

肢体再生的生物学基础

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第一节 引言

有些脊椎动物，通过一种芽基细胞的增殖，能够在一些切割伤的附肢部分组织或整个附肢进行再生，如指尖，四肢，鳍，尾，鹿茸和耳组织等地方。对于其中的一些结构，细胞再生的来源是不确定的，如哺乳动物的耳组织、指尖和鹿茸等；但是，其他一些结构，已有证据表明，芽基是通过伤口处一种成熟细胞去分化形成的，如鱼鳍和两栖动物的肢体以及尾尖的再生。两栖动物的尾部再生和蜥蜴的再生已经在第五章讨论过。这里，我们要重点讨论两栖动物的肢体再生和哺乳动物耳组织、鹿茸和指尖的再生。

第二节 两栖动物的肢体再生

一、肢体的再生活动

许多物种的幼虫、成体蝾螈 (urodeles) 以及早期青蛙和蟾蜍，蝌蚪等的肢体可以再生。Spallanzani (1768) 是第一个描述了成体蝾螈肢体的再生现象。图14.2表明成体蝾螈和幼体蜥蜴肢体再生的发展阶段 (Goss, 1969)，截肢表面会在几个小时内就被迁移的表皮覆盖。在受伤的表皮下，去分化的细胞聚集形成再生芽基。与此同时，受伤表皮增厚形成尖端表皮帽样组织 (apical epidermal cap, AEC)。AEC的外层形成保护层，而其基底层解剖和功能结构则与羊膜胚胎肢芽外胚层嵴尖 (apical ectodermal ridge, AER) 相似 (Christensen and Tassava, 2000)。在截肢后的几天里，毛细血管和神经的再生开始形成，并进入芽基细胞中。在芽基细胞的生长和增殖中，无论是AEC还是再生神经提供的生长和营养因子都起到至关重要的作用。

新生成的芽基迅速生长并生成一种圆锥形肢芽，其未分化细胞的形态学类似于胚胎的肢芽。随着进一步的发展，芽基不停分化并形成断肢的结构。在 PD (proximodistal, PD) 轴上，除了在腕骨或跗骨分化之前，指（趾）头开始分化外，分化和形态学发生则按照从近端到远端和从前面到后面的顺序进行。成体蝾

螈的肢芽和再生芽基在前后位轴 (anteroposterior , AP)上显示出一种独特的从前到后的顺序, 然而, 无尾两栖动物的芽基、胚胎肢芽还有羊膜动物的肢芽是按照从后到前的顺序生长发育的 (Shubin and Alberch, 1986)。在背腹部层面上 (dorsoventral , DV)的分化是同步进行的。其他的再生部分包括芽基的生长与未截断的肢体大小保持一致。无论截肢是指尖还是肱骨, 其再生残缺部分所需的时间是一样的。在幼体身上, 肢体再生的速度是在成体上的两倍甚至更多 (Voit et al.,1985)。

90%的成体蝾螈通过再生可以精确的复制原来的断肢。但是, 如果连续截肢再生的话, 其结果就会影响其形态学上的精确性。成体蝾螈的上臂在4次的截肢再生后, 有81%的再生肢体显示出了结构的异常, 如趾尖蹼, 骨骼元素数量在减少, 甚至会出现完全再生抑制 (Dearlove and Dresden, 1976)。

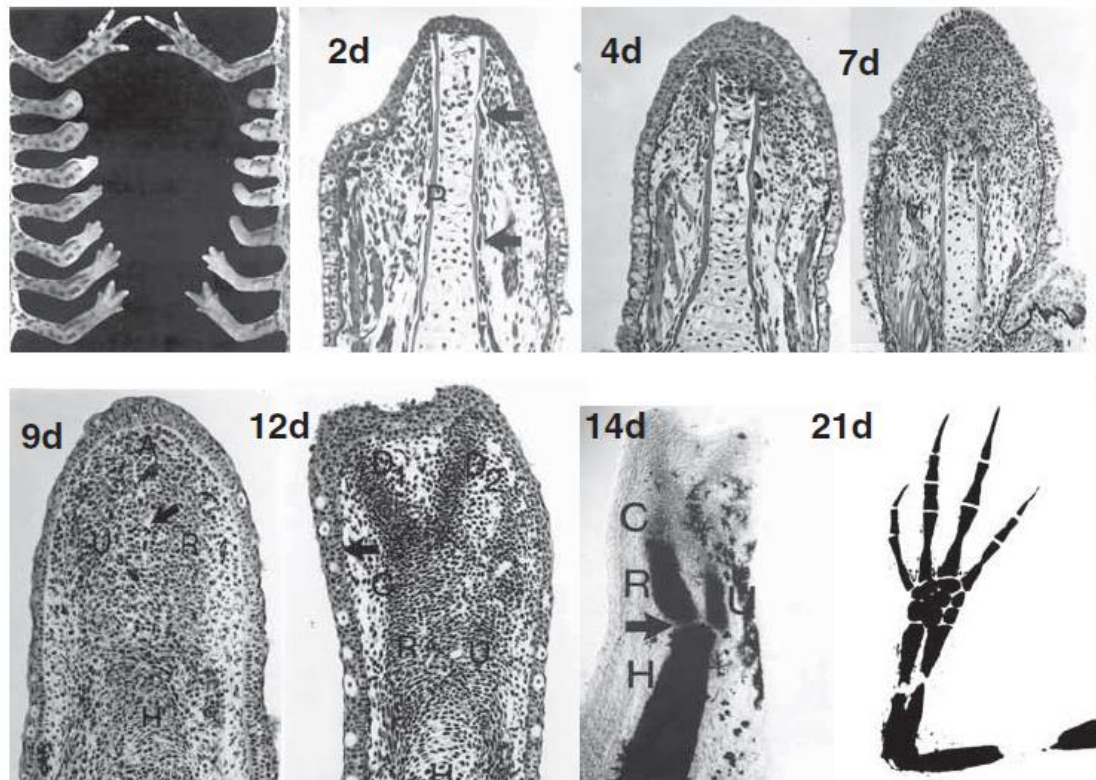


图 14-2 肢体再生的阶段。左上的图表明成虫蝾螈前肢在尺骨桡骨水平(左)和人类前肢尺骨桡骨水平(右)截断阶段的外部试图。过程是从上向下的。其余图片, 显示了苏木子染色和纵轴绿色荧光和甲基蓝后幼体蝾螈的肢体在截肢前段分化。P=骨膜外形, H=肱骨, R=桡骨, U=尺骨, C=腕骨部分, D1 和 D2=前指 1 和前指 2。在 2 天的那幅图中, 箭头指的是破骨细胞骨膜外形, 在第 9 天的是一个血管, 第 12 天的是重新形成的基底膜, 第 14 天的是肘关节。

