Journal of Nepal Dental Association (2010), Vol. 11, No. 2, Jul.-Dec., 193-198

Review Article

Recent understanding of biology of orthodontic tooth movement: A Review

Parajuli U[1], Mishra P[2], Bhattarai P[3]

[1]Resident, [2]Professor, [3]Associate Professor, Department of Orthodontics and Dentofacial Orthopaedics, NAMS, Bir Hospital

Abstract

Orthodontic treatment involves the application of force which has complex interaction with the paradental tissues. The Orthodontists should have a thorough understanding of biological aspects of orthodontic force (OF) and orthodontic tooth movement (OTM).This understanding will help us to apply appropriate mechanics so that we can maximize the rate of tooth movement with healthy biology and comfort to the patient. This review article attempts to compile the published articles related to OTM and amalgamate them so as to formulate a clear understanding of biology of OTM in a concise form. The articles were searched by conduction of an electronic search through Pub Med and Hinari and references from citations within the articles.

Key words: Orthodontic tooth movement, Biological response

Introduction

Accurate and precise control of tooth movement can be optimized with the proper use of mechanics and knowledge of the subsequent tissue response[1]. Orthodontic tooth movement (OTM) is characterized by remodelling changes in dental and paradental tissues, including dental pulp, periodontal ligament (PDL), alveolar bone, and gingiva. OTM differs markedly from physiological dental drift or tooth eruption. The former is uniquely characterized by the abrupt creation of compression and tension regions in the PDL while physiological tooth movement is a slow process[2]. An orthodontic appliance transfers mechanical stresses through the tooth to the periodontium where they are translated into physical, chemical, and electrical signals to cells that activate tissue remodeling to allow tooth movement. To interpret the biological responses to activation of any orthodontic appliance, each interface in the process must be thoroughly understood so that we can optimize the orthodontic treatment and make it comfortable to patients. Current literature has much data on molecular- and genetic-level cellular responses to orthodontic force.

Optimal orthodontic force

In 1932, Schwarz defined optimal orthodontic force (OF) as "the force leading to a change in tissue pressure that approximated the capillary vessels' blood pressure, thus preventing their occlusion in the compressed periodontal ligament." He said that the forces delivered as part of orthodontic treatment should not exceed the capillary bed blood pressure (20-25 g/cm^2 of root surface). If it exceeds this pressure, compression could cause tissue necrosis through "suffocation of the strangulated periodontium."[3] Schwarz's definition was slightly modified by Oppenheim[4], who advocated the use of the lightest force capable of bringing about tooth movement, and by Reitan[5], who demonstrated cell-free compressed areas within the pressure site even in cases where light forces were applied and also advocated the use of very light forces. The current concept of optimal force is based on the hypothesis that a force of a certain magnitude and temporal characteristics (continuous vs intermittent, constant vs declining) would be capable of producing a maximum rate of tooth movement without tissue damage and with maximum patient comfort. The optimal force for tooth movement may differ for each tooth and for each individual patient[6].

Different orthodontic forces and tissue responses

The forces delivered by orthodontic appliances are continuous, interrupted and intermittent. Although most fixed appliances are made to deliver light continuous

Correspondence
Dr. Umesh Parajuli, MDS Resident, Department of Orthodontics and Dentofacial Orthopaedics, NAMS, Bir Hospital
E-mail: drumeshparajuli@gmail.com

forces to affect OTM. The force of an orthodontic appliance which is meant to deliver a continuous force can subside rapidly and thus be interrupted after a limited period of time, such as in torquing movements by an edgewise archwire or labial movement of blocked-out maxillary lateral incisor with the help of ligation. It is not always possible to distinguish between continuous and interrupted movements and the latter act for only comparatively short durations[7]. Nevertheless, it appears that this kind of a force, that starts in a continuous mode and then becomes interrupted, is biologically favorable, particularly when it's initial magnitude is low. In such a case, hyalinized zones might develop in sites of compressed PDL, but, as soon as this necrotic tissue is eliminated and the tooth moves, the force decreases quickly. Finally, the archwire retains its passivity for a while, during which time (rest period) there will be an opportunity for calcification of the newly formed osteoid layer. This rest period between appliance activations is the time used by the tissues for reorganization. This rest can promote favorable cell proliferation for further tissue changes when the appliance is activated again[8].

