Expression of $\sigma^{54}$ (ntrA)-Dependent Genes Is Probably United by a Common Mechanism

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$\sigma^{54}$ IS REQUIRED TO TRANSCRIBE A DIVERSE SET OF GENES

In addition to the most abundant sigma factor, both gram-negative and gram-positive eubacteria employ alternative sigma factors that confer different promoter specificities on the core form of RNA polymerase (reviewed in reference 49). Several bacteriophages that infect each of these groups also encode sigma factors. Some of the alternative sigma factors allow transcription of genes whose products contribute to a common physiological response. For example, $\sigma^{32}$ of enteric bacteria allows transcription of genes whose products are needed for protection from heat shock and certain other stresses (42, 76). Sigma F of enteric bacteria (2) and $\sigma^{86}$ of Bacillus subtilis (47, 48), both of which confer the same promoter specificity on core polymerase, allow transcription of genes whose products are required for motility and chemotaxis. Some sigma factors allow transcription of genes whose products are required at a precise time, for example, at a particular time after bacteriophage infection (40, 64) or during the sporulation process of B. subtilis (73, 77). $\sigma^{54}$ differs from other alternative sigma factors in that it is needed for transcription of genes whose products have diverse physiological roles (9, 27, 62, 111).

$\sigma^{54}$ (encoded by ntrA [glnF, rpoN]) was identified as a positive regulatory factor needed for expression of the gene encoding glutamine synthetase, glnA, in enteric bacteria (39; reviewed in reference 74). It was later found to be required for expression of other genes whose products function in the assimilation of nitrogen. For example, $\sigma^{54}$ is required for transcription of genes encoding amino acid transport components and degradative enzymes (1, 117; reviewed in reference 78) and genes whose products are needed for biological nitrogen fixation (75; reviewed in references 28 and 44). It is required for transcription of the nitrogen fixation (nif) genes from a number of bacteria, including Klebsiella pneumoniae (28, 44), Rhodobacter capsulatus (63a, 79; reviewed in reference 45), and members of the genera Azotobacter (114, 125), Azospirillum (99), Rhizobium (44, 111), and Bradyrhizobium (31).

Recently, it has become apparent that $\sigma^{54}$-holoenzyme transcribes genes whose products have different physiological functions (Table 1). Examples of such genes are (i) the delta gene of rhizobia (111), which encodes a transport component for dicarboxylic acids; (ii) genes on the TOL (toluene) plasmid of Pseudomonas putida that encode proteins required for catabolism of toluene and xylene (27, 57, 101); (iii) genes encoding two of the components of a formate-degradative pathway in Escherichia coli (9); (iv) genes encoding hydrogenases responsible for the oxidation of molecular hydrogen in Alcaligenes eutrophus and Pseudomonas facilis (52, 105, 106, 127; J. Warrelmann, D. Romerma, and B. Friedrich, personal communication); (v) genes encoding the hook and filament proteins of Caulobacter flagella (85, 89, 94); and (vi) genes encoding pilins in Pseudomonas aeruginosa (59, 61) and Neisseria gonorrhoeae (84) that allow these organisms to adhere to human epithelial cells (121, 128, 131). In the first four cases and in the case of the Pseudomonas pilin gene, the requirement for $\sigma^{54}$ has been demonstrated directly by showing that transcription does not occur in mutant strains that lack this sigma factor (9, 27, 59, 106, 111; Table 1). In the latter two cases, the evidence for dependence on $\sigma^{54}$ is less direct (Table 1). Although it has been proposed that transcription from the major promoter for the puf photosynthetic operon of Rhodobacter capsulatus is $\sigma^{54}$ dependent (4), we think that this is unlikely because the proposed promoter lacks the minimal conserved features common to $\sigma^{54}$-dependent promoters (see below; reviewed in references 28 and 44). Moreover, disruption of the nifA gene, which was recently demonstrated to encode a $\sigma^{54}$ homolog in Rhodobacter capsulatus (63a), does not cause defects in photosynthesis (45; R. Kranz, personal communication).

