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The DREAM Complex: Controller of Late Stage Cellular Cycle

The Dream Complex, a group of proteins found in vertebrates, flies and worms, plays critical roll in regulating cell cycle and development. This group of nine proteins work together in the later phases of the cell cycle to turn off genes that cause DNA replication and to make the cell go dormant before the G0 phase. Strangely, oncogenes like Myb and FOXM1 which are part of this protein complex act as oncogenes and cause cellular proliferation in certain organs as well. The Dream Complex, like other proteins involved in cell cycle regulation, is also involved many different cancers. The protein Myb is found to be overexpressed in breast, lung, and pancreatic cancers, as well as other tumor suppressor proteins in the Dream complex like p130 are found to be underrepresented in cancer too (Decaprio). Labs across the country are now completely focused on understanding how this complex functions to create a better biological understanding of our bodies and to engineer the cancer treatments of tomorrow.

The Dream Complex is a fairly recent discovery, first described in *C. elegans* in 1988. For many years the scientific community thought that Rb was the sole regulator of the cell cycle, but they could not understand how that oncogene regulated late stages of the cycle. The Horvitz lab first showed other genes could regulate cell cycle by researching the vulva of these *C.elegans* when they discovered that the the vul class of genes encodes for EGF, EGF receptors and RAS (Horowitz). EGF is a growth factor that causes cells to proliferate and grow more cells(Lindsey), so when the vul (MUV) cells were overexpressed in *C. elegans* it caused many vulva to be

formed. Other proteins called lin54, lin52, and lin9 were found to oppose the EGF pathway and prevent the cells from over proliferating(Decaprio).

At the time, scientists did not know that these groups of proteins were part of a highly conserved complex found in many species, but that changed in 2002 when the Botchan lab replicated the chorian genes in *Drophila*(fruit flies) ovarian cells. (Botcham). The lab chose to use chorian genes because these genes are overexpressed in the s phase of cell cycle and respond to hormonal controls by telling cells to divide (Calvi) . Once they had amplified the chorian genes they performed an array and found a group of proteins including Myb, Mip 130, Mip 120, and Mip 140(Botchan) and E2F2. A BLAST of these genes showed that they were almost identical to the to the LIN genes previously identified by Horowitz. At about the same time, the Breham lab worked to find the entire DREAM complex by purifying protein complex that bound to RBF and GGF (Breham), both genes that are overexpressed in the s phase of cell development (Du 1996). The protein complex that they found to bind to those genes they called the *drosophila* RBF, E2F2 and Mip or DREAM for short(Decaprio) . Additional studies were done to make sure that the DREAM complex actually regulated late stage cell development by knocking out the genes identified to be in this complex(Dimova). The result was that many of the target genes that E2F2 and other proteins in the dream complex normally bound to were expressed in parts of the cell cycle that they are not usually expressed in. After this result came out, the Lin protein complex in worms was renamed to the DRM complex to mirror its functionality and much more research was done in sito to find a similar protein in mammals(Dimova). Eventually in 2004 the Gagrica lab found the mammalian equivalent of Lin and the Mip in sito by using a E2F probe, a group of proteins that are called Lin 9, 37, 52, 54 and RB4 as shown in figure 1.

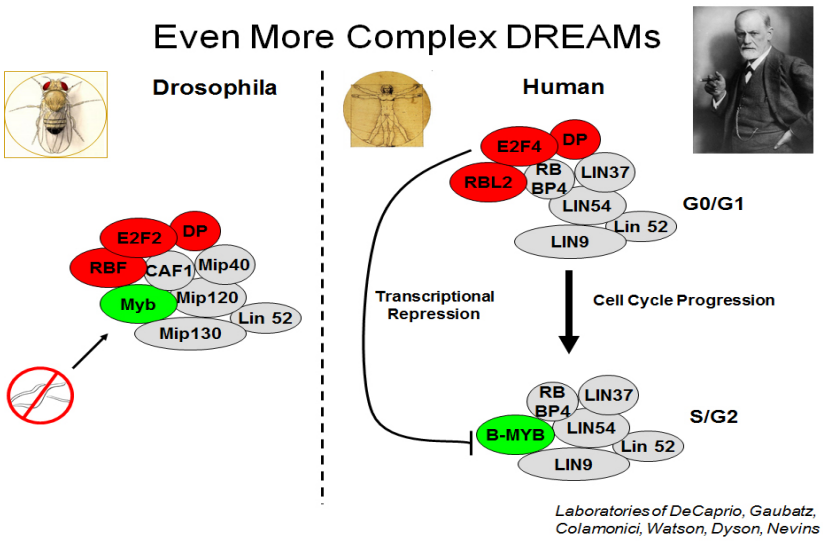

Figure 1: This shows the difference between *Drosophila* and human Dream complex. The grey proteins are the Muv core, red are tumor suppressors and the green is the oncogene, Myb

