THE ART AND DESIGN OF GENETIC SCREENS: MAMMALIAN CULTURE CELLS

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Given the wealth of sequence information from the Human Genome Project, many open reading frames urgently require assignment of function. Whereas genetic model organisms, such as yeast, *Drosophila* and *Caenorhabditis elegans*, have successfully been used in genetic screens for a long time, mammalian culture cells have only recently emerged as a suitable screening system to elucidate gene function. Diverse cellular activities, such as apoptosis, senescence, tissue-specific differentiation and oncogenic transformation, can be studied in cell culture. There is a plethora of functional assays that can provide a link between genes and physiology. The number of genetic elements to be tested necessitates the use of miniaturization strategies or robotic instrumentation for effective screens that use mammalian cell lines.

SENESCENCE
State in which normal cells irreversibly stop dividing.

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doi:10.1038/nrg1291

The current flood of sequence information from the various genome projects is accompanied by the lack of knowledge of how the newly discovered genes might function. Recent estimations indicate that >40% of all potential human open reading frames have not yet been assigned a function[1,2]; their integration into cellular pathways that are responsible for diseases is a particularly pressing need. Although methods for efficient DNA sequencing have now been established, assigning functions to genes is not straightforward. Genetic screens in various model organisms are a means to accomplish exactly this.

Some 40 years ago, the formulation of suitable growth media allowed continuously growing mammalian cells *in vitro*[3] to be efficiently established. In this way, the cell — biology's most fundamental entity that can undergo autonomous replication — became a valuable tool for geneticists. It has become probably the most widely used biological system for characterizing gene function and certainly the only practical approach to large-scale functional studies of mammalian genes. In this review, I provide an overview of how this minimal biological entity has been used in genetic screens and how recent developments can help make sense of the overwhelming amount of sequence data.

Cell culture versus model organisms

Yeast is an established genetic model for studying eukaryotic gene functions but accumulating data show that many human genes are not represented in yeast[4]. Importantly, there is no true counterpart in yeast for cellular processes such as apoptosis, SENESCENCE, tissue-specific differentiation and oncogenic transformation (FIG. 1). Because of the phylogenetic distance between other model organisms and mammals, often it might not be possible to extrapolate results from these systems to humans. Consequently, the above-mentioned effects can most accurately be observed in mammalian cells. Those and many other cellular alterations are not amenable for use in genetic selection procedures because they require the killing or growth inhibition of cells that fail to show a relevant biological effect. They can instead be determined using genetic screens (BOX 1).

Because of efficient mutagenesis protocols, the ease with which genetic material can be introduced into mammalian cells, their uniform genetic make-up and the use of defined growth conditions, mammalian cell culture cells are nowadays the most widely used biological system (TABLE 1). Among the pet objects of cell geneticists are the human 'HeLa' cells and the embryonic kidney '293' cells, which together had been cited in

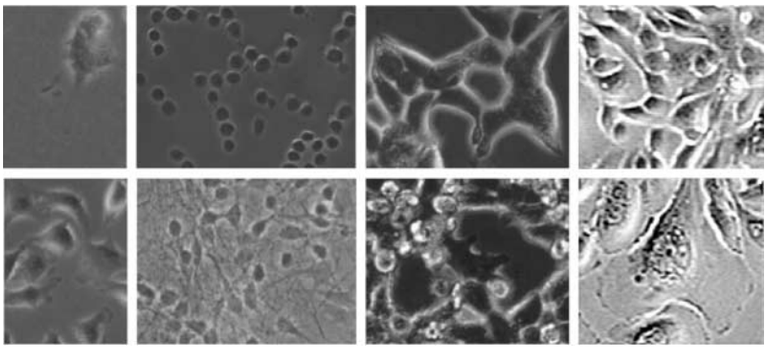


Figure 1 | **Possible fates of mammalian cells *in vitro*.** The proliferation of HeLa cells[122] is depicted in the lower left panel, followed by the differentiation of embryonic neuronal precursor cells to mature neurons (courtesy of M. Goetz, Max-Plank-Institute for Neurobiology, Martinsried, Germany), the induction of apoptosis in 293T cells by overexpressed caspase-2 (REF. 99) and the alteration of prostate epithelial cells after initiating senescence by serial passaging[123]. The corresponding parental cells are arranged in the same order in the upper panels.

more than 40,000 publications by the end of 2003. However, given that these cells are transformed, the physiological relevance of the data obtained using these cell lines should be determined in PRIMARY CELLS.

Mammalian culture cells: screen characteristics

Mammalian cell lines differ from other genetic systems in several important ways. They comprise a variety of different cell populations, both genetically and phenotypically. They can be derived from different tissues and their growth and differentiation status can therefore vary widely from cell line to cell line. This necessitates rigorous quality control over short periods of culture and a careful choice of the cells that are to be used in a given genetic screen. Aspects such as the genotype and the ability to show the desired cellular effect need to be considered.

In contrast to whole organisms, each particular mammalian cell line constitutes a phenotypically and genetically uniform population of individual cells that have been derived from one tissue.

Because the aim of cell biologists has been to produce genetically and phenotypically stable cell lines, cells in culture do not spontaneously enter a developmental pathway. Instead, differentiation has to be induced with special growth conditions. As an alternative, differentiated cell lines from a variety of different tissues can be obtained after immortalization *in vitro*[5] and from tumours, which, despite their tumour origin, most often reflect the properties of the original cells[6].

Mammalian cells from various differentiation stages can also circumvent the problem, often encountered in more complex organisms, of how to explain the multifarious phenotypes that sometimes arise as a result of simple genetic changes. Furthermore, many, possibly very informative, mutations in developing organisms can only be observed up to the first detectable phenotypic effect as they are often lethal.

