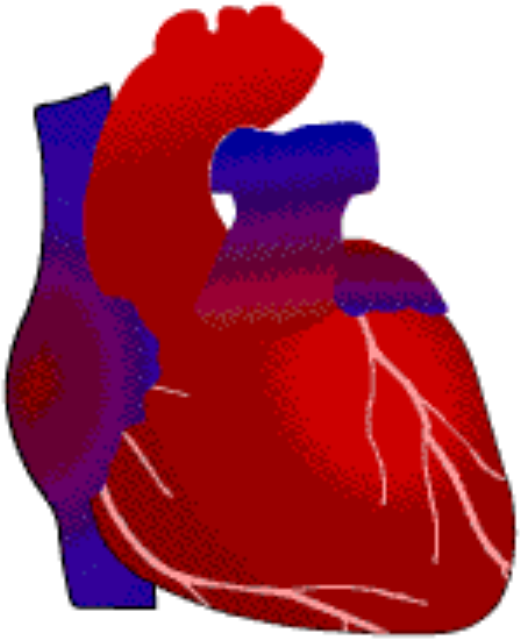
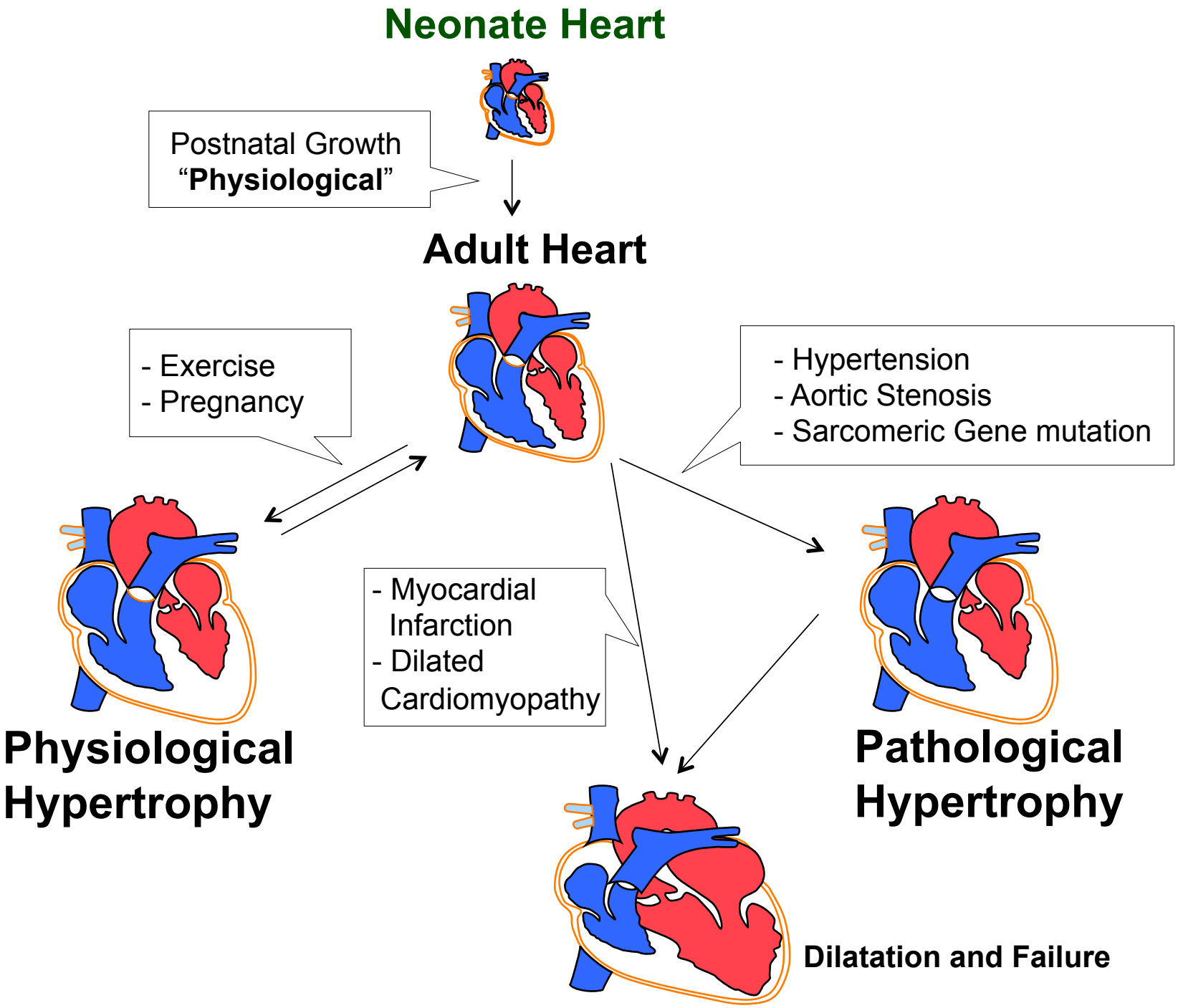
Transcriptional and Epigenetic Changes during Heart Disease

Danish Sayed

Unique Features of Heart

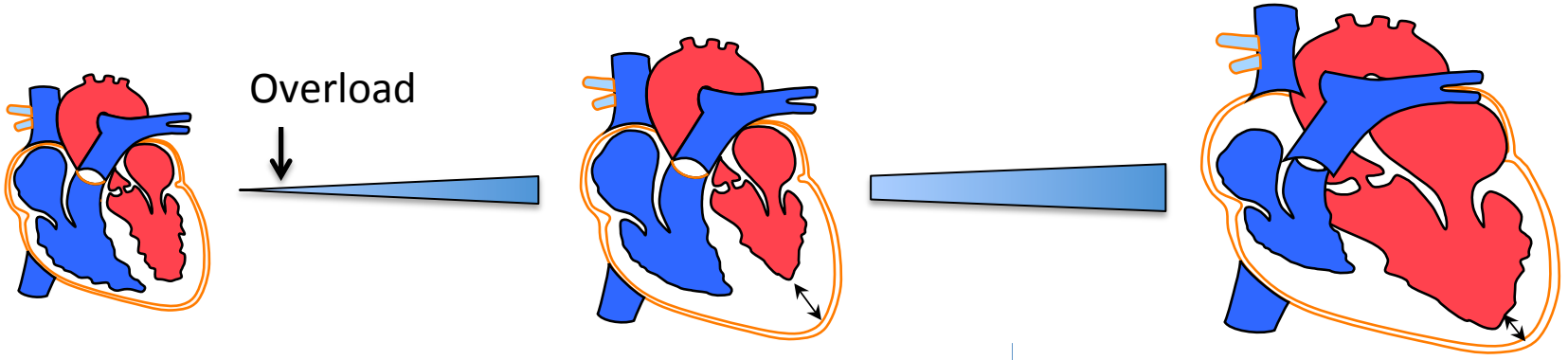


- Involuntary, rhythmic, cyclic contractions
- Terminally differentiated, postnatal myocytes increase in size not numbers for growth
- Number of non myocytes (e.g. fibroblasts) is more (60-70%) compared to number of myocytes (~30%).


Neonate Heart
Postnatal Growth
"Physiological"
Adult Heart
- Exercise
- Pregnancy
- Hypertension
- Aortic Stenosis
- Sarcomeric Gene mutation
- Myocardial Infarction
- Dilated Cardiomyopathy
Physiological Hypertrophy
Pathological Hypertrophy
Dilatation and Failure

Compensatory

Decompensation



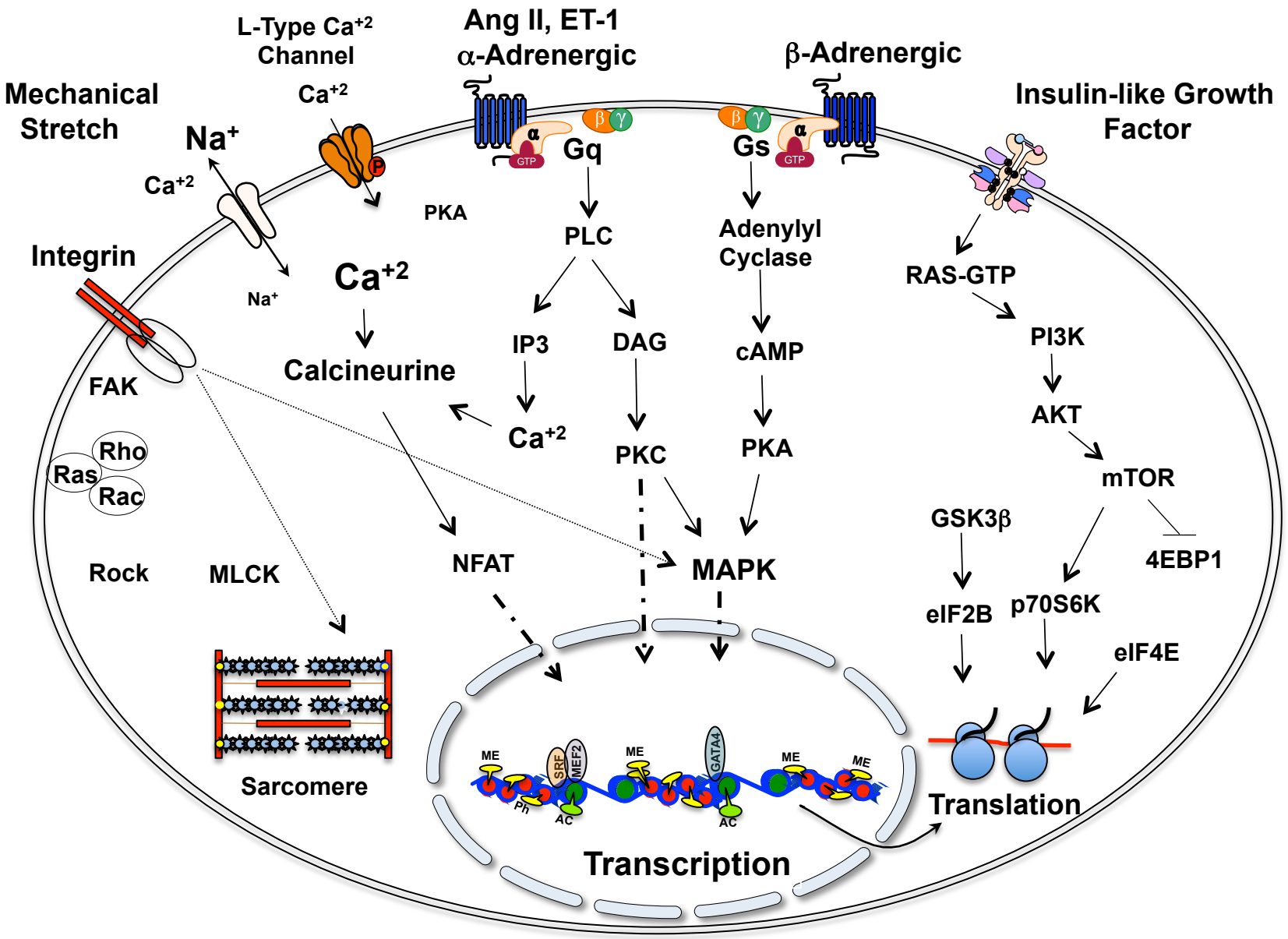
- Increase in cardiomyocyte size and mass, resulting in enlarged heart
- Increase in generalized gene expression, superimposed with significant increase in specialized genes, fetal gene program
- Increased wall thickness
- Switch to glucose metabolism for energy

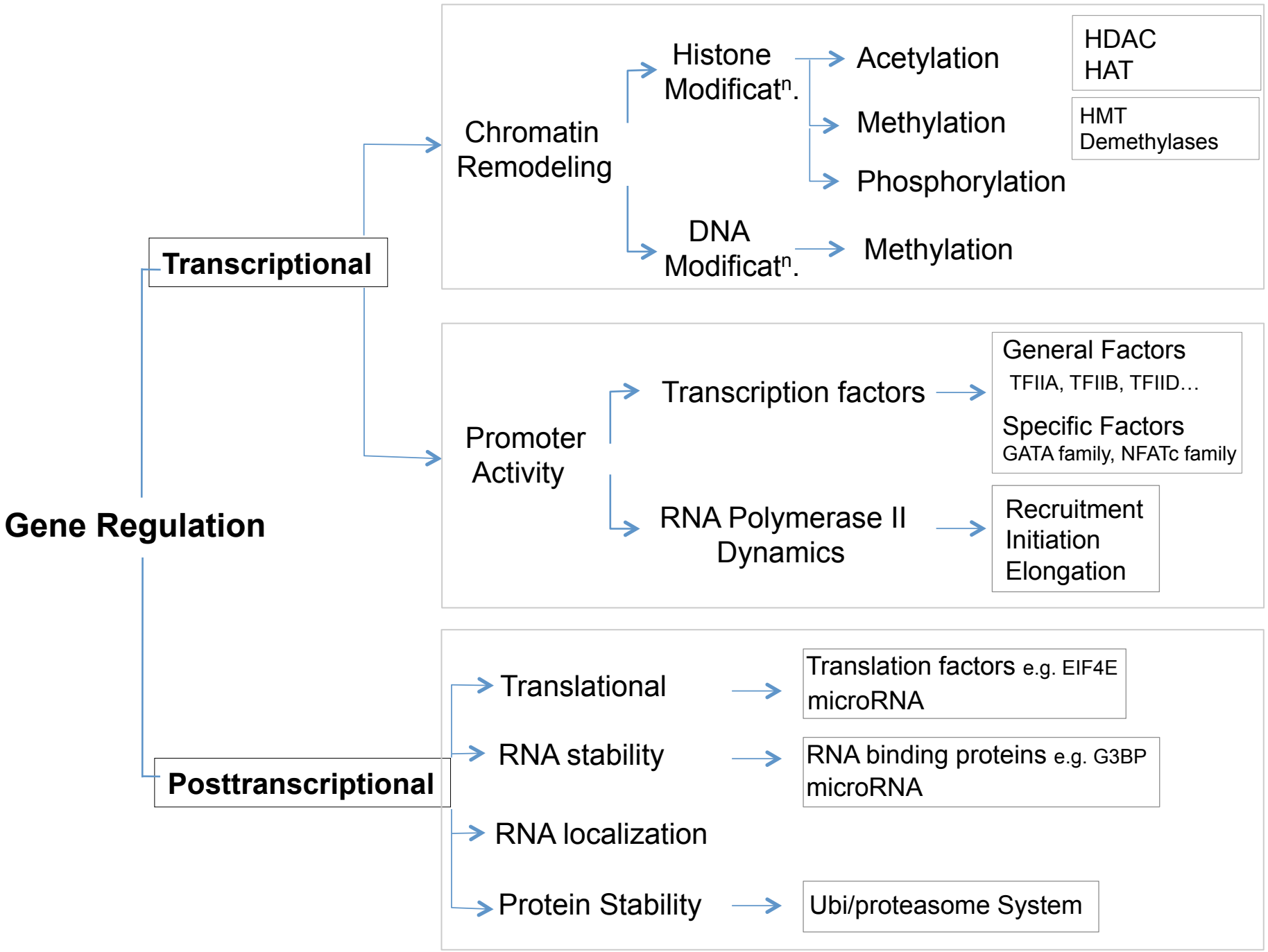
Maintained Cardiac Function and Output

- Chamber dilatation
- Decreased Ventricular Wall Thickness and Increased wall tension
- Altered Ca+2 handling
- Increased myocardial apoptosis

Compromised Cardiac function and Output

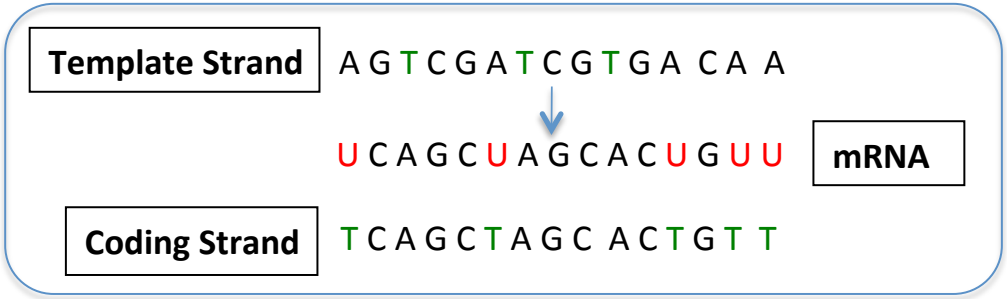
Modulators of Cardiac Hypertrophy




Gene Regulation
Transcriptional
Chromatin Remodeling
Histone Modificatn.
Acetylation
Methylation
Phosphorylation
HDAC
HAT
HMT
Demethylases
DNA Modificatn.
Methylation
Promoter Activity
Transcription factors
General Factors
TFIIA, TFIIB, TFIID…
Specific Factors
GATA family, NFATc family
RNA Polymerase II Dynamics
Recruitment
Initiation
Elongation
Posttranscriptional
Translational
Translation factors e.g. EIF4E
microRNA
RNA stability
RNA binding proteins e.g. G3BP
microRNA
RNA localization
Protein Stability
Ubi/proteasome System

Transcription

Definition- " Making a RNA copy from a sequence of DNA (a gene) "



- Transcription requires the action of **RNA polymerase**, that reads the template DNA sequence and generates complementary, anti-parallel transcript of Ribose Nucleic Acid

- The transcript is generated in 5' – 3' direction, therefore the DNA sequence is read in 3' – 5' direction by RNA polymerase.

- The transcript sequence is similar to the sequence of coding strand except Thymine (T) is replaced by Uracil (U).

- For successful transcription, the machinery requires the sequential assembly of various components at specific time and sites.

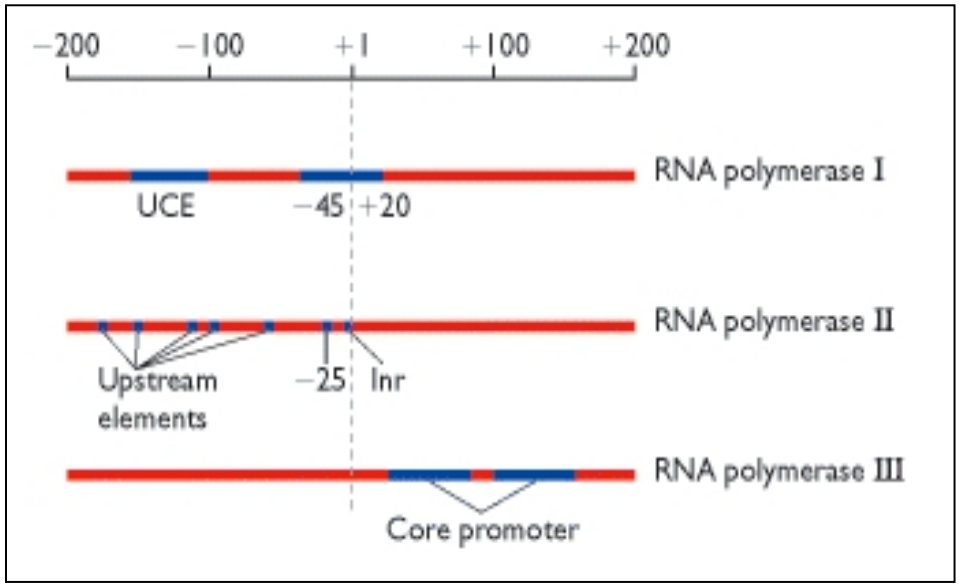
RNA polymerase II

- RNA polymerase II forms the center of the transcription machinery, and catalyzes the formation of mRNA precursors from DNA.
- Contains 12 subunits (RPB1-12), where RPB1 is the largest subunit containing the *C*arboxy *T*erminal *D*omain (CTD) that is required for the polymerase activity.
- CTD contains 52 repeats of heptapeptide (YSPTSPS), the phosphorylation status of which determines the activity state of the enzyme with respect to transcription.
- RNA pol II recruited to the gene promoters during the formation of preinitiation complex is hypo-phosphorylated at the CTD. Phosphorylation of serine 5 by CDK7 (component of TFIIH) initiates transcription and promoter clearance.
- Productive elongation of transcription by RNA pol II requires hyper-phosphorylation of serine 2, mediated by kinase Cdk9

Promoter

Definition: A DNA segment with specific sequence that is recognized by transcription factors and dictates the site of RNA polymerase binding for gene expression.

- Promoter region defines which gene will be transcribed by which RNA polymerase



Brown TA. Genomes. 2nd edition. Oxford: Wiley-Liss; 2002.

- RNA pol I (*transcribes 28S, 5.8S and 18S ribosomal RNA (rRNA) genes*) promoters are regions at the transcription start sites (– 45 to + 20) along with an upstream control element (- 100)

- RNA pol II (*transcribes all protein coding genes (mRNA), small nuclear RNA genes*) promoters can be found at variable distances upstream of the transcription start site. Along with core promoter regions (-25 or TATA box and Initiator sequence) genes can have several upstream promoter elements that can act as binding sites for enhancers or repressors of gene expression

- RNA pol III (*transcribes transfer RNAs, 5S rRNA, U6-snRNA, small nucleolar RNAs, small cytoplasmic RNA*) promoters usually located within the genes, with some exceptions.

DNA binding proteins during eukaryotic transcription

General Transcription factors

- TFIID: general transcription factor IID composed of *T*ATA-*B*inding *P*rotein (TBP) and *T*ranscription *A*ssociated *F*actors (TAF). TBP binds to the TATA promoters sequences along with the TAFs to initiate the formation of Pre-initiation complex.

- TFIIA: general transcription factor IIA binds and stabilizes pre-initiation complex.

- TFIIB: general transcription factor IIB required for the recruitment of RNA polymerase II to the transcription start site and the pre-initiation complex. Interacts with TBP and RNA pol II.