The Dream complex plays different roles in different parts of the cell cycle. In the G0 or dormant phase of the cycle, the Muv core binds with proteins p130 and E2F4 that are tumor suppressors(Boxem). Tagging both p130 and E2F4 with green florescent protein showed that p130 and E2F4 have adjacent binding sites in hundreds of genes across the genome, which means they must bind together as part of the larger DREAM complex(Decaprio). The genes coded for directly after these binding sites are usually only expressed in the G1, S or M phases of the cell cycle, showing that the binding of the Dream complex is what keeps these genes from being expressed. To start the cell cycle a ligase called INK4a removes the phosphate group form LIN52(Litovchick) dissociating p130 and E2F. .

This phosphorylation causes p130 and E2F4 to break off of the DREAM complex and stop blocking the genes which are then transcribed(Gaubatz).. The G1 portion of the cell cycle thus is initiated. When the cell has progressed into the S phase of development an oncogene called Myb attaches to the DREAM complex and once phosphoralyzed by Cyclin A, starts to turn on genes responsible for the late stages of cell development like CDKI, CENPE, and fLk1 (Gaubatz).As the cell progresses and enters the G2 phase, Myb dissociates from the complex and

another protein called FBOX is attached to the Dream complex (Decaprio). Both Myb and F-box are both oncogenes, but while Myb turns on genes for the G2 phase of the cell cycle Fbox binds with genes that are responsible for the m phase of cell division(Knight). Once the cell has gone through the M phase and split into two new cells the FOXM1 gene is phosphorylated again by anaphase producing complex and CDH. This extra phosphorylation causes the FBOX to dissociate and stop having genes that code for the G2 and M phases(Decaprio). At the same time DYRKA1 phosphorylates the s28 gene of LIN 52, one of the Muv proteins. This highly conserved phosphorylation prompts p130 and E2F4 to rejoin the DREAM complex and put the cell back into a dormant state(. The cycle of proteins attaching to the Muv core of the DREAM complex is shown in figure 2.

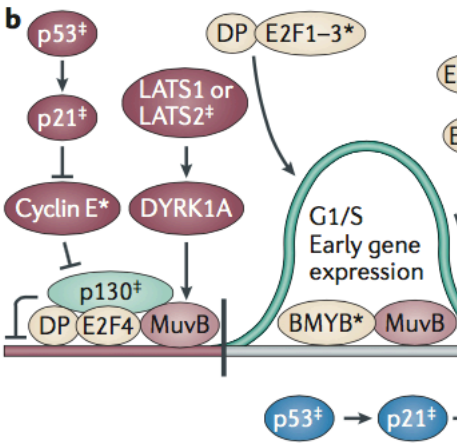

Figure 2: This shows the different proteins and times of the cell cycle that they attach to the Muv core to create the DREAM complex.

Since the DREAM complex plays such a key role in the cell cycle, mutations of the DREAM complex proteins also disrupt the cell cycle and often lead to cancer. The first mutation that affects the cell cycle is in the Lin 35 protein (Boxen and Van de Huevel) . This protein, part of the Muv core, acts as a negative inhibitor during the G1 phase slows down

cellular entry into development so that all other parts of the cell can grow before division. In experiments where Lin 35 is knocked out the cell cycle starts early and so immature cells are formed(Broxem). Another mutation that occurs in the DREAM complex is when E2F4 is knocked out. When this occurs cells do not divide or pass the G1 phase of development because the ligase that usually breaks down E2F is still present in the cell so it binds to other phosphorylated pieces of the Dream complex and breaks the complex apart (Decaprio).

Many more mutations of the DREAM complex are more closely associated with causing cancer. The phosphorylation of Lin52 to put the cell in the dormant GO part part of the cell cycle(quintessence) is crucial in preventing cancer. By using spectroscopy analysis the Litovchick lab was able to show if Lin52 or DYRK are mutated then phosphorylation on the s28 gene does not occur and cells do not enter their dormant stage. This means that the cells just keep proliferating through the cell cycle(Litovchick). Unrestricted cellular proliferation causes cancer tumors wherever the mutation has taken place. In his review article of the DREAM complex Decaprio states that there is debate now in the genetic community over whether or not DIRKA should be considered a tumor suppressor cell because its phosphorylation of Lin 52 keeps cells in correct differentiation timeframe. Work is now also being done to see if manually phosphorylating Lin52 could be used as a tumor therapy to put cancer cells in a dormant phase(Decaprio).

