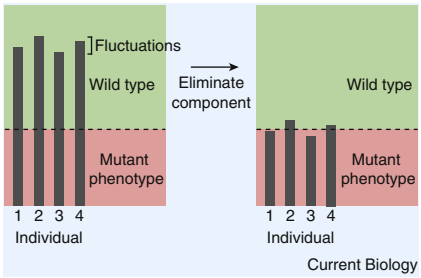

Figure 2. A conceptual framework to explain partial penetrance.

Removal of a component in a buffered system results in a partially penetrant phenotype. In the example shown, 50% (2/4) of the animals will display a mutant phenotype. See [14] for an intriguing recent report on the phenomenon of partially penetrant phenotypes.

(Figure 2). The enhancer studies fit nicely into this picture. Two enhancers ensure firing probability and/or sufficient mRNA output above a threshold even in the presence of perturbations, while removal of one enhancer decreases the firing probability and/or level of mRNA output to a threshold where fluctuations result in a significant impact in some but not all animals or cells (Figure 2). The bottom line of all this is quite simple and surely would have pleased Spemann and Waddington: having two copies of the same thing is good, but the adaptive advantage of such duplication may only be apparent under specific, perturbing conditions.

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DOI: 10.1016/j.cub.2010.07.035

Female Meiosis: Coming Unglued with Age

Chromosome abnormalities in humans are strikingly associated with increasing maternal age. Studies in mice implicate loss of chromosome cohesion as an important cause of age-related meiotic errors in the oocyte.

Patricia Hunt* and Terry Hassold

In the early 1930s, the noted British geneticist Lionel Penrose realized that Down syndrome babies are far likelier to be born to older women [1]. At the time, Down syndrome was known only as a form of mental retardation with characteristic phenotypic features. The understanding that the condition results from three copies of chromosome 21 (trisomy 21) would not be made for another 25 years [2,3]. In short, the recognition that advancing maternal age affects the likelihood of producing a normal, healthy child predated our understanding that chromosome abnormalities represent not only the leading cause of birth defects in humans, but also the major cause of pregnancy loss.

The meiotic errors that result in chromosome abnormalities are common in humans, and approximately 0.2–0.3% of newborn infants are trisomic [4]. However, this represents just the tip of a large iceberg, because most aneuploid conceptions die *in utero*. Indeed, studies of preimplantation embryos suggest that a large proportion, if not a majority, of fertilized human eggs have extra or missing chromosomes [5]. Because the vast majority of errors result from the fertilization of a chromosomally abnormal egg by a normal sperm, attention has focused on why human female meiosis is so error-prone.

How does maternal age factor into this equation? Hugely. Among women in their twenties, approximately 2–3% of clinically recognized pregnancies involve trisomic fetuses but, among women in their forties, this value skyrockets to over 35% (Figure 1). Given the importance of the age effect and the research attention devoted to it, it may seem odd that we know so little about its basis. Indeed, like the enigmatic smile on the Mona Lisa, the mechanism(s) by which maternal age induces its effects on chromosome segregation have remained a tantalizing mystery. However, three papers in this issue of *Current Biology* [6–8] lend strong support to a mechanism involving the ties that bind meiotic chromosomes. Physical connections — whether between sister chromatids during mitosis or between homologs and sister centromeres during meiosis — are essential for proper chromosome segregation and depend on a class of proteins known as the cohesins [9,10]. The production of haploid

