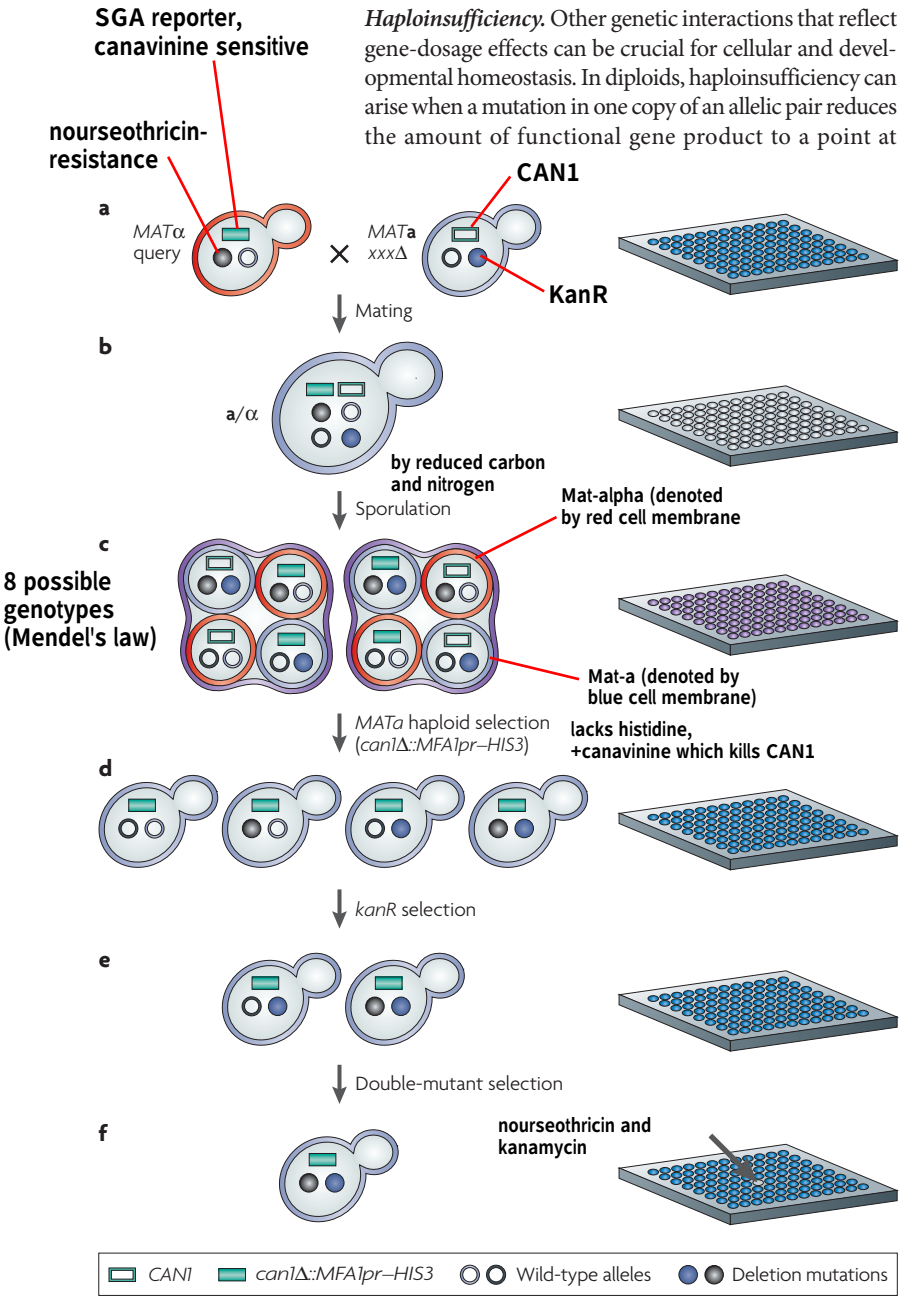

Figure 2 | **The synthetic genetic array (SGA) methodology. a** | A *MATα* strain carries a query mutation linked to a dominant selectable marker (represented as a filled black circle), such as the nourseothricin-resistance marker *natMX*, and the SGA reporter *can1Δ1::MFA1pr–HIS3* (in which *MFA1pr–HIS3* is integrated into the genome such that it deletes the ORF of the *CAN1* gene, which normally confers sensitivity to canavinine). This query strain is crossed to an ordered array of *MATa* deletion mutants (*xxxΔ*). In each of these deletion strains, a single gene is disrupted by the insertion of a dominant selectable marker, such as the kanamycin-resistance (*kanR*) module (the disrupted gene is represented as a filled blue circle). **b** | The resultant heterozygous diploids are transferred to a medium with reduced carbon and nitrogen to induce sporulation and the formation of haploid meiotic spore progeny. **c** | Spores are transferred to a synthetic medium that lacks histidine, which allows for selective germination of *MATa* meiotic progeny because these cells express the SGA reporter *can1Δ1::MFA1pr–HIS3*. To improve this selection, canavinine, which selects for *can1Δ1* and kills *CAN1* cells, is included in the selection medium. **d** | The *MATa* meiotic progeny are transferred to a medium that contains kanamycin, which selects for single mutants, equivalent to the original array mutants and double mutants. **e**,**f** | An array of double mutants is selected on a medium that contains both nourseothricin and kanamycin.

Haploinsufficiency. Other genetic interactions that reflect gene-dosage effects can be crucial for cellular and developmental homeostasis. In diploids, haploinsufficiency can arise when a mutation in one copy of an allelic pair reduces the amount of functional gene product to a point at which a phenotype is produced. Classically, a heterozygote is viewed as the wild type (that is, the mutant phenotype is recessive), and this is the case for most enzyme-coding genes[39]. However, for human transcription factors, over 65% of disease-causing mutations are dominant, and might reflect a haploinsufficient phenotype[39]. Haploinsufficiency can be particularly significant in the context of environmental or chemical interactions and has been exploited extensively to link inhibitory bioactive molecules to their targets, as heterozygote target-gene deletion mutants are often hypersensitive when compared with wild-type cells owing to their reduced target-gene dosage[40,41].

