down-regulation of ERK activity (by the enzyme Akt) could serve as a critical control point in LEC sprouting and also contribute to LEC differentiation and the maintenance of lymphatic identity (*3*).

It is likely that VEGR3 signaling outputs are further modulated by other signaling molecules expressed at the same time. The receptors for BMPs and transforming growth factor–β (TGFβ) emerge as interesting candidates in this context. Inducible, endothelial-specific deletion of TGFβ receptors (TGFβR2 and Alk5) suppresses LEC sprouting at the expense of LEC proliferation by altering the expression of VEGFR3 and another VEGF-C receptor called neuropilin-2 (*3*). BMP9, which binds with high affinity to a receptor complex composed of BMPR2 and Alk1, also affects lymphangiogenesis (*14*, *15*). Alk1 inhibition using a "ligand trap" blocked sprouting angiogenesis and decreased lymphangiogenesis (*15*, *16*), which suggests that BMP9 may be a potential target for promoting lymphangiogenesis without stimulating growth of blood vessels. Further studies are required to determine how BMPs and TGFβ affect lymphangiogenesis.

Other important recent discoveries in the field include the RNA binding protein called human antigen R (HuR) in maintaining the stability of mRNA encoding VEGF-C and -A. Depending on the cell type in which HuR is deleted, this results in impairment of lymphangiogenesis or angiogenesis (*3*). LEC mechanosensing is also emerging as a critical regulator of lymphangiogenesis and lymphatic valve formation, although many details are yet to be filled in (*17*, *18*). And exciting results suggest that cutaneous lymph capillaries are important for systemic blood pressure control by locally modulating skin electrolyte composition (*19*).

Unlike the blood system, much about the lymphatic system remains elusive. As our understanding of its development and pathology grows, we should begin to finally develop treatments for lymphedema and other disorders of lymphatic circulation—progressive, lifelong conditions that affect millions of people, for whom curative treatments are currently not available.

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Acknowledgments: We thank J. Bender, T. Hla, Y. Mukouyama, S.-W. Jin, and W. Sessa for permission to cite findings that were presented at the conference (*3*).

10.1126/science.1243452

CHEMISTRY

Copper's Contribution to Amination Catalysis

Copper complexes catalyze a remarkably broad range of organic reactions that form carbon-nitrogen bonds.

Sherry R. Chemler

Copper complexes are used as catalysts in modern synthetic chemistry because of their low cost, versatile reactivity, and broad tolerance for functional groups on substrates. Oxidation states in copper complexes can range from Cu^0 to Cu^{IV}, and the metal center can participate in either two-electron or single-electron processes, sometimes both in the same catalytic cycle (*1*–*6*). This perspective highlights copper's contribution to amination catalysis, specifically the formation of carbon-nitrogen (C–N) bonds by coupling amines or amides with aryl halides (ArX) and alkyl halides (Ullmann-Goldberg reaction), arenes and alkanes [carbon-hydrogen (C–H) bond amination], or alkenes (oxidative amination, aminooxygenation, carboamination, and diamination).

Formation of C–N bonds is one of the most common transformations in pharmaceutical synthesis. The Ullmann-Goldberg reaction, which couples aryl halides with anilines and amides (see the figure, panel A), has undergone extensive development since its initial discovery in 1906 (*4*, *7*). Initially, this reaction was performed with limited substrates at high temperatures (~210°C) with elemental copper (>13 mol %). Subsequent work showed that certain ligands and solvents (that can serve as ligands) substantially increase copper's catalytic activity and opened up a much broader substrate range. The reaction can be run at moderate temperatures (23° to 130°C) and in most cases with lower catalyst loading (1 to 20 mol %).

The mechanism of the Ullmann-Goldberg reaction is thought to initiate with the formation of an amido-copper(I) intermediate (see the figure, panel B) (*8*, *9*) that reacts with aryl halides. The resulting organocopper(III) intermediate undergoes reductive elimination to form the C–N bond and regenerate the [Cu^I] catalyst (*10*). Mechanisms involving oxidative addition into the Ar–X bond or single-electron transfer (SET), possibly via atom transfer, have been proposed for this step. The former creates the organocopper(III) intermediate directly, whereas the latter forms an aryl radical that can add to the resulting [Cu^{II}].

