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Natural and induced direct reprogramming: mechanisms, concepts and general principles — from the worm to vertebrates

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Elucidating the mechanisms underlying cell fate determination, cell identity maintenance and cell reprogramming *in vivo* is one of the main challenges in today's science. Such knowledge of fundamental importance will further provide new leads for early diagnostics and targeted therapy approaches both in regenerative medicine and cancer research. This review focuses on recent mechanistic findings and factors that influence the differentiated state of cells in direct reprogramming events, aka transdifferentiation. In particular, we will look at the mechanistic and conceptual advances brought by the use of the nematode *Caenorhabditis elegans* and highlight common themes across phyla.

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Introduction

Once, the stability of cellular identity was believed to be irreversible. However, changes in cell identity have been described in many different species, tissues and organisms as part of embryonic development [1–5], homeostasis [6,7], regeneration [8–12] or disease [13]. Besides instances of natural cell type conversion, landmark experiments have shown that the differentiated identity can be changed experimentally. For example, the expression programme of somatic nuclei can be reprogrammed when confronted with a different cytoplasmic environment through nuclear transfer or cell fusion, and small sets of transcription factors can force the conversion of differentiated cells into stem cell-like cells [14,15]. Reprogramming entails the erasure of an initial cellular identity and the adoption of a new identity. Transdifferentiation (Td) or direct reprogramming — as often used in the context of experimentally induced cell type conversion — more specifically describes the direct conversion of one fully differentiated cell type into another. This is defined as a functional swap between differentiated identities with distinguishable differences (functional, morphological and molecular) and where an unambiguous ancestor-descendant relationship is established between the initial and final cells [16]. Td occurs naturally and can be induced both *in vivo* and *in vitro* in several cell types [1–5,17–24].

The mechanisms and factors necessary to reprogram cells have been the focus of intense investigations, especially in the last decade. However, the analysis of the mechanisms underlying Td, or induced Td events as desirable in regenerative medicine, are impeded by the low efficiency of the process when induced experimentally (generally around 10% or lower), its lack of completeness (genetically and epigenetically) and the instability of the newly acquired cell identity in absence of the inductive cue(s) [25,26]. Thus, whether *in vitro* or *in vivo*, it remains difficult to predict Td events in cell populations and follow them at the single cell level while establishing an unambiguous lineal relationship between initial and final identities. Many studies have thus focused on deciphering means to improve the efficiency of the conversion, while relatively few have addressed the underlying mechanisms of the whole process.

In this context, *Caenorhabditis elegans* has proved a powerful model organism to study reprogramming events with its small number of cells (*i.e.* 959 somatic cells in hermaphrodite worms) and transparent body. These features have allowed the determination of its complete somatic cell lineage from the zygote to the adult [27,28], providing scientists with both unambiguous lineal relationships between cells and predictability of, at least, the natural Td events. Both instances of induced and natural direct reprogramming events have been described in *C. elegans*.

Induced direct reprogramming in *C. elegans*: *C. elegans* are post-mitotic organisms with a fixed number of somatic cells and, in most cases, once a somatic cell is terminally differentiated it cannot continue to proliferate or change its identity. The description of the complete somatic lineage showed essentially invariant division patterns and fates, and combined with blastomere ablation experiments, led to a deterministic view of *C. elegans* development [29]. However, several studies have revealed a

