

Published as: *Differentiation*. 2014 July ; 88(1): 3–8.

## Lessons from a Great Developmental Biologist

**Edward M. De Robertis**

Howard Hughes Medical Institute and Dept. of Biological Chemistry, University of California, Los Angeles, CA 90095-1662, Tel.: 1 310 306 1463, Fax 1 310 206 2008

Edward M. De Robertis: ederobertis@mednet.ucla.edu

### Abstract

The announcement that Sir John Gurdon had been awarded the 2012 Nobel Prize for Medicine or Physiology was received with great joy by developmental biologists. It was a very special occasion because of his total dedication to science and turning the Golden Rule of western civilization – love your neighbor as yourself – into a reality in our field. This essay attempts to explain how John became such a great scientific benefactor, and to review some of his discoveries that are less well known than the nuclear transplantation experiments. A few personal anecdotes are also included to illustrate the profound goodness of this unique man of science.

### Keywords

JB Gurdon; Xenopus; nuclear reprogramming; nuclear transplantation

## 1. Introduction

John B. Gurdon is probably the most beloved developmental biologist of our times. What has been the secret of his success? One answer would be a life tirelessly dedicated to science that was then invested in the common good of others in the field. Here I would like to describe some of the Gurdon scientific landmarks that are not generally known, some of the service John has given all of us, and relate a few stories about my interactions with my scientific mentor.

John Bertrand Gurdon belongs to an ancient English family. His first and middle name have been used repeatedly by many of his ancestors. I once saw a Gurdon genealogical tree; it included a Bertrand de Gurdon recorded circa 1200 and a Jean de Gurdon ca. 1400. The French origin of the names is due to the fact that the “de Gurdon” were of Norman ancestry. Personally, I quite like the nobiliary “de” but this preposition was dropped along the centuries. Many of the Gurdon ancestors were knights but John’s side of the family did not inherit the title (although even today his second cousin is a hereditary peer, or member of the House of Lords, and his children carry the Gurdon surname). In what must have been a great personal satisfaction, Sir John regained a knighthood on his own merits in 1995. His parents lived in India but returned to Britain where John and his sister Catherine were born. As children during the Second World War, they were sent to the countryside and shared in the generalized hardships of those times, which shaped a generation. John attended Eton and, famously against the recommendation of his biology teacher, studied Zoology at Oxford. An excellent personal historical account published in *Annual Reviews of Cell and*

Developmental Biology details these early years (Gurdon, 2006). John's first paper was on the description of an insect new to Britain (Gurdon, 1954), and he has kept a keen interest in butterflies which has driven his travels to exotic places all over the world.

## 2. An early start

It was a happy day for developmental biology when young Gurdon found an excellent Ph.D. mentor at Oxford. Michail Fischberg had an interesting scientific lineage: he studied with Ernst Hadorn in Zurich, who had in turn studied with Fritz Baltzer in Bern, who had himself studied under Theodor Boveri in Wurzburg. Much of European cell biology can be traced to Boveri (including Hans Spemann among others). Three months into his Ph.D. studies, Fischberg asked young Gurdon to re-investigate the Briggs and King nuclear transplantation experiments using eggs of the frog *Xenopus*. This South African frog was used for pregnancy testing and had been recently introduced by Pieter Nieuwkoop in Holland and by Fischberg in Britain for embryological research (reviewed in Gurdon and Hopwood, 2000). Fischberg had had the foresight of keeping a strain of *Xenopus* carrying only one nucleolus (as compared to wild-type frogs which have two nucleoli) that had been found spontaneously in the lab and provided an invaluable nuclear marker. At age 25 Gurdon published a paper in Nature entitled "Sexually mature individuals of *Xenopus laevis* from the transplantation of single somatic nuclei" (Gurdon et al., 1958). Later on, fertile frogs were obtained from differentiated intestinal nuclei (Gurdon and Uehlinger, 1966). This work demonstrated that differentiated nuclei could be reprogrammed and that genetic information is not lost during cell differentiation. This important discovery was not readily accepted and John spent many years reproducing the experiment in various settings. Finally, his perseverance was rewarded in 2012 with the Nobel Prize.

