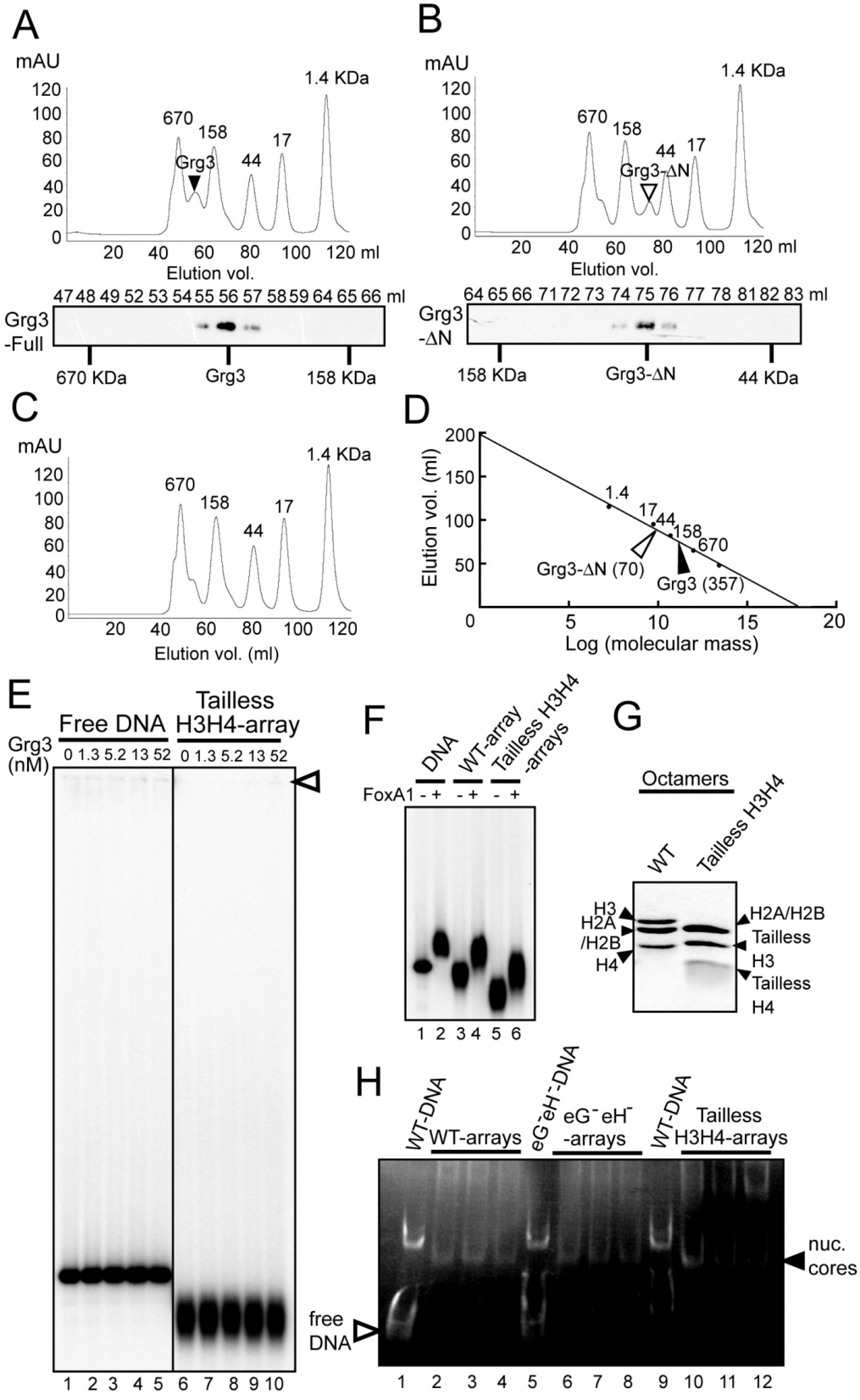
Supplemental Figures and Legends; Sekiya and Zaret, Cell D-07-00162 R