Cell culture provides a reductionist's view of cells in a two-dimensional arrangement (in culture dishes) in contrast to their normal multi-cellular environment in a three-dimensional geometry. However, this can be seen as a benefit rather than a disadvantage because it provides us with defined experimental settings in which to investigate the phenotypic consequences of genetic alterations.

One further important difference that sets mammalian cell lines apart from other genetic model organisms is the fact that they are asexual diploids (or polyploids). Because genes exist permanently in multiple copies and no meiotic recombination takes place, only SOMATIC-CELL GENETICS is possible in these systems. This lack of recombination can be overcome by using techniques that inhibit genes or proteins encoded by them and that allow knock-out cells to be created (see below).

Elements of screens in mammalian cells

As in other genetic model systems, screens in mammalian cells rely on mutagenesis, recognition of the altered phenotype or of the induced biochemical changes and characterization of the causative mutation. Below, I elaborate on the individual elements of such forward-genetic screens in mammalian cells. Reverse-genetic screens, on the other hand, test the effects of pre-defined gene mutations in cells. Few screens of this type have been performed in mammalian cells, although they are discussed in the section on 'other screens'.

The manipulation. Mutations are generated in cells *in vitro* mostly by introducing genetic material (BOX 2). This transfection is said to be stable under conditions that ensure the presence of the genes for an extended period of time in the cell, either as EPISOMALLY replicating plasmids or as DNA sequences that are integrated in the genome. Retroviruses are frequently used because they contain integrase, an enzyme that allows their efficient

PRIMARY CELLS
Culture cells with limited lifespan that are taken directly from an organism.

SOMATIC-CELL GENETICS
Area of genetics that deals with diploid, somatic cells and not germ-line cells.

EPISOMAL
In the context of transient transfection, it refers to a plasmid that is extra chromosomal.

FLUORESCENCE ACTIVATED CELL SORTING
(FACS). A method whereby dissociated and individual living cells are sorted, in a liquid stream, according to the intensity of fluorescence that they emit as they pass through a laser beam.

SPLICE DONOR SITES
DNA sequences that mediate splicing at the 5′ end of an intron.

Box 1 | **Screens versus selections**

The distinction between a selection and a screen can be drawn on the method of detection of the cellular consequences of the mutagenesis. A selection is based on the physical removal of those mutant cells that show a given cellular effect from the rest of the cell population. This can be accomplished by, for example, antibodies that recognize a novel epitope on the cell's surface and thereby immobilize them or by creating conditions in which only the mutant cell with a desired phenotype survives. Most often, this is achieved by the selective killing or growth inibition of the rest of the population. In a screen, on the other hand, mutants (single cells or cell populations) must be examined one-by-one and in each case a decision must be made about whether (or to what extent) the targeted phenotype is present. This can be done by FLUORESCENCE-ACTIVATED CELL SORTING (FACS) in which the inspection of the fluorescence is done in the millisecond range. Given the number of different genes in mammalian cells and the labour involved, most methods of distinguishing mutant cells in this system — although referred to as screens — are actually selections.

Table 1 | **Popular mammalian culture cells**

Cell name	Origin	Characteristics	Reference
HeLa	Human cervical carcinoma	Epithelial-like, aneuploid	124
293	Human embryonic kidney	Epithelial, aneuploid	125
MCF-7	Human breast	Epithelial, aneuploid	126
Saos-2	Human osteosarcoma	Epithelial, aneuploid	127
PC-3	Human prostrate carcinoma	Epithelial, aneuploid	128
Jurkat	Human T-cell leukaemia	Lymphoblasts, pseudodiploid	129
Cos-7	Monkey kidney	Fibroblasts, aneuploid	130
Vero	African green monkey	Fibroblasts, aneuploid	131
CHO	Chinese hamster ovary	Epithelial-like, pseudodiploid	132
L-929	Mouse connective tissue	Fibroblasts, aneuploid	133
3T3	Mouse connective tissue	Fibroblasts, aneuploid	134
BHK-21	Syrian baby hamster kidney	Fibroblasts, diploid	135
HUVEC	Human umbilical-vein endothelial cells	Epithelial, diploid	136
ES-D3	Mouse embryonic stem cells	Epithelial, diploid	137

insertion into the genome[7]. *Drosophila* transposons, which comprise elements that are similar to those found in retroviruses, can also be used to mutagenize the genomes of mammalian cells[8]. Using these approaches, the genetic elements are inserted randomly into the genome and, if linked to a strong promoter and a SPLICE DONOR SITE to remove intervening sequences, can be used to activate endogenous genes nearby. In one application, these approaches were used to generate a NORMALIZED LIBRARY of cell clones that overexpressed different genes, many of which were silent in the parental cells[9].

Other mutagenesis strategies aim to inhibit gene functions. As exons of active genes represent only the minority of sequence information in the mammalian genome, inserted foreign DNA is more likely to integrate within an intron. Consequently, the inserted sequences are engineered to contain strong SPLICING ACCEPTOR SITES that insert new exons into the mRNA and lead to a premature termination of protein synthesis[10]. Alternatively, when the inserted DNA sequences enclose a reporter plasmid (see below and FIG. 2), they allow us to determine the control of endogenous genes if their insertion site is near a transcriptional promoter[11].

Transfections are transient if the introduced genetic material does not integrate into the genome and is eventually lost or degraded. This straightforward approach has been mainly used to create dominant mutations by gene overexpression. However, transiently transfected DNAs can also inactivate endogenous genes, either through the expression of genetic suppressor elements[12], RIBOZYMES[13], RNA interference (RNAi) constructs[14] or antisense oligonucleotides[15,16].

