SESSION 7. EVOLUTION

What makes us human?

Genetic differences b/w humans and chimpanzees

Genome sequencing were completed in multiple species including human, chimpanzee, dog, pig, cow, … and can be compared in similarity and differences b/w genomes.

Advanced Human Abilities: Advances spoken and written language, abstract-level and creative thinking (concept generation), and reasoning.

→ What are significant genetic differences b/w us and primates?

→ The genetic elements are likely to affect brain function

Genetic differences b/w humans and chimpanzees

Humans vs Chimpanzees

35million single nucleotide differences / 3 billions ~ 1% differences

→ point mutations could affect regulation of transcription and processes such as splicing as well as affect protein sequence.

5 million indels ~ 3% difference

→ regulation of gene expression

Structural variations ~ ? % i.g., duplicated paralogs with a new function

➔ Such mutations may also be of interest when learning about functions that are specific to humans

Identification of genes with a function specific to humans

- Genes or protein sequences that are conserved during vertebrate or mammalian evolution, but where the human sequences have evolved at an unexpectedly high rate.
- **Noncoding RNA, HAR1F** (human accelerated region) is specifically expressed in the developing human neocortex (involved in function such as conscious thought and language).

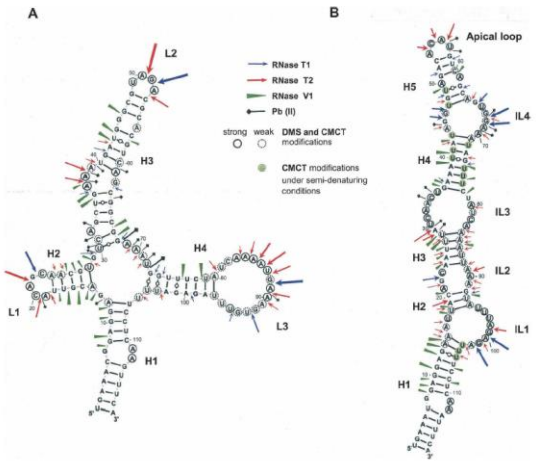
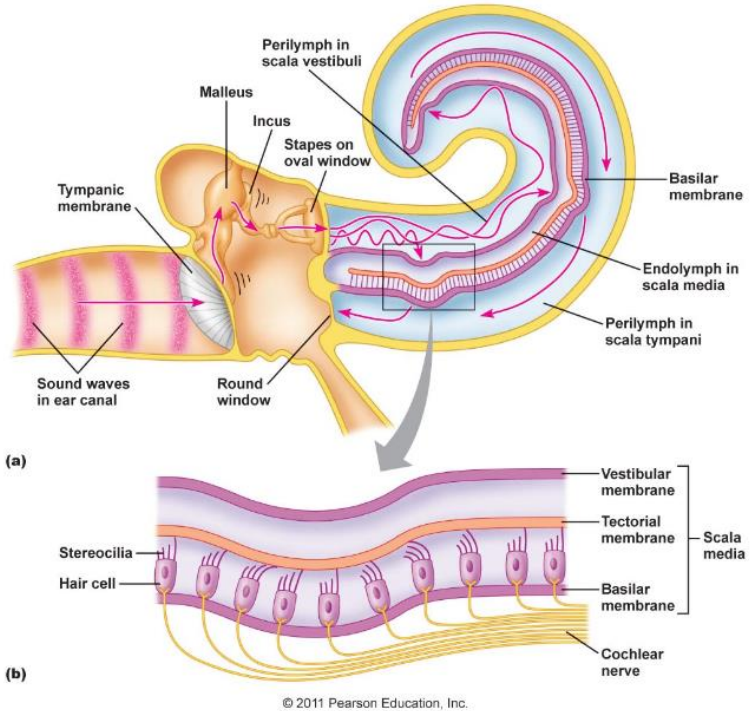


FIGURE 3. Two distinct experimentally supported secondary structure models for HAR1 RNAs. (*A*) The doverleaf-like model of the human HAR1 RNA. (*B*) The chimpanzee HAR1 RNA adopts a hairpin structure. The length and thickness of the symbols represent the intensity of the cleavages. Bases reactive to DMS or CMCT under native conditions are circled; weak reactivities are depicted by dotted circles. Bases modified by CMCT under semidenaturing conditions only are displayed with a green background. H, helix; IL, internal loop; L, loop.

