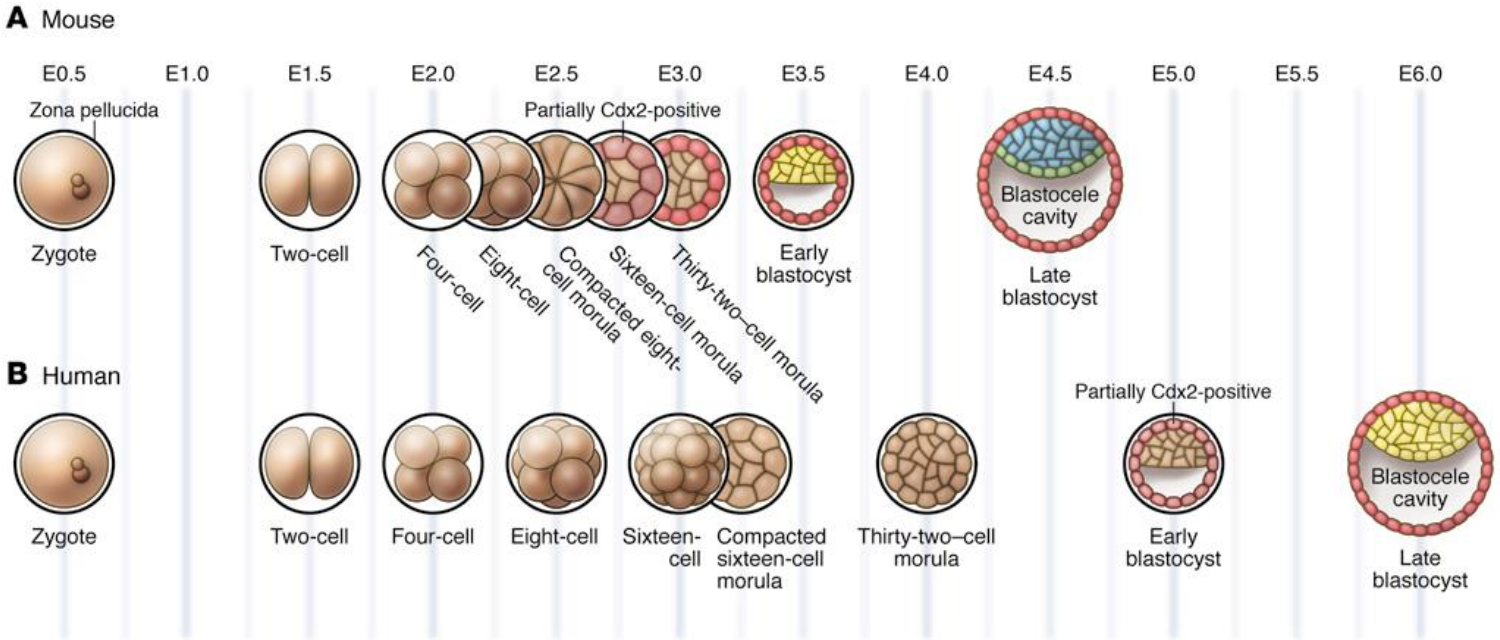
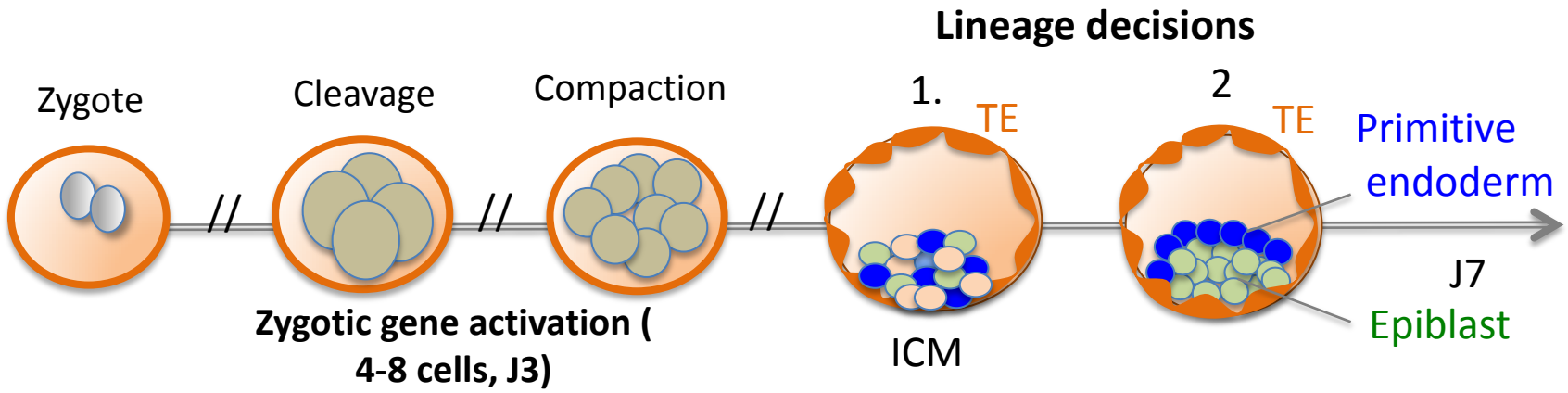
Research programs involving human early embryos



