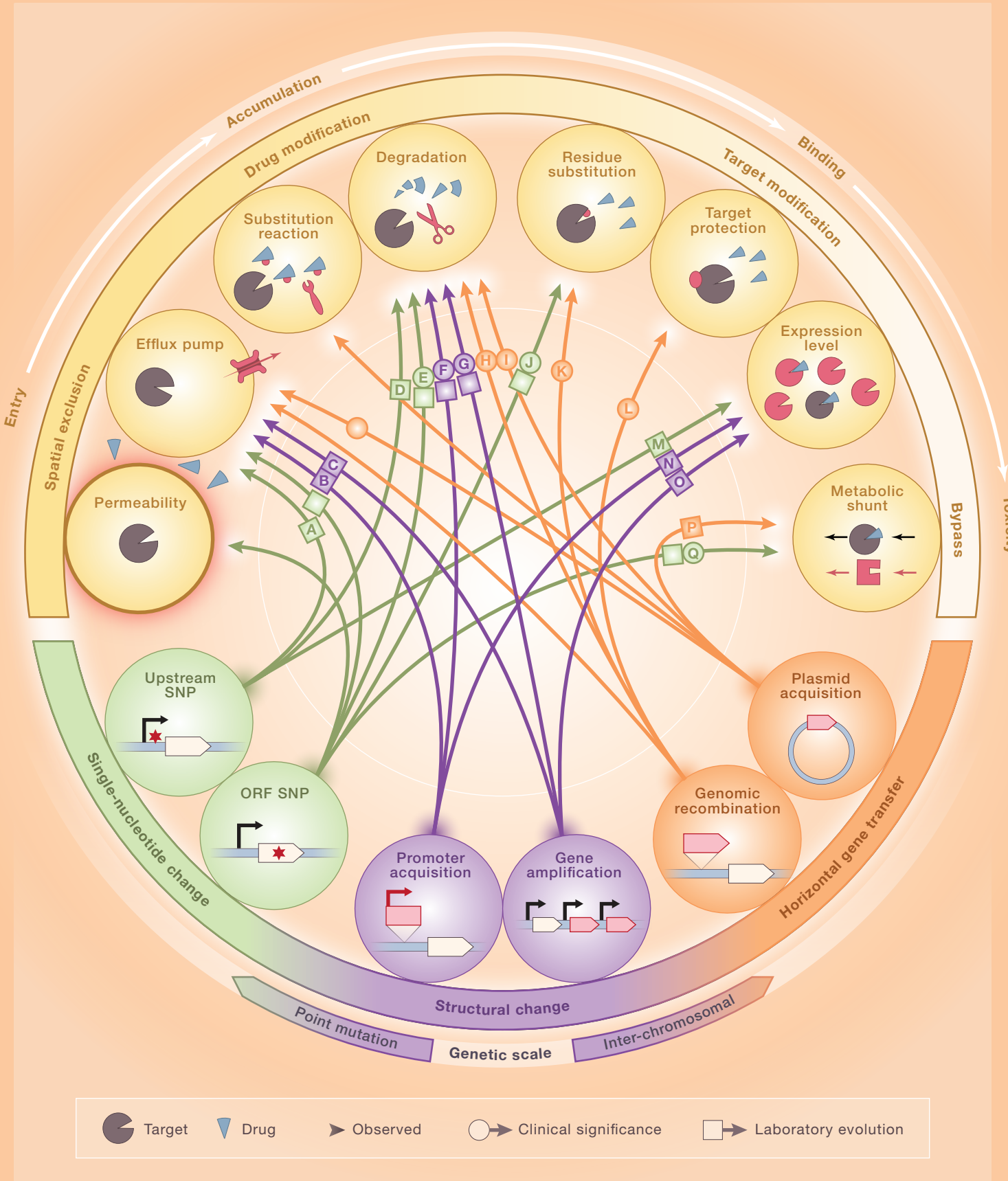
SnapShot: Antibiotic Resistance

Idan Yelin and Roy Kishony
Department of Biology, Technion - Israel Institute of Technology, Haifa 3200003, Israel

