Comparative genomics reveals long, evolutionarily-conserved, low-complexity islands in yeast proteins

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Abstract

Eukaryotic proteomes abound in low-complexity sequences, including tandem repeats and regions with significantly biased amino-acid compositions. We assessed the functional importance of compositionally-biased sequences in the yeast proteome using an evolutionary analysis of 2,838 orthologous ORF families from three *Saccharomyces* species (*S. cerevisiae, S. bayanus,* and *S. paradoxus*). Sequence conservation was measured by the amino-acid sequence variability and by the ratio of nonsynonymous to synonymous nucleotide substitutions (K_a/K_s) between pairs of orthologous ORFs. A total of 1,033 ORF families contained one or more long (at least 45 residues) low-complexity islands as defined by a measure based on the Shannon information index. Low-complexity islands were generally less conserved than ORFs as a whole; on average they were 50% more variable in amino-acid sequences and 50% higher in K_a/K_s ratios. Fast-evolving low-complexity sequences outnumbered conserved low-complexity sequences by a ratio of 10 to 1. Sequence differences between orthologous ORFs fit well to a selectively neutral Poisson model of sequence divergence. We therefore used the Poisson model to identify conserved low-complexity sequences. ORFs containing the 33 most conserved low-complexity sequences were overrepresented by those encoding nucleic-acid binding proteins, cytoskeleton components, and intracellular transporters. While a few conserved low-complexity islands were known functional domains (e.g., DNA/RNA-binding domains), most were uncharacterized. We discuss how comparative genomics of closely-related species can be employed further to distinguish functionally important, shorter, low-complexity sequences from the vast majority of such sequences likely maintained by neutral processes.

Introduction

Low-complexity or simple sequences comprise a large part of the proteome, but their evolutionary, structural, and functional roles are not well understood. (Here, a *low-complexity sequence* is a protein segment with low Shannon entropy whose composition is significantly different from the average amino-acid usage of a proteome (Wootton and Federhen 1996)). Eukaryotic proteomes abound in low-complexity sequences and protein repeats (Huntley and Golding 2000; Marcotte et al. 1999; Sim and Creamer 2002). For example, 21% of yeast proteins contain homopolymers of serine (S), glutamate (E), glutamine (Q), aspartate (D) or asparagine (N) (Golding 1999). By a less stringent definition of sequence complexity, 53% of proteins in yeast contain stretches of 10 or more residues at least half of which are single residue types (Sim and Creamer 2004). Since low-complexity sequences tend to be structurally disordered (Huntley and Golding 2002; Romero et al. 2001; Ward et al. 2004), eukaryotic proteomes include many disordered proteins. By a conservative computational estimate, long (≥30 residues) disordered segments occur in 33% of eukaryotic proteins and 2.0-4.2% of prokaryotic proteins (Ward et al. 2004). It has been suggested that the abundance of low-complexity, structurally disordered proteins in eukaryotes may be attributed to the multidomain nature of eukaryotic proteins as well as the increased complexity of transcriptional regulation of gene expressions in eukaryotes (Brocchieri and Karlin 2005; Huang et al. 1999; Rubin et al. 2000). For instance, disordered regions of proteins can function as flexible linkers between structured domains in modular proteins and can become folded on binding to their biological targets (Dunker et al. 1998; Dyson and Wright 2005; Tompa 2002). Among proteins containing long (at least 30 residues) disordered regions, nucleic-acid binding transcriptional regulatory proteins are over-represented and catalytic proteins are under-represented (Liu et al. 2002; Ward et al. 2004).

Nevertheless, it is unclear whether these significant functions for some disordered proteins, many of which are low-complexity sequences, imply that other low-complexity and disordered sequences play critical biological roles. The abundance of low-complexity sequences in eukaryotes could be due to a strong bias towards sequence duplication in eukaryotes (Mar Alba et al. 1999; Tompa 2003). The duplicated sequences underlying many low-complexity protein sequences may be adaptively neutral or even deleterious rather than maintained by natural selection (Huntley and Golding 2000; Lynch and Conery 2003). In fact, evolutionary comparisons showed that the majority of low-complexity sequences evolve faster than high-complexity sequences do (Huntley and Golding 2000; Sim and Creamer 2004). Similarly, evolutionary analyses showed that disordered proteins have faster evolutionary rates than ordered sequences (Brown et al. 2002). A small subset of low-complexity sequences, however, showed high sequence identity among putative orthologs from divergent species (Sim and Creamer 2004). Although there are evolutionarily conserved disordered regions with known functions (e.g., nucleic-acid binding domains), the functions of most conserved disordered regions are unknown (Brown et al. 2002; Liu et al. 2002).

Here, we extended these and other previous studies of the evolution of low-complexity sequences with a direct measure of evolutionary rates for low-complexity sequences. We estimated the evolutionary rates of low-complexity sequences (as the nonsynonymous to synonymous substitutions, or K_a/K_s ratios) using open reading frames (ORFs) whose orthology was verified by genome comparisons. This technique is more reliable than ortholog identification with procedures based on BLAST (Altschul and Gish 1996), or than the use of phylogenetically distant species (Huntley and Golding 2000; Sim and Creamer 2004). The genome-wide K_a/K_s comparisons for low-complexity sequences in three closely-related *Saccharomyces* species highlighted the functional importance of certain low-complexity sequences in the yeast proteomes. Our results corroborated earlier findings (Brown et al. 2002; Huntley and Golding 2000; Liu et al. 2002; Sim and Creamer 2004) that, on average, low-complexity sequences evolve faster than the ORFs in which they are found. In comparison with the evolutionary rates of the whole protein, about 24% of low-complexity sequences evolve significantly faster, while the rest showed no significant differences. About 2.4% of low-complexity sequences showed significant sequence conservation relative to the whole protein sequences.

Materials and Methods

Orthologous gene families

Nucleotide sequences of ORFs from the genomes of three *Saccharomyces sensu stricto* species (*S. cerevisiae, S. bayanus, S. paradoxus*) were downloaded from the *Saccharomyces* Genome Database (SGD) (http://genome-www.stardford.edu/Saccharomyces, (Kellis et al. 2003). (Other species listed in SGD were not used because of their low sequence identity to these three species.) According to an ortholog table (based on genome synteny) from SGD (Kellis et al. 2003), ORFs were grouped into orthologous gene families. We removed from the orthologous gene families any that contain more than one ORF from a single species (1-to-many orthology due to species-specific duplication), or those that contain ORFs with multiple stop codons. The protein sequences in each of the remaining orthologous gene families were aligned with CLUSTALW (Thompson et al. 1994) using its default parameters. These CLUSTALW protein alignments were the basis for nucleotide alignments and comparison of codon sequences.

