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With Sean Lind and Elizabeth Brown

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The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

by J. B. GURDON[1]

From the Embryology Laboratory, Department of Zoology, Oxford

WITH ONE PLATE

INTRODUCTION

AN important problem in embryology is whether the differentiation of cells depends upon a stable restriction of the genetic information contained in their nuclei. The technique of nuclear transplantation has shown to what extent the nuclei of differentiating cells can promote the formation of different cell types (e.g. King & Briggs, 1956; Gurdon, 1960*c*). Yet no experiments have so far been published on the transplantation of nuclei from fully differentiated normal cells. This is partly because it is difficult to obtain meaningful results from such experiments. The small amount of cytoplasm in differentiated cells renders their nuclei susceptible to damage through exposure to the saline medium, and this makes it difficult to assess the significance of the abnormalities resulting from their transplantation. It is, however, very desirable to know the developmental capacity of such nuclei, since any nuclear changes which are necessarily involved in cellular differentiation must have already taken place in cells of this kind.

The experiments described below are some attempts to transplant nuclei from fully differentiated cells. Many of these nuclei gave abnormal results after transplantation, and several different kinds of experiments have been carried out to determine the cause and significance of these abnormalities.

The donor cells used for these experiments were intestinal epithelium cells of feeding tadpoles. This is the final stage of differentiation of many of the endoderm cells whose nuclei have already been studied by means of nuclear transplantation experiments in *Xenopus*. The results to be described here may therefore be regarded as an extension of those previously obtained from differentiating endoderm cells (Gurdon, 1960*c*).

MATERIAL AND METHODS

The animals used for these experiments belong to the subspecies *Xenopus laevis laevis*. The transplantation technique has been carried out as described previously (Elsdale *et al.*, 1960), except that the donor tissue was exposed to

[1] *Author's Address*: Department of Zoology, Parks Road, Oxford, U.K.

[J. Embryol. exp. Morph., Vol. 10, Part 4, pp. 622–40 December 1962]

Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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[2] CREST, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan

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SUMMARY

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Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997) or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as *Stat3* (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), *c-myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and *β-catenin* (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

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We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity (see Table S1 in the Supplemental Data available with this article online). For β-catenin, c-Myc, and Stat3, we used active forms, S33Y-β-catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively. Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2ΔSH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.

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Cell

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From Idea to Scholarship

It's all about ideas, but…

You can't share an idea, only the expression of an idea.

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From Idea to Scholarship

Steps in the process:

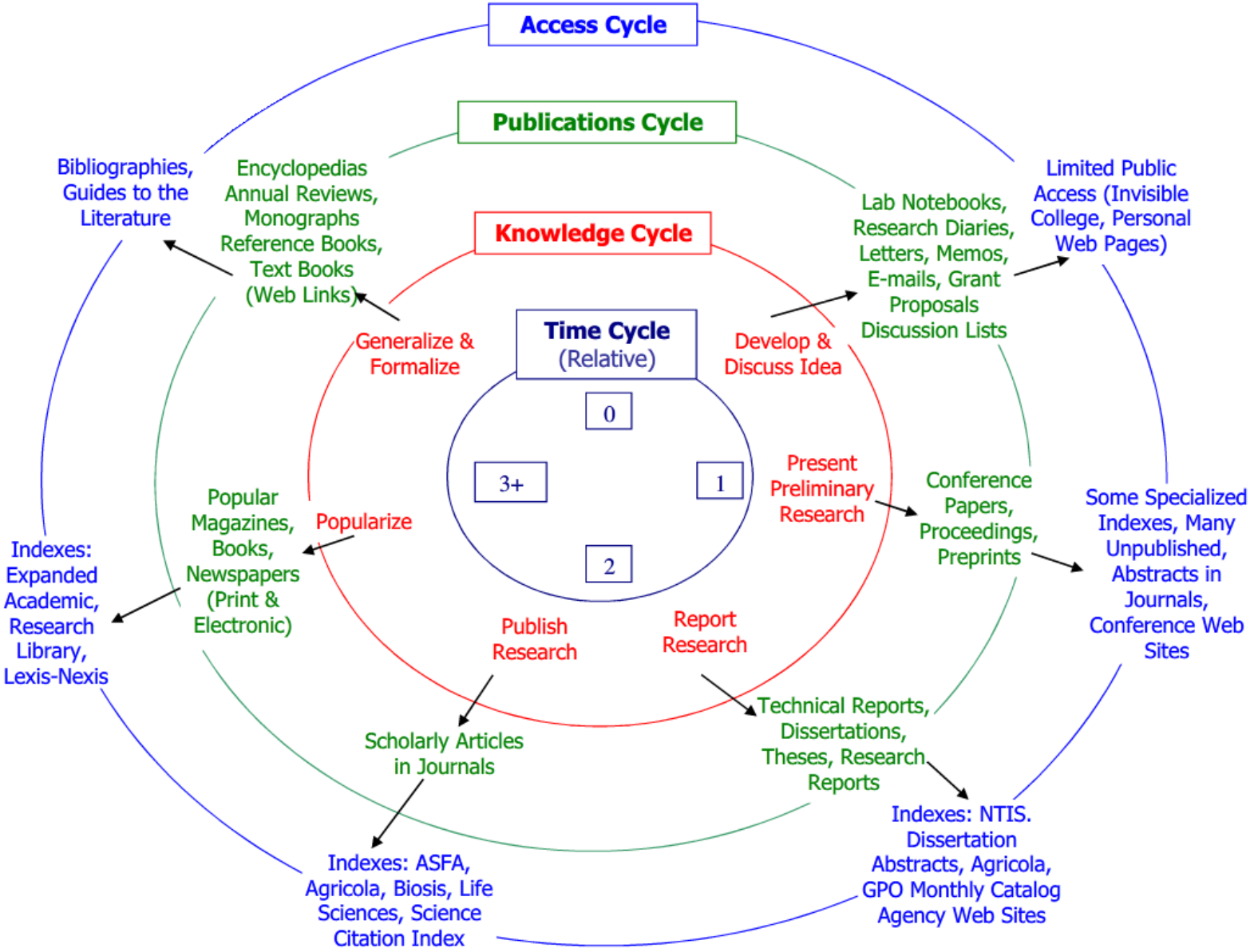
- Understanding that scholarship is a conversation
- Identifying the components of published scholarship
- Evaluating published scholarship
- Contributing your own voice to the conversation
- Disseminating your ideas