Supplemental Fig. 1. Purified Grg3 protein is a tetramer and fails to bind nucleosome arrays with tailless histones H3 and H4. (A-C) Absorbance readings of HiLoad 16/60 Superdex gel filtration eluates. Molecular size standards were loaded with (A) wild type Grg3, (B) Grg3-ΔN (deleted for the tetramerization domain; see Fig. 1A, B), or (C) no extra protein. Peaks of Grg3 and Grg3-ΔN in the eluted fractions are noted and Western blots below confirm the presence of the respective Grg3 protein within each peak. (D) Plot showing that the calculated molecular mass of Grg3 is ~357KDa, corresponding well to a tetramer of the predicted 84.7KDa protein, whereas the calculated mass of Grg3-ΔN is 70, corresponding well to the predicted mass of the 72.2KDa. (E) In EMSA, Grg3 protein that binds nucleosome arrays made with wild type histones (see Fig. 1D, 2A, 3C, 3D) is unable to bind arrays made with tailless histones H3 and H4. (F) However, FoxA1 protein binds the tailless arrays as well as wild type arrays, demonstrating DNA and array integrity. Note the tailless arrays migrate faster in EMSA than wild type arrays. (G) SDS-PAGE of octamers made with wild type or H3/H4 tailless histones. (H) EcoRI digestion assays of arrays with: wild type DNA and histones (WT-arrays, lanes 2-4), DNA with mutations of the eG and eH FoxA1 binding sites and wild type histones ($eG^{-}eH^{-}$ arrays, lanes 6-8), and wild type DNA with tailless H3/H4 histones (lanes 10-12). The corresponding nucleosome-free DNAs are shown in lanes 1, 5, and 9, respectively. Shifting of the free EcoRI fragment containing 5S rDNA in the array samples ("nuc. cores") demonstrates nucleosome assembly and proper positioning. We always perform histone titrations for assemblies. Each of the array lanes shown corresponds to nucleosome array assemblies of different histone:DNA ratios; lanes 2, 6, 10, 1:1; lanes 3, 7, 11, 1.2:1; lanes 4, 8, 12, 1.4:1. By assessing nucleosome deposition in this fashion, each instance arrays are made, different assembly reactions can be normalized for their extent and quality of octamer deposition. For the studies here, assemblies in lanes 2, 3, 7, 8, and 10 were used for experiments.

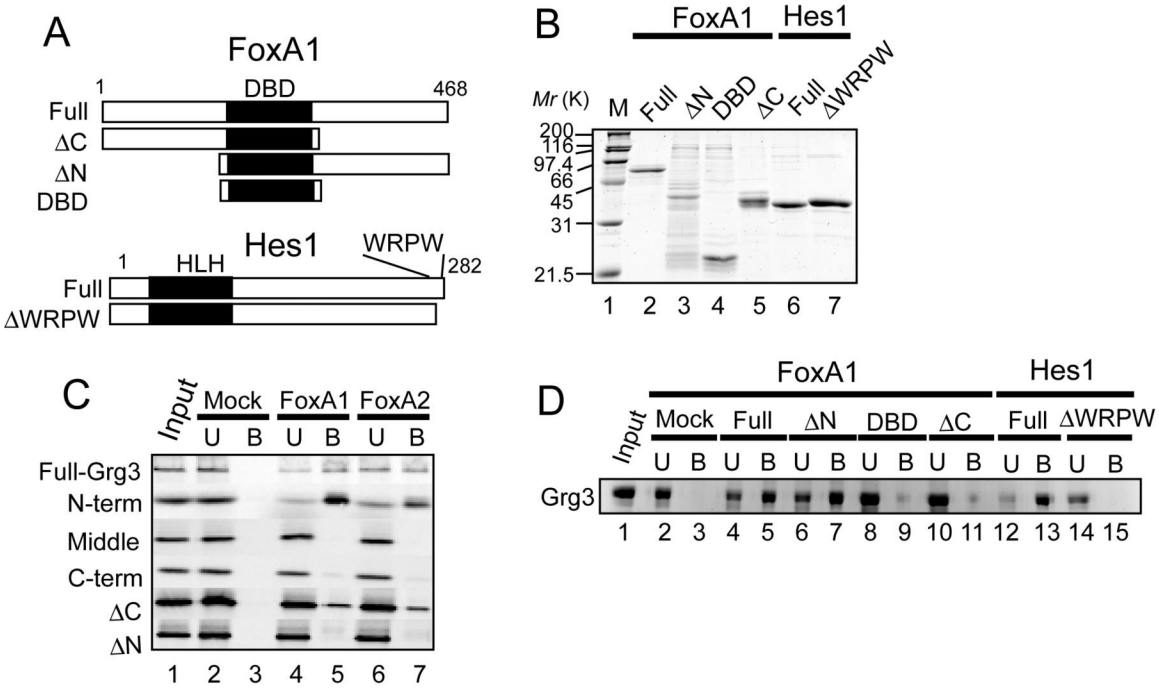
Sekiya - Zaret Supplementary Fig. 1



Supplemental Fig. 2. Domains of FoxA1 and Grg3 required for their interaction.

