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Human adaptation and evolution by segmental duplication

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Duplications are the primary force by which new gene functions arise and provide a substrate for large-scale structural variation. Analysis of thousands of genomes shows that humans and great apes have more genetic differences in content and structure over recent segmental duplications than any other euchromatic region. Novel human-specific duplicated genes, *ARHGAP11B* and *SRGAP2C*, have recently been described with a potential role in neocortical expansion and increased neuronal spine density. Large segmental duplications and the structural variants they promote are also frequently stratified between human populations with a subset being subjected to positive selection. The impact of recent duplications on human evolution and adaptation is only beginning to be realized as new technologies enhance their discovery and accurate genotyping.

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Introduction

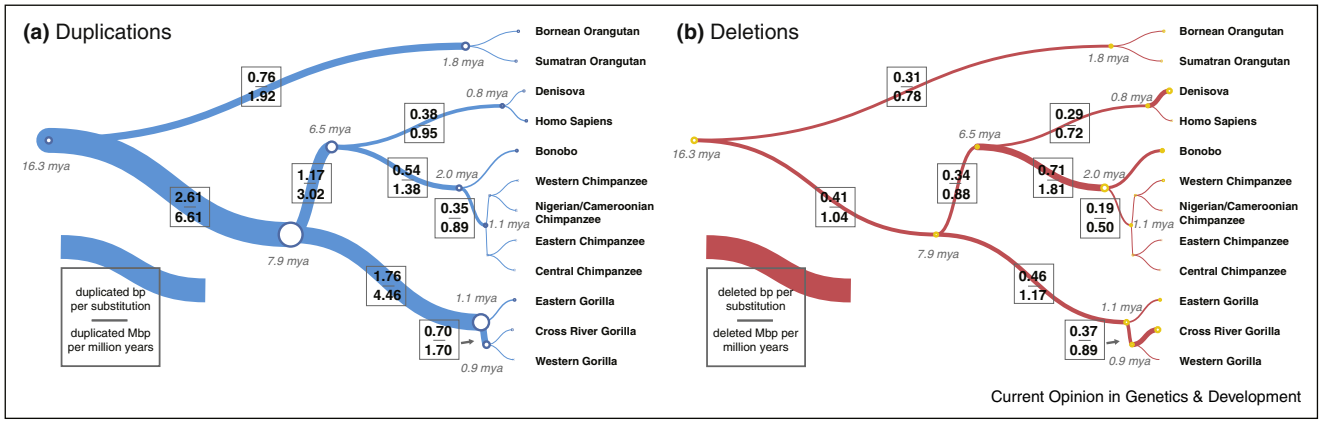
In this review, we will summarize recent advances in our understanding of primate segmental duplications (SDs; defined as large tracts of sequence (>1 kbp) sharing >90% identity [1]) and their impact in contributing to new genes and functions within the human lineage. Mutation by duplication has two, very different, consequences on an evolving genome [2,3]. First, it creates genetic redundancy liberating functional DNA from ancestral selective constraint. This can lead to the birth of novel genes and regulatory elements either instantaneously through duplicative transpositions or by subsequent mutational tinkering of the paralogous copy [4]. Second, by dint of its high sequence identity, duplication provides the substrate for subsequent rearrangement through the process of non-allelic homologous recombination [5••]. This mutational process is dynamic because the presence of SDs further increases the probability of subsequent rounds of duplications as a result of larger and more abundant tracts of identical sequences [6–8]. Duplications of genic material thus have the potential to radically change structure and content over extremely short periods of times. Here, we focus on gene innovation by duplication and emerging data regarding its importance to human adaptation.

Nonrandom evolution of great ape segmental duplication

The accumulation of SDs in the human–great ape lineage has been nonrandom in both time and space [9]. Comparative and sequence-identity analyses support a threefold excess of duplications in the common ancestor of human and great apes lineage (7–16 mya) in contrast to deletions, which have occurred in a more clocklike manner during evolution (Figure 1) [10]. The intrachromosomal burst and to a lesser extent interchromosomal duplications have been associated with particular segments identified as core duplicons [11,12] (Table 1). Using a repeat-graph approach, 24 core duplicons (~15 kbp) were originally identified as segments overrepresented in 437 duplication blocks within the human genome. Subsequent phylogenetic analyses revealed that the cores represent the focal point for the serial accumulation of SDs, resulting in increasingly larger duplication blocks (>250 kbp in size) composed of mosaic duplicated segments where evolutionarily younger duplicated segments are located at increasing distances from the core.

