



Dynamic Exchange at Regulatory Elements during Chromatin Remodeling Underlies Assisted Loading Mechanism

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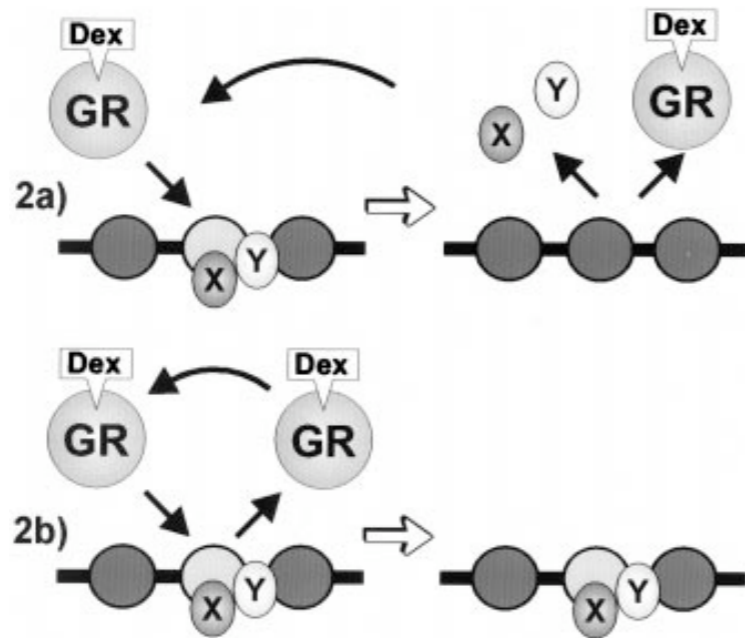
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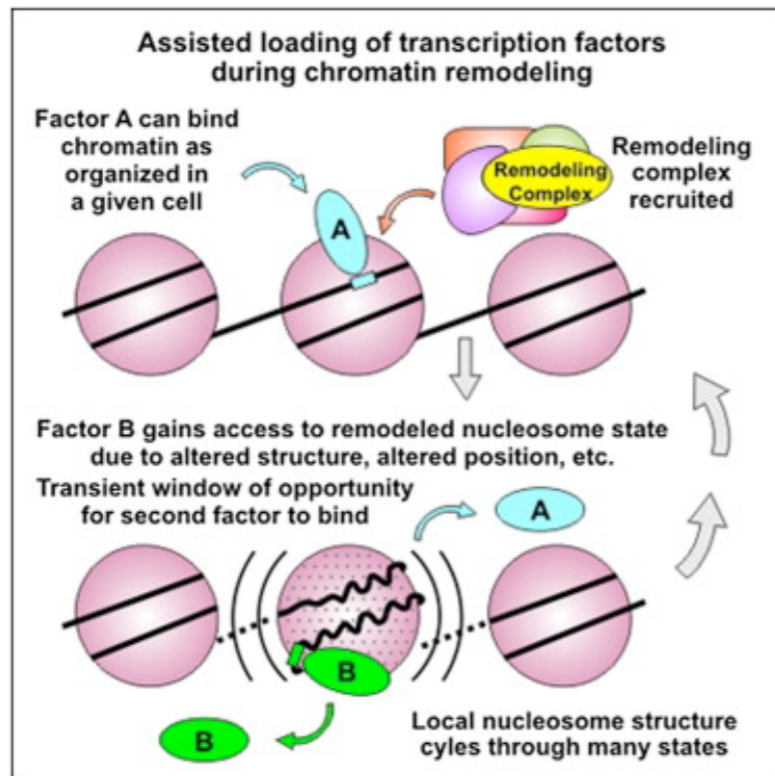
**Baikang Pei
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What's it about?



Hit-and-run model: Receptors transiently interact with DNA on the time scale of seconds and predict that the response elements may be sparsely occupied on average*

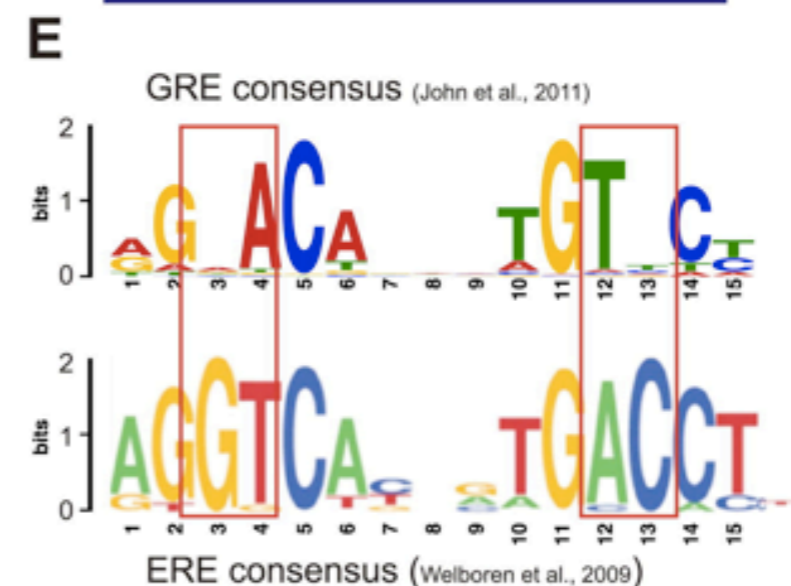
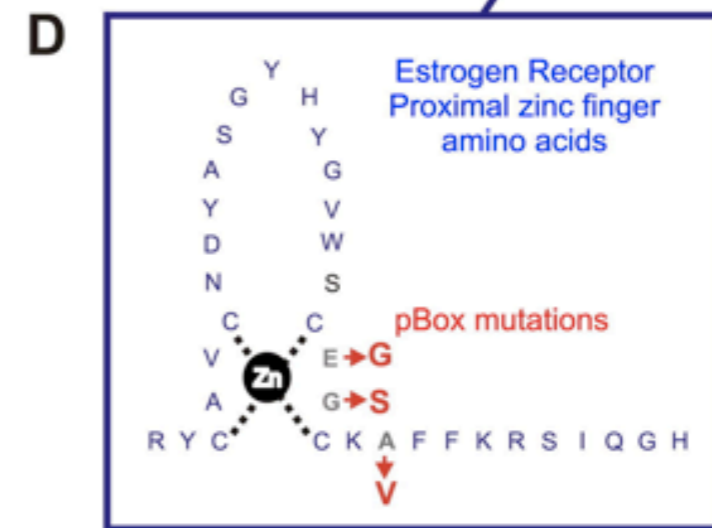
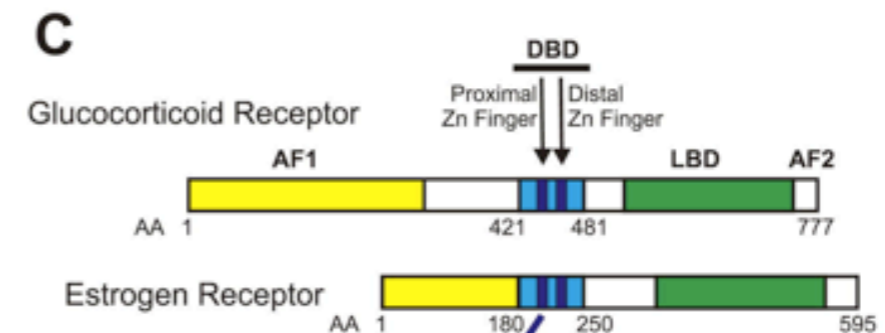
*J.G.MacNally et al. Science 287, 1262-1264 (2000)



Temporally sparse transcription factor-DNA interactions induce local chromatin reorganization, resulting in transient access for binding of secondary regulatory factors.

