HUMAN CYTOGENETICS: 46 CHROMOSOMES, 46 YEARS AND COUNTING

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Human cytogenetics was born in 1956 with the fundamental, but empowering, discovery that normal human cells contain 46 chromosomes. Since then, this field and our understanding of the link between chromosomal defects and disease have grown in spurts that have been fuelled by advances in cytogenetic technology. As a mature enterprise, cytogenetics now informs human genomics, disease and cancer genetics, chromosome evolution and the relationship of nuclear structure to function.

HUMAN GENETICS AND DISEASE

> "Before a renewed, careful control has been made of the chromosome number in spermatogonial mitoses of man we do not wish to generalize our present findings into a statement that the chromosome number of man is 2n = 46, but it is hard to avoid the conclusion that this would be the most natural explanation of our observations."[1]

The field of human cytogenetics was launched in 1956 with this hesitant statement. The serendipitous addition of water to a suspension of human mitotic cells[2], before they were fixed and dropped onto glass microscope slides, caused the chromosomes to spread apart from each other so that Tjio and Levan[1] could accurately count the full complement of 46 human chromosomes (FIG. 1). The number 46 was independently confirmed by Ford and Hamerton in the same year[3]. The prevailing dogma had held the count at 48 for more than 30 years, ever since the geneticist Thomas Painter had reported on his observations of testicular cells[4]. Establishing the correct number and this simple technological advance set off many discoveries that associated specific chromosomal abnormalities with disease in the late 1950s and quickly established the central role of cytogenetics in medicine.

In the ensuing years, human cytogenetics has been transformed by technological advances that have combined innovations in molecular biology, chemistry and instrumentation. Cytogeneticists can now extract far more information about the human genome than just chromosome number. Each chromosome can be easily recognized — even in the highly rearranged karyotypes of tumour cells — by colour-coded labels. The resolution and sensitivity of analyses have improved more than 10,000-fold in a very short time, first with the introduction of banding technology and later with fluorescence *in situ* hybridization (FISH). Extremely subtle alterations in chromosome composition can now be detected and analysed for their association with disease. Cytogeneticists have been freed from their early dependence on mitotic cells by techniques that make it possible to evaluate the karyotype of non-dividing cells. Other approaches yield quantitative information on chromosomal content and structure and allow cytogeneticists to isolate specific chromosomes for molecular analyses. The latest technology allows genome-wide screens for the loss or gain of chromosomal material to be conducted at unprecedented resolution. Most importantly, the cytogenetic map is cross-referenced to the human draft sequence at thousands of points. These connections greatly facilitate the translation of microscopically visible clues of the molecular basis of disease to the actual genes that are disrupted or altered in dosage.

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Figure 1 | **The picture that established 46 as the chromosome number in man.** Reproduced with permission from REF. 1 © (1956) Mendelian Society of Lund for the Scandinavian Association of Genetics.

This article outlines the history of the main technological advances that have occurred in human cytogenetics during the past 46 years. It highlights the impact that these advances have on our understanding of the molecular basis of human disease and of the structure, function and evolution of our chromosomes.

A late start, but rapid recovery

Flemming and Arnold first observed human chromosomes in the 1880s. It is therefore remarkable that such a fundamental aspect of human biology as chromosome number could have escaped the scientific community until 1956, three years after the structure of the DNA helix was elucidated[5]. Friedrich Vogel and Arno Motulsky[6] ascribe this delay to both technological and politico-social causes. They assert that most laboratory-based medical scientists at the time were uninterested in human genetics; they considered humans to be far too complex and preferred to focus on simpler model organisms that could be more easily manipulated. Also, many serious geneticists had dissociated themselves from human genetics during the eugenics movement in the early 1900s, which reached its nadir with the horrific practices of the Nazis. However, soon after the number 46 was firmly established, scientists readily applied the new cytogenetic technique to the investigation of phenotype–genotype correlations in humans and began to tap useful information from naturally occurring chromosomal rearrangements.

Human cytogeneticists were dealt a good hand by evolution. Had human chromosomes been as morphologically similar as those of mice, or as tiny and numerous as those of most birds, progress in cytogenetics would have been much slower. Fortunately, differences in the relative size of human chromosomes and the position of the centromeric constriction allowed cytogeneticists to match up the 23 pairs and classify them into seven groups (A to G) with relative ease[7].

Although crude, these early karyotypes allowed the discovery that some human disorders result from changes in chromosome number or appearance. In 1959, trisomy 21 was shown to be the cause of Down syndrome[8], and abnormalities in the number of sex chromosomes were shown to cause Turner syndrome (X0) and Klinefelter syndrome (XXY), two frequent disorders of sex differentiation[9,10]. It also became quickly apparent that most miscarriages were caused by abnormalities in chromosome number[11].

Work on the diminutive, but deadly, 'Philadelphia' chromosome established a new model for using cytogenetic clues to find genes that, when altered, cause human disease. In 1960, cytogeneticists recognized the Philadelphia chromosome as the cause of chronic myeloid leukaemia (CML)[12]. Thirteen years later, this chromosome was shown by Janet Rowley to be the product of a translocation between chromosomes 9 and 22 (REF. 13). The point at which these two chromosomes break and fuse was the obvious place to look for the molecular explanation of this disease. Indeed, by using the derivative chromosomes in molecular assays, the translocation was shown, in 1985, to create a new hybrid gene of *BCR* and *ABL* (breakpoint cluster region and v-abl Abelson murine leukaemia viral oncogene homologue 1)[14]. Subsequent studies showed that constitutive activation of BCR–ABL, a tyrosine kinase, affects many cellular pathways and leads to the cancer phenotype (reviewed in REF. 15). This understanding in turn led to the development of Gleevec (STI571), a drug that was designed to block the function of the BCR–ABL protein and that has proved to be a highly successful treatment for CML[16].