## 3. Service to others

The key to John Gurdon, I think, is that he interpreted the good fortune of making such an important discovery as a beginning graduate student as a call of duty to give back to others; *noblesse oblige*. To whom much is given, much is expected; this philosophy has rarely been followed so tirelessly. John's beneficial influence has been felt at many levels in our field of developmental biology. He has been an indefatigable supporter of scientific societies, in which scientists organize themselves for the common good of their field. He attends many of their large meetings and always makes it a priority to discuss as many posters as humanly possible with students. For decades John was the *éminence grise* behind the Company of Biologists (publishers of Development and Journal of Cell Science), who plow the journal profits back into the scientific community. He started, together with Igor Dawid, the *Xenopus* conferences that take place regularly every two years since 1984. In 1983 he founded a new institute in Cambridge, now aptly renamed the Gurdon Institute, the premier research center in our field. He served as Master of Magdalene College, Cambridge (a large job), and Governor of The Wellcome Trust, London. He was President of the International Society of Developmental Biologists, and served in many committees that keep the machinery of science running.

An early start, a good advisor, superb scientific publications, a record of selfless service for the common good, and finally his secret weapon: John is always working at his bench with his dissecting microscope, micromanipulator, and his beloved *Xenopus* oocytes. He has set the tone for the entire *Xenopus* field, one in which many principal investigators continue working with their own hands. Gurdon has trained many scientists (Fig. 1) and one can safely say the majority of the *Xenopus* development field is his F2, F3 or F4 progeny.

#### 4. A lifetime of discovery

John Gurdon has made many important contributions to cell and developmental biology in addition to demonstrating the totipotency of single nuclei transplanted into eggs. These landmark studies are described here in chronological order.

1964 – John Gurdon, together with his long-time friend Donald D. Brown of the Carnegie Institution, showed that when 1-nucleolus frogs were crossed, the resulting 25% of anucleolate tadpoles lacked ribosomal RNA synthesis (Brown and Gurdon, 1964). This key discovery led to the current realization that the function of the nucleolus is to produce large ribosomal RNA. In addition, this collaboration had the important effect of introducing the frog *Xenopus* to American molecular biology.

1968 – John explored the effects of microinjecting multiple nuclei (initially from brain) instead of a single one. He found that in eggs laid by the frog DNA was replicated; that in progesterone-matured oocytes chromosomes condensed but did not replicate DNA; and finally that nuclei microinjected into oocytes enlarged and synthesized RNA, but did not replicate DNA (Gurdon, 1968). These observations were later pursued by Yoshio Masui in Canada and led to the discovery of maturation-promoting factor (MPF) which provided the key to understanding the cell cycle and a Nobel Prize for Tim Hunt.

1970 – In very important work that started a new era in nucleocytoplasmic transport, Gurdon himself iodinated purified histone proteins and microinjected them into oocyte cytoplasm. He found that histones migrated into the oocyte nucleus where they accumulated even in the absence of DNA binding. This meant that the nuclear migration signal was part of the mature protein sequence. This important contribution is not well known because it was published as part of a review (Gurdon, 1970). However, his scientific progeny continued working in this new field of nuclear transport (Bill Bonner, Ron Laskey, myself, and most importantly my postdoctoral trainee Iain Mattaj), and eventually its molecular mechanism was elucidated. Gurdon's idea of microinjecting oocytes extracted surgically from the abdomen of the female frog with purified histones was crucial, because until then most work in amphibians had been carried out with fertilized eggs, which divide and differentiate rapidly. Gurdon generated a Modified Barth's Solution (a saline initially devised to study neural induction) that kept oocytes healthy for days and weeks. He realized that it was possible to use the oocyte as a living test tube to test the activity of purified macromolecules. This was a very fertile insight.