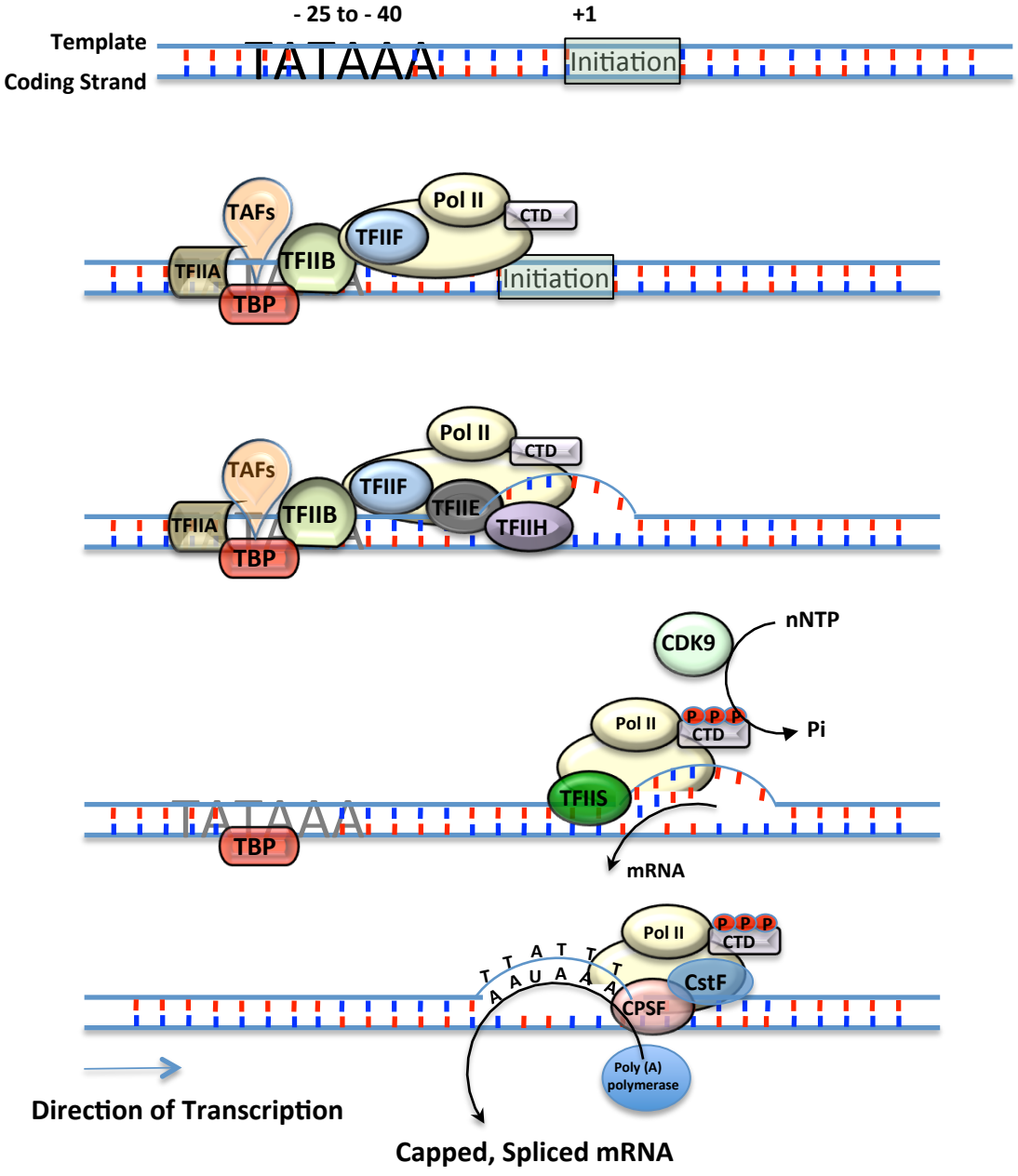
General Transcription factors, contd.

- TFIIF: general transcription factor IIF associates with RNA pol II and assists in recruitment, initiation and promotes elongation

- TFIIE: general transcription factor IIE required for the recruitment of TFIIH to the complex and initiates promoter clearance

- TFIIH: general transcription factor IIH contains 10 subunits that are involved in formation of open complex (helicase activity), DNA excision repair, promoter clearance transcription elongation (phosphorylation of CTD)

Steps of Eukaryotic Transcription



Core Promoter (Promoter + Initiator sequence)

DNA binding Proteins (General Transcription Factors TFIID, TFIIA, TFIIB and Transcription Associated Factors, RNA polymerase II enzyme and TFIIF).

Pre-Initiation Complex

Transcription factors TFIIE and TFIIH

Initiation Complex

Kinase activity of TFIIH (CDK7)
Phosphorylation of serine 5 of CTD

Promoter Clearance

Kinase activity P-TEFb (CDK9 and Cyclin T)
Hyper-phosphorylation of Serine 2 of CTD

Elongation Complex

Cleavage and polyadenylation specificity factor (CPSF)
Cleavage stimulation factor (CstF)
Signal Sequence in mRNA 5'-AAUAAA-3'

Termination Complex

DNA binding proteins during eukaryotic transcription

Specific Transcription Factors

- Proteins that bind to specific DNA sequence elements and function as transcription regulators of specific genes, under certain conditions of development or stress for e.g.

  – Serum Response factor (SRF): binds to the serum response element and required for the transcription of gene involved in cardiac differentiation and maturation.

  – Myocyte enhancer factor 2A- transcription activator that binds to MEF2 element found in most of the muscle specific genes

Methods to measure active transcription

- 3H Uridine Incorporation
- Nuclear Run Off assay
- High-throughput sequencing

mRNA abundance

- Microarray
- Quantitative PCR

3H Uridine incorporation

- Radiolabelled (3H) uridine is supplemented to cells (in vitro) and the incorporation measured, as an indicator of transcription.

- Measures mRNA transcription with no specificity to particular gene.

- The data is presented as Counts Per Minute (CPM) and normalized to DNA. (CPM/µg DNA)

Nuclear Run-Off Assay

- Technique is used to measure transcription of genes at given time, under given condition

- Isolated nuclei are supplemented with NTPs and radiolabelled (^{32}P) GTP and the nascent transcripts are elongated in vitro.

- The radiolabelled mRNAs are extracted and hybridized to gene/s of interest immobilized on membrane (dot blot).

- No new transcription initiation occurs, so only transcripts with RNA pol II attached and undergoing transcription in vivo are elongated in vitro.

High-throughput Sequencing

- aka ‘next generation sequencing’ or ‘deep sequencing’.
- ‘Depth’ in deep sequencing is the number of times a single base is read during the whole process.
- Massive parallel sequencing results are read by the computer and output is in the form of short (~35 -50 nucleotide) reads that can be aligned onto the reference genome.
- Platforms for deep sequencing are:
  - 454 (Roche)
  - SOLiD (Applied Biosciences)
  - Genome Analyzer (Illumina)
  - Helicos HeliScope

Applications

- Whole Genome sequencing (WGS)

- Transcriptome sequencing (RNA-Seq)

Uses

- Cellular genomics
  - sequencing of novel genome or re-sequencing of genome
  - gene expression profiles, alternate splicing
  - transcription patterns, epigenetics, etc.

- Metagenomics
  - sequencing and identifying organisms from mixed samples.

- Genomic Medicine
  - point mutations and gene variants associated with certain diseases, e.g. cancers
  - diagnostic tool for certain cancers, e.g. DNA methylation profiling in lung cancer

High-throughput Sequencing

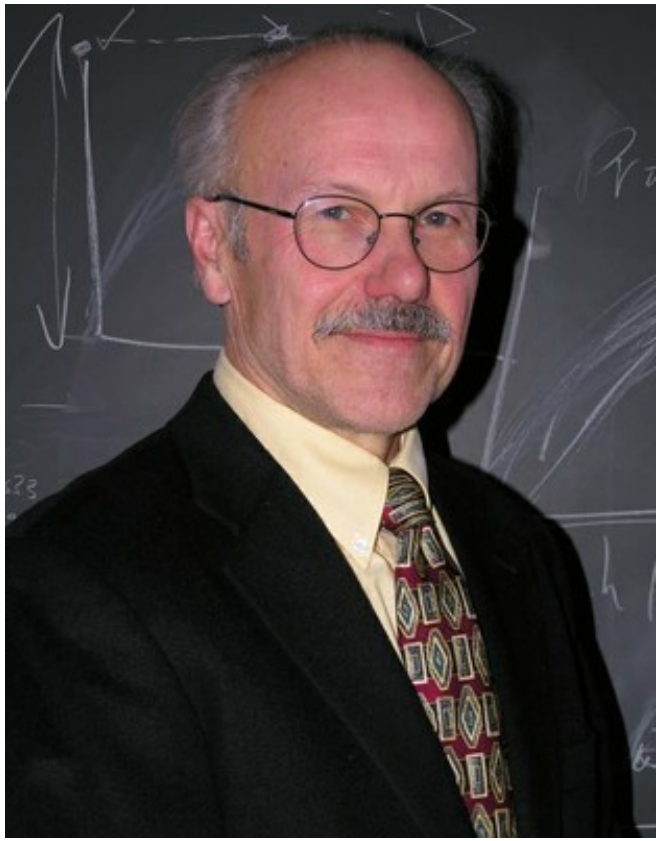
GRO-Seq –

- Transcripts elongated by nuclear run on assays are sequenced using deep sequencing.

- Elongating transcripts are labeled using BrdUTP in presence of Sarkosly (prevents RNA pol II binding to DNA) and immunoprecipited with anti-BrdU antibody, isolated converted to cDNA, deep sequenced and aligned to reference genome.

- Since there is no new recruitment of RNA pol II, only genes with bound RNA pol II are transcribed.

ChIP-Seq (chromatin immunoprecipitation)

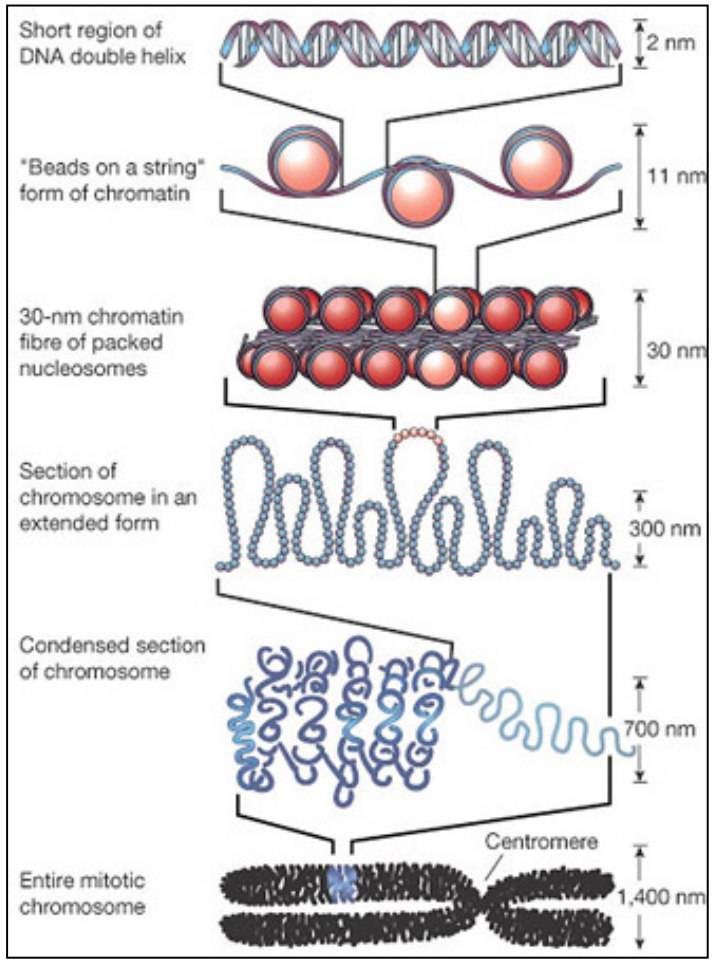


John Lis

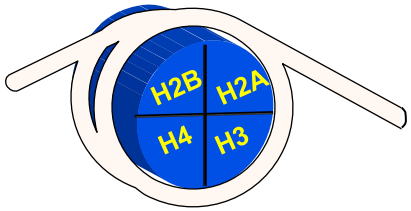
- In 1984, John Lis and David Gilmour for the first time immunoprecipitated bacterial DNA using RNA polymerase.
- 1985, same group reported the distribution of RNA pol II on heat shock genes in drosophila.
- Identified and showed RNA pol II pausing at the promoters in these genes.

Chromatin

Definition: “Mass of genetic material composed of DNA and proteins that condense to form chromosome during eukaryotic cell division”.



http://www.integratedhealthcare.eu/1/en/histones_and_chromatin/1497/



Nucleosome:

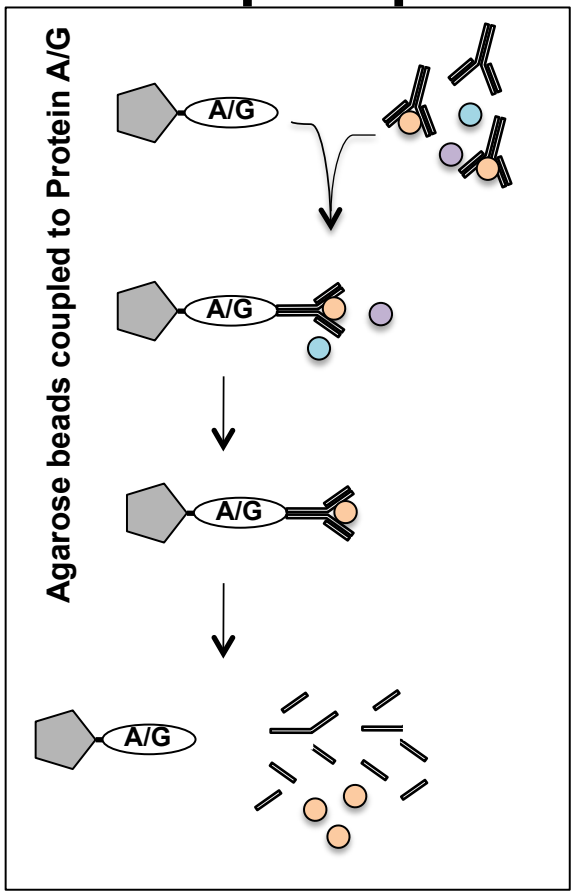
- basic unit of chromatin, which includes 146bp of DNA wrapped around an octamer of core Histones protein (two H2B, two H2A, H4 and H3).

- Two nucleosomes are connected by free DNA strand called “linker DNA”.

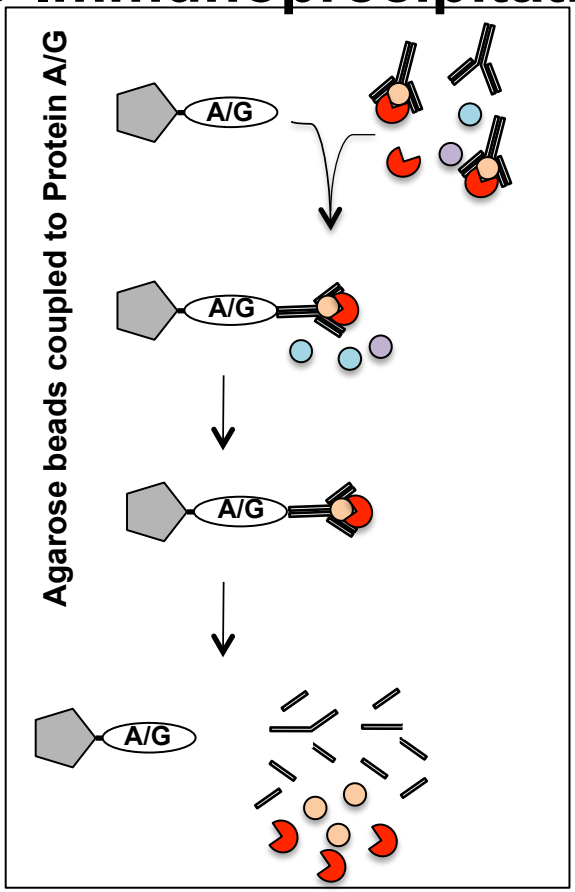
Immunoprecipitation

Definition: “Technique of precipitating a protein antigen out of solution using an antibody that specifically binds to that protein”.

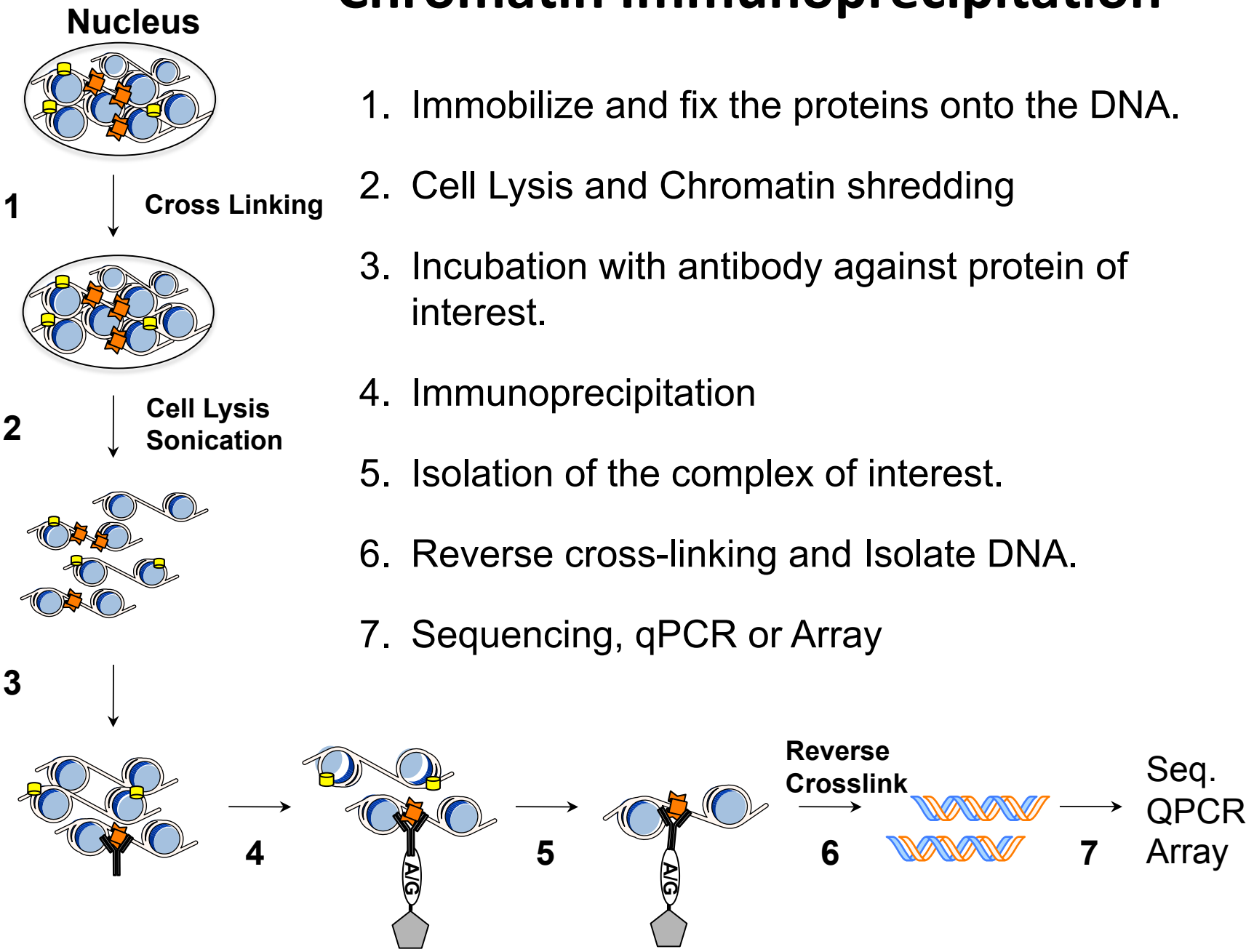
Immunoprecipitation



Co-Immunoprecipitation



Chromatin Immunoprecipitation



1. Immobilize and fix the proteins onto the DNA.
2. Cell Lysis and Chromatin shredding
3. Incubation with antibody against protein of interest.
4. Immunoprecipitation
5. Isolation of the complex of interest.
6. Reverse cross-linking and Isolate DNA.
7. Sequencing, qPCR or Array

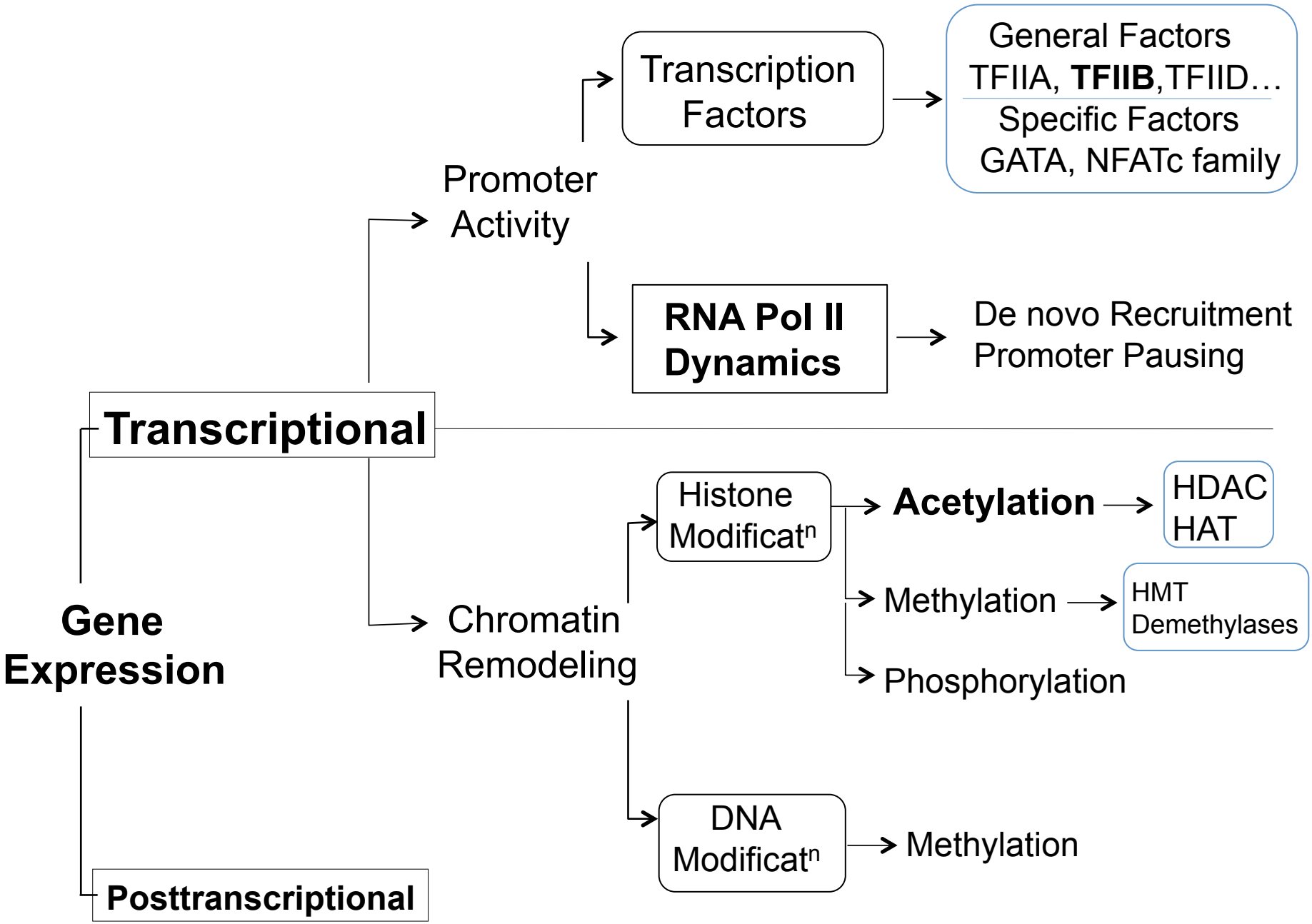
ChIP

What antigen (protein of interest) can be used for Immunoprecipitation?

