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REVIEW ARTICLE

Towards a compendium of essential genes – From model organisms to synthetic lethality in cancer cells

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Abstract

Essential genes are defined by their requirement to sustain life in cells or whole organisms. The systematic identification of essential gene sets not only allows insights into the fundamental building blocks of life, but may also provide novel therapeutic targets in oncology. The discovery of essential genes has been tightly linked to the development and deployment of various screening technologies. Here, we describe how gene essentiality was addressed in different eukaryotic model organisms, covering a range of organisms from yeast to mouse. We describe how increasing knowledge of evolutionarily divergent genomes facilitate identification of gene essentiality across species. Finally, the impact of gene essentiality and synthetic lethality on cancer research and the clinical translation of screening results are highlighted.

Keywords

Cancer research, CRISPR/Cas9, essential genes, genetic interaction, RNAi, screening, synthetic lethality

History

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Introduction

In the past decades, an increasing number of genomes have been completely sequenced (Adams, 2000; Hillier *et al*., 2008; Howe *et al*., 2013; Venter *et al*., 2001; Wood *et al*., 2002). With the increasing knowledge of the sequence composition of genomes, the next challenge has been to comprehensively analyze the function of encoded genes. Of particular interest have been genes that are essential for survival at the cellular or organismic level. Identifying the minimal set of genes necessary for sustaining life will allow better understanding of life itself and provide insight into the origin of diseases.

The search for essential genes has been extensively conducted in prokaryotic organisms, with the aim to identify novel targets for antibiotic therapy (Clatworthy *et al*., 2007) and critical building blocks for synthetic biology (Khalil & Collins, 2010). Due to the small size of prokaryotic genomes and their easy accessibility to genetic manipulation, essential genes have been identified for a broad panel of prokaryotic organisms (de Berardinis *et al*., 2008; Gerdes *et al*., 2003; Glass *et al*., 2006; Kobayashi *et al*., 2003). In eukaryotic organisms, multiple loss-of-function technologies have been developed to investigate gene functions, including chemical mutagenesis (Hrabé de Angelis *et al*., 2000), insertional mutagenesis (Bellen *et al*., 2004), RNAi technologies (Dietzl *et al*., 2007; Kamath *et al*., 2003) and CRISPR/Cas9 genome editing (Shalem *et al*., 2015; Wang *et al*., 2014). The effectiveness and also the limits of those screening technologies have determined the scope by which essential genes have been recovered.

In this review, we describe the development of screening technologies and their impact on discovery of essential genes for common eukaryotic model organisms. We illustrate how knowledge of gene essentiality contributes to understanding of human diseases and can be employed for anticancer therapy.

What is an essential gene?

Essential genes are defined as genes that are required for sustaining life (Juhas *et al*., 2011). The concept of gene essentiality and its limits was first discussed in 1963 (Gluecksohn-Waelsch, 1963). Presently, the general understanding of an essential gene is that it is required for survival and proliferation of single cell organisms. In multicellular organisms, loss of essential genes results in lethality during development or inability for reproduction. The estimated proportion of essential genes varies considerably between different species, and also among different publications (Table 1). Reasons for this discrepancy can be diverse and include differences in methods used to achieve loss-of-

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Table 1. Estimated proportion of essential genes in model organism.

Saccharomyces cerevisiae	
17–18.1%	(Giaever *et al*., 2002; Winzeler *et al*., 1999)
Caenorhabditis elegans	
13.9%	(Johnsen & Baillie, 1991)
1%	(Clark *et al*., 1988)
8.5%	(Kamath *et al*., 2003)
Drosophila melanogaster	
8-16.3%	(Bellen *et al*., 2004)
30%	(Dietzl *et al*., 2007)
Danio rerio	
5.4%	(Amsterdam *et al*., 2004)
9.3%	(Haffter *et al*., 1996)
Mus musculus	
13.3%	(Bradley *et al*., 2012)
Homo sapiens (core essential genes in a cancer cell line panel)	
1.4%	(Hart *et al*., 2014)

The percentage of essential genes is obtained by dividing the number of essential genes given in the indicated literature by the total number of protein coding genes in the respective genomes (retrieved from ENSEMBL database).

function, inability to perform genome-wide knockouts in many organisms and incomplete recovery of all phenotypes associated with gene essentiality. While there is a core set of essential genes that shows a stringently lethal phenotype upon loss, there is a larger group of genes on which survival depends on specific environmental conditions, in particular developmental stages or tissues. The impact of the environment on gene essentiality was extensively described for *Saccharomyces cerevisiae*, showing that under conditions other than rich in nutrients, the percentage and composition of essential genes varies (Giaever *et al*., 2002; Hillenmeyer *et al*., 2008). For instance, while 4769 homozygous deletion strains were considered non-essential in rich medium, only 205 strains (3% of the genome) were non-essential when growth was tested under multiple environmental conditions (Hillenmeyer *et al*., 2008). Furthermore, defects in genes related to the immune system can also lack any visible phenotypes under laboratory conditions, but quickly become essential upon challenge by infectious agents (Galiana-Arnoux *et al*., 2006; Gazit *et al*., 2006).

In multicellular organisms, gene essentiality can be restricted to specific tissues and developmental stages. For example, mice deficient in SLC2744/FATP4, a protein responsible for the cellular import of free fatty acids, die shortly after birth due to skin abnormalities (Herrmann *et al*., 2003). This lethal phenotype can be rescued by re-expression of the protein in the skin (Shim *et al*., 2009). In contrast, knockout of SLC2744/FATP4 in either adipose tissue or the intestine did not show any striking phenotype (Lenz *et al*., 2011; Shim *et al*., 2009). In another example, post-developmental knockdown of essential genes in *Caenorhabditis elegans* revealed a fraction of genes than can actually prolong life-span when their function is lost at a later developmental stage (Curran & Ruvkun, 2007).

