18 The Future of Transgenic Farm Animals

G.E. Seidel, Jr

Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, Colorado, USA

Animal scientists have dreamed of applying transgenic technology to improve production characteristics of farm animals for nearly two decades. Except for the special case of producing pharmaceutical products in milk, efforts have been disappointing. In retrospect, this is not surprising in view of our limited knowledge of gene regulation and function, particularly interactions and pleiotropic effects. Furthermore, insertion of constructs at random sites has much in common with random germline mutations that occur naturally; most such mutations have negative, if any, consequences for the organism (Crow, 1997). There also are serious non-molecular limitations to generating transgenic farm animals, including high costs of animals and their care, lack of inbred lines, long generation intervals, small litter size in some species, expense of adequate replication and failure to develop usable embryonic stem cells.

Over the next few years, transgenic techniques are more likely to be successful for obtaining basic information about farm-animal biology than for improving production characteristics such as growth or lactation rates. Ultimately the resulting information will lead to improved production traits, but the application phase often will not require transgenic procedures. Transgenic technology with farm animals is rapidly becoming more reliable and flexible at the same time as our knowledge of genes is increasing, in great part due to information from other species. This combination will lead to remarkably insightful findings over the next decade, and probably will result in several applications to production-animal agriculture. Finally, we must continue to share information and procedures, even when developed in the private sector, or with private-sector funding (usually with considerable public-sector input). Few organizations can afford to waste valuable resources on protracted litigation over intellectual property or circumventing inventions derived from obvious procedures that are either inappropriately patented, or appropriately patented but unavailable for licensing.

 Transgenic Animals in Agriculture
(eds J.D. Murray, G.B. Anderson, A.M. Oberbauer and M.M. McGloughlin)

Introduction

Hundreds of millions of dollars were invested in transgenic farm-animal research between 1983 and 1997, much of it by the private sector, primarily for producing pharmaceutical products. Questions that arise are: What have investors and taxpayers received for this investment? What will they receive in the future? and What returns are likely from additional investment? Future transgenic farm-animal research will be dependent on perceived answers to such questions.

Research in transgenic farm animals has a unique character. Thousands of person-years of effort, much of it from the private sector, have been expended without yielding any product. Huge emphasis has been placed on refining techniques, rather than on using techniques to answer biological questions or to develop potential practical applications. To some extent, this may be explained by the newness of the endeavour and by the daunting number of unknown quantities. Like embryo transfer, transgenic research is pushed along by the soaring imagination of people who would apply the technology. One other oddity of transgenic farm-animal research is the large number of review papers (Wall, 1996). There is nearly one review paper for every three data papers, a situation that probably arises because programme chairpersons are excited about new approaches and techniques, and transgenic approaches certainly are exciting to think about.

Transgenic research with farm animals generally is undertaken with one of three broad goals (Fig. 18.1). One goal is to create animals for special non-agricultural purposes such as producing pharmaceuticals in milk (Wright *et al*., 1991) or xenografts for replacing human tissues. When only a very few animals are needed to have a saleable pharmaceutical product, and the value of the product is high enough to justify costs both for creating and caring for the animals, this goal will be achievable and easily justified commercially.

A second goal is to produce either improved farm animals, for example, those that grow more efficiently, or improved animal products, for example, milk that yields more cheese. While such objectives start with creation of one or two animals, a resulting line of animals must survive and reproduce successfully with little or no further technological interference. This can be a formidable task and, because the line must be characterized, will not immediately result in a product that will cover the costs of developing the technology. The commercial advantage to the phenotype of individual transgenically altered animals usually will be less than 10% over herd mates (possibly excluding a transient spike of profit from novelty or exclusivity). Moreover, to be acceptable in production agriculture, the transgenic animals would have to be certified as healthy and not require special care. In the short term, few transgenic lines will meet the requirements for agricultural application. However, some decades from now, such applications may be relatively common.

