6.1 Future work

The DREAM complex initially detected in Drosophila embryonic cells appears to function in repressing transcription of genes involved in differentiation, rather than displaying any cell cycle regulatory effects (Korenjak *et al.,* 2004). Conversely, in mammalian cells Lin-9 was found to cooperate with Rb to induce expression of differentiation-responsive genes (Gagrica *et al*.,2004). As the HPV life cycle is tightly coordinated with keratinocyte differentiation, and E7 overexpression delays or inhibits cell differentiation, it is of obvious interest to investigate how E7 interactions with DREAM complexes affect this cell property.

Studies in which p130- and p107-associated DREAM complexes have been readily detected have failed to uncover an Rb-associated DREAM complex. Despite popular belief to the contrary, Rb/E2F complexes are rarely found on the promoters of cell cycle regulated genes in tissue culture cells (instead p130/E2F and p107/E2F complexes predominate). Rather Rb/E2F complexes are more associated with senescent or terminally differentiated cells, moreover, Rb cooperates with other transcription factors involved in cell differentiation to induce gene expression. Rb-associated DREAM complexes may therefore be more prevalent in primary cultures, particularly when differentiation is induced.

An obvious system in which to search for Rb/DREAM complexes is human primary keratinocytes. To investigate first whether DREAM complexes are important for their differentiation, we will knock-down LIN-9 expression to determine whether this affects the properties of keratinocytes in the Ca2+ differentiation assay. In this assay, differentiation can be monitored by screening for induction of involucrin and keratin 10 (Zhang *et al.,* 2006). We will then perform co-immunoprecipitation assays to identify which pocket proteins are associated with LIN-9 in control and differentiated

keratinocytes. If Rb is detected in these assays, we will further characterise the complex to determine whether other LINC components are present. We will also test the ability of E7 to disrupt these complexes following retrovirus-mediated transduction, and we will correlate these effects with respect to inhibition of differentiation by various E7 proteins.

The longer term goals of this project are to gain greater understanding of not only how E7 functions in cancer development but also in viral replication. For example, evidence that HPV-16 E7 binds avidly to Rb and targets it for destruction is likely to be of great significance for carcinogenesis by high risk types, but barely explains the significance of E7 functions encoded by low risk or cutaneous types. We would also like to begin to explore how crucial E7 functions could be blocked. These goals require a greater understanding of DREAM complex assembly and function and how this is affected by E7 proteins.

Structural studies have shown that the E7 and E2F binding sites in the Rb (and related p107/130) pocket do not overlap, and therefore E7 is not a very good competitor for E2F binding to the pocket. Indeed, it has been found that 16E7 can be found in a p107/E2F complex in CaSki cells (Arroyo *et al.*, 1993). It is known that Lin-9 also makes an interaction with the B pocket (of necessity at a site different from the E2F binding site, and it is possible that this interaction is a more significant target for E7. We will therefore make a detailed study of pocket protein and LINC complexes in CaSki cells, for example to address whether p107 is dissociated from LINC but still present in a complex with E2F-4. These studies will involve size separation of protein complexes on Sepharose 6B columns and detection by western blotting column fractions. This work will complement our analysis of DREAM complexes in embryonal cells.

As part of our study we plan to set up a binding assay for E7/p130. Bacterially expressed p130 pocket will be displayed on 96 well dishes and bound to soluble HPV16 E7 (or the other way round). ELISAs will be used to assay binding. We will test the ability of LINC components to block this interaction. This assay can in principle also be used to screen for molecules that block the interaction. Or maybe should set up p130 Lin-9 assay and test E7 ability to block.