Nick Dyson, PhD

The Dyson laboratory studies the role of the retinoblastoma tumor suppressor (pRB). pRB is expressed in most cell types and its functions enable cells to stop dividing. pRB is inactivated in many types of cancer; a change that is thought to be an important step in tumor progression. We have three main goals: we want to understand the molecular details of how pRB acts, we want to know how the inactivation of pRB changes the cell, and we are using these insights to target tumor cells.

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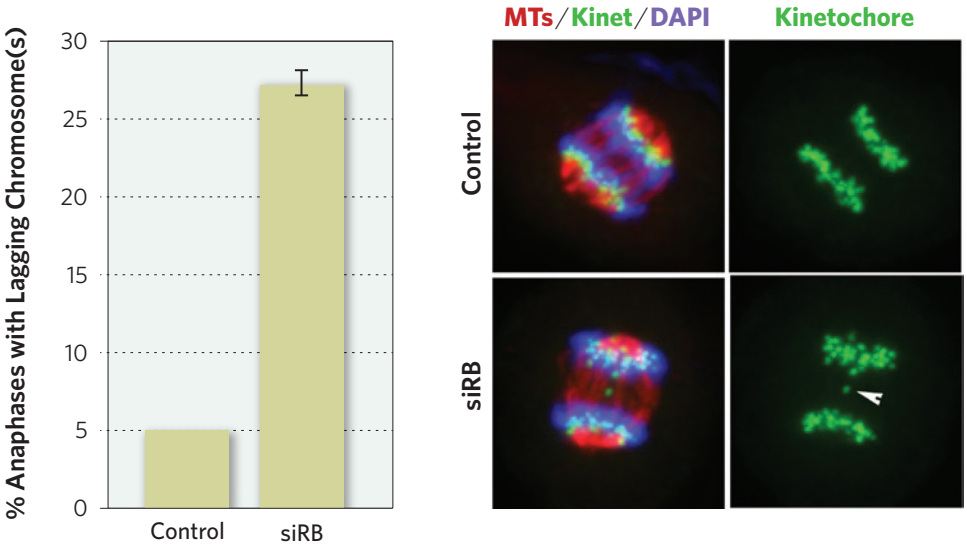
My laboratory investigates mechanisms that limit cell proliferation in normal cells and the ways that these controls are eroded in cancer cells. Our research focuses on the protein product of the retinoblastoma susceptibility gene *(RB1)* and its target, the E2F transcription factor. E2F controls the expression of a large number of target genes that are needed for cell proliferation. This transcriptional program is activated when normal cells are instructed to divide, but it is deregulated in tumor cells, providing a cellular environment that is permissive for uncontrolled proliferation. pRB has multiple activities but one of its most important roles is to limit the transcription of E2F targets. As a result, most tumor cells select for changes that compromise pRB function. Our research program spans four different aspects of pRB/E2F biology.

Dissecting the molecular functions of pRB

pRB's mechanism of action is an enigma. pRB has been linked to hundreds of proteins and implicated in many cellular processes. Purification of endogenous pRB complexes has been a major challenge and, consequently, it has been unclear which proteins are targeted by pRB at any given moment. Recently, we solved this problem and in collaboration with the Haas lab are using Mass Spectrometry to take detailed snapshots of pRB in action.

Proteomic profiles give a new perspective on the effects of RB1 mutation

E2F activity is typically measured by quantifying levels of RNA transcripts synthesized from genes that are controlled by E2F complexes. pRB inactivation changes the transcription of a vast number of genes and it has not been feasible to ask whether these transcriptional events impact protein levels. For over two decades it has been assumed that the RNA changes in *RB1* mutant cells are generally followed by similar changes in protein synthesis, and that the RNA signatures give a meaningful picture of a *RB1* mutant cell. To test this we generated RNA and protein profiles of tissues shortly after ablation of mouse *Rb1*. Remarkably, we discovered that the two types of data give strikingly different answers. Interestingly, mutation of *Rb1* has effects on protein levels that are far more extensive and very different from the changes predicted by RNA data. Unexpectedly, the most consistent proteomic change in *Rb1* mutant tissues was a decrease in mitochondrial proteins. Accordingly, *RB1* mutant cells have a proliferation disadvantage when grown in low-glucose conditions that put extra demands on mitochondrial function. In such conditions, pRB-deficient cells are more sensitive to mitochondrial poisons. These results reveal that the mutation of *Rb1/RB1* changes the cell in ways that had not previously been suspected.