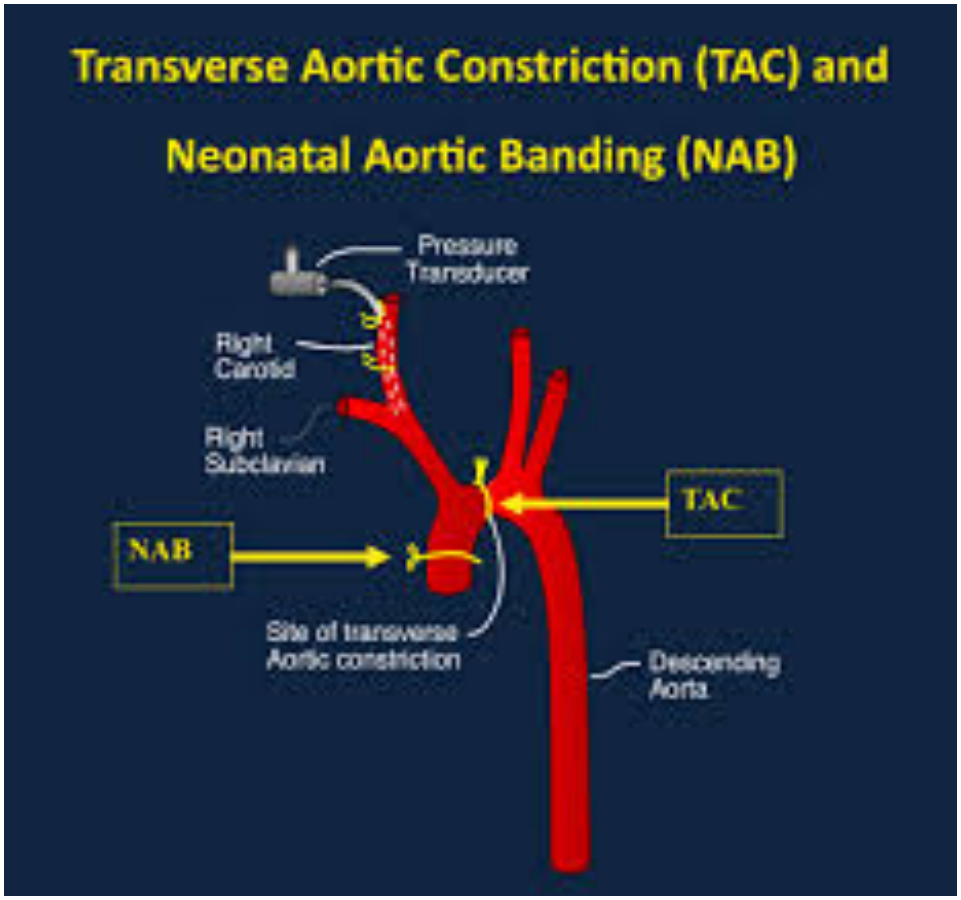
- Any protein that binds to DNA directly or indirectly, like Histones, RNA polymerase II, transcription factors, enhancers, associated proteins or any protein that forms complex or binds with DNA binding proteins.

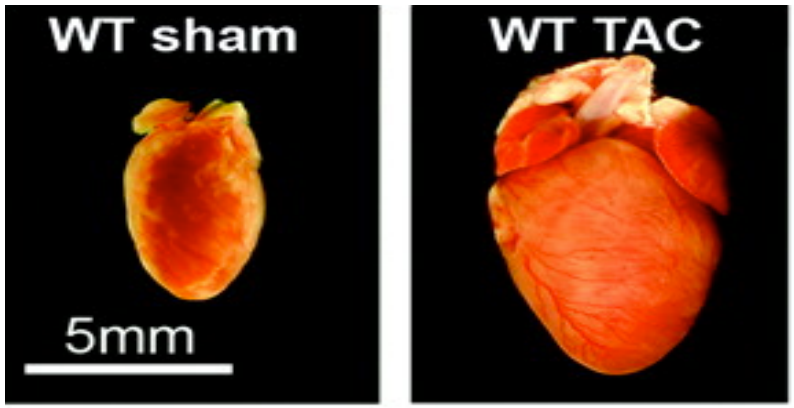
Uses-

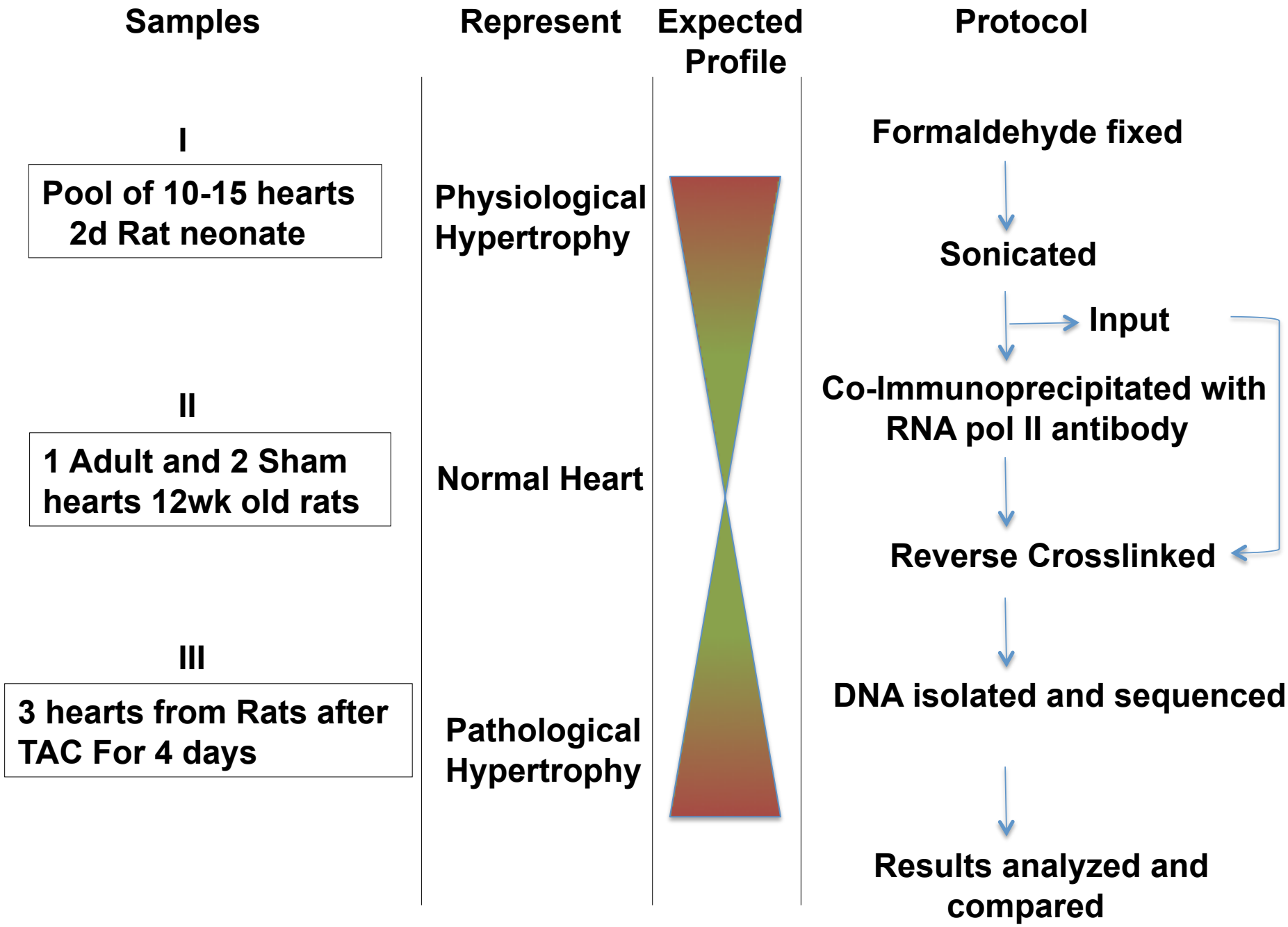
- Study protein-DNA interactions, protein binding sites
- Transcription factors and promoter interactions
- RNA pol II binding.
- Associated histone modifications with active vs. inactive gene transcription or repression.


Gene Expression
Transcriptional
Posttranscriptional
Promoter Activity
Transcription Factors
General Factors
TFIIA, TFIIB,TFIID…
Specific Factors
GATA, NFATc family
RNA Pol II Dynamics
De novo Recruitment
Promoter Pausing
Chromatin Remodeling
Histone Modificatn
Acetylation
HDAC
HAT
Methylation
HMT
Demethylases
Phosphorylation
DNA Modificatn
Methylation

Transverse Aortic Co-Arctation (TAC)






Samples
Represent
Expected Profile
Protocol
I
Pool of 10-15 hearts 2d Rat neonate
Physiological Hypertrophy
II
1 Adult and 2 Sham hearts 12wk old rats
Normal Heart
III
3 hearts from Rats after TAC For 4 days
Pathological Hypertrophy
Formaldehyde fixed
Sonicated
Input
Co-Immunoprecipitated with RNA pol II antibody
Reverse Crosslinked
DNA isolated and sequenced
Results analyzed and compared

Results

Output from the Genome analyzer

1. Sequence Analysis: The results in the form of *36-nt* reads (tags) are mapped onto the reference genome using ELAND (Efficient Local Alignment of Nucleotide Data) algorithm. Only unique tags with no more than two mismatches are mapped onto the genome.

2. Fragment density: The 3'- ends of the tags are extended in silico to length of 110-200 bp (fragments). To calculate the density of fragments along the genome, the genome is divided into *32-nt* bins (discrete locations). The number of fragments in each bin gives the fragment density of that bin. This data is stored in a binary analysis report (BAR) file and can be viewed in Affymetrix' Integrated Genome Browser (IGB)

Results contd.

3. Interval Analysis (“Peak Finding”): Region on the genome with start and end coordinates, that contains atleast three consecutive bins with fragment density more than the threshold (usually set at 10-20). Intervals are calculated and complied into BED files.

These results containing the fragment densities, number of bins, intervals, along with the gene coordinates is tabulated into Excel sheets, with overall statistics and comparisons between the samples.

