

Conditional alleles, inteins and degrons

To the editor:

In a Letter in the July issue (*Nat. Biotechnol.* 22, 871–876, 2004), Martin Zeidler *et al.* describe a new approach for generating temperature-sensitive alleles based on conditionally active inteins. The system described is undoubtedly a novel and valuable alternative to ‘classical’ temperature-sensitive alleles. The authors of the Letter, as well as Francine Perler in an accompanying News and Views article (*Nat. Biotechnol.* 22, 824–826, 2004), discuss in detail the advantages of the intein-based system over classical temperature-sensitive alleles. However, both Zeidler *et al.* and Perler neglect to mention that one alternative to the classical temperature-sensitive allele has already been described and successfully used.

This approach is based on the use of a ‘heat-inducible degron’ cassette that is

fused to the protein of interest and causes degradation of the degron together with the fused protein upon the temperature shift¹. It has been successfully used in both budding² and fission yeasts^{3,4}, and it can also be used in large-scale analyses⁵.

Juraj Gregan

*The Research Institute of Molecular Pathology (IMP), Dr. Bohr-Gasse 7, Vienna, A-1030, Austria
e-mail: gregan@imp.univie.ac.at*

1. Dohmen, R.J., Wu, P. & Varshavsky, A. *Science* **263**, 1273–1276 (1994).
2. Labib, K., Tercero, J.A. & Diffley, J.F. *Science* **288**, 1643–1647 (2000).
3. Lindner, K. *et al. Mol. Biol. Cell* **13**, 435–444 (2002).
4. Gregan, J. *et al. Mol. Biol. Cell* **14**, 3876–3887 (2003).
5. Kanemaki, M. *et al. Nature* **423**, 720–724 (2003).

has also upgraded its patent offices. Interviews carried out with officials at the patent offices in Mumbai and Chennai reveal that in the past 2 to 4 years, there has been substantial investment in patent office infrastructure in major cities, including the computerization of records, the establishment of libraries and the implementation of patent tracking systems. The number of employees in these offices has also increased tremendously. For instance, in Mumbai there were 7 employees in 1998–1999; today, there are 70, and more are expected. The time to process an Indian patent has been reduced from 5–7 years to 2 years on average.

By insisting on a move to product patents from process patents for all manufactured products, TRIPS shifts the compensation for innovation creation from secondary innovators to primary innovators. In an ideal world, this would motivate Indian firms to invest in the creation of innovations and become primary innovators. Conversely, they would be less inclined to invest in the independent development (or reengineering) and marketing of innovations already protected by preexisting foreign patents.

There are several reasons, however, why the situation is somewhat more complex. According to a recent report¹, the Indian pharmaceutical industry comprises about 23,000 manufacturing units. It is common knowledge that among these thousands of firms, at most only a hundred have the financial and technological wherewithal to undertake research and development (R&D) activities that can produce radical innovations. Furthermore, even these hundred or so elite Indian firms are dwarfed by Western multinationals in terms of financial resources for R&D investment. As external sources of finance, such as venture capital markets, remain rather sluggish, it is difficult to see how Indian companies can compete with Western counterparts as primary innovators. They simply do not have enough resources to invest in R&D.

At the same time, having stronger IP rights is not automatically going to induce foreign firms to increase their investment in India or their collaborations with Indian firms. For this to happen, several other necessary conditions for conducting business transactions need to be satisfied. These include a good ‘business climate,’ easy access to dependable infrastructure and the ability to conduct business transactions transparently and efficiently. In India (and many other developing countries), the main problems with conducting business

Biotech in post-TRIPS India

To the editor:

The Indian government’s substantial investment in patent infrastructure, together with recent modifications to Indian patent laws, reflects the country’s commitment, both in letter and spirit, to adhering to the principles laid down by the World Trade Organization’s (WTO, Geneva) agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS). For India’s biotech sector, however, a stronger patent system is unlikely to be sufficient to spark a boom of innovation. Indian companies simply cannot compete with the financial resources at the disposal of their Western counterparts. TRIPS implementation essentially signals the initiation of a race that is unfair.

TRIPS defines the minimum standards of the system of intellectual property (IP) rights that is to be enforced by the WTO in its 147 member states. Among the most important part of TRIPS is article 27, which calls for a uniform product patent regime in all manufacturing sectors and in all member countries. TRIPS is equivalent to a new law that commits India to cease the reengineering or independent development of products patented elsewhere.

From 1970 onwards, Indian IP law changed from granting product patents to

recognizing only process patents. This change was critical in facilitating the development of an Indian generic pharmaceutical industry and the emergence of an Indian public health care system that could cater to the poorer sections of society. With the implementation of TRIPS, however, India has been forced to modify its patent law of 1970; two amendments have already been made by parliament and currently a third amendment is under review. As well as process patents, the changes now allow the patenting of products including biopharmaceutical and pharmaceutical formulations, compositions, combinations, novel dosage forms and herbal extracts. What cannot be patented are new uses or properties of substances, intermediates used in the manufacture of drugs, traditional knowledge, knowledge in the public domain, business practices, medical practices and discoveries of living and non-living substances occurring in nature. Finally, there are a few loose ends, such as drug delivery systems, parts of microorganisms, functioning DNA sequences and parts of DNA sequences, whose patentability is not very clear.

To be more efficient and responsive in a post-TRIPS world, the Indian government

transactions are the difficulty in accessing correct and complete information on potential partners, suppliers or market possibilities and the uncertainty of ensuring a partner's commitment to formal contracts. The latter in particular, requires a judicial system that functions more efficiently and credibly. These conditions have nothing to do with TRIPS. Thus, the impact of TRIPS on either the commercial strategies of foreign companies or their strategic alliances with Indian companies is anyone's guess, as it is only one parameter among many that will be used in making foreign investment decisions.