1971 – The ability to culture oocytes allowed Gurdon to do a truly daring experiment: the microinjection of mRNA into oocytes. A peak of 9S RNA had been detected in rabbit reticulocytes and was thought it might be related to Globin synthesis. At that time, *in vitro*

systems for the translation of eukaryotic proteins (such as rabbit reticulocyte and wheat germ extracts) did not exist, and the existence of mRNA remained a hypothesis. Gurdon showed that microinjected mRNA could be translated into protein in frog oocytes (Gurdon et al., 1971). This experiment was a major breakthrough in molecular biology. In my opinion, it should have earned Gurdon the Nobel Prize, because the oocyte became the system of choice to translate eukaryotic proteins in order to study their biological activities. For example, *Xenopus* oocytes have been extensively used to analyze the function of neurotransmitter receptors. When years later Doug Melton introduced synthetic mRNAs transcribed with phage SP6 RNA polymerase, microinjection into oocytes and developing embryos became an extremely useful part of the molecular biology toolbox.

1977 – While carrying out microinjection experiments introducing multiple somatic nuclei, John realized that if the injection was targeted to the animal pole the injected material remained inside the giant oocyte nucleus, known as the germinal vesicle (GV). Microinjection of purified SV40 (Mertz and Gurdon, 1977) or 5S ribosomal DNA (Brown and Gurdon, 1977) into the GV resulted in transcription of these DNAs. This was the first transcription system available for eukaryotic genes and a major breakthrough. It was also found that DNA microinjected into the oocyte GV was transcribed and then translated into proteins (De Robertis and Mertz, 1977). Since a plasmid containing *Drosophila* histone genes was injected, this was the first time a gene cloned by recombinant DNA technology was expressed into protein (and John Gurdon did all the DNA microinjections for that study, my first Cell paper).

1984 – When mesoderm induction by growth factors was discovered by Jim Smith and Jonathan Slack, John Gurdon introduced the use of molecular markers such as muscle Actin genes to study embryonic induction by Activin (Mohun et al., 1984; Gurdon et al., 1985). The use of molecular markers led to important advances in our understanding of how cells perceive morphogen gradients. The Gurdon group went on to clone several key genes such as the twin-inducing homeobox gene *siamois* (Lemaire et al., 1995) and *eomesodermin*, a T-box gene induced very early during mesoderm formation (Ryan et al., 1996).

1988 – John Gurdon found that in order to obtain induction of muscle cells a certain number of cells interacting with each other was required. This a cell-cell interaction came to be known as the community effect (Gurdon, 1988; Gurdon et al., 1994), a new principle in cell-cell communication.

1977 to present – The microinjection of multiple somatic nuclei into oocytes (isolated from cells in which the plasma membrane had been partially removed with Lysolecithin) proved a very powerful tool to study nuclear reprogramming in the absence of any intervening DNA replication. The method of Dr. Shinya Yamanaka to reprogram somatic cells into iPS cells uses four transcription factors and requires many days and rapid cell replication. The power of oocytes to reprogram gene expression was first demonstrated by early experiments in which *Xenopus* kidney nuclei were transplanted into oocytes of the newt *Pleurodeles waltlii*. We found that the newt oocytes expressed *Xenopus* oocyte-specific genes and inactivated kidney-specific proteins (De Robertis and Gurdon, 1977). John Gurdon has relentlessly pursued the molecular mechanisms of nuclear reprogramming. For example, mammalian

somatic nuclei activate expression of the stem cell factor Oct-4 when placed in oocyte nuclei (Byrne et al., 2003). Differentiated cells resist reprogramming because epigenetic marks in DNA and chromatin tend to maintain pluripotency genes turned off. There is a battle for supremacy between the egg and the nucleus (Gurdon, 2013). Epigenetic memory depends on factors such as incorporation of variant Histone H3.3 into chromatin (Ng and Gurdon, 2008) and Histone H3 methylation (Murata et al., 2010). In their latest Science paper, the Gurdon team shows that a regulator of nuclear Actin reorganization is required for reprogramming (Miyamoto et al., 2013). The saga of nuclear reprogramming continues relentlessly to this day.

