

REVIEW ARTICLE

Understanding protein–protein interactions by genetic suppression

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Abstract

Protein–protein interactions influence many cellular processes and it is increasingly being felt that even a weak and remote interplay between two subunits of a protein or between two proteins in a complex may govern the fate of a particular biochemical pathway. In a bacterial system where the complete genome sequence is available, it is an arduous task to assign function to a large number of proteins. It is possible that many of them are peripherally associated with a cellular event and it is very difficult to probe such interaction. However, mutations in the genes that encode such proteins (primary mutations) are useful in these studies. Isolation of a suppressor or a second-site mutation that restores the phenotype abolished by the primary mutation could be an elegant yet simple way to follow a set of interacting proteins. Such a reversion site need not necessarily be geometrically close to the primary mutation site.

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Introduction

Protein–protein interactions form the basis of a large number of cellular processes. Genetic suppression has historically proved to be a powerful tool in identifying functional interactions (Hartman and Roth 1973; Jarvik and Botstein 1975). With advances in proteomics, more studies are now being directed towards unravelling the subtle network of interactions that govern cellular processes and development in organisms (Houry *et al.* 1999; Liu *et al.* 1999). However, the many bacterial genomes that have been sequenced in the recent past have not given us clues regarding the functions of more than half of their genes. Clearly the network of protein–protein interactions involving many players needs to be addressed, and it appears that the technology is lagging behind in this regard. Though a number of biochemical approaches have been successfully employed in determining protein–protein interactions *in vitro* (Chang and Flake 1972; Heyduk and Heyduk 1994; Zhong and Lin 1995), they essentially involve perturbation of the protein by insertion of a probe. Genetic suppression obviates the need to modify the protein and provides an alternative means to

unveil the functional relevance of interactions *in vivo*. However, one cannot undermine the value of a combined approach involving both biochemical and genetic means to establish physical interaction between proteins and their functional significance (Adams *et al.* 1989).

Suppression analysis in identifying interacting proteins

Classically, a mutant phenotype that is the result of a mutation rendering a gene product inactive can be restored to a wild-type phenotype by a compensatory mutation either within the same gene (intragenic) or in the gene that encodes an interacting partner (Hartman and Roth 1973). This can occur in two ways. A common mechanism is the restoration of the original contact points between interacting proteins, termed as the ‘lock and key’ model (Phizicky and Fields 1995; Prelich 1999). Such direct interaction calls for a high degree of allele specificity and these mutations define the amino acids that are in direct contact. Adjunct to this is that suppressors indicative of direct physical contact between two proteins or between two regions in the same protein tend to cluster in a small region of the gene rather than be distributed

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over a number of segments. Hence allele-specific suppression is an evidence of direct physical interaction between two proteins or within a single polypeptide. A second mode of restoring a primary mutation is by the formation of novel contacts between interacting proteins (Sandrock *et al.* 1997). In the following sections, examples of both types are described to illustrate the usefulness of a genetic approach in identifying interacting proteins and their functional relevance, particularly in multiprotein complexes (figure 1). Thus one can visualize a situation where restoration of a phenotype can be a simple assay one may follow to identify the interacting network with the primary mutation site.

Subunit contacts in transcription complexes

Prokaryotic transcription machinery

The transcription machinery in prokaryotes consists of DNA-dependent RNA polymerase comprising $\alpha_2\beta\beta'$ subunits (Burgess *et al.* 1987). The crystal structure of this enzyme is now known (Zhang *et al.* 1999). It shows inter-subunit interaction, geometry of the active site, etc. but till date one is unable to see how various factors may bind to the central enzyme molecule to regulate gene expression. However, as the antibiotic rifampicin binds RNA polymerase at a specific site on the β -subunit, rendering the enzyme inactive, it is possible to generate a rifampicin-resistant phenotype of bacteria. This property then can be easily assayed to find out the various modes of interaction of this antibiotic with the central enzyme (Yura and Ishihama 1979; Singer *et al.* 1993). Singer *et al.* (1993) dissected the rifampicin binding site on the β -subunit using genetic suppression. This site is comprised of two regions, cluster I and cluster III; a single primary mutation in cluster III confers rifampicin resistance and is both cold sensitive and temperature sensitive. Using these properties, second-site intragenic suppressors in cluster I region were isolated and characterized to be allele

