Reduce, reuse, reprogram

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Mouse lines with inducible reprogramming factors expressed from a single genomic locus will allow reprogramming studies in multiple cell types and defined genetic backgrounds.

Epigenetic reprogramming is a process in which the ectopic overexpression of defined transcription factors (typically Oct4, Sox2, Klf4 and c-Myc) in differentiated somatic cells initiates a complex and poorly understood process by which the cells regress developmentally into a state similar to that of embryonic stem cells (ESCs). This approach, which generates what are termed 'induced pluripotent stem' cells (iPSCs), has attracted considerable interest from the entire biomedical research community and has triggered many studies ranging from basic molecular and cellular research to efforts aimed at the generation of clinically relevant cells. Two papers published in this issue of *Nature Methods*, from groups led by Rudolf Jaenisch and by Konrad Hochedlinger, describe a system for making iPSCs that has the potential to drastically accelerate current efforts toward an understanding of the reprogramming process[1,2].

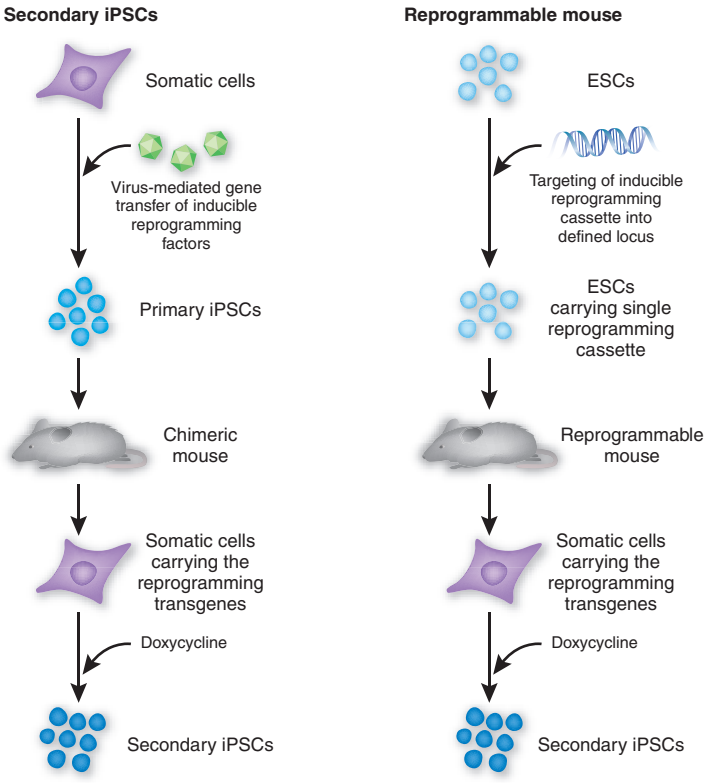

Figure 1 | Two inducible iPSC systems. Secondary systems (left) and the reprogrammable mouse (right) are shown.

The methods initially used to create iPSC lines involved virus-mediated gene transfer[3–6] and were as a consequence plagued by flaws related to the use of viral vectors. Early iPSC lines typically had considerable phenotypic variability, presumably because of the unpredictable locations of viral integration sites and different amounts of expressed reprogramming factors. Even though such cells were adequate for initial studies, it quickly became apparent that experiments seeking to probe deeper into the events and mechanisms underlying reprogramming required more precise tools.

Later versions of iPSC lines accordingly featured (i) genetically identical integration sites for the genes encoding the reprogramming factor and (ii) inducibility of the factors (**Fig. 1**). The so-called secondary iPSC system achieved these ends by using an inducible lentivirus system combined with an intermediate step in which newly reprogrammed iPSC lines were used to generate chimeric mice[7–9]. Subsequently, somatic cell types carrying the reprogramming factors were isolated from the mice. Such somatic cells were therefore identical to each other with respect to reprogramming factor integration sites, and, most importantly, the factors were inducible by doxycycline. The secondary iPSC system permitted a comprehensive comparison of reprogramming efficiency, even between different somatic cell types, and allowed the kinetics of reprogramming to be dissected. Critically, such methods permitted determination of the pivotal time window in which reprogramming factors are required. This system nevertheless suffered from two major drawbacks: first, reprogramming factor expression by the resulting cells remained somewhat unpredictable because of the random nature of the integration pattern and silencing of the viral transgenes; and second, the mouse strains essential to such studies were difficult to maintain and breed as the presence of multiple integrants made it challenging to follow the fate of every transgene.