## 5. Some personal anecdotes

John is a wonderful mentor who teaches by example. Recently, I asked him whether he still keeps working twice a week (hoping to add that I did too) and he answered: “Eddy, now I am free to work in the lab every day”. John is dedicated to doing good to others and is essentially a profoundly kind man. To celebrate my 60<sup>th</sup> birthday my students organized a symposium at EMBL in Heidelberg. John had another commitment that day, but generously found time to fly in from Cambridge in the morning, give his talk, and leave after lunch. It was possibly the happiest day of my life (Fig. 2).

John Gurdon is a spectacular speaker. I know two people who were inspired to enter science simply because they heard him speak. John trains his students in public speaking, and I have attempted to summarize how to give a seminar in imitation of the Gurdonian style in the sidebar “Tips for Talks” in my lab webpage <http://www.hhmi.ucla.edu/derobertis/>.

This brings me to the story of how I met John Gurdon, a life-changing moment. In 1972 I had completed my M.D. in Uruguay, and was on my first year of Ph.D. studies at the Leloir Institute in Buenos Aires. John had been invited to give a lecture in Brazil and the organizers asked the British Council (which is a kind of cultural branch of the British Foreign Office), to finance this trip. They agreed, but on condition that John gave a tour of lectures through several countries in Latin America. With his deep sense of duty and humility John, incredibly, accepted the challenge.

John was already immensely famous in our biochemical circles because of his recent mRNA microinjection work and great anticipation preceded his talk. When arriving for work that morning, I observed an archetypal Englishman standing between two centrifuges in the corridor and realized it had to be the seminar speaker. There were many people working in the institute at that moment but were shy of speaking English, and he was left standing there while our Director, Nobel laureate Luis Leloir, was occupied finishing an experiment. Seeing him all alone, I somehow gathered enough courage to approach him and offer to show my results (which were meager, just two tube SDS gels stained for RNA polymerase subunits). Gladly accepting, John came to my bench and we talked until the Director sent for him a half hour later. Gurdon gave us a spectacular seminar. Later in the afternoon I saw him leave, left alone once again, this time to brave the formidable Buenos Aires rush hour. Seeing that he was on foot, I jumped into the car (which my father had lent me that day and was of a rare German make called Borgward), drove around the block to the bus stop, and

casually pretended to be surprised of finding him standing there and gave him a ride to his hotel in the city center. This small gesture of politeness was to change my life.

A few years later I was looking for a postdoctoral position and one of my professors suggested Gurdon. I asked him why somebody as important as him would even consider lowly me. His answer was “because a few days after his visit the British Embassy sent a cultural attaché to talk to Dr. Leloir to say that if De Robertis ever wanted to train in a laboratory in Britain a fellowship would be made available to him”. Taken aback, I asked why I had not been told about this. “So that it did not go to your head” was the answer. I wrote, Gurdon accepted me into his lab just like that (Fig. 3), perhaps because he has a liking for rare automobiles. Later I learned that John was turning down many postdoctoral applications every year. I like to tell this story because it shows that John Gurdon took the time to put a good word in for a young student he hardly knew. A few months later our young family arrived in Cambridge, England, in 1975, where we stayed for six years. I have been an Anglophile ever since. Until we arrived I did not know that the Medical Research Council Laboratory was the best possible place to receive postdoctoral training. At that time Francis Crick, Max Perutz, Fred Sanger, Cesar Milstein, Aaron Klug, Sydney Brenner, and others that went on to earn Nobel prizes were working there. It was not a very large institute and we could talk to these luminaries in the cafeteria at teatime. For a young impressionable mind like mine, Cambridge was scientific heaven. John Gurdon was a kind and ever polite mentor; to learn one only needed to pay attention and attempt to imitate his example. His wonderful wife Jean was like a mother to the young families of postdoctoral fellows, she certainly took us under her wing.

Many years later I was very fortunate to be invited to Sir John’s Nobel ceremonies in Stockholm in December 2012, which lasted for almost a week. In addition to his lovely family, his other guests were Don Brown, Ron Laskey, Doug Melton, Laurence Korn, Marvin Wickens, Alan Colman, and the technicians that helped him carry out the famous nuclear transplantation experiments at Oxford, Ann Clarke and Valerie Moar (Fig. 4). It was a memorable experience to be there representing the many biologists that are indebted to John Gurdon.