There is evidence that the cores have been reused in different great ape lineages [13] and are preferential sites of rearrangements leading to large-scale inversion polymorphisms [14,15]. Interestingly, many of these cores are enriched in rapidly evolving human–great ape gene families [16–23]. Though the function of most of these gene families has not yet been determined, biochemical investigations into a few (e.g., *TBC1D3*) have suggested an association with cell proliferation [24]; others (e.g., *DUF1220* or *NBPF*) have been implicated in changes of brain size [21,25•,26•] while some (e.g., *NPIP*) show remarkable signatures of positive selection [19]. At the periphery of these core-mediated duplication blocks lie most of the human-specific segmental duplications (HSDs) [27,28]. HSDs are restricted to specific regions

Figure 1



Primate rates of duplication and deletion. Rates of fixed **(a)** duplications and **(b)** deletions are shown as a function of the number of substitutions along each branch of the great ape phylogeny. Branch widths are scaled proportionally to the number of duplicated base pairs per substituted base pair based on analysis of 97 human/ape genomes. A burst of duplicated base pairs appears to have occurred in the common ancestral branch leading to humans and African great apes, where duplicated base pairs were added at 2.6-fold the rate of substitution. In contrast, the rate of deletion in the great ape lineage is more clocklike along all branches (mean of 0.32 deleted base pairs per substitution) with the exception of the chimpanzee–bonobo ancestral lineage, where an approximate twofold increase in the rate of deletion is observed (0.71 deleted base pairs per substitution). Adapted from [10].

Table 1

Examples of genes/gene families mapped to core duplicons

Clade	Gene	Significant expression	Subcellular localization	Description	Possible function	Example disease-associated genomic hotspot
chr1	*NBPF*	Soft tissue	Cytoplasm	Neuroblastoma breakpoint gene family, DUF1220	Transcription factor regulated by NF-κB	1q21.1: Neuroblastoma, ASD, ID, Schizophrenia
chr2	*RANBP2*	Testis	Nuclear pore	RANBP2-like and GRIP domain- containing 5 isoform	Ran GTPase binding	2q13: ID
chr7_2	*PMS2L5*	Ubiquitous	Nuclear	Postmeiotic segregation increased 2-like 5	DNA mismatch repair	7q11.23: Williams–Beuren syndrome, ASD, ID
chr7_2	*SPDYE1*	Testis	Unknown	speedy/RINGO cell cycle regulator family member E1	Cell cycle regulator	7q11.23: Williams–Beuren syndrome, ASD, ID
chr7_3	*DPY19L2*	Testis	Unknown	dpy-19 like 2	Spermatogenesis	None
chr9_1	*SPATA31A1*	Exclusively in testis	Unknown	SPATA31 subfamily A, member 1	Unknown	None
chr9_2	*ZNF790*	Ubiquitous	Nuclear	Zinc-finger protein 790	DNA binding	None
chr11/chr2	*TRIM51*	Mammary gland	Unknown	Tripartite motif-containing 51	Unknown	2q11.2: ID, ADHD
chr15	*GOLGA*	Exclusively in testis	Unknown	Golgin-like protein, golgi autoantigen, golgin subfamily a	DNA binding	15q13.3: ASD, ID, Schizophrenia, Epilepsy
chr16	*NPIP*	Ubiquitous	Nuclear membrane	Nuclear pore complex interacting protein, morpheus gene family	Unknown	16p11.2: ASD, ID, Schizophrenia, Epilepsy
chr17_1	*LRRC37A*	Ubiquitous	Unknown	Leucine-rich repeat, c114 SLIT-like testicular protein	ATP-dependent peptidase activity	17q21.31: ID
chr17_2	*TBC1D3*	Testis	Cytoplasm	TBC1 domain family member	Cell growth and proliferation	17q12: ASD, ID, Schizophrenia
M1	*OR7E*	Unknown	Unknown	Olfactory receptor 7E pseudogenes	Receptors mediating sense of smell	8p23.1: ID
M5	*SMA*	Spinal cord	Lysosome	Spinal muscular atrophy associated gene	Hydrolase activity	5q13.2: Spinal Muscular Atrophy
M6	*CCDC127*	Ubiquitous	Unknown	Coiled-coiled domain containing 27	Unknown	None

ASD: autism spectrum disorder; ID: intellectual disability and associated developmental delay; ADHD: attention-deficit and hyperactivity disorder. Adapted from [11].

of the genome and appear to be enriched in genes associated with brain development and neuronal apoptosis. Approximately 25 of these regions are now associated with recurrent chromosomal rearrangements and neurodevelopmental disease [29•,30,31] suggesting that there has been a negative fitness effect with the propagation of these elements during evolution.