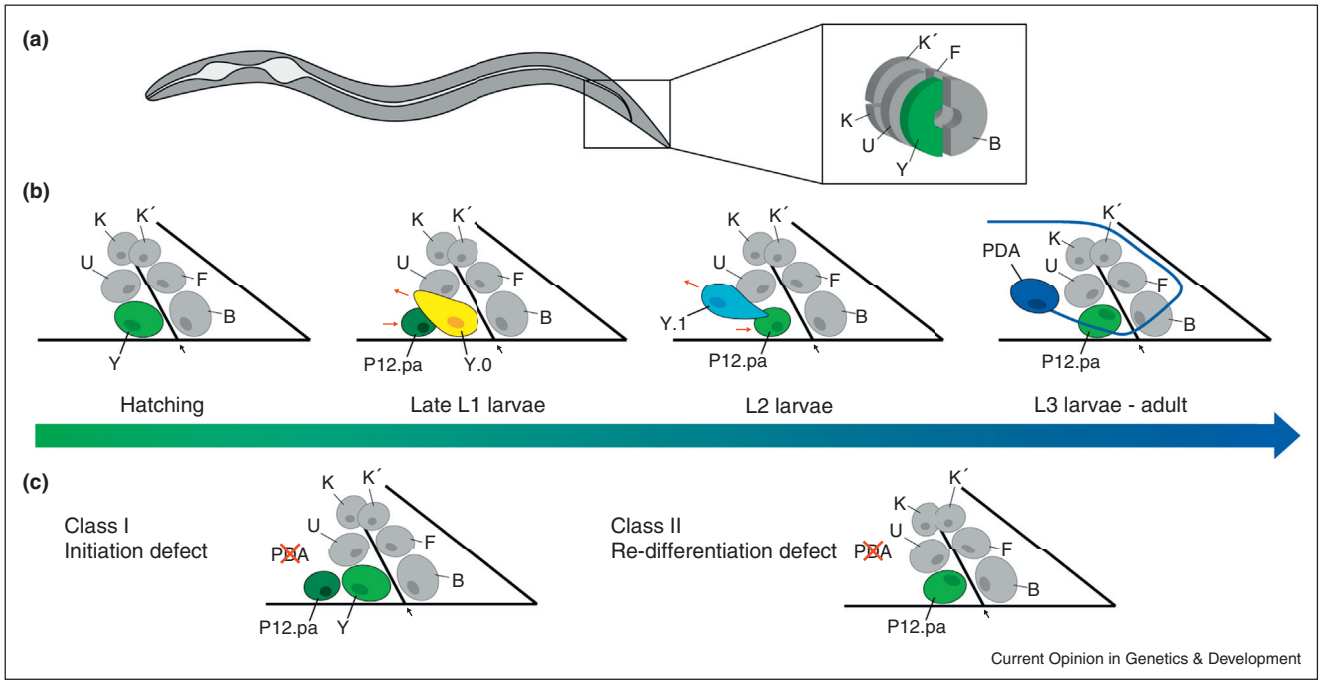
wider plasticity of embryonic blastomeres than suggested by their invariant lineages. Indeed, cell–cell interactions play an important role in their patterning and a change of position can lead to ectopic acquisition of an alternate fate due to aberrant intercellular signalling [30]. In addition, early embryonic blastomeres can exhibit pluripotency until the end of gastrulation (~100 cells), and can be induced to change their identity by overexpression (OE) of a cell fate determinant such as several transcription factors (TF) [31]. Furthermore, although cells at later developmental stages appear mostly refractory to direct reprogramming induced by a TF, instances of experimentally induced larval and germ cell reprogramming have been described [32,33••,34,35••]. Therefore, the worm, equipped with many genetic and engineering tools, stands out as a promising model to study induced direct reprogramming *in vivo*.

Natural transdifferentiation in *C. elegans*: Besides experimentally triggering direct reprogramming, one way to tackle the question of how cells lock or possibly unlock their identity is to study natural direct reprogramming *in vivo*. Determination of the cell lineage [27,28] suggested that natural Td could occur in *C. elegans*. This led to the study of the Y-to-PDA transition, where a rectal cell ('Y') fully converts into a motor neuron ('PDA') (Figure 1), a now well-characterised *bona fide* Td event [5]. Additionally, during sexual maturation male-specific interneurons ('MCM') derive from fully differentiated glia cells (AMso) *via* a cell division, a putative Td event [36]. Thus, *C. elegans* is also a powerful model to study natural direct reprogramming events, providing much needed cellular traceability and predictability at the single cell level.

Here, we will review our mechanistic understanding of direct reprogramming/ Td events at the cellular and molecular levels, with an emphasis on the conceptual advances brought by the use of *C. elegans* as a model system (see also Figure 2).

