to target the second allele, thereby generating homozygous knockout fetuses. Using this approach, each targeting event requires only 2.5 months, and a calf homozygous with respect to two targeted genes can be produced in less than 24 months (**Fig. 1a**).

The third and fourth rounds of marker selections present a challenge for sequentially targeting a second gene. To circumvent this problem, the authors recycled the same selectable markers[5], *neo*^{r} and *puro*^{r}, in their sequential gene targeting efforts, using the Cre-*lox*P system to remove the drug resistance markers used in the first and second rounds of gene targeting. This allows them to use limited selection markers to target multiple genes in a single animal. The *neo*^{r} and *puro*^{r} marker genes were not expressed in the cloned fetuses, probably owing to methylation of these genes in the donor cells during cloning reprogramming, as transgenes are often silenced by DNA methylation in transgenic animals[6]. Whether silencing of the marker genes will be a persistent feature of cloning or a random inactivation phenomenon has yet to be determined. The finding that the Cre recombinase plasmid integrated into the genome in most fetuses was also unexpected, but this problem might be solved with further technical improvements to the methodology.

Primary somatic cells have a finite lifespan in culture and would probably become senescent after one or two rounds of gene targeting. Kuroiwa *et al.* used serial cloning to rejuvenate primary somatic cells in order to accomplish multiple rounds of gene targeting. Cell rejuvenation in clones by rebuilding of eroded telomeres from aged donor cells in cattle[7,8] has been confirmed previously. Telomere reprogramming has also been found after serial cloning in cattle[9] and mice[10]. Notably, the pregnancy success rate after four rounds of fetal serial cloning was as high as 71%; however, the efficiency for term development is not yet known. The discrepancy between this study and previous serial cloning reports[9,10], which found progressive decreases in cloning efficiency, is probably due to the different source of donor cells (adult versus fetal cells).

Bigger, better, faster

Sequential targeting will undoubtedly be used to produce valuable livestock and create cattle resistant to deadly diseases, such as bovine spongiform encephalopathy (BSE). BSE is believed to be caused by prions, aberrant forms of a normal prion protein, PrP. Approximately 200,000 cattle have been diagnosed with BSE[11] and more than 130 people have died from BSE[12] since the first outbreak in 1996. This and previous studies[11,12] indicate that *PRNP* may be knocked out to create cows resistant to BSE. Although certain lines of PrP knockout mice are healthy, the health of PrP knockout cattle and the effectiveness of making such cattle resistant to BSE infection have not yet been tested.

Another benefit of mutating *PRNP* applies to using cattle as bioreactors. Medical products such as human immunoglobulin and human serum albumin produced in transgenic cattle must be quality-controlled to be prion-free. Mutating *PRNP* in these cattle could help ensure that bovine-derived recombinant protein products are free of prions.

Another area of livestock research that will benefit from the technique is xenotransplantation. The demand for organs suitable for human transplantation far exceeds the current donor supply. Research in the area of xenotransplantation has focused on the development of transgenic pigs that express human genes[13] or carry targeted mutations in detrimental genes[14]. The ability to create multiple genetic modifications could be used to disrupt active porcine endogenous retroviral sequences in the pig genome and eliminate cross-species retroviral infection (xenozoonosis). The potential to insert beneficial genes, mutate detrimental genes and eliminate the potential for xenozoonosis by sequential cloning are just a few of the potential applications of rapid, serial targeting.

Finally, sequential cloning in livestock may also benefit the development of large-animal models of human disease in areas where rodent models prove difficult to create or are ineffective at mimicking human disease phenotypes[15].

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Aging counts on chromosomes

Oscar Fernandez-Capetillo & André Nussenzweig

The acquisition of an abnormal number of chromosomes is a hallmark of many human cancers. A new study indicates that unequal segregation of genetic material to daughter cells during cell division can also lead to premature senescence and accelerated onset of a variety of aging phenotypes.

