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The Cartwheel Project

(Python) Tools for Regulatory Genomics

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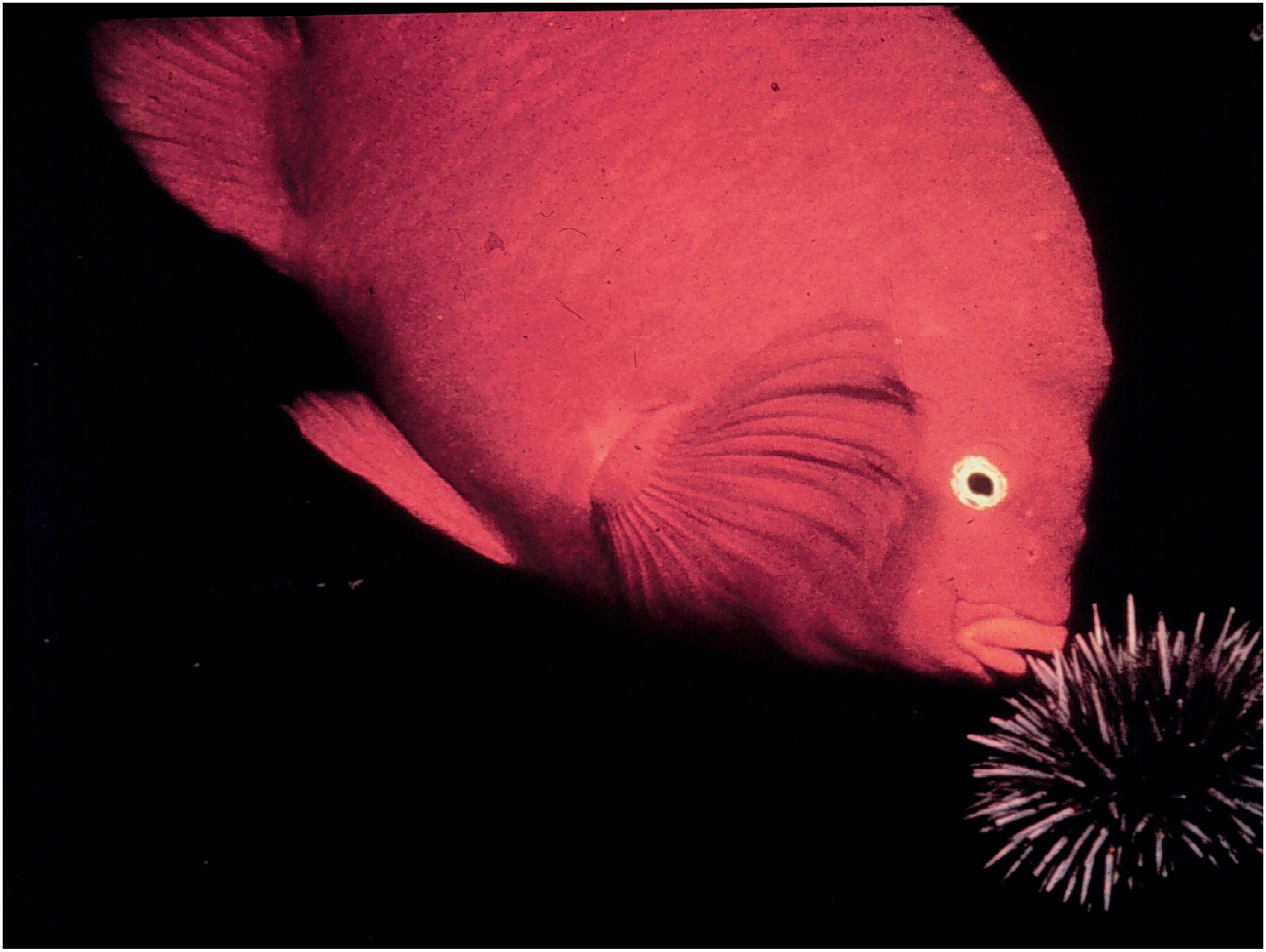
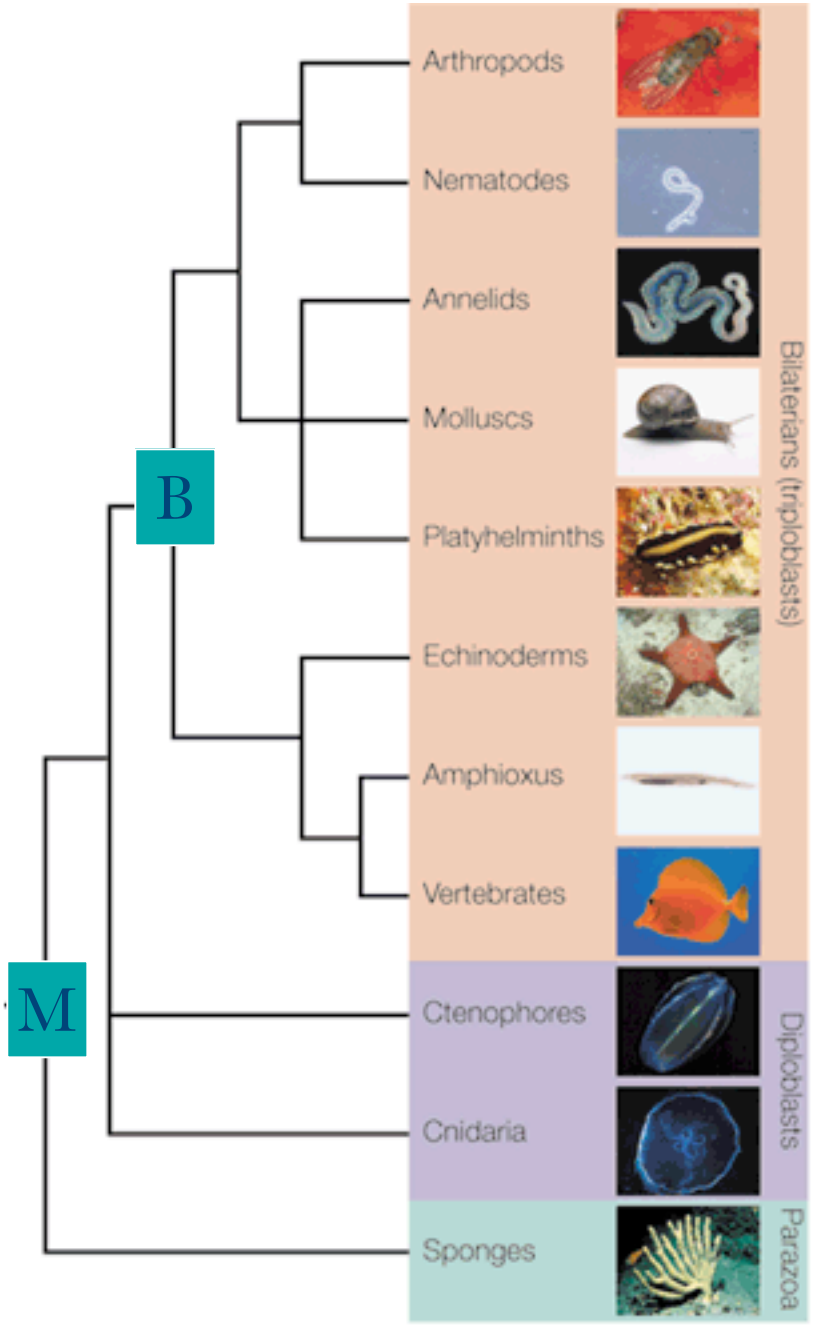
Outline

1. Introduction to regulatory genomics
2. A bioinformatics “in the medium” solution.
3. Digression: project maintenance.
4. Digression: sociological considerations.
5. Moving forward? Future plans.





