techniques or expertise that have slowed their wide adoption by organic chemists.

A few efforts have effectively used simple approaches to mine for new reactions (*12*, *13*), but none prior to the Robbins-Hartwig approach have enabled simultaneous and substantial variation of two or more reactants, a catalyst, and a ligand. In their method, new reactions can be discovered with no mechanistic hypothesis and no preconceived notion of what the new chemical reactions actually will be. They combined a large set of simple and widely available reactants, each bearing a single functional group, and injected the mixture into each well of a 384-well array. All of the substrates had a similar mass, so coupling products would be of higher mass than each reactant and readily spotted in subsequent analysis. They sampled ~50,000 reactant-catalyst-ligand combinations.

A simpler, hypothetical example with a 96-well plate (see the figure, panel A) outlines how a successful reaction is run and identified. In this example, after heating the well plate for several hours, 8 samples containing a portion of the contents of each row, and 12 samples containing a portion of the contents of each column, would be analyzed by gas chromatography–mass spectrometry and electrospray ionization mass spectrometry. In the example depicted, such analysis identified a characteristic mass of a new product **Z** produced by the catalyst in row 6 (metal **M6**) with the ligand in column 10 (**L10**).

In simple cases, evaluating the mass spectrum can directly allow the combination of reactants to be deduced (for example, if the mass of product **Z** equals the sum of two reactants). In cases where the reactants cannot be directly deduced (for example, some reactions might involve incorporation of only part of a substrate, elimination of functional groups, or combination of more than two molecules), the reactant identity can be deconvoluted by examining sets and then subsets of reactants (see the figure, panel B). This iterative analysis can identify not only the reactants that were incorporated into product **Z**, but also any reactant that acted as a catalyst or co-reagent.

Robbins and Hartwig identified interesting new reactions with their method. They also performed control experiments, which showed that several known reactions were not missed in their analysis. The method does have limitations. For example, consumption of a reactant in a fast reaction could prevent a slower, more interesting reaction involving that same reactant from being observed. Additionally, many very useful chemical reactions do not tolerate certain classes of functional groups, and any new reaction inhibited by a component of the reactant pool will be missed by this method. The use of an excess of catalyst to avoid a simple poisoning effect can address this concern to a large extent. A strength of the method is that it is biased to find chemical reactions that are widely tolerant of various functional groups.

Insight into the generality of the method will come from future applications that illustrate the frequency with which known reactions are inhibited by reactant components, masked by competing reactions that consume a necessary component, or not readily visible because of the complexity of chromatographic traces or mass spectrometric data. However, the preliminary indications look very encouraging. The method is clever and effective without being sophisticated, and can be easily adopted by others without special equipment or expertise. It can be used on virtually any scale, including more limited studies of particular reactions. Traditional approaches in mechanistic analysis and catalyst-ligand optimization will still be indispensable in developing the new reactions into robust methods that will join the organic chemist's repertoire.

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10.1126/science.1210735

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CELL SIGNALING

Getting to the Heart of Mechanotransduction

Stretching a heart muscle cell triggers signaling by reactive oxygen species to produce Ca^{2+} sparks.

Cecilia Hidalgo and Paulina Donoso

Mechanotransduction, the process of converting mechanical stimuli into cellular responses, enables cells to produce signals that regulate a wide range of physiological responses. In the beating heart, for example, the stretching of muscle cells causes the release of chemical signals that regulate heart function, and studies in mice and humans have suggested a connection between faulty stretch-sensing mechanisms and heart disease (*1*). The mechanisms underlying such processes, however, have been unclear. On page 1440 of this issue, Prosser *et al.* (*2*) use a novel method that involves precisely stretching single heart muscle cells (cardiomyocytes) that have been glued to microscopic glass rods to provide some clarity. They demonstrate that a moderate stretch during the cell's relaxed state (diastole) can trigger a burst of calcium "sparks." They also show that this process is defective in a life-threatening muscle disease.