Identification of low-complexity islands

Consensus sequences of the CLUSTALW protein alignments were used to calculate the sequence complexity of orthologous gene families. For each consecutive window of 45 amino-acid residues, a complexity score L was calculated as the Shannon information content (a reduction in maximum entropy, with sampling-error correction):

$$L = -\log_2 20 + \sum p_i \log_2 p_i - \frac{19}{2n \ln 2}, \qquad \textbf{(Equation 1)}$$

where the p_i's are amino-acid residue frequencies and n is the number of residues in the window (Schneider et al. 1986). (Low-complexity sequences have high L values.) We obtained the mean and variance of complexity scores from the score distribution of 45-residue windows across all orthologous gene families. A low-complexity window was defined as one whose complexity score was at least two standard deviations above the mean complexity score of all windows in all ORFs. A low-complexity island began with the first residue of a low-complexity window and extended to the last residue of a low-complexity window. Some low-complexity islands contained short (fewer than 45 residues) high-complexity segments. An example of a low-complexity island in a cell-wall protein is shown in Figure 1; it illustrates that the identification criterion is similar to the SEG filtering algorithm (Wootton 1994) with higher stringency.

Tests of sequence conservation

To identity which low-complexity islands were conserved, we compared amino-acid sequence diversity with both the genome-wide ORF diversity and with the average diversity of the ORFs containing the islands. To test for statistical significance, we assumed a selectively neutral Poisson process of amino acid substitutions during the evolution of an orthologous ORF family:

$$P(k \le D_{obs}) = \sum_{k=0}^{D_{obs}} \frac{{D_{exp}}^{k}}{k!} e^{-D_{exp}}, \qquad \textbf{(Equation 2)}$$

where k is the number of amino acid differences, D_{obs} is the observed number of substitutions of an island, and D_{exp} is the expected number of substitutions of the island based on the substitution rate of the entire ORF. When the probability of having less than or equal to the observed differences is low (P<0.001), island sequences were considered to be maintained by purifying selection, i.e., evolutionarily conserved. Note that this criterion, while allowing us to identity the most conserved islands, does not meet rigorous standards for the statistical significance of individual islands because adjustment for multiple tests was not applied. (For instance, to identify conserved islands at a 5% significance level among 1,500 independent tests, the critical

P value is 3.3×10^{-5}.) The Poisson test was applied to comparisons between pairs of orthologous ORFs, as well as to the more independent comparisons among all ORFs in an orthologous family at once based on phylogenetic reconstruction. For the tree-based test, the total numbers of substitutions of islands and those of the whole ORFs were obtained based on parsimony reconstruction using the PARS program of the PHYLIP package (Felsenstein 1989). (Because of the low overall proteome sequence difference (7-16%), the number of observed pairwise sequence differences as well as the number of substitutions based on parsimony reconstruction were close to the numbers of substitutions, and did not require correction for multiple hits at the same site.) The levels of evolutionary conservation of these islands were further verified using the K_a/K_s, the ratio of synonymous to nonsynonymous nucleotide substitutions. Total tree lengths of K_a and K_s were estimated based on maximum likelihoods using the CODEML program of the PAML package (Yang 1997).

Results

Proteome divergence and sequence complexity

From the 5,306 orthologous ORF families obtained from SGD, we used 2,838 orthologous gene families that were validated for sequence quality and 1:1 orthology. An unrooted tree based on the average protein sequence differences between orthologous ORFs from three pairs of species is shown in Figure 2A. Based on sequence differences, *S. cerevisiae* and *S. paradoxus* are more closely related to each other than either of them is to *S. bayanus* (Figure 2A). Protein sequence differences between pairs of orthologous ORFs closely fit a Poisson model of neutral amino-acid substitutions (Figure 2B).

Low-complexity sequences are less conserved than entire ORFs

The mean and standard deviation of the complexity scores (Equation 1) of 45-residue windows of ORFs in the 2,838 orthologous families were 0.31 bits and 0.23 bits, respectively. A cutoff value of L=0.77 (two standard deviations above the mean) was therefore used for delineating low-complexity islands. 1,033 orthologous gene families (36.4%) contained at least one of the 1,572 low-complexity islands of at least 45 residues. Evolutionary rates (including amino-acid differences, K_a, and K_s) of the low-complexity islands and the ORFs containing them were calculated for each pair of species. On average, amino-acid differences in the low-complexity islands were 50-70% higher than the amino acid differences of corresponding ORFs (Table 1). K_a of the low-complexity islands was on average 1.5-fold of that of the whole ORFs (Table 1; one-tailed, paired *t*-test highly significant), whereas K_s was only slightly higher (1.04-fold, paired t-test significant) in the low-complexity islands than in whole ORFs. At a significance level of p<0.001, less than 3% of the low-complexity islands were more conserved than ORFs as a whole, whereas close to 20% of the low-complexity islands evolved faster than the ORFs in which they were located (Table 2). About 80% of islands showed no significant difference in substitution rates to ORFs as a whole. Based on this evidence, we conclude that, for the most part, low-complexity segments in yeast are more variable and less conserved in protein sequences than the ORFs as a whole.

Conserved low-complexity sequences

We used the Poisson test (Equation 2) to identify conserved low-complexity islands. The number of conserved low-complexity islands varied depending on significance level and methods of comparison (Table 2). The power of this test increased with increased genome distance. At P<0.001, comparing the most closely related species (*S. cerevisiae/S. paradoxus*) yielded 0.39% conserved low-complexity islands. Comparison of more distantly related species (*S. cerevisiae/S. bayanus and S. paradoxus/S. bayanus*) showed about 1% conserved islands. Comparisons using the total tree lengths inferred by parsimony analysis resulted in 2.4% conserved low-complexity islands (Table 2). A total of 33 conserved (P<0.001) islands were identified based on the total tree lengths. The much lower K_a/K_s ratios of these islands relative to those of the whole ORFs further supported the amino-acid sequence conservation of these 33 low-complexity islands (Table 3). Table 4 lists the SGD annotations of the 31 ORFs whose product contain these conserved low-complexity islands. Ten genes (*CYC8, NPL3, SMC2, YAP3, HOS4, NOT3, CEF1, CRZ1, RLF2, FHL1*) encode proteins involved in transcription regulation or DNA/RNA-binding activities. Products of another eight ORFs (*EDE1, TAT1, VPS15,* YBR235W*, NUP49, MON1, SGM1, GMH1*) function in intracellular transport. Yet another seven genes (*SPC110, SCW11, MSB2, SPA2, STU2, CDC3, MDM1*) encode structural components of the cytoskeleton. Eight of the 33 conserved, low-complexity islands corresponded to known structural domains such as nucleic-acid binding folds (Figure 3; Table 5). Most conserved islands, however, did not contain known domains or motifs identified in SGD.

Although these sequences were highly repetitive and replete with tandem repeats, we observed only a few alignment gaps. The average gap density, defined as the percentage of alignment columns containing gaps, was 0.067 in all low-complexity islands and only 0.021 in these 33 conserved low-complexity islands.