EXCEL SHEET for GENES

Average Values

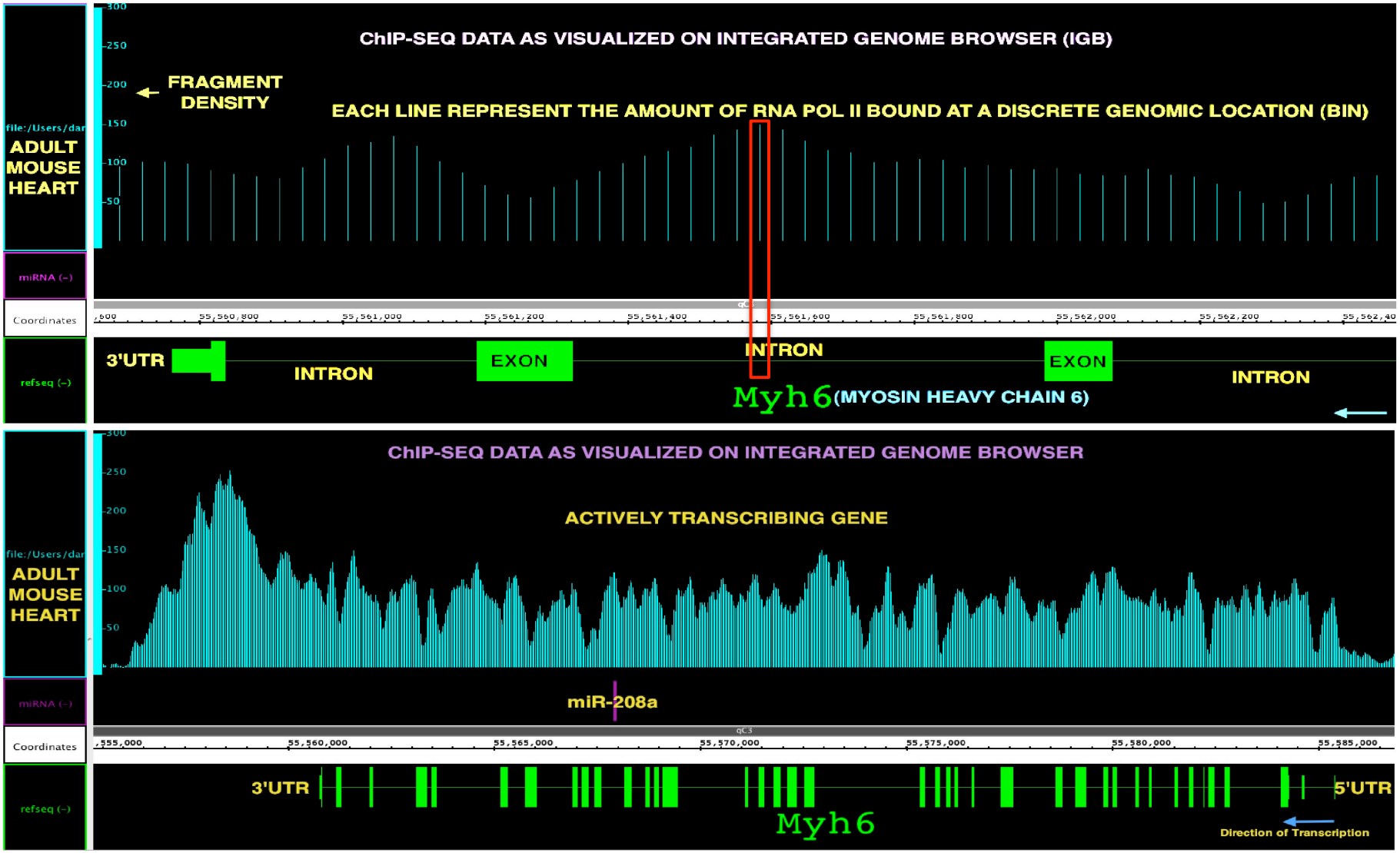
Interval details

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V
1	Gene ID	Gene Name	some	Gene Start	Gene End	Gene Len	Ger	1_A1::1 Avg Val	2_B1::1 Avg Val	3_H1::1 Avg Val	Ratio H1/A1	Ratio B1/A1	1_A1::1 Peak	2_B1::1 Peak	3_H1::1 Peak	#Intervals	Intervals	Interval Dists to Start	Interval Pos	1_A1::1	2_B1::1	3_H1::1
2	GeneID:20671	Sox17	1	4,481,009	4,486,494	5,485	-	5.045	2.492	4.784	0.948	0.494	33	13	27	2	1_A1::1::0, 3_H1::1::0	2878, 5086	in gene, in gene	1	0	1
3	GeneID:27395	Mrpl15	1	4,763,290	4,775,791	12,501	-	3.753	3.647	3.930	1.047	0.972	56	18	23	3	1_A1::1::2, 2_B1::1::0, 3_H1::1::	47, 47, -17	in gene, in gene, upstrea	1	1	1
4	GeneID:18777	Lypla1	1	4,797,974	4,836,816	38,842	+	2.364	1.968	1.970	0.833	0.832	38	15	15	2	1_A1::1::3, 3_H1::1::3	-54, -150	upstream, upstream	1	0	1
5	GeneID:21399	Tcea1	1	4,847,896	4,887,986	40,090	+	2.224	2.269	2.470	1.111	1.020	14	12	9	3	1_A1::1::4, 2_B1::1::1, 3_H1::1::	-152, -280, -248	upstream, upstream, ups	1	1	1
6	GeneID:108664	Atp6v1h	1	5,073,254	5,152,630	79,376	+	2.195	2.236	2.485	1.132	1.019	19	10	13	2	1_A1::1::5, 2_B1::1::2	-54, 80730	upstream, downstream	1	1	0
7	GeneID:654788	4732440D04Rik	1	6,199,939	6,209,341	9,402	-	5.547	5.777	4.598	0.829	1.041	64	41	25	3	1_A1::1::6, 2_B1::1::3, 3_H1::1::	4605, 4653, 4701	in gene, in gene, in gene	1	1	1
8	GeneID:12421	Rb1cc1	1	6,204,743	6,265,656	60,913	+	2.904	3.498	2.963	1.020	1.205	52	31	19	7	1_A1::1::6, 1_A1::1::7, 2_B1::1::	-7, 69033, -55, 41081, 6	upstream, downstream,	1	1	1
9	GeneID:240690	St18	1	6,720,132	6,851,021	130,889	+	1.286	1.285	1.282	0.997	0.999	5	4	4	1	1_A1::1::8	136988	downstream	1	0	0
10	GeneID:319263	Pcmtd1	1	7,079,231	7,163,709	84,478	+	2.543	2.157	2.280	0.897	0.848	64	22	17	4	1_A1::1::9, 2_B1::1::6, 2_B1::1::	33, 1, 86017, -95	in gene, in gene, downst	1	1	1
11	GeneID:59014	Rrs1	1	9,535,523	9,537,533	2,010	+	6.733	3.897	4.806	0.714	0.579	36	20	19	3	1_A1::1::11, 2_B1::1::8, 3_H1::1	-99, 2642, -115	upstream, downstream,	1	1	1
12	GeneID:76187	Adhfe1	1	9,538,161	9,568,049	29,888	+	3.373	4.309	3.007	0.891	1.277	34	24	27	4	1_A1::1::11, 2_B1::1::8, 2_B1::1	-2737, 4, 30463, -2753	upstream, in gene, down	1	1	1
13	GeneID:76982	3110035E14Rik	1	9,591,348	9,617,223	25,875	+	1.559	1.470	1.915	1.228	0.943	8	4	11	1	1_A1::1::12	29164	downstream	1	0	0
14	GeneID:17864	Mybl1	1	9,658,912	9,690,290	31,378	-	1.431	1.361	1.472	1.029	0.951	4	4	5	1	1_A1::1::13	-526	upstream	1	0	0
15	GeneID:70675	Vcpip1	1	9,713,273	9,738,463	25,190	-	3.068	2.946	3.356	1.094	0.960	52	21	35	4	1_A1::1::14, 2_B1::1::10, 2_B1::	31, 26111, -1, 31	in gene, downstream, up	1	1	1
16	GeneID:10003953(	LOC100039536	1	9,738,562	9,761,337	22,775	+	1.698	1.249	1.934	1.139	0.736	10	10	22	3	1_A1::1::14, 2_B1::1::11, 3_H1::	-130, -98, -130	upstream, upstream, ups	1	1	1
17	GeneID:170755	Sgk3	1	9,788,211	9,892,651	104,440	+	2.205	1.737	2.177	0.987	0.788	18	7	10	2	1_A1::1::15, 3_H1::1::11	77, 108845	in gene, downstream	1	0	1
18	GeneID:620986	EG620986	1	9,792,184	9,792,634	450	-	1.444	1.000	2.444	1.693	0.693	3	1	4	1	1_A1::1::15	4346	downstream	1	0	0
19	GeneID:240697	6030422M02Rik	1	9,901,800	9,931,035	29,235	+	2.485	2.627	3.718	1.496	1.057	15	21	25	4	1_A1::1::16, 2_B1::1::12, 3_H1::	32376, 32344, -4744, 32	downstream, downstrea	1	1	1
20	GeneID:26754	Cops5	1	10,014,911	10,027,987	13,076	-	2.755	2.722	2.661	0.966	0.988	36	17	13	4	1_A1::1::17, 2_B1::1::13, 2_B1::	-1933, 14755, -1709, -19	upstream, downstream,	1	1	1
21	GeneID:211660	Cspp1	1	10,028,299	10,126,849	98,550	+	2.552	3.336	2.769	1.085	1.307	48	27	42	5	1_A1::1::17, 2_B1::1::14, 2_B1::	1621, 1397, 97397, 165	in gene, in gene, in gene	1	1	1
22	GeneID:211673	Arfgef1	1	10,127,588	10,222,751	95,163	-	2.813	3.534	3.130	1.113	1.256	12	18	14	5	1_A1::1::18, 2_B1::1::15, 2_B1::	-289, 97055, 66207, 103	upstream, downstream,	1	1	1
23	GeneID:320492	A830018L16Rik	1	11,404,193	11,965,982	561,789	+	1.332	1.353	1.277	0.959	1.016	20	9	8	1	1_A1::1::22	44415	in gene	1	0	0
24	GeneID:10003973(	LOC100039733	1	12,647,457	12,764,674	117,217	+	3.051	1.872	2.698	0.884	0.614	25	11	18	5	1_A1::1::23, 1_A1::1::24, 1_A1::	35167, 61167, 112276, 3	in gene, in gene, in gene	1	0	1
25	GeneID:240725	Sulf1	1	12,708,626	12,850,453	141,827	+	2.860	2.102	2.674	0.935	0.735	25	11	13	5	1_A1::1::24, 1_A1::1::25, 1_A1::	-2, 51107, 146734, -82,	upstream, in gene, down	1	0	1
26	GeneID:240726	Slco5a1	1	12,856,630	12,981,216	124,586	-	1.728	1.518	1.633	0.945	0.878	12	11	11	2	1_A1::1::26, 3_H1::1::19	125856, 126752	downstream, downstrea	1	0	1
27	GeneID:17978	Ncoa2	1	13,129,240	13,364,164	234,924	-	2.654	2.965	2.508	0.945	1.117	35	23	21	4	1_A1::1::27, 2_B1::1::17, 2_B1::	1508, 235876, 1524, 14	in gene, downstream, in	1	1	1
28	GeneID:621685	EG621685	1	13,537,455	13,549,886	12,431	-	1.382	1.152	1.964	1.421	0.834	4	3	7	1	2_B1::1::19	-4130	upstream	0	1	0
29	GeneID:72265	Tram1	1	13,554,783	13,579,945	25,162	-	2.970	2.096	3.221	1.085	0.706	25	9	11	3	1_A1::1::28, 2_B1::1::19, 3_H1::	-183, 25929, -151	upstream, downstream,	1	1	1
30	GeneID:212442	Lactb2	1	13,615,979	13,650,590	34,611	-	2.339	2.660	2.607	1.115	1.137	32	9	22	2	1_A1::1::29, 3_H1::1::22	30, 62	in gene, in gene	1	0	1
31	GeneID:381246	Xkr9	1	13,658,852	13,691,804	32,952	+	1.288	1.233	1.271	0.987	0.957	3	3	4	2	1_A1::1::29, 3_H1::1::22	-8292, -8324	upstream, upstream	1	0	1
32	GeneID:21749	Terf1	1	15,795,739	15,833,510	37,771	+	2.434	2.376	2.148	0.882	0.976	50	13	13	3	1_A1::1::30, 2_B1::1::20, 3_H1::	5, 43253, -27	in gene, downstream, up	1	1	1
33	GeneID:226866	Gm106	1	15,843,943	15,882,803	38,860	-	1.825	1.909	1.981	1.085	1.046	5	8	9	1	2_B1::1::20	43811	downstream	0	1	0
34	GeneID:75799	4930444P10Rik	1	16,056,058	16,090,599	34,541	-	3.835	2.629	4.220	1.100	0.686	24	16	20	3	1_A1::1::31, 2_B1::1::21, 3_H1::	-3833, -3801, -3801	upstream, upstream, ups	1	1	1
35	GeneID:19989	Rpl7	1	16,091,379	16,094,488	3,109	-	8.031	4.977	7.794	0.970	0.620	95	31	68	3	1_A1::1::31, 2_B1::1::21, 3_H1::	56, 88, 88	in gene, in gene, in gene	1	1	1
36	GeneID:98711	Rdh10	1	16,095,963	16,122,631	26,668	+	2.122	2.065	2.378	1.121	0.973	11	11	15	3	1_A1::1::31, 2_B1::1::21, 3_H1::	-1531, -1563, -1563	upstream, upstream, ups	1	1	1
37	GeneID:66799	Ube2w	1	16,559,487	16,609,367	49,880	-	2.759	2.965	3.069	1.112	1.075	44	16	24	5	1_A1::1::32, 1_A1::1::33, 2_B1::	56311, 71, 56215, 5680	downstream, in gene, dc	1	1	1
38	GeneID:67923	Tceb1	1	16,631,917	16,646,946	15,029	-	3.232	3.280	3.471	1.074	1.015	18	17	15	4	1_A1::1::34, 2_B1::1::23, 2_B1::	-8366, 16162, -94, 1638	upstream, downstream,	1	1	1
39	GeneID:70397	Tmem70	1	16,655,272	16,668,356	13,084	+	3.011	2.924	3.120	1.036	0.971	33	20	14	4	1_A1::1::34, 2_B1::1::24, 2_B1::	40, -8232, 14184, 14456	in gene, upstream, down	1	1	1
40	GeneID:17087	Ly96	1	16,678,537	16,699,686	21,149	+	2.529	1.718	2.104	0.832	0.679	32	11	13	3	1_A1::1::35, 2_B1::1::25, 3_H1::	183, -9081, -8809	in gene, upstream, upstr	1	1	1
41	GeneID:17215	Mcm3	1	20,793,095	20,810,294	17,199	-	1.965	1.961	1.995	1.015	0.998	17	7	9	1	1_A1::1::36	-58	upstream	1	0	0
42	GeneID:74229	Paqr8	1	20,880,703	20,928,837	48,134	+	1.821	1.789	1.843	1.012	0.982	19	29	15	2	1_A1::1::37, 2_B1::1::26	-159, 44673	upstream, in gene	1	1	0
43	GeneID:71877	Efhc1	1	20,938,985	20,980,922	41,937	+	2.084	1.655	2.344	1.125	0.794	17	7	9	2	1_A1::1::38, 3_H1::1::29	2455, 46871	in gene, downstream	1	0	1
44	GeneID:170829	Tram2	1	20,991,459	21,069,306	77,847	-	2.392	1.988	2.802	1.171	0.831	21	10	22	7	1_A1::1::39, 1_A1::1::40, 1_A1::	64474, 10570, -262, 834	in gene, in gene, upstrea	1	0	1
45	GeneID:75712	Tmem14a	1	21,208,712	21,220,248	11,536	+	3.208	1.446	2.052	0.640	0.451	37	5	13	1	1_A1::1::42	120	in gene	1	0	0
46	GeneID:623356	EG623356	1	23,262,524	23,337,103	74,579	-	5.603	7.744	5.347	0.954	1.382	39	42	29	6	1_A1::1::43, 1_A1::1::44, 2_B1::	73967, 50223, 74255, 4	in gene, in gene, in gene	1	1	1
47	GeneID:387225	Mirn30a	1	23,279,107	23,279,177	70	+	1.500	6.500	4.000	2.667	4.333	2	7	4	3	1_A1::1::44, 2_B1::1::28, 3_H1::	7773, 10445, 7773	downstream, downstrea	1	1	1
48	GeneID:723964	Mirn30c-2	1	23,298,539	23,298,622	83	+	8.000	10.500	10.500	1.313	1.313	9	11	11	3	1_A1::1::44, 2_B1::1::28, 3_H1::	-11659, -8987, -11659	upstream, upstream, ups	1	1	1
49	GeneID:70155	Ogfrl1	1	23,373,263	23,390,014	16,751	-	2.143	1.965	2.568	1.198	0.917	9	6	11	1	1_A1::1::45	-386	upstream	1	0	0
50	GeneID:280645	B3gat2	1	23,768,765	23,854,704	85,939	+	2.591	3.231	3.239	1.250	1.247	15	18	18	2	2_B1::1::29, 3_H1::1::35	80995, 77315	in gene, in gene	0	1	1
51	GeneID:98366	Smap1	1	23,852,466	23,929,128	76,662	-	2.426	2.476	2.651	1.093	1.021	10	10	9	4	1_A1::1::46, 2_B1::1::29, 3_H1::	-184, 79368, 83048, -18	upstream, downstream,	1	1	1
52	GeneID:68002	1110058L19Rik	1	24,002,785	24,012,479	9,694	-	3.060	2.952	3.022	0.988	0.965	37	21	17	3	1_A1::1::47, 2_B1::1::30, 3_H1::	31, 31, 31	in gene, in gene, in gene	1	1	1
53	GeneID:68187	4921533L14Rik	1	24,018,520	24,093,413	74,893	-	1.793	2.445	1.827	1.019	1.364	6	11	8	3	1_A1::1::47, 2_B1::1::30, 3_H1::	80965, 80965, 80965	downstream, downstrea	1	1	1
54	GeneID:68421	Lmbrd1	1	24,685,383	24,823,146	137,763	+	2.419	2.209	2.302	0.952	0.913	35	21	19	5	1_A1::1::49, 1_A1::1::50, 2_B1::	-39, 55641, -55, 55641,	upstream, in gene, upstr	1	1	1
55	GeneID:213109	Phf3	1	30,859,187	30,920,101	60,914	-	3.101	4.559	3.035	0.979	1.470	13	34	11	4	1_A1::1::51, 2_B1::1::34, 2_B1::	63461, 62085, 38213, 6	downstream, downstrea	1	1	1
56	GeneID:638995	EG638995	1	30,929,497	30,930,631	1,134	+	9.944	5.528	5.611	0.564	0.556	29	11	15	2	1_A1::1::52, 3_H1::1::41	1383, 1255	downstream, downstrea	1	0	1
57	GeneID:19243	Ptp4a1	1	30,997,148	31,006,600	9,452	-	6.590	9.930	8.768	1.331	1.507	27	64	48	3	1_A1::1::53, 2_B1::1::36, 3_H1::	-152, 8968, 8936	upstream, in gene, in ge	1	1	1
58	GeneID:19076	Prim2	1	33,510,656	33,726,603	215,947	-	1.587	1.468	1.592	1.003	0.925	22	9	13	2	1_A1::1::55, 3_H1::1::44	-117, -85	upstream, upstream	1	0	1
59	GeneID:67503	1700001G17Rik	1	33,726,669	33,727,559	890	+	11.179	2.667	5.857	0.524	0.239	37	9	23	2	1_A1::1::55, 3_H1::1::44	51, 19	in gene, in gene	1	0	1
60	GeneID:19335	Rab23	1	33,776,741	33,798,342	21,601	+	2.311	2.821	3.105	1.344	1.221	23	11	12	3	1_A1::1::56, 2_B1::1::37, 3_H1::	27, 23195, 37883	in gene, downstream, dc	1	1	1
61	GeneID:213539	Bag2	1	33,802,329	33,814,595	12,266	-	2.883	3.167	3.900	1.353	1.099	22	14	27	4	1_A1::1::57, 2_B1::1::37, 2_B1::	-29, 14659, -109, -29	upstream, downstream,	1	1	1
62	GeneID:98403	Zfp451	1	33,818,598	33,871,272	52,674	-	2.717	3.036	2.949	1.085	1.117	29	18	17	5	1_A1::1::57, 1_A1::1::58, 2_B1::	56648, 40, 56568, 5664	downstream, in gene, dc	1	1	1
63	GeneID:13518	Dst	1	34,068,670	34,365,497	296,827	+	3.946	3.687	5.163	1.308	0.934	30	32	38	18	1_A1::1::59, 1_A1::1::60, 1_A1::	-5918, 109506, 156034,	upstream, in gene, in ge	1	1	1
64	GeneID:10004091(	LOC100040913	1	34,364,487	34,365,506	1,019	+	3.844	3.806	4.312	1.122	0.990	8	8	8	3	1_A1::1::63, 2_B1::1::42, 3_H1::	3417, 3577, 3481	downstream, downstrea	1	1	1
65	GeneID:69668	Ccdc115	1	34,493,521	34,496,517	2,996	-	6.775	5.174	5.098	0.752	0.764	43	23	21	3	1_A1::1::65, 2_B1::1::43, 3_H1::	-219, -187, -235	upstream, upstream, ups	1	1	1
66	GeneID:27993	Imp4	1	34,496,745	34,502,592	5,847	+	3.628	2.660	3.725	1.027	0.733	45	23	40	3	1_A1::1::65, 2_B1::1::43, 3_H1::	-9, -41, 7	upstream, upstream, in g	1	1	1
67	GeneID:19253	Ptpn18	1	34,516,591	34,530,629	14,038	+	2.811	1.953	2.465	0.877	0.695	15	10	10	1	1_A1::1::66	-63	upstream	1	0	0
68	GeneID:226970	Arhgef4	1	34,858,567	34,869,599	11,032	+	5.123	7.145	5.062	0.988	1.395	18	36	19	2	1_A1::1::67, 2_B1::1::44	8249, 10681	in gene, in gene	1	1	0
69	GeneID:214469	BC043098	1	34,870,071	34,899,819	29,748	-	4.331	4.350	4.326	0.999	1.004	21	20	14	5	1_A1::1::67, 1_A1::1::68, 2_B1::	33003, -181, 30571, -11	downstream, upstream,	1	1	1
70	GeneID:226971	Plekhb2	1	34,906,804	34,936,430	29,626	+	2.437	1.936	2.266	0.930	0.794	35	6	17	3	1_A1::1::68, 2_B1::1::45, 3_H1::	-6804, -6868, -6836	upstream, upstream, ups	1	1	1
71	GeneID:50785	Hs6st1	1	36,125,254	36,163,291	38,037	+	2.777	2.480	3.037	1.094	0.893	22	10	19	3	1_A1::1::72, 1_A1::1::73, 3_H1::	-86, 11482, -102	upstream, in gene, upstr	1	0	1

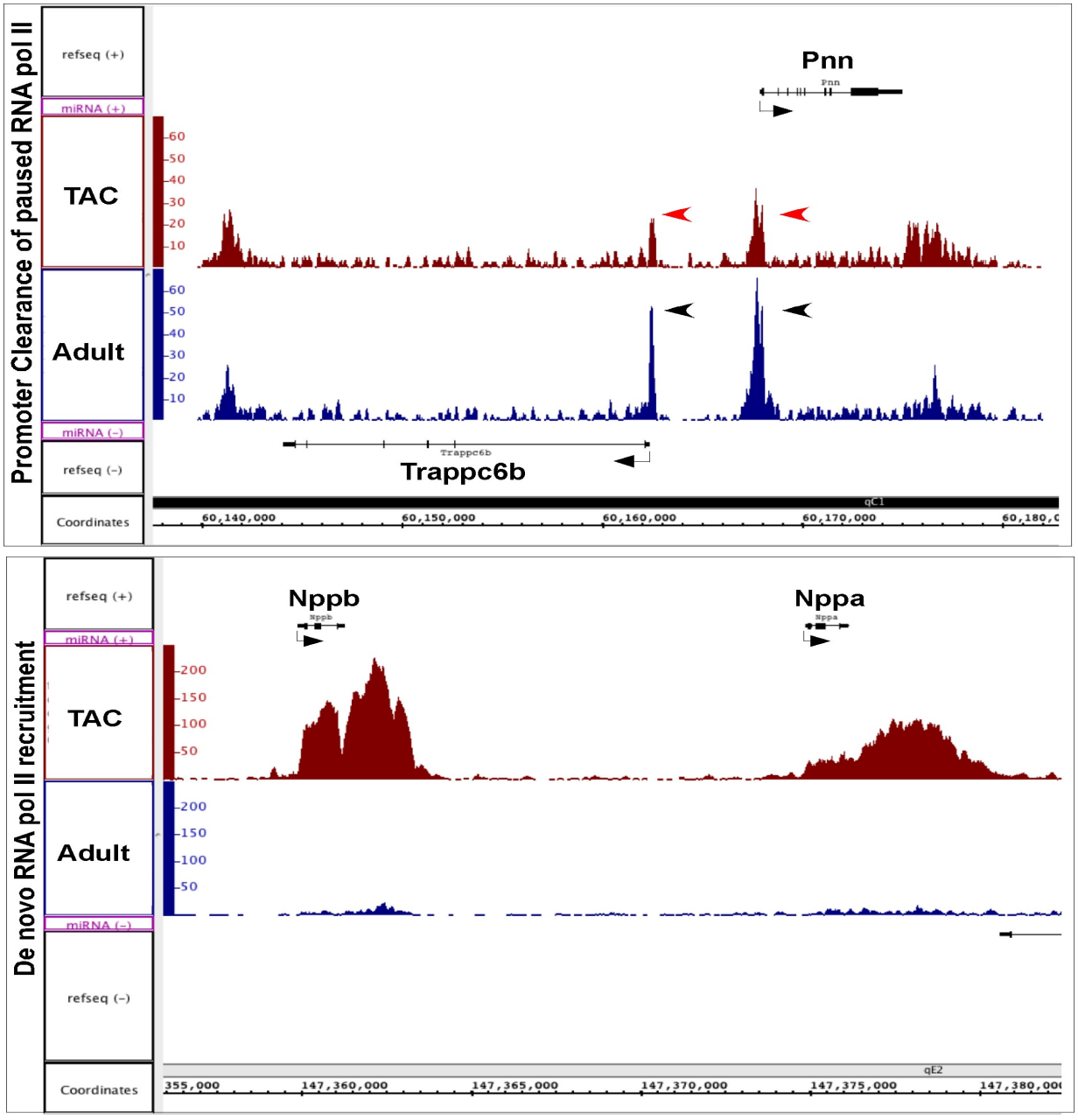
COMBINED TABLES FOR ADULT/NEONATE/TAC with AVERAGE VALUES FOR PROMOTER, IN-GENE and DOWNSTREAM REGIONS