Mechanotransduction has an important role in the myocardium, the heart's muscular tissue. Each contraction phase (systole) of the cardiac cycle causes sarcomeres (the basic unit of muscle) to shorten; the sarcomeres then lengthen again during diastole. In the early 1900s, European researchers Otto Frank and Ernest Starling showed that an increased length change during diastole produces a stronger contraction in the following systole. To better understand the mechanisms underlying cardiac mechanotransduction, Prosser *et al.* developed new tools to apply a controlled and moderate stretch (8%) to isolated rat or mice cardiomyocytes during diastole, and then measured intracellular levels of cal-

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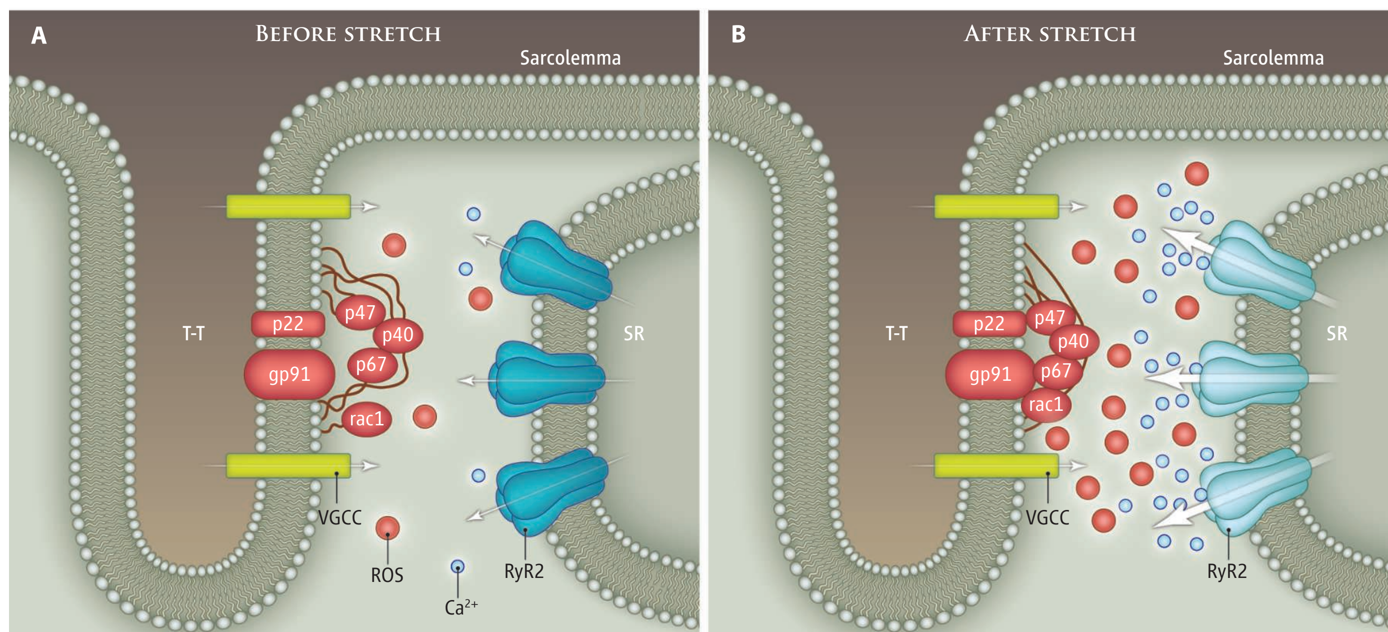

X-ROS signaling. In heart muscle cells, transverse tubule (T-T) membranes contain voltage-gated Ca^{2+} channels (VGCC, green). Influx of Ca^{2+} through the channels generates signals that open RyR2 channels (blue) in the neighboring sarcoplasmic reticulum (SR); the ensuing Ca^{2+} release promotes cell contraction. The T-T membrane also contains the NOX2 subunits p22 and gp91 (red). Before stretch (**A**), NOX2 activity, production of ROS (red dots), and Ca^{2+} sparks (blue dots) are low. A moderate stretch (**B**) causes an immediate activation of NOX2 by recruiting its regulatory subunits (p40, p47, p67, and rac1) to the T-T membrane via a mechanism that requires intact microtubules. The resulting increase in ROS production sensitizes RyR2 to activation by Ca^{2+}, presumably by changing RyR2 redox state, causing a burst of Ca^{2+} sparks. Returning the cell to its initial length returns X-ROS signaling to its initial state. [Figure adapted from (*2*)]