Cell



 DOI http://dx.doi.org/10.1016/j.cell.2018.02.018

See online version for legends and references

SnapShot: Antibiotic Resistance

Idan Yelin and Roy Kishony
Department of Biology, Technion - Israel Institute of Technology, Haifa 3200003, Israel

Cell

Bacterial mechanisms of drug resistance operate at sequential lines of defense tackling drug at entry, accumulation, target binding, or downstream toxicity. These mechanisms are encoded by genomic changes ranging in scale from point mutations, through assembly of preexisting genetic elements, to horizontal import of genes from the environment. A many-to-many relationship prevails between resistance mechanisms and the spectrum of genetic changes encoding them.

Mechanisms of Antibiotic Resistance

Resistance mechanisms counter the drug along its path from entry through accumulation and target binding to downstream toxicity (clockwise along the top arc). The outermost line of defense is prevention of the bare entry of the drug into the cell (Spatial exclusion ⊣ Entry). A change in the chemical composition or thickness of the bacterial cell envelope can impede the diffusion of antibiotics into the cell (Permeability). Additionally, cell membranes often contain drug dedicated or general pumps (Efflux pump). The next line of defense prevents drug accumulation by chemically targeting the drug (Drug modification ⊣ Accumulation): designated enzymes modify drug molecules (Substitution reaction) or hydrolyze them (Degradation). These reactions can either occur within the cell, or preemptively outside the cell if the enzymes are secreted. Even if drugs do accumulate unmodified in the bacterial cytoplasm, binding and inhibiting their target can be hampered by a change in the target (Target modification ⊣ Binding): chemical modification of the target itself (Residue substitution), binding of the target by a protective factor (Target protection), or change in target abundance (Expression level). Notably, while for some drugs overexpression of the target increases resistance, for others it is reduced expression that can confer resistance. Ultimately, the last line of defense can be avoiding the toxic effect of target binding (Bypass ⊣ Toxicity), by circumventing the need for the chemical reaction in which the target is involved, or by changing the chemical composition and functionality of the cell (Metabolic shunt).

Genotypic Basis of Resistance

Genetic changes range in scale from point mutations through recruitment of preexisting elements to horizontal import of genes (counterclockwise along the lower arc). At the finest genotypic scale, *de novo* point mutations (Single-nucleotide change, green) appearing upstream (Upstream SNP) or within genes (ORF SNP) can affect expression or alter RNA or protein structures of drug targets or resistance conferring genes. At the genomic scale, genetic elements can be shuffled across the genome (Structural change, purple) to assemble new combinations, which can affect expression of relevant genes by either introducing a strong promoter upstream of a previously unexpressed gene (Promoter acquisition) or by generating multiple copies of specific genes or gene cassettes (Gene amplification). At a scale exceeding the genome, new functionalities can be acquired by import of genetic systems to the bacterial cell (Horizontal gene transfer, orange), either integrating them into the genome (Genomic recombination) or maintaining them as extrachromosomal elements (Plasmid acquisition). While *de novo* point mutations and shuffling of genetic elements both appear frequently in laboratory evolution experiments (green or purple squares), horizontal gene transfer is ubiquitous in the natural environment and is often the culprit in acquirement of resistance in the clinic (orange circles).

