Novel and future applications of microarrays in toxicological research

Timothy W. Gant

Systems Toxicology Group,
Medical Research Council Toxicology Unit,
University of Leicester,
Lancaster Road,
Leicester, LE1 9HN
Tel: +44 116 2525579
Email: twg1@le.ac.uk

Abstract.

Microarray technologies have both fascinated and frustrated the toxicological community since their introduction about a decade ago. Fascination arose from the possibility offered by the technology to gain a profound insight into the cellular response to chemically mediated stress, and the potential that this genomic signature would be indicative of the biological mechanism by which that stress was induced. Frustrations have arisen primarily from technical factors such as data variance, the requirement for the application of advanced statistical and mathematical analysis and difficulties associated with actually recognising signature gene expression patterns, and discerning mechanisms. Toxicogenomics was predicted to make toxicological assessment and extrapolation easier, faster and cheaper. The reality has been that toxicogenomics is difficult. However, its potential when properly applied has been indicated by some well designed toxicogenomics studies, particularly in the differentiation of genotoxins from non-genotoxins. Technology waits though for no man. While the toxicological community has been working to apply transcriptomics (mRNA levels) in toxicology the technology has moved beyond this application into new arenas. Some have application to toxicology and are reviewed here, except transcriptomics which has been extensively written about before. Discussed are the application of microarray technologies applied to the genome *per se* (amplifications, deletions, epigenetic change), mRNA translation and its control mechanisms through miRNA. Which of the new genomics technologies will find most application in toxicology? In the opinion of this author there are three potentially major applications; 1) ArrayCGH in assessment and recognition of genotoxicity, 2) epigenetic assessment in developmental and transgenerational toxicology and, 3) miRNA assessment in all toxicology types but in particular developmental toxicology.

Keywords: Toxicogenomics, epigenetics, genotoxicity, microRNA, arrayCGH.

Introduction

Using microarrays to measure mRNA levels for genes across the whole or large parts of the genome has become a familiar technology in recent years. There are still many challenges associated with the interpretation of the data obtained, but the actual technology for data generation has matured with oligo based arrays in various formats becoming the *de facto* standard. Recent data from the USFDA has confirmed that the platforms are becoming robust, and that quantitatively similar data can be obtained across platforms [1].

The determination of mRNA levels for many genes simultaneously has been the major application of microarray technology [2]. However, to consider that this is their only application in regulatory and investigative toxicology is to take a myopic view of the potential application of this important methodology. The microarray format is as adaptable as its ancestor hybridization technologies and useful in all experimental applications involving hybridization. In this article we shall explore some of these applications, their role in making toxicological assessments, and position in the drug development pipeline.

As the use of microarrays for the determination of mRNA levels has been very well presented in a large number of articles it will not be addressed here. Rather this article will focus on some of the less explored applications of microarray technology and their application in toxicological assessment. The various applications will be presented in overview under separate sub-headings with the potential application to toxicological assessment dealt with at the end of each. The potential spectrum of microarray applications in toxicological assessment from the genome to assessment of mRNA translation is shown in figure 1. Also this article will be restricted to nucleic acid based arrays rather than those made with proteins or designed for high throughput transfections [3]. This focus though does not imply that non nucleic acid microarrays, such as those employing proteins as targets do not have application in the understanding of toxicology. Indeed there are profound opportunities for the use of non-nucleic acid microarrays in toxicological research.

What should be apparent is that although many articles have been written on the application of the microarrays in toxicology we are really still only at the initial stages of realising their true potential [4,5].

Array comparative genome hybridisation (ArrayCGH)

Outside of the determination of mRNA levels one of the first alternative applications of microarray technology was in the determination of gene changes (amplification and deletion) in the genome [6]. This method has recently been used to measure global variation in copy number through the human genome [7]. The process of determining chromosomal changes is essentially the same as that of measuring mRNA transcript levels, except that the probe is genomic DNA (gDNA) and not mRNA [8,9]. Hybridization and data collection occurs in a similar manner to that used for expression microarrays with (for a two colour system) probes from a control and test system hybridised to the same microarray to produce the familiar red and green spot image. The ratio of fluorescent dye in the hybridized spot indicates either an amplification or deletion in the genome. These data can be plotted against the chromosomal location of the probe to produce a map of the chromosome. An example of this can be seen in figure 2 where hybridisation of gDNA from a rat with a gene mutation giving rise to a Wilson's disease type was hybridised against that from a control Fischer rat. A deletion of one gene (cadherin 11) can be seen. The areas of the genome assayed in ArrayCGH are dependent on the targets on the microarray. Therefore targets with consensus sequences to gene coding regions will detect chromosomal changes in open reading frames, while for detection of changes in intragenic regions intronic probes are required. The resolution of the method depends on the number of probes used with the best resolution being provided by tiled probes such as those used in the study of Ekong *et al* in Axenfeld-Rieger syndrome [10].