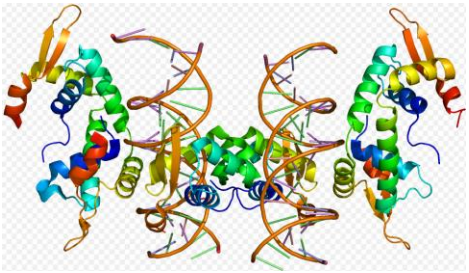
Identification of genes with a function specific to humans

- **Alpha tectorin** is specifically developed in the human lineage and localized in the tectorial membrane of the inner ear.
- Specialized or adjusted to human-specific development of speech.



A protein related to human speech: FOXP2

- **A protein related to human speech: FOXP2 gene**
- Foxp2 contains a forkhead box DNA binding domain, is expressed in CNS during development, and is likely to regulate a number of genes of importance for brain function.
- Foxp2 is fairly well conserved in all vertebrates and mutations in the Foxp2 gene cause speech deficiency.
- **R553H** in the Foxp2 is aa change in the DNA binding domain and **R328X** is nonsense mutation.
- Both copies are required for normal function (**dominant phenotype**).



FOXP2 in other animals

- FOXP2 seems to be related in synaptic plasticity (for motor-skill learning).
- Bird song has similarities and differences to human speech.
- Expression of FOXP2 in zebra finch was reduced to 50% of its normal level and the bird is not efficient for sound-imitation.

Comparing FOXP2 in different animals

- We first have to know which mutations affect the function of FOXP2 in neural development, synaptic plasticity, and speech.
- **Multiple sequence alignment** identifies mutations across different vertebrates.

FOXP2_HUMAN
FOXP2_GORGO (Gorilla)
FOXP2_MACMU (Rhesus mamaque)
FOXP2_PANTR (Chimpanzee)
FOXP2_HYLLA (Common gibbon)
FOXP2_PONPY (Orangutan)
FOXP2_MOUSE (mouse)
FOXP2_XENLA (African frog)

foxp2.fa

Multiple sequence alignment

- Global alignment for more than two sequences
- **ClustalW,** Muscle, and T-coffee for MSA
- ClustalW: based on pairwise alignments of each pair – **Progressive approach**

  step-wise procedure

  → 1) guide-tree construction from pairwise alignments

  → 2) Single + Subalignment

```
[jwnam@biglab-master Session7]$ clustalw2 foxp2.fa


 CLUSTAL 2.1 Multiple Sequence Alignments


Sequence format is Pearson
Sequence 1: FOXP2_HUMAN   715 aa
Sequence 2: FOXP2_GORGO   713 aa
Sequence 3: FOXP2_MACMU   714 aa
Sequence 4: FOXP2_PANTR   716 aa
Sequence 5: FOXP2_HYLLA   713 aa
Sequence 6: FOXP2_PONPY   713 aa
Sequence 7: FOXP2_MOUSE   714 aa
Sequence 8: FOXP2_XENLA   706 aa
Start of Pairwise alignments
Aligning...

Sequences (1:2) Aligned. Score:  99
Sequences (1:3) Aligned. Score:  99
Sequences (1:4) Aligned. Score:  99
Sequences (1:5) Aligned. Score:  99
Sequences (1:6) Aligned. Score:  99
Sequences (1:7) Aligned. Score:  99
Sequences (1:8) Aligned. Score:  95
Sequences (2:3) Aligned. Score:  100
Sequences (2:4) Aligned. Score:  100
Sequences (2:5) Aligned. Score:  98
Sequences (2:6) Aligned. Score:  98
Sequences (2:7) Aligned. Score:  99
Sequences (2:8) Aligned. Score:  95
Sequences (3:4) Aligned. Score:  100
Sequences (3:5) Aligned. Score:  100
Sequences (3:6) Aligned. Score:  99
Sequences (3:7) Aligned. Score:  98
Sequences (3:8) Aligned. Score:  95
Sequences (4:5) Aligned. Score:  100
Sequences (4:6) Aligned. Score:  99
Sequences (4:7) Aligned. Score:  99
Sequences (4:8) Aligned. Score:  95
Sequences (5:6) Aligned. Score:  99
Sequences (5:7) Aligned. Score:  99
Sequences (5:8) Aligned. Score:  95
Sequences (6:7) Aligned. Score:  99
Sequences (6:8) Aligned. Score:  95
Sequences (7:8) Aligned. Score:  95
Guide tree file created:   [foxp2.dnd]

There are 7 groups
Start of Multiple Alignment

Aligning...
Group 1: Sequences:   2      Score:15411
Group 2: Sequences:   3      Score:15416
Group 3: Sequences:   4      Score:15408
Group 4: Sequences:   2      Score:15421
Group 5: Sequences:   3      Score:15400
Group 6: Sequences:   7      Score:15379
Group 7: Sequences:   8      Score:14978
Alignment Score 118874

CLUSTAL-Alignment file created  [foxp2.aln]
```

