Animal Cloning & Cell Reprograming

Guest Editors

Michele Boiani and José B. Cibelli

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Preface

What scientists would like to tell you about reprogramming (if only they knew!)

In a famous Science editorial entitled *"Will society be prepared?",* Nobel Laureate Marshall Nirenberg envisioned the possibility that cells would be "programmed with synthetic messages within 25 years" (Nirenberg, 1967). Whether Nirenberg held messenger RNAs as such capable of (re) programming cells, is not known, but clearly he considered them as instrumental to achieve (re)programming.

Back in 1967 the term "programming" may have sounded new (it was probably taken from junior computer science). However, in biology the concept behind the terms "programming" and "reprogramming" was already familiar to entomologists (Willis, 1969; Kastern and Krishnakumaran, 1975). In the 1970s and 1980s, the term "reprogramming" reached vertebrates in the context of amphibian nuclear transfer (Hoffner and DiBerardino, 1977) and human-mouse heterokaryon experiments (Chiu and Blau, 1984). Oddly, in mice, the term "programming" came subsequent to "reprogramming" (McCue and Sherman, 1982).

The term reprogramming can indicate a natural change of cell fate, or an artificial change of gene expression imposed via an experiment. Gurdon and Simonsson define two types of experimental reprogramming: nuclear and cellular (Gurdon and Simonsson, 2003). Accordingly, nuclear reprogramming stands for "changes in gene activity that are induced experimentally by introducing nuclei into a new cytoplasmic environment" (Gurdon and Simonsson, 2003). This can be achieved by nuclear injection into an oocyte, or by fusion of a whole cell with an oocyte or with a pluripotent cell such as an embryonic stem (ES) cell. When the differentiation state of a whole cell is changed, without invasive micromanipulation, into one characteristic of a different cell type, this is referred to as direct reprogramming of a cell (Mitalipov and Wolf, 2009). It was not until the year 2006 that Nirenberg's vision became reality as Yamanaka and colleagues forced mouse fibroblasts to become induced pluripotent stem (iPS) cells by expressing the messages of four genes: *Oct4, Sox2, Klf4* and *c-Myc* (Takahashi and Yamanaka, 2006). In fact, the Yamanaka experiment opened the path to exceed Nirenberg's prediction, in that the requirement for messages (mRNAs) could be overcome by deploying the protein products of those messages (Zhou *et al.*, 2009).

In our role as guest editors of this Special Issue of *The International Journal of Developmental Biology (Int. J. Dev. Biol.),* we attempted to summarize the state of the art in the field of reprogramming by asking some leading scientists for their opinions. There are certainly multiple ways to structure an editorial that intends to introduce the topic of reprogramming (as it would be overambitious to think about, let alone pursue, all questions about reprogramming in only one Special Issue!). Therefore we have selected three of the many possible questions, and explain why, in our view, these questions should rank high on the reprogramming wish-list.

Natural programming and basic principles of reprogramming

The overarching question in the broad field of reprogramming is whether different mechanisms are responsible for different methods of reprogramming. It is conceivable that cell reprogramming as seen in ES and iPS cells is more reliant on cell surface receptors and signaling pathways compared to nuclear transfer into oocytes. The role of signaling pathways is also apparent in the reprogramming that is observed when primordial germ cells (PGCs) and spermatogonia are cultured *in vitro* to give rise to embryonic germ (EG) and germline pluripotent stem (gPS) cells, respectively (Resnick *et al.,* 1992; Matsui *et al.,* 1992; Ko *et al.,* 2009). It is still unclear if DNA replication is necessary for reprogramming to occur. Results of ES cell fusion experiments argue that DNA replication and cell division are not required for reprogramming (Do and Schöler, 2004), similar to the case of nuclear transplantation into the germinal vesicle of *Xenopus* oocytes and of the most recent mouse-human heterokaryon experiments (Bhutani *et al.*, 2010). Reprogramming in iPS cells appears to benefit from accelerated cell divisions (Hanna *et al.*, 2009), suggesting that DNA replication and cell division may be not be neutral or dispensable in this context after all, and that reprogramming methods operate in different ways.