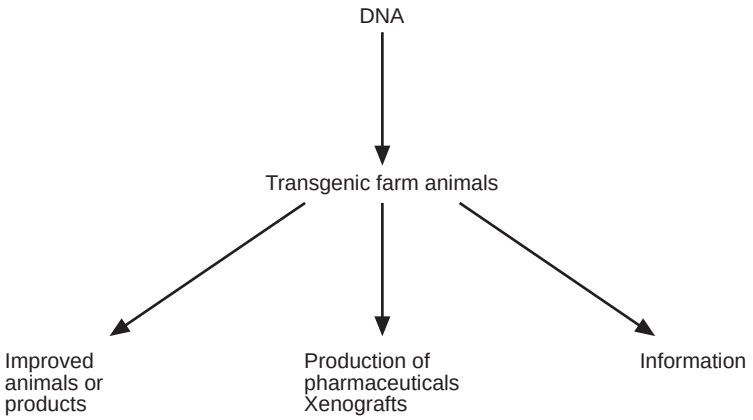

Fig. 18.1. Broad goals of transgenic research.

The third goal is to use transgenic procedures as a tool for basic research on the physiology of farm animals, for example, lactation, resistance to disease, or mechanisms of growth. Questions addressed in this paper include the following: Are transgenic farm animals a sensible approach to obtaining information, which then can be applied in a variety of ways, including making more appropriate transgenic farm animals? What sort of questions might be appropriate to ask using transgenic technology? Further: How useful might transgenic technology be for making improved farm animals? Are the problems insurmountable? In best-case situations, what traits might it be desirable to modify in farm animals using transgenic procedures?

A transgenic golden age?

The field of transgenic farm animals may be entering a 'golden age'. The power of transgenic technology has been proven in the mouse model; many hundreds of papers are published each year that effectively test hypotheses not easily tested by other methods (Wall, 1996). Also, we have accumulated an extensive foundation of technology for transgenic farm animals, both in making standard transgenic techniques more reliable (see other chapters in this volume), and in developing new techniques such as somatic-cell cloning (Campbell and Wilmut, 1997).

At this point in evolution, human society places great emphasis on *applications* of science and technology. Since use of transgenic technology in farm animals, almost by definition, is an application, this will be viewed favourably by both public and private funding sources. As a basis for developing applications, there currently is an explosion of information from

model systems such as the mouse, and from simpler systems such as *Drosophila*, *Caenorhabditis elegans* and prokaryotes (Miklos and Rubin, 1996). Moreover, tremendous opportunity exists for spin-off applications resulting from sequencing the human genome. As suggested earlier, transgenic technology has a charismatic quality about it that affects both scientists and administrators, including those at funding agencies. As public funding has levelled off, funding from private sources is more than making up the difference for transgenic farm animal research. Thus, there are good prospects for a 'golden age', particularly if communication and collaboration are optimized.

Basic Research with Transgenic Farm Animals

Tools for basic research evolve

One great thing about scientific research is that new techniques and new approaches constantly come along to solve old problems or create entirely new possibilities. Some of these innovations have been anticipated by those working in a given area, for example sequencing DNA, cloning adult animals or developing mammalian artificial chromosomes. Of course, it is difficult to anticipate when individual techniques will become available (Gomory, 1983).

Some techniques or concepts that affect their application cannot be anticipated, or at least not by the majority of people working in the field. For example, few reproductive physiologists foresaw the polymerase chain reaction, gametic imprinting, embryonic stem cells or transgenic technology itself. I do not mean to imply that researchers were entirely ignorant of these possibilities. For example, many of us struggled with the fact that parthenogenetic embryos develop encouragingly for a while, but never to term, and some had vague notions of possible mechanisms approximating gametic imprinting (Markert, 1982). But it took considerable research to build a sufficient body of evidence to understand how imprinting would both constrain and explain experimental outcomes.

Specific characteristics of transgenic research with farm animals

Transgenic research with farm animals is limited by high costs, long generation intervals, lack of highly inbred lines, and lack of good culture systems and usable embryonic stem cells and other techniques commonly used in transgenic research with laboratory species. Wall and Seidel (1992) and Wall (1996), and numerous others, have reviewed this area thoroughly and suggested many improvements in transgenic methodology. Frequently there are simpler ways to get the desired information than by making a

transgenic farm animal, for example, using radioisotopes to study metabolic pathways or laboratory-animal or cell-line models of farm animals. On the other hand, there are some distinct advantages to doing basic research with farm animals. For example, considerable amounts of tissue are available, and one can sample blood frequently, a process that can quickly be deleterious to small animals. There is also the ridiculous advantage that it is less expensive to care for sheep than rabbits in many research facilities.

