Transcription

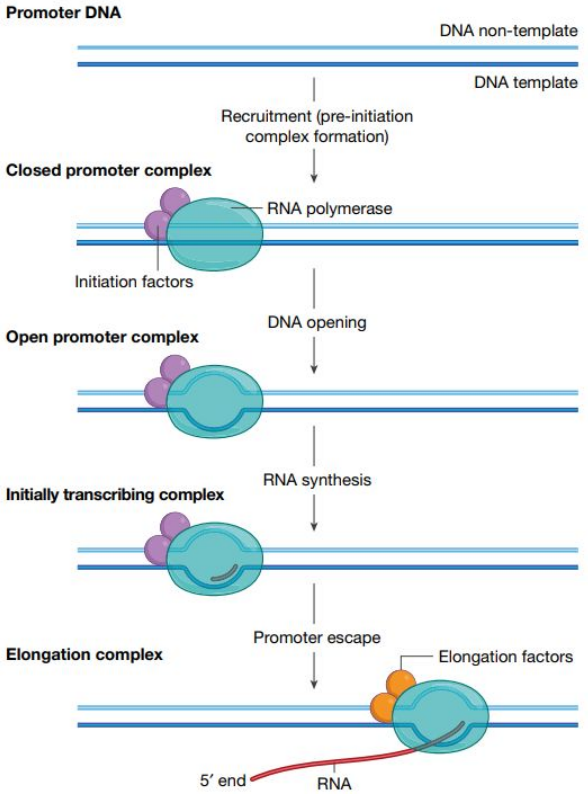
Eukaryotic transcription

RNAP forms PIC on the promoter

Promoter complex opens

DNA-dependent RNA synthesis then generates an initially transcribing complex

RNAP escapes promoter

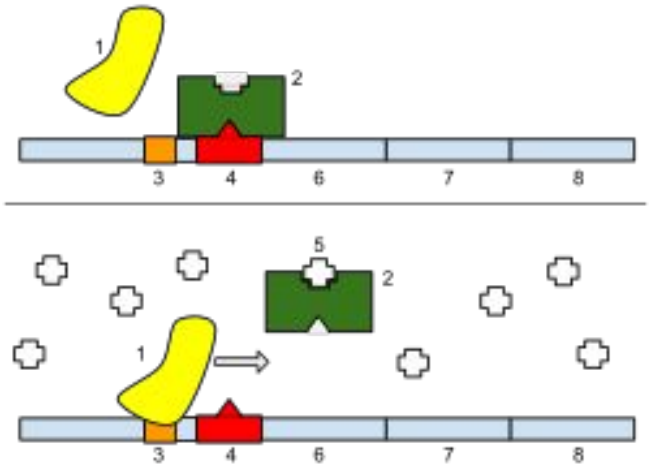


Cramer, 2019

Promoter

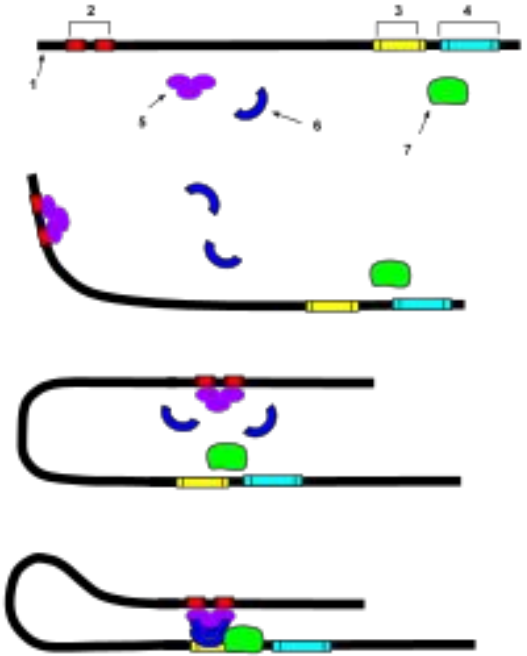
Region of transcription initiation

Located upstream of gene



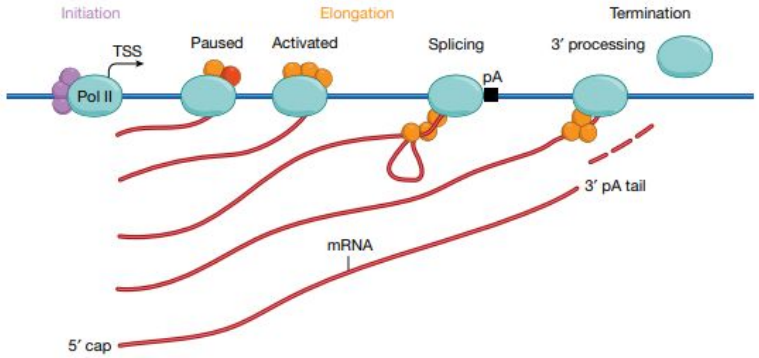
Enhancer

DNA region that binds transcription factors (TFs) to facilitate transcription



Eukaryotic transcription

RNAPII associates with different proteins during the transcription cycle

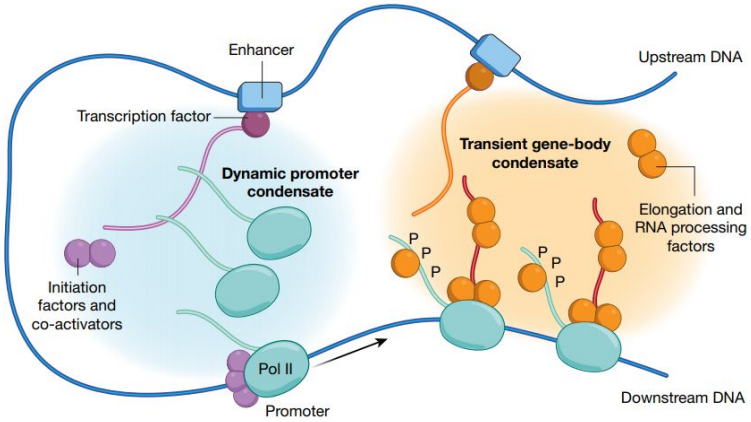


Cramer, 2019

Condensate model

TFs form condensates to recruit RNAPII to promoters

RNAPII shuttles between promoter and gene-body condensates



Cramer, 2019

Transcription factors and genome architecture

Linear view of genome is outdated - 3D structure matters

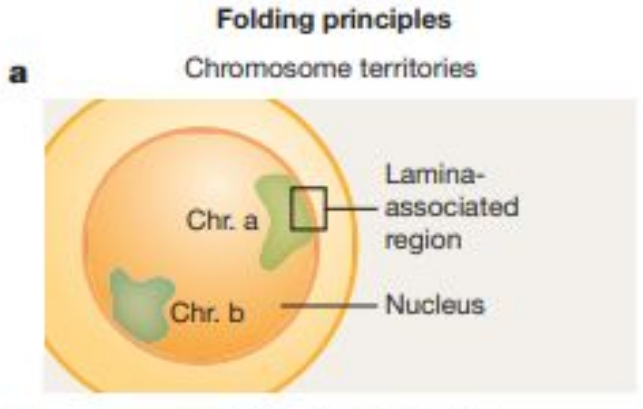
Transcription factors help shape 3D structure for altering functionality of genome

Chromosome territories

Chromosomes are organized into specific compartments in the nucleus

Interactions with nuclear lamina important for their maintenance

Territories rarely intersect



Stadhouder, 2019

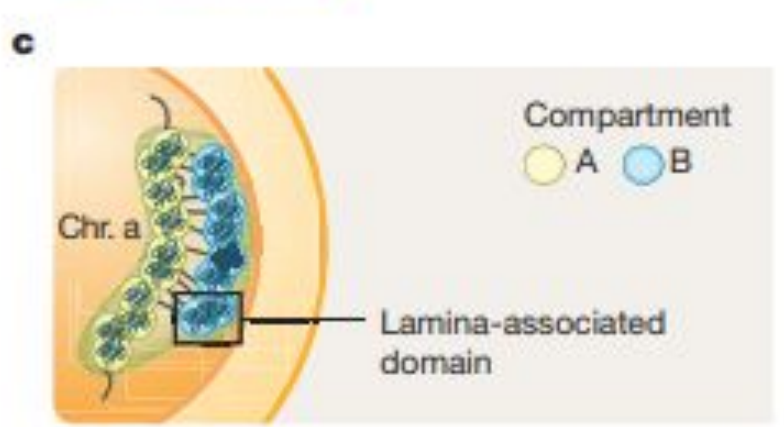
Compartments in territories

Each territory can be divided into A and B compartments

A is mostly euchromatic

B is lamina-associated and mostly heterochromatic

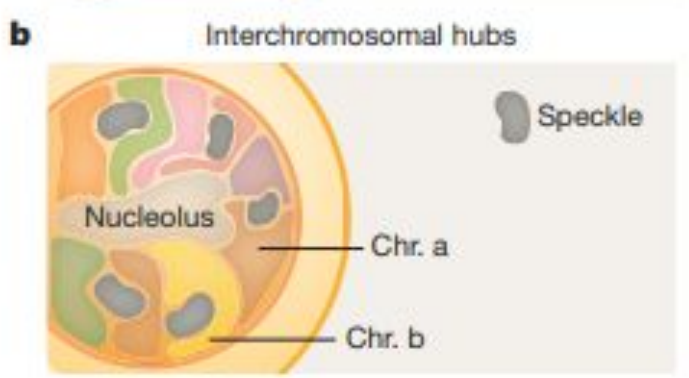
Transcription factors (TFs) can reposition genes between compartments



Stadhouder, 2019

Nuclear hubs

Higher order structures that form between different chromosomes

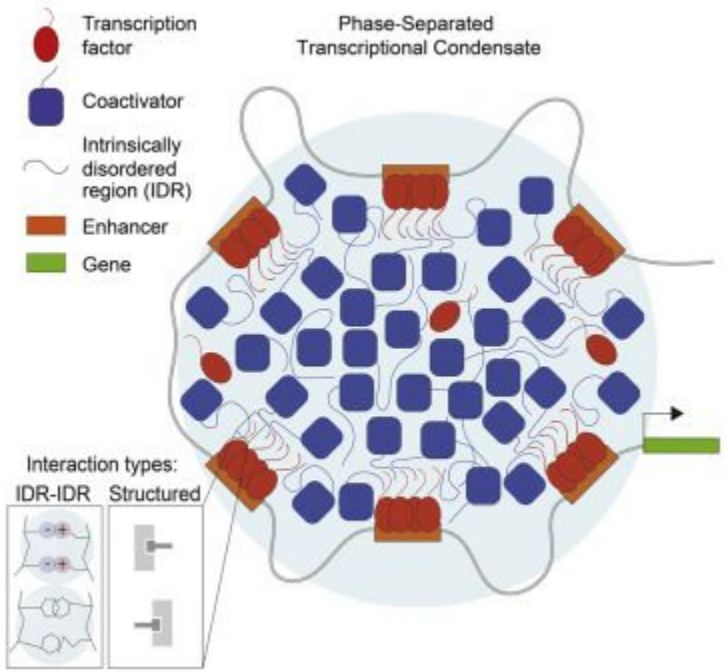


Stadhouder, 2019

TF-induced condensates

Transcription factors form phase-separated droplets via their activating domains

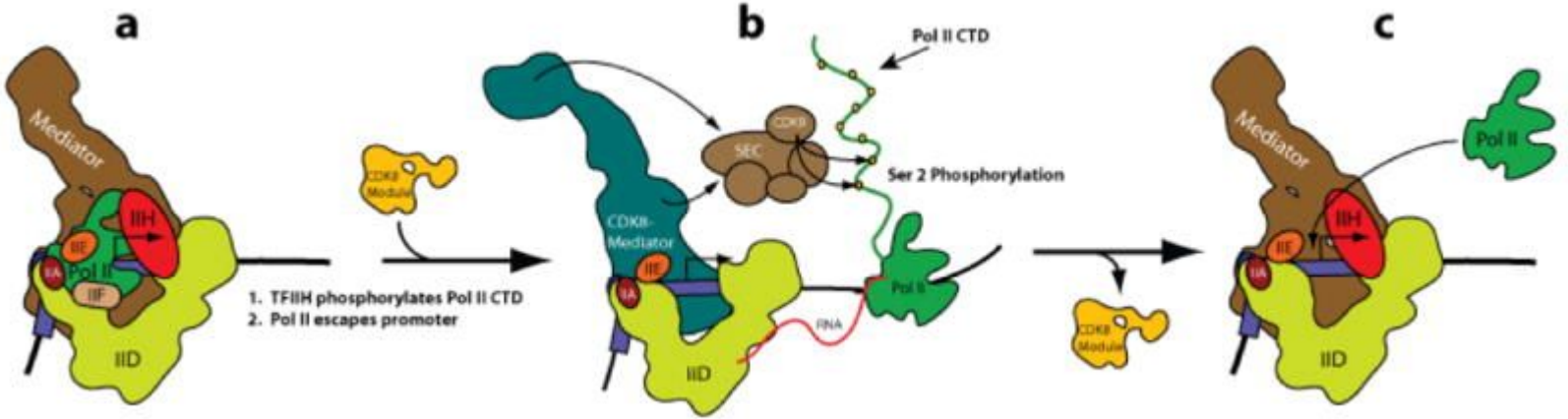
These droplets also contain Mediator



Boija, 2018

Mediator

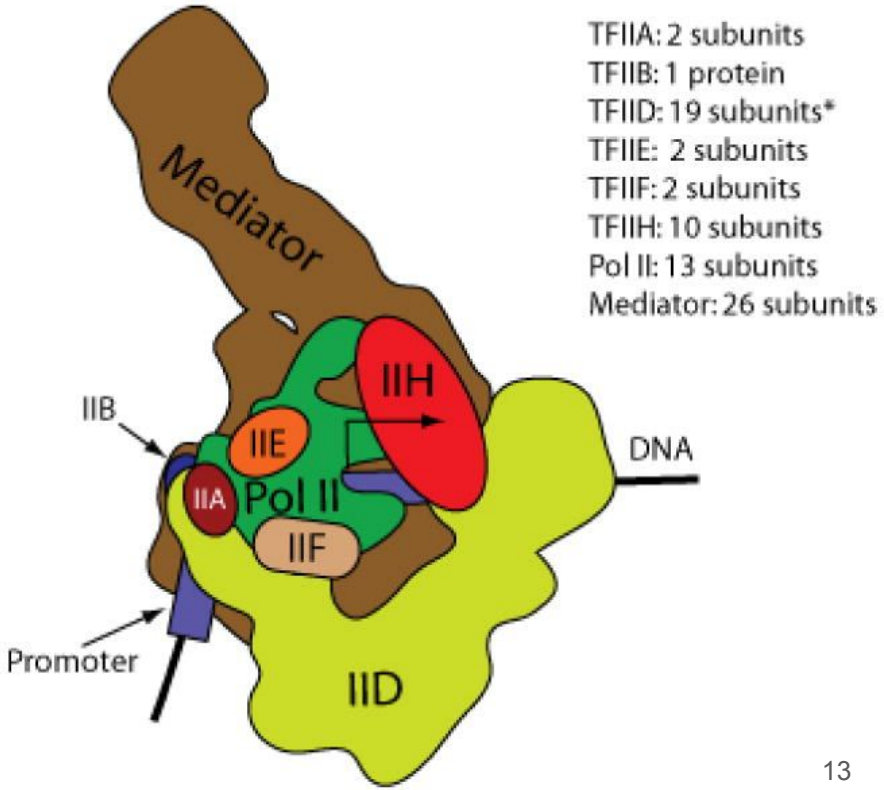
Communicates regulatory signals from DNA-bound TFs directly to the RNA polymerase II (pol II) enzyme