Fruit fly: 20,000 genes

Worm: 20,000 genes

Sea urchin: 22,000 genes

Most vertebrates, including humans: 25,000-45,000 genes

Unknown, but many genes in common, esp with vertebrates

Nature Reviews | Genetics

You can build a cathedral, …

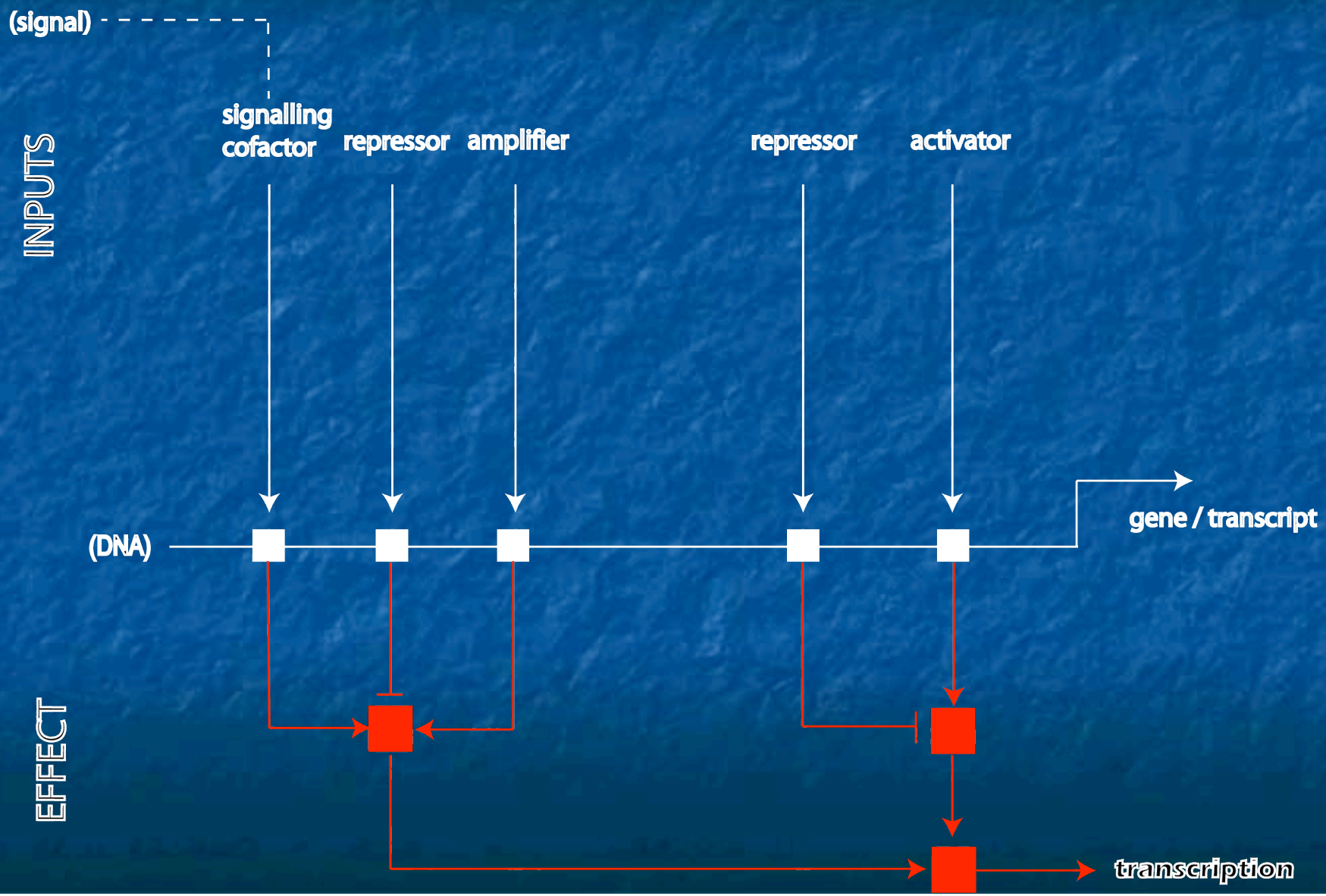
…and a train station, out of much the same material.

…because it's the *architectural plans* that make the difference.

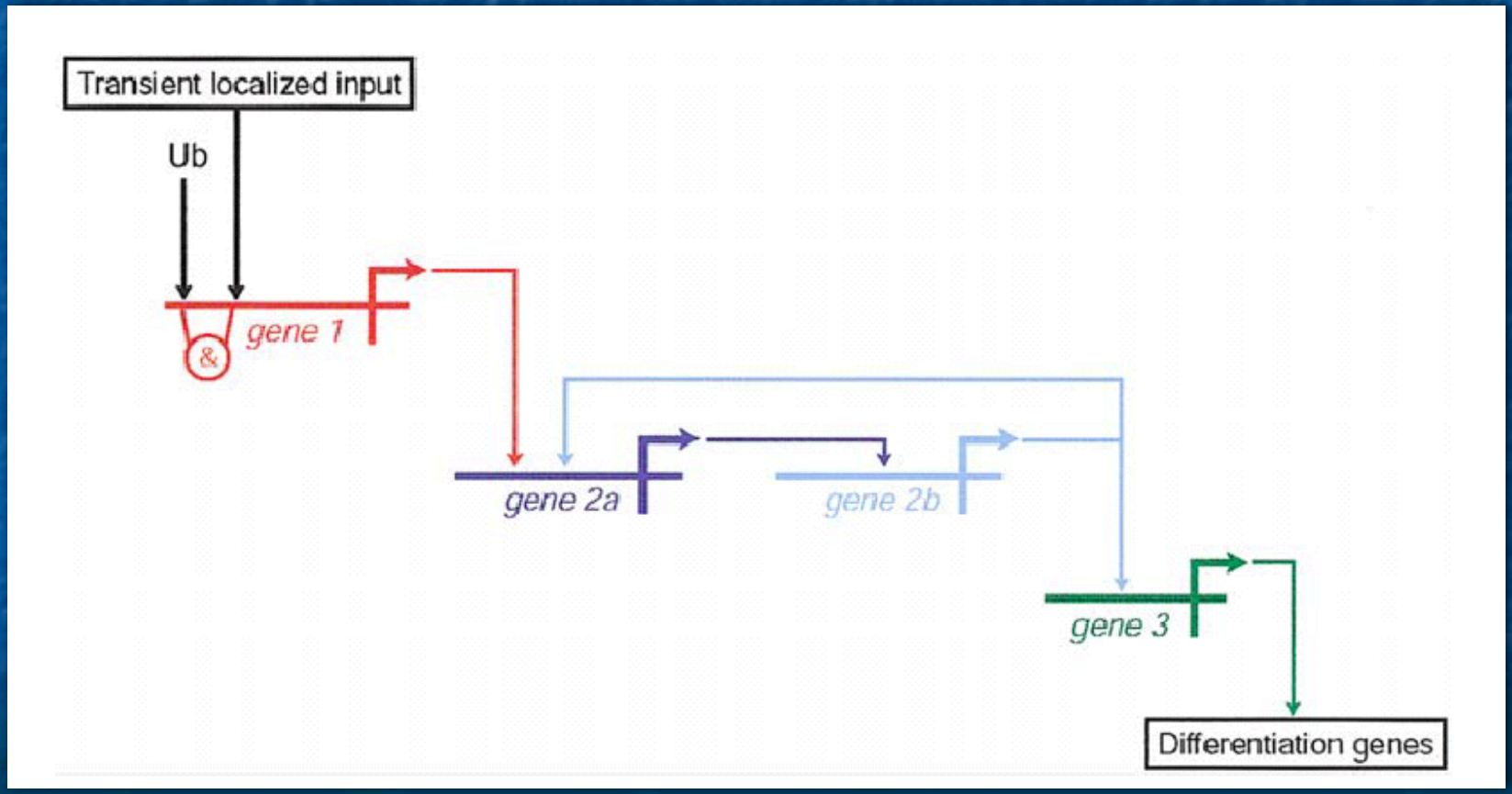
As with buildings, so with bodies: the developmental blue prints for animals are stored in our genomes, in both the genes and the **regulatory regions** that control gene expression.

