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Thermosensing Ability of Trg and Tap Chemoreceptors in *Escherichia coli*

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The thermosensing ability of the Trg and Tap chemoreceptors in *Escherichia coli* was investigated after amplifying these receptors in a host strain lacking all four known chemoreceptors (Tar, Tsr, Trg, and Tap). Cells with an increased amount of either Trg or Tap showed mostly smooth swimming and no response to thermal stimuli. However, when the smooth-swimming bias of the cells was reduced by adding Trg- or Tap-mediated repellents, the cells showed clear changes in the swimming pattern upon temperature changes; Trg-containing cells showed tumbling at 23°C but mostly smooth swimming at 32°C, while Tap-containing cells showed smooth swimming at 20°C but tumbling at 32°C. These results indicate that although both Trg and Tap have the ability to sense thermal stimuli, Trg functions as a warm receptor, as reported previously for Tar and Tsr, while Tap functions as a cold receptor.

Escherichia coli has the ability to sense temperature changes as thermal stimuli and to respond by changing its swimming pattern; a temperature increase induces smooth swimming and a temperature decrease induces tumbling (12). Further studies on the thermosensory transducing system in *E. coli* revealed that two major chemoreceptors, Tar and Tsr, which detect aspartate and serine, respectively, also function as thermoreceptors (4, 11, 13). These are transmembrane proteins with two functional domains in their role as chemoreceptors; one is a ligand-binding domain located in the periplasm and the other is a signaling domain located in the cytoplasm (for reviews, see references 2 and 16). Thus, it is suggested that a temperature change induces a conformational change in these two receptors and that this conformational change triggers the signaling for thermoresponse. In the simplest model of thermoreception by these receptors, two conformational states of these receptors are assumed: a low-temperature state and a high-temperature state (5).

E. coli has two other transmembrane receptors, Trg and Tap, which mediate the response to ribose and galactose and the response to dipeptides, respectively (2, 16). Since the amino acid sequences of Trg and Tap have significant homologies to those of Tar and Tsr (2), Trg and Tap also might function as thermoreceptors. In the absence of Tar and Tsr, however, cells showed mostly smooth swimming and no response to temperature changes (11). Since the amount of Trg and Tap in the cell is rather small compared with that of Tar and Tsr (16), the results did not necessarily imply that Trg and Tap had no ability to sense temperature changes.

We examined the thermoresponse in cells with elevated amounts of either Trg or Tap. The results indicate that these two receptors do have a thermoreceptor function.

MATERIALS AND METHODS

Bacterial strains and plasmids. All strains used are derivatives of *E. coli* K-12. A mutant with multiple defects in chemoreceptors, HCB339 [Δ(*tar-tap*)*5201* Δ*tsr7028 trg*::Tn*10*] (19), was obtained from H. C. Berg from Harvard University. AB1200 [Δ(*tar-tap*)*5201* Δ*tsr7028*] (8) was obtained from M. I. Simon from the California Institute of Technology. AW660 (*tsr-12 tar trg-1*) (7) was obtained from J. Adler from the University of Wisconsin.

Plasmids pCP31 (*trg*$^+$), pCP32 (*trg-19*), and pCP33 (*trg-8*) (15) were obtained from G. L. Hazelbauer from Washington State University. pVB8 (1), which carries a *tap*$^+$ gene under a *tac* promoter, was obtained from M. D. Manson from Texas A&M University.

Transformation to prepare plasmid-containing cells was done as described previously (9).

Chemicals. Phenol and glycerol were obtained from Wako Pure Chemicals, Osaka, Japan. Isopropyl-β-D-thiogalactopyranoside (IPTG) was obtained from Sigma Chemical Co., St. Louis, Mo.