## 6. Conclusion

It is most appropriate that the editors of *Differentiation* have prepared this special issue to honor Sir John Gurdon. His relentless pursuit of ever new scientific questions over so many decades is legendary. He is an exemplary human being who through a life of rectitude has given back so much to our field. The Gurdon Nobel Prize ennoble all developmental biologists.

## References

- Brown DD, Gurdon JB. Absence of ribosomal-RNA synthesis in the anucleolate mutant of *Xenopus laevis*. *Proc Nat Acad Sci US*. 1964; 51:139–146.
- Brown DD, Gurdon JB. High fidelity transcription of 5S DNA injected into *Xenopus* oocytes. *Proc Nat Acad Sci US*. 1977; 74:2064–2068.

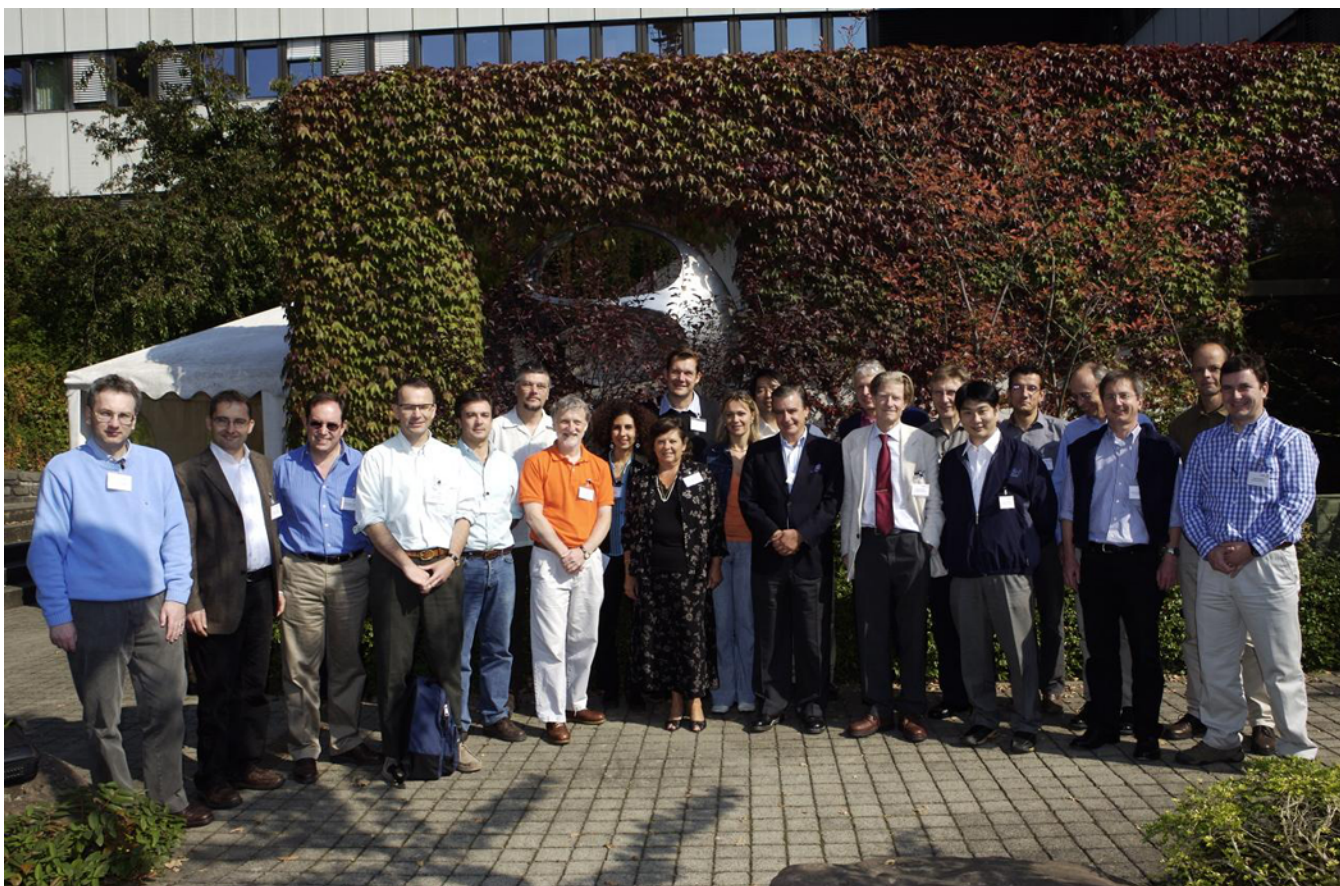
- Byrne JA, Simonsson S, Western PS, Gurdon JB. Nuclei of adult mammalian somatic cells are directly programmed to oct-4 stem cell gene expression by amphibian oocytes. *Curr Biol.* 2003; 13:1206–1213. [PubMed: 12867031]
- De Robertis EM, Gurdon GB. Gene activation in somatic nuclei after injection into amphibian oocytes. *Proc Nat Acad Sci US.* 1977; 74:2470–2474.
- De Robertis EM, Mertz JE. Coupled Transcription – Translation of DNA injected into *Xenopus* oocytes. *Cell.* 1977; 12:175–182. [PubMed: 198138]
- Gurdon JB. *Cephalcia fallen* Dalman (Hym., Pamphiliidae) new to Britain. *Ent Mon Mag.* 1954; 90:234.
- Gurdon JB. Changes in somatic cell nuclei inserted into growing and maturing amphibian oocytes. *J Embryol exp Morph.* 1968; 20:401–414. [PubMed: 5750105]
- Gurdon JB. Nuclear transplantation and the control of gene activity in development. *Proc Roy Soc B.* 1970; 176:303–314. [PubMed: 4395100]
- Gurdon JB. A community effect in animal development. *Nature.* 1988; 336:772–774. [PubMed: 3205305]
- Gurdon JB. From nuclear transfer to nuclear reprogramming: the reversal of cell differentiation. *Annu Rev Cell Dev Biol.* 2006; 22:1–22. [PubMed: 16704337]
- Gurdon JB. The egg and the nucleus: a battle for supremacy. *Development.* 2013; 140:2449–2456. [PubMed: 23715537]
- 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]
- Gurdon JB, Uehlinger V. “Fertile” intestine nuclei. *Nature.* 1966; 210:1240–1241. [PubMed: 5967799]