Chemical mutagenesis is used less frequently in mammalian cells because mutations induced in this way are difficult to determine as meiotic recombination cannot be used in mammalian cells to map the affected loci. Mutagenizing agents are therefore mostly chosen in combination with other screening methods, such as COMPLEMENTATION strategies (see below). Suitable mutagenesis reagents are the same as those used in screens in model organisms, such as ethyl methane sulphonate (EMS)[17] and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG)[18], as well as ICR-191, which is an especially potent mutagen that induces frame-shift mutations[19].

Other approaches to changing the genetic information in mammalian cell lines involve the fusion of tumour cells with other cells or with MICROCELLS that contain large DNA fragments or whole chromosomes. POLYETHYLENE GLYCOL or viruses are used to mediate the membrane fusion. Such fusions were used to generate a series of hybrid cell lines that contained various chromosomes or chromosome fragments and to screen them for growth or metastasis formation. For example, the ability of Wilms' tumour cells, a paediatric NEPHROBLASTOMA, to grow in nude mice was completely suppressed by introducing a normal human chromosome 11 (REF. 20). Using this laborious method, several tumour or METASTASIS SUPPRESSOR GENES were identified[21].

The detection. The set-up of the appropriate functional assay is a pivotal step for the success of genetic screens in mammalian cells. It must be tailored to the function in question and requires rigorous testing of the experimental setting, such as the signal background and the verification of positive controls.

Classical screens in other biological systems exploit the developmental changes that occur in the particular organism, be it in *Drosophila*[22], *C. elegans*[23,24] or

NORMALIZED LIBRARY
Ensemble of genes with equal numerical representation.

SPLICING ACCEPTOR SITES
DNA elements that mediate splicing at the 3′ end of an intron.

RIBOZYMES
RNAs with catalytic activity.

COMPLEMENTATION
Restoration of the wild type by non-allelic genes that act in trans.

MICROCELLS
Vesicles formed after treatment of cells with colchemid and cytochalasin b. They contain large DNA fragments or single chromosomes that are surrounded by a nuclear and a plasma membrane.

POLYETHYLENE GLYCOL
Highly hydrated polymer that can bring cell membranes into near molecular contact by making the water between them thermodynamically unfavourable.

NEPHROBLASTOMA
Tumour of the kidney.

METASTASIS SUPPRESSOR GENES
Genes that, when inactivated, lead to metastasis formation rather than conferring a growth advantage (as with tumour-suppressor genes).

LIPOFECTION
A technique for transfecting cells that uses lipids or lipid-related carrier molecules.

Box 2 | **Introduction of genetic material into mammalian cells**

The ability to introduce DNA into mammalian cells is central to the use of this system for genetic screens. The ever-growing list of transfection reagents that coat DNA so that it can be taken up by cells *in vitro* attests to the importance of this procedure[114]. With LIPOFECTION, a compact liposome/nucleic acid complex that associates with the cell membrane is generated, is taken up by endocytosis and is eventually released from the endosome by an unknown mechanism[115]. Calcium-phosphate co-precipitation, diethylaminoethyl-dextran (DEAE-dextran) and electroporation are more traditional, yet still effective, methods for transporting DNA into cell lines. With the calcium-phosphate co-precipitation method, the DNA is encapsulated in a calcium-phosphate precipitate that is taken up by the cells through endocytosis[116]. The positively-charged DEAE-dextran molecules associate with the negatively-charged backbone of nucleic acids and the resulting complexes adsorb to the cells and are endocytosed[117]. Electroporation uses an electrical pulse to perturb the membrane of cells and generates pores through which DNA enters cells[118]. The insertion of viral DNA into mammalian cells by infection has also been used for mutagenesis[102]. This method is most efficient and is preferred if no reporter plasmids (see FIG. 2) can be used to mark the transfected cells.

