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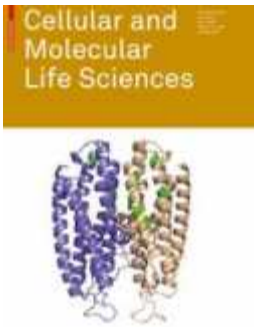

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MEGASATELLITES: A NEW CLASS OF LARGE TANDEM REPEATS DISCOVERED IN THE PATHOGENIC YEAST *CANDIDA GLABRATA*

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Abstract

Megasatellites are DNA tandem arrays made of large motifs, that were discovered in the yeast *Candida glabrata*. They are widespread in this species (40 copies) but are not found in any other hemiascomycete so far, raising the intriguing question of their very origin. They are found mainly in genes encoding cell wall products, suggesting that megasatellites were selected for a function linked to cell-cell adhesion or to pathogenicity. Their putative role in promoting genome rearrangements by interfering with DNA replication will also be discussed.

Megasatellites are a new class of DNA tandem repeats

In addition to its profund impact on evolutionary genomics or on our understanding of complex genetic networks, the systematic sequencing of whole eukaryotic genomes led to the discovery of new genetic elements. One of these discoveries was recently made in the genome of the opportunistic pathogen *Candida glabrata*. *C. glabrata* is a hemiascomycetous yeast, often involved in human candidiasis and bloodstream infections, particularly in immunocompromised patients [1,2]. *C. glabrata* is more resistant to fluconazole treatments than other pathogenic yeasts [3], and has become the second major causative agent of nosocomial infections due to yeast species. The *C. glabrata* genome of the reference strain (CBS138) was completely sequenced [4], and revealed that it was phylogenetically closer to *Saccharomyces cerevisiae* than to the other extensively studied pathogen *Candida albicans* [5]. We recently investigated the genome of *C. glabrata*, searching for minisatellites, a family of tandem DNA repeats whose motif size ranges from 9 nucleotides to usually less than 100 base pairs (reviewed in [6]). Besides the presence of numerous minisatellites, the *C. glabrata* genome also contains tandem repeats whose motif size is much longer, ranging from 135 to 417 nucleotides. We called this new family of large tandem repeats, megasatellites [7]. They

harbor two remarkable features: they are not found in any other sequenced living species besides *C. glabrata* and *Kluyveromyces delphensis* (two *Saccharomycetaceae* yeasts of the same clade [8]), and they are mainly found in genes proven, or suspected, to encode cell wall proteins, raising the possibility that megasatellites could be directly involved in regulating cell adhesion and pathogenicity. Altogether, 40 megasatellites were found in 33 genes in *C. glabrata* and classified in two large families, called SFFIT and SHITT, following the conservation of these five amino acids in each motif of the tandem repeat. Among these 40 megasatellites, 14 contain a SFFIT motif and 20 contain a SHITT motif, the number of motifs in each tandem array ranging from 3 to 32 and covering from 405 to 9,600 DNA base pairs (Figure 1). The remaining six motifs do not show obvious similarity with SFFIT and SHITT families. Megasatellites are distributed on each of the 13 chromosomes but show some preferential bias toward the subtelomeric regions, the right end of chromosome IX carrying seven such elements within 65 kb, a density significantly higher than the genome average (one megasatellite per 224 kb). Subtelomeric regions are highly flexible in *Saccharomyces cerevisiae*, exhibiting a high level of inter-chromatid and inter-chromosome recombination [9]. It is possible that *C. glabrata* subtelomeres share similar properties, and that subtelomeric megasatellites recombine with each other, although this remains to be demonstrated.

Possible involvement of megasatellites in pathogenicity

In *S. cerevisiae*, several genes involved in cell wall biogenesis contain minisatellites [10-12]. Some of them, called the *FLO* genes, play a direct role in flocculation and cellular adhesion. It was shown that cell adhesion and flocculation is directly correlated to the length of the minisatellite in *FLO1* [12], and that cell-cell adhesion leading to the formation of a biofilm at the surface of sherry wine was directly dependent on the size of a minisatellite in *FLO11* [13].

