In the recent *Genomics* supplement to *Drug Discovery Today*, Taylor presented an excellent summary of the possible applications of antisense technology to the analysis of gene function and drug target validation[1]. I would like to draw further attention to two important challenges that we still face when using antisense methods, which demand considerable input before the full potential of this technology is realized.

One important problem is finding accessible sites in mRNAs, and this directly determines success or failure of an antisense experiment. Considerable evidence suggests that a major determinant of accessibility is intramolecular folding in mRNAs (of course, secondary structure in antisense oligonucleotides can also affect binding). Messenger RNAs, being single-stranded, can form intramolecular base pairs between complementary sequences, and there might be further tertiary interactions. This results in ~90% of the molecule becoming inaccessible. Finding the ~10% accessible sites is not easy and some sites might also be more or less accessible than others. Our understanding of RNA folding is limited, and the theoretical tools currently available are not sufficient to predict the folding of RNAs as large as mRNA. Furthermore, the mechanisms of heteroduplex formation between RNA and oligonucleotides are not well understood.

Several methods have been developed to find the accessible sites empirically. Those of particular interest employ the use of oligonucleotide libraries[2] and oligonucleotide arrays[3]. Whereas the first method is easy to set up in a laboratory with basic molecular biology expertise, it has several limitations. In principle, the most accessible site on an mRNA molecule would be the best target. However, it is not possible to find out the most accessible site using oligonucleotide libraries. The use of oligonucleotide arrays appears more successful, but requires considerable technical skill and resources for setting up and is mainly limited to the pioneering laboratory[4]. More work is, therefore, still needed to develop approaches that are easy to use and that also produce desired results.

Another big challenge is the unwanted side effects of antisense reagents. Although some of these effects can be related to the type of chemistries used to produce antisense reagents (e.g. phosphorothioates can bind non-specifically with cellular proteins), there are others that are of a more intrinsic nature. The ability of antisense reagents to bind only with target mRNA has never been proven because it is difficult to analyze the global effects within a cell, and these reagents can bind with the wrong mRNA resulting in unpleasant side effects. For example, the activity of most antisense oligonucleotides is mediated by RNase H (an enzyme with a natural role in DNA replication), which cleaves the RNA part of the RNA–DNA heteroduplex. It requires only short regions of homology (~7 bp) whereas most antisense oligonucleotides are ~20 bp in length. Thus, there are 14 shorter 7mer sequences in a 20mer oligonucleotide, with each one potentially capable of recruiting RNase H at a complementary accessible site on a non-target mRNA. However, the use of shorter oligonucleotides could compromise affinity for the target. Considerable research is being conducted into the use of reagents with chemistries that do not recruit RNase H but work by blocking translation, and also into those with mixed backbone and mixed bases[5]. However, there is still much to be done in this area to improve specificity and avoid the side effects associated with antisense technologies.

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Muhammad Sohail
University of Oxford
Department of Biochemistry
South Parks Road
Oxford
UK OX1 3QU

Structural genomics: lessons to be learnt ▼

Over the past decade the pharmaceutical industry has embraced large-scale automation in perhaps two key areas: making and testing compounds. Indeed, the introduction of combinatorial chemistry and HTS into lead discovery allowed pharmaceutical company CEOs to boast to their shareholders about the numbers of compounds their companies could synthesize and test. In the 1990s, we became seduced by numbers and the profound belief that the 'factory' mentality could increase productivity in research. The ethos was: if we could make the compound cheaply and quickly then why not make and test it? There was less discussion on whether the compound was the right compound to make. It was assumed that the probability of finding an active compound was linear; if you made and screened 50-fold more compounds you would find 50-fold more hits. It is now questionable whether this approach improved productivity in finding new drugs, and many would argue that it did not. In the more far-sighted companies,

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this apparent failure has resulted in a backlash towards more 'intelligent screening' approaches where the knowledge of the target is used to pre-select compounds for screening.

By contrast, in the Human Genome Project, automation has been applied in a devastatingly effective manner. The landmark completion of the draft sequence of the human genome occurred much sooner than initially predicted[1]. Automation clearly accelerated the process of obtaining and analyzing the genome data. In this case, the factory-mentality was indeed highly effective.

We now live in the post-genomic era where the challenge is to make sense of these one-dimensional entities called genes. To this end, there is much interest in defining the three-dimensional shape of the proteins encoded for by the genomes. Many governments and commercial organizations are now funding structural genomics initiatives to obtain protein structures using X-ray crystallography and/or NMR. Here, again, there is a belief that automation will accelerate this process and many groups are engaged in out-bidding each other as to how many structures they will solve using their automated methods. It is also clear that some parts of the structure determination process will significantly improve using automation (e.g. X-ray data collection and analysis).

However, it must be remembered that structural biology, unlike gene sequencing, is not a 'linear process'. It resembles more the multi-disciplinary nature of lead discovery, with an interplay of molecular biology, biochemistry and biophysics all being required for the successful structure determination of a protein. There might be lessons for these structural genomics scientists to learn from the lead discovery experience. There are already clear analogies emerging between the two. The 'easier', but probably lower-value, protein structures are being solved, just as the 'easier' but ultimately low-value compounds were synthesized in the first generations of combinatorial chemistry. This is inevitable because the success of both activities were, and are, being measured by the number of structures or compounds that were generated. Here lies a potential pitfall in the relevance of structural genomics programs for drug discovery. These programs could generate many hundreds (if not thousands) of protein structures using their automated methods. However, the key question is: will these be the proteins of most therapeutic relevance, or the easiest and quickest to obtain?

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Harren Jhoti
Founder and CSO
Astex Technology
Cambridge
UK CB4 0WE