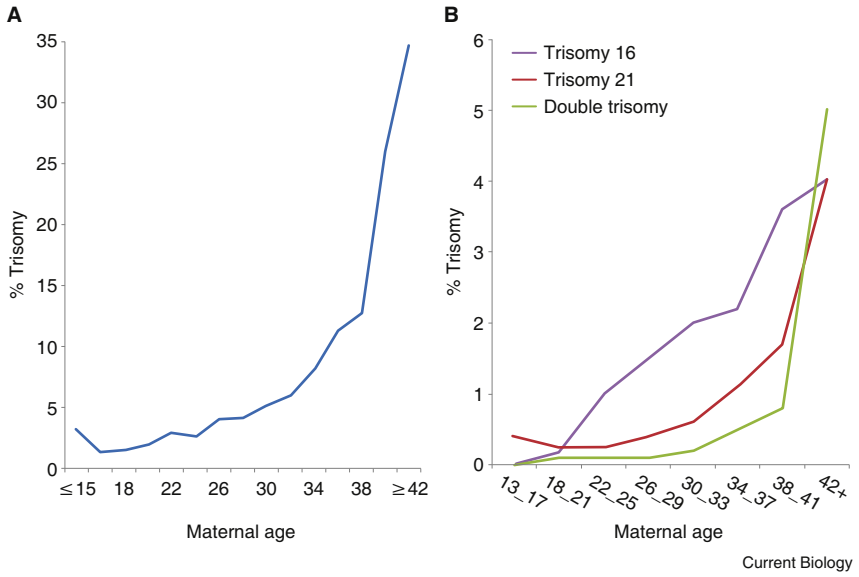

Figure 1. Maternal age affects the incidence of trisomy in clinically recognized pregnancy.
(A) In the human female, the risk of a trisomic pregnancy is increased around the time of menarche. The incidence decreases slightly by the late teens and a 'flat line' period of 10–15 years ensues during the prime reproductive years. This is followed by an exponential increase in incidence in the mid 30s. (B) The association with age varies among chromosomes. For example, trisomy 21 shows the characteristic exponential increase in the mid-thirties, but trisomy 16 (the most common trisomy in miscarriages) shows a linear increase with age and double trisomies are effectively limited to women in their 40s. (Modified from [20]; differences in the axes between (A) and (B) reflect the limited sample sizes for individual trisomies).

gametes requires a bit of cohesin magic: during meiosis I, crossovers and cohesion between arms lock homologs together until anaphase I, when degradation of all cohesin except that between sister kinetochores allows homologs to segregate. At anaphase II, the cohesion remaining between sister kinetochores is lost, allowing sisters to segregate (Figure 2A). These specialized chromosome acrobatics are accomplished by substituting meiosis-specific players (i.e., REC8, STAG3, and SMC1β) for three of the four components of the cohesin complex and by the protection at the first meiotic division of cohesin between sister centromeres by Shugoshin, a highly conserved family of proteins that also function in mitotic cell division [11].

Cohesins are assembled on chromosomes during S phase and degraded at anaphase during every cell division. But the situation in the egg is unique because S phase takes place during fetal development, while the cell division itself does not occur in humans until at least a dozen years later. Thus, a fundamental question is: does chromosome segregation in the human egg depend on a complex of cohesin proteins that is many years old and could age-related degradation of this complex at the first meiotic division be the basis of the human maternal age effect?

The paper by Revenkova *et al.* [6] in this issue bears directly on the first half of this question. Several years ago, these investigators created a mouse lacking a meiosis-specific cohesin (i.e., the SMC1β-deficient mouse) and provided the first direct evidence of an age-related decline in chromosome cohesion in mammalian oocytes [12]. Their current paper examines possible age-related cohesion deterioration using a mouse carrying a floxed *Smc1β* gene and *Cre* recombinase under the control of the *Gdf9* gene. The strategy is a clever one: the SMC1β protein is made and incorporated into the meiotic cohesin complex normally during meiotic prophase in the fetal ovary. However, because *Gdf9* is expressed shortly after birth, additional SMC1β protein cannot be made during the extended period of meiotic arrest or during oocyte growth. Thus, if protein turnover is critical for the maintenance of cohesion, these females should be in big trouble. Surprisingly, and in marked contrast to the conventional SMC1β-deficient mouse, the conditional knockout female is fully fertile and shows no signs of early reproductive senescence. Moreover, an analysis of metaphase I oocytes in young and old females provided no evidence of loss of cohesion. While this study does not rule out the possibility that new cohesin proteins get incorporated into the complex, it demonstrates beautifully that the cohesion established during premeiotic S in the fetal ovary is sufficient to maintain chromosome connections in the mouse, even in old oocytes.

What about the second half of the question — the possible association between age-related degradation of cohesin and increasing rate of aneuploidy? The other two cohesin papers in this issue [7,8] address this by analyzing much older, naturally aged females than those studied by Revenkova *et al.* [6]. In the first, Richard Schultz and Michael Lampson and colleagues [7] conducted live cell imaging and conventional cytological studies of oocytes from young and very old (>16 month old) B6D2F1/J females. They first assessed centromere cohesion, asking whether sister centromeres were locked together as closely in old as young oocytes. They weren't. In studies of cells at metaphase I and metaphase II they found that the distance between sister kinetochores was 25–50% greater in oocytes from older females, suggesting an age-related loss of centromere cohesion. An analysis of REC8, the cohesin component that is degraded at anaphase, revealed an interesting age-related difference: Although by western analysis total protein levels were similar, immunofluorescence staining of chromosome-associated REC8 was much less intense in oocytes of older females. This should predispose to meiotic errors involving the premature separation of homologs (Figure 2B) and sister chromatids (Figure 2C,D) and, indeed, live cell imaging studies confirmed this prediction.

