, Zuo-Yu Zhao, Deping Xu, Todd Jones, and William Gordon-Kamm

Plant Cell
Volume 28(9):1998-2015
October 10, 2016

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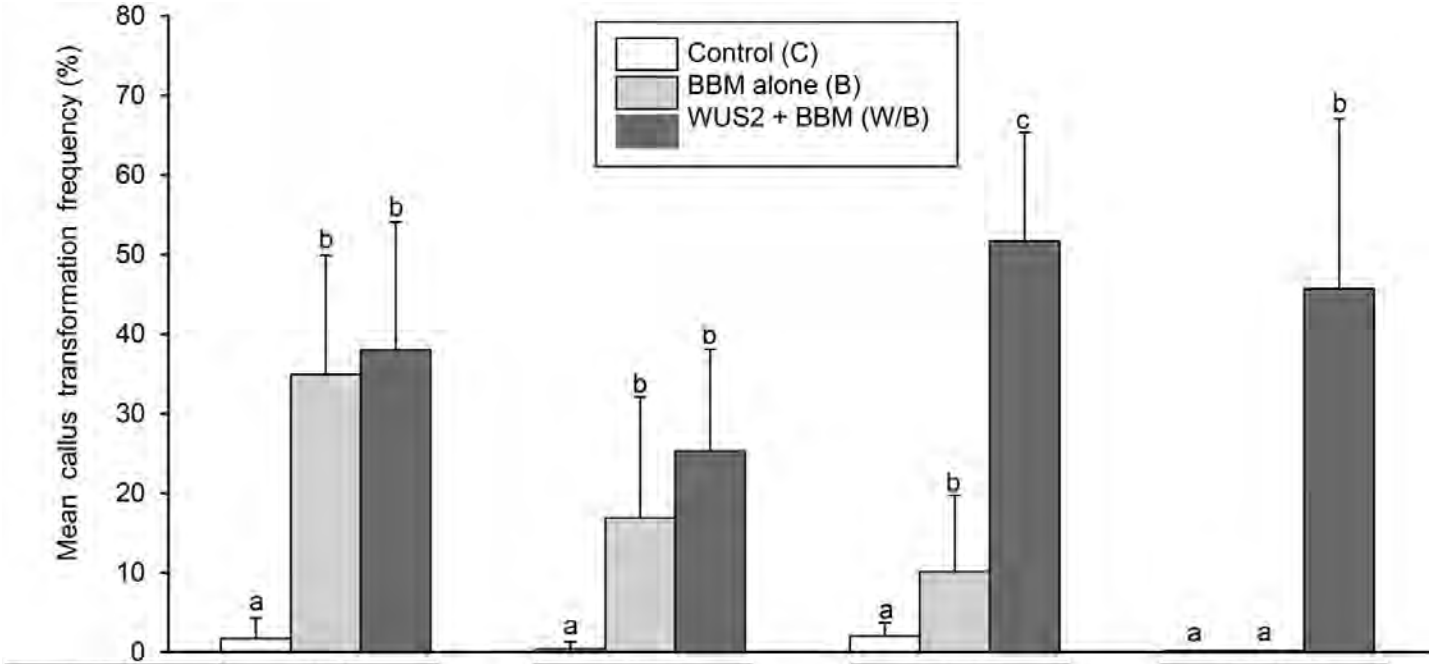
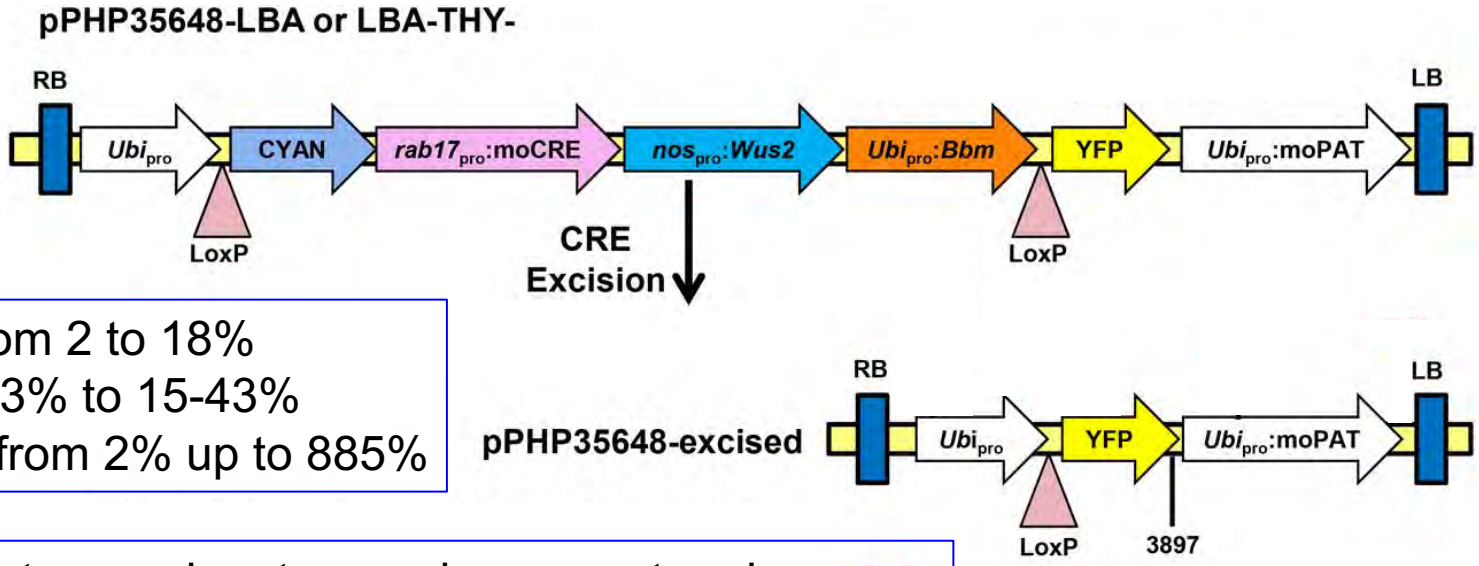
Ectopic Expression of Bbm and Wus2 Increased Transformation Frequencies in Four Maize Inbreds.



