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Genomically humanised mice: technologies and promise

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Abstract

Mouse models have become an invaluable tool for understanding human health and disease owing to our ability to exquisitely manipulate the mouse genome. Recent progress in genomic analysis has led to an increase in the number and type of disease-causing mutations detected, and has also highlighted the importance of non-coding regions. As a result there is increasing interest in creating ‘genomically’ humanised mouse models, in which entire human genomic loci are transferred into the mouse genome. The technical challenges to achieving this aim are large but are starting to be tackled with success.

The mouse is the model of choice for recapitulating genetic changes that give rise to human disease. Over the last 30 years mouse molecular genetics has been refined to allow production of an impressive panoply of mutants. These include additive transgenic, knock-out, and knock-in animals (all of which can be conditional or inducible), strains containing chromosomal rearrangements or large megabase-sized deletions and duplications, and even

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FURTHER INFORMATION
ENCylopedia Of DNA Elements, genome.ucsc.edu/ENCODE
International Knockout Mouse Consortium, www.knockoutmouse.org
KnockOut Mouse Project KOMP, www.komp.org

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transchromosomic mouse strains[1]. It is a fast developing area and new technologies are arising all the time, and include methods for modelling 'sporadic' disease such as cancer[2].

Almost all human disease models have been made to study changes in the coding genome. Typically this has been done by pronuclear injection, to generate transgenics that ectopically express a mutant protein, or by gene targeting in embryonic stem (ES) cells, for example, by creating a gene knock-in. As proteins with a human amino acid sequence can have different biochemical characteristics from their mouse orthologues, transgenics have often been made with human cDNAs, and targeting has involved placing human coding sequences into the orthologous mouse gene. This genetic 'humanising' strategy using coding sequences can result in a more accurate mouse model of disease than working with a mutant mouse protein. However, recent progress in genomic analysis has highlighted the importance of the non-coding genome (both transcribed and non-transcribed), making it clear that this category of sequence also needs to be taken into account when modelling disease. In particular, projects such as ENCODE (the ENCylopedia Of DNA Elements[3]) have discovered extensive transcription of the non-coding genome and human genome-wide association studies (GWASs) demonstrate that variation (including copy number variation) in non-coding regions confers susceptibility and resistance to disease in ways that we do not comprehend. As we learn more of the complexity of the genome it is apparent that understanding human biology, particularly with respect to disease models, will require humanised mouse models that address the potential roles of both coding and non-coding genomic sequence (Box 1).

Laboratories world-wide are developing the technology for creating such 'genomically' humanised mice, which are generated by transferring entire human genomic loci (including coding and non-coding regions) into the mouse genome. This is achieved either by the addition of human genomic sequences or by replacing regions of the mouse genome with equivalent human genomic sequence. However, the technical challenges remain daunting and while current approaches to optimise different strategies are proving successful, for example generation of mice carrying a whole human chromosome, they are far from routine.

Here we look at the different approaches that have been developed for creating genomically humanised mice, why genomic humanisation remains a challenge, and which new technologies are the most promising. We also speculate on the direction of future advances in this field.

YAC and BAC transgenics

The first technologies for producing genomically humanised mice made use of yeast artificial chromosomes (YACs) and bacterial artificial chromosomes (BACs). Transgenic lines can be created by pronuclear injection of YAC and BAC DNAs, with these DNAs being integrated at random chromosomal positions (Figure 1Aa). The key advantage of BACs and YACs is their size, ranging up to 300 kilobases (kb) for BACs and up to Megabases (Mb) for YACs, thus enabling inclusion of all or some of the upstream and downstream *cis*-sequences regulating expression of a gene of interest. YAC and BAC transgenic insertions show position-independent and copy-number-dependent expression more frequently than smaller transgenes do, and they more faithfully recapitulate the

anticipated expression profile[4, 5]. Compared to conventional cDNA transgenics, BAC and YAC transgenics also have the added advantage of containing low-copy-number integrations, which is important when studying the effect of gene dosage.

The extent of humanisation achievable with YACs can be increased by exploiting homologous recombination in yeast to join two existing YACs via a region of shared homology into a single larger recombinant YAC. The recombinant YAC can then be transferred into ES cells by fusion with yeast spheroplasts carrying the YAC, followed by selection for a drug-resistance selection marker (Figure 1Aa). ES cells can then be used to generate chimeras to achieve germline transmission. Transgenic mice containing the entire functional human TCRαβ gene loci were created in this way[6]. BACs can also be manipulated by homologous recombination in *E.coli,* for example to insert reporter genes[7].