The depletion of pRB results in a high frequency of lagging chromosomes. pRB was selectively depleted from RPE1 cells, a non-tranformed cell line, and the appearance of lagging chromosomes during anaphase was scored in cells stained to show the kinetochore (green), microtubules (red) or DNA (blue). Refer to Manning AL, et al for details.

The protein signatures may provide useful biomarkers in tumor samples and may reveal new ways to target tumor cells.

Targeting tumor cells with RB1 mutations

A long-term goal is to use information gleaned from molecular and mechanistic studies to improve cancer treatment. pRB is functionally compromised in most types of cancer, but mutation of the *RB1* gene is a hallmark event in three types of tumor (retinoblastoma, osteosarcoma and small cell lung cancer (SCLC)) suggesting that the complete elimination of pRB activity is especially significant in these forms of cancer. In collaboration with Dr. Anna Farago and members of the Haber/Maheswaran laboratories, we have generated patient derived xenograft models of SCLC and are testing ways to target these *RB1*- mutant tumors.

The biological consequences of eliminating E2F activity

Inhibition of E2F activity has been widely discussed as a potential therapeutic strategy for RB1 mutant tumors. To understand the consequences of global E2F inhibitors we have taken advantage of the relative simplicity of the *Drosophila* E2F/RB network and have performed a detailed analysis of *dDP* mutant animals. These mutants completely lack E2F function and, as expected, display extensive transcriptional changes. Proteomic profiles of *dDP* mutant animals reveal changes in protein levels that are different from, and even more extensive than, the transcriptional events. By integrating these RNA and protein profiles with ChIP data, we have identified a set of direct dE2F/dDP target genes are strongly altered in *dDP* mutant tissues. A genetic screen of these candidates has revealed, unexpectedly, that the upregulation of ATM expression is critical for *dDP* mutant phenotypes.

Selected Publications:

Wang H, Nicolay BN, Chick JM, Gao X, Geng Y, Ren H, Gao H, Yang G, Williams JA, Suski JM, Keibler MA, Sicinska E, Gerdemann U, Haining WN, Roberts TM, Polyak K, Gygi SP, **Dyson NJ**, Sicinski P. The metabolic function of cyclin D3-CDK6 kinase in cancer cell survival. *Nature.* 2017; 546 (7658):426-430.

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Miles WO, Lembo A, Volorio A, Brachtel E, Tian B, Sgroi D, Provero P and **Dyson NJ**. Alternative polyadenylation in triple-negative breast tumors allows NRAS and c-JUN to bypass PUMILIO post-transcriptional regulation. *Cancer Research.* 2016; 76(24):7231-7241.

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Nicolay BN, Danielian PS, Kottakis F, Lapek JD, Sanidas I, Miles WO, Dehnad M, Tschop K, Gierut J, Manning AL, Morris R, Haigis K, Bardeesy N, Lees JA, Haas W, and **Dyson NJ**. Proteomic analysis of pRb loss highlights a signature of decreased mitochondrial oxidative phosphorylation. *Genes and Development.* 2015; 29(17):1875-89.

Miles WO, Korenjak M, Griffiths LM, Dyer MA, Provero P, **Dyson NJ**. Post-transcriptional gene expression control by NANOS is up-regulated and functionally important in pRb-deficient cells. *EMBO J*. 2014; 33(19):2201-15.

Manning AL, Yazinski SA, Nicolay B, Bryll A, Zou L, **Dyson NJ**. Suppression of genome instability in pRB-deficient cells by enhancement of chromosome cohesion. *Molecular Cell.* 2014; 53(6):993-1004.