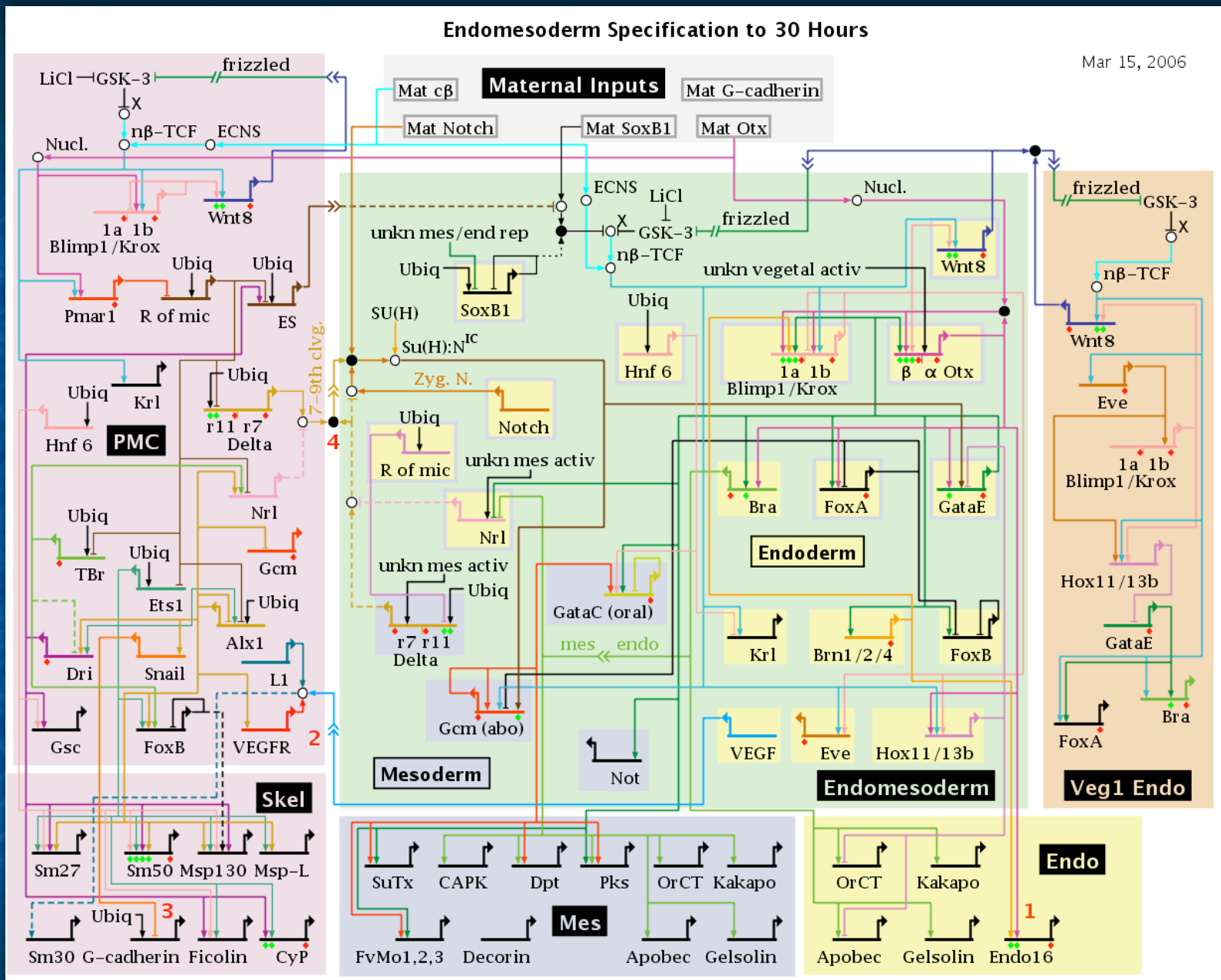
Finding these regulatory regions is important.

