Supplement

Experimental Procedures

Protein constructs and purification

For purification of NFκB:IRF-7:IRF-3, a freshly transformed colony was transferred into 10 ml LB broth containing 100 μg / ml ampicillin and grown overnight at 37°C to saturation. This overnight culture was used to inoculate 1 liter LB broth containing 100 μg / ml ampicillin and grown at 37°C to an OD_{600} of about 0.5. The culture was then induced with 0.4 mM isopropyl β-D-thiogalactoside for 4 hr. The cells were harvested, and the pellet was resuspended in 100 ml of 4°C cold lysis buffer (20 mM Tris, pH 8.0, 500 mM NaCl, 10% glycerol, 20mM imidazole) and broken by sonication. All subsequent steps were carried out at 4°C. After centrifugation at 25,000 x g for 30 min, the cleared supernatant was loaded at a flow rate of 1 ml / min onto a pre-equilibrated 5 ml HisTrap column (GE Healthcare). The column was washed with 40 column volumes of column buffer at a flow rate of 4 ml / min. The protein was eluted with 300 mM imidazole in buffer A (20 mM HEPES, pH 7.0, 100 mM NaCl, 1 mM dithiothreitol (DTT)) and then further purified on a 5 ml HiTrapSP column (GE Healthcare) in buffer A, using a 100-1000 mM NaCl gradient for elution. Size-exclusion chromatography on a HiLoad 16 / 60 Superdex column (GE Healthcare) in 20 mM HEPES pH 7.0, 250 mM NaCl, 1 mM DTT was used for the final purification step. After elution, the protein-containing fractions were analyzed by SDS-PAGE (gels stained with Coomassie blue)

and estimated to be ~95% pure. Fractions were pooled, concentrated in a Amicon Ultra concentrator (Millipore) to 20 mg / ml, flash frozen in aliquots, and stored at –80°C.

For crystallization of four IRF-3 domains bound to PRDII-IV, unlinked (monomeric) IRF-3 (residues 4-113) was expressed and purified as described previously (Panne *et al.*, 2004). Expression and purification of c-Jun (amino acids 263-324) and ATF-2 (amino acids 335-397) were also as described in that paper; likewise, the preparation of full-length HMGA1a, comprising amino acids 1-107 (formerly designated as HMGI) (Panne *et al.*, 2004). Recombinant NFκB contained human p50 (residues 39-350) and human RelA (residues 19-291); the two chains were coexpressed and purified as described (Chen *et al.*, 1999).

Electrophoretic mobility shift assays

For the NFκB:IRF-7:IRF-3 complex, binding reactions were assembled at 21°C in a total volume of 5μl in 10mM HEPES, pH7.0, 200 mM NaCl. For the IRF-3:DNA complex, binding reactions were assembled as above but in buffer 10 mM HEPES, pH 8.0, 500 mM NH_4OAc, 100 mM NaCl, 5 mM $MgCl_2$, 5% glycerol, 1 mM DTT. Binding reactions were loaded onto an actively running 7% polyacrylamide gel in 0.5x TBE (45 mM Tris, 45 mM borate, 1mM EDTA, pH 8.3) that had been pre-electrophoresed for 30 minutes at 4°C. Electrophoresis continued for a further 60 minutes at 4°C before the gel was stained with 0.5x TBE containing 0.5 μg / ml ethidium bromide.

Crystallization of the NFκB:IRF-7:IRF-3:DNA complex and x-ray data collection

Oligonucleotides were purchased from Integrated DNA Technologies. The complex of NFκB:IRF-7:IRF-3 in 20 mM HEPES, pH 7.0, 250 mM NaCl, 1 mM DTT was mixed with DNA to a final concentration of 200 μM DNA: 160 μM protein in the presence or absence of 160 μM HMGA1a. After incubation at room temperature for 10 minutes, the complex was further diluted in water to 120 μM DNA: 100 μM protein. Crystals in the presence or absence of HMGA1a were obtained with the hanging drop method. The complex (1 μl at 100 μM) was mixed with 1 μl well solution containing 100 mM HEPES, pH 7.0, 15% (w / v) PEG 4000 and equilibrated against 1 ml well solution. The largest crystals grew as plates to a maximum size of 0.2 x 0.07 x 0.07 mm^3 at room temperature. Crystals were cryoprotected by adding an equal volume of 50% ethylene glycol, 50 mM HEPES, pH7.0, 7.5% (w / v) PEG 4000 to the drop. Crystals were flash frozen in liquid nitrogen, and diffraction data were collected under cryogenic conditions (100 K) with radiation of 1.0 Å wavelength on station 8.2.1. at the Advanced Light Source, Berkeley, using a 70 μm collimated beam and a ADSC Q210 detector. The data were processed with HKL2000 (Otwinowski and Minor, 1997).

Crystallization of the IRF-3:DNA complex and x-ray data collection

Crystals containing the IRF-3 DNA-binding domains on a sequence-optimized 57-mer DNA from the INF-β enhancer were obtained during co-crystallization attempts using all enhanceosome components. Final concentrations of the components were 720 μM IRF-3, 180 μM NFκB, 180 μM ATF-2 / c-Jun and 200 μM DNA duplex in the presence or

absence of 200 μM HMGA1a in 10 mM HEPES, pH 8.0, 500 mM NH_4OAc, 100 mM NaCl, 5 mM $MgCl_2$, 5% glycerol, 1 mM Tris(2-carboxyethyl)phosphine hydrochloride. Crystals suitable for X-ray diffraction were obtained with the hanging drop method by mixing 1 μl complex solution with 1 μl well solution containing 100 mM Tris, pH 8.0, 20% (w / v) PEG 6000, 500 mM NH_4OAc, 100 mM NaCl, 5 mM $MgCl_2$, 5% glycerol and equilibrating the drop against 1 ml well solution. Crystals contained only IRF-3 and DNA. The largest crystals grew as thin plates to a maximum size of 0.3 x 0.06 x 0.03 mm^3 at room temperature. Crystals were stable in a cryoprotectant buffer equivalent to the well solution plus 20% glycerol. They were flash frozen in liquid nitrogen; diffraction data were collected under cryogenic conditions (100 K) at station 14C at the Advanced Photon Source, Argonne National Laboratory, using a Quantum 4 CCD detector and radiation of 0.9 Å. The data were processed and reduced with HKL2000 (Otwinowski and Minor, 1997).

Structure of the IRF-3:DNA complex

The structure was determined by molecular replacement using MOLREP (Vagin and Teplyakov, 1997) with an IRF-3:DNA complex as the search model (1T2K; Panne et al., 2004). After the first IRF-3-DNA complex was found, its position and orientation were fixed in a search for the second complex. This procedure was iterated until all four copies of IRF-3 had been found. DNA was built by superposing a standard B-DNA onto the molecular replacement solution. The phosphates were placed manually into the density, and their positions were restrained. The planarity of the base-pairs and the

sugar pucker were restrained to conform to standard B-DNA. The conformation of each of the four IRF-3 domains was restrained using non-crystallographic symmetry restraints in the core of the proteins, excluding the flexible loops L1, L2 and L3. The dihedral torsion angles of α-helices 1-3 were also restrained to those of standard α–helices. In later refinement cycles, these conformational restraints on the proteins and the DNA were removed. Iterative building and refinement were performed using the programs O and CNS 1.2.