- Gurdon JB, Lane CD, Woodland HR, Marbaix G. The use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. *Nature.* 1971; 233:177–82. [PubMed: 4939175]
- Gurdon JB, Fairman S, Mohun TJ, Brennan S. The activation of muscle-specific actin genes in *Xenopus* development by an induction between animal and vegetal cells of a blastula. *Cell.* 1985; 41:913–922. [PubMed: 4005952]
- Gurdon JB, Harger P, Mitchell A, Lemaire P. Activin signalling and the spatial control of response to embryonic induction. *Nature.* 1994; 371:487–492. [PubMed: 7935761]
- Gurdon JB, Hopwood N. The introduction of *Xenopus laevis* into developmental biology: of empire, pregnancy testing and ribosomal genes. *Int J Dev Biol.* 2000; 44:43–50. [PubMed: 10761846]
- Lemaire P, Garrett N, Gurdon JB. Expression cloning of *Siamois*, a new *Xenopus* homeobox gene expressed in dorsal vegetal cells of blastulae and capable of inducing a complete secondary axis. *Cell.* 1995; 81:85–94. [PubMed: 7720076]
- Mertz JE, Gurdon JB. Purified DNAs are transcribed after microinjection into *Xenopus* oocytes. *Proc Nat Acad Sci US.* 1977; 74:1502–1506.
- Miyamoto K, Teperek M, Yusa K, Allen GE, Bradshaw CR, Gurdon JB. Nuclear Wave1 is required for reprogramming transcription in oocytes and for normal development. *Science.* 2013; 341:1002–1005. [PubMed: 23990560]
- Mohun TJ, Brennan S, Dathan N, Fairman S, Gurdon JB. Cell type-specific activation of actin genes in the early amphibian embryo. *Nature.* 1984; 311:716–721. [PubMed: 6548550]
- Murata K, Kouzarides T, Bannister AJ, Gurdon JB. Histone H3 lysine 4 methylation is associated with the transcriptional reprogramming efficiency of somatic nuclei by oocytes. *Epigenetics Chromatin.* 2010; 3:4.10.1186/1756-8935-3-4 [PubMed: 20181087]
- Ng RK, Gurdon JB. Epigenetic memory of an active gene state depends on histone H3.3 incorporation into chromatin in the absence of transcription. *Nat Cell Biol.* 2008; 10:102–109. [PubMed: 18066050]
- Ryan K, Garrett N, Mitchell A, Gurdon JB. Eomesodermin, a key early gene in *Xenopus* mesoderm differentiation. *Cell.* 1996; 87:989–1000. [PubMed: 8978604]