Removable appliances deliver intermittent force which result in small compression zones in the PDL, short hyalinization periods, and lengthy rest periods when the appliance is removed intermittently. During this time, the tooth moves back to the tension side and remains in normal function. This mode of treatment can improve the paradental circulation and promote an increase in the number of PDL cells, because its fibers usually retain a functional arrangement[8]. Reitan defined this condition as "semi-hyalinization," meaning that in the compressed PDL not all fibers become compressed, and only some cells undergo necrosis. Consequently, osteoclasts might be formed directly along the bone surface subjacent to hyalinized tissue, and bone resorption is less disturbed by hyalinization. This situation might affect smooth and uniform movement of teeth[7].

The amount of force applied for orthodontic mechanotherapy is very important as it has effect on biological response. Light forces produce favorable tooth displacement, resulting in minimal discomfort and pain to the patient, but heavy forces (which exceed capillary blood pressure of 20-25 gm/cm2 of root surface) produce the classic 3-phase reaction–initial strain, lag phase, and progressive tooth movement[9]. Kohno et al[10] in his article reported that light forces can tip teeth without friction, with a constant rate of tooth movement, and without the 3 phases. In a study by Pilon et al[11] in dogs, in which application of 2 forces (50 and 100 cN) to second premolars resulted in the same rate of tooth movement. These findings suggest that, with increasing magnitudes of orthodontic forces, a constant rate of tooth movement would be reached, within a broad range of forces. This observation has led to the conclusion that force magnitude plays only a subordinate role in orthodontic tooth movement[12].

Theories of Orthodontic Tooth Movement

The biologic basis of force-induced tooth movement along with some concepts related to it was extensively investigated in the 19th century which led to the proposal of 2 main mechanisms for tooth movement—the application of pressure and tension to the PDL, and bending of the alveolar bone[13].

The pressure-tension theory

Sandstedt (1904)[13], Oppenheim (1911)[4], and Schwarz (1932)[3] after an extensive histological research led them to hypothesize that a tooth moves in the periodontal space by generating a "pressure side" and a "tension side." The histological presentation on the pressure side will be disorganization of PDL and diminution of fiber production. The cell replication decreases seemingly due to vascular constriction. On the tension side, stimulation produced by stretching of PDL fiber bundles results in an increase in cell replication. This enhanced proliferative activity leads eventually to an increase in fiber production[14]. The resorption of bone on the pressure side and apposition of bone on the tension side leads to tooth movement. The magnitude of applied force should not exceed 20-25 g/cm^2 of root surface[3]. If force exceeds this pressure; compression could cause tissue necrosis through "suffocation of the strangulated periodontium." Further greater force levels will result in physical contact between teeth and bone, yielding resorption in areas of pressure and undermining resorption or hyalinization in adjacent marrow spaces. The concept of pressure-tension in orthodontic tooth movement was evaluated mainly by histologic studies of the periodontium. The first sign of hyalinization is the presence of pyknotic nuclei in cells, followed by areas of acellularity, or cell-free zones. The resolution of the problem starts when cellular elements such as macrophages, foreign body giant cells, and osteoclasts from adjacent undamaged areas invade the necrotic tissue. These cells also resorb the underside of bone immediately adjacent to the necrotic PDL area and remove it together with the necrotic tissue. This process is known as undermining resorption[15].

The bone-bending theory

It was Farrar[16] in 1888, who suggested that alveolar bone bending plays a pivotal role in OTM. This hypothesis was later confirmed with the experiments of Baumrind[14] in rats and Grimm[17] in humans. They concluded that when an orthodontic appliance is activated, forces delivered to the tooth are transmitted to all tissues near force application which will bend bone, tooth, and the solid structures of the PDL. As bone is more elastic than

the other tissues it bends far more readily in response to force application. The active biologic processes that follow bone bending involve bone turnover and renewal of cellular and inorganic fractions. This theory is supported from Wolff's law because it could explain the relative slowness of en-masse tooth retraction; the rapidity of tooth movement toward an extraction site and the relative rapidity of tooth movement in children as compared to adults[14].