Segmental duplication and the evolution of a larger human brain

Over the last four years, a few interesting examples have highlighted the potential functional impact of SDs with respect to the evolution of the human brain. Of the three recently described examples, two — *SRGAP2C* and *ARHGAP11B* — are the direct consequence of HSDs [32,33,34••,35•]. It is noteworthy that the third, a recently described human-accelerated regulatory region (HARE5) of Wnt receptor *FZD8* (frizzled-8) [36•], is part of an 18 kbp primate-specific SD on chromosome 10 that likely arose after divergence of Old and New World primates, although the duplication of the locus occurred long before the burst of functional single-base-pair substitutions on the human lineage.

SRGAP2C

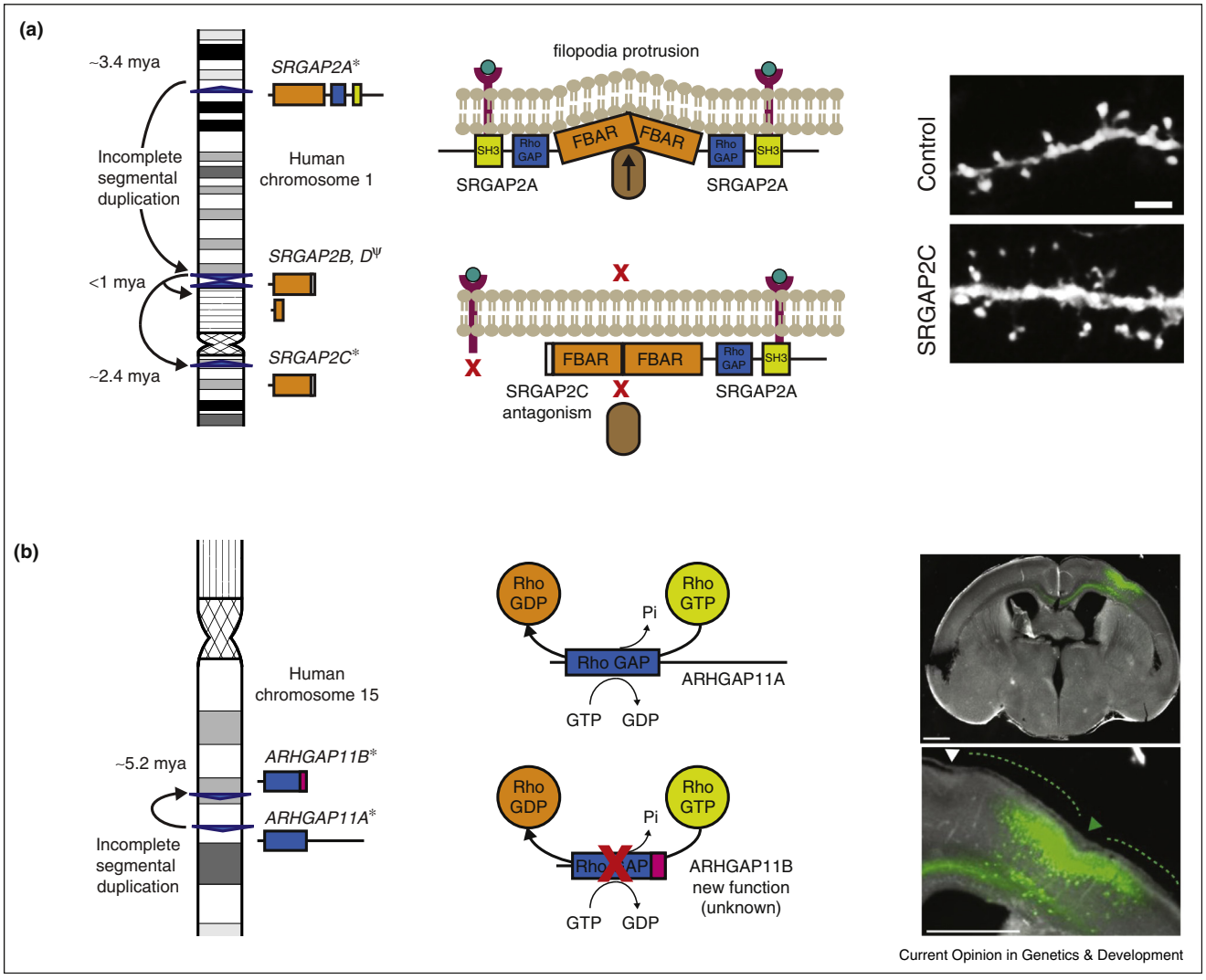
The cortical developmental gene *SRGAP2* (Slit-Robo Rho GTPase-activating protein 2) has duplicated three separate times uniquely in the human lineage with its paralogs dispersing across 85 Mbp on chromosome 1 (Figure 2a) [33]. Ancestral *SRGAP2A* is capable of homodimerizing via an FBAR domain at the cell surface where it induces membrane protrusions required for neuronal migration and morphogenesis in mammals [37]. Evolutionary reconstructions showed that a 258 kbp SD transposed from chromosome 1q32.1 to 1q21.1 ~3.4 million years ago (mya) where it spawned a truncated *SRGAP2B*, including the first 9 of 22 exons and putative regulatory sequences of the ancestral copy. Larger secondary duplications of *SRGAP2B* created an additional truncated 'granddaughter' paralog *SRGAP2C* (at chromosome 1p12 ~2.4 mya). Despite it being one of the most recent paralogs, *SRGAP2C* is fixed for copy number in all modern humans — unlike its predecessor *SRGAP2B*. This truncated paralog is co-expressed with the full-length ancestral *SRGAP2A* in the germinal layers of human embryonic cortex, where neural progenitors divide to produce postmitotic neurons, and the cortical plate, where neurons undergo terminal differentiation and synaptogenesis [32]. Transient