肢体再生的分子生物学是一种新兴的学科，它的出现是对组织和细胞水平的研究的补充。Geraudie and Ferretti (1998), 全面详尽地列出了所有肢体再生中被合成的分子，其中包括生长因子信号分子、细胞外基质成分和转录因子。

二、芽基的形成的机制

芽基是由位于截肢表面的细胞外基质降解形成，结果造成组织溶解和个体细胞的游离，进而导致显性表型的丢失和细胞的增殖 (Thornton, 1968)。不管其亲代细胞表型如何，芽基细胞呈现出肢芽间充质细胞的形态学表现。

细胞外基质被酸性水解酶降解，如组织蛋白酶 D、酸性磷酸酶 (Ju and Kirn, 1998)、P-葡萄糖醛酸酶、水解酶和羧酯酶等 (Schmidt, 1966), 以及由 MMP-s2 和 MMP-s9 (明胶酶) 和 MMP3/10a 和 b (溶剂质素) 等，酶在芽基形成阶段起重要作用 (Ju and Kirn, 1998; Yang et al., 1999; Park and Kirn, 1999; Vinarsky et al., 2005)。蝾螈的肢体再生与非再生之间的差异筛选分析发现在再生肢体的细胞外基质降解中，有一种新的很活跃的胶原蛋白的存在 (Vinarsky et al., 2005)。酸性水解酶是从死亡细胞和破骨细胞中释放出来的，在再生肢体中含量丰富。巨噬细胞、受伤的表皮和破骨细胞可在创伤组织中产生 MMPs，芽基细胞本身也会产生 MMPs。再生神经的轴突是生成 MMPs 的另一个潜在来源。在胚胎发育阶段，幼体的神经轴突分泌 MMPs 可以切断细胞外基质产生 MMPs 的通路 (Pittman and Buettner, 1989)。

芽基的细胞外基质可以维持芽基细胞处于未分化的状态 (Stocum, 1995)。图 14.5 展示了几个分子水平的肢体再生的过程。在胚胎芽肢中，纤维连接蛋白、肌腱和透明质酸酶表达显著上调，而层粘连蛋白的表达降至零。直到芽基开始分化，基底膜才会形成。在肢芽的间充质细胞中，胶原蛋白 II 表达也消失，仅有胶原蛋白 I 表达。而硫酸化的葡萄糖胺聚糖表达没有变化。一旦芽基形成，基质的降解便停止。随着芽基的分化，成熟肢体的细胞外基质重组。基质降解的停止可能涉及到金属蛋白酶抑制剂表达 (TIMPS) 的上调以及 MMPs 表达下调和酸性水解酶表达的下调。在再生肢体方面，无论是调节蛋白酶的活性还是蛋白酶抑制剂的表达尚无系统研究。

三、芽基细胞增殖与存活需要的表皮和神经源物质

芽基干细胞的存活和增殖需要受一些内分泌激素代谢影响，主要是胰岛素，生长激素，氢化可的松，和甲状腺素等 (Globus and Vethamany Globus, 1985)，但是也高度依赖一些 AEC 尖端表皮帽产生的特殊因子 (Thornton, 1986) 和芽基的神经物质 (Singer, 1965)。图 14.8 表明，一些表皮和神经的分子调节芽基细胞存活和增殖。它们应满足 4 个标准 (Brockes, 1984): (1)从 AEC 或者神经末梢分裂出来进入芽基; (2)切除能导致芽基细胞分子丢失 AEC 和神经物质; (3)分子能够代替 AEC 和神经物质来维持有丝分裂和/或促进再生完成; (4)分子的选择性中和作用可以取消芽基细胞的促有丝分裂的影响。很多候选分子至少要满足一条标准，只有一种是例外，就是转铁蛋白，能够满足全部 4 条标准。然而，多数的候选分子还尚未进行全部标准的实验检测。

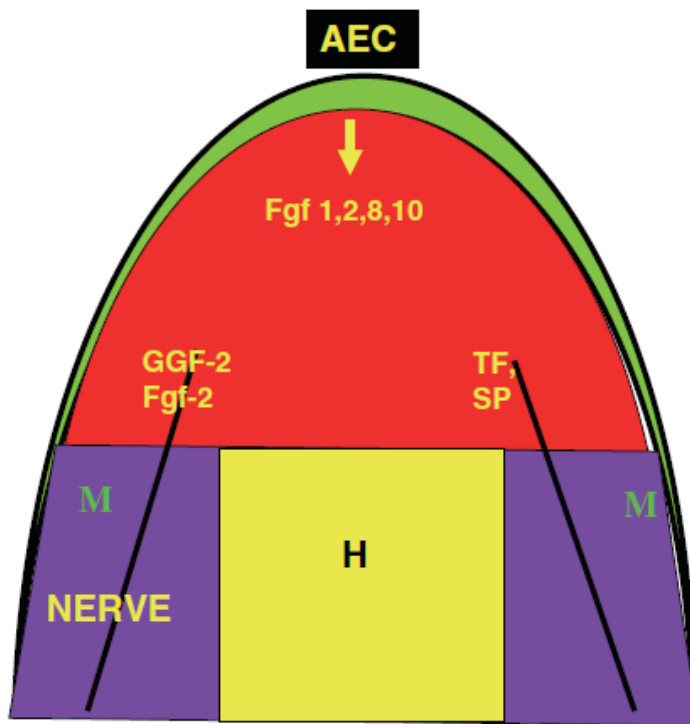


图 14.8 尖端表皮帽和神经产生的因子对芽基细胞的存活和增殖有至关重要的作用。FGF1, 2, 6, 10=成纤维细胞生长因子，GGF-2=神经胶质细胞生长因子-2，TF=转铁蛋白，SP=物质 P，H=肱骨，M=肌细胞

截肢肢体如果同时伴有切断脊神经III、IV和V而完全失去神经支配，显然不

能阻止受伤表皮迁移,组织溶解或者去分化。但是,去分化细胞不发生有丝分裂,芽基也不能够形成 (Tassava and Mescher, 1975)。去神经支配不改变蛋白质合成模式,但是抑制了 RNA 和蛋白质的合成 (Morzlock and Stocum, 1972; Singer, 1978)。只要纤维的数量达到某一临界值,感觉或运动神经纤维都支持再生 (Singer, 1952, 1965)。TUNEL 染色显示去分化细胞在截肢和去神经支配的肢体中会凋亡并被巨噬细胞清除 (Mescher et al., 2000)。在截肢和去神经支配的肢体,受伤表皮不能形成 AEC。