The rudimentary chromosome preparations of the early 1960s yielded other breakthroughs in human genetics. Lejeune recognized the first inherited deletion syndrome, Cri du Chat, in 1963; patients with severe mental retardation and a characteristic cat-like cry were all missing a portion of the short arm of chromosome 5 (REF. 17). In the same year, a patient with bilateral retinoblastoma was found to have a deletion of the long arm of a D-group chromosome[18]. Later work by Cavenee *et al.*[19] provided paradigm-setting proof of KNUDSON'S TWO-HIT HYPOTHESIS[20] by showing that the cancer arises owing to the loss of one allele of the *RB* (retinoblastoma) gene in 13q14 and mutation of the other allele. One of the first autosomal human genes to be mapped, the gene for the DUFFY BLOOD GROUP, was assigned to chromosome 1 because of the consistent way it tracked in families as a visible cytogenetic anomaly near the centromere of chromosome 1 (REF. 21).

Chromosomal barcodes

The power of cytogenetic analysis redoubled in the late 1960s with Torbjorn Caspersson's development of staining protocols that produced highly reproducible patterns of dark and light bands along the length of each chromosome[22]. These banding patterns became the barcodes with which cytogeneticists could easily identify chromosomes, detect subtle deletions, inversions, insertions, translocations, fragile sites and other more complex rearrangements, and refine breakpoints (FIG. 2).

KNUDSON'S TWO-HIT MODEL
First proposed by Alfred Knudson in 1971, this model indicates that successive hits, such as deletion or mutation, in both alleles of a tumour-suppressor gene are required to turn a normal cell into a cancer cell.

DUFFY BLOOD GROUP
An antigenic variant of a chemokine receptor that is expressed on red blood cells.

The bands appear only in metaphase chromosomes, and cycling cells are therefore required for this analysis. If cells can be caught in prometaphase — when chromosomes are in the very early stages of condensation — up to 2,000 bands can be discerned[23]; more typically, 400–800 bands are visible. The band-naming convention introduced in 1971 reflects the levels of resolution with which chromosomes can be analysed[24]. Despite the extensive use of these bands, their cause remains an enigma. They correlate with regional differences in base-pair composition, repetitive elements, replication timing and chromatin packaging and can be induced by many methods, but their molecular basis is not understood.

Cytogenetic information moved from the bench to the clinic in the late 1960s with the discovery that fetal cells could be obtained through AMNIOCENTESIS and could be checked for chromosomal abnormalities. Methods were quickly developed to induce fetal cells that had been derived from amniotic fluid to divide in culture and to obtain high-quality banded karyotypes. The same procedures are widely used today to provide prenatal diagnostic information to families.

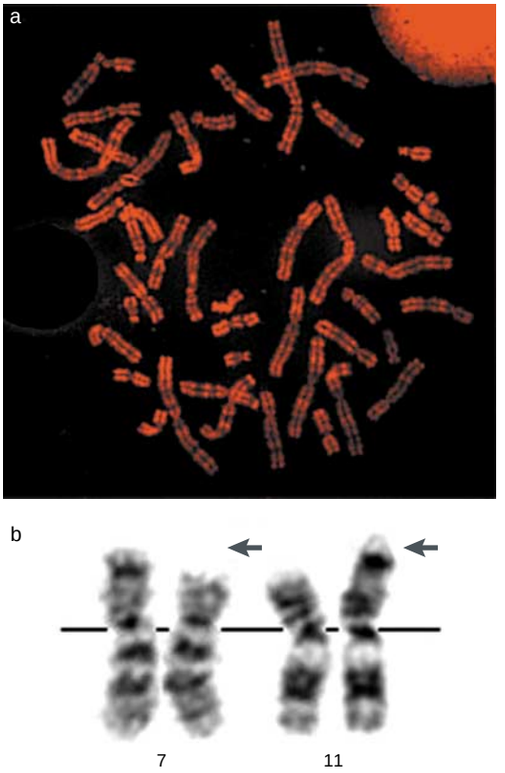

Figure 2 | **Cytogenetic banding patterns of human chromosomes. a** | An R-BANDED metaphase spread. **b** | G-BANDED chromosomes 7 and 11 from an individual with acute myeloid leukaemia, showing the subtle translocation that involves the terminal bands of the p (short) arms — t(7;11)(p15;p15). This translocation generates a hybrid gene of *NUP98* (nucleoporin 98 kDa) and *HOXA9* (homeobox gene A9), which results in leukaemogenesis. Panel **a** was provided by Cynthia Friedman, Fred Hutchinson Cancer Research Center; panel **b** was provided by Diane Norback and colleagues at the Waisman Cytogenetics Center at the University of Wisconsin.

AMNIOCENTESIS
A procedure in which a small sample of amniotic fluid is drawn out of the uterus through a needle inserted into the abdomen. The fluid is then analysed to detect genetic abnormalities in the fetus or to determine the sex of the fetus.

G-BANDS/R-BANDS
Chromosome banding pattern produced by Giemsa staining (G-bands); the reciprocal pattern (reverse or R-bands) can be produced with various other staining procedures.

Through the painstaking analysis of chromosome banding patterns, thousands of chromosomal abnormalities have been associated with inherited or *de novo* disorders, generating many leads to the underlying molecular causes of these disorders (see Online links box at the end of the article). Even today, when high-resolution genetic linkage analysis can be conducted easily, the discovery of a patient whose disorder is caused by a gross chromosomal abnormality is heralded as a valuable resource for locating the disease gene. Solid tumours also present a myriad of complex chromosomal aberrations — each is a possible clue to tumour initiation and progression. The challenge is to navigate from the visible morphological alteration to the sequence level. The next major advances in cytogenetics facilitated that process.

Moving from microscope to molecule

Once a rearranged chromosome has been identified, the next step is to position the translocation breakpoints or deletion boundaries relative to genes on molecular maps. This step can be accomplished by using techniques that physically separate abnormal and normal chromosomes so that they can be independently assayed for gene content. Three methods have been particularly useful in achieving this: somatic-cell-hybrid technology, fluorescence-activated cell (chromosome) sorting (FACS) and FISH (all discussed below). These techniques help researchers to zoom in on the defect from the cytogenetic to the molecular level, and, importantly, they have yielded rough maps for navigating the genome and for allowing more detailed molecular mapping and sequencing.

