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The *Drosophila period* Gene and Dye Coupling in Larval Salivary Glands: A Re-evaluation

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In 1987, Bargiello *et al.* (1) reported that mutations of the *period* (*per*) gene dramatically altered the extent of intercellular coupling in larval salivary glands, such that coupling was virtually absent in *per°* glands and was quite extensive in *per^s* compared to wild type. These results, together with early immunochemical data and sequence analysis of the PER protein (1, 2, 3), were interpreted as indications that the PER protein was a proteoglycan, localized at the cell boundaries in larval salivary glands, and acting there to regulate intercellular communication (1). Recent evidence suggests that the PER protein is unlikely to be a proteoglycan (4), and raises questions about its presence in salivary glands (5, 6). Therefore, we have re-evaluated the influence of *per* genotype on intercellular coupling in larval salivary glands.

Working in two different laboratories, we performed two extensive series of Lucifer Yellow injections into salivary glands from larvae of various *per* genotypes. The same four *per* strains were used by both groups: the arrhythmic *per°* mutant, the short-period *per^s* mutant, and two control strains—an isogenic *per^+* stock and a transgenic strain (*per°*; 13.2/13.2), wherein two copies of a fully functional 13.2 kb fragment of *per^+* DNA are inserted into a *per°* genetic background. The strains were coded so that experimenters were uninformed as to the genotype. As a historical note, after the first series of experiments (by K.F.F., J.C.H. and M.R.) had been completed, open discussions of their results stimulated a further series (by K.K.S. and D.C.S.).

Although the extent of dye coupling was evaluated by different criteria in the two laboratories, the results of both failed to show a correlation between *per* genotype and the extent of dye coupling in larval salivary glands (Tables IA, B). Although the median values of one data set (that in Table IB) reproduced the order *per^s > per^+ > per°* (with median values of 1.0, 0.8, and 0.5, respectively), the data displayed a wide range in the degree of coupling within each genotype, and mean values revealed no genotypic differences. Even within a single animal, the extent of dye spread could be highly variable; this was exemplified most prominently by two glands of a *per°*; 13.2/13.2 animal: one gland showed extensive dye coupling, and the other showed no transfer at all.

The variability reported here contrasts markedly with the striking differences and small variability within genotypes reported previously (1). In attempting to understand this discrep-

Table I

A: Dye transfer in larval salivary glands

Genotype	Anterior injections	Posterior injections
per°	4.8 (6)	3.5 (11)
per^+	1.0 (4)	4.2 (6)
per^s	3.0 (9)	2.0 (5)
per°; 13.2/13.2	1.8 (14)	3.4 (6)

B: Dye transfer in larval salivary glands

Genotype	All glands		Small		Large		Early		Late	
per°	0.9 ± 0.2	(36)	0.2 ± 0.1	(13)	1.3 ± 0.2	(23)	0.9 ± 0.3	(9)	0.9 ± 0.2	(27)
per^+	0.8 ± 0.1	(31)	0.7 ± 0.1	(22)	0.9 ± 0.2	(9)	0.7 ± 0.2	(11)	0.8 ± 0.1	(20)
per^s	0.8 ± 0.1	(46)	0.07 ± 0.04	(16)	1.3 ± 0.1	(30)	0.9 ± 0.1	(12)	0.8 ± 0.1	(34)
per°; 13.2/13.2	1.1 ± 0.2	(38)	0.5 ± 0.2	(11)	1.4 ± 0.2	(27)	1.1 ± 0.3	(11)	1.2 ± 0.2	(27)
Total	0.9 ± 0.07	(151)	0.4 ± 0.07	(62)	1.3 ± 0.08	(89)	0.9 ± 0.1	(43)	0.9 ± 0.08	(108)

A. In this series of experiments, dye transfer was scored as the number of cells filled 1–2 min after the beginning of each 30 s injection; the numbers of injections are in parentheses. These data are from cells whose resting potentials (pre-injection) were <-28 mV. Because anterior and posterior cells exhibit differences in electrical parameters (7), their scores were tabulated separately. In other cells whose resting potentials ranged from -27 mV to -12 mV ($n = 23, 9, 6,$ and 9, respectively, for the 4 genotypes), similar results were obtained: there were no systemic genotypic variations from the average dye transfer score of 3.5 cells.