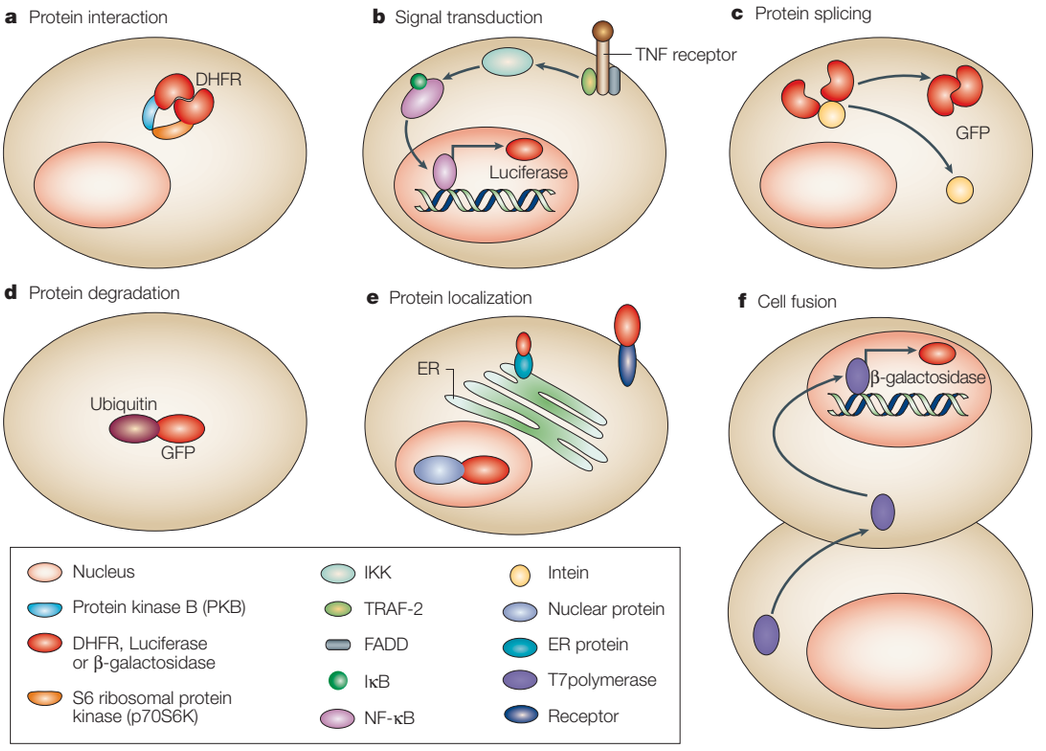


Figure 2 | **Examples of reporter plasmids.** Reporter plasmids encode proteins that can easily be traced and the activity of which is affected by the cellular effect under investigation. Some examples of general expression cloning approaches are given here. **a** | An interaction of fusion proteins with fragments of dihydrofolate reductase (DHFR) restores the activity of the reporter enzyme. **b** | The activity of a transcription factor or an entire signalling pathway leading to the activation of a transcription factor can be probed by a reporter plasmid that contains the promoter sequence for the DNA-binding moiety in front of the luciferase coding sequence. **c** | The GFP can be reconstituted by protein splicing when the splicing protein is assembled by interacting protein fusions. Other reporter plasmids target protein stability mediated by the proteasome and feature a GFP-ubiquitin fusion protein (**d**) or make it possible to assay the localization of GFP fusion proteins (**e**). Cell-fusion processes can be followed by trans-complementation of an activated β-galactosidase by a polymerase that binds to and activates the reporter plasmid in the target cell (**f**).

zebrafish[25,26]. Given that deviations from an invariant developmental process cannot readily be examined in mammalian cell culture, the number of phenotypes that are observable *in vitro* is limited (FIG. 1). Besides proliferation, the cells can undergo tissue-specific differentiation or apoptosis[27], or they might fall into a quiescent state that is often described as senescence[28]. All these changes are accompanied by observable morphological alterations, the genetic basis of which can be determined in screens (see below).

The absence of aberrations from a stereotyped developmental programme seen in other screening systems offers the possibility to pursue more focused screens in mammalian cell culture. The overwhelming complexity of biological systems has no shortage of more subtle biochemical changes. This is attested to by the multitude and diversity of functional read-outs used in genetic screens in cell lines[29]. For example, in one study, a soluble receptor molecule was used to clone its specific ligand[30], whereas another sought a secreted activity in supernatants of transfected cells and tested them in specific cellular assays[31]. Transporters were detected by the uptake of radiolabelled substrates[32], whereas other screens targeted the activity of an enzyme[33]. Screening for each of these activities requires an adjustment of the respective functional read-out.

Reporter plasmids have proved to be especially helpful as general tools to detect a great variety of cellular activities. Given that most mutagenesis strategies rely on the introduction of DNA into cells — a process that is still not 100% efficient — the reporter plasmids are co-transfected with an excess of the expression plasmids from a gene library. So, every reporter plasmid marks only mutagenized cells and allows the detection of the desired cellular effect.

The second functional aspect of reporter plasmids is that they encode protein products, the activity of which is affected in a specific way by the cellular effect of interest. Examples of reporter proteins include GFP, in which many variants allow us to elucidate various molecular changes that range from protein stabilization[34] and apoptosis induction by the FLUORESCENT RESONANCE ENERGY TRANSFER (FRET) EFFECT[35] to pH differences in various cellular organelles[36]. GFP-fusion proteins allow us to determine the subcellular localization of proteins and therefore make predictions about gene function[37,38]. Also, the activation of transcription factors has often been assessed by their binding to and

FLUORESCENT RESONANCE ENERGY TRANSFER EFFECT (FRET effect). A technique that is used to detect the closeness of fluorescent molecules.

activation of promoters that upregulate reporter proteins, such as GFP[39], chloramphenicol transacetylase[40], β-lactamase[41] or β-galactosidase, that have been either stably integrated into the genome[11] or are present extrachromosomally[42].

The β-galactosidase reporter enzyme has been used in an elegant transcomplementation screen to clone one of the human immunodeficiency virus (HIV) co-receptors[43]. The CD4 surface molecule is necessary but not sufficient for the entry of HIV into cells. At the time of this research, the existence of a co-receptor was still only postulated. In this screen, the target cells expressed the T7 RNA polymerase and the CD4 receptor molecule. These cells were transfected with a cDNA library and incubated with cells that contained the env protein of HIV and the gene for the reporter enzyme β-galactosidase that is linked to the T7 promoter. If a fusion between cells from those two populations occurred — mediated by CD4, env and the co-receptor — the T7 polymerase activated *in trans* the transcription of the enzyme the activity of which could be detected. *Fusin* — a gene that encodes a seven-transmembrane G-protein-coupled receptor — was identified in this way[43].

Another enzymatic complementation screen was set up to explore the association of proteins[44]. The potential interaction partners were linked to the α- or ω-fragments of β-galactosidase. When these fusion proteins associate, the fragments of β- galactosidase restore its enzymatic activity. The same principle was also used in the oligomerization and complementation of fragments of the enzyme dihydrofolate reductase[45], which also allows us to determine the cellular location of the interacting proteins[46]. In a similar approach, the MAMMALIAN TWO-HYBRID ASSAY was used to detect the activity of an assembled transcription factor[47].

In another approach to investigate protein–protein interactions, protein-splicing generates an intact GFP from two of its protein fragments[48]. This assay can also be used to observe the localization of proteins in relation to mitochondria[49]. All these experiments exemplify how an inventive application of reporter plasmids can be used to test for a variety of cellular functions.

Some screens in mammalian cells also encompass genotype screens. Rather than a phenotype, a defined genetic constitution of the cells is the initial aim in these screens. For example, both alleles of endogenous genes can be inactivated by homologous recombination (knock-out). This involves the successive introduction of two targeting vectors with different antibiotic-resistance genes into the cells followed by a genotype screen for mutant cell lines by Western or Southern blotting. This elaborate procedure was used, for example, to show that the *p21* (*CDKN1A*) gene mediates p53-induced cell-cycle arrest[50]. In a variation on this experiment, stem cells were mutagenized in only one allele and the mice that were derived from them were bred to generate homozygous knock-out animals. Only in some instances, in which the target gene mapped to the X chromosome, did the mutation of one allele result in a complete knock-out in XY male embryonic stem cells — these cells were taken directly for molecular analysis[51].

