The testis-specific octamer-binding protein of the sea urchin has a molecular weight of 85 kDa

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Submitted July 25, 1989

The octamer sequence ATTTGCAT is a regulatory element of many genes including histone H2B, U-snRNA and immunoglobulin genes and is recognized by a family of distinct octamer-binding proteins. All cell types of higher eukaryotes contain the Oct-1 protein (also called OTF-1, OBP100, NF-A1, NF III) of molecular weight 97 kDa (1). Lymphoid cells contain several additional octamer-binding proteins collectively known as Oct-2 (or as OTF-2, NF-A2), which are derived from a single gene by differential splicing (2-4). The Oct-2A proteins have a molecular weight of 58-61 kDa (4), while Oct-2B is 75 kDa in size (5). Recently, a novel protein, Oct-4 or NF-A3, has been identified in embryonal stem and germ cells which differs from Oct-1 and Oct-2 by its faster migration in bandshift gels (6 and P. Gruss, pers. com.). We have previously described a testis-specific protein of the sea urchin (here referred to as Oct-T), which was at the time of its discovery the second example of a tissue-specific octamer-binding protein (7). The Oct-T protein-DNA complex was identified by its slightly faster migration in bandshift gels as compared to the ubiquitous Oct-1 complex of the sea urchin. As shown in Figure 1A, the two complexes can be clearly resolved upon prolonged electrophoresis. The molecular weight of the sea urchin Oct-1 and Oct-T proteins was determined by Southwestern analysis of sea urchin gastrula and testis nuclear extracts (Fig. 1B). As reference we have used nuclear extracts of human BJA-B and HeLa cells. Clearly, the Oct-1 proteins of sea urchin and man show the same apparent molecular weight of 97 kDa, while the Oct-T protein is about 85 kDa in size. Hence, Oct-T differs from the previously characterized Oct-1 and Oct-2 proteins by its molecular weight and from Oct-4 by its clearly different electrophoretic mobility in bandshift assays. Oct-T is therefore a distinct member of the family of octamer-binding proteins. As previously discussed (7), Oct-T may be partly responsible for the testis-specific transcription of sperm H2B genes.

Figure 1. A) Bandshift assay. Nuclear extracts of sea urchin gastrula embryos and of human HeLa cells were analyzed with a radiolabelled octamer DNA probe as described (7). A 1000-fold molar excess of unlabelled octamer DNA was used as competitor in lane 2 and 4. B) Southwestern analysis. Nuclear proteins were separated by 10% SDS PAGE, transferred to

nitrocellulose filters and probed with a radiolabelled octamer dimer (2). Lanes 6,7,10 were exposed for a longer time than lanes 8,9. "Unspecific" denotes unspecific complexes (see ref. 2).

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