specific. Functional restoration by the cluster I mutants indicates that these two regions interact and contribute to formation of the rifampicin binding site. In a continued effort to understand regions of interaction within the β - β' subunit, intragenic allele-specific suppressors of elongation-deficient inviable *rpoB* mutants were isolated (Tavormina *et al.* 1996). One of the primary mutations conferring severe elongation and termination defects is located in the region Rif cluster II, a part of the highly conserved segment region D. The corresponding suppressors mapped to region B. The second primary β -subunit mutant used as a starting point was A676V, whose mutation was located in conserved region E. The allele-specific suppressors of this mutant fall within the conserved region H. Neither of the primary mutants nor their suppressors, in isolation, could support growth, thus augmenting the hypothesis that region B–region D and region E–region H interactions within the β -subunit are necessary for functional integrity of the polypeptide.

Eukaryotic transcription complex

Yeast RNA polymerase II (RNAP II) is a multisubunit complex comprising 10 polypeptides (Sentenac 1985). Isolation of suppressors of conditional-lethal mutants is a genetic means of identifying interacting proteins. Martin *et al.* (1990) convincingly demonstrated the contact region between the two largest subunits of RNAP II, namely RPB1 and RPB2, by this means. RPB2 suppressors of *rpb1-1* were allele-specific and were found to cluster in the C-terminal domain, indicating direct interaction, while RPB1 suppressors of *rpb2-2* were not clustered in the C-terminal domain alone, indicating that RPB1 could interact with RPB2 through C-terminal domain and another region identified as region C. Likewise, several genetic studies of yeast RNAP I and RNAP II illustrate the usefulness of this approach in identifying interacting proteins (Nonet and Young 1989; Archambault *et al.* 1990; Yano and Nomura 1991). Mutations in the largest subunit of RNAP II causing a temperature-sensitive phenotype were suppressed by overexpression of a 18-kDa subunit common to RNAPs I, II and III (Nonet and Young 1989). It appears that the suppression was achieved either through the primary mutation in the largest subunit of RNAP resulting in its increased dissociation from the holoenzyme or that the mutant largest subunit is unstable and can be stabilized upon formation of a large complex through interaction with the smaller subunits.

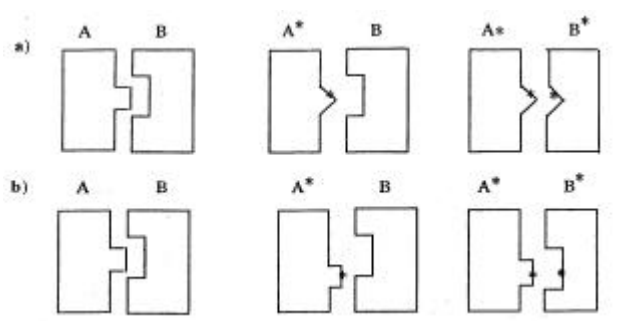


Figure 1. (a) A schematic representation of allele-specific conformational or stereospecific suppression of a mutation on protein A by a mutation on protein B, restoring the original points of contact. (b) Representation of suppression by formation of novel contact points, increasing the affinity of mutant protein A to mutant protein B.

