

Me, some fish & veg

Tessa Montague

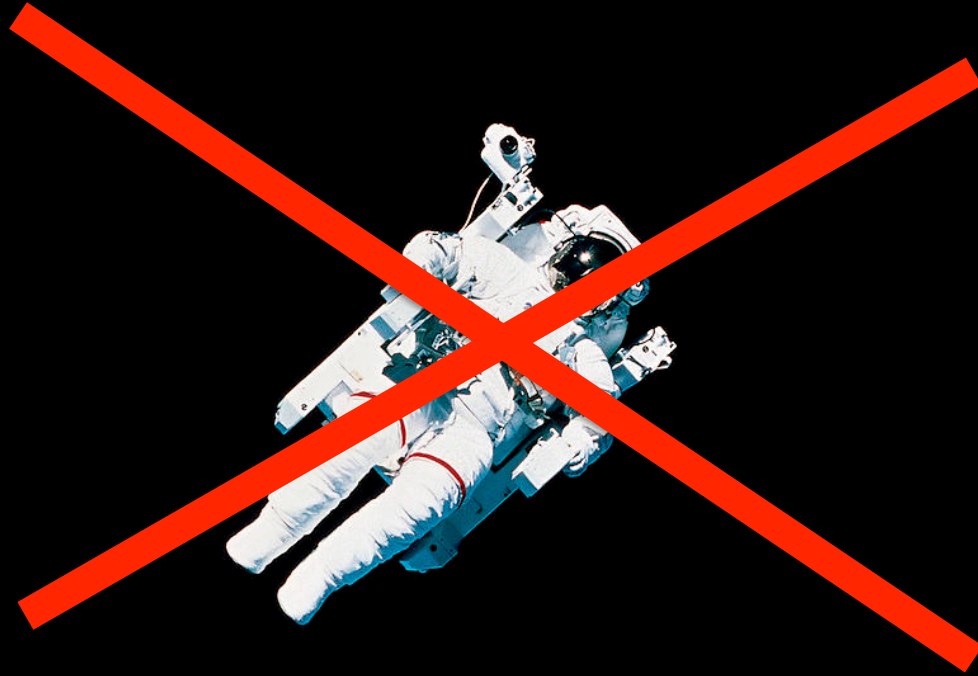
Graduate student, Schier Lab
Harvard University

Overview

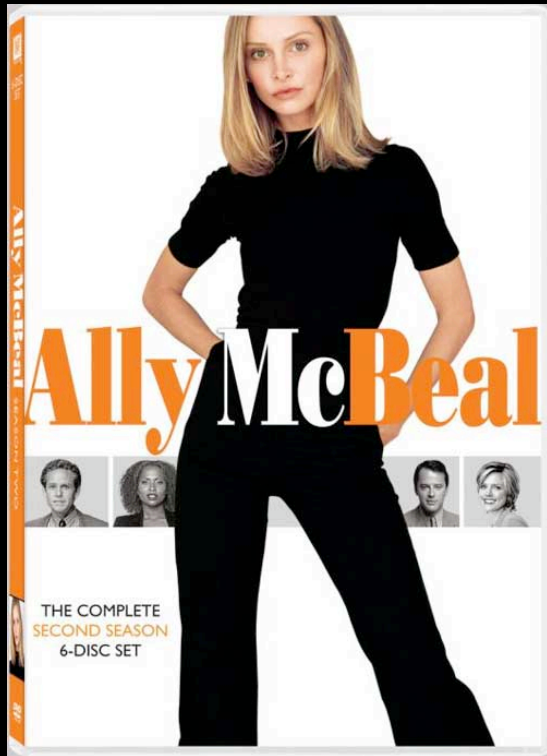
1. My path to science
2. Why zebrafish?
3. My research: veg
4. Introduction to the lab

Part I : My path to science

When I was younger I wanted to be...



Then I wanted to be a...