Genomically humanised YAC and BAC transgenic strains may be bred onto a null background for the gene of interest such that the humanised locus is the only version of the gene that is expressed (Figure 1Aa)[8]. Production of transgenic mice expressing only human antibodies has been achieved by breeding transgenic lines with knock-out alleles of heavy and light chain constant regions (for example, see Ref.[9]).

BACs can also be designed to integrate at specific and ubiquitously expressed chromosomal loci, such as the hypoxanthine-guanine phosphoribosyltransferase (*Hprt)* locus, by homologous recombination[10] or by site-specific recombination (SSR)[11] in ES cells. Site-specific integration avoids the problems due to deletions and concatemerisation that can occur during random integration by non-homologous recombination and, as the site of integration is predetermined, also achieves a more reproducible expression profile.

Targeted Genomic Replacement

Targeted integration of a human sequence into the equivalent region of the mouse genome in ES cells is the most precise method for humanisation, enabling a single copy of human sequence to reside at a natural site for its expression, while simultaneously replacing the corresponding mouse sequence. In principle it is possible to generate a homozygous humanised mouse strain by inter-crossing heterozygotes carrying the human replacement.

Traditionally, this approach has involved small sized changes (up to ~10 kb) replacing some or all of the exons and introns of a mouse gene with corresponding human sequence; the human sequence therefore remains under the control of mouse transcriptional regulatory sequences. Mice in which the genomic region encompassing exons 4–9 of the p53 gene was replaced by the orthologous human genomic region have been constructed by this approach and have a correct splicing pattern, producing a chimeric protein in which the p53 core domain is human. These animals have proved valuable for determining the tumorigenic role of human p53 mutations[12, 13]. However, replacements are now possible in which an entire mouse locus, including non-coding upstream and downstream sequences, is substituted by equivalent human sequence using constructs derived from BACs. In principle, this approach could be extended to encompass a larger region of shared synteny. There are two ways to achieve this aim (described below and shown in Figure 1 Ab, c): either directly by using

homologous recombination, or through a multi-step approach involving a combination of homologous recombination and SSR.

BAC-vector homologous recombination

Genomic replacements can be achieved by homologous recombination with a hybrid BAC vector (Figure 1Ab). This vector is assembled from mouse and human BAC clones by recombineering technology in *E.coli*, to create a construct with a large region of human sequence and a drug-resistance marker inserted between long regions of mouse genomic sequence (>100 kb in total). As a consequence of the length of the mouse sequences that form the homology arms this targeting vector results in efficient homologous recombination in ES cells[14]. Targeted integration is detected using quantitative PCR to assay for the reduction in copy number of mouse autosomal sequences from two to one. The advantage of this strategy is that it can be applied directly to unmodified ES cells and is essentially a single step procedure, although a second step to delete the selection marker using SSR is usually desirable.

A high throughput version of this technique has been developed, termed 'VelociGene', which allows rapid generation of large numbers of genetically modified mouse lines. So far, most mouse lines made using VelociGene contain null alleles, in that the entire mouse locus is replaced with a reporter cassette driven by the endogenous promoter. This high-throughput technology is being used by the NIH as part of their KnockOut Mouse Program (KOMP).

VelociGene has been successfully used to create a series of humanised knock-ins, to improve xenogeneic transplantation mouse models for studying *in vivo* human haematopoiesis and immune function. Four different cytokines have been humanised, in three separate targeting events. Both thrombopoietin (*TPO*), an essential cytokine for haematopoietic stem cell maintenance, and colony stimulating factor–1 (*CSF1*), a cytokine important for differentiation and function of human macrophages, were humanised in single targeting events[15, 16]. For both loci, the mouse promoter was left intact but the coding region and sequence extending to 3 kb downstream of the polyA signal was replaced with the human equivalent (changes of 5 kb and 18 kb, respectively). Granulocyte-macrophage colony-stimulating factor (*CSF2*) and Interleukin-3 (*IL3*) were humanised in the same targeting event due to their close proximity (<10 kb) in both the mouse and human genome[17]. The dual targeting construct replaced the mouse loci with the human equivalents (a change of 20 kb): While *IL3* retained the mouse promoter, *CSF2* is controlled by its human regulatory elements. The largest humanisation project carried out using VelociGene technology was the VelocImmune mouse, in which 6 Mb of the variable portion of the mouse immunoglobulin (Ig) loci were humanised, to allow production of human monoclonal antibodies for antibody therapeutics[18].