A special advantage of ruminants and swine is that methods to clone embryos by nuclear transplantation have progressed more rapidly than in most other species, including rodents. This is due in part to their commercial value, but there also may be a biological basis, for example, later activation of the embryonic genome. Recent developments of cloning by somatic cell nuclear transplantation in sheep (Campbell and Wilmut, 1997) are particularly attractive for many transgenic experimental needs. The efficiency of this promising methodology, however, needs to be improved.

Another reason for using farm animals for some kinds of basic research is the lack of models for some tissues. For example, mice simply lack hooves, a rumen or a shell gland. Moreover, even when laboratory-species models are feasible, transgenic work needs to be confirmed in farm animals because differences in physiology of some systems dictate confirmation in the animal species of interest. As transgenic technology becomes more widely applied among species, we are likely to be in for some surprises.

There also are reasons for not using farm-animal models for some kinds of basic research. It would be inappropriate, in my opinion, to attempt to unravel how primordial follicles are selected to begin differentiation in the ovary with a transgenic farm-animal model because of the expense. Similarly, regulation of gametic imprinting, studies on sperm–oocyte receptor mechanisms, or regulation of certain aspects of meiotic maturation might best be undertaken first in the less expensive and better-characterized small animal models than in farm animals.

To summarize, transgenic farm animals are not a panacea, even for obtaining basic information. On the other hand, as outlined earlier, transgenic approaches are sensible for some basic research objectives with farm animals.

Examples of basic research with transgenic farm animals

An elegant example of the power of transgenic approaches is presented by Pursel *et al*. in Chapter 10. They arranged for IGF-1 to be produced in muscle, which enabled study in a paracrine rather than an endocrine mode; this avoided the confusion of exposing all tissues of the body to high concentrations of IGF-1. This is by no means an isolated example of how transgenic technology can be used for basic research.

In some ways, the most striking recent example of basic research with transgenic farm animals has been to transfect fibroblasts with the transgenic

construct of interest, followed by fusing such a fibroblast with an oocyte to produce a transgenic animal with molecular properties already pre-characterized *in vitro* (Campbell and Wilmut, 1997; Schnieke *et al.*, 1997; also see various chapters in this volume). This, of course, circumvents one of the major disappointments in basic research in reproductive technology of farm animals, failure to develop usable embryonic stem cells (Chapter 4). Although much more needs to be done with this and parallel systems such as cultured primordial germ cells, these approaches likely can be used to make non-chimeric founder animals homozygous for the transgene, saving huge amounts of time in species with long generation intervals.

Applications of Transgenic Technology in Farm Animals

Considerations limiting application

As much as for basic research, the expense, long generation interval and long time lag from experimentation to observation of results in farm animals greatly limit commercial applications of transgenic technology (Wall, 1996). On top of these logistical constraints, there are special issues raised through commercialization such as: (i) safety to animals or consumers of the animals, especially regarding side effects; (ii) consumer acceptance, even if there are no known problems; (iii) possibilities of escape and contamination of wild-animal genomes; (iv) competition from simpler approaches and systems; and (v) problems with extreme phenotypes.

Transgenic procedures often produce extreme phenotypes, and nature tends to select against such extremes. There seems to be one best fit to the environment for most species, so with natural selection individuals within a species end up being similar in colour, size, shape and behavioural characteristics. Dramatic changes in physiology usually are incompatible with normal life cycles. For example, if cattle or horses were to superovulate naturally each reproductive cycle, the simultaneous development of multiple fetuses would lead to abortion. Very extreme phenotypes, such as sheep that produce an excess growth hormone, which leads to diabetes (Rexroad *et al.*, 1991), or excess growth in pigs, which may lead to arthritis (Pursel *et al.*, 1990), are not practicable or compensable by husbandry practices, and may be ethically inappropriate as well. Also, there is the serious limitation that animals with extreme phenotypes often fail to reproduce (Pursel *et al.*, 1990).

While there are costs (Box 18.1), there may be considerable benefits to extreme phenotypes, as long as they are not too extreme. Although animals with such phenotypes would not survive in nature, the farmers who use them in production agriculture may survive well economically.

One other constraint to development of new genetic variants, especially dramatic ones, is the need to study them in various genetic configurations.

Box 18.1. Examples of costs of extreme phenotypes in agriculture.

