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Received 12 April 1993; revised accepted 8 June 1993

A new look at the endocrine regulation of egg maturation in the decapod crustaceans

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Female reproduction, especially the egg maturation in the ovary of decapod crustaceans, is negatively controlled by an inhibitory neuropeptide from X-organ /sinus gland complex. Recent structural studies reveal that its primary structure is related to that of other eye stalk peptides such as crustacean hyperglycemic hormone and molt-inhibitory hormone. This peptide seems to inhibit yolk synthesis and uptake into the oocytes. Information on a gonad stimulatory hormone, purportedly originating from the neurosecretory cells of brain and thoracic ganglia, is still very preliminary. Recent evidence indicates that the methyl farnesoate, a sesquiterpenoid compound synthesized by the glandular mandibular organ, could stimulate egg maturation, in much the same way the corpora allata controls oogenesis in insects. Application potentials of endocrine manipulation to crustacean aquaculture are discussed.

MOST decapod crustaceans lay a large number of yolk-rich eggs which contain a high-density lipoprotein as the main yolk protein, often conjugated to carotenoid pigments[1–3]. Both structural and biochemical evidences indicate a biphasic vitellogenesis in Crustacea, with the ovary initiating the biosynthesis of yolk within the growing oocytes, followed by a selective sequestration of yolk precursor molecule from the haemolymph (see Adiyodi and Subramoniam[4]). However, divergent views have been expressed regarding the ultimate site of yolk precursor synthesis in the extra-ovarial sites. Equally unsettled is the problem of endocrine regulation of egg maturation in crustaceans. However, by the recent upsurge in crustacean aquaculture, endocrine manipulation of reproductive phenomena has gained new impetus all over the world. In this article we review the very recent progress made in the hormonal control of vitellogenesis in decapod crustaceans.

Gonad or vitellogenesis-inhibiting hormone

The existence of a gonad inhibitory principle in the eyestalk of decapod crustaceans was demonstrated 50 years ago by Panouse[5] with his observation of accelerated ovarian growth in eyestalk-ablated female shrimp *Palaemon serratus*. The nature of this eyestalk factor remained unknown for a long time despite its confirmation in the neurosecretory X-organ/sinus gland complex of several crustacean species[6]. The first isolation and partial characterization of GIH has been done in the crab *Cancer magister* using gel chromatography techniques. It was found to be a 2 kDa peptide with inhibitory effect on the ovarian growth of the shrimp *Crangon crangon*[7]. Quackenbush and Keeley[8] also purified a gonad-inhibiting hormone (GIH) from the crude eyestalk extract of the shrimp *Penaeus setiferus* using Sephadex G-25 chromatography and determined the molecular weight as 3300 Da. This peptide inhibited ^{14}C leucine incorporation into vitellogenin by the cultured ovary of *Uca pugilator*, while the incorporation of the radioactivity in the other proteins remained unaffected. In a further study, Quackenbush[9] used the GIH of *P. setiferus* to determine the effect on the yolk protein synthesis in the ovary and hepatopancreas of another penaeid shrimp, *P. vannamei*. By virtue of its specific inhibitory role in yolk synthesis, the GIH has recently been rechristened as vitellogenesis-inhibiting hormone (VIH)[10]. Van Deijnen[11] demonstrated the inhibition of oocyte growth in the shrimp *Atyaephra desmaresti* by sinus gland extract of the lobster *Homarus americanus*. Interestingly, almost all the VIH bioassays thus far made have been heterologous, implying lack of species-specificity of this peptide.