It is often informative to compare the data obtained from an ArrayCGH assay with that of expression level assayed by measuring mRNA levels which can indicate whether the genes have been amplified whole, and if the increased copy number is reflected in the amount of mRNA transcript. An example of this application is shown in figure 3 for doxorubicin resistance cells where the *BAK1* and *ABCB4* genes are shown amplified but not overexpressed, *MMP1* is overexpressed but not amplified but *ABCB1* is both amplified and overexpressed. Further *ABCB1* and *ABCB4* are adjacent on human chromosome 7 and so while both have probably been amplified as a cassette only *ABCB1* has been amplified in a manner which has led to its overexpression [11].

Many of the assays currently used for the assessment of genotoxicity are in actuality looking for the amplification or deletion of large sections of the genome. For example micronucleus formation essentially represents the amplification of a piece of DNA and indicates there should be a role for the use of arrayCGH in the detection of genetic change after xenobiotic exposure. One example study, though not using microarrays has shown the amplification of the SV40 sequence in cell lines after exposure to various clastogens and aneugens [12]. A much wider perspective could be gained from the use of arrayCGH.

There is a potential problem in the application of ArrayCGH in the detection of genotoxicity though and that is the number of cells affected. Established assays such as FISH, micronucleus and reversion assays for example work at a single cell level in that single affected cells are counted to determine the effect of the xenobiotic. Unless the effect of a genotoxic xenobiotic is very substantial effects at the genome level in a minority of cells could be so diluted by unaffected material as to not be detected by arrayCGH. However in tumours patterns of genomic alteration have been associated with disease outcomes, such as survival and genomic alteration in primary gastric adenocarcinomas [13] despite these tumours consisting of a heterogeneous cell population. These data suggest that dilution effects on minority cell populations may not severely disable the potential of the assay. Additionally the recent demonstration of the application of arrayCGH to single cells [14] however indicates that arrayCGH has potential in application to detection of genotoxicity using single cells. The information gained would be more informative than that achieved from established assays as it would show whether there was a gene or region specific effect.

Use of the Array CGH technique has demonstrated the diversity that exists of copy number in the human genome [7]. Such genetic change affects resistance and susceptibility to toxicity and understanding the effect of genome variation could have a fundamentally important application in the assessment of drug efficacy and safety. These data also indicate that there is an important need to use arrayCGH to characterise cells and animal strains used for testing purposes. This has not been carried out to date but could yield valuable data that would inform on the resistance and susceptibility of strains and species, and also act as a reference for confirming the genomic stability of cells used for testing purposes in culture.

Epigenetic modification.

The recent sequencing of the human epigenome for three chromosomes has brought this application of microarray technology into sharp focus [15]. For toxicology there is potential both for understanding how drugs and chemicals alter DNA methylation patterns in cells and how these might affect toxicity and, second, in elucidating how epigenetic changes affect gene expression and in turn susceptibility and resistance to toxicity. Most work in genetic susceptibility has focussed on polymorphic mechanisms, but the small amount of epigenetic work in toxicology published indicates that this is substantial contributory mechanism to the genetically modulated response to xenobiotic exposure [16].

One means of epigenetic modulation is via the differential cytosine methylation. This can be assessed using microarray technology by first breaking up the DNA using either a mechanical or enzymatic means followed by immunoprecipitation of those fragments methylated using an antibody against 5-methylcytosine. A microarray based comparison of the differences in DNA sequence content between the immunoprecipitated fractions indicates those regions of the chromosome where differential methylation has occurred [17].

There is however another potentially important role for epigenetics in toxicology; in the understanding the mechanisms of transgenerational toxicology. What is meant by transgenerational toxicology is genome alteration, which may give rise to a phenotype, and is present in progeny as a result of germline transmission from the exposed parents [18]. This is different from reproductive toxicity where the toxicity arises in the foetus as a result of direct xenobiotic exposure in the uterus. Transgenerational genome instability arising from chemical exposure has been indicated by minisatellite mutation in both individual sperm and somatic cells of first generation mice where the father has been exposed to radiation or genotoxins [19,20].

Apart from transgenerational effects modification of epigenetic status in the embryo through maternal exposure may have important long term effects for the foetus and subsequent generations. One such example is diethylstilboestrol where effects are then seen in the subsequent male and female lineages after exposure *in utero* [21]. The effects observed in the male lineage mice were an increased incidence of proliferative lesions of oestrogen target tissues and tumours of the reproductive tract.