Another concept that is closely related to gene essentiality is fitness. Fitness and fitness defects were originally used to describe changes of allele frequencies in population studies (Otto & Lenormand, 2002) (Figure 1). As opposed to gene essentiality, it is not measured on a single cell level, but is a

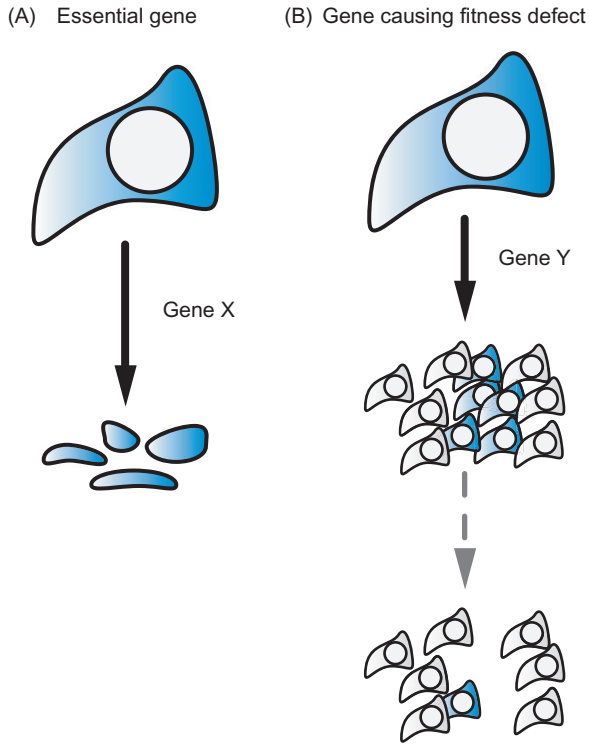

Figure 1. Model of essential genes and genes causing fitness defects. Loss-of-function of an essential gene X leads to cell death (A). In contrast, loss of a gene Y that is associated with a fitness defect leads to the gradual disappearance of the affected individuals from the population (B).

population-level phenotype. Among others, it describes the exponential growth rate of a given population relative to its wild-type counterpart (Giaever *et al*., 2002; Hillenmeyer *et al*., 2008). Compared to essential genes, loss of genes that are associated with fitness defects can show only mild or no phenotypes within one generation. However, in a heterogeneous and dynamic population, selective pressure against fitness defects will result in the disappearance of individuals carrying the unfavorable trait within consecutive generations, as shown for *C. elegans* (Ramani *et al*., 2012). Thus, genes that cause fitness defects can also be considered as essential in the context of population dynamics. Two studies in *C. elegans* and *S. cerevisiae* have shown that genes previously considered to be dispensable are actually associated with fitness defects (Breslow *et al*., 2008; Kamath *et al*., 2003). In summary, while the definition of gene essentiality is seemingly straightforward, unambiguously classifying a gene as essential is difficult and remains highly dependent on the context by which its function is measured.

Discovery of gene essentiality in single cell organisms

Saccharomyces cerevisiae, or budding yeast, was one of the first eukaryotic organisms in which essential genes were studied by a systematic approach and on a genome-wide scale (Winzeler *et al*., 1999). *Saccharomyces cerevisiae* genes have many orthologs in common with other eukaryotes and the high rate of homologous recombination in *S. cerevisiae* enables its rapid genetic modification (Baudin *et al*., 1993). Thus, it has been a favorable model for studying gene function on a larger scale. In a first set of experiments by Winzeler

et al. (1999), deletion strains for 2026 ORFs were created, of which 17% were found to be essential for growth and survival in rich medium. Using a competitive growth assay with a pool of homozygous deletions strains of non-essential genes, the authors additionally showed that 40% of the strains have fitness defects. The second large-scale deletion screen in *S. cerevisiae* already included 5916 genes (96% of all annotated ORFs), of which 18.7% turned out to be essential for growth in rich medium (Giaever *et al*., 2002). It was also observed that essential genes have more homologs in other organisms than their non-essential counterparts and that only 1% of essential genes had duplicates in the genome, as opposed to 8.5% of non-essential genes. While Winzeler *et al.* assessed phenotypes under two nutritional conditions (rich and low nutrients), Giaever *et al.* used five different conditions to demonstrate that gene essentiality and fitness both vary depending on the given environment. This observation was supported by another study in which a collection of ~11 000 homo- or heterozygous deletion strains were tested against 726 different drugs or environmental stresses (Hillenmeyer *et al*., 2008). The authors observed that 97% of all mutants exhibited growth defects under at least one condition, and therefore suggest that nearly all genes are required under a specific environmental condition.

A main challenge for analyzing the function of essential genes in *S. cerevisiae* is the difficulty to generate hypomorphic mutants. Several methods have been developed to address this issue: essential genes can be shut off by inducible transcriptional repression (Mnaimneh *et al*., 2004), by heterozygous deletion (Deutschbauer *et al*., 2005) or by mRNA perturbation (DamP) (Breslow *et al*., 2008).

Recently, a genome scale collection of deletion mutations was generated for *S. pombe* (Kim *et al*., 2010). *Saccharomyces cervisiae* and *S. pombe* are distantly related and differ in many cellular functions (Wood *et al*., 2002), thus allowing for comparison and identification of genetic functions that are common to eukaryotes in general. In *S. pombe*, 4836 genes could be deleted, corresponding to 98.4% of all ORFs. Of those, 1260 or 26.1% were found to be essential. Similar to budding yeast, the proportion of single copy genes or singletons was higher among essential than non-essential genes. Gene sets of essential genes in both organisms were enriched for specific cellular processes (synthesis of DNA, RNA, lipids and proteins, transcriptional initiation, ribosome assembly).

Studies of essential genes in multicellular organisms

Compared to *S. cerevisiae*, the comprehensive study of essential genes in multicellular eukaryotic organism presents a greater technical challenge. Historically, most efforts to obtain genotype–phenotype interactions in multicellular organisms relied on forward genetic screening strategies using chemical or insertional mutagenesis. A major advance in functional genomics was introduced by the complete genome sequencing of model organisms and the development of RNAi technologies. The combination of both enabled the targeted knockdown of genes, allowing for reverse arrayed screens. Here, we describe how gene essentiality was explored in the three common model organisms *Caenorhabditis elegans*, *Drosophila melanogaster* and *Danio rerio*.