**Fig. 1.**

The Gurdon group at the Laboratory of Molecular Biology, Cambridge, circa 1980. Top row: Doug Melton (graduate student), Marvin Wickens (postdoc), William Earnshaw (Laskey's postdoc), Eddy De Robertis, Ruth Longthorne (Eddy's technician), Richard Harland (Laskey's graduate student), Kazuko Nishikura (Eddy's first postdoc), Laurence Korn (postdoc), Stewart Weisbrod (postdoc), John Gurdon (at age 46) and Julian Wells (sabbatical visitor from Australia). Lower row: Sue Whytock (John's technician), two young ladies, Barbara Rodbard (John's excellent secretary) and Jeff Partington (postdoc). Long-time colleague Ron Laskey is missing from this photo. Note that John was the most handsome gentleman, and kept only a small group working directly with him.





**Fig. 2.** Sir John Gurdon with the author and some of his F2 from the De Robertis lab. The occasion was a symposium at EMBL to celebrate my 60<sup>th</sup> birthday in 2007. This is only part of the scientific progeny through just a single one of John Gurdon's many trainees, and serves to illustrate the enormous influence Sir John has had on developmental biology. The lineage of most workers in the *Xenopus* development field can be traced back to him. From left to right: Thomas Bürglin, Edgar Pera, Abraham Fainsod, Christof Niehrs, Zétó Belo, Luc Leyns, Iain Mattaj, Sandra Piccolo, Ana De Robertis, Stefano Piccolo, Ghislaine Agius, Yoshiki Sasai, Eddy De Robertis, Rolf Zeller, Sir John Gurdon, Chris Wright, Hiroki Kuroda, Eric Agius, Martin Blum, Herbert Steinbeisser, Michael Oelgeschläger and Juan Larraín.

**MRC**  
Medical Research Council

MRC Laboratory of Molecular Biology  
University Postgraduate Medical School  
Hills Road, Cambridge, CB2 2QH  
England

telephone Cambridge (0223) 48011

17th July, 1974

Dr. E.M. De Robertis,  
Instituto de Investigaciones Bioquimicas,  
Fundacion Campomar,  
OBLIGADO 2490,  
Buenos Aires (28),  
South America.

Dear Dr. De Robertis,

I do of course remember meeting you in Buenos Aires; I even remember  
your ~~Borgward~~ car!

Thank you very much for writing to me.

With best wishes,

Yours sincerely,

J.B. Gurdon

**Fig. 3.**  
Letter of acceptance to Gurdon's lab. Note that he remembered the little known Borgward  
car. John loves automobiles. For some years his first vehicle was a bicycle and the other one  
a Lotus sports car.



**Fig. 4.** The Gurdon party in Stockholm before the Nobel Banquet, December 2012. From left to right: Ann Laskey, Ron Laskey, Laurence Korn, Aurea Connolly (John's daughter), Lady Jean Gurdon, Sir John Gurdon, Catherine Thompson (John's sister), Alan Colman, Ann Clarke, Marvin Wickens, Valerie Moar, Douglas Melton, Edward De Robertis, Serena Connelly, Oliver Connelly and Edward Connelly.