In *C. glabrata* CBS138 strain, three *EPA* genes (functional homologues of the *FLO* genes, [14-17]) contain megasatellites (*EPA2*, *EPA11* and *EPA13*), but three other *EPA* genes contain simple minisatellites (*EPA1*, *EPA3* and *EPA15*), and three do not contain any kind of tandem repeat (*EPA6*, *EPA7* and *EPA8*). In addition, 30 other genes that are not part of the *EPA* family contain megasatellites. Some of the proteins encoded by these genes exhibit signatures of cell-wall proteins but experimental evidence of their function or their localization are lacking. Interestingly, in *S. cerevisiae*, three *FLO* genes (*FLO1*, *FLO5* and *FLO9*) contain a 135-bp motif, tandemly repeated 7 to 13 times [11]. This Threonine-rich motif shares no obvious similarity with any of the *C. glabrata* megasatellites. However, it has the same size as the SHITT motif. It is therefore possible that 45 amino acids (135 bp) is the optimal size for a tandem repeat in these cell-wall embedded proteins, and that the motif size is therefore under strong selection, whereas the sequence itself is not necessarily conserved.

In budding yeast, telomeric regions are silenced by a multiprotein complex containing the *SIR* genes, *RAP1*, *ESC1* and the Ku complex [18]. These genes are conserved in *C. glabrata*, to the exception of *SIR1*, which is involved in silencing the silent mating-type loci, but not in telomeric silencing [19]. The inactivation of *SIR3* and *RAP1* was shown to increase the level of expression of several *EPA* genes, including the megasatellite-containing *EPA2* gene [15], suggesting that the mechanism of subtelomeric silencing is probably similar in *C. glabrata* and in *S. cerevisiae*. Subtelomeric megasatellite-containing genes are therefore probably also silenced, although this remains to be shown. Therefore, at the present time, the possible role played by megasatellites in *C. glabrata* pathogenicity is unclear, and needs to be clarified in the future.

Megasatellites and genome rearrangements

Given the repeated nature of the large arrays formed by the megasatellites, one may wonder if they could behave like fragile sites and thus induce genome rearrangements. In humans, fragile sites are defined as chromatid constrictions or breaks visible on metaphasic chromosomes, when cells are grown in the presence of drugs that impair replication or DNA metabolism [6,20-22]. Although the precise molecular nature of all fragile sites is not known, some of them have been sequenced. *FRA3B* contains numerous transposons and LTRs found in direct and inverted orientations, *FRA10B* and *FRA16D* contain AT-rich minisatellites (42-bp and 33-bp motif size, respectively), *FRAXA*, *FRAXE*, *FRAXF*, *FRA11B* and *FRA16A* contain CGG trinucleotide repeats. Some of these fragile sites are associated to cancer. *FRA3B*, the most common fragile site in humans, often contains deletions in several gastrointestinal, colon, lung, breast and cervical cancers [23]. Loss of heterozygosity and a recurrent translocation were also observed at *FRA16D* in breast and prostate cancers and multiple myelomas [24]. Interestingly, chromosomal translocations and chromosome losses in *Candida albicans* are often associated to a large DNA tandem repeat, called the Major Repeat Sequence (MRS). It is a complex tandem repeat, found at nine different locations in the genome, and composed of a 2-kb motif tandemly repeated (RPS), itself including several tandem copies of smaller motifs (16 bp and 29 bp long). Most chromosome length polymorphisms in this yeast are due to size heterogeneity of the MRS [25]. When Muller and colleagues analyzed chromosomal translocations among different strains of *C. glabrata*, three major rearrangements, involving chromosomes IV, IX, XII and XIII, were found [26]. The three breakpoints corresponding to these three rearrangements were mapped and sequenced, but they are not located at the proximity of megasatellites, neither do they encompass any kind of repeated element. However, in the same study, it was shown that among 12 deletions, ranging in size from 130 bp to 12 kb, detected in the *C. glabrata* genome, two were located

within two megasatellites (one of them being the longest megasatellite of the genome). The probability of this happening by chance being low, it suggests that megasatellites might be involved in the mechanism leading to these two deletions. In a more recent study using a larger number of probes, 11 reciprocal and non-reciprocal translocations involving 11 out of the 13 *C. glabrata* chromosomes were found [27]. They also detected five segmental duplications leading to the formation of new chromosomes. In one of these, a SFFIT megasatellite is located less than 10 kb from a breakpoint, and could be involved in the rearrangement, although this was not formally proven. In conclusion, observations made on chromosomal plasticity in *C. glabrata* suggest that some of the rearrangements observed might be triggered by the presence of a megasatellite. However, the majority of megasatellites are not associated to chromosomal rearrangements and frequent rearrangements are observed far from any megasatellite, showing that megasatellites are not systematically involved in rearrangements. Large-scale studies of replication and recombination in *C. glabrata* are now needed to understand the precise role of megasatellites in chromosomal replication and instability.