Glucocorticoid Receptor & Estrogen Receptor pBox

- Both GR and ER are steroid hormone receptors
- pBox mutations: three amino acids changes in the ER
- GR and ER pBox interact with GRE consensus sequence



Lack of Competition and Assisted Loading

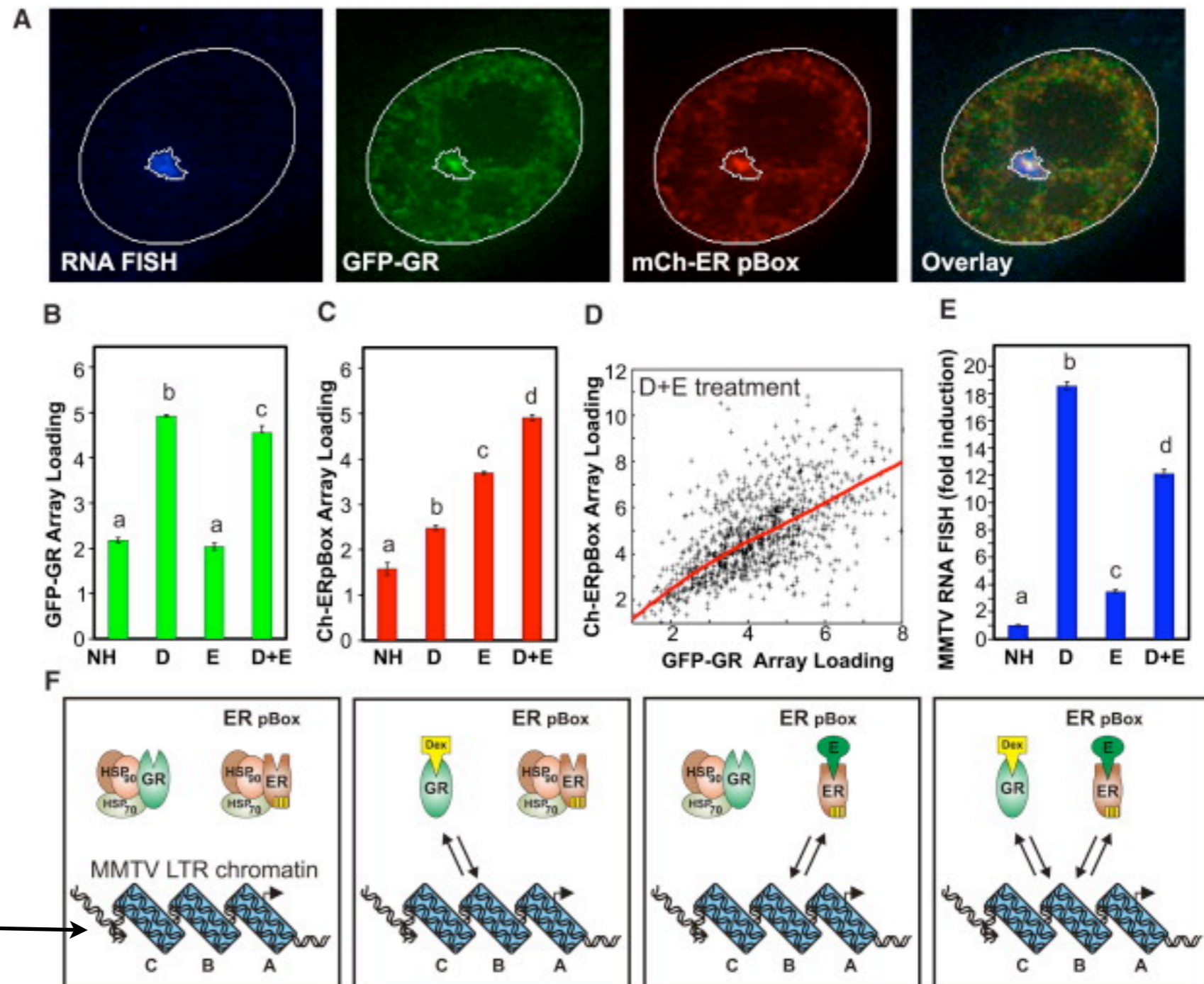
Design of Experiments

The 6644 mouse mammary carcinoma cell line was engineered to express GFP-GR and CH-ER pBox

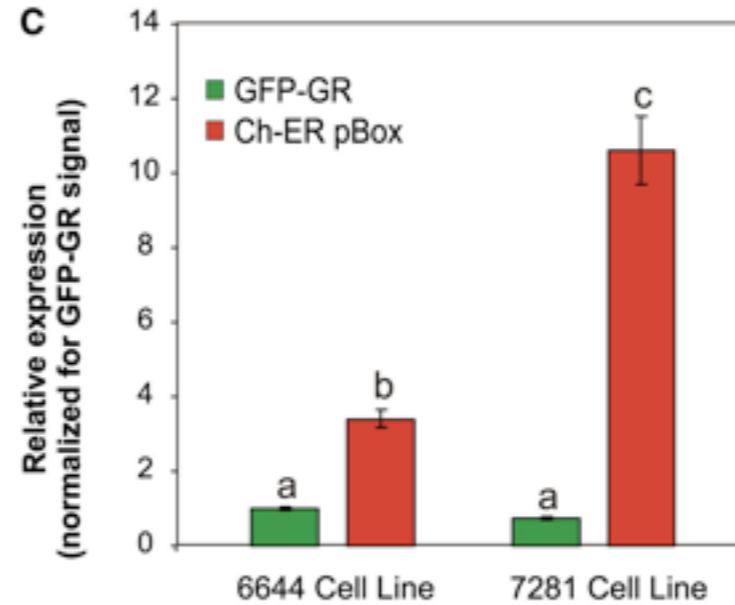
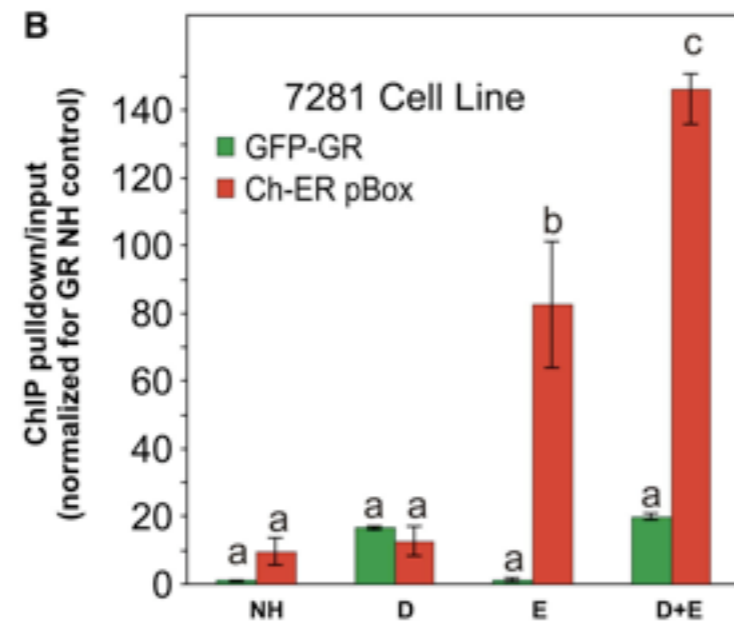
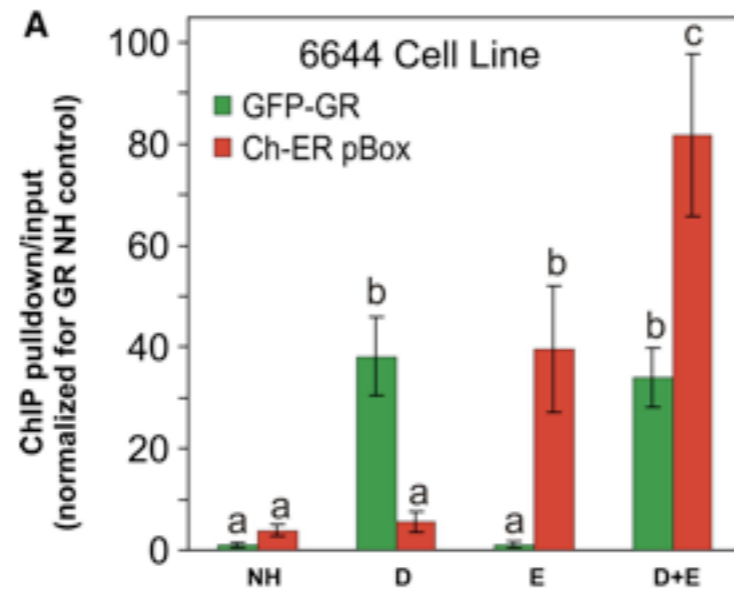
The 6644 cell line also contains Tandem repeat of MMTV LTR promoter-reporter cassettes