Somatic-cell hybrids are a fortunate quirk of cell biology. When rodent and human cells are fused in the laboratory, human chromosomes are preferentially ejected, but some are retained[25,26]. This phenomenon was capitalized on by the groups of Weiss and Ruddle, who were the first to use panels of hybrid cell lines, each retaining a different set of human chromosomes, to map genes and anonymous markers to specific chromosomes or portions thereof[27,28]. The chromosomal content of each line, established by cytogenetic analysis, is simply correlated with the results of hybridization assays, functional tests or PCR to assign a gene or marker of interest to a chromosome. Much more precise maps, which served as frameworks for the assembly of the human genome sequence, were generated using panels that contain different chromosomal fragments, such as aberrant chromosomes transferred from the cells of patients[29] or fragments that were experimentally produced by radiation[30].

Originally developed for cell analysis and separation, flow cytometry was adapted in 1979 for the quantitative analysis and sorting of human chromosomes by a team of investigators at the Lawrence Livermore National Laboratory in California[31]. In this technique, chromosomes are released into suspension from mitotic cells and stained with two fluorescent DNA dyes that have different base-pair specificities: this allows all but four human chromosomes (9–12)

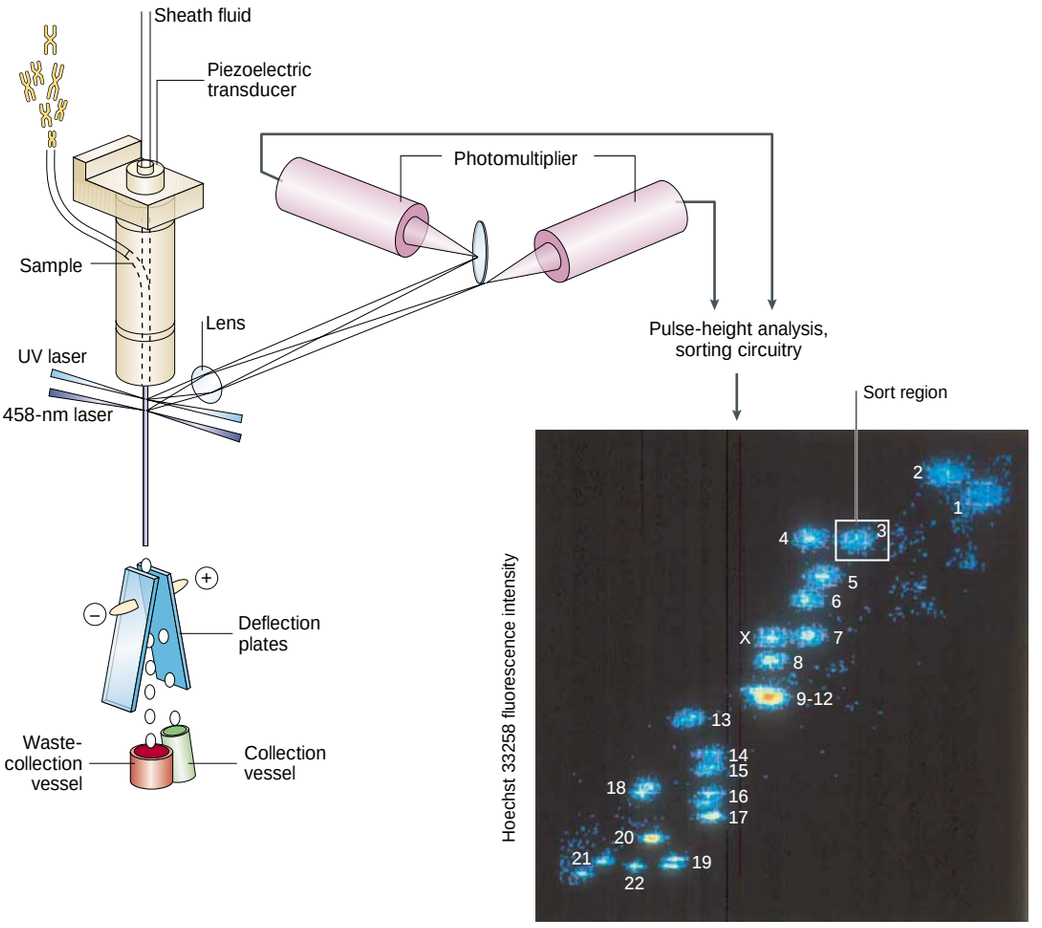

Figure 3 | **Schematic of the discrimination and sorting of human chromosomes by flow cytometry.** Chromosomes that are released from mitotic cells are stained with two DNA-binding dyes with different base-pair specificities, and the fluorescence intensities of each of several thousand chromosomes are measured in a two-laser flow cytometer. In the example shown, the two dyes are Hoechst 33258, which binds preferentially to A•T base pairs, and chromomycin A3, which binds to C•G base pairs. The resulting bivariate 'flow karyotype' (bottom right panel) resolves all chromosomes except for the 9–12 group. In this example, maternal and paternal homologues of both chromosomes 21 and 19 are resolved into separate peaks owing to differences in their DNA content. After measurement, droplets that contain desired chromosomes, such as chromosome 3 in this example (white box), can be deflected into tubes for molecular analyses. UV, ultraviolet. Diagram modified with permission from REF. 95 © (1986) Cold Spring Harbor Laboratory Press; plot provided by Ger van den Engh, Institute of Systems Biology.

to be resolved by a flow cytometer[32] (FIG. 3). The measurements give quantitative information on the extent of normal variation in chromosome size (some vary by 50% in DNA content) and the amount of DNA that is missing or gained in abnormal chromosomes[33,34]. Abnormal and normal chromosomes can also be separated for the molecular characterization of DNA-marker retention or loss[35]. Flow sorting was the key to the production of chromosome-specific DNA clone libraries[36,37], which have been important for constructing detailed, marker-dense physical maps of the genome, especially in the days when tackling the whole genome at once seemed too daunting. Flow sorting continues to be the technique of choice for producing chromosome-specific paints[38] (see below) and for characterizing sequences that are duplicated on more than one chromosome[39].

