LAWYERS' DELIGHTS AND GENETICISTS' NIGHTMARES: AT FORTY THE DOUBLE HELIX SHOWS SOME WRINKLES

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Abbreviations:
NIH, National Institutes of Health; DOE, Department of Energy; HGP, Human Genome Project; ELSI, Ethical, Legal and Social Issues; cDNA, complementary DNA; EPC, European Patent Convention; YAC, Yeast Artificial Chromosome.

SUMMARY

The NIH request to patent the base sequences of incomplete and uncharacterized fragments of DNA copied on messenger RNAs extracted from human tissues, the refusal opposed by the patent office, the appeal placed by NIH, have incited a violent controversy, fueled by rational as well as emotional elements.

In a compromising mode between liberism and protectionism, I propose that legal protection be considered only for those RNA/DNA sequences, either natural or artificial, which can generate practical applications per se, and not through their expression products.

Another controversy is developing around a popular tool for genomic research: the fidelity of YAC libraries being distributed worldwide for physical mapping is being questioned. Some of these libraries have been shown to be affected by substantial levels of co-cloning. Also in this case scientific as well as non-scientific components have to be considered.

Possible remedies for the underlying problems may be found in the proper use of kinetic, enzymatic and microbiological variables in the production of YAC. Also a sharper distinction between secular and scientific gratifications of research could help.

INTRODUCTION

A number of ethical, legal and social problems have been associated with recent developments of molecular genetics and in particular with the HGP, the initiative sponsored jointly by NIH/DOE in the US for the sequencing of a human genome. Analogous programs undertaken in other countries face similar complications.

It has therefore been appropriate that the HGP has set aside a substantial portion of its budget to the airing of the relevant issues, within a specially created program, ELSI (Drell, 1992). In other countries comparable measures are being taken by ad hoc committees and national bioethical institutions.

Among the issues indicated as requiring serious reflection are the societal abuse of genetic data, ranging from the forensic validity of DNA fingerprints (Devlin et al., 1993) to the presymptomatic diagnosis of disorders especially of behaviour (Wexler, 1992), the patenting of living organisms (Jaenichen and Schrell, 1993) and of genes (Roberts, 1992; Anderson, 1993). Although all of them deserve careful scrutiny, legal protection in the commercial use of living organisms and of genes represents a major issue in the field of human molecular genetics. Indeed leading actors in the play have felt a strong urgency for securing legal protection, such as patenting, for the commercial exploitation of modern human genetics. The resulting initiatives are exerting a strong impact on the HGP.

Recently, contrasting views over the appropriateness of patenting uncharacterized sequences of DNA derived from a commercial library of human brain cDNA (Zinder, 1993) have caused the positions championed by NIH Director Bernardine Healy and by the Director of the NIH Human Genome Research Center, Jim Watson, already orthogonal on many aspects of biomedical research, to move to a head-to-head collision route. The first consequence of the unavoidable clash has been the resignation of the director of the Center.

Since then even greater attention is being devoted to the discussions on the ownership of gene sequences, and in general to the problem of gene patenting, specially when the relevant basic research is supported by public funds.
Another issue raised by recent developments of the HGP is the quality of the gene libraries prepared by some groups, be them biotechnology companies or non-profit research institutions, and distributed to human genome laboratories all over the world for investigative purposes. Questions have been raised mainly for the variable occurrence of co-cloning in YAC libraries (Little, 1993) or for the contaminations affecting cDNA banks (Christiansen and Henikoff, 1992; Anderson, 1993). In these cases the problems are complex: they go beyond the scientific boundaries and raise ethical, political, commercial and legal issues, occasionally spiced by personal or chauvinistic idiosyncrasies.
Here I shall elaborate briefly on both subjects, gene patenting and co-cloning: as for the first I present a possible contribution to help channeling the discussion towards a workable settlement. In particular I shall outline a proposal relative to the patenting of "gene" sequences. For the second I shall discuss some technical options affecting co-cloning and some variables which may contribute to its reduction.