CONCLUSIONS

$\sigma^{54}$-Holoenzyme ($\sigma^{54}$ associated with core RNA polymerase) recognizes and binds to the major glnA promoter. (All promoters that are recognized by $\sigma^{54}$-holoenzyme are characterized minimally by a conserved GC doublet that lies

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| Gene  | Gene product(s) | Organisms\(a\) | Activator | Evidence\(d\) | Selected reference(s)\(f\) | Comments |
|-------|----------------|----------------|-----------|--------------|----------------------------|----------|
| glnA  | Glutamine synthetase | Enteric bacteria, including *Escherichia coli*, *Salmonella typhimurium*, and *Klebsiella pneumoniae*; *Pseudomonas aeruginosa* | NTRC       | (i), (iii), (v), (vi), (vii)| 38, 39, 51, 54, 66, 74, 75, 78 | In *Azotobacter vinelandii*, synthesis of glutamine synthetase appears to be constitutive and is normal in ntrA" mutants (114, 125). Similarly, synthesis of glutamine synthetase is normal in *ntrC"* (ntrA") mutants of *Alcaligenes eutrophus* (105) and in *nifK4"* (ntrA") mutants of *Rhodobacter capsulatus* (45; Kranz, personal communication). In *Rhizobium meliloti* and *Agrobacterium tumefaciens*, synthesis of glutamine synthetase II (the plantlike or eucaryotic form) appears to be under \(\sigma^{34}\) control because *ntrC"* mutants lack this enzyme (113). By contrast, synthesis of glutamine synthetase I (the bacterial enzyme, product of the glnA gene) is not affected in *ntrC"* mutants (113). In *Bradyrhizobium japonicum*, synthesis of glutamine synthetase I does not appear to be \(\sigma^{34}\) dependent because there is not a \(\sigma^{34}\)-dependent promoter upstream of the glnA transcriptional start site (18). |
| nas   | Assimilatory nitrate and nitrite reductases | *Klebsiella pneumoniae*; *Azotobacter vinelandii*; *Rhizobium meliloti*; *Agrobacterium tumefaciens*; *Azospirillum brasilense*; *Alcaligenes eutrophus* | NTRC       | (vi) | 17 | Since promoter sequences for nas genes (17) have not yet been identified, the possibility remains that the \(\sigma^{34}\) requirement for their transcription is indirect. |
| *nifH*, *nifD*, *nifK* | Dinitrogenase (*nifHDK*) and dinitrogenase reductase (*nifH1*), proteins required for synthesis of the iron-molybdenum cofactor of dinitrogenase (e.g., *nifDENV*) and for processing of dinitrogenase reductase to the active form (e.g., *nifM") | *Klebsiella pneumoniae*; *Azotobacter vinelandii*; *Azospirillum brasilense*; *Rhodobacter capsulatus* | NIFA       | (i)-(iii), (v)-(vii) | 75, 130; reviewed in ref. 28 and 44 | NIFA is inactivated by oxygen. In *K. pneumoniae*, this inactivation appears to require the NIFP product (30, 81), whereas in *Rhizobium* (8, 53) and *bradyrhizobium* (32, 33) it does not require a nif-specific product and in this sense appears to be direct. |
| *nifA* | Activator of transcription for *nif* and some *fix* genes (see below); also required for the formation of determinate symbiosis (production of normal, healthy nodules in normal number) by *Bradyrhizobium japonicum* (31) and probably by *Rhizobium meliloti* (123, 133) | *Klebsiella pneumoniae*; *Azospirillum brasilense*; *Rhodobacter capsulatus*; *Bradyrhizobium japonicum* | NTRC       | (i)-(vii) | 3, 130; reviewed in ref. 28 and 44 | Interestingly, transcription of *nifA* is dependent on \(\sigma^{34}\) in some organisms but not others. In *K. pneumoniae*, and probably in *A. brasilense* and *R. capsulatus*, *nifA* transcription is dependent on \(\sigma^{34}\) and NTRC and occurs under nitrogen-limiting conditions. Transcription of *nifA* in *B. japonicum* under symbiotic conditions also appears to be \(\sigma^{34}\) dependent, but NTRC is not the activator; neither the activator nor the relevant physiological signal for *nifA* transcription is known (124). In *A. vinelandii*, a free-living diazotroph, *nifA* transcription is not dependent on NTRC, and it is not known whether it is \(\sigma^{34}\) dependent (115, 125). |
### Expression of $\sigma^{44}$-Dependent Genes

