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Sensory transduction in *Escherichia coli:* A requirement for methionine in sensory adaptation

(bacterial chemotaxis/stimulus response/regulation of tumbling/protein methylation)

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ABSTRACT **Chemotaxis of *E. coli* is a behavioral response to a change in the concentration of a stimulatory compound. The response is transient; thus, *E. coli* undergoes sensory adaptation.**

In this communication, we show that L-methionine is required by *E. coli* for adaptation to increases in the concentration of chemical attractants, but is not required for the maintenance of the adapted state. When the concentration of the attractant is lowered to its initial level, cells regain their sensitivity to the attractant. This process of deadaptation does not require methionine. We suggest that the methylation of a membrane protein, a reaction previously shown to be involved in chemotaxis [Kort, E. N., Goy, M. F., Larsen, S. H. & Adler J. (1975) *Proc. Natl. Acad. Sci. USA* 72, 3939–3943] underlies these phenomena.

Sensory stimuli are first detected by interactions of the stimuli with receptor molecules. Through a series of steps, these interactions ultimately lead to a change in the output of the receptor cell, a process known as sensory transduction. Although the first and last steps of this sequence have been extensively studied, the molecular nature of the intermediate events linking them is poorly understood. In this and a subsequent article (in preparation) we describe one of these intermediate events in a simple sensory process, bacterial chemotaxis.

Bacterial chemotaxis is a behavioral response to changes in the chemical composition of the environment. Although this behavior appears simple when compared to the activities of higher organisms, its fundamental properties are strikingly similar to those of eukaryotic receptor cells. Like these cells, bacteria first record environmental stimuli through a change in state of specific receptor molecules, in this case called chemoreceptors (2, 3). This change in state is converted by the transduction machinery into a perturbation of the membrane potential (4), which is accompanied by a behavioral response.

Unstimulated bacteria swim in smooth lines, interrupted at random intervals by a tumbling motion that abruptly alters the direction of travel (5). When presented with a stimulus, specifically a change in the concentration of a chemical in the environment, the cells respond with a change in the frequency at which tumbling occurs (5–8). For example, the addition of an attractant leads to suppression of tumbling (6, 7). However, the response is transient: the tumbling frequency eventually returns to the pre-stimulus level even though there is no further change in the concentration of attractant (6, 9, 10). This decline in response, known as sensory adaptation, is characteristic of the transduction machinery of many sensory systems (11). Upon removal of the attractant, however, adapted cells rapidly regain their sensitivity and will respond again if the attractant is added back (10). This process may be considered the inverse of adaptation, and we refer to it as deadaptation.

Our study of the mechanism underlying sensory transduction is greatly facilitated by the availability of a chemical probe. Several years ago, it was discovered that L-methionine is absolutely required for chemotaxis (12). Moreover, it was observed that in the complete absence of methionine cells are unable to tumble (12–14). This observation provides a simple explanation for the methionine requirement: if cells cannot tumble they cannot carry out chemotaxis. However, three lines of evidence suggest that methionine is necessary not only for the occurrence of tumbling but also for the regulation of tumbling in response to chemical stimuli. First, attractants appear to increase the rate at which bacteria consume methionine, which implies that it is utilized during transduction (14). Second, when subjected to conditions which partially deplete their internal pools of methionine, cells can still tumble and respond to a stimulus but require abnormally long periods of time to adapt (13, 14); this observation suggests that methionine may be involved in sensory adaptation. Third, methionine was found to participate in the methylation of a membrane protein and this reaction has been implicated in the chemotactic response (1).

We have now extended and unified these observations. In this article, we demonstrate that part of the transduction process, specifically sensory adaptation, has an absolute requirement for methionine. In contrast, we find that maintenance of an adapted state and the occurrence of deadaptation are methionine independent. We suggest a role for the methylation reaction in sensory transduction that accounts for the phenomena described here.

EXPERIMENTAL STRATEGY

In principle we can determine whether methionine is required for adaptation by first depriving a methionine auxotroph of this compound, then subjecting the cells to a stimulus, and finally, establishing whether or not adaptation occurs. However, this procedure leads to difficulty in assessing the state of adaptation of the cells. At present, the resumption of a normal frequency of tumbling after a stimulus is the only measurable property which reflects completion of adaptation. Because strains auxotrophic for methionine cannot tumble in its absence, we have resorted to an indirect method to obtain the necessary information.