Scholarly Communication

"Scholarly Communication" = the sharing of ideas in academia and other areas of research

Scholarly communication as a conversation

- Not "everyday conversation"
- Complex exchanges about a well-defined topic between well-informed people
- Takes place over vast times and distances
- To effectively understand the conversation and contribute to it, one must "speak the language" of scholarship



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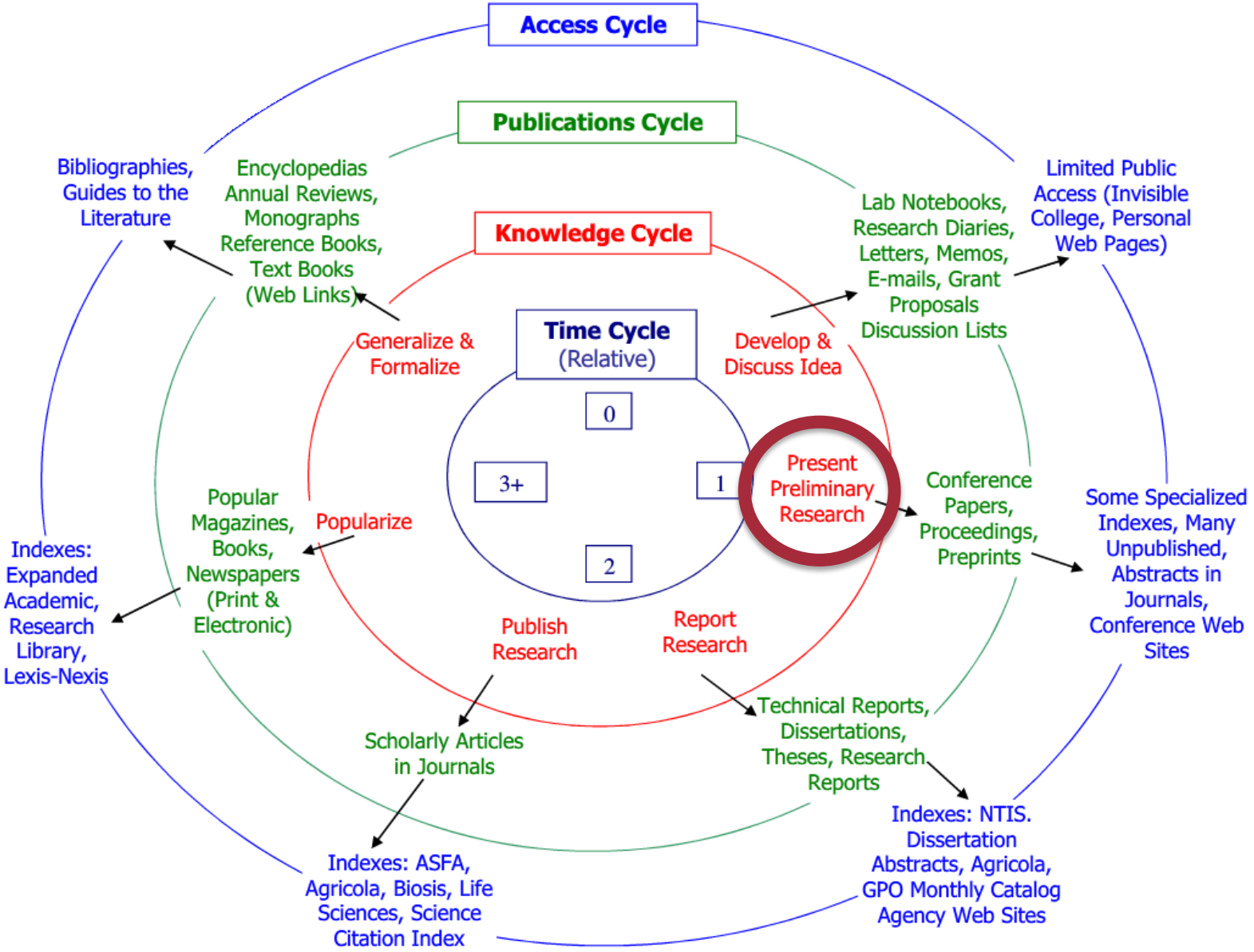