Anatomy of a gene regulatory network node

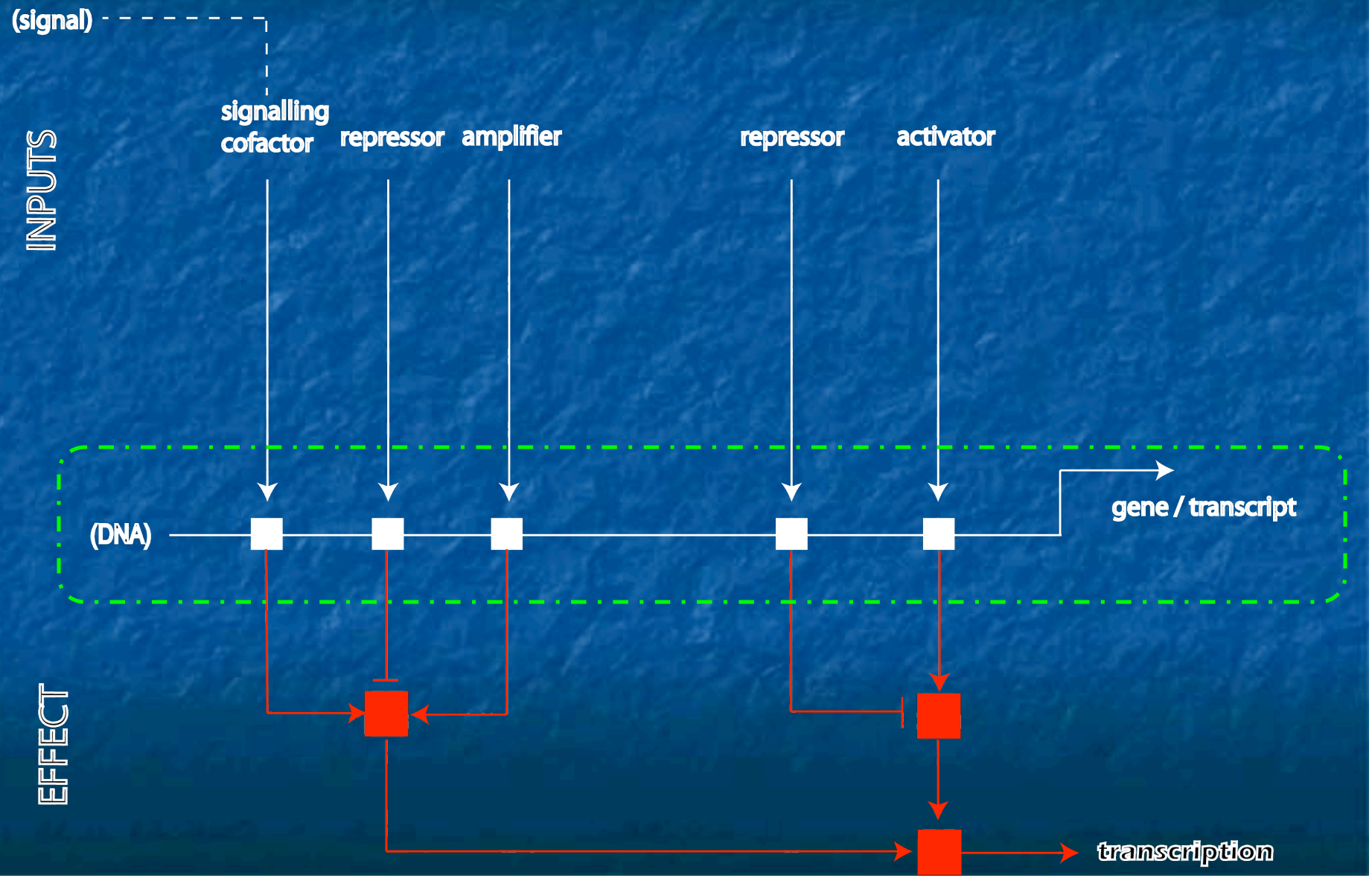


Transitioning to a developmental gene *network*: connecting the genes

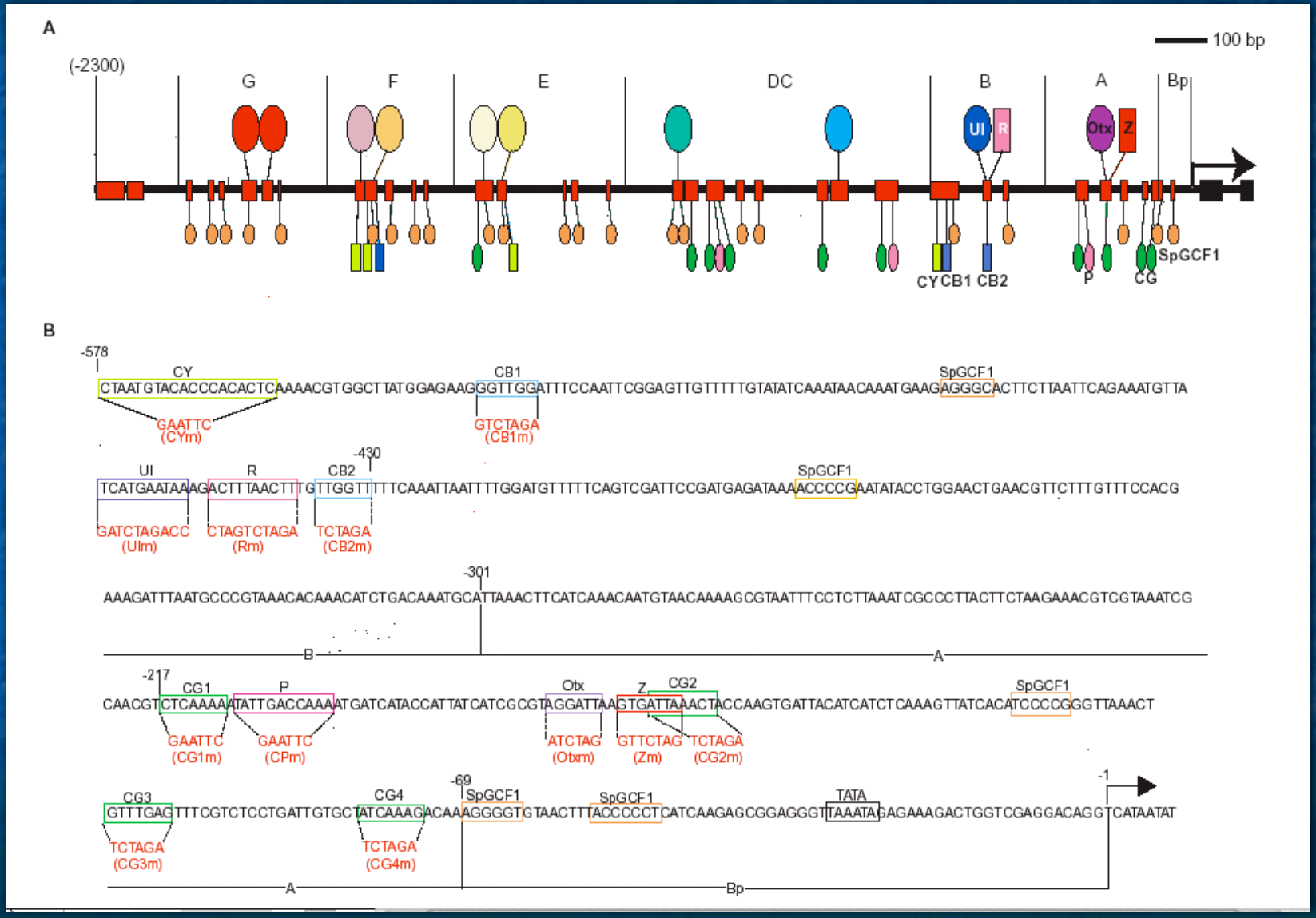




cis-Regulatory regions connect upstream genes to their regulators.



For example, this regulatory region…



Eukaryotic transcriptional initiation involves many general factors, as well as specific enhancers.