Bioelectric signals in orthodontic tooth movement

Bassett and Becker[18] proposed that, in response to applied mechanical forces, there is generation of electric potentials in the stressed tissues. These electric potentials might charge macromolecules that interact with specific sites in cell membranes or mobilize ions across cell membranes. Zengo et al[19] did a study on dogs in which they measured the electric potential in mechanically stressed dog alveolar bone during in-vivo and in-vitro experiments. They demonstrated that the concave side of orthodontically treated bone is electronegative and favors osteoblastic activity, whereas the areas of positivity or electrical neutrality–convex surfaces–showed elevated osteoclastic activity. Bioelectric signals can be divided into piezoelectricity and streaming potentials.

Piezoelectricity is production of electric current when a crystalline structure is deformed as electrons in a lattice are displaced from one part to other. The role of piezoelectricity in OTM is controversial because of the quick decay rate of current and production of current of equal magnitude in opposite direction[6]. The streaming potential has a long decay as compared to piezoelectric current and the mechanically stressed bone cells themselves, not the matrix, are the source of the electric current. Davidovitch et al[20] suggested that a physical relationship exists between mechanical and electrical perturbation of bone. The bending of bone causes 2 classes of stress-generated electrical effects. Their experiments with exogenous electrical currents in conjunction with orthodontic forces demonstrated enhanced cellular activities in the PDL and alveolar bone, as well as rapid tooth movement. Taken together, these findings suggest that bioelectric responses (piezoelectricity and streaming potentials) propagated by bone bending incident to orthodontic force application might function as pivotal cellular first messengers. They also suggested that OTM may be accelerated by the use of force in conjunction with other biologically potent means which can generate a local response and this study has demonstrated that electric currents, in that range of 10 to 20 microamperes, can be used successfully for this purpose[21].

Phases of Orthodontic Tooth Movement

Burstone[9] plotted the rates of tooth movement against time and suggested three phases of tooth movement—an initial phase, a lag phase, and a post lag phase. The initial phase is characterized by rapid tooth movement which is attributed to displacement of the tooth in the PDL space. In the lag phase there is slow rate of tooth movement which is due to the hyalinization of the PDL. The third phase of tooth movement gradually increases which commences after the removable of hyalinized zone.

In a study done by Pilon JJAM et al[11] on beagles, they divided the tooth movement curve into 4 phases. The first phase lasts 24 hours to 2 days and represents the initial movement of the tooth inside its bony socket. It is followed by a second phase, when tooth movement stops for 20 to 30 days. After the removal of necrotic tissue formed during the second phase, tooth movement is accelerated in the third phase and continues into the fourth phase. The third and fourth phases comprise most of the total tooth movement during orthodontic treatment. In a study done in beagles by von Bohl et al[22,23] they suggested that the hyalinization increases with increase in magnitude of force but hyalinization is independent of rate of tooth movement.

Pathways of Orthodontic Tooth Movement

Mostafa et al[24] on the basis of the most recent available information to explain how various stimuli affect bone cells proposed an integrated hypothetical model for the mechanism of tooth movement. They proposed pathways I and II which work concurrently. Pathway I represents a more physiologic response which is associated with normal bone growth and remodeling while Pathway II represents the production of a tissue inflammatory response generated by OF.

Pathway I

In this pathway OF creates vectors of pressure and tension which generates electric polarization.The electric stimulation elicits prostaglandin synthesis which they suggest that the primary biologic orthodontic event is polarization generated by the piezoelectric matrix properties. The prostaglandin synthesis and membrane electrical polarization by piezoelectric process act on the cell surface cyclic nucleotide pathway, generating changes in the levels of cAMP which is correlated with alteration in cell proliferation, differentiation and activation.

Pathway II

In pathway II the tissue injury generated by OF elicits a classic inflammatory response which is triggered along with the classic vascular and cellular infiltration.

The cellular infiltrates lymphocytes, monocytes, and macrophages contribute to the release of prostaglandin, hydrolytic enzymes and cAMP. This will lead to increased osteoclastic activity. There is also increased collagenage activity which suggest of increased bone remodeling.

Theoretical model

Henneman et al[25] in 2008 proposed a theoretical model to elucidate the complex cascade of events after the application of an orthodontic force to a tooth. In this model, the events are divided into four stages: matrix strain and fluid flow, cell strain, cell activation and differentiation, and remodeling.

Matrix strain and fluid flow

The application of an external force to a tooth strains the matrix of the PDL and evokes a fluid flow in the tissue which according to fluid flow theory induces canalicular fluid flow, which results in a shear stress on osteocytes[26,27,28,29]. This along with the microdamage in the bone after force application will attract osteoclasts to the site.