Protein–protein interactions in other multiprotein complexes

Bacterial chemotaxis

Detection of external signals and subsequent behavioural response to external stimuli play an important role in

bacterial adaptation to their environment. Most chemotactic responses in *Escherichia coli* are initiated by chemoreceptors called methyl-accepting chemotaxis proteins (MCPs), which are membrane associated, and the signal transduction proceeds through a series of phosphorylations involving soluble Che proteins (Hess *et al.* 1987; Wylie *et al.* 1988). CheW is a soluble protein that induces the MCPs to increase autokinasing of CheA protein, thus triggering a cascade of events culminating in clockwise flagellar rotation (Borkovich *et al.* 1989). Hence a logical prediction is that CheW interacts with CheA or MCPs or both and the possibility of such an interaction was genetically tested (Liu and Parkinson 1991). Indeed, *cheW* mutants were suppressible in an allele-specific manner by mutations in an MCP gene called *tsr*. Moreover, these suppressors were clustered, thus strengthening the hypothesis that the residues altered in the mutants were interacting directly with each other. Dissection of the signal transduction pathway by Che proteins through genetic analysis has identified interacting partners regulating chemotaxis (Sanna and Simon 1996).

Eukaryotic cytoskeleton

The functional relevance of interaction between the components of the cytoskeleton in *Saccharomyces cerevisiae* is best understood by probing the interaction *in vivo*, as exemplified by suppression analysis of the actin gene, *act1* (Adams *et al.* 1989). It is an essential gene, and temperature-sensitive conditional-lethal mutants of *act1* have been used to isolate suppressor mutants. These have been localized to genes encoding actin-binding proteins which are constituents of the actin-cytoskeleton apparatus. Each of the mutants individually shows disorganized actin cytoskeleton and cell morphology while the double mutant shows a wild-type phenotype, suggesting that the most likely mode of suppression is by compensatory changes in the interacting proteins. Besides the 'lock and key' mechanism which demands high allele specificity, suppression may occur by alteration of affinity between the interacting partners rather than restoration of the original contacts. *sac6* encodes an actin-filament-binding protein, fimbrin. Mutations in *sac6* were found to suppress an actin temperature-sensitive mutation. Had the suppressors been stereospecific, then the affinity between the two mutant proteins would be detectably higher than that between a mutant and its wild-type partner. Rather, the mutant SAC6 protein - wild-type protein interaction was found to be stronger. Moreover, the suppression was 'allele-restricted', as a set of actin mutants could suppress *sac6* and vice versa. This mechanism identifies interacting proteins with novel contacts, indicating that wild-type actin - wild-type SAC6 interactions are not of the maximal affinity and suggests that extremely strong interaction can be detrimental to cell growth (Sandrock *et al.* 1997).

Bacterial protein secretion

The protein secretory apparatus of *E. coli*, a multisubunit enzyme complex, comprises a number of proteins SecY, SecE, SecG, SecD, SecF and YajC (Duong and Wickner 1997), and the core of the translocase consists of SecY, SecE and SecG (Douville *et al.* 1995). Interactions between the components of the secretory apparatus and target proteins are critical for protein translocation and provide an insight into the mechanism of protein export. In the absence of biochemical methods to establish the function of proteins involved in the secretion process, the genetic approach provides an alternative. Bieker and Silhavy (1990) have employed an ingenious genetic strategy to identify interacting components of translocation. A hybrid fusion protein of LacZ and a target protein, in this case the maltoporin, LamB, was used as a starting point. This LacZ hybrid is secretion incompatible and jams the secretion machinery, giving rise to a *mal^s* (maltose-sensitive) phenotype upon induction with maltose. If the LacZ hybrid has a defective signal sequence, then the jamming of the translocation pathway is relieved and hence the cells remain viable on induction with maltose. Suppressors of the mutant signal sequence identified a protein translocator *prlA* (also called *secY*) which encodes a protein that aids the movement of the target protein across the inner membrane.