The French comedian Maurice Chevalier once said that "growing old isn't so bad when you consider the alternative." It is becoming clear that the accumulation of DNA damage has a key role in age-related cellular degeneration. Normal aging itself is associated with increased mutation rates and genomic instability, which may also contribute to the increased cancer risk that comes with age[1,2]. Furthermore, the inactivation of certain DNA repair pathways increases the rate at which aging phenotypes develop[1]. At the cellular level, one hallmark of these 'accelerated' aging models is the increase in various types of chromosomal abnormalities, including mutations, translocations, fusions and fragmented chromosomes. Damaged chromosomes are detected by a protein network that triggers either cell death (apoptosis) or a permanent arrest of cell division (senescence),

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thereby eliminating damaged cells that might otherwise pose a risk for malignant transformation. In addition to DNA damage, there are many other stimuli that trigger the apoptotic and senescence end-point, including oxidative stress, replicative exhaustion, telomere dysfunction and oncogene activation. On page 744 of this issue, Darren Baker and colleagues[3] illustrate that the accumulation of an abnormal number of chromosomes—even if these are structurally intact—also leads to cellular senescence and progeria in a mouse with a compromised mitotic checkpoint.

The mitotic checkpoint

Mitosis is the last gate at which the cellular checkpoint machinery can ensure that a proper genomic content is passed on to both daughter cells. The spindle or mitotic checkpoint[4,5] accomplishes this task by sensing the proper attachment of microtubules to protein complexes called kinetochores, as well as by monitoring the tension between the kinetochores and sister chromatids. The checkpoint signal emanating from the kinetochores delays entry into anaphase until each chromatid is properly attached to the microtubules. The proteins belonging to this checkpoint machinery include Bub1, Bub3, CENP-E, Mad1, Mad2, BubR1 (also called Mad3) and Mps1. By perhaps two independent mechanisms, the signal generated by these proteins leads to the inactivation of a complex with E3 ubiquitin ligase activity (anaphase-promoting complex) that promotes entry into anaphase[6].

Defects in the spindle assembly checkpoint provoke chromosome mis-segregation, aneuploidy and cell death. For example, mice lacking Mad2 have enhanced rates of chromosome gain and loss and do not survive beyond embryonic day 6.5 as a result of extensive apoptosis[7]. Similarly, recent studies have shown that targeted disruption of *Bub1b* (encoding BubR1) leads to early embryonic lethality in mice[8], and that severe depletion of BubR1 or Mad2 in human cancer cells inhibits tumor growth[9,10]. In contrast, heterozygosity with respect to *Bub1b* or *Mad2l1* (encoding Mad2) results in mis-segregation of chromosomes and an increase in aneuploidy without loss of viability. As a result, $Bub1b^{+/-}$ and $Mad2l1^{+/-}$ mice are more susceptible to tumorigenesis[11,12]. This suggests that although the complete absence of the mitotic checkpoint is incompatible with life, partial disruption can contribute to the development of cancer. Consistent with this idea, levels of mitotic checkpoints proteins are diminished, but not completely absent, in several human cancers[13].