MSA for FOXP2

- FOXP2.aln

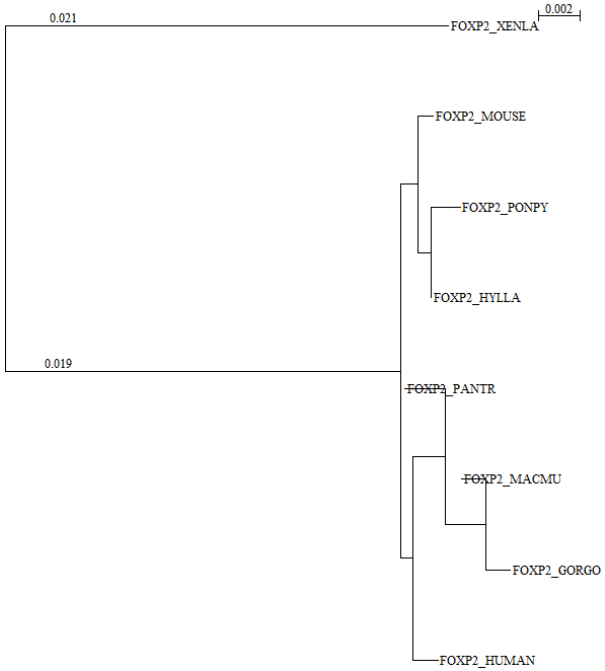
```
CLUSTAL 2.1 multiple sequence alignment


FOXP2_GORGO      MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
FOXP2_MACMU      MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
FOXP2_PANTR      MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
FOXP2_HUMAN      MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
FOXP2_HYLLA      MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
FOXP2_PONPY      MMQESVTETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
FOXP2_MOUSE      MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
FOXP2_XENLA      MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSSDTSSEVSTVELLHLQQQQALQA
                 *****.*******************************.**********************


FOXP2_GORGO      ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
FOXP2_MACMU      ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
FOXP2_PANTR      ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
FOXP2_HUMAN      ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
FOXP2_HYLLA      ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
FOXP2_PONPY      ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
FOXP2_MOUSE      ARQLLLQQQTSGLKSPKSSEKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
FOXP2_XENLA      ARQLLLQQQTSGLKSPKNNEKQRPLQVPVSMAMMTPQVITPQQMQQILQQQVLSPQQLQA
                 ****************..:**********:****************************


FOXP2_GORGO      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
FOXP2_MACMU      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
FOXP2_PANTR      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
FOXP2_HUMAN      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
FOXP2_HYLLA      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
FOXP2_PONPY      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
FOXP2_MOUSE      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
FOXP2_XENLA      LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
                 ************************************************************
```

- FOXP2.dnd

```
(
(
(
FOXP2_HUMAN:0.00122,
(
(
FOXP2_GORGO:0.00117,
FOXP2_MACMU:-0.00117)
:0.00196,
FOXP2_PANTR:-0.00196)
:0.00157)
:0.00059,
FOXP2_XENLA:0.04051)
:0.00083,
(
FOXP2_HYLLA:0.00000,
FOXP2_PONPY:0.00140)
:0.00066,
FOXP2_MOUSE:0.00074);
```