Dwarf wheat	Cannot compete with other plants
Large cows	Grazing insufficient for required nutrients
Multiplets in sheep	Lambs require extra feed
Docility	Protection from predators required
Twins in cattle	Major management changes required
Bovine somatotropin (BST) dairy cattle	For well managed herds *only*
Large beef carcasses	Do not fit standard transportation box

For example, all transgenic combinations of male, female, hemizygous, homozygous and controls should be studied for both beneficial and detrimental effects. This is as attractive an exercise as determining the factorial of a five-digit number without a calculator; unfortunately, homozygous transgenes frequently are lethal (Palmiter and Brinster, 1986). Also, there are cases in which a genetic change may be beneficial in one sex and detrimental in the other, or one sex may transmit the transgenic allele and not the other (Palmiter *et al*., 1984). A further problem is introgressing a transgene from a single founder to the homozygous state while minimizing inbreeding (Smith *et al*., 1987). Finally the transgene may be imprinted, causing further confusion.

Long-term prospects

The long-term prospects for application of transgenic technology are favourable. In a reversal of the law that everything that can go wrong will, things seem to be going unprecedentedly well. For example, as techniques for producing transgenic animals are becoming more efficient, the regulatory elements for genes are becoming easier to use (see Chapter 3). Indeed, relatively precise regulation of transgenes may be possible through feed additives or by injection (Pursel *et al*., 1997). Breakthroughs in other species also will be integrated rapidly into the farm-animal technology.

One driving force for rapid development of this technology, apart from the fact that against the odds it *can* be done, is the pressing need for more efficient food production due to rapid population growth throughout the world. Moreover, it is entirely possible that there will be specific consumer demand for transgenic farm-animal products, despite the current recoiling. Witness the wide acceptance of vaccination and synthetic vitamin pills. In a way, these are far more radical products of biotechnology than animals transgenically modified to be resistant to disease. They have been accepted by consumers because of their history of success. Once the value and safety of, for example, nutraceuticals have been demonstrated, consumers will

demand the products rather than hold them in suspicion. Moral scruples, as usual, will be sedated by convenience, economics and improved health.

Manipulation of genetic progress in non-production traits

One mistake that animal scientists are rightly accused of making is to emphasize production traits when low production is not a problem. More attention needs to be paid to non-production traits such as animal welfare, animal health, consumer acceptance and so on. A number of these non-production traits may be especially amenable to transgenic approaches. For example, some sheep are resistant to the spongiform encephalopathy, scrapie, because they have a particular allele (Westaway *et al.*, 1994). Homologous alleles might be transferred among breeds or even species, possibly making cattle resistant to bovine spongiform encephalopathy. Another example is the common practice of docking tails in lambs to minimize debris, faeces, etc. that accumulate, resulting in a haven for parasites. Genes that affect tail length have been identified in a number of species, and it is likely that appropriate alleles could be transferred to sheep transgenically to make tail docking unnecessary.

An intellectual exercise that illustrates important nuances in requirements for transgenic approaches to improving non-production traits is the elimination or diminution of odour from porcine faeces. Porcine faeces not only present a huge disposal problem, but the smell is also responsible for much ill will toward the swine industry. One could imagine a compound that could be fed to pigs that would end up in the faeces, neutralizing the odour. A more elegant solution would be to incorporate genes for such a compound in pigs that might, for example, be secreted into the bile or otherwise eliminated in the faeces, obviating the need to use feed additives. Such a compound should not decrease growth rates, carcass quality or other production traits, and conceivably might even enhance them. The manipulation also must have no detrimental effect on behaviour of the pig (e.g. reproductive behaviour or avoiding faeces); the flavour of the meat; the physiology of the pig; consumer safety (especially if eaten); or repulsion of faeces to pathogenic organisms or vectors such as insects. Clearly this is a rigorous set of requirements. Nevertheless, such chemicals may exist, and pigs with such transgenes conceivably could supplant pigs that did not have the odour-neutralizing chemical.

Manipulation of genetic progress in production traits

Use of transgenic procedures for production traits has been thoughtfully reviewed by Smith *et al.* (1987) and Hoeschele (1990) among others. Therefore, I will concentrate on some less conventional approaches here.

Transgenic technology might be used to decouple factors that inhibit animal production. For example, for continued egg production, hens eventually need to enter a starvation state that results in moulting of feathers; this somehow renews them reproductively, and they start egg production again. Currently, it is economically more practical to slaughter layers after peak egg production rather than invest in caring for them while they are unproductive. Reproduction in mammals similarly is affected negatively by a variety of situations. For example, lactation, weight loss, poor nutrition and season delay or inhibit reproduction in female mammals. We are starting to understand how these effects are regulated. In many cases, promoter regions of specific genes are inhibited or enhanced. Transgenic animals presumably could be made that did not have those inhibitory or enhancer elements in critical gene regulatory regions and, thus, would not be subject to such inhibitions. Of course, one would have to compensate with appropriate husbandry, for example, ensuring that an animal reaching puberty at a light weight eventually grows sufficiently to give birth normally. Such genetic changes could make animal agriculture much simpler and more profitable.