Allen, 2015

Mediator

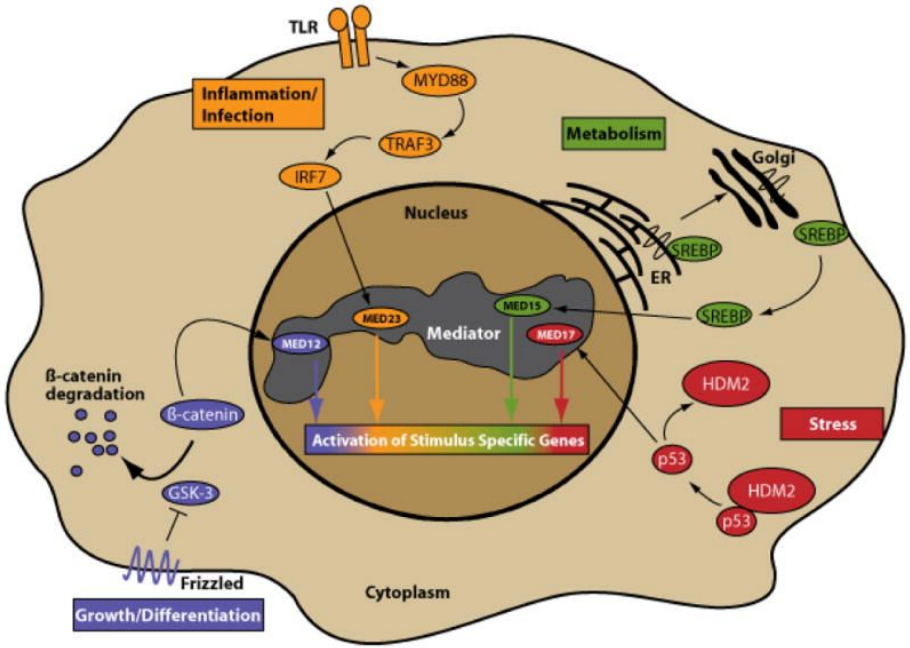
Huge multisubunit complex



Allen, 2015

Mediator

Different components of mediator are regulated via different signal pathways

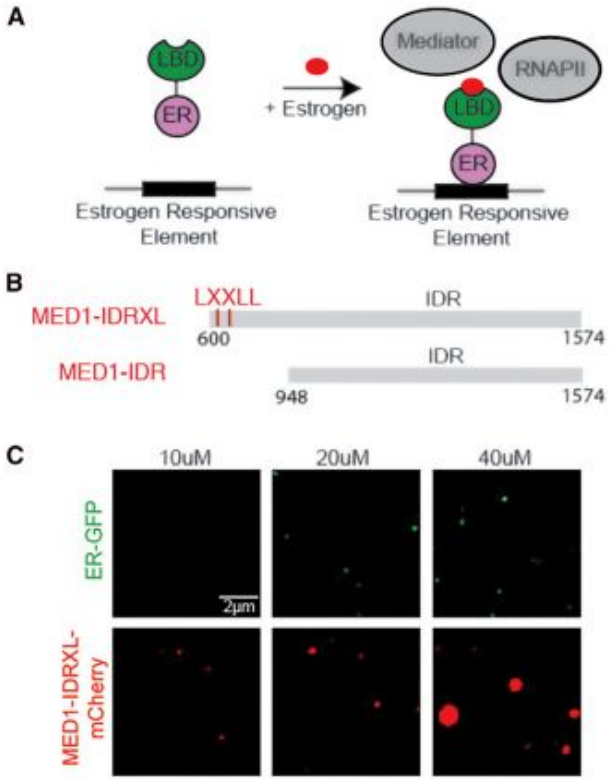


Allen, 2015

TF-induced condensates

Multiple different TFs form droplets with Mediator

Estrogen receptor forms droplets with Mediator in estrogen-dependent manner

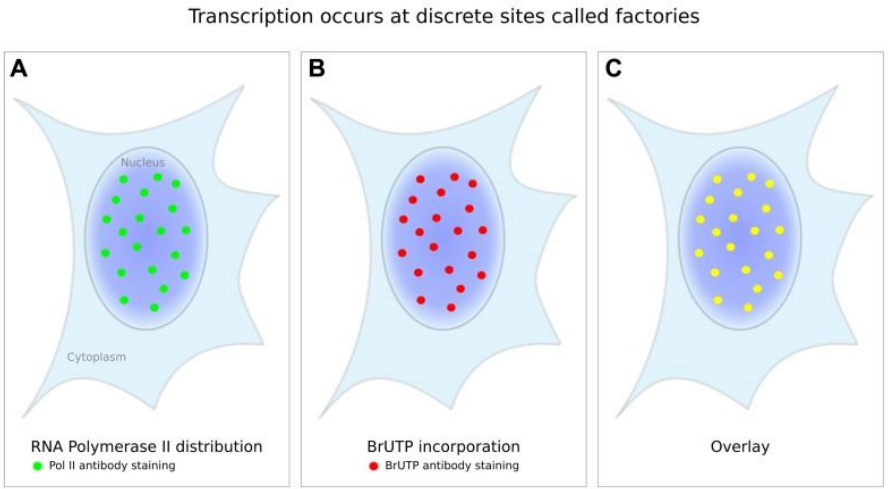


Boija, 2018

Transcription factories

Colocalization of RNAPII and UTP incorporation

100-8000 depending on cell state



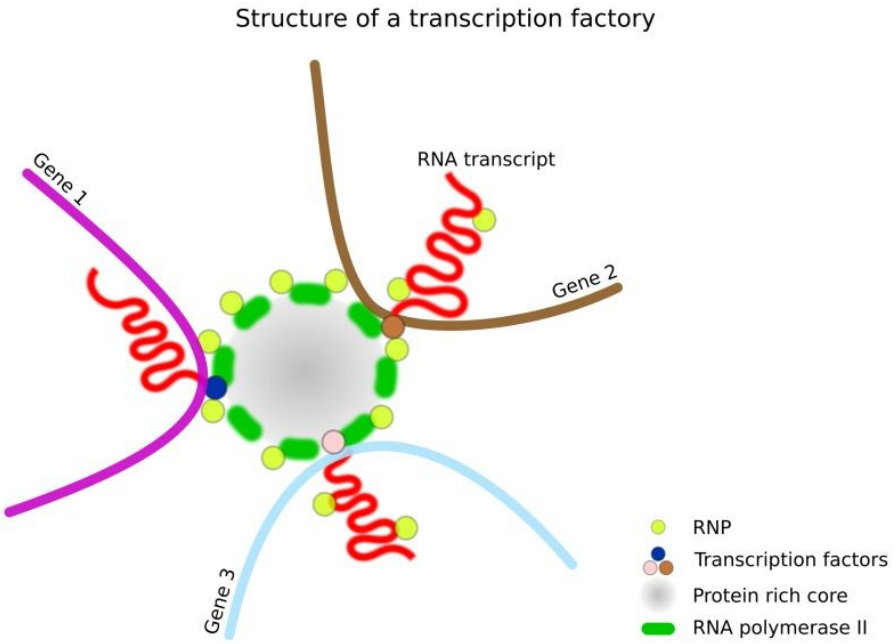
Rieder, 2012

Transcription factories

Contain 4–30 stationary RNA polymerase II molecules which are located on the surface of a protein-rich core

Contain co-activators, chromatin remodelers, transcription factors, histone modification enzymes, RNPs, RNA helicases, and splicing and processing factors

40 to 198 nm in size



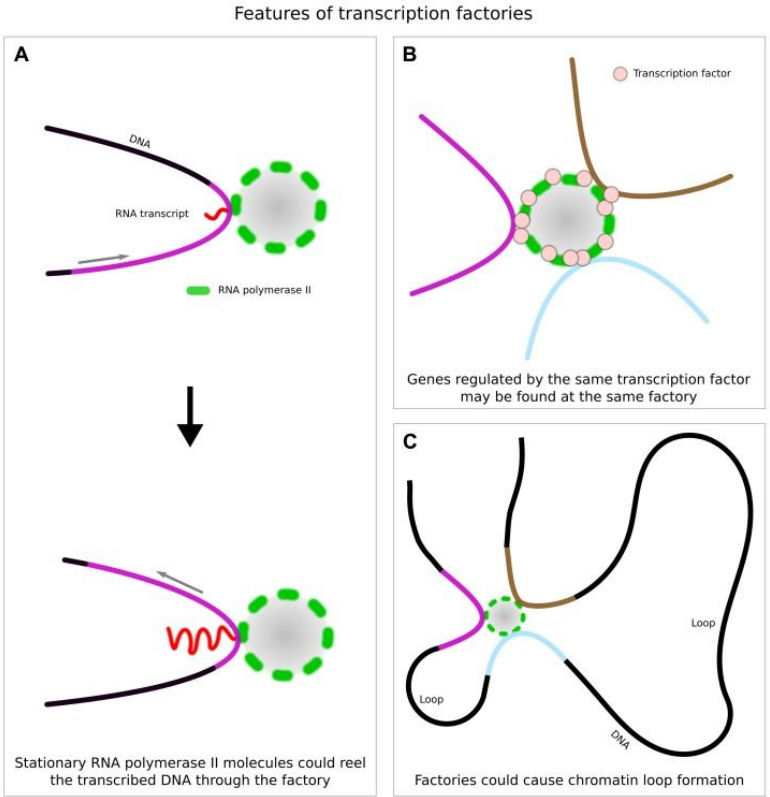
Rieder, 2012

Transcription factories

Transcription factories are probably stationary

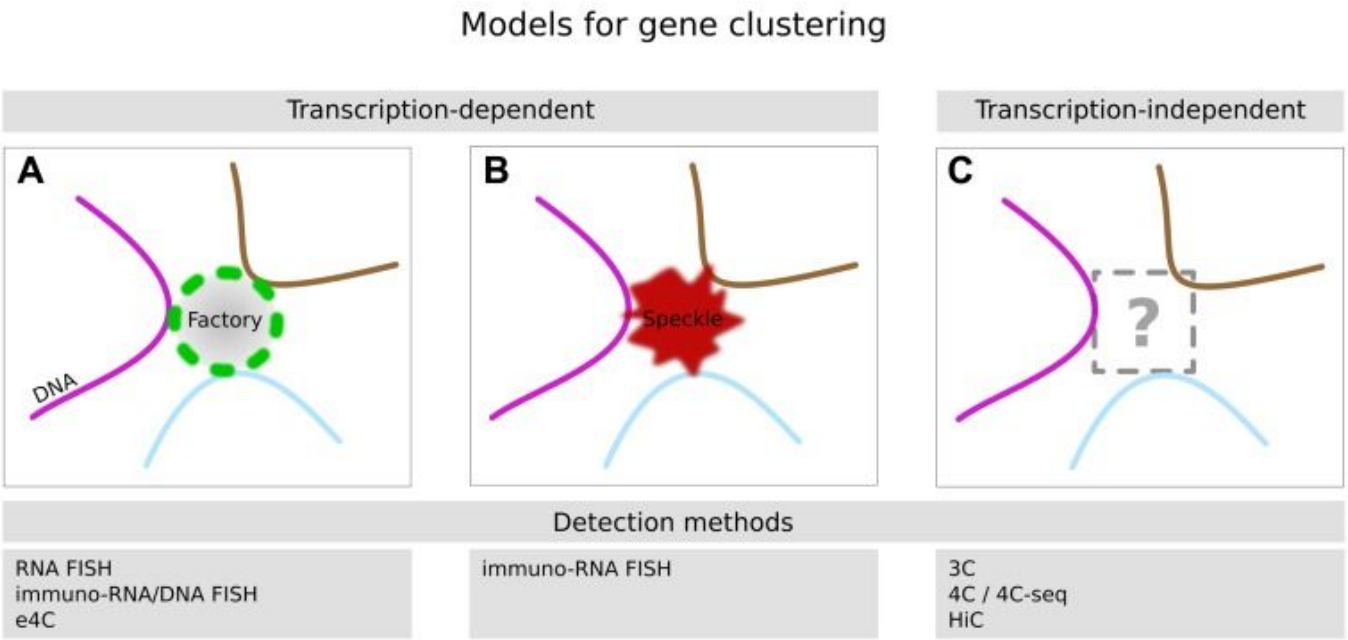
They may have TF preference

Can cause chromatin looping



Rieder, 2012

Several models of gene clustering

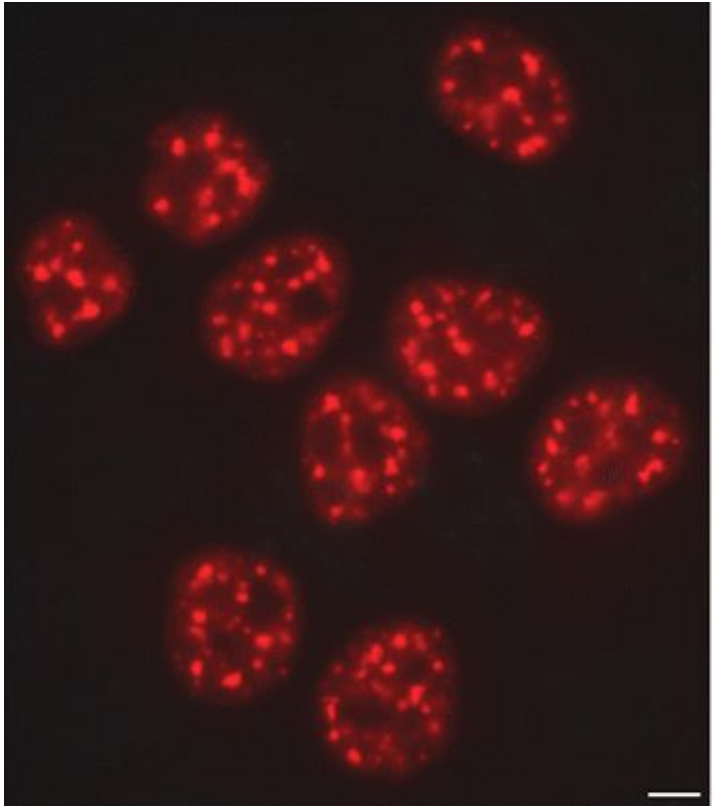


Rieder, 2012

Nuclear speckles

Contain splicing machinery (snRNP and SR proteins)