Identification of mutation. Once the cellular phenotype is determined, the search for the causative genetic element begins. In stark contrast to the multitude of functional read-outs, the detection of the mutation is performed in a relatively uniform manner and depends on the mutagenesis type. In transient transfections, the plasmids do not integrate into the genome of the host cell and are recovered by gentle lysis of the cells and subsequent introduction and amplification in bacteria[52]. Whenever the genetic information has stably integrated into the genome, it is used as a tag to define the gene that it influences. Usually, this involves the cloning of neighbouring sequences and the determination of the gene that is responsible for the cellular effect.

In sib-selection experiments, the plasmid pools are subdivided and narrowed down by an iterative test of further sub-pools until the active gene can be pinpointed[52].

More elaborate efforts are required to clone tumour-suppressor genes the activity of which is detected in cell-fusion experiments. First, the relevant chromosome is determined by correlating the tumorigenicity with the loss or retention of specific chromosomes. Following this, the candidate chromosome is introduced by microcell fusion and the location of the tumour-suppressor gene is mapped by introducing chromosomes with specific deletions[53]. Ultimately, candidate genes from a certain chromosome locus are tested for their activity in mammalian cells.

Traditional screening approaches

Dominant gain-of-function screens. Dominant gain-of-function screens[29] are the most frequently used type of screen in mammalian cells and constitute the basis of expression cloning (BOX 3). The screen begins with the introduction of a cDNA library of expression plasmids into mammalian cells. The desired clone (gene) is determined by phenotyping the cells. This approach has been used to screen the supernatant of transfected cells to isolate granulocyte–macrophage colony-stimulating factor (GM-CSF), a secreted growth factor, on the basis of its COLONY-FORMATION ACTIVITY[54]. The transcription activator GATA1 — a zinc-finger protein that is specific for erythroid cells — was determined by its property to bind a defined DNA sequence[55]. An enzyme in steroid hormone metabolism was cloned through its enzymatic activity[33] and several transporters were isolated by the accumulation of their radioactive substrate in the cells[32,56]. Other examples of successful expression cloning in cell culture include the isolation of transmembrane channels[57,58] and membrane-bound receptors[59–61].

Oncogenes are a well-known example of genes that have been successfully identified in dominant genetic screens. A functional expression screen was tested for genes isolated from human tumours that had overridden CONTACT INHIBITION and led to FOCUS FORMATION of rodent cell lines[62,63], both hallmarks of transformed cells. The active clone turned out to carry an *Hras* allele activated by a single point mutation that has been implicated in ~20% of all human cancers[64]. Many other oncogenes were later found using this procedure, such

MAMMALIAN TWO-HYBRID ASSAY
A technique that is used to detect protein–protein interactions in mammalian cells; it corresponds to the yeast two-hybrid assay, except that mammalian expression vectors are used. An intact transcription complex is generated if two fusion proteins interact: one that contains a DNA-binding domain and another that contains a transactivation domain.

COLONY-FORMATION ACTIVITY
Activity to sustain the growth of mammalian cells, as measured by the number of surviving cell colonies *in vitro*.

CONTACT INHIBITION
Inhibition of cell division following membrane contact of neighbouring cells, characteristic of untransformed cells.

FOCUS FORMATION
Generation of groups of transformed cells with distinctive morphology in cell cultures.

as the keratinocyte growth factor receptor[65], the serine kinase EST[66] and *NET1* (REF. 67).

Senescence could be one mechanism by which tumour cells are eliminated[68] and its investigation could therefore be of considerable importance. After narrowing down the candidate clones by SUBTRACTION HYBRIDIZATION, several genes were found to be potentially involved in senescence and have been screened for the induction of this cellular condition. One of them was identified as a polynucleotide phosphorylase that is involved in RNA degradation[69].

Recessive loss-of-function screens. Most often, selections are applied if recessive gene functions are assayed[70]. Occasionally, cells are mutated and selected for the absence of specific properties. Rather than screening a gene collection, candidate cDNAs that are suspected to be responsible for the defect are tested. Cell lines that are reconstituted with the affected gene can then be conveniently compared with wild-type cells for biochemical differences. This has been performed with a collection of cells that were unresponsive to the signal of the inducible transcription factor nuclear factor of κB (NF-κB) after tumour necrosis factor (TNF) treatment[71]. In the following screen, the cell clones were investigated for those that lacked a specific gene — in this case, the signal transducer receptor-interacting protein (RIP). The resulting cell clone was then compared with wild-type cells and it was concluded that RIP transmits a specific signal for apoptosis that is generated by one of the TNF death-domain receptors.

Complementation screens. In these screens, a cellular defect is restored by the transfection of a cDNA library. In one example, a dominant-negative β1-integrin-cytoplasmic domain inhibited integrin signalling. An expression library was screened first by fluorescence-activated cell sorting (FACS) analysis and then by sib selection for the reconstitution of the integrin activity. CD98 — a transmembrane protein that specifically associates with integrins and is known to be an early T-cell activation antigen — was identified in this way[72].

Using the same strategy, several groups described cell lines that proved to be unresponsive to the interferon-α or -γ signals and reported cloning the missing components of these signalling pathways, which turned out to be classical signal transducers such as Jak kinases, Stat transcription factors and receptor proteins[73,74]. A similar approach corrected the defect of the CHO cell line EM9 to withstand ionizing radiation and led to the isolation of *XRCC1* (REF. 75), a DNA-repair gene.