Lawyer



Doctor



CIA agent

My first research experience

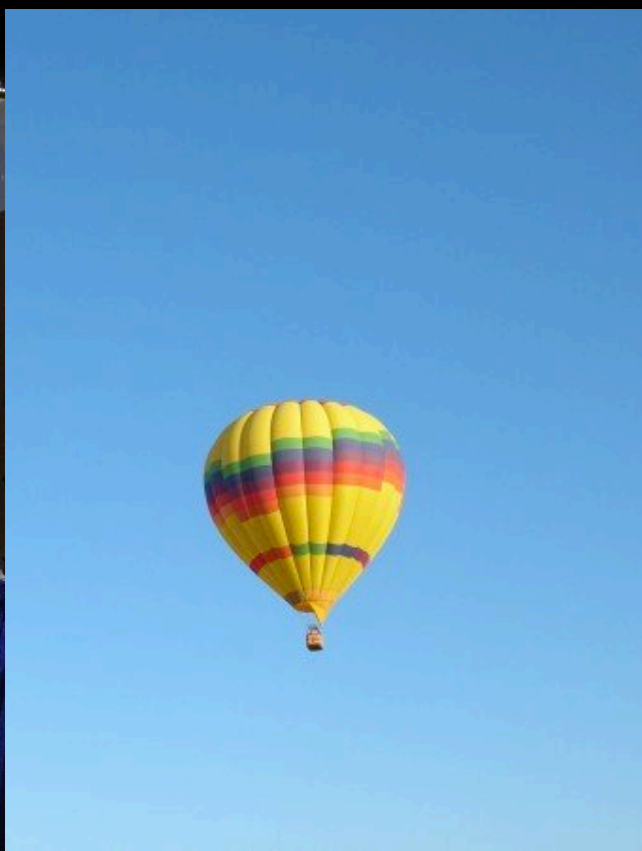






ISEF 2007





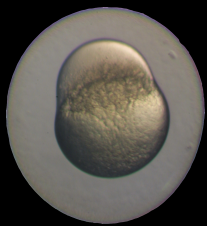
Cambridge University





Harvard MCB





Doing a PhD : new skills

```
tmontague - tmontague@rclogin07:~ - ssh - 123x53
File Edit Options Buffers Tools Python Help
target_strand = None

pattern = re.compile("(([\.\w]+):)?([\.\w]+)-([\.\w]+)")
isCoordinate = pattern.match(targetString)

if isCoordinate:
    # Target specified as coordinate
    if target_strand == None:
        target_strand = "+"
    elif target_strand == "-":
        sys.stderr.write("All targets must be on the same strand.\n")
        sys.exit(EXIT["GENE_ERROR"])

    ## CHROMOSOME LOCATION
    chrom = isCoordinate.group(2)
    i = 1

    for target in targetString.split(";"):
        m = pattern.match(target)

        if m:
            if m.group(2) != None and chrom != m.group(2):
                sys.stderr.write("Can't target regions on separate chromosomes (%s != %s).\n" % (chrom, m.group(2)))
                sys.exit(EXIT["GENE_ERROR"])

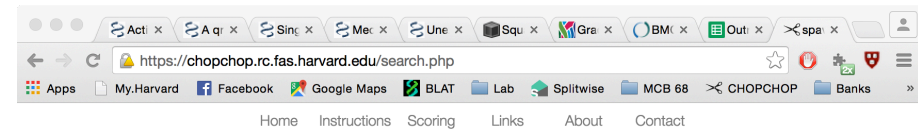
            startPos = m.group(3)
            endPos = m.group(4)

            startPos = startPos.replace(".", "").replace(":", "")
            endPos = endPos.replace(".", "").replace(":", "")

            startPos = int(startPos)
            endPos = int(endPos)
            targetSize += endPos - startPos + 1

            if (startPos >= endPos):
                sys.stderr.write("Start position (%s) must be smaller than end position (%s)\n" % (startPos, endPos))
                sys.exit(EXIT["GENE_ERROR"])

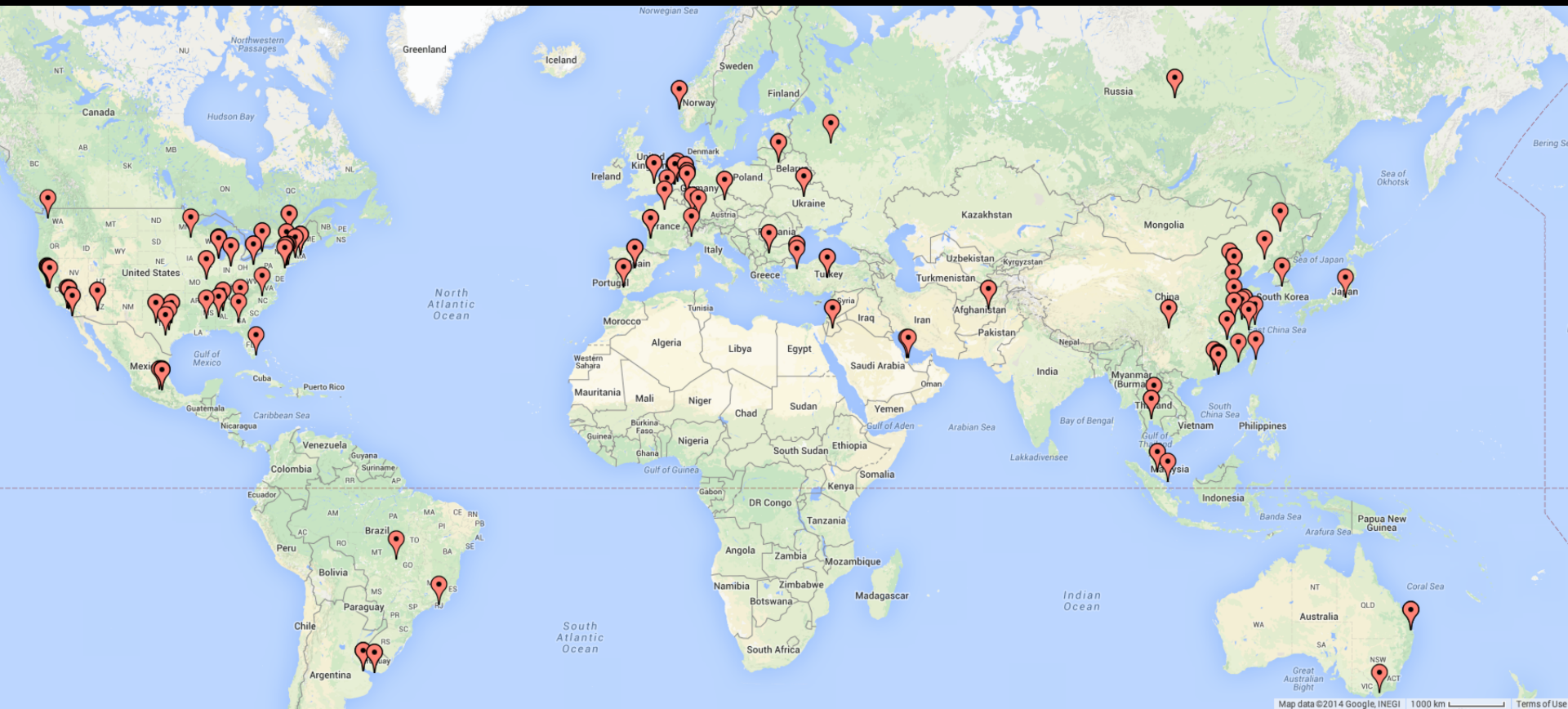
            targets.append("%s:%s-%s" % (chrom, max(0, startPos-padSize), endPos+padSize))
            visCoords.append([chrom, startPos, endPos, 0, 10, "+"])
            displayIndices.append(i)
            i += 1
        else:
            sys.stderr.write("All targets must be on the same strand.\n")
            sys.exit(EXIT["GENE_ERROR"])
```



Ranking	Target sequence	Genomic location	Exon	Strand	GC content (%)	Off-targets		
						0	1	2
1	CGGCTGCATTGCGAATGGAGAGG	chr5:71782845	1	-	61	0	0	0
2	GAGACACAAACGAAACCGTGTGG	chr5:71787509	2	+	52	0	0	0
3	AGGAAAGCCAACACCTTCAGAGG	chr5:71787566	2	+	52	0	0	0
4	AGCAAGCTTCACTTTGCCGGCGG	chr5:71787592	2	+	61	0	0	0
5	GGGTTGTAAGTCTCATCCAGAGG	chr5:71787714	2	-	52	0	0	0
6	TGAAGCTCTACCAACCTGAGCGG	chr5:71789623	3	+	57	0	0	0
7	GAGAGCGAAGTGAGGCGCAGCGG	chr5:71789667	3	-	70	0	0	0
8	AGTGGTGATGAGACACCAGGAGG	chr5:71789714	3	+	57	0	0	0
9	TGGATTTTGATCAGATTGGGTGG	chr5:71787628	2	+	43	0	0	1
10	GCATTGCGAATGGAGAGGTTTGG	chr5:71782840	1	-	52	0	0	0



In a two week period...



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During waking hours someone is running the web tool every minute!



Like

Comment



Humans of New York

"Who's influenced you the most in your life?"

"My principal, Ms. Lopez."

"How has she influenced you?"

"When we get in trouble, she doesn't suspend us. She calls us to her office and explains to us how society was built down around us. And she tells us that each time somebody fails out of school, a new jail cell gets built. And one time she made every student stand up, one at a time, and she told each one of us that we matter."

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Part II : Why zebrafish?

- easy to maintain

- lots of embryos!