Consider the following. A methionine auxotroph which has an external supply of methionine is stimulated by the addition of an attractant (see Fig. 1a). At first tumbling is suppressed and the cells swim smoothly, but after an interval, designated t_1, the cells have adapted, and resume tumbling at the pre-stimulus frequency. In a separate experiment (Fig. 1b), the external supply of methionine is removed and the cells are allowed to stand until spontaneous tumbling ceases. Methionine is now

Abbreviation: AiBu: α-aminoisobutyrate.

‡ The first two authors have contributed equally to this work and the order, therefore, was arbitrarily chosen.

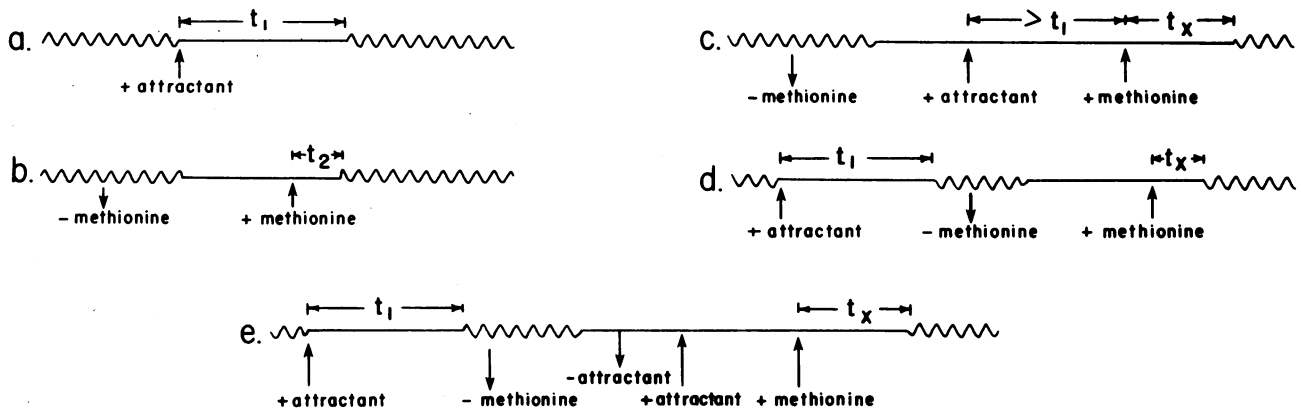

FIG. 1. Experimental strategies. The sawtooth lines (〰) represent motility with a normal frequency of tumbling and the smooth lines (—) motility with little or no tumbling. Attractant and methionine are added and removed as shown. See *Experimental Strategy*.

reintroduced. There is no immediate reaction to the addition and cells continue to swim smoothly, but after a relatively short interval, designated t_2, tumbling resumes.

We now combine the two experiments as follows (Fig. 1c). Methionine is removed and the cells are incubated until they have exhausted their intracellular pools of methionine and are no longer able to tumble. They are then subjected to an attractant stimulus. After a period of time greater than t_1 (the time necessary for the organisms to adapt to the stimulus in the presence of methionine), methionine is added and the time required for the cells to resume tumbling, designated as t_x, is measured. *If adaptation can occur in the absence of methionine, the cells will be fully adapted by the time methionine is readded and t_x will equal t_2. However, if all or part of the process of adaptation requires methionine, then that part cannot occur until this amino acid is added. Consequently t_x will be greater than t_2 and may approach t_1 in length.* In fact, it is possible for the interval t_x to be longer than t_1 because there may be some delay between the addition of methionine and restoration of the ability of the chemotactic machinery to begin the adaptation process.

We can also ask if methionine is required by *Escherichia coli* to maintain as well as to attain the adapted state. As in Fig. 1d, cells are stimulated with attractant in the presence of methionine and allowed to adapt. The external supply of methionine, but not the attractant, is now removed and the cells are incubated until spontaneous tumbling ceases. Methionine is readded and the time necessary for the resumption of a normal frequency of tumbling (t_x) is measured. If methionine is required to maintain the adapted state (recall that attractant has been present continuously), then deadaptation will occur after its removal and readaptation upon its readdition. Thus, t_x will be greater than t_2 and should approach or surpass t_1 in length. However, if methionine is not necessary for the maintenance of the adapted state, the cells will remain adapted despite its removal and t_x will equal t_2.