Inbred	PHN46			PH581			PHP38			PHH5G		
Treatment	C	B	W/B	C	B	W/B	C	B	W/B	C	B	W/B
Total ears	43	24	19	48	27	21	33	17	16	6	18	703
Total embryos	1716	948	758	1987	1130	815	1216	564	644	240	555	28120
Total Txn Calli	31	341	283	5	162	202	25	58	330	0	0	12851
Regeneration %	ND	ND	61	ND	ND	81	ND	ND	60	ND	ND	50
Single-copy %	ND	ND	60	ND	ND	33	ND	ND	25	ND	ND	67
Excision %	ND	ND	69	ND	ND	60	ND	ND	67	ND	ND	89
Quality Event %	ND	ND	9.6	ND	ND	4.0	ND	ND	5.2	ND	ND	13.7

Lowe et al. (2016) Plant Cell

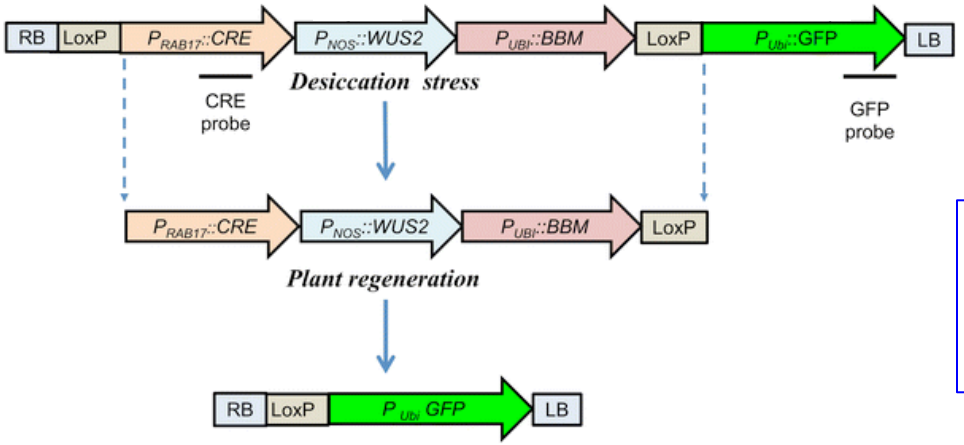
Lowe et al. (2016) Plant Cell 28, 1998-2015



In sorghum: from 2 to 18%
In rice: from 0-3% to 15-43%
In sugarcane: from 2% up to 885%

Explants: immature and mature embryos, mature leaves

Mookkan et al. (2017) Plant Cell Rep. 36, 1477–1491



Recovery of transgenic maize and sorghum plants from recalcitrant genotypes, without selectable marker