Elucidation of the primary structure of VIH

With the introduction of high-performance liquid chromatography (HPLC), efficient separation of biologically active peptides from the sinus gland of several crustaceans and their structural elucidation has been possible. In *H. americanus*, the VIH exists in two isoforms, whose complete primary structure has been elucidated[12]. Both are neutral peptides (pI = 6.8) with a free N-terminus and consist of a chain of 77 amino-acid residues, with 3 disulphide bridges and a molecular weight of 9137 Da. Interestingly, only one of the two isoforms inhibits oocyte growth in the shrimp *Palaemonetes varians*. Recently, Aguilar *et al.*[13] reported the identification and characterization of VIH from the Mexican crayfish *Procambarus bouvieri*. This neuropeptide has a molecular weight of 8388 Da and a blocked N-terminus. It consists of 72–74 amino-acid residues, with specific absence of Try, His and Met

Relationship with other SG neuropeptides, CHH and MIH

Recent structural elucidation of several SG neuropeptides, i.e. crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH) and VIH has revealed sequence homologies and has thus led to the recognition of a novel family of peptides. All have a molecular mass of around 8500 Da in common. Figure 1 depicts the alignment of CHH, MIH and VIH of *Carcinus maenas* and *H. americanus*. Carcinus MIH and Homarus VIH are more closely related to each other (48% homology) than each of them to CHH from the same species. The sequence homology of VIH with CHH is only 28%, consisting mostly of single dispersed identical positions. Recently, Aguilar *et al.*[13] also showed a close similarity between the molecular masses of CHH, MIH and VIH of the crayfish *P. bouvieri*. All three also lack Try, His and Met residues, have a molecular weight between 8300 and 8400 Da and have a blocked N-terminus. From the amino-acid composition, a high degree of homology has been assigned to VIH and CHH-I peptides. Interestingly, neither the CHH isoforms nor the MIH showed VIH activity, implying the distinctiveness of VIH in the crayfish.

In other species, structural similarities in the above three native neuropeptides are also reflected to a certain degree in the overlap of biological activity. There are indications that molt-inhibiting and hyperglycemic activity are associated with the same peptide, as demonstrated in the lobster *H. americanus*[12,14] and in the shore crab *C. maenas*[15]. In a similar way, immunological studies in the lobster indicated that VIH and CHH share common antigens.

Intriguingly, Tensen *et al.*[16] found a stimulatory effect on oocyte growth of the shrimp by 6.5 kDa peptides (VI / VII) of *H. americanus*, which not only showed HPLC retention times identical with those of glycemic peptides but also provoked a hyperglycemic response. Concerning the possible existence of such an ovarian stimulatory hormone in the sinus gland, it will be interesting to know whether this peptide works antagonistically to VIH or synergistically with other putative gonad stimulatory neurohormones.

Site of synthesis and mode of action

Using a polyclonal mouse antiserum raised against HPLC purified VIH, Meusy *et al.*[17] identified the VIH-producing cell system in the X-organ and detected VIH-containing axon terminals in the sinus gland of *H. americanus*. More recent results obtained from light and electron-microscopic immunocytochemical studies revealed co-localization of VIH and CHH in a variable number of the same group of X-organ neurosecretory perikarya of the same lobster