再生神经纤维和芽基细胞的关系是相辅相成的。再生的神经纤维进入芽基需要依赖几种芽基细胞产生的因子。体外研究证实,神经和芽基组织共同培养可促使神经细胞的轴突再生 (Richmond and Pollack, 1983)。一些已知的神经生长因子如脑源性神经营养因子 (BDNF), 神经营养因子 3 和 4 (NT 3.4), 胶质细胞衍生的神经营养因子 (GDNF), 肝细胞生长因子/离散因子 (HGF/SF) 可以代替部分芽基组织在促进轴突再生的过程中作用 (Tonge and Leclere, 2000)。这些因子中有几个均由施旺细胞产生,可以在哺乳动物再生末梢神经的过程中促进神经存活和轴突生长。轴突比芽基组织生长能力更强,提示其他不明来源的因子可能是由芽基细胞产生并促进神经存活和轴突生长。

四、刺激青蛙肢体的再生

无尾动物的后趾甲的再生,发生在他们分化的早期。但是,缺乏了一种从近端到远端肢体渐进分化的协调再生能力 (Guyenot, 1927; Dent, 1962)。这种无尾动物肢体再生中能力的渐增缺乏与发育的高级阶段中芽基细胞的细胞特征改变有关 (Korneluk and Liversage, 1984; Wolfe et al., 2000)。对爪蟾肢体再生缺乏的研究表明,组织溶解最小,芽基的形态是成纤维结构大于间充质结构,而且受伤表皮下的基膜和皮组织大量增殖, AEC 厚度减少,这减少了对神经和血管进入芽基 (Wolfe et al., 2000)。成纤维细胞的增殖和分化成不同软骨结节融合形成不同长度有结缔组织包围的对称软骨,但是没有肌肉 (图 14. 26)。其他青蛙的肢体都不能够再生 (Stocum, 1995)。

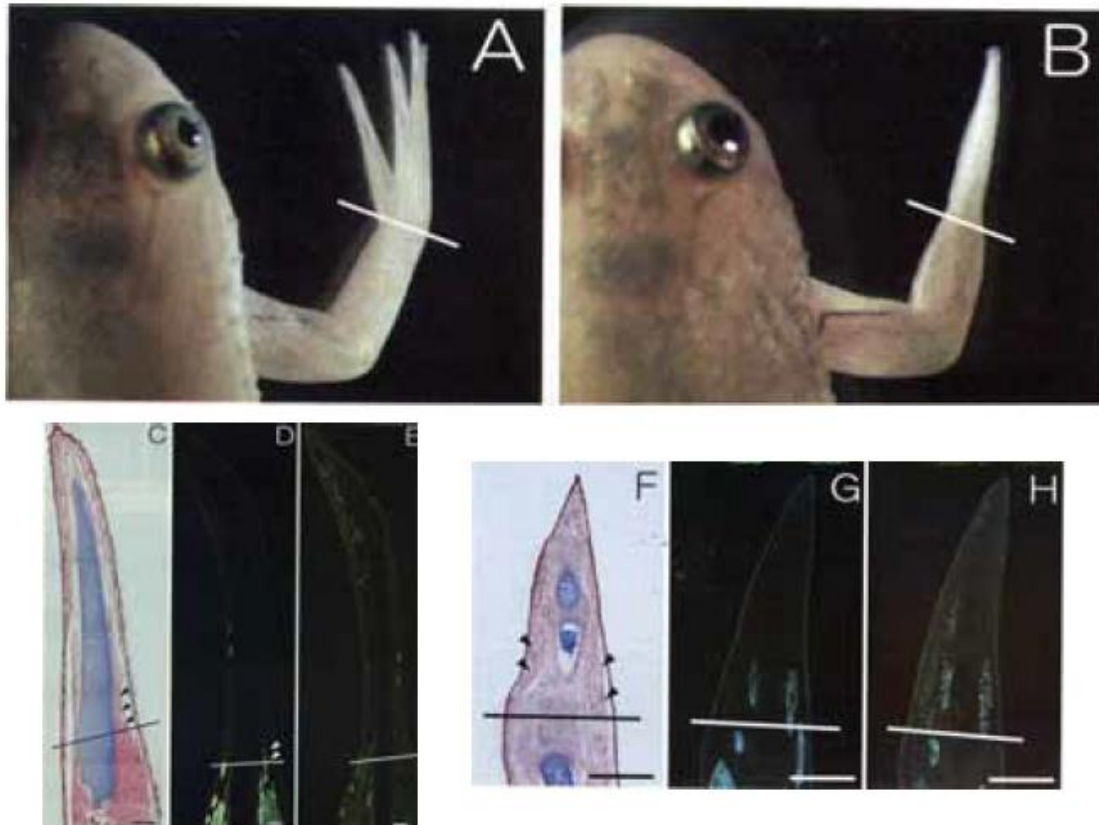


图 14-26 非洲蛙的肢体再生。(A) 未截肢的右前肢。(B) 截肢后通过腕骨的刺突再生。(C) 纵切面刺突用苏木紫或阿辛蓝染色。软骨染成蓝色，肌肉染成红色。划线显示截肢水平。(D, E) 刺突免疫染色对肌肉标记物肌球蛋白链 (D) 卫星细胞标记物 Pax-7 (E) (都是绿色)。刺突没有，箭头所指是一些刺突表面的肌纤维细胞。(F-H) 在 52 阶段后肢的鹿茸水平面的再生。与 C-E 染色相同，除了 G 用肌肉标记物 MF20 来染色。这二种都显示再生过程中肌肉和卫星细胞的存在。

在爪蟾肢体早期到晚期蝌蚪阶段中，这种再生活性的缺乏的转换都与神经支配需求或受伤表皮形成 AEC 的能力无关。将再生缺乏的肢芽移植到有再生活性的肢芽中，或者反过来，都不可能改变捐赠者肢芽的再生能力 (Sessions and Bryant, 1988; Filoni et al., 1991)。然而，其他证据显示，再生能力与免疫系统的成熟相关，而这可能是导致再生能力丢失的最大原因 (Harty et al., 2003; Mescher and Neff, 2005)。在再生能力强的早期的青蛙蝌蚪和幼虫中的炎症免疫应答是不存在的或者是极微量的。然而在成虫爪蟾中，它的炎症免疫应答同哺乳动物类似 (Robert and Cohen, 1998)。蝌蚪的抗原提呈细胞仅有 MHC II 蛋白。MHC I 蛋