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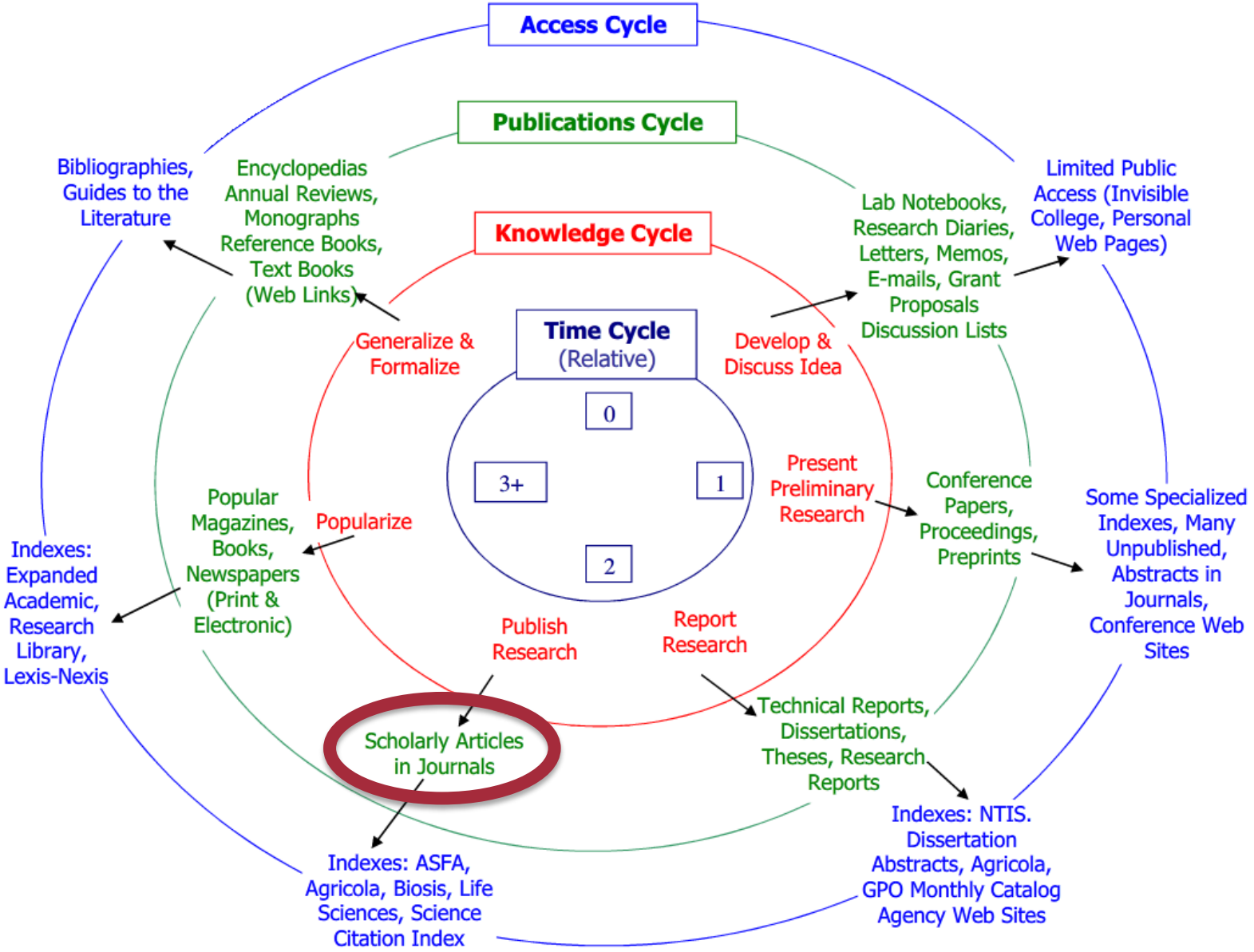

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The Peer-Reviewed Research Article

Peer review:

- the process of submitting one's scholarly work to rigorous evaluation by experts working in the same field.

