DENKER, H.-W.:

„Totipotency“ of stem cells: Plasticity vs. self-organization capacity

Comment on:
ISHIUCHI, T., ENRIQUEZ-GASCA, R., MIZUTANI, E., BOSKOVIC, A., ZIEGLER-BIRLING, C., RODRIGUEZ-TERRONES, D., WAKAYAMA, T., VAQUERIZAS, J.M., TORRES-PADILLA, M.E.: **Early embryonic-like cells are induced by downregulating replication-dependent chromatin assembly.**
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Abstract

*Recent progress in research on the regulation of stem cell potentiality is leading to increasingly divergent views how to classify early embryonic-type cells appropriately. In an article published in Nature Structural & Molecular Biology (2015), Ishiuchi et al. focus on stem cells resembling blastomeres from 2-cell stage embryos (2C-like cells), and they address these as **totipotent** on the basis of data on gene expression patterns and on the capacity to reactivate transcription of endogenous retroviruses, as well as observations on the embryo-forming capacity gained during reprogramming by nuclear transfer to oocyte cytoplasm. The present commentary addresses the question how this classification of 2C-like cells as totipotent relates to the recently expanding nomenclature used by various authors (terms like „naive“ vs. „primed“ states of pluripotency, „complete“ pluripotency, ground state, omnipotency, plenipotency). Emphasis is on the question how the observed phenomena are related to **plasticity** of cells or nuclei, and what the cellular basis may be for the capacity of early embryonic cells to develop **organismic wholeness**. It is concluded that testing the potentiality of 2C-like cells according to stringent biological criteria would be of considerable interest.*

In a recent article published in *Nature Structural & Molecular Biology*, Ishiuchi et al. (Ishiuchi et al. 2015) focus on (epi)genetically engineered stem cells resembling blastomeres from 2-cell stage embryos (2C-like cells) with regard to their gene expression patterns, to their capacity to reactivate transcription of endogenous retroviruses, as well as to their embryo-forming capacity gained during reprogramming by nuclear transfer to oocyte cytoplasm. Their data provide new insights into details of epigentic regulation of this capacity at the chromatin organization level, and how this can be manipulated experimentally. Interestingly, the authors show that this type of cells (described before to arise spontaneously in ES-cell cultures (Macfarlan et al. 2012)) can be induced to arise more frequently *in vitro* through down-regulation of the chromatin assembly activity of CAF-1.

A striking aspect of this article is that the potentiality level gained by inducing these 2C-like cells is being addressed as *totipotency*. Totipotency is defined by these authors as the phenomenon „that a full organism can be derived from a single cell“, in contrast to pluripotency which „refers to the ability of a cell to contribute to all three germ layers of the embryo but not to the extraembryonic lineages“ ((Ishiuchi et al. 2015) p. 662). I will not focus, in this comment, on the facts that the potential to form extraembryonic cells is *not* completely missing in mouse ES cells, that it can be upregulated (Morgani et al. 2013), and that it is indeed typically present in primate „pluripotent“ cells (Denker 2004). I will concentrate instead on the addressed potential to initiate the development of an organismic whole.

What really is a totipotent cell? Many authors would reserve this term for those cells that can develop into a *complete organism* autonomously. Increasingly often, however, this term is now being used in a less stringent sense when describing stem cells that have been shown to differentiate into derivatives of all germ layers. Indeed terminology in use to describe the properties of embryonic-type stem cells appears to undergo recently a process of evolution (*„naive“ and „primed“ state of pluripotency; „complete“ pluripotency; ground state; omnipotency or plenipotency; totipotency;* reviewed in (Denker 2014)). An increasing number of authors uses the term totipotency for ES or iPS cell lines that have the capacity to form derivatives of all germ layers, even if they have not been shown to fulfill the mentioned most stringent

criterium, i.e. the capacity to form a complete organism. Likewise the definition of *plasticity* of stem cells and how it may be related to potentiality is still a matter of debate. Another aspect seen differently by various authors is whether terms describing potentiality or plasticity should be used only when characterizing properties of *whole cells*, or whether they can also be used to describe properties of isolated *nuclei*, or chromatin organization.

Ishiuchi et al. (Ishiuchi et al. 2015) wish to apply a stringent definition for totipotency insofar as they do refer to the embryo-forming capacity in their introduction (*„fully totipotent").* As a testing strategy they use nuclear transfer to an oocyte cytoplasm. There are a number of points that should be kept in mind in this context, however. Nuclear transfer to an oocyte tests for properties not of the original cells in question but of nuclei isolated from them (and their dependence on chromatin configuration). Logically this demonstrates reprogrammability, i.e. *plasticity*, of the nuclei, meaning the ability to respond to the signals provided by the oocyte cytoplasm. It is problematical to talk about potentiality of a nucleus. The unit of life is the cell, and for clarity we should remain aware of the fact that it is a complex system. This should be reflected by the way we use terms describing properties of that system or parts of it. Potentiality should be seen as a property of complete cells. No nucleus can have any cellular potentiality of its own, nor can any cytoplasm. Potentiality arises exclusively from cooperation between the nucleus and cytoplasm. In this sense we should see the data presented by Ishiuchi et al. (Ishiuchi et al. 2015) as interesting new information about manipulation of nuclear plasticity by altering chromatin organization, with regard to the rates of success when deriving totipotent cellular constructs (in the strict sense, i.e. zygote equivalents) by nuclear transfer to oocyte cytoplasm. This is what the nuclear transfer test can show.