Bacterial growth. Cells were grown at 30°C with shaking in tryptone-glycerol broth consisting of 1% tryptone (Difco Laboratories, Detroit, Mich.), 0.5% NaCl, and 0.5% glycerol. For the growth of HCB339(pVB8), the broth was supplemented with 1 mM IPTG. This IPTG concentration gave a maximum swarm size for HCB339(pVB8) on tryptone swarm plates. After 4 to 5 h of cultivation, cells were harvested by centrifugation at room temperature. Cells were washed and resuspended in motility medium consisting of 10 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 10 mM sodium DL-lactate (pH 7.0), and 0.1 mM methionine. Cells (about 5×10^7 cells per ml) were kept at 30°C.

Measurement of thermoresponse. A drop of cell suspension was placed on a glass slide set on a temperature control apparatus as described previously (12). When necessary, 1.3 mM phenol or 7% glycerol was added to the suspension just before the experiment. Thermal stimuli were given by changing the temperature of the glass slide. Temperature changes were monitored by inserting a thin constantan-chromel thermocouple. Swimming cells were observed under a dark-field microscope and recorded on videotapes. Changes in the smooth-swimming fraction were measured photographically as described previously (13, 14).

For tethered cells, changes in rotation behavior after thermal stimulation were observed microscopically and analyzed as described previously (6).

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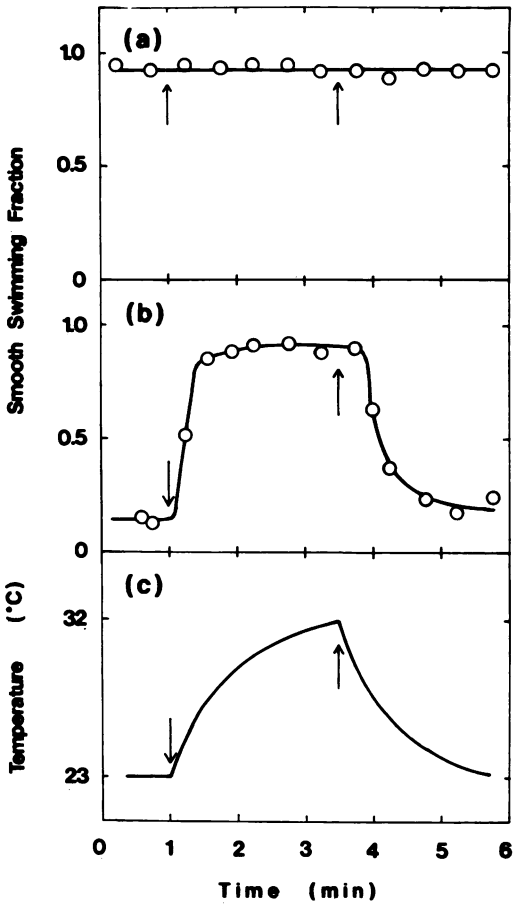

FIG. 1. Thermoresponse in HCB339(pCP31) (*trg*$^+$) cells. Cells in motility medium were incubated at 23°C for 3 min with or without 1.3 mM phenol, a Trg-mediated repellent. The temperature was increased to 32°C at the first arrow and decreased to 23°C at the second arrow. The fraction of smooth-swimming cells was measured as described in Materials and Methods. (a) No phenol; (b) 1.3 mM phenol; (c) time course of temperature change.