This allows for:

- Verification, validation, and confirmation
- Suggestions for improvement
- Inclusion of additional points of view
- Opportunities for future research…

Literature Review

Situating yourself within a larger conversation

What is that conversation?

Who are the key voices?

The Parts of a Scholarly Article

Bibliographic Elements

- Title
- Author
- Journal Title
- Publisher
- Publication date

Content

- Abstract
- Introduction
- Literature Review
- Methods/Procedures
- Results/Data
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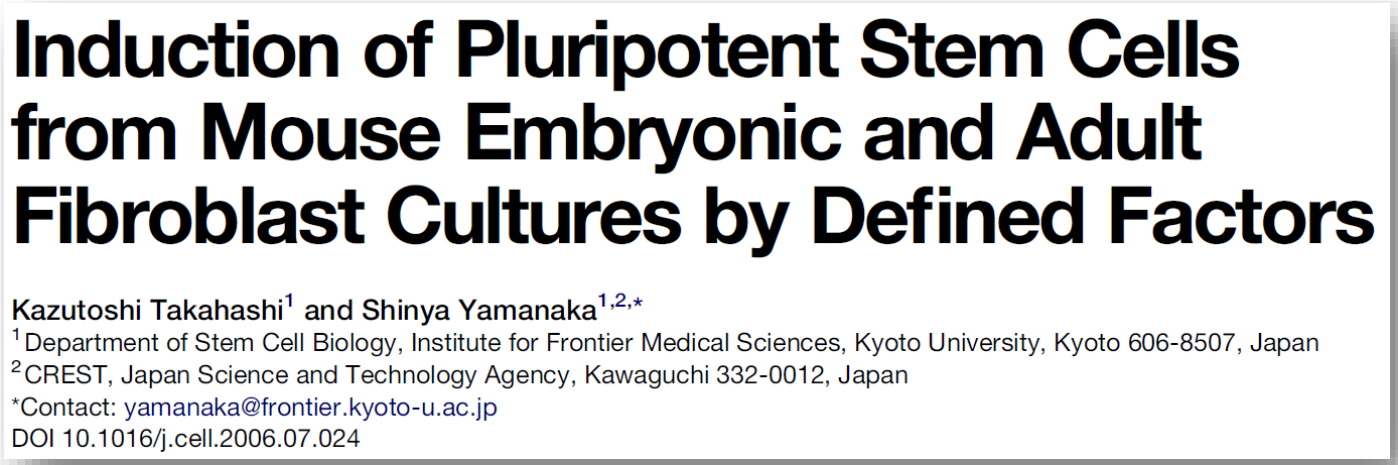

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Cell *126*, 663–676, August 25, 2006 ©2006 Elsevier Inc. **663**

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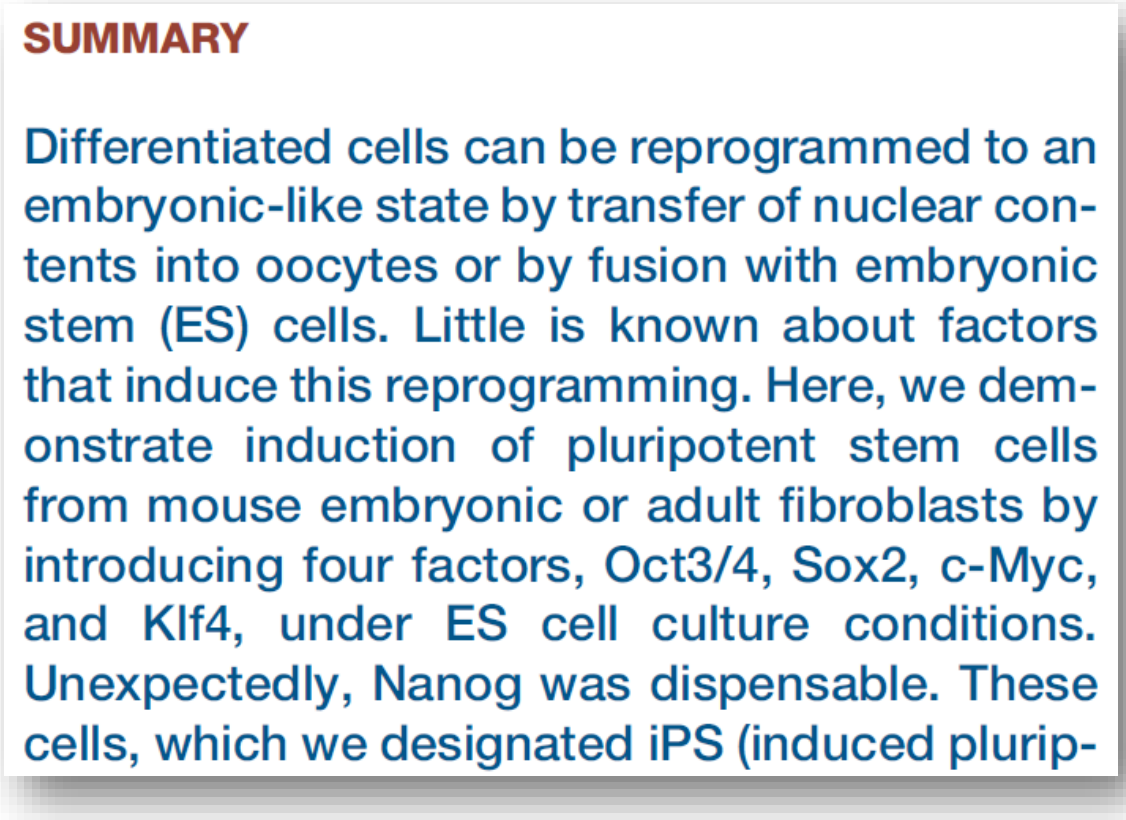