overexpression of *SRGAP2C* in culture and *in vivo* leads to human-specific features, including neoteny of dendritic spine maturation, promotion of longer spines at a greater density, and sustained radial migration in the developing mouse neocortex. Human-specific SRGAP2C, which lacks RhoGAP and SH3 functional domains, dimerizes with the ancestral SRGAP2A via its partial FBAR domain, in effect usurping functional full-length proteins from homodimerizing and assembling at the cell surface. This antagonistic function may have been present at the birth of *SRGAP2C* as a result of the incomplete SD that excluded the RhoGAP and SH3 functional domains. It is intriguing that these *SRGAP2* duplicates arose 2–3 mya, the estimated divergence time of *Australopithecus* to *Homo* and immediately prior to the paleontological estimate of human neocortex expansion [38]. This raises the exciting possibility that the *SRGAP2C* duplication played an important role in neural adaptive changes specific to the hominin lineage.

ARHGAP11B

Another potentially functionally relevant HSD gene is *ARHGAP11B*, the product of an incomplete duplication of *ARHGAP11A* (Rho-type GTPase-activating protein 11A) shortly after the chimpanzee and human lineages diverged ~5.2 mya (Figure 2b) [35•]. It was one of the initiating events that led to the formation of large complex HSDs responsible for mediating recurrent rearrangements contributing to 15q13.3 microdeletion syndrome associated with intellectual disability, epilepsy and schizophrenia [39]. Notably, all events were associated with the chromosome 15 core duplicon containing *GOLGA* [35•]. The truncated *ARHGAP11B* lacks the terminal 756 aa residues of *ARHGAP11A* and instead encodes a modified carboxyl terminus consisting of 47 functionally distinct residues. Unlike the full-length ARHAP11A or a shorter 250 aa isoform, the resulting 267 aa protein does not exhibit RhoGAP activity based on a RhoA/Rho-kinase-based cell transfection assay. *ARHGAP11B* may have evolved a completely novel function [34••].

