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Sir John Gurdon: Father of nuclear reprogramming

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Abstract

Sir John Gurdon founded the field of nuclear reprogramming. His work set the stage for the ever burgeoning area of stem cell biology and regenerative medicine. Here I provide personal reflections on times I shared with John Gurdon and professional reflections of the impact of his ground-breaking research on my own development as a scientist and on the field in general. His paradigm-shifting experiments will continue to provoke scientists to think outside the box for many years to come.

Keywords

Nuclear reprogramming; Cell plasticity; Cell fusion; Heterokaryons; Cloning

1. Personal reflections

John Gurdon is the quintessential gentleman. With a bashful nod of his head he will sidle up to you at the bar and ask apologetically if he might trouble you to ask you a small question or to explain two lanes on a figure of your student's poster. He gives you a wry smile, and in soft tones proceeds to ask the most penetrating question, one that never occurred to you, and that gets right to the heart of the issue. I first became aware of John Gurdon's nuclear transfer experiments when I was an undergraduate. I found his discovery thrilling and I knew then that I wanted to work in this area and address similar questions but had yet to figure out how. When I first met John in person at a FASEB meeting on Developmental Biology in Copper Mountain, Colorado, when I was a junior faculty member at Stanford, I was quite taken aback. John invited me to take a hike, a very steep hike as I recall, during which he asked me the most pointed and difficult questions about my nuclear reprogramming research using heterokaryons, which I tried my best to answer while gasping for air. With his typically impish grin and a flick of his thick red locks, he relentlessly pursued his targeted questions for hours on end, completely unfazed by the altitude. Another time at a meeting in Taos, I marveled that he emptied the salt and pepper shakers into mounds on his plate which he proceeded to devour together with a little food, due, I speculated, to his upbringing as his family had lived in India for quite some time. This

culinary fortitude I reasoned was the source of his amazing energy to repeatedly ski the black runs with moguls so high we could barely see one another.

Later, as a guest at his home, I watched with terror as he scaled the roof on a wobbly ladder to fix something that had gone amiss. He always had to do it himself, never hiring a helping hand. Similarly in the lab, he knew which petri dish contained which experiment and was intimately knowledgeable about each. I attended high table at Magdalene College where John was Master at his invitation, where I learned that he enjoyed challenging longheld customs, such as including me in the gathering as his college was the last all male college at Cambridge to admit women. We took a memorable car excursion into the countryside. When we arrived at a graveyard in Assington Village in Suffolk, John muttered in a rather matter of fact manner that virtually only Gurdons were buried there on the family property and that the graves spanned a period of roughly one-thousand years or so, and yes, other Gurdons had been independently knighted or served the country as members of the Peerage. It was a beautifully green spot, remote, understated, overgrown, lovely, and totally in character with John's nature. In 2012 I first witnessed the ruthlessly ambitious side of John. We were co-organizing a small meeting at Wiston House, a stately home near Sussex on rolling acres of lush green dotted with white sheep, inhabited until recently by a rather famous ghost. There I naively agreed to be John's croquet partner only to discover that the game was not about aiming one's ball through a series of hoops but instead decimating one's opponent by strategically and emphatically whacking his balls as far as humanly possible. John was quite the expert (Fig. 1).

Most notable was a recent trip in 2013 to an epigenetics meeting in Oxford, during which John invited me to accompany him to high table at Christ Church. It is not the spectacular library, the splendid chapel and impressive dining hall that he really wanted to show me but rather a "hole"—yes, a small round black hole outside on the grounds, which would have gone completely unnoticed, except that John pointed it out as very special to his development. As a junior fellow, his unbridled curiosity led him to lower himself into that black murky hole in order to see where it would lead him. He wound up riding in a canoe and floating underneath the entire city of Oxford, with occasional views of cars and pedestrians above—surfacing (covered with black muck) to face an awaiting bobby prepared to arrest him. These anecdotes reveal the essence of John. John is a man with insatiable curiosity, a love of adventure, a deep passion for understanding all things scientific, a gift for asking "the" penetrating question, a love of nature from small insects to large mountains, a man who does things for himself, with great charm, undaunted by challenges and utterly fearless.

2. Professional reflections

John's work exemplifies perseverance and strength of conviction. He interpreted his discovery that the transfer of specialized intestine nuclei into enucleated frog oocytes could give rise to all the tissues of swimming tadpoles as evidence of the remarkable plasticity of which nuclei, even from highly differentiated cell types, were capable (Gurdon et al., 1958). Notably, Briggs and King (Briggs and King, 1952) performed experiments of a similar nature a few years earlier (1952), but their interpretations were entirely different. They took

the low frequency at which such phenomena occurred as a sign of the limited plasticity of nuclear fate. And therein lies a clear example of Carl Jung's (Jung, 1933) profound insight: "It all depends on how we look at things". And typical of John, he chose not to focus on the low frequency, but rather on the fact that it was possible.

For decades John's finding of nuclear plasticity was viewed as a "frog phenomenon". The incidence was improved by him and others using other frog species, but no one was able to achieve the same results with mammals, for example mice, and not for lack of trying. Cell fusion in culture provided a means of showing that patterns of gene expression in one cell could be altered by transacting factors from another. Such experiments entailed combining cells of different species in order to distinguish their nuclei and gene products. Henry Harris in 1971 fused normal cells with malignant cells and found that the tumorigenic phenotype was repressed, providing the first demonstration for the existence of tumor suppressors acting in *trans* to repress an oncogenic phenotype (Harris et al., 1969). This ran counter to the prevailing dogma, as at the time, malignancy was thought to arise from activating mutations. Harris' conclusion relied on the reversion of the suppressed state by loss of chromosomes and re-emergence of the oncogenic state as a result.