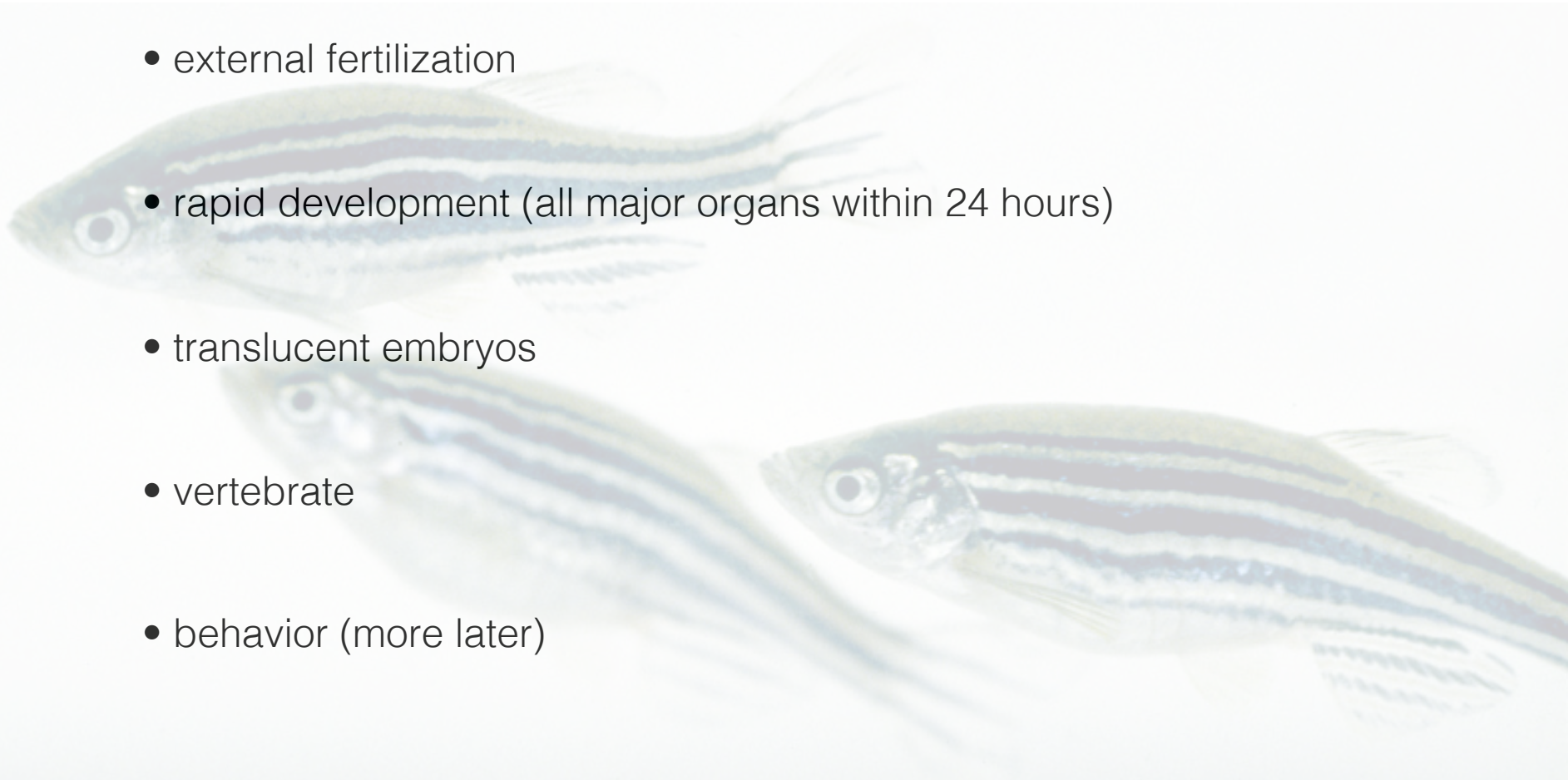
- external fertilization

- rapid development (all major organs within 24 hours)

- translucent embryos

- vertebrate

- behavior (more later)



Developmental Time Lapse

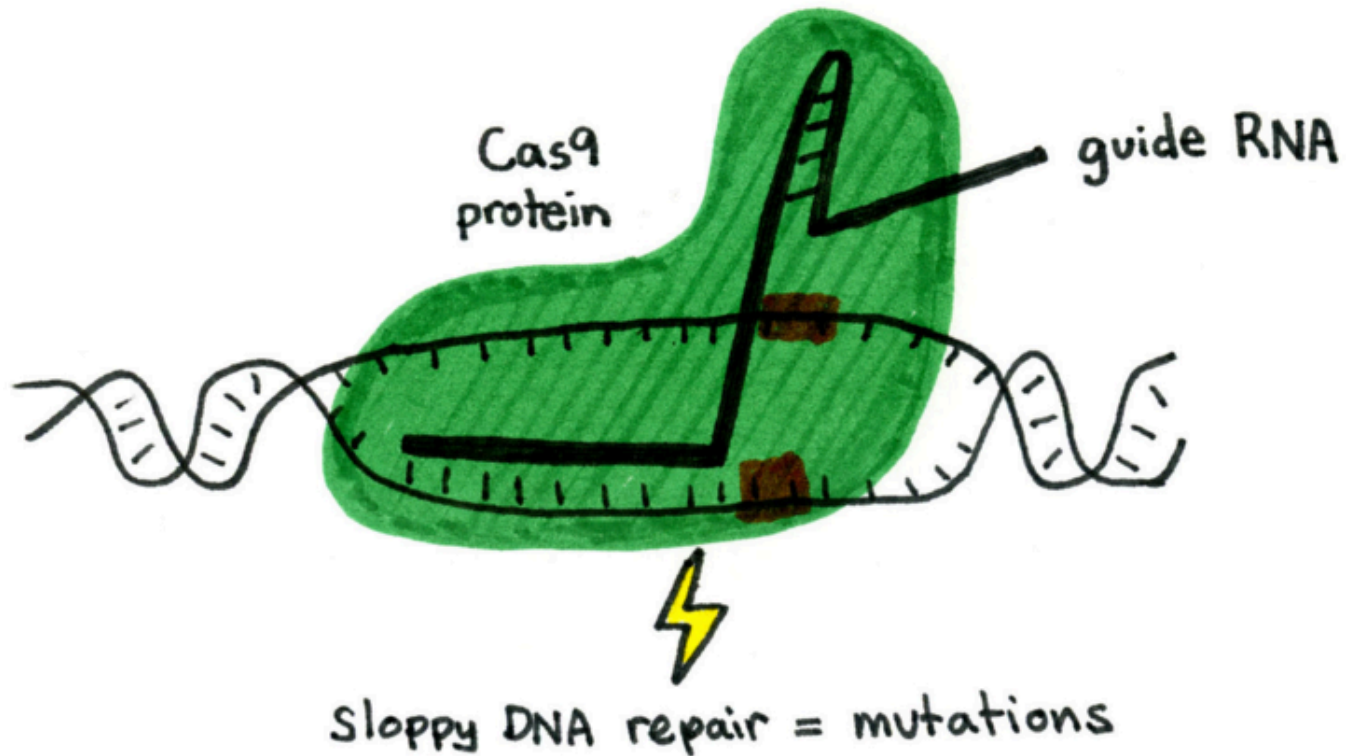
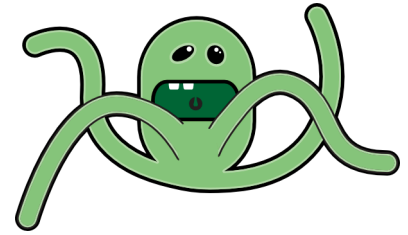




What happens when you cross a
zebrafish with a jellyfish?

Part III: My research

The CRISPR Craze

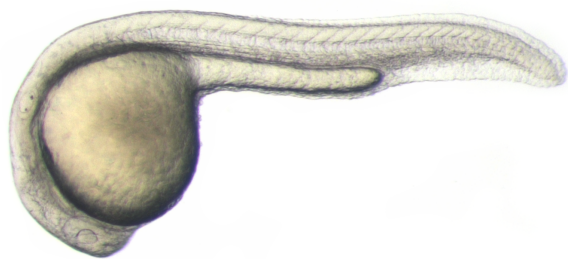


Human genome



- We have approximately 20,000 genes
- But each of us is a single knockout for 200 genes

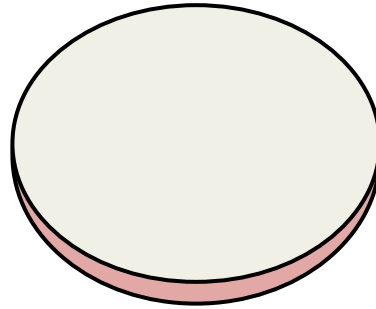
...And a double
knockout for 20 genes



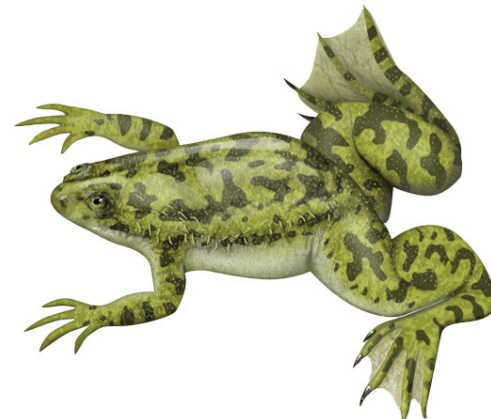
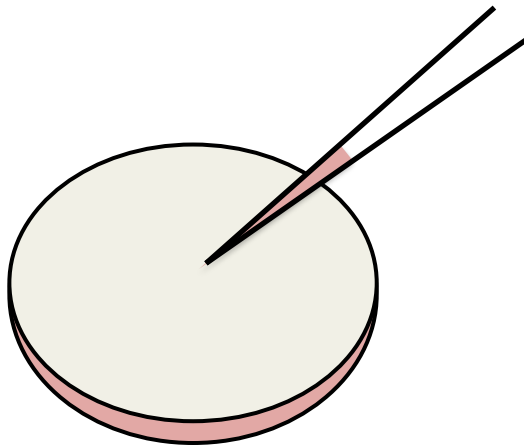