The FISHing trip

The next advance to revolutionize cytogenetics, FISH, provided a direct link between microscope and sequence. This technique allows the chromosomal and nuclear locations of specific DNA sequences to be seen through the microscope (FIG. 4). Each probe is a cloned piece of the genome that is conjugated to a reporter molecule, such as biotin. After denaturation, the probe is allowed to seek out its complement in the chromosomal DNA, and these locations are then marked with a fluorescent reagent, such as avidin-FITC, that binds to the reporter attached to the DNA probe. Although radioactively labelled DNA and RNA probes had been localized to cytogenetic bands since 1969 (REFS 40,41), the field advanced significantly when groups led by David Ward and Mels van der Ploeg replaced the isotopic labels with fluorescent ones[42,43]. Fluorescent tags are

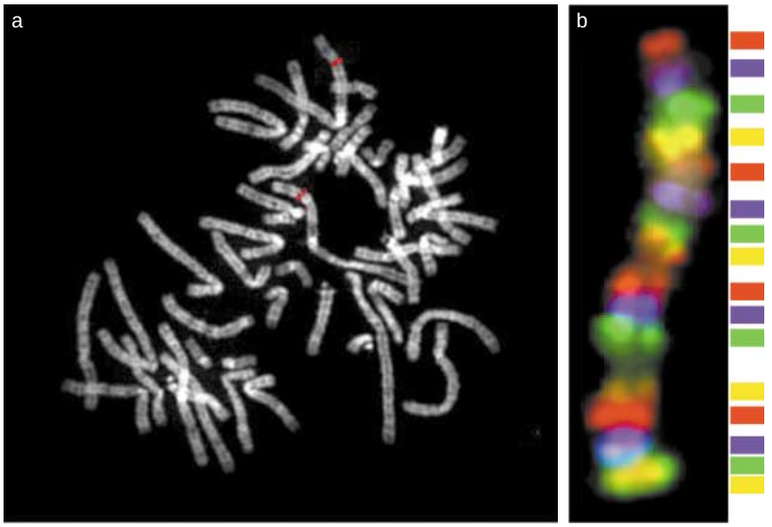

Figure 4 | **Cytogenetic localization of DNA sequences with fluorescence *in situ* hybridization (FISH). a** | FISH produces a fluorescent signal (red) at the sites of a specific DNA sequence; in this case, a 150-kb segment of chromosome 1. Reproduced with permission from *Nature* REF. 53 © (2001) Macmillan Magazines Ltd. **b** | Several probes, each corresponding to a defined genomic segment, can be simultaneously analysed and ordered with respect to each other using multicolour FISH. Reproduced with permission from REF. 96 © (2002) Springer Verlag. Provided by Ullrich Weier, Lawrence Berkeley National Laboratory.

safer and simpler to use, can be stored indefinitely, give higher resolution and opened up prospects for simultaneously locating several DNA sequences in the same cell by labelling them with different fluorochromes.

In less than 15 years, the sensitivity of FISH improved 10,000-fold. This remarkable achievement can be attributed to improvements in the probe labels that made them less bulky, simpler to incorporate into the probe and brighter; in the optics for fluorescence microscopy; and in more mundane, but crucial, aspects of the procedure, such as probe fragmentation and slide storage. By 1985, the first single-copy human gene, thyroglobulin, had been localized to a chromosome band by non-radioactive *in situ* hybridization[44]. This feat was an important milestone, even though thyroglobulin was one of the largest genes known at the time, and specificity was achieved by fastidiously removing all the interspersed repetitive elements from the probe before its use. Today, localizing segments as small as 10 kb is routine and 1 kb is achievable[45]. We now exploit the kinetics of DNA reassociation to pre-anneal the repetitive elements, so that only the unique/low-copy portions of the labelled probe are available for hybridization to chromosomes[41,46]. Using new probes that are based on PEPTIDE NUCLEIC ACID chemistry, the intensity of FISH spots is a reasonable measure of the local amount of complementary target. A good illustration is the study of telomere dynamics in normal and immortalized cells by quantitative analyses of TTAGGG-specific probes bound to the ends of chromosomes[47]. A clever modification of FISH (called COD-FISH) goes even further to reveal the absolute 3′–5′ direction of a particular sequence on the chromosome[48] and to detect inversions and sister-chromatid exchanges[49,50].

The interplay between genome mapping and cytogenetics escalated in the mid-1980s as FISH technology improved and cloned DNA reagents became available through the efforts of many genome mapping and sequencing groups. Using FISH, cytogeneticists could detect chromosomal abnormalities that involve small segments of DNA — if their probe was situated, fortuitously or by design, in the affected chromosomal segment[51]. Cytogeneticists were no longer limited to the resolution afforded by crude banding patterns. Conversely, FISH could be used to establish the order of DNA clones relative to bands, naturally occurring breakpoints and other clones (for example, REF. 52) (FIG. 4). These data were funnelled into the genome project as independent tests of the validity of maps constructed by other techniques[53]. In turn, as molecular biologists filled in the genome maps, large collections of molecular reagents in the form of cloned, mapped segments of the human genome (cosmids, BACS, PACS AND YACS) became available with which abnormal chromosomes could be characterized by FISH to identify affected genes. For example, FISH analyses identified clones that cross the two breakpoints of the PERICENTRIC INVERSION of chromosome 16 seen in patients with acute myelogenous leukaemia (AML). This finding set the stage for the identification of the two genes (*MYH11*, smooth muscle myosin heavy chain 11, and *CBFB*, the β-subunit of core-binding factor) that, when aberrantly fused, cause the leukaemic transformation[54,55]. Cytogenetic studies in Sam Latt's laboratory were crucial to the discovery that Angelman and Prader–Willi syndromes are disorders of IMPRINTING: rearrangements in 15q11–15q13 were invariably found in the maternal or paternal copy of this region, respectively[56], and FISH has been crucial in the identification of imprinted genes in this region (reviewed in REF. 57).