In *C. elegans*, the first screens that aimed at determining the number of essential genes relied on chemical mutagenesis with ethyl methanesulfonate (EMS) (Clark *et al*., 1988; Johnsen & Baillie, 1991). Based on the analysis of mutants in specific chromosomal regions including LGV(left) and unc-22 region, the authors estimated that the total number of essential genes in the *C. elegans* genome should range between 2850 and 3500 (Clark *et al*., 1988; Johnsen & Baillie, 1991). The first studies using RNAi in multicellular organisms were performed in *C. elegans*, by feeding animals with bacteria containing double stranded RNA or soaking animals in RNAi solution to achieve knockdowns (Fraser *et al*., 2000; Gönczy *et al*., 2000) (Figure 2A). Both screens started with the knockdown of genes on single chromosomes and steadily increased genomic coverage to genome scale (Kamath *et al*., 2003; Maeda *et al*., 2001; Sönnichsen *et al*., 2005). Roughly 800 genes were found to be critical for early embryonic development under laboratory conditions (Sönnichsen *et al*., 2005), which is only 4% of the *C. elegans* genome. The total number of essential genes was estimated to be around 1750 (Kamath *et al*., 2003). However, later studies indicate that the vast majority of non-essential genes show a measurable degree of fitness defect if measured over several generations, indicating that the number of essential genes might be underestimated (Ramani *et al*., 2012).

The use of transposable elements for insertional mutagenesis has been a major tool for studying genotype–phenotype interactions in *D. melanogaster*. Using P-element transposons, essential genes were identified by screening mutants on individual chromosomes (Bourbon *et al*., 2002; Deak *et al*., 1997; Oh *et al*., 2003; Peter *et al*., 2002). The number of identified essential genes ranged from 130 to 850. The effort to generate and classify P-element insertions in every gene is systematically conducted by the Berkeley Drosophila Gene Project. However, achieving genomic saturation with P-elements is difficult and so far, only 40% of all drosophila genes have been successfully disrupted (Bellen *et al*., 2004). The percentage of lethal genes found by the Berkeley Drosophila Gene Project ranged between 8 and 16.3%, depending on the study included (Bellen *et al*., 2004). The first genome-scale knockdown screen with dsRNA in *D. melanogaster* was performed in cultured blood cells and identified >400 genes that show a strong reduction of viability upon knockdown, many of which lacked mutant alleles (Boutros *et al*., 2004) (Figure 2A). In 2007, a genome-wide transgenic RNAi fly library was published and found that roughly 30% of the fly lines showed a lethal phenotype (Dietzl *et al*., 2007).

In *D. rerio*, chemical mutagenesis with N-ethyl-N-nitrosourea (ENU) is an effective tool to introduce germline point mutations. Hence, several studies used ENU to generate large collections of mutants (Driever *et al*., 1996; Haffter *et al*., 1996). However, major general drawbacks of this method included laborious positional cloning to retrieve the underlying mutation and low genomic saturation that can be achieved (Justice, 1999). In the most extensive ENU screen in *D. rerio*, mutants could be assigned to 375 genes, covering only a fraction of the zebrafish genome (Haffter *et al*., 1996).