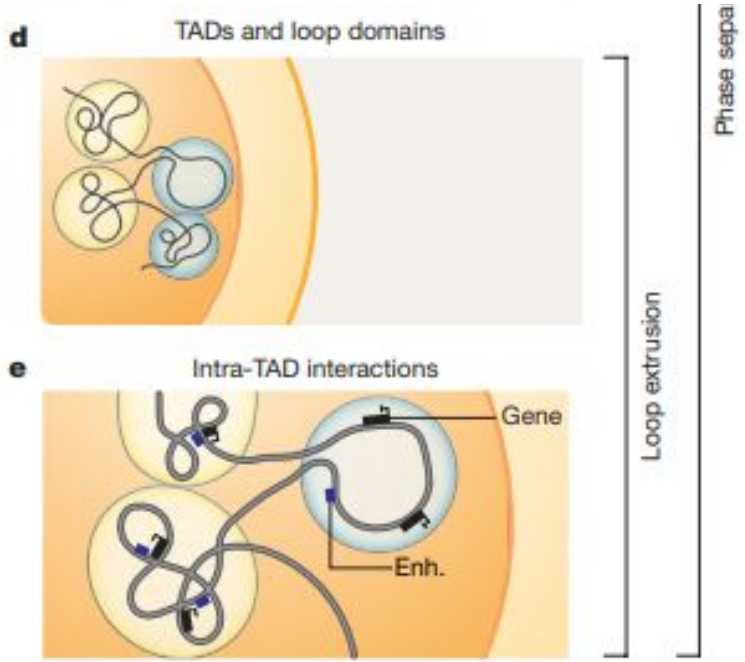
Some other proteins - translation factors, transcription factors



Spector, 2011

Interchromosomal domains

Topologically associating domains (TADs)

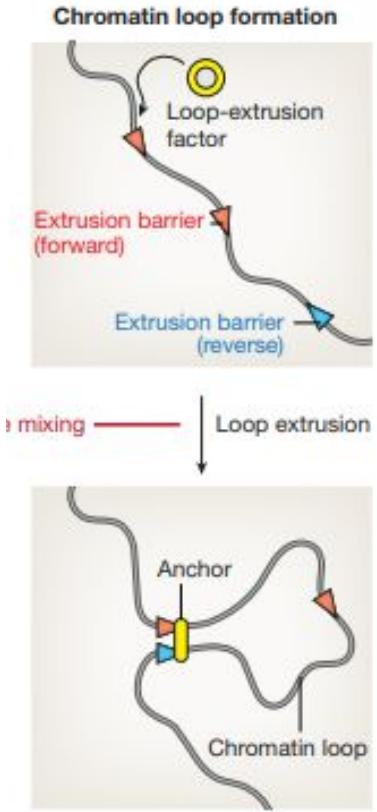


Stadhouder, 2019

TAD formation

Cohesin serves as a loop for DNA extrusion

CTCF is a stopping signal. Separates heterochromatin

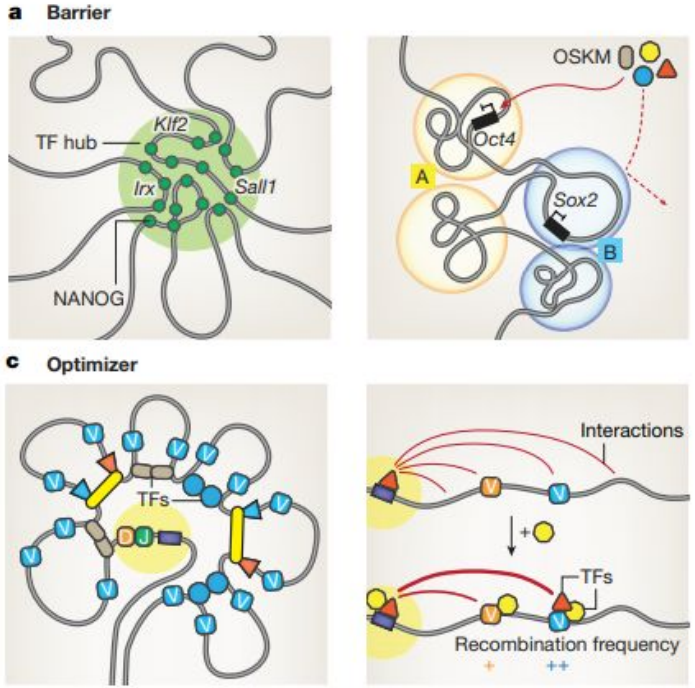


Stadhouder, 2019

Topology and gene functioning

A - maintaining expression levels to ensure stability of cell fate

C - TFs initiate locus contraction to ensure that all VDJ segments are equally available

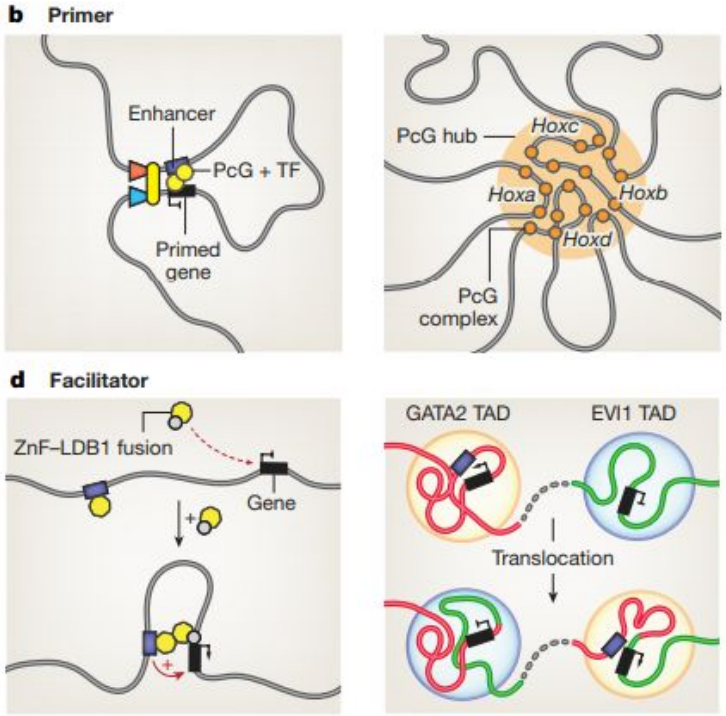


Stadhouder, 2019

Topology and gene functioning

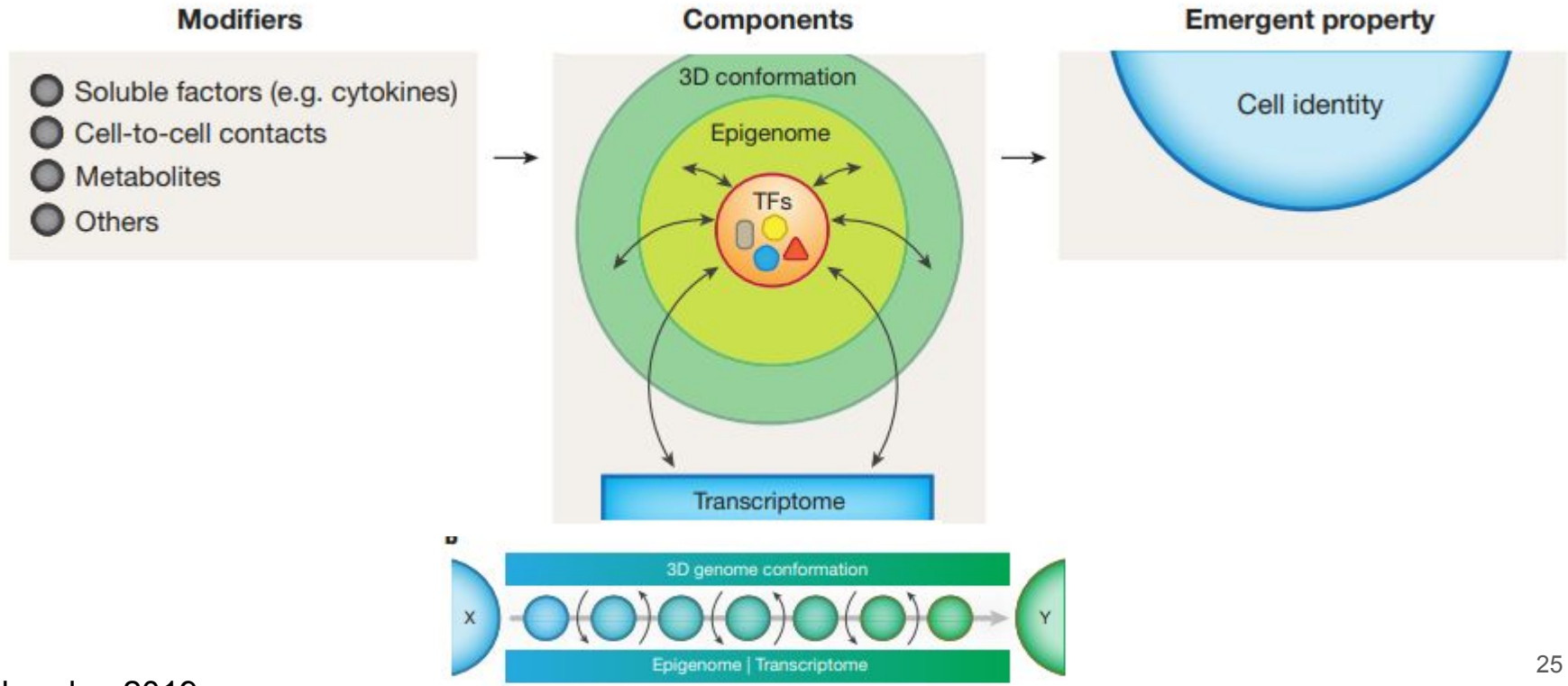
B - TFs may ensure that genes are primed for rapid activation

D - Genome architecture alteration alone can active genes without signal transmission



Stadhouder, 2019

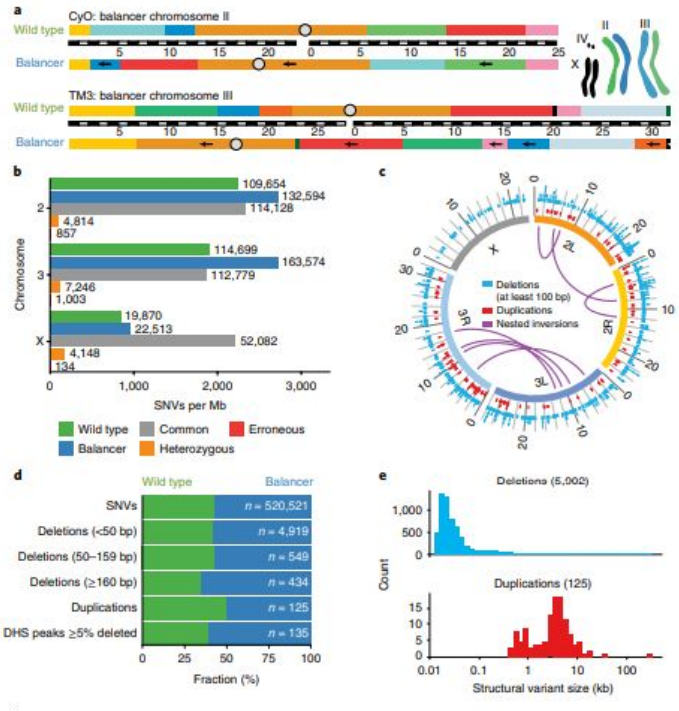
Overall regulatory scheme



Stadhouder, 2019

3D structure is not everything

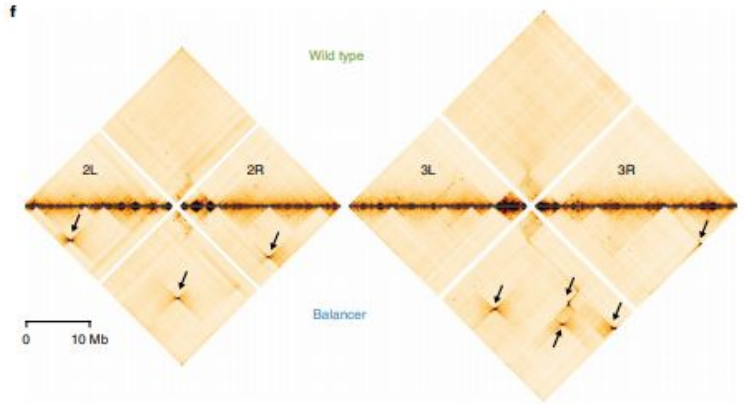
Drosophila lines with highly rearranged genome