The genome-wide view afforded by FISH has also revealed sequences that have been duplicated at distinct sites in the human genome; these sequences light up at more than the two expected sites and can be flagged for special attention during the assembly of the draft sequence[53]. Furthermore, many of these duplicated blocks have been implicated in chromosomal rearrangements that cause disease and are therefore of biological interest (reviewed in REF. 58).

Even more importantly, FISH opened up the nuclei of non-dividing cells to karyotype analysis. Conventional cytogenetics requires the capture of cells in mitosis, and many samples, particularly those from solid tumours, produce few, if any, analysable metaphases. Using FISH and chromosome-specific probes, cytogeneticists can enumerate chromosomes, simply by counting spots in each nucleus[59,60]. Deviations in spot number also signal gene deletion and amplification. Because DNA is packaged ~10,000-fold more loosely in interphase nuclei than in metaphase chromosomes, abnormalities that are not resolvable by metaphase FISH, such as the 1-Mb duplication that causes CHARCOT–MARIE–TOOTH SYNDROME[61], can be detected by interphase FISH (FIG. 5). Shifts in relative spot position reveal structural rearrangements, such as translocations and inversions[62] (FIG. 5).

PEPTIDE NUCLEIC ACID
(PNA). An analogue of DNA in which the backbone is a pseudopeptide rather than a sugar. PNA mimics the behaviour of DNA, but, because PNA has a neutral backbone, it binds complementary nucleic-acid strands more strongly and with greater specificity than an oligonucleotide.

COD-FISH
(Chromosome orientation and direction-fluorescence *in situ* hybridization). In this technique, single-stranded probes hybridize to one chromatid of a metaphase chromosome, because the most recently synthesized strand in each chromatid is specifically degraded before hybridization. A probe that recognizes the cytosine-rich strand of the telomeric repeat provides orientation by marking the 5′-end of each chromatid.

BAC, PAC AND YAC
Cloning vector system able to accomodate large genomic fragments. BACs and PACs are grown in bacteria; YACs are grown in yeast.

PERICENTRIC INVERSION
A structural alteration to a chromosome that results from breakage, inversion and reinsertion of a fragment that spans the centromere.

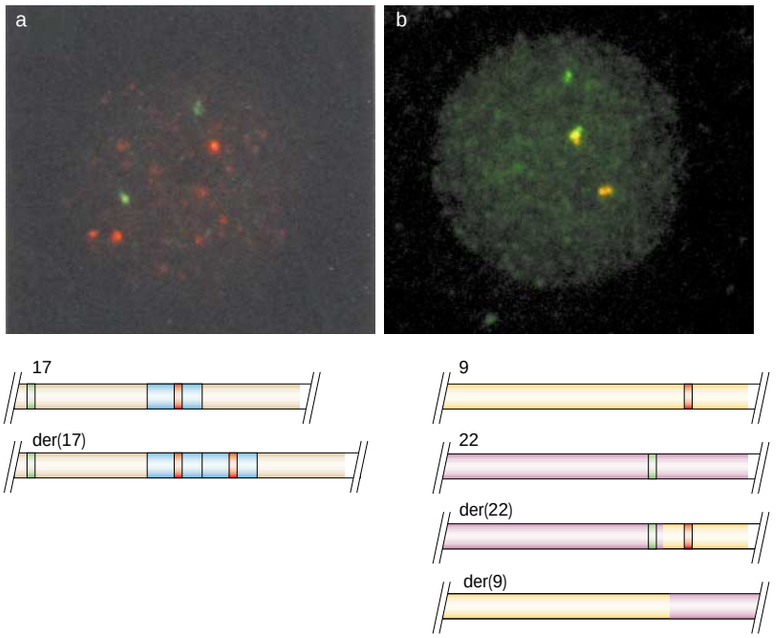

Figure 5 | **Using FISH to detect chromosomal abnormalities in interphase nuclei. a** | The duplication of a small portion of chromosome 17 that causes Charcot–Marie–Tooth syndrome is evident from the appearance of three, rather than two, red signals in this nucleus. The green spots mark a sequence outside the duplication. **b** | The translocation that creates a fusion of the *BCR* (breakpoint cluster region; on chromosome 22) and *ABL* (v-abl Abelson murine leukaemia viral oncogene homologue; on chromosome 9) genes in Philadelphia-chromosome-positive chronic myeloid leukaemia is evident from the close juxtaposition of one pair of green and red signals. These signals were generated using FISH (fluorescence *in situ* hybridization) probes for sequences located near these two genes, respectively. der(22) is the Philadelphia chromosome. Only the relevant portions of the normal and abnormal chromosomes are shown in the diagram below each panel. der, derivative. The photo in **a** is modified from REF. 61 © (1991) Elsevier Science; the photo in **b** is reproduced from REF. 62 © (1990) American Association for the Advancement of Science.

Interphase FISH has also made it possible to determine the relative times at which specific DNA sequences are replicated during the S phase of the cell cycle. Before replication, the probe generates a single dot on each chromosome, whereas two closely juxtaposed dots are visible after replication[63]. Using this approach, it was found that the order of replication is carefully orchestrated, and, for most loci, that the maternal and paternal alleles replicate in synchrony. By contrast, alleles of most imprinted loci are asynchronously replicated, with the expressed allele replicating earlier than the silenced one[64].

As the relationship between sequence proximity in interphase chromatin and separation along the DNA helix was elucidated, the order of DNA sequences could be inferred with 50–100-kb resolution by measuring the distances between fluorescent spots that mark DNA sequences of interest[65]. The ultimate in cytogenetic resolution is reached by wiping out nuclear organization altogether and conducting FISH on DNA fibres that have been affixed to glass (fibre-FISH)[66,67]. What is condensed to a small spot at the resolution of light microscopy in interphase becomes a long fluorescent line in fibre-FISH. Fibre-FISH is used to resolve ambiguities in the order of genes in a chromosomal region, to analyse the organization of tandem duplications and to detect small-scale rearrangements in chromosomes.