In females an increased incidence of uterine adenocarcinoma was observed. Transmission was only investigated through the female germ line. The mechanism of this effect was not investigated but previous work from this group has shown an effect of DES on DNA methylation patterns. Thus the hypothesis that altered methylation may be the mechanism is entirely plausible [22,23]. The demonstration of the inheritance to the F2 and F3 generations in the mouse of vinclozolin induced DNA methylation changes lends weight to the hypothesis [24].

Using microarrays containing probes for promoter regions combined with immunoprecipitation of methylated DNA offers profound opportunities for the study of epigenetic modification in terms of assessing contribution to xenobiotic susceptibility and resistance, and for recognition and understanding of mechanisms of transgenerational and reproductive toxicity. The modulation of epigenetic status is certainly a potentially important mechanism of toxicity, not least because of the inherited nature of the effects. The microarray method of analysis for epigenetic effects has a major role to play both in the future assessment of, and understanding of, such toxicities. The technique of single cell PCR amplification of minisatellites as a measure of transmissible genomic alteration [20] combined with a microarray based assessment of epigenetic change has enormous potential for the assessment of xenobiotic induced transmissible genomic change.

Chromatin Immunoprecipitation (ChIP) analysis.

The methodology of ChIP analysis is very similar to that used for epigenetic analysis. A microarray containing gene promoter region target sequences is required, as it is for the assessment of epigenetic analysis. Similarly an immunoprecipitation step is involved. The difference is that while the assessment of epigenetic status requires the use of an antibody against 5-methylcytosine, ChIP analysis requires an antibody against the transcription factor of interest. As there are two proteins involved, the antibody and the transcription factor, as well as the DNA fragment, it is necessary to chemically cross link the transcription factor to the DNA before immunoprecipitation [25].

Gene expression at the transcriptional level is controlled by the binding of transcription factors to the promoter regions of gene which may then recruit RNA polymerase II to initiate the transcription of the gene resulting in increased mRNA levels, which may be measured using microarray expression technology. An example

of a well known toxicant which alters gene expression by causing the translocation of an intracellular receptor and subsequent binding of a transcription factor is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Other examples would include the CAR receptor agonists such as Phenobarbital and retinoic acid receptor agonists. Data from the ChIP assay is able to show the gene promoter regions binding the transcription factor in one condition relative to another. As an example this assay was used to recognize and assess the binding sites of HNF6 in the liver, and elucidate the functions of GATA factor complexes [26] [27]. The limitation of this assay to date has been the availability of microarrays containing suitable promoter fragments. However there are now several companies supplying such microarrays which are likely to lead to an increased application of this assay method.

An assay such as ChIP is not likely to find application in the recognition of hazard. Rather this is an assay which may be utilised to understand mechanisms and by doing so make an informed assessment of risk. For example if, following a gene expression assessment, a novel chemical entity (NCE) is suspected to have Ah receptor type agonist properties from its gene expression profile, then a ChIP assay may be carried out with antibodies against the Ah receptor. The hypothesis would be that the NCE would cause increased immunoprecipitation of promoter regions containing Ah receptor binding sites and that the profile obtained in this assay would also be very similar to that obtained with TCDD. Such an assay would inform that the mechanism of action of the NCE was similar to that of TCDD and this was therefore likely to constitute a high risk level.

Transcription rate analysis.

The nuclear run-on assay has been employed to assess whether a gene is increased in its transcriptional rate in a certain circumstance eg: after chemical exposure. For example the nuclear run on assay has been used to show that transcription of the *ABCB1* gene can be increased after chemical exposure in the liver [28]. It should be possible to undertake such an analysis on a global scale using a microarray. Indeed a crude version of a microarray (the dot blot) has always been employed for this assay so the microarray would mean just an increase in the number of genes being probed.

The principle of the assay would then be that nuclei would be isolated from untreated and exposed cells or tissues. These nuclei would then be allowed to finish making

transcripts in an *in vitro* environment and incorporate a labelled nucleotide into the RNA made. The RNA would then be isolated from the nuclei in the same manner as for a nuclear run-on and hybridised to an expression type microarray consisting of gene coding regions. Where a gene is transcriptionally activated by exposure to the xenobiotic then this will cause an increased amount of transcript for that gene during the run-on *in vitro*. The net result will be that this differential transcription should be detected by increased hybridisation to the relevant target sequence on the microarray.

This assay would be a compliment to the ChIP type assay and would find use in toxicological assessment for an understanding of mechanisms of toxicity. For example with TCDD which leads to the transcriptional activation of genes through the Ah receptor an assay such as this should show increased transcription from the same battery of genes which are both differentially expressed, and transcriptionally regulated. Application of the ChIP assay should show increased Ah receptor binding to the promoter regions of these genes.

mRNA translation.