1. Understanding normal mammalian embryo development
2. Understanding errors in genetic and epigenetic programs
3. Providing research and therapeutic tools (derivation of human embryonic stem cells)

Rossant et al, J Clin Invest 2010

Exploring early human preimplantation development at cellular and molecular resolution



Gene expression profiling (microarray)
Single cell transcriptome analysis (RNA seq)
Epigenetic profiling
Imaging techniques (time-lapse)

Gamete reprogramming
Blastomere heterogeneity / Early asymmetry
Temporal EGA
Discrimination of germ layers specification

Ex vivo dynamic studies
(signaling response to GF or inhib)

Molecular determinants of lineage restriction

Research programs in France

1. Biomarkers for IVF outcome

- BCL2L10 expression in early human embryos : different distribution in viable embryos (mitochondria) and non-viable (nucleus). (*A. Aouacheria, JF Guérin, 2008).*
- Expression of anti- and pro-apoptotic BCL-2 family genes during human early embryonic development ; correlation of apoptosis-related genes with the onset of apoptotic events. (*S. Hamamah, 2007, 2012).*
- Expression of CD146 in embryos *(O. Lacroix, 2014)*

2. Genomic profiling

- Dissecting TE versus ICM transcriptional divergence during human embryonic development : the human trophectoderm expression signature*. (J. De Vos, 2007).*
- Genomic profile during early embryonic development : early embryonic development, particularly totipotent cells and highly pluripotent cells (hESCs), have quite distinct genetic programs (J *De Vos, S. Hamamah*)
- Single cell RNA seq of preimplantation embryos *(L. David, 2014)*