Recombinase-Mediated Cassette Exchange (RMCE) and Recombinase-Mediated Genomic Replacement (RMGR)

The second approach to 'humanisation by replacement' is to use SSR to achieve efficient exchange of chromosomal sequence with sequence on an incoming plasmid or BAC. This

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involves a strategy termed recombinase-mediated cassette exchange (RMCE). RMCE is a two stage process: firstly, a selection marker cassette that is flanked by heterospecific SSR sites is integrated into the genome by homologous recombination, deleting the region which is to be replaced. Secondly, another donor vector with the human genomic sequence flanked by the same SSR sites is introduced, in the presence of a recombinase (usually expressed from a co-transfected plasmid) resulting in the human sequence recombining into the genome (Figure 1Ac). RMCE has been applied in various cell lines, including ES cells[19, 20]. The advantage of RMCE is that once the initial selection marker cassette is integrated into the desired chromosomal position, the resulting cell line can be used recursively for unlimited rounds of cassette exchange via the inserted heterospecific sites, and the desired events are readily recovered by genetic selection.

In principle, RMCE could be applied to any gene in mouse ES cells. For example, RMCE was used to place a human cDNA for a cardiac sodium channel in exon 2 of the mouse orthologue[21]. For-large scale replacement of mouse genomic sequence with human sequence from a BAC clone, an elaboration of the RMCE strategy can be used called Recombinase-Mediated Genomic Replacement (RMGR)[22]. In RMGR, heterospecific SSR sites and linked positive and negative selection markers are integrated into ES cells by sequential rounds of homologous recombination to delineate the region for replacement. The human BAC clone is modified by recombineering to insert heterospecific SSR sites at the corresponding human sequences and following co-transfection with a Cre recombinase expressing plasmid, SSR events at both ends are selected (Figure 1Ac). This method was used to replace the 87 kb genomic region encompassing the α-globin regulatory domain of mouse with the equivalent human sequence of 117 kb. An additional round of recombineering of the BAC was used to delete the major regulatory element before introduction into ES cells and consequently create a mouse model of human α-thalassaemia[22]. Theoretically, RMGR could encompass replacements of any size up to the size limit of the BAC donor. Recently, Hasegawa and co-workers published a modified version of RMGR in which they flanked the mouse cytochrome P450 *Cyp3a* gene cluster (a region of 820 kb) with a pair of homospecific *loxP* sites that allowed deletion of this cluster in an intermediate step; these sites were present in addition to the heterospecific sites required to insert human *CYP3A4* and *CYP3A7*, contained within a 100 kb region of the donor BAC. The resulting humanised mouse has been created to study drug interactions[23].

Transchromosomic and chromosome-engineered mice

Copy number variation (CNV) is highly polymorphic in the human population and it is likely that many CNVs give rise to phenotypic effects because of differences in sequence dose between individuals. Genomic CNV can be modelled through chromosome engineering of the mouse genome[24, 25], but to model the effects of human CNVs that span extensive regions of non-coding sequence, genomically humanised mice carrying different copy numbers of the human sequence will need to be generated.

Humanised mouse models carrying a freely-segregating partial or whole human chromosome have been created by using microcell-mediated chromosome transfer (MMCT) into mouse ES cells[26-28]. These transchromosomic ES cells are then used in conventional

approaches to establish strains of transchromosomic mice that transmit the human chromosome through the germline (Figure 1Ad), for example, the Tc1 mouse carries a freely segregating human chromosome 21 and models trisomy 21 in humans[34].