白直到 55 段才会表达，并且直到变形结束一直在一个很低的水平。这些表达上的差异与三种不同淋巴细胞的连续性渗入发育中的胸腺相关，淋巴细胞来源于不同群体的前体细胞。这三种淋巴细胞分别渗入对应的变态前幼虫和小蛙/成蛙免疫系统 (Du Pasquier et al., 1989; Du Pasquier and Flajnik, 1999; Rollins-Smith et al., 1997; Turpen, 1998)。免疫系统的差别主要表现在他们对皮肤移植的反应。蝌蚪可以接受微小的组织相容性的错配的皮肤移植，然而青蛙却不能。从蝌蚪身上取下皮肤冰冻保存，当同一个蝌蚪变成青蛙后把这块皮肤移植给它，它仍然不接受移植 (Izutsu and Yoshizato, 1993)。这些不同反应与免疫细胞和受伤部位细胞因子的形成有关 (Harty et al., 2003; Mescher and Neff, 2005)。很有可能，成年炎症反应通过早期基膜和成纤维组织的免疫沉淀阻止了青蛙肢体再生组织相互作用。

已经做出许多努力来改进晚期无尾动物如蝌蚪，小蛙，成年蛙的肢体的再生能力。通过重复创伤和刺激组织溶解的增多或增加神经支持使芽基细胞的存活和增殖变多 (Singer, 1954; Polezhaev, 1972; Cecil and Tassava, 1986; Stocum, 1995)。这些实验总的结论是当再生能力几乎消失时，在晚期蝌蚪中引导芽基形成相对容易。然而，在小蛙和成年蛙肢体中引导芽基形成困难的多。然而，已有报道称被维甲酸 A 治疗过的芽基可以导致异常末肢再生 (Sharma and Niazi, 1979; Niazi et al., 1979; Cecil and Tassava, 1986)。

第三节 哺乳动物的附肢再生

有几种哺乳动物的附肢也能够在切割处再生。这些有趣的情况是在兔子和 MRL 鼠的耳朵，雄鹿鹿茸的再生，胎鼠和人的指尖再生等事例中发现的。成年鼠和人的指尖能够再生，但这个再生不是通过芽基形成得到的。

一、耳组织的再生

1. 兔子的耳组织

兔子的耳朵是由一片僵硬的纤维软骨覆盖皮肤组成。当耳朵上击穿一个 1-3 cm 的洞时，它们能够再生组织，与其他野生型哺乳动物比较，兔子的耳朵在受伤后，在洞的周围愈合 (Vorontsova and Liosner, 1960; Joseph and Dyson, 1966; Grimes and Goss, 1970)。兔子耳朵再生组织从缺陷边缘向内离心性的生长。软骨形成发生在再生组织形成 3 周左右，能把洞完全愈合则需 6-8 周时间。切除脚趾

移植到兔子耳洞中不会再生，这表明，耳组织周围再生环境并不能促进脚趾的再生 (Williams-Boyce and Daniel, 1980)。

组织学研究表明，兔子耳洞闭合是通过切割处组织的再生 (Goss and Grimes, 1975)。受伤边缘的间充质细胞迅速形成环形芽基并且分化成新的真皮和软骨，随着芽基细胞的生长耳洞闭合，表皮向下生长穿透受伤边缘的软骨和真皮。这种向下生长不能够在狗和羊的耳朵伤口处观测到，但是，却是成年蝾螈再生肢体的特征。再生的细胞有可能来源于一种干细胞或由真皮成纤维细胞或软骨分化而来。

兔子耳组织的再生需要耳部皮肤和软骨。如果用腹部的皮肤代替耳朵的皮肤，腹部皮肤上的洞不能够完整的闭合。在移植腹部皮肤前耳软骨被摘除，或者是软骨和耳皮肤都被摘除，那么在无软骨区的这个耳洞不会闭合了 (Goss and Grimes, 1972)。接受过 X 线照射的耳朵不会再生。但是，如果植入未经照射的软骨，其再生和正常耳朵再生很接近。未经照射的耳朵植入经照射的软骨显示 80% 能够闭合。但是，再生组织不会有软骨形成 (Grimes, 1974)。总的来说，这些数据表明，皮肤可以维持部分的再生，但不能完全再生，软骨是再生必须的原材料。这可能意味着软骨和真皮是芽基形成的主要细胞来源，软骨还起着一个诱发皮肤细胞形成芽基的作用，或者是二种细胞的协同作用。

2 鼠的耳组织

成年鼠的耳软骨不能再生。在耳朵上做切开的伤口后，一个纤维化瘢痕会在切开的软骨边缘形成，染色显示有大量的 I 型胶原形成 (Wagner et al., 2001)。然而，在新生鼠的耳朵上的相似的软骨切口的修复则需要软骨细胞的再生，这个过程表达 II 型胶原，再生软骨细胞来源于伤口处新生软骨细胞的增生。

在野生型 C57B1/6 小鼠中，耳洞的愈合就像标准的切开皮肤的再生：它们重复上皮形成，在边缘处形成瘢痕组织，并且在最小直径内闭合。MRL/lpr 鼠由于 fas 基因的突变而显示出狼疮样症状，这样的鼠被用作一种自身免疫性疾病的实验模型。有趣的是，在 MRL/lpr 鼠的耳洞至少需要 4 周才能完全闭合 (Heber-Katz et al., 2004)。图 14.30 说明这种闭合与兔耳洞闭合的过程非常相似。组织学研究表明，这种闭合是通过受伤边缘的芽基细胞增殖完成的。芽基再生耳组织的正常结构，包括支持的软骨 (Desquenue-Clark et al., 1998; Kench et al.,

1999; Heber-Katz et al., 2004)。在 MRL/MpJ 鼠的耳洞实验观察中得到类似的结果 (Masinde et al., 2001)。如兔的耳洞闭合，芽基的原始细胞无论是从一种干细胞转化而来还是由真皮成纤维细胞和软骨细胞去分化而来尚不清楚。有趣的是，MRL/MpJ 鼠背侧的皮肤伤口修复是通过皮肤的纤维化而来 (Rajnoch et al., 2003; Metcalfe. and Ferguson, 2005)。