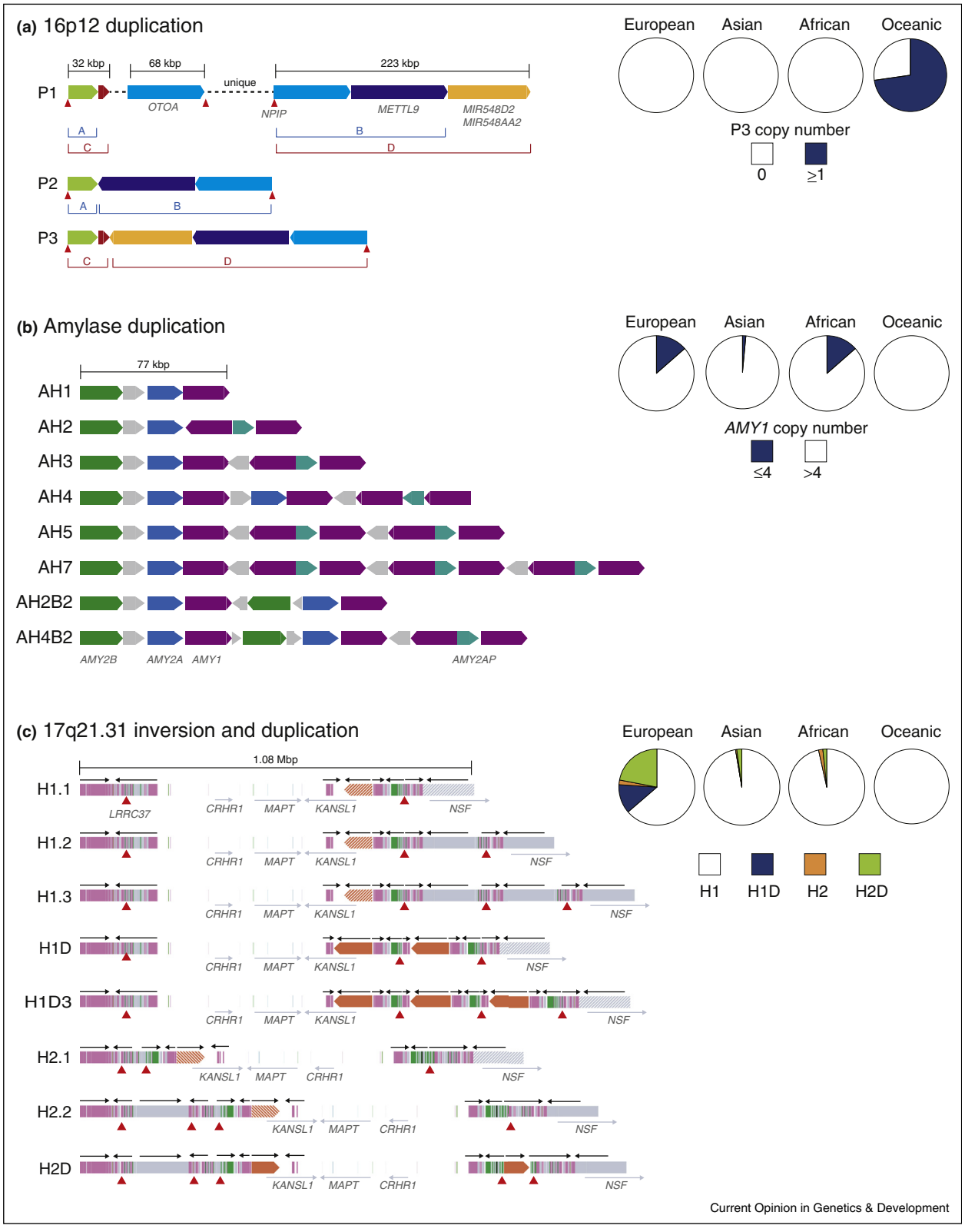
Performing an RNA-seq assay of cortical progenitor subpopulations in human and mouse, Florio and colleagues identified only *ARHGAP11B* when searching for transcripts unique to humans that show >10-fold expression difference between basal radial glial cell populations — isolated from the subventricular zone — compared with neuronal cells [34••]. In utero electroporation and expression of the gene in E13.5 mouse embryos resulted in a ~30% increase in the thickness of the subventricular zone due to greater basal radial glial mitotic divisions. Further, microinjection of *ARHGAP11B* into E13.5 mouse apical radial glial cells showed a subsequent increase in basal radial glial cells where the progeny lost apical contact (delamination). Remarkably, examination of the mouse brain slices at E18.5 demonstrated in half of the cases an increase in the cortical plate area and neocortical folding reminiscent of the gyrification common among primate species. These findings are consistent with the hypothesis that an increased number of radial glial neuron progenitor cells, possibly through increased symmetric cell divisions, would lead to a neuronal expansion in the cortical layers of the developing brain. While potentially exciting, caution should be exercised until transgenic mice are constructed that replicate these findings. There is concern that both

