Published in final edited form as:

Differentiation. 2014 July ; 88(1): 27–28. doi:10.1016/j.diff.2014.07.001.

Perspective for special Gurdon issue for differentiation: Can cell fusion inform nuclear reprogramming?

David Burns and Helen M. Blau*

Baxter Laboratory for Stem Cell Biology, Stanford University School of Medicine, Stanford, CA 94305, USA

Abstract

Nuclear reprogramming was first shown to be possible by Sir John Gurdon over a half century ago. The process has been revolutionized by the production of induced pluripotent cells by overexpression of the four transcription factors discovered by Shinya Yamanaka, which now enables mammalian applications. Yet, reprogramming by a few transcription factors remains incomplete and inefficient, whether to pluripotent or differentiated cells. We propose that a better understanding of mechanistic insights based on developmental principles gained from heterokaryon studies may inform the process of directing cell fate, fundamentally and clinically.

Keywords

Nuclear reprogramming; Cell fate changes; Cell fusion; Heterokaryons

The burgeoning aging population comprises a major and increasing problem worldwide and the need for regenerative medicine is acute. While autologous pluripotent cells can now be generated in abundance, recapitulating developmental cellular programs that lock down cell fate options to achieve a more stable mature differentiated state and avoid cancer, are needed.

Sir John Gurdon's ground-breaking experiments on somatic cell nuclear transfer (SCNT) in amphibians more than a half century ago, first demonstrated that the differentiated state could be reversed (Gurdon et al., 1958). The subsequent discovery by Shinya Yamanaka that pluripotency could be induced in specialized mammalian cells (iPSC) by the overexpression of only four transcription factors revolutionized the field by greatly enhancing the ability to reverse cell fates for modeling and treating human disease (Takahashi and Yamanaka, 2006).

To fully harness the potential of these findings for use in regenerative medicine, laboratories worldwide are now focusing on how to instruct induced pluripotent stem cells (iPSC) to become a desired mature differentiated state and evaluate their utility (Boulting et al., 2011; Lohle et al., 2012). An alternative approach entails transdifferentiation of one somatic cell to

express characteristics of another somatic cell (Marro et al., 2011; Vierbuchen et al., 2010). Yet a third strategy is to de-differentiate somatic cells to become tissue-specific stem cells dedicated to regenerating a particular tissue, overcoming current limitations in their abundance (Pajcini et al., 2010; Pomerantz and Blau, 2013). Can cell fusion aid in the identification of factors that reprogram cells to differentiated or stem-cell-like states?

Years ago, our laboratory showed that, contrary to the prevailing theory of the times, “terminally” differentiated human cells could be reprogrammed to adopt new fates. To demonstrate this plasticity of cell fate we employed cell fusion of two differentiated cell types in stable non-dividing heterokaryons (Blau et al., 1983; Blau et al., 1985) (Movie 1). Skewing the ratio of nuclei to favor the cell type of interest was often necessary for, reprogramming to occur, providing evidence that the stoichiometry of regulators was critical in determining the direction of reprogramming and that cell fate could be bi-directional. In other words, by tipping the balance of factors, a feature shared by SCNT and iPSC reprogramming (Yamanaka and Blau, 2010), gene expression typical of one differentiated cell type or fate could be altered to become characteristic of another. For example, when human hepatocytes were fused with an excess of mouse muscle cells, the activation of human muscle genes in the human liver cell could be observed. Moreover, we determined that the activation of genes encoding myogenic transcription factors followed a sequence that recapitulated muscle development (Pomerantz et al., 2009). However, due to technological limitations we could only analyze known candidate factors and a more comprehensive unbiased and detailed transcriptome-wide time-course and analysis of the overall extent of reprogramming were not possible. Now, due to recent advances both in fluorescence activated cell sorting (FACS) – which allows enrichment for purity and acquisition of thousands of heterokaryons – and in next generation sequencing and novel bioinformatics tools, we can revisit the potential of heterokaryons (Brady et al., 2013). Given these technological and methodological advances, we postulate that cell fusion based reprogramming may be fruitfully employed to help solve the major problem of obtaining specific desired cell fates that currently limits the use of iPSCs and transdifferentiated cells for regenerative medicine.