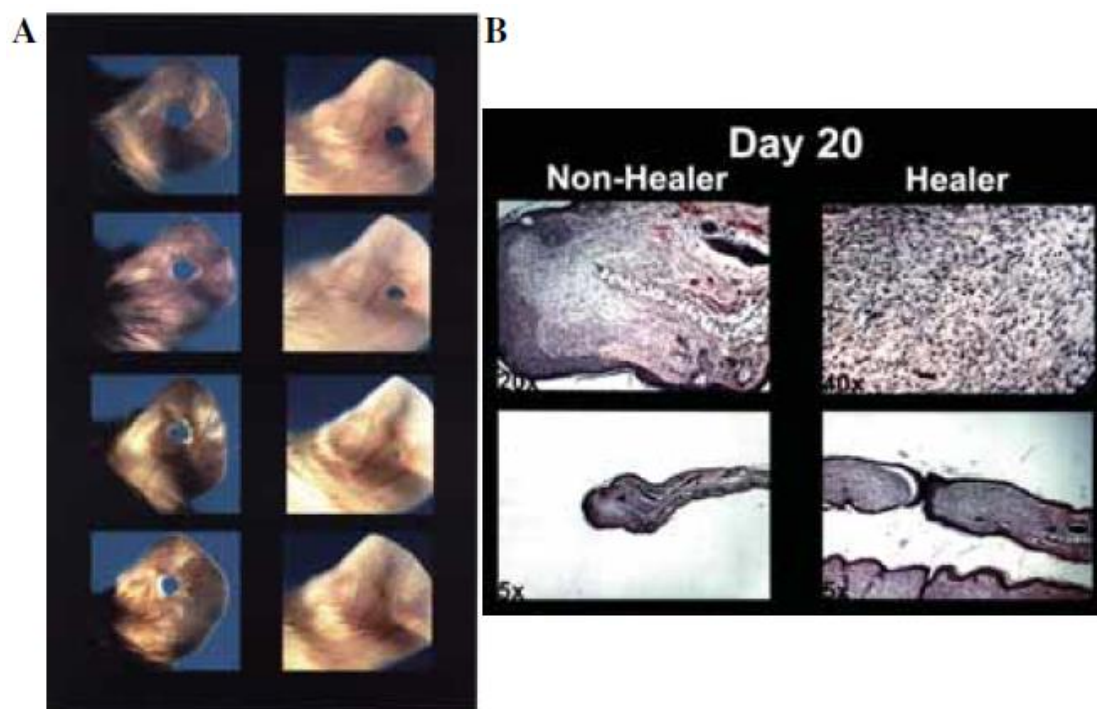


图 14-30 MRL 鼠与野生型鼠的耳组织再生。(A)在野生型鼠耳洞(左边)仅仅局部闭合，然而 MRL 鼠的耳洞(右侧)完全闭合。(B)左边野生型和右边 MRL 鼠耳洞愈合处切片显示野生型边缘有损伤瘢痕，MRL 鼠损伤边缘形成的芽基并且增长闭合伤口 (Courtesy of Dr. Ellen Heber-katz)。

在截肢的两栖动物肢体中，再生芽基的形成依赖 AEC 和下面的芽基间充质中间基膜的缺失，这种缺失导致这二者之间直接进行免疫沟通。在两栖动物肢体再生过程中，基膜是防止 MMPs 过早成熟。在再生兔耳中，基膜的状态还没有报道 (Goss and Grimes, 1975)，但是，MRL 鼠研究表明，像两栖动物四肢再生一样，鼠的耳洞闭合同基膜的缺乏有关 (Gourevich et al., 2003; Heber-Katz et al., 2004)。基膜最初是重新建立，但在受伤后的 5 天就会消失，然而，在野生型鼠中，它依然存在。这种差异的原因是 MRL 鼠的 MMP-2 和 MMP-9 的高表达和活跃，而这又是炎症细胞较多的原因，MRL 鼠的耳朵也具有少量的蛋白酶抑制剂 (TIMPs)。

二、鹿茸的再生

鹿茸是双生的，枝骨是从雄鹿的前额延伸出去的。在温带气候中，鹿茸的作用是在秋天交配季节用来显示雄性力量和作为争夺雌鹿的武器。鹿茸会在春天脱落，由于破骨细胞的作用，鹿茸的基底部骨生长的区域变狭窄，受到侵蚀而致 (Goss et al., 1992)。在夏季期，重新生成新鹿茸需要 4 个月的时间 (Goss, 1970, 1974, 1983, 1995; Price et al., 1996)。在哺乳动物中，鹿茸是唯一一种自然地反复的完整的在附件割处再生的例子，因此它是一个有价值的研究模型来帮助人们理解哺乳动物的附件是如何再生的。

雄鹿的第一对鹿角的发育类似于芽肢，幼鹿成熟过程中的睾酮素水平影响幼鹿形成对称芽基蒂，这些蒂是大量的被骨膜包被的海绵骨覆盖在额骨上的突起，突起的骨头凸出头骨的额骨。蒂骨膜底层的纺锤形间充质细胞形成芽基。实验中已经证实在手术割除骨膜后其结果是鹿角信息缺失，移植蒂骨膜到前额或者前脚上，其结果是在异位形成鹿茸 (Goss et al., 1964; Hartwig, 1968a, b; Hartwig and Schrudde, 1974; Goss and Powel, 1985)。蒂引导皮肤上的表皮变成鹿茸的表皮，因其多细毛而得名叫天鹅绒 (Goss, 1972)。

鹿茸再生通常发生在第一对鹿茸退掉以后。鹿茸纵向生长的速率随着成年鹿的大小而增长，如大麋鹿的鹿茸长的速度每天超过 2 厘米。脱鹿茸后会有一开放性伤口曝露蒂，间充质细胞来源形成的芽基再生鹿茸并不像第一对鹿茸的发育那么清楚。组织学研究表明，新鹿茸芽基细胞可能来源于蒂骨，骨膜和伤口周围的真皮 (Wislocki and Waldo, 1953; Goss, 1992, 1995)。不像两栖动物的肢体再生，鹿茸再生不需要神经支配 (Goss, 1985)。

不管源细胞是什么，组织学和分子学研究表明，鹿茸再生是一个改良型软骨形成的过程 (图 14.31)。一些区域分化后可以辨认出类似胚胎软骨长骨末梢部分的分化 (Price et al., 1996)。在不停生长的鹿茸分支的表皮下，间充质芽基形成一个帽结构，真皮插入间充质细胞和鹿茸表皮之间。近端的间充质细胞，软骨细胞排列成柱状后，分化成一个由近及远的序列。成熟的软骨细胞朝着鹿茸的基底肥大增长。

