Structure and expression of a single actin gene in *Volvox carteri*¹

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Summary. Southern blot analysis of *Volvox carteri* DNA indicated the presence of a single actin gene; the nucleotide sequence of that gene is reported here. In comparison with plant animal and fungal actins, the derived primary structure of 377 amino acids is highly conserved yielding similarity values of 79% to 94% (including non-identical, conservative exchanges). In contrast, the intron structure of the gene is highly unusual: in addition to one intron in the 5' untranslated region (ten nucleotides upstream of the initiator ATG), it has eight introns in the coding region, only three of which are in locations where introns have previously been reported. Transcription starts 26 nucleotides downstream of the putative TATA box and 70 nucleotides downstream of a conspicuous CCAAT motif. A potential polyadenylation signal, TGTAA, is located 366 nucleotides downstream of the terminator TAA. Northern hybridization indicates that the actin gene is transcribed throughout the *Volvox* life cycle with only a slight depression during the release of juveniles from mother spheroids. This pattern of gene expression suggests that actin may assume various functional roles in the differentiation and growth of *Volvox*.

Key words: *Volvox carteri* – Actin gene – Introns – Expression

Introduction

Actin, originally identified as a major component of the contractile apparatus of muscle cells, is now recognized as a major component of the cytoskeleton of all eucaryotic cells, and hence as one of the most ancient and ubiquitous of eucaryotic proteins (Stossel 1984). Actins are involved in many aspects of cell structure and behavior, including various forms of animal and protistan cell motility (Goldman et al. 1976), cytoplasmic streaming in plants (Kamiya 1981), mitosis and cytokinesis (Korn 1978), and possibly even the control of transcription (Scheer et al. 1984). These diverse functions are carried out by actins that are similar, but not identical, in structure. In all, six different actin isoforms have been identified in birds and mammals; these are the skeletal-striated-muscle, cardiac-striated-muscle, vascular-smooth-muscle, enteric-smooth-muscle, plus the β- and γ-cytoplasmic (non-muscle) types (Vandekerckhove and Weber 1979). Similarly, plant actin genes tend to fall into three discrete classes according to primary structure (Hightower and Mcgagh 1985), although the functional significance of these classes remains to be elucidated.

The clustering of amino acid interchanges between different actin isoforms, combined with the sequence conservation observed between widely divergent organisms, indicates that actin amino acid sequences have been subject to strong selective pressures. Indeed, strong evolutionary constraints are also indicated at the nucleotide sequence level, since actin genes from widely disparate organisms often cross-hybridize quite efficiently.

Our interest in characterizing selected structural genes of *Volvox carteri* is based on the intermediate phylogenetic position of this simple alga – between higher plants and animals, on one hand, and between lower and higher eucaryotes, on the other (Kirk 1988; Müller and Schmitt 1988; Rausch et al. 1989) – as well as on its conspicuous developmental patterns (Green and Kirk 1981). *V. carteri* is a multicellular green alga exhibiting simple, stereotyped patterns of cellular differentiation and morphogenesis that are altered dramatically by a number of single gene mutations; for that reason, it has been proposed as a model for exploring how fundamental developmental processes may be genetically preprogrammed (Starr 1970; Sessoms and Huskey 1973; Kirk and Harper 1986). Furthermore, the existence of several simpler, but closely re-
Materials and methods

Culture conditions and nucleic acid preparation. Volvox carteri f. nagariensis HK10 female strain (UTEX 1885) was grown synchronously in standard Volvox medium (SVM; Starr 1969) at 28°C under an 8 h dark/16 h light (10000–12000 lux) regimen (Starr and Jaenicke 1974). High molecular weight genomic DNA was prepared by a modified method of Bendich et al. (1979), as previously described (Mages et al. 1988). Stage-specific RNA was isolated from highly synchronized Volvox cultures according to Kirk and Kirk (1985) using cultures prepared as follows: ten selected juvenile spheroids were transferred to a Fernbach flask containing 800 ml SVM and incubated for about 144 h (three generations); cultures were then harvested at defined stages in the life cycle and used for RNA preparation.

Library screening, physical mapping and DNA sequencing. Screening of a λEMBL3-based genomic library of V. carteri (Mages et al. 1988) with chicken β-actin cDNA, clone pAl (Cleveland et al. 1980), resulted in six positive clones. Physical mapping with BamHII, EcoRI and HindIII and Southern hybridization (Southern 1975) yielded patterns congruent with the map shown in Fig. 1A. The 1.1 and 2.3 kb HindIII fragments containing the entire actin gene of λVac21 were subcloned using pUC8 (Vieira and Messing 1982), mapped in detail (Fig. 1B) and once more subeloned into M13mp18 and M13mp19 RF vectors (Norrander et al. 1983). DNA sequences of both strands were determined by the dieoxy chain termination procedure (Sanger et al. 1977) using synthetic primers, modified T7 DNA polymerase (Sequenase®; US Biochem. Corporation Cleveland, Ohio, USA) and gradient acrylamide gels (Heinrich 1986). DNA sequences were processed by the UWGCG program (Devereux et al. 1984) on a VAX computer.

Fig. 1 A–C. Restriction map and sequencing strategy for the Volvox carteri actin gene. A Region of genomic DNA contained in λVac21. The solid box indicates two adjacent HindIII fragments used for sequencing. B Expanded map of 3385 bp-combined HindIII fragments with restriction sites relevant for sequencing. Fragments were subcloned into M13 vectors and sequenced by dieoxy chain termination as indicated by arrows. C Schematic representation of the actin gene. Solid boxes indicate coding exons, open boxes represent transcribed untranslated regions and lines mark introns and flanking DNA. Transcription signals (CCAAT, TATAAA), the transcription start site (+1), the translational start (ATG) and stop (TAA) codons, and a potential polyadenylation signal (TGTTAA) are shown at their respective positions. Introns are numbered as I1 to I9. B, BamHI; E, EcoRI; HIII, HindIII; H, HpaI; P, PstI; PvuI; R, RsaI; S, SalI; Sa, Sau3AI; T, TaqI; X, XbaI

Fig. 2. A 3385 bp nucleotide sequence of two adjacent HindIII fragments containing the actin gene (Fig. 1) and the derived amino acid sequence. Numbers in the margin run sequentially and refer to nucleotides in each line. Upstream positions (negative numbers) and the transcription start site (+1) are shown. Presumptive promoter and putative polyadenylation sites are boxed, the translational start codon (ATG) is marked by an open triangle, splice junction borders are marked by solid triangles. Oligonucleotides used for amplification by PCR (numbers 3, 4, 5) and for S1 nuclease mapping (number 2) are delineated by horizontal arrows. The sequence data have been deposited in GenBank under accession number M33963.