Human artificial chromosomes (HACs) offer alternatives as vectors for gene delivery and to create animal models[29, 30]. HACs are non-integrating vectors that can be engineered to contain desired sequences and then moved by MMCT into mouse ES cells to make transchromosomic mice[31]. HACS are synthesised by two methods, either bottom-up (*de novo* synthesis) or top-down (engineered). The bottom-up approach involves introduction of two vectors, BAC or YAC, into cells permissive for recombination. One vector contains centromeric alphoid DNA, the other the genomic region of interest. Multiple copies of each are randomly assembled with no control on size or composition. The resultant HACs are usually circular and 1-10 Mb in size. The top-down approach involves shortening of human chromosomes by introduction of telomeric sequences via homologous recombination, to form mini-chromosomes. Efficiency of recombination is increased if this step is carried out in chicken DT40 cells which are unusually permissive for homologous recombination. More recently SSR sites have been introduced into engineered HACs, allowing introduction of any genomic region flanked by SSR sites in a donor vector (BAC or YAC). The latest generation of engineered HACs, designed with gene therapy and synthesis of animal models in mind contain multiple different SSR sites[32], allowing introduction of multiple genes of interest on the same vector, while the tet-O HAC[33] is conditional and once introduced can be selectively removed from cells (the current generation of HAC vectors are reviewed in ref [34]). The merits of creating transchromosomic mice are that they carry low or single copy number human chromosomes or HACs, they are generally freely segregating and thus do not disrupt endogenous sequences, and, presumably, the maximum size is that of a chromosome[34]. A possible disadvantage of this approach may be instability of the transchromosomes, though this has not been reported as a problem so far.

Mouse models for developing therapies

In addition to understanding pathogenic processes, genomically humanised mouse models are likely to be important for developing therapies, particularly gene therapies. In a recent example, a genomically humanised mouse strain was created by knocking into the ubiquitously expressed *Rosa26* locus a minigene for human factor 9 (*F9*), which included intron 1 and the Y155stop mutation, known to cause haemophilia B[35]. This humanised F9 mouse was then used to develop *in vivo* gene therapy, using zinc finger nucleases plus a promoterless therapeutic gene fragment consisting of wildtype *F9* cDNA exons 2–8 preceded by a splice acceptor site. The site-specific nucleases corrected the mutant *F9* gene by inserting the gene fragment into the first intron of *F9*. It is difficult to imagine how this therapeutic approach for haemophilia B could have been validated without the use of such mice.

Existing and new resources

Comparison between existing strains of mutant mice and humanised genomic models can be highly instructive for understanding species-specific biology[36, 37]. Additive multi-copy

transgenics will also continue to be necessary for modelling disorders, such as some late-onset neurodegenerative diseases, in which expression of human sequences needs to attain a critical level to manifest a phenotype[38]. Furthermore, existing mouse genetic resources can be used to understand the effects of genetic modifiers, by breeding humanised loci onto different inbred mouse lines [39] or into genetically sensitised mouse strains. Similarly, we may be able to dissect the effects of the environment on human genetic disease by altering the conditions in which genetically identical humanised mouse models are maintained.

Our ability to humanise mice is likely to increase greatly in the near future. New applications might include iterative application of the VelociGene technology along the length of a chromosome, to create contiguous regions of humanisation. Humanisation of multiple loci, linked or unlinked, could also be achieved in an ES cell line by extending RMGR technology. For example, targeting vectors already generated by the International Knockout Mouse Consortium could be adapted to insert heterotypic SSR sites and/or selection markers into the mouse genome, and corresponding human BACs overlapping these loci could be used as donors. Alternatively, BACs could joined by recombineering into a single mega-BAC to span the desired region. New SSR systems, such as Dre/*rox* recombination will undoubtedly be useful to implement this strategy[40].

If large-scale changes are required, it should also be possible to use chromosome engineering to apply RMGR on a megabase scale. In this scenario, human artificial chromosomes (HACs) maintained in DT40 cells could be first modified by homologous recombination to insert SSR sites and selection markers at the desired end points of the prospective replacement interval. Subsequently the HACs could be transferred by MMCT into mouse ES cells previously modified by targeted insertion of SSR sites and selection markers at the equivalent chromosomal positions. Expression of recombinase could then be induced to mediate the replacement event between the HAC and the mouse chromosome. This is an exciting prospect for modelling large CNVs and aneuploidy.

Current experience with transchromosomic mice is limited. Recognised issues include difficulty in obtaining germline transmission of the freely segregating human chromosome and its subsequent mosaicism in the animal[27, 41]. One way around these problems may be to engineer the human chromosome such that it is translocated onto a mouse chromosome, although inevitably this means deletion of some human and mouse sequences at the translocation breakpoints.