| Gene | Function/Details | Promoter/Target | References |
|------|----------------|-----------------|------------|
| fixABCX | The fixX gene of *Rhizobium trifoli* encodes a ferredoxin-like protein (55). Although the products of the other genes are not known, there is circumstantial evidence that they are involved in electron transport to dinitrogenase in aerobic diazotrophs, presumably under microaerobic conditions (discussed in ref. 43). | *Rhizobium melloti*<br>Bradyrhizobium japonicum | NIFA (i, vi)<br>NIFA (i, vi) | 123; reviewed in ref. 44<br>43 | fixABCX is a single operon in *R. melloti*. In *B. japonicum*, fixA and fixBC constitute separate operons (43). |
| dctA | C4-dicarboxylate transport component; required for growth on dicarboxylic acids in free-living state and apparently also symbiotically (110; reviewed in ref. 107) | *Rhizobia* (Rhizobium leguminosarum, *Rhizobium melloti*, *R. trifoli*)<br>DCTD (free living); unknown (symbiotic)<sup>9</sup> | NIFA (i, iii), (vi), (vii)<br>Alcaligenes eutrophus<br>(see Comments) (iii) | 107-109, 111; I. Jiang, B. Gu, L. M. Albright, and B. T. Nixon, J. Bacteriol., submitted for publication | The nomenclature is for *Rhizobium* spp. In the free-living state, induction apparently occurs in response to dicarboxylates external to the cell. Although the DCTB protein, which would be membrane bound on the basis of its amino acid sequence, is the primary sensor for the system; the DCTA protein itself appears to be involved because a haploid *dctA-lacZ* fusion is expressed at high levels in the absence of inducer (107, 109). Mutants *hno*<sup>−</sup> (motA<sup>−</sup>) of *A. eutrophus* have defects in dicarboxylate transport. |
| melA | Tyrosinase, required for synthesis of the pigment melanin | *Rhizobium leguminosarum*<br>biovare phaseoli (the melA gene is found only in this biovar) | NIFA (iv), (vi) | 46 | It is speculated that this tyrosinase may play a role in detoxification of plant phenolic compounds during senescence of root nodules (46). |
| *fdhF* and *hyd-3* (unlinked) | The selenoprotein of formate dehydrogenase F and the gas-evolving hydrogenase isozyme 3. These proteins are components of formate hydrogen lyase, which degrades formate to CO<sub>2</sub> and H<sub>2</sub> under anaerobic conditions (and in the absence of alternative electron acceptors such as nitrate). | *Escherichia coli*, probably<br>*Salmonella typhimurium*<br>Unknown; requirement for cis-acting sequences upstream of the *fdhF* promoter (between −144 and −100).<br>Upstream sequences mediating formate induction could not be separated physically from those mediating oxygen or nitrate repression (10). | NIFA (i)-(iii), (vii) | 9, 10 | Although the physiological function(s) of the formate hydrogen lyase pathway is not clear, a primary function may be decarboxylation of formate (reviewed in ref. 119). |
| hox | Soluble and membrane-bound hydrogenases that oxidize molecular hydrogen as a source of energy and/or reducing power. Oxygen is the terminal electron acceptor. | *Alcaligenes eutrophus*<br>HOXA, the product of a gene in the hoxC cluster; induction under conditions of energy limitation | NIFA (i, iii) | 30, 35, 36, 52, 71, 105, 106; Warrelmann, Romerma, and Friedrich, personal communication | The nomenclature is for the genus *Alcaligenes*. *hox* genes are clustered on a 450-kilobase megaplasmid, whereas the hno (motA<sup>−</sup>) gene is chromosomal. Expression of *hox* genes encoding a membrane-bound hydrogenase in *P. facilis* is $\sigma^{44}$ dependent. |