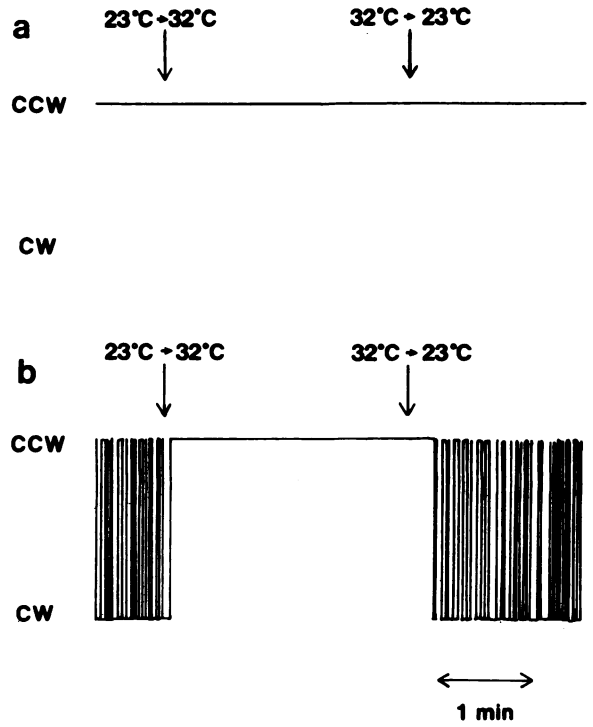

FIG. 2. Changes in rotational state of tethered cells of HCB339 (pCP31) (*trg*$^+$) in response to thermal stimuli. Cells were incubated for 3 min with or without 1.3 mM phenol, and at the times indicated by arrows, the temperature was changed as shown in Fig. 1c. CCW, Counterclockwise rotation; CW, clockwise rotation. (a) No phenol; (b) 1.3 mM phenol.

RESULTS

Thermosensing ability of Trg. Strains with normal levels of Trg only such as AB1200 were strongly biased toward smooth swimming (20) and showed no response to thermal stimuli (10). We therefore increased the amount of Trg with a multicopy plasmid, pCP31 (15). HCB339, which has defects in all four receptors, was used as the host. However, HCB339(pCP31) cells swam smoothly and showed no change in swimming pattern upon temperature changes between 22 and 32°C (Fig. 1a). A much larger change in temperature, between 10 and 38°C, also had no effect. Thus, even in the presence of an increased amount of Trg, the cells were strongly biased toward smooth swimming and showed no response to temperature changes.

To reduce the smooth-swimming bias and induce tumbling, we added phenol, a Trg-mediated repellent (20), to a final concentration of 1.3 mM. Phenol induced incessant tumbling in HCB339(pCP31) cells at 23°C (Fig. 1b). When the temperature was gradually increased to 32°C, the tumbling frequency of the cells gradually decreased, until at 32°C the cells showed mostly smooth swimming. This smooth swimming continued for more than 10 min, i.e., the cells showed no detectable adaptation. Tumbling was restored by decreasing the temperature from 32 to 23°C. Repeats of the temperature changes resulted in repeats of the changes in the swimming pattern of the cells (data not shown). Thus, HCB339(pCP31) cells showed a thermoresponse, provided the smooth-swimming bias of the cells had been reduced by the addition of phenol.

A thermoresponse in HCB339(pCP31) cells treated with phenol was also detected with tethered cells. Consistent with the strong smooth-swimming bias of free cells, tethered cells without phenol rotated only in the counterclockwise direction and showed no change in direction upon temperature changes (Fig. 2a). However, when phenol was added to 1.3 mM, they showed frequent changes in the direction of rotation at 23°C but rotated only in the counterclockwise direction at 32°C (Fig. 2b). The rotation mode of tethered cells only changed if the temperature was changed. Thus, the temperature-dependent changes in the swimming pattern observed in the phenol-treated HCB339(pCP31) cells were based on changes in the direction of flagellar rotation, indicating that the cells truly show a behavioral response.

Essentially the same results were obtained when HCB339 (pCP31) cells were treated with another Trg-mediated repellent, glycerol (14). Thus, phenol is not the only repellent permitting visualization of the thermoresponse in cells with Trg as the only receptor. From these results, we conclude that Trg has the ability to mediate thermal stimuli.