GENE PATENTING

The use of standard techniques for deciphering the correct sequences of genes and in general of DNA is not the most trivial task, but is also short of representing <u>per se</u> an inventive achievement deserving legal protection. Conversely, this may well be the case when innovative inventions are described and\or, in addition to being properly disclosed, useful, novel and non obvious, as canonically required, the resulting sequences have some chances of becoming themselves tradable commodities.

Thus it may be pertinent to recall here what the EPC stipulates under Art. 52: "Patentable Inventions.
(1) European patents shall be granted for any inventions which are susceptible of industrial application, which are new and which involve an inventive step.
(2) The following in particular shall not be regarded as inventions within the meaning of paragraph (1):
a. discoveries, scientific theories and mathematical methods; b. aestethic creations; c. schemes, rules and methods for performing mental acts, playing games or doing business, and programs for computers; d. presentation of information.
(3) (Omitted)
(4) Methods for treatment of human or animal body by surgery and therapy and diagnostic methods practiced on the human or animal body shall not be regarded as inventions which are susceptible of industrial applications within the meaning of paragraph (1). This provision shall not apply to products, in particular substances or compositions, for use in any of these methods".
Also relevant in this regard is Art. 53:
"Exceptions to patentability.
European patents shall not be granted in respect of:
a. inventions the publications or exploitation of which would be contrary to the public order or morality, provided that the exploitation shall not be deemed to be so contrary merely because it is prohibited by law or regulation in some or all the Contracting States;
b. plant or animal varieties or essentially biological processes for the production of plants and animals; this provision does not apply to microbiological processes or the products thereof." (Gaithwaite, 1991; Zinder, 1993, and references therein).
In most legislations there are a few cases of incompatibility with the granting of a patent, in spite of formal satisfaction of the canonical requirements: they include the preexistence in nature of the patentable item and its condition of living organism. Both have some

bearing on the issue of gene patenting, but I do not believe they should determine the banning of gene sequences from the range of patentable items. Some useful gene sequences have been produced in vitro without a known correspondence in vivo: the hybrid Escherichia coli trp-lac (tac) promotor being just one example (Amann e al., 1983). Additionally, genetic material is typical of living systems but it is certainly not the only determinant of their life: other (such as proteins) exist and are patentable.

What could then be the best course to follow in order to help an optimal conversion of products of human ingenuity such as base sequences (i. e. DNA and RNA) into beneficial goods and services? In the hope to simplify the underlying complex issues, I shall focus on the case of patenting "gene" sequences.

At the onset it should be remembered that not all the products of human ingenuity have always to enjoy legal protection (Paigen, 1993). Thus on one hand we might suggest that the best course is not to patent base sequences at all. Several elements can be listed in favor of such option: base sequences exist in nature, generally are forms of presentation of information, the use of human DNA sequences is most likely to be found in medicine, in some people's perception genes have unusual quasi-sacred features (see some of the "opposition proceedings" to the "Harvard onco-mouse" listed by Jaenichen and Schrell, 1993; Macer, 1992). In the absence of any legal constraints, information could circulate more freely and research would most likely be benefitted, but development and applications would probably end up being somehow affected. Failure to obtain legal protection either by accident or by choice, as in the cases of the monoclonal antibodies by Milstein and Kohler and of the anti-polio vaccine by Sabin, has not apparently caused major economical setbacks, possibly except to the actual discoverers.

On the other hand, one could take the opposite course, as it has been done by NIH in the case of the commercial human brain cDNA sequenced by Venter et al. (Zinder, 1993): namely

to apply for the patenting of any identified sequence, complete or not, with known or unknown function. This would provide strong protection to the inventors and their employers, with a probable lesser protection afforded ultimately to the tax payers, even if the bill of the relevant research has been undersigned by them; probably also the research activity would suffer some damage. The proponents of this course may suggest, in consonance with a hardly demonstratable or falsifiable opinion, that so far the legal protection of the intellectual property has played a positive role in the progress of our economy, and possibly of our civilization (ICSU Statement on Gene Patenting, 1992).