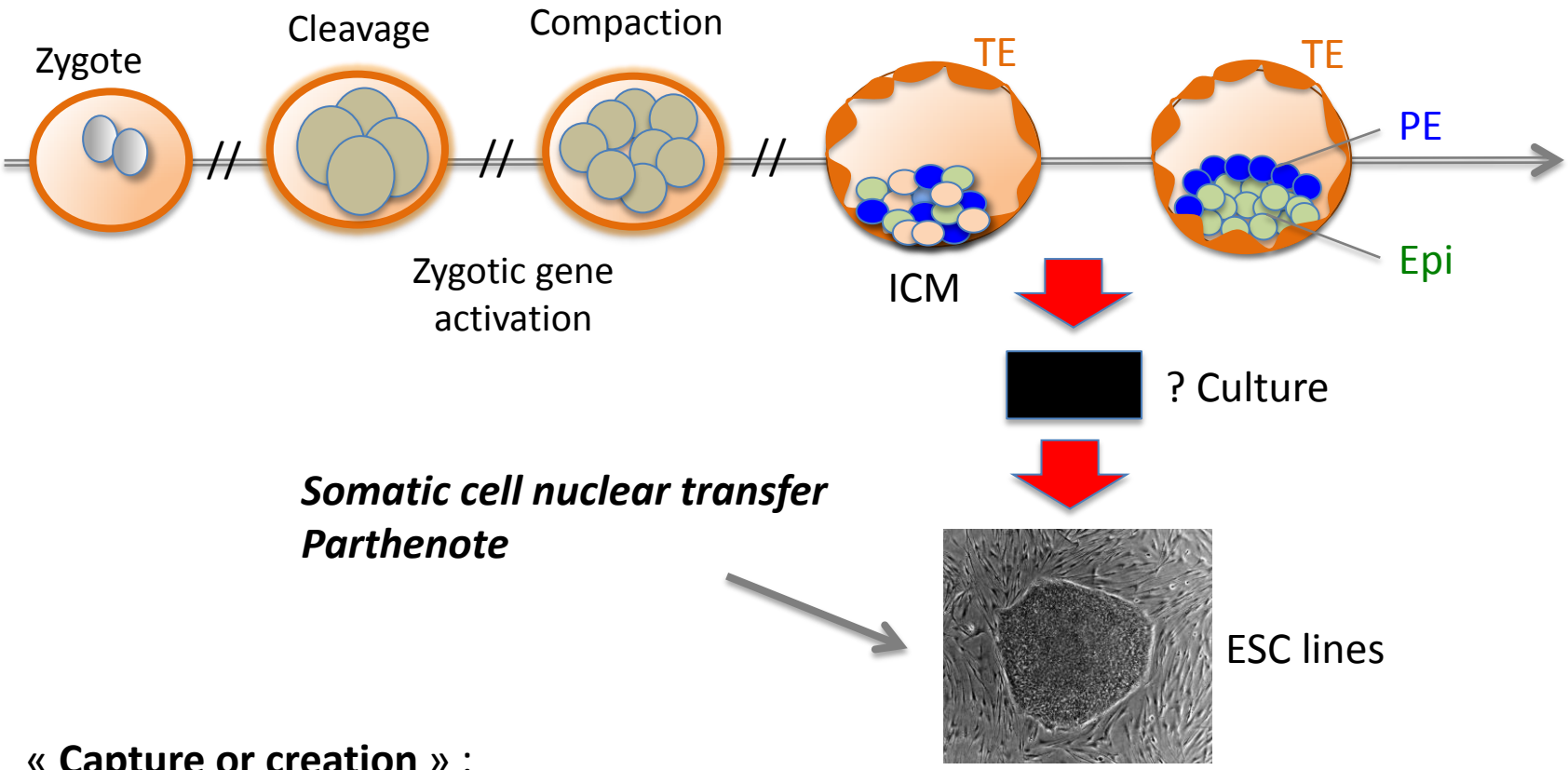
3**. Epigenetic reprograming during embryo development**

- X inactivation as a model of epigenetic reprograming in human embryos (*C. Patrat, 2007, 2011*)

4. **Understanding errors in genetic and epigenetic programs**

- mtDNA mutations during embryo development : mtDNA segregation (heteroplasmic ooocytes, individual blastomeres), copy number, and gene expression in embryos with mtDNA mutations (NARP, MELAS and MERF syndromes) (*JP Bonnefont, J Steffan, 2007*)
- Epigenetic status of embryonic genes in normal and arrested embryos. methylation status of the Nanog and Oct4 promoters and of the imprinted H19 maternal allele (Developmental failure after ICSI) *(A. Lefèvre, JF Guérin, 2008).*
- Chromosomal segregation in oocytes and embryos in relation with maternal age *(S. Hamamah 2007)*

The human preimplantation embryo : source of pluripotent stem cell lines



1. « **Capture or creation** » :
- stabilisation in culture of transitory states in the embryo
- « reprogramming » unrelated to in vivo ICM /Epi cells ?

2. We have differentiated these cells to specialized cells without knowledge of the relevant stages of human development in vivo

Dérivation de nouvelles lignées de CSEh

28 lignées – 4 équipes autorisées (couplées à un centre AMP)
Un dossier en cours d'examen

S. Viville (Strasbourg)
~ 261 embryons (de 70 DPI)
19 lignées, toutes porteuses mutations (Huntington, mucovisc, X fragile, neurofibromatose, polypose, MTMX)

Annelise Bennaceur /G. Tachdjian (Villejuif)
~ 168 embryons DPI /normaux
3 lignées (déséquilibre chromosom)

