J. Biol. Macromol., **3**(1) 3-6 (2003)

Review

Organization and regulation of the eukaryotic genome

Yukio Takii

Department of Food Science and Nutrition, School of Human Environmental Sciences, Mukogawa Women's University, Ikebiraki-cho, Nishinomiya, Hyogo 663-8558, Japan

Received February 22,2003

The death of offspring following nuclear transfer

One of the most scientific icons in the world, Dolly, was dead at age of six on February 14, 2003. She has suffered from arthritis showing symptoms of aging. The animals usually could live for 12 years. She has been widely recognized as the first cloned mammal from the embryos. The cell line and the offspring produced by nuclear transfer were well characterized (Fig. 1).

Dolly was born in 1996 at Roslin Institute, Scotland, as the offspring following nuclear transfer from an established cell line from the donated cells of 6 years old female sheep[1]. The issue is complicated because Dolly was created using genetic material from a six-year-old ewe, so has arguably been said to be 12 years old, not six.

Any research using cloned human embryos should be placed under a ban.

The UFO cult, *Raelian movement*, announced it had cloned a baby, named Eve, yet offered no evidence.

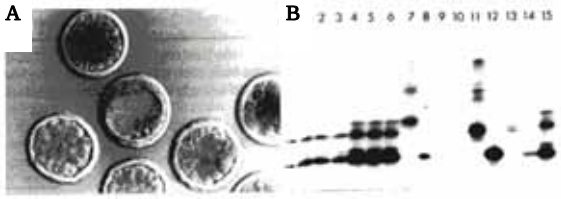

Fig.1 *In A*, group of embryos including single blastocyst after reconstruction. *In B*, autoradiogram showing the alleles generated following amplification of the micro satellite. Lanes 1-6 is a cell from generated by nuclear transfer. Lanes 7-15 is a cell from randomly chosen sheep as control. (*Nature,* 1996)

There followed a rush of outrage and condemnation and calls for immediate legislation. We may never know for sure how Eve came into this world. The figure of developed embryos by the *Clonaid* that was relevant to the UFO cult gave not any evidence (Fig.2) .

Fig.2. Human cells after nuclear transfer (presented by Clonaid)

Beyond that, there is the thorny debate over cloning embryos for therapeutic research. Scientists say this genetic research holds out the hope for some relief from a host of painful diseases, Parkinsons and Alzheimer's that are closely related to DNA polymorphisms.

The objections, many of them from other religious groups, invoke embryo farms and hatcheries where human life is created and then destroyed for a silver of knowledge. The language may be overwrought, but the position is intellectually honest. If you believe human life begins at conception, then cloning embryos to extract stem cells would be like breeding children for organs. No matter what it cured, it wouldn't be worth it.

A compromise came up that the president of the United States would ban any research using cloned embryos, but would allow federal funding for research on 60 stem cell lines derived from embryos that were not cloned. There is much to discover through research delimited in this way, but many scientists were not satisfied. Some complain that the president's policy did not leave enough stem cell lines to work with. They say the funding is needed to obtain more lines from non-cloned embryos.

One potential result of genetic research is finding a way to transplant organs that the body is less likely to reject. That is more likely to happen if the organs are obtained from embryos cloned from the patients themselves.

DNA polymorphisms

Herewith, it is important to emphasize the need to find a suitable probe to identify DNA polymorphisms. Lacking a probe to identify the DNA marker, a DNA region will remain unknown, no matter how variable it might be in its production of restriction fragments. Once a probe is found that reveals the variation, it still does not readily indicate where the variable site is located. To find the precise location of a disease gene, it is necessary to identify a marker that is very closely linked to it. Suppose that a marker recombines at a frequency of 5% with a gene. On the molecule level, this means that the marker and the gene are as many as 5 million base pairs apart.

A recombination frequency of 1% between a gene and its DNA marker would imply that the two are about 1 million base pairs apart. This greatly increases the chance of finding the precise DNA segment on which the gene resides and hence of cloning it.

Chromosome walking and the search for specific genes

One procedure that has been used to characterize a chromosome region and zero in on a gene is known as chromosome walking. This method enables us to locate a marker that is much closer to the gene than any we may already have and may lead us to the very gene itself. To walk along the chromosome, we start with a probe that is known to be closely linked to the gene we wish to locate, a human

disease gene. We then turn to a library of cloned DNA fragments that represent the entire genome. Using the probe, we scan the library, searching for a DNA sequence that overlaps a portion of the sequence in our probe.

Chromosome walking is a very laborious process, but facilitating it are refinements such as pulse field gel electrophoresis that permit the separation of larger fragments than is possible by ordinary methods.