Veg1 in the 1980/90s

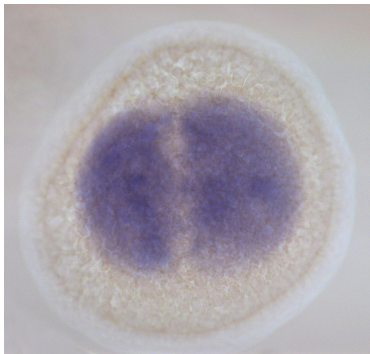
Xenopus
oocyte



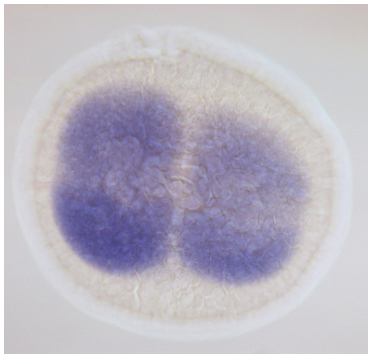
localized
vg1 mRNA



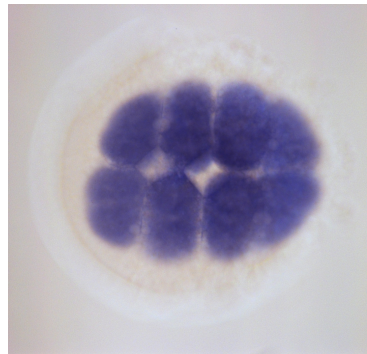
Vg1 in zebrafish



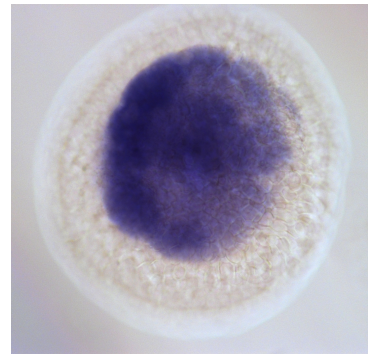
2 cell



