6. Results 4
Correlation between Replication Timing and Non-sequence Features of the Genome.

6.1 Introduction

Chapter five describes how microarrays can be used to assess replication timing. A correlation was observed between replication timing and sequence features of the genome. As described in section 1.3, replication timing has also been correlated with structural features of chromatin. This Chapter investigates the relationship between replication timing, transcriptional activity and histone modification of the genome. I also investigate how a change in chromatin, by a chromosomal translocation affects replication timing.

There is currently some controversy over whether there is a correlation between the time of replication and the transcriptional activity of regions of the genome. Experiments in yeast have shown no relationship between replication timing and transcriptional activity (Raghuraman, Winzeler et al. 2001), whilst analysis of the *Drosophila melanogaster* genome demonstrated no relationship with level of gene expression, but suggested a correlation with the probability of gene expression with genes that are transcribed being located within early replicating DNA (Schubeler, Scalzo et al. 2002). To investigate this link in the human genome, the expression of genes within a lymphoblastoid cell line was assayed and correlated with replication timing. This is described in section 6.2

Acetylation of histones within the nucleosomes has also been linked to transcriptionally active, early replicating regions of the chromosome (Grunstein 1997; Eberharter and Becker 2002; Vogelauer, Rubbi et al. 2002; Grewal and Moazed 2003). To investigate this correlation, DNA that had been immunoprecipitated with either of two antibodies, one anti-acetyl-Histone H3 (Upstate, USA) and a second anti-acetyl-Histone H4, ChIP grade for histone H4 acetylation (Upstate, USA), was applied to the array. Both antibodies are polyclonal and produced in rabbits. The Histone H3 recognises and is specific for acetylated human H3 of approx. 17kDa. The

Histone H4 antibody recognises acetylated histone proteins of approx 10kDa but is known to cross react with acetylated histone H2B and may cross react with other acetylated proteins.

The study was achieved in collaboration with the Microarrays, Transcriptional Regulation and Human Disease Group at the Sanger Institute. The chromatin immunoprecipitation was performed by Pawendeep Dharmi while I labelled and hybridised the DNA to the array. The results are reported in section 6.3.

Section 6.4 investigates chromosomal breakpoints and replication timing. Section 6.4.1 explores the effect a translocation between chromosomes 17 and 22 has on the replication timing of chromosome 22. The breakpoints had already been identified in our laboratory using an array painting technique (Fiegler, Gribble et al. 2003). This, and further resolution of the breakpoint by FISH showed that on chromosome 22 the breakpoint is within the clone bA46E17 (midpoint 11546117). Schleiermacher et *al* have shown that chromosomal breakpoints map to early replicating regions of the genome (Schleiermacher, Janoueix-Lerosey et al. 2003). This hypothesis is tested by mapping the position previously described breakpoints onto a normal replication timing profile (5.3.1) and is described in section 6.4.2.

6.2: Correlation between Replication Timing and Transcriptional Activity.

6.2.1 Correlation with Expression level on the 1 Mb Chip.

RNA was prepared from a cycling lymphoblastoid cell line (HRC 575) as described in section 2.6.1. The RNA was run on a 1% agarose gel to verify it was not degraded and is shown in Figure 6.1. The RNA was then labelled with Texas Red and applied to an Affymetrix U133A array in collaboration with Silvana Debenardi at the Molecular Oncology Unit, St Bartholomew's hospital.

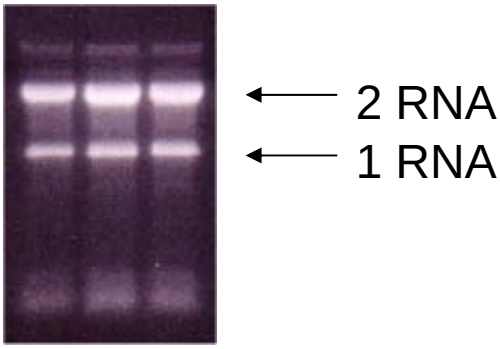


Figure 6.1: RNA prepared from a lymphoblastoid cell line.

The Affymetrix array contains oligonucleotides from approximately 13,000 human genes. Each gene loci is present as a pair of oligonucleotides, one contains the true gene sequence whilst the second oligonucleotide contains a mismatch. The Texas red ratio given by the sequence oligonucleotide is ratioed against the fluorescence obtained on the mismatched oligonucleotide. After hybridisation and sacanning the Affymetrix analysis program produces two values for each locus. Firstly, an expression level is given, this is obtained from the intensity of the fluorescence on the sequence oligonucleotide, secondly a ‘present’ or ‘absent’ call is given, a present call is obtained from the when the ratio of the intensities obtained from sequence: mismatch is above a set threshold.