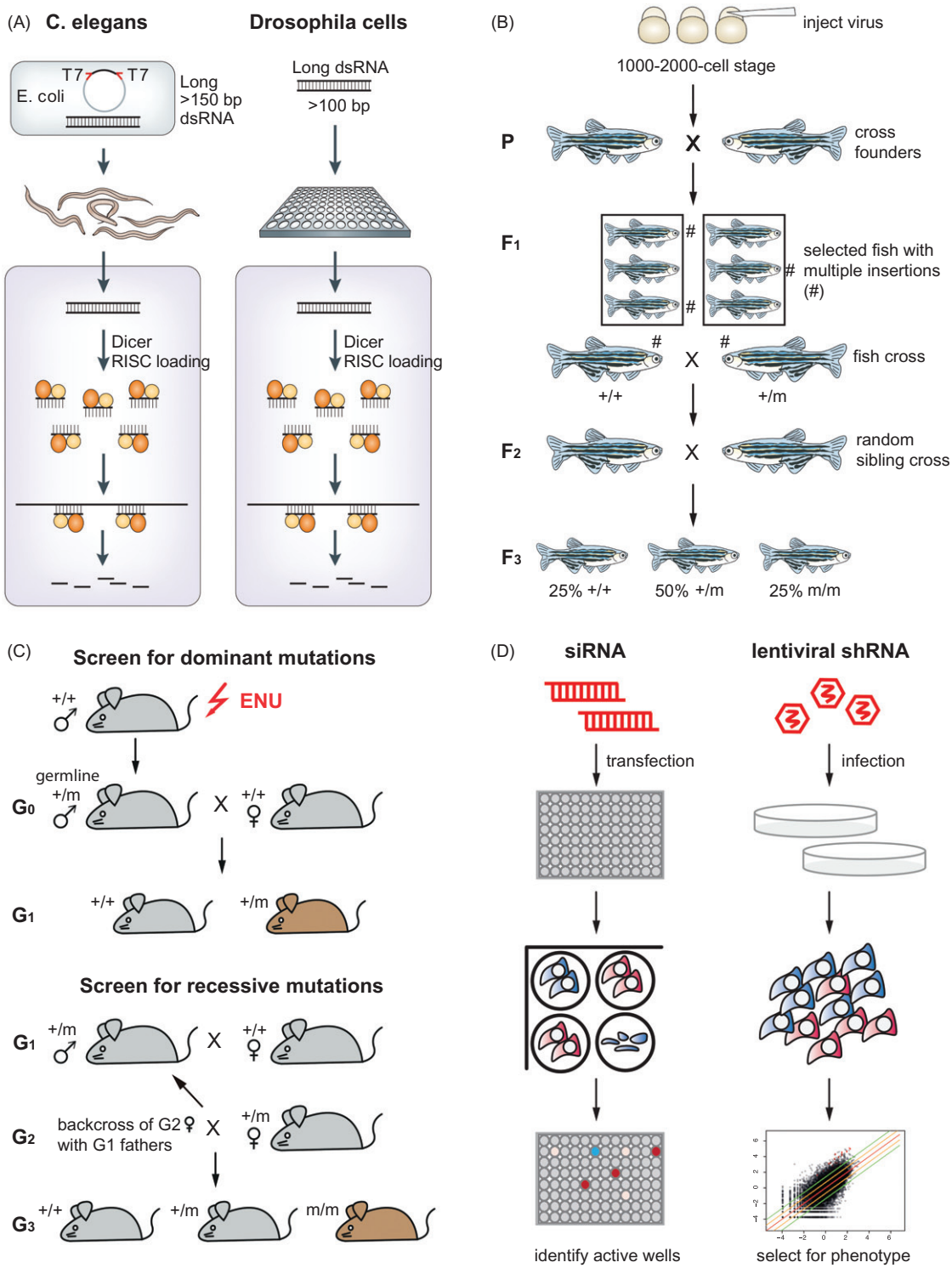

Figure 2. Screening strategies in different model organisms. (A) Schematic overview of RNAi screening approaches in *C. elegans* by ingestion of E.coli and in *Drosophila* cells by bathing (modified from Boutros & Ahringer (2008)). Long double-stranded RNAs are introduced into the respective organisms and diced intracellularly into small-interfering RNAs (siRNAs). This results in many different siRNAs targeting a single transcript. (B) Outline of insertional mutagenesis screen in zebrafish (adapted by permission from Macmillan Publishers Ltd.: Nature Reviews Genetics (Patton & Zon, 2001), (c) 2002). Embryos are injected with a retrovirus at a 1000–2000-cell stage. These embryos are raised (= founder generation P), mated and the mutations transmitted to the F_1 generation. Individual fish from the F_1 generation with multiple insertions are selected and further crossed with each other to generate the F_2 generation. Siblings of each F_2 family are crossed with each other to generate homozygous mutations. (C) Overview of screening approaches using ENU induced mutagenesis in mouse. For dominant mutations, ENU mutagenized males carrying mutations in their germ lines are crossed with wild-type females (G_0). Dominant mutations will be detected in the G_1 generation. For recessive mutations, males of the G_1 generation carrying mutations are crossed with wild type females. Then females of the resulting G_2 generation are backcrossed with G_1 males, thereby generating the G_3 generation that potentially carries individuals with homozygous mutations. (D) Arrayed versus pooled loss-of-function screens in cancer cell lines. siRNAs are used for arrayed screens in a multi-well plates. Each well harbors a distinct gene knockdown. Candidate genes are detected by measuring signal levels (e.g. luminescence) of individual wells. In contrast, pooled loss-of-function screens rely on viral infection of cells with shRNA vectors. Each vector contains a barcode allowing identification of the specific shRNA. Pools of cells with different gene knockdowns are generated and cultured for several doubling times. Depletion of cells with specific gene knockdowns is detected by sequencing of barcodes and measuring their relative abundance at different time points.

Based on the results, the authors estimated that the percentage of lethal genes is roughly 2400, which is approximately 10% of the complete genome. However, only mutants with specific organ dysfunctions were selected for genotyping while mutants with multiple, non-viable malformations were not considered. Thus, the number of essential genes is most likely underestimated. Another forward genetic screening approach in *D. rerio* relied on the use of insertional mutagenesis (Patton & Zon, 2001) (Figure 2B). Two large-scale retroviral insertion screens were conducted in zebrafish, but only few essential genes could be retrieved (Gaiano *et al*., 1996; Golling *et al*., 2002). An insertional screen for embryonic and early larval development identified 315 essential genes, but only achieved a genomic saturation of 25% (Amsterdam *et al*., 2004). Based on these numbers, the authors estimated that ∼1,400 genes would be essential for embryonic development (Amsterdam *et al*., 2004). Of the genes identified, a high proportion had homologs in yeast (72%) and human (99%), indicating that essential genes are phylogenetically conserved.

Essential genes in mouse

The mouse is the best studied mammalian model organism and identification of essential genes is of particular interest due to its close phylogenetic relationship to humans. Chemical mutagenesis with ENU has been the predominant screening tool to generate mouse mutants with novel phenotypes. ENU is a very powerful mutagen and predominantly creates single base mutations with the highest mutation rate in male spermatogonial stem cells (Balling, 2001; Russell *et al*., 1979). Chemical mutagenesis with ENU can cause both loss and gain of function mutations, and specific crossing strategies are required to obtain the desired mutations (Figure 2C). One of the first efforts to identify lethal genes in mouse used ENU to generate mutants and back-crossings to identify affected genomic loci, but without recovering the precise point mutation (Rinchik & Carpenter, 1993). A major drawback of ENU-based screens has been the laborious positional cloning necessary to identify underlying point mutations, which limited the rate by which novel mutants could be genotyped. In spite of this drawback, several large-scale ENU screens were initiated in the past to systematically generate, characterize and genotype novel mouse mutants (Hrabé de Angelis *et al*., 2000; Nolan *et al*., 2000a,b). While a large panel of phenotypic traits was documented for every mutant including fertility, other phenotypes of essential genes such as embryonic lethality were missed. Thus, only few essential genes were found in ENU screens. With the development of technologies for targeted gene disruption, many essential genes were identified by studying single gene functions in murine knockout models (Matsui *et al*., 1996; Varfolomeev *et al*., 1998).