Clinical cytogenetics laboratories now make significant use of FISH in both their diagnostic and their research work. FISH is routinely used to augment conventional banding analyses of chromosomal rearrangements. Cytogeneticists have at their disposal various commercially available probe kits that are tailored for specific questions, such as the diagnosis of syndromes caused by chromosomal abnormalities that are too subtle to detect reliably by banding. The FISH test for SMITH–MAGENIS SYNDROME, which uses a probe for a small deleted region of chromosome 17, is an excellent example[68]. In research, FISH features prominently in the cytogeneticists' process of finding recurrent translocation breakpoints or overlapping deletions among patients with similar phenotypic abnormalities.

Chromosome painting with a colourful palette

The thrill of seeing a single-copy gene fluoresce in a human cell was soon surpassed by the vivid image of 24 human chromosomes painted in different colours[69,70] (FIG. 6). This powerful development, called spectral karyotyping (SKY) or multiplex (M)-FISH, combines three significant advances. First was the production of chromosome-specific 'paints': collections of sequences derived from each chromosome (usually by flow sorting)[71,72]. These collections can be generated easily from small numbers of chromosomes using DEGENERATE OLIGONUCLEOTIDE-PRIMED PCR[38] or LINKER–ADAPTOR PCR[73]. When used as a probe, these collections label a chromosome end to end. (Region-specific paints can be generated if microdissected portions of chromosomes are used as a template[74].) Second was the combination of fluorochromes to produce 24 colour combinations, one for each chromosome[75]. Third were the advances in microscopic optics, filters and imaging systems for multicolour analyses. In the SKY system, the spectral characteristics of each pixel in the image are read out by an INTERFEROMETER[69]. In M-FISH, the spectral characteristics are evaluated by collecting images through a series of excitation and emission filters[70]. These imaging systems can be taught to classify each chromosomal segment automatically, and they offer the first real hope of automated karyotype analysis. So far, no system can classify banded chromosomes as robustly and accurately as a skilled cytogeneticist, despite the millions of dollars that have been invested in automated karyotype analysis since 1968.

SKY and M-FISH have proved to be extremely useful for detecting translocations and other complex aberrations (FIG. 6). For example, SKY has revealed amplification of regions on 11q, 21q and 22q that had not been detected before in AML patients with complex karyotypes; these defects could have a significant role in leukaemogenesis[76]. Even the karyotypes of tumours in mice can be deciphered[77]. M-FISH has been especially helpful in the study of radiation-induced damage and chromosome repair[78]. Although the breaks occur randomly, they are repaired in non-random patterns that reflect the proximity of the breaks in the nucleus during the repair process. So, SKY both has an impact on radiation dosimetry and gives insights into the organization of the human cell nucleus[79].

IMPRINTING
A genetic mechanism by which genes are selectively expressed from the maternal or paternal homologue of a chromosome.

CHARCOT–MARIE–TOOTH SYNDROME
An inherited degenerative peripheral nerve disorder that causes progressive muscle weakness and atrophy in the feet, legs, hands and forearms.

SMITH–MAGENIS SYNDROME
A rare condition that is associated with developmental delay, characteristic facial and other anatomical abnormalities, learning difficulties and behavioural problems, such as the tendency to harm oneself.

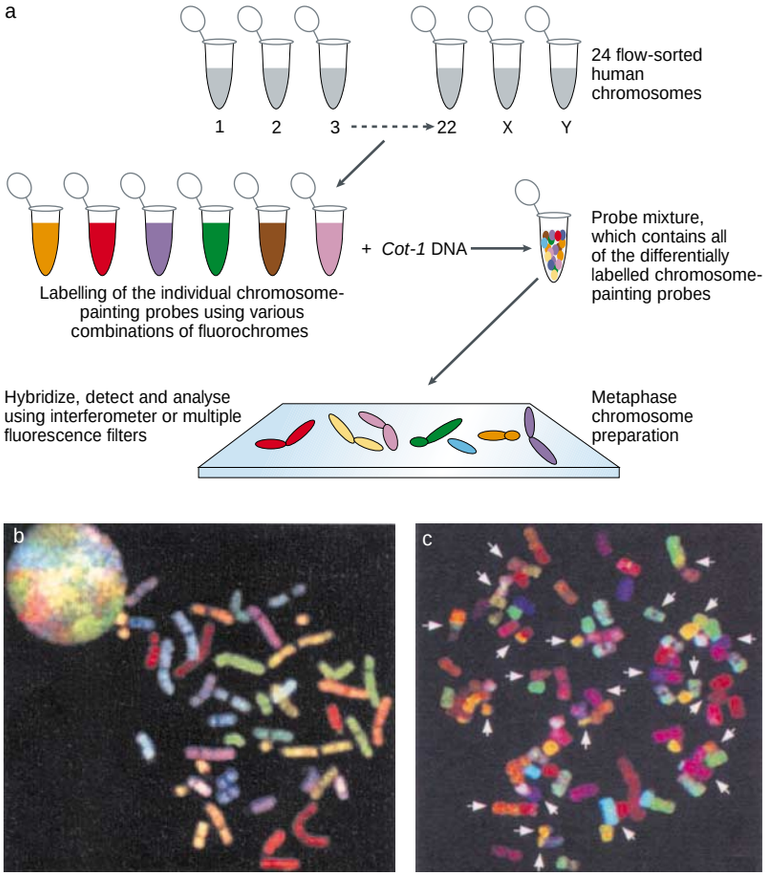

Figure 6 | **Spectral karyotyping and multicolour-FISH paint each human chromosome in one of 24 colours. a** | Outline of the spectral karyotyping (SKY) protocol. SKY and multicolour fluorescence *in situ* hybridization (M-FISH) differ only in the method used to measure the spectral characteristics of each pixel in the image (see main text). *Cot-1* DNA is enriched in repetitive sequences, and by binding to repetitive sequences in the fluorescently tagged probes, it suppresses their hybridization to target chromosomes. **b** | The application of SKY to normal interphase and metaphase human cells; the highly rearranged karyotype of a bladder cancer cell is shown in **c**. Arrows point to inter-chromosomal rearrangements. Panels **a** and **b** modified from REF. 97 © (2000) Cambridge University Press. Panel **c** reproduced from REF. 98 © (1999) Wiley.