The average expression level of each clone was correlated with the replication timing ratio for the 1Mb resolution array. Previous work by Bryan Young (Molecular Oncology, St Bartholemews hospital) had mapped Affymetrix data points within the clones present on the 1Mb array. Of the 3126 clones present on the 1Mb array only 1089 contained genes that were represented on the U133A Affymetrix chip. The replication timing ratio of each clone was plotted against the $\log_{10}$ of the average expression level of the clone (Figure 6.2).

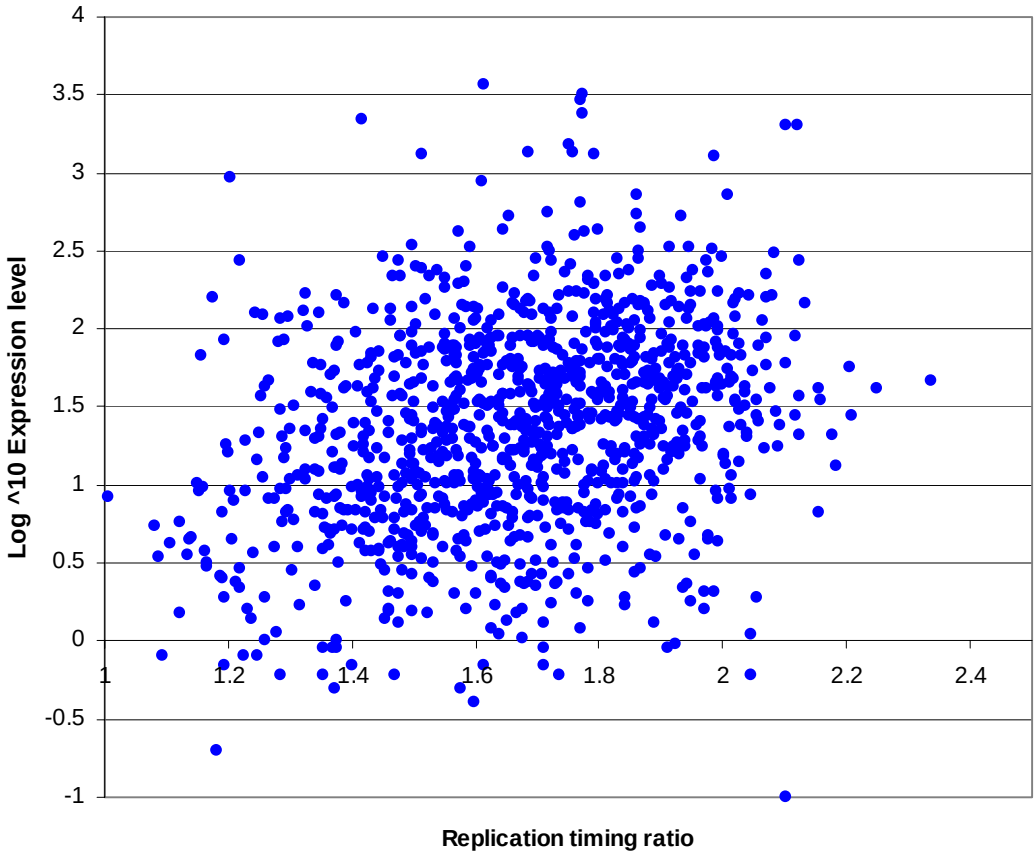


Figure 6.2: The correlation between replication timing ratio and $\log_{10}$ expression level of clones on the 1Mb array. $y = 0.057 + 0.86x$. $r = 0.30$.

These results show a weak correlation between the replication timing of a clone and the transcriptional activity of the genes within that clone. Hence early replicating clones are slightly more likely to be expressed at a higher level than those replicating later in S phase.

The expression level of the clone can also be plotted together with replication timing profiles reported in Chapter 5. An example of this is for chromosome 2 is shown in Figure 6.3. Other chromosome profiles are included in Appendix 8.