The two papers published in this issue[1,2] solve the problems of secondary

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reprogramming by reducing the number of transgenes to one; this transgene includes all four reprogramming factors, is inducible and is specifically targeted to a particular site in the genome (*Col1a1*), at which it is integrated via homologous recombination. Both groups conducted gene targeting with the reprogramming cassette in ESCs, and then a transgenic mouse line—the reprogrammable mouse—was created using standard techniques. All tissues in such mice as a result carry an inducible genetic cassette with all reprogramming factors integrated at a specific genomic site. Thus, effective breeding is possible, and highly controllable robust expression of reprogramming factors may be obtained in cells isolated from virtually any embryonic, fetal or adult tissue.

Many applications can be envisioned for the 'reprogrammable mouse', and both groups have conducted exploratory experiments that validate the system[1,2]. One observation made by both groups concerned differences in reprogramming efficiency between various cell types isolated from the reprogrammable mice. Generally, the rule appears to be that the more undifferentiated or proliferative the original cell population, the more efficient the reprogramming process and the smaller the required gene dosage of reprogramming factors. This observation confirms previous results obtained in less defined systems and provides valuable insight into the molecular process of reprogramming, as it appears that both cell division and its associated epigenetic remodeling, as well as the developmental 'distance' between the somatic cells to be reprogrammed and ESCs or iPSCs, are critical factors determining reprogramming efficiency. These results confirm models that envision epigenetic landscapes in which different cell types occupy discrete positions and in which the distance between such positions is determined by the developmental hierarchy of cellular lineages[10]. Thus, the efficiency of reprogramming a particular cell type may serve as a measure of its developmental potency.

The new system also allows the development of powerful tools permitting high-volume screening of small molecule libraries to seek materials that might specifically replace some or all reprogramming factors. As shown by Jaenisch and colleagues[1], removal of one factor from the reprogramming cassette, followed by isolation of reprogrammable cells, may be used to identify molecules or genes that can complement and therefore rescue the reprogramming activity of the defective cassette.

The study from Hochedlinger and colleagues documents that reprogrammable mice developed teratomas even in the absence of any doxycycline stimulation[2]. The most likely explanation for this is that even very low background activity of the reprogramming cassette can result in reprogramming, provided that the cassette is expressed in an appropriate cell type. Cells that can be reprogrammed *in situ* by such low levels of transgenes must therefore be extraordinarily close to ESCs or iPSCs in developmental capacity, as it takes so little to reprogram them. Given that such cells are exposed to very low, albeit adequate, amounts of reprogramming factors over a prolonged period of time and initiate teratoma formation, it is conceivable that they are undergoing a process that is similar to what happens endogenously as part of tumorigenesis. Thus, the reprogrammable mouse could also prove invaluable in tumor studies. It is also interesting that 'partially reprogrammed cell lines', which are thought to result from an aberrant reprogramming process, were not evident using the new system[2]. This poses the question of whether such intermediately stable states are of any relevance in an understanding of the reprogramming process. Perhaps such stable, partially reprogrammed cells really reflect inadequate expression of some reprogramming factors rather than a step in the reprogramming process *per se*.

The few experiments discussed here illustrate how a well-defined technological tool can drill deeply into the 'black box' of reprogramming and show that many more useful insights may emerge. It is clear that the reprogramming field urgently needs not only defined criteria for what cells may be considered as authentic iPSCs but will also benefit from the use of defined starting material for the reprogramming process. Only then will we be able to adequately compare data from different laboratories. Now that we are beginning to understand the molecular processes underlying pluripotency and reprogramming, the system described above, and other similar tools, are urgently needed to propel iPSC biology to the next level.

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Sorting cardiomyocytes: a simple solution after all?

Christine Mummery

Cardiomyocytes can be sorted to high purity upon staining them with a dye that labels mitochondria. This permits the preparation of pure populations of cardiomyocytes differentiated from stem cells.

Pluripotent stem cells are remarkable for their ability to form all cells of the body. At the same time this can be their most irksome property, as they will form a multitude of different cell types in a single culture. Although we can now steer stem cells sufficiently to differentiate into cells of one of the three germ lineages, obtaining pure populations of a particular cell type is still challenging. This is largely because there are few cell type–specific surface proteins to which antibodies are available for sorting. Such is the case for cardiomyocytes. Although there are many cardiomyocyte-specific proteins, such as transcription factors or sarcomeric pro-

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