Evolution of megasatellites

The last intriguing question concerning megasatellites relates to their mechanism(s) of formation. One simple way to propagate megasatellites is to duplicate the gene(s) that contain them. In *C. glabrata*, several megasatellites are found in paralogous gene families. The largest of these families encompasses 11 paralogues, each containing a SHITT megasatellite and five of them also containing a SFFIT repeat array (Rolland, Dujon and Richard, unpublished). However, six megasatellites, containing either SHITT or SFFIT motifs, are found in genes that are present in unique copies in the genome, raising the question of their very origin. If point mutations followed by replication slippage may explain how smaller tandem repeats,

like microsatellites, are born, it is hard to imagine the same mechanism responsible for the *de novo* creation of larger tandem arrays. Haber and Louis proposed that minisatellites are formed by replication slippage between two short (5 bp) sequences flanking a 10-20 nt unique sequence [28]. If most of the *S. cerevisiae* minisatellites are actually flanked by such short motifs [11], it does not seem to be the case for *C. glabrata* megasatellites.

In silico comparisons of megasatellites with each other show that some motifs found in a given gene are actually phylogenetically closer to motifs found in another gene, suggesting that some kind of genetic transfer exists between megasatellites (Rolland, Dujon and Richard, unpublished). This transfer may involve gene conversion, or alternatively one may imagine that SHITT and SFFIT motifs are able to "jump" from one megasatellite to another one, using mechanisms that may be related to transposition or retrotransposition (Figure 2). There is only one full-size retrotransposon in the *C. glabrata* genome, a gypsy-like element (Tcg3, gene name CAGL0G07183g, The Génolevures Consortium[1]), two degenerate copies and two solo LTRs (Cécile Neuvéglise, personal communication). Unless there is another source of reverse transcriptase in this genome, it is difficult to hypothesize that retrotransposition is involved in the spreading of megasatellites. In conclusion, the question of the origin of megasatellites is still completely open, but experiments designed specifically to answer this question using molecular tools available in this yeast species, should give some answers, and maybe explain why SHITT and SFFIT megasatellites are so widespread in *C. glabrata*.

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[1] http://www.genolevures.org/

[1] Bodey, G.P. et al. (2002) The epidemiology of Candida glabrata and Candida albicans fungemia in immunocompromised patients with cancer Am J Med 112, 380-5.

[2] Raad, I. et al. (2004) Management of central venous catheters in patients with cancer and candidemia Clin Infect Dis 38, 1119-27.

[3] Pfaller, M.A. and Diekema, D.J. (2004) Twelve years of fluconazole in clinical practice: global trends in species distribution and fluconazole susceptibility of bloodstream isolates Clinical Microbiology and Infection 10, 11-23.

[4] Dujon, B. et al. (2004) Genome evolution in yeasts Nature 430, 35-44.

[5] Dujon, B. (2006) Yeasts illustrate the molecular mechanisms of eukaryotic genome evolution Trends in Genetics 22, 375-387.

[6] Richard, G.F., Kerrest, A. and Dujon, B. (2008) Comparative genomics and molecular dynamics of DNA repeats in eukaryotes Microbiol Mol Biol Rev *72*, 686-727.

[7] Thierry, A., Bouchier, C., Dujon, B. and Richard, G.-F. (2008) Megasatellites: a peculiar class of giant minisatellites in genes involved in cell adhesion and pathogenicity in *Candida glabrata* Nucl. Acids. Res. 36, 5970-5982.

[8] Kurtzman, C.P. (2003) Phylogenetic circumscription of Saccharomyces, Kluyveromyces and other members of the Saccharomycetaceae, and the proposal of the new genera Lachancea, Nakaseomyces, Naumovia, Vanderwaltozyma and Zygotorulaspora FEMS Yeast Res 4, 233-45.

[9] Louis, E.J., Naumova, E.S., Lee, A., Naumov, G. and Haber, J.E. (1994) The chromosome end in yeast: its mosaic nature and influence on recombinational dynamics Genetics 136, 789-802.

[10] Bowen, S., Roberts, C. and Wheals, A.E. (2005) Patterns of polymorphism and divergence in stress-related yest proteins Yeast 22, 659-668.

[11] Richard, G.-F. and Dujon, B. (2006) Molecular evolution of minisatellites in hemiascomycetous yeasts Mol Biol Evol 23, 189-202.

[12] Verstrepen, K.J., Jansen, A., Lewitter, F. and Fink, G.R. (2005) Intragenic tandem repeats generate functional variability Nature Genetics 37, 986-990.