It is imperative that the lengths of the intervals t_1 and t_2 be made sufficiently different so as to avoid ambiguity in interpreting the results. The magnitude of t_2 is fixed. However, the length of t_1 is a function of the strength of the stimulus, and can be increased by increasing the potency of the attractant. Therefore, we chose a combination of attractants [10 mM L-aspartate + 50 mM α-aminoisobutyrate (AiBu)] for the stimulus. Each compound is used at a level more than 10-fold above the concentration which elicits half the maximal response so that the small amount of metabolism of the attractant that may occur during an experiment will not affect the duration of the response.

METHODS

Chemicals. L-threonine, L-leucine, L-histidine, L-methionine, L-aspartic acid, and AiBu were Calbiochem A grade. All other chemicals were reagent grade.

Bacteria. All experiments were performed with *E. coli* strain RP477*metF* (1). This strain is chemotactically wild type, Thr^-, Leu^-, His^-, and *metF*.

Growth and Manipulation of Bacteria. Cells were grown in tryptone broth (1) at 35° with rotary shaking to $OD_{590} = 0.5$ (approximately 5×10^8 bacteria per ml) and then transferred to a 30° room for the duration of the experiment. Ten milliliters of cells were washed twice by centrifugation with 4 ml of wash medium [10 mM potassium phosphate buffer at pH 7.0 containing 0.1 mM EDTA, threonine, leucine and histidine (0.1 mM each), and sodium D,L-lactate (10 mM)], and resuspended either in wash medium containing 1 μM methionine (for experiments shown in Fig. 1a–c) or wash medium containing 1 μM methionine and the desired attractant (for experiments shown in Fig. 1d and e). Cells resuspended with attractant were allowed to stand until fully adapted as judged by visual inspection of the tumbling frequency. Methionine was then removed from all cells by washing the cells twice in wash medium, always including attractant during the wash and final resuspension of the adapted cells (Fig. 1d and e). Final resuspension in all cases was to $OD_{590} = 0.3$. Cells were incubated with shaking in wash medium (Fig. 1a–c) or wash medium containing attractant (Fig. 1d and e) for 60 min to starve them for methionine. After starvation, all cells were diluted with the incubation media to $OD_{590} = 0.06$, again including attractant for adapted cells. They were then subjected to addition or removal of attractant and/or methionine, as illustrated in Fig. 1.

Assay. The time required for cells to resume tumbling was determined by the track counting procedure of Spudich and Koshland (9). In this assay, swimming cells are photographed so that paths (or tracks) of individual cells can be visualized and the fraction exhibiting tumbling can be determined at any given time. Exposures were 1 sec in duration and the photographs were taken with a stroboscopic light source set at 6 Hz so that each track consisted of six images. Experiments were carried out in a 30° room.

RESULTS

Measurement of Control Times t_1 and t_2. The interval t_1 (see Fig 1a and *Experimental Strategy*) was measured by adding attractant and determining the tumbling frequency at various times after the addition. As shown in Fig. 2a, t_1 is 9.5

