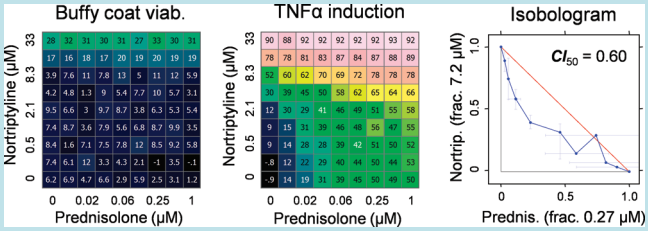
Spotlight

Selective Synergy



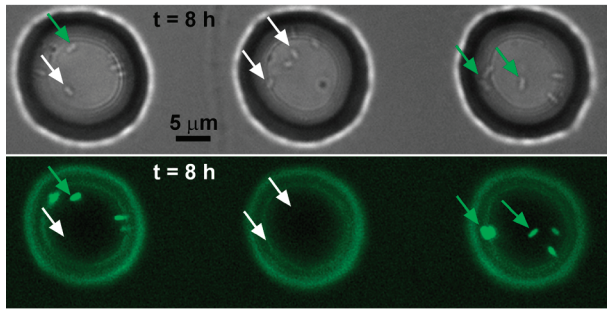
At the molecular level, diseases can be quite complex and may possess several potential targets for drug development. The synergistic combination of two or more drugs, each selective for a distinct target, is a promising strategy for treating such diseases, but not when unwanted side effects are synergized as well. Using molecular simulations and phenotypic screens, Lehár *et al.* (*Nat. Biotechnol.* 2009, 27, 659–666) assess the therapeutic selectivity of synergistic drug combinations.

Synergy and selectivity analyses were performed *in silico* with simulations of inhibition of bacterial metabolism and experimentally with 13 distinct phenotypic screens spanning diverse therapeutic areas. Both the simulations and the experimental data demonstrate that synergistic combinations have increased selectivity over single drugs, *i.e.*, side effects are *not* necessarily enhanced when drug combinations are more effective than their single agent counterparts. In the simulations, analysis of the ability of approximately 100,000 drug combinations to inhibit bacterial growth indicated that the top 1% of synergistic combinations were highly selective and nearly 4 times as potent as the single agents. Selective synergy was also observed in a comparably large set of phenotypic experiments. A key example was illustrated by the corticosteroid prednisolone and the antidepressant nortriptyline in an anti-inflammatory screen. The authors suggest that the higher expression of the nortriptyline receptor in cells that mediate the inflammatory response over cells that exhibit the toxicities associated with prednisolone treatment contributes to the increase in selectivity observed with the drug combination. This hypothesis was validated in a rat pain model, in which the anti-inflammatory effect was not accompanied by a rise in glucocorticoid-associated toxicity, a side-effect that often plagues glucocorticoid treatment regimens. In addition to the promising selectivity gains achieved through the use of drug combinations, the benefits associated with selective synergy can be expanded to various additional applications in bioengineering and biotechnology. Eva J. Gordon, Ph.D.

A Quota for Quorum Sensing

Bacteria use a process called quorum sensing (QS) to modulate their behavior as a function of the density of their population. Critical activities including motility, the acquisition of nutrients, and pathogenesis all rely on QS pathways. Most studies of QS assess the behavior of large numbers of bacteria at high density. Boedicker *et al. (Angew. Chem., Int. Ed.,* published online June 29, 2009; DOI: 10.1002/anie200901550) take a different approach, investigating QS by creating a high-density bacterial environment with a small number of bacteria confined to a very small volume.