Characteristic properties of Trg as a thermoreceptor. Trg mediates a chemoresponse to galactose and ribose by interacting with the ligand-bound galactose- or ribose-binding

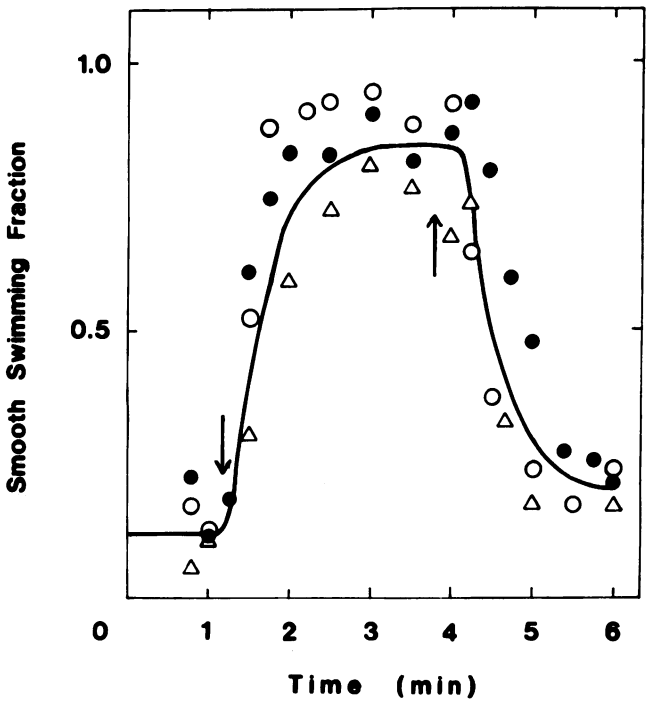

FIG. 3. Thermosensing by mutant Trg with altered galactose and ribose sensing. The response was measured in the presence of 1.3 mM phenol as described in the legend to Fig. 1. Symbols: ○, HCB339(pCP31) (*trg*$^+$); ●, HCB339(pCP32) (*trg-19*); △, HCB339 (pCP33) (*trg-8*).

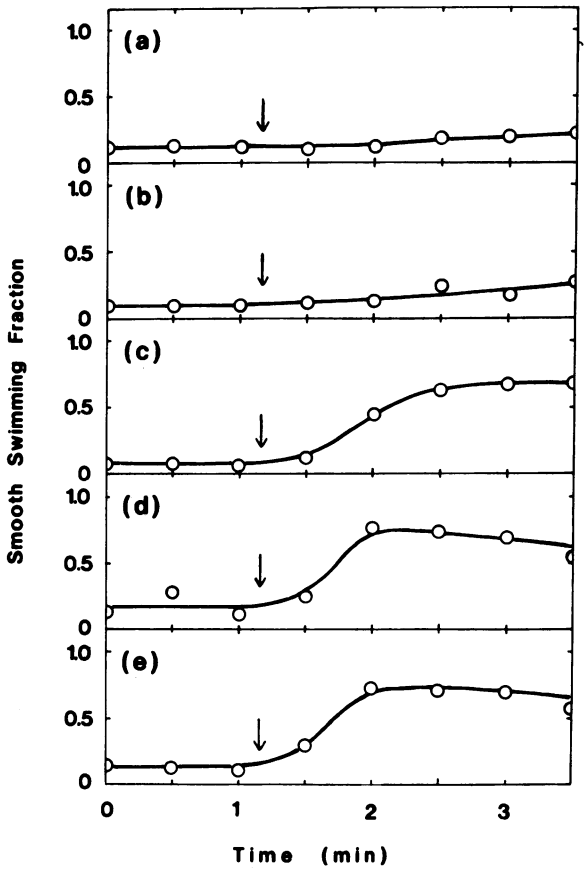

FIG. 4. Effect of starting temperature on the thermoresponse in phenol-treated HCB339(pCP31) (*trg*$^+$) cells subjected to a temperature change of fixed size. Cells were incubated for 3 min with 1.3 mM phenol at various starting temperatures, and at the time point indicated by the arrow, a temperature increase of 5.5°C was initiated. Starting temperatures were 15°C (a), 17.5°C (b), 19.5°C (c), 21.5°C (d), and 23.5°C (e).

proteins. Trg-8 and Trg-19 are mutant proteins with defects in both galactose and ribose sensing and in galactose sensing only, respectively (15). We previously reported that these mutant proteins showed normal sensing to phenol (20). Therefore, the thermosensing ability of Trg was examined in the presence of 1.3 mM phenol. Upon temperature changes between 23 and 32°C, HCB339 cells with Trg-8 or Trg-19 showed changes in their swimming pattern quite similar to those of cells with wild-type Trg (Fig. 3). Thus, as for phenol-sensing ability, the thermosensing ability is not affected by alterations in the galactose- and ribose-sensing abilities of Trg.