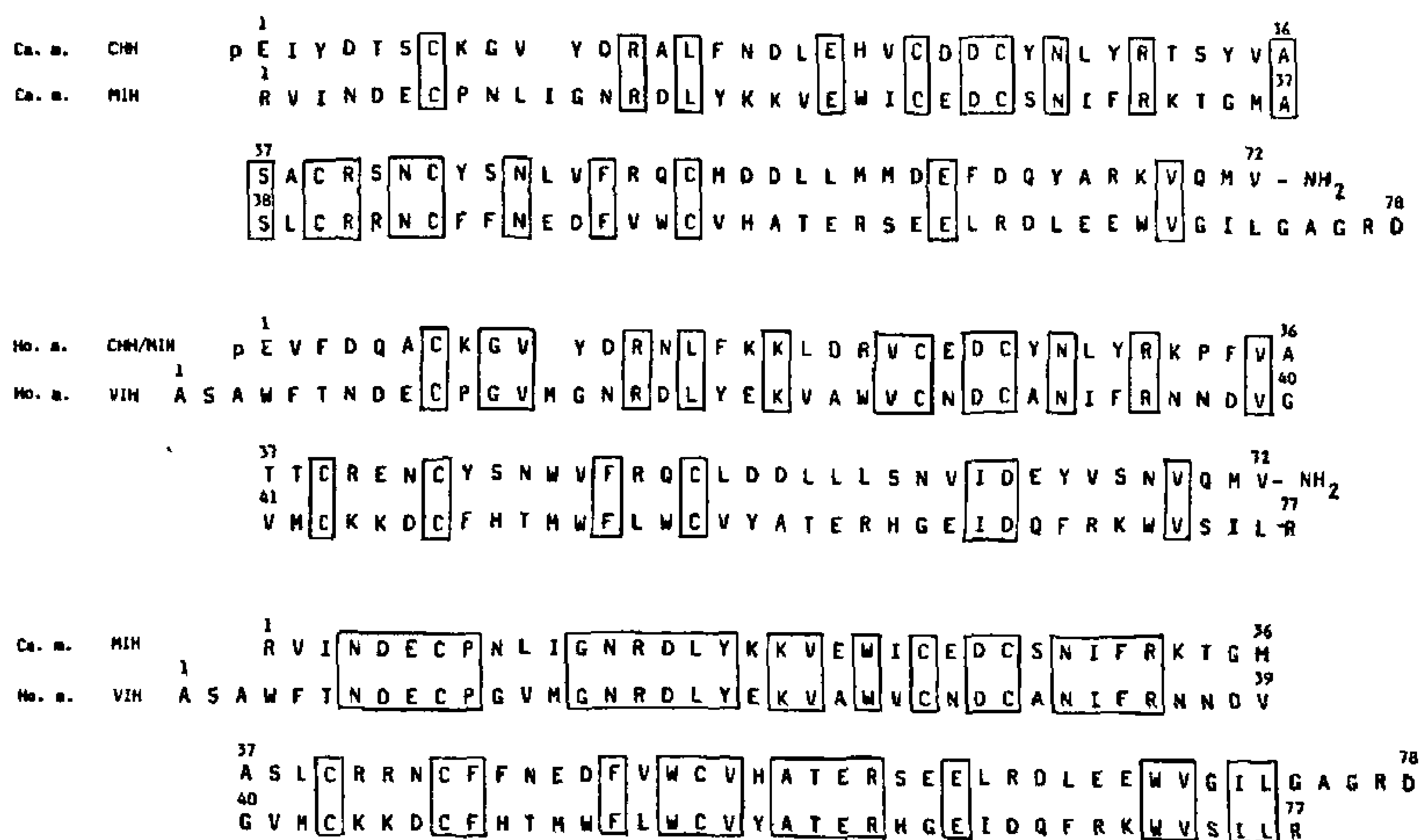

Figure 1. Alignment of sequences to show relationship between Carcinus CHH[35] and MIH[36], Homarus CHH/MIH[14] and vitellogenesis-inhibiting hormone (VIH)[12] and Carcinus MIH and Homarus VIH. According to Tensen *et al.*[37], the Homarus CHH shown here is the isoform A. The isoform B differs in seven residues. Identical positions are boxed. Reproduced from Keller[38]. Ca m = *Carcinus maenas*; Ho.a = *Homarus americanus*.

species[18]. *In situ* hybridization studies have also co-localized the CHH and VIH in the X-organ/sinus gland complex of this lobster[19]. These authors point to the possible existence of three groups of cells in the X-organ: one responsible for the synthesis of CHH, a second for the production of VIH and a third group capable of synthesizing both. That VIH and CHH are two different peptides has, however, been shown by the fact that preabsorption of the anti-VIH serum with purified CHH did not abolish immunoreactivity in axon terminals of the sinus gland.