The HPV, cervical cancer inducing virus, also mutates the Dream complex to cause cancer. The HPV E7 oncoproteins bind onto p130 and Lin 54 proteins and inactivate them. This makes it so Lin54 and p130 do not act as tumor suppressors to cell cycle dependent genes(Nor Rashid).Dr. Nor Rashid for the Imperial college of London tested this theory by analyzing the amount of DREAM complex in cervical cancer cells and found that the complex was very

underrepresented in the HPV cells than in normal cells and Rb and p53 tumor suppressors were underrepresented as well(Nor Rashid). Since the E7 HPV virus inhibits Rb which also helps control the G1 phase of the cell cycle, p53 a very important tumor suppressor (Zilfao), and the Dream complex cells will be unable to switch from an on position to an off position and then will cause cervical cancer.

Mutations in the Lin9 gene, part of the Muv core, also is associated in metastasis of different cancers. Hauser described that a loss of Lin9 leads to chromosomal instability which means that chromosomes are not correctly duplicated in offspring and cancer can ensue (Hauser). Cancers in which Lin9 is mutated are considered high grade cancers because they often metastasize to other parts of the body. Lin 9 mutations are even used in the Angenra genetic test to determine a breast cancer's risk of metastasizing(Tian).

Most of the mutations researched about the DREAM complex involve either an over or under expression of the oncogene BMyb. At Stanford for instance, Joe Lipsick runs a lab completely focused on understanding this one gene and its mutations. Myb is so widely researched because it is overexpressed in many different types of cancers including breast, lung, prostate, cervical and ovarian cancers(Whitfield). Since Myb turns on genes in the S and G2 phases of the cell cycle when cancer cells are proliferating rapidly they will usually have more Myb because the cells are rapidly going through cellular division(Whitfiteld). Myb is even used in different screenings today including Oncotype Dx, a test of levels of 21 genes to determine breast cancer risk(Peron), and in a test alone with three other Dream Sequence genes to determine risk for colon cancer (O'Connell). While having too much Myb in cells, not having Myb causes even greater problems. Low levels of Myb are associated with high levels of myeloid cells, which help tumors grow by promoting Angiogenesis, blood vessel growth to

tumors(Schmid). When there is no Myb expressed, genes that are expressed by Myb in the S and G2 phases of the cell cycle are never turned on. (Charrase) The absence causes almost immediate cell death and no cell proliferation occurs. Surprisingly, scientists researching Myb found that even though knocking out Myb completely kills all cells, knocking out Myb and certain proteins in the Muv complex cause about 5% of the cells to survive. Research is now being done to find out the best combinations of protein knockouts to form a cell without Myb that is still somewhat viable(Lipsick). For example, Chris LeBoa under the direction of Joe Lipsick has been growing a batches of flies with Myb, Mip 120 and another protein called F-box011 mutations that still have a 50% viability rate. With more research this knockout could be tested in vitro with Myb overexpressed human cancer cells to stop tumor cellular replication without destroying cells. In the future, if someone has a cancer with an overexpression of Myb, doctors may treat them with a drug that knocks out Myb and other proteins.

FOXM1, the protein in the DREAM complex that turns on gene in late G2 and M phases of the cell cycle, has also been studied in depth as an oncogene. FOXM1 is found to be overexpressed in glioblastoma, prostate cancer, lung cancer, esophageal cancer, pancreatic, cancer, and ovarian cancer (Decaprio). In gliomas the amount of FBOX present in the tumor is directly proportional to the grade of the cancer (Liu). The Liu laboratory hypothesizes that since the F-Box gene turns on a protein called Skp2 which in turn breaks apart a cell cycle regulator called p27. If p27 is degredated then the cells do not have one of the tumor suppressors required to stop proliferation and glioblastoma ensues(Liu). Liu's laboratory conclusion suggests researching FBOXM1 knockout as a possible treatment option for the cancer (Liu). The Kalin lab focused on FBOXM1 in prostate cancer. They found that increased levels of FOXM1 are associated with faster growing, more malignant, and deadly strains of prostate cancer(Kalin) as

shown by increased prostate size and more cellular mitosis of tumor cells in Figure 3 below.