Conclusion

As the technologies necessary for producing genomically humanised models develop further, it will become possible to introduce greater amounts of human genetic material into mice and other species. This prospect has promoted discussion about the ethical implications of such humanisations, for example in a report from the UK Academy of Medical Sciences, published in July 2011, which is freely available on-line[42].

A key question about genomically humanised mice concerns the extent to which the human DNA sequence is read correctly and efficiently by the mouse transcriptional machinery.

Little information is available, partly because there are relatively few genomically humanised models. It is reassuring that in the α-globin humanised mouse model, the human genes are expressed in an appropriate developmental stage- and cell type-specific manner[22]. However, in humanised heterozygotes human α-globin RNA is expressed at 40% of the level of endogenous mouse α-globin, possibly because the relevant mouse transcription factors bind cognate human sequences with sub-optimal efficiency or stability. Nevertheless, the humanised α-globin locus accurately recapitulates many of the important features of the human α-globin gene chromatin state, most notably chromosomal looping, transcription factor binding and polycomb (PcG) recruitment at α-globin CpG islands in non-erythroid cells, and subsequent eviction of PcG from the α-globin CpG island in mature erythroid cells[39]. Consistent with this observation, a recent study showed that when a human chromosome is placed in a mouse environment, the human DNA sequence directs a human rather than mouse pattern of chromatin modifications[43].

We have learned a significant amount from genetically humanised mice already. The tools now available, and those to come, will allow more accurate manipulation of the mouse genome with engineered human genomic material. This holds great promise for the better understanding of disease, development of effective therapies, more accurate models of drug metabolism as well as enhancing our understanding of mammalian and human genome function.

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Glossary

Knock-out mouse strain	A mouse strain in which the functional protein-coding capacity of a particular gene has been disrupted. This could be via a targeted deletion, gene trap, or targeted conditional knock-out.
Knock-in mouse strain	A mouse strain carrying a targeted replacement or insertion; for example, an exchange of nucleotide sequence to change the encoded protein sequence or an insertion to create a tagged protein.
Conditional mutation	A mutation whose expression is under experimental control. In a conditional knock-out, deletion of a critical exon flanked by recombination sites can be induced by expressing a recombinase, for example under a tissue-specific promoter.
Inducible mutation	A class of conditional mutation that is expressed by using a ligand-inducible recombinase. Inducible mutations allow recombinase-driven change to be induced at a particular timepoint by delivering the ligand into the animal by injection, diet or using viral vectors.
Transchromosomic mouse strain	A mouse strain into which either an entire chromosome or a chromosome fragment from another species has been transferred. This chromosome or chromosome fragment may be freely segregating or inserted into an existing mouse chromosome.
Pronuclear injection	A process by which DNA is added to the genome, generally to create transgenic mice. Linearised DNA is injected into one of the two pronuclei of the fertilized egg, is stably incorporated into the genome and the eggs develop to term in a pseudopregnant female mouse.

Gene targeting	A method of exchanging genetic information from a donor vector into a recipient genome, exploiting a DNA-repair mechanism that recognises homology between the donor DNA and the recipient locus. It results in the replacement of the original genomic sequence with the donor sequence.
Genome-wide association study (GWAS)	A whole-genome scan testing the statistical association between finely spaced polymorphic genetic markers and a trait under study. Success depends on large numbers of phenotypically well-classified DNA samples in order to have the statistical power to detect such associations.
Recombineering	Recombineering (derived 'recombination-mediated genetic engineering') uses recombination functions encoded by λ phage to mediate in-vivo recombination using short stretches of homologous sequence (30-50 nucleotides long) that can be readily appended to the termini of any desired DNA.
KOMP	An NIH-funded program contributing to the international mouse knockout consortium (IKMC) to generate a null mutation in every mouse gene. Other contributors are the Texas Institute for Genomic Medicine (TIGM), the North American Conditional Mouse Mutagenesis Program (NorCOMM) and the European Conditional Mouse Mutagenesis Programme (EUCOMM).
Xenogeneic transplantation	The transplantation of tissues or cells derived from one species into another. For example, the introduction of human stem cells into an immune-deficient mouse strain.
Microcell mediated chromosome transfer (MMCT)	A method for transferring entire chromosomes or chromosome fragments from a donor cell line into a recipient cell line.
Minigene	A gene construct that contains fewer exons and introns than its original full gene counterpart. Minigenes have been used to investigate regulatory sequences but may also be used because of their more convenient size.

