

Nicholas Dyson, PhD



Dyson Laboratory

Brian Brannigan
Nicholas Dyson, PhD
Marcello Stanzione, PhD
Jun Zhong, BS

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

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 RB, the protein product of the retinoblastoma susceptibility gene (*RB1*), and on E2F, a transcription factor regulated by RB. RB/E2F control the expression of a large number of genes that are needed for cell proliferation. This transcription 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. RB has multiple activities but one of its key roles is to limit the transcription of E2F targets. As a result, most tumor cells select for changes that compromise RB function. Our research program spans three areas of RB biology.

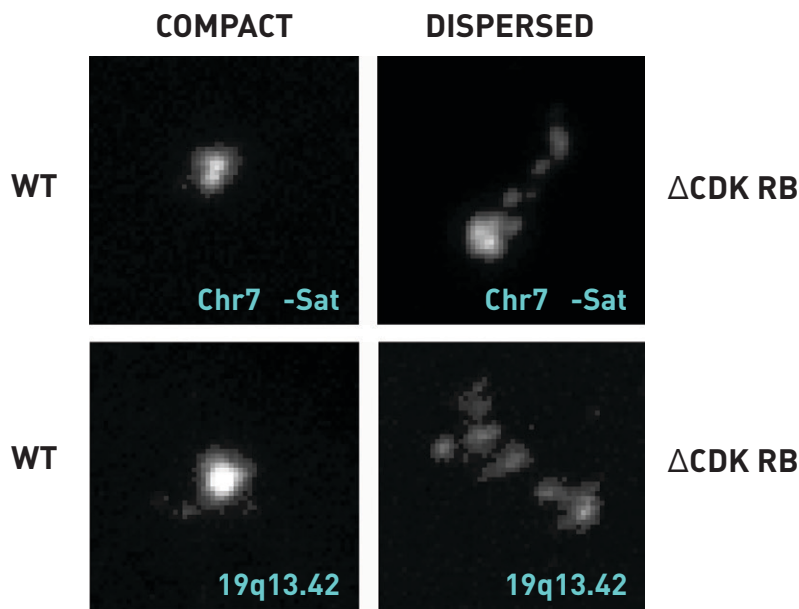
Dissecting the molecular functions of RB

RB's precise mechanism of action remains an enigma. RB has been linked to hundreds of proteins and has been implicated in many cellular processes. However, purification of endogenous RB complexes has been a major challenge and, consequently, it is uncertain which proteins physically interact with RB in any specific context. We solved this problem and, in collaboration with the Haas lab, have used Mass Spectrometry to take detailed snapshots of RB in action. We used this approach to test the hypothesis that RB's activity is tailored by mono-phosphorylation. Our data shows that the various mono-

phosphorylated forms of RB interact with different cellular proteins, regulate different sets of genes and have distinct functional properties (Sanidas et al 2019).

Active RB alters the organization of chromosomal domains

ChIP-seq experiments revealed that RB does not simply act at a few cell cycle-regulated promoters but targets thousands of sites that are distributed in euchromatin and heterochromatin. We have taken advantage of Oligopaint/FISH technology to visualize the impact of active RB on the nuclear organization of relatively large chromosomal regions (1-2 MB) that contain RB binding sites but lack canonical E2F-regulated, cell cycle genes. Induced expression of Δ CDK-RB (an active mutant protein that is impervious to CDK regulation) caused major changes in the organization of four different regions. Changes were quantified in both euchromatin and heterochromatin, but were most obvious with heterochromatic probes that typically gave a tight focal signal in cycling or quiescent cells. Following Δ CDK-RB expression these focal signals became diffuse, dispersed and scattered into multiple punctas (see Figure). Similar changes occurred following long-term palbocyclib treatment and in IMR-90 cells induced to enter senescence. These changes were time-dependent, and wash-out experiments suggest that they correlate with irreversible cell cycle exit. Interestingly, analysis of a



Consistent with the idea that RB is a master regulator of cell proliferation and that its activity leads to major changes in transcription, the expression of active RB (Δ CDK-RB) leads to changes in the organization of large chromosomal domains. Δ CDK-RB was induced in RPE1 cells, a non-transformed cell line, and the organization of a 4MB heterochromatic region (α -satellite) of chromosome 7 and 2.3MB euchromatic region of chromosome 19 was detected by FISH.

panel of mono-phosphorylation RB mutants revealed that some RB forms strongly induce these changes in G1-arrested cells while others do not, even though all repress E2F-dependent transcription. We infer that unphosphorylated RB does not simply suppress E2F-dependent transcription but drives changes in the nuclear organization of large chromosomal regions.

Targeting tumor cells with RB1 mutations

Our long-term goal is to use information gleaned from molecular studies to improve cancer treatment. RB is functionally compromised in most types of cancer, but the specific mutation of the RB1 gene is a hallmark of just three tumor types (retinoblastoma, osteosarcoma and small cell lung cancer (SCLC)). This implies that the complete elimination of RB function is especially important in these tumors. Together with Dr. Anna Farago, our clinical

collaborator, and with help from members of the Haber/ Maheswaran laboratories we have generated an extensive panel of patient derived xenograft (PDX) models of SCLC. These PDX models accurately reflect the genomic features and the drug sensitivities of the tumors from which they were derived (Drapkin et al 2018). We are using this panel of models to compare the effectiveness of different therapies, and to understand which SCLC tumors will respond best to each type of treatment (Farago et al 2019).

Selected Publications:

Farago AF, Yeap BY, Stanzione M, Hung YP, Heist RS, Marcoux JP, Zhong J, Rangachari D, Barbie DA, Phat S, Myers DT, Morris R, Kem M, Dubash TD, Kennedy EA, Digumarthy SR, Sequist LV, Hata AN, Maheswaran S, Haber DA, Lawrence MS, Shaw AT, Mino-Kenudson M, Dyson NJ, Drapkin BJ. Combination Olaparib and Temozolomide in relapsed small cell lung cancer. *Cancer Discovery*. 2019; Aug15.

Drapkin BJ, George J, Christensen CL, Mino-Kenudson M, Dries R, Sundaresan T, Phat S, Myers DT, Zhong J, Igo P, Hazar-Rethinam MH, LiCausi JA, Gomez-Caraballo M, Kem M, Jani KN, Azimi R, Abedpour N, Menon R, Lakis S, Heist RS, Büttner R, Haas S, Sequist LV, Shaw AT, Wong KK, Hata AN, Toner M, Maheswaran S, Haber DA, Peifer M, Dyson N, Thomas RK, Farago AF. Genomic and functional fidelity of small cell lung cancer patient-derived xenografts. *Cancer Discovery*. 2018; 8(5):600-615.

Sanidas I, Morris R, Fella KA, Rumde PH, Boukhali M, Tai EC, Ting DT, Lawrence MS, Haas W, Dyson NJ. A code of mono-phosphorylation modulates the function of RB. *Molecular Cell*. 2019;73(5): 985-1000.

Dick FA, Goodrich DW, Sage J, Dyson NJ. Non-canonical functions of the RB protein in cancer. *Nature Reviews Cancer*. 2018; 18(7):442-451.

Dyson NJ. RB1: a prototype tumor suppressor and an enigma. *Genes and Development*. 2016; 30(13): 1492- 502.