As described in the previous section, HCB339(pCP31) cells treated with 1.3 mM phenol showed apparently no adaptation to thermal stimuli. This suggests that under nonadaptive conditions, the absolute temperature but not the rate of temperature change is the thermal stimulus to the cells, as for chemoresponse in adaptation-deficient mutants (5). When the size of the temperature increase was fixed at 5.5°C but the starting temperature was varied, HCB339(pCP31) cells treated with 1.3 mM phenol showed a clear thermoresponse only when the final temperature was higher than 23°C (Fig. 4). These results indicate that under nonadaptive conditions, the absolute temperature itself is the thermal stimulus and temperatures higher than 23°C are effective in inducing a thermoresponse in cells containing only Trg.

The absence of adaptation is not due to the increased amount of Trg. AB1200 cells, which have a normal level of Trg only, also showed moderate tumbling at 23°C when 2.4 mM phenol had been added. The resultant tumbling continued for more than 10 min without any detectable adaptation, as in the chemoresponse to Trg-mediated attractants (3). The tumbling was reduced by increasing the temperature, and at 32°C, the cells showed mostly smooth swimming. The swimming pattern at 32°C did not change for more than 10 min, but tumbling was restored by a temperature decrease to 23°C.

Thermosensing ability of Tap. AW660, which has a normal level of Tap only (7), always showed smooth swimming and no change in swimming pattern upon temperature changes, as for cells with a normal level of Trg only. To increase the amount of Tap, HCB339 cells were transformed with pVB8, a plasmid carrying the *tap* gene under control of the *tac* promoter, and the amount of Tap in the cell was increased by cultivating the cells with 1 mM IPTG for 5 h.

Even after the amount of Tap was increased, HCB339 (pVB8) cells showed mostly smooth swimming and no detectable changes in the swimming pattern upon temperature changes between 20 and 32°C (Fig. 5a). To decrease the smooth-swimming bias of the cells, we added a Tap-mediated repellent, glycerol (14), to a final concentration of 7%. In contrast to the Trg-containing cells, glycerol-treated Tap-containing cells showed mostly smooth swimming at 20°C but vigorous tumbling at 32°C (Fig. 5b). Essentially the same results were obtained with cells treated with other Tap-