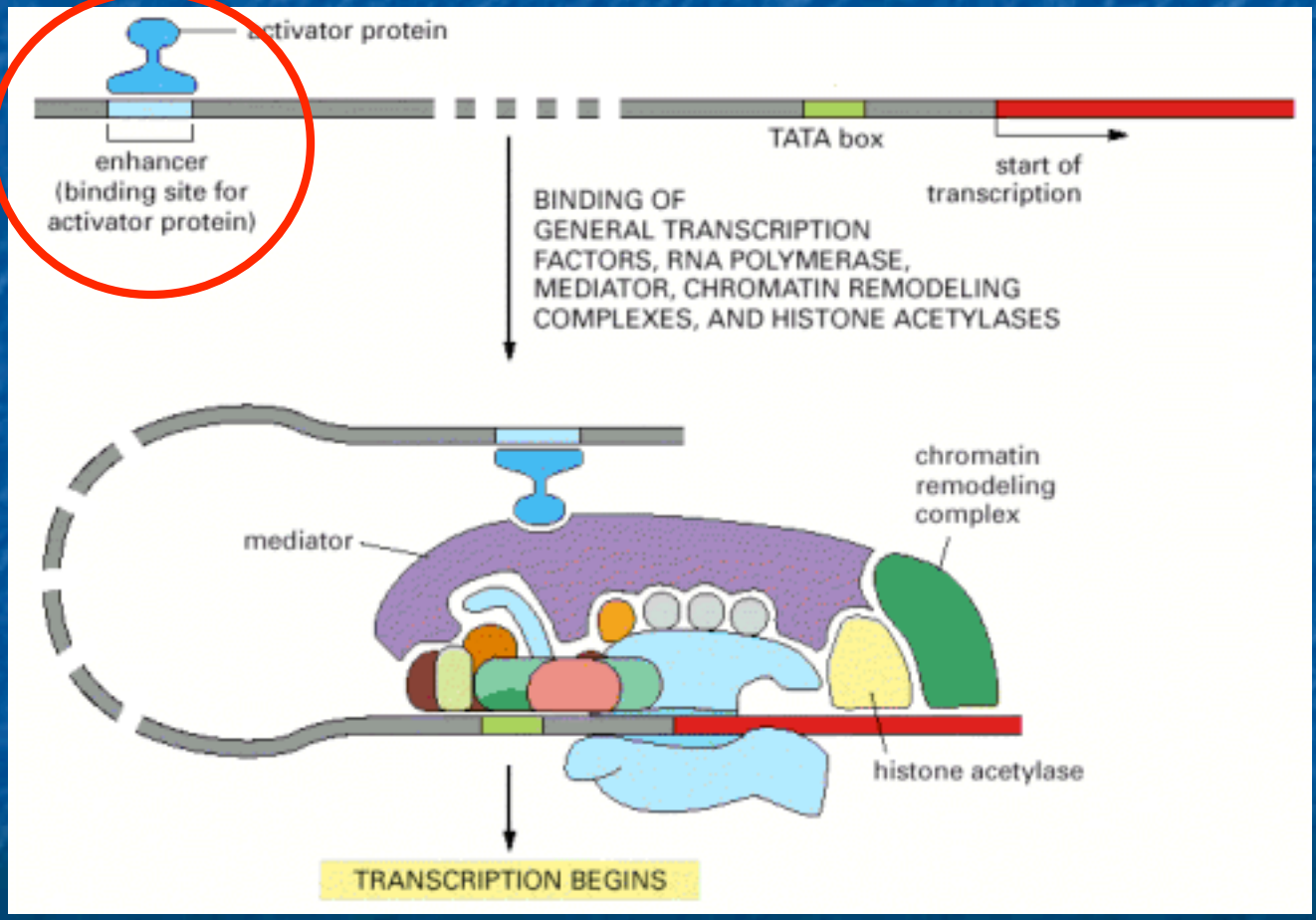
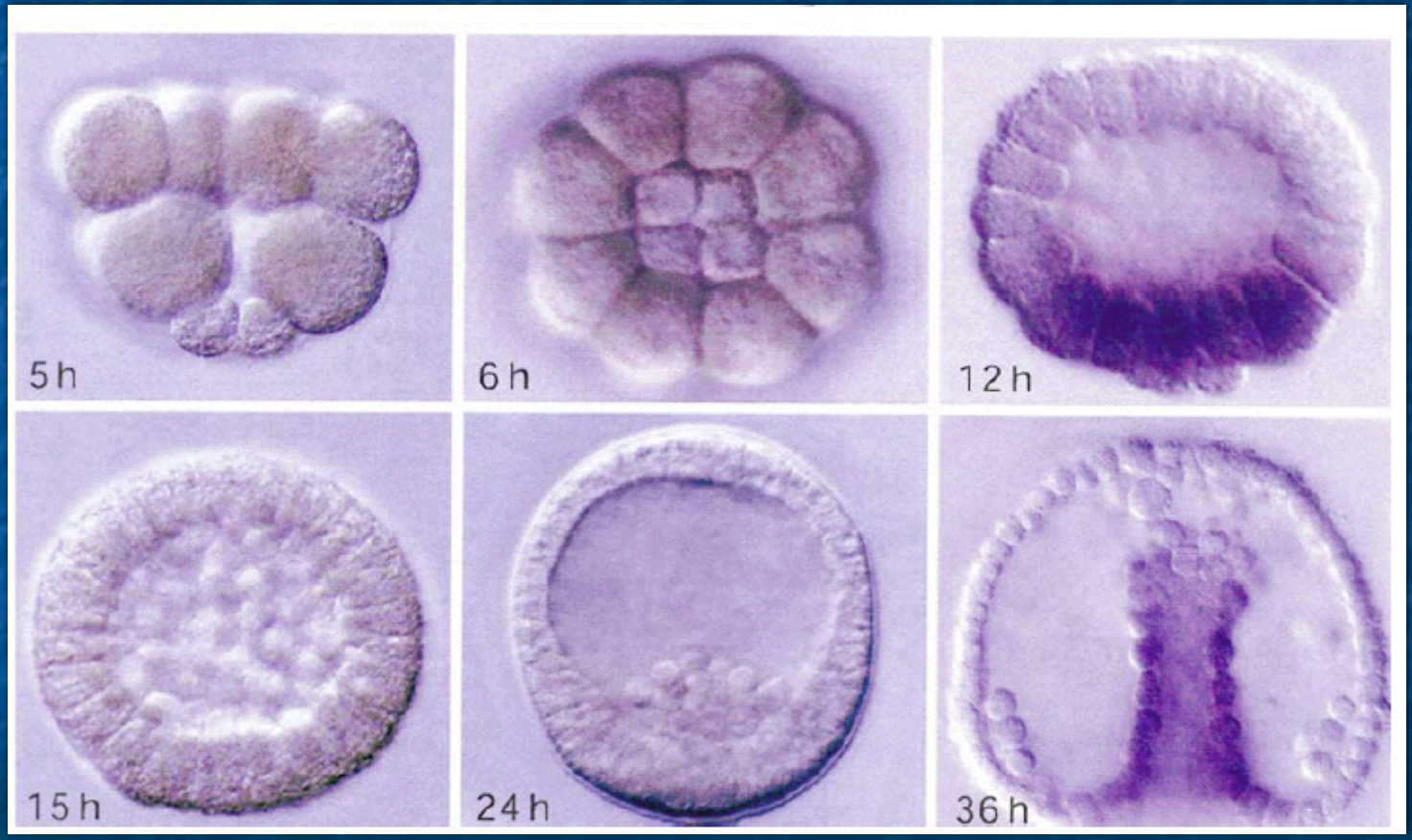
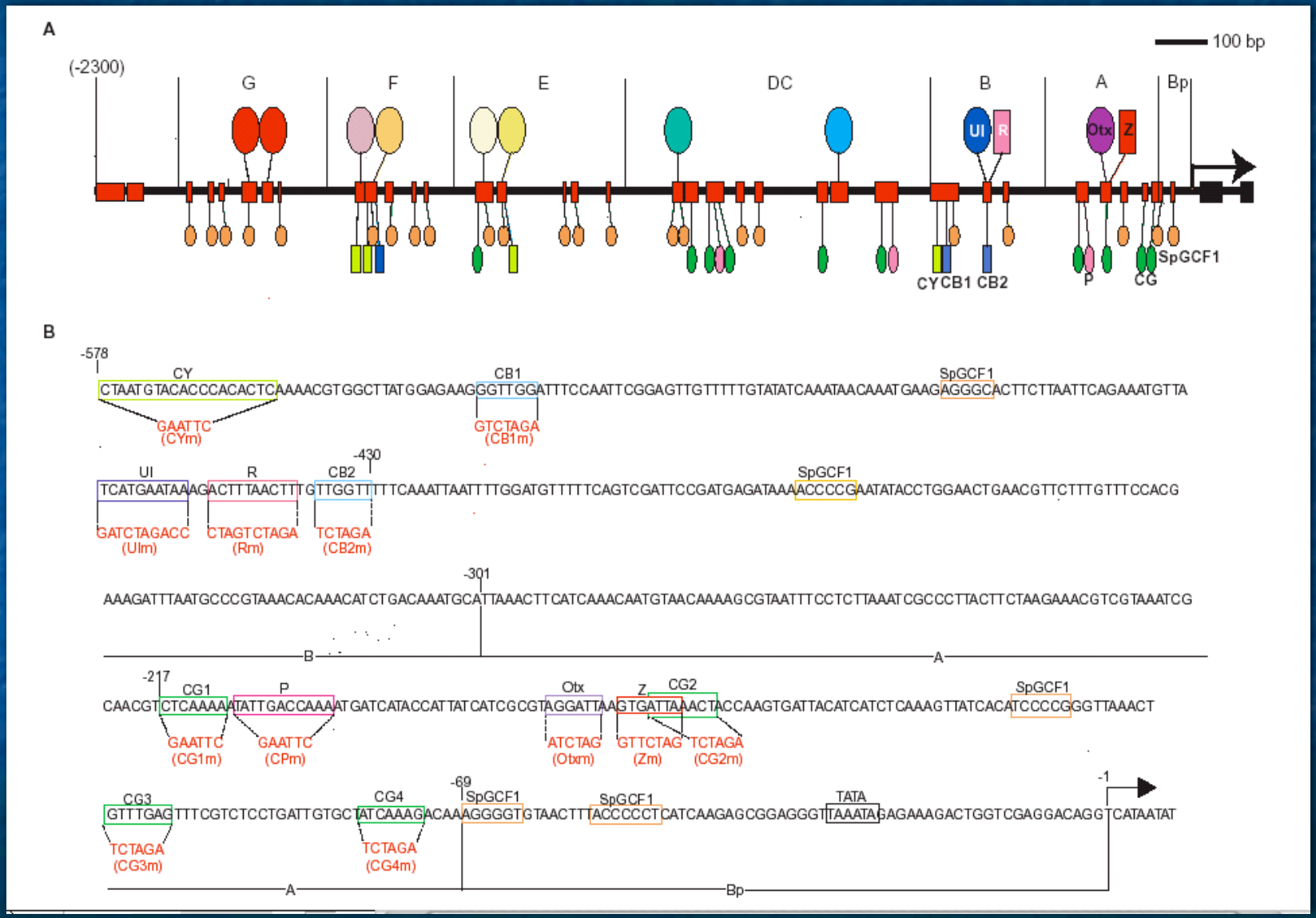


Figure from ***MBOC***

…drives this tissue-specific gene expression in the sea urchin embryo.



