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Directed Evolution of Bacterial Chemoreceptors

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The methyl-accepting chemotaxis proteins are a family of receptors in bacteria that mediate chemotaxis to diverse signals. We have developed a simple method for selecting bacteria that swim towards target attractants, which makes it possible to isolate novel chemoreceptors. The procedure is based on establishing a diffusive gradient in semi-soft agar and does not require that the attractant be metabolized or degraded. We have applied this method to evolve the *E. coli* aspartate receptor, Tar, to mediate chemotaxis to new attractants. We found that Tar is quite plastic and can be readily mutated to respond to diverse compounds. The overall change in specificity depended on the target attractant. In some cases the mutated receptors still showed significant sensitivity to aspartate, indicating that the receptors had a broadened specificity relative to wild-type Tar. In other cases, however, the Tar variants showed a dramatic decrease in their response to aspartate. This occurred in the absence of any counter-selection steps. For many of the receptors, the maximal sensitivity that was obtained could not be attributed solely to substitutions within the ligand binding pocket. The receptors that we have isolated, together with additional variants that may be obtained with our technique, provide new tools for exploring the molecular mechanisms of signal transduction by chemoreceptors. Our selection method will also be useful for constructing new receptors for the development of biosensors and for engineering bacteria for applications in biotechnology.