Discussion

Yeasts are at the forefront of comparative genomics of eukaryotes, and evolutionary events such as ancient genome duplications and numerous gene duplications and losses have been identified as a result of such efforts (reviewed in (Dujon 2005; Gianni and Edward 2005; Piskur and Langkjaer 2004). Whole-genome sequencing of closely-related species allows more accurate reconstruction of genome evolution than comparing genomes from phylogenetically distant species. This is because fewer multiple events affect the same genomic regions or sites during the relatively short time of divergence between closely-related species. Technically, sequences from closely-related species can be more reliably aligned using unsupervised procedures than sequences from distant species. This technical advantage is critical for the evolutionary study of low-complexity sequences, since compositionally-biased sequences are prone to misalignment when standard amino-acid substitution matrices are used (a problem known as “low-complexity corruption”) (Schaffer et al. 2001) (Coronado et al., in press). Here we used the comparative genomics resource available for multiple yeast species (Kellis et al. 2003) to systematically examine the evolution and function of low-complexity sequences that make up a large portion of the eukaryotic proteome (Golding 1999; Huntley and Golding 2000; Marcotte et al. 1999; Sim and Creamer 2002).

Are low-complexity sequences adaptive?

It is controversial whether low-complexity sequences are maintained by natural selection or by selectively neutral processes such as the birth-death process of DNA duplications (Malpertuy et al. 2003; Nei 2005). In the three yeast proteomes studied here, we found that the evolutionary rates of the low-complexity islands we identified were about 50% higher than those of the ORFs in which they were found. In addition, there were about 10 times as many low-complexity islands that evolve quickly rather than slowly at the tested significance levels (P<0.01 and P<0.001, Table 2). These results extend previous studies of evolutionary conservation of low-complexity sequences (Huntley and Golding 2000; Sim and Creamer 2004) that reported a similar lack of sequence conservation in low-complexity sequences. Those previous studies employed different criteria for low-complexity sequences, different measures of sequence conservation, and phylogenetically distant sequences. Disordered regions of protein, many of which are low-complexity sequences (Liu et al. 2002; Romero et al. 2001), evolve on average with either equivalent or faster rates than the flanking regions (Brown et al. 2002; Liu et al. 2002). The lack of sequence conservation of low-complexity sequences suggests that repetitive protein segments may be disposable parts of the proteome (Huntley and Golding 2000). It has been suggested that the abundance of low-complexity sequences in eukaryotes could be due to the smaller population sizes and, thereby, weaker selection in eukaryotes than in prokaryotes (Lynch and Conery 2003).

Nevertheless, we found that higher than expected numbers of low-complexity islands are conserved. These conserved low-complexity islands also showed fewer alignment gaps than non-conserved low-complexity islands. Low-complexity sequences may also be conserved in their amino-acid composition, sequence length, and higher-order structural propensities. Distinct classes of repetitive sequences (e.g., those enriched in Gln, Asn, and Asp) appeared associated with different functional classes of proteins (e.g., transcription factors) in yeast (Mar Alba et al. 1999). This suggests that certain amino acid compositions in proteins are selectively maintained. Similarly, certain classes of tandem triplet repeats (e.g., CAA, coding for Gln) were preferentially retained in coding regions, coding strands, and certain functional classes in the yeast genome (e.g., transcriptional regulators) (Malpertuy et al. 2003; Mar Alba et al. 1999; Young et al. 2000). Some amino acids (Gly, Gln, Ser, Asn, Pro, Asp, Glu, Lys) are over-represented in low-complexity and disordered regions of proteins (Liu et al. 2002; Romero et al. 2001; Sim and Creamer 2004). It has been suggested that these amino acids might be selected for forming functional yet natively disordered parts of proteins (Romero et al. 2001).

In this study we used 2,838 ortholgous ORF families from three *Saccharomyces sensu stricto* genomes (Kellis et al. 2003) to estimate the evolutionary rates of low-complexity sequences. For three reasons, we believe that our genome-verified, orthologous ORFs from closely-related species produces more reliable and more precise estimates of sequence conservation than previous studies of sequence conservation of low-complexity (Huntley and Golding 2000; Sim and Creamer 2004) or disordered (Brown et al. 2002; Liu et al. 2002) sequences. First, gene orthology among homologous ORFs identified from genome comparisons is more reliable than the output of BLAST or other searches based on sequence similarity. Evolutionary rates among orthologous genes are more uniform than among paralogous genes due to frequent function shifts in the latter. Sequence comparisons among paralogous sequences may underestimate (for recent duplications) or overestimate (for ancient duplications) evolutionary rates (Zmasek and Eddy 2001). Second, the availability of complete proteomes allowed a precise estimate of the overall rate of amino acid substitutions with an explicit model of neutral evolution. The proteome-wide amino-acid sequence differences fit a neutral model of amino-acid substitutions well (Figure 2). Conserved segments can thereby be identified as those that with significantly fewer differences than expected from the average proteome or average ORF differences. Finally, the availability of genome sequences offered an additional (although not independent) test of sequence conservation based on the nonsynonymous to synonymous (K_a/K_s) ratios (Hurst 2002). With the same level of amino acid variability (K_a), amino acid sequences are more conserved when accompanied by a high rate of synonymous substitutions (high K_s).

Putative roles of conserved low-complexity sequences

Despite the generally low sequence conservation of low-complexity sequences in the yeast proteome, the number of long, conserved low-complexity islands was greater than expected from neutral Poisson processes, both at P<0.01 and P<0.001 (Table 2). The length of our 33 most conserved low-complexity sequences identified in the present analysis ranged from 46 to 273 residues (Table 5). Conserved low-complexity sequences were previously identified in a survey of protein families from the COG database (Tatusov et al. 2003), which primarily consists of prokaryotic sequences (Sim and Creamer 2004). That work used an absolute measure of sequence conservation (40% sequence identity). A more informative measure would be the sequence identity of these low-complexity sequences relative to the identity of the full-length proteins.

The sequence conservation observed in these long, low-complexity islands is unlikely to be coincidental. At P<0.001 the expected number of false positives among 1,500 independent comparisons was 1.5 while the possibility of having 4 or more false positives was not significant (P=0.02 assuming a Poisson error rate). As expected, the sensitivity of the Poisson test increased with increasing genome distances (Eddy 2005) and with the use of tree-based methods (Table 2). Perhaps more convincingly, the K_a/K_s ratios provided further evidence of sequence conservation of these islands by showing that their synonymous substitution rates were comparable to, if not greater than, those of the ORFs (Table 3).