Lessons learned

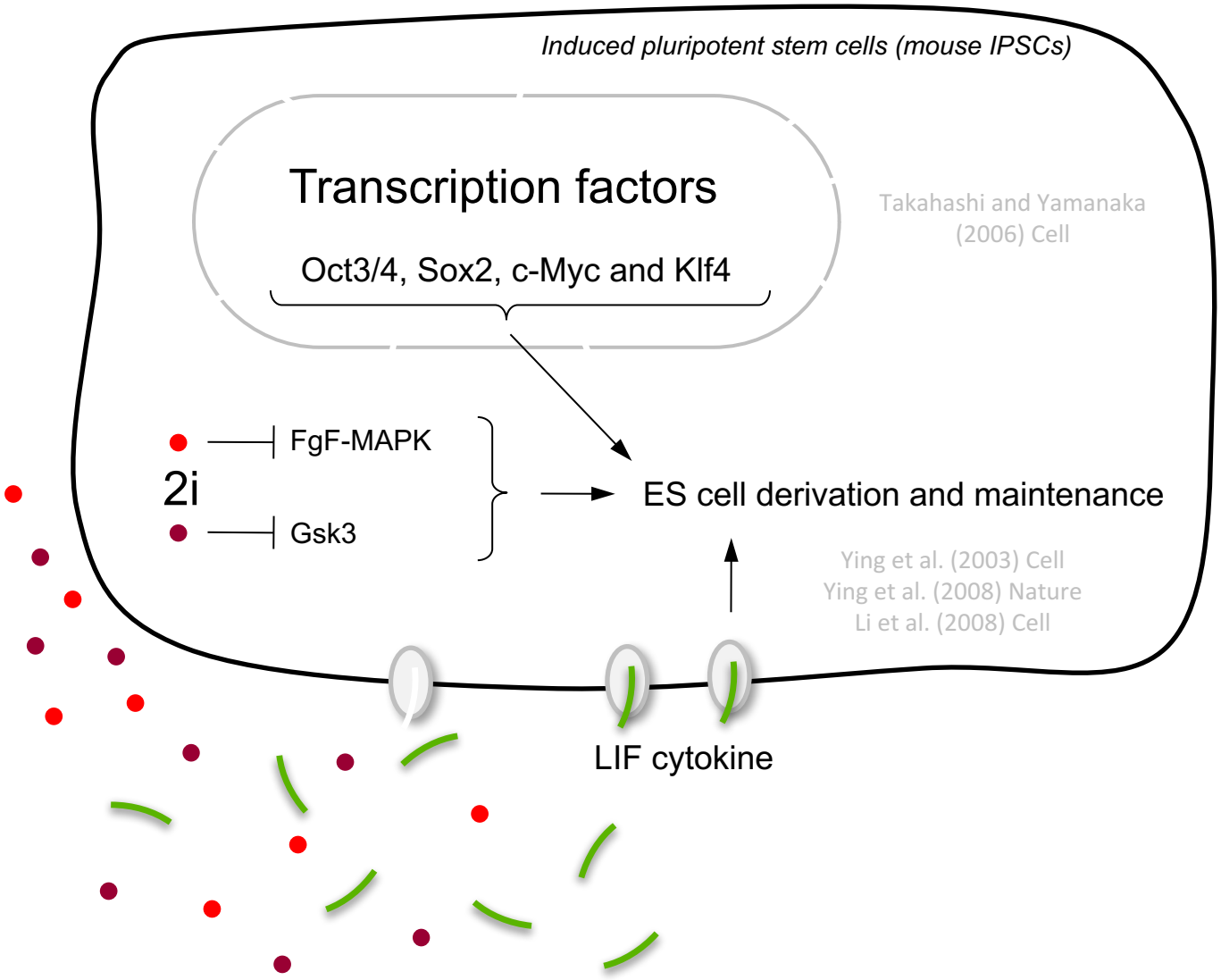
- Research in model species does lead to breakthroughs in crops
- The translation may be laborious, even for relatively simple ideas
- It is possible to transiently induce genes drivers of totipotency in plant cells, and thereby to boost regeneration of true-to-type plants

BUT IT'S ALL TRANSGENIC STUFF !