The engineered cells from which the nuclei have been taken cannot, however, be addressed as totipotent on the basis of this test alone. This could only be based on testing the properties of the original complete cells. According to the known concepts of developmental biology this type of test could be done by isolation (neutral environment, avoiding contacts to other types of cells, e.g. transfer to empty zonae) or by transplantation (chimera formation, tetraploid complementation). The latter types of experiment, although standard in many laboratories, are less stringent since

they allow for cell-cell interactions that are difficult to interpret. What transplantation type experiments can show is once again plasticity (as addressed above for nuclear transfer, but this time on a cellular level).

When testing entire 2C-like cells (Ishiuchi et al. 2015) with isolation type experiments, formation of a basic body plan (including a primitive streak where ordered gastrulation can occur) is to be seen as a reasonable criterium for totipotency (Denker 2014). This criterium is fulfilled autonomously by the zygote and early blastomeres („fully totipotent“ (Ishiuchi et al. 2015)), where this potential is most probably due to asymmetry cues that are derived from the oocyte/zygote cytoplasm (Gardner 2006; Zernicka-Goetz 2011). 2C-like cells derived from ES cells that have been propagated before in culture cannot be expected to possess such asymmetry cues directly segregated from oocyte cytoplasm anymore. However, according to many data from experimental embryology, ES cells can initiate self-organization (pattern formation), and in doing so they typically respond to exogenous asymmetry signals, the resulting patterns being influenced for example by extracellular matrix which can obviously provide artificial positional cues *in vitro* (Denker 2004; Denker 2014). Similarly, pattern development by ES cells is influenced by contact to other cells (as seen in chimera formation experiments). Perhaps it would it be reasonable to address this type of potentiality with a different term than totipotency (e.g. *omnipotency*), meaning a potentiality that is not endogenous to the cells and thus cannot be expressed autonomously (i.e. it will not be expressed in a neutral environment excluding external stimuli) but depends on externally provided signals. We should see that it remains unknown for the moment whether 2C-like cells might be able to initiate an early embryonic self-organization process independent of any external signaling/asymmetry cues, i.e. in a neutral environment (e.g. an empty zona).

In conclusion, notwithstanding terminological considerations the recent report by Ishiuchi et al. (Ishiuchi et al. 2015) could indeed provide a stimulus to investigate experimentally whether and under what circumstances induced 2C-like cells might be able to initiate a process of early embryonic pattern formation, comparable to early blastomeres. Or would their characteristic biological property have to be addressed better not as totipotency (in the strict sense) but rather as a maximal ability to

respond to external signals channelling differentiation, i.e. plasticity? It would be highly desirable to address these questions in the future by appropriate experiments, in the mouse and in nonhuman primate models.

References

Denker H.-W. (2004) Early human development: new data raise important embryological and ethical questions relevant for stem cell research. Naturwissenschaften 91:1-21.

Denker H.W. (2014) Stem cell terminology and 'synthetic' embryos: a new debate on totipotency, omnipotency, and pluripotency and how it relates to recent experimental data. Cells Tissues Organs 199:221-227.

Gardner R.L. (2006) The axis of polarity of the mouse blastocyst is specified before blastulation and independently of the zona pellucida. Hum Reprod 22:798-806.

Ishiuchi T., Enriquez-Gasca R., Mizutani E., Boskovic A., Ziegler-Birling C., Rodriguez-Terrones D., Wakayama T., Vaquerizas J.M. & Torres-Padilla M.E. (2015) Early embryonic-like cells are induced by downregulating replication-dependent chromatin assembly. Nat Struct Mol Biol 22:662-671.

Macfarlan T.S., Gifford W.D., Driscoll S., Lettieri K., Rowe H.M., Bonanomi D., Firth A., Singer O., Trono D. & Pfaff S.L. (2012) Embryonic stem cell potency fluctuates with endogenous retrovirus activity. Nature 487:57-63.

Morgani S.M., Canham M.A., Nichols J., Sharov A.A., Migueles R.P., Ko M.S. & Brickman J.M. (2013) Totipotent Embryonic Stem Cells Arise in Ground-State Culture Conditions. Cell Rep 3:1945-1957.

Zernicka-Goetz M. (2011) Proclaiming fate in the early mouse embryo. Nat Cell Biol 13:112-114.