John De Vos / Samir Hamamah (Montpellier)
~ 300 embryons
5 lignées
2 normales, et 3 porteuses mutations (mucovisc, von Hippel Lindau)

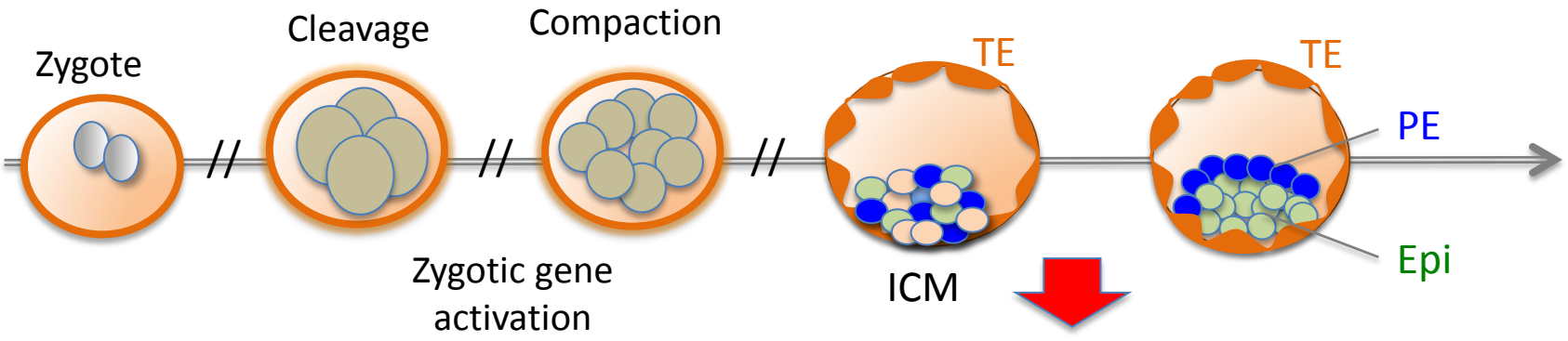
Pierre Savatier (Lyon)
~ 117 embryons
I lignée normale

Xxxx
Un dossier en cours d'examen

11 lignées utilisées dans les projets actuellement en cours

~ les nombres d'embryons utilisés sont donnés à titre indicatif

The human preimplantation embryo : source of pluripotent stem cell lines



Gene expression profiling *(J. De Vos)*
Single cell profiling *(L. David)*
Epigenetic profiling - X inactivation *(C.Patrat, C. Rougeulle)*
systems-level comparison of transcriptome data

1. « **Capture or creation** » :