A merodiploid of *prlA/prlA4* (suppressor allele) containing signal-sequence-defective LamB-LacZ shows a *mal^r* phenotype (maltose-resistant) because this mutant hybrid interacts only with PrlA4, thus jamming it, but does not interact with wild-type PrlA and hence confers the *mal^r* phenotype. Such a phenomenon was termed 'suppression directed inactivation' or SDI. The authors have used SDI to identify other components of the secretory apparatus by titration of essential components as described below. In *prlA4/prlA*, the mutant LamB-LacZ hybrid bound with PrlA4 is expected to be associated with putative secretory proteins, but since the cell is *prlA⁺*, there is no maltose sensitivity. But if a particular secretory protein (encoded by *sec* genes) is suspected to be involved in translocation, by titrating it out with the jammed complex the functional relevance of that particular *sec* gene product can be inferred. This can be achieved by using a conditional allele of the *sec* gene in the merodiploid. If the component is actually involved in translocation, it is sequestered by the jammed complex and not available to export wild-type LamB protein through PrlA, thus rendering the cell *mal^s*. Using the concept of SDI, SecE, a peripheral membrane protein with ATPase activity, was not found to be associated with PrlA. In the same manner, two other proteins, SecD and SecF, were identified to be associated with pre-translocation complex (a stage before processing of the signal sequence) (Bieker-Brady and Silhavy 1992). Regions of SecY (PrlA) which interact with signal sequence were also identified by isolation of

suppressor mutants mapping in *priA* (Osborne and Silhavy 1993).

Functional interactions in $F_0 - F_1$ ATPase

Genetic suppression has proved to be a very efficacious tool in understanding and dissecting functional interactions in multisubunit complexes. *E. coli* $F_0 - F_1$ ATP synthase synthesizes ATP coupled with an electrochemical gradient of protons. The F_1 unit is a multicomplex assembly comprising **a**, **b**, **g**, **d** and **e** subunits and the F_0 of a, b and c subunits. Missense mutations in **a**-subunit impair proton translocation. Working on the premise that, if two or more sites in a protein participate in critical interaction, mutations in one interacting region can be compensated for by a corresponding change in the other position, Hartzog and Cain (1994) isolated intragenic suppressors of **a**-subunit to pinpoint the determinants involved in protein-protein interactions. The **a**-subunit mutation G218K inhibits $F_0 - F_1$ ATP synthase activity to below detection limits and is suppressed by H245G. This suggests that these two segments of **a**-subunit are spatially in close proximity. In a similar study of $F_0 - F_1$ ATP synthase, intergenic suppressors of a frameshift mutation in the **g**-subunit were isolated by Beukelaer *et al.* (1995). The primary frameshift mutation was in **g**-subunit, resulting in alteration of the last 16 amino acid residues at the carboxy terminus. Such a mutant was unable to grow by oxidative phosphorylation and exhibited very low ATPase activity. This defect was suppressed by either R52C with or without a second mutation V77A, or by G150D in the **b**-subunit of the enzyme. The carboxy terminus of wild-type **g**-subunit is an **a**-helix while the frameshift mutation results in a **b**-sheet, and earlier work suggested interactions between this region of **b**- and **g**-subunits. The **b**-barrel domain of **b**-subunit contains the suppressor mutation R52C and hence compensated for the altered **b-g** interactions in the primary mutant. The mutant G150D may be suppressing through a different mechanism which affects conformational transmission, rather than by direct compensation of the primary mutant.

Problems

The examples discussed in this review illustrate the viability of a genetic approach in identification of interacting proteins and elucidation of the functional relevance of their interactions. The mechanism of suppression of the primary mutation provides a key to understanding the molecular basis of interactions between proteins. Suppressors may either restore the original contact (conformational and hence allele-specific) or may create a novel interaction compensating for the primary mutation in an indirect way. However, second-site mutants may not always reflect the proximity of these mutations to the

primary mutation site. Meunier and Rich (1998) have examined the second-site mutations of yeast cytochrome *c* oxidase subunit I and II and their results suggest that the compensatory mutations can be as far as 30 Å from the original mutation. However, all the suppressor mutations are located in the subunit interface, suggesting that these regions are critical for assembly or enzyme stability.