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1				Gene from -1000 to +5000 downstream			Promoter -300 to +300			In Gene +300 to End			Downstream End to +5000		
2	Gene.Name	Gene.Stra	Gene Length	Gene.AV A1	Gene.AV B1	Gene.AV H1	Prom.AV A1	Prom.AV B1	Prom.AV H1	InGene.AV A1	InGene.AV B1	InGene.AV H1	Down.AV A1	Down.AV B1	Down.AV H1
3	Xkr4	-	457016	0.311	0.351	0.277	1.250	0.700	0.950	0.303	0.350	0.272	0.669	0.382	0.669
4	LOC100038975	+	46966	0.309	0.350	0.352	0.100	1.250	0.350	0.280	0.345	0.341	0.631	0.331	0.446
5	LOC664792	+	944	0.138	0.344	0.298	0.000	0.250	0.000	0.238	0.000	0.762	0.127	0.268	0.312
6	Rp1h	-	16249	0.338	0.328	0.266	0.150	0.250	0.200	0.375	0.321	0.313	0.287	0.350	0.159
7	Sox17	-	5485	4.950	2.850	5.747	11.750	3.350	6.150	4.405	1.853	4.307	4.471	3.803	7.522
8	Mrpl15	-	12501	3.651	2.731	3.584	27.300	7.000	12.850	2.673	3.123	3.380	3.185	1.420	3.191
9	LOC620009	+	1459	3.064	2.090	3.573	2.000	1.650	1.900	1.351	0.730	1.865	3.236	2.452	4.045
10	Lypla1	+	38842	2.068	1.340	1.632	25.850	7.950	12.000	1.590	1.069	1.351	2.032	2.369	2.057
11	Tcea1	+	40090	2.113	2.388	2.387	20.150	9.900	11.950	1.592	1.604	1.961	3.497	7.210	3.968
12	LOC619829	-	1455	1.487	1.026	1.957	1.400	0.750	1.550	0.432	0.405	0.514	1.701	1.153	2.255
13	Rgs20	-	109410	0.534	0.592	0.402	1.500	0.550	0.900	0.365	0.397	0.229	3.975	4.879	4.102
14	Atp6v1h	+	79376	1.743	1.929	2.124	13.600	5.950	8.650	1.585	1.572	1.884	2.930	7.134	5.287
15	Oprk1	+	14373	0.337	0.392	0.216	0.300	0.350	0.000	0.385	0.442	0.181	0.159	0.255	0.223
16	Npbwr1	-	3691	0.444	0.378	0.230	0.000	0.100	0.500	0.514	0.449	0.355	0.478	0.255	0.140
17	4732440D04Rik	-	9402	4.824	5.541	4.788	4.050	1.650	2.000	5.028	5.674	3.982	4.936	6.274	6.962
18	Rb1cc1	+	60913	2.896	3.925	3.029	31.400	21.200	15.200	2.347	2.986	2.452	5.382	12.592	7.777
19	EG620393	+	35678	0.288	0.309	0.242	0.850	0.650	0.500	0.269	0.305	0.248	0.350	0.242	0.159
20	LOC664946	-	472	0.394	0.443	0.172	0.600	0.900	0.500	0.833	0.333	0.000	0.344	0.446	0.064
21	LOC100039302	+	623	0.409	0.466	0.216	0.350	0.700	0.000	0.273	1.000	0.000	0.382	0.414	0.287
22	St18	+	130889	0.287	0.355	0.214	0.250	0.000	0.100	0.288	0.357	0.211	0.229	0.408	0.287
23	Pcmtd1	+	84478	2.309	1.755	1.942	29.900	9.750	12.150	1.836	1.390	1.682	3.841	5.815	3.936
24	Sntg1	-	937069	0.294	0.325	0.211	1.000	0.250	0.450	0.293	0.324	0.210	0.382	0.414	0.255
25	LOC100039474	-	586	0.193	0.203	0.048	0.250	0.000	0.250	0.000	0.000	0.000	0.191	0.210	0.032
26	LOC620444	-	342	0.302	0.352	0.231	0.000	0.450	0.050	0.000	0.000	0.500	0.382	0.318	0.280
27	EG665007	+	341	0.201	0.603	0.151	0.000	0.450	0.000	0.000	1.000	0.000	0.255	0.656	0.159
28	LOC665103	-	1034	0.271	0.380	0.063	0.500	0.000	0.000	0.000	0.417	0.000	0.318	0.408	0.032
29	LOC100039464	+	2922	0.161	0.214	0.143	0.000	0.000	0.000	0.361	0.120	0.301	0.096	0.318	0.096
30	Rrs1	+	2010	7.845	5.984	6.239	22.100	12.100	15.950	4.630	2.981	4.056	7.643	6.580	6.268
31	Adhfe1	+	29888	2.737	4.267	2.443	18.150	11.750	11.650	2.370	3.701	2.186	2.325	6.185	2.045
32	LOC620542	+	1129	2.116	5.643	1.545	1.750	2.350	0.650	0.000	0.000	0.000	2.471	7.038	1.943
33	3110035E14Rik	+	25875	1.024	0.707	1.350	1.250	1.000	0.900	0.744	0.552	1.061	2.446	1.497	2.771
34	Mybl1	-	31378	0.794	0.672	0.795	3.250	1.150	2.650	0.525	0.567	0.572	0.573	0.783	0.777
35	Vcpip1	-	25190	2.948	3.360	3.710	18.350	9.050	17.700	2.279	2.271	2.728	4.325	8.338	6.866
36	LOC100039536	+	22775	0.944	0.726	0.891	19.050	9.300	18.100	0.401	0.394	0.336	0.497	0.662	0.573
37	EG620986	-	450	3.010	1.044	2.217	1.800	0.550	2.200	0.167	0.500	0.000	3.344	1.083	2.420
38	Sgk3	+	104440	1.718	1.038	1.755	8.450	0.750	3.100	1.579	0.988	1.566	3.459	2.140	5.306
39	LOC100039643	+	292	3.247	1.889	4.207	2.000	1.150	1.250	4.000	0.000	0.000	3.446	2.127	4.955
40	6030422M02Rik	+	29235	2.355	2.173	2.916	0.850	0.450	1.050	1.477	1.299	2.051	7.822	7.605	8.382
41	LOC100039596	-	4077	0.649	0.468	0.443	0.700	0.700	0.700	0.479	0.479	0.420	0.414	0.395	0.255
42	4930418G15Rik	-	40506	0.479	0.478	0.347	0.750	1.050	1.500	0.410	0.445	0.298	0.930	0.605	0.637
43	Cops5	-	13076	2.898	3.536	3.020	15.250	7.200	6.950	1.887	2.230	2.135	3.369	6.293	4.325
44	Cspp1	+	98550	1.767	2.353	2.042	11.950	7.150	7.400	1.556	2.165	1.864	3.624	5.178	4.376
45	**Arfgef1**	**-**	**95163**	**2.665**	**3.600**	**3.062**	**13.000**	**3.500**	**7.750**	**2.417**	**3.173**	**2.814**	**4.739**	**11.439**	**6.433**
46	Cpa6	-	395216	0.277	0.281	0.171	0.700	0.150	0.100	0.274	0.277	0.172	0.478	0.510	0.083
47	C030045D06Rik	+	40835	1.349	0.685	0.823	0.150	0.050	0.200	1.349	0.659	0.797	1.318	0.917	1.185
48	A830018L16Rik	+	561789	0.279	0.310	0.201	0.250	1.550	1.000	0.278	0.309	0.201	0.414	0.331	0.083
49	LOC621353	+	713	0.213	0.403	0.313	0.350	0.500	0.750	0.357	0.000	0.000	0.191	0.318	0.287
50	LOC100039733	+	117217	1.838	0.933	1.693	0.000	0.500	0.000	1.835	0.925	1.668	2.382	1.293	2.611
51	Sulf1	+	141827	2.414	1.546	2.404	14.850	4.500	8.850	2.271	1.443	2.240	4.828	4.115	6.121
52	Slco5a1	-	124586	0.942	0.691	0.908	2.800	2.400	1.900	0.744	0.543	0.659	5.064	3.847	6.427
53	Prdm14	-	13735	0.518	0.542	0.372	1.250	2.000	1.750	0.594	0.570	0.356	0.255	0.255	0.223
54	Ncoa2	-	234924	2.032	2.515	1.947	10.050	6.900	6.400	1.982	2.442	1.898	3.013	4.873	3.503
55	EG621685	-	12431	0.315	0.234	0.352	1.450	0.950	1.000	0.118	0.079	0.132	0.382	0.420	0.287
56	Tram1	-	25162	2.833	1.862	3.333	18.200	6.450	10.100	2.212	1.508	2.857	4.057	3.121	5.038
57	Lactb2	-	34611	1.803	2.217	2.240	16.100	5.050	10.300	1.552	1.972	2.003	1.783	3.631	3.057
58	Xkr9	+	32952	0.401	0.347	0.197	1.200	0.600	0.200	0.363	0.341	0.216	0.567	0.401	0.032
59	EG433273	-	1052	0.575	0.543	0.317	1.000	0.750	0.450	0.000	0.200	0.440	0.605	0.605	0.255

Pol II binding sites on Myh6 (Alpha Myosin Heavy Chain) gene as visualized on Integrated genome Browser

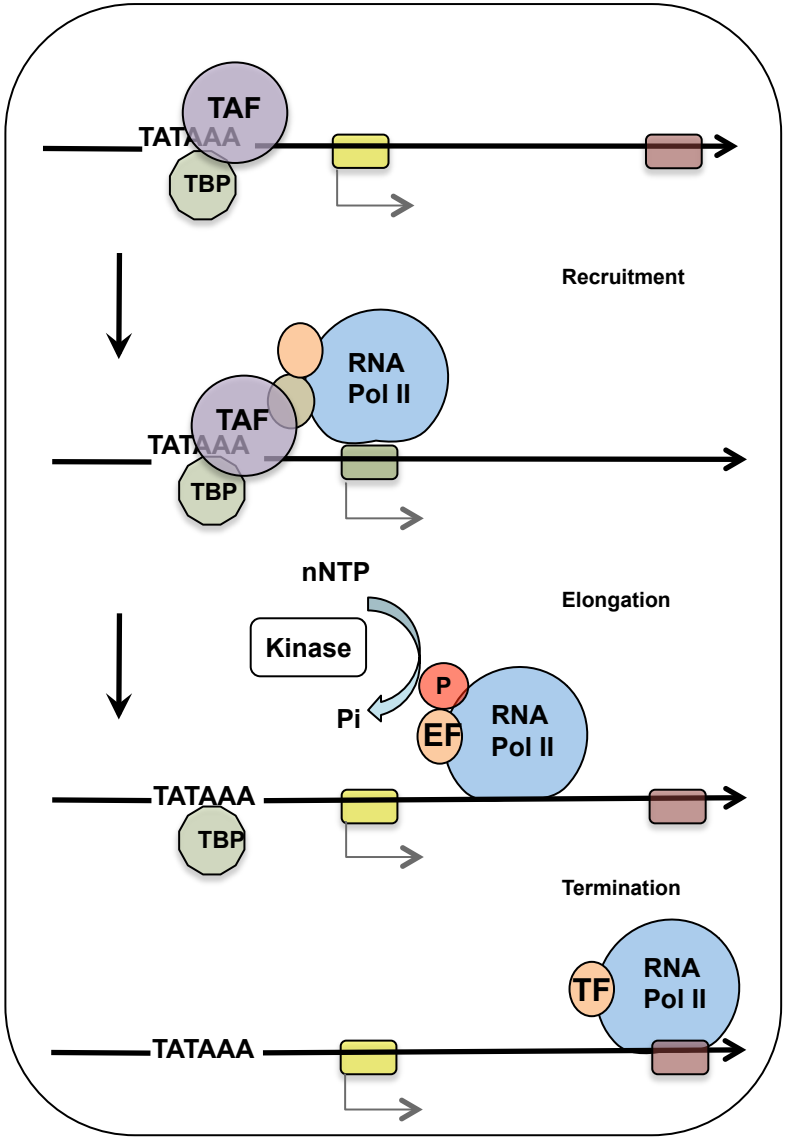


Two modes of gene transcription during growth



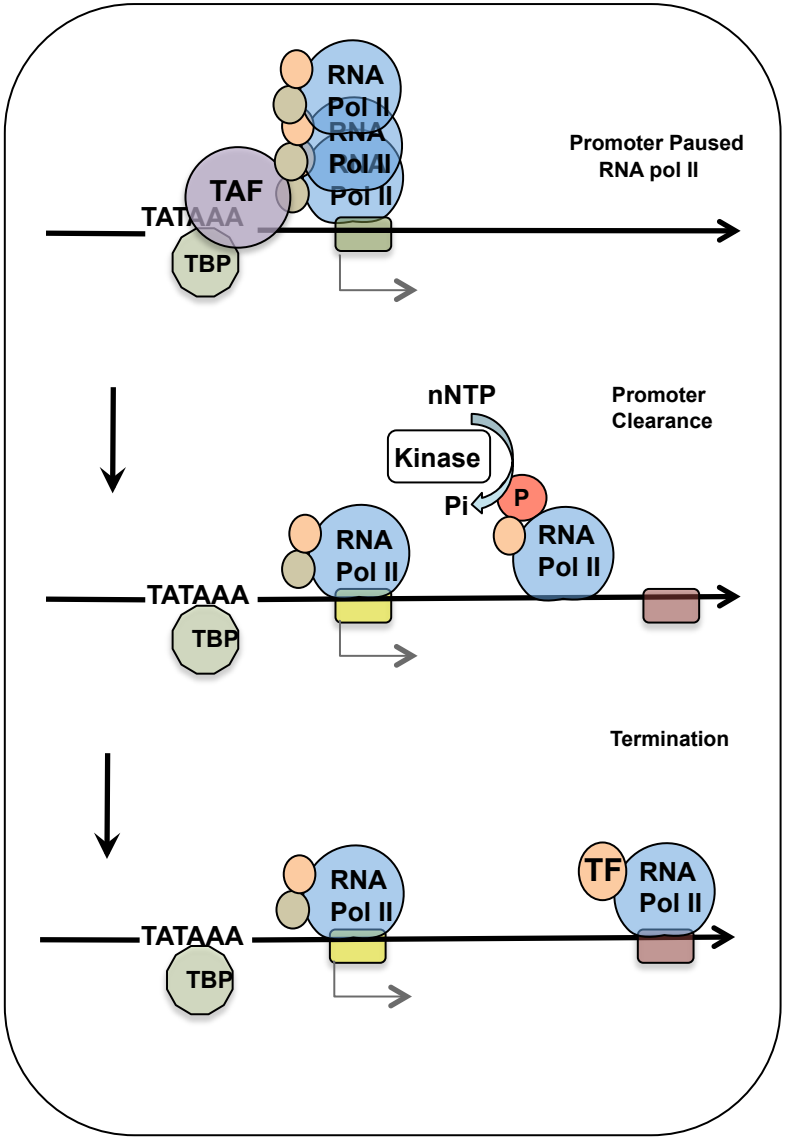
Transcription during cardiac hypertrophy

Central Dogma



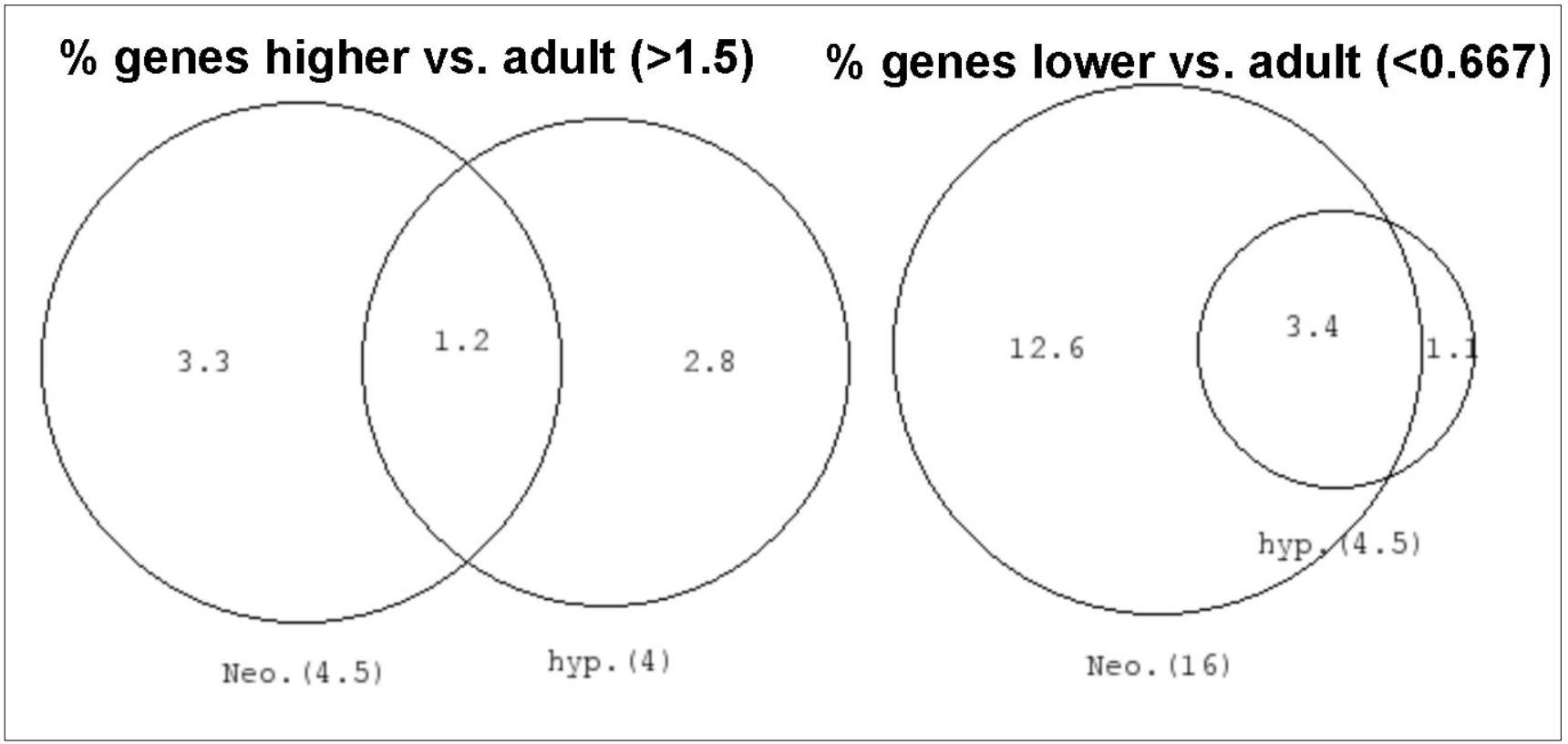
De Novo RNA Pol II Recruitment

Recently identified

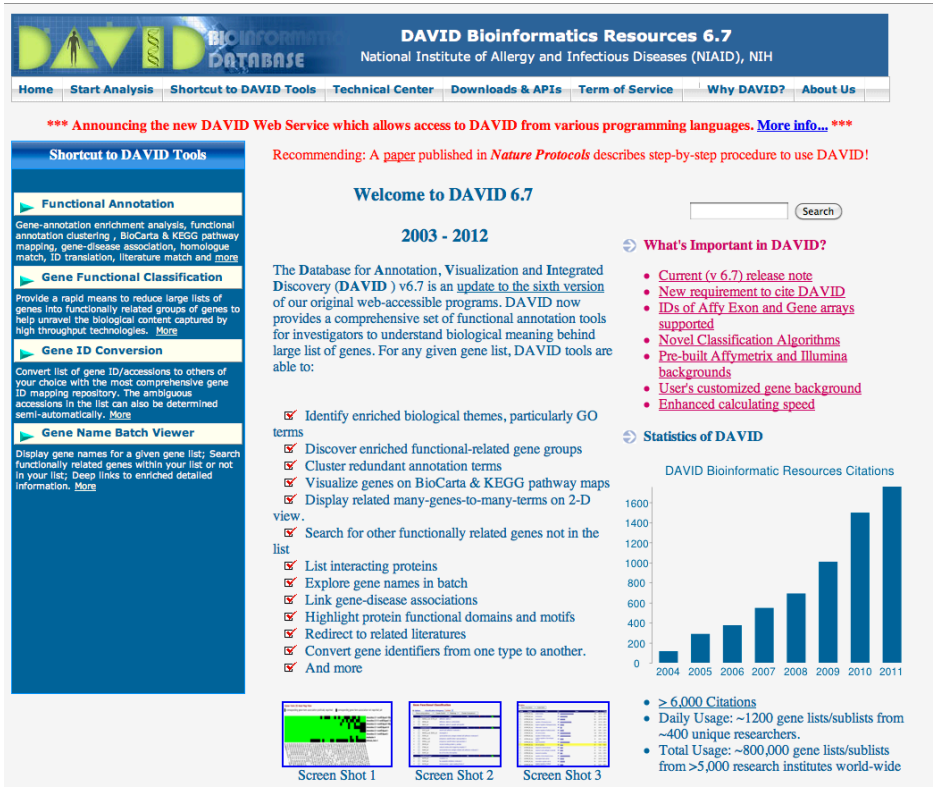


Promoter Clearance of paused RNA pol II

De Novo recruitment of RNA pol II during hypertrophy



Gene Ontology



Genes that require De Novo Pol II recruitment during Hypertrophy

DAVID Bioinformatics Resources 6.7
National Institute of Allergy and Infectious Diseases (NIAID), NIH

*** Announcing the new DAVID Web Service which allows access to DAVID from various programming languages. More info... ***

Functional Annotation Chart

Help and Manual

Current Gene List: sublist
Current Background: Mus musculus
30 DAVID IDs

⊞ **Options**

Rerun Using Options | Create Sublist

12 chart records

Download File

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
☐	KEGG_PATHWAY	Hypertrophic cardiomyopathy (HCM)	RT		15	50.0	4.3E-19	1.5E-17
☐	KEGG_PATHWAY	Dilated cardiomyopathy	RT		15	50.0	1.7E-18	2.9E-17
☐	KEGG_PATHWAY	Regulation of actin cytoskeleton	RT		16	53.3	1.4E-14	1.6E-13
☐	KEGG_PATHWAY	Cardiac muscle contraction	RT		10	33.3	8.0E-11	7.0E-10
☐	KEGG_PATHWAY	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	RT		7	23.3	1.5E-6	1.1E-5
☐	KEGG_PATHWAY	Tight junction	RT		8	26.7	3.5E-6	2.0E-5
☐	KEGG_PATHWAY	Focal adhesion	RT		8	26.7	4.3E-5	2.1E-4
☐	KEGG_PATHWAY	Leukocyte transendothelial migration	RT		6	20.0	2.8E-4	1.2E-3
☐	KEGG_PATHWAY	Adherens junction	RT		4	13.3	6.4E-3	2.5E-2
☐	KEGG_PATHWAY	Renal cell carcinoma	RT		3	10.0	4.8E-2	1.6E-1
☐	KEGG_PATHWAY	Calcium signaling pathway	RT		4	13.3	7.0E-2	2.1E-1
☐	KEGG_PATHWAY	Viral myocarditis	RT		3	10.0	8.1E-2	2.2E-1

Genes that show promoter pol II pausing in adult hearts

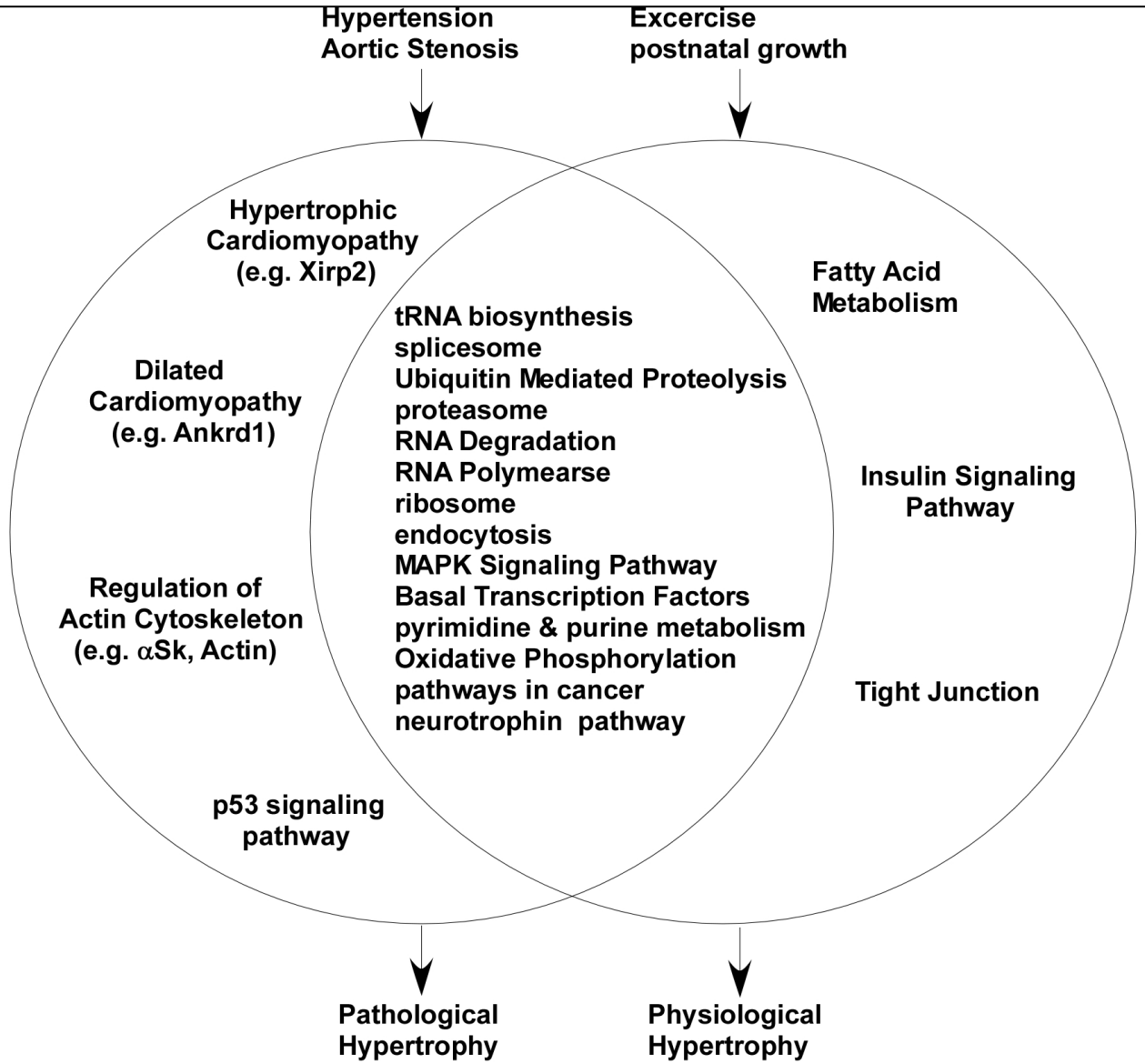
Genes that exhibited promoter-proximal pol II pausing in the adult *versus* neonatal heart (paused genes cutoff: average pol II density of TSS/in-gene >6.2) and those that exhibited a release of pol II pausing during TAC were analyzed for functional categories using DAVID v6.7

Only the top 20 functional categories are listed. The full list is published in supplementary Table 1S.