Simultaneous or individual treatment of Dex and E2, which are respective ligands of GR and ER.

Tandem repeat of MMTV LTR promoter-reporter cassettes



Assisted Loading from ChIP Assay

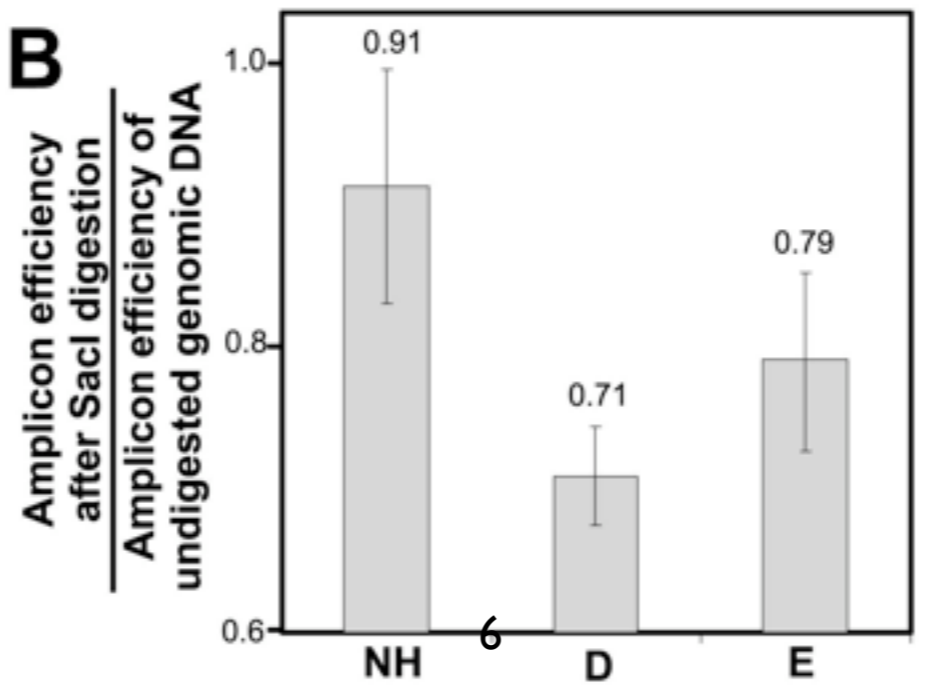
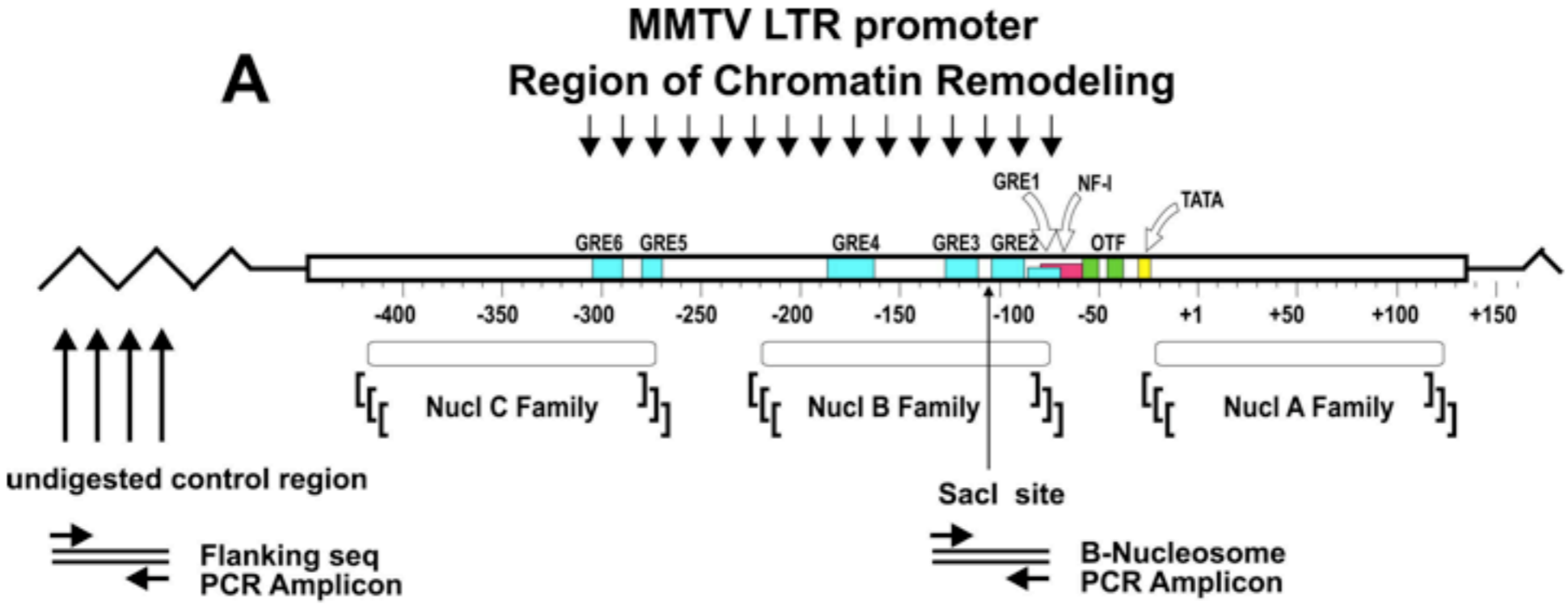


