Supplementary Information

Pearson *et al*. 10.1073/pnas.0810219106

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Supporting Text. DNA enhancer sequence boundaries, mutant site sequence changes, evolutionary sequence alignments of epidermal wound enhancers, oligonucleotide sequences used in preparation of clones and in EMSA assays.

Reporter Construct Generation. All reporter constructs were generated by PCR-amplification from genomic or plasmid DNA and cloning either into the TA-cloning vectors pCR-II (Invitrogen) or pGEM-T/pGEM-T Easy (Promega), then cloned into DsRed H-Stinger (1), within the polylinker. All constructs were cloned in the direction that maintains the relative proximal-distal orientation to the HSP70 promoter as found in the native gene; i.e., fragments from 5′ of the gene are "forward oriented", whereas intronic fragments are "reverse oriented".

Construct Boundaries and Site Mutations. Internal deletions and site mutations were generated by PCR sewing. Deletion sequences indicate bounds of deleted region, mutation sequences show altered nucleotides in red. The number of independent P-element insertion lines that were generated is indicated in parentheses.

***Ddc* .47-Based Constructs.** *Ddc* .47 (2) (3 lines): AAAACCCTGTTT to AGCGGGCAGCG

Ddc Gap1 (2 lines): deleted ACGAGATCGC to ATCAAATTAAG

Ddc Gap2 (4 lines): deleted AACTAATTTC to AGTTACTGAT

Ddc Gap3 (12 lines): deleted AGCGCCCAAT to GGACTGCGAT

Ddc Gap123 (3 lines): deleted ACGAGATCGC to GGACTGCGAT

Ddc .47 AP1m (8 lines): changed nucleotides CGAGTCCCCCATAA.TTACTCCCCCAGCG

***ple*-WE1-Based Constructs.** *ple*-WE1 (9 lines): TTGGTTTGCA to CGAGGGCTGG

ple-WE1 APm (7 lines): changed nucleotides GTGTGGTGGAGCAC.GCACGGCGCTGACA

ple-WE1 CREBm (4 lines): changed nucleotides ACGTGGATCAAAAT.GCACGGCGCTGACA

ple-WE1 EXDm (4 lines): changed nucleotides GCACGGCGCTGACA.AAAATCCCTGCCA

ple-WE1GRHm (7 lines): changed nucleotides CACCCGGGAAAGTTG

ple-WE1 HOXm (7 lines): changed to GGAATGGTACTA. CAATACCATACAATGGCCAGCAA. CTCGTCCGGAACGCACATGGTTGCC. CTCTTGGTTGTATTTACCGGTTGCGTTTGGTTGAACCATGAATGGTATTT

*kkv*2 (6 lines): AAGTGCCAGT to GAGTCCTGTC

***kkv*-WE1-Based Constructs.** *kkv*-WE1 (4 lines): CAACAAAGGA to TGGGTGTGTT

kkv-WE1SubA (9 lines): CAACAAAGGAT to CTCGAAAGAT

kkv-WE1SubB (8 lines): GCTTACTCCG to ATCAAACCGC

kkv-WE1 AP1m (3 lines): changed to GGGTGGTGGATGGC.AAGTGGAGGACTCG.GGAAATCCGCCACAA

kkv-WE1 GRHm (1 line): changed to CAACCTTGGGTCGGC.ATACCTTGGGCTATC.AGACTTTGGGTTTAAAAACAGTTCGATCCCCAAGCTTT.TATAGCCAGAGTTG

*msn*2 (6 lines): AGCACTGGCC to GTCTCGTGGA

msn-WE1 (7 lines): GAGTGTAGCC to ATTGACAGCA

msn-WE2 (5 lines): CCACTGCCAAC to ATTGACAGCA

The following sets of primers were used for generating the PCR fragments containing the ORFs for reticulocyte lysate produced *Drosophila* GRH-N protein, *Drosophila* FOS-B, *Drosophila* FOS-D and *Drosophila* JUN.

:GRH, 5′-GCGCTCGAGGCCACCATGTCCACATCCAC-3′ and 5′-CGCAAGCTTCTGATTGGGCAGCTCCGT-3′;

DFOS-B, 5′-GCGCTCGAGGCCACCATGACGCTGGACAGCTACAA-3′ and 5′-TAAAAGCTTTAAGCTGACCAGCTTGGA-3′;

DFOS-D, 5′-GCGCTCGAGGCCACCATGATTGCACTAAA-3′ and 5′-TAAAAGCTTTAAGCTGACCAGCTTGGA-3′;

DJUN, 5′-GCGCTCGAGGCCACCATGAAAACCCCCGTTTCCG-3′ and 5′-CGCAAGCTTTTGGTCTGTCGAGTTCGGC-3′

Electrophoretic Mobility Shift Assays. 500 picomoles of each of two single-stranded oligonucleotides were annealed in a final volume of 100 μl in annealing buffer (10 mM Tris·HCl pH7.5, 20 mM NaCl) and labeled with polynucleotide kinase (New England BioLabs,) in the presence of [^{32}P]ATP for 30 min at 37 °C. The double-stranded probes were purified by QIAquick Nucleotide Removal Kit (Qiagen). Ten to 20 fmol of radiolabeled DNA probes and 1 μl of GRH protein from in vitro transcription/translation reactions were added to a binding buffer containing 25 mM Hepes (pH7.9), 100 mM KCl, 1 mM DTT, 1% polyvinylalcohol, 1% Nonidet P-40, 0.1% BSA, 10% glycerol, 20 μg/ml poly(dI-dC) in a volume of 10 μl. Proteins were incubated with DNA for 30 min at 4 °C and electrophoresed through 4% native polyacrylamide gel containing 0.5× TBE at 4 °C. Gels were dried and autoradiographed with the use of intensifying screens.