Ghavi-Helm, 2019

TAD disruption

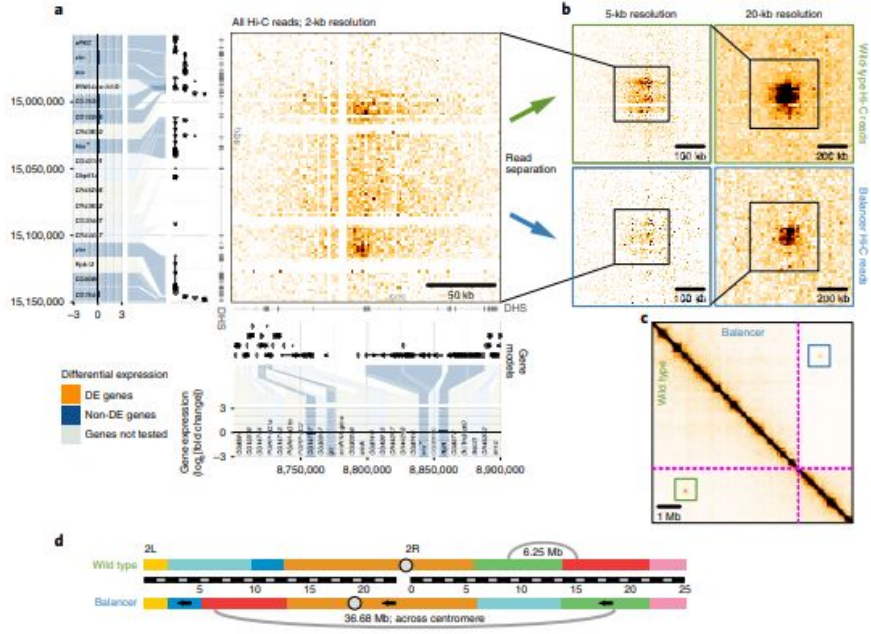
Disrupted both long and short-range interactions



Ghavi-Helm, 2019

Gene expression changes

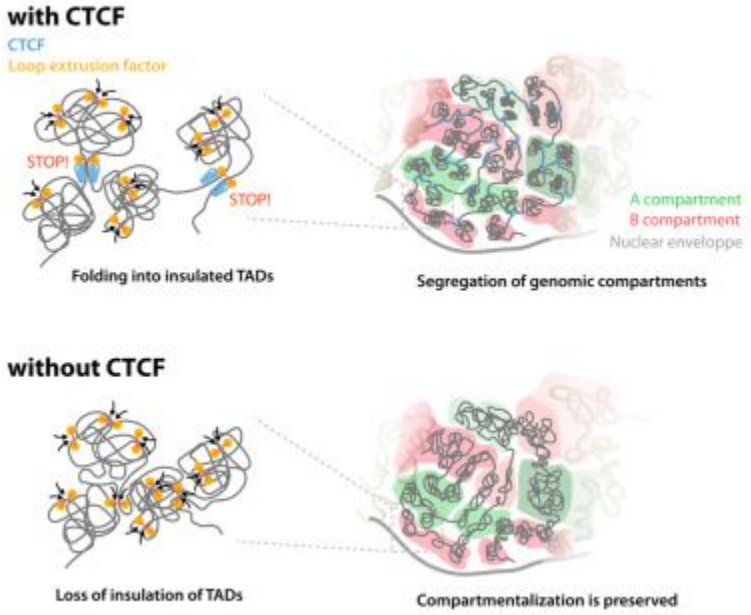
Gene expression near rearrangements was mostly unaltered



Ghavi-Helm, 2019

CTCF loss

CTCF degradation leaves genomic compartmentalization mostly unaffected

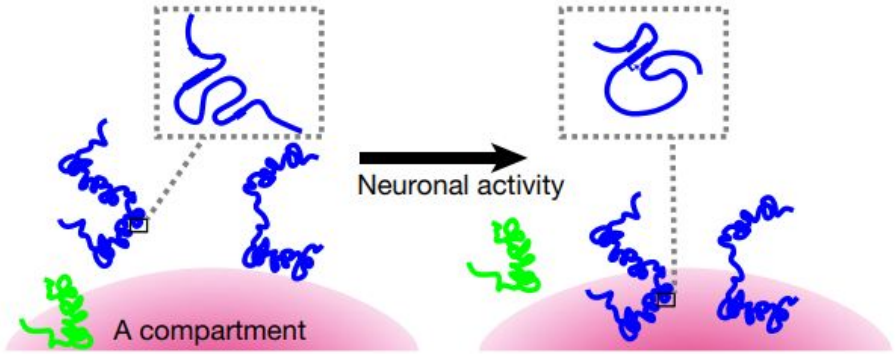


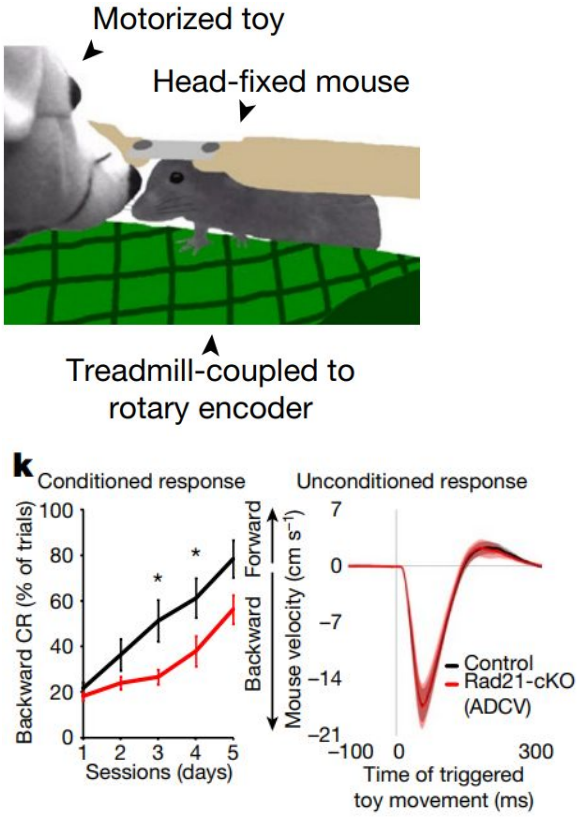
Nora, 2017

Manipulating 3D architecture to learn

Motor learning causes chromatin rearrangement

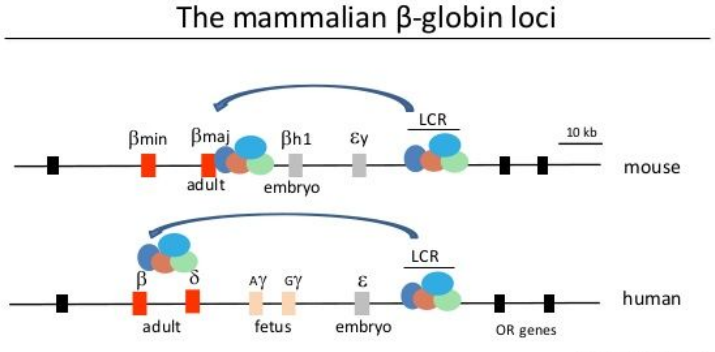
Depletion of cohesin impairs learning





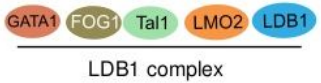
Yamada, 2019

Manipulating 3D architecture for therapy

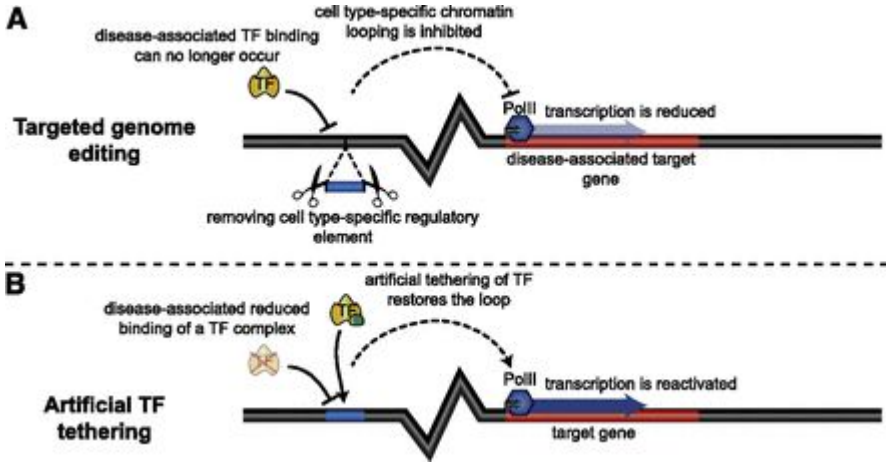


Tolhuis et al, Mol. Cell, 2002
Palstra et al, Nat. Genet., 2003

Locus control region (LCR) enhancer loops to genes determining which one is active as development proceeds



Drissen *et al, Genes Dev.*, 2004
Vakoc *et al, Cell*, 2005
Song *et al. Mol. Cell*, 2007
Yun *et al, NAR*, 2014



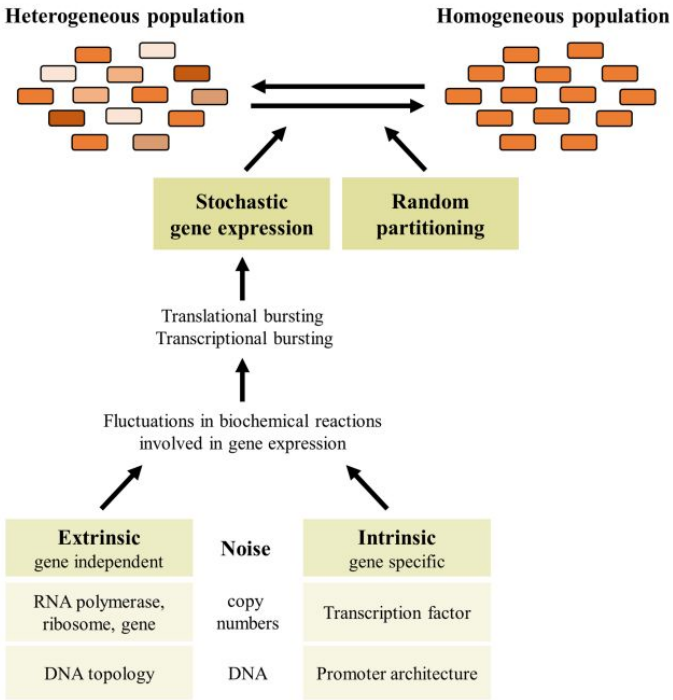
van den Heuvel, 2015

Why do we need biological replicates?

Cell-to-cell variation

Living cells are not homogenous even in the same environment

There are different reasons for that



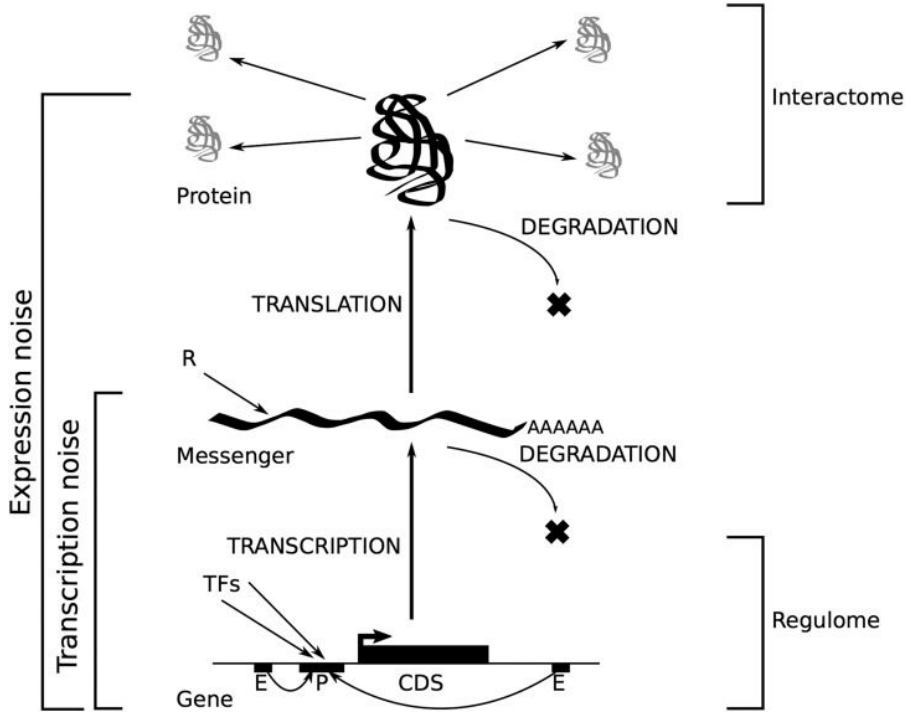
Engl, 2018

Expression noise

Biochemical reactions rely on interacting molecules

Diffusion and binding are stochastic

Isogenic cells in the same environment can be different



Barroso, 2018

Stochasticity in cells

Intrinsic stochasticity is “generated by the dynamics of the system from the random timing of individual reactions” and extrinsic stochasticity is “generated by the system interacting with other stochastic systems in the cell or its environment.” (Shahrezaei and Swain, 2008)

Transcription noise

Intrinsic - gene-specific (promoter architecture, transcription factors)

Extrinsic - cell-specific (availability of RNAP, DNA topology, ribosomes, copy number variation)