The SET mechanism can be favored if the reaction is promoted by light at temperatures where the thermal reaction is not observed (*11*). Ultraviolet light excites an electron on the copper(I) amide to a higher-energy state, which in turn reduces the aryl halide to an aryl radical that combines with the resulting copper(II) amide to form a copper(III) intermediate. This strategy was also used to couple amines and alkyl halides (*12*). Reaction temperatures can be reduced with these photoreactions, and the stereochemical outcome could be altered, such as net retention of configuration at carbon instead of the usual inversion of configuration (see the figure, panel C) (*12*). In this reaction, the

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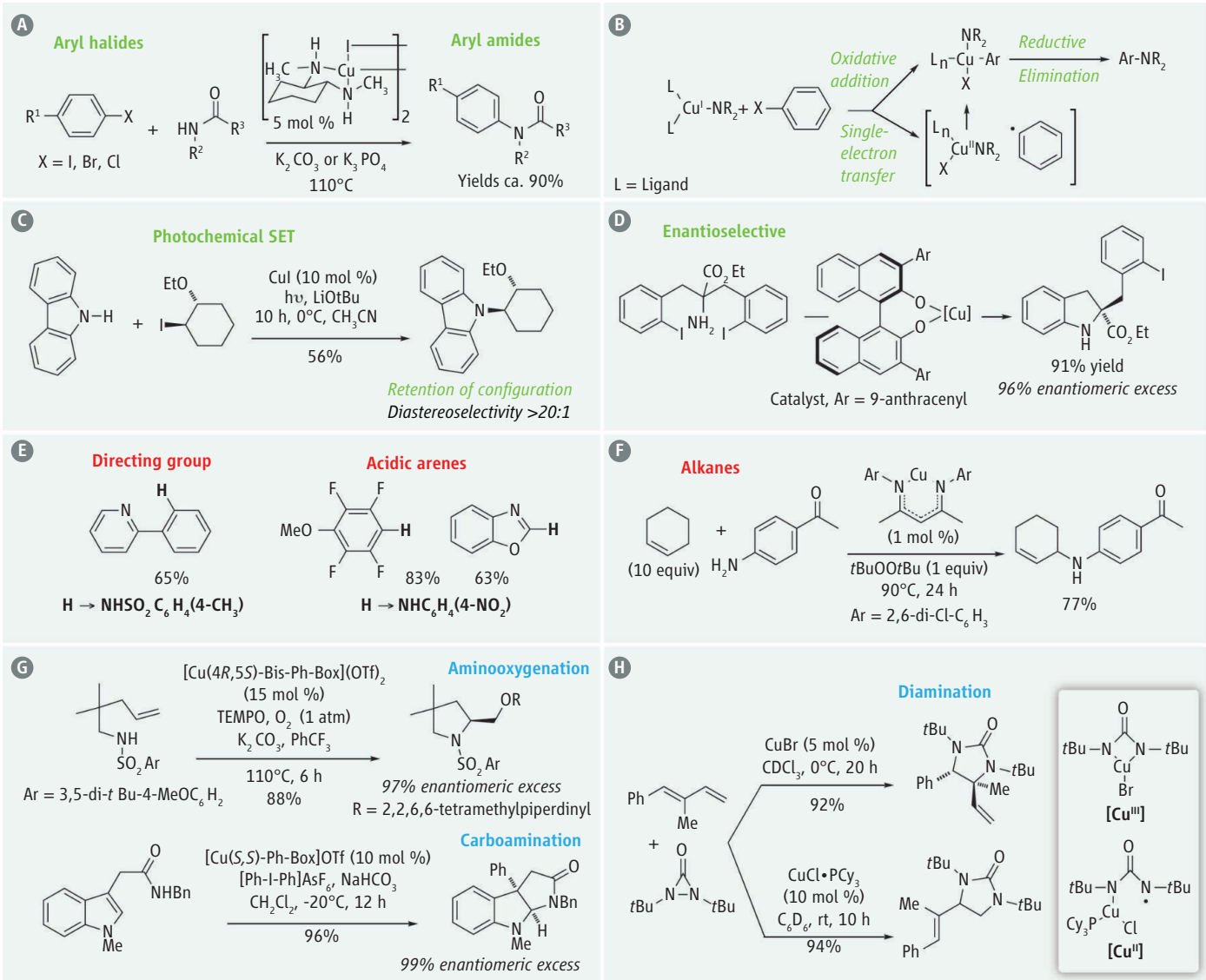

