Identification of a fourth Nuclear Factor I gene in chicken by cDNA cloning: NFI-X

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Nuclear Factor I/TGGCA proteins, which bind as dimers to the palindromic consensus sequence 5'-YTGGCANNNTGCCAR-3' (1), act as transcription factors and stimulate adenovirus DNA replication in vitro (2, 3).

Recently, we have identified three independent non cross-hybridizing chicken Nuclear Factor I genes (NFI-A, NFI-B and NFI-C). This allowed us to categorize the so far published NFI sequences of other species into four groups (4 and references therein). The fourth group consisted of only one member, the hamster NFI-X sequence (5). In addition, genomic Southern blot analysis with a NFI-C probe revealed weak additional bands, which could not be explained by restriction enzyme cuts within the probe region, indicating the existence of a further NFI-C related gene in chicken (4). This observation prompted us to look for the chicken homologue of the hamster NFI-X gene.

Here, we report the sequence of a full length cDNA clone encoding a chicken NFI-X protein (cNFI-X). This clone was isolated by screening a chicken promacrophage cDNA library with a PCR amplified X specific DNA probe (Figure 1). Rehybridization of the original NFI-C genomic Southern blot with an NFI-X specific probe reveals that the previously weak signals now appear strong and vice versa strong signals on the C type blot are now weak (Figure 2). This cross-hybridization between cNFI-X and cNFI-C is due to a 87% similarity on the nucleotide level within the probe region.

Comparison of the amino acid sequence of the chicken and hamster NFI-X proteins shows 92% identity. This high degree of similarity further corroborates our previous finding that Nuclear Factor I/TGGCA proteins are highly conserved in vertebrates (4).

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Figure 1. Restriction map and isolation strategy of cNFI-X. A chicken X specific DNA probe has been amplified by PCR with primers ON13 and ON35 using chicken BM2 cDNA library as template exactly as described previously (4). ON13 (4) corresponds to amino acid 10–18 and ON35 to position 222–228 of the hamster NFI-X sequence (5). After cloning of this fragment in pBluescript and verification by sequencing, this fragment served as a probe for screening the BM2 cDNA library (4). ON35: 5'-GGAATTCAC(A/T/C)A(G/A)(T/C)TC(A/T/C)GT(A/G/T/C)AC(A/G)TTCCA-3'.

Figure 2. Genomic Southern blot analysis. A previously published genomic Southern blot hybridized with a chicken NFI-C probe (4; Fig. 5B) has been rehybridized with a chicken NFI-X probe as indicated in Figure 1. Hybridization was carried out under stringent conditions (4).

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