Continued on following page
| Gene | Gene product(s) | Organism(s) | Activator | Evidence | Selected reference(s) | Comments |
|------|----------------|-------------|-----------|----------|-----------------------|----------|
| **Structural gene encoding pilin (designated pilA in *Pseudomonas aeruginosa* and pilE in *Neisseria gonorrhoeae*).** | A pilin of the N-methylphenylalanine class, the monomeric subunit of pil; synthesized as a precursor called prepilin | *Pseudomonas aeruginosa*<br>*Neisseria gonorrhoeae*<br>*Bacteroides nodosus*<br>*Moraxella bovis* | Unknown. The only condition known to result in greatly increased transcription of the pilA gene of *P. aeruginosa* appears to be the inability to repress (depolymerize) pil (60). | (i), (iii), (vii) | 59-61, 97a, 131 | Gonooccocal pil greatly enhance adherence of the bacterium to host epithelial cells (121, 128), and this increased adherence apparently accounts for the increased virulence of piliated strains (67, 68). Similarly for the pili of *P. aeruginosa* (131). |
| **xylCAB and xylS** | Enzymes (upper pathway) that catabolize toluene to benzene and xylene to the corresponding methylbenzoates (xylCAB operon) and activator of transcription for the operon encoding enzymes (lower or meta pathway) that further catabolize benzene to methylbenzoates to products that can enter the tricarboxylic acid cycle (xylS) | *Pseudomonas putida* (TOL plasmid pWWO) | XYL; induction by toluene, xylene, and their alcohol catabolic products | (i), (iv), (vi) | 27, 34, 56-58, 62, 91, 101, 118, 132 | These pathways are being manipulated for the degradation of toxic aromatic compounds (102). |
| **Gene encoding carboxypeptidase G2** | Carboxypeptidase G2, which hydrolyzes the C-terminal glutamate moiety from folic acid and analogs such as methotrexate | *Pseudomonas putida* | Unknown. When the organism is grown in the presence of glutamate and folate as carbon sources, enzyme synthesis does not occur until the glutamate has been depleted (unpublished results cited in ref. 87). | (i) | 86, 87 | This enzyme is used in cancer chemotherapy (86). |
| **fbG operon (fliK, flaN, fliK, figl)** | Hook and filament proteins of the flagellum | *Caulobacter crescentus* | Unknown; requirement for upstream sequences (designated ftr) at fbG (at -- 100); similar sequences also occur upstream (at -- 100) of both flik and figl. | (i), (ii), (v), (vii) | 21, 85, 89, 90, 94, 97 | The nomenclature is for *C. crescentus*. A single flagellum is synthesized by the *Caulobacter* predivisional cell at the pole opposite to the stalk; it is inherited by the daughter swarmer cell (reviewed in ref. 20 and 92). Transcription of each of the genes listed depends on expression of flagellar regulatory and structural components in a hierarchical manner and occurs at a specific time in the cell cycle (21, 85, 97). It is not yet clear whether activators for fliK-holoenzyme, which would be expected to play a role in the regulatory hierarchy, also play a role in cell cycle control or whether this control occurs by an independent mechanism. It is not clear whether fliK (sigma F), which is required for transcription of any of the *Caulobacter* flagellar or chemotaxis genes in *E. coli* and *E. coli* (2, 47, 48), is required for transcription of any of the *Caulobacter* flagellar or chemotaxis genes. Mutants *spoN* ("ntrA") of *P. aeruginosa* lack flagella and lack flagellin as detected immuno-logically. |
EXPRESSION OF \(\sigma^{4}\)-DEPENDENT GENES

between 11 and 14 base pairs [bp] upstream of the transcriptional start and a conserved GG doublet that lies exactly 10 bp farther upstream [reviewed in references 28, 44, and 62; D. Popham and S. Kustu, manuscript in preparation].) However, recognition complexes between \(\sigma^{4}\)-holoenzyme and the glnA promoter (closed complexes) are nonproductive transcriptionally because the DNA remains double stranded (74, 100, 116). Initiation of transcription requires an activator protein called NTRC, also known as NRI (encoded by \(ntrC\) [glnG]) [reviewed in reference 66]. NTRC binds upstream of the promoter to sites that have the properties of transcriptional enhancers (95, 103). It catalyzes the isomerization of closed complexes between \(\sigma^{4}\)-holoenzyme and the glnA promoter to transcriptionally productive open complexes in which the DNA strands are locally denatured in the region of the transcription start site (100, 116). The isomerization reaction requires ATP (100, 116).