Copper catalysis of carbon-nitrogen bond formation. Examples and mechanistic details are shown for reactions that form amides from (**A** to **D**) aryl and alkyl halides, and (**E** and **F**) activated C-H bonds. In (**G**), nucleophilic addition of amine nucleophiles to alkenes is shown for aminooxygenation and carboamination. In (**H**), an example of diamination in shown. Conditions in (D) were 10 mol % CuI, 20 mol % chiral ligand, Cs_2CO_3, dioxane, room temperature (rt) for 10 hours (h). Conditions in (E) were 20 mol % copper(II) acetate, 1 atm O_2. For the first example, *p*-toluenesulfonamide, dimethylsulfoxide, 48 hours, 160°C were used, and for the other examples 4-$NOC_6H_4NH_2$, dimethylformamide, potassium *tert*-butoxide, TEMPO, 40° or 80°C, 24 hours were used.

stereochemistry of the alkyl amine product is controlled by the chirality of the carbon adjacent to the halogenated carbon.

A chiral copper catalyst can be used to control the absolute stereochemistry in the thermal Ullmann-Goldberg reaction manifold (see the figure, panel D) (*13*). The desymmetrization of an achiral substrate that contains both amine and aryl halide components was achieved, forming a chiral indoline. The researchers proposed that the enantioselectivity was determined in the oxidative addition step (*13*).

Direct amination of a C–H bond bypasses the need for adding a halide to an existing molecule. Oxygen gas is frequently used as an environmentally benign stoichiometric oxidant in these reactions. At present, copper-catalyzed C–H aminations are limited to acidic arenes (those functionalized with electron-withdrawing groups) and those functionalized with directing groups usually adjacent (ortho) to the C–H bond (see the figure, panel E) (*6*, *10*). Copper-catalyzed C–H aminations can occur either via two-electron or SET mechanisms, depending largely upon the substrate's structure and the oxidants and ligands used. Copper-catalyzed intra- and intermolecular net C–H aminations of activated alkenes (vinyl arenes) that involve nitrogen radical intermediates have also been reported (*14*).

The C–H amination of alkanes under oxidizing conditions (*15*) can occur and proceed either as concerted, C–H insertion of copper nitrenes ([Cu]=NR), or cascades in which C–H atom abstraction is followed by radical rebound processes. In both cases, weaker C–H bonds, such as those adjacent to phenyl rings and alkenes, can be targeted (see the figure, panel F). Asymmetric catalysis has been achieved in a few allylic amination cases; yields and enantioselectivities are promising but moderate (yields up to 44%; enantioselectivities up to 70%) (*16*).

Copper catalysts enable the addition of amine nucleophiles to alkenes by acting as an electrophile to accept π-bond electrons.

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Copper(II)-2,2′-isopropylidenebis[(4*R*,5*S*)-4,5-diphenyl-2-oxazoline]ditriflate {[Cu(4*R*,5*S*)-bis-Ph-box](OTf)$_2$} catalyzes intramolecular additions of sulfonamides to terminal alkenes with concomitant addition of the stable oxygen radical (2,2,6,6-tetramethylpiperidine-1-yl)oxyl (TEMPO) (see the figure, panel G) (*17*). The mechanism involves concerted intramolecular addition of R_2N-[Cu^{II}] across the alkene followed by C-[Cu^{II}] homolysis and subsequent carbon radical coupling with TEMPO. A different ring-forming alkene amino-functionalization strategy involves electrophilic addition of a chiral organocopper(III) complex (formed in situ by oxidation of a chiral Cu^{I} complex with [Ph-I-Ph]AsF_6) (where Ph is phenyl) to the electron-rich alkene of an indole (see the figure, panel G) (*18*). Amine addition to the resulting iminium ion forms a new C–N bond, and reductive elimination provides the chiral C–C bond.