The second paper by Mary Herbert and her colleagues [8] took a slightly different approach but reached remarkably similar conclusions. Examining 2 month old and 14 month old females from a 'long-lived' strain of mouse (C57BL/Icrfa^{t}) and focusing on live cell imaging, they saw increases in inter kinetochore distances, reductions in REC8 signals, and increases in anaphase defects in oocytes from old females. In addition, they observed an age-related depletion of SGO2, the

protein necessary for preventing the degradation of centromere cohesin at anaphase I, suggesting another route to aneuploidy.

The age-dependent loss of cohesin reported in these studies of naturally aged mice supports previous observations from studies of cohesin mutants in mice and *Drosophila* [13,14]. It also provides a plausible explanation for a wide spectrum of nondisjunctional events — not just abnormalities involving whole chromosomes at the first meiotic division, but abnormalities involving mis-segregation of sister chromatids (Figure 2B–D). This is an important consideration, since human trisomies are thought to originate in a variety of ways [15].

A role for premature cohesion loss in meiotic errors seems indisputable, but can we conclude that cohesin degradation is the basis of the human maternal age effect? Clearly chronological age — the shear *amount* of time spent in prophase arrest — is an insufficient explanation, and in this respect the naturally aged mouse models provide important insight: although both studies report a linear age-related decline in chromosome associated cohesins, the expected increase in aneuploidy is only evident in reproductively senescent females. This suggests that physiological rather than chronological age is the ultimate culprit, an intellectually satisfying conclusion for several reasons. First, if time alone were the critical factor, the 12+ years preceding puberty in the human should ensure a maternal age effect by the time of puberty. In addition, although it is widely assumed that there is a magic bullet — a single cause of the maternal age effect — in fact, human studies suggest multiple age effects, some of which are unlikely to involve abnormalities in cohesins [16]. Indeed, the fact that the error rate is increased in the human female at both extremes of reproductive age (Figure 1A), that different human chromosomes exhibit strikingly different age curves (Figure 1B), and that data from mouse studies implicate other aspects of physiological aging [17,18] combine to suggest that factors other than chronological age play a significant role.

The studies in this issue beg for direct studies of cohesins in human oocytes and, importantly, a recent study provides just such data [19]. The results, however, provide some surprising counterpoints to the mouse data.