The high-density bacterial environment was generated using microfluidic techniques such that as few as one to three bacterial cells contained within a droplet in a well could be monitored. QS was evaluated using a fluorescent reporter for a QS-controlled gene. In some of the wells containing two cells each, QS was initiated and QS-dependent growth occurred. However, the process was highly variable, and the variability appeared to be inherent property of the bacteria, as it could not be attributed to any outside factors. In contrast, QS was always initiated in groups starting with seven cells, suggesting that behavior of small groups of cells is more random than large groups. Notably, on occasion an individual cell initiated QS before dividing, in stark contrast to the prevailing view that QS is exclusively a group behavior. In addition, when small groups of cells that were dependent on QS for growth were confined, only in those cells where QS was initiated did growth occur. Taken together, the results indicate that even small numbers of cells, when con-



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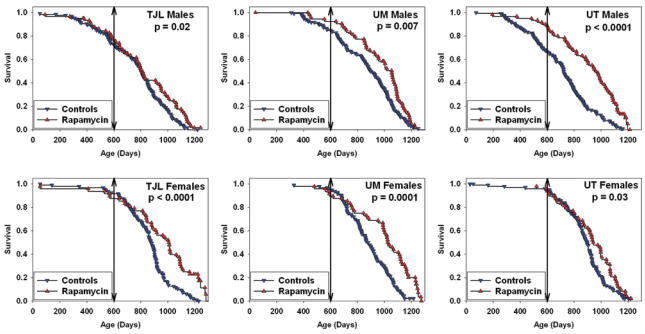
fined to a small volume, can participate in QS activities. Strikingly, even a single cell was able to initiate QS, offering evidence for autocrine signaling by QS pathways. These results have important practical implications, for example in situations where cells are confined to small volumes such as during pathogenesis. **Eva J. Gordon, Ph.D.**

Drinking from a Fountain of Rapamycin

The small molecule drug rapamycin is well-known as an immunosuppressive agent used to prevent rejection in organ transplant patients. Rapamycin inhibits the protein TOR, a kinase involved in many aspects of cell function including growth, survival, and gene regulation. Recent studies have shown that the lifespan of several invertebrate species can be extended by inhibiting the TOR signaling pathway, but it is unclear whether the same holds true for mammalian species. In an impressive study across three institutions, Harrison *et al.* (*Nature* 2009, *460*, 392–395) demonstrate that rapamycin-fed mice do in fact enjoy longer lives than their regular chow-fed counterparts.



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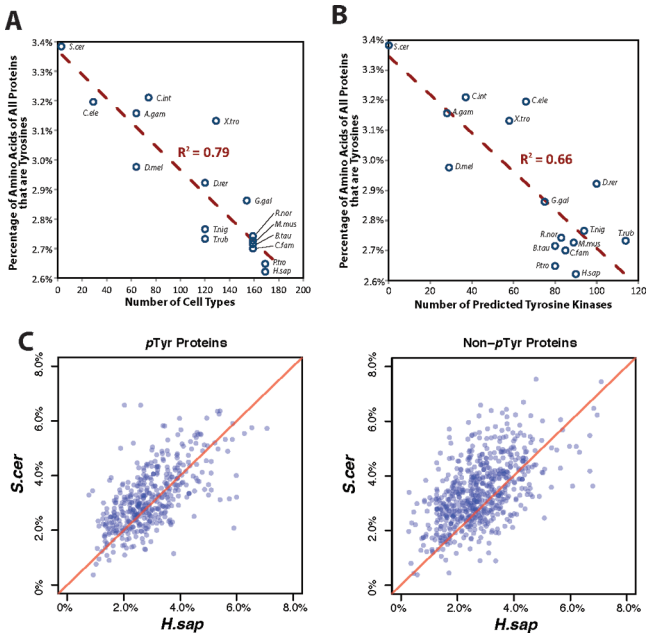
The study was part of the National Institute on Aging Interventions Testing Program. Mice were fed rapamycin in laboratories in Maine, Michigan, and Texas, starting when they were 600 days old. Notably, this corresponds to a human age of approximately 60. At all three sites and in both males and females, both the median and the maximal lifespan was increased in the mice whose diet included rapamycin. The mice whose lives were extended were highly heterogeneous progeny from a 4 strain cross, so it is unlikely that life extension comes from merely postponing a few specific diseases. In fact, the causes of death among the rapamycin-fed and control mice were not altered; the rapamycin-fed mice just lived longer. Mice fed rapamycin did exhibit reduced phosphorylation of the ribosomal protein subunit S6, indicating that the rapamycin was indeed acting as a kinase inhibitor in the animals. Precisely how rapamycin extends lifespan, however, remains elusive, though delaying death from cancer or slowing mechanisms of aging are both plausible mechanisms. The fact that the treatment began late in life is notable, as similar life-extension as a result of diet restriction is not effective when started after 550 days of age. From a clinical standpoint, antiaging agents that could be initiated later in life have distinct advantages over other life-extension agents. **Eva J. Gordon, Ph.D.**