Recently, another eukaryotes, fungi, have been recognized as suitable sources of gene structure and evolution[4]. Of these, *Aspergillus* sp. was selected as useful recipient of genes of glucoamylase and 1,2-D-mannosidase[5]. In our research amylase was selected for gene walking studies in the nucleotide sequences of *Aspergillus oryzae*[6] (Fig. 3).

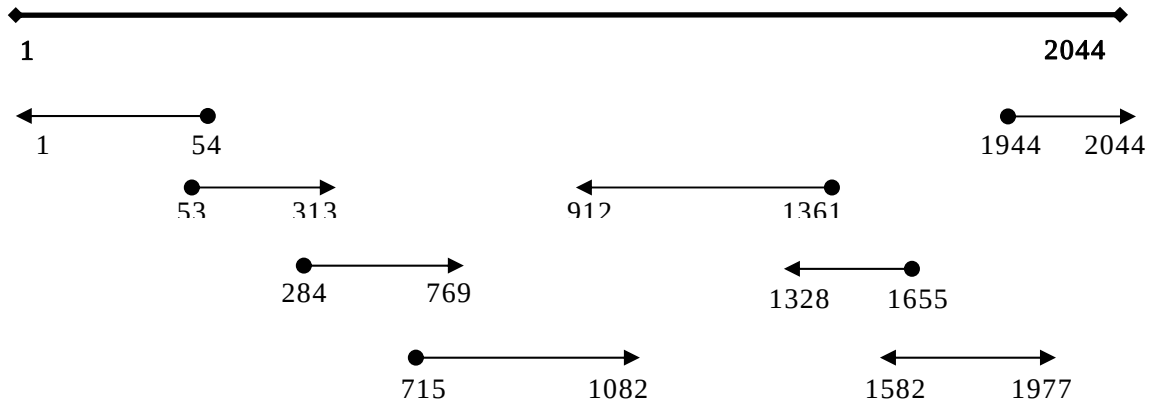

Fig. 3. Analysis of *A. oryzae* MIBA316 alpha-amylase gene (2044bp) by gene walking.

Most genes characterized to date are from Ascomycetes, Basidiomycetes or Deuteromycetses, though a number of genes have recently been characterized from Zygomycetes and Oomycetes. Both saprophytes and pathogen produce a large range of extra cellular enzymes for the degradation of carbohydrates.

Genome arrangements and gene amplification

Many fungi produce a large range of extra cellular enzymes for the degradation of carbohydrates, proteins and lipids. Some of these enzymes have been exploited for use in the food industry[7].

Digestion of raw starch requires a complex mixture of different types of enzyme. Isolation of genes for amylase from *A.oryzae* has revealed the existence of gene families[7]. Sequence, intron position and its length are highly conserved.

The amylase is encoded by three genes, two of these are identical in the coding region, diverging greatly only in the 3'-non-coding region, and a third gene has only two nucleotide differences in the coding region. It is possible that this is a

recent amplification event, and that the selection of strains carrying this amplification. Chromosomal translocation events have been studied in detail in fungi, and often result from mutagenic treatment, especially UV irradiation. Some translocation lead to a duplication of a large section of chromosome, and such strains are usually very unstable, giving rise to stable, faster growing sectors, which have lost the duplication. It is now clear that fungal strains carrying large segments of amplified DNA can be mitoticallystable.

Such amplification ion can arise by mutation and selection for a particular phenotype, or by transformation to insert extra copies of a gene sequence.

In conclusion

The least permissive approach would be to ban all forms of research on cloned embryos but reconsider the ban after some limited number of years. The most permissive would be to allow existing regulations to monitor and adapt to the changing science. The worst, however, would be to allow the specter the baby to shut down the scientific debate.

References

1) Campbell, K.H.S., McWhir, J., Ritchie,W.A.and Wilmut,I. *Nature*, **380**, 64-66 (1996).

2)Devlin,T.M.: In *Textbook of biochemistry with clinical correlations* (3rd ed.). Wiley-Liss, New York,Chichester,Brisbane,Toronto, Singapore (1992).

3) Lewin, B. *Genes V*. Oxford University Press, Oxford, New York, Tokyo (1994).

4)Takii, Y. *J.Biol.Macromol*. **1**(1), 3-7 (2001)

5)Ichisima,E., et al. *Biochem. J*. **339**, 589-595 (1999)

6) Kariya,M. Master Degree Thesis of Mukogawa Women's University (2003)

7)Sakaguchi,K.,Takagi,M.,Horiuchi, H.,and Gomi,K.*Applied Molecular Genetics of Filamentous Fungi,* eds. Kinghorn, J.R. and Turner, G. pp. 54-99 . Blackie(1992)