A fungal minisatellite

Several minisatellites, or variable number of tandem repeats, have been described in the nucleotide sequences of mammalian[1], insect[2], fish[3] and bird[4] genomes. We present here sequence data of a minisatellite found in the lager brewing yeast *Saccharomyces carlsbergensis*. We were surprised to detect the minisatellite while investigating the degree of conservation between open reading frames (ORFs) in the yeasts *S. cerevisiae* and *S. carlsbergensis*[5]. This is, to our knowledge, the first discription of a minisatellite nucleotide sequence from a fungus.

The minisatellite consists of 13 tandem repeats of 12 base pairs (Fig. 1), and is placed inside an ORF that is homologous to YCL010c from *S. cerevisiae*[6]. Alignment of the putative products from the two ORFs shows 70% identity at the amino-acid level. The *S. carlsbergensis* clone, 16BD1 (from C. Newlon and C. Yang) only supported the sequencing of supposedly the first 145 amino acids compared with 146 amino acids of YCL010c. Deletion of 12 of the 13 repeats in the *S. carlsbergensis* ORF allows alignment of the putative product to that of the *S. cerevisiae* ORF without gaps.

Alignment of the nucleotide sequence from the two ORFs shows two frame shifts. Deletion of one base pair, just downstream of the minisatellite, and insertion of one base pair further downstream, restores the frame. There is no stop codon created between them. The presence of the minisatellite within the *S. carlsbergensis* genome has been confirmed by cloning and partial sequencing of an independently obtained fragment.

The high degree of homology between the two ORFs indicates that they are derived from a common ancestor, and the fact that only 12 of 13 repeats need be deleted to restore the alignment suggests that expansion of an existing DNA structure created the minisatellite. However, the possibility that the structure was created before the divergence of the sibling species cannot be ruled out, as a deletion covering 12 of the repeats might have occurred in the *S. cerevisiae* lineage.

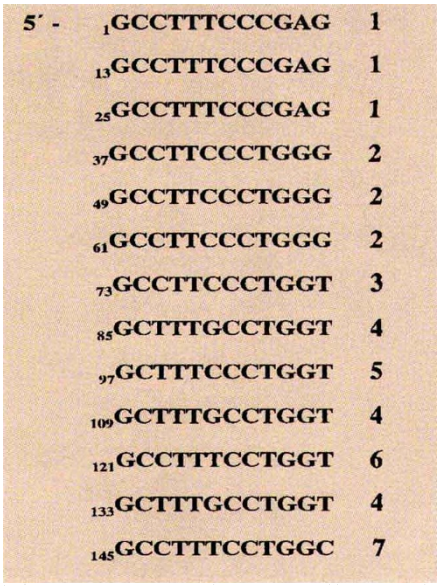

Figure 1 **DNA sequence of the Watson strand[6] (polarity 5′→3′) of the 156-base-pair minisatellite found in the brewing yeast, *Saccharomyces carlsbergensis*. The 13 repeats consist of 12 base pairs each and are aligned to give the highest homology between repeats. The numbers on the right represent the seven different repeat motifs found. The organization of the minisatellite shows a decreasing internal order going from top to bottom. Note that all repeats, except for those of type 1, contain the four-base-pair motif CCTG/CAGG (base pairs 8–11) which has been described in several minisatellites from various species[1–4]. EMBL accession number is Z86109.**

The 156 base pairs that compose the minisatellite align over the entire length, with 71% identity and only 3 gaps, to the pl18 minisatellite[4] from the bird *Phylloscopus trochilus*. Such a high degree of homology would normally indicate a close relationship between the two organisms. However, in this case it reflects that 8 of 12 base pairs of the consensus repeats in the two minisatellites are identical. Thus, a high degree of homology between minisatellite sequences does not necessarily represent a close phylogenetic relationship between two species. This emphasizes our general lack of knowledge about the origin of minisatellite DNA.

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The one that got away

In his interesting News and Views article on our work[1], Paul Calvert compared the diffusion in thin polymer films to fish (unperturbed chains) swimming among reeds (fully stretched chains from either the air or substrate interfaces). We feel that this metaphor is misleading. What our results[2] and those of others[3] show is that the diffusion coefficient is markedly reduced even when the thickness of the polymer film is more than 20 times the radius of gyration of the polymer. It is remarkable that surface effects could propagate so far into an amorphous polymer.

To form 'reeds', the chains must be anchored to the surface and the anchoring density would have to be quite high to overcome the entropic penalty of stretching the chains away from their unperturbed gaussian state[4]. Achieving high grafting densities is quite difficult. In a melt, this entropic penalty is not offset by any favourable enthalpic interactions[5].

Previous studies have shown that the gaussian conformation of the chain is retained even after grafting occurs[6]. Consequently, high grafting densities are unlikely. Even if a high grafting density could be achieved, the entropic penalty for a free chain to enter the grafted layer is simply too large. Thus, the 'fish' would be excluded from the reeds. Finally, if the reed picture were correct, thin polymer films would be optically anisotopic. This is not the case. Measurements from various laboratories suggest that, if anything, the chains have a preference for orienting parallel to the surface.

Each of these arguments dispels the image of polymer fish swimming through a bed of polymer reeds. But the question still remains as to why the diffusion coefficient is suppressed at such large thicknesses. The phase diagram of polymer mixtures is also altered at comparable film thickness[7]. The Gibbs–DiMarzio theory of glasses treats the glass transition as being a second-order phase transition[8]. By analogy with the critical point for polymer mixtures, also a second-order transition, the glass transition in thin films may also be perturbed by finite size effects at similar distances. The diffusion coefficient is related to the difference between the glass transition and measurement temperatures. How much would the glass transition have to be changed to explain our results?

Based on bulk parameters for polystyrene, the diffusion coefficient would decrease by a factor of 2 at 140 °C by an