What are we looking for?



What are we looking for?

- **Regulatory regions** consist of multiple binding sites that function combinatorially.
- **Binding sites** are 8-30 bp segments of DNA to which transcription factors bind specifically.

Why is this a hard problem?

- Genomes are *big*, regulatory regions are *small*.

  40-80 kb of DNA *per gene*; ~500 bp regulatory regions…

- No obvious statistical signature, unlike protein coding regions.

- No good way to *test* predictions without doing experiments (slow, expensive, difficult).

Comparative sequence analysis

- Look for regions conserved between two or more species.
- Basic logic: if a given gene is expressed in similar places in two organisms, molecular mechanisms (including *sequence)* driving expression may not have diverged.


seqcomp, 50bp -- closeup on (19791, 22214) x (23429, 26358)
+19792
+22215
+19792
+22215
+23430
+26359
TATCAAAATTAAGTAATAATAATTATTATTTTTGTGTCGAATTTCAGGAGTGTTCCTGTGCTCGAAAGGATGCCGAACAAGTTCGGGA
20140
20150
20160
20170
20180
20190
20200
20210
20220
TCACCGAATGATATTTATATTCATTATTTTCTTCATTCTTGTAGGTGTATTTCTGTGTTCGAAAGGATGCCGAACAAGTACGGGGGAC
0
24380
24390
24400
24410
24420
24430
24440
24450

The simplest sequence matching algorithm on the planet:

```
for i in range(0, len(seq1) - w):
    for j in range(0, len(seq2) - w):
        m = count_identity(seq1[i:i+w],
                           seq2[j:j+w])
        if m >= threshold:
            record_match(i, j, m)
        # forget reverse-complement for now
```


seqcomp, 50bp -- closeup on (19791, 22214) x (23429, 26358)
+19792
+22215
+19792
+22215
+23430
+26359
TATCAAAATTAAGTAATAATAATTATTATTTTTGTGTCGAATTTCAGGAGTGTTCCTGTGCTCGAAAGGATGCCGAACAAGTTCGGGA
20140
20150
20160
20170
20180
20190
20200
20210
20220
TCACCGAATGATATTTATATTCATTATTTTCTTCATTCTTGTAGGTGTATTTCTGTGTTCGAAAGGATGCCGAACAAGTACGGGGGAC
0
24380
24390
24400
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24430
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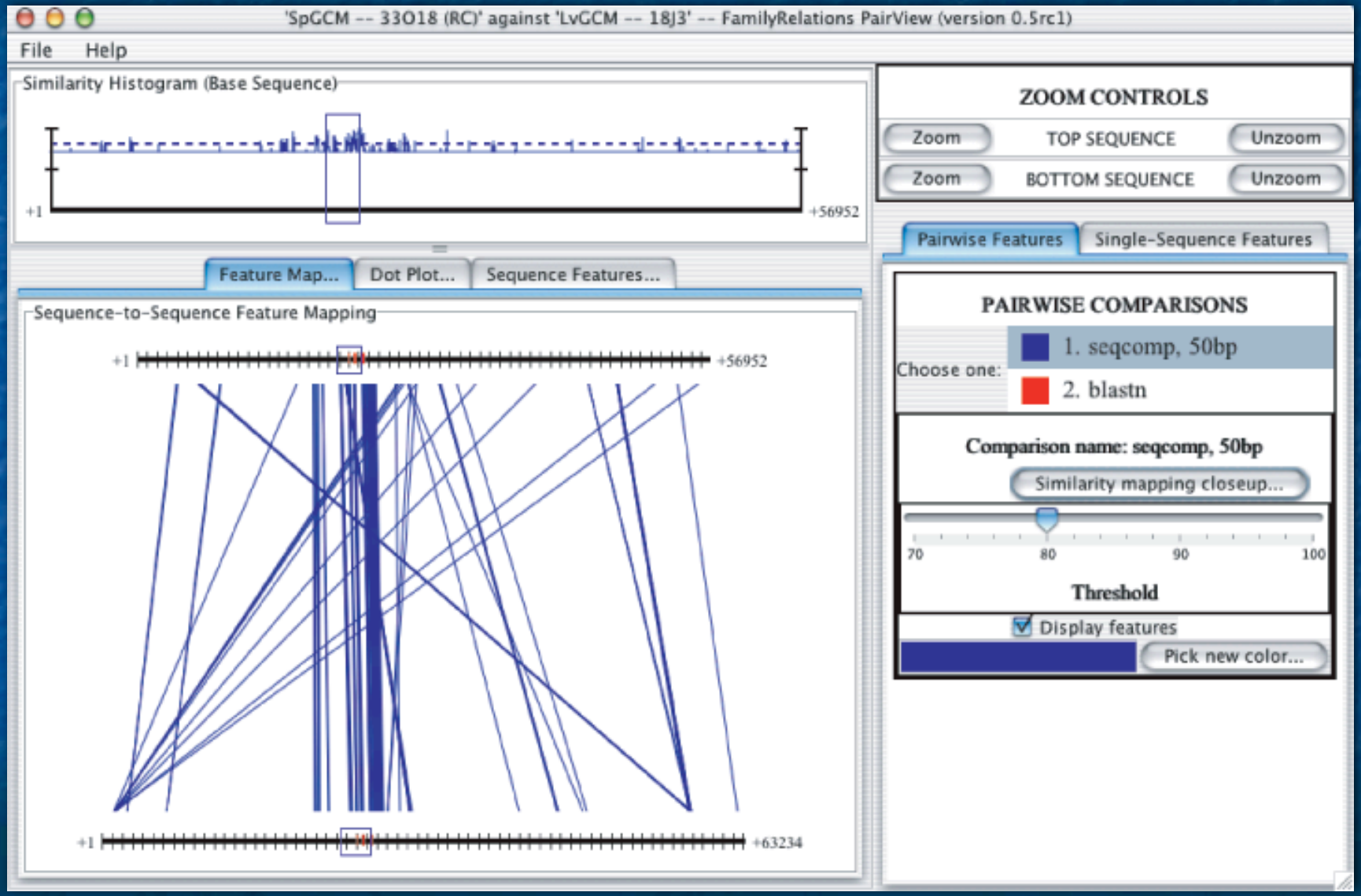
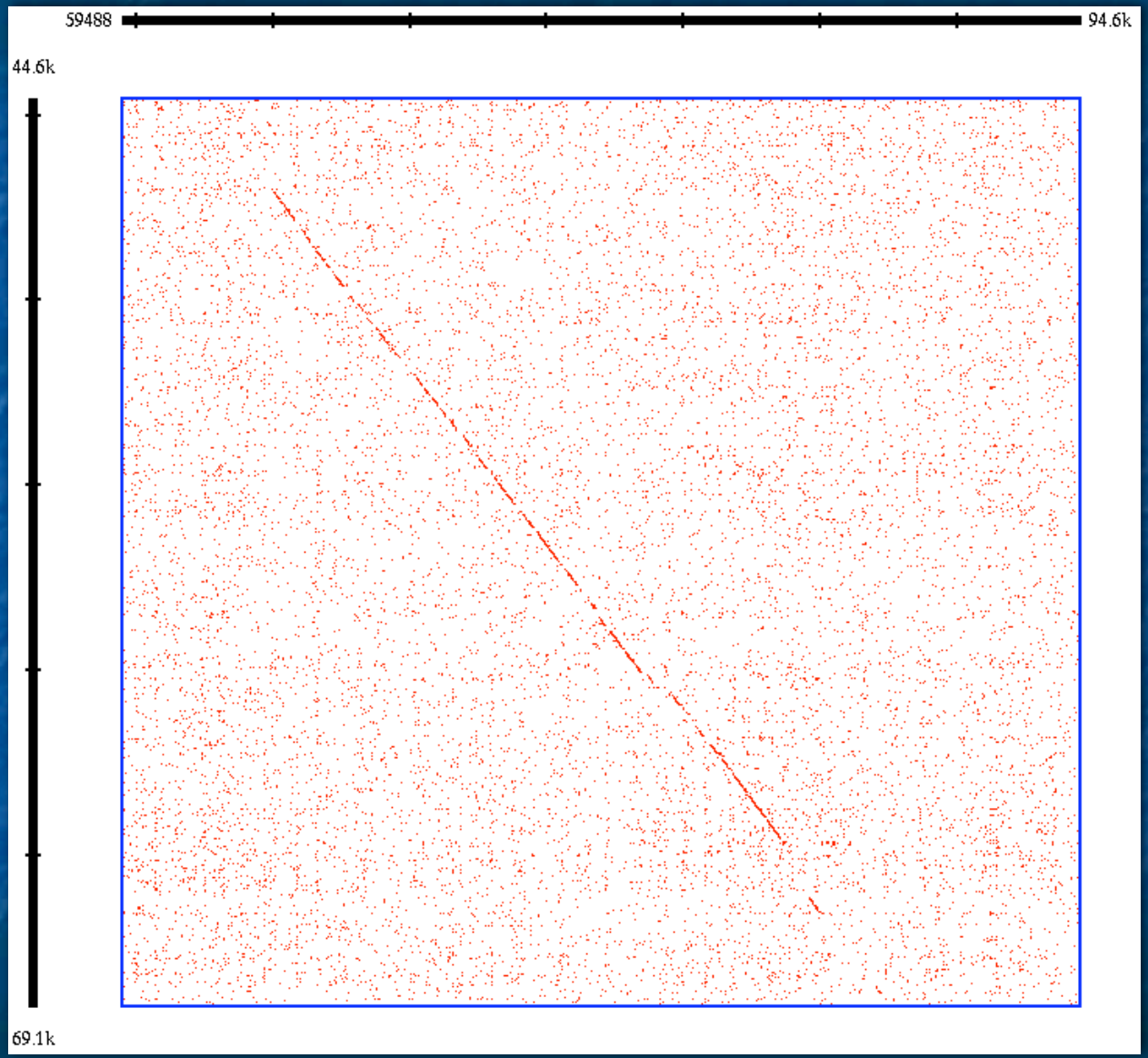
24450