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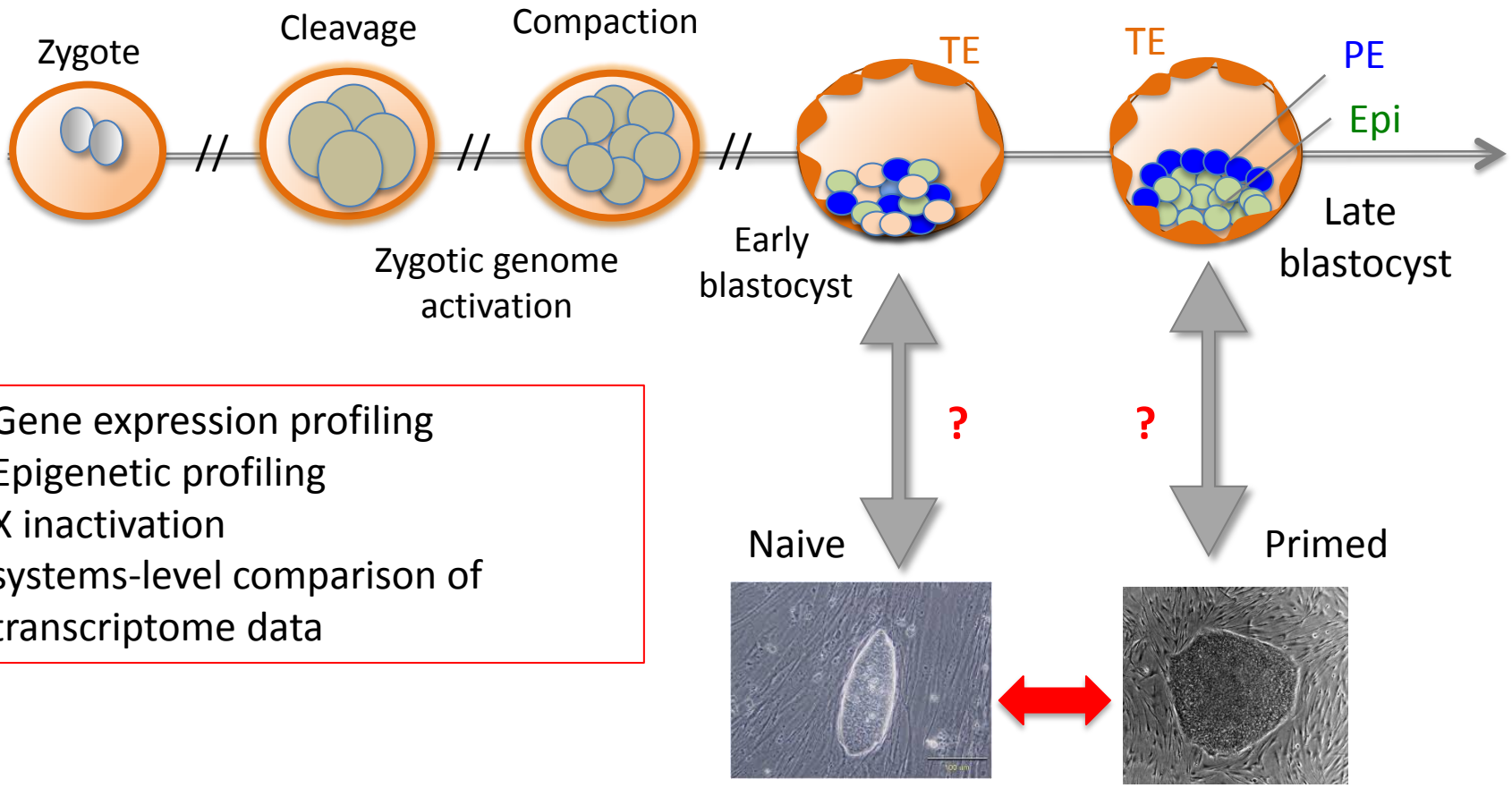
Pluripotent stem cell lines : what relevance to embryo development?