Assisted loading is observed in ChIP assay results, as in image analysis;

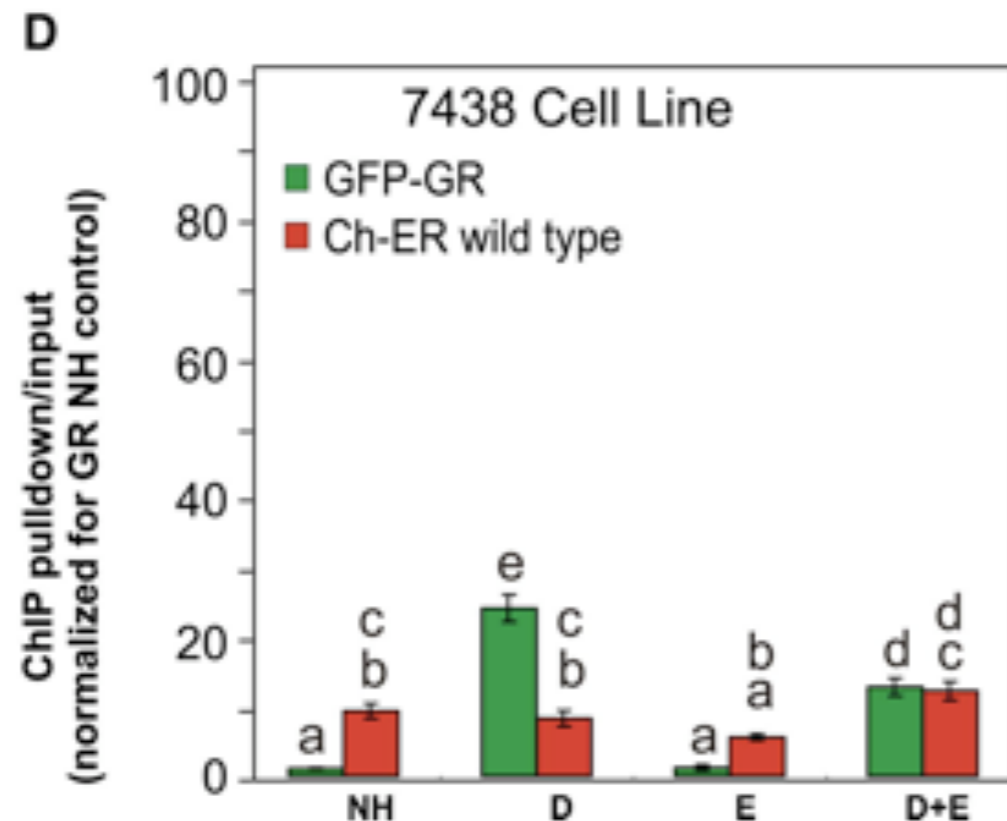
Assisted loading phenomenon indicates the GREs are not saturated, and lack of receptors is not the reason

ER is less efficient in recruiting cofactors required for GRE binding

GR Produces More Chromatin Access than ER pBox



Protein-Protein Interactions? Not Likely

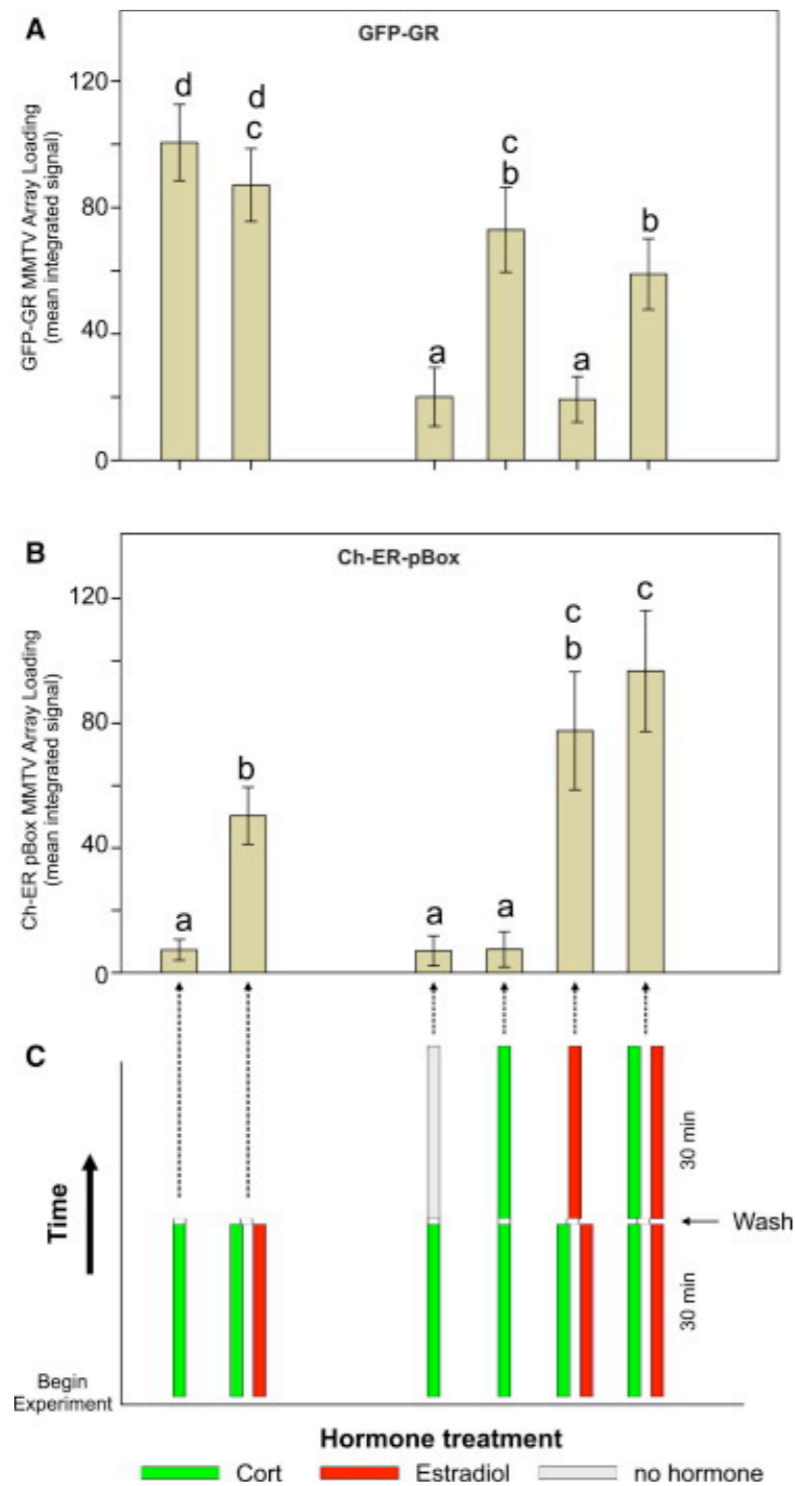


7438 cell line is same as 6644 cell line, except it expresses Ch-ER wild type, but not ER pBox