Figure 2



Gene duplication and neuroanatomical adaptations. **(a)** *SRGAP2A* encodes a 1071 aa protein with three protein domains shown as boxes: FBAR (orange), RhoGAP (blue), and SH3 (yellow). An incomplete SD from chromosome 1q32.1 created *SRGAP2B* at 1q21.1 encoding a partial FBAR domain (458 aa) and seven unique residues [33]. A subsequent duplication from *SRGAP2B* created *SRGAP2C* at 1p12.1 and *SRGAP2D* at 1q21.1 (which later partially deleted and represents a pseudogene). SRGAP2A homodimerizes and assembles at the cell membrane surface via its SH3 domain and induces filopodia protrusion by interacting with a protein complex including F-actin (brown oval) [37]. Truncated SRGAP2C is capable of heterodimerizing with SRGAP2A via its FBAR domain but lacks the RhoGAP and SH3 domains in turn antagonizing the function of the ancestral gene by not allowing it to assemble at the cell membrane surface. *SRGAP2C* expression induces long thin spines in mouse-cultured cortical neurons that phenocopies *SRGAP2A* deficiency in mice. Pictured are segments of dendrites from cortical neurons (20DIV) expressing EGFP alone (control) or EGFP and *SRGAP2C* (*SRGAP2C*) imaged two days after transfection. Reprinted with permission from [32]. **(b)** *ARGHAP11A* encodes a 1023 aa protein with a RhoGAP domain (blue). It was partially duplicated at chromosome 15q13.3 resulting in a paralog *ARHGAP11B* encoding a truncated RhoGAP domain (220 aa) and 47 unique residues at the C terminus (pink box) [35•]. Via its RhoGAP domain, ARHGAP11A and a truncated alternative isoform encoding 250 aa (not pictured) show RhoGAP activity, evidenced by dephosphorylation of myosin phosphatase target protein 1, unlike ARHGAP11B, which does not exhibit RhoGAP activity. *ARHGAP11B* overexpression leads to an increase in basal progenitors in the mouse neocortex possibly inducing cortical folding. Pictured are coronal sections of an E18.5 mouse telencephali in utero electroporated at E13.5 with *ARHGAP11B* and GFP expression plasmids. Phase contrast and GFP fluorescence of one section along the rostro-caudal axis. Scale bars, 500 μm. Green and white dashed lines and triangles indicate gyrus- and sulcus-like structures in and adjacent to the electroporated area, respectively. Reprinted with permission from [34••].

Figure 3



Population diversity of duplications in modern human populations. SDs are indicated as colored blocks with directionality shown. Pie charts show frequencies of paralogs/alleles across European (E), Asian (As), African (Af), and Oceanic (O) populations. Core duplicons (red triangles) are depicted below structural paralogs or haplotypes. **(a)** A ~ 255 kbp Oceanic-specific SD (P3) was identified and shown to be made up of disperse

microinjection as well as electroporation may have induced artefacts associated with cell proliferation.