Synthetic lethal screens. This type of screen detects mutations that are lethal only in combination with a given gene mutation[76]. Instrumental to it are cells with a plasmid that contains a specific gene in its wild-type form, whereas the genomic DNA harbours its mutant version. The genome is again mutagenized and the cells are screened for those in which the plasmid is not spontaneously lost as a result of segregation of the plasmid. These cells contain a mutation that is incompatible with the mutated genomic allele, which indicates a functional interaction between them. In its first application, it was shown that this system works for genes in nucleotide synthesis pathways[77].

This approach is especially interesting as it promises to specifically target tumour cells that have arisen through the activation of known oncogenes.

Other screens. All of the above assays use forward genetics — they start with a mutagenesis step and are followed by the detection of a phenotype. The underlying genetic alteration(s) is then sought and correlated with a molecular function. Reverse-genetic screens have also been performed in cell lines. They begin with a known gene that is altered and re-introduced into the cell or organism to test its effects. This approach is among the most popular to test deletion mutants for mapping functional domains in proteins[78].

In other experiments of this type — often referred to as 'directed evolution'[79] — researchers try to optimize genes for a pre-defined activity. Techniques for random mutagenesis, such as DNA SHUFFLING[80], are used to generate novel alleles for their subsequent *in vitro* screen in cell lines. For example, in one report, the cytokine IL-12 has been improved 128-fold for human T-cell proliferation[81]. In another study, the activity of the epidermal growth factor (EGF) was enhanced 123-fold[82].

New approaches

Targeting proteins in screens. As many disease models can be recreated in cell culture, the pharmaceutical industry has been using cultured cells extensively in drug screens. In a typical setting for such an experiment, the cells are kept in multiwell plates, treated with a panel of different chemical compounds and assayed for specific cellular changes associated with a disease[83]. With the advent of newer, more specific chemical reagents, it has been suggested that these compounds be used as baits to determine the interacting proteins (and ultimately the genes that encode them) that are thought to be responsible for the observed effect. Although the specificity of this approach, called 'chemical genetics'[84], has been questioned[85], it has already yielded several interesting genes and insights into cellular pathways. For example, the drug monastrol, a novel 1,4,-dihydropyrimidine-based compound, was found to arrest cells in mitosis with a MONOPOLAR SPINDLE. It turned out to interact with and inhibit the mitotic motor protein kinesin Eg5, which is required for spindle bipolarity[86].

Another approach uses an antibody library to target intracellular proteins for destruction. It makes use of the relatively straighforward generation of complex antibody libraries by synthetic means[87]. A chromophore is coupled with the antibodies, or the target proteins are directly fused to GFP[88] or a fluorescein-binding domain[89,90]. They are then excited by a laser after their introduction into cells to generate short-lived reactive oxygen species that destroy the proteins[91], thereby creating knock-out cells. This approach has been used, for example, in an assay to identify β-1 integrin as a crucial determinant for the invasiveness of fibrosarcoma cells[92].

SUBTRACTION HYBRIDIZATION
A technique that is used to specifically enrich the DNA species that are present in one sample by hybridizing with nucleic acids of another sample and removing the associated double-stranded molecules.

DNA SHUFFLING
An *in vitro* technique that uses the artificial recombination between genes to accelerate the mutation rate to select for pre-defined qualities.

MONOPOLAR SPINDLE
Aberrant mitotic spindle arrangement that shows a monoastral microtubule array (instead of the normal bipolar spindle).

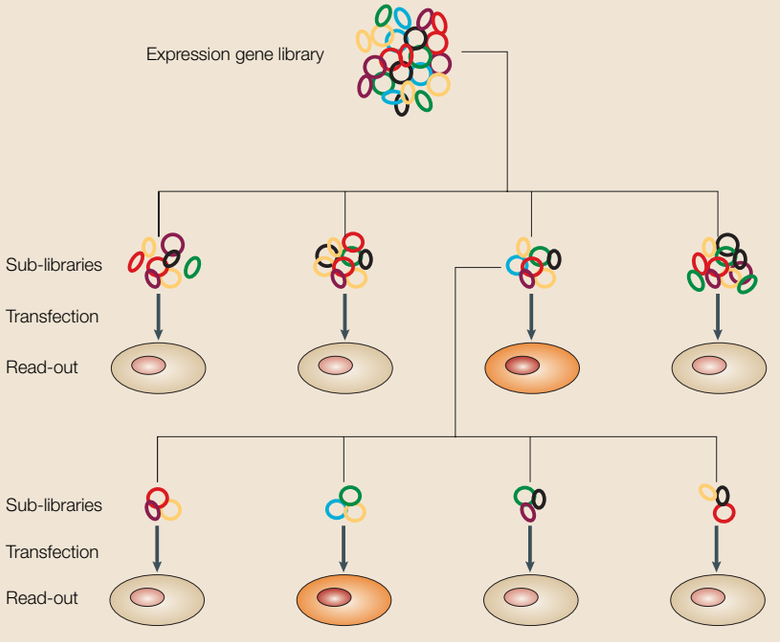
As with chemical genetics, the associated protein must then be identified using the antibody to biochemically isolate the antigen and only then can the corresponding gene be determined.

Expression screening goes genomic with array technologies. With the availability of the complete human and mouse genomes, systematic genome-wide genetic screens became feasible and are poised to augment the tools for functional genomics in an important way. Whereas correlative methods — such as DNA microarrays that investigate differentially expressed genes in the whole genome — have found many genes that could potentially be involved in a given process, functional genetic screens promise to detect only those genes that are truly responsible for the cellular effect. Such screens necessitate the evaluation of many hundreds of thousands of read-outs. Consequently, they require a massive parallel investigation of genes in a high-throughput format that can be accomplished by either miniaturization or automation.

