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MOLECULAR CELL BIOLOGY

Pluripotent cell isolation for regenerative medicine

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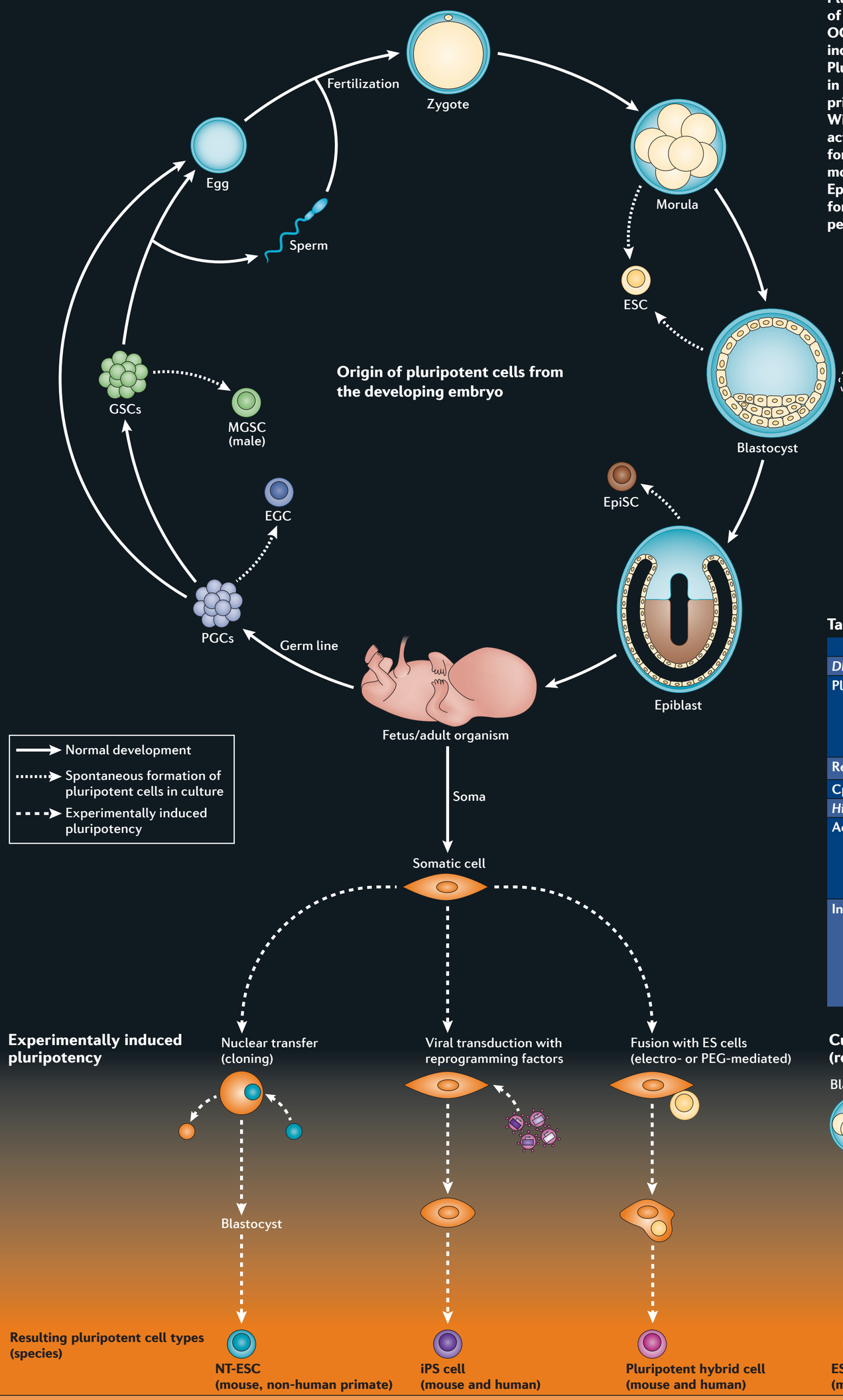
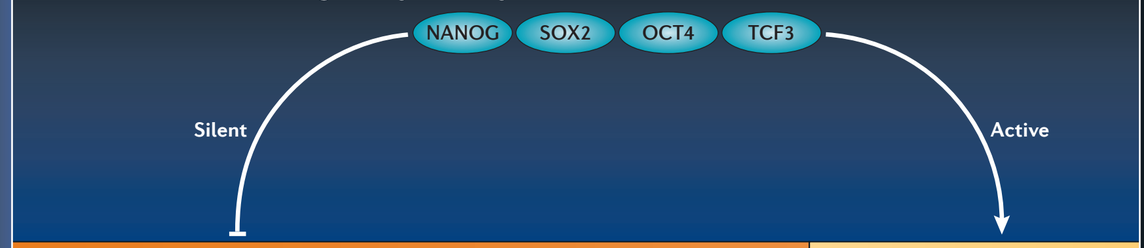
Pluripotent cells offer great promise to the future of regenerative medicine and tissue engineering. Nuclear transfer, direct reprogramming and cell fusion can be used to experimentally induce pluripotency in somatic cells. To date, no naturally occurring pluripotent cell has been identified in the mammalian soma, and cells with pluripotent potential in the early embryo or germ lineage are difficult to isolate from patients. This makes methods of experimentally induced pluripotency in readily available somatic cells (such as skin biopsies) invaluable for the generation of patient-specific stem cells.