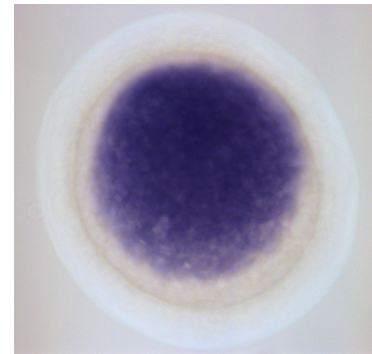
4 cell



8 cell



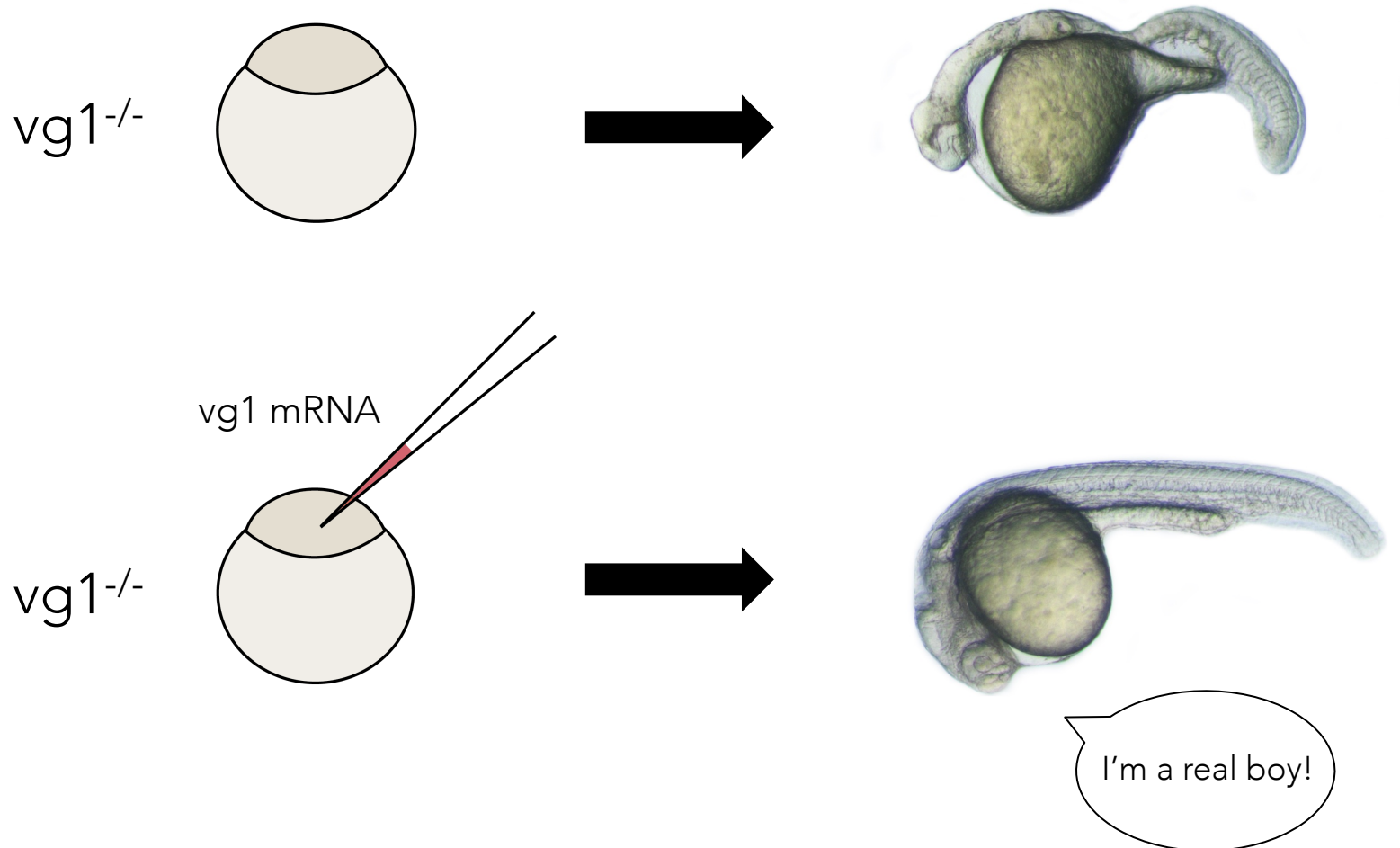
128 cell



1000 cell

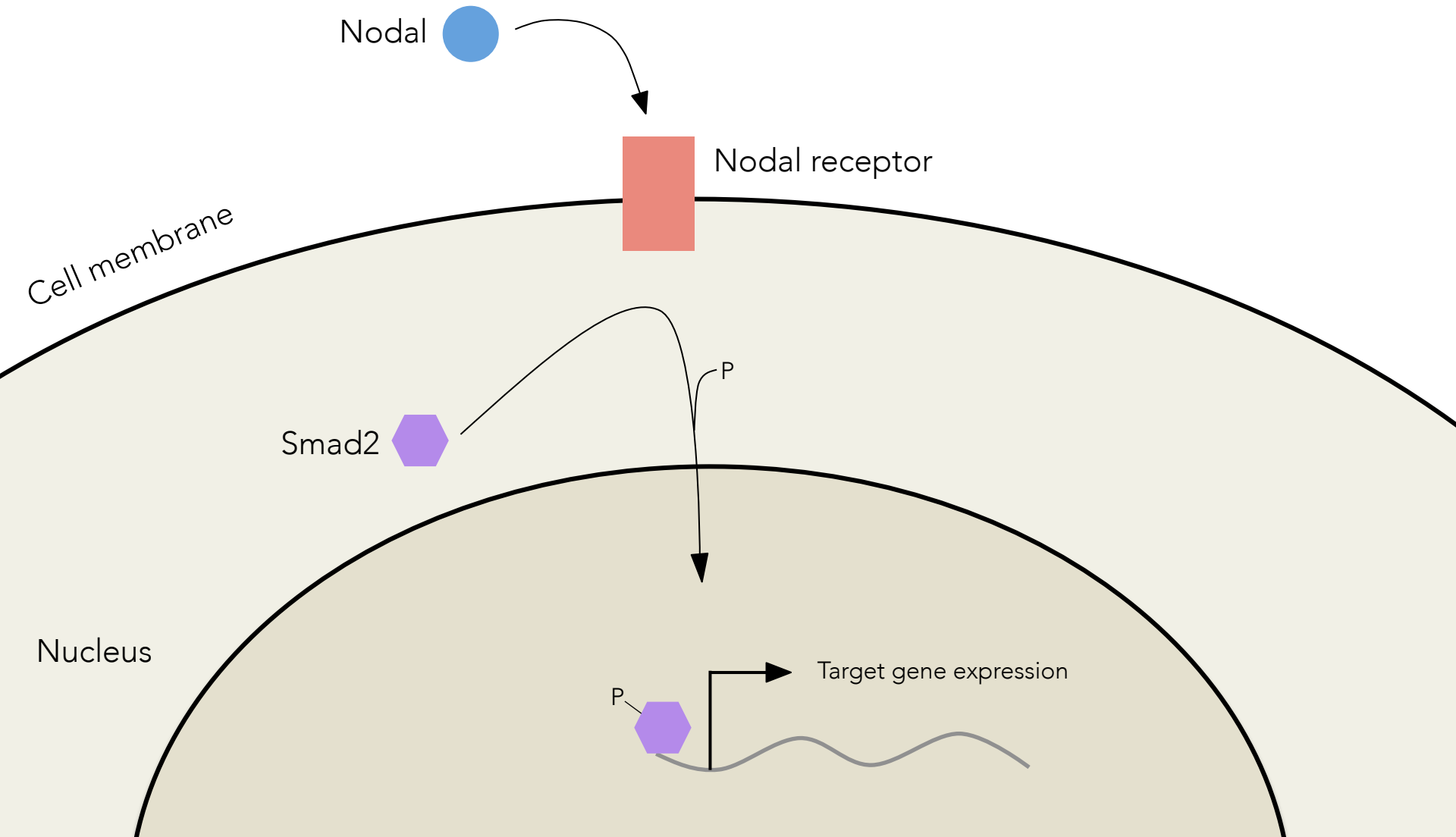


Vg1 mRNA rescues the phenotype

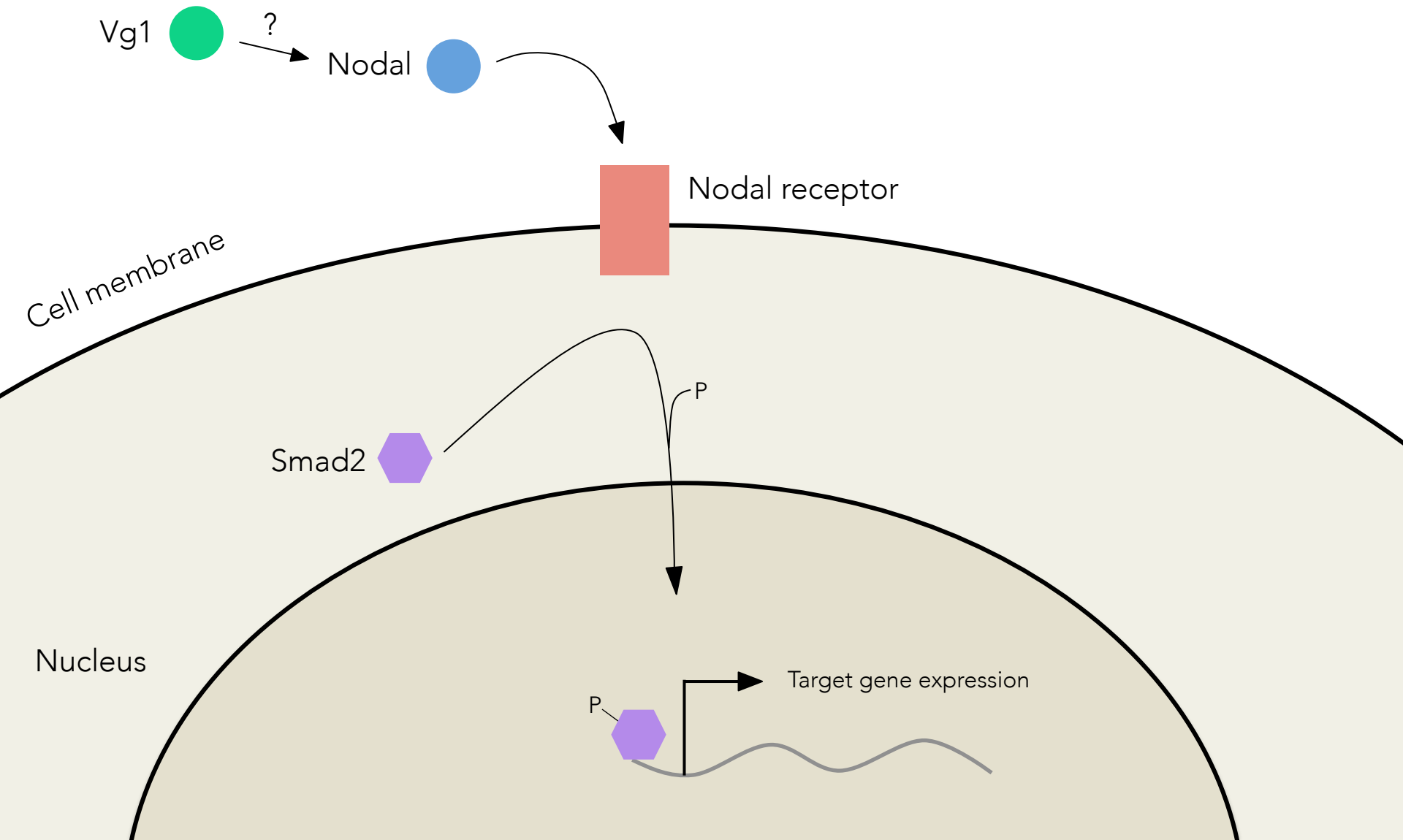


So what does V_{g1} do?