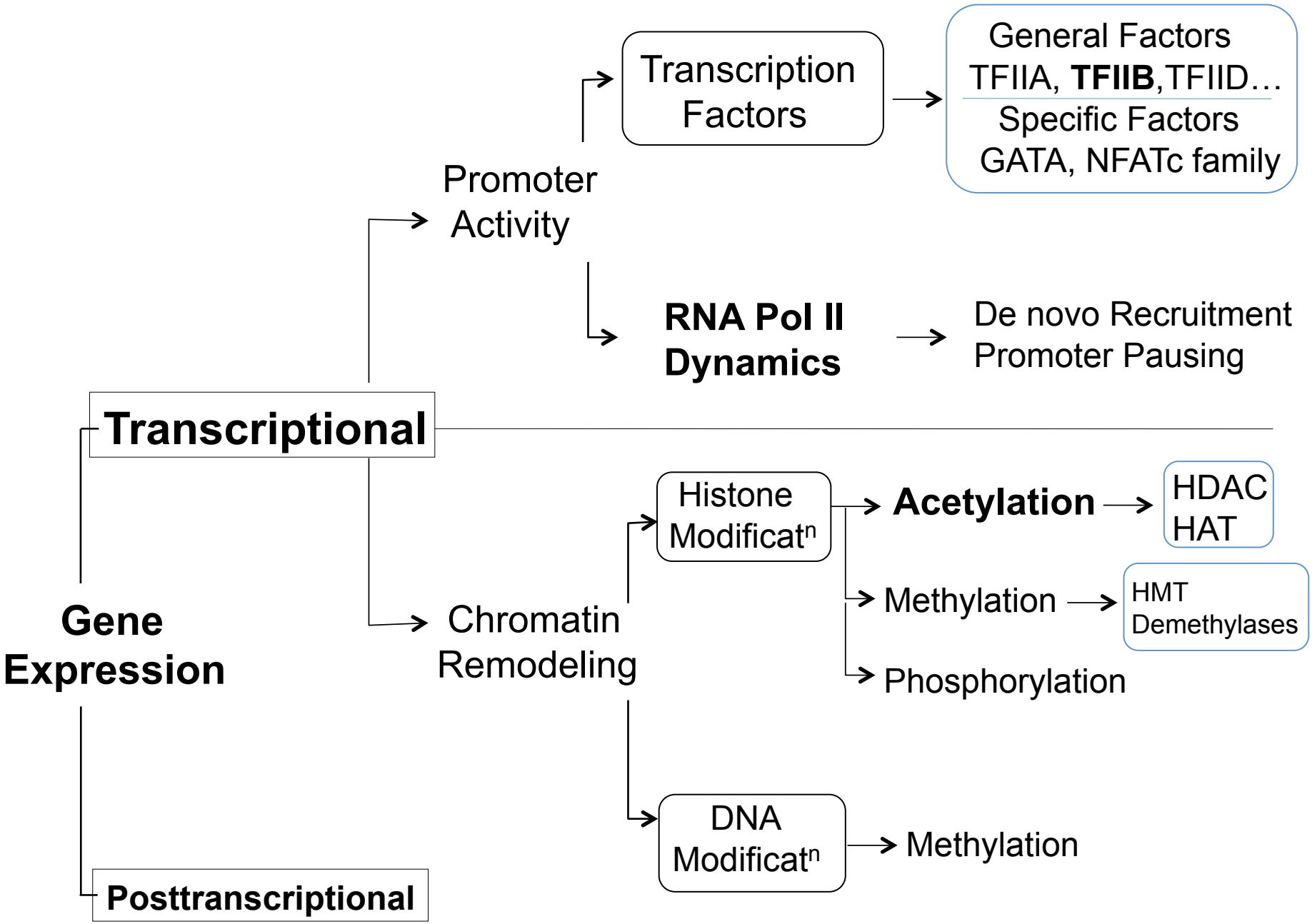
Functional annotation of genes paused in the adult heart			Functional annotation of genes exhibiting reduced pausing during TAC		
Functional pathway	No. of genes	*p* value	Functional pathway	No. of genes	*p* value
Aminoacyl-tRNA biosynthesis	36	1.9E-18	Aminoacyl-tRNA biosynthesis	28	9.1E-13
Splicesome	61	1.1E-12	Splicesome	51	1.3E-11
Nucleotide excision repair	27	8.1E-9	Ubiquitin-mediated proteolysis	51	6.7E-10
Pyrimidine metabolism	43	1.2E-7	Lysosome	41	5.8E-7
Ubiquitin-mediated proteolysis	54	2.8E-7	Nucleotide excision repair	21	1.4E-6
Lysosome	49	3.1E-7	Ribosome	32	4.7E-6
RNA degradation	30	8.8E-7	Proteasome	20	3.1E-5
Valine, leucine, and isoleucine degradation	24	5.8E-6	Endocytosis	54	4.3E-5
RNA polymerase	17	9.5E-6	RNA degradation	23	4.6E-5
Purine metabolism	53	8.3E-5	SNARE interactions in vesicular transport	17	7.4E-5
Glycosylphophatidylinositol anchor biosynthesis	15	8.3E-5	Cell cycle	34	1.6E-3
Endocytosis	63	2.2E-4	Pyrimidine metabolism	27	2.3E-3
Ribosome	33	3.8E-4	Huntington disease	44	2.6E-3
Proteasome	21	3.9E-4	Purine metabolism	39	2.6E-3
SNARE interactions in vesicular transport	18	5.3E-4	Basal transcription factors	13	2.7E-3
Fatty acid metabolism	20	6.1E-4	Oocyte meiosis	30	4.3E-3
Oocyte meiosis	39	7.7E-4	ErbB signaling pathway	23	1.2E-2
Cell cycle	41	1.9E-3	Fatty acid metabolism	14	1.6E-2
Huntington disease	54	2.6E-3	RNA polymerase	10	1.7E-2
Glycosaminoglycan degradation	12	2.7E-3	Homologous recombination	10	1.7E-2

Genes induced by de novo pol II recruitment during cardiac growth

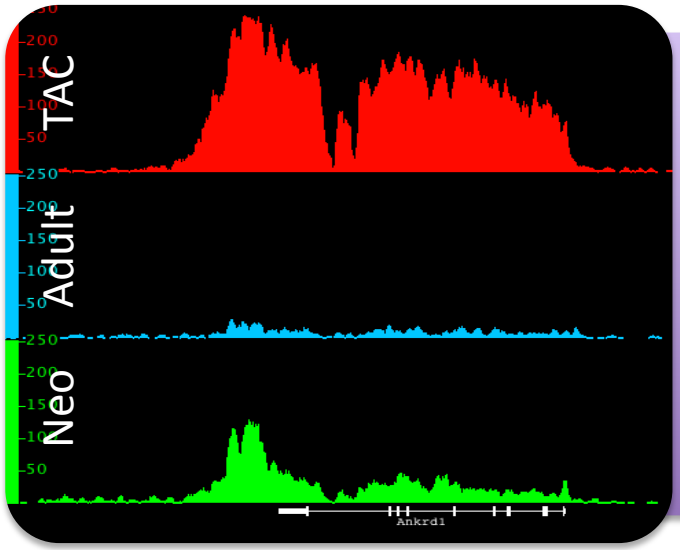
Functional annotation of genes exhibiting *de novo* pol II recruitment during TAC (>2x)			**Functional annotation of genes exhibiting higher neonatal/adult pol II recruitment (>2x)**		
Functional pathway	**# of genes**	***P* value**	**Functional pathway**	**# of genes**	***P* value**
Hypertrophic cardiomyopathy (HCM)	10	8.4E-6	Dilated cardiomyopathy	15	1.2E-13
Dilated cardiomyopathy	10	1.8E-5	Hypertrophic cardiomyopathy (HCM)	13	1.8E-11
Systemic Lupus erythematosus	10	4.4E-5	Cardiac muscle contraction	12	1.6E-10
Focal adhesion	12	3.9E-4	Arrhythmogenic right ventricular cardiomyopathy	7	8.7E-5
ECM-receptor interaction ←	8	4.1E-4	Calcium signaling pathway	8	2.6E-3
Chemokine signaling pathway ←	11	7.8E-4	Tight Junction	6	1.1E-2
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	6	7.8E-3	Vascular smooth muscle contraction	5	3.1E-2
Cardiac muscle contraction	6	9.1E-3			
Hematopoietic cell lineage	6	1.2E-2			
Natural killer cell mediated cytotoxicity ←	7	1.5E-2			
Regulation of actin cytoskeleton	9	2.7E-2			
p53 signaling pathway ←	5	2.7E-2			
Fc epsilon RI signaling pathway	5	4.6E-2			



Venn diagram demonstrating the functional gene categories in physiological and pathological hypertrophy and their overlap as identified by our high resolution anti-pol II and anti-H3K9ac ChIP-Seq results. A therapeutic goal is suppress the genes that are uniquely activated during pathological hypertrophy while preserving those that are involved in essential cell functions and overlap with physiological hypertrophy.


Gene Expression
Transcriptional
Posttranscriptional
Promoter Activity
Transcription Factors
General Factors
TFIIA, TFIIB,TFIID…
Specific Factors
GATA, NFATc family
RNA Pol II Dynamics
De novo Recruitment
Promoter Pausing
Chromatin Remodeling
Histone Modificatn
Acetylation
HDAC
HAT
Methylation
HMT
Demethylases
Phosphorylation
DNA Modificatn
Methylation

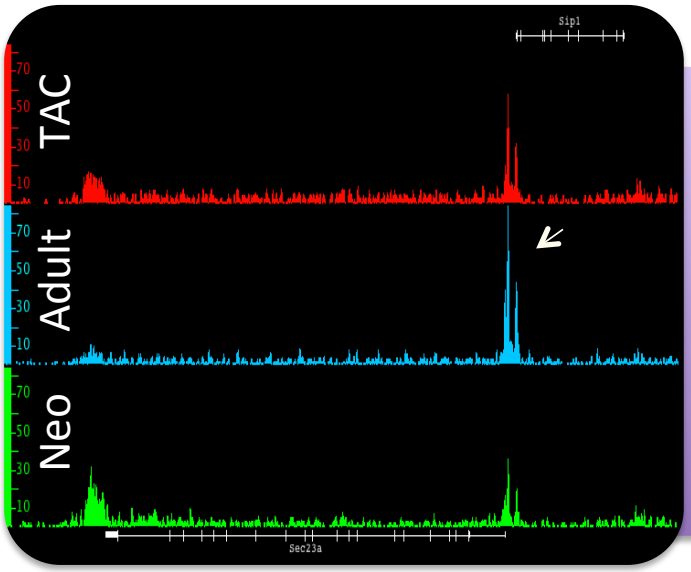
General factors that mediate the two modes of gene transcription



De novo pol II recruitment

TATA binding protein (TBP)

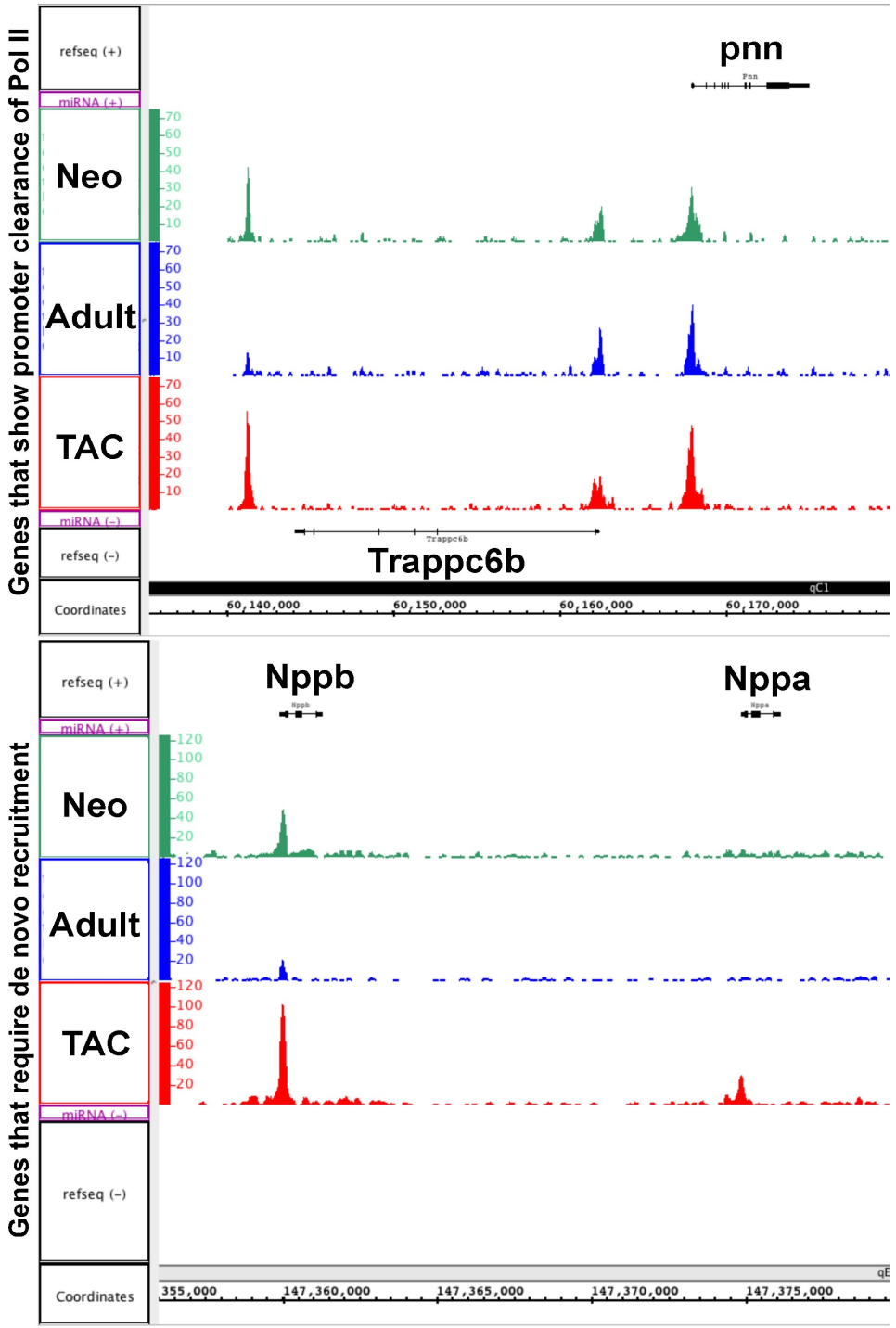
Transcription factor IIB **(TFIIB)**



Promoter Clearance of pol II

Cyclin dependent kinase **(CDK9)**

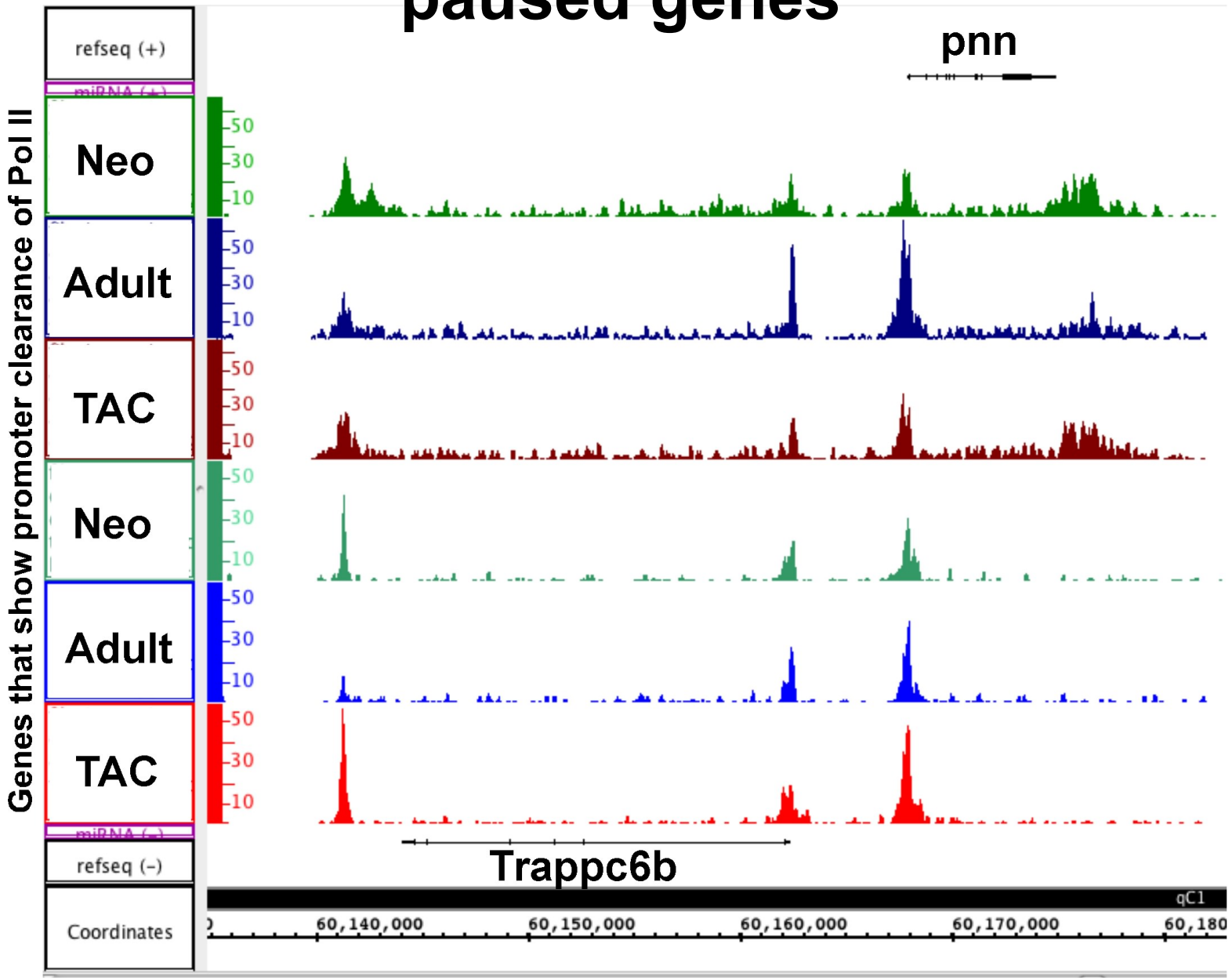
Negative elongation factor (NELF)



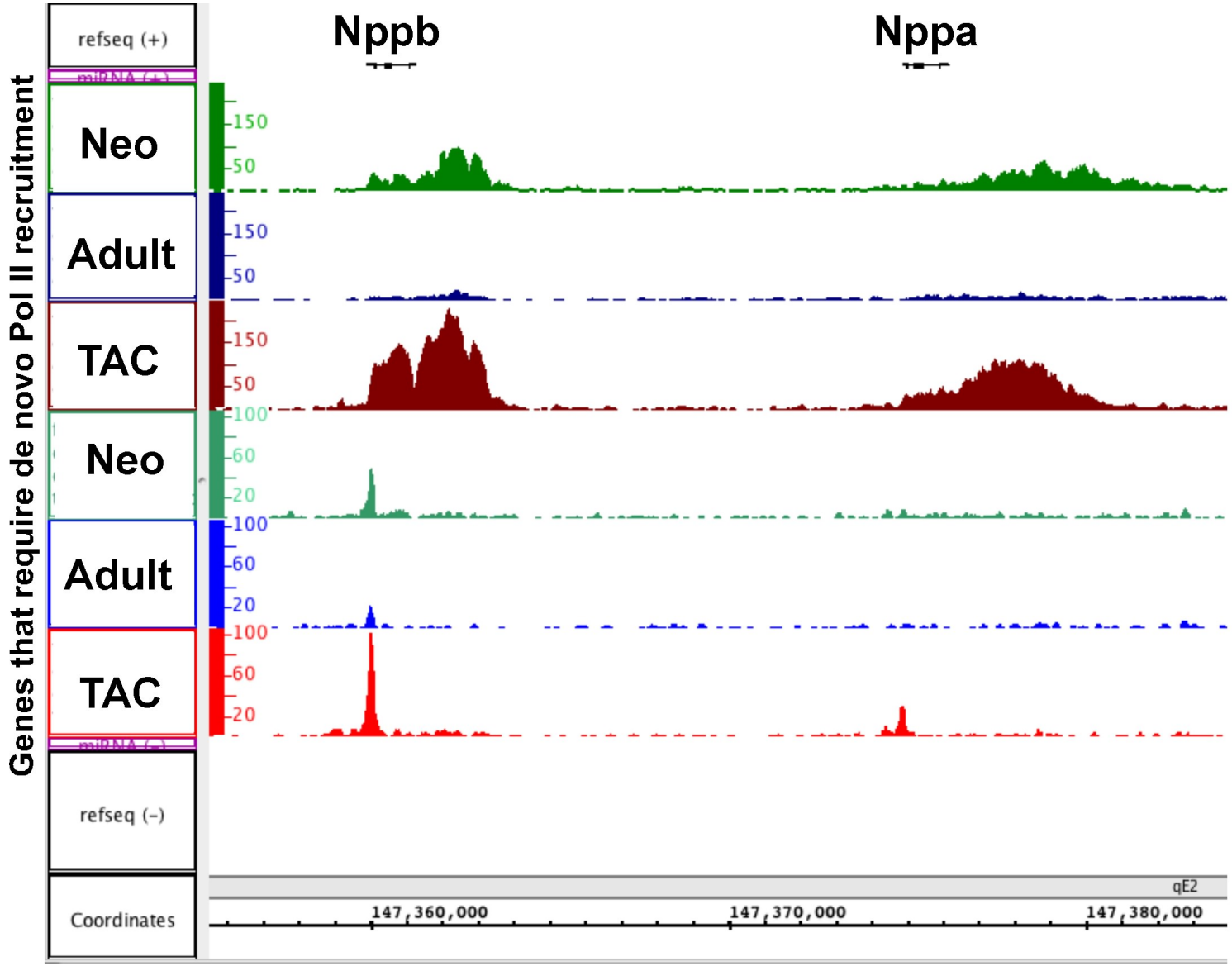
TFIIB dynamics during cardiac growth

- To understand the TFIIB distribution and dynamics during cardiac hypertrophy, we performed ChIP-Seq using TFIIB antibody