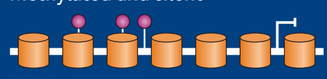
Pluripotency is defined as the ability of a cell to differentiate into any cell type of the three germ layers. The acquisition of pluripotency in somatic cells requires the activation of a transcriptional regulatory network (this involves NANOG, SOX2, OCT4, TCF3 and possibly others), a phenomenon which has been observed using three distinct methods of experimentally induced pluripotency in somatic cells. These methods include nuclear transfer, direct reprogramming and cell fusion. Pluripotent cells have also been shown to arise from *ex vivo* cultures of early embryonic cells and cells of the germ lineage, in which members of the pluripotency network are normally active, including cells of the morula, inner cell mass, epiblast, primordial germ cells and germline stem cells. Several functional criteria are commonly used to assess pluripotency. With increasing stringency these criteria are: *in vitro* differentiation into cells that represent all three germ lineages, active pluripotency transcriptional regulators (demethylated OCT4 and NANOG promoters), teratoma formation, chimaera formation with subsequent germline transmission (for mouse cells only), and the tetraploid complementation assay (for mouse cells only). All of the cells described here meet the first three criteria, and all except fusion-derived hybrid cells and EpiSCs can generate chimaeras following injection into a blastocyst. Only ESCs, NT-ESCs and iPS cells have successfully formed embryos through tetraploid complementation. As chimaera formation and tetraploid complementation can only be performed in mice, teratoma formation is the most stringent test for pluripotency that is available in the human system.

Model of the core ESC regulatory circuitry



Neurogenesis	Ectoderm	Mesoderm	Ectoderm/ endoderm	Endoderm	Extra-embryonic tissue/placenta	ESC transcription factors	ESC signalling
NEUROG1	PAX6 MEIS1 HOXB1 LHX5 OTX1	DLX5 HAND1 ONECUT1	ISL1	ATBF1	ESX1L	OCT4 SOX2 NANOG STAT3 HESX1 ZIC3	TCF3 FGF2 LEFTY2 SKIL* *Active and silent

Table of the epigenetic characteristics of somatic and pluripotent cells

	Somatic cells	Pluripotent cells
DNA methylation		
Pluripotency gene promoters	Methylated and silent  OCT4, NANOG	Unmethylated and active  OCT4, NANOG
Repetitive elements	Methylated	Methylated
CpG islands	Rare methylated CpG islands	Low numbers of methylated CpG islands
Histone modifications		
Active genes	H3K4me1–H3K4me3 H3K36me1–H3K36me3 Lys acetylation 	H3K4me1–H3K4me3 H3K36me1–H3K36me3 Lys acetylation 
Inactive genes	H3K9me1–H3K9me3 H3K27me1–H3K27me3 H4K20me1–H4K20me3 	H3K4me1–H3K4me3 H3K27me1–H3K27me3 Concomitant repressive and active histone marks at silent developmental regulatory genes 