M-FISH has also sparked a new industry of probe development to monitor many loci at once for subtle aberrations. The best example is the use of probes that mark the unique sequence near each telomere to detect subtle rearrangements of the ends of chromosomes[80]. With this technique, as many as 7% of patients with previously unexplained mental retardation have been found to have chromosome abnormalities that had gone undetected in previous analyses[81].

One of the most thriving areas of cytogenetics today is the study of the chromosomal rearrangements that occurred during evolution[82,83]. During each speciation event, some cards in the genome deck are moved. These events can be reconstructed with FISH. Such studies have revealed, for example, that the evolutionary rate of chromosomal translocations is ten times greater between the mouse and the rat genomes than between those of humans and cats or chimpanzees[84]. Comparative cytogenetics is also crucial for disease-gene mapping. The use of dogs to identify genes that cause human disease is a case in point[85]. At least half of the inherited disorders that are recognized in various dog breeds correspond to specific human diseases, including various forms of cancer, deafness, heart disease, blindness and epilepsy. With extensive dog pedigrees, it is feasible to genetically map the canine disease to a region of the dog genome. Comparative cytogenetic maps of the human and dog genomes, produced by hybridizing human chromosome paints to dog chromosomes[86], show where to dig in the human genome for candidate genes, which can then be tested for mutations in dogs and/or humans.

CGH-arrays — a surrogate for chromosomes

The next transformation of cytogenetics came with the realization that genome-wide scans for the loss or gain of chromosomal material could be conducted without even looking directly at the subject's chromosomes. The technique that made this possible is called comparative genome hybridization (CGH) and was developed by a team led by Ollie and Anna Kallioniemi, Dan Pinkel and Joe Gray[87]. In this approach, the genomic DNA of test and reference samples is isolated, fragmented, labelled in red and green, respectively, and allowed to compete for hybridization sites in sets of normal chromosomes (FIG. 7). As in regular FISH, interspersed repetitive elements are taken out of the picture by pre-annealing the probes with unlabelled DNA that is enriched for repetitive sequences. The ratio of red-to-green fluorescence is measured along the length of each chromosome. The chromosomal regions that are equally represented in the test and reference samples appear orange, but those deleted or amplified in the test sample appear more red or more green. CGH is particularly important in cancer cytogenetics, in which it is used to identify chromosomal regions that are recurrently lost or gained in tumours. For example, CGH led the way to the identification of *PIK3CA*, the catalytic subunit of phosphatidylinositol 3-kinase (PI3K), as an oncogene in ovarian cancer[88]. DNA-amplification techniques have also been developed to find genetic alterations in small samples of rare cells[89], such as rogue cells found in blood that have escaped a primary tumour and might foreshadow metastasis.

The current excitement in cytogenetics revolves around the promise of array-CGH[90] (FIG. 7). In this technique, metaphase chromosomes are replaced by an array of thousands of BAC clones, each of which contains an ~150-kb segment of the human genome. An array of 3,000 BACs can be constructed that samples the genome, on average, once every megabase pair[53]. Array-CGH is therefore the equivalent of conducting thousands of FISH experiments at once, but without the need to count dots to measure the copy number of each test locus. CGH provides better quantification of copy number and more precise information on the breakpoints of segments that are lost or gained than does conventional CGH. More importantly, each clone is an entry point to the genomic sequence in which

DEGENERATE OLIGONUCLEOTIDE-PRIMED (DOP) PCR/LINKER–ADAPTOR PCR
DOP-PCR uses partially degenerate primers to amplify sequences at dispersed sites in a sample. In linker-adaptor PCR, the DNA sample is digested with a restriction enzyme, the ends are ligated to an adaptor oligonucleotide, and the ligated fragments are amplified using PCR primers that are complementary to the linker-adaptor oligonucleotide. Both techniques generate large pools of fragments that almost completely represent the starting sample.

affected genes can be identified. Although CGH is insensitive to changes that are present at low frequency in the cells being analysed, it is expected that array-CGH will enable many groups to evaluate large numbers of tumours for recurrent changes using a common platform. These analyses should generate prognostic markers, identify new tumour-suppressor genes or oncogenes and, ultimately, lead to a better understanding of the cancer process. In addition, I predict that some prenatal diagnostic tests that now rely on banding and conventional FISH will also be supplanted by custom arrays. It is hoped that technological advances,