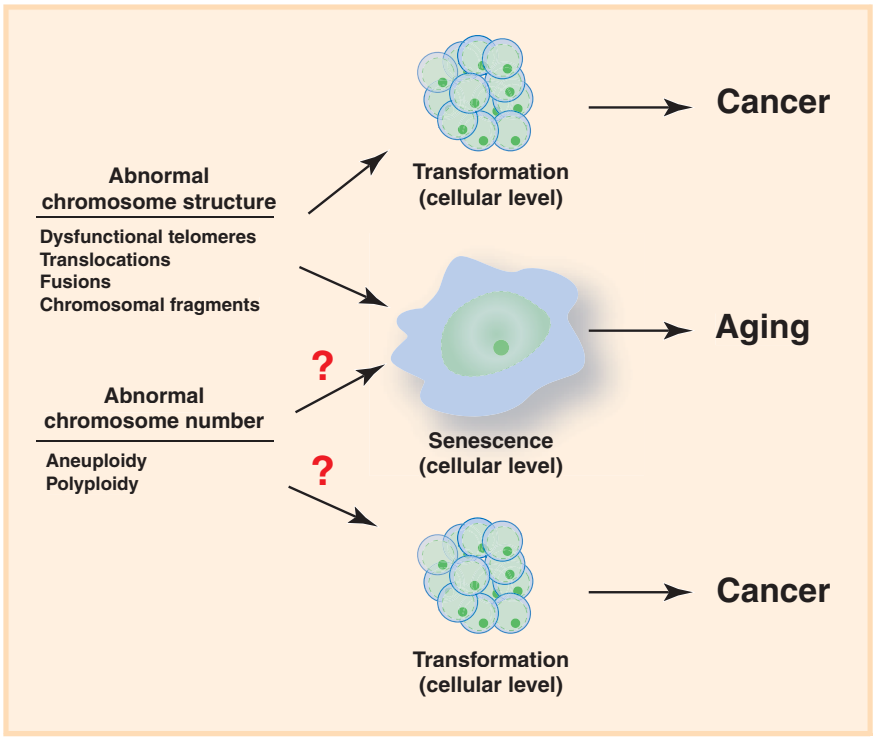

Figure 1 The consequences of chromosomal instability. The model proposes that abnormalities in chromosomal numbers might have biological consequences similar to those triggered by abnormalities in chromosomal structure: cancer and aging.

Stand up and be counted

The study by Baker and colleagues[3] takes this issue of dosage a step further by developing a hypomorphic mouse model (with genotype $Bub1b^{H/H}$) in which BubR1 levels are minimal. $Bub1b^{-/H}$ mice, which express BubR1 at 4% of normal levels, die at birth, whereas $Bub1b^{H/H}$ mice (which express BubR1 at 11% of normal levels) initially appear normal but show a short lifespan and premature onset of several progeroid features, including cataracts, weight loss, muscle atrophy, thinning of the skin, reduced ability to repair wounds and infertility. Consistent with other models of accelerated aging, senescent cells accumulated in tissues as well as in mouse embryonic fibroblast (MEF) cultures from $Bub1b^{H/H}$ mice. There were no detectable defects in chromosomal repair or associated structural aberrations, despite the fact that the canonical senescence response proteins p53, p21, p16 and p19 were activated. What, then, is the lesion that triggers senescence, and ultimately aging? The only discernable defect in $Bub1b^{H/H}$ MEFs was a high rate of chromosomal mis-segregation and an accumulation of aneuploid cells, and this defect was even more profound in $Bub1b^{-/H}$ MEFs. Furthermore, BubR1 levels decreased during normal aging in several tissues from wild-type mice. Taken together, these results suggest that instability of chromosome number, in addition to chromosomal structure, might trigger senescence and aging (**Fig. 1**). It is noteworthy that in contrast to most human progeroid disorders, which have defects in DNA repair[1], Down syndrome is caused by chromosome dosage imbalance (trisomy for chromosome 21) and is also associated with accelerated aging phenotypes.

Aneuploidy in cancer and aging

It is formally possible that senescence is not triggered by aneuploidy *per se*, but rather by an unidentified senescence-suppressor function mediated by BubR1. After all, haploinsufficiency of BubR1 also leads to a weakened spindle checkpoint and to frequent chromosomal mis-segregation with no increase in senescence or aging[12]. On the other hand, the senescence response may be triggered only when abnormalities of chromosome number increase beyond a certain threshold. This checkpoint arrest would have to be overridden in cancer cells by an antiapoptotic mutation, since these often have high rates of chromosomal gains and losses. Notably, a p53-dependent checkpoint that eliminates polyploid cells has been recently identified, and the existence of an aneuploidy checkpoint

has also been suggested[14]. Given that the tumor suppressors p53 and p19 are upregulated in *Bub1b*$^{H/H}$ senescent cells, it would be interesting to determine whether loss of these in *Bub1b*$^{H/H}$ mice would relax cell cycle control, promote tumorigenesis and perhaps alleviate some of the phenotypes associated with aging.

Whether aneuploidy is a specific driving force in the development of cancer is controversial. With the demonstration that severe disruption of *Bub1b* leads to aging-related phenotypes, the study by Baker *et al.*[3] now adds aneuploidy and aging to the debate about the relationship between biological cause and consequence. Regardless of these hierarchical considerations, it is apparent that although silencing of the mitotic checkpoint (by pharmacological agents, for example) may be beneficial for inhibiting the growth of cancer cells, the price we may pay is premature aging.