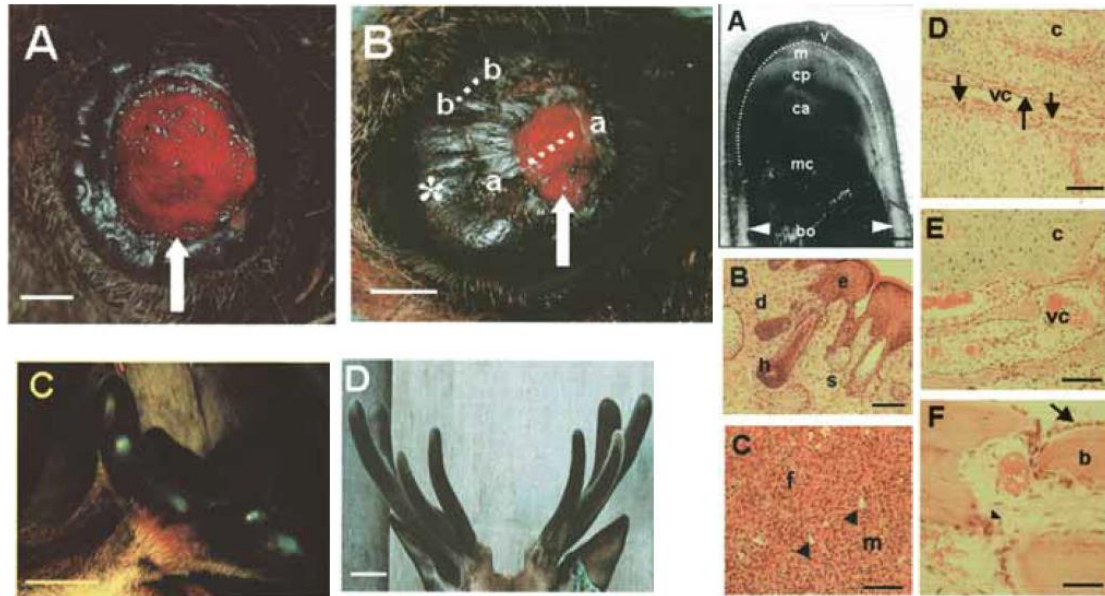


图14-31 红鹿茸的再生。(左)解剖学示意图。(A) 切除鹿茸后24小时。(B) 切除鹿茸后7天的芽基。白色箭头显示表皮边缘再生。虚线a-a和b-b显示那一块区域被切断。星号显示鹿茸枝桠的位置。(C和D) 分别显示4周和8周的生长情况。(右)鹿茸尖的组织溶解。(A)纵切面显示尖端分化到底部分化来源的鹿茸芽基区域。V=鹿茸表皮，m=间充质，cp=软骨祖细胞区域，ca=未钙化的软骨，mc=钙化的软骨，bo=骨软骨膜，f=纤维端，m=间充质段。箭头指示血管。(D)未钙化软骨，c=血管分开分化的软骨细胞(vc)。箭头显示血管周围组织。(E)钙化的，肥大的软骨(c)和大血管(vc)。(F)骨，箭显示成骨细胞，箭头指示一个破骨细胞。

过度肥大的软骨细胞基质的钙化，随后由破骨细胞降解并且由成骨细胞分泌的骨基质代替。正常骨组织再生通过血管侵入无血管的软骨模型中进行骨化，血管周围的间充质细胞开始转化成成骨细胞和骨髓细胞分泌骨基质。相比之下，鹿茸的软骨模板从一开始就有许多血管及周围的成骨细胞，成骨细胞使骨基质形成从一开始就是一个完整的模板的组成部分。这种骨化的机制与鹿茸骨不含有骨髓这个情况有关。

分子方面，鹿茸再生和软骨发育或骨折愈合相类似。I型胶原在鹿茸生长的尖端的间充质细胞中是主要表达基因 (Price et al., 1996)。硫酸化的GAGs，II型胶原，蛋白多糖和蛋白聚糖蛋白在软骨细胞分化中表达，X型胶原在肥大缘故细胞中表达 (Moello et al., 1963; Frasier et al., 1975; Price et al., 1994,1996)。破骨细胞改变鹿茸软骨模型首次出现在血管周围组织中，表明他们原始来源于单核细胞，与这些附件的软骨有相同表型 (Faucheux et al., 2001)。

三、小鼠和人指尖的再生

1. 哺乳动物的胚胎肢芽能够再生，但是这种再生能力随着末稍分化而失去

多项研究表明，大鼠和小鼠胚胎肢芽能够在其早期发育中再生。但是，会在肢芽分化时失去这种能力。在子宫内，鼠前肢被截肢后呈现各种水平的再生发育，在截肢表面那些未分化的细胞形成一个结节。Deuchar (1976) 从子宫取出 11.5 天的小鼠胚胎，在基底部截去前肢，然后在旋转管培养胚胎 44 个小时。11.5 天的肢芽是未分化的阶段，相当于由 Wanek 等人构建的 2/3 小鼠肢芽形成阶段 (1989)，其中 29/32 截肢胚胎能够再生性状和大小都类似的肢芽，他们会有 AER，并可持续分化，体外研究中发现未分化的再生大鼠和小鼠肢芽也被 Chan (1991) 等人和 Lee 和 Chan (1991) 报道过。

早期小鸡肢芽不能够在截肢后重组 AER 所以不能再生。当早期肢芽末梢带有 AER 被植入到近端水平可以再生肢体 (Hampe, 1959)；来源于晚期阶段芽肢 (24-25 阶段) 细胞则有骨再生能力 (Hayamizu et al., 1994)。在截肢小鸡的 24-25 阶段肢芽中，FGF-2 和 FGF-4 能够代替 AER 诱导肢体再生过程 (Taylor et al., 1994; Kostakopoulou et al., 1996, 1997)。FGF-2 和 FGF-4 可以诱导肢芽中 *Msx1*, *Shh*, 和 *HoxD13* 等基因的上调 (Hayamizu et al., 1994; Kostakopoulou et al., 1996)。

小鼠肢芽的再生在远端的区域芽肢分化的过程中变得很严格 (Wanek et al., 1989; Reginelli et al., 1995)。在 7/8 阶段 (胚胎 12.5 天)，小鼠子宫内截肢后的一个或二个肢体末梢再生是通过指骨或趾骨再生形成胚胎脚板，但是不能通过近端来再生 (Wanek et al., 1989)。小鼠的 11 阶段 (胚胎 14.5 天) 时，肢体末梢的再生能力非常有限 (Reginelli et al., 1995)。