All of the above microarray based assays give information about the upstream events that can lead to differential mRNA levels. The subject of the determination of gene expression by measurement of mRNA levels is not going to be covered in this article for the reasons explained in the introduction. One of the criticisms though often levelled at the assessment of mRNA levels is that measurement has little application in mechanistic toxicological assessment because 'only proteins matter' and there is no evidence that the mRNA is translated into protein in a quantitative manner. Thus, it could be that a gene is increased in its transcriptional regulation resulting in an increased mRNA level, but that this does not *per se* lead to an increase in its protein level. Addressing this question is difficult because of limitations in technologies. Proteomic techniques do not have the dimensionality of genomic techniques due the inherent problems of 2D gel resolution. Even when a protein can be measured the problems of quantification and of quantification methods, the necessity of measuring actual number of molecules (which is usually not done) and other technical factors make any quantitative comparison of data from genomics and proteomics techniques notoriously difficult, or impossible. Biochemically the new investigation area of the role of microRNA (miRNA) in the cell (see section following) has shown that these species can target mRNA molecules for storage in P bodies within the cytoplasm

[29]. These can be potentially retrieved and used for rapid translation and thus there is a possibility of an increase in protein level without new transcription. This is most likely a fast response mechanism though and in the longer term new transcription becomes necessary. Data from our laboratory (Alexandra Paun personal communication in collaboration with Richard Currie of Syngenta CTL) has indicated that phenobarbital causes an increase in translation in rat liver which is dose and time dependent. Using the technique below we have shown that many of the genes which are transcriptionally increased in expression are also translated more actively indicating that both transcription and translation control mechanisms are important in the cellular response to xenobiotics.

There therefore appears to be a fundamental requirement to understand the effect of chemicals on mRNA translation. In particular if chemical exposure can lead to mRNA species being differentially translated as the cells increase their requirement for particular proteins to counter xenobiotic effects.

The assay mentioned above which has been developed in our laboratory and elsewhere [30] seeks to resolve both the questions of 'is the mRNA translated?' and 'is the mRNA differentially translated?' The assay relies on separation from whole RNA the mRNA with ribosomes attached by density, the polysomal fraction. The mRNA without attached ribosomes combines with the ribosomal RNA itself is the monosomal fraction (figure 4). An assumption is made mRNA found in the polysomal fraction is being actively translated, and there is a linear relationship between the number of ribosomes bound to the mRNA and the amount of protein formed. With this assumption the basis of the assay is in fact very simple. A cytoplasmic cell extract is prepared in which the ribosomes are stabilised on the mRNA through the use of the translation inhibitor cycloheximide. This is then layered onto a continuous sucrose density gradient and centrifuged. The proteins fail to enter the gradient and remain in a layer at the top. The mRNA species band through the gradient according to their density, and their density is dependent on the number of ribosomes bound. Due to its higher density the polysome fraction finds density equilibrium at higher sucrose concentrations, and the monosome fraction at the lower sucrose concentrations. These gradients can then be unloaded using a collection apparatus with continuous monitoring at 254 nm allowing the profile of the separation to be determined and the fractions collected. The RNA can be isolated from the collected fractions and obtained is a series of RNA fractions from the heavy polysomal layers through to the lighter polysomal layers and the monosomes.

Either alone, or by pooling, the mRNA content of each of the fractions can then be assessed for mRNA content and diversity using a microarray containing consensus sequences for gene coding regions. Careful reference of the ratio of RNA in the monosome layers of two systems compared with that in the polysome layers allows the question 'is an mRNA species differentially translated in this system?' to be addressed [31]. The question of 'is an mRNA translated' is answered more simply by comparing the proportion in the polysome fraction with that in the monosome fraction. As the RNA has been fractionated in this method care needs to be taken both with the amount used for hybridization and the method employed for normalization of the data. We use the assumption of majority non differential translation as the basis for normalisation. To achieve this on a two colour microarray the monosome fractions of the two samples are hybridised together on one microarray, and the polysomes from the same two samples on another.

It is not possible to normalize between polysomal fractions from control and treated tissues because this fails to correct for the amount of mRNA present in the sample for any particular species which could be altered due to differential transcription. Comparison of the polysomal fractions only would lead to the massive likelihood of calling a gene differentially translated between two samples when it is in fact just being differentially expressed.