Driving development with chemicals

genetic reprogramming

chemical reprogramming



Defined medium to produce naive human pluripotent stem cells
Gafni et al. (2013) Nature

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Novel bioactive molecules to improve *in vitro* culture methods

- To induce reversible phenotypes
- To circumvent genetic redundancy and lethality
- To target specific branches of signalization pathways

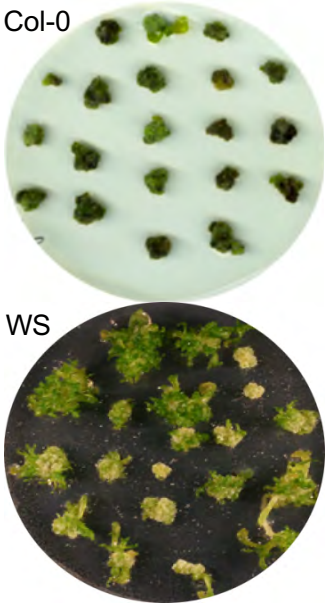
- To improve IVC protocols
- To study developmental switches

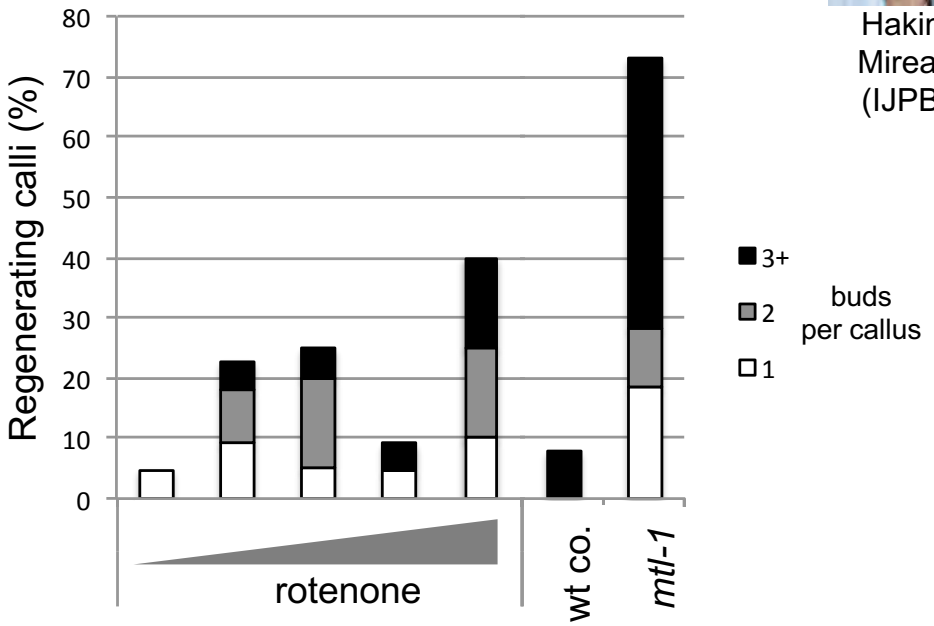
- Rational choice of potentially favorable perturbators
- Large-scale screens with no *a priori* knowledge (structure, function)

A mitochondrial defect boosts shoot initiation

Hakim Mireau (IJPB)

Shoot meristems on Arabidopsis calli





Partial genetic or pharmalogical inhibition of the mitochondrial electron transport chain (complex I) enhances organogenesis

Reproduced in crops for better organogenesis or somatic embryogenesis (INRA labs)

Inhibitors of enzymes altering epigenetic marks

Trichostatin A, inhibitor of histone deacetylases (HDACs), promotes totipotency in the male gametophyte (Li et al., 2014; *Arabidopsis thaliana*, *Brassica napus*)

BIX-01294, inhibitor of histone (H3K9) methylation, promotes stress-induced microspore totipotency and enhances embryogenesis initiation (Berenguer et al., 2017; *Brassica napus*, *Hordeum vulgare*)

But what happens after the early stages?

Is it a good idea to use pleiotropic inhibitors of epigenetic regulatory mechanisms to boost regeneration?

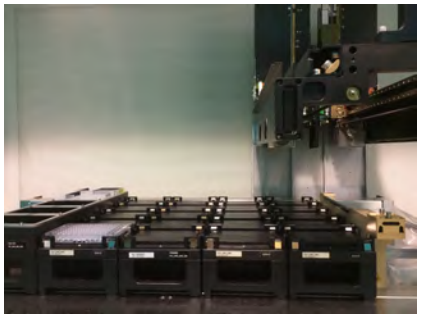
Compound library screens

Novel molecules led to important discoveries

- Pyrabactin: perception of abscissic acid (Park et al., 2009)
- Bikinin: induction of brassinosteroid pathway (De Rybel et al., 2009)
- Naxillin: lateral root development (De Rybel et al., 2012)
- Phényl-adénine: induction of adventive caulogenesis adventive (Motte et al., 2013)

Practical aspects

- Library choice
  - large, selected backbones, combinatorial synthesis
  - targeted bioactivity (kinases, phosphatase, chromatin, etc.)
- Sampling of library in sterile conditions (long cultures on rich media!)