Cell strain

After force application both matrix strain and fluid flow in the PDL and the bone cause deformation of cells. Mediators such as cytokines are produced through integrin signaling and other transduction pathways which in turn activate several types of cells[30].

Cell activation and differentiation

The activation of PDL and bone cells lead to the production of mediators under the effect of mechanical stimulation. At the resorption side, soluble factors such as colony-stimulating factor, receptor activator of nuclear factor kappa β ligand (RANKL), osteoprotegerin, and bone morphogenic proteins regulate osteoclast differentiation has been shown in vitro[31]. These factors are produced by osteocytes present in the alveolar bone and by osteoblasts and fibroblast present in the PDL. Colony-stimulating factor is of importance during the first steps of differentiation. RANKL, and its receptor RANK, stimulate the further differentiation of osteoclasts. Osteoprotegerin(OPG) is a decoy receptor for RANKL that prevents its binding to RANK and thereby further differentiation.[32] During orthodontic tooth movement, increased levels of osteopontin (OPN) have been found at the resorption side which help in attachment of osteoclast to the bone surface through specific integrins (α v β 3). Bone formation at the apposition side of the tooth is a combination of ECM synthesis and mineralization. In vitro studies show that loading of PDL cells results in an increased production of alkaline phosphatase, osteocalcin, and other non-collagenous matrix proteins. After mechanical stimulation in vitro, osteoblasts produce NO, which is, among others, a mediator of bone formation. Several mediators produced by activated PDL and bone cells stimulate ECM synthesis and reduce its degradation. Examples are members of the transforming growth factor- β (TGF-β) superfamily[33]. Increased levels of transforming growth factor- β, cathepsins B and L, and interleukin-1 beta were also found in the crevicular fluid of orthodontically moved teeth in humans and at the apposition side of rat teeth after orthodontic force application. In addition, ECM breakdown is also inhibited by the tissue inhibitors of matrix metalloproteinases (MMPs) produced by PDL cells.

Remodeling

The resorption side, has a picture in which the PDL tissue and alveolar bone is degraded to create space for the moving tooth while new PDL tissue is simultaneously formed to maintain the attachment. The migration and differentiation of the osteoclast is followed by the release of hydrogen ions at the ruffled border, which dissolve the anorganic matrix and, after the organic matrix is resorbed by enzymes such as cathepsins and MMPs[34]. The attachment of the principal fibres of the PDL to the bone is lost. The non-functional fibres that mainly contain type I collagen are degraded and replaced by a loose connective tissue mainly containing type III collagen[11]. The apposition side shows stretching of the principal fibres and remodelling of the PDL. New bone is formed by the activated osteoblasts that first produce new ECM and then mineralize this in a unidirectional manner. New PDL matrix is formed to maintain the width of the PDL and the attachment of the tooth to the alveolar bone. This new PDL contains thick principal fibres of mainly type I collagen for correct attachment of the tooth to the bone. Remarkable increase of collagen type I was found at the apposition side compared with the resorption side after orthodontic force application in rats[35].

Molecular genetics in Orthodontic Tooth Movement

Discoveries in the molecular biology and genetics of bone and connective tissue physiology permit appreciation of the complexity and regulatory sophistication of OTM[36]. Mechanical activation of bone cells is linked to many genes, which produce various enzymes, such as glutamate/ aspartate transporter, inducible nitric oxide synthase, and prostaglandin G/H synthetase. Currently, 96 genes are identified in human osteogenesis. Functionally, 44 are grouped as growth factors (GFs), 30 as extracellular matrix (ECM) proteins, and 8 as cell adhesion molecules. Many genes control the complex process of osteogenesis, the TF Cbfa1 is the earliest expressed and most specific marker of bone formation. Other bone-forming genes encode proteins for growth factors (GFs), bone morphogenetic proteins (BMPs), transforming growth factor-beta (TGF-β), and GF-associated internal signaling molecules. A family of molecules known as “homeobox” proteins (specialized