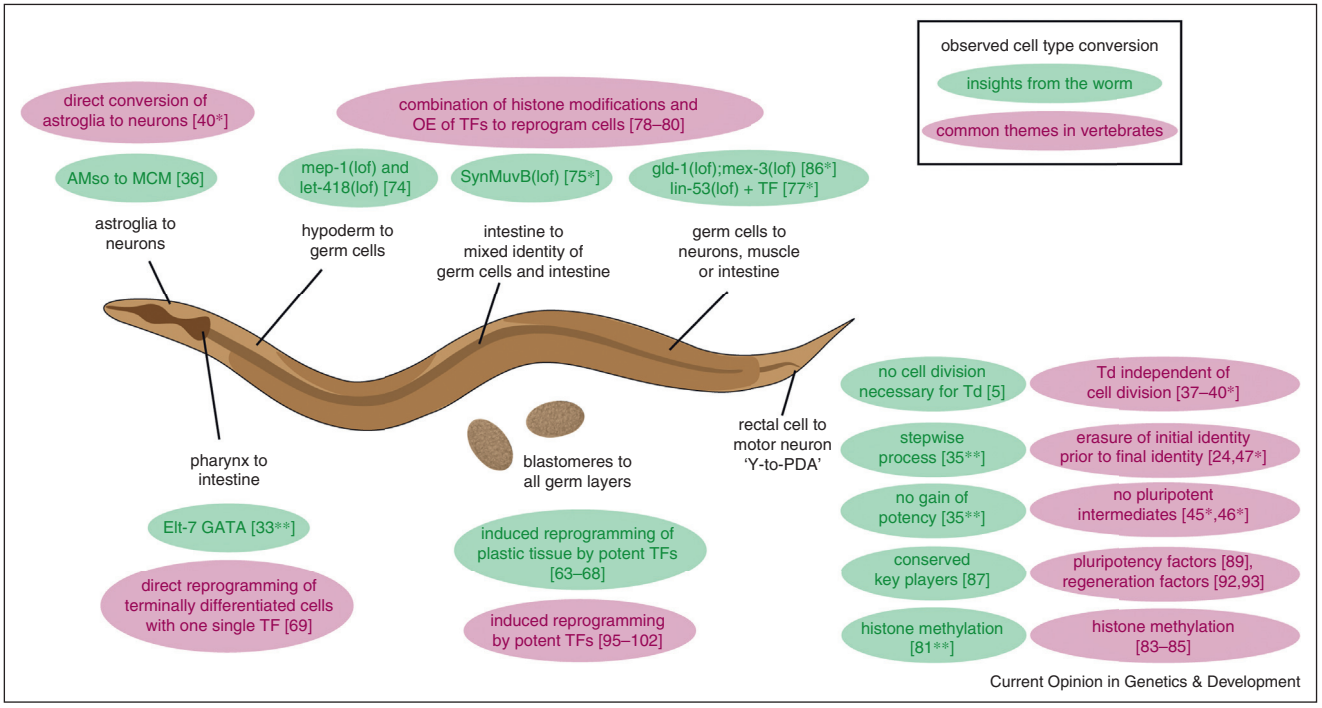
Cell division or not? The induced or latent plasticity of differentiated cells has raised a number of questions requiring a detailed study at the single cell level that have been difficult to address. How does the conversion proceed at the cellular level? For instance, a number of natural or induced direct reprogramming events occur along with cell division [20,23,36], but is it necessary? The use of *C. elegans* has allowed to address this issue unambiguously at the single cell level: Live lineage tracing as

Figure 1



Y-to-PDA natural transdifferentiation. **(a)** In *C. elegans*, the rectal tube contains six epithelial cells (Y, B, U, F, K/Ka and K′) that form a toroid structure, the hindgut, that is used for defecation. **(b)** Between the L1 and L2 larval stages, a fully differentiated rectal cell named 'Y' retracts from the rectum, migrates anterodorsally and transdifferentiates into the motor neuron named 'PDA'. The lasting integrity of the rectal tube is ensured by the concomitant addition of the P12.pa cell that takes both Y empty position and role in the rectum. **(c)** Td mutants (see Table 1) are subdivided in different classes: class I mutants show a Td initiation defect and class II mutants a re-differentiation defect, whereas class III mutants show both types of defect. Y.0, dedifferentiated intermediate; Y.1, early neural cell intermediate. Red arrows, direction of Y migration. Black arrows, rectal slit. Anterior is to the left and ventral to the bottom.