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Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997)

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Teratoma Formation and Histological Analysis

ES cells or iPS cells were suspended at 1×10^7 cells/ml in DMEM containing 10% FBS. Nude mice were anesthetized with diethyl ether. We injected 100 μl of the cell suspension (1×10^6 cells) subcutaneously into the dorsal flank. Four weeks after the injection, tumors were surgically dissected from the mice. Samples were weighed, fixed in PBS containing 4% formaldehyde, and embedded in paraffin. Sections were stained with hematoxylin and eosin.

Bisulfite Genomic Sequencing

Bisulfite treatment was performed using the CpGenome modification kit (Chemicon) according to the manufacturer's recommendations. PCR primers are listed in Table S9. Amplified products were cloned into pCR2.1-TOPO (Invitrogen). Ten randomly selected clones were sequenced with the M13 forward and M13 reverse primers for each gene.

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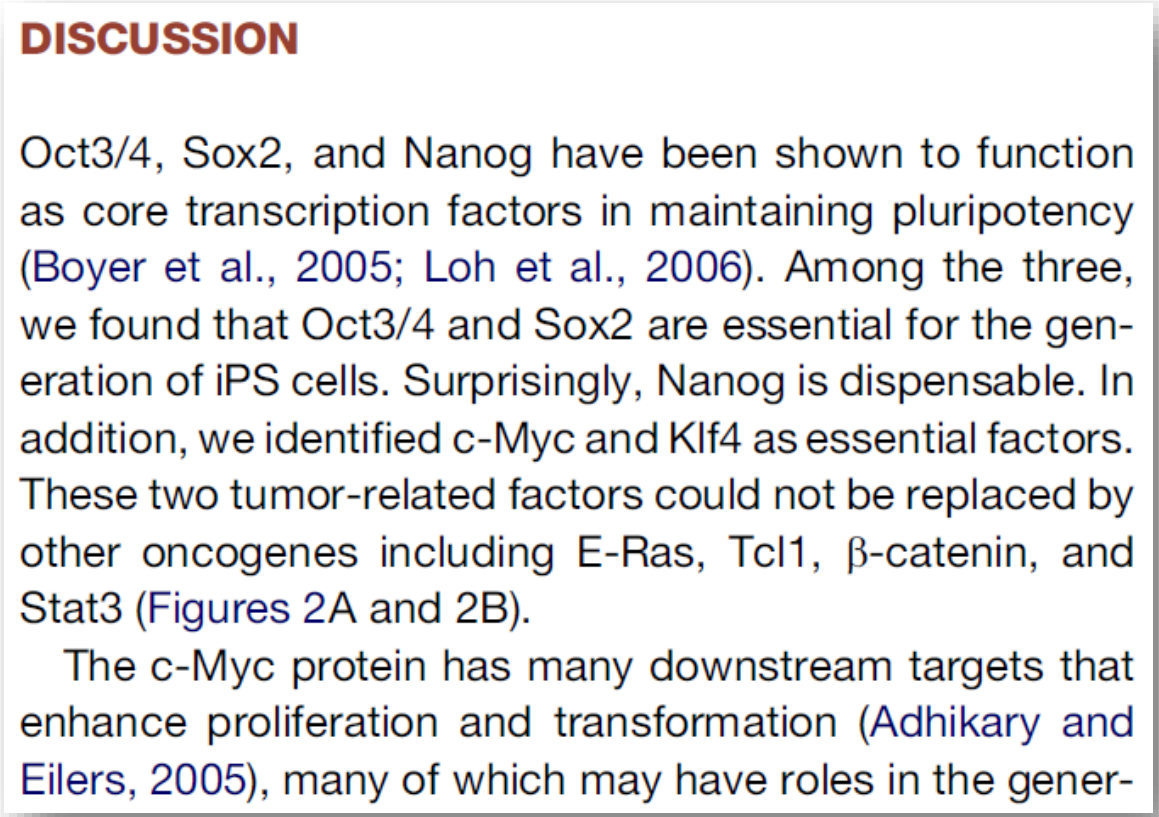

DISCUSSION

Oct3/4, Sox2, and Nanog have been shown to function as core transcription factors in maintaining pluripotency (Boyer et al., 2005; Loh et al., 2006). Among the three, we found that Oct3/4 and Sox2 are essential for the generation of iPS cells. Surprisingly, Nanog is dispensable. In addition, we identified c-Myc and Klf4 as essential factors. These two tumor-related factors could not be replaced by other oncogenes including E-Ras, Tcl1, β-catenin, and Stat3 (Figures 2A and 2B).