The approach of density RNA fractionation with microarrays has been used in fundamental biology to understand translational changes during spermatogenesis and cancer progression but remains underutilised in toxicology [32,33]. However a few studies have indicated its potential application for example the translational response to redox stress in yeast and in mammalian cells to UV light [34,35]. Shenton *et al* showed that in yeast hydrogen peroxide (H_2O_2) to concentrations of 2.0 mM causes a suppression of global translation. Using microarrays in the manner described above the effect of H_2O_2 on specific genes was determined. At low concentrations (0.2mM) of H_2O_2 there was specific upregulation of the translation of transcripts associated with membrane transport and iron regulation amongst others and at 2.0 mM H_2O_2 transcripts associated with ribosome biogenesis were upregulated perhaps indicating the importance to the cell of maintaining its translational capability in the face of a substantial toxic insult [35].

For the toxicity profiling with the aim to identify types of potential toxicity in novel agents there is not requirement to assay for translation. Whether a gene is translated, or not, is of no consequence for this analysis where it is only the pattern of gene expression that is required for database matching. However for an understanding of the mechanisms of toxicity and appropriate risk assessment first understanding whether there is a change in the protein complement of the treated system and whether this is *per se* regulated is of primary importance.

miRNA

One of the main control methods of gene translation occurs through the recently recognised microRNA species (miRNA) [36]. These miRNA species are mainly transcribed from the genome by RNA polymerase II though some transcription from RNA polymerase III may occur [37,38]. The immature transcripts then undergo processing in the nucleus and cytoplasm to give rise to a 21-23nt miRNA molecule which, by interaction the RISC complex can control the translation of mRNA species against which it has a degree of homology [39].

For toxicology there are some interesting aspects to these miRNA species. Firstly they are transcribed from polycistronic regions and the control of the transcription appears to be very similar to that used for protein coding genes [37]. This implies that these genes may well be differentially regulated under the influence of chemicals which could then substantially change the translational profile of the cell. Second each miRNA species has an effect on the translation of many mRNA species and so a change in its level of expression could substantially affect the protein complement of the cell by altering the mRNA translation and lead to profound responses to chemical exposure. Finally, because of the potential control of the polycistronic regions at the transcriptional level they form an expression profile in a similar manner to mRNA species. Therefore the pattern of their expression could be potentially used to identify the type of toxicity which may be associated with the xenobiotic exposure. Such miRNA profiling has been shown to differentiate cancer types and the same could occur for different xenobiotic exposures [40].

To assess the expression of the miRNA species using microarrays there are some technical challenges to overcome. First, if there is a desire to assess the mature miRNA forms only then it is necessary to use a RNA tailing method for the labelling because the miRNA species are too short for conventional labelling techniques.

Conventional random primer labelling techniques can though be used to label the immature forms of the miRNA. Second, as the miRNA species are short it is often necessary to place targets on the microarray which contain modified nucleotides called locked nucleic acid nucleotides. These have the effect of making the hybridization more like an RNA/RNA hybridisation which has a higher melting temperature and thus gives rise to an increased stability and sensitivity [41].

For the recognition of toxicity by profiling the miRNA species probably have little to offer over conventional expression profiling. However, their expression is fundamental to the control of the protein complement of the cells, and as this may be material both to the understanding of mechanisms of toxicity as well as differential susceptibility. There remains much work to be done to determine if miRNAs are differentially expressed between populations, the effect of this on mRNA translation and ultimately susceptibility or resistance to chemical toxicity. To date there has been no application of this technology in toxicology. A search of PubMed (May 4, 2007) for **mir OR miRNA OR microRNA** yielded 227 papers. When the search term was combined with **toxicology** there were no hits.

Expert opinion.

The technologies described above give profound opportunities for the further understanding, recognition and assessment of toxicity. Recent years have seen a steady improvement in the quality of microarray technology as well as its breadth of application, and for many laboratories the use of microarray technology is now routine. In genotoxicity there is a recognised need to improve the predictive power of genotoxicity assays, particularly with respect to their relevance to actual carcinogenicity and non-genotoxic carcinogenic potential. The application of ArrayCGH could inform not only on the potential of a compound for aneugen or clastogen action but also where the effect occurs on the genome. This is relevant because an increase in DNA instability in a known area of genomic area of instability could be of potentially lesser relevance than, for example, oncogene amplification. Epigenetic modification is achieving importance driven by understanding in cancer where changes in the epigenetic DNA profile are recognised as having a role the causing phenotypic changes in the tumour. The role of epigenetics in relation to chemical toxicity is little explored to date but as relationships between epigenetic change and phenotype are defined then the assessment of epigenetic change in response to xenobiotics will become essential. This will be particularly so if a

relationship between epigenetic change and phenotypic change in the progeny of exposed individuals is established. Of particular relevance for investigative toxicology will be the new application of microarray technology in the field of translation because it is becoming clear there is an important role for altered translation in toxic responses, particularly stress responses. This may be controlled by miRNA species which have promoter structures which may allow for transcriptional regulation by xenobiotics. Alteration of specific miRNA transcript levels can quickly result in the increase in the specific mRNA translation which may have been stored in an intracellular P-body. Recent data from our laboratory has indicated that under the influence of certain chemicals some mRNA species are not only increased in transcription but also in translation. For genes in a rapid response network this seems a biochemically sensible approach. This then leads to the probable requirement to assess differential miRNA expression in cells under the influence of xenobiotics.