GO annotations showed that the ORFs containing these conserved low-complexity sequences were over-represented by ORFs functioning in transcriptional regulation (10/31, or 32%; *CYC8, NPL3, SMC2, YAP3, HOS4, NOT3, CEF1, CRZ1, RLF2, FHL1*), consistent with earlier findings that sequence repetitiveness and structural disorder are associated with macromolecular binding activities (Liu et al. 2002; Malpertuy et al. 2003; Mar Alba et al. 1999; Ward et al. 2004). The other two major functional classes of ORFs containing conserved low-complexity sequences included eight genes (26%; *EDE1, TAT1, VPS15,* YBR235W*, NUP49, MON1, SGM1, GMH1*) coding for proteins involved in intracellular transport and seven (23%; *SPC110, SCW11, MSB2, SPA2, STU2, CDC3, MDM1*) coding for structural components of cytoskeleton. Although it is unlikely that low-complexity islands correspond exactly to biologically functional units, a conserved low-complexity island in Yap3p matches almost precisely with a known structural domain, the DNA-binding bZIP domain (Figure 3). The conserved island in Stu2p coincides with the tRNA-binding arm of the molecule. Six other low-complexity islands do not coincide but overlap with known domains or motifs: an island in Ede1p and another in Sgm1p each containing a t-SNARE domain important for membrane fusion and endo- or exocytosis, an island in Tat1p and another in the unnamed YBR235W ORF product each containing a amino-acid permease motif, an island in Vps15p forming a part of HEAT repeats involved in intracellular transport, and an island in Npl3p associated with an RNA binding domain. Computational prediction showed a high propensity for structural disorder in these highly conserved low-complexity islands (Table 5). It would be intriguing to experimentally test the structural and functional roles of these long, conserved low-complexity islands.

Detecting short, conserved motifs using comparative genomics

Overall, we observed 10 times as many fast-evolving, low-complexity sequences than conserved low-complexity sequences at every level of statistical significance (Table 2). Because we targeted only long (at least 45 residues) low-complexity sequences, shorter conserved sequences, such as mini- or micro-repeat units (Tompa 2003), may also exist. The statistical power of sequence conservation detection with comparative genomics increases with the number of genomes, the level of genome divergence and the length of conserved sites (Cooper et al. 2003; Eddy 2005). To identify short conserved motifs within low-complexity sequences, one would have to increase the number of genomes or to compare more divergent genomes (as long as reliable alignments can be obtained). Assuming a Poisson model of neutral amino-acid substitutions, the probability of observing no sequence difference (P_0) in N (not necessarily consecutive) amino acids between two proteomes differing by D (per site) is $P_0 = e^{-DN}$. By this formula, the shortest conserved segments showing no sequence difference at $P=0.001$ in comparisons between the *S. cerevisiae* and *S. bayanus* proteomes (D=0.16) is 43 amino acids long. The identification of conserved segments 10 amino acids long at the same significance level would require the comparison of two proteomes that differ by 69%, the use of more genomes, or both. (At large distances sequence difference needs to be corrected for multiple hits.)

In summary, we showed through comparative genomics in yeast proteomes that long (at least 45 residues), conserved low-complexity sequences are rare but exist at a frequency greater than chance would predict. If there are other highly conserved, low-complexity sequences, their biological roles would rely predominantly upon shorter (fewer than 45 residues) conserved motifs or upon features other than their primary sequences (e.g., amino-acid composition, sequence length, and structural propensity). We expect the comparative genomics of closely-related species to play an increasingly prominent role in revealing selectively-maintained and functionally-important low-complexity sequence motifs and disordered proteins. Once sufficient numbers of closely-related genomes become available, the functional importance of protein sequences at the single residue level can be tested based upon their evolutionary conservation. Adaptively-maintained low-complexity or disordered sequences could thereby be distinguished from the (probably much more common) low-complexity and disordered sequences that are maintained in the proteome by neutral processes.

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Figure Legends

Figure 1. Identification of low-complexity islands. The example used is an orthologous gene family containing the *S. cerevisiae* ORF YGR279C (coding for Scw4p, a soluble cell-wall protein). **(A)** Plot of (negative) Shannon complexity score in consecutive windows of 45 residues of Scw4p. A low-complexity island extends from the first residue of the first low-complexity window to the last residue of the last low-complexity window. **(B)** Sequence alignment of the same protein molecule with low-complexity island shaded. For the second sequence, only amino-acid differences between two sequences are shown; the dots (".") represent the same residue as in the first sequence. The majority of amino-acid substitutions occurred inside the low-complexity island in this molecule (ORF K_a=0.0313, island K_a=0.076). For method comparison, low-complexity islands identified using SEG (Wootton 1994) (with window length 45, trigger complexity 3.4, extension complexity 3.74) are underlined.

Figure 2. Proteome sequence divergence. **(A)** A phylogeny of three yeast species, based on the average amino-acid sequence differences (shown in percentages) between orthologous ORFs. **(B)** Each panel is a histogram of protein sequence differences between orthologous ORFs from two species. These counts were not significantly different from the simulated values (bold lines) obtained by assuming a selectively neutral Poisson process of amino-acid substitutions.

Figure 3. Identification of conserved low-complexity sequences. **(A)** A conserved low-complexity island (*shaded portion*) was identified as segments of low amino-acid sequence diversity relative to that of the whole ORF (Equation 2). Sequence conservation was further tested using the K_a/K_s ratios. Using the example of Yap3p (a bZIP transcription factor), the K_a and K_s values (for each of the 3 ORF pairs, using a window size of 45-amino acids) were plotted along with the complexity scores ($-L$). **(B)** Sequence alignment showed that the conserved low-complexity island (red residues) coincides with the region characterized by SGD as a bZIP DNA binding domain.

Table 1. Evolutionary rates of low-complexity sequences in yeasts

	S. cer* vs. *S. par	***S. cer* vs. *S. bay***	***S. par* vs. *S. bay***
ORF			
No. of pairs	2786	2581	2535
Amino-acid diff.	7.81%	16.1%	15.5%%
K_s^*	0.3277	0.7734	0.7128
K_a^*	0.0509	0.1235	0.1157
K_a^*/K_s^*	0.1553	0.1597	0.1623
Low-complexity Islands			
No. of pairs	1537	1418	1386
Amino-acid diff.	12.9%	25.5%	24.3%
K_s	0.3422	0.8052	0.7451
K_a	0.0735	0.1813	0.1718
K_a/K_s	0.2149	0.2252	0.2306

*Average values.

Table 2. Levels of sequence conservation of low-complexity islands

		***S. cer* vs.**	***S. cer* vs.**	***S. par* vs.**	***Tree-length***
		S. par	***S. bay***	***S. bay***	***Test*[a]**
No. of islands		1537	1418	1386	1387
Fast-evolving	$P<10^{-3}$	206 (13.4%)	266 (18.8%)	248 (17.9%)	332 (23.9%)
Islands	$P<10^{-2}$	379 (24.7%)	436 (30.7%)	411 (29.7%)	494 (35.6%)
Similar-rate	$P<10^{-3}$	1325 (86.2%)	1136 (80.1%)	1125 (81.2%)	1022 (73.6%)
Islands	$P<10^{-2}$	1131 (73.6%)	933 (65.8%)	926 (66.8%)	820 (59.0%)
Conserved	$P<10^{-3}$	6 (0.39%)	16 (1.1%)	13 (0.94%)	33 (2.4%)
Islands	$P<10^{-2}$	27 (1.8%)	49 (3.5)	49 (3.1%)	73 (5.3%)

[a] Poisson test based on tree lengths (total number of substitutions inferred using the PARS program of PHYLIP; see Methods).