Although such a large distance between the primary mutation site and its suppressor raises a doubt about the validity of this method in estimating the distance geometry between them, one cannot ignore that the suppression itself indicates functional complementation even if they are far apart. We have recently observed such suppression in assembly-defective *E. coli* RNA polymerase (Sujatha *et al.* 2001) and it appears that more such cases will emerge at least in the cases of multisubunit proteins. Do they indicate long-range interactions in the protein moiety? Mapping of many such long-range interactions in a functional protein complex like the transcription assembly may throw new light on participating genes in the background of available genome sequence.

References

- Adams A. E. M., Botstein D. and Drubin D. G. 1989 A yeast actin-binding protein is encoded by SAC6, a gene found by suppression of an actin mutation. *Science* **243**, 231–233.
- Archambault J., Schappert K. T. and Freisen J. D. 1990 A suppressor of an RNA polymerase II mutation of *Saccharomyces cerevisiae* encodes a subunit common to RNA polymerases I, II and III. *Mol. Cell. Biol.* **10**, 6123–6131.
- Beukelaer C. J. D., Omote H., Iwamoto-Kihara R., Maeda M. and Futai M. 1995 **b-g** subunit interaction is required for catalysis by H^+ -ATPase (ATP synthase). *J. Biol. Chem.* **270**, 22850–22854.
- Bieker K. L. and Silhavy T. J. 1990 PriA (SecY) and PriG (SecE) interact directly and function sequentially during protein translocation in *E. coli*. *Cell* **61**, 833–842.
- Bieker-Brady K. and Silhavy T. J. 1992 Suppressor analysis suggests a multistep, cyclic mechanism for protein secretion in *E. coli*. *EMBO J.* **11**, 3165–3174.
- Borkovich K. A., Kaplan N., Hess J. F. and Simon M. 1989 Transmembrane signal transduction in bacterial chemotaxis involves ligand-dependent activation of phosphate group transfer. *Proc. Natl. Acad. Sci. USA* **86**, 1208–1212.
- Burgess R. R., Erickson B., Gentry D., Gribskov M., Hager D., Lesley S., Shickland M. and Thompson N. 1987 In RNA polymerase and the regulation of transcription, Elsevier Science.
- Chang F. N. and Flake G. J. 1972 The specific cross-linking of two proteins from the *Escherichia coli* 30S ribosomal subunit. *J. Mol. Biol.* **68**, 177–180.
- Douville K., Price A., Eichler J., Economou A. and Wickner W. 1995 SecYEG and SecA are the stoichiometric components of preprotein translocase. *J. Biol. Chem.* **270**, 20106–20111.
- Duong F. and Wickner W. 1997 Distinct catalytic roles of the SecYE, SecG and SecDFYajC subunits of preprotein translocase holoenzyme. *EMBO J.* **16**, 2756–2768.
- Hartman P. E. and Roth J. R. 1973 Mechanisms of suppression. *Adv. Genet.* **17**, 1–105.
- Hartzog P. E. and Cain B. D. 1994 Second site suppressor mutations at glycine 218 and histidine 245 in the **a**-subunit of