The above techniques are though an addendum to, and not a replacement for the more established expression profiling applications which will remain the first choice microarray technique for both the assessment of toxicity and investigation of mechanisms. For further mechanistic understanding there is a need to understand the regulation of genes under the control of xenobiotics. ChIP and microarray based analysis of transcription rate will indicate genes which are similarly transcriptionally controlled. Combined with bioinformatics analysis of the control regions involved these assays will give knowledge on the network regulation of genes at the transcriptional level, and those that are important for regulating cellular responses to xenobiotics. Analysed together with other data, in particular that of mRNA levels it should be possible to further identify gene expression biomarkers specific for certain xenobiotic types from the whole cohort of genes differentially expressed in response to xenobiotic exposure. This in turn may ease the difficulties in analyzing mRNA transcriptome data for the purposes of identifying hazard, and quantifying risk.

For the drug development pipeline the following deliverables are highly desirable:

1. The more rapid identification of potential toxicity in early stage development prior to Phase I
2. Recognition of the potential involvement of genetic polymorphisms which may give rise to adverse drug reactions and

3. Some understanding of the mechanism of toxicity such that rational evaluation of risk can be made particularly with reference to species and strain responses.

To achieve the above the use of mRNA profiling analysed with data from established endpoints such as histopathology and clinical chemistry will be the first microarray based assay employed. These data should then be analysed by various methods such as phenotypic and pathway mapping and alongside other data sets from similar chemicals in order to discern possible mechanisms of toxicity. The use of transcriptomics has highlighted the variability in biological background which is of highly relevant to the assessment of toxicity. *In vitro* established and primary cells which may be genetically unstable, easily contaminated and very responsive to changes in environment are particularly problematic in this regard. For this reason it is probably sensible to use inbred animal strains where there is control on the genetic background to reduce variability in the data. Reduction of variability is essential for high dimension data sets. It is conceivably possible though that the time period required for assessment *in vivo* can be reduced such that for example the same data can be gained from a 4 or 5 day test as could be achieved with a 28 day or longer test. This would result in a considerable cost saving with the added advantage of mechanistic understanding. ArrayCGH has been used to indicate the degree of variability in the human genome [7] and should be applied to inbred animal strains and cells used for toxicology testing to show genetic variability. These data can then be built into the risk assessment of xenobiotics which may indicate particular genome changes which give rise to differential susceptibility. ArrayCGH could also be used *in vivo* or *in vitro* in the same biological samples used for transcriptomics to indicate any genomic change associated with the xenobiotic exposure. Careful analysis of the regions affected could indicate whether these are likely to be important for toxicity.

Similarly the role of epigenetics in either conferring differential susceptibility or in mechanisms of toxicity should be explored. The data available indicating the involvement of epigenetic modification with the transgenerational toxicity of DES suggests this is an area of importance. Once again the samples from a short exposure could be used with appropriate microarray techniques to assess epigenetic change. Single cell minisatellite analysis could be carried out to show any genome instability which may be associated with epigenetic change. Finally the role of translation as a primary response to cellular stress is becoming apparent. Translation and miRNA analysis on the same samples from the short term exposure studies

could be carried out to discern if any xenobiotic related effect on translation has occurred, and if this is mediated through differential miRNA expression.

For mechanistic understanding which may be necessary for an appropriate risk assessment then ChIP and transcriptional analysis could be carried out. Data from the transcriptome and translational assays would indicate if there was a requirement for these assays.

Therefore microarrays have potential application both in recognition and understanding of toxicity from xenobiotics. The challenge has been, is now, and for the foreseeable future will be, not so much use of the technology but data interpretation. These technologies have exciting potential in toxicology but also bring with then the challenge of data analysis and then integration for real success to be achieved.

Acknowledgements

The author would like to thank the members of his laboratory, Robyn Perkins, Alexandra Paun, Richard Currie (Syngenta CTL) and the laboratory of Prof. Anne Willis and Dr Martin Bushell (University of Nottingham) for their work and stimulating thought. The four unknown reviewers are also thanked for their thoughtful and relevant comments.