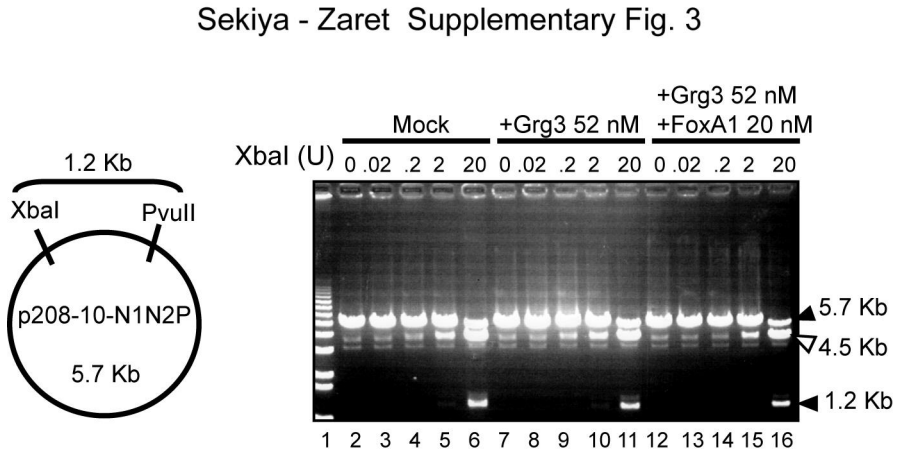
(A) Deletion mutant series of FoxA1, and Hes1. Numbers refer to amino acid positions. DBD, Forkhead DNA binding domain; HLH, basic helix-loop-helix DNA binding domain. (B) SDS-PAGE analysis of 3 μg of each protein, detected by Commassie staining. (C) Association of FoxA1 and FoxA2 with Grg3 in vitro. In vitro translated ^{35}S labeled Grg3 proteins, as indicated (see Fig. 1 for constructs) were incubated with FoxA1-conjugated, FoxA2-conjugated, or mock-conjugated Sepharose beads (see Cirillo et al., 2002 for details). The bound proteins were analyzed by SDS-PAGE and autoradiography. U, unbound material in first wash; B, bound material after various washes. (D) Association of full length Grg3 with a deletion mutant series of FoxA1 and Hes1. Full length, recombinant Grg3 protein was incubated with the Sepharose-conjugated FoxA1 and Hes1, and deletion mutants as indicated, or mock-coupled Sepharose. The bound proteins were analyzed by SDS-PAGE and silver staining.

Sekiya - Zaret Supplementary Fig. 2



Supplemental Fig. 3. Grg3 and FoxA1 do not inhibit XbaI digestion of the *alb1* enhancer in free DNA.

(Left) Plasmid used to make DNA for nucleosome arrays (see Fig. 1C). XbaI and PvuII each cut the plasmids once, as shown. (Right) Plasmid DNA was digested to completion with PvuII and with a titration of XbaI to 20 units per reaction, the same concentration as used in the XbaI digests of nucleosome arrays with Grg3 and FoxA1 in Fig. 4B. No difference was observed in the efficiency of generating the 1.2 kb XbaI-PvuII fragment on free DNA in the presence or absence of Grg3 and/or FoxA1, whereas on nucleosome arrays, the same concentrations of Grg3 and FoxA1 completely blocked XbaI cleavage (Fig. 4B, lanes 6, 7).



Supplemental Fig. 4. Creation of a nucleosomal array with two central Hes1 binding sites.

(A) Strategy to construct nucleosome arrays with Hes1 sites. 53 bp of the *alb1* enhancer N1 region spanning the eG and eH FoxA1 binding sites were replaced by 53 bp from Hes1 promoter, which contains two Hes1 binding sites (Takebayashi et al., 1994). (B) Hes1 binds specifically to Hes1-arrays. EMSA was performed with FoxA1, Hes1 proteins and albumin and Hes1 arrays. Note that FoxA1 binds readily to the albumin arrays and poorly to the Hes1 arrays (compare lanes 1-4 with 11-14), whereas Hes1 binds poorly to the albumin arrays and readily to the Hes1 arrays (compare lanes 5-10 with 15-20).