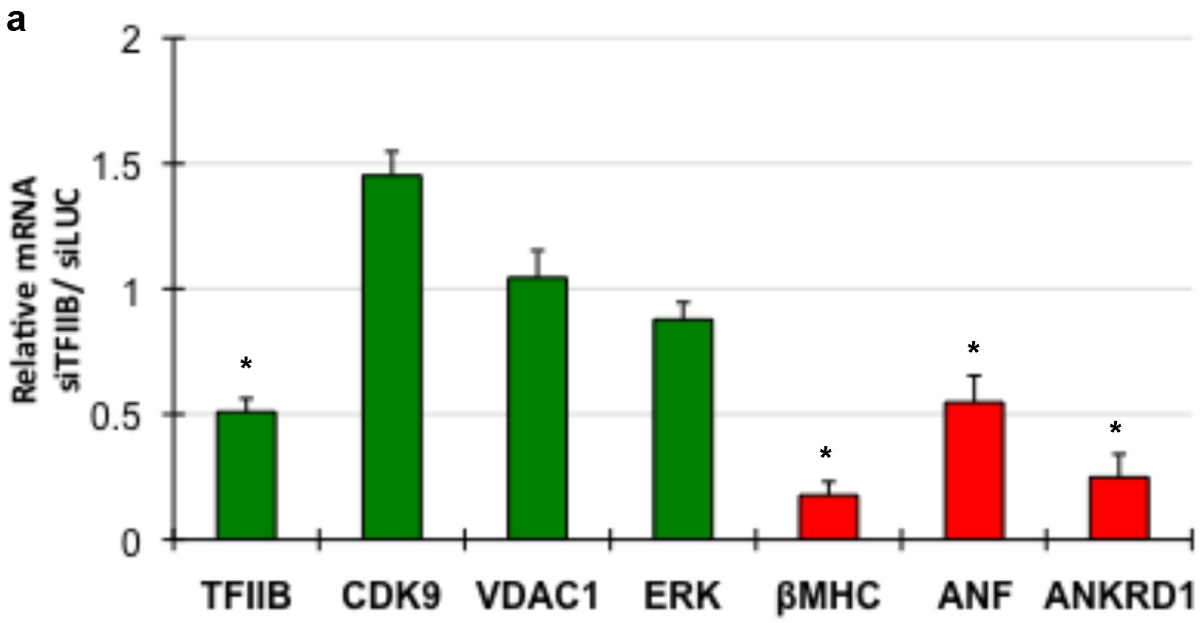
Alignment of TFIIB and Pol II on paused genes



Alignment of TFIIB and Pol II on Specialized genes



Will inhibiting TFIIB effect only cardiomyopathy-related genes?



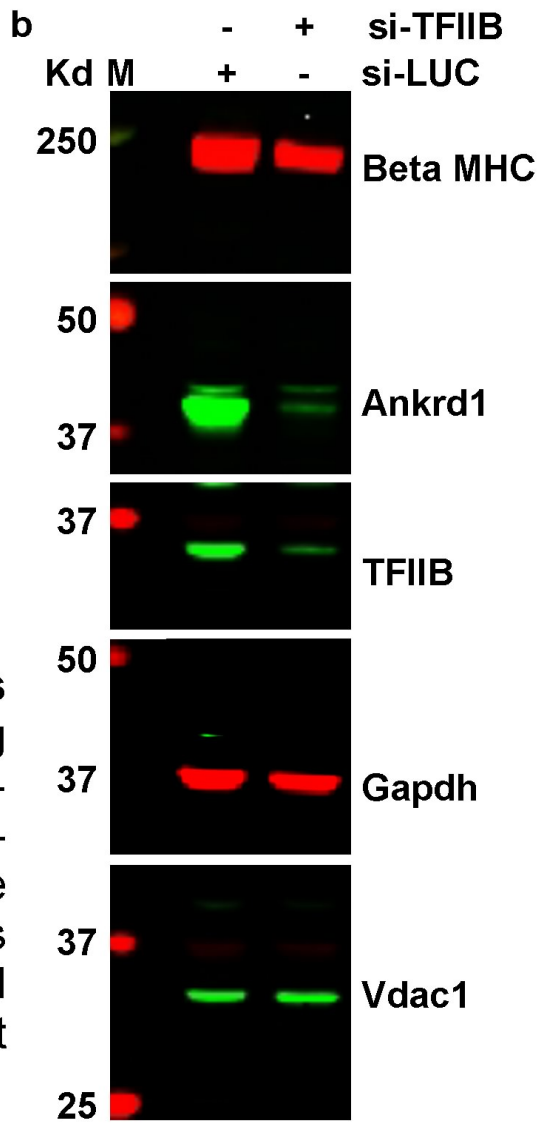
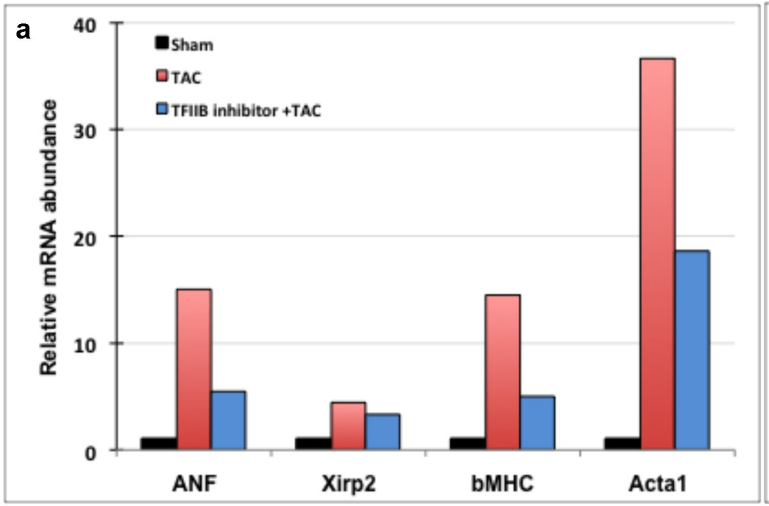
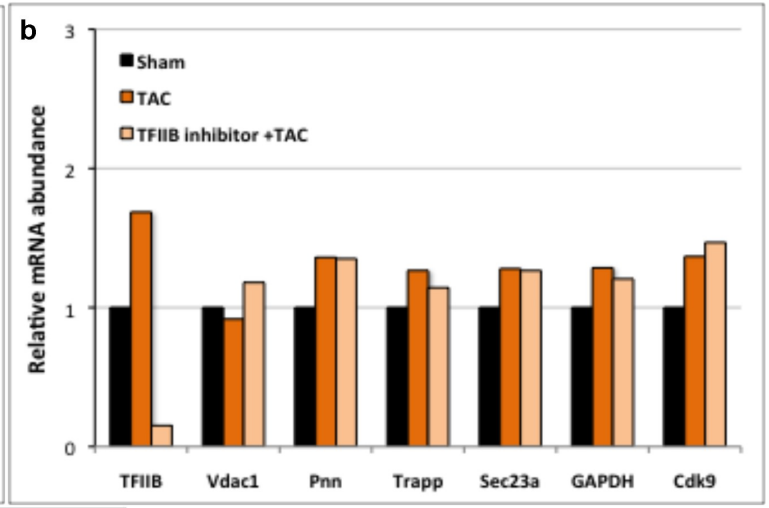


Fig 2. TFIIB inhibition restricts the expression of genes that require de novo pol II recruitment in hypertrophying cultured cardiomyocytes. a. Neonatal myocytes were cultured under growth conditions and supplemented with adenovirus expressing shRNA against TFIIB (si-TFIIB) or Luciferase gene (si-LUC) for 24hrs extracting RNA for qPCR for the genes indicated. Error bars represents SE, and * <0.01. **b**. parallel experiments as described in a, were conducted to extract protien lysaye for western blot analysis.

In vivo Inhibition of TFIIB





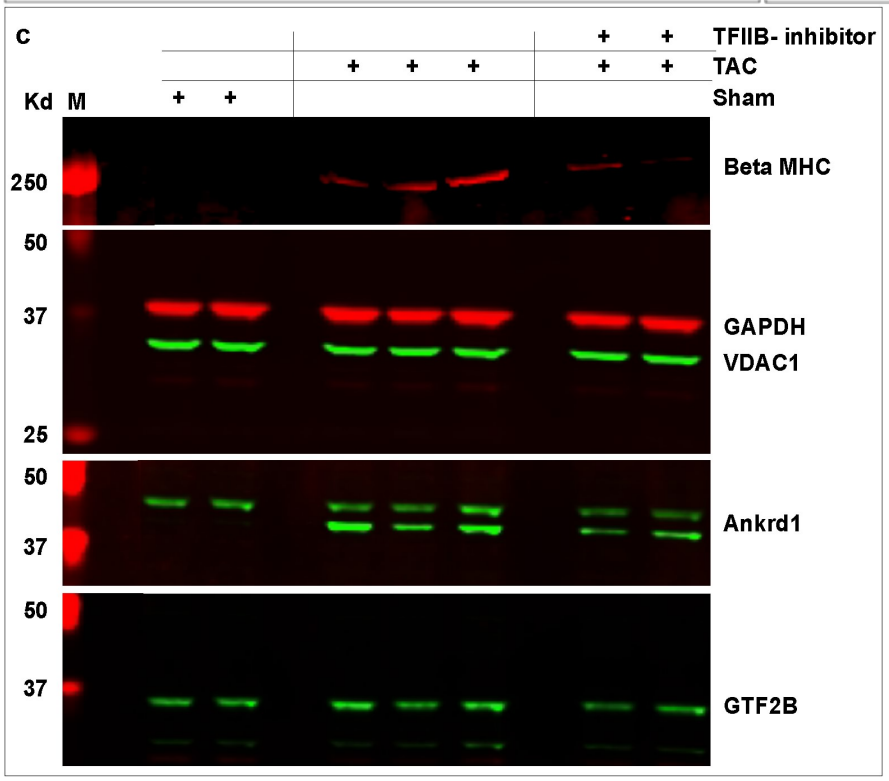


Fig 3. In-vivo inhibition of TFIIB represses the expression of selective genes. C57/Blk mice were subjected to TAC or sham operations. 1day post TAC mice were either injected with In vivo LNA-TFIIB inhibitor or control LNA oligo. Hearts were harvested 7days post TAC and RNA extracted for qPCR and protein for western blot analysis. a. qPCR results for genes that are regulated by de novo pol II recruitment. b. qPCR results for genes that are regulated by promoter clearance during cardiac hypertrophy. c. Western blot analysis of the protein lysate from mice hearts as described above of selected genes.

Transcription during cardiac hypertrophy

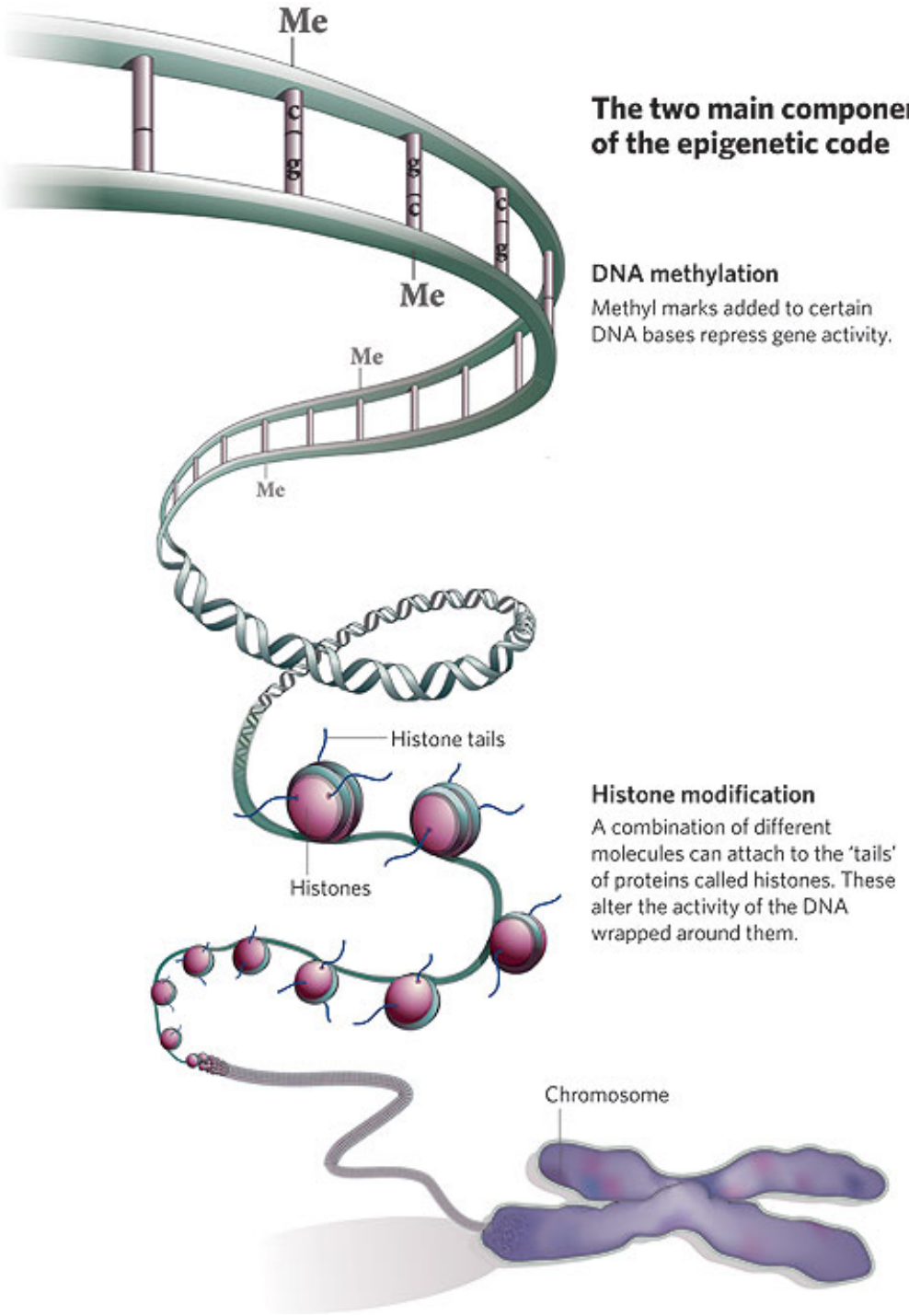
- Release of promoter paused RNA pol II and de novo RNA pol II are the two modes of gene transcription during cardiac hypertrophy.
- These two modes regulate distinct set of genes. Promoter clearance controls incremental increase in essential/housekeeping genes, de novo pol II recruitment regulates induction of specialized genes
- Manipulation of TFIIB levels can serve as a potential adjuvant therapeutic target for selectively regulating cardiomyopathy-related genes during pathological cardiac hypertrophy.

Epigenetics

http://www.pbs.org/wgbh/nova/body/epigenetics.html

What is epigenetics?

- Study of heritable, self perpetuating, reversible changes in gene activity, not caused by changes in DNA sequence.
- Epi – 'Over or on top of' – genetics



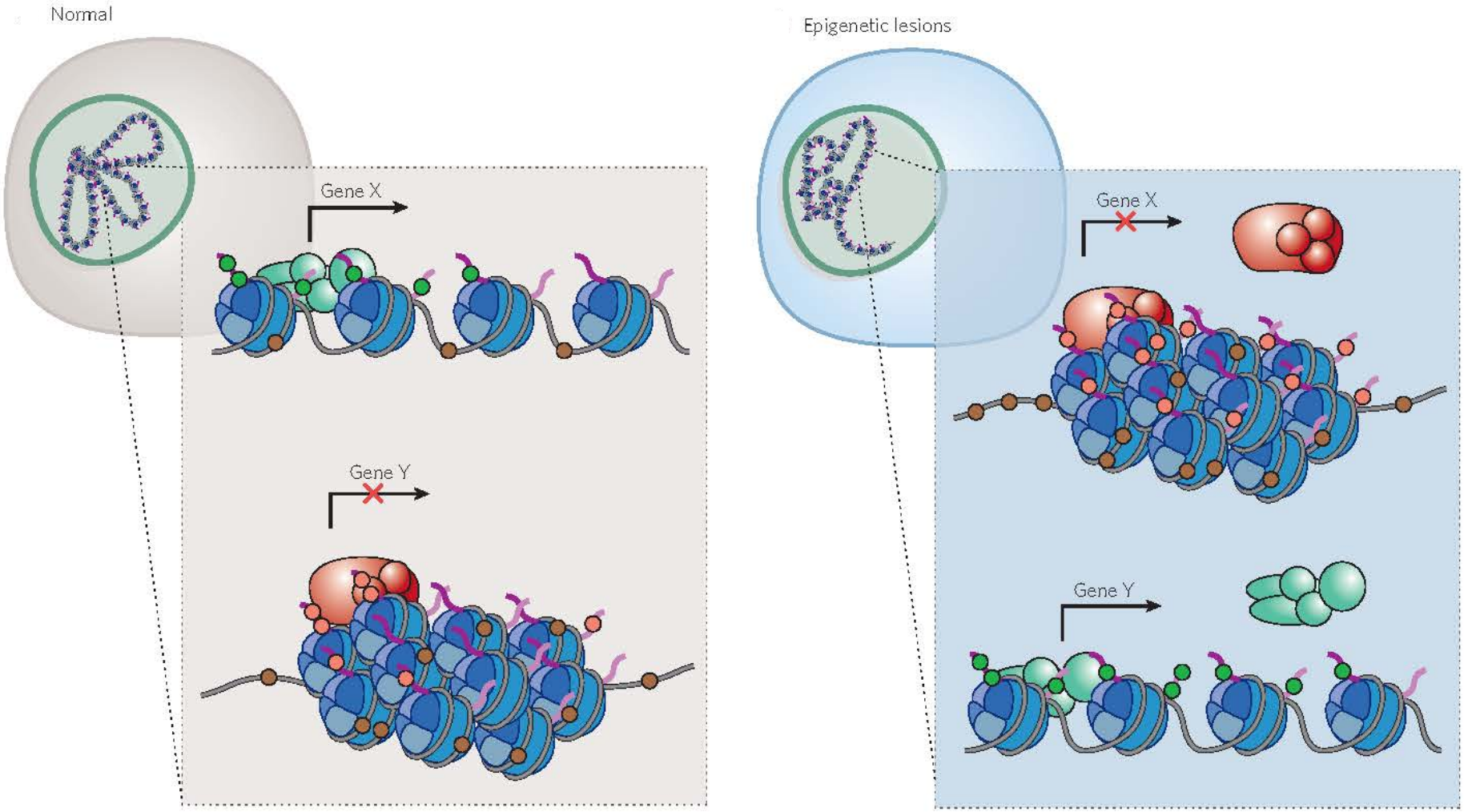
The two main components of the epigenetic code

DNA methylation

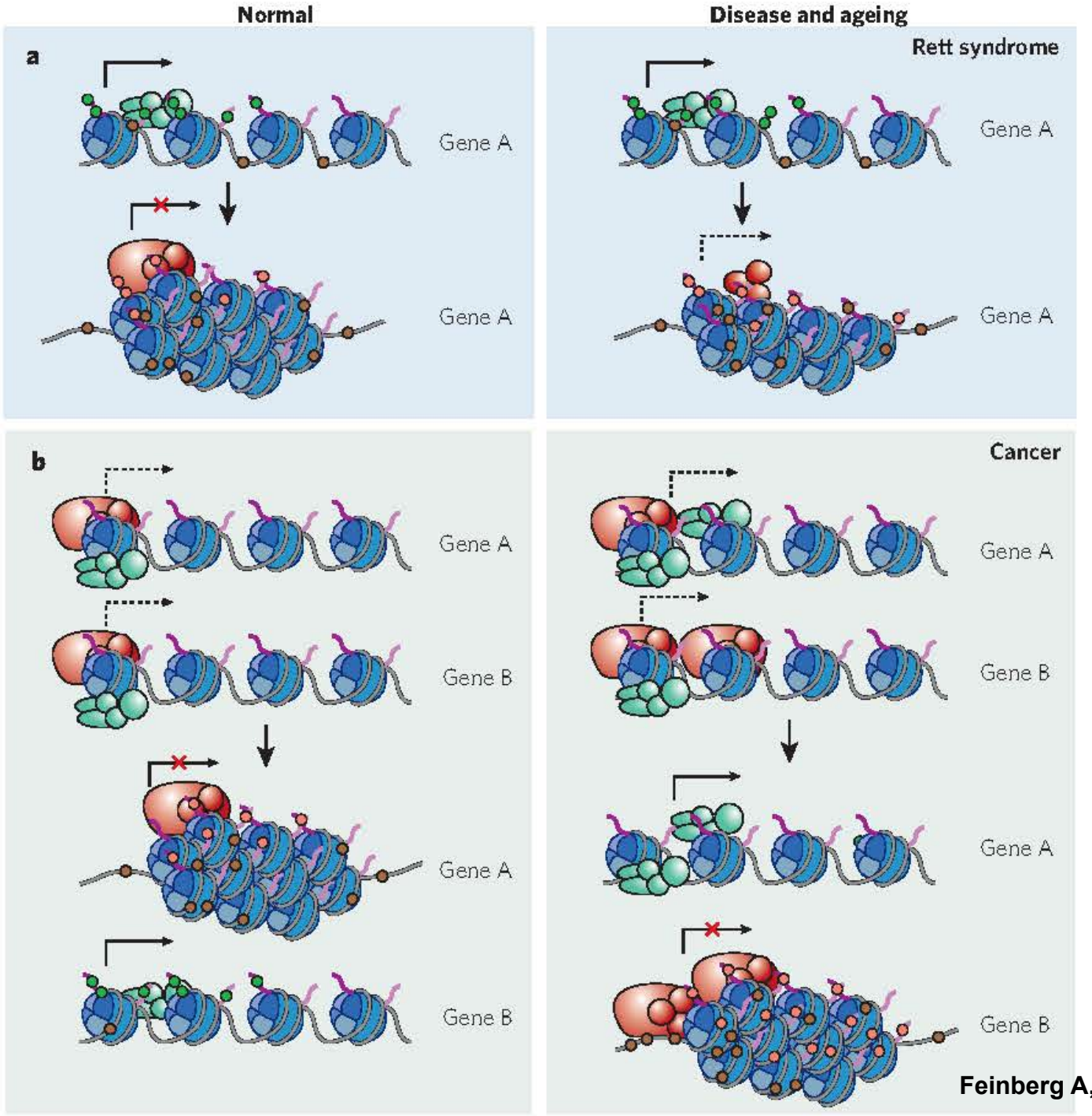
Methyl marks added to certain DNA bases repress gene activity.