Signaling through Tyrosine: Less Is More

Expansion of certain protein families, for example, those involved in signaling mechanisms and tissue development like tyrosine kinases, likely enabled the evolution of more complex species. Surprisingly, genomic analyses reveal that the number of tyrosine residues present in the proteins of a given species actually correlates *negatively* with the complexity of the species. Tan *et al.* (*Science* published online June 25, 2009; DOI: 10.1126/science.1174301) discover and explore this phenomenon by examining tyrosine loss in human proteins that are and are not tyrosine-phosphorylated. By comparing these proteins with their orthologs in yeast, which do not possess conventional tyrosine kinases, the relevance of the emergence of phosphotyrosine signaling on tyrosine content could be evaluated.



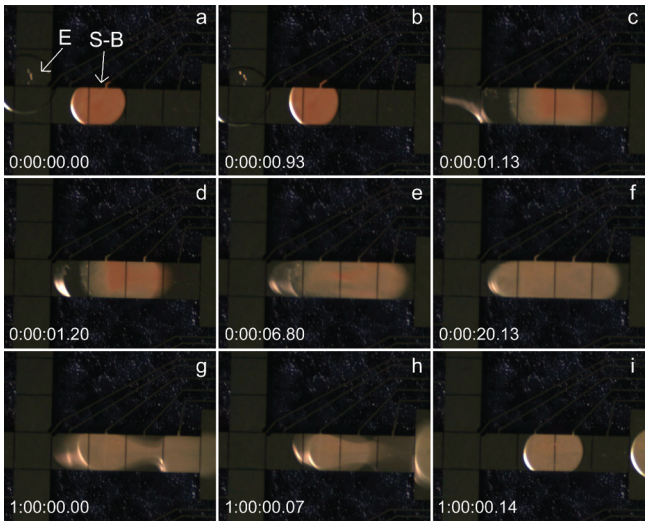
From Tan, C. S., *et al.*, *Science*, June 25, 2009; DOI: 10.1126/science.1174301. Reprinted with permission from AAAS.

The examination revealed that human proteins possess significantly fewer tyrosines than yeast proteins, and strikingly, this is especially true for proteins that do not contain phosphorylated tyrosines. The authors propose that this phenomenon arose with emerging complex signaling mechanisms in order to reduce superfluous tyrosine phosphorylation events that did not contribute positively to an evolving signaling network. Thus, elimination of ty-

rosine residues may have functioned as systems-level adaptive mutations that favored the materialization of phosphotyrosine signaling pathways. Notably, similar negative correlations exist with other amino acids that can be phosphorylated, methylated or glycosylated, suggesting that such systems-wide adaptive changes may be a general evolutionary selection mechanism. Intriguingly, it is possible that this type of selection mechanism could also play a hand in the development of complex diseases such as cancer. **Eva J. Gordon, Ph.D.**

Makings of a Microfluidic Golgi

Heparan sulfate (HS) is a complex molecule to untangle. The Golgi organelle both modifies these glycosaminoglycan (GAG) sequences of glucuronic acid and *N*-acetylglucosamine and adds them to serine residues of the core peptide of proteoglycans (PG). The enzymes within the Golgi do not react completely with their substrates, which can create complex patterns of GAG sequences. The resulting PGs, however, mediate critical molecular and cellular recognition processes such as blood coagulation, angiogenesis, and cellular growth and differentiation. As a tool to study the biosynthesis of HS, Martin *et al.* designed a clever microfluidic device that could mimic the series of reactions that occur within the Golgi (*J. Am. Chem. Soc.* 2009, DOI: 10.1021/ja903038d).