One special set of transgenic applications is to move genes from one species to another. Here are three of a wide spectrum of examples:

1. Tolerance to larkspur. Larkspur is a plant common to pastures in the foothills of the western USA. At certain times of the year its consumption is lethal to cattle, so the economic impact of this poisonous plant is significant. Sheep, on the other hand, are minimally affected by eating larkspur, possibly because they have an efficient enzyme for detoxifying the alkaloid in larkspur that is lethal to cattle. Simply replacing the bovine gene with the ovine gene for this enzyme might make cattle tolerant to larkspur.
2. Visual indicator of oestrus in farm animal species. When oestrogen concentrations in blood are high in baboons, they sport a bright red posterior, indicating that they are in oestrus. Likely this response is governed by only one or two genes. If pigs, for example, could be made to have bright posteriors when they were in oestrus, timing artificial insemination would be easier.
3. Omega-3 fatty acids in fish. Fish, although it has high concentrations of fat, appears to be a health-promoting food, and eating certain fish actually may decrease coronary disease in humans. It may be possible, for example, to modify pigs so that this healthful substance will be present in high concentrations in pork, although the pig may or may not smell and taste more like a fish than a pig.

Building on sex differences

An interesting aspect of animal husbandry is that what is good for the goose is not necessarily good for the gander. In most farm animals, the ideal

female in a herd or flock has what are termed good maternal traits such as mothering ability, appropriate milk or egg production, small to moderate size, high fertility and early puberty. On the other hand, males and females to be slaughtered for meat should have a different set of traits. These are termed terminal-cross traits and include desirable carcass characteristics such as juiciness and tenderness, rapid growth rates, and moderate to large animal size. To some extent, maternal and terminal traits are antagonistic, and therefore inappropriate to have in the same animal or same breed. While these differences are, in fact, exploited by having different lines of animals for different purposes, gross inefficiencies still result. For example, in a maternal line, half of the offspring are of the 'wrong' sex. Because only a few males are needed for breeding, most males that have the maternal-cross characteristics have suboptimal carcasses and are, therefore, a by-product. To some extent this might eventually be circumvented with sexed semen, a product that is not available commercially at this time.

One could enhance these sex differences transgenically. To continue the above example, extra growth could be designed into the male. By adding androgen-response elements to the regulatory regions of growth genes and inseminating sexed semen, one could specify that the growth gene would be activated either by secretion of testosterone as the testis matures or, if the animal is castrated or female, by an implant with androgenic properties. Exploiting such naturally occurring methods of regulating genes would seem a high priority for transgenic research.

Sex differences also might be exploited by adding genes to the Y chromosome which, therefore, would only be expressed in males of the line (Wall and Seidel, 1992). The Y chromosome is nature's artificial chromosome. It is one of the smallest chromosomes in most species and has few genes; most of the chromosome has no known function. Also, most of the chromosome is hemizygous, which simplifies many aspects of application. The Y chromosome would seem to be a good place to add cassettes of genes. Another potentially exploitable fact is that XYY males are fertile and usually have normal XY sons. This allows for the possibility of transplanting an entire Y chromosome, custom modified in a cell line, to a one-cell embryo to serve as a vector.

Collegiality and Intellectual Exchange

Nature of the scientific enterprise

Science is a social enterprise, and most scientists love to discuss their findings. They genuinely do walk on the shoulders of giants. Scientists attend scientific meetings, participate in e-mail discussion groups, and even tolerate peer review of their work by granting agencies and journals. In many ways, the main currency of this social enterprise is communication,

and the most rigorous form of communication in science is the refereed journal article, which involves increasing numbers of collaborators and co-authors compared with a decade or two ago. Scientists have special rules regarding ownership of ideas and the ethics of using the ideas of others. For example, while plagiarism is condemned, it is considered honourable and even flattering if someone uses another's findings, as long as proper attribution is made.