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Box 1. Why humanise mice?

Genetic humanisation

Few proteins are 100% conserved between human and mouse[44], and differences in orthologous sequences can have functional consequences. For example, mouse serum amyloid P (SAP) binds to amyloid fibrils with only ~3% of the avidity of the human protein although mouse and human SAP are ~70% conserved[45]. Similarly, mutant superoxide dismutase 1 (*SOD1*) is causative for the human neurodegenerative disease amyotrophic lateral sclerosis; the human and mouse proteins share 83% identity, but a tryptophan residue at codon 32 (W32) is found only in humans, where it appears to potentiate SOD1 aggregation and human specific SOD1–SOD1 interaction which may contribute to motor neuron death in humans and in mice with mutant human *SOD1* transgenes[46].

Similarly, wild-type mice expressing mouse CD81 and occludin (OCLN) are non-permissive to hepatitis C virus (HCV) entry[47]. However, animals expressing two human orthologues of these two proteins are permissive for HCV infection, while remaining fully immunocompetent. This model greatly eases studies of the immune response to HCV because previously humans and chimpanzees were the only two species known to be permissive for HCV infection[47].

Humanisation also gives insight into gene evolution. FOXP2 transcription factor is important for human speech and language. When this protein was humanised in mice it produced abnormal behavioural and other phenotypes in cortico-basal ganglia circuits, suggesting that humanised FOXP2 protein may take on a new function(s) in these regions that is important for the evolution of human language and speech[48]. These phenotypes were not found in a *Foxp2* knock-out, indicating they arose from the function of the wildtype human protein.

For a small number (<200) of human protein-coding genes no mouse orthologue has been found[49], and thus one approach to learn more about the biology of these human genes is to introduce them into mice.

Genomic humanisation

Although genetic humanisation has given us great biological insight, *genomic* humanisation will be necessary to investigate the functional importance of non-coding regions and therefore to fully model aneuploidy, to study disorders in which species-specific splicing patterns play a role, or to determine the functions of untranslated sequences. For example, the different effects of disrupting *Hotair* orthologues in human and mouse, indicate that this long non-coding RNA has human function(s) that may not easily be determined from non-genomically-humanised mice[50,51].

Likewise, genomically humanised mice containing a caspase 12 (*CASP12)* variant responded in a gender-specific manner when infected with *Listeria monocytogenes*[52], leading to the identification of an oestrogen receptor element (ERE) in intron 7, which appears to be responsible for oestrogen-modulated expression of the *CASP12* variant being studied. Treatment of the male humanised *CASP12* mice with 17-β-oestradiol (E2)

conferred increased resistance to infection, leading to suggestion of the therapeutic use of E2. The oestrogen-response element is not found in mouse intron 7 and wild-type male mice do not respond to oestrogen at the *Casp12* locus[52]. Without the use of genomically humanised mice, this ERE and potential therapy would not have been discovered.

Thus the non-coding genome must be taken into account in studying gene function and genomic humanisation will be essential to create an optimal set of models of human disease.