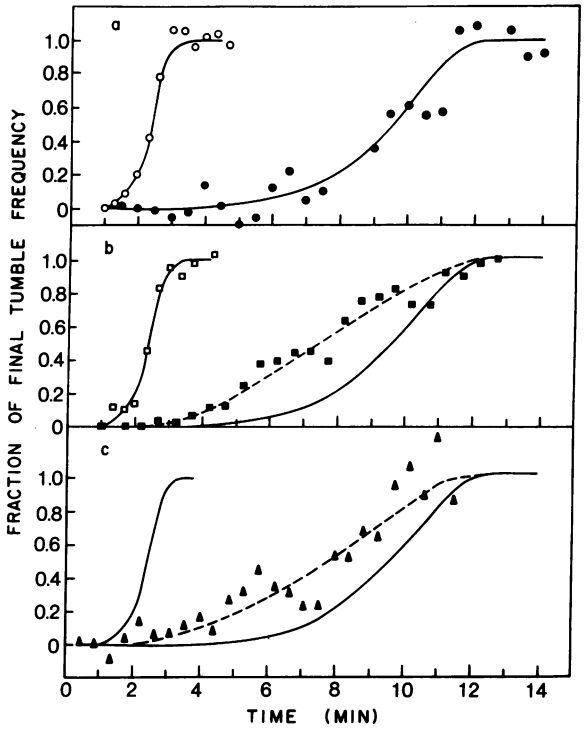

FIG. 2. Dependence of adaptation on the presence of methionine. Experiments are described in the *text*. Methionine starvation was ended by adding methionine to 0.1 mM final concentration. In all experiments, 0 time refers to the time at which the last addition was made. Times given in the text for t_1, t_2, and t_x are those at which the number of tracks with a tumble has reached half its final value. Typically, the final tumbling frequency is such that 50–60% of the tracks show a tumble. We find that about 10% of the tracks show tumbles even immediately after potent attractant stimuli. This probably represents cells mechanically incapable of swimming smoothly or which become transiently stuck to the slide and are incorrectly scored as tumbles. Therefore, we have subtracted this baseline value from the results presented. (a) t_1 (●—●) was determined by adding attractant to cells given methionine 30 min earlier (see Fig. 1a): t_2 (○—○) was measured by adding methionine to methionine starved cells (see Fig. 1b). (b) Methionine-starved cells were first given attractant and then 15 min later methionine was added (■---■) (see Fig. 1c). In another experiment (see Fig. 1d), cells were adapted to attractant prior to removal of methionine and then incubated in methionine-free media containing attractant (see *text*). After 60 min of incubation methionine was added (□—□). Solid lines are redrawn from (a). (c) Cells were adapted to attractant prior to the removal of methionine and then incubated in methionine-free medium containing attractant. After 60 min of incubation attractant was removed and then added back. Twenty-five min after readdition of attractant the cells were given methionine (▲---▲) (see Fig. 1e). Solid lines are redrawn from (a).

min when cells are stimulated with a mixture of aspartate and AiBu. The interval t_2 (see Fig. 1b and *Experimental Strategy*) was measured similarly by adding methionine and found to be 2.4 min (Fig. 2a).

Methionine Is Required for Adaptation. The experiment demonstrating this requirement is illustrated in Fig. 1c. Cells were starved for methionine and then stimulated with aspartate and AiBu as the attractants. After 15 min (1.7 times t_1), methionine was added and t_x, the period of time necessary for tumbling to resume one half its normal frequency, was measured. Under these conditions, we find t_x to be 7.5 min in length (Fig. 2b). This value is much greater than t_2 and approaches t_1 in magnitude, a result consistent with the conclusion that at least part of the adaptation process requires methionine and cannot occur normally in its absence. However, t_x is somewhat shorter than t_1, which implies that another part of the adaptation process does occur in the absence of methionine.

Methionine Is Not Required to Maintain the Adapted State. Do cells which have adapted to a stimulus in the presence of methionine maintain that state of adaptation when methionine (but not the stimulating attractant) is removed? As illustrated in Fig. 1d, cells are stimulated and allowed to adapt in medium containing methionine. Subsequently methionine is removed and the cells are incubated for 60 min in the presence of the attractant alone. Methionine is then added back and t_x is measured. If the cells can maintain the state of adaptation despite the removal of methionine, then t_x will equal t_2; if not, then the organism must readapt upon addition of methionine and t_x will approximate t_1. As seen in Fig. 2b, t_x is identical to t_2; this demonstrates that the presence of methionine is not required to maintain the adapted state.

Methionine Is Not Required for Deadaptation. Having shown that methionine is required to attain but not to maintain the adapted state, we can now ask whether its presence is necessary for deadaptation when the stimulatory chemical is removed (Fig. 1e). Cells are stimulated and allowed to adapt in medium containing methionine. Methionine, but not attractant, is removed and the cells are incubated for 60 min. Attractant is then washed away and subsequently readded. This procedure takes several minutes, and, because *E. coli* normally requires less than 5 sec to deadapt (10), the interval between removal and readdition should be more than sufficient to allow deadaptation. As before, methionine is reintroduced and t_x is measured. If deadaptation can occur in the absence of methionine, then the cells will have to readapt to the attractant so that t_x will approximate t_1. However, if *E. coli* requires the presence of methionine to deadapt, the organism will remain adapted despite the removal of the attractant and no stimulation will occur upon reintroduction of the attractant. Thus t_x will equal t_2. We find t_x to be approximately 7.9 min in length (see Fig. 2c), and this demonstrates that deadaptation does not require the presence of methionine.