Ch-ER WT protein does not interact with MMTV promoter following the treatment of estrogen, which indicates the binding of ER pBox with GRE is sequence specific, but not due to protein-protein interaction

The decrease of GR binding after simultaneous treatment of Dex and E2 is due to sequestration of cofactors

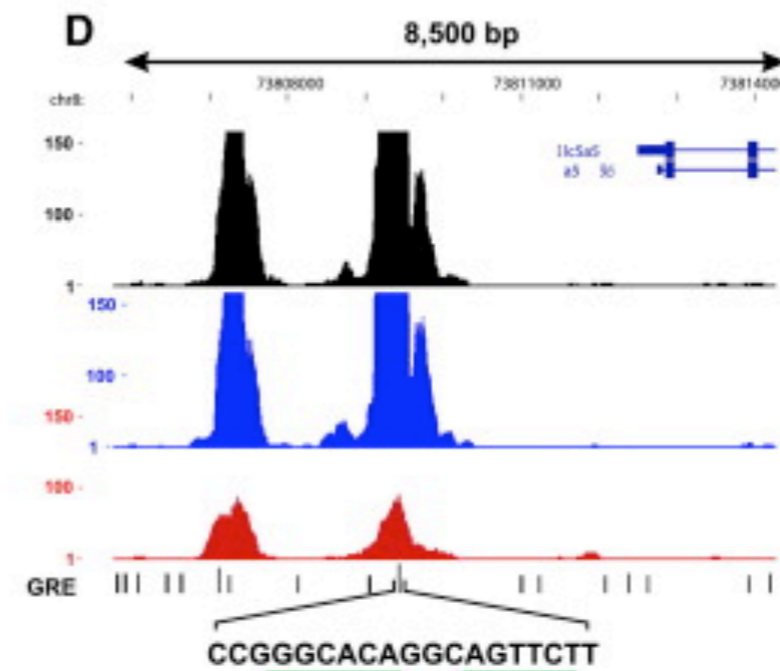
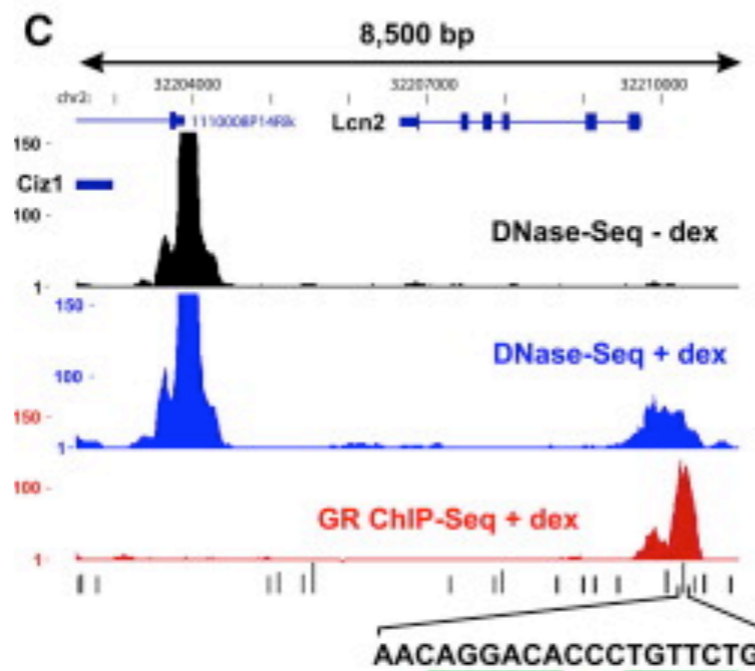
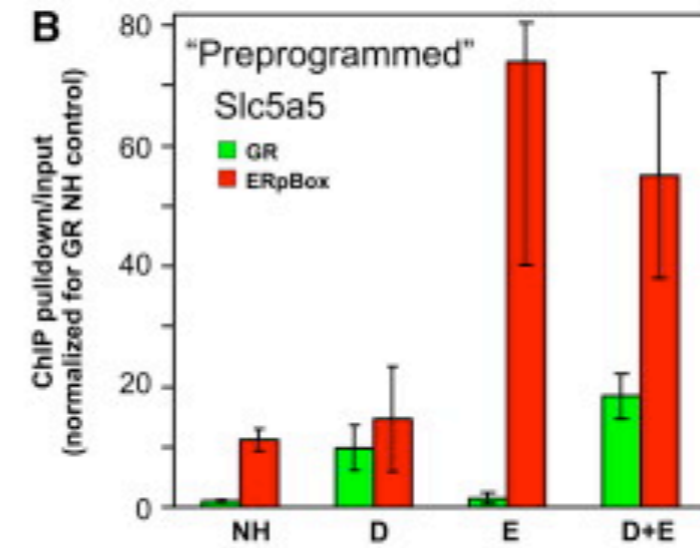
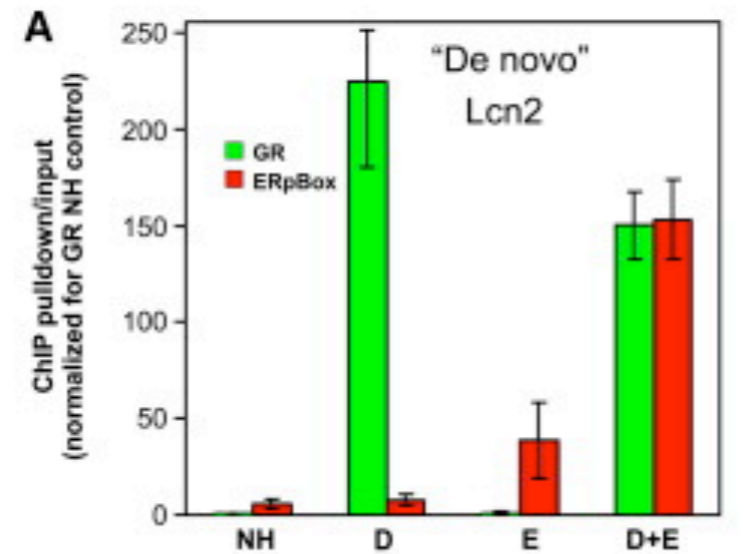
Another Evidence for no PPI



Cort: ligand of GR, can be washed out of cell

Following the simultaneous treatment - wash - simultaneous treatment, the GR binding and ER pBox binding changes in opposite direction

