CANCER BIOLOGY

Phosphorylation of BRCA2 regulates homologous recombination

Mutations in *BRCA2* cause a predisposition to early-onset breast cancers. Cells lacking BRCA2 are hypersensitive to DNA-damaging agents and it is thought that tumours are caused by defects in DNA repair pathwaths that require homologous recombination. BRCA2 probably mediates homologous recombination by binding to RAD51, however it is not clear how this interaction is regulated. Esashi *et al.* show that phosphorylation of Ser3291 of BRCA2 inhibits RAD51 binding to the C-terminus of BRCA2 [2].

RAD51 initiates homologous recombination by forming filaments and mediating strand exchange. The authors made nine GST–BRCA2 fusions encompassing the entire gene and found that fragments 2, 3 and 9 bound to RAD51. Fragments 2 and 3 contained BRC repeats, which bind RAD51, whereas fragment 9 contained a novel RAD51-binding site. It was shown that this fragment was phosphorylated by cyclin-dependent kinases.

The authors made three truncations of fragment 9 and found that only TR2 bound to RAD51. This fusion contained five potential S/T phosphorylation sites, so the authors made a series of Glu-substituted mutants to mimic phosphorylation. Only mutants containing Glu at position 3291 did not bind RAD51, showing that phosphorylation of Ser3291 inhibits binding to RAD51.

Phosphorylation of Ser3291 was examined *in vivo* using a phosphorylation-specific antibody. Phosphorylation increased as cells progressed from G2 to M, and decreased after cells were treated with ionising radiation. Overexpression of the TR2 fragment reduced the efficiency of recombinational DNA repair. These data show that phosphoryation of Ser3291 was functionally important to regulate the RAD51–BRCA2 interaction.

It was proposed that when Ser3291 is dephosphorylated, it accepts RAD51 monomers from the BRC repeats. BRCA2 then facilitates the transfer of RAD51 to single-stranded DNA. However, further research is required to understand how the interactions between the BRC repeats and RAD51 are regulated.

2 Esashi, F. *et al.* (2005) CDK-dependent phosphorylation of BRCA2 as a regulatory mechanism for recombinational repair. *Nature* 434, 598–604

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This study validates the relevant memory paradigm in the *Drosophila* model of FXS. Moreover, it raises the possibility that mGluR antagonists could similarly improve cognitive functions in Fragile X patients. In response to mGluR activiation, *FMR1* mRNA is translated into its gene product FMRP, which binds a subset of mRNAs and acts as a regulator of translation. As many similarities are found between symptoms observed in Fragile X patients and phenotypes observed in the fly, and that deregulation of mGluR activity appears to be an evolutionarily conserved feature, this model allows for easy access to investigate the intricate relationship between mGluR, mGluR antagonists and the gene product of *dfmr1*.

1 McBride, S.M. et al. (2005) Pharmacological rescue of synaptic plasticity, courtship behavior, and mushroom body defects in a *Drosophila* model of Fragile X syndrome. *Neuron*. 45, 753–764

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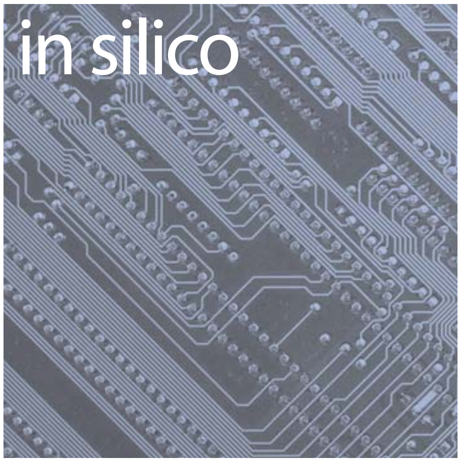

Same shape, different way

Bacterial resistance to available antibiotics is quickly becoming a public health threat. Wyeth is conducting a drug discovery program targeted at the ZipA–FtsZ protein–protein interaction that is required for proper cell division. Unfortunately, the most active compound identified from a fluorescence polarization HTS was found to have toxic side-effects and to have intellectual property (IP) issues, soon after its X-ray crystal structure bound with ZipA was solved. Instead of abandoning the 3-D data, Rush *et al.* chose to use the X-ray coordinates of the active compound as a prototype to find other molecules with similar shape [1]. To this end, they developed a new similarity algorithm, called rapid overlay of chemical structures (ROCS).

ROCS perceives similarity between two molecules based on their molecular shape only. It approximates their volumes with Gaussian functions instead of hard spheres, thereby resulting in mathematical equations that are analytic and differentiable, which allow for fast and robust global optimization of volume overlap by varying their relative orientation. A similarity function then measures the 'shape distance' between the pair of molecules at optimal overlap of volume. Molecules that ROCS deems near the prototype are similar in shape, but not necessarily built from the same atoms in the same way.

Wyeth's corporate 3-D database of available lead-like compounds was searched by ROCS with two molecular shape queries derived from the prototype. Hits with different scaffolds than the prototype were overlaid on the X-ray coordinates of the prototype bound with ZipA, and those hits with unfavorable intermolecular contacts were discarded, leaving 29 candidates. A final fluorescence polarization assay yielded three non-toxic active molecules with μM-range K_D. One of the three molecules was co-crystallized with ZipA and its binding mode pose was found to be very similar to the pose of the prototype. Two novel scaffolds without obvious IP concerns were extracted from the three molecules.

This study showed that the volume approach of ROCS can 'hop' via the 3-D molecular shape of a known active to scaffolds built from unexpected 2-D chemical structure. These new scaffolds can lead to drug candidates that are out of patented areas of chemistry space. Extensions to the ROCS algorithm might include the scoring of the receptor–ligand contacts based on the matching of classified atoms or functional groups, as in Masek's original MSC method. An additional application of ROCS could be to overlay the training set of molecules for CoMFA methodology, which is a well-known source of difficulty in CoMFA analyses.

1 Rush, *et al.* (2005) A shape-based 3-D scaffold-hopping method and its application to a bacterial protein–protein interaction. *J. Med. Chem.* 2005, 48, 1489–1495

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