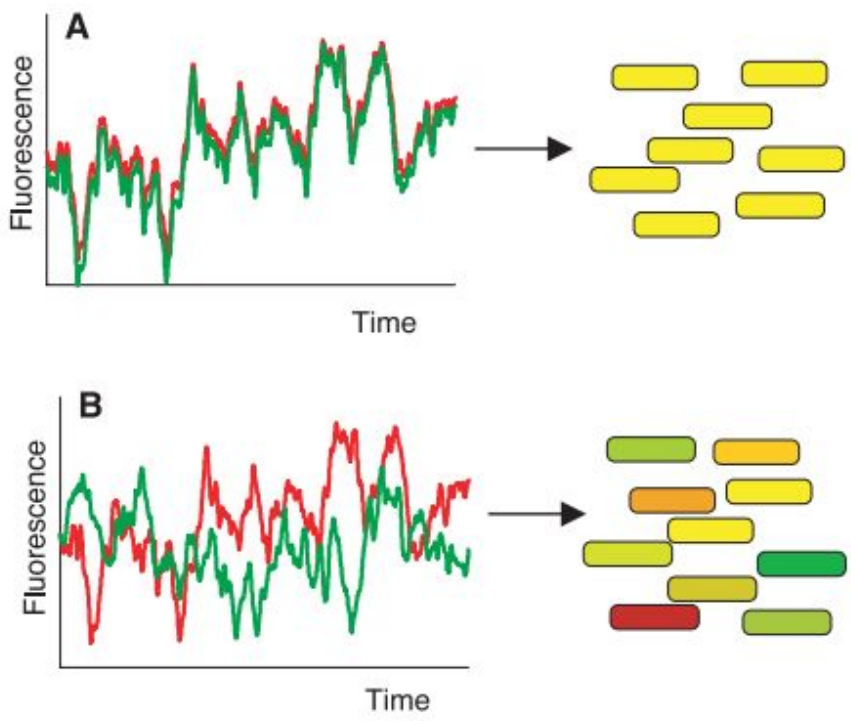
Extrinsic gene independent	**Noise**	**Intrinsic** gene specific
RNA polymerase, ribosome, gene	copy numbers	Transcription factor
DNA topology	DNA	Promoter architecture

Engl, 2018

Measuring transcription noise

A - extrinsic noise. Cells may differ but all genes are equally affected

B - intrinsic noise. Genes differ



Elowitz, 2002

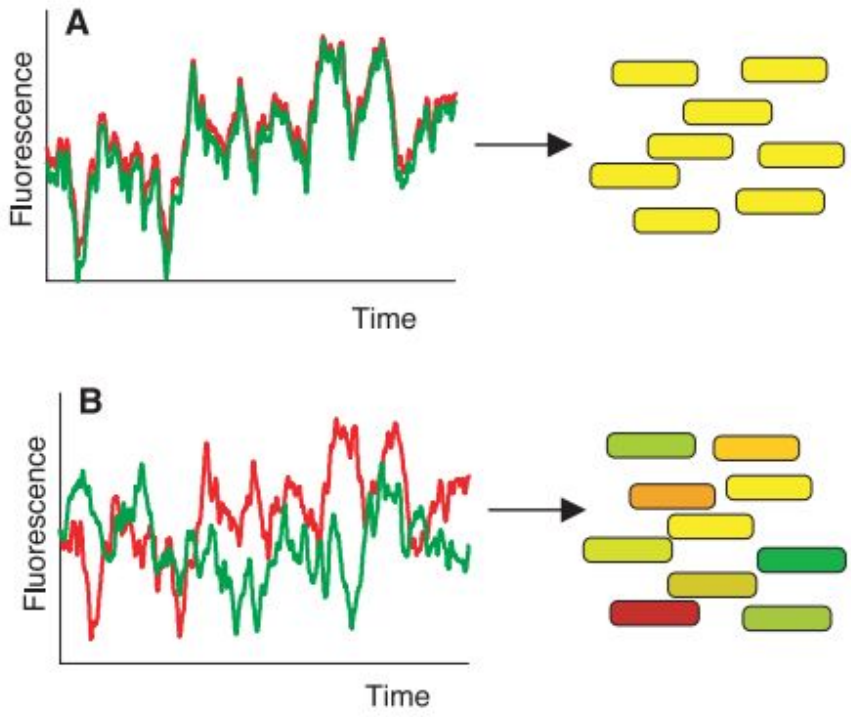
Measure different noises

YFP and CFP controlled by lac promoter

Genes are equidistant from oriC

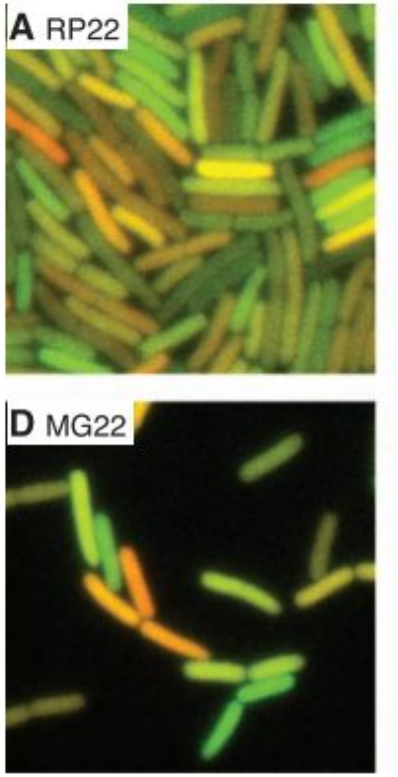
Fluorescent intensity difference tells about intrinsic noise

Total noise ^ 2 = Intrinsic noise ^ 2 + Extrinsic noise ^ 2



Distinguishing between extrinsic and intrinsic noise

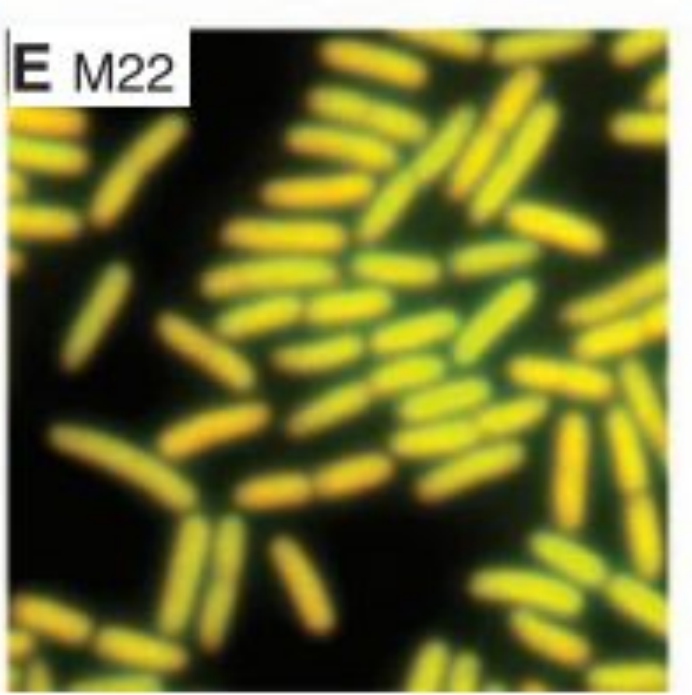
Wild-type (i.e. lacI+) strains are noisy



Elowitz, 2002

Distinguishing between extrinsic and intrinsic noise

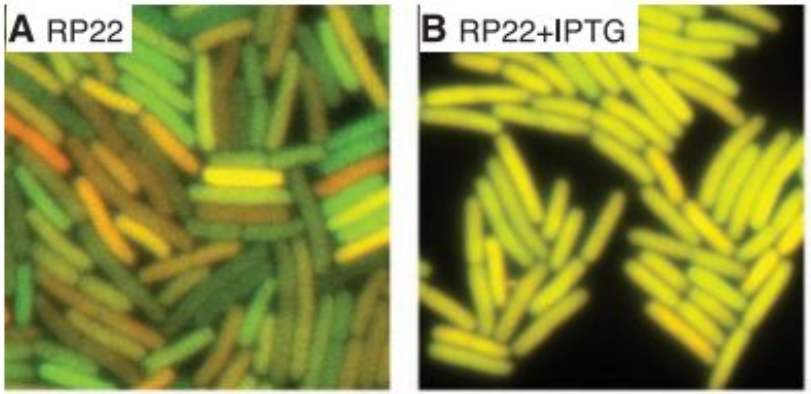
Strain without lacI is not noisy



Elowitz, 2002

Distinguishing between extrinsic and intrinsic noise

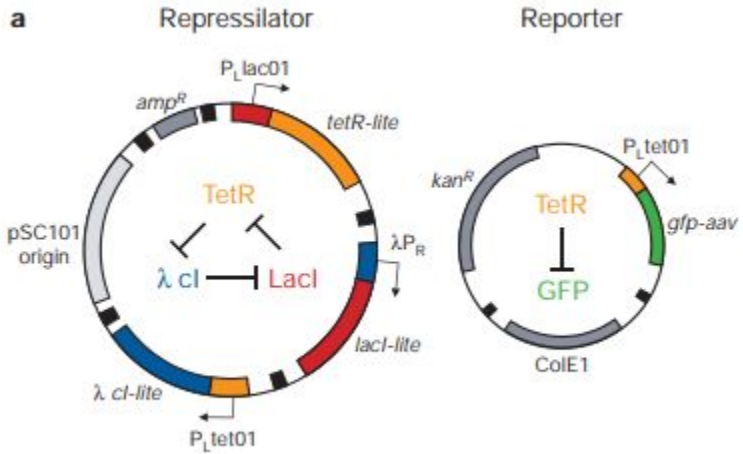
Blocking lacI with IPTG reduces noise



Elowitz, 2002

Repressilator

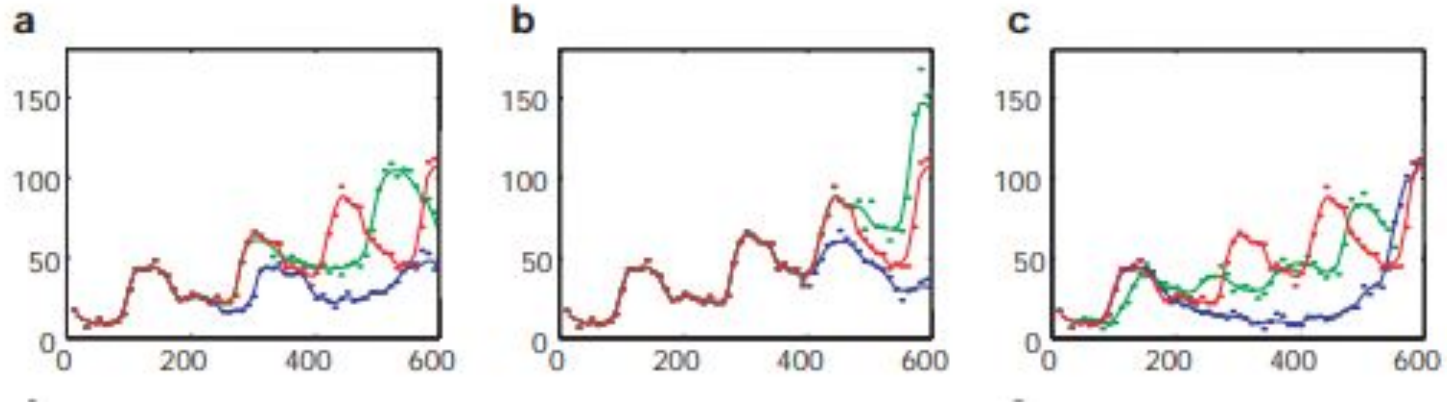
Oscillatory behaviour because of mutually repressed genes