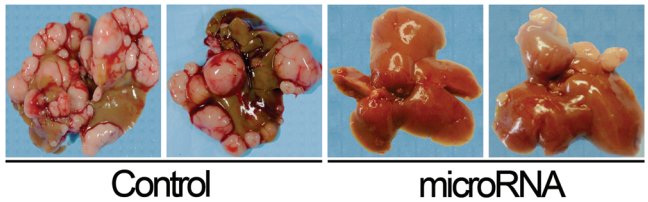


As a proof-of-concept, they demonstrated that the system performed the final reaction in HS synthesis, the modification of a GAG sequence by 3-*O*-sulfotransferase (3-OST).

The researchers constructed a digital microfluidic chip, a 2D platform that controls droplet flow and mixing with electronic pulses instead of using channels. Using the device, the researchers combined and mixed a 400 nL droplet of suspended magnetic nanoparticles with immobilized GAG substrate with the same volume of another droplet containing 3-OST and the sulfate source, adenosine 3′-phosphate 5′-phosphosulfate (PAPS). They allowed the drops to incubate for one hour, long enough to modify at least one 3-*O*-sulfonation site on each chain, consistent with natural HS. The resulting droplet was then removed from the chip and added to a solution of fluorescently labeled anticoagulant protein antithrombin III (ATIII), which binds to HS but not the substrate. Thirty percent of the modified nanoparticle surface area bound to ATIII compared to 0.5% of the substrate nanoparticles, similar to results for HS nanoparticles modified off-chip. They also demonstrated that the reaction droplet could be split for use in a further reaction. These results represent the first enzymatic reaction of an immobilized substrate in a digital microfluidic device. The researchers are currently developing the system as a lab-on-a-chip for the synthesis and screening of customized glycans. **Sarah A. Webb, Ph. D.**

Slowing Liver Cancer with a Little RNA

MicroRNAs may be the smallest of non-coding RNA regulators, but a quick PubMed query can tell you that their impact is anything but tiny. Numerous studies demonstrate that single miRNAs can have multiple mRNA targets and misregulation of just one particular miRNA can vastly impact a cell's fate. Now, with abundant resources for profiling miRNA levels, cancer researchers are finding that some miRNAs change drastically between a normal tissue and a tumor sample. In cases when the miRNA is overabundant, some methods have sought to quell the miRNA's effect by blocking its effects with an antisense approach. Now, a new study by Kota *et al.* (*Cell* 2009; 137, 1005–1017) takes a look at the opposite case, where a miRNA is underexpressed in a liver cancer.



Using a mouse model for hepatocellular carcinoma, the authors found that miRNA-26a was down several fold in liver tumor tissue versus normal liver. Once they confirmed that this downregulation is also present in matched human tissue samples, they went on to ask what mRNA targets might be tweaked by this loss. An interesting clue came from overexpressing miRNA-26a in a liver cancer cell line. Concomitant with abundance of this miRNA came an arrest of the cell cycle, pointing the way toward candidates associated with the cell cycle and checkpoints. With bioinformatics predictions, they found and then validated two Cyclins, D2 and E2, as targets of

miRNA-26a. Armed with this knowledge *in vitro*, they dove back into the mouse model and developed an elegant viral system to express both GFP and the miRNA-26a precursor in vivo. After induction of the liver cancer phenotype, the mice were given either a GFP alone virus or one that also made the miRNA. Three weeks later, the livers were assessed for tumors and in 80% of the cases, there was significant loss of tumor progression. Further characterization showed that the cell cycle was arrested in the miRNA-treated tumors and apoptosis was also increased, but normal liver cells were spared any toxic effects. Though the liver represents one of the better targets for a therapeutic RNA approach, this study demonstrates that reconstituted expression of miRNA can have profound effects.
Jason G. Underwood, Ph.D.