Finally, diamination is one of the more sought-after alkene difunctionalization reactions, and it can occur as an intermolecular copper-catalyzed reaction of dienes with diaziridinones with complementary regioselectivity by either a two-electron or single-electron mechanism, depending upon the ligands used (see the figure, panel H) (*19*). Oxidative addition of [Cu^{I}] into the diaziridinone gives a new [Cu^{III}] species that can be in equilibrium with a [Cu^{II}] species by Cu–N homolysis. Electrophilic addition of the copper(III) species to the alkene occurs at the more electron-rich, internal alkene, generating the internal diamine. This preference can be changed by addition of the bulky PCy_3 (tricyclohexylphosphine) ligand, which shifts the equilibrium to the copper(II)-aminyl radical species that favors addition to the terminal alkene carbon.

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10.1126/science.1237175

GENETICS

The Maturing Brain Methylome

DNA methylation patterns in the developing and adult mammalian brain point to a role in synaptic development and maturation.

Harrison W. Gabel and Michael E. Greenberg

The methylation of DNA in mammalian genomes regulates gene expression, guiding differentiation and maintaining cellular identity within tissues. However, it may have a distinct function in the brain. On page 629 of this issue, Lister *et al*. (*1*) present a comprehensive analysis of DNA methylation and hydroxymethylation at single-base resolution in the mammalian frontal cortex. The authors chart out striking postnatal alterations in neuronal methylation profiles that occur as synapses develop and are refined, from the fetal to adult stage. The patterns suggest that DNA methylation is important in the maturation of neurons in the developing brain.

DNA methylation represses gene expression in all mammalian cells. The methylation of cytosines in the context of cytosine-guanine dinucleotides (mCG) is a stable repressive mark on DNA. However, the Tet family of enzymes converts methylcytosine to hydroxymethylcytosine (hmC), an oxidized form that can be demethylated (*2*, *3*). hmC is enriched in stem cells and neurons, suggesting that they might be particularly susceptible to changes in DNA methylation state (*2*, *4*). Intriguingly, stem cells and brain tissue also contain substantial cytosine methylation outside of the CG context [mCH, where H is adenine (A), thymine (T), or cytosine (C)], which is rare in most somatic cells (*5*, *6*). Although the role of hmC and mCH in stem cells has been extensively investigated at base-pair resolution (*5*, *7*), there has been limited examination of DNA methylation at high resolution in the brain.

Using high-throughput sequencing to profile the mouse and human cortex from the fetal to adult stage, Lister *et al*. revealed a conserved, genome-wide increase in mCH amounts in the brain after birth. Although the period during which mCH accumulates differs between mice and humans (several weeks versus several years, respectively), the increase coincides with the peak of synaptogenesis and synaptic pruning in the brain for each organism (see the figure). Thus, the acquisition of mCH may be linked to neuronal maturation. High amounts of mCH were found in neurons compared to low amounts in nonneuronal (glial) cells. Although the average percentage methylation detected in neurons at CH is quite low (~2 to 6%) and quite high at CG (~80%), because CG is rare in mammalian genomes relative to CH, a similar number of total mCH and mCG events occur in neurons. Indeed, in adult human neurons, the total number of mCH sites surpasses that of mCG sites. Rather than a minor addition to the methylation modifications in the genome ("methylome"), mCH is likely a major substrate for gene regulation in the maturing brain.

hmC builds up in neurons with a timing similar to that of mCH (*8*), raising the possibility that, in addition to occurring at CG, hydroxymethylation also takes place at CH in the brain. Lister *et al*. used another high-throughput sequencing method (*9*) to profile hmC at single-base resolution in mouse fetal and adult frontal cortex. Surprisingly, even though hmC and mCH both accumulate as the brain matures, hydroxymethylation occurs almost exclusively in the hmCG context. If mCH is converted to hmCH at all, it must be extraordinarily short-lived, as it is essentially undetectable at steady-state amounts.

To better understand how methylation contributes to transcriptional regulation, Lister *et al*. performed integrated analysis

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Copper's Contribution to Amination Catalysis

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Science **341**, 624 (2013);
DOI: 10.1126/science.1237175