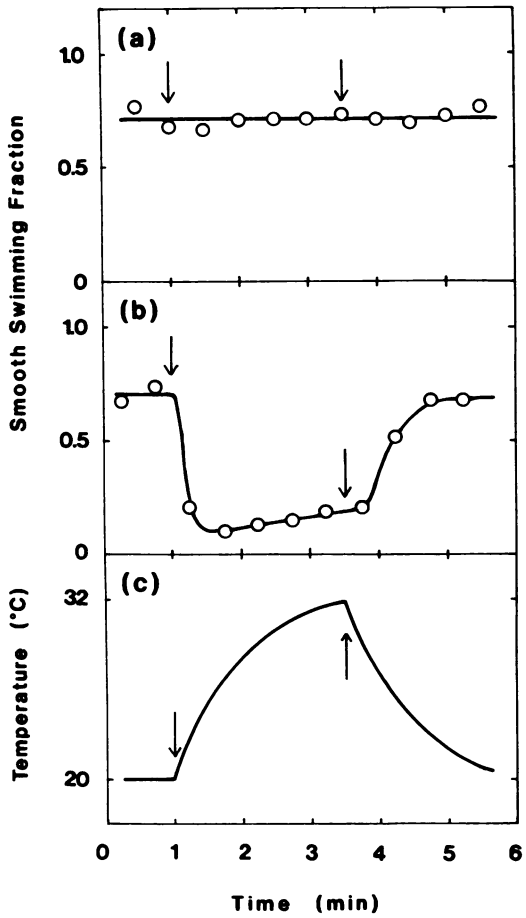

FIG. 5. Thermoresponse in HCB339(pVB8) (*tap*$^+$) cells. Cells in motility medium were incubated at 20°C for 3 min with or without 7% glycerol, and then the temperature was changed. (a) No glycerol; (b) 7% glycerol; (c) time course of temperature change. Arrows are described in the legend to Fig. 1.

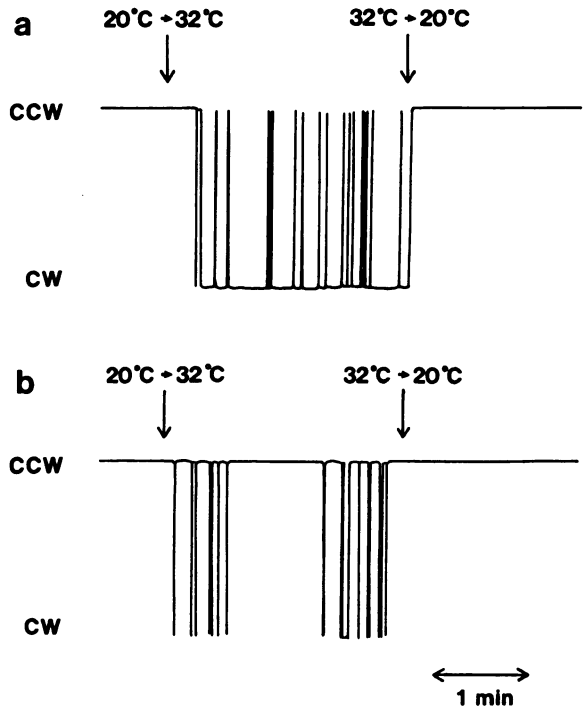

FIG. 6. Changes in rotational state of tethered cells of HCB339(pVB8) (*tap*$^+$) in response to thermal stimuli. At the time points indicated by arrows, the temperature was changed between 20 and 32°C. (a) Rotation of a tethered cell treated with 7% glycerol. (b) Rare example of a tethered cell that showed some clockwise rotation in the absence of glycerol. CCW, Counterclockwise; CW, clockwise.

mediated repellents, such as 34 mM acetate at pH 6.0 or 10 mM phenol (20). The tumbling frequency observed at 32°C gradually decreased with time, indicating that the cells showed slow adaptation to thermal stimuli.

Tethered cells of HCB339(pVB8) treated with 7% glycerol also responded to temperature changes, rotating exclusively counterclockwise at 20°C and mostly clockwise at 32°C (Fig. 6a). In the absence of glycerol, most cells rotated only counterclockwise at any temperature. However, a small fraction of cells showed some changes in the direction of rotation at 32°C but rotated only counterclockwise at 20°C (Fig. 6b).

Thus, we conclude that Tap, like Trg, has the ability to function as a thermoreceptor but that the sign of the signals elicited by Tap and Trg upon a thermal stimulus is opposite.

Thermoresponse of Tap- or Trg-containing cells in growth medium. HCB339(pVB8) cells produced small but definite swarms on a tryptone swarm plate, and the swarm size was maximum when the amount of Tap in the cells was increased by the addition of 1 mM IPTG to the plate. Although the cells were expected to tumble significantly for the production of definite swarms, the cells in motility medium showed almost no tumbling (Fig. 5a). However, we noticed that cells grown for 5 h with 1 mM IPTG showed considerable tumbling in the growth medium. Furthermore, when the smooth-swimming cells in motility medium were diluted into an equal volume of the supernatant obtained from a 5-h-old culture, we observed a clear restoration of tumbling. Thus, it is likely that some repellents for Tap are accumulated during cultivation.