The c-Myc protein has many downstream targets that enhance proliferation and transformation (Adhikary and Eilers, 2005), many of which may have roles in the gener-

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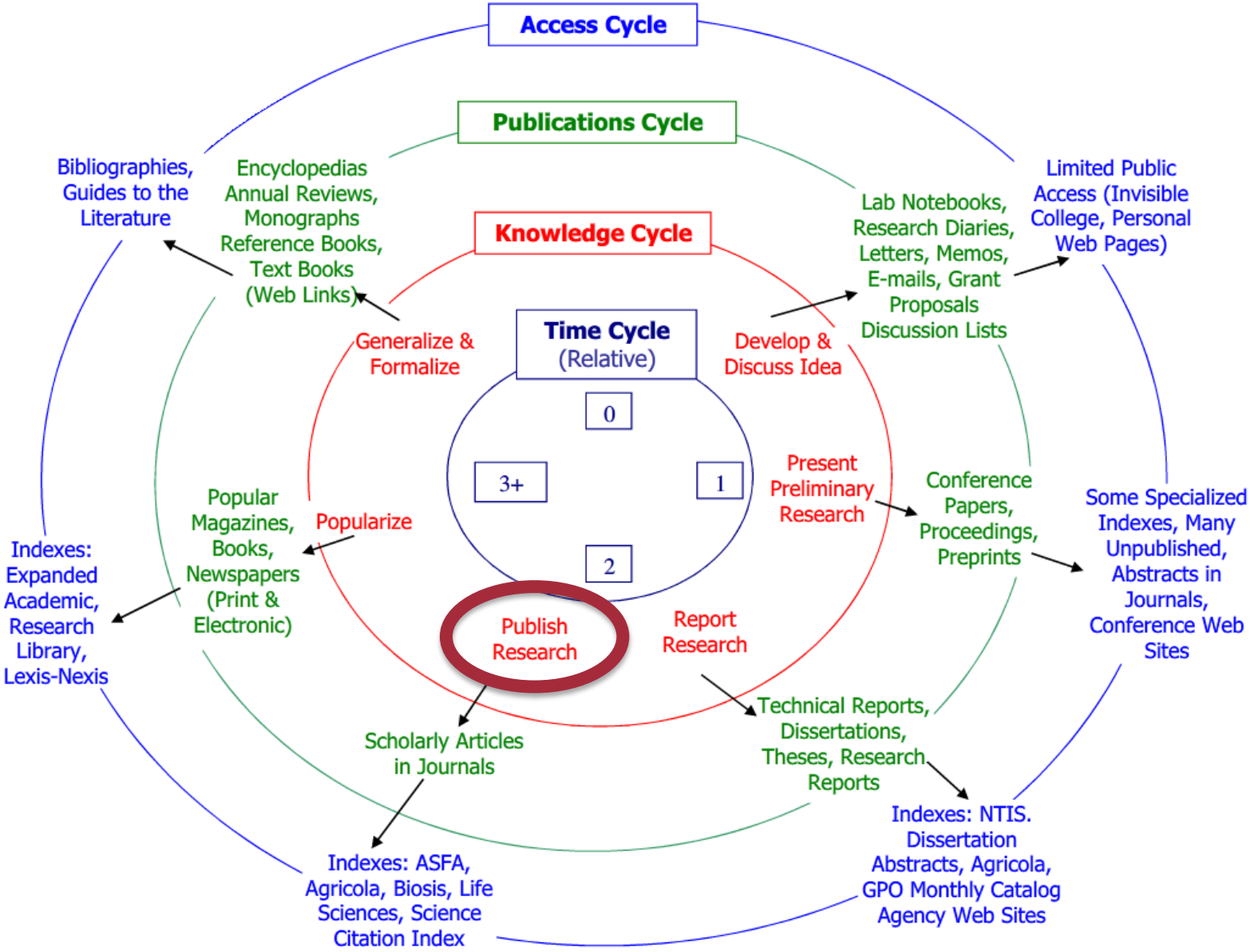
The Importance of Citation and References

- Citation and references allow the reader to reconstruct the scholarly conversation, even if that conversation has happened over vast distances and over long periods of time
- What were the ideas that led to this idea?
- How have the arguments changed over time?
- Have certain theories or practices been debunked?
- Within the larger scholarly conversation, who do you agree with? Who do you disagree with?