Elowitz, 2000

Repressilator

Oscillatory behaviour rapidly falls apart :(



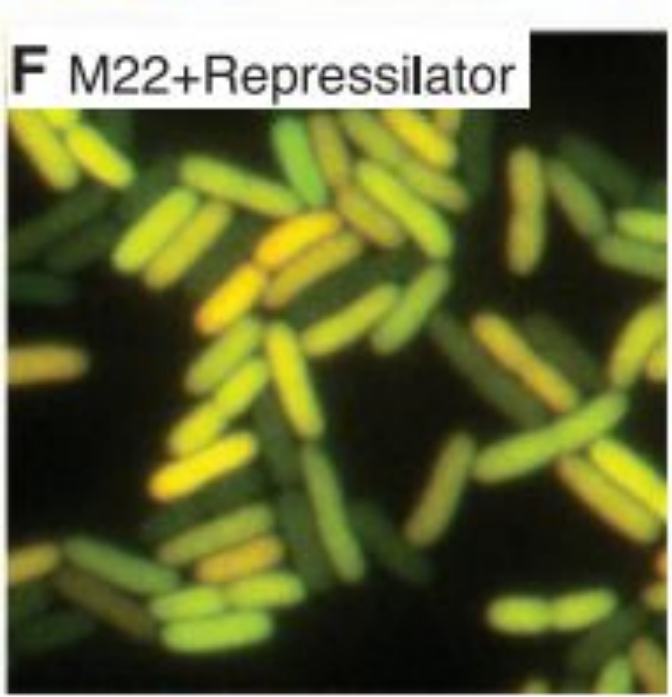
Elowitz, 2000

Distinguishing between extrinsic and intrinsic noise

Repressilator induces noise

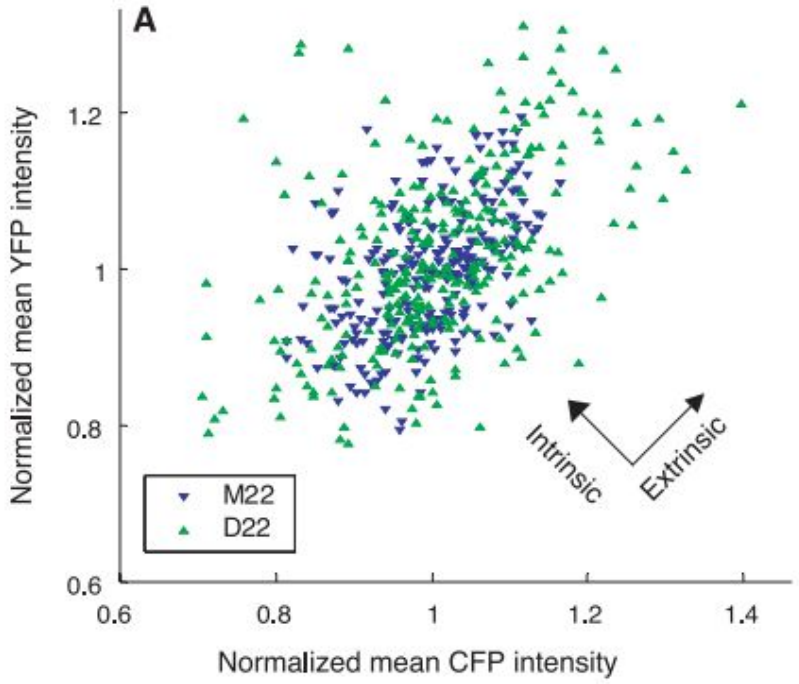
Also note increased total noise

Intrinsic noise is increased when not in a steady state



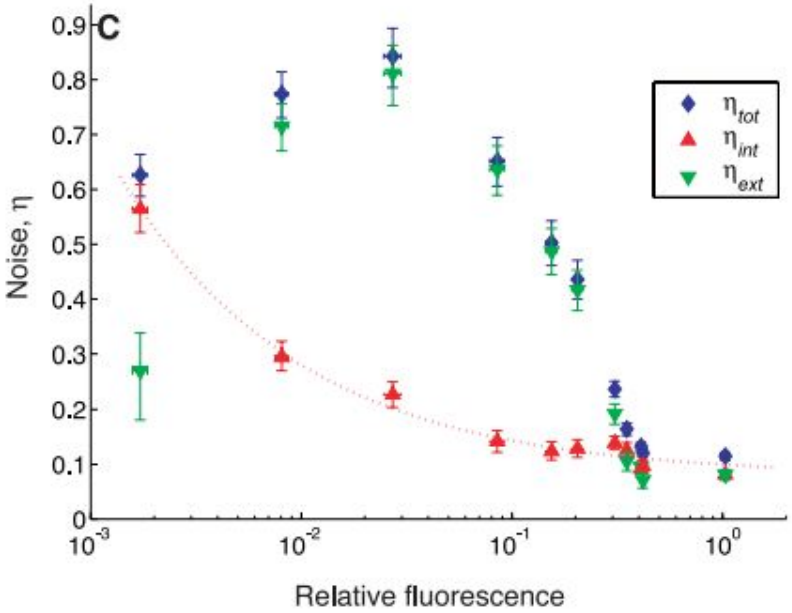
Elowitz, 2002

Noisy vs non-noisy strain

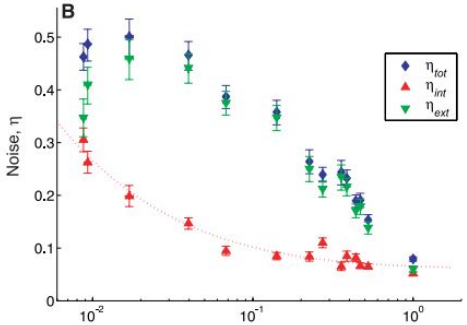


Elowitz, 2002

RecA decreases intrinsic noise



D22 (recA-, lacI-)

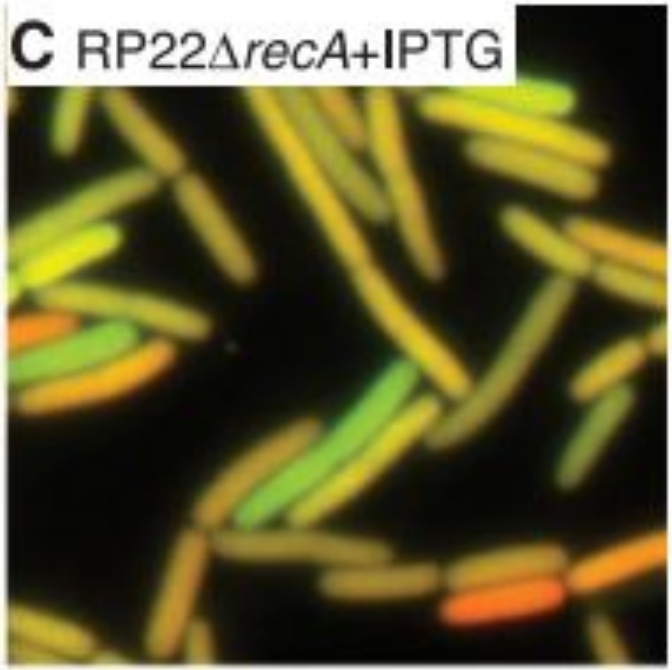


M22 (recA+, lacI-)

Elowitz, 2002

Distinguishing between extrinsic and intrinsic noise

recA deletion increases intrinsic noise

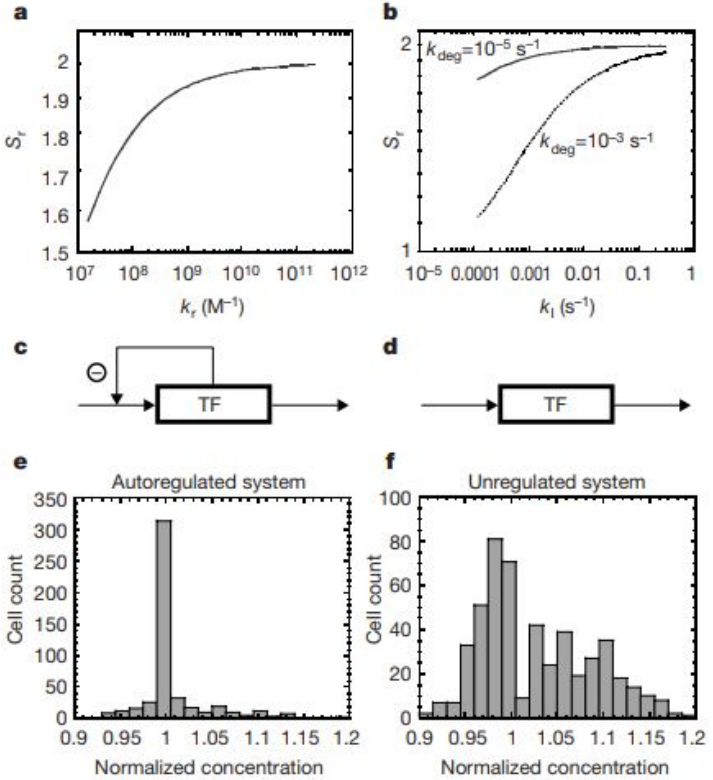


Elowitz, 2002

Feedback regulation

Feedback regulation decreases noise

May be the reason why it is so popular in gene regulatory networks

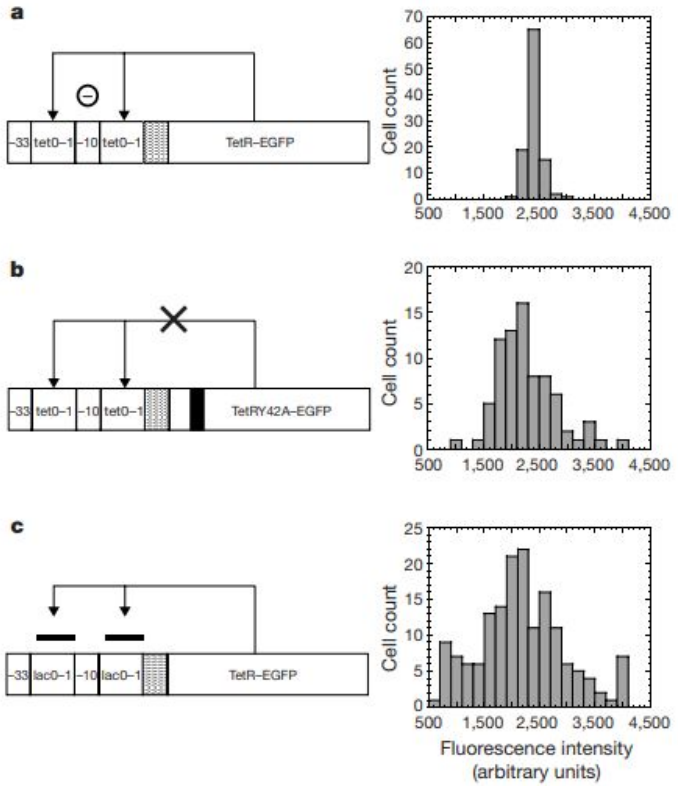


Becskei, 2000

Feedback regulation

Repressing feedback regulation increases noise

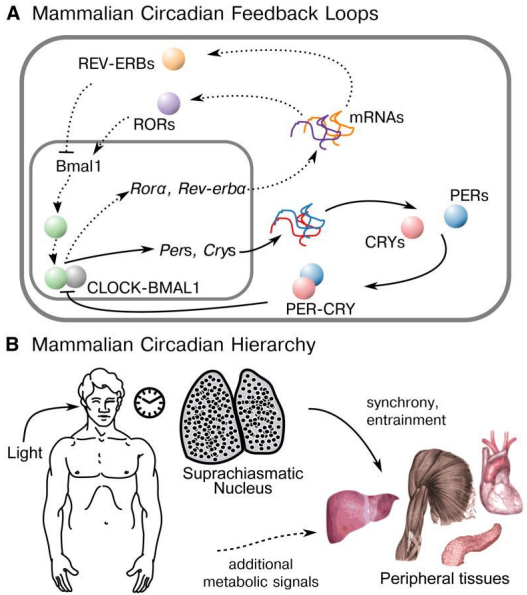
Can be done via mutating repressor or deleting operators

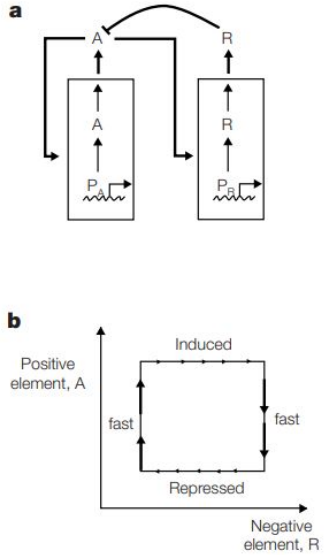


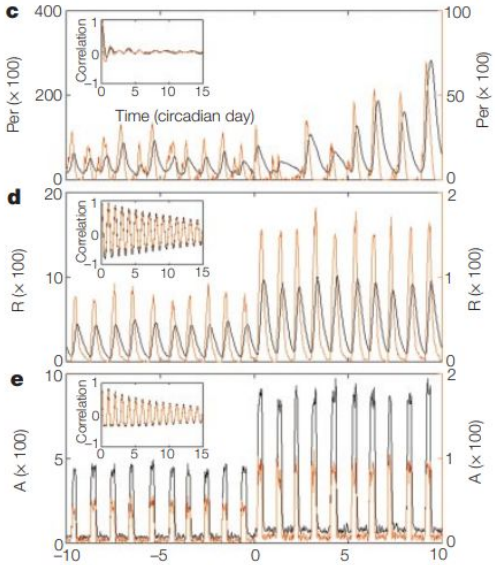
Becskei, 2000

Fighting noise in circadian clocks

Circadian systems should be noise resistant



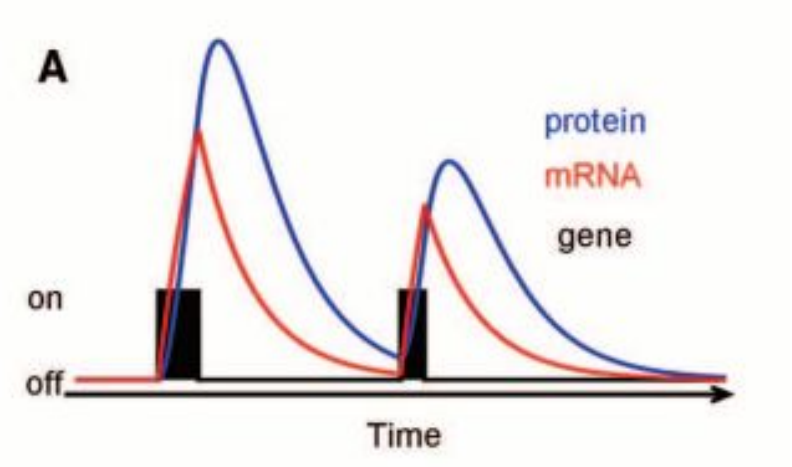




Abel, 2006; Barkai, 2000

Transcription bursts

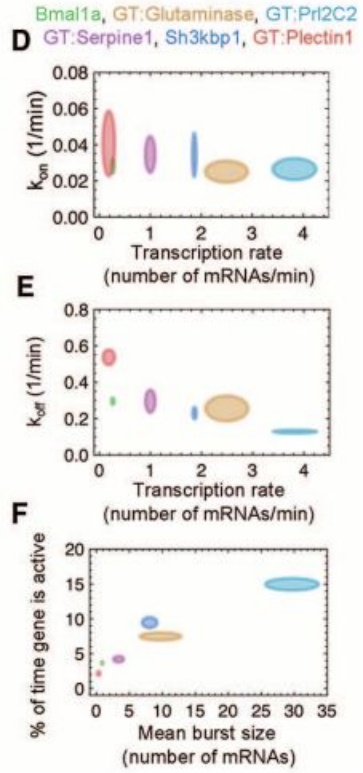
In prokaryotes and eukaryotes, most genes appear to be transcribed during short periods called transcriptional bursts, interspersed by silent intervals