Phylogenetic tree from dendrogram

- NJPlot: http://doua.prabi.fr/software/njplot



foxp2.py: identification of mutation specific to human

clustalw2 foxp2.fa -output=fasta → foxp2.fasta

```
>FOXP2_GORGO
MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
QQQQQQQQQ---HPGKQAKEQQQQQQQQQQLAAQQLVFQQQLLQMQQLQQQQHLLSLQRQ
GLISIPPGQAALPVQSLPQAGLSPAEIQQLWKEVTGVHSMEDNGIKHGGLDLTTNNSSST
TSSTTSKASPPITHHSIVNGQSSVLNARRDSSSHEETGASHTLYGHGVCKWPGCESICED
FGQFLKHLNNEHALDDRSTAQCRVQMQVVQQLEIQLSKERERLQAMMTHLHMRPSEPKPS
PKPLNLVSSVTMSKNMLETSPQSLPQTPTTPTAPVTPITQGPSVITPASVPNVGAIRRRH
SDKYNIPMSSEIAPNYEFYKNADVRPPFTYATLIRQAIMESSDRQLTLNEIYSWFTRTFA
YFRRNAATWKNAVRHNLSLHKCFVRVENVKGAVWTVDEVEYQKRRSQKITGSPTLVKNIP
TSLGYGAALNASLQAALAESSLPLLSNPGLINNASSGLLQAVHEDLNGSLDHIDSNGNSS
PGCSPQPHIHSIHVKEEPVIAEDEDCPMSLVTTANHSPELEDDREIEEEPLSEDLE
>FOXP2_MACMU
MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
QQQQQQQQQ--HPGKQAKEQQQQQQQQQQLAAQQLVFQQQLLQMQQLQQQQHLLSLQRQ
GLISIPPGQAALPVQSLPQAGLSPAEIQQLWKEVTGVHSMEDNGIKHGGLDLTTNNSSST
TSSTTSKASPPITHHSIVNGQSSVLNARRDSSSHEETGASHTLYGHGVCKWPGCESICED
FGQFLKHLNNEHALDDRSTAQCRVQMQVVQQLEIQLSKERERLQAMMTHLHMRPSEPKPS
PKPLNLVSSVTMSKNMLETSPQSLPQTPTTPTAPVTPITQGPSVITPASVPNVGAIRRRH
SDKYNIPMSSEIAPNYEFYKNADVRPPFTYATLIRQAIMESSDRQLTLNEIYSWFTRTFA
YFRRNAATWKNAVRHNLSLHKCFVRVENVKGAVWTVDEVEYQKRRSQKITGSPTLVKNIP
TSLGYGAALNASLQAALAESSLPLLSNPGLINNASSGLLQAVHEDLNGSLDHIDSNGNSS
PGCSPQPHIHSIHVKEEPVIAEDEDCPMSLVTTANHSPELEDDREIEEEPLSEDLE
>FOXP2_PANTR
MMQESATETISNSSMNQNGMSTLSSQLDAGSRDGRSSGDTSSEVSTVELLHLQQQQALQA
ARQLLLQQQTSGLKSPKSSDKQRPLQVPVSVAMMTPQVITPQQMQQILQQQVLSPQQLQA
LLQQQQAVMLQQQQLQEFYKKQQEQLHLQLLQQQQQQQQQQQQQQQQQQQQQQQQQQQQ
QQQQQQQQQQQHPGKQAKEQQQQQQQQQQLAAQQLVFQQQLLQMQQLQQQQHLLSLQRQ
GLISIPPGQAALPVQSLPQAGLSPAEIQQLWKEVTGVHSMEDNGIKHGGLDLTTNNSSST
TSSTTSKASPPITHHSIVNGQSSVLNARRDSSSHEETGASHTLYGHGVCKWPGCESICED
FGQFLKHLNNEHALDDRSTAQCRVQMQVVQQLEIQLSKERERLQAMMTHLHMRPSEPKPS
PKPLNLVSSVTMSKNMLETSPQSLPQTPTTPTAPVTPITQGPSVITPASVPNVGAIRRRH
SDKYNIPMSSEIAPNYEFYKNADVRPPFTYATLIRQAIMESSDRQLTLNEIYSWFTRTFA
YFRRNAATWKNAVRHNLSLHKCFVRVENVKGAVWTVDEVEYQKRRSQKITGSPTLVKNIP
TSLGYGAALNASLQAALAESSLPLLSNPGLINNASSGLLQAVHEDLNGSLDHIDSNGNSS
PGCSPQPHIHSIHVKEEPVIAEDEDCPMSLVTTANHSPELEDDREIEEEPLSEDLE
```

foxp2.py: identification of mutat

```
At position  304
FOXP2_HUMAN          N
FOXP2_PANTR          T
FOXP2_MOUSE          T
FOXP2_GORGO          T
FOXP2_MACMU          T
FOXP2_PONPY          T
FOXP2_HYLLA          T
FOXP2_XENLA          T
```

```
import re

inFile = open('foxp2.fasta','r')

nonhuman = dict()

id = ''
seq = ''

for line in inFile.readlines():
        line = line.strip()
        #print line
        if re.search('^>', line):
                if id != '':
                        if not 'HUMAN' in id:
                                nonhuman[id[1:]] = seq
                        else:
                                id_human = id[1:]
                                seq_human = seq
                        id = line
                        seq = ''
                else:
                        id = line
        else:
                seq += line
if not 'HUMAN' in id:
        nonhuman[id[1:]] = seq
else:
        id_human = id[1:]
        seq_human = seq

inFile.close()

for i in xrange(0,len(seq_human)):
        unique = 1
        for id in nonhuman.keys():
                if seq_human[i] == nonhuman[id][i]:
                        unique = 0
        if unique:
                pos = i + 1
                print "At position ", pos
                aa = seq_human[i]
                print id_human, '\t', aa
                for ID in nonhuman.keys():
                        print ID,'\t', nonhuman[ID][i]
```

→ Human-specific T303N

- T303N and N325S are positively selected for language and speech
- No N325S in our res. due to *Xenopus*