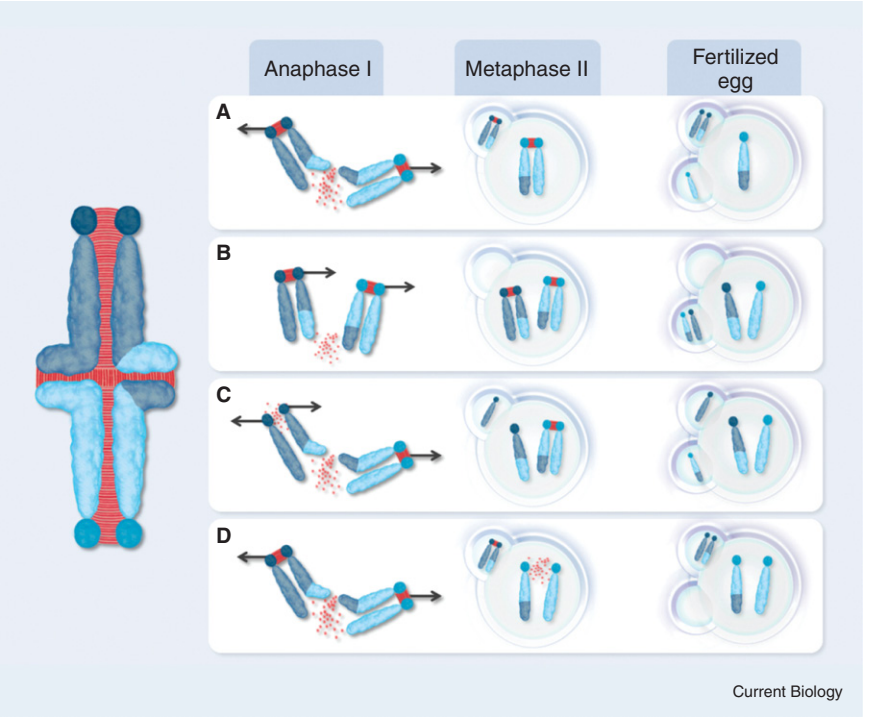

Figure 2. Potential effects of premature cohesin loss on meiotic chromosome segregation.
The large schematic on the left depicts a pair of homologs with a single distal crossover. (A) During normal meiosis, cohesion (denoted in red) is lost sequentially; arm cohesion is lost at anaphase I, allowing homologs to segregate to opposite spindle poles (arrows), but cohesion between sister centromeres is retained to facilitate the orientation and segregation of sister chromatids at anaphase II. (B) Premature loss of arm cohesion would make homologs with a single crossover near the end of the chromosome arm particularly vulnerable to error; i.e., loss of their physical connection would allow homologs to segregate independently, and segregation to the same spindle pole would result in both copies of the chromosome in the metaphase II arrested egg and none in the polar body (or vice versa). Normal segregation of sister chromatids at the second meiotic division following fertilization would result in a trisomic conceptus. (C, D) Premature loss of the connections between sister centromeres could create a variety of problems, depending upon the timing of the loss. (C) Loss occurring during the first meiotic division would allow sister kinetochores to act independently. In this example, loss of sister centromere cohesion in one homolog results in the proper segregation of one homolog and the premature segregation of sister chromatids at anaphase I in the other. This results in a missing chromatid in the polar body and an extra chromatid in the egg (or vice versa). This imbalance can be corrected at the second meiotic division if the single chromatid is segregated to the polar body but if, as depicted, it remains in the egg, the conceptus would be trisomic. (D) Loss of centromere cohesion at anaphase I or during metaphase II arrest would dramatically increase the likelihood of an error at the second meiotic division because sister chromatids would no longer be constrained to segregate from each other.

Specifically, in studies of oocytes from 18–34 year old women, the authors where unable to identify age-related changes in immunolocalization patterns of REC8, STAG3, or SMC1β. Thus, direct evidence linking age-related cohesin degradation to human oogenesis is lacking, and this study underscores the important point: although premature loss of cohesion is almost certainly an important contributor, we still don't understand the physiological basis of maternal age-related aneuploidy. In short, these studies provide new and exciting insight with direct relevance to humans but, for the time being, the Mona Lisa smile on the face of maternal age remains intact.

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DOI: 10.1016/j.cub.2010.08.011

Visual System: How Does Blindsight Arise?

Some patients can discriminate unseen visual stimuli within a field defect caused by damage to the primary visual cortex. The pathways for this 'blindsight' have never been established, but recent studies implicate hitherto overlooked cells in the thalamic LGN.

Alan Cowey

The primary visual cortex, or V1, is the major cortical destination of the input from the eyes and contains a 'map' of the image on the retina. Hardly surprising, then, that when it is partly destroyed, as often happens following stroke or traumatic injury to the back of the brain, the patient has a visual field defect in which he is clinically blind — part of the map has been deleted. Why the term 'clinically blind'? Why not just blind? The answer lies in a controversy that began almost a century ago between two eminent British neurologists. Gordon Holmes [1] concluded that, in the absence of part of the striate cortex, the blindness is complete, the field defect is absolute. But George Riddoch [2], contemporary and colleague, disagreed and argued that such patients could perceive motion within their otherwise blind field. This controversy, like old volcanoes, has rumbled on ever since and pervades much of the research on what is now called blindsight.

Several investigators have studied the role of V1 in monkeys, the visual pathways of which closely resemble our own. They have found an ever increasing range of residual visual sensitivity and discrimination within the visual field defect caused by removing part, or even all, of V1 (see [3] for review) — not just reflexes such as the pupillary response to light, but also learned voluntary responses to the orientation, shape, brightness, size and motion of visual stimuli. Unsurprisingly, monkeys, unlike human patients, were considered to have genuine residual vision and this was attributed to the many other pathways from the eye into the brain, as shown schematically in Figure 1.

But a huge puzzle remained: why don't patients have the same abilities given that they too have these other pathways? The answer lies in the different ways in which monkeys and patients had been tested for decades. Patients were asked whether they saw anything in their field defects and with the exception of motion — and perhaps not even that — they said "No". But monkeys were not asked this question. Instead, and in order to get a reward, they had to choose between two visual stimuli — to make forced-choice decisions. This difference was highlighted when several investigators [4,5], using forced-choice guessing, demonstrated that patients were just as good as monkeys, and Cowey and Stoerig [6] showed that monkeys categorized visual stimuli that they could detect as being not like a light, but invisible. In both cases the subjects were showing 'blindsight', excellent forced choice performance in the face of denial of consciously seeing anything.

Once the relevance of investigations on monkeys to human blindsight was established the search was renewed in monkeys for the pathways that underlie it. Did all the pathways shown in Figure 1 contribute or only one or a few? Early work [7] showed that monkeys with part of V1 removed could still move their eyes to targets confined to the field defect but that this ability was destroyed if the corresponding part of the superior colliculus, which also has a 'map' of the retina, was subsequently extirpated. This still remains strong evidence that the pathway from the eye to the superior