已经有报道称新生儿附属鼠肢体截肢后，可以通过接合骨部分进行再生 (Mizell, 1968; Mizell and Isaacs, 1970)，在截肢水平面实际上是通过踝骨和趾骨再生的 (Fleming and Tassava, 1981)。因此，新生鼠也能再生末梢肢体，但再生肢体不具有原肢的骨架结构

2. 哺乳动物的末梢肢体再生是通过 *Msx1* 和 BMP4 的调节

Msx1 涉及羊膜动物肢芽的生长和两栖动物肢体再生的调节。*Msx1* 在肢芽顶端的间充质和再生芽基中均有表达。但是，他的表达依赖于 AER 和 AEC 中 FGFs

的表达,就像通过 BMPs (Pizette et al., 2001), TGF β (Ganan et al., 1996), 和 RA 的通路调节一样 (Wang and Sassoon, 1995)。在这个阶段,再生发生仅仅是发育中的末梢指骨部分,因为末梢指骨是唯一持续表达 *Msx1* 的地方 (Reginelli et al., 1995)。

体内研究截肢的第 11 阶段指骨和器官培养 (Han et al., 2003)表明,尖端表皮再生是 *Msx1* 和 *BMP4* 基因在末梢指骨的压缩软骨周围的芽基的尖端间充质细胞中的联合表达所致 (图 14.33)。如果截肢近端无这二种基因表达,那么指骨不能再生。此外, *Msx2* 基因在尖端表皮和其下的间充质中也表达。体内和体外研究发现,截肢后再生的 *Msx2* 基因表达的频率是 90%。定向 *Msx1* 基因的突变鼠再生频率在体内是 38%,体外是 28%。与 *BMP4* (1,000ng/ml) 联合培养的突变体的再生频率可以恢复到 86%,但是,用 *BMP4* 的抗体, *Noggin* (200ng/ml),则将再生频率减少到 8%,野生型培养中没有 *Msx1* 表达,再生频率为 18%。野生型鼠的指尖在再生末梢指骨中软骨化的部分表达 *Ihh*,而 *HoxC13* 在甲床表皮中表达。但是,有些 *Msx1* 突变的鼠不能再生指尖,说明 *Msx1* 和 *Msx2* 的功能性表达有重叠的部分, *BMP4* 是 *Msx1* 和 *Msx2* 的下游靶位基因。

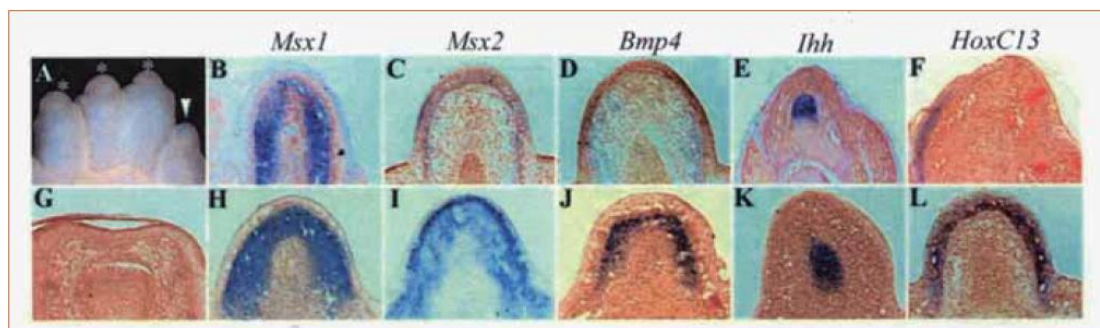


图14-33 体内实验证明鼠胎儿指尖通过末梢指节截断再生。(A)E17.5 指夹,侧面影像。(B-F),在E14.5时截肢四天后指尖的再生过程。*Msx-1*在末梢指骨的间充质周围表达, *Msx-2*在尖端表皮表达, *Bmp-4*在尖端间充质中低水平表达, *Ihh*在重建的末梢指骨中表达, *Hoxc13*在指甲中表达。(G)若指尖截断在近端,则末梢指尖不能再生。(H-L)在末梢指骨截断两天后原位杂交发现, *Msx1*, *Msx2*, *Bmp-4*和 *Ihh*高水平表达。

体外实验中,人类胚胎末梢指骨同样可以成功再生 (Zaaijer, 1958; Rajan and Hopkins, 1970; Zeitinger and Holbrook, 1997; Allan et al., 2005). Allan et al. (2005)发现 *Msx1* 基因的表达是在人类胚胎末梢指骨指甲的下方,从怀孕开始的 53-67天开始形成。在指骨被截断四天后,表皮表达角蛋白 K14 和 K19,近伤口有成

纤维细胞样的细胞大量增殖，在迁移表皮内 *Msx1* 基因高表达，而培养的末梢指骨的近端没有增殖反应，骨端的皮肤收缩；远端截肢部位的增殖同胎龄相关。

3. 成年哺乳动物维持指尖的再生能力最远可达到最后一个趾尖关节

成年哺乳动物末梢指骨的再生能力首次在人类身上得到验证。有一些孩子们的指尖断指后的再生病例报道 (Douglas, 1972; Illingworth, 1974; Rosenthal et al., 1979)。手指和脚趾末端的指骨再生也在成年人身上发生 (Neumann, 1988) (图 14.34)。值得注意的是，再生仅仅发生在伤口曝露的情况下，而不是遵循了普通医疗实践中，截肢表面皮肤闭合的情况。由于开放性伤口的表皮明显愈合，说明上皮间充质的相互作用是成人和其他哺乳动物指尖再生的必要条件，就如同两栖动物肢体再生。



图 14-34 成人及儿童指尖再生。(A)左，七岁的女孩的指尖在一次自行车事故中被截断，没有进行缝合和换敷料治疗。右，指尖在八周内再生 (Courtesy of Dr. Christopher Allen)。(B) 一个七十六岁老人的 3, 4, 5 指尖在 29 岁时的一次机械事故中被截断后，未经任何治疗再生。划线部分显示的是当时截断的部位。

胎鼠的再生末梢肢体的能力可以保持到成年。Borgens (1982) 将 4 周大的老鼠的中趾近端截断，截趾的中趾在 4 周时间内再生并且看似正常，其外观和形态学结构均正常。如果截肢水平面是近侧端的关节，则趾再生不会发生，人类的指