Ironically, it was precisely these features that made a demonstration of reprogramming by trans-acting activators of silent genes so difficult to prove. Nonetheless, significant advances were made by laboratories worldwide in a series of elegant fusion experiments by Borris Ephrussi (Davidson et al., 1966; Fougere et al., 1972), Mary Weiss (Weiss and Chaplain, 1971), and Nils Ringertz (Carlsson et al., 1970), to name a few. Some of the problems in achieving gene activation were features of the times: it was difficult to grow cells in culture that were not transformed (transformed cell lines, as we now know, are not as easily reprogrammed and quite different epigenetically); species-specific markers of nuclei were hard to come by and assays of gene expression relied primarily on electro-phoretic differences among proteins of two different species (as monoclonal antibodies and PCR did not yet exist). Moreover, the very cell division and chromosome loss (which were key to Harris' demonstration of repressors) confounded evidence of activators, as what might appear as *de novo* expression and activation of a gene could well have resulted from loss of a repressor. Thus, finding a system in which cell division did not occur was crucial to the demonstration that such activating factors that functioned in *trans* existed.

Inspired by Gurdon and the pioneering work of the above colleagues, I decided to take on the challenge of reprogramming nuclei in mammalian cells. Gurdon's work showed that attention to the cell milieu was crucial. I reasoned that muscle, a naturally non-dividing multinucleate intracellular milieu, could serve as the appropriate microenvironment in which to observe muscle gene activation in human nuclei of disparate cell types. In heterokaryons with a skewed nuclear ratio that favored muscle, this proved true (Blau et al., 1983; 1985; Chiu and Blau, 1984; 1985). I recall that over the years John Gurdon encouraged and provoked me with questions that led to new insightful reprogramming experiments.

The first demonstration that mammalian specialized nuclei could give rise to an entire organism was shown by Ian Wilmut in Edinburgh with "Dolly the Sheep" so named to honor Dolly Parton, as the cell derived from mammary tissue (Wilmut et al., 1997). Notably

this reprogramming of nuclei was not due to enucleation and then transplantation of the nucleus into an oocyte, but instead entailed electro-fusion of an enucleated oocyte with a mammary cell and then transplantation of the fusion product into the oviduct. All the same, it showed that mammals were capable of the ‘frog phenomenon’ since an entire sheep could be generated from a mammary cell nucleus. Inspired by the sheep result, in 1998 Teru Wakayama and colleagues in Hawaii became determined to achieve reprogramming in mice. They succeeded and therein lies another major lesson. Others before him had failed because they transplanted the specialized nucleus into an enucleated fertilized egg, not an oocyte. The reason was that the two cell types seemed similar, both being mononucleate and early developmental stages. However, they could not have been more different in terms of their reprogramming potential. A technical difficulty was also a major reason that the oocyte was not used, as it was so fragile that touching it with a needle inevitably led to its demise, whereas the fertilized oocyte (egg) was much more hardy. The nature of the recipient cell proved to be crucial, as the egg was not naïve and reprogramming had been initiated. Thus, once Wakayama developed a pipette that delivered piezo electric pulses that could delicately enucleate the oocyte, he achieved nuclear transfer into this fragile recipient. His work met with success and entire mice were the first cloned (Wakayama et al., 1998) (Figs. 1 and 2).

And what did John Gurdon do in the interim? He continued to ask profound questions. The hallmark of his seminars was to select one particular question of interest—a question that sounded extremely simple (the sign of a clear and deep thinker) —usually illustrated with a hand-drawn cartoon. He would then proceed to describe his hypothesis together with a range of alternate solutions and interpretations, which he would systematically rule out with a series of simple and elegant experiments. One such example that remains especially poignant to me was the notion of a “community effect” which John conceived of and first described in *Nature* (Gurdon, 1988). His work revealed that the ability of a cell to respond to induction, for instance by differentiating as muscle, was enhanced by, or even dependent on, other neighboring cells differentiating in the same way at the same time (Gurdon et al., 1993). He speculated that the “community effect” was responsible for the formation of specific tissues from sheets of cells and creating sharp borders between them. Even upon inhibiting cell movement and division, he could induce changes in differentiation of cells in close proximity to one another. This fundamental principle could explain morphogenesis resulting from embryonic induction and the demarcation of juxtaposed tissues, with relevance to regenerative medicine as well.

However, it was not until Shinya Yamanaka (Takahashi and Yamanaka, 2006) (Takahashi et al., 2007) achieved the ultimate demonstration of mammalian cell plasticity by showing that the overexpression of just four transcription factors could reprogram virtually any mouse or human cell to pluripotency did John Gurdon’s pioneering work become fully appreciated and appropriately celebrated with a shared Nobel Prize. Clearly, John Gurdon’s intellect, creative thinking, and paradigm-changing experiments will continue to stimulate scientists to innovate for decades to come.

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Fig. 1.
John inserting a somatic nucleus into an oocyte.

Fig. 2.
John Gurdon and the author.