Figure 2



Summary of current knowledge in cell reprogramming that was gained by studying *C. elegans*, and how it also applies to vertebrates.

well as DNA staining and quantification showed that no cell division is necessary for the natural conversion of the 'Y' rectal cell into the 'PDA' motor neuron in hermaphrodite worms [5,35••]. Similarly, induced direct reprogramming of post-mitotic pharyngeal cells in live *C. elegans via* transient OE of the ELT-7 TF does not seem to involve cell division [33••]. These studies showed that cell division is not a requirement for natural or induced direct reprogramming *in vivo*.

Although observed at the population level, later studies have also highlighted that cell division appears dispensable in several induced direct reprogramming settings in mammals as well. For instance, the TF-mediated conversions of pancreatic exocrine cells into pancreatic insulin-producing cells *in vivo* [37] or into cells with hepatic properties *in vitro* [38,39], as well as the conversion of astroglia into neurons [40•] appear to occur in absence of cell division.

Thus, cell division is dispensable for cell type conversion and this appears to be a common theme to both natural and experimentally induced Td, whether *in vivo* or in cell culture. Other Td events occur concomitantly to a cell division [20,23,36], and the importance and relative contribution of cell division and/or putative downstream events in these instances remain to be elucidated. Several hypotheses can be made in favour of a putative requirement of cell division in some cases. For instance, it may be that cell division is the critical step to allow at least one daughter to change its identity. Or that it rather facilitates direct reprogramming, possibly by ensuring efficient epigenetic reprogramming. For example, recent studies show that, following nuclear transfer in oocytes, the mitotic chromatin is highly responsive to reprogramming factors compared to interphase nuclei indicating that temporal access to cytoplasmic factors during mitosis might promote Td [41]. Finally, it is conceivable that cell division allows the asymmetric segregation of important Td players that would then ensure that the initial identity is erased only in one of the daughter cells.

Cell fusion as a natural mechanism? Some cells have changed their identity through fusion with cells in a higher or lower differentiated status, in particular after being grafted into a host organism [15,42–44]. Work in our lab has examined if cell fusion, or cell engulfment, a process commonly seen in *C. elegans* during programmed cell death, could underlie the conversion of the rectal Y cell into a PDA neuron. However, live imaging and EM analyses showed that neither cell fusion nor engulfment of Y were observed, and Td occurs unaffected in worms with defective apoptosis or engulfment machinery [5], strongly suggesting that it does not underlie Y cell type conversion. If swapping an identity is not enforced through fusion to another cell, and cell division is not a strict requirement, how does it proceed?

Step-by-step and unipotent. Does the conversion proceed directly through parallel loss and gain of the initial versus the final identities, or can it transit through different discrete cellular steps? In addition, can a cell change its identity without reverting to a pluripotent ground state? Studies on induced direct reprogramming in mammals, where no evidence of cell division has been found, assumed that absence of cell division implied absence of reversion to a dedifferentiated intermediate [37]. What did single cell analyses over the course of a Td event taught us?

An EMS mutagenesis screen for mutants with PDA defects revealed several genes necessary for Y-to-PDA Td which are subdivided in different classes (Table 1 and Figure 1c). For instance, in class I mutants, Y is formed, but never leaves its position in the rectal tube, and never becomes a PDA motor neuron. In fact, three epithelial cells are visible in the anterior part of rectal tube, instead of two normally, indicating that the initiation of Td is blocked in these mutants. In class II mutants, Y develops normally in the beginning: Y has a fully differentiated rectal cell identity, which is lost upon retraction from the rectum and migration towards its final position. Nevertheless, PDA is not formed, because re-differentiation into a subtype-specific neuron is impaired. Thus, these series of genetic mutants affect differently Td, suggesting a process occurring in multiple steps [35••].