The mode of VIH action on the reproductive cells is not adequately understood. Jugan and Soyez[20] demonstrated that a sinus gland extract inhibited the binding of colloidal-gold-labelled vitellin on oocyte microvilli of the prawn *Macrobrachium rosenbergii*. Subsequently, Jugan[21] showed that VIH inhibited the receptor-mediated endocytosis of yolk precursor protein by oocytes under *in vitro* conditions. Additional experiments are needed to understand the exact cellular interactions of VIH with oocytes during vitellogenesis.

Gonad stimulatory hormone

The first evidence for a gonad stimulatory principle in the central nervous system of crustaceans was obtained by Otsu[22], who noticed precocious ovarian development in the crab *Potamon dehaani* after implantation of thoracic ganglia. Subsequent studies in this respect employed not only the implantation of thoracic ganglia and brain, but also aqueous extracts from these nervous tissues to induce ovarian maturation[23–25]. Surprisingly, no real attempt has been made so far to study the chemical nature of the active hormone. Recently, Richardson *et al.*[26] studied the effect of 5-hydroxy tryptamine on ovarian development in the fiddler crab *U. pugilator*. These authors speculate that this biogenic amine could release the gonad stimulatory neurohormone from brain/thoracic ganglia.

In recent years attention has been focused on another gland, the mandibular organ, as a source of gonad-stimulating factors in decapod Crustacea. Hinsch[27] first reported augmentation of ovarian development after the implantation of mandibular organs in the spider crab *Libinia emarginata*. Laufer *et al.*[28] identified a sesquiterpenoid compound, methyl farnesoate (MF), in the mandibular organ (MO) as well as in the haemolymph of the spider crab. MF, the immediate precursor of the insect JH III, has been shown to be present in several decapod crustacean species[29]. In addition, the MO of decapod crustaceans are structurally similar to corpora allata of insects[30]. More interestingly, the activity of the MO appears to be controlled by eyestalk hormones, as evidenced by the finding that eyestalk ablation results in the hypertrophy of this gland[29]. An increase in the blood vitellogenin level following methyl farnesoate injection

into the reproductively active female crab may also indicate its influence on the yolk-precursor-synthesizing tissues such as hepatopancreas[31]. Clearly, MF is the crustacean juvenoid, probably involved in the stimulation of vitellogenesis. Farnesoic acid (FA), a precursor to MF is also secreted in large amounts by MO in brachyuran crabs such as *Scylla serrata*[32]. FA may be a prehormone which could undergo conversion to MF or even JH III in the target tissues. Elucidation of endocrine functions of these sesquiterpenoid products in the ovarian maturation could be highly rewarding in commercial aquaculture production. That the decapod ovary is receptive to insect JH has been shown recently by Sasikala and Subramoniam[33], who found enhanced yolk deposition in the crab *Paratelphusa hydrodromous* after injection of JH III.

Conclusions

The regulatory role of eyestalk neuropeptides on the ovarian muturation in crustaceans is still far from clear. Although the recent structural studies on VIH have revealed its similarity with other neurohormones such as CHH and MIH, its physiological role as well as the mode of action on the target organs are poorly understood. The main impediment in this regard is the lack of authentic bioassay systems to test its inhibitory, and/or restraining[34] functions. Further complication to resolve this problem arises from the discordant views concerning the mode of vitellogenesis, including the site of synthesis of vitellogenin in crustaceans. A careful perusal of existing information on crustacean vitellogenesis may indicate the diversity of pattern, imposed probably by environmental conditions in which the animal has to carry out its reproductive processes to perpetuate its progeny. Therefore, for crustacean species at least, an understanding of the environmental cues inducing the endocrine centres to initiate reproduction becomes very important. Until then, no generalization could be made regarding the hormonal control of ovarian development for crustaceans.