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.diff.2014.07.001>.

At present, reprogramming to either a pluripotent or transdifferentiated state is often inefficient or incomplete (Lister et al., 2009; Lister et al., 2011; Passier and Mummery, 2010; Wang et al., 2013). These limitations are likely due to current approaches. The ectopic overexpression of a handful of transcription factors was highly successful in the groundbreaking Yamanaka strategy (Takahashi and Yamanaka, 2006). However, introducing transcription factors typical of the desired end state, whether it is pluripotent, hepatic, cardiac or neuronal, abbreviates the natural reprogramming and differentiation processes imbued by exposure to the panoply of endogenous factors typical of cells undergoing SCNT, cell fusion, or normal differentiation. Thus, although attractive in their simplicity, we hypothesize that current approaches could benefit from an understanding of the sequential activation of transcription factors by cell signaling pathways, that ultimately leads to the desired mature differentiated cell “end state”. We propose that to fully enlist the

potential of nuclear reprogramming for regenerative medicine, a more thorough understanding of these temporal mechanisms may prove useful in order to more closely mimic and recapitulate human development.

Would a heterokaryon renaissance be useful? We postulate that, as in development, reprogramming cells entails a sequence of coordinated events of transiently expressed regulators that act in concert to induce a molecular program that results in a change of cell phenotype. By fusing cells of two types, we have shown that a time course of reprogramming can be recapitulated (Pomerantz et al., 2009). As a result, crucial transient activators and repressors can be identified during the reprogramming process (Bhutani et al., 2010; Brady et al., 2013). Furthermore, because cell fusion based reprogramming is very efficient (Bhutani et al., 2010; Pasque et al., 2011), the kinetics of regulators that act upon one another is revealed by transcriptome based profiling using RNA-seq (Brady et al., 2013). Global gene expression combined with recently developed methods for revealing sites of open chromatin and transcription factor binding sites, such as ATAC-seq (Buenrostro et al., 2013), DNase hypersensitivity and ChIP-seq (Gerstein et al., 2012) enable novel insights. Importantly from these next generation sequencing methods and bioinformatics algorithms, the temporal relationship of molecular events that give rise to either differentiated, transdifferentiated, or dedifferentiated cells can be generated en route to the desired cell fate. This information may help to overcome current problems plaguing the field of nuclear reprogramming for the purposes of regenerative medicine. It is our hope that advances resulting from heterokaryon studies will enhance our understanding and ability to direct cell fate and ultimately aid in the treatment of the ailments afflicting an ever-increasing aging population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

- Bhutani N, Brady JJ, Damian M, Sacco A, Corbel SY, Blau HM. Reprogramming towards pluripotency requires AID-dependent DNA demethylation. *Nature*. 2010; 463:1042–1047. [PubMed: 20027182]
- Blau HM, Chiu CP, Webster C. Cytoplasmic activation of human nuclear genes in stable heterokaryons. *Cell*. 1983; 32:1171–1180. [PubMed: 6839359]
- Blau HM, Pavlath GK, Hardeman EC, Chiu CP, Silberstein L, Webster SG, Miller SC, Webster C. Plasticity of the differentiated state. *Science*. 1985; 230:758–766. [PubMed: 2414846]
- Boulting GL, Kiskinis E, Croft GF, Amoroso MW, Oakley DH, Wainger BJ, Williams DJ, Kahler DJ, Yamaki M, Davidow L, Rodolfa CT, Dimos JT, Mikkilineni S, MacDermott AB, Woolf CJ, Henderson CE, Wichterle H, Eggan K. A functionally characterized test set of human induced pluripotent stem cells. *Nat Biotechnol*. 2011; 29:279–286. [PubMed: 21293464]
- Brady JJ, Li M, Suthram S, Jiang H, Wong WH, Blau HM. Early role for IL-6 signalling during generation of induced pluripotent stem cells revealed by heterokaryon RNA-Seq. *Nat Cell Biol*. 2013; 15:1244–1252. [PubMed: 23995732]
- Buenrostro JD, Giresi PG, Zaba LC, Chang HY, Greenleaf WJ. Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nat Methods*. 2013; 10:1213–1218. [PubMed: 24097267]