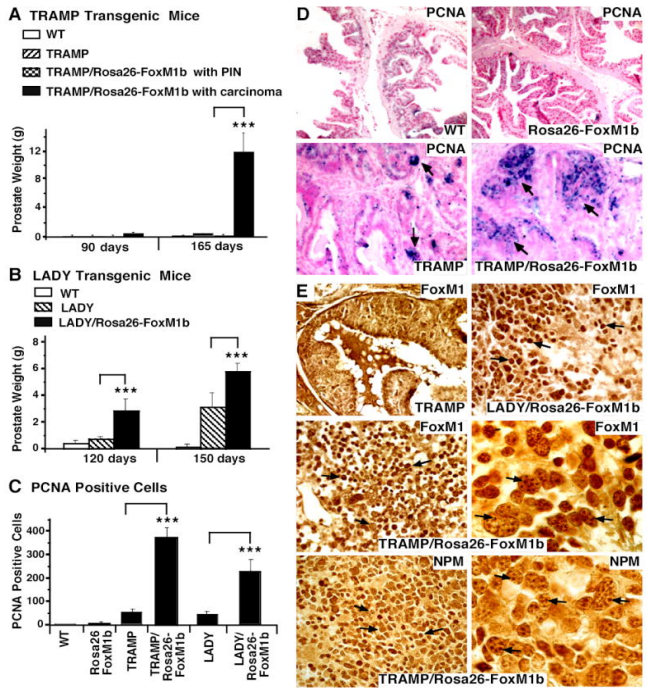

Figure 3: The graphs on the left side of the figure show the prostate weight of mice with pancreatic cancer without and with FBOX over-amplification. The ones with the over amplification are significantly larger. The photos on the right show prostate cancer strains the left sided ones do not have over amplification of Fbox while the ones on the right side to

The Kalin lab was also able to show that in mice they could stop the proliferation of FOXM1 by inducing RNA transfection that inhibited the FOXM1(Kalin). Even without creating a brand new treatment method, FBOX expression can be used to screen prostate cancer patients for the severity of their disease. Seventy percent of men over the age of 70 have some sort of prostate cancer, but only a few of these cases are quick growing and metastasize (Cancer.org). If patients with prostate cancer were screened to see the expression of F-Box. If doctors saw an overexpression of FOXM1 they would know the cancer is more likely to be malignant and recommend the patient for surgery, but a regular expression of FOXM1 would show them that the cancer is slow growing and is probably best to just watch. Like the Liu lab, the Kalin lab is interested in continuing research and concludes, “FoxM1 is a novel target for prostate cancer treatment”(Kalin).

All studies published relating FOXM1 to different cancers all come to the

conclusion that increased amounts of the oncogene FOX make cancers more deadly and faster growing. Each study though associates FOXM1 proliferation with affecting different proteins to make the cancer worse. The Dibbs lab concludes that in esophageal cancer FOXM1 triggers a kinase called PLK1 that starts a positive feedback loop signaling more FOXM1 to be produced and turn on more cellular proliferation genes(Dibbs). Currently there are PLK inhibitors in phase two clinical trials (Ingelheim) that could possibly also work to prevent PLK1 –FOXM1 positive feedback loop.

FOXM1 also causes cancer when it is mutated. Since FOXM1 turns on genes that regulate the M section of the cell cycle, when FOXM1 is mutated incorrect mitosis occurs and chromosomes are matched incorrectly (Loukii). Incorrect matching leads to cells that are not diploid and other unmatched genes that do not code for the correct proteins. The plurality of mutations lead to unregulated cells and a whole host of different cancers. The Carter lab was able to come up with a model to calculate chromosomal instability based on FOXM1 mutations(Carter) that can be used to judge a person's cancer risk.

Even though many labs conduct research on the DREAM complex, much still needs to be learned about the complex and its function in cells. It is still unknown if the DREAM regulates more genes than just cell cycle dependent ones. Researchers are still also looking into the pathways that, attach Myb and FOXM1 to the DREAM complex. Beyond basic biology questions, many more questions have been opened up about how to create therapeutic solutions for cancer using the DREAM complex. Research is being done to see if inducing quiescence, like what DKRA1A does when it

phosphorylates Lin52 is a way to restrict tumor growth or if creating Myb or FOXM1 inhibitors can reduce the progression and mortality of different kinds of cancers.

The DREAM complex plays a crucial role in regulating later stages of the cell cycle. By binding to p130 and E2F2 the DREAM complex keeps the cell in a dormant quiescent stage but later in the cell cycle when the Muv core of the DREAM complex binds to Myb and FOXM1 they turn on genes that play crucial roles in the G2 and mitosis of the cell cycle. Mutations in the DREAM complex are associated with many different cancers, but much more research needs to be done to assign causality of any specific cancer to a mutation in the DREAM complex or to create cancer treatments for through inhibiting DREAM complex proteins.

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