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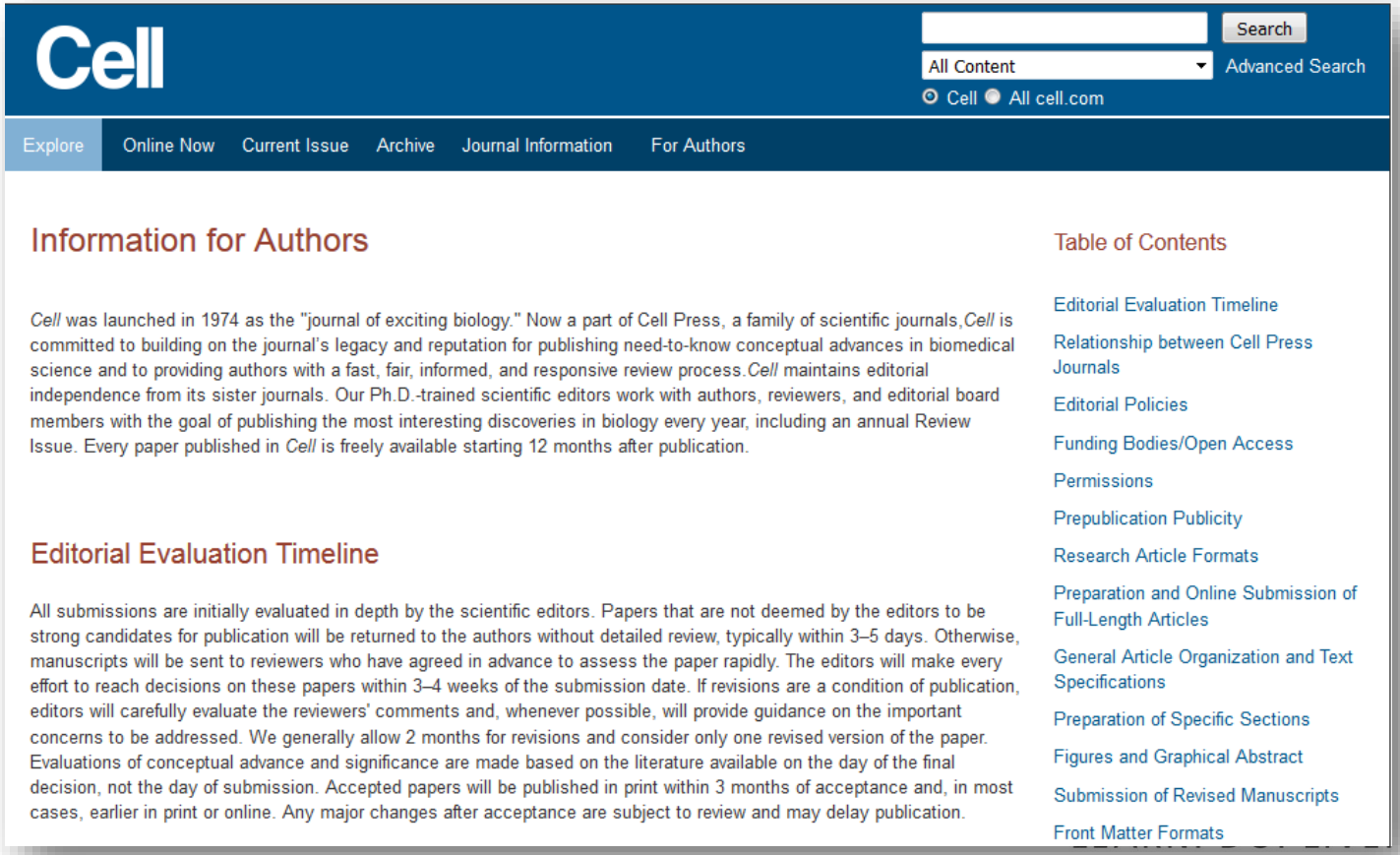

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WITH ONE PLATE

INTRODUCTION

AN important problem in embryology is whether the differentiation of cells depends upon a stable restriction of the genetic information contained in their nuclei. The technique of nuclear transplantation has shown to what extent the nuclei of differentiating cells can promote the formation of different cell types (e.g. King & Briggs, 1956; Gurdon, 1960*c*). Yet no experiments have so far been published on the transplantation of nuclei from fully differentiated normal cells. This is partly because it is difficult to obtain meaningful results from such experiments. The small amount of cytoplasm in differentiated cells renders their nuclei susceptible to damage through exposure to the saline medium, and this makes it difficult to assess the significance of the abnormalities resulting from their transplantation. It is, however, very desirable to know the developmental capacity of such nuclei, since any nuclear changes which are necessarily involved in cellular differentiation must have already taken place in cells of this kind.