Variation and selection within human populations

SDs show extensive copy number variation in the human species [27,28,40,41•,42•,43,44] and contribute more variant base pairs between any two individuals than any other single source, including single-nucleotide polymorphisms (SNPs), indels and structural variants [45••]. Genomic analysis of a diversity panel of 236 sequenced human genomes identified 1036 copy number variants (CNVs) stratified between human populations [45••]. Using the Vst statistic, analogous to Fst for multiallelic or multicopy states, found that duplications were 1.8 times more likely than deletions to exhibit population stratification but less likely to be tagged by adjacent SNPs (R^2 = 0.03 Vst of duplications vs. Fst of flanking SNPs) making them more difficult to detect. This is in agreement with other studies showing the majority of multicopy duplications correlating less well with flanking SNP genotypes [43,46]. Among this set were five duplications identified in Oceanic populations that were shared with the archaic Denisovans. A subset of these constituted a larger 255 kbp SD mapping to chromosome 16p12.1 (Figure 3a). Using shared SNPs, this SD was determined to have arisen in Denisovans ~400 thousand years ago (kya) followed by introgression into the Oceanic ancestral population ~40 kya, where it has since nearly fixed either through positive selection or genetic drift. The duplication is associated with the chromosome 16 core duplicon containing *NPIP* [19], appears to be exclusive to living Oceanic human populations and represents the first example of an SD arising in a sibling species of humans that was later introduced to our lineage likely by introgression. While interesting, is there any evidence that SDs and their associated structural variants may be substrates for more recent selection and adaptation?

Amylase and adaptation to a starch-rich diet

Recent duplications of the salivary amylase gene (*AMY1*) are frequently cited as an example of adaptive evolution in the human lineage [47,48]. Expansion of *AMY1* and the concomitant increase in salivary enzyme production may improve our ability to digest starch-rich foods, a potentially beneficial trait related to the diet of early modern humans as they switched from a hunter-gatherer to a primarily agricultural society ~10 kya. Genotyping modern humans using qPCR and microarray, Perry and colleagues determined that copy number ranged from 2 to 15, noting stark differences between populations with low and high starch diets [47]. Further comparisons in closely related nonhuman primates and archaic hominins show these extreme expansions of gene copy are unique to modern humans and not seen in Neanderthal and Denisovan genomes [45••]. More refined copy number estimates and insights into the structural variation of this locus are beginning to emerge [45••,49,50••], with diverse haplotypes identified ranging in size from 77 kbp to ~200 kbp including variability (2–6 copies) of nearby pancreatic amylase genes (*AMY2B* and *AMY2A*) (Figure 3b) [50••].

17q21.31 duplication and inversion

The expansion of chromosome 17q21.31 duplications and the associated inversion polymorphism represent another potential example of recent human adaptation. Stefansson and colleagues originally reported a 900 kbp inversion polymorphism flanked by SDs enriched in European and Mediterranean populations [51]. Within the Icelandic population [51] and in European-Americans [52], the inverted haplotype (H2) has been associated with increased fecundity of H2 carrier females and an overall increase in global recombination, potentially explaining the increase in frequency. Subsequent sequencing and characterization of the predominant H2 duplicated (H2D) haplotype in individuals of European descent showed that the H2 haplotypes were virtually identical having expanded ~17–48 kya [53,54]. The high European allele frequency (~36%) of such a young haplotype was consistent with the original suggestion of positive selection. This finding was even more remarkable in light of the fact that the H1 and H2 haplotypes diverged

(Figure 3 Legend Continued) segments from chromosome 16p12.1. The duplication is present in the genome of the ancient hominin, Denisova, but not observed in any other modern human populations possibly as a result of introgression back into the human lineage. The Oceanic-specific P3 formed as a result of interspersed duplications of P1 (represented in the human reference genome GRCh38) and P2 and included the *NPIP* core duplicon. P3 frequency was estimated based on genome sequence read-depth from the Human Genome Diversity Project (HGDP) cohort (E = 59, As = 45, Af = 36, O = 21) [41•]. The P3 duplication has been identified in all Papuan individuals. **(b)** Diverse duplication structures exist at the amylase locus, with at least eight predicted haplotypes representing varying copies of *AMY2B*, *AMY2A*, *AMY1*, and *AMY2AP* (figure adapted from [50••]). Overall copy number estimates of *AMY1* in 1000 Genomes Project [41•] and HGDP cohorts [44] were calculated and low-copy ($CN \leq 4$) versus high-copy ($CN > 4$) frequencies were determined (E = 145, As = 204, Af = 133, O = 21). European and African populations show overall lower copy numbers of *AMY1* compared with Asian and Oceanic. **(c)** Structural diversity of the chromosome 17q21.31 haplotype (figure adapted from [54]). Various forms of the directly orientated haplotype (H1) have been identified, including European-enriched haplotypes that show duplications of the promoter and first exon of *KANSL1* (H1D). The inverted haplotype (H2) exists in a simpler form (H2.1) found among the San Khoisan and in more complex duplicated forms in European/Mediterranean haplotypes, including a smaller duplication of the promoter and first exon of *KANSL1* (H2D). Allele frequencies of H1, H1D, H2, and H2D are shown based on sequence data from 1000 Genomes Project and HGDP cohorts (E = 628, As = 733, Af = 820, O = 27) [54]. H2.1 is predicted to represent the ancestral haplotype (2.3 mya), H1 is now the dominant haplotype worldwide, and the increased frequencies of H1D, H2, and H2D in European populations are the result of positive selection or extraordinary genetic drift. This is remarkable in light of the fact that the European H2 haplotype is predisposed to microdeletion due to the accumulation of directly oriented SDs. The less complex pattern of SDs observed among African H2.1 allele carriers suggest that the ancestral H2 haplotype is not predisposed to disease [54].