A new perspective of neuroendocrine research in decapod crustaceans is the application of its results to aquaculture. In this respect, an interesting dichotomy is that the experimental studies including the structural elucidation of neuropeptides have been mostly done in lobsters, crayfishes and crabs, but the empirical application of eyestalk ablation techniques in crustacean aquaculture has been made on penaeid shrimps. This certainly warrants an investigation of the neuropeptides of commercially important penaeid species. Although VIH seems to be non-species-specific, our unpublished results on the CHH of *Penaeus indicus* indicate that it does not elicit hyperglycemia when injected into the crayfish *Orconectes limosus*. However, it exhibits immunological compatibility with anti-Orconectes CHH serum, as evidenced by ELISA and immunocytochemistry.

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ACKNOWLEDGEMENTS: Unpublished observations reported in this paper are supported by DFG, Germany, T. Subramoniam is grateful to the Indian National Science Academy and DFG for the exchange of Scientist Visitorship to Bonn.

Received 18 November 1992; revised accepted 7 May 1993

RESEARCH ARTICLE

Petrology of tectonically segmented Central Indian Ridge

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Distribution and mineralogy of various rock types along the 4200-km-long slow-spreading Central Indian Ridge, between Owen fracture zone in the north and Indian Ocean triple junction in the south, is studied in the light of ridge segmentation, and associated stress regime. To understand such phenomena along an extremely low magmatic budget spreading axis, rock samples from nine sites were examined. Rocks at these sites differ markedly in mineralogical composition and texture, but, surprisingly, not geochemically. Nature of segmentation of the ridge (length and offset) by transform faults appears to have influenced the variable extent of melting of the source rock and depth of magma generation below each ridge segment. We conclude that segmentation plays a significant role in facilitating polybaric fractional crystallization and the resultant mineralogical and textural variations in the erupted rocks.

THE old understanding of magma chamber configuration being a 'relatively large, essentially molten, steady-state reservoir in which melt accumulates and undergoes magmatic differentiation prior to eruption or emplacement'[1] has undergone a distinct change to 'relatively small (< 1 km thick, 1–3 km wide), tectonically bounded, lenticular magma chambers surrounded by semi-molten low-seismic-velocity zone (LVZ)[2]. Recent reports suggest that proximity to structural offsets such as transform faults could lead to a lower magmatic temperature[3], a wide range of magma composition[4] and the resultant eruption of the enriched basalt[5]. Thus, it appears that transform fault separates coherent geochemical units reflecting differences in crystal fractionation and extents of melting. A concept of 'spreading cell-deval' has been proposed to account for an observation[5] of such small magma chambers. (A small ridge portion bounded by two transform faults is known as a spreading cell, which also describes the boundary of smaller magma chambers[2,4,5].)

In continuation to such understanding, we examine here the effects of small and large transform faults on the petrology of Mid-Ocean Ridge Basalts (MORB) and magmatic processes along the less explored, slow-accreting Central Indian Ridge (CIR). The geologic settings and petrology of rocks dredged from nine sites along the 4200-km-long ridge system [from the Owen fracture zone (OFZ) in the northwest to the south up to Indian Ocean triple junction (IOTJ)] are described. Bathymetric data from these sites (Figure 1), along a number of profiles across the ridge system, were collected by narrow-beam echo-sounder and Seabeam systems, onboard the research vessels, viz. *ORV Sagar Kanya* (India), *RV Sonne* (Germany) and *Academician Vernadskiy* (Russia). These sites correspond to three tectonic environments: zone I, sites located at or near large offset (> 55 km) of the ridge by transform faults (sites A, D, F and G); zone II, sites located at and around small offsets (10–55 km) of the ridge by transform faults (sites C, E and H); and zone III, midway along relatively undisturbed rift valley floor of individual tectonic segment (RVF, sites B and I). The average water depth of the sampled sites is 3225 m (range 4664 m at A to 1700 m at D, Table 1). A little more than 4500 kg of rocks were recovered from 33 dredge operations at the nine sites. For seven of these sites petrological examination was made, while for sites A and D the earlier description was followed[6].