Indeed, detailed analysis of a class II mutant as a model to identify the cellular steps of Td showed that a mixed Y/PDA identity is never seen. Instead, Y loses first its rectal identity, *sensu stricto* a dedifferentiation, before it re-differentiates into the PDA neuron in a stepwise manner. Interestingly, none of the intermediates of the process can be forced to adopt another identity, nor revert to Y original identity after ectopic expression of different cell fate determinants that are able to reprogram early *C. elegans* blastomeres [35••]. This suggests that *in vivo* the dedifferentiation of a cell is not necessarily coupled with an increase of its cellular potential or the reversion into a pluripotent state. This is reminiscent of the behaviour of cells during axolotl limb regeneration where each adult tissue produces regenerating cells with restricted potential corresponding to their tissue of origin rather than complete dedifferentiation to a pluripotent state [45•,46•].

Thus, these data highlight two salient features of Y Td: a stepwise identity change involving the erasure of the initial identity before the establishment of the final identity, and the absence of reversal to a pluripotent or a progenitor state. Both appear to be conserved and have been described in several induced reprogramming events in mammals. For example, a genome-wide transcriptomic analysis over the time course of the C/EBPα-induced pre-B to macrophage cell conversion shows no evidence for a mixed identity intermediate. Rather, the transcriptomic programme of the first identity appears to get extinguished before the programme for the second identity is turned on [47•]. In addition, no reversal to a pluripotent or a progenitor state (retro-differentiation) was observed in this case, nor was it during the induced conversion of amniotic cells to endothelial cells [48].

Factors involved in cell identity conversion

On a molecular level, direct cell identity conversion has historically been triggered using a variety of inducing cues, including drugs such as 5-azacytidine [49,50], copper-deficient diet [51,52] or the disruption of the cells'

Table 1

Key players in Y-to-PDA Td. Overview of Td factors with their biological activity, mammalian orthologues, molecular identity, the percentage of PDA defects in null/loss-of-function mutants at 20 °C and their PDA defect class, with class I: Td initiation defect, class II: re-differentiation defect, class III: class I + II

Biological activity	Td factor	Mammalian orthologue	Molecular identity	PDA defects	Class	Reference
Transcription factors (TF)	EGL-5	HOX9-13	homeodomain TF	100.0%	I	[5]
	SEM-4	SALL4	zing finger TF	100.0%	I	[5,87]
	UNC-3	EBF1-4	COE (Collier, Olf, EBF)-type TF	88.8%	II	[35••]
	CEH-6	POU3F2,4	POU family homeodomain protein	23.4%[a]	I	[87]
	SOX-2	SOX1-3	HMG-box transcription factor	12.5%[a]	I	[87]
Repressor complex associated factor	EGL-27	MTA1	transcriptional modulator	83.6%	I	[87]
Histone 3/chromatin modifiers	JMJD-3.1	JMJD3	H3K27me3/me2 demethylase	13.8%	II	[81••]
	WDR-5.1	WDR5	Set1 complex subunit	11.0%	III	[81••]
	ASH-2	ASH2L	Set1 complex subunit	4.9%	III	[81••]
	DPY-30	DPY30	Set1 complex subunit	4.6%	I	[81••]
	RBBP-5	RBBP5	Set1 complex subunit	3.3%	III	[81••]
	SET-2	SET1A/SET1B	H3K4 methylase	1.8%	III	[81••]
	CFP-1	CFP1	Set1 complex subunit	1.6%	n.d.	[81••]

n.d., not determined.
[a] Loss-of-function as obtained *via* RNAi, as no viable mutants are available.