~2–3 mya and the H2 haplotype predisposes to recurrent microdeletions associated with Koolen-de Vries syndrome [55,56] and, thus, subject to some modest negative selection. A total of eight complex human structural haplotypes have been characterized to date ranging in size from 1 to 1.5 Mbp with considerable expansions of SDs occurring in the last million years of human evolution [53,54] (Figure 3c). The *LRRC37A* core duplicon resides near many of the breakpoints, including the inversion, flanking SDs, and disease-associated microdeletion [57]. Although the molecular basis for the selection of the European H2 locus is not known, it is interesting that the region contains several genes important in neural function, including *MAPT* — associated with several neurodegenerative disorders including Alzheimer's disease, *CRHR1* — a cortical-releasing hormone receptor, and *KANSL1* — the gene responsible for the Koolen-de Vries syndrome [58]. It is interesting to note that the H2 haplotype was recently associated with increased intracranial volume indicating larger brain size [59].

Summary & future directions

Although the selective disadvantage of interspersed SDs is well established, examples of their selective benefit have been slower to emerge. Examples such as *SRGAP2C* and *ARHGAP11B* are interesting because, in both cases, the SDs were incomplete and potentially functional at birth due to truncations facilitating antagonistic interactions with the parental gene (*SRGAP2A*) or leading to rapid neofunctionalization (*ARHGAP11B*). Examples such as the 17q21.31 inversion and the amylase gene family highlight the continued importance of SDs to more recent adaptations. Despite these intriguing examples, several challenges remain. First, many of the regions are still not properly assembled in human and other great ape genomes due to their high sequence identity and association with other larger more complex duplications. Although the catalog of human SDs is nearly complete [41•], recent estimates suggest that as much as 40 Mbp of euchromatic sequence may be missing from the current human reference due to structural variation [60] of which at least 4.2 Mbp are duplicated (>3 copies) and are copy number polymorphic [45••]. This has meant that underlying genes have only been discovered through targeted efforts via sequencing of clones, full-length cDNA, and *de novo* genome assembly using long-read methods to correct the reference genome and distinguish paralogs [61,62,63•]. Second, our understanding of the patterns of human variation in these regions is currently in its infancy. An important step forward will be the development of robust genotyping assays capable of inferring both the sequence content and structure of these regions [28,42•,50••]. Finally, functional studies assaying duplications are not well established. While CRISPR/Cas9 technology has facilitated knockouts within human induced pluripotent stem cells (iPSCs), the high degree of sequence identity between paralogs makes such an undertaking nontrivial often promoting the formation of large-scale rearrangements [64•]. Though less straightforward due to the megabase pairs of highly rearranged sequence frequently associated with HSDs, genome editing can also be used to 'knock in' HSDs in species where these genes do not exist. Generating the genomic context to establish equivalence and proof of functional effect *in vivo* has not yet been achieved for any HSD. Nevertheless, efforts can be made to 'humanize' chimpanzee iPSCs as well as mice. The ultimate test of function lies in identifying mutations of HSD genes associated with human disease (e.g., natural gene knockouts in humans). Current whole-genome sequencing efforts will make it possible to assay variation in a number of disease cohorts. Though limitations exist when using short reads to assay variation between highly similar paralogs, recent advances in synthetic long read methods via barcoding (e.g., 10X Genomics) may prove effective in improving variant calling and characterizing the complexity of structural change. Notwithstanding these challenges, the emerging data suggest a disproportionate role for SDs not only in human disease but also human evolution.

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- •• of outstanding interest

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