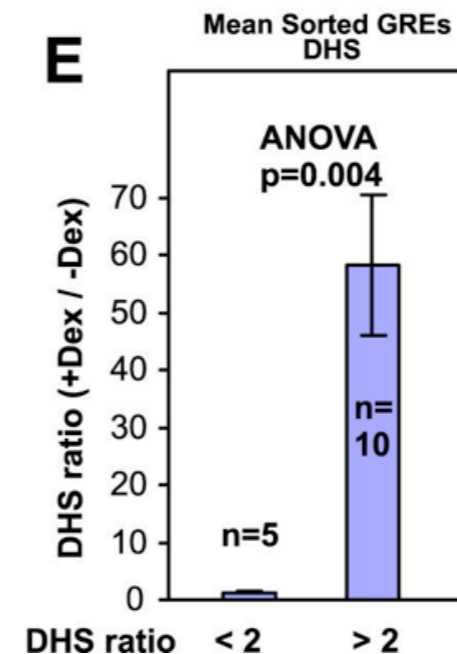
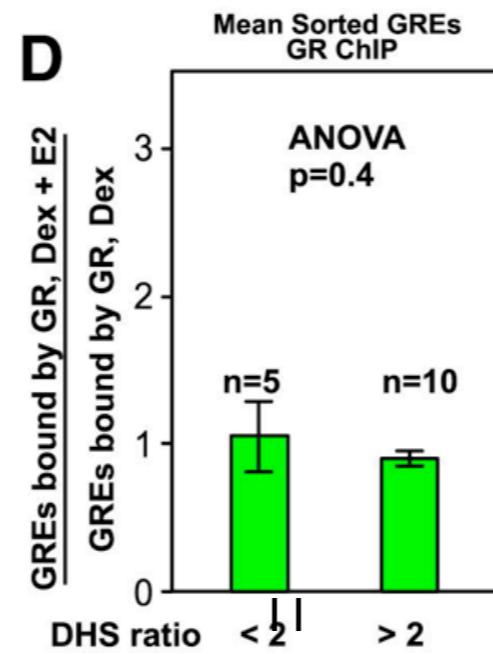
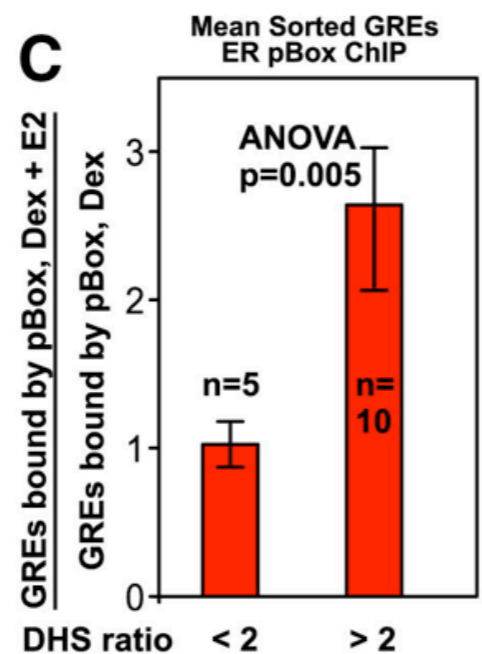
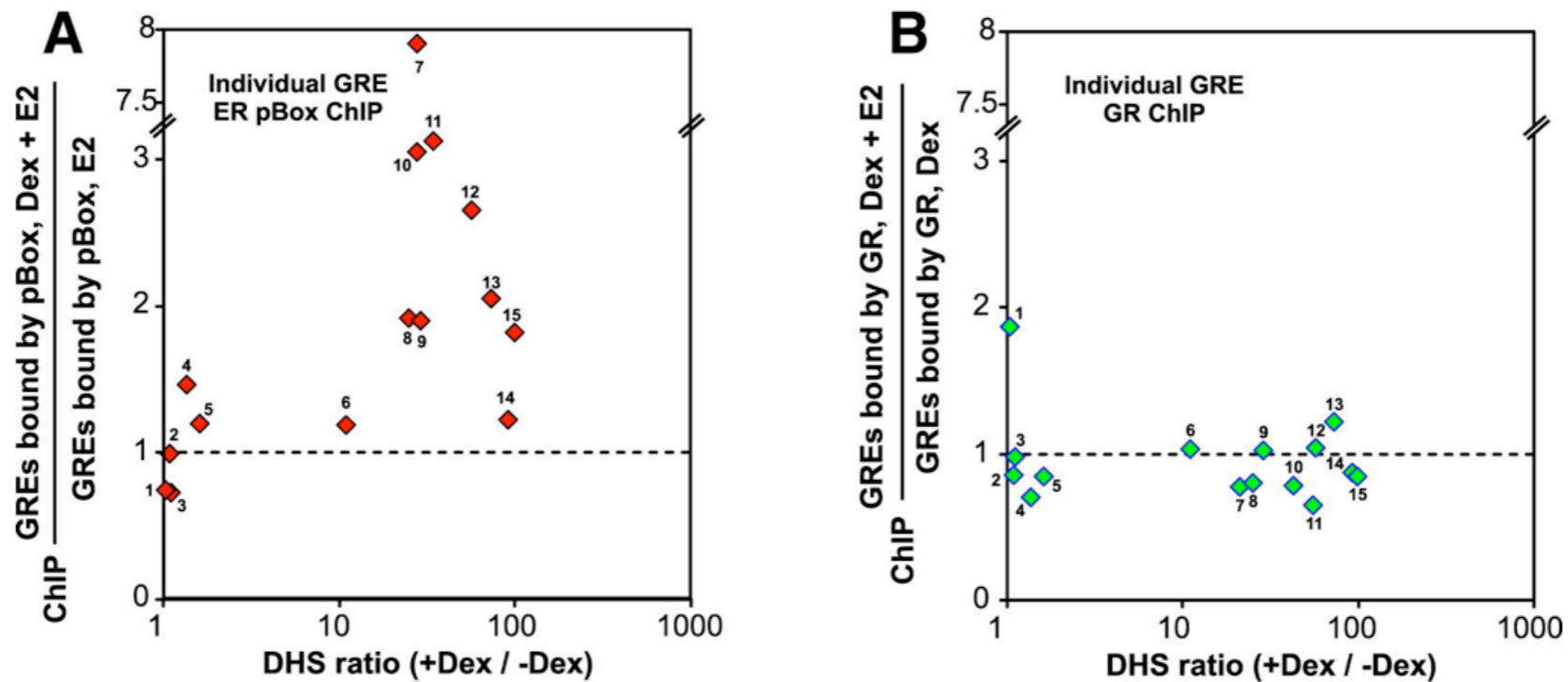
Assisted Loading at Endogenous Response Elements