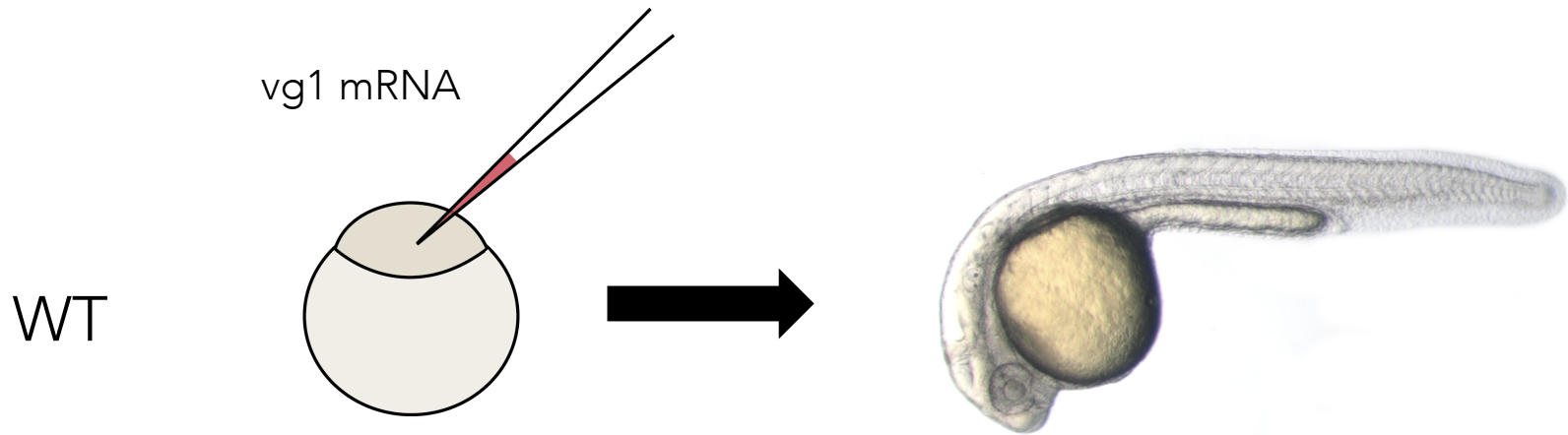
The Nodal signaling pathway



Hypothesis 1: Vg1 activates Nodal



Overexpression of *vg1*...



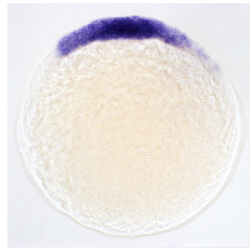
...Does nothing

Nodal target gene expression

Nodal



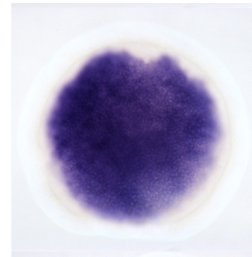
Target gene
expression



Nodal



Target gene
expression



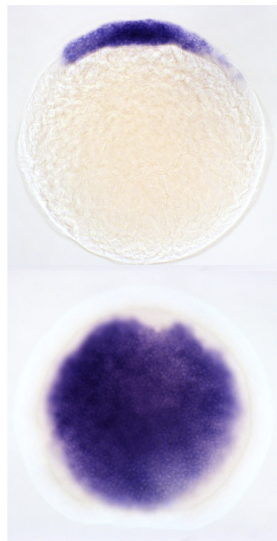
Nodal

↓

↓

Target gene
expression

WT

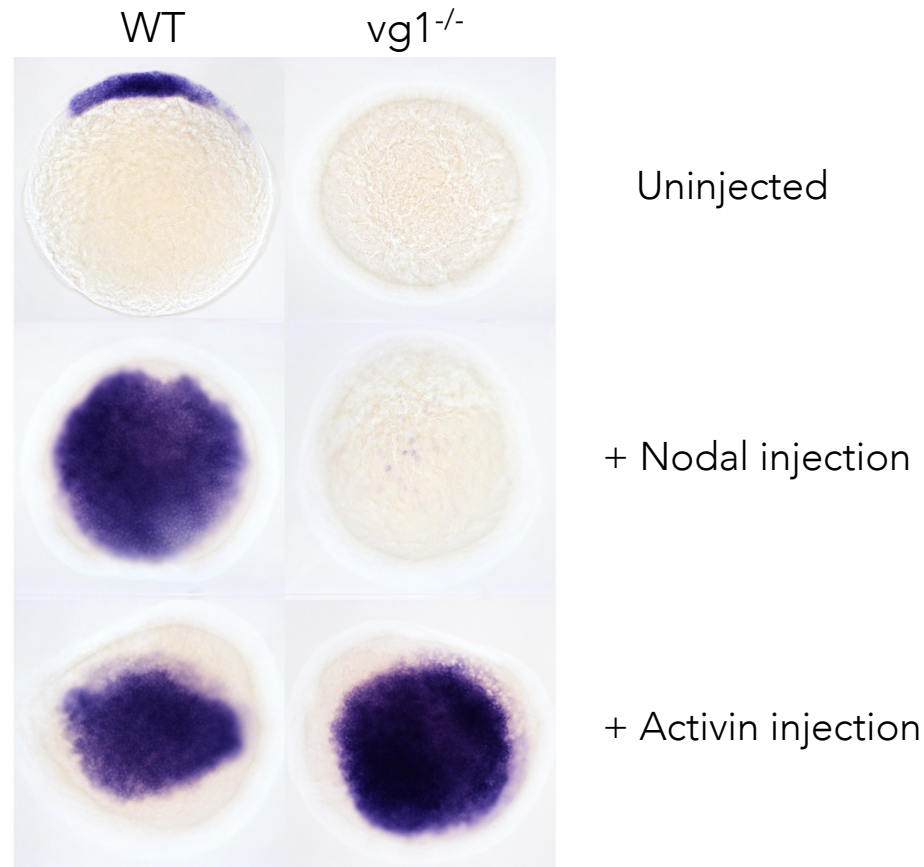
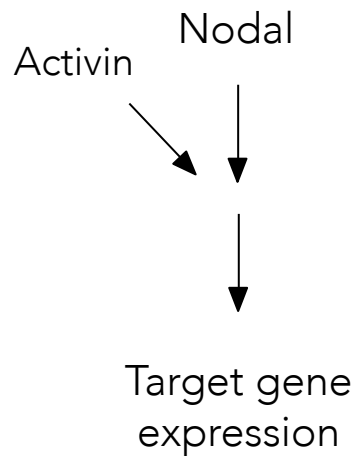


Normal (uninjected)