Because HCB339(pVB8) cells in the 5-h-old culture showed considerable tumbling, their thermoresponse could be examined without further addition of exogenous repellent. We found that the cells showed tumbling at 32°C but mostly smooth swimming at 23°C. Under these conditions, the adaptation to the thermal stimulus was again slow. Thus, the thermoresponse observed in HCB339(pVB8) cells in growth medium is indistinguishable from that of the cells treated with a known Tap-mediated repellent.

In contrast to Tap-containing cells, HCB339(pCP31) cells in both motility medium and growth medium showed mostly smooth swimming and no response to thermal stimuli. Thus, it does not appear that any Trg-mediated repellent was detectably accumulated during the cell growth. Consistent with this, HCB339(pCP31) cells produced almost no swarms on the tryptone swarm plate.

DISCUSSION

By using cells with an increased amount of Trg or Tap, we showed that both chemoreceptors have the ability to sense thermal stimuli, as reported previously with Tar and Tsr (11, 13). This is not surprising because the amino acid sequences of all four chemoreceptors have a significant homology. In contrast to Tar and Tsr, however, cells with either Trg or Tap only had a strong bias toward smooth swimming even in

the presence of an increased amount of these receptors, and therefore, the thermoresponse in these cells was visualized only in the presence of their respective repellents. This peculiar property observed in these cells may have some relation to a severe defect in the adaptation ability of the cells not only to thermal stimuli as described here but also to the respective chemical stimuli as reported before (20). The nonadaptive property of these cells does not result from the increased amount of Trg or Tap, since cells with a normal level of Trg only also showed no adaptation (3).

Upon a temperature change, the Trg- and Tap-containing cells showed the opposite direction of thermoresponse even in the presence of a shared repellent such as glycerol. This clearly indicates that Trg and Tap but not other cellular components required for the sensory transduction for the repellents have the ability to sense temperature changes as thermal stimuli.

The swimming pattern of the Trg- and Tap-containing cells was determined simply by the temperature of the medium, indicating that these cells under nonadaptive conditions sense the absolute temperature as the thermal stimulus. The results are essentially the same as those reported previously with an adaptation-deficient mutant containing Tar and Tsr (5). Thus, it is concluded that the signaling states of all four receptors either for smooth swimming or for tumbling are determined by the absolute temperature, as in chemoreception, in which chemoreceptors detect the absolute concentration of chemoeffectors (2, 16). In Trg-containing cells, temperature changes below 23°C had almost no effect (Fig. 4), indicating that the effective change in the signaling state of Trg seems to occur at temperatures higher than 23°C. Similarly, the effective change in the signaling state of Tap seems to occur at temperatures higher than 20°C (Fig. 5). Thus, it is likely that these receptors have at least two conformations corresponding to two signaling states: a low-temperature form and a high-temperature form.

For Trg, the high-temperature form corresponds to the signaling state for smooth swimming and the low-temperature form corresponds to the signaling state for tumbling, as reported previously with Tar and Tsr (5). In contrast, the high-temperature form of Tap corresponds to the signaling state for tumbling and the low-temperature form corresponds to that for smooth swimming. Thus, if we assume that Trg, Tar, and Tsr correspond to the warm receptor, Tap corresponds to the cold receptor.

Although cells with Tap only showed small but definite swarms on a tryptone swarm plate, they showed mostly smooth swimming in motility medium. We found that the cells in a 5-h-old culture in tryptone broth showed significant tumbling and that the supernatant of the 5-h-old culture contained unidentified Tap-mediated repellent(s). Thus, although Tap itself has little ability to adapt to any stimulus, the presence of a Tap-mediated repellent appears to be helpful to produce a significant size of swarm. This phenomenon may be related to adaptationless taxis (17, 18).

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