DNA sequences in exons of many regulatory genes) also helps control osteoblast differentiation. Msx2 could be another regulator of Cbfa1 expression. The homeobox protein Hoxa-2 controls second branchial arch patterning and might suppress both Cbfa1 expression and bone formation. At least 24 genes and 60 proteins are implicated in positive and negative regulation of osteoclastogenesis and osteoclast function. A series of TFs controls osteoclast differentiation. Roberts et al[37] considered bone resorption at the PDL surface the rate-limiting step in OTM. Harada and Rodan[38] specified osteoprotegerin (OPG), cathepsin K, and chloride channel 7 (ClCN7) as rate-limiting agents for osteoclast differentiation and function. OPG blocks the TF receptor activator of nuclear factor kappa B (RANK) and RANK ligand (RANKL) docking, cathepsin K destroys bone matrix proteins, whereas chloride channel 7 maintains osteoclast neutrality by shuffling chloride ions through the cell membrane. Recently the ENCODE (ENCyclopedia Of DNA Elements) project has started identifying "all structural and functional elements of the human genome." Such researches will eventually permit correlation of patient genotype with clinical presentation and laboratory-derived protein profiles. This will allow orthodontists to identify biological promoters and inhibitors of OTM and plan molecular intervention to maximize adaptive response during OTM[39].

Conclusion

- The recent advances in researches related to OTM and bone remodeling have helped us better understand the molecular, cellular and genetic level interaction involved during OTM.
- Understanding OTM facilitates the orthodontists to apply biomechanics which is efficient for tooth movement and patient comfort.
- There is a need of more researches in genetic level so that gene level intervention could be done in OTM making it efficient and patient friendly.

References

1. Reitan K, Rygh P. Biomechanical principles and reactions. In:Graber RM, Vanarsdall RL, editors. Orthodontics—current principles and techniques. 2nd ed. St Louis: Mosby; 1994.
2. Reitan K. Tissue behavior during orthodontic tooth movement. Am J Orthod 1960; 46:881-90.
3. Schwarz AM. Tissue changes incident to orthodontic tooth movement. Int J Orthod 1932; 18:331-52.
4. Oppenheim A. Human tissue response to orthodontic intervention of short and long duration. Am J Orthod Oral Surg. 1942; 28: 263–301.
5. Reitan K. Clinical and histologic observations on tooth movement during and after orthodontic treatment. Am J Orthod. 1967; 53: 721–745.
6. Proffit WR. Biologic basis of orthodontic therapy. In: Proffit WR, Fields HW, editors. Contemporary orthodontics. 3rd ed. St Louis: Mosby; 2000.
7. Thilander B, Reitan K, et al: Tissue reactions in orthodontics. In: Graber TM, Vanarsdall RL, editors. Orthodontics: current principles and techniques. 3rd ed. St Louis: Mosby; 2000.
8. Gianelly AA. Force induced changes in the vascularity of the periodontal ligament. Am J Orthod 1969; 55:5-11.
9. Burstone CJ. The biomechanics of tooth movement. In: Kraus BS, Riedel RA, editors. Vistas in orthodontics. Philadelphia: Lea & Febiger; 1962.
10. Kohno T, Matsumoto Y, Kanno Z, Warita H, Soma K. Experimental tooth movement under light orthodontic forces— rates of tooth movement and changes of the periodontium. J Orthod 2002; 29:129-35.
11. Pilon JJAM, Kuijpers-Jagtman AM, Maltha JC. Magnitude of orthodontic forces and rate of bodily tooth movement: an experimental study in beagle dogs. Am J Orthod Dentofacial Orthop 1996; 110:16-23.
12. Owman-Moll P, Kurol J, Lundgren P. Effects of doubled orthodontic force magnitude on tooth movement and root resorption. Eur J Orthod 1996; 18:141-50.
13. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop 2006; 129: 469.e1 – 469.e32.
14. Baumrind S. A reconsideration of the property of the pressure tension hypothesis. Am J Orthod 1969; 55:12-22.
15. Kardos BT, Simpson LO. A new periodontal membrane biology based on thixotropic concepts. Am J Orthod 1980; 77:508-15.
16. Farrar JN. Irregularities of the teeth and their correction. Vol 1. New York: DeVinne Press; 1888: 658.
17. Grimm FM. Bone bending, a feature of orthodontic tooth movement. Am J Orthod 1972; 62:384-93.
18. Basset CAL, Becker RO. Generation of electric potentials by bone in response to mechanical stress. Science 1962; 137: 1063-5.
19. Zengo AN, Bassett CA, Pawluk RJ, Prountzos G. In vivo bioelectric potentials in the dentoalveolar complex. Am J Orthod 1974; 66:130-9.
20. Davidovitch Z, Finkelson MD, Steigman S, Shanfeld JL, Montgomery PC, Korostaff E. Electric currents, bone remodeling and orthodontic tooth movement. I—the effect of electric currents on periodontal nucleotides. Am J Orthod 1980; 77:14- 32.
21. Davidovitch Z, Finkelson MD, Steigman S, Shanfeld JL, Montgomery PC, Korostaff E. Electric currents, bone remodeling and orthodontic tooth movement. II—increase in rate of tooth movement and periodontal cyclic nucleotide levels by combined force and electric current. Am J Orthod 1980; 77: 33-47.
22. von Böhl M, Maltha JC, Von Den Hoff JW, Kuijpers-Jagtman AM. Focal hyalinization during experimental tooth movement in beagle dogs. Am J Orthod Dentofacial Orthop 2004; 125: 615-23.