Table 3. Conserved low-complexity islands

			Complexity	Conservation Tests[a]			
ORF Name	Gene	Island	L_{island}/L_{orf}[c]	P[d]	Isle	ORF	$K_{s\ [isle]}/$
	Name	Position[b]		$(\times 10^{-4})$	K_a/K_s	K_a/K_s	$K_{s\ [ORF]}$
YBL047C	*EDE1*	699-745	2.42	7.4	0.0092	0.084	0.77
YBR069C	*TAT1*	103-156	4.68	5.6	0.0023	0.088	3.9
YBR097W	*VPS15*	636-687	3.40	2.9	0.0071	0.082	1.1
YBR112C	*CYC8*	2-69	2.18	1.1	0.017	0.095	0.74
YBR235W	(none)	74-141	3.31	6.4	0.0001	0.042	1.3
YCR016W	(none)	39-108	1.73	3.3	0.056	0.15	1.0
YDR356W	*SPC110*	745-790	1.64	6.8	0.030	0.093	0.67
YDR432W	*NPL3*	268-432	3.17	0.23	0.0043	0.056	0.86
YFR031C	*SMC2*	176-233	2.28	1.5	1.0×10^{-4}	0.058	1.5
YGL172W	*NUP49*	335-381	1.52	1.3.	1.0×10^{-4}	0.097	1.0
YGL124C	*MON1*	252-302	3.84	4.4	0.013	0.11	1.2
YGL028C	*SCW11*	28-75	1.40	9.2	0.014	0.14	1.7
YGR014W	*MSB2*	1122-1227	1.18	0.002	0.13	0.39	1.2
YGR130C	(none)	674-804	2.09	0.18	0.029	0.14	1.9
YHL009C	*YAP3*	154-225	3.77	0.006	0.047	0.22	1.1
YIL112W	*HOS4*	834-950	3.07	7.0	0.037	0.10	1.3
YIL038C	*NOT3*	40-85	2.88	1.3	1.0×10^{-4}	0.087	0.75
YJR134C	*SGM1*	599-707	1.60	3.7	0.046	0.13	1.3
YKR030W	*GMH1*	184-273	3.27	0.20	0.0030	0.068	2.0
YLL021W	*SPA2*	294-395	2.61	0.87	0.11	0.21	0.73
		1420-1503	2.07	5.7	0.042	0.21	3.6
YLR045C	*STU2*	681-793	2.50	0.83	1.0×10^{-4}	0.086	2.2
YLR273C	*PIG1*	5-77	3.23	1.7	0.11	0.19	0.74
YLR314C	*CDC3*	491-568	2.97	0.72	0.0025	0.070	2.0
YML104C	*MDM1*	723-773	2.79	0.13	0.0026	0.11	3.5
YMR124W	(none)	843-888	2.23	0.63	0.0078	0.13	2.6
YMR213W	*CEF1*	148-196	3.34	0.0006	1.0×10^{-4}	0.13	1.8
YNL091W	*NST1*	520-797	3.14	1.9	0.023	0.11	3.1
YNL027W	*CRZ1*	385-462	2.23	6.2	0.017	0.10	1.7
YOR171C	*LCB4*	25-83	2.54	0.50	0.011	0.089	0.58
YPR018W	*RLF2*	375-451	2.17	0.11	0.019	0.14	2.1
YPR104C	*FHL1*	84-129	2.49	4.7	0.011	0.11	1.7
		684-733	2.48	0.07	0.013	0.11	1.0

[a] Number of amino acid substitutions, K_a, and K_s were estimated based on total tree lengths using PHYLIP and PAML (see Methods).

[b] Alignment positions.

[c] Complexity score (L, Equation 1) of island relative to that of ORF.

[d] P: probability of having fewer than observed number of amino-acid substitutions in islands than expected from ORF average differences (Equation 2).

Table 4. Annotation of *S. cerevisiae* ORFs containing conserved low-complexity islands

ORF Name	Gene Name	GO Annotation[a] (Mol. Function; Biol. Process; Cell. Component)
YBL047C	*EDE1*	Unknown; Endocytosis; Actin cortical patch, bud neck, bud tip
YBR069C	*TAT1*	Amino acid transporter; Amino acid transport; Plasma membrane
YBR097W	*VPS15*	Ser/Thr kinase; Phospohorylation, retention in Golgi, vacuole transport; Golgi membrane, mitochondrion
YBR112C	*CYC8*	Transcription repressor/coactivator; Chromotin remodeling; Nucleus
YBR235W	(none)	Ion transporter; Unknown; Unknown
YCR016W	(none)	Unknown; Unknown; Nucleolus
YDR356W	*SPC110*	Cytoskeleton constituent; Microtubule nucleation; Spindle pole
YDR432W	*NPL3*	mRNA binding; mRNA export from nucleus; Cytoplasm, nucleus
YFR031C	*SMC2*	DNA binding, ATPase; Mitosis; Mitochondrion, nuclear condensin complex
YGL172W	*NUP49*	Component of nuclear pore complex; Ribosome transport; Nuclear pore
YGL124C	*MON1*	Unknown; Autophagy, vesicle docking; Vacuolar membrane
YGL028C	*SCW11*	Glucan 1,3-beta-glucosidase; Cytokinesis; Cell wall
YGR014W	*MSB2*	Osmosensor; Establish cell polarity, response to osmotic stress; plasma membrane, site of polarized growth
YGR130C	(none)	Unknown; Unknown; Cytoplasm
YHL009C	*YAP3*	Basic leucine zipper (bZIP) transcription factor; Regulation of transcription; Nucleus
YIL112W	*HOS4*	NAD-dependent histone deacetylase; Negative regulation of meiosis; Histone deacetylase complex
YIL038C	*NOT3*	3’-5’-exoribonuclease; Poly(A) shortening, regulation of transcription; CCR4-NOT core complex, cytoplasm
YJR134C	*SGM1*	Unknown; Unknown; Golgi apparatus
YKR030W	*GMH1*	Unknown; Transport; Integral to Golgi membrane
YLL021W	*SPA2*	Cytoskeletal regulatory protein binding; Actin filament organization; Bud neck, polarisome
YLR045C	*STU2*	Microtubule binding; Mitotic spindle organization and biogenesis; Kinetochore, spindle, cell cortex
YLR273C	*PIG1*	Protein phosphatase type-1 regulator; Glycogen biosynthesis; Protein phosphatase type-1 complex
YLR314C	*CDC3*	Phosphatidylinositol binding, component of cytoskeleton; Cell wall organization and biogenesis, cytokinesis; Septin ring, spore wall
YML104C	*MDM1*	Structural constituent of cytoskeleton; Mitochondrion biogenesis, nuclear migration; Cytoplasm
YMR124W	(none)	Unknown; Unknown; Cytoplasm
YMR213W	*CEF1*	RNA splicing factor; Nuclear mRNA splicing; Spliceosome complex
YNL091W	*NST1*	Unknown; Salt stress; Cytoplasm
YNL027W	*CRZ1*	Transcription factor; Calcium-mediated signaling; Cytoplasm and nucleus
YOR171C	*LCB4*	D-erythro-sphingosine kinase; Calcium-mediated signaling, spingolipid metabolism; Golgi, ER
YPR018W	*RLF2*	Transcription regulator; Nucleosome assembly; Chromatin assembly complex
YPR104C	*FHL1*	Transcription factor; rRNA processing; Nucleolus

[a] Based on the *Saccharomyces* Genome Database (SGD) queries.