- F₁–F₀ ATP synthase in *E. coli*. *J. Biol. Chem.* **269**, 32313–32317.
- Hess J. F., Oosawa K., Matsumura P. and Simon M. I. 1987 Protein phosphorylation is involved in bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA* **84**, 7609–7613.
- Heyduk E. and Heyduk T. 1994 Mapping domains involved in macromolecular interactions: a novel protein footprinting approach. *Biochemistry* **33**, 9643–9650.
- Houry W. A., Frishman D., Eckerskorn C., Lottspeich F. and Hartl F. U. 1999 Identification of *in vivo* substrates of the chaperonin GroEL. *Nature* **402**, 147–154.
- Jarvik J. and Botstein D. 1975 Conditional lethal mutations that suppress genetic defects in morphogenesis by altering structural proteins. *Proc. Natl. Acad. Sci. USA* **72**, 2738–2742.
- Liu J. and Parkinson J. S. 1991 Genetic evidence for interaction between the CheW and Tsr proteins during chemoreceptor signaling by *Escherichia coli*. *J. Bacteriol.* **173**, 4941–4951.
- Liu Y., Patricelli M. P. and Cravatt B. F. 1999 Activity based protein profiling: the serine hydrolases. *Proc. Natl. Acad. Sci. USA* **96**, 14694–14699.
- Martin C., Okamuram S. and Young R. 1990 Genetic exploration of interactive domains in RNA polymerase II subunits. *Mol. Cell. Biol.* **10**, 1908–1914.
- Meunier A. S. and Rich P. R. 1998 Second-site reversion analysis is not a reliable method to determine distances in membrane proteins: An assessment using mutations in yeast cytochrome co-oxidase subunits I and II. *J. Mol. Biol.* **283**, 727–730.
- Nonet M. L. and Young R. A. 1989 Intragenic and extragenic suppressors of mutations in the heptapeptide repeat domain of *Saccharomyces cerevisiae* RNA polymerase II. *Genetics* **123**, 715–724.
- Osborne R. S. and Silhavy T. J. 1993 PrlA suppressor mutations cluster in regions corresponding to three distinct topological domains *EMBO J.* **12**, 3391–3398.
- Phizicky E. M. and Fields S. 1995 Protein–protein interactions: methods for detection and analysis. *Microbiol. Rev.* **59**, 94–123.
- Prelich G. 1999 Suppression mechanisms: themes from variations. *Trends Genet.* **15**, 261–266.
- Sandrock T. M., O’Dell J. L. and Adams A. E. M. 1997 Allele-specific suppression by formation of new protein–protein interactions in yeast. *Genetics* **147**, 1635–1642.
- Sanna M. G. and Simon M. I. 1996 Isolation and *in vitro* characterization of CheZ suppressors for the *Escherichia coli* chemotactic response regulator mutant CheYN23D. *J. Biol. Chem.* **271**, 7357–7361.
- Sentenac A. 1985 Eukaryotic RNA polymerases. *CRC Crit. Rev. Biochem.* **18**, 31–90.
- Singer M., Jin D. J., Walter W. A. and Gross C. A. 1993 Genetic evidence for the interaction between cluster I and cluster III rifampicin resistant mutations. *J. Mol. Biol.* **231**, 1–5.
- Sujatha S., Ishihama A. and Chatterji D. 2001 Functional complementation between mutations at two distant positions in *E. coli* RNA polymerase as revealed by second-site suppression. *Mol. Gen. Genet.* **264**, 531–538.
- Tavormina P. L., Reznikoff W. S. and Gross C. A. 1996 Identifying interacting regions in the beta subunit of *Escherichia coli* RNA polymerase. *J. Mol. Biol.* **258**, 213–223.
- Wylie D., Stock A., Wong C. Y. and Stock J. 1988 Sensory transduction in bacterial chemotaxis involves phosphotransfer between Che proteins. *Biochem. Biophys. Res. Commun.* **151**, 891–896.
- Yano R. and Nomura M. 1991 Suppressor analysis of temperature-sensitive mutations of the largest subunit of RNA polymerase I in *Saccharomyces cerevisiae*: a suppressor gene encodes the second-largest subunit of RNA polymerase I. *Mol. Cell. Biol.* **11**, 754–764.
- Yura T. and Ishihama A. 1979 Genetics of bacterial RNA polymerases. *Annu. Rev. Genet.* **13**, 59–97.
- Zhang G., Campbell E. A., Minakin L., Richter C., Severinov K. and Darst A. S. 1999 Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 Å resolution. *Cell* **9**, 811–824.
- Zhong M. and Lin L. A. 1995 Method for probing the topography and interactions of proteins: footprinting of myoglobin. *Proc. Natl. Acad. Sci. USA* **92**, 2111–2115.