From the perspective of Western firms, the implementation of TRIPS in India may encourage them to introduce new brand drugs because such products will now enjoy patent protection—a situation not possible since 1970. This will not mean, however, that high-priced, Western-manufactured products can be directly shoehorned into the Indian market. As K.S.N. Prasad, CEO of Shantha Biotechnics (Hyderabad, India), puts it: “Though TRIPS gives exclusive rights to Western companies to market their brand products in India—eliminating competition from local companies that copy inventions—these multinationals are unlikely to benefit from selling their products at high prices because Indian consumers simply cannot afford the high costs of drugs developed and manufactured abroad. Therefore, it will be necessary for Western and Indian companies to enter into strategic alliances so that novel

drugs can be manufactured under license for local consumption. Such alliances will lead to a win-win situation for all, both biotech companies and the public.”

To sum up, Indian biotech firms basically have three choices in the short-term as business innovation strategies²: first, they can focus on products that are either off-patent already or soon to be off-patent (essentially the generics market); second, they can collaborate with Western multinationals and biotech companies (two areas that are likely to witness an increase in collaborations are clinical trials and R&D outsourcing); or third, they can focus on innovations that the multinationals will not be interested in; that is, mainly ‘tropical’ or developing world diseases.

Shyama V. Ramani¹, Preeti Pradhan² & Mahesh Ravi¹

¹Department of Economics, Institut National de la Recherche Agronomique; UMR INRA-UPMF, BP 47, 38040 Grenoble cedex 9, France.

²Department of Economics, University of California, 543 Evans Hall, c/o Jaeger, Berkeley, CA 94720, USA.
e-mail: shyamar@grenoble.inra.fr

1. Ames, G. & Sunil, P. Indian pharmaceutical industry: market, regulatory, import and investment regime (Pacific Bridge, Inc., Washington, DC, 2002). http://www.pacificbridgemedical.com/publications/Indian_Pharma_Industry_2002.pdf
2. Ramani, S.V. & Maria A. TRIPS and its possible impact on the biotech based segment of the Indian pharmaceutical industry. *Economic and Political Weekly*, in the press (January 2005).

(cell dissociation buffer, CDB) and enzymatic (collagenase/trypsin, CT)—eventually compromised the genetic integrity of the hES cell lines that had previously been passaged by manual methods. Chromosomal abnormalities dominated the BG01 hES cell cultures after as few as 23 passages after changing over to the CDB passaging method. The BG01 cell line maintained a normal karyotype for 42 manual passages but developed trisomy 12 and 17 in all cells (5 cells out of 20 analyzed contained an additional X chromosome) as early as 23 passages after changing from the manual to the nonenzymatic CDB passaging method (Supplementary Fig. 1 online). The BG02 hES cell line maintained a normal karyotype for 45 manual passages, but trisomy 17 was observed 25 passages after switching to CDB passaging. The BG02 line, which demonstrated a normal karyotype after 12 manual passages, was found to have trisomy 12, 14, 17 and an extra copy of the X chromosome in all cells (3 cells out of 20 analysed contained an extra copy of chromosome 20), when studied 56 passages after switching to the enzymatic CT passaging method (Supplementary Fig. 2 online).

Because manually passaged BG02 hES cells maintained a normal karyotype through 105 passages, we then investigated whether limited CDB or CT passaging of hES cell colonies could be used to reduce or limit changes in karyotype. Limited disaggregation of BG02 hES cell colonies resulted in normal karyotypes for 13 and 15 passages in CDB and CT treatments, respectively (Table 1). However, trisomy for chromosome 17 was observed by CDB passage 23, indicating that chromosomal abnormalities eventually arose using current enzymatic/nonenzymatic passaging methods.

Abnormal karyotype was also associated with significant changes in expression of candidate genes implicated in maintaining pluripotency^{9–11} as well as of other genes related to early developmental lineage restriction. Differential gene expression analyses by real-time PCR was conducted on four BG02 hES cell groups. We tested the effect of passage number (early versus late) in both manual and enzymatic (CT) passaged cells. Gene expression of the aneuploid late CT was compared to the normal karyotype groups (early manual, late manual and early CT), and the late CT exhibited a higher expression of genes associated with pluripotency, including *POU5F1*, *SOX2*, *LEFTY2* (also

Preserving the genetic integrity of human embryonic stem cells

To the editor:

The limited number of human embryonic stem (hES) cell lines^{1–7} heightens the need to maintain their genetic integrity. A report by Draper *et al.* published in last January's issue (*Nat. Biotechnol.*, 22, 53–54, 2004) and a related correspondence from Buzzard and colleagues in the April issue (*Nat. Biotechnol.* 22, 381–382) suggest that hES cell lines propagated *in vitro* for even a few months can develop an abnormal karyotype. We report here data from our laboratory that throw more light on the genetic stability of hES cell lines and its relation to how cells are maintained in culture.

In our laboratory, US National Institutes of Health (NIH, Bethesda, MD, USA)–

registered hES cell lines BG01 and BG02 were propagated by manual dissection of the hES cell colonies³ and have normal karyotypes at passages 41, 50, 62, 74 and 105. These results confirm previous observations from Buzzard and colleagues, indicating that the difficult and laborious manual passaging of hES cells will retain a stable karyotype, even after 100 passages. Faster and easier means of passaging hES cells are available, but we found that they can promote chromosomal aneuploidy, especially trisomy 12 and/or 17, in conjunction with aberrant gene expression.

Two means of disaggregating hES cell colonies into single cell suspensions for bulk hES cell passaging^{1,6,8}—nonenzymatic