physiological environment [53]. However, studies of cell differentiation during organisms' development had unravelled a number of TFs that were either crucial to direct cells towards a lineage or a specific fate [54,55], or to maintain that fate over time [56,57]. These studies have highlighted the potential for such nuclear effectors to be key drivers of the determination and expression of a specific cellular identity. Therefore and prior to the use of a cocktail of pluripotency TFs by Takahashi and Yamanaka to reprogram differentiated cells back to a pluripotent state [14], a number of *in vitro* studies had used cell fate determinant TFs to change the cellular identity. *In vitro* cell type conversions have thus been described following ectopic expression of MyoD [58], GATA-1 [59] or PPARγ plus C/EBPα OE [60], among others. Induced direct conversions *in vivo* were further described early on in *Drosophila* using Pax-6 OE [61], or in the worm (Table 2) [31]. Following on their observations that MyoD can convert certain mammalian cells into muscles *in vitro* [62], Krause and Weintraub tested if the worm homologue of MyoD, called *hlh-1*, could do the same *in vivo*. They found that indeed OE of exogenous *hlh-1* in *C. elegans* embryo led most of its cells to adopt muscle characteristics [63]. Several other studies also showed that forced ectopic expression of a single TF is sufficient to force most blastomeres before the 8E stage to adopt a specific cell identity and that *C. elegans* blastomeres can be reprogrammed into all three germ layers: END-1 (endoderm) [64], ELT-2 (intestine) [63], PHA-4 (pharyngeal) [32,65], LIN-26 (epithelial) [66], ELT-1 or ELT-3 (epidermis) [67] or UNC-30 (GABAergic neurons) [68] can all induce cell fate changes (Table 2A). Interestingly, the ability of these TFs to convert most cells is restricted to a specific early developmental time window after which the efficiency decreases dramatically, to be close to zero in larvae: as cells mature or further differentiate, they become increasingly refractory to *in vivo* reprogramming [32,34]. However, ectopic expression of the ELT-7 GATA TF can reprogram fully differentiated pharyngeal cells to become intestinal cells even after mid-embryogenesis. Of note, several cells in the worm initially exhibited intestinal features after a pulse of exogenous ELT-7, but these characteristics were lost after 72 h in most cells [33••]. This suggests that terminally differentiated post-mitotic cells can be forced to (at least partially) reprogram *in vivo* by OE of a single TF, but stable Td may depend on the cellular context. Similarly and more recently, *in vivo* direct reprogramming has been achieved in the last few years in a number of vertebrate models by OE of single or combination of TFs (for a review, see [69]). Thus, lineage reprogramming can be triggered efficiently by TFs *in vivo*, but the efficiency drops with developmental ageing, and for a given inducing cue, the extend of the response and the stability of the new identity differs depending on the tissues.

How can a single or a handful of TFs change a whole expression programme, and access inactive regions of the chromatin? It has been suggested that 'pioneer TFs' — TFs capable to initiate changes in chromatin structure and activity by directly binding their cognate DNA sites on a nucleosome, even in chromatin that is locally compacted by linker histones — are important drivers of the conversion [70,71]. However, besides TFs, histone modifiers

Table 2

Factors or mutants used in *C. elegans* to induce a cell identity conversion. (A) Inductive factors that lead to cell identity conversion when induced ectopically at a specific time point. E, stage defined by number of cells in the E lineage. (B) Depletion of genes leading to cell identity changes alone or in combination with overexpression (OE) of specific transcription factors (TF).

(A) Inductive factors	Stage of induction	Starting identity	Final identity	Reference
ELT-7	All stages	Pharynx	Intestine	[33••]
HLH-1	2E to 8E	Blastomere	Muscle	[63]
END-1	2E to 8E	Blastomere	Endoderm	[64]
LIN-26	2E to 8E	Blastomere	Epithelium	[66]
ELT-2	4E to 8E	Blastomere	Intestine	[63]
ELT-1	4-cell stage to 8E	Blastomere	Hypoderm	[67]
ELT-3	4-cell stage to 8E	Blastomere	Hypoderm	[67]
PHA-4	4E to 8E	Blastomere	Pharynx	[32,65]
UNC-30	adult worm	Non-GABAergic neurons Pharyngeal muscle cells	GABAeric neurons	[68]

(B) Cell identity swap or loss in mutant background	Starting identity	Final identity	Reference
gld-1(lof); mex-3(lof)	Germ cells	Neurons, muscle, intestine	[86•]
lin-53(RNAi) +CHE-1 OE +UNC-3 OE +UNC-30 OE	Germ cells	Neurons	[77•]
mep-1(RNAi) or *let-418(RNAi)*	Hypodermis, intestine	Germ cells	[74]
lin-35(lof) and other *SynMuvB(lof)*	Intestine	Mixed identity of germ cells and intestine	[75•]

Note that, for all cases described in the table, the completeness of the conversion has not been assessed. lof, loss-of-function. n.d., not determined.