[13] Fidalgo, M., Barrales, R.R., Ibeas, J.I. and Jimenez, J. (2006) Adaptive evolution by mutations in the FLO11 gene Proc Natl Acad Sci U S A 103, 11228-11233.

[14] Castano, I., Pan, S.-J., Zupancic, M., Hennequin, C., Dujon, B. and Cormack, B.P. (2005) Telomere length control and transcriptional regulation of subtelomeric adhesins in Candida glabrata Molecular Microbiology 55, 1246-1258.

[15] De Las Penas, A., Pan, S.J., Castano, I., Alder, J., Cregg, R. and Cormack, B.P. (2003) Virulence-related surface glycoproteins in the yeast pathogen Candida glabrata are encoded in subtelomeric clusters and subject to RAP1- and SIR-dependent transcriptional silencing Genes Dev 17, 2245-58.

[16] Frieman, M.B., McCaffery, J.M. and Cormack, B.P. (2002) Modular domain structure in the *Candida glabrata* adhesin Epa1p, a β1,6 glucan-cross-linked cell wall protein Molecular Microbiology 46, 479-492.

[17] Zupancic, M., Frieman, M.B., Smith, D., Alvarez, R.A., Cummings, R.D. and Cormack, B.P. (2008) Glycan microarray analysis of *Candida glabrata* adhesin ligand specificity Molecular Microbiology 68, 547-559.

[18] Taddei, A., Hediger, F. and Gasser, S.M. (2004) The function of nuclear architecture: a genetic approach Annual Review of Genetics 38, 305-345.

[19] Fabre, E., Muller, H., Therizols, P., Lafontaine, I., Dujon, B. and Fairhead, C. (2005) Comparative genomics in hemiascomycete yeasts: evolution of sex, silencing, and subtelomeres Mol Biol Evol 22, 856-873.

[20] Debacker, K. and Kooy, R.F. (2007) Fragile sites and human disease Hum Mol Genet 16 Spec No. 2, R150-8.

[21] Glover, T.W., Arlt, M.F., Casper, A.M. and Durkin, S.G. (2005) Mechanisms of common fragile site instability Hum Mol Genet 14 Spec No. 2, R197-205.

[22] Sutherland, G.R., Baker, E. and Richards, R.I. (1998) Fragile sites still breaking TIG 14, 501-506.

[23] Durkin, S.G., Ragland, R.L., Arlt, M.F., Mulle, J.G., Warren, S.T. and Glover, T.W. (2008) Replication stress induces tumor-like microdeletions in FHIT/FRA3B Proc Natl Acad Sci U S A 105, 246-51.

[24] Popescu, N.C. (2003) Genetic alterations in cancer as a result of breakage at fragile sites Cancer Letters 192, 1-17.

[25] Magee, P.T. (2007) in: Candida comparative and functional genomics, pp. 7-26 (d'Enfert, C. and Hube, B., Eds.) Caister Academic Press.

[26] Muller, H. et al. (2008) Genomic polymorphism in the population of *Candida glabrata*: gene copy-number variation and chromosomal translocations Fungal Genetics and Biology In press.

[27] Polakova, S., Blume, C., Zarate, J.A., Mentel, M., Jorck-Ramberg, D., Stenderup, J. and Piskur, J. (2009) Formation of new chromosomes as a virulence mechanism in yeast Candida glabrata Proc Natl Acad Sci U S A 106, 2688-93.

[28] Haber, J.E. and Louis, E.J. (1998) Minisatellite origins in yeast and humans Genomics 48, 132-135.

Figure legends

Figure 1: Schematic representation of *C. glabrata* SHITT and SFFIT megasatellites.

Gene names are indicated to the left (http://www.genolevures.org). Vertical lines near gene names indicate paralogous gene families. The region of the gene containing the megasatellite is represented by colored boxes. Orange: SHITT motifs. Green: SFFIT motifs. The number in boxes corresponds to the number of tandemly repeated motifs (no number indicates the presence of only one motif). Purple: degenerate SFFIT motifs. The degenerate SFFIT motifs found within CAGL0J11924g and CAGL0J05170g are not identical, although they both probably come from a SFFIT motif. Light grey: sequence of variable length, that is sometimes tandemly repeated and found interspersed within some of the SHITT and SFFIT megasatellites. Dark grey: Glycine- and Serine-rich motifs, of variable length, found only in CAGL0L00227g and CAGL0J01774g. Their size (in amino-acids) is indicated when too long to be drawn to scale. Otherwise, boxes are drawn to scale. Numbers shown before and after boxes represent the number of amino-acids before and after the repeated motifs. Given the lower sequence coverage and unprecise assembly of subtelomeric regions, it is possible that the number of motifs shown for the 11 subtelomeric megasatellite-containing genes is different from what is represented here (11 genes, from CAGL0A04873g to CAGL0L00227g). (1) The subtelomeric sequence is interrupted within a megasatellite.