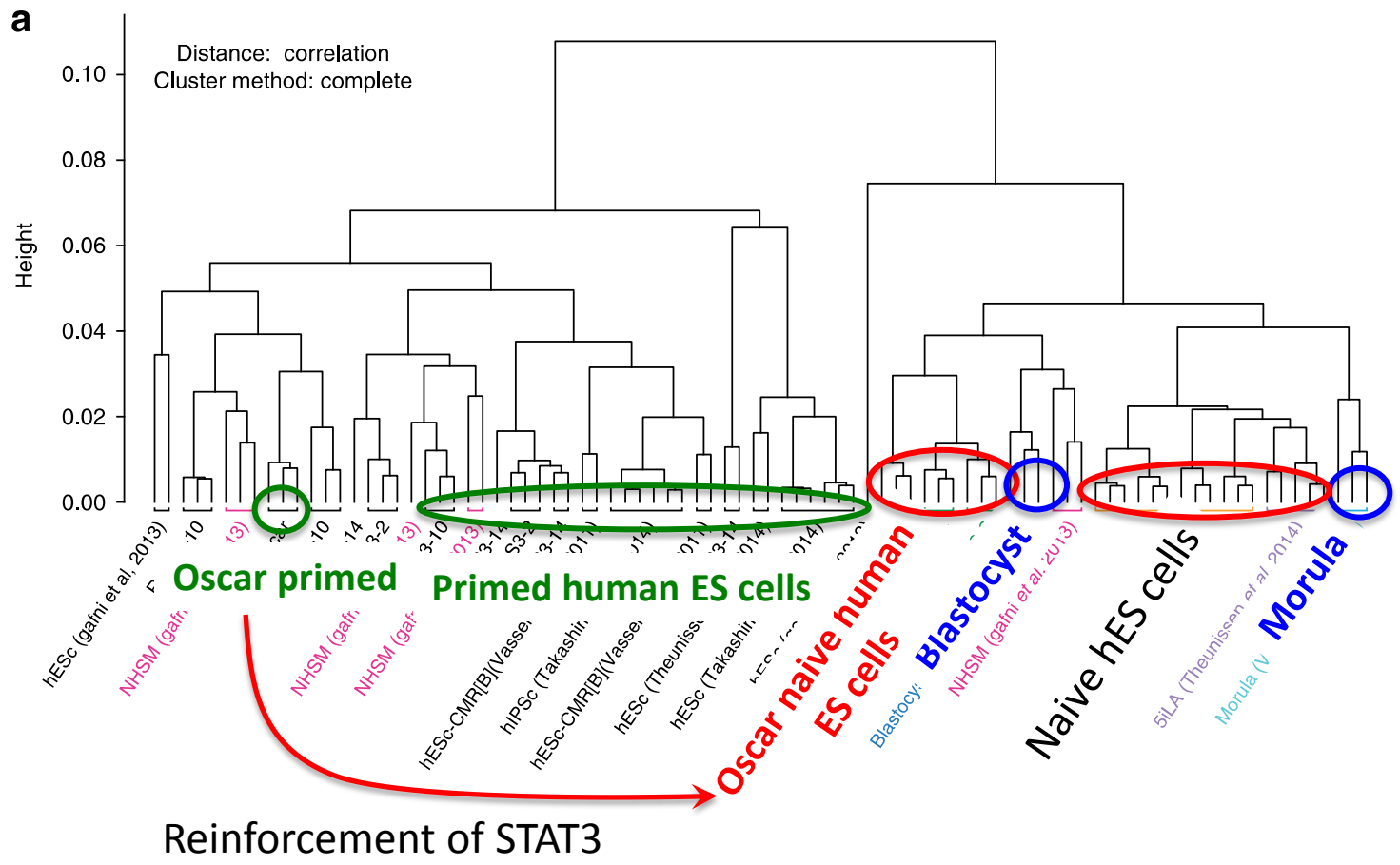


Gene expression profiling
Epigenetic profiling
X inactivation
systems-level comparison of transcriptome data

1. Do naive and primed ES cells represent discrete stages of early embryo development ?
2. Does in vitro plasticity reflect in vivo heterogeneity?
3. Can we identify in developing embryos specifically expressed genes that will help deriving relevant cell lines ?