cium ions (Ca^{2+}) and reactive oxygen species (ROS) before, during, and after stretch. They observed that stretch initiated, within milliseconds, a burst of Ca^{2+} sparks—highly localized and temporary increases in intracellular Ca^{2+} concentration—and a nearly instantaneous increase in the rate of ROS production. Both Ca^{2+} spark generation and ROS production immediately returned to baseline levels after the cell was restored to its initial length. They demonstrated that the stretch-induced burst of Ca^{2+} sparks requires ROS; introducing an antioxidant molecule prevented the sparks, whereas a mild oxidant enhanced them.

Prosser *et al*. also studied the behavior of single cardiomyocytes from mice that have a genetic mutation that causes a muscle disease similar to human Duchenne muscular dystrophy. Myocytes from these *mdx* mice display greater nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity—and higher cellular ROS levels—than myocytes from wild-type mice (*3*). Prosser *et al*. report that moderately stretching *mdx* cells produced Ca^{2+} waves instead of sparks; waves are typical responses of abnormal cardiac Ca^{2+} signaling.

Prosser *et al*. propose that the process they call X-ROS signaling produces these results. Past studies had described increased Ca^{2+} spark generation in response to stretching of single cardiomyocytes (*4*–*6*), and implicated ROS in the enhanced Ca^{2+} sensitivity of *mdx* cardiomyocytes (*3*, *7*), but the molecular mechanisms were unresolved. Now, using pharmacological and molecular techniques, Prosser *et al*. show that moderate diastolic stretch activates the enzyme complex NADPH oxidase 2 (NOX2), which they found colocalized with markers for the transverse tubule (T-T) system formed by invaginations of the muscle fiber's plasma membrane. NOX2, in turn, directly mediates ROS-dependent Ca^{2+} spark generation (see the figure). The finding that dystrophic heart muscle has an excessive X-ROS signaling response advances our knowledge of the mechanisms underlying abnormal Ca^{2+} signaling in this disease.

Prosser *et al*. also propose that stretch-induced ROS production increases Ca^{2+} spark generation by sensitizing ryanodine receptor type 2 (RyR2) channels located in the nearby sarcoplasmic reticulum (SR), the intracellular membrane network that surrounds myofibrils. The SR releases and recaptures Ca^{2+} in each contraction-relaxation cycle that underlies the heartbeat. The cycle starts with Ca^{2+} entry into cardiomyocytes through voltage-activated channels located in the T-T system. Next, Ca^{2+} entry stimulates the opening of RyR2 Ca^{2+} release channels. This cellular response, known as Ca^{2+}-induced Ca^{2+} release (CICR), causes muscle contraction. The cycle ends with relaxation, which occurs when intracellular Ca^{2+} returns to resting levels (*8*). The Ca^{2+} sensitivity of RyR2 channels is a key feature in CICR regulation. Alterations in RyR2 Ca^{2+} sensitivity, which is influenced by cellular factors and RyR2 redox state (*9*, *10*), may underlie subcellular changes in Ca^{2+} signaling that contribute to disease (*11*).

Although researchers reported more than a decade ago that the Ca^{2+} sensitivity of single RyR2 channels is redox-dependent (*12*), Prosser *et al*. demonstrate that a Ca^{2+} spark burst results from the very fast and reversible X-ROS signaling, which requires an intact microtubule network. Previous studies indicated that activation of cardiac NOX2 increases RyR2 S-glutathionylation, a reversible redox modification that enhances RyR2 activity and hence promotes SR Ca^{2+} release (*13*). Tachycardia (accelerated heart rate) and exercise augment these effects (*14*), suggesting a direct correlation between increased heart activity, NOX2 activation, increased RyR2 S-glutathionylation, and enhanced Ca^{2+} release. It remains unclear, however, if the Ca^{2+} spark burst induced by controlled stretch entails RyR2 S-glutathionylation. In addition, the cellular mechanisms that so efficiently return ROS production and RyR2 activity to baseline levels after stretch remain unclear, as do the molecular mechanisms that enable extremely fast microtubule-dependent NOX2 activation. For example, do angiotensin receptors mediate this response, as they mediate the slow stretch response of the myocardium (*15*, *16*)?

