Cloning and analysis of the macronuclear gene for histone H1 from Euplotes eurystomus

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Euplotes is a hypotrichous ciliate that contains both a macronucleus (MAC) and a micronucleus in the same cell. The DNA in the MAC is composed of small, linear highly amplified fragments, each of which contains one gene, two telomeres and all the necessary regulatory elements. Although there is a large amount of condensed chromatin in the MAC, it appears not to be organized in 30 nm fibers as seen in higher eukaryotes (9). Since histone H1 is responsible for the formation of the 30 nm fiber, analysis of this protein in Euplotes may provide some insight into the unique chromatin structure in the MAC.

Histone H1 from the Euplotes MAC has been previously identified, purified and partially characterized (7). The N terminus was sequenced and was used to construct an oligonucleotide probe. This probe identified a single gene on Southern blots of MAC DNA and was successfully used to screen a MAC DNA library (5). The double-stranded portion of the gene is 1310 bp and contains a 405 bp open reading frame, coding for a 134 aa protein (the N-terminal Met is removed in the mature protein). There are two putative TATA boxes within 55 bp upstream of the coding region, but no CCAAT boxes, which are seen upstream in other ciliate histone genes (1, 6). Quantitative Southern blots estimate 9 x 10^4 copies of this gene per MAC (data not shown).

A search of the SwissPro database (using the GCG programs, (4)) reveals that 20 of the top 25 matches were to histone H1 proteins. Despite an identity of about 30%, little or none of the homology was to the 80 aa globular domain, the most conserved domain among H1. However, the search matched Euplotes H1 with Tetrahymena H1, which also does not contain a globular domain. The Euplotes H1 does not contain the SPKK (2) or KTPKKAKKP (3) repeat motifs seen in some higher eukaryotic H1, that are believed to form beta turns and interact with the minor groove. On the other hand, it does contain a number of repeats of a 9-11 aa motif (see Figure 1) some of which could form beta turns. The beta turn would center on the sequence KKA(G/A)ARK. A computer search (4) revealed that all of the HI repeats contain at least one phosphorylation motif for either cAMP dependent kinase [(R,K)(R,K)X(S,T)] or protein kinase C [(S,T)X(R,K)]. In addition, many contain sites that could be phosphorylated by other kinases (8). These would provide an appropriate opportunity for a phosphorylation event to modify the binding of the H1 to DNA. Seven of the repeats are on the N-terminal portion of the protein, separated from the last two repeats by a 19 aa segment rich on A and P (the segment which has the greatest potential for forming α-helices and is double underlined in Figure 1).

Although the evolutionary relationship of Euplotes H1 to other H1 is in question, this protein displays many of the properties and performs many of the functions associated with H1 (7).

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REFERENCES

CONSENSUS: KKAGAAPARXSGKAKKAKK

Figure 1. Peptide sequence of the Euplotes histone H1 with the 9—11 aa repeat motifs aligned. The consensus of the repeat motif is aligned above the sequence. The fraction to the right of each repeat designates the number of matches (underlined) with the 8 aa that make up the consensus. The number to the left of each repeat is the aa number at the beginning of the that line.

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