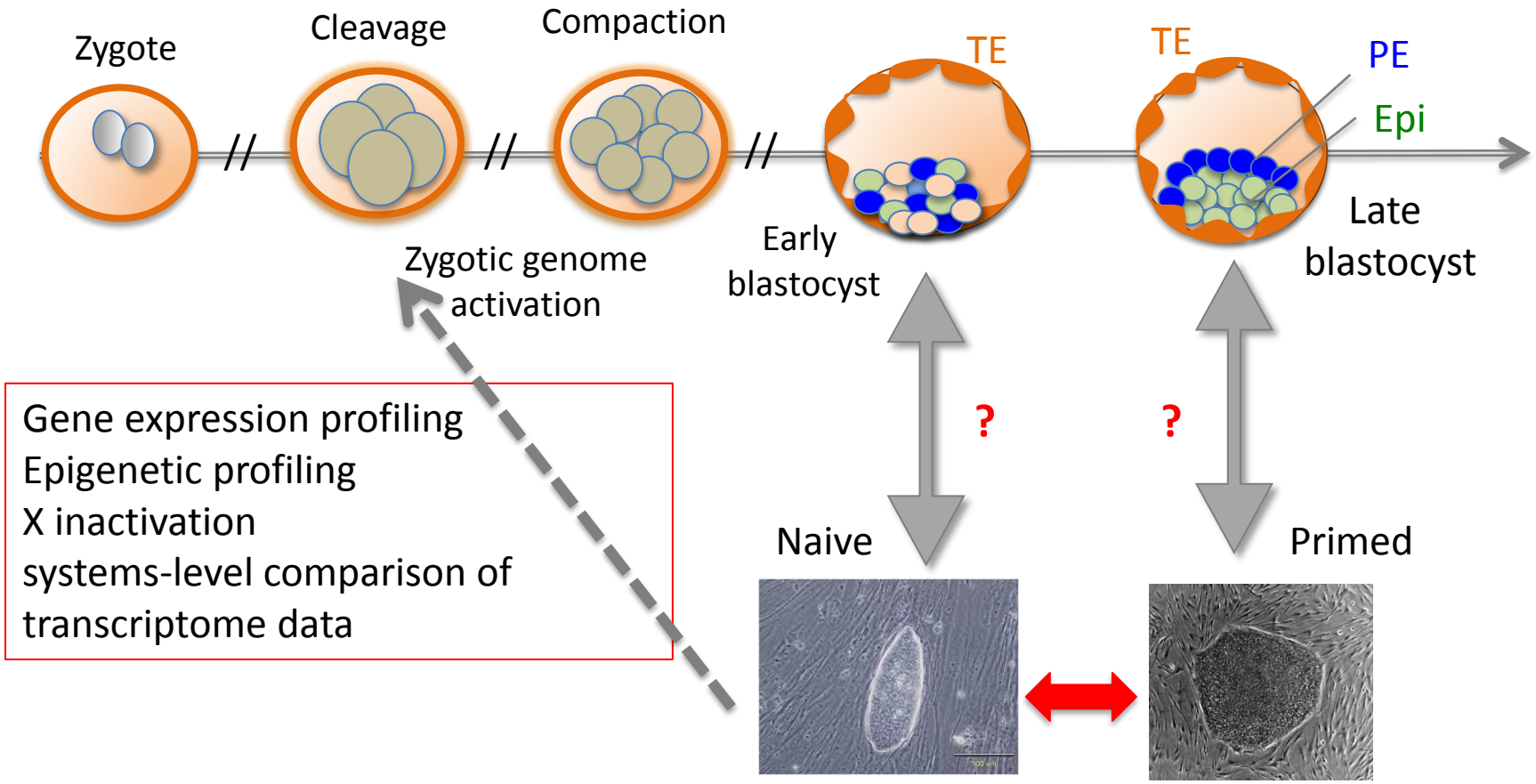
How close to human embryos are naive and primed human ES cells

Correlation clustering of transcriptome data



Labo P. Savatier, Nat Comm May 2015

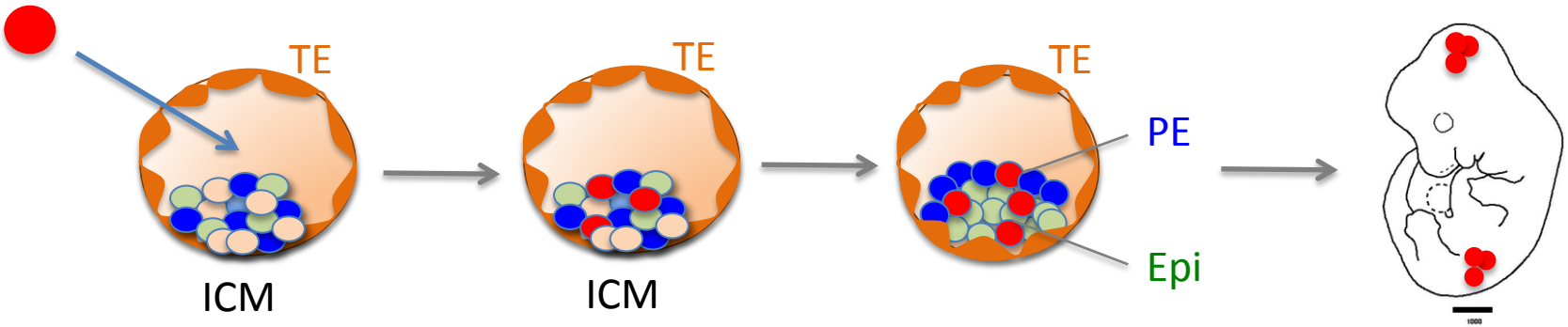
Pluripotent stem cell lines : what relevance to embryo development?



1. Do naive and primed ES cells represent discrete stages of early embryo development ?
2. Does in vitro plasticity reflect in vivo heterogeneity?
3. How our knowledge of the embryo molecular determinants can help deriving more relevant pluripotent cell lines ?