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Prosser *et al*. propose that X-ROS signaling regulates Ca^{2+} signaling in the heart under normal conditions; in contrast, it may trigger abnormal rhythms in an injured or diseased heart. Afuture challenge will be to test whether the changes they observed in isolated heart cells also occur in the beating heart.

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17. Research was supported by FONDECYT-FONDAP (grants 1501006 and BNI P-09-015F) and FONDECYT (grant 1110257).

10.1126/science.1212183

EVOLUTION

Mother Tongue and Y Chromosomes

A global picture is emerging of sex-specific transmission of language change in quite different regions and continents.

Peter Forster[1,2] and Colin Renfrew[3]

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Some 6000 different languages are spoken in the world today, and tracing the prehistory of languages and of language change by means of genetic markers has long been a goal (*1*). However, this has proven to be a more challenging task than simply tracing colonizations. Nevertheless, a number of genetic studies over the past few years have started to address language and language change before recorded history. A correlation is emerging that suggests language change in an already-populated region may require a minimum proportion of immigrant males, as reflected in Y-chromosome DNA types. By contrast, the female lineages, as indicated by mitochondrial DNA (mtDNA) types, do not reflect the survivor language but represent more ancient settlement.

Chaubey *et al*. have found that in the Indian subcontinent, the Austroasiatic languages spoken by tribes such as the Munda show a high proportion (75%) of immigrant Y-chromosome DNA (a type called O2), which is generally found among peoples of East Asia, but predominantly (75 to 100%) local Indian mtDNA types (*2*) (in humans, mtDNA is inherited from the mother) (see the figure). Similarly, the study found that another major language family, Tibeto-Burman in northeastern India, coincides with a high proportion of immigrant East Asian Y-chromosome O3 types, which are generally found in populations of southeastern Asia. The East Asian mtDNA is rarer in Tibeto-Burman speakers residing in India.

A compatible observation has recently been made by Stoneking and Delfin (*3*), who noted that in East Asia, a patrilocal residence pattern (where women rather than men change residence upon marriage) favors geographic stability of the male lineage. This may reflect the migration of men and women (and their language) to an already populated area, but once settled, the women intermarry with men from neighboring populations and move to their villages. In this scenario, the immigrant men remain, and the language therefore stays in the same place as the immigrant Y chromosomes. And in Africa, Wood *et al*. (*4*) and de Filippo *et al*. (*5*) found that Bantu and other Niger-Congo languages correlate well with Y-chromosome types (indicating male lineages), whereas mtDNA types (associated with maternal descent) correlate not with language but with geography in Bantu-speaking areas.

Sex-specific transmission of language change may be a feature when the mechanism of change is by farming/language dispersal (*6*). This dispersal hypothesis describes how language change can be linked to the early spread of food—domesticated animals and plants—by farmers speaking a protolanguage. In the Americas, language replacement in the course of postulated farming dispersal has been found to correlate in the Uto-Aztecan language family with Y-chromosomal DNA variation but not with mtDNA variation (*7*).

As for the immigrant Indo-European and the presumably indigenous Dravidian languages in India, there is one major candidate genetic marker for immigration from the northwest. It is a Y-chromosome DNA

Language transmission. Glossogenetic studies relate Y-chromosome DNA types with language in the indicated regions. Such correlation is not observed for mtDNA, which is inherited from the mother. "Melanesian" designates non–Malayo-Polynesian languages in New Guinea, where Malayo-Polynesian is spoken in coastal pockets. The hatched region shows Bantu as a branch within the Niger-Congo language family.

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Science

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Science **333** (6048), 1388-1390.
DOI: 10.1126/science.1212183

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