Suter, 2011

Transcription bursts

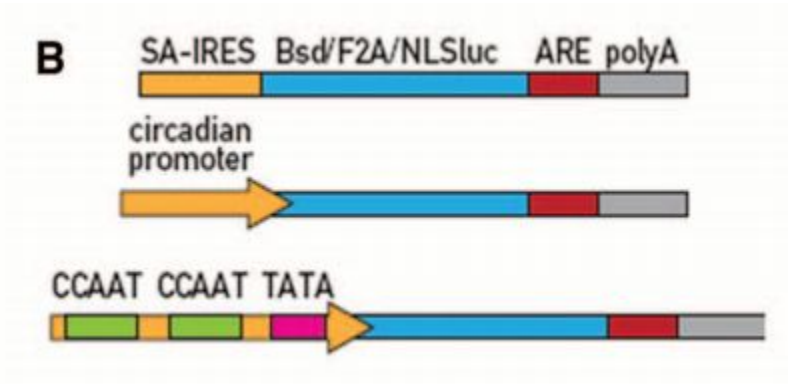
Burst kinetics is gene-specific

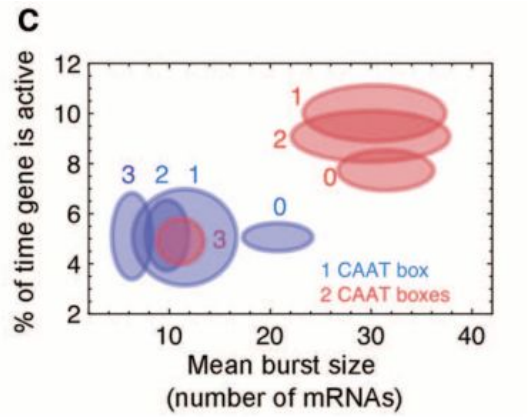


Suter, 2011

Transcription bursts

Promoter structure influences burst kinetics



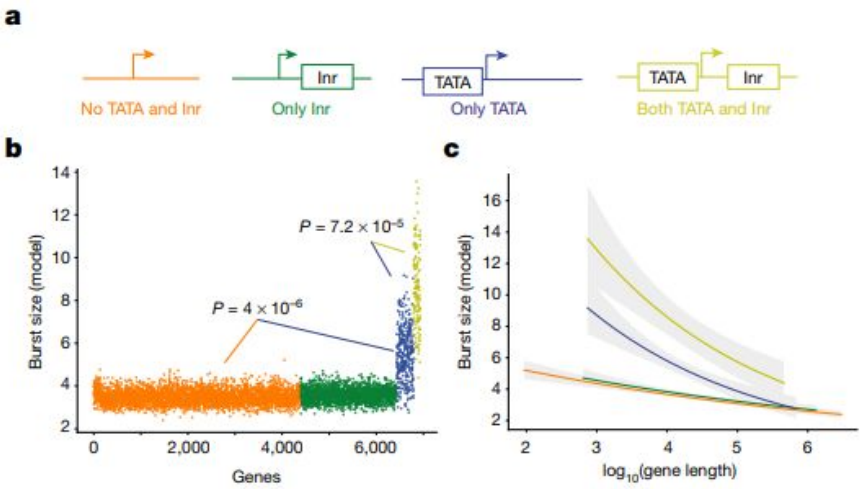


Suter, 2011

Core promoter regulates bursts

TATA increases bursts

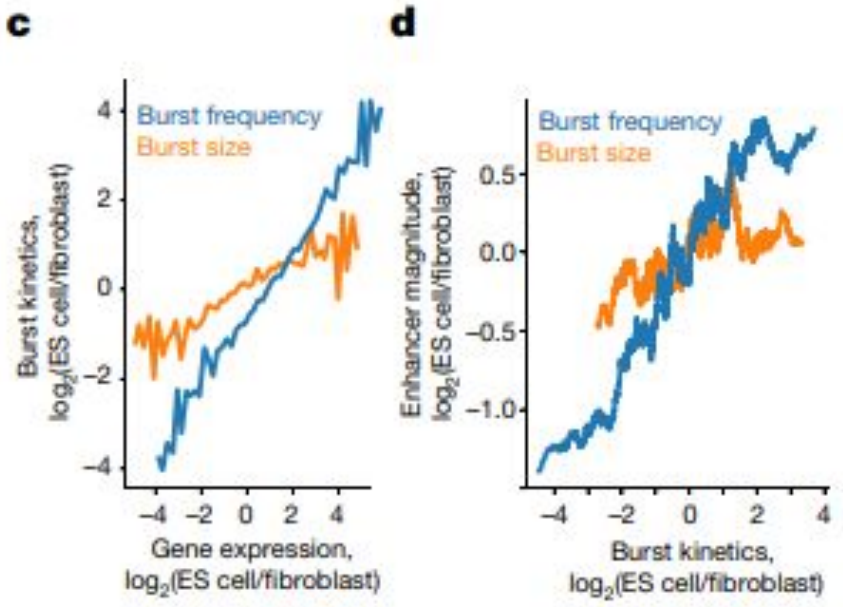
Initiator increases bursts if TATA is present



Larsson, 2019

Enhancers also regulate bursts

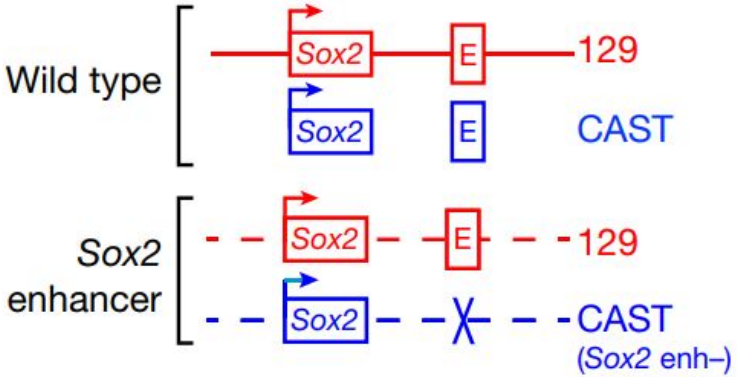
Transcription bursts are cell type-specific

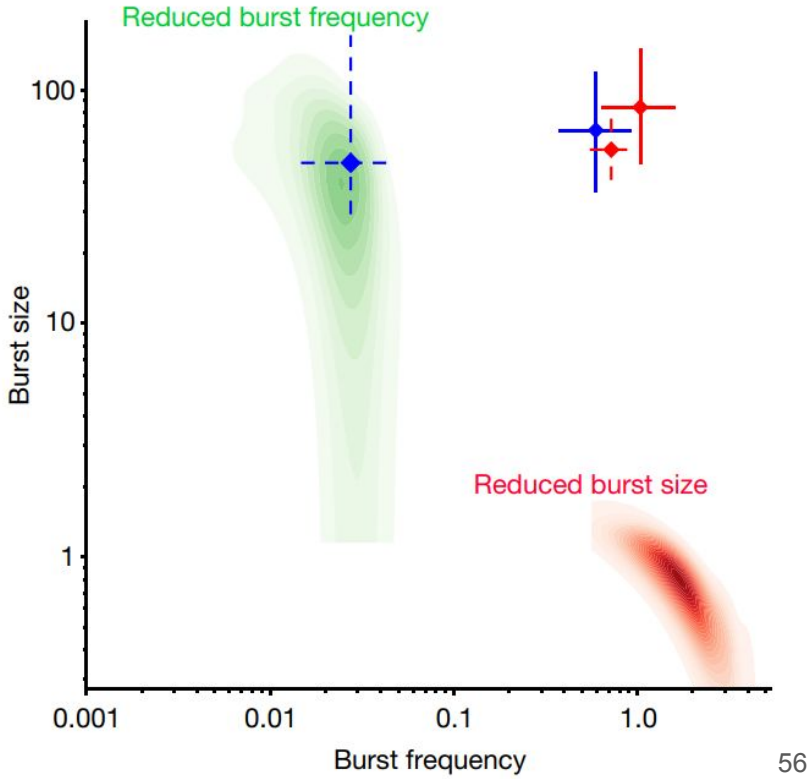


Larsson, 2019

Enhancers also regulate bursts

Mutating enhancer can alter burst kinetics





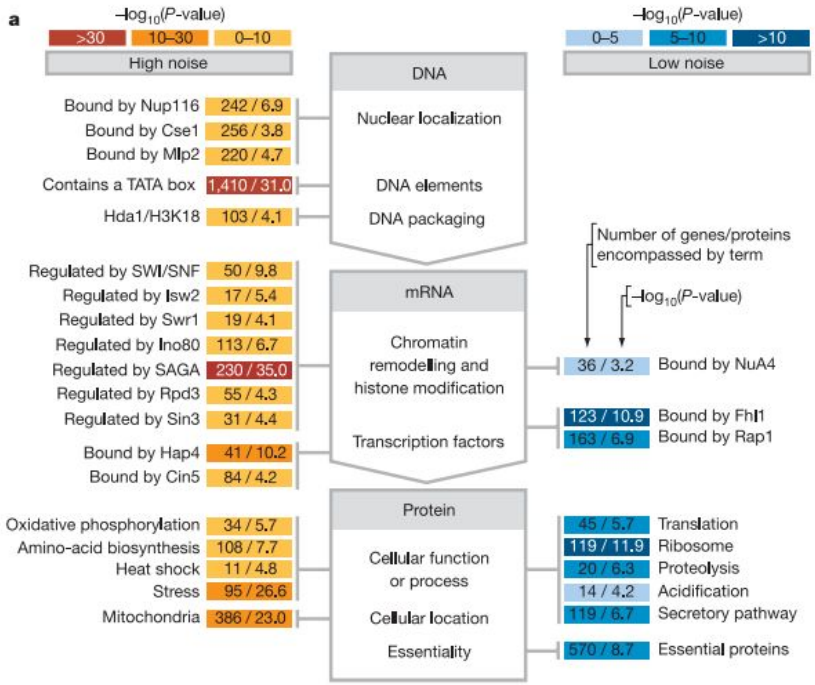
Larsson, 2019

Noise is random but noisiness is not

Biological noise differs highly across biological groups

Housekeeping genes tend to be less noisy

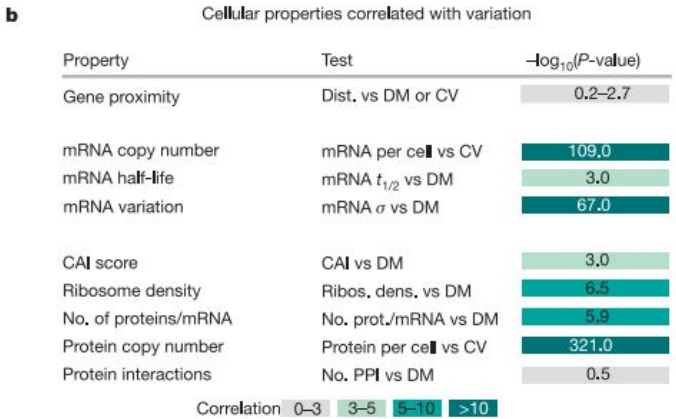
Chromatin remodeling tends to induce noise in target genes



Newman, 2006

Noise is random but noisiness is not

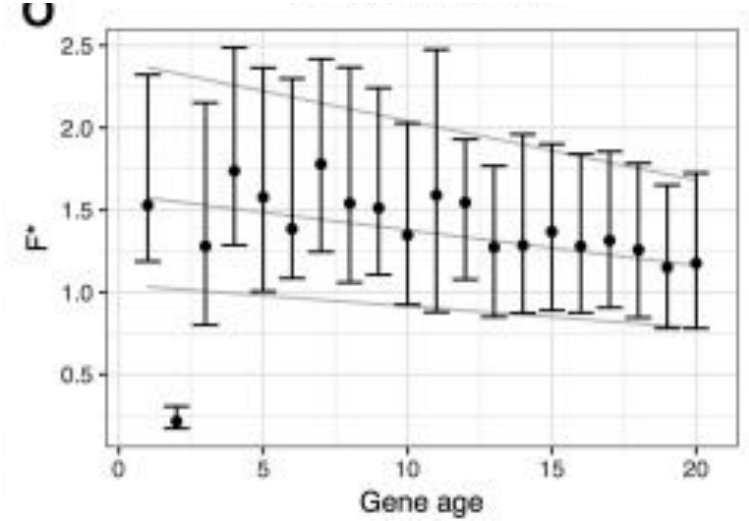
mRNA or protein copy number and the variation in mRNA expression are very strongly correlated with CV and DM values, respectively