As it is often the case, it seems possible to compromise between the Schylla of an excessive protectionism and the Carybdis of an anarchic laissez-faire. In brief it is proposed here that base sequences could be considered for any form of legal protection if and only if they satisfy the canonical requirements per se, and not because of their (protein) products. For example, "gene" sequences corresponding to promotors, enhancers, ribosome binding sites, replication origins, diagnostic probes, antisense and similar sequences, could be eventually protected, obviously after they have been shown to possess all the required qualifications. Conversely sequences coding for even the most useful proteins would not be eligible for patent, if this could be granted to their products. In the latter case the gene (coding sequence) would be considered an element of the relevant synthetic procedure. In view of the adequate protection enjoyed by both the production process and the product, it may be fair that the particular gene sequence would be let free for further research and development, possibly leading to other uses.

This may provide a somehow limited satisfaction, but almost all the parties involved would enjoy some of it. With a caveat: possibly it may also reduce the delights several patents law firms are getting out of the HGP.

CO-CLONING

The term "co-cloning" is used to describe rearrangements of cloned sequences caused by the apposition of sequences non contiguous in the original genome. Such wrong appositions are obviously detrimental to the proper use of the affected clones in the study of genes and specially of genomes: it could suggest wrong organizations of genes and spurious linkage of sequences which derive even from different chromosomes. It is generally possible to detect co-cloning in the material one elects to use before much effort has been waisted in the investigation of the cloned sequences. Occasionally its discovery can be complicated, and certainly it is time consuming and frustrating. To the point that some people have come to refer to co-cloning as the geneticists' nightmare (Anderson, 1993).

Co-cloning requires that two or more non contiguous sequences end up in the same host cell and eventually in the same YAC. This could be mainly due to two causes :

1. _in vitro_ co-ligation of inserts,
2. _in vivo_ recombination of two or more YACs or parts thereof, following co-transformation of a single cell (see also Green et al., 1991).

The first cause can be removed with relative ease: one can exploit either an enzymatic step, the dephosphorylation of the inserts rather than of the vector's arms. In this way the former cannot ligate among themselves while being ligatable to the vector (Sgaramella et al., 1990). In addition, to reduce co-ligation, one can impose a kinetic control on the reaction by using a vast molar excess of vector over insert (Azevedo et al., 1993).

But after all this is done, steps have to taken to avoid co-transformation of the same cell by two or more single-insert constructs: this could also contribute to co-cloning. It is sufficient that the two constructs recombine _in vivo_, e. g. at Alu sequences (Green et al., 1992). Also in this case an appropriate excess of recipient cells would probably help

reducing the frequency of co-transformation and thus of co-cloning. It is somehow peculiar that the problem of co-cloning has been acknowledged by the YAC pioneers with a crescendo which started at an acceptably low level of 10% (Schlessinger, 1990), to end at an annoying 60 % (Green et al., 1991; Foote et al., 1992; Little, 1992), in concomitance with a careful analysis of the causes (Green et al., 1991), but with preciously few suggestions for possible prevention.

Being unquestionable that due credit should be recognized to the researchers that have developed the YAC libraries and distributed them to the scientific community (Evans, 1993), it would seem wise to see whether co-cloning is reduceable if not avoidable, and still the brilliant results reported so far with co-cloned YACs are obtained, and possibly with lesser efforts. The adoption of tricks such as those briefly commented above, or of other more sophisticated, provided they do not affect seriously the size of the YAC and the overall simplicity of the procedure, is a problem worth considering.

As in the case of gene patenting, probably also here some of the complications are rooted in an excessively strong emphasis placed on secular values such as economic gratification or public recognition, which are known traditionally to coexist uneasily with the correct performance of science. The interfering effects of these values, although widespread and strong now more than before, may not impede the reaching of the goal of the HGP within 2005, as anticipated by the NIH\DOE program: everybody should share that hope. Also those who, if that were the case, by year 2006 may eventually welcome the return of old-fashioned human molecular genetics among the various "genomics".

By that time hopefully also these minor wrinkles of the double helix model, then in its fifties, might have been smoothed.

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