SUPPLEMENTAL INFORMATION

MATERIALS AND METHODS

Nuclear extracts preparation

Nuclear extracts from SW-13 and HeLa cells were obtained by harvesting the exponentially growing cells (10^8). The pelleted cells were washed with cold PBS and resuspended in 1 ml of lysis buffer (10mM HEPES [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 1mM phenylmethylsulfonyl fluoride and proteinase inhibitor cocktail). After dounce homogenizing with a glass homogenizer the nuclei were pelleted and extracted for 30 min at 4°C with 1 ml of extraction buffer (20 mM HEPES [pH 7.9], 0.42 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 20% glycerol, 1mM phenylmethylsulfonyl fluoride and proteinase inhibitor cocktail).

Electrophoretic mobility shift assay (EMSA)

For EMSA, the nuclear extracts from SW-13 and HeLa cells or the in vitro translated TEF-1 were used. The probes are listed in Figure 1B and Figure 3D. Nuclear extracts (3 μg) and in vitro translated products were pre-incubated with 0.2 μg and 0.02 μg of the non-specific carrier DNA poly (dI.dC) (Sigma), respectively, for 30 min at 4°C. 1.6 μl Binding buffer (200 mM Tris-Hcl [pH 7.5], 500 mM NaCl, 30 mM EDTA, 20% glycerol, 0.5% NP-40) and annealed [γ-^{32}P]ATP labelled oligonucleotide probes (0.25 ng) were then added to a final volume of 20 μl. For competition assays, 100-fold excess of the unlabelled probe was added and incubated at 4°C for 15 min prior to the addition of the labeled probe. The protein/DNA complexes formed were resolved by non-

denaturing polyacrylamide gel electrophoresis. For supershift assays, 2 μg of antibodies were added to the reaction mixture and pre-incubated for 30 min at 4°C before resolving on the gel.

DNA affinity protein purification and mass spectrometry

Forward and reverse complementary 5′ biotinylated oligonucleotides (Fig. 1B) were used to achieve concatamerization of protein binding sites using the self primed PCR technique [1,2]. The PCR reaction mixture consisted of 10 pmol of wild type or mutant oligonucleotides, *Tgo* DNA polymerase (Roche), 1 mM dNTPs and 2.5 mM $MgCl_2$. The PCR cycling conditions were as follows: 95°C for 3 min, 30 cycles of 95°C for 30 s, 54°C for 40 s and 72°C for 2 min followed by 72°C for 10 min. The product size ranged from 0.3 to 10 kb. Streptavidin Magnesphere Paramagnetic Particles (Promega) were washed with the wash buffer (20 mM HEPES [pH 7.9], 100 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 20% glycerol, 1mM phenylmethylsulfonyl fluoride and proteinase inhibitor cocktail) thrice. 75 μl of the PCR products were added to 300 ul beads and incubated for 15 min at 4°C. The beads were then washed to remove the unbound DNA. Nuclear extracts were diluted with binding buffer and pre-incubated with poly (dI.dC) (0.015 μg/μg protein) for 30 min at 4°C followed by its addition to the paramagnetic beads and incubation on ice for 1 h. The beads were collected and washed thrice. The bound proteins were eluted with the binding buffer containing 600 mM NaCl and resolved by SDS-PAGE and stained with silver nitrate. The protein band was excised from the gel and identified by MALDI-TOF mass spectrometry carried out at the Lerner

Research Institute, Mass Spectrometry Laboratory for Protein Sequencing, the Cleveland clinic foundation, Cleveland, Ohio.

REFERENCES

[1] Hemat, F. and McEntee, K. (1994) A rapid and efficient PCR-based method for synthesizing high-molecular-weight multimers of oligonucleotides. Biochem Biophys Res Commun. 205, 475-81.

[2] Xue, H.H. et al. (2004) GA binding protein regulates interleukin 7 receptor alpha-chain gene expression in T cells. Nat Immunol. 5, 1036-44.