Since the establishment of homologous recombination in embryonic stem cells as a tool for targeted gene deletion (Thomas & Capecchi, 1987), this technology has been further developed to enable generation of knockout cells on a larger scale (Skarnes *et al*., 2011). Consequently, large efforts aiming at systematically generating knockout mouse models for every gene were started (Bradley *et al*., 2012; White *et al*., 2013). A subset of mice mutants with knockouts of 472 secreted proteins have been screened for specific phenotypes, and 8% of those showed pre-weaning lethality (Tang *et al*., 2010). So far, roughly 3000 genes were identified to be essential upon knockout, which accounts for ∼13% of the murine genome (Georgi *et al*., 2013).

Bioinformatic resources

With the wealth of data available from both large loss-of-functions screens and genome sequencing projects, web-based depositories for genotype–phenotype interactions have been developed. The Online Essential Gene Database OGEE integrates results from large-scale screens from 16 prokaryotic and 8 eukaryotic organisms (Chen *et al*., 2012). The database offers annotations to each essential protein-coding gene, including corresponding expression profile, duplication status or involvement in embryonic development. Another repository is the Database of Essential Genes (DEG), which since its first publication in 2004 has been updated several times (Luo *et al*., 2013). The most recent release, DEG 11, includes essential genomic elements beyond protein-coding genes, such as promotors or non-coding RNAs. For common model organism such as *M. musculus*, *C. elegans* or *D. melanogaster*, community databases exist that systematically collect available phenotypes for every gene of the respective organisms (Blake *et al*., 2014; Dos Santos *et al*., 2015; Harris et al., 2014) and are therefore a useful resource for detailed information on essential genes. The growing amount of data also enables comparative genomics approaches to explore common features of essential genes across different species (Figure 3). For example, the propensity of genes to be lost in evolution was studied for a set of eukaryotic organisms (Krylov *et al*., 2003). Essential genes were found to be associated with a low propensity to be lost during evolution, to accumulate fewer substitutions in the protein sequence and to be highly expressed. Furthermore, essential/lethal genes are found to be highly connected in protein networks (Jeong *et al*., 2001) and have a high degree of phylogenetic retention (Gustafson *et al*., 2006). In contrast, non-essential genes were more frequently targeted by many transcriptional factors, indicating that they are more dynamically regulated (Yu *et al*., 2004). Based on functional data from yeast, essential genes were initially thought to be predominantly singletons. However, later studies could show that both singletons and duplicates are equally represented among essential genes in other organisms (Liang & Li, 2007; Liao & Zhang, 2007). Comparative genomics also allows dissecting characteristics of potentially essential genes in humans, which are not directly amenable to experimental studies. Analysis of the evolutionary and population genetics property of 2472 human orthologs of murine essential genes showed that they have less variants and are more frequently haploinsufficient (Georgi *et al*., 2013). Whether human orthologs of murine essential genes are more frequently associated with human diseases is under debate, as some studies found associations (Dickerson *et al*., 2011; Georgi *et al*., 2013) while others did not (Park *et al*., 2008). In summary, comparative genomic approaches help to

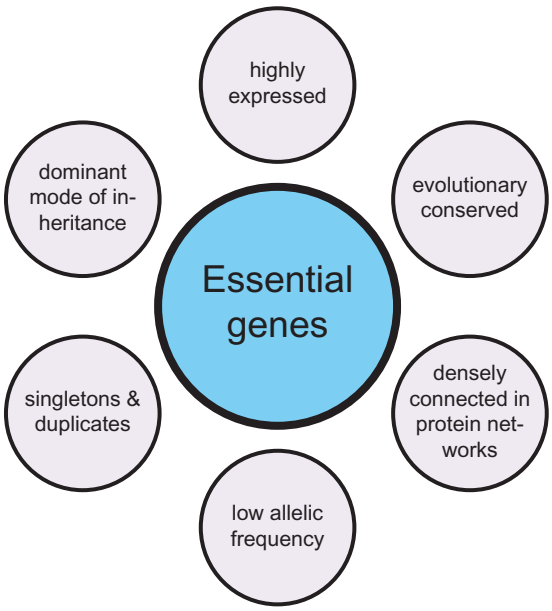

Figure 3. General characteristics of essential genes. General characteristics of essential genes found across different species by comparative genomics are presented.

understand the global structural features of essential genes and thus allow sequence-based prediction of essential genes in organisms without data from functional genomics experiments.

Genetic interactions in model organisms

To systematically study the functions and interaction partners of genes on a larger scale, synthetic genetic arrays have been developed in *S. cerevisiae* as a powerful tool (Boone *et al*., 2007). In these experiments, every gene from a panel of query genes is deleted in combination with a gene from a second panel, resulting in large set of mutants with the loss of two genes. The growth behavior of all mutants is then individually measured and compared to each other. Three possible outcomes can result from a combinatorial loss-of-function: if the observed phenotype is the same as the single knockout, no genetic interaction is assumed. If the knockout of one gene can compensate for the loss of the other gene, then a positive or alleviating genetic interaction is present. If the knockout of one gene aggravates the phenotype caused by the loss of the second gene, a negative genetic interaction is found. If two genes share the same genetic interaction pattern across a large panel of genes, it can be assumed that they are functionally related.

Synthetic genetic arrays were first performed with deletion strains of non-essential genes and could uncover several novel genetic interactions (Tong *et al*., 2001, 2004). Based on these results, large-scale screens were conducted in *S. cerevisiae* mutants that harbor a conditional repressed essential gene and the loss of a query gene (Davierwala *et al*., 2005). The effect of the genetic interaction between 575 essential genes and 30 query genes on growth behavior of mutants was analyzed. Similar to non-essential genes, essential genes also tend to share similar interaction partners if they are functionally related. However, the density of genetic interaction, i.e. the number of interaction partners was five times higher in essential genes, underlining that they are central hubs within the cellular network. Furthermore, the function of previously unknown essential genes could be assigned due to similarity of interaction partners, e.g. PGA1, which is required for specific functions of the endoplasmic reticulum.