尖再生也是如此 (Neufeld and Zhao, 1995)。尽管胎鼠的指尖再生末梢指骨是由软骨完成的，成人指尖则是由骨沉积到剩余骨后直接再生的。成纤维细胞在截肢部位参与再生指骨，他们可能改造真皮，骨膜，结缔组织，骨和脂肪的形成。甲母质，甲床和甲板则从表皮再生而来。小鼠指尖的血管供应较丰富而利于再生 (Said et al., 2004)，甲上皮和其他一些上皮间充质为再生提供细胞来源 (Mohammad et al., 1999)。Han et al. (2003) 报道在新生鼠的截断残留的末梢指骨中，*Msx1*，*Msx2*，*BMP4* 和 *HOXC13* 在甲母质下的结缔组织和甲床都有表达，鉴于 *Msx1* 和 *BMP4* 在胎鼠指尖再生的间充质细胞中有表达，指甲下的结缔组织可能是末梢指尖再生的细胞来源。

小 结

两栖动物能够通过切割处再生来代替它们的肢体和下颚。鹿、麋鹿和驼鹿可再生鹿茸，兔子和 MRL 鼠可再生耳组织。小鼠，兔子和人均能再生指尖。这些现象提供了研究附件再生能力的模型。

我们知道很多两栖动物肢体再生的机制。再生完成需要得益于截断面真皮、软骨和肌肉细胞去分化的芽基形成。去分化是由蛋白酶介导的细胞外基质的降解和细胞逆分化到一个类胚胎型的结构。芽基细胞重新进入细胞周期是由一个凝血酶素诱导激活一个尚不明身份的蛋白所致，类似于晶体再生时 PECs 重新进入细胞周期。我们对芽基细胞中表达的 *Notch* 信号转导途径介导的去分化机制尚没有一个清楚的结论，但是也可能涉及其他信号途径。体外研究中，小鼠肌纤维细胞的去分化是通过三倍体嘌呤取代二倍体嘌呤，这意味着这些分子中可能存在与再生肢体相同作用的因子。芽基细胞需要尖端表皮帽 (Apical epidermal Cap, AEC) 中的生长因子和营养因子促使他们存活，及促进神经的增殖，并需要 AEC 和神经提供的 FGFs。此外，芽基细胞的维持还需要神经角质生长因子-2 (GGF-2)、P 物质和神经来源的转铁蛋白。

芽基从一开始就是一个自我组织的实体。其发育的特点是被植入异位时也不能改变其发育轨迹。这种自我组织的机制可能是由于局部细胞的交互作用导致插层反应，在截肢肢体结构边缘存在早期芽基所致。肢体被看做一个三维立体的“正常邻居”的地图，其中每个细胞都知道自己的位置及和其他细胞的相对位置。

当肢体被截断后，细胞分化继承了他们在 PD 轴的定位和在肢体周长上的定位。这些定位构成了再生的肢体 PD 轴和周长上的边界。远端边界形成的机制尚不清楚，可能涉及随机选取直接相邻 AEC 细胞的分类定位。近端和远端边界细胞的相互作用，可以诱导任何没有 PD 轴定位的插层细胞的增殖。植入实验证明，标记的细胞沿着早期芽基的 PD 轴生长。体内和体外研究，利用 RA 治疗的粘附定量实验，证明定位是细胞表面的编码蛋白决定的。*Prod1* 基因调控 PD 轴定位，在哺乳动物中，类似的分子是 CD59，它与 *Prod1* 基因的区别是 CD59 受 RA 调控表达。在芽基自我组织过程中，模式基因通过细胞相互作用被激活，它同已知的发育中胚胎芽肢相类似。在 PD 轴上，*HoxD10* 基因、*Meis1* 基因和 *Meis2* 基因都参与了柱骨的定位构建，*HoxA13* 基因参与肢身的定位构建。在 AP 轴，*Shh* 基因在建立组织模式和定位中起重要作用，*Lmx-1* 基因在背侧组织模型的生长中起重要作用。

无尾动物的两栖动物随着它们从蝌蚪到青蛙的转变过程，他们失去再生的能力。在幼蛙和成年蛙中诱导芽基形成很困难，有证据表明，改变组织环境，特别是免疫系统，是改变再生能力的主要原因。因此，改进成年蛙肢体再生应答的途径可能是抑制它们自身的免疫系统。再生机能不全伴随着 FGF-8，FGF-10，BMP4 和 HGF 的表达缺乏，已发现在再生机能活跃和再生机能缺乏的肢体中基因表达有显著差别，这些基因的鉴定，随着它们表达模式和功能作用的深入研究，将为再生医学提供新的理论指导。

两栖类 Urodele 的上下颞骨也通过未分化细胞形成的芽基再生。上颞骨的再生是完整的，包括牙齿的再生和牙蕾的修复，但是下颞骨的再生会缺少舌头和舌骨。颞骨的再生不同于肢体的再生是因为它不需要依赖神经。尽管颞骨芽基有顶端受伤表皮增厚，但是我们不知道颞骨的再生是否依赖表皮。

一直以来，哺乳动物的附件再生研究的比较少。兔子和 MRL 鼠的耳组织再生研究发现重复穿孔的组织、皮肤和软骨，通过一种环状边缘芽基进行再生。但是，形成芽基的细胞类型尚不明确。在 MRL 鼠中，已经确定有 16 个遗传位点同再生相关联。目前，还不明确耳组织的再生是否需要神经和受伤表皮组织。鹿茸的骨结构再生来源于骨膜的间充质干细胞和伤口周围的真皮细胞形成的芽基。芽基细胞增殖和分化成一个软骨模板。RA 是间充质干细胞增殖的重要刺激源，

软骨细胞的分化速率是依靠 *Ihh/PhrP/PPR* 途径调控，随着鹿茸的生长，破骨细胞骨的结构形成改建，破骨细胞的分化通过 *PTHrp/PPR* 和 *RANKL/RANK* 途径调节。

在截肢后，如果顶端外胚层脊（Apical ectodermal ridge，AER）能复位，早期鼠和小鸡的肢芽能够再生。在鼠的胚胎后期阶段，末梢指骨的再生的能力受限，同肢体的再生不同。末梢指骨再生需要间充质芽基的形成。这种芽基的形成与分化都是依靠 *BMP4* 基因的促进。在成功指骨再生中，*Msx1* 基因表达，而 *BMP4* 基因似乎是它的下游靶位。截肢后，培养人的胎儿指骨同样产生再生反应，高表达 *Msx1* 基因，而且，成年鼠和人类可维持这种末梢指骨再生的能力，指尖再生是通过直接的骨沉积，而不是通过芽基。已证明截肢后，试着诱导成年末梢骨的再生，通过近端骨刺激、胰蛋白酶和电流作用都失败。近年有报道牵张力的刺激可以激活并维持肢体的再生能力。其机理现尚需进一步研究。

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