Chapter Eight – Conclusions, future work and projections into the future

1. Conclusions

This thesis encompasses two distinct bodies of work in advancing transposon technology, focused on the DNA transposon *PiggyBac* in mammalian genetics and genomics. The first part of my research work focused on the establishment of a genome-wide insertional mutagenesis strategy utilising efficient intra-genomic mobilisation of the PB transposon to conduct recessive genetic screens in *Blm*-deficient mouse ES cells. This technological development with the PB transposon is a further step forward towards the goal of unbiased genome-wide mutagenesis in mammalian systems. Combined with the methodology established using *Blm*-deficient ES cells for parallel conversion of heterozygous mutants to homozygosity on a genome-wide scale, the mutant library should cover a broader range of genes to facilitate genome-function assignment in many biological pathways of interests.

The second part concerns the development of novel reporter systems which enable phenotypic screens for the discovery of components of the miRNA biogenesis and effector pathways together with the established mutagenic strategy. This type of non-hypothesis driven genetic screen has so far not been conducted for the study of the mammalian miRNA pathways, although the siRNA pathway has been investigated using such an approach in *C. elegans*, *Drosophila* and mouse ES cells (Kim *et al.*, 2005; Dorner *et al.*, 2006; Trombly *et al.*, 2009). The two reporter systems established complement each other in the potential of their scope to probe two different branches of the miRNA downstream effector pathways. Upon completion of screens using both systems, it is hoped that some currently unanswered questions regarding the determinants and differential regulation of these two branches of the effector pathway can be revealed through the identification of novel factors. Although only preliminary screening results with one of the systems are described in this thesis, experiments are ongoing towards the completion of the large-scale screening work.

The final section of this thesis describes an independent body of research focusing on discovering new aspects of the *piggyBac* transposon for basic research as well as clinical

applications. The cargo capacity of the PB transposon was addressed for its use in large genomic DNA delivery. Up to 100 kb of genomic DNA cargo can be integrated and excised from the mouse genome using the PB transposon system. To our knowledge, this is the only transposon to possess such a large cargo capacity. This work could have exciting applications in correcting human genetic disorders in conjunction with the current developments in patient-specific induced pluripotent stem cells or adult stem cell derivation and transplantation.

2. Future work

The work described in this thesis has raised several interesting questions and opened up several lines of exciting research work which will be followed up in the near future. Firstly, large-scale phenotypic screening using the two developed reporter systems is still ongoing to identify the novel components within the miRNA biogenesis and effector pathways. The identification of potential novel factors can open up biological investigations into the function of these factors within the pathway. This work may reveal new aspects in the miRNA biogenesis and miRNA-mediated gene silencing.

Secondly, there are still a big scope for improvement of the current homozygous mutant conversion system using *Blm*-deficient ES cells. Although *Blm*-deficiency elevates the heterozygous to homozygous conversion rate, there is still a significant proportion of cells within the mutant pools that are irrelevant heterozygous cells. A method has been developed by Yue Huang in our laboratory to use a double-selection system to enrich the homozygous mutants by eliminating the heterozygous cells through the drug selection (Huang, *et al* and Horie *et al*, submitted). This method can successfully isolate homozygous mutants from their counterparts in a clone-by-clone fashion. However, the mixed mutant pooling method for assessing thousands of mutants at a time can not yet be coupled to this double-selection system due to the presence of aneuploid ES cells or/and cells with two-copy mutagen integrations. Through such a strong double-selection scheme, aneuploid cells or cells possessing two copies of the transposons can dominate the mutant pools. Thus methods to

allow the homozygous enrichment within a mixed mutant pool will further improve the efficiency of isolating relevant homozygous mutants.

Thirdly, the future investigation into the kinetics of *piggyBac* intra-genomic mobilisation may reveal the fundamental characteristics of DNA transposon mobilisation. PB transposition is highly efficient within the mammalian genome, and local hopping is not observed in cell culture or *in vivo* when a sufficient amount of transposase is supplied. In contrast to *piggyBac*, another DNA transposon from a separate family, *Sleeping Beauty*, is much less efficient than *piggyBac* during intra-genomic mobilisation and also shows a severe local hopping effect (Keng et al., 2005). However, *piggyBac* intra-genomic local hopping has recently been shown in Chapter Three of this work and by Wang and co-workers (Wang et al., 2008b). This observation poses the question as to whether the general mechanism of transposon intra-genomic mobilisation is dependent on continuous cycles of integration and excision gradually moving away from the donor site, until the transposition reaction is terminated by either a lack of sufficient transposase or integrations into "difficult-to-excise" genomic contexts. Understanding the transposition kinetics not only helps us to gain further understanding of the fundamental biology in DNA transposons, it may also highlight safety issues in using DNA transposons in clinical applications as every excision may bring the possibility of a mutagenic transposon foot-print.

Finally, much of the *piggyBac* transposon technology developed in mouse ES cells can be directly transferred to human cell-based research as *piggyBac* transposition is host independent. Although mice bear a high resemblance to human genetically, physiologically and anatomically, there are still major differences at the molecular, cellular and physiological levels. Furthermore, many disease models using mice can not capture the disease characteristics seen in human patients. Recent rapid developments in human ES cell culture and *in vitro* differentiation conditions allow the investigation of cellular pathways in these non-transformed human cell lines. Forward genetic screens can be conducted in human ES cells or iPS cells to uncover biological pathways, for example, the highly human-specific pathways that human immune deficiency virus (HIV) and influenza utilise for infection. The

derivation of induced human iPS cells and adult stem cells from patients opens up the possibilities of investigating these diseases in “authentic” pathological scenarios at molecular level and curing the disease using such matched cells after correction. Therefore, useful genetic tools and methodologies established in mice and cell cultures will play a vital role in helping us to understand the physio-/pathological mechanisms in the human system and providing therapeutic avenues for human patients.