The experiments described below are some attempts to transplant nuclei from fully differentiated cells. Many of these nuclei gave abnormal results after transplantation, and several different kinds of experiments have been carried out to determine the cause and significance of these abnormalities.

The donor cells used for these experiments were intestinal epithelium cells of feeding tadpoles. This is the final stage of differentiation of many of the endoderm cells whose nuclei have already been studied by means of nuclear transplantation experiments in *Xenopus*. The results to be described here may therefore be regarded as an extension of those previously obtained from differentiating endoderm cells (Gurdon, 1960*c*).

MATERIAL AND METHODS

The animals used for these experiments belong to the subspecies *Xenopus laevis laevis*. The transplantation technique has been carried out as described previously (Elsdale *et al.*, 1960), except that the donor tissue was exposed to

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Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

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SUMMARY

Differentiated cells can be reprogrammed to an embryonic-like state by transfer of nuclear contents into oocytes or by fusion with embryonic stem (ES) cells. Little is known about factors that induce this reprogramming. Here, we demonstrate induction of pluripotent stem cells from mouse embryonic or adult fibroblasts by introducing four factors, Oct3/4, Sox2, c-Myc, and Klf4, under ES cell culture conditions. Unexpectedly, Nanog was dispensable. These cells, which we designated iPS (induced pluripotent stem) cells, exhibit the morphology and growth properties of ES cells and express ES cell marker genes. Subcutaneous transplantation of iPS cells into nude mice resulted in tumors containing a variety of tissues from all three germ layers. Following injection into blastocysts, iPS cells contributed to mouse embryonic development. These data demonstrate that pluripotent stem cells can be directly generated from fibroblast cultures by the addition of only a few defined factors.

INTRODUCTION

Embryonic stem (ES) cells, which are derived from the inner cell mass of mammalian blastocysts, have the ability to grow indefinitely while maintaining pluripotency and the ability to differentiate into cells of all three germ layers (Evans and Kaufman, 1981; Martin, 1981). Human ES cells might be used to treat a host of diseases, such as Parkinson's disease, spinal cord injury, and diabetes (Thomson et al., 1998). However, there are ethical difficulties regarding the use of human embryos, as well as the problem of tissue rejection following transplantation in patients. One way to circumvent these issues is the generation of pluripotent cells directly from the patients' own cells.

Somatic cells can be reprogrammed by transferring their nuclear contents into oocytes (Wilmut et al., 1997) or by fusion with ES cells (Cowan et al., 2005; Tada et al., 2001), indicating that unfertilized eggs and ES cells contain factors that can confer totipotency or pluripotency to somatic cells. We hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells.

Several transcription factors, including Oct3/4 (Nichols et al., 1998; Niwa et al., 2000), Sox2 (Avilion et al., 2003), and Nanog (Chambers et al., 2003; Mitsui et al., 2003), function in the maintenance of pluripotency in both early embryos and ES cells. Several genes that are frequently upregulated in tumors, such as *Stat3* (Matsuda et al., 1999; Niwa et al., 1998), *E-Ras* (Takahashi et al., 2003), *c-myc* (Cartwright et al., 2005), *Klf4* (Li et al., 2005), and *β-catenin* (Kielman et al., 2002; Sato et al., 2004), have been shown to contribute to the long-term maintenance of the ES cell phenotype and the rapid proliferation of ES cells in culture. In addition, we have identified several other genes that are specifically expressed in ES cells (Maruyama et al., 2005; Mitsui et al., 2003).

In this study, we examined whether these factors could induce pluripotency in somatic cells. By combining four selected factors, we were able to generate pluripotent cells, which we call induced pluripotent stem (iPS) cells, directly from mouse embryonic or adult fibroblast cultures.

RESULTS

We selected 24 genes as candidates for factors that induce pluripotency in somatic cells, based on our hypothesis that such factors also play pivotal roles in the maintenance of ES cell identity (see Table S1 in the Supplemental Data available with this article online). For β-catenin, c-Myc, and Stat3, we used active forms, S33Y-β-catenin (Sadot et al., 2002), T58A-c-Myc (Chang et al., 2000), and Stat3-C (Bromberg et al., 1999), respectively. Because of the reported negative effect of Grb2 on pluripotency (Burdon et al., 1999; Cheng et al., 1998), we included its dominant-negative mutant Grb2ΔSH2 (Miyamoto et al., 2004) as 1 of the 24 candidates.

2012 Nobel Prize in Physiology or Medicine:

Sir John B. Gurdon of the Gurdon Institute in Cambridge, England

- Gurdon's article was first published in 1962 in *The Journal of Embryology and Experimental Morphology*

The Developmental Capacity of Nuclei taken from Intestinal Epithelium Cells of Feeding Tadpoles

by J. B. GURDON[1]

From the Embryology Laboratory, Department of Zoology, Oxford

WITH ONE PLATE

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- Yamanaka's article was published in 2006 in *Cell*.

Cell

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