Synthetic genetic arrays were also performed in cultured *drosophila* cells using combinatorial knockdown with siRNA and image-based analysis of morphological features of cells (Fischer *et al*., 2015; Horn *et al*., 2011). In the publication by Horn *et al.*, 93 genes involved in MAPK, JNK and p38 signaling were knocked down in pairwise combinations, resulting in positive and negative genetic interactions. For example, single knockdown of Ras85D resulted in a reduced cell number, indicating that this gene is essential. However, the effect of Ras85D knockout could be compensated by the knockdown of a second gene, CG13197 (Horn *et al*., 2011). The same approach was performed on a larger scale by Fischer *et al.*, by combined knockdown of a panel of 1367 genes involved in key cellular process (chromatin biology, cell cycle regulation, protein homeostasis) against a panel of 72 query genes. By grouping genes according to the pattern of their genetic interaction, genes could be assigned to known functional groups and directionality of genetic interaction could be inferred. Using this approach, novel links between the ERK signaling and chromatin remodeling could be discovered.

Essential genes in cancer cells

The identification of essential genes in tumor cells is of outstanding interest in cancer research, as they present potential targets for novel therapeutic interventions. The first evidence that cancer cells may depend on specific mutated genes for proliferation and survival was shown in Kras mutant colorectal cancer cells (Shirasawa *et al*., 1993). It was shown that the targeted deletion of mutant Kras resulted in a significant growth defect of cancer cells in nude mice. This observation was generalized under the concept of oncogene addiction (Weinstein & Joe, 2008), which proposes that cancer can become dependent on specific mutated genes (oncogenes). These oncogenes then take over an essential role within a specific pathway that is not found in normal cells.

Thus, in pursuit of those conditionally essential genes, many loss-of-function screens have been performed. The two main screening strategies that are used to identify candidate genes in cancer cells are arrayed screens using siRNA and pooled screens using shRNA (Figure 2D). A pilot study tested the effect of siRNA-mediated knockdown of 21 genes in transformed and non-transformed mammalian cells, measuring cell viability as outcome (Harborth *et al*., 2001). While the chosen siRNA library was small, the study highlighted that screening with siRNAs was feasible in mammalian cell lines. Three years later, a first large-scale RNAi screen using 5305 siRNAs was performed to identify genes that regulate cell division in HeLa cells (Kittler *et al*., 2004). Although not primarily focusing on essential genes, this study showed that knockdown of previously known essential genes such as ribosomal proteins or proteasome core units result in a lethal phenotype.

One of the first shRNA screens was performed in diffuse B-cell lymphoma using a retroviral library targeting 2500 genes, uncovering an essential role of NFkappaB pathway members for cell survival (Ngo *et al*., 2006).Two years later, several screens that used large pools of shRNAs to identify essential genes in cancer cells were published. The screen by Silva *et al.* used shRNA pools of different scales to knockdown genes in five breast cancer cell lines (Silva *et al*., 2008). Among the identified essential genes were several cell cycle regulators and components of the protein translation machinery. The sensitivity of cells towards knockdown of essential genes varied between cell lines and this observation could be confirmed using drugs with the same targets. Similar findings were obtained from another study that used 8204 shRNAs targeting 2924 genes in four cell lines (Schlabach *et al*., 2008). The number of depleted genes varied significantly between cell lines, from as low as 2.5% of the gene panel to 23.8%. Another shRNA screen used the TRC library developed by the RNAi consortium (Moffat *et al*., 2006), which contains 170 000 shRNAs targeting 17 200 human genes, to screen for essential genes in 12 cancer cell lines (Luo *et al*., 2008). Two sets of essential genes could be discriminated: one set of global essential genes that was found in all cell lines and enriched for cellular processes including mRNA processing, translation and proteasomal degradation. In addition, another set of essential genes was identified that was specific to selected cell lines and often included oncogenes. One example of such a lineage-specific essential gene is IRF4, which was identified to be essential in multiple myeloma (Shaffer *et al*., 2008). IRF4 is not genetically altered itself, indicating that essential genes do not necessarily correlate with mutation status in cancer. Another example is Brd4, which was found to be essential in a genetically defined model of acute myeloid leukemia and is a regulator of Myc (Zuber *et al*., 2011).

Due to the promising findings of the first screens, larger efforts were conducted to identify global and lineage-specific essential genes across larger sets of cell lines. The largest of such efforts to date, termed Project Achilles, assessed gene essentiality for 11 000 genes across 216 human cancer cell lines by pooled lentiviral shRNA screens (Cowley *et al*., 2014). In ovarian cancer, 54 lineage-specific essential genes were identified (Cheung *et al*., 2011), among which PAX8 was further validated and found to be also amplified in ovarian cancer. Another large-scale screen used pooled shRNAs to target 16 000 genes, but focused on a large panel of cell lines for a few selected tumor entities (Marcotte *et al*., 2012). A total of 72 different breast, pancreatic and ovarian cancer cell lines were screened and a large set of essential genes was detected which overlaps with previous shRNA screens, indicating that the method is robust (Koh *et al*., 2012). In total, a core set of 291 genes were discovered to be essential across many cell lines (Hart *et al*., 2014).

Synthetic lethality in cancer

A concept of genetic interaction that has major implication for cancer therapy is synthetic lethality, with the potential to target the selected loss of tumor suppressor genes or addiction to oncogenes in cancer cells (Chan & Giaccia, 2011; Kaelin, 2005). Briefly, synthetic lethality occurs in cells that survive with an altered gene function in either gene A or gene B, but does not survive if the function of gene A and B are both altered (Figure 4). Within the framework of gene essentiality, synthetic lethality can also be understood as an essential functional relationship between two genes.