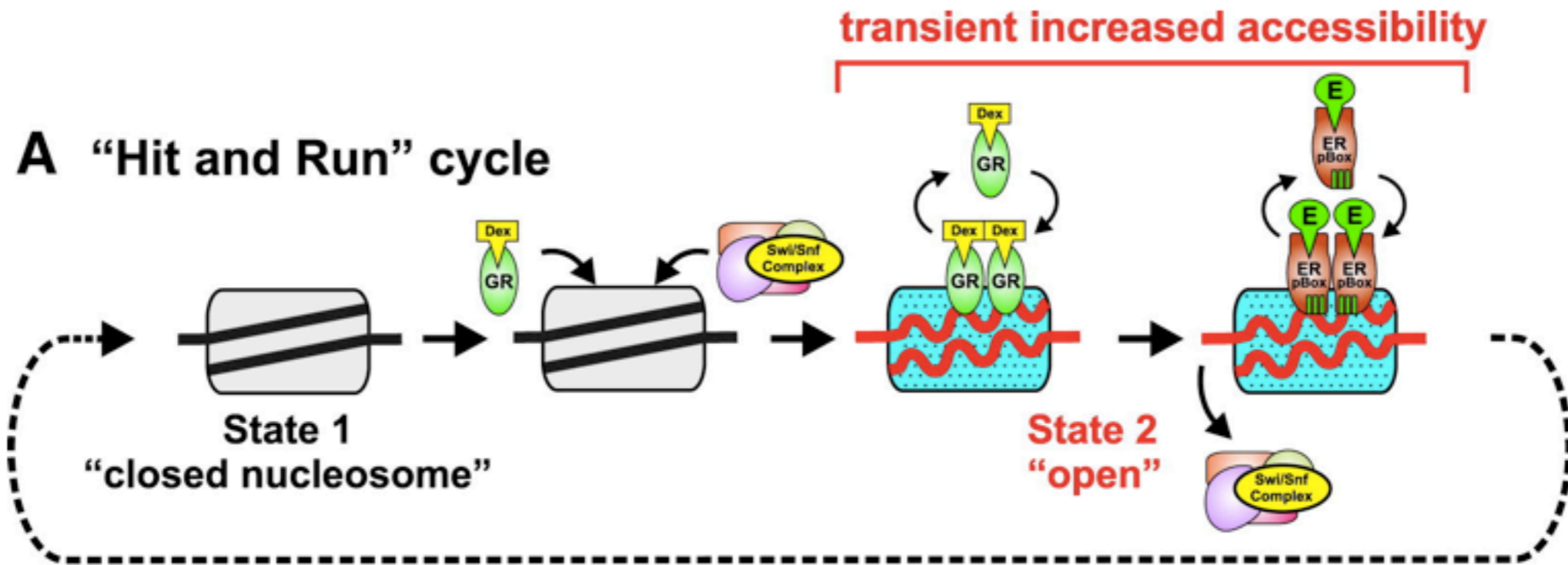
Assisted Loading at Endogenous Response Elements

Internal Locus ID #	Gene Name description	Unique Internal locus description	DHS peak seq tag count - dex	DHS peak seq tag count + dex	DHS ratio +dex/-dex	GR ChIP + dex	GR ChIP + dex + E2	GR ChIP Ratio +dex+E2/+dex	ERpBox ChIP + E2	ERpBox ChIP + dex + E2	ERpBox ChIP Ratio +dex+E2/+E2
Preprogrammed sites											
1	Slc5a5	chr8_73809380	462	475	1.03	0.0001243	0.00023276	1.872566193	0.00093363	0.00069478	0.74
2	ND	chr7_112937750	34	37	1.09	0.0019598	0.00167482	0.854591125	0.00129272	0.00128267	0.99
3	Spp2/Glrp1	chr1_90306468	107	119	1.11	0.00152132	0.0014933	0.98158272	0.00372546	0.00272714	0.73
4	Tbl1xr1	chr3_22143881	114	155	1.36	0.00138335	0.000967	0.699023533	0.00076966	0.00112488	1.46
5	ND	chr6_28982040	41	66	1.61	0.00345054	0.00292163	0.846717549	0.00169957	0.00202999	1.19
de novo sites											
6	Sys1/Sdc4	chr2_164146892	45	494	10.98	0.00959871	0.00992899	1.034408297	0.00789394	0.00941958	1.19
7	Tns1 Intron 4	chr1_73948020	2	42	21.00	0.00454624	0.0035061	0.771210164	0.00026992	0.00210237	7.79
8	Slc44a3 50kb Pr	chr3_121597000	9	224	24.89	0.00646772	0.00515919	0.797682902	0.0013308	0.00255533	1.92
9	Tsku	chr7_98267828	12	347	28.92	0.01080488	0.01104406	1.022136347	0.00491957	0.00935684	1.90
10	Rlbp1l1	chr4_9187915	1	43	43.00	0.00186196	0.0014597	0.783958934	0.00049665	0.00150721	3.03
11	Lcn2	chr2_32210200	1	55	55.00	0.00273052	0.00178083	0.6521947	0.00062448	0.00204808	3.28
12	Ggta1 Intron 1	chr2_35262340	1	57	57.00	0.00284432	0.00295558	1.039116842	0.00165691	0.00419515	2.53
13	Cugbp2 alt pr1 Intr	chr2_7160037	1	73	73.00	0.00102724	0.00125195	1.218755595	0.00036985	0.00075955	2.05
14	Rpl29	chr9_106271900	1	92	92.00	0.00225594	0.00197216	0.874207234	0.00132128	0.00162055	1.23
15	Slc25a33	chr4_148657510	2	199	99.50	0.00920885	0.00780574	0.847634561	0.00399993	0.00726651	1.82

Assisted Loading at Endogenous Response Elements

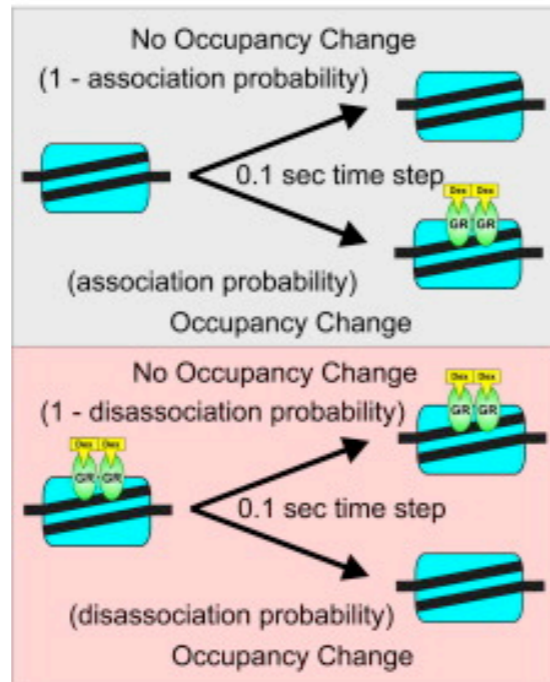


Proposed Model

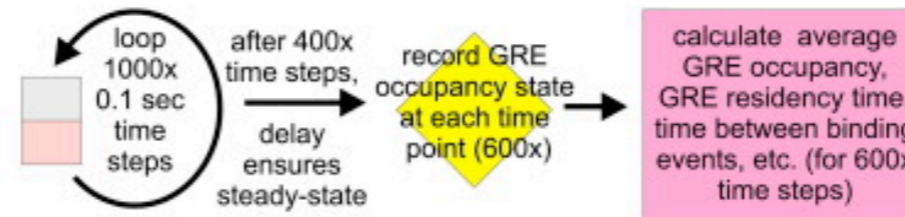


Math Model and Simulation

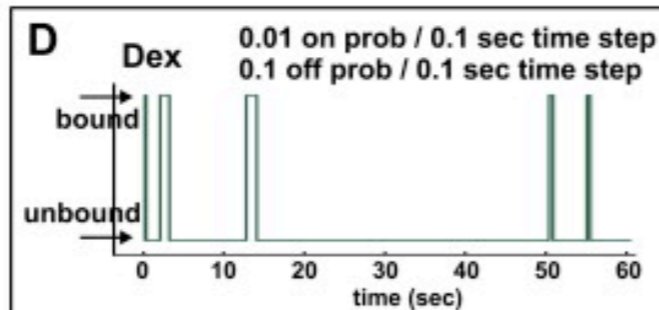
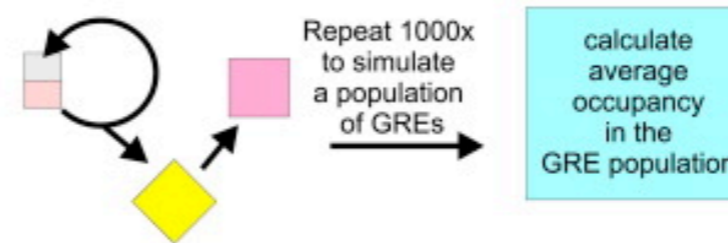
A Stochastic state-change calculation module



