Basic adaptation of perennial plants to environment
Determines species range
Subject to climate change

Seasonal dormancy

- Photoperiod
- Temperature
- Water
- Carbohydrates
- Hormones
  - Auxin
  - Gibberellic acid
  - Abscisic acid
  - Ethylene

Factors affecting dormancy

- Photoperiod sensing
- Temperature sensing
- Cellular signalling
- Growth regulation/hormonal signalling
- Epigenetic modification
  - Chromatin remodelling
  - 5-methylcytosine (5mC)

Questions

- How does gene expression change over the course of bud dormancy?
- How does the epigenome – specifically DNA methylation (5mC) – change during bud dormancy?
- How does 5mC content of different gene features affect gene expression?
Approach

- *Populus trichocarpa* (Nisqually-1) vegetative lateral buds (5th or 4th node below terminal) sampled in August, December, March (3 biological reps)
- Assess genome-wide cytosine DNA methylation (5mC) using MeDIP-seq
- Analyze transcriptome using RNA-seq with same samples

Results: outline

- Gene expression
- DNA methylation (5-methylcytosine, 5mC)
- Gene expression-5mC relationship
- Candidate regulatory gene examples
  > expression trends
  > 5mC trends

Distinctive seasonal gene expression profiles

- Spring buds (ecodormant) have more genes in the 1-5 FPKM range.
- Fall (paradormant) and winter (endodormant) buds have more genes with >= 10 FPKM.

Bioinformatic analysis

RNA-seq
- Illumina HiSeq1000 (2 x 100 bp)
- TopHat/CuffDiff for ID of differentially-expressed genes (*P. trichocarpa* V3 genome assembly)

MeDIP-seq
- Illumina GAIIx (1 x 36 bp)
- Methylation-enriched genome regions (1 kb tiled windows, gene promoters, gene bodies) determined by comparison to non-IP control @ 10% FDR (*P. trichocarpa* V2.2 genome assembly)

Gene expression

Transcriptome changes over the course of seasonal dormancy

ENDO with largest number of differentially-expressed genes

Pairwise comparisons
**Cluster 2: predominant in PARADORMANCY**

- Gene Ontology overrepresentation: 16 categories related to lipid metabolism
- 286 genes

**Cluster 3: predominant in ENDODORMANCY**

- Gene Ontology overrepresentation: 92 categories
- 653 genes
- Abiotic stress response
- Carbohydrate catabolism
- Transcriptional regulation

**Cluster 3: GO overrepresentation (biological process)**

- Carbohydrate metabolism
- Abiotic stress response
- Transcriptional regulation

**Cluster 3: predominant in ENDODORMANCY**

- Examples of other categories not overrepresented, but relevant to dormancy:
Cluster 4: predominant in ECODORMANCY

No overrepresented Gene Ontology categories

Examples of genes in this cluster:
- Potri.005G244100 similar to Alpha-expansin
- Potri.008G163900 similar to Transcriptional regulator SUPERMAN
- Potri.013G053200 similar to Syntaxin-related protein KNOLLE (Syntaxin 111)
- Potri.006G142600 nutrient reservoir activity
- Potri.007G013500 similar to histone H4 from Lycopersicon esculentum

Examples of genes in this cluster:
- 5mC changes during dormancy transitions
- MeDIP-seq
  - Illumina GAIIx (1 x 36 bp)
  - Methylation-enriched genome regions (1 kb tiled windows, gene promoters, gene bodies) determined by comparison to non-IP control @ 10% FDR (P. trichocarpa V2.2 genome assembly)

Promoters, gene bodies have similar 5mC trends

No clear gene expression – PROMOTER 5mC relationship among season predominant clusters

Cytosine DNA methylation

5mC changes during dormancy transitions

Genome 5mC has no consistent seasonal trends

• 3 biological replicates/ season
• Large variance

607 597 574
1484 1113 1012

ENDO PARA ECO
No clear gene expression – GENE BODY 5mC relationship among season predominant clusters

Promoter and gene body 5mC – expression correlation weak or absent

Data from all seasons pooled
• Higher gene expression weakly correlated ($r = -0.17$) with lower promoter 5mC in rank-based correlation tests ($p=0$)
• No correlation between gene body 5mC and gene expression ($p=0.52$).

Identification of candidate regulatory genes

FORWARD APPROACH
• Hierarchical clustering of differentially-expressed genes
• Lists of season-predominant genes
• Examination of expression vs. methylation

REVERSE APPROACH
• Genes with known or putative roles in dormancy in Populus spp. or other plant species
• Poplar homologs
• Examination of expression vs. methylation

~1100 genes ~100 genes

Candidate regulatory genes

PARA-predominant

ENDO-predominant

APETALA2 (AP2) / ETHYLENE-RESPONSIVE ELEMENT BINDING PROTEIN (EREBP)

Transcription factors
• Unique to plants
• 37 annotated in P. trichocarpa V3)
• Regulation of multiple processes
  >Dormancy induction
  >Drought/freezing tolerance

AP2 Expression-5mC comparison
PARA-predominant

5mC

solid line= promoter, dotted line= gene body
Summary

- **Future work**: statistical evaluation of methylome variation, further interrogation of candidate dormancy-associated genes
- **Extensive transcriptome reorganization during bud dormancy**
- **Lipid, carbohydrate metabolism extensively reconfigured**
- **Distinct expression profile in endodormancy relative to paradormancy and ecodormancy**
- **Largest number of genes (653) upregulated during endodormancy**
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