Properties of the Methionine Requirement. The results presented above (experiment of Fig. 1c) support the idea that part of the adaptation process requires methionine and does not proceed normally when methionine is removed. However, an objection can be raised: it is possible, in principle, that the similarity between t_x and t_1 is fortuitous and arises from some unknown effect of the presence of the attractant *per se* on the mechanisms that normally make methionine available for tumbling. For example, the presence of the attractant might hinder the uptake or metabolism of methionine. The possibility of such an artifact can be ruled out by the demonstration that methionine is not required to maintain the adapted state (experiment of Fig. 1d). The cells in this experiment have attractant present when methionine is readded, just as do the cells in the experiment of Fig. 1c. However, they have not been stimulated in the absence of methionine. Under these conditions t_x equals t_2 (Fig. 2b), thereby proving that the mere presence of the attractant does not cause t_x to be longer than t_2. Thus, it is a change in concentration and not simply the presence of the attractant that causes t_x to approximate t_1. Furthermore, we can relate the magnitude of t_x directly to the chemotactic potency of the stimulus by varying t_1 through the use of different attractants. As seen in Fig. 3, the quantity t_x/t_1 is constant for all values of t_1, making a fortuitous result exceedingly unlikely.

Is methionine necessary merely to increase the rate of adaptation, or is its requirement absolute? By varying the interval

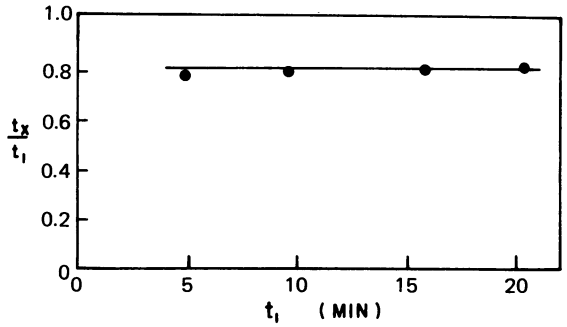

FIG. 3. Dependence of t_x/t_1 on t_1. t_x was measured as in Fig. 2b by adding the appropriate attractant and then, after a period of time greater than t_1, adding methionine. t_1 was measured for each attractant as described in Fig. 2a. Attractants used: 50 mM AiBu (t_1 = 4.8 min); 10 mM aspartate + 50 mM AiBu (t_1 = 9.5 min); 0.1 mM serine (t_1 = 15.5 min); 0.17 mM serine (t_1 = 20.3 min).

between the addition of the attractant and the addition of methionine (Fig. 4), we show t_x/t_1 to be independent of the length of this interval. Thus, a major part of the adaptation process does not occur at all in the absence of methionine and the requirement must be absolute.

It should be noted, however, that t_x is always somewhat shorter than t_1. It appears that adaptation is composed of at least two subprocesses, one methionine-dependent and the other methionine-independent. Furthermore, because t_x/t_1 is constant regardless of the length of t_1 (Fig. 4), the duration of both subprocesses is proportional to the potency of the chemotactic stimulus.

DISCUSSION

When a rapidly adapting sensory system, as described here for bacteria, adapts fully to a stimulus, the output of that system returns to pre-stimulus levels. For *E. coli*, this entails restoration of the tumbling frequency to the level which existed prior to addition of attractant. However, the transduction machinery itself does not return to the "ground" state but remains in an "excited" state for as long as the attractant is present. If this were not so, the response would depend only on the final concentration of attractant reached and would be independent of the concentration present initially. Thus, the length of response to the addition of sufficient AiBu to give 50 mM final concentration would be the same regardless of whether the cells had been previously adapted to 0, 0.5, or 5 mM of the same compound. Because this is clearly not true (9, 10), the presence of

