Structure determination of Oct-4, a key regulator in embryonic stem cell differentiation

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Derived from inner cell mass of the blastocyst, embryonic stem cells (EC) are capable of differentiating indefinitely in vitro, and forming all three germ cell layers (pluripotency). These properties make ECs extremely valuable, for example in novel therapies in regenerative medicine. Four transcription factors (Oct-4, Sox2, c-Myc, Klf4) are required to retain pluripotency and allow a normal embryonic development. [1,2]

In order to elucidate the structural properties that allow a wide variety of DNA motifs to be recognized, we have crystallized the Oct-4 DNA binding domain in complex with the PORE binding motif. Crystals are produced in a reproducible manner and several datasets have been collected at beam lines X11 and X12 at EMBL Hamburg and BM14 and ID14.4 at ESRF Grenoble. The datasets included both native datasets and efforts to derivatize the crystals, with high-resolution limits up to 2.8 Å. The presence of a strong pseudosymmetry resulted in mis-indexing of the initial datasets in space group P4322. The correctly identified space group was P2221. Subsequently, a molecular replacement solution was found and is under refinement (R/Rfree 0.30/0.38; Figure 1).

Figure 1: DNA binding domain of Oct-4 complexed with PORE DNA motif. A cartoon presentation of the Oct-4 DNA binding domain complexed with PORE motif from an osteopontin enhancer. Different protein subunits are coloured with light and dark grey.

References