B. In this series of experiments, dye transfer was scored on a rating scale of 0–3 by two individuals who were blind as to the genotype, and the two scores for each injection were averaged. The numbers of injections are in parentheses. Larvae were maintained at 25°C in a 12 h light:12 h dark cycle. Data in the 'Early' column are from glands dissected between 1 and 3 h after 'lights-on'; data in the 'Late' column are from glands dissected within 2.5 h of 'lights-off.' Salivary glands were classified as small or large prior to injections.

ancy, we have compared subsets of our data with regard to location of injection (anterior *vs.* posterior gland regions in Table IA), large *versus* small glands (Table IB), and glands injected at different times of day (early *vs.* late in Table IB). These restricted data sets revealed significantly weaker coupling in smaller glands than in larger glands, but no evidence for an effect of *per* genotype. Thus, while other variables affect coupling in this tissue, we conclude that the *per* gene itself does not detectably influence the extent of intercellular coupling in larval salivary glands.

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Gating and Single Channel Properties of Gap Junction Channels in Hepatopancreatic Cells of *Procambarus clarkii*

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The crustacean hepatopancreas is responsible for major metabolic events in the organism, including enzyme secretion, absorption and storage of nutrients, molting, and vitellogenesis (1, 2). The multifunctional role of the hepatopancreas requires that its constituent cells be precisely coordinated so that the organ can produce appropriate responses. Hepatopancreocytes are connected by large gap junctions (3, 4), which are specialized transmembrane channels involved in direct cell-to-cell communication. In the crayfish, the morphological organization of gap junction plaques (as revealed by freeze-fracture methodology), as well as the extent of electrical coupling between neighboring cells (as revealed by coupling coefficient measurements with two microelectrodes) are modulated by the molting cycle and by the molting hormone crustecdysone (5, 6). These observations suggest that gap junctional communication may be involved in the function of crustacean hepatopancreatic cells.
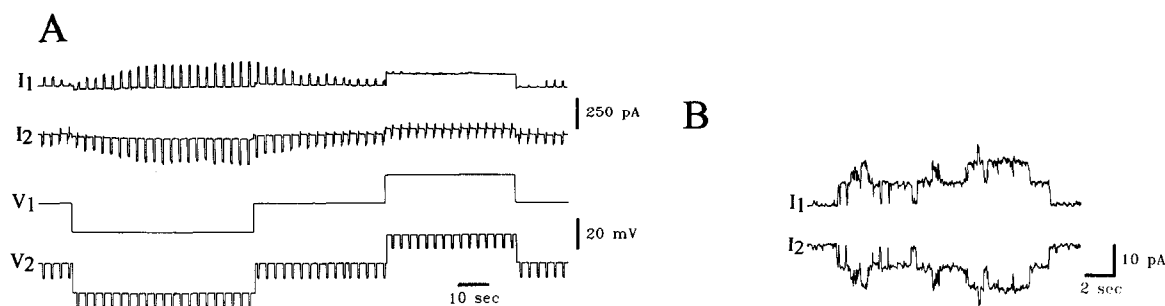

Figure 1. *(A) Inside-outside voltage dependence of junctional conductance between hepatopancreatic cell pairs. At the beginning of the experiment, both cells of the pair were held at a common holding potential of 0 mV (V_1 and V_2). Junctional conductance was determined by applying 10 mV pulses to cell 2. This protocol evoked a current, both in the pulsed cell (I_2), and in the non-pulsed cell (I_1). Although the latter current is only junctional, the current appearing in cell 2 is the sum of junctional (I_j) and non-junctional currents (I_{nj}). When both cells were hyperpolarized to -20 mV, I_j increased with time indicating the opening of gap junction channels. This effect is reversible when cells were held back to the initial holding potential. In contrast, when cells were depolarized to positive values ($+20$ mV), I_j was no longer detected indicating closure of gap junction channels. I_j could be resolved once again when the cells were clamped to 0 mV. (B) Single gap junction channel activity in hepatopancreatic cell pairs. To visualize single gap junction channels, a difference of potential was elicited across the junctional membrane by holding one cell of a pair at 0 mV and the second cell at -20 mV. Single gap junction channel openings and closures are recognized by simultaneous transitions of identical but opposite polarities in both cells of the pair. Upward transitions in I_1 indicate channel opening. Most current transitions correspond to a junctional conductance of about 250 pS. A similar junctional conductance value was observed when the pulsed cell was held at a positive voltage of 20 mV.*