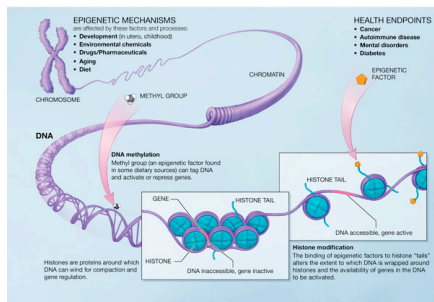


Protein Engineering

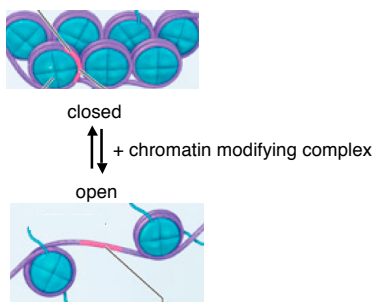
20.109 Module 2 Day 2
Thursday Oct 9th, 2008

Textbook version of DNA condensation

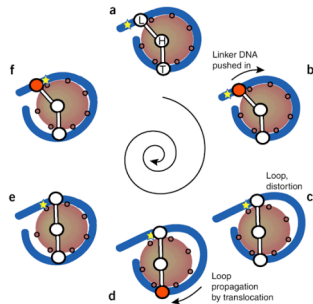


<http://nihroadmap.nih.gov/epigenomics/epigeneticmechanisms.asp>

Chromatin "epigenetics"

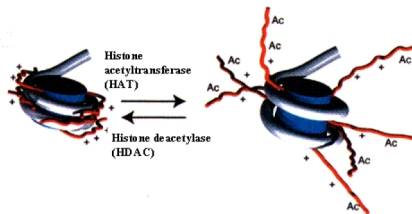


Chromatin "epigenetics": chromatin remodeling

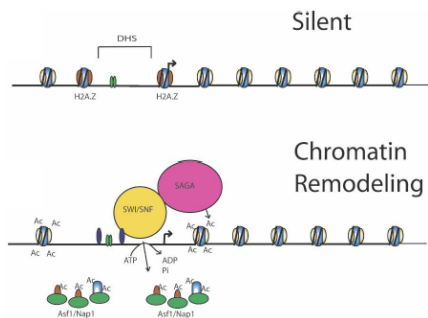


Nature Structural & Molecular Biology 12, 732 - 733 (2005)

Chromatin "epigenetics": histone modifications



Regulation of Chromatin Structure



from Workman G&D 2006 review

S. cerevisiae as great model organism



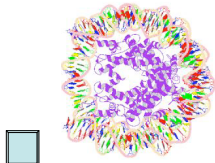
- Genomic stability/recombination balance
- Fully sequenced
- Two mating types
- Many phenotypes (sugar use, aa biosynth, mating type)
- Quick growth
- Easy/cheap growth
- Techniques to manipulate (molecular biology)
- Evolutionary conservation with more complex eukaryotes

S. cerevisiae nucleosomes: made from histones

Protein	Gene
H2A } dimer	HTA2/HTA1 (H istone T wo A)
H2B } dimer	HTB2/HTB1 (H istone T wo B)
H3 } dimer	HHT1/HHT2 (H istone H Three)
H4 } dimer	HHF1/HHF2 (H istone H Four)

x2

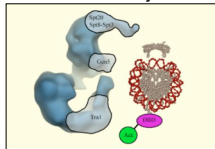
+ linker histone
H1 (encoded by **HHO1**)
 + histone variants, e.g.
H2AZ (encoded by **HTZ1**)



<http://www.ncbi.nlm.nih.gov/Structure/mdb/mmdbsrv.cgi?form=6&db=t&Dopt=s&uid=17269>

SAGA: a histone modifying complex

Spt-Ada-Gcn5-acetyltransferase



19 proteins, only 6 are essential for viability

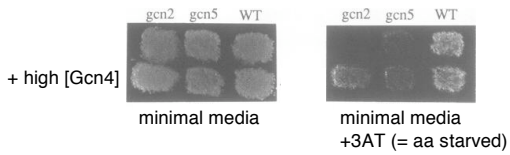
Subunit	size, chromosome, null p-type
Possible subunits to TAP tag	
Sgf73	1.974 kb=657aa, Chr. VII, viable
Sgf29	0.779 kb=259aa, Chr. III, viable
Sgf11	0.3 kb=99aa, Chr. XVI, viable
Ubp8	1.416 kb=471aa, Chr. XIII, viable
Sus1	gene with intron, Chr. II, viable

OR

Unknown	ORF
1	YHR033W
2	YOR302W
3	YJR097W
4	YBL028C
5	YDR034W-B
6	YGR067C
7	YKL037W
8	YER067W

GCN5: general control nonderepressible

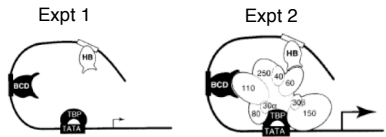
"general control" = amino acid starvation--> derepression of many biosynthetic genes by Gcn4



"Thus, GCN5 is a new member of the recently revealed general class of transcriptional regulators that collaborate with certain specific DNA binding activators to promote high levels of transcription."

EMBO J. (1992) 11: 4145

TAF: TBP associated factors

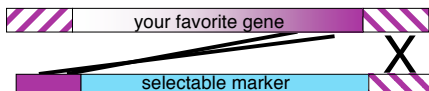


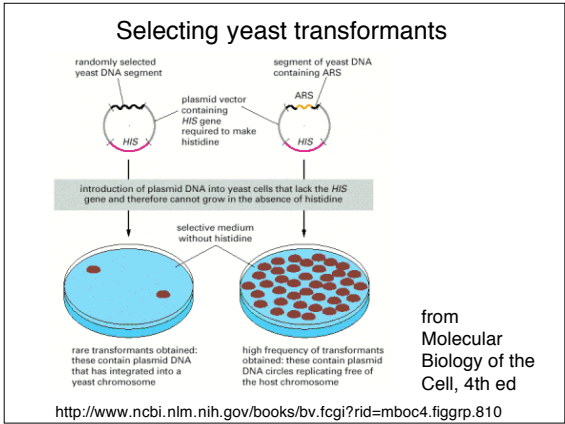
Component	Expt 1	Expt 2
TF (bicoid)	+	+
TF (hunchback)	+	+
TBP } TFIID	+	+
TAFs	-----	+
<i>In vitro</i> transcript?	little	lots

TIBS (1996) 21:338

Less well-characterized SAGA subunits

Tra subunit	
Tra1 <i>φ</i>	11,235 kb=3744aa, Chr. VIII, <i>inviable</i>
Other subunits	
Sgf73 <i>φ</i>	1,974 kb=657aa, Chr. VII, <i>viable</i>
Sgf29 <i>φ</i>	0,779 kb=259aa, Chr. III, <i>viable</i>
Sgf11 <i>φ</i>	0,3 kb=99aa, Chr.XVI, <i>viable</i>
Ubp8 <i>φ</i>	1,416 kb=471aa, Chr. XIII, <i>viable</i>
Sus1 <i>φ</i>	gene with intron, Chr. II, <i>viable</i>





Summary

FRONT RUNNERS

epigenetics" = chromatin modifiers = remodelers/histone modifiers

S. cerevisiae SAGA complex

Spt-Ada-Gcn5-acetyltransferase+TAFs

Selecting yeast transformants