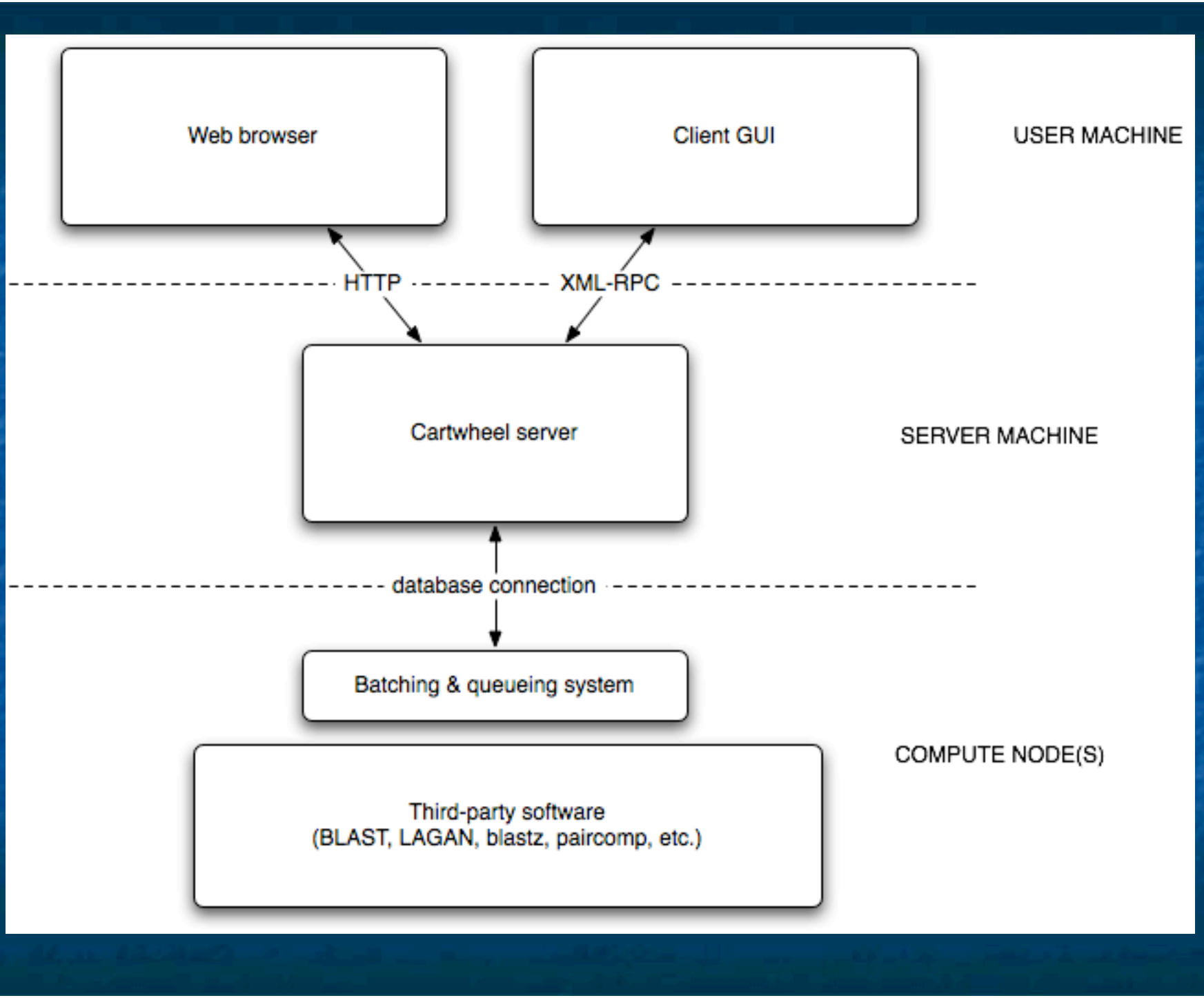
FamilyRelations / pair view




59488
94.6k
44.6k
69.1k

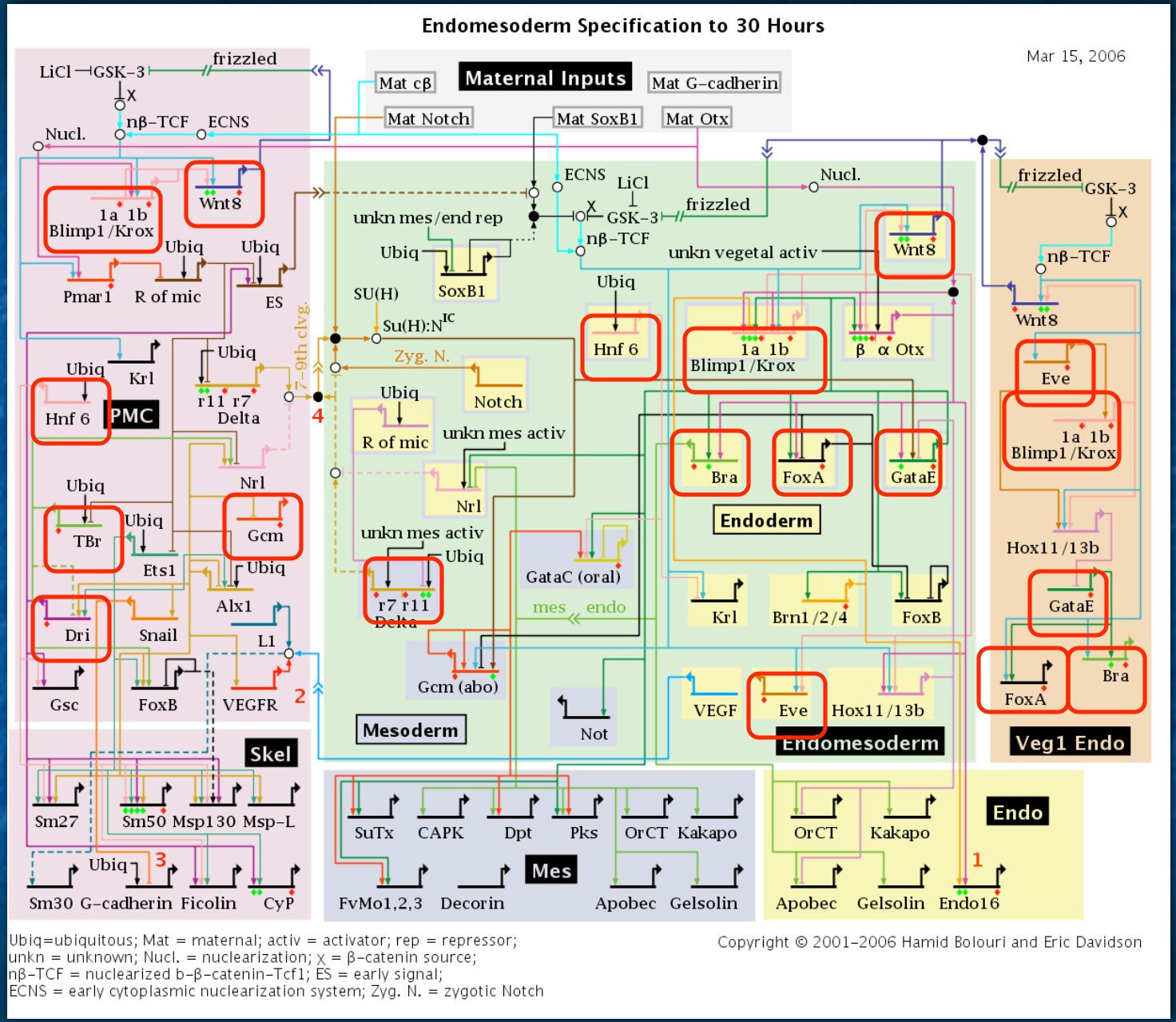
Cartwheel

- A system that lets biologists:
  - establish sequence analyses with custom params;
  - run on them on someone else's compute server;
  - visualize and interact with the results via a client GUI
- Aimed at *bench biologists.*
- Intended to be extensible.


Web browser
Client GUI
USER MACHINE
HTTP
XML-RPC
Cartwheel server
SERVER MACHINE
database connection
Batching & queueing system
COMPUTE NODE(S)
Third-party software
(BLAST, LAGAN, blastz, paircomp, etc.)

Cartwheel technology (all OSS)

- Server:
  - Linux
  - Python, Quixote (1.x), SCGI
  - PostgreSQL db, psycopg
  - Home-grown O/R adapter “cucumber”
  - Several subpackages (paircomp, motility) w/Python interfaces
- Client:
  - Started with Jython; switched to FLTK (C++). **Cross platform.**
  - Uses XML-RPC to communicate with server.


Endomesoderm Specification to 30 Hours
Mar 15, 2006
Maternal Inputs
Mat cβ
Mat G-cadherin
Mat Notch
Mat SoxB1
Mat Otx
LiCl
GSK-3
X
frizzled
nβ-TCF
ECNS
Nucl.
1a 1b Blimp1/Krox
Wnt8
Pmar1
R of mic
Ubiq
ES
SU(H)
Su(H):N^IC
Zyg. N.
7-9th clvg.
4
Hnf 6
PMC
Krl
r11 r7 Delta
Notch
unkn mes/end rep
SoxB1
unkn mes activ
Nrl
TBr
Gcm
Ets1
Alx1
Dri
Snail
L1
Gsc
FoxB
VEGFR
2
r7 r11 Delta
GataC (oral)
mes
endo
Gcm (abo)
Not
Mesoderm
unkn vegetal activ
β α Otx
Bra
FoxA
GataE
Endoderm
Brn1/2/4
VEGF
Eve
Hox11/13b
Endomesoderm
Veg1 Endo
Skel
Sm27
Sm50
Msp130
Msp-L
Sm30
G-cadherin
Ficolin
CyP
3
SuTx
CAPK
Dpt
Pks
OrCT
Kakapo
FvMo1,2,3
Decorin
Apobec
Gelsolin
Mes
Endo16
1
Endo
Ubiq=ubiquitous; Mat = maternal; activ = activator; rep = repressor;
unkn = unknown; Nucl. = nuclearization; χ = β-catenin source;
nβ-TCF = nuclearized b-β-catenin-Tcf1; ES = early signal;
ECNS = early cytoplasmic nuclearization system; Zyg. N. = zygotic Notch
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Comparative Sequence Analysis *works*

This approach (not just Cartwheel…) has turned regulatory region search from a 2-yr technically difficult task into a 3-mo summer student project.

Cartwheel statistics

- Built progressively 2002-2004; several iterations.
- Server: 6k LoC (server); GUI: ~10k LoC
- ~600 total users, 50-100 labs, 30-40 institutions
- ~20 publications that used it
- 10-20 users/month

(Yes, scalability has never been an issue…)

Digression: maintenance

- I'm the primary programmer (~95%).
- I do other things (experiments, PhD thesis, etc.)