appear to play a role, either to restrict cellular plasticity or to facilitate it. As mentioned above, at mid-embryogenesis *C. elegans* blastomeres become quite resistant to cell fate-changing factors, an event interpreted as a loss of plasticity [32,34,35••] that can only be postponed by elimination of the Polycomb repressor complex (PcG) or Notch signalling [72•,73]. Depletion of Zn-finger protein MEP-1 and NuRD complex subunit LET-418, both found in a complex with histone deacetylase HDAC-1, de-repress germline-specific genes in larval somatic cells [74]. Also, loss of chromatin remodelling in mutants of the retinoblastoma (Rb) pathway lead to somatic expression of P granules, an attribute of germ cells, in the intestine [75•]. In the same line, in mammals, removal of the DNA methyltransferase DNMT1 in murine embryonic β cells leads to their conversion into glucagon producing α cells [76].

Sometimes, a combination of ectopic OE of a TF and knock down of factors altering chromatin activity or structure is required for cell conversion. For instance, RNAi-mediated depletion of *lin-53* (human RBBP7 and RBBP8), or of the Polycomb homologues *mes-2* and *-3*, lead to somatic differentiation of the germ cells when combined with the ectopic expression of a cell-fate TF determinant, and may act by priming germ cells for reprogramming [77•]. In line with this, inhibitors of histone modifiers have been used in combination with OE of one or more TFs to improve the efficiency of pluripotent reprogramming of mammalian differentiated cells [78–80].

Chromatin-modifying activities can also promote the reprogramming of the differentiated identity in combination with TFs, rather than hinder it. During Y-to-PDA Td the SET1 complex, through its H3K4 methylation activity, and JMJD-3.1, a H3K27 demethylase — though dispensable for the process *per se* — act in a stepwise manner paralleling the gradual cellular transformation to maintain the efficiency of the conversion to 100%. Their activity becomes crucial to achieve a perfect Y-to-PDA Td every time when the worm faces stresses. To ensure such robustness, they act in conjunction with step-specific TFs, maybe by facilitating, stabilising or reinforcing their action and transcriptional output [81••].

A positive role for histone modifiers has also been observed during *in vitro* induced direct reprogramming, where non-cardiac mesoderm have been transformed into cardiomyocytes using OE of the Gata4 and Tbx5 TFs in combination with Baf60c, a Swi/Snf-like BAF chromatin remodelling complex member [82]. It has been postulated that Baf60c potentiates the function of Gata4 and Tbx5, partly by allowing binding of Gata4 to cardiac loci. As modulation of histone methylation is involved in other somatic reprogramming [83–85], cell identity changes might be deeply influenced by activities that impact on histones or chromatin activity. We propose that the chromatin modifiers' main role in this process would be to impact on transcriptional output, whether by safeguarding a transcriptional programme and hence a cellular identity against changes, or by facilitating/enhancing the activity of key TFs to initiate a different transcriptional programme.

Further studies emphasised that it is the control of gene expression generally that matters: control of RNA translation and storage or degradation has been shown to be key for the maintenance of germ cell identity in *C. elegans*. For example, the translational regulators GLD-1 and MEX-3 are necessary for the maintenance of totipotency in the germ cells, and loss-of-function double mutants show ectopic differentiation into neurons, muscles and intestinal cells inside the gonad and loss of germ cell characteristics such as P granules [86•]. Focusing on the aberrant muscle differentiation of germ cells in these mutants, the authors found that it was dependent on the PAL-1/Caudal transcriptional regulator, and its downstream target HLH-1/MyoD, which expression is normally repressed in germ cells. While it may be that germ cells are particularly sensitive to translational regulation as they stock large quantities of maternal RNA that will be used in the first steps of embryonic development, this study points once again at the importance of lineage specification TFs and the control of a cell's expression programme.

While entirely changing the identity of a cell, especially in a short time frame, likely involves multiple cellular processes, like targeting key mRNA and proteins of the initial identity to degradation and membrane remodelling, the current data point to nuclear events as the driving factors of cell conversion. Interestingly, Y-to-PDA Td players identified through our unbiased EMS screen all act in the nucleus (Table 1) [unpublished data M.C. Morin & S.J.]. TFs are likely to play a major role in driving and executing the cell identity change, while other factors such as histone modifiers will facilitate and enhance this role. Of note, a single TF may be sufficient in a certain cell type or at a given differentiation state, but not in other contexts or as cells age. These findings suggest that the maintenance of cell identity is reinforced by one or several secondary mechanisms appearing later, or is reinforced by the progressive accumulation of key players of the maintenance machinery.

