The Impact of Rapamycin Mediated Disruption of Nutrient Sensing on Genome Function

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Supervisor: Dr. Christopher Eskiw
How do cells sense nutrients?

- **The mammalian Target of Rapamycin (mTOR) pathway**
  - A major hub involved in nutrient sensing within the cell
  - Receives signals from glucose, amino acids, ATP levels
  - Influences cell growth, proliferation, autophagy
  - Well characterized at the biochemical level

- **Rapamycin inhibits mTOR**, proposed mimetic of decreased nutrient availability

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Genome Function?
What is rapamycin?

- **Rapamycin**
  - A commonly used immunosuppressant
  - Approved for use in humans

- **Extends lifespan in**:
  - Yeast
  - Flies
  - Worms
  - Mice
  - Potential treatment for the premature ageing disease Hutchinson Gilford Progeria Syndrome
  - Humans?
What impact does rapamycin-mediated disruption of nutrient sensing have on human genome function?
Does rapamycin impact fibroblast growth?

+500nM Rapamycin

Healthy Human Foreskin Fibroblasts

http://www.nzblackcurrants.com/assets/images/cell-culture-image.jpg
Rapamycin decreases fibroblast proliferation

Marker of proliferation: Ki-67

Incorporated during DNA synthesis/S phase: 5’-ethynyl-2’deoxyuridine (EdU) assay
Therefore...

- Rapamycin causes a decrease in cell proliferation without causing cell death.
- Rapamycin appeared to induce the cells into a quiescent-like state.

Is rapamycin treatment mimicking quiescence?
Chromosome territory positioning

Bolzer, et al., 2005

Rapid chromosome territory relocation by nuclear motor activity in response to serum removal in primary human fibroblasts

Ishita S Mehta, Manelle Amira, Amanda J Harvey and Joanna M Bridger
Rapamycin induced chromosome territory re-positioning similar to that of quiescence

Blue = H33342
Red = Chromosome Territory

<table>
<thead>
<tr>
<th>Chr</th>
<th>Pro</th>
<th>Qui</th>
<th>Rap</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Periphery</td>
<td>Interior</td>
<td>Interior</td>
</tr>
<tr>
<td>10</td>
<td>Interior</td>
<td>Periphery</td>
<td>Periphery</td>
</tr>
<tr>
<td>X</td>
<td>Periphery</td>
<td>Periphery</td>
<td>Periphery</td>
</tr>
</tbody>
</table>
Therefore...

- Chromosomes 10 and 18 reposition to similar nuclear locations in response to both rapamycin treatment and quiescence induction.

- Changes in genome organization are indicative of changes in genome function.

Does rapamycin induce the same changes in genome function that quiescence does?
Are transcript profiles between rapamycin treated and quiescent induced fibroblasts similar?
qPCR validation of RNAseq data

- \(\Delta \Delta CT\) method using 5 normalizing genes identified from RNA-sequencing data:
  - PRDX5, EFEMP2, FAU, SPARC and FKBP10

Blue: qPCR fold change
Red: RNA-seq fold change

Anything that was >5 fold by RNAseq was >5 fold by qPCR
What biological pathways are changing as a result of rapamycin treatment and quiescence induction?
Pathway enrichment: genes $\geq 5$ up-regulated in response to quiescence induction

<table>
<thead>
<tr>
<th>Gene Set</th>
<th>Genes From Network</th>
<th>Nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complement and coagulation cascades</td>
<td>9</td>
<td>CFH, CFD, SERPING1, C3, C1R, BDKRB2, C1S, C2, CFB</td>
</tr>
<tr>
<td>Extracellular matrix organization</td>
<td>13</td>
<td>LTB4, NCAM1, CTSK, VCAN, MFAP4, FMOD, LUM, DCN, BMP4, BMP2, TNXB, FBLN1, COL14A1</td>
</tr>
<tr>
<td>Staphylococcus aureus infection</td>
<td>7</td>
<td>CFH, CFD, C3, C1R, C15 C2, CFB</td>
</tr>
<tr>
<td>Metabolism of xenobiotics by cytochrome P450</td>
<td>5</td>
<td>ADH1B, ADH1A, AKR1C2, ALDH3A1</td>
</tr>
<tr>
<td>Pertussis</td>
<td>5</td>
<td>SERPING1, C3, C1R, C1S, C2</td>
</tr>
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**Pathway enrichment: genes ≥5 down-regulated in response to quiescence induction**