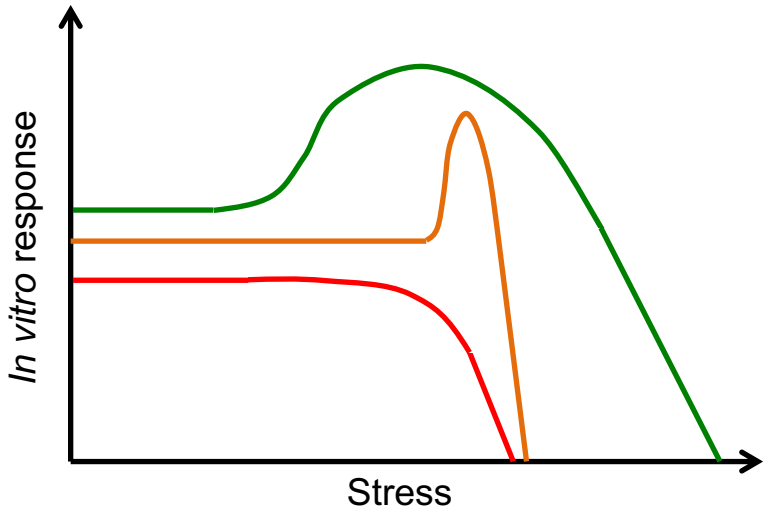
- Miniaturization of *in vitro* response assays
- Automation of read-out (image acquisition, simple plug-ins)

Stress used to enhance SE for decades

Temperature, salt, osmoticum, UV, hypoxia

- How is it relayed at the cell level?
  - mechanical constraints?
  - redox pathways (ROS)?
  - which subcellular compartments are involved?
- Are all stresses relayed through the same mechanisms?

- Why does it work only sometimes, only with some explants?



dsRNA applied on plant tissues induces RNAi

nature plants

ARTICLES

PUBLISHED: 9 JANUARY 2017 | VOLUME: 3 | ARTICLE NUMBER: 16207

Clay nanosheets for topical delivery of RNAi for sustained protection against plant viruses

Neena Mitter[1]*, Elizabeth A. Worrall[1], Karl E. Robinson[1], Peng Li[2], Ritesh G. Jain[1], Christelle Taochy[1,3], Stephen J. Fletcher[1,3], Bernard J. Carroll[3], G. Q. (Max) Lu[2,4] and Zhi Ping Xu[2]*

Silencing detectable

Long-term release

Useful in the field?

Active on in vitro tissues?

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5. Perspectives

Embryogenèse somatique végétale – que sait-on vraiment ?

- Gènes clés et modules fonctionnels ont été identifiés
- Certaines connexions sont bien caractérisées dans les espèces modèles, mais beaucoup restent inconnues
- Compréhension croissante de l'homéostasie des méristèmes (tige, racine, cambium)
- Progrès dans la dissection de l'embryogenèse zygotique, mais étude génétique et accès problématiques

- Pas de modélisation exhaustive de la morphogénèse
- L'analyse détaillée de la réponse des explants aux niveaux cellulaire et moléculaire commence seulement à pointer des voies d'améliorations méthodologiques, au-delà d'approches empiriques
- Les modèles mathématiques ne permettent pas d'expliquer et encore moins de prédire la réponse *in vitro* d'explants

- La cascade précise d'événements aboutissant à l'initiation de l'ES est inconnue
- Cause(s) de la dépendance génotypique inconnue(s), probablement multiples
- Difficile de comprendre comment les connexions entre modules évoluent / diffèrent selon les clades

Quelles directions ?

- Mieux comprendre les étapes de la morphogénèse
  - microscopie, hybridation *in situ* (modèles et espèces cultivées)
  - marqueurs (génétiques, épigénétiques, structuraux)
  - fenêtre compatible avec transition développementale
- Optimiser les perturbations productives
  - moment clé, dose, transitoire
  - phytohormones, stress, inhibiteurs spécifiques, peptides, RNAi
  - combinatoire
- Rapprocher entreprises, labos R&D, chercheurs
  - ASF, RegenCrop, GIS Biotechnologie Vertes, etc.
  - contacts directs entre acteurs
  - formations mutuelles