Table 5. Sequences and characteristics of long, conserved low-complexity sequences in *S. cerevisiae*

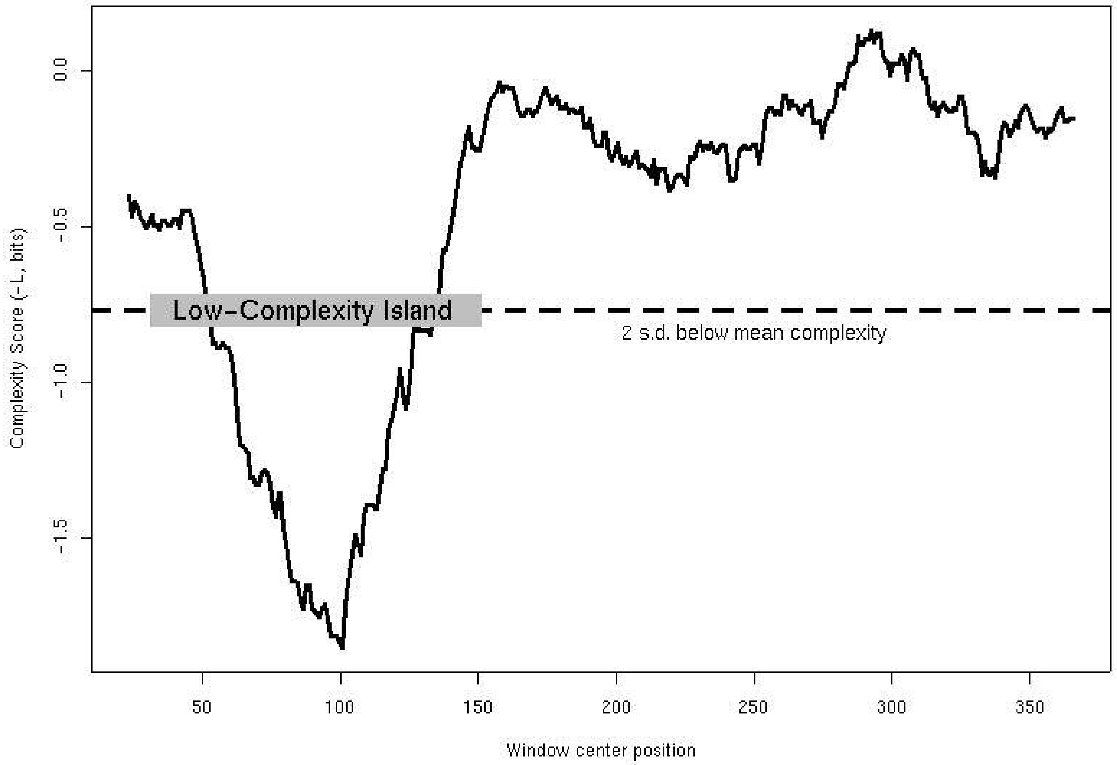
ORF Name	Gene Name	Island Length	Island Sequence	Predicted Disorder[a]	Domain/Motif
YBL047C	*EDE1*	47	aesklnelttdlqesqtknaelkeqitnlnsmtaslqsqlnekqqqv	100%	t-SNARE
YBR069C	*TAT1*	54	vmislgtgigtgllvgngqvlgtagpaglvlgygiasimlyciiqaagelglcy	0%	amino acid permease
YBR097W	*VPS15*	52	rvsliqtisgisillgtvtleqyilplliqtitdseelvvisvlqslkslfk	0%	HEAT repeat
YBR112C	*CYC8*	64	npggeqtimeqpaqqqqqqqqqqqqqqqqaavpqqpldpltqstaetwlsiaslaetlgdgdra	0%	poly (Q)
YBR235W	(none)	68	alnvlsilmflrfgfilgqlgiictiglllllsytinllttlsisaistngtvrgggayymisrslgpe	0%	amino acid permease
YCR016W	(none)	70	lnitthlstgnltkkekkriingesksstkkgkrvskpgtkkkeklskdeknskknkilkdqlrylieff	94%	
YDR356W	*SPC110*	46	rekeelnensnnirimedkmtrikknylseitslqeenrrleerli	100%	
YDR432W	*NPL3*	158	rlnniefrgsvitverddnpppirrsnrggfrgrggfrggfrggfrggfsrggfggprggfggprggyggysrggyggysrggyggsrggydsprggydsprggysrggyggprndygpprgsyggsrggydgprgdygpprdayrtrdaprersptr	93%	RNA binding; GGX repeats
YFR031C	*SMC2*	58	drrekaertmskketklqenrtllteeiepkleklrnekrmflefqstqtdlekteri	76%	
YGL172W	*NUP49*	47	llksesatsqylkqdlkkissfkslidedlldtqtfsvllqqlltpg	40%	
YGL124C	*MON1*	51	sergessnellnqldflysyilsslserqllrlfskrenfdlrnylestdf	0%	
YGL028C	*SCW11*	48	evvtrvhtasttnvvtdfystttevviaptveflisdsvtftttlipq	100%	
YGR014W	*MSB2*	104	tdgmgtaksmaamvdssipltgllhdsnsnsggssdgssssnsnsgssgsgsnsnsgvssssgnsyqdagtleyssksnsnvstssksk kkiiglvigvvvggc	91%	poly (S)
YGR130C	(none)	131	ikqenanektqlsaitkrlederraheeqlkleaeerkrkeenllekqrqeleeqahqaqldheqqitqvkqtyndqltelqdklateekeleavkrertrlqaekaieeqtrqknadealkqeilsrqhk	100%	
YHL009C	*YAP3*	72	dskakkkaqnraaqkafrerkearmkelqdkllesernrqsllkeieelrkanteinaenrlllrsgnenfs	90%	bZIP domain
YIL112W	*HOS4*	117	eklksisplsmephspkkaksveiskiheetaaerearlkeeeeyrkkrlekkrkkeqellqklaedekkrieeqekqkvlemerlekatlekarkmerekemeeisyrravrdlyp	71%	
YIL038C	*NOT3*	46	qkdklesdlkrevkklqrlreqikswqsspdikdkdslldyrrsve	67%	

Table 5. (Continued)