Science has a broadly international character since nationality is irrelevant to ideas. International collaboration takes a number of forms for a number of reasons, including circumventing or exploiting local constraints or sources of funding, costs of doing research, resources available, animal diseases, laws and even culture. International research often is encouraged by funding agencies and others, sometimes because there is a prestige element. I, and many others, believe that goodwill is *the* most important international commodity.

Decreasing scientific collegiality

In recent years, I have perceived a decreased collegiality amongst scientists, including those doing transgenic research. There has always been, in any human endeavour, a balance between competition and collaboration. This has particular significance in science because informal collaboration, including discussing new approaches with potential competitors, is part of the creative process, and also is a validating process. The balance between competition and informal collaboration may have changed to give more weight to competition, in part because sources of funding are less public and more private. Other factors influencing the degree of trust and communication among colleagues is the larger scientific community (more competitors), introduction into the process of the influence of uninformed public opinion through more rapid and efficient publicity given to new breakthroughs, and an increased potential for private gain.

Decreased collegiality sometimes results in gross inefficiencies. For example, scientists on the cutting edge frequently communicate their findings with each other well before they are published in formal journal articles. Those who rely on such articles generally are up to a year behind those who regularly communicate informally.

Inefficiency is perhaps the least of the harm as generation of new ideas is retarded by lessening of informal communication; for example, lack of informal constructive criticism increases inadvertent bad science. Another example of gross inefficiency is removing from general use the best, most efficient techniques by patenting them without then providing reasonable licensing terms, with the result that costs are driven up or fear of litigation becomes the basis of experimental design. Sequelae include slaughtering hundreds of animals for projects for which patented *in vitro* approaches are

more sensible. To the extent that research funding is used for litigation rather than for the advancement of science, resources, especially time and intellectual energy are wasted.

Possible solutions

There are no simple solutions to these problems. Nor are the problems so different from those that have occurred with other human endeavours throughout history.

Communication could be encouraged by designing participatory, rather than passive scientific meetings. Another idea is the research consortium. Such consortia have been set up for companies working with high-speed computing and, in the USA, amongst the large automobile manufacturers to develop more efficient, safer automobiles. I suspect that private companies doing transgenic research would be much better off collectively if some types of research were done under the umbrellas of such consortia where findings were exchanged, thus making the whole field more efficient. Timing to commercialization, particularly of agricultural transgenic products, might be reduced by years, making certain transgenic endeavours profitable, instead of commercial failures.

Perhaps there should also be changes to the patent system. Patents currently are clumsy, time-consuming, and expensive to formulate and use. Inventors in the USA may wait 1 year from publication of their results before filing for a patent without losing potential patent rights. Such time delays are not permitted in all countries. Delays ideally might even be longer than 1 year in some circumstances. Perhaps it would be workable to have a two-phase time delay. For example, the right to patent for basic research use might expire after 1 year and for commercial applications after 2 years after publication. The principle would be to get information published expeditiously without having to give up the right to patent. Of course, most commercial entities would want to apply for patents sooner rather than later. It seems to me that misuse of 'submarine' patents and similar approaches aimed at undermining competitors (Petroski, 1998) are unethical when the health and nutrition of people and animals are at stake.

One other clearly unfair happenstance is that we give undue credit to timing of discoveries. For example, if one group files a patent application 1 day before another group, or publishes a paper 1 week before another group, priority is given to the first group in establishing ownership rights both legally and intellectually. Obviously, if ideas are to be used as commodities to gain wealth, there must be rules to define ownership of intellectual property as there must for real property, but the group that is 1 day ahead is not necessarily more deserving, and rushing to publication results in sloppy science. A rational example of how dating is dealt with on a routine basis is that when citing literature, we use the year, not the

precise date that a publication appeared. Providing only this degree of precision is a way of saying that two groups publishing a particular concept within a year have substantially equivalent intellectual priority. One might broaden this in certain legal and intellectual situations by saying that any work reported within a 12-month period was, for practical purposes, simultaneous, and that a certain sharing of rights and credit would be appropriate.

Collegiality and sharing information contributes to making scientific work rewarding and, to the extent that these do not occur, less pleasant and less efficient. With all that science has to offer humanity, and with all of the problems that plague us, it seems to me that we have responsibilities to make scientific endeavours as efficient as possible. Therefore, we are obligated to invest a certain amount of energy in making it desirable to share information and collaborate as appropriate. Organizations that encourage cross collaboration and intra- and inter-organizational collegiality will thrive.

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