Histone modification

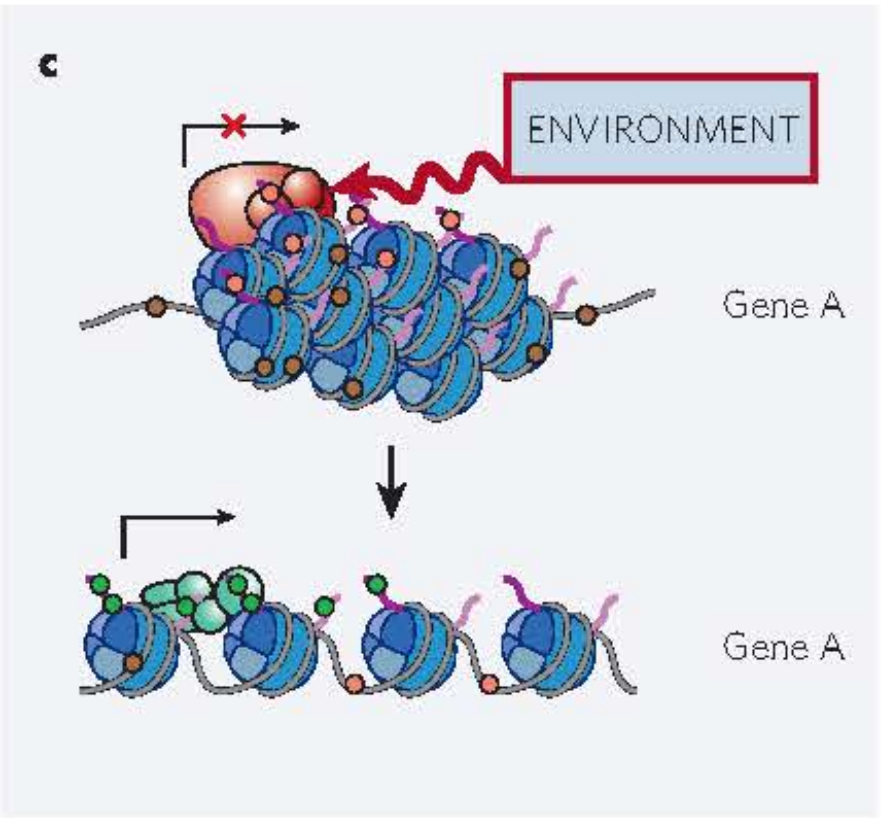
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.

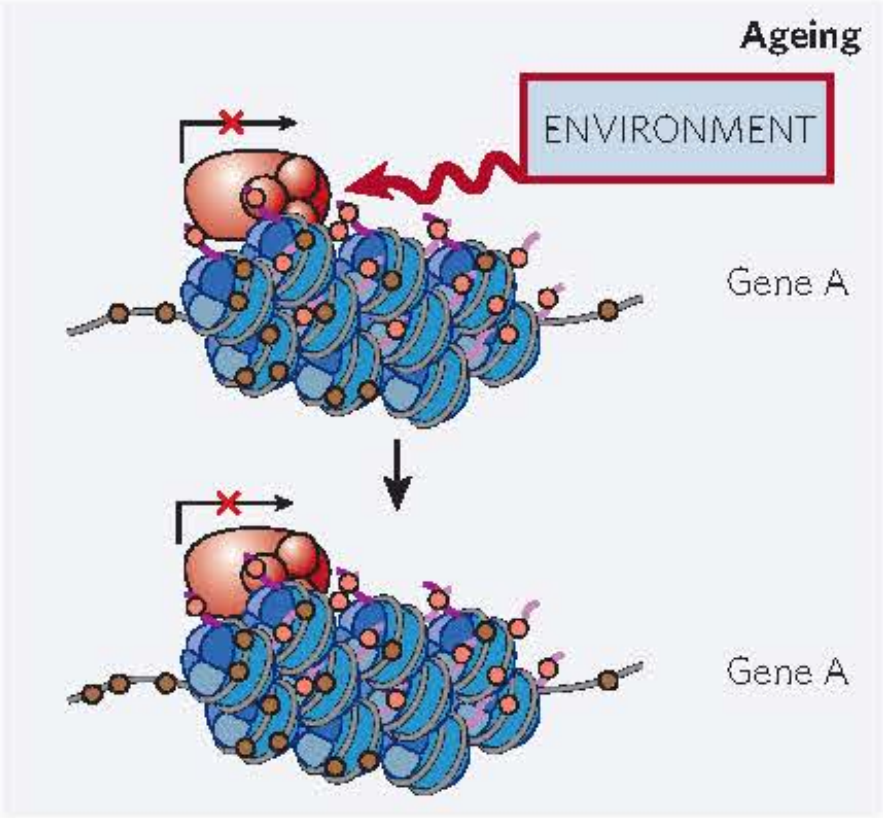


Feinberg A, Nature/Vol 447/2007



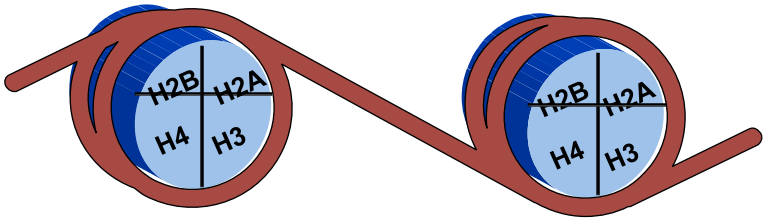
Feinberg A, Nature/Vol 447/2007



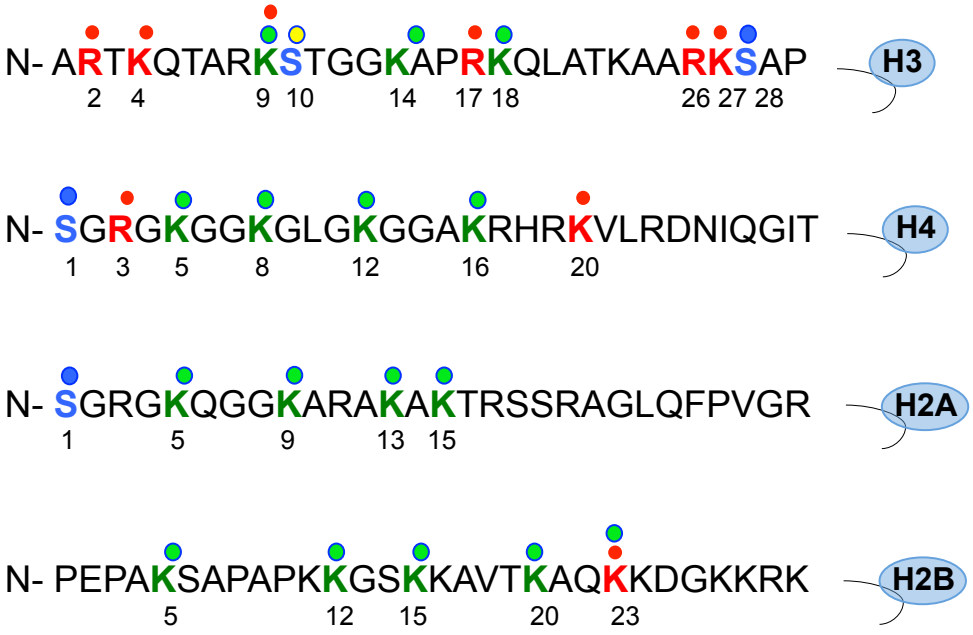


Feinberg A, Nature/Vol 447/2007

Histone Modifications

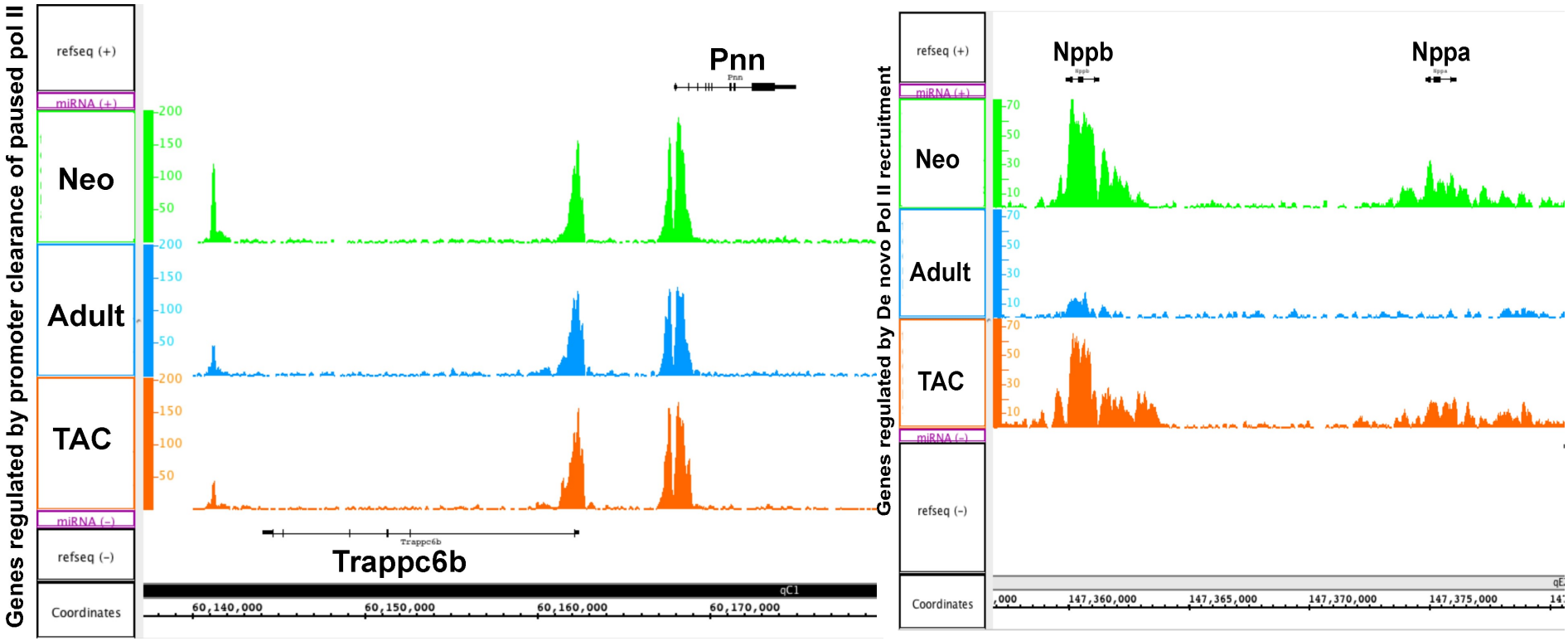


Nucleosome

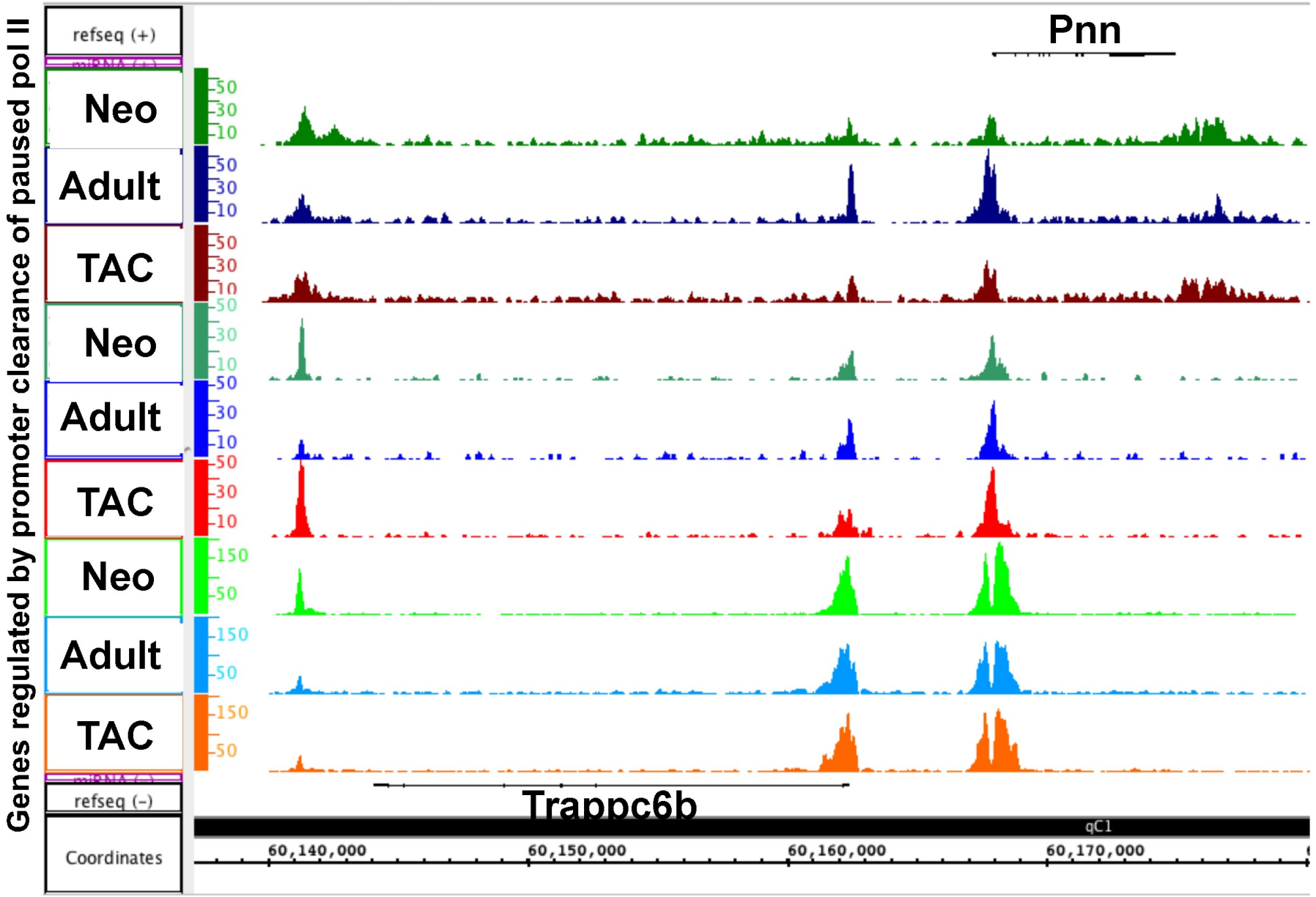


Acetylation is associated with activation, while methylation can be associated with activation or inhibition depending on location of modification as well as number added; mono, di or tri methylation

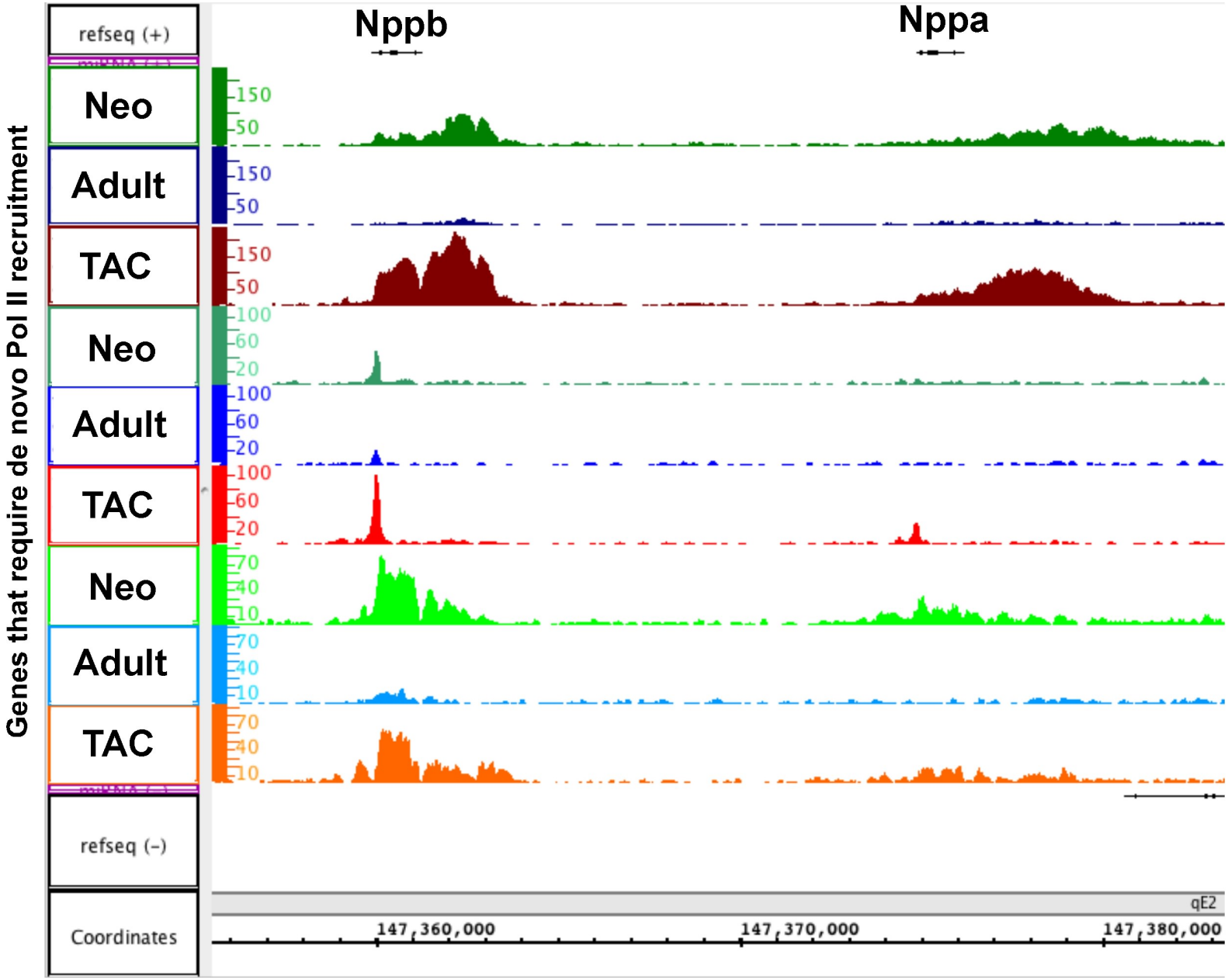
H3K9Ac status of cardiac genome during growth



Aligning RNA pol II, TFIIB and H3K9Ac.



Aligning RNA pol II, TFIIB and H3K9Ac.



Will regulating H3K9Ac effect only selective genes during cardiac hypertrophy

- Essential genes with paused promoter RNA pol II have acetylated H3K9 in adult and the hypertrophied hearts, similar trend as TFIIB
- Specialized genes require de novo pol II H3K9Ac during cardiac hypertrophy for transcription.
- Preventing de novo H3K9 acetylation will effect only specialized genes, sparing the essential genes during cardiac hypertrophy
- Modulators – Inhibitors of HAT e.g. Curcumin, Histone deacetylators

Conclusion

- Gene regulation during cardiac hypertrophy can be broadly divided into two sets, incremental increase in essential/housekeeping genes and specialized genes that show significant induction

- Two modes, promoter clearance of pol II and de novo recruitment of pol II, TFIIB and H3K9Ac differentially regulate the two groups during cardiac hypertrophy

- Manipulation of TFIIB levels or H3K9Ac status at gene promoters can serve as potential therapeutic targets to selectively and collectively control induction of specialized genes during cardiac growth

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