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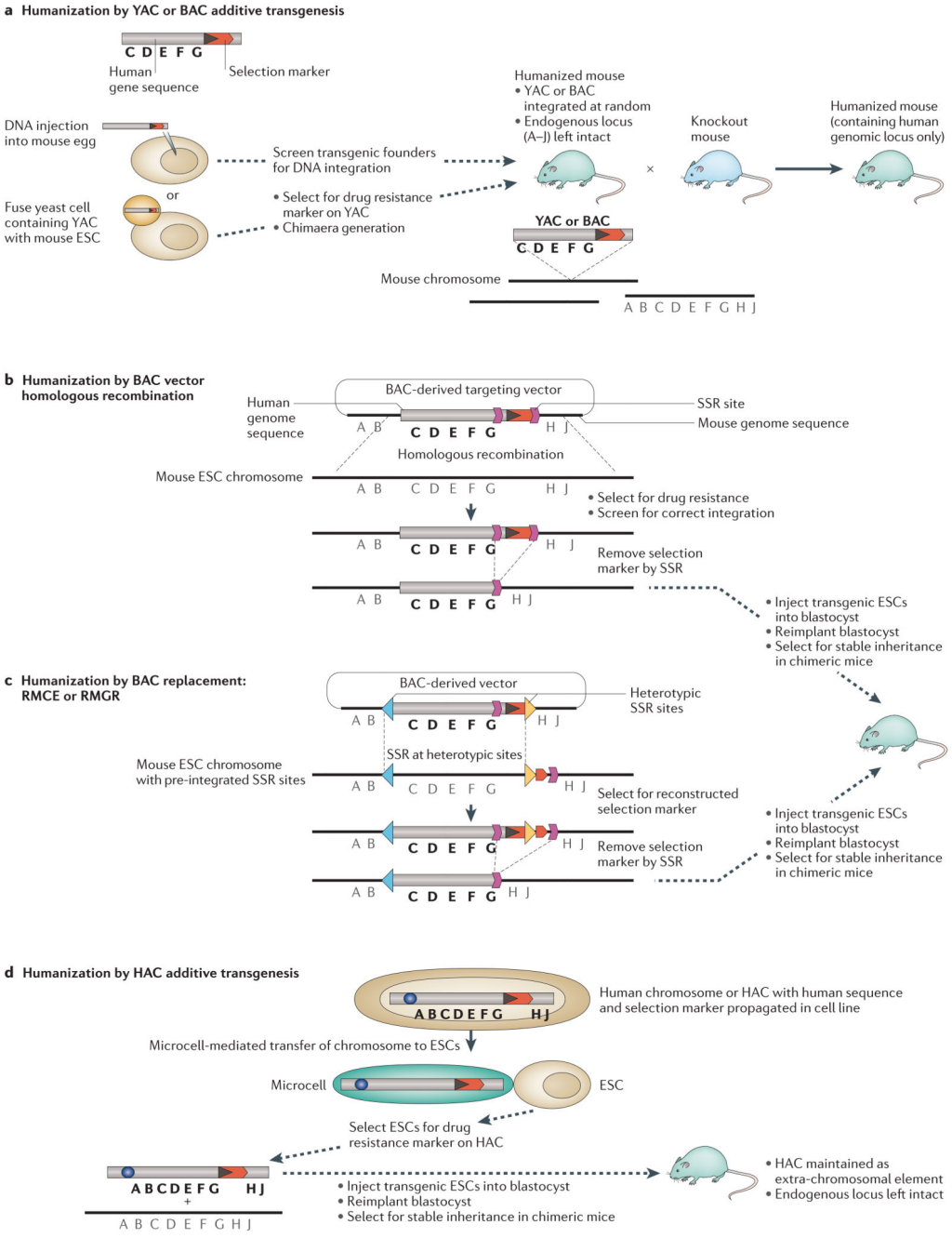

Figure 1. Methods of humanised mouse synthesis

There are multiple ways of introducing the human genomic region of interest into the mouse germline. (**a**) Traditionally this has been via an additive process, where a YAC or BAC vector is introduced via pronuclear injection or cell fusion, resulting in random incorporation into mouse genome, while the endogenous mouse locus is unmodified. (**b, c**) An alternative is the specific targeting and replacement of genomic loci, either using (**b**) homologous recombination or (**c**) the SSR-based technologies RMCE and RMGR. Homologous recombination with a genomic fusion (mouse-human) BAC vector results in the endogenous

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mouse locus being replaced by equivalent human sequence, using the large regions of homology provided by the BAC vector for increased targeting efficiency (**b**). RMCE and RMGR require prior modification of the mouse genome, to introduce heterotypic SSR sites to flank the region of interest. A BAC vector containing equivalent human genomic region flanked by same SSR sites then acts as a donor for the swap of genetic material mediated by expression of recombinase (**c**). (**d**) A non-integrative approach to creating a humanised mouse is by the introduction of a HAC into ES cells via MMCT. The HAC vector is synthesised via a top-down or bottom-up approach, where the genomic region of interest is introduced by homologous recombination or SSR. The HAC is mitotically stable and maintained as an extra-chromosomal element, leaving the mouse genome unmodified.

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