Resulting pluripotent cell types (species)	**NT-ESC (mouse, non-human primate)**	**iPS cell (mouse and human)**	**Pluripotent hybrid cell (mouse and human)**	**ESC (mouse and human)**	**EpiSC (mouse)**	**EGC (mouse and human)**	**MGSC (mouse)**
Kinetics of pluripotency acquisition	Hours to days	>14 days (mouse)	Hours to days	Direct *in vitro* explant culture	Direct *in vitro* explant culture	Direct culture-induced formation *in vitro*	Spontaneous transformation (with variable timing) *in vitro*
***In vitro* growth-factor dependence**	LIF, BMP (mouse)	LIF, BMP (mouse) FGF, activin (human)	LIF, BMP	LIF, BMP (mouse) FGF, activin (human)	FGF, activin	SCF, LIF, FGF	GDNF, then LIF
Molecular mechanisms	Unknown factors in ooplasm mediate reprogramming	Expression of four reprogramming transcription factors triggers a series of stochastic events that result in fully reprogrammed cells	Unknown factors in ESC or ECC cytoplasm and/or nucleus mediate reprogramming. NANOG overexpression increases efficiency	Pluripotent cells can be directly isolated from the inner cell mass of the blastocyst. Addition of the MEK inhibitor PD98059 results in increased efficiency	Pluripotent cells can be directly isolated from the epiblast	Unknown	Unknown
Advantages	No genetic modifications required. Nuclear transfer can be performed using readily available skin cells as nuclear donors to generate patient-specific NT-ESCs	Simple, robust method that can be performed on readily available skin cells to generate patient-specific iPS cells	No genetic modifications required	No genetic modifications required	No genetic modifications required	No genetic modifications required	No genetic modifications required
Disadvantages	Highly inefficient, often owing to incomplete reprogramming and technical limitations. NT has not yet been performed successfully with human cells. Human oocytes are scarce. NT is technically challenging	Requires genetic modification of target cells by the introduction of integrating viruses that encode known oncogenes	Resulting hybrid cell is tetraploid, precluding therapeutic application	Cells of the inner cell mass are transient populations that do not exist in adult organisms, prohibiting the generation of patient-specific cells. However, single blastomeres may be prospectively isolated, allowing the morula to develop further	Cells of the epiblast are transient populations that do not exist in adult organisms, prohibiting generation of patient-specific cells	PGCs are transient populations that do not exist in adult organisms, prohibiting the generation of patient-specific EGCs	The inacessibility of GSCs complicates the generation of patient-specific cells. Spontaneous *in vitro* transformation to MGSC is highly inefficient

Abbreviations

ATBF1, AT-binding transcription factor-1; BMP, bone morphogenetic protein; DLX5, distal-less homeobox-5; ECC, embryonic carcinoma cell; EGC, embryonic germline cell; EpiSC, epiblast stem cell; ESC, embryonic stem cell; FGF, fibroblast growth factor; GDNF, glial-cell-derived neurotrophic factor; GSC, germline stem cell; HOXB1, homeobox B1; ICM, inner cell mass; iPS cell, induced pluripotent stem cell; ISL1, islet-1; LIF, leukaemia inhibitory factor; MEK, mitogen-activated protein kinase and extracellular signal-regulated kinase kinase; MGSC, multipotent germline stem cell; NT, nuclear transfer; OCT4, octamer-binding transcription factor-4; PEG, polyethylene glycol; PGC, primordial germ cell; SCF, stem cell factor; SOX2, SRY-related high-mobility group (HMG)-box protein-2; STAT3, signal transducer and activator of transcription-3; TCF3, transcription factor-3.

Contact information

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For further reading, a glossary and additional abbreviations, see www.nature.com/nrm/posters/stemcellreprogramming

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www.nature.com/nrm/series/stemcells