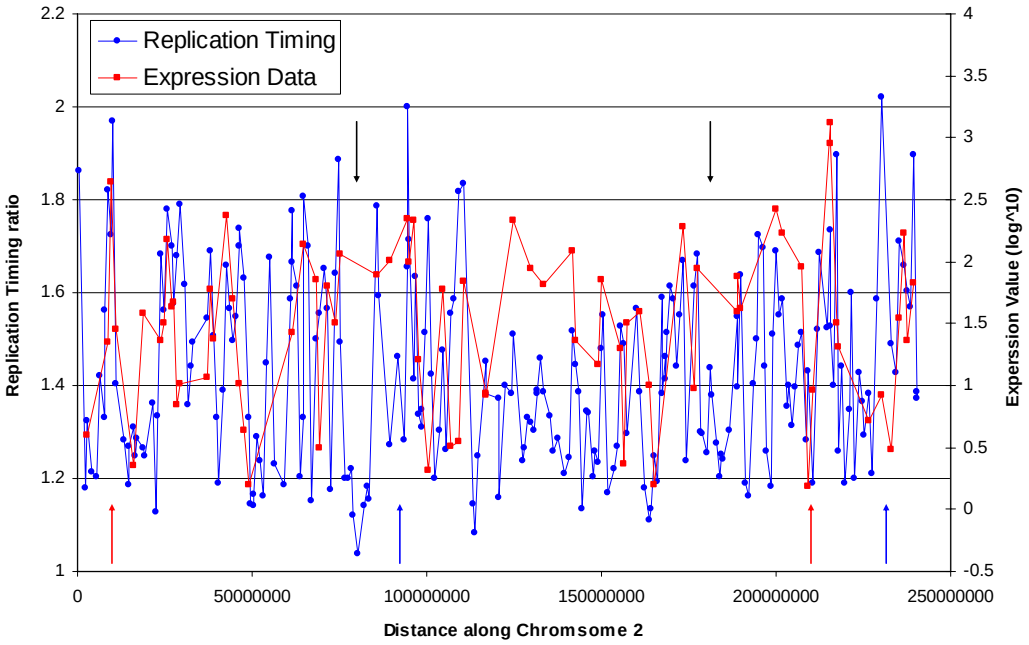


Figure 6.3: Replication timing and expression level profiles on Chromosome 2. Red arrows: regions where replication timing and transcriptional activity appear to correlate, Blue arrows: regions where replication timing and transcriptional activity do not correlate. Black arrow: regions that are late replicating, but are not represented on the U133A Affymetrix array.

The replication timing and expression level profiles of chromosome 2 show some regions where the two features appear to correlate (8.5-16Mb and 209-217Mb along chromosome 2), and regions where the two features are disparate (85-95Mb and 227-236Mb along chromosome 2).

6.2.2 Correlation with Expression level on the Tile path arrays.

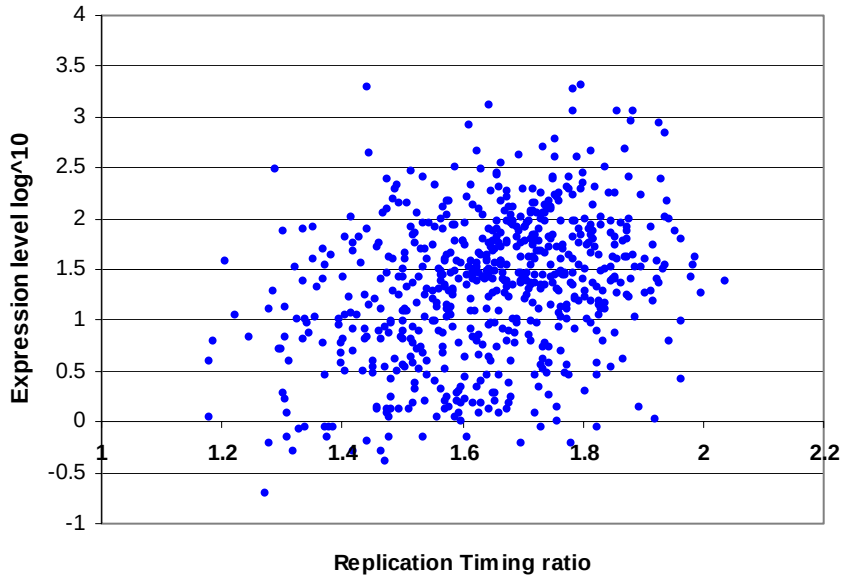
Analysis of replication timing and gene expression level was performed on tile path arrays for chromosomes 1, 6 and 22. This allowed the correlation between replication and transcription on a small, a medium and a large sized chromosome.

