Nano-Enhanced Epigenetics Research (NE2R) in Nebraska
Key Step in Controlling Genetic Information is the Transcription of the Genome
Key Genomic Data to be Obtained for Genomic Response to Stress

- TF and their binding sites
- Histone Modifications and modifying enzymes
- Chromatin remodeling complexes
The complexity of genomes of multicellular organisms

The System:

- 20,000 – 40,000 genes.
- ~ Two thousand transcription factors (TFs).
- Hundreds of thousands of potential TF binding sites (TFBSs).
- Many complex interactions of proteins with DNA, with other proteins, and post-translational protein modifications across the whole genome.

**Approach:** Combined large-scale experimental genomics data and large-scale, sophisticated data analysis, complex network models

**Extremely limited whole genome chromatin data for any species other than yeast.**
The Interactive Research System

Comparative Analysis: ANIMAL MODELS
Nutrition/Development

Primary Relevance: PLANT MODEL
Agricultural

System: Cellular Stress Responses

Specific Aims:
1. Chromatin Expression Networks
2. Chromatin Structural Analyses
3. Targeted Intracellular Chromatin Manipulations

Tools:
- Microarray and Computational Capacity
- Crystallography
  - Atomic Force Microscopy
  - 2-Photon Laser Confocal
- Nano-Engineered Delivery Systems

Researchers:
- Avramova
- Cerutti
- Christman
- Fromm
- Ladunga
- Mackenzie
- Soukup
- Viljoen
- Wang
- Zempleni
- Avramova
- Borgstahl
- Cerutti
- Christman
- Fromm
- Hallworth
- Lyubchenko
- Zempleni
- Avramova
- Baesu
- Dzenis
- Gladyshev
- Hallworth
- Mackenzie
- Subramanian
- Turner
- Comparative Genomics (new)
- Epigenetics (new)
- Chromatin Remodeling (new)
- Systems Biology (new)
- Cell Biomechanics (new)
- Nanodevice Engineering (new)
Building new interactions:

- Nano-technologies for targeted cellular manipulations
- Animal Biology
- Plant Biology
- Chromatin Analytical Technologies
ChIP: Chromatin Immunoprecipitation (ChIP)

Specific histone modification or Bound Protein of interest

Crosslink protein to DNA and fragment DNA

Specific Antibody

Immunoprecipitate with antibodies to target modification or bound protein

Enriched chromatin after immunoprecipitation
ChIP-chip: Chromatin Immunoprecipitation (ChIP) hybridized to microarrays (chip)

Enriched chromatin after immunoprecipitation

Solexa massively parallel DNA sequencing system:
- attachment of randomly fragmented genomic DNA to a planar surface
- amplification to create an ultra-high density sequencing unit with > 10 million clusters, each with ~1,000 copies of template per sq. cm.
- templates are sequenced using four-color DNA sequencing-by-synthesis technology with reversible, fluorescent terminators
Computational biology and Predictive modeling

- Chromatin Biology
- Biomolecular Engineering
- Molecular Analytical Technologies

Outstanding opportunities for innovative student training
Broadened faculty expertise

- Bin Yu, PSI and School of Biological Sciences: Small RNA biology, epigenetic processes in Arabidopsis
- Jeff Mower, PSI and Department of Agronomy/Horticulture: Plant comparative genomics and genome evolution
- David Holding, PSI and Department of Agronomy/Horticulture: Plant protein biochemistry and quantitative genetics
- Chi Zhang, PSI and School of Biological Sciences: Computational biology
- Jung Yul Lim, Engineering Biomechanics
Enhanced local grantsmanship and infrastructure

- Next generation Ilumina sequencing capability, NSF-MRI awarded, Fromm as PI
- NSF-REU in interdisciplinary research, awarded
- NSF-IGERT, full proposal pending, Mackenzie
- NSF Plant Genome Research Program Mackenzie as PI, Wang as coPI
- New RO1, renewed RO1, new R21, Zempleni
- Environmental Electron Microscopy
- Rapid Atomic Force Microscopy (pending)
Schematics for nucleosome particles with different number of DNA supercoiling turns around the histone core

<table>
<thead>
<tr>
<th>Nucleosome schematics</th>
<th>1</th>
<th>1.25</th>
<th>1.5</th>
<th>1.75</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of DNA turns</td>
<td>0</td>
<td>90</td>
<td>180</td>
<td>270</td>
<td>360</td>
</tr>
<tr>
<td>Rotation angle for long arm, deg</td>
<td>0</td>
<td>90</td>
<td>180</td>
<td>270</td>
<td>360</td>
</tr>
<tr>
<td>Length of wrapped DNA, bp</td>
<td>86</td>
<td>108</td>
<td>130</td>
<td>151</td>
<td>173</td>
</tr>
</tbody>
</table>
AFM image taken in air of the nucleosome sample deposited and dried on APS-mica.

Nucleosomes marked 1, 2 and 3 have about 1.7, 1.4 and 1.0 turns of DNA wrapped around the core particle.
Research Advancements

Avramova: Effects of ATX1 on chromatin modification patterns

Cerutti: novel, plant-specific Ser/Thr protein kinase (MUT9) that modifies Thr3 of histone H3 (H3T3ph)

Fromm: whole-genome chromatin analysis, responses to drought

Zempleni/Lyubchenko: AFM analysis of H4 biotinylation influences on nucleosome structure

Wang: statistical methods related to second generation sequencing technology and work flow design for analyzing data from ChIP-seq and RNA-seq experiments

Zempleni/Borghstahl: successful expression of holocarboxylase synthetase
Center for Plant Science Innovation at
University of Nebraska

- Metabolic Biochemistry
- Abiotic and Biotic Stress
- Agricultural Biotechnology & Crop Transgene Production Technologies
- Cellular Genetics/Genomics
- Evolution and Computational Biology
Center for Plant Science Innovation at University of Nebraska

Agricultural Biotechnology & Crop Transgene Production Technologies

- Engineering of biofuels from crops and algae
- Genetic engineering and testing of agricultural traits
- Intl ag crops
- Systems biology
- Plant evolution, bioinformatics, computational biology
- Modern plant breeding & genomics
Center for Plant Science Innovation at University of Nebraska

EPSCoR Funding 2004: Nutritional Genomics and Metabolic Biochemistry

EPSCoR Funding 2007: Epigenetics, Engineering and Computation

Future Directions:
- Algal Biofuels
- Student Training in modern plant genomics

Agricultural Biotechnology

Intl ag crops

Systems biology

Genetic engineering and testing of agricultural traits

Plant evolution, bioinformatics, computational biology

Production Technologies
Future Directions

Nutritional Genomics and Bioenergy
– Crops improved for nutrition and bioenergy potential
  • Novel lipid composition
  • Discovery of novel metabolic intermediates
  • Modified growth patterns
  • New sources (algae)

Integration with Engineering, nutrigenomics, biofuels development
Future Directions

New plant signaling strategies

– Phytohormone and pathogen research
– ROS signaling
– RNA and epigenetic signaling

Expansion to student training effort