Consequently,

- Web site became *very* fragile.

"Test-obsessed"

Need to test Web sites => built twill.

Need to run tests flexibly => use nose.

Need to target more tests => built figleaf.

Need to record Web tests => built scotch.

(yes, I got a little sidetracked…)

Automated testing rocks.

Probably ~30% of the Web app covered.

- unwarranted confidence in basic structure
- *very* easy to upgrade packages, transfer servers (~1 day to upgrade postgresql, switch to new version of Debian on a VMware server)

Sociological considerations

User population is computationally naïve.

Most tools in this area are either *very simple* or *virtually impossible to use.*

We provided a simple Web interface, an intuitive GUI interface, and a tutorial.

Biologists prefer making their own judgements.

…and they hate high false positives.

Do other programmers use it?

Provided simple interfaces (XML-RPC) for establishing, manipulating, downloading analyses.

Tried to make things easily extensible.

No documentation, of course.

Result: no one uses my code.

My small libraries are moderately popular (paircomp, motility) because they have a simple Python API.

Planning for the future

Technology update:

- I love Quixote for Web apps
- FLTK sucks (ugly!) => QT or KWWidgets (testing!)
- I hear Python is up to 3000 now??
- SQLAlchemy is a very nice O/R mapping system
- ExtJS javascript toolkit.

New functionality thoughts

Cartwheel originally built around targeted subsequencing of genomes.

Now we have a lot of big genomes sequenced!

⇒ Need to import information, interact with already-established alignments.

"for" loops don't cut it -- move to pygr for genome manipulation/interaction?

New functionality thoughts

Motif searching is the new black.

Biologists want to search for binding sites (fuzzy 6-22 bp) and ask questions like:

- do these sites correlate well with conservation?
- do they cluster with themselves/others?
- are they near my favorite gene(s)?

Idea is to expand Cartwheel into a tool for regulatory genomics hypothesis generation and exploration.

Concluding thoughts

Write end-user software that *works* over software that is flexible.

Tutorials, ease of use are both *really* important (pick your target audience - devs? users?)

If you're unlucky, your project will hang around for longer than you thought and you will have to think about maintenance.

Test.

Acknowledgements

Eric Davidson (old boss)
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