To test the DNA binding activity of *Drosophila* JUN and FOS proteins, 10–20 fmol of radiolabeled DNA probes and 1 μl of DJUN protein or 1 μl of DFOS-D or 1.5 μl of DFOS-B protein (equimolar amounts) from in vitro transcription-translation reactions were added to a binding buffer containing 25 mM Hepes (pH7.9), 100 mM KCl, 1 mM $MgCl_2$, 1 mM DTT, 0.1% Nonidet P-40, 0.1% BSA, 10% glycerol, 20 μg/ml poly(dI-dC) in a volume of 10 μl. After incubation at 4 °C for 30 min, the reactions were then resolved by electrophoresis on 5% native polyacylamide gels containing 0.5× TBE at 4 °C. Gels were dried and autoradiographed.

Oligonucleotide Probes for EMSA Assays. The following oligonucleotides (IDT) were annealed to their respective complementary sequences, radiolabeled and then used as probes in EMSA assays: *Ddc* wild type GRH site, 5′-CGGACTGCGATTGAACCGGTCCTGCGGAATTGG-3′; *Ddc* mutant GRH site, 5′-CGGACTGCGATTCCCAAGGTCCTGCGGAATTGG-3′; *ple* wild type GRH site, 5′-GTGATTCAGCACCCAAACGAGTTGATCTTGGAAAG-3′; *ple* mutant GRH site, 5′-GTGATTCAGCACCCGGGAAAGTTGATCTTGGAAAG-3′; *Ddc* wild-type AP1-like site, 5′-TGTTTCGAGTGACTCATAATTGGGGGA-3′; *Ddc* mutant AP1-like site, 5′-TGTTTCGAGTCCCCCATAATTGGGGGA-3′.

Details of the Hapten Labels and Detection Methods for Multiplex in Situ Hybridizations to Wounded Embryos. Transcripts from the *msn* gene were detected with an RNA probe synthesized from a

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full-length *msn* cDNA clone. The *msn* probe was labeled with biotin-UTP, and then detected with HRP-coupled streptavidin followed by a reaction with tyramide-Cy3. Transcripts from the *ple* gene were detected with an RNA probe synthesized from clone containing exons 5 and 6 from the *ple* locus. The *ple* probe was labeled with FITC-UTP, and then detected with HRP-coupled mouse anti-FITC antibodies followed by a reaction with tyramide-Alexa Fluor 405. Transcripts from the *Ddc* gene were detected with an RNA probe synthesized from a clone containing exon 3 of the *Ddc* locus. The *Ddc* probe was labeled with digoxigenin-UTP, then detected with HRP-coupled sheep anti-digoxigenin antibodies followed by a reaction with tyramide-fluoroscein. Transcripts from the *kkv* gene were detected with an RNA probe synthesized from a *kkv* full-length cDNA. The *kkv* probe was labeled with dinitrophenol-UTP, and then detected with rabbit anti-dinitrophenol antibodies followed by incubation with donkey anti-rabbit antibodies coupled to Alexa Fluor 647 fluorophore (Invitrogen).

***Drosophila* Species Sequence Alignments of the Four Wound Enhancers from the Ddc, ple, msn and kkv Genes.** Sequences were cloned by molecular biological techniques or identified by Discontiguous MegaBLAST of NCBI Trace archives, were trimmed to approximate common boundaries and aligned by T-Coffee (2). Alignments were then adjusted based on evolutionary proximity using Lalign (www.ch.embnet.org/software/LALIGN_form.html), and hand alignments were done for small sections of poorly aligned sequences.

1. Barolo S, Castro B, Posakony JW (2004) New Drosophila transgenic reporters: Insulated P-element vectors expressing fast-maturing RFP. *Biotechniques* 36:436-441.
2. Mace KA, Pearson JC, McGinnis W (2005) An epidermal barrier wound repair pathway in Drosophila is mediated by grainy head. *Science* 308:381–385.
3. Notredame C, Higgins DG, Heringa J (2000) *J Mol Biol* 302:205–217.42.

Table S1. Table of quantitative responses of wound enhancers after wounding

Line	% none	% weak	% moderate	% strong
Ddc APm.2	100	0	0	0
Ddc APm.3	100	0	0	0
Ddc APm.4	100	0	0	0
Ddc APm.6	100	0	0	0
Ddc APm.9	100	0	0	0
ple APm.2	100	0	0	0
ple APm.3	100	0	0	0
ple APm.4	100	0	0	0
ple APm.8	100	0	0	0
ple APm.9	100	0	0	0
ple GRHm.2	95	5	0	0
ple GRHm.3	93	7	0	0
ple GRHm.4	93	7	0	0
ple GRHm.6	57	29	7	7
ple GRHm.8	52	22	22	4
ple WE1.1	0	6	27	64
ple WE1.4	8	33	51	0
Ple CREBm.1	11	19	19	52
ple CREBm.2	40	13	13	33
Ple CREBm.3	10	19	35	35
ple CREBm.4	17	29	29	25
ple EXDm.5	55	6	35	3
Ple EXDm.2	3	10	62	24
Ple EXDm.3	8	23	62	8
Ddc 0.47.2	0	9	50	41
Ddc 0.47.5	11	11	44	33

A subset of independent transformant lines were wounded in parallel and allowed to recover as described in *Materials and Methods*, and wound responses were judged in a single blind test as follows: No Fluorescent protein around wound = None; ambiguous response or weak wound-specific response (1–3 cell radius from wound site) = Weak; moderate wound-specific response (more than 3 cell radius from wound site) = Moderate; Substantial wound-specific response (Fluorescent wound responses from entry and exit wounds overlap) = Strong. Only lines with greater than 10 successful wounded embryos were included in quantifications.

Other Supporting Information Files

SI Appendix