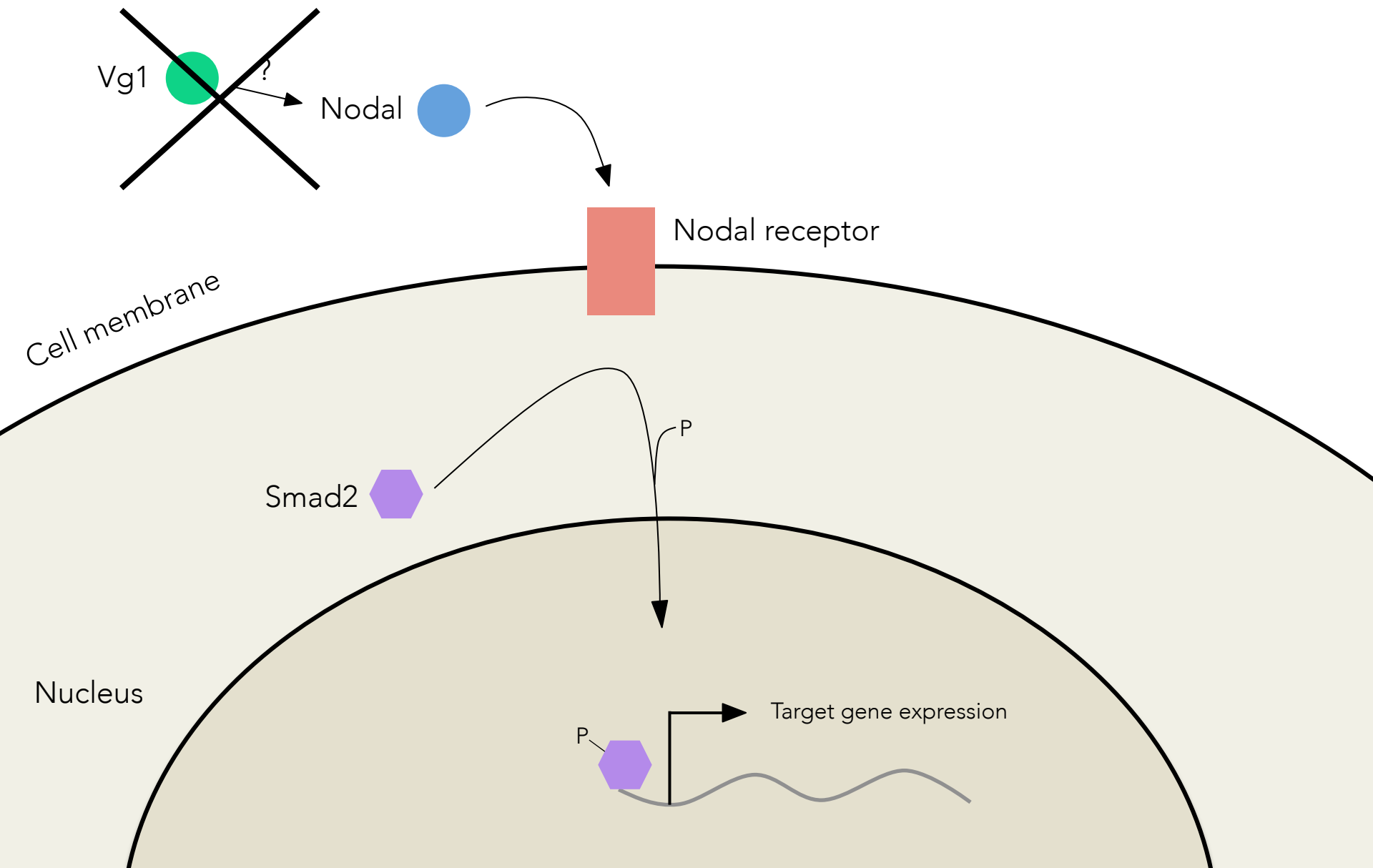
+ Nodal injection

Target gene
expression

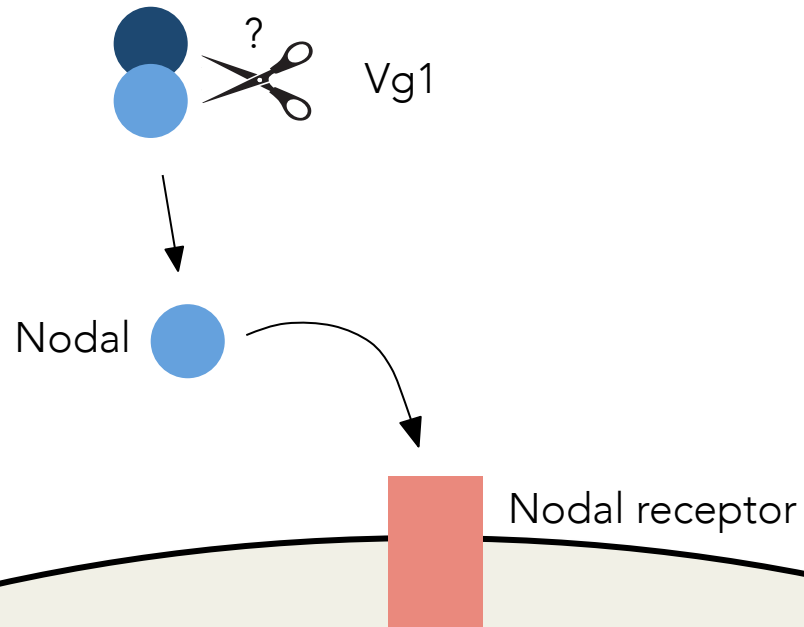
Nodal can't induce gene expression without Vg1