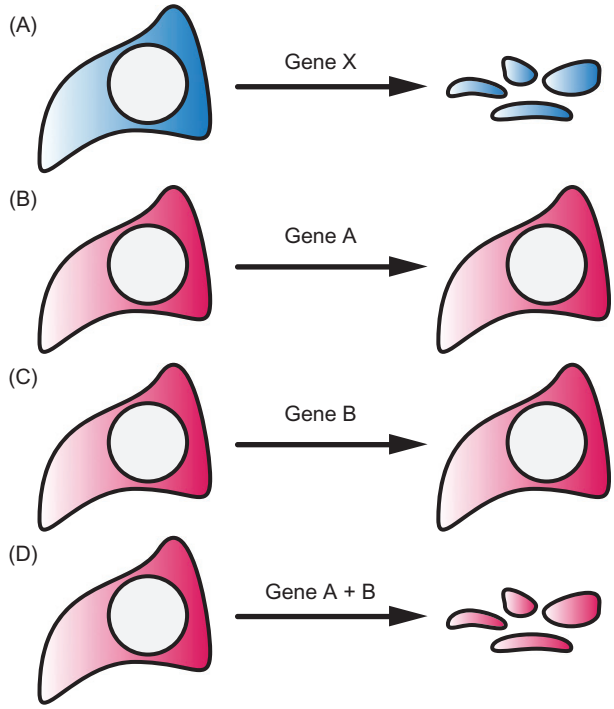

Figure 4. Concept of synthetic lethality. Loss-of-function of the essential gene x leads to immediate cell death (A), while loss-of-function of either gene A or gene B does not have a phenotypic effect. In contrast, combined loss-of-function of gene A and B results in a synthetic lethal interaction.

Since most oncogenes or tumor suppressor genes are not directly amenable to pharmacological therapy, there is an urge to identify genes that become essential due to their functional interaction with oncogenes or tumor suppressors (Garber, 2002; Kaelin, 2005). In addition, genes that gain essentiality when specific cellular pathways are blocked by anticancer drugs are also of particular interest for combinatorial drug treatment. Knowing those genes would considerably enlarge the repertoire of cancer therapy and allow more selective killing of cancer cells.

A first proof-of-principle experiment in a mammalian cell model used a hypoxanthine–guanine phosphoribosyl transferase (HPRT1) deficient cell line that expresses HPRT1 and a GFP reporter on an episomal plasmid (Simons *et al*., 2001). HPRT1 is non-essential under normal conditions and the episomal plasmid is consequently lost. However, when the biosynthetic pathway leading to guanine monophosphate production is perturbed by specific inhibitors, HPRT1 becomes essential and only cells that were able to retain the expression plasmid survived.

Subsequently, several studies exploited the concept of synthetic lethality to identify genes with chemosensitizing potential. For example, an arrayed siRNA screen was performed by Whitehurst *et al.* with the small lung cancer cell line NCI-H1155, measuring viability of gene knockdowns in the presence of sub-lethal concentrations of paclitaxel (Whitehurst *et al*., 2007). The authors identified 87 genes that

render the lung cancer cells sensitive to treatment with this microtubule inhibitor. In another arrayed siRNA screen, breast cancer cells were treated with a PARP-inhibitor and a number of kinases were identified to act synthetically lethal with the inhibitor (Turner *et al*., 2008). Using the same approach, synthetic lethal interactions were found for a multiplicity of drugs, including inhibitors of PLK1 (Liu-Sullivan *et al*., 2011; van der Meer *et al*., 2014), DNA-PK (Dietlein *et al*., 2014), ATR (Mohni *et al*., 2014) or EGFR (Astsaturov *et al*., 2010). A different approach making use of synthetic lethality aims at identifying genes that are essential in a specific genetic background, i.e. the presence of a gain or loss of function mutation. Targeting a panel of genes, either with RNAi or selective drugs, would reveal candidates that act synthetically lethal with the respective mutation. One of the first studies based on this concept screened 23 550 compounds to identify drugs that selectively kill cells transformed by different combinations of oncogenes, but not their isogenic non-transformed counterparts (Dolma *et al*., 2003). The authors found that specific combinations of oncogenes increased topoisomerase expression, rendering cells sensitive to topoisomerase inhibitors. A further early study aimed at identifying kinases that are required by clear renal cancers lacking the von Hippel-Lindau tumor suppressor (Bommi-Reddy *et al*., 2008). A small shRNA screen was performed and revealed several kinases that act synthetically lethal, of which some could be confirmed using drugs.

Later, several genome-scale RNAi screens have been performed using isogenic cell lines, which either have a RAS mutation (Schlabach *et al*., 2008) or loss of TP53 (Krastev *et al*., 2011) background. In another screen, Vizeacoumar *et al.* used genome-scale shRNA libraries to systematically identify negative genetic interactions across five isogenic cell lines with loss of function of major tumor suppressors (Vizeacoumar *et al*., 2013). An alternative approach is to compare genetic interactions in multiple cell lines harboring the desired mutational background to a panel of cells without this background. Two hallmark papers used this approach to identify synthetic lethal interactions with mutated KRAS using a large set of cell lines (Barbie *et al*., 2009; Scholl *et al*., 2009), hereby identifying TBK1 and STK33 as negative interactors. TBK1 activates anti-apoptotic signals via NfKappaB (Barbie *et al*., 2009), while STK33 suppresses mitochondrial apoptosis through S6K1 and BAD (Scholl *et al*., 2009). However, different results were obtained with pharmacological inhibitors, indicating that the interaction might be independent of the kinase activity of STK33 (Babij *et al*., 2011; Luo *et al*., 2012; Weïwer *et al*., 2012).

With the wealth of data from functional screens and mutation data from sequencing projects at hands, integrated approaches to identify synthetic lethal interactions were also possible. For example, RNAi profiles of cancer cells were compared to genome-wide copy number abberations, hereby identifying 56 genes for which reduction in growth only occurred if cells also harbored a copy number loss of the respective gene (Nijhawan *et al*., 2012). Another study combined results from a Wnt pathway activity readout with data on lethal phenotypes identified by pooled shRNA screens for a set of 85 cell lines (Rosenbluh *et al*., 2012). The authors found out that cancer with high active Wnt levels rely on YAP1 that forms a complex with TBX5 and mediates expression of anti-apoptotic genes BCL2L1 and BIRC5.