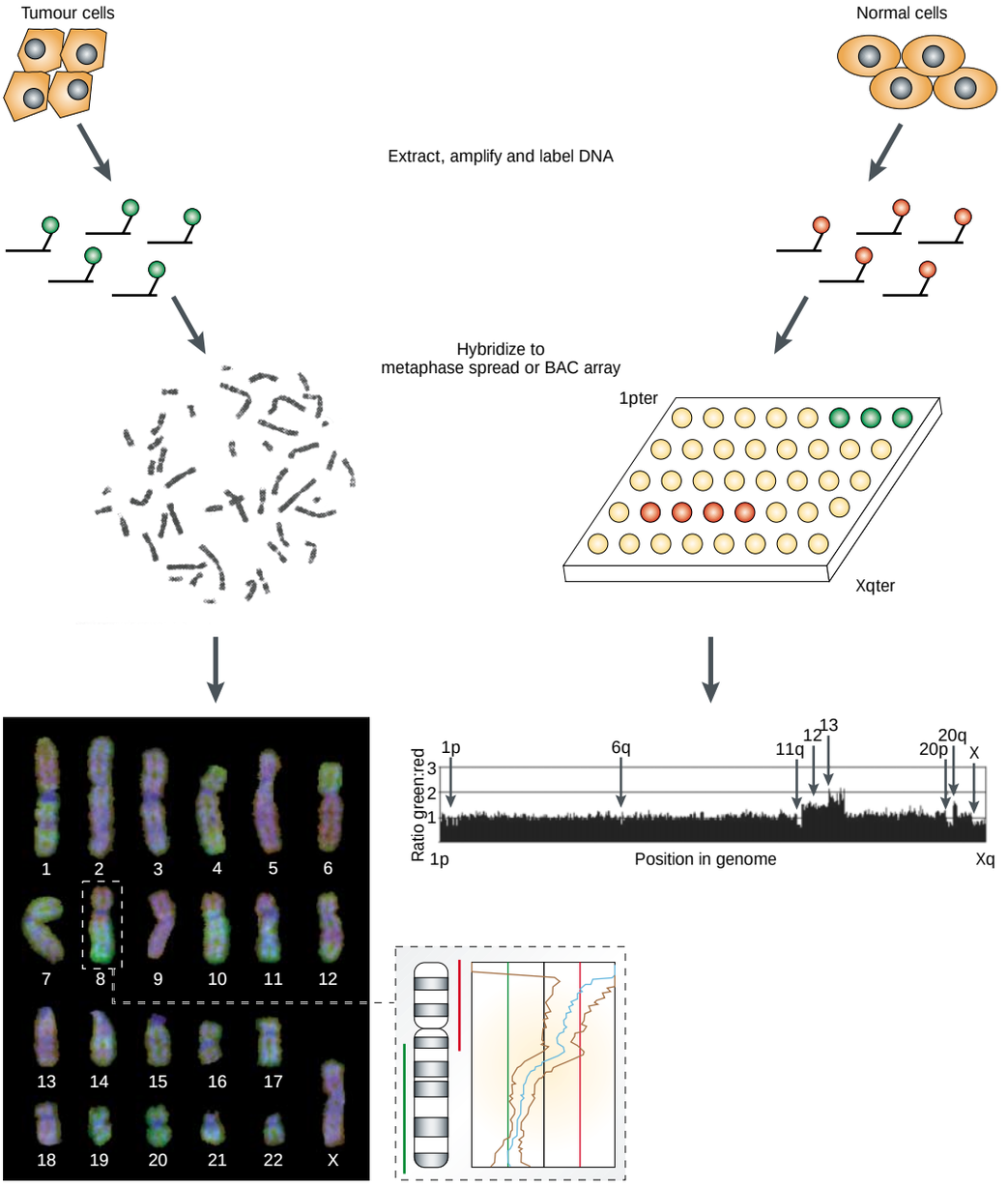

Figure 7 | **Comparative genome hybridization.** This technique reveals the loss or gain of chromosomal regions in test samples (for example, derived from a tumour) relative to normal controls. DNA in the test and reference samples is labelled with green and red fluorochromes, respectively, and allowed to compete for hybridization sites on either metaphase chromosomes (left) or an array of BAC (bacterial artificial chromosome) clones that represent thousands of small DNA segments distributed across the genome (right). Areas on the chromosome, or spots on the array, that are more green than average are present in extra copies in the test sample; those that are more red than average are deleted in the test sample. The bottom left panel shows the outcome of conventional comparative genome hybridization using the prostate-cancer cell line PC-3 as a test sample; the loss and gain of several chromosomal regions are evident as red and green areas, respectively. Inset: the profile of the average green:red ratio of ten chromosomes shows 8p loss and 8q gain in these cells. The bottom right panel shows copy-number losses of 1p, 6q, 11q and 20p, and gains of 12, 13 and 20q in the ML-2 cancer cell line. Bottom left panel provided by Ilona Holcomb, Fred Hutchinson Cancer Research Center; bottom right panel reproduced with permission from *Nature* REF. 53 © (2001) Macmillan Magazines Ltd.

INTERFEROMETER
A device that uses an interference pattern to determine wave frequency, length or velocity.

TYRAMIDE CHEMISTRY
A labelling system that uses a hybridization probe that is directly or indirectly labelled with peroxidase. The peroxidase catalyses the localized deposition of a reactive tyramide-labelled tag (for example, biotin or fluorescent dyes).

such as array-CGH, will reduce the time and cost of cytogenetic analyses so that they can be accessed by more families.

What next?

Of course, cytogenetic technology has its limitations. Ideally, each cytogenetics lab should have at hand a bank of clones that represent sequences that are distributed once every megabase-pair across the genome, so that any chromosomal abnormality could be analysed at the molecular level with ease and efficiency using conventional FISH or array-CGH. Efforts have been made to assemble and distribute such a reagent set[53]. Navigating from the microscope to the DNA sequence and back again would be further facilitated by increased FISH sensitivity, so that probes as short as most PCR products could be reliably detected. Signal-amplification techniques, such as those that involve TYRAMIDE CHEMISTRY[91] or rolling-circle amplification[92], are pushing at this limitation. The study of duplications by FISH would be more informative with a clearer understanding of how variation in target size and divergence affects signal intensity. Strategies for robust allele-specific FISH might allow determination of the position and copy number of maternal or paternal alleles. The adaptation of FISH for flow cytometric analysis of cells would greatly benefit our understanding of tumour biology, by opening up the possibility of assessing aneuploidy, as measured by the fluorescence of chromosome-specific probes, in conjunction with other phenotypic characteristics of cells, such as cell-surface antigens. Finally, techniques to mark endogenous loci in live cells and new applications of genome-wide array technology (such as in REFS 93,94) are needed to investigate how chromosome and nuclear structure relates to gene regulation, replication and repair.

Recurrent reincarnation

Human cytogenetics is flourishing at the age of 46 years and does not risk being supplanted by younger and more precise molecular techniques. Chromosomal abnormalities are nature's guide to the molecular basis of far too many unexplained human disorders, particularly solid tumours. Furthermore, cytogenetics continues to reinvent itself to aid explorations of chromosome structure, function and evolution. The cytogenetics lab is a bustling enterprise of service and research, with a still expanding set of tools. Banding techniques, which are unchanged from the 1970s apart from the introduction of digital image handling, are now combined with state-of-the-art multicolour FISH and molecular analysis. From their vantage through the microscope, the cytogeneticists' view of the genome is still unrivalled in its scope, detail and colour.

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acute myelogenous leukaemia | Angelman syndrome | Charcot–Marie–Tooth syndrome | chronic myeloid leukaemia | Cri-du-Chat syndrome | Down syndrome | Klinefelter syndrome | Prader–Willi syndrome | retinoblastoma | Turner syndrome

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