On chromosome 1, 647 of the 1961 tile path clones contained Affymetrix U133A data points. On chromosome 6, 430 of the 1651 tile path clones contained Affymetrix

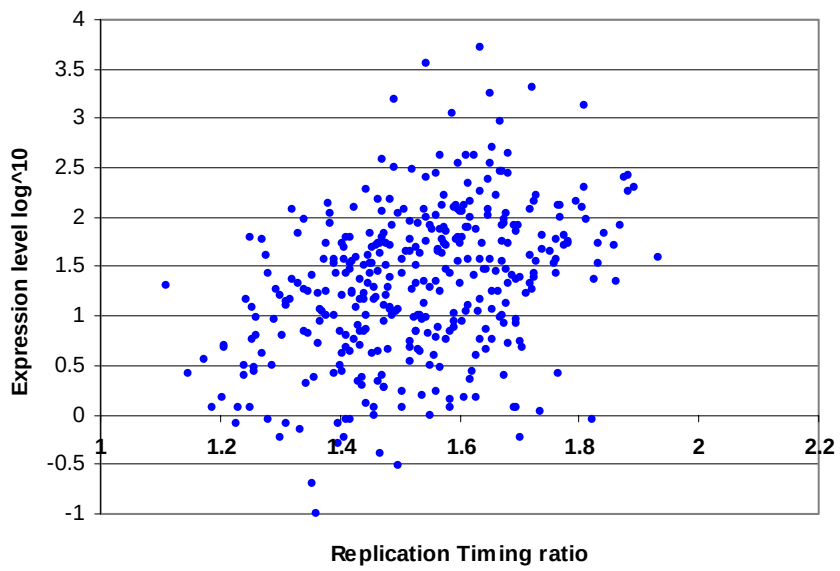
U133A data points and on chromosome 22, 147 of the 444 tile path clones contained Affymetrix U133A data points.

The correlation between replication timing and transcriptional activity was plotted for each chromosome as shown in Figure 6.4, The statistics for the linear regression line of each graph is shown in Table 6.1.

A



B



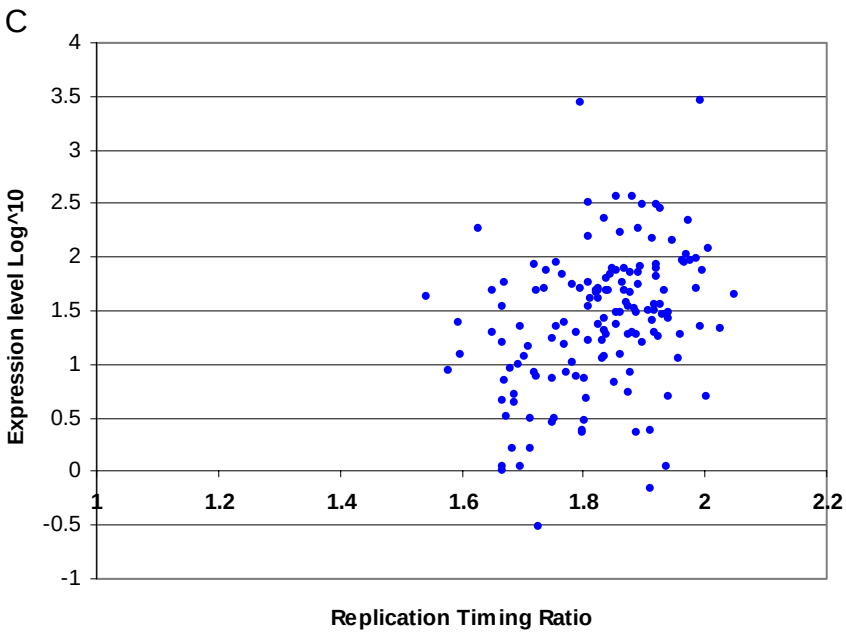


Figure 6.4: Replication timing ratio plotted against expression level (Log_{10}) for; A: Chromosome 1, B: Chromosome 6 and C: Chromosome 22.

Table 6.1: Regression features of Replication timing versus expression levels at tile path resolution.

Chromosome	**Intercept**	**Regression coefficient**	**Correlation coefficient**
1	-1.02	1.43	0.33
6	-1.02	1.84	0.38
22	- 2.54	2.16	0.34

As before, the replication timing and expression level of the chromosome was plotted against chromosome position, for the three chromosomes examined at tile path resolution. This is shown in Figure 6.5.