Although there is a constantly growing amount of data from structural and functional studies, only few identified genes have been exploited as drug targets in a clinical setting. Initial successes by targeting lineage specific essential genes (BCR-Abl fusion protein in chronic myeloid leukemia by imatinib mesylate (Kantarjian *et al*., 2002)) have raised high hopes for a more efficient cancer therapy. However, while the repertoire for targeted therapy is constantly enlarging and improving overall survival rates of many cancers, the initial success of BCR-Abl imatinib remains an exception rather than the rule. Potential reasons for this development include inherent limitations of cell lines as a cancer model (Wilding & Bodmer, 2014), tumor heterogeneity (Gillies *et al*., 2012) and the involvement of the tumor microenvironment (Straussman *et al*., 2012).

Clinical translation of synthetic lethality has been (in part) successful in two cases: PARP inhibitors in BRCA mutated breast cancer and the combination of retinoid acid and arsenic trioxide for treating promyelocytic leukemia. In 2005, two parallel publications described that in breast cancer cells with deficient BRCA1 and BRCA2, the Poly(ADP-ribose) polymerase PARP1 takes over an essential function in repairing DNA lesions (Bryant *et al*., 2005; Farmer *et al*., 2005). Pharmacological inhibition of PARP1 was highly effective in eradicating BRCA1 and BRCA2 deficient cancer cells. With a strong biological rationale behind, PARP inhibitors quickly went to phase I (Fong *et al*., 2009) and phase II (Audeh *et al*., 2010; Tutt *et al*., 2010) trials for treatment of BRCA1/2 mutated breast and ovarian cancer, with promising results regarding response rate and clinical benefit. Several consecutive trials, including phase 3 trials, have been started, but the results so far are mixed and recent findings suggest that the significance of BRCA germline mutations in determining therapy response needs to be reassessed (Scott *et al*., 2015).

The combination of all-trans retinoic acid (ATRA) and arsenic trioxide in treatment of promyelocytic leukemia (PML) is another example for clinical translation of synthetic lethality. The standard treatment of PML has been a combination of retinoic acid and anthracyclines, with overall high success rates. However, several studies show that ATRA and arsenic trioxide can strongly synergize to eradicate PML in *in vitro* and *in vivo* models (Lallemand-Breitenbach *et al*., 1999; Shao *et al*., 1998). Both compounds bind at different moieties of the PML-RARA fusion protein, thereby synergistically accelerating its degradation. A phase 3 clinical trial showed that combination of both substances is most likely superior to standard therapy and associated with less hematological toxicity, but higher hepatic toxicity (Lo-Coco *et al*., 2013). In summary, while systematic screens in cancer cell lines have yielded a wealth of data on essential genes and synthetic lethality, translating these findings into novel clinical therapy still remains a major challenge.

Novel methods for discovery of essential genes

RNAi has been the main workhorse for targeted identification of novel gene functions for almost a decade, with constant

refinement of design and application. However, there are also limits and disadvantages inherent to RNAi technology. These include off-target effects (Ma *et al*., 2006), toxicity and incompleteness of generating knockdown for selected genes (Boutros & Ahringer, 2008). Knockout efficiency is also dependent on biological sources of variability, such as AGO2 expression levels (Hart *et al*., 2014). Additionally, RNAi exclusively targets the mRNA of transcribed genomic regions, creating only loss-of-function. This however precludes analysis of non-coding genomic regions and gain-of-function phenotypes.

Recently, the development of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 system of *S. pyogenes* into a genome editing tool (Cong *et al*., 2013; Mali *et al*., 2013) has opened new avenues for functional genomics. In brief, a synthetic small guide RNA targets the modified Cas9 endonuclease to a complementary sequence in the genome, where it introduces a double strand break. In most cases, these breaks are repaired by non-homologs end joining, which is error-prone and frequently results in loss-of-function deletion or insertions. CRISPR/Cas9 can be used in a wide range of species with high efficiency (Friedland *et al*., 2013; Gratz *et al*., 2013; Hwang *et al*., 2013), making it an universal tool.

The simplicity of the CRISPR/Cas9 technology allows high-throughput screening in a similar scale as pooled shRNAs. CRISPR/Cas9 screens were performed in cancer cells and murine embryonic stem cells to identify resistance mechanisms towards toxins or drugs (Koike-Yusa *et al*., 2014; Shalem *et al*., 2014; Zhou *et al*., 2014). In addition, screens have been conducted to identify essential genes in haploid and diploid leukemia cells (Wang *et al*., 2014) and colorectal cancer cells (Hart *et al*., 2015). Interestingly, identified hits between shRNA and CRISPR screens were partly non-overlapping, indicating that complete loss of function may result in different phenotypes (Hart *et al*., 2015; Shalem *et al*., 2014). In addition, the number of essential genes identified by a CRISPR/Cas9 screen was higher than by a shRNA screen in HCT116 (Hart *et al*., 2015, 2014). It is thus anticipated that large-scale loss of function screens will be repeated using CRISPR/Cas9 technology. In addition to loss-of-function of protein coding genes, CRISPR/Cas9 also allows targeting and functional characterization of long non-coding RNAs and non-transcribed regions (Ho *et al*., 2015; Kearns *et al*., 2015; Yin *et al*., 2015). Moreover, modifications of the CRISPR/Cas system for transcriptional activation of genes will in the future enable identification of gene essentiality and genetic interaction with gain-of-function CRISPR/Cas9 libraries (Gilbert *et al*., 2014; Konermann *et al*., 2014). CRISPR/Cas9 also enables reconstruction of point mutations frequently found in cancer (Antal *et al*., 2015), thereby allowing screening for synthetic lethality in very specific genetic backgrounds. In face of all those possibilities opened up by novel screening methods, technical standardizations of screening procedures and bioinformatics analysis pipelines are essential to obtain comparable results across different screens.

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