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<tr>
<th>Gene Set</th>
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<tr>
<td>Mitotic Prometaphase</td>
<td>15</td>
<td>AURKB, KIF2C, CDCA8, CDK1, MAD2L1, ZWINT, NCAPH, BUB1, CENPM, CENPF, BIRC5, CDC20, CCNB1, CCNB2, PLK1</td>
</tr>
<tr>
<td>Cell Cycle</td>
<td>14</td>
<td>E2F2, PKMYT1, PTTG1, CDC45, CCNA2, CDK1, MCM5, MAD2L1, SFN, BUB1, CDC20, CCNB1, CCNB2, PLK1</td>
</tr>
<tr>
<td>Aurora B Signalling</td>
<td>9</td>
<td>KIF23, AURKA, AURKB, KIF2C, CDCA8, NCAPH, BUB1, BIRC5, KIF20A</td>
</tr>
<tr>
<td>FOXM1 Transcription Factor Network</td>
<td>9</td>
<td>AURKB, CCNA2, CDK1, FOXM1, CENPF, BIRC5, CDC20, PLK1</td>
</tr>
<tr>
<td>Mitotic Metaphase and Anaphase</td>
<td>13</td>
<td>PTTG1, AURKB, KIF2C, CDCA8, UBE2C, MAD2L1, ZWINT, BUB1, CENPM, CENPF, BIRC5, CDC20, PLK1</td>
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Pathway enrichment: genes ≥5 up-regulated in response to rapamycin treatment

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<tr>
<th>Gene Set</th>
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<tbody>
<tr>
<td>Cytokine-Cytokine Receptor Interaction</td>
<td>13</td>
<td>IL6ST, IL11, IL1B, INHBA, CXCL1, CXCL3, CXCL2, LIF, IFNA1, IL6, BMP2, IL8, TGFBR1</td>
</tr>
<tr>
<td>Salmonella Infection</td>
<td>8</td>
<td>IL1B, ROCK2, PKN2, CXCL1, CXCL3, CXCL2, IL6, IL8</td>
</tr>
<tr>
<td>Pathways in Cancer</td>
<td>12</td>
<td>FG F7, PTGS2, TPR, FG F2, MDM2, LAMC2, ITGA5, IL6, BMP2, IL8, TGFBR1, ITGA2</td>
</tr>
<tr>
<td>Legionellosis</td>
<td>6</td>
<td>IL1B, CXCL1, CXCL3, CXCL2, IL6, IL8</td>
</tr>
<tr>
<td>Signalling by SCF-KIT</td>
<td>8</td>
<td>FG F7, NRG1, FG F2, EREG, MDM2, NR4A1, HBEGF, JAK2</td>
</tr>
</tbody>
</table>
Pathway enrichment: genes ≥5 down-regulated in response to rapamycin treatment

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<tr>
<td>GPCR Ligand Binding</td>
<td>4</td>
<td>MCHR1, F2, GNG13, ADM2</td>
</tr>
<tr>
<td>Formation of Fibrin Clot (Clotting Cascade)</td>
<td>2</td>
<td>F2, PF4V1</td>
</tr>
<tr>
<td>Thrombin Signalling Through Proteinase Activated Receptors (PARs)</td>
<td>2</td>
<td>F2, GNG13</td>
</tr>
<tr>
<td>Gastrin-CREB Signalling Pathway via PKC and MAPK</td>
<td>3</td>
<td>MCHR1, F2, GNG13</td>
</tr>
</tbody>
</table>
Rapamycin ≠ Quiescence
Are these changes in gene expression equating to the protein level?

- Are changes in gene expression conserved at the protein level?
  - IL-6
  - IL-8
  - LIF

![Bar graphs showing IL-6 and IL-8 levels](image)

**Figure 4**
What is the mechanism behind these rapamycin induced changes in genome function?
What is the mechanism mediating rapamycin-induced changes in genome function?

- Analysis of promoter regions of genes up-regulated in response to rapamycin
Could the transcription factor STAT5A/B be mediating rapamycin-induced changes in gene expression?

- Transcription factor motif search
- Revealed STAT5A/B binding sites in promoter regions of up-regulated genes (34.2%)
Is STAT5A/B binding the promoters of genes changing expression?

Immunofluorescence for Phosphorylation STAT5A/B

Chromatin Immuno-Precipitation (ChIP)

STAT5A/B

% Input

- Pro
- Rap
Conclusions

- Treatment of human fibroblasts with rapamycin decreases proliferation and induces chromosome territory re-positioning of chromosomes 10 and 18.

- Bioinformatic analyses revealed enrichment for cytokine-cytokine signaling pathway in response to rapamycin, particularly those from the IL-6 signalling cascade.

- STAT5A/B is part of the mechanism driving rapamycin-mediated changes in gene expression.
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