23. von Böhl M, Maltha JC, Von den Hoff H, Kuijpers-Jagtman AM. Changes in the periodontal ligament after experimental tooth movement using high and low continous forces in beagle dogs. Angle Orthod 2004; 74:16-25.
24. Mostafa YA, Weaks-Dybrig M, Osdoby P. Orchestration of tooth movement. Am J Orthod 1983; 83:245-50.
25. Henneman S, Von den Hoff JW, Maltha JC. Mechanobiology of tooth movement. Eur J Orthod 2008; 30:299–306.
26. Weinbaum S, Cowin S C, Zeng Y. A model for the excitation of osteocytes by mechanical loading-induced bone fluid shear stresses. J Biomech. 1994; 27: 339 – 360.
27. Mak A F, Huang D T, Zhang J D, Tong P. Deformation-induced hierarchical flows and drag forces in bone canaliculi and matrix microporosity. J Biomech 1997; 30: 11 – 18.
28. Cowin S C, Weinbaum S, Zeng Y. A case for bone canaliculi as the anatomical site of strain generated potentials. J Biomech 1995; 28: 1281 – 1297.
29. Bonewald LF.Mechanosensation and transduction in osteocytes. Bonekey Osteovision 2006; 3 (10):7-15.
30. Tuncer BB, Ozmeric N, Tuncer C, Teoman I, Cakilci B, Yucel A, et al. Levels of interleukin-8 during tooth movement: Angle Orthod 2005;75:539-44.
31. Kurata K, Heino T J, Higaki H, Vaananen H K. Bone marrow cell differentiation induced by mechanically damaged osteocytes in 3D gel embedded culture. J Bone Miner Res 2006; 21: 616 – 625.
32. Toygara HU, Kircellib BH, Bulutc S, Sezgind N, Tasdelene B. Osteoprotegerin in gingival crevicular fluid under long-term continuous orthodontic force application. Angle Orthod 2008; 78(6):988-993.
33. Nahm D S, Kim H J, Mah J, Baek S H. In vitro expression of matrix metalloproteinase-1, tissue inhibitor of metalloproteinase-1 and transforming growth factor-beta1 in human periodontal ligament fibroblasts. Eur J Orthod 2004; 26: 129 – 135.
34. Rody WJ, King GJ, Gu G. Osteoclast recruitment to sites of compression in orthodontic tooth movement. Am J Orthod Dentofacial Orthop 2001; 120:477-89.
35. Nakagawa M, Kukita T, Nakasima A, Kurisu K. Expression of the type I collagen gene in rat periodontal ligament during tooth movement as revealed by in situ hybridization. Arch of Oral Biol 1994; 39: 289 – 294.
36. Masella RS,Meister M.Current concepts in the biology of orthodontic tooth movement. Am J Orthod Dentofacial Orthop 2006; 129:458-68.
37. Roberts WE, Huja S, Roberts JA. Bone modeling: biomechanics, molecular mechanisms, and clinical perspectives. Sem Orthod 2004; 10:123-61.
38. Harada S, Rodan GA. Control of osteoblast function and regulation of bone mass. Nature 2003; 423:349-54.
39. The ENCODE Project Consortium. The ENCODE (ENCyclopedia Of DNA Elements) Project. Science 2004; 306: 636-9.