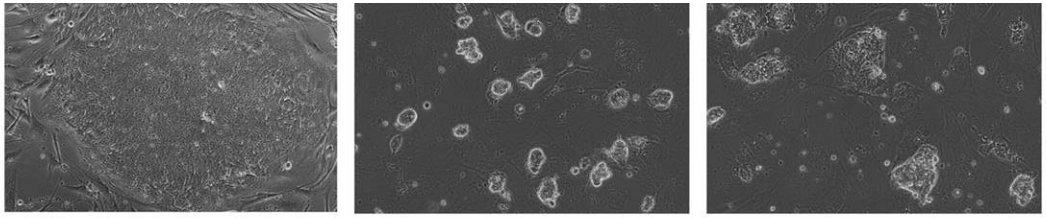
Question : how to assess functional pluripotency in vivo ?

i.e., to demonstrate the ability of cells to reenter embryogenesis ?



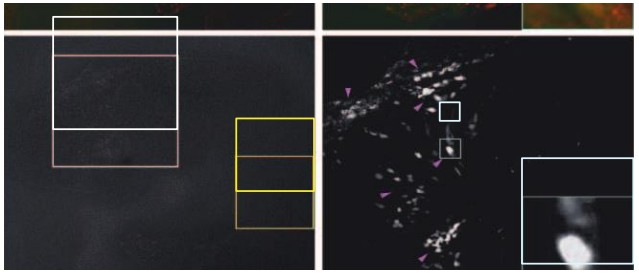
Interspecies chimeric embryos
(microinjection of human ES/iPS in mouse embryos)

in vitro, integration into the ICM



Masaki H, Development 2015

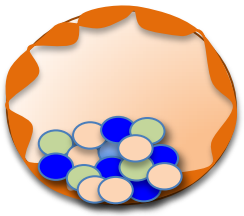
Contribution to germ layers derivatives after in utero transfer in the host



Gafni O, Nature 2013

What if stem cells turn into « embryos » in a dish?

(Editorials Cell 2011, Nature Methods oct 2015)



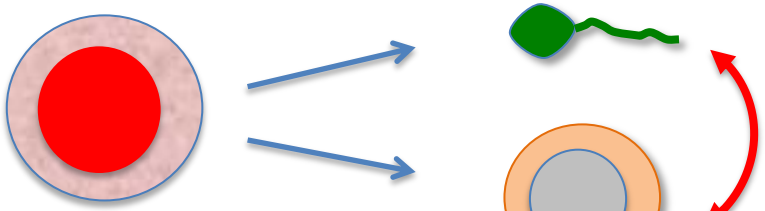
Pluripotent stem cells

iPS

« Anarchic » teratoma

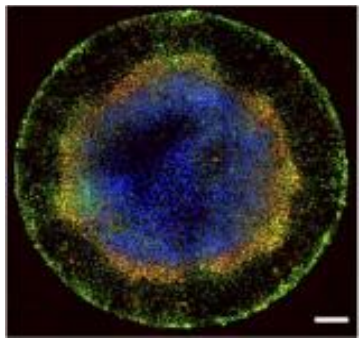
	Matrigel Required	Teratoma Formation in <8 Weeks	Formation of Cysts	N
Kidney Capsule	-	+	+	3
Intramuscular (i.m.)	-	+	-	3
Subcutaneous (s.c.)	+	-	-	6
Intraperitoneal (i.p.)	-	+	+	6
Testis	-	+	+	3
Liver	-	+	+	3
Epididymal Fat Pad	-	+	+	6

Inducing human germ cells from human pluripotent stem cells



PGC like cell

CDX2/BRA/SOX2



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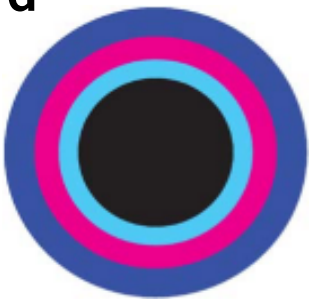

Nat Methods, Aout 2014

Ordered gastrulation-like events and body plan from human ES cells on molds micropatterned substrates ?