Two strategies have recently been developed to potentially cover all expressed genes in genetic screens (FIG. 3). In contrast to traditional screens that use batch transfections, both approaches detect the effect of single expression plasmids that have been introduced into a separate population of cells. They provide sensitivity and help to clone even those cDNAs that exert only a minor effect on a subpopulation of transfected cells.

The first method[93] uses expression plasmids spotted on glass arrays that are subsequently covered by lipofection reagents and overlayed with cells. The cells take up and express the plasmids and lead to a pattern of transfected cells that corresponds to the pattern of the DNA array. Approximately 30–80 cells are transfected with each printed plasmid spot in this procedure. As the DNA spots are only ~150 μm in diameter, several hundred plasmids can be tested on one slide. This cell microarray allows the read-out to be performed in a high-throughput format with optical detection methods using microscopes. Its potential has been shown by isolating several positive controls in model screens for many possible cellular responses, such as cell adhesion, apoptosis and kinase signalling[93]. In addition, detection of receptors for pharmacologically relevant compounds highlights the possible use of this assay as a substitute for protein arrays. In contrast to conventional protein chips, this approach makes it possible to investigate the polypeptides in their native configuration.

Box 3 | **Expression cloning**



Expression cloning is the most frequently used method for cloning genes in mammalian cells. It was developed according to the rationale that many gene functions can only be observed in the context of a mammalian cell[29,119].

During expression cloning, an expression library is introduced into cells in either a batch transfection or in the form of sib selection. The former requires the subsequent selection of those cells that show a desired cellular change. The transfected DNAs — mostly extrachromosomal, autonomously replicating plasmids — are then isolated by HIRTH EXTRACTION[120], amplified in bacteria and again transfected into mammalian cells. Plasmids that encode the desired protein are enriched by repeated rounds of this procedure.

Sib selection can, despite its name, be considered to be a screen. In this assay, the expression gene library is subdivided into several pools that are individually transfected into cells (see figure). They can then be inspected for the cellular effect and the sib-selection process is started by subdividing the positive plasmid pool into several smaller pools. These are again transfected into cells and the positive pool is determined. After several repetitions, the active pool is small enough to test single genes to determine the underlying gene[121]. Consequently, the selection in this assay takes place at the level of the plasmid pools, not at the cellular level.

HIRTH EXTRACTION
Gentle lysis of cells that releases extrachromosomal plasmids in the supernatant.

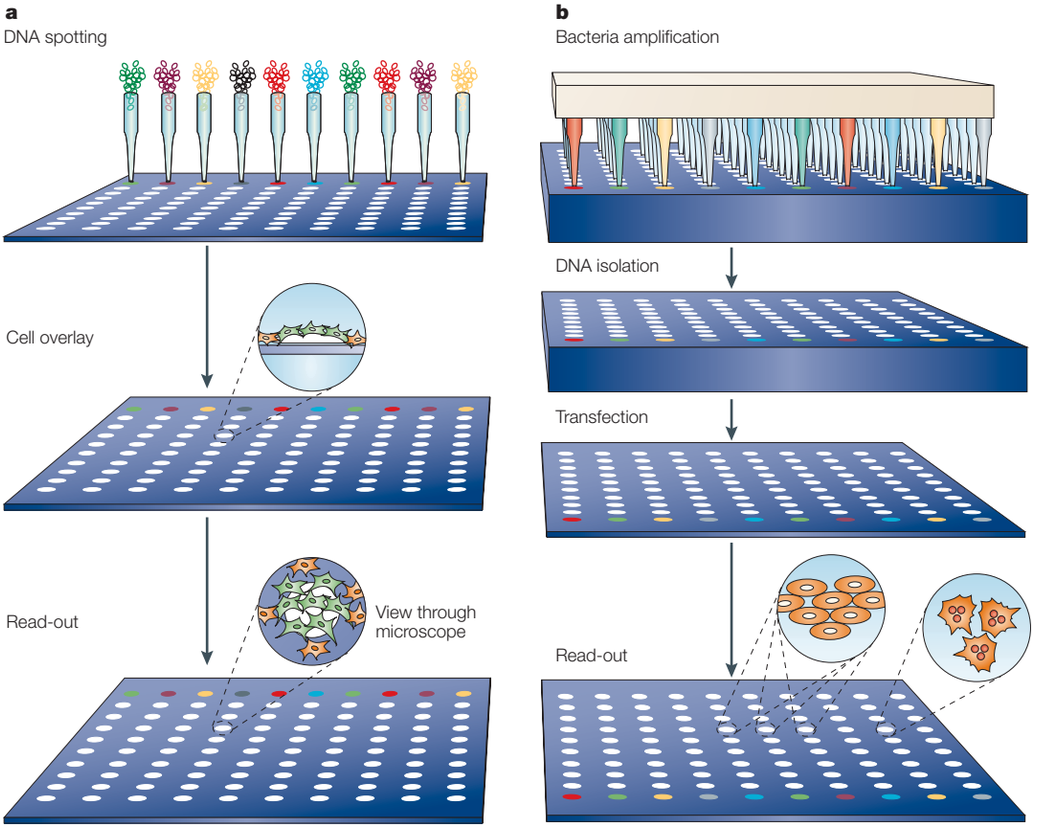


Figure 3 | **Two alternative approches to genome-wide screens in mammalian cells. a** | Cell microarrays are generated by spotting individual expression plasmids on a slide that is subsequently overlaid with cells that take up the spotted plasmid DNA. This generates pre-defined patterns of transfected cells. The cellular effects of the expressed genes can be detected by microscopic inspection of the cells. **b** | The robotic single cDNA investigation (RISCI) assay involves one DNA amplification, one DNA preparation, one transfection and one read-out for every individual gene in a 96-well format. The different colours indicate that a different plasmid is processed in each well. All steps are performed by robots to allow high throughput.