Figure 2: Different mechanisms can lead to megasatellite spreading in the *C. glabrata* genome.

Left: gene A with no megasatellite may acquire a motif by retrotransposition or another mechanism, followed by expansion of the motif into a megasatellite. Right: a gene already containing a megasatellite may duplicate itself, leading to the formation of two paralogues, each of them containing an identical megasatellite. Contractions and expansions may now

occur, independently in both tandem repeats, and point mutations may accumulate in one or several motifs, leading to slightly different motif sequences (in green). These new motifs may propagate (or disappear) by intergenic (or intragenic) gene conversion. New motifs may also propagate by "jumping" into a megasatellite encoded by a non-paralogous gene (bottom).

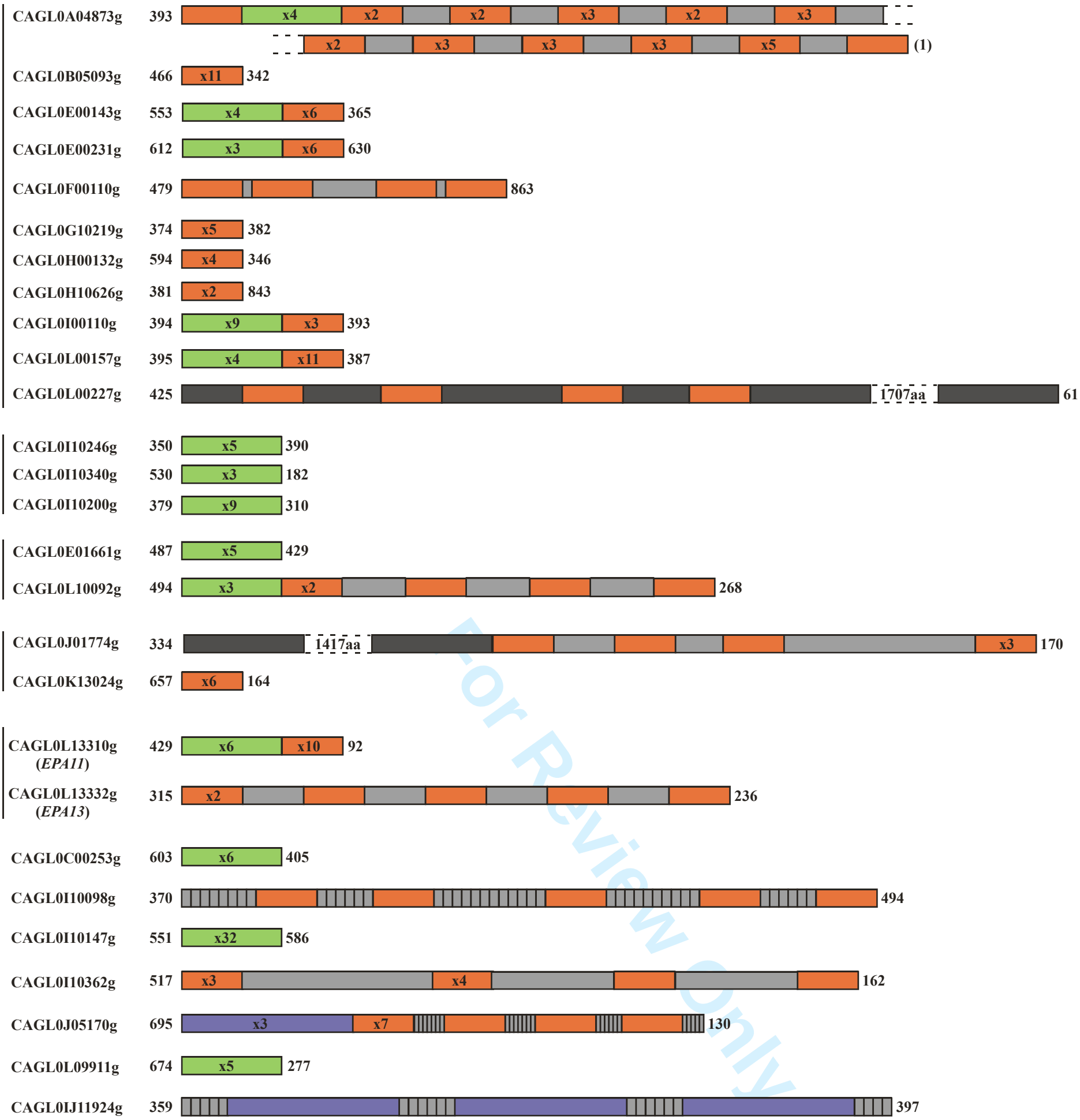

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Figure 1

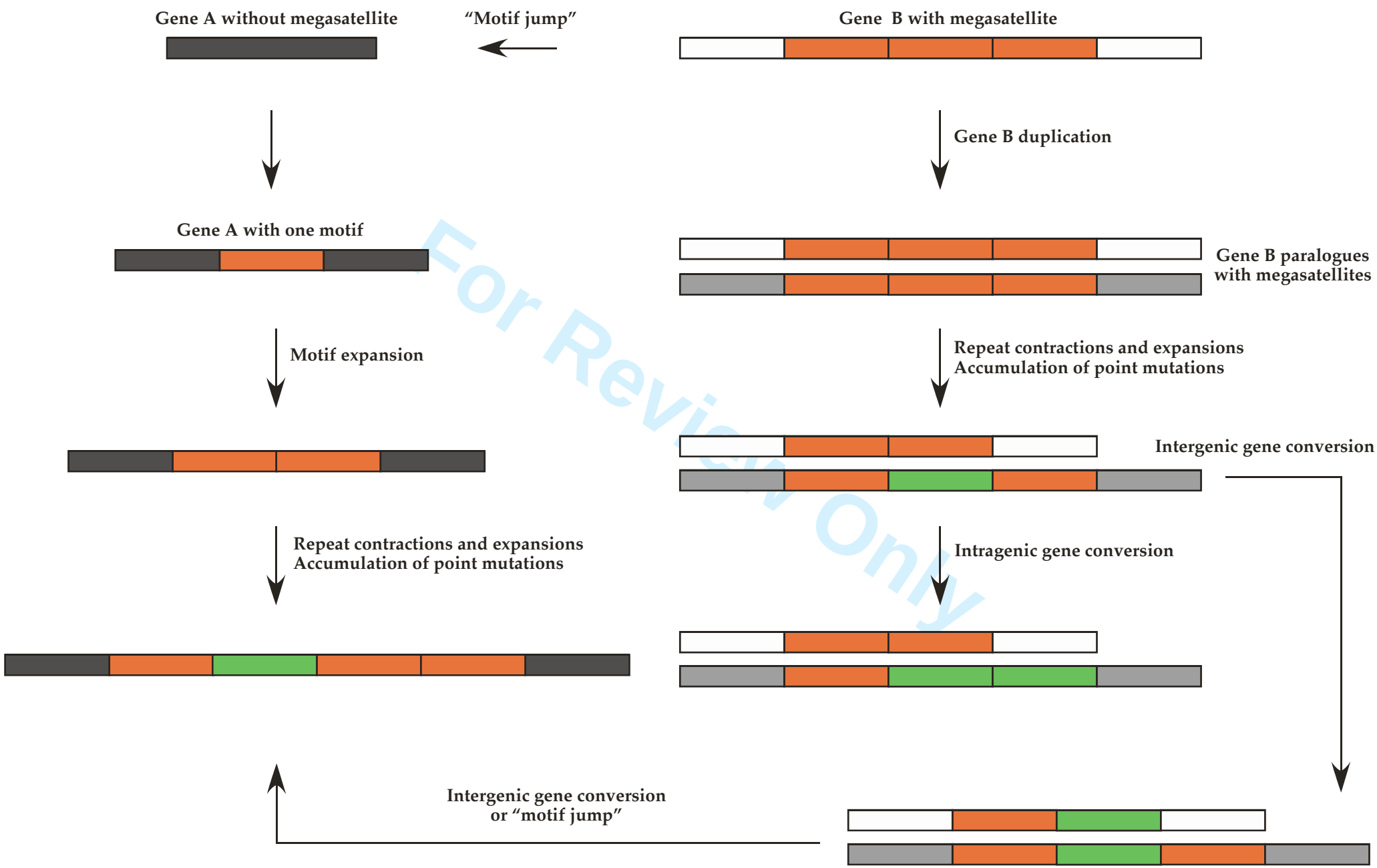

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Figure 2