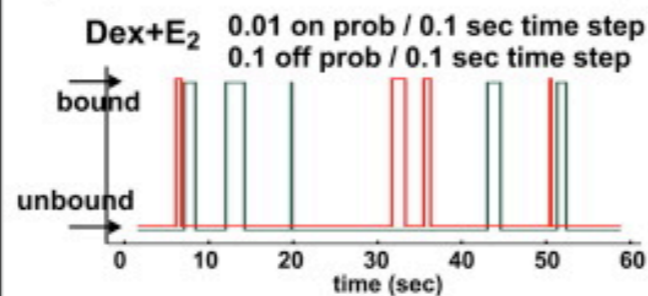
B Single GRE occupancy over time calculation module



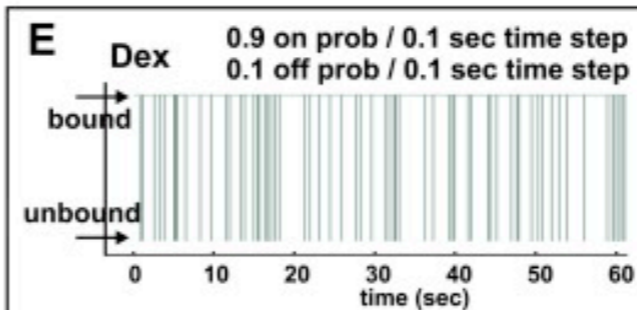
C GRE population average calculation module



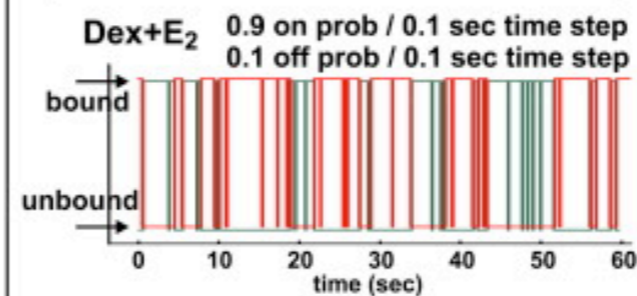
Mean GR/GRE occupancy time = 0.99 sec
 Mean # GR binding events / min = 5.4
 Mean time between binding events = 10.1 sec
 Mean fract occupancy of GREs by GR / time step = 0.089



Mean GR/GRE occupancy time = 0.99 sec
 Mean # GR binding events / min = 5.0
 Mean time between binding events = 11.1 sec
 Mean fract occupancy of GREs by GR / time step = 0.083

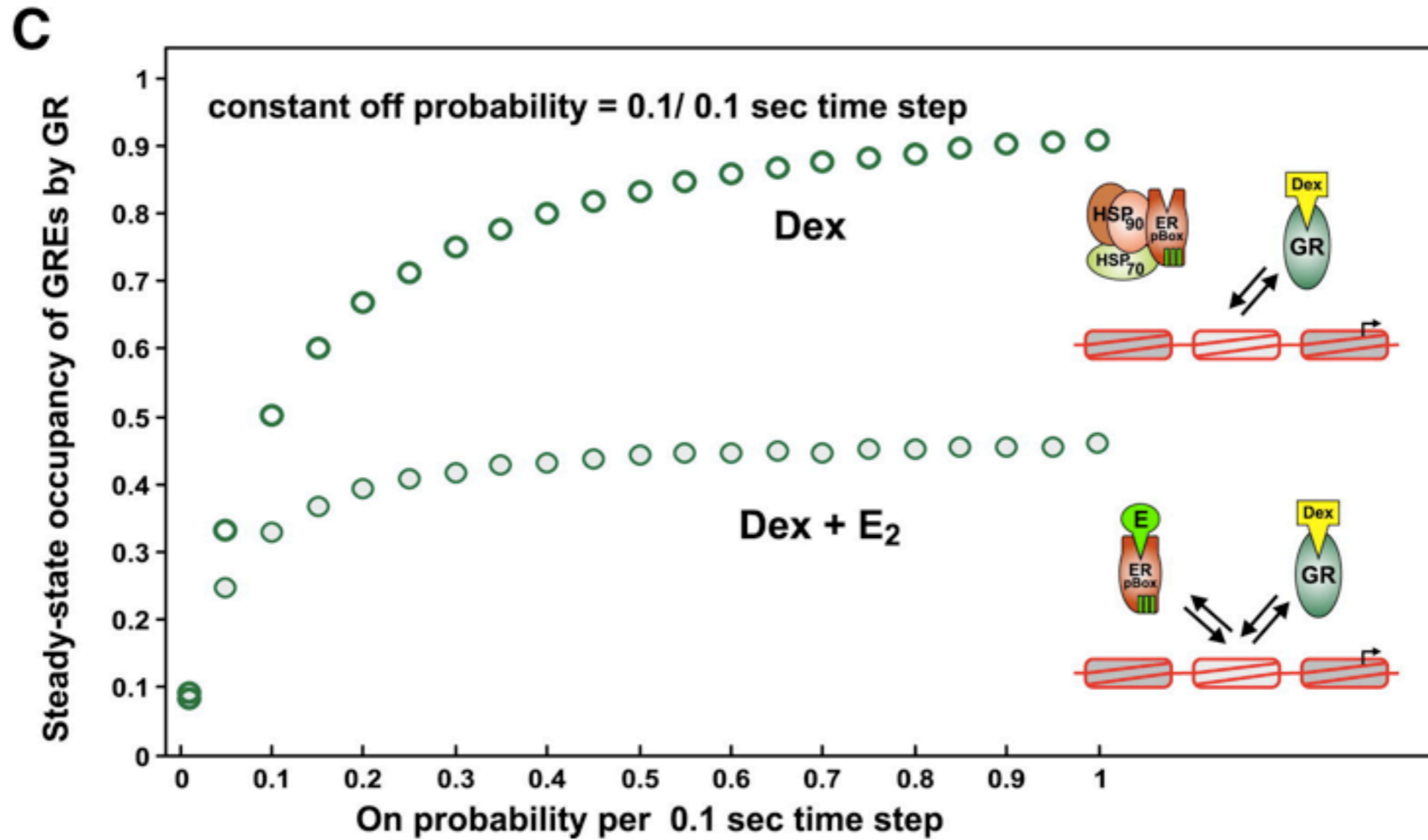


Mean GR/GRE occupancy time = 0.99 sec
 Mean # GR binding events / min = 53
 Mean time between GR binding events = 0.14 sec
 Mean fract occupancy of GREs by GR / time step = 0.90



Mean GR/GRE occupancy time = 0.99 sec
 Mean # GR binding events / min = 27
 Mean time between GR binding events = 1.2 sec
 Mean fract occupancy of GREs by GR / time step = 0.45

Math Model and Simulation



Conclusions

- TFs don't compete for binding sites
- Hit-and-run model coupled with chromatin remodeling for assisted loading