ORF Name	Gene Name	Island Length	Island Sequence	Predicted Disorder[a]	Domain/Motif
YJR134C	*SGM1*	109	lvnklstelkrlegelsaskelydnllkektkandeilrlleendkfnevnkqkddllkrveqmqskletslqllgekteqveelendvsdlkemmhqqvqqmvemqgk	100%	t-SNARE
YKR030W	*GMH1*	90	sfliillclyfiqflllpiinlqnwislligns1ycfaighyfiltfygynqlpflknlnfillptlglsiiylislfgidlskklsfyn	0%	
YLL021W	*SPA2*	102	dkvkeltdlnsdlhlqiedlnaklasltsekekekkeekeekekeknlkinytidesfqkellslnsqigelsienenlkqkisefelhqkkndnhndlkit	100%	
		76	dekhysddddssyqfvpmkheeqeqeqnrseeeeseddde eeedsdfdvdtfdienpdntlselllylehqtmdvi	17%	
YLR045C	*STU2*	51	erqsllekmnntenykiemikenemlreqlkeaqsklnekniqlrskeidv	100 %	tRNA binding
YLR273C		68	hgkklkpslklaktistssfvssttsnsfspledstsassstsssssgksvrfaahlytvkkfntkla	75%	
YLR314C	*CDC3*	78	skqleektlheaklakleiemktvfqqkvsekekklqksetelfarhkemkekltkqlkaledkkkqlelsinsaspn	100%	
YML104C	*MDM1*	51	qiekelellrhlilkadltnnqmqlkilkksqrtllkelemkellkqqymv	100%	
YMR124W	(none)		qlnlitenkelmneltlvstelaesikreteleerirlyetnnsap		
YMR213W	*CEF1*	49	ekemlaeararllntqgkkatrkirermleeskriaelqkrrelkqagi	100%	
YNL091W	*NST1*	273	phhhyhststhsedelseeeyisdielphdphkhfhrdddildgdedepeeedenegddeedtydsgldetdrleegrkliqiaitkllqsrimasyhekqadnnrlkllqeleeekrkkrekeekkqkkrekekekkrlqqlakeeekrkreeekerlkkeleeremrrreaqrkkveeakrkkdeerkrrleeqqrreemqekqrkqkeelkrkreeekkrireqkrleqeklqkekeeeerqrliaedalrkqklneeqtsanilsakpf	100%	
YNL027W	*CRZ1*	75	lsdidrsfediingrklklkksrrrssqtsnnsftsrrssrsrsispdekaksisanreklemadllpssendn	69%	
YOR171C	*LCB4*	59	mfnkhgqlrsgdslsllsclsclddgtlssdggsfdeddslellplnttipfnrilnak	49%	
YPR018W	*RLF2*	77	dpfstkgtgfnydydsdvewvneeeegevdnlesgeeeeeeeddedvpsegefdgfldseensdldglpcakrkfvg	64%	poly(E)
YPR104C	*FHL1*	46	npvtddngnlklelpdnldnadfskllefdakndealfnsnellsh	0%	
		50	knpqhlnlilaaavnaatakvtkgevkqlvnpettaaaalaakaqhskpi	100%	poly(A)

[a]Proportion of residues predicted to be structurally disordered using DISORDER2 (Ward et al. 2004) at 5% error rate.

Figure 1. Identification of low-complexity islands
(A)



(B)

```
S.cer   MRLSNLIASASLLSAATLAAPANHEHKDKRAVVTTTVQKQTTIIVNGAASTPVAALEENA
S.par   ...............TA.......G.......I................P.Q........

S.cer   VVNSAPAAATSTTSSAASVATAAASSSENNSQVSAAASPASSSAATSTQSSSSSQASSSS
S.par   ..............A..P...T...T.DVK...............A..L...........

S.cer   SSG-EDVSSFASGVRGITYTPYESSGACKSASEVASDLAQLTDFPVIRLYGTDCNQVENV
S.par   ..SS........................................................

S.cer   FKAKASNQKVFLGIYYVDQIQDGVNTIKSAVESYGSWDDVTTVSIGNELVNGNQATPSQV
S.par   .............V..........D...................................

S.cer   GQYIDSGRSALKAAGYTGPVVSVDTFIAVINNPELCDYSDYMAVNAHAYFDKNTVAQDSG
S.par   ............................................................

S.cer   KWLLEQIQRVWTACDGKKNVVITESGWPSKGETYGVAVPSKENQKDAVSAITSSCGADTF
S.par   ....D.......................................................

S.cer   LFTAFNDYWKADGAYGVEKYWGILSNE
S.par   ...........................
```

