Extended E-Motifs and Cytosine Accessibility

Kathryn Iverson
SoCalBSI 2008

Dr. Ian Haworth and Dr. Rebecca Romero
University of Southern California
School of Pharmacy
Outline

- **E-Motif Structure**
  - Dynamics simulation
  - Results
- **Cytosine accessibility**
  - Methods
  - Future directions
Fragile X

One of the most common forms of inherited mental retardation. 1 in 2000 boys and 1 in 4000 girls are afflicted and 1 in 260 women are carriers.

Characterized by the expansion of GCC repeats in the 5’ untranslated region of the FMR1 gene

No synthesis of FMR1 protein, perhaps because of hairpins forming in DNA due to extended GCC repeats and possible E-Motif structure

http://www.conquerfragilex.org/about.php
Hairpins Formed by GCC Repeats

Mismatched cytosines fold back in the 5’ direction

Cytosines may interact extra-helically, as demonstrated by mechlorethamine cross-linking

Stacking of G-C base pairs in the helix

1,4
Conformation
1,7 Conformation
## GCC Hairpin Stability

<table>
<thead>
<tr>
<th></th>
<th>- Mechlorethamine</th>
<th>+ Mechlorethamine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High pH (~8)</strong></td>
<td><strong>Intra-helical ↔ Extra-helical</strong></td>
<td><strong>1,4 Cross-linked Extra-helical Cytosines</strong></td>
</tr>
<tr>
<td><strong>Low pH (7.5)</strong></td>
<td><strong>1,7 Protonated Extra-helical Cytosines</strong></td>
<td><strong>No Cross-linking</strong></td>
</tr>
</tbody>
</table>

**Methods**

- Use the E-Motif Creator to generate PDB files
- Calculate restraints for each structure in both the 1,4 and 1,7 conformations
- Run dynamics simulations with AMBER using constraints
Results

1,7 mismatched cytosines come close enough to interact

Mismatched cytosines in minor grove

1,4 structure may be a transient structure that is “captured” by mechlorethamine
Cytosine Accessibility in DNA
Bisulfite Probes

- **Probe DNA structure**
- **Experimentally detect cytosine accessibility in E-Motifs**
- **Extra-helical cytosines would be hyper-reactive**
- **Useful in methylation analysis**
ACCESSIBILITY ALGORITHMS

- Looking at accessibility of cytosines to bisulfite probes
- Detection of bisulfite interactions
- Probe many different DNA structures
- Building an expandable framework for future projects
Coiled DNA

- DNA around the histone
- Looking at accessibility of cytosines to bisulfite probes
Methods

Read in sequence

Coil → NASDAC → Moltool

Format output
Moltool

- Calculates all potential bisulfite interaction geometries for a given cytosine
- Run for each cytosine in the sequence
Future Directions

- Run on many sequences
- Look for patterns within and between sequences
- Identify possible sites of methylation
Acknowledgements

Thank you to Dr. Ian Haworth and Dr. Rebecca Romero for their guidance and advice throughout the project.

USC Health Sciences Center and School of Pharmacy

Program leaders and faculty of SoCalBSI

SoCalBSI participants

Cal State LA

Funding provided by:

The National Science Foundation

The National Institute of Health

Economic and Workforce Development
Questions?