- Gerstein MB, Kundaje A, Hariharan M, Landt SG, Yan KK, Cheng C, Mu XJ, Khurana E, Rozowsky J, Alexander R, Min R, Alves P, Abyzov A, Addleman N, Bhardwaj N, Boyle AP, Cayting P, Charos A, Chen DZ, Cheng Y, Clarke D, Eastman C, Euskirchen G, Frietze S, Fu Y, Gertz J, Grubert F, Harmanci A, Jain P, Kasowski M, Lacroute P, Leng J, Lian J, Monahan H, O'Geen H, Ouyang Z, Partridge EC, Patacsil D, Pauli F, Raha D, Ramirez L, Reddy TE, Reed B, Shi M, Slifer T, Wang J, Wu L, Yang X, Yip KY, Zilberman-Schapira G, Batzoglou S, Sidow A, Farnham PJ, Myers RM, Weissman SM, Snyder M. Architecture of the human regulatory network derived from ENCODE data. *Nature*. 2012; 489:91–100. [PubMed: 22955619]
- Gurdon JB, Elsdale TR, Fischberg M. Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei. *Nature*. 1958; 182:64–65. [PubMed: 13566187]
- Lister R, Pelizzola M, Downen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature*. 2009; 462:315–322. [PubMed: 19829295]
- Lister R, Pelizzola M, Kida YS, Hawkins RD, Nery JR, Hon G, Antosiewicz-Bourget J, O'Malley R, Castanon R, Klugman S, Downes M, Yu R, Stewart R, Ren B, Thomson JA, Evans RM, Ecker JR. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011; 471:68–73. [PubMed: 21289626]
- Lohle M, Hermann A, Glass H, Kempe A, Schwarz SC, Kim JB, Poulet C, Ravens U, Schwarz J, Scholer HR, Storch A. Differentiation efficiency of induced pluripotent stem cells depends on the number of reprogramming factors. *Stem Cells*. 2012; 30:570–579. [PubMed: 22213586]
- Marro S, Pang ZP, Yang N, Tsai MC, Qu K, Chang HY, Sudhof TC, Wernig M. Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. *Cell Stem Cell*. 2011; 9:374–382. [PubMed: 21962918]
- Pajcini KV, Corbel SY, Sage J, Pomerantz JH, Blau HM. Transient inactivation of Rb and ARF yields regenerative cells from postmitotic mammalian muscle. *Cell Stem Cell*. 2010; 7:198–213. [PubMed: 20682446]
- Pasque V, Jullien J, Miyamoto K, Halley-Stott RP, Gurdon JB. Epigenetic factors influencing resistance to nuclear reprogramming. *Trends Genet*. 2011; 27:516–525. [PubMed: 21940062]
- Passier R, Mummery C. Getting to the heart of the matter: direct reprogramming to cardiomyocytes. *Cell Stem Cell*. 2010; 7:139–141. [PubMed: 20682439]
- Pomerantz JH, Blau HM. Tumor suppressors: enhancers or suppressors of regeneration? *Development*. 2013; 140:2502–2512. [PubMed: 23715544]
- Pomerantz JH, Mukherjee S, Palermo AT, Blau HM. Reprogramming to a muscle fate by fusion recapitulates differentiation. *J Cell Sci*. 2009; 122:1045–1053. [PubMed: 19295131]
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006; 126:663–676. [PubMed: 16904174]
- Vierbuchen T, Ostermeier A, Pang ZP, Kokubu Y, Sudhof TC, Wernig M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature*. 2010; 463:1035–1041. [PubMed: 20107439]
- Wang T, Wu H, Li Y, Szulwach KE, Lin L, Li X, Chen IP, Goldlust IS, Chamberlain SJ, Dodd A, Gong H, Ananiev G, Han JW, Yoon YS, Rudd MK, Yu M, Song CX, He C, Chang Q, Warren ST, Jin P. Subtelomeric hotspots of aberrant 5-hydroxymethylcytosine-mediated epigenetic modifications during reprogramming to pluripotency. *Nat Cell Biol*. 2013; 15:700–711. [PubMed: 23685628]
- Yamanaka S, Blau HM. Nuclear reprogramming to a pluripotent state by three approaches. *Nature*. 2010; 465:704–712. [PubMed: 20535199]