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Resolving schizophrenia's CATCH22

Assen Jablensky

Schizophrenia is a severely disabling disorder with a lifetime risk of ~1%, which accounts for 2.8% of the global burden of disease. A new study provides evidence that a third gene in the 22q11 region is associated with schizophrenia, further confounding an already complicated story.

A genetic contribution to the etiology of schizophrenia is well established, and environmental exposures, from neurodevelopmental insults to psychosocial factors, are also suspected to have a role. The search for associated genes and pathogenetic pathways is hampered by complex genetics, lack of a pathognomonic neuropathology or neurophysiology, and a phenotype defined solely by psychopathological criteria (*e.g.*, delusions and hallucinations). More than 30 genome scans and numerous association studies have resulted in multiple suggestive linkage findings and tentative associations[1,2]. Further insights are being gained through studies bridging linkage and association studies with gene expression and functional effects in experimental animals that model aspects of the disease phenotype. On page 725 of this issue, Jun Mukai and colleagues[3] report on a new candidate gene, *ZDHHC8*, in the ~3-Mb region on chromosome 22q.11.2, which is heterozygously deleted in DiGeorge/velocardiofacial syndrome, also known by the particularly apt acronym CATCH22 (cardiac abnormality/abnormal facies, T-cell deficit, cleft palate, hypocalcemia; OMIM 188400).

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From association to knockout

The 22q.11 microdeletion region is associated with increased risk for a range of neuropsychiatric phenotypes, including schizophrenia, rapidly cycling affective bipolar disorder, learning disability, attention

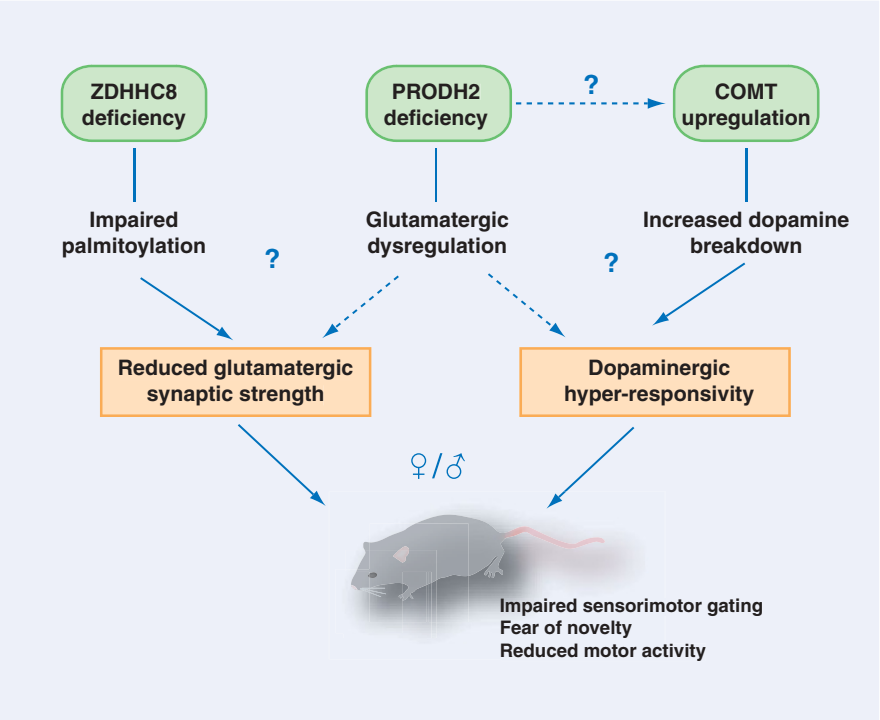

Figure 1 A deficiency in any of three schizophrenia-associated genes on 22q11 can result in behavioral impairments in knockout or heterozygous mice. Further dissection of independent and synergistic effects of these genes in animal models, and endophenotype refinement in individuals with schizophrenia and in controls, will be required to fully delineate the causative pathways.