The other approach — robotic single cDNA investigation (RISCI) — probes gene activities in mammalian cells by using high-throughput robots for each step[94,95]. Single plasmids are grown in bacteria in a 96-well format and the plasmid DNA is isolated using a special protocol to attain pure DNA[96]. The DNA is transfected into cells and the functional read-out performed. As the cells that are transfected by the individual plasmids are separated in the 96-well plate, it is possible to detect even secreted proteins or perform enzymatic assays. So far, this screen has been used to detect the prototypical read-out that is unsuitable for selection: the induction of apoptosis. The results showed that many isolated genes mediate signals for apoptosis within the cell. This is in agreement with the general observation that genes that transmit a signal for apoptosis can also induce cell death on overexpression. One gene from this screen encodes ANT1 (REF. 97), a subunit of the mitochondrial PT-pore[98]; another gene is *cybL/SDHC*, a component of complex II of the respiratory chain and a tumour-suppressor gene[99]. Both genes transmit a variety of pro-apoptotic signals. By contrast, Spike (a novel BH3 protein of the endoplasmic reticulum[100]) and the metastasis suppressor gene *KAI1* (REF. 101) convey specific pro-apoptotic signals.

A different version of this screening approach was set up without the use of robots. An arrayed virus library was created in PACKAGING CELLS and various cell lines were infected yielding several interesting genes for osteoblast differentiation, capillary formation and loss of endothelial morphology[102].

The above approaches were all first designed as screens for dominant traits that were observed following the overexpression of genes. However, they are also suitable for uncovering recessive gene activities.

RNAi is a new technique that uses double-stranded RNA (dsRNA) to inhibit endogenous mRNAs in a sequence-specific manner[103]. Its recent application in mammalian cell cultures[14] promises to have a strong impact on the way in which genes can be repressed, especially in high-throughput assays. The cell microarray is suitable for RNAi experiments[104,105] and was recently used in an RNAi screen to search for genes that modulate the pro-apoptotic signal of the tumour-necrosis-factor-related apoptosis-inducing ligand (TRAIL) receptor, a member of the tumour necrosis

PACKAGING CELLS
Cell lines that express one or more viral proteins and, in combination with additionally transfected components, release *in trans* reconstituted infectious particles.

INTRACELLULAR SECOND MESSENGER
Signalling molecule in cells the concentration of which changes on the binding of an extracellular ligand to a plasma membrane-bound receptor.

factor receptor (TNFR) family[106]. Although in this experiment only a limited number of genes were repressed, both negative and positive regulators of TRAIL could already be determined.

High-throughput screening methods promise to achieve a qualitative difference compared with existing methods by increasing the sheer quantity of read-outs. In the end, however, who can remember hundreds of genes that are responsible for a given cellular phenotype? The human mind is only able to comprehend complex issues if a network of information can be established. For this reason, it will only be the combination of these technologies with the classical molecular characterization and physiological integration of single genes that will be most informative.

Prospects

High-throughput screens, such as those outlined above, take advantage of the Human Genome Project with its complete annotation of all expressed genes. The mammalian gene collection project[107], or its counterpart in the mouse[108], will comprise all expressed cDNAs and will greatly enhance investigations by RISCI or cell microarrays. Moreover, a consortium will soon generate RNAi constructs for every expressed human gene[109]. As with the mammalian gene collection, this would make a saturation screen possible, circumvent the redundancy of the present gene libraries and increase the throughput. Such complete and annotated master gene libraries could convert both screens from forward to reverse screens. In combination with new and innovative functional screens, it could greatly contribute to our understanding of gene functions.

With procedures at hand to achieve high throughput in genetic assays for mammalian cell culture cells, the limiting factor is still the read-out — that is, the detection of the cellular changes. However, many experimental approaches that address this bottleneck have recently been described, mainly initiated by the pharmaceutical industry or at least influenced by its need to generate novel targets. These different approaches could usher in a convergence of the methods used by geneticists and pharmaceutical screening experts to elucidate the function of the genome by amassing many physiological data points. High content screening[110], for example, refers to the use of high-resolution microscopes that are used to scan the cells for phenotypic changes in an automated fashion. Rather than focusing on single biochemical alterations in the cell, it uses a range of powerful optical read-outs for multiple cellular targets in parallel.

Another application, multiplexed array technology[111], labels various cells with different tags, combines them in one experiment and correlates their reactions with the original label. Moreover, the detection of cellular activities could be sped up by using high-throughput FACS analysis with its myriad of possible applications that range from cell-cycle analysis and organelle physiology to the detection of INTRACELLULAR SECOND MESSENGERS[112].

Microfabricated devices, often referred to as 'lab-on-a-chip', use interdisciplinary technologies, such as microfluidics and nanotechnology, to analyse samples on the smallest scale. They could provide new and rapid detection techniques for biochemical sensors[113]. Because of their tiny dimensions, they are pre-destined for high-throughput approaches. Beyond such technical improvements, biological advances could also enrich screens in the future. Stem cells can differentiate into a variety of specialized cell types and hold great potential in the treatment of human diseases. However, the factors that direct their differentiation are not well understood. So, stem cells could considerably widen the use of screens for discrete differentiation steps.

With all these developments underway, it is safe to say that genetic screens in cell culture cells are destined to generate an immense amount of useful data and greatly help to elucidate the functions of the genome. Consequently, it can be predicted that screens in mammalian cell culture have a bright future.

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Acknowledgements

I thank H. Hermeking and A. Gewies for comments on the manuscript. The work in my laboratory was supported by Roche, the Deutsche Forschungsgemeinschaft and Bayrisches Staatsministerium. I apologize to all colleagues whose work was not cited owing to space limitations.

Competing interests statement

The author declares that he has no competing financial interests.

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