The combination of two heterozygous mutations might lead to a genetic interaction in which the diploid hemizygote double mutant shows an extreme synergistic phenotype, such as synthetic lethality. This combinatorial double-mutant effect has been referred to as complex haploinsufficiency[42]. A screen of 4,800 complex hemizygote yeast strains, in which an actin-null allele was combined with the non-essential gene-deletion collection, identified 208 genes showing deleterious complex haploinsufficient (CHI) interactions and many of the double mutants showed actin-based morphology defects. Thus, CHI genetic-interaction screens can provide extensive functional information if carried out on a global scale.

Quantitative mapping of epistatic relationships

Synthetic methodologies allow a quantitative assessment of the relative fitness of double-mutant meiotic progeny. This means that, in addition to Fisher's general idea of epistasis, other more specific ones, including Bateson's classical definition in which one allele masks the effects at another locus (BOX 1), can be examined globally. In the Fisher model, the double-mutant growth rate should deviate from the expected multiplicative value that is associated with the combined single-mutant phenotypes, and this can potentially be examined in detail. In particular, so-called aggravating interactions, in which the double-mutant fitness is lower than expected, might reflect separate but compensatory pathways. Synthetic-lethal double mutants obviously deviate from the multiplicative; however, synthetic slow-growing double mutants with fitness rates that are less than either single mutant but equal to the expected multiplicative double-mutant fitness would not be scored as showing a genetic interaction. Using Fisher's quantitative definition of epistasis may be important for identifying true interactions and thereby revise genetic networks that have not applied this model[43].

In contrast to aggravating interactions, so-called alleviating interactions occur when the double-mutant fitness is greater than expected, such as cases in which the fitness defect of a double mutant is no greater than for either of the single mutants. This often occurs when genes function in the same non-essential pathway or complex. Indeed, a quantitative analysis of an SGA interaction map[44] that focused on genes involved in endoplasmic reticulum (ER) to Golgi transport seems to support this idea, because genes in the same pathway deviated from the expected multiplicative double-mutant phenotype and displayed a level of fitness resembling the single-mutant phenotypes[25]. Thus, genes