As is true for glnA, transcription of a number of other \(\sigma^{4}\)-dependent genes requires an activator. For example, transcription of \(nif\) genes in a variety of bacteria requires the activator protein NIFA (5, 13, 31, 79, 115, 123; reviewed in reference 44). Similarly, transcription of the \(dctA\) gene is dependent on DCTD (108, 109), transcription of the \(xylCAB\) and \(xylS\) genes requires XYLXR (references 27, 57, 101, and 118 and references cited therein), and transcription of the \(hox\) (hydrogen oxidation) genes is dependent on the product of a gene in the \(hox\) locus (30, 36, 71, 106).

Like NTRC, other activators of \(\sigma^{4}\)-holoenzyme (NIFA [6, 7, 11, 14, 15, 88; reviewed in reference 44] and DCTD [B. T. Nixon, personal communication]) bind to sites located at least 80 bp away from the promoters they regulate. In several systems for which the specific activator has not yet been identified (\(E. coli\) formate hydrogen lyase, \(C. crescentus\) hook protein, and \(P. aeruginosa\) pilin), a requirement for upstream sequences that could serve as activator binding sites has nevertheless been demonstrated (10, 89, 90, 97a). It therefore seems likely that transcription by \(\sigma^{4}\)-holoenzyme will prove to depend on an activator in these other cases, and it is an attractive speculation that this will be true in every case (9, 100, 111).

The activator proteins whose sequences are known (NTRC, NIFA, DCTD, and XYLXR) show a high degree of sequence similarity within their central domains (domain D of reference 29), each of which contains a putative ATP-binding site (regions 1 and 3 in reference 37; 58, 109). The central domain spans \(\sim\)240 amino acid residues [16, 29], of which 30% are identical in each of the four activators—NTRC and NIFA from \(K. pneumoniae\), DCTD from \(R. leguminosarum\), and XYLXR from \(P. putida\) [58, 109].

Eleven independent mutant forms of \(Salmonella\) NTRC that are specifically defective in the ability to activate transcription (129) have amino acid substitutions within the central domain; moreover, the substitutions affect residues that are identical in the four activators, including residues that constitute the proposed ATP-binding site (D. Weiss and S. Kustu, unpublished data). These results indicate that the central domain of NTRC is specifically required for formation of open complexes between \(\sigma^{4}\)-holoenzyme and the glnA promoter. Sequence similarity among the activators is consistent with the simple hypothesis that they all function in a similar manner. Interestingly, truncated forms of the NIFA protein from \(Rhizobium\) \(meliloti\) that retain only the central domain appear to retain the ability to activate transcription from the \(Rhizobium\) \(nifH\) promoter (53).
FUNCTION OF ACTIVATOR PROTEINS IS CONTROLLED BY DIFFERENT PHYSIOLOGICAL SIGNALS AND DIFFERENT MECHANISMS

Control of transcription at σ^4-dependent promoters appears to be accomplished primarily by modulation of the activity state of activator proteins (see below; Table 1) and their abundance (57; reviewed in references 28, 44, and 74). The amount of σ^4 does not vary much, at least under different conditions of nitrogen availability (19, 26, 83, 111). Each activator allows σ^4-holoenzyme to initiate transcription in response to a distinct physiological signal, such as (i) limitation of combined nitrogen (NTRC as activator [reviewed in references 66, 78]); (ii) low oxygen tension (NIFA as activator [8, 25, 28, 32, 33, 44, 50, 72, 81, 126]); (iii) availability of dicarboxylic acids, presumably external to the cell (DCTD as activator [107, 109]); (iv) availability of toluene, xylene, or their alcohol catabolic products (XYLR as activator [34, 57, 91, 101, 132]); (v) energy limitation (product of a gene in the boxC locus as activator [30, 35, 36, 71]); and (vi) presence of formate under anaerobic conditions (activator unknown [9, 10]). It will be of interest to understand the nature of the signals that regulate transcription of the Caulobacter hook and flagellin genes, expression of which is controlled by a complex regulatory hierarchy (12, 20, 21, 22, 92, 97) and occurs in a cell cycle-dependent manner (85, 97).