References

- 1 GUO L, LOBENHOFER EK, WANG C *et al.*: Rat toxicogenomic study reveals analytical consistency across microarray platforms. *Nature Biotechnology* (2006) **24**(9):1162-1169.
- 2 HOLLOWAY AJ, VAN LAAR RK, TOTHILL RW, BOWTELL DD: Options available-from start to finish-for obtaining data from DNA microarrays II. *Nature Genetics* (2002) **32**:481-489.
- 3 MACBEATH G: Protein microarrays and proteomics. *Nature Genetics* (2002) **32**:526-532.
- 4 VAN INGEN C: Biology's century: just the beginning for microarrays. *Nature Genetics* (2002) **32**:463.
- 5 SLONIM D: From patterns to pathways: gene expression data analysis comes of age. *Nature Genetics* (2002) **32**:502-508.
- 6 ARRETT M, SCHEFFER A, BEN-DOR A *et al.*: Comparative genomic hybridization using oligonucleotide microarrays and total genomic DNA. *PNAS* (2004) **101**(51):17765-17770.
- 7 REDON R, ISHIKAWA S, FITCH K *et al.*: Global variation in copy number in the human genome. *Nature Genetics* (2006) **444**(23):444-454.
- 8 PINKEL D, ALBERTSON D: Comparative genomic hybridization. *Annual reviews in genomics and human genetics* (2005) **6**:331-354.
- 9 BASTIAN B, OLSHEN A, LEOBIT P, PINKEL D: Classifying Melanocytic Tumors Based on DNA Copy Number Changes. *Am. J. Path.* (2003) **163**(5):1765-1770.
- 10 EKONG R, JEREMIAH S, JUDAH D *et al.*: Chromosomal anomalies on 6p25 in iris hypoplasia and Axenfeld- Rieger syndrome patients defined on a purpose-built genomic microarray. *HUMAN Mutation* (2004) **24**(1):76-85.
- 11 TURTON NJ, JUDAH DJ, RILEY J *et al.*: Gene expression and amplification in breast carcinoma cells with intrinsic and acquired doxorubicin resistance. *Oncogene* (2001) **20**:1300-1306.
- 12 FAHRIQ R, STEINKAMP-ZUCHT A: Induction or suppression of SV40 amplification by genotoxic carcinogens, non-genotoxic carcinogens or tumor promoters. *Mutation Research* (1996) **356**(2):217-224.
- 13 WEISS MM, KUIPERS EJ, POSTMA C *et al.*: Genomic alterations in primary gastric adenocarcinomas correlate with clinicopathological characteristics and survival. *Cellular Oncology* (2004) **26**:307-317.
- 14 FIEGLER H, GEIGL J, LANGER S *et al.*: High resolution array-CGH analysis of single cells. *Nucl. Acids Res.* (2007) **35**(3):e14 11-10.
- 15 ECKHARDT F, LEWIN J, CORTESE R *et al.*: DNA methylation profiling of human chromosomes 6, 20 and 22. *Nature Genetics* (2006) **38**(12):1378-1385.
- 16 WATSON R, GOODMAN J: Epigenetics and DNA methylation come of age in toxicology. *Toxicol. Sci.* (2002) **67**(1):11-16.
- 17 VAN STEENSEL B: Mapping of genetic and epigenetic regulatory networks using microarrays. *Nature Genetics* (2005) **37**:518-524.
- 18 BARBER R, DUBROVA Y: The offspring of irradiated parents, are they stable? *Mutation Research* (2006) **598**:50-60.