Specific Resistance Mechanisms Can Be Acquired by Diverse Genetic Changes

Acquirement of resistance is defined by a specific type of genotypic change leading to a specific type of resistance mechanism (arrows indicate observed acquisition events). Any one specific resistance mechanism can often be acquired by multiple types of genetic changes (multiple color arrows often lead to the same type of resistance mechanism). We demonstrate this principle by notable examples from both clinic and laboratory evolution pertaining to each of the four lines of defense. At the outermost defense line, preventing drug entry, chloramphenicol resistance through increased expression of a specific efflux pump can be acquired in at least three different ways: a point mutation in the promoter of the gene encoding the pump (A), integration of a new promoter upstream of the gene (B), or gene amplification (C). At the second line, tackling drug accumulation, resistance to β-lactams, often mediated through drug degradation by β-lactamase enzymes, can be attained by increased expression level of a chromosomally encoded β-lactamase acquired by upstream SNP (D), promoter acquisition (F) or gene amplification (G). Additionally, the β-lactamase gene itself can be acquired by integration into the genome (H) or by plasmid acquisition (I). Moreover, adaptation to specific β-lactams may involve changes in β-lactamase by accumulation of *de novo* point mutations in the gene itself (E). At the third line of defense, preventing target binding, residue substitution that lowers target affinity to the drug is a common evolutionary pathway often underlying resistance to various drugs, notable examples of residue substitution in both clinical and laboratory settings (J) are mutations in gyrase leading to quinolone resistance, or in dihydrofolate reductase (DHFR) leading to trimethoprim resistance, and changes in penicillin-binding proteins resulting in resistance to β-lactams. These latter changes can also be acquired by genomic recombination (K) either substituting the entire gene (e.g., *mecA* in methicillin-resistant *Staphylococcus aureus*, MRSA) or parts of it (e.g., *penA* of *Neisseria gonorrhoeae*). Targets can also be protected by yet an additional protein or enzyme horizontally acquired (L), as is the case for *fusB* based fusidic acid resistance in *S. aureus*. Additionally, resistance can be provided through increased expression of the target by an upstream point mutation (M), promoter acquisition (N), or gene amplification (O). The effect of increased expression can vary, and while it confers resistance in some cases (e.g., DHFR), it has the opposite effect in others (e.g., gyrase). At the last line of defense, resistance can be attained by making the target expendable. This can be achieved by acquiring a new metabolic function via horizontal gene transfer (P). For example, changes in peptidoglycan and LPS metabolism provide vancomycin or colistin resistance, respectively, by replacing their molecular targets. Metabolic changes which confer resistance can also result from *de novo* changes in specific metabolic pathways encoded in the bacterial genome (Q).

REFERENCES

Ameyama, S., Onodera, S., Takahata, M., Minami, S., Maki, N., Endo, K., Goto, H., Suzuki, H., and Oishi, Y. (2002). Antimicrob. Agents Chemother. *46*, 3744–3749.

Chevereau, G., Dravecká, M., Batur, T., Guvenek, A., Ayhan, D.H., Toprak, E., and Bollenbach, T. (2015). PLoS Biol. *13*, e1002299.

Hiramatsu, K., Cui, L., Kuroda, M., and Ito, T. (2001). Trends Microbiol. *9*, 486–493.

Jaurin, B., and Normark, S. (1983). Cell *32*, 809–816.

Liu, Y.-Y., Wang, Y., Walsh, T.R., Yi, L.-X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., et al. (2016). Lancet Infect. Dis. *16*, 161–168.

Palmer, A.C., and Kishony, R. (2014). Nat. Commun. *5*, 4296.

Salverda, M.L.M., De Visser, J.A.G.M., and Barlow, M. (2010). FEMS Microbiol. Rev. *34*, 1015–1036.

Toprak, E., Veres, A., Michel, J.-B., Chait, R., Hartl, D.L., and Kishony, R. (2011). Nat. Genet. *44*, 101–105.

Weigel, L.M., Clewell, D.B., Gill, S.R., Clark, N.C., McDougal, L.K., Flannagan, S.E., Kolonay, J.F., Shetty, J., Killgore, G.E., and Tenover, F.C. (2003). Science *302*, 1569–1571.

Zampieri, M., Enke, T., Chubukov, V., Ricci, V., Piddock, L., and Sauer, U. (2017). Mol. Syst. Biol. *13*, 917

  DOI http://dx.doi.org/10.1016/j.cell.2018.02.018