Newman, 2006

Noisiness is important in evolution

Older genes are less noisy



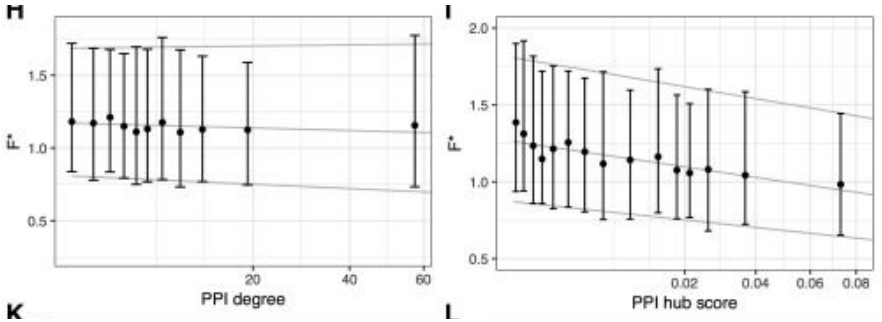
Barroso, 2018

Noisiness is important in evolution

Genes that encode highly connected proteins are less noisy

Protein position in PPI network defines transcriptional noise

Noisiness and mean expression are separate parameters in evolution

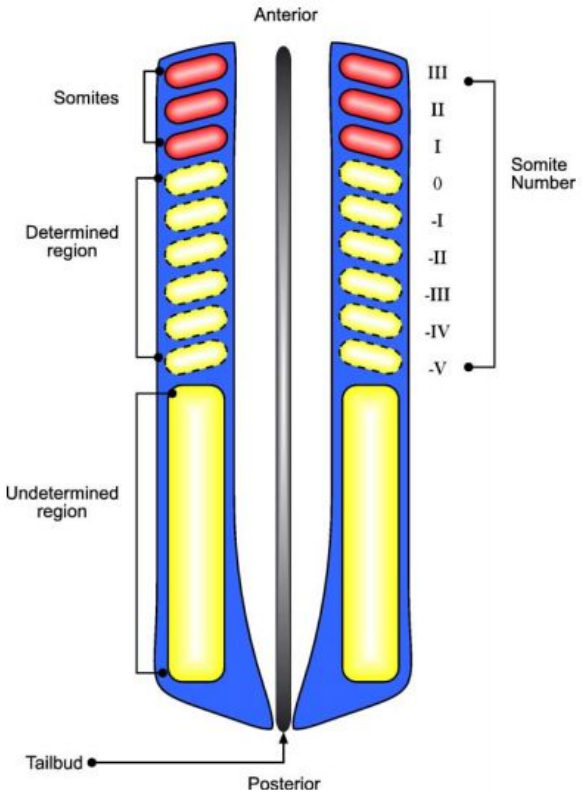


Barroso, 2018

Cell fate vs noise

Somite differentiation

Highly uniform process despite potential noise sources



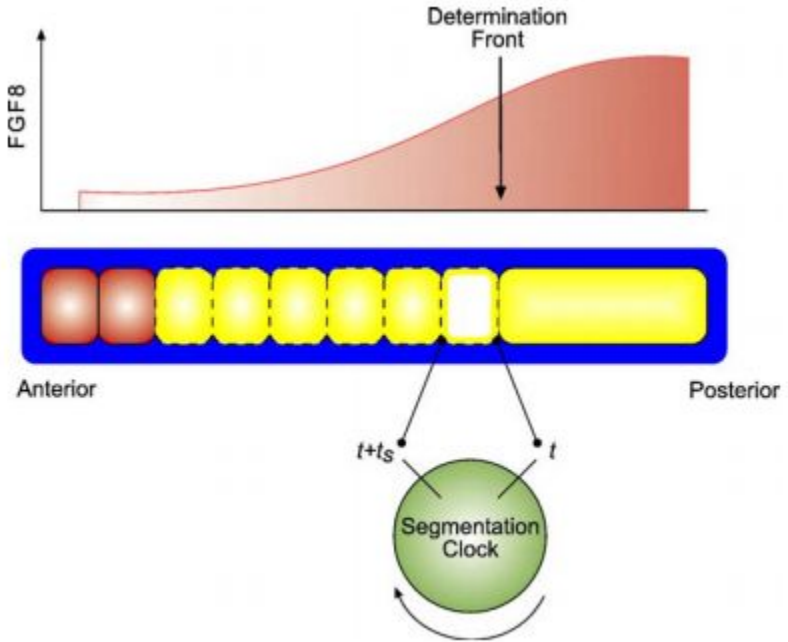
Baker, 2006

Clock and wavefront model

Longitudinal positional information gradient down the AP

Interaction with cellular oscillator

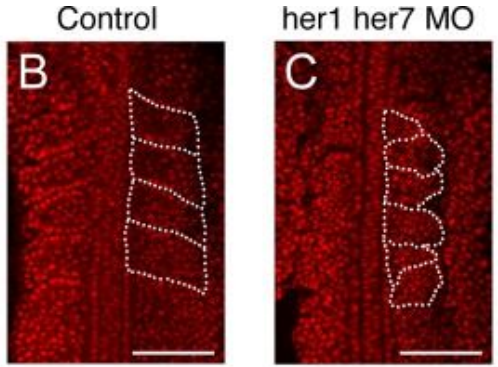
Clock determines the time of catastrophe - rapid change of cell state

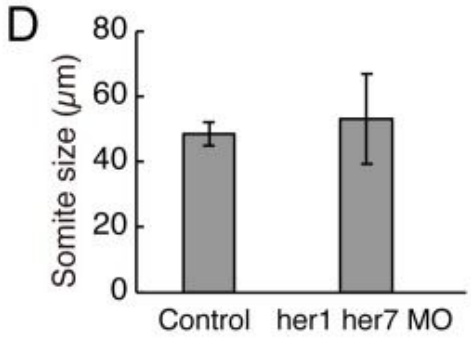


Baker, 2006

Breaking clock disrupts somites

Zebrafish mutants with disrupted clock have larger variation in somite size



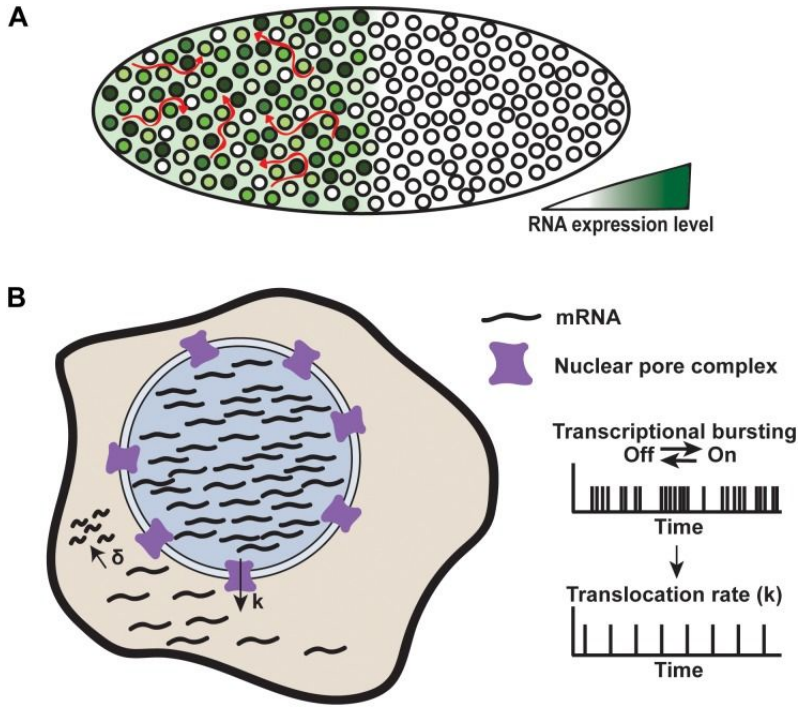


Naoki, 2019

Other ways to buffer noise

A - RNA levels are buffered across syncytium

B - transcription is noisy but translocation is not



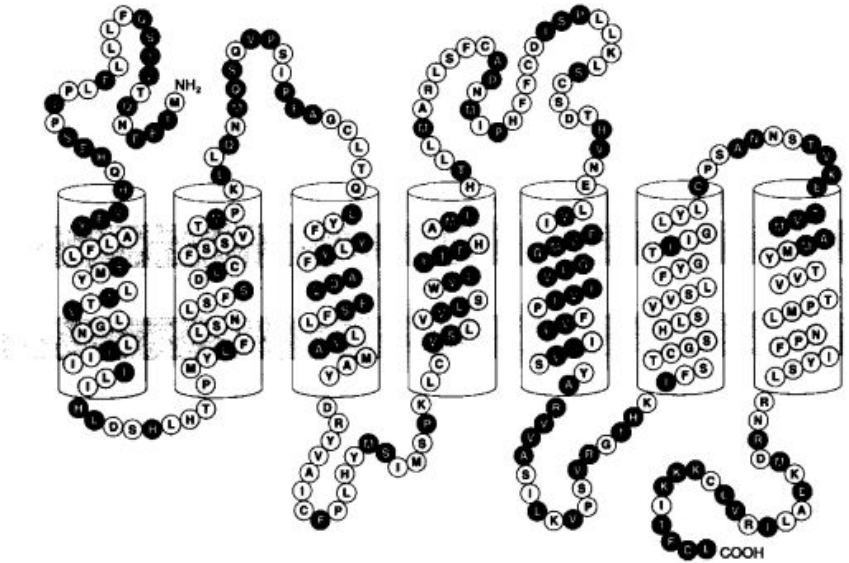
Urban, 2018

Olfactory receptors (ORs)

Encoded by a huge multigene family

Mice have ~1000 functional genes (3% of genome)

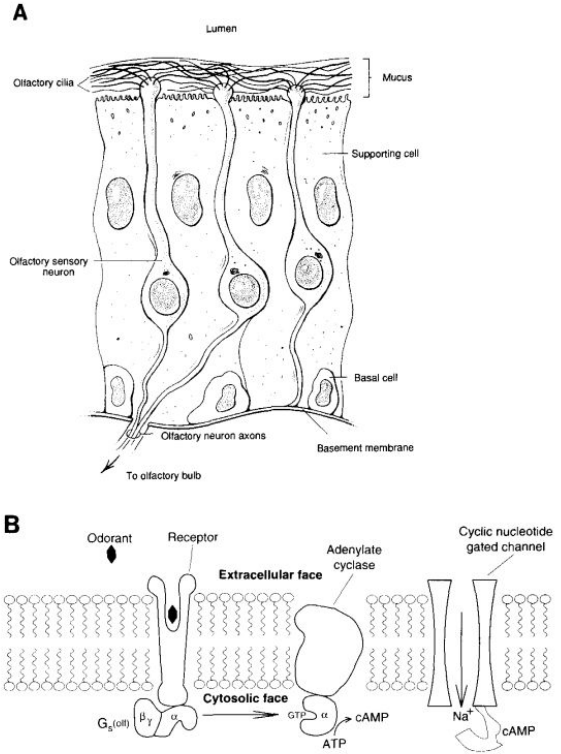
Humans have around 400



Buck, 1991

Olfactory sensory neurons

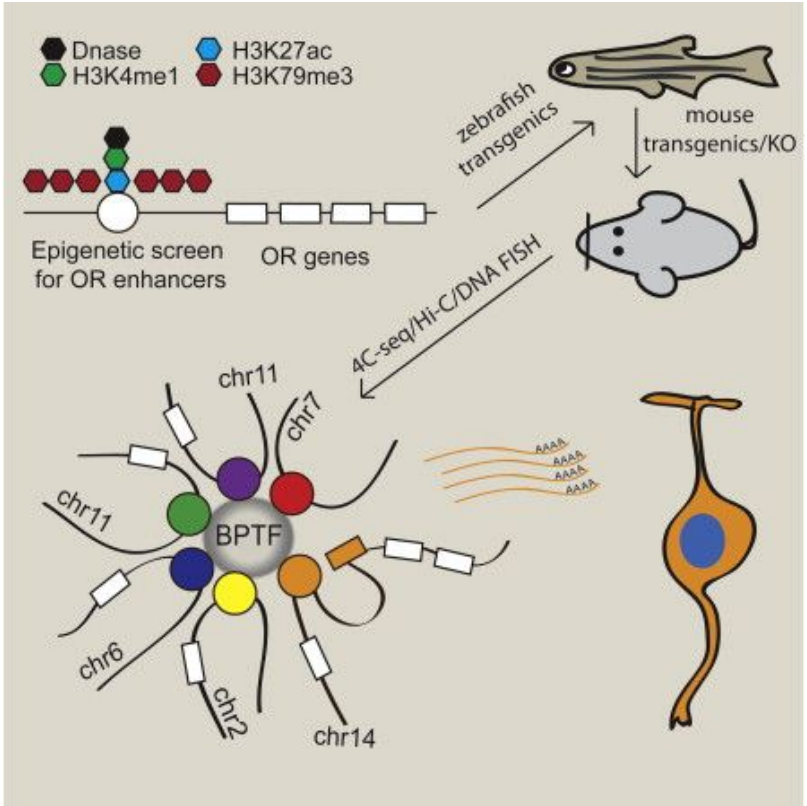
Each mature olfactory sensory neuron expresses one olfactory receptor gene in monoallelic and stochastic fashion



Buck, 1991

Greek islands

OR gene choice is controlled by multiple enhancers scattered across genome

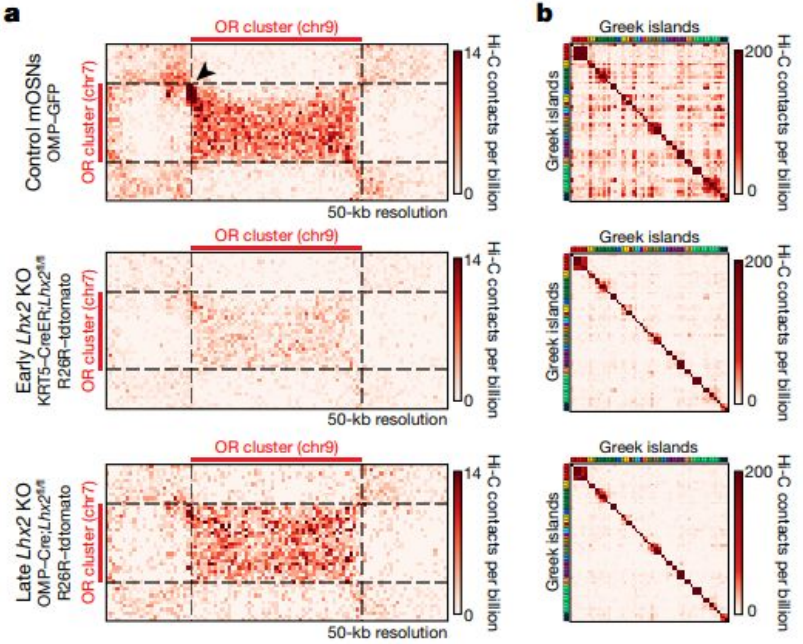


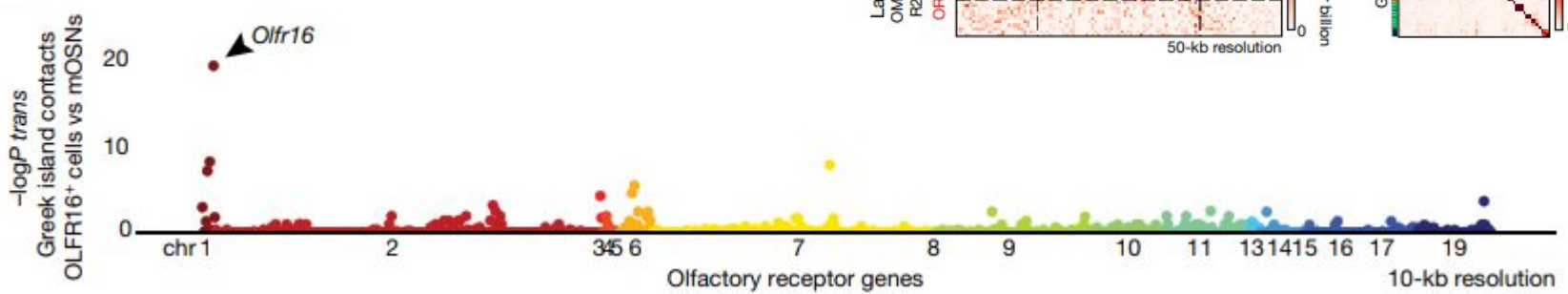
Markenscoff-Papadimitriou, 2014

Greek islands and ORs

Greek islands are bound by LHX2 and LDB1

Greek islands only interact with active OR genes





Monahan, 2019

Questions?