Conserved key players and the dedifferentiated state

What does dedifferentiation entail? Our unbiased genetic screens for genes affecting Y-to-PDA Td have identified the *C. elegans* homologues of SOX2, OCT4 (CEH-6), SALL4 (SEM-4) and MTA1 (EGL-27) as crucial for the natural reprogramming of Y, and mutations in each of these genes completely block the initiation of cell conversion. Several evidences suggest that all these factors act through a multiproteic complex in the worm:

these genes are expressed in the same rectal-epithelial cells; they act at the very same step, the initiation of the Y-to-PDA Td; and co-immunoprecipation experiments showed an association of EGL-27/MTA1, CEH-6/OCT, SEM-4/SALL4 and SOX-2 [87]. In addition, not only are these genes conserved, but their association is also conserved in mammals where they form the NODE complex [88].

The identity of these exciting players came somewhat as a surprise as these genes are known, individually and collectively, to promote or maintain the pluripotent state of ES cells in mammals [89] as well as to be involved in vertebrate regeneration [90,91]. Two of them, SOX2 and OCT4 are used in the OSKM cocktail that triggers the reprogramming of differentiated cells into induced pluripotent stem-like cells (iPS) [14]. By contrast, in the Y-to-PDA process, the Y cell loses its identity to pass through a dedifferentiated state that is not coupled to a broad gain, if any, in cellular potential [35••]. Thus, the initial identity can be erased without inducing an aberrant or pluripotent state, and acquisition of a new identity as well as the cellular potential associated with each cellular step in the process are tightly regulated. This is reminiscent of lens regeneration in the newt, where the dedifferentiated cells originating from pigmented iris express very early in the process pluripotency factors such as SOX2, but remain committed to a lens fate as transplantation experiments demonstrated [92,93]. These factors could act as direct transcriptional repressors, for example of the initial identity programme, or as transcriptional activators as postulated in Ref. [94], to trigger the expression of factors necessary to erase the initial identity and remodel the Y cell, or both depending on co-factors.

During evolution, these factors or their combination may have gained a new function, namely to promote not only a dedifferentiated state but a pluripotent one. But what are the implications for induced direct reprogramming? Passage through a dedifferentiated state could be the key to efficient reprogramming as seen in the worm. Indeed, reprogramming efficiency of astroglia into GABAergic neurons is further enhanced by prior dedifferentiation of astroglia *via* expansion under neurosphere conditions [40]. A few groups have successfully tried to improve the fairly low overall efficiency of induced Td in mammalian cell cultures by using (transient expression of) pluripotency factors in combination with a given reprogramming environment and/or lineage-specific factors [95–102]. Several hypotheses have been postulated to account for the resulting improved efficiency, such as facilitation of the re-activation of lineage-specific repressed genes [69] or reversal to a very transient pluripotent state [103,104]. However, in light of their action in natural Td in *C. elegans*, we propose that their key activity here may be to promote dedifferentiation *sensu strictu*, that is the erasure of the initial identity, while the accompanying widening of the cellular potential may be a *sine qua non* consequence in mammals.

Conclusion

During the past decades, *C. elegans* has evolved more and more into a powerful model organism to study cellular plasticity, cell reprogramming and maintenance of cell identity. Many conserved mechanisms and conceptual insights brought by the nematode led to novel understandings in the field of natural Td and induced direct cell reprogramming. A number of conserved themes between different direct reprogramming events, whether *in vivo* or *in vitro* and whether natural or induced, have thus been uncovered across phyla. In addition, while it remains unclear to which extend different cell type conversion will all use the same mechanisms, findings in the worm suggest that general principles underlying cellular plasticity might be at play.

Further studies will hopefully provide a further in-depth understanding of the molecular mechanisms behind these events with their promising implications in cancer therapy and regenerative medicine.

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