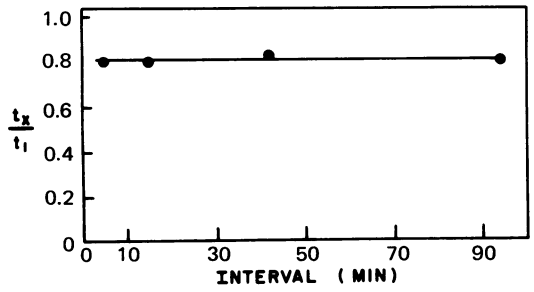

FIG. 4. Dependence of t_x/t_1 on the interval between additions of attractant and methionine. Values for t_x were determined as described in Fig. 2b by adding attractant (10 mM aspartate + 50 mM AiBu) and then adding methionine the indicated number of min later. t_1 was measured as described in Fig. 2a. These data have been normalized due to slight (10%) day-by-day variations in the value of t_1.

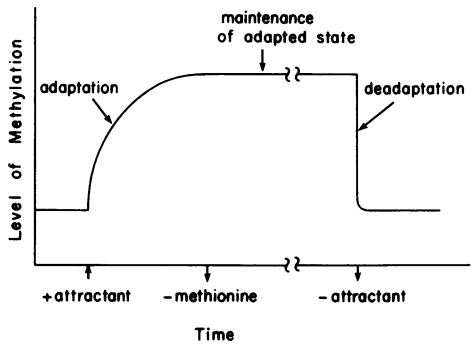

FIG. 5. Model for the role of methionine in adaptation. Ordinate shows the extent of methylation of a component of the transduction machinery. Methionine and attractant are added and removed as indicated.

attractant must effect a sustained change in the transduction machinery despite the return of the tumbling frequency to its pre-stimulus level. This property must be considered in any mechanism proposed for sensory transduction.

In this communication, we establish that methionine or some derivative of methionine is necessary for the operation of the machinery which transduces chemical stimuli into behavioral responses. In particular, we find that methionine is necessary for adaptation to an attractant stimulus but is required neither to maintain a state of adaptation once it has been reached nor for the deadaptation process that occurs when attractant is removed. What is the biochemical mechanism underlying these phenomena? We suggest that methionine is necessary to alter the state of the transduction machinery by methylating a component of that machinery. Numerous examples exist in nature of similar covalent modifications that control enzymatic activity and specificity and hence regulate many of the metabolic processes of the cell (15). This possibility is supported by earlier work implicating the methylation of a membrane protein in the chemotactic response (1).

Our model for the function of methionine in the adaptation process is presented in Fig. 5. In the unstimulated or ground state some component of the transduction machinery is methylated to a low basal level. Upon addition of attractant the extent of methylation increases until a new level, representing the adapted state, is reached. The extent of methylation reflects the absolute concentration of the stimulatory compound as detected by the chemoreceptors. Thus, adaptation requires the presence of methionine. In the presence of methionine, the methyl linkages are not stable, and the methyl groups undergo continual turnover. This reaction may account for the use of methionine by the transduction machinery (14). Furthermore, the rate of turnover is proportional to the level of methylation, and therefore, as previously reported (14), consumption of methionine will be increased by the presence of attractants. Turnover requires the presence of methionine, and ceases in its absence. Thus, when methionine is removed there is no demethylation, so that maintenance of the "excited" level, and hence the state of adaptation, requires only the continued presence of the stimulatory compound, and not that of methionine. However, even in the absence of methionine, demethylation to the basal level occurs upon removal of the stimulatory compound. Thus, deadaptation does not require the presence of methionine.[§]

[§] We have not attempted to account for the observation that methionine is required for spontaneous tumbling in unstimulated cells. This point will be discussed elsewhere (M. F. Goy, M. S. Springer, and J. Adler, manuscript in preparation).

This model is supported in detail by biochemical evidence. Specifically, the methylation reaction described earlier (1) exhibits exactly the properties discussed in the preceding paragraph (M. F. Goy, M. S. Springer, and J. Adler, manuscript in preparation). However, this represents only part of the adaptation mechanism. The model requires additional components to detect whether the level of methylation is constant or changing and thus determine whether a behavioral response is appropriate. Several two-process mechanisms have been proposed for sensory adaptation (6, 10, 16), and by extending our model to include such a mechanism we can fulfill this requirement. In this form, the model illustrates the intimate relationship between sensory adaptation and sensory transduction. We feel that the two phenomena are really the same process.

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