Function of the activators themselves is apparently controlled by a variety of mechanisms. NTRC is synthesized in an inactive form, and its activity is regulated positively and negatively by phosphorylation and dephosphorylation, respectively, of its NH_2-terminal domain (65, 93). This domain (~120 amino acids) appears to control the ability of the central domain to activate transcription by σ^4-holoenzyme (65, 96, 112). The degree of phosphorylation of NTRC is increased under nitrogen-limiting conditions. NTRC is a member of a two-component regulatory system (70, 96, 112) and is phosphorylated by its partner NTRB, also known as NRRI (encoded by ntrB [glnL]). Like NTRC, the DCTD protein of rhizobia is a member of a two-component regulatory system, and therefore it is probably activated by phosphorylation by its partner DCTB (109); phosphorylation is thought to increase in response to availability of external dicarboxylates. Unlike NTRC and DCTD, the NIFA protein is apparently synthesized in an active form (8, 13, 33). In K. pneumoniae, it is inactivated by the NIFL protein (mechanism unknown) in response to molecular oxygen or combined nitrogen (50, 81; reviewed in references 28 and 44). In Bradyrhizobium japonicum (32, 33) and Rhizobium meliloti (8), NIFA is inactivated at high oxygen tensions by a mechanism(s) that does not involve NIFL or any other nif-specific protein. Finally, the XYL protein of P. putida appears to be activated directly by binding low-molecular-weight substrates of the xylene catabolic pathway (27, 34, 57, 91, 101, 132). Thus, function of the activator proteins that control transcription by σ^4-holoenzyme is highly regulated. For different activator proteins, it is regulated by different mechanisms.

THE SEQUENCE OF σ^4 HAS UNIQUE FEATURES

As discussed above, σ^4 confers on core RNA polymerase the ability to bind specifically to a promoter (a minimal definition of a sigma factor) but it apparently does not confer the ability to form open complexes. In this regard it is interesting that σ^4 shows little amino acid sequence similarity to other sigma factors (63, 80, 82, 111) (of which ~15 have now been identified [49]), whereas these share several regions of amino acid sequence similarity with each other (41, 49, 120). Rather, σ^4 has a glutamine-rich region at its amino terminus that resembles the glutamine-rich region required for activation of transcription by mammalian transcription factor Sp1 (23). (In different organisms, between 15 and 25% of the first 50 residues of σ^4 are glutamine [63a, 80, 82, 114].) S. Sasse-Dwight and J. D. Gralla have determined that the glutamine-rich region of σ^4 is specifically required for NTRC-dependent isomerization of closed to open complexes at the glnA promoter (personal communication). Small deletions in this region allow the formation of closed recognition complexes at glnA but prevent the formation of open complexes.

CONCLUSIONS

σ^4 has physiologically diverse roles and in this way resembles the most abundant sigma factor in eubacterial cells (σ^70 and its homologs) rather than other alternative sigma factors. A direct line of evidence for physiological diversity is that mutant strains which lack σ^4 have pleiotropic phenotypes. For example, such mutant strains of A. eutrophus (105; Warrelmann, Romerrman, and Friedich, personal communication), which were designated hno-, for ‘hydrogen, nitrate and other things’ (52, 105), fail to express not only hydrogenases required for utilization of molecular hydrogen as an energy source but also enzymes required for utilization of urea and formamide as nitrogen sources and a dicarboxylate transport system; they have defects in several additional functions as well. σ^4-Deficient strains of E. coli, R. meliloti, and P. aeruginosa also have physiologically pleiotropic phenotypes (9, 59, 111; Table 1). The properties of mutant strains that lack σ^4 indicate that this sigma factor is not essential for bacterial viability under all conditions (39, 59, 105, 111, 114, 125). As discussed above, however, σ^4 is required for several important biological processes. It is required for the autotrophic growth of P. facilis (106, 127) and for that of A. eutrophus with molecular hydrogen as an electron donor (52, 105, 106). σ^4 is required for biological nitrogen fixation in a variety of gram-negative bacteria (Table 1) and, in addition, is needed for the establishment of stable symbiotic relationships between Bradyrhizobia (31), rhizobia (123, 133), and their plant hosts (Table 1). σ^4 would appear to be required for the formation of functional swarmer cells in C. crescentus (Table 1) and for virulence of N. gonorrhoeae (67, 68, 121, 128) and P. aeruginosa (59, 131).

Transcription by σ^4-holoenzyme appears to be controlled by a common mechanism: use of an activator protein and ATP to catalyze formation of transcriptionally productive open complexes (100). It is the activator proteins that allow σ^4-holoenzyme to respond to diverse physiological signals. The selective advantage to use of σ^4 and this mechanism, if any, remains to be determined (27, 111).

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