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Animal cloning

To many, it remains questionable whether the mammalian oocyte can perform reprogramming to a full extent, since the existence of any normal e.g. healthy clones has often been disputed. Primordial oocytes grow in the ovary for 19 days (mouse) and 3 months (human) to form one of the largest cells of the body and the only one that will become totipotent when fertilized. Oocytes have probably the highest amounts of reprogramming factors, which they can exchange with the nucleus very quickly (Catez *et al.*, 2004). In turn, these factors may override preexisting circuits that hinder fast reprogramming of somatic nuclei. These circuits are not equally refractory to be reprogrammed across somatic cell types, some cell types being easier to reprogram than others. Considering that ES, EG and iPS cells do not exist *in vivo*, whereas oocytes do, reprogramming by oocytes is the best model to follow when it comes to understanding the natural mechanisms of reprogramming. It has been proposed that oocytes can be used for reprogramming even if they are of suboptimal or poor quality. This possibility is particularly appealing in humans, where paucity of fresh human oocytes can be offset, in part, with the use of oocytes discarded from Assisted Reproductive Technology (ART) programs after fertilization failure or thawing of zygotes that were stored in liquid nitrogen for too long to be safely implanted: the technique for nuclear transfer in zygotes has been worked out (Greda *et al.*, 2006; Egli *et al.*, 2007). However, even with an increased supply of oocytes, the method of nuclear transfer requires special skills and it is not amenable to substantial upscaling in humans, while the situation is more promising in farm species. The availability of ES and EG cells provides the second most effective means to induce pluripotency by fusion with somatic cells, after nuclear transfer into oocytes. Unlike the nuclear transfer method, cell fusion has been applied successfully in humans to derive pluripotent cell lines (Cowan *et al.*, 2005; French *et al.*, 2008; Fan *et al.*, 2011). However, technical problems remain with this technique since the somatic genome of the donor cell and the pluripotent genome of the host cell mingle after fusion, making it more difficult to conclude that the somatic component was stably reprogrammed, compared to other experimental settings in which the nuclei do not mingle (e.g. heterokaryon). Efforts are underway to remove the ES cell chromosomes selectively from the fusion hybrids (Matsumura *et al.*, 2007). The answer to the question of whether an oocyte can achieve full reprogramming is pending, but one may wonder which system can accomplish full reprogramming if the oocyte cannot.

Cell reprogramming

Lastly, we put forward the question of how reprogramming can be assessed independently of the generation of a cloned animal. From the pluripotency perspective, the gold standard of ultimate reprogramming is the formation of a complete organism containing functional cells of every kind (Gurdon and Melton, 2008). However this is not only problematic to achieve in humans, but it entails more than potency: it entails also epigenesis that is, the ability to build structured multicellular complexity in which the properties of the whole exceed the mere sum of the individual cell properties (see Denker 2009 for deeper reflections on the significance of pattern formation to cell potency). For practical purposes, it is not strictly required that the differentiation possibilities have to build a whole organism in order to prove that the reprogrammed cell was pluri/totipotent; and reprogramming may be complete also if it accomplishes a full, stable transition from one cell lineage to another, without necessarily going through a naive state of pluri/totipotency (Vierbuchen *et al.*, 2010).

From the studies that have been mentioned in this preface, it appears that programming and reprogramming can be part of the normal life cycle, that experimental reprogramming can be accomplished by more or less invasive means, and that the reprogrammed cell state can vary with regard to the spectrum of differentiation possibilities (potency). We would like to end this preface by wholeheartedly thanking the Editor in Chief of *The International Journal of Developmental Biology* and his editorial team, the board of reviewers, and the authors. There are other scientists in the field, besides those who joined this Special Issue, who would be worth asking for their opinions on reprogramming. It is however hard to convey the views and research of all those working in the field, in one single volume. We are proud of the work done, and trust that our readers will share our view that this Special Issue takes, albeit not yet the full path, a significant step in the direction of deepening current knowledge on reprogramming.

Michele Boiani and José B. Cibelli

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