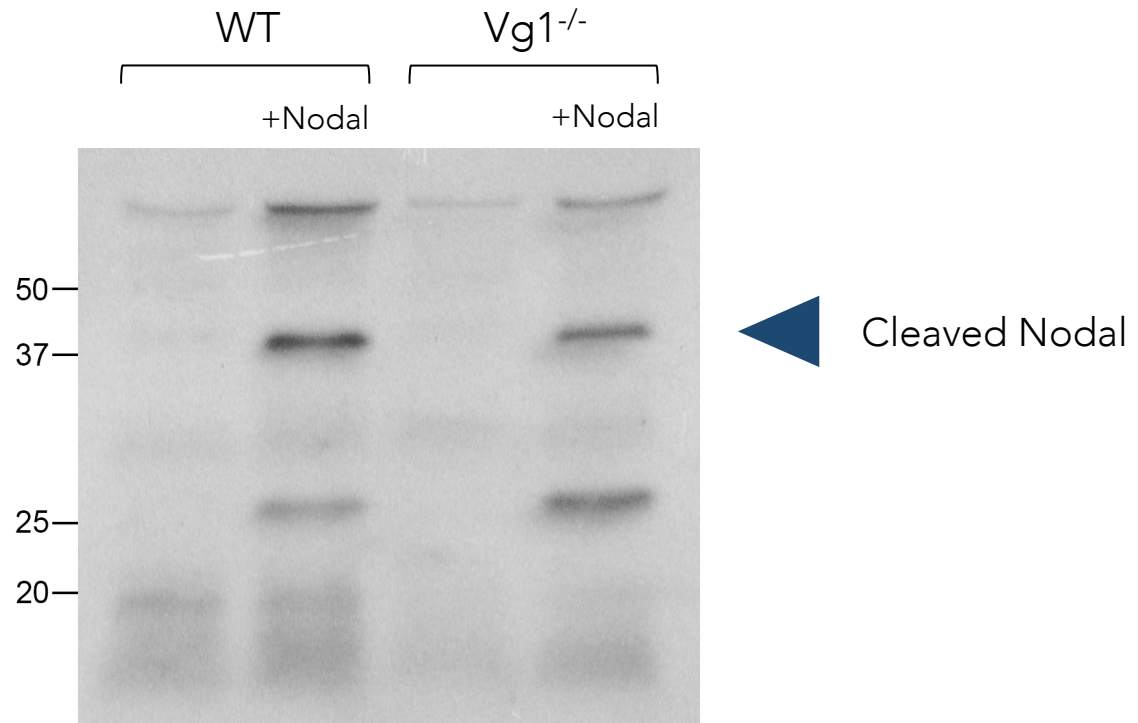
Hypothesis 1: Vg1 activates Nodal



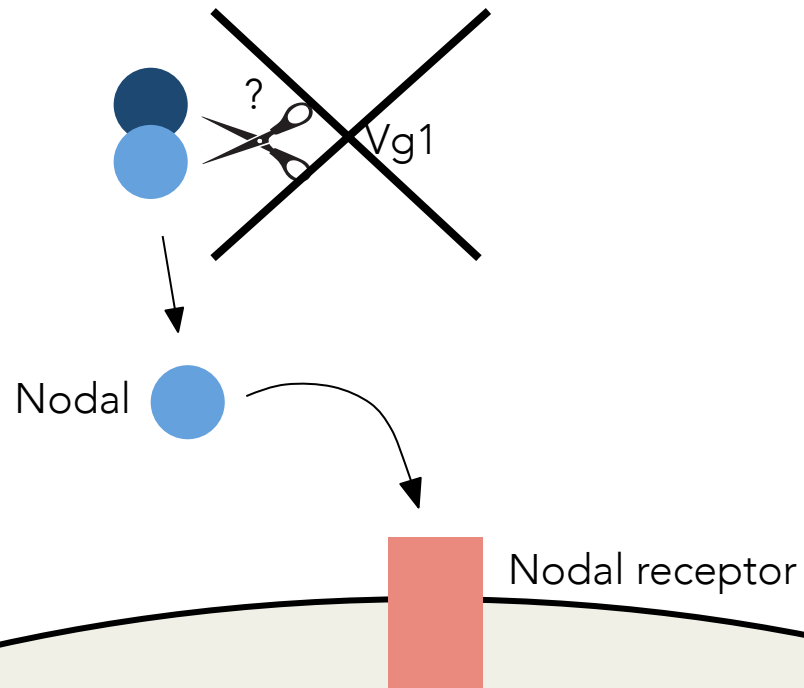
Hypothesis 2: Vg1 cleaves Nodal



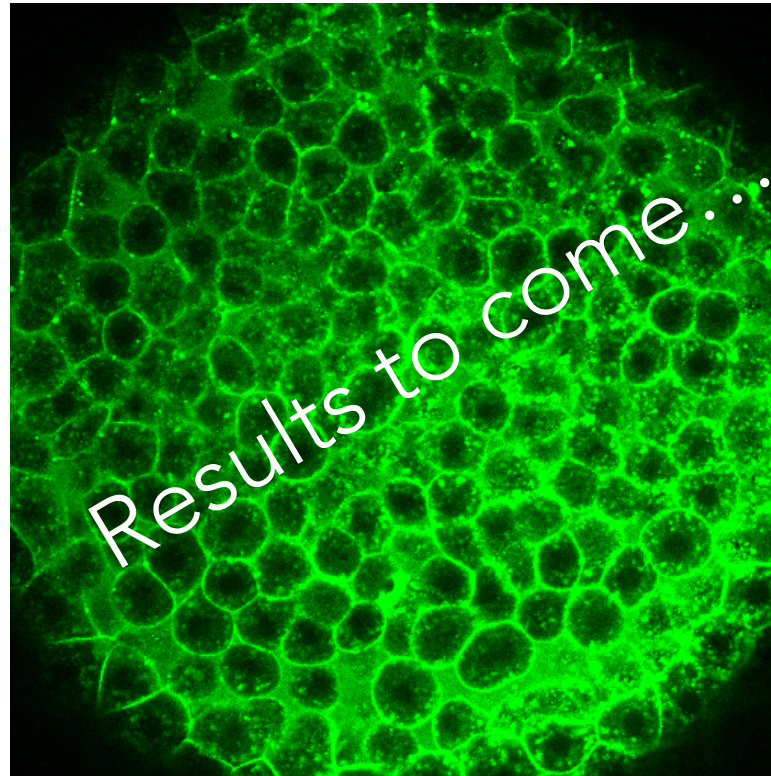
Nodal is cleaved normally without Vg1



Hypothesis 2: Vg1 cleaves Nodal

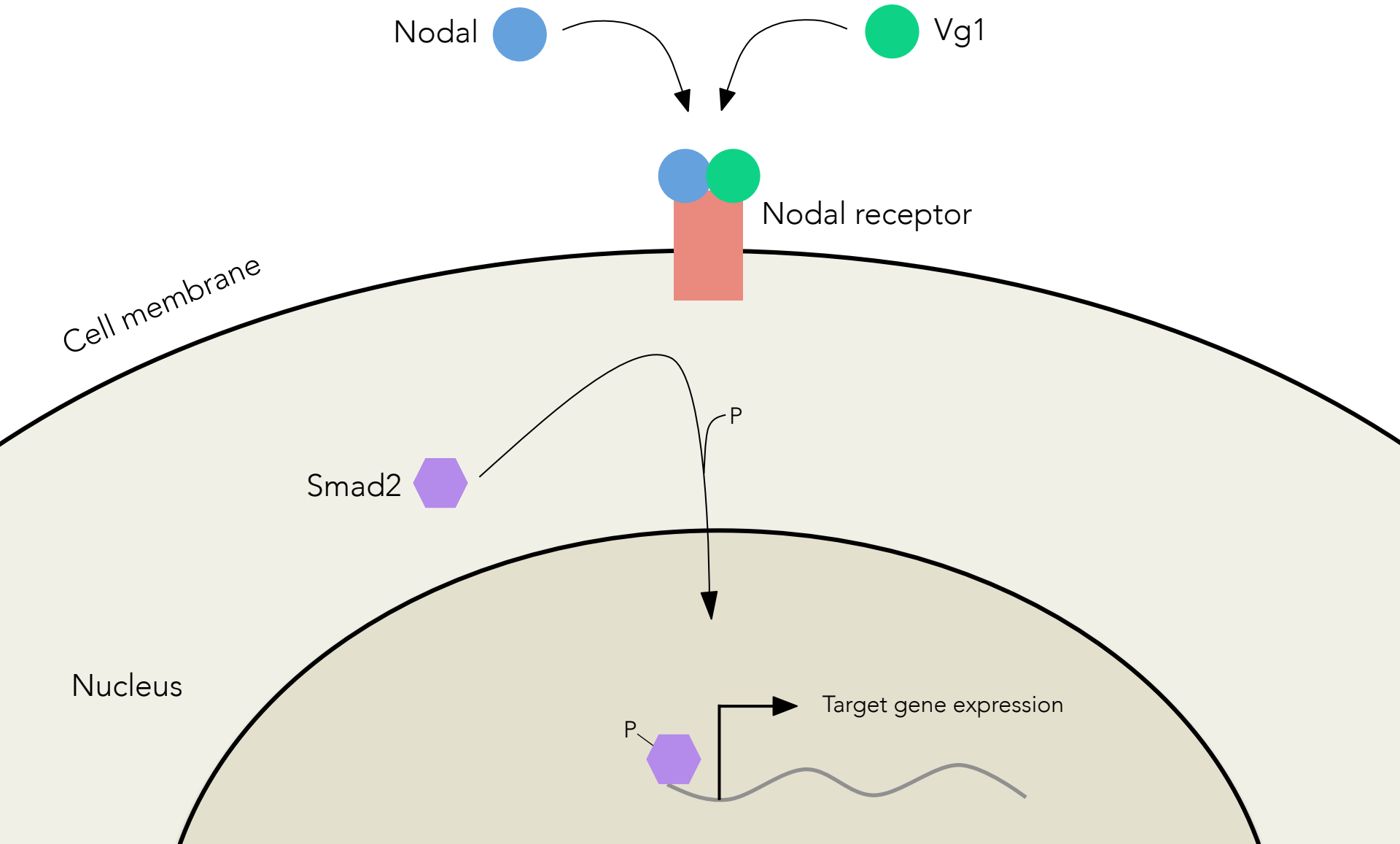


Hypothesis 3: Vg1 controls secretion of Nodal



Katherine Rogers

Our favorite hypothesis



To be continued...

Acknowledgements

- Alex Schier
- The Schier Lab
- Andrea Pauli
- CHOPCHOP: Eivind Valen, James Gagnon, José Cruz (George Church Lab)



Your lab next week



Prey capture



Response to acoustic stimuli



Light startle response



Optomotor response



Light preference