Figure 2. Protein sequence divergence between orthologous ORF pairs

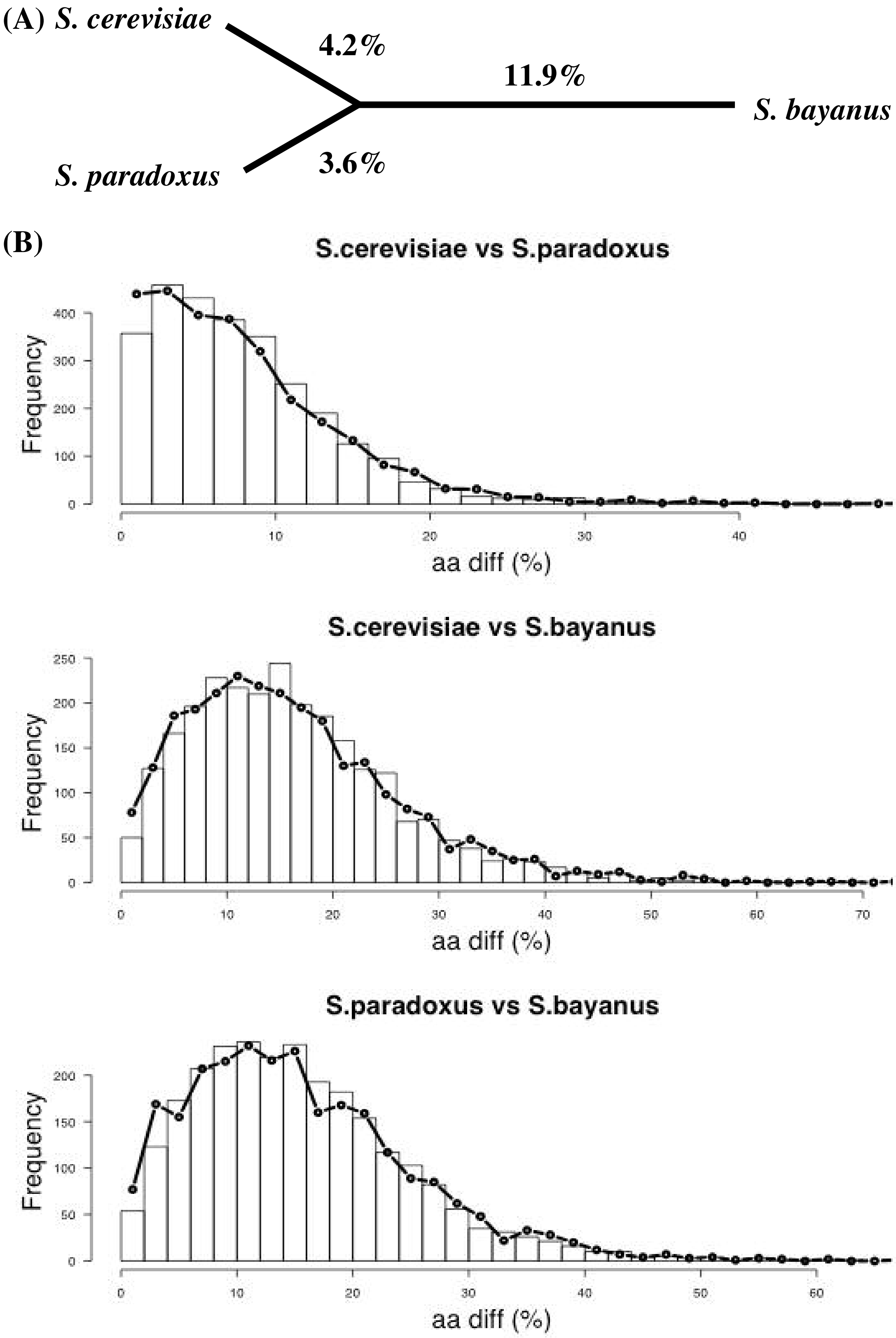
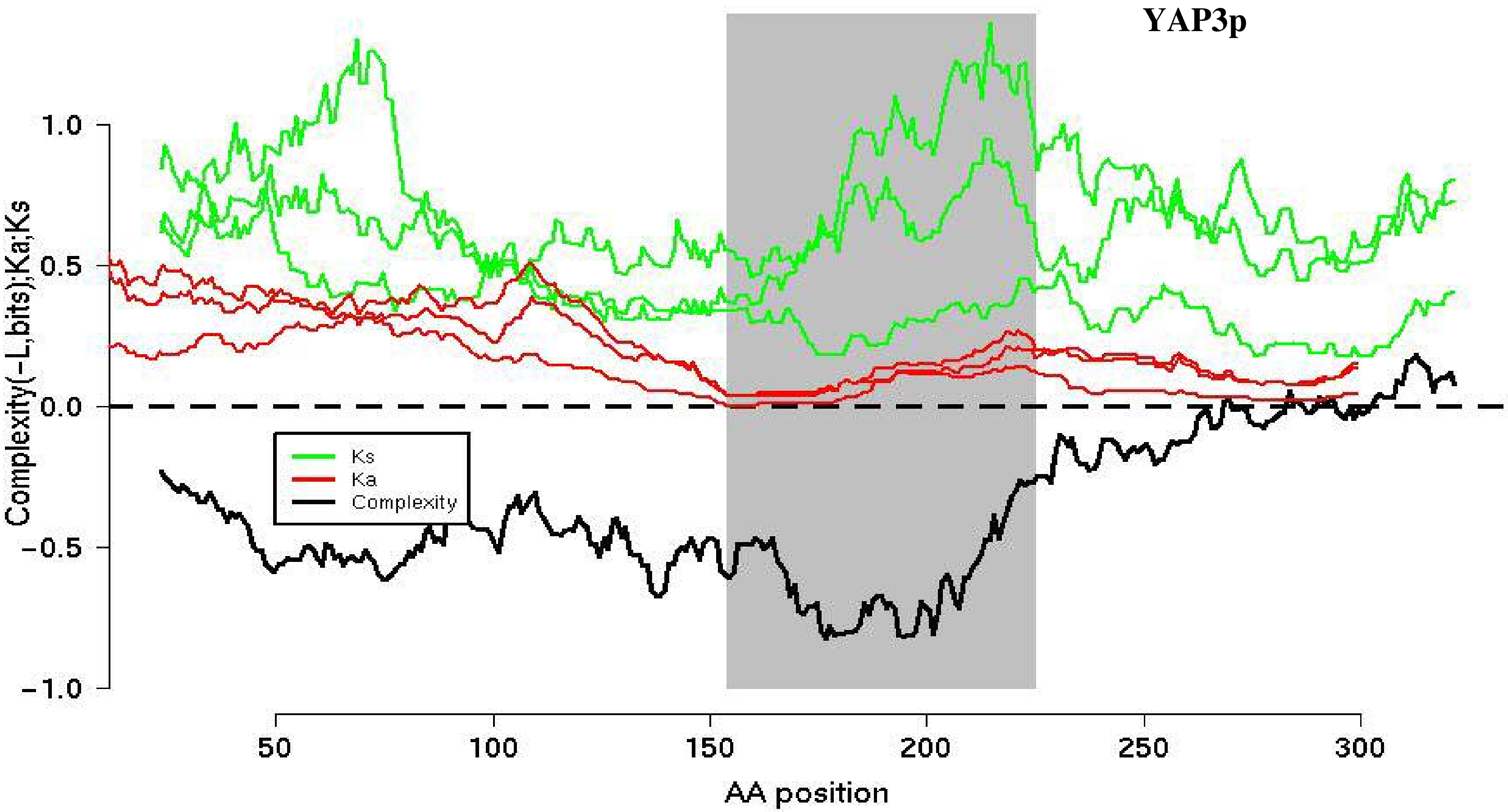


Figure 3. Identification of conserved low-complexity segments
(A)



(B)

```
Scer   MTPSNMDDNTSGFMKFINPQCQEEDCCIRNSLFQEDSKCIKQQPDLLSEQTAPFPILEDQCPALNLDRSNNDLLLQNNISFPKGSDLQAIQL
Spar   .P..DV.N..N..I...........F.PE.....N.NE.....R.I.N.........N....T.D.SG..D..M...E.PLSESTNF.TV..
Sbay   .DT.PS.A.--..IT..DS.Q.GQNH.NES.I..DNNQ.AEE.QN..ND..SS....SG...V...NE.G...MFHSG.PL..ANT...S..

                                                               Basic motif for DNA binding Leucine Zipper
Scer   TPISGDYSTYVMADNNNNDNDSYSNTNYFSKNNGISPSSRSPSVAHNENVPDDSKAKKKAQNRAAQKAFRERKEARMKELQDKLLESERNRQSLLKEIEEL
Spar   ..S...C.SFA.....K....N.TD..C...KKD......T...P...DA.E.................................................
Sbay   ..S...CASF-.DE.K..YSNNNKDIS...EKKDS.SG..T.P.YS-DDLSG........................L...E.......K............

Scer   RKANTEINAENRLLLRSGNENFS---KDIEDDTNYKYSFPTKDEFFTSMVLESKLNHKGKYSLKDNE-IMKRNTQYTDEAGRHVLTVPATWEYLYKLSEER
Spar   ..V.................K.P...R.LI...DH.................G...N..M.......V...Q..........Q................D.
Sbay   ................N.S.KATKSRR.LI..S.S..........S...I..G..DD.SA..P....PV..H.A.....S..................NN.

Scer   DFDVTYVMSKLQGQECCHTHGPAYPRSLIDFLVEEATLNE
Spar   ..........................N.....A...A...
Sbay   .........E.....R..S.......T...S.....VSK.
```