- 19 VILARINO-GUELL C, SMITH A, DUBROVA Y: Germline mutation induction at mouse repeat DNA loci by chemical mutagens. *Mutation Research* (2003) **526**:63-73.
- 20 BARBER R, HICKENBOTHAM P, HATCH T *et al.*: Radiation-induced transgenerational alterations in genome stability and DNA damage. *Oncogene* (2006) **25**(56):7336-7342.
- 21 NEWBOLD R, PADILLA-BANKS E, JEFFERSON W: Adverse effects of the model environmental estrogen diethylstilbestrol are transmitted to subsequent generations. *Endocrinology* (2006) **147**:S11-17.
- 22 LI S, WASHBURN K, MOORE RE *et al.*: Developmental exposure to diethylstilbestrol elicits demethylation of estrogen-responsive lactoferrin gene in mouse uterus. *Cancer Res.* (1997) **57**:4356-4359.
- 23 LI S, HANSMAN R, NEWBOLD R *et al.*: Neonatal diethylstilbestrol exposure induces persistent elevation of c-fos expression and hypomethylation in its exon-4 in mouse uterus. *Mol. Carcinogen.* (2003) **38**:78-84.
- 24 ANWAY M, CUPP A, UZUMCU M, SKINNER M: Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* (2005) **308**(5727):1466-1469.
- 25 POLLACK J, VISHWANATH I: Characterizing the physical genome. *Nature Genetics* (2002) **32**
- 26 RUBINS N, FRIEDMAN J, LE P *et al.*: Transcriptional Networks in the Liver: Hepatocyte Nuclear Factor 6 Function Is Largely Independent of Foxa2. *Mol. Cell. Biol.* (2005) **25**(16):7069-7077.
- 27 GRASS J, JUNG H, KIM S-I *et al.*: Distinct Functions of Dispersed GATA Factor Complexes at an Endogenous Gene Locus. *Mol. Cell. Biol.* (2006) **26**(19):7056-7067.
- 28 GANT TW, SILVERMAN JA, BISGAARD HC *et al.*: Regulation of 2-acetylaminofluorene- and 3-methylcholanthrene--mediated induction of multidrug resistance and cytochrome P450IA gene family expression in primary hepatocyte cultures and rat liver. *Mol. Carcinogen.* (1991) **4**(6):499-509.
- 29 SHIVDASANI R: MicroRNAs: regulators of gene expression and cell differentiation. *Blood* (2006) **108**(12):3646-3653.
- 30 MAZAN-MAMCZARZ, KAWAI T, MARTINDALE J, GOROSPE M:
- 31 MAZAN-MAMCZARZ KKT MJLGM: En masse analysis of nascent translation using microarrays. *BioTechniques* (2005) **39**(1):61-+.
- 32 IGUCHI N, TOBIAS J, HECHT N: Expression profiling reveals meiotic male germ cell mRNAs that are translationally up- and down-regulated. *P. Natl. Acad. Sci. USA* (2006) **103**(20):7712-7717.
- 33 PROVENZANI A, FRONZA R, LORENI F *et al.*: Global alterations in mRNA polysomal recruitment in a cell model of colorectal cancer progression to metastasis. *Carcinogenesis.* (2006) **27**(7):1323-1333.
- 34 KRYSTYNA M-M, KAWAI T, MARTINDALE J, GOROSPE M: En masse analysis of nascent translation using microarrays. *BioTechniques* (2005) **39**(1):61-67.
- 35 SHENTON D, SMIRNOVA J, SELLEY J *et al.*: Global translational responses to oxidative stress impact upon multiple levels of protein synthesis. *Journal of Biological Chemistry* (2006) **281**(39):29011-29021.
- 36 KIM V, NAM J-W: Genomics of microRNA. *Trends in Genetics* (2006) **22**(3):165-176.

- 37 LEE Y, KIM M, HAN J *et al.*: MicroRNA genes are transcribed by RNA polymerase II. *EMBO Journal* (2004) **23**:4051-4060.
- 38 BORCHERT G, LANIER W, DAVIDSON B: RNA polymerase III transcribes human microRNAs. (2006) **13**(12):1097-1101.
- 39 CULLEN B: Transcription and Processing of Human microRNA precursors. *Molecular Cell* (2004) **16**:861-865.
- 40 LU J, GETZ G, MISKA E *et al.*: MicroRNA expression profiles classify human cancers. *Nature* (2005) **435**:834-838.
- 41 CASTOLDI M, SCHMIDT S, BENES V *et al.*: A sensitive array for microRNA expression profiling (miChip) based on locked nucleic acids (LNA). *RNA* (2006) **12**(5):913-920.

Figure 1

The applications of microarrays within genomics and their association with the other main 'omic sciences of proteomics and metabolomics.

Figure 2.

Genomic change in rat model of Wilson's disease showing a change in copy number of one gene cadherin 11 which is labelled and shown in grey. Each datapoint is derived from the competitive hybridisation to a probe of DNA from the fisher rat and the Wilson's disease rat. This fisher rat is the genetic background of the disease model. The probes are aligned on the x axis according to their position on the chromosome.

Figure 3. Genes various combinations of gene amplification and/or expression in MCF7 cells resistant to doxorubicin compared with the wild type in each case. Those with acquired resistance have been derived from exposure of a population of naïve cells to increasing concentrations of doxorubicin and have thus acquired resistance. It is hypothesised though that those cells with an acquired resistance arise through selection of cells which have an intrinsic resistance due to genome alteration. Those cells labelled as intrinsically resistant here are representative of this population of cells having been derived without drug exposure (an antibody selection technique was utilised [11]). The similarity of their differential gene expression and genome alteration compared with wild type progenitors suggests supports the hypothesis that cells with an acquired resistance are indeed toxin selected intrinsically selected cells from a majority sensitive population.

Figure 4. Separation of monosomes and polysomes on a sucrose gradient. Translationally inhibited mRNA species are separated on a polysome gradient. Those mRNA species with more ribosomes attached sediment at the higher densities with the UV trace showing the separation between monosomes and polysomes.